Supplementary Materials:

Materials and Methods

Splenectomy. Adult mice were anethestized with isofluorane via a drop vapoizer and subjected to splenectomy or mock splenectomy (peritoneum entered, spleen mobilized but not removed) as previously reported (15).

Bone marrow derived mast cell engraftment. BMMC were grown in IL-3 10ng/ml and SCF 12.5mg/ml (Peprotech) for 4-6 weeks in complete DMEM medium, resulting in a population >97% $Kit^{+}/Fc\varepsilon RI^{+}$ MC, as described (15). $1x10^{7}$ donor cells were washed into serum-free DMEM and transferred by tail vein. Engraftment was assessed as specified in the text, including tail vein phlebotomy, cardiac puncture, flow cytometry of blood, bone marrow, and disaggregated spleen, and histology. Assessment of complete blood count and leukocyte differential was accomplished using an Advia 120 Hematology System (Siemens) and appropriate species-specific standards and software as optimized previously (5).

Polymerase chain reaction. The following primers were employed to genotype NF-$E2^{-/-}$ mice and to detect engrafted NF-E2 bone marrow:

WT (product length 530bp)

AAC TTG CCG GTA GAT GAC TTT AAT

CAC CAA ATA CTC CCA GGT GAT ATG

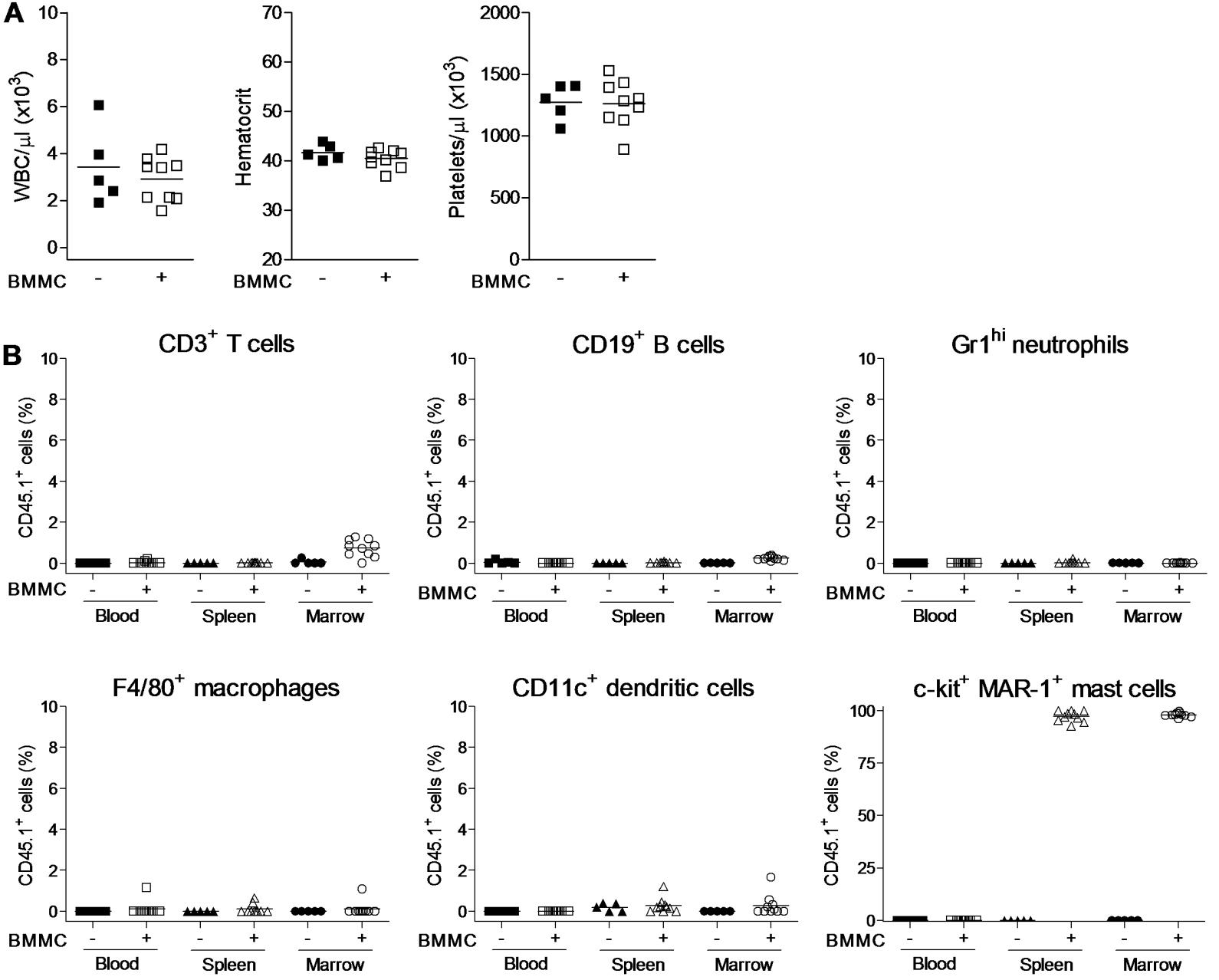
NF-$E2^{-/-}$ (product length 1.5kb)

