Supplementary Materials

Figure S1

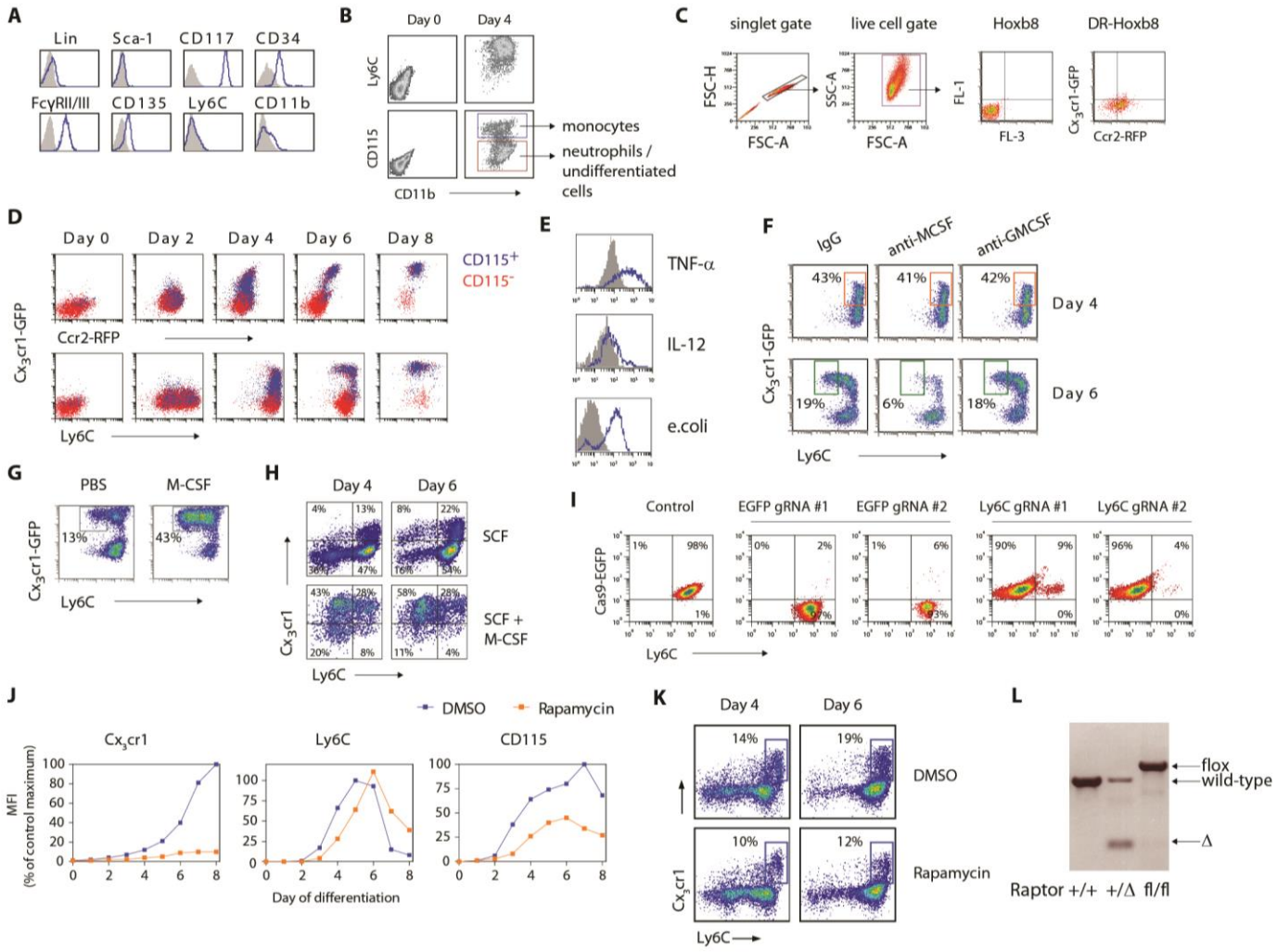

Figure S1. Phenotypic and functional analysis of DR–ER–Hoxb8 cells and their response to mTOR inhibition. A) Expression of myeloid progenitor markers in undifferentiated ER-Hoxb8 cells. Gray shade represents isotype control. B) FACS plot illustrating monocytes ($CD115^{+}$) and granulocytes / undifferentiated cells ($CD115^{-}$) derived from wild-type ER–Hoxb8 progenitors upon estrogen withdrawal. C) FACS plot of gating strategy and baseline fluorescence in DR–ER–Hoxb8 cells and wild-type ER–Hoxb8 cells prior to differentiation. D) Longitudinal analyses of Cx_3cr1-GFP, CCR2-RFP and Ly6C expression in DR–ER–Hoxb8 cells during the course of differentiation. Monocytes are identified by CD115 staining (blue). E) Intracellular staining of TNF-α and IL-12 production (top and middle panel) 16 hours after LPS stimulation (1 µg/mL) and FACS analysis of fluorescent *Escherichia coli* ($1x10^6$ particles per $1x10^5$ cells) uptake by $Ly6C^{hi}$ monocytes (lower panel). Gray shade represents untreated controls. F) FACS plots of DR-ER-Hoxb8 cells differentiated in the presence of neutralizing antibodies to M-CSF or GM-CSF (10 µg/mL). Boxes depict $Ly6C^{hi}$ monocytes in the upper panels and $Ly6C^{lo}$ monocytes in the lower panels. G) FACS analysis of DR-ER-Hoxb8 cells

differentiated in the presence of M-CSF (20 ng/mL) or 6 days. Boxes depict the percentage of $Ly6C^{lo}$ monocytes. H) FACS plot of sorted bone marrow CMP (Lin^{-} $Sca\text{-}1^{-}$ $c\text{-}kit^{+}$ $CD127^{-}$ FcγR II/III^{mid} $CD34^{+}$) cultured in low-dose SCF (10 ng/mL) with or without the addition of M-CSF (20 ng/mL) for 4 or 6 days. I) FACS plot of Cas9-EGFP ER-Hoxb8 cells with or without introduction of the indicated gRNA. Cells were analyzed on Day 4 of differentiation. J) Longitudinal analysis of monocyte marker expression in DR–ER–Hoxb8 cells in the presence or absence of rapamycin (500 nM). Results were normalized to the peak expression (MFI) in DMSO-treated cells. K) FACS plots of monocyte differentiation from freshly isolated bone marrow CMP in low dose SCF (10 ng/mL) with or without rapamycin (500 nM). L) Genotype confirmation of Raptor $^{fl/fl}$ and $^{+/\Delta}$ ER–Hoxb8 cells by conventional PCR and agarose gel electrophoresis.

