Annexin A1 mediates hydrogen sulfide properties in the control of inflammation

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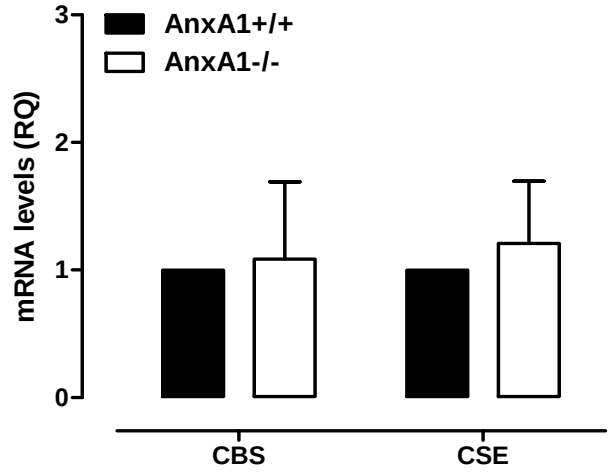
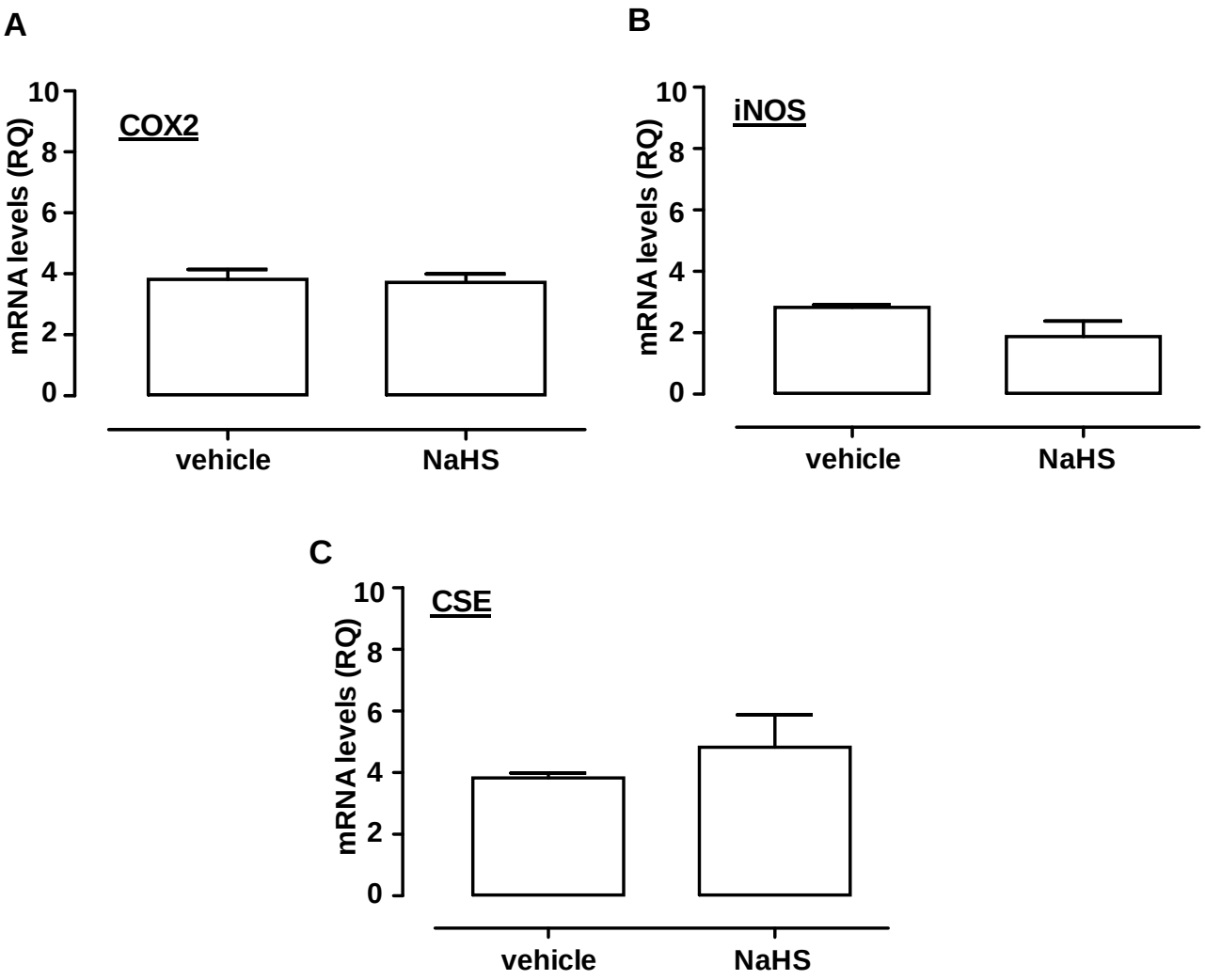
JPET/2014/217034 - Supplemental material

