Lysine acetyltransferase Tip60 is required for hematopoietic stem cell maintenance

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Key points

- Conditional deletion of *lysine acetyltransferase 5* (*Tip60*) in murine hematopoietic system leads to HSC loss, both in fetal and adult stage.

- c-Myc is the most enriched transcription factor with genome-wide binding of Tip60 in murine hematopoietic stem and progenitor cells.

- Tip60 activates Myc target genes critical for HSC maintenance through acetylation of H2A.Z at the target genomic regions.

Abstract

Hematopoietic stem cells (HSC) have the potential to replenish the blood system for the lifetime of the organism. Their two defining properties, self-renewal and differentiation, are tightly regulated by the epigenetic machineries. Here, using conditional gene knockout models, we demonstrate a critical requirement of lysine acetyltransferase 5 (*Kat5*, also known as *Tip60*) for murine HSC maintenance both in the embryonic and adult stages, which depends on its acetyltransferase activity. Genome-wide chromatin and transcriptome profiling in murine hematopoietic stem and progenitor cells revealed that Tip60 co-localizes with c-Myc and that *Tip60* deletion suppress the expression of Myc target genes, which are associated with critical biological processes for HSC maintenance, cell-cycle and DNA repair. Notably, acetylated H2A.Z (acH2A.Z) was enriched at the Tip60-bound active chromatin and *Tip60* deletion induced a robust reduction in the acH2A.Z / H2A.Z ratio. These results uncover a critical epigenetic regulatory layer for HSC maintenance at least in part through Tip60 dependent H2A.Z acetylation to activate Myc target genes.

Introduction

Hematopoietic stem cells (HSCs) possess two defining properties, a self-renewal and multi-lineage differentiation ability, under various tightly regulated epigenetic mechanisms [1]. Chromatin modifying enzymes play crucial roles in regulating gene expression for HSC maintenance [2-5]. Histone acetylation, a reversible-covalent post-translational modification (PTM), is one of the most studied chromatin modifications, which is catalyzed by lysine acetyltransferases (KATs) and mediates unique and specific effects on gene transcription by altering the degree of chromatin condensation [6, 7]. According to structural homology and acetylation mechanisms, KATs are classified into five representative families: GNAT, MYST, p300/CBP, SRC, and TAF1 [8]. The MYST family is defined by the proteins containing a C_2HC-type zinc finger and an acetyl-CoA binding domain, and consists of five members: *Tip60/Kat5*, *Moz/Kat6a*, *Morf/Kat6b*, *Hbo1/Kat7*, and *Mof/Kat8*. Previous studies of various KAT deletions in mice detailed their importance in the maintenance or differentiation of HSCs. Homozygous deletion and catalytic mutant mice of *Moz*/*Kat6a* revealed its essential role in both fetal and adult HSC maintenance as a repressor of p16 expression to prevent HSC senescence [5, 9]. *Mof*/*Kat8* is critical for adult, not fetal HSC maintenance, and the catalytically inactive mutant neither restores H4K16ac levels nor rescues colony forming ability in adult hematopoiesis [10]. The Hbo1-Brd1 complex is required for transcription of genes regulating erythroid development [11]. Each lysine acetyltransferase has a specific regulatory role in hematopoiesis despite some redundancy in their substrate specificities [12].

Lysine acetyltransferase 5, Kat5 (also known as Tat-Interactive Protein 60, Tip60) plays a key role in DNA damage response and repair, as well as gene-specific transcriptional regulation [13]. It is part of the evolutionarily conserved Nucleosomal Acetyltransferase of H4 (NuA4) protein complex, which acetylates nucleosomal histones H3, H4, H2A, and H2A variants [14]. Homozygous global *Tip60* deletion in mice lead to pre-implantation lethality at embryonic day 3.5 [15], indicating its requirement for

78   embryonic development. In the hematopoietic system, a conditional deletion of *Tip60* in regulatory T

79   (Treg) cells impairs their function in peripheral immune organs by suppressing the transcriptional

80   activity of FOXP3 [16]. However, the role of Tip60 in HSCs has been largely unknown. Here, we

81   demonstrate a critical requirement for *Tip60* in murine HSC maintenance using murine conditional

82   *Tip60* deletion models. Genome-wide transcriptome and chromatin profiling reveal that Tip60 and c-

83   Myc co-localize at active chromatin loci to activate transcription of their target genes. Notably, Tip60

84   deletion reduces acetylation level of H2A.Z at the target genes promoters. We thus propose a new

85   epigenetic mechanism in HSC maintenance: Tip60-mediated H2A.Z acetylation for the activation of

86   Myc target genes.

Materials and methods

Mice

Tip60^{flox} 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 (supplemental Figures 1A-B). Additional details are described in the supplemental information. 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.

Inducible *Tip60* deletion

For *in vivo Tip60* deletion, *Mx1-cre*; *Tip60$^{f/f}$* mice were injected with 300 µg pIpC (GE Healthcare) per body for three consecutive days. For *in vitro Tip60* deletion, LSK or c-Kit$^+$ cells were sorted from fetal liver of *Rosa26 Cre-ERT2*; *Tip60$^{f/f}$* embryos (E13.5-15.5) by FACS Aria (BD Biosciences), cultured in Stemline II (Sigma-Aldrich) supplemented with 5% FBS, murine recombinant SCF 100ng/µL, IL-3 6 ng/µL, IL-6 10ng/µL, IL-11 20 ng/µL (Peprotech), and 4-Hydroxytamoxifen (4-OHT) (Sigma-Aldrich) 400 nM for 72 hours, and collected for analysis.

Flow cytometry

Single-cell suspensions were analyzed by flow cytometry. Cells stained with antibodies were analyzed or sorted using LSRII flow cytometer or FACS Aria (BD Biosciences). Flow Jo 7.5 (Tree Star) was used for data analysis. The antibodies used in this study are described in the supplemental information.

Retroviral transduction and Tip60 KAT rescue

FLAG-tagged human TIP60 wild-type and catalytically inactive mutant constructs [17] were cloned into MSCV vector ($TIP60^{wt}$ and $TIP60^{mut}$, respectively). $TIP60^{wt}$ and $TIP60^{mut}$ retroviruses were produced in BOSC23 cells. FACS-sorted LSK cells obtained from $Tip60^{f/f}$; $Vav\text{-}iCre$ embryos (CD45.2$^+$) at E13.5 were seeded in Retronectin (Takara)-coated plates containing retroviral supernatants for 24 hours. 2×10^3 transduced cells were injected into lethally (900 rads) irradiated congenic mice (CD45.1$^+$) along with wild type WBM cells from congenic mice (CD45.1$^+$ CD45.2$^+$). Transduction of plasmid DNAs were verified by Sanger sequencing of the PCR-amplified genomic products amplified by the following primers: Tip60KAT-F: 5'- GTG TTT CCT TGA CCA TAA GAC ACT GTA CTA T -3' and Tip60KAT-R: 5'- GGT CTG GGA CCA GTA GCT TCG ATA G -3'.

Bone marrow transplantation assay

For competitive transplantation assay, unfractionated fetal liver cells (1×10^6 cells) from $Tip60^{f/+}$, $Tip60^{+/\Delta}$, and $Tip60^{\Delta/\Delta}$ E14.5 embryos were injected into lethally irradiated (900 rads) congenic mice along with competitor WBM cells (1×10^5 cells). $Mx1\text{-}Cre$; $Tip60^{f/f}$ and $Tip60^{f/f}$ mice, which were injected 800 μg of pIpC per body every other day for 7 days, whole BM (WBM) cells (1×10^6 cells) were collected 10 days after the last injection, and then injected into lethally irradiated congenic mice along with competitor WBM cells (2×10^5 cells). For reciprocal transplantation assays, unfractionated WBM cells from $Mx1\text{-}Cre$; $Tip60^{f/f}$ and $Tip60^{f/f}$ mice were injected into lethally irradiated (900 rads) congenic mice along with competitor WBM cells (1×10^6 and 2×10^5, 5×10^5 and 5×10^5 cells, respectively), and the recipients were injected with 800 μg of pIpC per body every other day for 4 days after engraftment. To exclude cell extrinsic effect caused by $Tip60$ deletion, unfractionated CD45.1$^+$ WBM cells (1×10^6 cells) were injected into lethally irradiated $Tip60^{f/f}$ and $Tip60^{f/f}$; $Mx1\text{-}Cre$ mice. After transplantation, chimerism analysis was performed as described in the supplemental information.

