Lysine acetyltransferase Tip60 is required for hematopoietic stem cell maintenance

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Supplemental information

Supplemental materials and methods

Mice

Tip60^{f} mice are generated by inserting *LoxP* sites flanking introns 2 and 11 of the mouse *Tip60* gene. Cre-recombinase-mediated excision was designed to remove exons 3 to 11, which includes the chromo-finger, zinc finger, and acetyl Co-A binding domains. Embryonic stem cell clones with correct homologous recombination were injected into *C57/Bl6* blastocysts, which transmitted the targeted allele via germline following implantation. Germline chimeras were analyzed by Southern blotting using Probe (491 bp), which was generated by PCR with the following primers: Probe F 5'- CTG CCC TCA CCC CAA ACC TAC CAT C -3' and Probe R 5' AGG AAG CGG CAG ACG GGA AAC AGC G -3' (supplemental Figures 1A and 1B). The neomycin resistance cassette flanked by FRT recombination sites were removed by crossing with a mouse expressing a *Flp recombinase* transgene. The details were described before [1]. *Tip60$^{f/+}$* mice were crossed to *Mx1-Cre*, *Vav-iCre* [2] and *Rosa26-CreERT2* strain for further experiments. *Mx1-Cre*; *Tip60$^{f/f}$* mice and *Vav-iCre*; *Tip60$^{f/+}$* were crossed to *Rosa26-stop-tdTomato* reporter mice (*Rosa-tdTomato*) to measure reporter activity which is reflective of *Tip60* deletion efficiency. CD45.1^{+} congenic mice (*B6.SJL*) were crossed to C57BL/6 mice to generate CD45.1^{+}CD45.2^{+} congenic mice. *B6.SJL*, *Rosa26-creERT2* and *Rosa-tdTomato* mice were purchased from Jackson Labs. Experiments were performed on 8-12 weeks old mice. All mice were housed in a sterile barrier facility within the Comparative Medicine facility at the National University of Singapore. All mice experiments performed in this study were approved by Institutional Animal Care and Use Committee.

PCR

Tail DNA from embryos or mice were used for PCR with the following primer: For genotyping: gTip60-F 5'- TAC AAT GTG GCC TGC ATC TTG ACT-3' and gTip60-R 5'- TCC TTT ATT TCT GGC GGC TCT CC -3', which amplify a 188 bp *Tip60* allele band and a 274 bp *Tip60^{f}* allele band. The *Mx1-Cre*, *Vav-iCre*, *Rosa26-CreERT2* and *Rosa-tdTomato* mice genotyping protocols are available from Jackson Labs. To measure *Tip60* genome abundance in genomic DNA, cells were sorted into lysis buffer and DNA was extracted using QIAamp DNA Micro Kit (Qiagen). The *Tip60^{f}* allele specifically, Tip60flox-F 5'- TCA ACG ATC GCA CGG GAG G -3' and Tip60flox-R 5'- GAT CAT CTC CGT ACA ACT CGA G -3' were used. For reference, *36b4* gene was amplified using 36b4-F 5'- ACT GGT CTA GGA CCC GAG AAG -3' and 36b4-R 5'- TCA ATG GTG CCT CTG GAG ATT -3'. Real time PCR was performed using SYBR green detection reagent on a Sequence Detection System 7700 (Applied Biosystems). The abundance of *Tip60* gene normalized to *36b4* gene was calculated using the *ΔΔ*CT method. To measure Tip60 mRNA, RNA was extracted using RNeasy kit (QIAGEN), reverse transcribed using the QuantiTech Reverse Transcription kit (QIAGEN), and quantitatively assessed using an ABI7500 (Applied Biosystem). For each sample, transcript levels of tested genes were normalized to β-actin using the delta CT method. The highest expression was arbitrarily set to 1 and expressions in the other samples were normalized to this value. All experiments were performed with three technical replicates. qRT-PCR was performed on cDNA using following primers: Tip60-F: 5'- GCC TGG ACG GAA GCG GAA ATC TAA T -3', Tip60-R: 5'- AAA CAC TTG GCC AGA AGA CAC AG -3', β-actin-F: 5'- GGT CCA CAC CCG CCA CCA G -3', β-actin-R: 5'- TTG CTC TGG GCC TCG TCA CC -3'. For ChIP-qPCR, PCR was carried out with GoTaq qPCR mastermix (Promega) and analysed with a Corbett Rotagene system. Relative enrichment is calculated over the intergenic control. The primers used are as follows: Ncl promo-ChIP-F: 5'-CCC AAC

TGC TTC CCA CTT TCT C-3', Ncl promo-ChIP-R: 5'-GTG GGG GAG TGT CTG TAG TAC C-3', Rps9 promo-ChIP-F: 5'-CGG GAA GAG TTA GGT AGC CAT G-3', Rps9 promo-ChIP-R: 5'-CTT GAC CCT CCA AAC CTC ACG-3', NC (control intergenic region) -ChIP-F: 5'-CCC TCA CAC AGC TGA AAT CGC-3', NC-ChIP-R: 5'-CCT GGC ACT GCA GAT GTA CTC-3'.

Apoptosis assay

Cells stained for cell-surface markers were incubated with Annexin-V FITC antibody (BD Biosciences) according to the manufacturer's instructions. After washing, the cells were stained with DAPI (Invitrogen) and analyzed by LSRII flow cytometer.

Cell-Cycle and DNA content analysis

Cells stained for cell-surface markers were fixed and permeabilized using fixing and permeabilization buffers (BD Biosciences), and stained with Ki-67-FITC antibody (BD Pharmingen) according to the manufacturer's instructions. After washing, the cells were incubated with DAPI (Invitrogen) or Hoechst 33258 (Invitrogen), and RNaseA, and then analyzed by LSRII flow cytometer.

Cell lines

BOSC23 (ATCC, CRL11270) and HEK293T (ATCC, CRL3216) cells were maintained in DMEM (Biowest) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biowest). MCF10A (ATCC, CRL10317) cells were cultured in DMEM/F12 medium supplemented with 5% horse serum and epithelial growth factor (EGF) (20 ng/ml), hydrocortisone (0.5 mg/ml), cholera toxin (100 ng/ml), insulin (10 μg/ml). HPC-7 cells [3] were grown in SCF, and 32D (DSMZ, AC441) cells were grown in murine IL-3 (10 ng/ml).

Flow cytometry

Single-cell suspensions were analyzed by flow cytometry. Antibodies conjugated with phycoerythrin (PE), PE-CY7, fluorescein isothiocyanate (FITC), allophycocyanin (APC), APC-CY7, and Pacific Blue obtained from BD Pharmingen, BioLegend, and eBioscience were used. Dead cells were stained with PI, DAPI, or Fixable Viability Dye (eBioscience). Stained cells were analyzed with an LSRII flow cytometer (BD Biosciences) or sorted using a FACS Aria (BD Biosciences). Flow Jo 7.5 (Tree Star) was used for data acquisition and analysis.

Antigen	Specificity	Cat no.	Company	Clone	Dilution	Fluorochrome
CD11b (Mac1)	mouse	17-0112-82	eBioscience	M1/70	1:200	APC
CD11b (Mac1)	mouse	25-0112-82	eBioscience	M1/70	1:200	PE-CY7
Gr-1/Ly6G	mouse	17-5931-81	eBioscience	RB6-8C5	1:200	APC
Gr-1/Ly6G	mouse	25-5931-82	eBioscience	RB6-8C5	1:200	PE-CY7
CD3e	mouse	25-0031-82	eBioscience	145-2C11	1:200	PE-CY7
CD4	mouse	25-0041-82	eBioscience	GK1.5	1:200	PE-CY7
CD8a	mouse	25-0081-82	eBioscience	53-6.7	1:200	PE-CY7
B220/CD45R	mouse	553092	BD Pharmingen	RA3-6B2	1:100	APC

B220/CD45R	mouse	25-0452-82	eBioscience	RA3-6B2	1:100	PE-CY7
CD19	mouse	25-0193-82	eBioscience	1D3	1:100	PE-CY7
Ter119	mouse	25-5921-82	eBioscience	TER-119	1:100	PE-CY7
NK1.1	mouse	25-5941-82	eBioscience	145-2C11	1:200	PE-CY7
CD117/c-kit	mouse	553356	BD Pharmingen	2B8	1:100	APC
Sca-1/Ly-6A/E	mouse	560654	BD Pharmingen	D7	1:100	APC-CY7
CD150 (SLAM)	mouse	115904	Biolegend	TC15-12F12.2	1:100	PE
CD48	mouse	11-0481-82	eBioscience	HM48-1	1:100	FITC
CD48	mouse	25-0481-80	eBioscience	HM48-1	1:100	PE-CY7
CD34	mouse	553733	BD Pharmingen	RAM34	1:50	FITC
CD16/32	mouse	553145	BD Pharmingen	2.4G2	1:100	PE
CD127/IL7Ra	mouse	11-1271-85	eBioscience	A7R34	1:100	FITC
CD135/Flt3	mouse	12-1351-81	eBioscience	A2F10	1:100	PE
CD45.1	mouse	110721	Biolegend	A20	1:200	Pacific Blue
CD45.2	mouse	109805	Biolegend	104	1:200	FITC

Chimerism analysis

Cells from peripheral blood were stained with anti-CD45.1 and CD45.2 antibodies after RBC lysis to distinguish donor-derived cells from the host cells, as well as lineage-specific antibodies Mac1, Gr1, B220, CD19, CD4 and CD8 to identify myeloid, B, and T lineages.

