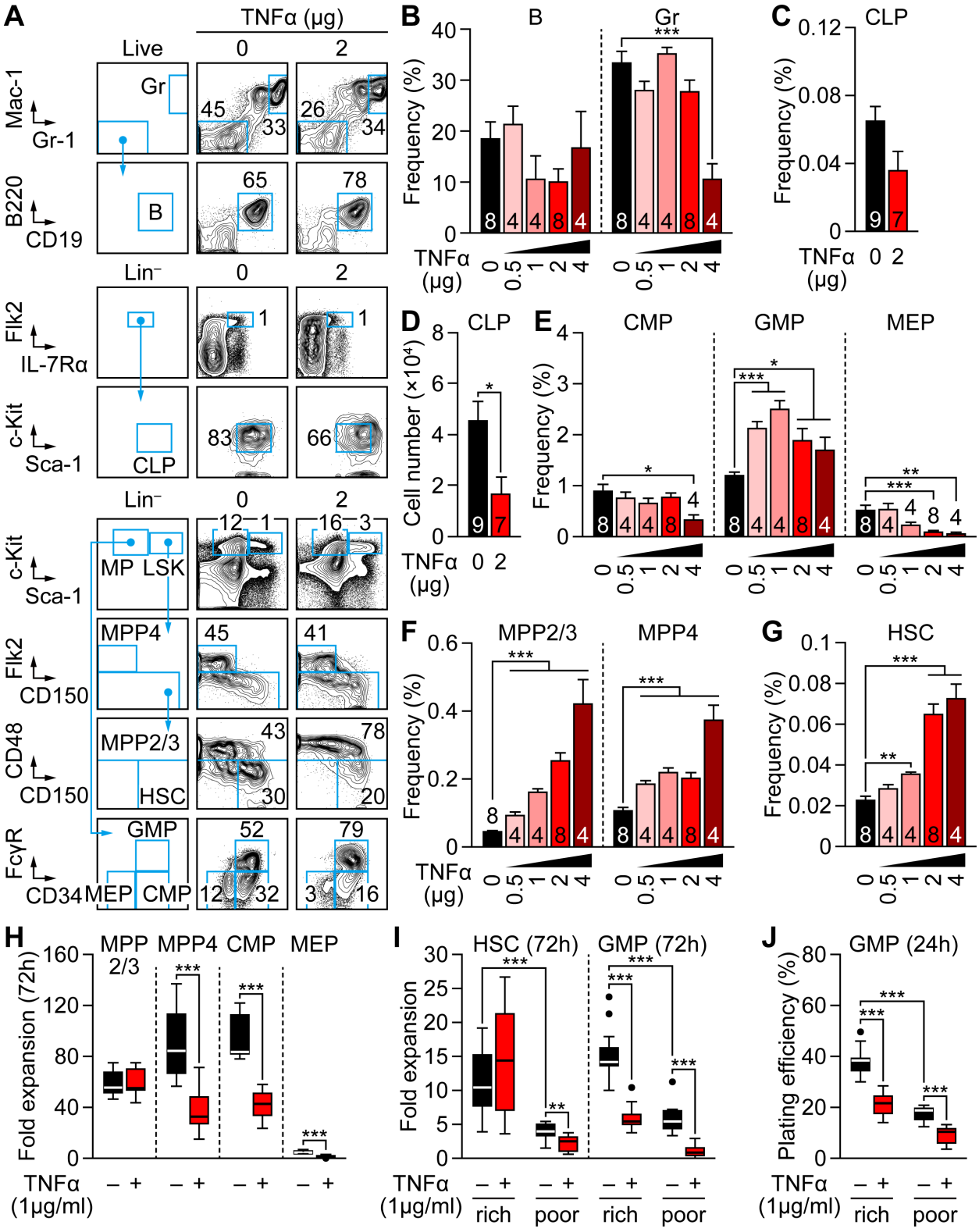
A
TNFα (μg)
Live
0
2
Mac-1
Gr-1
Gr
45
33
26
34
B220
CD19
B
65
78
Lin⁻
Flk2
IL-7Rα
1
1
c-Kit
Sca-1
CLP
83
66
MP LSK
12
1
16
3
MPP4
45
41
CD150
MPP2/3
HSC
43
30
78
20
CD48
FcγR
CD34
GMP
MEP
CMP
52
12
32
79
3
16
B
Gr
Frequency (%)
TNFα (μg)
C
CLP
D
Cell number (×10⁴)
E
CMP
GMP
MEP
F
MPP2/3
MPP4
G
HSC
H
MPP 2/3
MPP4
CMP
MEP
Fold expansion (72h)
TNFα (1μg/ml)
I
HSC (72h)
GMP (72h)
rich
poor
J
GMP (24h)
Plating efficiency (%)

Figure S1. Differential effects of TNFα on HSCs and their progeny, related to Figure 1

(**A**) Gating strategy used to identify BM Grs and B cells (top), lymphoid progenitors (CLP) (middle), myeloid progenitors (CMP, GMP, MEP) and early stem and progenitor population (HSC, MPP2/3, MPP4) (bottom) in WT mice ± TNFα injections.

(**B**) Frequencies of BM B cells and Grs ± TNFα (n = 4-8 mice/group from 5 independent experiments).

(**C-D**) Lymphoid progenitors: (C) frequency and (D) absolute numbers of BM CLPs ± TNFα (n = 7-9 mice/group from 9 independent experiments).

(**E-G**) Frequencies BM stem and progenitors ± TNFα (n = 4-8 mice/group from 5 independent experiments): (E) myeloid progenitors, (F) MPPs, and (G) HSCs.

(**H**) Expansion of the indicated BM populations after 72h culture ± TNFα (n = 9-12 pools of 350-500 cells/group from 3-4 independent experiments). Results are expressed as fold expansion compared to the number of plated cells/condition.

(**I**) Expansion of BM HSCs and GMPs after 72h culture in cytokine-rich (IL-3, SCF, TPO, EPO, GM-CSF, IL-11 and Flt3-L) or -poor (SCF and G-CSF) media ± TNFα (n = 12 pools of 300-500 cells/group from 4 independent experiments). Results are expressed as fold expansion compared to the number of plated cells/condition.

(**J**) Plating efficiency in –TNFα methylcellulose of BM GMPs after 24h culture in cytokine-rich or -poor media ± TNFα (n = 9 pools of 100 cells/group from 3 independent experiments). Colonies are scored after 7 days.

