## Supplemental methods

### Cytospin

Ba/F3 and 32D cells were spun onto glass slides for 5 min at 800 rpm using CytoSpin III Cytocentrifuge (Shadon). The cells were stained with the “Hemacolor® Rapid staining of blood smear” kit (Merck) according to manufacturer’s instructions.

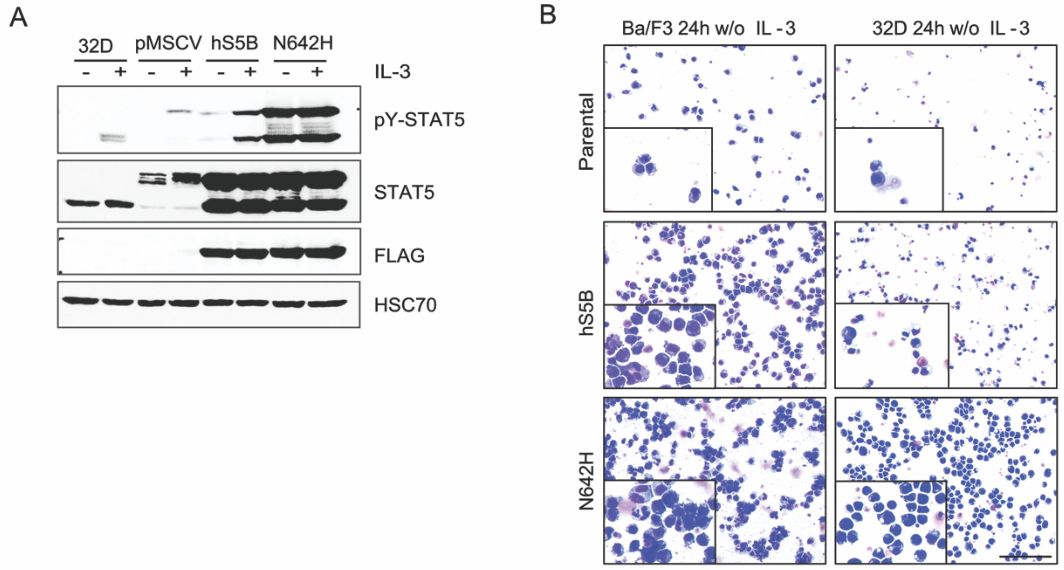
### qPCR

mRNA was isolated from the cells using Trizol reagent (LifeTechnology). cDNA synthesis and qPCR were performed using RevertAid H Minus First Strand cDNA Synthesis Kit and 2xSYBR green Master Mix (Thermo Scientific), respectively. Primers used for qPCR were listed in Supplemental Table 5.

### Electroporation

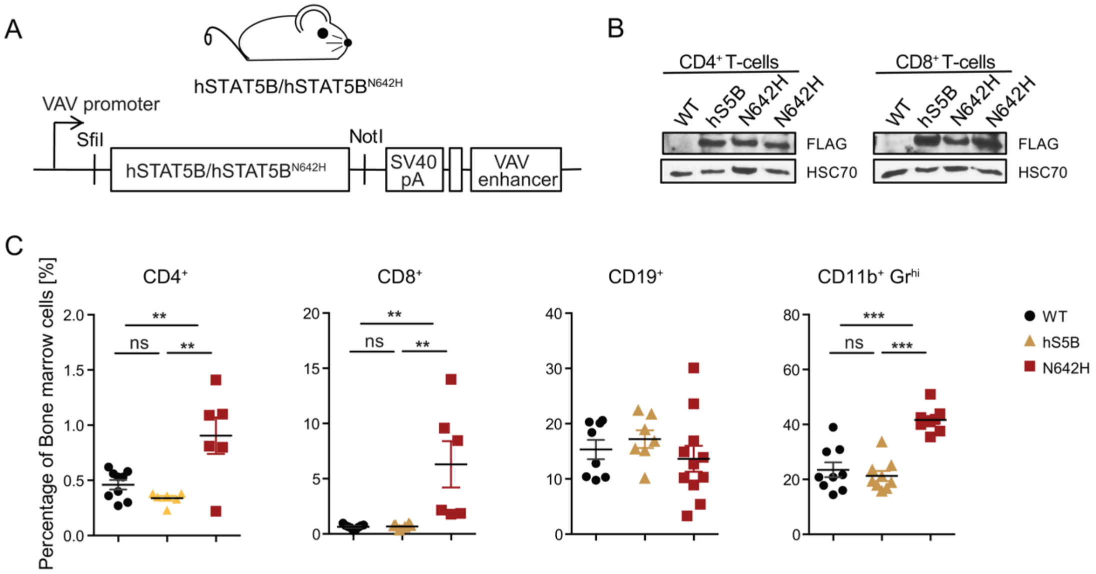
$5\times10^6$ Ba/F3 or 32D cells were electroporated in 800 µl plain RPMI containing 20 µg of DNA. 32D cells were electroporated at 250 V and 960 µF and Ba/F3 cells were electroporated at 300 V and 960 µF. Following electroporation, cells were incubated with 20% FCS and 2 ng/ml IL-3 for 2 days.

## Supplemental figure

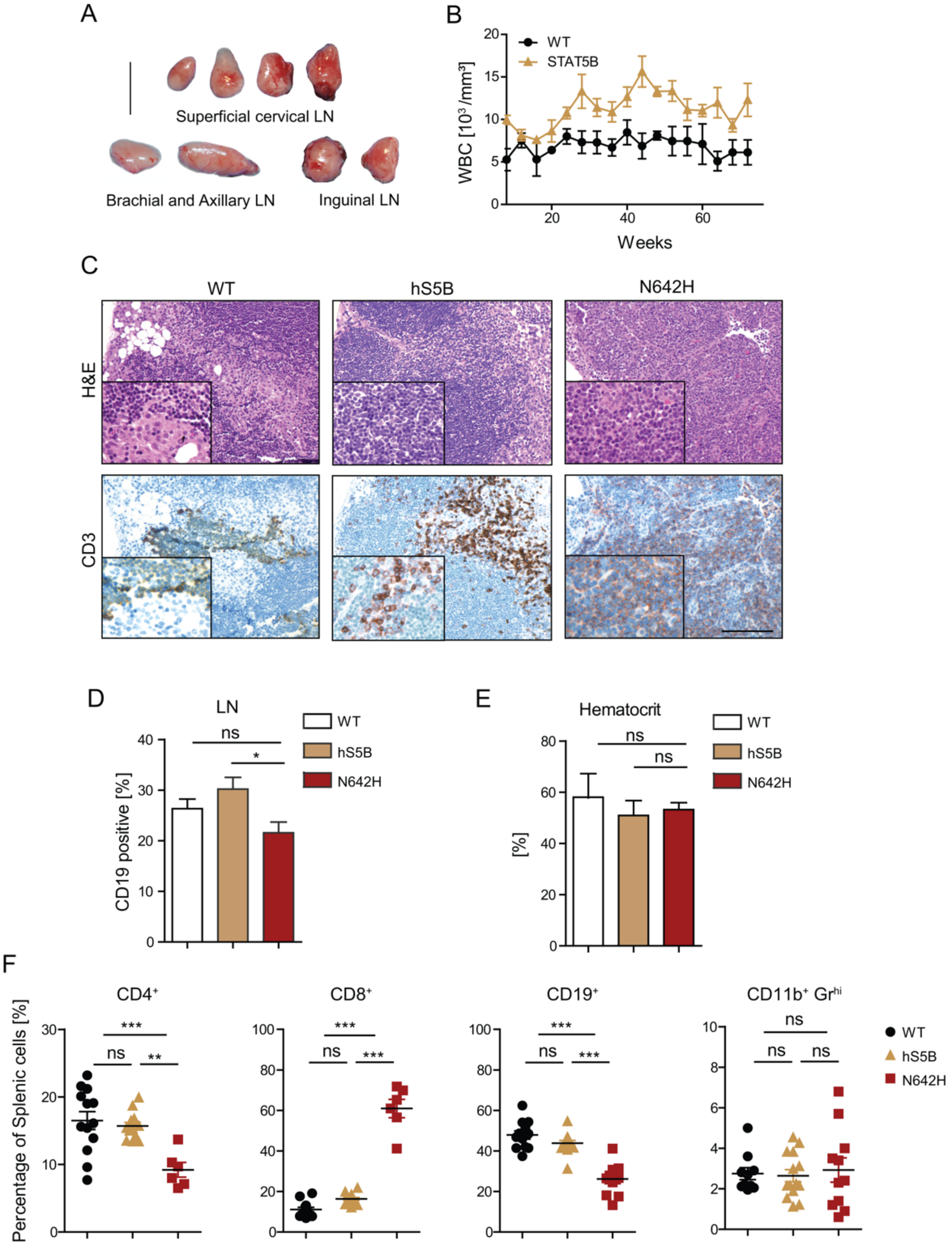


Supplement Figure 1

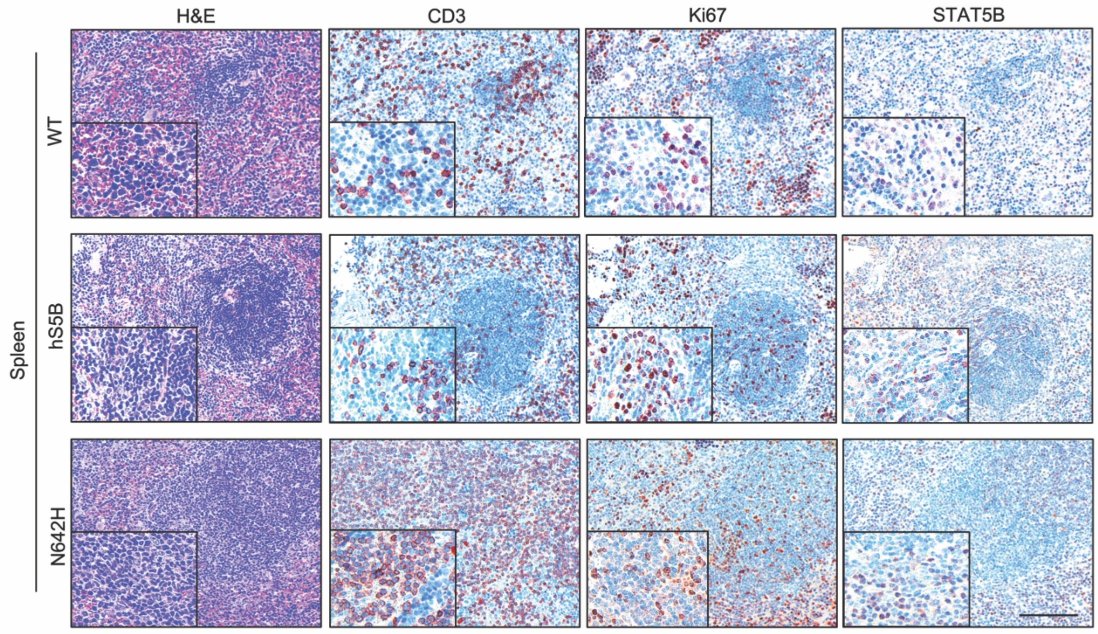
**Supplemental Figure 1. hSTAT5B$^{N642H}$ promotes cytokine-independent growth in lymphoid and myeloid cell lines***.* **(A)** 32D cells stably expressing hSTAT5B (hS5B) and hSTAT5B$^{N642H}$ (N642H) were cultivated with and without IL-3. Western blot (WB) analysis of 32D cells showed hSTAT5B$^{N642H}$ was tyrosine phosphorylated in the absence of IL-3. Parental and pMSCV-transfected 32D cells were used as controls. **(B)** Microscopic view of Hemacolor®-stained parental, hSTAT5B and hSTAT5B$^{N642H}$ expressing Ba/F3 and 32D culture after 24h IL-3 starvation. Black bar denotes 100 µm. Data presented are representative of three independent experiments



**Supplemental Figure 2. hSTAT5B$^{N642H}$ drives hematopoietic expansion *in vivo*.** **(A)** Schematic of the *Vav1* vector used to generate two transgenic mouse models, hSTAT5B and hSTAT5B$^{N642H}$. **(B)** Western blot analysis of FLAG and HSC70 in CD4$^+$ T-cells and CD8$^+$ T-cells isolated from WT, hSTAT5B (hS5B) and hSTAT5B$^{N642H}$ (N642H) mice**.** **(C)** The percentages CD3$^+$ CD4$^+$ T-cells, CD3$^+$ CD8$^+$ T-cells, myeloid cells (CD11b$^+$, Gr1$^+$) and B-cells (CD19$^+$) in the bone marrow (BM) of hSTAT5B$^{N642H}$, hSTAT5B transgenic mice and WT mice ($n \geq 5$, all 7 weeks-old) were quantified using flow cytometry. All data are presented as mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (1way ANOVA and Bonferroni corrected $P$ value).

