Supplemental Methods

Study Population

Human subjects all signed written informed consent and were enrolled in studies approved by the Weill Cornell Medicine Institutional Review Board. Two subjects were enrolled in the Weill Cornell Ancillary Substudy of the SPIROMICS Bronchoscopy study. Details of the human subjects used in RNA-sequencing are summarized in Supplemental Table III.

Airway Epithelium Sampling, Basal Cell (BCs) Purification and Culture

The large airway epithelium (1^{th}-5^{th} generation bronchi) were collected by bronchoscopic brushing from the healthy nonsmokers, asymptomatic smokers and chronic obstructive pulmonary disease (COPD) smokers as shown in Supplemental Table III.

BCs purification from the airway epithelium of healthy nonsmokers, asymptomatic smokers and COPD smokers was carried out as described previously [1]. Briefly, the 14 brushes of the airway epithelial cells were digested with 0.05% trypsin/ethylenediaminetetraacetic acid (EDTA; Gibco, Grand Island, NY) for 5 min and then seeded into the T25 flasks (Corning, Corning, NY) at the density 1.5~2.5 × 10^4 alive cells/cm^2. The numbers of the total and alive cells initially isolated from the 14 brushes varied between different individuals, ranging from 0.32/0.1 ~ 3.55/1.1 × 10^6 (total/alive) cells. Fewer cells were initially isolated from the brushes in COPD smokers (total cells 0.77±0.44 × 10^6, alive cells 0.39±0.14 × 10^6) compared to nonsmokers (total cells 1.27±0.70 × 10^6, alive cells 0.68±0.25 × 10^6). No difference between nonsmokers *vs* asymptomatic smokers (total cells 0.84±0.25 × 10^6, alive cells 0.48±0.14 × 10^6; Supplemental Table III) and asymptomatic smokers *vs* COPD smokers. The cell viabilities of the initially isolated cells from nonsmokers, asymptomatic and COPD smokers are similar (~60%; Supplemental Table III). Only BCs attached and proliferated in the flask. BCs (passage 0) from different phenotypes grew similarly and after 7~8 days culture in BEGM, they were ~80% confluent

for harvest.

Greater than 95% of the cultured cells were BCs, positive for BCs marker (keratin 5, KRT5) and negative for ciliated cell (β-tubulin IV), secretory cell (mucin 5AC), neuroendocrine cell (chromogranin A) and mesenchymal cell (N-cadherin) markers [1]. Watson et al. [3] has reported that the mouse airway BCs are comprised of multipotent basal/stem cells and committed precursors. To better characterize our cultured human airway BCs, we performed immunofluorescence co-staining of the widely expressed BCs markers (EGFR and ITGB4; Supplemental Figure 15 A-B), putative basal/stem cell markers (DLL1, SNAI2 and DLK2; Supplemental Figure 15 D-F) and basal luminal precursor marker (KRT8; Supplemental Figure 15C) with the typical BCs marker KRT5 on the cultured BC (passage 1 or 2) from healthy nonsmokers. We found that 92.4 ± 2.2% of our cultured BCs were $KRT5^+$. The % of $KRT5^+$ cells was a little bit lower than our previous report (>95%) [1], which may be due to the sensitivities of different staining methods. Moreover, 93.7 ± 1.2% and 92.0 ± 1.8% of the culture BCs were EGFR and ITGB4 positive, which is consistent with that EGFR and ITGB4 are widely expressed in the airway BCs. The cultured BCs are also heterogeneous. 27.5 ± 10.4%, 33.0 ± 14.4% and 86.8 ± 4.7% of the culture BCs were positive for putative basal/stem cell markers DLK2, SNAI2 and basal luminal precursor marker KRT8, respectively, while no cells were positive for DLL1 (Supplemental Figure 15C-G). Based on the expression of BCs markers, we speculate that, our cultured nonsmoker BCs contain both putative basal/stem cell and basal luminal precursor populations. Unlike the approximately equal numbers of putative basal/stem cells and basal luminal precursors in the mouse airway BCs populations, the cultured human airway BCs have decreased proportion of putative basal/stem cells and increased proportion of basal luminal precursors. Lacking expression of DLL1 in human airway BCs may reflect the difference between human and mouse airway BCs.

For *in vitro* studies, BCs derived from human airway epithelium (passage 0) were washed with phosphate buffered saline (PBS; Gibco) and disaggregated with 0.05% trypsin/EDTA at 37°C, 5 min. The trypsinization was stopped by 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffered saline (Lonza, Basel, Switzerland) supplemented with 15% fetal bovine serum (FBS; Gibco). The passage 0 BCs were pelleted by 250 × g centrifugation, washed with PBS and seeded into T75 flasks at a density of 3,000 cells/cm^2. The passaged BCs (passage 1) reached 80% confluent after 5 to 6 days in culture and were used for the *in vitro* experiments.

To compare the BCs proliferation between different phenotypes, the BCs (passage 1) from healthy nonsmokers, asymptomatic smokers and COPD smokers were seeded into the 24-well plate (5×10^3 cells/well; Corning). These BCs were trypsinized and the cell numbers were counted by the hemocytometer at day 2, 4 and 6. Doubling times of the cultured BCs from non-smokers, asymptomatic and COPD smokers were 30.9 ± 0.9, 35.7 ± 3.5 and 37.2 ± 6.0 hr, receptively (Supplemental Figure 16) [2]. Though no difference of the BCs proliferation rate between nonsmokers, asymptomatic and COPD smokers, there was an increased variation from nonsmokers to COPD smokers, suggesting the heterogeneity in the COPD BCs populations. (Supplemental Figure 16)

All the BCs were cultured in the bronchial epithelial cell growth medium (BEGM; Lonza), the antibiotics from the manufacturer were replaced with penicillin-streptomycin (50 μg/ml; Gibco), amphotericin B (1.25 μg/ml; Gibco) and gentamicin (50 μg/ml; Sigma-Aldrich, St. Louis, MO).

RNA Sequencing

The airway epithelium from bronchoscopic brushing and the BCs purified from the airway epithelium were homogenized in Trizol reagent (Life Technologies, Rockville, MD) and total RNA was extracted using RNeasy MinElute Cleanup Kit (Qiagen, Valencia, CA). Agilent

Technologies 2100 Bioanalyzer (Santa Clara, CA), and NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) were used to assess the RNA integrity and concentration. RNA-sequencing was carried out on the Illumina HiSeq 2500 in the New York Genome Center. The library was prepared using (0.5 μg total RNA) TruSeq RNA Library Prep Kit v2. Illumina HiSeq paired-end reads were aligned to GRCh37/hg19 human reference genome and RefSeq gene definitions (2014-06-02) using STAR (2.3.1z13_r470). Cufflinks (2.2) was used to convert aligned reads into fragments per kilobase of exon per million fragments sequenced (FPKM) using RefSeq gene definitions. The data are available in NCBI Gene Expression Omnibus (GEO accession number: GSE101353, under superSeries GSE128709).

Microarray of Human Small Airway Epithelium (SAE)

Research subjects were evaluated at the Weill Cornell Medical College Clinical Translational and Science Center and the Department of Genetic Medicine Clinical Research Facility under IRB-approved protocols. All subjects were recruited from the general population in New York City by posting advertisements in local newspapers and on electronic bulletin boards and all individuals provided written consent prior to enrollment. After enrollment all subjects underwent a detailed screening visit and assessment of medical history, physical exam, complete blood count, coagulation studies, liver function tests, urine analysis, chest X-ray, high resolution chest CT scan, EKG, and pulmonary function tests. The human SAE (10^{th}-12^{th} generation bronchi) were collected by bronchoscopic brushing from healthy nonsmokers, COPD smokers and COPD smokers who were willing to quit smoking. All subjects that met the inclusion criteria participated in a protocol that involved 4 bronchoscopies at baseline, 3, 6 and 12 months. All COPD smokers were encouraged to quit smoking immediately following the baseline bronchoscopy with the aid of varenicline (Chantix, targeting alpha-4 beta-2 nicotinic receptor in the brain) to help them stop smoking and counseling. Current COPD smokers were defined as self-reported

current smokers with verification of current smoking status by urinary levels of nicotine and its derivative cotinine. After each bronchoscopy total RNA was extracted using the TRIzol method and subsequent clean-up was performed on RNeasy columns (Qiagen). RNA quantity was assessed by Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE) and RNA quality assessed by Bioanalyzer (Agilent Technologies, Santa Clara, CA) [4]. Total RNA was prepared for microarray transcriptome analysis using the 3'IVT Express Kit (Affymetrix, Santa Clara, CA), and assessed using Affymetrix HG U133 Plus 2.0 microarray Gene Chips (Affymetrix), as previously described [5]. The data are available in NCBI Gene Expression Omnibus (GEO accession number: GSE128708, under superSeries GSE128709).

Air-liquid Interface (ALI) Culture

Passage 1 nonsmoker BCs were harvested by trypsin digestion and seeded into 0.4 μm pore sized transwell inserts (Corning) pre-coated with Type IV collagen (Sigma-Aldrich) at a density 6×10^5 cells/cm^2. The cells were cultured in a 1:1 mixture of Dulbecco's modification of Eagle's medium (DMEM; Cellgro, Manassas, VA) and F-12 medium (Gibco) plus 5% fetal bovine serum (FBS; HyClone, Logan, UT). The next day, the medium was changed to ALI differentiation medium with 1:1 mixture of DMEM and F-12 medium plus 2% Ultroser G serum substitute (USG; Bioserpa, Cergy-Saint-Christophe, France). After 2 days in culture, the BCs in transwell inserts were 100% confluent. The apical surface of the BCs was exposed to air to establish the ALI (day 0) by removal of the upper chamber medium. The cells were cultured in 37°C, 8% CO_2 to day 5, then moved to 37°C, 5% CO_2 until harvested. The ALI medium on the basolateral side was changed every 2 days.

Effects of BMP4 of Human Airway BCs

To determine to the effects of BMP4 on the BCs proliferation, the BCs (passage 1 or 2)

from healthy nonsmokers were seeded into the 12-well plate (1.2×10^4 cells/well) with or without recombinant human BMP4 (10 ng/ml; R&D Systems, Minneapolis, MN) stimulation. The BCs were trypsinized and the cell numbers were counted by Countess™ Automated Cell Counter (Invitrogen, Carlsbad, CA) at days 2 and 4.

The effects of BMP4 on BCs transcriptome were assessed by adding BMP4 (10 ng/ml) to the BEGM medium of passage 1 BCs *vs* control with the culture continued for 48 hr. The effects of BMP4 on airway BCs differentiation were assessed by adding BMP4 (2 or 10 ng/ml) to the basolateral side of airway BCs from day 0 of ALI culture until harvested. BMP4 was added every other day when changing the ALI medium on the basolateral side. HCl (4 μM), the solvent of BMP4, has no effect on BC proliferation, transcriptome or differentiation. For some experiments, Epidermal growth factor (EGF, 10 ng/ml; Sigma-Aldrich) was added to the basolateral side of airway BCs from day 0 of ALI culture until harvested. To study the receptors that mediate BMP4-induced BCs remodeling, Type I BMP receptor inhibitor DMH1 (5 μM; R&D Systems) was applied to the basolateral side of ALI culture with or without BMP4/EGF (10 ng/ml) stimulation. Dimethyl sulfoxide (DMSO; Sigma-Aldrich), the solvent for DMH1, was used as the negative control.

We used siRNAs in both primary BCs culture and ALI differentiation models to suppress the expression of BMP receptors or BMP4 downstream signaling. In the BCs culture, we used 5 siRNAs (Qiagen), including BMP receptor - BMPR1A, BMPR2 and ACVR1 siRNAs, BMP4 downstream signaling - SMAD4 siRNA and the negative control siRNA. We first seeded nonsmoker BCs into the 24-well plate (4×10^4 alive cells/well) in 1 ml BEGM. After that, we mixed each siRNA (final concentration 5 nM) with 3 μl HiPerFect transfection reagent (Qiagen) in 100 μl bronchial epithelial cell growth basal medium (BEBM; Lonza). The siRNA were incubated for 5 to 10 min at room temperature to form the siRNA-transfection complex. Lastly, we

added the siRNA-transfection complex into the cultured BCs in 24-well plate and incubated them for 48 hours until harvest. BMP4 (10 ng/ml) was added into the medium when seeding the cells into 24-well plate, right before we applied the siRNAs. The transfection efficiency of siRNAs was assessed by the Taqman analysis. As shown in Supplemental Figure 11 A-C and Supplemental Figure 12, the negative control siRNA has no significant effect on the expression of all the genes. When compared to the negative control siRNA, the BMPR1A, BMPR2, ACVR1 and SMAD4 siRNAs decreased their own expression by 62/72%, 74/84%, 63/75% and 60/71% (no BMP4/BMP4 treatment), respectively.

We selected BMPR1A siRNA (ThermoFisher Scientific, San Jose, CA) and related negative control siRNA (ThermoFisher Scientific) in the ALI differentiation culture. For each transwell insert in 24-well plate, we first diluted the lipofectamine™ RNAiMAX transfection reagent (1.5 µl; ThermoFisher Scientific) and the siRNA (final concentration – 25 nM) in 25 µl Opti-MEM medium (ThermoFisher Scientific), separately. Then we mixed these two dilutions and incubated them for 5 mins at room temperature to form the siRNA-lipid complex. Finally, we mixed the siRNA-lipid complex with 2×10^5 BCs in 150 µl DMEM and F12 medium + 5% FBS, and seeded them into transwell insert (ALI day-2). The next day (ALI day -1), the medium was changed as normal. At ALI day 0, medium from apical side was aspirated and basolateral side medium was changed with DMEM/F12 medium + 2%USG without or with BMP4 (10 ng/ml) stimulation. BMP4 was added 2 days later after applying siRNAs to the BCs in ALI culture. The samples were harvested for Taqman analysis at day 7 of ALI. The transfection efficiency of siRNAs was assessed by the Taqman analysis and as shown in Supplemental Figure 11D, the negative control siRNA has no significant effects on the expression of BMPR1A, while the BMPR1A siRNA decreases the BMPR1A expression by 87% in both no BMP4 or BMP4 treated groups (Supplemental Figure 11D).

Gene Expression Analysis by TaqMan

To assess mRNA expression, the cultured BCs and the airway epithelium in ALI were washed with PBS and homogenized in Trizol reagent (Life Technologies). Total RNA was purified by RNeasy MinElute Cleanup Kit (Qiagen) and double-stranded cDNA was synthesized using 1 μg total RNA in 50 μl volume reaction system by TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA). 2 μl of the 1:10 diluted cDNA in 25 μl volume PCR reaction was performed on the 7500 Sequence Detection System (Applied Biosystems). The 18S ribosomal RNA (Life Technologies) was used as the endogenous control and relative gene expression was assessed by the ΔΔCt method [6, 7].

Western Analysis

Activation of BMP4 downstream signaling in BCs was assessed by Western blot analysis. BMP4 (10 ng/ml) was applied to the basolateral side of BCs at day 0 of ALI culture for various time points (30 min, 1, 5 and 24 hr). The BMP4 solvent HCl (4 μM) and EGF (10 ng/ml; EGF has no effects on BMP4 downstream signaling) were used as negative controls for each time point. The BCs were lysed in radioimmunoprecipitation lysis (RIPA) buffer (Sigma-Aldrich) containing protease/phosphatase inhibitor cocktail (1:100 dilution; Cell Signaling Technology, Beverly, MA) and the concentration of the protein was quantified by BCA protein assay (ThermoFisher Scientific). After boiling for 10 min, 10 μg protein of each sample with 4X NuPAGE LDS sample buffer (Invitrogen) and 50 mM dithiothreitol (DTT; Sigma-Aldrich) was loaded into the NuPAGE 4~12% Bis–Tris gradient gels (Invitrogen). The protein was then transferred to the 0.2 μm pore size polyvinylidene difluoride (PVDF) membranes (Invitrogen). The membranes were blocked by 4% bovine serum albumin (BSA; Jackson ImmnoResearch, West Grove, PA) for 1 hr and incubated with the phospho-Smad1 (Ser463/465)/Smad5 (Ser463/465)/Smad8 (Ser465/467) (D5B10) rabbit monoclonal antibody (1:1,000 dilution; Cell

Signaling Technology) overnight at 4°C. The next day, the membranes were washed and incubated with anti-rabbit antibody conjugated to horseradish peroxidase (1:5,000 dilution, GE Healthcare Biosciences, Piscataway, NJ) for 1 hr at room temperature and then exposed to X-ray film by adding ECL Western blot analysis detection reagents (GE Healthcare Biosciences). After washing with restore Western stripping buffer (ThermoFisher Scientific), the same membranes were used again for the control protein Smad1 (D59D7) rabbit monoclonal antibody incubation (1:1,000 dilution; Cell Signaling Technology).