RNA/ChIP-sequencing

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 Next ChIP-Seq library prep reagent set and multiplexed (New England Biolabs). Each library was sequenced on an Illumina Hiseq2000 sequencer. The details of chromatin immunoprecipitation and sequence analysis are described in the supplemental information.

ChIP-Seq similarity analysis

Similarity analysis was performed using normalized pointwise mutual information (NPMI) [18]. After normalization, NPMI ranged from 1 for complete co-occurrence (correlation limit), 0 for independent peaks profiles, and –1 when peaks did not occur together (anticorrelation limit). NPMI values were clustered using Euclidean distance and Ward linkage in R. All data sets except Tip60 profiling are accessible through an intuitive Web browser interface (CODEX). http://codex.stemcells.cam.ac.uk/

Statistical analysis

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

Additional details are described in the supplemental information.

Data availability

The RNA-seq and ChIP-seq data described in this study have been deposited in GEO under accession number GEO: GSE120705.

Results

Tip60 deletion leads to rapid HSC loss in both fetal and adult stages

To study the role of *Tip60* in HSCs, we generated mice in which a loxP-flanked *Tip60* allele (exons 3 to 11, which includes the chromo, zinc finger and acetyl Co-A binding domains) (supplemental Figures 1A-B) [16] was conditionally deleted by improved Cre (iCre) recombinase whose expression was under the control of the *Vav1* promoter (*Tip60^f/f*; *Vav-iCre*) (supplemental Figures 2A-C) [19]. *Tip60^f/+*; *Vav-iCre* and *Tip60^f/f* mice were crossed, and among the 76 viable progenies obtained, none of the 19 (25%) expected *Tip60^f/f*; *Vav-iCre* (*Tip60^Δ/Δ*) mice were born (Figure 1A). In a retrospective analysis, *Tip60^Δ/Δ* embryos were viable at E14.5, however, they became anemic at E17.5 (Figure 1B), suggesting that the cause of the lethality of *Tip60^Δ/Δ* embryos was hematopoietic failure, mainly anemia (supplemental Figure 2D). *Tip60* deletion led to a decrease in the size of the fetal liver, accompanied by 6 to 9-fold reductions in the number of fetal liver cells at E14.5 and E17.5 (Figure 1C and supplemental Figure 2E). The subpopulations of hematopoietic stem and progenitor cells (HSPCs) were dramatically reduced (Figures 1D-E). To assess the repopulating ability, we performed competitive transplantation using whole fetal liver cells of E14.5 embryos, which demonstrated complete loss of hematopoietic reconstitution by *Tip60^Δ/Δ* cells (Figure 1F and supplemental Figures 2F-G). There were few *Tip60^Δ/Δ* HSCs present in the bone marrow (BM) of the recipients at 16 weeks post-transplantation, suggesting an impaired long-term repopulating ability of fetal *Tip60^Δ/Δ* HSCs (Figure 1G). Notably, heterozygous deletion of *Tip60* (*Tip60^+/Δ*) did not affect hematopoietic reconstitution, which indicated that there was no gene dosage effect of *Tip60* in HSC function.

To determine the role of Tip60 in adult HSCs, we generated *Tip60^f/f* and *Tip60^f/+* mice wherein expression of Cre is driven by the interferon-inducible promoter of the gene encoding Mx1. In this

model, the *Tip60* gene was excised by injecting polyinosinic-polycytidylic acid (pIpC), leading to a

reduction of Tip60 protein in HSPCs (supplemental Figures 3A-C) [20]. *Tip60* deletion induced a

dramatic decrease in cellularity and numbers of HSPCs in BM (Figures 2A-B), whereas heterozygous

Tip60 deletion did not, again demonstrating the absence of a gene dosage effect of Tip60 in adult

hematopoiesis as well. In competitive transplantations, *Tip60*$^{Δ/Δ}$ whole BM (WBM) cells did not

reconstitute hematopoiesis (Figure 2C and supplemental Figure 3D), with few or no *Tip60*$^{Δ/Δ}$ HSCs were

detected in the BM 16 weeks after the transplantation (Figure 2D), indicating an impaired long-term

repopulating ability in adult stage as well. Short-term *in vivo* homing assays demonstrated no significant

difference in engraftment efficiency (Figure 2E).

Tip60 is a critical cell-intrinsic regulator of HSC

Since Cre under Mx1 promoter is activated by pIpC in various tissues [20], reciprocal competitive

transplantations were conducted to exclude a cell-extrinsic effect of *Tip60* deletion. First, WBM cells

from *Tip60*$^{f/f}$; *Mx1-Cre* or *Tip60*$^{f/f}$ mice were transplanted into lethally irradiated congenic mice and

pIpC was injected into the recipients 6 weeks post-transplantation. Peripheral blood (PB) chimerism

analysis revealed a continuous decrease in *Tip60*$^{f/f}$; *Mx1-Cre* cells in all lineages over time, but not in

control (*Tip60*$^{f/f}$) cells (Figure 2F and supplemental Figure 3E). Secondly, WBM cells from congenic

mice were transplanted into lethally irradiated recipients, *Tip60*$^{f/f}$; *Mx1-Cre* and *Tip60*$^{f/f}$ mice, which

were subsequently injected with pIpC. The chimerism of donor-derived cells was sustained in both

groups for 8 weeks. However, all the *Tip60*$^{f/f}$; *Mx1-Cre* recipients died within 3 months (supplemental

Figure 3F) with clinical presentations of severe dermatitis, hair loss, and deteriorating general condition.

PB analysis demonstrated stable blood counts, which were derived from donor mice (data not shown),

suggesting that the hematopoietic defect was not the cause of death. Given that Tip60 plays a critical

role in response to DNA double-strand breaks (DSBs) [17, 21], *Tip60* deletion may have exacerbated the

197 genotoxicity of irradiation, leading to fatal failure of non-hematopoietic organs wherein *Tip60* is deleted.

198 In summary, we conclude that Tip60 is a critical cell-intrinsic regulator for HSCs.

Tip60 acetyltransferase activity is essential for HSC function

200 Since our conditional deletion model ablates all the functional domains of Tip60, we investigated

201 whether its acetyltransferase activity is required for HSC function. We overexpressed both wild-type

202 TIP60 and acetyltransferase defective TIP60 (Q377E/G380E) [17] in HEK293T cells and confirmed that

203 both proteins were expressed at similar levels (supplemental Figure 4A). *Tip60$^{\Delta/\Delta}$* LSK cells purified

204 from E13.5 fetal livers were transduced with retroviruses expressing wild-type TIP60 (TIP60^{wt}),

205 TIP60^{mut}, or empty vector (Empty) and subsequently transplanted into lethally irradiated congenic mice

206 along with wild-type WBM cells (Figure 3A and supplemental Figure 4B). The *Tip60$^{\Delta/\Delta}$* LSK cells

207 transduced with empty vector did not recover long-term hematopoiesis, while Tip60^{wt} rescued the defect

208 successfully (Figure 3B). Notably, the transduction of TIP60^{mut} did not rescue the defect to any

209 detectable level. Furthermore, we confirmed that both TIP60 wild-type and mutant could interact with

210 key components of the NuA4 complex, including p400, RUVBL1, RUVBL2, and TRRAP

211 (supplemental Figures 4C-E), suggesting that the catalytic mutations in TIP60 did not affect its binding

212 to the complex. However, we cannot fully exclude the possibility that the TIP60 mutant could have

213 some loss or gain of additional protein interactions that may account for its inability to rescue HSC

214 regenerative capacity. Collectively, these results demonstrate a specific requirement of Tip60

215 acetyltransferase activity for HSCs regenerative ability.

