Obesity-Induced Increase in Cystatin C Alleviates Tissue Inflammation

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Supplementary Figure 1

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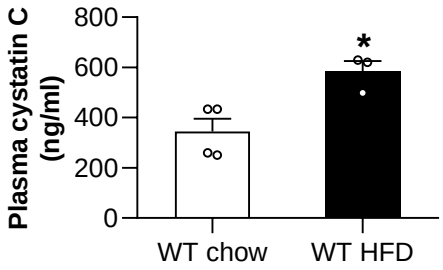

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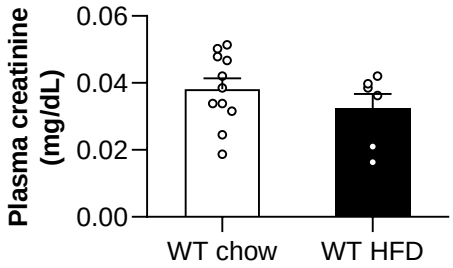
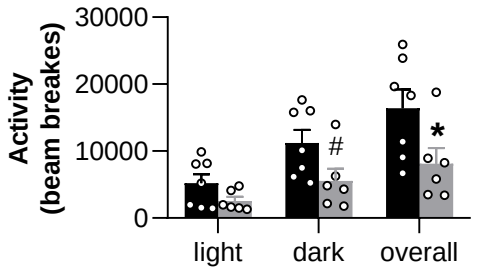


Increased circulating cystatin C levels in HFD-fed mice

(A) Plasma levels of cystatin C in portal blood of WT mice (n=4 chow-fed mice and n=3 HFD-fed mice). (B) Plasma levels of creatinine in systemic blood of chow- and HFD-fed WT mice (n=11 chow-fed mice and n=6 HFD-fed mice). Values are expressed mean±SEM. *p<0.05 (Student's *t*-test).

Supplementary Figure 2

A



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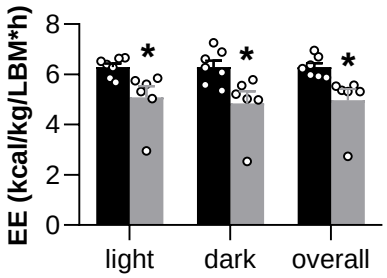

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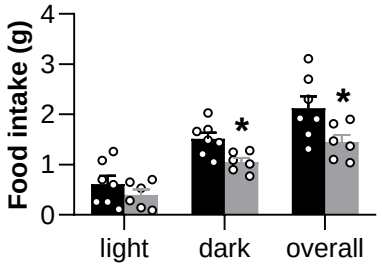
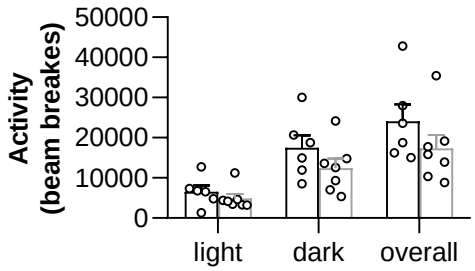


Decreased locomotor activity, energy expenditure and food intake in HFD-fed CysC KO mice

(A) Locomotor activity, (B) energy expenditure and (C) food intake of HFD-fed WT and CysC KO mice (n=7 WT and n=6 CysC KO mice). Values are expressed as mean±SEM. (A) #p=0.06. *p<0.05 (Student's *t*-test).

Supplementary Figure 3

A



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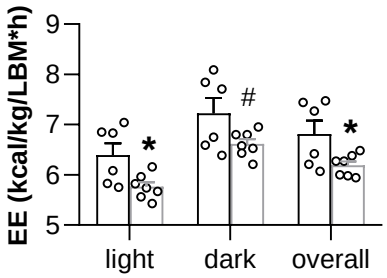

