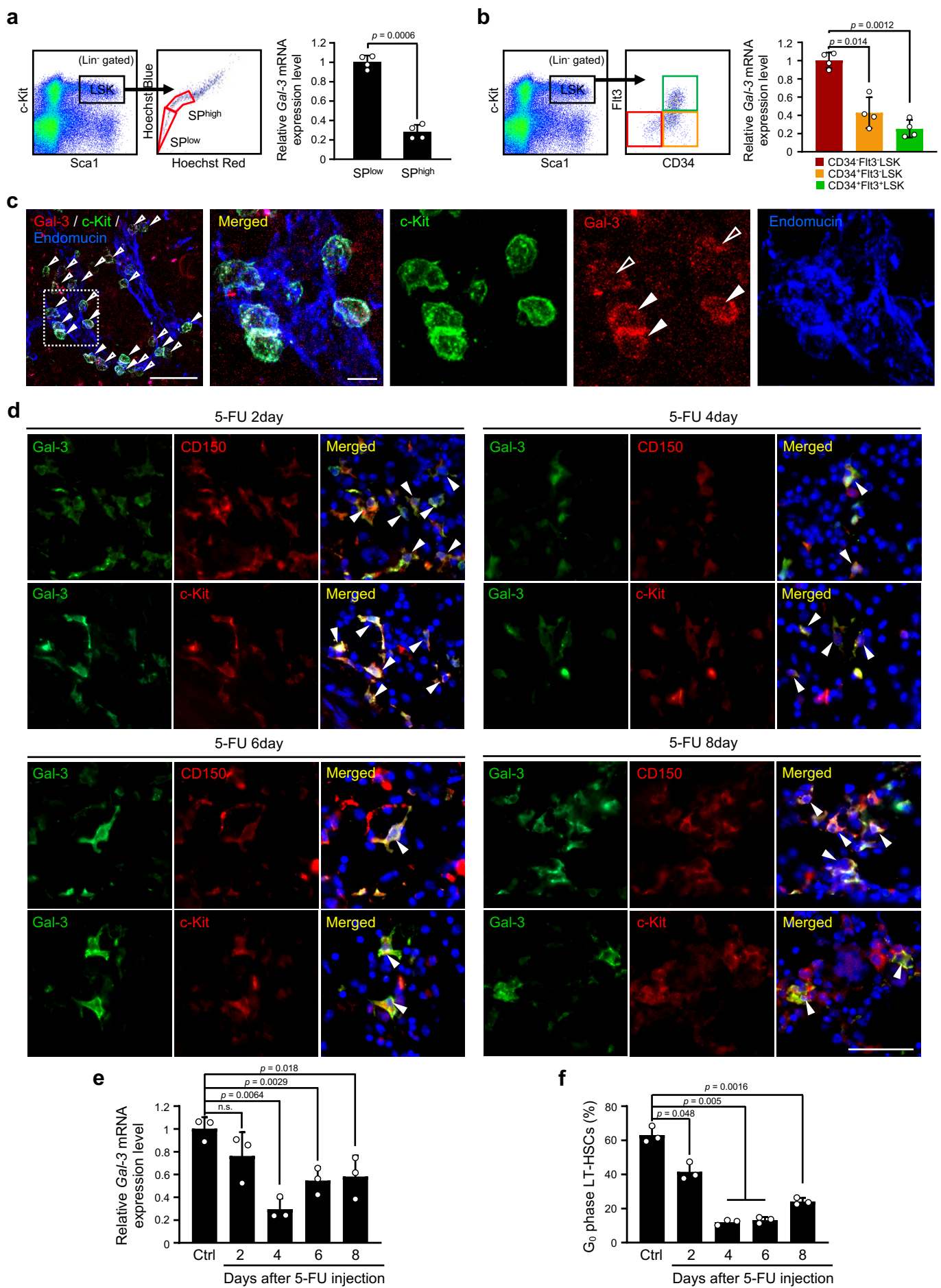
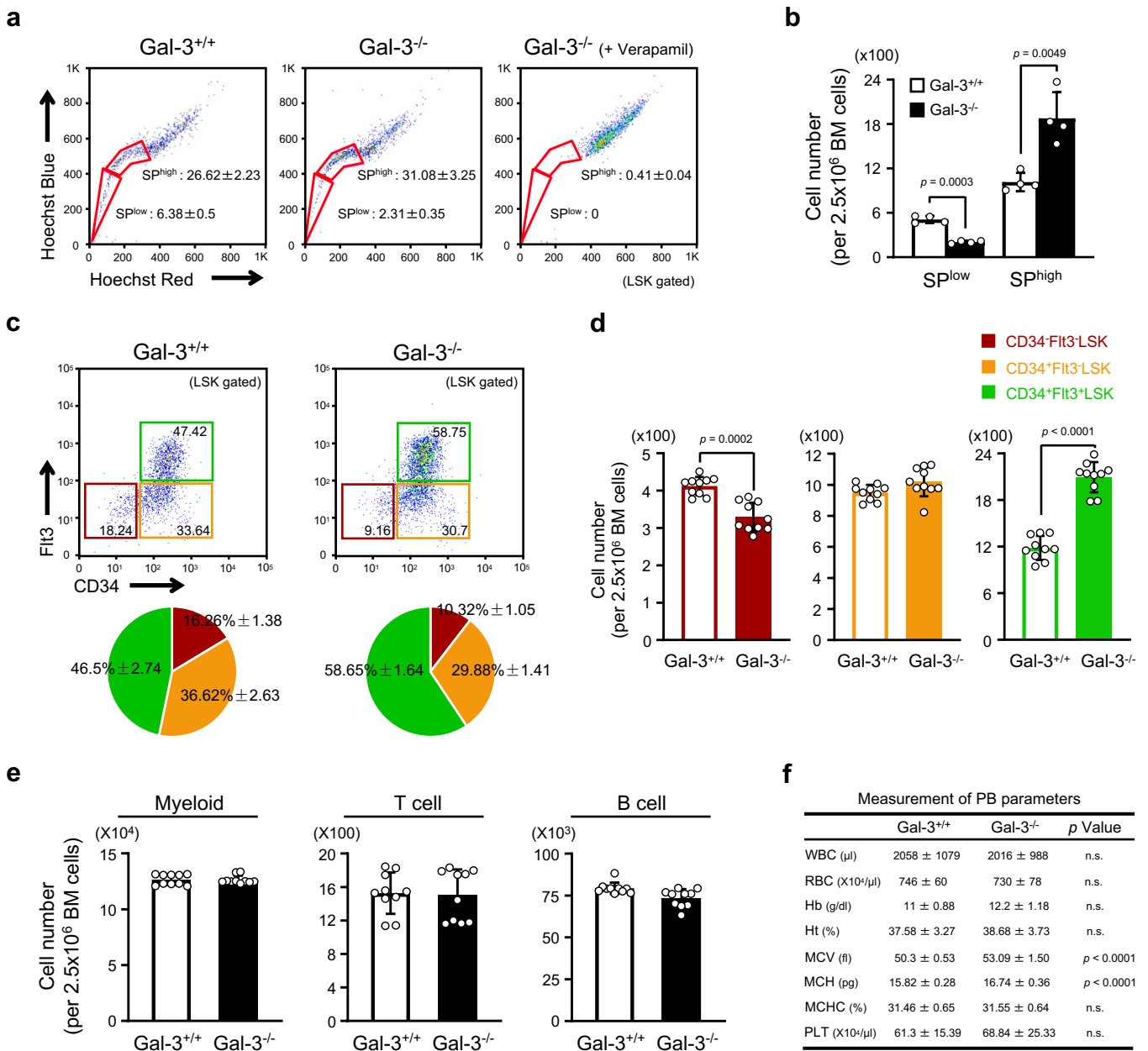




