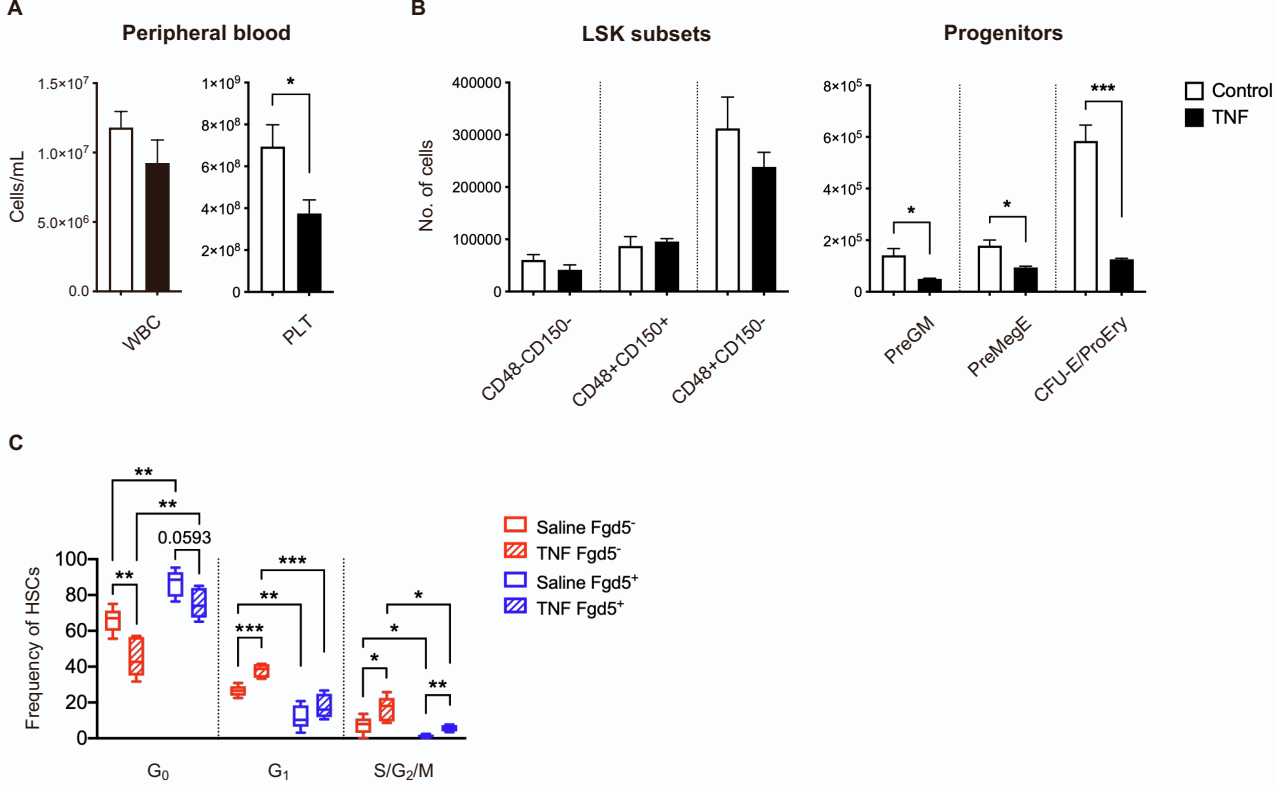


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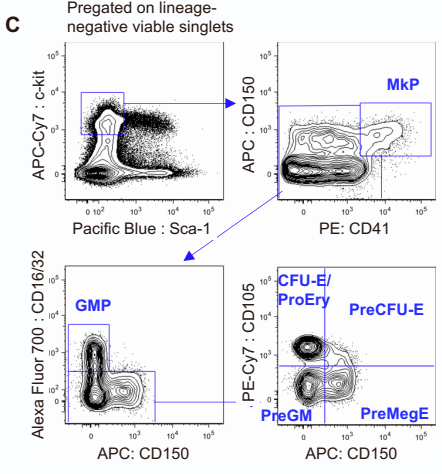
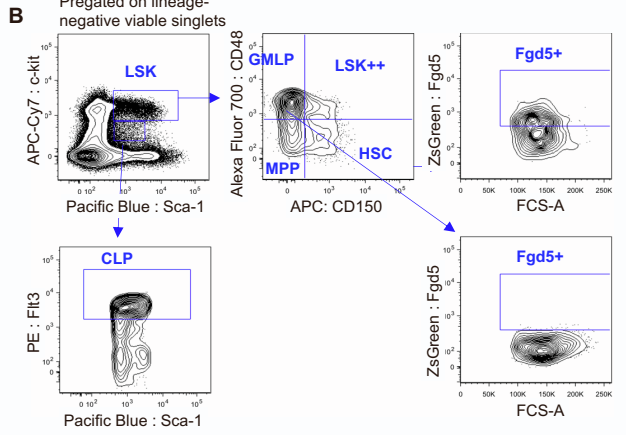
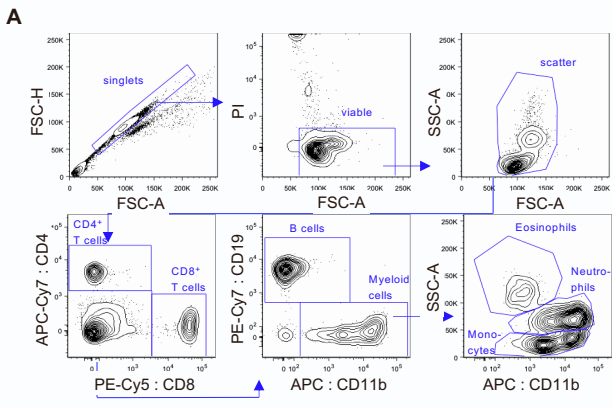
**Supplemental information**

**Temporal dynamics of TNF-mediated  
changes in hematopoietic stem cell  
function and recovery**

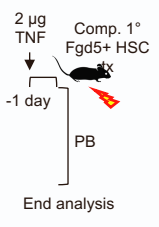
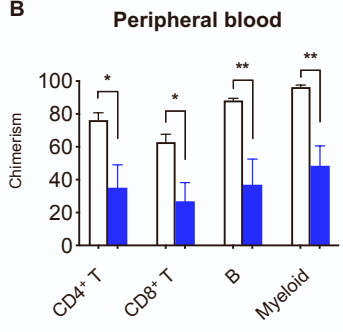
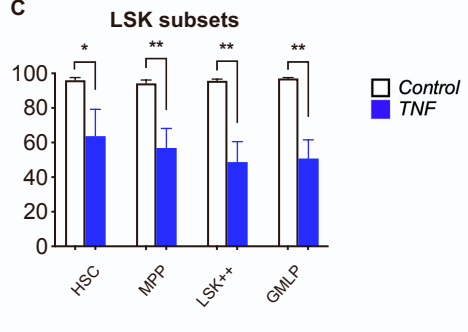
**Alexandra Rundberg Nilsson, Isabel Hidalgo, David Bryder, and Cornelis Jan Pronk**



**Supplementary Figure 1.**



**Supplementary Figure 2.**

**A****B****C**

**Supplementary Figure 3.**

## SUPPLEMENTAL INFORMATION

### Supplemental figure legends

#### **Supplementary Figure 1. [Acute TNF stimulation results in reduced PB platelet levels and BM myeloid-erythroid progenitors, and increased HSC proliferation.]**

**Related to Figure 1.** A-C depict results one day post TNF administration. **(A)** PB WBC and PLT cell concentrations. **(B)** BM cell numbers for various subsets within the LSK compartment (left) and of progenitor populations (right). TNF-treated mice n = 5, control-treated mice n = 4. Error bars represent +SEM. **(C)** Cell cycle distribution of Fgd5<sup>-</sup> and Fgd5<sup>+</sup> HSCs from saline- and TNF-treated mice. Saline n = 5, TNF n = 5. Whiskers represent min to max. Comparisons were done using unpaired two-tailed student's t-tests, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

#### **Supplementary Figure 2. [Flow cytometry gating strategy for PB and BM cellular subsets.]**

**Related to Figures 1-3.** Representative flow cytometry gating for **(A)** PB populations, **(B)** BM HSC and lymphoid progenitor populations, and **(C)** BM myeloid, erythroid, and megakaryocytic progenitor populations.

