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Supplemental Information

Control of Hematopoietic Stem and Progenitor Cell Function through Epigenetic Regulation of Energy Metabolism and Genome Integrity

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SUPPLEMENTAL INFORMATION

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Zhenhua Yang[1], Kushani Shah[1], Alireza Khodadadi-Jamayran[1], and Hao Jiang[1, 2]

INVENTORY OF SUPPLEMENTAL INFORMATION

- **Supplemental figures 1-7 and legends**
- **Supplemental tables 1-3 and legends. Table S2 is supplied as an Excel file.**
- **Supplemental experimental procedures**
- **Supplemental references**

Supplemental Figure 1

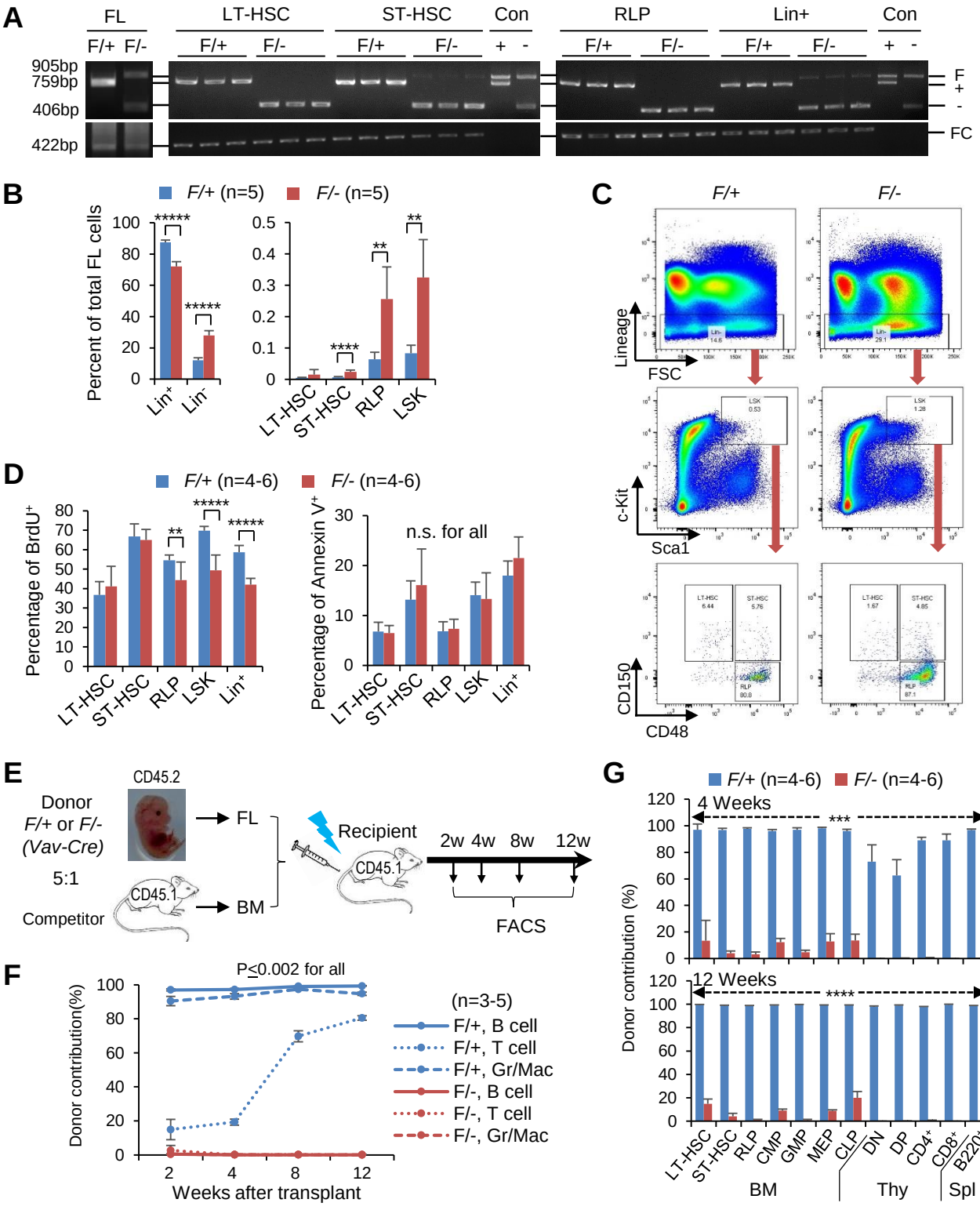


Figure S1. Dpy30 deficiency in fetal liver results in anemia and defective HSC function.

Vav-Cre; Dpy30$^{F/+}$ (*Fl*/+) and *Vav-Cre; Dpy30*$^{F/-}$ (*Fl*/-) littermate fetuses were used in all panels.

(A) Genomic PCR to detect *Dpy30* deletion in total fetal liver and FACS sorted fetal liver cell populations as indicated at E14.5. Each lane was from an embryo. The calculated sizes of the PCR products (shown on the right) are consistent with the mobility of the bands. Note that the recombination in Lin+ cells was inefficient. Con (+) and Con (-) were from *Dpy30*$^{F/+}$ and *Dpy30*$^{F/-}$ (no Cre) fetal liver cells, respectively.

(B) Percentage of different cell populations in fetal liver. n=5 each.

(C) Representative FACS analysis of fetal liver cells at E14.5.

(D) BrdU incorporation (left) and annexin V staining (right) assays for different cell populations in fetal liver at E14.5. n=4-6.

(E) Scheme for the competitive transplantation system using whole fetal liver cells from *Vav-Cre*; *Dpy30*$^{F/+}$ and *Vav-Cre*; *Dpy30*$^{F/-}$ as donors and wild type BM as competitors.

(F) Donor contribution to indicated peripheral blood cells was determined by FACS at different times post transplantation following the scheme in (e). n=3-5 mice.

(G) Donor contribution to different cell populations in bone marrow (BM), thymus (Thy) and spleen (Spl) at 4 weeks (top) and 12 weeks (bottom) after transplantation. n=4-6 mice.

Data are shown as mean ± SD for all bar graphs and (F). n.s., not significant, **P<0.01, ***P<0.001, ****P<0.0001, ***** P<0.00001, 2-tailed Student's *t*-test.

Related to Figure 1.

