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Generation of transgenic mouse models with expression of constitutively active STAT5A

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Master of Science (M.Sc.) **Harini Nivarthi**
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Referrent	**Prof. Dr. Med. Matthias Eder, Department of Hematology/Oncology MHH, Hannover**
Koreferrent	**Prof. Dr. Hans-Jörg Jacobsen Institut für Pflanzengenetik Leibniz Universität, Hannover**
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“Always look on the bright side of life”

- Monty Python, Life of Brian, 1979

ABSTRACT

STAT5 is a member of the JAK-STAT signaling pathway and plays an important role in proper differentiation and function of hematopoietic cells. Persistent activation of STAT5 is associated with development of many hematopoietic cancers. A constitutively active mutant of STAT5A ($cS5^F$) was shown to induce multi-lineage leukemia in a mouse bone marrow transplant model. However, use of retroviral vectors is associated with disadvantages. Therefore, a reliable, genetic mouse model with $cS5^F$ was required to study STAT5-mediated hematopoietic neoplasms and to question cooperation with other cancer drivers. We have generated and analyzed three transgenic mouse models with different levels of $cS5^F$ expression.

The first mouse model was generated for inducible $cS5^F$ expression, under the regulation of the endogenous *Stat5a* promoter, using BAC recombineering (icS5 mice). The mice were bred with $RosaCreER^{T2}$ mice which show Cre activity ubiquitously upon treatment with tamoxifen. We could demonstrate inducible expression of $cS5^F$ in the cells of hematopoietic and epithelial origin. Since the expression level of $cS5^F$ in these mice was very low, there were no discernible abnormalities in the hematopoietic system of these mice. However, co-operative mono-allelic loss of PTEN with $cS5^F$ expression led to a considerable increase in the total white blood cell (WBC) counts and mice are monitored for tumor development.

Two more transgenic mouse models were developed in which $cS5^F$ is expressed under the hematopoietic specific vav-promoter (vcS5 mice). The two lines showed different number of transgene integrations and proportional levels of $cS5^F$ expression, specifically in hematopoietic organs. Hematological analysis of the vcS5 mice showed increased WBC counts in both lines at 12 weeks of age. Interestingly, the platelet numbers and the hematocrit values remained unchanged. FACS analysis of the vcS5 mice with high $cS5^F$ expression showed highly elevated percentage of $CD8^+$ T-cells in peripheral lymphoid organs. The mice are currently monitored for development of hematopoietic neoplasms.

In summary, we have generated three transgenic mouse models, which will provide valuable tools to understand dose-dependent functions of STAT5A in hematopoietic cells and their transformation in association with other signaling pathways.

Keywords – STAT5, Transgenic, Mice

ZUSAMMENFASSUNG

STAT5 ist ein Signalmolekül des JAK-STAT Signalweges und spielt eine essentielle Rolle in der Differenzierung von hämatopoetischen Zellen und deren physiologischen Funktionen. Eine konstitutive Aktivierung von STAT5 wird jedoch mit einer malignen Transformation von hämatopoetischen Zellen assoziiert. In diesem Zusammenhang wurde gezeigt, dass die retrovirale Expression einer konstitutiv-aktiven STAT5A Mutante ($cS5^F$) zu der Entstehung einer “Multilineage“-Leukämie in einem murinen Knochmarkstransplantationsmodel führt. Die Verwendung von retroviralen Vektoren in Transplantationsmodellen weist allerdings einige Einschränkungen auf. Aus diesem Grund ist ein transgenes Mausmodell, das $cS5^F$ spezifisch in hämatopoetischen Zellen exprimiert, wesentlich für die Untersuchung von STAT5A-induzierten hämatopoetischen Neoplasmen als auch für die Identifizierung von potentiell mit STAT5A kooperierenden Onkogen-/Tumorsuppressor-Netzwerken.

Für die vorliegende Arbeit wurden daher drei verschiedene transgene Mausmodelle generiert, die unterschiedliche $cS5^F$ Expressionslevel aufweisen. Das erste Mausmodel wurde für eine induzierbare $cS5^F$ Expression unter der Kontrolle des endogenen *Stat5a* Promotors mittels eines BAC-Transgens generiert (icS5 Mäuse). Durch die Verpaarung von icS5 Mäusen mit der $RosaCreER^{T2}$ Mauslinie, welche nach Tamoxifen Behandlung die Cre Rekombinase ubiquitär exprimiert, konnte gezeigt werden, dass eine $cS5^F$ Expression in hämatopoetischen Zellen als auch in Epithelzellen induziert werden kann. Auf Grund einer sehr geringen Transgenexpression konnten allerdings keine Anomalien des hämatopoetischen Systems beobachtet werden. Durch einen zusätzlichen monoallelischen Verlust von PTEN führte die geringe $cS5^F$ Expression jedoch zu einer deutlichen Erhöhung der Leukozytenzahl (WBC). Des Weiteren wird diese Mauslinie zurzeit hinsichtlich einer malignen Transformation von hämatopoetischen Zellen untersucht.

Die zwei weiteren transgenen Mausmodelle exprimieren $cS5^F$ unter der Kontrolle des für hämatopoetische Zellen-spezifischen vav-Promotor (vcS5 Mäuse). Diese Mauslinien haben unterschiedlich viele Kopien des Transgenkonstrukts in ihrem Genom integriert und zeigen eine hierzu proportionale Expression von $cS5^F$. Im Alter von 12 Wochen weisen beide vcS5 Mauslinien eine Erhöhung des WBC bei unveränderter Thrombozytenzahl und Hämatokrit auf. Weiterhin zeigte die FACS Analyse der hochexprimierenden vcS5 Mauslinie eine erhebliche Erhöhung der $CD8^+$ T-Zellpopulation in peripheren lymphoiden Organen. Zurzeit wird diese Mauslinie

ebenfalls hinsichtlich einer malignen Transformation von hämatopoetischen Zellen überwacht.
Zusammengefasst wurden im Zuge dieser Arbeit drei cS5^{F}-transgene Mausmodelle generiert, welche in zukünftigen Studien zum besseren Verständnis der Funktionen von STAT5A in Abhängigkeit des Expressionslevels in hämatopoetischen Zellen herangezogen werden können. Des Weiteren ermöglichen diese Mausmodelle die Untersuchung der mit STAT5A kooperierenden Signalwege in der malignen Transformation von hämatopoetischen Zellen.
Schlagwörte – STAT5, Transgene, Mäuse

TABLE OF CONTENTS

ABBREVIATIONS

A	Ampere
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANOVA	Analysis of variance
ATLL	Adult T cell lymphoma/leukemia
B-ALL	B cell ALL
Bcl	B cell lymphoma
BM	Bone marrow
BMMC	Bone marrow-derived mast cell
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
CD	Cluster of differentiation
cDNA	Complementary DNA
CFU-E	Colony forming unit erythroid
CLP	Common lymphoid progenitor
CML	Chronic myeloid leukemia
CMML	Chronic myelo-monocytic leukemia
CMP	Common myeloid progenitor
CRLF2	Cytokine receptor-like factor 2
CTCL	Cutaneus T cell lymphoma
DC	Dendritic cell
ddH_2O	Double distilled water
DEPC	Diethylpyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DS-ALL	ALL associated with Down syndrome
DTT	Dithiothreitol
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EGF	Epithelial growth factor

EPO	Erythropoietin
ER	Endoplasmatic reticulum
ER^{T2}	Mutated estrogen receptor
ET	Essential thrombocythemia
FCS	Fetal calf serum
FLT3-ITD	Internal tandem duplications of fms-like tyrosine kinase receptor-3
GAB2	Grb2-associated binding protein 2
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GAS	γ-interferon-activated sequence
G-CSF	Granulocyte colony stimulating factor
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GFP	Green fluorescent protein
GH	Growth hormone
GM-CSF	Granulocyte-macrophage colony stimulating factor
GMP	Granulocytic-monocytic progenitor
GTP	Guanosine triphosphate
HA	Homology Arm
HCl	Hydrogen chloride
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HS	hypersensitive
HSC	Hematopoietic stem cell
HSC70	Heat shock protein 70
HTLV	Human T cell lymphoma virus
Ig	Immunoglobulin
IL	Interleukin
IP	immunoprecipitation
IP_3	Inositol triphosphate
IRES	Internal ribosome entry site
IRP	Iron-regulatory protein
JAK	Janus kinase
JMML	Juvenile myelomonocytic leukemia
kb	Kilobases

KCl	Potassium chloride
kDa	Kilodalton
KH_2PO_4	Monopotassium phosphate
$KHCO_3$	Potassium hydrogen carbonate
KIT-ITD	Internal tandem duplications of KIT receptor
KOH	Potassium hydroxide
LB medium	Luria-Bertani medium
LEC	Liver endothelial cell
LT	Long-term
m/v	Mass/volume
MAPK	Mitogen-activated protein kinase
MCP	Mast cell progenitor
MCS	Multiple cloning site
MEP	Megakaryocytic-erythroid progenitor
$MgCl_2$	Magnesium chloride
MGF	Mammary gland factor
$MgSO_4$	Magnesium sulfate
$MnCl_2$	Manganese(II) chloride
MOPS	3-(N-morpholino) propanesulfonic acid
MPD	Myeloproliferative disorders
MPP	Multi-potent progenitor
mRNA	Messenger RNA
µF	Microfarad
Na_2HPO_4	Disodium hydrogen phosphate
Na_3VO_3	Sodium orthovanadate
NaCl	Sodium chloride
NaF	Sodium fluoride
NaH_2PO_4	Sodium dihydrogen phosphate
NaOH	Sodium hydroxide
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor κB
NH_4Cl	Ammonium chloride
NK cell	Natural killer cell
NP-40	Nonyl phenoxypolyethoxylethanol

OD	Optical density
OSM	Oncostatin M
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
pBS	Plasmid Bluescript
PBST	Phosphate buffered saline Tween-20
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PDGFRβR	Platelet-derived growth factor receptor-β
PI3K	Phosphatidylinositol-3 kinase
PIAS	Protein inhibitor of inactivated Stat
PIP_2	Phosphatidylinositol bisphosphate
PIP_3	Phosphatidylinositol triphosphate
PMF	Primary myelofibrosis
pMSCV	Plasmid murine stem cell virus
PMSF	Phenylmethylsulfonyl fluoride
PRL	Prolactin
PTEN	Phosphatase and tensin homolog
PV	Polycythemia vera
RbCl	Rubidium chloride
RNA	Ribonucleic acid
Rnase	Ribonuclease
rpm	Revolutions per minute
RT	Real-time
RTK	Receptor tyrosine kinase
SCF	Stem cell factor
SDS	Sodium dodecyl sulfate
SH2	SRC homology-2
SOCS	Suppressor of cytokine signaling
SRC	sarcoma
SSC	Saline-sodium citrate
ST	Short-term
STAT	Signal transducer and activator of transcription
$Stat5^{null}$	Complete STAT5 knockout

$Stat5^{\Delta N}$	N-terminal truncated STAT5
SUMO	Small Ubiquitin-like modifier
Tx	Tamoxifen
TAE	Tris-acetate-EDTA
T-ALL	T cell ALL
TFB	Transformation buffer
Tfh	Follicular helper T cell
TfR-1	Transferrin-receptor 1
Th cell	T helper cell
Th17	T cells which produce IL-17
TPO	thrombopoietin
T_{regs}	Regulatory T cells
TSLP	Thymic stromal lymphopoietin
U	Unit
UTR	Untranslated region
UV	ultraviolet
V	Voltage
VEGF	Vascular endothelial growth factor
WBCs	White blood cells
WT	Wild-type
ZNF	Zink finger

1. INTRODUCTION

1.1. Overview of the JAK-STAT signaling pathway

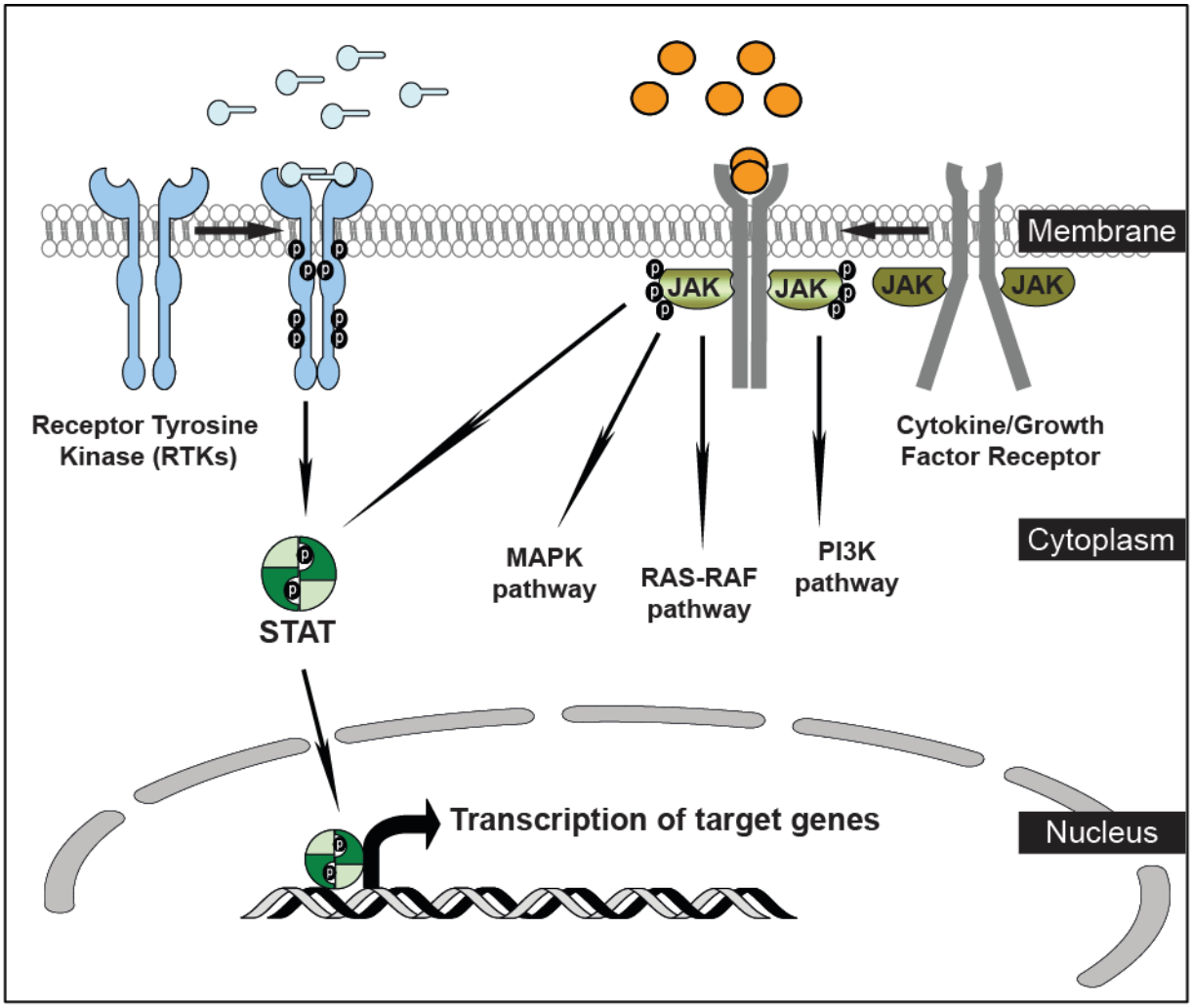

Figure 1. Overview of the JAK-STAT Signaling Pathway. The JAK-STAT signaling pathway is induced by the binding of cytokines/growth factors to the corresponding receptors, which leads to a conformation change in the corresponding receptors. This leads to mutual phosphorylation and activation of the JAK kinases associated with the receptors, which can further phosphorylate the respective STAT molecules. The phosphorylated, dimerized STAT molecules can translocate to the nucleus, where they bind DNA and activate transcription of the target genes. STAT molecules can also be activated by Receptor Tyrosine Kinases (RTKs), which have inherent kinase activity in their cytoplasmic domains, and can phosphorylate STATs. JAKs can also activate other signaling pathways, such as - the PI3K-AKT pathway, RAS-RAF pathway and the MAPK pathway by associating with different adaptor molecules.
Source: Self-made

The JAK-STAT signaling pathway is an elegant system of rapid intra-cellular signaling, in response to different cytokines and growth factors. It consists of two major signaling components – Janus Kinase (JAK) and Signal Transducer and Activator of Transcription (STAT). The JAKs are associated with the cytoplasmic domain of membrane bound receptors for cytokines/growth factors. Upon binding of the ligand, the receptors dimerise; which leads to a conformational change in their cytoplasmic domain, bringing the associated JAKs in close proximity to each other. This in turn, causes activation of the JAKs and mutual phosphorylation of the associated JAK molecules. The active phosphorylated JAKs can phosphorylate their respective STAT substrates, at a crucial tyrosine residue. Dimers of active phosphorylated STATs can translocate to the nucleus where they bind to specific DNA sequences and regulate expression of the target genes (Figure 1) [Leonard and O'Shea, 1998].

STATs can also be directly phosphorylated by other receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) and cellular kinases, such as c-src. The phosphorylation and the consequent activation of STATs are tightly regulated by the phosphatases like, SHP1/2, TC-PTP and CD45 [Bourdeau et al., 2007; Valentino and Pierre, 2006; Wu et al., 2009; Xu and Qu, 2008].

Negative regulation of the JAK-STAT signaling pathway is also mediated by suppressor of cytokine signaling (SOCS) proteins, which inhibit the activation of JAKs and down-regulate the expression of the receptors, by targeting them to degradation [Alexander and Hilton, 2004a]. The protein inhibitors of activated STAT (PIAS) also play an important role in the down-regulation of the JAK-STAT signaling, by blocking the DNA binding function of the STAT molecules [Shuai, 2006; Valentino and Pierre, 2006].

Mammalian genomes encode four different JAKs (JAK1, JAK2, JAK3 and Tyk2) and seven different STAT molecules (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B

and STAT6). The JAK-STAT signaling pathway plays a crucial role in many different physiological processes including embryonic development, cell survival, proliferation, differentiation, modulation of immune responses and transformation [Alexander and Hilton, 2004b; Imada and Leonard, 2000b]

1.2. Molecular Structure and Biology of STAT5

1.2.1. Structure of STAT5 and interacting partners

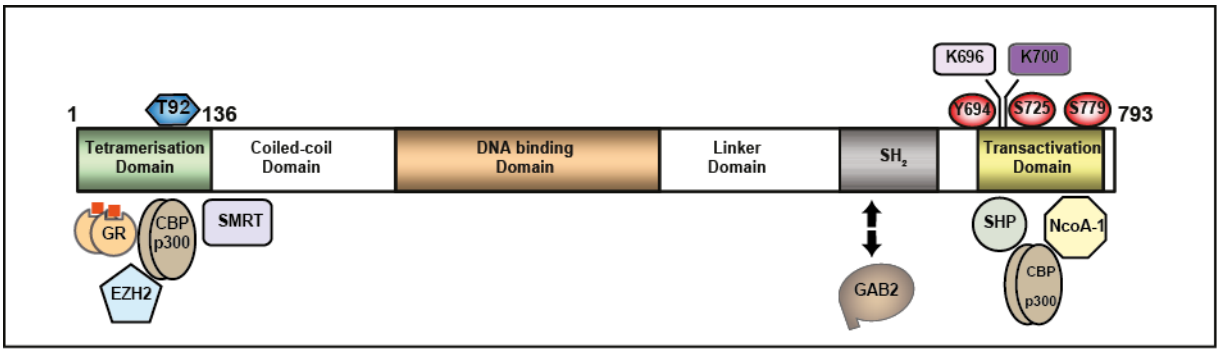

Figure 2. Schematic representation of molecular structure of STAT5A. The figure shows the structural domains of the STAT5A molecule. The important residues that are post-translationally modified are also highlighted. Phosphorylation of Tyrosine 694 is crucial for activation of the molecule. Serines 725 and 779 are aslo phosphorylated and are essential for the transforming capability of STAT5A. Threonine 92 is glycosylated and is required for the interaction of STAT5A with CBP/p300. The Lysines 696 and 700 can be sumoylated. However, K696 is also amenable to acetylation, and the presence of an acetyl- or SUMO- moeity on this critical amino acid determines the status of tyrosine phosphorylation at the Y696 residue.
The N-terminal region is an interaction site for the gluco-corticoid receptor (GR), the histone acetyl transferase CBP/p300 and EZH2 (catalytic subunit of the polycomb repressive complex2). STAT5A can also interact with SMRT, a nuclear co-repressor, at the N-terminal domain. STAT5A interacts with GAB2 (probably via an adapter protein), and induces the activation of the PI3K-AKT pathway. At the C-terminal region, STAT5A has interaction sites with co-factors - CBP/p300, and NCoA-1. It is also a docking site for the SHP phosphatases.
Source: Self-made

STAT5 has an N-terminal oligomerization domain, which is essential for the tetramerization of the STAT5 molecule [Moriggl et al., 2005]. This is followed by an alpha-helical coiled coil domain that is essential for interaction of STAT5 to different regulatory proteins. The DNA binding domain allows STAT5 to bind specific DNA

elements. The SH2 (Src-homology 2) domain determines the specificity of STAT5 to the cognate receptor and its association to the activating JAK. It also interacts with the phosphorylated tyrosine residue of the dimerization partner, stabilizing the dimer conformation of activated STAT5. The SH2 domain is attached, via a linker, to the transactivation domain, which interacts with different co-factors of STAT5 and is responsible for the activation of transcription of the target genes [Kisseleva et al., 2002; Kornfeld et al., 2008] (Figure 2). Constitutively active mutant forms of STAT5 have been identified, which upon phosphorylation, retain their active state for considerably long time, compared to the wild type STAT5 [Onishi et al., 1998].

STAT5 is amenable to many post-translational modifications, which regulate its function. The phosphorylation of the Tyrosine 694 is indispensable for the activation of STAT5 [Imada and Leonard, 2000a]. The N-terminus of STAT5 contains a glycosylation site, at a Threonine residue (T92), which is essential for the interaction of STAT5 with CBP/p300 [Gewinner et al., 2004]. The phosphorylation of two Serine moieties at the C-terminus (S725 and S779) is essential for the transforming potential of STAT5A [Friedbichler et al., 2010]. Recently, there have been reports showing the acetylation and sumoylation of the STAT5 molecules at two lysine residues, close to the Tyrosine phosphorylation site (K696 and K700 in STAT5A and K701 and K705 in STAT5B). In STAT5A, while K700 can only be sumoylated, K696 can undergo sumoylation and acetylation. Moreover, acetylated K696 promotes phosphorylation of STAT5A, but sumoylation of the same site inhibits it, providing an interesting mechanism for regulation of STAT5A activity [Van Nguyen et al., 2012].

The function of STAT5 depends on its ability to interact with various co-factors. STAT5 has two interaction sites with the histone acetylase CBP/p300, one at the N-

and another at the C-terminus [Ferbeyre and Moriggl, 2011; Pfitzner et al., 1998]. STAT5 also interacts with another chromatin modifying enzyme EZH2 via its N-terminal region. This interaction is essential for the repression of the Igκ locus during B-cell development, and is associated with the tetramer complex of STAT5 [Mandal et al., 2011]. The N-terminus is also an important site for interaction of STAT5 with co-factors like the glucocorticoid receptor (GR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). Interaction of STAT5 with GR is very important for expression of genes that regulate body growth and metabolism in liver [Engblom et al., 2007b; Kornfeld et al., 2008]. SMRT is a nuclear co-repressor which inhibits STAT5 mediated transcription [Nakajima et al., 2001]. STAT5 also interacts with Grb2-associated binding protein2 (GAB2) leading to activation of the PI3K-AKT pathway and the RAS-MAPK pathway. However, the exact mechanism of this interaction has not been elucidated, and probably involves another adaptor molecule [Nyga et al., 2005]. At the c-terminus, STAT5 has docking sites for the SHP phosphatases [Xu and Qu, 2008], which dephosphorylate STAT5. STAT5 also interacts with another nuclear co-factor (NCoA-1), via its α-helical region in the transactivation domain [Litterst et al., 2003].

1.2.2. Biology and functions of STAT5

The Signal Transducer and Activator of Transcription 5 (STAT5) was first discovered in mammary glands and was labeled as mammary gland-specific transcription factor (MGF). Its activity was shown to be induced by lactogenic hormones and MGF was described as the inducer of the transcription of the milk proteins like ß-casein and Whey Acidic Protein [Wakao et al., 1992]. About two years later, MGF was identified as a member of the cytokine inducible STAT family of transcription factors, activated by

tyrosine phosphorylation [Wakao et al., 1994]. Within a short time, another isoform of STAT5 was discovered and the two isoforms have been called STAT5A and STAT5B [Azam et al., 1995b]. The genes encoding the two isoforms are located adjacent to each other on chromosomes #11 and #17, in mice and humans, respectively. The two genes have unique as well as redundant functions in different tissues; notable among them are the mammary glands, liver and the hematopoietic system [Ferbeyre and Moriggl, 2011]. The functions of STAT5A and STAT5B in different organs have been assessed using a variety of transgenic mice (Table 1).

STAT5A plays a very important role in the development of the mammary gland during pregnancy. STAT5A (but not STAT5B) knockout mice show normal ductal architecture, but fail to develop lobular alveolar units during late pregnancy and lactation [Wagner and Rui, 2008]. STAT5B is the major isoform expressed in the liver and signals downstream of the growth hormone (GH). Disruption of STAT5 in hepatocytes leads to decreased circulating levels of insulin-like growth factor 1 (IGF1), leading to retardation in body growth. The mice also develop GH resistance and severe non-alcoholic hepato-steatosis [Engblom et al., 2007a; Hennighausen and Robinson, 2008; Mueller et al., 2011]. STAT5 plays a crucial role in the development of various lineages of the hematopoietic system. It also regulates the maintenance and survival of the hematopoietic stem cells (HSCs). Persistent activation of STAT5 has been associated with a variety of hematopoietic neoplasms [Bunting, 2007; Ferbeyre and Moriggl, 2011].

1.2.3. Table1. STAT5 transgenic mice

Strain	Description	References
Loss of STAT5 function		
***Stat5a*$^{-/-}$**	**Disruption of the promoter sequences and the first 3 exons of *Stat5a***	**[Liu et al., 1997]**
***Stat5b*$^{-/-}$**	**Interruption of *Stat5b* at codon 181**	**[Udy et al., 1997]**
***Stat5a*$^{\Delta N}$**	**Disruption of the N-terminus of *Stat5a***	**[Teglund et al., 1998]**
***Stat5b*$^{\Delta N}$**	**Disruption of the N-terminus of *Stat5b***	**[Teglund et al., 1998]**
***Stat5a/b*$^{\Delta N}$**	**Disruption of the N-termini of *Stat5a* and *Stat5b* genes**	**[Teglund et al., 1998]**
***Stat5ab*$^{fl/fl}$**	**Targeting of the entire *Stat5a/Stat5b* locus with loxP sites**	**[Cui et al., 2004]**
***Stat5*^{null}**	**Germ line deletion of *Stat5a/b* locus in *Stat5ab*$^{fl/fl}$ oocytes with MMTV-Cre mice [Wagner et al., 1997]**	**[Cui et al., 2004]**
***Stat5*^{DKI}**	**Replacement of *Stat5a* and *Stat5b* with corresponding double point mutations (I28A and F81A), to create Double Knock In (DKI) mice**	**[Lin et al., 2012]**

Gain of Stat5 function

***Stat5b*-tg**	**Over-expression of wild type Stat5b under H-2Kb promoter and IgM enhancer, preferential expression in T-cells, B-cells and NK-cells**	**[Kelly et al., 2003a]**
***Stat5b-CA*-tg**	**Over-expression of constitutively active Stat5b-1*6 [Onishi et al., 1998] under the Lck proximal promoter and IgM enhancer**	**[Burchill et al., 2003]**
Eμ-cS5^F	**Over-expression of cS5^F in B-cells under the regulation of Eμ enhancer**	**[Joliot et al., 2006]**
TetO-cS5^F	**Expression of cS5^F under regulation of doxycycline inducible - 'Tet- on' promoter**	**[Creamer et al., 2010]**
SL/Kh strain	**Integration of endogenous murine leukemia virus in the second intron of STAT5A.**	**[Tsuruyama et al., 2002]**

1.3. Hematopoietic system

Hematopoiesis (derived from the Greek words 'hema – blood' and 'poieo – create') is the process of generation of blood cells. All the cells of the hematopoietic lineage are derived from HSCs. The HSCs can be identified as cells that are positive for the cell surface markers Sca-1 and c-kit; and are negative for any of the differentiated lineage markers (lineage$^-$, Sca-1$^+$ and c-kit$^+$; 'LSK' cells) . This population includes the so-called 'Long-term' and the 'Short-term' HSCs (LT-HSCs and ST-HSCs); and the multi-potent progenitors (MPPs). These progenitors can give rise to the Common

Lymphoid Progenitors (CLP) and the Common Myeloid Progenitors (CMP) which can further divide to give rise to the lymphoid and myeloid lineages, respectively [Orkin and Zon, 2008; Weissman, 2000] .

The hematopoietic cells can be broadly classified into two major types (Figure 3):-

- Myeloid cells – The myeloid cells consist of erythroid cells, megakaryocytes, granulocytes, macrophages, mast cells, dendritic cells and platelets. The erythroid cells are the transporters of oxygen in the blood. The other myeloid cells are usually associated with the innate immune system and perform diverse functions, including phagocytosis, modulation of the adaptive immune response and blood clotting. They are derived from common myeloid progenitors (CMPs).
- Lymphoid cells – The lymphoid cells consist of T-cells, B-cells and NK-cells. They represent the cells of adaptive immune response. These cells develop from the common lymphoid progenitors (CLPs).

STAT5 plays a crucial and indispensable function in the development and differentiation of a variety of hematopoietic cells (Figure 3).

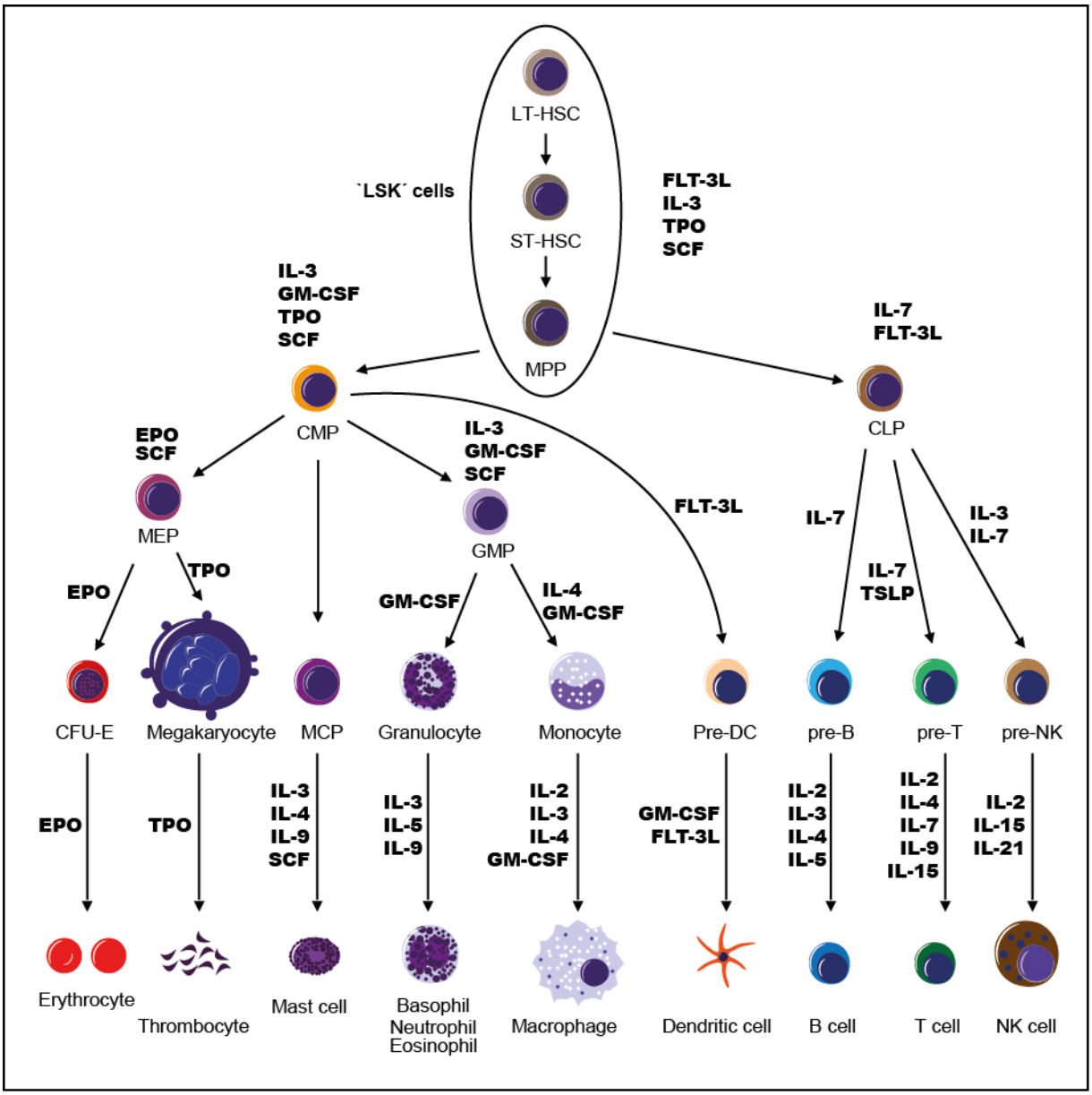

Figure 3. Schematic overview of hematopoiesis. The HSCs give rise to all the cells of the hematopoietic lineage. The figure shows the cytokines and growth factors which signal via STAT5, highlighting the indispensable role of STAT5 in hematopoietic differentiation. CFU-E: Colony Forming Unit- Erythroid, DC: Dendrtic Cells, EPO: Erythropoietin, GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor, IL: Interleukin, MCP: Mast Cell Progenitor, SCF: Stem Cell Factor, TPO: Thrombopoietin, TSLP: Thymic Stromal Lymphopoietin

Source: Self-made

1.3.1. STAT5 functions in hematopoietic stem cells

The first STAT5 knockout mice generated lack the N-termini of both STAT5 proteins [Teglund et al., 1998] and are now recognized as *Stat5*$^{\Delta N}$ mice expressing hypomorphic alleles. The N-terminus of STAT5 is the docking platform for the glucocorticoid receptor and it provides the oligomerization domain for STAT dimer and tetramer interaction on chromatin with different chromatin modifying enzymes [Ferbeyre and Moriggl, 2011] (Figure 2). *Stat5a/b*$^{\Delta N}$ mice have normal numbers of HSCs and the cells are capable of engrafting into lethally irradiated hosts [Bunting et al., 2002]. However, their reconstitution ability is highly reduced in competitive transplantation assays, suggesting a defect in the ability of the HSCs to 'self-renew' [Bradley et al., 2002]. Once *Stat5a/b*-deficient mice (*Stat5*^{null}) [Cui et al., 2004] were available, competitive reconstitution experiments with fetal liver cells showed a very drastic defect in their repopulation capacity in the absence of STAT5 [Li et al., 2007; Yao et al., 2006]. The differentiation and 'self-renewal' capabilities of progenitor cells is also reduced in *Stat5a/b*$^{\Delta N}$ mice, as assayed by the number and size of colony forming units [Bunting et al., 2002]. The N-terminus of STAT5 is essential for the suppression of the microRNAs miR15/16 and the induction of anti-apoptotic genes *Bcl-2* and *Bcl-x*$_L$, which are required for survival of HSCs [Li et al., 2010a; Li et al., 2007]. The above data clearly indicate a crucial role for STAT5 in the maintenance and renewal of HSCs. A role for activated STAT5 (pYSTAT5) is now emerging in hematopoietic cancer stem cells. Down regulation of STAT5 expression, by RNA interference or deletion of STAT5, impairs the long-term expansion of leukemic stem/progenitor cells in primary acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) [Schepers et al., 2007; Scherr et al., 2006]. STAT5B activity has been shown to be linked to leukemia initiating cells in MN1 and HOXA9 expressing AML cell lines [Heuser et al., 2009]. In AML patients, STAT5 activation by the mutant receptor tyrosine kinase, FMS-like

tyrosine kinase-3 with internal tandem duplications (FLT3-ITD), leads to high expression of the pro-survival gene Mcl-1 which promotes the survival of leukemic stem cells (LSCs) [Yoshimoto et al., 2009]. STAT5 activation in the HSCs of patients with truncated granulocyte colony stimulating factor (G-CSF) receptor provides them with a clonal advantage that can progress to AML or myeloproliferative neoplasm (MPN). These patients suffer from severe congenital neutropenia and are treated with exogenous G-CSF to boost neutrophil numbers [Liu et al., 2008]. Interestingly, the activation of STAT5 by cytokines in the stem cells of AML patients shows a high degree of heterogeneity and does not correlate with the surface expression of the cytokine receptors [Han et al., 2009]. Further studies are required to understand the differential activation of STAT5 by cytokines in LSCs compared to normal HSCs.

1.3.2. STAT5 as a key regulator for myelo- and erythropoiesis

STAT5 is the key signaling molecule downstream of a variety of myeloid cytokines and growth factors including interleukins (IL-3, IL-5) [Azam et al., 1995a; Mui et al., 1995], thrombopoietin (TPO) [Bacon et al., 1995], erythropoietin (EPO) [Gouilleux et al., 1995], stem cell factor (SCF) [Ryan et al., 1997], fms-like tyrosine kinase-3 ligand (Flt3L) [Zhang et al., 2000], G-CSF [Tian et al., 1996] and granulocyte macrophage colony stimulating factor (GM-CSF) [Mui et al., 1995].

Stat5a/b$^{\Delta N}$ embryos display anemia that can be partially rescued by ectopic expression of the survival genes *Bcl-2* or *Bcl-*X_L*, in vitro* [Dolznig et al., 2006]. However, the *Stat5*^{null} embryos die during definitive erythropoiesis on pure C57Bl/6 or Balb/c backgrounds [Cui et al., 2004]. Interestingly, in a mixed background, STAT3 activity can compensate for the function of STAT5 in erythropoiesis and a few mice survive. Compound deletion of STAT5 and STAT3 is required to provide conclusive proof for

the compensation of STAT5 requirement by STAT3 during definitive erythropoiesis. However, the surviving *Stat5*^{null} mice are severely sick and display dwarfism, autoimmune disorders and neutrophil infiltration in organs [Hoelbl et al., 2006]. The defect in erythropoiesis in *Stat5*^{null} erythroid progenitors is due to their inability to absorb iron efficiently; as they have reduced expression of the transferrin receptor (CD71) and iron regulatory protein-2, which are direct targets of STAT5 [Kerenyi et al., 2008]. The key role of STAT5 in erythropoiesis is highlighted by the fact that the expression of constitutively activated STAT5 (cS5) in *Jak2*$^{-/-}$ and *EpoR*$^{-/-}$ fetal liver cells leads to the development of functional erythroblasts in transplantation and colony forming assays [Grebien et al., 2008].

The progenitors derived from the bone marrow of *Stat5*$^{\Delta N}$ mice are deficient in the ability to give rise to myeloid colonies [Bunting, 2007; Teglund et al., 1998]. The mast cells in these mice are not only drastically reduced [Shelburne et al., 2003], but they also show functional defects in degranulation upon IgE binding [Barnstein et al., 2006]. This phenotype is aggravated in *Stat5*^{null} mice, and re-expression of STAT5A in fetal liver cells restores their capability to differentiate into functional mast cells *in vitro* [Li et al., 2007]. The *Stat5*$^{\Delta N}$ mice also suffer from thrombocytopenia, due to defects in TPO signaling [Bradley et al., 2002; Bunting et al., 2002]. Notably, high levels of persistently active STAT5 were observed in the bone marrow megakaryocytes in mice [Harir et al., 2008]. The physiological relevance of this observation is yet to be elucidated. STAT5 is also essential for the differentiation of eosinophils upon IL-5 treatment, in mice and in humans [Buitenhuis et al., 2003; Zhu et al., 2004]. STAT5 plays an interesting ambivalent role in granulopoiesis. While STAT5 is required cell intrinsically for the survival of granulocytes, it represses G-CSF production in liver endothelial cells (LECs). During inflammation, STAT5 is rapidly degraded in the LECs to induce G-CSF production [Fievez et al., 2007]. Mice lacking STAT5 in hematopoietic cells have reduced numbers

of neutrophils. Especially under myelosuppressive conditions, these mice are unable to produce higher numbers of neutrophils and to respond to GM-CSF [Kimura et al., 2009].

1.3.3. STAT5 signaling in lymphocytes

STAT5 is crucial for signaling by major lymphoid cytokines like IL-2 [Wakao et al., 1995], IL-4, IL-7 [Lin et al., 1995], IL-9 [Demoulin et al., 1996], IL-15 and IL-21 [Giliani et al., 2005; Habib et al., 2002]. It is also activated by the cytokine thymic stromal lymphopoietin (TSLP) [Isaksen et al., 1999] which plays a role in B-cell development and T-helper 2 cell (Th2) polarization [Kang and Der, 2004]. *Stat5*^{null} mice exhibit a severe combined immunodeficiency phenotype reminiscent of γ_c [Cao et al., 1995], JAK3 [Thomis et al., 1995] and IL-7Rα [Maki et al., 1996] deficient mice [Yao et al., 2006]. Thus, STAT5 plays a crucial role at various steps during the differentiation of lymphocytes (Figure 4) [Heltemes-Harris et al., 2011b].

