Erythromycin inhibits neutrophilic inflammation and mucosal disease by upregulating DEL-1

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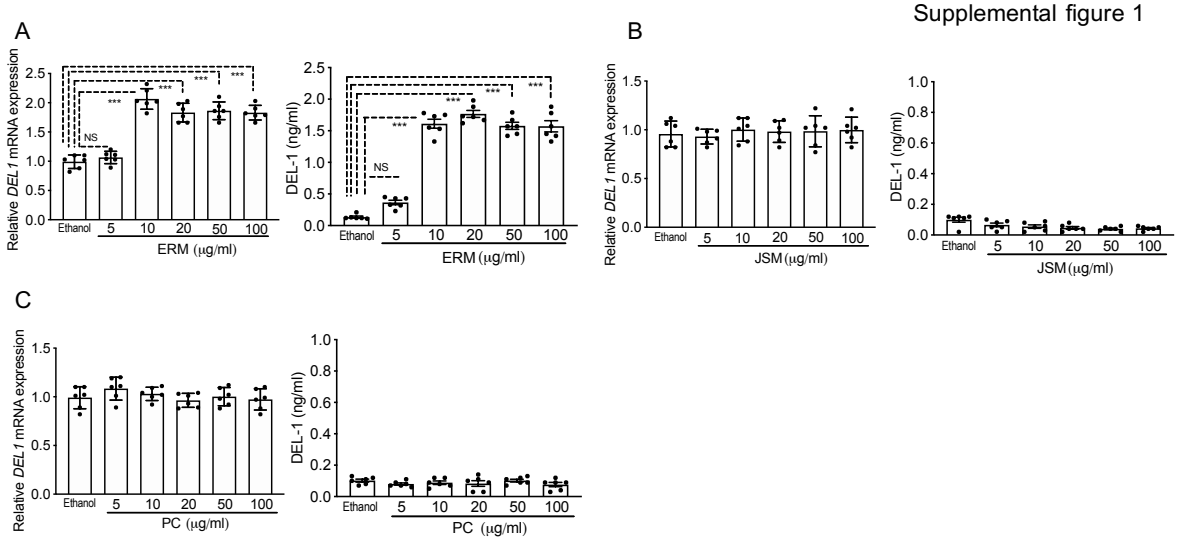
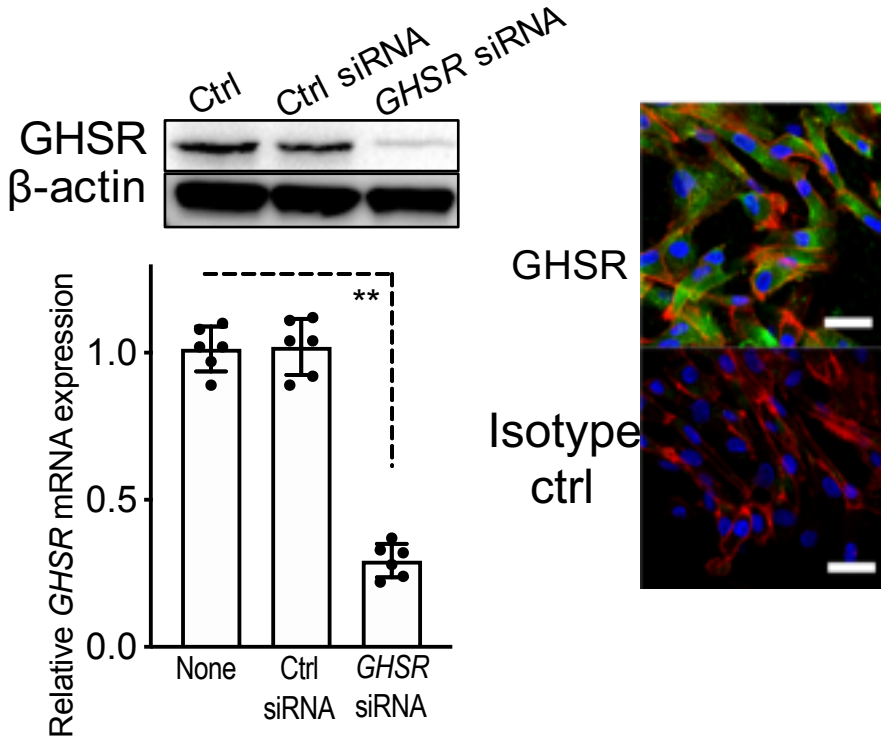


Supplementary figure 1: Dose-response experiments of macrolides and PC used in the study.

