Supplemental Figure Legends

Figure S1. Identification of tissue resident macrophage populations that clear apoptotic cells *in vivo*, related to Figure 1. (A) Example of gating strategy used to identify AC-engulfing cells. Control bone marrow chimeras generated with WT CD45.1$^+$ bone marrow were compared to chimeras generated with tdTomato$^+$CD45.1$^+$ bone marrow. **(B)** Quantification of AC-engulfing (tdTomato$^+$CD45.1$^-$) cells in different tissues. Data from Figure 1D is represented relative to other cells in the same tissue. Data are the combined results from three independent experiments. **(C)** CD45.1$^-$tdTomato$^+$ ACs were injected IP and one hour later peritoneal cells were harvested and analyzed by flow cytometry. Data are representative of two independent experiments. **(D)** All cells from peritoneal or pleural lavage were plated *ex vivo* and incubated with CD45.1$^+$tdTomato$^+$ ACs before analysis by flow cytometry. Data are representative of two independent experiments. **(E)** Representative flow cytometric analysis of tissues from bone marrow chimeras. Cells were gated on CD45.1$^-$ cells or AC-engulfing cells (CD45.1$^-$tdTomato$^+$), as indicated. Data are representative of three independent experiments. **(F)** Quantification of tdTomato$^+$ ACs in different tissues. Cells from bone marrow chimeras were assayed for Caspase-3/7 activation. Shown is the percentage of total cells that were tdTomato$^+$ and positive for activated Caspase -3/7. Data are the combined results of two independent experiments.

Figure S2. AC-engulfing macrophages do not express TLR9, related to Figure 2. (A) Representative histograms of TLR expression by gated macrophages harvested from *Tlr9*$^{HA:GFP}$, *Tlr7*$^{FLAG:tdTomato}$, or WT mice. Red pulp macrophages are F4/80^{hi}CD11b$^-$ cells. Kupffer cells are live CD45$^+$CD64$^+$MHCII$^+$ cells. F4/80^{mid} macrophages are live CD11b$^+$F4/80^{mid}MHCII$^+$Ly6C$^-$CD11c$^-$. Data are representative of at least three independent experiments. **(B)** TNF production by macrophages from WT mice stimulated *ex vivo* with TLR ligands. Data are representative of at least three independent experiments. **(C)** Tim-4$^+$ pMacs were isolated from WT and *Rosa*^{TLR9} mice. Macrophages were cultured overnight then incubated with CD45.1$^+$tdTomato$^+$ ACs at indicated ratios and analyzed by flow cytometry. Data are representative of two experiments. **(D)** Representative flow cytometric analysis of peritoneal cells at different time points after pristane injection**. (E)** *Lyz2cre*$^{+/-}$*Rosa*$^{lsl\text{-}Tlr9}$ or littermate control *Lyz2cre*$^{-/-}$*Rosa*$^{lsl\text{-}Tlr9}$ were injected intraperitoneally with pristane. 14d after injection peritoneal cells were harvested and analyzed by flow cytometry. The number of total peritoneal exudate cells, neutrophils (CD11b$^+$Ly6G$^+$Ly6C^{mid}), and Ly6C$^+$ monocytes (CD11b$^+$Ly6C$^+$Ly6G$^-$) are shown. Data are the combined results of five independent experiments with total n = 48 for *Lyz2cre*$^{-/-}$ and n = 51 for *Lyz2cre*$^{+/-}$ p-value determined by t-test performed on log-transformed data to account for the non-normal distribution of the data. **(F and G)** *Lyz2cre*$^{+/-}$*Rosa*$^{lsl\text{-}Tlr9}$ or littermate control *Lyz2cre*$^{-/-}$*Rosa*$^{lsl\text{-}Tlr9}$ mice were injected intraperitoneally with

pristane and analyzed 9 months after injection. (F) Peritoneal cells were harvested and analyzed by flow cytometry. The number of total peritoneal exudate cells, $CD11b^+$ cells, T cells ($CD3\varepsilon^+CD11b^-CD19^-$), Tim-$4^+$ macrophages, and neutrophils ($CD11b^+Ly6G^+Ly6C^{mid}$) are shown. (G) Serum samples were collected and tested by ELISA for anti-dsDNA IgG. Data are the combined results of four independent experiments with total n = 38 for *Lyz2cre*$^{-/-}$ and n = 43 for *Lyz2cre*$^{+/-}$; p-value determined by t-test performed on log-transformed data to account for the non-normal distribution of the data.

Figure S3. AC-engulfing macrophages removed from their tissue environment generate inflammatory responses to ACs, related to Figure 3. **(A)** IL-6 production by Tim-4^+ pMacs cultured overnight (programmed) or for 60h (deprogrammed) before stimulation with TLR ligands or ACs. Data are representative of at least three independent experiments **(B)** Tim-4^+ pMacs from WT, *Timd4*$^{-/-}$, or *Unc93b1*$^{-/-}$ mice were cultured overnight or for 60h then incubated with $CD45.1^+tdTomato^+$ ACs at indicated ratios and analyzed by flow cytometry. Data are representative of at least two experiments. **(C)** TNF production by WT BMMs stimulated with TLR ligands or ACs. Data are representative of at least three independent experiments. **(D)** RNA sequencing analysis of programmed and deprogrammed Tim-4^+ pMacs stimulated with ACs. Data are presented as fold stimulated over unstimulated of all significantly changed genes plotted as $\log_2$ fold changes. To minimize contamination from AC mRNA, genes whose expression was greater than two fold higher in RNA isolated from ACs alone relative to macrophage RNA were considered AC-derived and excluded. **(E)** TNF production by peritoneal cells cultured in complete media alone or complete media plus 25% or 50% omentum supernatant for 60h before stimulation with TLR ligands or ACs. Data are representative of at least three independent experiments. **(F and G)** Mice were injected intraperitoneally with lentivirus expressing Gata6 or empty vector. One week after injection peritoneal cells were harvested and cultured for 60h and (F) analyzed for Gata6 expression or (G) stimulated with TLR ligands and analyzed for TNF production. Data are representative of two independent experiments

Figure S4. BMMs gain AC-clearance programming in tissue environments related to Figures 3 and 4. **(A - D)** mRNA expression in deprogrammed relative to programmed Tim-4^+ pMacs measured by RNA sequencing. Results of two independent experiments are shown with replicates averaged. (A) Peritoneal macrophage signature genes. (B) Macrophage core genes. (C) classical (M1) and alternative (M2) macrophage associated genes. **(E)** mRNA expression in deprogrammed relative to programmed Tim-4^+ pMacs quantified using a Nanostring nCounter. Results from two independent experiments are shown. **(F)** $CD45.1^+$ BMMs were labeled ± CFSE and injected IP. After three weeks peritoneal cells were harvested and analyzed for CFSE levels. Data are representative of two independent experiments.

