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Supplemental Information

High-Throughput Drug Screening Identifies a Potent Wnt Inhibitor that Promotes Airway Basal Stem Cell Homeostasis

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Figure S1. Related to Figure 1.

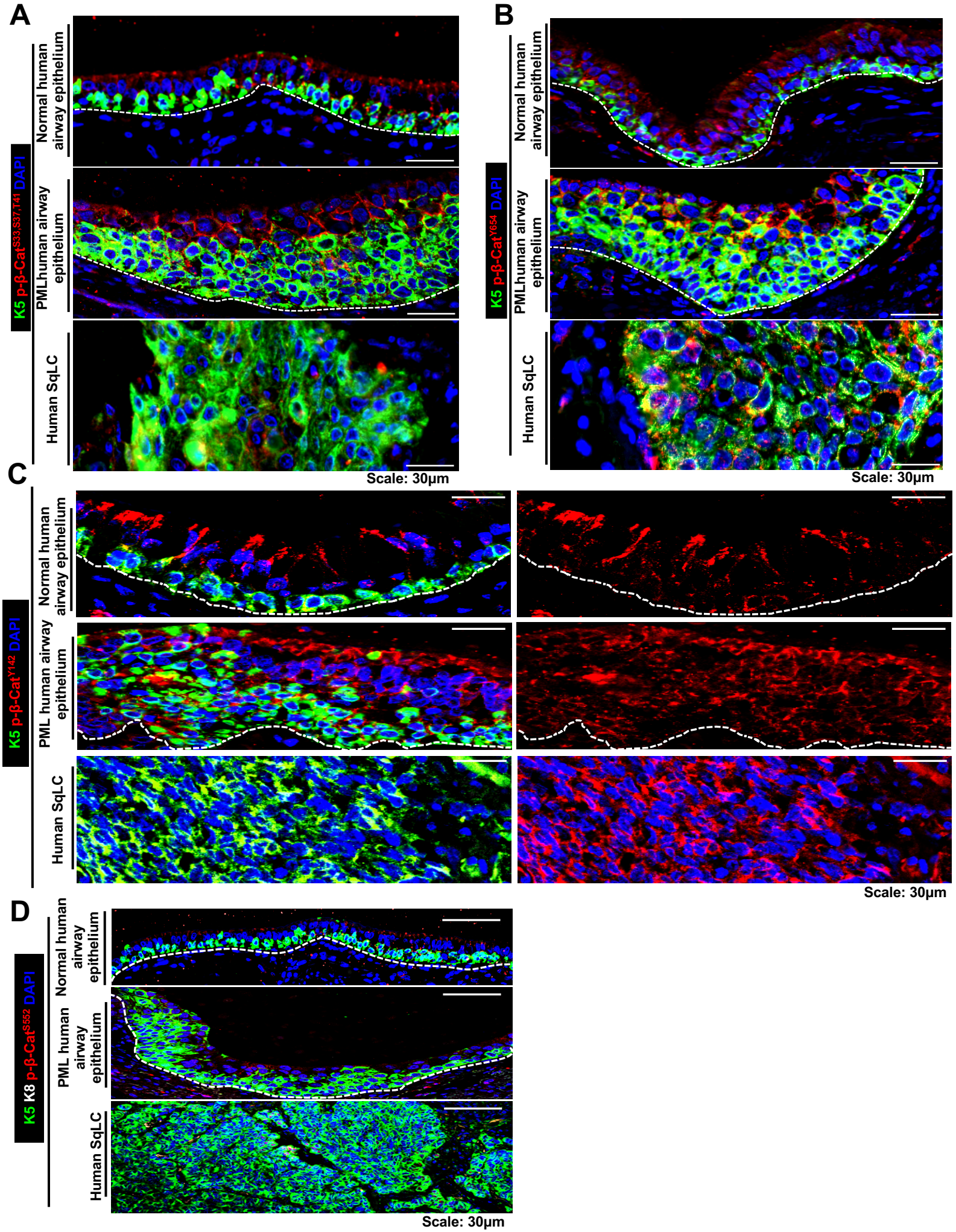
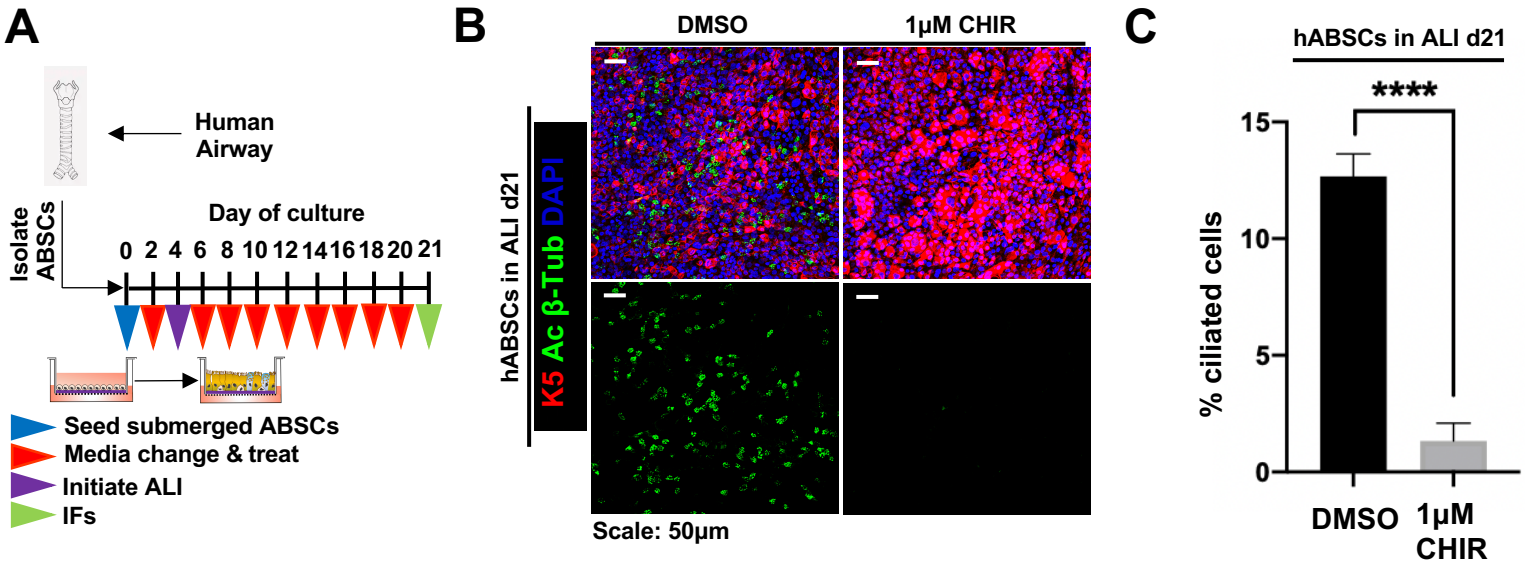