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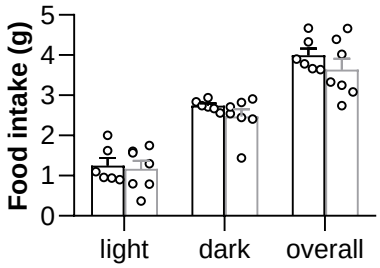
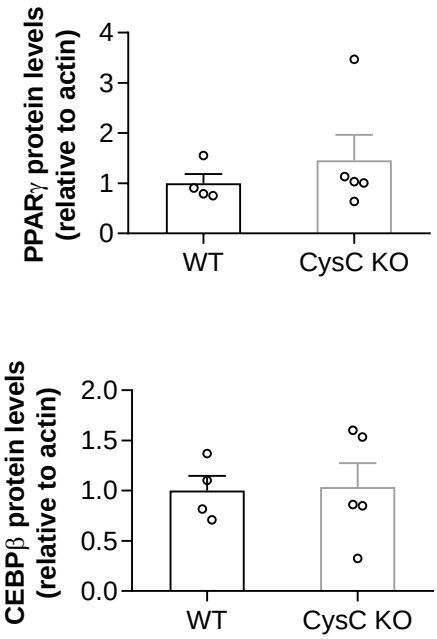


Similar locomotor activity and food intake, but decreased energy expenditure in chow-fed CysC KO mice

(A) Locomotor activity, (B) energy expenditure and (C) food intake of chow-fed WT and CysC KO mice (n=6 WT and n=7 CysC KO mice). Values are expressed as mean±SEM. $^{\#}$p=0.06, *p<0.05 (Student's *t*-test).

Supplementary Figure 4

A



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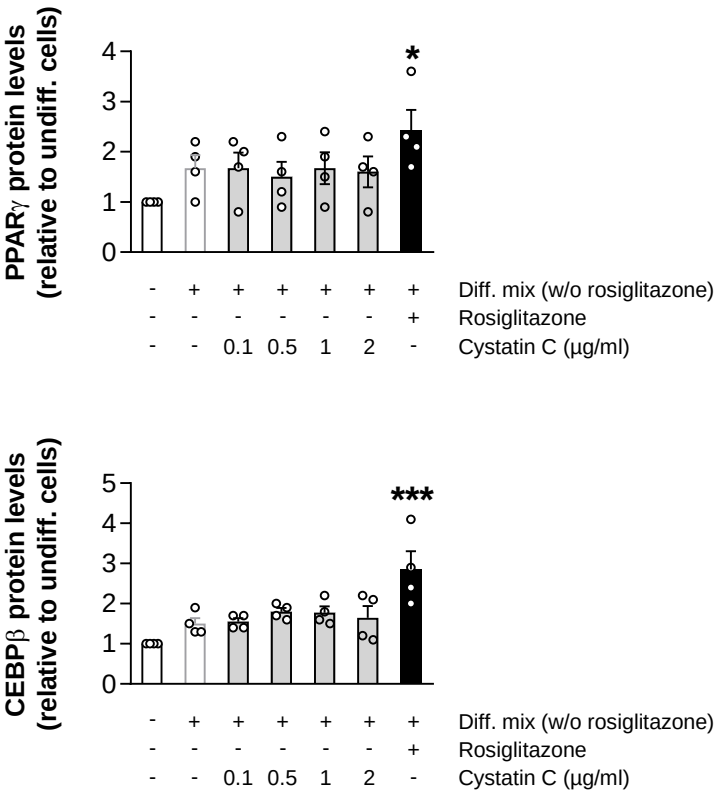
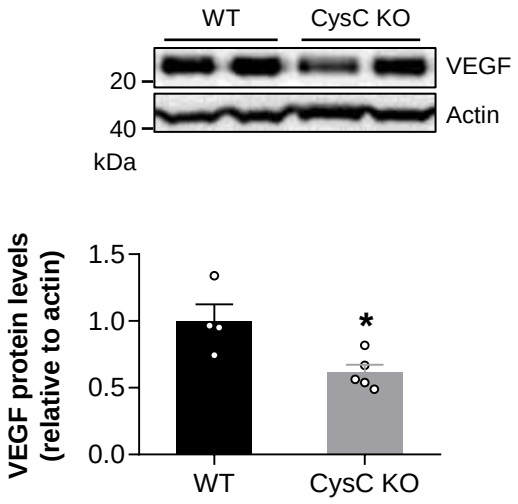


