Supplemental Methods

Sampling and Single Cell Isolation

The small airway epithelium (SAE) was brushed from 10th-12th generation bronchi by fiberoptic bronchoscopy of 3 healthy nonsmokers and 3 asymptomatic smokers (Supplemental Table S1) using a protocol approved by Weill Cornell Medical College Institutional Review Board. The pooled cells were prepared for single cell sequencing based on standard methods [1]. The pooled cells were washed with Small Airway Growth Basal Medium (Lonza, Walkersville, MD) and incubated with ammonium chloride potassium lysing buffer (Gibco, Grand Island, NY) for 3 min to remove the red blood cells. The cells were then digested by trypsin/ethylenediaminetetraacetic acid (EDTA, 0.05%; Gibco) for 5 min, and neutralized by N'-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (HEPES; Lonza) with 15% fetal bovine serum (Gibco). The cells were washed again and resuspended in 1 ml phosphate buffered saline, pH 7.4 (PBS; Gibco) with 0.01% bovine serum albumin (BSA; Jackson ImmunoResearch, West Grove, PA). Trypan blue (Gibco)-treated cell suspensions were assessed using a hemocytometer and the numbers of viable cells quantified. In all cases, >80% of the cells were single cells, with 70 to 80% cell viability. Cell suspensions were filtered through a 35 µm nylon mesh cell strainer snap cap (Falcon/Becton Dickinson Labware, Franklin Lakes, NJ), stained with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI, 1 µg/ml; Molecular Probes, Basel, Switzerland), and loaded on an Influx™ cell sorter (BD Biosciences, San Jose, CA; Flow Cytometry Core, Weill Cornell Medicine Flow Cytometry Core Facility). The single viable cells were sorted, resuspended in PBS/0.01% BSA and the sorted single cell suspension counted using Countess™ Automated Cell Counter (Invitrogen, Carlsbad, CA) adjusted to the final concentration of 100 to 120 cells/µl.

Single Cell RNA-sequencing and Analysis

Drop-seq-based single cell RNA-sequencing was performed in the Weill Cornell Genomics Core Facility following the protocol from McCarroll [2, 3]. The single cells and barcoded beads were encapsulated into oil-based droplets using a co-flow microfluidics device (FlowJEM, Toronto, Canada). Single cells in the droplets were lysed immediately and the mRNA released from the cell hybridized to the barcoded primers on the surface of the beads. The droplets were then harvested and broken with perfluorooctanol. cDNA synthesis and library preparation were performed [2], and cDNA libraries were sequenced on Illumina HiSeq 2500 instrument (Illumina, San Diego, CA). The 6 samples (nonsmoker 1-3, smoker 1-3) were processed at 6 different days, the library preparation and sequencing were performed on 4 technical batches: batch 1 – nonsmoker 1 + smoker 1; batch 2 – nonsmoker 2; batch 3 – nonsmoker 3 + smoker 2; batch 4 – smoker 3. The single cell data are available in Gene Expression Omnibus (GEO) site with accession number: GSE123405.

Clustering was performed using Seurat, an R package for single cell analysis[14]. Raw digital expression matrices containing transcript counts for each gene in each cell were generated separately for each sequencing experiment[15]. Raw data was filtered as: (1) genes expressed in no less than 10 cells and cells with no less than 200 genes detected were kept for subsequent analysis; and (2) cells were filtered out that had unique gene counts over 10^4 or <200 and the % mitochondrial genes for each cell >0.25. The quality control for the single-cell RNA-sequencing data in each individual after filtering was shown in Supplemental Table S9.

A total of 11,702 cells from the 6 samples were combined and normalized by the total number of unique molecular identifiers (UMI) per cell, multiplied by a scaling factor (10^4) and then log transformed. Cell-cell variation was regressed out in gene expression driven by the number of detected molecules per cell as well as percent mitochondrial gene content. The mean expression and dispersion (variance/mean) were calculated for each of the 19,748 genes detected across the entire dataset to identify the most variable genes. Genes were placed into bins based on average expression and a z score calculated for dispersion within each bin to identify outlier genes whose expression values were highly variable compared to genes with similar average expression. A lower cutoff of 0.1 for average gene expression and 1 for dispersion was used to identify 1,952 highly variable genes. Principal component analysis (PCA) was used to assess selected genes from 11,702 cells. The identified highly variable genes were used as input to the PCA to ensure robust identification of the primary structure of the data [4]. Twenty statistically significant principal components (PCs) were calculated. Two approaches were used to determine significant PCs for downstream analysis. First, standard deviations of the PCs were plotted to identify the cutoff where a clear elbow existed in the plot. Second, the PCs score of cells and genes were visualized to explore correlated gene sets. The PCs that had lowest sum weight scores for mitochondrial and ribosomal genes were selected. The PCs were used to project cells onto a two dimensional map using t-Distributed Stochastic Neighbor Embedding (t-SNE) [5] with perplexity parameter set to 30, allowing cells with similar expression signature genes and similar PC loading to localize near each other. To identify distinct cell clusters, a K-nearest neighbor (KNN), graph based on the Euclidean distance in PCA space, was constructed to iteratively group cells together. The resolution parameter in FindClusters function in Seurat package was set to 0.5. With this approach, 11 distinct clusters were identified and the cell numbers of each cell population from each individual were shown in Supplemental Table S10. These clusters were compared using a Wilcoxon rank-sum test to identify up-regulated signature genes in each cluster. The criteria used to define signature genes for each cluster included: (1) genes detected at a minimum of 10% of cells in the cluster and (2) genes with mean expression increased by 0.25

(log scale) in the cluster compared to all other clusters [6]. Ambient RNA contamination was estimated using SoupX (https://omictools.com/soupx-tool). The fraction of ambient RNA contamination in nonsmokers was 6.2% while smoker data had 5.4% ambient RNA contamination. We also did the t-SNE plots on each sample (Supplemental Figure S9A), as well as on the 4 technical batches (Supplemental Figure S9B). Most of the cell populations in either different individual or the technical batches overlapped quite well, suggesting our samplings and experimental processing were consistent and no big variations between different individuals or batches.

To evaluate the effects of gender to the gene expression of human SAE, we -analyzed our previous microarray and RNA-seq datasets of the bulk human SAE. We found that gender had little effect, while smoking dominates changes in gene expression of human SAE (Supplemental Table S11).

Imputation Method for Gene-Gene Interactions

The Markov Affinity-based Graph Imputation of Cells (MAGIC) computational approach was used for recovering missing values of mRNA capture [7]. MAGIC starts with count matrix representing observed transcript counts of genes and cells. Using a graph-based method, a distance matrix was calculated in the Seurat package (SNN matrix). A Gaussian kernel function was applied to convert the distance matrix to an affinity matrix as a weighted adjacency matrix. A Markov transition matrix was created by row normalizing the affinity matrix, and the imputed expression values were calculated by multiplying the Markov transition matrix powered to diffusion time (t) by the distance matrix. Genes associated with monogenetic lung disorders [8], idiopathic pulmonary fibrosis (IPF) [9-11] and lung cancers [12-16] were collected from the literature, and chronic obstructive pulmonary disease (COPD)-related genes taken from the COPDGene study (http://www.copdgene.org/). Imputed data is presented in violin and box plots throughout the text. Imputed data was not used for tSNE plots, heatmaps, dot plots or any other data representations.