AAC TTG CCG GTA GAT GAC TTT AAT

GCC CGG TTC TTT TTG TCA AGA CCG

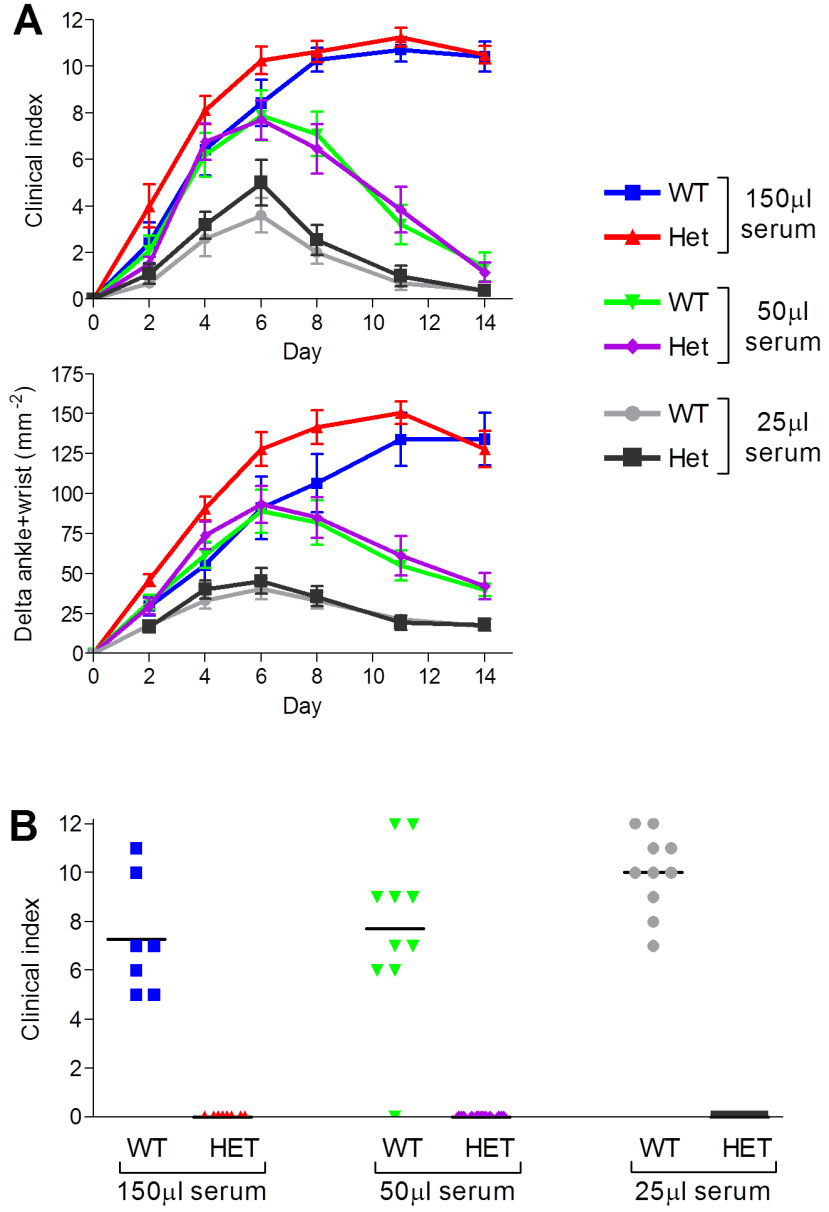
To monitor engraftment of W/Wv mice after marrow transfer, initial PCR products from harvested bone marrow of recipient mice (3-4 weeks post-engraftment) were resolved, and

regions of agarose gel corresponding to the 1.5kb fragment were cut, eluted, and subjected to a second round of PCR.


A
WBC/μl (x10^3)
Hematocrit
Platelets/μl (x10^3)
BMMC
B
CD3+ T cells
CD19+ B cells
Gr1hi neutrophils
F4/80+ macrophages
CD11c+ dendritic cells
c-kit+ MAR-1+ mast cells
CD45.1+ cells (%)
Blood
Spleen
Marrow

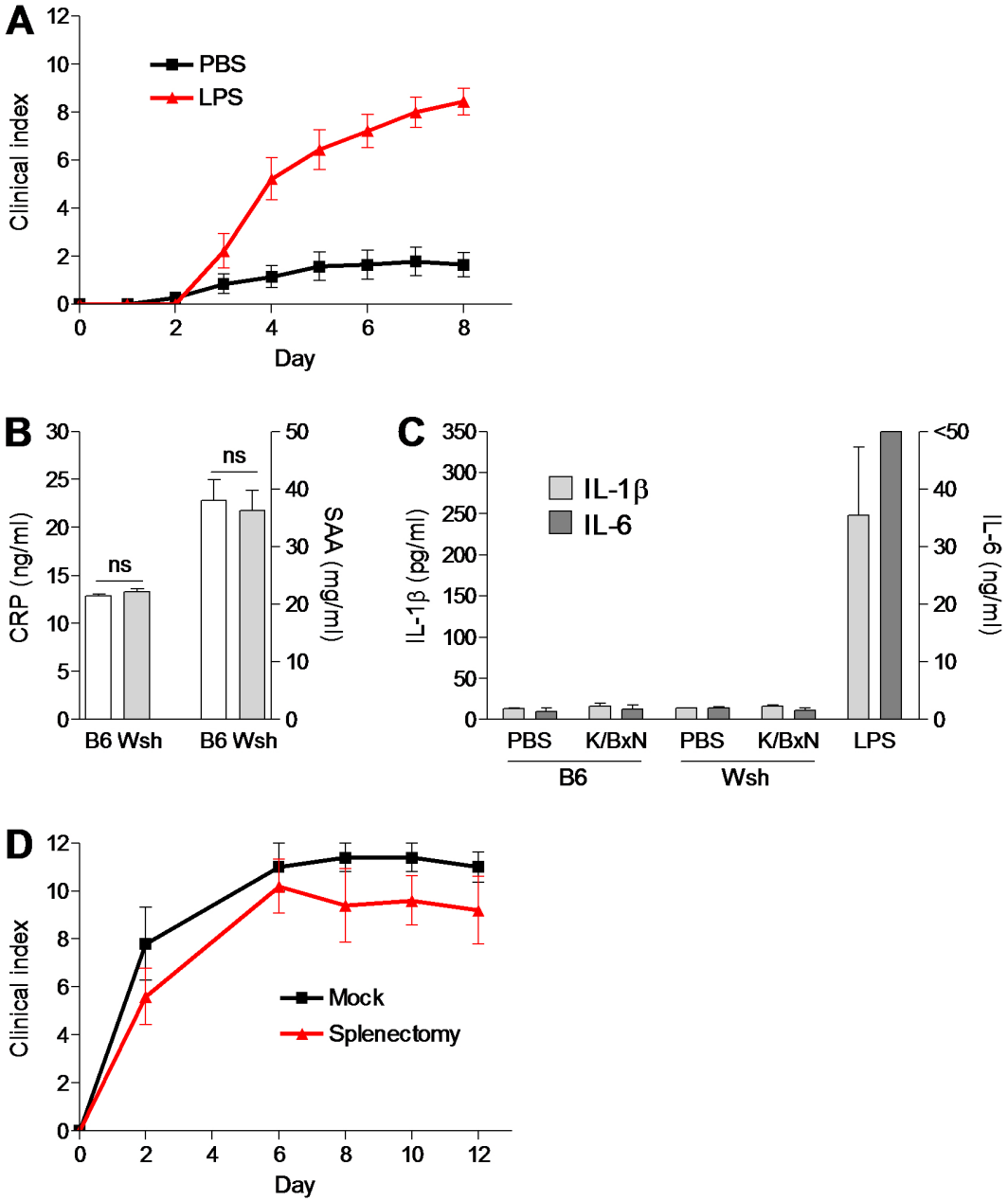
Supplemental Figure 1. BMMC engraftment of W/Wv mice is not contaminated by engraftment of other hematopoietic lineages.

