Supplementary Information

Supplementary Table 1: Primers and RNAi list

Oligonucleotides		
Negative Control DsiRNA	Integrated DNA Technologies	
Mouse: ON-TARGETplus Ptgs2 SMARTpool siRNA	GE Healthcare Dharmacon	L-056799-01-0005
Mouse: ON-TARGETplus Dusp4 SMARTpool siRNA	GE Healthcare Dharmacon	L-061306-00-0005
Mouse: ON-TARGETplus Dnmt3a SMARTpool siRNA	GE Healthcare Dharmacon	L-065433-01-0005
Mouse: IDT Mapk1 DsiRNA	Integrated DNA Technologies	mm.Ri.Mapk1.13.3
Mouse: IDT Mapk3 DsiRNA	Integrated DNA Technologies	mm.Ri.Mapk3.13.3
Mouse: IDT Cd36 DsiRNA	Integrated DNA Technologies	mm.Ri.Cd36.13.2
Mouse: IDT Dnmt3a DsiRNA	Integrated DNA Technologies	mm.Ri.Dnmt3a.13.2
Mouse: IDT Tgfb1 DsiRNA	Integrated DNA Technologies	mm.Ri.Tgfb1.13.2
Mouse: IDT MAT2A DsiRNA	Integrated DNA Technologies	mm.Ri.MAT2A.13.3
Mouse: IDT Ptger2 DsiRNA	Integrated DNA Technologies	mm.Ri.Ptger2.13.3
Mouse: IDT Ptger4 DsiRNA	Integrated DNA Technologies	mm.Ri.Ptger4.13.3
Human: ON-TARGETplus Ptgs2 SMARTpool siRNA	GE Healthcare Dharmacon	L-006672-01-0005
Mouse: *Cd36* Forward ATGGGCTGTGATCGGAACTG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Cd36* Reverse TTTGCCACGTCATCTGGGTTT	PrimerBank	www.pga.mgh.harvard.edu/primerbank

Mouse: *Hprt* Forward TCAGTCAACGGGGGACATAAA	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Hprt* Reverse GGGGCTGTACTGCTTAACCAG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Dnmt3a* Forward GATGAGCCTGAGTATGAGGATGG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Dnmt3a* Reverse CAAGACACAATTCGGCCTGG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Ptgs2* Forward TGCACTATGGTTACAAAAGCTGG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Ptsg2* Reverse TCAGGAAGCTCCTTATTTCCCTT	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Tgfb1* Forward CTCCCGTGGCTTCTAGTGC	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Tgfb1* Reverse GCCTTAGTTTGGACAGGATCT	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Ptger2* Forward TCCCTAAAGGAAAAGTGGGACC	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Ptger2* Reverse GAGCGCATTAACCTCAGGACC	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Ptger4* Forward ACCATTCCTAGATCGAACCGT	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Ptger4* Reverse CACCACCCCGAAGATGAACAT	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Mat2a* Forward GCTTCCACGAGGCGTTCAT	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Mat2a* Reverse AGCATCACTGATTTGGTCACAA	PrimerBank	www.pga.mgh.harvard.edu/primerbank

Mouse: *Rbcn* Forward CAGGGTGTAGTGCATGGTTCT	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Rbcn* Reverse CCGCCAAGATCCATTCCCG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Dusp4* Forward CGTGCGCTGCAATACCATC	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Mouse: *Dusp4* Reverse CTCATAGCCACCTTTAAGCAGG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Human: *TGFB1* Forward CAATTCCTGGCGATACCTCAG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Human: *TGFB1* Reverse GCACAACTCCGGTGACATCAA	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Human: *PTSG2* Forward CTGGCGCTCAGCCATACAG	PrimerBank	www.pga.mgh.harvard.edu/primerbank
Human: *PTSG2* Reverse CGCACTTATACTGGTCAAATCCC	PrimerBank	www.pga.mgh.harvard.edu/primerbank
DUSP4 MeDIP Forward CACCCGCAACGATCTCTAC	Integrated DNA Technologies	
DUSP4 MeDIP Reverse AACGGAGACCTAGAGGAAGAA	Integrated DNA Technologies	

Supplementary Table 2: Antibodies, catalogue number, source and dilutions used

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-Cox2	Cayman Chemicals	Cat# 160126 (1:1000 dilution)
Rabbit anti-Dnmt3a	Cell Signaling Technology	Cat# 3598S (1:1000 dilution)
Rabbit IgG-HRP linked	Cell Signaling Technology	Cat# 7074S (1:5000 dilution)