Tip60 maintains HSC genome integrity

217 To elucidate the cellular mechanisms underlying the loss of *Tip60$^{\Delta/\Delta}$* HSCs, we assessed apoptosis and

218 cell-cycle status. *Tip60* deletion induced apoptosis in fetal CD45$^+$ cells and adult HSCs, exhibiting

219   increased AnnexinV$^+$DAPI$^-$ cells and cleaved caspase-3 levels (Figures 4A-B). Cell-cycle analysis

220   revealed fewer quiescent $Tip60^{\Delta/\Delta}$ HSCs (G$_0$: Ki67$^-$Hoeschst$^-$) with a concordant increased percentage of

221   cells in cycling phase (S-G$_2$/M: Ki67$^+$Hoeschst$^+$) compared to control (Figure 4C). Moreover, DNA

222   content analysis, using fetal LSK cells of $Tip60^{f/f}$; $Rosa26$-$CreERT2$ and $Tip60^{f/f}$ embryos at E14.5,

223   subsequently treated with 4-hydroxytamoxifen (4-OHT) in culture for 5 days, revealed an increase in

224   cells with 4N or greater than 4N DNA content (Figure 4D). Such increase was not seen in LSK cells

225   from $Rosa26$-$CreERT2$ embryos, suggesting that this effect is specific to the deletion of $Tip60$

226   (supplemental Figure 4F). Microscopic examination revealed that $Tip60^{\Delta/\Delta}$ cells grew in size and became

227   multi-nucleated, exhibiting abnormal nuclear morphology including multi-lobulated nuclei and

228   micronuclei (supplemental Figure 4G). Given that the Tip60 complex has been shown to be important

229   for DNA double-stranded breaks (DSBs) repair process [17, 21], we investigated DNA damage in $Tip60^{\Delta/\Delta}$

230   HSCs. Remarkably, alkaline comet assay demonstrated increased tail moment in $Tip60^{\Delta/\Delta}$ HSPCs

231   (Figure 4E), suggesting that $Tip60$ deletion impaired DNA repair, which was further verified by the

232   accumulation of γH2AX signals, a marker for unrepaired DSB loci (Figure 4F). Collectively, these

233   results indicate that $Tip60$ deletion induces aberrant cell-cycle progression, and apoptosis that is possibly

234   caused by DNA breaks in HSCs.

235   **Tip60 is required for expression of Myc target genes**

236   To identify genes regulated by Tip60 in a cell-intrinsic manner, we performed RNA-seq using LSK cells

237   purified from $Tip60^{f/f}$; $Rosa26$-$CreERT2$ and $Tip60^{f/f}$ embryos that were treated with 4-OHT for 72 hours

238   (supplemental Figures 5A-B). A total of 1,278 genes exhibited significant change in expression levels,

239   with 847 genes up-regulated and 431 genes down-regulated (Log$_2$ fold change > 0.5 and false discovery

240   rate (FDR) < 0.05) in $Tip60^{\Delta/\Delta}$ cells compared to control ($Tip60^{f/f}$) (supplemental Figure 5C). Gene set

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241   enrichment analysis (GSEA) using Molecular Signature Database (MSigDB) revealed that E2f and Myc

242   target genes were down-regulated in *Tip60$^{\Delta/\Delta}$* cells (supplemental Figure 5D). Given that Tip60

243   functions mainly as a transcriptional co-activator for several sequence-specific transcription factors [22-24],

244   it is plausible that a variety of transcription factors are involved in the underlying mechanisms for the

245   rapid loss of *Tip60$^{\Delta/\Delta}$* HSCs. Hence, an open-ended GSEA was conducted using all available gene sets of

246   transcription factor targets. Interestingly, multiple Myc-associated gene sets were specifically enriched

247   in control (Figures 5A-B). While c-Myc at RNA and protein levels were not suppressed (supplemental

248   Figures 5E, F), most Myc target genes were transcriptionally inactivated upon *Tip60* deletion. Among

249   the 124 differentially expressed Myc target genes (supplemental Figure 5G), we observed a significant

250   over-representation of Tip60 targets in the downregulated group compared to the upregulated group (*p*-

251   value $< 10^{-5}$; Fisher's exact test) (supplemental Figure 5H). Our results demonstrate that Tip60 acts as a

252   co-activator of Myc target genes.

253   Cell-cycle regulators play a pivotal role in HSC maintenance by tuning the balance between quiescence

254   and self-renewal [25]. The DNA repair process is critical as well, exemplified by the recent studies which

255   demonstrated that HSCs are susceptible to DNA damage due to intrinsic and extrinsic stress factors,

256   such as aging, replication, and genotoxic and oxidative stresses [26-28]. Of note, the genes which were

257   inactivated by *Tip60* deletion are associated with cell-cycle and DNA repair. Therefore, we conclude

258   that Tip60 maintains HSC functional integrity, mainly cooperating with Myc family transcription factors

259   to activate the multiple genes which are involved in essential cellular processes for HSC maintenance.

260   **Tip60 and Myc co-activate target genes**

261   To better understand the involvement of Tip60 in the active transcription of Myc target genes, we

262   performed genome-wide ChIP-seq assays in the murine hematopoietic progenitor cell line HPC-7 [29],

263 using rabbit polyclonal antibodies that specifically recognize endogenous Tip60 [23]. The majority of 4347

264 of high-confidence Tip60-bound genomic loci identified were located at the proximal promoter regions

265 (−1kb, +100bp from transcriptional start sites; TSS, 66.7 %), including the promoters of previously

266 reported Tip60 target genes (*Ncl*, *Rps9*, and *Cdkn1b*) [23, 30, 31] (supplemental Figures 6A-B). To define the

267 transcription factors that Tip60 co-localizes with, a correlation analysis of genome-wide Tip60 and 10

268 hematopoietic transcription factors (c-Myc, Tal1, Gata2, Lyl1, Lmo2, Runx1, Fli1, Meis1, Gfi1b, and

269 Spi1) occupancy was performed as previously described [18]. Notably, c-Myc was the most enriched

270 transcription factor in the Tip60-bound regions (Figure 5C), which corroborates the findings from our

271 RNA-seq analysis. Given that Myc binding peaks are not more than those of other transcription factors,

272 a specificity of Tip60 co-localization with c-Myc does not reflect abundance of c-Myc binding peaks.

273 Co-localization of c-Myc and Tip60 at the promoters of the target genes was validated by ChIP-qPCR

274 (supplemental Figure 6C). Consistent with previous studies in non-hematopoietic cells [23], we

275 demonstrated that Tip60 interacts with c-Myc by reciprocal co-immunoprecipitation experiments in

276 murine hematopoietic progenitor cells, 32D (Figure 5D).

277   To delineate the chromatin conformations, we generated heatmaps of ChIP-seq peak signals

278 between -4 kb to 4 kb from TSS region for Tip60, active promoter histone mark H3K4me3, repressive

279 histone mark H3K27me3, and DNase 1 hypersensitive sites (DHSs)[18], and ranked the gene order based

280 on RNA expression levels (supplemental Figure 6D). Remarkably, the Tip60 binding intensity

281 correlated with the RNA levels of the corresponding genes, and its occupancy overlapped with an

282 H3K4me3 enrichment and DHSs, but not with the H3K27me3. Given that c-Myc and N-myc bind

283 specifically to a common consensus sequence [32, 33], we conclude that Tip60 co-localizes with Myc

284 proteins at the transcriptional regulatory elements and active chromatin regions to activate genes in

285 HSPCs.

Tip60 maintains acetylated H2A.Z at Myc target genes

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287 Tip60 has been demonstrated to acetylate histones H3, H4, H2A, and H2A variants as well as non-

288 histone proteins [13, 17]. H2A.Z is an evolutionarily conserved variant of the canonical H2A. In

289 *Saccharomyces cerevisiae*, ESA1, the yeast ortholog of Tip60, also mediates the acetylation of Htz1, the

290 ortholog of H2A.Z [34, 35]. Drosophila Tip60 acetylates lysine 5 of H2Av, a functional homolog of the

291 mammalian H2A.Z isoform [36]. H2A.Z is enriched around TSS across the different species [37, 38], whereas

292 an acetylated form of H2A.Z (acH2A.Z) is enriched at active promoter region [39] and confers nucleosome

293 destabilization and open chromatin conformation [40]. We evaluated global histone acetylation levels upon

294 overexpression of Tip60 in the hematopoietic progenitor cells 32D and found an increased H2A.Z

295 acetylation levels as well as H4K16, known histone substrate of Tip60 (Supplemental Figure 7A). Given

296 that H2A.Z has been linked with actively transcribed regions, we further evaluated the changes in

297 acH2A.Z along with an active enhancer and promoter mark (H3K27ac) and a repressive mark

298 (H3K27me3), evoked by *Tip60* deletion by performing ChIP-seq (supplemental Figure 7B). Consistent

299 with the findings from a previous study [41], both acH2A.Z and H2A.Z were enriched at the active

300 chromatin marked by H3K27ac (Figure 6A), and acH2A.Z exhibited a pronounced bimodal enrichment

301 around TSS at highly expressed genes, while exhibiting less enrichment at poorly expressed genes and

302 no enrichment at untranscribed genes (supplemental Figure 7C). Remarkably, we observed that the

303 transcription levels for genes which were highly enriched in acH2A.Z and H2A.Z marks around TSS are

304 significantly higher than all transcribed genes (supplemental Figure 7D). We also observe a similar trend

305 for genes with H3K27ac enrichment, whereas those with H3K27me3 marks exhibit a significant

306 decrease in their transcription levels. Moreover, the ratio of acH2A.Z/H2A.Z around TSS correlated

307 with RNA levels of the corresponding genes (Pearson correlation $R^2 =0.7$), and most of the Tip60-bound

308 genes (82.0%) were highly expressed and had a higher acH2A.Z/H2A.Z ratio (Figure 6B).