CD45.1 B6 BMMC were engrafted into W/Wv mice for 10 weeks prior to harvest; sham engrafted mice were maintained as control (n=5 sham, n=10 engrafted, pooled from 2 independent experiments). (**A**) Automated white blood cell count, hematocrit, and platelet count. As reference, WBB6 hematocrit values are shown in Supplementary Figure 4 D-F. (**B**) Flow cytometry of fresh blood, femoral bone marrow, and mechanically disrupted splenocytes to identify $CD45.1^+$ cells expressing specific lineage markers.


A
Clinical index
Day
Delta ankle+wrist (mm-2)
WT
Het
150µl serum
50µl serum
25µl serum
B
Clinical index
WT
HET
150µl serum
50µl serum
25µl serum

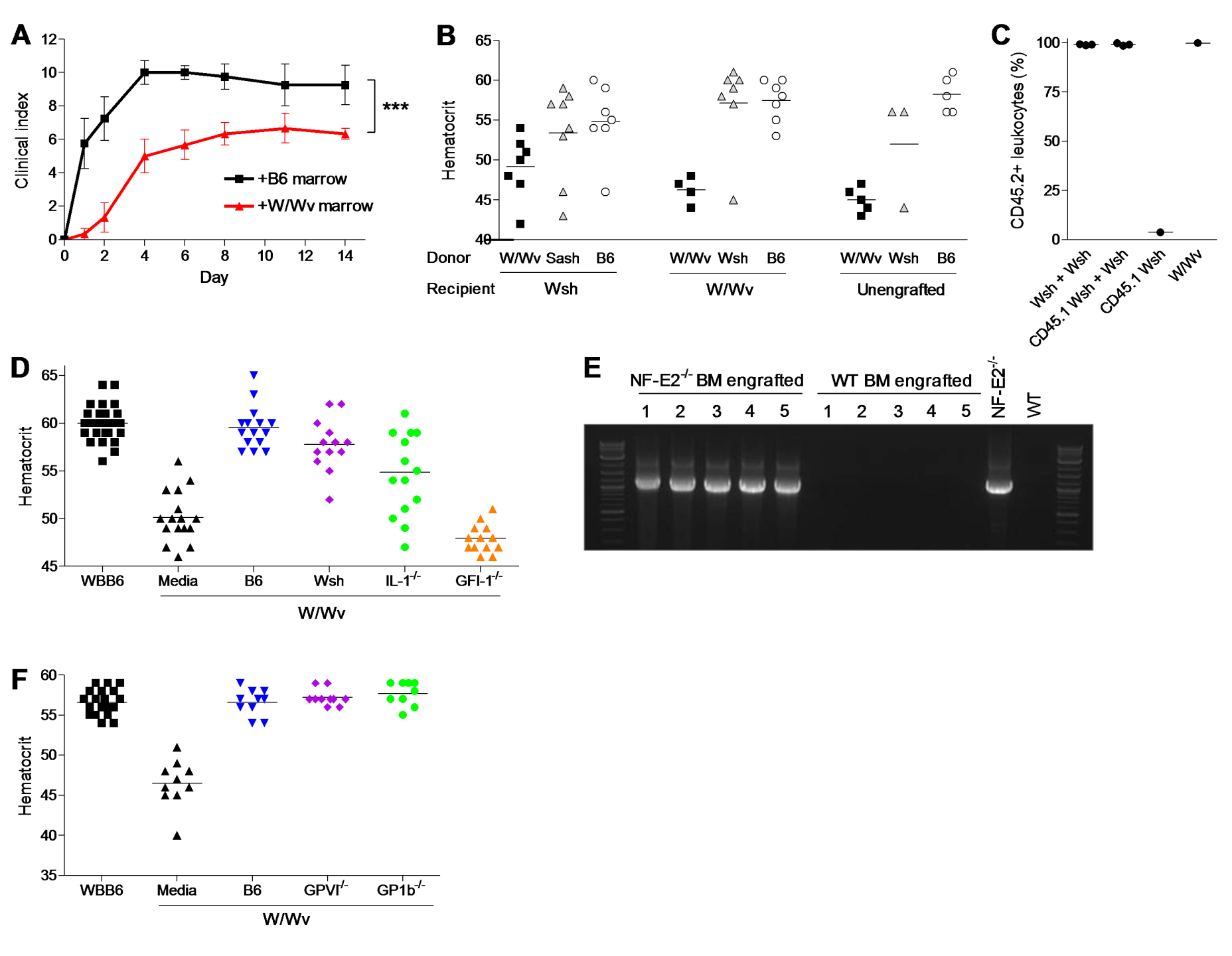
Supplemental Figure 2. Arthritis susceptibility in Cre-Master mice is not dependent of the intensity of the arthritogenic stimulus.

Male *Cpa3*$^{+/+}$ (WT) and *Cpa3*$^{Cre/+}$ (Het) were treated with K/BxN serum 150μL (n=7-8/group), 50μL (n=10-13/group) or 25μL (n=10-11/group) i.p. on days 0 and 2. Results pooled from 4 identical experiments using a single batch of pooled K/BxN serum. (**A**) Clinical scoring (top) and ankle and wrist thickness change (bottom) assessed over 2 weeks. (**B**) Acute arthritis development (flare) assessed 30min after injection of K/BxN serum.


A
Clinical index
PBS
LPS
Day
B
CRP (ng/ml)
SAA (mg/ml)
ns
ns
B6 Wsh
B6 Wsh
C
IL-1β (pg/ml)
IL-6 (ng/ml)
IL-1β
IL-6
PBS
K/BxN
PBS
K/BxN
LPS
B6
Wsh
D
Clinical index
Mock
Splenectomy
Day

Supplemental Figure 3. Susceptibility of Kit$^{Wsh/Wsh}$ mice does not reflect an intrinsic inflammatory phenotype.

(**A**) Male W/Wv mice were treated with K/BxN serum 150μL i.p. on days 0 and 2. Immediately following the day 0 injection, mice received a single dose of *S. abortus equi* LPS (Sigma, 50μg i.p. n=9) or LPS-free PBS (Gibco; n=14) ($p<0.0001$). (**B**) The inflammatory markers C reactive protein and serum amyloid A (SAA) were measured by ELISA in serum from healthy age-matched B6 and Wsh mice (n=13-21/group; p=ns; one Wsh outlier (SAA 250,000ng/ml) was excluded from the analysis). (**C**) B6 and Wsh animals were treated with PBS or K/BxN serum, and levels of IL-1β and IL-6 determined in serum obtained by cardiac puncture 4h later (n=3-6 mice/group, p=ns). B6 mice treated with LPS (n=4) served as the positive control. Similar results were obtained from samples obtained by retro-orbital phlebotomy 1h after injection (not shown). (**D**) Wsh mice were subjected to splenectomy or mock splenectomy, rested at least 2 weeks, and then treated with K/BxN serum 150μL i.p. on days 0 and 2 to induce arthritis (n=5/group, p=ns).