1.3.3.1. STAT5-regulated B-cell development

B-cell development is dependent on IL-7 signaling, as indicated by the complete lack of mature B-cells in IL-7$^{-/-}$and IL-7R$\alpha^{-/-}$ mice as reviewed by [Malin et al., 2010a]. Expression of constitutively active STAT5B (*Stat5B-CA*) in the lymphoid cells can rescue B-cell development in IL-7R$\alpha^{-/-}$ mice. The expansion of the pro-B-cells is associated with the expression of the STAT5 target genes *cyclin D2, pim-1 and Bcl-x_L*, suggesting a role for STAT5 in the survival and proliferation of pro-B-cells [Goetz et al., 2004]. B-cell development in *Stat5*$^{\Delta N}$ mice is only mildly affected [Sexl et al., 2000], but *Stat5*^{null} mice show a drastic reduction in mature B-cell numbers due to a

developmental block at the pre-pro-B-cell stage [Dai et al., 2007; Hoelbl et al., 2006; Yao et al., 2006]. Earlier studies suggested a direct transcriptional regulation of Pax5 and Ebf1 by STAT5 [Dai et al., 2007; Hirokawa et al., 2003]. However, this observation has been contradicted by later studies. In fact, the levels of Pax5 and Ebf1 were found to be quite normal in BCL-2 rescued STAT5 deficient pro-B-cells, suggesting a more permissive role of STAT5 in B-cell development. Indeed, the apoptosis of STAT5 deficient pro-B-cells is due to aberrant expression of the Igκ light chain, as STAT5 represses the recombination of this locus [Malin et al., 2010b].

TSLP induces pYSTAT5 and promotes the differentiation of fetal liver cells to immature B-cells. The TSLP receptor complex includes the IL-7Rα and early studies implied that STAT5 activation by TSLP in a pre-B-cell line is independent of the JAK kinases [Levin et al., 1999]. However, recent publications have shown that activation of STAT5 by TSLP in $CD4^+$ T-cells and mouse embryonic fibroblasts requires the activation of JAK1 and JAK2 [Wohlmann et al., 2010].

STAT5 was reported to play an important role in the generation of memory B-cells. The memory B-cells in the germinal centers of human tonsils were shown to express significant levels of pYSTAT5. Knockdown of STAT5 by shRNA decreased proliferation of human Burkitt lymphoma cell line. Moreover, overexpression of constitutively active STAT5B in primary human B-cells dramatically increased their survival and expansion in culture, by directly up-regulating the expression of the pro-survival gene *Bcl-6* [Scheeren et al., 2005]. However, STAT5 seems to be dispensable for activation of B-cells in mice, as deletion of STAT5 in mature B-cells (using CD23-Cre and Aicda-Cre transgenic lines) does not affect the normal numbers of follicular and marginal zone B-cells. The B-cells lacking STAT5 are also able to differentiate into plasma cells that can develop to functional memory B-cells [Malin et al., 2010b].

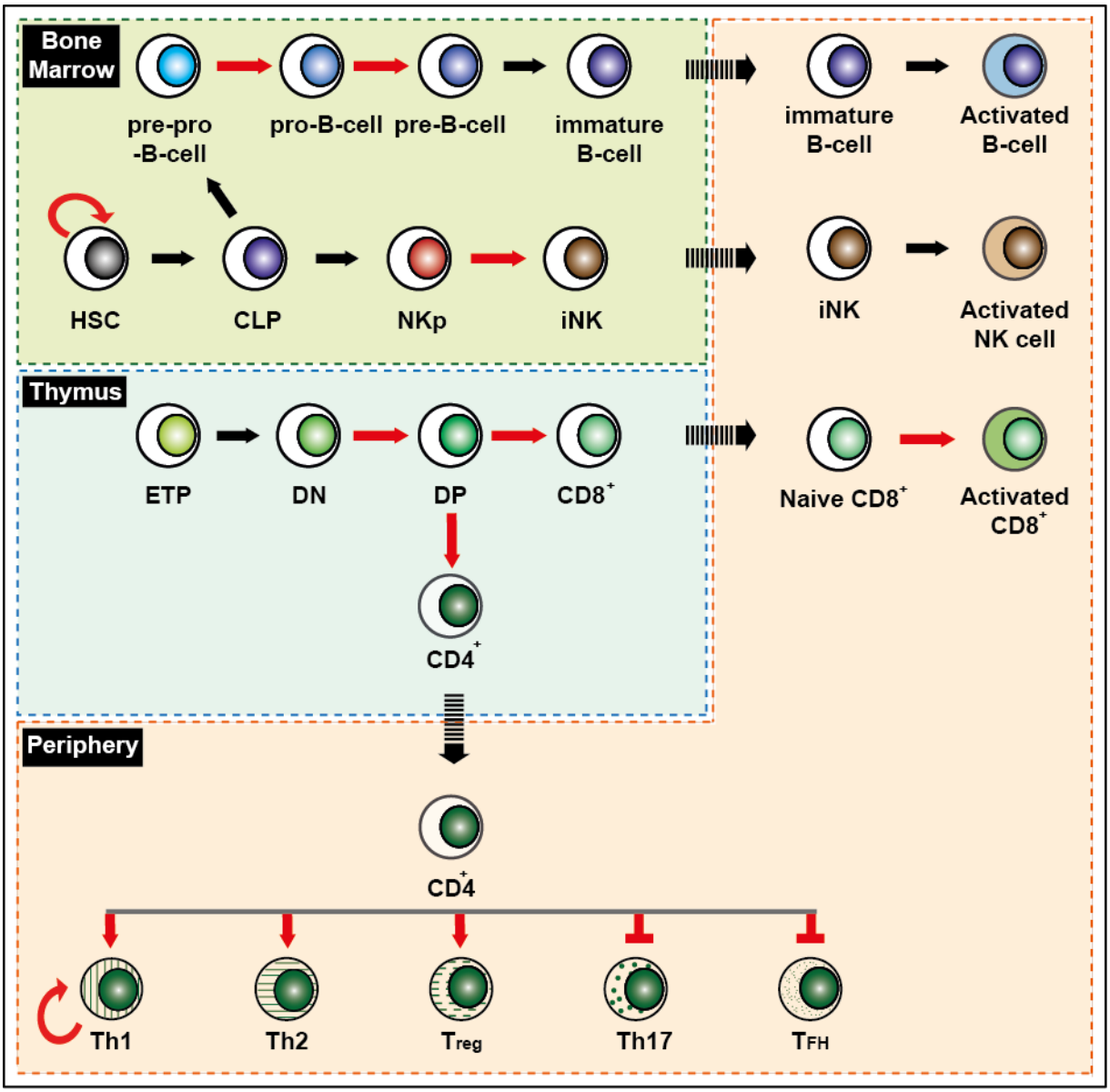

Figure 4. Role of STAT5 in lymphocyte differentiation. The figure is a schematic representation of differentiation of lymphocytes. The STAT5 regulated steps are marked in red. STAT5 is essential for the survival and expansion of the pro-B-cells, in the bone marrow. The differentiation of NK cells from the progenitors is dependent on STAT5. In the thymus, STAT5 is indispensable for the generation of $CD4^+$ and $CD8^+$ cells from the ETPs (early thymic progenitors). IL-2 mediated activation of Stat5 is required for the activation and proliferation of $CD8^+$ cells. The $CD4^+$ cells can differentiate into five different lineages, in the periphral lymphoid organs. STAT5 mediates Th1 differentiation by regulating chromatin modification of the IFN-γ locus.The concerted action of Stat5 and Gata-3 leads to the differentiation of the Th2 subset. Stat5 directly regulates FoxP3 transcription which is the driver of T_{reg} differentiation. STAT5 represses the differentiation of Th17 and T_{FH} cells.
Source: Self-made

1.3.3.2. STAT5-regulated T-cell development

STAT5 plays an important role in the differentiation and function of T-cell subsets. While the Th1 subset is essential for cell mediated immunity accomplished by the cytolytic activity of $CD8^+$ T-cells; the Th2 subset is crucial for mounting a humoral response against extracellular pathogens [Zhu and Paul, 2008]. The *Stat5*$^{\Delta N}$ mice show a mild reduction in the number of $CD8^+$ T-cells in the periphery, but normal thymocyte and γδ T-cell numbers [Moriggl et al., 1999]. However, the analysis of *Stat5*^{null} mice showed a massive reduction of thymocyte numbers, which results in a severe reduction in $CD8^+$ T-cells and a complete absence of γδ T-cells [Hoelbl et al., 2006; Yao et al., 2006]. Deletion of STAT5 in $CD4^+CD8^+$ double positive thymocytes also leads to a severe reduction of $CD8^+$ T-cells [Hoelbl et al., 2006]. Interestingly, STAT5 regulates differentiation of $CD8^+$ T-cells in a dose dependent manner [Ermakova et al., 2011]. In fact, the IL-7-STAT5 mediated signaling pathway, which leads to the induction of the transcription factor Runx3 and survival signals by *Bcl-2*, can even circumvent the requirement for the T-cell receptor (TCR) signaling for differentiation of $CD8^+$ T-cells [Park et al., 2010]. Transgenic mice with ectopic expression of wild type STAT5B (*Stat5b-tg*) in lymphoid cells show an expansion of $CD8^+$ T-cells [Kelly et al., 2003a]; while those that express STAT5B-CA (*Stat5b-CA-tg*) show an increase in the number of $CD8^+$ and γδ T-cells [Burchill et al., 2003]. It has been shown that peripheral T-cells of *Stat5*$^{\Delta N}$ mice are highly deficient in proliferation upon stimulation with IL-2 and IL-4 despite normal TCR activation [Moriggl et al., 1999]. Recently, new transgenic mice have been generated, which have two point mutations, in the N-terminal domain, (I28A and F81A, called Double Knock In, DKI mice) in both STAT5A and STAT5B genes. These mice are incapable of forming STAT5 tetramers, but can still form dimers. The

Stat5^{DKI} mice have reduced numbers of CD8^{+} T-cells, and show impaired IL-2 response; implying the requirement of STAT5 tetramers for proper differentiation and function of CD8^{+} T-cells [Lin et al., 2012]. Moreover, CD8^{+} T-cells from untreated HIV^{+} patients show decreased expression of STAT5 mRNA and protein and are also deficient in their ability to activate STAT5 upon IL-7 stimulation [Vranjkovic et al., 2010].

Naive CD4^{+} T-cells can undergo five distinct cellular fates: Th1, Th2, T_{regs} (regulatory T-cells), Th17 and T_{FH} (Follicular helper T-cells) [Fazilleau et al., 2009; Lee et al., 2011; Wilke et al., 2011; Zhu and Paul, 2008]. Differentiation of Th1 cells is induced by the combined action of IL-12 and IFN-γ, which leads to further amplification of the signal by induction of IFN-γ, leading to the induction of the Th1 specific transcription factor T-bet [Schoenborn and Wilson, 2007; Zhu and Paul, 2008]. It has been reported that JAK3 mediated STAT5 activation regulates chromatin remodeling at the *Ifng* locus leading to its increased accessibility to T-bet, implying an essential role for STAT5 during the early stage of Th1 differentiation [Shi et al., 2008].

Th2 differentiation is dependent on TCR stimulation, IL-4 and IL-2 signaling. IL-4 leads to the activation of the transcription factors GATA3, STAT6 and STAT5, while IL-2 signaling predominantly activates STAT5. These transcription factors interact in a complex manner to determine cell fate decisions of Th subsets [Zhu, 2010]. Early studies showed that IL-2 can induce the expression of the high affinity IL-2Rα chain, also known as CD25. This induction is mediated by STAT5, along with GATA family proteins [John et al., 1996]. Moreover, IL-2 signaling 'primes' T-cells to Th2 differentiation by inducing and maintaining the expression of the IL-4Rα chain via STAT5 [Liao et al., 2008]. Profound defects in the differentiation of Th2 cells were identified in *Stat5a*$^{-/-}$ mice [Kagami et al., 2001]. STAT5A and GATA3 directly bind the promoter regions of *Il1rl1* [Guo et al., 2009] which encodes the receptor IL-33Rα. Upon binding its ligand, IL-33Rα induces the production of IL-13, which further amplifies

the Th2 responses [Oboki et al., 2010]. Interestingly, GATA3 can up-regulate CD25 expression [Hwang et al., 2002] and STAT5 maintains GATA3 expression in Th2 cells [Guo et al., 2009]. STAT5 is also activated in naive $CD4^+$ T-cells upon TSLP treatment, where it promotes their survival and proliferation [Rochman et al., 2010]. STAT5 also assists Th2 differentiation by epigenetic modification of the *Il4/Il13* gene locus and is required for IL-4-induced Th2 priming [Zhu et al., 2003].

STAT5 deficient mice are incapable of generating $CD4^+CD25^+$ regulatory T-cells (T_{regs}). STAT5 is a direct transcriptional regulator of Foxp3 and CD25, the key molecules required for T_{regs} differentiation [Yao et al., 2007]. Interestingly, tetramerization of STAT5 is essential for the differentiation of regulatory T-cells as the *Stat5*^{DKI} mice are also deficient in T_{regs} [Lin et al., 2012].

Two new subsets of T-cells, Th17 and T_{FH} have been described recently [Korn et al., 2009]. Th17 cells have been shown to play an important role in the regulation of auto immune disorders. Addition of IL-2 to *in vitro* differentiation culture systems, leads to inhibition of Th17 differentiation. IL-2 deficient mice also have higher number of Th17 cells. Moreover, mice lacking STAT5 in T-cells (*Stat5*$^{fl/fl}$;CD4-Cre) develop more Th17 cells, implying that IL-2 inhibits Th17 differentiation in a STAT5 dependent manner [Laurence et al., 2007]. In fact, STAT5 displaces STAT3 from the promoter region of *Il17* and directly suppresses the transcription. The balance between the amount of STAT5 and STAT3 determines the differentiation of a cell to Th17 lineage [Yang et al., 2011]. However, the molecular mechanism that regulates the activating vs. repressing functions of STAT5 at different promoters remains in the dark [Levy and Marie, 2012]. Considering the opposing roles of STAT5 in the development of T_{regs} and Th17 cells, it has been suggested that the auto-immune phenotype seen in the *Stat5*$^{\Delta N}$ and *Stat5*^{null} mice could be due to a skewed ratio of T_{regs} to Th17 cells.

As the name suggests, T_{FH} cells are seen in the follicular region of the germinal centers and regulate the development of antigen specific B-cells. These cells are identified to be $CXCR5^+$ and produce abundant levels of IL-21 [Fazilleau et al., 2009]. Two independent groups have recently reported that STAT5 negatively regulates differentiation and function of T_{FH} probably by repressing the transcription factor *Bcl-6* [Johnston et al., 2012; Nurieva et al., 2012].

The cytokines IL-7 and IL-15 (both of which can signal via STAT5) are essential for the survival and maintenance of $CD8^+$ memory T-cells [Osborne and Abraham, 2010]. Michael Farrar and colleagues were the first to provide direct evidence for the role of STAT5 in generation of memory T-cells. They showed that transgenic mice expressing either wild type STAT5B or constitutively active STAT5B in the lymphoid compartment have markedly increased numbers of $CD8^+$ memory T-cells [Burchill et al., 2003; Kelly et al., 2003a]. Recent experiments have shown that $CD8^+$ T-cells transduced with retroviruses expressing cS5 are able to expand dramatically more than the control cells in a LCMV infection model [Hand et al., 2010], implying an important role for STAT5 in the generation and/or maintenance of memory T-cells.

1.3.3.3. STAT5-regulated NK cell development

The development of NK cells is regulated primarily by the cytokines IL-2, IL-15 and IL-21. $IL\text{-}15^{-/-}$ and $IL\text{-}15R\alpha^{-/-}$ mice show a drastic reduction in the number of peripheral NK cells [Zwirner and Domaica, 2010]. $Stat5^{\Delta N}$ mice display reduction in the number of NK cells [Moriggl et al., 1999]. $Stat5^{null}$ mice show reduced NK cell numbers and impaired cytolytic activity of whole splenocyte cultures [Imada et al., 1998]. Moreover, conditional deletion of STAT5 in NK cell progenitors (using a novel Ncr1-cre mouse) abrogates their ability to differentiate into immature NK cells [Eckelhart et

al., 2011]. A STAT5 binding site has been identified in the promoter of the human perforin gene suggesting a role for STAT5 in regulating cytotoxic functions of NK cells and of T-cells [Yu et al., 1999]. A case study of two male siblings has been reported who have a four nucleotide deletion (CTCC, position 424-427) in the STAT5B mRNA. These patients suffer from growth hormone insensitivity, as STAT5B is indispensable for growth hormone signaling in the liver. Notably, the patients also suffer from lymphopenia and particularly from reduced numbers of NK cells [Pugliese-Pires et al., 2010].

1.4. Role of STAT5 in hematopoietic cancer development and progression

1.4.1. Persistent activation of STAT5 in hematopoietic neoplasms

STAT5 has been shown to play a crucial role in the generation of a variety of hematopoietic neoplasms (Table 2). Aberrant activation of STAT5 can render the proliferation of many hematopoietic cells factor independent [Grebien et al., 2008; Moriggl et al., 2005], which is a hallmark of oncogenic transformation. Moreover, a persistently activated mutated STAT5A ($cS5^F$) [Onishi et al., 1998] has been used to show its role in the generation of hematopoietic malignancies in mouse bone marrow transplant models [Moriggl et al., 2005]. Interestingly, the leukemogenic potential of $cS5^F$ is critically dependent on the phosphorylation of two serine molecules in the C-terminus [Friedbichler et al., 2010]. Intriguingly, most of the activating mutations in patients have been found in upstream kinases and receptors, but not in STAT5 itself (Figure 6). The best studied of these mutations is the BCR/ABL translocation that leads to a persistent activation of STAT5.

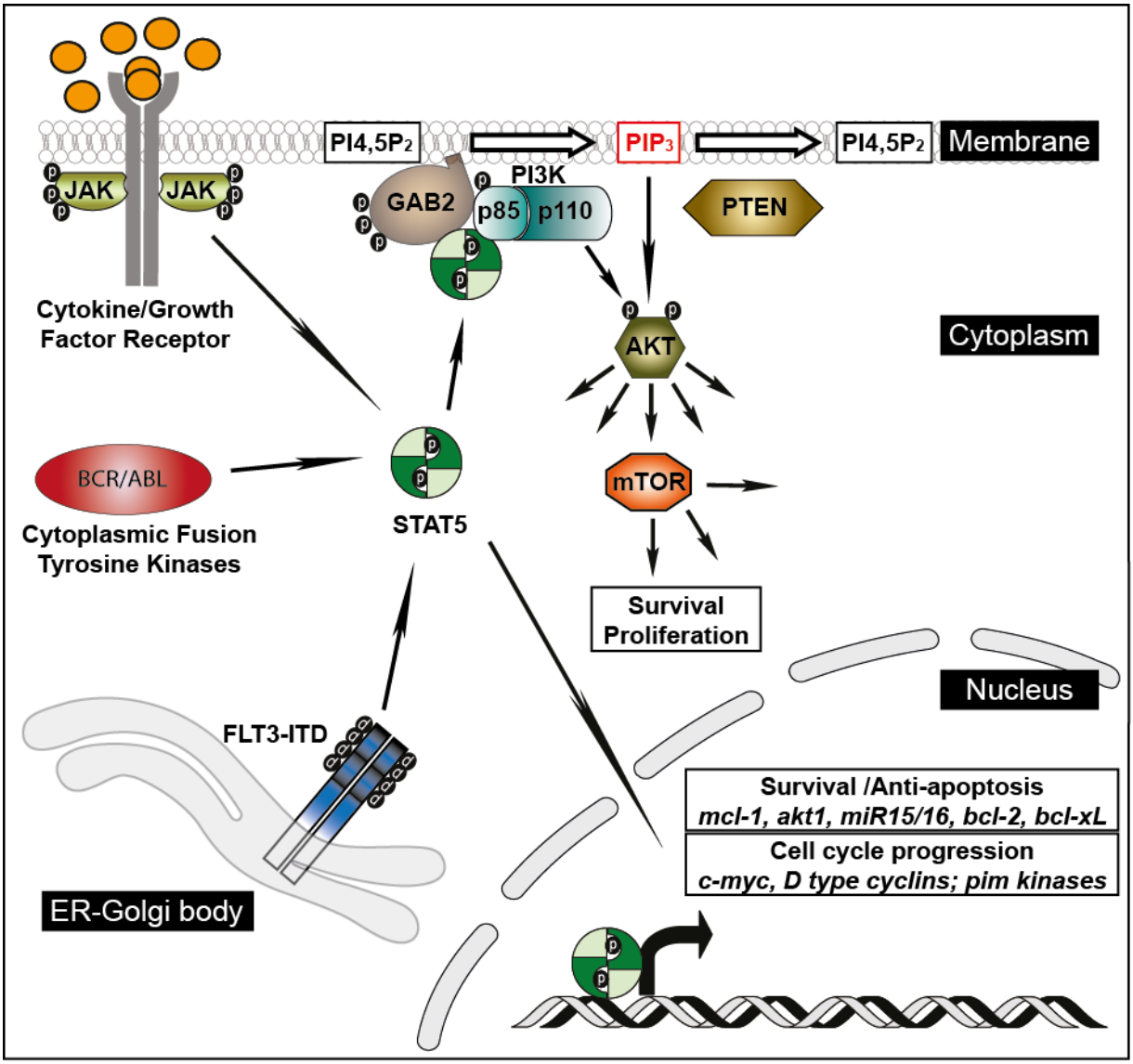

Figure 5. STAT5 Activation and Oncogenic Function. STAT5 can be activated by many different mechanisms. It is phosphorylated by receptor tyrosine kinases and JAK kinases associated with the cytokine and growth factor receptors, at the membrane. In the cytoplasm, it can be activated by oncogenic fusion tyrosine kinases such as BCR/ABL. Other oncogenic receptor tyrosine kinases such as FLT3-ITD phosphorylate STAT5 at the endoplasmic reticulum (ER) – Golgi body. STAT5 is primarily a transcription factor and upon activation it migrates to the nucleus where it stimulates the transcription of its target genes. Stat5 targets include anti-apoptotic genes, such as *bcl-2, bcl-xL, mcl-1* and *survivin*. It induces expression of *D type cyclins, c-myc* and *akt1*, genes that promote cell cycle progression. Some targets of STAT5, like *egfr, pim kinases* and *c-myc*, have been implicated in oncogenic pathways. In the cytoplasm, activated STAT5 can interact with GAB2 which leads to the activation of the PI3K-AKT pathway, via generation of phosphatidyl inositol trisphosphate (PIP3). The AKT pathway also results in the transcription of survival and proliferation genes, which further augments the transforming potential of STAT5. This pathway is negatively regulated by the PTEN phosphatase.
Source: Self-made

1.4.2. Table 2. Mutations leading to persistent STAT5 activation in hematopoietic neoplasms

Mutation	Disease	Reference
Fusion Tyrosine Kinases		
BCR/ABL (p185)	**Acute lymphoblastic leukemia (ALL)**	**[Chan et al., 1987; Okuda et al., 1996]**
BCR/ABL (p210)	**Chronic myeloid leukemia (CML)** **Acute myeloid leukemia (AML)** **Erythroleukemia**	**[Carlesso et al., 1996]** **[Price et al., 1988]** **[Okuda et al., 1996]**
BCR/JAK2	**Chronic myeloid leukemia (CML)** **Acute myeloid leukemia (AML)**	**[Griesinger et al., 2005]** **[Cirmena et al., 2008; Cuesta-Dominguez et al., 2012]**
TEL/ABL	**Acute lymphoblastic leukemia (ALL)**	**[Okuda et al., 1996; Papadopoulos et al., 1995]**
TEL/JAK2	**Acute lymphoblastic leukemia (ALL)**	**[Ho et al., 1999; Lacronique et al., 1997; Peeters et al., 1997]**
ETV6/LYN	**Myelofibrosis**	**[Takeda et al., 2011; Tanaka et al., 2010]**
E ML1/ABL	**Acute lymphoblastic leukemia (ALL)**	**[De Keersmaecker et al., 2005]**
TEL/PDGFRβ	**Chronic myelo-monocytic leukemia (CMML)**	**[Golub et al., 1994; Wilbanks et al., 2000]**
FIP1L1/PDGFRα	**Eosinophilia**	**[Buitenhuis et al., 2007; Cools et al., 2003]**
ZNF198/FGFR1	**Stem cell leukemia lymphoma syndrome**	**[Heath and Cross, 2004; Xiao et al., 1998]**

Receptor Tyrosine Kinases		
FLT3-ITD	**Acute myeloid leukemia (AML)**	**[Hayakawa et al., 2000; Nakao et al., 1996]**
KIT-ITD	**Acute myeloid leukemia (AML)**	**[Corbacioglu et al., 2006]**
KIT^{D816V}	**Acute myeloid leukemia (AML) Mastocytosis**	**[Harir et al., 2008; Nagata et al., 1995]**
Truncated G-CSFR	**Acute myeloid leukemia (AML)**	**[Dong et al., 1997; Liu et al., 2008]**
JAK mutations		
JAK1^{V658F}, JAK1^{A634D}, JAK1^{R879C}, JAK1^{V658F}	**Acute lymphoblastic leukemia (ALL)**	**[Flex et al., 2008; Hornakova et al., 2009; Jeong et al., 2008]**
JAK2^{V617F}	**Polycythemia vera, Myelofibrosis, Essential thrombocythemia**	**[Aboudola et al., 2007; Kralovics et al., 2005]**
JAK2^{T875N}	**Megakaryoblastic leukemia**	**[Mercher et al., 2006]**
JAK2$^{R683S/G}$	**Megakaryoblastic leukemia**	**[Kearney et al., 2009]**
JAK2 exon 12	**Polycythemia vera, Myelofibrosis,**	**[Scott et al., 2007]**
JAK3^{A572V}	**Acute myeloid leukemia (AML) Megakaryoblastic leukemia**	**[Walters et al., 2006]**
Other transforming events		
IL-2 and IL-2R amplifications	**Cutaneous T-cell lymphoma (CTCL) Sezary syndrome**	**[Vermeer et al., 2008; Zhang et al., 1996]**
HTLV-1 infection	**Adult Tcell-lymphoma/leukemia (ATLL)**	**[Migone et al., 1995; Takemoto et al., 1997; Tomita et al., 2006]**
Amplification of IL-21 signaling	**Hodgkin's lymphoma**	**[Scheeren et al., 2008]**
IL-7Rα mutations	**Childhood ALL**	**[Shochat et al., 2011; Zenatti et al., 2011]**

It has been shown that STAT5 is not only required for initiation of the leukemia but it is indispensable for leukemia maintenance, thereby identifying STAT5 as a target for leukemia therapy [Hoelbl et al., 2006; Hoelbl et al., 2010; Kovacic et al., 2012]. The expression of STAT5 mRNA and protein differs in the transformation stages of diseased myeloid cells in CML patients. In fact, higher STAT5 levels contribute to resistance to tyrosine kinase inhibitors [Warsch et al., 2011]. STAT5 has been implicated in leukemia/lymphomas induced by a variety of fusion tyrosine kinases, such as TEL/JAK2, NPM/ALK, TEL/ABL and TEL/PDGFRβ (Table 2). A comprehensive study with these fusion tyrosine kinases showed that STAT5 mediated over-expression of Rad51 (involved in dsDNA break repair by homologous recombination repair mechanism) is one of the important contributors for the resistance of the malignant cells against DNA damage inducing drugs like cisplatin and mitomycin [Slupianek et al., 2002].

Activating mutations in JAK kinases, which lead to persistent activation of STAT5, have been identified in multiple hematopoietic neoplasms (Table 1). However, the activation of STAT5 is not limited to JAK kinases localized at the cell membrane, but certain mutated growth factor receptors such as KIT-ITD and FLT-3ITD can activate STAT5 at the endoplasmic reticulum (ER)-Golgi membrane network [Choudhary et al., 2009]. In a similar manner, the STAT5 pathway has also been successfully hijacked by the human T-cell leukemia virus (HTLV) to induce adult T-cell leukemia/lymphoma [Nicot et al., 2001]. STAT5 activation was identified in cutaneous T-cell lymphoma and Sézary syndrome. IL-2 signaling pathway components are amplified in the genome of the malignant cells from these patients [Vermeer et al., 2008]. Recent reports have also shown activating mutation in IL-7Rα in childhood ALL [Shochat et al., 2011; Zenatti et al., 2011]

Recently, STAT5 has been reported to be present in the mitochondria of murine lymphoma cell line (LSTRA) and a murine hematopoietic progenitor cell line (Ba/F3), where it binds the D-loop regulatory region of mitochondrial DNA. The authors hypothesize that this might be the mechanism for a shift in metabolism of these cancer cells, known as Warburg effect [Chueh et al., 2010]; but further proof from other types of cancer is needed to understand the physiological relevance of this observation. STAT5 can transcribe genes implicated in mitochondrial function, such as *c-myc, akt1, glut1, Bcl-2 and Bcl-x_L*, which indicates that STAT5 might also regulate the metabolism of cancer cells [Ferbeyre and Moriggl, 2011].

1.5. STAT5-PI3K-AKT interaction

The PI3K (PhosphatidyiIiositol-3-Kinase), belongs to a large group of lipid kinases, which are activated by a variety of receptor tyrosine kinases and intracellular proteins, such as Protein Kinase C, Rac, Rho and Src. As is evident by its name, activated PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3), on the cell membrane. PIP_3 is a substrate of the Phospholipase C (PLC), which cleaves it to generate the second messenger inositol triphosphate (IP3). However, membrane bound PIP_3 can bind proteins containing the Pleckstrin Homology (PH) domain. This leads to recruitment of AKT, also known as Protein Kinase B (PKB), to the membrane; where it is subsequently phosphorylated by the PI3K [Hay, 2005; Hennessy et al., 2005]. Activated AKT can phosphorylate a myriad of downstream signaling molecules to regulate many different physiological processes:

- AKT promotes cell survival and inhibits apoptosis [Song et al., 2005].
- AKT mediates cell cycle progression [Chang et al., 2003].
- AKT facilitates growth and anabolic metabolism [Gonzalez and McGraw, 2009].
- AKT promotes tumor angiogenesis [Chen et al., 2005].

One of the important downstream signaling molecules in the PI3K-AKT pathway is mTOR (mechanistic target of rapamycin). It was first identified as the target of the drug rapamycin by genetic screens in yeast [Cafferkey et al., 1993]. However, it has been shown that rapamycin inhibits mTOR by binding to a suppressor of mTOR pathway (FKBP12) and activating it [Sabatini et al., 1994]. mTOR is a serine/threonine kinase, and is a direct target of PI3K. It can interact with several protein to form 2 distinct complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2). mTORC1 integrates the signals from nutrients, energy, growth factors and stress. It is activated by a variety of signals including Insulin, amino acids and oxidative stress. mTORC2 is also regulated by the same signals, but its major functions involves modulation of cellular cytoskeleton [Laplante and Sabatini, 2012].

The PI3K/AKT pathway is negatively regulated by the lipid phosphatase PTEN (Phosphatase and Tensin Homolog), which dephosphorylates PIP_3 to PIP_2. Loss of function of PTEN leads to persistent activation of the AKT signaling, which has been shown to result in the development of cancers in a variety of tissues. In fact, it is one of the most commonly mutated or deleted tumor suppressor genes in human cancers [Carracedo et al., 2011]. Various mouse models have been developed to study the loss of PTEN in different tissues [Suzuki et al., 2008].

1.5.1. Table 3. Co-activation of STAT5-AKT in hematopoietic neoplasms

Disease	Description	Reference
Chronic Myelomonocytic Leukemia (CMML)	**Complete transformation of Ba/F3 cells by TEL/PDGFβR fusion protein requires the engagement of the PI3K and PLCγ and activation of STAT5**	**[Sternberg et al., 2001]**
Childhood AML	**KIT-ITD expression in Ba/F3 cells leads to constitutive activation of the PI3K and STAT signaling pathways. Synergistic inhibition of cell growth by imatinib and rapamycin**	**[Corbacioglu et al., 2006]**
Mastocytosis	**Constitutive activation of STAT5 and AKT signaling in patients with KIT^{D816V} mutation**	**[Harir et al., 2008]**
Ph^+ ALL	**Imatinib resistance in Ph+ ALL cells is due to down-regulation of PTEN due to promoter hypermethylation**	**[Montiel-Duarte et al., 2008]**
AML	**FLT3-ITD induced Factor independent growth is due to concomitant activation of STAT5 and the PI3K-AKT pathways**	**[Masson et al., 2009]**
CML	**Down-regulation of PTEN by BCR/ABL contributes to early development of leukemia**	**[Peng et al., 2010]**
MPN	**Elevated phosphorylation of STAT5 and AKT in the megakaryocytes of patients with the JAK2 V617F mutation**	**[Grimwade et al., 2009]**
MPN	**Synergistic signaling by STAT5 and AKT-mTOR pathways in leukemic cells**	**[Li et al., 2010b]**

Interestingly, a cross-talk was discovered between the STAT5 and the PI3K pathway, when Harir *et al*. reported the presence of pYSTAT5 in the cytoplasm of murine and human leukemic cells. They also showed that pYSTAT5 forms a complex with the

myristoylated, membrane anchored and scaffolding protein GAB2 (Grb2-associated binding protein2) and PI3K in the cytoplasm. This leads to activation of the PI3K-AKT pathway [Harir et al., 2007]. Earlier, it had been shown that concomitant knockdown of BCR/ABL and GAB2 induces stronger inhibition of the growth of primary CML patient cells, compared to respective individual knock down [Scherr et al., 2006]. Moreover, while GAB2 and STAT5 have non-redundant functions in the maintenance of HSCs, they were shown to act synergistically to promote self-renewal [Li et al., 2010c]. Importantly, simultaneous inhibition of STAT5 and AKT-PI3K pathway resulted in a drastic reduction in the survival and proliferation of leukemic cells; advocating the use of combination drugs to inhibit these pathways as a therapeutic approach for human MPNs [Corbacioglu et al., 2006; Li et al., 2010b].

In lymphomas, elevated IL7-Rα signaling was shown to mediate resistance to mTOR inhibitors in pre-B ALL cell lines [Brown et al., 2007]. A recent report indicates that the concerted action of STAT5-PI3K-mTORC1 signaling pathways leads to upregulation of telomerase expression in leukemic cells obtained from patients suffering from adult T-cell leukemia (ATL) by transcriptional and post-transcriptional mechanisms [Yamada et al., 2012].

To further analyze the role of STAT5 and to identify its transforming partners, we decided to generate transgenic mice with inducible expression of constitutively active STAT5A, under the endogenous promoter. To attain this, we needed a large vector that could accommodate the entire STAT5A regulatory locus. Therefore, we decided to use BAC recombineering techniques, to generate a BAC transgenic mouse model.

1.6. BAC recombineering technology

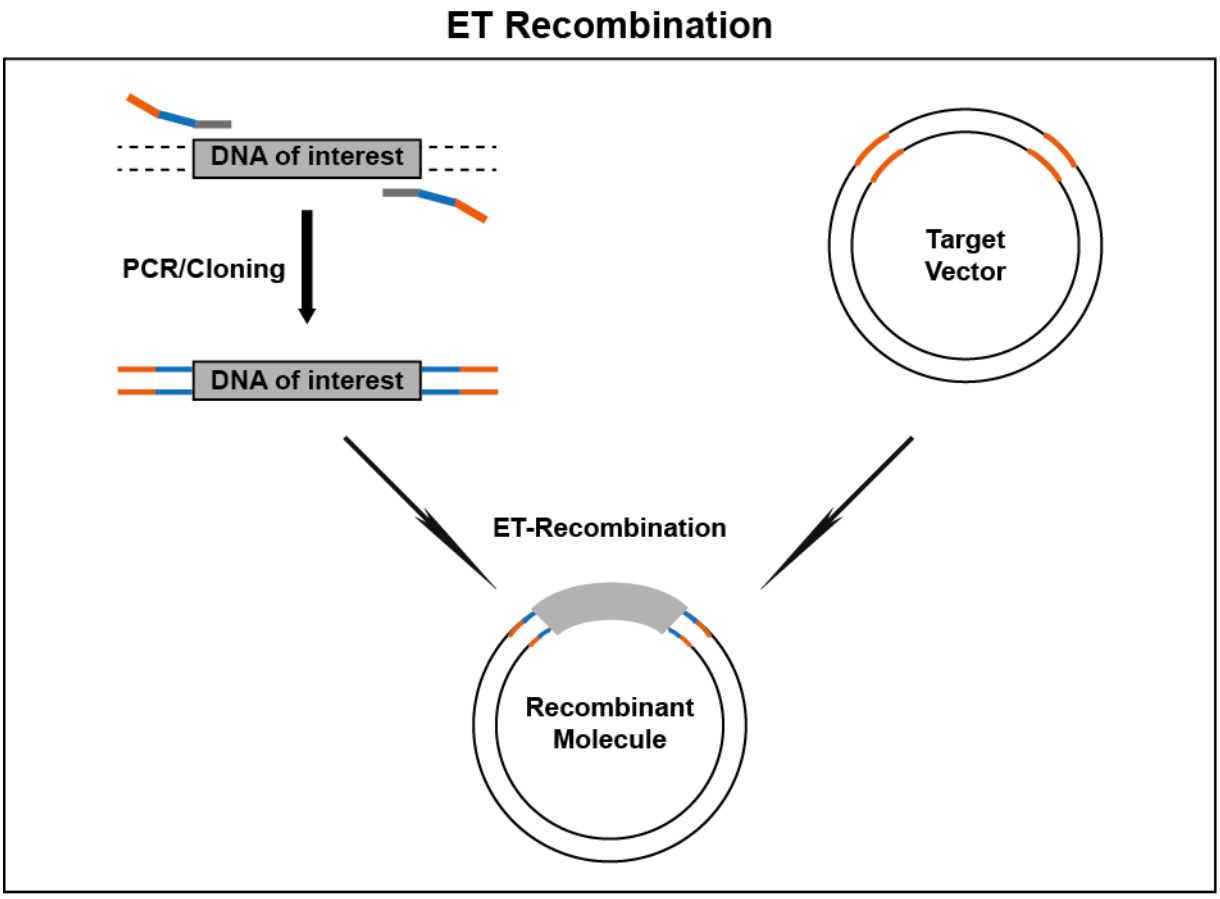

Figure 6. ET-recombination. Recombination of the DNA of interest into the target molecule occurs via homologous `arms´ (shown in orange), and is mediated by the phage derived enzymes - *recE* and *recT*.
Source: Self-made

Generation of transgenic mice is associated with many technical hurdles. While cloning of the vector, pronuclear injections and derivation of transgenic mice is relatively well-established, expression of the transgenic construct is not always as desired. The random, stochastic integration of the the transgenic construct is highly influenced by the positional effects of the chromatin. Moreover, the mice display mosaic expression of the transgene, which might even be silenced over time [Giraldo and Montoliu, 2001]. One way to avoid this problem is to use insulators or other DNA sequences which prevent the influence of the neighboring genes and regulatory

elements at the site of integration [Antoniou et al., 2003; Pikaart et al., 1998]. Another possibility is to target the vector into the endogenous locus with the desired expression pattern. However, the homologous recombination into a specific locus is a very rare event in somatic cells.

The use of Bacterial Artificial Chromosomes (BAC) allows one to overcome these problems. BAC recombineering was pioneered by the lab of A. Francis Stewart and Neil G. Copeland, using the phage derived recombinases *recE* and *recT* (hence also known as ET recombination). Recombination occurs through homologous arms (HA) and very large stretches of DNA can be recombined specifically and precisely [Lee et al., 2001; Muyrers et al., 1999; Zhang et al., 1998] (Figure 7).

The advantages of using BAC vectors over conventional plasmid targeting vectors: -

- As BAC vectors can be very large in size (up to hundreds of kilo bases), they can accommodate very large loci, which can include all (or at least most) of the regulatory elements required for controlled expression of the gene of interest.
- Using BAC vectors can overcome the chromatin positional effects to allow stable and reliable expression of the transgenic construct.
- The expression levels of the gene of interest are usually proportionate to the number of integrations.

Therefore, we used BAC recombineering techniques to generate a $cS5^{F}$ transgenic mouse model, under the regulation of endogenous promoter.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Primers and oligonucleotides

Primers were obtained from Eurofins MWG/Operon. Sequences are written 5' to 3'.

