Supplemental Methods

Isolation and processing of organs, whole blood and serum. Bone marrow cells were removed from the leg bones of mice by flushing the bone marrow cavity with cold PBS and filtering the cells over a 70 μm Falcon cell strainer. Spleens were removed and weighed for analysis of splenomegaly. Splenocytes were harvested from whole spleens after digestion in DNase I (Roche) and collagenase (Roche) at 37°C for 30 minutes. Spleen digests were filtered over a 70 μm strainer. Liver leukocytes were harvested by filtering the bulk liver through a 70 μm strainer. After centrifugation, the liver cell pellet was resuspended in 30% Percoll and layered over 70% Percoll prior to being spun at room temperature at 1000 G for 20 minutes. The liver leukocytes were harvested at the Percoll interface. Peritoneal cells were harvested by washing the intact peritoneal cavity with 3 mLs of cold FBS and collecting the cellular exudate. Whole blood for analysis by flow cytometry was harvested by cheek bleed immediately prior to mouse euthanasia. After the tissue and blood cells were harvested, red blood cell lysis was performed using ACK lysis buffer from Lonza prior to cell counting. Cells were counted on a Countess™ Automated Cell Counter from ThermoFisher Scientific to calculate the number of cells harvested from each organ. The cells were then placed in culture or subjected to cell surface marker immunofluorescence staining for FACS analysis. Complete blood counts were obtained from whole blood from cheek bleeds and analyzed using a Hemavet analyzer or a Sysmex XT-2000iV Automated Hematology Analyzer. Serum was obtained by removing whole blood by cardiac puncture after euthanasia, allowing clot formation to occur at 4°C, and removal of the serum after centrifugation. Serum was frozen at -80°C until ELISAs were performed. ELISAs were performed on sera collected at euthanasia by terminal bleed or from cell culture supernatants where indicated. BD OptEIA ELISA sets for murine IL-12 (p70) were obtained from BD Biosciences. *In vitro* cell cultures were performed after leukocytes were harvested from mouse tissues as described above. Leukocytes were cultured in DMEM (Gibco) supplemented with 10% heat-inactivated FBS (Atlanta Biologicals) and Penicillin-Streptomycin-L-Glutamine (Cellgro). Cells were maintained at 37°C in 5% CO_2 and 95% humidity and stimulated as described in the main text methods. Mixed *in vitro* cultures: Bone marrow and splenocytes from TLR9-sufficient and TLR9-deficient Yet40 (IL-12 reporter) mice were stimulated with or without 10 μg/mL CpG1826 in isolation or in mixed cultures for 20 hours. Thereafter, cell culture supernatants were analyzed for IL-12 production by ELISA and cells were analyzed for YFP expression by flow cytometry.

Cellular immunophenotyping. Fluorescently-labeled cells were analyzed on a BD LSRII, Miltenyi MacsQuant, or BD FACS Aria II. FACS data were analyzed using FlowJo software, which was used to create all flow cytometry plots. All populations were gated on forward and side scatter to limit inclusion of dead cells, debris and doublets. Live cells were identified by excluding cells staining positive for Live-Dead reagents obtained from ThermoFisher Scientific. Inflammatory monocytes were identified as $Ly6G^-Ly6C^+Ccr2^+$ or $Ly6G^-Ly6C^+CD115^+$ cells. Myeloid progenitors were identified as CMPs ($Lin^-c\text{-}Kit^+CD105^-CD16/32^{low}CD115^-$), GMPs ($Lin^-c\text{-}Kit^+CD105^-CD16/32^{high}CD115^-$), MDPs ($Lin^-c\text{-}Kit^+CD105^-CD115^{high}$), or common monocyte progenitors (cMoPs; $Lin^-c\text{-}Kit^+CD105^-CD115^{high}Ly6C^+$). The lineage panel included antibodies against B220, CD4, CD5, CD8a, CD11b, CD11c, CD90.2, DX5, Gr-1 (Ly6C/Ly6G), NK1.1, and Ter119, except for identification of cMoPs where Ly-6G was used instead of GR-1. Fluorescently labeled antibodies were obtained from Biolegend, eBioscience, BD Biosciences, and R&D Systems.

Detailed methods for the microarray analysis. Mice were treated with 4 doses of PBS (N=3) or CpG1826 (N=3), as described. Twenty-four hours after the last injection, CMPs from the spleen and bone marrow were isolated and sorted directly into lysis buffer using a FACS Aria. RNA was isolated from sorted CMPs using the Qiagen RNAeasy Micro kit per the manufacturer's instructions. Further analysis and processing of the RNA for quality, amplification, and hybridization to the mouse Affymetrix ST 2.0 Chip were performed by the NAPcore at the Children's Hospital of Philadelphia as previously described (20). Too few CMPs from the spleens of PBS-treated mice were obtained to generate enough RNA for further analysis. Normalization of the individual chips was performed with robust multichip averaging using Affymetrix software. The microarray dataset was filtered to include only probesets with annotations to gene names in the Refseq and ENSEMBL databases, which resulted in 10,527 transcripts for analysis. To assess whether transcriptional differences could segregate the three groups we performed Principal Component Analysis (PCA) using these 10,527 transcripts and looked for clustering of samples. The 10,527 transcripts were further filtered using a $\log_2$ intensity value of 5 as an expression floor to eliminate non-expressed genes and a minimum $\log_2$ difference between row values of 0.5 to select genes that varied across the samples. These filtering parameters resulted in 3,110 transcripts used for the unsupervised hierarchical clustering analysis that is shown in Figure 6C using the GenePattern platform. The above-mentioned filtering parameters were also used for 2-way comparison analyses. 2-way comparisons (1 – CMPs isolated from bone marrow versus spleen of CpG-treated mice; 2 – CMPs isolated from the bone marrow of PBS-treated versus CpG-treated mice) were completed using publically available software from the Broad Institute to generate lists of differentially expressed genes (DEGs) with an FDR cut-off of 0.1 using the Benjamini-Hochberg procedure. DEGs were further analyzed with IPA Upstream Analysis software to generate the predicted regulators of the transcriptional changes between spleen and bone marrow CMPs isolated from CpG-treated mice using a p value less than 0.0001 and an absolute value Z score greater than or equal to 2.

Quantitative RT-PCR. Mice were treated with 4 doses of PBS or CpG1826, as described in the main text methods. Inflammatory monocytes ($Ly6G^{-}Ly6C^{hi}Ccr2^{+}$) were sorted using a BD FACS Aria II. RNA was isolated using the Qiagen RNAeasy Micro kit per the manufacturer's protocol. cDNA was made with 50 ng of RNA per sample using the Superscript III First-Strand Synthesis System according to manufacturer's instructions (Invitrogen). Quantitative PCR was performed using TaqMan gene expression assays (Applied Biosystems) with probes for mouse TLR9 (Mm00446193_m1) and 18S rRNA (Mm03928990_g1). Real-time PCR reactions were performed on the StepOnePlus™ Real-Time PCR System using the Taqman mastermix per the manufacturer's instructions (Applied Biosystems). The delta–delta CT method was used to quantify results. Values for each gene were normalized to 18S rRNA and one of the spleen inflammatory monocyte samples was set at 1 for fold-change comparisons.

Supplemental Figures

Figure S1. Identification of TLR9 responsive cells in multiple tissues. Yet40 (IL-12 reporter) mice were treated with or without 4 doses of CpG1826. Cells were isolated from multiple tissues and stimulated *ex vivo* with or without CpG prior to YFP analysis by flow cytometry. Plots show representative data of at least two separate experiments with 4 mice per experiment.

Figure S2. Surface phenotyping of YFP^{+}Ly6C^{mid}Ccr2^{+} cells. Yet40 mice were treated with or without 4 doses of CpG1826. Cells were isolated from multiple tissues and stimulated *ex vivo* with or without CpG prior to YFP analysis by flow cytometry. Further characterization of the cell surface markers expressed by YFP^{+}Ly6C^{mid}Ccr2^{+} cells was performed and representative FACS plots are shown. Plots show representative data of 3 experiments (N = 3).

Figure S3. Identification of inflammatory monocytes in multiple tissues. C57BL/6 mice were treated with or without 4 doses of CpG1826. Cells were isolated from multiple tissues and analyzed by flow cytometry. Inflammatory monocytes were identified as Ly6G^{-}Ly6C^{+}Ccr2^{+}. The expression of CD115 by inflammatory monocytes (iMonos) in red was compared to CD115 expression on other cells in gray. Plots show representative data of 7-8 mice per group.

Figure S4. Inflammatory monocytes express TLR9 and are activated by CpG to produce IL-12 in a cell-intrinsic manner. (A) Mice were treated with or without 4 doses of CpG1826 and spleen Ly6G^{-}Ly6C^{hi}Ccr2^{+} inflammatory monocytes were sorted for analysis of TLR9 expression by QT-PCR. (B-D) Bone marrow and spleen cells were isolated from TLR9-sufficient (TLR9$^{+/+}$) or TLR9-deficient (TLR9$^{-/-}$) Yet40 (IL-12 reporter) mice. (B) Cells were stimulated with or without CpG and YFP expression was measured by flow cytometry in Ly6C^{hi}Ccr2^{+}CD11b^{+} inflammatory monocytes. (C) Cells from congenically-labeled TLR9-sufficient and TLR9-deficient Yet40 mice were stimulated in mixed cultures and YFP expression was measured by flow cytometry. Plots are representative histograms of 3 separate experiments. (D) Supernatants from TLR9-sufficient, TLR9-deficient, and mixed cultures were measured for IL-12 by ELISA. The graphs show representative data of 3 separate experiments analyzed by one-way ANOVA with Bonferroni post-test analyses.

