

Supplementary Figure Legends:

Supplemental Figure 1. p21-tdT⁺ HSCs were in an inactive state of cell division

(A) Generation of p21-tdTomato knock-in allele. (B) FACS gating strategies for indicated cells. (C) Representative flow cytometric analysis showing the percentages of p21-tdT⁺ cell populations in Lin⁺, Lin⁻, LKS⁻, CMP, GMP, MEP, and MkP from wild type (WT) and p21-tdTomato mice. (D-E) Representative flow cytometric analysis (D) and the histograms (E) showing the percentages of p21-tdT⁺ cell populations in CD3⁺, B220⁺, CD11b⁺ and Ter119⁺ cells from wild type and p21-tdTomato mice. (F) The kinetics of cell division in p21-tdT⁺ and p21-tdT⁻ HSCs during the 4-day culture period.

Supplemental Figure 2. p21-tdT⁺ HSCs possess enhanced self-renewal capacity

(A) Representative images of single-cell culture clones. Scale bars, 5mm. (B) Representative images of typical clones from single-cell culture. Scale bars, 500 μ m. (C) Representative images of cell population cultures. Scale bars, 200 μ m. (D) Percentages of different colony types in primary culture on day 8. (E-F) Representative flow cytometric analysis (E) and the histograms (F) showing the percentages of donor-derived CD45.2 cells in the BM of recipient mice at 18h after transplantation (n = 5 recipient mice per condition). (G) Percentages of donor-derived LT-HSCs in the BM of recipient mice in 4th month after primary transplantation (n = 4 recipient mice per condition). (H) Representative flow cytometric analysis showing the gating strategies for the indicated donor-derived cells. Data are represented as mean \pm SD. ns, No Significance; *, $P < 0.05$; **, $P < 0.01$; unpaired two-tailed Student's t test for D.

Supplemental Figure 3. Zbtb18 is highly expressed in p21-tdT⁺ HSCs

(A) Heatmap showing ATAC-seq results for p21-tdT⁻ and p21-tdT⁺ HSCs from p21-tdTomato mice. (B) TSS profile and heatmap of CUT&Tag sequencing results showing binding of H3K4me1, H3K4me2, H3K4me3 histone modifications in p21-tdT⁻ and p21-tdT⁺ HSCs.

Supplemental Figure 4. p21 interacts with ZBTB18 to repress the transcription of *cKit*

(A) Representative gating strategy for flow cytometric analysis of cKit expression in p21-tdT⁻ and p21-tdT⁺ HSCs (LKS⁺CD150⁺CD48⁻) from p21-tdTomato mice. (B) Representative gating strategy for flow cytometric analysis of p21 expression in cKit-high and cKit-low HSCs (LKS⁺CD150⁺CD48⁻) from p21-tdTomato mice. (C) Western blotting analysis showing the expression of indicated proteins in the reporter assays of 293T cells. (D) The summary about the mechanism and function of p21 in HSCs is as follows: p21 can interact with ZBTB18, co-repressing the expression of cKit with decreased chromatin accessibility. Therefore, p21-tdT⁺ HSCs, characterized by low expression of cKit, demonstrate enhanced long-term and self-renewal abilities compared to p21-tdT⁻ HSCs.

Table S1. Differentially expressed genes in p21-tdT⁻ and p21-tdT⁺ HSCs

Table S2. ATAC-seq upregulated genes in p21-tdT⁺ HSCs (76)

Table S3. Differentially expressed genes of H3K27ac CUT&Tag sequencing in p21-tdT⁻ and p21-tdT⁺ HSCs

Table S4. Zbtb18 CUT&Tag sequencing upregulated genes in p21-tdT⁺ HSCs (8968)

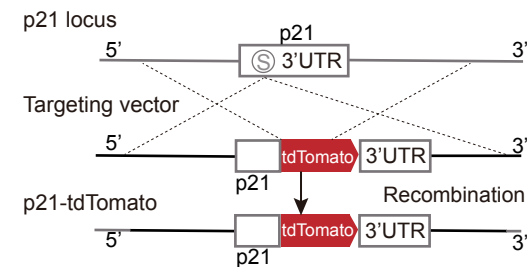
Table S5. H3K4me3 CUT&Tag downregulated genes in p21-tdT⁺ HSCs (663)