Name/Description	Sequence
Southern analysis	
cS5-5'HA probe-FP	GGCTGTTTACCTGGGGGA
cS5-5'HA probe-FP	GAGAGAAGGGCAGCCTCGAG
cS5-hCD2 probe-FP	GGTGCAGTCTCCAAAGAG
cS5-hCD2 probe-RP	GATATCCTGATCATCGGTC
cS5-ind probe-FP	GATGCTGGGAGAGAGCTTAAG
cS5-ind probe-FP	GGATGCCCTGAATCACTCTTG
Stat5a-exon 14 FP	GGCAGGGTGCCATTTGCTGTG
Stat5a-exon 14 RP	CCGGTTGAACTGGGACCAGGA
Genotyping primers	
cS5-gen-FP	AGGCGACCATCATCAGCGAGC
cS5-gen-RP	GAATGGAGAAATCTCGCGTCG
Cre-gen-FP	CGGTCGATGCAACGAGTGATGAGG
Cre-gen-RP	CCAGAGACGGAAATCCATCGCTCG
EMSA	
B-casRe sense	AGATTTCTAGGAATTCAAATC
B-casRe antisense	GATTTGAATTCCTAGAAATCT

Real time PCR

Gapdh FP	AGAAGGTGGTGAAGCAGGCATC
Gapdh RP	CGGCATCGAAGGTGGAAGAGTG
Ccnd2 FP	AGAAGGGGCTAGCAGATGA
Ccnd2 RP	AGGATGATGAAGTGAACACA
Bcl-2 FP	ACTGAGTACCTGAACCGGCATC
Bcl-2 RP	GGAGAAATCAAACAGAGGTCGC
Mcl-1 FP	GGAGATCATCTCGCGCTACTTG
Mcl-1 RP	ATCCTGCCCCAGTTTGTTACG
c-Myc FP	GTGCTGCATGAGGAGACACCG
c-Myc RP	ATGGAGATGAGCCCGACTCCG

2.1.2. Antibodies

Western Blotting

Antibody	Company	Dilution
Anti-FLAG M2 monoclonal antibody (mouse)	SIGMA, F 3165	1:10000
pYSTAT5 (rabbit)	Zymed, # 71-6900	1:1000
α-STAT5a rabbit polyclonal antiserum	self-generated against the extreme C-terminus	1:5000
HSC70 mouse monoclonal IgG	Santa Cruz, B-6: sc-7298	1:10000
ECL™ Anti-mouse IgG Horseradish Peroxidase linked whole antibody (sheep)	GE Healthcare	1:10000
ECL™ Anti-rabbit IgG Horseradish Peroxidase linked whole antibody (from donkey)	GE Healthcare	1:10000

FACS analysis

Flourochrome	Ebiosciences	BD pharmingen
FITC	CD4, c-kit, Sca-1, CD11b	
PE	CD8α, IL7Rα, FcRγ	CD19, hCD2
PerCP-cy5.5	CD3e, CD8α, CD11b, Streptavidin	
PE-Cy7	IL7Rα	
APC	CD8α, GR1, CD49b	c-kit,
Biotin		mouse linage panel, CD90.2
Unconjugated		Fc-Block™

EMSA

Antibody	Company	Dilution
Anti-FLAG M2 monoclonal antibody (mouse)	SIGMA, F 3165	1:20
α-STAT5 (C-17)	Santa Cruz	1:20

2.1.3. Recipes for buffers and solutions

Working with bacteria

Antibiotics

All antibiotics were purchased from Sigma.

Antibiotic	**Final concentration**
Ampicillin	100 µg/ml
Kanamycin	25 µg/ml
Chloramphenicol	12.5 µg/ml

LB medium

20 g LB Broth in 1 liter of H_2O

autoclave

LB agar

1 tablet LB agar in 50 ml H_2O

autoclave

TFBI

30 mM potassium acetate

100 mM RBcl

10 mM $CaCl_2$

50 mM $MnCl_2$

15% glycerol

adjust pH to 5.8 with acetic acid

and filter sterilize

TFBII

10 mM MOPS

75 mM $CaCl_2$

10 mM RBcl

15% glycerol

adjust pH to 6.5 with KOH

and filter sterilize

DNA analysis

50x TAE for agarose gels, 1 liter

2 M Tris-Base

57.1 ml acetic acid

0.5 M EDTA (pH 8.0)

6x DNA loading dye, 50 ml

125 mg Bromphenol blue

125 mg Xylencyanol FF

15 ml glycerol

fill up with H_2O to 50 ml

Southern Blotting

Hybridization buffer, 500 ml

171 ml 1 M Na_2HPO_4

79 ml of 1 M NaH_2PO_4

175 ml 20% SDS

1 ml of 0.5 M EDTA (pH 8)

Washing buffer, 2 liters

54.7 ml 1 M Na_2HPO_4

25.28 ml 1 M NaH_2PO_4

100 ml 20% SDS

4 ml 0.5 M EDTA

20x SSC

0.3 M NaCl

0.3 M Nacitrate

Adjust pH to 7.0 with HCl

RNA analysis

10x MOPS buffer

400 mM MOPS

100 mM sodium acetate

10 mM EDTA

filll up to 1 liter with DEPC treated H_2O

DEPC treated H_2O

1 ml DEPC

dissolve with constant stirring

autoclave

RNA sample buffer

10 ml deionized Formamide

3.5 ml Formaldehyde

2 ml of 10x MOPS buffer

RNA loading dye

50% Glycerol

0.2% Bromophenol blue

1 mg/ml Ethidium bromide

Protein analysis

IP-buffer stock solution

25 mM HEPES pH 7.5

25 mM Tris-HCl pH 7.5

150 mM NaCl

10 mM EDTA

0.1% Tween-20

0.5% NP-40

10 mM beta-glycerolphosphate

6x Laemmli buffer, 20 ml

2 g DTT

4 ml 20% SDS

1 ml of 1 M Tris (pH 6.8)

8 ml Glycerol

12 mg of bromophenol blue

fill up with H_2O to 20 ml, store at -20°C

Proteinase- and phosphatase-inhibitors were added fresh to the IP-buffer:

Inhibitor	Stock concentration	Concentration in IP-buffer
Proteinase Inhibitor Cocktail (PIC)	1/2 tablet/ml	1 tablet for 50 ml
Aprotinin	1 mg/ml	10 µg/ml
PMSF	100 mM	1 mM
NaF	500 mM	1 mM
Na_3VO_4	100 mM	1 mM
Leupeptin	1 mg/ml	10 µg/ml

Composition of SDS-polyacrylamide gels.

Compound	8% Separating gel	Stacking gel
1.5 M Tris pH 8.9	5 ml	-
1 M Tris pH 6.7	-	0.8 ml
30% Bis-acrylamide	5.3 ml	0.8 ml
10% SDS	200 µl	60 µl
10% APS	200 µl	60 µl
Temed	10 µl	4 µl
ddH_2O	9.2 ml	4.3 ml

Western blotting

10x Tris-Glycine buffer

0.25 mM Tris

1.92 M Glycine

Ponceau S solution

0.1% Ponceau S

5% Acetic acid

For 1x running buffer:

1x Tris-Glycine buffer
0.1% SDS
Fill up with ddH_2O
adjust pH to 8.3

For 1x transfer buffer:

1x Tris-Glycine buffer
20% Methanol
Fill up with ddH2O

Stripping buffer, 100 ml

1 M Tris-HCl (pH 6.8)
0.675 ml β-Mercaptoethanol
10 ml 20% SDS

1x PBST

.1% Tween in 1x PBS

EMSA

Binding buffer (5X)

50 mM Tris
5 mM DTT
1 mM PMSF
0.5mM EDTA
250 mM NaCl
25% Glycerol
0.5% KPO4

BSA solution

10 µg BSA
20 mM KPO4
50 mM NaCl
0.1 mM EDTA
5% glycerol

Annealing buffer (10x)

5X AmpliTaq Buffer
7.5 mM $MgCl_2$

10 x TBE (Tris borate EDTA) buffer (1 liter)

108 gm Tris
22 gm Boric acid
20 mM EDTA

Working with mice

1x PBS

137 mM NaCl

2.7 mM KCl

8 mM Na_2HPO_4

1.46 mM KH_2PO_4

Fill up to 1L with ddH_2O

Pronuclear injection buffer

10 mM Tris

0.1 mM EDTA

100 mM NaCl

adjust pH to 7.5, critical

Tail digestion buffer

500 mM KCl

100 mM Tris (pH 8.3)

0.2 mg/ml Gelatine

1% NP-40

1% Tween-20

filter sterilize

Erythrocyte lysis buffer

150 mM NH_4Cl

10 mM $KHCO_3$

0.125 mM EDTA

Adjust pH to 7.2-7.4 with HCl

Fill up to 1L with ddH_2O

filter sterilize

Tamoxifen (Tx) – 10 mg/ml of Tx (Sigma) was dissolved in oil, by rolling at room temperature for 4 hours

2.2. Methods

2.2.1. Working with Bacteria

Preparation of competent *E.coli*

E.coli from glycerol stocks (strain *DH10β*) were streaked on a non-selective LB plate and incubated overnight at 37°C. A single colony was incubated into 2.0 ml LB and shaken overnight at 37°C. This overnight culture was inoculated in 250 ml of LB + 20 mM $MgSO_4$ and incubated (with shaking) till the OD_{600} of 0.6 (about 3-3.5 hours). The cells were pelleted by centrifugation at 5000g for 5 mins at 4°C. The pellet was gently re-suspended in 100 ml (1/2.5 of original volume) of TFBI and incubated on ice for 5 mins. The cells were pelleted again by centrifugation at 5000g for 5 mins at 4°C, and then re-suspended in 10 ml (1/25 of original volume) of cold TFBII. The cells were incubated on ice for 1 hour. 100 µl of bacteria was aliquoted per tube (chilled) and flash frozen in liquid nitrogen. The aliquots were stored at -80°C.

E.coli transformation

Competent *E.coli* cells were thawed on ice before transforming DNA was added. 10 ng of plasmid DNA (10 ng/µl) or 20 µl of ligation mix was added to the bacterial cells and incubated on ice for 30 mins. Heat shock was performed at 42°C for 60 seconds to facilitate uptake of DNA by the bacteria. The cells were cooled on ice for 2 mins and 1 ml of non-selective LB medium was added. The cells were incubated at 37°C with shaking at 600 rpm for 1 hour, to allow recovery of the bacteria. The cells were pelleted at 5000g for 2 mins and the pellet was re-suspended in LB medium. Bacteria were plated on selective LB agar plates containing the appropriate antibiotic and incubated at 37°C overnight.

2.2.2. DNA analysis

Quick plasmid DNA mini preparation

Plasmid DNA mini preparation was performed by using buffers from the High Pure Plasmid Isolation Kit (Roche). For one mini preparation, a single bacterial colony was picked from the selective LB-plate and inoculated in 3 ml liquid LB-medium with the appropriate antibiotic. The cultures were incubated in a shaker at 37°C, overnight. From these cultures 2 ml was used for mini preparation, the rest was stored for making glycerol stocks or for midi/maxi- preparation. The cells were pelleted at 5000g for 5 mins at 4°C. The supernatant was removed carefully and the bacterial pellet was re-suspended in 250 µl of Suspension Buffer + RNase. Then, 250 µl of Lysis Buffer was added and the tube was mixed gently by inverting to prevent shearing of the genomic DNA. After incubation for 5 mins at room temperature, 350 µl of chilled Binding Buffer was added and the tube was mixed gently by inverting. The tube was incubated on ice for 5 mins and then centrifuged for 10 mins at 13000g. The supernatant was collected in a fresh tube (about 900 µl), 600 µl isopropanol was added to precipitate the plasmid DNA and centrifuged at 13000g for 15 mins at 4°C. The pellet was washed with 200 µl of 70% ethanol, centrifuged at 12000g for 10 mins at 4°C and air-dried. The DNA was dissolved in 20 µl of sterile distilled water (Ampuwa).

Plasmid DNA midi- and maxi preparation

For plasmid midi preparation (maxi preparation), 50 µl (200 µl) of a bacterial culture or one single colony was used to inoculate 50 ml (200 ml) of LB medium containing the appropriate antibiotic.

The culture was incubated overnight at 37°C, with shaking.

The cells were pelleted at 5000g for 5 mins at 4°C. Bacterial lysis and plasmid DNA isolation was performed using the Genopure Plasmid Midi and Maxi Kit (Roche) following the 'Procedure for High Copy Number Plasmids' as described in the manufacturer's manual. The purified plasmid DNA was dissolved in 100 µl of sterile ddH_2O and stored at -20°C.

Photometric measurement of DNA concentration

DNA concentration was measured using a BioPhotometer plus (Eppendorf). A DNA solution with an OD_{260} of 1 contains 50 µg/ml of double stranded DNA. 1 µl of the DNA solution was diluted with 99 µl ddH_2O and the absorbance (optical density, OD) was detected at 260 nm.

Preparing glycerol stocks

Glycerol stocks of bacteria were made in 30% glycerol and stored at -80°C.

Restriction digest of DNA

All restriction endonucleases were obtained from Fermentas or NEB.

Plasmid DNA was mixed with respective restriction enzyme and 1x appropriate digestion buffer. The mixture was incubated at optimum temperature of the respective restriction enzyme (usually 37°C) for appropriate time (1 hour for 1 µg DNA with 10U of enzyme).

In case of double digestion, a suitable buffer was chosen (according to Fermentas DoubleDigest™ Engine) or a serial digestion was performed.

The digested fragments were separated on a 0.8-1.5% agarose gel, depending on the size of the expected fragments.

Ethanol precipitation of DNA

To precipitate DNA, one-tenth volume of sodium acetate buffer (3 M, pH 5.2) and 2.5 volumes of cold 100% ethanol were added and incubated in the -20°C freezer for at least 1 h. The sample was centrifuged for 15 mins at 13000g in a 4°C cold micro-centrifuge. The supernatant was discarded and the pellet was washed with 200 µl of cold 70% ethanol (centrifugation for 5 mins at 4°C). The supernatant was removed and the pellet was air-dried. The DNA pellet was re-suspended in an appropriate volume of sterile ddH_2O.

Agarose gel electrophoresis

Agarose was dissolved in 1x TAE to obtain 1-2% w/v agarose gels, by boiling the mixture in a microwave. After cooling, ethidium bromide was added (final concentration - 0.5 µg/ml). The gel was poured in a gel apparatus, the combs were added and the gel was allowed to solidify. Then the gel was transferred into an electrophoresis apparatus filled with 1x TAE. DNA samples were mixed with 6x loading dye and run at 60-120 V, together with 10 µl of 1 kb DNA ladder (Fermentas).

DNA extraction from agarose gels

DNA-bands of interest were cut from the agarose gel with a clean scalpel and purified by using the GenElute™ Gel Extraction Kit (Sigma), according to the manufacturer's instructions. The plasmid DNA was eluted with 50 µl of warm sterile ddH_2O and stored at -20°C.

DNA ligation

Ligation of DNA-fragments was done with the T4 DNA ligase (Roche). Vector DNA (100 ng) and insert DNA (3x molar concentration of the vector) were mixed with 1x

T4 DNA ligase buffer (Roche) and 1 µl of T4 DNA ligase (Roche) in a final volume of 20 µl, followed by overnight incubation at 4°C. The entire ligation mix was used to transform competent *E.coli* cells.

Polymerase chain reaction

PCR was performed to amplify DNA fragments and to introduce new sequences at the ends (restrictions sites, LoxP sites and FLAG tag). Phusion High-Fidelity DNA Polymerase (Biozym) was used to avoid mutations in the amplified DNA. The PCR reaction was performed on an Eppendorf Mastercycler.

The annealing temperature was adjusted according to the melting temperature (T_m) of the primers used.

Recipe for one PCR reaction:

Component	**Volume (µl)**	**Final concentration**
5x Phusion HF Buffer	10	1x
10 mM dNTPs (Fermentas)	1	200 µM
10 µM primer mix (forward + reverse)	1	0.5 µM
Template DNA	1 (10 ng)	0.2 ng/µl
Phusion DNA Polymerase (1 U/µl)	0.5	0.02 U/µl
H_2O	36.5	
Total volume	**50**	

DNA sequencing

The sequencing was performed on a 48 capillary ABI 3730 DNA Genetic Analyser at the service department of the Institute of Molecular Pathology (IMP) in Vienna, Austria. 5 µM primer solution and a minimum of 150 ng plasmid DNA were used for each sequencing reaction. The sequence data was verified using the Chomas Lite software, version 2.01, and analyzed with the Clone Manager Suite 7 program.

2.2.3. Working with Bacterial Artificial Chromosome (BAC)

BAC maxi preparation

BAC constructs were purified using the NucleoBond® BAC 100 kit (Macerney-Nagel), according to the manufacturer's instructions. 500 ml of bacterial culture was used for the BAC preparation. Extreme care was taken to prevent shearing of the BAC. As the BAC DNA is susceptible to shearing at -20°C, it was stored only at 4°C for up to a week. After a week, fresh preparation was made.

Preparation of BAC containing competent cells

The DH10β bacterial strain with the desired BAC was streaked on a plate containing chloramphenicol (Most BACs, including RP23-362J7, contain a chloramphenicol resistance cassette). A single colony was inoculated in 5 ml of LB medium at 37°C overnight, with shaking. 1 ml of the culture was transferred to 50 ml LB medium and the cells were grown at 37°C, with shaking till the OD_{600} reached 0.6 (3-3.5 hours). The cells were cooled on ice for 10 mins, followed by centrifugation at 5000g, for 10 mins at 4°C. The supernatant was discarded and the cells were re-suspended in ice-cold 10% glycerol. The washing with ice-cold glycerol was repeated two more times.

After the final wash, the cells were re-suspended in ~800 µl of 10% glycerol. The cells were aliquoted into chilled eppendorf tubes (50 µl in each tube) and flash frozen in liquid nitrogen.

Electroporation of BAC containing *E.coli*

Electroporation was done using a Bio-Rad Gene Pulser.

The competent bacteria were thawed on ice and transferred to ice-cold cuvettes. Electroporation was done using the following settings: 2.3 kV, 25 µF and 200 Ω. 1 ml of antibiotic free LB medium was added and the cells were allowed to recover for 1.5 hours by incubation at 37°C, with shaking. The cells were pelleted and plated on LB agar plates supplemented with the appropriate antibiotic(s).

ET recombination

ET recombination was performed as described before [Muyrers et al., 1999].

Competent cells were made from the bacteria containing the BAC. The cells were electroporated with 100 ng of pR6K.αβγBAD plasmid, which encodes for the recombinases RecE and RecT that mediate BAC recombination. The cells were plated on LB agar plates supplemented with chloramphenicol (for the BAC) and ampicillin (for pR6K.αβγBAD plasmid). Competent cells were then made with the bacteria containing the BAC and the pR6K.αβγBAD plasmid. The competent cells were electroporated with 100 ng of the linearized construct (NotI restriction followed by gel purification) generated for homologous recombination. The cells were plated on LB agar plates containing three antibiotics: - chloramphenicol (BAC), ampicillin (pR6K.αβγBAD) and kanamycin (cS5^{F}-IRES-hCD2 construct). The cells were incubated over night at 37°C. BAC DNA was isolated from the cells and recombination was confirmed by restriction digestion.

Excision of kanamycin resistance cassette

The FRT flanked kanamycin resistance cassette was excised using the 705-Flp plasmid which expressing the *flippase* enzyme. It is based on the pSC101 temperature sensitive origin, which replicates at 30°C, but is lost at 37°C. The kanamycin resistance cassette was excised by electroporation of the cells containing the recombinant BAC with the 705 Flp-plasmid. The final BAC was confirmed by Southern blotting.

BAC purification

The recombinant BAC, after the excision of the kanamycin resistance cassette, was linearized with NotI enzyme and purified by Sepharose 4B-CL chromatography. The cotton block of a 5 ml pipette was pushed (by air-pressure) to the tapering tip of the pipette and the pipette was clamped vertically to a stand. CL4b sepharose was gently added into the pipette till it was packed almost to the top (the column should never dry out). A reservoir was set up at the top with a 10 ml syringe and the column was equilibrated with 30 ml of injection buffer. The digested BAC DNA was loaded with a dye onto the top of the column. The reservoir was refilled with 10 ml of equilibration buffer. 24 fractions of 500 µl each were collected, and analyzed for the presence of the BAC DNA by photometric analysis and by electrophoresis. The fraction with highest concentration of the BAC was used for pronuclear injections.

2.2.4. Southern blotting

Southern blotting was first developed by the British biologist Edwin Southern in 1975 [Southern, 1975]. This technique allows one to detect specific DNA sequences by using

a PCR generated probe that is radiolabelled. The radio-labelled probe is used to hybridize against a membrane to which electrophoresis separated DNA fragments have been transferred.

Amplification of the DNA-probe

The respective probe was amplified with the AmpliTaq DNA. The amplified DNA fragment was separated on a 2% agarose gel and purified with the GenElute™ Gel Extraction Kit (Sigma) as described above.

Precipitation of genomic DNA

A piece of the respective tissue was digested with 150 µl tail digestion buffer containing 1:10 dilution of proteinaseK (10 mg/ml, Sigma) and incubated overnight at 56°C, with shaking. Each sample was diluted with 450 µl of ddH_2O and 1/3 volume of 6M NaCl (200 µl) was added. The samples were mixed by shaking at 1000 rpm for 5 mins at room temperature. Phenol:chloroform extraction was performed by mixing in 500 µl of UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (Invitrogen) and centrifugation for 10 mins at 13000g. The upper aqueous phase containing the genomic DNA was transferred into a new tube and 0.6 volumes of isopropanol was added. Centrifugation was done for 20 mins at 13000g, the supernatant was discarded and the DNA pellet was washed with 300 µl of cold 70% ethanol. After the ethanol was discarded, the pellets were air-dried and the DNA was re-suspended in 50-100 µl of ddH_2O by gently flicking the tubes to avoid breaks in the genomic DNA. DNA concentration was determined photometrically.

Restriction digest of genomic DNA

About 10 µg of genomic DNA was mixed with 40 U of the respective restriction enzyme and 1x restriction buffer in a total volume of 40 µl. The samples were incubated at 37°C for 24 hours.

Agarose gel electrophoresis for Southern blotting

The digested DNA samples were run on a 0.8% agarose gel for 6-12 hours. The gel picture was taken with a fluorescent scale to allow calculation of the size of the radio-active DNA fragments. The gel was first washed in 0.25 M HCl with shaking for 10 mins, followed by 0.4 M NaOH (twice, for 15 mins each). Finally, the gel was washed in 20x SSC for 10 mins.

DNA transfer

To transfer the DNA onto a nylon membrane, “blotting apparatus” was assembled:

About 300 ml of 20x SSC was poured in a tray. A glass plate was placed across the tray and a Whatman filter paper soaked in 20x SSC was placed on the plate, so that the ends of the paper dropped into the 20x SSC, to form a paper wick. The agarose gel, with a nylon membrane (Porablot NY amp, Macherey-Nagel) on top, was placed on top of the glass plate. The gel and the nylon membrane were sandwiched between 2 Whatman papers soaked in 20x SSC on each side. Air bubbles were removed and a stack of paper towels were placed on top with a weight to allow optimal blotting. The tray was filled with 20x SSC and the blotting was performed for 24 h.

Pre-hybridization

The transferred DNA was denatured by heating the membrane at 80°C for one hour in an oven. The DNA was crosslinked to the membrane by exposure to UV light using a UV Stratalinker 2400 (Stratagene).

Pre-hybridization of the membrane was performed in hybridization tubes with 25 ml of hybridization buffer and rolling in an oven at 65°C for 2 h.

Probe preparation

The probe generated by PCR was radio-labelled using Prime-It II Random Primer Labeling Kit (Agilent Technologies).

100 ng of DNA, dissolved in 24 µl of ddH_2O, was used as a template for generating the radioactive labeled probe. 10 µl of primer-mix (10 µM) was added to the template and the DNA was denatured at 95°C for 5 mins. After the mixture was spun briefly and had cooled down to room temperature, 10 µl of 5x dCTP buffer, 5 µl of α-^{32}P-dCTP (PerkinElmer) and 1 µl of Exo (-) Klenow-polymerase (5 U/µl) were added. The reaction was incubated at 37°C for 2 h. 2 µl of Stop Mix was added to stop the reaction.

Probe purification

The labeled probe was purified using the Micro Bio-Spin® 6 Chromatography Columns (Bio-Rad) according to the manufacturer's instructions. The resin was compacted by spinning at 2800 rpm for 1 min. The column was placed in a new tube and the labeled DNA was added to the resin. The purified labeled DNA was collected in the collection tube by centrifugation at 2800 rpm for 3 mins.

Probe hybridization

The labeled DNA-probe was denatured at 95°C for 5 mins and added to the membrane in the hybridization tubes. Hybridization was performed overnight at 65°C.

Washing and exposure

The membrane was washed 3 times for 20 mins at 65°C with 25 ml of pre-warmed washing buffer. Then it was transferred into a Southern blot cassette and exposed to a Kodak BioMax MR film (Sigma-Aldrich) for at least 3 days at -80°C before it was developed.

2.2.5. RNA analysis

RNA isolation

RNA extraction was performed by using TRIzol® Reagent (Invitrogen) according to the manufacturere's instructions.

Pieces of tissues or cell pellets were homogenized using a Polytron PT 1200E homogenizer (Kinematica AG). 1.5 ml of TRIzol was added for 1.5 mg of tissue or 10^7cells. The cells were lysed by pipetting up and down and vortexing until a homogenous suspension had formed and incubated for 5 mins at room temperature. 300 µl of chloroform (200 µl per 1 ml Trizol) was added and the sample was mixed by inverting the tube. After incubation for 5 mins at room temperature, centrifugation was done at 13000g for 10 mins at 4°C. The upper aqueous phase was transferred into a new tube and RNA was precipitated by adding an equal volume of isopropanol. The tube was mixed by inverting, incubated for 10 mins at room temperature and centrifuged at 13000g for 15 mins at 4°C. The supernatant was discarded and the RNA pellet was washed with 300 µl of 75% ethanol (in DEPC-treated H_2O), followed

by centrifugation at 7500g for 5 mins at 4°C. The ethanol was removed and the RNA pellet was air-dried for 5-10 mins. The RNA pellet was re-suspended in 50-100 µl of DEPC-treated water and incubated on a heat block at 55°C for 10 mins for better solubility. The RNA concentration was measured and samples were stored at -80°C.

Photometric measurement of RNA concentration

RNA concentration was measured using a BioPhotometer plus (Eppendorf). A RNA solution with an OD_{260} of 1 contains 40 µg/ml of double stranded DNA. 1 µl of the RNA solution was diluted with 99 µl ddH_2O and the absorbance (optical density, OD) was detected at 260 nm.

RNA electrophoresis

Electrophoresis was done to ensure the quality of the purified RNA by separating it on a 0.8% agarose gel in 1x MOPS. 1 µl of RNA sample was mixed with 5 µl of RNA sample buffer and heated at 65°C for 5 mins to denature secondary RNA structures. 1 µl of RNA loading dye (containing ethidium bromide) was added and the samples were loaded on the agarose gel and run at 80-100 V to obtain sufficient separation of RNA. In case of non-degraded RNA, the 28S rRNA and the 18S rRNA are visible as two clear bands, where the 28S rRNA band is twice as intense as the 18S rRNA band.

cDNA synthesis

First strand cDNA synthesis was performed using the RevertAid™ hour Minus First Strand cDNA Synthesis Kit (Fermentas), according to the manufacturer's instructions. 1 µg of template RNA was mixed with 1 µl of oligo $(dT)_{18}$ primers (0.5 µg/µl) and filled

up to 12 µl with DEPC-treated water. This mixture was incubated at 70°C for 5 mins and chilled on ice.

Then the following components were added:

5x reaction buffer	4 µl
Ribolock Ribonuclease inhibitor (20 U/µl)	1 µl
10 mM dNTP mix	2 µl
RevertAid hour Minus M-MuLV reverse transcriptase (200 U/µl)	1 µl
Total volume	**20 µl**

The mixture was incubated at 42°C for 1 h. The reaction was stopped by heating at 70°C for 10 mins and samples were chilled on ice. The cDNA was stored at -80°C.

Quantitative real-time PCR

cDNA prepared from 50 ng of RNA was used per reaction. Samples were always applied as triplicates on 96 well plates. The compounds were always mixed on ice.
Real-time (RT) PCR was done on an Eppendorf RealPlex cycler. SYBR Green, a fluorescent dye which binds to double stranded DNA was used to detect amplification.

The cycle number at the theshold, when the increase in fluorescence becomes exponential (c_t value), is measured. The c_t values were normalized to the housekeeping gene murine *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*). This was achieved by using the "ΔC(t) method" (gene-specific expression level relative to that of an endogenous housekeeping gene).

Recipe for one RT-PCR reaction:

Component	Volume (µl)
cDNA (1:5 dilution)	5
10x Taq buffer	2.5
DMSO (Sigma)	2.25
25 mM dNTPs	0.2
10 µM primer mix (forward + reverse)	1
SYBR Green (Roche, 1:100 dilution in DMSO)	0.25
Taq DNA polymerase (Eppendorf)	0.1
H_2O	13.7
Total volume	**20**

RT-PCR reactions were run under following conditions:

Cycle step	Temperature	Time	Cycles
Initial denaturation	94°C	2 mins	1
	94°C	30 seconds	
DNA amplification	55°C	30 seconds	40
	72°C	2 mins	
	95°C	15 seconds	
Detection of melting temp.	60°C	15 seconds	
	95°C	15 seconds	end

2.2.6. Protein analysis

Protein isolation

Protein extraction was done using IP-buffer (ice cold) supplemented with proteinase- and phosphatase-inhibitors to generate complete IP-buffer. For protein extraction from single-cell suspensions, 2 volumes of IP-buffer was added to the cell-pellet. Cells were lysed by vortexing at short intervals in between incubations on ice. For protein extraction from tissues, a small piece of tissue (0.5 x 0.5 centimeter) was homogenized on ice in 1 ml of complete IP-buffer with a Polytron PT 1200E homogenizer (Kinematica AG). The samples were rotated for 1 hour (cell suspensions) or 2 hours (homogenized tissue) at 4°C, followed by centrifugation at 13000g for 30 mins at 4°C to remove the cell debris. The supernatant was transferred into a new tube; protein concentration was determined and the samples were stored at -80°C.

Measurement of protein concentration

Protein concentration was determined by using the Bio-Rad Protein Assay which is based on the Bradford method. The dye reagent concentrate was diluted to 1:6 with ddH_2O and filtered through Whatman paper. 1 µl of protein extract was mixed with 1 ml of filtered reagent in a cuvette and incubated for 5 mins at room temperature. 1 µl of IP buffer mixed with 1 ml of filtered reagent was used to set the blank. BSA-standards in the concentrations 4, 8, 12, 16 and 20 µg/µl were used to generate the standard curves. Absorbance at 595 was measured on a BioPhotometer plus (Eppendorf), which automatically determined protein concentration based on the BSA-standard curve.

SDS-PAGE

Proteins were separated on sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE) according to their molecular weight. Protein lysate was mixed with IP-buffer and 6x loading dye (freshly added β-mercaptoethanol to 3% concentration), to a final volume of 21 µl per well. The protein samples were denatured by heating at 95°C for 10 mins. 8% separating gel was used to separate the proteins. The gel was run at 70-100 V until sufficient separation of the desired proteins was obtained.

Protein ladder (PageRuler Prestained Protein Ladder, Fermentas) was used to determin the size of the protein bands detected.

Protein transfer

Protein transfer was performed by 'wet blotting' onto a nitrocellulose membrane (Hybond-c extra, Amersham Biosciences). Protein transfer was performed at a constant current of 120 mA overnight at 4°C.

Immunodetection

Blocking

The transfer apparatus was disassembled and the membrane was stained with Ponceau S (a reversible red dye that binds proteins) solution for 5-10 mins at room temperature, to determine successful transfer of the proteins to the membrane. Destaining was done by washing the membrane twice in ddH_2O. The membrane was blocked with 5% BSA-PBST-buffer by rolling for 1 hour at room temperature.

Antibody incubation

The membrane was incubated with primary antibody diluted in 1% BSA-PBST by rolling overnight at 4°C. Then, the membrane was washed 3 times with 1x PBST for 10 mins each. Incubation with the secondary antibody which was conjugated to horseradish peroxidase (HP) was performed by rolling for one hour at room temperature. Finally, the membrane was washed 3 times with 1x PBST for 10 mins.

Detection

Protein bands were detected by using either the ECL™ Plus Western Blotting Detection System (GE Healthcare) or the Pierce® ECL Western Blotting substrate (Thermo Scientific). The membrane was incubated with the reagent following the manufacturer's instructions. Then a CL-x Posure™ film (Thermo Scientific) was placed on top of the membrane and exposed in the dark until an appropriate protein signal was detected.

Western blot stripping

In order to perform a second procedure of immunoblotting, the membrane was stripped to remove the bound antibodies. The membrane was incubated with stripping buffer by rolling for 30 mins at 50°C. Then the membrane was washed thee times in 1x PBST for 15 mins each and could be blocked again with 5% BSA-PBST.

Note: Expression of the 'house-keeping' gene, HSC70 (Heat shock cognate 70) was used as loading control in all Western blots.

2.2.7. Electrophoretic Mobility Shift Assay (EMSA)

EMSA is a technique used to study the interaction between DNA binding proteins (e.g. transcription factors) and respective DNA elements *in vitro*. Respective DNA oligomers are radioactively labelled and allowed to migrate along an electric current. In the presence of interacting proteins, a DNA-protein complex is formed and the migration becomes slower resulting in a 'shift' in the location of the DNA band on the gel. Specific proteins in the complex can be identified by adding antibodies against them, which lead to further deceleration of the migration ('super shift').

Labelling of the probe

Classical STAT5 responsive DNA elements from the bovine beta-Casein promoter were used as probes. Single stranded DNA oligonucleotides applied for EMSA analysis were annealed at equimolar concentrations (~100 µM/oligomer) in annealing buffer, by heating to 95°C for 10 mins, followed by slow cooling to room temperature and finally by incubation on ice for 15 mins. The probe was diluted to a 2.5 µM concentration for labelling and stored at -20°C. 5 pmoles of the probe was used for labelling with 5 µl of ATP ^{32}P (Amersham Biosciences, 8000 cpm/µl), with 10 U of PNK, Polynucleotide Kinase (Roche). The oligo nucleotides were purified with Micro Bio-Spin® 6 Chomatography Columns (Bio-Rad) according to the manufacturer's instructions and stored at -20°C.

Reaction mixture

The following reaction mixture was used to set up the EMSA reaction:

Component	Volume (µl)
Protein	10 µg
BSA solution	2 µl
Poly dI:dC (Roche)	2 µl
5X Binding buffer	4 µl
Probe	2 µl
Total volume	**20 µl**

The reaction mixture was incubated at room temperature for 5 mins before loading on a gel to allow the generation of complexes.

Electrophoresis

The complexes were separated on a 4% acryl amide gel made in 0.25X TBE buffer. Orange G dye was loaded in one of the wells to monitor the migration. Once the dye reached close to the edge of the gel, the gel was transferred onto a Whatmann filter paper and dried on a vacuum drier at 80°C for 2 h. Then it was transferred into an autoradiography cassette and exposed to a Kodak BioMax MR film (Sigma-Aldrich) over night at -80°C. The film was developed after 24 hours.

2.2.8. Cell culture

The cells were maintained in DMEM medium supplemented with 10% FCS (Fetal Calf serum), penicillin/streptomycin (10U/ml) and L-glutamine (2 mM) – all from PAA. The cells were grown in an incubator at 37°C, 5% CO_2.

Transfection

The cells were transfected with 10 µg of plasmid using the reagent Lipofectamine™ (Invitrogen), according to the manufacturer's instructions.

2.2.9. Flow Cytometric Analysis

Cells were incubated with mouse Fc Block (1:100), for 5 mins at 4°C, to prevent non-specific binding of the antibodies. The cell suspension was incubated with specific antibodies conjugated to the respective fluorochomes (1:100) for 15 mins at 4°C. The cells were washed once with PBS, followed by centrifugation at 1200 rpm for 5 mins. The cell pellet was re-suspended in PBS and subjected to FACS analysis on BD FACSCanto II, BD biosciences.

2.2.10. Working with animals

All mice were maintained at the IMP/IMBA Animal Facility according to the Austrian laboratory animal law. Mice were kept on a daily cycle of 14 hours light and 10 hours dark. Pups were genotyped by DNA analysis from a small piece of tail and weaned at the age of 18-21 days. Age matched littermates were used as control mice in all experiments.

Mouse strains used –

Strain	Reference
$RosaCreER^{T2}$	[Hameyer et al., 2007]
$Pten^{fl/fl}$	[Suzuki et al., 2001]

Genotyping

PCR conditions:

Cycle step	Temperature	Time	Cycles
Initial denaturation	94°C	5 mins	1
Denaturation	94°C	30 seconds	
Annealing	55°C	30 seconds	35
Extension	72°C	1 minute	
Final extension	72°C	5 mins	1
	25°C	hold	

Genotyping PCR was performed to detect the mice carrying the transgene. Tail biopsies were digested with 150 µl of tail digestion buffer supplemented 1:20 with proteinase K (10 mg/ml). Samples were incubated shaking at 56°C overnight and diluted with 600 µl of sterile ddH_2O. 5 µl of the solution was used for each genotyping reaction. Genotyping PCR was performed on an Eppendorf Mastercycler.

After amplification, 30 µl from each PCR reaction was separated on 1% agarose gel to detect mice positive for the transgene.

Tamoxifen treatment

The mice were injected with 100 µl of tamoxifen (Tx) (10 mg/ml), per day for 5 consecutive days, intraperitoneally. The control mice received same volume of oil.

Preparation of single-cell suspensions

Spleen, thymus and lymph nodes were meshed through a 70 µm nylon cell strainer (BD Biosciences), which was flushed with medium to obtain a single cell suspension from these organs. The bone marrow was flushed with medium using a syringe with a needle (27G). The suspension was centrifuged at 1200 rpm for 5 mins. The

supernatant was removed, the cell pellets from thymus and lymph nodes were washed with 1x PBS. The splenocyte pellet was re-suspended in 1x erythocyte lysis buffer and incubated 10 mins at room temperature. The reaction was stopped by adding an excess of 1x PBS. The suspension was centrifuged at 1200 rpm for 5 mins. The supernatant was discarded, the pellet was washed with 1x PBS. The cells were counted manually, and used for FACS analysis, or flash frozen for protein/RNA analysis.

Blood analysis

Mice were bled from the tail vein and blood parameters were measured on an animal blood counter (Scil vet abc).

2.2.11. Statistics

Statistical analysis was performed by using the GraphPad Prism 5 software. All values are represented as means plus or minus standard error of the mean (SEM).

2.2.12. Figures

All figures in the ‘Introduction’ section of this manuscript were self-made using the programs – Microsoft PowerPoint 2012, Adobe Illustrator CS5 and PowerPoint Toolkit-Biology (Motifolio).

3. RESULTS

3.1 Cloning of cS5^{F}-FLAG-IRES-hCD2 construct

The first step was to generate a plasmid construct to target the cS5^{F} for homologous recombination into the BAC. We chose the BAC RP23-362J7, which encompasses the entire STAT5A and STA5B locus, and parts of STAT3 locus.

The 3' homologous arm (with appropriate restriction sites and the LoxP site) was amplified from the BAC RP23-362J7, by PCR. The cS5^{F} gene (with the Kozak sequence at the 3' and the FLAG tag at the 5') was also amplified by PCR from the pMSCV- cS5^{F} plasmid (previously generated in the lab of Dr. Richard Moriggl). The 'IRES-hCD2t-SV40polyA-FRT-Kan-FRT' cassette was cloned from the pQS-CD19-hCD2t plasmid [Delogu et al., 2006]. The 5' homologous arm was also PCR amplified from the BAC (including the restriction sites and the LoxP site). The entire construct was assembled in the pQS1 plasmid (Figure 7). The orientation of the HA sequences was designed such that the recombined BAC contains the cS5^{F} sequence in the anti-sense 'off' orientation (see section 6.1. for sequence).

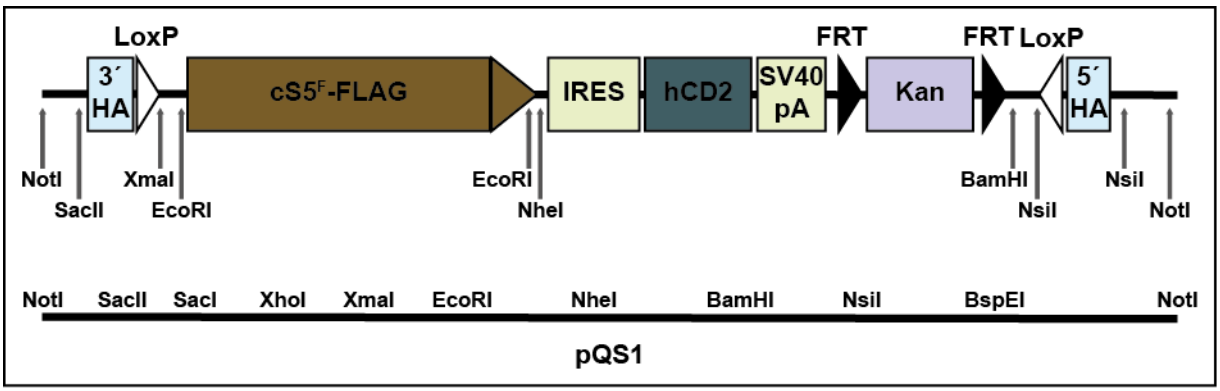

Figure 7. cS5^{F}-IRES-hCD2 construct. Schematic representation of cS5^{F} construct. The 3´homologus arm, with the LoxP site, was PCR amplified from the BAC RP23-362J7. cS5^{F}-FLAG was amplified from the plasmid pMSCV-cS5^{F}. IRES-thCD2-SV40polyA-FRT-Kan-FRT cassette was cloned from pQS1-CD19-hCD2t construct. The 5´homologous arm with the LoxP site was PCR amplified from the BAC. The entire cassette can be excised from pQS1 by NotI digestion.