Supplemental Figure 2

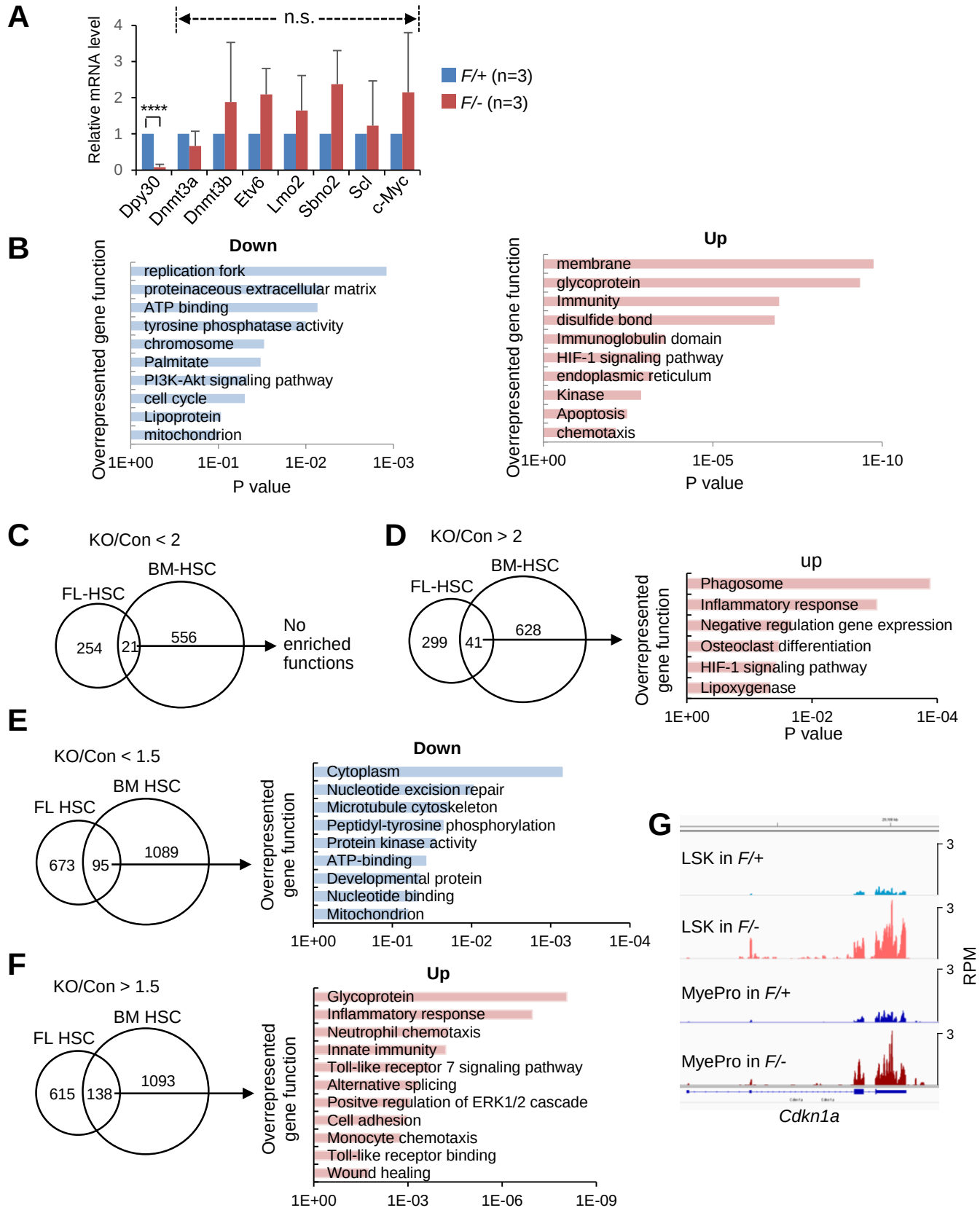


Figure S2. Analysis of genes significantly altered by Dpy30 loss in HSCs.

(A) Relative mRNA levels of indicated genes in the HSCs sorted from fetal livers of *Vav-Cre; Dpy30*$^{F/+}$ and *Vav-Cre; Dpy30*$^{F/-}$ littermate fetuses at E14.5 were determined by RT-qPCR and normalized to *Actb*. Data are shown as mean ± SD. n=3 each. n.s., not significant, **** P<0.0001, by 2-tailed Student's *t*-test.

(B) Gene ontology analysis by DAVID for genes that were downregulated or upregulated over 2 fold in HSCs from 3 pairs of fetal littermates.

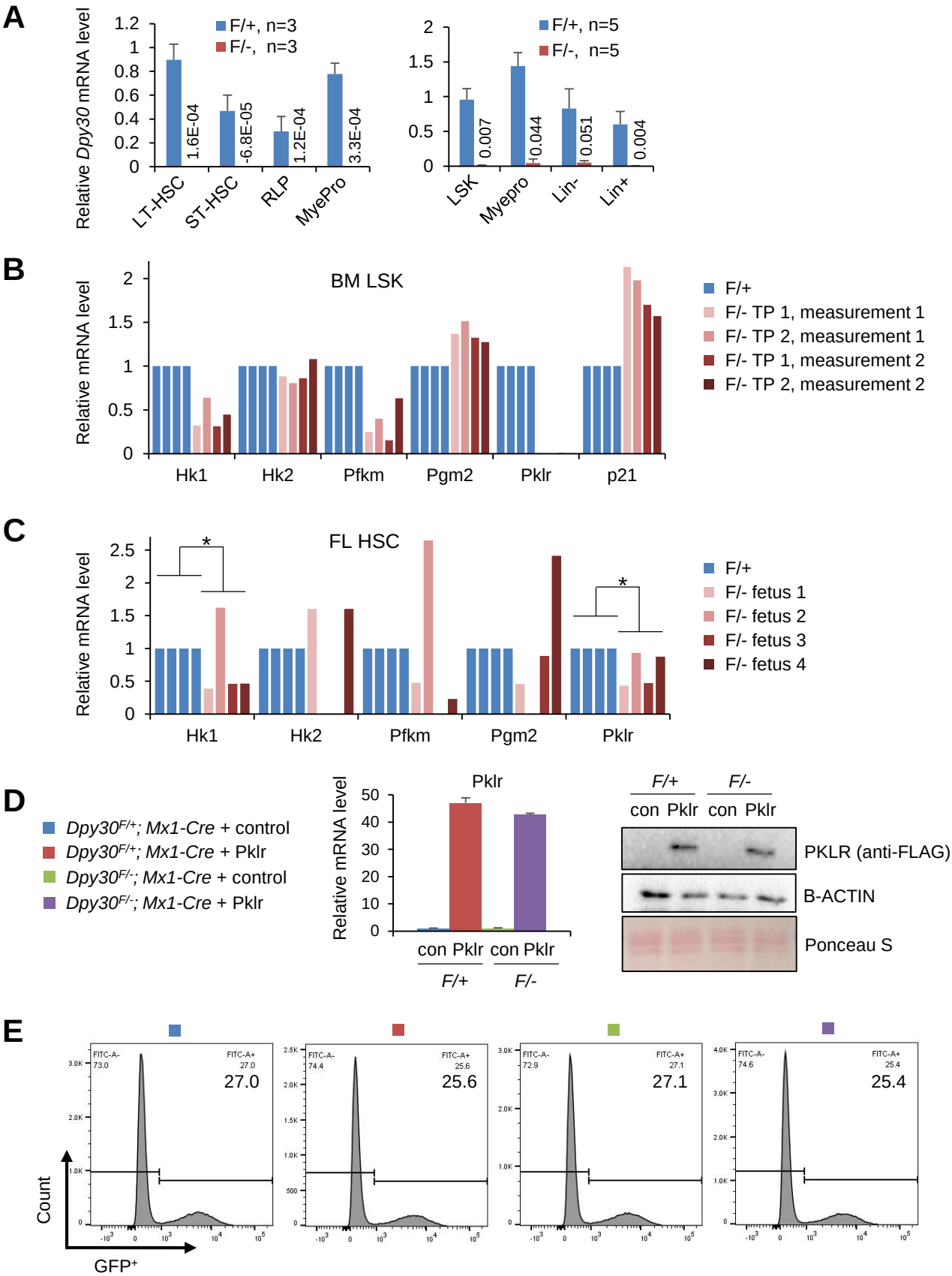
(C and D) Venn diagram for genes that were downregulated (C) or upregulated (D) over 2 fold in HSCs from 3 pairs of fetal littermates and 2 pairs of BM HSCs (left). Gene ontology analysis by DAVID of the shared genes are shown (right). In panels (C-G), the BM HSC data are based on RNA-seq results in donor (*Mx1-Cre; Dpy30*$^{F/+}$ or *Mx1-Cre; Dpy30*$^{F/-}$) - derived HSCs in the BM chimera recipients in two independent BM transplantations (TP1 and TP2) from our previous work (Yang et al., 2016).

(E and F) Venn diagram for genes that were downregulated (E) or upregulated (F) over 1.5 fold in HSCs from 3 pairs of fetal littermates and 2 pairs of BM HSCs (left). Gene ontology analysis by DAVID of the shared genes are shown (right).

(G) RNA-seq profiles at *Cdkn1a* (*p21*) from one BM chimera transplant assay.

Related to Figure 1.