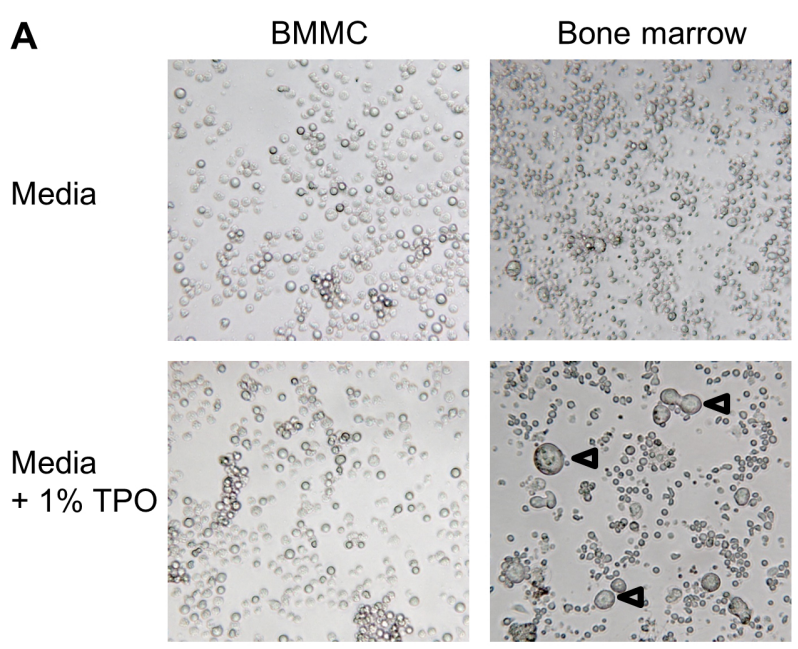

A
Clinical index
Day
***
+B6 marrow
+W/Wv marrow
B
Hematocrit
Donor
W/Wv Sash B6
W/Wv Wsh B6
W/Wv Wsh B6
Recipient
Wsh
W/Wv
Unengrafted
C
CD45.2+ leukocytes (%)
Wsh + Wsh
CD45.1 Wsh + Wsh
CD45.1 Wsh
W/Wv
D
Hematocrit
WBB6
Media
B6
Wsh
IL-1-/-
GFI-1-/-
W/Wv
E
NF-E2-/- BM engrafted
WT BM engrafted
1 2 3 4 5 1 2 3 4 5
NF-E2-/-
WT
F
Hematocrit
WBB6
Media
B6
GPVI-/-
GP1b-/-
W/Wv

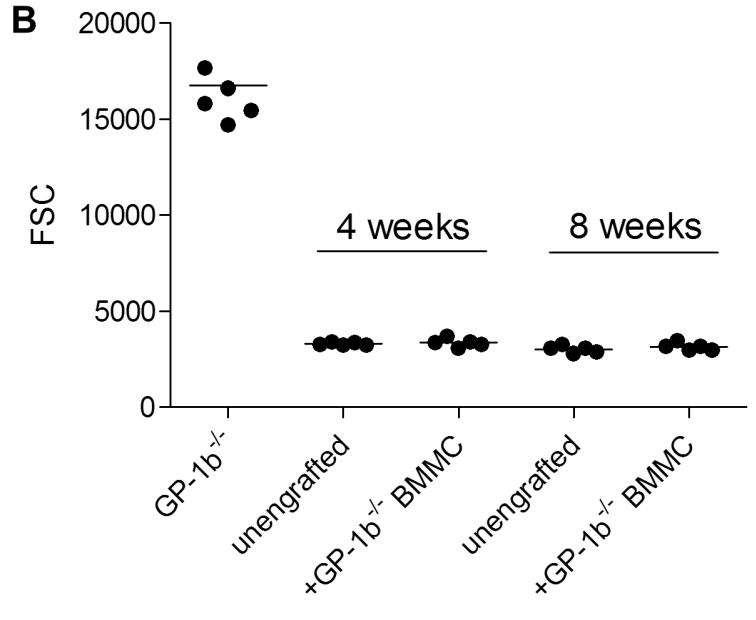
Supplemental Figure 4. Confirmation of engraftment in criss-cross transplantation (Figure 2) and marrow transfer experiments (Figure 3, 4).

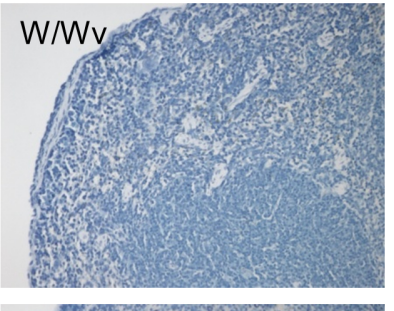
(**A**) Irradiated B6 mice engrafted with B6 or W/Wv marrow followed by arthritis initiation, as per Figure 2 (n=3-4/group, p=0.0004). (**B**) Hematocrit of recipient animals at termination of experiment, demonstrating appropriately lower hematocrit values in Wsh mice engrafted with W/Wv marrow and correction of anemia in W/Wv mice engrafted with Wsh or B6 marrow. (**C**) Flow cytometry of blood leukocytes in subgroup of 3 $CD45.1^+$ Wsh mice engrafted with W/Wv marrow, confirming absence of chimerism of circulating leukocytes. Controls include WT (CD45.2) Wsh engrafted with WT Wsh marrow, and single $CD45.1^+$ Wsh and $CD45.2^+$ W/Wv mice. (**D**) Hematocrit values of unirradiated recipient animals determined by standardized blood centrifugation at termination of experiment described in Figure 3B (3 weeks after marrow engraftment) demonstrating correction of anemia in W/Wv mice engrafted with recipient marrow, aside from those receiving marrow from GFI-1 mice, which (while not anemic at baseline) exhibit a recognized defect in engraftment of the erythrocyte lineage upon marrow transfer (1). WBB6 hematocrit values shown for reference. (**E**) Detection of NF-E2 engraftment in recipient W/Wv mice (related to Figure 4B) evidenced by an agarose gel demonstrating identification of a 1.5kb NF-E2 band from the bone marrow of 5 W/Wv mice engrafted with NF-$E2^{-/-}$ marrow but none in unengrafted controls. (**F**) Confirmation of $GPVI^{-/-}$ and $GP1b^{-/-}$ marrow engraftment (related to Figure 4C) as described in (**D**).

