

Supplementary information

Neonatal pneumococcal colonisation caused by Influenza A infection alters lung function in adult mice.

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Supplementary methods

Flow cytometric analysis of lung epithelial cells

Single lung cells were isolated by disaggregation of lung tissue by collagenase digestion (Liberase TM; Roche; 1 Wunsch U/3mL Hank's buffered saline solution (HBSS)/lung for 45 min at 37°C with agitation) followed by hypotonic lysis of red blood cells (10mM KHCO₃, 150mM NH₄Cl, 0.1mM EDTA Na₂ for 90 sec at RT). Single cells were then resuspended at 5 x 10⁷ cells/mL in HBSS plus 2% (v/v) fetal bovine serum (FBS; Invitrogen) containing a cocktail of antibodies (anti-CD45, anti-CD31, anti-EpCAM, anti-MHCII) and relevant isotype controls (Biolegend). Labeled cells were washed in HBSS-2% FBS, resuspended at 1 x 10⁷ cells/mL and held on ice for flow cytometric analysis. Viability was determined by propidium iodide (PI; 1 µg/mL) staining, and doublets excluded by forward/side scatter (height) vs. forward/side scatter (width) gating. Type II alveolar (AT2) cells were identified by the phenotype CD45⁻ CD31⁻ EpCAM⁺ MHCII⁺.