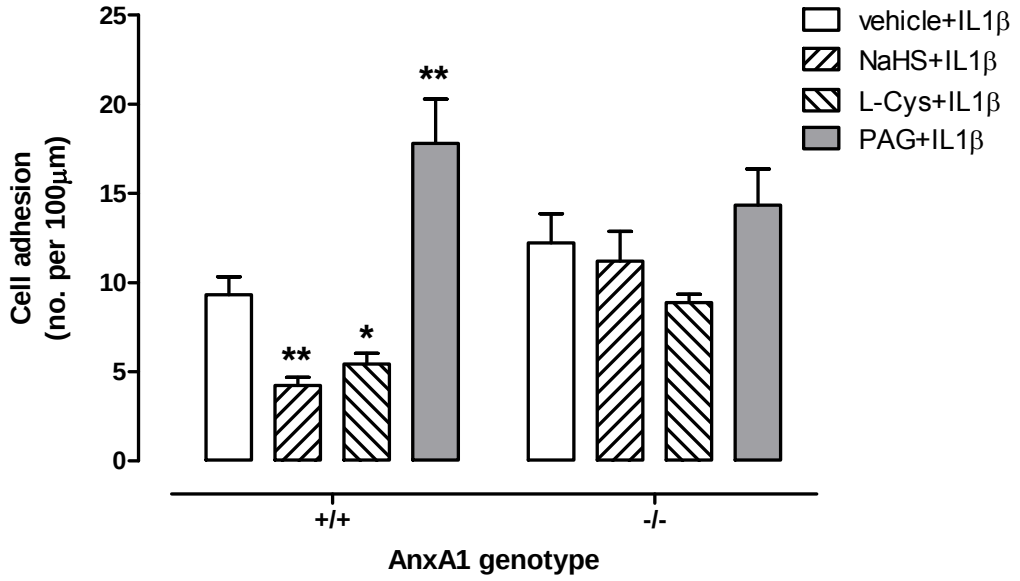
Supplemental material



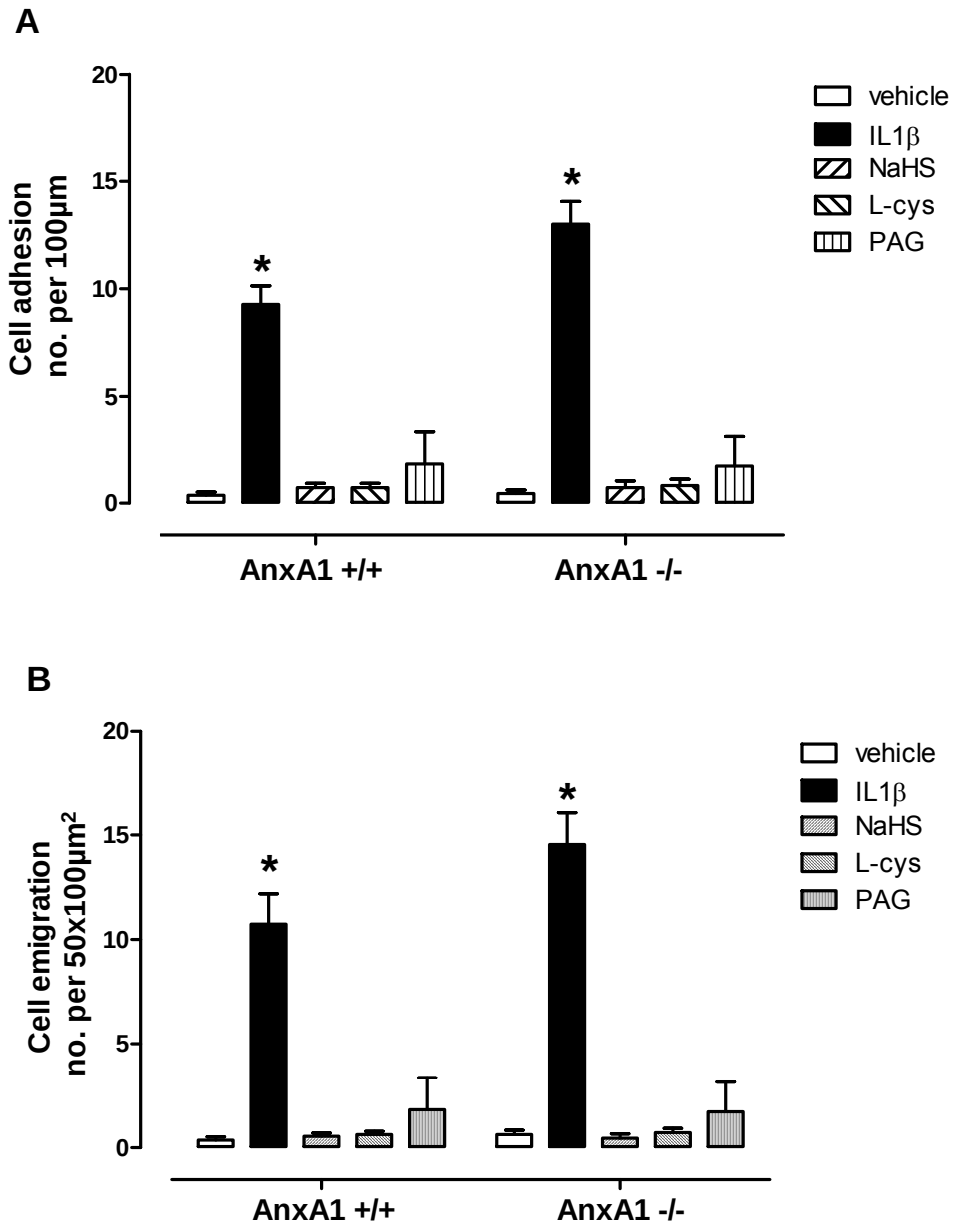
Supplemental figure 1. Expression of mRNA levels in liver harvested from AnxA1+/+ and AnxA1-/- mice. mRNA levels expressed as relative quantity (RQ). No statistical difference were assessed by using Student's t test (*$p<0.05$, **$p<0.01$; *vs AnxA1+/+; n=6).



