

Supplementary methods.

Fluorescent activated cell sorting (FACS) and flow cytometry

Bone marrow was isolated by crushing mouse tibiae, femora, ilia, and spine in PBS/2% FCS. Erythrocyte lysis was performed using ACK lysis buffer (Lonza) at 4°C for 2 min. Cells were resuspended in FACS buffer (PBS, 2% FCS, 0.02% NaN₃, 1mM EDTA) and incubated at 4°C for 20 min with biotinylated antibodies against lineage markers (B220, Gr-1, Ter119, CD3, CD11b, CD19, CD41, CD127). Magnetic depletion was performed after incubation with Streptavidin-coupled magnetic beads (Roti®-MagBeads, Carl Roth). Lineage-negative cells were stained for at least 30 min at 4°C with fluorochrome-conjugated antibodies against Sca-1, CD117, CD16/32, CD150, CD105, and CD45.2. Fluorochrome-labeled streptavidin was used to detect remaining biotin-labeled lineage markers. Peripheral blood was analyzed for donor contribution after erythrocyte lysis by staining against CD45.1 and CD45.2. Cells sorted from in vitro cultures were collected in FACS buffer and then stained against CD16/32, CD11b, and CD115. For intracellular staining of phospho-ERK, cells were starved for 24 h in SFEM and then stimulated with 100ng/ml CSF-1. Cells were fixed with 2% paraformaldehyde (PFA) at room temperature for 10min and permeabilized with 100% ice-cold methanol at 4°C for 10 min. Cells were subsequently stained for phospho-ERK at 4°C for 30 min. Cell sorting was performed on a BD FACSAriaIII using a 70µm nozzle. Flow cytometry was performed on a BD LSR Fortessa and data analyzed using BD FACSDiva or FlowJo (Tree Star) software. Antibody clones used and suppliers are listed in Table S1.

Virus production and transductions

Third generation vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped lentivirus was produced in human embryonic kidney (HEK) 293T cells and titrated using NIH-3T3 fibroblasts. For transductions of primary progenitors, FACS-purified cells (5x10E4 cells/ml) were cultured overnight in a flat bottom 96-well plate. Lentivirus was then added at a multiplicity of infection of 50-100. Cells were further incubated for 48 h before purifying transduced cells by FACS.

qRT-PCR

For C10 gene expression analysis, 20000 cells were sorted directly into RLT lysis buffer and RNA was prepared using the RNeasy Micro+ kit (Quiagen) according to manufacturer's instructions. cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen). qRT-PCRs were performed in triplicates on a LightCycler 96 (Roche). SYBR Green probes used are listed in Table S2.

Phagocytosis assay

Phagocytic activity was measured by flow cytometry using pHrodo Red E. Coli BioParticles (Life Technologies) according to manufacturer's instructions.

Table S1: List of antibodies used in this study

<u>Antibody</u>	<u>Clone No.</u>	<u>Supplier</u>
CD3e	145-2C11	eBioscience
CD19	eBio1D3	eBioscience
CD41	eBioMWRag30	eBioscience
Ter119	TER-119	eBioscience
B220	RA3-6B2	eBioscience
Gr-1	RB6-8C5	eBioscience
CD11b	M1/70	eBioscience
CD127	A7R34	eBioscience
CD16/32	2.4G2	Becton Dickinson
Sca-1	D7	BioLegend
CD117	2B8	eBioscience
CD105	MJ7/18	BioLegend
CD150	TC15-12F12.2	BioLegend
CD45.1	A20	eBioscience
CD45.2	104	eBioscience
Ly6G	1A8	BioLegend
F4/80	BM8	eBioscience
pErk1/2	197G2	Cell Signaling Technology

Table S2: List of qRT-PCR primers.

<u>Gene</u>	<u>fw Primer (5' --> 3')</u>	<u>rev Primer (5' --> 3')</u>
Actb	TTCACCACCACAGCTGAGAG	ATAGTGATGACCTGGCCGTC
Cebpa	GTGGAGACGCAACAGAAGGT	TGTCCAGTTCACGGCTCAG
Csf1r	GTCAGAAGCCCTTCGACAAA	GCTGCTTGCTTTGTCCTAGG
Emr1	CTGTAACCGGATGGCAAAC	GGTTCTGAACAGCACGACAC
Fcgr1	TTCGTTGAACACGGTTCTCTATG	CACGCCATCGCTTCTAACTT
Fos	GCAGAGCATCGGCAGAAG	GTTGATCTGTCTCCGCTTGG
Ifitm1	TATGCCTACTCCGTGAAGTCTAG	CGTGAGGATGGTGAAGAACAG
Irf8	GGGCAGGGAGAGTCATCAT	GAGCAACAGTCAGCAGGTAAG
Itgam	TCCTGCGCCTCAATTATACA	TGAGATCGTCTTGGCAGATG
Lyz2	ACTCCTCCTGCTTTCTGTCACT	GTAGCCAGCCATTCCATTCCTT
Mmp12	GCAGCATTCCAATAATCCAAAGTC	TTCCACCAGAAGAACCAGTCT
Rag1	ACAGTCTCCAGTAGTTCCAGAG	TCTTGAAACGATTCCCACAGATG
CD19	TCCTTCTCCAATGCTGAGTCTTA	GTCCATCATCCTGCCAACTG
Il7r	GGTCATCTTAGCCCATGTGC	CAGGAAACTTTCGGGATTGA
Pax5	CGACTCCTCGGACCATCA	CCTGTCTCATAATACCTGCCAAG

Table S3: qRT-PCR ddCt values.