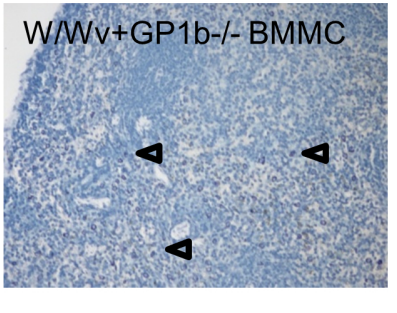
Supplemental Reference

1. Kim W, Klarmann KD, Keller JR. Gfi-1 regulates the erythroid transcription factor network through Id2 repression in murine hematopoietic progenitor cells. *Blood*. 2014;124(10):1586–1596.


A
BMMC
Bone marrow
Media
Media
+ 1% TPO

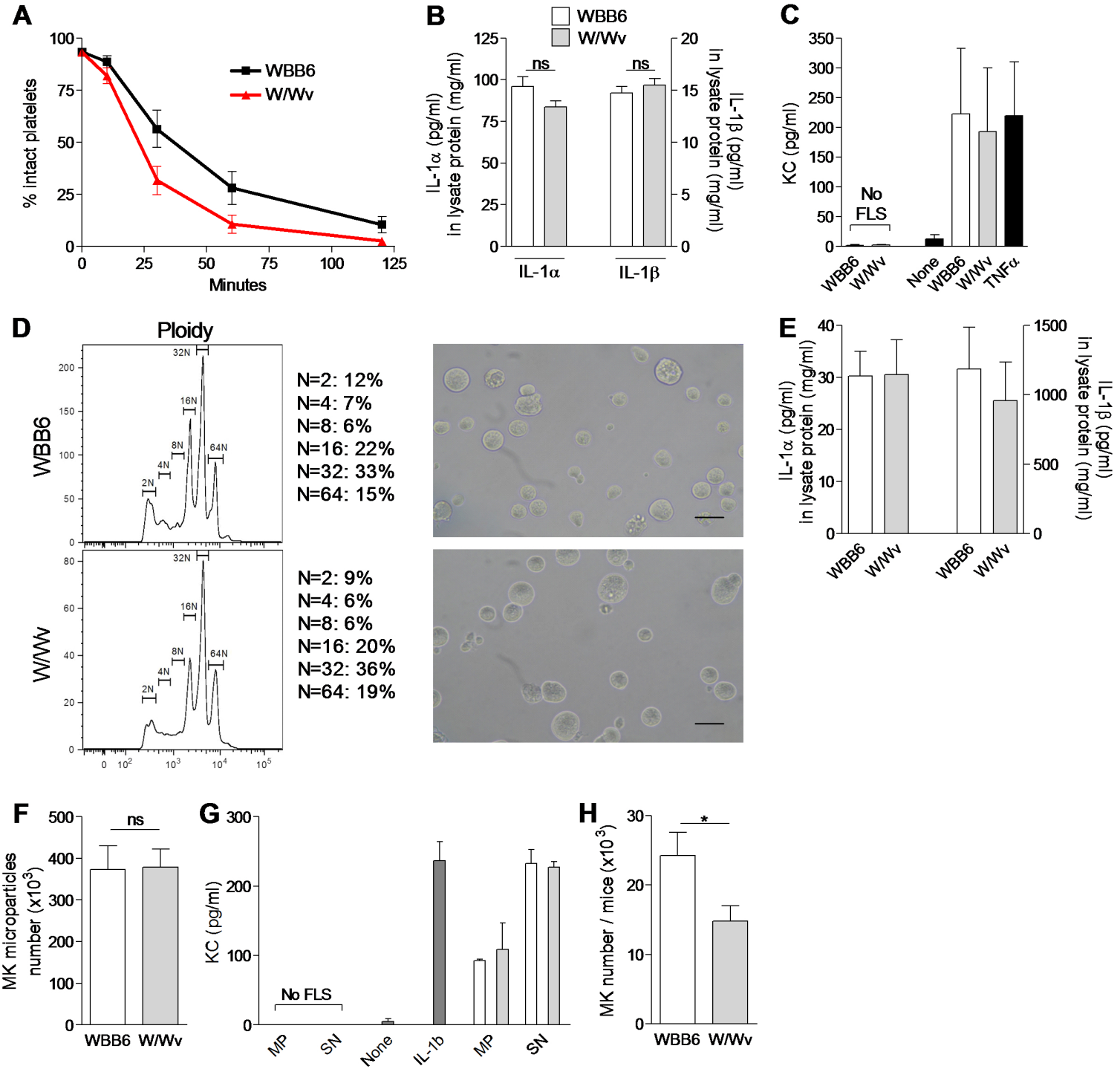

B
20000
15000
10000
5000
0
FSC
4 weeks
8 weeks
GP-1b-/-
unengrafted
+GP-1b-/- BMMC
unengrafted
+GP-1b-/- BMMC


W/Wv


W/Wv+GP1b-/- BMMC

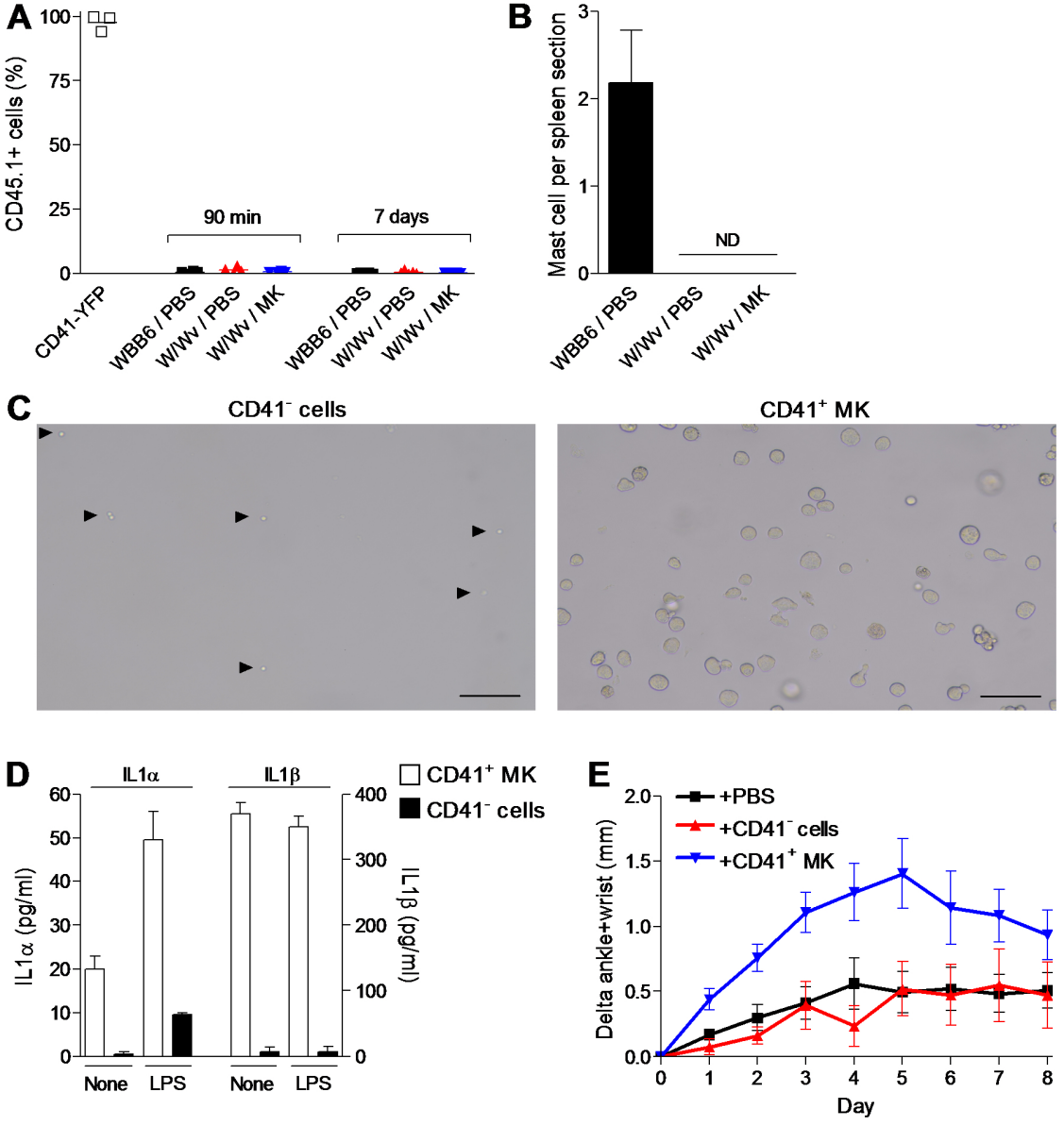
Supplemental Figure 5. BMMC engraftment is not contaminated with megakaryocyte co-engraftment.