Figure S5. Programmed macrophages have a higher activation threshold for TLR7 and TLR9 responses, related to Figure 4 and Figure 5. (A) IL-6 production by programmed and deprogrammed Tim-4$^+$ pMacs stimulated with increasing doses of R848. Data are representative of at least three independent experiments**. (B)** TNF production by programmed and deprogrammed WT Tim-4$^+$ pMacs stimulated with 2 fold dilutions of G + PolyU with a starting concentration of 0.2mM G + 20µg/ml PolyU. Data are representative of two independent experiments. **(C)** TNF production by programmed and deprogrammed WT Tim-4$^+$ pMacs stimulated with increasing doses of Sa19. Data are representative of at least three independent experiments **(D)** IL-6 production by programmed and deprogrammed Tim-4$^+$ pMacs from *Rosa*^{TLR9} mice stimulated with increasing doses of CpG ODN. Data are representative of at least three independent experiments. **(E)** TNF production by programmed and deprogrammed WT Tim-4$^+$ pMacs stimulated with TLR ligands. PD CpG = phosphodiester CpG ODN. Data are representative of at least three independent experiments. **(F)** TNF production by programmed and deprogrammed Tim-4$^+$ pMacs from *Rosa*^{TLR9} mice stimulated with increasing doses of PD CpG ODN. Data are representative of two independent experiments.

Figure S6. Inflammatory cues induce TLR9 expression in AC-engulfing macrophages, related to Figure 6. (A) IL-6 production by Tim-4$^+$ pMacs treated overnight with the indicated cytokines before stimulation with TLR ligands or ACs. Data are representative of at least three independent experiments **(B)** Tim-4$^+$ pMacs from *Tlr9*$^{HA:GFP}$ mice were cultured overnight with IFNβ or untreated for 60hrs and TLR9 levels in lysates were measured by anti-HA immunoprecipitation and immunoblot. An anti-tubulin immunoblot was performed on lysates. Shown are the indicated lanes of the same membrane. Data are representative of two independent experiments. **(C)** Tim-4$^+$ pMacs were isolated from WT mice and cultured overnight ± IFNγ or for 60 hours then incubated with CD45.1$^+$tdTomato$^+$ ACs at the indicated ratios and analyzed by flow cytometry. Data are representative of at least two independent experiments. **(D-E)** IL-6 production by programmed Tim-4$^+$ pMacs incubated overnight with IFNβ or by untreated deprogrammed Tim-4+ pMacs stimulated with increasing doses of CpG (D) or R848 (E). Data are representative of three independent experiments.

Figure S7. KLF2 and KLF4 imprint an AC-clearance program on macrophages, related to Figure 7. (A) Transcription factors with at least 2-fold ($p < 0.05$) changes in expression in deprogrammed Tim-4$^+$ pMacs relative to programmed Tim-4$^+$ pMacs as measured by RNA sequencing. Results of two independent experiments are shown with replicates averaged. **(B)** Cas9 expressing BMMs were transduced with vectors encoding guide RNAs targeting the indicated transcription factors. To determine efficiency of cutting, genomic DNA was analyzed by qPCR using primers that bind the cas9 cut site. Data was

normalized to an unrelated site in genomic DNA. **(C and D)** BMMs were transduced with retrovirus encoding KLF2, KLF4, Gata6 or empty vector. (C) The expression of *Gata6* and *Klf4* mRNA was determined by qPCR and is expressed relative to *Actb* mRNA. (D) The expression of *Klf2* was quantified using a Nanostring nCounter. Data are the combined results of three independent experiments. n = 3-4 per group from independent experiments.