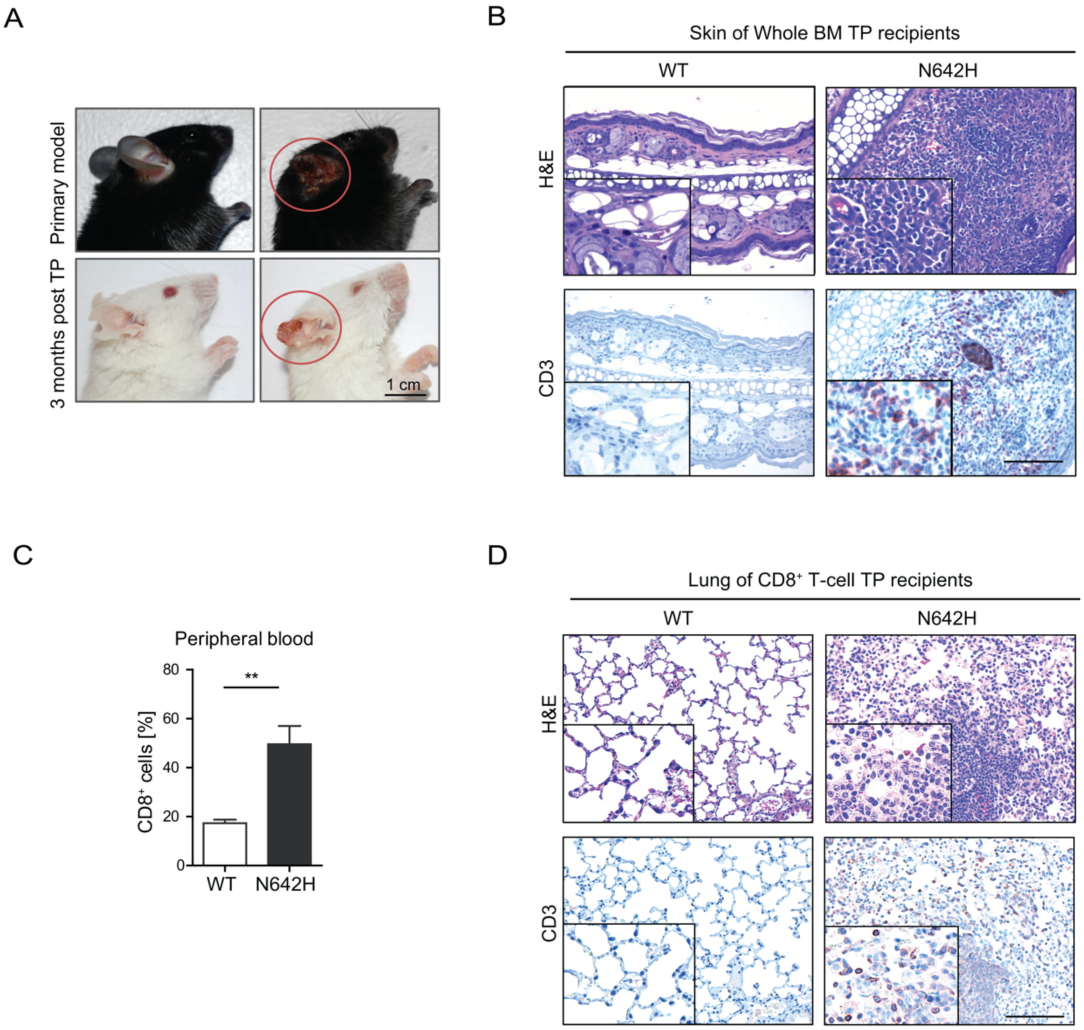


**Supplemental Figure 3. hSTAT5B$^{N642H}$ transgenic mice suffer from an aggressive form of leukemia/lymphoma. (A)** Macroscopic view of superficial cervical, inguinal, brachial and axillary lymph nodes (LN) of 8 week-old hSTAT5B$^{N642H}$ mice. Scale bar denotes 1 cm. **(B)** Quantification of white blood cell (WBC) count of WT (*n*=4) and hSTAT5B (hS5B) (*n*=8) mice over 80 weeks using animal blood counter VetABC. **(C)** Histological analysis of $CD3^+$ cells in the LN of hSTAT5B, $hSTAT5B^{N642H}$ and WT mice showed severe T-cell infiltration in the LN of $hSTAT5B^{N642H}$ mice. Black bar denotes 100 µm. **(D)** The relative number of B-cells ($CD19^+$) in the LN of $hSTAT5B^{N642H}$ (N642H), hSTAT5B transgenic and WT mice were quantified using flow cytometry. **(E)** Quantification of percentage of hematocrit of WT and hSTAT5B 8 week-old mice using animal blood counter VetABC. $n$=8 WT and $n$=8 hSTAT5B, $n$=8 $hSTAT5B^{N642H}$. **(F)** The relative number of T-cell ($CD4^+$ or $CD8^+$), B-cells ($CD19^+$) and myeloid cells ($CD11b^+$, $Gr1^+$) in the spleen of $hSTAT5B^{N642H}$, hSTAT5B transgenic and WT mice were quantified using flow cytometry. $n$=13 WT and $n$=12 hSTAT5B, $n$>5 $hSTAT5B^{N642H}$. All data are presented as mean ± SD. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (1way ANOVA and Bonferroni corrected $P$ value).

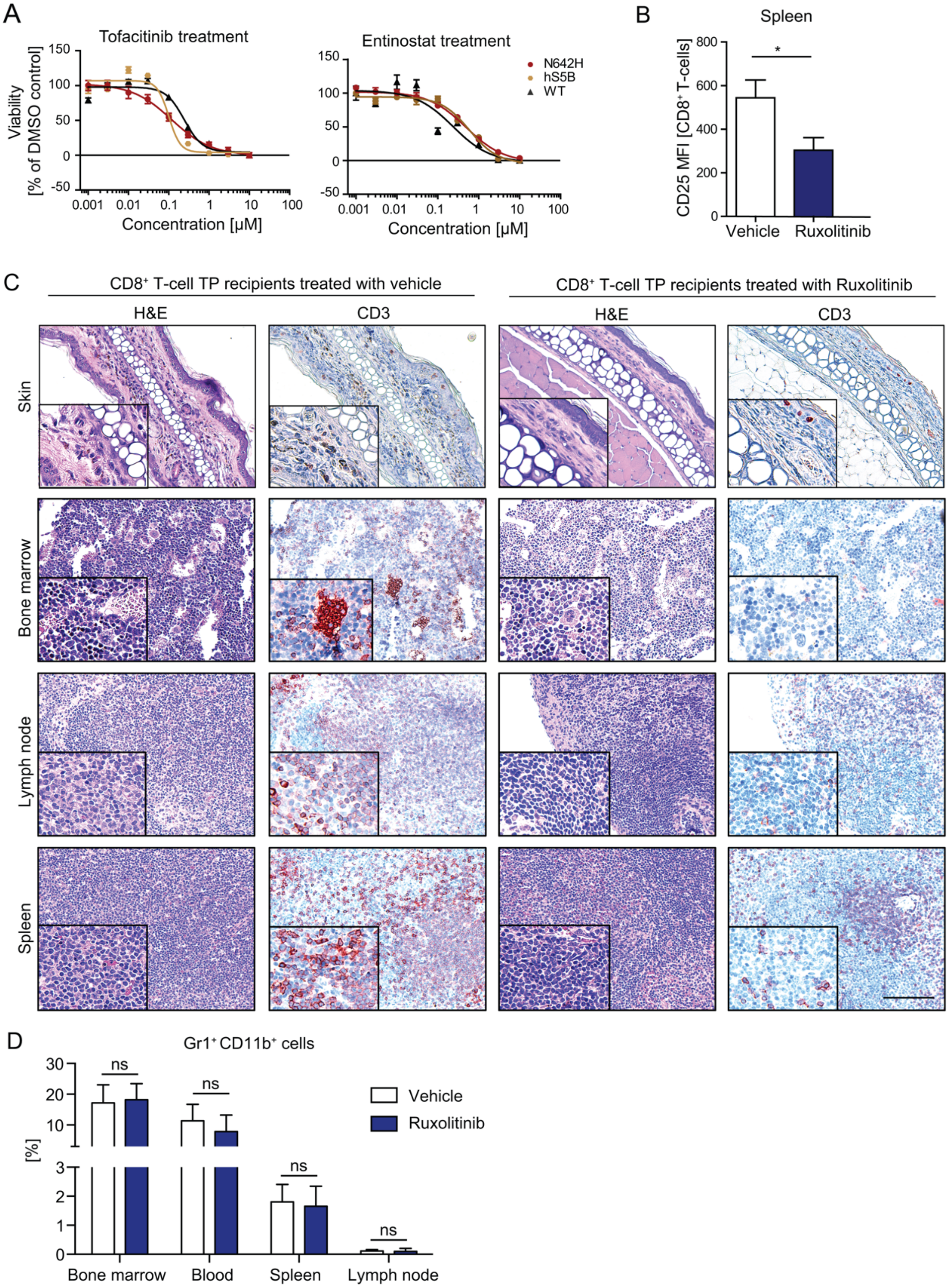


Supplement Figure 4

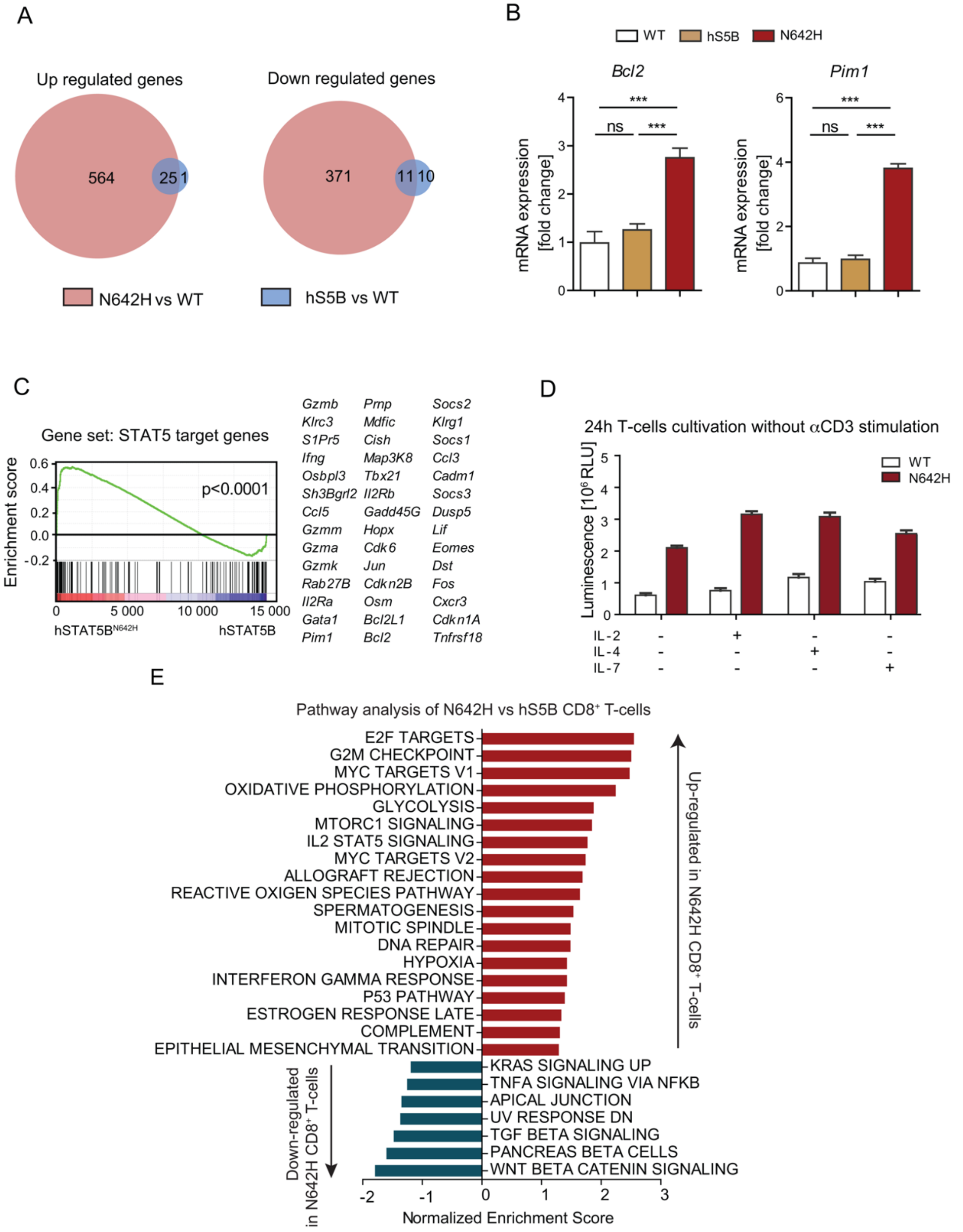
**Supplemental Figure 4. Splenomegaly is caused by T-cell infiltration and proliferation in hSTAT5B$^{N642H}$ transgenic mice.** Histological analysis of the spleen from hSTAT5B (hS5B), hSTAT5B$^{N642H}$ (N642H) and WT mice performing immunostaining with CD3, Ki76 and STAT5B specific antibodies. Black bar denotes 100 μm. All analyses were performed with 8 to 10-week-old mice. Data are representative of three independent experiments.