Gene	control (x10E-4)	c-Src CA (x10E-4)	fold over control (average)
Cebpa	20.6	15000.4	720.96
	24.5	17471.5	
Csf1r	n.d.	133.7	-
	n.d.	139.4	
Emr1	n.d.	n.d.	7.36
	0.1	0.8	
Fcgr1	5.5	2597.1	635.49
	2.8	2670.2	
Fos	5.6	1461.0	198.12
	10.0	1621.0	
Ifitm1	0.6	20.2	39.43
	0.5	23	
Irf8	970.6	10316.8	11.00
	937.5	10680.7	
Itgam	1.7	35.1	19.51
	2.4	44.4	
Lyz2	492.1	21213.8	45.54
	455.9	21961.9	
Mmp12	n.d.	370.3	1499.09
	0.3	516.5	
Rag1	85.8	10.5	0.25
	85.8	32.5	
CD19	691.1	174.6	0.26
	740.7	191.0	
Il7R	333.8	182.6	0.56
	318.0	185.2	
Pax5	74.2	5.5	0.11
	52.1	9.0	

Shown are ddCt (2 technical replicates) and average fold values of one representative experiment (of three independent experiments).

Figure S1

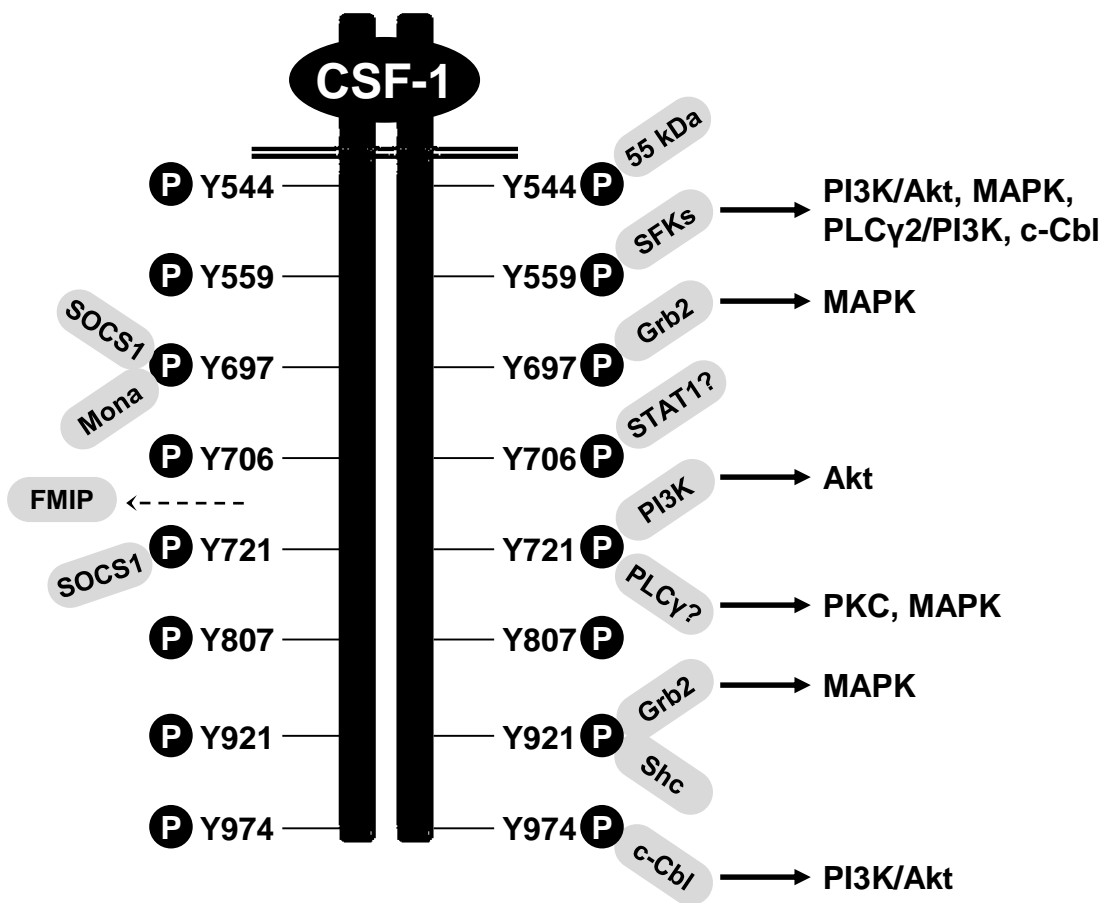


Figure S1: CSF-1R signaling.
Shown are adaptor proteins known to bind the 8 functional CSF-1R tyrosine residues and their activated downstream signaling pathways.