Rat anti-Mac2	Cederlane	Cat# CL8942AP (1:10,000 dilution)
Rabbit anti-β-actin-HRP	Cell Signaling Technology	Cat# 5125S (1:5000 dilution)
Rabbit anti-MAT2A	Novus Biologicals	Cat# NB110-94158 (1:1000 dilution)
Rabbit anti-Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Cell Signaling Technology	Cat# 4370S (1:1000 dilution)
Rabbit anti-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Cell Signaling Technology	Cat# 9102S (1:1000 dilution)
Cox2 (D5H5) XP (R) Alexa 488	Cell Signaling Technology	Cat# 13596S (1:100 dilution)
PE anti-mouse F4/80	Biolegend	Cat# 123110 (1:100 dilution)
APC anti-mouse F4/80	Biolegend	Cat# 123116 (1:100 dilution)
PB anti-mouse Ly6G	Biolegend	Cat# 127612 (1:100 dilution)
Alexa fluor 647 anti-ERK1/2 Phospho (Thr202/Tyr204)	Biolegend	Cat# 675504 (1:100 dilution)
Alexa fluor 488 anti-ERK1/2 Phospho (Thr202/Tyr204)	Biolegend	Cat# 675507 (1:100 dilution)
PE anti-mouse LAP (TGF-β1)	Biolegend	Cat# 141404 (1:100 dilution)
APC anti-mouse LAP (TGF-β1)	Biolegend	Cat# 141406 (1:100 dilution)
APC anti-mouse Ly-6G/Ly-6C (Gr-1)	Biolegend	Cat# 108412 (1:100 dilution)
FITC Annexin V	Biolegend	Cat# 640906 (1:50 dilution)
Rabbit IgG	Sigma-Aldrich	Cat# PP64B
Alexa fluor 64 goat anti-rabbit IgG	Invitrogen	Cat# A21244 (1:100 dilution)

Alexa fluor 488 goat anti-rat IgG	Invitrogen	Cat# A11006 (1:100 dilution)
Alexa fluor 594 goat anti-rabbit IgG	Invitrogen	Cat# A11037 (1:100 dilution)
Rabbit anti-DUSP4	Abcam	Cat# Ab216576 (1:100 dilution)
Rabbit anti-COX2	Abcam	Cat# Ab188183 (1:100 dilution)
Rabbit anti-TGFβ1	Abcam	Cat# Ab92486 (1:100 dilution)

Supplementary Table 3: List of chemical reagents used, catalogue number and their source

Chemicals, Peptides, and Recombinant Proteins		
Dulbecco's Modified Eagle Media (DMEM)	Corning	Cat# 10-013-CV
Roswell Park Memorial Institute (RPMI) 1640 Media	Corning	Cat# 10-040-CV
Opti-MEM	GIBCO	Cat# 31985-070
1X PBS	Corning	Cat# 21-040-CV
CellStripper	Corning	Cat# 25-056-CI
Modified DMEM	GIBCO	Cat# 21013024
Heat-Inactivated Fetal Bovine Serum	GIBCO	Cat# 10438-026
Fetal Bovine Serum, dialyzed	GIBCO	Cat# 26400064
Penicillin/Streptomycin	Corning	Cat# 30-002-CI
HISTOPAQUE-1077	Sigma-Aldrich	Cat# 10771-100ML
Novex 4-20% Tris-Glycine Mini Gels, 15-well	Invitrogen	Cat# XP04205BOX
4X Laemmli Buffer	Bio-Rad	Cat# 1610747
PKH26 Fluorescent Cell Linker	Sigma-Aldrich	Cat# PKH26GL-1KT
Diluent C	Sigma-Aldrich	Cat# CGLDIL
U0126	Tocris Bio-Techne	Cat# 1144
LY3200882	Selleckchem	Cat# S8772