**Supplemental Figure 5. hSTAT5B$^{N642H}$ driven disease is transplantable. (A)** Phenotypic representation of BM transplanted recipient in comparison to the primary disease model. **(B)** Histological analysis of the CD3$^{+}$ cells in the skin of hSTAT5B$^{N642H}$ whole BM transplanted NSG recipients. **(C)** Flow cytometry analysis showed the quantity of CD8$^{+}$ T-cells in the peripheral blood of CD8$^{+}$ T-cell transplanted Ly5.1/CD45.1 recipients. Black bar denotes 100 μm. In this analysis, n=6 WT and n=5 hSTAT5B$^{N642H}$ recipients were used **(D)** Histological analysis of the CD3$^{+}$ cells in the lungs of hSTAT5B$^{N642H}$ (N642H) CD8$^{+}$ T-cell transplant recipients in comparison to the WT $CD8^+$ T-cell transplanted counter parts. Transplanted mice were analyzed at terminal state. Black bar denotes 100 μm. All data are presented as mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (unpaired two-tailed Student *t*-tests).

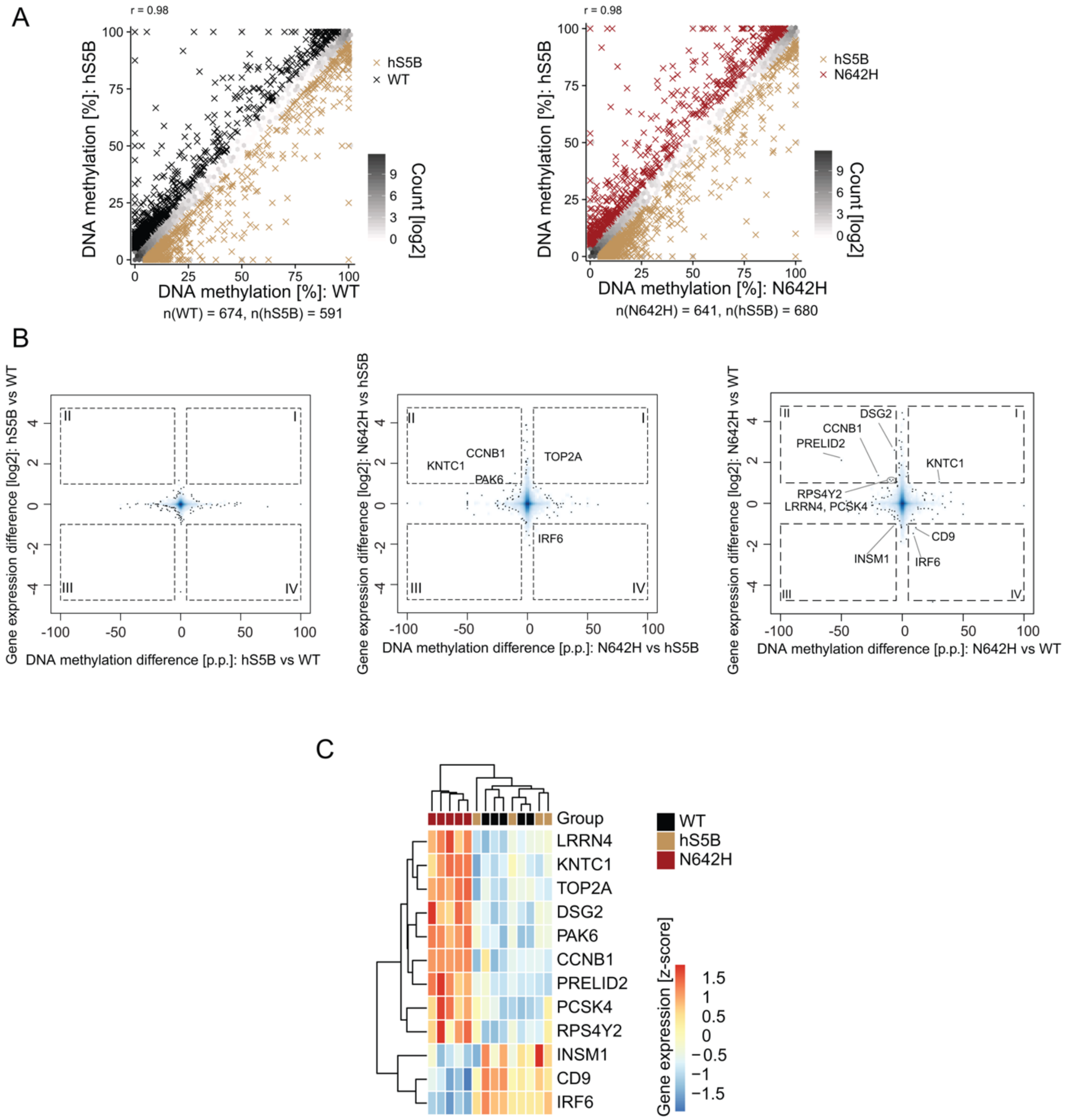


**Supplemental Figure 6. Potential therapy for hSTAT5B$^{N642H}$ driven T-cell disease. (A and B)** Dose-response curves of WT, hSTAT5B (hS5B) and hSTAT5B$^{N642H}$ (N642H) expressing T-cells in response to 72h treatment with JAK inhibitors (left) and HDAC inhibitor entinostat (right) after 72h of treatment analyzed using CTG viability assay. $IC_{50}$ values were determined using Graphpad Prism software. Error bars indicate ± SEM. **(B)** hSTAT5B$^{N642H}$ CD8$^{+}$ T-cells recipient mice were treated with ruxolitinib at the dosage of 45 mg/kg twice a day for 30 days. Flow cytometry analysis of CD25 expression on splenic CD8$^{+}$ T-cells of hSTAT5B$^{N642H}$ CD8$^{+}$ T-cell transplanted recipient mice after ruxolitinib or vehicle treatment. **(C)** H&E and CD3 staining of the skin, bone marrow, lymph nodes and spleen of CD8$^{+}$ T-cells transplanted mice after ruxolitinib treatment. **(D)** Flow cytometry analysis of Gr1$^{+}$ CD11b$^{+}$ cells in the blood, bone marrow, lymph nodes and spleen of CD8$^{+}$ T-cells transplanted mice after ruxolitinib treatment. Black bar denotes 100 μm. $n \geq 5$. All data are presented as mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (unpaired two-tailed Student's $t$ test).



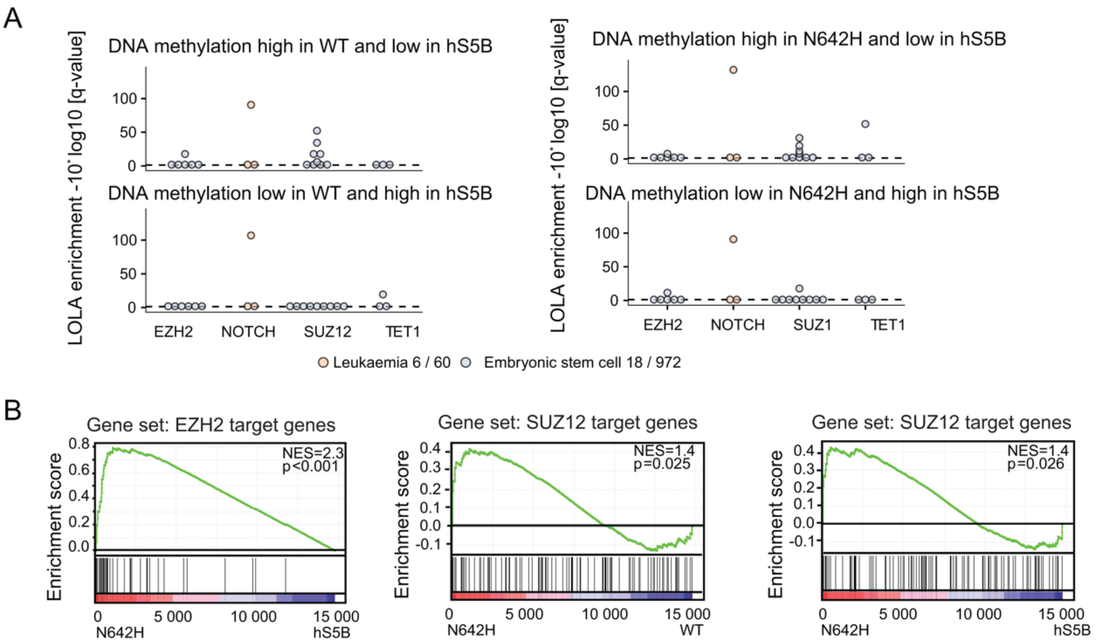
**Supplemental Figure 7. The expression of hSTAT5B$^{N642H}$ leads to the up-regulation of STAT5B target genes and cell cycle promoting genes. (A)** Venn diagrams showing genes differentially regulated (more than 2-fold difference) in hSTAT5B (*n*=4) and hSTAT5B$^{N642H}$ (*n*=5) expressing CD8$^{+}$ T-cells compared with

WT counterpart (*n*=5). **(B)** mRNA expression of *Bcl2* and *Pim1* in purified CD8$^+$ T-cells of 8 week-old WT, hSTAT5B$^{N642H}$ (N642H) and hSTAT5B (hS5B) transgenic mice were quantified using quantitative PCR. $n \geq 3$. **(C)** GSEA of genes that were ranked by signal/noise ratio according to their differential expression between hSTAT5B and hSTAT5B$^{N642H}$ CD8$^+$ T-cells. STAT5 target gene set are marked as vertical bars, and the enrichment score is shown in green. The list of significantly up-regulated STAT5 target genes was given using GSEA tool. **(D)** Comparison of T-cell viability between T-cells isolated form the LN of WT or hSTAT5B$^{N642H}$ mice under IL-2, IL-4 or IL-7 stimulation. Viability was measured with CTG viability assay*.* T-cells were isolated from 7 week-old mice and *n=3* per genotype. **(E)** Top enriched gene sets associated with up or down-regulated genes in hSTAT5B$^{N642H}$ CD8$^+$ T-cells sorted by normalized enrichment scores obtained by GSEA tool using hallmark gene sets. All analyses were performed using 13 week-old mice. All data are presented as mean ± SD. $n \geq 6$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (unpaired two-tailed Student *t*-tests).

