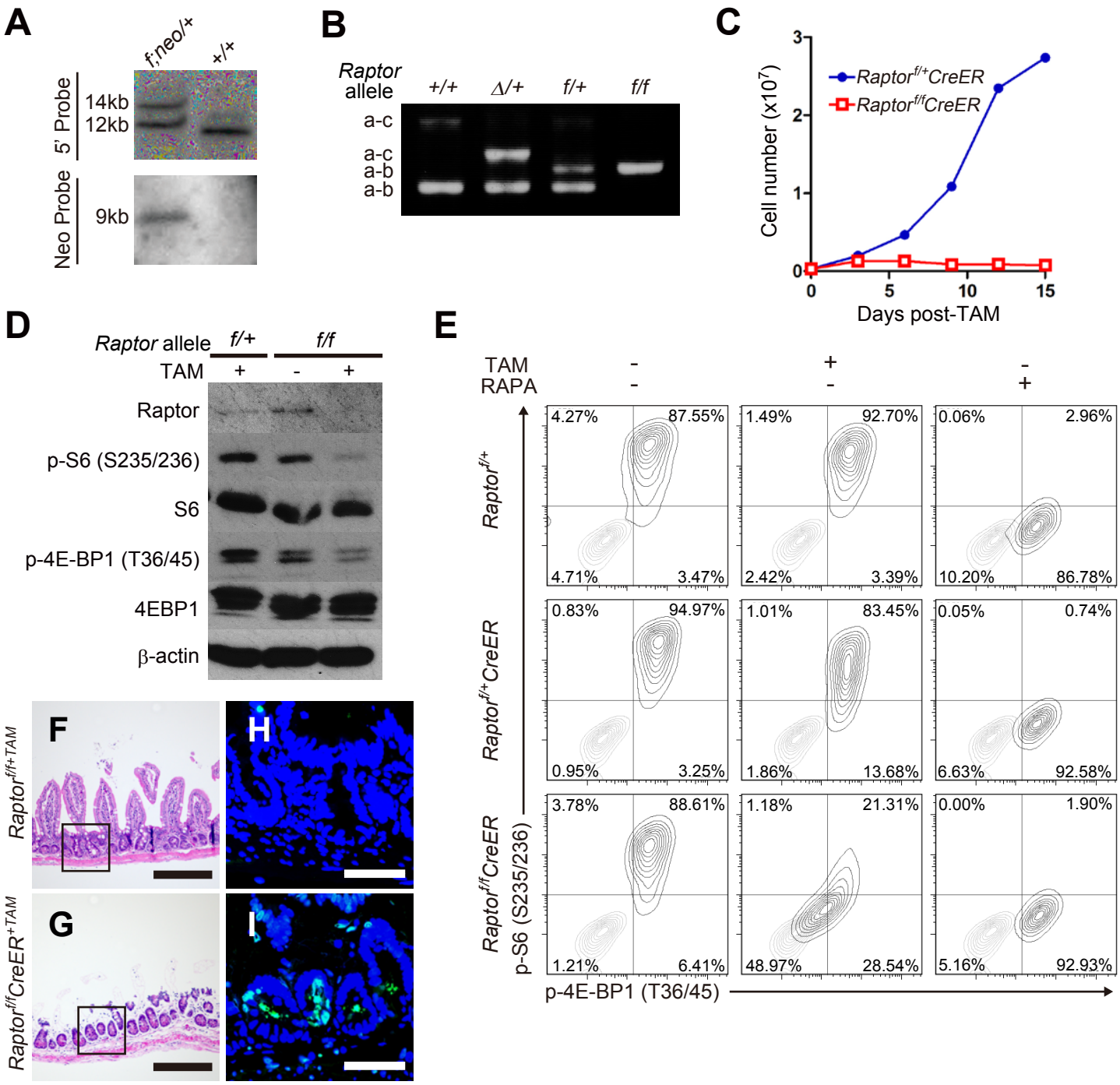


Supplemental Figure 1. Generation of *Raptor* conditional mutant mice

(A) Southern blot verifying the recombination of the targeting vector at the *Raptor* genomic locus. ES clone DNA was digested by *SacI* or *EcoRI* and hybridized to a digoxigenin-labeled 5' probe and a Neo probe, respectively.

(B) Genotyping of mouse tail DNA by PCR. The wild-type Raptor allele (+), the *Raptor* allele after excision of the Neo cassette (f), and the *Raptor* allele lacking exon 2 (Δ) were detected by the indicated combinations of primers (a, b, c). See Figure 1A for the primer locations.

(C) Growth curves of *Raptor*$^{f/+}$*CreER* mouse embryonic fibroblasts (MEFs) and *Raptor*$^{f/f}$*CreER* MEFs after TAM addition *in vitro*.

