

1 **Supplementary Figures**

2 **Fig. S1: Cytokine and growth factor profile in cantharidin blisters from young and aged**
3 **donors.**

4 Quantification by multiplex ELISA of **A)** IL-6, **B)** IL-10, **C)** CCL2, **D)** CCL3, **E)** CCL4, **F)**
5 **CCL7**, **G)** CCL8, **H)** CXCL1, **I)** CXCL5, **J)** osteopontin (OPN), **K)** M-CSF, and **L)** platelet-
6 derived growth factor (PDGF)-BB. Data from young are shown in black, aged in red. Each
7 symbol represents a sample from a single participant. Data are shown on log scales. 2-way
8 ANOVA on log-transformed data with Sidak's multiple comparisons post-test to show age-
9 dependent changes. * $p < 0.05$, *** $p < 0.001$.

10

11 **Fig. S2: Blister gating strategy**

12 Representative plots for flow cytometric gating are shown for a healthy aged participant at
13 24h. The same gating strategy (denoted by the arrows) was used for all volunteers at both
14 time points for the characterization study. Particularly, HLA-DR⁺/CD14⁺ mononuclear
15 phagocytes were gated out according to these criteria for more detailed analysis of this cell
16 type. Briefly, doublets were excluded using **A)** Forward Scatter (FSC) and **B)** Side Scatter
17 (SSC) height and width characteristics. **C)** Debris was excluded based on forward and side
18 scatter. **D)** Leukocytes were gated on CD45 and **E)** lineage (Lin, CD3/CD19/CD56) negative
19 cells were taken forward. **F)** HLA-DR⁻ populations contained **G)** CD16^{hi}SSC^{hi} neutrophils
20 (PMNs) and **I)** CD16⁻SSC^{hi}Siglec-8⁺ eosinophils. **H)** The PMN population was examined for
21 apoptosis using Annexin V and subsequently divided into **J)** apoptotic and necrotic/dead cells
22 using a viability dye. HLA-DR⁺ cells (**F)** were gated on CD14 and CD16 to identify
23 Dendritic Cells (DCs) and CD14^{hi} monocytes/macrophages (mononuclear phagocytes, MPs).

24

25 **Fig. S3: PMN function and phenotype**

26 Flow cytometry analysis to enumerate **A)** MPs and **B)** PMNs in cantharidin blisters harvested
27 24 and 48 hours after challenge (n = 6 young, 7 old). Paired flow cytometry analysis of
28 PMNs in whole blood (WB) and 24 h cantharidin blisters (CB 24h) from young (black) and
29 aged (red) participants of **C)** CD16, **D)** CD11b, **E)** CD66b and **F)** CD62L expression. Two-
30 way ANOVA with Tukey's correction in A-B), two-tailed paired Student's *t* tests on log
31 transformed data in C-E) and Wilcoxon test on untransformed data in F). *p* values are given.

32

33 **Fig. S4: Fas-driven PMN apoptosis, Fas and TNF receptor expression are all unchanged**
34 **with age.**

35 **A)** Fas-inducible neutrophil apoptosis measured by cell viability over a 24 h time course.
36 Shown are means and s.d. (n = 3 per group). Multiple unpaired *t* tests with Holm-Sidak
37 correction. Flow cytometric analysis of 24 h cantharidin blister PMN expression of **B)**
38 TNFR1, **C)** TNFR2, and **D)** Fas. Data are shown on a logarithmic scale, with geometric
39 means and geometric s.d. (n = 5 per group). Two-tailed unpaired Student's *t* tests on log
40 transformed data (ns: *p* > 0.05).

41

42 **Fig. S5: Correlations between TNF- α and PMN migration into inflammatory blisters**

43 Scatter plots showing **A)** donor neutrophil clearance (expressed as 24 h – 48 h PMN numbers
44 per blister) against 24 h MP infiltrates (n = 6 young, 7 old) and TNF- α levels vs total PMNs
45 in 24 h blisters of **B)** young and **C)** aged donors. Shown are linear regression with 95%
46 confidence bands. Pearson *r* was calculated and is shown alongside *p*-values (n = 13 young, n
47 = 12 aged).

48

49 **Fig. S6: Extended data on phagocytosis.**

50 Representative cytopins from **A)** 24 h cantharidin blisters and **B)** an *ex vivo* efferocytosis
51 assay using cultured MPs. Arrows denote efferocytosis events. Scale bars are 10 μ m. **C)**
52 Cytochalasin B (10 μ M) was used as an actin polymerization inhibitor to block
53 internalization. **Data were paired and normalized to control, set to 1 (n = 4).** **D)** Flow
54 cytometric analysis of Annexin V binding to 24 h blister PMNs gated as live (CD16^{hi}Annexin
55 V⁻), CD16^{hi} early apoptotic (CD16^{hi}Annexin V⁺) and CD16^{lo} late apoptotic (CD16^{lo}Annexin
56 V⁺). Two-way ANOVA with Tukey's correction (n = 5 young [black], n = 7 aged [red]). **E)**
57 Representative ImageStreamX images from *ex vivo* phagocytosis assays using 24 h cultured
58 MPs (stained with CD14, red). Shown are images of unchallenged control MPs (Ctrl), MPs
59 challenged with CFSE-labelled ACs at a 3:1 AC:MP ratio (AC), MPs challenged with 10:1
60 fluorescently labelled latex beads that were either opsonised in serum (OLB) or left in their
61 native state (LB).