HMVECs were treated with the indicated concentrations of macrolides (ERM (A) and JSM (B)) and PC (C) for 3h to determine *Del1* mRNA expression by qPCR (left panels) or 6h to determine DEL-1 protein levels in culture supernatants by ELISA (right panels). mRNA data were normalized against *Gapdh* mRNA and expressed as fold induction relative to control vehicle ethanol, which was assigned an average value of 1. Data are presented as the mean ± SD. ***P < 0.0001 by the One-way ANOVA followed by Tukey's multiple comparison test, NS: not significant.

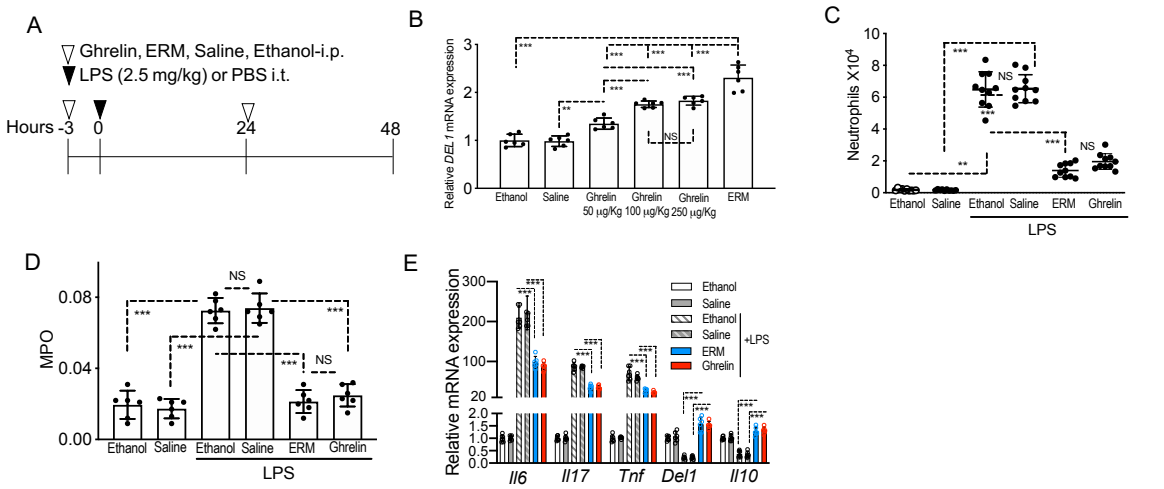
Supplemental figure 2



Supplementary figure 2: HMVEC expresses GHSR

HMVECs were treated with siRNA to *GHSR* or control siRNA and 24h later were assessed for expression of GHSR at the protein (upper left panel) and mRNA level (lower left panel) by WB and qPCR, respectively (n=6 sets of cultures/group). The mRNA data were normalized against *Gapdh* mRNA and expressed as fold induction relative to untreated control ('none'), which was assigned an average value of 1. Immunofluorescence analysis of GHSR in HMVECs (right panel). The cells were labeled with GHSR (green), Nuclei (blue) and F-actin (red). Lower panel represents HMVECs stained with isotype control antibody to assess background. Scale bars, 10 μm.

Supplemental figure 3



Supplementary figure 3: Ghrelin upregulates DEL-1 mRNA and suppress neutrophil infiltration in BALF.

(A) Experimental design. *E. coli* LPS (2.5mg/kg) was administrated intratracheally. ERM, ghrelin, ethanol (vehicle control for ERM) or saline (vehicle control for ghrelin) (n = 10 mice/group) was administrated i.p. 3h before and 24h after LPS administration. Samples were collected 48h after LPS administration. (B) *Del1* mRNA transcription in the lung tissue was analyzed by qPCR 24h after i.p. injection the indicated concentrations of ghrelin, ERM (20 mg/kg), or controls (ethanol, or saline). Data were normalized against *Gapdh* mRNA and expressed as fold induction relative to treatment with ethanol or saline control, each of which was assigned an average value of 1. Data are presented as the mean ± SD; (n = 6 mice/group). (C, D) Neutrophil counts (C) and MPO activity (D) in the bronchoalveolar lavage fluid (BALF) 48h after LPS challenge, or not, in mice which received i.p. injection with ERM (20 mg/kg), or ghrelin (100 µg/ml), or their respective vehicle control, according to the scheme in panel A. (B; n = 10 mice/group, C; n = 6 mice/group). (E) The mRNA levels of pro-inflammatory cytokines (*Il6*, *Il17*, and *Tnf*), *Del1,* and *Il10* in the lung tissue of the same groups of mice described in C and D was determined by qPCR 48h after LPS challenge (n = 6 mice/group). Data were normalized against *Gapdh* mRNA and expressed as fold induction relative to treatment with ethanol control, which was assigned an average value of 1. One-way ANOVA followed by Tukey's multiple comparison test, **P < 0.001, ***P < 0.0001

between indicated groups, NS: not significant.