Cholesterol-25-hydroxylase promotes efferocytosis and resolution of lung inflammation

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SUPPLEMENTAL INFORMATION

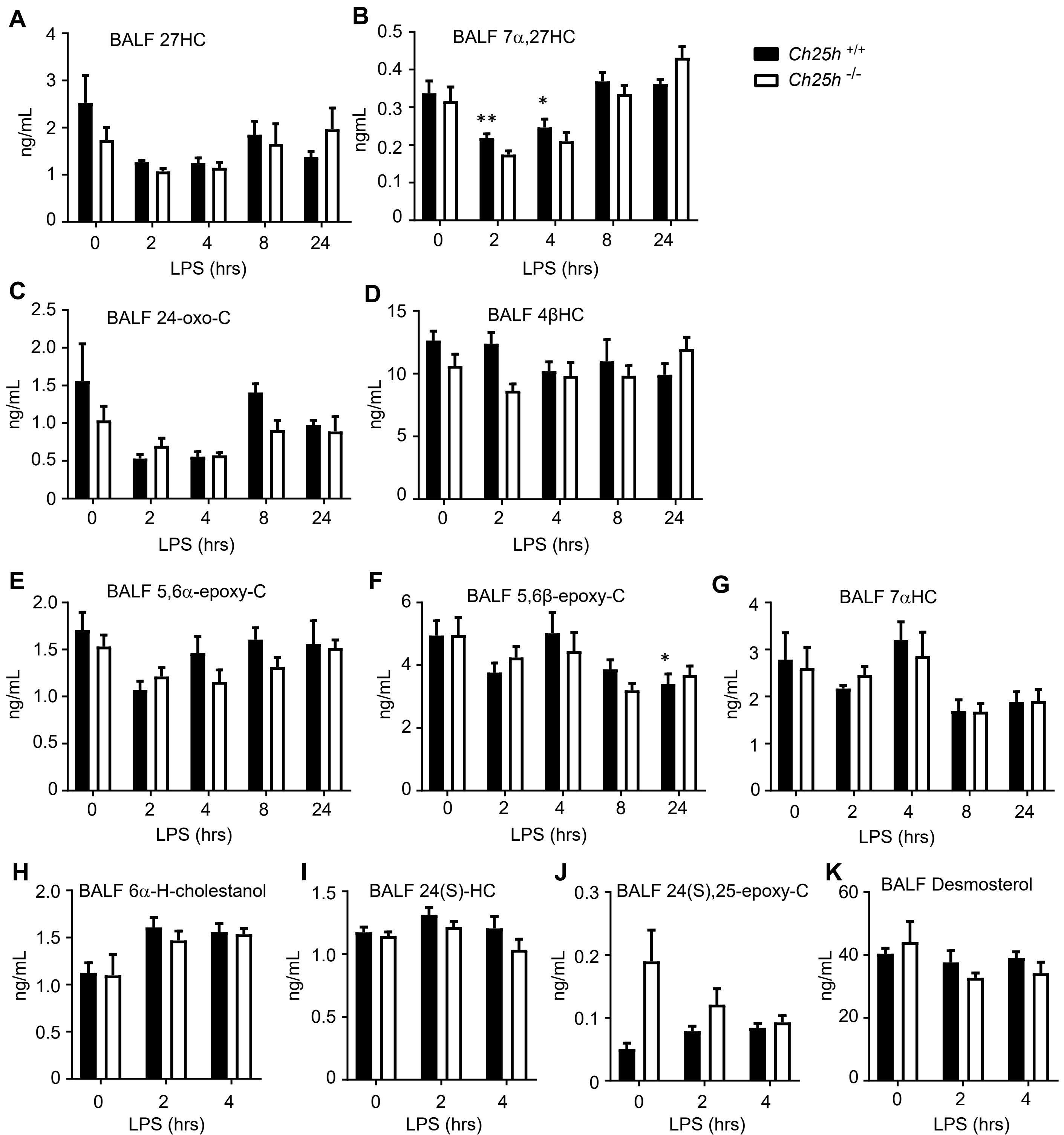

Figure S1. Oxy(sterol) profile in LPS-exposed murine airspace. (**A-K**) *Ch25h*$^{+/+}$ and *Ch25h*$^{-/-}$ mice (n=5-7/genotype/timepoint for panels A-G; n=2/genotype for 0 hrs and n=5/genotype for 2 and 4hrs for panels H-K) were exposed to inhaled LPS and then the oxysterols and sterols indicated were quantified in bronchoalveolar lavage fluid (BALF) at various post-exposure time points. C = cholesterol; HC = hydroxycholesterol. Data are mean +/- s.e.m. *, $P<0.05$; **, $P<0.01$ compared to *Ch25h*$^{+/+}$ 0 hrs, by one-way ANOVA with Dunnett's multiple comparison test.

