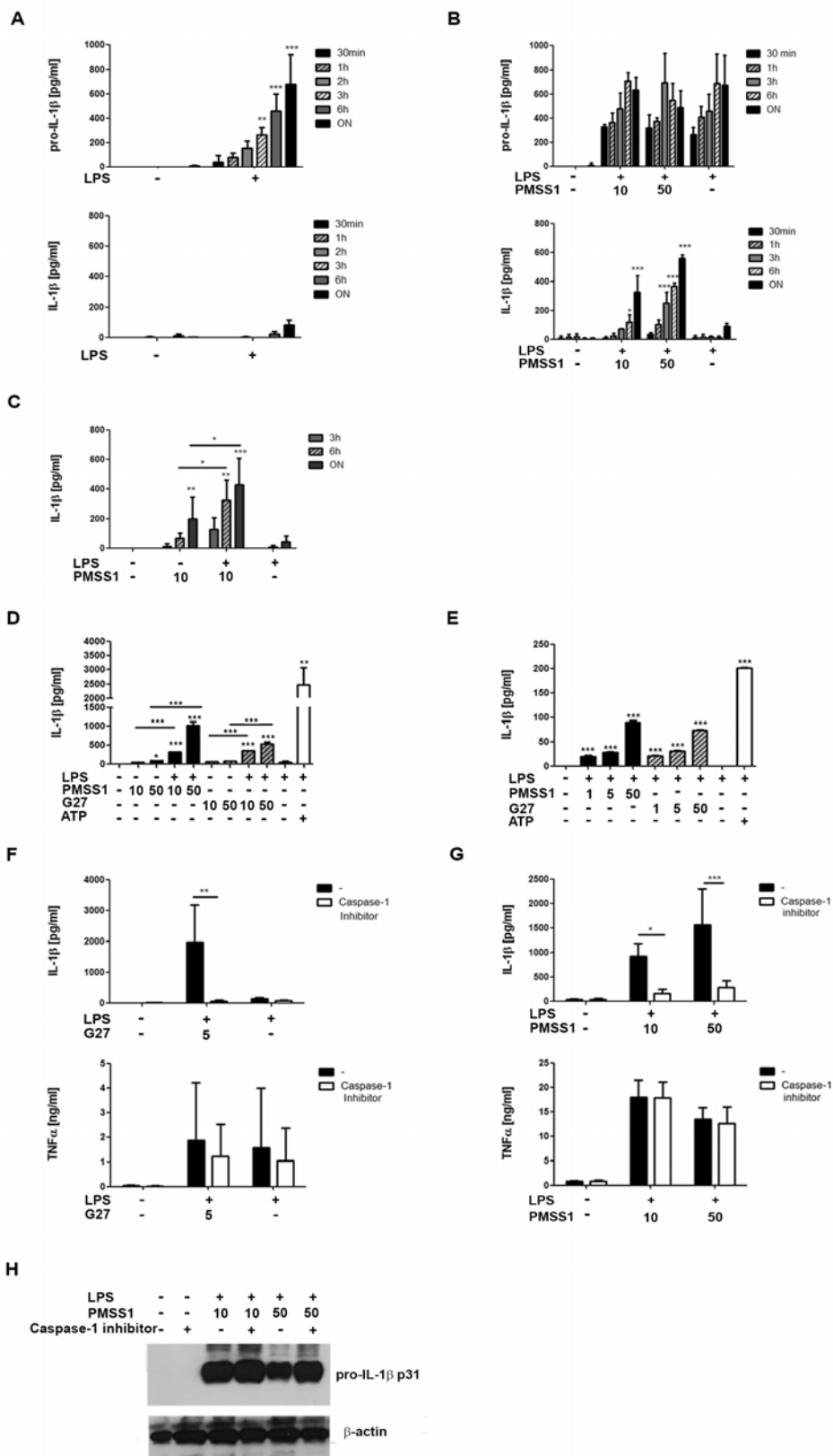


Suppl. Figure 1



2 (A) Time course experiment showing pro-IL-1 β and IL-1 β levels in BMDCs stimulated with
3 LPS (10 ng/ml) for the indicated times. Data are presented as mean \pm S.D. of three independent
4 experiments. LPS stimulated BMDCs were compared to non-stimulated control cells of the
5 respective time-point. ($p < 0.0001$ ANOVA, ** $p \leq 0.01$, *** $p \leq 0.001$ Bonferroni-corrected for pair
6 wise comparison).