Supplementary Fig. 1

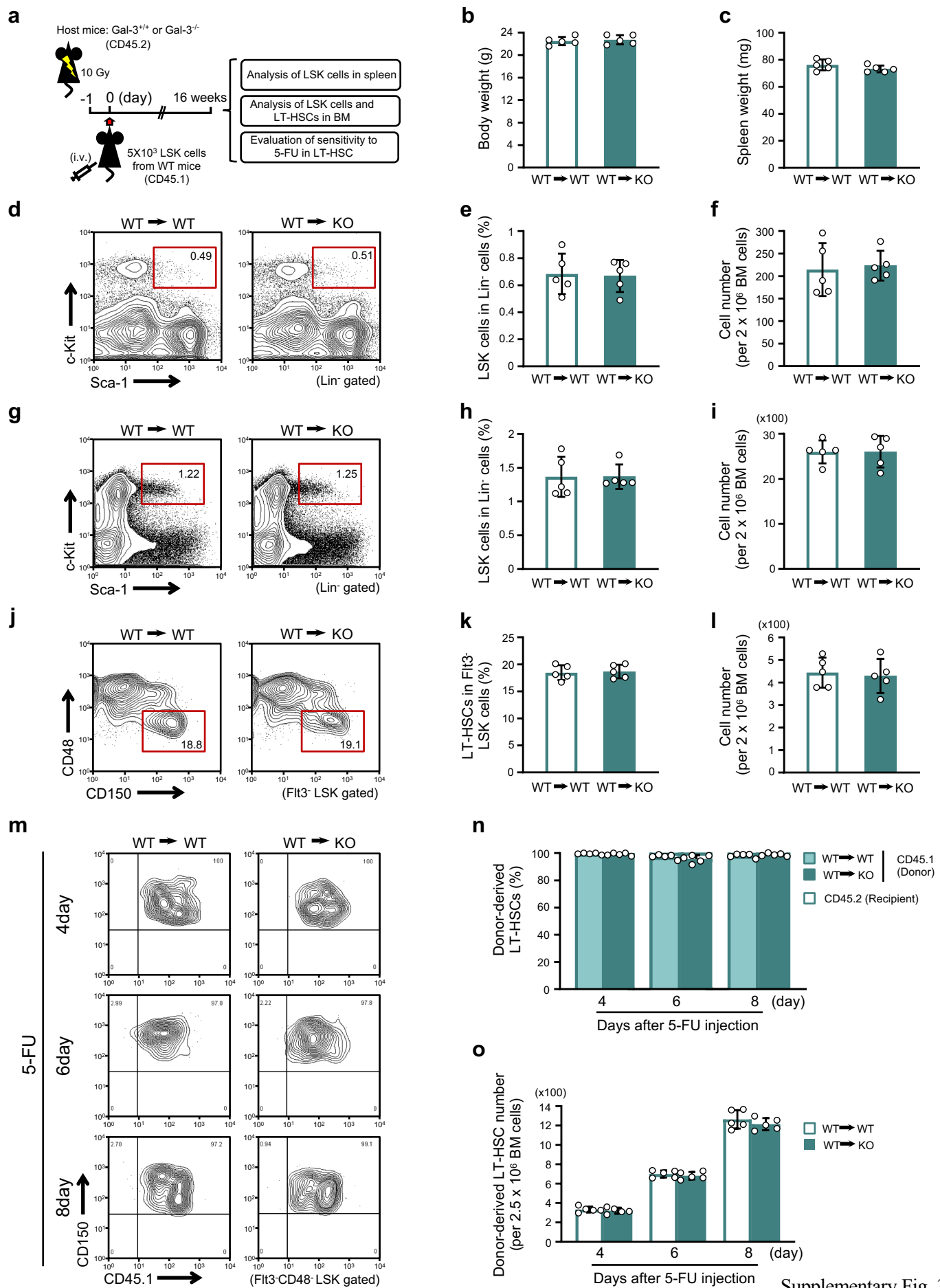
Supplementary Fig. 1: High Gal-3 expression in HSCs is closely related to slow cell cycling or quiescence.

- (a) Relative expression level of *Gal-3* mRNA in BM-derived SP^{low} and SP^{high} LSK cells from WT mice ($n = 4$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (b) Relative expression level of *Gal-3* mRNA in CD34⁻Flt3⁻, CD34⁺Flt3⁻ and CD34⁺Flt3⁺ LSK cells from WT mice ($n = 4$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (c) Immunofluorescence staining of Gal-3 (red), c-Kit (green) and Endomucin (blue) in the BM sections from WT mice. Dashed box indicates areas shown at higher magnification in right panels. Transparent and white arrows indicate focal Gal-3-negative and Gal-3-positive cells. A representative image is shown ($n = 3$ biologically replicates). Scale bar, 50 μ m and 10 μ m (inset).
- (d) Immunofluorescence staining of Gal-3 (green) and CD150 or c-Kit (red) in BM sections from WT mice at different time points after 5-FU treatment. DAPI (blue) was used to detect nuclei. Arrows indicate focal co-expression of Gal-3 and CD150 or c-Kit in the cells. A representative image is shown ($n = 3$ biologically replicates at each time point). Scale bar, 50 μ m.
- (e) Relative expression level of *Gal-3* mRNA in LT-HSCs (CD150⁺CD48⁻Flt3⁻LSK) in BM of WT mice at different time points after 5-FU treatment. The control group (Ctrl) was treated with PBS ($n = 3$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D. and n.s., not significant.
- (f) The percentage of WT LT-HSCs (CD150⁺CD48⁻Flt3⁻LSK) in G₀ phase at different time points after 5-FU treatment. The Ctrl was treated with PBS ($n = 3$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..