309   Notably, *Tip60* loss reduced acH2A.Z levels at the promoters of the Tip60-bound Myc genes and

310   the Tip60-bound distal regions, whereas neither H3K27ac nor H3K27me3 enrichment levels were

311   altered (Figure 6C and supplemental Figure 7E). H2A.Z abundance within nucleosomes at Tip60-bound

312   promoters was not decreased; indicating that reduced acetylation of H2A.Z is not a direct consequence

313   of H2A.Z eviction. Myc target genes (816 genes), which we obtained from Myc-associated gene sets

314   (Figure 5A), demonstrated reduced acH2A.Z/H2A.Z ratios around TSS and significant downregulation

315   of their RNA levels (217 genes), including various genes related to cell-cycle and DNA repair (Figure

316   6D and supplemental Figure 7F). We conducted additional analysis for Tal1 and PU.1 (Spi-1) target

317   genes obtained from public database (supplemental Figure 7G). We chose Tal1 as a representative of the

318   genes that showed minimum correlation with Tip60 in the genome-wide occupancy analysis and PU.1

319   (Spi-1) as a representative of genes that showed high correlation (supplemental Figure 5C). A higher

320   proportion of Myc target genes (69.8%) exhibited decreased acH2AZ/H2AZ ratio and RNA expression

321   compared to Tal1 (33.02%) and PU.1 target genes (28.47%). We thus propose that the Tip60-acH2A.Z

322   epigenetic axis contributes to HSC survival through activation of Myc target genes (Figure 6E).

Discussion

The lysine acetyltransferase Tip60 is the catalytic subunit of the multiprotein complex NuA4 and acts mainly in this large molecular complex. Previous studies revealed a critical requirement for three subunits of the complex in normal hematopoiesis; ablation of the gene encoding E1A-binding protein p400 [42], transformation/transcription domain-associated protein (Trrap) [43], and RuvB-like AAA ATPase 1 (Ruvbl1) [44], resulted in rapid loss of adult HSCs from BM due to apoptosis. These subunits are required for the complex integrity, assembly and activity [45-48], suggesting that the NuA4 complex is important for HSC maintenance. However, the role of Tip60, specifically the catalytic function, in normal hematopoiesis remains undefined. In the present study, we established *Tip60* conditional knockout mice in two different Cre strains (Vav-iCre and the Mx1-Cre) to study the role of Tip60 in both fetal and adult hematopoiesis. Importantly, we demonstrated that the acetyltransferase activity of Tip60 is critical for long-term regenerating ability of murine HSCs.

The conditional deletion of *Tip60* in murine hematopoietic cells evoked catastrophic DNA damage in HSCs at both fetal and adult stages, thereby resulting in rapid apoptosis. It is noteworthy that conditional deletion of *Tip60* in Treg cells increased their number in the thymus, while they were decreased in both the spleen and the lymph nodes [16]. These results highlight that loss of Tip60 does not always result in cell lethality. Given that recent studies employing DNA repair-deficient mouse models indicate that HSCs are vulnerable to intrinsic and extrinsic DNA damage [26-28], *Tip60* deletion provoked apoptosis in HSCs due to cellular susceptibility to DNA damage. Therefore, the essentiality of Tip60 for cell survival could be cell context-dependent.

Tip60 deletion had a clear impact on functional HSCs loss. All the transplantation assays in this study were followed up at least for 16 weeks, thus excluding contribution from progenitor cells-derived

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345   hematopoiesis. Indeed, bulk RNA-seq was performed using hematopoietic stem and progenitor cells,

346   therefore, it is a possibility that gene expression changes in Myc and E2F targets could be a consequence

347   of decreased number of proliferating cells. Nonetheless, the specific co-binding of Tip60 and Myc on

348   the target gene promoters demonstrated by ChIP-seq analysis provides evidence for a direct role of

349   Tip60 in the regulation of c-Myc target genes. Intriguingly, *Tip60* deletion did not suppress c-Myc RNA

350   and protein levels, indicating that down-regulation of Myc target genes by Tip60 deletion is not a direct

351   consequence of c-Myc levels. In line with a previous study [23], we also demonstrated interaction of Tip60

352   and c-Myc in hematopoietic progenitor cells. These results provide substantial evidence for a direct role

353   of Tip60 in the regulation of Myc target genes. Noting that the HSC phenotype evoked by simultaneous

354   *c-Myc* and *N-myc* deletion in a previous study [49] resembles those of the *Tip60* conditional knockout mice,

355   we conclude that Tip60 maintains HSC survival by interacting with c-Myc to co-activate target genes.

356   We demonstrated that the catalytically inactive TIP60 mutant was not able to rescue the impaired HSC

357   function although it maintained critical components of NuA4 complex, indicating that Tip60

358   acetyltransferase activity was specifically required. H2A.Z has been validated to be a substrate of Tip60

359   [34-36], and has various functions in different species, including gene activation and repression, DNA

360   repair, heterochromatin formation, and chromosome segregation [50-52]. The diverse functions are

361   influenced by PTMs, including acetylation, SUMOylation, ubiquitination, and methylation of lysines [53].

362   Among them, acetylation is the most studied PTM as active enhancer and promoter marks [39, 40, 54, 55]. We

363   extended these findings to murine HSPCs by performing genome-wide ChIP-seq analysis to investigate

364   how Tip60 impacts H2A.Z acetylation and chromatin structure. Notably, *Tip60* deletion resulted in a

365   global reduction in acH2A.Z / H2A.Z ratio around TSSs as well as to some extent in distal enhancer

366   regions of the Tip60-bound genes, and reduced expression of a portion of these corresponding genes,

367   particularly Myc targets. These data indicate that Tip60-mediated H2A.Z acetylation may serve as a

prerequisite for active gene transcription although how acetylated H2A.Z facilitates this machinery has remained largely enigmatic. Tip60 has also been described to acetylate H3, H4 and non-histone proteins besides H2A.Z [13]. Additional targets besides H2A.Z may be involved in the regulation of genes for HSC survival, and therefore further work is necessary to study the detailed mechanisms.

Additionally, we cannot exclude the possibility that Tip60-acH2A.Z epigenetic axis may involve other transcription factors besides c-Myc. Recent studies have suggested a putative association between H2A.Z and transcription factor E2F1 and co-activator Brd2 in active gene transcription [56, 57]. It will be of future interest to identify and investigate how Tip60 cooperates with different transcription factors to facilitate gene transcription through acetylation of H2A.Z. In summary, our study highlights the importance of Tip60 in maintaining proper cell-cycle progression and DNA repair in murine HSCs at both fetal and adult stages, which is at least in part mediated through Tip60-dependent acetylation of H2A.Z to activate Myc target genes.

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392 **Authorship contributions**

393 Conceptualization, AN, SKB, JL, DB, and DGT; Methodology, AN, HSK, QZ, YZ, VK, MO, and DB;

394 Formal Analysis, AN, HSK, QZ, JL, VEA, RH, SZ, RTM, CQW, and JP; Investigation, AN, HSK, TB,

395 RSW, and DB; Resources, DR, SJ, BG and JL; Writing – Original Draft, AN and HSK; Writing –

396 Review & Editing, AN, HSK, DB, and DGT; Visualization, AN, HSK, QZ and VEA; Supervision, DGT,

397 Funding Acquisition, DGT.

398 **Disclosure of Conflicts of Interest**

399 The authors declared no competing no financial interests.

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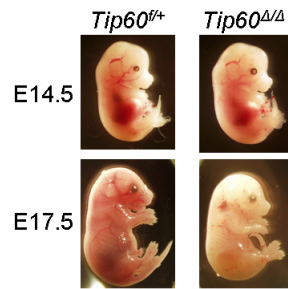498 Biol. 2013;33(6):1164-1174.