Airway Epithelial Morphology, Immunohistochemistry and Immunofluorescence Staining

To determine the localization and effect of smoking on BMP4 protein expression in the airway epithelium, biopsies of airway epithelium from nonsmokers, asymptomatic smokers and COPD smokers obtained from bronchoscopy were assessed by immunohistochemistry and immunofluorescence staining using previously described protocols [6, 7]. In brief, the paraffin-embedded biopsy sections were deparaffinized and rehydrated by a series wash of xylene and graded ethanol. To enhance protein immune-reactivity, the samples were steamed in the citrate buffer solution (ThermoFisher Scientific) for 20 min, followed by cooling at room temperature for 20 min. For immunohistochemistry staining, the endogenous peroxidase in the tissue was quenched with 0.3% H_2O_2 (Sigma-Aldrich) incubation for 30 min and followed by blocking with goat serum for 20 min using Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). The samples were incubated with the rabbit anti-human BMP4 primary antibody (1.1 μg/ml; Abcam, Cambridge, MA) at 4°C overnight. The next day, a biotinylated secondary antibody (anti-rabbit IgG; Vectastain Elite ABC kit), ABC Reagent (Vectastain Elite ABC kit) and AEC substrate kit (Dako, Carpinteria, CA) were sequentially applied to the samples, 30 min for each. The samples were stained with Mayer's hematoxylin (Polysciences, Warrington, PA) and mounted with Faramount mounting medium (Dako). Images were captured by Nikon Microphot-

SA microscope (Nikon, Melville, NY) with an Olympus DP70 camera (Olympus, Center Valley, PA). For immunofluorescence staining, after cooling in the citrate buffer, the samples were permeabilized with 0.1% triton X-100 (Sigma-Aldrich) for 10 min and then blocked with 10% goat serum (Sigma-Aldrich) for 45 min. The following primary antibodies were applied: rabbit anti human BMP4 (8.8 μg/ml; Abcam), mouse anti human β-tubulin IV antibody (Biogenex, San Ramon, CA) and mouse anti human keratin 6 (KRT6, 2 μg/ml; LifeSpan Biosciences, Seattle, WA). After incubation with the primary antibodies overnight at 4ºC, Alexa Fluor 488 goat anti mouse IgG (Molecular Probes, Carlsbad, CA) and Alexa Fluor 546 goat anti rabbit IgG (Molecular Probes) labeled secondary antibodies were used to visualize the antibody binding. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Molecular Probes). Images were captured by Zeiss Axiovert 200M microscope (Zeiss, Jena, Germany) and analyzed by AxioVision Rel 4.8 software (Zeiss).

The cultured BCs and airway epithelium derived from BCs in ALI were pre-fixed by 4% paraformaldehyde and assessed by immunofluorescence staining as described above. The following primary antibodies were used: rabbit anti-human dynein axonemal intermediate chain 1 (DNAI1, 4 μg/ml; Sigma-Aldrich), rat antihuman secretoglobin family 1A member 1 (SCGB1A1, 5 μg/ml; R&D systems), mouse anti-human keratin 14 (KRT14, 5 μg/ml; R&D Systems), rabbit anti-human KRT14 (2 μg/ml; Sigma-Aldrich), mouse anti-human involucrin (IVL, 2 μg/ml; ThermoFisher Scientific), mouse anti-human keratin 5 (0.08 μg/ml; ThermoFisher Scientific), rabbit anti-human EGFR (5 μg/ml; Abcam), rabbit anti-human integrin β4 (ITGB4, 4 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-human keratin 8 (KRT8, 0.7 μg/ml; Abcam), mouse anti-human delta like canonical notch ligand 1 (DLL1, 10 μg/ml; R&D Systems), rabbit anti-human delta like non-canonical notch ligand 2 (DLK2, 4 μg/ml;

ThermoFisher Scientific) and rabbit anti-human snail family transcriptional repressor 2 (SNAI2, 1:50 dilution, Cell Signaling Technology)

Immunohistochemistry staining of BMP4 in the biopsies of asymptomatic (n=6) and COPD smokers (n=6) were analyzed to evaluate the BMP4 protein distribution among different cell types in human airway epithelium. A total of 444 airway epithelial cells were counted. To quantify the ciliated cell and squamous cell differentiation in the ALI cultures, the IVL, KRT14 and DNAI1 positive cells were counted based on the immunofluorescence staining of the cyto-spins from the airway epithelium in ALI after 14 or 28 days' culture. A minimum of 118 cells were counted for each sample. To quantify the cultured BCs, the BCs marker positive cells were counted based on the immunofluorescence staining on the cytopsins of BCs, a minimum of 360 cells were counted for each BCs marker.

Cigarette Smoking Extract (CSE)

The preparation of CSE was described in our previous papers [8]. Briefly, the CSE stock was made by bubbling one Marlboro Red commercial cigarette through 12.5 ml DMEM/F12+2% Ultroser G (USG) differentiation medium, filtered through the 0.22 μm pore size filter (Millipore, Burlington, MA) and frozen in aliquots in -20°C. The absorbance of the CSE stock was measured at 320 nm in the DU-640 Spectrophotometer (Beckman, Fullerton, CA) and the optical density of 1 was defined as 100%. The 3% CSE was diluted with differentiation medium from the CSE stock based on the reading of the optical density.

Statistics

ANOVAs were applied to test for significant differences between measured gene expression levels and cell counts for all comparisons among groups in the *in vivo* and *in vitro* experiments, where measured expression levels and cell counts were checked for outliers and major deviations from normality before analysis. For each expression and cell count variable, ANOVA

models were fit simultaneously considering all groups within the experiment, where these were either single factor ANOVAs with two or more groups or ANOVAs that additionally included a repeated measure (ordered) factor to account for repeated observations over time. For all tests between pairs of groups for all ANOVA models with more than two groups, Tukey contrasts were used to test for differences.

Supplemental References

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Supplemental Table I. Expression of BMPs and BMP Antagonists in Human Airway Epithelium and Basal Cells

	Airway epithelium[1]						Basal cells[2]						Airway epithelium *vs* basal cells		
	Expression (FPKM)[3]			p values[4]			Expression (FPKM)[3]			p values[4]			p values[4]		
Genes	NS	S	COPD-S	NS *vs* S	NS *vs* COPD-S	S *vs* COPD-S	NS	S	COPD-S	NS *vs* S	NS *vs* COPD-S	S *vs* COPD-S	NS	S	COPD-S
BMP2	0.2±0.1	0.3±0.1	0.3±0.2	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	5.5±5.2	4.1±2.6	4.9±5.1	$9.3x10^{-1}$	$1.0x10^{0}$	$1.0x10^{0}$	$5.2x10^{-3}$	$2.2x10^{-1}$	$6.2x10^{-2}$
BMP4	1.0±0.7	3.5±1.1	2.8±1.4	$7.2x10^{-5}$	$1.0x10^{-2}$	$7.6x10^{-1}$	0.8±0.5	1.2±1.4	1.5±1.5	$9.6x10^{-1}$	$5.5x10^{-1}$	$9.9x10^{-1}$	$1.0x10^{0}$	$3.5x10^{-4}$	$7.6x10^{-2}$
BMP5	0.1±0.2	0.1±0.1	0.1±0.1	$8.2x10^{-1}$	$1.0x10^{0}$	$9.5x10^{-1}$	0.0±0.0	0.0±0.0	0.1±0.0	$1.0x10^{0}$	$6.6x10^{-1}$	$9.6x10^{-1}$	$3.1x10^{-2}$	$9.0x10^{-1}$	$8.3x10^{-1}$
BMP6	0.0±0.1	0.1±0.2	0.1±0.1	$6.8x10^{-1}$	$1.0x10^{0}$	$8.8x10^{-1}$	0.1±0.1	0.2±0.1	0.1±0.1	$8.0x10^{-1}$	$1.0x10^{0}$	$8.8x10^{-1}$	$8.4x10^{-1}$	$9.8x10^{-1}$	$9.7x10^{-1}$
BMP7	0.9±0.3	1.6±0.3	2.0±0.9	$4.9x10^{-2}$	$7.0x10^{-4}$	$6.8x10^{-1}$	0.8±0.5	0.6±0.3	0.7±0.6	$9.7x10^{-1}$	$1.0x10^{0}$	$1.0x10^{0}$	$9.8x10^{-1}$	$1.6x10^{-3}$	$1.2x10^{-5}$
NOG	0.0±0.0	0.0±0.0	0.0±0.0	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	0.1±0.1	0.1±0.1	0.1±0.1	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	$4.7x10^{-6}$	$5.0x10^{-5}$	$6.7x10^{-5}$
FST	0.3±0.2	0.4±0.3	0.4±0.2	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	41.4±22.6	42.3±32.6	6.0±46.0	$1.0x10^{0}$	$3.5x10^{-1}$	$6.2x10^{-1}$	$1.6x10^{-3}$	$9.8x10^{-3}$	$2.1x10^{-5}$
FSTL3	0.5±0.2	0.5±0.2	0.5±0.2	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	39.2±11.0	38.6±7.7	35.6±12.0	$1.0x10^{0}$	$8.3x10^{-1}$	$9.6x10^{-1}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$
CHRD	0.0±0.0	0.0±0.0	0.0±0.0	$7.4x10^{-1}$	$1.0x10^{0}$	$8.7x10^{-1}$	0.0±0.0	0.0±0.0	0.0±0.0	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	$9.2x10^{-1}$	$2.7x10^{-1}$	$7.5x10^{-1}$
CHRDL2	0.0±0.0	0.0±0.0	0.0±0.0	$9.8x10^{-1}$	$1.0x10^{0}$	$1.0x10^{0}$	0.0±0.0	0.0±0.0	0.0±0.0	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	$1.5x10^{-1}$	$7.9x10^{-2}$	$1.6x10^{-1}$
TWSG1	16.0±2.8	14.8±5.4	16.0±2.9	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	42.3±7.3	37.1±7.9	42.8±9.1	$4.0x10^{-1}$	$1.0x10^{0}$	$3.8x10^{-1}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$

[1] Airway epithelium: nonsmokers n=10, smokers n=10, COPD smokers n=9

[2] Airway basal cells: non-smokers n=20, smokers n=9, COPD smokers n=13

[3] Average ± standard deviation (SD); FPKM - fragments per kilobase of transcript per million mapped reads

[4] Tukey contrast for variable pairs within full ANOVA; NS - nonsmokers; S - smokers; COPD-S – COPD smokers

Supplemental Table II. Expression of BMP4 Receptors in Human Airway Epithelium and Basal Cells

BMP-related receptor	Airway epithelium[1]						Basal cells[2]						Airway epithelium vs basal cells		
	Expression (FPKM)[3]			p values[4]			Expression (FPKM)[3]			p values[4]			p values[4]		
	NS	S	COPD-S	NS *vs* S	NS *vs* COPD-S	S *vs* COPD-S	NS	S	COPD-S	NS *vs* S	NS *vs* COPD-S	S *vs* COPD-S	NS	S	COPD-S
BMPR1A	9.3±1.2	7.4±1.6	7.1±1.6	$2.3x10^{-2}$	$8.1x10^{-3}$	$1.0x10^{0}$	11.0±1.4	10.3±1.1	11.2±1.0	$8.3x10^{-1}$	$1.0x10^{0}$	$7.1x10^{-1}$	$2.0x10^{-2}$	$1.1x10^{-4}$	$<1.0x10^{-7}$
BMPR1B	11.1±1.4	18.3±4.0	17.3±2.1	$<1.0x10^{-7}$	$6.0x10^{-7}$	$9.3x10^{-1}$	4.2±1.8	4.9±1.7	4.4±1.5	$9.6x10^{-1}$	$1.0x10^{0}$	$1.0x10^{0}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$
BMPR2	6.5±0.7	6.1±0.6	6.4±1.0	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	13.0±2.0	13.0±1.6	13.0±2.4	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$
ACVR1	9.0±1.1	9.3±0.9	9.5±1.5	$1.0x10^{0}$	$1.0x10^{0}$	$1.0x10^{0}$	15.7±4.1	17.5±3.7	16.5±5.4	$8.3x10^{-1}$	$1.0x10^{0}$	$9.9x10^{-1}$	$1.0x10^{-4}$	$6.4x10^{-5}$	$4.2x10^{-4}$
ACVR2A	5.1±0.7	5.1±1.1	5.5±0.7	$1.0x10^{0}$	$7.7x10^{-1}$	$7.2x10^{-1}$	2.8±0.5	2.9±0.5	3.0±0.6	$1.0x10^{0}$	$9.7x10^{-1}$	$1.0x10^{0}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$	$<1.0x10^{-7}$
ACVR2B	2.1±0.2	1.8±0.2	1.9±0.3	$2.4x10^{-2}$	$2.8x10^{-1}$	$9.2x10^{-1}$	1.0±0.2	1.2±0.3	1.0±0.2	$6.1x10^{-1}$	$1.0x10^{0}$	$5.0x10^{-1}$	$<1.0x10^{-7}$	$2.0x10^{-7}$	$<1.0x10^{-7}$

[1] Airway epithelium: nonsmokers n=10, smokers n=10, COPD smokers n=9

[2] Airway basal cells: nonsmokers n=20, smokers n=9, COPD smokers n=13

[3] Average ± standard deviation (SD); FPKM - fragments per kilobase of transcript per million mapped reads

[4] Tukey contrast for variable pairs within full ANOVA; NS - nonsmokers; S - smokers; COPD-S – COPD smokers

Supplemental Table III. Study Populations Source of Biologic Samples[1]

	Source of airway epitelium[2]			Source of basal cells[3]		
Parameter	**Healthy nonsmokers**	**Smokers**	**COPD smokers**	**Healthy nonsmokers**	**Smokers**	**COPD smokers**
n	10	10	9	20	9	13
Gender (M/F)[4]	8/2	8/2	9/0	7/13	8/1	12/1
Ethnicity (B/W/O)[5]	5/1/4	7/2/1	7/0/2	8/4/8	5/1/3	5/3/5
Age	36 ± 11	43 ± 10	49 ± 4	35 ± 12	38 ± 11	60 ± 7
Smoking history						
Pack-yr	-	26 ± 11	31 ± 6	-	27 ± 20	35 ± 14
Packs per day	-	0.9 ± 0.4	1.2 ± 0.5	-	1.2 ± 0.7	0.8 ± 0.2
Urine cotinine (ng/ml)	-	1533 ± 1153	2145 ± 682	-	1879 ± 967	1124 ± 685
Lung functions[6]						
FEV1	103 ± 14	107 ± 14	91 ± 16	102 ± 13	104 ± 14	72 ± 20
FVC	110 ± 12	113 ± 13	110 ± 19	104 ± 12	109 ± 13	100 ± 22
FEV1/FVC	78 ± 5	77 ± 6	66 ± 3	82 ± 4	78 ± 4	58 ± 11
TLC	100 ± 15	95 ± 9	97 ± 12	98 ± 12	101 ± 14	103 ± 17
DLCO	94 ± 12	85 ± 5	71 ± 12	92 ± 12	88 ± 6	66 ± 17
GOLD stage (I/II/III)	-	-	7/2/0	-	-	5/7/1
Cells initially isolated from the brushes						
Total cells ($10x10^6$)	-	-	-	1.27 ± 0.70	0.84 ± 0.25	0.77 ± 0.44
Alive cells ($10x10^6$)	-	-	-	0.68 ± 0.25	0.48 ± 0.14	0.39 ± 0.14
Cell Viability (%)				57.78 ± 11.75	59.85 ± 11.40	56.37 ± 20.04

[1] All data are presented as mean ± standard deviation
[2] Samples of brushed airway epithelium [1]
[3] Samples of basal cells purified from brushed airway epithelium [1]
[4] Male/female
[5] Black/White/Other
[6] FEV1, FVC, TLC and DLCO are presented as % predicted, FEV1/FVC as % observed; levels are pre-bronchodilators in nonsmokers and healthy smokers, and post-bronchodilators in COPD smokers

Supplemental Figure Legends

Supplemental Figure 1. RNA-seq assessment of the expression of BMPs**,** BMP antagonists and BMP4 receptors in the total airway epithelium and BCs. **A.** BMPs. **B.** BMP antagonists. **C.** BMP4 receptors. The data are presented in dot plots and each dot represents an individual. The gene symbols are shown on top of each dot plot. *Blue dots* – healthy nonsmokers (airway epithelium n=10, BCs n=20), *yellow dots* – asymptomatic smokers (airway epithelium n=10, BCs n=9), *red dots* – COPD smokers (airway epithelium n=9, BCs n=13). The related p values are shown in the Supplemental Tables I and II.

Supplemental Figure 2. Expression of BMP4 in the human airway epithelium *in vivo* and *in vitro*. **A.** RNA-seq assessment of BMP4 expression in human airway epithelium and BCs of nonsmokers (*blue dots* - airway epithelium n=10, BCs n=20), asymptomatic smokers (*yellow dots* – airway epithelium n=10, BCs n=9) and COPD smokers (*red dots* – airway epithelium n=9, BCs n=13); p values are indicated in the figures, n.s. - not significant. **B.** TaqMan assessment of BMP4 mRNA expression in ALI culture at different time points (day 0, 3, 5, 7, 14 and 28; n=3).