Supplementary Fig. 2: Gal-3 deficiency in LT-HSC does not alter the frequency of mature blood cells.

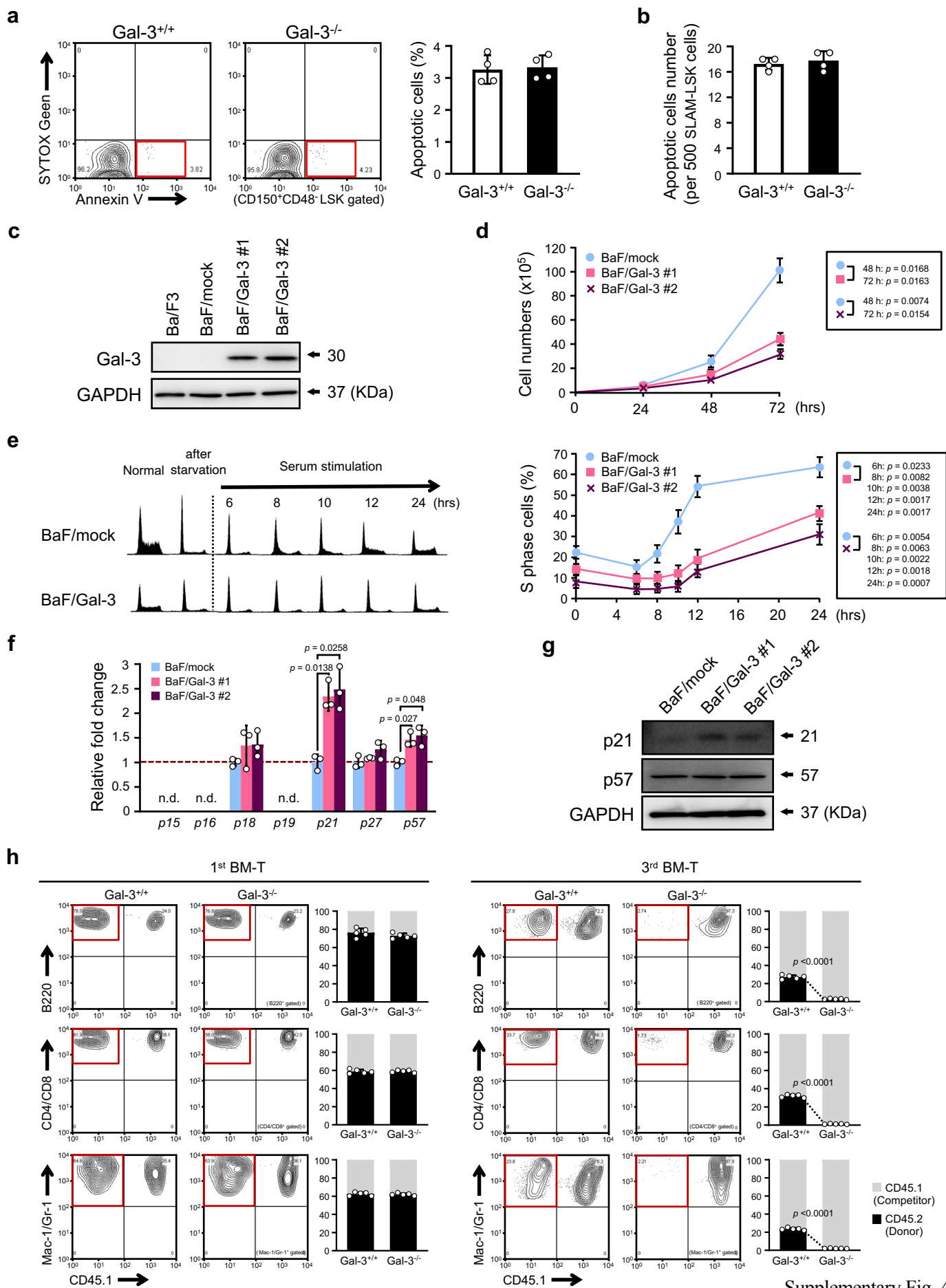
- (a) Representative flow cytometric analysis of SP^{low} and SP^{high} LSK populations in BM of Gal-3^{+/+} or Gal-3^{-/-} mice. The indicated values represented the percentage among LSK population, and disappearance of SP cells caused by Verapamil treatment (25 µg/ml). $n = 4$ biological replicates per genotype, compared by two-sided t test. Data are presented as mean values $\pm$ S.D. and exact p values: SP^{low}: $p = 0.00035$ vs Gal-3^{-/-}; SP^{high}: $p = 0.18$ vs Gal-3^{-/-}.
- (b) Absolute number of SP^{low} and SP^{high} LSK cells in BM of Gal-3^{+/+} or Gal-3^{-/-} mice ($n = 4$ biological replicates per genotype, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (c) (Top) Representative flow cytometric analysis of CD34⁻Flt3⁻, CD34⁺Flt3⁻ and CD34⁺Flt3⁺ LSK populations in BM of Gal-3^{+/+} or Gal-3^{-/-} mice. (Bottom) Pie charts represent average frequencies of the 3 subsets within the LSK population ($n = 10$ biological replicates per genotype, compared by two-sided t test). Data are presented as mean values $\pm$ S.D. and exact p values: CD34⁻Flt3⁻ LSK cells: $p < 0.0001$ vs Gal-3^{-/-}; CD34⁺Flt3⁻ LSK cells: $p = 0.00018$ vs Gal-3^{-/-}; CD34⁺Flt3⁺ cells: $p < 0.0001$ vs Gal-3^{-/-}.
- (d) Absolute number of CD34⁻Flt3⁻, CD34⁺Flt3⁻ and CD34⁺Flt3⁺ LSK cells in BM of Gal-3^{+/+} or Gal-3^{-/-} mice ($n = 10$ biological replicates per genotype, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (e) Absolute number of myeloid cells (Mac-1/Gr-1⁺), T cells (CD4/CD8⁺) and B cells (B220⁺) in BM of Gal-3^{+/+} or Gal-3^{-/-} mice ($n = 10$ biological replicates per genotype).
- (f) Comparison of blood cell counts in Gal-3^{+/+} and Gal-3^{-/-} mice ($n = 12$ biological replicates per genotype, compared by two-sided t test). Data are presented as mean values $\pm$ S.D.. WBCs: white blood cells; RBCs: red blood cells; Hb: hemoglobin; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLTs: platelets. n.s., not significant.



