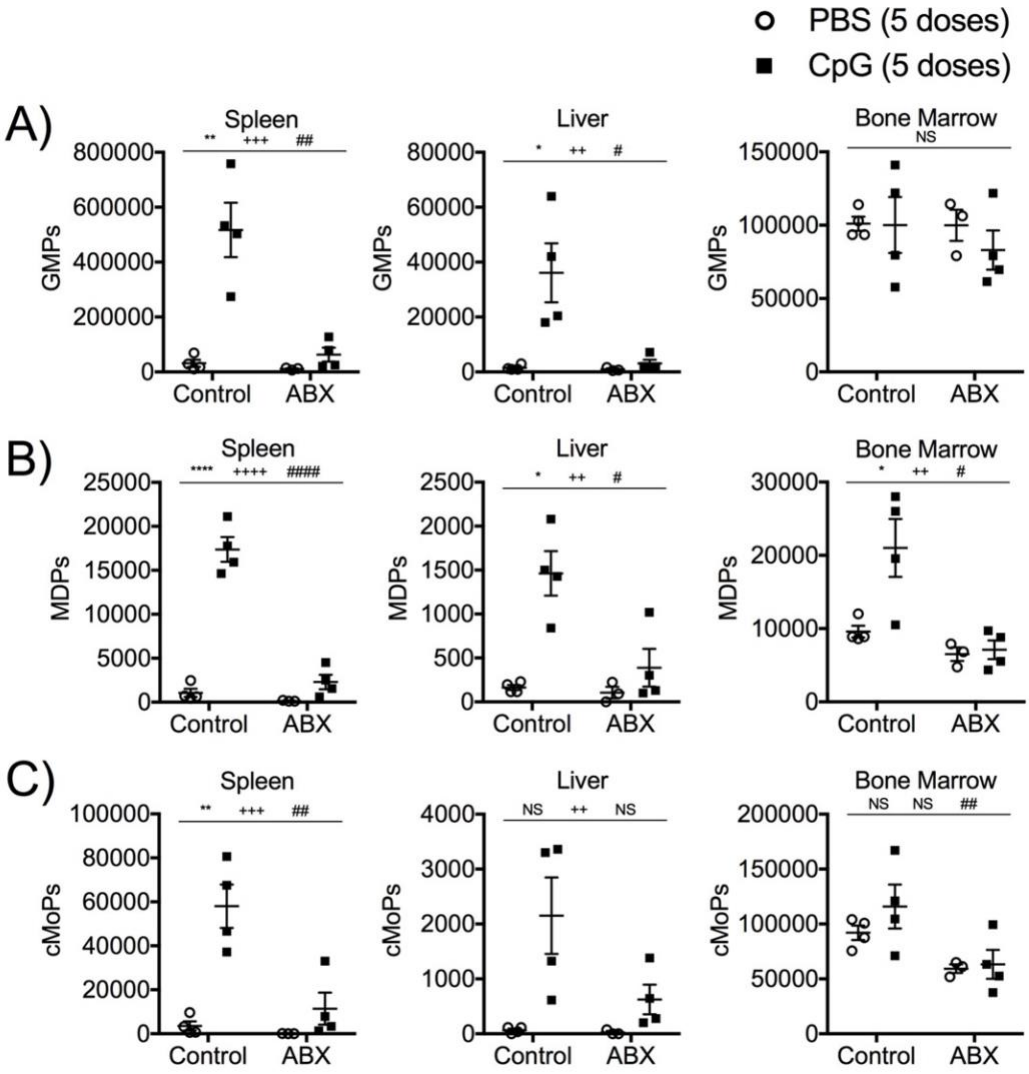
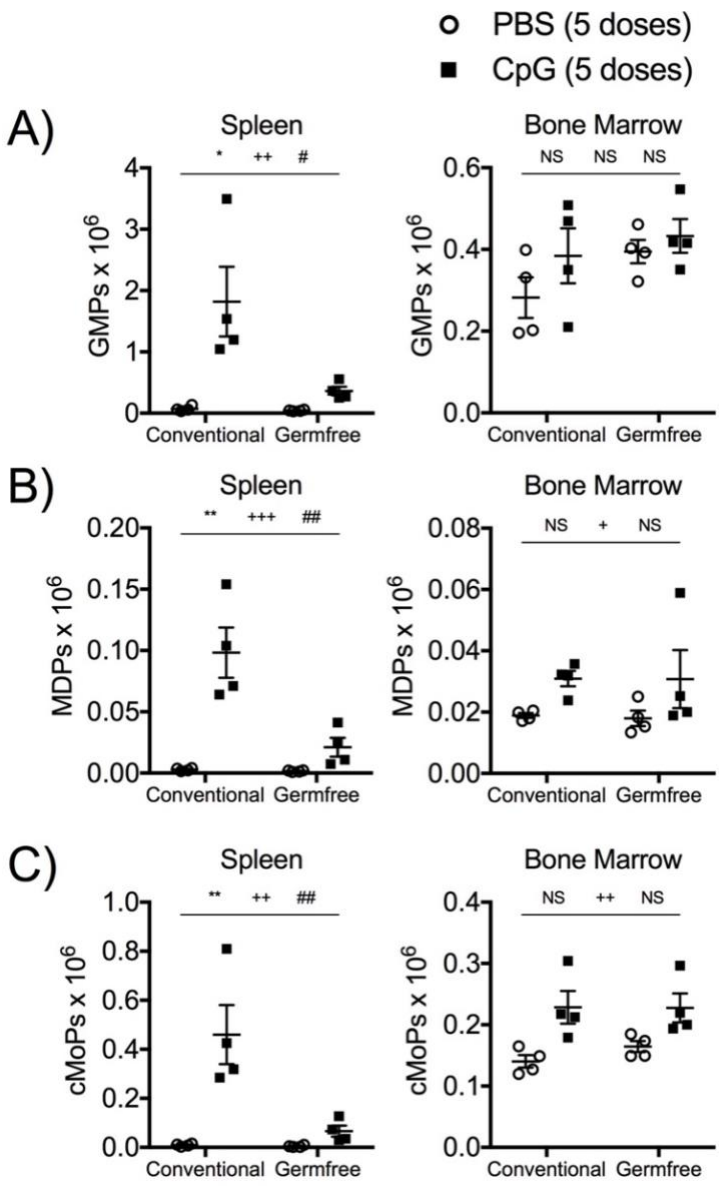
Supplementary Materials:

Fig. S1.



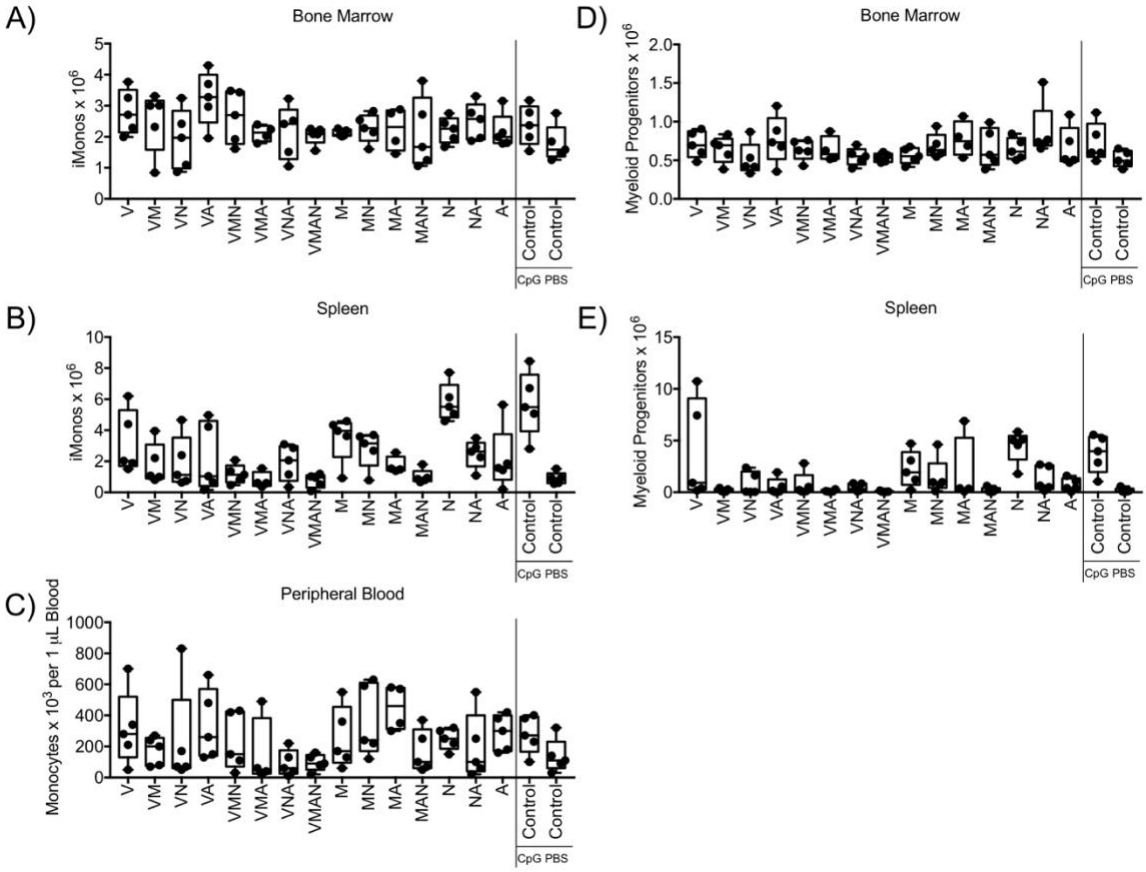
Supplemental Figure S1. Related to Figure 1. Antibiotic-treated mice do not accumulate extramedullary myeloid progenitors downstream of repeated TLR9 activation *in vivo*. C57BL/6 mice were pretreated with antibiotic (ABX) or control water *ad libitum* for 3 weeks and continued throughout the course of the experiment. Five doses of PBS or the TLR9 agonist CpG1826 (50 μg/injection) were subsequently administered intraperitoneally to antibiotic-treated and control mice over the course of 10 days. Mice were sacrificed twenty-four hours after the fifth injection. Numbers of spleen (left), liver (middle), and bone marrow (right) myeloid progenitors were enumerated [**A** - granulocyte-monocyte progenitors (GMPs); **B** – Monocyte-Dendritic cell Progenitors (MDP); **C** – committed Monocyte Progenitor (cMoP)]. Each graph displays representative data from one of three independent experiments (N=4 mice per group). Individual symbols represent one mouse and the horizontal lines represent mean values. Analysis was performed by two-way ANOVA (P values are denoted as follows: * Interaction term; + control vs. antibiotic-treated; # PBS vs. CpG *in vivo* treatments; NS = not significant).

Fig S2.