No effect of recombinant CysC on 3T3-L1 adipocyte differentiation

(A) Quantification of protein levels of PPARγ and CEBPβ in iWAT of chow-fed WT (n=4) and CysC KO (n=5). Protein levels were normalized to WT mice. (B) Confluent 3T3-L1 adipocytes were treated for three days with or without a differentiation (Diff.) mix containing 500 µM isobutylmethylxanthine, 1 µM dexamethasone and 1.7 µM insulin, rosiglizatone (1µM) and different concentrations of recombinant CysC as indicated. Thereafter, cells were treated for three days with or without a second Diff. mix containing 0.5 µM insulin and different concentrations of recombinant CysC as indicated. Six days after induction of differentiation, cells were lysed and Western blots were performed. Shown are quantified protein levels of PPARγ and CEBPβ. n=4 biological replicates. Values are expressed as mean±SEM. *$p<0.05$, ***$p<0.001$ (ANOVA, compared to undifferentiated cells).

Supplementary Figure 5

A



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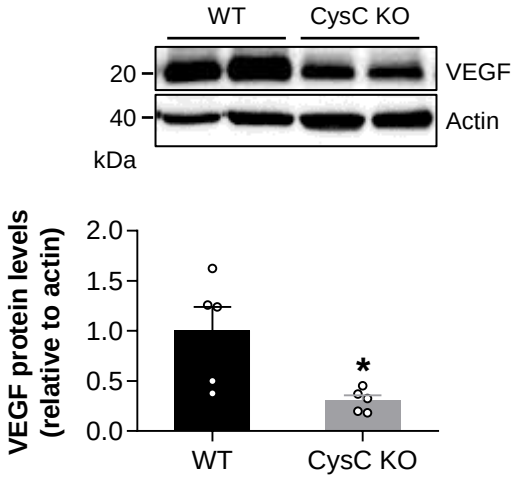
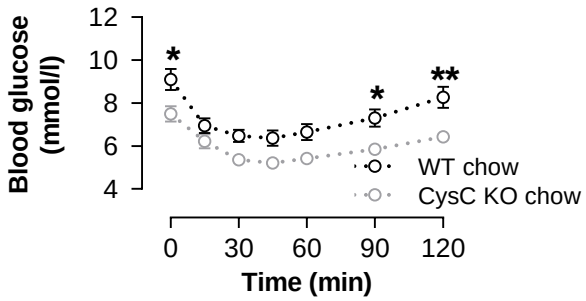


Decreased VEGF levels in skeletal muscle of HFD-fed CysC KO mice

Representative Western blots and quantification of protein levels of VEGF in iWAT (A; n=4 WT and n=5 CysC KO) and in skeletal muscle of HFD-fed mice (B; n=5 mice per group). Protein levels were normalized to WT mice. Values are expressed as mean±SEM. *p<0.05 (Student's *t*-test).

Supplementary Figure 6

A



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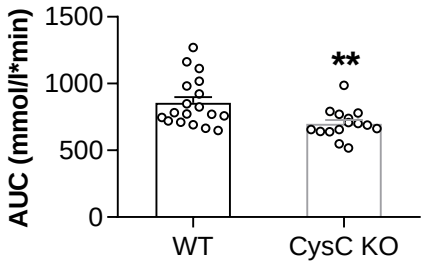

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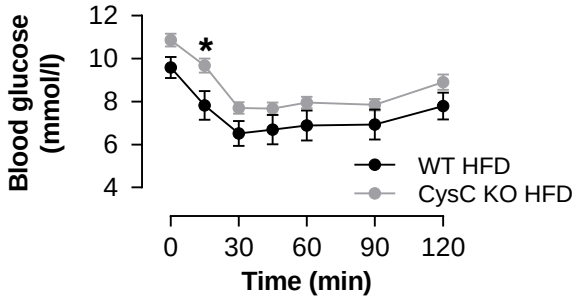