Supplemental Figure 3. Microarray assessment of BMP4 expression in the human small airway epithelium. The total human small airway epithelium were harvested at different time points (month 0, 3, 6 and 12). *Blue dots* – healthy nonsmokers (n=21), *red dots* – COPD smokers (n=21), *yellow dots* – COPD smokers who quit smoking from month 0~12 (n=5), *pink dots* – COPD smokers who quit smoking from month 0~3, but re-started to smoking after month 3 (n=5). ** $p<0.01$, *** $p<0.001$.

Supplemental Figure 4. Immunohistochemistry staining of BMP4 protein expression in human airway epithelial biopsy samples from nonsmokers (n=4), asymptomatic smokers and COPD smokers with normal and abnormal morphology (n=4 for each). For the asymptomatic smokers

and COPD smokers, the *left column* are from areas of relatively normal morphology, while the *right column* are from areas of abnormal morphology. Scale bar – 20 μm.

Supplemental Figure 5. BMP4 suppresses BCs proliferation. 1.2×10^4 nonsmoker BCs (n=3) were seeded to each well of 12-well plate with or without BMP4 stimulation. The BCs expanded and the total BCs number were counted at day 2 and 4. *Blue dots* – untreated control, *red dots* – BMP4 stimulation. The cell numbers were normalized to the cell number of the control group at day 2. ** $p<0.01$.

Supplemental Figure 6. BMP4 suppresses normal ciliated and secretory cell differentiation. **A.** TaqMan expression assessment of ciliated cell related genes (*top left* - FOXJ1, *top right* - DNAI1) and secretory cell related genes (*bottom left* - SCGB1A1, *bottom right* - TFF3) in ALI cultured at different time points with or without BMP4 (2 and 10 ng/ml) treatment. *Blue dots* - control, *yellow dots* - BMP4 (2 ng/ml) and *red dots*- BMP4 (10 ng/ml) treatment. ** $p<0.01$, *** $p<0.001$, n.s. – not significant. **B.** Immunofluorescence staining of the ciliated cell marker DNAI1 (*red*) on the airway epithelium in cytospin slides after 28 days ALI culture with BMP4 (10 ng/ml) stimulation from the basolateral side (*right*) *vs* untreated control (*left*). Scale bar - 50 μm. Nuclei are stained with DAPI (*blue*). **C.** Quantification of $DNAI1^+$ cells from the immunofluorescence staining in panel **B.** *Blue column* - Control*, red column* - BMP4 stimulation, n=3, p value is shown in the figure. **D.** TaqMan assessment of fold-change (log2) in the expression of the ciliated cell (MCIDAS, DNAH5 and IFT172) and secretory cell (SPDEF and TFF3) early differentiation-related genes in BCs with BMP4 (10 ng/ml) treatment for 48 hr *vs* untreated control, n=3 or 4. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplemental Figure 7. BMP4-induced differentiation towards squamous metaplasia. **A.** TaqMan expression assessment of squamous cell related genes (*left* - IVL, *right* - KRT14) in ALI culture at different time points with or without BMP4 (2 and 10 ng/ml) treatment. *Blue dots* -

control, *yellow dots* - BMP4 (2 ng/ml) and *red dots* - BMP4 (10 ng/ml) treatment. * $p<0.05$, *** $p<0.001$, n.s. – not significant. **B.** Representative immunofluorescence staining of the squamous cell marker IVL (*top*) and KRT14 (*bottom*) on the airway epithelium in cytospins after 14 days ALI culture with BMP4 (10 ng/ml) stimulation from the basolateral side (*right*) *vs* untreated control (*left*). The IVL and KRT14 protein are shown in *green* in the figure. Scale bar - 50 μm, nuclei are stained with DAPI (blue). **C.** Quantification of IVL^+ cells from the immunofluorescence staining in panel **B** (*top*), n=3. **D.** Quantification of $KRT14^+$ cells from the immunofluorescence staining in panel **B** (*bottom*), n=3. In **C.-D.**, *Blue column* - Control*, red column* - BMP4 stimulation, p values are shown in the figure. **E.** TaqMan assessment of fold-change (log2) in the expression of the squamous cell related genes (IVL, KRT14, KRT6A, KRT6B and SFN) in BCs with BMP4 (10 ng/ml) treatment for 48 hr *vs* untreated control, n=3 or 4. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. - not significant.

Supplemental Figure 8. Basolateral-stimulation of BMP4 induces abnormal phenotypes in the fully differentiated airway epithelium. TaqMan assessment of fold-change (log2) in the expression of various markers on the airway epithelium from ALI culture (day 28~35) with or without BMP4 (10 ng/ml) stimulation from basolateral side. *Green columns* – ciliated cell markers: DNAI1, FOXJ1 and MCIDAS; *yellow columns* – secretory cell markers: SCGB1A1, SPDEF, MUC5B and TFF3; *red columns* – squamous cell markers: IVL, KRT14 and KRT6A, n=3 or 4. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. - not significant.

Supplemental Figure 9. Apical-stimulation of BMP4 has no effect on the fully differentiated airway epithelium. TaqMan assessment of fold-change (log2) in the expression of various markers on the airway epithelium from ALI culture (day 28~35) with or without BMP4 (10 ng/ml) stimulation from apical side. *Green columns* – ciliated cell markers: DNAI1 and FOXJ1; *yellow columns* – secretory cell markers: SCGB1A1, MUC5B and TFF3; *red columns* – squamous cell

markers: IVL and KRT14. ** $p<0.01$ for DNAI1 by untreated control *vs* apical BMP4 stimulation. No changes for the rest of the markers.

Supplemental Figure 10. BMPR1A receptor mediated BMP4-induced airway epithelial remodeling. TaqMan assessment of the squamous cell marker IVL (**A.**) and ciliated cell marker FOXJ1 (**B.**) in the airway epithelium from ALI culture (day 7). The BCs in ALI were treated with BMP4 (10 ng/ml) stimulation (from ALI day 0~7) while siRNA silencing of BMPR1A (from ALI day -2~7). Negative control siRNA were used as negative control. The data are normalized to untreated control. *** $p<0.001$.

Supplemental Figure 11. BMP4 receptors silencing by siRNA in BCs and airway epithelium in ALI. **A.-C.** Normalized BMP receptor (**A.** BMPR1A, **B.** BMPR2 and **C.** ACVR1) gene expression in BCs with BMP4 (10 ng/ml) stimulation *vs* untreated control for 48 hr with silencing of BMPR1A, BMPR2 and ACVR1 by siRNAs, n=3. **D.** Normalized BMPR1A expression of the airway epithelium in ALI culture (day 7) with BMP4 (10 ng/ml) stimulation *vs* untreated control while BMPR1A (yellow) silenced by siRNA. In **A.-D.**, data are normalized to the untreated control. *** $p<0.001$. % of the decreased expression of the receptors by their own siRNA are shown in the figure.

Supplemental Figure 12. Smad signaling suppression by siRNA in airway BCs. Normalized gene expression of Smad4 in BCs with BMP4 (10 ng/ml) stimulation *vs* untreated control for 48 hr while silencing of Smad4 by siRNA, n=4. Negative control siRNA was applied to both untreated control *vs* BMP4 (10 ng/ml) stimulation groups. Data are normalized to the untreated control. *** $p<0.001$. % of the decreased expression of the Smad4 by its own siRNA are shown in the figure.

Supplemental Figure 13. DMH1 reverses CSE-suppressed ciliated and secretory cell differentiation. **A.** TaqMan assessment of fold-change (log2) in the expression of ciliated cell markers

FOXJ1 (*left*) and DNAI1 (*right*) in the airway epithelium derived from BCs in ALI culture at day 14. **B.** TaqMan assessment of fold-change (log2) in the expression of secretory cell markers SCGB1A1 (*left*) and TFF3 (*right*) in the airway epithelium derived from BCs in ALI culture at day 14. The BCs were exposed to 3% CSE stimulation from the basolateral side *vs* untreated control in the absence or presence of BMP4 type I receptor inhibitor DMH1 (5 μM). DMSO was used as negative control, n=4. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

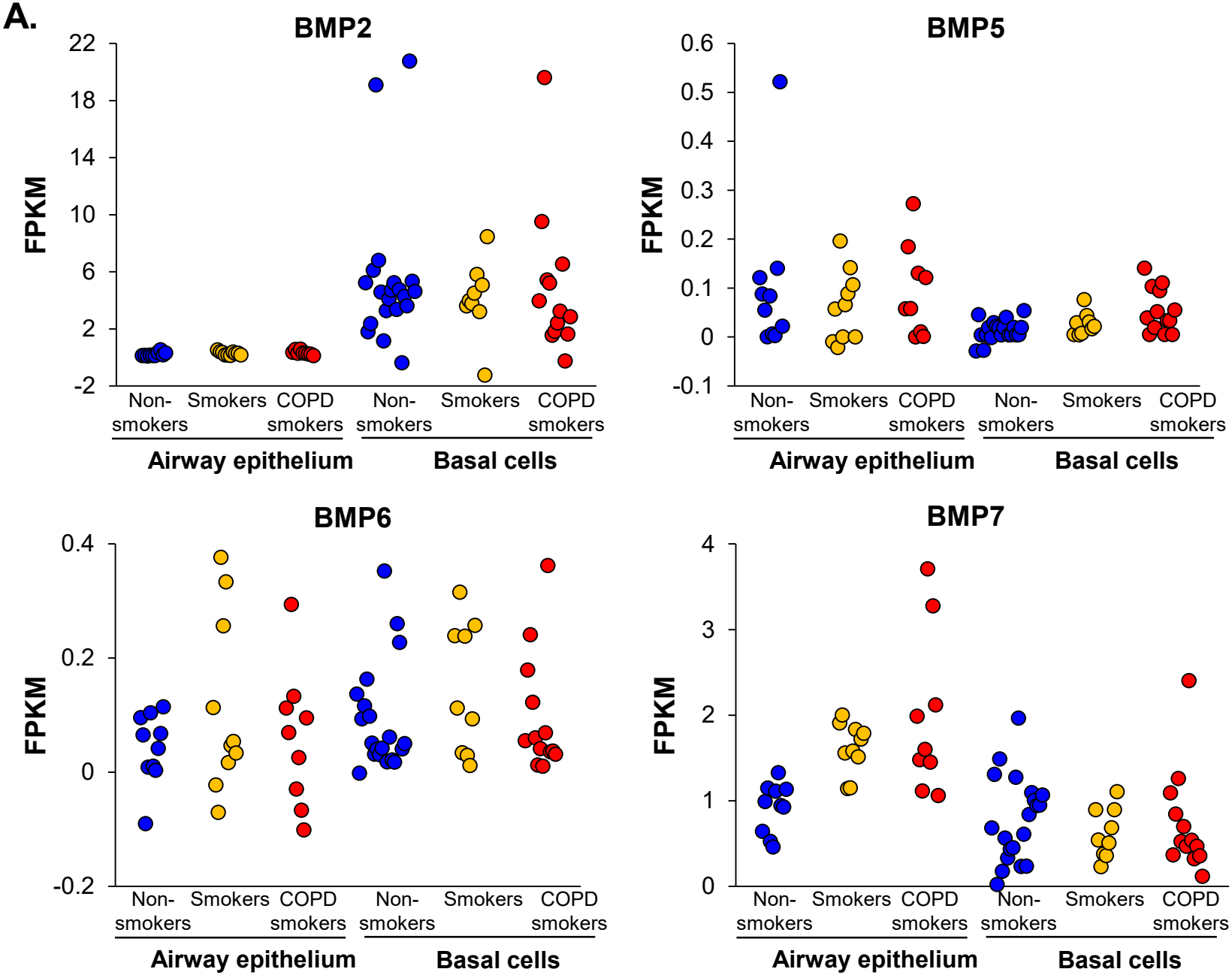
Supplemental Figure 14. DMH1 reverses EGF-induced abnormal phenotypes of the airway epithelium. **A.** TaqMan assessment of fold-change (log2) in the expression of various markers (squamous cell markers – IVL and KRT14; ciliated cell markers – FOXJ1 and DNAI1; secretory cell marker – SCGB1A1) on the airway epithelium in ALI culture at day 14. The BCs were exposed to EGF (10 ng/ml) stimulation from the basolateral side *vs* untreated control in the absence or presence of BMP4 type I receptor inhibitor DMH1 (5 μM). DMSO was used as negative control. *Red columns* – EGF treatment; *yellow columns* – EGF+DMSO; *blue columns* – EGF+DMH1. *** $p<0.001$. **B.** Normalized expression of amphiregulin (AREG) in the airway epithelium from ALI culture for 14 days with BMP4 (10 ng/ml) stimulation from the basolateral side (*red column*) *vs* untreated control (*blue column*), n=5. n.s. - not significant.

Supplemental Figure 15. Characterization of the cultured human BCs from healthy nonsmokers. **A.-F.** Immunofluorescence co-staining of the BCs marker KRT5 (*green*) with the other widely expressed BC markers (**A.)** EGFR and (**B.)** ITGB4, (**C.)** basal luminal precursor marker KRT8 and putative basal/stem cell markers (**D.)** DLK2, (**E.)** DLL1 and (**F.)** SNAI2. All other BCs markers, except KRT5, are shown in *red* color. *Left* – KRT5, *middle* – other BCs markers, *right* – overlapping of KRT5 with other BCs markers. Scale bar – 20 μm. **G.** Quantification of BCs marker positive cells in the cultured nonsmoker BCs. *Blue* – negative, *red* – positive. Markers and related % of positive cells are shown in the figure, n=3.

Supplemental Figure 16. Doubling time of the proliferating BCs from nonsmokers, asymptomatic smokers and COPD smokers. *Blue dots* – nonsmokers (n=3), *yellow dots* – asymptomatic smokers (n=4), *red dots* – COPD smokers (n=4). n.s. – not significant.

Supplemental Figure 17. Diagram of the mechanism for the smoking associated BMP4-induced abnormal phenotypes in human airway epithelium. In conjunction with decreased junctional barrier integrity, the smoking-induced BMP4 is secreted from the ciliated and intermediate cells, interacts and skews the BCs differentiation toward the smoking related phenotypes, including suppression of normal mucociliary epithelium and induction of squamous metaplasia.

A.