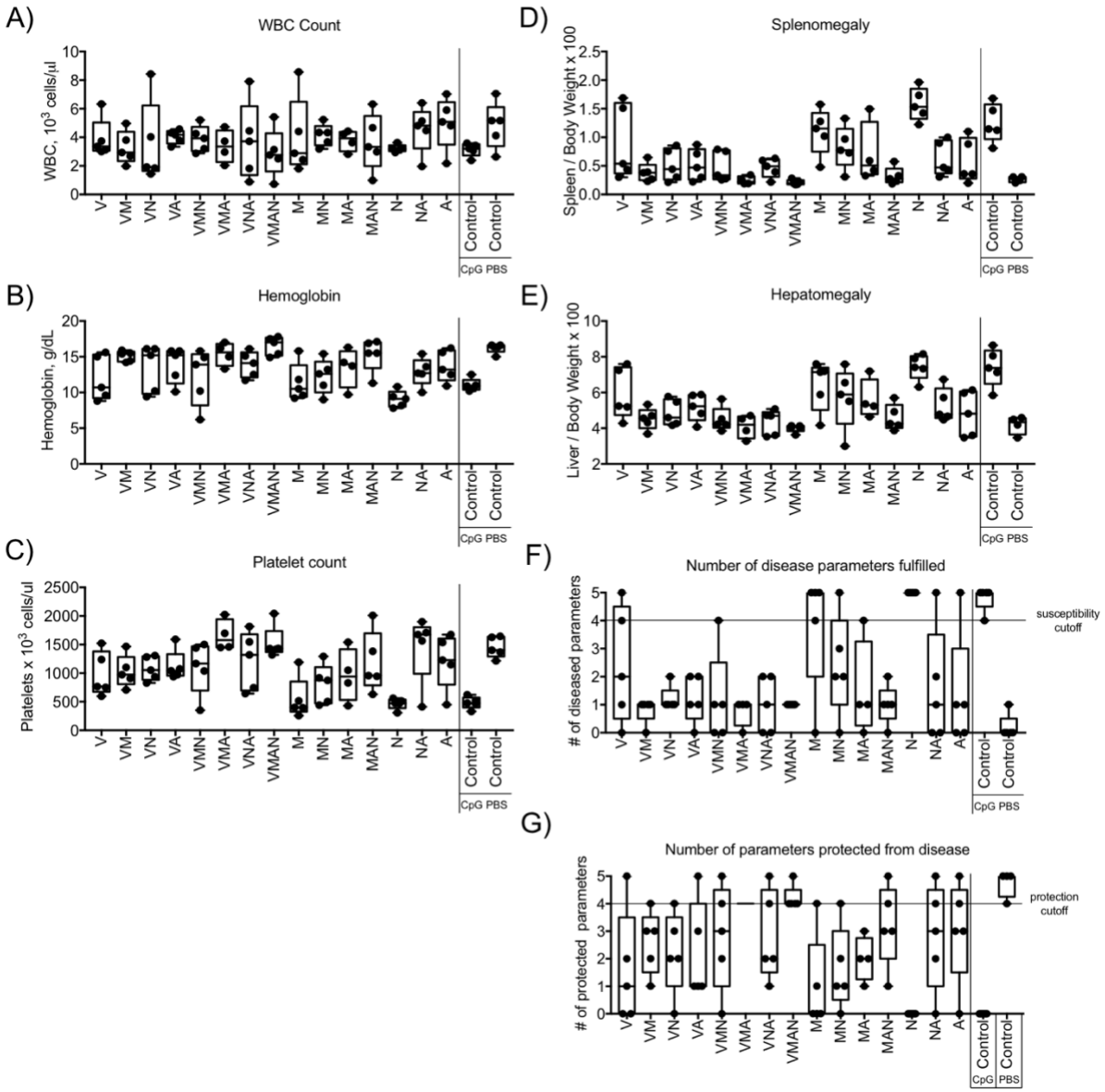
Supplemental Figure S2. Related to Figure 2. Germ-free mice do not accumulate extramedullary myeloid progenitors downstream of repeated TLR9 activation *in vivo*. Conventionally-housed and germ-free C57BL/6 mice were treated with five doses of PBS or CpG1826 over the course of 10 days. Mice were sacrificed twenty-four hours after the fifth injection. Numbers of spleen (left) and bone marrow (right) myeloid progenitors were enumerated [**A** - granulocyte-monocyte progenitors (GMPs); **B** – Monocyte-Dendritic cell Progenitors (MDP); **C** – committed Monocyte Progenitor (cMoP)]. Each graph displays representative data from one of two independent experiments (N=4 mice per group). Individual symbols represent one mouse and the horizontal lines represent mean values. Analysis was performed by two-way ANOVA (P values are denoted as follows: * Interaction term; + conventionally-housed vs. germ-free; # PBS vs. CpG *in vivo* treatments; NS = not significant).

