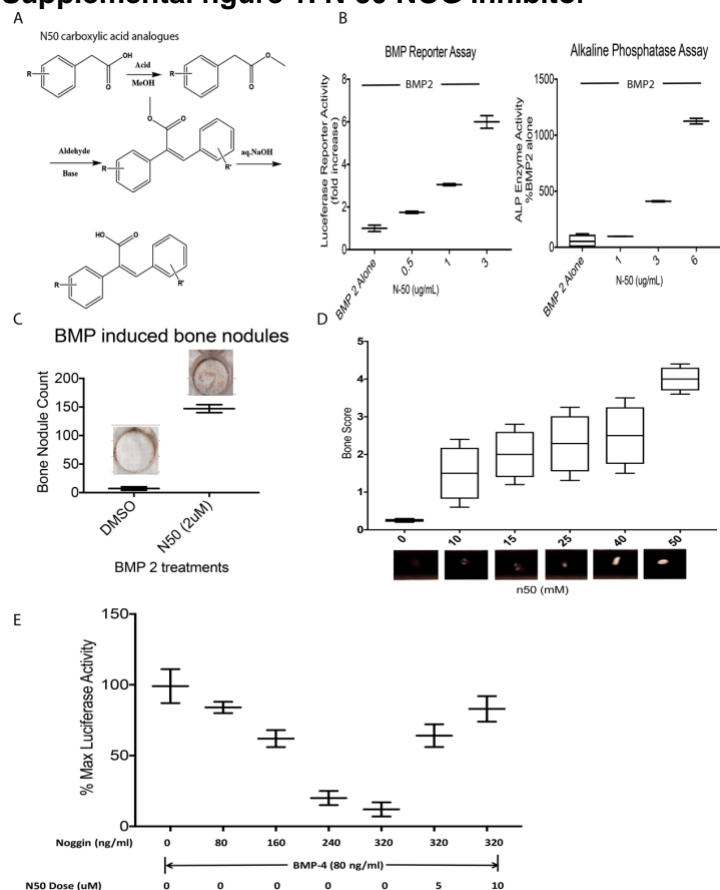
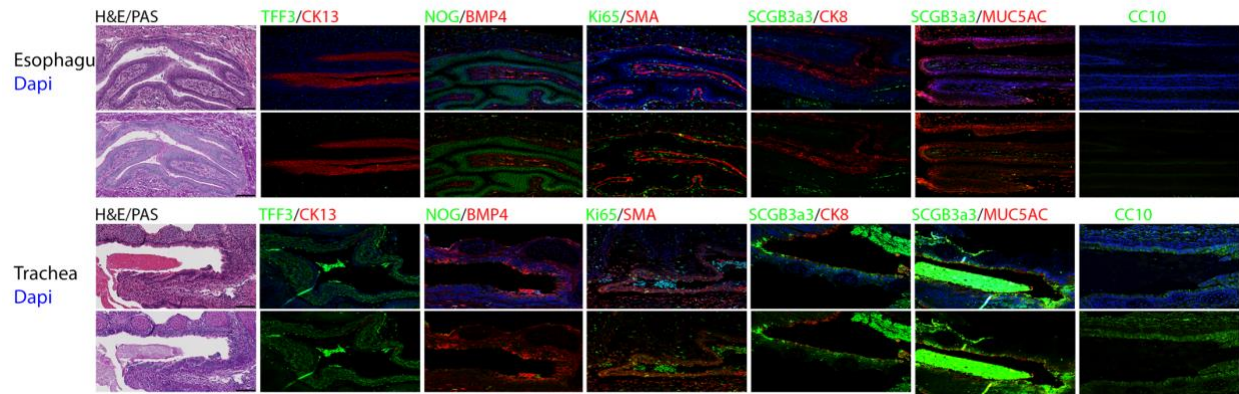