Supplementary Fig. 3

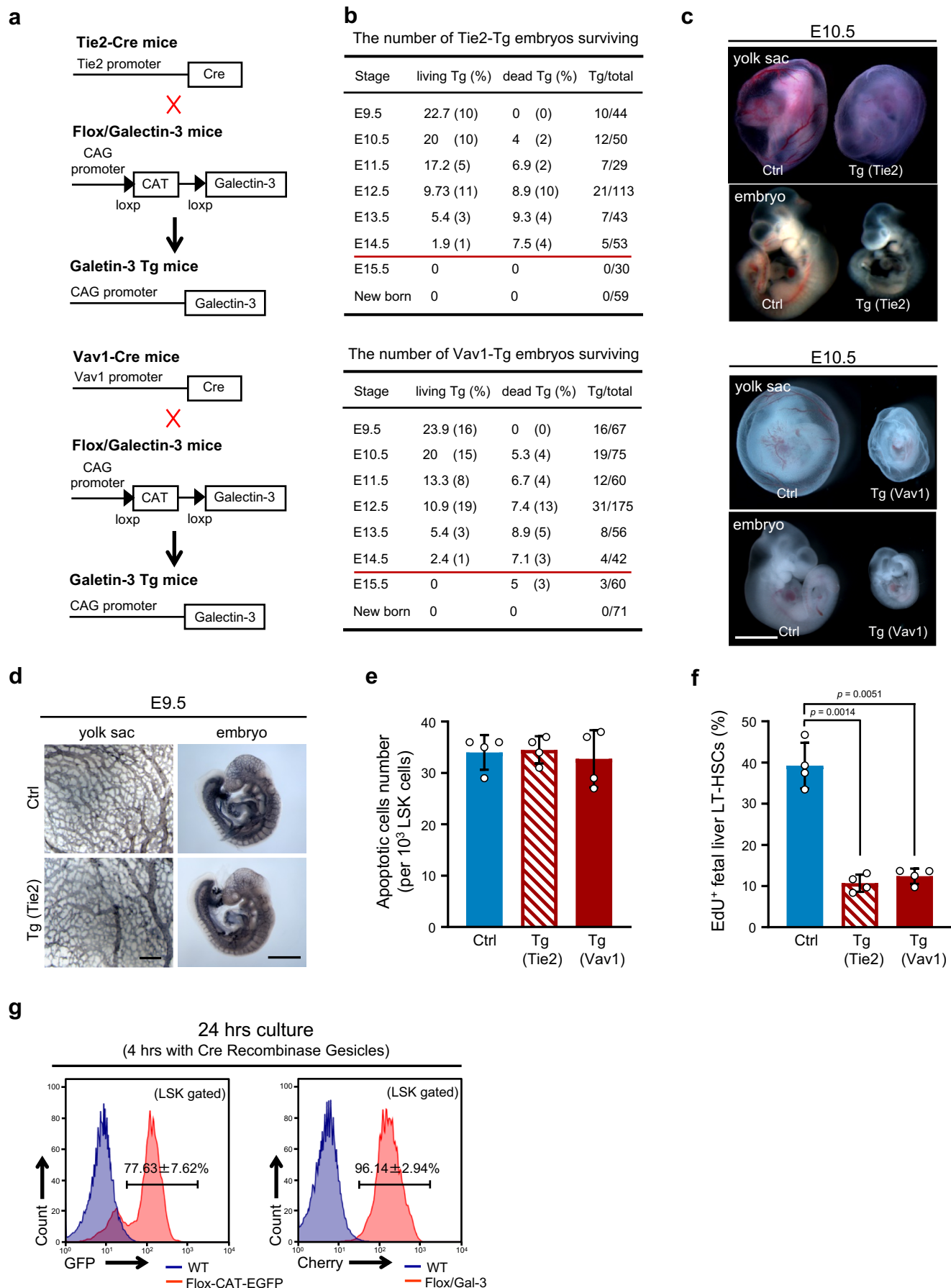
Supplementary Fig. 3: Gal-3 deficiency in BM stromal cells does not affect HSC number and function.

- (a) Experimental schema for the BM-T chimeric mouse model. Donor (CD45.1)-derived WT LSK cells were transplanted into lethally-irradiated Gal-3^{+/+} (WT) or Gal-3^{-/-} (KO) mice (CD45.2). WT donors into WT recipients (WT→WT); WT donors into KO recipients (WT→KO).
- (b) Weight of body after BM-T (*n* = 5 biological replicates per genotype).
- (c) Weight of spleen after BM-T (*n* = 5 biological replicates per genotype).
- (d) Representative flow cytometric analysis of donor-derived LSK cell frequencies (red box) in spleens of recipient mice (WT or KO).
- (e) The percentage of donor-derived LSK cells in the Lin⁻ population in spleens of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (f) Absolute number of donor-derived LSK cells in spleens of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (g) Representative flow cytometric analysis of donor-derived LSK cell frequencies (red box) in BM of recipient mice (WT or KO).
- (h) The percentage of donor-derived LSK cells in the Lin⁻ population in BM of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (i) Absolute number of donor-derived LSK cells in BM of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (j) Representative flow cytometric analysis of donor-derived LT-HSC (CD150⁺CD48-Flt3-LSK) frequencies (red box) in BM of recipient mice (WT or KO).
- (k) The percentage of donor-derived LT-HSCs (CD150⁺CD48-Flt3-LSK) in the Flt3-LSK population in BM of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (l) Absolute number of donor-derived LT-HSCs (CD150⁺CD48-Flt3-LSK) in BM of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (m) Representative flow cytometric analysis of donor-derived LT-HSC (CD45.1⁺CD150⁺CD48-Flt3-LSK) frequencies in BM of recipient mice (WT or KO) at different time points after 5-FU treatment.
- (n) The percentage of donor-derived LT-HSCs (CD45.1⁺CD150⁺CD48-Flt3-LSK) in the Flt3-LSK population in BM of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.
- (o) Absolute number of donor-derived LT-HSCs (CD45.1⁺CD150⁺CD48-Flt3-LSK) in BM of recipient mice (WT or KO). *n* = 5 biological replicates per genotype.