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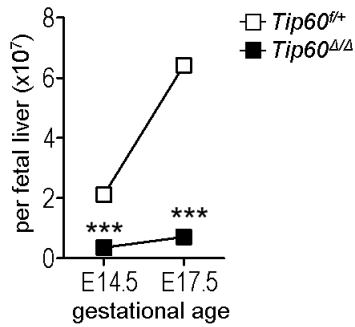516   Maintain Poised Chromatin in ESCs. Cell Rep. 2016;14(5):1142-1155.

517

A

Vav-iCre	+	+	−	−	Total
Tip60	f/+	f/f	f/+	f/+	
E11.5	4	6	7	7	24
E14.5	22	18	17	19	76
E17.5	9	7	2	5	23
Weaned	30	0	25	21	76

B

Tip60^{f/+} *Tip60^{Δ/Δ}*

E14.5

E17.5

C

per fetal liver (x10^7)

□ *Tip60^{f/+}*
■ *Tip60^{Δ/Δ}*

E14.5   E17.5
gestational age

*** ***

D

E14.5 fetal liver

Tip60^{f/+}   *Tip60^{Δ/Δ}*

c-Kit-APC

LK 5.4%   LSK 0.19%   0.66%   0.16%

Sca1-APC-CY7

CD16/32-PE

GMP 0.65%   0.22%
MEP 3.4%   CMP 0.65%   0.10%   0.19%

CD34-FITC

E14.5 fetal liver

Tip60^{f/+}   *Tip60^{Δ/Δ}*

Flk2-PE

MPP 0.12%   0.12%
LT-HSC 0.01%   ST-HSC 0.05%   0.004%   0.002%

CD34-FITC

E

LT-HSC   ST-HSC   MPP   CMP   GMP   MEP

per fetal liver (x10^3)   per fetal liver (x10^3)   per fetal liver (x10^3)   per fetal liver (x10^5)   per fetal liver (x10^5)   per fetal liver (x10^5)

E14.5 embryos
□ *Tip60^{f/+}* (n=3)
■ *Tip60^{Δ/Δ}* (n=4)

**   **   *   ***   ***   ***

F

% donor cell in PB

ns   ns   ns   ns

□ *Tip60^{f/+}*
▨ *Tip60^{+/Δ}*
■ *Tip60^{Δ/Δ}*

*** *** *** ***

4   8   12   16
after transplant (W)

G

% donor HSC in BM

ns

□ *Tip60^{f/+}*
▨ *Tip60^{+/Δ}*
■ *Tip60^{Δ/Δ}*

***

16weeks
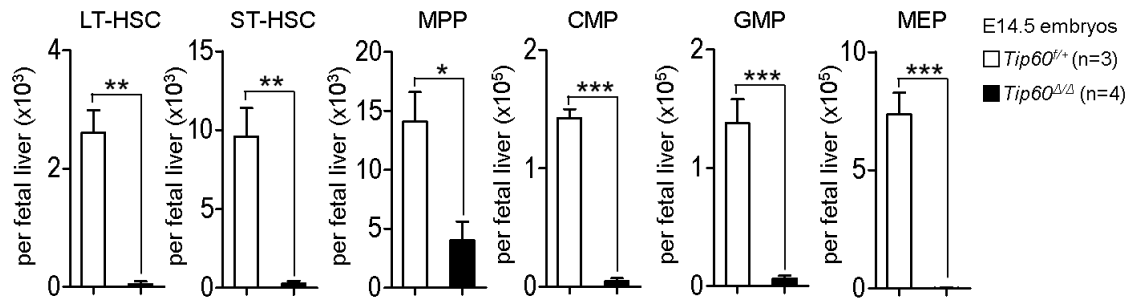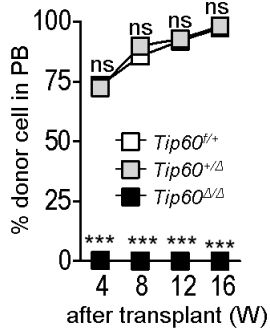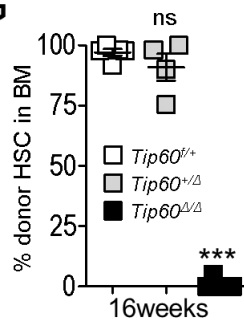
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**Figure 1. Genetic deletion of *Tip60* leads to fetal hematopoietic failure.**

A, Genotype distribution of offspring and embryos derived from extensive *Tip60^{f/f}* × *Tip60^{fl+}*; *Vav-iCre* breeding. **B,** Representative images of control (*Tip60^{f/+}*) and *Tip60^{Δ/Δ}* (*Tip60^{f/f}*; *Vav-iCre*) embryo at E14.5 and E17.5. **C,** Average absolute numbers of nucleated cells from control or *Tip60^{Δ/Δ}* fetal liver at different gestational ages; n = 3–5 embryos for each genotype for each gestational age. **D,** Representative dot plots of flow cytometry analysis of control and *Tip60^{Δ/Δ}* E14.5 fetal liver cells and frequencies of the gated populations are shown. **E,** Absolute numbers of HSPC subpopulations from control and *Tip60^{Δ/Δ}* E14.5 embryos from the same littermates. LSK (Lin⁻c-Kit⁺Sca-1⁺), LK (Lin⁻c-Kit⁺Sca-1⁻), LT-HSC (CD34⁻Flk2⁻LSK), ST-HSC (CD34⁺Flk2⁻LSK), MPP (CD34⁺Flk2⁻LSK), GMP (CD34⁺CD16/32⁺LK), CMP (CD34⁺CD16/32⁻LK), and MEP (CD34⁻CD16/32⁻LK). **F,** 1,000,000 whole fetal liver cells from control, *Tip60^{+/Δ}*(*Tip60^{f/+}*; *Vav-iCre*), and *Tip60^{Δ/Δ}* E14.5 embryos were transplanted into lethally irradiated congenic mice (CD45.1⁺) along with 100,000 congenic whole bone marrow (WBM) (CD45.1⁺CD45.2⁺) cells. Donor chimerism of the recipients' peripheral blood (PB) of are shown. Statistical analyses were performed versus *Tip60^{f/+}* using ANOVA with Bonferroni's post-test. **G,** Donor chimerism of the recipients' BM HSCs (CD34⁺ Flk2⁻LSK) at 16weeks after the transplantation. The values are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ns; not significant.