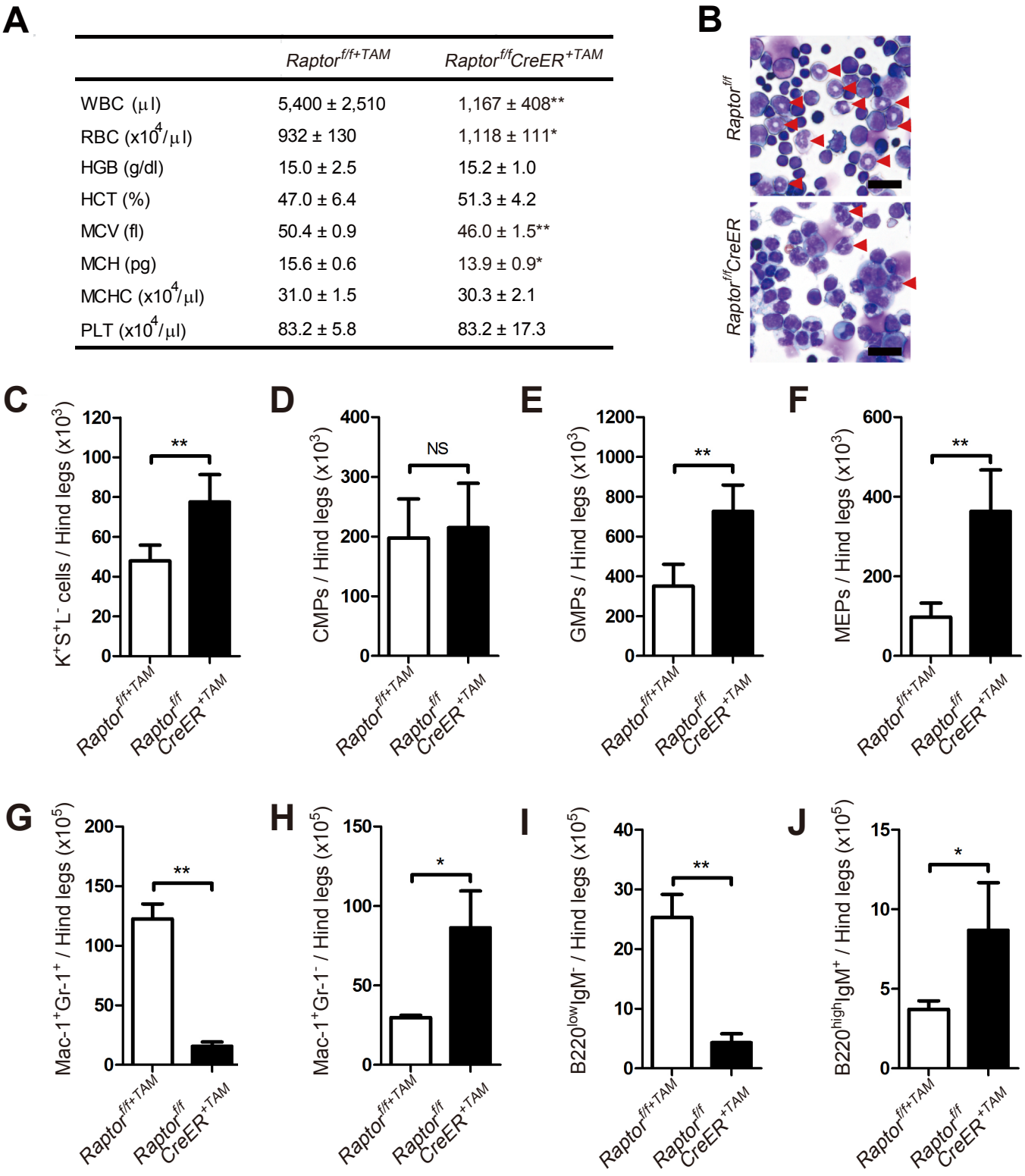
(D) Phosphorylation of downstream targets of mTORC1 in *Raptor*$^{f/+}$*CreER* or *Raptor*$^{f/f}$*CreER* MEFs at 4 days post-TAM. β-actin, loading control.

(E) Flow cytometric analysis of intracellular phospho (p)-S6 and p-4E-BP1 of MEF cells. Data shown are levels of p-S6(S235/236) and p-4E-BP1(T36/45) in *Raptor*$^{f/+}$, *Raptor*$^{f/+}$*CreER*, and *Raptor*$^{f/f}$*CreER* MEFs at 6 days post-TAM or 24 hours post-rapamycin (RAPA) treatment.

(F-I) Histology of small intestine from control and *Raptor*-deficient mice. Samples were obtained 10 days post-TAM. Sections were subjected to hematoxylin-eosin staining (F and G) or TUNEL staining (H and I). (H) and (I) show higher magnifications of the areas in adjacent sections corresponding to the boxed regions in (F) and (G), respectively. Scale bars, 200 μm (F, G), 50 μm (H, I)

A

	Raptor$^{f/f+TAM}$	*Raptor*$^{f/f}$*CreER*$^{+TAM}$
WBC (μl)	5,400 ± 2,510	1,167 ± 408**
RBC (x10^4/μl)	932 ± 130	1,118 ± 111*
HGB (g/dl)	15.0 ± 2.5	15.2 ± 1.0
HCT (%)	47.0 ± 6.4	51.3 ± 4.2
MCV (fl)	50.4 ± 0.9	46.0 ± 1.5**
MCH (pg)	15.6 ± 0.6	13.9 ± 0.9*
MCHC (x10^4/μl)	31.0 ± 1.5	30.3 ± 2.1
PLT (x10^4/μl)	83.2 ± 5.8	83.2 ± 17.3



