JEM

Supplemental material

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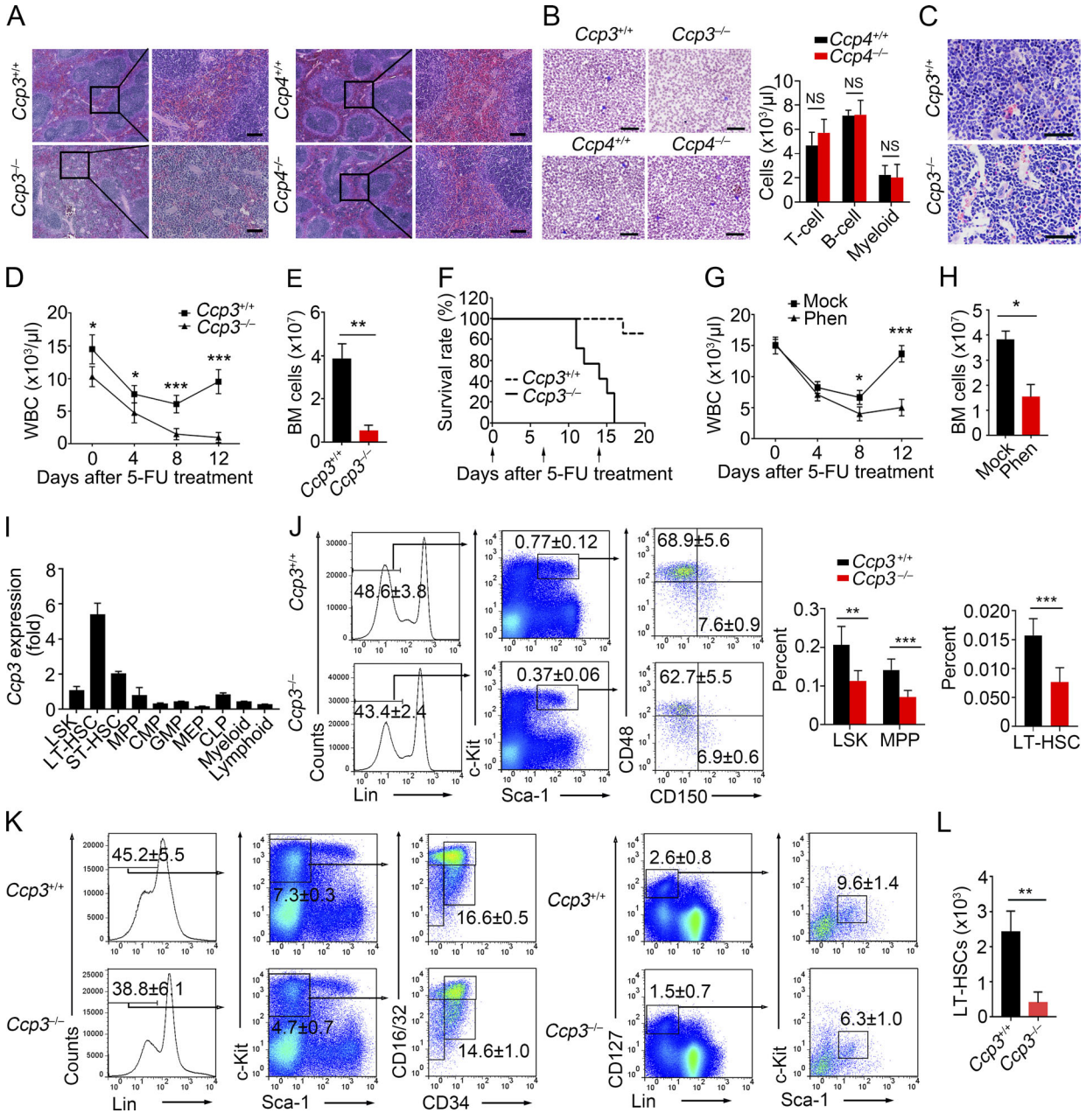

Figure S1. ***Ccp3* deletion impairs HSC expansion. (A)** H&E staining of spleen sections from *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice (left) and *Ccp4*$^{+/+}$ and *Ccp4*$^{-/-}$ mice (right). Scale bars, 50 µm. **(B)** Left: Peripheral blood smears with Wright's staining of *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ (top) and *Ccp4*$^{+/+}$ and *Ccp4*$^{-/-}$ mice (bottom). Scale bars, 30 µm. Right: T cells (CD3^{+}), B cells (CD19^{+}), and myeloid cells (CD11b^{+}Gr-1^{+}) of peripheral blood from *Ccp4*$^{+/+}$ and *Ccp4*$^{-/-}$ mice were analyzed with flow cytometry (n = 6). **(C)** H&E staining of BM sections from femurs of *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice. Scale bars, 50 µm. **(D and E)** *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice were injected i.p. with 5-FU (150 mg/kg). Peripheral blood cells were counted every 4 d (D), and BM cells were counted 2 wk after 5-FU treatment (E). n = 5. **(F)** *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice were injected i.p. with 5-FU (150 mg/kg) every 7 d for three rounds, and survival rates were calculated (n = 7). **(G and H)** WT mice were injected i.p. with 5-FU (150 mg/kg) and then with phenanthroline (1.8 mg/kg) or PBS as a control every other day. Peripheral blood cells were counted every 4 d (G), and BM cells were counted 2 wk after 5-FU treatment (H). n = 4. **(I)** *Ccp3* mRNA expression levels in indicated lineages were detected by qPCR. Results were normalized to endogenous *Actb* gene (n = 4). **(J)** Flow cytometry gating strategies for LSKs (Lin^{-}Sca-1^{+}c-Kit^{+}), MPPs (Lin^{-}Sca-1^{+}c-Kit^{+}CD48^{+}CD150^{-}), and LT-HSCs (Lin^{-}Sca-1^{+}c-Kit^{+}CD48^{-}CD150^{+}) from *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice. Total percentages of LSKs, MPPs, and LT-HSCs in a femur were counted (n = 6). **(K)** Flow cytometry gating strategies for HSPCs (Lin^{-}Sca-1^{-}c-Kit^{+}), CMPs (Lin^{-}Sca-1^{-}c-Kit^{+}CD34^{+}CD16/32^{-}; left), and CLPs (Lin^{-}CD127^{+}Sca-1^{low}c-Kit^{low}; right) from *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice. **(L)** *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice were injected i.p. with 5-FU (150 mg/kg). 1 wk later, LT-HSCs were analyzed by flow cytometry (n = 4). Results are shown as means ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Two-tailed Student's t test. Data in J are pooled from three independent experiments. Data in A–I, K, and L are representative of three independent experiments.