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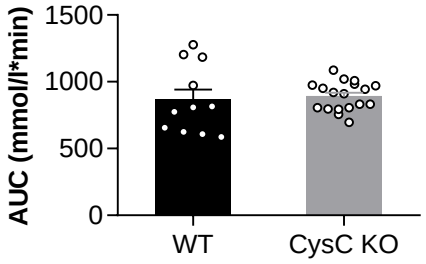

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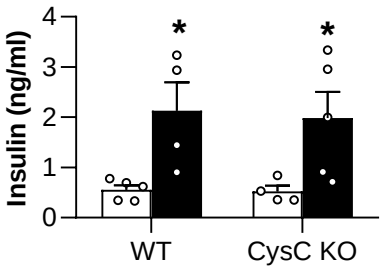
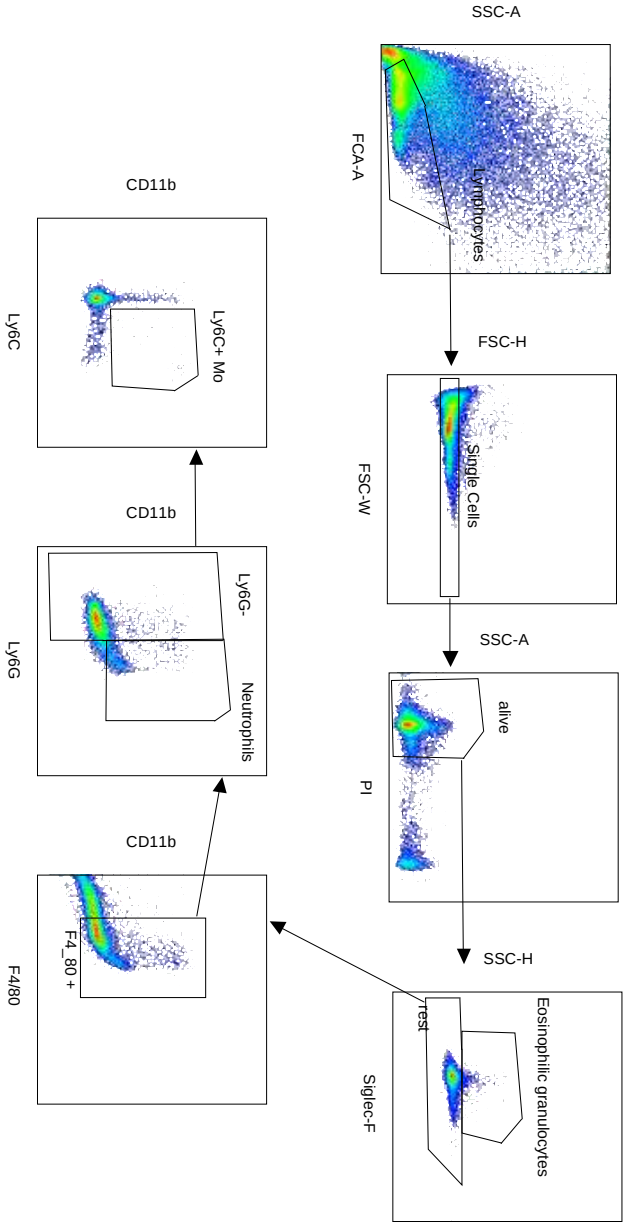


Insulin tolerance in chow- and HFD-fed WT and CysC KO mice

Intraperitoneal insulin tolerance test (ipITT) in chow- (n=18 WT and n=15 CysC KO mice) and HFD-fed (n=11 WT and n=18 CysC KO mice) WT and CysC KO mice (A-D). (E) Circulating insulin levels of chow- and HFD-fed WT mice (chow n=5, HFD n=4) as well as CysC KO mice (chow n=4, HFD=5). Values are expressed as mean±SEM. *p<0.05, **p<0.01. Student's *t*-test (B, D, E), two-way ANOVA (A, C).