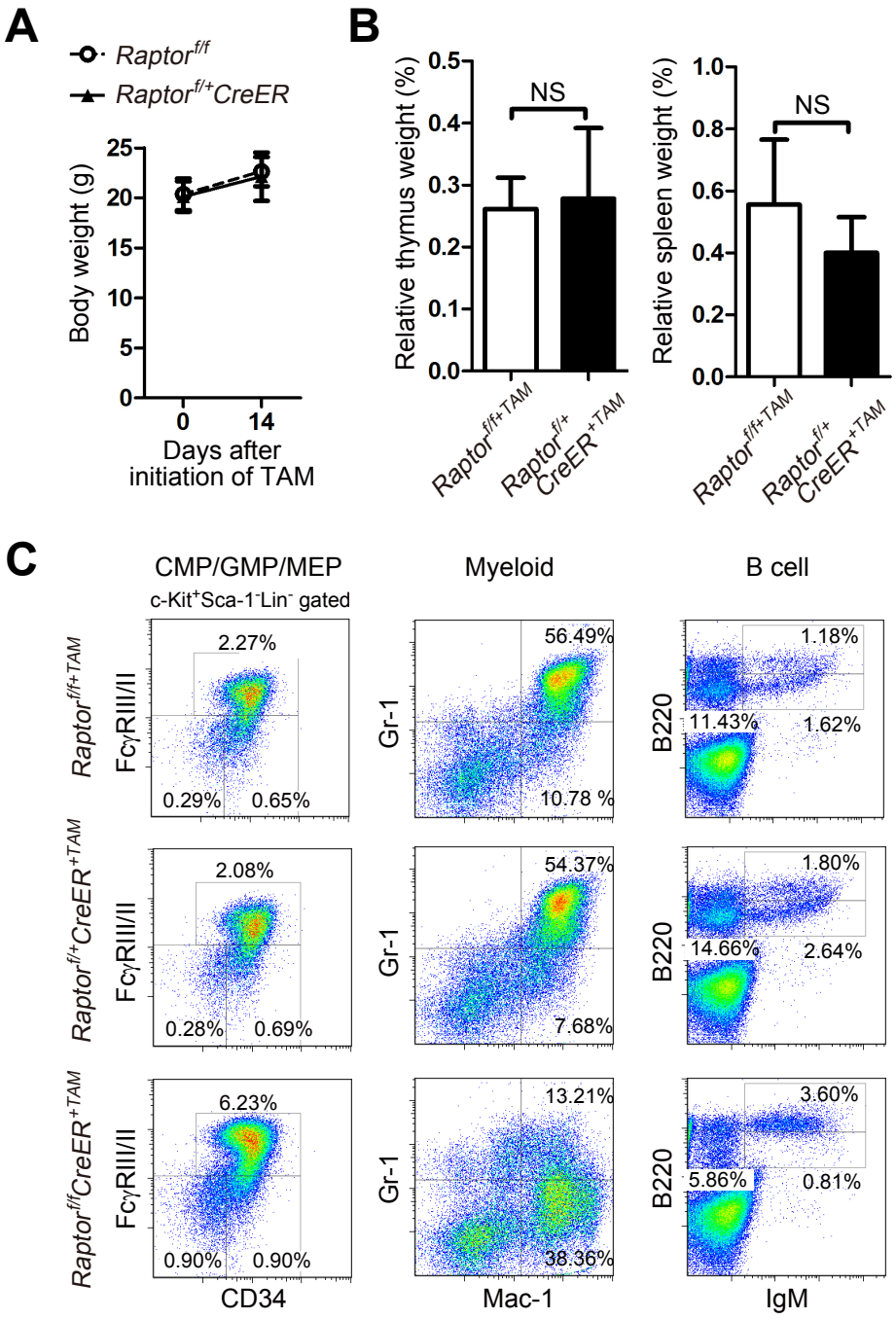
Supplemental Figure 2. Deletion of *Raptor* results in hematopoietic abnormalities

(A) Peripheral blood cell counts of *Raptor*$^{f/f+TAM}$ and *Raptor*$^{f/f}$*CreER*$^{+TAM}$ mice 10 days post-TAM. Data shown are mean ± SD (n = 5). WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet.

(B) Morphological analysis of BM-MNCs isolated from hind legs of the *Raptor*$^{f/f+TAM}$ and *Raptor*$^{f/f}$*CreER*$^{+TAM}$ mice 10 days post-TAM and stained with May-Grünwald-Giemsa. The number of granulocytes (red arrowheads) was reduced in *Raptor*-deficient BM. Scale bar, 20 μm.

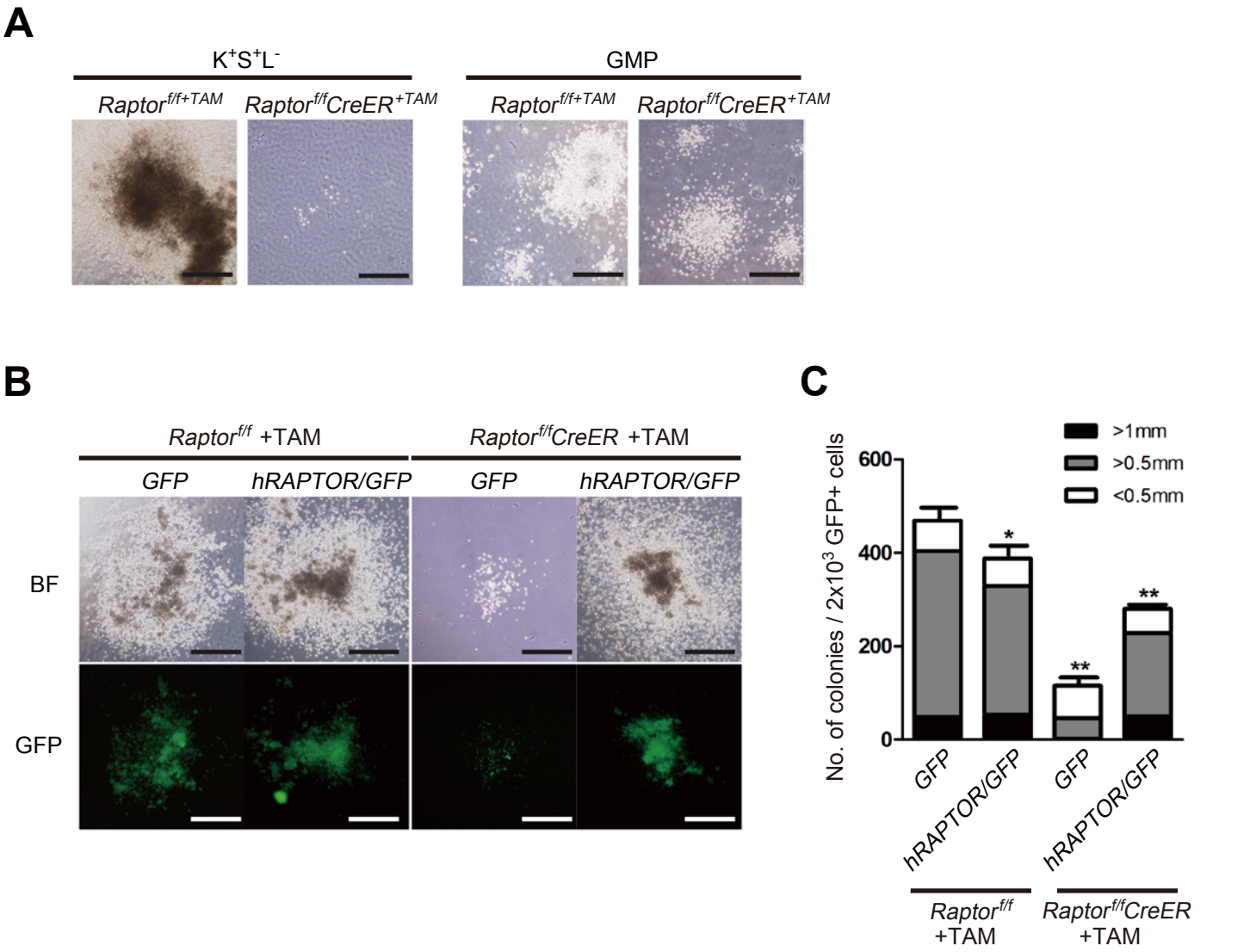
(C-J) Absolute number of HSCs/MPPs (C, c-Kit$^+$Sca-1$^+$Lin$^-$; K$^+$S$^+$L$^-$), CMPs (D, Lin$^-$c-Kit$^+$Sca-1$^-$FcgRIII/II$^-$CD34$^+$), GMPs (E, Lin$^-$c-Kit$^+$Sca-1$^-$FcgRIII/II$^+$CD34$^+$), MEPs (F, Lin$^-$c-Kit$^+$Sca-1$^-$FcgRIII/II$^-$CD34$^-$), myeloid lineage cells (G and H, Mac-1/Gr-1), and B lineage cells (I and J, B220/IgM) in bone marrow. Values in panels are the mean percentage ± SD of the specified subpopulation among total BM-MNCs (n = 4).