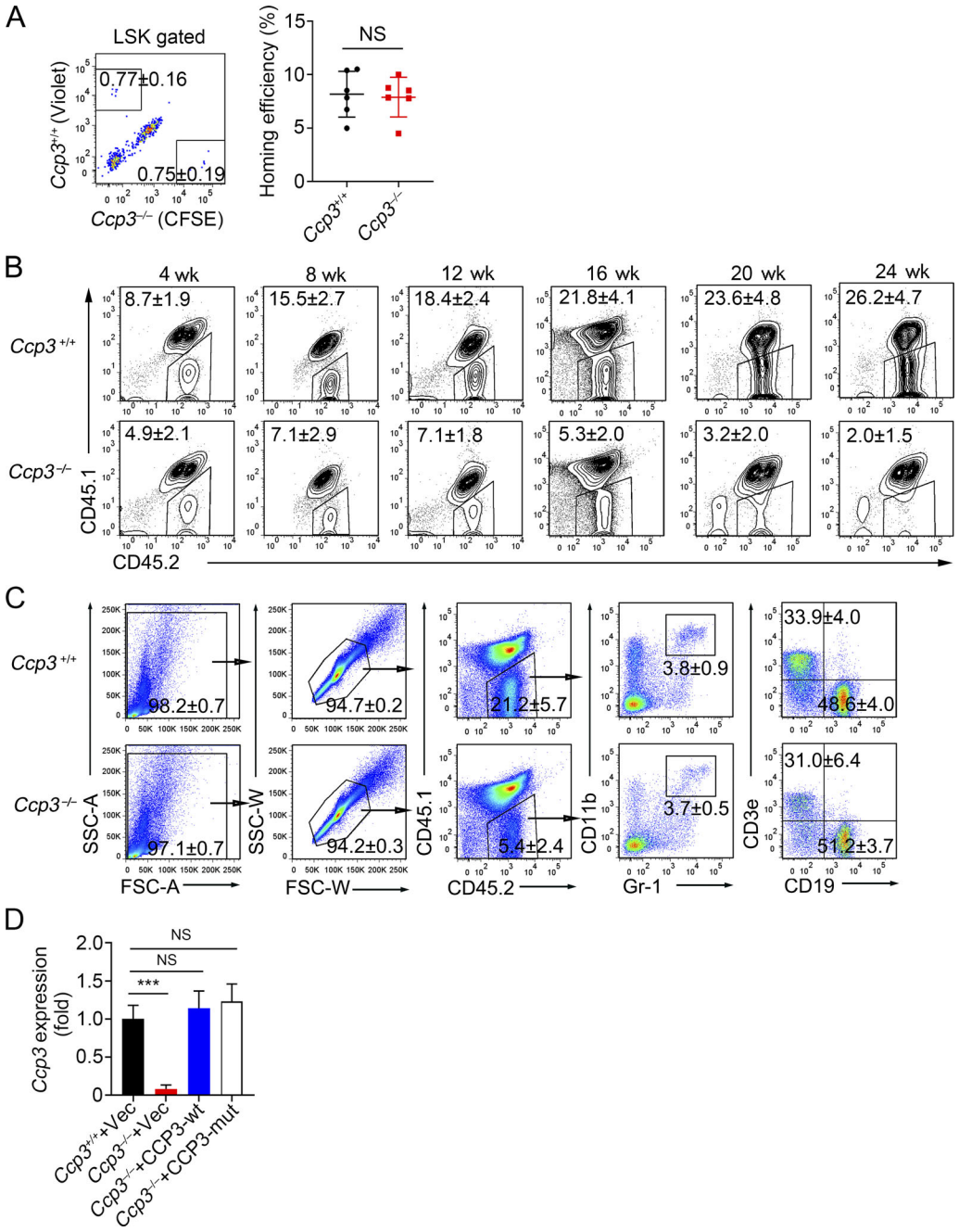

Figure S2. **CCP3 plays an intrinsic role in HSC self-renewal. (A)** LT-HSC homing assay. 2 × 10^3 LT-HSCs from *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice were sorted and stained with CellTrace violet and CellTrace CFSE, respectively, and then injected into lethally irradiated mice through tail vein injection. Homing of LT-HSCs in BM was analyzed by flow cytometry 18 h after transplantation, and homing efficiencies were calculated (n = 6). **(B and C)** 1 × 10^2 LT-HSCs from *Ccp3*$^{+/+}$ or *Ccp3*$^{-/-}$ mice were sorted and mixed with 5 × 10^5 BM helpers (CD45.1$^+$CD45.2$^+$) and then cotransplanted into lethally irradiated CD45.1 mice (n = 5). **(B)** Peripheral blood cells were analyzed by flow cytometry every 4 wk. **(C)** Peripheral myeloid cells (CD11b$^+$Gr-1$^+$), T cells (CD3$^+$), and B cells (CD19$^+$) derived from *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ LT-HSCs were further detected. **(D)** CCP3-wt or enzymatic inactive CCP3 (CCP3-mut) were overexpressed in *Ccp3*$^{-/-}$ LT-HSCs with pMYs retrovirus. GFP$^+$ cells were sorted and CCP3-wt or CCP3-mut overexpression levels were analyzed by qPCR (n = 4). Results are shown as means ± SD. ***, P < 0.001. Two-tailed Student's t test. Data are representative of three independent experiments.