BMP2
FPKM
22
18
14
10
6
2
-2
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells
BMP5
FPKM
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells
BMP6
FPKM
0.4
0.2
0
-0.2
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells
BMP7
FPKM
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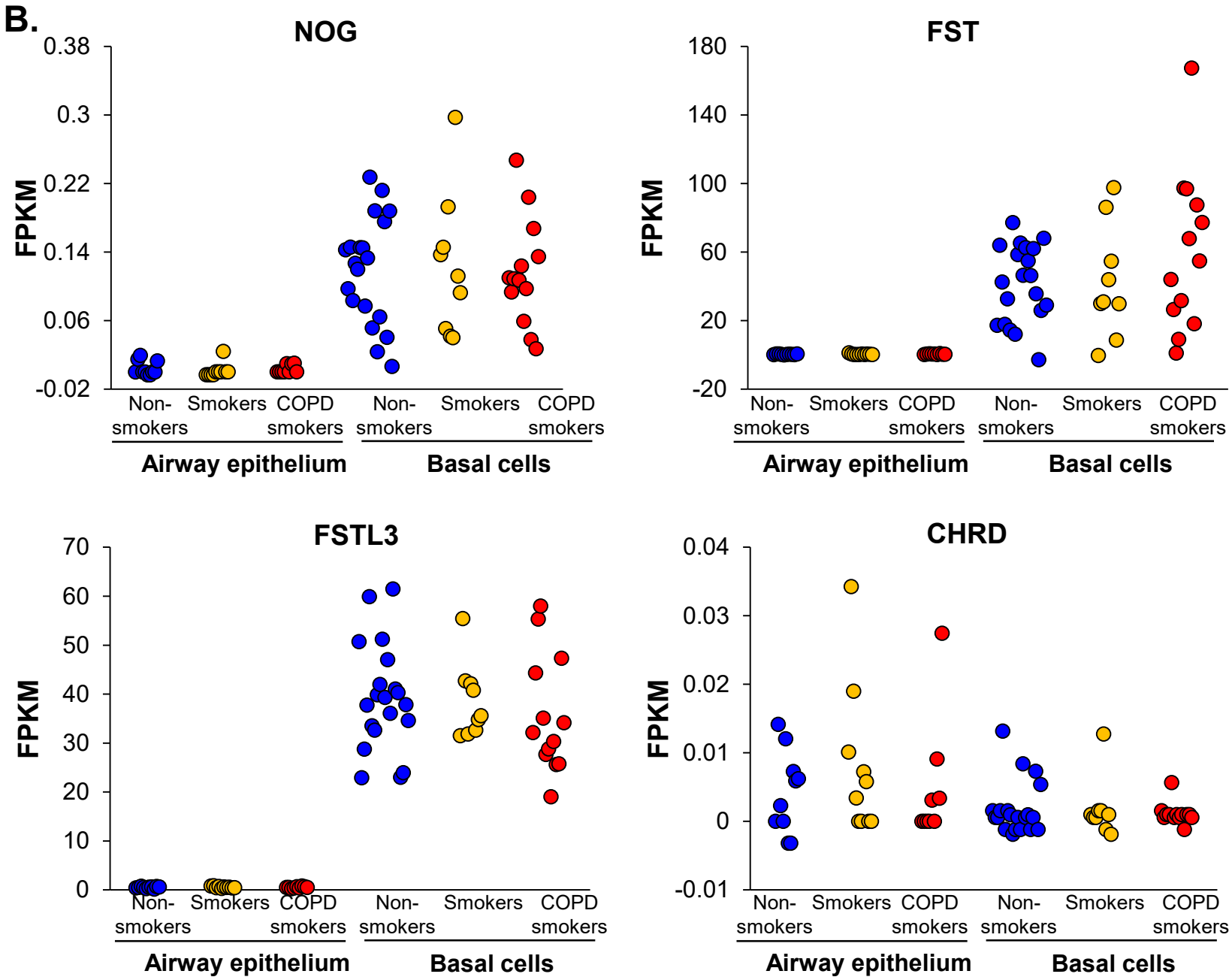
2
1
0
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells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NOG
FST
FSTL3
CHRD
FPKM
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0.22
0.14
0.06
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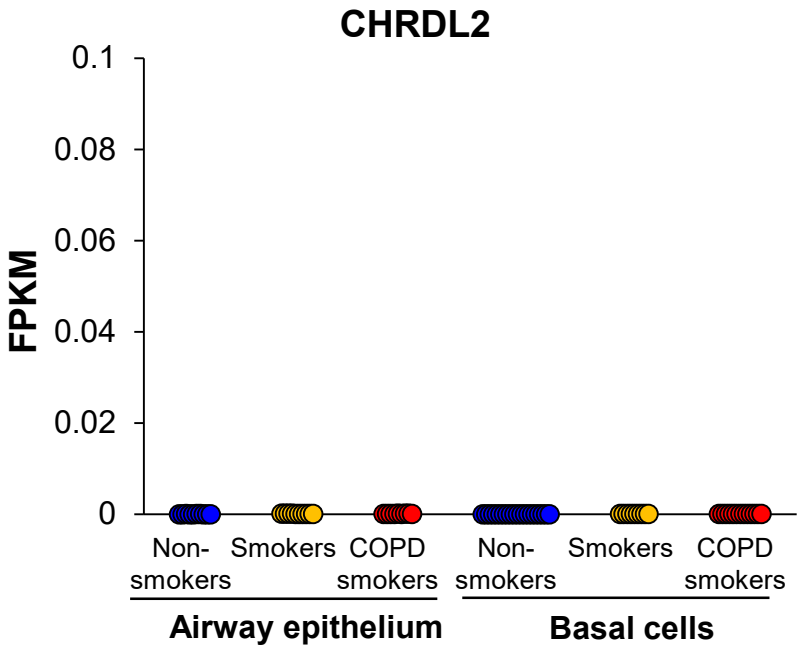
180
140
100
60
20
-20
70
60
50
40
30
20
10
0
0.04
0.03
0.02
0.01
0
-0.01
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells


CHRDL2
FPKM
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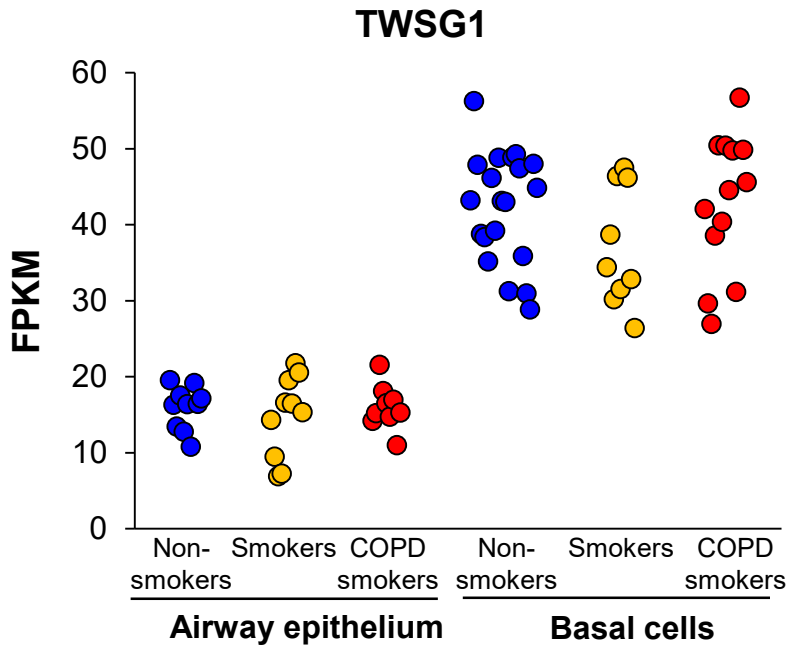
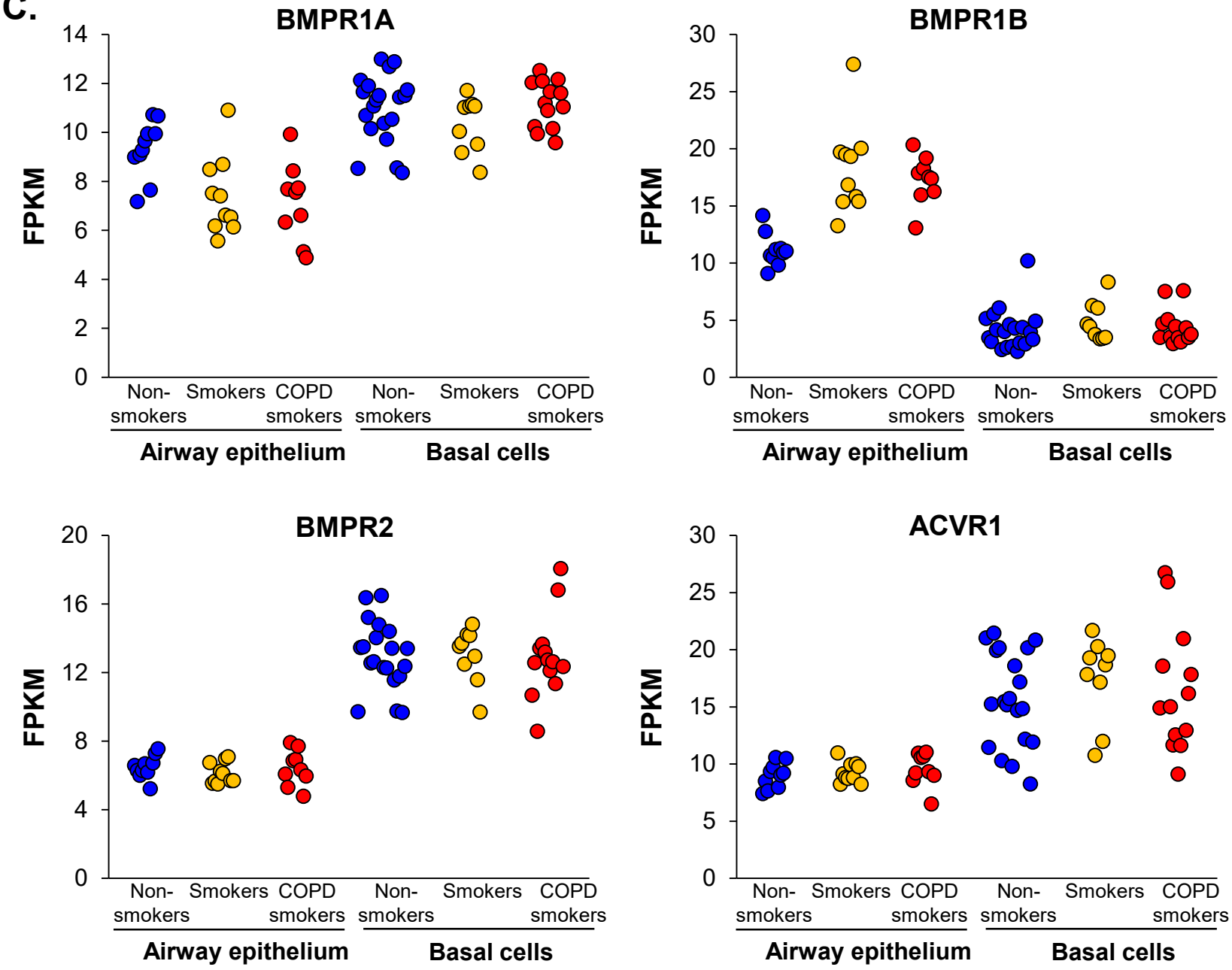
0.08
0.06
0.04
0.02
0
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells


TWSG1
FPKM
60
50
40
30
20
10
0
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells

C.


BMPR1A
FPKM
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10
12
14
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells
BMPR1B
FPKM
0
5
10
15
20
25
30
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells
BMPR2
FPKM
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12
16
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Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells
ACVR1
FPKM
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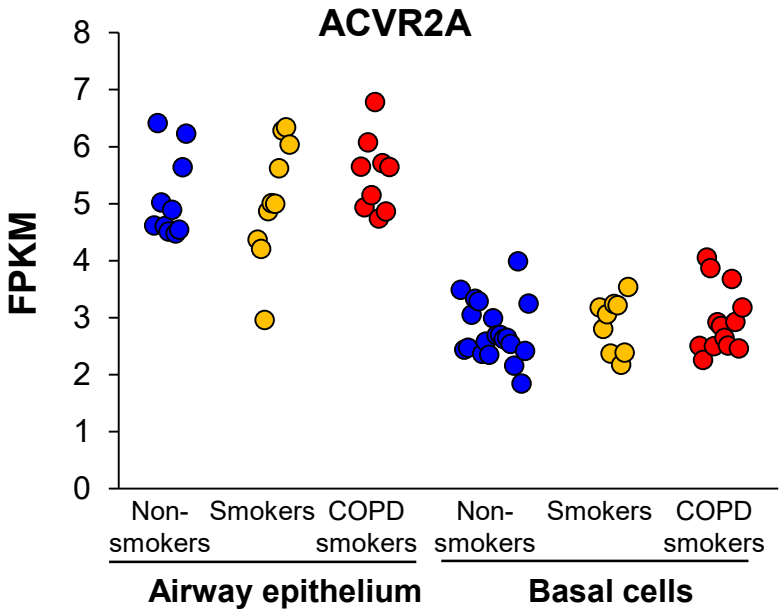
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells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FPKM
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5
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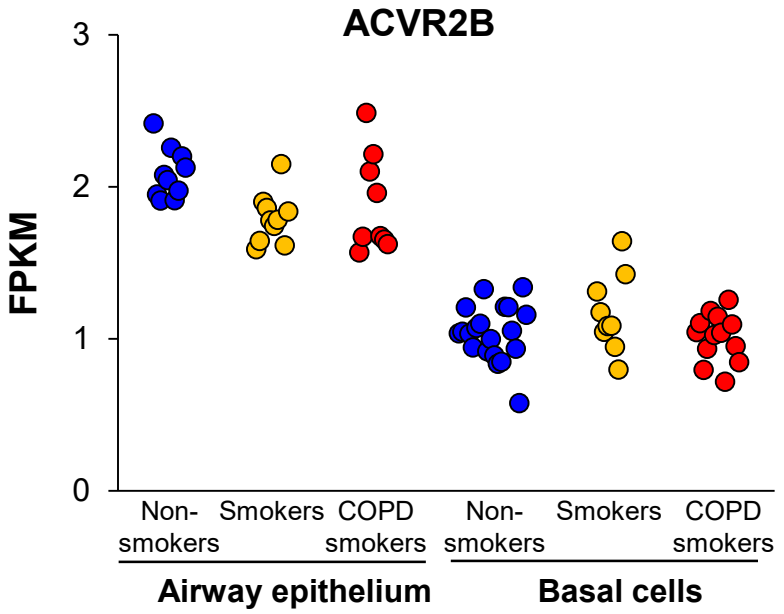
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells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FPKM
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2
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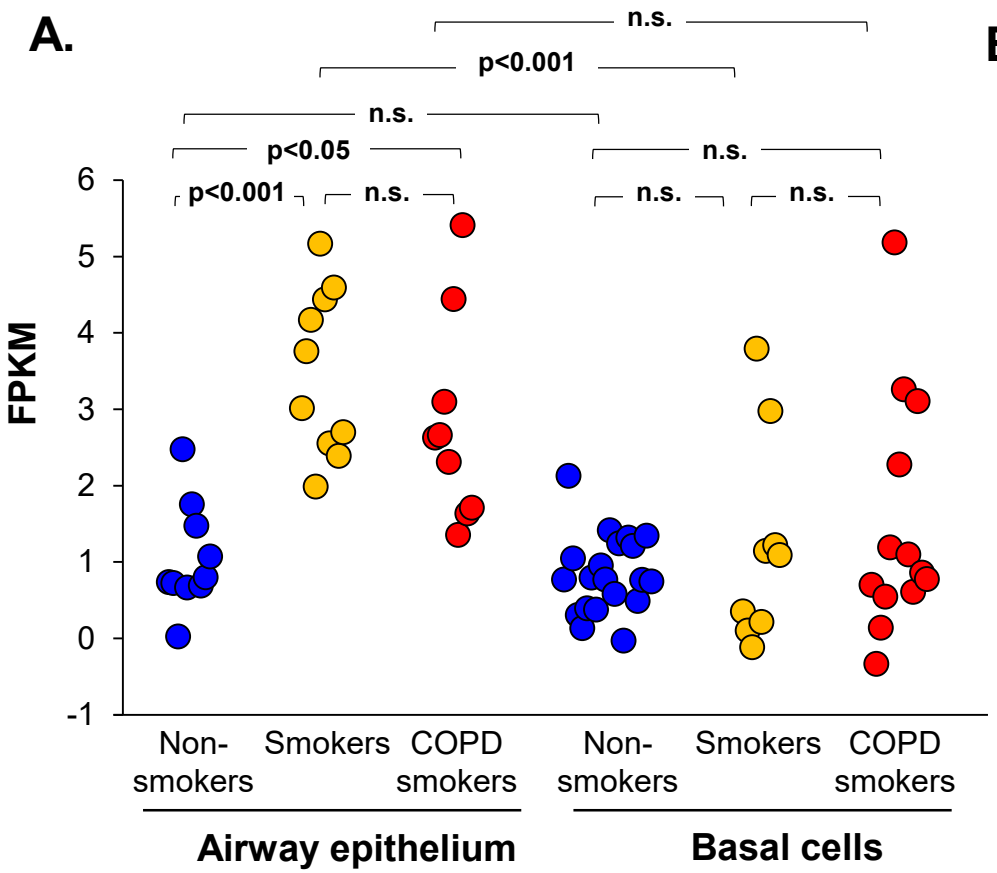
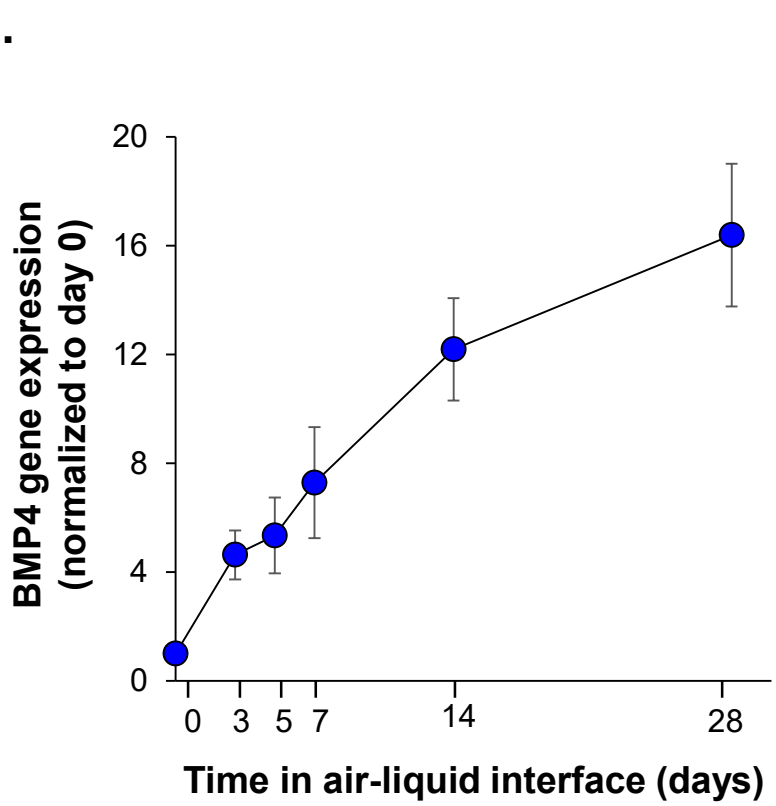
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells


A.
n.s.
p<0.001
n.s.
p<0.05
n.s.
p<0.001
n.s.
n.s.
n.s.
FPKM
6
5
4
3
2
1
0
-1
Non-smokers
Smokers
COPD smokers
Non-smokers
Smokers
COPD smokers
Airway epithelium
Basal cells


B.
BMP4 gene expression (normalized to day 0)
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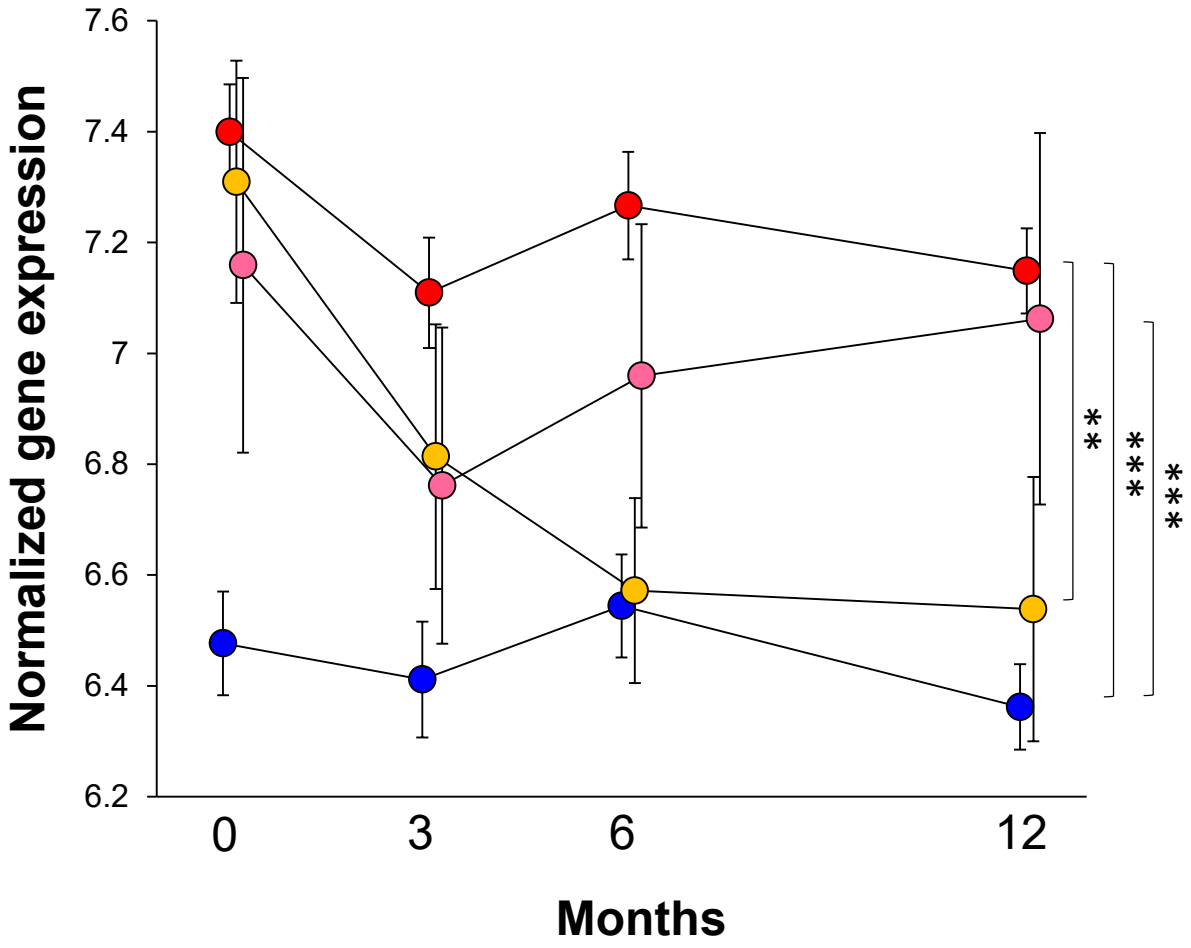
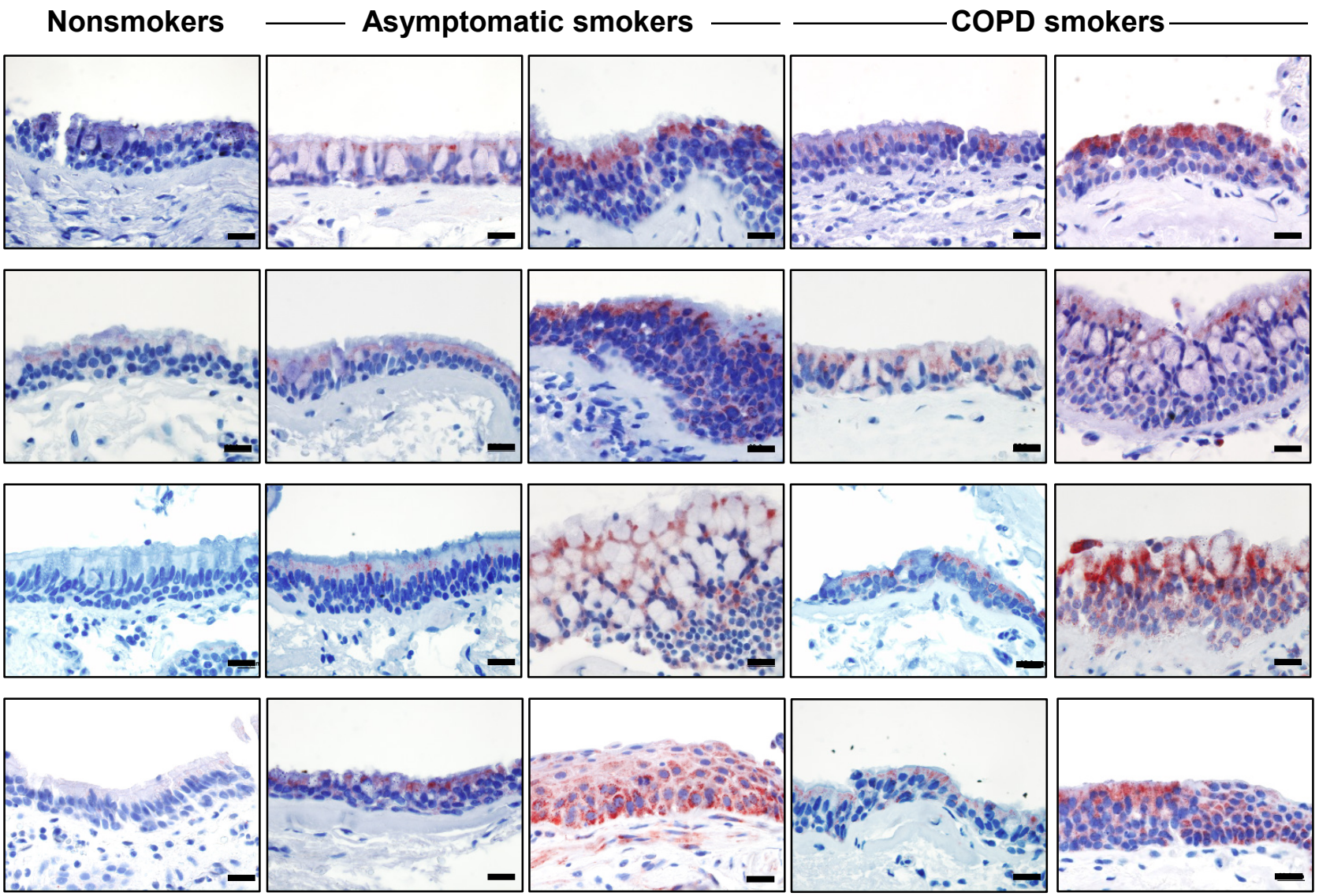
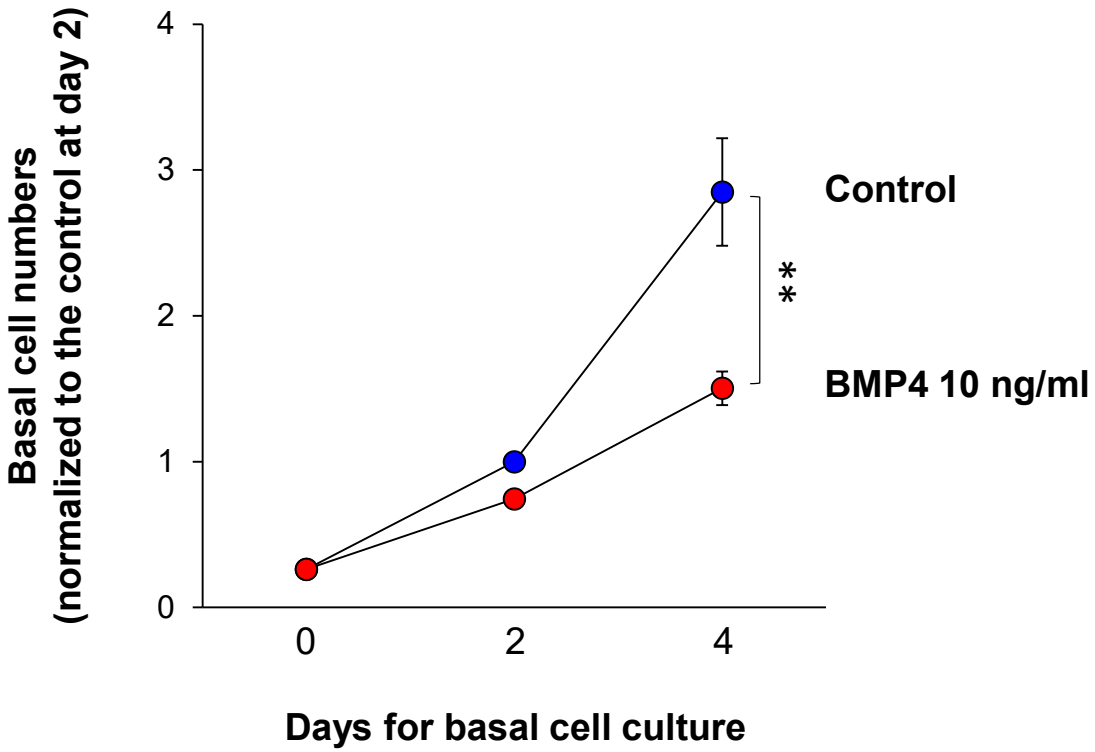
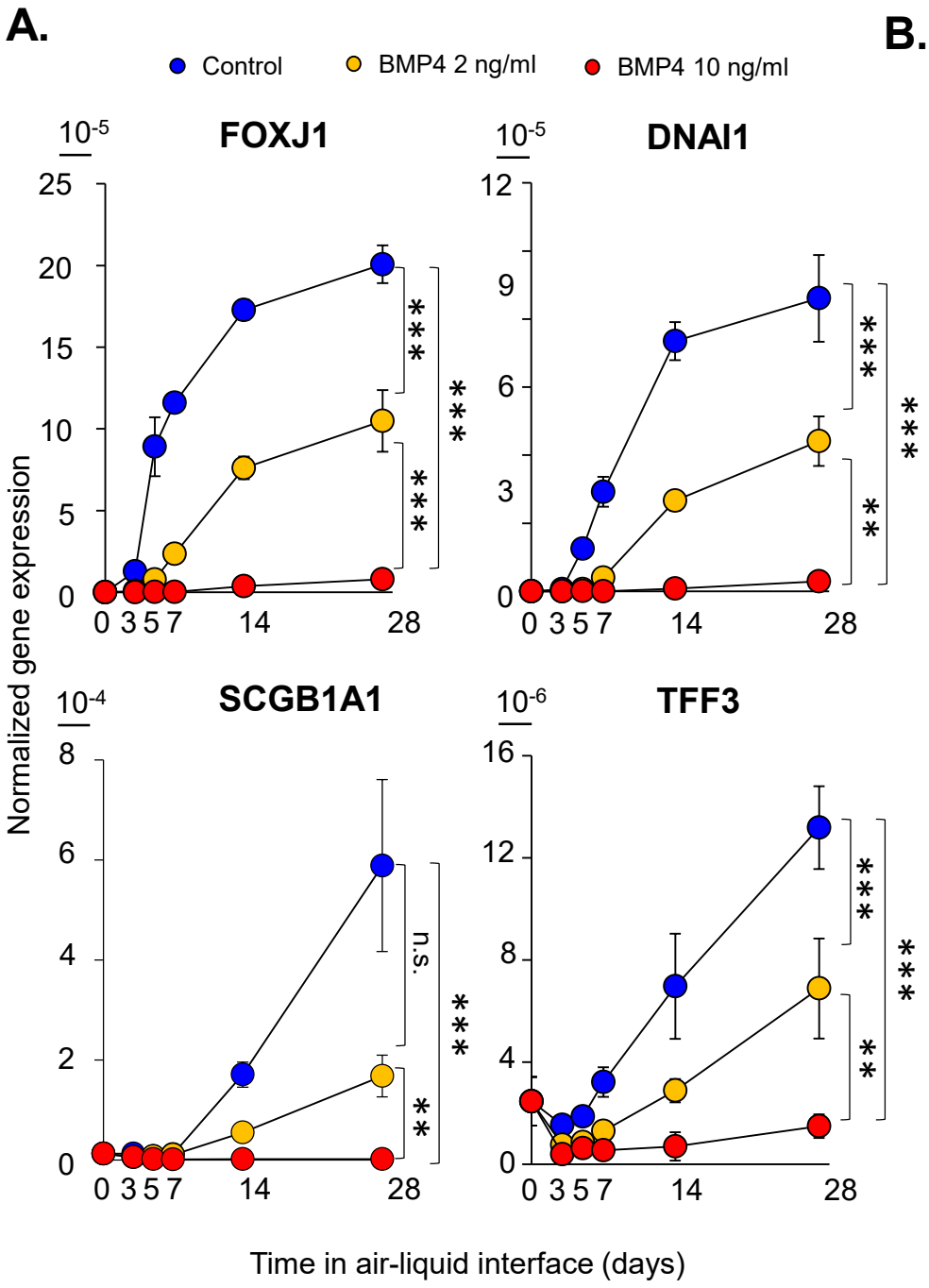
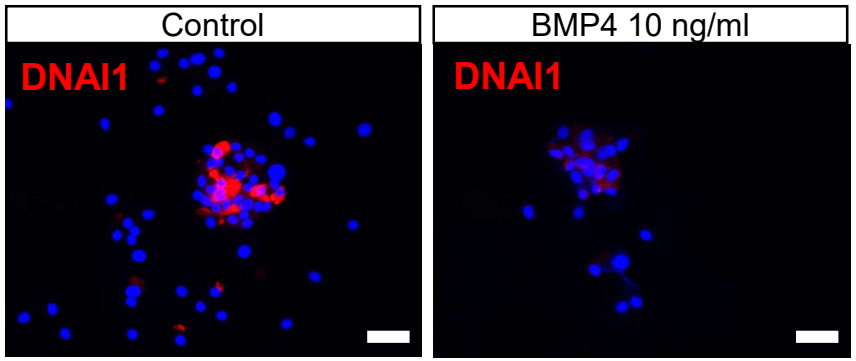
16
12
8
4
0
0
3
5
7
14
28
Time in air-liquid interface (days)

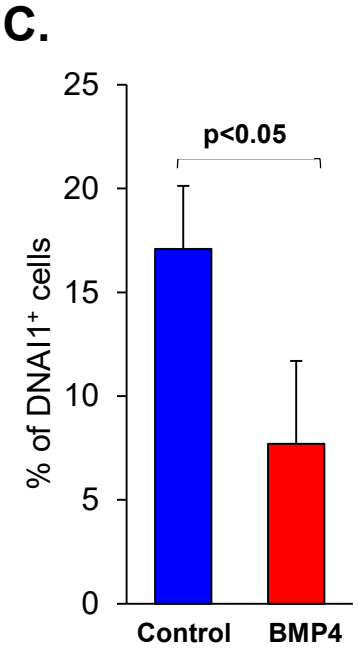

Normalized gene expression
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
0
3
6
12
Months
**
***
****

