

Figure S2. **Efficiency of  $CCSP^{iCre}$  recombination.** (A) Confocal image of lung sections from  $CCSP^{iCre/+}; ROSA^{mTmG/mTmG}$  mice showing plasma membrane outlined in green. Lumens of an airway and a vessel, and region of alveoli, are indicated. Scale bar=100  $\mu m$ . (B) High magnification image of lung section in (A), showing plasma membrane outline. Scale bar=50  $\mu m$ . (C) Confocal image of immunostained lung section from same mouse as in (A) using GFP antibody to increase sensitivity of the detection of recombination. Arrowheads show type 2 alveolar cells. Scale bar=50  $\mu m$ .



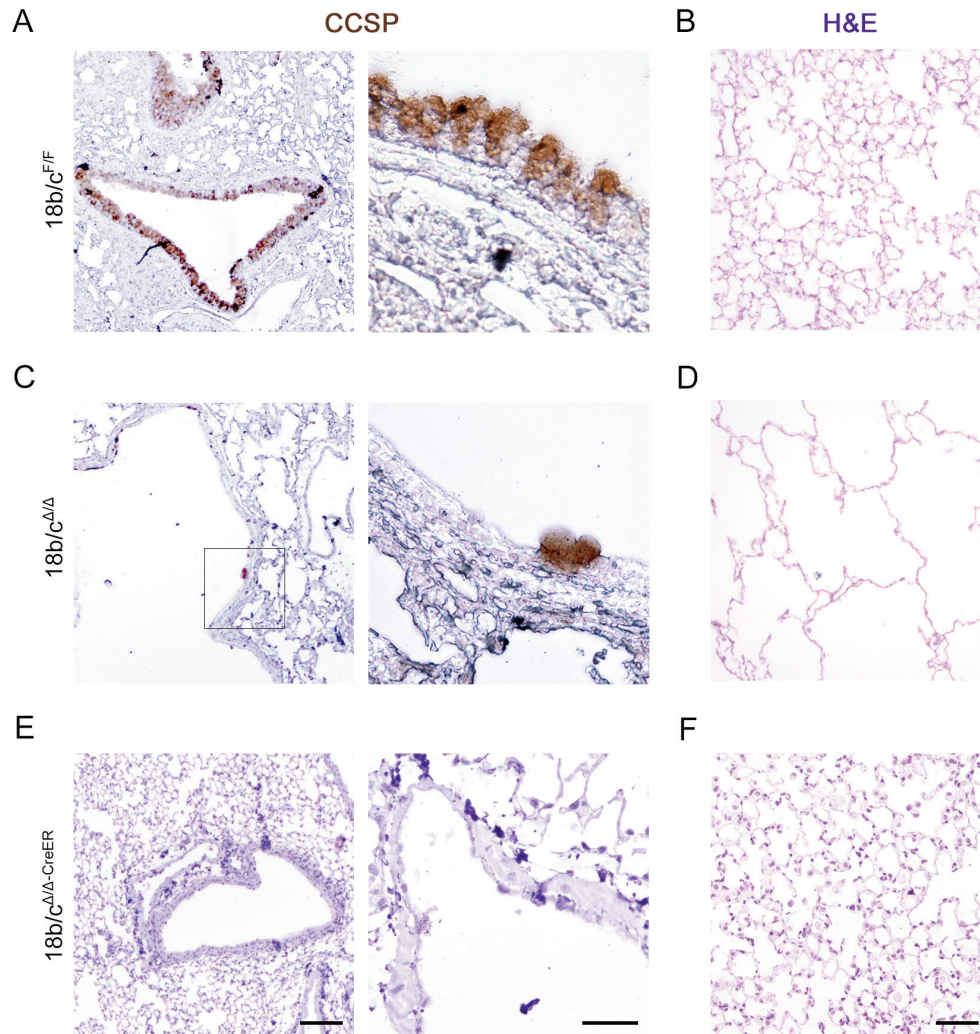
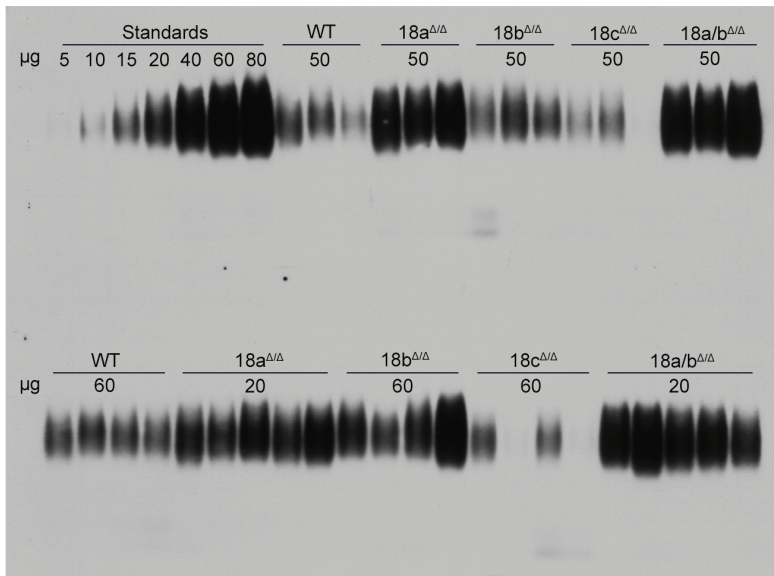