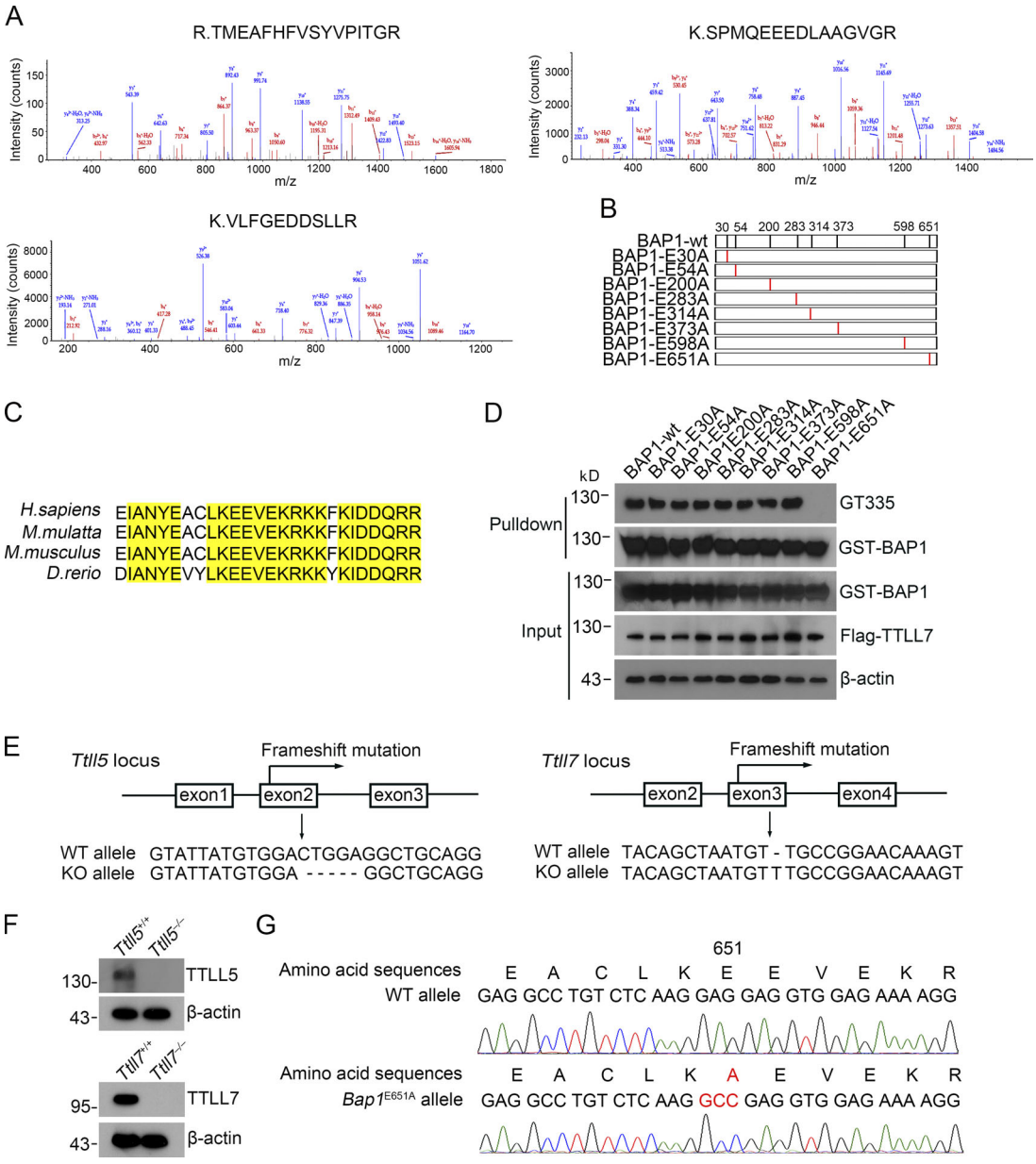

Figure S3. **BAP1 is glutamylated at E651 by TTLL5 and TTLL7. (A)** Recombinant CCP3-wt and enzymatic inactive CCP3 (CCP3-mut) were immobilized in Affi-Gel 10 resin and went through BM cell lysates. Eluted fractions were resolved by SDS-PAGE and stained by silver staining. A differential band around 95 kD in the CCP3-mut lane was cut for mass spectrometry. Tandem mass spectrometry profiles of representative BAP1 peptide sequences are shown. **(B)** Schematic representation of eight putative glutamate-rich sites in BAP1. **(C)** Amino acid alignment of BAP1 from different species. Conserved sequences are highlighted in yellow. **(D)** Flag-tagged TTLL7 were transfected into HEK293T cells for 48 h. Cell lysates were incubated with recombinant WT BAP1 or various indicated mutants at 37°C for 2 h, followed by incubating with GST beads at 4°C for 1 h and Western blotting. Protein glutamylation was examined by immunoblotting with GT335 antibody. **(E)** Diagram of strategy for *Ttll5* and *Ttll7* knockout generation via CRISPR-Cas9 technology. 5-bp deletions of *Ttll5* exon 2 and 1-bp insertion of *Ttll7* exon 3, forming frameshift mutation, were identified by PCR and TA clone for DNA sequencing. **(F)** Western blot to confirm deficiency of TTLL5 and TTLL7. **(G)** *Bap1*^{E651A} knock-in mice were generated by CRISPR-Cas9 approach. Glutamine 651 of BAP1 was mutated to alanine (*Bap1*^{E651A}). Mutant mice were identified by PCR and TA clone for DNA sequencing. Data in A, D, F, and G are representative of three independent experiments.