Figure S5. Identification of inflammatory monocytes in Ccr2$^{-/-}$ mice. C57BL/6 and Ccr2$^{-/-}$ mice were treated with or without 4 doses of CpG1826. (A) Inflammatory monocytes were identified in the spleens and livers of mice by gating on Ly6G^{-}Ly6C^{hi}CD115^{+} cells. (B) Baseline numbers of Ly6G^{-}Ly6C^{hi}CD115^{+} inflammatory monocytes were enumerated from multiple tissues of C57BL/6 and Ccr2$^{-/-}$ mice. Plots show representative data of 4 mice per group.

Figure S6. CpG-induced systemic inflammation and peripheral inflammatory monocyte accumulation occur independent of IFNα and IFNγ. (A) C57BL/6 and IFNAR1$^{-/-}$ mice were treated with and without 4 doses of CpG1826 before analyzing splenomegaly, serum IL-12, and enumeration of Ly6C^{hi}Ccr2^{+} inflammatory monocytes in tissues (N = 3 mice per group). (A) C57BL/6 and IFNγ$^{-/-}$ mice were treated with and without 4 doses of CpG1826 before analyzing splenomegaly, serum IL-12, and enumeration of Ly6C^{hi}Ccr2^{+} inflammatory monocytes in tissues (N = 3-6 mice per group). Graphs were analyzed by two-way ANOVA. (p-values denoted as follows - * *in vivo* treatment with vs. without CpG, # WT vs. IFNγ$^{-/-}$/IFNAR1$^{-/-}$, and + interaction term)

Figure S7. Identification of myeloid progenitors in multiple tissues. C57BL/6 mice were treated with PBS or CpG1826 for 4 doses. Cells were isolated from multiple tissues and analyzed by flow cytometry for expression of myeloid progenitor cell surface

markers. Plots show representative data from 3 separate experiments with 10-12 mice per group.

Figure S8. cMoPs are increased in extramedullary tissues during CpG-induced inflammation. C57BL/6 mice were treated with or without 4 doses of CpG1826. (A) Representative plots used to identify bone marrow, spleen, and liver Ly6C$^+$ cMoPs within the Lin$^-$cKit$^+$CD105$^-$CD115$^+$ myeloid progenitor cell gate are shown. (B) cMoPs were enumerated in the bone marrow, spleen, and liver. Plots show representative data and graphs show compiled data from 4-8 mice per group. Graphs were analyzed by Mann-Whitney *U* tests.

Figure S9. Myeloid progenitor cell culture schematic and phenotype of progeny. (A) This schematic shows the *in vivo* treatment of mice with PBS or CpG prior to sorting myeloid progenitor cells used in myelopoiesis assays. Myelopoiesis assays proceeded for 12 days with supplementation of growth media with myeloid specific growth factors on Day 0 and Day 7. In some experiments, cellular progeny from myelopoiesis assays were stimulated with or without CpG during the last 24 hours of culture. (B) The cellular progeny from myelopoiesis assays were stained with the myeloid cell markers Ly6C and CD11b with representative flow plots shown for cultures of myeloid progenitors isolated from the bone marrow (B) or spleen (C). Plots show representative data from at least 2 experiments with 3 mice per experiment.

Figure S10. Ly6C^{hi} cells differentiate into Ly6C^{mid} cells during *in vitro* myelopoiesis assays. (A) Bone marrow CMPs were sorted from C57BL/6 mice and cultured with myeloid specific growth factor supplementation on Days 0, 7, and 14. CMP progeny were harvested on Days 4, 7, 10, 14, and 18 for analysis of Ly6C and CD11b expression. (B-D) Bone marrow CMPs were sorted from C57BL/6 mice and cultured with myeloid specific growth factor supplementation on Days 0 and 7 before CMP progeny were sorted into Ly6C^{hi}CD11b$^+$, Ly6C^{mid}CD11b$^+$, and CD11b$^-$ cell populations on Day 10. 2000 CMP progeny of the indicated phenotype were then cultured from Day 10 to 24 with additional myeloid specific growth factor supplementation on Days 10 and 17. (B) On Day 24, the cultures were analyzed for expression of Ly6C and CD11b and representative flow cytometry plots are shown. (C) The total number of each type of CMP progeny produced from these cultures was enumerated. (D) The expression of additional cell surface markers is shown for Day 24 CD11b$^-$ cell progeny with Ly6C^{hi}CD11b$^+$ cells marked in red, Ly6C^{mid}CD11b$^+$cells marked in blue, and CD11b$^-$ cells marked in green. Plots show representative data and graphs show compiled data from 4 mice with separate myelopoiesis assays run from CMPs sorted from individual mice.

Figure S11. YFP$^+$ CMP progeny express markers similar to those expressed by Ly6C^{mid}Ccr2$^+$ TLR9 responsive cells identified *in vivo*. CMPs from Yet40 (IL-12 reporter) mice were cultured as described in Figure S10A. CMP progeny were harvested 24 hours after stimulation with or without 10 μg/mol CpG1826 on Days 4, 7, 10, 14, and 18. (A) Representative FACS plots show the identification of YFP$^+$ CMP progeny. (B) Representative FACS plots show the cell surface expression of Ly6C, CD11b, CD11c, MHC class II, CD80, and CD86 on YFP$^+$ and YFP$^-$ CMP progeny from the gates indicated in (A). Plots show representative data from 2 separate experiments (total N = 7). Separate myelopoiesis assays were run from CMPs sorted from individual mice.

Figure S12. PCA analysis of 10,527 transcripts reveals distinct clustering of CMP populations from spleen and bone marrow as well as differences induced by CpG

exposure. PCA analysis was performed using all 10,527 transcripts from the microarray dataset, as described in the methods. A two-dimensional plot of the top two components capturing variation between samples is shown with the x-axis explaining 45% of the dataset variance, and the y-axis explaining 12.25% of the dataset variance. Bone marrow CMPs isolated from a PBS-treated mouse (blue points), bone marrow CMPs isolated from a CpG-treated mouse (red points) and splenic CMPs isolated from a CpG-treated mouse (green points) form three distinct clusters along these axes with the x-axis separating spleen CMPs from bone marrow CMPs, and the y-axis separating all three populations from each other.

Supplemental Tables

Table S1. The top 30 upregulated and top 30 downregulated differentially expressed genes (DEGs) between spleen and bone marrow CMPs isolated from CpG-treated mice.

Upregulated Genes	Absolute Fold Change
Elane	4.516155
Hba-a2	3.490993667
Ifi205	3.351694333
Pyhin1	3.169947667
Ctsg	3.121305
Egln3	2.848421333
Emb	2.840471
Prss57	2.807495
Hspa1b	2.787002
Cebpe	2.778084667
Tifab	2.773028333
Prtn3	2.711546
Bex6	2.677333
Ly6d	2.501624333
Il1r1	2.479228667
Scin	2.453412333
F13a1	2.448659
Parp8	2.418605667
Gm885	2.347355667
Hk3	2.338814667
St8sia4	2.323179333
Satb1	2.304795667
Pcp4l1	2.299667
Ccl9	2.299426667
Mpo	2.294403333
Cxcr2	2.282835333
Kmo	2.24102
Il18rap	2.208517667
Cd34	2.207703333
Il7r	2.195504667

Downregulated Gene	Absolute Fold Change
Ikzf1	-3.223376788
Dtx3	-3.21723753
Stard3	-3.085680079
Fcho2	-3.0609996
Gemin5	-2.910332651
Ttll4	-2.889318862
Dnajc21	-2.859656631
Pfkfb2	-2.808836224
Hectd1	-2.781035194
Rnaset2a	-2.755175137
Bag2	-2.734860723
Ddx5	-2.695781103
Rnaset2b	-2.685813533
Spop	-2.679102465
Btbd2	-2.671751306
Zbed3	-2.659725374
Ddx21	-2.650949482
Zfp187	-2.623677994
Nnt	-2.576896746
Wdr75	-2.572093641
Ptch1	-2.56857012
Zfp955b	-2.56747759
Drosha	-2.561755383
Casp8	-2.519291474
Mrpl12	-2.508283607
Ifnar2	-2.496953716
Heatr5a	-2.493614269
Dusp23	-2.487549813
Mef2c	-2.485733959
Akap10	-2.46562913

Table S2. Differentially expressed genes between bone marrow CMPs isolated from PBS- and CpG- treated mice. Highlighted genes are shared DEGs between the two comparator groups listed in Table S1 and S2.

Upregulated Genes	Absolute Fold Change
Csf2rb2	2.692477
Ccl5	2.678479
H2-Eb1	2.465277
H2-Aa	2.280898
Ly6a	2.250188333
H2-DMb2	2.232294667
Ly86	1.436957333

Downregulated Genes	Absolute Fold Change
Slc36a1	-2.31523463
Mllt6	-2.25521688
Klhl24	-1.967556308
Dyrk3	-1.849582118
Lst1	-1.408684162
Mdga1	-1.396890615
Gm10409	-1.226617602
Gm10409	-1.226617602
Gm10409	-1.118690863
Tgtp2/1	-1.087893845
Tmem50b	-1.001285317

Table S3. Predicted upstream regulators of gene expression changes between spleen and bone marrow CMPs isolated from CpG-treated mice.