Data are mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

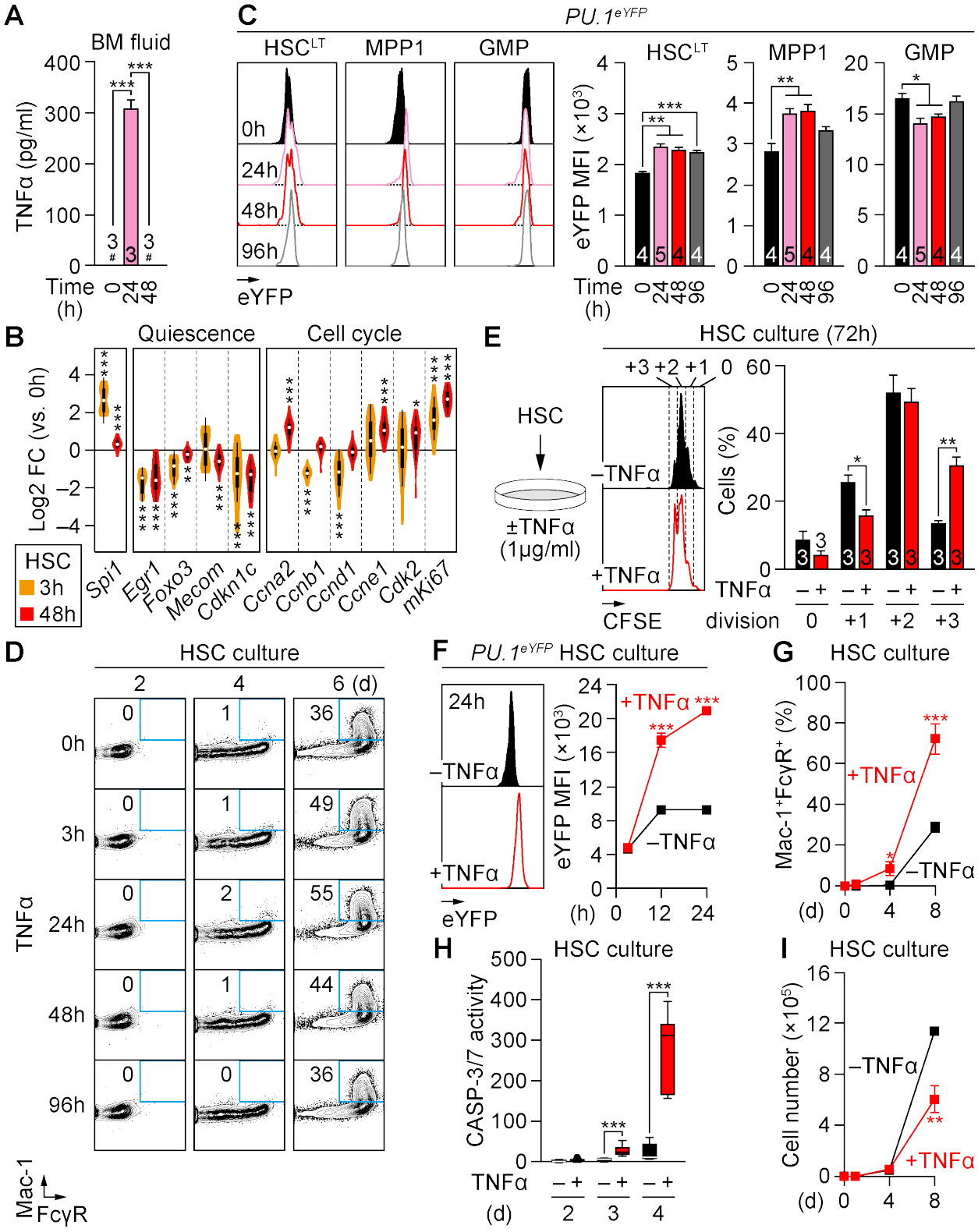
A
BM fluid
TNFα (pg/ml)
Time (h)
B
Quiescence
Cell cycle
Log2 FC (vs. 0h)
HSC
3h
48h
Spi1
Egr1
Foxo3
Mecom
Cdkn1c
Ccna2
Ccnb1
Ccnd1
Ccne1
Cdk2
mKi67
C
PU.1eYFP
HSCLT
MPP1
GMP
0h
24h
48h
96h
eYFP
eYFP MFI (×10³)
D
HSC culture
TNFα
Mac-1
FcγR
E
HSC culture (72h)
HSC
±TNFα (1μg/ml)
–TNFα
+TNFα
CFSE
Cells (%)
division
F
PU.1eYFP HSC culture
G
HSC culture
Mac-1+FcγR+ (%)
H
HSC culture
CASP-3/7 activity
I
HSC culture
Cell number (×10⁵)

Figure S2. Cell cycle activation and myeloid priming in TNFα-exposed HSCs, related to Figure 2

(**A**) ELISA measurement of TNFα concentration in BM fluid of WT mice ± TNFα (n = 3 biological replicates/group from 3 independent experiments); $^{\#}$ undetectable or less than 8 pg/ml.

(**B**) Fluidigm qRT-PCR analyses of *Spi1* (PU.1), quiescence- and cell cycle-associated gene expression in BM HSCs ± TNFα (n = 22-24 pools of 100 cells/group from 4 mice in 2 independent experiments). Results are expressed as log2 fold changes compared to 0h HSCs (* vs. 0h).

(**C**) PU.1 level in the indicated BM *PU.1-eYFP* populations ± TNFα (n = 4-5 mice/group from 2 independent experiments).

(**D**) Representative flow cytometry plots showing *in vitro* myeloid differentiation from BM HSCs ± TNFα.

(**E**) Experimental design and divisional history of CFSE-labelled BM HSC after 72h culture ± TNFα (n = 3 pools of 1000 cells/group from 3 independent experiments).

(**F**) PU.1 level in BM *PU.1-eYFP* HSCs after 24h culture ± TNFα (n = 3 pools of 1000 cells/group from 3 independent experiments).

(**G**) *In vitro* myeloid differentiation of BM HSCs cultured ± TNFα (n = 5 pools of 1000 cells/group from 5 independent experiments). Results are shown as percentage of Mac-1^{+}/FcγR^{+} mature myeloid cells.

(**H**) CASP-3/7 activity in BM HSCs cultured ± TNFα (n = 9-12 pools of 200 cells/group from 3-4 independent experiments). Results are expressed as fold changes compared to –TNFα HSCs on day 2 (set to 1).

(**I**) Expansion of BM HSCs cultured ± TNFα (n = 3 pools of 1000 cells/group from 3 independent experiments). Results are expressed as absolute numbers.