Table S6. Overlapped genes of upregulated genes in Zbtb18 CUT&Tag-seq and downregulated genes in RNA-seq, H3K4me3 and H3K27ac CUT&Tag-seq in p21-tdT⁺ HSCs (47)

sFigure 1

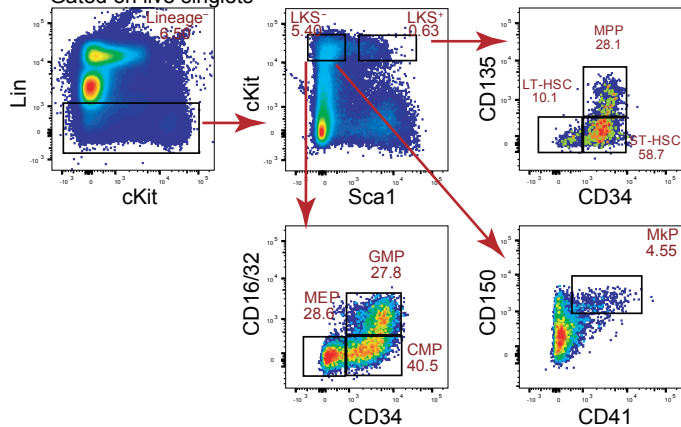
A

p21-tdTomato knockin strategy

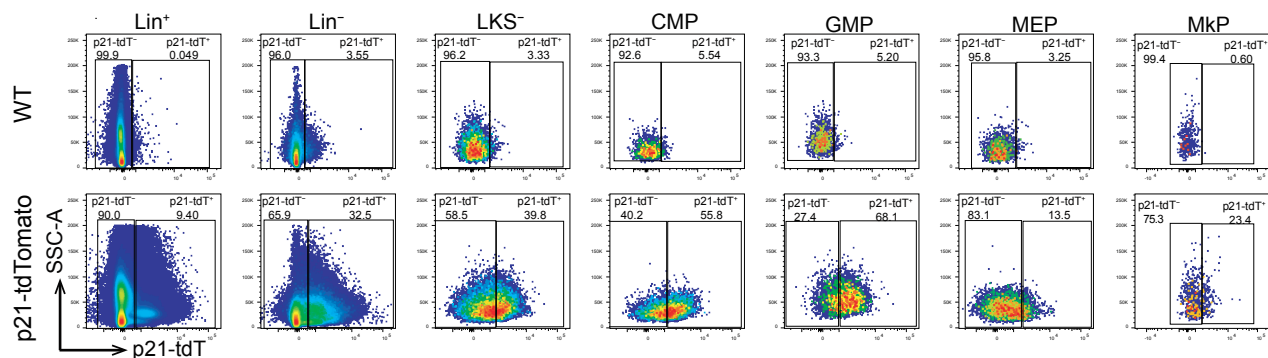


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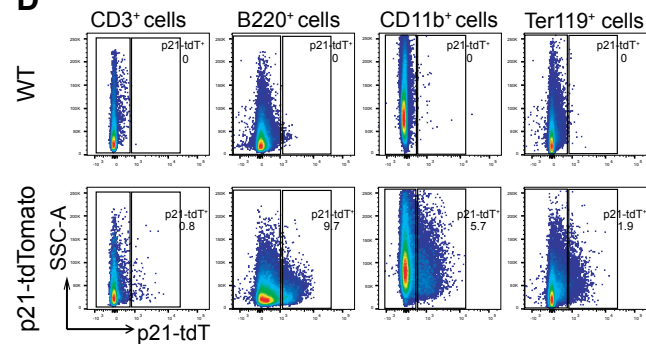
Gated on live singlets



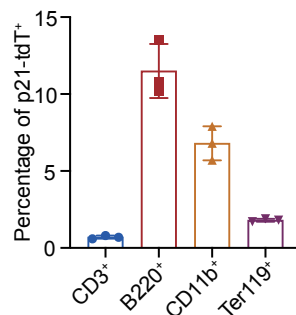
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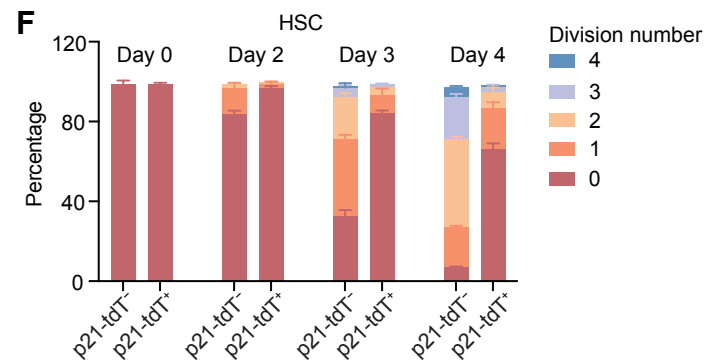
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E

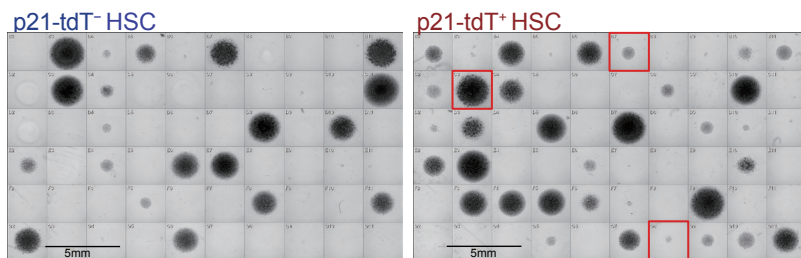


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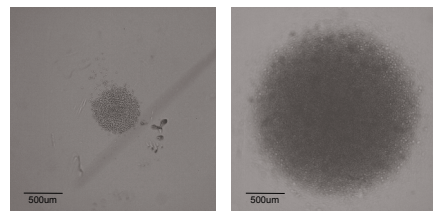
sFigure 2

