Figure S1

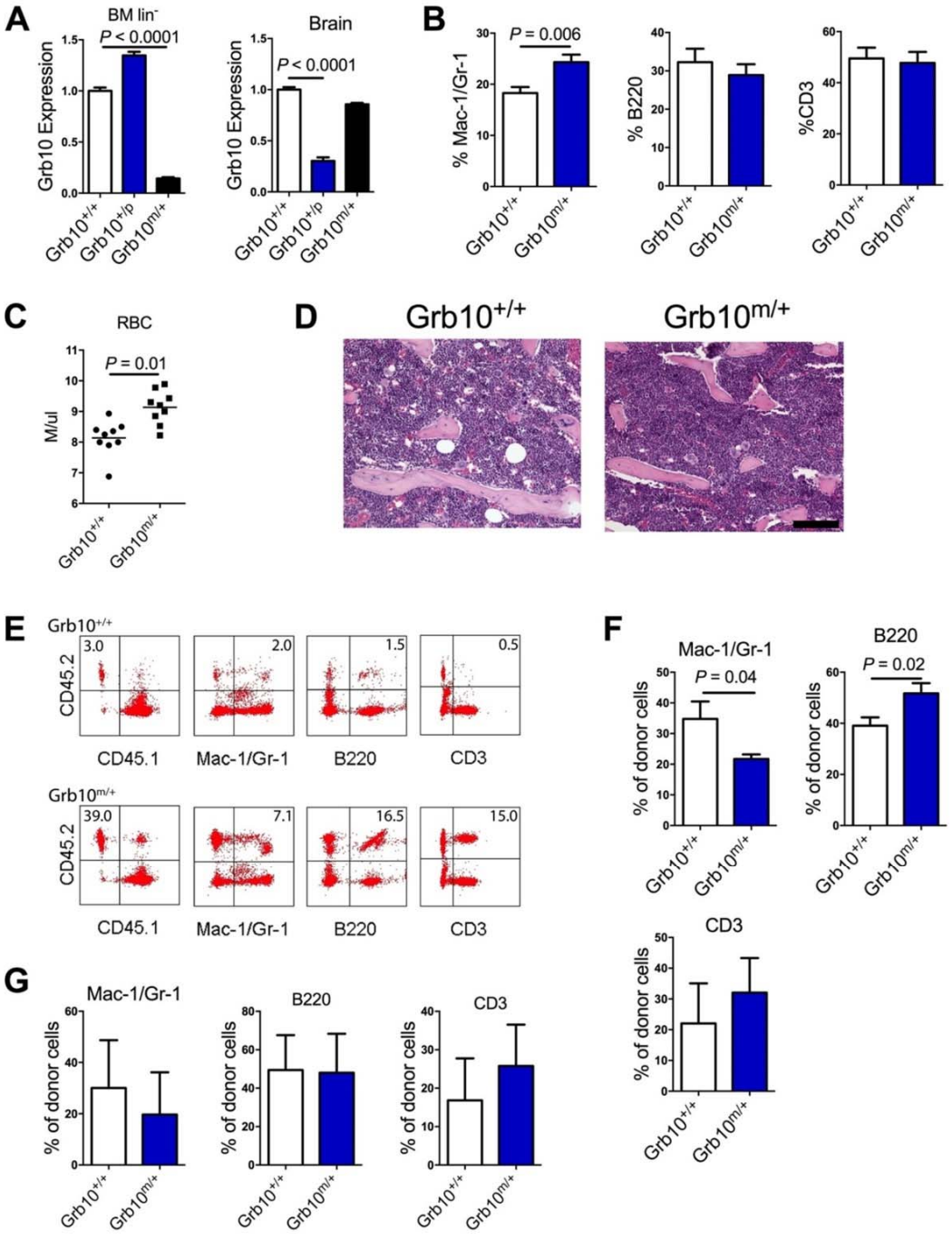
A
BM lin-
P < 0.0001
Grb10 Expression
Grb10+/+
Grb10+/p
Grb10m/+
Brain
P < 0.0001
B
P = 0.006
% Mac-1/Gr-1
% B220
%CD3
C
RBC
P = 0.01
M/ul
D
Grb10+/+
Grb10m/+
E
Grb10+/+
CD45.2
CD45.1
Mac-1/Gr-1
B220
CD3
3.0
2.0
1.5
0.5
Grb10m/+
39.0
7.1
16.5
15.0
F
Mac-1/Gr-1
P = 0.04
B220
P = 0.02
% of donor cells
CD3
G
Mac-1/Gr-1
B220
CD3
% of donor cells