Statistical Analysis

To identify signature genes in each of the 11 clusters, gene expression from each cluster was compared to the gene expression from all other cells of remaining clusters by using the Seurat “FindAllMarkers” function. The test used to identify markers was the two-sided, combined likelihood-ratio test with three degrees of freedom, designed for the sampling distributions of single cell gene expression as described in McDavid et al [6]. The criteria to define marker genes included: (1) marker genes differed by at least 0.25 (log-scale) between the mean expression in each group of cells; and (2) that genes were only tested if they were detected in a minimum fraction of 0.1 cells in either of the cell groups. Bonferroni correction was used to adjust p values by multiplying by the total gene number of the dataset.

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Supplemental Table S1. Demographics of Nonsmokers and Smokers[1, 2]

Parameter	Nonsmokers	Smokers	p value
n	3	3	
Gender (M/F)	0/3	3/0	p>0.1
Age (yr)	26 ± 8	42 ± 14	p>0.1
Race (B/W/H/O)[2]	1/1/0/1	1/0/1/1	p>0.8
Body Mass Index	23 ± 4	23 ± 4	p>0.8
Smoking history			
Age of initiation	NA	20 ± 1	NA
Pack years	NA	12 ± 6	NA
Urine nicotine (ng/ml)[3]	0	844 ± 616	NA
Urine cotinine (ng/ml)[3]	0	936 ± 458	NA
Carboxyhemoglobin (%)	1.6 ± 0.1	2.3 ± 0.2	p>0.1
Pulmonary function parameters[4]			
FVC	115 ± 15	102 ± 13	p>0.5
FEV1	110 ± 12	100 ± 10	p>0.4
FEV1/FVC	83 ± 5	80 ± 4	p>0.4
DLCO	89 ± 8	92 ± 10	p>0.1
TLC	107 ± 12	103 ± 6	p>0.7
Cough Score[5]	0.7 ± 0.6	1.3 ± 0.6	p>0.3
Sputum Score[5]	0.7 ± 0.6	1.3 ± 0.6	p>0.3
SAE cell differential (%)			
Epithelial	96.6 ± 1.1	98.9 ± 0.2	p>0.06
Inflammatory	3.4 ± 1.1	1.1 ± 0.2	p>0.06
Ciliated	59.9 ± 2.0	57.7 ± 9.9	p>0.7
Secretory	10.3 ± 2.5	9.6 ± 5.1	p>0.8
Undifferentiated	24.0 ± 0.7	29.8 ± 5.8	p>0.2
Basal	2.4 ± 1.0	1.8 ± 1.0	p>0.4

[1] Data are presented as mean ± standard deviation, p values of numeric parameters calculated using a 2-tailed Student's t-test, p value of categorical parameters calculated using a Fisher's exact test.

[2] Abbreviations: B=Black, W=White, H=Hispanic, O=Other, NA=not applicable; FVC - forced vital capacity, FEV1 - forced expiratory volume in 1 sec, TLC - total lung capacity, DLCO - diffusing capacity of the lung for carbon monoxide, SAE=small airway epithelium.

[3] Undetectable urine nicotine <2 ng/ml; cotinine < 5 ng/ml.

[4] Pulmonary function testing parameters are given as % of predicted value with the exception of FEV1/FVC which is reported as % observed

[5] Cough and sputum score were each evaluated on a scale of 0-4: 0 = not at all; 1 = only with chest infections; 2 = a few days a month; 3 = several days a week; 4 - most days a week [17]

Supplemental Table S2. Entire List of Signature Genes for the Cell Populations Identified by Unsupervised Clustering in Human Small Airways of Healthy Nonsmokers

Basal			Intermediate			Club			Mucous			Ciliated			Ionocyte			Neuroendocrine			T cell			Antigen presenting			Mast			NCL^{high}		
Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value	Gene symbol	Fold-change (log$_e$)	Adjusted p value

See attached xls file for complete table

Supplemental Table S3. Top 20 Signature Genes Expressed by Each of the Major Cell Populations in the Human Small Airway Epithelium of Healthy Nonsmokers[1]

Categories	Basal	Intermediate	Club	Mucous	Ciliated
Defense[2]					
against pathogens, particulates			C3, LCN2, AGR2, CXCL17, CXCL1	TFF3, MUC5AC, BPIFB1, SCGB1A1, MUC5B, LYZ	
against toxins	MGST1, FMO2		MGST1, ALDH1A1	GALNT7,	
antiproteases	SPINT2	SLPI	SLPI, WFDC2, SERPINB3	WFDC2	
barrier function	PERP, CLDN1			CEACAM6	
Proteases			PRSS23, CTSC	CAPN8	
Cytoskeleton	KRT15, HSPB1, KRT5	KRT19, KRT5	KRT7, KRT19		
Protein synthesis	RPLP1, RPL32, RPL31, RPL34, RPL35, RPL7A	RPS4X, RPL3, RPL10A, RPS18, RPS24, RPL12, RPL7, RPS6, RPL5, RPS8, RPS2, RPS3A, RPL4		RRBP1	
Growth factors	ADIRF, IL33	IL33	TNFSF10		
Transcription factors and regulation		EPAS1	ELF3	XBP1, CREB3L1	FHAD1
Receptors	RACK1		ALCAM	PIGR, ADRA2A	CDHR3
Maintenance of ionic balance				SLC31A1	CAPS
Cell respiration	MT-CO3, ATP5G2				
Calcium regulation		S100A2		S100P	
Metalloprotein			CP		
Proliferation				MSMB, SCGB3A1	
Signal transduction				TSPAN8	
Ciliary architecture					DNAAF1, DNAH12, RSPH1, CFAP43, TPPP3, SPAG17, CFAP157, DHAH5, CFAP45, CETN2, SNTN
Protein folding					HSP90AA1
Unknown	MIR205HG	MIR205HG	FAM3D, CYP2B7P		LRRIQ1, ERICH3, CCDC170, C20orf85, CCDC146

[1] The signature genes, ordered by p values (from smallest to 0.05), for each cell population were generated by comparing cells from each cell population with all other cells using Seurat "FindAllMarkers" function. The signature genes were expressed in >10% of the cells in the corresponding cell populations, and the average expression levels of the expressing cells in the corresponding cell population *vs* all other expressing cells were >0.25 (log). Bonferroni corrected $p<0.05$ was used as the cutoff.

[2] Defense-related genes include those against pathogens, particulates, toxins, proteases and barrier function.