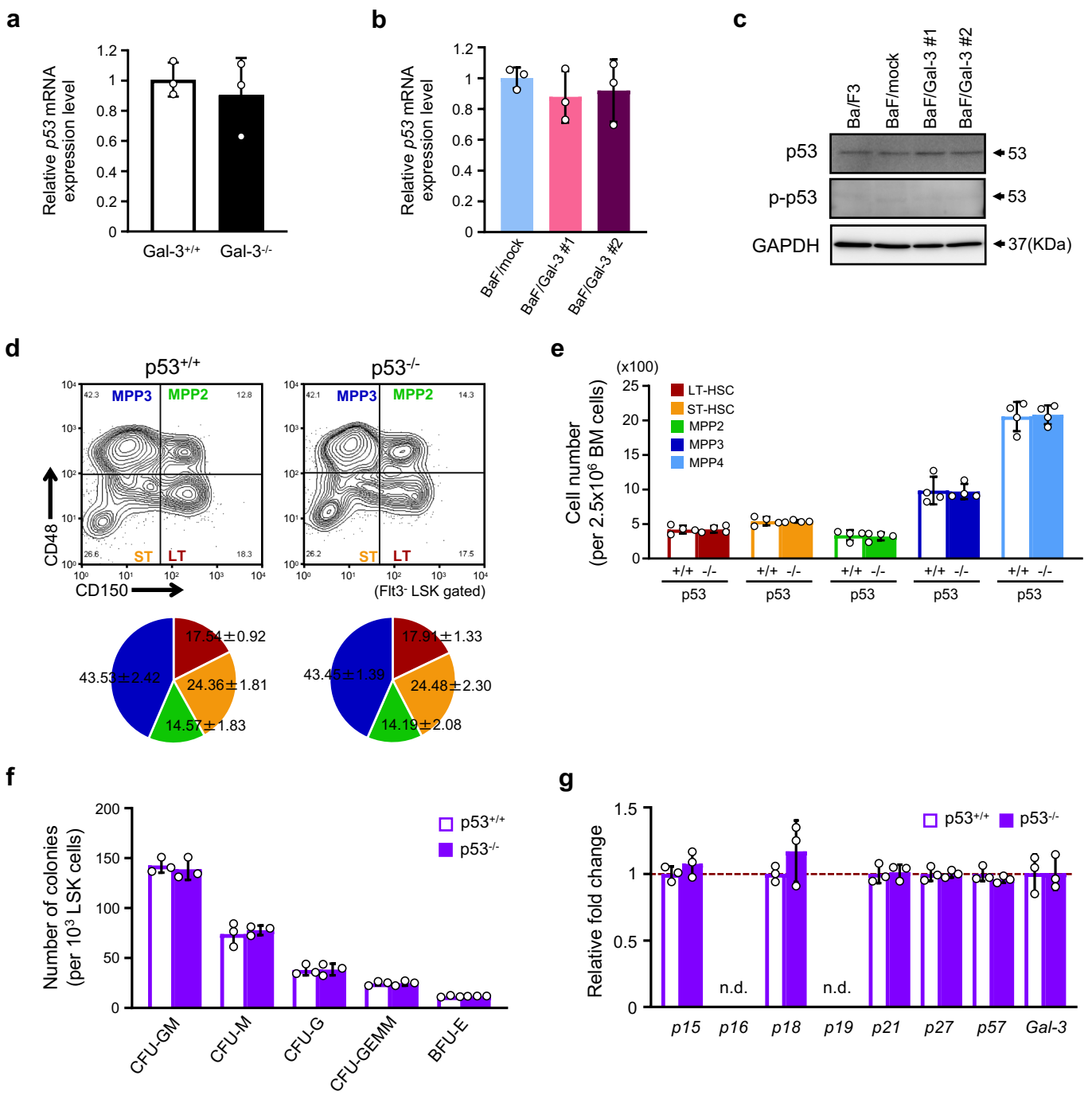
Supplementary Fig. 4: Overexpression of Gal-3 suppresses cell-cycle progression in Ba/F3 cells.

- (a) (Left) Representative flow cytometric analysis of apoptotic Gal-3^{+/+} or Gal-3^{-/-} LT-HSCs (CD150⁺CD48⁻LSK, red box) using Annexin V and SYTOX Green staining. (Right) Bar graph showing the percentage of apoptotic cells (Annexin V⁺/SYTOX Green⁺) in LT-HSCs ($n=4$ biological replicates per genotype).
- (b) Absolute number of apoptotic cells in Gal-3^{+/+} or Gal-3^{-/-} LT-HSCs (CD150⁺CD48⁻LSK). $n=4$ biological replicates per genotype.
- (c) Western blotting of Gal-3 in Ba/F3, BaF/mock and BaF/Gal-3 cells (#1, #2).
- (d) Growth curves of BaF/mock and BaF/Gal-3 cells (#1, #2). Cells (1×10^4) were seeded in 6 well dishes and counted after 24, 48, and 72 hrs ($n=3$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (e) Cell cycle analysis of BaF/mock and BaF/Gal-3 cells (#1, #2) by staining with PI. (Left) Cells were harvested and cultured in under serum-free conditions for 12 hrs. After starvation, both were stimulated with serum and IL-3 for 24 hrs, and cell cycle time points measured. (Right) Percentages of S phase cells were measured at each time point ($n=3$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (f) Relative expression level of mRNA for *p16* and *p21* family members in BaF/mock and BaF/Gal-3 cells (#1, #2) ($n=3$ biological replicates, compared by two-sided t test). Data are presented as mean values $\pm$ S.D. and n.d., not determined.
- (g) Western blotting of p21 and p57 in BaF/mock and BaF/Gal-3 cells (#1, #2).
- (h) Analysis of donor-derived B cells (B220⁺CD45.1⁻), T cells (CD4/CD8⁺CD45.1⁻) and myeloid (Mac-1/Gr-1⁺CD45.1⁻) in peripheral blood from primary and third BM-T recipients. Flow cytometric plots showed the relative contribution of mature blood cells from Gal-3^{+/+} or Gal-3^{-/-} donor-derived LT-HSCs (red boxes). Bar graph showing the percentage of donor- or competitor-derived B cells, T cells and myeloid cells in peripheral blood ($n=5$ biological replicates per genotype, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..