For A, C-J, *P < 0.05, **P < 0.01 (Student's t test). NS, not significant.



Supplemental Figure 3. Normal hematopoiesis in *Raptor*$^{f/+}$*CreER*$^{+TAM}$ mice

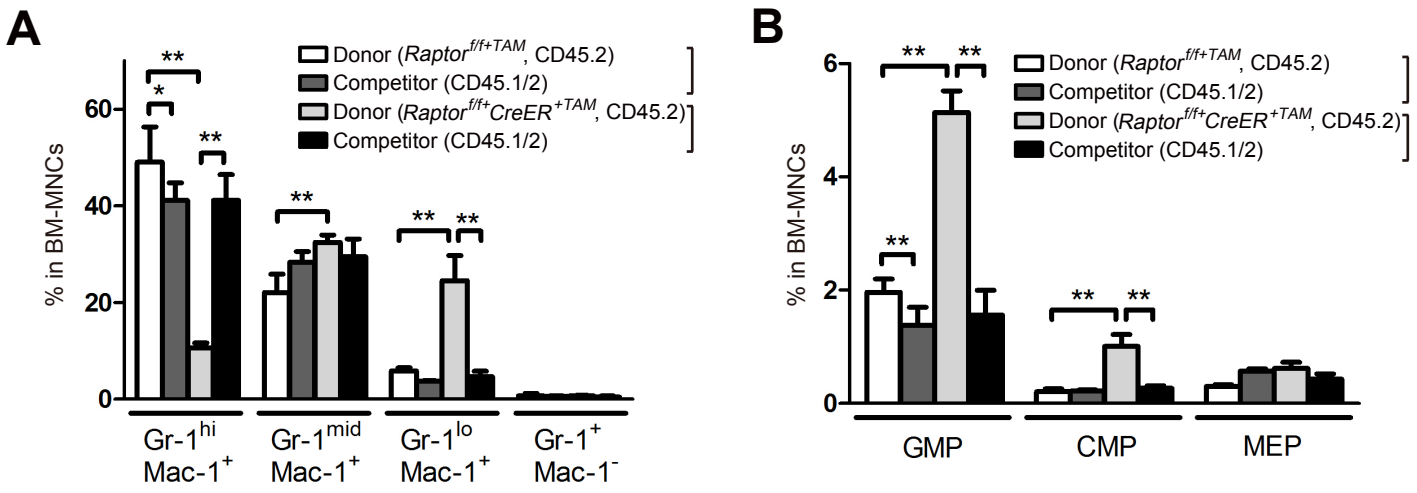
(A) Body weight of *Raptor*$^{f/f+TAM}$ and *Raptor*$^{f/+}$*CreER*$^{+TAM}$ mice. Data shown are the mean body weight ± SD (n = 4).
(B) Organ weights of *Raptor*$^{f/f+TAM}$ and *Raptor*$^{f/+}$*CreER*$^{+TAM}$ mice at 10 days post-TAM. Data shown are the mean relative organ weight (as % of total body weight) ± SD (n = 3). NS, not significant.
(C) Flow cytometric analysis of BM-MNCs from *Raptor*$^{f/f+TAM}$, *Raptor*$^{f/+}$*CreER*$^{+TAM}$, and *Raptor*$^{f/f}$*CreER*$^{+TAM}$ littermates. Images shown are representative data for CMPs (Lin$^-$c-Kit$^+$Sca-1$^-$FcgRIII/II$^-$CD34$^+$), GMPs (Lin$^-$c-Kit$^+$Sca-1$^-$FcgRIII/II$^+$CD34$^+$), MEPs (Lin-c-Kit$^+$Sca-1$^-$FcgRIII/II$^-$CD34$^-$), myeloid lineage cells (Mac-1/Gr-1), and B lineage cells (B220/IgM).



Supplemental Figure 4. Colony-forming ability of *Raptor*-deficient K$^+$S$^+$L$^-$ and GMP cells