Supplemental Table S4. Top 20 Signature Genes Expressed by Each of the Minor Cell Populations in the Human Small Airway Epithelium of Healthy Nonsmokers[1]

Categories	Ionocyte	Neuroendocrine	T cell	Mast	Antigen presenting	NCL^{high}
Cytokine	RARRES2, IGF1		CCL5, IL32			
Cell surface molecules			CD2, PTPRC, TRBC, CD52, B2M, HLA-B, HLA-C, HLA-E, TRAC, IL7R, HLA-A, CD3D, CD3E, TRBC1, CD3G	KIT, CD52	HLA-DRA, HLA-DRB1, CD74, HLA-DPB1, HLA-DPA1, HLA-DQB1, HLA-DQA1, HLA-DMB, HLA-DRB5, HLA-DQB2	
Protein secretion	SEC11C	RTN1, CHGA		SRGN, LAPTM5		
Protein degradation		UBB				HSP90AB1
Cytoskeleton		TUBA4A, MAP1B, TUBA1A	TMSB4X, EVL	VIM, CAPG	TMSB10	
Signal transduction	STAP1, CLNK	CALM1, CALM3, GNG13, FSTL5, CALM2, GNAL, RIC8B		TYROBP		
Fat metabolism				APOE		
Transcription factors	ASCL3, TFCP2L1, FOXI1					
Receptors	ADGRF5					
Maintenance of ionic balance	ATP6V1G3, CLCNKB, ITPR2, GABRB2, ATP6V0B	ATP1B1, STOML3				
Defense						
against pathogens, particulates					LYZ	
against toxins	DGKI				CYBB	
against protease					CST3	

Supplemental Table S4. Top 20 Signature Genes Expressed by Each of the Minor Cell Populations in the Human Small Airway Epithelium of Healthy Nonsmokers[1] (cont., page 2)

Categories	Ionocyte	Neuroendocrine	T cell	Mast	Antigen presenting	NCL^{high}
Glucose and insulin homeostasis	APLP2					
Mitosis	HEPACAM2					
Development	SEMA3C					
Proliferation					AIF1, LST1	
Lysosomal targeting					PSAP	
Cellular ion regulation					FTL	
Cellular energy homeostasis		CKB				
Chromatin regulation						NCL, HMGB2
Unknown	LINC01187, TMEM61	CALM1P1, CALM1P2, S100A5, HSP90AB3P	GIMAP7	VWA5A, SLC45A3, DTNBP1	HLA-DRB6, MNDA	PTMAP2, PTMAP5, HSP90AA2P, HSP90AA5P, RPSAP55, RPSAP28, NPM1P4, HSP90AB3P, RP11-538P18.1, NPM1P8, RP11-253E3.1, CTA-351J1.1, NPM1P43, RPL37AP8, AB019441.29, HSP90AA6P, FAUP1

[1] The signature genes, ordered by p values (from smallest to 0.05), for each cell population were generated by comparing cells from each cell population with all other cells using Seurat "FindAllMarkers" function. The signature genes-were expressed in >10% of the cells in the corresponding cell populations, and the average expression levels of the expressing cells in the corresponding cell population *vs* all other expressing cells-were >0.25 (log). Bonferroni corrected $p<0.05$ was used as the cutoff.

[2] Defense-related genes include those against pathogens, particulates, toxins and proteases.

Supplemental Table S5. Differentially Expressed Genes in Nonsmokers *vs* Smokers in the Major Human Small Airway Epithelial Cell Populations

Basal				Intermediate				Club				Mucous				Ciliated			
Down-regulated genes		Up-regulated genes		Down-regulated genes		Up-regulated genes		Down-regulated genes		Up-regulated genes		Down-regulated genes		Up-regulated genes		Down-regulated genes		Up-regulated genes	
Gene symbol	Adjusted p value[1]	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value	Gene symbol	Adjusted p value

[1] Adjusted p value means Bonferroni corrected p value and that down- and up-regulation is in smokers compared to nonsmokers

See attached xls file for complete table

Supplemental Table S6. Categories of the Top 25[1] Differentially Expressed Genes in Nonsmokers *vs* Smokers in the Major Human Small Airway Epithelial Cell Populations

	BC		Intermediate		Club		Mucous		Ciliated	
Categories	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**
Defense against pathogens, particulates	SCGB1A1*		SCGB1A1* MUC5B* C3* LCN2 LTF* LYPD2* TNFAIP2 B2M		C3* MUC5B* LCN2 LTF* SCGB1A1* LYPD2* TNFAIP2 SAA1* FCGBP* CXCL6* CCL20*		SCGB1A1* MUC5B* LYPD2* FCGBP* ITLN1 HLA-B	BPIFB1* MUC5AC AGR2* B3GNT6* CXCL17 PTMA	SCGB1A1* SAA1* HLA-DRA ALCAM CXCL1 B2M	MUC5AC
against toxins		ALDH3A1* TXNIP	GDA*	ALDH3A1* CYP1B1* TXNIP PRDX1*		ALDH3A1* CYP1B1* NQO1* CYP26A1* PRDX1* TXNIP AKR1C2*	CY4B1 ODC1	ALDH3A1* CYP1B1*	HE3ST1 CYP4B1	ALDH3A1* NQO1* AKR1C2* AKR1C1* ADH7* GSTA2* PRDX1* GPX2* ABHD2* AKR1B10* CES1 TKT* TXNRD1* TALDO1*
antiproteases	SLPI		SLPI		SLPI PI3*	SERPINB1		WFDC2	SLPI	
barrier functions		PERP		PERP		PERP		CEACAM5* CEACAM6*		
Proteases							KLK11 KLK10			
Anti-apoptosis		IER3		IER3		IER3				
Cytoskeleton		KRT17*	RHOV	KRT17*	RHOV	KRT18		EZR		KRT8

Supplemental Table S6. Categories of the Top 25 Differentially Expressed Genes in Nonsmokers *vs* Smokers in the Major Human Small Airway Epithelial Cell Populations[1] (cont., page 2)

	BC		Intermediate		Club		Mucous		Ciliated	
Categories	Down[2]	Up[2]	Down[2]	Up[2]	Down[2]	Up[2]	Down[2]	Up[2]	Down[2]	Up[2]
		KRT15		KRT18				ACTG1		
		KRT5						KRT18		
		TPM1								
Protein synthesis	RPS29	RPS3A		RPS18		RPS18		RPS6	RPLP1	
		RPL3		RPS2		RPLP0			RPS29	
		RPS2		RPLP0		RPS2				
		RPLP0		RPL18A						
Growth factors		NTS		NTS		NTS				SPP1*
		CTGF*								
Transcription factors		JUN		JUN		JUN	PAX5	JUN		
and regulation		TSC22D1		ATF3			HES1			
		FOS		TSC22D1						
Receptors		F3	PIGR	F3	PIGR		PILRB	CD24	PIGR	
							ADRA2A**	CD55	ITGA2	
							PTGFR		CD74	
Maintenance of	CLCA2	AQP5							PIEZO2	S100A10*
ionic balance										
Cell respiration	MT-CO3		MT-ND3			MT-CO1	MT-ND2		MT-ND3	
	MT-ND3					MT-CO2	MT-ND3		MT-ATP6	
Cellular ion regulation		FTL*			MT3	FTH1*		S100A6		FTL*
						FTL*		FTL*		FTH1*
Metalloprotein			CP		CP					
Proliferation	SCGB3A1		SCGB3A1	H19*	SCGB3A1	H19*	SCGB3A1	TPT1	SCGB3A1	
					TMEM45A*					
Signaling transduction		CYR61		SDPR*		SFRP2*		TSPAN1*		
		SDPR*		TSPAN1*						
		THSD4		PPP1R15A						
Ciliary architecture	RSPH1									TUBA1A*
Protein folding					HSP90AA1					
Extracellular matrix				LAMB3						
Cell migration			MALAT1	IER2,	MALAT1	ANXA2			MALAT1	
				SNHG5						
Cellular membrane										
composition										
Stem cell marker					PROM1					
Neuro-regulation		MT-RNR2		MT-RNR2		MT-RNR2		MT-RNR2	SLITRK6	