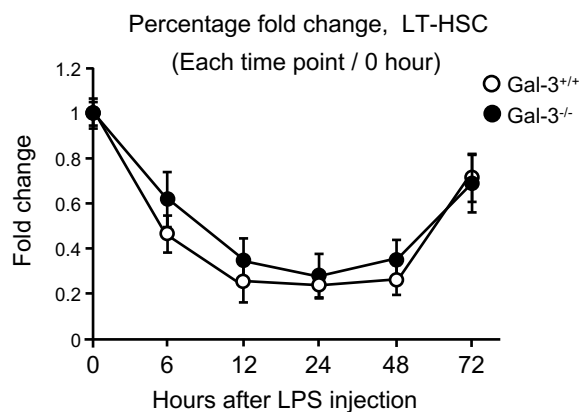
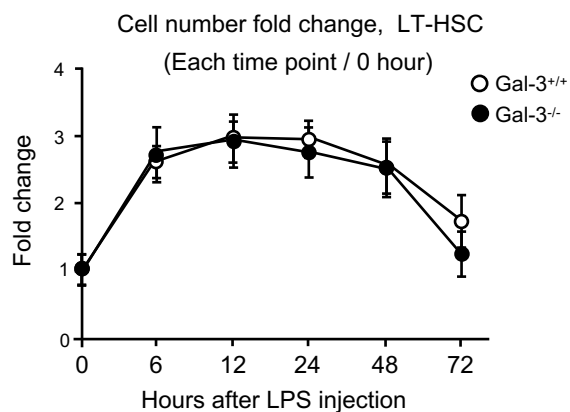
Supplementary Fig. 5: Early embryonic lethality in Gal-3 transgenic mice caused by anemia.

- (a) Schematic representation of the construct used to generate conditional transgenic mice. Overexpressed Gal-3 in Tie2-expressing cells or Vav-1-expressing cells. Black triangles in the middle figure indicate the loxp sites.
- (b) Survival of Gal-3 Tg mice (Tie2-cre-Flox/Gal-3 or Vav-1-Cre-Flox/Gal-3) at different gestational ages. No Gal-3 Tg embryos survived beyond E15.5.
- (c) Severe anemic phenotype displayed in E10.5 Gal-3 Tg embryos and yolk sac. Embryos were obtained by mating Tie2-Cre (Top) or Vav-1-Cre (Bottom) with Flox/Gal-3 (Ctrl) mice. A representative image is shown ($n = 5$ biological replicates per genotype). Scale bar, 1 mm.
- (d) Whole mount staining of CD31 in the yolk sac and embryos of control (Ctrl, Flox/Gal-3) and Gal-3 Tg embryos (Tie2-Cre background) at E9.5. A representative image is shown ($n = 5$ biological replicates per genotype). Scale bar, 200 μ m (yolk sac) and 1 mm (embryos).
- (e) Apoptosis of LT-HSCs (CD150⁺CD48⁻LSK) from E12.5 Ctrl (Flox/Gal-3) and Gal-3 Tg fetal liver (Tie2-cre-Flox/Gal-3 or Vav-1-Cre-Flox/Gal-3) assessed by staining with Annexin V and SYTOX Green. Bar graph shows the absolute number of apoptotic cells in fetal liver-LT-HSCs ($n = 4$ biological replicates per genotype)
- (f) Percentage of EdU-positive cells in fetal liver LT-HSCs (CD150⁺CD48⁻LSK). These cells were sorted from E12.5 Ctrl (Flox/Gal-3) and Gal-3 Tg embryos (Tie2-cre-Flox/Gal-3 or Vav-1-Cre-Flox/Gal-3) and labeled with EdU for 2 hrs ($n = 4$ biological replicates per genotype, compared by two-sided t test). Data are presented as mean values $\pm$ S.D..
- (g) Determination of Cre recombinase activity in ex vivo experiments. Purified LSK cells from the BM of Flox-CAT-EGFP or Flox/Gal-3 mice were treated with Cre Recombinase Gesicles for 4 hrs. One day later, fluorescent protein (GFP or mCherry) expression levels were estimated by flow cytometry.



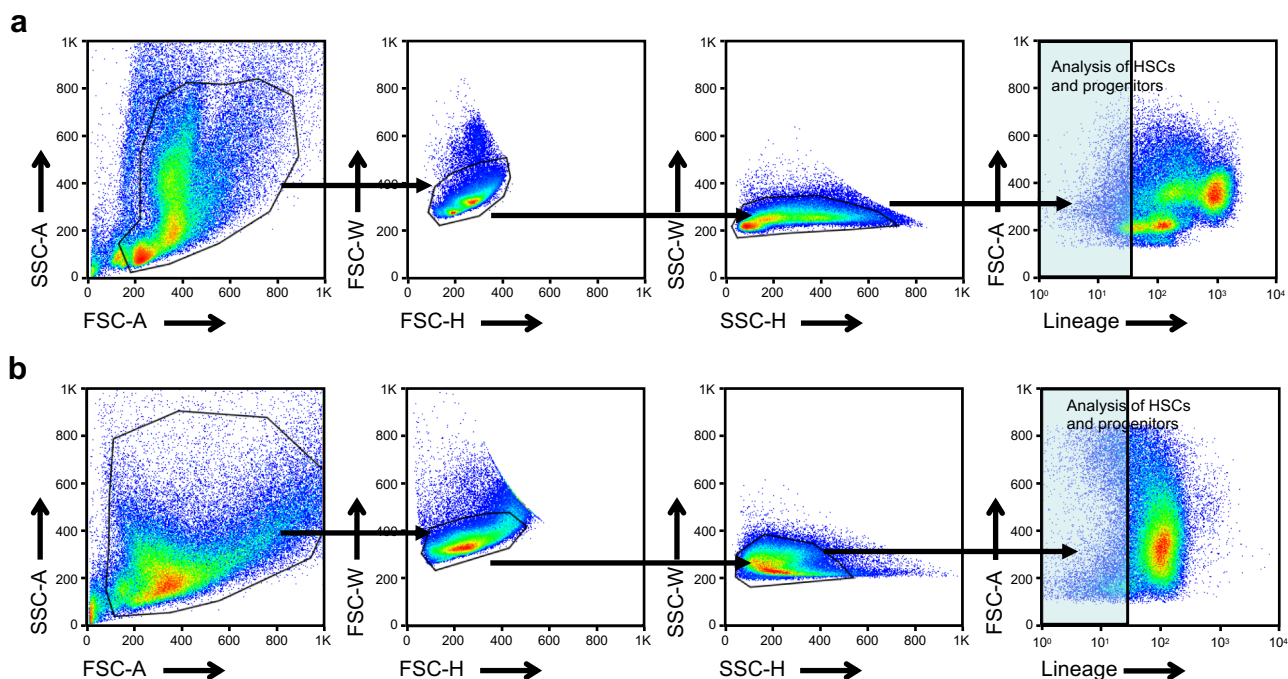
Supplementary Fig. 6: Gal-3 regulates *p21* transcription in LT-HSC independent of p53.