Figure S2

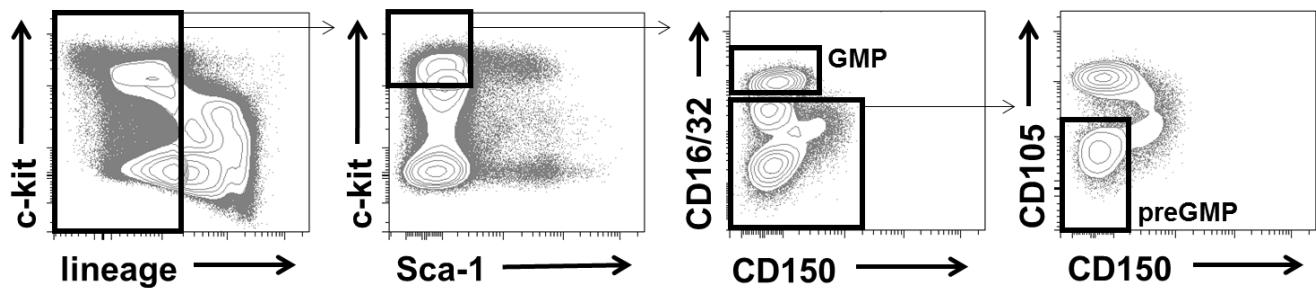


Figure S2: FACS gating scheme to sort BM-derived GMPs and preGMPs.
GMPs are gated as $\text{lin}^{\text{low}} \text{c-kit}^{\text{pos}} \text{Sca-1}^{\text{neg}} \text{CD16/32}^{\text{hi}}$, preGMPs are gated as $\text{lin}^{\text{low}} \text{c-kit}^{\text{pos}} \text{Sca-1}^{\text{neg}} \text{CD16/32}^{\text{neg/lo}} \text{CD105}^{\text{neg}} \text{CD150}^{\text{neg}}$.

Figure S3

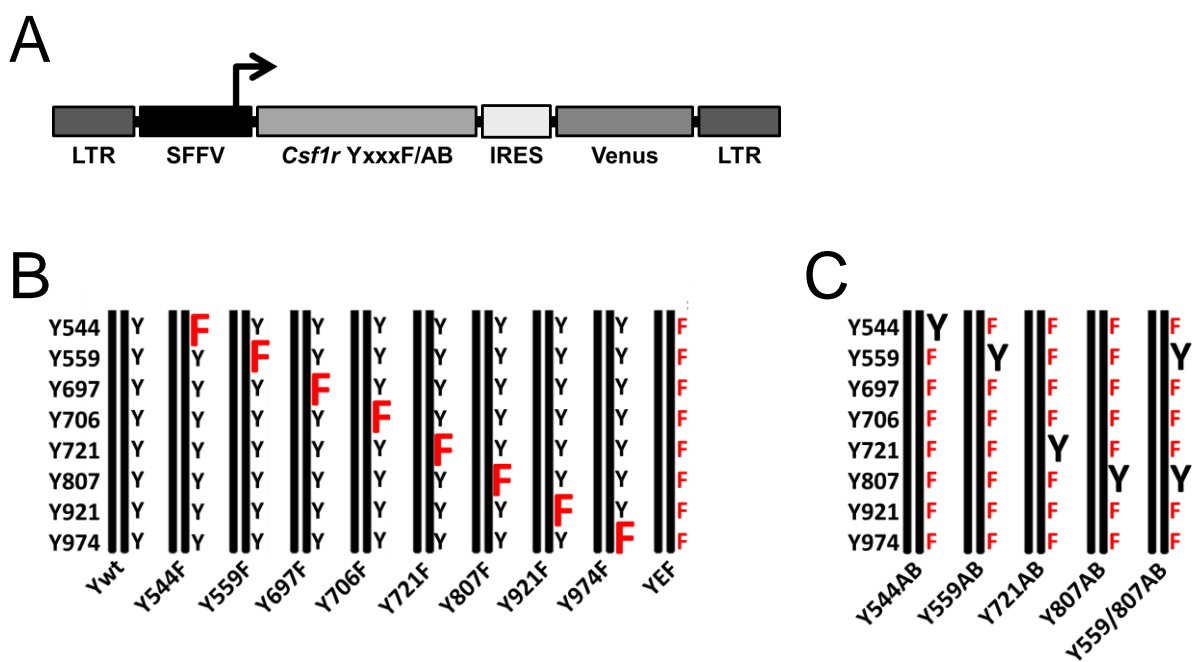


Figure S3: CSF-1R signaling and mutants analyzed.