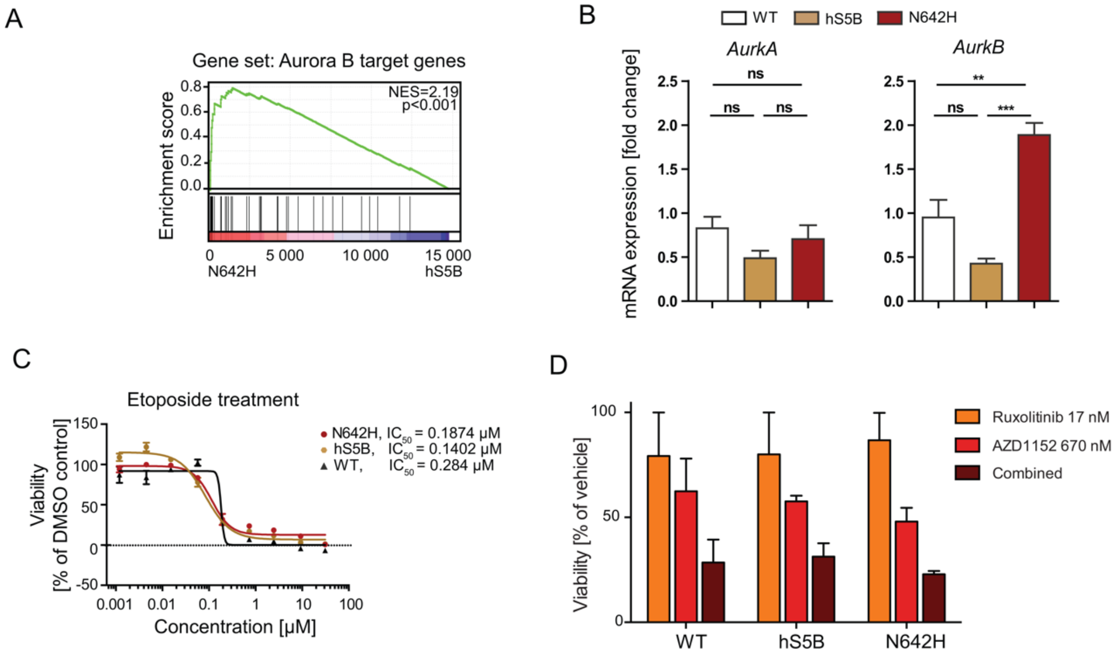


**Supplemental Figure 8: DNA methylation pattern of CD8$^+$ T-cells. (A)** Scatterplot contrasting mean DNA methylation levels in WT vs hSTAT5B (hS5B) (left) and hSTAT5B vs hSTAT5B$^{N642H}$ (N642H) (right) CD8$^+$ T-cells in all CGIs covered in at least one sample per genotype (*n*=2/genotype). The density of data points in each plot region is indicated by color intensity (absolute difference ≥ 5 percentage points). **(B)** Density scatterplot contrasting hSTAT5B/WT or

$hSTAT5B^{N642H}$/hSTAT5B or $hSTAT5B^{N642H}$/WT differences in DNA methylation at CGIs proximal to transcription start sites (TSS, maximum distance = 10 kb) with expression differences of the corresponding genes. Genes with strong differences in DNA methylation (absolute difference ≥ 5 percentage points) and gene expression (|log2FC| ≥ 1) are highlighted. **(C)** Heat map visualizing the relative expression levels of twelve genes with strong differences in both DNA methylation and gene expression in all comparisons between WT, hSTAT5B, and $hSTAT5B^{N642H}$ cells. Colors indicate expression levels and have been scaled such that red colors indicate the strongest and blue color the lowest expression per gene. Each column represents one dataset. Columns and rows have been rearranged by hierarchical clustering with complete linkage.



**Supplemental Figure 9: DNA methylation status of specific binding sites. (A)** Region set enrichment analysis for CGIs with lower DNA methylation in hSTAT5B (hS5B) than in WT (top left) or lower DNA methylation in WT than in hSTAT5B (bottom left) and for CGIs with lower DNA methylation in hSTAT5B than in hSTAT5B$^{N642H}$ (top right) or lower DNA methylation in hSTAT5B$^{N642H}$ than in hSTAT5B (bottom right). Enrichment was determined using Locus Overlap Analysis (LOLA). Each dot represents one ChIP-seq experiment for a given transcription factor from the CODEX database. The vertical dashed line represents the significance threshold (FDR-adjusted p-value ≤ 0.05). **(B)** Enrichment blot of EZH2 target genes in HSCs and SUZ12 target genes in embryonic stem cells. The barcode blot indicates the position of gene in the gene set. Red represents positive Pearson's correlation with hSTAT5B$^{N642H}$ CD8$^+$ T-cells and blue color represents positive Pearson's correlation with WT CD8$^+$ T-cells. The gene set was obtained from Molecular signature database (1).



**Supplemental Figure 10. The enrichment of EZH2 target genes reveals novel targets for the treatment of hSTAT5B$^{N642H}$-driven disease. (A)** Enrichment blot of AURKB target genes. The barcode blot indicates the position of gene in the gene set. Red represents positive Pearson's correlation with hSTAT5B$^{N642H}$ (N642H) CD8$^{+}$ T-cells and blue color represents positive Pearson's correlation with WT CD8$^{+}$ T-cells. The gene set was obtained from Molecular signature database (1). **(B)** mRNA expression of *AurkA* and *AurkB* from CD8$^{+}$ T-cells of 13 week-old mice. $n \geq 3$. **(C)** Dose-response curve of WT T-cells and T-cells expressing hSTAT5B (hS5B) or hSTAT5B$^{N642H}$ in response to 72h treatment with etoposide, TOP2A inhibitor analyzed using CTG assay (n=4 per genotype). IC$_{50}$ values were determined using Graphpad Prism software. **(D)** Effect of low concentration of ruxolitinib in combination with AZD1152 on cell viability of WT T-cells and T-cells expressing hSTAT5B or hSTAT5B$^{N642H}$ after 72h treatment, analyzed using CTG assay. Error bars indicate ± SD. All data are presented as mean ± SD. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; (1way ANOVA and Bonferroni corrected *P* value). Data presented in D is a representation of 2 independent experiments.

**Supplemental Table 1:** $IC_{50}$ values of drugs tested on WT, hSTAT5B and $hSTAT5B^{N642H}$ T-cells

| Compound | $IC_{50}$ [µM] | | |
|---|---|---|---|
| | WT | STAT5B | $STAT5B^{N642H}$ |
| Tofacitinib | 0.24 | 0.1 | 0.12 |
| Entinostat | 0.24 | 0.59 | 0.49 |
| JQ1 | 0.16 | 0.15 | 0.09 |
| Etoposide | 0.28 | 0.14 | 0.18 |
| Ruxolitinib | 0.21 | 0.16 | 0.11 |
| 5-Azacitidine | 0.32 | 0.52 | 0.36 |

**Supplemental Table 2:** Top 50 up-regulated genes in $hSTAT5B^{N642H}$

| | Average normalized fold of expression | | |
|---|---|---|---|
| Gene name | WT | hSTAT5B | $hSTAT5B^{N642H}$ |
| MYO6 | -0.870653602 | -0.498452588 | 1.269415672 |
| FAT1 | -0.761441206 | -0.649424566 | 1.280980859 |
| IGJ | -0.69783193 | -0.724025384 | 1.277052237 |
| GM1060 | -0.992803179 | -0.298555646 | 1.231647695 |
| GZMB | -0.799522949 | -0.596843026 | 1.27699737 |
| IL12RB2 | -0.764926485 | -0.645588998 | 1.281397684 |
| OSBPL3 | -0.86526464 | -0.504880599 | 1.269169119 |
| FASL | -0.963219414 | -0.349297197 | 1.242657172 |
| AA467197 | -0.693033977 | -0.725862473 | 1.273723955 |
| KIRREL | -0.820819737 | -0.564862115 | 1.27270943 |
| KLRC1 | -0.909685102 | -0.427346524 | 1.251562321 |

| | | | |
|---|---|---|---|
| S100A6 | -0.95569918 | -0.351553249 | 1.236941779 |
| GLDC | -0.688135612 | -0.712748907 | 1.258334737 |
| PIM1 | -0.913018037 | -0.426528614 | 1.254240928 |
| SEPT8 | -0.874618733 | -0.481759321 | 1.26002619 |
| LPIN1 | -0.913210576 | -0.424227733 | 1.252592763 |
| GZMM | -0.984326233 | -0.295293396 | 1.22056095 |
| ENTPD1 | -0.908631227 | -0.427253114 | 1.250433718 |
| LY6C1 | -0.938540434 | -0.138063695 | 1.04899139 |
| CCR5 | -0.974576774 | -0.316160173 | 1.227504913 |
| AQP9 | -1.0079714 | -0.259639493 | 1.215682994 |
| LGALS3 | -0.688118674 | -0.713828978 | 1.259181857 |
| STC2 | -0.93104298 | -0.39338042 | 1.245747316 |
| PTGER3 | -0.709555663 | -0.698671591 | 1.268492936 |
| IFNG | -0.78255179 | -0.612404947 | 1.272475748 |
| IFITM1 | -0.675732458 | -0.693844389 | 1.230807968 |
| CCR2 | -0.952146931 | -0.344587153 | 1.227816654 |
| RP23-320E6.1 | -0.901587823 | -0.432360979 | 1.247476607 |
| CCNB2 | -0.576418121 | -0.839645961 | 1.248134889 |
| D630039A03RIK | -0.669353607 | -0.737911651 | 1.259682928 |
| NM_010889 | -0.895026601 | -0.408847023 | 1.222104219 |
| PSRC1 | -0.771462836 | -0.624335659 | 1.270931363 |
| RCN1 | -0.883898301 | -0.46326955 | 1.254513941 |
| CCL5 | -0.919678005 | -0.377836216 | 1.221946977 |
| FAM19A3 | -0.866826417 | -0.484716546 | 1.254599655 |
| F2R | -0.959269028 | -0.338342192 | 1.229942782 |
| MTAP6 | -0.690826898 | -0.726484031 | 1.272014123 |

| | | | |
|---|---|---|---|
| KLRB1C | -0.703594545 | -0.689476272 | 1.255175563 |
| DYRK4 | -0.940041418 | -0.357057716 | 1.225687591 |
| AC101743.4 | -0.457475743 | -0.930351737 | 1.201757133 |
| 5830411N06RIK | -0.910031966 | -0.415371126 | 1.242328867 |
| IL2RB | -0.96424611 | -0.328061141 | 1.226695023 |
| PRELID2 | -0.674940544 | -0.699792961 | 1.234774913 |
| ZCCHC18 | -0.903380784 | -0.434500373 | 1.250981083 |
| SKA1 | -0.539026647 | -0.85720637 | 1.224791743 |
| CDC2A | -0.659246971 | -0.733384018 | 1.245954185 |
| SH3BGRL2 | -0.564636582 | -0.816520235 | 1.217852769 |
| KLRE1 | -0.907338358 | -0.388287079 | 1.217968021 |
| S1PR5 | -0.812132194 | -0.528598217 | 1.235010768 |
| NPAS2 | -0.756083451 | -0.642495202 | 1.270079612 |

