

Supplemental information

Neutrophil “plucking” on megakaryocytes

drives platelet production and boosts

cardiovascular disease

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Supplemental Material

Supplemental Tables

Table S1

Gene Symbol(s)	Associated Platelet Index	rsID (where available)	Chr (GRCh37)	BP (GRCh37)	REF (GRC37)	ALT (GRC37)	Minor Allele	Minor Allele Frequency	Estimate of Additive Allelic Effect (REF=Baseline, ALT=Effect)	Standard Error of Estimator	P-value	Genome-wide significant (P < 5e-8)
CXCR4	PLT	rs11679328	2	136884461	C	T	T	0.063912	0.0255603	0.00431502	3.20E-09	yes
CXCR4	MPV	rs527361487	2	136870091	T	C	C	0.000534	0.171358	0.0551363	0.0019	no
CXCR4	PDW	rs58400844	2	136873341	T	G	G	0.000192	-0.379426	0.0985573	0.00012	no
CXCR4	PCT	rs11679328	2	136884461	C	T	T	0.0639	0.0288911	0.00434531	3E-11	yes
CYBA , ZC3H18 , IL17C	PLT	rs8050943	16	88664312	C	T	C	0.0958932	-0.0307737	0.00359595	1.10E-17	yes
CYBA , ZC3H18 , IL17C	MPV	rs17190682	16	88728280	C	A	A	0.1615	0.0136534	0.00281058	1.20E-08	no
CYBA , ZC3H18 , IL17C	PDW	rs184838760	16	88700790	G	C	C	0.002857	-0.121779	0.0233407	1.80E-07	no
CYBA , ZC3H18 , IL17C	PCT	rs8050943	16	88664312	C	T	C	0.0958938	-0.0347098	0.00361942	8.8E-22	yes
CYBB/NOX2	PLT	rs537265994	X	37678143	A	G	G	6.60E-05	0.572828	0.170216	0.00076	no
CYBB/NOX2	MPV	rs141420609	X	37676772	A	G	G	0.00431	0.0529468	0.0148427	0.00036	no
CYBB/NOX2	PDW	rs782790403	X	37597482	C	T	T	0.000552	-0.175953	0.0511628	0.00058	no
CYBB/NOX2	PCT	rs373038990	X	37628597	C	G	G	0.000164	-0.228592	0.0835409	0.00062	no
FYN	PLT	rs1321169	6	112082342	T	A	A	0.361312	0.00912616	0.00218799	0.00003	no
FYN	MPV	rs9487736	6	112168309	G	A	A	0.1353	0.0146566	0.00300959	0.000011	no
FYN	PDW	rs72938270	6	111951212	A	G	G	0.142959	-0.0154492	0.00311147	0.00000069	no
FYN	PCT	rs7772036	6	112060799	G	A	A	0.424867	0.0109885	0.00213889	0.00000029	no
ITGAL/LFA1	PLT	rs34114657	16	30584430	T	C	C	0.224234	-0.0155328	0.00256237	1.30E-09	yes
ITGAL/LFA1	MPV	rs117391625	16	30504832	C	T	T	0.08971	-0.0223826	0.00365946	9.60E-10	yes
ITGAL/LFA1	PDW	rs36063822	16	30571910	C	T	T	0.21725	0.016161	0.00264723	1.00E-09	yes
ITGAL/LFA1	PCT	rs145456050	16	30551991	G	A	A	0.255824	0.0138015	0.00244131	1.60E-08	no
ITGB2	PLT	rs235317	21	46275047	A	C	C	0.289817	-0.00974131	0.00233574	3.00E-05	no
ITGB2	MPV	rs58244480	21	46279172	A	C	C	0.005639	0.0880889	0.0158115	1.70E-05	no
ITGB2	PDW	rs150428568	21	46321915	G	A	A	0.002123	0.0899021	0.0254768	0.00042	no
ITGB2	PCT	rs760453	21	46340512	G	A	A	0.453753	-0.00817902	0.00215168	0.00014	no
JAK2	PLT	rs7865719	9	5082333	A	G	G	0.488499	-0.0411012	0.0021031	4.70E-85	yes
JAK2	MPV	rs36051895	9	4981866	T	T	T	0.287963	0.013654	0.00227246	1.90E-09	yes
JAK2	PDW	rs138462199	9	5131703	G	C	C	0.004486	-0.0678819	0.017479	1.00E-04	no
JAK2	PCT	rs7865719	9	5082333	A	G	G	0.488461	-0.0423782	0.0021524	2.70E-89	yes
LYN	PLT	rs12676105	8	56795439	C	T	C	0.49527	-0.0183217	0.00211325	4.30E-18	yes
LYN	MPV	rs6985353	8	56750016	G	A	A	0.493843	0.0268047	0.00205647	7.80E-39	yes
LYN	PDW	rs9990008	8	56768236	G	A	A	0.442775	0.0151557	0.0021735	3.10E-12	yes
LYN	PCT	rs7828258	8	56867945	C	T	C	0.497286	-0.0142013	0.00213685	3.00E-11	yes
MPO , B2RAP1	PLT	rs2526378	17	56404349	A	G	G	0.448025	0.0177287	0.00211932	6E-17	yes
MPO , B2RAP1	MPV	rs569218599	17	56370008	G	C	C	0.000533	0.208811	0.0683914	0.0017	no
MPO , B2RAP1	PDW	rs142495996	17	56386194	C	T	T	0.005622	0.0777308	0.0171257	0.0000057	no
MPO , B2RAP1	PCT	rs2526378	17	56404349	A	G	G	0.44802	0.0221213	0.00213497	3.7E-25	yes
NCF1	PLT	rs373258753	7	74250185	C	T	T	0.007173	-0.0430935	0.014212	0.0024	no
NCF1	MPV	rs2953661	7	74245012	A	C	A	0.304845	0.0123401	0.00242336	3.50E-07	no
NCF1	PDW	rs801011	7	74236723	C	T	C	0.295267	-0.0103465	0.00242851	0.00002	no
NCF1	PCT	rs587735842	7	74248493	T	C	C	0.042119	-0.0192024	0.00543292	0.00041	no
NCF2	PLT	rs183508282	7	183508282	G	A	A	0.000223	-0.404832	0.103305	8.80E-05	no
NCF2	MPV	rs545993414	1	183520480	G	C	C	0.000155	-0.435677	0.125678	0.00052	no
NCF2	PDW	rs12122217	1	183545665	C	T	T	0.328095	-0.0075548	0.00227318	0.00089	no
NCF2	PCT	rs798680	1	183524855	C	A	C	0.128987	0.0154025	0.00324918	2.10E-06	no
NCF4	PLT	rs773415145	22	37259449	A	G	G	0.000566	-0.223368	0.0577185	0.00011	no
NCF4	MPV	rs539979592	22	37257053	G	A	A	5.3E-05	0.702725	0.188881	0.0002	no
NCF4	PDW	rs6000440	22	37242512	T	G	G	0.000355	-0.35866	0.078016	0.0000053	no
NCF4	PCT	rs185926103	22	37299399	A	T	T	0.001056	-0.158006	0.0387346	0.000045	no
NOX1	PLT	rs5921667	X	100102109	C	T	C	0.363418	0.00722827	0.00182935	0.000078	no
NOX1	MPV	rs2213514	X	100093907	T	C	T	0.352507	-0.0118012	0.00178937	4.2E-11	yes
NOX1	PDW	rs5921666	X	100101992	T	C	T	0.36292	-0.013932	0.00188476	7.9E-14	yes
NOX1	PCT	rs148027650	X	100158639	G	A	A	5.4E-05	0.63051	0.186876	0.00074	no
PTPN11/SHP2	PLT	rs11066283	12	11284076	A	G	G	0.4069	0.0854866	0.00214961	0	yes
PTPN11/SHP2	MPV	rs7978851	12	112928451	G	A	A	0.177193	-0.0184797	0.00263361	2.30E-12	yes
PTPN11/SHP2	PDW	rs7978851	12	112928451	G	A	A	0.177187	-0.0308388	0.00281689	6.80E-28	yes
PTPN11/SHP2	PCT	rs10850034	12	112817521	T	A	A	0.360339	0.0978521	0.00235319	0	yes
RAC1 , DAGLB	PLT	rs836468	7	6436722	T	A	A	0.310244	-0.00971227	0.00227998	2.00E-05	no
RAC1 , DAGLB	MPV	rs141698020	7	6490693	G	A	A	0.042899	-0.0340956	0.00514549	3.4E-11	yes
RAC1 , DAGLB	PDW	rs190913482	7	6422627	A	C	C	0.006536	-0.0544125	0.0140886	0.00011	no
RAC1 , DAGLB	PCT	rs13235365	7	6456091	C	T	T	0.275278	-0.0101228	0.00238628	2.20E-05	no
RAC2	PLT	rs75375644	22	37638621	C	A	A	0.031455	0.033405	0.00614946	5.60E-08	no
RAC2	MPV	rs75375644	22	37638621	C	A	A	0.031445	-0.0452821	0.00588884	4.00E-14	yes
RAC2	PDW	rs75375644	22	37638621	C	A	A	0.031453	-0.0591788	0.00626771	3.70E-21	yes
RAC2	PCT	rs7556560	22	37611220	C	T	T	0.438903	-0.0077412	0.00215164	0.00032	no
SDF-1/CXCL12	PLT	rs17156360	10	44922040	C	T	T	0.132215	0.0126046	0.00310811	5.00E-05	no
SDF-1/CXCL12	MPV	rs529382271	10	44880064	G	A	A	0.003044	-0.123685	0.027674	8.40E-06	no
SDF-1/CXCL12	PDW	rs197452	10	44870240	C	T	T	0.149415	0.0108648	0.00304233	0.00046	no
SDF-1/CXCL12	PCT	rs35213189	10	44914195	C	A	A	0.439976	-0.0119011	0.00214195	2.80E-08	no
SIRPA	PLT	rs8045612	20	1931001	C	T	T	0.264357	-0.0428032	0.00240523	7.60E-71	yes
SIRPA	MPV	rs4814776	20	1921523	C	A	A	0.330883	-0.0713871	0.00218804	1.80E-233	yes
SIRPA	PDW	rs4814776	20	1921523	C	A	A	0.330885	-0.0183454	0.00228954	1.10E-15	yes
SIRPA	PCT	rs11906768	20	1924066	T	C	C	0.26855	-0.0859245	0.00239674	1.80E-281	yes
SOD1/CuZnSOD	PLT	rs150651634	21	33042819	G	A	A	0.005902	0.0415007	0.0146263	0.0045	no
SOD1/CuZnSOD	MPV	rs147709416	21	33040475	G	T	T	0.000135	-0.360693	0.116455	0.0022	no
SOD1/CuZnSOD	PDW	rs540906387	21	33026655	C	G	G	0.000131	0.354686	0.116493	0.0003	no
SOD1/CuZnSOD	PCT	rs113757809	21	33015188	G	A	A	0.044312	0.0178834	0.00520851	0.00059	no
SOD2/MnSOD	PLT	rs755905938	6	160152057	C	A	A	0.000577	0.269381	0.0871724	6.10E-05	no
SOD2/MnSOD	MPV	rs539852371	6	160133211	C	T	T	0.087744	-0.0168516	0.00411719	4.30E-05	no
SOD2/MnSOD	PDW	rs535331958	6	160084079	G	A	A	0.000755	-0.239052	0.0569058	2.70E-05	no
SOD2/MnSOD	PCT	rs755905938	6	160152057	C	A	A	0.000576	0.281164	0.0875634	3.20E-05	no