(A) Lentiviral construct used to deliver CSF-1R mutants into *Csf1r*^{-/-} progenitors. (B) CSF-1R single tyrosine mutants analyzed. (C) CSF-1R add back mutants analyzed. (LTR=long terminal repeat, SFFV=spleen focus-forming virus promoter, IRES=internal ribosome entry site, Y=tyrosine, F=phenylalanine, AB=add back).

B

Figure S4: Rescue of CSF-1 responsiveness by re-introducing wt CSF-1R into *Csf1r*^{-/-} progenitors.

(A) Representative FACS plots showing CSF-1R expression on untransduced *Csf1r*^{-/-}, wild type, and *Csf1r*^{-/-} cells rescued with wt CSF-1R via lentiviral transduction. Dashed lines represent thresholds of negative and endogenous CSF-1R expression. Bold lines represent CSF-1R level gate set for sorting transduced cells. (B) Wt cells and *Csf1r*^{-/-} cells rescued with wt CSF-1R generate adherent CD11b^{pos} F4/80^{pos} macrophage colonies in response to CSF-1. Pictures are representative snapshots from a time-lapse experiment. CD11b and F4/80 were detected by live in-culture staining using anti-Cd11b-PE and anti-F4/80-AlexaFluor647 antibodies, respectively. Original magnification x10. BF=Brightfield.

Figure S5

Example genealogies of *Csf1r*^{-/-} GMPs rescued with:

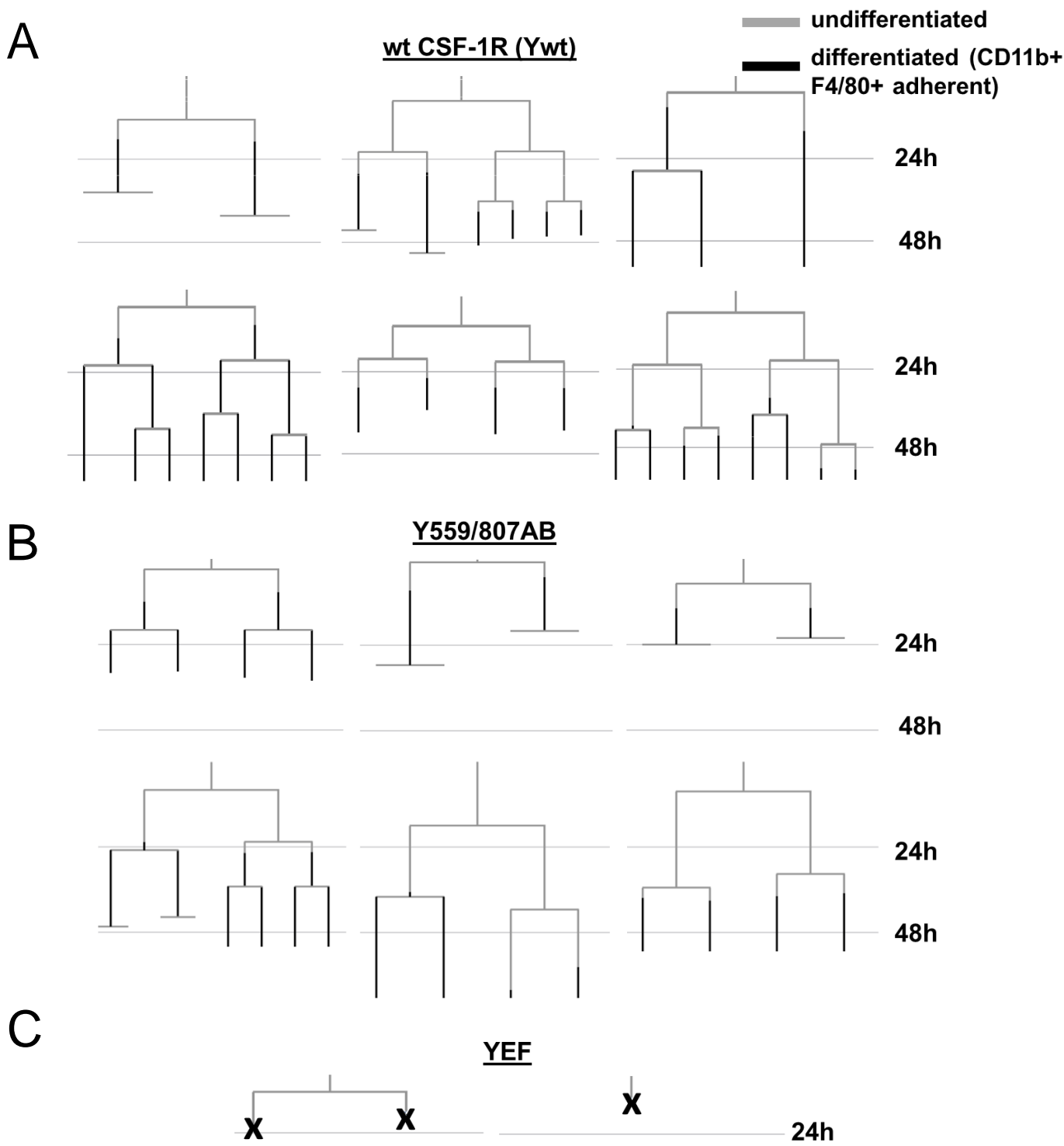


Figure S5: Macrophage instruction by CSF-1 is rescued by wt CSF-1R and the Y559/807AB mutant.