62

63 **Fig. S7: Extended data on MP phenotype.**

64 Comparison between 24 h cantharidin blister (CB 24h) MPs, isolated blood MPs cultured for
65 24 h (MPs 24h), and isolated monocytes cultured for 7 days with M-CSF (20 ng/ml) to yield
66 monocyte-derived macrophages (MDMs). Flow cytometric analysis of **A)** CD14, **B)** TIM-4,
67 **C)** MerTK, **D)** CD36, and **E)** CD51 (ITGAV) expression. One-way ANOVA with Sidak's
68 correction (n = 4-9 per group). **F)** Representative ImageStreamX images from an *ex vivo*
69 efferocytosis assay using 24 h cultured MPs (stained with CD14, red) and autologous, blood-
70 derived apoptotic ACs (green) at a ratio of 3:1 ACs:MPs. Shown are images of a CFSE-
71 stained apoptotic cell (AC), an AC-negative MP (MP), and MPs that have bound to an AC
72 (<0) or that have ingested an AC (>0). Summary data of MPs that have associated with ACs
73 (internal and external) between MPs isolated from young (black) and aged (red) donors (n = 3
74 per group). Two-tailed unpaired Student's *t* test *p* value given). Flow cytometric analysis on

75 24 h cantharidin blister MPs between young (black) and aged (red) for expression of **G)**
76 **CD14**, **H) CD16**, **I) HLA-DR**, **J) CD163**, **K) CD206** (mannose receptor), **L) CD36**, and **M)**
77 **CD51** (ITGAV, **Integrin α V**). Two-tailed unpaired Student's *t* tests on log-transformed data,
78 except CD36 and CD51 where Mann-Whitney tests were used. **All tests showed no**
79 **significant change**. Flow cytometric analysis of **N) TIM-4** (**n = 8 per group**) and **O) MerTK**
80 expression on 24 h cultured MPs isolated from young (n = 8) and aged (n = 5) donors. Two-
81 tailed unpaired Student's *t* tests on log-transformed data (*p* values given).

82

83 **Fig. S8: Representative image of nuclear translocation experiments of NF κ B and P-**
84 **STAT3.**

85 Isolated blood monocytes were cultured for 24 h before stimulation with vehicle (Ctrl), 1
86 ng/ml LPS (LPS), 3:1 AC:MP CFSE-labelled autologous ACs (AC), or simultaneous addition
87 of LPS and ACs (LPS + AC). MPs were stained with CD14-AF647 (red), DAPI (blue),
88 phospho-STAT3 (P-STAT3, green), and NF κ B subunit p65 (p65, yellow). Overlays show
89 CD14, DAPI and P-STAT3, and CD14, DAPI and NF κ B respectively. Scale bar denotes 7
90 μ m. Representative of six independent experiments.

91

92 **Fig. S9: p300 blockade using A-485**

93 **A) Flow cytometry was used to confirm TIM-4 expression in MPs cultured with 10 μ g/ml**
94 **LPS with or without 3 μ M losmapimod and the indicated dose of A-485 (in nM). The**
95 **experiment was performed five times with five different donors and summary data of all**
96 **experiments are shown. B) Normalised TIM-4 expression of the same experiments is shown**
97 **alongside CD14 expression on the same cells (n = 5). C) ChIP to confirm Histone3 Lysine 27**
98 **acetylation in MPs treated with 3 μ M losmapimod with either C646 and SGC-CBP30 (2.5**