A

B
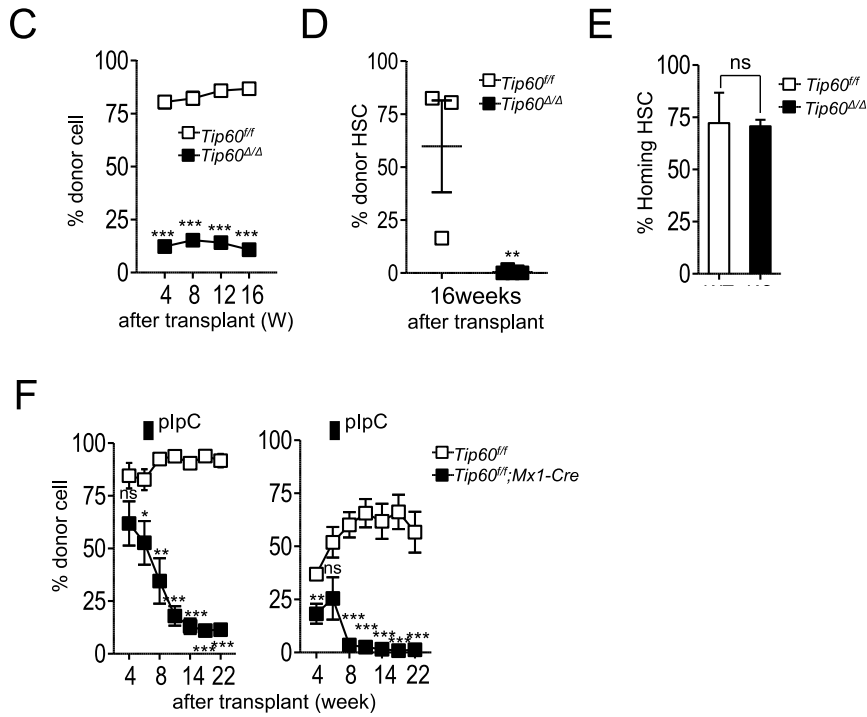

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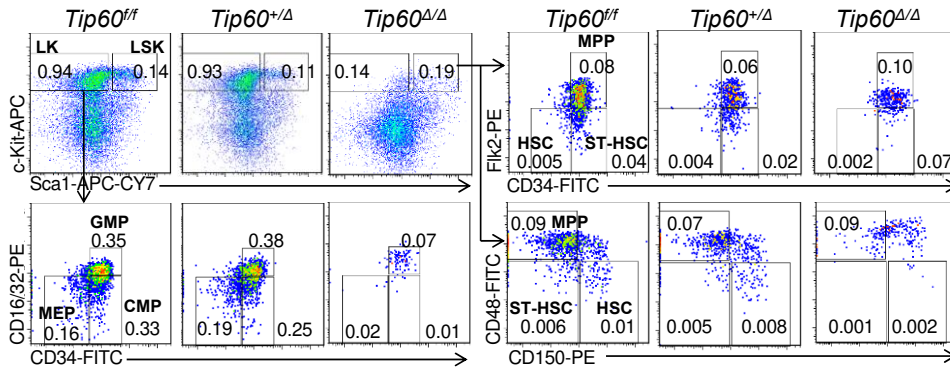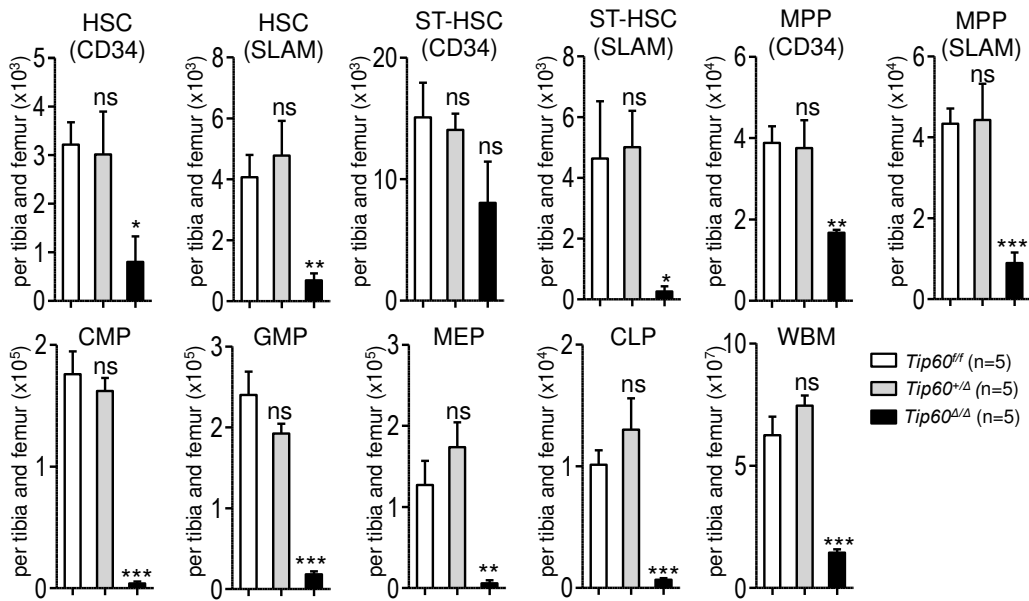
Figure 2. Tip60 is required for adult HSC maintenance in a cell-intrinsic manner.

A, 6-10 weeks old, male, *Tip60^{f/f}*, *Tip60^{f/+}*; *Mx1-Cre* and *Tip60^{f/f}*; *Mx1-Cre* mice were injected with

pIpC for 3 consecutive days. BM cells were analyzed for the frequency of HSPCs by flow cytometry 5

days after the last injection. LSK (Lin$^-$c-Kit$^+$Sca-1$^+$), LK (Lin$^-$c-Kit$^+$Sca-1$^-$), HSC (CD34, CD34$^-$Flk2$^-$;

SLAM, CD150$^+$CD48$^-$LSK), ST-HSC (CD34, CD34$^+$Flk2$^-$; SLAM, CD150$^-$CD48$^-$LSK), MPP (CD34,

CD34$^+$Flk2$^+$; SLAM, CD150$^-$CD48$^+$LSK), GMP (CD34$^+$CD16/32$^+$LK), CMP (CD34$^+$CD16/32$^-$LK),

MEP (CD34$^-$CD16/32$^-$LK) and CLP (Lin$^-$IL7R$^+$c-Kit$^+$Sca1$^+$Flk2$^+$). Representative dot plots and

frequencies of HSPC subpopulation from *Tip60^{f/f}*, *Tip60^{+/Δ}*, and *Tip60^{Δ/Δ}* mice are shown. **B,** Absolute

numbers of HSPCs and WBM cells from *Tip60^{f/f}*, *Tip60^{+/Δ}*, and *Tip60^{Δ/Δ}* mice are shown. **C,** 1×10^6

WBM cells from control *Tip60^{f/f}* and *Tip60^{Δ/Δ}* mice (CD45.2$^+$) were transplanted into lethally irradiated

recipient mice (CD45.1$^+$) along with 2×10^5 congenic WBM cells (CD45.1$^+$CD45.2$^+$). Donor chimerism

of the recipients' PB are shown. **D,** Donor chimerism of the recipients' BM HSCs (CD150$^+$CD48$^-$LSK)

at 16weeks after the transplantation. **E,** CFSE (carboxyfluorescein succinimidyl ester)-labelled HSCs

were injected into lethally irradiated congenic mice and homing efficiency was analyzed 16 hours after

the injection (n=3). **F,** Left, 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$^+$). Right, 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 into the recipients 6 weeks after the transplantation. Donor

chimerism in all nucleated cells 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

B

44

45
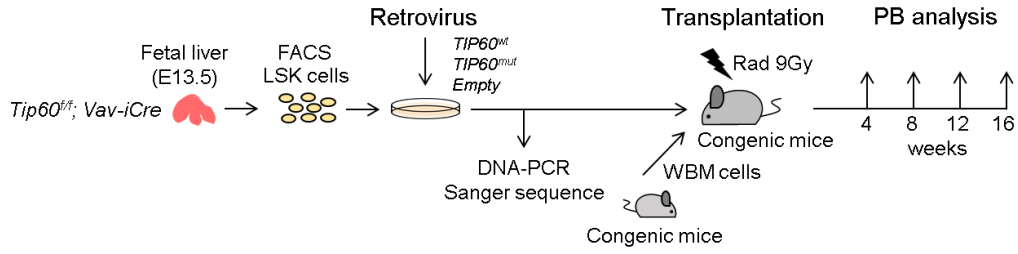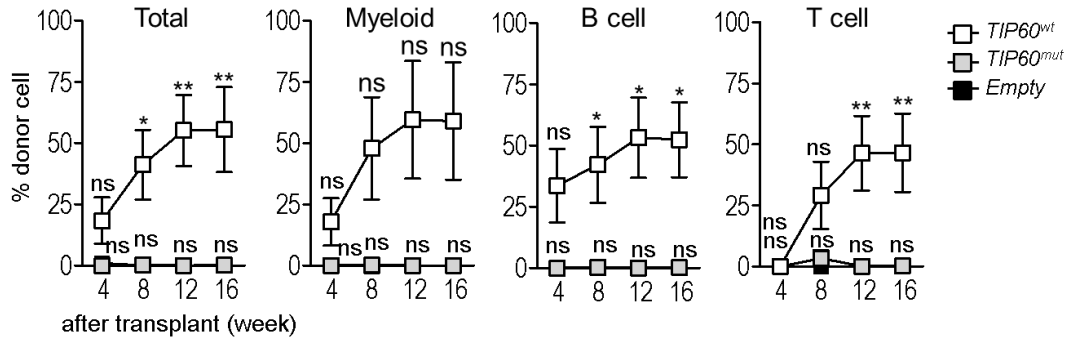
Figure 3. Tip60 acetyltransferase activity is critical for HSC function.