3.2. Functional test of cS5^F construct (in vitro)

To confirm the expression of cS5^F and hCD2 from this construct, the entire cassette was cloned into the retroviral pMSCV backbone. NIH-3T3 cells were transfected with this plasmid and expression of hCD2 was confirmed by FACS analysis (Figure 8A). An empty pMSCV vector was used as control.

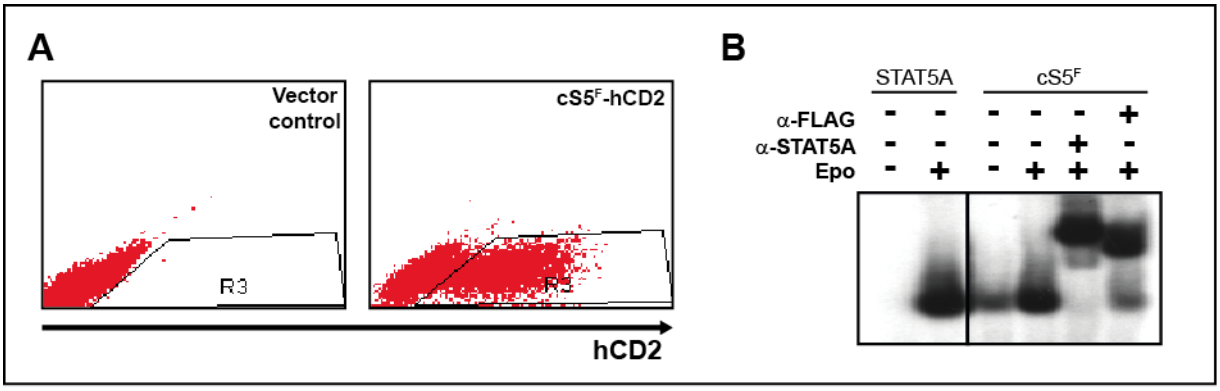

Figure 8. Functional test of cS5^F and hCD2 expression. A. NIH-3T3 cells were transfected with the pMSCV-cS5^F-hCD2 or empty pMSCV vector, with lipofectamine. hCD2 expression was detected in cells transfected with pMSCV-cS5^F-hCD2, by FACS analysis. **B.** 293T cells were transfected with the pMSCV-cS5^F-hCD2 vector and a construct expressing the EpoR. Control cells were transfected with pMSCV-wtSTAT5A. The cells expressing wt STAT5A showed activated STAT5A upon stimulation with Epo. However, the cells expressing cS5^F showed STAT5 DNA binding in the absence of Epo stimulation. The activation of cS5^F could be further induced by Epo. The complex is supershifted by antibodies against STAT5 and the FLAG epitope.

The DNA binding ability of cS5^F was tested by transfecting 293T cells with the pMSCV-cS5^F-hCD2 construct and along with an expression plasmid of EpoR (Erythropoietin Receptor). The control cells were transfected with a retrovirus expressing wild type (wt) STAT5A. The cells were treated with Epo, and DNA binding ability of cS5^F was determined by EMSA. While the DNA binding activity of wt-STAT5A was stimulated by Epo, cS5^F binding was seen in unstimulated cells. The cS5^F was hyper activated by Epo stimulation. The cS5^F complex can be super-shifted with antibodies against STAT5 and the FLAG epitope (Figure 8B).

3.3. ET recombination into the BAC RP23-362J7

After the construct was cloned, and the expression of the proteins was confirmed, recombination of the construct in to the BAC was done. The ET recombination was performed as described before [Muyrers et al., 1999]. The kanamycin resistance cassette was then excised by electroporation of bacterial cells containing the BAC with the 705-Flp plasmid. After electroporation, the cells were allowed to recover at 30°C for 1.5 hours with shaking to allow recombination of the FRT locus. The cells were plated at 37°C overnight which leads to loss of the 705-Flp plasmid. The final BAC harbors the 'cS5^{F}-FLAG-IRES-hCD2' construct, flanked by LoxP sites, in an antisense orientation within the endogenous STAT5A locus. The homologous arms allow specific recombination of the BAC, where in the start codon of the first exon of STAT5A was replaced by the construct. This allows expression of the cS5^{F} construct under the regulation of the endogenous promoter upon Cre recombination (Figure 9A).

The final BAC construct was confirmed by two independent Southern blot strategies. The BACs were digested with two different enzymes (KpnI and SpeI). Radioactive probes against the 5' HA and hCD2 sequences were used for probing the membrane. The 5'HA probe recognizes DNA fragments of different sizes in the endogenous BAC (~14 kb by KpnI restriction and ~10 kb by SpeI restriction) and the recombinant BAC (~5 kb by KpnI restriction and ~14 kb by SpeI restriction). As hCD2 sequence does not exist in the endogenous BAC, the hCD2 probe recognizes a band only in the recombinant BAC (~5 kb by KpnI restriction and ~16 kb by SpeI restriction). DNA fragments of the right size were detected in all cases and the recombination of the construct in to the BAC was confirmed (see section 6.2. for sequence).

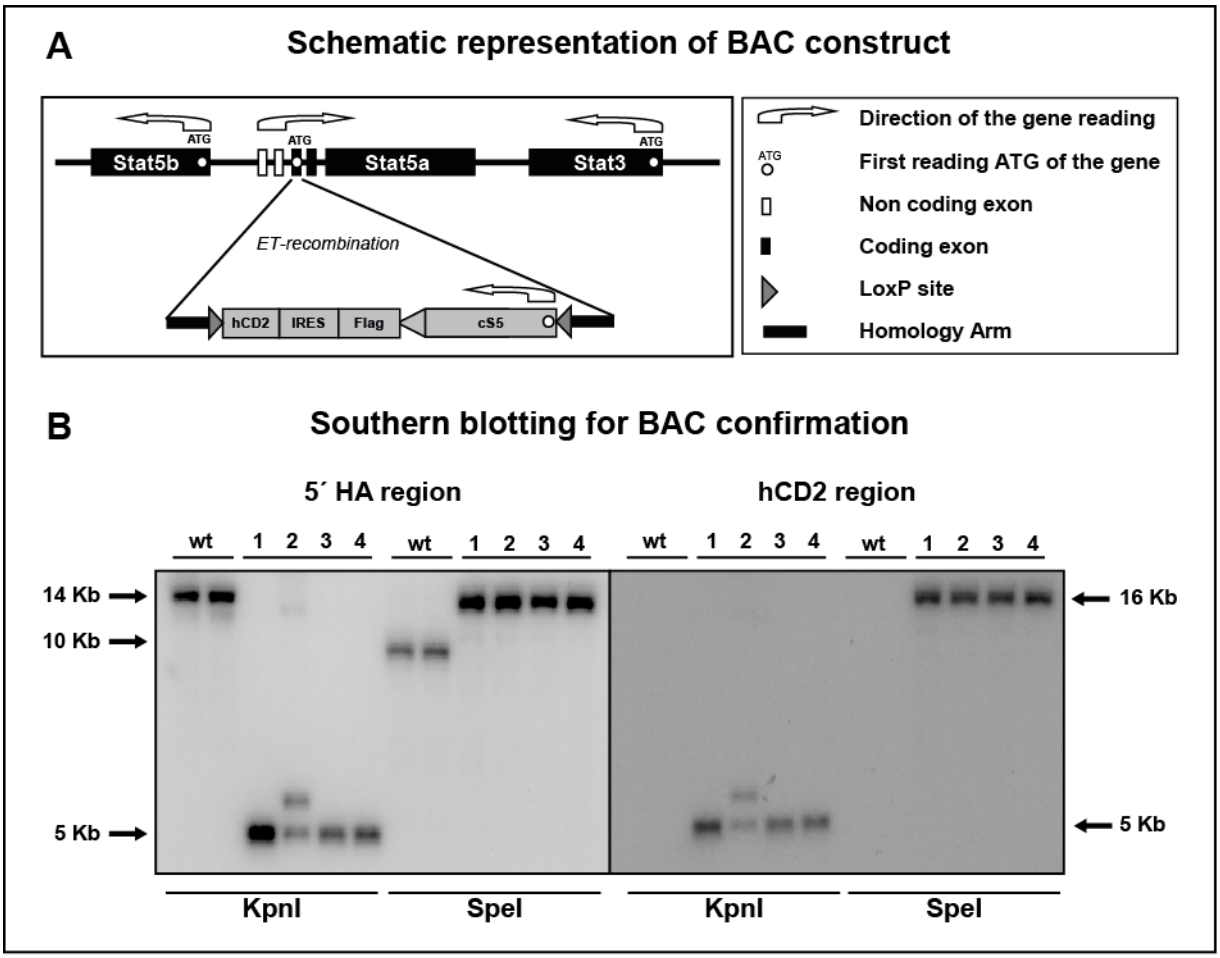

Figure 9. A. **Schematic representation of the BAC construct**. The endogenous STAT5A locus in the transgenic BAC is replaced by the $cS5^F$ construct in the `off´orientation. The transgene can be switched `on´ upon recombination by Cre recombinase; leading to the expression of $cS5^F$ under the regulation of the endogenous promoter. **B. Southern blotting**. 2 bacterial colonies with wild type BAC and 4 colonies with recombinant BAC were digested with two different enzymes (KpnI and SpeI). The membranes were probed with radioactively labelled probes against the 5´homologous arm region and the hCD2 region. Fragments of expected size were seen, confirming the BAC construct.

3.4. Generation of icS5 transgenic mice

Once the recombined BAC was generated and confirmed by Southern blotting the BAC construct was linearized with *NotI* restriction digestion and purified as described above. Pronuclear injection was done at the University of Veterinary Medicine, Vienna, Austria. 1-2 pl (2 ng/µl) of the purified, linearized transgene was injected into the male pro-nuclei of fertilized eggs from C57BL/6 mice x SV129 F1 mice. Embryos were implanted into pseudo-pregnant females. 52 mice were born from the injected

oocytes, of which 9 were found to be positive for the transgene by PCR. Transgenic pups were transferred to the IMP/IMBA (Research Institute of Molecular Pathology/Institute of Molecular Biotechnology) Animal Facility, Vienna Biocenter, Vienna. 5 of these mice showed germ line transmission of the transgene.

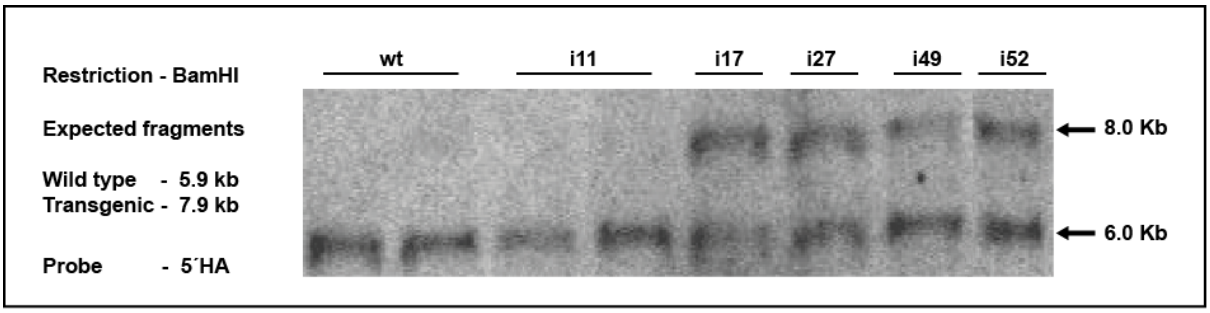

Figure 10. Southern blot analysis of transgenic pups. Southern analysis was performed with the tail DNA of the pups of the transgenic lines which showed germline transmission. 5´HA sequence was used as probe. Fragments of expected size of the endogenous locus was seen in all mice. The transgenic band was not seen in line icS5-11, suggesting that only a broken piece of the BAC has integrated in this line. Intensity of the endogenous and transgenic bands suggests a copy number of two in all 4 lines.

Southern blot analysis was performed to confirm the presence of the transgene. The genomic DNA was restricted with BamHI enzyme and the 5'HA sequence was used as a probe. This allows detection of the endogenous or wild type (~5.9 kb) and the transgenic locus (~7.9 kb) within the same blot.

As the line icS5-11 did not show a band with the 5'HA probe, it suggests that only parts of the BAC have integrated in this line. This line was excluded from further analysis. Comparison of the intensity of the endogenous and the transgenic bands suggests that there are 2 copies of the BAC construct integrated in the other 4 transgenic lines (Figure 10).

3.5. Recombination of cS5^{F} construct in icS5 mice

As the cS5^{F} construct is flanked by LoxP sites in the opposite orientation, it can be induced upon recombination by the Cre recombinase. Towards this, all 4 transgenic lines of icS5 mice were crossed with RosaCreER^{T2} mice [Hameyer et al., 2007], in which the Cre-Estrogen Receptor (ER) fusion protein is expressed under the regulatory elements of the ubiquitously expressed *Rosa* locus. Moreover, the ER harbors two point mutations which inhibit the binding of the endogenous estrogen, but allow activation of the ER by an estrogen antagonist – tamoxifen (Tx). Therefore, the treatment of the mice with Tx can lead to the activation of the Cre, which results in the induction of the gene of interest.

The icS5;RsCreER^{T2} mice were treated with Tx and recombination of the transgene into the 'on' orientation was checked by Southern blot, one week after the final injection. Genomic recombination could be detected in all the organs tested: - liver, lung, kidney, bone marrow and splenocytes (Figure 11), in 3 of the 4 transgenic lines. As no significant recombination could be detected in line icS5-49, no further experiments were done with this line.

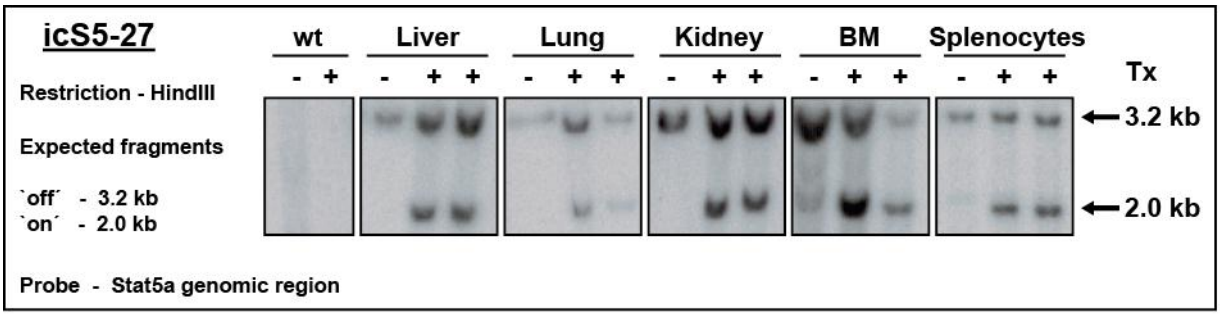

Figure 11. Genomic recombination of cS5^{F} construct. Upon treatment of icS5;RsCreER^{T2} mice with Tx, recombination of the genomic locus was seen in different organs, as detected by Southern blotting, in 3 out of the 4 transgenic lines. No recombination was seen in line i49. However, lines i17 and i52 showed similar recombination as seen in line i27 (data not shown).

3.6. Expression of the transgenic protein

Once the recombination of the $cS5^F$ construct in the genomic locus was confirmed, determination of the expression of the protein levels became important. Towards this, icS5;$RsCreER^{T2}$ mice of all four transgenic lines were injected with Tx, and the liver was harvested from these mice one week after the last injection. Protein extracts were made from the liver as described before. 50 µg of protein was loaded per well and Western blot analysis of liver extracts was performed using antibodies against total STAT5A and the FLAG epitope.

When probed with antibody against STAT5A, no induction of the transgene was detected in the line icS5-49. This is to be expected, as we did not see any induction of the transgene in this line. In the line icS5-17, two protein bands were detected, but they did not migrate at the same size as STAT5A in the wild type mice. Expression of $cS5^F$ was seen in the line icS5-27 and icS5-52 (Figure 12A).

The liver extracts (100 µg) from the icS5-27 and icS5-52 were subjected to Western blot analysis and the membrane was probed with an antibody against the FLAG epitope. While both the transgenic lines showed expression of $cS5^F$, best expression levels were seen in the line icS5-27 (Figure 12B). All further experiments were performed with the line icS5-27, henceforth called as icS5.

To ensure the expression of $cS5^F$ in cells of the hematopoietic lineage, single cell suspensions were made from the spleens of icS5;$RsCreER^{T2}$ mice after induction with Tx (control - 4 mice and Tx treated - 5 mice). 30 µg of protein was loaded per well and expression of $cS5^F$ in splenocytes was detected by Western blotting with antibodies against total STAT5A and the FLAG epitope (Figure 12C).

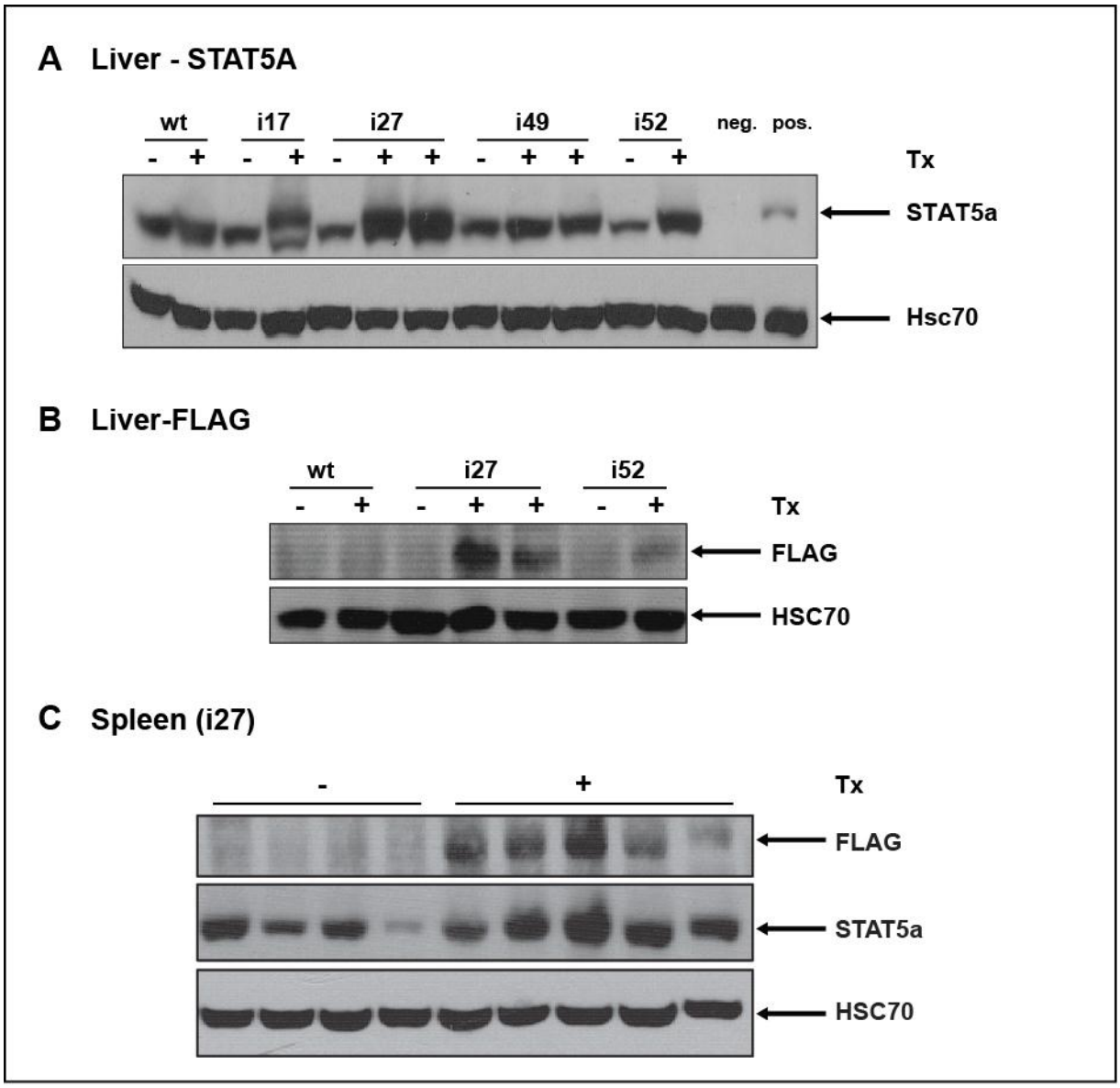

Figure 12. Expression of the transgenic protein. cS5^F expression was detected by Western blotting using antibodies against the FLAG epitope and total STAT5A. **A.** Lines icS5-27 and icS5-52 showed expressionof cS5^F in the liver extacts of mice induced by Tx as detected by antibody against STAT5A. **B.** The liver extracts were probed with antibody against the FLAG epitope. The line icS5-27 expressed higher levels of cS5^F, compared to the line icS5-52. **C.** The splenocytes from icS5-27 also showed expression of the transgene upon induction with Tx.

3.7. Expression of the surrogate marker hCD2

The recombination of the genomic locus into the 'on' orientation leads to the transcription of the transgenic construct under the regulatory elements of the endogenous *Stat5a* promoter. As the surrogate marker hCD2 is transcriptionally

linked to the cS5^{F}, expression of the transgene is coupled with the expression of the hCD2 on the surface of the cells. To detect the expression of hCD2, the cells of the hematopoietic organs spleen, thymus, lymph nodes, mesenteric lymph nodes and bone marrow of icS5 mice, were analyzed by FACS, one week after the last Tx injection. Expression of hCD2 could be detected in all the hematopoietic organs (Figure 13). In the spleen the expression was seen on different cell lineages- such as CD4^{+} T-cells, CD8^{+} T-cells and CD19^{+} B-cells. However, we did not detect any induction of hCD2 expression in CD11b^{+} myeloid cells in the spleen by Tx. Interestingly, in the bone marrow, hCD2 expression was seen on the early hematopoietic progenitors, 'LSK' cells, upon induction by Tx.

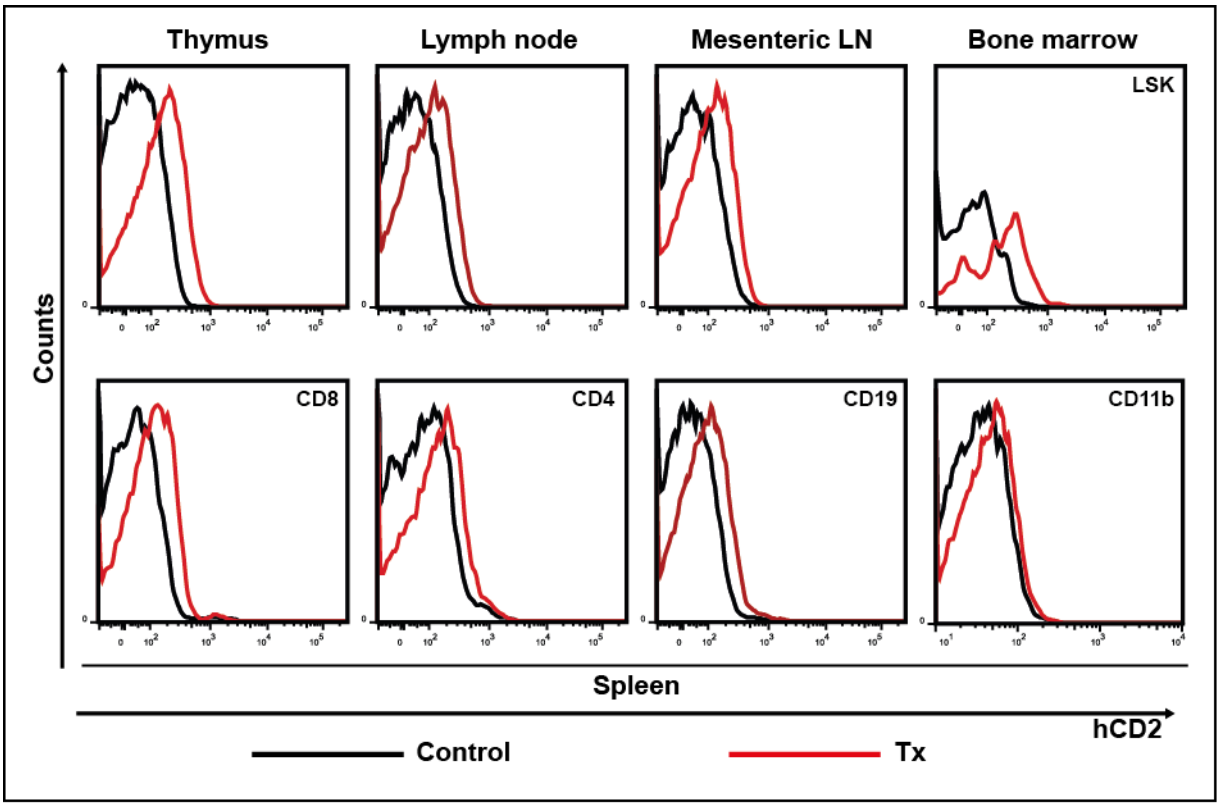

Figure 13. hCD2 expression upon induction of icS5;RsCreER^{T2} mice. FACS analysis of the cells showed low levels, but consistent expression of the hCD2 transgene in different hematopoietic organs - spleen, thymus, lymph nodes, mesenteric lymph nodes and bone marrow. The black line represents hCD2 expression in control mice, while the red line represents that in Tx induced mice. In the spleen, hCD2 was detected in T-cells (CD8^{+} and CD4^{+}) and B-cells (CD19^{+}). However, no expression was seen in myeloid cells (CD11b^{+}). In the bone marrow, hCD2 expression can be seen in the early progenitor (LSK) cells after Tx induction.

3.8. Elevated expression of STAT5 targets in icS5 mice

Expression of the surrogate marker and even of the transgenic protein does not automatically imply that the protein is functional. To determine the functionality of the expressed $cS5^{F}$ protein in the transgenic mice, we decided to look at the mRNA levels of the transcriptional targets of STAT5. Towards this, mRNA was isolated from the liver of icS5;$RsCreER^{T2}$ induced with Tx (liver from the mice induced with oil were used as controls). cDNA was prepared and expression of the bonafide targets of STAT5, such as *c-Myc, Bcl-2, Ccnd2* and *Mcl-1*, were checked by real time PCR. The expression of the STAT5 target genes were up-regulated in Tx treated mice compared to the controls (Figure 14), implying that the transgenic $cS5^{F}$ protein is transcriptionally functional and active.

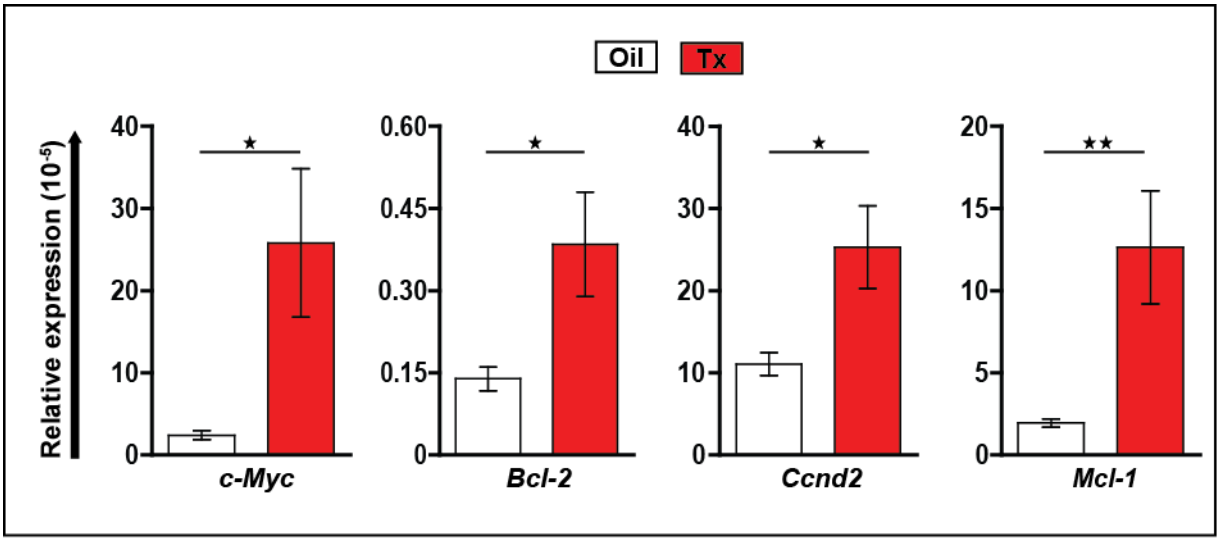

Figure 14. Elevated expression of Stat5 targets in icS5 mice. Real time PCR analysis of the liver of icS5;$RsCreER^{T2}$ mice shows increased mRNA levels of bonafide Stat5 targets - *c-Myc, Bcl-2, Ccnd2* and *Mcl-1*. 6-7 mice were used per group for the analysis. Statistical comparison was done by unpaired t-test. *-p< 0.05, **-p< 0,01.

3.9 Disturbed hematopoiesis in icS5 mice

In the previous experiments, we harvested organs from Tx induced icS5;RsCreER^{T2} mice, one week after Tx injection, to analyze the recombination and expression of the transgenic construct. At this time point we made an interesting observation that the icS5;RsCreER^{T2} mice had striking atrophy in the thymii, which was macroscopically obvious (Figure 15A).There was a drastic reduction (about 8-10 fold) in the total thymic cellularity, (Figure 15B). Therefore, we analyzed the T-cell compartment in different lymphoid organs by FACS.

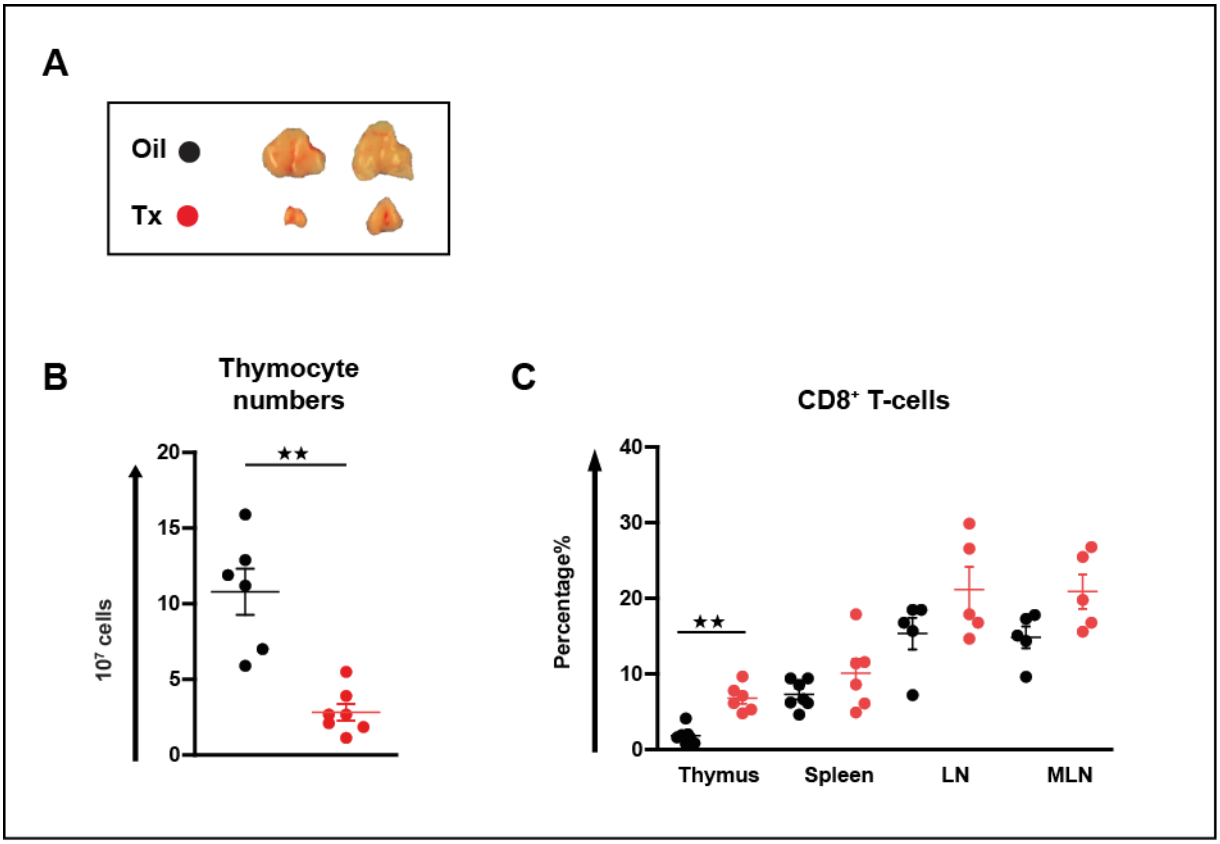

Figure 15. Disturbed hematopoiesis in icS5;RsCreER^{T2} mice after tamoxifen treatment. **A.** The icS5;RsCreER^{T2} mice showed highly atrophic thymii 1 weeks after induction with Tx. The figure legend for the graphs is also shown. **B.** The thymic atrophy is also reflected in the drastic reduction of total thymic cellularity. **C.** However, the percentage of CD8^{+} T-cells is significantly higher in the thymii of icS5;RsCreER^{T2} mice compared to the control mice. There is a tendency towards increased CD8^{+} T-cells in other lymphoid organs, such as spleen, lymph nodes (LN) and mesenteric lymph nodes (MLN).
Statistical comparison was done by unpaired t-test. **-p< 0.005

Interestingly, we found that there was a significant increase in the percentage of $CD8^+$ T-cells in the thymus of the Tx induced icS5;RsCreER^{T2} mice compared to the control mice (from 1.77% to 6.61%). Moreover, there was a clear tendency for an elevation in the $CD8^+$ T-cells, in other lymphoid organs, such as spleen, lymph nodes (LN) and mesenteric lymph nodes (MLN).

Since STAT5A plays a crucial role in the HSCs, we analyzed the early progenitor cells in the bone marrow. We stained for the following populations: LSK cells (lin-; c-kit+; Sca1+), common lymphoid progenitor (CLP – lin^-; Sca-1^{lo}; c-kit^{lo}; IL-7Rα^+), common myeloid progenitor (CMP – lin^-; Sca-1^-; IL-7Rα^-; c-kit^+; $CD34^+$; FcγR^{lo}), granulocyte-macrophage progenitor (GMP - lin^-; Sca-1^-; IL-7Rα^-; c-kit^+; $CD34^+$; FcγR^{lo}) and megakaryocyte-erythoid progenitor (MEP - lin^-; Sca-1^-; IL-7Rα^-; c-kit^+; $CD34^+$; FcγR^{lo}).

We found that the percentage of LSK cells was decreased by a factor of 2 in iS5;RsCreER^{T2} mice compared to the control mice. There was no difference in the relative percentages of CLPs and CMPs. However, we noticed a significant increase in the percentage of MEPs coupled with a slight reduction in the percentage of GMPs.

This suggested that cS5^F may preferentially promote the development of MEPs at the cost of GMPs (Figure 16).

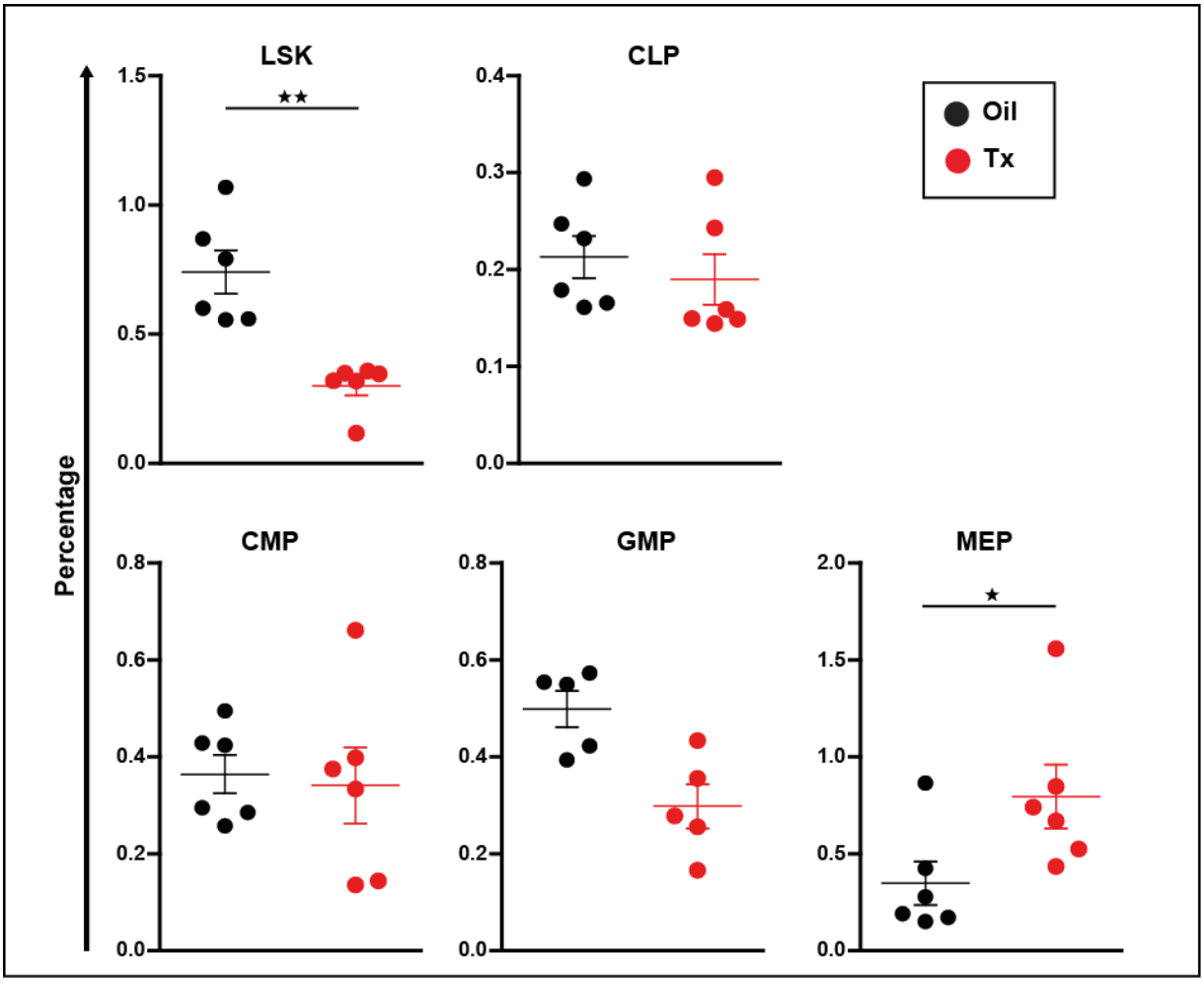

Figure 16. Anomalies in the bone marrow progenitor cells of icS5;RsCreER^{T2} mice. Single cell suspensions were made from the bone marrow of icS5;RsCreER^{T2} mice, 1 week after treatment with Tx, and FACS analysis was performed to analyze different populations of the progenitor cells. About 2 fold reduction was seen in the percentage of LSK (lin-;Sca-1+;c-Kit+) cells. While there was no difference in the percentage of common lymphoid progenitors (CLP) and common myeloid progenitor (CMP) populations, cS5^{F} expression seemed to skew the differentiation towards megakaryocyte-erythroid prgenitors (MEPs) at the cost of granulocyte-macrophage progenitors (GMPs).
Statistical analysis was done by unpaired t-test. *-p< 0.05, **-p< 0.005

During the analysis, we came across an article which described the specific toxic effects of RosaCreER^{T2} in the hematologic tissue. However, the mice recover one month after the Tx injections [Higashi et al., 2009]. To avoid the artifacts introduced by the toxicity of CreER^{T2}, we decided to analyze the mice at a later time point, and to include Tx induced RosaCreER^{T2} mice as controls in further experiments.

3.10. Normal hematopoiesis in icS5 mice after tamoxifen induction

Mice in all four groups were given respective treatment. First, expression of the transgenic protein was confirmed in the splenocytes of the induced mice (one mouse per group), 5 weeks after induction. 30 µg of protein extract was loaded per well, and subjected to Western blotting. The membrane was probed with an antibody against the FLAG epitope, and expression of the transgenic protein was detected only in the iS5;RsCreER^{T2} mouse induced with Tx (Figure 17A). After another week, hematopoietic organs were harvested from the mice, and the total number of cells in the bone marrow, thymus and spleen were counted. The cells were stained with respective antibodies and subjected to FACS analysis.

The Tx induced icS5 mice express hCD2 on the cell surface. The Mean fluorescence Intensity (MFI) of hCD2 expression in the white blood cells (WBC) in the induced mice was nearly twice as much as that in the control mice (Figure 17B). However, no significant differences were seen in the total cellularity of bone marrow, thymus and spleen (Figure 17C). There was also no significant difference in the percentage of LSK cells in the bone marrow (Figure 17D). The percentage of CD4$^+$ and CD8$^+$ T-cells in thymus remained unchanged within all the experimental groups (Figure 17E). The percentages of granulocytes (defined as CD11b$^+$ GR1$^+$) and T-cells (CD4$^+$ and CD8$^+$) in the spleen of the icS5 mice are also comparable to those in the control mice treated with Tx (Figure 17F).

In conclusion the different hematopoietic lineages in icS5 mice remain largely unchanged, at 6 weeks, after Tx injection.