Figure S2

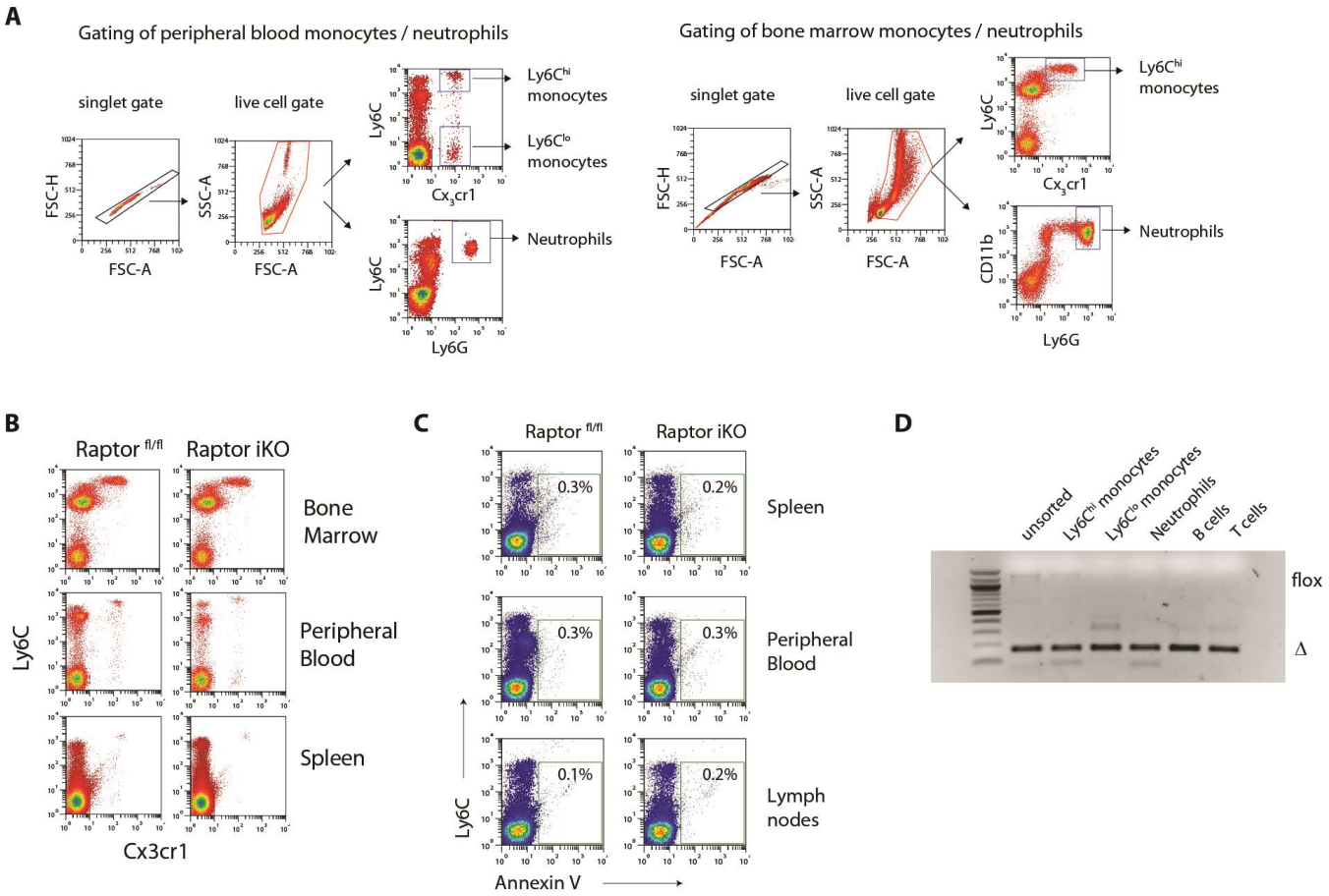

Figure S2. Raptor deletion impairs monocyte development without causing apoptosis. A) Gating strategy for the analysis of peripheral blood and bone marrow myeloid cells. B) FACS plot of baseline myeloid cell populations in Raptor fl/fl and iKO mice prior to tamoxifen treatment. C) FACS analysis of annexin V staining the peripheral blood, spleen, and lymph nodes from Raptor $^{fl/fl}$ or iKO mice two weeks after tamoxifen treatment. Box indicates percentage of annexin V positive events. D) PCR analysis of Raptor deletion in sorted bone marrow cells from Raptor iKO mice 2 weeks after tamoxifen treatment.