Data are mean ± SEM, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

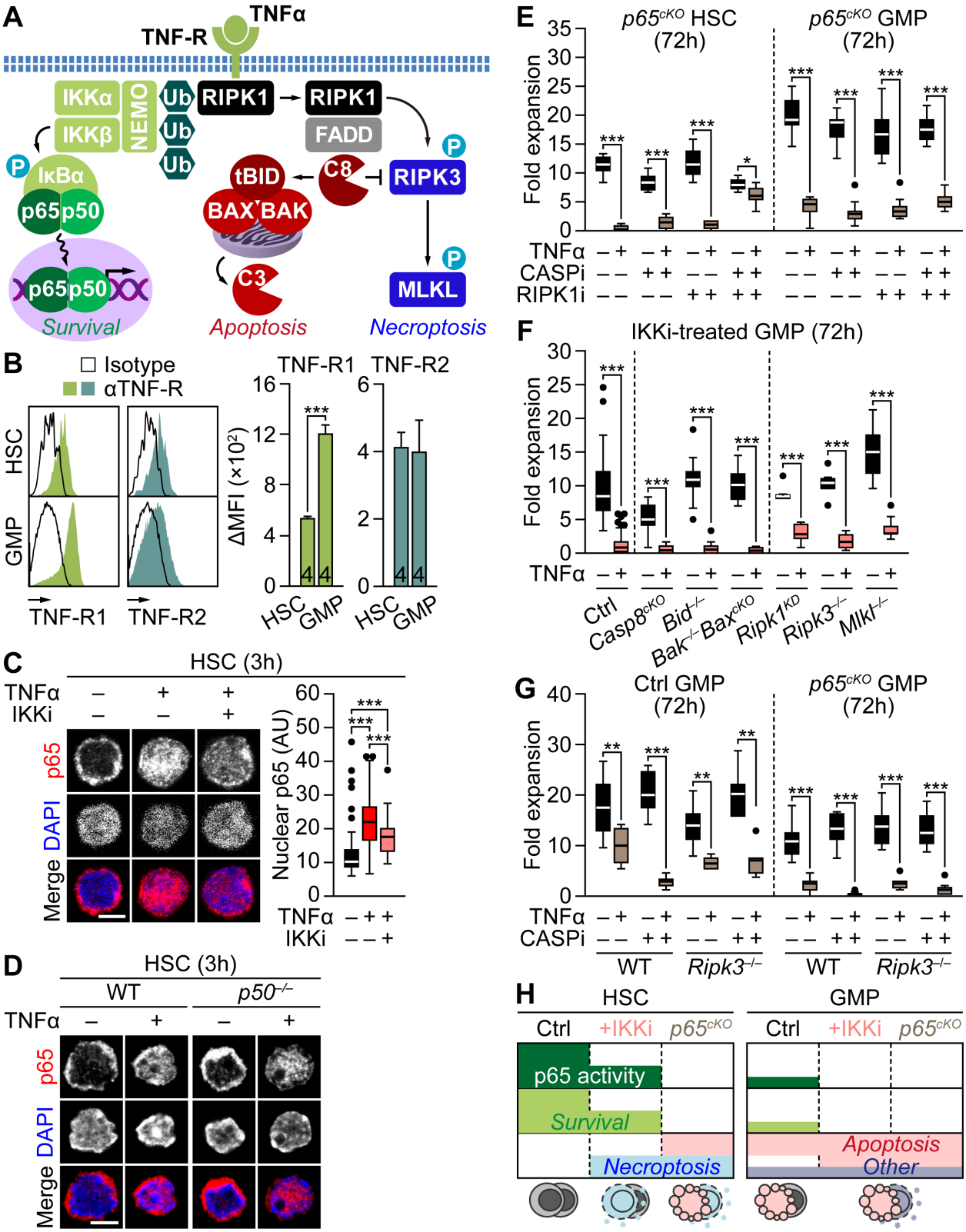
A
TNFα
TNF-R
IKKα
IKKβ
NEMO
Ub
RIPK1
RIPK1
FADD
C8
RIPK3
IκBα
p65p50
tBID
BAX BAK
C3
MLKL
Survival
Apoptosis
Necroptosis
B
Isotype
αTNF-R
HSC
GMP
TNF-R1
TNF-R2
ΔMFI (×10²)
C
HSC (3h)
TNFα
IKKi
p65
DAPI
Merge
Nuclear p65 (AU)
D
HSC (3h)
WT
p50−/−
E
p65cKO HSC (72h)
p65cKO GMP (72h)
Fold expansion
TNFα
CASPi
RIPK1i
F
IKKi-treated GMP (72h)
Ctrl
Casp8cKO
Bid−/−
Bak−/−Baxcko
Ripk1KD
Ripk3−/−
Mlkl−/−
G
Ctrl GMP (72h)
p65cKO GMP (72h)
WT
Ripk3−/−
H
HSC
GMP
Ctrl
+IKKi
p65cKO
p65 activity
Survival
Necroptosis
Apoptosis
Other

Figure S3. Differential activation of NF-κB and programmed cell death pathways in HSCs and GMPs, related to Figure 3

(**A**) Schematic of signaling pathways regulated by TNFα; C8, caspase-8; tBID, truncated BID; C3, caspase-3. Ub, ubiquitin; P, phosphate.

(**B**) TNFα receptor-1 (TNF-R1) and -2 (TNF-R2) expression in BM HSCs and GMPs. Results are expressed as ΔMFI obtained by subtracting MFI values of isotype control from MFI values of TNF-R1 or -R2 antibody (αTNF-R, n = 4 mice per group from 2 independent experiments).

(**C**) p65 nuclear localization in BM HSCs after 3h culture ± TNFα and IKKi; scale bar, 5 μm. Results are expressed as arbitrary units (AU) corresponding to total fluorescence of p65 in the nucleus (n = 50-51 cells per group from 1 experiment).

(**D**) p65 localization in BM WT and $p50^{-/-}$ HSCs after 3h culture ± TNFα; scale bar, 5 μm.

(**E**) Expansion of BM $p65^{cKO}$ HSCs and GMPs after 72h culture ± TNFα, pan-caspase inhibitor (CASPi, 20 μM zVAD-fmk) and RIPK1 kinase inhibitor (RIPK1i, 10 μM GSK'963) (n = 8-9 pools of 300 cells/group from 3 independent experiments). Results are expressed as fold expansion compared to the number of plated cells/condition.

(**F**) Expansion of the indicated IKKi-treated BM GMPs after 72h culture ± TNFα (n = 9-63 pools of 300-500 cells/group from 21 independent experiments). Results are expressed as fold expansion compared to the number of plated cells/condition.

(**G**) Expansion of the indicated BM GMPs after 72h culture ± TNFα and CASPi (n = 6-9 pools of 300 cells/group from 3 independent experiments). Results are expressed as fold expansion compared to the number of plated cells/condition.

(**H**) Schematic illustrating relationship between p65/NF-κB activity and programmed cell death in HSCs and GMPs. HSCs survive TNFα challenge when they can fully engage p65-dependent pro-survival pathways. Partial inhibition of NF-κB leads to HSC susceptibility to necroptosis-mediated killing, whereas complete inhibition results in their death by both apoptosis and necroptosis. In contrast, GMPs only weakly activate p65-dependent pro-survival pathways upon TNFα exposure and primarily die from apoptosis, although they can also engage alternative form of cell death distinct from necroptosis when apoptosis is blocked. NF-κB blockade further increases GMP susceptibility to TNFα cytotoxicity.

