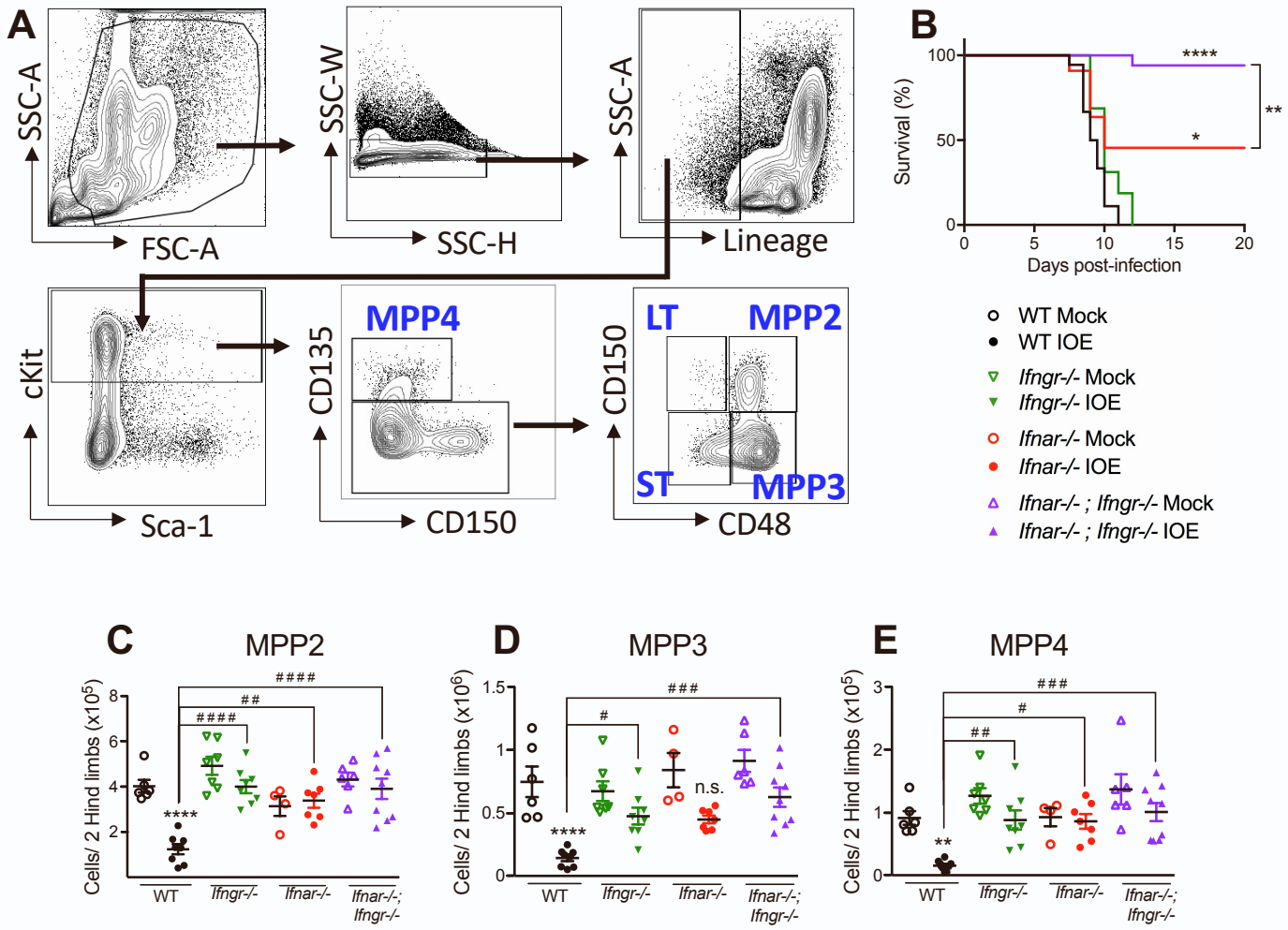
Stem Cell Reports, Volume *16*

Supplemental Information

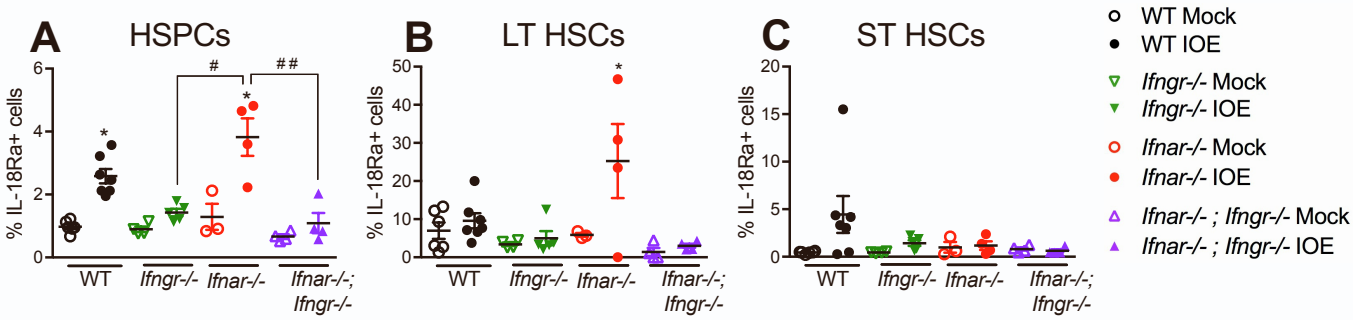
IL-18R-mediated HSC quiescence and MLKL-dependent cell death limit hematopoiesis during infection-induced shock

Jennifer E. Howard, Julianne N.P. Smith, Gabrielle Fredman, and Katherine C. MacNamara

