

## Online Supplemental Information

### ATP12A promotes mucus dysfunction during Type 2 airway inflammation

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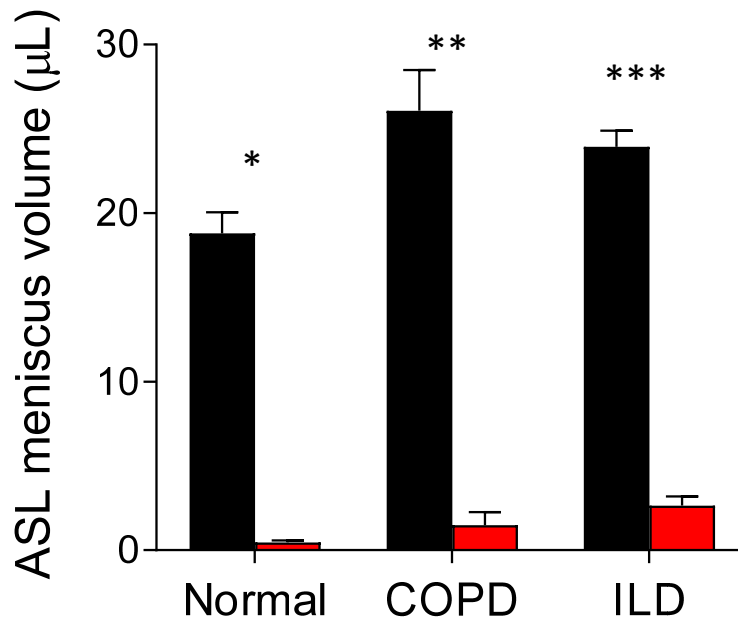
UPMC Montefiore Hospital, NW628

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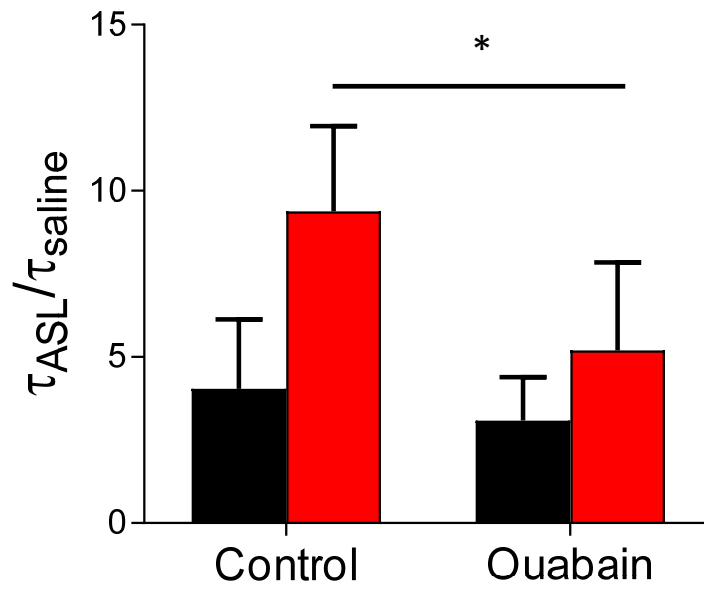
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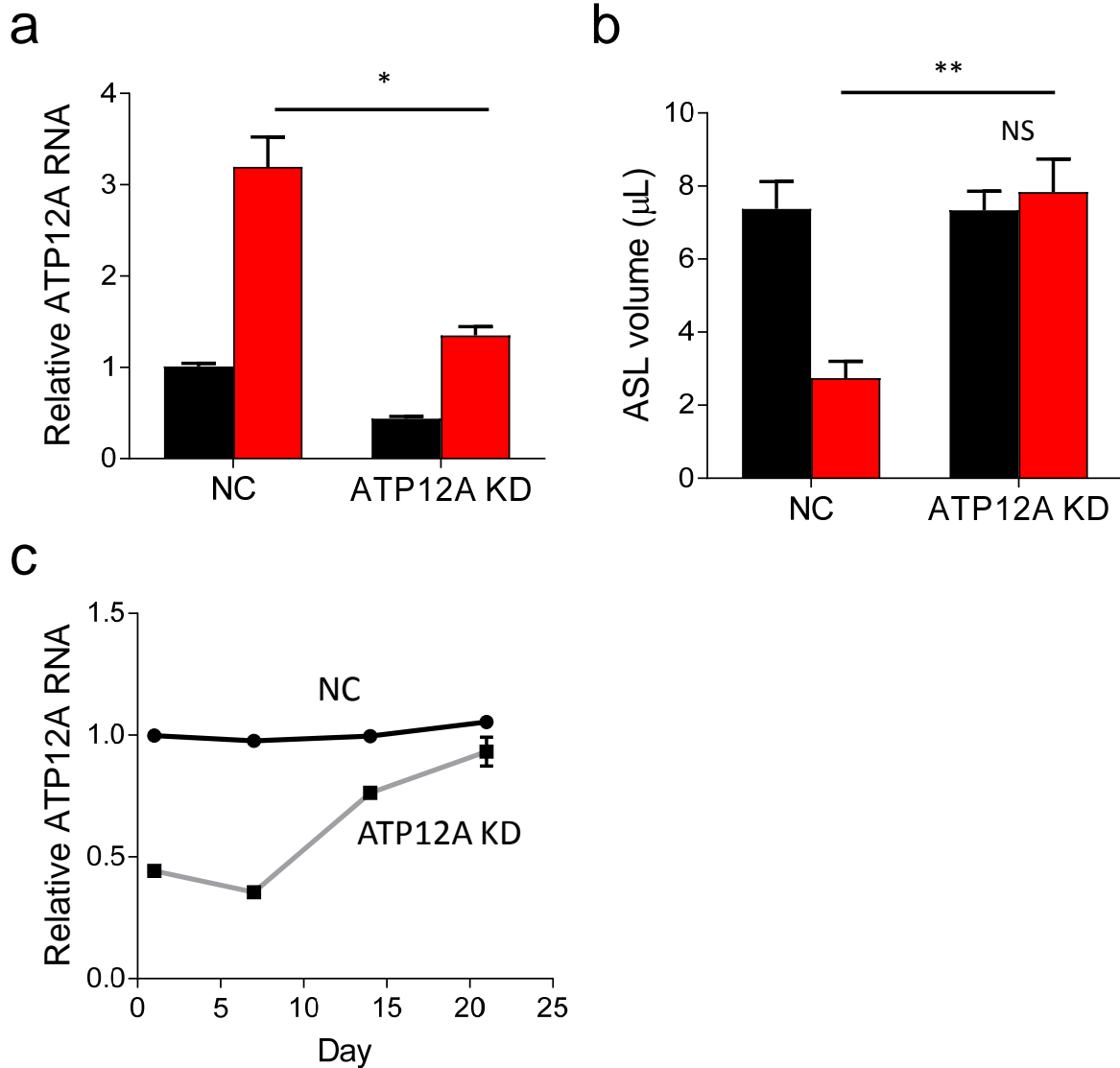
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Supplemental Figure S1. Airway surface liquid meniscus volume is decreased by IL13 across multiple disease states. Cultures were obtained from lung transplant recipients with COPD and ILD. ‘Normal’ lungs were obtained from donor organs that were not deemed suitable for transplant. Data is expressed as mean ASL meniscus volume ( $\mu\text{L}$ )  $\pm$  SEM. Normal: \* $p=0.005$  by unpaired Student’s *t*-test,  $n=2$  tissue donors, 4-6 replicates per donor. COPD: \*\* $p=0.01$  by unpaired Student’s *t*-test,  $n=2$  tissue donors, 4-6 replicates per donor. ILD: \*\*\* $p<0.001$  by unpaired Student’s *t*-test,  $n=8$  tissue donors, 4-6 replicates per donor. Control conditions are expressed in black and IL-13 treated conditions are in red.