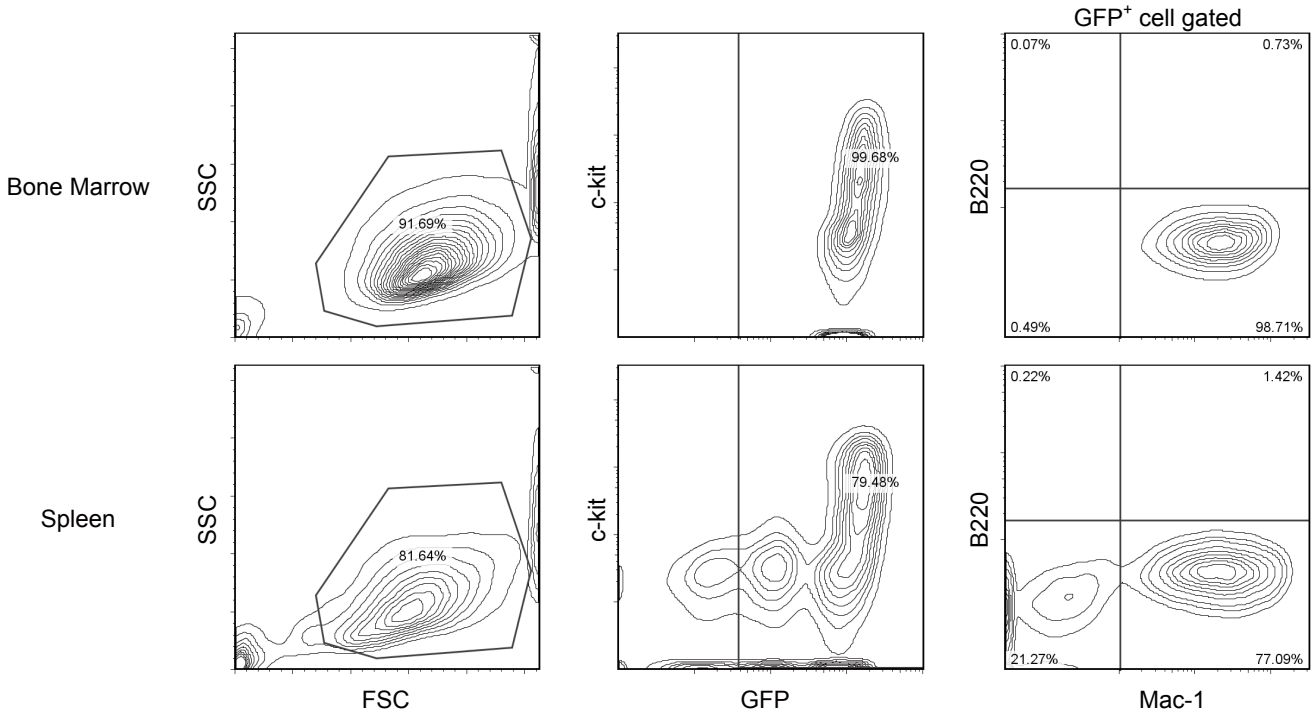
(A) Morphology of representative colonies formed by K$^+$S$^+$L$^-$ and GMP cells from *Raptor*$^{f/f+TAM}$ and *Raptor*$^{f/f}$*CreER*$^{+TAM}$ (10 days post-TAM) in semi-solid medium. Scale bar, 0.5 mm.

(B and C) Restoration of colony-forming ability of *Raptor*-deficient K$^+$S$^+$L$^-$ cells by expression of exogenous human RAPTOR. K$^+$S$^+$L$^-$ cells from *Raptor*$^{f/f}$ and *Raptor*$^{f/f}$*CreER* mice were infected with retroviruses carrying *GFP* alone or *hRAPTOR/GFP*. GFP$^+$ cells were collected and cultured with TAM for 10 days to delete Raptor, and colony-forming ability was assayed. (B) Representative colony morphology. BF, bright field microscopy. Scale bar, 0.5 mm. (C) Mean colony number ± SD (n = 3). For C, *$P < 0.05$, **$P < 0.01$ (Student's *t* test, vs. *Raptor*$^{f/f}$+TAM *GFP*).



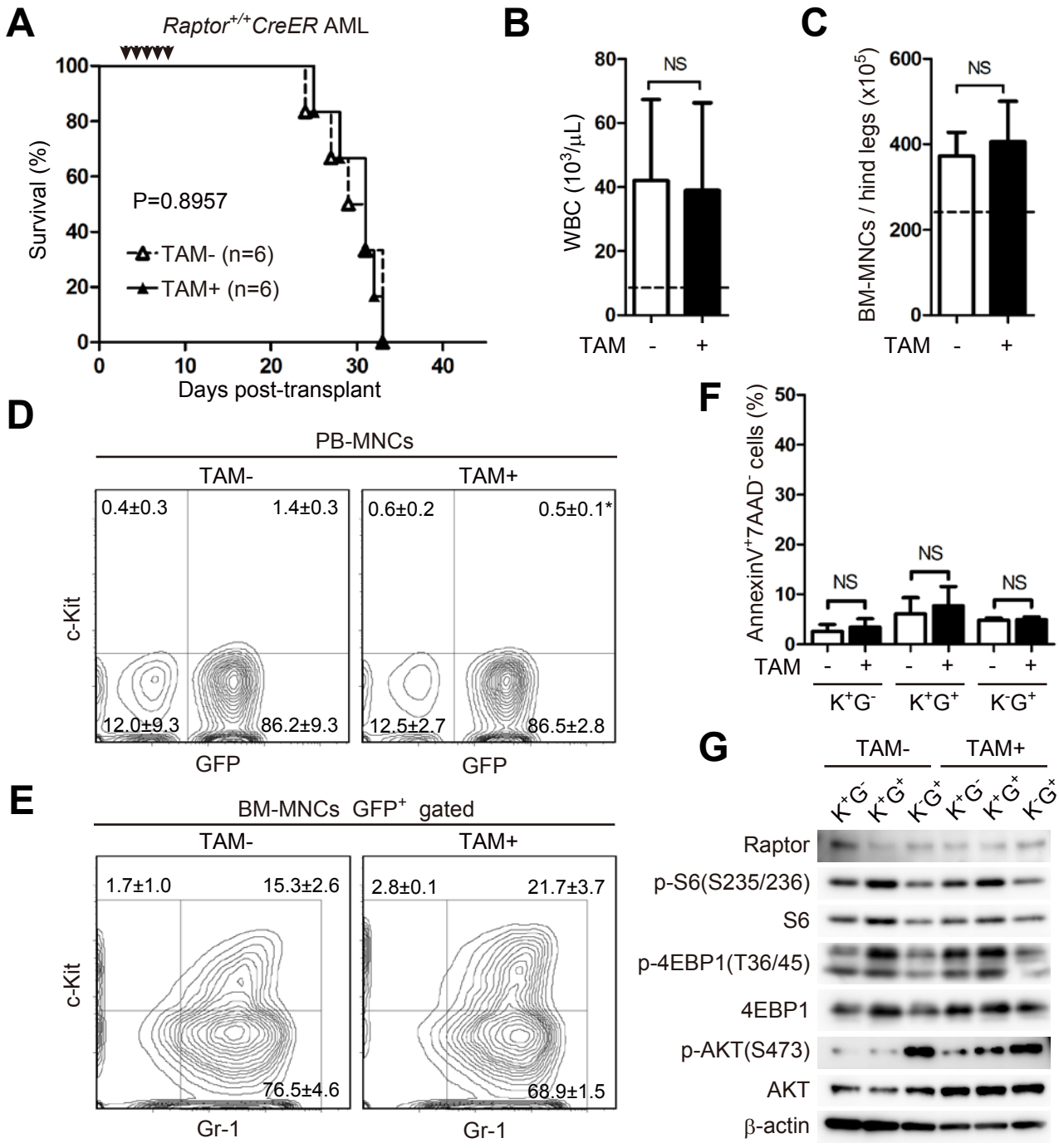
Supplemental Figure 5. Effects of *Raptor* deficiency on myeloid cell differentiation in vivo