Figure S1. Related to Figure 1.

A. Representative IF images of p-β-catenin^{S33,S37,T41} (red) in human NL airway epithelium, PMLs, and SqLC patient samples. **B.** Representative IF images of p-β-catenin^{Y654} (red) in human NL airway epithelium, PMLs, and SqLC patient samples. **C.** Representative IF images of p-βcatenin^{Y142} (red) in human NL airway epithelium, PMLs, and SqLC patient samples. **D.** Representative IF images of p-β-catenin^{S552} (red) in human NL airway epithelium, PMLs, and SqLC patient samples.

Figure S2. Related to Figure 2.



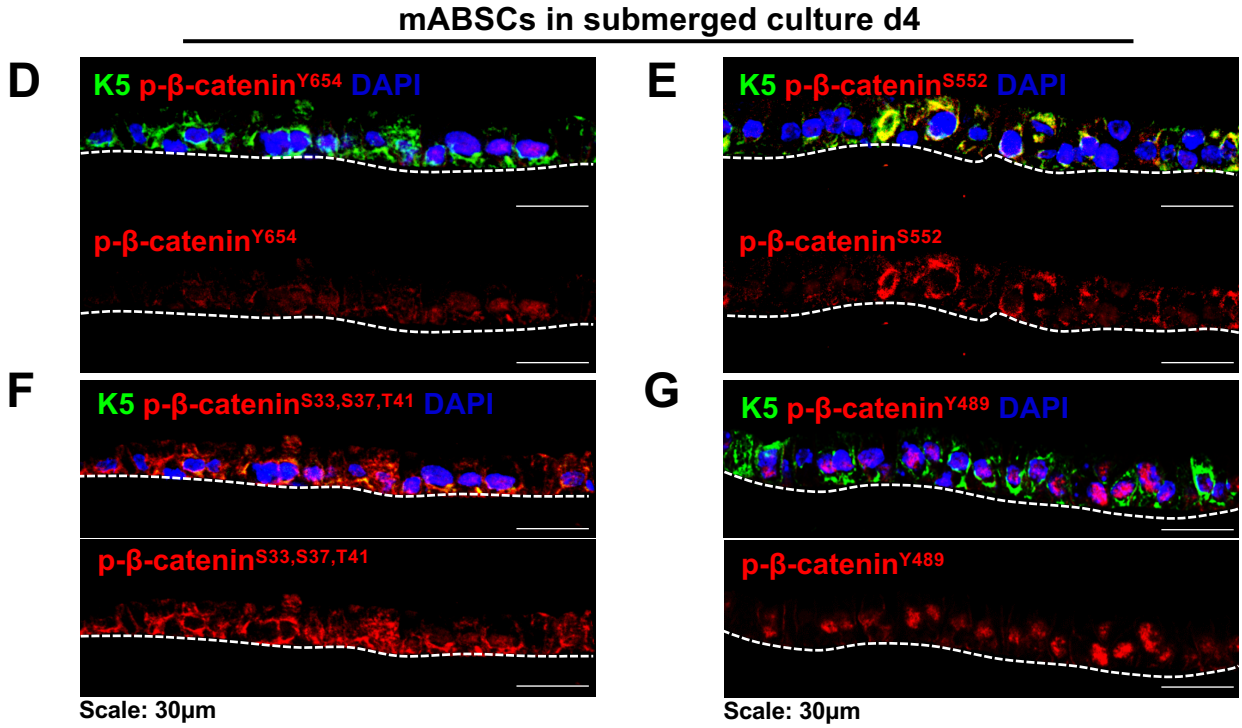


Figure S2. Related to Figure 2.

A. Experimental schematic outlining time of seeding and treatment of hABSCs under submerged and ALI culture conditions with DMSO or CHIR for 21 days. **B.** Representative IF images of hABSCs on ALI day 21 treated with DMSO or CHIR. **C.** Quantification of percentage of ciliated cells from hABSC cultures under ALI conditions for 21 days treated with DMSO or CHIR. 4 fields used for quantification. **D.** Representative IF images of mABSCs cultured under submerged conditions assessing p-βcatenin^{Y654} expression and localization. **E.** Representative IF images of mABSCs cultured under submerged conditions assessing p-β-catenin^{S552} expression and localization. **F.** Representative IF images of mABSCs cultured under submerged conditions assessing p-β-catenin^{S33,S37,T41} expression and localization. **G.** Representative IF images of mABSCs cultured under submerged conditions assessing p-β-catenin^{Y489} expression and localization. n = 3-6. **** $p < 0.0001$ by Student's t test. All error bars represent mean ± SEM.

Figure S3. Related to Figure 3.