Supplemental Figure 3



Supplemental Figure 3, continued

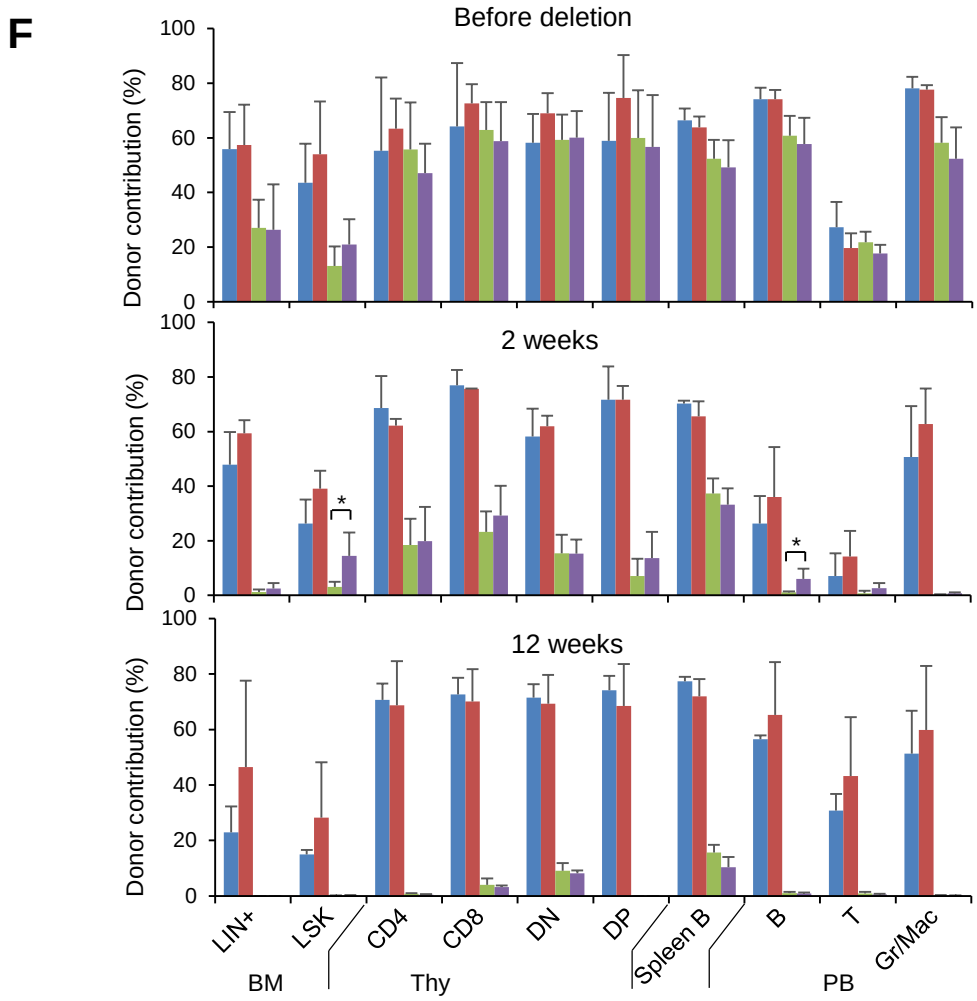


Figure S3. Downregulation of key glycolytic genes by Dpy30 loss.

(A) Relative mRNA levels of *Dpy30* by RT-qPCR and normalized to *Actb*, in FACS sorted BM cell populations from *Mx1-Cre; Dpy30$^{F/+}$* (*F/+*) and *Mx1-Cre; Dpy30$^{F/-}$* (*F/-*) mice after pIpC injections.

(B and C) Relative mRNA levels of indicated genes in the donor (*Mx1-Cre; Dpy30$^{F/+}$* or *Mx1-Cre; Dpy30$^{F/-}$*) - derived LSK cells in the BM chimera recipients in two independent BM transplantations (TP1 and TP2) from previous work (Yang et al., 2016) for (B), and from fetal livers of *Vav-Cre; Dpy30$^{F/+}$* and *Vav-Cre; Dpy30$^{F/-}$* littermate fetuses at E14.5 for (C), by RT-qPCR and normalized to *Actb* , and the data are shown for each measurement.

(D) Relative expression of FLAG-*Pklr* from retrovirally-transduced BM cells determined by RT-qPCR (left) and immunoblotting (right) prior to the transplant. The *Pklr* mRNA levels were normalized to *Actb* and relative to the level in the *Dpy30$^{F/+}$; Mx1-Cre* + control, and are shown as mean ±SD of technical duplicate. Anti-FLAG antibody was used as our anti-PKLR antibody failed to react with mouse PKLR. Legends are the same in (D-F).

(E) FACS analysis of the retroviral transduction efficiency of BM cells, shown by the percentage of GFP-positive population.

(F) Donor contribution to indicated cell populations in bone marrow (BM), thymus (Thy), spleen, and peripheral blood (PB) before deletion (top), 2 weeks (middle) and 12 weeks (bottom) after pIpC-injections in the transplant recipients following scheme in Figure 2G. n=4 each.

Data are shown as mean ±SD. *P<0.05, **P<0.01, ***P<0.001, by 2-tailed Student's *t*-test.

Related to Figure 2.

Supplemental Figure 4

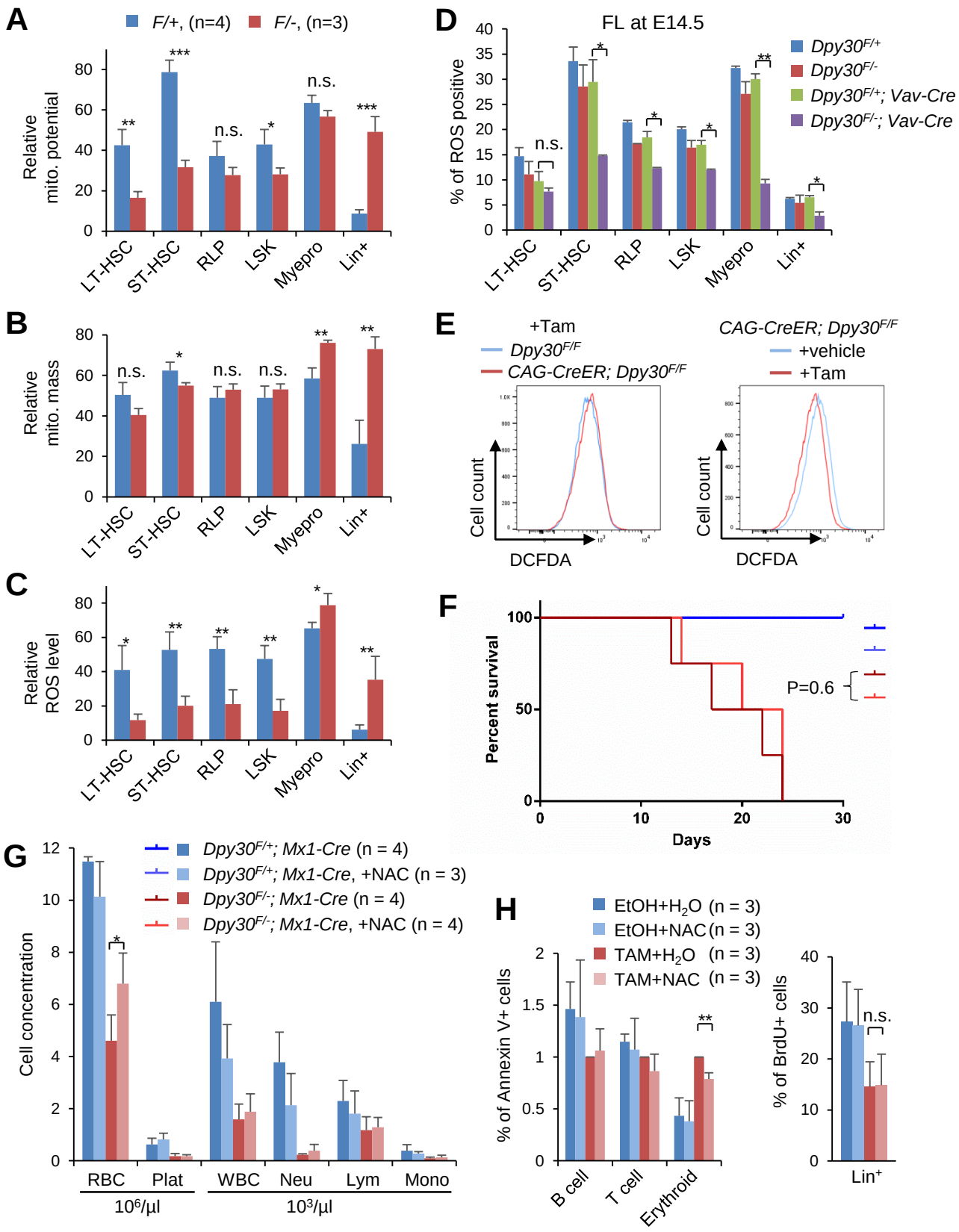


Figure S4. Dpy30 deficiency impairs mitochondrial function in different types of cells.

(A-C) Mitochondrial membrane potentials (A), mitochondrial mass (B) and intracellular ROS levels (C) in different BM cell populations from pIpC-injected *Mx1-Cre; Dpy30$^{F/+}$* (*F/*+) and *Mx1-Cre; Dpy30$^{F/-}$* (*F/*-) mice were determined by FACS. Because these values were vastly different for MyePro and Lin$^+$ cells (much higher) compared to the cell populations, gating criterion for these values were the same for LT-HSC, ST-HSC, RLP, and LSK cells, but different for MyePro, and that for Lin$^+$ cells was also different from all other cells.

(D) Intracellular ROS levels in different cell populations of fetal livers of indicated genotypes at E14.5. n=2 each.

(E) Primary MEFs of indicated genotypes were treated with vehicle (ethanol) or 4-OH tamoxifen (Tam), and intracellular ROS levels were determined by FACS.