Figure S3

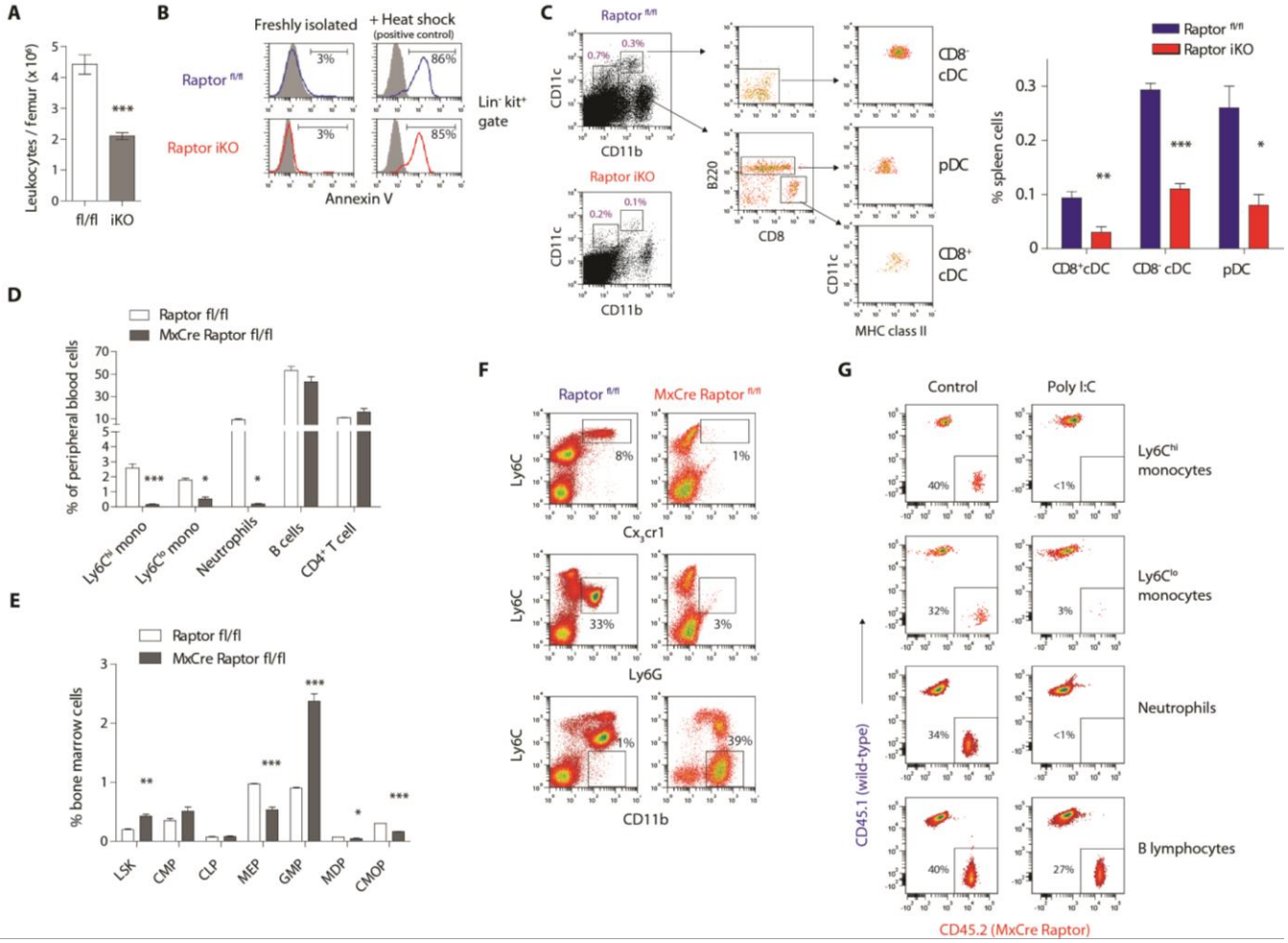

Figure S3. Impaired development of myeloid progenitors in Raptor-deficient mice. A) Comparison of total bone marrow cell count in Raptor $^{fl/fl}$ and Raptor iKO mice (n = 5 per group). B) Annexin V staining of c-kit+ progenitor cells from Raptor $^{fl/fl}$ and Raptor iKO mice. Cells heat-shocked at 45°C for 30 min were used as positive control. C) Gating strategy and quantification of splenic DC subsets in Raptor $^{fl/fl}$ and Raptor iKO mice two weeks after tamoxifen treatment (n = 3 per group). D) Quantification of peripheral blood cell subsets (n = 5 per group) and E) bone marrow progenitors in Mx-Cre Raptor $^{fl/fl}$ mice (n = 3 per group) two weeks after poly I:C treatment. F) FACS analysis of bone marrow myeloid cells in Mx-Cre Raptor $^{fl/fl}$ mice and controls two weeks after poly I:C treatment. Boxes indicate Ly6C^{hi} monocytes (upper), neutrophils (middle) and Ly6C^{lo} monocyte / macrophages (lower panel). G) FACS plots of peripheral blood cells in CD45.1^{+} recipient mice transplanted with bone marrow cells from CD45.1^{+} wild-type and CD45.2^{+} MxCre Raptor $^{fl/fl}$ mice. Analysis was performed two weeks after poly I:C treatment (see schematic in Fig 3E). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Figure S4

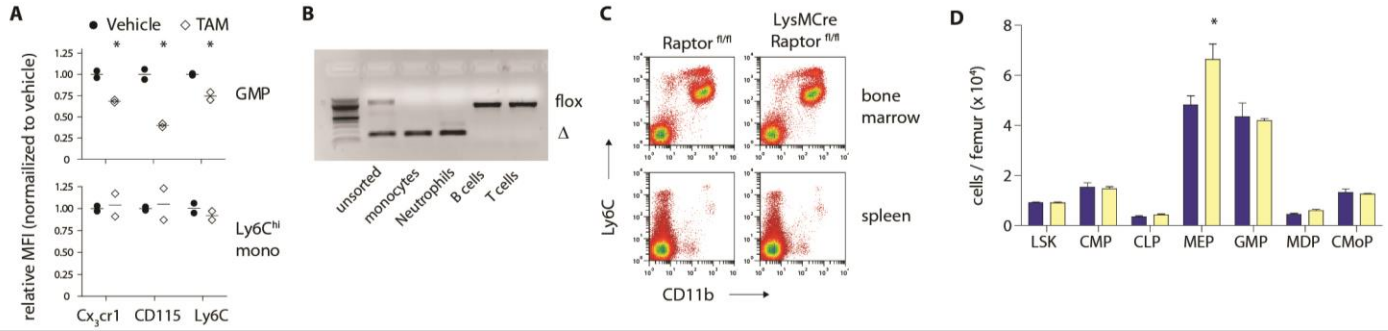

Figure S4. Myeloid-specific deletion of Raptor does not affect monocyte or neutophil homeostasis. A) FACS analysis of sorted GMP and Ly6C^{hi} monocytes from Raptor iKO mice cultured in medium containing SCF and M-CSF (10 ng/mL) ± 4OH-tamoxifen (1 μM) for 4 days. B) PCR confirmation of myeloid-specific Raptor deletion in LysMCre mice. C) FACS plots of bone marrow and spleen myeloid cells in LysMCre Raptor $^{fl/fl}$ and control mice. D) Quantification of bone marrow progenitors from LysMCre $^{+/+}$ Raptor $^{fl/fl}$ and control mice (n = 4 per group). Bars represent mean ± SEM. * $p < 0.05$