Supplemental figure 1. N-50 NOG inhibitor



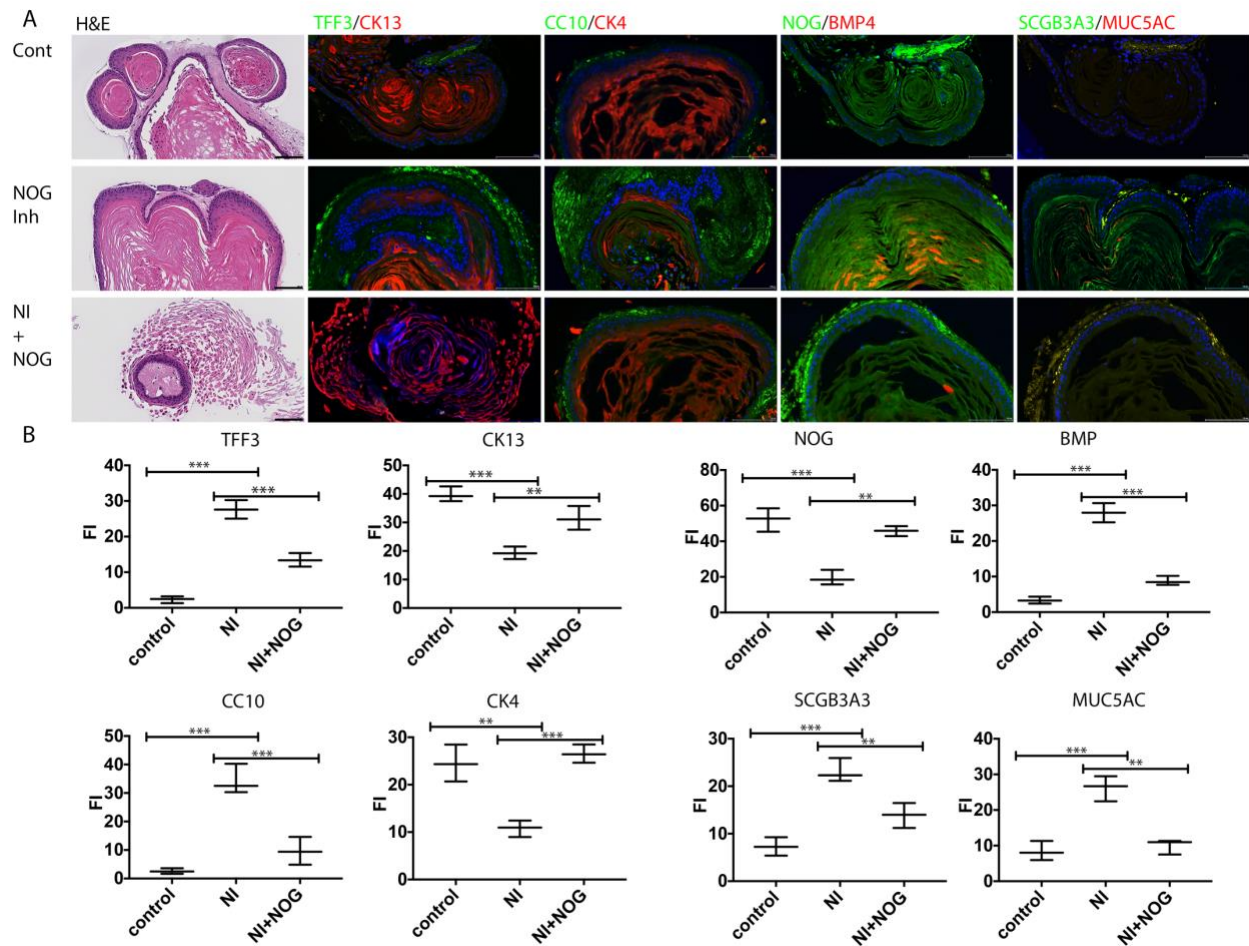
A. All novel compounds were custom synthesized (AA ChemBio, San Diego, CA) and were at least .99% pure based on mass spectrometric analyses. We used the compound N50 whose chemical formula is 3-(3-chlorophenyl)-2-(pyridin-2-yl)acrylonitrile. A brief compound synthesis scheme is given. **B.** N-50 potentiates BMP activity in the absence of exogenously added noggin. N-50 produced a dose-dependent increase in the BMP-induced response compared to the BMP-alone control shown in blue in both the luciferase reporter assay and a standard alkaline phosphatase assay. Since N-50 was specifically designed to be unable to interact with BMP-receptors the most likely interpretation is that N-50 blocks endogenously induced noggin to enhance the effect of both exogenously applied and endogenously induced BMPs. Activities were determined in triplicates. **C.** Activity of N-50 in bone nodule formation by BMP-induced secondary rat calvarial osteoblast differentiation. The treatment with a suboptimal dose of BMP-2 (25 ng/ml) and N-50 compound (2 μ M) induces formation of mineralized bone nodules dramatically compared to treatment with BMP-2 and diluent (DMSO) alone as detected by von Kossa staining. Thus, concurrent exposure to N-50 compound enabled an ineffective dose of BMP-2 to facilitate bone nodule formation. Activities were determined in triplicates. **D.** Activity of N-50 in ectopic bone formation in vivo. We tested the ability of N-50 to enhance ectopic bone formation in rats when suboptimal doses of BMP-2 on a collagen disc were implanted subcutaneously. Using the suboptimal BMP-2 dose, we tested co-delivery with various doses of N-50 and observed substantial enhancement of bone formation compared with BMP-2 alone. Representative X-ray images are shown beneath the bar graph. **E.** N50 rescues noggin-mediated inhibition of BMP-4 responsive luciferase reporter activity. The pre-osteoblastic C2C12 cells were treated with or without BMP-4 (80 ng/ml) in the presence or absence of different amounts of recombinant noggin. At a noggin dose of 320 ng/ml a ~90% inhibition of BMP-4 activity was observed. Treatment of cells concurrently with 5 or 10 μ M N50 reversed the inhibitory effect of noggin under these conditions. Average of triplicate are plotted