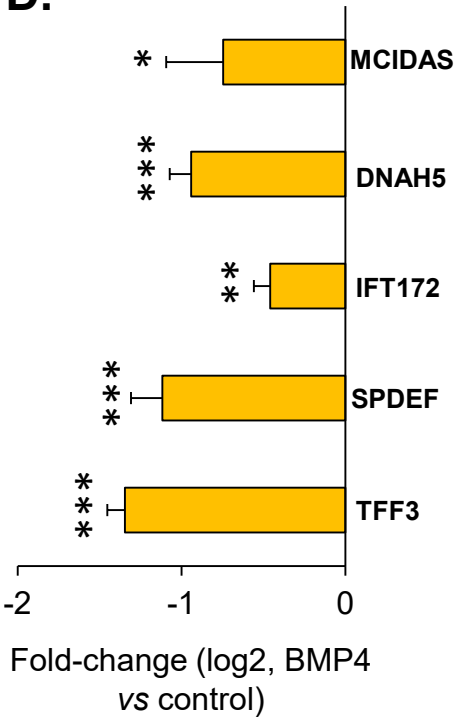

Nonsmokers
Asymptomatic smokers
COPD smokers

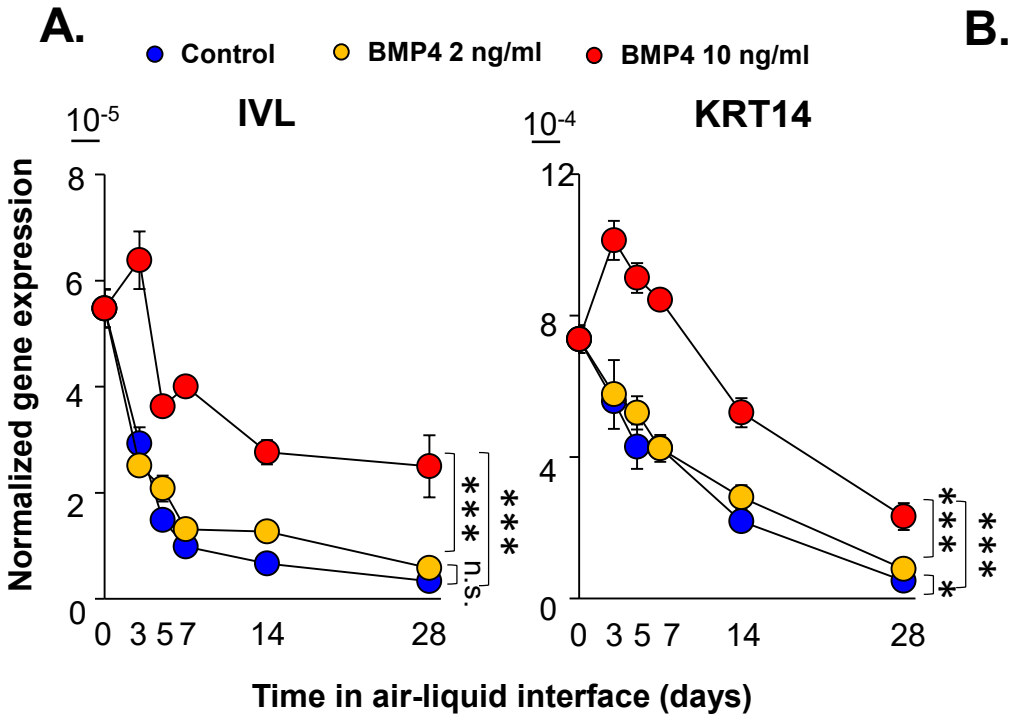

Basal cell numbers
(normalized to the control at day 2)
4
3
2
1
0
0
2
4
Days for basal cell culture
Control
BMP4 10 ng/ml
**

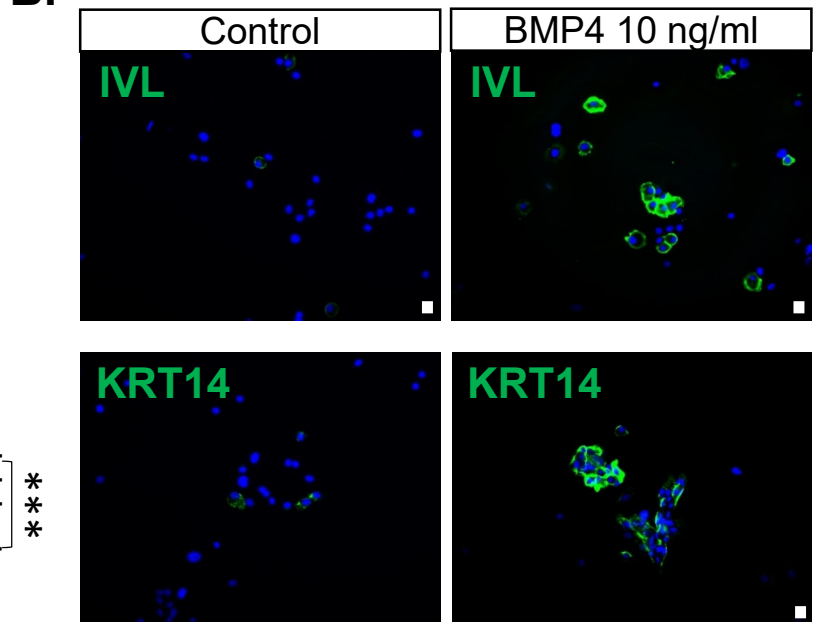

A.
Control
BMP4 2 ng/ml
BMP4 10 ng/ml
FOXJ1
DNAI1
SCGB1A1
TFF3
Normalized gene expression
Time in air-liquid interface (days)
n.s.

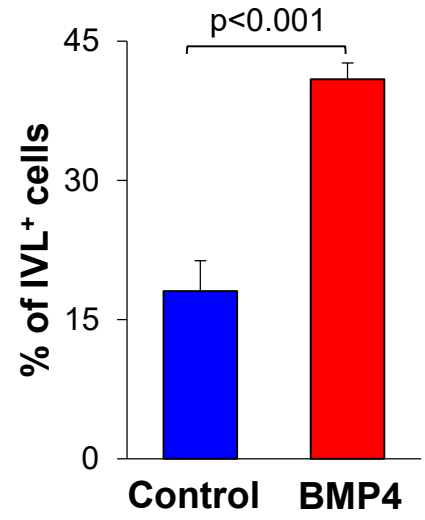

B.
Control
BMP4 10 ng/ml
DNAI1
DNAI1

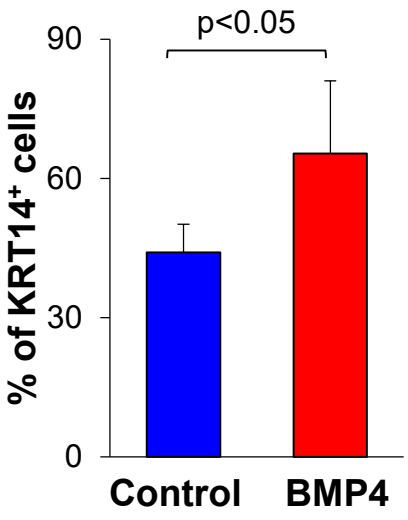

C.
p<0.05
% of DNAI1+ cells
Control
BMP4

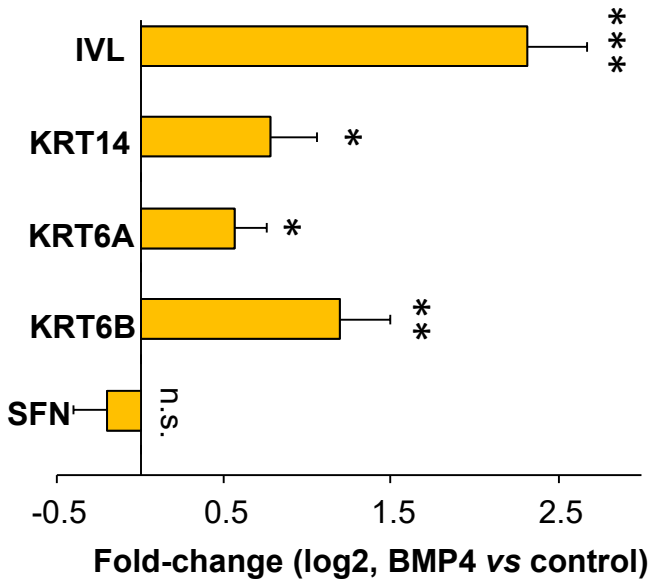

D.
MCIDAS
DNAH5
IFT172
SPDEF
TFF3
Fold-change (log2, BMP4 vs control)


A.
Control
BMP4 2 ng/ml
BMP4 10 ng/ml
IVL
KRT14
10-5
10-4
Normalized gene expression
0 3 5 7 14 28
Time in air-liquid interface (days)
n.s.