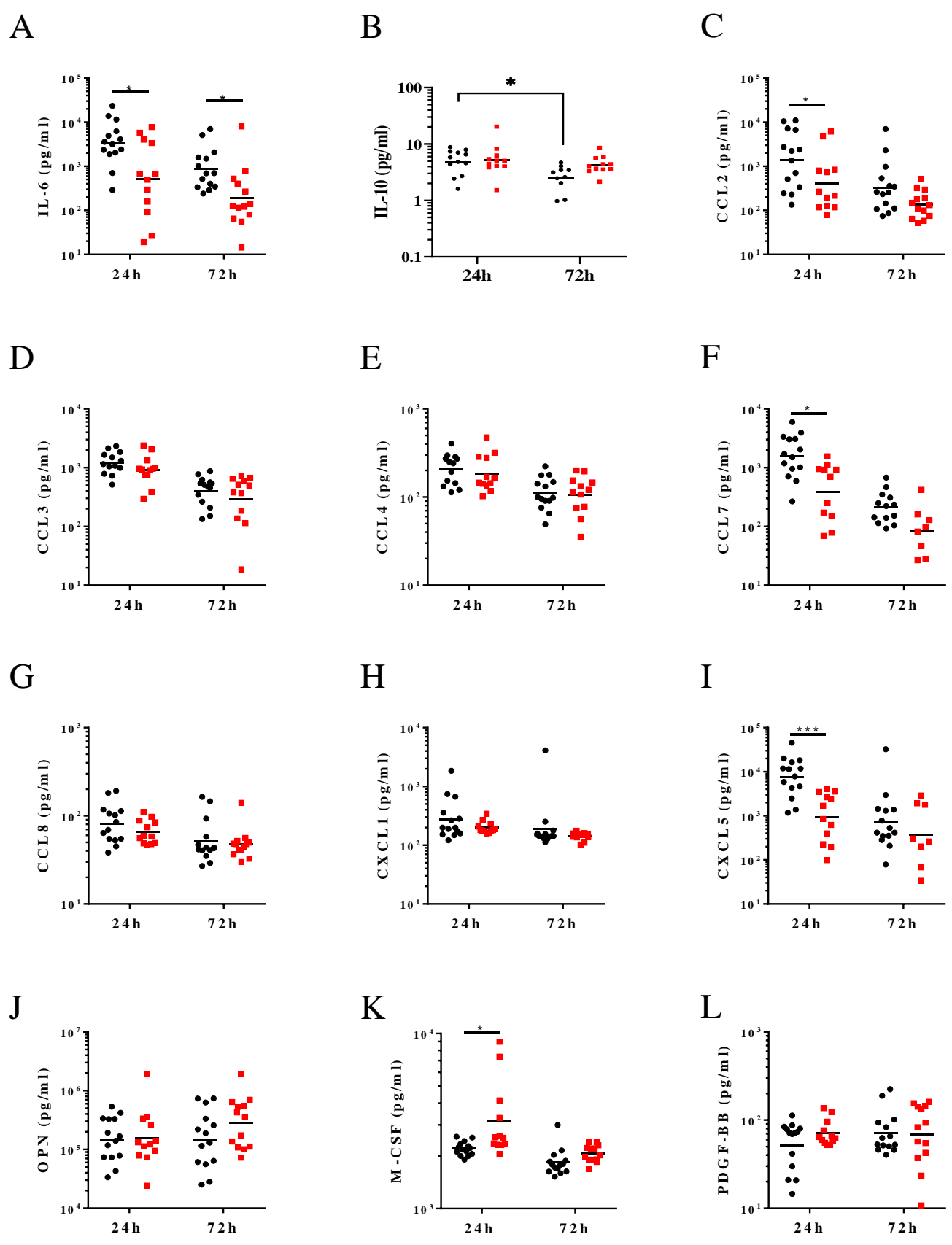
99 μM and $5 \mu\text{M}$ respectively) or A-485 (200 nM). Results are normalised by setting
100 losmapimod samples to 1 (n = 2 pooled donors).

101

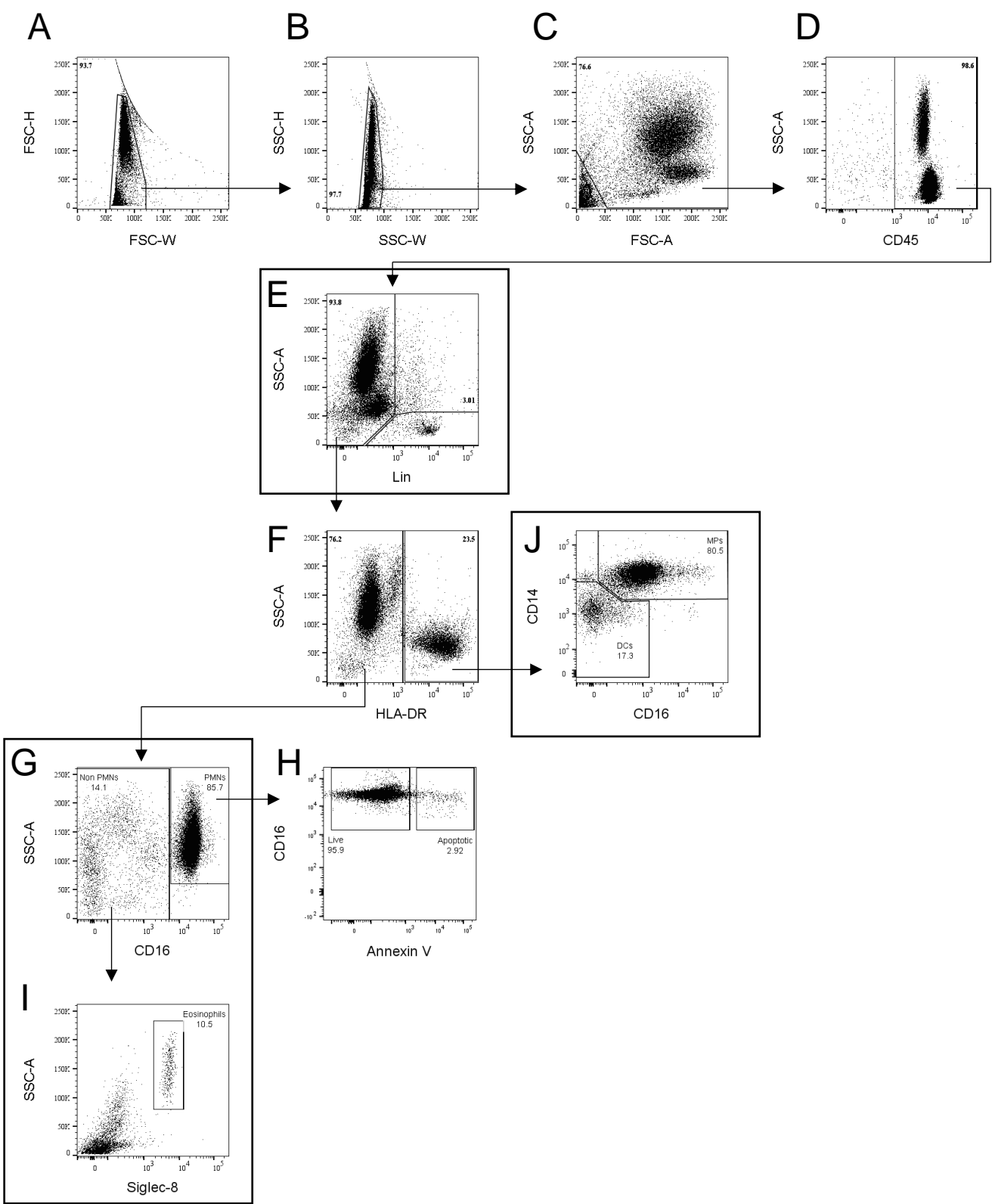
102 **Fig. S10: Extended data on losmapimod-treated individuals.**