Data are mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

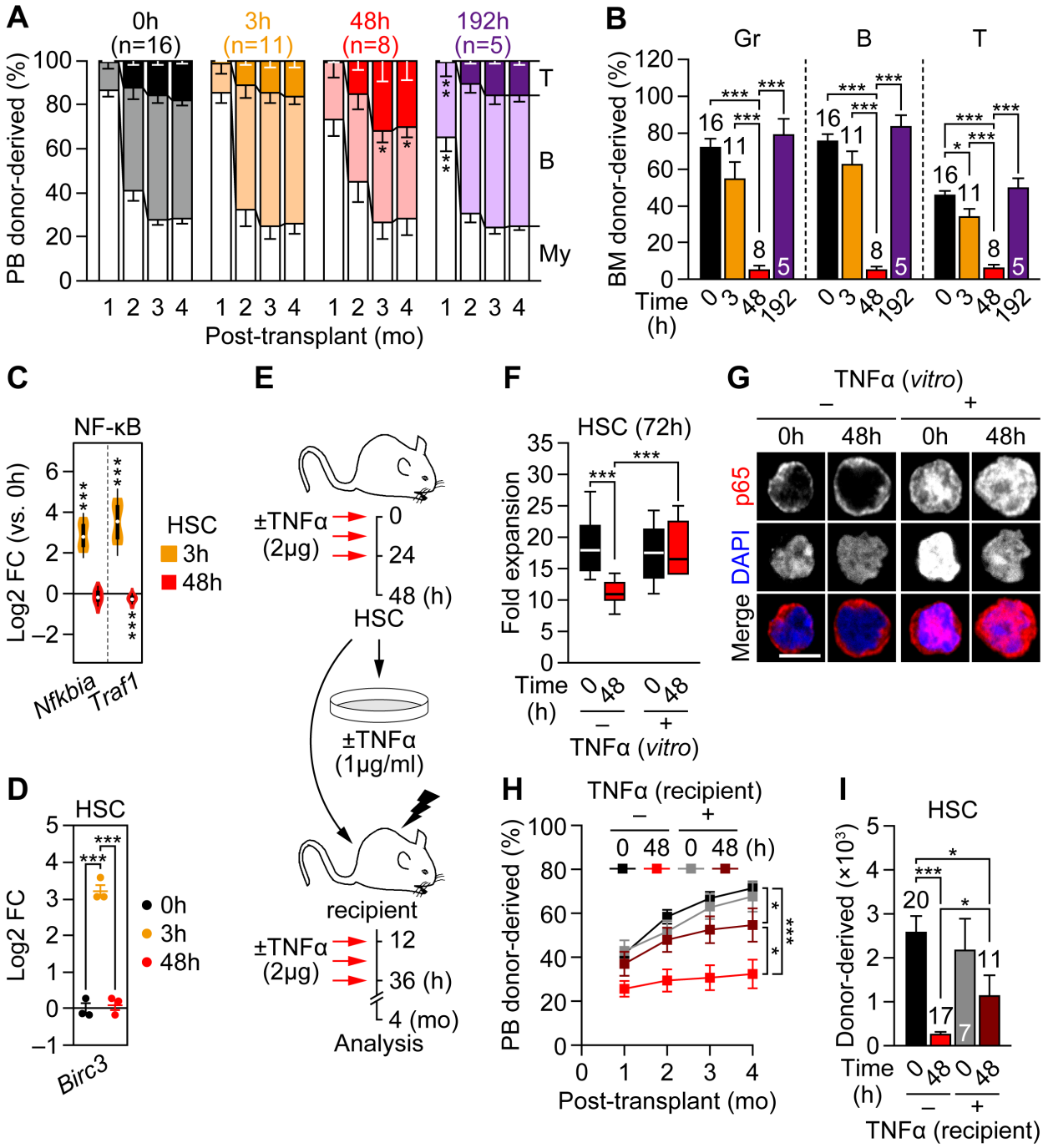
A
0h (n=16)
3h (n=11)
48h (n=8)
192h (n=5)
PB donor-derived (%)
T
B
My
Post-transplant (mo)
B
Gr
B
T
BM donor-derived (%)
Time (h)
C
NF-κB
Log2 FC (vs. 0h)
HSC
3h
48h
Nfkbia
Traf1
D
HSC
Log2 FC
0h
3h
48h
Birc3
E
±TNFα (2μg)
0
24
48 (h)
HSC
±TNFα (1μg/ml)
recipient
±TNFα (2μg)
12
36 (h)
4 (mo)
Analysis
F
HSC (72h)
Fold expansion
Time (h)
TNFα (vitro)
G
TNFα (vitro)
0h
48h
0h
48h
p65
DAPI
Merge
H
TNFα (recipient)
PB donor-derived (%)
Post-transplant (mo)
I
HSC
Donor-derived (×10³)
Time (h)
TNFα (recipient)

Figure S4. Engraftment potential of TNFα-treated HSCs, related to Figure 5

(**A-B**) Analyses of recipient mice transplanted with BM HSCs ± TNFα (n = 5-16 mice/group from 3 independent experiments; experimental scheme shown in Fig. 5A): donor-derived lineage distribution in (A) PB (* vs. WT) and (B) BM 4 months post-transplantation; My, myeloid.

(**C**) Fluidigm qRT-PCR analyses of NF-κB target gene expression in BM HSCs ± TNFα (n = 22-24 pools of 100 cells/group from 4 mice in 2 independent experiments). Results are expressed as log2 fold changes compared to 0h HSCs (* vs. 0h).

(**D**) Quantitative RT-PCR analyses of *Birc3* expression in BM HSCs ± TNFα (n = 3 biological replicates/group from 3 independent experiments). Results are expressed as log2 fold changes compared to 0h HSCs.

(**E-I**) *In vitro* and *in vivo* TNFα supplementation of 0h and 48h TNFα-treated BM HSCs: (E) experimental design; (F) expansion after 72h culture ± TNFα supplementation (n = 8-9 pools of 300 cells/group from 3 independent experiments; results are expressed as fold expansion compared to the number of plated cells/condition); (G) representative images of p65 localization after 3h culture ± TNFα supplementation (scale bar, 5 μm); and (H) donor-derived chimerism in PB and (I) absolute numbers of donor-derived BM HSCs at 4 months post-transplantation in recipient ± TNFα supplementation (n = 7-20 mice/group from 5 independent experiments).