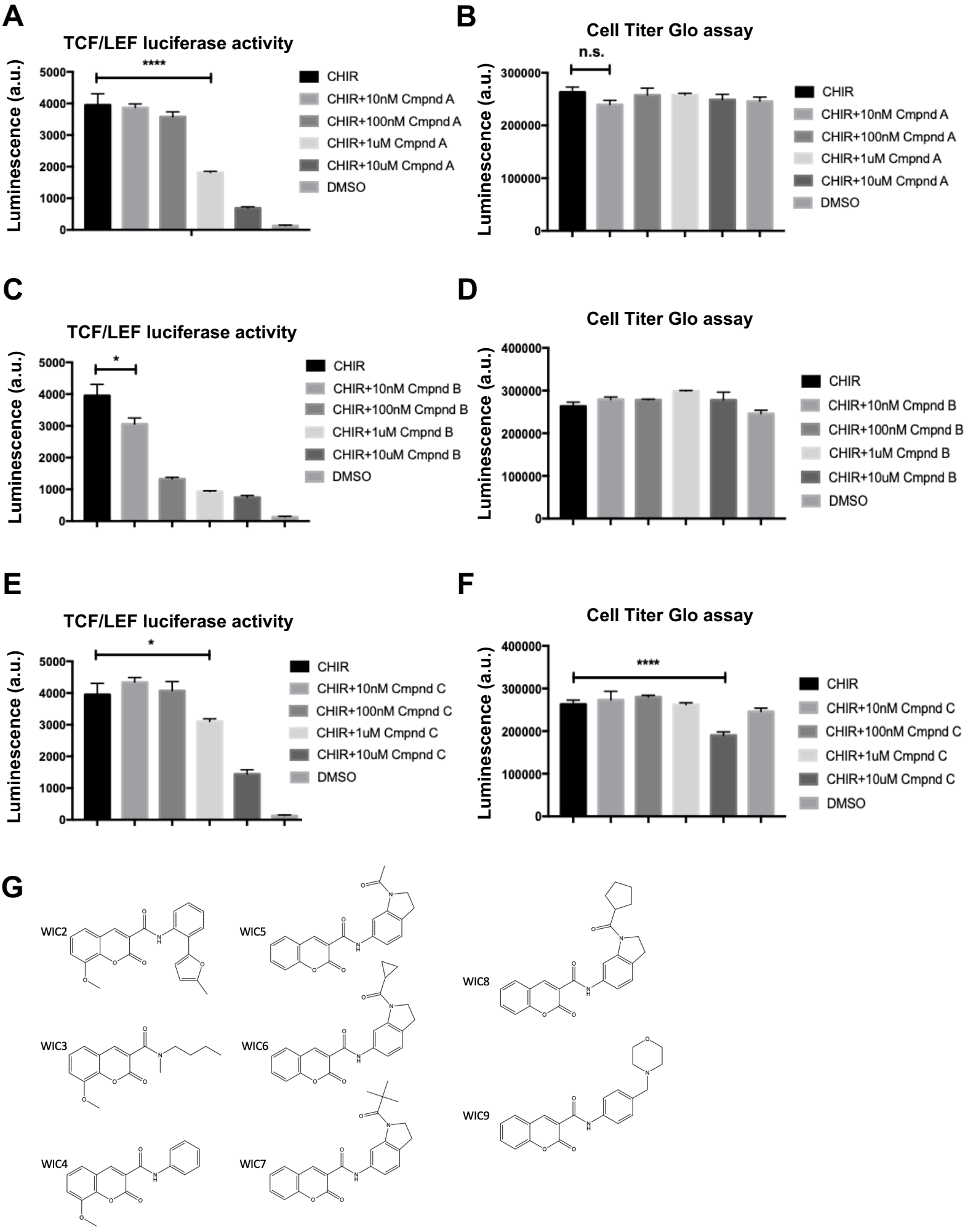
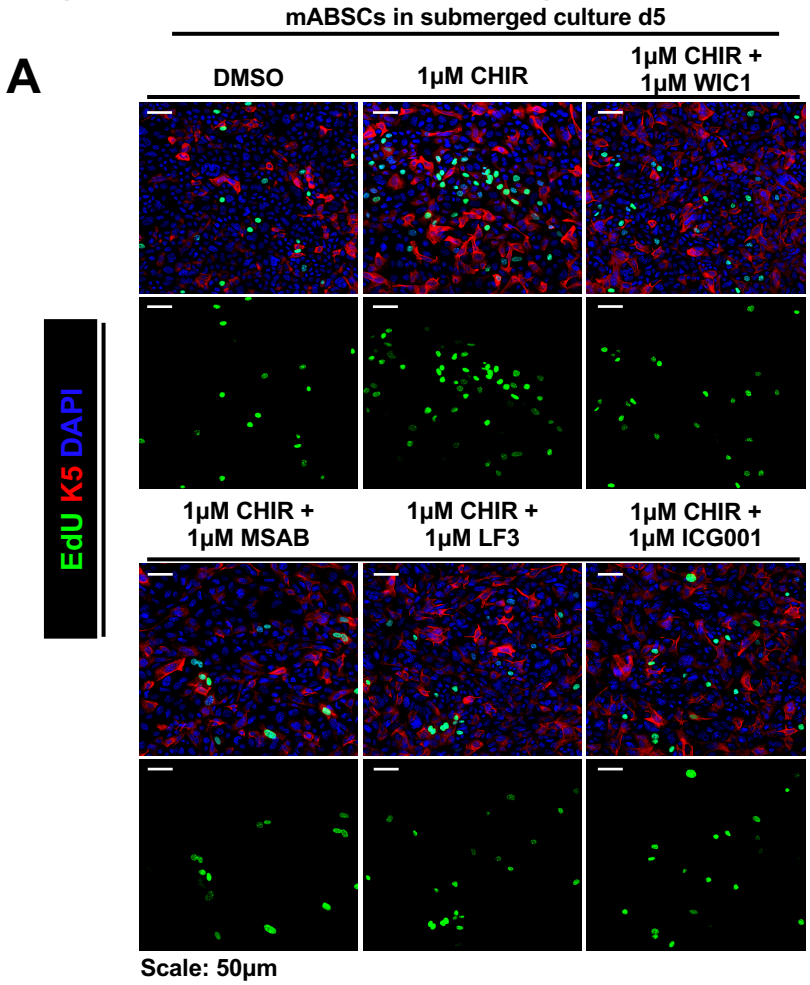
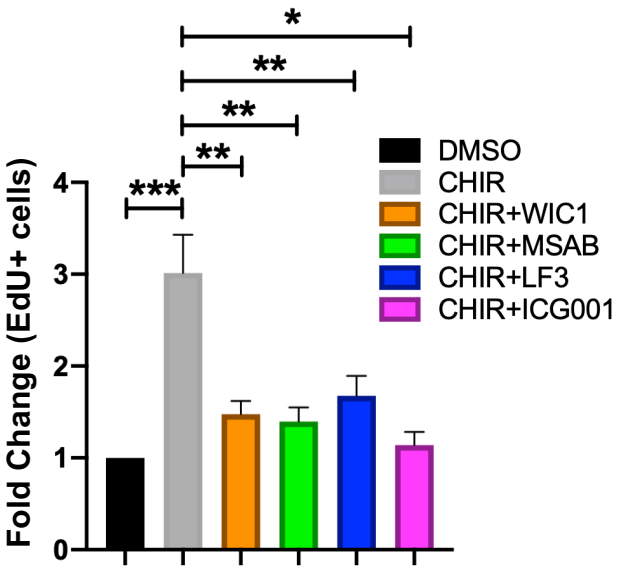