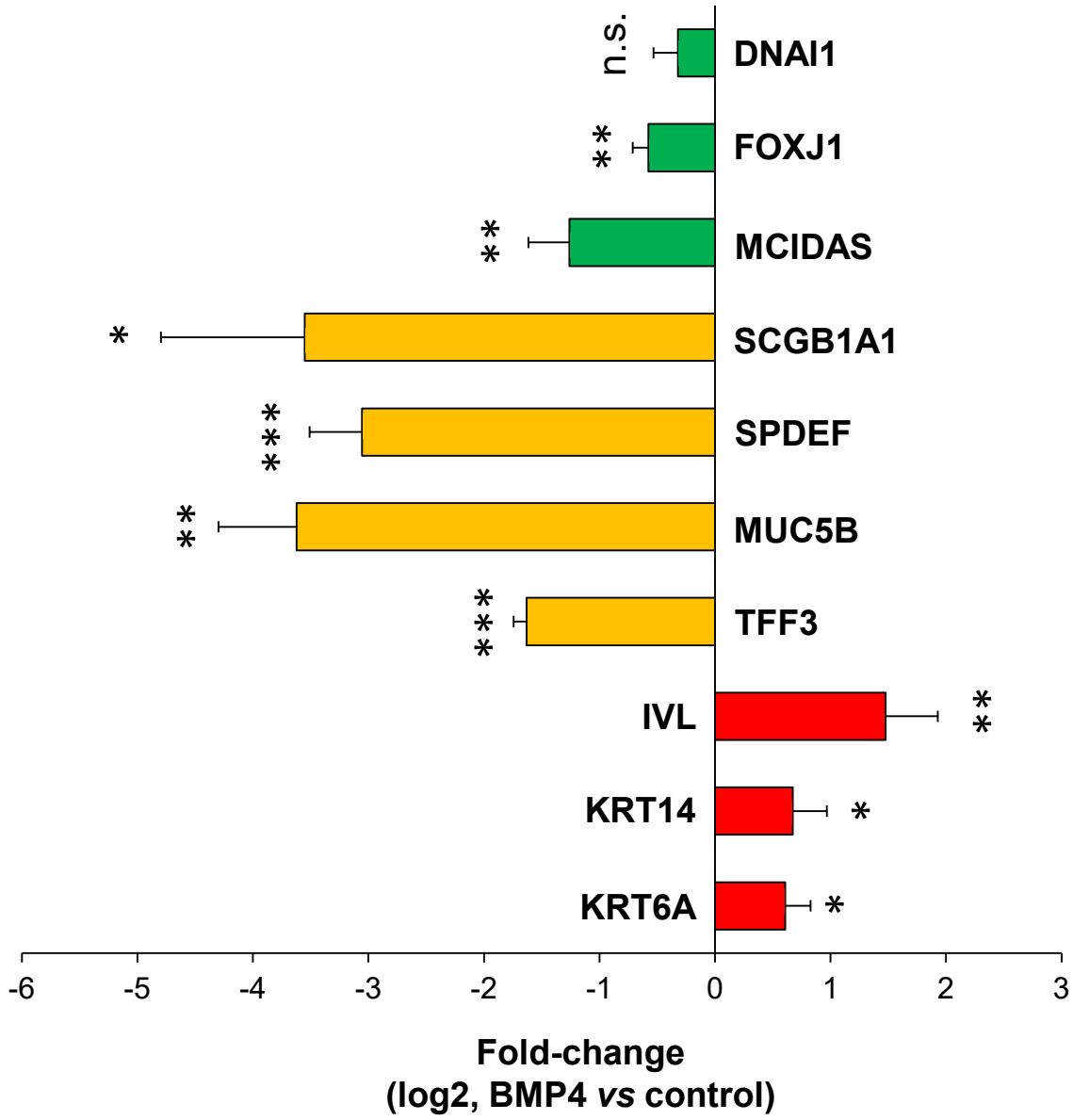

B.
Control
BMP4 10 ng/ml
IVL
KRT14


C.
p<0.001
% of IVL+ cells
Control
BMP4

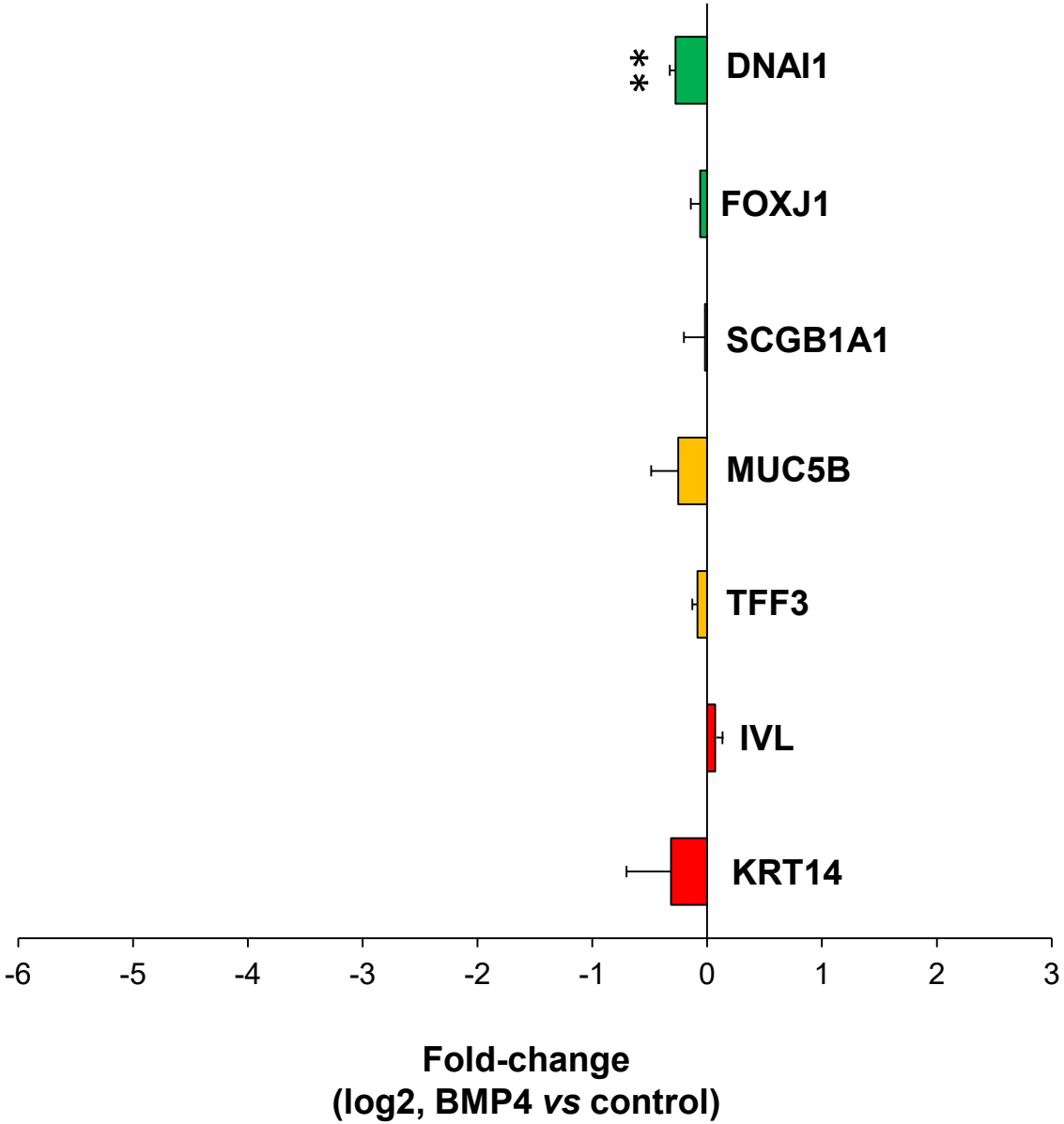

D.
p<0.05
% of KRT14+ cells
Control
BMP4


E.
IVL
KRT14
KRT6A
KRT6B
SFN
n.s.
Fold-change (log2, BMP4 vs control)

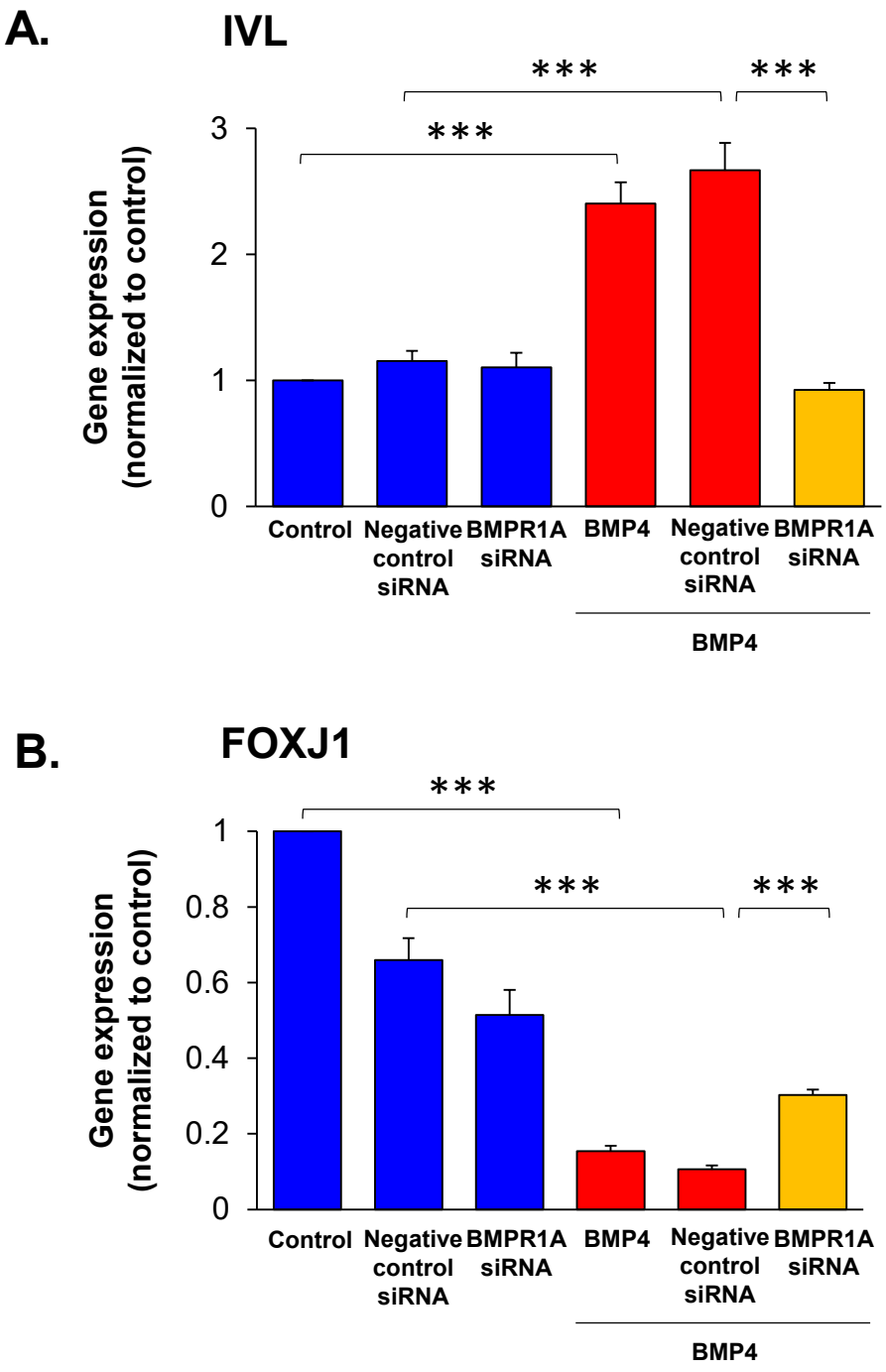
Supplemental Figure 8

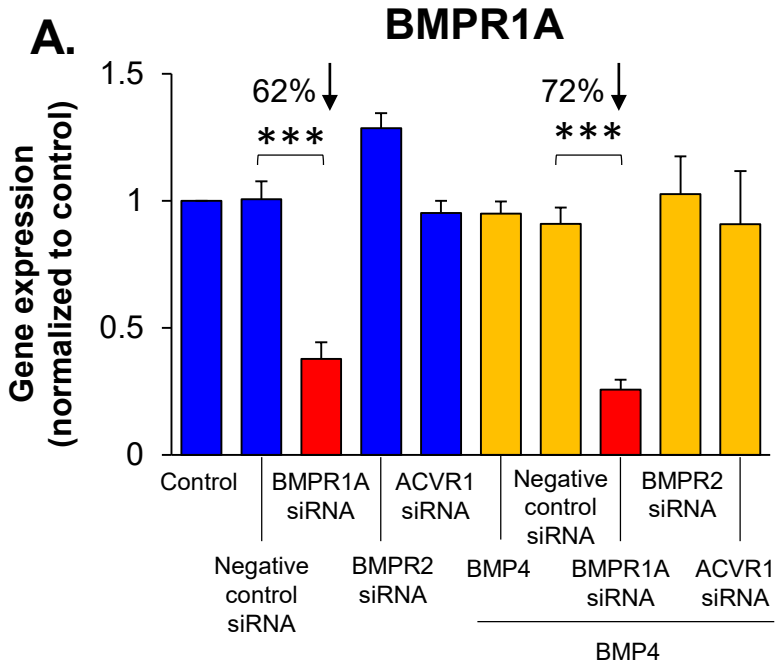


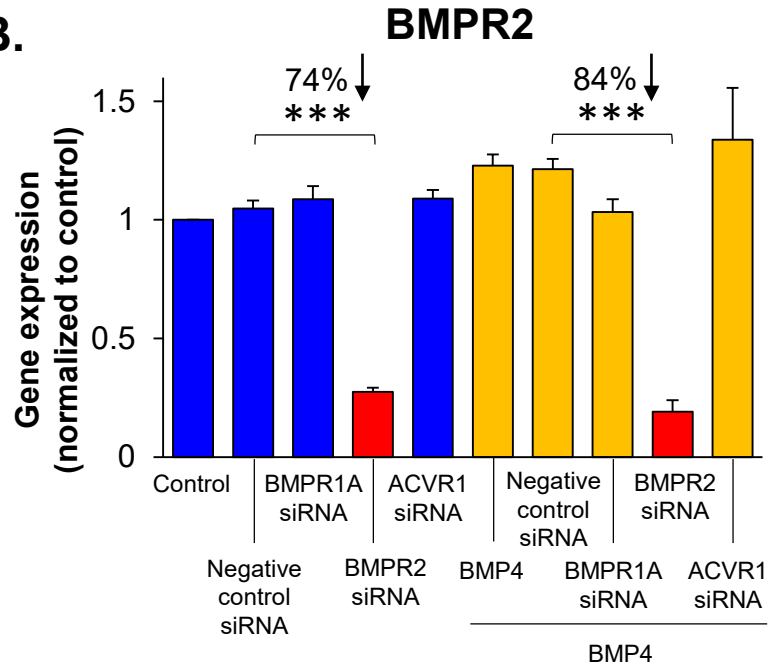
Supplemental Figure 9

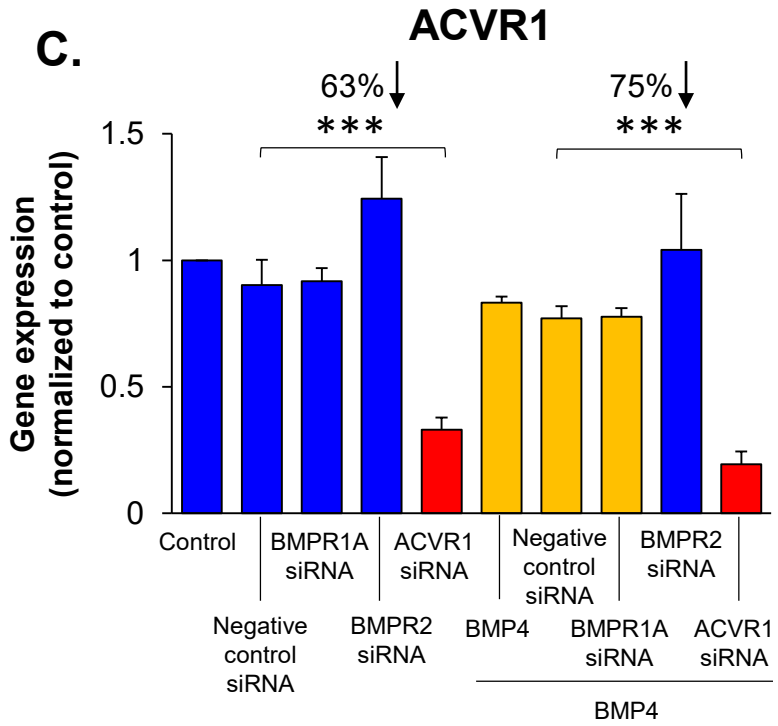


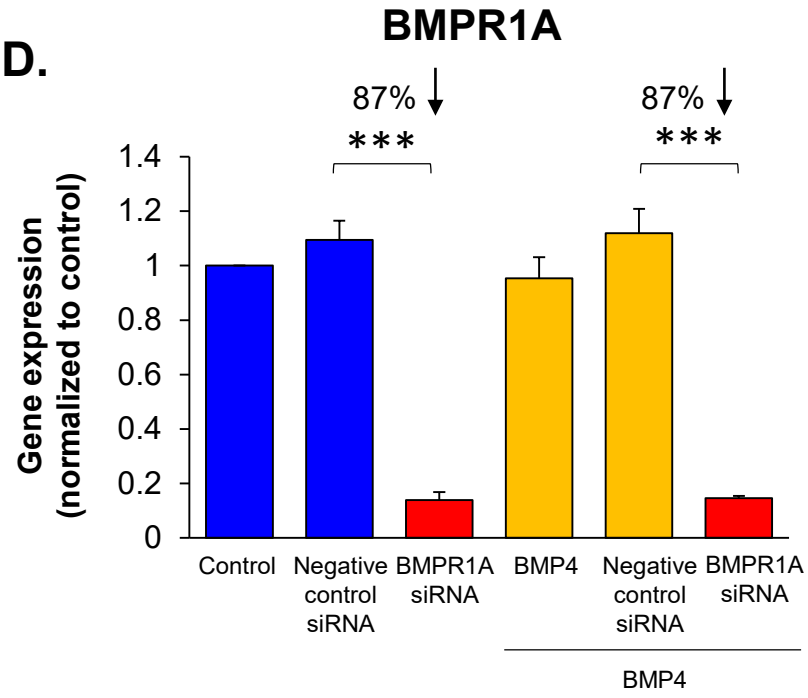
Supplemental Figure 10





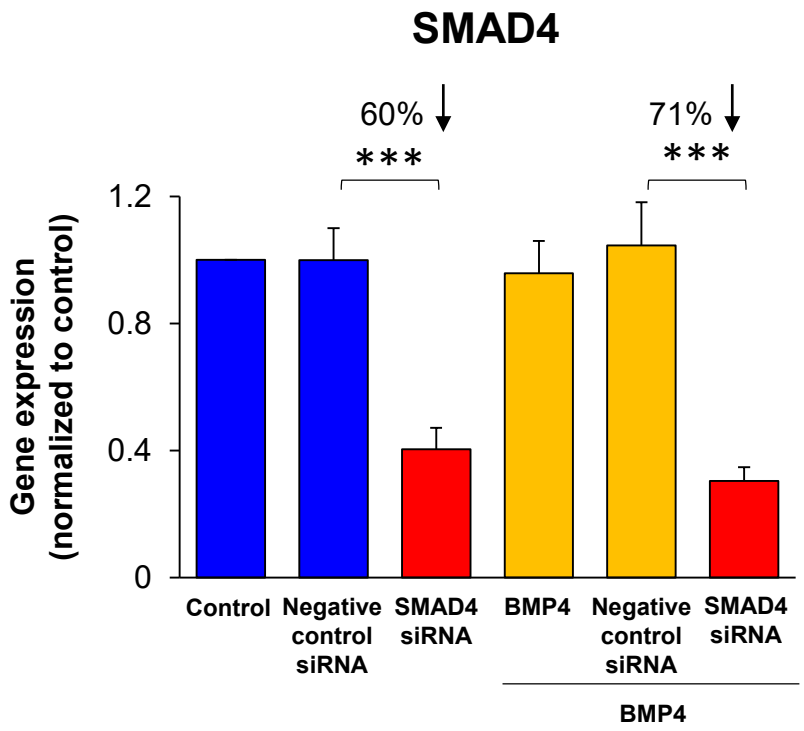


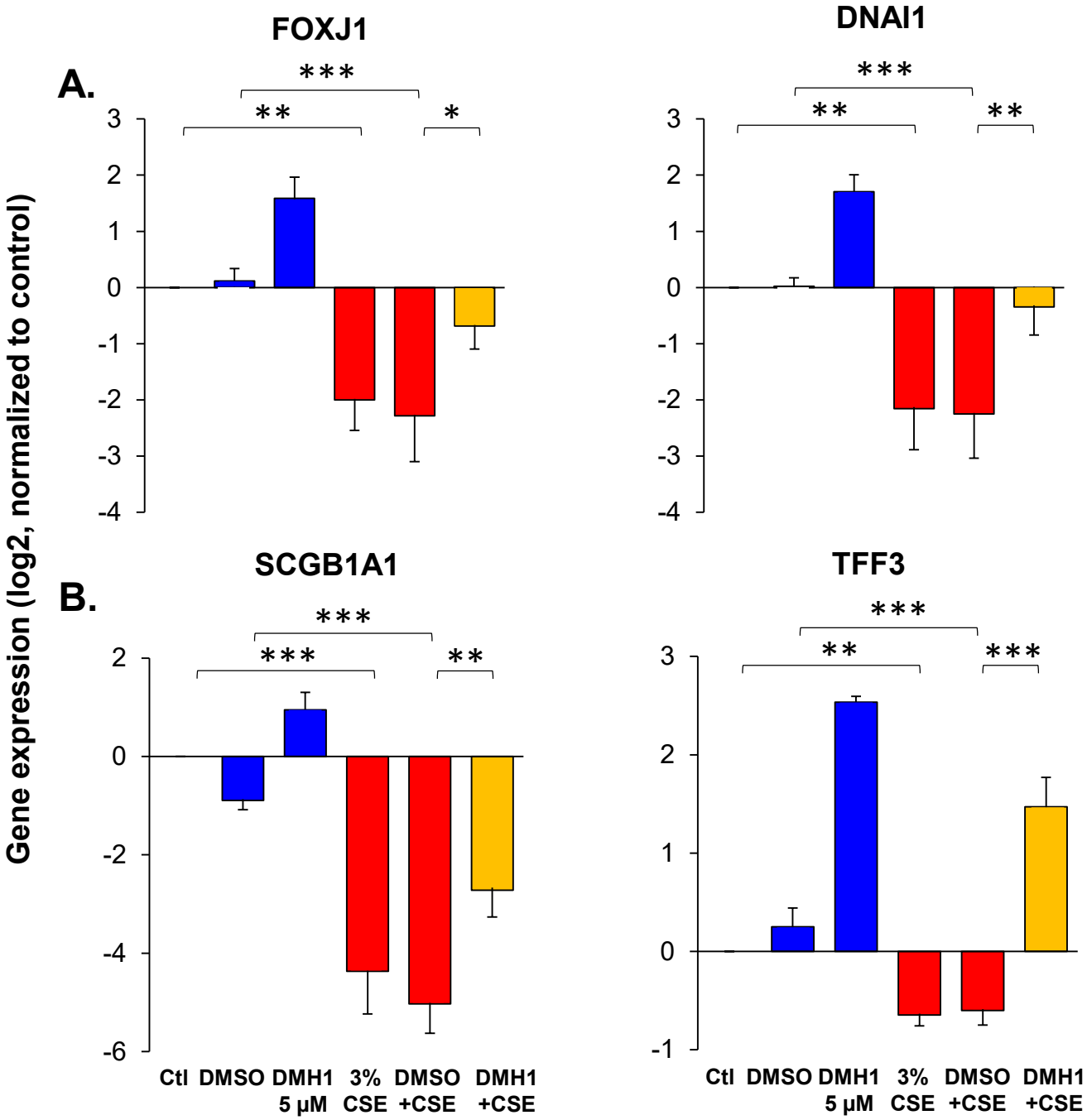




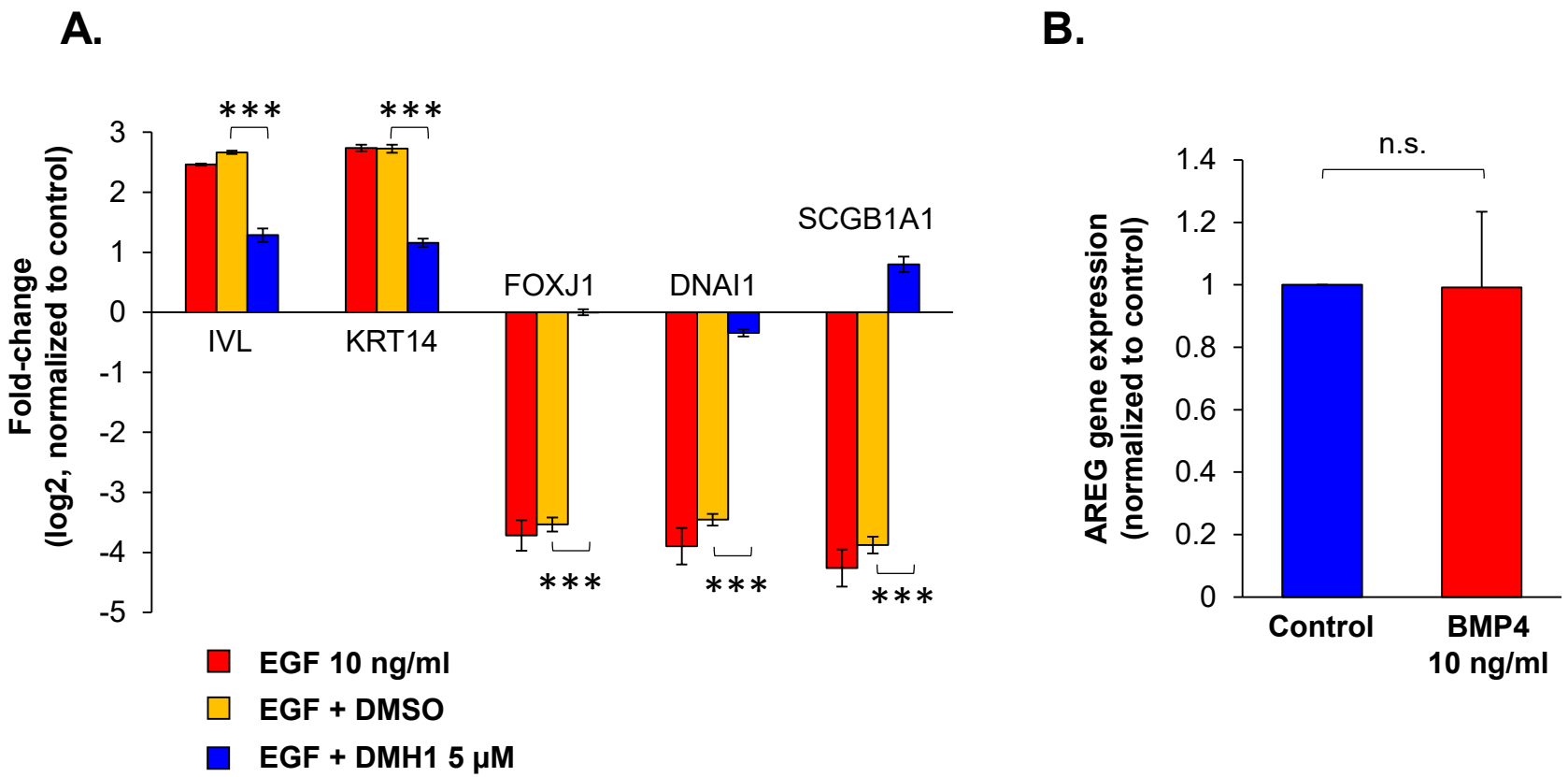
Supplemental Figure 11

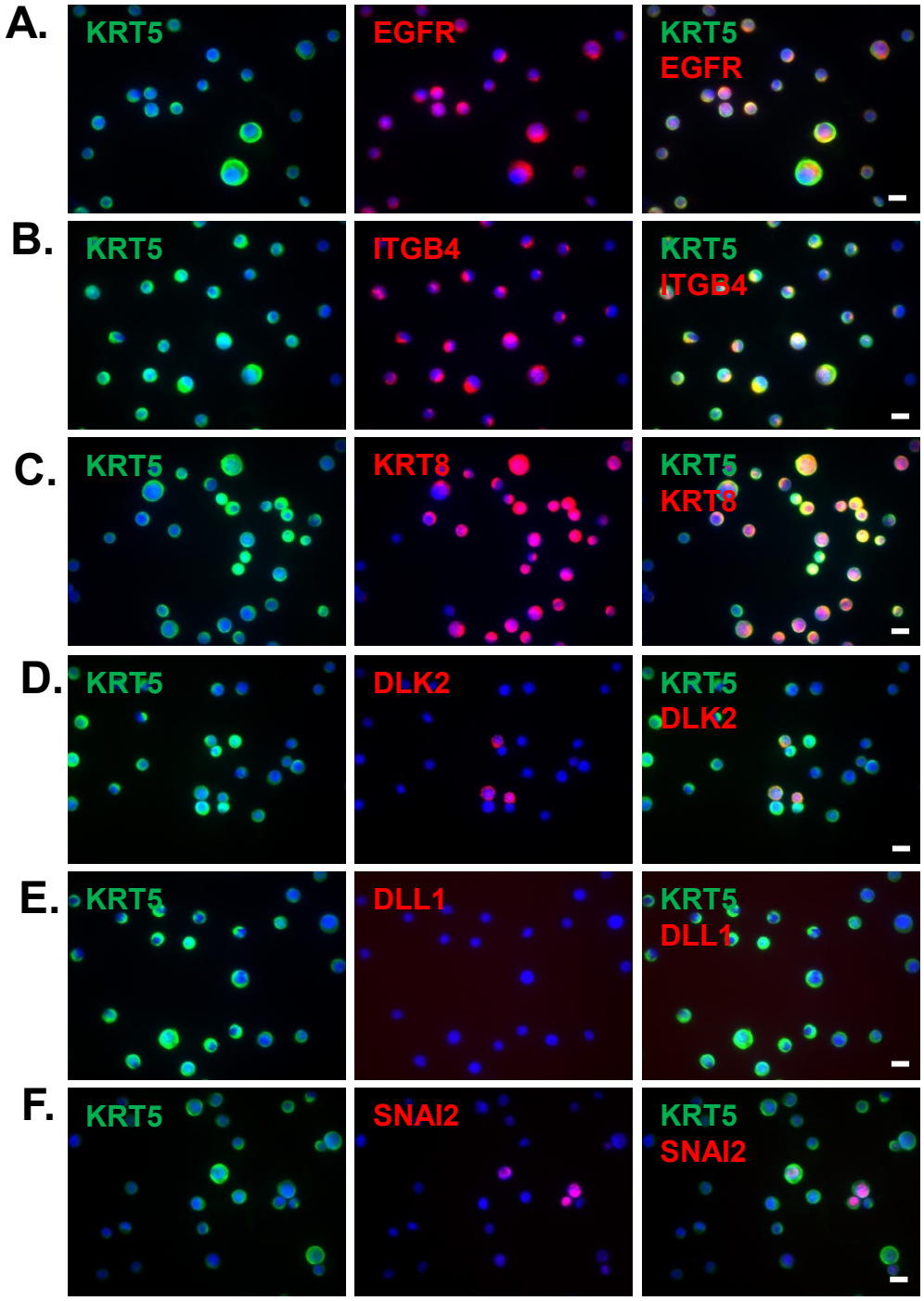
Supplemental Figure 12

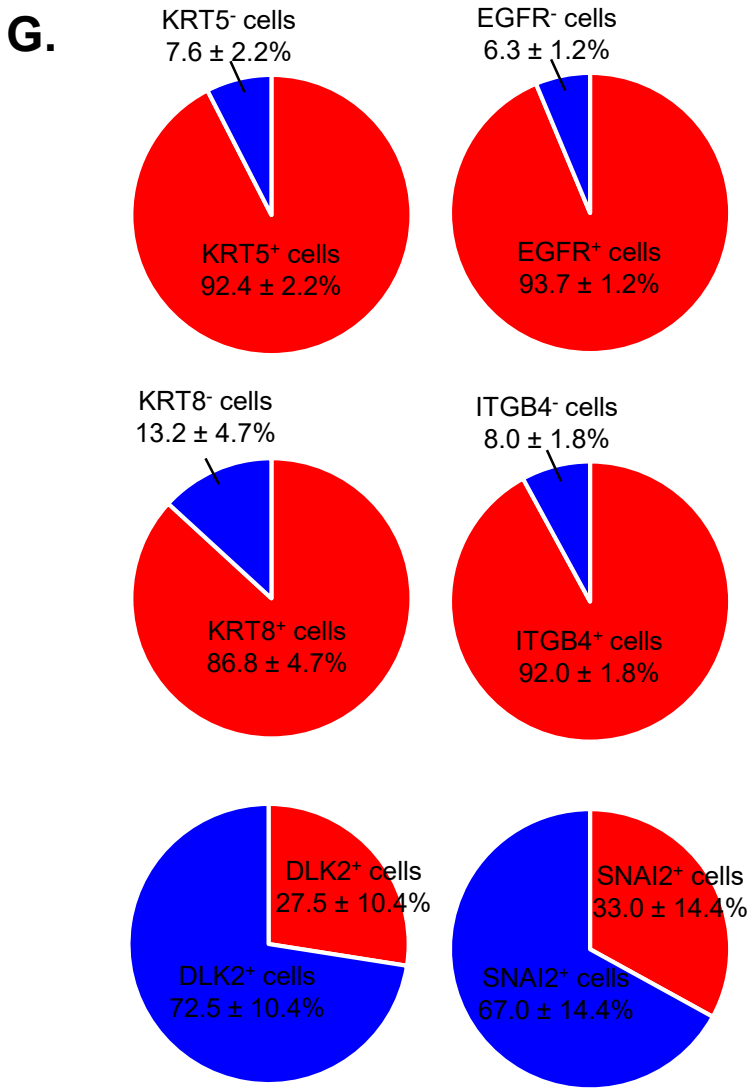



A.
FOXJ1
DNAI1
B.
SCGB1A1
TFF3
Gene expression (log2, normalized to control)
***
**
*
Ctl
DMSO
DMH1 5 µM
3% CSE
DMSO +CSE
DMH1 +CSE

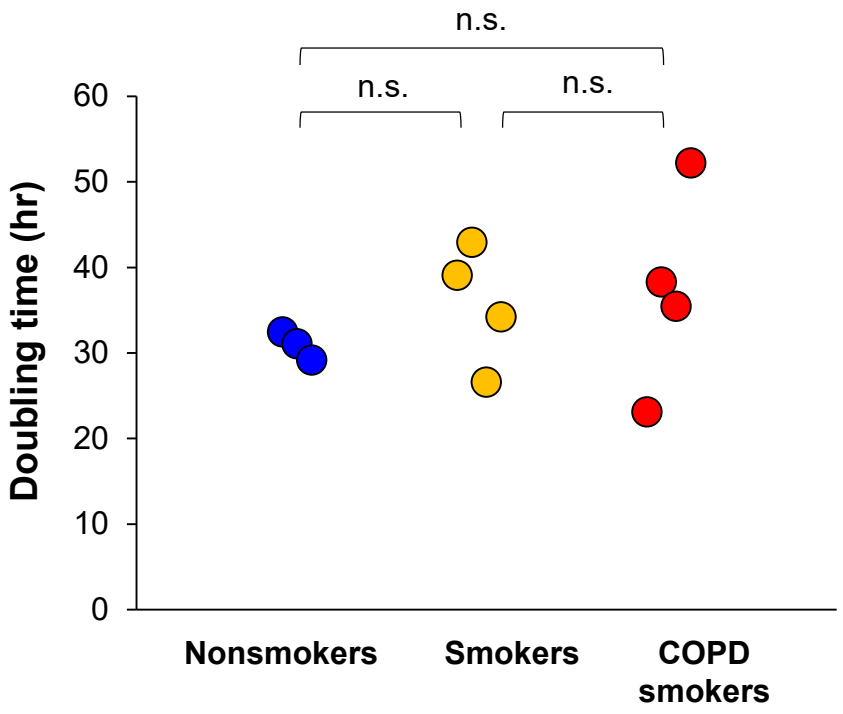
Supplemental Figure 14

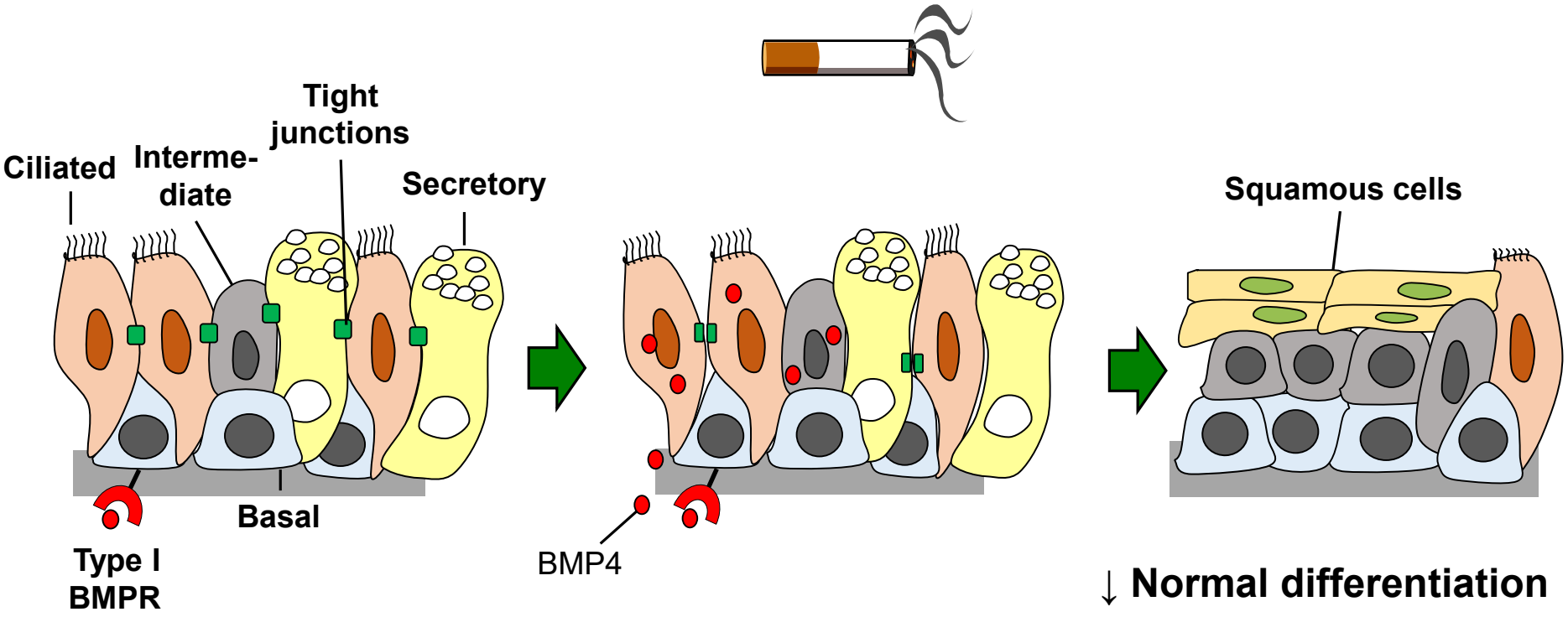



A.
KRT5
EGFR
KRT5
EGFR
B.
KRT5
ITGB4
KRT5
ITGB4
C.