103 **A)** Representative plot showing the dose-dependent relationship between 24h *ex vivo* LPS
104 stimulation and TNF- α (measured by cytometric bead array) in blood pre- and post-
105 losmapimod (n = 11, multiple paired *t* tests). **B)** Plot showing the dose-dependent relationship
106 between 24h *ex vivo* LPS stimulation and IL-6 (measured by cytometric bead array) in blood
107 pre and post losmapimod (n = 11, means and s.d.). Multiple paired *t* tests per row. **C)**
108 Representative photographs of 24 h and 72 h cantharidin blisters on an untreated (Aged) and
109 losmapimod-treated (Losmapimod) individual. Arrows denote 72 h blisters. and **D)** Total
110 blister exudate volume (mean, two-way ANOVA with Tukey's correction).

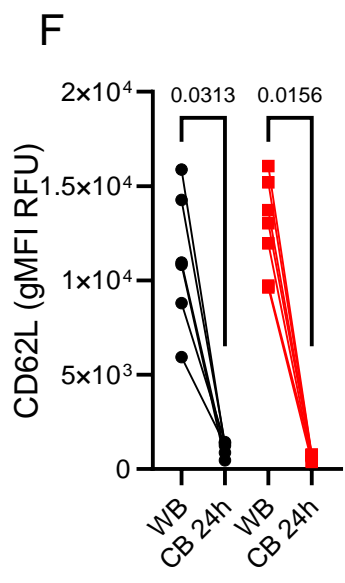
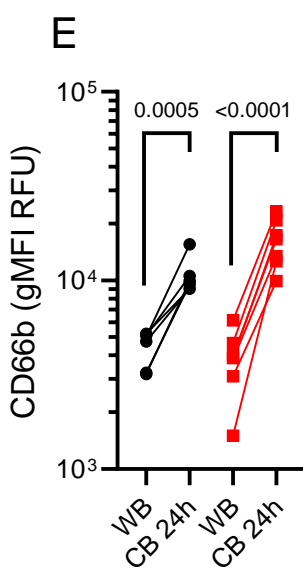
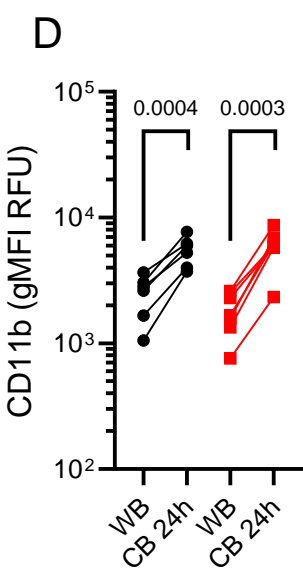
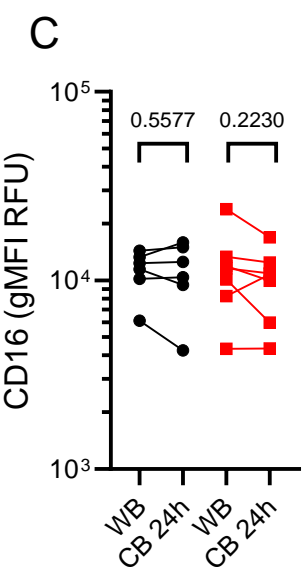
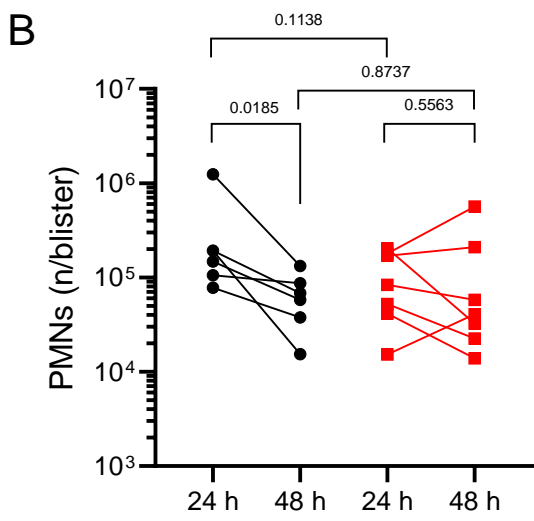
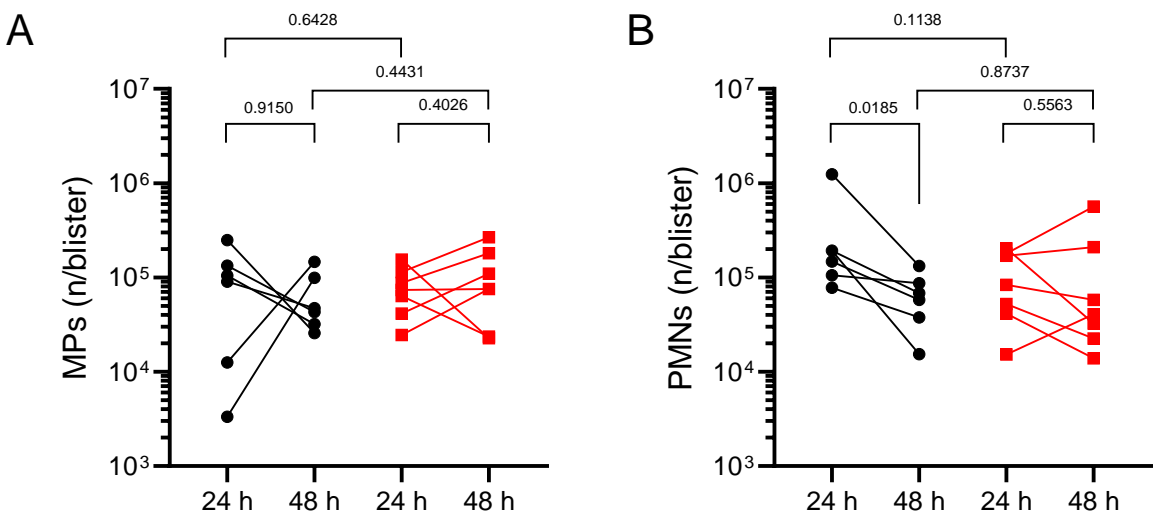
Supplementary Fig 1