(A and B) Competition analyses of in vivo development of myeloid lineage (A) and progenitor cells (B) in the absence of Raptor. Tester (1×10^6 *Raptor*$^{f/f}$ or *Raptor*$^{f/f}$*CreER*; CD45.2) and competitor (1×10^6; CD45.1/2) BM-MNCs were transplanted into recipient mice (CD45.1). At 8 weeks after transplantation, *Raptor* deficiency was induced by TAM treatment. At 2 weeks post-TAM, the frequencies of tester and competitor cells in the indicated BM subpopulations were analyzed by flow cytometry. Data shown are the mean percentage ± SD of a given subpopulation among total BM-MNCs (n = 3). **P* < 0.05, ***P* < 0.01 (Student's *t* test).



Supplemental Figure 6. Generation of a *Raptor*-deficient murine AML model

Representative flow cytometric analysis of *Raptor*$^{f/f}$*CreER*$^{\text{-TAM}}$ AML cells in BM and spleen. AML cells (GFP^{+}; ie, MLL-AF9–expressing cells) expressed Mac-1 but not B220.



Supplemental Figure 7. Effect of Cre activity on MLL-AF9–driven AML

(A) Survival of *Raptor*$^{+/+}$*CreER* AML mice. *Raptor*$^{+/+}$*CreER* AML mice were established as illustrated in Figure 4A. BM-MNCs from these animals were transplanted into a fresh set of recipients (along with rescue cells) and these animals were treated with oil diluent as the control (TAM–) or with TAM (TAM+). *P* value, log-rank test.

(B) Number of WBC in PB. PB were obtained from the *Raptor*$^{+/+}$*CreER* AML mice 14 days after control or TAM treatment (TAM- or TAM+). Data shown are mean number ± SD (n = 5). The horizontal dotted line is the mean value of the number of WBC in normal adult mice (8 weeks old, n = 5).

(C) Number of BM-MNCs in BM. Samples were obtained from the *Raptor*$^{+/+}$*CreER* AML mice 14 days after control (TAM-) or TAM treatment (TAM+). Data shown are mean number ± SD (n = 3). The horizontal dotted line is the mean value of the number of BM-MNCs in normal adult mice (8 weeks old, n = 5).

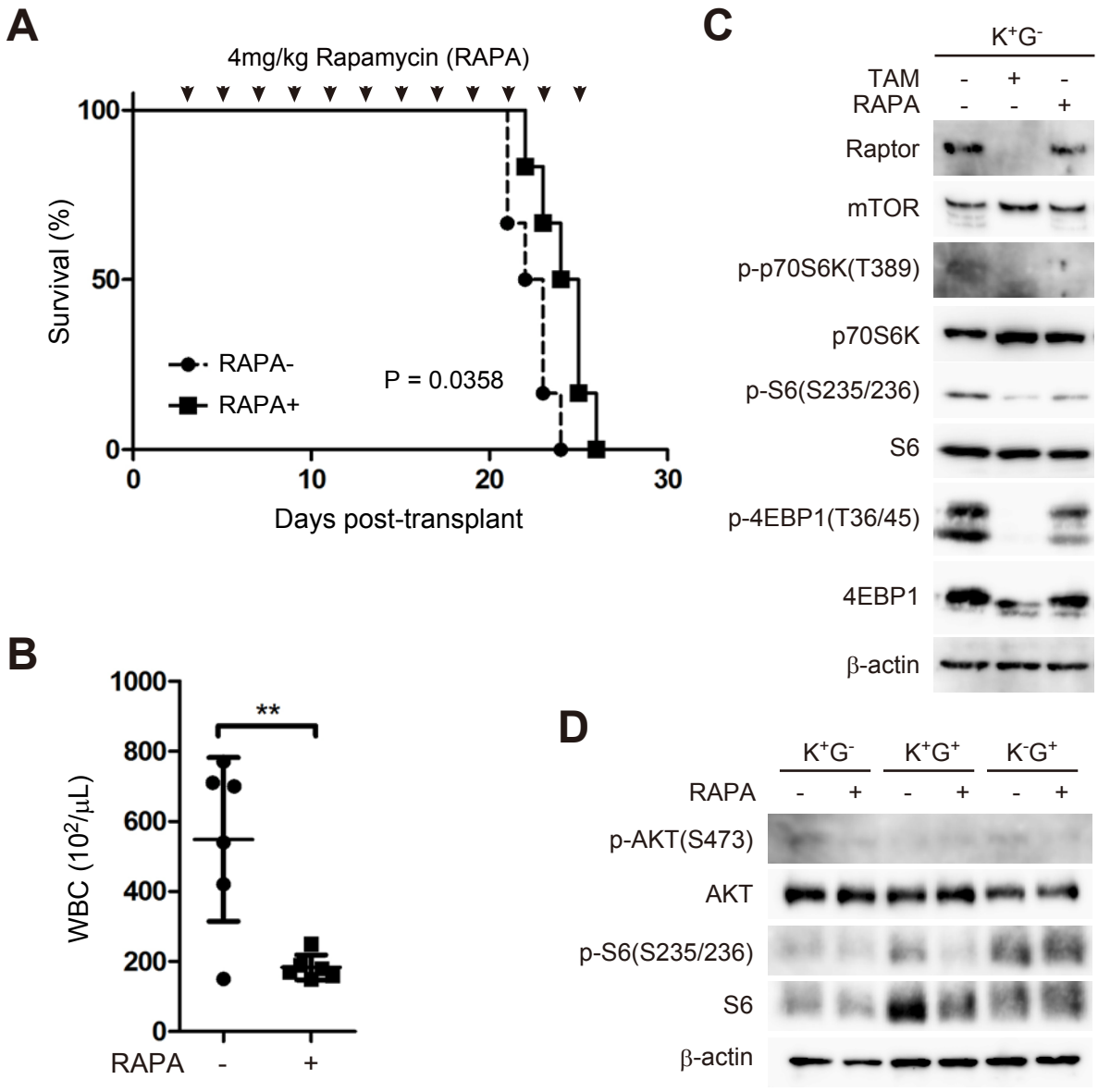
(D) Flow cytometric analyses of AML cells in PB. PB were obtained from the *Raptor*$^{+/+}$*CreER* AML mice 14 days post-TAM. Representative data are shown for GFP/c-Kit expression in PB-MNCs. Values in panels are the mean percentage ± SD for the indicated subpopulations (n = 4).