Activated Regulator in Spleen CMPs	Molecule Type	z-score	p-value
MKL2	transcription regulator	3.9	2.48E-16
MKL1	transcription regulator	3.9	9.33E-14
IL10RA	transmembrane receptor	3.1	8.44E-07
KCNK9	ion channel	2.9	8.58E-07
MYC	transcription regulator	2.6	3.15E-08
Cdc42	enzyme	2.4	9.85E-05
NCSTN	peptidase	2.4	8.90E-14
ADAMTS12	peptidase	2.4	6.90E-06
EPO	cytokine	2.2	1.10E-08
SRF	transcription regulator	2.1	2.09E-10
GFI1	transcription regulator	2.1	1.13E-07

Suppressed Regulator in Spleen CMPs	Molecule Type	z-score	p-value
SPI1	transcription regulator	-4.2	3.31E-14
CEBPA	transcription regulator	-4.0	5.43E-08
IL6	cytokine	-3.5	2.89E-14
CEBPE	transcription regulator	-3.3	2.49E-11
HOXA9	transcription regulator	-3.2	8.72E-05
TNF	cytokine	-3.2	5.74E-19
TGFB1	growth factor	-3.1	4.22E-13
HOXA7	transcription regulator	-3.0	6.90E-06
SOX4	transcription regulator	-2.9	3.04E-13
ETS1	transcription regulator	-2.9	5.22E-06
TGM2	enzyme	-2.7	2.45E-08
CD38	enzyme	-2.5	1.14E-15
IFNG	cytokine	-2.4	3.20E-14
CSF3	cytokine	-2.3	3.94E-14
SMARCA4	transcription regulator	-2.2	5.77E-05
IL2	cytokine	-2.2	4.10E-10
TCF3	transcription regulator	-2.1	2.42E-06
NFKBIA	transcription regulator	-2.1	8.21E-07

Figure S1

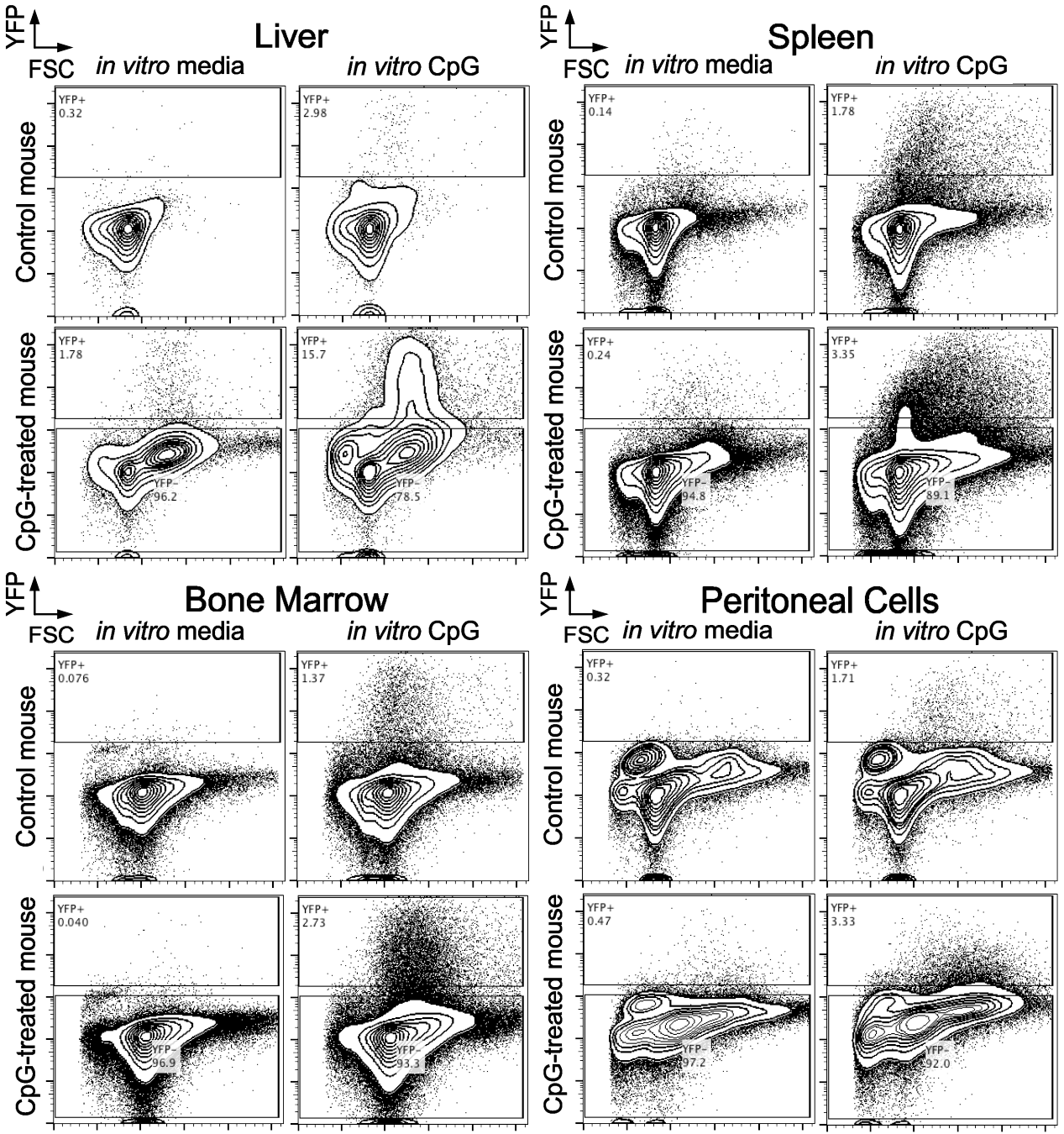

Liver
Spleen
Bone Marrow
Peritoneal Cells
YFP
FSC
in vitro media
in vitro CpG
Control mouse
CpG-treated mouse
YFP+ 0.32
YFP+ 2.98
YFP+ 1.78
YFP- 96.2
YFP+ 15.7
YFP- 78.5
YFP+ 0.14
YFP+ 1.78
YFP+ 0.24
YFP- 94.8
YFP+ 3.35
YFP- 89.1
YFP+ 0.076
YFP+ 1.37
YFP+ 0.040
YFP- 96.9
YFP+ 2.73
YFP- 93.3
YFP+ 0.32
YFP+ 1.71
YFP+ 0.47
YFP- 97.2
YFP+ 3.33
YFP- 92.0