Figure S3. **Double deletion of Munc18b and Munc18c in the airway epithelium.** Representative lung sections of Munc18b/c<sup>F/F</sup> (A, B), Munc18b/c<sup>Δ/Δ</sup> (C, D) and Munc18b/c<sup>Δ/Δ-CreER</sup> (E, F) mice. A, C and E show airways immunostained with CCSP at a low magnification (left column, scale bar=200 μm) and at a high magnification (right column, scale bar=25 μm). B, D and F show alveolar regions stained with hematoxylin and eosin. Scale bar=50 μm.

A



B

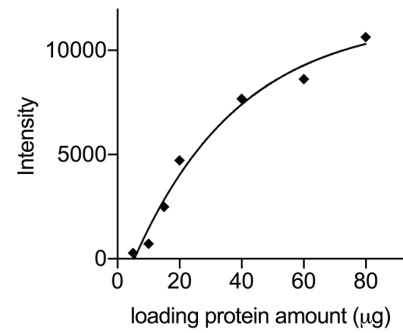
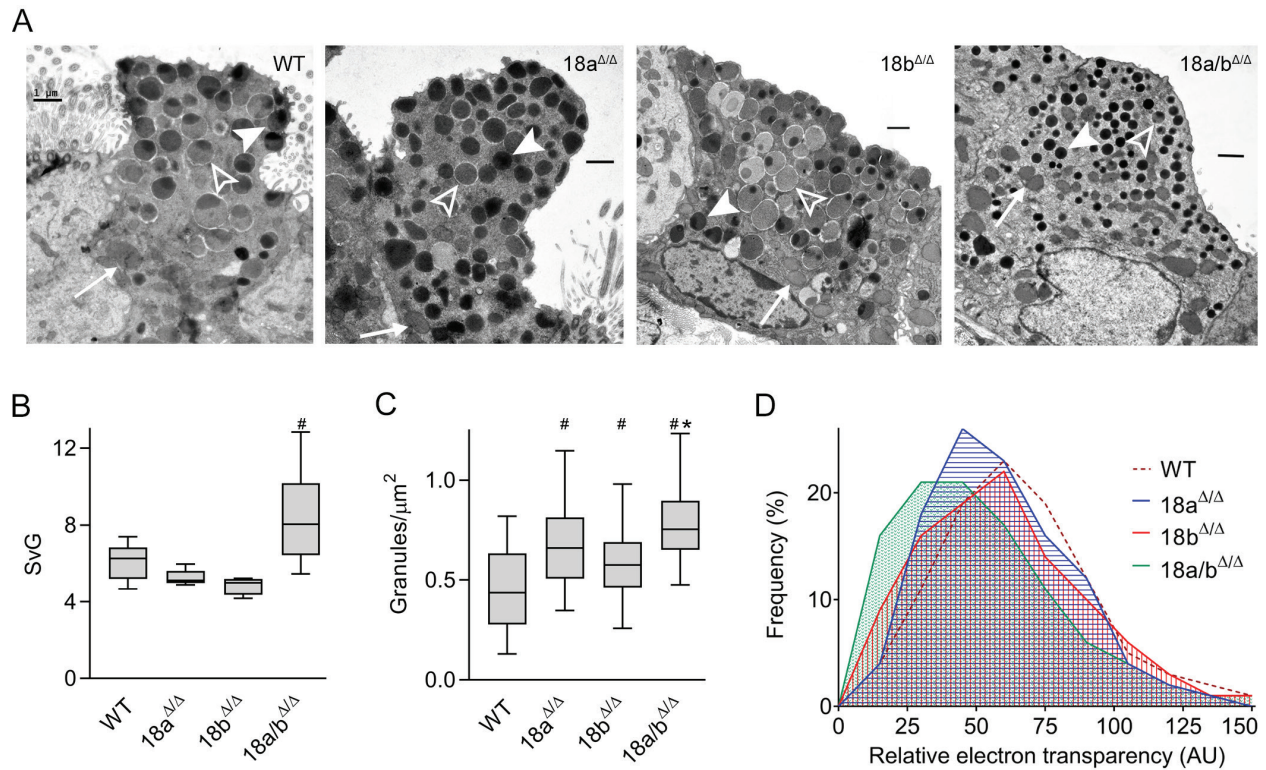
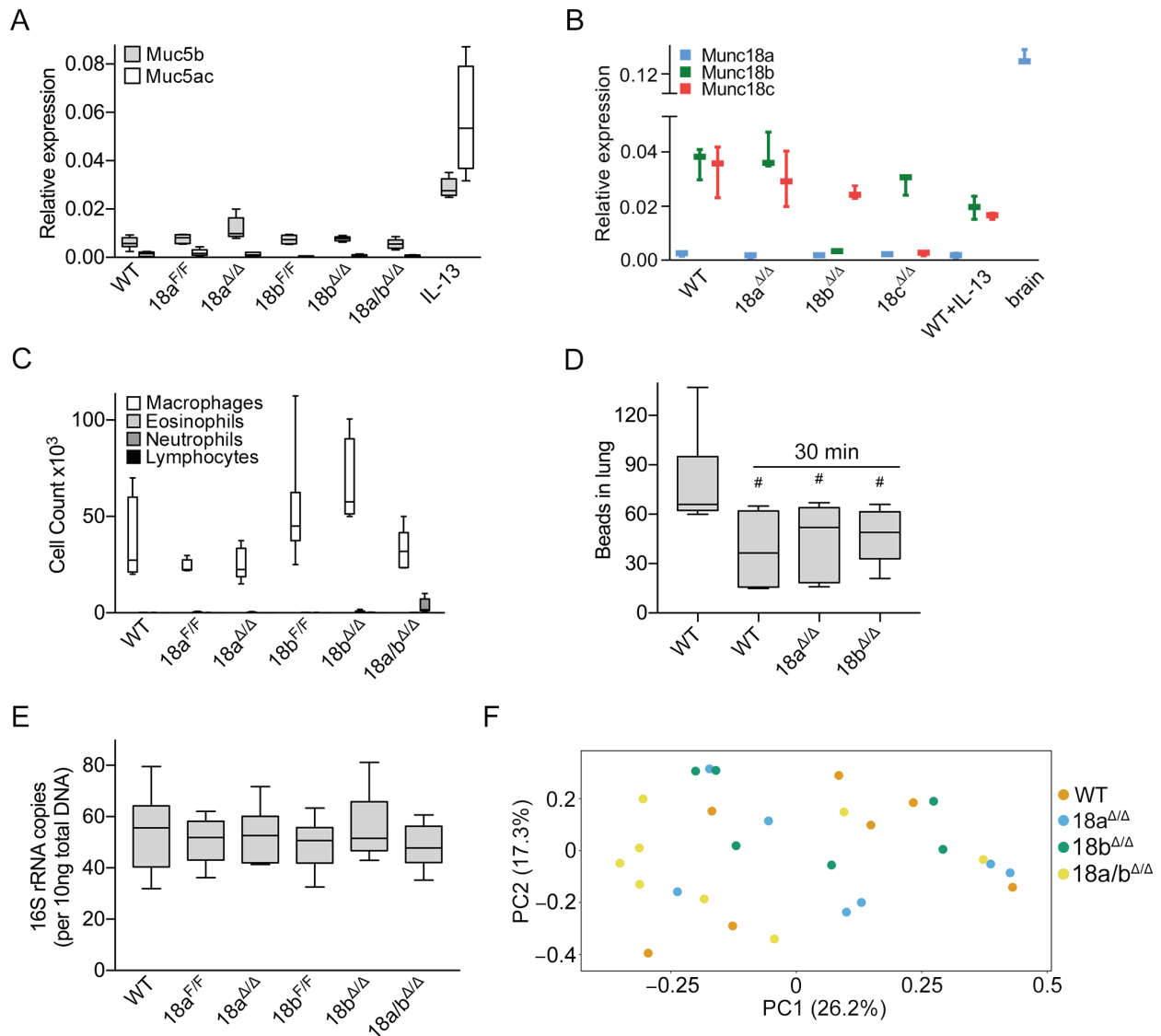