**Supplemental Table 3:** Top 50 down-regulated genes in hSTAT5B$^{\text{N642H}}$

| | Average normalized fold of expression | | |
|---|---|---|---|
| Gene name | WT | hSTAT5B | hSTAT5B$^{\text{N642H}}$ |
| TMIE | 0.801148288 | 0.584630342 | -1.268852562 |
| KCNA2 | 0.641371626 | 0.778150102 | -1.263891708 |
| IL6ST | 0.777078692 | 0.6172273 | -1.270860532 |
| CNN3 | 0.861893931 | 0.502210608 | -1.263662417 |
| GPRC5B | 0.771267267 | 0.618468046 | -1.266041703 |
| XKRX | 0.853850414 | 0.505963655 | -1.258621339 |
| FMNL3 | 0.876210915 | 0.475863282 | -1.256901541 |
| CPM | 0.798484096 | 0.581889559 | -1.263995743 |

| | | | |
|---|---:|---:|---:|
| ENG | 0.827986762 | 0.530785402 | -1.252615083 |
| CCR9 | 0.818778336 | 0.528045519 | -1.241214752 |
| TIMP2 | 0.742039526 | 0.651997767 | -1.263637739 |
| FAM101B | 0.81248802 | 0.548576231 | -1.251349005 |
| KCNC1 | 0.790985068 | 0.577510822 | -1.252993726 |
| NTRK3 | 0.677983617 | 0.729401315 | -1.261504669 |
| CHST15 | 0.770815638 | 0.607903299 | -1.257138278 |
| ACTN1 | 0.77380593 | 0.603150473 | -1.256326309 |
| ST8SIA1 | 0.758460612 | 0.606813927 | -1.243911754 |
| IRF6 | 0.776274883 | 0.593290298 | -1.250907121 |
| MTSS1 | 0.827493473 | 0.524239538 | -1.246885104 |
| EPHX1 | 0.827379832 | 0.507024044 | -1.232999068 |
| BCL6 | 0.969071588 | 0.306283485 | -1.214098376 |
| ART2A | 0.611444324 | 0.729630867 | -1.195149018 |
| TBC1D30 | 0.463861125 | 0.937481055 | -1.213845969 |
| LDLRAP1 | 0.775842197 | 0.594774477 | -1.251661779 |
| 2610019F03RIK | 0.689245969 | 0.690694299 | -1.241801408 |
| NAB2 | 0.815649746 | 0.517162277 | -1.229379568 |
| SEPP1 | 0.866029178 | 0.465859756 | -1.238716983 |
| MFHAS1 | 0.812477393 | 0.525113032 | -1.232567819 |
| MAML3 | 0.853499966 | 0.470462426 | -1.229869907 |
| E430029J22RIK | 0.770945943 | 0.582827431 | -1.237207888 |
| AC159809.2 | 0.608972694 | 0.783133743 | -1.235479689 |
| CD209E | 0.819305262 | 0.451555297 | -1.1805495 |
| CXXC5 | 0.919120177 | 0.372813547 | -1.217371014 |
| AFAP1 | 0.696071529 | 0.656611795 | -1.221360965 |

| | | | |
|---|---|---|---|
| ITGAE | 0.936758427 | 0.338155214 | -1.207282597 |
| TLR12 | 0.811762638 | 0.524233903 | -1.231149761 |
| CYP2S1 | 0.503115697 | 0.874163952 | -1.202446859 |
| APP | 0.728626442 | 0.620110438 | -1.224714793 |
| ART4 | 0.699393059 | 0.644005493 | -1.214597453 |
| GRIA3 | 0.829587886 | 0.485624957 | -1.218087851 |
| RNF19A | 0.839272792 | 0.509928439 | -1.247215543 |
| CNGA1 | 0.815415353 | 0.511841031 | -1.224888178 |
| AMPD1 | 0.719700342 | 0.64504694 | -1.235737894 |
| PLEKHG2 | 0.705903026 | 0.64038242 | -1.218208962 |
| TSPAN9 | 0.99054511 | 0.244061536 | -1.185794339 |
| RGS10 | 0.733607925 | 0.60868815 | -1.220558445 |
| ABCA3 | 0.688468274 | 0.650657463 | -1.208994244 |
| MAP3K9 | 0.479833169 | 0.862278296 | -1.169655806 |
| NHSL1 | 0.924426576 | 0.358349035 | -1.211105804 |
| INADL | 0.789685904 | 0.54395365 | -1.224848824 |