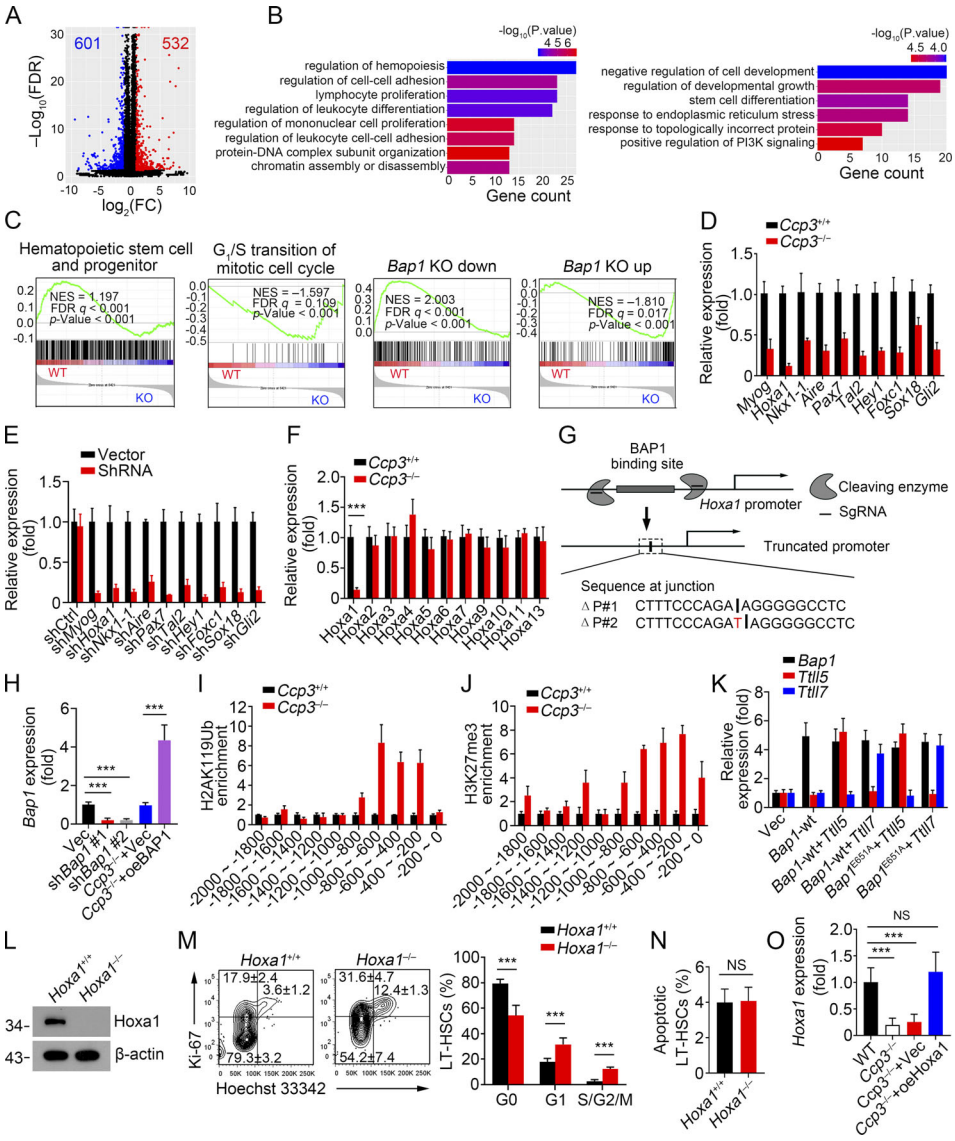

Figure S4. **BAP1 targets on *Hoxa1* promoter to enhance *Hoxa1* expression. (A)** Volcano plot of differentially expressed genes in $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs. Genes whose expression changed more than twofold and whose false-discovery rate q value was <0.05 were selected as differentially expressed genes. **(B)** GO analysis of differentially expressed genes in $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs. **(C)** GSEA of genes in $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs enriched in selected gene sets, which were related to HSC and progenitor (first), G_1/S transition mitotic cell cycle (second), downregulated genes in BAP1-deficient LSKs (third), and upregulated genes in BAP1-deficient LSKs (fourth). Gene sets related to HSC and progenitor and G_1/S transition mitotic cell cycle were from the Molecular Signatures Database. Gene sets related to downregulated and upregulated genes in BAP1-deficient LSKs were based on transcriptomic data from GEO accession no. GSE40541. **(D)** RNA were extracted from $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs ($n = 4$), and the top 10 downregulated genes were further analyzed by qPCR. Results were normalized to expression of endogenous *Actb* gene. **(E)** The top 10 downregulated genes in $Ccp3^{-/-}$ LT-HSCs were knocked down by LMP microRNA-adapted retroviral system, followed by qPCR. Results were normalized to expression of empty vector infection ($n = 4$). **(F)** Genes in HoxA cluster in $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs were analyzed by qPCR. Results were normalized to expression in $Ccp3^{+/+}$ LT-HSCs ($n = 4$). **(G)** Schematic of deletion of BAP1 binding region of *Hoxa1* promoter with CRISPR-Cas9 technology. Indicated region deletions were identified by PCR and DNA sequencing. **(H)** LT-HSCs were infected with LMP retrovirus carrying shRNA against BAP1. BAP1 was overexpressed in $Ccp3^{-/-}$ LT-HSCs with pMYs retrovirus, followed by qPCR. Results were normalized to expression of empty vector infection ($n = 4$). **(I)** Enrichment assessment of H2AK119Ub on indicated regions of *Hoxa1* promoter in $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs. Enrichments were detected by qPCR with indicated primers and normalized to IgG enrichment value ($n = 4$). **(J)** Enrichment assessment of H3K27me3 on indicated regions of *Hoxa1* promoter in $Ccp3^{+/+}$ and $Ccp3^{-/-}$ LT-HSCs. Enrichments were detected by qPCR with indicated primers and normalized to IgG enrichment value ($n = 4$). **(K)** BAP1-wt or BAP1-mut with TTLL5 or TTLL7 was co-overexpressed in $Ttll5^{-/-}$;$Ttll7^{-/-}$ LT-HSCs with pMYs retrovirus, followed by qPCR. Results were normalized to expression of empty vector infection ($n = 4$). **(L)** sgRNA targeting *Hoxa1* was designed according to an online tool and screened for efficiency. sgRNA targeting *LacZ* was used as a control. LT-HSCs sorted from Cas9 knock-in mice were infected with lentivirus including sgRNA and Cre recombinase expression, followed by cotransplantation with helper cells into lethally irradiated recipient mice. 1 mo later, GFP^+ BM cells were sorted to analyze Hoxa1 protein levels through Western blotting. **(M)** Cell cycle analysis of $Hoxa1^{+/+}$ and $Hoxa1^{-/-}$ LT-HSCs ($n = 6$). **(N)** Apoptosis analysis of $Hoxa1^{+/+}$ and $Hoxa1^{-/-}$ LT-HSCs ($n = 6$). **(O)** *Hoxa1* was overexpressed in $Ccp3^{-/-}$ LT-HSCs, followed by qPCR. Results were normalized to expression of WT LT-HSCs ($n = 4$). Results are shown as means ± SD. *** $P < 0.001$ (two-tailed Student's *t* test). Data in M and N are pooled from three independent experiments. Data in D–F, H–L, and O are representative of three independent experiments. FDR, false-discovery rate; FC, fold change; NES, normalized enrichment score.