Homing assay

Lineage negative cells were purified from *Tip60*$^{f/f}$ and *Tip60*$^{f/f}$; *Mx1-Cre* mice 5 days after the first pIpC injection (300μg for 3 consecutive days) and were labeled with CellTrace™ CFSE (Invitrogen). 1×10^6 cells were injected to lethally irradiated congenic mice and the number of transplanted $CD150^+CD48^-$LSK cells present in the BM was determined at 16 hours post-injection.

Co-immunoprecipitation

For 32D, 1 x 10^7 cells were washed once with ice-cold PBS and lysed in M-PER™ mammalian protein extraction reagent (Thermo Scientific) supplemented with 150 mM NaCl, 1 mM PMSF and EDTA-free cOmplete™ protease inhibitor cocktail (Roche) 48h after transduction. The lysate was incubated at 4°C with constant rotation for 10 minutes and followed by centrifugation at 14,000 x *g* for 10 min at 4°C. Supernatant was collected and protein concentration was quantified by Bradford assay (Biorad). For co-immunoprecipitation of FLAG-TIP60, 30 µl of anti-FLAG M2 magnetic beads (Sigma) was added to the sample and incubated at 4°C overnight with constant rotation. For co-immunoprecipitation of c-myc, 3 µg c-Myc antibody (Cell Signaling Technology, CST5605) was incubated with 30 µl protein A Dynabeads (Invitrogen) at 4°C with constant rotation for 4 hours, then beads-bound antibody was added to the sample and incubated at 4°C overnight with constant rotation. On the next day, beads-bound immunoprecipitates were washed twice with buffer WG (50 mM Tris, pH 8.0, 300 mM NaCl, 10 mM NaF, 1 mM EDTA, 1% Triton-X100, 0.2% Sarkosyl, 10% glycerol) supplemented with protease

inhibitor, and twice with buffer D (20 mM Tris, pH 8.0, 300 mM NaCl, 0.5 mM EDTA, 10% glycerol, 0.5% NP-40) supplemented with protease inhibitor. For MCF10A, 2 x 10^7 cells were lysed lysed in M-PER™ mammalian protein extraction reagent (Thermo Scientific) supplemented with 1 mM PMSF and EDTA-free cOmplete™ protease inhibitor cocktail (Roche) 48h after transduction. Protein concentration was quantified, and 2 mg lysates were used for co-immunoprecipitation of FLAG-tagged Tip60. The antibody-lysate mixture was incubated at 4°C overnight with constant rotation and then washed thrice in wash buffer (20 mM Tris, pH 8.0, 300 mM NaCl, 0.5 mM EDTA, 10% glycerol, 0.5% NP-40). Proteins were eluted from the beads with Laemmli sample buffer and analyzed with Western blotting or mass spectrometry.

Western blotting

1×10^6 *Tip60$^{f/f}$* and *Tip60$^{\Delta/\Delta}$* FACS-sorted CD45$^+$ cells from fetal liver (E14.5), LSK cells from adult bone marrow, or 32D cells 48h after transduction were lysed in 100 µl of 1×Laemmli sample buffer and boiled at 95°C for 10 min. Proteins were separated on 10% SDS-PAGE gels. Immunoblots were incubated with primary antibody overnight at 4°C, followed by a secondary horseradish peroxidase (HRP)-conjugated antibody for 1h at room temperature. The antibodies used are caspase-3 (Cell Signaling Technology, CST9662), cleaved caspase-3 (Cell Signaling Technology, CST9661), FLAG M2 (Sigma, A8592), p400 (Santa Cruz, sc-66497), RVB1 and RVB2 [5], c-Myc (Cell Signaling Technology, CST5605), TIP60 (K-17) (Santa Cruz, sc-5727), TIP60 (N-17) (Santa Cruz, sc-5725), acetyl H2A.Z (abcam, ab18262), H2A.Z (abcam, ab4174), acH4K16 (MERCK, 07-329), histone 4 (H4) (Cell Signaling Technology, CST2592), acetyl-histone 3 (ac-H3) (Active Motif, 39139), histone 3, (H3) (Cell Signaling Technology, CST9715), and β-actin (C4) (Santa Cruz, sc-47778).

Chromatin immunoprecipitation

Briefly, cells were fixed with 1% formaldehyde and then neutralized with glycine at a final concentration of 0.125M. Fixed chromatin were fragmented using a Bioruptor sonicator (Diagenode) at high power in a constantly circulating 4°C water bath to an average size of 500 base pairs. Sonicated chromatin was incubated with antibody overnight at 4°C. The antibodies used are Tip60 [6], H2A.Z (abcam, ab4174), acH2A.Z (abcam, ab18262), H3K27ac (abcam, ab4729), and H3K27me3 (CST, 9733BC). Protein A and Protein G Dynabeads (Life Technologies) were added to the mixture and incubated for 2 hours at 4°C. Antibody:chromatin complexes were collected with a magnet and washed two times with a solution of 50 mM HEPES-KOH, pH 7.6, 500 mM LiCl, 1 mM EDTA, 1% NP-40 and 0.7% Na-Deoyxcholate. After a TE wash, samples were eluted, RNase and proteinase K treated and purified using a QIAGEN MinElute PCR purification kit. Pull-down DNA (2-10 ng) was used for library construction using ThruPLEX kits (Rubicon Genomics).

RNA/ChIP-sequencing

RNA was isolated using RNeasy mini columns (Qiagen). RNA-seq libraries were prepared using Illumina Tru-Seq Stranded Total RNA with Ribo-Zero Gold kit protocol, according to the manufacturer's instructions (Illumina, San Diego, California, USA). Libraries were validated with an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), diluted and applied to an Illumina flow cell using the Illumina Cluster Station. ChIP-seq libraries were prepared using ThruPLEX kits (Rubicon

Genomics). Each library was sequenced on an Illumina Hiseq2000 sequencer at Duke-NUS Genome Biology Facility and Beijing Genomics Institute.

RNA-seq data analysis

Raw reads were mapped to mouse genome assembly GRCm38 (mm10) with UCSC gene annotation using STAR. Raw gene expression was obtained with the FeatureCounts, normalization was performed and volcano plots were generated using the DESeq2. For identification of enriched gene sets or pathways, the mouse gene symbols converted to human equivalent homolog genes based on the annotation from Ensemble. Gene Set Enrichment Analysis (GSEA) was performed with the converted gene expression profiling information using the tool for preranked gene list (http://www.broad.mit.edu/gsea/).

ChIP-seq data analysis

ChIP-seq reads for both pulldown and input were first aligned using the Burrows-Wheeler algorithm (BWA) to mm10. The mapped files were normalized to each other to extract bigwig files using BEDtools. The aligned ChIP-seq bam files were converted into tag directory using MakeTagDirectory function in HOMER, and subsequently ChIP-seq signal was calculated on proximal promoter region of each gene using annotatePeaks.pl function in HOMER with -hist -ghist setting. Promoter region was defined as from -1 kb to +100 bp from transcription start site (TSS). Active promoter was identified as promoter region overlapping H3K27ac peak. Distal Tip60-bound regions were defined as Tip60 peaks annotated as "intergenic" which is the category that encompasses the peaks that do not fall into the TSS, TTS, exons (CDS), 5'UTR, 3'UTR, CpG islands, repeats, or introns categories using HOMER. acH2A.Z/H2A.Z ratio was calculated based on the output in R program. ChIP-seq heatmap was generated from the aligned bam files using ngs.plot. The cloud plots and scatter plot were generated using R package.

Comet assay

DNA damage was measured using the CometAssay Kit (Trevigen). *Tip60*$^{f/+}$, *Tip60*$^{f/f}$, or *Tip60*$^{\Delta/\Delta}$ LSK cells from fetal liver or CD150^{+}CD48^{-}LSK cells from adult bone marrow were purified and suspended in PBS at a concentration of 1×10^{5} cells/ml. 50 µl of cell suspension was mixed with 450 µl of molten Comet LM agarose and spread onto coated microscope slides. After the agarose solidified, cells were lysed in Lysis Solution for 1 hour at 4°C. Slides were then incubated in Alkaline Unwinding Solution, pH > 13 for 1 hour at 4°C. Electrophoresis was carried out with pre-chilled Alkaline Electrophoresis Solution at 21 V for 25 min. Comets were visualized using 1×SYBR Gold under epifluorescence microscopy examination (Nikon). Images were analyzed using NIH Image J program. DNA damage was determined by the tail moment and quantified for at least 60 cells from each experimental group. The tail moment is defined as the product of the tail length and the tail DNA percentage of the total DNA. Gamma-irradiated mouse whole bone marrow cells (300 rads) were used as positive control.