## **Supplemental Table 4:** LOLA analysis of DNA methylation

| Differentially methylated regions | ChIP-seq reference data | | | Enrichment statistics | | | | |
|---|---|---|---|---|---|---|---|---|
| User set | File name | Cell type | Antibody | Description | Log-odds | P-value | Q-value | -10*log10 (q-value) |
| DNA methylation high in hSTAT5B$^{N642H}$ and low in hSTAT5B | GSM1014542_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.30 | 0.07401 | 0.28575 | 5.4 |
| | GSM327668_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.28 | 0.22298 | 1.00000 | 0.0 |
| | GSM905445_Ezh2.bed | Embryonic stem cell | Ezh2 | 129-C57Bl/6 ES cell differentiated *in vitro* by removal of LIF for 7 days | 1.20 | 0.41673 | 1.00000 | 0.0 |
| | GSM1215218_Ezh2.bed | Embryonic stem cell | Ezh2 | C57BL/6-CBA ES cells | 0.92 | 0.88092 | 1.00000 | 0.0 |
| | GSM1059004_Ezh2.bed | Embryonic stem cell | Ezh2 | ES cells expressing control shRNA targeting GFP | 0.92 | 0.90285 | 1.00000 | 0.0 |
| | GSM1199182_Ezh2.bed | Embryonic stem cell | Ezh2 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 0.69 | 1.00000 | 1.00000 | 0.0 |
| | GSM859512_Notch1.bed | Leukemia | Notch1 | intracellular Notch1-IC over-expressing tumors | 2.68 | 0.00000 | 0.00000 | 132.1 |
| | GSM732916_Notch1.bed | Leukemia | Notch1 | NULL | 0.42 | 0.99993 | 1.00000 | 0.0 |
| | GSM732913_Notch1.bed | Leukemia | Notch1 | NULL | 0.29 | 1.00000 | 1.00000 | 0.0 |
| | GSM1041374_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.46 | 0.00365 | 0.00077 | 31.1 |
| | GSM1019771_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.38 | 0.01229 | 0.01118 | 19.5 |
| | GSM1199188_Suz12.bed | Embryonic stem cell | Suz12 | Cells were lysed in ChIP buffer (50 mM Tris- | 1.34 | 0.03500 | 0.06856 | 11.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | HCl pH 7.9; 150 mM NaCl; 1% Triton X | | | | |
| | GSM1169018_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.33 | 0.04232 | 0.09916 | 10.0 |
| | GSM1169019_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.27 | 0.08641 | 0.38215 | 4.2 |
| | GSM700554_Suz12.bed | Embryonic stem cell | Suz12 | ES cells expressing control shRNA | 1.18 | 0.19905 | 1.00000 | 0.0 |
| | GSM288360_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.19 | 0.38450 | 1.00000 | 0.0 |
| | GSM700553_Suz12_Pcl3_shRNA.bed | Embryonic stem cell | Suz12 | ES cells expressing shRNA targeting Pcl3 | 1.08 | 0.49381 | 1.00000 | 0.0 |
| | GSM307144_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 0.90 | 0.87446 | 1.00000 | 0.0 |
| | GSM611192_Tet1C.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 C-terminus | 2.37 | 0.00039 | 0.00001 | 51.8 |
| | GSM659799_Tet1.bed | Embryonic stem cell | Tet1 | N/A | 0.66 | 0.99483 | 1.00000 | 0.0 |
| | GSM611194_Tet1N.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 N-terminus | 0.41 | 1.00000 | 1.00000 | 0.0 |
| DNA methylation low in STAT5B$^{N642H}$ and high in hSTAT5B | GSM1014542_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.35 | 0.03406 | 0.06856 | 11.6 |
| | GSM327668_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.35 | 0.13375 | 0.77539 | 1.1 |
| | GSM905445_Ezh2.bed | Embryonic stem cell | Ezh2 | 129-C57Bl/6 ES cell differentiated *in vitro* by removal of LIF for 7 days | 1.33 | 0.21238 | 1.00000 | 0.0 |
| | GSM1215218_Ezh2.bed | Embryonic stem cell | Ezh2 | C57BL/6-CBA ES cells | 1.07 | 0.59011 | 1.00000 | 0.0 |
| | GSM1059004_Ezh2.bed | Embryonic stem cell | Ezh2 | ES cells expressing control shRNA targeting GFP | 1.02 | 0.71316 | 1.00000 | 0.0 |
| | GSM1199182_Ezh2.bed | Embryonic stem cell | Ezh2 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 0.67 | 1.00000 | 1.00000 | 0.0 |
| | GSM859512_Notch1.bed | Leukemia | Notch1 | intracellular Notch1-IC over-expressing tumors | 2.28 | 0.00001 | 0.00000 | 90.9 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GSM732916_Notch1.bed | Leukemia | Notch1 | NULL | 0.30 | 1.00000 | 1.00000 | 0.0 |
| | GSM732913_Notch1.bed | Leukemia | Notch1 | NULL | 0.21 | 1.00000 | 1.00000 | 0.0 |
| | GSM1041374_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.36 | 0.01595 | 0.01667 | 17.8 |
| | GSM700554_Suz12.bed | Embryonic stem cell | Suz12 | ES cells expressing control shRNA | 1.22 | 0.10682 | 0.54192 | 2.7 |
| | GSM1019771_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.21 | 0.11564 | 0.58958 | 2.3 |
| | GSM1169019_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.22 | 0.14795 | 0.95314 | 0.2 |
| | GSM307144_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.43 | 0.16882 | 1.00000 | 0.0 |
| | GSM700553_Suz12_Pcl3_shRNA.bed | Embryonic stem cell | Suz12 | ES cells expressing shRNA targeting Pcl3 | 1.19 | 0.19374 | 1.00000 | 0.0 |
| | GSM1169018_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.17 | 0.23296 | 1.00000 | 0.0 |
| | GSM288360_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.27 | 0.23880 | 1.00000 | 0.0 |
| | GSM1199188_Suz12.bed | Embryonic stem cell | Suz12 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 1.13 | 0.32472 | 1.00000 | 0.0 |
| | GSM611192_Tet1C.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 C-terminus | 1.42 | 0.19500 | 1.00000 | 0.0 |
| | GSM659799_Tet1.bed | Embryonic stem cell | Tet1 | N/A | 0.53 | 0.99980 | 1.00000 | 0.0 |
| | GSM611194_Tet1N.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 N-terminus | 0.38 | 1.00000 | 1.00000 | 0.0 |
| DNA methylation high in WT and low STAT5B$^{N642H}$ | GSM1014542_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.49 | 0.00257 | 0.00036 | 34.4 |
| | GSM905445_Ezh2.bed | Embryonic stem cell | Ezh2 | 129-C57Bl/6 ES cell differentiated *in vitro* by removal of LIF for 7 days | 1.58 | 0.03116 | 0.05913 | 12.3 |
| | GSM327668_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.43 | 0.05729 | 0.17878 | 7.5 |
| | GSM1215218_Ezh2.bed | Embryonic stem cell | Ezh2 | C57BL/6-CBA ES cells | 1.15 | 0.38742 | 1.00000 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GSM1059004_Ezh2.bed | Embryonic stem cell | Ezh2 | ES cells expressing control shRNA targeting GFP | 1.04 | 0.64442 | 1.00000 | 0.0 |
| | GSM1199182_Ezh2.bed | Embryonic stem cell | Ezh2 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 0.77 | 0.99993 | 1.00000 | 0.0 |
| | GSM859512_Notch1.bed | Leukemia | Notch1 | intracellular Notch1-IC over-expressing tumors | 2.46 | 0.00000 | 0.00000 | 126.4 |
| | GSM732916_Notch1.bed | Leukemia | Notch1 | NULL | 0.29 | 1.00000 | 1.00000 | 0.0 |
| | GSM732913_Notch1.bed | Leukemia | Notch1 | NULL | 0.21 | 1.00000 | 1.00000 | 0.0 |
| | GSM1041374_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.61 | 0.00005 | 0.00000 | 71.1 |
| | GSM1019771_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.50 | 0.00041 | 0.00001 | 51.7 |
| | GSM700554_Suz12.bed | Embryonic stem cell | Suz12 | ES cells expressing control shRNA | 1.47 | 0.00087 | 0.00003 | 44.6 |
| | GSM1169019_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.35 | 0.01934 | 0.02382 | 16.2 |
| | GSM700553_Suz12_Pcl3_shRNA.bed | Embryonic stem cell | Suz12 | ES cells expressing shRNA targeting Pcl3 | 1.33 | 0.02358 | 0.03473 | 14.6 |
| | GSM1169018_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.33 | 0.02696 | 0.04389 | 13.6 |
| | GSM1199188_Suz12.bed | Embryonic stem cell | Suz12 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 1.29 | 0.04384 | 0.10459 | 9.8 |
| | GSM288360_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.28 | 0.20392 | 1.00000 | 0.0 |
| | GSM307144_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.18 | 0.47422 | 1.00000 | 0.0 |
| | GSM611192_Tet1C.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 C-terminus | 1.32 | 0.27642 | 1.00000 | 0.0 |
| | GSM659799_Tet1.bed | Embryonic stem cell | Tet1 | N/A | 0.65 | 0.99753 | 1.00000 | 0.0 |
| | GSM611194_Tet1N.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 N-terminus | 0.38 | 1.00000 | 1.00000 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| DNA methylation high in hSTAT5B$^{N642H}$ and low in WT | GSM327668_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.16 | 0.43134 | 1.00000 | 0.0 |
| | GSM1014542_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 0.99 | 0.76311 | 1.00000 | 0.0 |
| | GSM905445_Ezh2.bed | Embryonic stem cell | Ezh2 | 129-C57Bl/6 ES cell differentiated *in vitro* by removal of LIF for 7 days | 0.80 | 0.96168 | 1.00000 | 0.0 |
| | GSM1215218_Ezh2.bed | Embryonic stem cell | Ezh2 | C57BL/6-CBA ES cells | 0.71 | 0.99682 | 1.00000 | 0.0 |
| | GSM1059004_Ezh2.bed | Embryonic stem cell | Ezh2 | ES cells expressing control shRNA targeting GFP | 0.66 | 0.99985 | 1.00000 | 0.0 |
| | GSM1199182_Ezh2.bed | Embryonic stem cell | Ezh2 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 0.58 | 1.00000 | 1.00000 | 0.0 |
| | GSM859512_Notch1.bed | Leukemia | Notch1 | intracellular Notch1-IC over-expressing tumors | 2.63 | 0.00000 | 0.00000 | 123.1 |
| | GSM732916_Notch1.bed | Leukemia | Notch1 | NULL | 0.44 | 0.99983 | 1.00000 | 0.0 |
| | GSM732913_Notch1.bed | Leukemia | Notch1 | NULL | 0.27 | 1.00000 | 1.00000 | 0.0 |
| | GSM1041374_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.18 | 0.22017 | 1.00000 | 0.0 |
| | GSM1169019_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.17 | 0.27316 | 1.00000 | 0.0 |
| | GSM1169018_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.14 | 0.33296 | 1.00000 | 0.0 |
| | GSM1019771_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.12 | 0.35377 | 1.00000 | 0.0 |
| | GSM288360_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.06 | 0.64628 | 1.00000 | 0.0 |
| | GSM1199188_Suz12.bed | Embryonic stem cell | Suz12 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 1.03 | 0.66013 | 1.00000 | 0.0 |
| | GSM307144_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.02 | 0.73098 | 1.00000 | 0.0 |
| | GSM700553_Suz12_Pcl3_shRNA.bed | Embryonic stem cell | Suz12 | ES cells expressing shRNA targeting Pcl3 | 0.97 | 0.81911 | 1.00000 | 0.0 |
| | GSM700554_Suz12.bed | Embryonic stem cell | Suz12 | ES cells expressing control shRNA | 0.97 | 0.83610 | 1.00000 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GSM611192_Tet1C.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 C-terminus | 2.60 | 0.00008 | 0.00000 | 66.7 |
| | GSM659799_Tet1.bed | Embryonic stem cell | Tet1 | N/A | 0.72 | 0.98348 | 1.00000 | 0.0 |
| | GSM611194_Tet1N.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 N-terminus | 0.40 | 1.00000 | 1.00000 | 0.0 |
| DNA methylation high in WT and low in hSTAT5B | GSM1014542_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.43 | 0.01267 | 0.01136 | 19.4 |
| | GSM905445_Ezh2.bed | Embryonic stem cell | Ezh2 | 129-C57Bl/6 ES cell differentiated *in vitro* by removal of LIF for 7 days | 1.47 | 0.09176 | 0.42086 | 3.8 |
| | GSM327668_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.26 | 0.24275 | 1.00000 | 0.0 |
| | GSM1059004_Ezh2.bed | Embryonic stem cell | Ezh2 | ES cells expressing control shRNA targeting GFP | 1.05 | 0.61359 | 1.00000 | 0.0 |
| | GSM1215218_Ezh2.bed | Embryonic stem cell | Ezh2 | C57BL/6-CBA ES cells | 0.99 | 0.77329 | 1.00000 | 0.0 |
| DNA methylation high in WT and low in hSTAT5B | GSM1199182_Ezh2.bed | Embryonic stem cell | Ezh2 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 0.72 | 1.00000 | 1.00000 | 0.0 |
| | GSM859512_Notch1.bed | Leukemia | Notch1 | intracellular Notch1-IC over-expressing tumors | 2.29 | 0.00001 | 0.00000 | 90.6 |
| | GSM732916_Notch1.bed | Leukemia | Notch1 | NULL | 0.40 | 0.99998 | 1.00000 | 0.0 |
| | GSM732913_Notch1.bed | Leukemia | Notch1 | NULL | 0.27 | 1.00000 | 1.00000 | 0.0 |
| | GSM1041374_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.57 | 0.00036 | 0.00001 | 52.3 |
| | GSM1019771_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.45 | 0.00254 | 0.00036 | 34.4 |
| | GSM1169018_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.38 | 0.01846 | 0.02235 | 16.5 |
| | GSM1199188_Suz12.bed | Embryonic stem cell | Suz12 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 1.36 | 0.02015 | 0.02516 | 16.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GSM700554_Suz12.bed | Embryonic stem cell | Suz12 | ES cells expressing control shRNA | 1.25 | 0.06840 | 0.25574 | 5.9 |
| | GSM288360_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.32 | 0.17404 | 1.00000 | 0.0 |
| | GSM700553_Suz12_Pcl3_shRNA.bed | Embryonic stem cell | Suz12 | ES cells expressing shRNA targeting Pcl3 | 1.20 | 0.17562 | 1.00000 | 0.0 |
| | GSM1169019_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.15 | 0.29485 | 1.00000 | 0.0 |
| | GSM307144_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.08 | 0.63973 | 1.00000 | 0.0 |
| | GSM611192_Tet1C.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 C-terminus | 1.53 | 0.11509 | 0.58958 | 2.3 |
| | GSM659799_Tet1.bed | Embryonic stem cell | Tet1 | N/A | 0.70 | 0.99088 | 1.00000 | 0.0 |
| | GSM611194_Tet1N.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 N-terminus | 0.40 | 1.00000 | 1.00000 | 0.0 |
| DNA methylation high in STAT5B and low in WT | GSM1014542_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.23 | 0.17368 | 1.00000 | 0.0 |
| | GSM327668_Ezh2.bed | Embryonic stem cell | Ezh2 | N/A | 1.24 | 0.30004 | 1.00000 | 0.0 |
| | GSM905445_Ezh2.bed | Embryonic stem cell | Ezh2 | 129-C57Bl/6 ES cell differentiated *in vitro* by removal of LIF for 7 days | 0.90 | 0.88413 | 1.00000 | 0.0 |
| | GSM1059004_Ezh2.bed | Embryonic stem cell | Ezh2 | ES cells expressing control shRNA targeting GFP | 0.86 | 0.95805 | 1.00000 | 0.0 |
| | GSM1215218_Ezh2.bed | Embryonic stem cell | Ezh2 | C57BL/6-CBA ES cells | 0.83 | 0.96337 | 1.00000 | 0.0 |
| | GSM1199182_Ezh2.bed | Embryonic stem cell | Ezh2 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 0.62 | 1.00000 | 1.00000 | 0.0 |
| | GSM859512_Notch1.bed | Leukemia | Notch1 | intracellular Notch1-IC over-expressing tumors | 2.52 | 0.00000 | 0.00000 | 106.9 |
| | GSM732916_Notch1.bed | Leukemia | Notch1 | NULL | 0.38 | 0.99996 | 1.00000 | 0.0 |
| | GSM732913_Notch1.bed | Leukemia | Notch1 | NULL | 0.28 | 1.00000 | 1.00000 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GSM1041374_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.26 | 0.09065 | 0.41779 | 3.8 |
| | GSM1169019_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.22 | 0.17392 | 1.00000 | 0.0 |
| | GSM1169018_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.19 | 0.22200 | 1.00000 | 0.0 |
| | GSM1019771_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.14 | 0.30745 | 1.00000 | 0.0 |
| | GSM1199188_Suz12.bed | Embryonic stem cell | Suz12 | Cells were lysed in ChIP buffer (50 mM Tris-HCl pH 7.9; 150 mM NaCl; 1% Triton X | 1.13 | 0.35967 | 1.00000 | 0.0 |
| | GSM307144_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.20 | 0.47061 | 1.00000 | 0.0 |
| | GSM700553_Suz12_Pcl3_shRNA.bed | Embryonic stem cell | Suz12 | ES cells expressing shRNA targeting Pcl3 | 1.07 | 0.54624 | 1.00000 | 0.0 |
| | GSM700554_Suz12.bed | Embryonic stem cell | Suz12 | ES cells expressing control shRNA | 1.06 | 0.54898 | 1.00000 | 0.0 |
| | GSM288360_Suz12.bed | Embryonic stem cell | Suz12 | N/A | 1.09 | 0.59090 | 1.00000 | 0.0 |
| | GSM611192_Tet1C.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 C-terminus | 1.94 | 0.01297 | 0.01140 | 19.4 |
| | GSM659799_Tet1.bed | Embryonic stem cell | Tet1 | N/A | 0.51 | 0.99975 | 1.00000 | 0.0 |
| | GSM611194_Tet1N.bed | Embryonic stem cell | Tet1 | ES cell expressing scrabbled control shRNA. Antibody to Tet1 N-terminus | 0.36 | 1.00000 | 1.00000 | 0.0 |

**Supplemental Table 5:** DNA methylation status at EZH2 binding site

| Chromosom | Start | End | Target gene | Differentially methylated | EZH2 target |
|---|---|---|---|---|---|
| chr1 | 20819889 | 20820297 | MCM3 | 0.081994315 | Not bound |
| chr1 | 20890435 | 20890811 | MCM3 | 1.911259612 | Not bound |
| chr1 | 191821250 | 191821971 | NEK2 | -0.289590773 | Bound |
| chr1 | 191906597 | 191907697 | NEK2 | -0.079683446 | Not bound |
| chr2 | 71367519 | 71367773 | HAT1 | -0.328074202 | Not bound |
| chr2 | 71389037 | 71389586 | HAT1 | -0.712346064 | Not bound |
| chr2 | 71453142 | 71453517 | HAT1 | -0.555555556 | Not bound |
| chr2 | 72476060 | 72477161 | CDCA7 | -1.921243901 | Not bound |
| chr2 | 119546917 | 119547155 | NUSAP1 | 4.857024525 | Not bound |
| chr2 | 119547472 | 119547988 | NUSAP1 | 0.175233943 | Bound |
| chr2 | 119594232 | 119594717 | NUSAP1 | -1.532116121 | Not bound |
| chr2 | 119618173 | 119618652 | NUSAP1 | 0 | Not bound |
| chr2 | 119662622 | 119662879 | NUSAP1 | 0.146942264 | Bound |
| chr2 | 119674934 | 119675628 | NUSAP1 | 1.667495566 | Not bound |
| chr2 | 132247528 | 132247857 | PCNA | 1.146266353 | Not bound |
| chr2 | 132262954 | 132263752 | PCNA | 0.04653081 | Not bound |
| chr2 | 155074085 | 155074476 | AHCY | 1.823727139 | Bound |
| chr2 | 155133431 | 155133879 | AHCY | 0.297424093 | Bound |
| chr2 | 172345330 | 172345900 | AURKA | -1.966563736 | Not bound |
| chr2 | 172370597 | 172371333 | AURKA | -0.615172595 | Not bound |
| chr2 | 172440317 | 172440790 | AURKA | 0.631820639 | Not bound |
| chr3 | 27153414 | 27154068 | ECT2 | 0.583915529 | Not bound |
| chr3 | 27182878 | 27183286 | ECT2 | -1.498472116 | Not bound |
| chr3 | 137864038 | 137865781 | H2AFZ | -1.840631785 | Not bound |
| chr3 | 137867582 | 137868040 | H2AFZ | 0.286924613 | Not bound |
| chr3 | 137918409 | 137918684 | H2AFZ | 0.313627234 | Not bound |
| chr5 | 33629016 | 33629950 | TACC3 | -0.332267772 | Not bound |
| chr5 | 33651872 | 33652808 | TACC3 | 0.297619048 | Not bound |
| chr5 | 33657578 | 33658835 | TACC3 | -0.330915718 | Bound |