Fig. S3



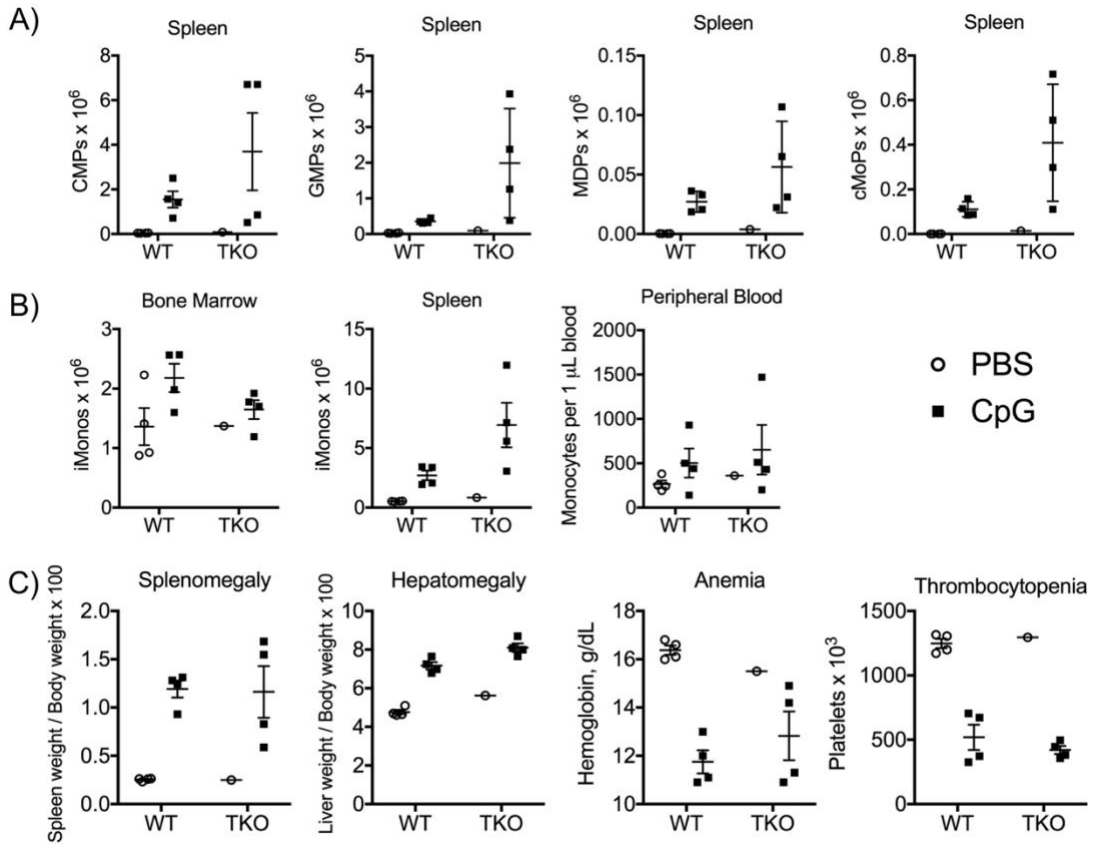
Supplemental Figure S3. Related to Figures 5 and 6. Inflammation-induced accumulation of extramedullary medullary monocytes and myeloid progenitors vary by antibiotic treatment. A factorial study design was conducted to treat cohorts of mice with every combination of the four antibiotic cocktail found to protect mice from murine MAS (V = Vancomycin, M = Metronidazole, N = Neomycin, A = Ampicillin). C57BL/6 mice were pretreated with each antibiotic combination for 3 weeks prior to treatment of mice with five doses the TLR9 agonist CpG1826 over the course of 10 days to induce murine MAS. Mice were sacrificed twenty-four hours after the fifth injection. Numbers inflammatory monocytes (iMonos) were enumerated from the bone marrow (A) and spleen (B). Peripheral blood monocytes were enumerated from analysis of whole blood from cheek bleeds (C). Numbers of total myeloid progenitors (CMPs + GMPs + MDPs + cMoPs) were enumerated from the bone marrow (D) and spleen (E).