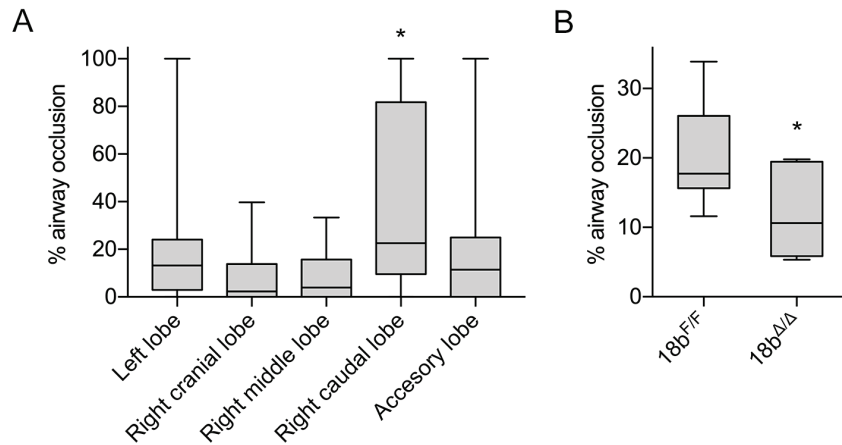
Figure S4. **Muc5b immunoblot of naïve Munc18 conditional deletant mice excerpted in Figure 4 C. (A)** Agarose gel electrophoresis and PVDF membrane transfer developed with ECL and imaged using X-ray film of lung lysates from naïve WT and Munc18 conditional deletant mice probed for Muc5b. Labels on gel show genotype and protein amount loaded. Each lane is an individual mouse sample. Standard lanes come from a Munc18a<sup>Δ/Δ</sup> lung homogenate. **(B)** Band intensity was determined for a range of standard loading amounts and the curve was fitted using a 4-parameter logistic curve.