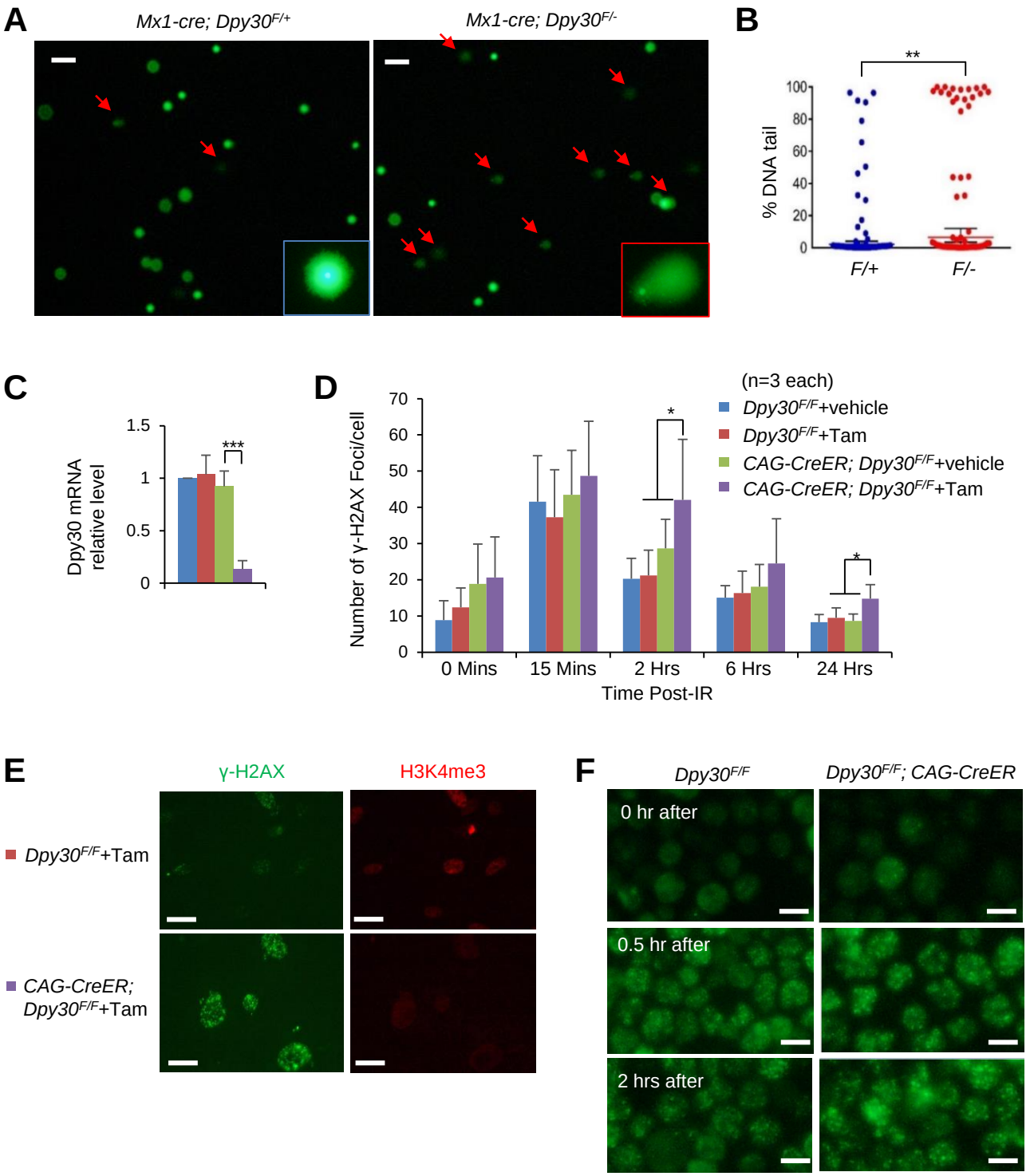
(F and G) Effects of antioxidant administration on hematopoiesis of Dpy30-deficient animals. Legends in (G) apply to (F) and (G). Indicated number of pIpC-injected *Mx1-Cre; Dpy30$^{F/+}$* and *Mx1-Cre; Dpy30$^{F/-}$* mice were on NAC-containing drinking water or not, and were examined for survival (F) and peripheral blood profiling (G),

(H) Lin$^+$ BM cells from three *CAG-CreER; Dpy30$^{F/F}$* mice were treated with either EtOH control or tamoxifen (TAM), and water control or NAC in culture for two days, and subjected to assays for apoptosis by Annexin V staining and for cell proliferation by in vitro BrdU incorporation.

Data are shown as mean $\pm$SD for all panels. Number of animals are indicated. n.s., not significant, *P<0.05, **P<0.01, ***P<0.001, by 2-tailed Student's *t*-test, except by 1-factor ANOVA with post hoc *t* test for (D) and log-rank test for (F).

Related to Figure 3.

Supplemental Figure 5



Supplemental Figure 5, continued

G

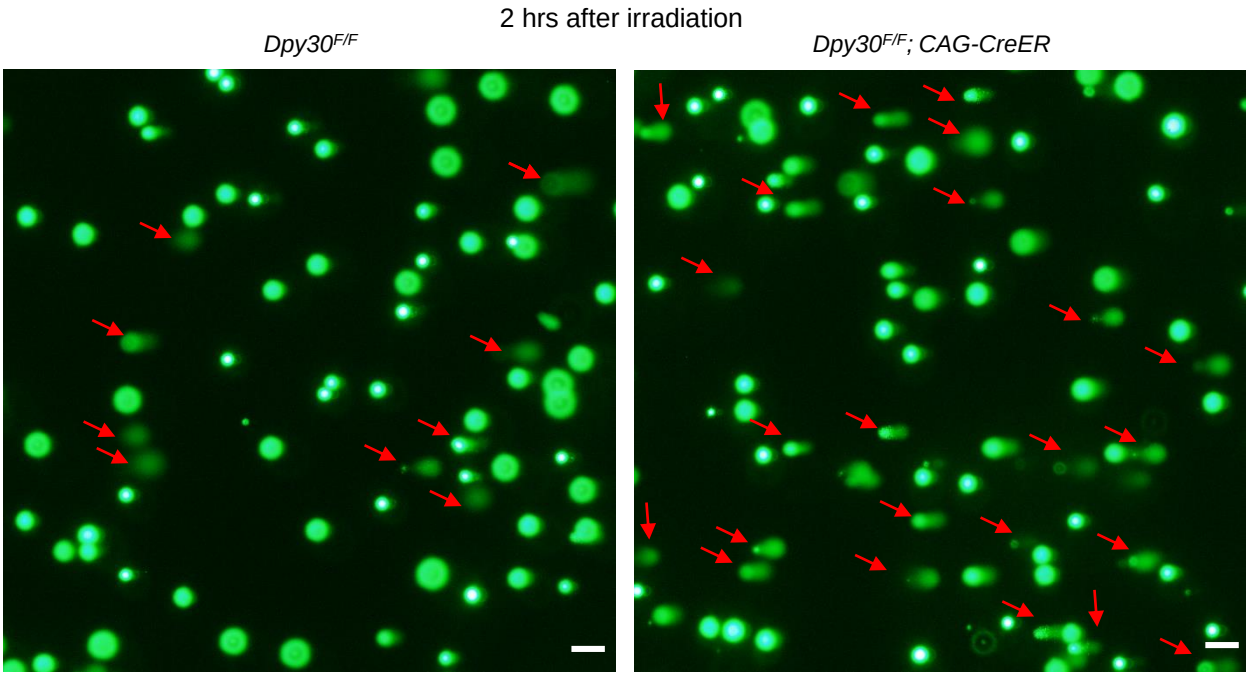


Figure S5. Dpy30 deficiency results in increase in DNA damage and impairs DNA repair.
(A and B) Comet assay using BM LSK cells pooled from 3 mice each, with representative images in (A) and quantification of the relative tailing level of cells in (B). Red arrows point to comet-shaped cells with significant DNA damage. The representative zoom-in images of an undamaged (blue box) and damaged (red box) cells are shown in the insets. In (B), each dot represents a cell analyzed by the OpenComet software, and 100% means maximum tail DNA (most damage) and 0% means tail DNA (no damage).
(C-E) Primary MEFs of indicated genotypes were treated with vehicle (ethanol) or 4-OH tamoxifen, and subjected to ionizing radiation at 0 min, followed by γ-H2AX staining at indicated times after radiation. Relative *Dpy30* mRNA levels were determined by RT-qPCR and normalized to *Actb*, n=2 for each of the left two bars and n=4 for each of the right two bars (C). The γ-H2AX foci numbers per cell from 20-60 cells in each repeat of 3 biological repeats are plotted (D). Representative images of indicated cells at 2 hrs post irradiation are shown (E).
(F and G) Representative images of γ-H2AX staining (F) and comet assay (G) for LSK cells from indicated animals at indicated times post irradiation following the scheme in Figure 4B. (G) is from 2 hrs after irradiation.
Data are shown as mean ±SD. *P<0.05, **P<0.01, ***P<0.001, by 2-tailed Student's *t*-test for (B) and 1-factor ANOVA with post hoc *t* test for (C) and (D). Scale bars, 50 μm (S5A), 10 μm (S5E and S5F), 20 μm (S5G).
Related to Figure 4.