Table S1: GWAS analysis of platelet traits (related to Figure 4)

Associations of blood traits (PLT=platelet counts; PDW=platelet distribution width; MPV=mean platelet volume; PCT=plateletcrit) with 20 selected candidate genes in UK Biobank. The gene symbol is either a gene name with a possible alternative name (separator “/”) or a small gene cluster (separator “,”). rsID

is always the rs-number of the SNP with the smallest p-value in the respective candidate gene region. Information about chromosomes, positions and reference as well as alternative alleles is with respect to Genome Reference Consortium Human Build 37 (GRCh37). GWAS summary statistics are calculated with UK biobank data (Vuckovic et al, Cell, Volume 182, Issue 5, 2020) and are downloaded from ftp://ftp.sanger.ac.uk/pub/project/humgen/summary_statistics/UKBB_blood_cell_traits. Genome wide significantly regulated traits are highlighted in bold.

Table S2

Clinical characteristics of patients for reticulated platelets analysis

	Control patient	STEMI patient	
Patients count	10	10	
Age	68.7 ± 11.56	67.2 ± 12.05	
Male, n [%]	8 [80]	6 [60]	
Family anamnesis, n [%]	3 [30]	1 [10]	
Diabetes, n [%]	4 [40]	1 [10]	
Hypertension, n [%]	9 [90]	3 [30]	
CAD, n [%]	10 [100]	9 [90]	
Smoke, n [%]	6 [60]	4 [40]	
		day1	day5
Hemoglobin, g/dl	13.71 ± 1.62	12.98 ± 1.36	11.78 ± 1.64
Hematocrit, %	38.13 ± 12.46	39.41 ± 4.62	36.06 ± 4.06
Leukocyte, 10 ⁹ /L	7.95 ± 2.23	13.17 ± 6.51	9.99 ± 2.81
Erythrocyte, 10 ¹² /L	4.53 ± 0.45	4.22 ± 0.50	3.81 ± 0.54

Clinical characteristics of patients for CXCR4 analysis

	Control patient	STEMI patient	
Patients count	5	7	
Age	75 ± 12.75	59 ± 11.15	
Male, n [%]	1 [20]	4 [57.1]	
Family anamnesis, n [%]	0 [0]	1 [14.29]	
Diabetes, n [%]	1 [20]	2 [28.57]	
Hypertension, n [%]	5 [100]	4 [57.1]	
CAD, n [%]	4 [80]	7 [100]	
Smoke, n [%]	2 [40]	5 [83.3] *	
		day1	day5
Hemoglobin, g/dl	13.52 ± 3.36	12.24 ± 2.22	11.18 ± 2.96
Hematocrit, %	40.42 ± 9.81	37.24 ± 6.50	34.3 ± 8.14
Leukocyte, 10 ⁹ /L	7.8 ± 1.47	13.21 ± 4.66	8.94 ± 2.05
Erythrocyte, 10 ¹² /L	4.31 ± 1.14	4.21 ± 0.62	3.81 ± 0.87