Supplemental Table S6. Categories of the Top 25 Differentially Expressed Genes in Nonsmokers *vs* Smokers in the Major Human Small Airway Epithelial Cell Populations[1] (cont., page 3)

	BC		Intermediate		Club		Mucous		Ciliated	
Categories	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**	**Down[2]**	**Up[2]**
		SLITRK6								
Muscle contraction							TNNT3**			
Unknown	MTRNR2L1		SAA2* BICDL2		MTRNR2L3 SAA2* ALPL	LY6D	CTD-2531D15.4 SNHG25 ALPL MTRNR2L3 MT-TV MT-TP	PSCA NEAT1	MTRNR2L1 ABCA13* MTRNR2L3 OSBPL6* EPB41L2*	TMEM190* PSCA LDLRAD1 MUCL1*

*Differentially expressed genes identified in the total small airway epithelium

**Differentially expressed genes identified in the total small airway epithelium with opposite direction

[1] Pseudogenes were excluded. Only 9 and 18 gene were down-regulated in BC and intermediate cells, respectively.

[2] Up or down-regulated genes in smokers compared to nonsmokers. These genes were expressed in >10% of the cells in the corresponding cell populations in both nonsmokers and smokers, and the average expression levels of the expressing cells in the corresponding cell population in nonsmokers *vs* smokers were >0.25 (up-regulated genes) or <-0.25 (down-regulated genes). Bonferroni corrected $p<0.05$ was used as the cutoff.

Supplemental Table S7. Cell Numbers of Ciliated Cell Sub-populations in Each Individual

Sub-populations	Nonsmoker-1	Nonsmoker-2	Nonsmoker-3	Smoker-1	Smoker-2	Smoker-3
1	109	0	9	5	0	3
2	102	28	96	109	45	48
3	4	3	2	117	56	24
4	6	29	111	31	8	25

Supplemental Table S8. Smoking-induced Changes in Human Small Airway Cell-specific Expression of Genes-related to Lung Diseases

	BC		Intermediate		Club		Mucous		Ciliated		Ionocytes		T cells		Mast	
Lung diseases	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**	**Down[1]**	**Up[1]**
Monogenic lung disorders	RSPH1*					TGFBR2			OFD1*	C21orf59*		SFTPB*			EFEMP2*	
a		THSD4*		THSD4 PID1		TGFB2										
IPF			MUC5B*	CDKN1A	MUC5B*	CDKN1A	MUC5B*						MUC5B*			
Lung cancers		TP63*	B2M*	SLC34A2 EGFR MCL1 PTP4A1 PHF3 MYC KLF5 TPM3 NFE2L2	B2M FGFR3*	MDM2		EZR* TPM3*	B2M* CD74*	EZR*			B2M ZFP36L1		TP73* DOT1L* PDE4DIP* MAP2K1*	

[1] Up or down-regulated genes in smokers compared to nonsmokers. Bonferroni corrected p<0.05 was used as the cutoff.

* For those genes, the average expression levels of the expressing cells in the corresponding cell population in nonsmokers *vs* smokers were >0.25 (up-regulated genes) or <-0.25 (down-regulated genes)

Supplemental Table S9. Quality Control for the Single-cell RNA-sequencing Data in Each Individual[1]

Phenotype	Total reads	Mean reads/cell	Number of gene detected	Median genes/cell	Median UMI count/cell
Nonsmoker-1	112,793,786	91,405	29,816	736	1,390
Nonsmoker-2	131,807,942	107,686	31,800	896	1,517
Nonsmoker-3	67,651,975	29,842	30,250	769	1,259
Smoker-1	113,288,982	43,690	31,544	816	1,393
Smoker-2	47,156,371	23,472	28,539	830	1,465
Smoker-3	128,445,800	54,082	31,840	727	1,247

[1] Table reflects quality control data after applying filters during processing; UMI = unique molecular identifiers.

Supplemental Table S10 . Cell Numbers of Different Cell Populations in Each Individual

Cell type	Nonsmoker-1	Nonsmoker-2	Nonsmoker-3	Smoker-1	Smoker-2	Smoker-3
Basal	109	122	206	395	1112	417
Intermediate	266	248	577	513	444	590
Club	405	252	586	213	70	253
Mucous	18	141	11	542	153	255
Ciliated	221	60	218	262	109	100
Ionocytes	20	11	54	82	32	40
Neuroendocrine	0	0	146	0	0	0
T cell	171	244	436	418	45	651
Antigen-presenting	22	21	33	76	31	24
Mast	2	4	0	91	13	44
NCL^{high}	0	121	0	1	0	1

Supplemental Table S11. Effect of Gender *vs* Smoking on Small Airway Epithelium Gene Expression[1]

	Microarray[2]		RNA-Seq[3]	
Comparison[1]	**n of subjects[2]**	**n of genes[4]**	**n of subjects**	**n of genes[4]**
Nonsmoker M *vs* nonsmoker F	38 M *vs* 22 F	30	10 M *vs* 10 F	42
Smoker M *vs* smoker F	53 M *vs* 20 F	23	20 M *vs* 3 F	16
M *vs* F	91 M *vs* 42 F	40	30 M *vs* 13 F	68
Smoker *vs* nonsmoker	73 S *vs* 63 NS	3,408	23 S *vs* 20 NS	2,454

[1] Analysis of previously acquired datasets; M = males, F = females, NS = healthy nonsmokers, S = asymptomatic smokers.

[2] Small airway epithelial samples processed on Affymetrix HG-U133 Plus 2.0 microarrays (Affymtrix); previously published (GEO accession # 77658).

[3] Small airway epithelial samples processed on Illumina Hi Seq 2500 (Illumina); a subset of the samples has been previously published (GEO accession # 92661).

[4] N of genes differentially expressed when comparing the groups on a genome-wide basis [in microarray: n=14,465 genes (present in at least 20% of the samples in each group , one probe per gene, chosen based on Affymetrix specificity and sensitivity scores); in RNA-Seq: n=16,140 genes (FPKM >0.125)]; p value corrected for multiple tests (Benjamini-Hochberg) <0.05 considered significant.