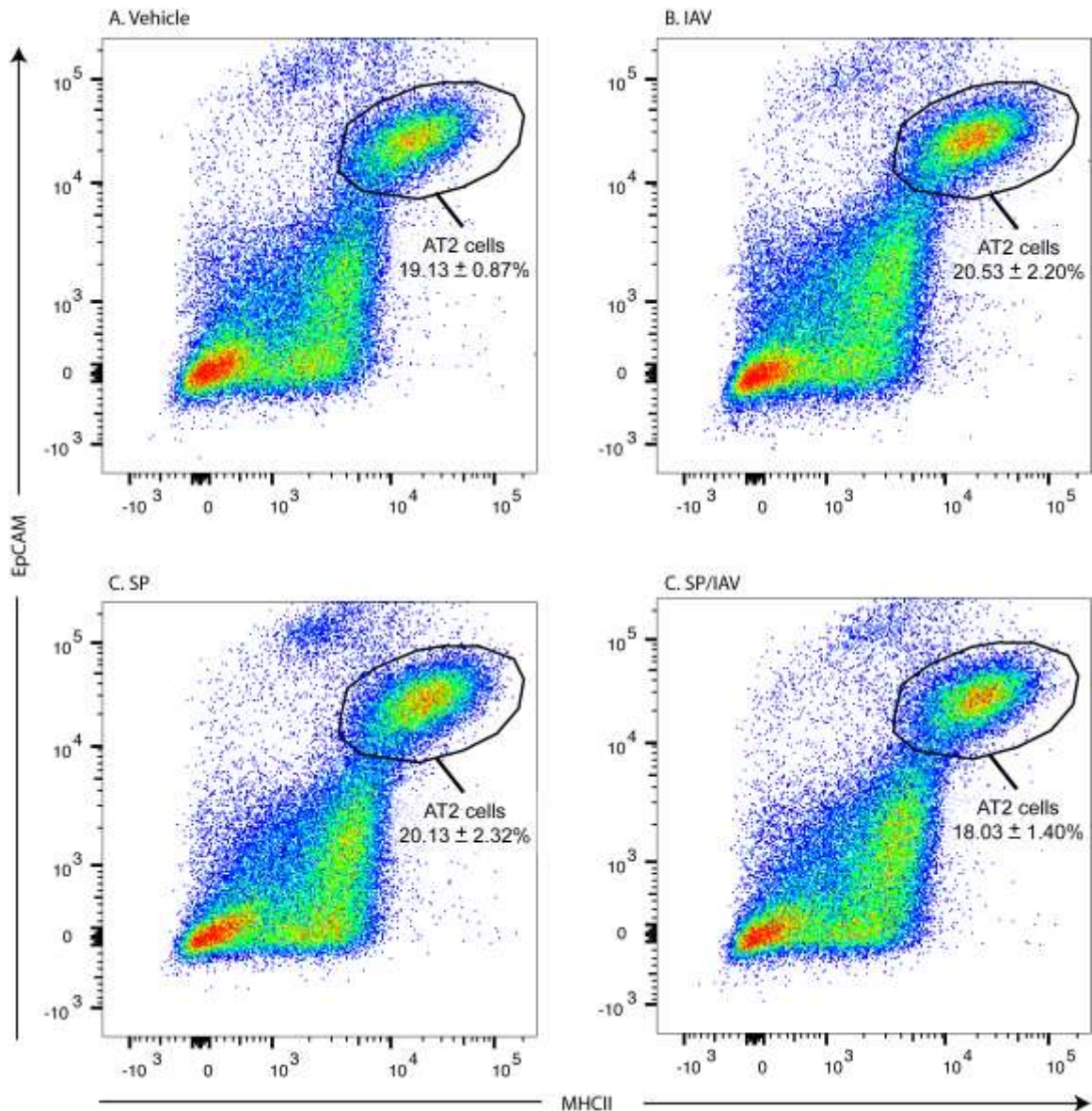


Figure S1. The relative abundance of At2 cells does not alter between groups. Representative images of FACS plots of viable, non-hematopoietic ($CD45^-$), non-endothelial ($CD31^-$) cells from PBS vehicle (A), IAV (B), SP (C) and SP/IAV treated (D) mice shows no difference in the relative abundance of $EpCAM^+ MHCII^+$ AT2 cells between groups. Quantification is shown as mean \pm sem (n=3).

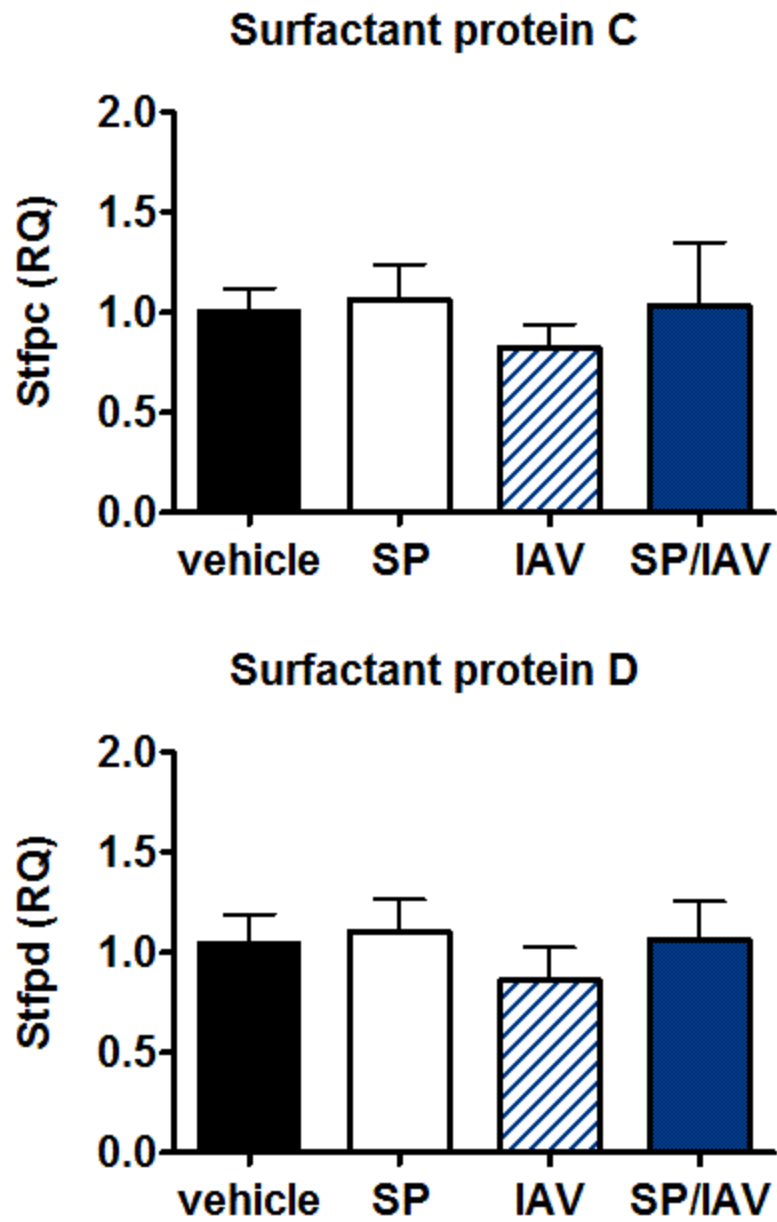


Figure S2. Neonatal co-infection does not alter surfactant protein gene expression in adult lung Taqman PCR analysis for gene expression of surfactant protein C and D in lung tissue is expressed as fold-change compared to vehicle-treated control and normalised to GAPDH housekeeping gene (n = 6).