(**A**) 4 week-old B6 BMMC and fresh positive control B6 bone marrow were washed into MK culture media containing 1% thrombopoietin (TPO) and cultured for 2 weeks to test for development of MK, defined by size (arrowheads) and ploidy (ploidy not shown). No MK were observed in BMMC cultures (data reflect 3 independent experiments). (**B**) 4 week-old $GP1b^{-/-}$ BMMC were engrafted by tailvein into 5 W/Wv mice. Left, tail bleeding was performed at 4 and 8 weeks after engraftment to assess for increase in platelet size. Right, harvest at 8 week confirmed successful BMMC engraftment in all 5 mice by toluidine blue staining of splenic sections.


A
% Intact platelets
Minutes
WBB6
W/Wv
B
WBB6
W/Wv
IL-1α (pg/ml) In lysate protein (mg/ml)
IL-1β (pg/ml) In lysate protein (mg/ml)
ns
ns
IL-1α
IL-1β
C
KC (pg/ml)
No FLS
WBB6
W/Wv
None
WBB6
W/Wv
TNFα
D
Ploidy
WBB6
W/Wv
2N
4N
8N
16N
32N
64N
N=2: 12%
N=4: 7%
N=8: 6%
N=16: 22%
N=32: 33%
N=64: 15%
N=2: 9%
N=4: 6%
N=8: 6%
N=16: 20%
N=32: 36%
N=64: 19%
E
IL-1α (pg/ml) In lysate protein (mg/ml)
IL-1β (pg/ml) In lysate protein (mg/ml)
WBB6
W/Wv
WBB6
W/Wv
F
MK microparticles number (x10³)
ns
WBB6
W/Wv
G
KC (pg/ml)
No FLS
MP
SN
None
IL-1b
MP
SN
H
MK number / mice (x10³)
*
WBB6
W/Wv

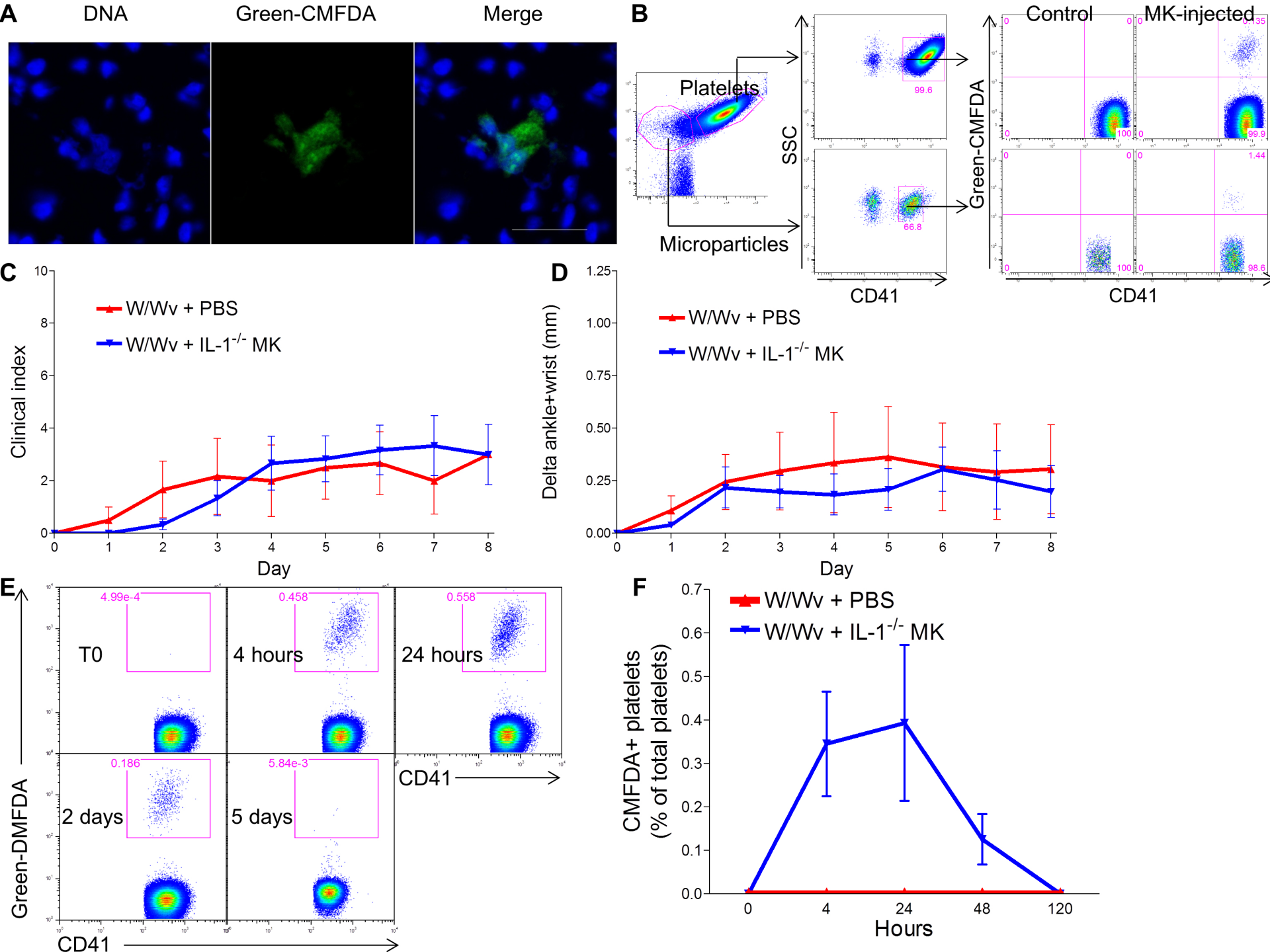
Supplemental Figure 6. Evidence that arthritis resistance in W/Wv mice is due to megakaryocytopenia.