Supplementary Figure 7

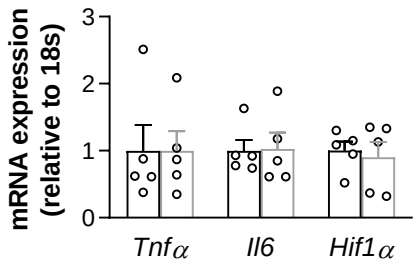


Gating strategy on cells in eWAT

Lymphocytes and single cells were initially identified by size. PI negative cells were identified as alive. Following exclusion of Eosinophils we gated on F4/80. Afterwards Ly6G negative cells were gated on macrophages based on CD11b and Ly6C expression. PI: Propidium iodide.

Supplementary Figure 8

A



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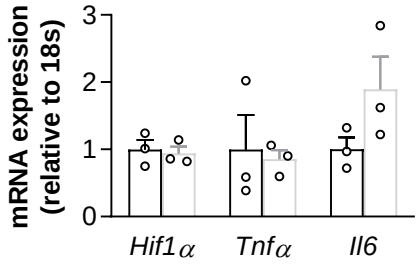

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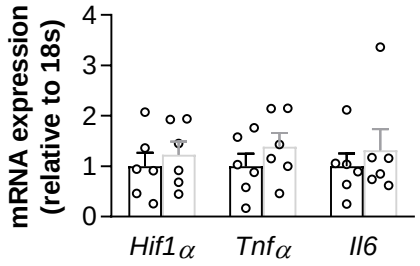
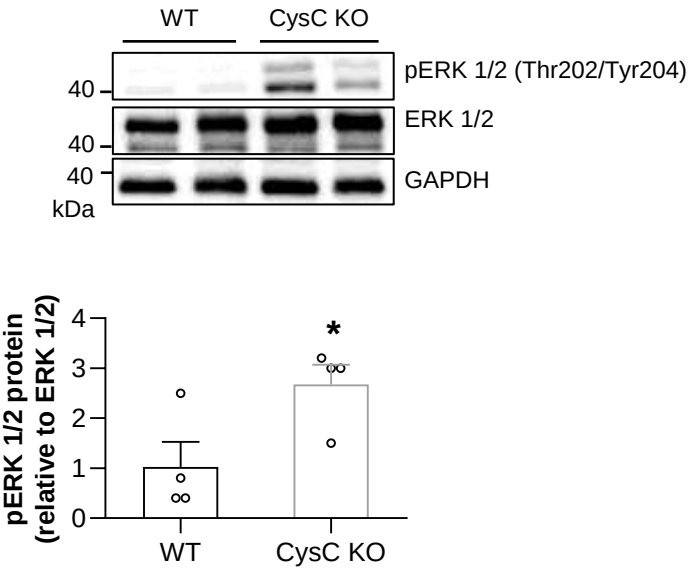


Similar expression of pro-inflammatory cytokines in WAT of chow-fed WT and CysC KO mice

mRNA expression levels of pro-inflammatory cytokines in (A) eWAT (n= 5 mice per group), (B) Liver (n=3 per group) and (C) skeletal muscle (n=6 per group) of chow-fed WT and CysC KO. Values are expressed as mean±SEM.

Supplementary Figure 9

A



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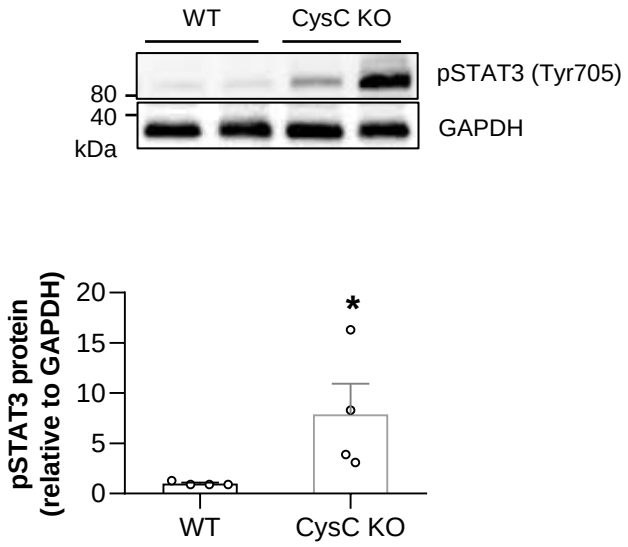
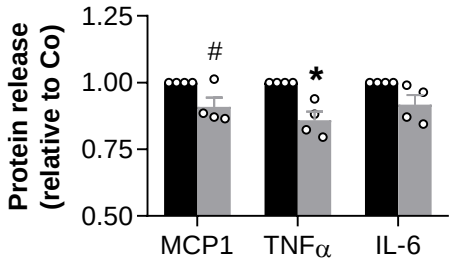