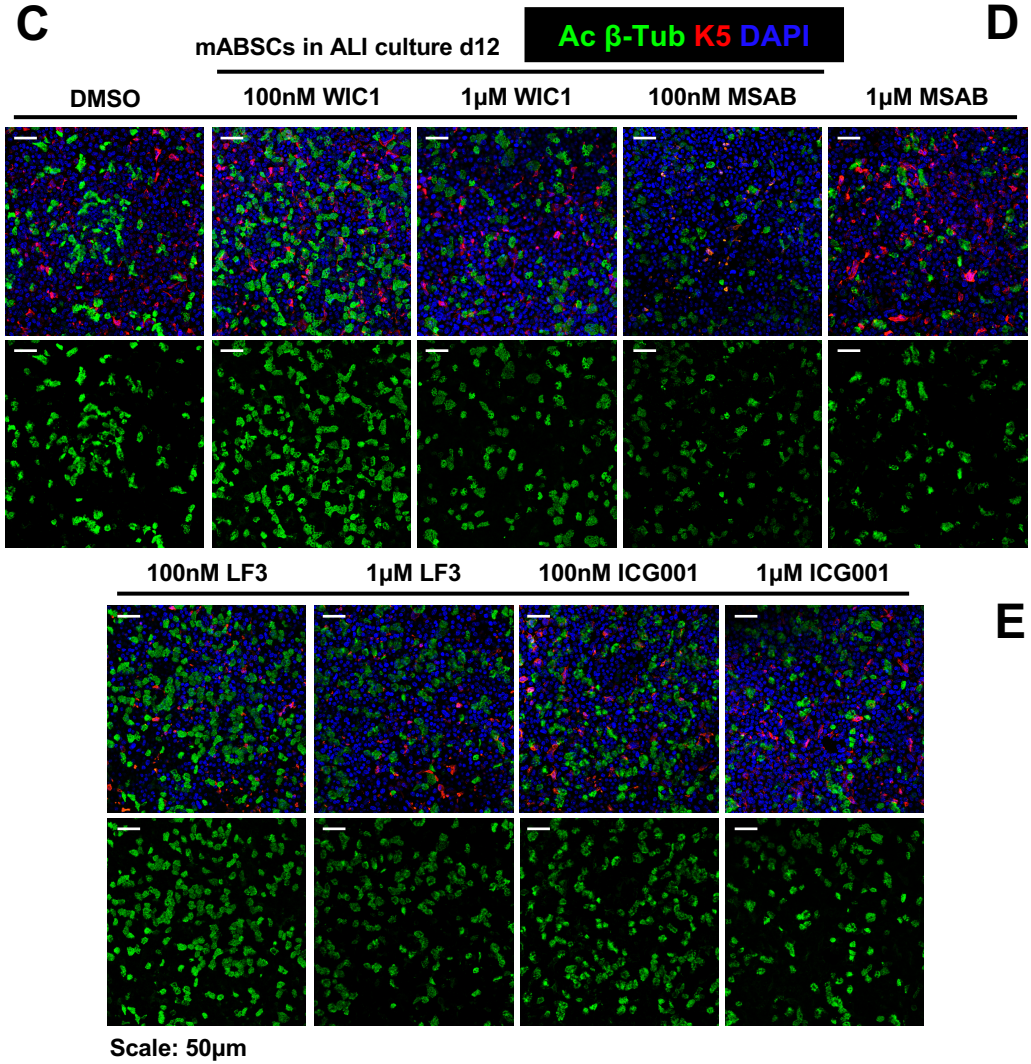
Figure S3. Related to Figure 3.