DMSO	Sigma-Aldrich	Cat# D2650
β-mercaptoethanol	Bio-Rad	Cat# 1610710
Hoechst 33342	Thermo Scientific	Cat# 62249
Bafilomycin A1	Sigma-Aldrich	Cat# B1793
Recombinant Mouse TGFβ1	Biolegend	Cat#763104
Dexamethasone	Calbiochem	Cat# 265005
Zymosan A	Sigma-Aldrich	Cat# Z4250
Lipofectamine RNAiMax	Life Technologies	Cat# 13778-150
Neomycin	Sigma-Aldrich	Cat# N1142
40 µm Nylon Cell Strainers	BD Falcon	Cat# 352340
Power SYBR Green PCR Master Mix	Applied Biosystems	Cat# 4367659
MAT2A Inhibitor (PF-9366)	BioVision	Cat# B2244-5
S-(5'-Adenosyl)-L-Methionine Chloride	Sigma-Aldrich	Cat# A7007
L-Glutamine	Sigma-Aldrich	Cat# G8540
L-Cystine	Sigma-Aldrich	Cat# C6727
Sodium Pyruvate	Sigma-Aldrich	Cat# S8636
Prostaglandin E2 ELISA Kit	Cayman Chemicals	Cat#514010
Legend MAX™ Total TGFβ1 ELISA Kit	Biolegend	Cat# 436707
Methylamp Methylated DNA Capture Kit	EPIGENTEK	Cat# P-1015-48
REAGENT or RESOURCE		
0.45-mm nitrocellulose membranes	Bio-Rad	Cat# 1620115
Hoechst 33342	Thermo Scientific	Cat# 62249
Lipofectamine RNAiMax	Life Technologies	Cat# 13778-150
Perm buffer II	BD Biosciences	
Critical Commercial Assays		
PureLink RNA Mini Kit	Thermo Fisher Scientific	Cat# 12183025
TUNEL Kit	Roche	Cat# 12156792910
Supersignal West Pico Chemiluminescence Kit	Thermo Fisher Scientific	Cat# 34080

Extended Data Fig. 1. Additional experiments documenting the efferocytosis-*Ptgs2*/COX2-TGFβ1 pathway in macrophages. Related to Fig. 1.a-d, BMDMs were incubated ± ACs, after which noninternalized ACs were removed by rinsing. The cells were assayed for *Ptgs2* mRNA after an additional 1 h incubation and COX2 protein after 3 h (**a**); PGE_2 in the media after 3 h (**b**); and TGFβ1 in the media after 18 h (**c**). For (**d**), experiments similar to those in panel a were analyzed for mouse and human *Ptgs2/PTGS2* or *Tgfb1/TGFβ1* mRNA to prove that mRNA being measured is not residual human mRNA derived from human apoptotic Jurkat cells. **e**, BMDMs were transfected with scrambled RNA (Scr) or siRbcn and then, after 72 h, assayed for *Rbcn* mRNA. **f**, BMDMs were transfected with Scr or siRbcn or treated with vehicle or bafilomycin A1 and then incubated with PKH26-labeled ACs for 45 min, followed by rinsing and quantification of percent $PKH26^+$ macrophages of total macrophages. **g**, BMDMs were transfected with Scr or siPtgs2 and then, after 72 h, assayed for *Ptgs2* mRNA. **h**, BMDMs were incubated ± ACs for 45 min, after which noninternalized ACs were removed by rinsing. The cells were assayed for *Ptges* mRNA after an additional 1 h incubation (left). **i**, BMDMs were transfected with scrambled RNA (Scr) or siPtges and then, after 72 h, assayed for *Ptges* mRNA. **j-k**, BMDMs were transfected with Scr, siPtger4, or siPtger2 and then, after 72 h, assayed for *Ptger4* or *Ptger2* mRNA, respectively. **l**, BMDMs were transfected with Scr or siTgfb1. After 72 h, the cells were incubated ± ACs for 45 min, after which noninternalized ACs were removed by rinsing. After an additional 1 h of incubation, the cells were assayed for *Ptgs2* mRNA. **m**, BMDMs were transfected with Scr or si*Tgfb1* and then, after 72 h, assayed for *Tgfb1* mRNA. All mRNA data are expressed relative to the first control group. Values are means ± SEM. *ns*, not significant ($P > 0.05$); n = 3 biological replicates. Two-sided *P* values were determined by a Student's t-test for two groups or one-way ANOVA with Fisher's LSD posthoc analysis for three or more groups.

Extended Data Fig. 2. Additional experiments documenting the role of SAM in the efferocytosis-*Ptgs2*/COX2-TGFβ1 pathway. Related to Fig. 2. All AC incubations were 45 min, and *Ptgs2* or *Tgfb1* were assayed 1 or 6 h after AC removal, respectively. **a**, BMDMs pretreated 2 h with vehicle or the MAT2A inhibitor PF9366 were incubated with pHrodo-labeled ACs and quantified for the percent pHrodo-AC^+ macrophages.