Supplemental Figure S2. Inhibition of ATP12A's function with apical ouabain (50  $\mu$ M) reduced the IL-13 mediated hyperviscosity as measured by fluorescence recovery after photobleaching (FRAP). Data shown are mean  $\tau_{ASL}$  relative to  $\tau_{saline} \pm$  SEM, n = 6 tissue donors with 4-6 experimental replicates per donor, \* $p < 0.05$  by unpaired Student's *t*-test).



**Supplemental Figure S3.** ATP12A knockdown by dicer substrate siRNA is protective against IL-13 mediated decreases in ASL meniscus volume. Control conditions are expressed in black and IL-13 treated conditions are in red. (a) Relative ATP12A mRNA with siRNA knockdown ± IL-13. HBE cells were transfected with ATP12A siRNA as they were seeded onto the transwell filter inserts. IL-13 (10 ng/mL) was added to the basolateral growth media on days 4-7 and the cultures were studied on day 7. Data expressed is mean ATP12A mRNA level relative to the untreated NC per individual tissue donor ± SEM. \* $p < 0.0001$  by unpaired Student's t-test,  $n = 24$  replicates from 2 tissue donors. (b) ATP12A siRNA knockdown is protective against decreases in ASL meniscus volume. Data expressed is mean ASL meniscus volume ( $\mu\text{L}$ ) ± SEM \*\* $p < 0.0001$  by unpaired Student's t-test,  $n = 12$  experimental replicates from 2 tissue donors. (c) ATP12A siRNA KD was most pronounced in the first week of ALI culture and did not persist until full cellular differentiation (average 21 days). Data shown is mean relative ATP12A RNA level with and without siRNA KD ± SEM over time,  $n = 4$  experimental replicates per time point from an individual tissue donor.