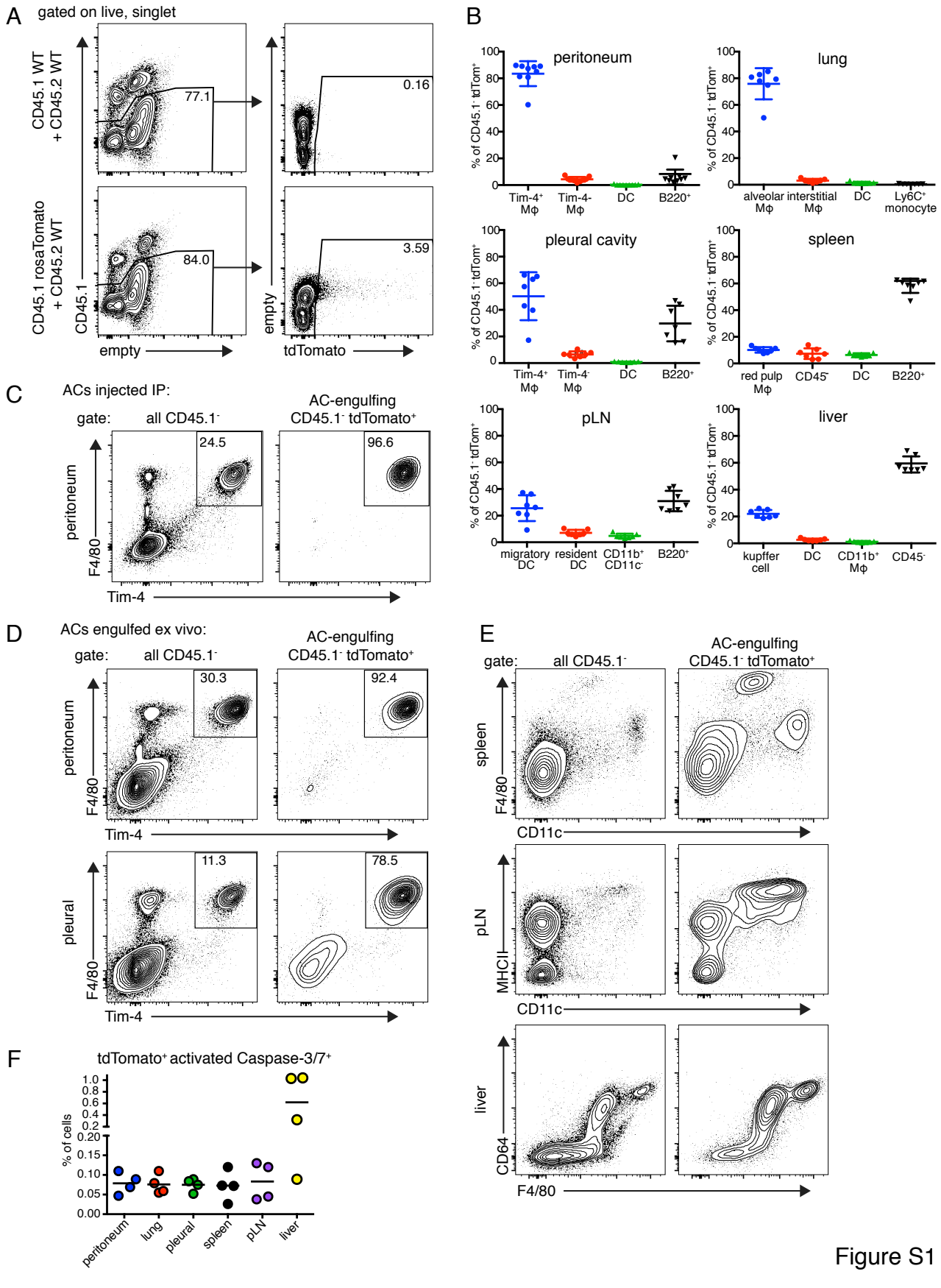
A
gated on live, singlet
CD45.1 WT + CD45.2 WT
CD45.1 rosaTomato + CD45.2 WT
CD45.1
empty
77.1
84.0
0.16
3.59
tdTomato
B
peritoneum
lung
pleural cavity
spleen
pLN
liver
% of CD45.1- tdTom+
Tim-4+ Mφ
Tim-4- Mφ
DC
B220+
alveolar Mφ
interstitial Mφ
Ly6C+ monocyte
red pulp Mφ
CD45-
migratory DC
resident DC
CD11b+ CD11c-
kupffer cell
CD11b+ Mφ
C
ACs injected IP:
gate:
all CD45.1-
AC-engulfing CD45.1- tdTomato+
24.5
96.6
peritoneum
F4/80
Tim-4
D
ACs engulfed ex vivo:
30.3
92.4
11.3
78.5
pleural
E
spleen
CD11c
pLN
MHCII
liver
CD64
F4/80
F
tdTomato+ activated Caspase-3/7+
% of cells
peritoneum
lung
pleural
spleen
pLN
liver

Figure S1

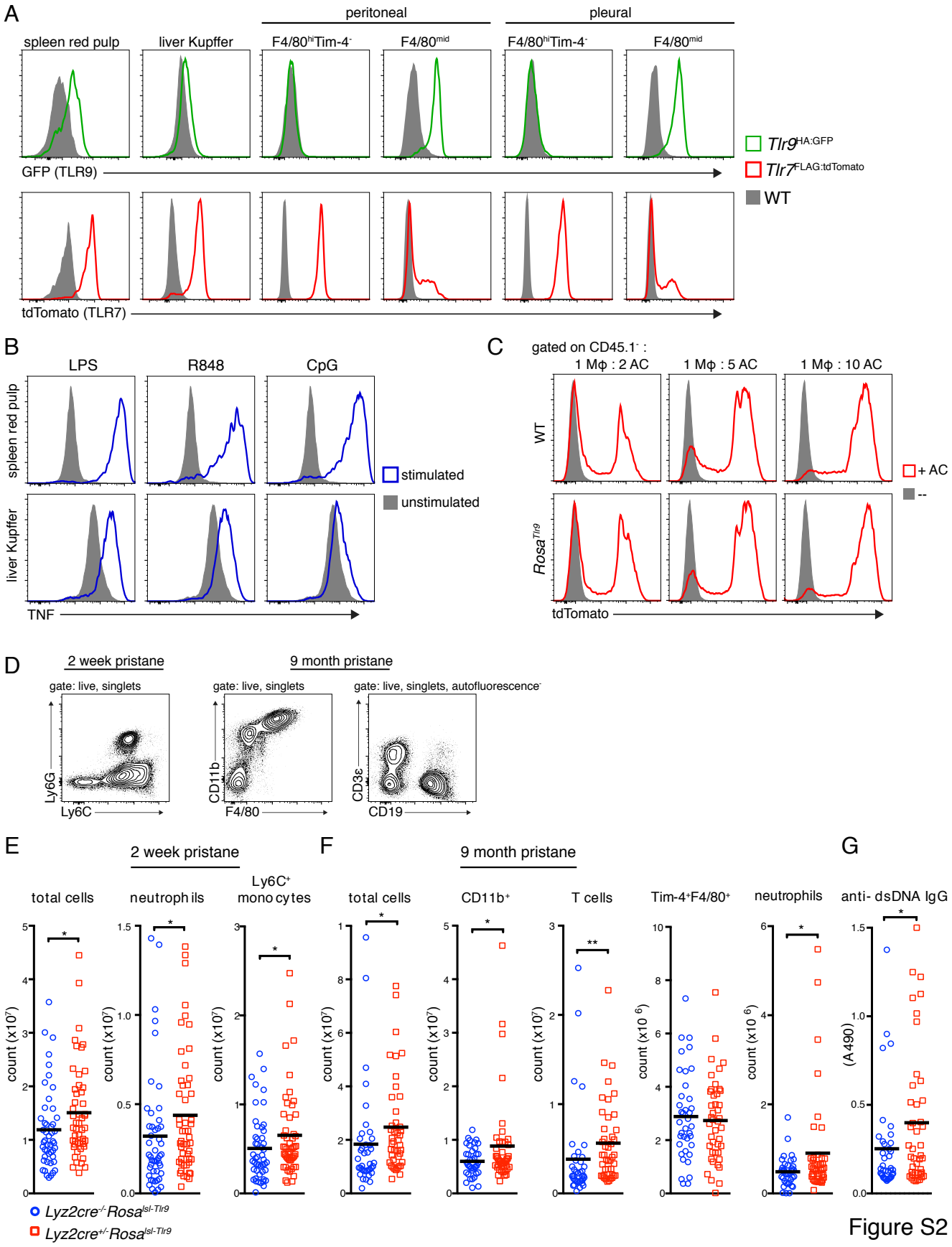
A
peritoneal
pleural
spleen red pulp
liver Kupffer
F4/80hiTim-4-
F4/80mid
F4/80hiTim-4-
F4/80mid
GFP (TLR9)
tdTomato (TLR7)
Tlr9HA:GFP
Tlr7FLAG:tdTomato
WT
B
LPS
R848
CpG
spleen red pulp
liver Kupffer
stimulated
unstimulated
TNF
C
gated on CD45.1- :
1 Mϕ : 2 AC
1 Mϕ : 5 AC
1 Mϕ : 10 AC
WT
RosaTlr9
+ AC
--
tdTomato
D
2 week pristane
9 month pristane
gate: live, singlets
gate: live, singlets
gate: live, singlets, autofluorescence-
Ly6G
Ly6C
CD11b
F4/80
CD3ε
CD19
E
2 week pristane
total cells
neutrophils
Ly6C+ monocytes
count (x10^7)
F
9 month pristane
total cells
CD11b+
T cells
Tim-4+F4/80+
neutrophils
count (x10^6)
G
anti- dsDNA IgG
(A490)
Lyz2cre-/- Rosalsl-Tlr9
Lyz2cre+/- Rosalsl-Tlr9