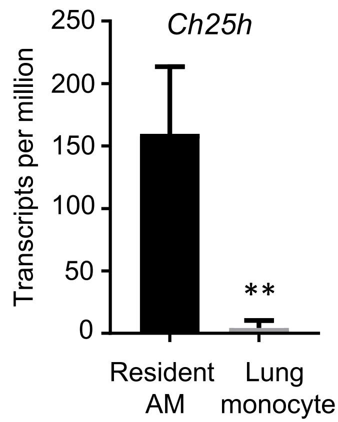

Figure S2. Ch25h expression and its regulation in the murine lung. Naïve C57BL/6 mice (n=3) underwent bronchoalveolar lavage and resident alveolar macrophages (AM) were then isolated by fluorescence-assisted cell sorting ($CD64^+F4/80^+CD11c^{high}CD11b^{low}$). Alternatively, naïve mice underwent enzymatic lung digestion, and lung parenchymal monocytes were sorted. *Ch25h* mRNA expression was evaluated by RNA-Seq. Data are mean +/- s.e.m. **, $P<0.01$ by unpaired t-test.

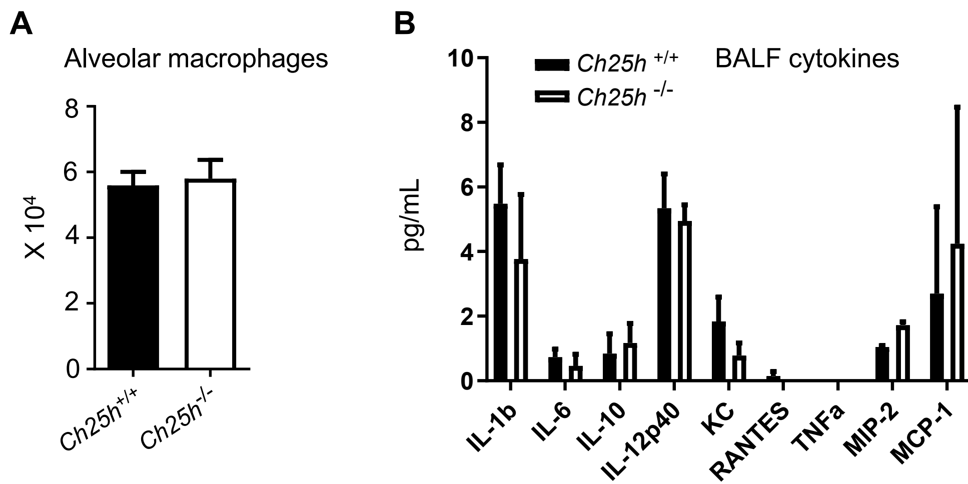

Figure S3. Normal alveolar macrophage and cytokine profile in naïve Ch25h-null lung. BALF was collected from naïve *Ch25h*$^{+/+}$ and *Ch25h*$^{-/-}$ mice (n=3/genotype) and analyzed for (**A**) alveolar macrophage count (manual count of cytospun cells) and (**B**) cytokine levels by multiplex assay. Data are mean +/- s.e.m.

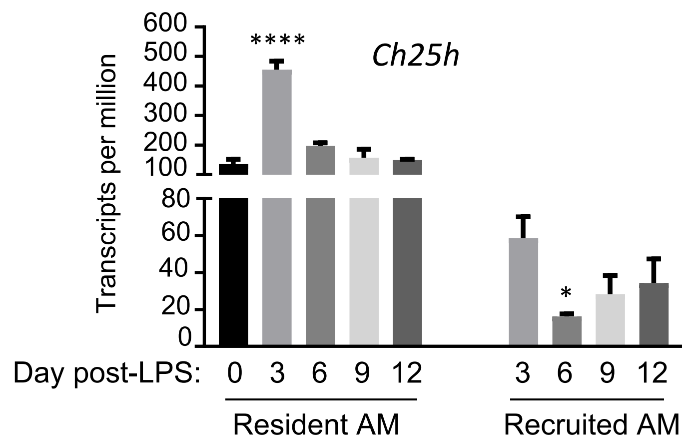

Figure S4. *Ch25h* is differentially regulated in resident and recruited alveolar macrophages after LPS inhalation. C57BL/6 mice were treated i.t. with 20 μg of *E. coli* LPS. Alveolar macrophages (AMs) harvested by bronchoalveolar lavage at various time points were analyzed by RNA-Seq after initial FACS-sorting into resident AM ($CD64^{+}F4/80^{+}CD11c^{high}CD11b^{low}$) and recruited (i.e., blood monocyte-derived) AM ($CD64^{+}F4/80^{+}CD11c^{low}CD11b^{high}$) populations, as previously described (1). *Ch25h* mRNA expression over time is shown for both populations. Data are mean +/- s.e.m. N=3 mice per time point. *, $P<0.05$; ****, $P<0.0001$ in comparison to day 0 (resident AM) or day 3 (recruited AM) by one-way ANOVA with Dunnett's multiple correction test.