Table S2: Clinical characterization of STEMI patients (related to Figure 5)

Supplemental Figures

Figure S1

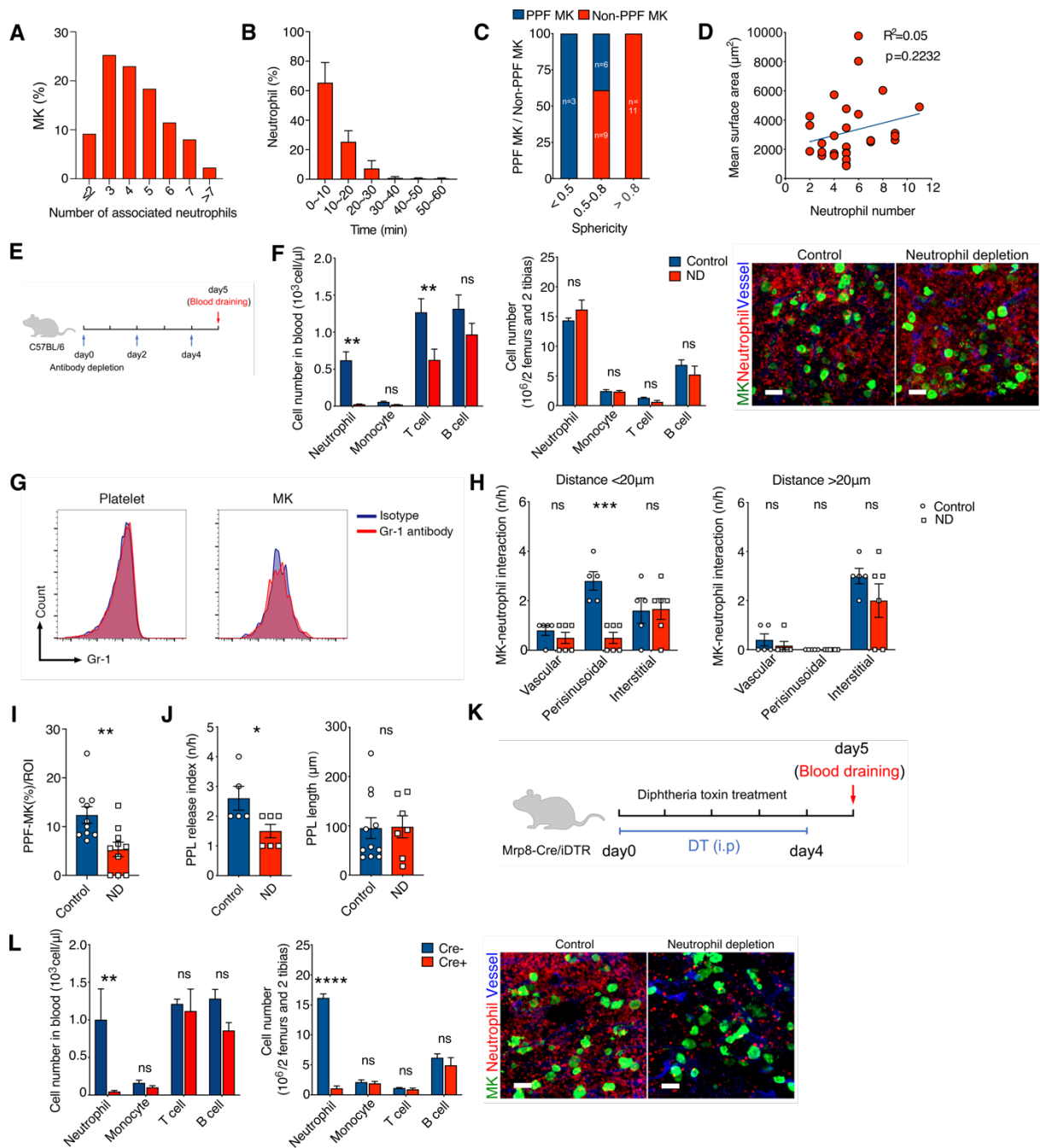


Figure S1: Characterization of MK-neutrophil interactions in vivo (related to Figure 1 and 2)

(A) Frequency distribution of MK-neutrophil interactions over 1h is shown (n=4 animals per group, 7 movies per group, 29 MKs were observed and analyzed). (B) Frequency distribution of interaction times over 1h is shown (n=4 animals per group, 7 movies per group). (C) Ratio of PPL forming MKs to non-PPL forming MKs grouped by sphericity indices (n=4 animals, 7 movies). (D) Correlation of MK surface area to occurring MK-neutrophil interactions ($p=0.22$), (n=4 animals, 7 movies). (E-F) Gr-1 induced neutrophil depletion model. (E) Treatment scheme and quantification of (F) leukocyte subpopulation in the peripheral blood and BM by flow cytometer (n=4 animals per group) and whole mount BM staining.

Scale bar represents 50 μ m. **(G)** Gr-1 staining of platelet and MKs, a representative histogram is shown (n=3 independent experiments). **(H-J)** Analysis of MK-neutrophil interaction in Gr-1 treated neutropenic and control antibody treated dual reporter mice (*Pf4-cre/Confetti/Lyz2-eGFP*) by video analysis. **(H)** Quantification of interaction frequencies within different compartments by distance to the PPL budding site (control: n=4 animals, ND: n=3 animals). **(I)** Frequency of PPL forming MKs in each region of interest (ROI), each symbol indicates one ROI area, multiple ROI per mice were compared (Gr-1 treatment n=4 animals; control AB treatment n=4 animals) **(J)** Analysis of PPL release from individual MKs per hour and maximal PPL lengths (Gr-1 treatment n=3 animals; control AB treatment n=4 animals). **(K-L)** Diphtheria toxin induced neutropenia in *Mrp8-cre(+)*/iDTR and littermate control *Mrp8-cre(-)*/iDTR mice. **(K)** Treatment scheme and **(L)** leukocyte subpopulations in the peripheral blood and BM were quantified by flow cytometer (n=4 animals per group). Whole mount BM staining (bar represents 50 μ m). Bars represent mean \pm SEM; symbols indicate individual animals; p-values are indicated, **<0.01, ***<0.001, ****<0.0001, n.s. not significant. P-values were determined using unpaired (I, J) Student's t- test, two-way (F, H, L) ANOVA multigroup test and (D) Pearson's correlation coefficient.