Figure S2

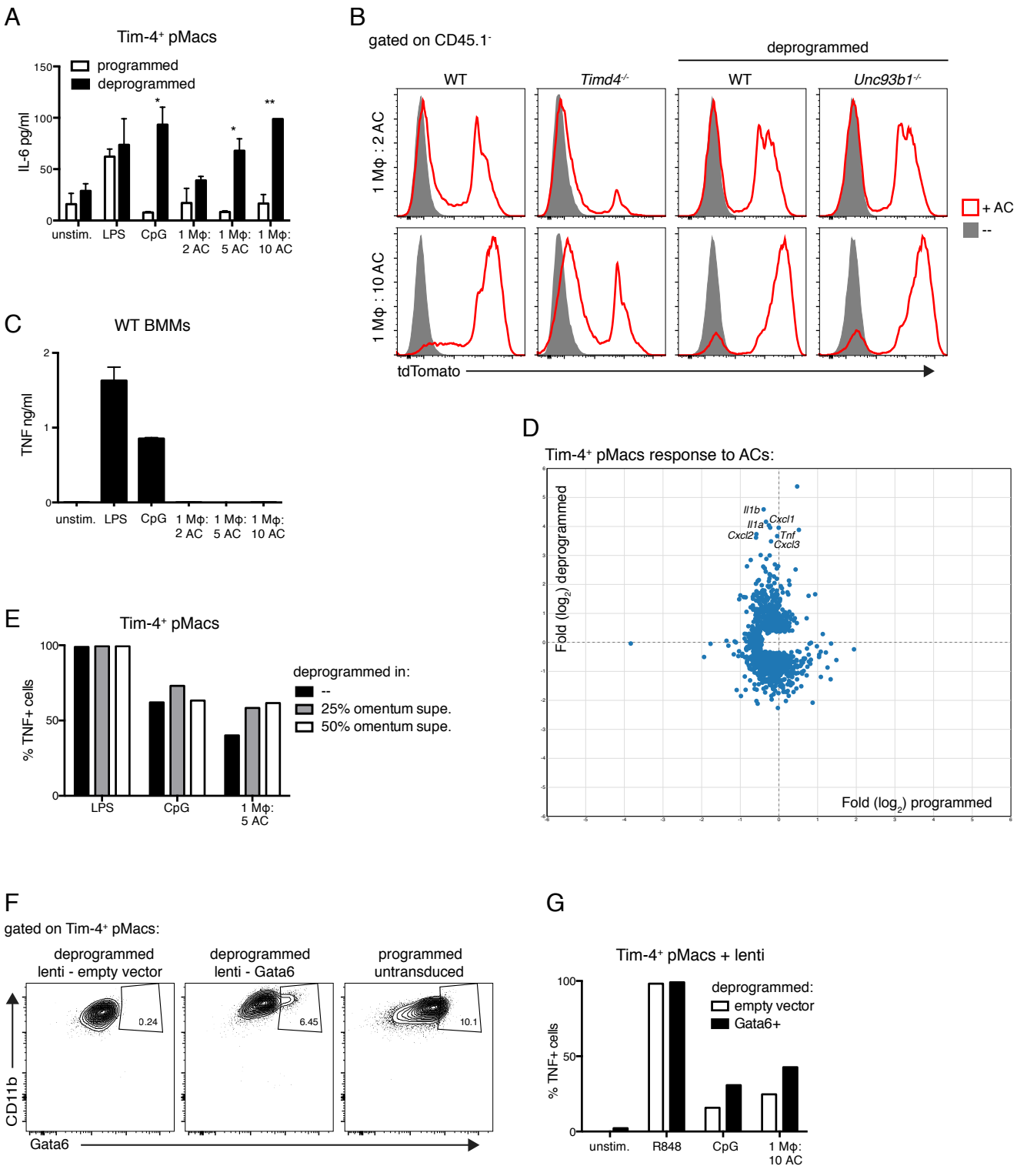
A
Tim-4+ pMacs
programmed
deprogrammed
IL-6 pg/ml
150
100
50
0
unstim.
LPS
CpG
1 Mϕ: 2 AC
1 Mϕ: 5 AC
1 Mϕ: 10 AC
B
gated on CD45.1-
deprogrammed
WT
Timd4-/-
WT
Unc93b1-/-
1 Mϕ : 2 AC
1 Mϕ : 10 AC
+ AC
--
tdTomato
C
WT BMMs
TNF ng/ml
2
1
0
unstim.
LPS
CpG
1 Mϕ: 2 AC
1 Mϕ: 5 AC
1 Mϕ: 10 AC
D
Tim-4+ pMacs response to ACs:
Fold (log2) deprogrammed
Fold (log2) programmed
Il1b
Il1a
Cxcl1
Cxcl2
Tnf
Cxcl3
E
Tim-4+ pMacs
% TNF+ cells
100
50
0
LPS
CpG
1 Mϕ: 5 AC
deprogrammed in:
--
25% omentum supe.
50% omentum supe.
F
gated on Tim-4+ pMacs:
deprogrammed lenti - empty vector
deprogrammed lenti - Gata6
programmed untransduced
0.24
6.45
10.1
CD11b
Gata6
G
Tim-4+ pMacs + lenti
% TNF+ cells
100
50
0
deprogrammed:
empty vector
Gata6+
unstim.
R848
CpG
1 Mϕ: 10 AC