Figure S2

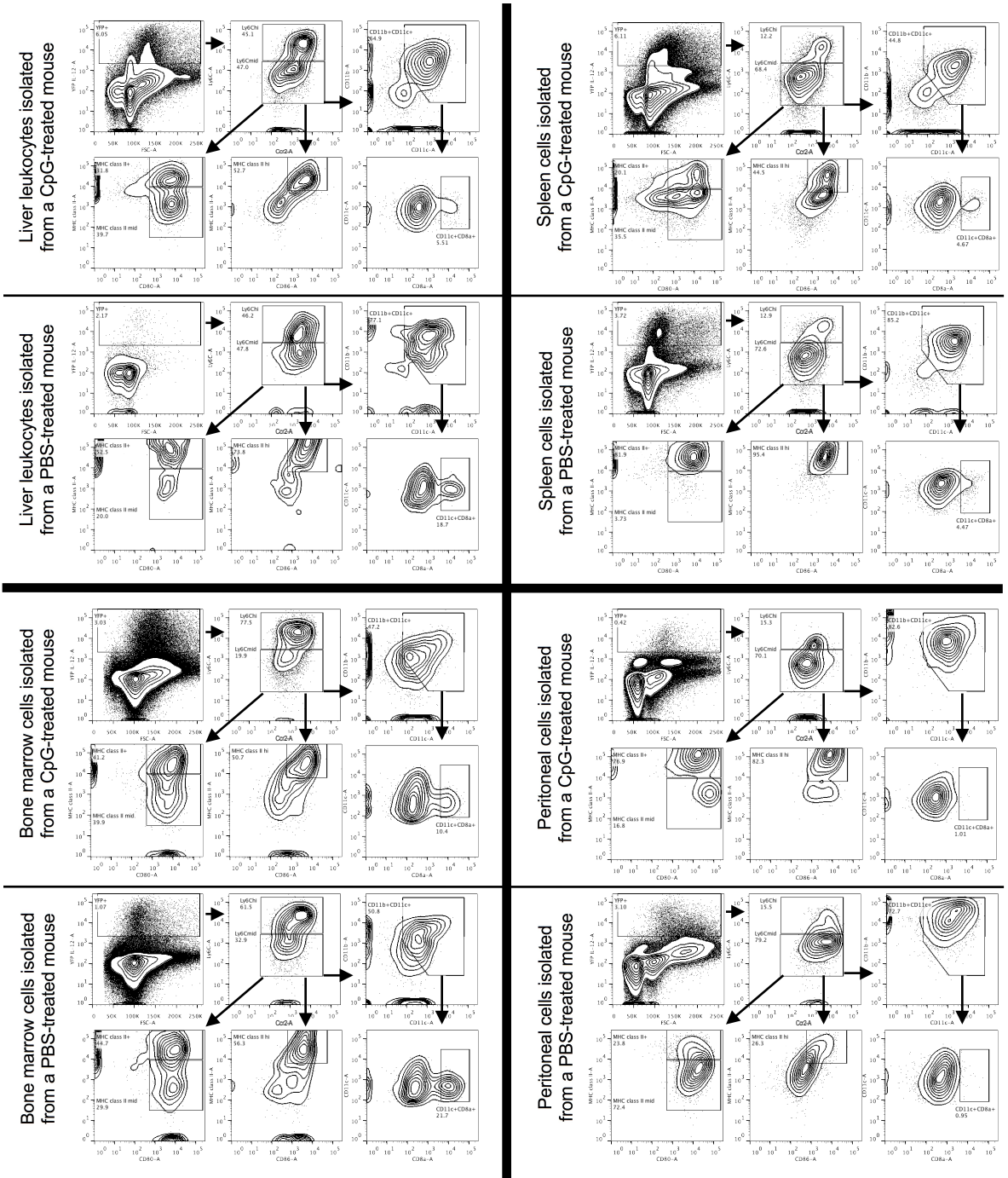

Liver leukocytes isolated from a CpG-treated mouse
Liver leukocytes isolated from a PBS-treated mouse
Spleen cells isolated from a CpG-treated mouse
Spleen cells isolated from a PBS-treated mouse
Bone marrow cells isolated from a CpG-treated mouse
Bone marrow cells isolated from a PBS-treated mouse
Peritoneal cells isolated from a CpG-treated mouse
Peritoneal cells isolated from a PBS-treated mouse
YFP+
Ly6Chi
Ly6Cmid
CD11b+CD11c+
MHC class II+
MHC class II mid
MHC class II hi
CD11c+CD8a+
FSC-A
Cx3r1-A
CD80-A
CD86-A
CD8a-A

Figure S3

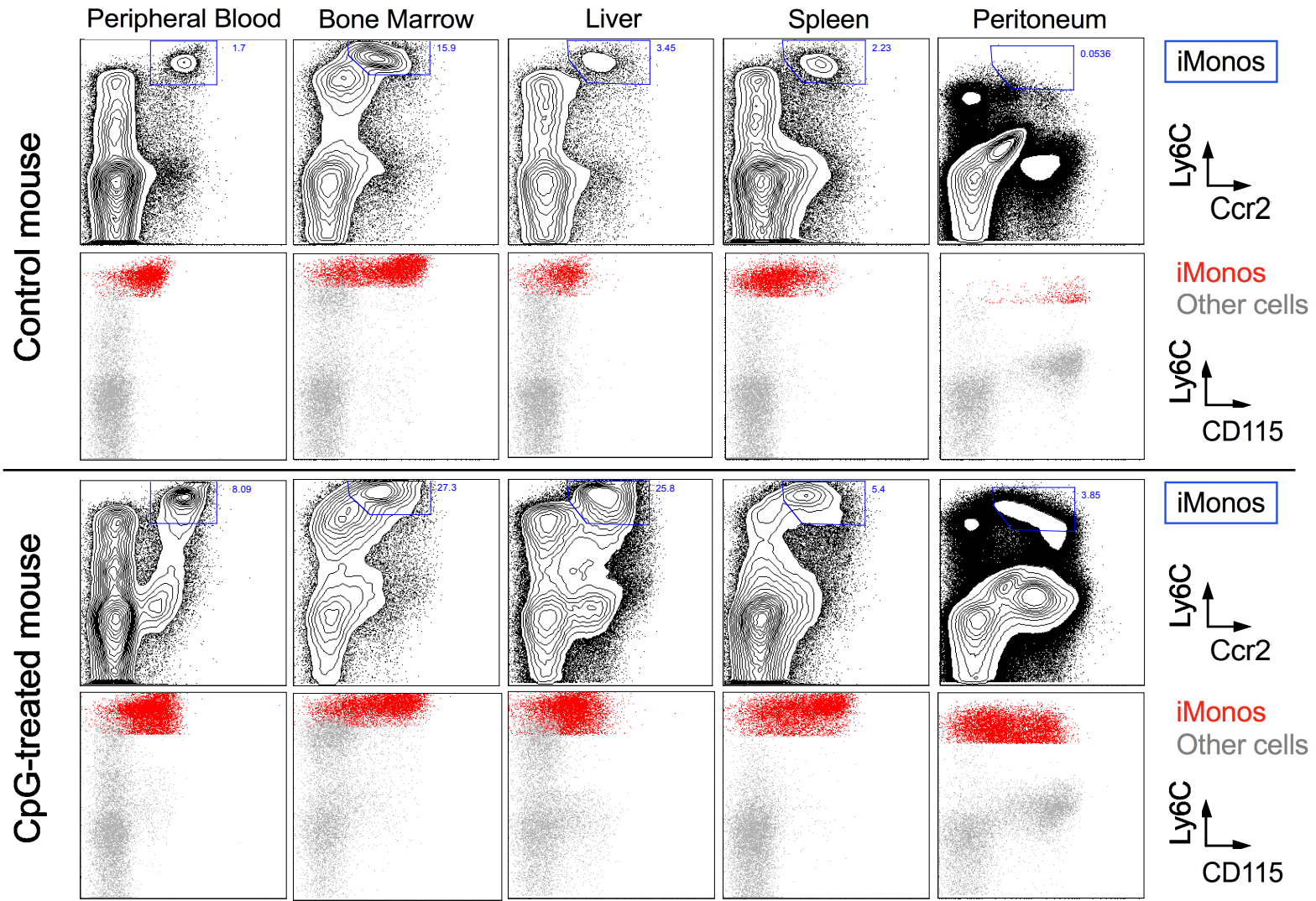

Peripheral Blood
Bone Marrow
Liver
Spleen
Peritoneum
Control mouse
CpG-treated mouse
1.7
15.9
3.45
2.23
0.0536
8.09
27.3
25.8
5.4
3.85
iMonos
Ly6C
Ccr2
iMonos
Other cells
Ly6C
CD115

Figure S4

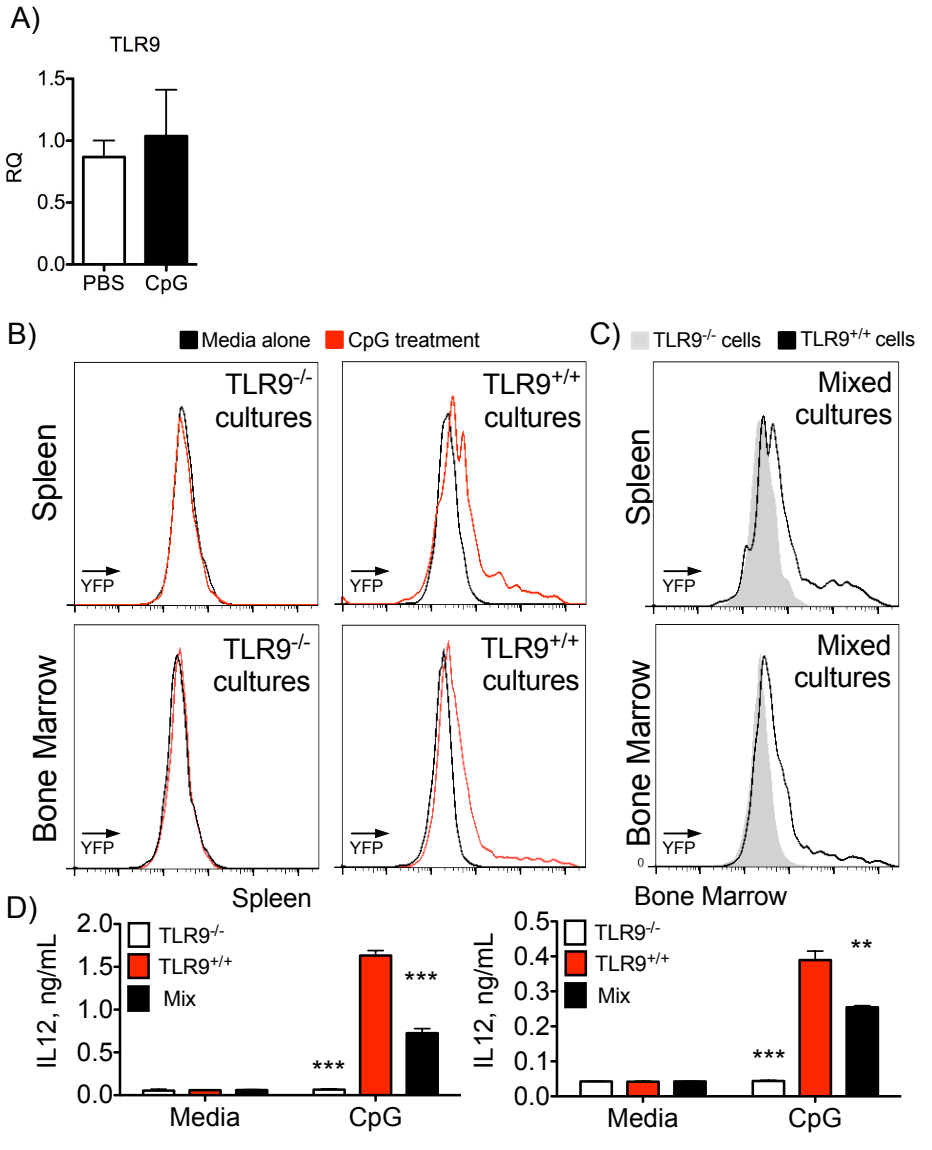
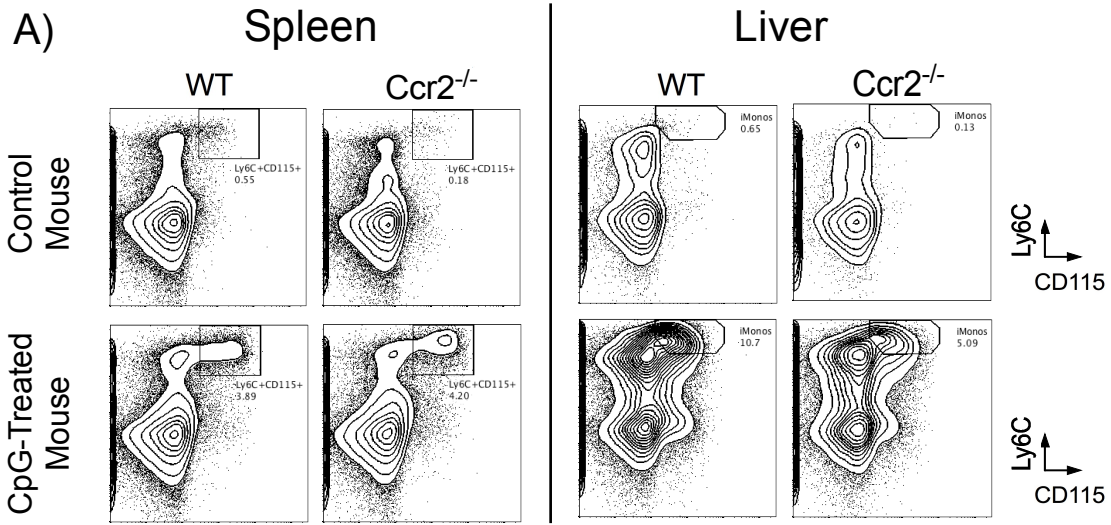