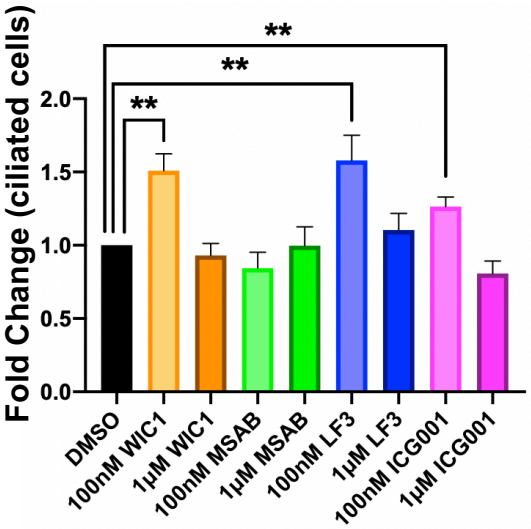
A. Bar graph representing TCF/LEF luciferase reporter activity in BEAS2B cells treated for 24 hours with DMSO, CHIR, or CHIR with varying concentrations of Compound A (Cmpnd A). **B.** Bar graph representing Cell Titer Glo assay to assess toxicity of BEAS2B cells treated for 24 hours with DMSO, CHIR, or CHIR with varying concentrations of Cmpnd A. **C.** Bar graph representing TCF/LEF luciferase reporter activity in BEAS2B cells treated for 24 hours with DMSO, CHIR, or CHIR with varying concentrations of Cmpnd B. **D.** Bar graph representing Cell Titer Glo assay to assess toxicity of BEAS2B cells treated for 24 hours with DMSO, CHIR, or CHIR with varying concentrations of Cmpnd B. **E.** Bar graph representing TCF/LEF luciferase reporter activity in BEAS2B cells treated for 24 hours with DMSO, CHIR, or CHIR with varying concentrations of Cmpnd C. **F.** Bar graph representing Cell Titer Glo assay to assess toxicity of BEAS2B cells treated for 24 hours with DMSO, CHIR, or CHIR with varying concentrations of Cmpnd C. **G.** Chemical structures of WIC2-WIC9 identified from SAR studies. n = 3-6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p<0.0001$ by Student's t test. All error bars represent mean ± SEM.