Data are mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

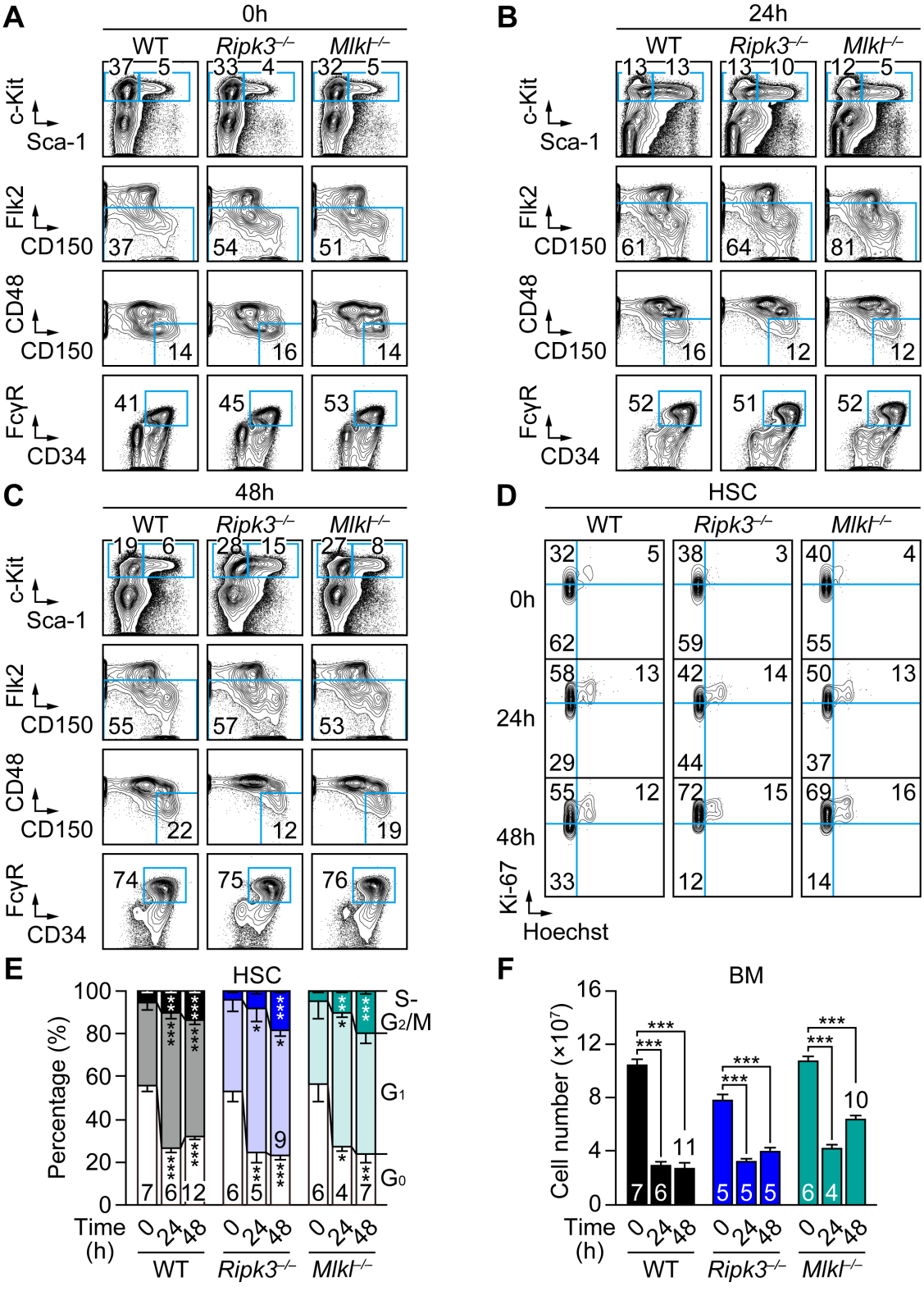
A
0h
WT
Ripk3−/−
Mlkl−/−
c-Kit
Sca-1
Flk2
CD150
CD48
CD150
FcγR
CD34
B
24h
C
48h
D
HSC
0h
24h
48h
Ki-67
Hoechst
E
HSC
Percentage (%)
S-G2/M
G1
G0
Time (h)
F
BM
Cell number (×10^7)

Figure S5. Analyses of necroptosis-deficient mice after TNFα injection, related to Figure 5
(**A-C**). Representative flow cytometry plots of BM HSCs and GMPs in WT, *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice at (A) 0h, (B) 24h and (C) 48h post-TNFα injection.
(**D**) Representative flow cytometry plots of BM HSCs cell cycle distribution in WT, *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice ± TNFα; G_0, Ki-67$^-$/Hoechst^{lo}; G_1, Ki-67$^+$/Hoechst^{lo}; S-G_2/M, Ki-67$^+$/Hoechst^{hi}.
(**E**) BM HSCs cell cycle distribution in WT, *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice ± TNFα (n = 4-12 mice/group from 6 independent experiments; * vs. 0h).
(**F**) BM cellularity in WT, *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice ± TNFα (n = 4-11 mice/group from 5 independent experiments).
Data are mean ± SEM, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

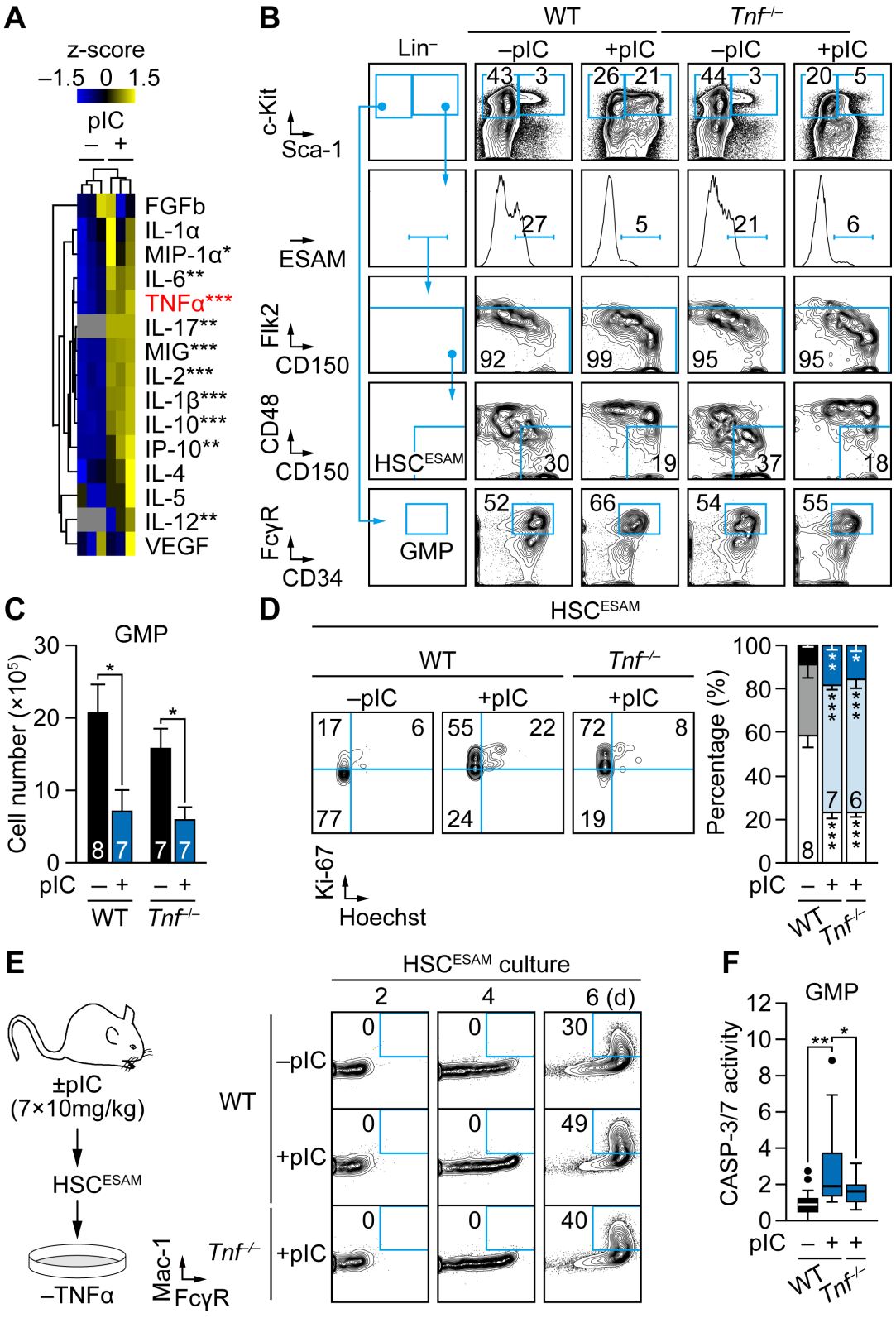
A
z-score
−1.5 0 1.5
pIC
− +
FGFb
IL-1α
MIP-1α*
IL-6**
TNFα***
IL-17**
MIG***
IL-2***
IL-1β***
IL-10***
IP-10**
IL-4
IL-5
IL-12**
VEGF
B
WT
Tnf−/−
Lin−
−pIC
+pIC
−pIC
+pIC
c-Kit
Sca-1
43 3
26 21
44 3
20 5
ESAM
27
5
21
6
Flk2
CD150
92
99
95
95
CD48
CD150
HSCESAM
30
19
37
18
FcγR
CD34
GMP
52
66
54
55
C
GMP
Cell number (×10⁵)
30
20
10
0
*
*
8 7 7 7
pIC − + − +
WT
Tnf−/−
D
HSCESAM
WT
Tnf−/−
−pIC
+pIC
+pIC
17 6
77
55 22
24
72 8
19
Ki-67
Hoechst
Percentage (%)
100
80
60
40
20
0
8
7 6
****
pIC − + + +
WT
Tnf−/−
E
±pIC
(7×10mg/kg)
HSCESAM
−TNFα
HSCESAM culture
2
4
6 (d)
WT
−pIC
+pIC
Tnf−/−
+pIC
0
0
30
0
0
49
0
0
40
Mac-1
FcγR
F
GMP
CASP-3/7 activity
12
10
8
6
4
2
0
**
*
pIC − + + +
WT
Tnf−/−