Figure S5

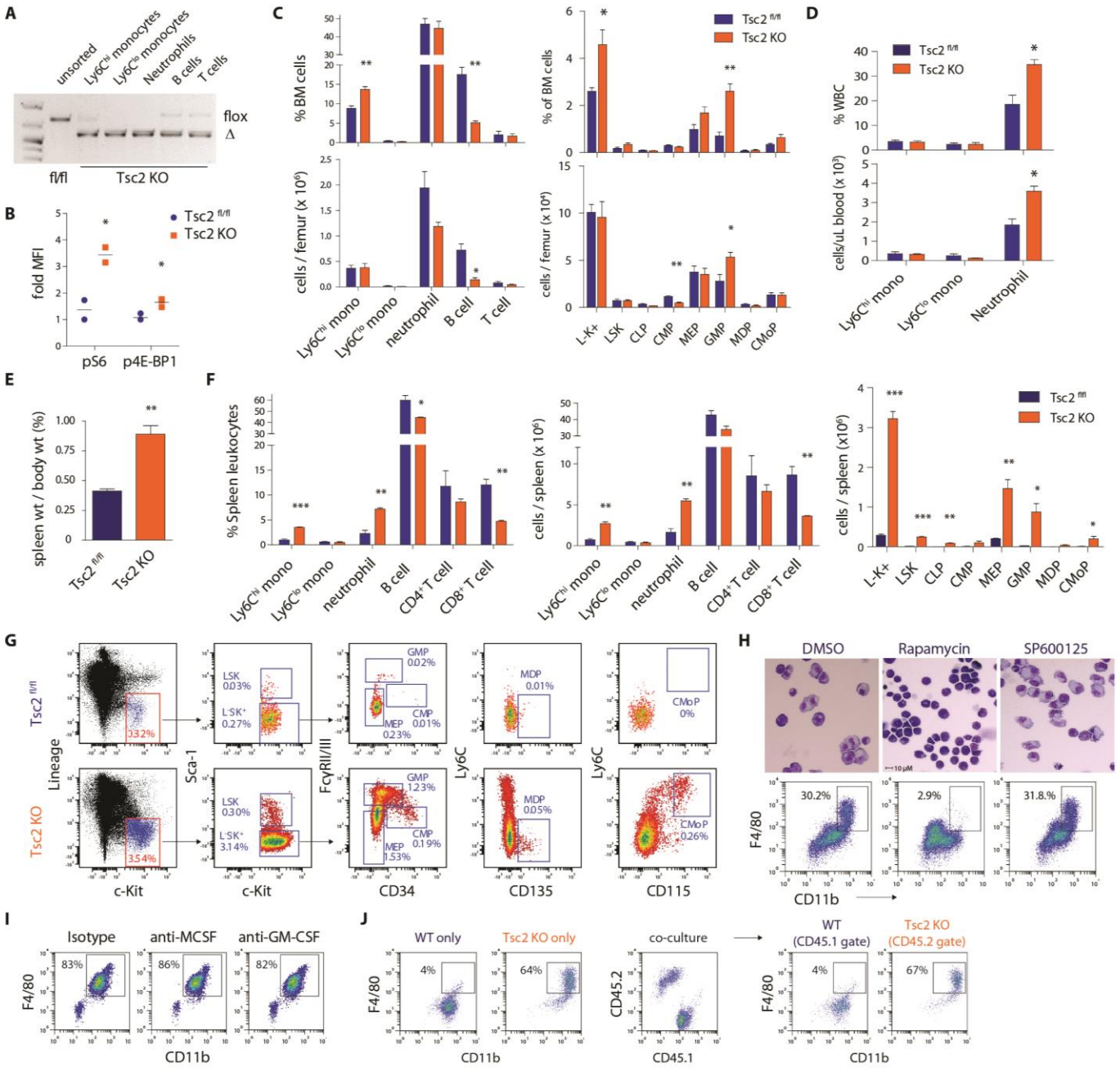

Figure S5. Deletion of Tsc2 augments myelopoiesis via mTORC1 activation. A) PCR analysis of Tsc2 deletion in sorted bone marrow cells from Tsc2 KO after poly I:C treatment. B) Intracellular flow cytometry analysis of phospho-S6 and phospho-4E-BP1 in total bone marrow cells (n =2 per group). C) Quantification of bone marrow cell subsets and D) peripheral blood myeloid cells in Tsc2 KO vs. control mice (n = 3 per group). E) Measurement of spleen weight / total body weight ratio and F) quantification of spleen cell subsets in Tsc2 KO mice and controls (n = 3 per group). G) FACS plots illustrating the gating scheme for spleen myeloid progenitor cell analysis. Boxes indicate progenitor cell subsets and percentage of total bone marrow cells. For panels B-G, animals were analyzed 4 weeks after poly I:C treatment. H) FACS plots and Giemsa-Wright

staining of cells differentiated from sorted Tsc2 KO LSK cells cultured in the presence of rapamycin (100 ng/mL) or the JNK inhibitor SP600125 (5 μM) for 6 days. I) FACS plots of sorted Tsc2 KO LSK cells cultured in the presence of indicated antibodies for 8 days. J) FACS plots of control LSK cells (CD45.1) and Tsc2 KO LSK cells co-cultured in the presence of SCF for 8 days. Boxes in panel H-J indicate $CD11b^+$ $F4/80^+$ macrophages. Bars represent mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p< 0.001$