Representative pedigrees of instructed *Csf1r*^{-/-} colonies after re-introducing the (A) wt CSF-1R (Ywt) or (B) 559/807AB mutant. (C) *Csf1r*^{-/-} colonies harboring the YEF mutant die before making two consecutive cell divisions. Black bars represent differentiated cells. Note that cellular differentiation onsets are very synchronous within colonies.

Figure S6

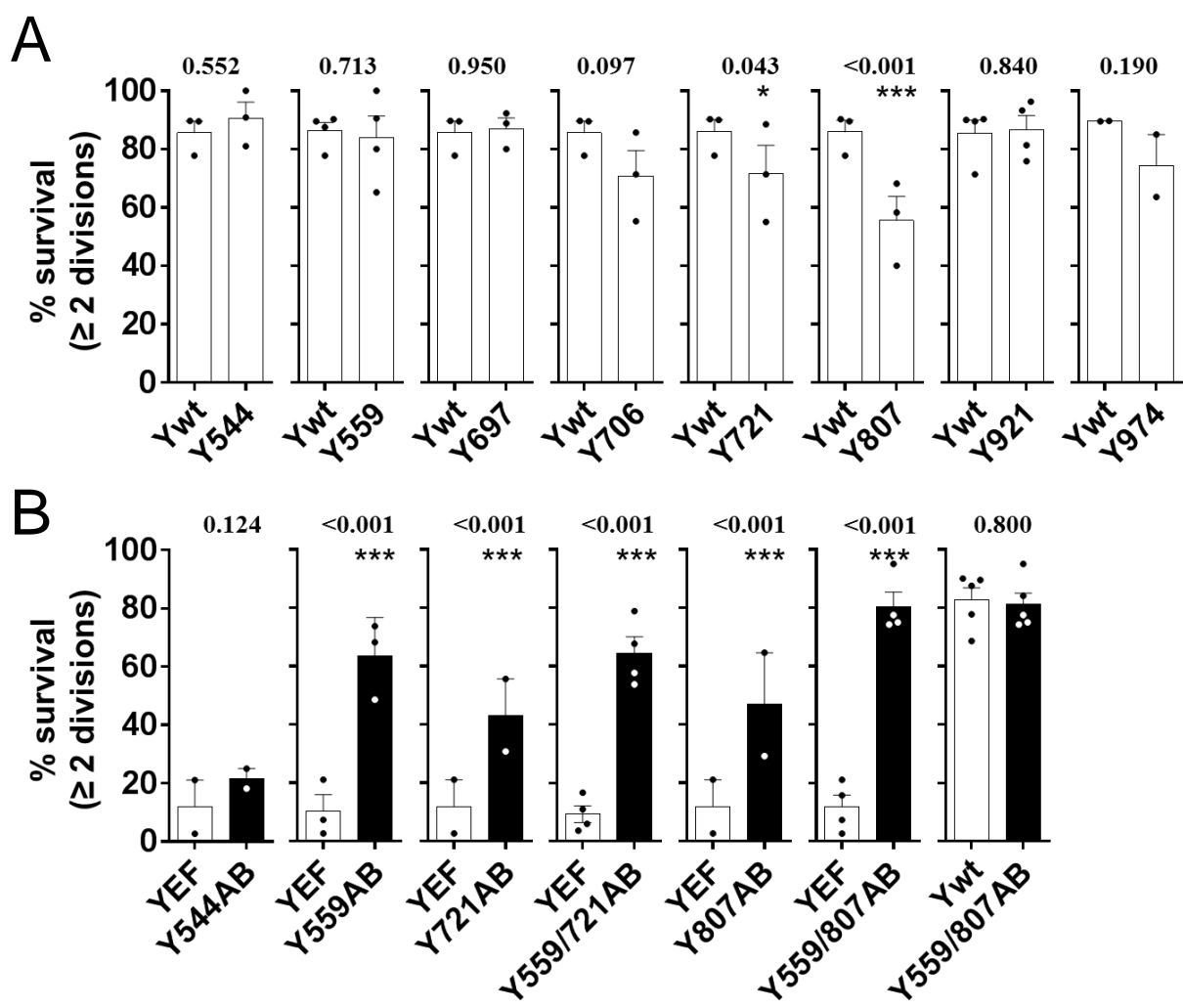


Figure S6 (related to Figure 4B): **Time lapse imaging reveals contribution of CSF-1R tyrosine residues to CSF-1-mediated survival.**

(A) Pair-wise comparisons of Ywt to CSF-1R single tyrosine mutants. (B) Pair-wise comparison of YEF or Ywt to CSF-1R add back mutants. Each dot represents one time-lapse experiment. Total movies/colonies analyzed: Ywt (9/247), Y544F (3/55), Y559F (4/75), Y697F (3/45), Y706F (3/52), Y721F (3/81), Y807F (3/141), Y921F (4/114), Y974F (2/51), YEF (4/140), Y544AB (2/64), Y559AB (3/138), Y721AB (2/67), Y559/721AB (4/99), Y807AB (2/72), Y559/807AB (4/115). Black bars represent CSF-1R add back (AB) mutants. p-values are displayed and depicted as *<0.05, **<0.01, ***<0.001 compared to Ywt (single tyrosine mutants) or YEF (add back mutants).