(**A**) W/Wv and WBB6 platelets were activated with xlCRP, and conversion to microparticles was determined by flow cytometry; n=14 W/Wv and 11 WBB6 mice, pooled from 3 experiments; p=0.03 by two-way ANOVA. (**B**) IL-1α and IL-1β content of lysed resting platelets from W/Wv and WBB6 mice. n=5 W/Wv mice and 10 WBB6 mice, p=ns, reflecting two similar experiments. (**C**) W/Wv and WBB6 microparticles obtained by activating freshly-harvested platelets with xlCRP 5μg/ml 60min were incubated 18h with cultured FLS, n=2 mice/group/experiment, pooled from 3 independent experiments. (**D**) Left, ploidy analysis in MK from W/Wv compared to MK from WBB6. Right, images show MK from WBB6 and W/Wv bone marrow, enriched as described in the Material and method section. Scale bar 50μm. Results are representative of 3 independent experiments. (**E**) IL-1α and IL-1β content of lysed MK from W/Wv and WBB6 bone marrow, n=2 mice per group, reflecting two similar experiments. (**F**) WBB6 and W/Wv MK were stained with Green-CMFDA and cultured 18h. MK microparticles ($CMFDA^+CD41^+$ <1μm) were analyzed by flow cytometry. Results show the number of MK microparticles produced by $50x10^3$ MK. Representative of 4 independent experiments. (**G**) KC production by WT FLS stimulated by microparticles or supernatant from WBB6 or W/Wv MK cultures. IL-1β 10ng/ml were used as positive controls, n=2. (**H**) Number of MK obtained per WBB6 or W/Wv mouse (i.e. 2 femurs and 2 tibias). Represent 5 experiments with n=1-3 mice/group/experiment.


A
CD45.1+ cells (%)
100
75
50
25
0
90 min
7 days
CD41-YFP
WBB6 / PBS
W/Wv / PBS
W/Wv / MK
WBB6 / PBS
W/Wv / PBS
W/Wv / MK
B
Mast cell per spleen section
3
2
1
0
ND
WBB6 / PBS
W/Wv / PBS
W/Wv / MK
C
CD41⁻ cells
CD41⁺ MK
D
IL1α
IL1β
CD41⁺ MK
CD41⁻ cells
IL1α (pg/ml)
IL1β (pg/ml)
60
50
40
30
20
10
0
400
300
200
100
0
None
LPS
None
LPS
E
+PBS
+CD41⁻ cells
+CD41⁺ MK
Delta ankle+wrist (mm)
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
Day

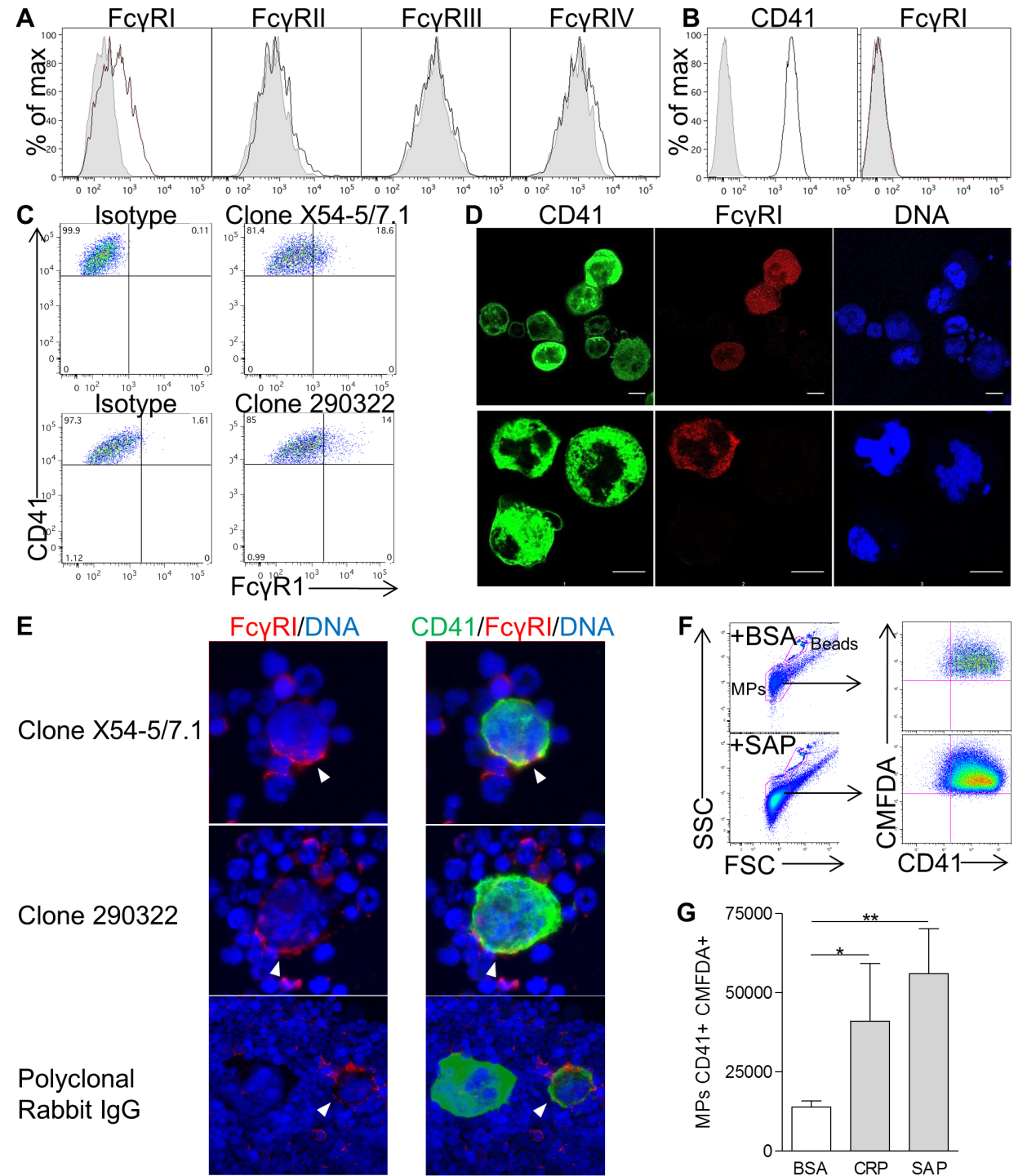
Supplemental Figure 7. Megakaryocyte engraftment is uncontaminated by co-engraftment of circulating hematopoietic lineages or mast cells (Figure 6F).