Figure S2

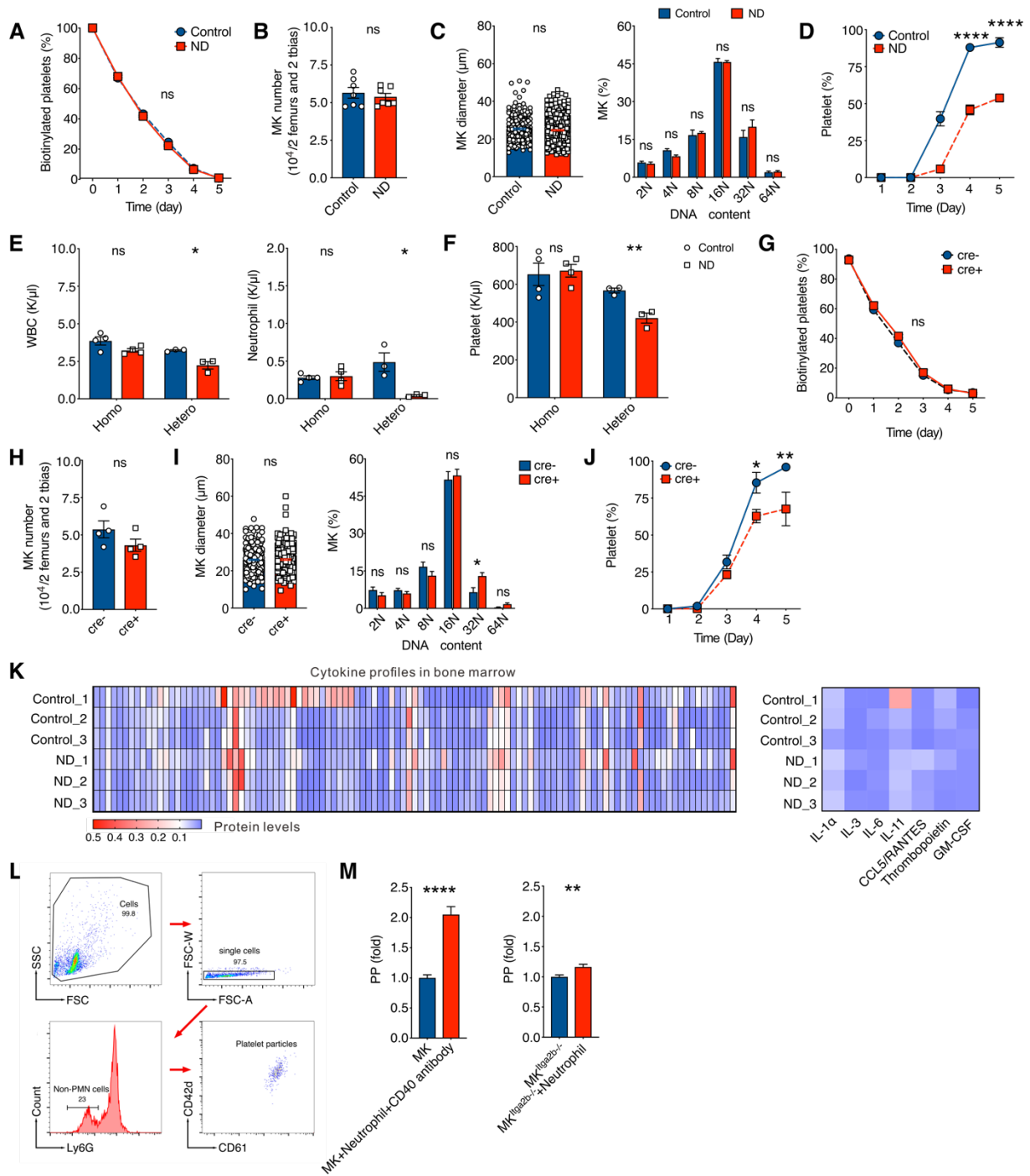


Figure S2: Thrombopoiesis under neutropenia and in vitro (related to Figure 2)

(A-D) Characterization of platelets and MKs in GR-1 or isotype control antibody treated mice are shown. (A) Platelet life span following biotin pulse labelling, (B) MK numbers (each symbol indicates individual MK from n=7 animals per group), (C) MK diameters (each symbol indicates individual MK) and MK ploidy (n=4 animals per group). (D) Platelet count recovery following antibody induced double depletion of platelets and neutrophils in GR-1 induced neutropenic mice. (n=4 animals per group). (E-F) Gr-1 depletion in Ly6G deficient homozygous (homo, n=4 animals) and littermate control heterozygous (hetero, n=3 animals) catchup mice are shown. (E) Leukocyte, neutrophil and (F) platelet counts are shown. (G-J) Platelet and MK

characterization in *Mrp8-cre*/iDTR mice with diphtheria toxin treatment. **(G)** Platelet life span following biotin pulse labelling, **(H)** MK numbers, **(I)** MK diameters (n=4 animals per group, each symbol indicates individual MKs analyzed in total) and MK ploidy (n=4 animals per group) are shown. **(J)** Platelet count recovery following double depletion of platelets and neutrophils in diphtheria toxin treated *Mrp8-cre*/iDTR mice (n=4 animals per group). **(K)** Cytokine levels of BM interstitial fluid were determined by a cytokine profile assay in Gr-1 treated neutropenic or control antibody treated mice. Cytokines related to thrombopoiesis are shown on the right (n=3 animals per group). **(L)** Flow cytometry gating strategy of platelet particles (PP) from co-culture supernatants. **(M)** In vitro analysis of PP production in co-culture supernatant after indicated treatments (n=3 independent experiments). Bars represent mean±SEM; symbols indicate individual animals; p-values are indicated, **<0.01, ****<0.0001, n.s. not significant. P-values were determined using unpaired (E, F (left panel), G) Student's t-test and two-way (C, D, F (right panel)) ANOVA multigroup test.