Figure S6

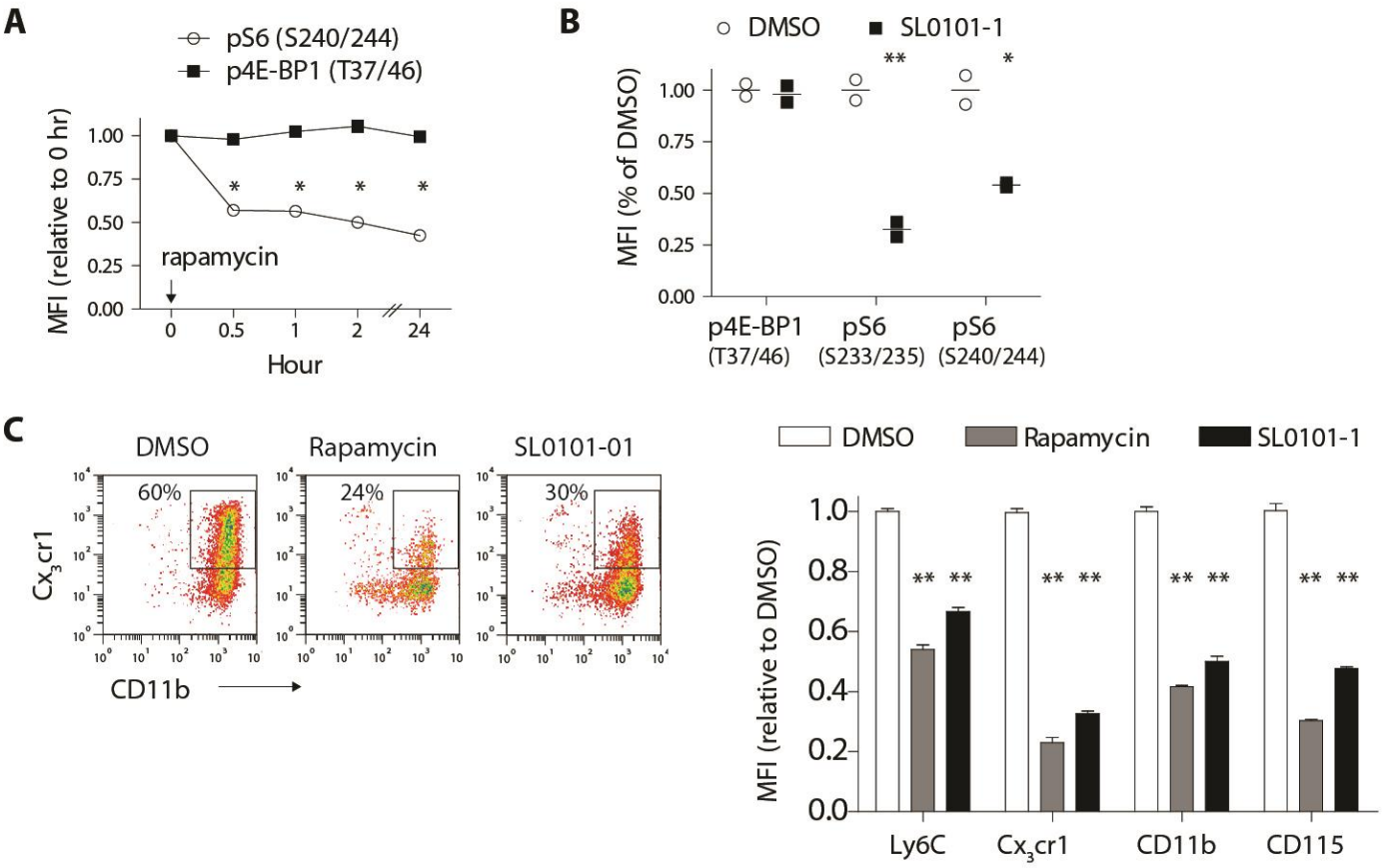

Figure S6. mTORC1 modulates myeloid development via S6K1. A) Longitudinal analysis of phospho-S6 and phospho-4E-BP1 staining in ER-Hoxb8 cells treated with rapamycin (100 nM). Values represent average MFI of duplicate samples normalized to pre-stimulation control (0 hr). B) Intracellular staining of phospho-S6 and phospho-4E-BP1 in ER-Hoxb8 cells treated with DMSO or SL0101-01 (10 nM) for 24 hr (n = 2 per condition). C) FACS plots and quantification of DR-ER-Hoxb8 cells differentiated in the presence of DMSO, rapamycin (100 nM) or SL0101-01 (10 nM) for 4 days. Box in FACS plots (upper panel) indicates the percentage of $Ly6C^{hi}$ monocytes. Bar graph depicts relative expression of monocyte markers normalized to DMSO-treated cells. Bars present mean ± SEM of triplicate samples. * $p < 0.05$, ** $p < 0.01$

