

Delayed resolution of lung inflammation in *Il-1rn*^{-/-} mice reflects elevated IL-17A/G-CSF expression

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ONLINE DATA SUPPLEMENT

Figure E1: Expression of IL-1 pathway components after aerosolized LPS exposure. A) BAL protein concentrations in unexposed mice (zero hours) and mice 4, 8, 24 and 48 hours after exposure to LPS. (n=4-10 mice at each time) B) Relative quantity of mRNA expression in mouse lungs in unexposed mice (zero hours) and 4, 8, 24 and 48 hours after LPS exposure. (n=3-6 mice per time point)

Figure E2: BAL cytokine protein concentration in *Il-1r1*^{-/-} mice after LPS compared with C57BL/6 controls. A) IL-6, at 4 (*p<0.0001) and 8 hours (*p<0.01). B) TNF- α , 4 hours (*p<0.001). C) IL-1RA, at 48 hours (*p<0.01). (n=10-12 mice per time point)

Figure E3: Cytokine expression in the BAL of *Il-1rn*^{-/-} mice after LPS. A) IL-6 protein concentration in *Il-1rn*^{-/-} mice and littermate controls (*Il-1rn*^{+/+}). B) TNF- α was decreased exclusively at 4 hours (*p<0.05). C) IL-1RA, at 4 (*p<0.001), 8 (*p<0.001), 24 (*p<0.001) and 48 hours (*p<0.01) after LPS in *Il-1rn*^{-/-} mice. (n=10-19 mice per time point)

Figure E4: Baseline bone marrow composition in *Il-1r1*^{-/-}, C57BL/6 and *Il-1rn*^{-/-} mice. A) Percentage of GR1⁺ cells in *Il-1rn*^{-/-}, C57BL/6 and *Il-1r1*^{-/-} in unstimulated bone marrow. B) Percentage of macrophage lineage cells (F4-80+CD11b⁺) in *Il-1rn*^{-/-} mice, *Il-1r1*^{-/-} mice and C57BL/6 mice. C) Absolute numbers of macrophage lineage cells. (n=3-4 mice per genotype)

Figure E5: Cell counts and bone marrow composition in *Il-1rn*^{-/-} mice pretreated with rhIL-1RA. Forty-eight hours after LPS exposure the: A) BAL total WBC, B) Peripheral WBC, C) Peripheral neutrophil count and D) GR1⁺ cells in the bone marrow in *Il-1rn*^{-/-} mice pretreated two hours prior to LPS with rhIL-1RA versus those pretreated with PBS i.t.. (n=3-5 mice per treatment)

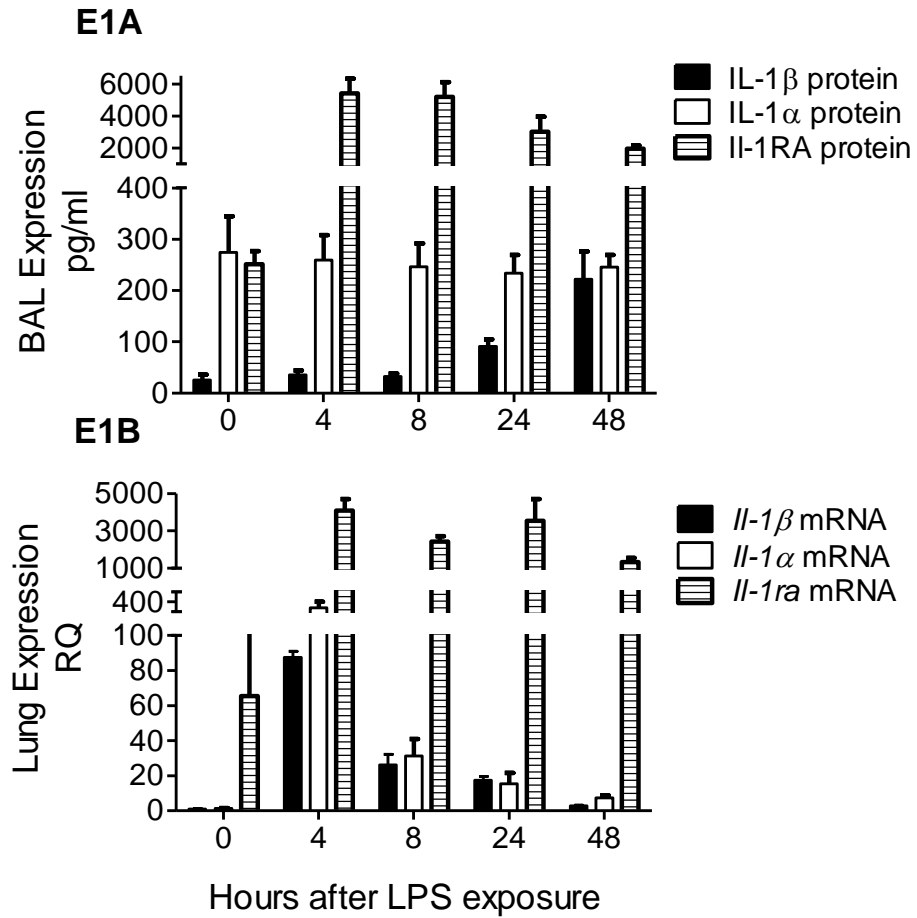
Figure E6: IL-17A expression in *Il-1r1*^{-/-} mice. A) IL-17A protein concentration in BAL of *Il-1r1*^{-/-} mice compared with C57BL/6 mice (n=9-12 mice per time point). B) *Il-17a* mRNA expression overall in *Il-1r1*^{-/-} mouse lungs, at 4 hours (*p<0.05). (n=7-9 mice per time point)