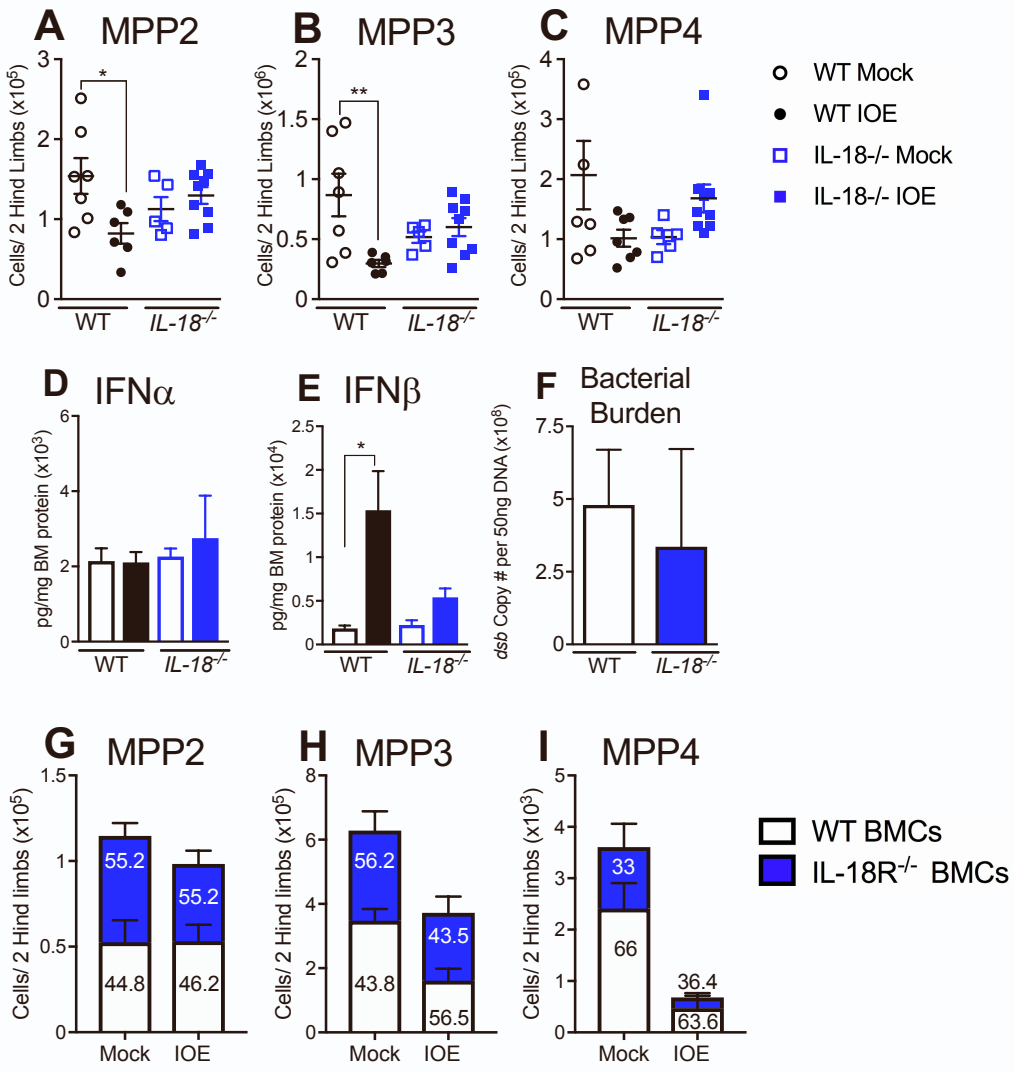


Suppl. Figure 1. (See figure 1) (A) Gating strategy used to identify HSPCs, LT HSCs, ST HSCs, and multipotent progenitor cells MPP2-4. (B) Mice infected with 10^5 copies of IOE were followed for 20 days. Data represent the survival curves of WT (n=18), *Ifnar*$^{-/-}$ (n=12), *Ifngr*$^{-/-}$ (n=16), and *Ifnar*$^{-/-}$*; Ifngr*$^{-/-}$ mice (n=17). (C-E) Absolute cellularity of MPP2 (CD135- CD150+ CD48+), MPP3 (CD135- CD150- CD48+), and MPP4s (CD135+ CD150-) in WT, *Ifngr*$^{-/-}$, *Ifnar*$^{-/-}$, and *Ifnar*$^{-/-}$*; Ifngr*$^{-/-}$ mice, n=4-9 mice.