Figure S1 (related to Figure 2). Phenotype of *Grb10 $^{m/+}$* mice and *Grb10 $^{+/+}$* mice

(A) Expression of Grb10 in BM lin$^-$ cells and in the brain of *Grb10 $^{+/P}$* mice and in *Grb10 $^{m/+}$* mice (n=3/group). (B) Percentages of PB myeloid cells (Mac-1/Gr-1$^+$), B cells (B220$^+$) and T cells (CD3$^+$) in the PB of *Grb10 $^{m/+}$* mice and *Grb10 $^{+/+}$* mice (n = 8 mice/group). (C) Scatter plots of peripheral blood red blood cell counts (RBC) in 8 week *Grb10 $^{m/+}$* mice and *Grb10 $^{+/+}$* mice (n=9 mice/group). (D) Representative H&E staining of femurs from *Grb10 $^{+/+}$* mice and *Grb10 $^{m/+}$* mice (20x, scale bar=100 μm). (E) Representative flow cytometric analysis of donor derived CD45.2$^+$ cells in the PB of recipient CD45.1$^+$ mice transplanted competitively with 5 x 10^4 BM cells from *Grb10 $^{+/+}$* mice or *Grb10 $^{m/+}$* mice (and 2 x 10^5 host BM cells) at 20 weeks after BM transplantation. Percentages of donor Mac-1/Gr-1(myeloid), B220(B cell) and CD3 (T cell) cells are shown in the upper right quadrants. (F) Mean percentages of Mac-1/Gr-1$^+$, B220$^+$ and CD3$^+$ cells as a proportion of total engrafted donor cells in the PB of recipient mice transplanted with *Grb10 $^{+/+}$* or *Grb10 $^{m/+}$* donor BM cells at 20 weeks after transplantation (n = 8/group, Mann-Whitney test). (G) Mean percentages of Mac-1/Gr-1$^+$, B220$^+$ and CD3$^+$ cells as a proportion of total engrafted donor cells in the PB of secondary recipient mice transplanted with *Grb10 $^{+/+}$* or *Grb10 $^{m/+}$* donor BM cells, at 12 weeks after secondary transplantation (n = 10-12/group).