Supplemental Figure 6

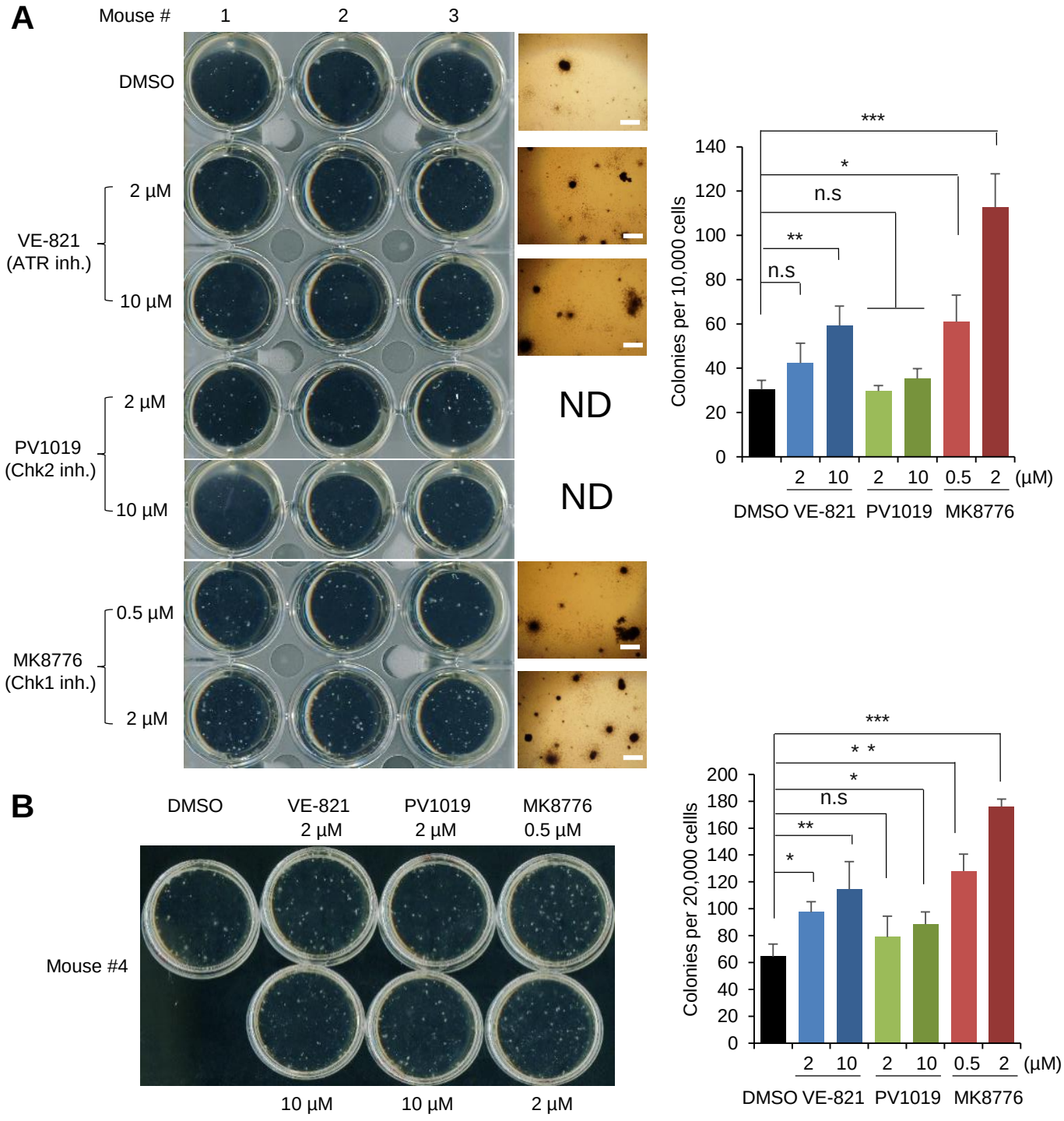


Figure S6. DDR Inhibition partially rescues the function of the Dpy30-deficient hematopoietic cells. Lin$^-$ BM cells from *CAG-CreER; Dpy30$^{F/F}$* mice were treated with indicated agents in liquid culture for two days and subjected to colony formation assays in two independent sets of experiment in (A) and (B). In (A), 3 mice (#1, 2, 3) were used and 10,000 cells were seeded. In (B), 2 mice (#4, 5) were used and 20,000 cells were seeded. Representative image of the plates and colonies are shown on the left, and quantification for all colonies is shown on the right. ND, not done.
Data are shown as mean ±SD for all bar graphs. *P<0.05, **P<0.01, ***P<0.001, by 1-factor ANOVA with post hoc *t* test. Scale bars, 300 μm.
Related to Figure 5.

Supplemental Figure 7

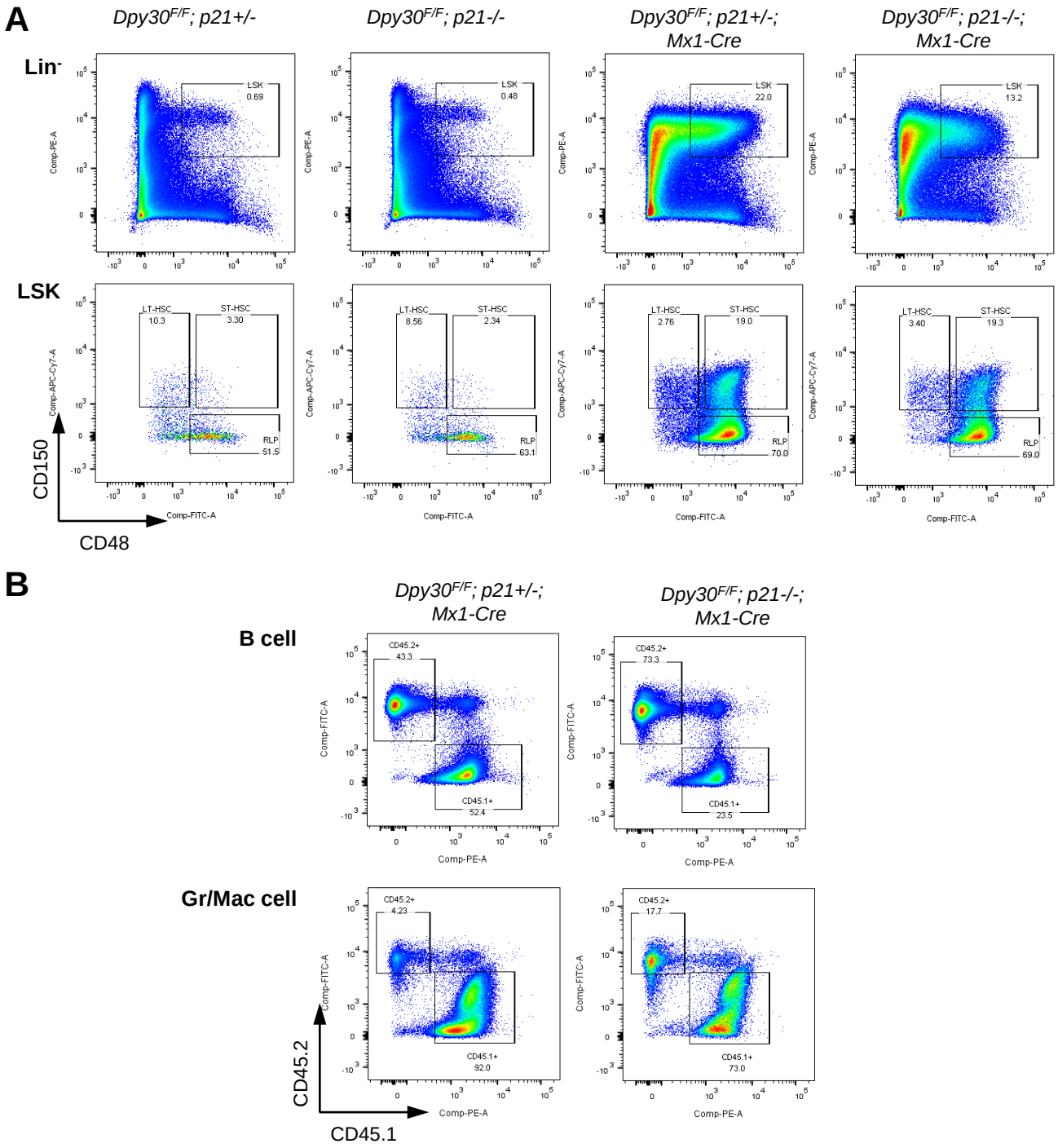


Figure S7. *P21* deletion partially rescues the function of the *Dpy30* KO hematopoietic cells.
(A) Representative FACS analysis of BM from pIpC-injected mice of indicated genotypes.
(B) Representative FACS analysis of B cells in spleen and granulocytes & macrophages in peripheral blood from the recipient mice that received donors of indicated genotypes (top) based on the scheme in Figure 6B.
Related to Figure 6.