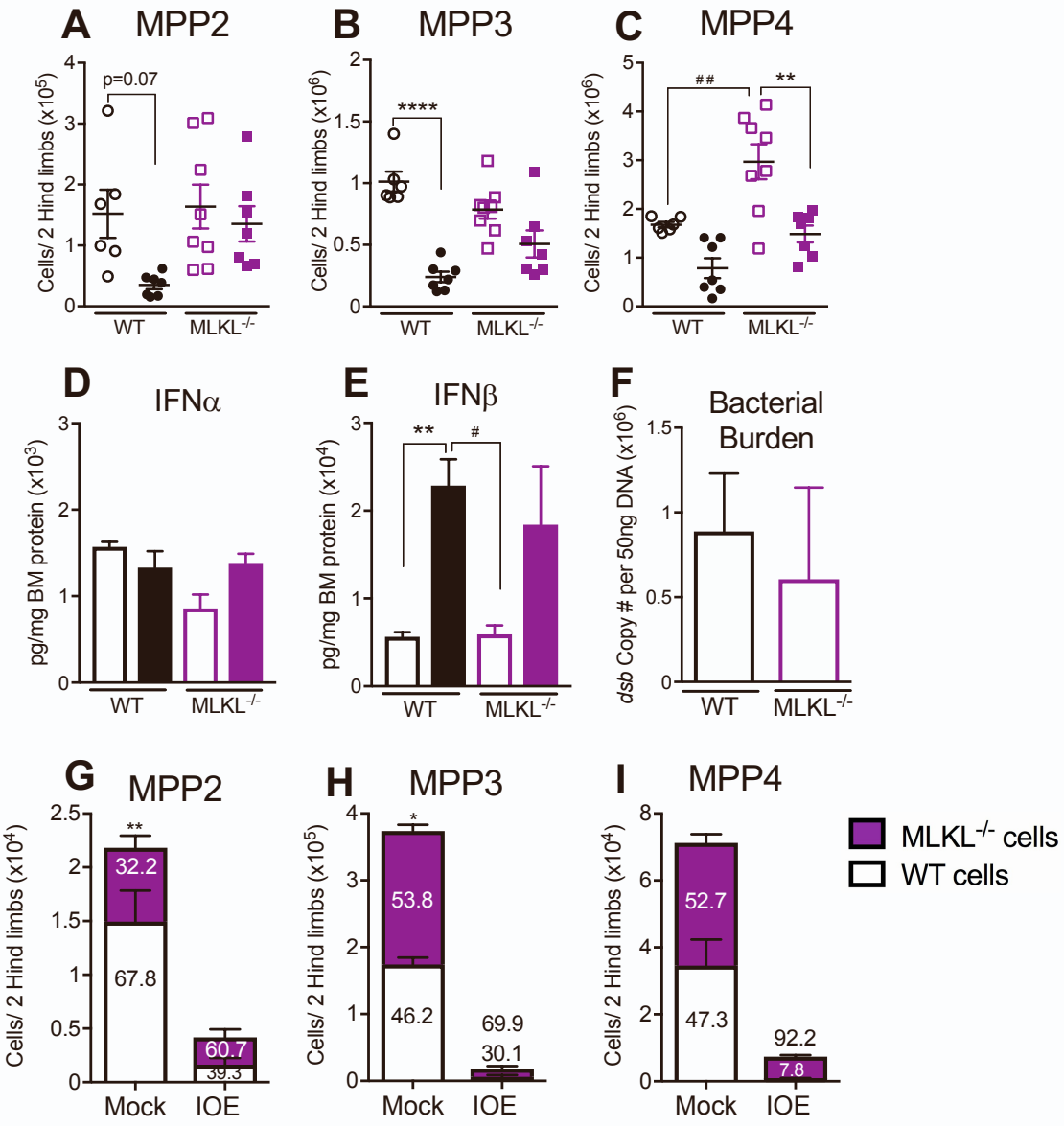
Suppl. Figure 2



Suppl. Figure 2. (See figure 2) WT, *Ifngr*$^{-/-}$, *Ifnar*$^{-/-}$, and *Ifnar*$^{-/-}$*; Ifngr*$^{-/-}$ mice were infected with 10^5 copies of IOE and BM was analyzed at 7dpi. (A) Expression of IL-18Rα (alpha chain) on HSPCs was determined by flow cytometric staining. The percent of IL-18Rα + cells is shown for (B) LT HSCs and (C) ST HSCs in mock- and IOE-infected mice day 7 post-infection; n=3-7 samples/group.



Suppl. Figure 3. (See figure 4 and 6) WT mice and IL-18-deficient mice were inoculated with 10^5 copies of IOE and BM was analyzed at 7dpi. (A) Absolute cellularity of MPP2, (B) MPP3, and (C) MPP4s. (D-E) BM protein concentrations of IFNa and IFNb. (F) Bacterial burden from homogenized spleen of IOE infected mice, measured by qPCR for the IOE gene *dsb*. **P<0.05, **P<0.001, ***P<0.0001.*



Suppl Figure 4. (See figure 7) WT and *Mlkl*$^{-/-}$ mice were inoculated with 10^5 copies of IOE and BM was analyzed at 7dpi. (A) Absolute cellularity of MPP2, (B) MPP3, and (C) MPP4s. (D-E) BM protein concentrations of IFNa and IFNb. (F) Bacterial burden from homogenized spleen of IOE infected mice, measured