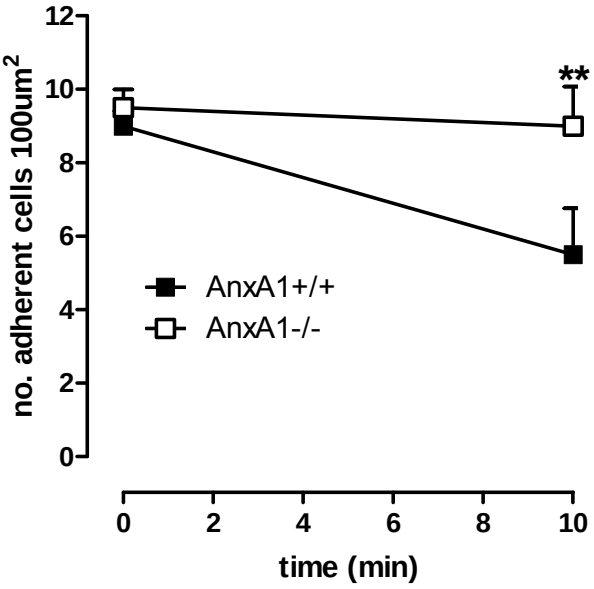
Supplemental figure 2. Expression of COX2 (A), iNOS (B) and CSE (C) mRNA levels in unstimulated AnxA1+/+ BMDMs treated with vehicle or NaHS alone (100μM, 1h). NaHS does not affect basal levels of gene products for COX2, iNOS and CSE. Statistical analysis was made by using Student's t-test (n=3).