Figure S7

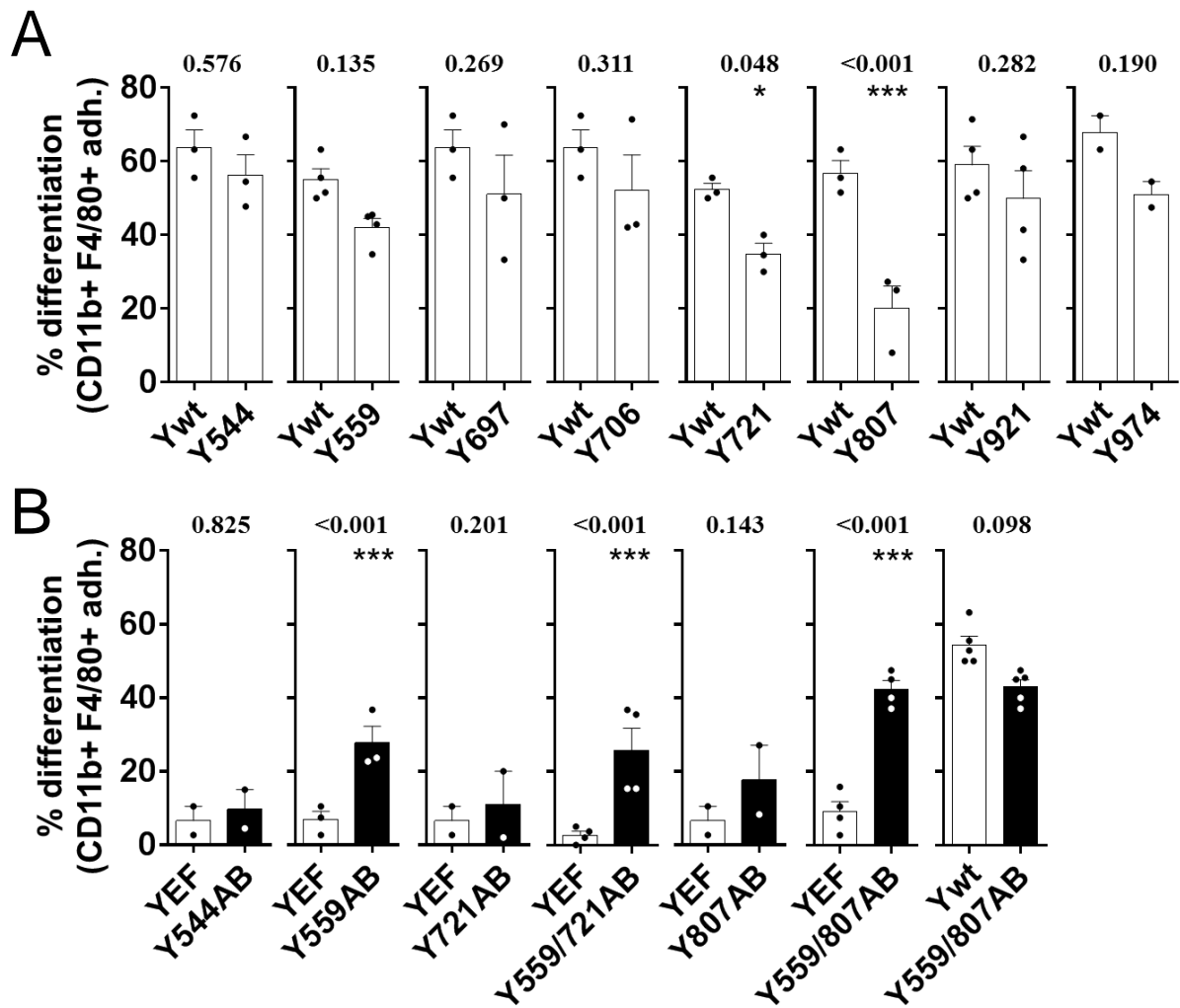


Figure S7 (related to Figure 4C): **Time lapse imaging reveals contribution of CSF-1R tyrosine residues to CSF-1-mediated differentiation.**

(A) Pair-wise comparisons of Ywt to CSF-1R single tyrosine mutants. (B) Pair-wise comparison of YEF or Ywt to CSF-1R add back mutants. Each dot represents one time-lapse experiment. Total movies/colonies analyzed: same as Figure S6. p-values are displayed and depicted as *<0.05, **<0.01, ***<0.001 compared to Ywt (single tyrosine mutants) or YEF (add back mutants).