(**A**) Circulating leukocytes in WBB6 injected with PBS, W/Wv injected with PBS or W/Wv injected with MK were analyzed for their expression of CD45.1 90 minutes and 7 days after the 1st injection of K/BxN serum (n=4-5/group). (**B**) Spleen sections from 5 mice/group were stained with toluidine blue and mast cells were counted. (**C-E**) MK were enriched as described in the Material and method sections and magnetically sorted based on CD41 expression. (**C**) Images show the negative fraction containing some contaminating CD41^{-} cells (arrowheads) and the CD41^{+} fraction containing highly pure MK. Scale bars represent 200μm. (**D**) Detection of IL1α and IL1β by ELISA in lysate from the negative fraction and from 1x10^{4} CD41^{+} MKs cultured 18H without or with 1μg/ml LPS. (**E**) PBS, CD41^{-} cells and 2x10^{5} CD41^{+} MKs were injected i.v. into W/Wv mice one hour prior arthritis induction. Arthritis was assessed over 8 days. Results are expressed as ankles and wrist change, n=4-5/group. W/Wv injected with PBS vs. W/Wv injected with CD41^{+} MK, p=0.0029. W/Wv injected with CD41^{-} cells vs. W/Wv injected with CD41^{+} MK, p=0.0047. W/Wv injected with PBS vs. W/Wv injected with CD41^{-} cells, p=ns.


A
DNA
Green-CMFDA
Merge
B
Platelets
Microparticles
SSC
CD41
Green-CMFDA
Control
MK-injected
99.6
66.8
100
99.9
1.44
98.6
C
W/Wv + PBS
W/Wv + IL-1-/- MK
Clinical index
Day
D
W/Wv + PBS
W/Wv + IL-1-/- MK
Delta ankle+wrist (mm)
Day
E
T0
4 hours
24 hours
2 days
5 days
4.99e-4
0.458
0.558
0.186
5.84e-3
Green-DMFDA
CD41
F
W/Wv + PBS
W/Wv + IL-1-/- MK
CMFDA+ platelets (% of total platelets)
Hours

Supplemental Figure 8. IL-1-deficient megakaryocytes are unable to restore arthritis in W/Wv mice.

(**A-B**) W/Wv mice were injected i.v. with $2x10^5$ IL-$1^{-/-}$ MK stained with CMFDA. (**A**) Detection of $CMFDA^+$ MKs in lung section 75 minutes after the engraftment. DNA was visualized with DraqV. Scale bars represent 20μm. (**B**) Detection of $CMFDA^+CD41^+$ MPs and platelets in non-injected control mice or 75 minutes after MK injection. **(C-D)** W/Wv mice were injected i.v. with PBS or $2x10^5$ IL-$1^{-/-}$ MK. After 60 minutes, and again on day 2, mice were treated with K/BxN serum 150μL i.p. as described in the Figure 6F. (**C**) clinical scoring on a 0-12 scale. W/Wv injected with PBS vs. MK, p=ns. (**D**) Ankle and wrist thickness change. W/Wv injected PBS vs. MK, p=ns, n=6/group pooled from 2 experiments. (**E-F**) Some W/Wv mice were injected with IL-$1^{-/-}$ MK previously stained with Green-CMFDA prior arthritis induction. (**E**) Detection of circulating $CMFDA^+CD41^+$ platelets over time by flow cytometry. (**F**) Graph shows the percentages of $CMFDA^+CD41^+$ platelets among total $CD41^+$ circulating platelets, n=3 mice per group.


A
FcγRI
FcγRII
FcγRIII
FcγRIV
% of max
B
CD41
FcγRI
C
Isotype
Clone X54-5/7.1
Isotype
Clone 290322
CD41
FcγR1
D
CD41
FcγRI
DNA
E
FcγRI/DNA
CD41/FcγRI/DNA
Clone X54-5/7.1
Clone 290322
Polyclonal
Rabbit IgG
F
+BSA
Beads
MPs
+SAP
SSC
FSC
CMFDA
CD41
G
MPs CD41+ CMFDA+
75000
50000
25000
0
BSA
CRP
SAP

Supplemental Figure 9. FcγRI is expressed by a population of megakaryocyte and its activation via pentraxins CRP and SAP induces microparticle production.

(**A**) Flow cytometry analysis of FcγRI (clone X54-5/7.1), FcγRII (Clone 93 in the presence of the blocking anti-FcγRIII, clone 3G8), FcγRIII (clone 3G8) and FcγRVI (clone 9E9) on CD41+ MK (black line histograms). Filled grey histograms represent isotype-matched controls. Histograms are representative of 3 independent experiments. (**B**) FcγRI expression on CD41+ platelets, representative of 2 experiments. (**C**) Frequency of the FcγRI+ population using 2 different anti-FcγRI antibodies. Representative of 4 experiments. (**D**) Confocal microscopy of MK stained with anti-CD41 (green, clone MWReg30), anti-FcγRI (clone 290322 revealed by a donkey anti-rat AF594). DNA is visualized using Draq5. Scale bars = 20μm, representative of 3 experiments. (**E**) Bone marrow cells were stained with Draq5 (DNA, blue), anti-CD41 (green), and 3 different anti-FcγRI (red). Upper photos: clone X54-5/7.1, middle photos: clone 290322. Lower photos: polyclonal rabbit IgG. (**F-G**) MK are stained with green-CMFDA and cultured for 18h in the presence of 1μM of pentraxins CRP and SAP. 1μM BSA is used as a protein control. (**F**) CD41+ CMFDA+ microparticles (<1μm) are detected by flow cytometry and quantified using 1μm counting beads (Invitrogen). (**G**) Quantification of CD41+ CMFDA+ microparticles produced by $2x10^3$ MKs, n=6.