FIGURE S2

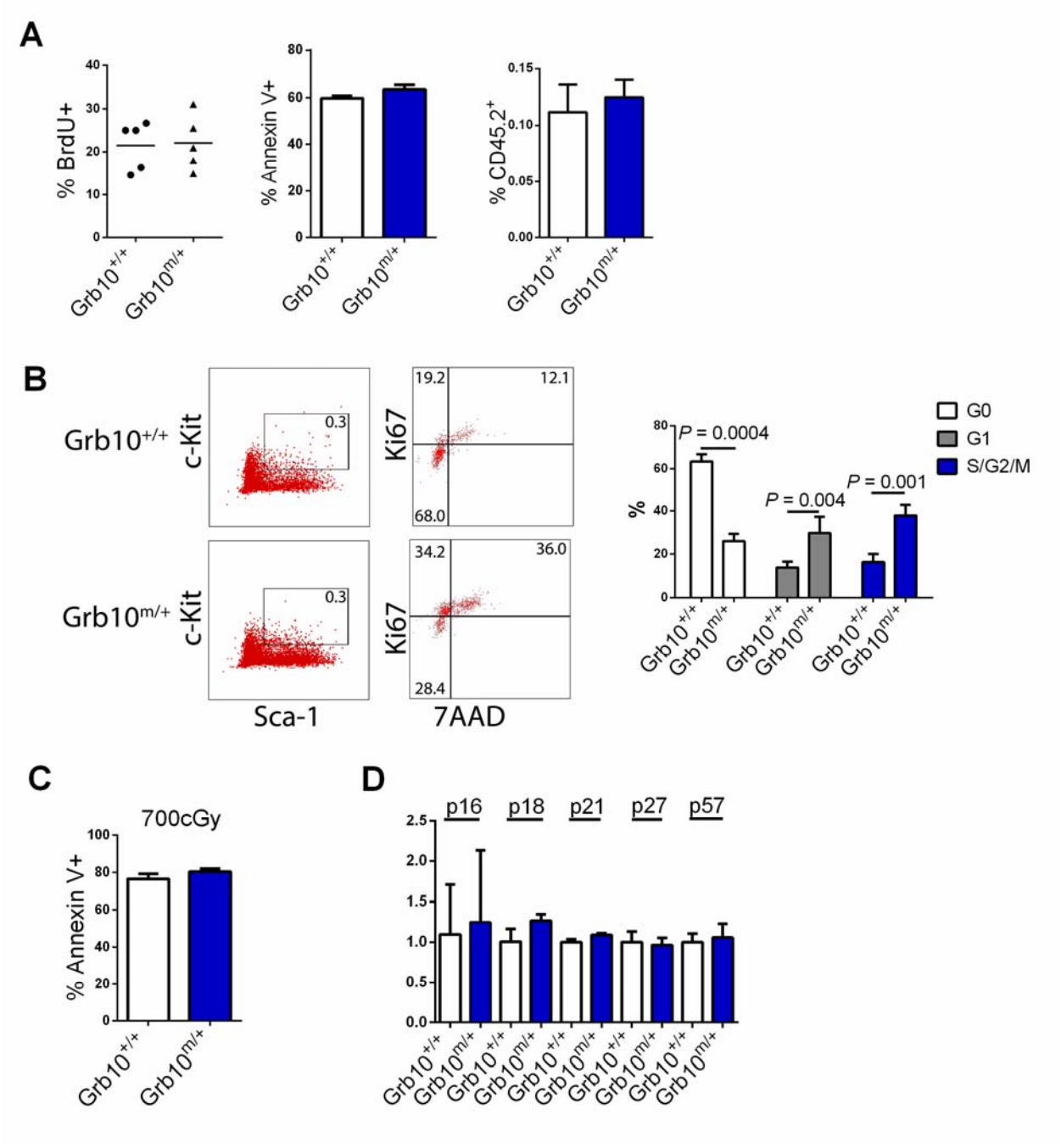
A
% BrdU+
% Annexin V+
% CD45.2+
Grb10+/+
Grb10m/+
B
Grb10+/+
Grb10m/+
c-Kit
Sca-1
Ki67
7AAD
0.3
19.2
12.1
68.0
34.2
36.0
28.4
P = 0.0004
P = 0.004
P = 0.001
%
G0
G1
S/G2/M
C
700cGy
% Annexin V+
D
p16
p18
p21
p27
p57

Figure S2 (related to Figure 4). Grb10 deletion promotes HSC cycling following irradiation

(A) Mean percentage of $BrdU^+$ BM $SLAM^+KSL$ cells (left, n = 5 mice /group) and Annexin V^+ KSL cells (center, n = 8 mice/group) in *Grb10* $^{m/+}$ mice and *Grb10* $^{+/+}$ mice at steady state. At right, mean levels of donor $CD45.2^+$ cells detected at 18 hours following transplantation of BM $sca\text{-}1^+lin^-$ cells from *Grb10* $^{+/+}$ mice and *Grb10* $^{m/+}$ mice in recipient $CD45.1^+$ mice (n=6/group). (B) At left, a representative flow cytometric plot is shown of BM KSL cells at 24 hours following 700 cGy in *Grb10* $^{m/+}$ mice and *Grb10* $^{+/+}$ mice. At center, a representative flow cytometric plot of cell cycle analysis in BM KSL cells is shown. At right, mean percentages of cells in G_0, G_1 and G_2/S/M phase in each group (n=4 mice/group). (C) Mean percentage of Annexin V^+ BM KSL cells in *Grb10* $^{+/+}$ mice and *Grb10* $^{m/+}$ mice at 24 hours following 700 cGy (n = 8 mice/group). (D) Expression of cyclin dependent kinase inhibitors in BM KSL cells from *Grb10* $^{+/+}$ mice and *Grb10* $^{m/+}$ mice at 6 hours following 300 cGy irradiation (n = 6/group).