**Figure S5. Assessment of granule size, number and density by electron microscopy.** **(A)** Representative EM images of secretory cells from mice treated with a low dose (0.2  $\mu\text{g}$ ) of IL-13. Arrow – atypical mitochondrion (Evans et al., 2004); open arrowhead – lucent mucin granule with dense core; filled arrowhead – condensed mucin granule with dense core. Scale bar=1  $\mu\text{m}$ . **(B)** Sv (surface density related to volume density) of granules measured by stereology, with greater Sv indicating smaller size. WT vs 18a/b<sup>ΔΔ</sup>,  $P < 0.0001$ , Student's two-tailed  $t$  test. **(C)** Number of granules per cytoplasmic area. 18a<sup>ΔΔ</sup> vs WT,  $P < 0.0001$ ; 18b<sup>ΔΔ</sup> vs WT,  $P < 0.0001$ ; 18a/b<sup>ΔΔ</sup> vs WT,  $P < 0.0001$ ; 18a/b<sup>ΔΔ</sup> vs 18a<sup>ΔΔ</sup>,  $P = 0.002$ ; 18a/b<sup>ΔΔ</sup> vs 18b<sup>ΔΔ</sup>,  $P < 0.0001$ , Student's two-tailed  $t$  test. **(D)** Frequency distribution of electron density of mucin granules based on a 0 to 255 relative scale (only values between 0-150 are shown). 18a<sup>ΔΔ</sup> vs WT,  $P < 0.0001$ ; 18b<sup>ΔΔ</sup> vs WT,  $P < 0.0001$ ; 18a/b<sup>ΔΔ</sup> vs WT,  $P < 0.0001$ , Kolmogorov-Smirnov test. #,  $P < 0.05$  vs WT; \*,  $P < 0.05$  vs other deletants.



**Figure S6. Assessment of mucin gene expression, inflammation, mucociliary clearance function and bacterial infection in Munc18 conditional deletant mice.** **(A)** qRT-PCR of lung tissue for Muc5b and Muc5ac relative to  $\beta$ -actin ( $n=5$  mice per group). Mice treated with IL-13 are plotted for comparison. **(B)** qRT-PCR of sorted epithelial cells for Munc18a, b and c relative to  $\beta$ -actin ( $n=3$  mice per group). Brain tissue was added as a positive control for confirmation of Munc18a assay. **(C)** Total inflammatory cell numbers from the lung lavage fluid of naïve mice ( $n=5-9$  mice per group). **(D)** Fluorescent  $4\ \mu\text{m}$  polystyrene microspheres were instilled into the lungs, and microspheres present in the lungs were counted at the time of instillation and after 30 min ( $n=5-8$  mice per group). WT (0 min) vs WT (30 min),  $P=0.0127$ , vs 18a<sup>Δ/Δ</sup> (30 min),  $P=0.0295$ , vs 18b<sup>Δ/Δ</sup> (30 min),  $P=0.0087$ , Mann-Whitney test. **(E)** qPCR of lung tissue for copies of bacterial 16S per 10 ng of total DNA ( $n=7$  per group) **(F)** Principal components analysis of bacterial communities using ribosome gene 16S rDNA gene sequencing ( $n=7$  per group). #,  $P<0.05$  vs WT.



**Figure S7. Assessment of airway mucus occlusion (A)** Distribution of airway mucus occlusion throughout the lung lobes. A WT mouse was treated with IL-13 (2  $\mu$ g daily for 4 days) to induce mucin production, and then stimulated with aerosolized 150 mM (30 mg/ml) Mch to stimulate mucin secretion. Lobes were sectioned every 500  $\mu$ m, stained with PAFS, and a ratio of the area of mucus to the area of lumen (fractional occlusion) was obtained. Right caudal lobe vs left lobe,  $P=0.0043$ , vs right cranial lobe,  $P=0.0002$ , vs right middle lobe,  $P=0.0008$ , vs accessory lobe,  $P=0.0025$ , Tukey test. **(B)** Munc18 deletant and floxed littermate mice were treated as in (A), and fractional occlusion in the right caudal lobe was determined. ( $n=7-8$  mice). 18b <sup>$\Delta/\Delta$</sup>  vs 18b<sup>F/F</sup>,  $P=0.0336$ , Student's two-tailed  $t$  test. \*,  $P<0.05$ .

**Table S1. Number of embryos harvested at various days of embryonic (E) development after generating a full KO mouse**

<b>Stage</b>	<b>Total</b>	<b>+/+</b>	<b>+/-</b>	<b>-/-</b>
E3.5 <sup>A</sup>	42	8	22	12
E10.5	46	11	29	6
E11.5	49	16	33	0
E12.5	38	14	24	0
E13.5	16	6	10	0
E15.5	29	12	17	0

<sup>A</sup>embryos grown in vitro