Figure S6. TNFα drive myelopoiesis from HSCs but induces apoptosis in GMPs during inflammation, related to Figure 6

(**A**) Heatmap of cytokine levels in BM fluid of WT mice ± pIC (n = 3 biological replicates from 3 mice in 1 experiment). Differentially expressed cytokines are highlighted with asterisks (* vs. – pIC) and TNFα is shown in red.

(**B**) Gating strategy used to identify HSC^{ESAM} and GMPs in WT and *Tnf*$^{-/-}$ mice ± pIC.

(**C**) Absolute number of BM GMPs in WT and *Tnf*$^{-/-}$ mice ± pIC (n = 7-8 mice/group from 5 independent experiments).

(**D**) Cell cycle distribution in BM HSC^{ESAM} from WT and *Tnf*$^{-/-}$ mice ± pIC (n = 6-8 mice/group from 4 independent experiments; * vs. WT –pIC).

(**E**) Experimental design and *in vitro* myeloid differentiation representative flow cytometry plots of BM HSC^{ESAM} from WT and *Tnf*$^{-/-}$ mice ± pIC.

(**F**) CASP-3/7 activity in BM GMPs from WT and *Tnf*$^{-/-}$ mice ± pIC (n = 12-14 pools of 200 cells/group from 3 independent experiments). Results are expressed as fold changes compared to PBS-treated WT GMPs (set to 1).

Data are mean ± SEM, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

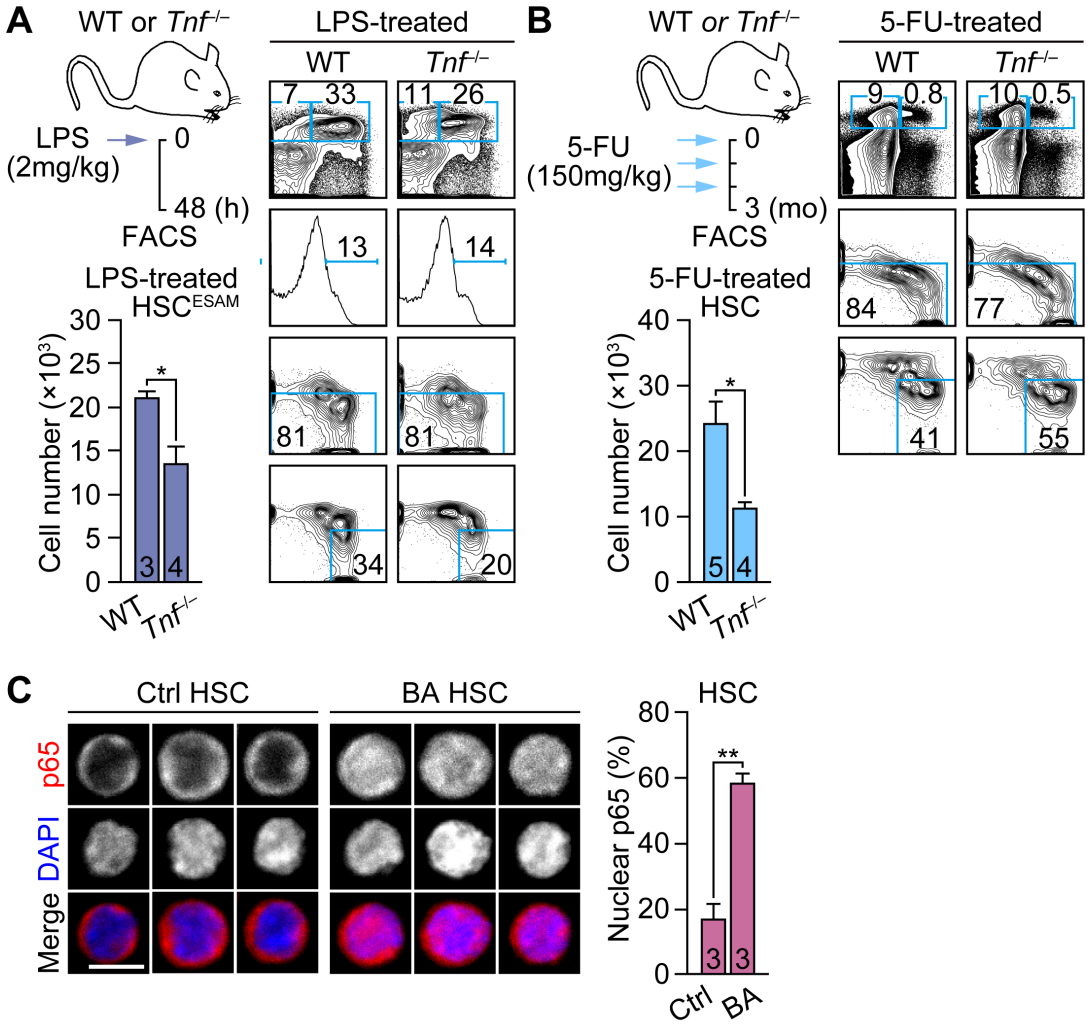

Figure S7. Protective role for TNFα in HSC maintenance during inflammation and leukemia development, related to Figures 6 and 7

(**A**) Experimental design and quantification of BM HSC^{ESAM} in LPS-treated WT and *Tnf*$^{-/-}$ mice (n = 3-4 mice/group from 1 experiment).