7 (B) Time course experiment showing pro-IL-1 β and IL-1 β levels in BMDCs stimulated with LPS
8 (10 ng/ml) for 3 h and infected with the *H. pylori* strain PMSS1 (MOI 10 and 50) for the
9 indicated times. Data are presented as mean \pm S.D. of three independent experiments. LPS pre-
10 stimulated and *H. pylori* infected BMDCs were compared to only LPS-stimulated control cells of
11 the respective time-point. ($p < 0.0001$ ANOVA, ** $p \leq 0.01$, *** $p \leq 0.001$ Bonferroni-corrected for
12 pair wise comparison).

13 (C) Time course experiment showing mature IL-1 β levels in BMDCs infected with the *H. pylori*
14 strain PMSS1 (MOI 10) for the indicated times. Cells were pre-stimulated with LPS (10 ng/ml)
15 for 3 hours where indicated. Data are presented as mean \pm S.D. of four independent experiments.
16 *H. pylori*-infected cells were compared to uninfected control cells, while infected and LPS pre-
17 stimulated PBMCs were compared to only LPS-stimulated control cells (asterisks shown on top
18 of the bars). ($p < 0.0001$ ANOVA, ** $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ Bonferroni-corrected for pair
19 wise comparison).

20
21 (D) Bone marrow macrophages were infected with the *H. pylori* strains PMSS1 or G27 o/n at
22 MOI 10 and 50 and the secretion of IL-1 β was measured in the supernatants by ELISA. For pre-
23 stimulation 10 ng/ml LPS were used. One hour stimulation with ATP (5 mM) was used as a
24 positive control. Data are presented as mean \pm S.D, of three independent experiments. *H. pylori*-

25 infected cells were compared to uninfected control cells, while infected and LPS pre-stimulated
26 BMMs were compared to only LPS-stimulated control cells (asterisks shown on top of the bars)
27 ($p < 0.0001$ ANOVA, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ Bonferroni-corrected for pair wise
28 comparison).

29 (E) IL-1 β levels detected in the supernatants of neutrophils infected with the *H. pylori* strain
30 PMSS1 or G27 o/n at MOI 1, 5 and 50. 50 ng/ml LPS were used to pre-stimulate cells where
31 indicated. As a positive control ATP (5 mM) was used. Data are presented as mean \pm S.D. of one
32 experiment performed in triplicates. *H. pylori*-infected and LPS pre-stimulated neutrophils were
33 compared to only LPS-stimulated control cells (asterisks shown on top of the bars) ($p < 0.0001$
34 ANOVA, $***p \leq 0.001$ Bonferroni-corrected for pair wise comparison).

35 (F) The levels of IL-1 β and TNF α were measured in the supernatants of human PBMCs infected
36 o/n with G27 *H. pylori* strain at MOI 5. Cells were pre-stimulated with 5 ng/ml of LPS. The
37 caspase-1 inhibitor (10 μ M) was added 30 minutes prior to infection. Results are presented as
38 mean \pm S.D. from three different donors. Inhibitor-treated cell were compared to non-treated cells
39 ($p = 0.0032$ ANOVA, $**p \leq 0.01$ Bonferroni-corrected for pair wise comparison).