Increased ERK and STAT3 phosphorylation in adipose tissue of LPS-treated CysC KO mice

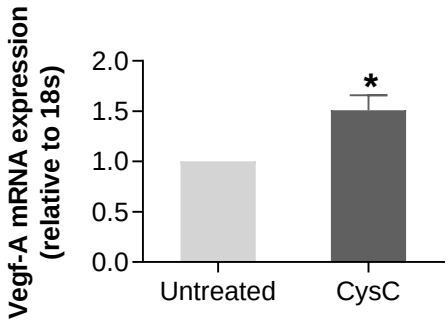
Phosphorylation levels of selected IL-6 downstream targets ERK 1/2 (A) and STAT3 (B) (n=6 mice per group). Values are expressed mean±SEM. *p<0.05 (Student's *t*-test).

Supplementary Figure 10



Decreased release of pro-inflammatory mediators from CysC-treated RAW cells
MCP1, TNFα and IL-6 protein levels were determined in the supernatant of LPS-stimulated RAW 264.7 cells in the absence (controls (Co), black bars) or presence of CysC (grey bars). Protein release was normalized to RNA concentration and is shown relative to Co cells. n=4 independent experiments. $^{\#}p<0.1$, $^{*}p<0.05$ (One sample *t*-test).

Supplementary Figure 11



Increased *Vegf-A* expression in cystatin C-treated eWAT explants

mRNA expression of *Vegf-A* in eWAT explants harvested from HFD-fed mice and treated with or without recombinant cystatin C (n=5 independent experiments). Values are expressed mean±SEM. *p<0.05 (Student's *t*-test).

Supplementary Tab. 1 mRNA primer used for experiments in human individuals

mRNA	*Order number*
HIF1α	Hs00153153_m1
TNFα	Hs01113624_g1
IL-6	Hs00985639_m1
CysC	Hs00264679_m1
HPRT1 mRNA	Hs01003267_m1

Primers were provided by Applied Biosystems, Darmstadt, Germany

Supplementary Tab. 2 mRNA primer used for experiments in mice

mRNA	*Order number*
Hif1α	Mm00468869_m1
Tnfα	Mm00443258_m1
Il-6	Mm00446190_m1
Vegf-A	Mm01281449_m1

Primers were provided by Applied Biosystems, Rotkreuz, Switzerland

Supplementary Tab. 3 Order number of primary antibodies used

Target	*Order number*	*Company*
Actin	MAB1501	Millipore, Billerica, MA, USA
CEBPβ	sc-150	Santa Cruz Biotechnology, Dallas, TX, USA
PPARγ	sc-7196	Santa Cruz Biotechnology
pSTAT3 (Tyr705)	D3A7	Santa Cruz Biotechnology
VEGF	sc-7269	Santa Cruz Biotechnology
ERK 1/2	9102	Cell Signaling Technology, Danvers, MA, USA
pERK 1/2 (Thr202/Tyr204)	9101	Cell Signaling Technology
GAPDH	10494-1-AP	Proteintech, Rosemont, IL, USA

Supplementary Tab. 4 Dye and fluorochrome-coupled antibodies used for flow cytometry

Antibody	*Company*
fluorescein isothiocyanate (FITC)–anti-Annexin	BioLegend Way, San Diego, CA, USA
phycoerythrin (Pe)/Cy7–anti-CD11b (clone M1/70)	BioLegend Way
PE–anti-Ly6G	BioLegend Way
APC-Cy7–anti-Ly6C (clone HK1.4)	BioLegend Way
Biotin–anti-Siglec-F	BioLegend Way
Alexa Fluor 700–streptavidin	BioLegend Way
APC–anti-F4/80	BioLegend Way