Supplemental Figure Legends

Supplemental Figure S1. Violin plots of the expression of TP63 and MKI67 in the cells populations identified in Figure 1. The violin plots were constructed using imputed data. **A.** TP63. **B.** MKI67.

Supplemental Figure S2. Human small airway ionocyte transcriptome and comparison with the ionocytes from large airways. **A.** Gene Ontology (https://david.ncifcrf.gov/) analysis of the signature genes of human small ionocytes. **B.** Venn diagram of signature genes of ionocytes in human small airways *vs* large airways. The large airway ionocyte data is from Montoro et al [18]. Shown are the number of genes uniquely enriched in human large and/or small airways. Examples of the signature genes are indicated. **C.** Violin plot of POSTN expression in the cell populations from nonsmoker human small airway epithelium. The violin plots were constructed using imputed data. SAE = small airway epithelium, LAE = large airway epithelium.

Supplemental Figure S3. Expression of genes-associated with monogenic lung disorders in the cell populations of the healthy human small airway epithelium. **A-F.** Genes associated with monogenic lung disorders divided by different categories: **A, B.** Primary ciliary dyskinesia; **C.** Cystic fibrosis and other bronchiectasis; **D.** α1-antitrypsin deficiency, Birt-Hogg Duke syndrome, cutis laxa, Ehlers-Danlos syndrome, lymphangioleiomyomatosis, Loey-Diez syndrome, and Marfan syndrome; **E.** Familial fibrosis, Fibrosis and hypothyroidism, Hermansky-Pudlak syndrome, pulmonary alveolar proteinosis, surfactant deficiency; **F.** Pulmonary hypertension with arteriovenous malformations and hereditary hemorrhagic telangiectasia, pulmonary hypertension with hereditary hemorrhagic telangiectasia, pulmonary hypertension, syndromic hypoventilation, hypereosinophilic syndrome, and hyper IgE syndrome. The genes are shown on the x-axis. The identities of the cell populations are shown on the y-axis. The size of the dots represents the fractions of the expressing cells in each cell population, and the color intensity represents the average expression level. **G-L.** Violin plots of the selected monogenic lung disorder genes in the cell populations from nonsmoker human SAE. The violin plots were constructed using imputed data. **G.** SERPINA1 (α1-antitrypsin deficiency); **H.** CFTR (cystic fibrosis); **I.** SCNN1B (bronchiectasis); **J.** RSPH9 (primary ciliary dyskinesia); **K.** DOCK8 (hyper IgE syndrome); and **L.** DTNBP1 (Hermansky-Pudlak syndrome).

Supplemental Figure S4. Violin plots of expression of genes associated with risk for chronic obstructive pulmonary disease (COPD) in the cell populations from healthy human small airway epithelium. The violin plots were constructed using imputed data. **A, B.** Definite COPD risk genes; **C-E.** Probable COPD risk genes. **A.** FAM13A; **B.** DSP; **C.** ARMC2; **D.** CFDP1; and **E.** TET2.

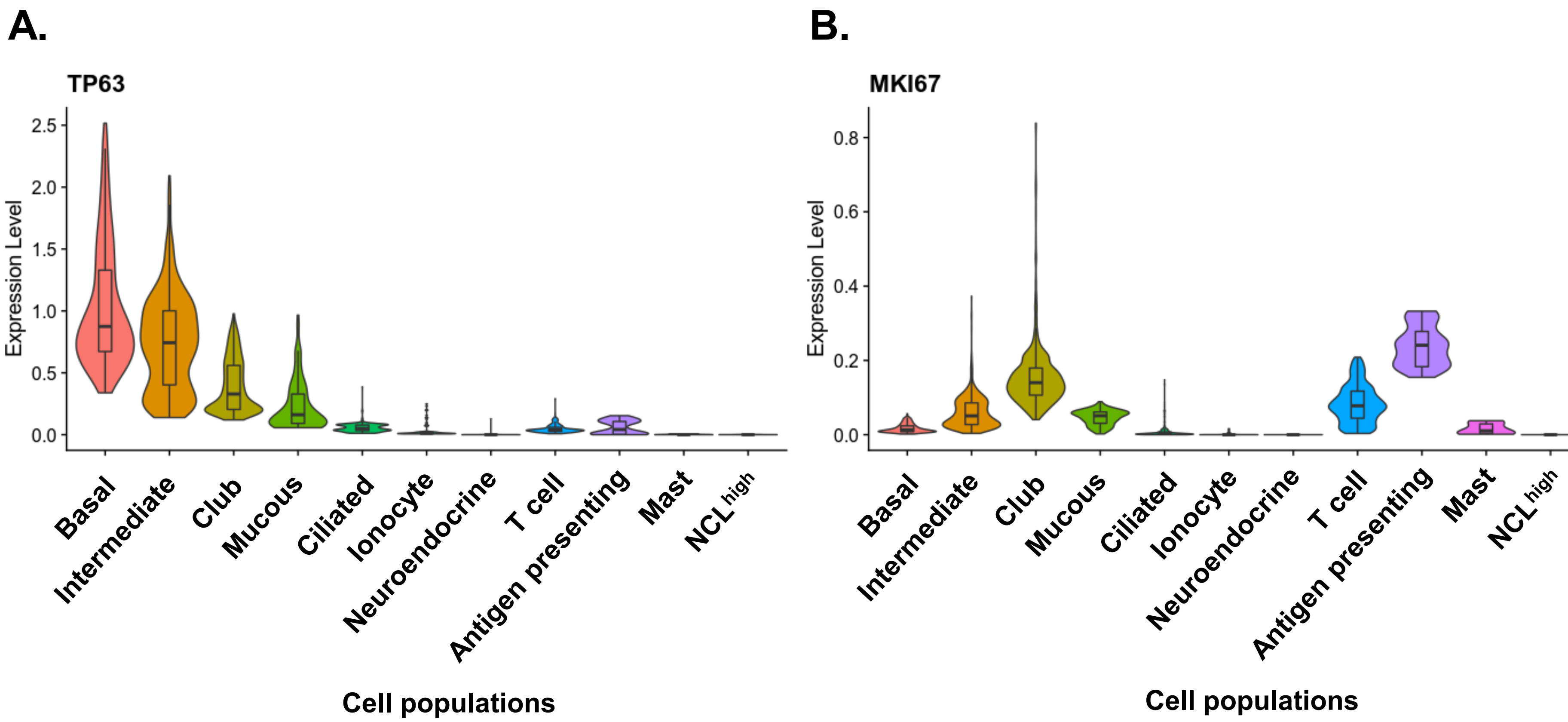
Supplemental Figure S5. Expression in the healthy human small airway epithelium cell populations of genes associated with risk for idiopathic pulmonary fibrosis (IPF). Genes associated with IPF are divided by 3 categories: **A.** alveolar stability and telomere length; **B.** immunity and inflammation; and **C.** common genetic variation. The gene symbols are listed on the x-axis, the identities of the cell populations are shown in y-axis. The size of the dots represents the fractions of the expressing cells in each cell population, and the color intensity represents the average expression level. **D-I.** Violin plots of genes related to risk for IPF in the cell populations from healthy human SAE. The violin plots were constructed using imputed data. **D.** MUC5B; **E.** MUC2; **F.** TGFB1; **G.** CDKN1A; **H.** HSPA1L; and **I.** HLA-DRB1.