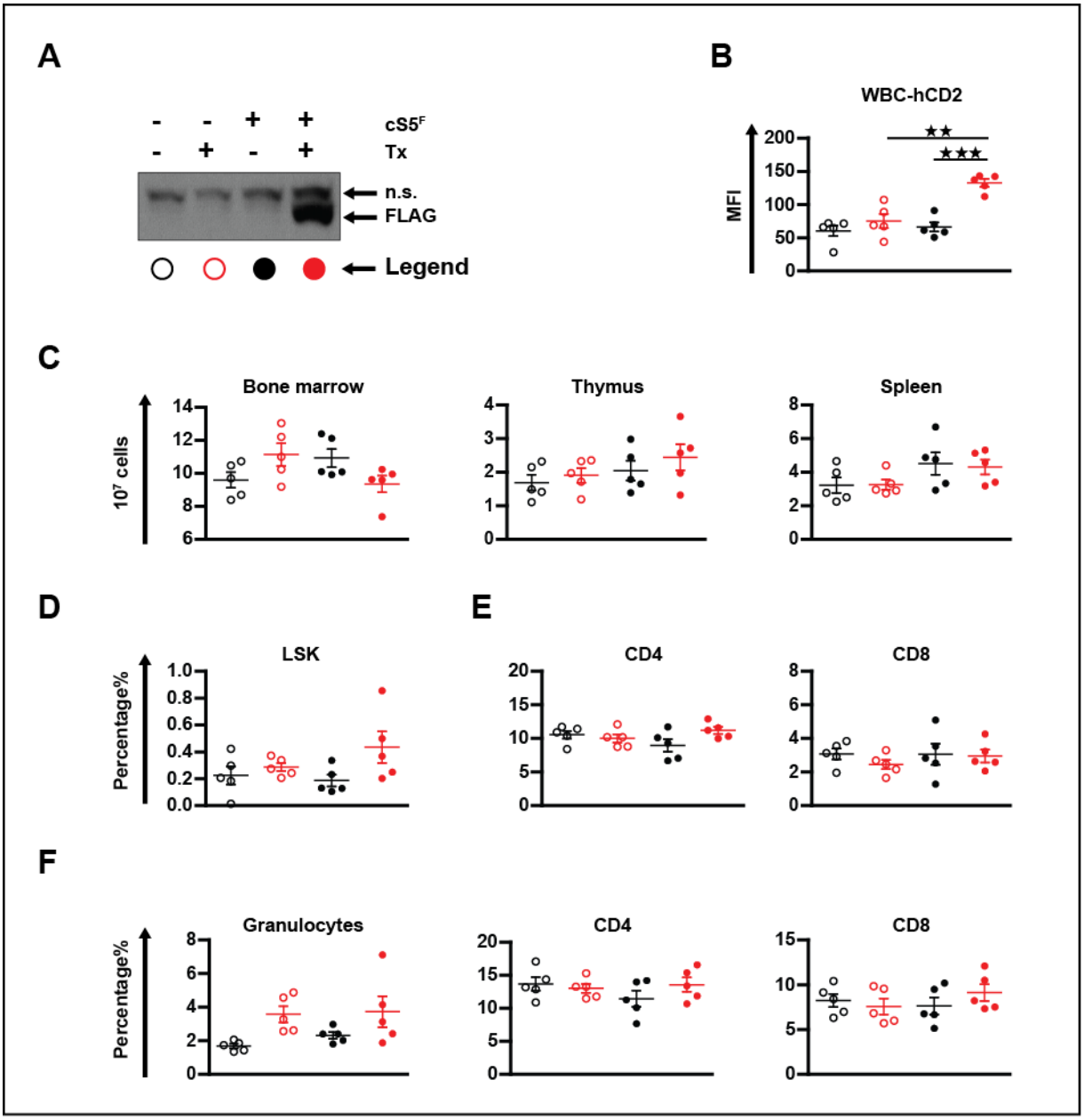

Figure 17. Normal hematopoiesis in i27cS5 mice. A. 5 weeks after Tx induction, expression of the transgenic construct in the splenocytes was checked by western blot, using an antibofy against the FLAG epitope. The colour legend for the graphs in other panels is also shown. Red colour repsents Tx treatments and the closed circles represent presence of the $cS5^F$ transgene. **B.** The expression of hCD2 is higher in the white blood cells (WBC) of icS5 mice 6 weeks after induction with Tx as measured by the increase in the Mean Flourescence Intensity (MFI) in FACS analysis. **C.** There is no significant difference in the total cellularity of the bone marrow, thymus and spleen in Tx induced icS5 mice compared to the control mice. **D.** The percentage of LSK cells in the bone marrow is not changed significantly in Tx induced icS5 mice compared to the control mice. **E.** The percentage of $CD4^+$ and $CD8^+$ T-cells in thymus are similar in all the groups. **F.** The percentage of splenic $CD4^+$ and $CD8^+$ T-cells and granulocytes in icS5 mice are comparable to that in the control mice treated with tx.
Statistical analysis was performed by Tukey's test. (n.s. - non-specific)

3.11. Increased WBCs in icS5;RsCreER^{T2};Pten$^{fl/+}$ mice

As described before, STAT5 and PI3K signaling pathways have been shown to be simultaneously active in the hematopoietic neoplasms. Tx induced deletion of Pten in RsCreER^{T2};Pten$^{fl/fl}$ leads to the development of lymphomas, carcinomas of the gastro-intestinal (GI) tract, prostate and endometrial cancers. However, loss of one allele of PTEN is not sufficient to drive carcinogenesis in these mice for up to 1 year after induction [Lu et al., 2007]. To study co-operative effects of persistent signaling of STAT5A and the PI3K-AKT pathway, we bred the icS5;RsCreER^{T2} mice into a *Pten*$^{fl/+}$ background (Figure 18A). Upon treatment of the mice with Tx, one allele of PTEN is lost systemically and expression of cS5^{F} is induced randomly in 50% of the cells. Since we have two copies of the BAC in the mouse, we expect higher recombination frequency of cS5 in these mice.

As the Tx induced CreER^{T2} is toxic at early time points after treatment, analysis of these mice at late stages for carcinogenesis will be free of the undesirable side effects. Moreover, the RsCreER^{T2};Pten$^{fl/+}$ mice, treated with Tx (or oil) were used as controls. The mice groups (n>15) were bled regularly and monitored for development of cancer.

The simultaneous loss of PTEN and over-expression of cS5^{F} leads to elevated WBC counts in the icS5;RsCreER^{T2};Pten$^{fl/+}$ mice compared to the mice in the control groups (Figure 18B). However, the mice did not succumb to a disease for up to 6 months post Tx induction. The mice are currently being monitored for development of cancer.

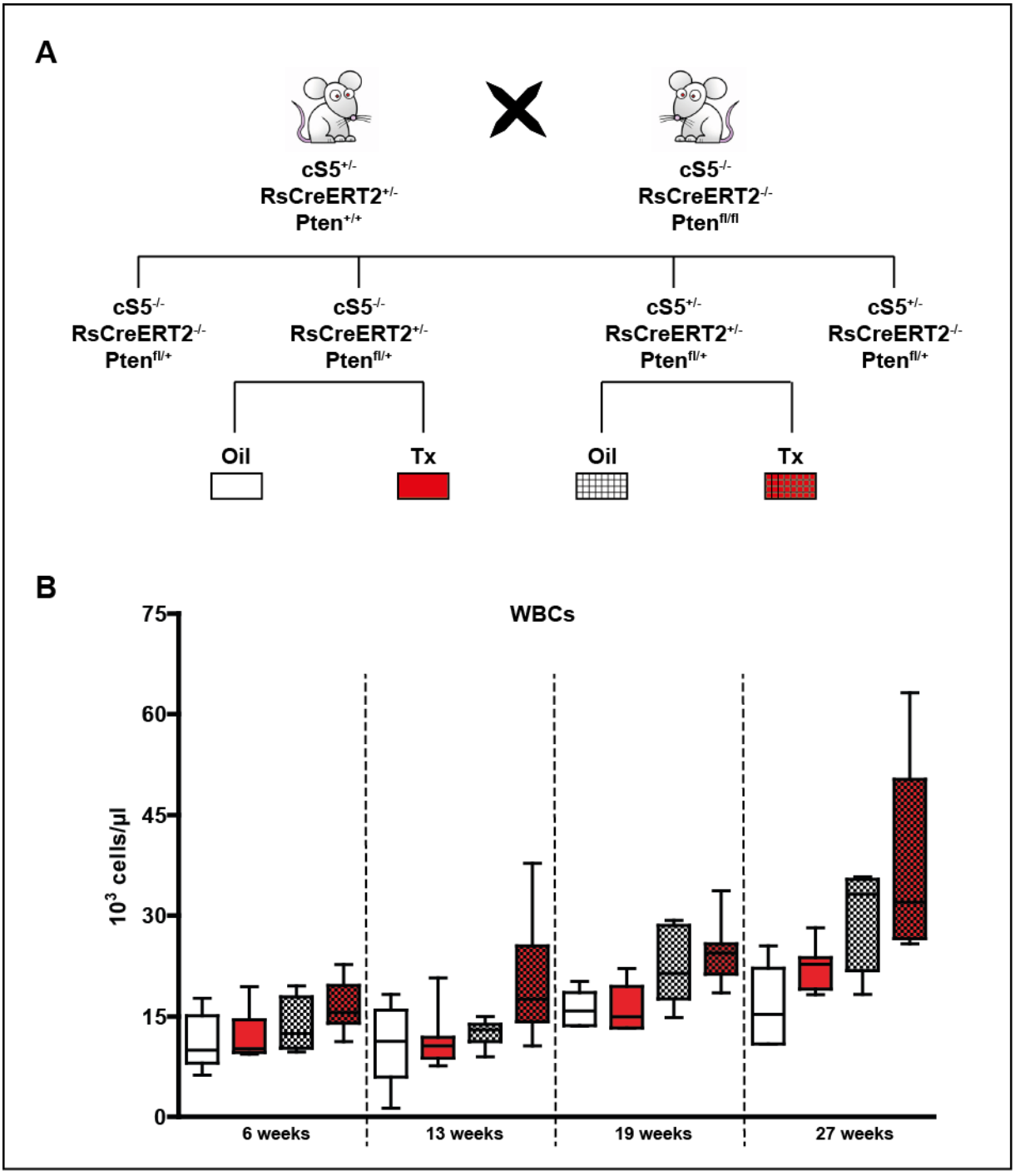

Figure 18. Increased WBCs in icS5 mice lacking one allele of Pten. A. Schematic representation of the mating scheme used to generate icS5;RsCreER^{T2};Pten$^{fl/+}$ mice. RsCreER^{T2};Pten$^{fl/+}$ mice treated with Tx (or oil) were used as controls. **B.** There is a progressive increase, over time, in the WBC counts in the blood of the icS5;RsCreER^{T2};Pten$^{fl/+}$ mice induced with Tx, compared to the mice in the control groups. (n>15 per group).

The icS5;RsCreER^{T2} mice did not develop any hematopoietic phenotype for up to 6 weeks after Tx induction. Moreover, no lethality was observed in the icS5;RsCreER^{T2};Pten$^{fl/+}$ mice, 6 months after Tx induction. We rationalized that very low level of expression of cS5^{F}, as seen in the icS5;RsCreER^{T2} mice does not lead to any major perturbation of the hematopoietic compartment, at least within our analysis time point. In addition, the unwanted toxicity mediated by RosaCreER^{T2} mice created certain limitations to the use of these mice. Since, our aim was to develop a mouse model for STAT5A mediated leukemia; we decided to generate another transgenic mouse model with higher expression levels of cS5^{F}. To attain this, we used a plasmid-based approach to generate transgenic mouse models for over-expression of cS5^{F} under the hematopoietic specific vav-promoter.

The VAV proteins are guanine nucleotide exchange factors (GEF) and are essential for the activation of the RHO-RAC GTPases. Expression of VAV1 is relatively confined to the hematopoietic system [Bustelo, 2000]. It plays an important role in the development and progression of various hematologic malignancies [Oberley et al., 2012]. The promoter of the vav1 gene was analyzed for regulatory sequences and the minimal promoter element required to drive the expression of the gene of interest in the hematopoietic system was identified [Ogilvy et al., 1998]. The modified vav-hematopoietic vector drives stable and strong expression of the transgene specifically in the hematopoietic cells [Ogilvy et al., 1999a; Ogilvy et al., 1999b].

Therefore, we decided to generate vav-cS5^{F} transgenic mice, with expression of cS5^{F} specifically in the hematopoietic system. We hoped to derive transgenic lines with different copy numbers leading to variable levels of cS5^{F} expression.

3.12. Generation of vav-cS5^{F} mice (vcS5)

Note: The experiments described in section 3.12. were performed by **Susanne Winkler**, a diploma student in the University of Vienna, under my supervision.

The cS5^{F}-FLAG sequence was cloned between the SfiI and NotI sites to replace the hCD4 sequence in the vav-hCD4 plasmid received from Prof. Jerry Adams; WEHI, Australia; to direct expression of cS5^{F} specifically in hematopoietic cells [Ogilvy et al., 1998; Ogilvy et al., 1999a; Ogilvy et al., 1999b] (Figure 19A; see section 6.3. for sequence). The plasmid was linearized with HindIII digestion and pronuclear injections were performed at the University of Veterinary Medicine, Vienna, Austria. 42 pups were born, of which 2 were identified to be transgene positive (vcS5-1 and vcS5-19). Both the lines showed germ line transmission of the transgene.

Southern analysis showed that line v19 has very low copy numbers (probably only 1 copy- henceforth referred to as vcS5-19^{lo}), whereas the line vcS5-1 (vcS5-1^{hi}) has high copy numbers of transgene integration (Figure 19B). The number of integrated copies was also reflected in the amount of protein expressed. While both the lines showed expression of the transgene in hematopoietic tissues like spleen, thymus and LN (thymus and lymph nodes- data not shown), line vcS5-1^{hi} showed much higher expression compared to the line vcS5-19^{lo}. Importantly, transgene expression was not detected in the non-hematopoietic tissues, such as liver, kidney and gut (kidney and gut - data not shown) (Figure 19C).

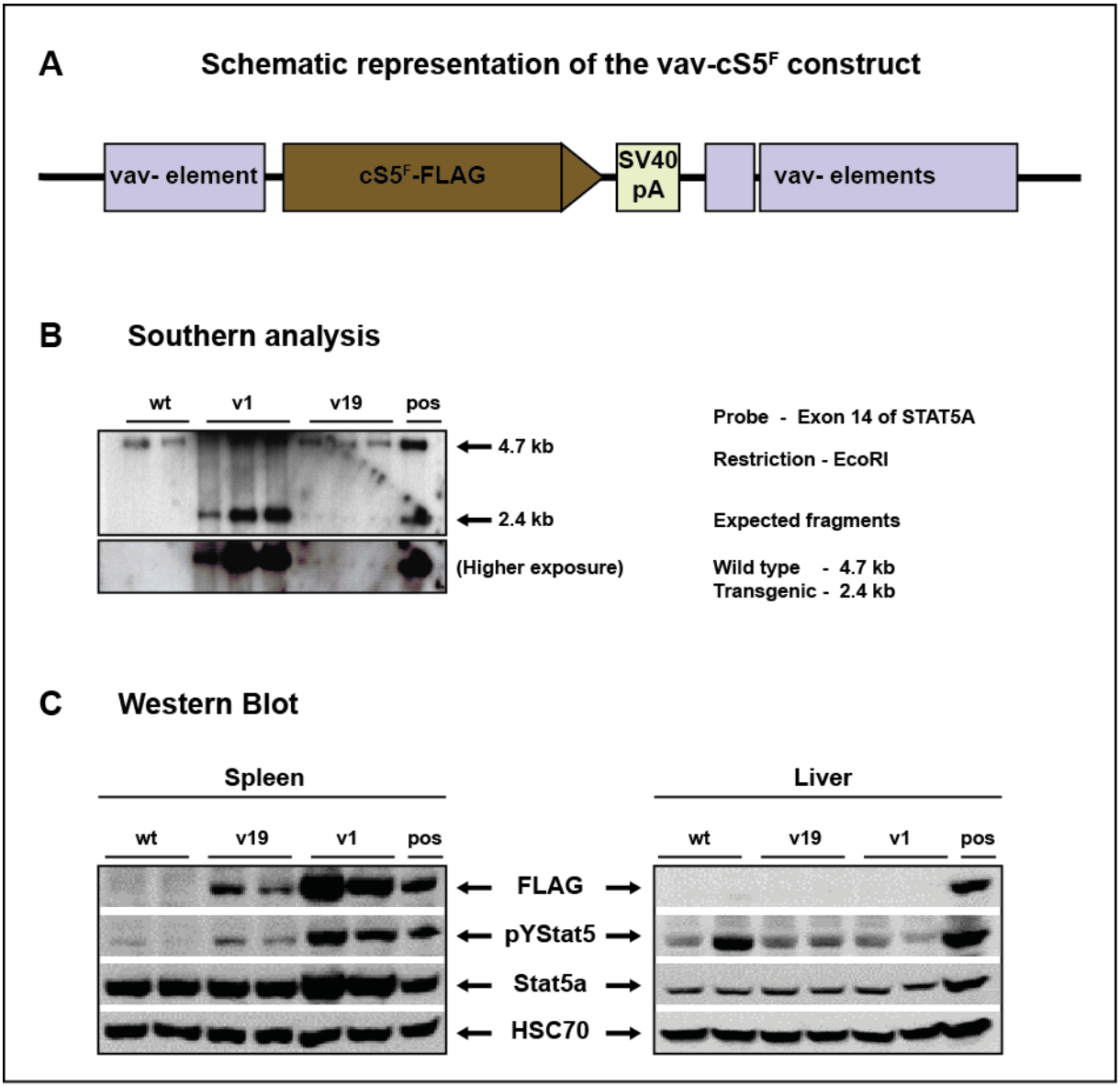

Figure 19. A. vav-cS5^F construct. The cS5^F-FLAG construct was cloned under the regulatory elements of the vav-promoter. **B. Southern analysis of the transgenic pups of lines v1 and v19.** Fragments of expected size were seen. Comparison of intensity with the endogenous band suggests a single copy integration in v19, but multiple copies in v1. Positive control (pos) is genomic DNA from the inducible cS5^F mice (icS5). **C. Western blot from organs of vav-cS5^F mice.** Organs were harvested from 6 weeks old mice and the protein extracts were subject to western blot analysis. Expression of the transgene could be detected in the splenocytes of the transgenic mice with antibodies against the FLAG, pYStat5 and total Stat5. v1 showed higher expression levels of cS5^F compared to v19. No transgene expression was detected in the liver. As positive control (pos), protein extract from NIH-3T3 cells transduced with a retrovirus expressing the cS5^F-FLAG construct was used.

3.13. Increased $CD8^+$ T-cells in vcS5-1^{hi} mice at 6 weeks

Once the vcS5 mice were generated and specific expression of the $cS5^F$, in the hematopoietic lineage, was confirmed; the mice were analyzed for a phenotype in the hematopoietic organs.

The mice were bled and the WBC counts were measured. At 6 weeks there was no difference in the WBC counts between the groups of mice (Figure 20B). FACS analysis was done for different lineages in the hematopoietic organs. The percentage of $CD8^+$ T-cells in the vcS5-1^{hi} mice is increased (2-3 fold) compared to the wild type mice, in blood and spleen (Figure 20C). Simultaneously, there is a 2 fold decrease in the percentage of $CD4^+$ T-cells in the blood and the spleen. In the thymus, this change in $CD8^+$ and $CD4^+$ is milder. As a result, the CD8:CD4 ratio in these mice is elevated up to 5 times in the blood and the spleen compared to the wild type mice. There were no statistically significant differences in the percentages of granulocytes and B-cells between the groups at 6 weeks of age (Figure 20D). We also did not find any differences in the percentage of LSK cells in the bone marrow in the mice within the groups (Figure 20E).

Interestingly, at 6 weeks of age, the vcS5-19^{lo} mice were indistinguishable from the wild type mice. There was no significant change in any of the lineages in the hematologic organs in the vcS5-19^{lo} mice compared to the wild type mice.

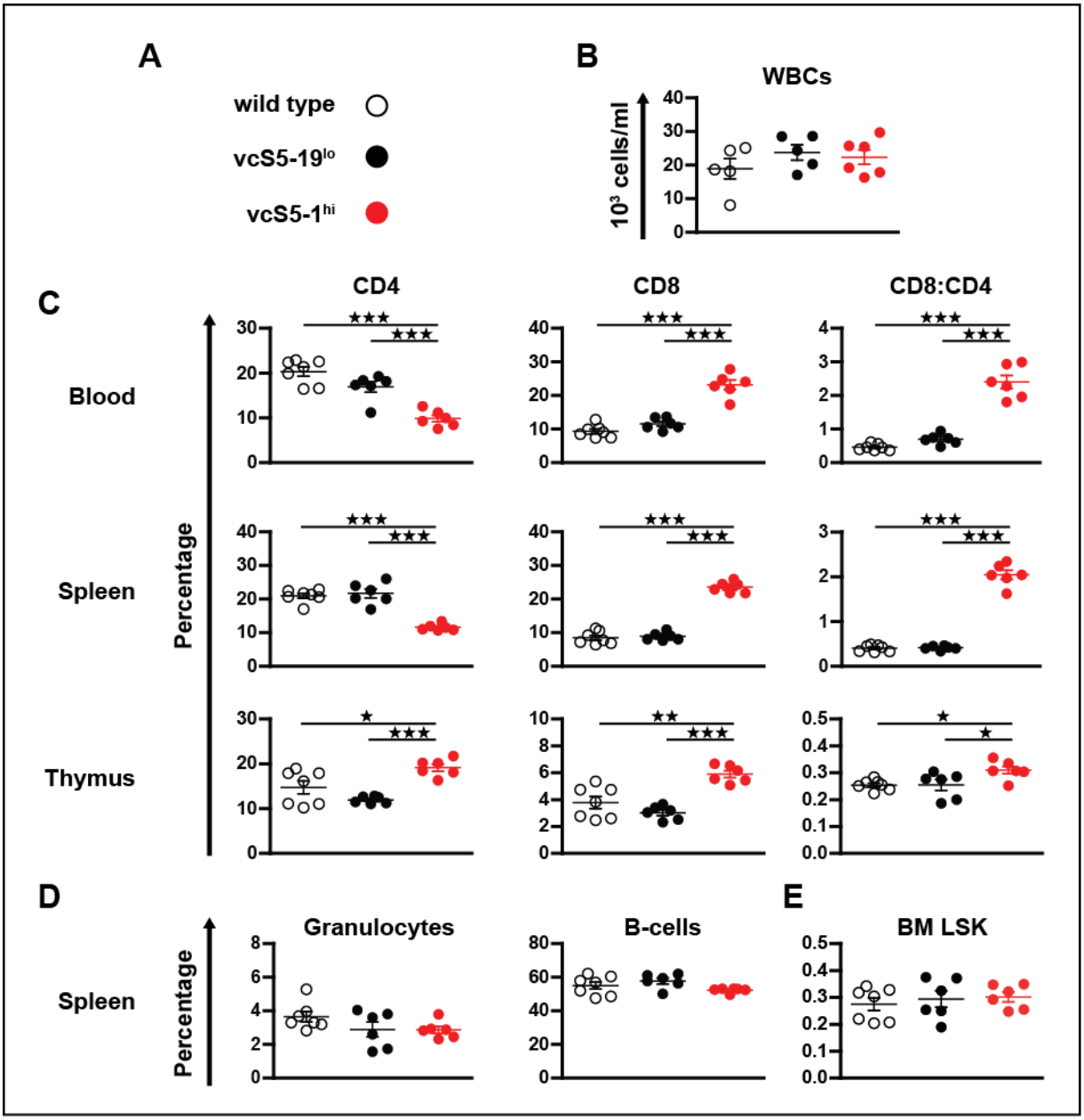

Figure 20. vcS5-1^{hi} mice have elevated CD8^{+} T-cells at 6 weeks of age. A. The legend for the graphs; representing the wild type (open circles), vcS5-19^{lo} (closed black circles) and vcS5-1^{hi} (closed red circles) mice. **B.** There is no significant difference in the WBC counts among the three groups of mice **C.** The vcS5-1^{hi} mice show increased levels of CD8^{+} T-cells, coupled with a reduction in the CD4^{+} T-cells, in all the three organs - blood, spleen and thymus. This difference results in a drastic increase in the ratio of CD8^{+} to CD4^{+} T-cells (wild type mice have a ratio of 0.5). However, the vcS5-19^{lo} mice seem indistinguishable from the wild type mice. **D.** No differences were seen in the percentages of granulocytes and B-cells among the groups at 6 weeks of age. **E.** No difference was detected in the percentage of LSK cells in the BM, within the groups.
Statistical analysis was done by Tukey test. * - $p < 0.05$, **- $p < 0.005$, ***- $p < 0.0001$

3.14. Steady increase in CD8^{+} T-cells in vcS5-1^{hi} mice at 12 weeks

Next, we analyzed the mice at 12 weeks of age, to detect any changes in the hematopoietic lineages over time, due to over-expression of cS5^{F}. Mice were bled from the tail vein and blood parameters were determined. We found significant increase in the WBCs, in both vcS5-1^{hi} and vcS5-19^{lo} mice, compared to the wild type mice (Figure 21). However, the total number of platelets remains unchanged. There was also no difference in the percentage of hematocrit in the vcS5 mice compared to the wild type mice.

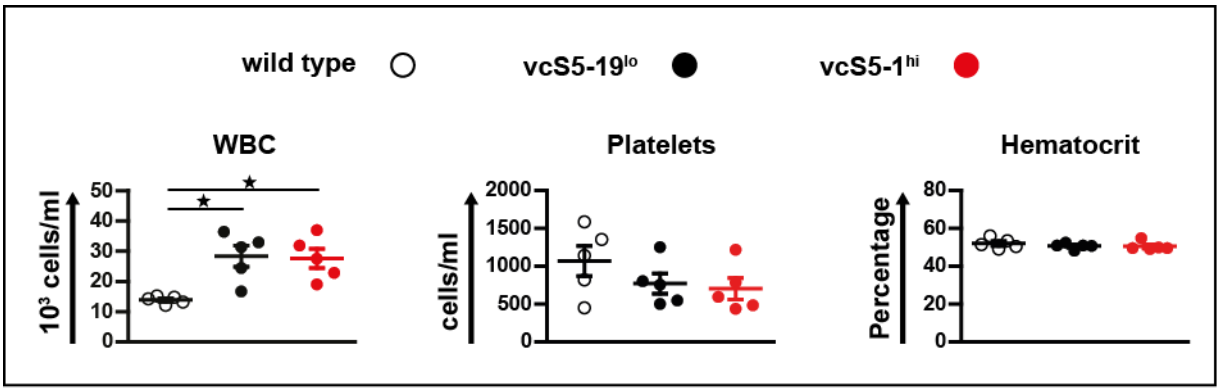

Figure 21. Hematologic analysis of vcS5 mice at 12 weeks. The legend for the graphs representing the wild type (open circles), vcS5-19^{lo} (closed black circles) and vcS5-1^{hi} (closed red circles) mice. The total WBC counts are significantly increased in both the transgenic lines, compared to the wild type mice. However, there is no change in the number of platelets. The percentage of hematocrit is also unchanged between the groups of mice.
Statistical analysis was done by Tukey test. * - $p< 0.05$, ** - $p< 0.005$, *** - $p< 0.0001$

Hematologic organs were harvested from the mice and subjected to FACS analysis. The splenic cellularity of vcS5-19^{lo} mice is increased by 30%, while that of vcS5-1^{hi} mice is increased by around 50% compared to the wild type mice.We did not see any difference in the cellularity of the thymus and the bone marrow at this age (Figure 22B).

FACS analysis showed that the levels of CD8^{+} T-cells in the lymphoid organs of vcS5-1^{hi} mice, at 12 weeks, were considerably higher than at 6 weeks of age in all the lymphoid organs. In blood, the CD8^{+} T-cells increase from 23.15% to 39.3% and in spleen from 23.56% to 43.36%. However, the decrease in the percentage of CD4^{+} T cells is not very drastic over time. In blood, the CD4^{+} T-cells decrease from 9.86% to 7.76% and in the spleen from 11.61 to 10.13% (Figure 22C). In the vcS5-19^{lo} mice, there was no significant difference in the percentages of hematopoietic lineages compared to the wild type mice. Interestingly, we found a significant reduction in the percentage of CD19^{+} B-cells in vcS5-1^{hi} mice in the spleen at this time point. However, there was no difference in the percentage of splenic granulocytes (Figure 22D). We did not find any difference in the percentage of LSK cells in the bone marrow of these mice compared to the wild type mice (Figure 22E).

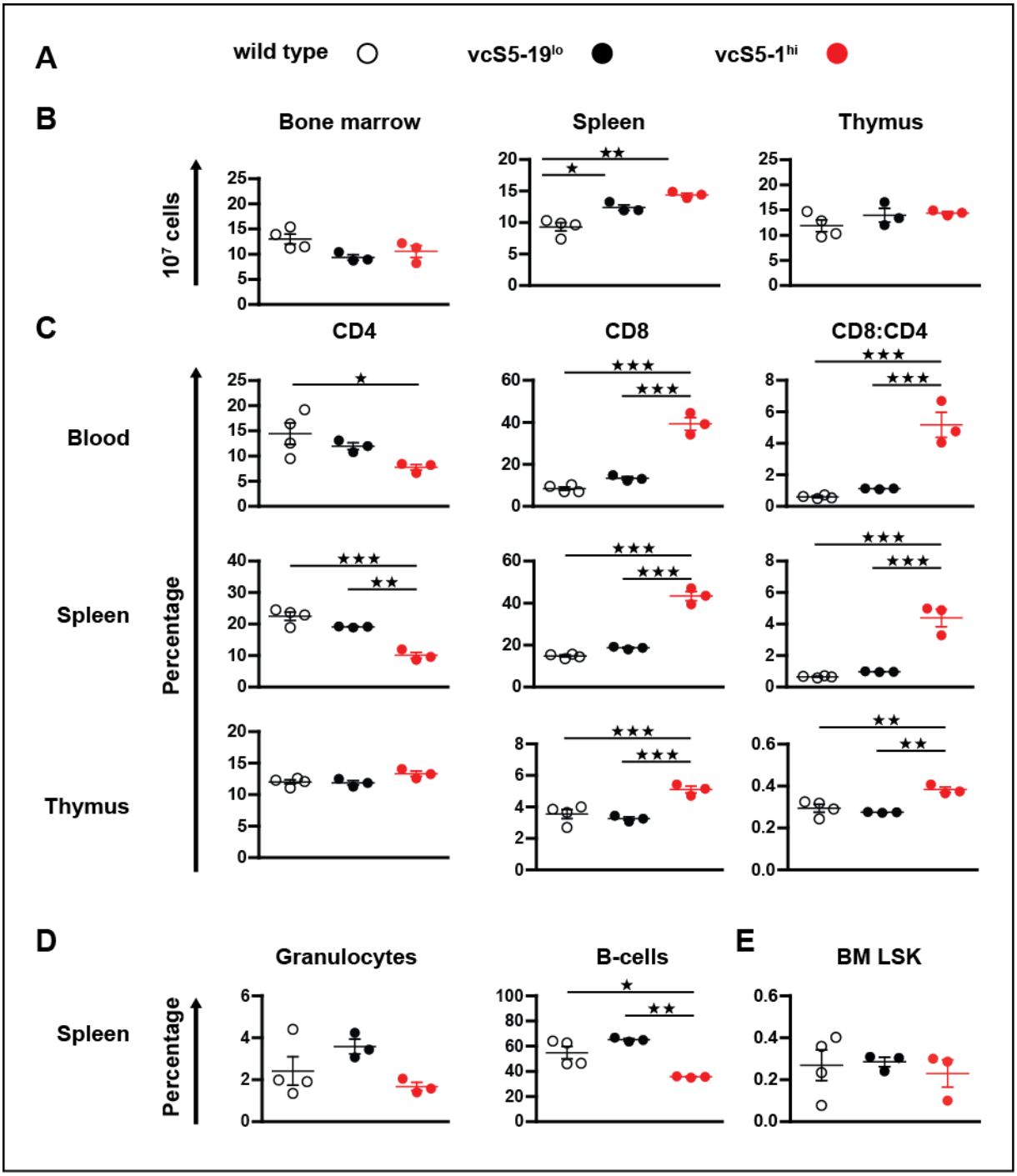

Figure 22. Steady increase in CD8⁺ T-cells in vcS5-1^{hi} mice. A. The legend for the graphs representing the wild type (open circles), vcS5-19^{lo} (closed black circles) and vcS5-1^{hi} (closed red circles) mice. **B.** The splenic cellularity increases with the number of integration of the vcS5 construct. There was no change in the cellularity of thymus and bone marrow within the groups. **C.** There is a constant increase in the percentage of CD8⁺ T-cells in the blood, spleen and thymus of the vcS5-1^{hi} mice, compared to the wild ype mice and the vcS5-19^{lo} mice, which is reflected in the drastic increase in CD8:CD4 ratio in these mice. **D.** There is a 40-50% reduction in the CD19⁺ B-cells in vcS5-1^{hi} mice, compared to the wild type and vcS5-1^{lo} mice. **E.** No difference was detected in the percentage of LSK cells in the BM, within the groups. Statistical analysis was done by Tukey test. * - $p < 0.05$, ** - $p < 0.005$, *** - $p < 0.0001$

In summary, we have successfully generated three transgenic mouse lines for different levels of expression of constitutively active STAT5A (see Table 4).

The first one (icS5 mice) is an inducible model for the expression of $cS5^F$, in which expression of the transgene can be induced by using the $RosaCreER^{T2}$ transgenic mice. These mice will allow expression of relatively physiological levels of $cS5^F$, in a variety of tissues, based on the inducible cre-transgenic line used. They will provide an interesting tool to study the functions of STAT5A in various physiological processes in different tissues.

In the second model, we have derived two transgenic lines where $cS5^F$ is expressed specifically in the hematopoietic lineage under the vav-promoter. The two lines have different integration numbers of the vector, which leads to different levels of expression of $cS5^F$. Interestingly, the mice with high $cS5^F$ expression have highly increased number of $CD8^+$ T-cells in all the lymphoid organs. However, apart from increased WBC counts, mice with low $cS5^F$ expression are similar to the wild type mice in the cellular composition of their hematologic organs.

Analysis of these mice, over time will be essential to determine the effect of different levels of $cS5^F$ expression over tumor initiation. As these mice show $cS5^F$ expression in all lineages of the hematopoietic system, they are predisposed to hematopoietic neoplasms of different origins, such as leukemia, lymphoma and MPN.

3.15. Table 4. Summary of transgenic mouse models

Transgenic line	Expression pattern	Expression levels	Phenotype
icS5 mice	**Inducible, based on the cre transgenic (RosaCreER^{T2})**	**Low**	**Normal hematopoiesis.** **Increased white blood cells upon an additional loss of one allele of Pten.**
vcS5-19^{lo} mice	**Hematopoietic cells**	**Intermediate**	**Increased number of WBCs and splenic cellularity at 12 weeks of age.**
vcS5-1^{hi} mice	**Hematopoietic cells**	**High**	**Increased number of WBCs and splenic cellularity at 12 weeks of age.** **Drastic increase in CD8:CD4 ratio starting at 6 weeks of age.**

4. DISCUSSION

The indispensable role of STAT5 in hematopoiesis has been well established. STAT5 is a downstream signaling molecule of many cytokines and growth factors that regulate the differentiation of many different lineages of the hematopoietic system (Figure 3). Many of the transcriptional targets of STAT5 are genes that promote cell survival and proliferation. *C-Myc* is an established proto-oncogene that plays an important role in many physiological processes [Pelengaris et al., 2002; Wilson et al., 2004]. Other targets of STAT5, such as – *Bcl-2, Mcl-1* [Kelly and Strasser, 2011; Quinn et al., 2011], *Ccdn2* (cyclin d2) [Johnson and Walker, 1999] and *Pim-1* [Shah et al., 2008], have also been shown to promote cell survival and play an important role in oncogenesis. Therefore, it is not very surprising that STAT5 plays an oncogenic role in the development of hematopoietic neoplasms.

Early reports showed persistent activation of the JAK-STAT signaling pathways in leukemic cells [Danial et al., 1995; Gouilleux-Gruart et al., 1996; Migone et al., 1995]. Constitutive activation of STAT5 by the BCR/ABL fusion kinase was first reported in 1996 [Shuai et al., 1996]. Since then, there have been numerous studies to understand the role of STAT5 in leukemia and lymphomas. Studies with STAT5-deficient mice have clearly shown that Abelson mediated transformation of cells is not possible in the absence of STAT5 [Hoelbl et al., 2006]. Many driving mutations of hematopoietic neoplasms lead to the activation of STAT5 (see Table 2). To study the oncogenic functions of STAT5 bone marrow transplantation experiments have been done with hematopoietic progenitor cells transduced with retrovirus expressing a constitutively active mutant version of STAT5A ($cS5^F$). In this experimental model, expression of $cS5^F$ leads to an early onset of multi-lineage leukemia which is lethal within 8-10

weeks of transplantation [Friedbichler et al., 2010; Harir et al., 2007; Li et al., 2010b; Moriggl et al., 2005]. Interestingly, bone marrow transplantation experiments with over-expression of a very similar STAT5B mutant gives rise to leukemia with a much longer latency (~4 months) and only 50% penetrance (K. Friedbichler; unpublished data). The picture that has emerged has very convincingly established the crucial requirement of STAT5A in the initiation of hematopoietic cancers.

However, the bone marrow transplantation experiments suffer from certain drawbacks. Retroviral expression leads to extremely high levels of $cS5^F$ expression (up to 40 fold increase – R. Moriggl, unpublished data). Moreover, retroviral integrations are mutagenic and can lead to oncogenic transformation of the hematopoietic cells [Baum, 2007]. In addition, the experimental set up requires semi-lethal irradiation of the recipient mice, which drastically alters the chemical and physiological environment of the bone marrow. Therefore, a genetically reliable transgenic mouse model for oncogenic STAT5A became necessary to understand the molecular nature of STAT5A driven hematopoietic transformation.

A few attempts have been made in this direction (see Table 1). Considering the crucial role of STAT5 in the development of lymphocytes, the earliest transgenic mice were designed for over-expression of wild type STAT5A or STAT5B, or constitutively active STAT5B in the lymphoid compartment. The constitutively active version of STAT5B used in the generation of these mice is a double point mutant (H299R and S710F, originally called caSTAT51*6) [Onishi et al., 1998]. These mice displayed deregulation of the development of B-cells and T-cells [Burchill et al., 2003; Goetz et al., 2004; Goetz et al., 2005; Kelly et al., 2003a]. Although, these mice develop lymphoblastic lymphoma, it happens at a very old age and with a low penetrance (8-20%) [Bessette et al., 2008; Kelly et al., 2003b]. However, the H299R mutation has an inhibitory effect on

STAT5A activity, as it is unable to genetically complement for the lack of STAT5 in hematopoietic cells [Moriggl et al., 2005]. Recently another transgenic mouse model has been established, in which, $cS5^F$ is expressed under the regulation of the doxycycline inducible, 'Tet-on' promoter. In this study the TetO-$cS5^F$ mice were bred with transgenic mice expressing the repressing 'rtTA' specifically in the mammary gland (WAP-rtTA). The authors used this elegant system to show that STAT5A induces the transcription of an alternate splice variant of *Akt1* by binding to a distinct promoter [Creamer et al., 2010].

Recent reports have made it clear that STAT5 is also indispensable for the maintenance and progression of the transformed cells. Deletion of STAT5 leads to elimination of myeloid and lymphoid leukemic cells, *in vitro* and *in vivo* [Hoelbl et al., 2010]. Activated STAT5 is associated with poor prognosis [Heltemes-Harris et al., 2011a; Heuser et al., 2009]. In fact, STAT5 activation can be used as a clinical biomarker in patient with MPN, such as - juvenile myelomonocytic leukemia (JMML), chonic myelomonocytic leukemia (CMML) and AML subtype M4/5 [Kotecha et al., 2008]. In addition, STAT5 protects leukemic cells from apoptosis induced by DNA damaging drugs [Slupianek et al., 2002]. Moreover, increased levels of STAT5, in BCR/ABL^+ cells, mediate resistance to imatinib [Warsch et al., 2011] and pharmacological inhibition of STAT5 activity leads to decreased survival in these cells [Nelson et al., 2011]. The recent observation that BCR/ABL can directly phosphorylate STAT5, independent of the JAKs, has made STAT5 a very attractive target in CML [Hantschel et al., 2012].

Considering the decisive role of STAT5 in both myeloid and lymphoid transformation, we decided to generate a transgenic mouse model in which the constitutive activity of STAT5 could be induced and maintained at physiological levels, in the cells/and

tissues where it is naturally expressed. Therefore, we decided to express $cS5^F$ under the endogenous promoter of STAT5A. This required the generation of a BAC transgenic mouse model, as the construct had to be large enough to accommodate the entire STAT5A locus. The $cS5^F$ gene was FLAG tagged at the C-terminal end to allow its distinction from the endogenous protein. The construct is followed by an internal ribosome entry site (IRES) and truncated hCD2. This couples the expression of hCD2 with that of $cS5^F$; and as hCD2 is expressed on the surface, expression of $cS5^F$ can be detected by the expression of hCD2 by FACS analysis. To make the expression of $cS5^F$ inducible, we took advantage of the 'Cre-LoxP' system. The entire '$cS5^F$-FLAG-IRES-hCD2' construct was flanked by LoxP sites, in the opposite orientation. This cassette was used to replace the start codon of the endogenous STAT5A in the BAC (Figure 8).