Fig. S4



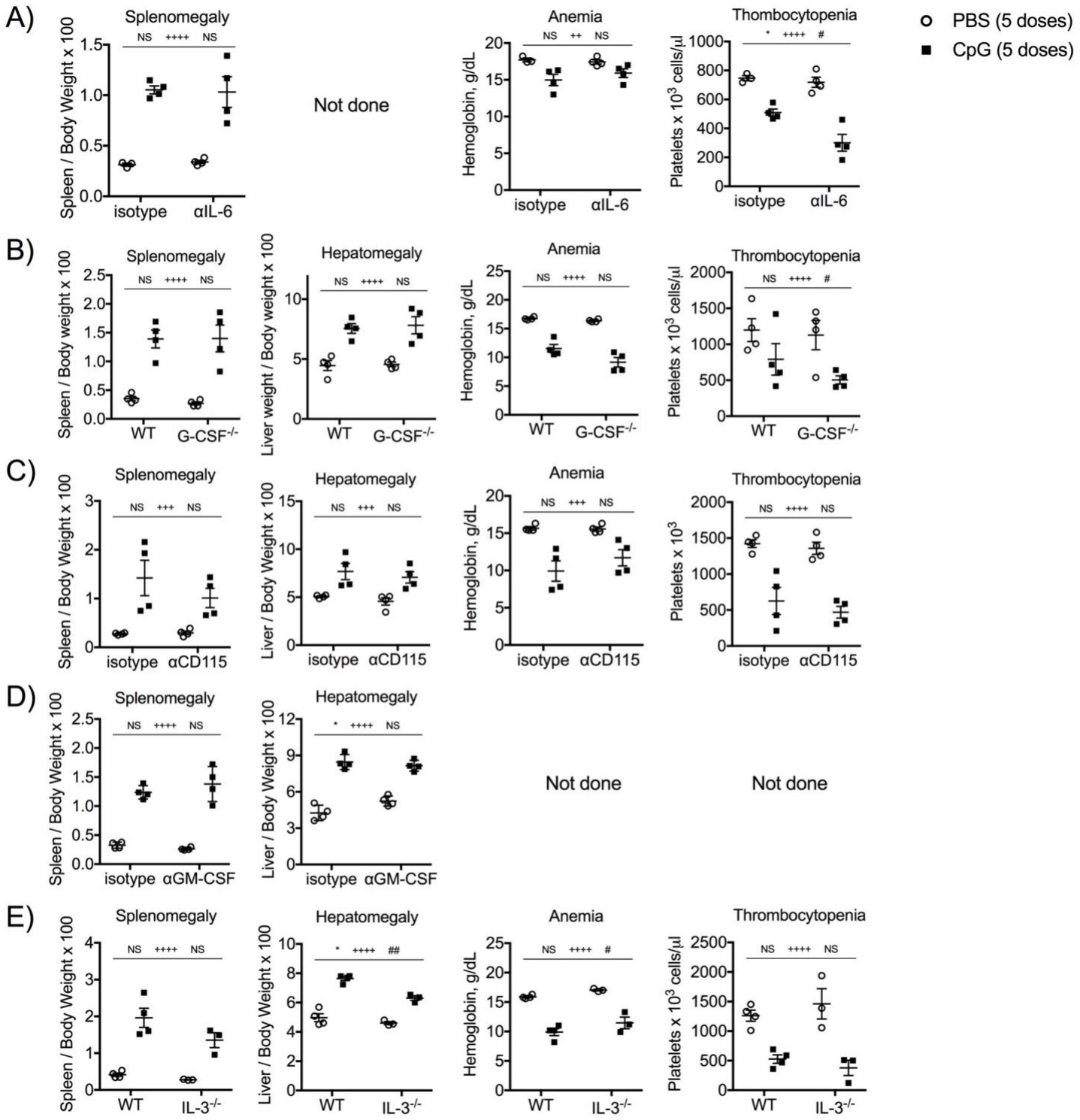
Supplemental Figure S4. Related to Figures 5 and 6. TLR9-induced MAS disease parameters by antibiotic treatment. A factorial study design was conducted to treat cohorts of mice with every combination of the four antibiotic cocktail found to protect mice from murine MAS (V = Vancomycin, M = Metronidazole, N = Neomycin, A = Ampicillin). C57BL/6 mice were pretreated with each antibiotic combination for 3 weeks prior to treatment of mice with five doses the TLR9 agonist CpG1826 over the course of 10 days to induce murine MAS. Mice were sacrificed twenty-four hours after the fifth injection, and markers of disease were measured including white blood cell (WBC) count (A), hemoglobin (B), platelet count (C), splenomegaly (D), and hepatomegaly (E). (F) Individual mice within each antibiotic treatment group were categorized based on MAS disease outcomes into mice that were susceptible to disease based on meeting 4/5 disease parameters (splenomegaly, hepatomegaly, anemia, leukopenia, and thrombocytopenia) within 20-30% of CpG-treated Control mice or worse. (G) Individual mice within each antibiotic treatment group were categorized based on MAS disease outcomes into mice that were protected from disease based on meeting 4/5 disease parameters (splenomegaly, hepatomegaly, anemia, leukopenia, and thrombocytopenia) within 20-30% of PBS-treated Control mice or better.

Fig. S5.



Supplemental Figure S5. LPS-signaling via TLR4/Caspase is not required for TLR9-enhanced myelopoiesis or induction of murine MAS. TLR4$^{-/-}$ mice were bred to Caspase-1/11$^{-/-}$ to make TLR4$^{-/-}$ Caspase-1/11$^{-/-}$ triple knockout mice that are unable to be activated by LPS at the cell surface (TLR4 activation) or upon encountering LPS within the cytoplasm of cells (Caspase-11 activation). C57BL/6 wild-type mice and triple knockout mice were treated with five doses the TLR9 agonist CpG1826 (closed symbols) or PBS (open symbols) over the course of 10 days to induce murine MAS. Mice were sacrificed twenty-four hours after the fifth injection, and markers of disease were measured. (A) Myeloid progenitors were enumerated from the spleens of sacrificed mice (CMPs = Common Myeloid Progenitors, GMPs = Granulocyte-Monocyte Progenitors, MDPs = Monocyte-Dendritic cell Progenitors, cMoPs = committed Monocyte Progenitors). (B) Bone marrow and spleen inflammatory monocytes (iMonos) and total peripheral blood monocytes were enumerated. (C) Markers of murine MAS disease were measured including splenomegaly, hepatomegaly, anemia, and thrombocytopenia.

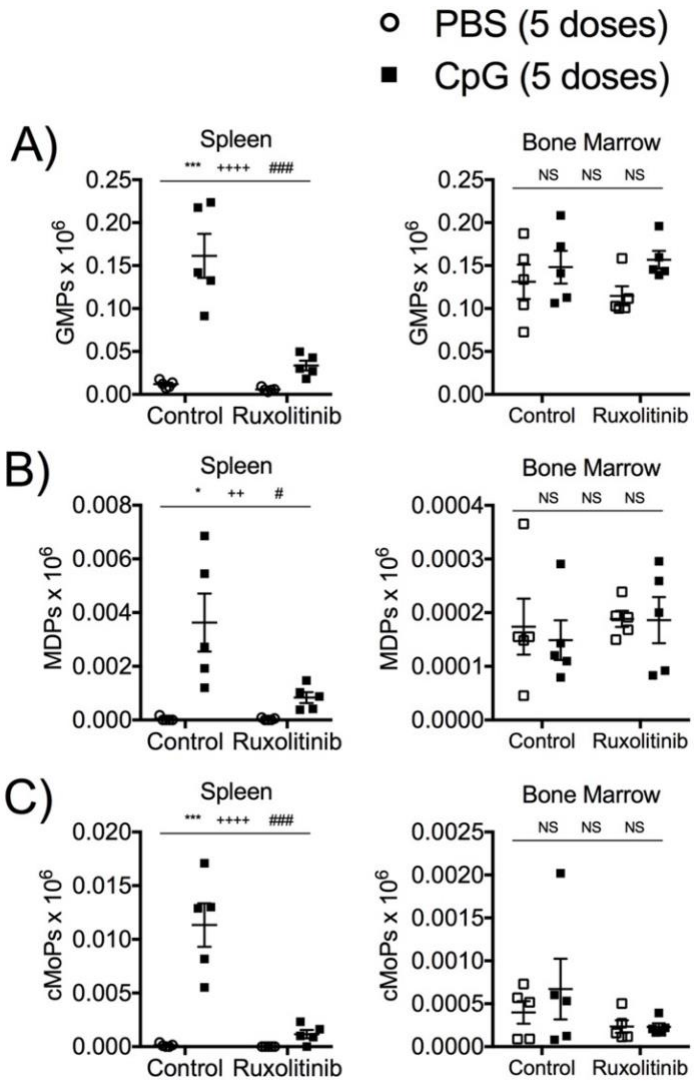
Fig. S6.