Figure S3

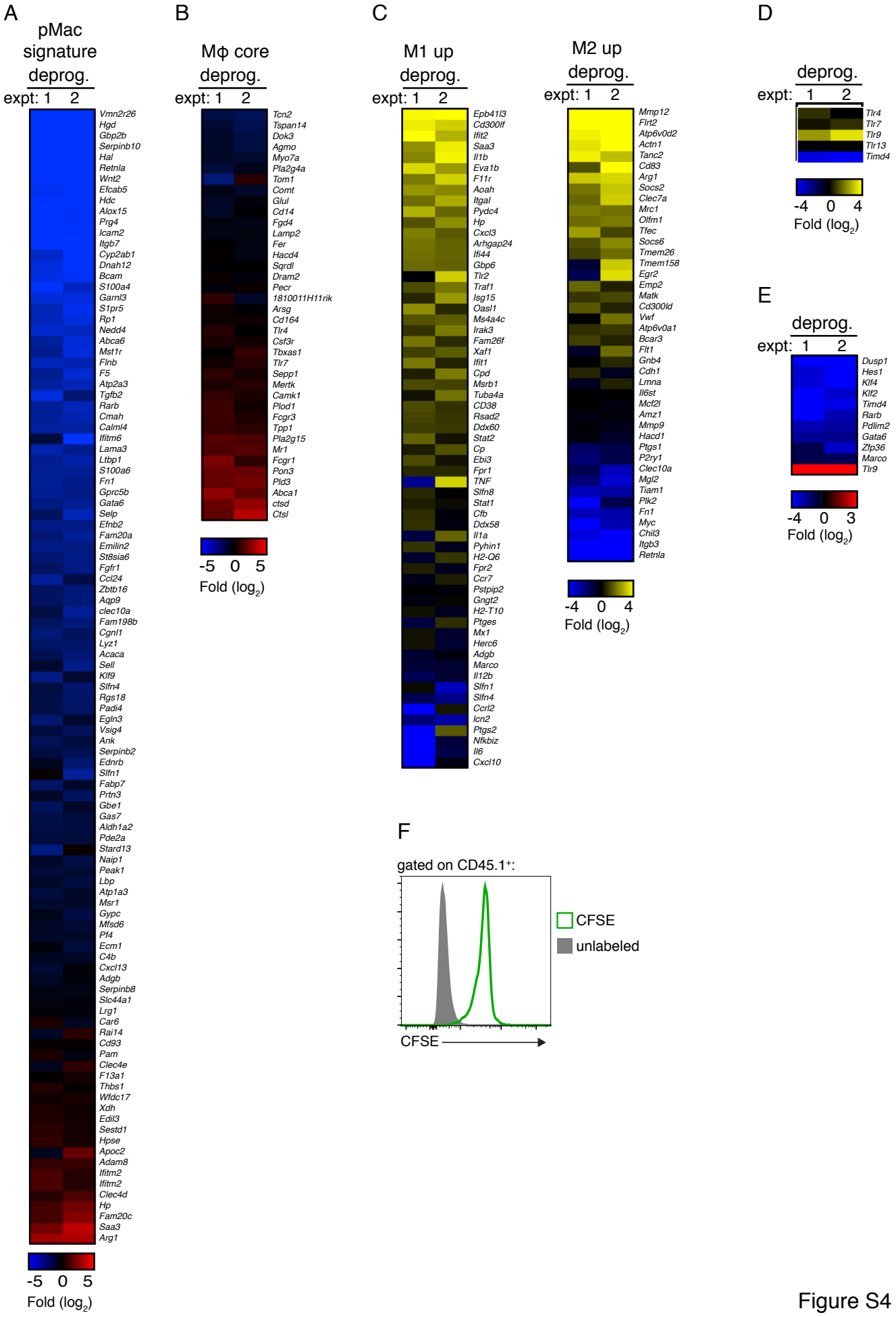
A
pMac signature deprog.
expt: 1 2
Vmn2r26
Hgd
Gbp2b
Serpinb10
Hal
Retnla
Wnt2
Efcab5
Hdc
Alox15
Prg4
Icam2
Itgb7
Cyp2ab1
Dnah12
Bcam
S100a4
Garnl3
S1pr5
Rp1
Nedd4
Abca6
Mst1r
Flnb
F5
Atp2a3
Tgfb2
Rarb
Cmah
Calml4
Ifitm6
Lama3
Ltbp1
S100a6
Fn1
Gprc5b
Gata6
Selp
Efnb2
Fam20a
Emilin2
St8sia6
Fgfr1
Ccl24
Zbtb16
Aqp9
clec10a
Fam198b
Cgnl1
Lyz1
Acaca
Sell
Klf9
Slfn4
Rgs18
Padi4
Egln3
Vsig4
Ank
Serpinb2
Ednrb
Slfn1
Fabp7
Prtn3
Gbe1
Gas7
Aldh1a2
Pde2a
Stard13
Naip1
Peak1
Lbp
Atp1a3
Msr1
Gypc
Mfsd6
Pf4
Ecm1
C4b
Cxcl13
Adgb
Serpinb8
Slc44a1
Lrg1
Car6
Rai14
Cd93
Pam
Clec4e
F13a1
Thbs1
Wfdc17
Xdh
Edil3
Sestd1
Hpse
Apoc2
Adam8
Ifitm2
Ifitm2
Clec4d
Hp
Fam20c
Saa3
Arg1
-5 0 5
Fold (log2)
B
Mϕ core deprog.
expt: 1 2
Tcn2
Tspan14
Dok3
Agmo
Myo7a
Pla2g4a
Tom1
Comt
Glul
Cd14
Fgd4
Lamp2
Fer
Hacd4
Sqrdl
Dram2
Pecr
1810011H11rik
Arsg
Cd164
Tlr4
Csf3r
Tbxas1
Tlr7
Sepp1
Mertk
Camk1
Plod1
Fcgr3
Tpp1
Pla2g15
Mr1
Fcgr1
Pon3
Pld3
Abca1
ctsd
Ctsl
-5 0 5
Fold (log2)
C
M1 up deprog.
expt: 1 2
Epb41l3
Cd300lf
Ifit2
Saa3
Il1b
Eva1b
F11r
Aoah
Itgal
Pydc4
Hp
Cxcl3
Arhgap24
Ifi44
Gbp6
Tlr2
Traf1
Isg15
Oasl1
Ms4a4c
Irak3
Fam26f
Xaf1
Ifit1
Cpd
Msrb1
Tuba4a
CD38
Rsad2
Ddx60
Stat2
Cp
Ebi3
Fpr1
TNF
Slfn8
Stat1
Cfb
Ddx58
Il1a
Pyhin1
H2-Q6
Fpr2
Ccr7
Pstpip2
Gngt2
H2-T10
Ptges
Mx1
Herc6
Adgb
Marco
Il12b
Slfn1
Slfn4
Ccrl2
Icn2
Ptgs2
Nfkbiz
Il6
Cxcl10
M2 up deprog.
expt: 1 2
Mmp12
Flrt2
Atp6v0d2
Actn1
Tanc2
Cd83
Arg1
Socs2
Clec7a
Mrc1
Olfm1
Tfec
Socs6
Tmem26
Tmem158
Egr2
Emp2
Matk
Cd300ld
Vwf
Atp6v0a1
Bcar3
Flt1
Gnb4
Cdh1
Lmna
Il6st
Mcf2l
Amz1
Mmp9
Hacd1
Ptgs1
P2ry1
Clec10a
Mgl2
Tiam1
Plk2
Fn1
Myc
Chil3
Itgb3
Retnla
-4 0 4
Fold (log2)
D
deprog.
expt: 1 2
Tlr4
Tlr7
Tlr9
Tlr13
Timd4
-4 0 4
Fold (log2)
E
deprog.
expt: 1 2
Dusp1
Hes1
Klf4
Klf2
Timd4
Rarb
Pdlim2
Gata6
Zfp36
Marco
Tlr9
-4 0 3
Fold (log2)
F
gated on CD45.1+:
CFSE
unlabeled
CFSE