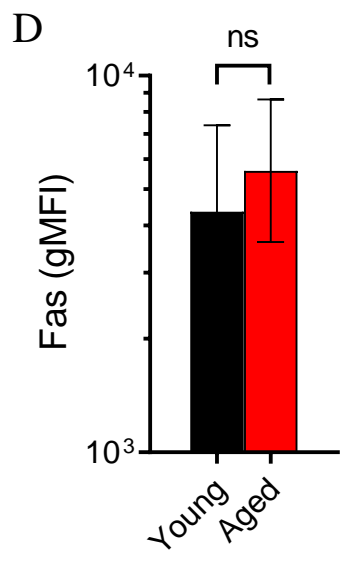
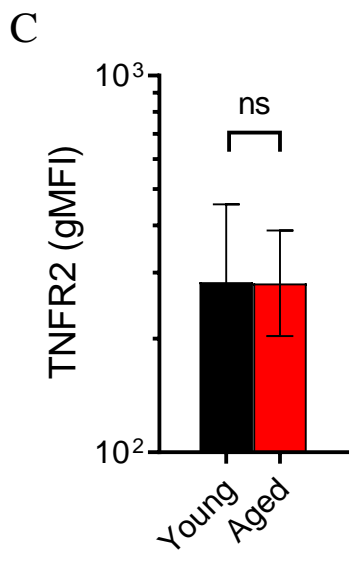
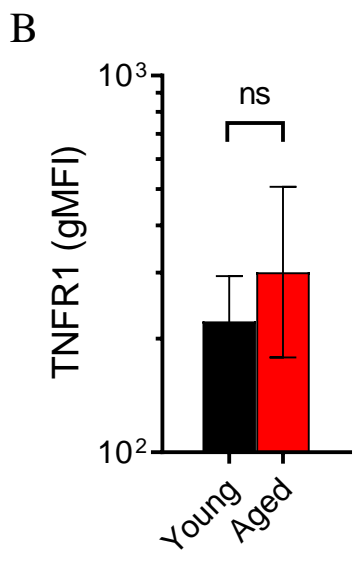
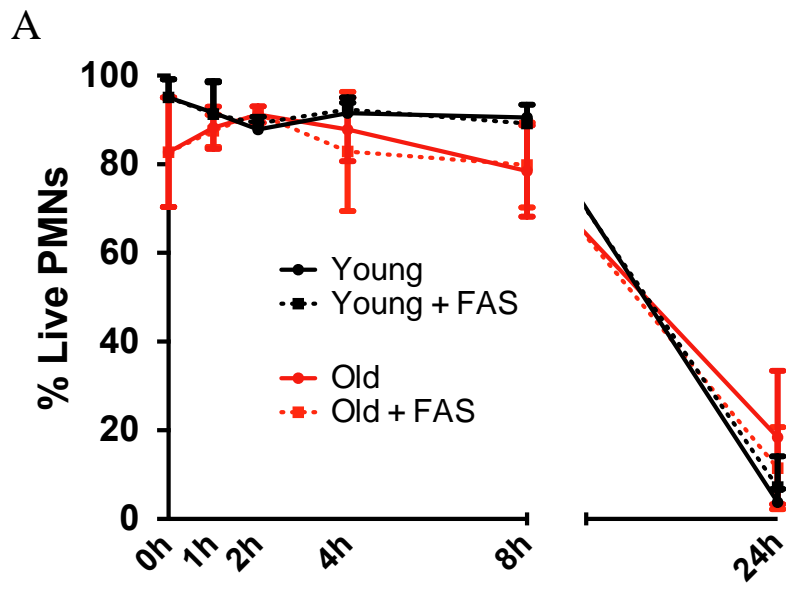
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