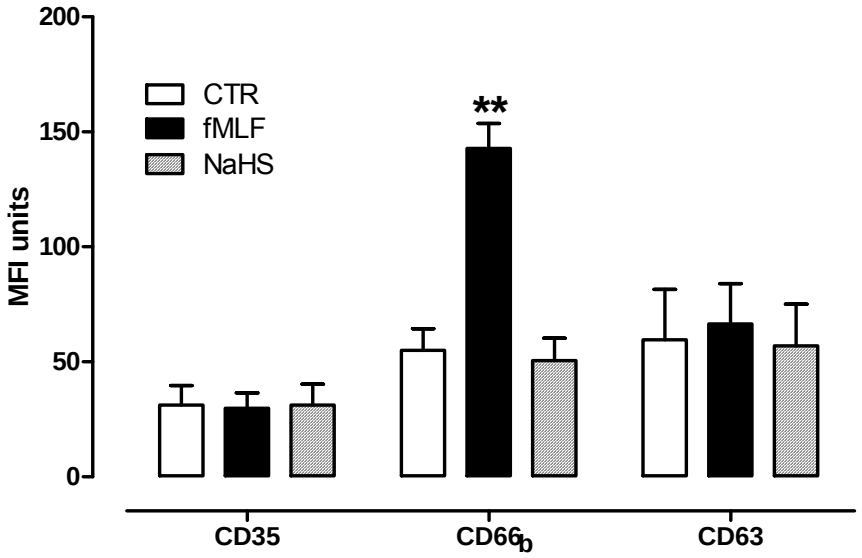
Supplemental figure 3. Intravital microscopy analysis of postcapillary venules in AnxA1+/+ or AnxA1-/- mice showing adherent leukocytes. Mice were pre-treated with vehicle, NaHS (100μmol/kg s.c.) or L-cysteine (L-cys, 1000μmol/kg s.c.) 1h before stimulation with IL1β (10ng/mouse i.p., 2h) and number of adherent leukocytes were analysed (expressed as no. cells per 50x100μm^2). D,L-propargylglycine (PAG, 10mg/kg i.p.) was given 30 minutes before IL1β injection. Statistical analysis was made by using two-way ANOVA (*p<0.05, **p<0.01; * vs vehicle; n=6)



Supplemental figure 4. Intravital microscopy analysis of AnxA1+/+ and AnxA1-/- mouse mesenteric microcirculation in non-inflamed condition. Administration of NaHS (100μmol/kg s.c.), L-cysteine (L-cys, 1000μmol/kg s.c.) or D,L-propargylglycine (PAG, 10mg/kg, i.p.) did not affect cell trafficking baseline expressed as cell adhesion (A) or emigration (B) in AnxA1+/+ or AnxA1-/- mice. Statistical analysis was made by using two-way ANOVA (*$p<0.05$ vs vehicle; n=6).



Supplemental figure 5. Intravital microscopy analysis of AnxA1+/+ and AnxA1-/- mouse mesenteric microcirculation inflamed by IL1β injection (10ng/mouse i.p., 2h). Administration of NaHS (1μM, 10 minutes superfusion on the postcapillary venule selected) induced detachment of adherent leukocytes in AnxA1+/+, but not in AnxA1-/-, mice. Statistical analysis was made by using Student's t-test (**$p<0.01$, $n=6$).



Supplemental figure 6. Modulation of CD35, CD66b and CD63 surface expression by H_2S. The graph shows CDs surface expression in human PMN pretreated with vehicle (CTR) or NaHS (100μM). fMLP (0.1μM, 30 minutes) was used as positive control for PMN activation/degranulation. As displayed, NaHS does not affect PMN surface expression of CD35, CD66b or CD63, markers for secretory vesicles, specific granules and azurophilic granules, respectively. Statistical analysis was made by using one-way ANOVA (**$p<0.01$ vs CTR; n=4).