Figure E7: IL-17 family expression in *Il-1rn*^{-/-} mouse lungs after LPS. A) IL-17A protein in the BAL of *Il-1rn*^{-/-} mice compared with littermate controls (*Il-1rn*^{+/+}) after LPS significant exclusively at 48hrs (*p<0.05). (n=7-17 mice per time point) B) Relative expression of *Il-17f* mRNA—measured by real time PCR—in *Il-1rn*^{-/-} mouse lungs, at 48 hours (*p<0.05) after LPS, compared with littermate controls (*Il-1rn*^{+/+}). C) *Il-17c* mRNA lung expression is only significantly different at 0 hours (p<0.05). D) *Il-23a* mRNA and E) *Il-22* mRNA lung expression by genotypes. (n=4-7 mice per time point)

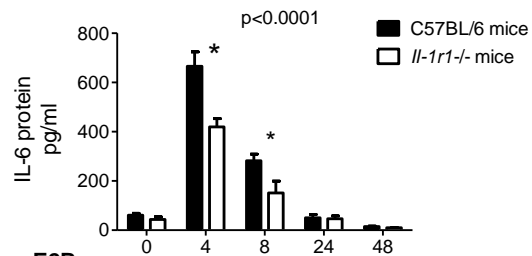
Figure E8: Cellular sources of IL-17A in *Il-1rn*^{-/-} mouse lungs after LPS. A) Percentage of IL-17A+ $\gamma\delta$ T cells and B) IL-17A+CD3⁺ cells, both overall and at 48 hours, compared with C57BL/6 lungs. C-F) Representative dot plots of myeloid cells positive for IL-17A in the lungs of C&E) C57BL/6 or D&F) *Il-1rn*^{-/-} mice. G) Representative dot plots illustrating the percentage

of TCR β +IL-17A+ cells in the lungs of C57BL/6 and H) *Il-1rn*^{-/-} mice at 48 hours after LPS. (n=4-6 mice per time point)

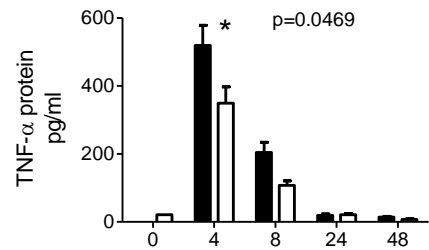
Figure E9: Cell count and cytokine expression in *Il-1rn*^{-/-} mice after IL-17A blockade. *Il-1rn*^{-/-} mice pretreated 24 hours prior to LPS with 100 μ g i.p. IL-17A mAb (monoclonal antibody) with 48 hour: A) BAL G-CSF protein (n=5 mice), B) Plasma G-CSF protein (n=4 mice) or C) BAL total WBC compared with those *Il-1rn*^{-/-} mice pretreated with 100 μ g i.p. isotype control Ab (n=6 mice). D) Absolute number in the bone marrow of GR1+ cells or E) Macrophage lineage cells (F4-80+CD11b+) between those mice pretreated 24 hours prior to LPS with IL-17A mAb i.p. or those pretreated with isotype control Ab. (n=6 mice)



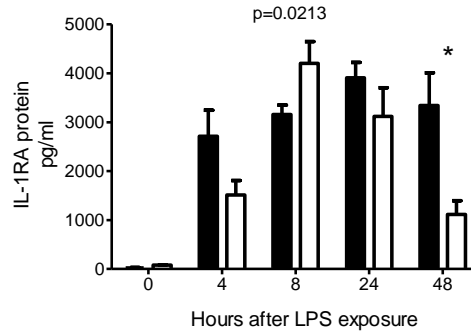
E2A

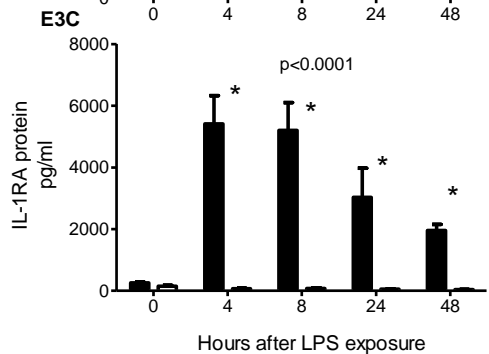
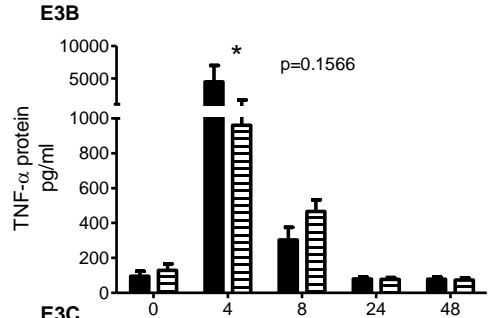
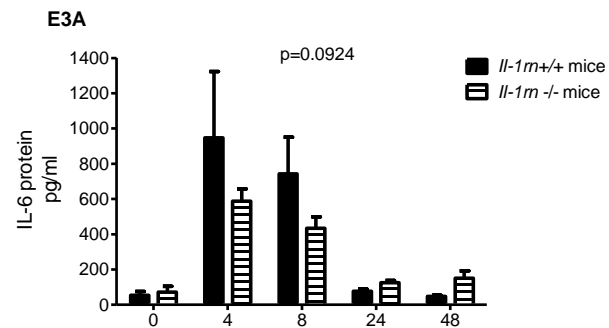


E2B



E2C





Hours after LPS exposure