Immunofluorescence staining

CD150^{+}CD48^{-}LSK cells were sorted onto Poly-L-Lysine-coated glass slides (Matsunami Glass Industry) by FACS Aria, fixed, and permeabilized. Immunofluorescence staining was performed using γ-H2AX (Millipore, JBW301) antibody, and

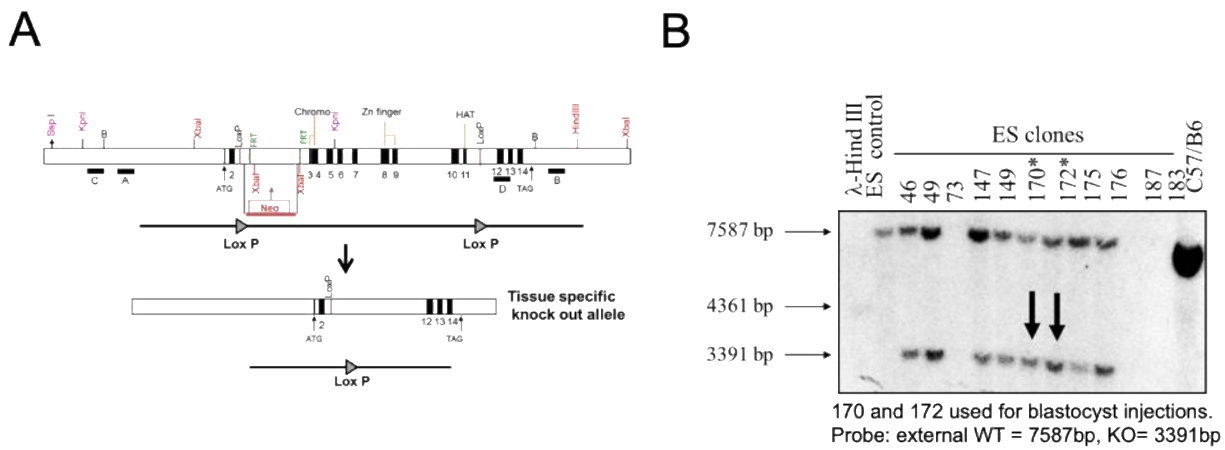
counterstained with DAPI to detect nuclei. Images were acquired on Zeiss LSM510 Meta Upright Confocal Microscopy (× 63 objectives). An average of 40 cells were scored from three independent experiments (n=3).

Mass spectrometry analysis

Quadruplicate IP samples for each condition were separated on a 12% NuPAGE Bis-Tris precast gel (Thermo Fisher Scientific) for 310 min at 170 V in MOPS buffer. The gel was fixed using the Colloidal Blue Staining Kit (Thermo Fisher Scientific) and each sample as a single piece. For in-gel digestion prior to MS analysis, samples were destained in destaining buffer (25 mM ammonium bicarbonate, 50% ethanol) and reduced in 10 mM DTT for 1 hour at 56 °C followed by alkylation with 55 mM iodoacetamide (Sigma) for 45 min in the dark. Tryptic digest was performed in 50 mM ammonium bicarbonate buffer with 2 µg trypsin (Promega) at 37 °C overnight. Peptides were desalted on StageTips and analysed by nanoflow liquid chromatography on an EASY-nLC 1200 system coupled to a Q Exactive HF Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). Peptides were separated on a C18-reversed phase column (25 cm long, 75 µm inner diameter) packed in-house with ReproSil-Pur C18-QAQ 1.9 µm resin (Dr Maisch). The column was mounted on an Easy Flex Nano Source and temperature controlled by a column oven (Sonation) at 40 °C. A 105-min gradient from 2 to 40% acetonitrile in 0.5% formic acid at a flow of 225 nl/min was used. Spray voltage was set to 2.2 kV. The Q Exactive HF was operated with a TOP20 MS/MS spectra acquisition method per MS full scan. MS scans were conducted with 60,000 at a maximum injection time of 20 ms and MS/MS scans with 15,000 resolution at a maximum injection time of 50 ms. The raw MS files were processed with MaxQuant version 1.5.2.8 using the LFQ quantitation option on unique peptides with at least 2 ratio counts with the ‘match between runs’ option activated. Quantification providing LFQ intensities was performed on unique peptides only. Carbamidomethylation was set as fixed modification while methionine oxidation and protein N-acetylation were considered as variable modifications. Search results were filtered with a false discovery rate of 0.01. The resulting protein groups table was further processed with an in-house script to exclude contaminants (based on the MaxQuant contaminant list), reverse hits and proteins that were only identified by a modified peptide. Missing values were imputed based on normally distributed values corresponding to the percentage of missing values in each sample for proteins that were quantified in at least 3 out of 4 replicates in at least one biological condition.

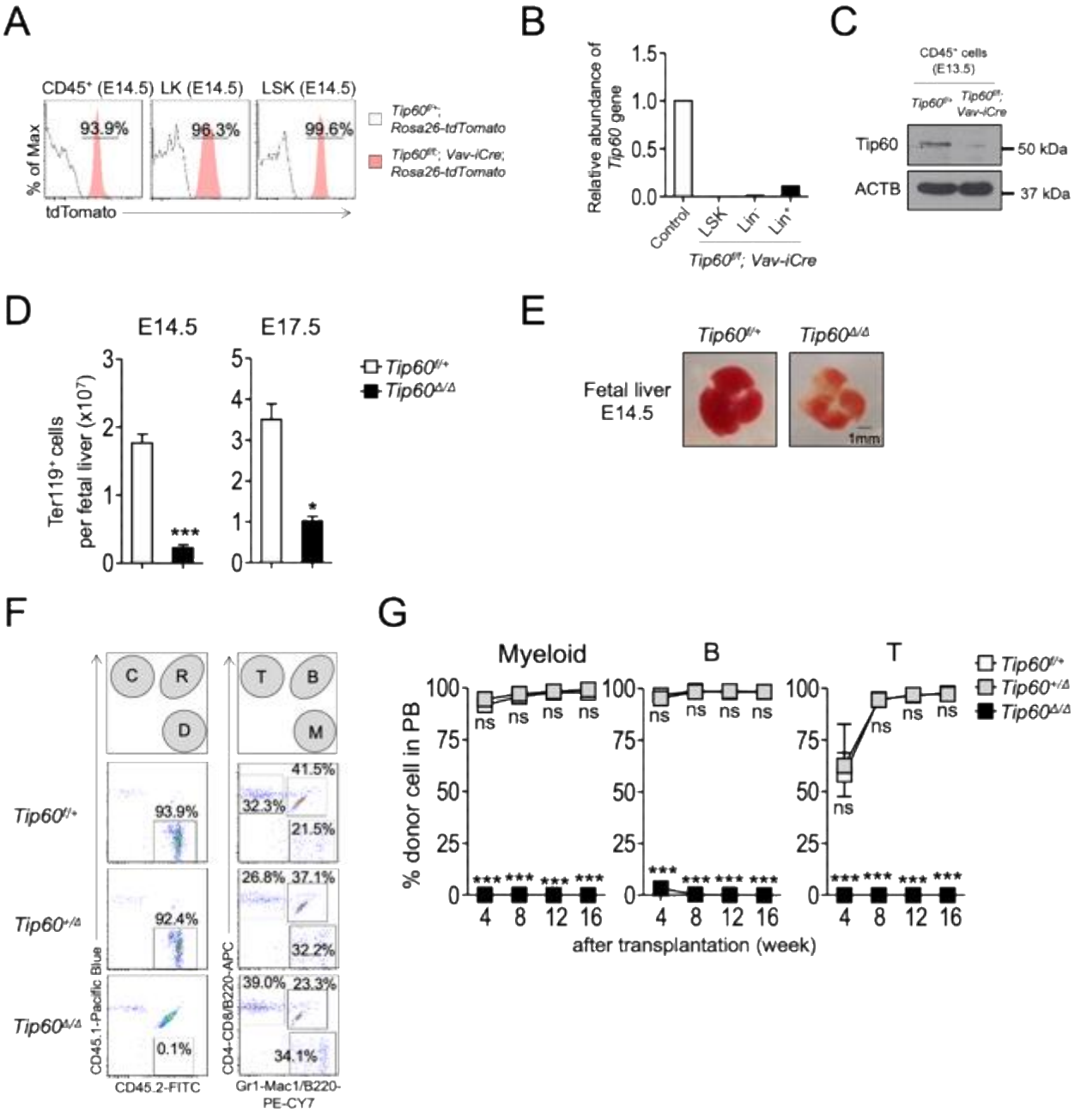
Statistical analysis

The statistical significances were assessed by Student’s t-test using the GraphPad software unless otherwise specified.



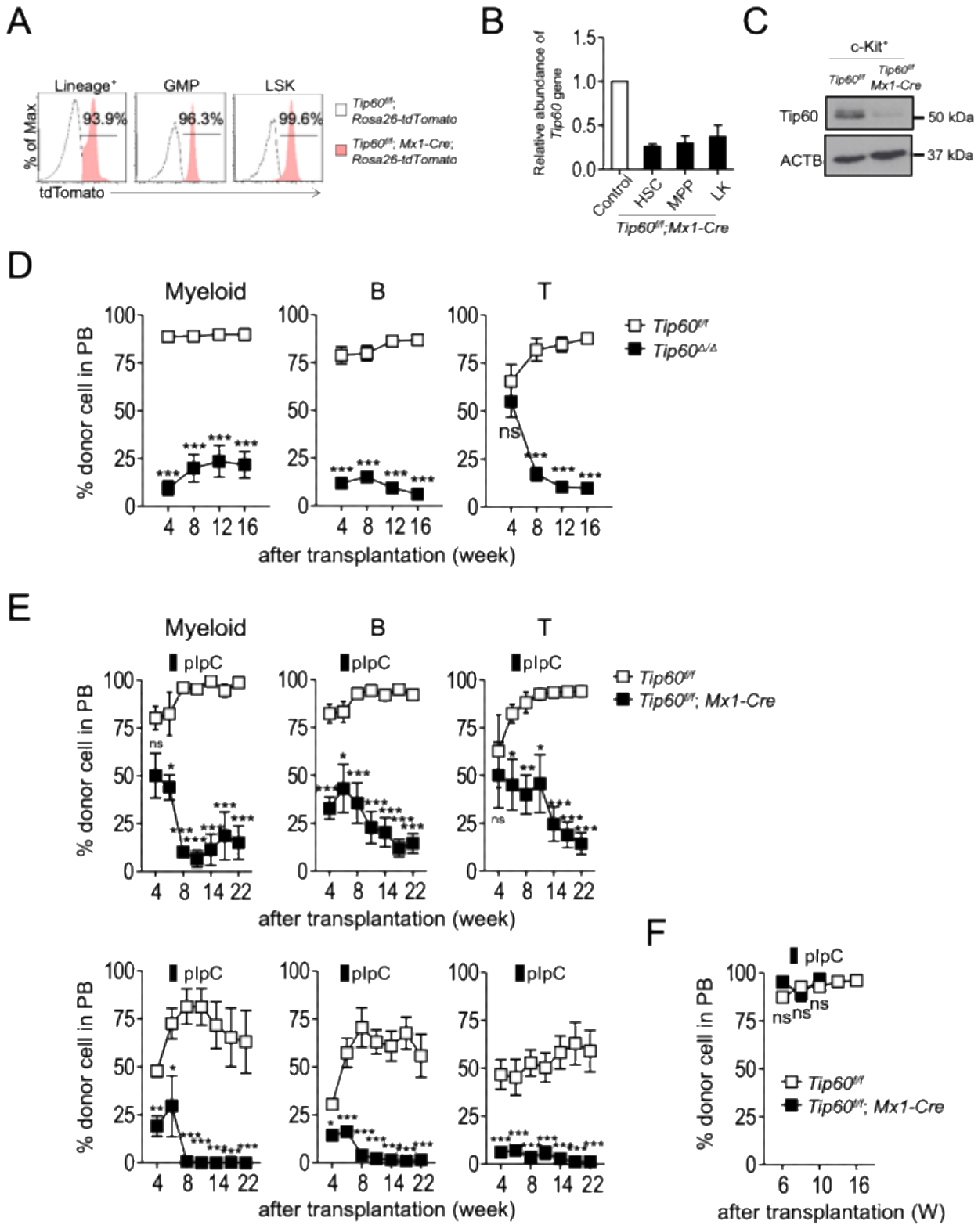
Supplemental Figure 1 | Generation of *Tip60* conditional knockout mice.