Figure S3

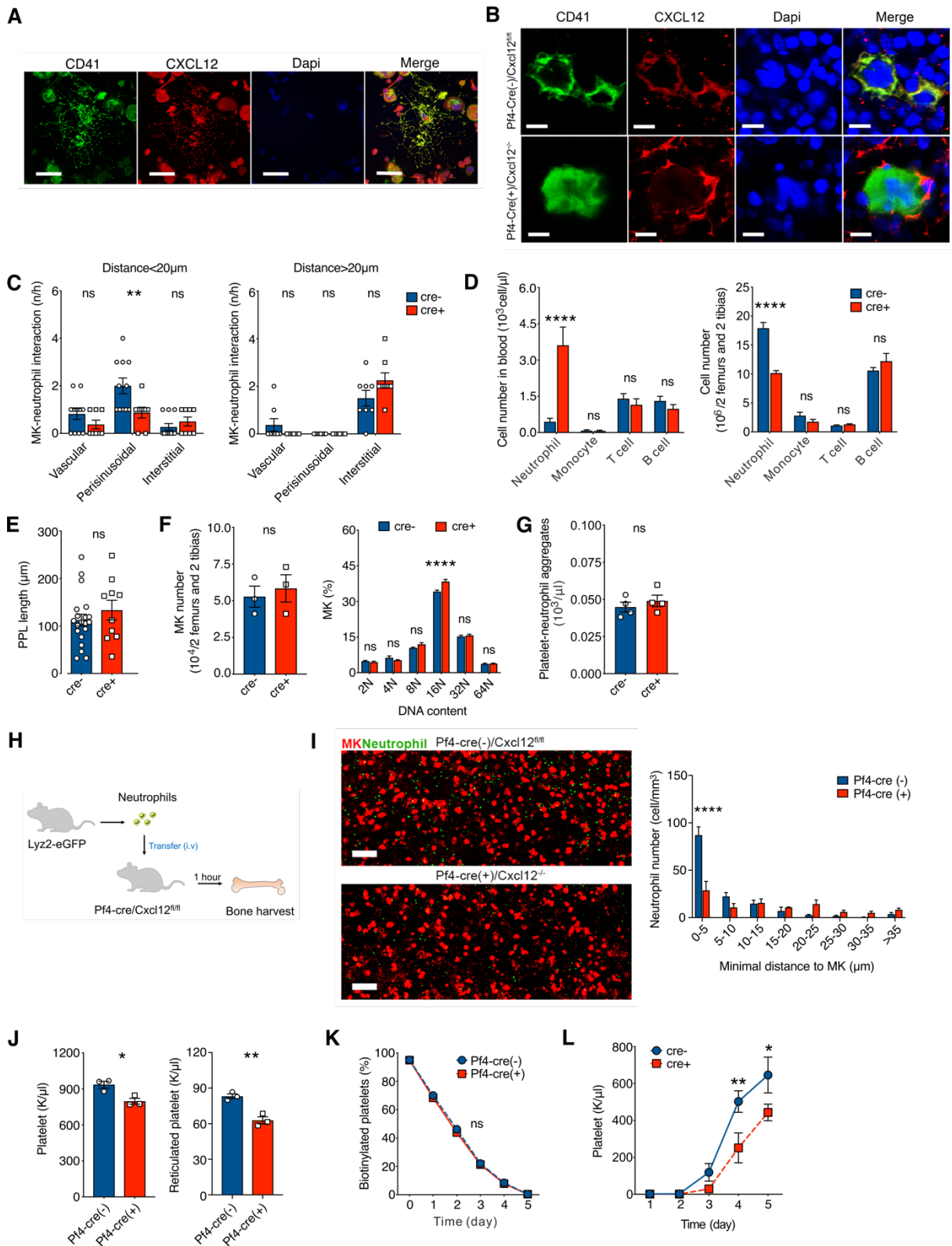


Figure S3: CXCR4-CXCL12 axis regulates thrombopoiesis (related to Figure 3)

(A) Representative confocal images of in vitro cultured, fetal liver cell derived megakaryocytes, scale bars represent 30 μm (n=3 independent experiments). (B) Whole mount staining for CXCL12 in *Pf4-cre(+)/cxcl12^{-/-}* deficient mice or littermate controls (*Pf4-cre(-)/cxcl12^{fl/fl}*). Representative confocal

images of three independent experiments are shown. Scale bars represent 10 μ m. **(C-E)** Two-Photon-Imaging video analysis of *Mrp8-cre/Cxcr4* mice. **(C)** Quantification of MK-neutrophil interaction frequencies within different compartments by distance to the PPL budding site (multiple movies per mouse were included in analysis, symbols indicate individual MKs; *Mrp8-cre(+)/Cxcr4 $\Delta\Delta$* n=3 animals; *Mrp8-cre(-)/Cxcr4^{fl/fl}* n=4 animals). **(D)** Flow cytometry based analysis of leukocyte subpopulation in the blood and BM (*Mrp8-cre(-)*: n=5 animals; *Mrp8-cre(+)*: n=4 animals), **(E)** Analysis of PPL lengths (symbols indicate individual MKs; *Mrp8-cre(+)/Cxcr4 $\Delta\Delta$* n=3 animals; *Mrp8-cre(-)/Cxcr4^{fl/fl}* n=4 animals). **(F)** MK numbers and MK ploidy (n=3 animals per group). **(G)** Analysis of platelet-neutrophil aggregates in the peripheral blood of *Mrp8-cre/Cxcr4* mice (n=4 animals per group). **(H)** Scheme of adoptive neutrophil transfer experiment is shown. **(I)** Distribution analysis of adoptively transferred neutrophils was done following whole mount BM staining. Representative 2D pictures of stained bones are shown (scale bar represents 100 μ m). Minimal MK-neutrophil distance analysis between MKs and neutrophil (n=3 animals per group). **(J)** Analysis of platelet and reticulated platelet counts are shown. **(K)** Platelet life span after biotin pulse labeling (n=3 animals per group). **(L)** Platelet count recovery following antibody induced immune-thrombocytopenia in *Mrp8-cre(+)/Cxcr4 $\Delta\Delta$* and littermate control mice (n=4 animals per group). Bars represent mean \pm SEM; symbols indicate individual animals; MKs or proplatelets; p-values are indicated, * <0.05 , ** <0.01 , **** <0.0001 , n.s. not significant. P values were determined using unpaired ((E, F (left panel), G, J) Student's t-test and two-way (C, D, F (right panel), I, K, L) ANOVA multigroup test.