After pronuclear injection, we derived five transgenic lines which showed germ line transmission. Southern blot was performed to confirm the presence of the transgene. One of the transgenic lines had an incomplete BAC integrated (icS5-11) and was excluded from further analysis. A Southern blot strategy was designed to allow detection of both the endogenous and the transgenic DNA fragment. As there are two copies of the endogenous locus in every mouse, comparison of the intensity of the transgenic band to that of the endogenous band allowed us to deduce that the number of BAC copies integrated into the genome in the other transgenic lines was 2 (Figure 9).

The four transgenic lines were bred with the $RosaCreER^{T2}$ mice [Hameyer et al., 2007]. The cre activity can be induced by treatment with Tx, leading to recombination of the transgenic construct into an 'on' orientation. As the *Rosa* locus is ubiquitously

expressed, the recombination of the transgene should also, ideally, take place in many different tissues. The mice were treated with Tx and organs were harvested 1 week after the last injection, to check for genomic recombination, protein and marker expression and analysis of STAT5A target genes. We were gratified to see genomic recombination of the construct in all major organs tested, specifically in the cells of the hematopoietic tissues (Figure 11). While genomic recombination was detected in three of the transgenic lines analyzed, upon Tx induction, only two of them showed significant expression of the transgenic protein in liver, as detected by Western blotting against the FLAG tag and total STAT5A (Figure 12A). The transgenic line with the highest expression of $cS5^{F}$ (called icS5) was used for functional and phenotypic analysis. Expression of $cS5^{F}$ protein was also detected in the splenocytes of the icS5 mice upon cre induction by Tx (Figure 12B).

We were also able to detect the expression of hCD2 in the cells of different lineages of the hematopoietic system (Figure 13). The expression levels of hCD2 are very low and the MFI of hCD2 is increased only by a factor of 2. This is probably because the expression of the construct is regulated by the endogenous STAT5A promoter, which is not a very strong promoter. Interestingly, we did not detect hCD2 expression in $CD11b^{+}$ myeloid cells. This can also be explained by the fact that there are no reports of any STAT5A functions in terminally differentiated myeloid cells, suggesting very low or no activity of the endogenous STAT5A promoter in these cells. To analyze the *in vivo* transcriptional capability of the $cS5^{F}$, real time PCR was used to measure the levels of mRNA of the some bonafide targets of STAT5, such as *c-Myc, Bcl-2, Ccnd2* and *Mcl-1*. We detected elevated levels of mRNA of STAT5A targets, in liver, upon induction with Tx, assuring us of transcriptional function of the transgenic $cS5^{F}$ protein (Figure 14).

When we first analyzed the icS5;RsCreER^{T2} mice for expression of the transgene, 1 week after Tx injection, we observed that these mice had highly atrophic thymi, with drastic reduction in the total thymocyte numbers. We analyzed other hematopoietic organs and found an increase in the number of CD8^{+} T-cells. Moreover, we analyzed the bone marrow progenitors in these mice and were surprised to observe decreased hematopoietic progenitors (LSK cells) in the bone marrow, as retroviral transduction of bone marrow cells leads to increased percentage of LSK cells (R. Moriggl, unpublished data). Detailed literature search revealed an article which showed that induction of RosaCreER^{T2}, with Tx, is highly toxic, especially in the hematopoietic system [Higashi et al., 2009; Huh et al., 2010]. These mice showed drastic thymic atrophy and reduction in bone marrow progenitor cells. The authors showed that the Cre-ER^{T2} fusion protein translocates to the nucleus, upon Tx binding and indulges in illegal recombination at cryptic LoxP sites. This causes chromosomal abnormalities in the cells, ultimately leading to cellular apoptosis. However, the RosaCreER^{T2} mice recover from the Cre mediated toxicity in about 1 month after the treatment. Therefore, we decided to study the icS5 induced phenotype at a later time point and to include the Tx treated RosaCreER^{T2} mice as control groups for all subsequent analysis.

5 weeks after the injection, organs were harvested from 1 mouse per group to check for the expression of the transgenic protein (Figure 15A). While we could detect expression of hCD2 (Figure 15B), we found no significant differences in any of the hematopoietic lineages. The total cellularity of the spleen, thymus and the bone marrow is unchanged (Figure 15C). There is no difference in the percentages of CD4^{+} and CD8^{+} T-cells in the thymus and the spleen (Figure 15E and F). A slight increase was detected in the granulocytes; however, this increase is due to the effect

of Tx on RosaCreER^{T2}, as it was also detected in the control mice treated with Tx (Figure 15F). Although not statistically significant, there is a tendency for increased percentage of LSK cells in Tx treated icS5;RosaCreER^{T2} mice (Figure 15D). This suggests that there might be differences in the biological properties of HSCs in these mice. A detailed analysis would require assessment of the 'self-renewal' and repopulation capabilities of the early progenitors of these mice. This can be done, *in vitro*, by performing colony forming assays with the bone marrow progenitors and, *in vivo*, by competitive bone marrow repopulation experiments [Li et al., 2010c].

The icS5;RsCreER^{T2} mice provide an interesting tool to study the role of persistent STAT5A signaling, while maintaining relatively physiological levels of STAT5 expression. The toxicity associated with Cre-ER^{T2} is an unfortunate side effect. Moreover, Tx itself has many toxic side effects, including carcinogenicity, teratogenicity, genotoxicity and reproductive toxicity [Carthew et al., 2000; Cunha et al., 1987; Potter et al., 1994]. However, these limitations can be circumvented by using appropriate measures. It is important to use the RsCreER^{T2} mice subjected to the same induction treatment given to the experimental mice as proper experimental controls. It is also important to delay any analysis of these mice to at least a month after the Tx treatment to allow the mice to recover, especially when studying the immune system. Indeed, we have a collaborative effort where the icS5;RsCreER^{T2} mice are being used to analyze the role of persistent STAT5A signaling in intestinal epithelial cells, in mouse models for gut injury and colon cancer.

Theoretically, icS5 mice can be used with any Cre transgenic mice in which the activity of Cre is inducible. However, similar to the RosaCreER^{T2} mice, other inducible systems are also fraught with undesirable side effects. A commonly used inducible Cre transgenic mouse model, especially for expression in the hematopoietic

system, is the Mx1-Cre, which can be induced by type I interferons (or by using polyI:C, and artificial RNA molecule, that mimics a viral infection and induces production of type I IFN) [Kuhn et al., 1995]. While the immune-modulatory effects of interferon have been well established [Theofilopoulos et al., 2005], two recent reports have shown that IFN-α induces proliferation in quiescent HSCs [Essers et al., 2009; Sato et al., 2009]. This observation has serious implications for a number of studies performed with the Mx1-Cre mice, to analyze the development and differentiation of hematopoietic progenitors. Moreover, interferons are also known to induce cellular senescence [Li et al., 2008; Song et al., 2008]. Another inducible mouse model uses tetracycline (or its derivative –doxycycline) responsive promoters to either induce or repress the expression of the transgene [Gossen and Bujard, 1992; Gossen et al., 1995]. The original drawbacks in the 'tet' system have been largely circumvented by ingenious remodeling – such as, use of better tet-responsive transactivators [Urlinger et al., 2000] and bidirectional tetO-CMV promoters [Krestel et al., 2001]. However, tetracycline leads to changes in the gut microbiota, which has immune-modulatory functions, especially in the induction and maintenance of peripheral immune tolerance [Ochoa-Reparaz et al., 2009].

STAT5 has been shown to be associated with the PI3K-AKT pathway in hematopoietic neoplasms (see Table 3). Therefore, we decided to couple $cS5^{F}$ expression with increased PI3K-AKT signaling. To attain this we bred the icS5;$RsCreER^{T2}$ mice into a $Pten^{fl/+}$ background, so that induction with Tx leads to loss of one allele of Pten , along with over-expression of $cS5^{F}$. As PTEN is a negative regulator of the PI3K-AKT pathway, we wanted to test the hypothesis that $cS5^{F}$ overexpression can act synergistically with PTEN haplo-insufficiency in the generation of hematopoietic cancers. We used $RsCreER^{T2}$; $Pten^{fl/+}$ mice as controls, as this allowed us to simultaneously control for both the loss of one allele of PTEN

and Tx induced toxicity in RosaCreER^{T2} mice. Once the mice were generated, they received the respective treatment at the age of 6 weeks. The mice were bled regularly from the tail vein and blood parameters were measured. There is a significant increase in the WBC counts in the mice that have cS5^{F} expression coupled with PTEN haplo-insufficiency compared to the mice that have lost only one allele of Pten. The contribution of mono-allelic loss of tumor suppressor genes to STAT5 mediated hematopoietic cancers is discussed later in this manuscript.

To avoid the complications associated with the use of inducible mouse models, we decided to use a more straight forward approach to make a cS5^{F} over-expression model, using a plasmid construct. Moreover, as the low levels of cS5^{F} expression could be the reason for lack of a hematopoietic phenotype in icS5;RsCreER^{T2} mice, after 6 weeks of induction, we decided to use a system that would make it possible to have higher expression levels of cS5^{F}. Therefore, we decided to use the hematopoietic specific vav-promoter, which has been used successfully in the past to generate transgenic mouse models for other oncogenic genes [Ogilvy et al., 1999a; Ogilvy et al., 1999b; Turner et al., 2003]. Towards this, we cloned the cS5^{F}-FLAG construct under vav-promoter (Figure 19A). Pronuclear injections were performed and 2 positive transgenic mice were derived, called vcS5 mice. Both the mice showed germ line transmission and fortunately, there is a difference in the number of integrations in these mice, as detected by Southern blotting (Figure 19B). More importantly, this difference in copy numbers is reflected in the level of cS5^{F} expressed in the mice, as the high copy transgenic line (vcS5-1^{hi}) has much higher levels of cS5^{F} expression compared to the low copy transgenic line (vcS5-19^{lo}). Finally, Western blot analysis of different organs from these mice, revealed specific expression of cS5^{F} in the hematopoietic tissues. While expression of the transgene was detected in spleen,

thymus and lymph nodes, no expression was detected in the non-hematopoietic tissues that were analyzed, i.e. - liver, gut and kidney. However, the expression of the transgene in all the hematopoietic lineages is yet to be performed. This can be done either by Western blot analysis of the purified cell populations or by FACS analysis by intracellular staining of pYSTAT5. These two transgenic lines will allow us to study the dose-dependent effects of $cS5^{F}$ and to evaluate the hypothesis that progression of hematopoietic neoplasms is associated with the levels of pYSTAT5.

Analysis of the mice at the age of 6 weeks and later at 12 weeks (Figure 20 and 21), revealed a steady increase in the percentage of $CD8^{+}$ T-cells in $cS5\text{-}1^{hi}$ mice, in all the lymphoid organs, over time. This is in accordance to the fact that STAT5A has been shown to be the master regulator of $CD8^{+}$ T-cells. Other transgenic mouse models with over-expression of either wild type or constitutively active STAT5, in the lymphoid lineage, show increased $CD8^{+}$ T-cells [Burchill et al., 2003; Joliot et al., 2006; Kelly et al., 2003a]. This increase is coupled with a reduction in the percentage of $CD4^{+}$ T-cells, leading to a drastic increase in the ratio of CD8:CD4 T-cells. One possibility is that $cS5^{F}$ skews the differentiation of thymic double positive cells specifically towards $CD8^{+}$ lineage. Another explanation could be that the $CD8^{+}$ T-cells in transgenic mice might proliferate faster than the $CD4^{+}$ T-cells, as STAT5 mediates IL-2 induced proliferation of $CD8^{+}$ T-cells. While there was no difference in the percentages of granulocytes and B-cells in the spleen at 6 weeks, there is a significant reduction in the percentage of B-cells in the mice at 12 weeks. It is not clear whether this reduction is a result of the apparent decrease of percentage of B-cells due to such high numbers of $CD8^{+}$ T-cells; or if it is a specific function of STAT5, which directs differentiation of T-cells (specifically $CD8^{+}$ T-cells) at the cost of B-cell differentiation. Analysis of the B-cell differentiation stages (Hardy fractions) [Hardy et al., 1991] in the

vcS5 bone marrow might provide an insight into this question. We did not see any significant difference in the percentages of granulocytes in the hematopoietic organs. Moreover, the number of platelets and the hematocrit in these mice is also comparable to the wild type mice. There was also no difference in the percentage of LSK cells in the bone marrow of these mice. However, further analysis of the progenitor cells is still to be performed. Similar to the icS5 mice, colony forming assays and bone marrow repopulation assays are essential to study the effect of $cS5^{F}$ expression on the self-renewal and repopulation ability of the HSCs. The analysis of both the transgenic lines will allow us to study the dose dependent effects of STAT5 activity in HSCs.

Most interestingly, the transgenic line vcS5-19^{lo}, was indistinguishable from the wild type litter mates at 6 weeks of age, even though considerable expression of the $cS5^{F}$ was detected by Western blot. This could also explain the lack of hematopoietic anomalies in the icS5 mice, as these mice had much lower level of $cS5^{F}$ expression compared to the vcS5-19^{lo} mice. The only difference we could detect was that at the age of 12 weeks the vcS5-19^{lo} mice had much higher levels of WBCs compared to the wild type mice and a moderate increase in splenic cellularity. Interestingly, as there is no difference in the percentages of any of the lineages in the blood and the spleen by FACS, it appears that this increase is due to an increase in the number of cells of all lineages. It is, of course, of primary importance to increase the number of mice analyzed to confirm this intriguing observation. However, this leads to a very interesting question regarding the differential effect of STAT5 on the hematopoietic lineages based on the level of expression. It has been shown that reduction in the level of STAT5 by loss of one allele, has an effect on the number of CD8+ T-cells [Ermakova et al., 2011]. We have also observed that haplo-insufficieny of STAT5,

specifically in T-cells (using *Stat5*$^{fl/+}$;LckCre mice) leads to reduced number of CD8^{+} T-cells compared to wild type litter mates. This reduction in number manifests itself as impairment of contact hypersensitivity response in these mice to small molecule haptens such as di-Nitro-Flouro-Benzene (DNFB) (H. Nivarthi, unpublished data). STAT5A has an important role in the development of NK-cells and different T-cell subsets [Eckelhart et al., 2011; Heltemes-Harris et al., 2011b]. STAT5 inhibits the differentiation of Th17 cells by physically displacing STAT3 from *il17* promoter and repressing transcription. Therefore, the ratio of STAT5 to STAT3 expression determines the development of Th17 cells [Yang et al., 2011]. The analysis of different T-cell subsets in the vcS5 mice will allow elucidation of the molecular mechanisms of this repression. The two transgenic lines of vcS5 mice provide an elegant system to address the question of the dose-dependent effects of STAT5 in different hematopoietic lineages.

Currently, we are monitoring the vcS5 mice for development of hematopoietic cancers. The mice do not develop a disease for up to 5 months of age (mice in breeding cages). This observation is in stark contrast to the fact that in bone marrow transplantation experiments cS5^{F} induced multi-lineage leukemia is fatal within 8-10 weeks. This observation also argues that the leukemia in bone marrow transplantation experiments is due to a combined effect of cS5^{F} expression and mutations introduced by retroviral integrations. The mutagenic effects of retroviruses by modulating the expression of the genes at the site of integration have been conclusively established [Baum, 2007; Modlich et al., 2009]. In case of non-transforming situations, the retroviral vectors can still change the clonal expansion of the HSCs over time [Kustikova et al., 2005]. No studies have been performed to identify retroviral integration sites in the transformed cells in the cS5^{F} transplantation experiments.

Identification of clonal populations with specific retroviral integration sites would help to identify genes that might contribute to STAT5A induced leukemia. Taken together, these data imply that STAT5A is a rather weak oncogene and probably requires activation of synergistic oncogenic signaling pathways for full transforming potential.

According to an interview of Prof. Vogelstein, over the last years, sequencing data from hundreds of human tumors has shown that 90% of the mutations occur in tumor suppressor genes [Kaiser, 2010]. Indeed, in other transgenic mice with expression of STAT5B-CA or $cS5^{F}$ in the lymphoid lineage, B-cell and T-cell lymphomas develop with a shorter latency and higher penetrance when coupled with a 'second-hit' such as lack of p53, *Pax5* or *Ebf1* [Heltemes-Harris et al., 2011a; Joliot et al., 2006]. As has been discussed before, we have also seen an increase in the number of WBCs in icS5 mice when accompanied with PTEN haplo-insufficiency. These data support the hypothesis that STAT5A mediated transformation requires activation of other co-operating signaling pathways.

Moreover, STAT5 is a clinical bio-marker for many hematopoietic neoplasms and mediates resistance to drugs [Kotecha et al., 2008; Slupianek et al., 2002; Warsch et al., 2011]. This suggests that STAT5A activation is directly associated to disease prognosis. Patients suffering with Down's syndrome are predisposed to development of ALL and mutations in JAK2 ($JAK2^{R683S/G}$) and cytokine receptor like factor 2 (*CRLF2*) have been identified in these patients. Over-expression of CRLF2 and JAK2 mutations leads to elevated levels of pYSTAT5 [Hertzberg et al., 2010; Mullighan et al., 2009a; Mullighan et al., 2009b; Russell et al., 2009; Yoda et al., 2010], further supporting the hypothesis that pYSTAT5 levels are directly linked to disease progression.

In MPN patients, whole genome sequencing has led to the identification of genes that are haplo-insufficient due to loss of one allele. These include genes such as *SOCS2,*

TET2, *EZH2*, *IKFZ1*, *FOXP1*, *ETV6/TEL*, *CUX1* and *JARID2* [Etienne et al., 2007; Jager and Kralovics, 2011; Klampfl et al., 2011; Puda et al., 2012; Vainchenker et al., 2011]. Many of these genes have already been reported to have functions in the development of normal and pathogenic hematopoiesis. *SOCS2* is an important negative regulator of the JAK2-STAT5 signaling pathway [Alexander and Hilton, 2004a]. Loss of *SOCS2* function results in elevated pYSTAT5 levels which can lead to disease progression in $JAK2^{V617F}$ mediated MPN [Quentmeier et al., 2008]. The gene *Ikaros* (*IKZF1*) is a transcription factor that regulates cell fate decisions by chromatin remodeling, and loss of *Ikaros* is usually associated with development of ALL [Jager et al., 2010; Payne and Dovat, 2011]. *FOXP1* regulates B-cell development and is associated with development of lymphomas [Fuxa and Skok, 2007; Tzankov et al., 2010]. *ETV6* (more popularly known as *TEL*) is also essential for the development of B-cells, and is a part of many transforming translocations, which lead to generation of lymphomas [De Braekeleer et al., 2012]. The role of *CUX1* in the hematopoietic cells has also been recently reported [Vadnais et al., 2012].

One of the most relevant questions in MPN development is the contribution of somatic mutations to clonal expansion of the mutant cells, leading to aggravation in the disease. It has been shown that increasing the gene dosage of the $JAK2^{V617F}$ mutant protein leads to a proportional increase in the activation of STAT5 [Akada et al., 2010]. We would like to breed the vcS5 mice into a haplo-insufficient background of the above mentioned genes (*PTEN, SOCS2, IKFZ1, ETV6, FOXP1* and *CUX1*), to see if there is an increase of the activated levels of STAT5 due to the loss of these genes. We would also like to generate compound mice that have mono-allelic loss of the above mentioned genes with mice expressing $JAK2^{V617F}$ [Marty et al., 2010]. Competitive repopulation assays will allow us to analyze the effect of the loss of

these genes on clonal selection and/or clonal expansion of the $cS5^{F}$ or $JAK2^{V617F}$ expressing hematopoietic cells.

Expression of STAT5 is associated with cancer in a variety of other tissues [Ferbeyre and Moriggl, 2011]. Although, STAT5 has been considered a classical oncogene, it has been shown to have hepato-protective functions [Blaas et al., 2010; Friedbichler et al., 2012]. Another recent study has revealed that STAT5 signaling in human lung cancer is associated with better prognosis. In fact, the authors recommend the use of STAT5 as a clinical bio-marker for lung cancer prognosis [He et al., 2012]. However, the study of STAT5 in other organs has been limited by the facts that *Stat5*^{null} mice show prenatal lethality and that tightly regulated tissue specific Cre transgenic mice are not available for many tissues. We would like to breed the vcS5 mice into the *Stat5*^{null} background, to rescue these mice. If successful, the vcS5;*Stat5*^{null} mice will provide an elegant system to study the role of STAT5 in a variety of tissues and cancers.

In summary, we have generated three interesting transgenic mouse models of persistent STAT5A activation, with distinct expression pattern and expression levels. These mice will provide a valuable tool to study pathological functions of STAT5A, especially in the development of hematopoietic cancers. As STAT5A co-operates with additional signaling pathways, these mice will furnish us with the means to identify the genes that act synergistically with STAT5A. These studies will provide valuable insight into the molecular mechanisms governing the transformation of hematopoietic cells which could lead to identification of pathways that are amenable to therapeutic interventions.

5. REFERENCES

1 Aboudola, S., Murugesan, G., Szpurka, H., Ramsingh, G., Zhao, X., Prescott, N., Tubbs, R. R., Maciejewski, J. P., and Hsi, E. D. (2007). Bone marrow phospho-STAT5 expression in non-CML chronic myeloproliferative disorders correlates with JAK2 V617F mutation and provides evidence of in vivo JAK2 activation. Am J Surg Pathol *31*, 233-239.

2 Akada, H., Yan, D., Zou, H., Fiering, S., Hutchison, R. E., and Mohi, M. G. (2010). Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. Blood *115*, 3589-3597.

3 Alexander, W. S., and Hilton, D. J. (2004a). The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. Annu Rev Immunol *22*, 503-529.

4 Alexander, W. S., and Hilton, D. J. (2004b). The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immuneresponse. Annual Review of Immunology *22*, 503-529.

5 Antoniou, M., Harland, L., Mustoe, T., Williams, S., Holdstock, J., Yague, E., Mulcahy, T., Griffiths, M., Edwards, S., Ioannou, P. A., Mountain, A., and Crombie, R. (2003). Transgenes encompassing dual-promoter CpG islands from the human TBP and HNRPA2B1 loci are resistant to heterochromatin-mediated silencing. Genomics *82*, 269-279.

6 Azam, M., Erdjument-Bromage, H., Kreider, B. L., Xia, M., Quelle, F., Basu, R., Saris, C., Tempst, P., Ihle, J. N., and Schindler, C. (1995a). Interleukin-3 signals through multiple isoforms of Stat5. EMBO J *14*, 1402-1411.

7 Azam, M., Erdjumentbromage, H., Kreider, B. L., Xia, M., Quelle, F., Basu, R., Saris, C., Tempst, P., Ihle, J. N., and Schindler, C. (1995b). Interleukin-3 Signals through Multiple Isoforms of Stat5. Embo Journal *14*, 1402-1411.

8 Bacon, C. M., Tortolani, P. J., Shimosaka, A., Rees, R. C., Longo, D. L., and O'Shea, J. J. (1995). Thrombopoietin (TPO) induces tyrosine phosphorylation and activation of STAT5 and STAT3. FEBS Lett *370*, 63-68.

9 Barnstein, B. O., Li, G., Wang, Z., Kennedy, S., Chalfant, C., Nakajima, H., Bunting, K. D., and Ryan, J. J. (2006). Stat5 expression is required for IgE-mediated mast cell function. J Immunol *177*, 3421-3426.

10 Baum, C. (2007). Insertional mutagenesis in gene therapy and stem cell biology. Curr Opin Hematol *14*, 337-342.

11 Bessette, K., Lang, M. L., Fava, R. A., Grundy, M., Heinen, J., Horne, L., Spolski, R., Al-Shami, A., Morse, H. C., 3rd, Leonard, W. J., and Kelly, J. A. (2008). A Stat5b transgene is capable of inducing CD8+ lymphoblastic lymphoma in the absence of normal TCR/MHC signaling. Blood *111*, 344-350.

12 Blaas, L., Kornfeld, J. W., Schramek, D., Musteanu, M., Zollner, G., Gumhold, J., van Zijl, F., Schneller, D., Esterbauer, H., Egger, G., Mair, M., Kenner, L., Mikulits, W., Eferl, R., Moriggl, R., Penninger, J., Trauner, M., and Casanova, E. (2010). Disruption of the growth hormone--signal transducer and activator of transcription 5--insulinlike growth factor 1 axis severely aggravates liver fibrosis in a mouse model of cholestasis. Hepatology *51*, 1319-1326.

13 Bourdeau, A., Dube, N., Heinonen, K. M., Theberge, J. F., Doody, K. M., and Tremblay, M. L. (2007). TC-PTP-deficient bone marrow stromal cells fail to support normal B lymphopoiesis due to abnormal secretion of interferon-{gamma}. Blood *109*, 4220-4228.

14 Bradley, H. L., Hawley, T. S., and Bunting, K. D. (2002). Cell intrinsic defects in cytokine responsiveness of STAT5-deficient hematopoietic stem cells. Blood *100*, 3983-3989.

15 Brown, V. I., Hulitt, J., Fish, J., Sheen, C., Bruno, M., Xu, Q., Carroll, M., Fang, J., Teachey, D., and Grupp, S. A. (2007). Thymic stromal-derived lymphopoietin induces proliferation of pre-B leukemia and antagonizes mTOR inhibitors, suggesting a role for interleukin-7Ralpha signaling. Cancer Res *67*, 9963-9970.

16 Buitenhuis, M., Baltus, B., Lammers, J. W., Coffer, P. J., and Koenderman, L. (2003). Signal transducer and activator of transcription 5a (STAT5a) is required for eosinophil differentiation of human cord blood-derived CD34+ cells. Blood *101*, 134-142.

17 Buitenhuis, M., Verhagen, L. P., Cools, J., and Coffer, P. J. (2007). Molecular mechanisms underlying FIP1L1-PDGFRA-mediated myeloproliferation. Cancer Res *67*, 3759-3766.

18 Bunting, K. D. (2007). STAT5 signaling in normal and pathologic hematopoiesis. Front Biosci *12*, 2807-2820.

19 Bunting, K. D., Bradley, H. L., Hawley, T. S., Moriggl, R., Sorrentino, B. P., and Ihle, J. N. (2002). Reduced lymphomyeloid repopulating activity from adult bone marrow and fetal liver of mice lacking expression of STAT5. Blood *99*, 479-487.

20 Burchill, M. A., Goetz, C. A., Prlic, M., O'Neil, J. J., Harmon, I. R., Bensinger, S. J., Turka, L. A., Brennan, P., Jameson, S. C., and Farrar, M. A. (2003). Distinct effects of STAT5 activation on CD4+ and CD8+ T cell homeostasis: development of CD4+CD25+ regulatory T cells versus CD8+ memory T cells. J Immunol *171*, 5853-5864.

21 Bustelo, X. R. (2000). Regulatory and signaling properties of the Vav family. Mol Cell Biol *20*, 1461-1477.

22 Cafferkey, R., Young, P. R., McLaughlin, M. M., Bergsma, D. J., Koltin, Y., Sathe, G. M., Faucette, L., Eng, W. K., Johnson, R. K., and Livi, G. P. (1993). Dominant missense mutations in a novel yeast protein related to mammalian phosphatidylinositol 3-kinase and VPS34 abrogate rapamycin cytotoxicity. Mol Cell Biol *13*, 6012-6023.

23 Cao, X., Shores, E. W., Hu-Li, J., Anver, M. R., Kelsall, B. L., Russell, S. M., Drago, J., Noguchi, M., Grinberg, A., Bloom, E. T., and et al. (1995). Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity *2*, 223-238.

24 Carlesso, N., Frank, D. A., and Griffin, J. D. (1996). Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl. J Exp Med *183*, 811-820.

25 Carracedo, A., Alimonti, A., and Pandolfi, P. P. (2011). PTEN level in tumor suppression: how much is too little? Cancer Res *71*, 629-633.

26 Carthew, P., Edwards, R. E., Nolan, B. M., Martin, E. A., Heydon, R. T., White, I. N., and Tucker, M. J. (2000). Tamoxifen induces endometrial and vaginal cancer in rats in the absence of endometrial hyperplasia. Carcinogenesis *21*, 793-797.

27 Chan, L. C., Karhi, K. K., Rayter, S. I., Heisterkamp, N., Eridani, S., Powles, R., Lawler, S. D., Groffen, J., Foulkes, J. G., Greaves, M. F., and et al. (1987). A novel abl protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia. Nature *325*, 635-637.

28 Chang, F., Lee, J. T., Navolanic, P. M., Steelman, L. S., Shelton, J. G., Blalock, W. L., Franklin, R. A., and McCubrey, J. A. (2003). Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia *17*, 590-603.

29 Chen, J., Somanath, P. R., Razorenova, O., Chen, W. S., Hay, N., Bornstein, P., and Byzova, T. V. (2005). Akt1 regulates pathological angiogenesis, vascular maturation and permeability in vivo. Nat Med *11*, 1188-1196.

30 Choudhary, C., Olsen, J. V., Brandts, C., Cox, J., Reddy, P. N., Bohmer, F. D., Gerke, V., Schmidt-Arras, D. E., Berdel, W. E., Muller-Tidow, C., Mann, M., and Serve, H. (2009). Mislocalized activation of oncogenic RTKs switches downstream signaling outcomes. Mol Cell *36*, 326-339.

31 Chueh, F. Y., Leong, K. F., and Yu, C. L. (2010). Mitochondrial translocation of signal transducer and activator of transcription 5 (STAT5) in leukemic T cells and cytokine-stimulated cells. Biochem Biophys Res Commun *402*, 778-783.

32 Cirmena, G., Aliano, S., Fugazza, G., Bruzzone, R., Garuti, A., Bocciardi, R., Bacigalupo, A., Ravazzolo, R., Ballestrero, A., and Sessarego, M. (2008). A BCR-JAK2 fusion gene as the result of a t(9;22)(p24;q11) in a patient with acute myeloid leukemia. Cancer Genet Cytogenet *183*, 105-108.

33 Cools, J., DeAngelo, D. J., Gotlib, J., Stover, E. H., Legare, R. D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J. D., Cross, N. C., Tefferi, A., Malone, J., Alam, R., Schrier, S. L., Schmid, J., Rose, M., Vandenberghe, P., Verhoef, G., Boogaerts, M., Wlodarska, I., Kantarjian, H., Marynen, P., Coutre, S. E., Stone, R., and Gilliland, D. G. (2003). A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med *348*, 1201-1214.

34 Corbacioglu, S., Kilic, M., Westhoff, M. A., Reinhardt, D., Fulda, S., and Debatin, K. M. (2006). Newly identified c-KIT receptor tyrosine kinase ITD in childhood AML induces ligand-independent growth and is responsive to a synergistic effect of imatinib and rapamycin. Blood *108*, 3504-3513.

35 Creamer, B. A., Sakamoto, K., Schmidt, J. W., Triplett, A. A., Moriggl, R., and Wagner, K. U. (2010). Stat5 promotes survival of mammary epithelial cells through transcriptional activation of a distinct promoter in Akt1. Mol Cell Biol *30*, 2957-2970.

36 Cuesta-Dominguez, A., Ortega, M., Ormazabal, C., Santos-Roncero, M., Galan-Diez, M., Steegmann, J. L., Figuera, A., Arranz, E., Vizmanos, J. L., Bueren, J. A., Rio, P., and Fernandez-Ruiz, E. (2012). Transforming and tumorigenic activity of JAK2 by fusion to BCR: molecular mechanisms of action of a novel BCR-JAK2 tyrosine-kinase. PLoS One *7*, e32451.

37 Cui, Y., Riedlinger, G., Miyoshi, K., Tang, W., Li, C., Deng, C. X., Robinson, G. W., and Hennighausen, L. (2004). Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. Mol Cell Biol *24*, 8037-8047.

38 Cunha, G. R., Taguchi, O., Namikawa, R., Nishizuka, Y., and Robboy, S. J. (1987). Teratogenic effects of clomiphene, tamoxifen, and diethylstilbestrol on the developing human female genital tract. Hum Pathol *18*, 1132-1143.

39 Dai, X., Chen, Y., Di, L., Podd, A., Li, G., Bunting, K. D., Hennighausen, L., Wen, R., and Wang, D. (2007). Stat5 is essential for early B cell development but not for B cell maturation and function. J Immunol *179*, 1068-1079.
40 Danial, N. N., Pernis, A., and Rothman, P. B. (1995). Jak-STAT signaling induced by the v-abl oncogene. Science *269*, 1875-1877.
41 De Braekeleer, E., Douet-Guilbert, N., Morel, F., Le Bris, M. J., Basinko, A., and De Braekeleer, M. (2012). ETV6 fusion genes in hematological malignancies: A review. Leuk Res.
42 De Keersmaecker, K., Graux, C., Odero, M. D., Mentens, N., Somers, R., Maertens, J., Wlodarska, I., Vandenberghe, P., Hagemeijer, A., Marynen, P., and Cools, J. (2005). Fusion of EML1 to ABL1 in T-cell acute lymphoblastic leukemia with cryptic t(9;14)(q34;q32). Blood *105*, 4849-4852.
43 Delogu, A., Schebesta, A., Sun, Q., Aschenbrenner, K., Perlot, T., and Busslinger, M. (2006). Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. Immunity *24*, 269-281.
44 Demoulin, J. B., Uyttenhove, C., Van Roost, E., DeLestre, B., Donckers, D., Van Snick, J., and Renauld, J. C. (1996). A single tyrosine of the interleukin-9 (IL-9) receptor is required for STAT activation, antiapoptotic activity, and growth regulation by IL-9. Mol Cell Biol *16*, 4710-4716.
45 Dolznig, H., Grebien, F., Deiner, E. M., Stangl, K., Kolbus, A., Habermann, B., Kerenyi, M. A., Kieslinger, M., Moriggl, R., Beug, H., and Mullner, E. W. (2006). Erythroid progenitor renewal versus differentiation: genetic evidence for cell autonomous, essential functions of EpoR, Stat5 and the GR. Oncogene *25*, 2890-2900.
46 Dong, F., Dale, D. C., Bonilla, M. A., Freedman, M., Fasth, A., Neijens, H. J., Palmblad, J., Briars, G. L., Carlsson, G., Veerman, A. J., Welte, K., Lowenberg, B., and Touw, I. P. (1997). Mutations in the granulocyte colony-stimulating factor receptor gene in patients with severe congenital neutropenia. Leukemia *11*, 120-125.
47 Eckelhart, E., Warsch, W., Zebedin, E., Simma, O., Stoiber, D., Kolbe, T., Rulicke, T., Mueller, M., Casanova, E., and Sexl, V. (2011). A novel Ncr1-Cre mouse reveals the essential role of STAT5 for NK cell survival and development. Blood.
48 Engblom, D., Kornfeld, J. W., Schwake, L., Tronche, F., Reimann, A., Beug, H., Hennighausen, L., Moriggl, R., and Schutz, G. (2007a). Direct glucocorticoid receptor-Stat5 interaction in hepatocytes controls body size and maturation-related gene expression. Genes & Development *21*, 1157-1162.
49 Engblom, D., Kornfeld, J. W., Schwake, L., Tronche, F., Reimann, A., Beug, H., Hennighausen, L., Moriggl, R., and Schutz, G. (2007b). Direct glucocorticoid receptor-Stat5 interaction in hepatocytes controls body size and maturation-related gene expression. Genes Dev *21*, 1157-1162.
50 Ermakova, O., Piszczek, L., Luciani, L., Cavalli, F. M., Ferreira, T., Farley, D., Rizzo, S., Paolicelli, R. C., Al-Banchaabouchi, M., Nerlov, C., Moriggl, R., Luscombe, N. M., and Gross, C. (2011). Sensitized phenotypic screening identifies gene dosage sensitive region on chromosome 11 that predisposes to disease in mice. EMBO Mol Med *3*, 50-66.
51 Essers, M. A., Offner, S., Blanco-Bose, W. E., Waibler, Z., Kalinke, U., Duchosal, M. A., and Trumpp, A. (2009). IFNalpha activates dormant haematopoietic stem cells in vivo. Nature *458*, 904-908.
52 Etienne, A., Carbuccia, N., Adelaide, J., Bekhouche, I., Remy, V., Sohn, C., Sainty, D., Gastaut, J. A., Olschwang, S., Birnbaum, D., Mozziconacci, M. J.,

and Chaffanet, M. (2007). Rearrangements involving 12q in myeloproliferative disorders: possible role of HMGA2 and SOCS2 genes. Cancer Genet Cytogenet *176*, 80-88.

53 Fazilleau, N., Mark, L., McHeyzer-Williams, L. J., and McHeyzer-Williams, M. G. (2009). Follicular helper T cells: lineage and location. Immunity *30*, 324-335.

54 Ferbeyre, G., and Moriggl, R. (2011). The role of Stat5 transcription factors as tumor suppressors or oncogenes. Biochim Biophys Acta *1815*, 104-114.

55 Fievez, L., Desmet, C., Henry, E., Pajak, B., Hegenbarth, S., Garze, V., Bex, F., Jaspar, F., Boutet, P., Gillet, L., Vanderplasschen, A., Knolle, P. A., Leo, O., Moser, M., Lekeux, P., and Bureau, F. (2007). STAT5 is an ambivalent regulator of neutrophil homeostasis. PLoS One *2*, e727.

56 Flex, E., Petrangeli, V., Stella, L., Chiaretti, S., Hornakova, T., Knoops, L., Ariola, C., Fodale, V., Clappier, E., Paoloni, F., Martinelli, S., Fragale, A., Sanchez, M., Tavolaro, S., Messina, M., Cazzaniga, G., Camera, A., Pizzolo, G., Tornesello, A., Vignetti, M., Battistini, A., Cave, H., Gelb, B. D., Renauld, J. C., Biondi, A., Constantinescu, S. N., Foa, R., and Tartaglia, M. (2008). Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. J Exp Med *205*, 751-758.

57 Friedbichler, K., Kerenyi, M. A., Kovacic, B., Li, G., Hoelbl, A., Yahiaoui, S., Sexl, V., Mullner, E. W., Fajmann, S., Cerny-Reiterer, S., Valent, P., Beug, H., Gouilleux, F., Bunting, K. D., and Moriggl, R. (2010). Stat5a serine 725 and 779 phosphorylation is a prerequisite for hematopoietic transformation. Blood *116*, 1548-1558.

58 Friedbichler, K., Themanns, M., Mueller, K. M., Schlederer, M., Kornfeld, J. W., Terracciano, L. M., Kozlov, A. V., Haindl, S., Kenner, L., Kolbe, T., Mueller, M., Snibson, K. J., Heim, M. H., and Moriggl, R. (2012). Growth-hormone-induced signal transducer and activator of transcription 5 signaling causes gigantism, inflammation, and premature death but protects mice from aggressive liver cancer. Hepatology *55*, 941-952.

59 Fuxa, M., and Skok, J. A. (2007). Transcriptional regulation in early B cell development. Curr Opin Immunol *19*, 129-136.

60 Gewinner, C., Hart, G., Zachara, N., Cole, R., Beisenherz-Huss, C., and Groner, B. (2004). The coactivator of transcription CREB-binding protein interacts preferentially with the glycosylated form of Stat5. J Biol Chem *279*, 3563-3572.

61 Giliani, S., Mella, P., Savoldi, G., and Mazzolari, E. (2005). Cytokine-mediated signalling and early defects in lymphoid development. Curr Opin Allergy Clin Immunol *5*, 519-524.

62 Giraldo, P., and Montoliu, L. (2001). Size matters: use of YACs, BACs and PACs in transgenic animals. Transgenic Res *10*, 83-103.

63 Goetz, C. A., Harmon, I. R., O'Neil, J. J., Burchill, M. A., and Farrar, M. A. (2004). STAT5 activation underlies IL7 receptor-dependent B cell development. J Immunol *172*, 4770-4778.

64 Goetz, C. A., Harmon, I. R., O'Neil, J. J., Burchill, M. A., Johanns, T. M., and Farrar, M. A. (2005). Restricted STAT5 activation dictates appropriate thymic B versus T cell lineage commitment. J Immunol *174*, 7753-7763.

65 Golub, T. R., Barker, G. F., Lovett, M., and Gilliland, D. G. (1994). Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell *77*, 307-316.