A, Cre-recombinase-mediated excision was designed to remove exons 3–11, including the chromo-finger, zinc finger, and acetyl-CoA binding domains, by recombineering wherein LoxP sites were inserted into introns 2 and 11 of *Tip60*. **B,** Correctly targeted embryonic stem cells were injected into C57/Bl6 blastocysts, which, after implantation, transmitted the targeted allele via germline. Correct targeting was verified by external probe. *Hind*III and *Pac*I double digestion resulted in 7587 bp for wild type allele and 3391 bp for correctly targeted allele. Following verification of targeting via Southern blotting and removal of the neomycin-resistance gene, mice were bred to the genotypes that were used in the experiments.


A
CD45+ (E14.5)
LK (E14.5)
LSK (E14.5)
93.9%
96.3%
99.6%
% of Max
tdTomato
Tip60f/+; Rosa26-tdTomato
Tip60f/f; Vav-iCre; Rosa26-tdTomato
B
Relative abundance of Tip60 gene
Control
LSK
Lin-
Lin+
Tip60f/f; Vav-iCre
C
CD45+ cells (E13.5)
Tip60f/+
Tip60f/f; Vav-iCre
Tip60
ACTB
50 kDa
37 kDa
D
E14.5
E17.5
Ter119+ cells per fetal liver (x10^7)
Tip60f/+
Tip60Δ/Δ
E
Tip60f/+
Tip60Δ/Δ
Fetal liver E14.5
1mm
F
C
R
D
T
B
M
Tip60f/+
Tip60+/Δ
Tip60Δ/Δ
93.9%
92.4%
0.1%
41.5%
32.3%
21.5%
26.8%
37.1%
32.2%
39.0%
23.3%
34.1%
CD45.1-Pacific Blue
CD45.2-FITC
CD4-CD8/B220-APC
Gr1-Mac1/B220-PE-CY7
G
Myeloid
B
T
Tip60f/+
Tip60+/Δ
Tip60Δ/Δ
% donor cell in PB
ns
***
after transplantation (week)

Supplemental Figure 2 | *Tip60* loss leads to fetal hematopoietic failure.

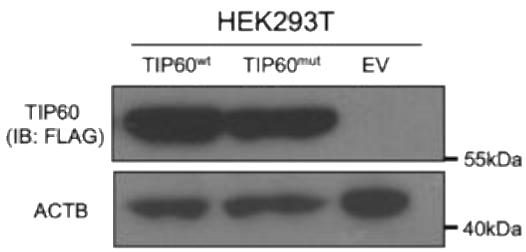
A, Expression of Tomato in $CD45^+$, LK ($Lin^-c\text{-}Kit^+Sca\text{-}1^-$), and LSK ($Lin^-c\text{-}Kit^+Sca\text{-}1^+$) cells from fetal liver of *Tip60*$^{f/+}$; *Rosa26-tdTomato* and *Tip60*$^{f/f}$; *Vav-iCre*; *Rosa26-tdTomato* embryo at E14.5. **B,** The efficiency of *Tip60* gene ablation was analyzed by quantitative genomic PCR in fetal LSK, Lin^-, and Lin^+ cells. The abundance of Tip60 gene normalized to 36B4 gene was calculated using the $\Delta\Delta$CT method. Relative values of *Tip60*$^{f/f}$; *Vav-iCre* to control (*Tip60*$^{f/f}$ whole bone marrow cells) are shown (n=4). **C**, Tip60 protein levels of $CD45^+$ cells of *Tip60*$^{f/f}$ and *Tip60*$^{f/f}$; *Vav-iCre* embryos were analyzed by Western blotting. **D,** Absolute numbers of mature erythroid ($Ter119^+$) per fetal liver from control (*Tip60*$^{f/+}$) and *Tip60*$^{\Delta/\Delta}$ (*Tip60*$^{f/f}$; *Vav-iCre*) embryos at E14.5 and E17.5 are shown. **E,** Representative images of control and *Tip60*$^{\Delta/\Delta}$ fetal liver at E14.5. **F,** 1×10^6 whole fetal liver cells from control, *Tip60*$^{+/\Delta}$ (*Tip60*$^{f/+}$; *Vav-iCre*), and *Tip60*$^{\Delta/\Delta}$ embryos at E14.5 were transplanted into lethally irradiated congenic mice ($CD45.1^+$) along with 1×10^5 congenic whole bone marrow (WBM) cells ($CD45.1^+CD45.2^+$). Top, cartoons showing a chimerism analysis of the recipients' peripheral blood (PB) by flow cytometry. When staining for CD45.1 and CD45.2, CD45.1 and CD45.2 double positive cells are recipient-derived cells (R). CD45.2 only positive cells are donor-derived cells (D), while CD45.1 only positive cells are competitor-derived cells (C). When staining for B220/CD4-CD8 and Mac1-Gr1/B220, the double positive population is $B220^+$ B lineage cells (B). CD4-CD8 only positive are T lineage cells (T), while Mac1-Gr1 only positive cells are from the myeloid lineage (M). Bottom, representative flow cytometry dot plots for identification of donor cell, myeloid, B and T cell of the recipients' PB of *Tip60*$^{f/+}$, *Tip60*$^{+/\Delta}$, and *Tip60*$^{\Delta/\Delta}$ 16 weeks after transplantation. Percentages of the gated populations are shown. **G,** Donor chimerism in each lineage cells of the recipients' PB. The values are presented as mean ± SEM. Statistical analyses were performed against *Tip60*$^{f/+}$. *$p < 0.05$, ***$p < 0.001$.



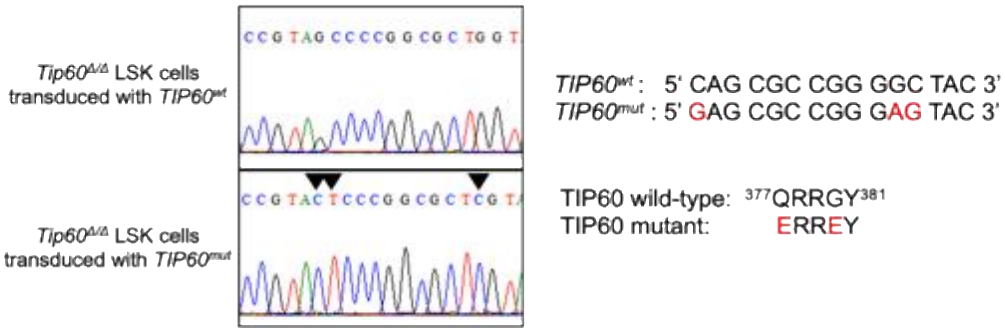
Supplemental Figure 3 | Tip60 is required for adult HSC maintenance in a cell-intrinsic manner.