Figure S4

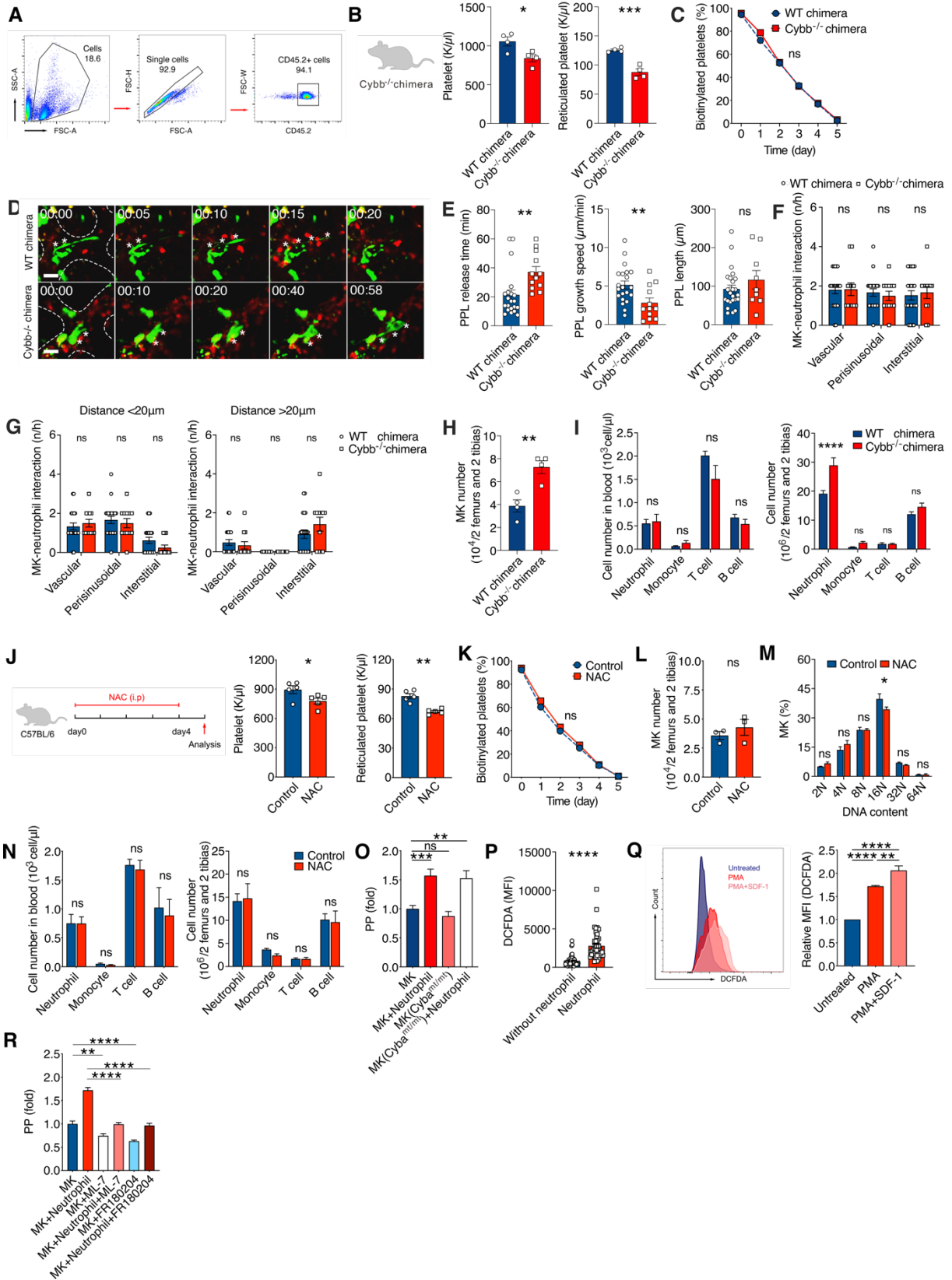


Figure S4: Neutrophil derived ROS augments platelet biogenesis in vivo and in vitro (related to Figure 4)

(A) Gating strategy to verify the efficiency of chimerism (in accordance with the ratio of CD45.2 positive cells) of *Cybb*^{-/-} Chimera is shown. (B) Analysis of platelet and reticulated platelet counts in *Cybb*^{-/-} Chimera (n=4 animals). (C) Platelet Lifespan as measured by clearance of biotinylated platelets. (D-G) In-vivo multiphoton visualization of MK-neutrophil interactions in *Cybb*^{-/-} Chimera. (D) Image sequence showing PPL formation in *Cybb*^{-/-} Chimera. Scale bar represents 20µm, Timeline (min) is indicated. (E) Analysis of proplatelet growth speeds, release time and PPL length (n=3 animals per group). (F) Quantification of MK-neutrophil interaction frequencies within different compartments and (G) within different distance categories. (H) Megakaryocyte numbers and (I) characterization of the leukocyte subpopulation ratio in blood and bone marrow by flow cytometry (n=4 animals). (J-N) N-Acetylcysteine or vehicle treatment in C57BL/6 mice over 5 days (n=5 animals per group), (J) Treatment scheme is shown (left). Quantification of platelet and reticulated platelet counts is shown. (K) Platelet life span following biotin pulse labelling. Analysis of (L) MK numbers and (M) MK ploidy. (N) Leukocyte subpopulations in the BM and peripheral blood are shown (n=3 animals per group). (O) Quantification of PP release in vitro co-culture assay with MKs isolated from *Cyba*^{mt/mt} or littermate control mice (n=3 independent experiments). (P) Mean fluorescence intensity of DCFDA in MKs co-cultured with or without neutrophils (individual MKs from three independent experiments are shown). (Q) ROS production in neutrophils following stimulation with indicated agonists (i.e. 200ng/ml SDF-1; 50nM PMA) was determined by 2',7'-Dichlorofluorescein-Diacetat (DCFDA). (R) In vitro quantification of PP release following ERK, MLC inhibition or vehicle treatment (n=3 independent experiments). Bars represent mean±SEM; symbols indicate individual animals or MKs; p-values are indicated, *<0.05, **<0.01, ***<0.001, ****<0.0001, n.s. not significant. P-values were determined with unpaired (B, E, H, J, L, P) Student's t-test, one-way (O, Q, R) or with two-way ANOVA (C, F, G, I, K, M, N) multigroup test.

Figure S5

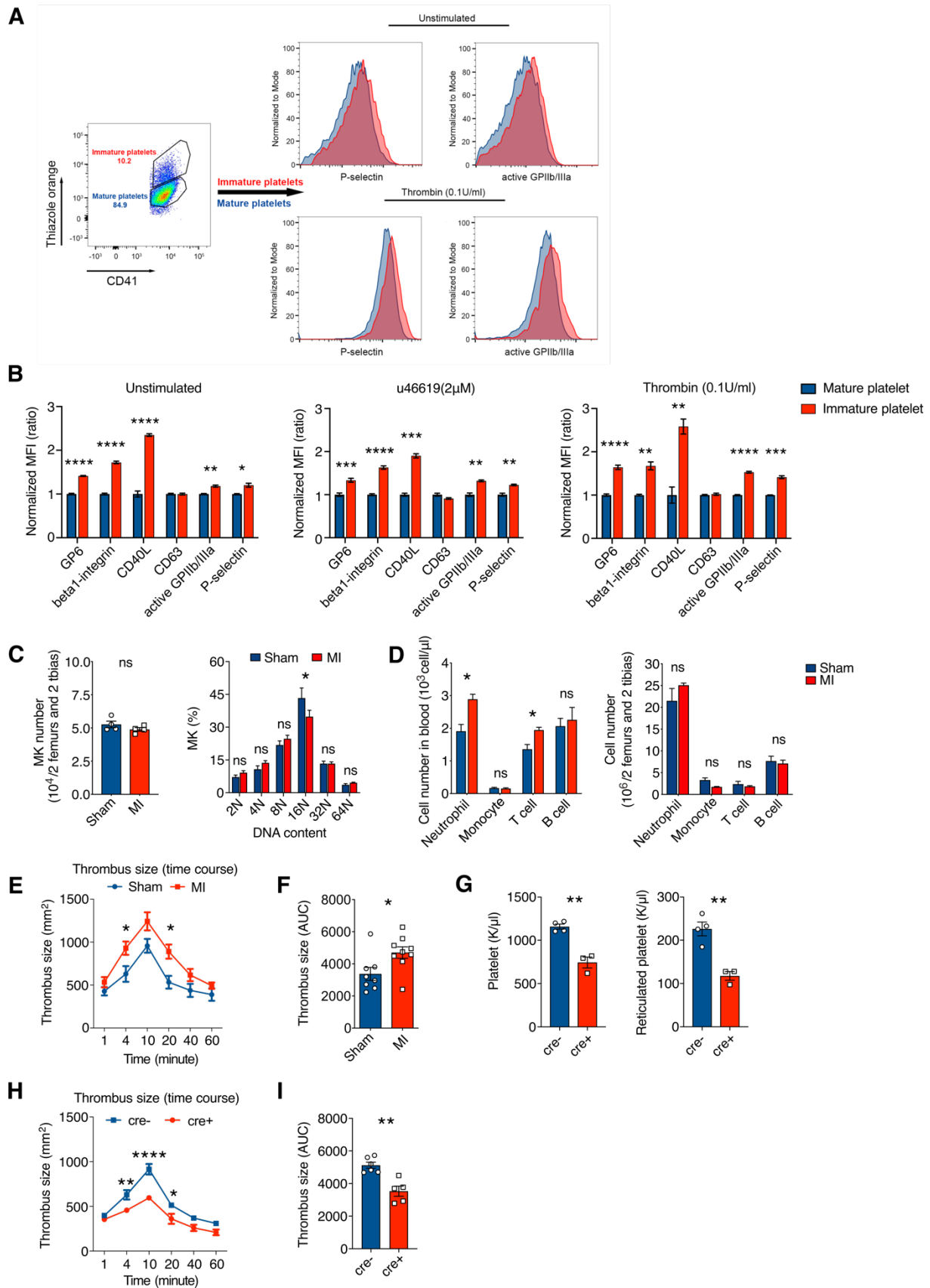


Figure S5: Increased reticulated platelet counts drive risk of thrombosis in MI (related to Figure 5 and 6)