Figure S8

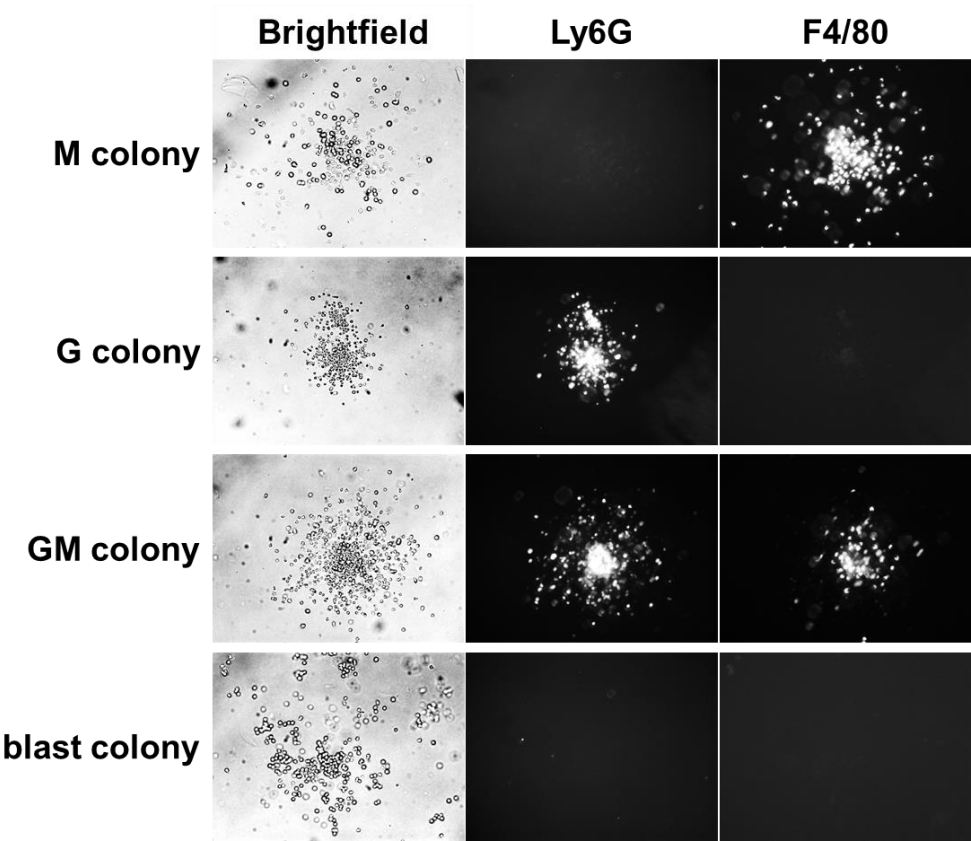


Figure S8: In culture antibody staining.
Representative examples of colony types identified in methylcellulose by live in-culture antibody staining. Ly6G detects granulocytic/neutrophilic cells, F4/80 detects macrophages. Pictures were acquired with a Zeiss Axiovert200M equipped with a 10x Fluar objective (NA 0.5) and a Zeiss AxioCam HRm camera. Ly6G and F4/80 were detected by live in-culture staining using anti-Ly6G-PE and anti-F4/80-AlexaFluor647 antibodies, respectively. Original magnification x10.

Figure S9

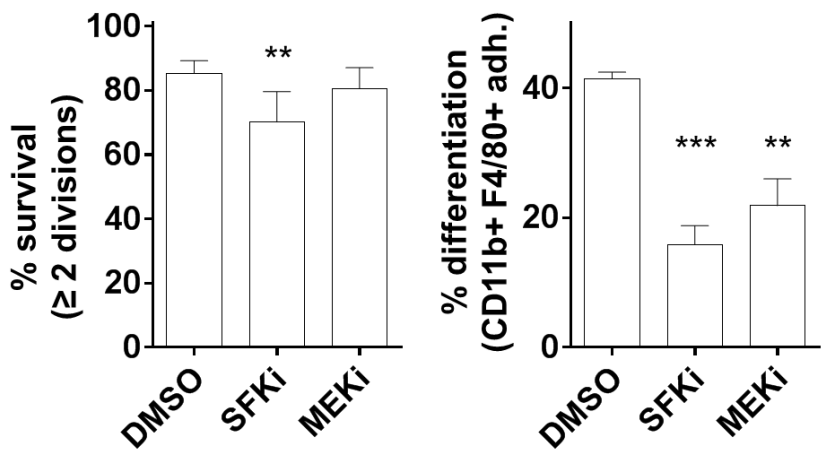


Figure S9: **Blocking SFK or MEK/ERK signaling inhibits Y559-mediated differentiation.**

Csf1r^{-/-} preGMPs were transduced with the Y559/807AB mutant and imaged during CSF-1-mediated differentiation. Compared to the DMSO control, both an SFK (2μM SU6656) and MEK (10μM U0126) inhibitor blocked the differentiation mediated through Y559 (n = 3). p-values are shown as *<0.05, **<0.01, ***<0.001 compared to DMSO.