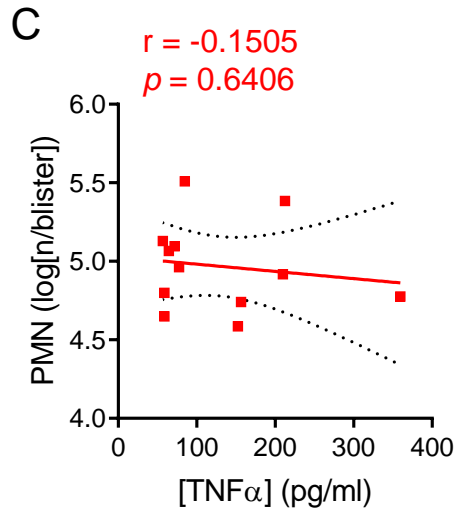
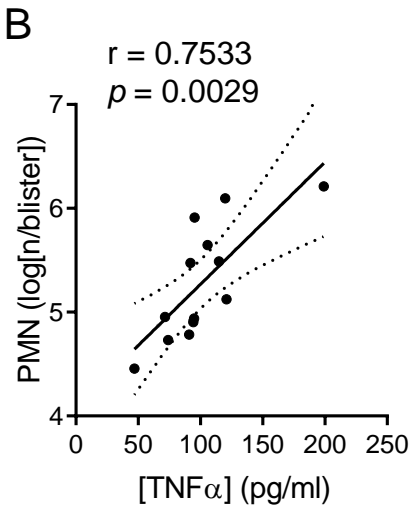
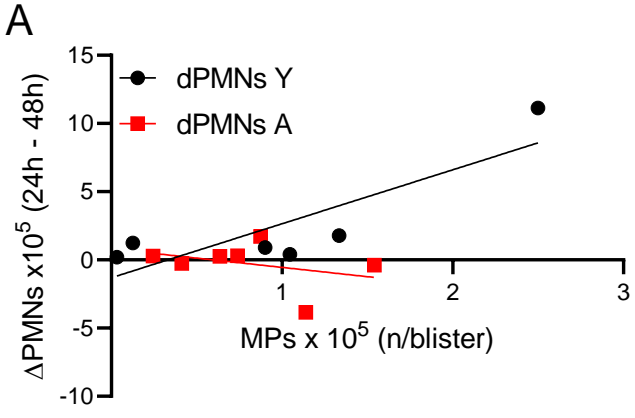
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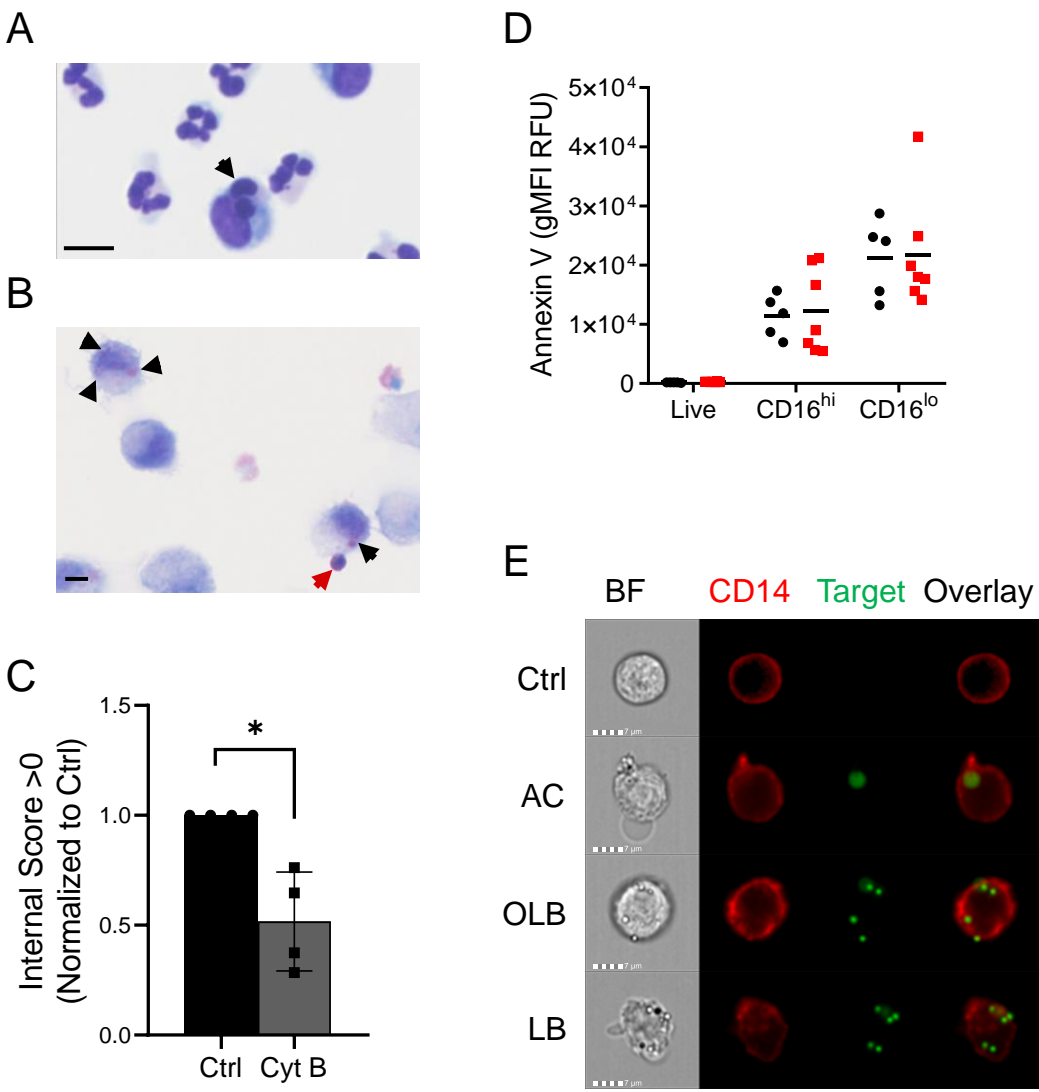