#### **Supplementary Figure 3. [Active TNF signaling reduces HSC repopulation capacity.]**

**Related to figure 2.** **(A)** Experimental strategy to assess the activity of Fgd5<sup>+</sup> HSCs receiving one dose of TNF one day prior to transplantation. Results are depicted in B-C. **(B)** PB and **(C)** BM HSPC chimerism at 20 weeks post transplantation. WT n = 6, TNF n = 5. Error bars represent +SEM. Comparisons were done using unpaired two-tailed student's t-tests, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

Supplementary Table 2. [Downregulated pathways in TNF-exposed HSCs identified through gene set enrichment analysis.] Related to Table 1.

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
Rho GTPase cycle	11/123	1.0541317779794091E-5	0.007790033839267833	5.677541208791209	65.0658031592915	PREX1;ARHGAP10;STARD8;MCF2;RAC2;ARHGAP18;ARHGEF18;PIK3R2;SRGAP3;RHOQ;RHOBTB2
Hemostasis pathway	22/468	2.7818417090565628E-5	0.010278905114964	2.8970392616667353	30.38939799759139	DGKG;GUCY1A3;NFE2;SELP;TRPC6;DOCK8;PDGFB;PIK3R2;ITPR3;IGF1;MAPK14;GNG2;CDK5;MMRN1;INPP5D;RAC2;GNB4;SIRPA;ESAM;PRKACB;ZFPM1;SH2B1
Lipid and lipoprotein metabolism	22/489	5.3575924703435054E-5	0.013197536118612836	2.763740185581727	27.179756210012027	ABCA1;CERS4;HSD3B7;CDK19;MTMR3;CHD9;GBA;INPL1;PRKAG2;PIK3R2;CROT;MED13L;SMPD2;GM2A;INPP5D;LPCAT2;ANGPTL4;HADH;PRKACB;ABCD1;PPARGC1B;ABCG1
PI3K class IB pathway in neutrophils	6/43	9.562137148561107E-5	0.017666048381966646	9.273028130170987	85.8229344338371	PREX1;CYTH4;INPP5D;RAC2;PRKACB;PDK1
GATA3-mediated activation of Th2 cytokine expression	4/20	3.539340622779106E-4	0.048218533026207135	14.228260869565217	113.06345113427675	NFATC2;NFATC1;MAPK14;PRKACB
G alpha (12/13) signaling events	7/77	3.914901192926154E-4	0.048218533026207135	5.725438596491228	44.919216389146435	PREX1;GNG2;MCF2;RAC2;GNB4;ARHGEF18;PIK3R2

**Supplementary Table 4. [Upregulated pathways in TNF-exposed HSCs identified through gene set enrichment analysis.] Related to Table 1.**