SUPPLEMENTAL TABLES

Table S1: Cell surface phenotypes used in this work

Cell population	Cell surface phenotype
Lin$^-$ (Fetal liver)	CD3$^-$ CD4$^-$ CD5$^-$ CD8$^-$ B220$^-$ Gr1$^-$ TER119$^-$
Lin$^-$ (Bone marrow)	CD3$^-$ B220$^-$ CD11b Gr1$^-$ TER119$^-$
Lin$^+$	Cells excluding Lin$^-$
LSK	Lin$^-$Sca1$^+$cKit$^+$
LT-HSC	LSK CD48$^-$CD150$^+$
ST-HSC	LSK CD48$^+$CD150$^+$
RLP	LSK CD48$^+$CD150$^-$
MyePro	Lin$^-$Sca1$^-$cKit$^+$ (L$^-$S$^-$K$^+$)
CLP	Lin$^-$Sca1^{low}cKit^{low}IL7R$^+$CD135$^+$
CMP	Lin$^-$Sca1$^-$cKit$^+$CD34$^+$FcRγ$^-$
MEP	Lin$^-$Sca1$^-$cKit$^+$ CD34$^-$FcRγ$^-$
GMP	Lin$^-$Sca1$^-$cKit$^+$ CD34$^+$FcRγ$^+$
B cell	B220$^+$
T cell	CD3ε$^+$
DN	CD4$^-$CD8$^-$
Erythroid	Ter-119$^+$
DP	CD4$^+$CD8$^+$
Gr/Mac	Gr1$^+$ or Mac1$^+$

Table S2. Gene expression analyses in fetal liver HSCs in control and *Dpy30* KO background. This Table is separately attached as an Excel file. There are 14 tabs in this file. Fetal liver (FL) HSCs from 3 pairs of *Vav-Cre; Dpy30$^{F/+}$* (control) and *Vav-Cre; Dpy30$^{F/-}$* (KO) fetuses, each pair from the same litter, at E14.5 were sorted out for RNA-Seq. Gene expression data for the bone marrow (BM) HSCs are from our previous work (Yang et al., 2016) and are included here for convenience of comparison. The BM data are from two transplant (TP1 and TP2) recipients at two weeks after pIpC induction. These TP recipients received *Mx1-Cre; Dpy30$^{F/+}$* (control) and *Mx1-Cre; Dpy30$^{F/-}$* (KO) donor BM cells and were then injected with pIpC. Compared to control, genes up- or down-regulated more than 2 (or 1.5) fold in both BM TPs are shown in tabs "BM up>2 (or 1.5)" and "BM down>2 (or 1.5)". All normalized FL HSC RNA-Seq data with three fetuses of each genotype are listed in tab "All genes in FL HSC". Genes up- or down-regulated in FL HSCs with mean value more than 2 fold and $p < 0.05$ are listed in tabs "FL down >2" and "FL up>2". Genes up- or down-regulated more than 1.5 fold in all three littermate pairs are listed in tabs "FL down >1.5" and "FL up>1.5". Tabs "BM & FL down>2 (or 1.5)" show overlapping genes between tabs "BM down>2 (or 1.5)" and "FL down >2 (1.5)". Tabs "BM & FL up>2 (1.5)" show overlapping genes between tabs "BM up>2" and "FL up>2".

Table S3: Primers and Oligos

App.	Species	gene	Forward primer (5'-3')	Backward primer (5'-3')
expression	mouse	*Actb*	CCTTCAACACCCCAGCCATGTACG	GGCACAGTGTGGGTGACCCCGTC

expression	mouse	*Acsl6*	ATGCAGAGGAACTCAACTACTG	CTTTGTCCTGTCCTCGTCTTT
expression	mouse	*Cdkn1a*	CTGGGAGGGGACAAGAG	GCTTGGAGTGATAGAAATCTG
expression	mouse	*Dnah1*	GCTGGTACAAGAGGTCATTAGG	CTCGAGCTCCAAGGACATTAC
expression	mouse	*Dnmt3a*	AGCGTCACACAGAAGCATATC	GGCGGTAGAACTCAAAGAAGAG
expression	mouse	*Dnmt3b*	TCTGAGATCTCTGCTGACAAAC	GTGGTACATGGCCTTCCTATAA
expression	mouse	*Dpy30*	ACCCTCACTCTGAGTACGGG	GGACTGTAGATCCACCTTCTGT
expression	mouse	*Etv6*	TGCTCTTCTCACCATTCTTCC	GTCCTCTGGACACAGTTATCTTC
expression	mouse	*Hk1*	CTGGAATCTCCCATCGCTTTAG	GGAAACACCACTCCGACTTT
expression	mouse	*Hk2*	CTCGGCCTTGTGAGTAGATAGA	CGCGGGTGAATGAGGTATTT
expression	mouse	*Igdcc4*	CAAAGCCCTGTGTCCTCTAAC	GCATGTTCCTGAGCCATATCT
expression	mouse	*Lmo2*	TGCGGGTGAAAGACAAAGT	GTCGGAGTTGATGAGAAGGTATC
expression	mouse	*Myc*	CCACCAGCAGCGACTCTGA	TGCCTCTTCTCCACAGACACC
expression	mouse	*Ntpcr*	CAGGAAGGGAAGAGGATAGGA	TCGGGCTTTCCAGGTAGA
expression	mouse	*Pfkm*	GCATCCCATTTGTGGTCATTC	GTCACAGGTTGTGCAGATAGT
expression	mouse	*Pgk1*	CACAGAAGGCTGGTGGATTT	CTTTAGCGCCTCCCAAGATAG
expression	mouse	*Pklr*	GAGTCGGAGGTGGAAATTGT	CCTGGGTGATATTGTGGTAGTC
expression	mouse	*Pkn3*	GGACACAGAAGAGGAGGTATTTG	CTTCTGGAGGAGCTTCTGAATG
expression	mouse	*Sbno2*	CTTGGATGTGGAGAAGGACTG	CGAAGGTGTCGGAGAAGTATTG
expression	mouse	*Scl*	CACCCACCAGACAAGAAACTA	CTCCTCCTGGTCATTGAGTAAC
expression	mouse	*Zcchc3*	GAAGACCCTCTTCATCCTCTTC	GAACCTGTCGGTCACTTTCA
expression	mouse	*Hbb*	GAAGCGATTCTAGGGAGCAG	GGAGCAGCGATTCTGAGTAGA
expression	mouse	*mt-Co2*	GCCGACTAAATCAAGCAACA	CAATGGGCATAAAGCTATGG
App.	**Species**	**gene**	**Forward primer (5'-3')**	**Backward primer (5'-3')**
shRNA	mouse	*Scramble*	CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG	AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG
shRNA (1#)	mouse	*Acsl6*	CCGGTGCACGGTAATCGTCGATAAACTCGAGTTTATCGACGATTACCGTGCATTTTTG	AATTCAAAAATGCACGGTAATCGTCGATAAACTCGAGTTTATCGACGATTACCGTGCA
shRNA (2#)	mouse	*Acsl6*	CCGGGCAAAGGAGAAGGAGAGATATCTCGAGATATCTCTCCTTCTCCTTTGCTTTTTG	AATTCAAAAAGCAAAGGAGAAGGAGAGATATCTCGAGATATCTCTCCTTCTCCTTTGC
shRNA (1#)	mouse	*Dnah1*	CCGGCGAGCATATCAATCCTGACAACTCGAGTTGTCAGGATTGATATGCTCGTTTTTG	AATTCAAAAACGAGCATATCAATCCTGACAACTCGAGTTGTCAGGATTGATATGCTCG
shRNA (2#)	mouse	*Dnah1*	CCGGCGAGGCGTCATCAGAAATTAACTCGAGTTAATTTCTGATGACGCCTCGTTTTTG	AATTCAAAAACGAGGCGTCATCAGAAATTAACTCGAGTTAATTTCTGATGACGCCTCG
shRNA (1#)	mouse	*Igdcc4*	CCGGGCGAGCAAATCATTGGCTTCTCTCGAGAGAAGCCAATGATTTGCTCGCTTTTTG	AATTCAAAAAGCGAGCAAATCATTGGCTTCTCTCGAGAGAAGCCAATGATTTGCTCGC
shRNA (2#)	mouse	*Igdcc4*	CCGGCCATTTACCAAGTACGAGTTTCTCGAGAAACTCGTACTTGGTAAATGGTTTTTG	AATTCAAAAACCATTTACCAAGTACGAGTTTCTCGAGAAACTCGTACTTGGTAAATGG
shRNA (1#)	mouse	*Ntpcr*	CCGGGCGGTTTGAGTAAATTCTAAACTCGAGTTTAGAATTTACTCAAACCGCTTTTTG	AATTCAAAAAGCGGTTTGAGTAAATTCTAAACTCGAGTTTAGAATTTACTCAAACCGC
shRNA (2#)	mouse	*Ntpcr*	CCGGCCAAACACAGAGTGTGTATCACTCGAGTGATACACACTCTGTGTTTGGTTTTTG	AATTCAAAAACCAAACACAGAGTGTGTATCACTCGAGTGATACACACTCTGTGTTTGG
shRNA (1#)	mouse	*Pkn3*	CCGGCGCTATCAAGGCGTTGAAGAACTCGAGTTCTTCAACGCCTTGATAGCGTTTTTG	AATTCAAAAACGCTATCAAGGCGTTGAAGAACTCGAGTTCTTCAACGCCTTGATAGCG
shRNA (2#)	mouse	*Pkn3*	CCGGGACACAGAAGAGGAGGTATTTCTCGAGAAATACCTCCTCTTCTGTGTCTTTTTG	AATTCAAAAAGACACAGAAGAGGAGGTATTTCTCGAGAAATACCTCCTCTTCTGTGTC
shRNA (1#)	mouse	*Zcchc3*	CCGGGACGACATCTACGCGGTCATTCTCGAGAATGACCGCGTAGATGTCGTCTTTTTG	AATTCAAAAAGACGACATCTACGCGGTCATTCTCGAGAATGACCGCGTAGATGTCGTC
shRNA (2#)	mouse	*Zcchc3*	CCGGGCAGCCCAAGACGTGCTTTAACTCGAGTTAAAGCACGTCTTGGGCTGCTTTTTG	AATTCAAAAAGCAGCCCAAGACGTGCTTTAACTCGAGTTAAAGCACGTCTTGGGCTGC
App.	**Species**	**gene**	**Forward primer (5'-3')**	**Backward primer (5'-3')**
genotyping	mouse	*Dpy30*	TTCAGCACCAGCACTTGGATTTGG (within 5' arm)	CCAACTGACCTTGGGCAAGAACAT (targets "-" allele)
genotyping	mouse	*Dpy30*		GCACAGCAAGTCCCTGTAATA (within exon 4)
genotyping	mouse	*Dpy30*	CCAGGACTAAAGGCGCATAA (targets "FC" allele)	GCCAAGGCTACACAGAGAAA (within 3' arm)
App.	**Species**	**gene**	**Forward primer (5'-3')**	**Backward primer (5'-3')**
ChIP	mouse	*Hk1* (TSS-273bp)	TCAGAGGGAAGTGGGATAGAG	CAGCCCACTAGGTTTCATCTT
ChIP	mouse	*Pfkm* (TSS+30bp)	CCAAGCTCAAGACAAGAGGAA	GCTCATCGCCTCTTGGTAAT
ChIP	mouse	*Pklr* (TSS+29bp)	CAGATAAGACCAGCAGTTAGGG	GTAGCTCGTTCTCTTGGACAG
ChIP	mouse	*Punc* (TSS-4260bp) (*Neg)*	AGCCATGCCATCTCTCTGGTTTCT	CACTTGATGTGCCAGCCAGTTGAA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transplantation

