



**Supplementary Figure 3.** Gad67 expression by PNECs is required for mucus overproduction following early life OVA exposure. (A) Lineage tracing of *Gad2*-expressing lineages. *Gad2*<sup>CreERT2/+</sup>;*Rosa(lacZ)* mice received tamoxifen injection at P10, P14, and P18. At P21, lungs and brain tissues were processed for X-gal staining and histological assessment. N= 3. Arrows point to  $\beta$ -gal<sup>+</sup> cells in mouse brains at P21. No labeling was found in lungs. (B) CGRP staining of PNECs in *Shh*<sup>Cre-</sup>;*Gad1*<sup>ff</sup> littermate controls and *Shh*<sup>Cre+</sup>;*Gad1*<sup>ff</sup> mutant mice at P21. Loss of GAD1 in airway epithelium had no effect on PNEC development. Arrowheads point to PNECs. (C) Representative images of PAS staining of lung sections from *Shh*<sup>Cre-</sup>;*Gad1*<sup>ff</sup> littermate controls and *Shh*<sup>Cre+</sup>;*Gad1*<sup>ff</sup> mutant mice with and without OVA exposure. Arrows point to mucin<sup>+</sup> cells. N=2 for *Shh*<sup>Cre+</sup>;*Gad1*<sup>ff</sup> mice. N=4 for *Shh*<sup>Cre-</sup>;*Gad1*<sup>ff</sup> mice. Scale bars, 100 $\mu$ m.