A, Expression of Tomato in Lineage$^+$, GMP, and LSK cells from of bone marrow (BM) of *Tip60$^{f/f}$*; *Rosa26-tdTomato* and *Tip60$^{f/f}$*; *Mx1-Cre*; *Rosa26-tdTomato* mice. The mice were injected with pIpC for 3 consecutive days and analyzed 5 days after the first injection. **B,** Efficiency of *Tip60* ablation was assessed by quantitative genomic PCR in BM HSC ($CD150^+CD48^-$LSK), MPP ($CD150^-CD48^+$LSK), and LK (Lin^-c-$Kit^+Sca\text{-}1^-$) cells 5 days after the first pIpC injection. The abundance of Tip60 gene normalized to 36B4 gene was calculated using the *ΔΔ*CT method. Relative values of *Tip60 $^{f/f}$*; *Mx1-Cre* to control (*Tip60$^{f/f}$* WBM cells) are shown (n=4). **C,** Tip60 protein levels of BM c-Kit$^+$ cells purified from *Tip60$^{f/f}$* and *Tip60$^{f/f}$*; *Mx1-Cre* mice 5 days after the last of the pIpC injections for three consecutive days were analyzed by Western blotting. **D,** 1×10^6 WBM cells from control (*Tip60$^{f/f}$*) and *Tip60$^{Δ/Δ}$* mice were transplanted into lethally irradiated recipient mice along with 2×10^5 congenic WBM cells. Donor chimerism in myeloid, B and T cells of the recipients' PB are shown. **E,** The upper panels, 1×10^6 WBM cells from *Tip60$^{f/f}$* or *Tip60$^{f/f}$; Mx1-Cre* mice ($CD45.2^+$) were transplanted into lethally irradiated recipient mice ($CD45.1^+$) along with 2×10^5 congenic WBM cells ($CD45.1^+CD45.2^+$). The lower panels, 5×10^5 WBM cells from *Tip60$^{f/f}$* or *Tip60$^{f/f}$; Mx1-Cre* mice ($CD45.2^+$) were transplanted into lethally irradiated recipient mice ($CD45.1^+$) along with 5×10^5 congenic WBM cells ($CD45.1^+CD45.2^+$). pIpC was injected to the recipients 6 weeks after the transplantation. Donor chimerism in each lineage cells of the recipients' PB are shown. **F,** Control (*Tip60$^{f/f}$*) and *Tip60$^{f/f}$*; *Mx1-Cre* mice were irradiated lethally and transplanted with 1×10^6 congenic normal WBM cells. pIpC was injected into the recipients 6 weeks after the transplantation. Donor chimerism of the recipients' PB are shown. The values are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns; not significant.

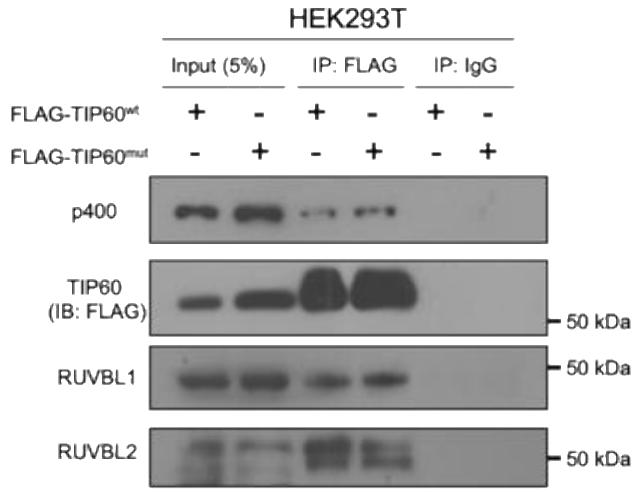
A


HEK293T
TIP60wt
TIP60mut
EV
TIP60
(IB: FLAG)
55kDa
ACTB
40kDa

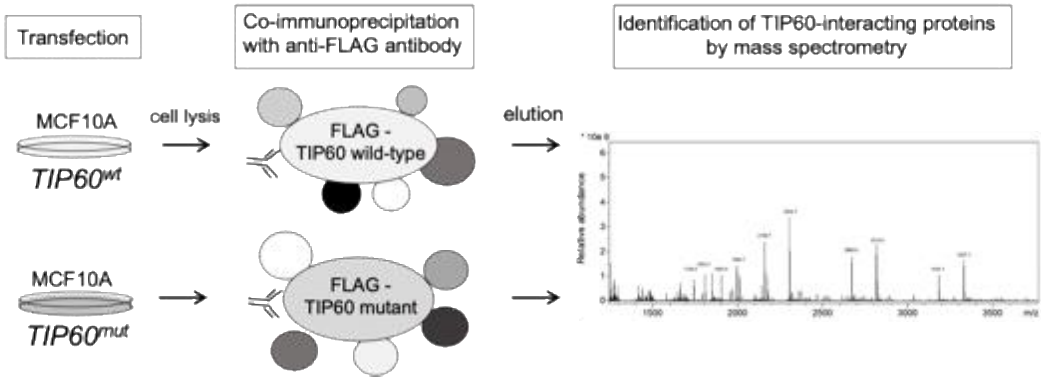
B


Tip60Δ/Δ LSK cells
transduced with TIP60wt
Tip60Δ/Δ LSK cells
transduced with TIP60mut
TIP60wt : 5' CAG CGC CGG GGC TAC 3'
TIP60mut : 5' GAG CGC CGG GAG TAC 3'
TIP60 wild-type: 377QRRGY381
TIP60 mutant: ERREY

C


HEK293T
Input (5%)
IP: FLAG
IP: IgG
FLAG-TIP60wt
FLAG-TIP60mut
p400
TIP60
(IB: FLAG)
50 kDa
RUVBL1
50 kDa
RUVBL2
50 kDa

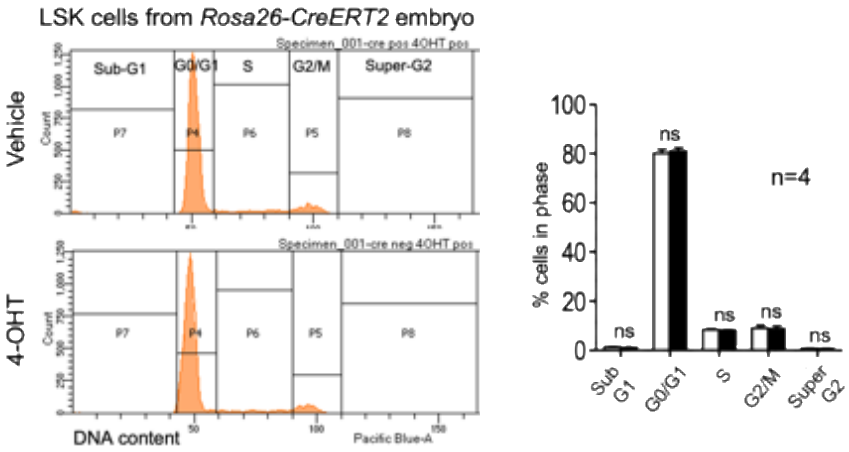
D


Transfection
Co-immunoprecipitation
with anti-FLAG antibody
Identification of TIP60-interacting proteins
by mass spectrometry
MCF10A
TIP60wt
cell lysis
FLAG -
TIP60 wild-type
elution
MCF10A
TIP60mut
FLAG -
TIP60 mutant

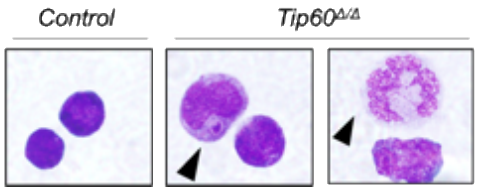
E

Gene names	Protein names	Mol. weight [kDa]
KAT5 (TIP60)	Histone acetyltransferase KAT5 (TIP60)	53.076
ACTR2	Actin-related protein 2	44.76
CECR5	Cat eye syndrome critical region protein 5	43.588
EP400	E1A-binding protein p400	335.8
H2AFV;H2AFZ	Histone H2A.V;Histone H2A.Z;Histone H2A	13.509
HDAC2	Histone deacetylase 2	51.998
HIST1H2B	Histone H2B	18.804
HIST1H4A	Histone H4	11.367
HSPA8	Heat shock cognate 71 kDa protein	70.897
LAMC2	Laminin subunit gamma-2	130.97
LIMA1;TRMT1	LIM domain and actin-binding protein 1	85.225
PPHLN1	Periphilin-1	44.682
RCN2	Reticulocalbin-2	36.876
RPL36A	60S ribosomal protein L36a	12.469
RPL6	60S ribosomal protein L6	32.728
TRIM28	Transcription intermediary factor 1-beta	88.549
TRRAP	Transformation/transcription domain-associated protein	436.13

F



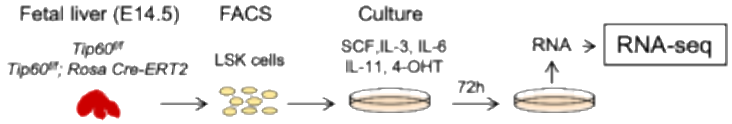
G



Supplemental Figure 4 | Tip60 mutant complex integrity and morphological change after *Tip60* deletion.

A, HEK293T cells were transfected with FLAG-TIP60 wild-type (*TIP60*^{wt}), FLAG-TIP60 mutant (*TIP60*^{mut}), or empty vector (EV) plasmid. Cells were harvested 72 hours after the transfection, whole cell lysates were immunoblotted with anti-FLAG antibody. **B,** Transduction of *TIP60*^{wt} and *TIP60*^{mut} was verified by Sanger sequencing of the PCR-amplified genomic products. Arrows indicate the three point-mutations corresponding to two amino acid substitutions in the acetyl CoA binding domain, resulting in lack of acetyltransferase activity. **C,** HEK293T cell lysates were used for co-immunoprecipitation using FLAG-M2 beads. Proteins present in immunoprecipitates (IP) or cell lysates of transfected cells (input) were separated by SDS-PAGE and immunoblotted with p400, FLAG, RUVBL1, and RUVBL2 antibodies. **D,** MCF10A cells were transduced with *TIP60*^{wt}, *TIP60*^{mut} or EV. 2 mg of whole cell lysates were used for immunoprecipitation (IP) using FLAG-M2 affinity gel overnight at 4°C. Immunoprecipitates were washed and bound proteins were eluted with 1x SDS-PAGE sample loading buffer. The proteins were subjected to mass spectrometry analysis. **E,** List of top common proteins that co-immunoprecipitated with both FLAG-TIP60 wild-type and mutant in MCF10A cells identified by IP-MS studies. **F,** Left, DNA content analysis by DAPI staining of LSK cells purified from *Rosa26-CreERT2* embryos at E14.5, treated with or without 4-hydroxytamoxifen (4-OHT) in culture for 5 days. Right, percentages of cells in each phase are graphed. The values are presented as mean ± SEM. **G,** LSK cells were sorted from fetal liver of *Tip60*$^{f/f}$ and *Rosa26 Cre-ERT2*; *Tip60*$^{f/f}$ embryos (E14.5), cultured with 4-OHT for 7 days, and collected for Giemsa staining. *Tip60*$^{\Delta/\Delta}$ cells grew in size and exhibited abnormal nuclear morphology including multi-lobulated nuclei and micronuclei (arrows).