For competitive transplantation assays, the donor FLs were separated from E14.5 embryos (CD45.2$^+$, *Vav-Cre; Dpy30$^{F/+}$* and *Vav-Cre; Dpy30$^{F/-}$*) and suspended in phosphate-buffered saline (PBS) supplemented with 3% heat-inactivated fetal bovine serum (FBS), followed by removal of red blood cells. 1 × 10^6 donor FLs (CD45.2$^+$) and 2 × 10^5 whole BM cells (C57Bl/6J, CD45.1$^+$) at a ratio of 5:1 were transplanted via tail vein into lethally irradiated recipient mice (CD45.1$^+$). For mixed chimera assays, 1 × 10^6 whole FLs as donors prepared from E14.5 embryos (CD45.2$^+$, *Mx1-Cre; Dpy30$^{F/+}$* and *Mx1-Cre; Dpy30$^{F/-}$*) were mixed with 2 × 10^5 whole BM cells (C57Bl/6J, CD45.1$^+$) at a ratio of 5:1, and transplanted into recipient mice in the same way as described above. Recipients were then injected with 150 µl pIpC (InvivoGen) at 1 mg/ml 4 times over 10 days starting at 5 weeks post transplantation. The first two injections were 2 days apart and last injections were 4 days apart. Reconstitution levels were assessed at different time points after pIpC injection. Prior to transplant, all of the recipient mice were lethally irradiated with a split dose of 1100 Rads with 3 hours apart. Donor contribution was determined by flow cytometry at different time points and calculated by CD45.2$^+$/(CD45.1$^+$ + CD45.2$^+$) × 100% within the indicated cell type except for the Pklr rescue assay, where the donor contribution was calculated by GFP$^+$/(GFP$^+$ + CD45.1$^+$) × 100%. For the Pklr rescue assay, the mouse *Pklr* cDNA was cloned with N-terminal FLAG tag into pMSCV-IRES-GFP (Addgene, #20672) between EcoRI and XhoI sites to generate the FLAG-*Pklr*-expressing plasmid. To generate the retroviruses, 293T cells were transiently transfected with either empty pMSCV-IRES-GFP vector or the FLAG-*Pklr*-expressing construct with pCL-Eco plasmid using Lipofectamine 2000 (Invitrogen), and viruses in the supernatants were harvested two days after transfection. Four days after injecting both *Dpy30$^{F/+}$; Mx1-Cre* and *Dpy30$^{F/-}$* ; *Mx1-Cre* mice (6-8-week old) with 150 mg/kg 5-fluorouracil (5-FU, Sigma-Aldrich), BM cells were flushed from these mice and were incubated overnight in RPMI 1640 with 20% fetal calf serum, 20 ng/ml SCF, 20 ng/ml Flt-3, 10 ng/ml IL-3 and 10 ng/ml IL-6 (all from Peprotech, Rocky Hill, NJ) at 37ºC to promote cell cycle entry. Cells were then spinoculated with retroviral supernatant in the presence of 8 µg/ml polybrene (Clontech, Mountain View, CA) for 90 minutes at 500 x g at 30°C. The spinoculated cells were analyzed for transduction efficiency (GFP positivity) by flow cytometry, and cells containing 1 × 10^5 GFP$^+$ cells (CD45.2$^+$) and 2 × 10^5 helper BM cells (CD45.1$^+$) were transplanted via tail vein into lethally irradiated recipient mice (CD45.1$^+$).

Flow cytometry for analysis and cell isolation

Single cell suspensions were prepared from FL, BM, thymus, spleen, or peripheral blood and stained with antibodies as previously described (Yang et al., 2016). FACS analysis was performed on LSRFortessa (Becton Dickinson), and data were analyzed using FlowJo software (Tree Star) as previously described (Yang et al., 2016). Cells were fixed, permeablized and immunostained with anti-phospho-AMPKα (Thr172) antibody (Cell Signaling Technology, #2535), or phospho-KAP-1 (S824) (Bethyl Laboratories, #A300-767A). Mean fluorescence intensity was determined. To measure mitochondrial membrane potential and mitochondrial mass, the HSC staining was modified to make the channels available for tetramethyl rhodamine methyl ester (TMRM) and Mitotracker Deep Red staining (all from Molecular Probes), respectively. After antibody staining cells were incubated with 25 nM TMRM or 1nM Mitotracker Deep red for 15 min at 37ºC followed by flow-cytometry on LSRFortessa (Becton Dickinson), respectively. To measure cellular ROS levels, cells were incubated with 5 mM 2`-7`-dichlorofluorescein diacetate (DCFDA) at 37ºC for 15 min after being stained with the antibodies as described. ROS levels in the gated populations were quantified using flow cytometry. To measure the glucose uptake, 3×10^4 sorted BM LSK cells were incubated with 100 µM 2-NBDG at 37ºC for 1 hour in RPMI-1640 (Life Technologies, no glucose) containing 10% FBS, 100 ng/mL SCF and 100 ng/mL Flt-3. Cellular glucose uptake levels were quantified using flow cytometry. FACS sorting from BM cells was performed as we previously described (Yang et al., 2016). FACS sorting from FLs was performed on LSRAria II (Becton Dickinson). Briefly, 2-3 embryos were pooled and stained with lineage cocktail

containing biotin-conjugated antibodies against CD3e (145-2C11), CD4 (GK1.5), CD5 (53-7.3), CD8 (53-6.7), CD45R (RA3-6B2), Gr1 (RB6-8C5) and Ter119 (TER-119), and the following antibodies to discriminate FL HSC: Sca1 (D7)-PE-Cy7; CD117 (c-Kit 2B8)-PE; CD48 (HM48.1)-FITC; CD150 (TC15-12F12.2)-Pacific blue. All fluorescence-conjugated antibodies were obtained from BioLegend Inc.