Supplemental Figure S6. Related to Figure 8. Inhibition of individual myeloid-specifying cytokines does not prevent TLR9-mediated cytokine storm immunopathology. (A) WT C57BL/6 and G-CSF$^{-/-}$ mice were treated with five doses of PBS or CpG1826, and immunopathology downstream of TLR9-mediated cytokine was measured twenty-four hours after the last injection. **(B)** WT C57BL/6 mice were treated with anti-CD115 (the M-CSF receptor) or isotype control antibodies in combination with five doses of PBS or CpG1826 over the course of ten days, and immunopathology downstream of TLR9-mediated cytokine was measured twenty-four hours after the last injection. **(C)** WT C57BL/6 mice were treated with anti-GM-CSF or isotype control

antibodies in combination with five doses of PBS or CpG1826 injections over the course of ten days, and immunopathology downstream of TLR9-mediated cytokine was measured twenty-four hours after the last injection. **(D)** WT C57BL/10J and IL-3$^{-/-}$ mice were treated with five doses of PBS or CpG1826, and immunopathology downstream of TLR9-mediated cytokine was measured twenty-four hours after the last injection. **(E)** WT C57BL/6 mice were treated with anti-IL-6 or isotype control antibodies in combination with five doses of PBS or CpG1826 injections over the course of ten days, and immunopathology downstream of TLR9-mediated cytokine was measured twenty-four hours after the last injection. Individual symbols represent one mouse and the horizontal lines represent mean values. Analysis was performed by two-way ANOVA (P values are denoted as follows: * Interaction term; + control vs. antibiotic-treated; # PBS vs. CpG *in vivo* treatments; NS = not significant).

Fig. S7.



Supplemental Figure S7. Related to Figure 9. Ruxolitinib-treated mice do not accumulate extramedullary myeloid progenitors downstream of repeated TLR9 activation *in vivo*. C57BL/6 mice were treated with five doses of PBS or CpG1826 over the course of 10 days. Mice received control chow or chow containing the JAK1/2 inhibitor ruxolitinib starting after the second injection, and were sacrificed twenty-four hours after the fifth injection. Numbers of spleen (left) and bone marrow (right) myeloid progenitors were enumerated [**A** - granulocyte-monocyte progenitors (GMPs); **B** – Monocyte-Dendritic cell Progenitors (MDP); **C** – committed Monocyte Progenitor (cMoP)]. Each graph displays representative data from one of two independent experiments (N=5 mice per group). Individual symbols represent one mouse and the horizontal lines represent mean values. Analysis was performed by two-way ANOVA (P values are denoted as follows: * Interaction term; + control vs. ruxolitinib-treated; # PBS vs. CpG *in vivo* treatments; NS = not significant).

Table S1. Related to Figure 7. Results of GSEA using gene sets relevant to myelopoiesis and innate immunity. (NES = Normalized Enrichment Score; FDR = false discovery rate)

Gene Set	NES	FDR
IFNα/β signaling	-1.92	0.003
IFNγ signaling	-1.67	0.028
cytokine signaling in immune system	-1.5	0.088
Negative regulators of RIG-I/MDA5 signaling	-1.41	0.129
Activated TLR4 signaling	-1.35	0.155
IL-3, 4, and GM-CSF signaling	-1.01	0.802
IL-1 signaling	-0.97	0.815
NOTCH1 intracellular domain regulates transcription	-0.92	0.833
IL-2 signaling	-0.88	0.846
Signaling by Notch	-0.84	0.84
PI3K AKT activation	-0.66	0.961
Inflammasomes	1.3	0.101

Table S2. Related to Figure 7. Differentially expressed genes between myeloid progenitors from antibiotic-treated mice and control mice. (FDR = false discovery rate)

Entrez Gene Name	Gene Symbol	FDR
small nucleolar RNA, C/D box 13	Snord13	0.042
interferon induced protein 44	IFI44	0.057
olfactory receptor 695	Olfr695	0.059
peptidylprolyl isomerase C	PPIC	0.059
C-type lectin domain family 4 member A	CLEC4A	0.063
family with sequence similarity 159 member A	FAM159A	0.063
carbonic anhydrase 1	CA1	0.076
nucleoporin 210	NUP210	0.087
BCL2 interacting protein 3 like	BNIP3L	0.094
monoamine oxidase A	MAOA	0.094
RNA binding motif protein 3	RBM3	0.105
growth differentiation factor 3	GDF3	0.109
predicted gene 12977	Gm12977	0.109
aminopeptidase puromycin sensitive	NPEPPS	0.109
chromosome 7 open reading frame 50	C7orf50	0.111
echinoderm microtubule associated protein like 5	EML5	0.111
hexosaminidase subunit alpha	HEXA	0.111
La ribonucleoprotein domain family member 1	LARP1	0.111
pre-mRNA processing factor 8	PRPF8	0.111
ring finger protein 11	RNF11	0.111
carbonyl reductase 1	CBR1	0.115
chitinase-like 3	Chil3/Chil4	0.115