A, Experimental scheme for *in vivo* repopulating assays: *Tip60$^{\Delta/\Delta}$* (*Tip60$^{f/f}$*; *Vav-iCre*) LSK cells at E13.5 (CD45.2$^+$) were transduced with *TIP60* wild-type (*TIP60^{wt}*), catalytically inactive *TIP60* mutant (*TIP60^{mut}*) (Figure S4), and empty vector (Empty) retrovirally, and transplanted into lethally irradiated recipient mice (CD45.1$^+$) along with congenic WBM cells (CD45.1$^+$CD45.2$^+$). Transduction of plasmid DNA was verified by Sanger sequencing of the PCR-amplified genomic products. **B,** Chimerism of the recipients' PB was monitored at different times for 16 weeks after the transplantation. Percentages of donor cells in total (all nucleated cell), myeloid (Gr1$^+$Mac1$^+$), B (CD19$^+$B220$^+$), and T cells (CD3$^+$CD4$^+$) of the recipients' PB are shown. The values are presented as mean ± SEM. Statistical analyses were performed versus *Empty* using ANOVA with Bonferroni's post-test.

A

CD45+ (E14.5)   Adult HSC

% Annexin-V+ DAPI-

□ *control*
■ *Tip60^{Δ/Δ}*

B

CD45+ (E14.5)   Adult c-Kit+
control *Tip60^{Δ/Δ}*   *control* *Tip60^{Δ/Δ}*

cleaved caspase-3 — 15kDa

caspase-3 — 37kDa

C

Fetal LSK
control
29.0   34.0
G1   **S-G2/M**
G0
32.6   4.36

Tip60^{Δ/Δ}
13.9   60.0
17.4   8.72

Ki-67 / Hoechst

HSC distribution (%)

□ *control*
■ *Tip60^{Δ/Δ}*

** G0   ns G1   * S-G2/M

Adult HSC
control
10.1   8.05
78.8   2.99

Tip60^{Δ/Δ}
29.3   33.2
35.3   2.11

Ki-67 / Hoechst

HSC distribution (%)

□ *control*
■ *Tip60^{Δ/Δ}*

*** G0   ** G1   *** S-G2/M

D

control
Sub-G1   2.2
G0/G1   66
S   15.1
G2/M   16.9
Super-G2   0.0

Tip60^{Δ/Δ}
Sub-G1   2.9
G0/G1   38
S   9.1
G2/M   37
Super-G2   13

Count / DAPI

% cells in phase

□ *control*
■ *Tip60^{Δ/Δ}*

ns Sub-G1   ** G0/G1   ns S   * G2/M   * Super-G2

E

Fetal LSK   Adult HSC
control
Tip60^{Δ/Δ}
30 μm

Fetal LSK   Adult HSC

Tail moment per cell

□ *control*
■ *Tip60^{Δ/Δ}*

** (Fetal LSK)   ** (Adult HSC)

F

DAPI   -H2AX   Merge

Adult HSC
control
Tip60^{Δ/Δ}
10 μm

% HSC scored

□ *control*
■ *Tip60^{Δ/Δ}*

ns 0   ns 1   ns 2   * 3   * 4

1

2   **Figure 4. Tip60 loss leads to apoptosis and impairs cell cycle progression in HSCs.**

3   **A,** AnnexinV and DAPI staining of CD45$^+$ cells from E14.5 embryos of control (*Tip60^{fl+}*) (n=3) and

4   *Tip60$^{Δ/Δ}$* (*Tip60$^{f/f}$*; *Vav-iCre*) (n=4) and BM CD150$^+$CD48$^-$LSK cells from control (*Tip60$^{f/f}$*) (n=3) and

5   *Tip60$^{Δ/Δ}$* (*Tip60$^{f/f}$*; *Mx1-Cre*) mice (n=3) (pIpC injection on day 1, 2, and 3, and analysis on day 5).

6   Percentages of AnnexinV$^+$DAPI$^-$ cells are shown. **B,** Immunoblotting of caspase-3 and cleaved caspase-

7   3 using CD45$^+$ cells from E14.5 embryos of control and *Tip60$^{Δ/Δ}$* and BM c-Kit$^+$ cells from control

8   (*Tip60$^{f/+}$*) and *Tip60$^{Δ/Δ}$* mice. **C,** Cell-cycle status of fetal LSK (E14.5) (left) and adult

9   CD150$^+$CD48$^-$LSK cells (right) were analyzed by flowcytometry. Representative dot plots are shown on

10   the left, with average percentages of cells in each phase are graphed on the right. **D,** Left, DNA content

11   analysis by DAPI staining of LSK cells purified from *Tip60$^{f/f}$* and *Tip60$^{f/f}$*; *Rosa26-CreERT2* embryos at

12   E14.5, treated with 4-hydroxytamoxifen (4-OHT) in culture for 5 days. Right, percentages of cells in

13   each phase are graphed. The values are presented as mean ± SEM. **E,** Assessment of DNA breaks by

14   alkaline comet assay. Left, images of control (*Tip60$^{f/+}$*) and *Tip60$^{Δ/Δ}$* fetal LSK cells, and control

15   (*Tip60$^{f/f}$*) and *Tip60$^{Δ/Δ}$* adult CD150$^+$CD48$^-$LSK cells. Right, quantitative values of tail DNA moment. **F,**

16   Left, images of immunostaining of γ-H2AX in control (*Tip60$^{f/f}$*), and *Tip60$^{Δ/Δ}$* CD150$^+$CD48$^-$LSK cells.

17   Right, percentages of cells (Y-axis) which have corresponding number of γ-H2AX foci (X-axis) are

18   graphed. The values are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns; not

19   significant.

A

Gene set	Size	NES	FDR q-value
SCHUHMACHER_MYC_TARGETS_UP	70	2.429	0.000
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	44	2.332	0.000
DANG_MYC_TARGETS_UP	123	2.331	0.000
SCHLOSSER_MYC_TARGETS_REPRESSED_BY_SERUM	127	2.296	0.000
SANSOM_APC_TARGETS_REQUIRE_MYC	165	2.271	0.000
MYCMAX_01	196	2.242	0.000
ODONNELL_TARGETS_OF_MYC_AND_TFRC_DN	35	2.211	0.000
YU_MYC_TARGETS_UP	35	2.168	0.000
DANG_REGULATED_BY_MYC_UP	59	2.074	0.001
MORI_EMU_MYC_LYMPHOMA_BY_ONSET_TIME_UP	84	2.009	0.002
COLLER_MYC_TARGETS_UP	22	1.904	0.006
MENSSEN_MYC_TARGETS	44	1.866	0.009

B

C

D
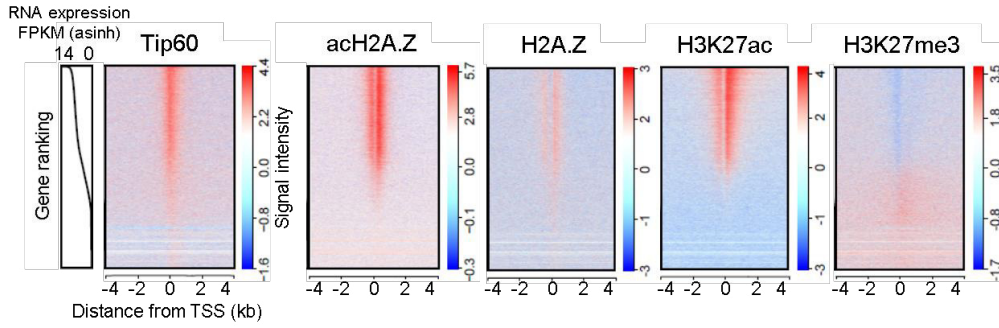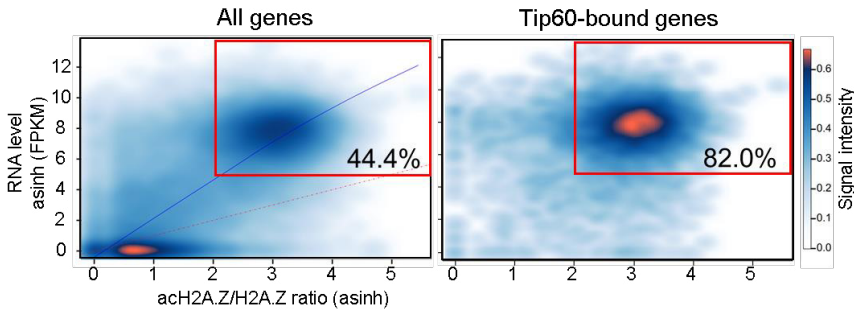

20

Figure 5. Tip60 regulates Myc target genes.