Colony formation assays

Lin$^-$ FL cells were enriched by staining with lineage cocktail containing biotin-conjugated antibodies against CD3e (145-2C11), CD4 (GK1.5), CD5 (53-7.3), CD8 (53-6.7), CD45R (RA3-6B2), Gr1 (RB6-8C5) and Ter119 (TER-119), and the following anti-biotin microbeads (Milteni Biotec, Cat#:130-090-485). Lin$^-$ BM cells were enriched by Lineage Cell Depletion Kit (Milteni Biotec, Cat#:130-090-858). pLKO.1-based lentiviral constructs expressing scramble control (Addgene plasmid 1864) or short hairpins (shRNAs) targeting indicated genes were transfected into 293T cells, respectively. Viral particles were produced as we described (Yang et al., 2014). Lin$^-$ BM and Lin$^-$ FL cells were harvested as described above and incubated overnight prior to infecting with unconcentrated viruses in the presence of 4 μg/ml of polybrene (Sigma). Two days after infection, puromycin (1 μg/ml) was added, and 2 more days later, cells were used for various assays including RNA isolation and colony formation assay. Indicated numbers of cells were plated in duplicate or triplicate into 1.1 ml Mouse Methylcellulose Complete Media (STEMCELL Technologies, M3434). Colonies were scored at 7-10 days after plating.

DNA damage assays

Primary mouse embryonic fibroblasts (MEFs) were isolated from E13.5 embryos of *Dpy30$^{F/F}$* or *CAG-CreER; Dpy30$^{F/F}$* genotype, cultured in MEF culture medium (Dulbecco's Modified Eagle Medium [DMEM] supplemented with L-glutamine, 10% FBS, 0.1 mM non-essential amino acids, and 55μM β-mercaptoethanol, all from Invitrogen), and passage was kept at minimum. MEFs were treated with vehicle (ethanol) as control or 0.5 μM of 4-OH tamoxifen for 4 days to induce *Dpy30* deletion. Lin$^-$ BM cells were treated with vehicle (ethanol) as control or 0.5 μM of 4-OH tamoxifen for 48 hrs, and were either FACS sorted for LSK cells before irradiation and assays for γ-H2AX staining and comet assay, or directly used for irradiation and flow cytometry analysis for phospho-Kap1. To induce DNA damage, cells were irradiated with 3.2 Gy (X-RAD 320 irradiator; dose rate, 0.8 Gy/min). BM cells were cytospun before immunostaining. Immunostaining was performed using primary antibody for H3K4me3 described before (Jiang et al., 2011; Yang et al., 2015) followed by Alexa Fluor 555 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed secondary antibody (ThermoFisher Scientific, # A-21428), and primary antibody for phospho-H2AX (Ser139) (Millipore, #05-636), followed by Alexa Fluor 488 Goat anti-Mouse IgG (H+L) Cross-Adsorbed secondary antibody (ThermoFisher Scientific, # A-11001). Comet Assay was performed using the OxiSelect Comet Assay Kit according to the manufacturer's instructions (Cell Biolabs). Images were acquired by a Nikon Ti-S microscope using a FITC filter, and the comet images were analyzed in two ways. For comet assays in Figures 5A and 5B, the percentage of tail DNA of individual cells was determined by the OpenComet (Gyori et al., 2014) plugin of ImageJ. The graph and two-tailed Student's *t*-test were performed using the GraphPad Prism software. For all other comet assays, we performed visual scoring method that closely correlates with computer scoring method (Collins, 2004).

For rescue assays by DDR inhibitors in culture, Lin$^-$ BM cells from *CAG-CreER; Dpy30$^{F/F}$* mice were treated with DMSO control or indicated inhibitors in liquid culture containing vehicle control (ethanol) or 1 μM of 4-OH tamoxifen for two days and subjected to colony formation assays. ATM inhibitor KU55933 (Sigma, #SML1109) was used at 10 μM in DMSO, and ATR inhibitor VE-821 (Sigma, #SML1415), Chk2 inhibitor PV1019 (Sigma, #220418), and Chk1 inhibitor MK8776 (Cayman Chemical, #891494-63-6) were used at indicated concentration in DMSO. For rescue assays by DDR inhibitors in vivo, mice were injected with pIpC, and either vehicle (DMSO) or KU55933 (10 mM, 50 μl per mouse) was injected intraperitoneally one day prior to the first pIpC injection, and was repeated every two days until the end of the experiment.

NAC effects

For NAC administration in vivo, *Mx1-Cre; Dpy30*$^{F/+}$ and *Mx1-Cre; Dpy30*$^{F/-}$ mice were injected with pIpC as previously described (Yang et al., 2016), and NAC (Sigma, Cat# A7250) was meanwhile supplied in drinking water at 2 mg/ml. Fresh NAC drinking water was replace every two days until the end of the experiment. For NAC treatment in cultured cells, Fresh Lin^+ BM cells were enriched as described (Yang et al., 2016). The cells were then incubated with 1 μM of 4-OH tamoxifen or/and 1 μM of NAC for 2 days as indicated.

Proliferation and apoptosis

HSPC proliferation and apoptosis were determined as previously described (Yang et al., 2014; Yang et al., 2016) with modifications. Briefly, to analyze the cell cycle status of FLs, 1.5 mg Brdu was intraperitoneally injected into E14.5 pregnant mice 1 hour prior to sacrifice. Cells were stained with cell surface antibodies as described above and then processed with BrdU staining using the FITC-BrdU Flow kit (BD Pharmingen) following the manufacturer's instructions. The cells were washed by PBS and then stained with antibodies labeled with various fluorochromes as described. For HSC cycle analysis, fresh BM cells were stained with SLAM cell surface markers as described. Cells were then fixed and permeabilized by the buffers from FITC-BrdU Flow kit (BD Pharmingen) and then stained with FITC-labeled anti-Ki67 antibody (BD Biosciences) followed by 7-AAD. Percentages of the cells at G0, G1, and S/G2/M phases were quantified by flow cytometry. For apoptosis assay, cells were harvested and stained with antibodies as described. After washing twice with cold PBS containing 3% heat-inactivated FBS, the cells were then incubated with FITC-Annexin V (BD Pharmingen) and 7-amino-actinomycin D (7-AAD) for 15 minutes in binding buffer (10mM HEPES, 140 mM NaCl and 2.5mM $CaCl_2$) at room temperature in dark. The stained cells were analyzed immediately by flow cytometry.

RNA extraction, chromatin immunoprecipitation (ChIP) and quantitative PCR (qPCR)

Total RNAs were isolated using RNeasy Plus Mini (for non-HSCs) or Micro (for HSCs) Kit (Qiagen) as described (Yang et al., 2016). ChIP assays and qPCR were performed as described(Yang et al., 2016). Primers used are listed in **Table S3**. Relative expression levels were normalized to *Actb*. For ChIP results, percent input was first calculated from ChIP qPCR results as described(Jiang et al., 2011), and ChIP enrichment fold was calculated as the ratio of the percent input value for each locus over that for the negative control site in the control cells (*Mx1-Cre*; *Dpy30*$^{F/+}$). RNAs were reverse transcribed with SuperScript III (Invitrogen). qPCR was performed with SYBR Advantage qPCR Premix (Clontech) on a ViiA7 Real-Time PCR System (Applied Biosystems). Primers used are listed in **Table S3**. Relative expression levels were normalized to *Actb*. RNA-sequencing and analyses were performed as described before (Yang et al., 2016).

Immunoblotting

Whole fetal liver cells and bone marrow cells were isolated after removing red blood cells by ACK lysis buffer. The cells were then lysed by lysis buffer (1% SDS, 10mM EDTA and 50mM Tris-Cl pH 7.5) with fresh added Protease Inhibitor Cocktail (Roche) and boiled for 10 min. Proteins were resolved on SDS-PAGE followed by immunoblotting for DPY30, H3, H3K4me1, H3K4me2, and H3K4me3, all using antibodies described before (Jiang et al., 2011; Yang et al., 2015), and for β-Actin (Santa Cruz Biotechnology, sc-47778), FLAG (Sigma-Aldrich, F3165), phospho-AMPKα (Thr172) (Cell Signaling Technology, #2535), phospho-ACC (Ser79) (Cell Signaling Technology, #3661), and phospho-H2AX (Ser139) (Millipore, #05-636). Band intensities were quantified by ImageJ.

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