| | | | | | |
|---|---|---|---|---|---|
| chr5 | 33692519 | 33692773 | TACC3 | 7.400055752 | Not bound |
| chr5 | 33695268 | 33695842 | TACC3 | 0.807956618 | Bound |
| chr5 | 33721593 | 33723909 | TACC3 | -0.029648768 | Bound |
| chr6 | 8209078 | 8209710 | RPA3 | 0.447874045 | Not bound |
| chr6 | 8258939 | 8259647 | RPA3 | -0.452192735 | Not bound |
| chr6 | 66535130 | 66535719 | MAD2L1 | -2.26007642 | Not bound |
| chr6 | 88841539 | 88843023 | MCM2 | 0.434944866 | Not bound |
| chr6 | 88874242 | 88875232 | MCM2 | -10.25249892 | Bound |
| chr6 | 88898587 | 88898835 | MCM2 | 1.445297667 | Not bound |
| chr6 | 88901959 | 88902760 | MCM2 | 0.623889341 | Not bound |
| chr7 | 80198190 | 80198815 | PRC1 | -0.101163361 | Bound |
| chr7 | 80232412 | 80232922 | PRC1 | -0.614239503 | Not bound |
| chr7 | 80246218 | 80246618 | PRC1 | -0.248772244 | Not bound |
| chr7 | 80261133 | 80261518 | PRC1 | -1.53710692 | Not bound |
| chr7 | 80269579 | 80269844 | PRC1 | 1.318083344 | Not bound |
| chr7 | 80294257 | 80294862 | PRC1 | 0 | Not bound |
| chr7 | 80324099 | 80324365 | PRC1 | 2.083333333 | Not bound |
| chr7 | 80339932 | 80340448 | PRC1 | -0.83105039 | Not bound |
| chr7 | 80363080 | 80363296 | PRC1 | 0 | Not bound |
| chr7 | 80370617 | 80371559 | PRC1 | 0.620695746 | Bound |
| chr7 | 102441613 | 102442102 | RRM1 | 1.413637614 | Bound |
| chr7 | 102512908 | 102513374 | RRM1 | 0.4135878 | Bound |
| chr8 | 13287140 | 13288476 | TFDP1 | 0.736707017 | Bound |
| chr8 | 13338535 | 13339396 | TFDP1 | 0.594743839 | Not bound |
| chr9 | 70421361 | 70421597 | CCNB2 | 0.266193638 | Not bound |
| chr9 | 70503067 | 70503877 | CCNB2 | -0.128997261 | Not bound |
| chr10 | 111472969 | 111473826 | NAP1L1 | 0.104891576 | Not bound |
| chr10 | 111506138 | 111506897 | NAP1L1 | -0.435992834 | Not bound |
| chr11 | 68967718 | 68968283 | AURKB | -0.82577107 | Not bound |
| chr11 | 69039957 | 69040362 | AURKB | 0.617360508 | Not bound |
| chr11 | 69045641 | 69046110 | AURKB | -0.46107311 | Not bound |
| chr11 | 69059294 | 69060850 | AURKB | -1.197594644 | Not bound |
| chr11 | 69070707 | 69071062 | AURKB | -0.820660934 | Not bound |

| | | | | | |
|---|---|---|---|---|---|
| chr11 | 69088414 | 69089149 | AURKB | -0.631137376 | Not bound |
| chr11 | 69095157 | 69095360 | AURKB | 1.291666667 | Not bound |
| chr11 | 69117978 | 69118214 | AURKB | 2.490329453 | Not bound |
| chr11 | 69122149 | 69123102 | AURKB | 0.52396829 | Bound |
| chr11 | 98936172 | 98936439 | TOP2A | 6.193568336 | Bound |
| chr11 | 98937355 | 98938138 | TOP2A | 1.267700759 | Bound |
| chr11 | 98959605 | 98960112 | TOP2A | -0.884518404 | Bound |
| chr11 | 98960435 | 98961001 | TOP2A | 0.334593987 | Not bound |
| chr11 | 98982169 | 98983029 | TOP2A | -1.141416983 | Bound |
| chr11 | 99023930 | 99024182 | TOP2A | 4.232500768 | Not bound |
| chr11 | 99041012 | 99041815 | TOP2A | 2.740718795 | Bound |
| chr11 | 106920094 | 106920955 | KPNA2 | -0.066803139 | Not bound |
| chr11 | 106999065 | 106999677 | KPNA2 | -2.528131486 | Not bound |
| chr11 | 107012601 | 107012831 | KPNA2 | -1.160322682 | Not bound |
| chr11 | 107027746 | 107028594 | KPNA2 | -0.524302036 | Not bound |
| chr13 | 24761560 | 24762170 | GMNN | -0.714749523 | Not bound |
| chr13 | 24801094 | 24801950 | GMNN | -0.298877611 | Not bound |
| chr13 | 24802080 | 24802349 | GMNN | -0.130779796 | Not bound |
| chr13 | 24831403 | 24832195 | GMNN | 0.154445991 | Not bound |
| chr13 | 24845055 | 24845340 | GMNN | -1.067821068 | Not bound |
| chr19 | 6015454 | 6015722 | CDCA5 | -0.561326523 | Not bound |
| chr19 | 6046737 | 6047482 | CDCA5 | 0.58846546 | Not bound |
| chr19 | 6057666 | 6058189 | CDCA5 | -0.137362637 | Not bound |
| chr19 | 6061019 | 6061919 | CDCA5 | -0.06902404 | Not bound |
| chr19 | 6084755 | 6085356 | CDCA5 | 1.516116932 | Not bound |
| chr19 | 6117859 | 6118613 | CDCA5 | 1.395758731 | Bound |
| chr19 | 6140555 | 6141406 | CDCA5 | -2.048640416 | Not bound |
| chr19 | 6184251 | 6184665 | CDCA5 | 4.63911381 | Not bound |

**Supplemental Table 6:** Antibodies for flow cytometry

| **Antigen** | **Flourochrome** | **Clone** | **Catalog No** |
|---|---|---|---|
| Fc-Block™ | Unconjugated | 93 | 14-0161-82 (ebioscience) |
| CD3 | eFluor® 450 | 17A2 | 48-0032-82 (ebioscience) |
| CD3e | PerCP Cy5.5 | 145-2C11 | 45-0031-82 (ebioscience) |
| CD19 | PE | eBio1D3 (1D3) | 12-0193-82 (ebioscience) |
| CD19 | eFluor® 450 | eBio1D3 (1D3) | 48-0193-82 (ebioscience) |
| CD4 | FITC | GK1.5 | 11-0041-82 (ebioscience) |
| CD4 | PE | GK1.5 | 12-0041-82 (ebioscience) |
| CD8a | FITC | 53-6.7 | 11-0081-82 (ebioscience) |
| CD8a | PE | 53-6.7 | 12-0081-82 (ebioscience) |
| CD11b | FITC | M1/70 | 11-0112-82 (ebioscience) |
| CD11b | eFluor® 450 | M1/70 | 48-0112-82 (ebioscience) |
| CD25 | APC | PC61.5 | 17-0251-81 (ebioscience) |
| CD44 | PE | IM7 | 12-0441-82 (ebioscience) |
| CD117 (c-Kit) | PE-Cy5 | 2B8 | 15-1171-83 (ebioscience) |
| CD45.1 (Ly5.1) | PE | A20 | 12-0453-82 (ebioscience) |
| CD45.2 (Ly5.2) | APC | 104 | 17-0454-82 (ebioscience) |
| CD45R (B220) | eFluor® 450 | RA3-6B2 | 48-0452-82 (ebioscience) |
| CD45R (B220) | PerCP Cy5.5 | RA3-6B2 | 45-0452-82 (ebioscience) |

|  |  |  |  |
|---|---|---|---|
| Ly6.G (Gr1) | APC | RB6-8C5 | 17-5931-82 (ebioscience) |
| Ly6.G (Gr1) | eFluor® 450 | RB6-8C5 | 48-5931-82 (ebioscience) |
| Ly-6A/E (Sca-1) | PE-Cy7 | D7 | 25-5981-82 (ebioscience) |
| Strepdavidin | APC-eFluor® 780 |  | 47-4317-82 (ebioscience) |
| TER-119 | eFluor 450 | TER-119 | 48-5921-82 (ebioscience) |
| CD62L | APC | MEL-14 | 17-0621-81 (ebioscience) |
| NK1.1 | PE-Cyanine7 | PK136 | 25-5941-82 (ebioscience) |
| CD135 | Unconjugated | A2F10.1 | 13-1351-82 (ebioscience) |
| CD127 | Unconjugated | A7R34 | 13-1271-82 (ebioscience) |
| CD16/CD32 | PE | 93 | 12-0161-82 (ebioscience) |
| CD93 (AA4.1) | APC | AA4.1 | 17-5892-82 (ebioscience) |
| Hamster anti-Mouse CD3e | Unconjugated | 145-2C11 | 51-01082J (BD) |
| Rat anti-Mouse CD45R | Unconjugated | RA3-6B2 | 51-01122J (BD) |
| Rat anti-Mouse Ly-6G/C | Unconjugated | RB6-8C5 | 51-01212J (BD) |
| Rat anti-Mouse CD11b | Unconjugated | M1/70 | 51-01712J (BD) |
| Rat anti-Mouse TER-119 | Unconjugated | TER-119 | 51-09082J (BD) |
| CD34 | FITC | RAM34 | 553733 (BD) |
| CD150 | APC | TC15-12F12.2 | 562373 (BD) |
| CD48 | PE | HM48-1 | 557484 (BD) |
| CD34 | APC | MEC14.7 | 119310 (Biolegend) |

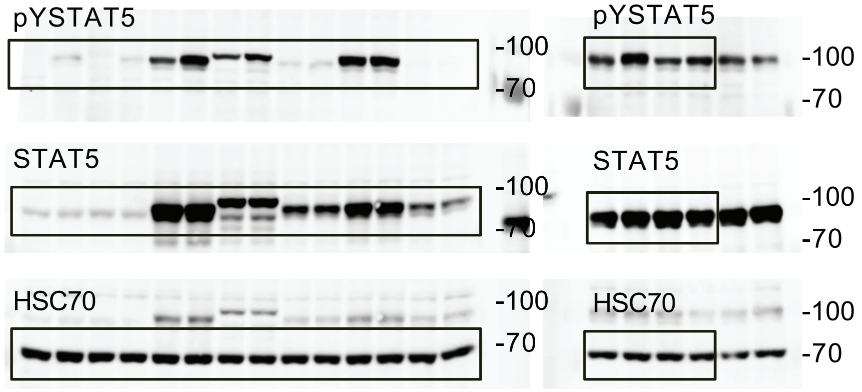
**Supplemental Table 7:** qPCR primers

| | Forward primer | Reverse primer |
|---|---|---|
| *Bcl2* | GGTCTTCAGAGACAGCCAGG | GATCCAGGATAACGGAGGCT |
| *Pim1* | TGTTCTCGTCCTTGATGTCG | GGAGCCCTACAGGAGGACC |
| *Aurk a* | CAATGCTTGCTGACAGACACCCC | CACAGGCAGGAACCTGCTCC |
| *Aurk b* | ACGCGTAAATGCACACAAGCCCTG | AGATCTAGCTGGACAGAGAGG |
| Negative region | TACCCCTTCCAACTCTGACTGAGC | TTCCCTCCAGGATGTGACTGTG |
| P.R.*Ccnd2* | TCAACACGTGGTCAGAGTGG | GGGAAAGTTAAAGCGCGTCC |
| P.R.*Cis* | GTCCAAAGCACTAGACGCCTG | TTCCCGGAAGCCTCATCTT |
| P.R. *Cdkn2a* | TGTACAGAATCCTAGCACTGATACAGCAAC | GATCCCAACAACCCTAGCTCAAACAAC |
| CpGi *Aurb-01* | TAAGCATTCTGTACCTGTGTCC | GTGGAGAAGAGCAAGTTCTGA |
| CpGi *Aurb-02* | CTGCGTCAGTTTCCCACTTG | GAGAGGTGTACGAAACAGCAAC |