A)
TLR9
RQ
1.5
1.0
0.5
0.0
PBS
CpG
B)
Media alone
CpG treatment
TLR9-/- cultures
TLR9+/+ cultures
Spleen
Bone Marrow
YFP
C)
TLR9-/- cells
TLR9+/+ cells
Mixed cultures
D)
Spleen
Bone Marrow
IL12, ng/mL
TLR9-/-
TLR9+/+
Mix
Media
CpG
***
**

Figure S5


A)
Spleen
Liver
WT
Ccr2-/-
WT
Ccr2-/-
Control Mouse
CpG-Treated Mouse
Ly6C+CD115+ 0.55
Ly6C+CD115+ 0.18
Ly6C+CD115+ 3.89
Ly6C+CD115+ 4.20
iMonos 0.65
iMonos 0.13
iMonos 10.7
iMonos 5.09
Ly6C
CD115
Ly6C
CD115

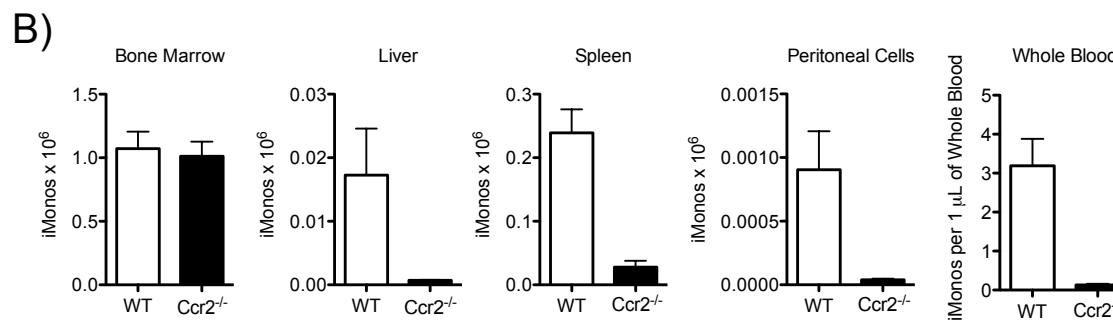

B)
Bone Marrow
Liver
Spleen
Peritoneal Cells
Whole Blood
iMonos x 10^6
iMonos x 10^6
iMonos x 10^6
iMonos x 10^6
iMonos per 1 µL of Whole Blood
1.5
1.0
0.5
0.0
0.03
0.02
0.01
0.00
0.3
0.2
0.1
0.0
0.0015
0.0010
0.0005
0.0000
5
4
3
2
1
0
WT
Ccr2-/-
WT
Ccr2-/-
WT
Ccr2-/-
WT
Ccr2-/-
WT
Ccr2-/-

Figure S6

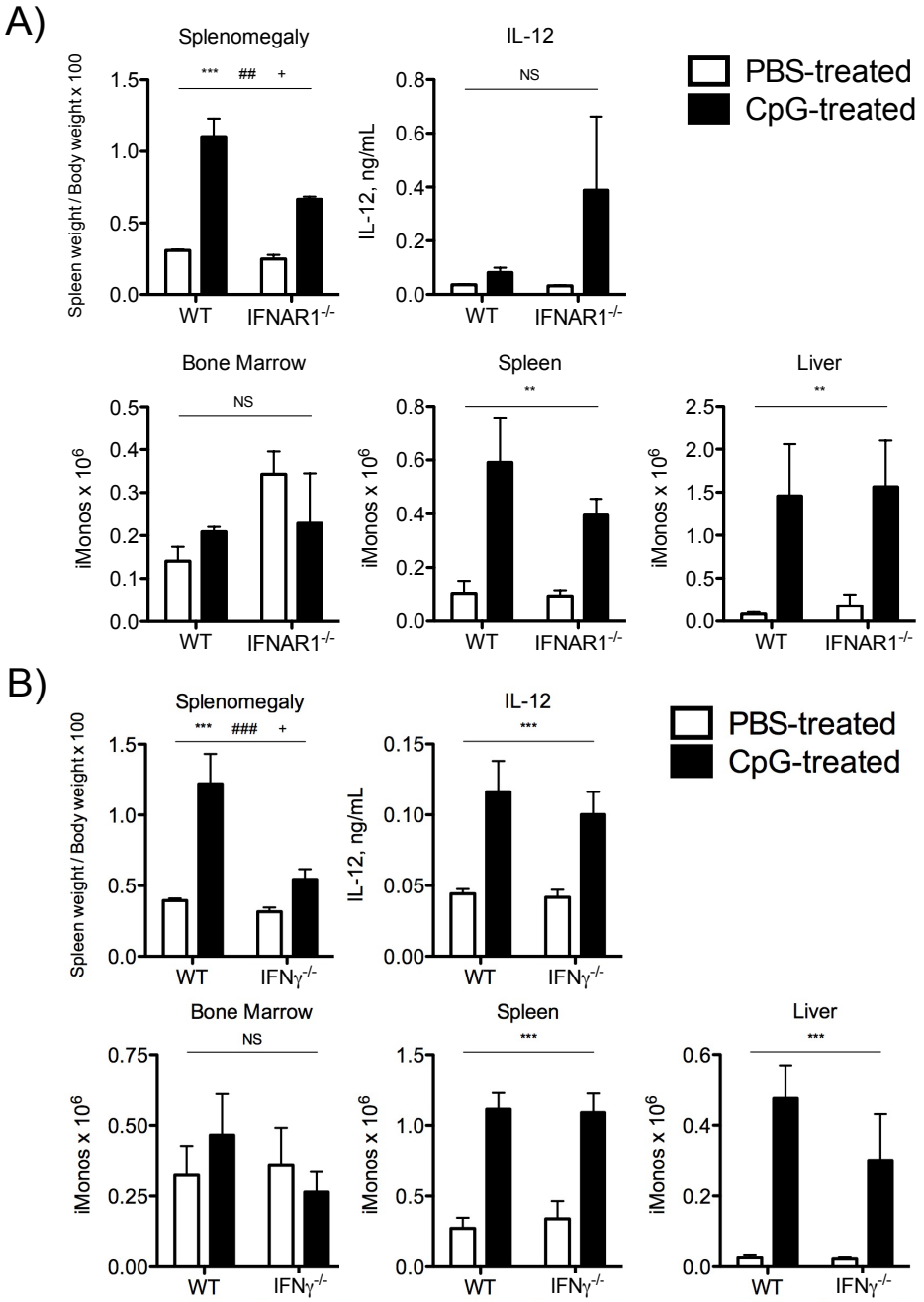

A)
Splenomegaly
*** ## +
Spleen weight / Body weight x 100
1.5
1.0
0.5
0.0
WT
IFNAR1-/-
IL-12
NS
IL-12, ng/mL
0.8
0.6
0.4
0.2
0.0
WT
IFNAR1-/-
PBS-treated
CpG-treated
Bone Marrow
NS
iMonos x 10^6
0.5
0.4
0.3
0.2
0.1
0.0
WT
IFNAR1-/-
Spleen
**
iMonos x 10^6
0.8
0.6
0.4
0.2
0.0
WT
IFNAR1-/-
Liver
**
iMonos x 10^6
2.5
2.0
1.5
1.0
0.5
0.0
WT
IFNAR1-/-
B)
Splenomegaly
*** ### +
Spleen weight / Body weight x 100
1.5
1.0
0.5
0.0
WT
IFNγ-/-
IL-12
***
IL-12, ng/mL
0.15
0.10
0.05
0.00
WT
IFNγ-/-
PBS-treated
CpG-treated
Bone Marrow
NS
iMonos x 10^6
0.75
0.50
0.25
0.00
WT
IFNγ-/-
Spleen
***
iMonos x 10^6
1.5
1.0
0.5
0.0
WT
IFNγ-/-
Liver
***
iMonos x 10^6
0.6
0.4
0.2
0.0
WT
IFNγ-/-