Scale bar, 50 μm. **b-c**, BMDMs treated with scrambled RNA (Scr) or siMat2a were incubated ± ACs and then assayed for *Ptgs2* or*Tgfb1*. **d**, BMDMs treated with Scr or siMat2a were assayed for *Mat2a*. **e**, BMDMs treated with vehicle or SAM were assayed for SAM content. **f**, BMDMs cultured in methionine-free media with D-FBS and pretreated for 2 h with vehicle or bafilomycin A1 (Baf) were incubated 1 h with PKH26-labeled ACs whose proteins were labeled with $^{13}C_5{}^{15}N$-methionine. AC^+ and AC^- macrophages were sorted and assayed for SAM content (**g**) and percent $^{13}C_5{}^{15}N$-SAM of total SAM (**h**). **i**, Control or DNMT3A-KO BMDMs were incubated with PKH26-labeled ACs and then quantification for the percent PKH26-AC^+ macrophages. Scale bar, 50 μm. **j**, Control and DNMT3A-KO BMDMs were immunoblotted for DNMT3A and β-actin. **k**, HMDMs treated with Scr or siDNMT3A were assayed for *DNMT3A*. **l**, BMDMs were incubated with IgG-coated RBCs for 45 min and then assayed 3 h later for COX2 MFI by flow cytometry. **m-n**, Control and DNMT3A-KO BMDMs treated with LPS or LPS + IFNγ for 4 h were assayed for *Il6*, *Ptgs2,* or COX2 (n). **o-p**, BMDMs treated with Scr or siDnmt3a were incubated for 4 h with vehicle and LPS + IFNγ or IL4 and assayed for *Nos2* or *Arg1*. **q**, Control and DNMT3A-KO BMDMs were incubated ± ACs for 45 min and then assayed for SAM. **r**, BMDMs pretreated for 2 h with vehicle or bafilomycin A1 were incubated ± ACs whose proteins were labeled with $^{13}C_5{}^{15}N$-methionine and then assayed 1 h after AC removal for $^{13}C_5$-methylcytosine in DNA. **s**, BMDMs treated with Scr or siCreb1 were assayed for *Creb1*. All mRNA data are expressed relative to the first control group. Values are means ± SEM. *n.s.*, not significant ($P > 0.05$); n = 3 biological replicates for all bar graphs except i (n = 6). Two-sided *P* values were determined by the Student's t-test for two groups or one-way ANOVA with Fisher's LSD posthoc analysis for three or more groups.

Extended Data Fig. 3. Experiments documenting the role of ERK, CD36, and DUSP4 in the efferocytosis-*Ptgs2*/COX2-TGFβ1 pathway. Related to Fig. 4. AC incubations were 45 min, and *Ptgs2* or *Tgfb1* were assayed 1 or 6 h after AC removal, respectively. **a**, BMDMs incubated ± ACs were immunoblotted for p-ERK1/2, ERK1/2, and β-actin. **b**, BMDMs pretreated with vehicle or bafilomycin A1 were incubated ± PKH26-labeled ACs for 45 min and assayed by flow cytometry for p-ERK1/2 in $PKH26^+$

(AC^+) and $PKH26^-$ (AC^-) macrophages. **c-d**, BMDMs transfected with scrambled RNA (Scr) or siMapk1 and siMapk3 were incubated ± ACs for 45 min and immunoblotted for ERK1/2, COX2, and β-actin or assayed for *Ptgs2* or *Tgfb1*. **e**, WT or MerTK-KO BMDMs were incubated with PKH26-labeled ACs and assayed by flow cytometry for p-ERK1/2 or, after a 3-h chase, COX2. (**f**) BMDMs treated with Scr or si*Cd36* were assayed for *Cd36*. **(g)** BMDMs treated with Scr or siCd36 were incubated with pHrodo-labeled ACs and, after an 18-h chase, assayed by flow cytometry for TGFβ1 in $pHrodo^+$ (AC^+) and $pHrodo^-$ (AC^-) macrophages. **h**, BMDMs pretreated for 2 h with vehicle or U0126 (MEK inhibitor) were incubated ± ACs and assayed for *Dusp4*. **i**, BMDMs treated with Scr, siDnmt3a, siDusp4, or siDnmt3a + siDusp4 were assayed for *Dnmt3a* or *Dusp4*. **j-k**, Control or DNMT3A-KO BMDMs transfected with Scr or siDusp4 as indicated were incubated ± ACs and assayed for *Ptgs2* or *Tgfb1*. **l-m**, Control or DNMT3A-KO BMDMs treated with Scr or siDusp1 as indicated were incubated ± ACs and assayed for *Ptgs2* or *Tgfb*. **n-o**, BMDMs treated with Scr, siMat2a, siDusp4, or siMat2a + siDusp4 were incubated ± ACs and assayed for *Tgfb1*. **p-q**, BMDMs treated with Scr, siMapk1/3, siDusp4, or siMapk1/3 + siDusp4 were incubated ± ACs and assayed for *Tgfb1*, *Mapk3*, *Mapk1*, and *Dusp4* after a 6-h chase. Data are expressed relative to the first control group. Values are means ± SEM. *n.s.*, not significant ($P > 0.05$); n = 3 biological replicates. Two-sided *P* values were determined by the Student's t-test for two groups or one-way ANOVA with Fisher's LSD posthoc analysis for three or more groups.