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
<b>Myc Targets V1</b>	35/200	1.0604112613714109E-19	5.196015180719913E-18	8.743102668475803	381.99017698961836	EIF4A1;SF3B3;ODC1;NOLC1;DDX21;GSPT1;UBE2L3;SRM;SYNCRIP;G3BP1;DHX15;USP1;EIF4H;PSMD1;SNRPB2;CCT3;CCT2;SSB;NCBP1;IFRD1;PA2G4;RAD23B;RSL1D1;PSMA4;PSMC6;TCP1;CANX;HNRNPD;MCM5;ETF1;TARDBP;ABCE1;EIF3D;KPNB1;EIF3B
<b>Interferon Gamma Response</b>	30/200	3.651428090324361E-15	5.963999214196456E-14	7.195085629188384	239.19096626997057	CD274;MVP;TNFAIP2;NLRC5;ADAR;IFIT1;ICAM1;IL18BP;SOCS3;CCL5;NAMPT;CASP4;TNFSF10;ST8SIA4;PIM1;PDE4B;STAT2;STAT3;PARP14;SOD2;NFKB1;SELP;NFKBIA;PLSCR1;ZNF1;SERPING1;PSME2;LCP2;CD69;LY6E
<b>mTORC1 Signaling</b>	30/200	3.651428090324361E-15	5.963999214196456E-14	7.195085629188384	239.19096626997057	PSMD12;INSIG1;RPN1;USO1;ATP2A2;VLDLR;HMGCR;STIP1;SERP1;NAMPT;SSR1;ACTR3;CYB5B;XBP1;HSPA5;HMGCS1;HSPA4;EDEM1;IFRD1;M6PR;IMMT;CCT6A;SQLE;SLC7A5;PSMA4;PSMC6;PSME3;CANX;ETF1;CALR
<b>TNF-alpha Signaling via NF-kB</b>	24/200	2.635517446603224E-10	3.2285088720889497E-9	5.488636363636363	121.06159753095606	PTGER4;PFKFB3;TNFAIP2;RNF19B;TRAF1;SOD2;TANK;RELA;GEM;NFKB1;ICAM1;RHOB;NFKB2;EHD1;NFKBIA;SOCS3;CCL5;NAMPT;BCL3;PDE4B;CD69;NFKBIE;PDLIM5;BIRC3
<b>Unfolded Protein Response</b>	18/113	4.596353223270804E-10	4.5044261588053884E-9	7.563742690058479	162.62491318708126	EIF4A1;XBP1;HSPA5;EDEM1;EIF4A3;NOLC1;IFIT1;SDAD1;HERPUD1;SLC7A5;SPCS3;SERP1;DKC1;SSR1;HYOU1;CALR;ATF6;EIF4G1
<b>Inflammatory Response</b>	21/200	3.781082707334731E-8	3.08788421099003E-7	4.692008744231236	80.18957470268029	PTGER4;IL1R1;ITGB3;ATP2A2;AHR;SLC7A1;RELA;NFKB1;ICAM1;NFKBIA;IL18RAP;CCL5;NAMPT;TNFSF10;PDE4B;FFAR2;LCP2;CD69;SLC28A2;CD55;LY6E
<b>G2-M Checkpoint</b>	19/200	7.976554252623375E-7	5.583587976836363E-6	4.180497807142451	58.70083256954046	SLC12A2;HSPA8;TGFB1;CUL5;ODC1;NOLC1;SLC7A1;RAD23B;GSPT1;SQLE;SYNCRIP;SLC7A5;UCK2;DKC1;G3BP1;BCL3;HNRNPD;MCM5;KPNB1
<b>IL-2/STAT5 Signaling</b>	18/199	3.1353563363179708E-6	1.920405755994757E-5	3.9523224882340906	50.08686487338141	CYFIP1;XBP1;GPR65;ODC1;AHR;TRAF1;RHOB;SOCS2;SELP;UCK2;PLSCR1;MUC1;MAPKAPK2;TNFSF10;PIM1;ENPP1;LTB;TGM2
<b>IL-6/JAK/STAT3 Signaling</b>	11/87	1.1614789764841096E-5	6.32360776085793E-5	5.70139852674282	64.78631014850518	SOCS3;TGFB1;IL1R1;ITGB3;STAT2;STAT3;PIM1;LTB;IL2RG;IL13RA1;PF4
<b>Myc Targets V2</b>	9/58	1.3557415513554027E-5	6.643133601641474E-5	7.21595547309833	80.88059175477478	SUPV3L1;MYBBP1A;NDUFAF4;NOLC1;MPHOSPH10;MCM5;PA2G4;WDR43;SRM
<b>Complement</b>	16/200	5.0030344592495686E-5	2.0429057375269071E-4	3.44119743406985	34.07776815292405	USP14;DOCK4;HSPA5;LRP1;PREP;FN1;EHD1;PLSCR1;COL4A2;CCL5;CASP4;PIM1;SERPING1;ADAM9;LCP2;CD55
<b>Allograft Rejection</b>	16/200	5.0030344592495686E-5	2.0429057375269071E-4	3.44119743406985	34.07776815292405	SPI1;TGFB1;GPR65;IL2RG;IL27RA;ICAM1;FGR;IL18RAP;CCL5;BCL3;ST8SIA4;LCP2;LTB;ABCE1;EIF3D;PF4
<b>Apoptosis</b>	14/161	5.95897689755456E-5	2.2460759075397957E-4	3.7607385811467444	36.58456517910965	APP;PSEN2;SOD2;RELA;RHOB;DNAJA1;CASP4;TNFSF10;PMAP1;TIMP3;CTNBN1;ETF1;CD69;BIRC3