Supplemental figure 2. TFF3, SCGB3A3 and CC10 are specific markers for respiratory epithelium.



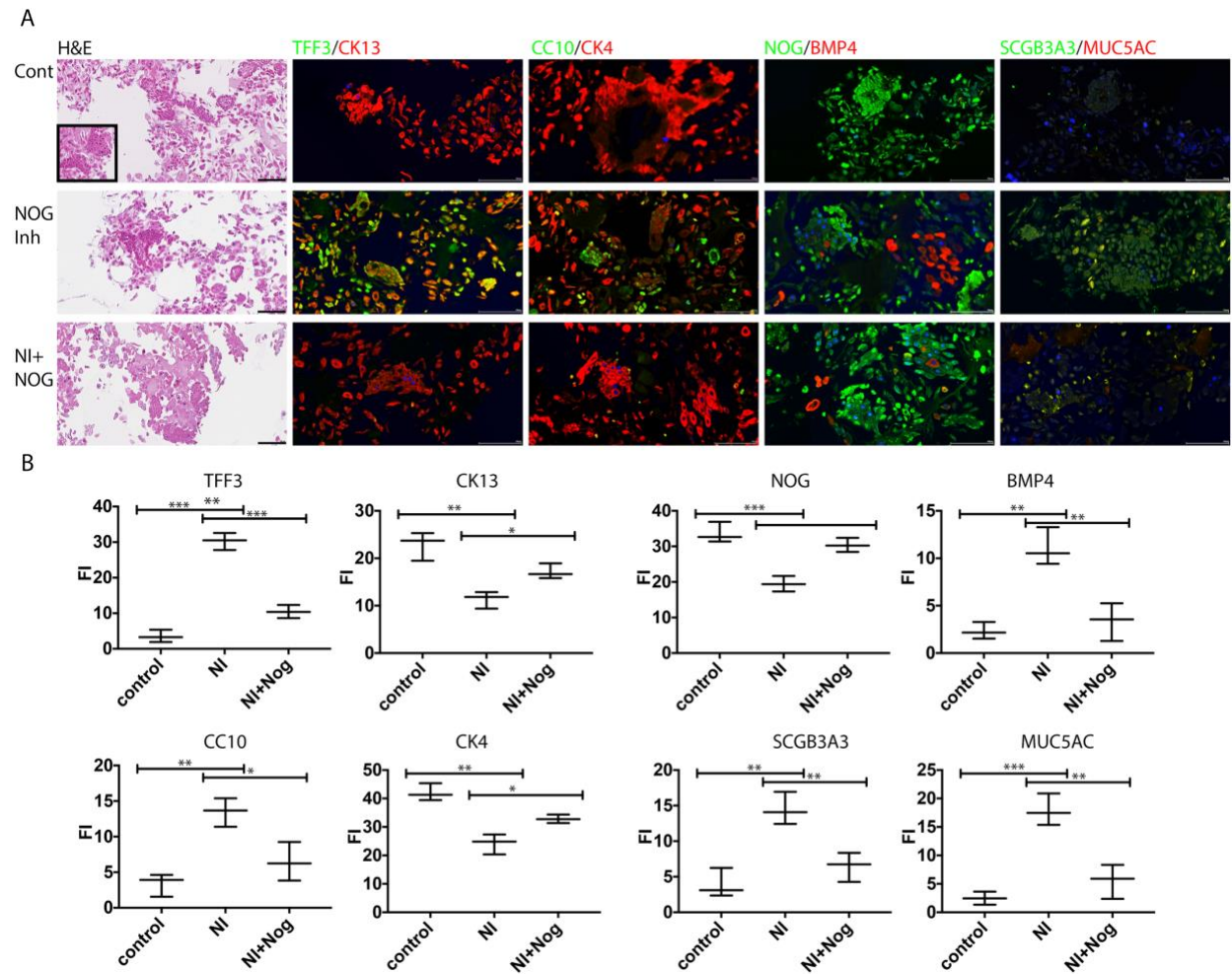
Expression of respiratory and esophageal markers in P1 murine trachea and esophagus. Paraffin sections of P1 esophagus and trachea were evaluated for expression of TFF3, NOG, MKI67, SCGB3A3, CC10 (green), CK13, BMP4, MUC5AC, SMA, CK8. All images were obtained at 20X. Scale bar 100 μ m