FIGURE S3

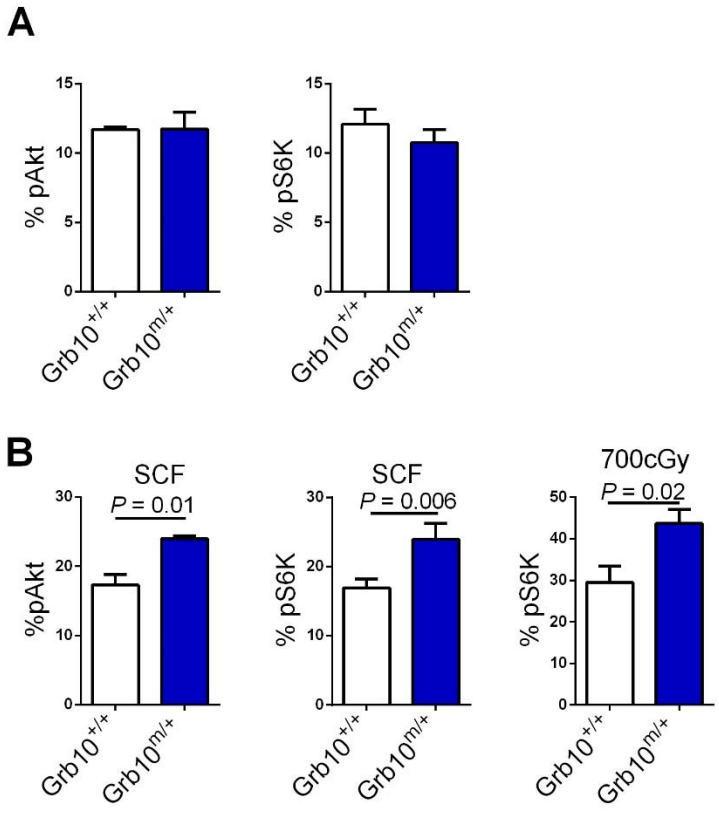

Figure S3 (related to Figure 4). Grb10 deletion augments Akt and mTOR activation after SCF stimulation or irradiation (A) Phosphorylation of Akt and S6 kinase (S6K), a direct target of mTORC1, in BM KSL cells from *Grb10* $^{+/+}$ mice and *Grb10* $^{+/+}$ mice in steady state (n = 4-8/group). (B) Phosphorylation of Akt in BM KSL cells from *Grb10* $^{+/+}$ mice and *Grb10* $^{m/+}$ mice at 5 minutes of SCF treatment (left); phosphorylation of S6K in BM KSL cells from *Grb10* $^{+/+}$ mice and *Grb10* $^{m/+}$ mice at 5 minutes of SCF treatment (center), and at 24 hours after 700 cGy TBI (at right, n = 4–8/group).

Table S1 (related to Figure 1). Upregulated and downregulated genes in BM KSL cells following high dose irradiation