KRT5
KRT8
KRT5
KRT8
D.
KRT5
DLK2
KRT5
DLK2
E.
KRT5
DLL1
KRT5
DLL1
F.
KRT5
SNAI2
KRT5
SNAI2


G.
KRT5- cells
7.6 ± 2.2%
KRT5+ cells
92.4 ± 2.2%
EGFR- cells
6.3 ± 1.2%
EGFR+ cells
93.7 ± 1.2%
KRT8- cells
13.2 ± 4.7%
KRT8+ cells
86.8 ± 4.7%
ITGB4- cells
8.0 ± 1.8%
ITGB4+ cells
92.0 ± 1.8%
DLK2+ cells
27.5 ± 10.4%
DLK2+ cells
72.5 ± 10.4%
SNAI2+ cells
33.0 ± 14.4%
SNAI2+ cells
67.0 ± 14.4%

Supplemental Figure 16




Ciliated
Interme-
diate
Tight
junctions
Secretory
Basal
Type I
BMPR
BMP4
Squamous cells
↓ Normal differentiation
↑ Squamous metaplasia

Supplemental Video Legend

Supplemental Video 1. Effects of BMP4 and inhibition of BMP4 receptor on the cilia beating in the airway epithelium *in vitro*. The airway epithelium in ALI (day 28) were derived from the normal nonsmoker BCs. The BCs were exposed to BMP4 (10 ng/ml) stimulation from the baso-lateral side *vs* untreated control in the absence or presence of BMP4 type I receptor inhibitor DMH1 (5 μM). DMSO was used as the negative control. **A.** control; **B.** DMSO; **C.** DMH1 (5 μM); **D.** BMP4 (10 ng/ml); **E.** BMP4+DMSO and **F.** BMP4+DMH1.