A



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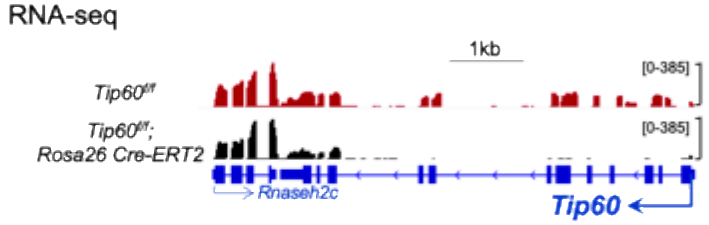

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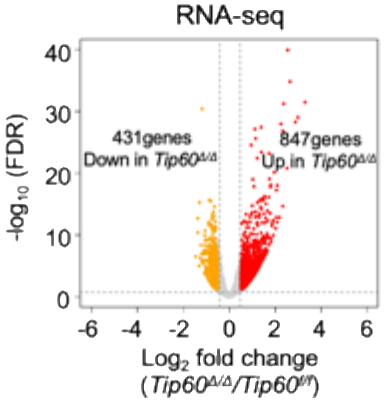


D

Enrichment in *Tip60$^{f/f}$*

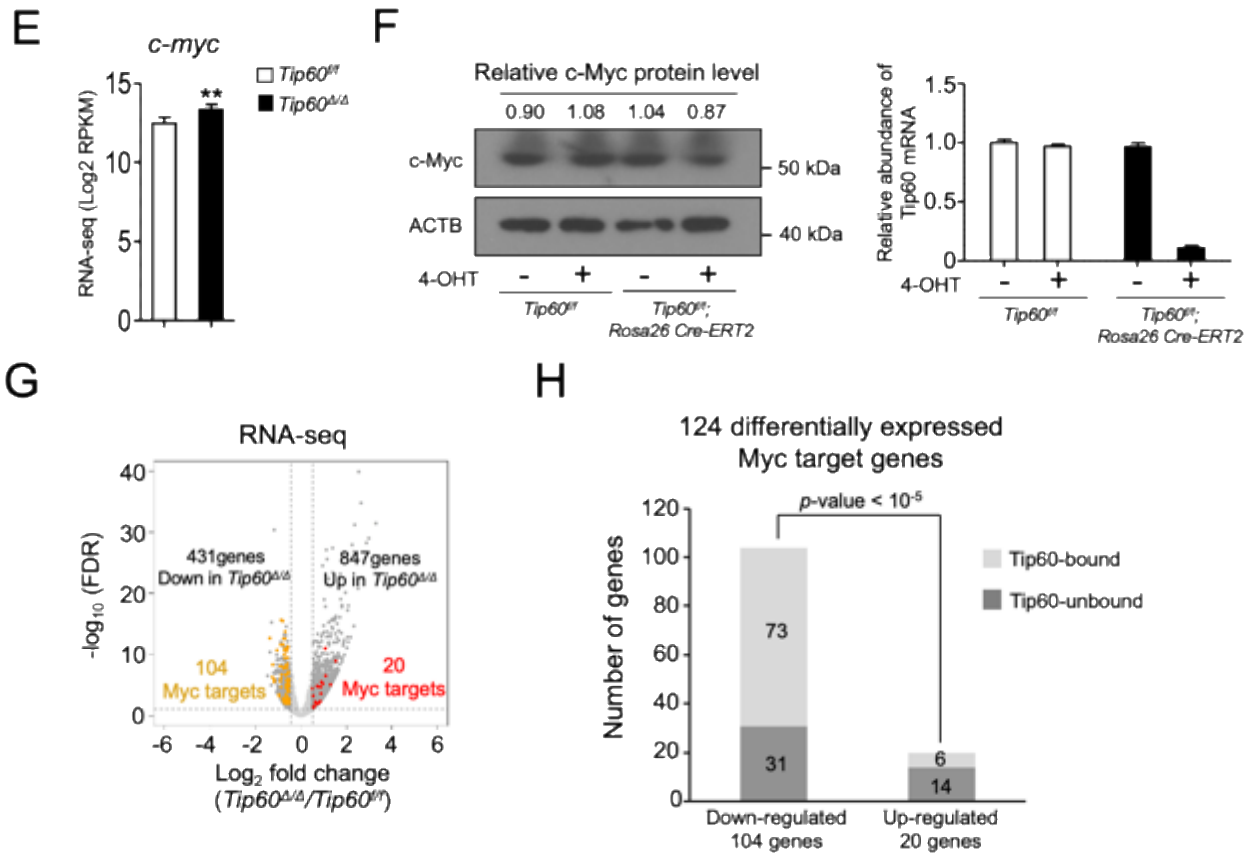
HALLMARK gene set	Size	NES	FDR q-value
MYC_TARGETS_V1	175	3.09	0.000
E2F_TARGETS	172	3.08	0.000
G2M_CHECKPOINT	178	2.79	0.000
MYC_TARGETS_V2	48	2.55	0.000
UNFOLDED_PROTEIN_RESPONSE	103	1.81	0.001
DNA_REPAIR	134	1.70	0.002
OXIDATIVE_PHOSPHORYLATION	183	1.45	0.030
MTORC1_SIGNALING	180	1.37	0.047
HEME_METABOLISM	169	1.28	0.087
FATTY_ACID_METABOLISM	143	1.12	0.241

KEGG gene set	Size	NES	FDR q-value
RIBOSOME	83	3.17	0.000
AMINOACYL_TRNA_BIOSYNTHESIS	32	2.13	0.003
DNA_REPLICATION	33	2.11	0.002
CELL_CYCLE	113	2.05	0.003
MISMATCH_REPAIR	22	1.93	0.005
HOMOLOGOUS_RECOMBINATION	25	1.74	0.002
BASE_EXCISION_REPAIR	33	1.74	0.036
SPLICESOME	93	1.67	0.031
RNA_POLYMERASE	27	1.65	0.050
ONE_CARBON_POOL_BY_FOLATE	17	1.65	0.053

Enrichment in *Tip60$^{Δ/Δ}$*

HALLMARK gene set	Size	NES	FDR q-value
EPITHELIAL_MESENCHYMAL_TRANSIT	187	-2.00	0.000
COAGULATION	120	-1.87	0.000
MYOGENESIS	194	-1.76	0.002
KRAS_SIGNALING_UP	184	-1.73	0.003
INFLAMMATORY_RESPONSE	184	-1.71	0.003
ANGIOGENESIS	33	-1.70	0.003
ALLOGRAFT_REJECTION	174	-1.60	0.011
APICAL_SURFACE	180	-1.58	0.013
TNFA_SIGNALING_VIA_NFKB	169	-1.57	0.012
HEDGEHOG_SIGNALING	143	-1.56	0.011

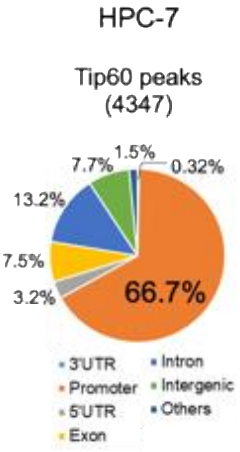
KEGG gene set	Size	NES	FDR q-value
ECM_RECEPTOR_INTERACTION	81	-2.17	0.000
AXON_GUIDANCE	126	-2.04	0.000
METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	39	-1.97	0.001
AUTOIMMUNE_THYROID_DISEASE	27	-1.93	0.001
CELL_ADHESION_MOLECULES_CAMS	104	-1.93	0.001
CYTOKIEN_YTOKINE_RECEPTOR_INTERACTION	218	-1.91	0.001
FOCAL_ADHESION	187	-1.90	0.001
GRAFT_VERSUS_HOST_DISEASE	16	-1.86	0.003
INTESTINAL_IMMUNE_NETOWORK_FOR_IGA_PRODUCTION	32	-1.71	0.026
TYPE1_DIABETES_MELLITUS	21	-1.67	0.041



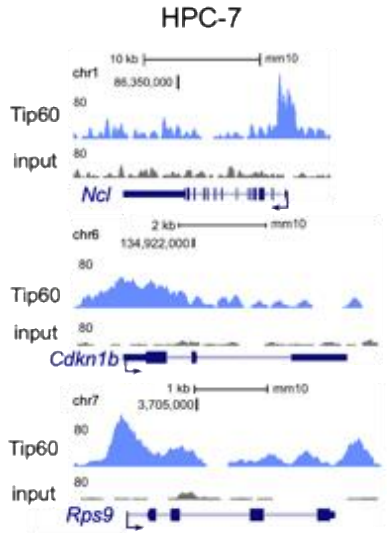
Supplemental Figure 5 | RNA-seq analysis and c-Myc expression of *Tip60$^{f/f}$* and *Tip60$^{Δ/Δ}$* LSK cells.