Figure S4

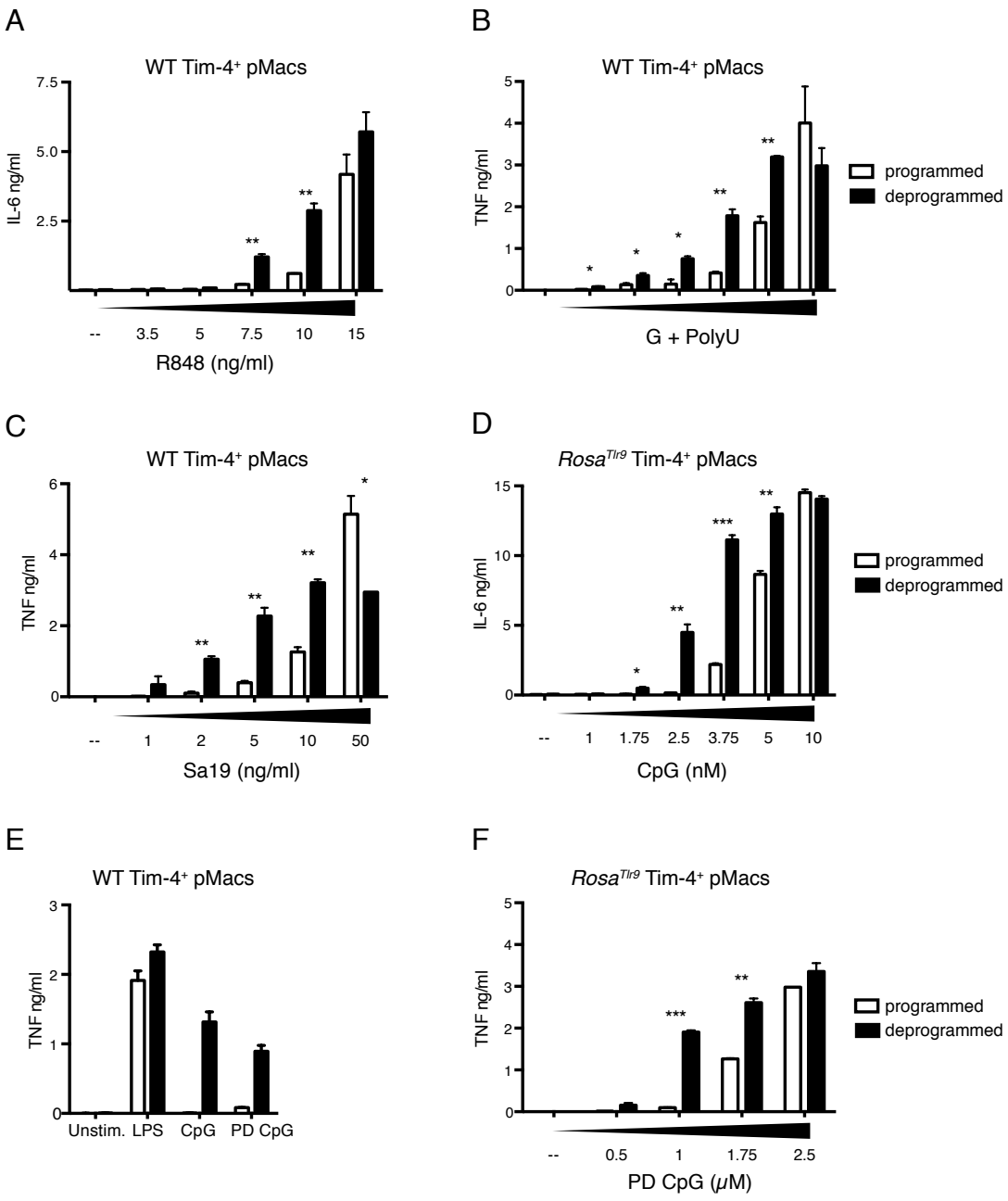
A
WT Tim-4+ pMacs
IL-6 ng/ml
7.5
5.0
2.5
**
**
--
3.5
5
7.5
10
15
R848 (ng/ml)
B
WT Tim-4+ pMacs
TNF ng/ml
5
4
3
2
1
0
*
*
*
**
**
G + PolyU
programmed
deprogrammed
C
WT Tim-4+ pMacs
TNF ng/ml
6
4
2
0
**
**
**
*
--
1
2
5
10
50
Sa19 (ng/ml)
D
RosaTlr9 Tim-4+ pMacs
IL-6 ng/ml
15
10
5
0
*
**
***
**
--
1
1.75
2.5
3.75
5
10
CpG (nM)
programmed
deprogrammed
E
WT Tim-4+ pMacs
TNF ng/ml
3
2
1
0
Unstim.
LPS
CpG
PD CpG
F
RosaTlr9 Tim-4+ pMacs
TNF ng/ml
5
4
3
2
1
0
***
**
--
0.5
1
1.75
2.5
PD CpG (μM)
programmed
deprogrammed