<b>Androgen Response</b>	10/100	2.1517321329566838E-4	7.531062465348393E-4	4.364822312190733	36.85685296802441	RPS6KA3;SPCS3;STEAP4;HMGCS1;INSIG1;UAP1;PA2G4;HMGCR;PDLIM5;FKBP5
<b>Epithelial Mesenchymal Transition</b>	14/200	5.729734291110886E-4	0.0018717132017628895	2.9662058371735793	22.14175132712898	ITGB1;TGFB1;TPM4;LRP1;ITGB3;FN1;TNFRSF11B;GEM;RHOB;GLIPR1;COL4A2;COL4A1;TIMP3;TGM2
<b>UV Response Up</b>	12/158	6.81570548091752E-4	0.002046316548097335	3.2325425993985966	23.568826236413006	SULT1A1;NFKBIA;CYB5B;DNAJA1;STIP1;NXF1;RPN1;DDX21;SOD2;HSPA13;ICAM1;RHOB
<b>Protein Secretion</b>	9/96	7.099465575031571E-4	0.002046316548097335	4.056217345872518	29.40887724311624	RPS6KA3;COPB2;TMED10;SNAP23;USO1;M6PR;ANP32E;TMED2;ATP1A1
<b>Coagulation</b>	11/138	7.56346569193817E-4	0.0020589434383609466	3.402900448802926	24.45668248774843	ARF4;LRP1;GDA;ITGB3;PREP;FN1;ADAM9;SERPING1;TIMP3;ISCU;PF4
<b>Cholesterol Homeostasis</b>	7/74	0.002597997750445961	0.0067000994616764255	4.084296825730503	24.31387710928505	FDPS;SQLE;PLSCR1;HMGCS1;CTNNB1;HMGCR;HSD17B7
<b>Apical Junction</b>	11/200	0.012826455296009972	0.03142481547522443	2.279285660624403	9.92912772222282	ITGB1;CD274;INSIG1;NF1;ADAM9;MSN;TNFRSF11B;MADCAM1;PIK3CB;TRAF1;ICAM1
<b>Fatty Acid Metabolism</b>	9/158	0.018902871112150863	0.04410669926168535	2.360829774252593	9.368814750853424	HSP90AA1;HMGCS1;ODC1;DLST;SERINC1;HSD17B7;GLUL;PRDX6;DLD



## **SUPPLEMENTAL INFORMATION**

### **Supplemental table legends**

**Supplementary Table 2. [Downregulated pathways in TNF-exposed HSCs identified through gene set enrichment analysis.] Related to Table 1. BioPlanet gene sets, adj. p-value < 0.05.**

**Supplementary Table 4. [Upregulated pathways in TNF-exposed HSCs identified through gene set enrichment analysis.] Related to Table 1. MSigDB\_Hallmark gene sets, adj. p-value < 0.05.**