Figure S7

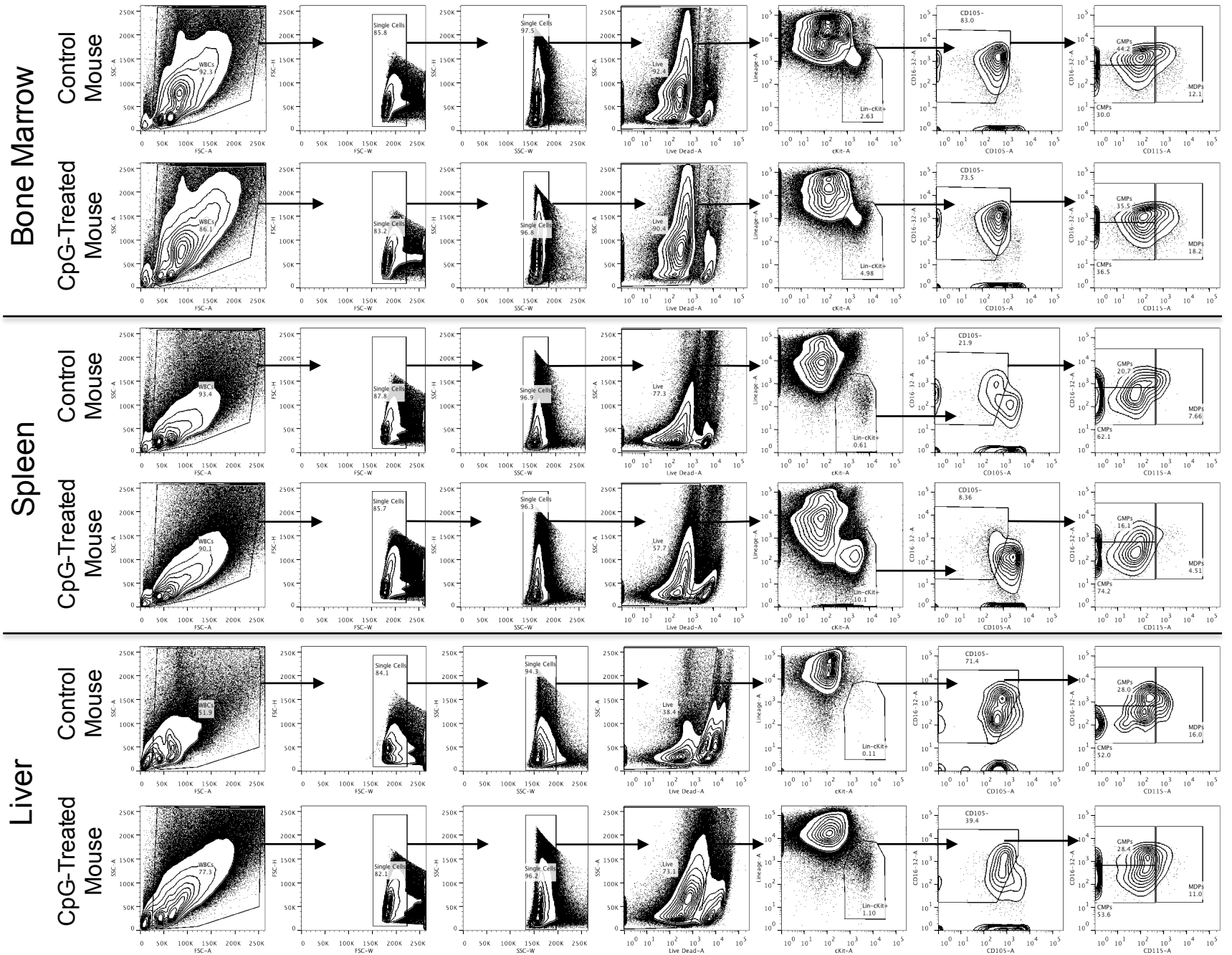
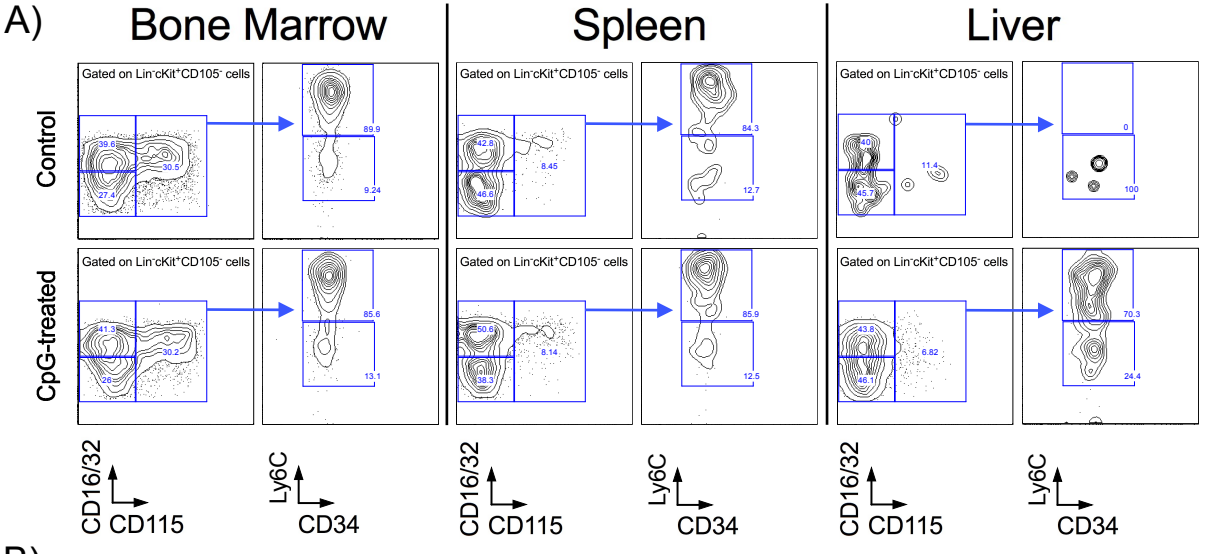

Bone Marrow
Control Mouse
CpG-Treated Mouse
Spleen
Control Mouse
CpG-Treated Mouse
Liver
Control Mouse
CpG-Treated Mouse
WBCs 92.3
Single Cells 85.8
Single Cells 97.5
Live 92.4
Lin-cKit+ 2.63
CD105- 83.0
GMPs 44.2
MDPs 12.1
CMPs 30.0
WBCs 86.1
Single Cells 83.2
Single Cells 96.8
Live 90.4
Lin-cKit+ 4.98
CD105- 73.5
GMPs 35.5
MDPs 18.2
CMPs 36.5
WBCs 93.4
Single Cells 87.8
Single Cells 96.9
Live 77.3
Lin-cKit+ 0.61
CD105- 21.9
GMPs 20.7
MDPs 7.66
CMPs 62.1
WBCs 90.1
Single Cells 85.7
Single Cells 96.3
Live 57.7
Lin-cKit+ 10.1
CD105- 8.36
GMPs 16.1
MDPs 4.51
CMPs 74.2
WBCs 91.5
Single Cells 84.1
Single Cells 94.3
Live 38.4
Lin-cKit+ 0.11
CD105- 71.4
GMPs 28.0
MDPs 16.0
CMPs 52.0
WBCs 77.3
Single Cells 82.1
Single Cells 96.2
Live 73.1
Lin-cKit+ 1.10
CD105- 39.4
GMPs 28.4
MDPs 11.0
CMPs 53.6

Figure S8


A)
Bone Marrow
Spleen
Liver
Control
CpG-treated
Gated on Lin-cKit+CD105- cells
CD16/32
CD115
Ly6C
CD34