Figure S7

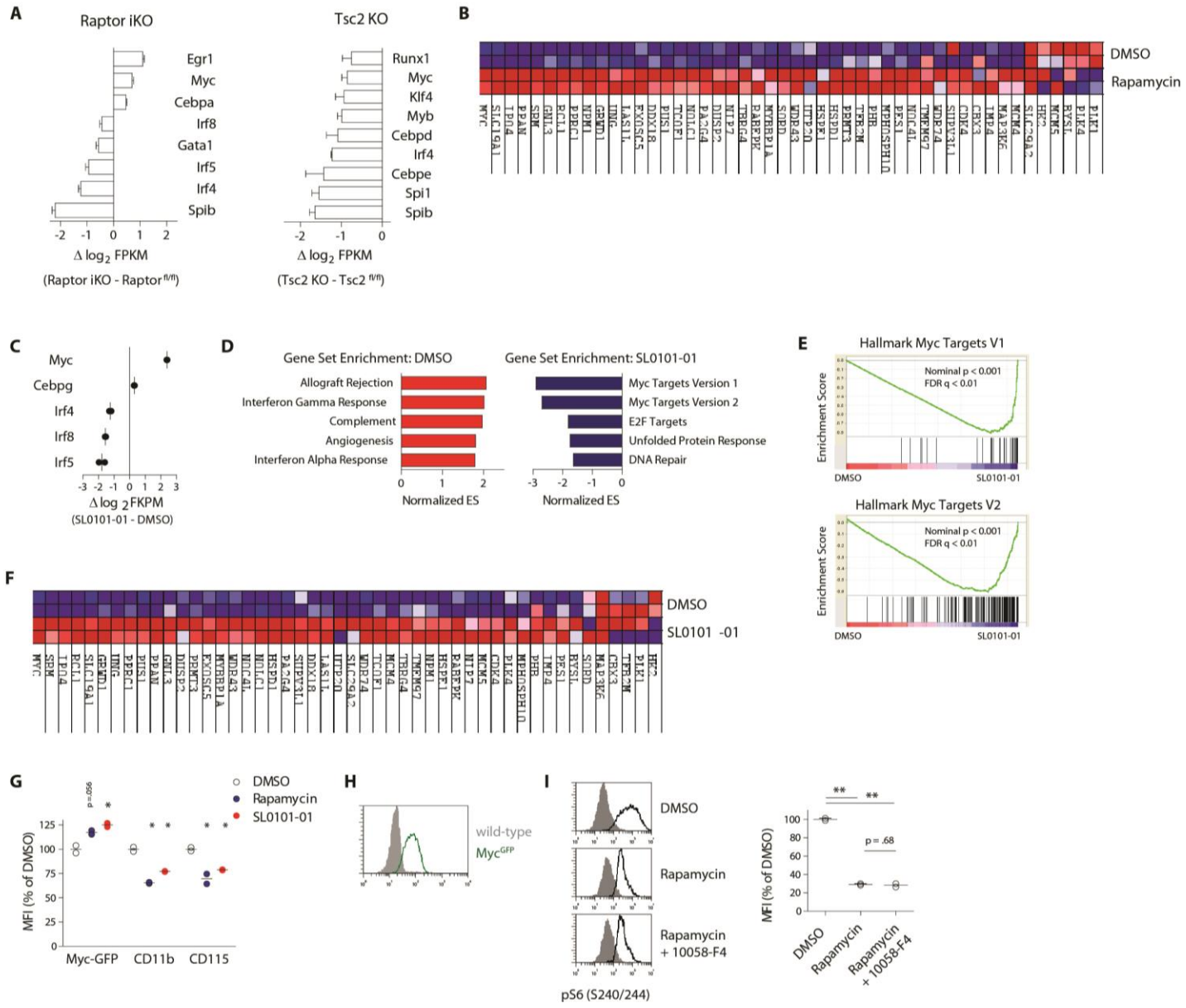

Figure S7. mTORC1-S6K signaling modulates the expression of Myc and its target genes. A) Bar graph display of myeloid transcription factors differentially expressed in GMPs of Raptor $^{fl/fl}$ vs. Raptor iKO and Tsc2 $^{fl/fl}$ vs. Tsc2 KO mice on RNA-seq analysis (n = 3 per group). Bars represent mean ± SEM. Positive values indicate greater expression in the KO group while negative values indicate greater expression in the control group. B) Heat map display of Myc target gene set V1 (from GSEA) in ER-Hoxb8 cells differentiated in the presence of DMSO vs. Rapamycin (100 nM) for 4 days (n = 2 per group). C-F) RNA-seq analysis of myeloid transcription factors in ER-Hoxb8 cells differentiated for 4 days in the presence of DMSO vs. SL0101-01 (10 nM). C) Display of transcription factors with significant difference between DMSO vs. SL0101-01 treatment. D) Top 5 enriched gene sets in DMSO vs. SL0101-01-treated ER-Hoxb8 cells based on normalized enrichment score (NES) from GSEA. E) Enrichment plot of Myc target gene sets from GSEA. F) Heat map display of Myc

target gene set V1 (from GSEA) in ER-Hoxb8 cells differentiated in the presence of DMSO vs. SL0101-01 for 4 days (n = 2 per condition). G) Quantification of GFP and myeloid marker expression in sorted CMP from Myc^{GFP} mice cultured in the presence of DMSO, rapamycin (100 nM) or SL0101-01 (10 nM) for 3 days (n = 2 per condition). J) FACS analysis of baseline GFP expression in wild-type and Myc^{GFP} ER-Hoxb8 cells. I) Histogram and quantification of intracellular pS6 staining in ER-Hoxb8 cells differentiated in the presence of DMSO, rapamycin (100 nM) or rapamycin + 10058-F5 (50 nM) for 4 days (n = 2 per condition). * $p < 0.05$, ** $p < 0.01$

Figure S8

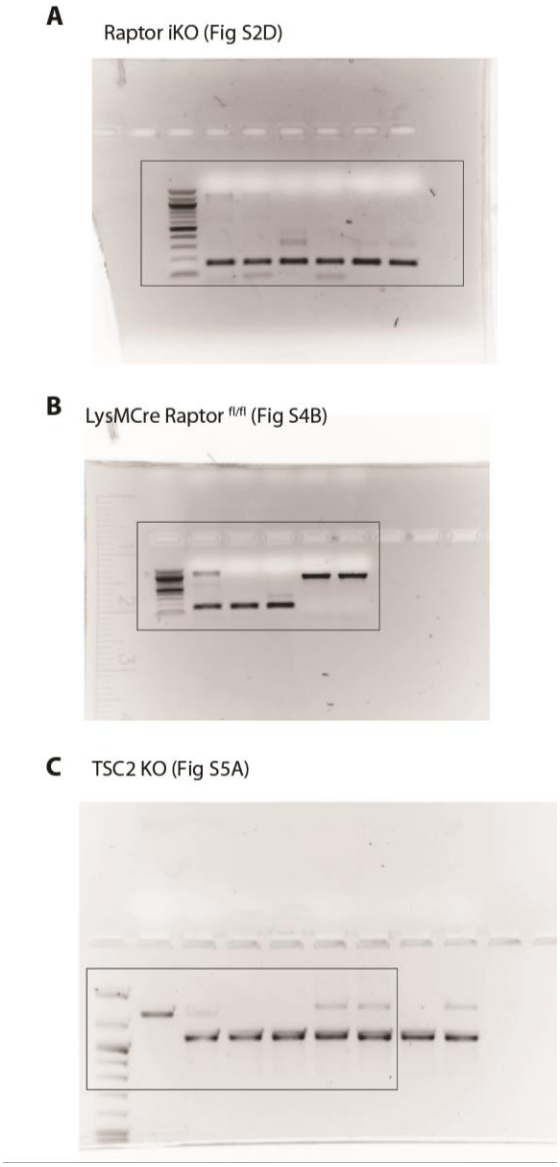

Figure S8. Unmodified images from gel electrophoresis. Boxes indicate the area cropped for figure display.