Extended Data Fig. 4. In vivo evidence of AC-induced COX2-TGFβ1 pathway. Related to Fig. 5. a-b, Wild-type (WT) C57BL/6J mice were transplanted with bone marrow from *Vav1Cre*$^{+/-}$ (Control) or *Dnmt3a*$^{fl/fl}$ *Vav1Cre*$^{+/-}$ (H-DNMT3A-KO) mice and, after 4 weeks, injected with PBS or dexamethasone (DEX). After 4h, the thymus was harvested and immunostained with anti-annexin V to document the initial increase in apoptosis after PBS or DEX injection, n=4 mice per group (**a**). For **b** (left), thymi were immunostained with Mac2 (green) and DNMT3A (Red). White arrows indicate non-macrophage DNMT3A, and brown arrows indicate macrophage DNMT3A. For **b** (right), documentation of co-localized p-ERK1/2, COX2, and Mac2. Representative image from n=4 mice per group. Scale bar, 50 μm. **c-d**, Wildtype (WT) C57BL/6J mice transplanted

with bone marrow from control or H-DNMT3A-KO mice were injected i.p. with 1 mg/mL Zymosan A1. After 12 h, peritoneal exudate cells were analyzed by flow cytometry for $F4/80^+$ and $COX2^+$ cells (n=4 mice/group) LAP-$TGF\beta1^+$ cells (n= 4 mice/group). **e-m**, *Ldlr*$^{-/-}$ (LDLR-KO) mice were transplanted with bone marrow from control or H-DNMT3A-KO mice and, after 4 weeks, fed a western-type diet (WD) for 12 weeks. Aortic root sections were immunostained for Mac2 and DNMT3A. Brown arrows indicate macrophage DNMT3A. Representative image from n=8 mice per group (**e**); body weight, n=10 mice/group (**f**); and the plasma or blood was assayed for the indicated metabolic and immune cell parameters, n=10 mice/group (**g-l**) and the lipoprotein-cholesterol profile by FPLC (**m**). Scale bar, 50 μm. Values are means ± SEM; *n.s.*, not significant ($P > 0.05$). Two-sided *P* values were determined by the Student's t-test for two groups or one-way ANOVA with Fisher's LSD posthoc analysis for three or more groups.

Extended Data Fig. 5. DNMT3A mediates efferocytosis and resolution in vivo. Related to Fig. 6. a, BMDMs were pretreated with vehicle or a TGFβ1R inhibitor for 2 h and with vehicle or recombinant TGFβ1 for 1 h, as indicated. The macrophages were then incubated with PKH26-labeled ACs for 45 mins, followed by rinsing and quantification of percent PKH26-AC^+ macrophages of total macrophages, n=4 biological replicates. **b-f**, Wildtype (WT) C57BL/6J mice were transplanted with bone marrow from control or H-DNMT3A-KO mice and, after 4 weeks, injected with PBS or dexamethasone (DEX). After 18 h, the thymi were weighed, n= 7 and 9 mice for PBS and DEX groups respectively (**b**); immunostained for DAPI, TUNEL, and Mac2 (**c**); assayed for $F4/80^+$ cells, n=5 mice per group (**d**); and assayed for TNF-a, n=3 and 5 mice for PBS and DEX groups respectively and IL-6 by ELISA, n=3 and 4 mice for PBS and DEX groups respectively (**e-f**). The image in b illustrates thymic macrophages with cytoplasmic TUNEL as an example of efferocytosing thymic macrophages. Scale bar, 100 μm. **g**, The peritoneal exudates were assayed for $Ly6G^+$ polymorphonuclear cells (PMN) 18 hours after Zymosan A1 injection, n=3 mice per group. **h**, Wild-type mice received 200 ng/mL recombinant TGFβ1 i.p. or vehicle control 15 and 20 hours after Zymosan injection and then assayed for the number of PMNs 4 hours later, n=3 mice/group. **i**, *Ldlr*$^{-/-}$ (LDLR-KO) mice were transplanted with bone marrow from control

or H-DNMT3A-KO mice and, after 4 weeks, fed a western-type diet (WD) for 12 weeks. Aortic root sections were quantified for lesional area, n=8 mice/group. Values are means ± SEM; *n.s.*, not significant ($P > 0.05$). Two-sided *P* values were determined by the Student's t-test for two groups or one-way ANOVA with Fisher's LSD posthoc analysis for three or more groups.