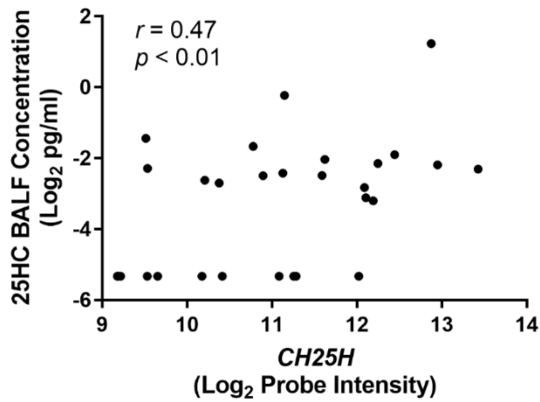

Figure S5. Alveolar macrophage *CH25H* expression and 25HC bronchoalveolar lavage fluid (BALF) concentrations are correlated in acute respiratory distress syndrome. BALF was collected within 48 hours of ARDS onset from subjects previously enrolled in a phase-II trial of omega-3 fatty acids for the treatment of ARDS (50). AM gene expression was assessed through genome-wide microarrays in patients (n = 30) who had AMs purified from the BALF through an antibody-based negative selection approach (82). BALF 25HC concentrations were quantified by mass spectrometry. A Pearson's test was used to generate a correlation coefficient between normalized $\log_2$ *CH25H* probe intensity and $\log_2$ 25HC BALF concentrations. The individual values are depicted.

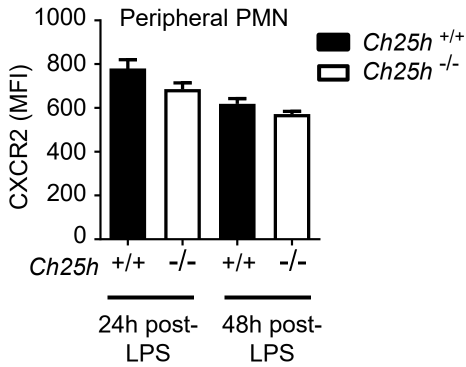

Figure S6. Unchanged chemokine receptor display on circulating *Ch25h*$^{-/-}$ PMNs. CXCR2 expression on peripheral blood ($CD45^+Ly6G^+$) neutrophils (PMNs) was quantified by flow cytometry in the indicated strains (n=4/genotype/timepoint) 24h and 48h post-inhalation of LPS. Data are mean +/- s.e.m. *, P<0.05 by unpaired t-test.

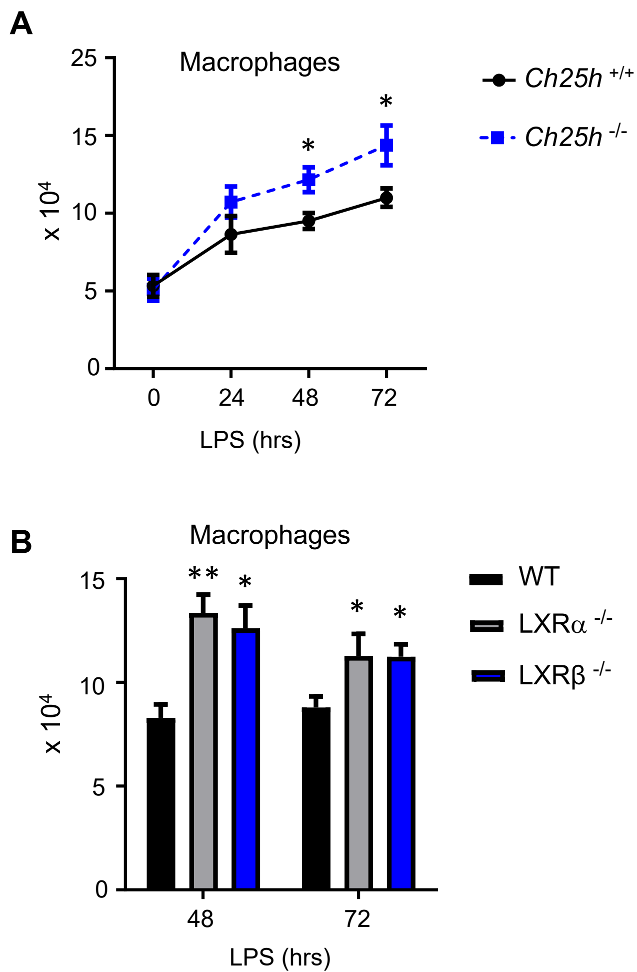

Figure S7. Increased recruitment of macrophages to the airspace of *Ch25h*$^{-/-}$ and LXR-deficient mice. Mice of the indicated genotypes (N=5-15/genotype/timepoint) were exposed to LPS aerosol (300 ug/ml, 30 min) and macrophages quantified in bronchoalveolar lavage fluid at the post-LPS time points shown. Data are mean +/- s.e.m. *, P<0.05, **, P<0.01. Unpaired t-test was used for panel A, one-way ANOVA with Dunnett's post-hoc test comparing KO strains to WT for each time point was used for B.