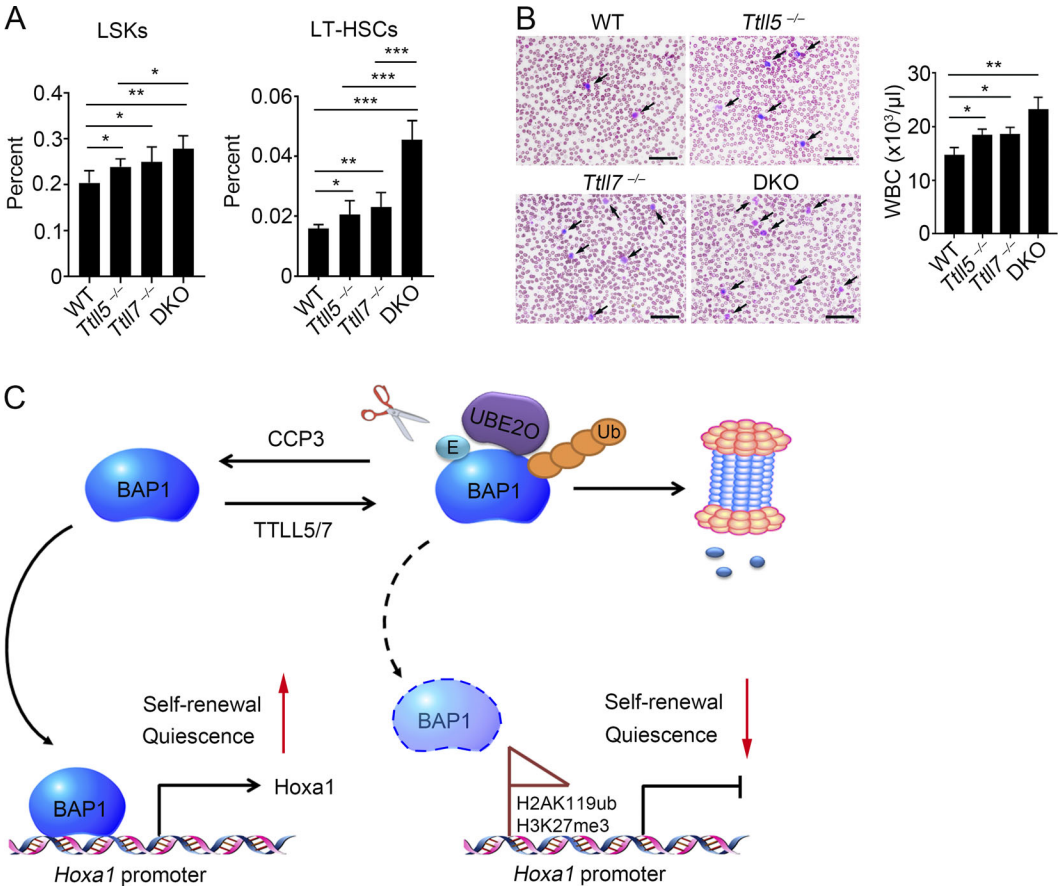

Figure S5. ***Ttll5* and *Ttll7* deficiencies promote HSC self-renewal. (A)** LSKs and LT-HSCs from WT, *Ttll5*$^{-/-}$, *Ttll7*$^{-/-}$, and *Ttll5*$^{-/-}$;*Ttll7*$^{-/-}$ (DKO) mice were detected by flow cytometry, and percentages per femur were calculated (n = 6). **(B)** Left: Peripheral blood smears with Wright's staining of WT, *Ttll5*$^{-/-}$, *Ttll7*$^{-/-}$, and DKO mice. The arrows indicate peripheral white blood cells. Right: Numbers of peripheral blood cells in indicated mice were calculated (n = 6). **(C)** Work model of BAP1 glutamylation in regulating HSC self-renewal. Results are shown as means ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Two-tailed Student's t test. Data in A and B are pooled from three independent experiments.

Table S1. **Hematopoietic cell counts in peripheral blood of *Ccp3*$^{+/+}$ and *Ccp3*$^{-/-}$ mice**

Parameter	*Ccp3*$^{+/+}$ (n = 5)	*Ccp3*$^{-/-}$ (n = 5)	P value
WBC (x10^6/ml)	22.3 ± 4.5	10.0 ± 1.6	0.001
Lymph (x10^6/ml)	16.6 ± 3.9	6.5 ± 2.5	0.003
Mon (x10^6/ml)	0.7 ± 0.1	0.5 ± 0.1	0.005
Gran (x10^6/ml)	5.0 ± 0.7	2.9 ± 1.3	0.023
RBC (x10^9/ml)	10.2 ± 1.4	7.7 ± 1.1	0.018
HGB (mg/ml)	157.0 ± 13.6	128.2 ± 19.7	0.043

Hematopoietic parameters were analyzed using an XFA6030 automated hemocytometer (Slpoo). Cell numbers and percentages were counted for each population. Data are shown as means ± SD. Lymph, lymphoid cells; Mon, monocytes; Gran, granulocyte; HGB, hemoglobin.

Table S2. **sgRNA sequences used in this study**

Target gene	sgRNAs
Ccp3	5′-GGAGTATCAGCTAGGAAGAT-3′
Ccp4	5′-GCCTATACCTTCCCAGCCCC-3′
Ttll5	5′-GGGATCACCCATGTATTATG-3′
Ttll7	5′-GCCGGAACAAAGTTTGAAAT-3′
Bap1^{E651A}-up	5′-GCCCCTAAGGTATACAATGT-3′
Bap1^{E651A}-down	5′-CAGCTGTCCTTGGGCAGTAG-3′
Hoxa1	5′-ATCCTTGGCAGTGGCGACTC-3′
LacZ	5′-TGCGAATACGCCCACGCGAT-3′

sgRNAs were designed according to an online tool (http://crispr.mit.edu/) and purchased from Sangon. sgRNA targeting *LacZ* was used as a control.

Table S3. **Sequences of primers used for genotyping and qPCR**

Primers	Sequences