- (a) Relative expression level of *p53* mRNA in LT-HSCs (CD150⁺CD48⁺Flt3⁺LSK) in BM of Gal-3^{+/+} or Gal-3^{-/-} mice ($n = 3$ biological replicates per genotype).
- (b) Relative expression level of *p53* mRNA in BaF/mock or BaF/Gal-3 cells (#1, #2). $n = 3$ biological replicates.
- (c) Western blotting of p53 and p53 phosphorylation (p-p53) in BaF3, BaF/mock and BaF/Gal-3 cells (#1, #2).
- (d) (Top) Representative flow cytometric analysis of LT-HSC, ST-HSC, MPP2 and MPP3 populations in BM of p53^{+/+} or p53^{-/-} mice. (Bottom) Pie charts represent average frequencies of the 4 subsets within the Flt3⁺ LSK population ($n = 4$ biological replicates per genotype).
- (e) Absolute number of LT-HSCs, ST-HSCs, MPP2, MPP3 and MPP4 in BM of p53^{+/+} or p53^{-/-} mice ($n = 4$ biological replicates per genotype).
- (f) Colony forming potential of LSK cells from p53^{+/+} or p53^{-/-} mouse BM in CFU assays ($n = 3$ biological replicates per genotype).
- (g) Relative expression level of mRNA for *p16* and *p21* family members in BM LT-HSCs (CD150⁺CD48⁺Flt3⁺LSK) from p53^{+/+} or p53^{-/-} mice ($n = 3$ biological replicates per genotype). n.d., not determined.

a**b**

Supplementary Fig. 7: Response of Gal-3 KO LT-HSCs to hematopoietic stress caused by LPS-treatment.

- (a) Fold-changes in percentages of LT-HSCs (CD150⁺CD48⁺Flt3⁺LSK) in BM of Gal-3^{+/+} or Gal-3^{-/-} mice after LPS injection ($n = 3$ biological replicates per genotype at each time point).
- (b) Fold-changes in numbers of LT-HSCs (CD150⁺CD48⁺Flt3⁺LSK) in BM of Gal-3^{+/+} or Gal-3^{-/-} mice after LPS injection ($n = 3$ biological replicates per genotype at each time point).



Supplementary Fig. 8: Gating strategies used for cell analysis and sorting.

- (a) Gating strategy to analyze and BM cells from Gal-3^{+/+}, Gal-3^{-/-}, Flox/Gal-3 and p53^{-/-} mice (Fig.1 a-c, Fig.2 a-d, f, g, i, Fig.3 a-g, i, Fig.4 h-m, Fig.5 a-c, e-i, k, l, n, o, Fig.6 e, g, h, Supplementary Fig.1 a, b, e, f, Supplementary Fig.2 a-e, Supplementary Fig.3 d-o, Supplementary Fig.4 a, b, h, Supplementary Fig.5 g, Supplementary Fig.6 a, d-g, Supplementary Fig.7 a, b).
- (b) Gating strategy to analyze and fetal liver cells from WT, control (Flox/Gal-3) and Gal-3 Tg (Tie2-Cre-Flox/Gal-3 or Vav-1-Cre-Flox/Gal-3) embryos (Fig.4 a-f, Supplementary Fig.5 e, f,).

Fig. 6 b

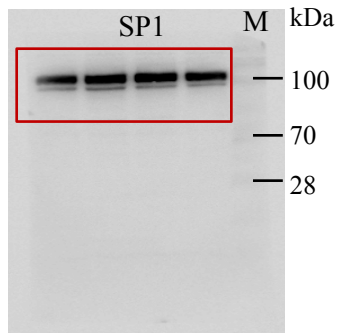


Fig. 6 c

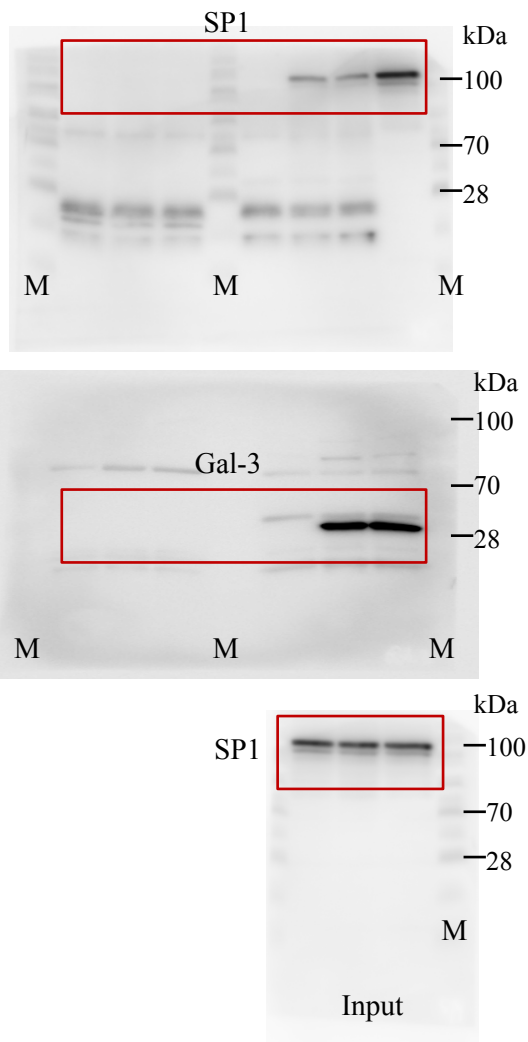
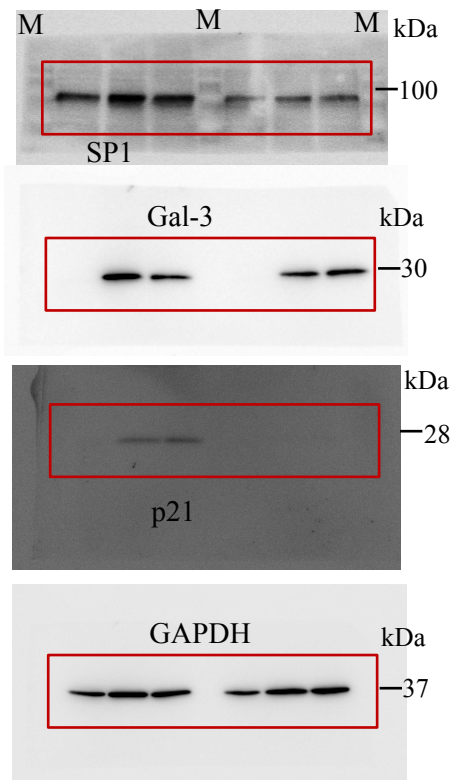
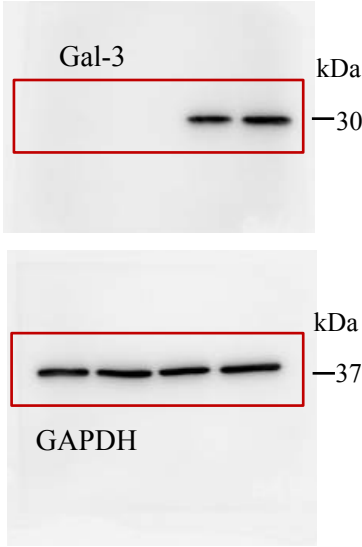