Supplemental figure 3. NOG blocks the effects of N-50 on murine EOU



P1-P3 esophagus were used to culture mEOU in the presence of NOG inhibitor N-50, or NOG plus NOG inhibitor for 48 hours, at which point the wells were washed and the cultures allowed to proceed for 8 more days. (A) Samples were fixed, imbedded in paraffin and slides tested for expression of respiratory markers CC10, TFF3, and SCGB3A3. All images were obtained at 20X. Scale bar 100 μ m X. (B) Fluorescence intensity of CK13, TFF3, CC10, CK4, NOG, SCGB3A3 and BMP was evaluated using Image J. 10 litters were used to set up 3 experimental repeats. n=3-8, One way ANOVA with Kruskal Wallis post hoc test was performed to compare all groups. Error bars (\pm SEM), *= p <0.05, **= p <0.005, ***= p <0.0009.

Supplemental figure 4. NOG blocks the effects of N-50 on human EOU



Esophagus from pediatric human organ donors were used to culture hEOU in the presence of NOG inhibitor N-50, or NOG plus NOG inhibitor for 48 hours, at which point the wells were washed and the cultures allowed to proceed for 8 more days. (A) Samples were fixed, imbedded in paraffin and slides tested for expression of respiratory markers CC10, TFF3, and SCGB3A3 All images were obtained at 20X. Scale bar 100 μ m. (B) Fluorescence intensity of CK13, TFF3, CC10, CK4, NOG, SCGB3A3 and BMP was evaluated using Image J. n=3-8, 2 donors were used to set up 3 experimental repeats. n=3-8, One way ANOVA with Kruskal Wallis post hoc test was performed to compare all groups. Error bars (\pm SEM), * p <0.05, ** p <0.005, *** p <0.0009. Black squares represent other areas from same slide, same power who were combined to show more representative organoid units.

Table S1

Bisulfite sequencing-specific primers			
<i>NOG</i> exon, forward: GYGGAGGGAAGTGTTTTAGAATTAG			
<i>NOG</i> exon, reverse: TCCTCTCCCRATCTACTAAAAAAAC			
<i>NOG</i> promoter, forward: TGGTGGGGGAGAGAGAAAATTG			
<i>NOG</i> promoter, reverse: ACAACRCCCAAACCTATACCC			
<i>NOG</i> upstream sequence, forward: TTAGTGYGGAGTTAGATGGGG			
<i>NOG</i>	upstream	sequence,	reverse:
CRAAATTTAAAACCAAAAAAAAAAAAAAACTTCAAC			