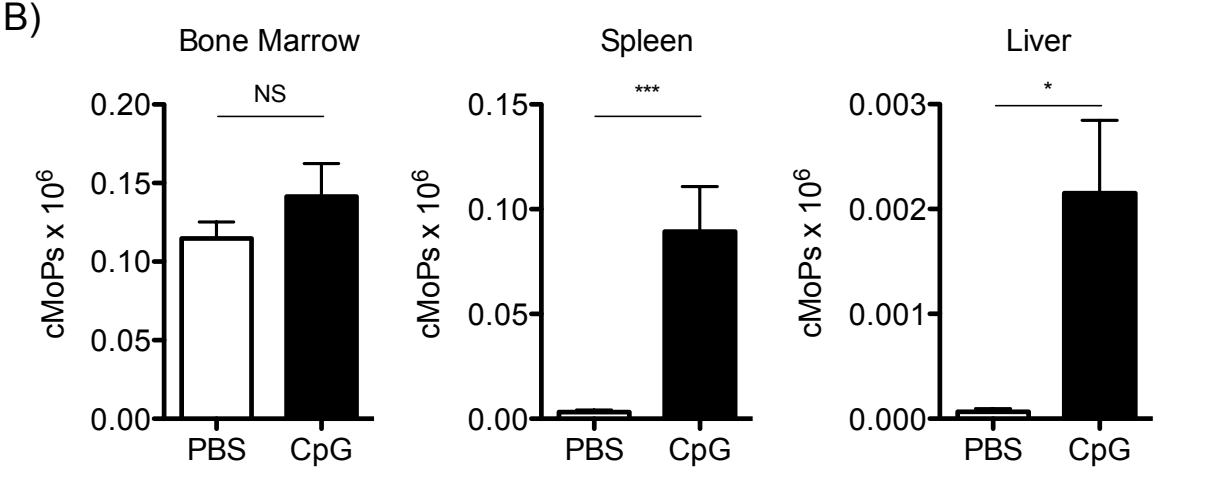
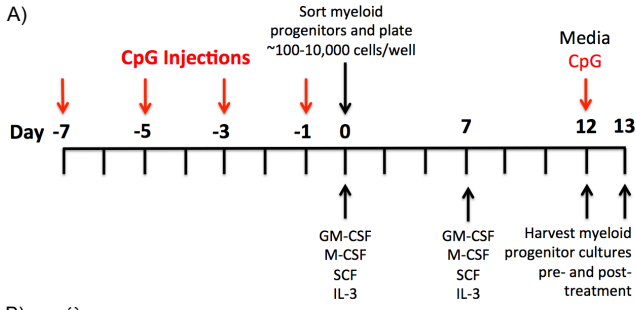
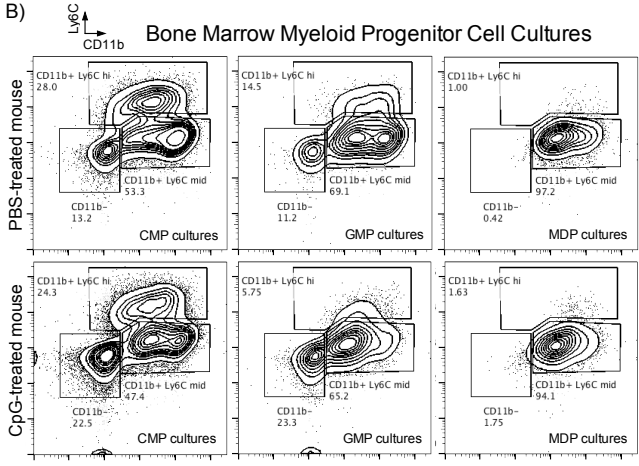

B)
Bone Marrow
Spleen
Liver
NS
***
*
cMoPs x 10^6
PBS
CpG

Figure S9


A)
Sort myeloid progenitors and plate ~100-10,000 cells/well
CpG Injections
Media CpG
Day -7
-5
-3
-1
0
7
12
13
GM-CSF M-CSF SCF IL-3
GM-CSF M-CSF SCF IL-3
Harvest myeloid progenitor cultures pre- and post-treatment


B)
Ly6C
CD11b
Bone Marrow Myeloid Progenitor Cell Cultures
PBS-treated mouse
CpG-treated mouse
CD11b+ Ly6C hi
CD11b+ Ly6C mid
CD11b−
CMP cultures
GMP cultures
MDP cultures
28.0
53.3
13.2
14.5
69.1
11.2
1.00
97.2
0.42
24.3
47.4
22.5
5.75
65.2
23.3
1.63
94.1
1.75

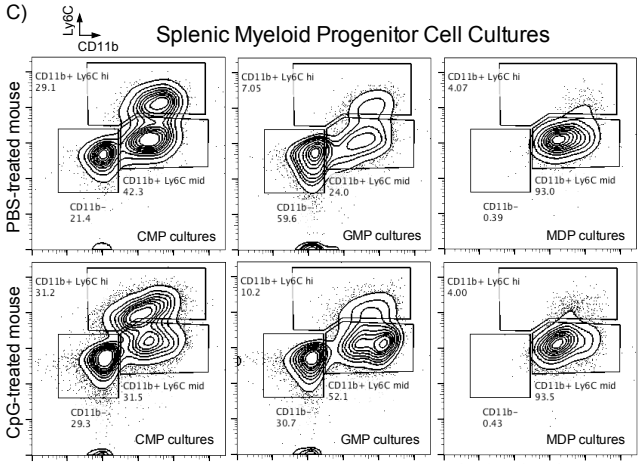

C)
Ly6C
CD11b
Splenic Myeloid Progenitor Cell Cultures
PBS-treated mouse
CpG-treated mouse
CD11b+ Ly6C hi
CD11b+ Ly6C mid
CD11b−
CMP cultures
GMP cultures
MDP cultures
29.1
42.3
21.4
7.05
24.0
59.6
4.07
93.0
0.39
31.2
31.5
29.3
10.2
52.1
30.7
4.00
93.5
0.43

Figure S10

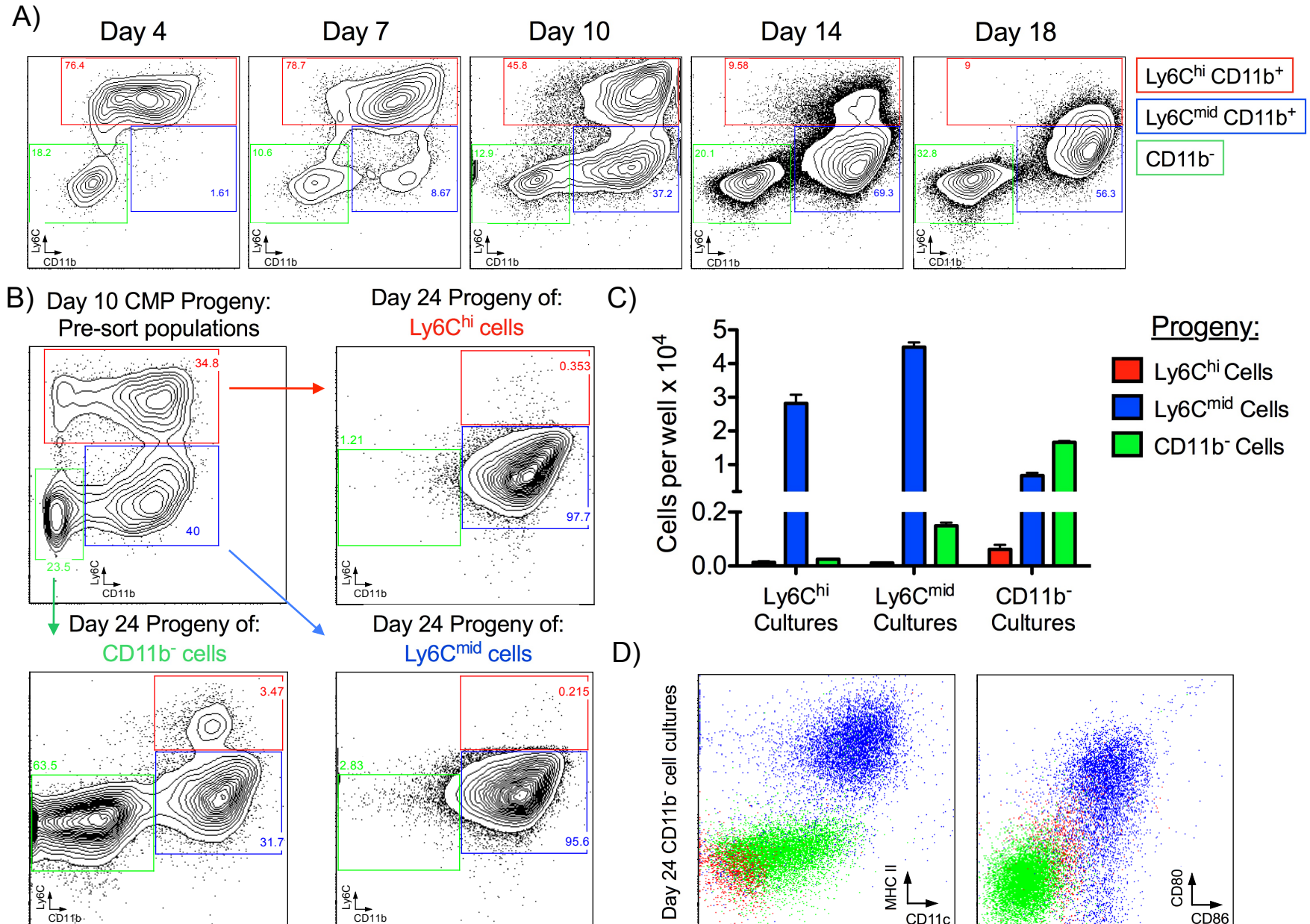

A)
Day 4
Day 7
Day 10
Day 14
Day 18
76.4
18.2
1.61
78.7
10.6
8.67
45.8
12.9
37.2
9.58
20.1
69.3
9
32.8
56.3
Ly6C
CD11b
Ly6C^hi CD11b^+
Ly6C^mid CD11b^+
CD11b^-
B) Day 10 CMP Progeny:
Pre-sort populations
34.8
40
23.5
Day 24 Progeny of:
Ly6C^hi cells
0.353
1.21
97.7
Day 24 Progeny of:
CD11b^- cells
3.47
63.5
31.7
Day 24 Progeny of:
Ly6C^mid cells
0.215
2.83
95.6
C)
Cells per well x 10^4
5
4
3
2
1
0.2
0.0
Ly6C^hi Cultures
Ly6C^mid Cultures
CD11b^- Cultures
Progeny:
Ly6C^hi Cells
Ly6C^mid Cells
CD11b^- Cells
D)
Day 24 CD11b^- cell cultures
MHC II
CD11c
CD80
CD86