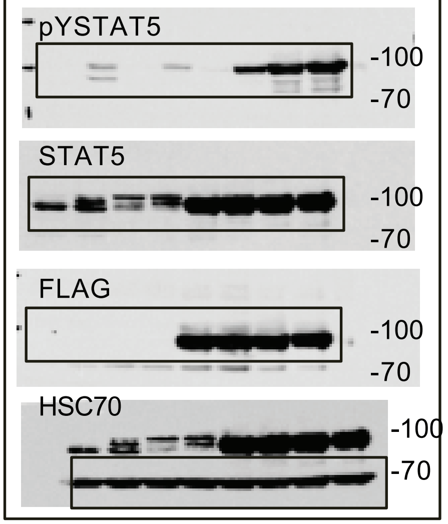
## Reference:

1. **Kamminga, L.M., Bystrykh, L.V., de Boer, A., Houwer, S., Douma, J., Weersing, E., Dontje, B., and de Haan, G. 2006. The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. *Blood* 107:2170-2179.**

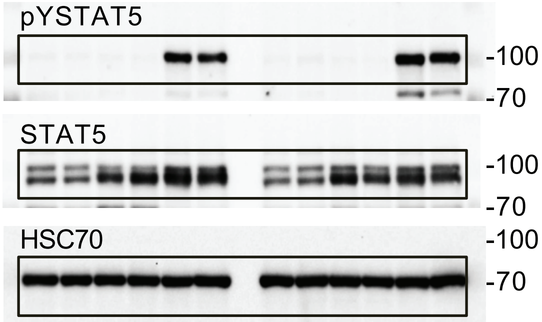
Full unedited blots for figure 1B



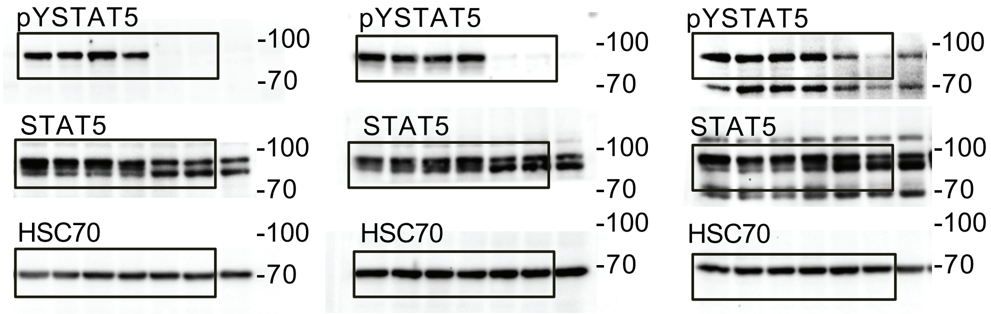
Full unedited blots for figure 1C



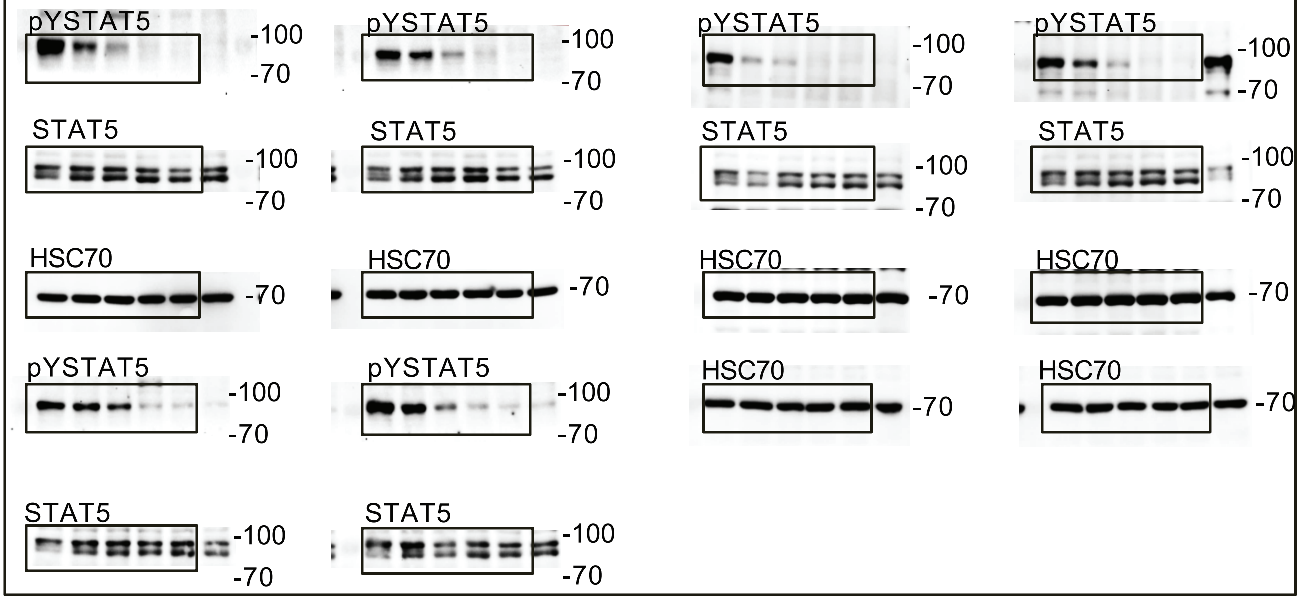
Full unedited blots for figure 2B



Full unedited blots for figure 6A

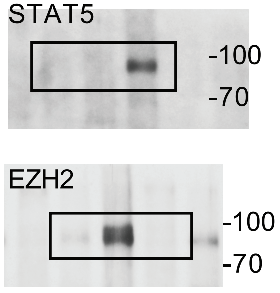


Full unedited blots for figure 6C

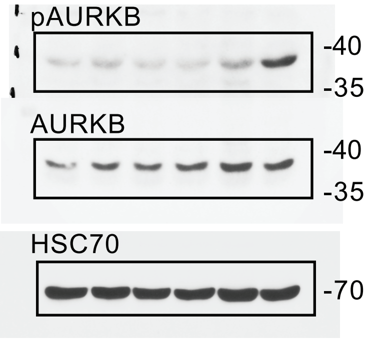


Supplement Figure 12

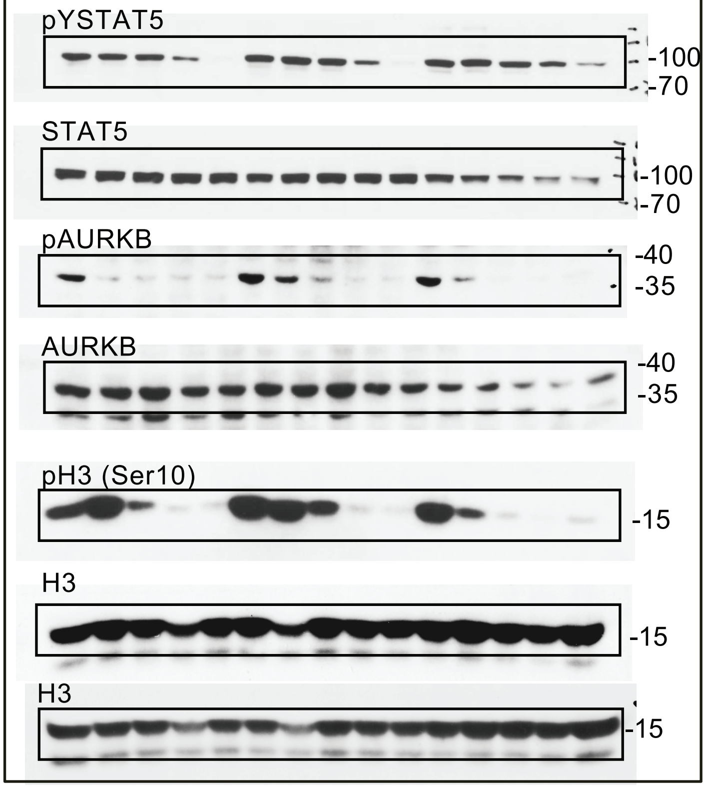
Full unedited blot for figure 8E



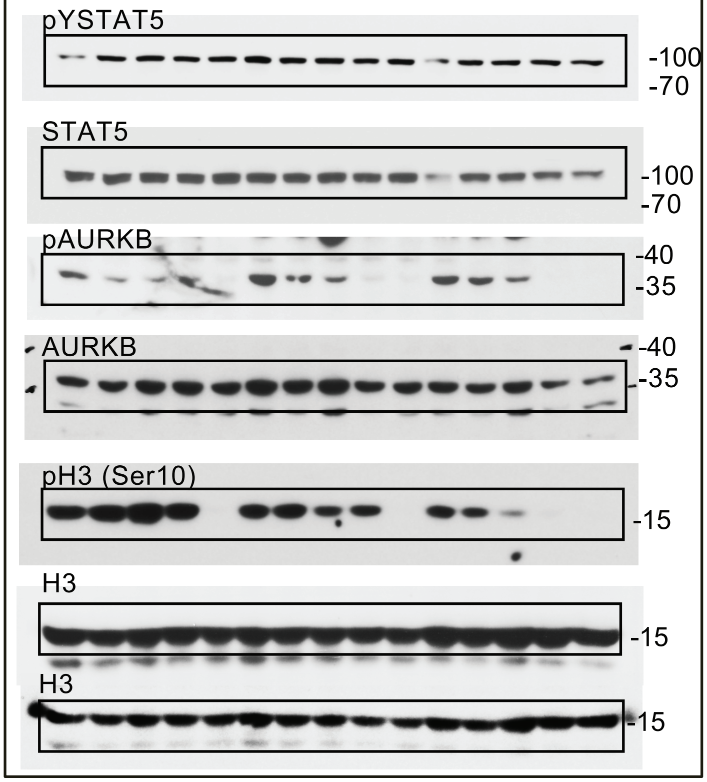
Full unedited blot for figure 9A



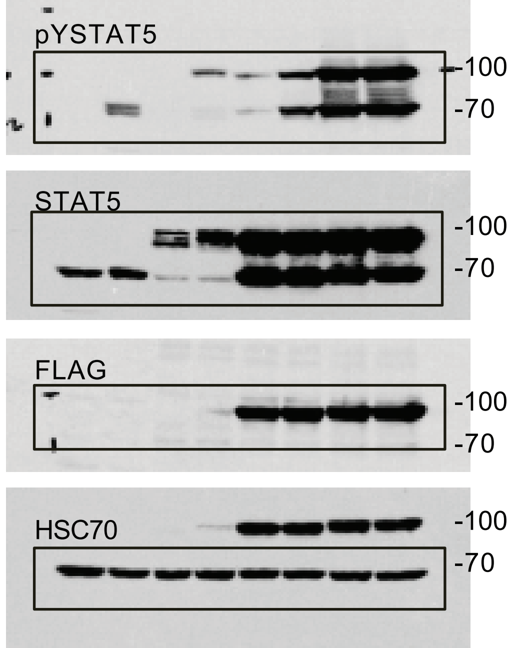
Full unedited blot for figure 9C



Full unedited blot for figure 9D



Full unedited blot for supplemental figure 1A



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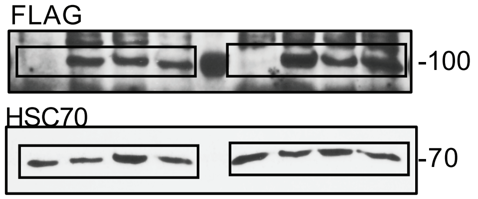

Supplement Figure 12