A, Experimental scheme for *in vitro Tip60* deletion in HSPC. LSK cells were purified from *Tip60$^{f/f}$ and Tip60$^{f/f}$; Rosa26 Cre-ERT2* fetal liver at E14.5, cultured in media supplemented with SCF, IL-3, IL-6, IL-11, and 4-OHT for 72 hours, and harvested for analysis. **B,** Genome browser view of the *Tip60* locus obtained from RNA-seq analysis. **C,** Volcano plot showing average $\log_2$ fold change against $-\log_{10}$ FDR for all genes in *Tip60$^{f/f}$* versus *Tip60$^{Δ/Δ}$* from RNA-seq analysis. The red dots represent the significantly upregulated genes ($\log_2$ fold change > 0.5 and FDR < 0.05); the orange dots represent the significantly downregulated genes ($\log_2$ fold change < -0.5 and FDR < 0.05). **D,** Left, lists of enriched gene sets in *Tip60$^{f/f}$* over *Tip60$^{Δ/Δ}$* LSK cells, right, enriched gene sets in *Tip60$^{Δ/Δ}$* over *Tip60$^{f/f}$* from GSEA. **E,** RNA-seq analysis of *c-myc* expression. **F,** c-Myc protein levels measured by Western blotting (left) and Tip60 mRNA levels by qPCR (right) in Lin$^-$ c-Kit$^+$ cells purified from *Tip60$^{f/f}$* and *Tip60$^{f/f}$; Rosa26 Cre-ERT2* fetal liver (E14.5) after 72 hour 4-OHT treatment. **G,** Volcano plot showing average $\log_2$ fold change against $-\log_{10}$ FDR for all genes in *Tip60$^{f/f}$* versus *Tip60$^{Δ/Δ}$*. The red dots represent the significantly upregulated Myc target genes ($\log_2$ fold change > 0.5 and FDR < 0.05); the orange dots represent the significantly downregulated Myc target genes ($\log_2$ fold change < -0.5 and FDR < 0.05). **H,** Association between gene expression change (up-regulated vs. down-regulated) and Tip60 binding (Tip60-bound vs. unbound) in the differentially expressed Myc target genes. The values in the graph are presented as mean ± SEM. *p*-value was estimated by Fisher's exact test. **p < 0.01.

A


HPC-7
Tip60 peaks
(4347)
1.5%
0.32%
7.7%
13.2%
7.5%
3.2%
66.7%
3'UTR
Intron
Promoter
Intergenic
5'UTR
Others
Exon

B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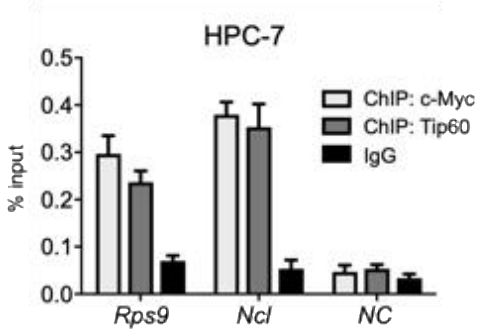
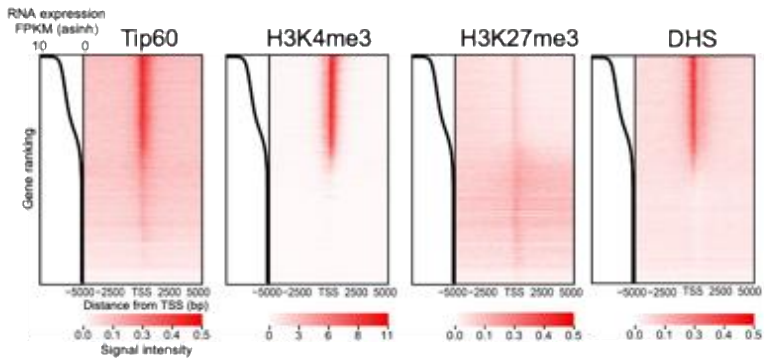
chr1
10 kb
mm10
86,350,000
Tip60
input
Ncl
chr6
2 kb
mm10
134,922,000
Tip60
input
Cdkn1b
chr7
1 kb
mm10
3,705,000
Tip60
input
Rps9