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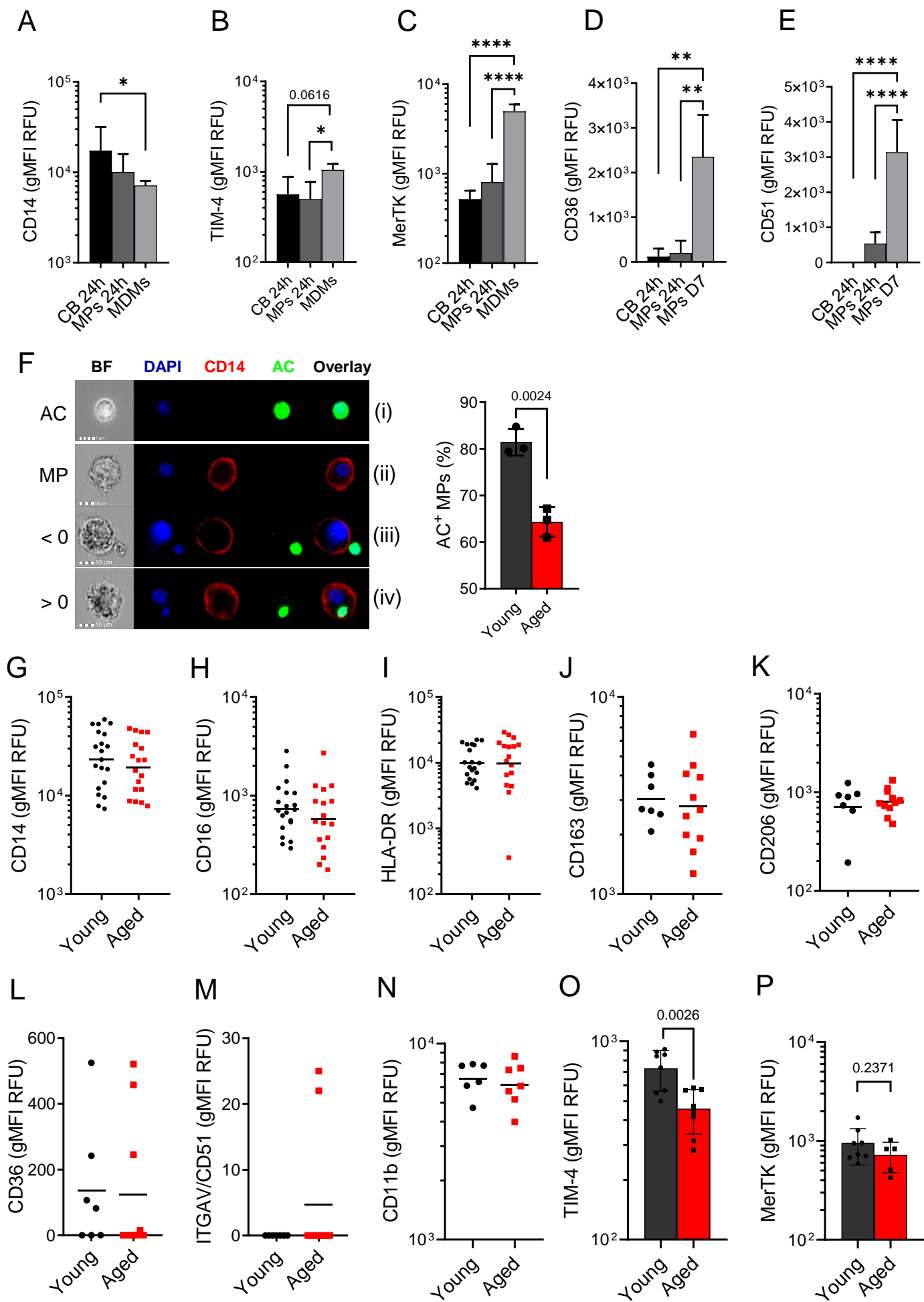
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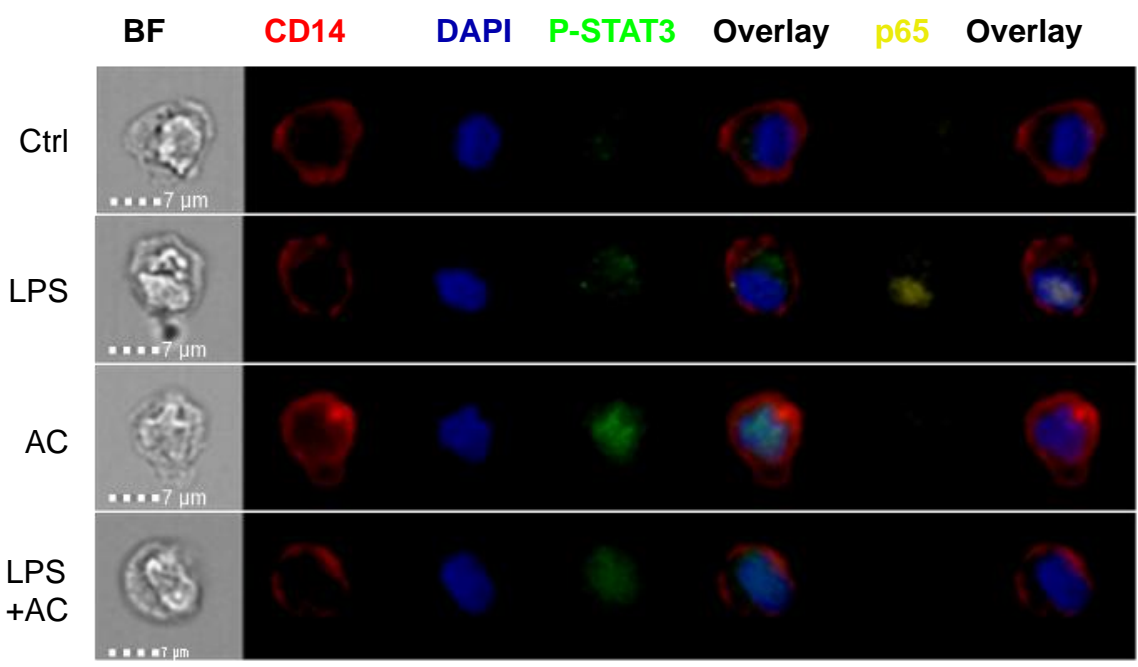
Supplementary Fig 6