Figure S4. Related to Figure 4.









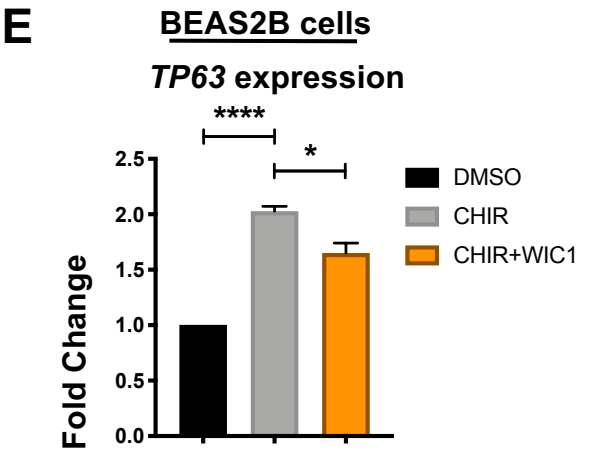


Figure S4. Related to Figure 4.

A. Representative IF images of mABSC proliferation *in vitro* under submerged conditions on day 4 treated with DMSO, 1μM CHIR, or 1μM CHIR + 1μM of either WIC1, MSAB, LF3, or ICG001 using click-iT EdU assay. **B.** Quantification of proliferating K5+ EdU+ mABSCs under submerged conditions on day 4 treated with DMSO, 1μM CHIR, or 1μM CHIR + 1μM of either WIC1, MSAB, LF3, or ICG001 using click-iT EdU assay. 5-9 fields used for quantification. **C.** Representative IF images of mABSCs *in vitro* under ALI culture conditions for 12 days treated with DMSO or indicated concentrations of WIC1, MSAB, LF3, or ICG001. **D.** Quantification of percentage of ciliated cells from mABSC cultures under ALI conditions for 12 days treated with indicated concentrations of WIC1, MSAB, LF3, or ICG001. 5 fields used for quantification. **E.** Bar graph representing qPCR data assessing mRNA expression of *TP63* in BEAS2B cells treated with DMSO, 5μM CHIR, or 5μM CHIR + 1μM WIC1 for 48 hours. n = 3-6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p<0.0001$ by Student's t test. All error bars represent mean ± SEM.