Fig. 6 d

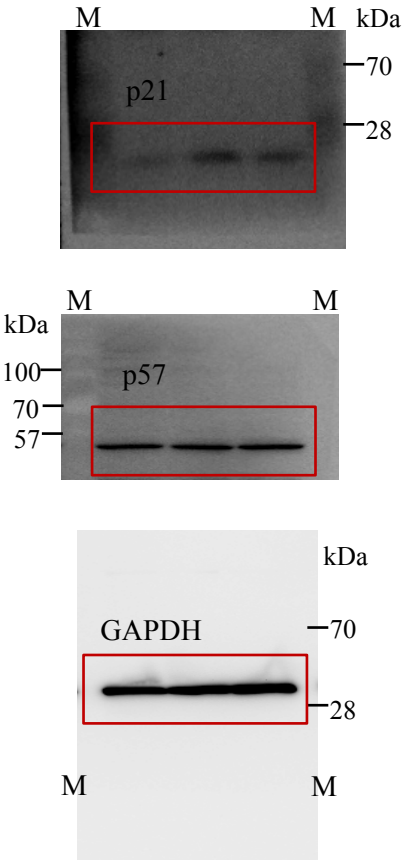


Supplementary Fig. 9: Full scans of Western blots shown in main figures.
Cropped areas are marked by red box. M: Marker

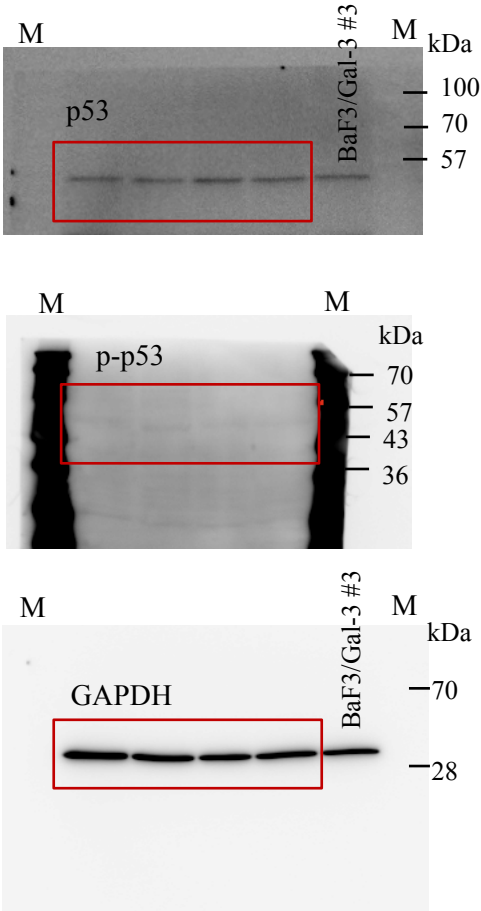
Supplemental Fig. 4 c



Supplemental Fig. 4 g



Supplemental Fig. 6 c



Supplementary Fig. 10: Full scans of Western blots shown in supplementary figures.
Cropped areas are marked by red box. M: Marker

Supplementary Table 1

Sequences of gene-specific primers for real-time PCR

Gene name	Forward primer	Reverse primer
<i>Gal-3</i>	5'-tgccctatgacctgccctt-3'	5'-tctctctcgtgttacacacaa-3'
<i>p15</i>	5'-agatcccaacgcctgaac-3'	5'-cccatcatcatgacctggatt-3'
<i>p16</i>	5'-cgtaccccgattcaggtgat-3'	5'-ttgagcagaagagctgctacgt-3'
<i>p18</i>	5'-ttcaaagaccgatgcgtatcc-3'	5'-ctatgtcagtatcagccagcaaa-3'
<i>p19</i>	5'-gccgcaccggaatcct-3'	5'-ttgagcagaagagctgctacgt-3'
<i>p21</i>	5'-taggggaattggagtcaggc-3'	5'-agagtgcgaagacagcgacaa-3'
<i>p27</i>	5'-agatacagagtggcaggaggt-3'	5'-tcttaattcggagctgtttacgtc-3'
<i>p53</i>	5'-aaaggatgcccagtctacag-3'	5'-tatggcgggaagtagactgg-3'
<i>p57</i>	5'-accaatcagccagcagaaca-3'	5'-agttgaagtccagcggttc-3'
<i>GAPDH</i>	5'-tggcaaagtggagattgttgc-3'	5'-aagatggtgatgggcttcccg-3'

p16 and *p21* family primer sequences were as previously described [Yamakawa, D. et al. Visualization of proliferative vascular endothelial cells in tumors in vivo by imaging their partner of sld5-1 promoter activity. *Am. J. Pathol.* 188, 1300-1314 (2018)].

Supplementary Table 2Sequences of gene-specific primers for generation of different *p21* promoters

Promoter fragment	Forward primer	Reverse primer
-302 to -1	5'-cccttgagagacaaggtgga-3'	5'-tttcccagacaaactgagc-3'
-602 to -302	5'-ccaacatagggcggaattta-3'	5'-cttgagctcctcactgatgc-3'
-902 to -602	5'-gtgtcctcgcctcatcta-3'	5'-cacgcacgtacacagacaca-3'
-1102 to -902	5'-agaggagcccgcactgtag-3'	5'-gttccgagtggatggatgt-3'
-1402 to -1102	5'-gcgaggaggtgactcattgt-3'	5'-atactgtgcccgccaaatag-3'
-1702 to -1402	5'-gcttctggtgccaatgttt-3'	5'-aaacagggcacttggttcac-3'
-2002 to -1702	5'-tgtgtgtggtgatgagtga-3'	5'-tccaaggactggaagagtgg-3'
-2302 to -2002	5'-aaggagtgggttgctcgtg-3'	5'-tgtctgatatcgctgtgga-3'
-2602 to -2302	5'-cccgaaccagcagatttat-3'	5'-ccacacctgggctattctct-3'
-2902 to -2602	5'-caggctggtctgaacctgt-3'	5'-aggcattcaaggtcgttttg-3'