Supplementary Fig 7

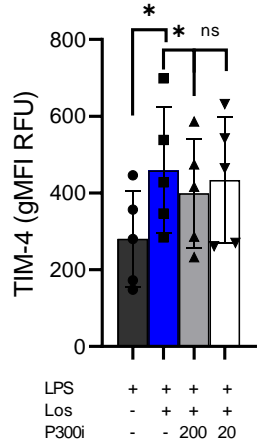


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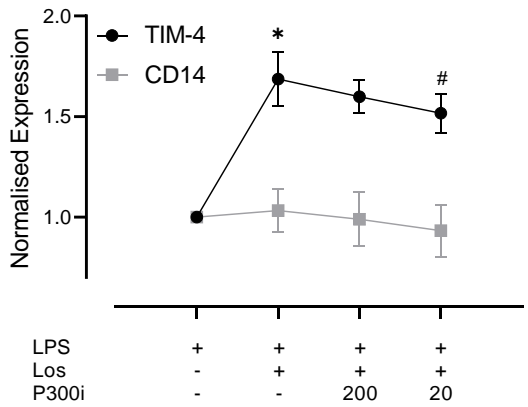


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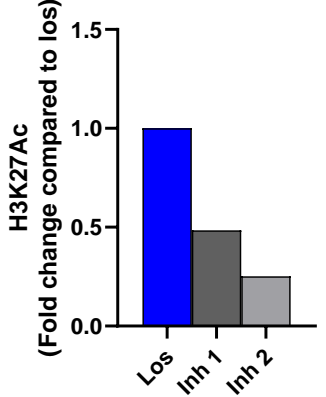
A



B



C



Supplementary Fig 10