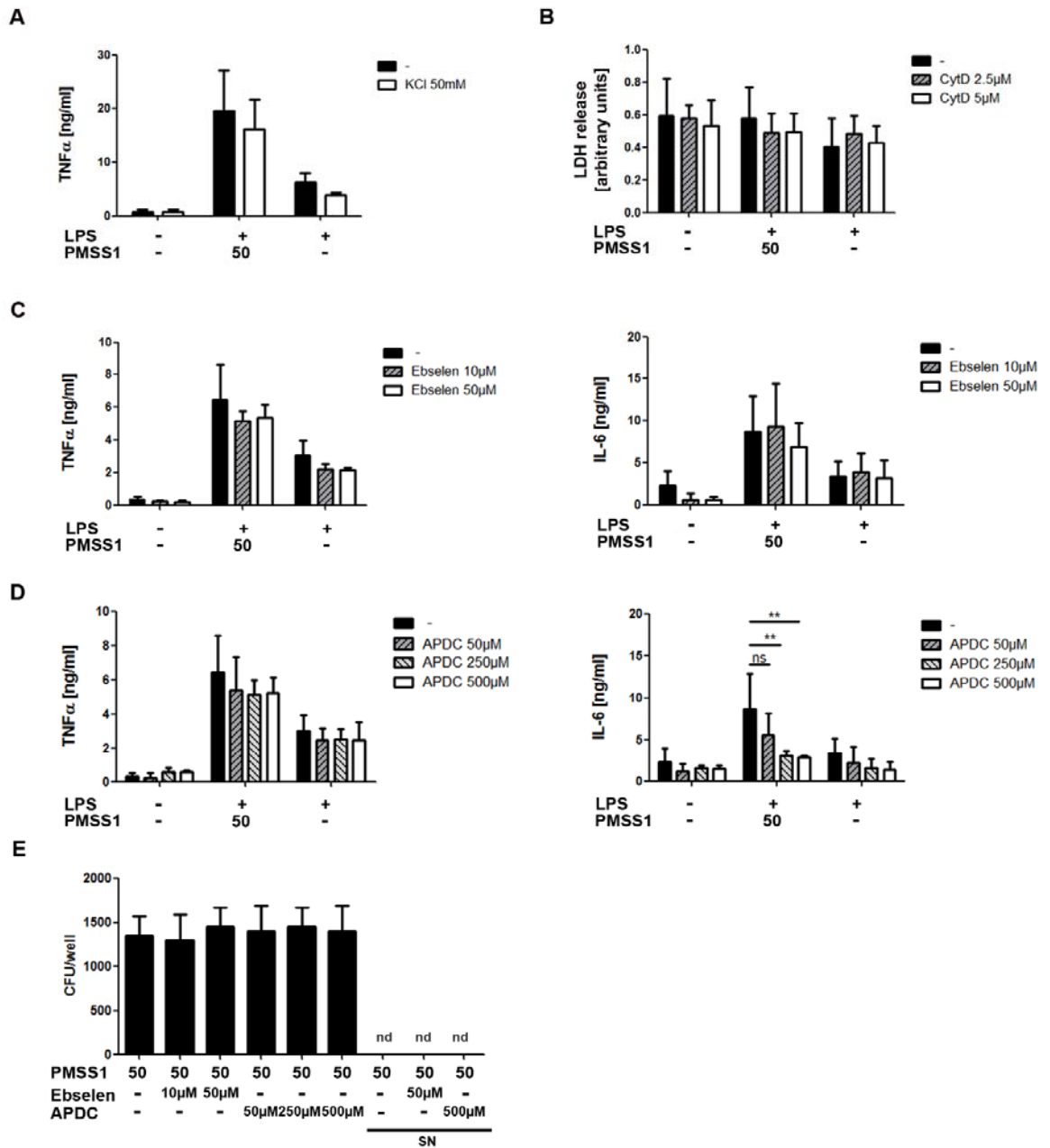
40 (G) BMDCs were infected o/n with PMSS1 at MOI 10 and 50 and the levels of IL-1 β and TNF α
41 were analyzed in the supernatants by ELISA. For pre-stimulation 10 ng/ml LPS were used.
42 Caspase-1 inhibitor (10 μ M) was added 30 minutes before infection. Results are presented as
43 mean \pm S.D. of three independent experiments. Inhibitor-treated cell were compared to non-
44 treated cells ($p = 0.0002$ ANOVA, $*p \leq 0.05$, $***p \leq 0.001$ Bonferroni-corrected for pair wise
45 comparison).

46 (H) BMDCs were infected o/n at MOI 10 and 50 with PMSS1 and the levels of pro-IL-1 β were
47 analyzed in cell lysates by Western Blot. β -actin was used as loading control. For pre-stimulation

48 10 ng/ml LPS were used. Caspase-1 inhibitor (10 μ M) was added 30 minutes prior to infection.

49 One representative blot is shown.

Suppl. Figure 2



50

51 (A) TNF α secretion measured in the supernatants of BMDCs infected o/n with *H. pylori* PMSS1
52 at MOI 50. For pre-stimulation 10 ng/ml LPS was used. 50 mM KCl were added 30 minutes
53 before infection. Results are presented as mean \pm SD of three independent experiments.

54 (B) BMDCs were infected for 6 hours with *H. pylori* PMSS1 at MOI 50 and LDH released was
55 measured in the supernatants. For pre-stimulation 10 ng/ml LPS was used. Cytochalasin D (2.5
56 and 5 μ M) was added 30 minutes before infection. Results are presented as mean \pm S.D. of three
57 independent experiments.