A

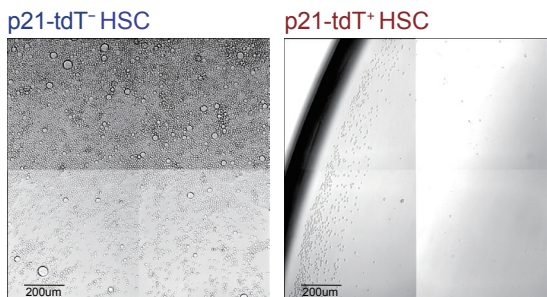


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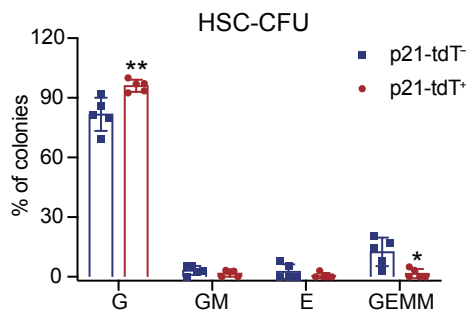
Typical clones



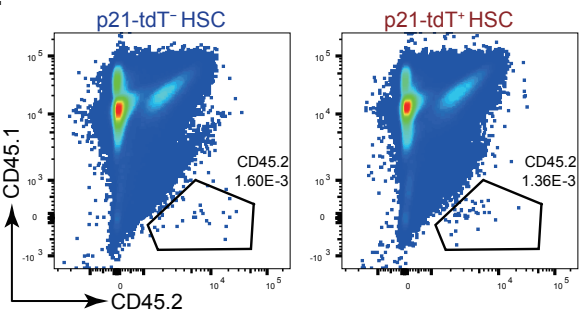
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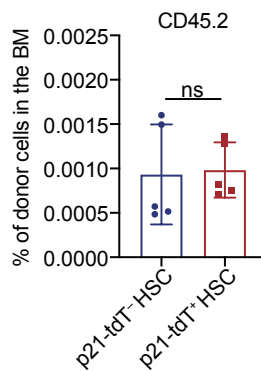
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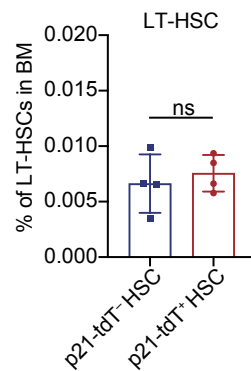
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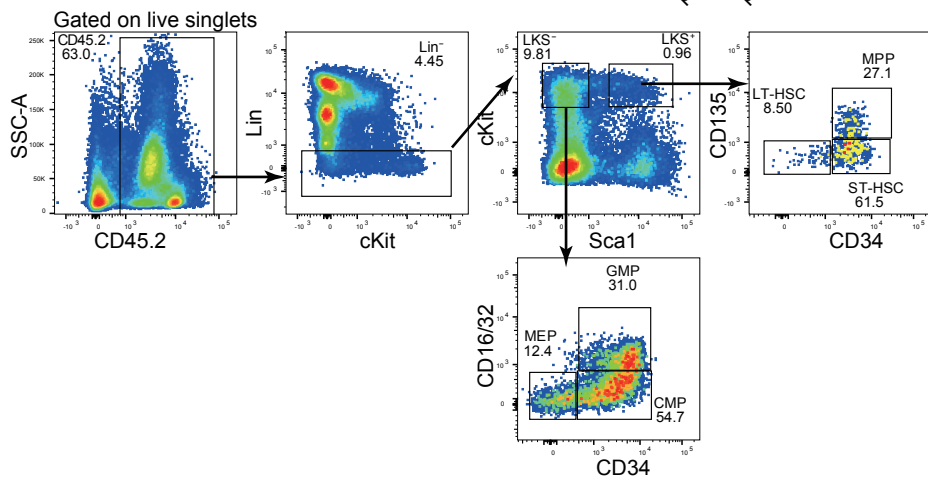
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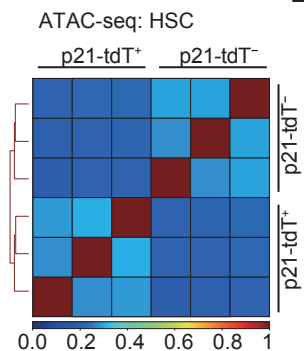


H



sFigure 3

A



B