Ccp3 KO (Forward)	5′-TCAGCTGATTCTATTGGTGACCC-3′
Ccp3 KO (Reverse)	5′-TGACCTCACAGTGGTATGGC-3′
Ccp4 KO (Forward)	5′-AGGCTGTGTGCATTTCATTATC-3′
Ccp4 KO (Reverse)	5′-AGAAGATCACAGTTGACCTGAAC-3′
Ttll5 KO (Forward)	5′-GCCAAGTATGAGGTAGGGACA-3′
Ttll5 KO (Reverse)	5′-GGTCATACCCAGATCCCCTT-3′
Ttll7 KO (Forward)	5′-GTCACCGTTCGTAGCTTTAACC-3′
Ttll7 KO (Reverse)	5′-CTCCAGAACCCTACACTGCTTT-3′
Bap1^{E651A} (Forward)	5′-CTTGAGTGGAGAGAAGTACTC-3′
Bap1^{E651A} (Reverse)	5′-ATAGTTGTGGGTCCTTCGCTG-3′
Hoxa1 KO (Forward)	5′-ATGGAGGAAGTGAGAAAGTTGGC-3′
Hoxa1 KO (Reverse)	5′-TGGTGGTGGGGCGAGCTGATCTG-3′
Ccp1 qPCR (Forward)	5′-TGGAAAGCTATCAGCCCTGG-3′
Ccp1 qPCR (Reverse)	5′-GAGCTGGCGTCTGAAGGATG-3′
Ccp2 qPCR (Forward)	5′-TCGAGAACCCCGAGAACTCTT-3′
Ccp2 qPCR (Reverse)	5′-TGCTCCTCTCCCACAATCTCT-3′
Ccp3 qPCR (Forward)	5′-TGACTTGGATGAGGATTCCTTCA-3′
Ccp3 qPCR (Reverse)	5′-GGGAAGAATGGGTCACCAATAG-3′
Ccp4 qPCR (Forward)	5′-CCAGCAGTGCCTATACCTTCC-3′
Ccp4 qPCR (Reverse)	5′-TGCTCAGATCAGTTTCCAAGTC-3′
Ccp5 qPCR (Forward)	5′-CTGCTCATTCTCGTCTTCAGG-3′
Ccp5 qPCR (Reverse)	5′-ATCGAGTCCTAATGCAAGGGA-3′
Ccp6 qPCR (Forward)	5′-AGGCAGGCAATGATACAGGAA-3′
Ccp6 qPCR (Reverse)	5′-GGTTACCACTTTCAAAGCAAGCA-3′
Bap1 qPCR (Forward)	5′-CTCCTGGTGGAAGATTTCGGT-3′
Bap1 qPCR (Reverse)	5′-GAGTGGCACAAGAGTTGGGAA-3′
Hoxa1 qPCR (Forward)	5′-CCTGGAGTGATGTGGTCCAG-3′
Hoxa1 qPCR (Reverse)	5′-AGCAACCACTGTAGTCCAGC-3′
Myog qPCR (Forward)	5′-GAGACATGAGTGCCCTGACC-3′
Myog qPCR (Reverse)	5′-AGGCTTTGGAACCGGATAGC-3′
Nkx1-1 qPCR (Forward)	5′-GACACTATGGACGGACGAGC-3′
Nkx1-1 qPCR (Reverse)	5′-CGGCGTCTCCTACTGTTGAA-3′
Aire qPCR (Forward)	5′-AGACCATGGCAGCTTCTGTC-3′
Aire qPCR (Reverse)	5′-ATAGTGACCTGGGCTCCCTT-3′
Pax7 qPCR (Forward)	5′-TCAAGCCAGGAGACAGCTTG-3′
Pax7 qPCR (Reverse)	5′-TAGGCTTGTCCCGTTTCCAC-3′
Tal2 qPCR (Forward)	5′-GTTCCCAGCTCCTAGCAAGA-3′
Tal2 qPCR (Reverse)	5′-CACCGCTCCCTGGTATTTGT-3′
Hey1 qPCR (Forward)	5′-TAACCGGAGACTGAGCGTGA-3′
Hey1 qPCR (Reverse)	5′-TCGTTGGGGACATGGAACAC-3′
Foxc1 qPCR (Forward)	5′-AGTCGTGGTTAAGAGCGAGG-3′
Foxc1 qPCR (Reverse)	5′-ATGATGGTCTCCACGCTGAA-3′
Sox18 qPCR (Forward)	5′-GCTAGCAGCGCGGTCTATTA-3′
Sox18 qPCR (Reverse)	5′-TGGCATCTTTAGGCCACCAG-3′