C-type lectin domain family 4, member b2	Clec4b2	0.115
carboxypeptidase A3	CPA3	0.115
family with sequence similarity 13 member B	FAM13B	0.115
isocitrate dehydrogenase (NADP(+)) 2, mitochondrial	IDH2	0.115
interferon induced transmembrane protein 3	IFITM3	0.115
immunoglobulin kappa chain variable 8-27	Igkv8-27	0.115
ketohexokinase	KHK	0.115
kelch like family member 24	KLHL24	0.115
multiple inositol-polyphosphate phosphatase 1	MINPP1	0.115
2'-5'-oligoadenylate synthetase 3	OAS3	0.115
poly(ADP-ribose) polymerase family member 14	PARP14	0.115
pleckstrin homology like domain family B member 3	PHLDB3	0.115
prostaglandin E synthase 3	PTGES3	0.115
transforming growth factor beta 1	TGFB1	0.115
TIA1 cytotoxic granule associated RNA binding protein	TIA1	0.115
transmembrane protein 97	TMEM97	0.115
vascular endothelial zinc finger 1	VEZF1	0.115
zinc finger and BTB domain containing 16	ZBTB16	0.115
family with sequence similarity 173 member A	FAM173A	0.117
NHP2 ribonucleoprotein	NHP2	0.117
scinderin	SCIN	0.117
cell death-inducing DFFA-like effector a	CIDEA	0.118
formin like 2	FMNL2	0.118
mucin 13, cell surface associated	MUC13	0.118
dehydrogenase/reductase 3	DHRS3	0.127
fructose-bisphosphatase 1	FBP1	0.133
RIKEN cDNA A930041C12 gene	A930041C12Rik	0.134
adaptor related protein complex 2 mu 1 subunit	AP2M1	0.134
FXYD domain-containing ion transport regulator 2	Fxyd2	0.134
predicted gene 428	Gm428 (includes others)	0.134
LIM zinc finger domain containing 1	LIMS1	0.134
matrilin 1, cartilage matrix protein	MATN1	0.134
chromosome 12 open reading frame 57	C12orf57	0.138
heterogeneous nuclear ribonucleoprotein A1	Hnrnpa1	0.138
protein disulfide isomerase family A member 6	PDIA6	0.138
argininosuccinate synthase 1	ASS1	0.145
cartilage associated protein	CRTAP	0.145
small nucleolar RNA, C/D box 104	Snord104	0.145
RIKEN cDNA 5730408K05 gene	5730408K05Rik	0.148
SET and MYND domain containing 4	SMYD4	0.148
proteasome subunit beta 7	PSMB7	0.149

B-cell CLL/lymphoma 11A	BCL11A	0.154
ALG8, alpha-1,3-glucosyltransferase	ALG8	0.162
TATA-box binding protein associated factor 15	TAF15	0.162
charged multivesicular body protein 6	CHMP6	0.167
leucine rich repeat containing 59	LRRC59	0.167
single-pass membrane protein with coiled-coil domains 4	Smco4	0.168
calreticulin	CALR	0.171
glutathione S-transferase mu 3	GSTM3	0.171
host cell factor C1	HCFC1	0.171
MARVEL domain containing 2	MARVELD2	0.171
minichromosome maintenance complex component 4	MCM4	0.171
SUB1 homolog, transcriptional regulator	SUB1	0.171
transmembrane BAX inhibitor motif containing 6	TMBIM6	0.171
heat shock protein family D (Hsp60) member 1	HSPD1	0.173
predicted gene, 36856	Gm36856	0.178
predicted gene, 16758	Gm16758	0.181
3-oxoacid CoA-transferase 1	OXCT1	0.181
hypoxia up-regulated 1	HYOU1	0.184
MARVEL domain containing 1	MARVELD1	0.193
nuclear transport factor 2 like export factor 1	NXT1	0.193
ATP binding cassette subfamily E member 1	ABCE1	0.196
basal cell adhesion molecule (Lutheran blood group)	BCAM	0.196
predicted gene 12247	Gm12247	0.196
microRNA 421	mir-95	0.196
naked cuticle homolog 2	NKD2	0.196
olfactory receptor 472	Olfr472	0.196
protocadherin beta 1	PCDHB1	0.196
peptidase, mitochondrial processing alpha subunit	PMPCA	0.196

Table S3. Key Resources Table.