by qPCR for the IOE gene *dsb*. (G-I) The absolute cellularity of MPP2, MPP3, and MPP4s of either WT or *Mlkl*$^{-/-}$ donor origin in the BM of WT recipients, overlaid with percentages of each genotype, n=4-8 mice/group. **P<0.05, **P<0.001.*

Suppl. Table 1

Molecule	Flour	Clone	Vendor
7-AAD		Cat #: 420404	BioLegend
Annexin V	PB	Cat #: 640918	BioLegend
CD135	PE	AF210	BioLegend
CD135	PE Cy5	AF210	BioLegend
CD150	BV711	TC15-12F12.2	BioLegend
CD150	PE Cy7	TC15-12F12.2	BioLegend
CD45.1	PE	A20	BioLegend
CD45.1	PB	A20	BioLegend
CD45.2	BV711	104	BioLegend
CD48	FITC	HM48-1	BioLegend
CD48	APC	HM48-1	BioLegend
CD48	FITC	HM48-1	BioLegend
cKit	PerCP Cy5.5	2B8	BioLegend
cKit	PB	2B8	BioLegend
DAPI		CAS #: 28718-90-3	Krackler Scientific
IL-18Rα	PE	P3TUNYA	eBioscience
Ki67	FITC	16-A8	BioLegend
Ki67	PE	16-A8	BioLegend
Sca-1	PE Cy7	D7	BioLegend
Lineage:			
CD11b	FITC	M1/70	BioLegend
CD11b	Biotin	M1/70	BioLegend
Gr-1	FITC	RB6-8C5	BioLegend
Gr-1	Biotin	RB6-8C5	Invitrogen
Ter119	FITC	TER-119	BioLegend
Ter119	Biotin	TER-119	Invitrogen
B220	FITC	R3A-6B2	BioLegend
B220	Biotin	RA3-6B2	Invitrogen
CD3e	FITC	17A2	BioLegend
CD3e	Biotin	145-2C11	Invitrogen
Streptavidin	APC Cy7	405208	BioLegend