58 (C) and (D) TNF α and IL-6 secretion in supernatants from BMDCs infected for 6 hours with *H.*
59 *pylori* PMSS1 at MOI 50 detected by ELISA. Cells were pre-stimulated with 10 ng/ml of LPS.
60 The inhibitor ebselen (C) and APDC (D) were added 30 minutes prior to infection at the
61 indicated concentrations. Results (mean \pm S.D.) from three independent experiments are shown.

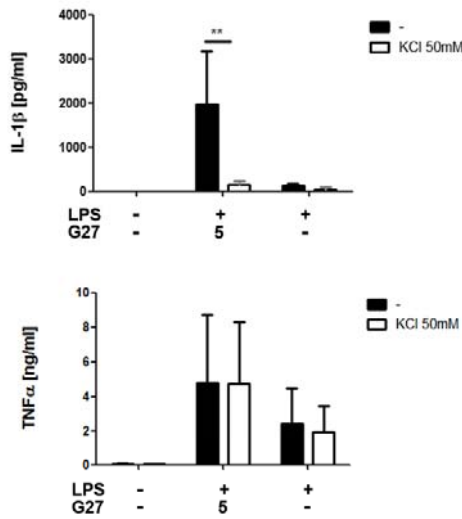
62 (E) Bacterial uptake of ROS inhibitor-treated cells. Cells were treated with the inhibitors 30 min
63 prior infection and infected for 1h. Gentamycin containing medium was added and cells were
64 lysed after 45 minutes of incubation. Corresponding supernatants were also plated as control for
65 antibiotic treatment. Results (mean \pm S.D.) from one experiment performed in triplicate is shown.

66 SN, supernatant; nd, not detected.

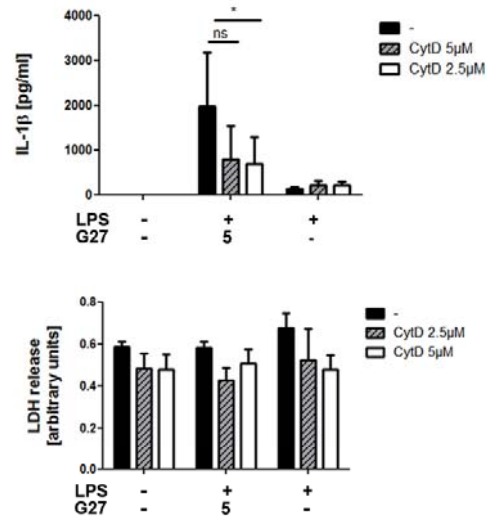
67

Suppl. Figure 3

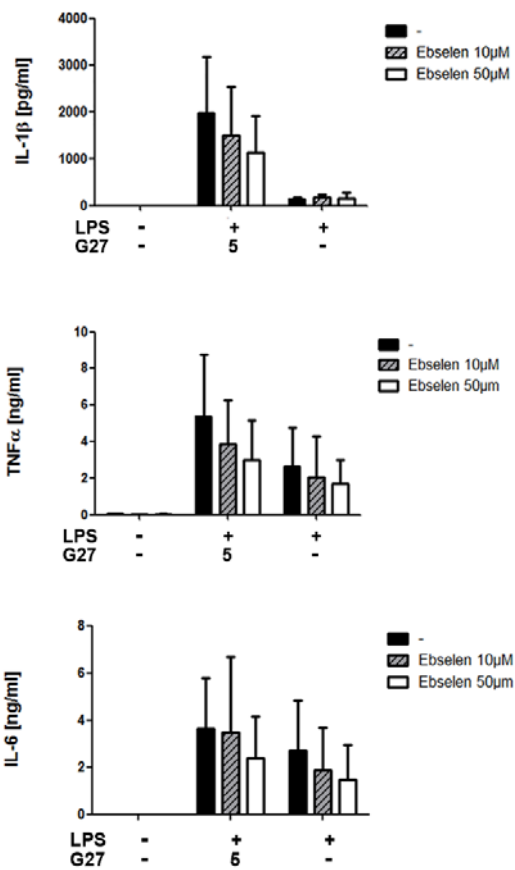
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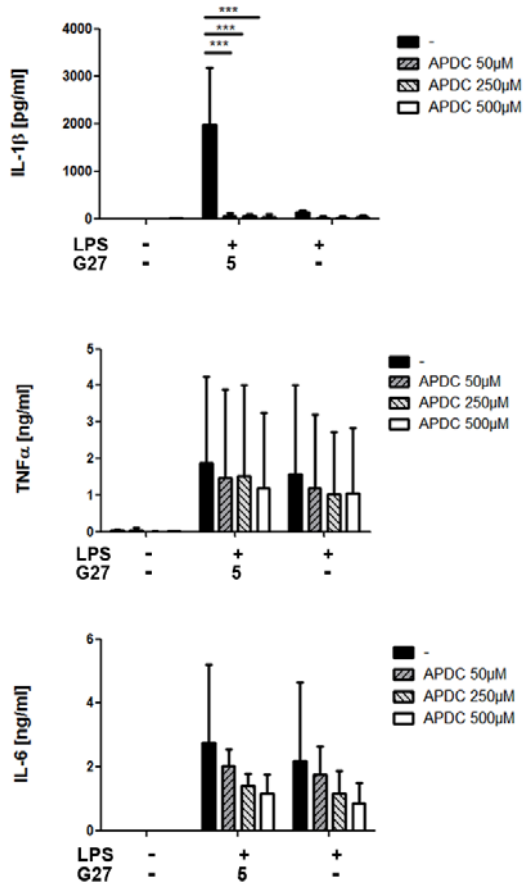
B