Figure S10

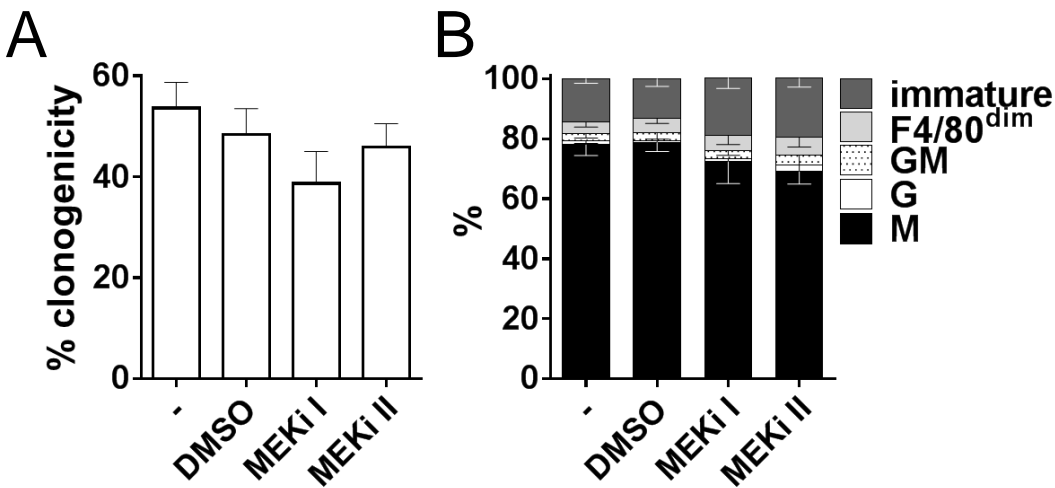


Figure S10: **Blocking MEK/ERK signaling does not block constitutive active c-Src effect.** Related to Figure 5. preGMPs were transduced with constitutive active c-Src and after 3 days sorted as single cells into 384-well plates containing SCF, IL3, IL6, and 10% FCS. After 5-7 days in culture, colonies were enumerated and identified using lineage specific markers (F4/80 for M and Ly6G for G). Immature colonies consisted of cells negative for both markers. (A) Clonogenicity and (B) lineage output are shown (n=3). No statistical difference was detected. MEKi I = 1 μ M PD0325901, MEKi II = 10 μ M U0126.

Figure S11

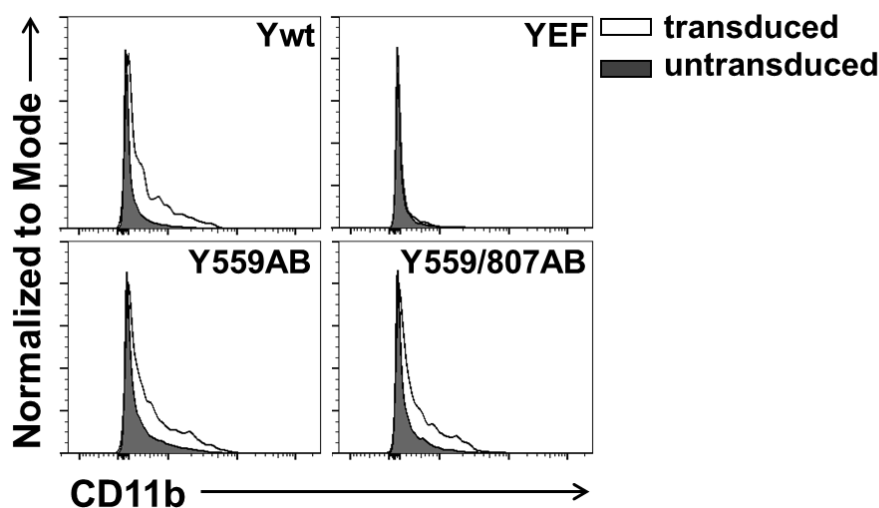


Figure S11: CSF-1R Y559AB mutant leads to CD11b expression in pro-B-cells. C10 cells were transduced with the indicated CSF-1Rs and cultured in presence of CSF-1 for 6 days. Cells were then analyzed for CD11b expression by flow cytometry. Representative FACS plots are shown (n=3). CD11b upregulation was dependent on CSF-1 (data not shown).

Video 1 (related to Figure 4A): Example time lapse movie of differentiating ivGMPs

In this example, ivGMPs were cultured in medium containing CSF-1 and G-CSF to allow M and G differentiation. Phase contrast and fluorescent images were acquired every 2 min and 3 h, respectively. Primary image data is strongly accelerated in example movie, cells from 17 h - 69 h after start of the culture and movie are shown. Time is labelled as d – hh:mm:ss. Colonies generating adherent cells expressing CD11b and F4/80 were considered as M colonies (e.g. emerging upper right colony, but not lower left). Surface marker expression was detected via in-culture CD11b-PE and F4/80-AlexaFluor647 antibody staining.