Table S3. **Sequences of primers used for genotyping and qPCR (*Continued*)**

Primers	Sequences
Gli2 qPCR (Forward)	5′-GGTGTGGACTCATTGCCTGA-3′
Gli2 qPCR (Reverse)	5′-TGCACCAAATTTACTGCCTG-3′
18S (Forward)	5′-AACCCGTTGAACCCCATT-3′
18S (Reverse)	5′-CCATCCAATCGGTAGTAGCG-3′
Actb (Forward)	5′-GGCTGTATTCCCCTCCATCG-3′
Actb (Reverse)	5′-CCAGTTGGTAACAATGCCATGT-3′
Ttll1 qPCR (Forward)	5′-GAAGTGGGTCACTGACATTGAG-3′
Ttll1 qPCR (Reverse)	5′-ACGTTGCGAATGGTTTGCAC-3′
Ttll2 qPCR (Forward)	5′-GAGTTCACACCCCTGACATTC-3′
Ttll2 qPCR (Reverse)	5′-GCATTTGTACCTACCCACGAGT-3′
Ttll4 qPCR (Forward)	5′-TGGATGAGAACCTGAAACCCT-3′
Ttll4 qPCR (Reverse)	5′-TGGGGCTGCTGGAACTAGA-3′
Ttll5 qPCR (Forward)	5′-ACTCCCCAGCTCCCATCTG-3′
Ttll5 qPCR (Reverse)	5′-GGGGCATTGTCAGGAACGG-3′
Ttll6 qPCR (Forward)	5′-CTAACTGCCGGTATGACAGCG-3′
Ttll6 qPCR (Reverse)	5′-AGTAGTCGGTCCAATAGAGAGTC-3′
Ttll7 qPCR (Forward)	5′-CTCTGCCTCAAGATGGGGTTA-3′
Ttll7 qPCR (Reverse)	5′-GTTCCGGCAACATTAGCTGTAA-3′
Ttll9 qPCR (Forward)	5′-TGGAGTGTCGAAAGGAAAAGAGA-3′
Ttll9 qPCR (Reverse)	5′-TGCTCATCCATGTAGGTGTGG-3′
Ttll11 qPCR (Forward)	5′-CCTGACCAACTACTCCCTGAA-3′
Ttll11 qPCR (Reverse)	5′-GGGATGTCTGACTGGTAGAAAAC-3′
Ttll13 qPCR (Forward)	5′-GGCCTGAAGGAAGTAGGGGA-3′
Ttll13 qPCR (Reverse)	5′-CATGCCAGGGAAGTGGTTGA-3′
Hoxa2 qPCR (Forward)	5′-TACGAATTTGAGCGAGAGATTGG-3′
Hoxa2 qPCR (Reverse)	5′-GTCGAGGTCTTGATTGATGAACT-3′
Hoxa3 qPCR (Forward)	5′-TCAGCGATCTACGGTGGCTA-3′
Hoxa3 qPCR (Reverse)	5′-GAGGCAAAGGTGGTTCACCC-3′
Hoxa4 qPCR (Forward)	5′-GAAAGCACAAACTCACAGCCC-3′
Hoxa4 qPCR (Reverse)	5′-GTCTCGGGTTTACTTAGGGAAG-3′
Hoxa5 qPCR (Forward)	5′-CTCATTTTGCGGTCGCTATCC-3′
Hoxa5 qPCR (Reverse)	5′-ATCCATGCCATTGTAGCCGTA-3′
Hoxa6 qPCR (Forward)	5′-CACCCTCGGGCAATAACAAG-3′
Hoxa6 qPCR (Reverse)	5′-GCCGTCAGGTTTGTACTGCT-3′
Hoxa7 qPCR (Forward)	5′-TCCAGAATCGGCGCATGAAG-3′
Hoxa7 qPCR (Reverse)	5′-ACGCTTTTCCAACTGTCCTG-3′
Hoxa9 qPCR (Forward)	5′-GGCCTTATGGCATTAAACCTGA-3′
Hoxa9 qPCR (Reverse)	5′-ACAAAGTGTGAGTGTCAAGCG-3′
Hoxa10 qPCR (Forward)	5′-GGCAGTTCCAAAGGCGAAAAT-3′
Hoxa10 qPCR (Reverse)	5′-GTCTGGTGCTTCGTGTAAGGG-3′
Hoxa11 qPCR (Forward)	5′-TCTTCGCGCCCAATGACATAC-3′
Hoxa11 qPCR (Reverse)	5′-GGCTCAATGGCGTACTCTCT-3′
Hoxa13 qPCR (Forward)	5′-TGGAAAGCTATCAGCCCTGG-3′