C



D



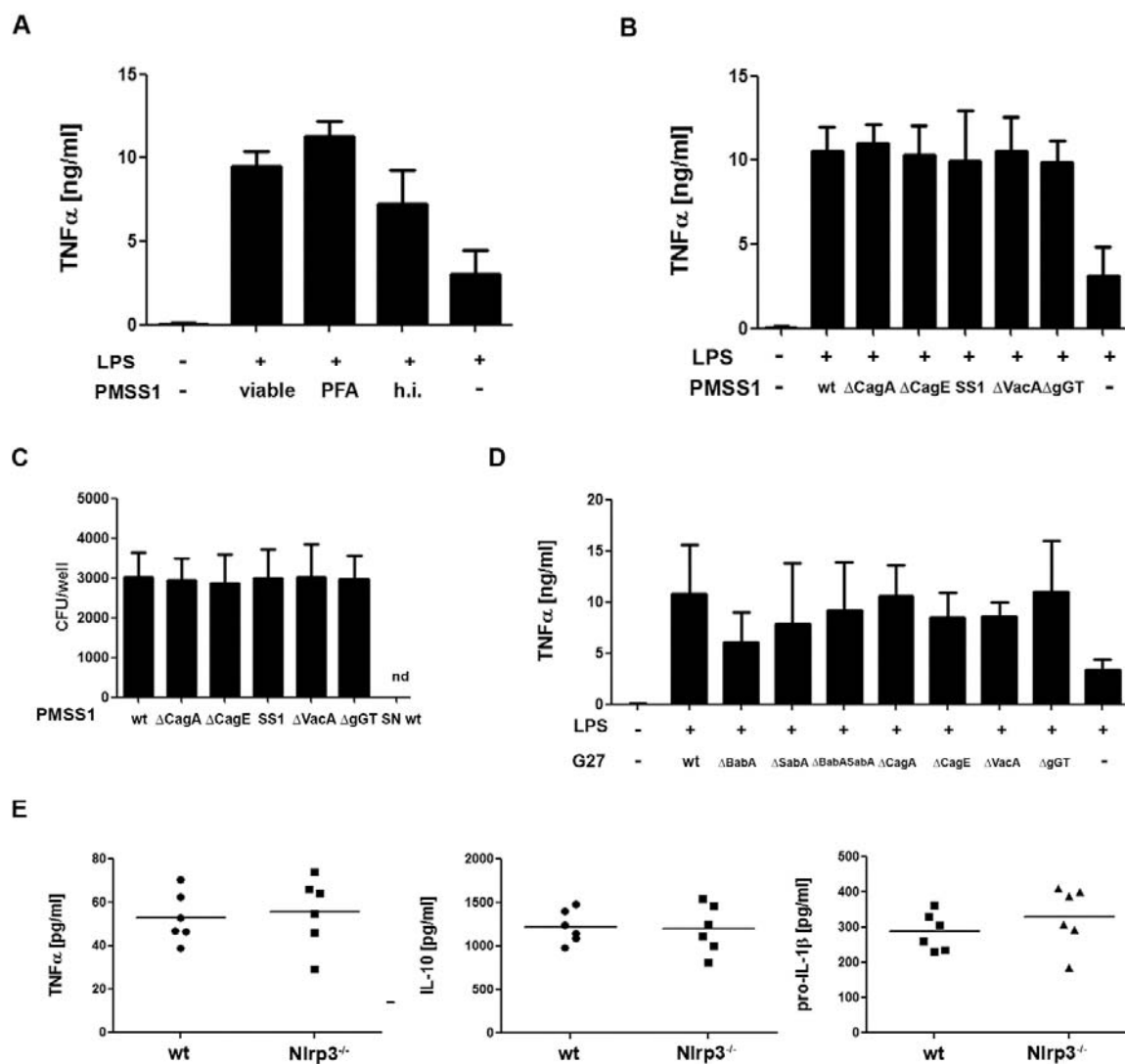
69 (A) Human PBMCs were infected for 6 hours with the *H. pylori* G27 strain at MOI 5 and the
70 levels of IL-1 β and TNF α were analyzed in the supernatants by ELISA. Cells were pre-stimulated
71 with LPS (5 ng/ml). 50 mM KCl was added 30 minutes before infection. Results are presented as
72 mean \pm S.D. of three different donors. Inhibitor-treated cell were compared to non-treated cells
73 (p=0.0021 ANOVA, **p \leq 0.01 Bonferroni-corrected for pair wise comparison).