Supplemental Figure S6. Violin plots of examples of small airway epithelium cell-specific expression of "driver" genes which participate in the development of lung cancer. The violin plots were constructed using imputed data. **A.** EGFR; **B.** KRAS; **C.** MET; **D.** TP53; **E.** KIF5B; and **F.** SOX2.

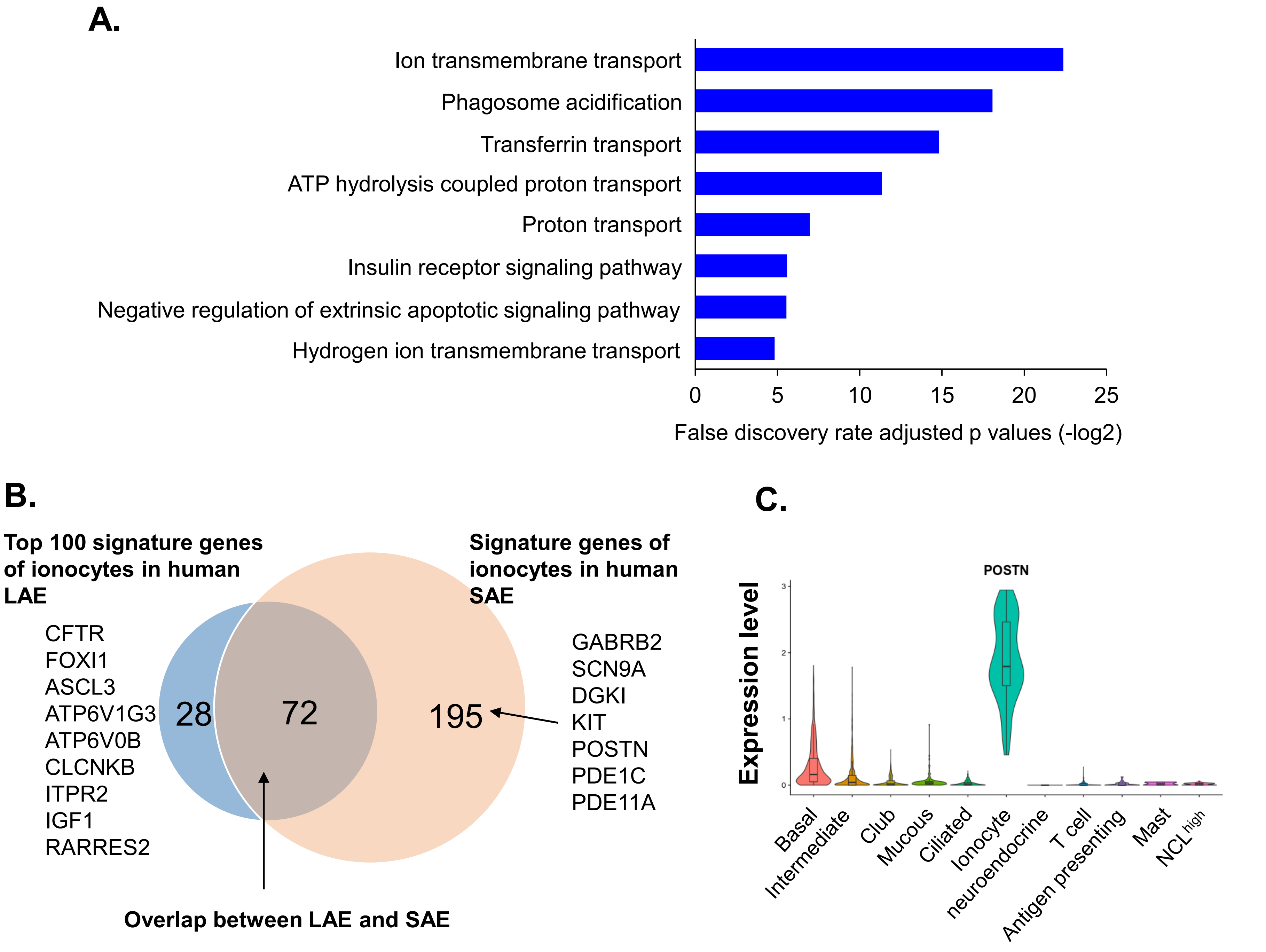
Supplemental Figure S7. Cigarette smoking-associated dysregulated genes in the minor cells populations from human small airway epithelium of nonsmokers *vs.* smokers . Volcano plots show the down-regulated (left) and up-regulated genes (right) in smokers in ionocytes, T cells, mast cells, and antigen presenting cells. Y-axis represents the negative p value (log) and the x-axis represents the fold-change (log). The cutoff is shown as dotted lines. Fold-change (log) >0.25 for up-regulated genes or < –0.25 for down-regulated genes, p value <0.05 with Bonferroni correction. NS = nonsmokers, S = smokers.

Supplemental Figure S8. Impact of cigarette smoking on gene expression in the ciliated cell sub-populations of the human SAE. **A.** Unsupervised t-SNE clustering identifies 4 unique ciliated cell sub-populatFhions in nonsmokers (left) *vs* smokers (right). **B.** Fractions of ciliated cell sub-populations in nonsmokers *vs* smokers in each individual, nonsmoker (n=3) *vs* smoker (n=3). **C.** Dot plots of gene expressions in the ciliated cell sub-populations. The ciliated cell sub-populations are shown on the y-axis, and the gene symbol and detailed categories of the genes are shown on the x-axis. The size of the dots represents the fraction of the expressing cells in each cell population. The color represents the average gene expression in positive cells.