(**B**) Experimental design and quantification of BM HSCs in 5-FU-treated WT and *Tnf*$^{-/-}$ mice (n = 4-5 mice/group from 1 experiment; representative of 3 independent experiments).

(**C**) p65 localization in BM HSCs from Ctrl and *Scl-tTA:TRE-BCR/ABL* (BA) mice (n = 3 biological replicates/group from 3 independent experiments); scale bar, 5 μm.

Data are mean ± SEM, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Table S1. Fluidigm gene expression levels in TNFα-exposed HSCs, related to Figure 2.

	HSC (TNFα)	
Genes	**3h**	**48h**
Axin2	0.72 ± 0.09*	4.17 ± 0.49***
Bax	1.77 ± 0.12***	1.56 ± 0.11***
Bbc3	2.63 ± 0.37***	0.75 ± 0.13*
Bcl2	0.62 ± 0.04***	1.54 ± 0.15**
Bcl2l1	1.00 ± 0.11	2.04 ± 0.17***
Birc2	2.95 ± 0.19***	1.14 ± 0.05*
Bmi	1.06 ± 0.09	1.01 ± 0.06
Cbx7	1.07 ± 0.05	0.72 ± 0.04***
Ccl3	0.37 ± 0.03***	0.95 ± 0.06
Ccna2	1.04 ± 0.06	2.26 ± 0.18***
Ccnb1	0.45 ± 0.02***	1.09 ± 0.05
Ccnd1	0.49 ± 0.05***	0.99 ± 0.07
Ccne1	1.83 ± 0.25*	2.39 ± 0.23***
Cd34	0.99 ± 0.12	1.19 ± 0.08*
Cd48	0.90 ± 0.14	3.81 ± 0.41***
Cdc20	0.44 ± 0.02***	1.04 ± 0.05
Cdk2	1.43 ± 0.23	1.81 ± 0.19
Cdkn1a	1.15 ± 0.05*	0.97 ± 0.02
Cdkn1b	1.09 ± 0.08	1.31 ± 0.06***
Cdkn1c	0.56 ± 0.09***	0.40 ± 0.04***
Cebpa	0.63 ± 0.06***	0.99 ± 0.10
Csf1r	0.40 ± 0.03***	0.96 ± 0.06
Csf2ra	1.76 ± 0.16***	0.91 ± 0.06
Csf3r	1.61 ± 0.08***	1.18 ± 0.07*
Dnmt1	0.87 ± 0.05*	1.91 ± 0.11***
Dnmt3a	3.03 ± 0.35***	1.13 ± 0.05
Ebf1	1.11 ± 0.15	1.10 ± 0.12
Egr1	0.34 ± 0.02***	0.44 ± 0.05***
Epor	1.36 ± 0.21	4.89 ± 0.32***
Evi1	1.22 ± 0.14	0.71 ± 0.05***
Ezh1	0.73 ± 0.03***	0.83 ± 0.03***
Ezh2	1.83 ± 0.14***	2.40 ± 0.17***
Flt3	1.20 ± 0.09	0.93 ± 0.05
Fn1	73.49 ± 9.91***	4.84 ± 0.44***
Fos	0.63 ± 0.03***	0.82 ± 0.04***
Foxo3	0.56 ± 0.04***	0.88 ± 0.04**
Fzd2	0.34 ± 0.05***	0.84 ± 0.06*
Gata1	0.69 ± 0.07***	3.53 ± 0.25***
Gata2	1.23 ± 0.10*	1.48 ± 0.06***
Gfi1	0.26 ± 0.04***	0.60 ± 0.06***
Gfi1b	0.34 ± 0.03***	2.20 ± 0.11***
Gli1	9.16 ± 1.28***	0.91 ± 0.14

Hes1	0.68 ± 0.18*	2.11 ± 0.54
Hes5	0.39 ± 0.04***	0.81 ± 0.09*
Hey1	0.32 ± 0.07***	0.67 ± 0.06***
Hhip	0.19 ± 0.03***	1.32 ± 0.16
Hif1a	1.93 ± 0.18***	1.67 ± 0.10***
Hmga2	0.66 ± 0.09**	1.02 ± 0.07
Hoxa9	1.06 ± 0.12	0.71 ± 0.06***
Id1	0.27 ± 0.02***	0.87 ± 0.06
Ikzf1	2.60 ± 0.31***	1.30 ± 0.08**
Il1b	0.47 ± 0.04***	1.15 ± 0.09
Il6	0.37 ± 0.02***	0.94 ± 0.06
Il6ra	1.76 ± 0.15***	1.49 ± 0.15**
Il7r	0.34 ± 0.02***	0.84 ± 0.05*
Irf8	1.20 ± 0.15	1.34 ± 0.19
Jun	0.82 ± 0.06*	1.36 ± 0.09**
Lrp5	1.26 ± 0.12*	0.89 ± 0.03**
Mcl1	2.50 ± 0.34***	1.02 ± 0.10
Meis1	0.96 ± 0.07	1.24 ± 0.06**
Mfng	0.91 ± 0.06	0.84 ± 0.03***
Mki67	3.64 ± 0.47***	6.51 ± 0.48***
Mpl	0.99 ± 0.07	0.89 ± 0.03*
Myc	1.57 ± 0.15**	1.02 ± 0.05
Nfe2l2	1.27 ± 0.09*	1.11 ± 0.06
Nfkbia	7.82 ± 0.64***	0.92 ± 0.05
Notch1	0.47 ± 0.03***	0.90 ± 0.08
Pax5	0.90 ± 0.11	1.06 ± 0.13
Pdk4	0.27 ± 0.01***	0.89 ± 0.06
Pmaip1	476.89 ± 159.58**	16.03 ± 5.91*
Ppargc1a	0.14 ± 0.03***	0.49 ± 0.06***
Prkdc	1.02 ± 0.07	1.84 ± 0.11***
Ptch1	0.97 ± 0.06	1.02 ± 0.05
Rad51	2.09 ± 0.28**	3.46 ± 0.41***
Rpa1	1.65 ± 0.14***	1.83 ± 0.13***
Runx1	1.28 ± 0.13	1.48 ± 0.05***
Spi1	6.99 ± 0.63***	1.28 ± 0.05***
Slamf1	3.75 ± 0.55***	1.95 ± 0.11***
Smad7	1.35 ± 0.21	1.30 ± 0.11
Tcf3	1.48 ± 0.07***	1.11 ± 0.03*
Tnf	1.29 ± 0.07***	0.98 ± 0.05
Traf1	13.42 ± 1.49***	0.84 ± 0.03***
Vwf	0.54 ± 0.04***	1.58 ± 0.09***
Xiap	1.27 ± 0.09*	1.29 ± 0.08*
Xrcc5	1.54 ± 0.13***	1.35 ± 0.10**
Xrcc6	0.85 ± 0.03***	1.35 ± 0.06***
Zfpm1	0.58 ± 0.06***	1.92 ± 0.09***