66 Gonzalez, E., and McGraw, T. E. (2009). The Akt kinases: isoform specificity in metabolism and cancer. Cell Cycle *8*, 2502-2508.
67 Gossen, M., and Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci U S A *89*, 5547-5551.
68 Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. (1995). Transcriptional activation by tetracyclines in mammalian cells. Science *268*, 1766-1769.
69 Gouilleux-Gruart, V., Gouilleux, F., Desaint, C., Claisse, J. F., Capiod, J. C., Delobel, J., Weber-Nordt, R., Dusanter-Fourt, I., Dreyfus, F., Groner, B., and Prin, L. (1996). STAT-related transcription factors are constitutively activated in peripheral blood cells from acute leukemia patients. Blood *87*, 1692-1697.
70 Gouilleux, F., Pallard, C., Dusanter-Fourt, I., Wakao, H., Haldosen, L. A., Norstedt, G., Levy, D., and Groner, B. (1995). Prolactin, growth hormone, erythropoietin and granulocyte-macrophage colony stimulating factor induce MGF-Stat5 DNA binding activity. EMBO J *14*, 2005-2013.
71 Grebien, F., Kerenyi, M. A., Kovacic, B., Kolbe, T., Becker, V., Dolznig, H., Pfeffer, K., Klingmuller, U., Muller, M., Beug, H., Mullner, E. W., and Moriggl, R. (2008). Stat5 activation enables erythropoiesis in the absence of EpoR and Jak2. Blood *111*, 4511-4522.
72 Griesinger, F., Hennig, H., Hillmer, F., Podleschny, M., Steffens, R., Pies, A., Wormann, B., Haase, D., and Bohlander, S. K. (2005). A BCR-JAK2 fusion gene as the result of a t(9;22)(p24;q11.2) translocation in a patient with a clinically typical chronic myeloid leukemia. Genes Chromosomes Cancer *44*, 329-333.
73 Grimwade, L. F., Happerfield, L., Tristram, C., McIntosh, G., Rees, M., Bench, A. J., Boyd, E. M., Hall, M., Quinn, A., Piggott, N., Scorer, P., Scott, M. A., and Erber, W. N. (2009). Phospho-STAT5 and phospho-Akt expression in chronic myeloproliferative neoplasms. Br J Haematol *147*, 495-506.
74 Guo, L., Wei, G., Zhu, J., Liao, W., Leonard, W. J., Zhao, K., and Paul, W. (2009). IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. Proc Natl Acad Sci U S A *106*, 13463-13468.
75 Habib, T., Senadheera, S., Weinberg, K., and Kaushansky, K. (2002). The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. Biochemistry *41*, 8725-8731.
76 Hameyer, D., Loonstra, A., Eshkind, L., Schmitt, S., Antunes, C., Groen, A., Bindels, E., Jonkers, J., Krimpenfort, P., Meuwissen, R., Rijswijk, L., Bex, A., Berns, A., and Bockamp, E. (2007). Toxicity of ligand-dependent Cre recombinases and generation of a conditional Cre deleter mouse allowing mosaic recombination in peripheral tissues. Physiol Genomics *31*, 32-41.
77 Han, L., Wierenga, A. T. J., Rozenveld-Geugien, M., van de Lande, K., Vellenga, E., and Schuringa, J. J. (2009). Single-Cell STAT5 Signal Transduction Profiling in Normal and Leukemic Stem and Progenitor Cell Populations Reveals Highly Distinct Cytokine Responses. Plos One *4*.
78 Hand, T. W., Cui, W., Jung, Y. W., Sefik, E., Joshi, N. S., Chandele, A., Liu, Y., and Kaech, S. M. (2010). Differential effects of STAT5 and PI3K/AKT signaling on effector and memory CD8 T-cell survival. Proc Natl Acad Sci U S A *107*, 16601-16606.
79 Hantschel, O., Warsch, W., Eckelhart, E., Kaupe, I., Grebien, F., Wagner, K. U., Superti-Furga, G., and Sexl, V. (2012). BCR-ABL uncouples canonical

JAK2-STAT5 signaling in chronic myeloid leukemia. Nat Chem Biol *8*, 285-293.

80 Hardy, R. R., Carmack, C. E., Shinton, S. A., Kemp, J. D., and Hayakawa, K. (1991). Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J Exp Med *173*, 1213-1225.

81 Harir, N., Boudot, C., Friedbichler, K., Sonneck, K., Kondo, R., Martin-Lanneree, S., Kenner, L., Kerenyi, M., Yahiaoui, S., Gouilleux-Gruart, V., Gondry, J., Benit, L., Dusanter-Fourt, I., Lassoued, K., Valent, P., Moriggl, R., and Gouilleux, F. (2008). Oncogenic Kit controls neoplastic mast cell growth through a Stat5/PI3-kinase signaling cascade. Blood *112*, 2463-2473.

82 Harir, N., Pecquet, C., Kerenyi, M., Sonneck, K., Kovacic, B., Nyga, R., Brevet, M., Dhennin, I., Gouilleux-Gruart, V., Beug, H., Valent, P., Lassoued, K., Moriggl, R., and Gouilleux, F. (2007). Constitutive activation of Stat5 promotes its cytoplasmic localization and association with PI3-kinase in myeloid leukemias. Blood *109*, 1678-1686.

83 Hay, N. (2005). The Akt-mTOR tango and its relevance to cancer. Cancer Cell *8*, 179-183.

84 Hayakawa, F., Towatari, M., Kiyoi, H., Tanimoto, M., Kitamura, T., Saito, H., and Naoe, T. (2000). Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. Oncogene *19*, 624-631.

85 He, Y., Zhou, Z., Hofstetter, W. L., Zhou, Y., Hu, W., Guo, C., Wang, L., Guo, W., Pataer, A., Correa, A. M., Lu, Y., Wang, J., Diao, L., Byers, L. A., Wistuba, II, Roth, J. A., Swisher, S. G., Heymach, J. V., and Fang, B. (2012). Aberrant expression of proteins involved in signal transduction and DNA repair pathways in lung cancer and their association with clinical parameters. PLoS One *7*, e31087.

86 Heath, C., and Cross, N. C. (2004). Critical role of STAT5 activation in transformation mediated by ZNF198-FGFR1. J Biol Chem *279*, 6666-6673.

87 Heltemes-Harris, L. M., Willette, M. J., Ramsey, L. B., Qiu, Y. H., Neeley, E. S., Zhang, N., Thomas, D. A., Koeuth, T., Baechler, E. C., Kornblau, S. M., and Farrar, M. A. (2011a). Ebf1 or Pax5 haploinsufficiency synergizes with STAT5 activation to initiate acute lymphoblastic leukemia. J Exp Med *208*, 1135-1149.

88 Heltemes-Harris, L. M., Willette, M. J., Vang, K. B., and Farrar, M. A. (2011b). The role of STAT5 in the development, function, and transformation of B and T lymphocytes. Ann N Y Acad Sci *1217*, 18-31.

89 Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y., and Mills, G. B. (2005). Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov *4*, 988-1004.

90 Hennighausen, L., and Robinson, G. W. (2008). Interpretation of cytokine signaling through the transcription factors STAT5A and STAT5B. Genes Dev *22*, 711-721.

91 Hertzberg, L., Vendramini, E., Ganmore, I., Cazzaniga, G., Schmitz, M., Chalker, J., Shiloh, R., Iacobucci, I., Shochat, C., Zeligson, S., Cario, G., Stanulla, M., Strehl, S., Russell, L. J., Harrison, C. J., Bornhauser, B., Yoda, A., Rechavi, G., Bercovich, D., Borkhardt, A., Kempski, H., te Kronnie, G., Bourquin, J. P., Domany, E., and Izraeli, S. (2010). Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood *115*, 1006-1017.

92 Heuser, M., Sly, L. M., Argiropoulos, B., Kuchenbauer, F., Lai, C., Weng, A., Leung, M., Lin, G., Brookes, C., Fung, S., Valk, P. J., Delwel, R., Lowenberg, B., Krystal, G., and Humphries, R. K. (2009). Modeling the functional heterogeneity of leukemia stem cells: role of STAT5 in leukemia stem cell self-renewal. Blood *114*, 3983-3993.

93 Higashi, A. Y., Ikawa, T., Muramatsu, M., Economides, A. N., Niwa, A., Okuda, T., Murphy, A. J., Rojas, J., Heike, T., Nakahata, T., Kawamoto, H., Kita, T., and Yanagita, M. (2009). Direct hematological toxicity and illegitimate chromosomal recombination caused by the systemic activation of CreERT2. J Immunol *182*, 5633-5640.

94 Hirokawa, S., Sato, H., Kato, I., and Kudo, A. (2003). EBF-regulating Pax5 transcription is enhanced by STAT5 in the early stage of B cells. Eur J Immunol *33*, 1824-1829.

95 Ho, J. M., Beattie, B. K., Squire, J. A., Frank, D. A., and Barber, D. L. (1999). Fusion of the ets transcription factor TEL to Jak2 results in constitutive Jak-Stat signaling. Blood *93*, 4354-4364.

96 Hoelbl, A., Kovacic, B., Kerenyi, M. A., Simma, O., Warsch, W., Cui, Y., Beug, H., Hennighausen, L., Moriggl, R., and Sexl, V. (2006). Clarifying the role of Stat5 in lymphoid development and Abelson-induced transformation. Blood *107*, 4898-4906.

97 Hoelbl, A., Schuster, C., Kovacic, B., Zhu, B., Wickre, M., Hoelzl, M. A., Fajmann, S., Grebien, F., Warsch, W., Stengl, G., Hennighausen, L., Poli, V., Beug, H., Moriggl, R., and Sexl, V. (2010). Stat5 is indispensable for the maintenance of bcr/abl-positive leukaemia. EMBO Mol Med *2*, 98-110.

98 Hornakova, T., Staerk, J., Royer, Y., Flex, E., Tartaglia, M., Constantinescu, S. N., Knoops, L., and Renauld, J. C. (2009). Acute lymphoblastic leukemia-associated JAK1 mutants activate the Janus kinase/STAT pathway via interleukin-9 receptor alpha homodimers. J Biol Chem *284*, 6773-6781.

99 Huh, W. J., Mysorekar, I. U., and Mills, J. C. (2010). Inducible activation of Cre recombinase in adult mice causes gastric epithelial atrophy, metaplasia, and regenerative changes in the absence of "floxed" alleles. Am J Physiol Gastrointest Liver Physiol *299*, G368-380.

100 Hwang, E. S., White, I. A., and Ho, I. C. (2002). An IL-4-independent and CD25-mediated function of c-maf in promoting the production of Th2 cytokines. Proc Natl Acad Sci U S A *99*, 13026-13030.

101 Imada, K., Bloom, E. T., Nakajima, H., Horvath-Arcidiacono, J. A., Udy, G. B., Davey, H. W., and Leonard, W. J. (1998). Stat5b is essential for natural killer cell-mediated proliferation and cytolytic activity. J Exp Med *188*, 2067-2074.

102 Imada, K., and Leonard, W. J. (2000a). The Jak-STAT pathway. Mol Immunol *37*, 1-11.

103 Imada, K., and Leonard, W. J. (2000b). The Jak-STAT pathway (vol 37, pg 1, 2000). Molecular Immunology *37*, 403-403.

104 Isaksen, D. E., Baumann, H., Trobridge, P. A., Farr, A. G., Levin, S. D., and Ziegler, S. F. (1999). Requirement for stat5 in thymic stromal lymphopoietin-mediated signal transduction. J Immunol *163*, 5971-5977.

105 Jager, R., Gisslinger, H., Passamonti, F., Rumi, E., Berg, T., Gisslinger, B., Pietra, D., Harutyunyan, A., Klampfl, T., Olcaydu, D., Cazzola, M., and Kralovics, R. (2010). Deletions of the transcription factor Ikaros in myeloproliferative neoplasms. Leukemia *24*, 1290-1298.

106 Jager, R., and Kralovics, R. (2011). Molecular pathogenesis of Philadelphia chromosome negative chronic myeloproliferative neoplasms. Curr Cancer Drug Targets *11*, 20-30.
107 Jeong, E. G., Kim, M. S., Nam, H. K., Min, C. K., Lee, S., Chung, Y. J., Yoo, N. J., and Lee, S. H. (2008). Somatic mutations of JAK1 and JAK3 in acute leukemias and solid cancers. Clin Cancer Res *14*, 3716-3721.
108 John, S., Robbins, C. M., and Leonard, W. J. (1996). An IL-2 response element in the human IL-2 receptor alpha chain promoter is a composite element that binds Stat5, Elf-1, HMG-I(Y) and a GATA family protein. Embo Journal *15*, 5627-5635.
109 Johnson, D. G., and Walker, C. L. (1999). Cyclins and cell cycle checkpoints. Annu Rev Pharmacol Toxicol *39*, 295-312.
110 Johnston, R. J., Choi, Y. S., Diamond, J. A., Yang, J. A., and Crotty, S. (2012). STAT5 is a potent negative regulator of TFH cell differentiation. J Exp Med *209*, 243-250.
111 Joliot, V., Cormier, F., Medyouf, H., Alcalde, H., and Ghysdael, J. (2006). Constitutive STAT5 activation specifically cooperates with the loss of p53 function in B-cell lymphomagenesis. Oncogene *25*, 4573-4584.
112 Kagami, S., Nakajima, H., Suto, A., Hirose, K., Suzuki, K., Morita, S., Kato, I., Saito, Y., Kitamura, T., and Iwamoto, I. (2001). Stat5a regulates T helper cell differentiation by several distinct mechanisms. Blood *97*, 2358-2365.
113 Kaiser, J. (2010). A Skeptic Questions Cancer Genome Projects. In Sciencemag, (AAAS).
114 Kang, J., and Der, S. D. (2004). Cytokine functions in the formative stages of a lymphocyte's life. Curr Opin Immunol *16*, 180-190.
115 Kearney, L., Gonzalez De Castro, D., Yeung, J., Procter, J., Horsley, S. W., Eguchi-Ishimae, M., Bateman, C. M., Anderson, K., Chaplin, T., Young, B. D., Harrison, C. J., Kempski, H., So, C. W., Ford, A. M., and Greaves, M. (2009). Specific JAK2 mutation (JAK2R683) and multiple gene deletions in Down syndrome acute lymphoblastic leukemia. Blood *113*, 646-648.
116 Kelly, J., Spolski, R., Imada, K., Bollenbacher, J., Lee, S., and Leonard, W. J. (2003a). A role for Stat5 in CD8+ T cell homeostasis. J Immunol *170*, 210-217.
117 Kelly, J. A., Spolski, R., Kovanen, P. E., Suzuki, T., Bollenbacher, J., Pise-Masison, C. A., Radonovich, M. F., Lee, S., Jenkins, N. A., Copeland, N. G., Morse, H. C., 3rd, and Leonard, W. J. (2003b). Stat5 synergizes with T cell receptor/antigen stimulation in the development of lymphoblastic lymphoma. J Exp Med *198*, 79-89.
118 Kelly, P. N., and Strasser, A. (2011). The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. Cell Death Differ *18*, 1414-1424.
119 Kerenyi, M. A., Grebien, F., Gehart, H., Schifrer, M., Artaker, M., Kovacic, B., Beug, H., Moriggl, R., and Mullner, E. W. (2008). Stat5 regulates cellular iron uptake of erythroid cells via IRP-2 and TfR-1. Blood *112*, 3878-3888.
120 Kimura, A., Rieger, M. A., Simone, J. M., Chen, W. P., Wickre, M. C., Zhu, B. M., Hoppe, P. S., O'Shea, J. J., Schroeder, T., and Hennighausen, L. (2009). The transcription factors STAT5A/B regulate GM-CSF-mediated granulopoiesis. Blood *114*, 4721-4728.
121 Kisseleva, T., Bhattacharya, S., Braunstein, J., and Schindler, C. W. (2002). Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene *285*, 1-24.

122 Klampfl, T., Harutyunyan, A., Berg, T., Gisslinger, B., Schalling, M., Bagienski, K., Olcaydu, D., Passamonti, F., Rumi, E., Pietra, D., Jager, R., Pieri, L., Guglielmelli, P., Iacobucci, I., Martinelli, G., Cazzola, M., Vannucchi, A. M., Gisslinger, H., and Kralovics, R. (2011). Genome integrity of myeloproliferative neoplasms in chronic phase and during disease progression. Blood *118*, 167-176.

123 Korn, T., Bettelli, E., Oukka, M., and Kuchroo, V. K. (2009). IL-17 and Th17 Cells. Annu Rev Immunol *27*, 485-517.

124 Kornfeld, J. W., Grebien, F., Kerenyi, M. A., Friedbichler, K., Kovacic, B., Zankl, B., Hoelbl, A., Nivarti, H., Beug, H., Sexl, V., Muller, M., Kenner, L., Mullner, E. W., Gouilleux, F., and Moriggl, R. (2008). The different functions of Stat5 and chromatin alteration through Stat5 proteins. Front Biosci *13*, 6237-6254.

125 Kotecha, N., Flores, N. J., Irish, J. M., Simonds, E. F., Sakai, D. S., Archambeault, S., Diaz-Flores, E., Coram, M., Shannon, K. M., Nolan, G. P., and Loh, M. L. (2008). Single-cell profiling identifies aberrant STAT5 activation in myeloid malignancies with specific clinical and biologic correlates. Cancer Cell *14*, 335-343.

126 Kovacic, B., Hoelbl, A., Litos, G., Alacakaptan, M., Schuster, C., Fischhuber, K. M., Kerenyi, M. A., Stengl, G., Moriggl, R., Sexl, V., and Beug, H. (2012). Diverging fates of cells of origin in acute and chronic leukaemia. EMBO Mol Med *4*, 283-297.

127 Kralovics, R., Passamonti, F., Buser, A. S., Teo, S. S., Tiedt, R., Passweg, J. R., Tichelli, A., Cazzola, M., and Skoda, R. C. (2005). A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med *352*, 1779-1790.

128 Krestel, H. E., Mayford, M., Seeburg, P. H., and Sprengel, R. (2001). A GFP-equipped bidirectional expression module well suited for monitoring tetracycline-regulated gene expression in mouse. Nucleic Acids Res *29*, E39.

129 Kuhn, R., Schwenk, F., Aguet, M., and Rajewsky, K. (1995). Inducible gene targeting in mice. Science *269*, 1427-1429.

130 Kustikova, O., Fehse, B., Modlich, U., Yang, M., Dullmann, J., Kamino, K., von Neuhoff, N., Schlegelberger, B., Li, Z., and Baum, C. (2005). Clonal dominance of hematopoietic stem cells triggered by retroviral gene marking. Science *308*, 1171-1174.

131 Lacronique, V., Boureux, A., Valle, V. D., Poirel, H., Quang, C. T., Mauchauffe, M., Berthou, C., Lessard, M., Berger, R., Ghysdael, J., and Bernard, O. A. (1997). A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science *278*, 1309-1312.

132 Laplante, M., and Sabatini, D. M. (2012). mTOR signaling in growth control and disease. Cell *149*, 274-293.

133 Laurence, A., Tato, C. M., Davidson, T. S., Kanno, Y., Chen, Z., Yao, Z., Blank, R. B., Meylan, F., Siegel, R., Hennighausen, L., Shevach, E. M., and O'Shea J, J. (2007). Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity *26*, 371-381.

134 Lee, E. C., Yu, D., Martinez de Velasco, J., Tessarollo, L., Swing, D. A., Court, D. L., Jenkins, N. A., and Copeland, N. G. (2001). A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. Genomics *73*, 56-65.

135 Lee, H. M., Bautista, J. L., and Hsieh, C. S. (2011). Thymic and peripheral differentiation of regulatory T cells. Adv Immunol *112*, 25-71.

136 Leonard, W. J., and O'Shea, J. J. (1998). Jaks and STATs: biological implications. Annu Rev Immunol *16*, 293-322.
137 Levin, S. D., Koelling, R. M., Friend, S. L., Isaksen, D. E., Ziegler, S. F., Perlmutter, R. M., and Farr, A. G. (1999). Thymic stromal lymphopoietin: a cytokine that promotes the development of IgM+ B cells in vitro and signals via a novel mechanism. J Immunol *162*, 677-683.
138 Levy, D. E., and Marie, I. J. (2012). STATus report on tetramers. Immunity *36*, 553-555.
139 Li, G., Miskimen, K. L., Wang, Z., Xie, X. Y., Brenzovich, J., Ryan, J. J., Tse, W., Moriggl, R., and Bunting, K. D. (2010a). STAT5 requires the N-domain for suppression of miR15/16, induction of bcl-2, and survival signaling in myeloproliferative disease. Blood *115*, 1416-1424.
140 Li, G., Miskimen, K. L., Wang, Z., Xie, X. Y., Tse, W., Gouilleux, F., Moriggl, R., and Bunting, K. D. (2010b). Effective targeting of STAT5-mediated survival in myeloproliferative neoplasms using ABT-737 combined with rapamycin. Leukemia *24*, 1397-1405.
141 Li, G., Wang, Z., Miskimen, K. L., Zhang, Y., Tse, W., and Bunting, K. D. (2010c). Gab2 promotes hematopoietic stem cell maintenance and self-renewal synergistically with STAT5. PLoS One *5*, e9152.
142 Li, G., Wang, Z., Zhang, Y., Kang, Z., Haviernikova, E., Cui, Y., Hennighausen, L., Moriggl, R., Wang, D., Tse, W., and Bunting, K. D. (2007). STAT5 requires the N-domain to maintain hematopoietic stem cell repopulating function and appropriate lymphoid-myeloid lineage output. Exp Hematol *35*, 1684-1694.
143 Li, Q., Tang, L., Roberts, P. C., Kraniak, J. M., Fridman, A. L., Kulaeva, O. I., Tehrani, O. S., and Tainsky, M. A. (2008). Interferon regulatory factors IRF5 and IRF7 inhibit growth and induce senescence in immortal Li-Fraumeni fibroblasts. Mol Cancer Res *6*, 770-784.
144 Liao, W., Schones, D. E., Oh, J., Cui, Y. Z., Cui, K. R., Roh, T. Y., Zhao, K., and Leonard, W. J. (2008). Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor alpha-chain expression. Nature Immunology *9*, 1288-1296.
145 Lin, J. X., Li, P., Liu, D., Jin, H. T., He, J., Rasheed, M. A., Rochman, Y., Wang, L., Cui, K., Liu, C., Kelsall, B. L., Ahmed, R., and Leonard, W. J. (2012). Critical Role of STAT5 Transcription Factor Tetramerization for Cytokine Responses and Normal Immune Function. Immunity *36*, 586-599.
146 Lin, J. X., Migone, T. S., Tsang, M., Friedmann, M., Weatherbee, J. A., Zhou, L., Yamauchi, A., Bloom, E. T., Mietz, J., John, S., and et al. (1995). The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity *2*, 331-339.
147 Litterst, C. M., Kliem, S., Marilley, D., and Pfitzner, E. (2003). NCoA-1/SRC-1 is an essential coactivator of STAT5 that binds to the FDL motif in the alpha-helical region of the STAT5 transactivation domain. J Biol Chem *278*, 45340-45351.
148 Liu, F., Kunter, G., Krem, M. M., Eades, W. C., Cain, J. A., Tomasson, M. H., Hennighausen, L., and Link, D. C. (2008). Csf3r mutations in mice confer a strong clonal HSC advantage via activation of Stat5. J Clin Invest *118*, 946-955.

149 Liu, X., Robinson, G. W., Wagner, K. U., Garrett, L., Wynshaw-Boris, A., and Hennighausen, L. (1997). Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev *11*, 179-186.
150 Lu, T. L., Chang, J. L., Liang, C. C., You, L. R., and Chen, C. M. (2007). Tumor spectrum, tumor latency and tumor incidence of the Pten-deficient mice. PLoS One *2*, e1237.
151 Maki, K., Sunaga, S., and Ikuta, K. (1996). The V-J recombination of T cell receptor-gamma genes is blocked in interleukin-7 receptor-deficient mice. J Exp Med *184*, 2423-2427.
152 Malin, S., McManus, S., and Busslinger, M. (2010a). STAT5 in B cell development and leukemia. Curr Opin Immunol *22*, 168-176.
153 Malin, S., McManus, S., Cobaleda, C., Novatchkova, M., Delogu, A., Bouillet, P., Strasser, A., and Busslinger, M. (2010b). Role of STAT5 in controlling cell survival and immunoglobulin gene recombination during pro-B cell development. Nat Immunol *11*, 171-179.
154 Mandal, M., Powers, S. E., Maienschein-Cline, M., Bartom, E. T., Hamel, K. M., Kee, B. L., Dinner, A. R., and Clark, M. R. (2011). Epigenetic repression of the Igk locus by STAT5-mediated recruitment of the histone methyltransferase Ezh2. Nat Immunol *12*, 1212-1220.
155 Marty, C., Lacout, C., Martin, A., Hasan, S., Jacquot, S., Birling, M. C., Vainchenker, W., and Villeval, J. L. (2010). Myeloproliferative neoplasm induced by constitutive expression of JAK2V617F in knock-in mice. Blood *116*, 783-787.
156 Masson, K., Liu, T., Khan, R., Sun, J., and Ronnstrand, L. (2009). A role of Gab2 association in Flt3 ITD mediated Stat5 phosphorylation and cell survival. Br J Haematol *146*, 193-202.
157 Mercher, T., Wernig, G., Moore, S. A., Levine, R. L., Gu, T. L., Frohling, S., Cullen, D., Polakiewicz, R. D., Bernard, O. A., Boggon, T. J., Lee, B. H., and Gilliland, D. G. (2006). JAK2T875N is a novel activating mutation that results in myeloproliferative disease with features of megakaryoblastic leukemia in a murine bone marrow transplantation model. Blood *108*, 2770-2779.
158 Migone, T. S., Lin, J. X., Cereseto, A., Mulloy, J. C., O'Shea, J. J., Franchini, G., and Leonard, W. J. (1995). Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I. Science *269*, 79-81.
159 Modlich, U., Navarro, S., Zychlinski, D., Maetzig, T., Knoess, S., Brugman, M. H., Schambach, A., Charrier, S., Galy, A., Thrasher, A. J., Bueren, J., and Baum, C. (2009). Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. Mol Ther *17*, 1919-1928.
160 Montiel-Duarte, C., Cordeu, L., Agirre, X., Roman-Gomez, J., Jimenez-Velasco, A., Jose-Eneriz, E. S., Garate, L., Andreu, E. J., Calasanz, M. J., Heiniger, A., Torres, A., and Prosper, F. (2008). Resistance to Imatinib Mesylate-induced apoptosis in acute lymphoblastic leukemia is associated with PTEN down-regulation due to promoter hypermethylation. Leuk Res *32*, 709-716.
161 Moriggl, R., Sexl, V., Kenner, L., Duntsch, C., Stangl, K., Gingras, S., Hoffmeyer, A., Bauer, A., Piekorz, R., Wang, D., Bunting, K. D., Wagner, E. F., Sonneck, K., Valent, P., Ihle, J. N., and Beug, H. (2005). Stat5 tetramer formation is associated with leukemogenesis. Cancer Cell *7*, 87-99.
162 Moriggl, R., Topham, D. J., Teglund, S., Sexl, V., McKay, C., Wang, D., Hoffmeyer, A., van Deursen, J., Sangster, M. Y., Bunting, K. D., Grosveld, G.

C., and Ihle, J. N. (1999). Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. Immunity *10*, 249-259.

163 Mueller, K. M., Kornfeld, J. W., Friedbichler, K., Blaas, L., Egger, G., Esterbauer, H., Hasselblatt, P., Schlederer, M., Haindl, S., Wagner, K. U., Engblom, D., Haemmerle, G., Kratky, D., Sexl, V., Kenner, L., Kozlov, A. V., Terracciano, L., Zechner, R., Schuetz, G., Casanova, E., Pospisilik, J. A., Heim, M. H., and Moriggl, R. (2011). Impairment of hepatic growth hormone and glucocorticoid receptor signaling causes steatosis and hepatocellular carcinoma in mice. Hepatology *54*, 1398-1409.

164 Mui, A. L., Wakao, H., Harada, N., O'Farrell, A. M., and Miyajima, A. (1995). Interleukin-3, granulocyte-macrophage colony-stimulating factor, and interleukin-5 transduce signals through two forms of STAT5. J Leukoc Biol *57*, 799-803.

165 Mullighan, C. G., Collins-Underwood, J. R., Phillips, L. A., Loudin, M. G., Liu, W., Zhang, J., Ma, J., Coustan-Smith, E., Harvey, R. C., Willman, C. L., Mikhail, F. M., Meyer, J., Carroll, A. J., Williams, R. T., Cheng, J., Heerema, N. A., Basso, G., Pession, A., Pui, C. H., Raimondi, S. C., Hunger, S. P., Downing, J. R., Carroll, W. L., and Rabin, K. R. (2009a). Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet *41*, 1243-1246.

166 Mullighan, C. G., Zhang, J., Harvey, R. C., Collins-Underwood, J. R., Schulman, B. A., Phillips, L. A., Tasian, S. K., Loh, M. L., Su, X., Liu, W., Devidas, M., Atlas, S. R., Chen, I. M., Clifford, R. J., Gerhard, D. S., Carroll, W. L., Reaman, G. H., Smith, M., Downing, J. R., Hunger, S. P., and Willman, C. L. (2009b). JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A *106*, 9414-9418.

167 Muyrers, J. P., Zhang, Y., Testa, G., and Stewart, A. F. (1999). Rapid modification of bacterial artificial chromosomes by ET-recombination. Nucleic Acids Res *27*, 1555-1557.

168 Nagata, H., Worobec, A. S., Oh, C. K., Chowdhury, B. A., Tannenbaum, S., Suzuki, Y., and Metcalfe, D. D. (1995). Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc Natl Acad Sci U S A *92*, 10560-10564.

169 Nakajima, H., Brindle, P. K., Handa, M., and Ihle, J. N. (2001). Functional interaction of STAT5 and nuclear receptor co-repressor SMRT: implications in negative regulation of STAT5-dependent transcription. EMBO J *20*, 6836-6844.

170 Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., Kashima, K., Sonoda, Y., Fujimoto, T., and Misawa, S. (1996). Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia *10*, 1911-1918.

171 Nelson, E. A., Walker, S. R., Weisberg, E., Bar-Natan, M., Barrett, R., Gashin, L. B., Terrell, S., Klitgaard, J. L., Santo, L., Addorio, M. R., Ebert, B. L., Griffin, J. D., and Frank, D. A. (2011). The STAT5 inhibitor pimozide decreases survival of chronic myelogenous leukemia cells resistant to kinase inhibitors. Blood *117*, 3421-3429.

172 Nicot, C., Mulloy, J. C., Ferrari, M. G., Johnson, J. M., Fu, K., Fukumoto, R., Trovato, R., Fullen, J., Leonard, W. J., and Franchini, G. (2001). HTLV-1 p12(I) protein enhances STAT5 activation and decreases the interleukin-2 requirement for proliferation of primary human peripheral blood mononuclear cells. Blood *98*, 823-829.

173 Nurieva, R. I., Podd, A., Chen, Y., Alekseev, A. M., Yu, M., Qi, X., Huang, H., Wen, R., Wang, J., Li, H. S., Watowich, S. S., Qi, H., Dong, C., and Wang, D. (2012). STAT5 negatively regulates T follicular helper (Tfh) cell generation and function. J Biol Chem.
174 Nyga, R., Pecquet, C., Harir, N., Gu, H., Dhennin-Duthille, I., Regnier, A., Gouilleux-Gruart, V., Lassoued, K., and Gouilleux, F. (2005). Activated STAT5 proteins induce activation of the PI 3-kinase/Akt and Ras/MAPK pathways via the Gab2 scaffolding adapter. Biochem J *390*, 359-366.
175 Oberley, M. J., Wang, D. S., and Yang, D. T. (2012). Vav1 in hematologic neoplasms, a mini review. Am J Blood Res *2*, 1-8.
176 Oboki, K., Ohno, T., Kajiwara, N., Saito, H., and Nakae, S. (2010). IL-33 and IL-33 receptors in host defense and diseases. Allergol Int *59*, 143-160.
177 Ochoa-Reparaz, J., Mielcarz, D. W., Ditrio, L. E., Burroughs, A. R., Foureau, D. M., Haque-Begum, S., and Kasper, L. H. (2009). Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol *183*, 6041-6050.
178 Ogilvy, S., Elefanty, A. G., Visvader, J., Bath, M. L., Harris, A. W., and Adams, J. M. (1998). Transcriptional regulation of vav, a gene expressed throughout the hematopoietic compartment. Blood *91*, 419-430.
179 Ogilvy, S., Metcalf, D., Gibson, L., Bath, M. L., Harris, A. W., and Adams, J. M. (1999a). Promoter elements of vav drive transgene expression in vivo throughout the hematopoietic compartment. Blood *94*, 1855-1863.
180 Ogilvy, S., Metcalf, D., Print, C. G., Bath, M. L., Harris, A. W., and Adams, J. M. (1999b). Constitutive Bcl-2 expression throughout the hematopoietic compartment affects multiple lineages and enhances progenitor cell survival. Proc Natl Acad Sci U S A *96*, 14943-14948.
181 Okuda, K., Golub, T. R., Gilliland, D. G., and Griffin, J. D. (1996). p210BCR/ABL, p190BCR/ABL, and TEL/ABL activate similar signal transduction pathways in hematopoietic cell lines. Oncogene *13*, 1147-1152.
182 Onishi, M., Nosaka, T., Misawa, K., Mui, A. L., Gorman, D., McMahon, M., Miyajima, A., and Kitamura, T. (1998). Identification and characterization of a constitutively active STAT5 mutant that promotes cell proliferation. Mol Cell Biol *18*, 3871-3879.
183 Orkin, S. H., and Zon, L. I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. Cell *132*, 631-644.
184 Osborne, L. C., and Abraham, N. (2010). Regulation of memory T cells by gammac cytokines. Cytokine *50*, 105-113.
185 Papadopoulos, P., Ridge, S. A., Boucher, C. A., Stocking, C., and Wiedemann, L. M. (1995). The novel activation of ABL by fusion to an ets-related gene, TEL. Cancer Res *55*, 34-38.
186 Park, J. H., Adoro, S., Guinter, T., Erman, B., Alag, A. S., Catalfamo, M., Kimura, M. Y., Cui, Y., Lucas, P. J., Gress, R. E., Kubo, M., Hennighausen, L., Feigenbaum, L., and Singer, A. (2010). Signaling by intrathymic cytokines, not T cell antigen receptors, specifies CD8 lineage choice and promotes the differentiation of cytotoxic-lineage T cells. Nat Immunol *11*, 257-264.
187 Payne, K. J., and Dovat, S. (2011). Ikaros and tumor suppression in acute lymphoblastic leukemia. Crit Rev Oncog *16*, 3-12.
188 Peeters, P., Raynaud, S. D., Cools, J., Wlodarska, I., Grosgeorge, J., Philip, P., Monpoux, F., Van Rompaey, L., Baens, M., Van den Berghe, H., and Marynen, P. (1997). Fusion of TEL, the ETS-variant gene 6 (ETV6), to the

receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood *90*, 2535-2540.

189 Pelengaris, S., Khan, M., and Evan, G. (2002). c-MYC: more than just a matter of life and death. Nat Rev Cancer *2*, 764-776.

190 Peng, C., Chen, Y., Yang, Z., Zhang, H., Osterby, L., Rosmarin, A. G., and Li, S. (2010). PTEN is a tumor suppressor in CML stem cells and BCR-ABL-induced leukemias in mice. Blood *115*, 626-635.

191 Pfitzner, E., Jahne, R., Wissler, M., Stoecklin, E., and Groner, B. (1998). p300/CREB-binding protein enhances the prolactin-mediated transcriptional induction through direct interaction with the transactivation domain of Stat5, but does not participate in the Stat5-mediated suppression of the glucocorticoid response. Mol Endocrinol *12*, 1582-1593.

192 Pikaart, M. J., Recillas-Targa, F., and Felsenfeld, G. (1998). Loss of transcriptional activity of a transgene is accompanied by DNA methylation and histone deacetylation and is prevented by insulators. Genes Dev *12*, 2852-2862.

193 Potter, G. A., McCague, R., and Jarman, M. (1994). A mechanistic hypothesis for DNA adduct formation by tamoxifen following hepatic oxidative metabolism. Carcinogenesis *15*, 439-442.

194 Price, C. M., Rassool, F., Shivji, M. K., Gow, J., Tew, C. J., Haworth, C., Goldman, J. M., and Wiedemann, L. M. (1988). Rearrangement of the breakpoint cluster region and expression of P210 BCR-ABL in a "masked" Philadelphia chromosome-positive acute myeloid leukemia. Blood *72*, 1829-1832.

195 Puda, A., Milosevic, J. D., Berg, T., Klampfl, T., Harutyunyan, A. S., Gisslinger, B., Rumi, E., Pietra, D., Malcovati, L., Elena, C., Doubek, M., Steurer, M., Tosic, N., Pavlovic, S., Guglielmelli, P., Pieri, L., Vannucchi, A. M., Gisslinger, H., Cazzola, M., and Kralovics, R. (2012). Frequent deletions of JARID2 in leukemic transformation of chronic myeloid malignancies. Am J Hematol *87*, 245-250.

196 Pugliese-Pires, P. N., Tonelli, C. A., Dora, J. M., Silva, P. C., Czepielewski, M., Simoni, G., Arnhold, I. J., and Jorge, A. A. (2010). A novel STAT5B mutation causing GH insensitivity syndrome associated with hyperprolactinemia and immune dysfunction in two male siblings. Eur J Endocrinol *163*, 349-355.

197 Quentmeier, H., Geffers, R., Jost, E., Macleod, R. A., Nagel, S., Rohrs, S., Romani, J., Scherr, M., Zaborski, M., and Drexler, H. G. (2008). SOCS2: inhibitor of JAK2V617F-mediated signal transduction. Leukemia *22*, 2169-2175.

198 Quinn, B. A., Dash, R., Azab, B., Sarkar, S., Das, S. K., Kumar, S., Oyesanya, R. A., Dasgupta, S., Dent, P., Grant, S., Rahmani, M., Curiel, D. T., Dmitriev, I., Hedvat, M., Wei, J., Wu, B., Stebbins, J. L., Reed, J. C., Pellecchia, M., Sarkar, D., and Fisher, P. B. (2011). Targeting Mcl-1 for the therapy of cancer. Expert Opin Investig Drugs *20*, 1397-1411.

199 Rochman, Y., Kashyap, M., Robinson, G. W., Sakamoto, K., Gomez-Rodriguez, J., Wagner, K. U., and Leonard, W. J. (2010). Thymic stromal lymphopoietin-mediated STAT5 phosphorylation via kinases JAK1 and JAK2 reveals a key difference from IL-7-induced signaling. Proc Natl Acad Sci U S A *107*, 19455-19460.

200 Russell, L. J., Capasso, M., Vater, I., Akasaka, T., Bernard, O. A., Calasanz, M. J., Chandrasekaran, T., Chapiro, E., Gesk, S., Griffiths, M., Guttery, D. S., Haferlach, C., Harder, L., Heidenreich, O., Irving, J., Kearney, L., Nguyen-

Khac, F., Machado, L., Minto, L., Majid, A., Moorman, A. V., Morrison, H., Rand, V., Strefford, J. C., Schwab, C., Tonnies, H., Dyer, M. J., Siebert, R., and Harrison, C. J. (2009). Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood *114*, 2688-2698.

201 Ryan, J. J., Huang, H., McReynolds, L. J., Shelburne, C., Hu-Li, J., Huff, T. F., and Paul, W. E. (1997). Stem cell factor activates STAT-5 DNA binding in IL-3-derived bone marrow mast cells. Exp Hematol *25*, 357-362.

202 Sabatini, D. M., Erdjument-Bromage, H., Lui, M., Tempst, P., and Snyder, S. H. (1994). RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell *78*, 35-43.

203 Sato, T., Onai, N., Yoshihara, H., Arai, F., Suda, T., and Ohteki, T. (2009). Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. Nat Med *15*, 696-700.

204 Scheeren, F. A., Diehl, S. A., Smit, L. A., Beaumont, T., Naspetti, M., Bende, R. J., Blom, B., Karube, K., Ohshima, K., van Noesel, C. J., and Spits, H. (2008). IL-21 is expressed in Hodgkin lymphoma and activates STAT5: evidence that activated STAT5 is required for Hodgkin lymphomagenesis. Blood *111*, 4706-4715.

205 Scheeren, F. A., Naspetti, M., Diehl, S., Schotte, R., Nagasawa, M., Wijnands, E., Gimeno, R., Vyth-Dreese, F. A., Blom, B., and Spits, H. (2005). STAT5 regulates the self-renewal capacity and differentiation of human memory B cells and controls Bcl-6 expression. Nat Immunol *6*, 303-313.

206 Schepers, H., van Gosliga, D., Wierenga, A. T., Eggen, B. J., Schuringa, J. J., and Vellenga, E. (2007). STAT5 is required for long-term maintenance of normal and leukemic human stem/progenitor cells. Blood *110*, 2880-2888.

207 Scherr, M., Chaturvedi, A., Battmer, K., Dallmann, I., Schultheis, B., Ganser, A., and Eder, M. (2006). Enhanced sensitivity to inhibition of SHP2, STAT5, and Gab2 expression in chronic myeloid leukemia (CML). Blood *107*, 3279-3287.

208 Schoenborn, J. R., and Wilson, C. B. (2007). Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol *96*, 41-101.

209 Scott, L. M., Tong, W., Levine, R. L., Scott, M. A., Beer, P. A., Stratton, M. R., Futreal, P. A., Erber, W. N., McMullin, M. F., Harrison, C. N., Warren, A. J., Gilliland, D. G., Lodish, H. F., and Green, A. R. (2007). JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med *356*, 459-468.

210 Sexl, V., Piekorz, R., Moriggl, R., Rohrer, J., Brown, M. P., Bunting, K. D., Rothammer, K., Roussel, M. F., and Ihle, J. N. (2000). Stat5a/b contribute to interleukin 7-induced B-cell precursor expansion, but abl- and bcr/abl-induced transformation are independent of stat5. Blood *96*, 2277-2283.

211 Shah, N., Pang, B., Yeoh, K. G., Thorn, S., Chen, C. S., Lilly, M. B., and Salto-Tellez, M. (2008). Potential roles for the PIM1 kinase in human cancer - a molecular and therapeutic appraisal. Eur J Cancer *44*, 2144-2151.