Table S3. **Sequences of primers used for genotyping and qPCR (*Continued*)**

Primers	Sequences
Hoxa13 qPCR (Reverse)	5′-GAGCTGGCGTCTGAAGGATG-3′

Primers were designed with Primer 5 and purchased from Sangon.

Table S4. **Sequences of primers used in chIP assays**

Loci	Sequences
−200 ~ 0 (Forward)	5′-TCCAAGTCAGCTCCGGG-3′
−200 ~ 0 (Reverse)	5′-GAATGTACAGTGCGCAAGAG-3′
−400 ~ −200 (Forward)	5′-AGAGATTTTCGGCCCACAAGA-3′
−400 ~ −200 (Reverse)	5′-GCTGACTTGGAGCACTGGG-3′
−600 ~ −400 (Forward)	5′-TCTGCGCACGTCCCTCTA-3′
−600 ~ −400 (Reverse)	5′-GCCGAAAATCTCTGCGTGG-3′
−800 ~ −600 (Forward)	5′-TTCCCAGAGAGCTGGGTTCGTA-3′
−800 ~ −600 (Reverse)	5′-GGACGTGCGCAGAGGATTGA-3′
−1000 ~ −800 (Forward)	5′-AGCATGCTCCTGGGTCTCTA-3′
−1000 ~ −800 (Reverse)	5′-GAACCCAGCTCTCTGGGAAA-3′
−1200 ~ −1000 (Forward)	5′-TGTCCCTCCCACCTGCC-3′
−1200 ~ −1000 (Reverse)	5′-GGCCATCTGCCAACTCTTAG-3′
−1400 ~ −1200 (Forward)	5′-CCAAGCTTAGAGTTGACGTGAC-3′
−1400 ~ −1200 (Reverse)	5′-GGTGTTGAGGACCGGCAG-3′
−1600 ~ −1400 (Forward)	5′-TTGCATTCTTCTTCCCTTCCT-3′
−1600 ~ −1400 (Reverse)	5′-AGATGTCACGTCAACTCTAAGC-3′
−1800 ~ −1600 (Forward)	5′-CTACTCCCCGAAAGTGGGTT-3′
−1800 ~ −1600 (Reverse)	5′-TCTATAGGAAGGGAAGAAGAATGC-3′
−2000 ~ −1800 (Forward)	5′-GAGAGAGGCCCTCACCCG-3′
−2000 ~ −1800 (Reverse)	5′-ACCCACTTTCGGGGAGTAGTAT-3′

Primers were designed with Primer 5 and purchased from Sangon.

Table S5. **Sequences for shRNAs used in this study**

shRNA target gene	Sequences
Myog #1	5′-AGGAATTTAGCTGACTCCTTAA-3′
Hoxa1 #1	5′-CGGCCCTGGCCACGTATAATAA-3′
Hoxa1 #2	5′-AGCCACGTATAATAACTCCTTA-3′
Nkx1-1 #1	5′-CCGTTCCTACAGAAGAGAAAT-3′
Nkx1-1 #2	5′-GCCATGTCCCAGAACAAGCAT-3′
Aire #1	5′-CCCTTCCTCTTGGAAACGGAAT-3′
Aire #2	5′-CCGACCTGGAGTCCCTCCTCAA-3′
Pax7 #1	5′-CGTCCAGGTCTGGTTCAGTAA-3′
Pax7 #2	5′-GGCTCTTCAAGGTCTGGACAA-3′
Tal1 #1	5′-CGGACAAACTGTCCTGTACATA-3′
Tal1 #2	5′-CCCTGTGTATCTGTCATTGTAT-3′
Hey1 #1	5′-ACCGACGAGACCGAATCAATAA-3′
Hey1 #2	5′-CCGCCACTATGCTCAATGTTAA-3′
Foxc1 #1	5′-CGCCTCTCACCTGTAAGATATT-3′
Foxc1 #2	5′-CCCTATATGTCTGAATACTTTA-3′
Sox18 #1	5′-ACGAATTTGACCAGTATCTCAA-3′
Gli2 #1	5′-GGCACCAACCCTTCAGACTAT-3′
Gli2 #2	5′-GGCCAGTATCCAGGATATAAT-3′
Bap1 #1	5′-CCGTCTGTGATTGATGATGATA-3′
Bap1 #2	5′-CCCACGTCACCTTCCTGAGAAA-3′

Target sequences for RNA interference were designed according to MSCV-LTRmiR30-PIG system instructions.