(E) Flow cytometric analyses of AML cells in BM. Representative data are shown for c-Kit/Gr-1 expression in GFP^{+}-gated BM-MNCs. Values in panels are the mean percentage ± SD for the indicated subpopulations (n = 3).

(F) Apoptosis. Data shown are the mean percentage ± SD of Annexin-V^{+}7AAD^{-} cells in the indicated AML cell subpopulations (n = 4).

(G) Phosphorylation of mTOR signaling pathway proteins. Lysates were prepared from the indicated AML cell subpopulations and immunoblotted to detect the indicated proteins.

For B-F, *P < 0.05 (Student's *t* test). NS, not significant.



Supplemental Figure 8. Effects of rapamycin treatment on MLL-AF9–driven AML

(A) Survival of rapamycin-treated AML mice. *Raptor$^{f/f}$CreER* AML cells were transplanted into a fresh set of recipients (along with rescue cells) and these animals were treated with vehicle as the control (RAPA-) or with 4 mg/kg rapamycin (RAPA+) every other day starting 3 days after transplantation. *P* value, log-rank test.

(B) Number of WBC in PB. PB was obtained from the mice treated with vehicle (RAPA-) or with 4 mg/kg rapamycin (RAPA+) 20 days after transplantation. Data shown are mean number ± SD (n = 6). $^{**}P < 0.01$ (Student's *t* test).

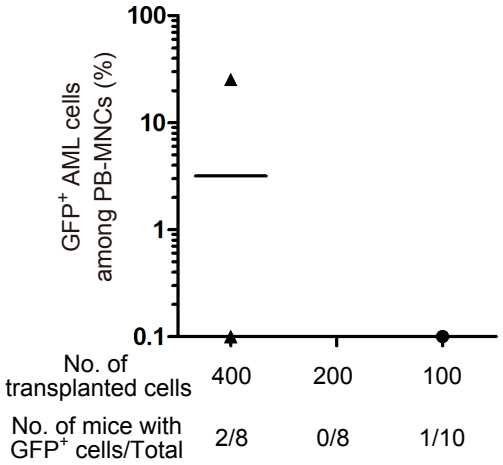
(C) Phosphorylation of mTOR signaling pathway proteins. Lysates were prepared from the K^+G^- AML cells of vehicle-, rapamycin-, or TAM-treated mice 20 days after transplantation (13 days post-TAM) and immunoblotted to detect the indicated proteins.

(D) Phosphorylation of AKT. Lysates were prepared from the indicated AML subpopulations of mice treated with vehicle (RAPA-) or rapamycin (RAPA+) 20 days after the transplant and immunoblotted to detect the indicated proteins.

A

	Recipient mice	
	without GFP$^+$ cells	with GFP$^+$ cells
WBC (μl)	3,052 ± 2,438	2,143 ± 1,345
RBC (x10^4/μl)	901 ± 353	907 ± 63
HGB (g/dl)	11.2 ± 0.4	10.9 ± 0.9
HCT (%)	43.8 ± 1.6	43.7 ± 2.3
MCV (fl)	48.7 ± 1.4	48.3 ± 1.4
MCH (pg)	12.4 ± 0.5	12.0 ± 0.4
MCHC (x10^4/μl)	25.5 ± 1.0	24.8 ± 1.4
PLT (x10^4/μl)	67.6 ± 19.5	62.0± 18.3