Figure S5

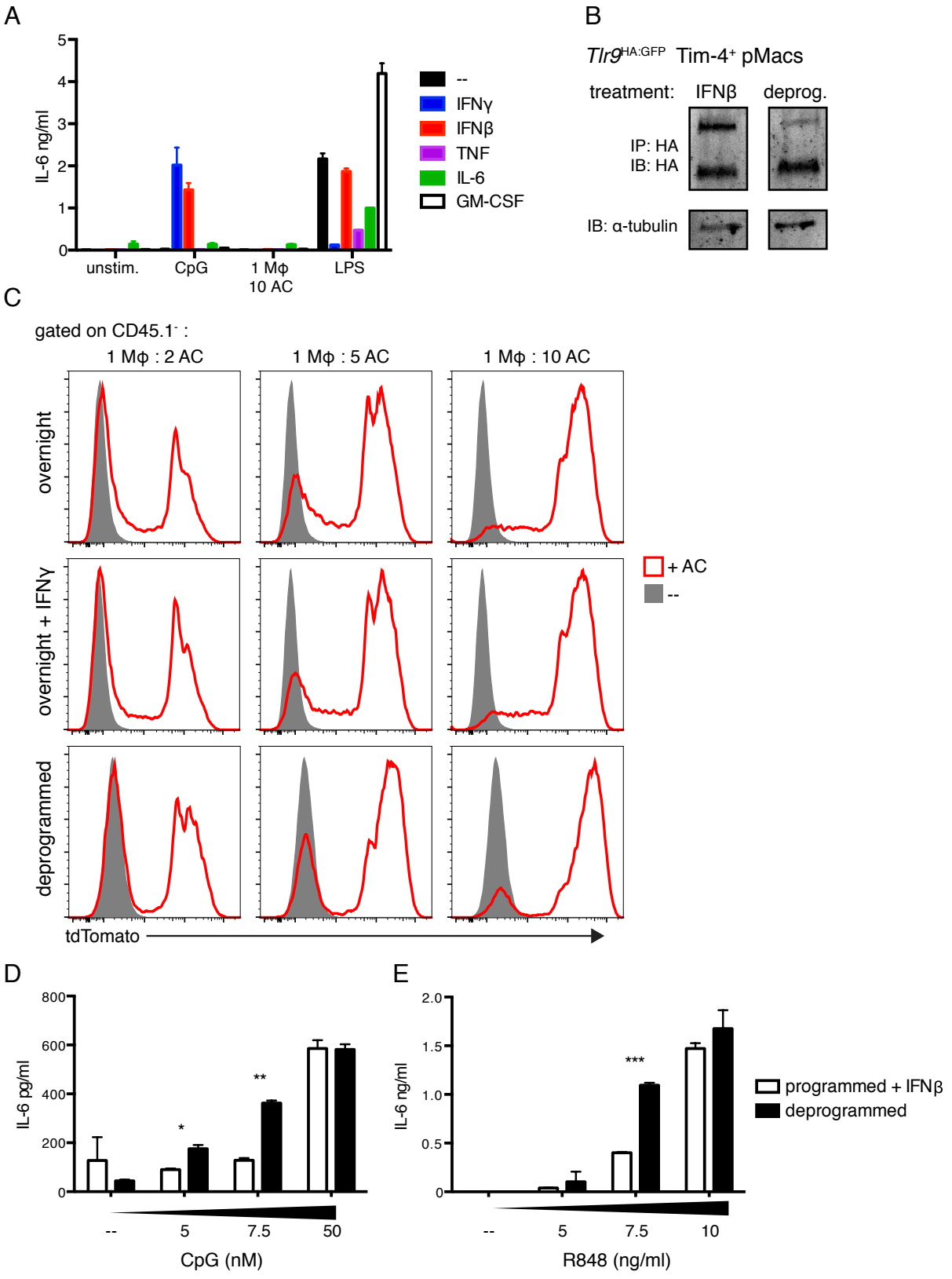
A
IL-6 ng/ml
unstim.
CpG
1 Mϕ 10 AC
LPS
--
IFNγ
IFNβ
TNF
IL-6
GM-CSF
B
Tlr9HA:GFP Tim-4+ pMacs
treatment:
IFNβ
deprog.
IP: HA
IB: HA
IB: α-tubulin
C
gated on CD45.1- :
1 Mϕ : 2 AC
1 Mϕ : 5 AC
1 Mϕ : 10 AC
overnight
overnight + IFNγ
deprogrammed
+ AC
--
tdTomato
D
IL-6 pg/ml
CpG (nM)
E
IL-6 ng/ml
R848 (ng/ml)
programmed + IFNβ
deprogrammed