Figure S11

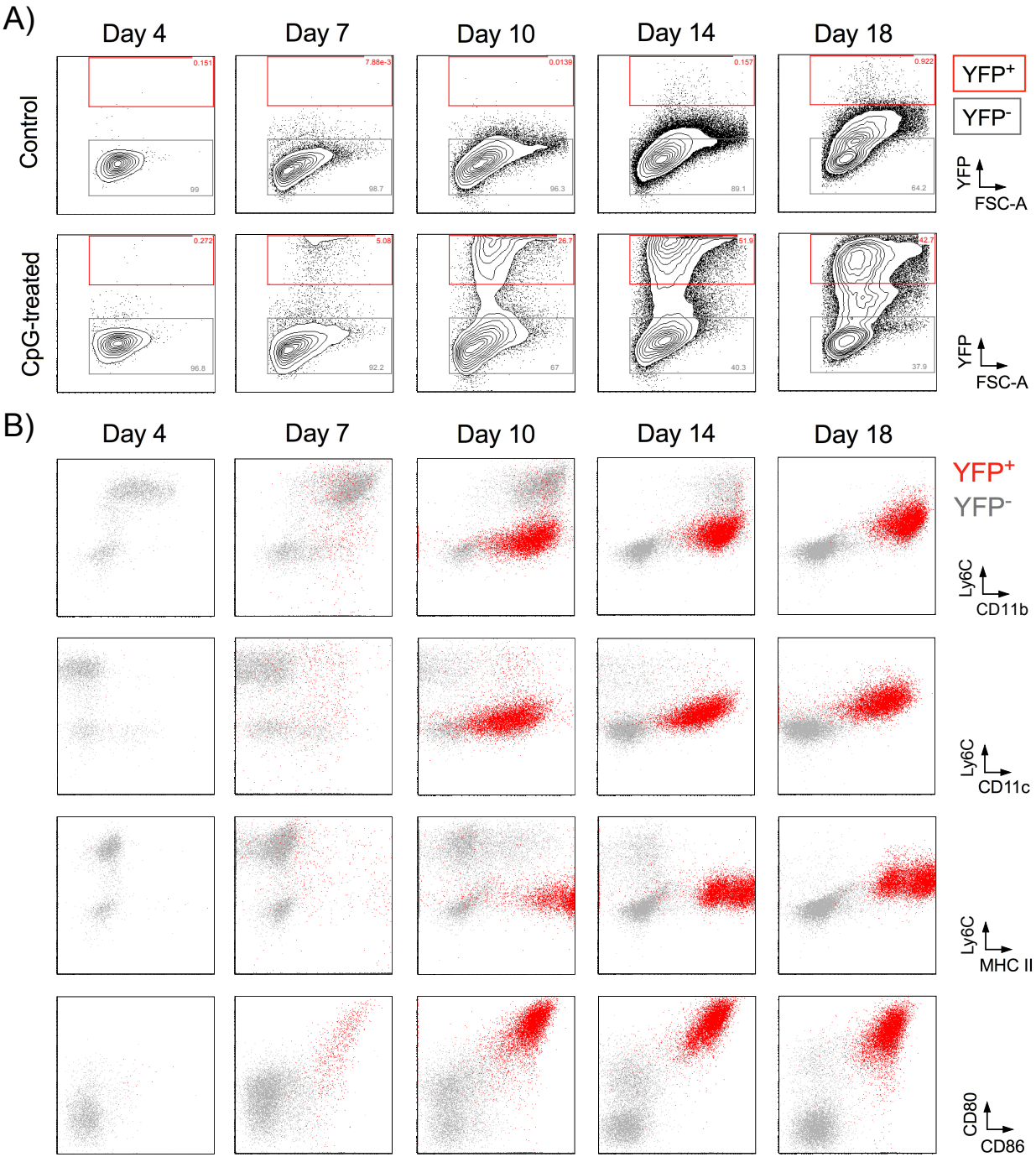
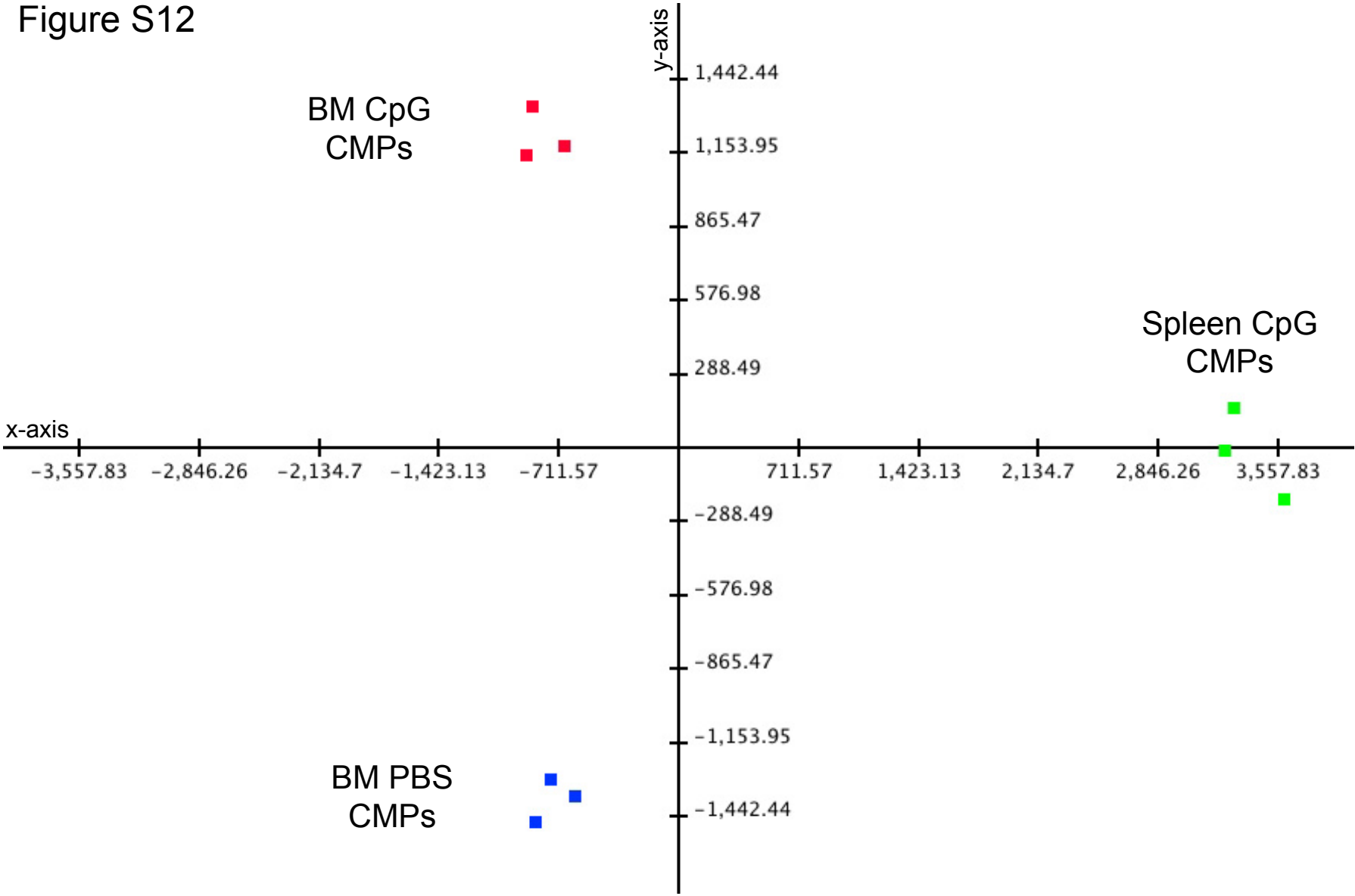

A)
Day 4
Day 7
Day 10
Day 14
Day 18
Control
CpG-treated
0.151
99
7.88e-3
98.7
0.0139
96.3
0.157
89.1
0.922
64.2
0.272
96.8
5.08
92.2
26.7
67
51.9
40.3
42.7
37.9
YFP+
YFP-
YFP
FSC-A
B)
Day 4
Day 7
Day 10
Day 14
Day 18
YFP+
YFP-
Ly6C
CD11b
Ly6C
CD11c
Ly6C
MHC II
CD80
CD86

Figure S12


y-axis
1,442.44
1,153.95
865.47
576.98
288.49
-288.49
-576.98
-865.47
-1,153.95
-1,442.44
x-axis
-3,557.83
-2,846.26
-2,134.7
-1,423.13
-711.57
711.57
1,423.13
2,134.7
2,846.26
3,557.83
BM CpG CMPs
Spleen CpG CMPs
BM PBS CMPs