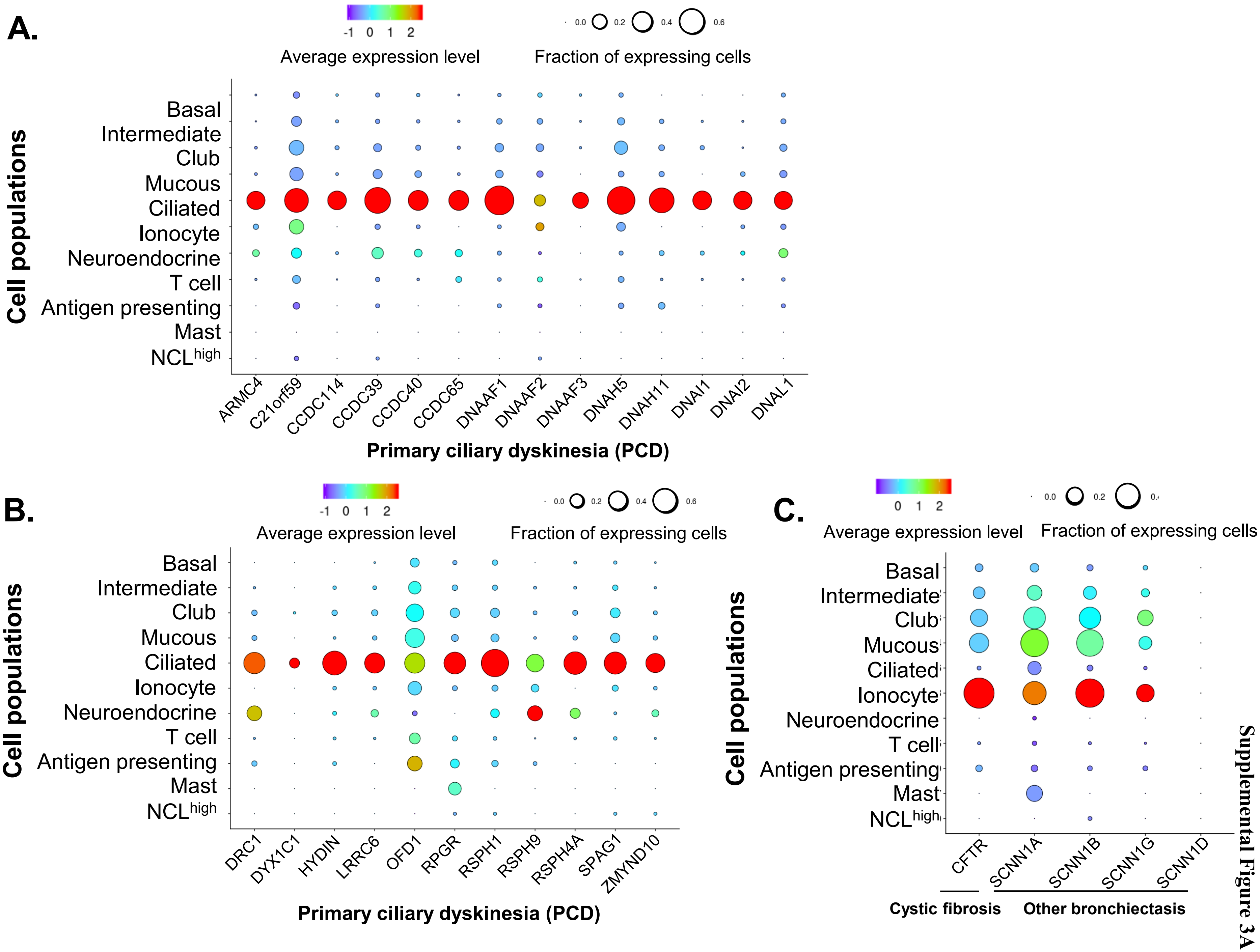
Supplemental Figure S9. Single-cell RNA sequencing identifies 11 unique cell populations from human SAE of 6 individuals. **A.** t-SNE plots of the single cells from each individuals. **B.** t-SNE plots of the single cells from 4 different technical batches. Batch 1 – nonsmoker 1 + smoker 1; batch 2 – nonsmoker 2; batch 3 – nonsmoker 3 + smoker 2; batch 4 – smoker 3. NS = nonsmokers, S = smokers.