B

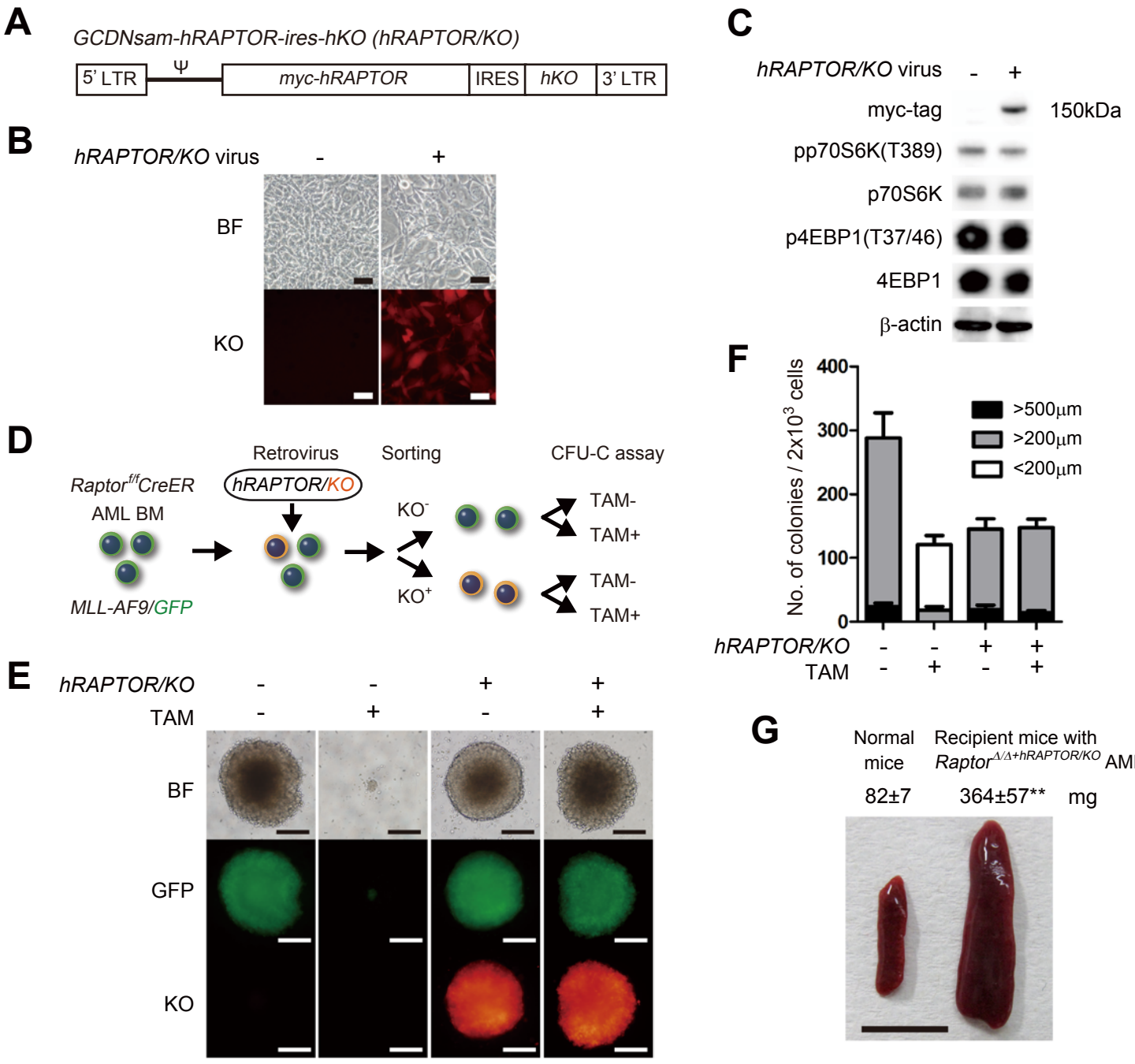


Supplemental Figure 9. Characterization of surviving *Raptor*$^{\Delta/\Delta}$ (long-term *Raptor*-deficient) AML cells in PB

Recipient mice were transplanted with 100–400 K$^+$G$^-$ AML cells from *Raptor*$^{f/f}$*CreER*$^{+TAM}$ AML mice at 14 days post-TAM. At 100 days after transplantation, surviving GFP$^+$ leukemia cells (*Raptor*$^{\Delta/\Delta}$ AML cells) in both PB and BM were analyzed in 26 recipient mice.

(A) PB cell counts. See Supplemental Figure 2A for definitions. GFP$^+$ cells were present in the BM of 7 recipients (with GFP$^+$ cells), but not in the BM of the other 19 recipients (without GFP$^+$ cells).Data shown are the mean ± SD for recipients without GFP$^+$ cells (n = 19) or with GFP$^+$ cells (n = 7).

(B) Frequency of *Raptor*$^{\Delta/\Delta}$ AML cells (GFP$^+$) in PB of recipients with GFP$^+$ cells. GFP$^+$ cells were present in the PB of 3 recipients. Data shown are the percentage of GFP$^+$ cells among PB-MNCs. Horizontal line, mean percentage of GFP$^+$ cells of two cases with GFP$^+$ cells. Numbers of mice with GFP$^+$ cells / total number of recipients are shown at the bottom of the panel.



Supplemental Figure 10. Retroviral transduction of the hRAPTOR gene into *Raptor*$^{\Delta/\Delta}$ AML cells

(A) Diagram of the GCDNsam-*hRAPTOR*-ires-*hKO* (*hRAPTOR/KO*) construct.

(B-F) Confirmation of the ability of *hRAPTOR/KO* to restore Raptor function.

(B) KO fluorescence in NIH3T3 cells infected (or not) with retrovirus carrying *hRAPTOR/KO*. BF, bright-field microscopy; KO, fluorescence microscopy to detect Kusabira-Orange. Scale bars, 50 μm.

(C) Expression of Myc-tagged hRAPTOR protein and phosphorylation of mTOR signaling pathway proteins in NIH3T3 cells infected with *hRAPTOR/KO* retrovirus. Lysates prepared from the cells in (B) were immunoblotted to detect the indicated proteins.

(D) Experimental design to restore the colony-forming ability of *Raptor*-deficient AML cells by *hRAPTOR/KO* retrovirus infection. *Raptor*$^{f/f}$*CreER* AML cells (GFP$^+$) were infected with *hRAPTOR/KO* retrovirus. KO$^-$ and KO$^+$ cells were collected separately and cultured in semi-solid medium containing TAM to perform colony-forming assays.

(E and F) Restoration of colony-forming ability of *Raptor*-deficient AML cells as described in (D).

(E) Bright-field (BF), GFP fluorescence, and KO fluorescence of colonies. Scale bars, 200 μm.

(F) Mean number ± SD of colonies in (E) (n = 3). Colony diameters are indicated.

(G) Splenomegaly at 26-35 days after transplantation in mice transplanted with *Raptor*$^{\Delta/\Delta+hRAPTOR/KO}$ AML cells. Values shown above representative samples are the mean spleen weight ± SD of untreated normal mice and recipient mice transplanted with *Raptor*$^{\Delta/\Delta+hRAPTOR/KO}$ AML cells (n = 4).

Scale bar, 10 mm. $^{**}P < 0.01$ (Student's *t* test).