Figure S6

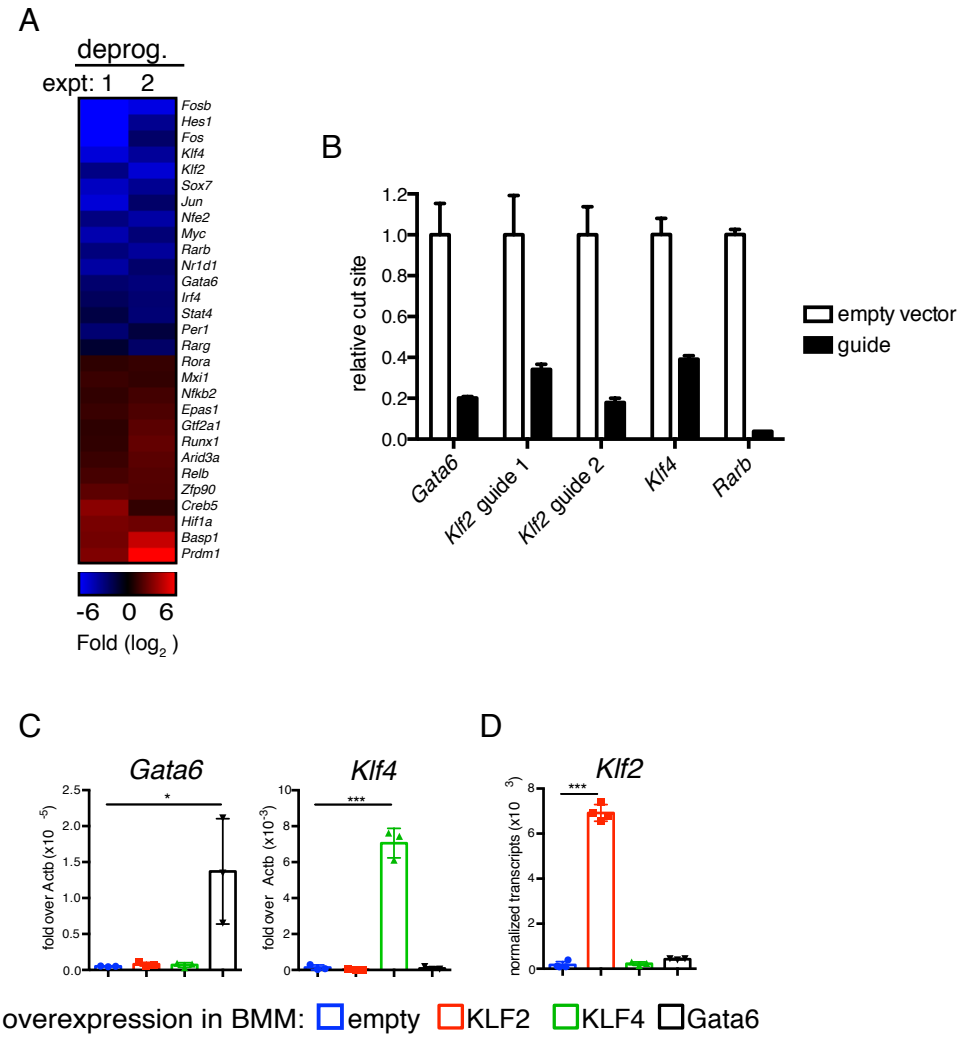
A
deprog.
expt: 1 2
Fosb
Hes1
Fos
Klf4
Klf2
Sox7
Jun
Nfe2
Myc
Rarb
Nr1d1
Gata6
Irf4
Stat4
Per1
Rarg
Rora
Mxi1
Nfkb2
Epas1
Gtf2a1
Runx1
Arid3a
Relb
Zfp90
Creb5
Hif1a
Basp1
Prdm1
-6 0 6
Fold (log2)
B
relative cut site
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Gata6
Klf2 guide 1
Klf2 guide 2
Klf4
Rarb
empty vector
guide
C
Gata6
Klf4
fold over Actb (x10 -5)
fold over Actb (x10-3)
*
***
D
Klf2
***
normalized transcripts (x10 3)
overexpression in BMM: empty KLF2 KLF4 Gata6

Figure S7

Table S1 related to STAR Methods: Cas9 guide testing primers

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Gata6 guide test F: AGTGGATGGCCTTGACTGAC	This paper	N/A
Gata6 guide test R: GTCGAGCTGCGGCGTCCT	This paper	N/A
KLF2 guide 1 test F: GTGCGAGCGCGGCCTCCAG	This paper	N/A
KLF2 guide 1 test R: TCTAACAACTAGGCCCTCAA	This paper	N/A
KLF2 guide 2 test F: GAAATGAACCCGAGGCGG	This paper	N/A
KLF2 guide 2 test R: GCTCGGCCTTCACTAGCC	This paper	N/A
KLF4 guide 1 test F: CGGCCCGGCGGGAAGGG	This paper	N/A
KLF4 guide 1 test R: ACACGCACTTAAGGCCAACT	This paper	N/A
Rarb guide 2 test F: CAGTGCCTGCGAGGGGT	This paper	N/A
Rarb guide 2 test R: GTTTGCCCTTGGTCACTCAT	This paper	N/A
Cas9 editing ctl F: TGCAATCGCAAGAGGAAAG	This paper	N/A
Cas9 editing ctl R: AGGCTATTGATTTTCCCGGT	This paper	N/A