A, Left, a plot of false discovery rate (FDR) versus normalized enrichment score (NES) based on GSEA from RNA-Seq data of control (*Tip60^{f/f}*) versus *Tip60^{Δ/Δ}* LSK cells. Distribution of all transcription factor target gene sets obtained from the MSigDB (c2 and c3) is shown. The red dots indicate the gene sets for Myc, and the gray for the non-Myc transcription factors. The dashed line represents the FDR cutoff. Right, list of enriched Myc-associated gene sets. **B,** Representative GSEA plots for Myc target gene sets. **C,** Correlation analysis of genome-wide occupancy for Tip60 and 10 transcriptional factors (c-Myc, Tal1, Gata2, Lyl1, Lmo2, Runx1, Fli1, Meis1, Gfi1b, and Spi1) in a murine hematopoietic progenitor cell line HPC-7 cells, using normalized pointwise mutual information (NPMI). The color intensity represents the correlation efficiency of each two transcriptional factors. A total 3651 c-Myc binding peaks, as well as 4347 Tip60, 7596 Tal1, 2796 Gata2, 3432 Lyl1, 5202 Lmo2, 5992 Runx1, 18796 Fli1, 7690 Meis1, 3279 Gfi1b, and 18249 Spi1 peaks were identified. **D,** Left, Hematopoietic progenitor cells 32D were transduced with FLAG-TIP60. Whole cell lysates were prepared from the transfected cells and subjected to immunoprecipitation with FLAG–M2 beads. Proteins present in IP or whole cell lysates (input) were separated by SDS-PAGE and immunoblotted with antibodies for FLAG and c-Myc. Right, Interaction was confirmed by reciprocal co-IP.
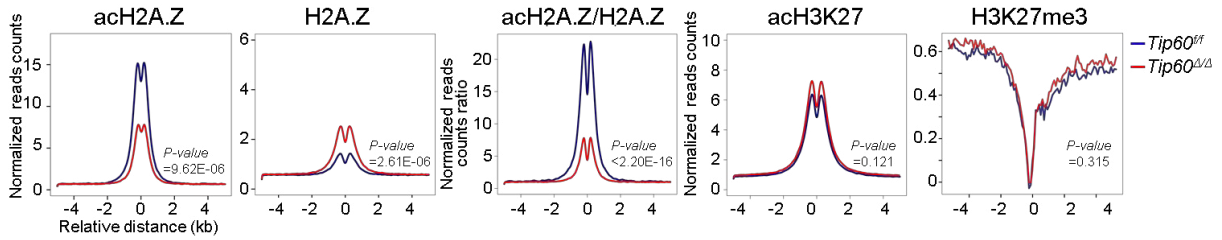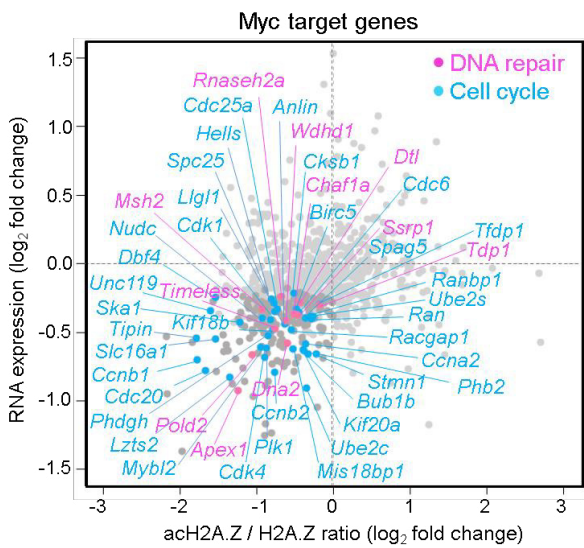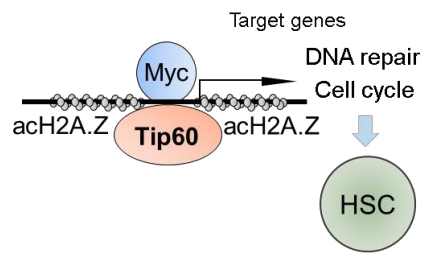
A

RNA expression FPKM (asinh)

Tip60, acH2A.Z, H2A.Z, H3K27ac, H3K27me3

Gene ranking

Signal intensity

Distance from TSS (kb)

B

All genes — 44.4%

Tip60-bound genes — 82.0%

RNA level asinh (FPKM)

acH2A.Z/H2A.Z ratio (asinh)

Signal intensity

C

acH2A.Z, H2A.Z, acH2A.Z/H2A.Z, acH3K27, H3K27me3

Normalized reads counts

Normalized reads counts ratio

Relative distance (kb)

P-value =9.62E-06

P-value =2.61E-06

P-value <2.20E-16

P-value =0.121

P-value =0.315

Tip60^{f/f}
Tip60^{Δ/Δ}

D

Myc target genes

- DNA repair
- Cell cycle

RNA expression (log$_2$ fold change)

acH2A.Z / H2A.Z ratio (log$_2$ fold change)

Rnaseh2a, Cdc25a, Anlin, Wdhd1, Hells, Cksb1, Dtl, Spc25, Chaf1a, Cdc6, Msh2, Llgl1, Birc5, Ssrp1, Tfdp1, Nudc, Cdk1, Spag5, Tdp1, Dbf4, Ranbp1, Unc119, Timeless, Ube2s, Ska1, Kif18b, Ran, Tipin, Racgap1, Slc16a1, Ccna2, Ccnb1, Dna2, Stmn1, Phb2, Cdc20, Bub1b, Phdgh, Pold2, Ccnb2, Kif20a, Lzts2, Apex1, Plk1, Ube2c, Mybl2, Cdk4, Mis18bp1

E

Target genes
DNA repair
Cell cycle

Myc

Tip60

acH2A.Z   acH2A.Z

HSC

37

Figure 6. Tip60 maintains the acetylation of H2A.Z to activate Myc target genes.

A, An integrated view of Tip60 occupancy and acH2A.Z, H2A.Z, H3K27ac and H3K27me3 enrichment around transcriptional start sites (TSS$\pm$5 kb) in ChIP-seq analysis using wild type fetal c-Kit$^+$ cells. Genes were ordered according to the RNA expression levels (line plots) from high (top) to low (bottom). The color scale represents changes in signal intensity for each antibody. **B,** Cloud plots represent all (left) and Tip60-bound genes (right) according to the corresponding acH2A.Z/H2A.Z ratio and the RNA expression level. Correlation analysis between the acH2A.Z/H2A.Z ratio and the RNA expression level is shown. The blue line represents polynomial regression curve ($R^2 = 0.7$). The red line represents theoretical linear regression curve (Y=X). Genes with high acH2A.Z/H2A.Z ratio (> 2) acH2A.Z and high RNA level ($\log_2$ FPKM value > 5) are squared in red. Tip60 bound genes were identified based on Tip60 enrichment at the proximal promoter region (-1 kb, $+100$ bp from TSS). **C,** Mean-plot of acH2A.Z, H2A.Z, acH2A.Z/H2A.Z ratio, acH3K27 and H3K27me3 enrichment based on relative distance from Tip60-bound proximal promoter sites in both control (purple) and *Tip60$^{\Delta/\Delta}$* fetal c-Kit$^+$ cells (red). Statistical analyses were performed using Mann-Whitney-Wilcoxon method. **D,** 816 Myc target genes were identified to be transcribed in both control and *Tip60$^{\Delta/\Delta}$* fetal LSK cells in the RNA-seq analysis. X-axis indicates $\log_2$ fold change in acH2A.Z/H2A.Z ratio at TSS ($\pm$1kb) induced by *Tip60* deletion and Y-axis indicates $\log_2$ fold change in RNA expression level. DNA repair related genes (blue) and cell-cycle related genes (pink) with significant downregulation in their RNA levels ($p < 0.05$) are labelled. Myc target genes are obtained from the gene sets described in Figure 5A. DNA repair and cell-cycle related genes belong to Gene Ontology category GO:0006281 and GO:0007049, respectively. **E,** Tip60 maintains HSC thorough acetylation of H2A.Z to activate Myc target genes, regulating cell-cycle and DNA repair processes.