Table S1. Results from small molecule library screen using DR-ER-Hoxb8 cells *

Plate	Well	Compound	Optimized Concentration	Ly6C MFI	Cx3cr1 MFI	CD115 MFI	Ccr2 MFI
	Control	DMSO	0.10%	100%	100%	100%	100%
1	A2	TG003	10 μM	35%	29%	35%	66%
1	A3	AG-879	10 μM	128%	46%	50%	108%
1	A4	Chelerythrine chloride	1 μM	117%	119%	108%	110%
1	A5	Canertinib (hydrochloride)	1 μM	147%	61%	82%	78%
1	A6	CHIR99021	10 μM	36%	17%	4%	19%
1	A7	Bisindolylmaleimide IV	1 μM	148%	62%	74%	98%
1	A8	NU 6102	10 μM	95%	31%	49%	87%
1	A9	SU 6656	1 μM	106%	142%	83%	100%
1	A10	ABT-869	1 μM	86%	79%	52%	87%
1	A11	U-0126	10 μM	105%	116%	67%	196%
1	B2	PKC 412	10 μM	146%	113%	109%	94%
1	B3	1-NA-PP1	1 μM	106%	82%	105%	90%
1	B4	Tunicamycin	10 mM	116%	86%	88%	98%
1	B5	SB 431542	10 μM	91%	37%	85%	63%
1	B6	BIO	1 μM	109%	77%	85%	77%
1	B7	Ro 31-6045	10 μM	72%	68%	101%	323%
1	B8	KN-62	10 μM	147%	36%	78%	59%
1	B9	LY364947	10 μM	81%	62%	92%	111%
1	B10	CAY10622	10 μM	106%	60%	77%	87%
1	B11	Staurosporine	1 μM	21%	25%	24%	40%
1	C2	Doramapimod	1 μM	43%	48%	66%	83%
1	C3	Torin 1	100 nM	46%	12%	17%	66%
1	C4	AZD 7762	1 μM	119%	121%	76%	104%
1	C5	PD 173074	1 μM	91%	36%	87%	86%
1	C6	Imatinib (mesylate)	1 μM	85%	42%	57%	69%
1	C7	NSC 663284	1 μM	120%	43%	83%	79%
1	C8	KN-93	1 μM	112%	60%	100%	74%
1	C9	SB 203580 (hydrochloride)	10 μM	32%	15%	60%	50%
1	C10	17β-hydroxy Wortmannin	1 μM	92%	30%	50%	117%
1	C11	KN-92 (hydrochloride)	10 μM	67%	58%	83%	75%
1	D2	Paclitaxel	1 μM	131%	133%	125%	110%
1	D3	Bisindolylmaleimide II	1 μM	77%	27%	55%	188%
1	D4	GSK 1059615	1 μM	34%	22%	53%	85%
1	D5	Valproic Acid (sodium salt)	10 μM	107%	47%	107%	73%
1	D6	Sunitinib Malate	-	cell death at all tested concentrations			
1	D7	D 4476	10 μM	74%	37%	90%	76%
1	D8	CGP 57380	10 μM	61%	13%	43%	55%
1	D9	CAY10621	1 μM	81%	65%	104%	112%
1	D10	CAY10626	100 nM	48%	25%	51%	71%
1	D11	AS-605240 (potassium salt)	10 μM	24%	25%	65%	72%
1	E2	Erlotinib	1 μM	43%	71%	58%	71%
1	E3	BIBF 1120	1 μM	51%	86%	61%	74%
1	E4	Ruxolitinib	10 μM	52%	21%	31%	51%
1	E5	PD 0325901	1 μM	60%	50%	58%	81%
1	E6	Gefitinib	1 μM	90%	39%	68%	69%
1	E7	NU 7026	10 μM	55%	18%	37%	78%
1	E8	Iso-Olomoucine	10 μM	111%	65%	94%	81%
1	E9	YM-201636	1 μM	68%	24%	35%	96%
1	E10	SU 6668	10 μM	104%	69%	86%	99%

* Results are expressed as percentage of mean fluorescence intensity relative to DMSO control

Plate	Well	Compound	Optimized Concentration	Ly6C MFI	Cx3cr1 MFI	CD115 MFI	Ccr2 MFI
1	E11	PD 166326	1 μM	88%	88%	70%	128%
1	F2	Necrostatin-5	1 μM	104%	90%	110%	101%
1	F3	SMI-4a	10 μM	129%	53%	106%	83%
1	F4	Necrostatin-1	10 μM	113%	51%	92%	82%
1	F5	SB 203580	10 μM	44%	13%	36%	49%
1	F6	PP2	1 μM	102%	102%	112%	115%
1	F7	Gö 6983	1 μM	85%	23%	39%	229%
1	F8	(S)-Glycyl-H-1152 (hydrochloride)	1 μM	114%	66%	93%	98%
1	F9	ZM 447439	1 μM	124%	149%	156%	137%
1	F10	CAY10572	1 μM	122%	39%	60%	145%
1	F11	O-1918	10 μM	108%	75%	125%	100%
1	G2	NVP-BEZ235	10 μM	28%	20%	26%	59%
1	G3	CAY10657	1 μM	113%	55%	67%	90%
1	G4	SB 505124	10 μM	68%	78%	79%	52%
1	G5	VX-702	10 μM	27%	51%	57%	61%
1	G6	3-Methyladenine	10 μM	84%	61%	81%	82%
1	G7	H-9 (hydrochloride)	10 μM	88%	55%	78%	87%
1	G8	Ro 31-7549 (acetate)	1 μM	76%	28%	41%	89%
1	G9	AS-041164	10 μM	98%	65%	85%	88%
1	G10	N,N-Dimethylsphingosine	1 μM	79%	71%	91%	104%
1	G11	Y-27632 (hydrochloride)	10 μM	57%	72%	59%	113%
1	H2	Phthalazinone pyrazole	1 μM	86%	37%	46%	62%
1	H3	AS-703026	10 μM	110%	102%	81%	79%
1	H4	INK128	100 nM	31%	11%	22%	53%
1	H5	Emodin	10 μM	150%	46%	72%	106%
1	H6	Bisindolylmaleimide I	1 μM	105%	38%	49%	165%
1	H7	Indirubin-3'-monoxime	1 μM	109%	60%	63%	89%
1	H8	ST638	10 μM	99%	37%	51%	67%
1	H9	PP242	1 μM	41%	21%	28%	69%
1	H10	LY294002	10 μM	20%	13%	18%	36%
1	H11	Leelamine	1 μM	85%	91%	81%	111%
2	A2	PD 98059	10 μM	86%	142%	70%	124%
2	A3	Leelamine (hydrochloride)	1 μM	91%	128%	112%	118%
2	A4	SC-1	10 μM	22%	14%	24%	64%
2	A5	Triciribine	100 nM	64%	35%	34%	83%
2	A6	SB 415286	10 μM	83%	49%	72%	58%
2	A7	DRB	10 μM	40%	19%	38%	68%
2	A8	Lavendustin C	10 μM	120%	58%	71%	107%
2	A9	H-89	1 μM	89%	73%	55%	70%
2	A10	CAY10576	10 μM	88%	67%	97%	77%
2	A11	Myricetin	10 μM	110%	85%	77%	86%
2	B2	PD 169316	1 μM	12%	16%	41%	71%
2	B3	Arachidonic Acid Leelamide	10 μM	76%	87%	91%	134%
2	B4	(R)-Roscovitine	1 μM	150%	100%	98%	88%
2	B5	Erbstatin Analog	1 μM	84%	94%	79%	106%
2	B6	AG-17	1 μM	90%	63%	64%	139%
2	B7	RG-13022	10 μM	87%	51%	47%	140%
2	B8	SP 600125	1 μM	55%	65%	58%	87%
2	B8	ZM 336372	1 μM	79%	80%	81%	103%
2	B9	HA-1077 (hydrochloride)	10 μM	77%	57%	40%	161%

