Supplementary Methods

Genotyping: DNA was isolated from tail snips using a Wizard SV Genomic DNA Purification System Kit (A2360; Promega, Madison, WI). Primers used for genotyping (Integrated DNA Technologies [IDT], Coralville, IA) were as follows: SPC Cre 1, 5'-TTCGGCTATACGTA-ACAGGG-3' and SPC Cre 2, 5'-TCGATGCAACGAGTGATGAG-3'. Genotyping was performed using PCR, with an initialization at 95°C for 3 min to activate hot-start Taq (Qiagen, Germantown, MD), followed by denaturation at 95°C for 30 sec, annealing at 55°C for 45 sec, extension/elongation at 72°C for 5 min, and a final hold at 4°C. To visualize all bands, a 1.7% agarose gel was utilized.

To confirm MyD88 floxed status, genotyping was conducted periodically. Primers used (from IDT) were as follows: MyD88 fl/fl F: 5'-GTTGTGTGTGTCCGACCGT-3' and MyD88 fl/fl R: 5'-GTCAGAAACAACCACCACCATGC3'. The genotyping protocol involved initialization at 95°C for 3 min to activate hot-start Taq, followed by denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension/elongation at 72°C for 60 sec, final elongation at 72°C for 5 min, and then a final hold at 4°C. Wild-type (WT) mice exhibited a band at 266 bp and floxed mice had a band at 353 bp. The primers used to genotype the CCSP mice (IDT) were as follows: scgb1a1 common (Club cells): 5'-ACTCACTATTGGGGGTGTGG -3', Scgb1a1 mutant reverse 5'-CCAAAAGACGGCAATATGGT-3', Scgb1a1 WT reverse 5'- AGGCTCCT-GGCTGGAATAGT-3'. Genotyping was performed using an initialization at 94°C for 2 min, followed by 31 PCR cycles of 94°C for 30 sec, then 60.1°C for 30 sec, and then 72°C for 45 sec. After the 31 cycles, the protocol proceeded at 72°C for 5 min and then a final hold at 4˚C. Genotyping was also conducted for the CCSP $Cre^{+/-}$ mice to confirm $Cre^{+/-}$ and MyD88 $floxed^{+/-}$ status similarly to the SPC $Cre^{+/-}$ mice.

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Muc5ac immunoblot: Generated lysates were centrifuged at 23,755 x g for 30 min at 4°C and the resulting supernatants were collected and evaluated. After determining total protein concentration in each supernatant using a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL), 40 µg total protein from each sample was electrophoresed over a 0.8% agarose gel. The gel was then incubated in a 10 mM dithiothreitol (DTT)/3.0 M saline/0.3 M sodium citrate (SCC) buffer (pH 7.0) at room temperature for 20 min, and then rinsed in water before undergoing vacuum transfer to a 0.45-µm nitrocellulose membrane (BioRad). For Muc5ac assessment using UEA-1 detection, gels were rinsed with SCC (3.0 M sodium chloride, 0.3 M sodium citrate, pH 7.0) buffer without DTT, before undergoing vacuum transfer to a corresponding membrane. All membranes were rinsed with a solution of Tris-buffered saline (TBS, pH 7.5) and then coated with a solution of TBS containing 5% non-fat milk (blocking buffer) for 1 hr at room temperature to prevent non-specific binding events. Each membrane was then incubated in a solution of blocking buffer containing lectin UEA-1 L8146 (at 1 mg/ml; 1:1000 dilution; Sigma) overnight at 4°C. After several gentle washes with TBS, blocking buffer containing rabbit anti-lectin UEA-1 (U4754; 1:1000 dilution; Sigma) was applied to detect Muc5ac. Each membrane was then incubated at room temperature for 1 hr. After several gentle washes with TBS, secondary antibody IRDye 800CW goat anti-rabbit IgG (#D11215-02, Li-Cor, Lincoln, NE; 1:10,000 dilution) in blocking buffer was applied to each UEA-1/Muc5ac membrane and the blots were incubated at room temperature for an additional 1 hr.

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