C57BL/6 mice underwent MI I/R or sham treatment, analysis was performed after 48h (n=4 animals per group). **(A)** Analysis of P-selectin and GPIIb/IIIa expression on immature (reticulated platelets) vs. mature platelets. Gating strategy and representative histogram plots following thrombin (0.1U/ml) stimulation are shown, respectively. **(B)** Quantification of platelet surface and activation marker expression on immature or mature platelets under unstimulated condition and following stimulation with U46619 (2 μ M) or thrombin (0.1U/ml) (n=3 animals). **(C, D)** Characterization of blood and BM following MI. Analysis of **(C)** MK numbers, MK maturation and **(D)** leukocyte subpopulations within the blood and BM. **(E-F)** Fe-(III) chloride carotid artery thrombosis was induced 48h after myocardial ischemia reperfusion (I/R) injury and sham treatment in C57BL/6 mice. **(E)** Thrombus size and **(F)** total thrombus burden over 60min was quantified by video analysis in C57BL/6 mice. (Sham: n=8 animals, MI: 9 animals). **(G)** Platelet and reticulated platelet counts were determined after 48h I/R and sham treatment (*Mrp8-cre(-)/Cxcr4^{fl/fl}* : n=4 mice; *Mrp8-cre(+)/Cxcr4^{ΔΔ}*:n=3 mice). **(H-I)** Fe-(III) chloride carotid artery thrombosis was induced 48h after MI I/R and sham treatment in *Mrp8-cre/Cxcr4^{fl/fl}* mice. **(H)** Thrombus size and **(I)** total thrombus burden (over 60min) was analyzed by video analysis. (*Mrp8-cre(-)/Cxcr4^{fl/fl}*: n=6 animals; *Mrp8-cre(+)/Cxcr4^{ΔΔ}*: n=5 animals). Bars represent mean \pm SEM, symbols indicate individual animals; p-values are indicated, *<0.05, **<0.01, ***<0.001, ****<0.0001, n.s. not significant. P-values were determined using unpaired (C (left panel), F, G, I) Student's t-test or two-way (B, C (right panel), D, E, H) ANOVA multigroup test.

Figure S6

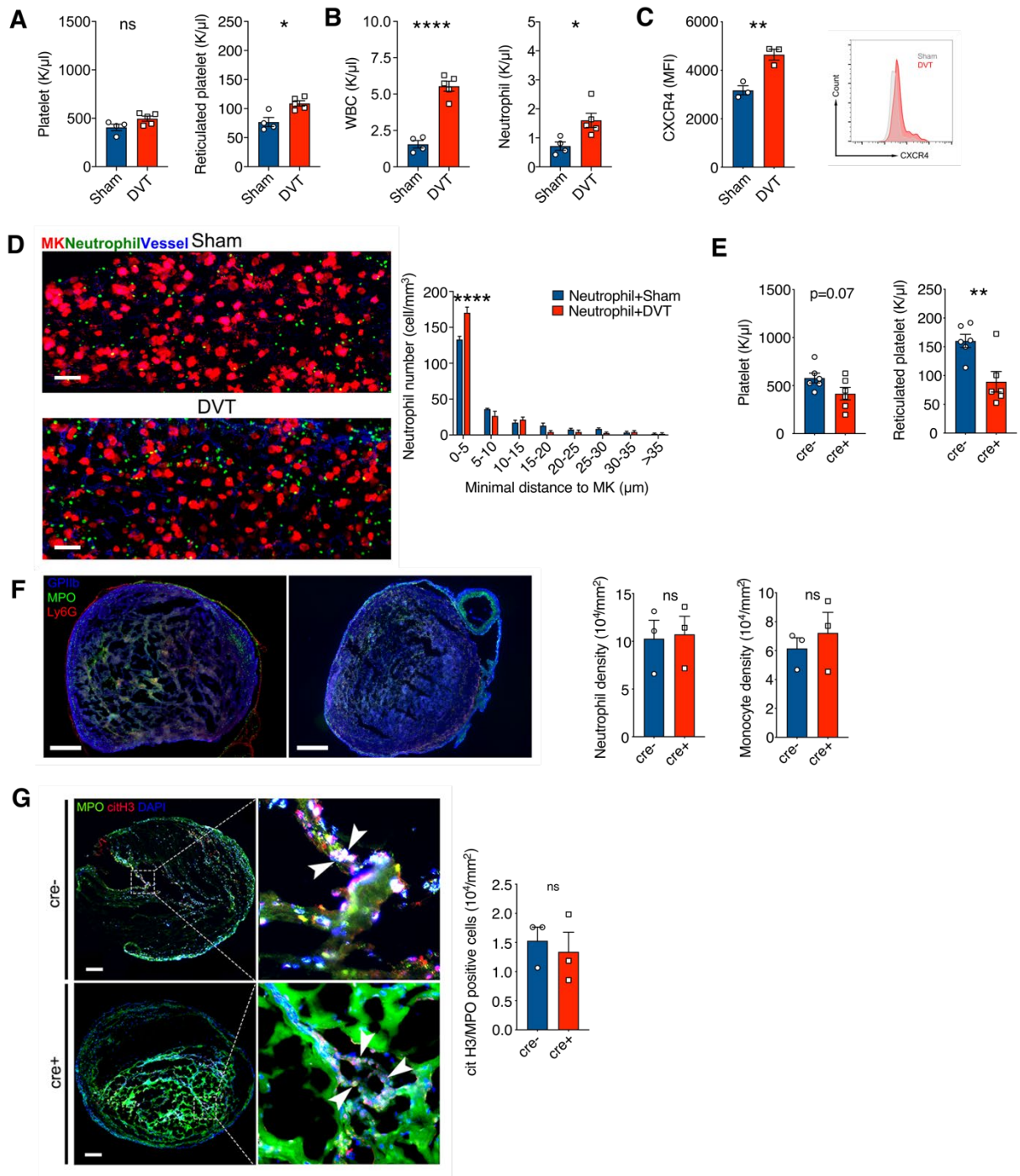


Figure S6: Thrombopoiesis during venous thrombosis (related to Figure 6)