Plate	Well	Compound	Optimized Concentration	Ly6C MFI	Cx3cr1 MFI	CD115 MFI	Ccr2 MFI
2	B10	NH125	1 μM	70%	91%	100%	72%
2	B11	Rapamycin	100 nM	48%	46%	66%	100%
2	C2	TGX-221	10 μM	42%	37%	37%	75%
2	C3	Lauric Acid Leelamide	10 μM	102%	36%	59%	93%
2	C4	BAY-43-9006	10 μM	99%	61%	59%	102%
2	C5	Kenpaullone	1 μM	48%	35%	32%	69%
2	C6	H-8 (hydrochloride)	10 μM	160%	55%	69%	107%
2	C7	RG-14620	1 μM	137%	115%	109%	101%
2	C8	5-Iodotubercidin	1 μM	90%	22%	17%	59%
2	C9	AG-370	10 μM	132%	86%	79%	93%
2	C10	TWS119	10 μM	69%	37%	49%	102%
2	D2	(S)-H-1152 (hydrochloride)	1 μM	105%	73%	70%	121%
2	D3	AS-252424	10 μM	39%	34%	81%	72%
2	D4	CAY10561	1 μM	88%	139%	90%	111%
2	D5	Olomoucine	10 μM	72%	168%	110%	124%
2	D6	LFM-A13	10 μM	95%	113%	95%	108%
2	D7	AG-490	1 μM	78%	68%	47%	74%
2	D8	SB 202190	10 μM	10%	14%	25%	42%
2	D9	Wortmannin	1 μM	73%	43%	49%	95%
2	D10	NSC 210902	10 μM	111%	89%	86%	104%
2	E2	AS-605240	10 μM	33%	21%	41%	63%
2	E3	CAY10505	10 μM	73%	86%	93%	59%
2	E4	AS-604850	10 μM	5%	74%	91%	68%
2	E5	AG-494	1 μM	92%	74%	63%	122%
2	E6	SC-514	10 μM	59%	66%	65%	103%
2	E7	AG-82	10 μM	72%	85%	69%	125%
2	E8	CAY10571	10 μM	25%	19%	62%	47%
2	E9	AG-1296	1 μM	114%	115%	93%	114%
2	E10	CAY10577	10 μM	106%	82%	92%	109%
2	F2	D-erythro-Sphingosine C-18	1 μM	131%	91%	106%	70%
2	F3	PI-103	100 nM	124%	67%	92%	79%
2	F4	PI3-Kinase α Inhibitor 2	1 μM	31%	17%	35%	87%
2	F5	AG-825	10 μM	85%	70%	68%	112%
2	F6	Apigenin	10 μM	68%	56%	68%	122%
2	F7	AG-99	10 μM	27%	68%	29%	220%
2	F8	Nilotinib	10 μM	97%	61%	82%	104%
2	F9	Janex 1	10 μM	50%	29%	56%	106%
2	F10	CAY10578	10 μM	96%	93%	77%	101%
2	G2	OSU03012	1 μM	121%	71%	106%	69%
2	G3	Sphingosine Kinase Inhibitor 2	1 μM	92%	104%	76%	103%
2	G4	CAY10567	10 μM	97%	53%	99%	62%
2	G5	AG-1478	1 μM	67%	71%	73%	108%
2	G6	AG-18	10 μM	40%	55%	41%	157%
2	G7	AG-213	10 μM	42%	59%	46%	165%
2	G9	CAY10574	10 μM	37%	29%	50%	25%
2	G10	PD 184161	1 μM	91%	123%	110%	99%
2	H2	JNJ-10198409	100 nM	101%	93%	92%	109%
2	H3	Piceatannol	10 μM	109%	82%	118%	101%
2	H4	ML-9	1 μM	71%	90%	84%	107%
2	H5	SB 216763	10 μM	38%	169%	110%	113%
2	H6	CAY10554	10 μM	40%	69%	65%	93%
2	H7	AG-183	10 μM	62%	105%	87%	137%
2	H8	L-threo-Sphingosine C-18	10 μM	100%	84%	80%	88%
2	H9	CAY10575	10 μM	106%	54%	85%	65%
2	H10	CCT018159	10 μM	106%	79%	100%	76%

Table S2. Effects of mTOR inhibitors on the expression of monocyte markers in DR-ER-Hoxb8 cells *.

Compound	Concentration	Ly6C	CX_3CR1-GFP	CD115	CCR2-RFP
DMSO (Control)	-	100%	100%	100%	100%
CAY10626	100 nM	48%	25%	51%	71%
INK128	100 nM	31%	11%	22%	53%
NVP-BEZ235	1 μM	28%	20%	26%	59%
PP242	1 μM	41%	21%	28%	69%
Rapamycin	100 nM	48%	46%	66%	100%
Torin 1	100 nM	46%	12%	17%	66%

* Results are expressed as percentage of mean fluorescence intensity relative to DMSO control

Table S3. List of antibodies and sources

Target antigen	Clone	Source
B220	RA3-6B2	Biolegend
CD117	2B8	Biolegend
CD11a	M17/4	Biolegend
CD11b	M170	Biolegend
CD11c	N418	Biolegend
CD127	A7R34	Biolegend
CD135	A2F10	Biolegend
CD34	HM34	Biolegend
CD45.1	A20	Biolegend
CD45.2	104	Biolegend
CD64	X54-5/7.1	Biolegend
CX3CR1	SA011F11	Biolegend
Ia/Ie	M5/114.15.2	Biolegend
Lineage Cocktail	multiple	Biolegend
Ly6C	HK1.4	Biolegend
Ly6G	1A8	Biolegend
Isotype controls	Various	Biolegend
F4/80	BM8	Ebioscience
IL12	C17.8	Ebioscience
TNF alpha	MP6-XT22	Ebioscience
CD16/32	2.4G2	BD
phospho-4EBP1 (T37/46)	236B4	CST
phospho-S6K1 (T389)	108D2	CST
phospho-S6 (S240/244)	D68F8	CST
phospho-Akt (S473)	D9E	CST
MCSF	5A1	Bio X Cell
GM-CSF	MP1-22E9	Bio X Cell

* Source of antibodies include Biolegend (San Diego, CA), BD Biosciences (San Jose, CA), Ebioscience (San Diego, CA), Cell Signaling Technology (CST; Danvers, MA) and Bio X Cell (West Lebanon, NH).