212 Shelburne, C. P., McCoy, M. E., Piekorz, R., Sexl, V., Roh, K. H., Jacobs-Helber, S. M., Gillespie, S. R., Bailey, D. P., Mirmonsef, P., Mann, M. N., Kashyap, M., Wright, H. V., Chong, H. J., Bouton, L. A., Barnstein, B., Ramirez, C. D., Bunting, K. D., Sawyer, S., Lantz, C. S., and Ryan, J. J. (2003). Stat5 expression is critical for mast cell development and survival. Blood *102*, 1290-1297.

213 Shi, M., Lin, T. H., Appell, K. C., and Berg, L. J. (2008). Janus-kinase-3-dependent signals induce chromatin remodeling at the Ifng locus during T helper 1 cell differentiation. Immunity *28*, 763-773.
214 Shochat, C., Tal, N., Bandapalli, O. R., Palmi, C., Ganmore, I., te Kronnie, G., Cario, G., Cazzaniga, G., Kulozik, A. E., Stanulla, M., Schrappe, M., Biondi, A., Basso, G., Bercovich, D., Muckenthaler, M. U., and Izraeli, S. (2011). Gain-of-function mutations in interleukin-7 receptor-alpha (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med *208*, 901-908.
215 Shuai, K. (2006). Regulation of cytokine signaling pathways by PIAS proteins. Cell Res *16*, 196-202.
216 Shuai, K., Halpern, J., ten Hoeve, J., Rao, X., and Sawyers, C. L. (1996). Constitutive activation of STAT5 by the BCR-ABL oncogene in chronic myelogenous leukemia. Oncogene *13*, 247-254.
217 Slupianek, A., Hoser, G., Majsterek, I., Bronisz, A., Malecki, M., Blasiak, J., Fishel, R., and Skorski, T. (2002). Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. Mol Cell Biol *22*, 4189-4201.
218 Song, G., Ouyang, G., and Bao, S. (2005). The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med *9*, 59-71.
219 Song, L. L., Alimirah, F., Panchanathan, R., Xin, H., and Choubey, D. (2008). Expression of an IFN-inducible cellular senescence gene, IFI16, is up-regulated by p53. Mol Cancer Res *6*, 1732-1741.
220 Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol *98*, 503-517.
221 Sternberg, D. W., Tomasson, M. H., Carroll, M., Curley, D. P., Barker, G., Caprio, M., Wilbanks, A., Kazlauskas, A., and Gilliland, D. G. (2001). The TEL/PDGFbetaR fusion in chronic myelomonocytic leukemia signals through STAT5-dependent and STAT5-independent pathways. Blood *98*, 3390-3397.
222 Suzuki, A., Nakano, T., Mak, T. W., and Sasaki, T. (2008). Portrait of PTEN: messages from mutant mice. Cancer Sci *99*, 209-213.
223 Suzuki, A., Yamaguchi, M. T., Ohteki, T., Sasaki, T., Kaisho, T., Kimura, Y., Yoshida, R., Wakeham, A., Higuchi, T., Fukumoto, M., Tsubata, T., Ohashi, P. S., Koyasu, S., Penninger, J. M., Nakano, T., and Mak, T. W. (2001). T cell-specific loss of Pten leads to defects in central and peripheral tolerance. Immunity *14*, 523-534.
224 Takeda, Y., Nakaseko, C., Tanaka, H., Takeuchi, M., Yui, M., Saraya, A., Miyagi, S., Wang, C., Tanaka, S., Ohwada, C., Sakaida, E., Yamaguchi, N., Yokote, K., Hennighausen, L., and Iwama, A. (2011). Direct activation of STAT5 by ETV6-LYN fusion protein promotes induction of myeloproliferative neoplasm with myelofibrosis. Br J Haematol *153*, 589-598.
225 Takemoto, S., Mulloy, J. C., Cereseto, A., Migone, T. S., Patel, B. K., Matsuoka, M., Yamaguchi, K., Takatsuki, K., Kamihira, S., White, J. D., Leonard, W. J., Waldmann, T., and Franchini, G. (1997). Proliferation of adult T cell leukemia/lymphoma cells is associated with the constitutive activation of JAK/STAT proteins. Proc Natl Acad Sci U S A *94*, 13897-13902.
226 Tanaka, H., Takeuchi, M., Takeda, Y., Sakai, S., Abe, D., Ohwada, C., Sakaida, E., Shimizu, N., Saito, Y., Miyagi, S., Iwama, A., and Nakaseko, C. (2010). Identification of a novel TEL-Lyn fusion gene in primary myelofibrosis. Leukemia *24*, 197-200.

227 Teglund, S., McKay, C., Schuetz, E., van Deursen, J. M., Stravopodis, D., Wang, D., Brown, M., Bodner, S., Grosveld, G., and Ihle, J. N. (1998). Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell *93*, 841-850.
228 Theofilopoulos, A. N., Baccala, R., Beutler, B., and Kono, D. H. (2005). Type I interferons (alpha/beta) in immunity and autoimmunity. Annu Rev Immunol *23*, 307-336.
229 Thomis, D. C., Gurniak, C. B., Tivol, E., Sharpe, A. H., and Berg, L. J. (1995). Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science *270*, 794-797.
230 Tian, S. S., Tapley, P., Sincich, C., Stein, R. B., Rosen, J., and Lamb, P. (1996). Multiple signaling pathways induced by granulocyte colony-stimulating factor involving activation of JAKs, STAT5, and/or STAT3 are required for regulation of three distinct classes of immediate early genes. Blood *88*, 4435-4444.
231 Tomita, M., Kawakami, H., Uchihara, J. N., Okudaira, T., Masuda, M., Matsuda, T., Tanaka, Y., Ohshiro, K., and Mori, N. (2006). Inhibition of constitutively active Jak-Stat pathway suppresses cell growth of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. Retrovirology *3*, 22.
232 Tsuruyama, T., Nakamura, T., Jin, G., Ozeki, M., Yamada, Y., and Hiai, H. (2002). Constitutive activation of Stat5a by retrovirus integration in early pre-B lymphomas of SL/Kh strain mice. Proc Natl Acad Sci U S A *99*, 8253-8258.
233 Turner, S. D., Tooze, R., Maclennan, K., and Alexander, D. R. (2003). Vav-promoter regulated oncogenic fusion protein NPM-ALK in transgenic mice causes B-cell lymphomas with hyperactive Jun kinase. Oncogene *22*, 7750-7761.
234 Tzankov, A., Zlobec, I., Went, P., Robl, H., Hoeller, S., and Dirnhofer, S. (2010). Prognostic immunophenotypic biomarker studies in diffuse large B cell lymphoma with special emphasis on rational determination of cut-off scores. Leuk Lymphoma *51*, 199-212.
235 Udy, G. B., Towers, R. P., Snell, R. G., Wilkins, R. J., Park, S. H., Ram, P. A., Waxman, D. J., and Davey, H. W. (1997). Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci U S A *94*, 7239-7244.
236 Urlinger, S., Helbl, V., Guthmann, J., Pook, E., Grimm, S., and Hillen, W. (2000). The p65 domain from NF-kappaB is an efficient human activator in the tetracycline-regulatable gene expression system. Gene *247*, 103-110.
237 Vadnais, C., Davoudi, S., Afshin, M., Harada, R., Dudley, R., Clermont, P. L., Drobetsky, E., and Nepveu, A. (2012). CUX1 transcription factor is required for optimal ATM/ATR-mediated responses to DNA damage. Nucleic Acids Res *40*, 4483-4495.
238 Vainchenker, W., Delhommeau, F., Constantinescu, S. N., and Bernard, O. A. (2011). New mutations and pathogenesis of myeloproliferative neoplasms. Blood *118*, 1723-1735.
239 Valentino, L., and Pierre, J. (2006). JAK/STAT signal transduction: regulators and implication in hematological malignancies. Biochem Pharmacol *71*, 713-721.
240 Van Nguyen, T., Angkasekwinai, P., Dou, H., Lin, F. M., Lu, L. S., Cheng, J., Chin, Y. E., Dong, C., and Yeh, E. T. (2012). SUMO-specific protease 1 is

critical for early lymphoid development through regulation of STAT5 activation. Mol Cell *45*, 210-221.

241 Vermeer, M. H., van Doorn, R., Dijkman, R., Mao, X., Whittaker, S., van Voorst Vader, P. C., Gerritsen, M. J., Geerts, M. L., Gellrich, S., Soderberg, O., Leuchowius, K. J., Landegren, U., Out-Luiting, J. J., Knijnenburg, J., Ijszenga, M., Szuhai, K., Willemze, R., and Tensen, C. P. (2008). Novel and highly recurrent chromosomal alterations in Sezary syndrome. Cancer Res *68*, 2689-2698.

242 Vranjkovic, A., Crawley, A. M., Patey, A., and Angel, J. B. (2010). IL-7-dependent STAT-5 activation and CD8+ T cell proliferation are impaired in HIV infection. J Leukoc Biol.

243 Wagner, K. U., and Rui, H. (2008). Jak2/Stat5 signaling in mammogenesis, breast cancer initiation and progression. Journal of Mammary Gland Biology and Neoplasia *13*, 93-103.

244 Wagner, K. U., Wall, R. J., St-Onge, L., Gruss, P., Wynshaw-Boris, A., Garrett, L., Li, M., Furth, P. A., and Hennighausen, L. (1997). Cre-mediated gene deletion in the mammary gland. Nucleic Acids Res *25*, 4323-4330.

245 Wakao, H., Gouilleux, F., and Groner, B. (1994). Mammary-Gland Factor (Mgf) Is a Novel Member of the Cytokine Regulated Transcription Factor Gene Family and Confers the Prolactin Response. Embo Journal *13*, 2182-2191.

246 Wakao, H., Harada, N., Kitamura, T., Mui, A. L., and Miyajima, A. (1995). Interleukin 2 and erythropoietin activate STAT5/MGF via distinct pathways. EMBO J *14*, 2527-2535.

247 Wakao, H., Schmittney, M., and Groner, B. (1992). Mammary Gland-Specific Nuclear Factor Is Present in Lactating Rodent and Bovine Mammary Tissue and Composed of a Single Polypeptide of 89 Kda. Journal of Biological Chemistry *267*, 16365-16370.

248 Walters, D. K., Mercher, T., Gu, T. L., O'Hare, T., Tyner, J. W., Loriaux, M., Goss, V. L., Lee, K. A., Eide, C. A., Wong, M. J., Stoffregen, E. P., McGreevey, L., Nardone, J., Moore, S. A., Crispino, J., Boggon, T. J., Heinrich, M. C., Deininger, M. W., Polakiewicz, R. D., Gilliland, D. G., and Druker, B. J. (2006). Activating alleles of JAK3 in acute megakaryoblastic leukemia. Cancer Cell *10*, 65-75.

249 Warsch, W., Kollmann, K., Eckelhart, E., Fajmann, S., Cerny-Reiterer, S., Holbl, A., Gleixner, K. V., Dworzak, M., Mayerhofer, M., Hoermann, G., Herrmann, H., Sillaber, C., Egger, G., Valent, P., Moriggl, R., and Sexl, V. (2011). High STAT5 levels mediate imatinib resistance and indicate disease progression in chronic myeloid leukemia. Blood.

250 Weissman, I. L. (2000). Stem cells: units of development, units of regeneration, and units in evolution. Cell *100*, 157-168.

251 Wilbanks, A. M., Mahajan, S., Frank, D. A., Druker, B. J., Gilliland, D. G., and Carroll, M. (2000). TEL/PDGFbetaR fusion protein activates STAT1 and STAT5: a common mechanism for transformation by tyrosine kinase fusion proteins. Exp Hematol *28*, 584-593.

252 Wilke, C. M., Bishop, K., Fox, D., and Zou, W. (2011). Deciphering the role of Th17 cells in human disease. Trends Immunol *32*, 603-611.

253 Wilson, A., Murphy, M. J., Oskarsson, T., Kaloulis, K., Bettess, M. D., Oser, G. M., Pasche, A. C., Knabenhans, C., Macdonald, H. R., and Trumpp, A. (2004). c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev *18*, 2747-2763.

254 Wohlmann, A., Sebastian, K., Borowski, A., Krause, S., and Friedrich, K. (2010). Signal transduction by the atopy-associated human thymic stromal lymphopoietin (TSLP) receptor depends on Janus kinase function. Biol Chem *391*, 181-186.
255 Wu, L., Bijian, K., and Shen, S. H. (2009). CD45 recruits adapter protein DOK-1 and negatively regulates JAK-STAT signaling in hematopoietic cells. Mol Immunol *46*, 2167-2177.
256 Xiao, S., Nalabolu, S. R., Aster, J. C., Ma, J., Abruzzo, L., Jaffe, E. S., Stone, R., Weissman, S. M., Hudson, T. J., and Fletcher, J. A. (1998). FGFR1 is fused with a novel zinc-finger gene, ZNF198, in the t(8;13) leukaemia/lymphoma syndrome. Nat Genet *18*, 84-87.
257 Xu, D., and Qu, C. K. (2008). Protein tyrosine phosphatases in the JAK/STAT pathway. Front Biosci *13*, 4925-4932.
258 Yamada, O., Ozaki, K., Akiyama, M., and Kawauchi, K. (2012). JAK-STAT and JAK-PI3K-mTORC1 Pathways Regulate Telomerase Transcriptionally and Posttranslationally in ATL Cells. Mol Cancer Ther *11*, 1112-1121.
259 Yang, X. P., Ghoreschi, K., Steward-Tharp, S. M., Rodriguez-Canales, J., Zhu, J., Grainger, J. R., Hirahara, K., Sun, H. W., Wei, L., Vahedi, G., Kanno, Y., O'Shea, J. J., and Laurence, A. (2011). Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. Nat Immunol.
260 Yao, Z., Cui, Y., Watford, W. T., Bream, J. H., Yamaoka, K., Hissong, B. D., Li, D., Durum, S. K., Jiang, Q., Bhandoola, A., Hennighausen, L., and O'Shea, J. J. (2006). Stat5a/b are essential for normal lymphoid development and differentiation. Proc Natl Acad Sci U S A *103*, 1000-1005.
261 Yao, Z., Kanno, Y., Kerenyi, M., Stephens, G., Durant, L., Watford, W. T., Laurence, A., Robinson, G. W., Shevach, E. M., Moriggl, R., Hennighausen, L., Wu, C., and O'Shea, J. J. (2007). Nonredundant roles for Stat5a/b in directly regulating Foxp3. Blood *109*, 4368-4375.
262 Yoda, A., Yoda, Y., Chiaretti, S., Bar-Natan, M., Mani, K., Rodig, S. J., West, N., Xiao, Y., Brown, J. R., Mitsiades, C., Sattler, M., Kutok, J. L., DeAngelo, D. J., Wadleigh, M., Piciocchi, A., Dal Cin, P., Bradner, J. E., Griffin, J. D., Anderson, K. C., Stone, R. M., Ritz, J., Foa, R., Aster, J. C., Frank, D. A., and Weinstock, D. M. (2010). Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A *107*, 252-257.
263 Yoshimoto, G., Miyamoto, T., Jabbarzadeh-Tabrizi, S., Iino, T., Rocnik, J. L., Kikushige, Y., Mori, Y., Shima, T., Iwasaki, H., Takenaka, K., Nagafuji, K., Mizuno, S. I., Niiro, H., Gilliland, G. D., and Akashi, K. (2009). FLT3-ITD up-regulates MCL-1 to promote survival of stem cells in acute myeloid leukemia via FLT3-ITD-specific STAT5 activation. Blood *114*, 5034-5043.
264 Yu, C. R., Ortaldo, J. R., Curiel, R. E., Young, H. A., Anderson, S. K., and Gosselin, P. (1999). Role of a STAT binding site in the regulation of the human perforin promoter. J Immunol *162*, 2785-2790.
265 Zenatti, P. P., Ribeiro, D., Li, W., Zuurbier, L., Silva, M. C., Paganin, M., Tritapoe, J., Hixon, J. A., Silveira, A. B., Cardoso, B. A., Sarmento, L. M., Correia, N., Toribio, M. L., Kobarg, J., Horstmann, M., Pieters, R., Brandalise, S. R., Ferrando, A. A., Meijerink, J. P., Durum, S. K., Yunes, J. A., and Barata, J. T. (2011). Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet *43*, 932-939.
266 Zhang, Q., Nowak, I., Vonderheid, E. C., Rook, A. H., Kadin, M. E., Nowell, P. C., Shaw, L. M., and Wasik, M. A. (1996). Activation of Jak/STAT proteins

involved in signal transduction pathway mediated by receptor for interleukin 2 in malignant T lymphocytes derived from cutaneous anaplastic large T-cell lymphoma and Sezary syndrome. Proc Natl Acad Sci U S A *93*, 9148-9153.

267 Zhang, S., Fukuda, S., Lee, Y., Hangoc, G., Cooper, S., Spolski, R., Leonard, W. J., and Broxmeyer, H. E. (2000). Essential role of signal transducer and activator of transcription (Stat)5a but not Stat5b for Flt3-dependent signaling. J Exp Med *192*, 719-728.

268 Zhang, Y., Buchholz, F., Muyrers, J. P., and Stewart, A. F. (1998). A new logic for DNA engineering using recombination in Escherichia coli. Nat Genet *20*, 123-128.

269 Zhu, J. (2010). Transcriptional regulation of Th2 cell differentiation. Immunol Cell Biol *88*, 244-249.

270 Zhu, J., Cote-Sierra, J., Guo, L., and Paul, W. E. (2003). Stat5 activation plays a critical role in Th2 differentiation. Immunity *19*, 739-748.

271 Zhu, J., and Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. Blood *112*, 1557-1569.

272 Zhu, Y., Chen, L., Huang, Z., Alkan, S., Bunting, K. D., Wen, R., Wang, D., and Huang, H. (2004). Cutting edge: IL-5 primes Th2 cytokine-producing capacity in eosinophils through a STAT5-dependent mechanism. J Immunol *173*, 2918-2922.

273 Zwirner, N. W., and Domaica, C. I. (2010). Cytokine regulation of natural killer cell effector functions. Biofactors *36*, 274-288.

6. APPENDICES

6.1. Sequence of icS5 targeting construct

Features –

7	296	3' Homologous arm
297	330	LoxP F
346	365	B-gl-5'UTR
366	370	Kozak
371	2749	$cS5^{F}$
2750	2773	FLAG Tag
2841	3416	IRES
3417	4256	thCD2 (truncated human CD2)
4487	4527	FRT site (Deleted neomycin sequence by Flp)
4528	4561	LoxP R
4562	4761	5' Homologous arm

```
   1  ccgcggcttc ttctgcagct cctgcaccag gccctccagg agttgggtgg
  51  cctgacctcg gtcctgggga ttatccaagt caatagcatc ccttggaaca
 101  taaaaagagg ggagataaag catcaagagt gattcagggc atcccagtcc
 151  cacaagactt gctcaccaca gctgtataag catcaaagtc tagatgagtc
 201  tcccctctga tagctccagt ttccctcttc ccctttccca gagacaccac
 251  cccttccgca caaggacaga tagaaccaga gtcataccaa accacaataa
 301  cttcgtatag catacattat acgaagttat cccgggtttg aattcgactc
 351  acaaccccag aaacaccacc atggcgggct ggattcaggc ccagcagctt
 401  cagggagatg ccctgcgcca gatgcaagtg ttgtatgggc agcatttccc
 451  catcgaggtc cggcactacc tggcccagtg gatcgagagc cagccgtggg
 501  atgctattga cttggataat ccccaggacc gaggtcaggc cacccaactc
 551  ctggagggcc tggtgcagga gctgcagaag aaggcggagc accaggtggg
 601  ggaagatggg tttttgctga agatcaagct ggggcactat gccacacagc
 651  tccagaacac gtatgaccgc tgtcccatgg agctggttcg ctgtatccgt
 701  cacattctgt acaacgaaca gaggctggtt cgcgaagcca acaattgcag
 751  ctcccctgct ggtgtcctgg ttgacgccat gtcccagaag caccttcaga
 801  tcaaccaaag gtttgaggag ctgcgcctga tcacacagga cacggagaac
 851  gagctgaaga agctgcagca gacccaagag tacttcatca tccagtacca
 901  ggagagcctg cggatccaag ctcagtttgc ccagctgggc cagctgaacc
 951  cccaggagcg catgagcagg gagacggccc tccagcagaa gcaagtgtcc
1001  ctggagacct ggctgcagcg agaggcacag acactgcagc agtaccgagt
1051  ggagctggct gagaagcacc agaagaccct gcagctgctg cggaagcagc
1101  agaccatcat cctggacgac gagctgatcc agtggaagcg gagacagcag
1151  ctggccggga acgggggtcc ccccgagggc agcctggacg tgctgcagtc
1201  ctggtgtgag aagctggccg agatcatctg gcagaaccgg cagcagatcc
1251  gcagggctga gcacctgtgc cagcagctgc ccatcccagg ccccgtggag
1301  gagatgctgg ctgaggtcaa cgccaccatc acggacatca tctcagctct
1351  ggtcaccagc acgttcatca tcgagaagca gcctcctcag gtcctgaaga
1401  cccagaccaa gtttgcggcc accgtgcgcc tgctggtggg gggaaagctg
1451  aatgtgcaca tgaacccccc gcaggtgaag gcgaccatca tcagcgagca
```

1501 gcaggccaag tccctgctca agaatgagaa cacccgcaat gagtgcagcg
1551 gcgagatcct gaacaactgt tgcgtcatgg agtaccacca ggccactggc
1601 acgctcagcg cccacttcag aaacatgtca ctgaaaagaa tcaagcgcgc
1651 cgacaggcgt ggtgcagagt cggtgacgga ggagaagttc acagtcctgt
1701 ttgagtctca gttcagcgtt ggcagcaacg agctggtgtt ccaggtgaag
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2001 acctcgagga ctacaacagc atgtctgtgt cctggtccca gttcaaccgg
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6.2. Sequence of icS5 BAC

Features -

77581	77583	St5a Stop
99794	100083	3'HA
100084	100117	loxPF
100158	102536	$cS5^F$
102537	102560	FLAG
102628	103203	IRES
103204	104043	Truncated human CD2
104315	104348	LoxPR
104349	104546	5'HA

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191051 cccaatcctg ggtcaaggat ggacaggtcg ttattgtgcc catttgaagg
191101 ccagtataat gtggtcaagt ggccaaaatt ctagacacgc ctgtggtggt
191151 tttaaagaaa atggccccca tcaactcgta gggagtggcc tatcaggagg
191201 tgtggccttg ttggaggaag tgcgtcactg ggggtgggct ttgaggtttc
191251 aaatgaggcc cagtgttgct ctctcttcct gctgtctgtc aatcaggatg
191301 taaggctctc agccacctct ccagcaccat gtctgcctgc ctgtcaccat
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191401 ttcccttata agagttgctg tgggggggcta gagagatggc tcagtggata
191451 aagagcactg actgttctca gacgacctga gttcaaatcc tagaaaccac
191501 atggtggctc acaaccatcc gtaatgagat ctgataccct tcttctggtg
191551 tgtctgaaga cagttacagt gtacttacat ataataaata aatctttaaa
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191951 gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgcgcgc actggctttg
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192101 gcacaggcag gtggatctct aaatttgagg ccagcctgtt ctacagaggg
192151 agtctcagga cagccaaggc tacacagaga aaccctgctt cataaaacaa
192201 caatagcaat ataatattta atttgaggag aaaaaaagaa cttaagctgg
192251 gattcacagg gactggaaat atagctaact tggtaaagtt tttgcctaga

192301 atattaaagc cataaattcc aactcaacac tgcattaaac tgggcgtggt
192351 ggcctgacgg taatcccagc tctctggaag tattggcagg aggatcaaga
192401 attcaaagtt ggactagaga gatggctcag tggttaagag cactgactgt
192451 tctcccagag gtcctgagtt caattcccag caaccacatg gtgactcaca
192501 gccatgggag taatgggatt cgatgccccc ttctggttgt ctgaaaacag
192551 ctaagatgta ctcatataca ttaaataaat aaacttttaa aaaaaaataa
192601 agaattcaaa gtcagtatta gctatctatc aagttcaaag tcatcctgaa
192651 ctaacaccat gattcaaaaa agggggggagg ggggcgggca ggaagggggt
192701 taggaggagt ggctggagag ccctcagcac ttactgccat ttcagaaggc
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192901 cacatggcag ctcacaaact gtaactccag ttccaggaga tctaagcctc
192951 acacacagag acacacacag gtaaaatatc aaagcactta aaataagaaa
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193101 tttgtgtatt tggttttgct tttctgagat aaagtcagta aattcagaca
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193251 tctgtagaag ggggtgggtt gcagtctgtc tgtctgtatg tatgtctgtc
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194051 gagagagaga gagaaagaaa gagagagaaa gaaagaaaga aagaaagaaa
194101 gaaagaaaga aagaaagaaa gaaagaaaga aagaaaagac gtgtagctca
194151 gactggcctt gaacttgtta tgtagctgta gctgtgggtg accttgtcct
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194451 cctagggctg caacaactag tctgcacgac cacacctcgg cagctgtggt
194501 ttttacttga gtctgaatct gctcctgaat ggaaagcaag cactctttcc
194551 acacacacct ccctgtcttt ctcgacgatc ttgccaccct ggccttaact
194601 ccctccatct gccaacctga gccagcaact caacaagtta ccttttaggt
194651 aacttgttcc tttaagaaca ttccgcccca cccccgggtc ctcccagtca
194701 ggccggattt cctgcccaag ccctgccttt taaagtcatc acttcaactt
194751 tcaggactgc gaccagacac actgagggac cctgaggggt cccggggtgc
194801 tgaaagtaag cagaaatagg ctgtgacagg tgtatatata tacccagggc
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194951 ggagggagga gctgtcagag ctgttcttct agctcgagaa gcaaggatgc
195001 cagttagtag gatgccagat tctttttggt ttttcgagac agggtttctc
195051 tgtgtagccc tggctgtcct ggaactcact ctgtagacca ggctggcctc
195101 gaactcagaa atccacctgc ctctgcctcc cgagtgctgg gattaaaggc

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195151  gtgcgccacc acgcccggcc agggtgccag attcttgagg gtctctcttc
195201  catattgcct gcctaacagg gccctggctt cctaccaccg tcgtctcctt
195251  cctctgtttt ctgtatgcgg cggagtgtgt gggaagacat ggttatcccc
195301  ctcacacccc attccattcc attcctctcc caggagctcc ttggagatgc
195351  ttctgctgtg gctgctgctg ctgctgctcc tcctcgtgcc cctgctggcc
195401  atactctggc agcagaggtc tcggggtgcc aggccatgct ggctgatcag
195451  tctccaacac cgagtggcct gggggatgct gggctgggca gccgcctggc
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195551  caggccctca tgtggtgtct gaagaaagcc cagggctcct gctgtctccc
195601  cagggaggac acaggtgtgt ttctggcacc tgggggaggt gacccaagga
195651  gagaaaatag cccacaaggc agtcagattc tagaactcaa gggccccttc
195701  atttaccacc aatatgatgt ggcctggtgc agagccctac catggcttac
195751  aacctgacac ctattaggat gggttttcct ttgctgggcc ctggtctagc
195801  cttcacctca tttcccaggc cctttctggg ttcctttctc aaggctctcc
195851  acagtcttct cacttcctga ggagtttctg attttttttg gggggggggt
195901  ctacagatat gagaaccttc cggaatcatc ttccactgac ccaaaccagc
195951  cacactcagg agcaagaaag tgaagagacg ctcccatccc ctgcctcccc
196001  ccagtaccat ggagacgcct ctttgcaggt aaacagaggt acctccacct
196051  cacaacagca gacccctccc aaaaggaggt acaaagaagg cccgctatgg
196101  tagctggggt ctggttctaa ggtctgaatg acaggccctc ctgtctccct
196151  ccaggccacc ctgctgggtc taataaccct aaacaaggcc tacccagaag
196201  cgctggctcc aggaagtact gcctgtgtga cccctacatc gccatggccc
196251  tgctctgtcc cctggcttgg gcacgccctg ggccgggtaa gccccgatgg
196301  agccaaggac ccccggaccc tgctgctgga ggcattgata tccccggggc
196351  tgagggtact tgaggcaagg actgcagtgg agctcctgga tgtctttgtg
196401  ggcttggagg ctgatggtga agaactggct gaggccatag cagctgggat
196451  tctgggaacg cttctcccca aacgagccgc tgagctgaag gaggccctag
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196551  gtggtgactc tggactcagg aggacaggca gaagctgtgg ctgcccttag
196601  ggtcctgtgg tgccaagggc tagccttctt ctctcctgct tatgctgcct
196651  caggaggtca gcttgatgcg gggggggatt ggatggatgc aggtggggct
196701  gggggcagat gtcatacagc tagctaccat gcattgaggc acatacactt
196751  accttcttca accctcctca gtgcctggga tggtatgggt tactagctct
196801  gactaacagc cgagcacact aaggttcact gagacaaagt gtctcaggca
196851  gactcaggtg gtaccaggat ttgtttccac ctctgatccc tgccagaacc
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197001  agaggagccg ggcctgctgt cccgagtgag gcgttacttt ctgctgttgc
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197101  ctataggagt ggtggcccta aacctgtggc cagagcgacc ccaaggatcc
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197301  tgattctcct actaactggc tcttgtctac tctgatgaga accccggcct
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197551  tgaatgttcg aggagaagtt actgatgaga ctgtattctc tgtggccctg
197601  gcccaagcag tggggcagtg gccaggggcc aagctgttgg accatgtctg
197651  tgtggagagc agagttctgg gtaagcatct ctaccatctc ctacctccgt
197701  ttcttgggtc tggctatcca ctgacccatg ttcgccctga ccagagacat
197751  ctaggaagga gattaatcac acccttcccc actgttccta aagaaagtcc
197801  ctctcttagg gctagagaga tggttcagcg gttaagagca ctgactgctc
197851  ttccaaaggt cctgagttca aatcccagca accacatggt ggctcacaac
197901  catttgtaat gagatctggc ccccctcttc tggtgtatct gatggcagca
197951  acagtgtatt tacatgaaag agaaagaaag aaaagaaaga aagaaagaaa
```

```
198001  gaaagaaaga aagaaagaaa gaaagaaaga aagaaaggaa ggaaggaaga
198051  aaaaagaaag aaagaaagaa aggaagaaag gaagagaggg ggagggaggg
198101  aggaagtccc tctctatatc taacttccat ccctacagtc tccacctctt
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198251  gggtgtcagc cctcgaagct gccttgtcct cagaaagtct gctttcctgt
198301  ggctttatcc tcagactcct gtgagggttc tgccccacac tacgaagtgt
198351  ttgtagagct gaggggggttg aggaatctgt cggaggaaaa tcgagacaag
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198451  tcccagttgt catggagatg agagagttaa gggaagagga tgggccttag
198501  ggaggggggcg gcacccccctt tcagagaatc tagaaaggaa gtatgggtcc
198551  cacaacacac caaaatccta agtgcatgtg gccttggata cggtcatgca
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198651  tgcccttagc ttgacaactg cctgcaggaa gcctccgcac agtacaagtc
198701  cttgcgattc cggggcagtg tgggtcctgc caaagtccat ctggtgaggc
198751  cagggagctt cagggttctc cgggaagcac tggcagcctt ctcctcctcc
198801  tcctgccgcc ctcctgaaat gcctcgggtc atcaggctca ggcacctggc
198851  ccagctcctg cagaagaggg tgatatccta accaaggcag ccagccttcc
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198951  gaccagatgt cacataacag tggtctgatt gggtccaatt ggactcattg
199001  gcaagtttta agatgccggc ttggagcaca cagctgaacc catcatcgat
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199401  agaaagaaag aaagaaagaa agaaagaaag aaagaaagaa agaaagaaag
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199501  accacctgaa gggctgaggc tgtggcccac agtggcccag tgttcagtgc
199551  tcttcttacc agatcccccc atccctccat cctcctctcc cctgcttact
199601  ctatcattct gttccctgga tcaaccccca aataaaatta aatatatata
199651  aatattaatg tatgtatttt atgttatctg tgtgtgtctg catgtgtgca
199701  tgtgcaccac gcatatgtga acgccctcag aggccagaag aggagagtga
199751  attc
```

6.3. Sequence of vav-cS5 construct (*Sfi*I to *Not*I)

Features –

1	13	SfiI
45	2450	$cS5^F$
2173	2173	$cS5^F$ mutation
2424	2447	FLAG Tag
2467	2471	NotI

```
   1  ggcccgtacg gccgaattcg actcacaacc ccagaaacac caccatggcg
  51  ggctggattc aggcccagca gcttcaggga gatgccctgc gccagatgca
 101  agtgttgtat gggcagcatt tccccatcga ggtccggcac tacctggccc
 151  agtggatcga gagccagccg tgggatgcta ttgacttgga taatccccag
```

```
 201  gaccgaggtc aggccaccca actcctggag ggcctggtgc aggagctgca
 251  gaagaaggcg gagcaccagg tgggggaaga tgggtttttg ctgaagatca
 301  agctggggca ctatgccaca cagctccaga acacgtatga ccgctgtccc
 351  atggagctgg ttcgctgtat ccgtcacatt ctgtacaacg aacagaggct
 401  ggttcgcgaa gccaacaatt gcagctcccc tgctggtgtc ctggttgacg
 451  ccatgtccca gaagcacctt cagatcaacc aaaggtttga ggagctgcgc
 501  ctgatcacac aggacacgga gaacgagctg aagaagctgc agcagaccca
 551  agagtacttc atcatccagt accaggagag cctgcggatc caagctcagt
 601  ttgcccagct gggccagctg aacccccagg agcgcatgag cagggagacg
 651  gccctccagc agaagcaagt gtccctggag acctggctgc agcgagaggc
 701  acagacactg cagcagtacc gagtggagct ggctgagaag caccagaaga
 751  ccctgcagct gctgcggaag cagcagacca tcatcctgga cgacgagctg
 801  atccagtgga agcggagaca gcagctggcc gggaacgggg gtccccccga
 851  gggcagcctg gacgtgctgc agtcctggtg tgagaagctg gccgagatca
 901  tctggcagaa ccggcagcag atccgcaggg ctgagcacct gtgccagcag
 951  ctgcccatcc caggccccgt ggaggagatg ctggctgagg tcaacgccac
1001  catcacggac atcatctcag ctctggtcac cagcacgttc atcatcgaga
1051  agcagcctcc tcaggtcctg aagacccaga ccaagtttgc ggccaccgtg
1101  cgcctgctgg tgggggggaaa gctgaatgtg cacatgaacc ccccgcaggt
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1201  agaacacccg caatgagtgc agcggcgaga tcctgaacaa ctgttgcgtc
1251  atggagtacc accaggccac tggcacgctc agcgcccact tcagaaacat
1301  gtcactgaaa agaatcaagc gcgccgacag gcgtggtgca gagtcggtga
1351  cggaggagaa gttcacagtc ctgtttgagt ctcagttcag cgttggcagc
1401  aacgagctgg tgttccaggt gaagaccctg tccctccctg tggtcgttat
1451  cgtccatggc agccaggacc acaatgctac tgccaccgtg ctgtgggaca
1501  atgcctttgc tgagccgggc agggtgccat ttgctgtgcc tgacaaggtg
1551  ctgtggccgc agctgtgtga agcgctcaac atgaaattca aggctgaagt
1601  acagagcaac cggggcttga ccaaggagaa cctcgtgttc ctggcacaga
1651  aactgttcaa catcagcagc aaccacctcg aggactacaa cagcatgtct
1701  gtgtcctggt cccagttcaa ccgggagaac ttgcccggct ggaactacac
1751  cttctggcag tggttcgacg gggtgatgga ggtgctgaag aagcaccata
1801  agccccattg gaatgatggg gctatcctgg gtttcgtgaa caagcaacag
1851  gcccacgacc tgctcatcaa caagccggac gggaccttcc tgctgcgctt
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2051  gttcccagac cgacccaagg acgaggtctt tgccaagtat tacactcctg
2101  tacttgcgaa agcagttgac ggatacgtga agccacagat caagcaagtg
2151  gtccctgagt tcgtcaatgc attcacagat gccggagcca gcgccaccta
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2251  tgtacccacc caaccctgac cctgtccttg accaagatgg cgagtttgac
2301  ctggatgaga gcatggatgt tgccaggcac gtggaagaac ttttacgccg
2351  gcccatggac agtctcgacg cccgcctctc cccacctgct ggtctcttca
2401  cctccgctag aagctccctg tccgactaca aggacgacga tgacaagtga
2451  gaattcctgc agcccggggg atccactagt tctagagcgg ccgc
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7. CURRICULUM VITAE

Harini Nivarthi

Personal Information

Date of Birth	15.08.1980
Place of Birth	Adoni, India
Nationality	Indian
E-mail	niv.hari@gmail.com

Educational Qualifications

2008- to date Doctoral studies	Institute of Technical chemistry, Hannover University, Hannover Ludwig Boltzmann Institute for Cancer Research (LBI-CR), Vienna (Austria)
2003-2007 PhD (Discontinued)	Department of Pediatric Hematology/Oncology, Hannover Medical School (MHH), Hannover (Germany)
2001-2003 Masters of Science (M.Sc.)	Madurai KAMARAJ University (MKU), Madurai (India)
1998-2001 Bachelor of Science (B.Sc.)	Biochemistry from Delhi University, Delhi (India)

Research Experience

PhD: Since 2008

PhD at Ludwig Boltzmann Institute for Cancer Research, Vienna (Austria)

Projects:

1. Generation of transgenic mouse models for constitutively active STAT5A
2. STAT5 regulates T-cell development and function in a dose dependent manner
3. STAT1/STAT3 ratio determines colon cancer progression

PhD (Discontinued): October 2003 – September 2007

Department of Pediatric Hematology/Oncology, Hannover Medical School, Hannover (Germany)

Project: Identification of transcription factors required for differentiation of dendritic cells from hematopoietic stem cells.

Master's Thesis: January 2003-July 2003

School of Biotechnology, Madurai Kamaraj University, Madurai (India)

Project: Development of a system to determine the frequency of recombination of DNA A of Mung bean Yellow Mosaic Virus (MYMV)

Summer Training: June 2002

National Centre of Biological Sciences (NCBS), Bangalore (India)

Project: Cloning of *Drosophila itpr* (Inositol-trisphosphate receptor) and a point mutant of the same into a baculovirus expression vector. Dissection of *Drosophila* larval brains to screen the expression pattern of some GAL4 lines.

Achievements

1. Scholarship for 2 years of M.Sc. education (2001-2003), by Department of Biotechnology, Government of India.
2. Qualification of the National Eligibilty Test (NET) conducted by Council of Scientific and Industrial Research and University Grants commission, Government of India.
3. Graduate Stipendium for 2 years (2003-2005), Graduiertenfoerderungsgesetz (GradFoeG) Niedersachsen (Germany).
4. Abstract acheivement award at ASH 2011, San Diego (USA)

Additional skills/activities

1. Student representative of the LBI-CR (November 2009-October 2010)
2. Member of organizing committee of the lecture series 'Meet The Expert', organized by Ludwig Boltzmann Gesellschaft (LBG), (November 2010 to date)

Langauges

English: Advanced, fluent

German: Beginners

Telugu: Mother tongue

Hindi: Advanced, fluent

Publications

Kornfeld JW, Grebien F, Kerenyi MA, Friedbichler K, Kovacic B, Zankl B, Hoelbl A, Nivarthi H, Beug H, Sexl V, Muller M, Kenner L, Mullner EW, Gouilleux F and Moriggl R. Front Biosci. 2008 May 1;13:6237-54. Review. The different functions of Stat5 and chromatin alteration through Stat5 proteins.

Books

Jak-Stat Signaling: From Basics to Disease, edited by Prof. Thomas Decker and Prof. Mathias Müller (In print)

Chapter: Stat5 as a Hematopoietic Master Regulator for Differentiation and Neoplasia Development.
Nivarthi H, Friedbichler K and Moriggl R.

Poster presentations

- Poster presentation at Young Scientists Association (YSA) of Medical University of Vienna, PhD symposium 2008, Vienna (Austria)

  Role of STAT5 during Septic or Inflammatory Shock.
  Nivarthi H, Tsyrulnyk A, Hoelbl A, Friedbichler K, Bauer A, Sexl V, Yao Z, O'Shea J, Tuckermann J, Kolbe T, Müller M and Moriggl R.

- Poster presentation at FEBS-JAK-STAT meeting 2010, Vienna (Austria)

  Generation of a Transgenic Mouse Model with Inducible Oncogenic Stat5a Activation
  Nivarthi H, Tsyrulnyk A, Kenner L, Rülicke T, Müller M and Moriggl R.

- Poster presentation at American Society of Hematology (ASH) 2011, San Diego (USA)

  Generation of a Transgenic Mouse with Inducible Constitutively Active Stat5a
  Nivarthi H, Tsyrulnyk A, Warsch W, Wang Z, Winkler S, Kenner L, Müller M, Baum C, Bunting KD, Sexl V, Rülicke T and Moriggl R.

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Name: Harini Nivarthi

Anschrift: Rennweg 90/13, 1030, Wien

Telefon: 06503011018

Mail: niv.hari@gmail.com

Erklärung

Thema der dissertation:
Generation of transgenic mouse models with expression of constitutively active STAT5A

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