(A) Quantification of platelet, reticulated platelets, (B) white blood cells (WBC) and neutrophil counts 48 hours after induction of venous thrombosis (DVT). (C) CXCR4 surface expression was determined on peripheral neutrophils of DVT and sham treated mice by flow cytometer (n=3 animals per group), a representative histogram blot is shown. (D) Adoptive transfer experiments of neutrophils in DVT or sham treated C57BL/6 mice. Representative 2D pictures of whole-mount-stained bones are shown (scale bar represents 100 μ m). Minimal MK-neutrophil distance between MKs and neutrophils (n=3 animals per groups). (E) Platelet and reticulated platelet counts in peripheral blood were shown (*Mrp8-cre(-)/Cxcr4^{fl/fl}*)

n=6 animals; *Mrp8-cre(+)/Cxcr4^{Δ/Δ}* n=6 animals). Leukocyte count and thrombus composition analysis 48h after induction of thrombosis. (F) Immunohistological staining of vena cava thrombi harvested from *Mrp8-cre(+)/Cxcr4^{Δ/Δ}* or *Mrp8-cre(-)/Cxcr4^{fl/fl}* control mice (n=3 animals per group). Representative images of stained thrombi are shown, scale bar indicates 100μm. Quantification of neutrophil (MPO+/Ly6G+) and monocytes (MPO+/Ly6G-) densities by IMARIS software. (G) NET formation in thrombi was quantified by immunofluorescence microscopy. Representative images are shown, scale bar indicates 200μm. Symbols indicate individual animals; p-values are indicated, *<0.05, **<0.01, ****<0.0001, n.s. not significant. P-values were determined using unpaired (A, B, C, E, F, G) Student's t-test or two- way (D) ANOVA multigroup test.

Figure S7

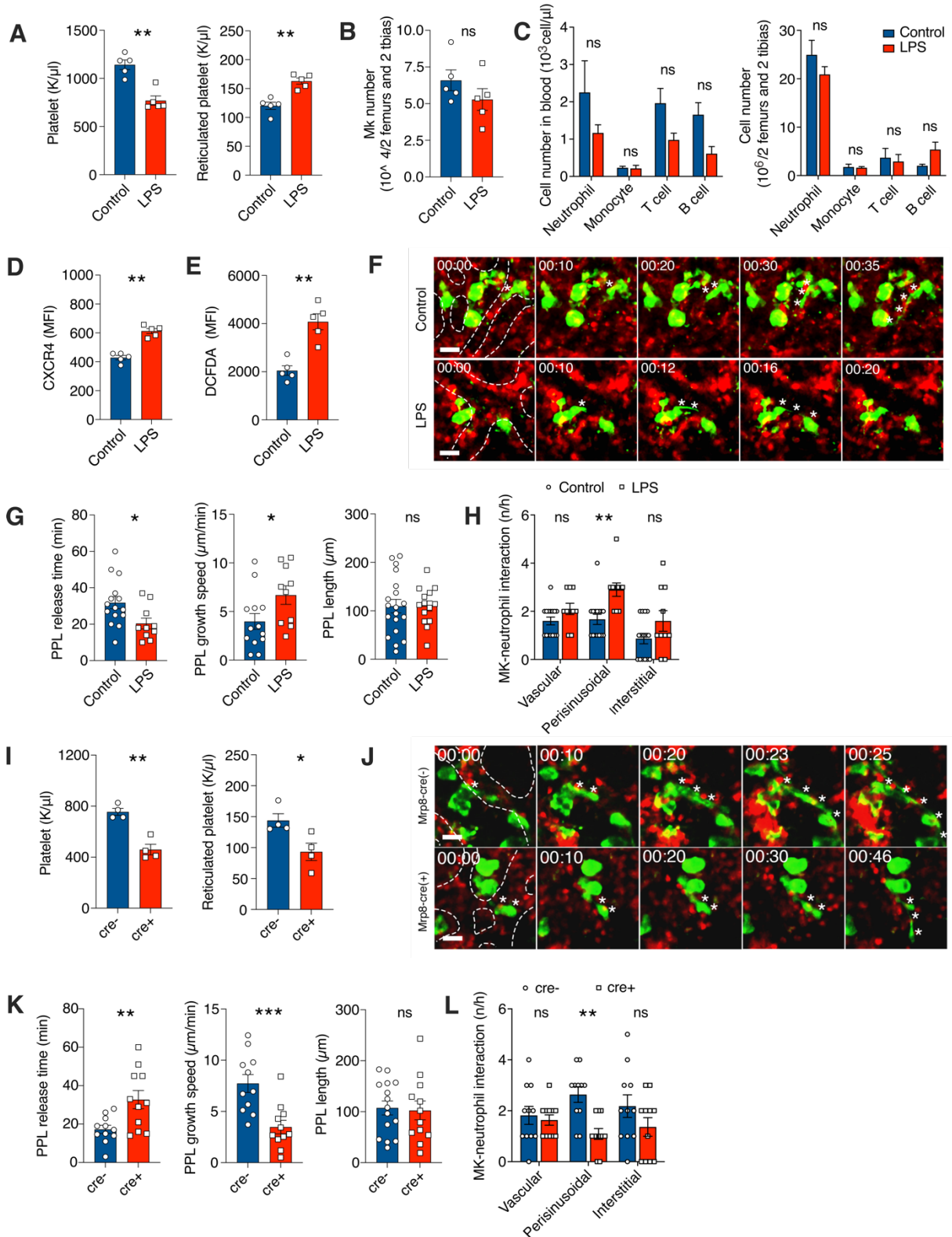


Figure S7: Neutrophil plucking in LPS induced inflammation (related to Figure 6)

(A-E) LPS model 36h after intraperitoneal LPS application (0,1 mg/kg Bodyweight). (n=4). (A) Platelet and reticulated platelet counts. (B) Megakaryocyte number (C) Characterization of the leukocyte subpopulation ratio in blood and BM by flow cytometry. (D) Neutrophil expressed CXCR4 and (E) ROS (measured by DCFDA staining) was determined by flow cytometry. (F-H) In vivo multiphoton

visualization of MK-neutrophil interactions 36 hours after LPS treatment. (F) Image sequence showing PPL formation under LPS induced inflammation. Scale bar represents 20 μ m, Timeline (min) is indicated. (G) Analysis of proplatelet growth speeds, release time and PPL length (n=3 animals per group). (H) Quantification of MK-PMN interaction frequencies within different compartments (n=3 animals per group). (I) Platelet and reticulated platelet counts in *Mrp8-cre/Cxcr4* mice 36h after intraperitoneal LPS injection (0,1mg/kg Bodyweight) (n=4). (J) Image sequence showing PPL formation under LPS induced inflammation. Scale bar represents 20 μ m, Timeline (min) is indicated. (K) Analysis of proplatelet growth speeds, release time and PPL length (n=3 animals per group). (L) Quantification of MK-neutrophil interaction frequencies within different compartments (n=3 animals per group). Bars represent mean \pm SEM; symbols indicate individual animals or MKs; p-values are indicated, *<0.05, **<0.01, ***<0.001, ****<0.0001, n.s. not significant. P-values were determined with unpaired (A, B, D, E, G, I, K) Student's t-test or with two-way ANOVA (C, H, L) multigroup test.