74 (B) IL-1 β and LDH levels detected in the supernatants of PBMCs after 6 hour infection with *H.*
75 *pylori* G27 at MOI 5. For pre-stimulation 5 ng/ml LPS were used. Cytochalasin D was added at
76 the indicated concentrations 30 minutes before infection. Results expressed as mean \pm S.D. from
77 three different donors are shown. Inhibitor-treated cell were compared to non-treated cells
78 (p=0.0030 ANOVA, *p \leq 0.05 Bonferroni-corrected for pair wise comparison).

79 (C) and (D) Human PBMCs were infected for 6 hours with the *H. pylori* G27 strain at MOI 5,
80 and IL-1 β , TNF α and IL-6 secretion was measured in the supernatants by ELISA. Cells were pre-
81 stimulated with LPS (5 ng/ml). Ebselen (C) and APDC (D) were added 30 minutes prior to
82 infection at the indicated concentrations. Results are presented as mean \pm SD of three different
83 donors. Inhibitor-treated cell were compared to non-treated cells (p \leq 0.0001 ANOVA,
84 ***p \leq 0.001 Bonferroni-corrected for pair wise comparison).

85

Suppl. Figure 4



86

87 (A) BMDCs were incubated o/n with viable, heat inactivated (h. i.) or PFA-fixed *H. pylori*

88 PMSS1 (MOI 50) and TNF α secretion was measured in the supernatants by ELISA. For pre-

89 stimulation 10 ng/ml LPS were used. Results are presented as mean \pm S.D. of three independent

90 experiments.

91 (B) TNF α secretion levels were measured in the supernatants of BMDCs infected o/n with the *H.*
92 *pylori* wild type strains PMSS1 or SS1 or the PMSS1 isogenic mutant strains deficient for CagA,
93 CagE, VacA or gGT at MOI 50. Cells were pre-stimulated with LPS (10 ng/ml). Data (mean \pm
94 S.D.) from three independent experiments are shown.

95 (C) Uptake of *H. pylori* wild type strains PMSS1 or SS1 or the PMSS1 isogenic mutant strains
96 deficient for CagA, CagE, VacA or gGT by BMDCs measured in cell lysates after gentamycin
97 treatment or in corresponding supernatants to control for antibiotic treatment efficiency. Data
98 (mean \pm S.D.) from one representative experiment performed in triplicate are shown. SN,
99 supernatant; nd, not detected.

100 (D) PBMCs were infected o/n with *H. pylori* G27 or the indicated isogenic mutants at MOI 5 and
101 TNF α secretion was measured in the supernatants by ELISA. For pre-stimulation 5 ng/ml LPS
102 was used. Results from four different healthy donors are presented as mean \pm S.D.

103 (E) TNF α , IL-10, and pro-IL-1 β protein levels detected in gastric mucosa extracts of wild type
104 and Nlrp3^{-/-} mice infected one month with *H. pylori* PMSS1. Data from one representative
105 experiment is shown. Horizontal lines indicate the mean.

106

107

108