Fluidigm gene expression analyses of BM HSCs ± TNFα (n = 9-24 pools of 100 cells/group from 4 mice in 2 independent experiments). Results are mean ± SEM and are expressed as fold changes compared to levels in 0h HSCs. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Table S3. HSC-specific TNFα signature genes, related to Figure 4

	HSC fold change			
Genes	**vitro 3h**	**vitro 12h**	**vivo 3h**	**+TNFα mean**
Cd274	3.23	6.98	4.55	8446
Pfkfb3	3.05	3.27	18.56	4743
Serpina3f	3.12	14.97	64.1	4369
Gbp3	3.06	4.15	10.8	3764
Cxcl9	4.1	267.14	119.9	3100
RP23-428M4.4	7.74	152.23	160.79	2919
Birc3	3.84	7.91	10.38	2874
Magee2	4.45	58.19	71.43	2456
Cd69	7.31	19.99	28.82	2370
Ccl22	6.96	92.58	8.34	2256
Ccl9	3.86	9.99	8.45	2222
Gbp5	4.65	9.94	15.03	1861
Loxl2	4.38	41.83	13.93	1522
Pdcd1lg2	3.67	19.91	22.16	1139
Tnfrsf9	3.52	29.48	105.63	1088
Nfkb2	3.85	6.91	7.66	824
RP23-307N14.2	10.93	53.19	48.8	801
3110043O21Rik	5.3	5.07	3.22	709
Fcer2a	4.29	42.01	23.03	655
Cd82	3.81	21.43	5.89	649
4930523C07Rik	3.82	4.87	4.26	604
Gm26809	3.84	13.92	28.66	565
Cd83	15.27	27.31	28.09	440
Zbtb46	4.99	10.23	4.91	439
Tnfaip8l1	4.02	4.45	9.03	312
Cxcl11	9.19	40.05	169.16	278
Abtb2	4.82	8.96	5.13	267
C77370	7.66	16.49	334.14	259
Ffar2	3.4	5.81	10.18	231
Nod2	20.74	77.47	5.05	171
Serpina3i	4.53	22.85	371.25	160
Dpysl5	4.9	12.34	17.75	160
Psd	3.65	5.88	3.67	147
Tmod2	4.68	10.6	4.5	133
Slc39a4	4.49	3.58	3.5	91
S1pr3	5.12	26.04	8.13	91
Rnd1	3.52	4.42	19.2	83
Ackr1	6.09	17.83	103.95	72
Madcam1	24.73	12.26	54.32	71
Spic	4.7	6.25	3.53	57
RP23-211F21.4	8.29	6.04	7.96	35
Gm15674	8.75	4.91	6.07	27

Ptger2	3.72	25.7	22.29	23
Stab1	8.71	3.8	5.97	10

Genes commonly upregulated across all three types of TNFα treatment in BM HSCs but not BM GMPs (44 genes, FDR < 0.1, fold change > 3, n = 3). Results are expressed as fold changes compared to levels in respective –TNFα control HSC groups. Genes are ordered by mean normalized read counts of all the +TNFα HSC groups.

Table S4. GMP-specific TNFα signature genes, related to Figure 4

	GMP fold change			
Genes	**vitro 3h**	**vitro 12h**	**vivo 3h**	**+TNFα mean**
Cybb	3.47	9.06	10.46	14996
Car2	7.49	13.32	5.82	3860
Il1f9	9.04	4.22	12.03	2980
Saa3	4.47	7.49	684.75	2527
Ccl3	3.38	6.93	4.13	2129
Tnip3	5.71	12.58	51.22	2101
Nrp2	3.53	8.25	11.65	1772
Ptx3	9.56	23.74	190.61	1387
Pde4b	4.55	3.38	4.5	1122
Sdc4	3.21	4.16	60.91	1090
Tnf	3.97	3.4	4.72	697
Ralgds	3.95	5.51	11.45	498
Cx3cr1	4.98	10.07	5.72	493
Cp	4.13	15.25	38.85	303
Inhba	3.67	3.76	144.97	284
Arl5c	4.45	4.27	10.52	264
Phlda1	3.46	3.86	6.02	245
Pilrb2	3.78	3.97	7.28	196
Bcl2a1d	3.99	4.08	7.46	182
Fpr2	6.14	24.08	284.37	178
Il6	3.5	7.18	4.21	166
Lta	10.48	10.58	13.36	163
Bcl2a1b	5.43	4.05	5.11	161
Pilrb1	4.15	3	16.21	147
Ccl4	4.45	9.31	10.72	134
Emr4	7.39	46.56	8.16	117
Bcl2a1a	6.75	4.42	11.98	116
Rasgrp1	4.03	3.52	14.71	113
Ankrd33b	3.8	4.99	17.06	81
Mmp14	3.2	30.15	39.24	79
Gem	4.22	3.79	4.5	62
Sh2d4a	9.04	13.28	22.12	51
Adora2a	7.41	4.67	3.02	17

Genes commonly upregulated across all three types of TNFα treatment in BM GMPs but not BM HSCs (33 genes, FDR < 0.1, fold change > 3, n = 3). Results are expressed as fold changes compared to levels in respective–TNFα control GMP groups. Genes are ordered by mean normalized read counts of all the +TNFα GMP groups.

Table S5. Common TNFα signature genes, related to Figure 4

	HSC fold change			GMP fold change			
Genes	**vitro 3h**	**vitro 12h**	**vivo 3h**	**vitro 3h**	**vitro 12h**	**vivo 3h**	**+TNFα mean**
Cxcl10	4.79	79.83	155.57	4.79	15.46	23.13	8520
Tnfaip3	5.76	9.42	10.55	6.99	6.28	3.89	2117
Gpr84	6.47	24.47	6.47	9.26	11.15	41.15	2064
Nfkbia	6.61	13.24	11.05	6.34	6.87	5.53	1733
Prdm1	4.29	21.2	4.97	3.26	3.44	4.16	1720
Traf1	4.63	19.71	10.21	7.7	14.64	24.34	1259
Irg1	21.27	36.95	30.67	3.13	51.69	187.46	1013
Icam1	4.92	3.93	7.13	6.29	4.46	14.61	984
Vcam1	14.25	82.52	5.55	4.11	9.27	4.75	954
Il1b	18.87	18.8	5.55	13.98	17.58	7.27	734
Nfkbie	11.5	9.48	7.33	6.88	4.86	3.34	560
Cxcl2	6.85	12.27	125.91	14.32	6.26	4.55	514
Atf3	4.34	35.67	11.12	3.81	8.28	4.1	473
Il1rn	4.2	12.63	32.17	3.16	10.41	6.04	323
Relb	7.5	16.28	18.07	4.01	7.27	45.89	263
Fas	3.12	9.04	4.18	5.02	4.94	15.07	180
Cxcl16	8.4	21.24	6.47	5.85	8.45	4.1	172
Cd40	6.46	69.66	40.37	5.67	6.6	41.82	76

Genes commonly upregulated across all three types of TNFα treatment in both BM GMPs and HSCs (18 genes, FDR < 0.1, fold change > 3, n = 3). Results are expressed as fold changes compared to levels in respective –TNFα control HSC and GMP groups. Genes are ordered by mean normalized read counts of all the +TNFα HSC and GMP groups.