C

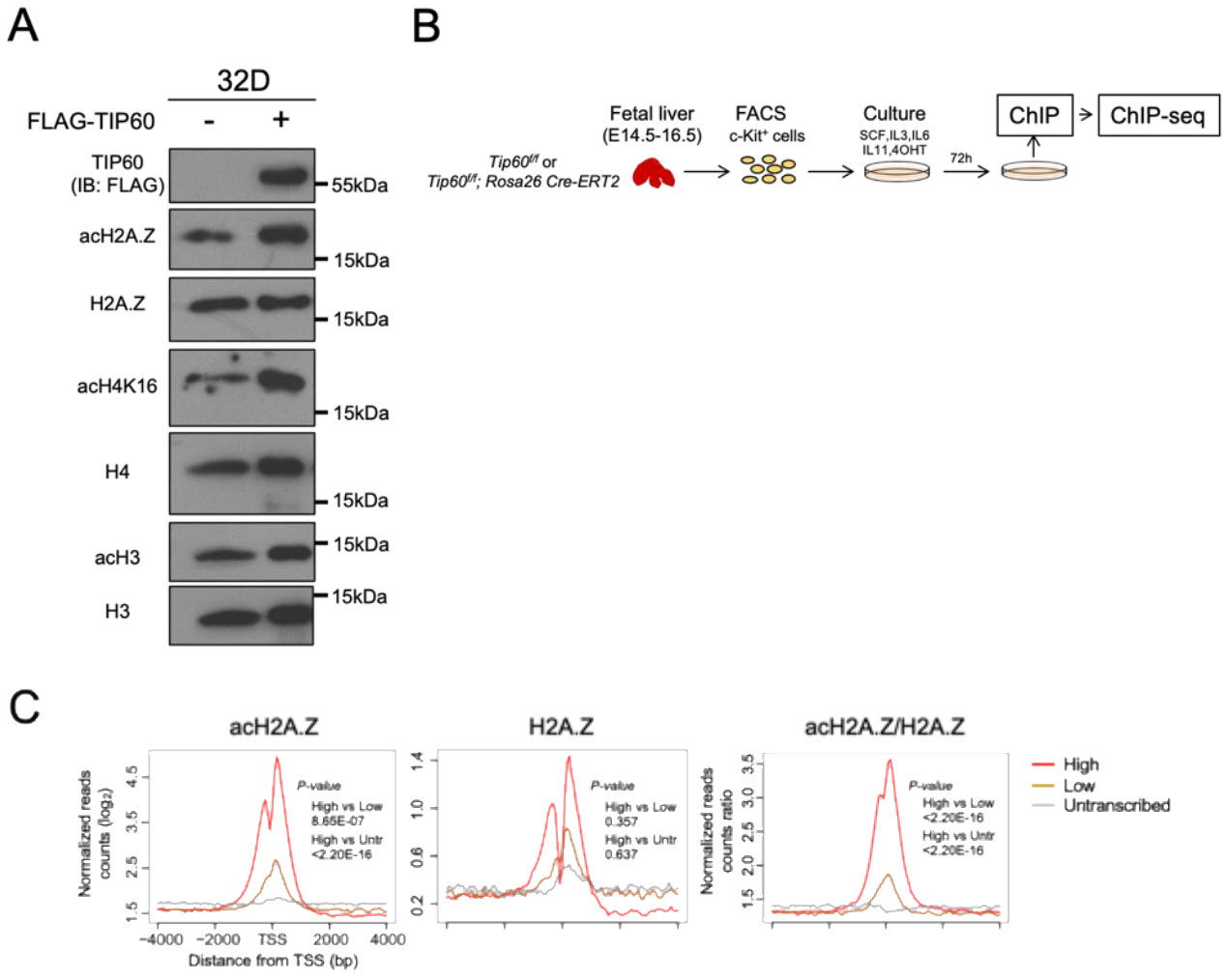

HPC-7
% input
0.5
0.4
0.3
0.2
0.1
0.0
ChIP: c-Myc
ChIP: Tip60
IgG
Rps9
Ncl
NC

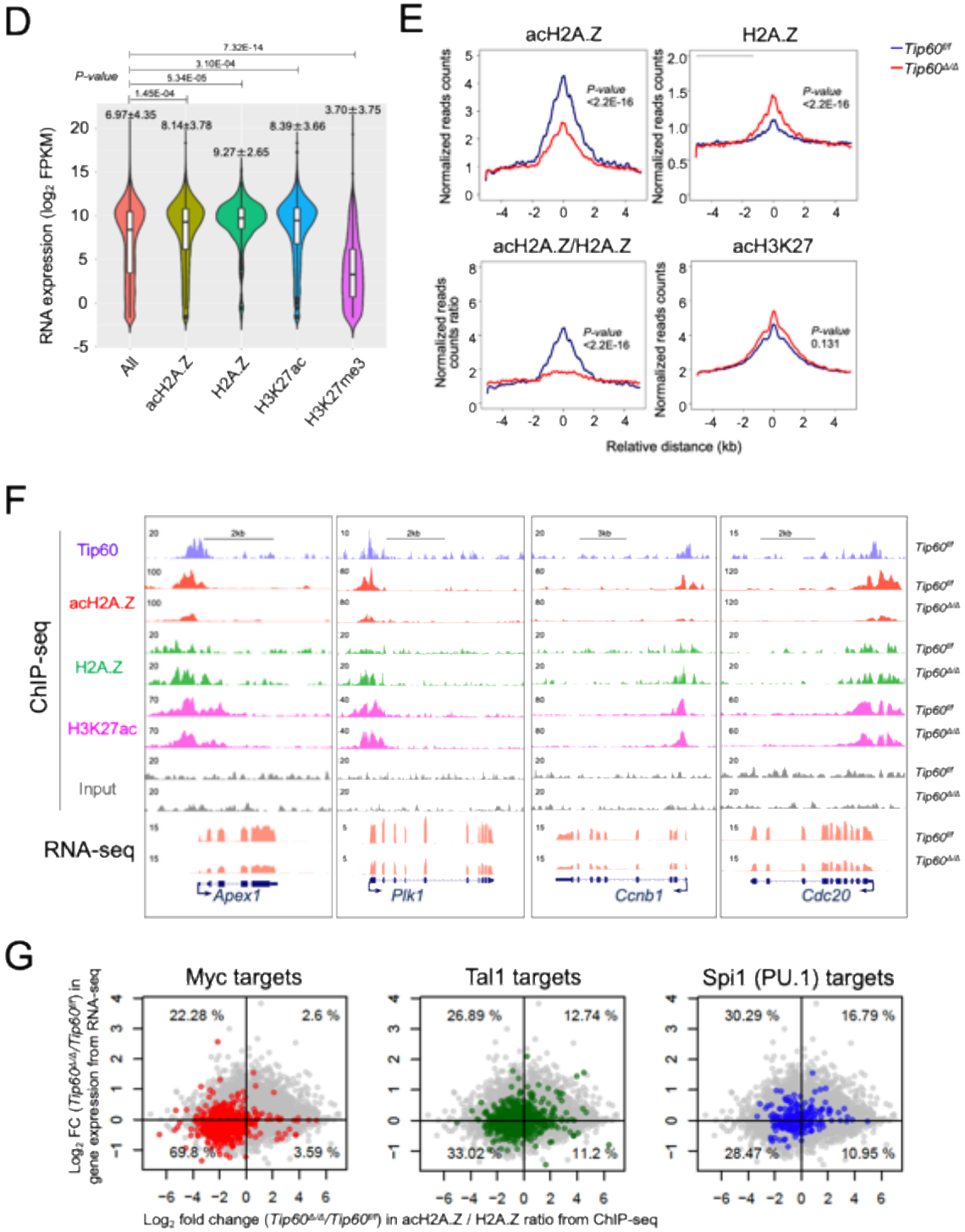
D


HPC-7
RNA expression
FPKM (asinh)
Tip60
H3K4me3
H3K27me3
DHS
Gene ranking
-5000 -2500 TSS 2500 5000
Distance from TSS (bp)
Signal intensity

Supplemental Figure 6 | Tip60 ChIP-seq and ChIP-qPCR in HPC-7 cells.

A, Distribution of Tip60 binding peaks in HPC-7 cells relative to gene annotations. **B,** Representative Tip60 binding peaks in the previously reported Tip60 target gene loci (*Ncl*, *Cdkn1b*, and *Rps9*). **C,** c-Myc and Tip60 ChIP-qPCR in the *Rps9* and *Ncl* gene promoters in the murine multipotent progenitor cell line HPC-7 cells. Data are presented as mean ± SD from three independent experiments. NC: control intergenic region. **D,** Heatmaps of ChIP-seq signals (Tip60, H3K4me3, and H3K27me3) and DNase I hypersensitive site (DHS) around transcriptional start sites (TSSs ± 5 kb), which are ranked according to gene expression levels after an *asinh* transformation.


A
32D
FLAG-TIP60 - +
TIP60 (IB: FLAG)
55kDa
acH2A.Z
15kDa
H2A.Z
15kDa
acH4K16
15kDa
H4
15kDa
acH3
15kDa
H3
15kDa
B
Fetal liver (E14.5-16.5)
FACS c-Kit+ cells
Culture SCF,IL3,IL6 IL11,4OHT
72h
ChIP
ChIP-seq
Tip60f/f or Tip60f/f; Rosa26 Cre-ERT2
C
acH2A.Z
H2A.Z
acH2A.Z/H2A.Z
Normalized reads counts (log2)
Normalized reads counts ratio
P-value
High vs Low 8.65E-07
High vs Untr <2.20E-16
High vs Low 0.357
High vs Untr 0.637
High vs Low <2.20E-16
High vs Untr <2.20E-16
−4000 −2000 TSS 2000 4000
Distance from TSS (bp)
High
Low
Untranscribed


D
P-value
7.32E-14
3.10E-04
5.34E-05
1.45E-04
6.97±4.35
8.14±3.78
9.27±2.65
8.39±3.66
3.70±3.75
RNA expression (log2 FPKM)
All
acH2A.Z
H2A.Z
H3K27ac
H3K27me3
E
acH2A.Z
H2A.Z
acH2A.Z/H2A.Z
acH3K27
Normalized reads counts
Normalized reads counts ratio
P-value <2.2E-16
P-value 0.131
Relative distance (kb)
Tip60fl/fl
Tip60Δ/Δ
F
ChIP-seq
RNA-seq
Tip60
acH2A.Z
H2A.Z
H3K27ac
Input
Apex1
Plk1
Ccnb1
Cdc20
G
Myc targets
Tal1 targets
Spi1 (PU.1) targets
22.28 %
2.6 %
69.8 %
3.59 %
26.89 %
12.74 %
33.02 %
11.2 %
30.29 %
16.79 %
28.47 %
10.95 %
Log2 FC (Tip60Δ/Δ/Tip60fl/fl) in gene expression from RNA-seq
Log2 fold change (Tip60Δ/Δ/Tip60fl/fl) in acH2A.Z / H2A.Z ratio from ChIP-seq

Supplemental Figure 7 | Changes in global histone acetylation in HSPCs induced by *Tip60* deletion.

A, Western blot showing global histone acetylation levels in murine hematopoietic progenitor cell line 32D cells 48h after transduction of FLAG-TIP60. **B,** Experimental scheme for *in vitro Tip60* deletion in murine HSPCs. c-Kit$^+$ cells were purified from fetal liver of *Tip60$^{f/f}$ and Tip60$^{f/f}$; Rosa26 Cre-ERT2* embryos by FACS, cultured in media supplemented with SCF, IL-3, IL-6, IL-11, and 4-OHT for 72 hours, and harvested for analysis. **C,** Mean-plot for H2A.Z (left), acH2A.Z (middle), and acH2A.Z/H2A.Z ratio (right) enrichment around TSSs (±4kb). Genes were split into three groups according to RNA expression level: red, high with $\log_2$ expression (FPKM)>7, 10467 genes; brown, low with 0.02< $\log_2$ expression (FPKM) <=7, 5630 genes; gray, untranscribed with $\log_2$ expression (FPKM) <0.02, 8013 genes. The average signal intensity of the specific group is plotted. *p*-value was estimated by Mann-Whitney-Wilcoxon method. **D,** Violin plots showing the gene expression distributions in *Tip60$^{f/f}$* fetal LSK cells from RNA-seq for all genes (24,110 genes) and genes enriched with peaks for acH2.A.Z (1,426 genes), H2A.Z (985 genes), H3K27ac (1,300 genes) and H3K27me3 (5,521 genes) around TSS (±4kb) obtained from the ChIP-seq analysis of *Tip60$^{f/f}$* fetal c-Kit$^+$ cells. Corresponding boxplot represents mean and the 25th to 75th percentiles of the distribution. Mean expression levels (log2 FPKM) ± SD are indicated. *p*-value was estimated by Mann-Whitney-Wilcoxon method. **E,** Mean-plot of acH2A.Z, H2A.Z, the acH2A.Z/H2A.Z ratio, and acH3K27 at Tip60-bound distal enhancer regions. The X axis presents the relative distance to Tip60 peaks, while the Y axis presents the normalized reads counts/reads counts ratio (for acH2A.Z/H2A.Z) in both control (dark blue) and *Tip60$^{\Delta/\Delta}$* fetal c-Kit$^+$ cells (red). *p*-value was estimated by Mann-Whitney-Wilcoxon method. **F,** ChIP-seq (Tip60, acH2A.Z, H2A.Z, and H3K27ac) and RNA-seq peak of both control (*Tip60$^{f/f}$*) and *Tip60$^{\Delta/\Delta}$* cells at the representatives of the selected Myc target genes (*Apex1*, *Plk1*, *Ccnb1*, and *Cdc20*). **G,** Log_2 fold change in acH2A.Z/H2A.Z ratio at promoter regions (X-axis) from ChIP-seq and $\log_2$ fold change in gene expression level (Y-axis) of Myc target genes (red), Tal1 (green), Spi1 (PU.1) (blue), and whole genes (grey) from RNA-seq. Myc, Tal1, and Spi1 (PU.1) target genes were obtained from the gene sets described in Figure 5A, ENCSR000DIA, and GSE58128, respectively.

Reference

1. Fisher JB, Horst A, Wan T, et al. Depletion of Tip60 from In Vivo Cardiomyocytes Increases Myocyte Density, Followed by Cardiac Dysfunction, Myocyte Fallout and Lethality. PLoS One. 2016;11(10):e0164855.
2. de Boer J, Williams A, Skavdis G, et al. Transgenic mice with hematopoietic and lymphoid specific expression of Cre. Eur J Immunol. 2003;33(2):314-325.
3. Pinto do OP, Kolterud A, Carlsson L. Expression of the LIM-homeobox gene LH2 generates immortalized steel factor-dependent multipotent hematopoietic precursors. EMBO J. 1998;17(19):5744-5756.
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