Gene	Description	Fold-Change (550cGy vs. 0cGy)	p-value
Hba-a1	hemoglobin alpha, adult chain 1	9.16117	0.001
Ahsp	alpha hemoglobin stabilizing protein	7.51942	0.001218
Mt2	metallothionein 2	7.06283	0.003
Klf1	Kruppel-like factor 1 (erythroid)	6.91808	0.000256
Atp1b2	ATPase, Na+/K+ transporting, beta 2 polypeptide	5.54998	0.005169
Hspa1b	heat shock protein 1B	5.49593	0.002139
Col5a1	collagen, type V, alpha 1	5.3232	0.002098
Mt1	metallothionein 1	5.30729	0.001357
Tjp1	tight junction protein 1	5.11938	3.94E-09
Podxl	podocalyxin-like	4.97906	6.83E-06
Epdr1	ependymin related protein 1 (zebrafish)	4.58322	5.75E-05
Ank1	ankyrin 1, erythroid	4.19122	0.000132
Socs2	suppressor of cytokine signaling 2	3.64415	0.000803
Grb10	growth factor receptor bound protein 10	3.33681	5.85E-07
Gypa	glycophorin A	3.32025	0.01439
Sfrp4	secreted frizzled-related protein 4	3.29086	0.000819
Tcrg	Mus musculus 2 days neonate thymus thymic cells cDNA	3.2588	0.001539
Gja1	gap junction protein, alpha 1	3.25332	6.49E-05
Selp	selectin, platelet	3.24308	0.000483
Fam132a	family with sequence similarity 132, member A	3.22703	0.00131
Slamf1	signaling lymphocytic activation molecule family member 1	3.20324	0.000204
Asb17	ankyrin repeat and SOCS box-containing 17	3.14976	0.004065
Pvt1	plasmacytoma variant translocation 1	3.14739	0.002714
Spta1	spectrin alpha, erythrocytic 1	3.11081	6.72E-07
Slc38a5	solute carrier family 38, member 5	3.09002	0.001255
Pklr	pyruvate kinase liver and red blood cell	3.0684	0.027695
Gata1	GATA binding protein 1	3.06067	2.98E-05
Hbb-b2	hemoglobin, beta adult minor chain	3.04464	1.86E-05
Plek	pleckstrin	-3.00879	4.17E-06
Trim30d	tripartite motif-containing 30D	-3.08386	0.000544
Ldhb	lactate dehydrogenase B	-3.1654	2.77E-05
Rora	RAR-related orphan receptor alpha	-3.26513	0.003363
Robo4	roundabout homolog 4 (Drosophila)	-3.27413	0.000239
Vegfa	vascular endothelial growth factor A	-3.28679	0.00354
Ndn	necdin	-3.3007	0.002251

Alcam	activated leukocyte cell adhesion molecule	-3.35081	2.92E-05
Cd33	CD33 antigen	-3.41538	0.000259
Adrb2	adrenergic receptor, beta 2	-3.45489	0.000445
Ptms	parathymosin	-3.46324	2.95E-07
Itgb5	integrin beta 5	-3.46925	7.76E-05
Zbtb20	zinc finger and BTB domain containing 20	-3.54392	5.88E-06
Il1r2	interleukin 1 receptor, type II	-3.56333	0.000948
Pdgfrb	platelet derived growth factor receptor, beta polypeptide	-3.6034	0.000796
Ltbp3	latent transforming growth factor beta binding protein 3	-3.67145	0.000105
Clcf1	cardiotrophin-like cytokine factor 1	-3.70842	0.000191
Nr4a3	nuclear receptor subfamily 4, group A, member 3	-3.72911	0.001252
Itsn1	intersectin 1 (SH3 domain protein 1A)	-3.89208	0.000239
Sdc4	syndecan 4	-4.01356	4.79E-06
Flt3	FMS-like tyrosine kinase 3	-4.18661	9.14E-05
Lif	leukemia inhibitory factor	-4.29672	0.000113
Gcnt2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	-4.37489	0.000195
Satb1	special AT-rich sequence binding protein 1	-4.39525	1.25E-05
Ccl4	chemokine (C-C motif) ligand 4	-4.78784	0.000473
Serpinf1	serine (or cysteine) peptidase inhibitor, clade F, member 1	-5.46444	1.37E-07
Dntt	deoxynucleotidyltransferase, terminal	-6.3669	2.25E-07
Ccl3	chemokine (C-C motif) ligand 3	-6.80125	9.32E-06
Wfdc18	WAP four-disulfide core domain 18	-7.46518	1.29E-08