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Mus musculus Strains		
Wild-type C57BL/6	The Jackson Laboratory	000664
G-CSF$^{-/-}$	The Jackson Laboratory	002398
Wild-type C57BL/10J	The Jackson Laboratory	000665
IL-3$^{-/-}$	The Jackson Laboratory	023816
TLR4$^{-/-}$	The Jackson Laboratory	007227
Caspase-1/11$^{-/-}$	The Jackson Laboratory	016621
Chemicals, Peptides, and Recombinant Proteins		
ACK lysis buffer	Lonza	10-548E
LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit	ThermoFisher Scientific	L34957
Sucrose	Avantor	4097-06
Percoll	Sigma	GE17-0891-01
Brefeldin A	Sigma	B7651
Ampicillin	Sigma	A9518
Metronidazole	Sigma	M3761
Neomycin	Sigma	N1876
Vancomycin	Sigma	V2002
DNase I	Sigma	10104159001
Collagenase A	Sigma	10103586001
Anti-mouse CD16/32 (Fc block), clone 2.4G2	Bio X cell	BE0307
Anti-mouse CSF1R (CD115), clone AFS98	Bio X cell	BE0213
Anti-mouse GM-CSF, clone MP1-22E9	Bio X cell	BE0259
Anti-mouse IL-6, clone MP5-20F3	Bio X cell	BE0046
Anti-mouse IFNAR-1, clone MAR1-5A3	Bio X cell	BE0241
Control antibody for anti-IFNAR-1 experiment, MOPC-21	Bio X cell	BE0083
Control antibody for anti-M-CSF experiment, clone LFT-2	Bio X cell	BE0090
Control antibody for anti-GM-CSF experiment, clone LFT-2	Bio X cell	BE0090
Control antibody for anti-IL-6 experiment, clone SFR8-B6	ATCC	ATCC® HB-152™
Recombinant murine IL-3	Peprotech	213-13
Recombinant murine M-CSF	Peprotech	315-02
Recombinant murine GM-CSF	Peprotech	315-03
Recombinant murine SCF	Peprotech	250-03
Control chow	Incyte Corporation	MTA required
Ruxolitinib chow	Incyte Corporation	MTA required
Oligonucleotides		
CpG1826 (special order with a phosphothioate backbone)	Integrated DNA Technologies	
Sequence: T*C*C*A*T*G*A*C*G*T*T*C*C*T*G*A*C*G*T*T		
Flow Cytometry Antibodies		
PE anti-mouse B220 Antibody (clone RA3-6B2)	Biolegend	103207
PE anti-mouse CD4 Antibody (clone RM4-5)	BD Biosciences	553049
PE anti-mouse CD8 Antibody (clone 53-6.7)	Biolegend	100708
APC/Cy7 anti-mouse CD11b Antibody (clone M170)	BD Biosciences	557657
PE anti-mouse CD11b Antibody (clone M170)	eBiosciences	12-0112-82
PE anti-mouse CD11c Antibody (clone HL3)	BD Biosciences	557401
APC/Cy7 anti-mouse CD16/32 Antibody (clone 93)	Biolegend	101328
PE anti-mouse CD49b Antibody (clone DX5)	Biolegend	108908
PE anti-mouse CD90.2 Antibody (clone 53-2.1)	BD Biosciences	553006
PerCP/Cy5.5 anti-mouse CD105 Antibody (clone MJ7/18)	Biolegend	120416

APC anti-mouse CD115 Antibody (clone AFS98)	eBiosciences	17-1152-82
PE/Cy7 anti-mouse CD115 Antibody (clone AFS98)	eBiosciences	25-1152-82
APC anti-mouse Ccr2 Antibody (clone 475301)	R&D Systems	FAB5538A-100
PE anti-mouse Ccr2 Antibody (clone 475301)	R&D Systems	FAB5538P-100
APC anti-mouse c-Kit/CD117 Antibody (clone 2B8)	BD Biosciences	553356
PE anti-mouse GR-1 Antibody (clone RB6-8C5)	Biolegend	108408
PE anti-mouse IL-12 (p40/p70) Antibody (clone C15.6)	BD Biosciences	554479
PacBlue anti-mouse Ly6C Antibody (clone HK1.4)	Biolegend	128014
FITC anti-mouse Ly6G Antibody (clone 1A8)	BD Biosciences	561105
PE anti-mouse Ly6G Antibody (clone 1A8)	BD Biosciences	551461
PE anti-mouse NK1.1 Antibody (clone PK136)	Biolegend	108708
PE-Cy7 anti-mouse pSTAT1 (clone A15158B)	Biolegend	686408
PE anti-mouse Sca-1 Antibody (clone D7)	Biolegend	108108
PE anti-mouse Ter119 Antibody (clone TER-119)	Biolegend	116208

Commercially available kits

Cytofix/Cytoperm™	BD Biosciences	554714
OptEIA™ TMB Substrate Reagent Set	BD Biosciences	555214
OptEIA™ Mouse IL-3 ELISA Set	BD Biosciences	555228
OptEIA™ Mouse IL-6 ELISA Set	BD Biosciences	555240
OptEIA™ Mouse IL-12 ELISA Set	BD Biosciences	555256
OptEIA™ Mouse IFNγ ELISA Set	BD Biosciences	551866
OptEIA™ Mouse GM-CSF ELISA Set	BD Biosciences	555167
Mouse M-CSF Quantikine ELISA Kit	R&D Systems	MMC00
Mouse G-CSF Quantikine ELISA Kit	R&D Systems	MCS00
RNeasy Plus Micro Kit	Qiagen	74034
GeneChip™ Mouse Gene 2.0 ST Array	ThermoFisher Scientific	902119

Software

Prism v. 7.0a	GraphPad	http://www.graphpad.com
FlowJo v10.2	FlowJo	http://www.flowjo.com
SPOT 5.1 Basic Software	SPOT Imaging	http://www.spotimaging.com/software/
Partek Genomics Suite	Partek	http://www.partek.com/pgs
Gene Set Enrichment Analysis	Broad Institute	http://software.broadinstitute.org/gsea/index.jsp
R version 3.2.3	R Project	https://cran.r-project.org/

Ingenuity Pathway Analysis Qiagen Bioinformatics
https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/

Transcriptome Analysis Console ThermoFisher Scientific
http://www.thermofisher.com/us/en/home/life-science/microarray-analysis/microarray-analysis-instruments-software-services/microarray-analysis-software/affymetrix-transcriptome-analysis-console-software.html

Gene Pattern Broad Institute
http://software.broadinstitute.org/cancer/software/genepattern/