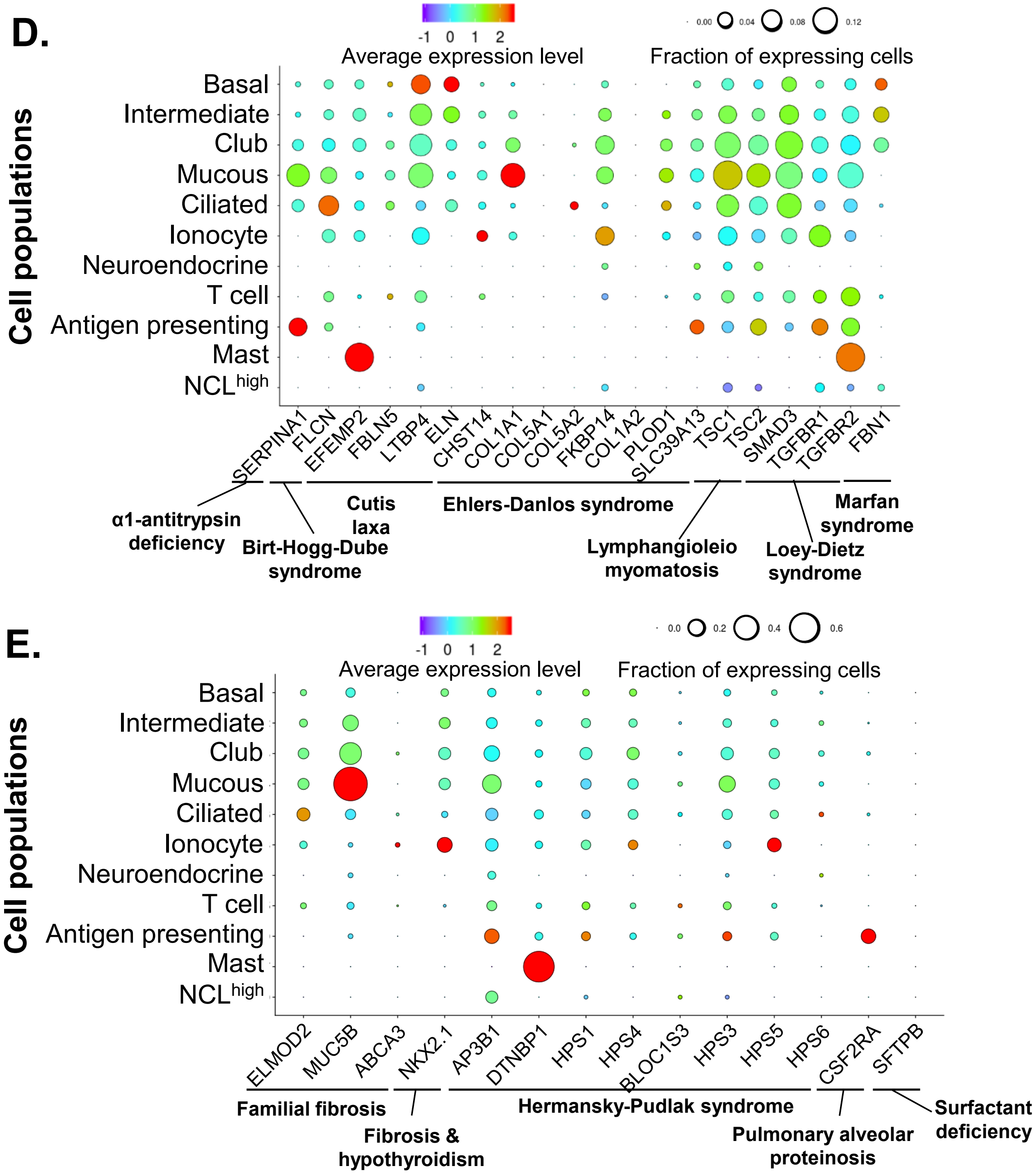
Supplemental Figure 1



Supplemental Figure 2

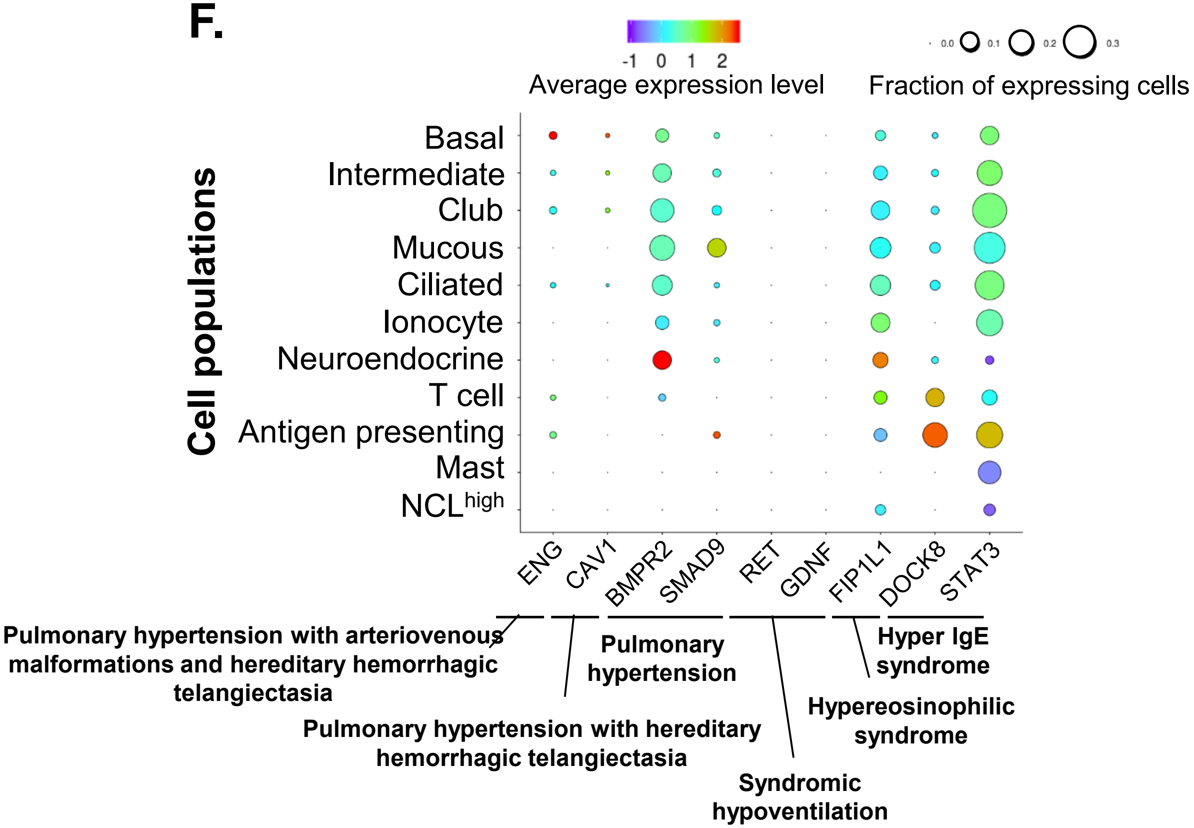


Supplemental Figure 3A

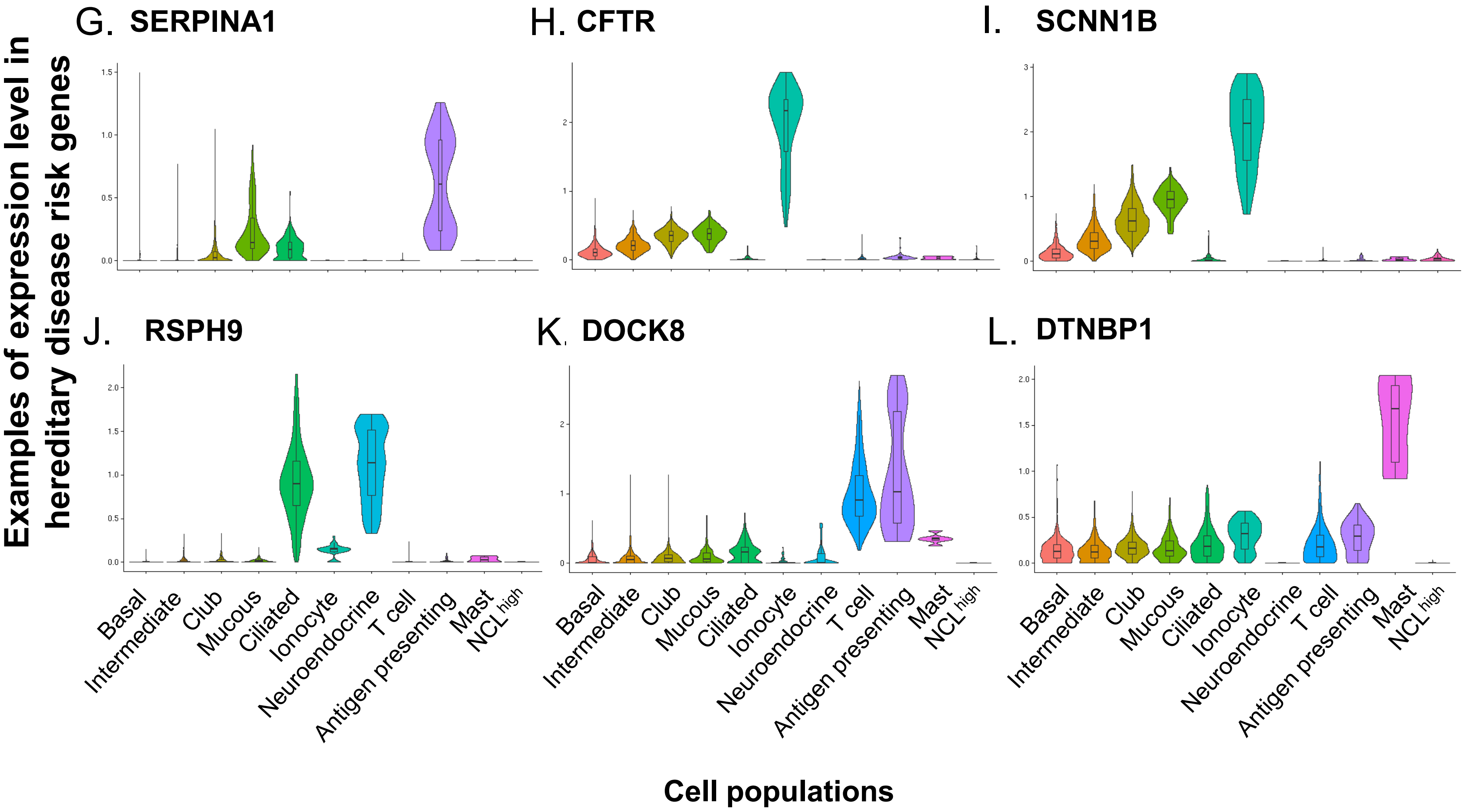


Supplemental Figure 3B

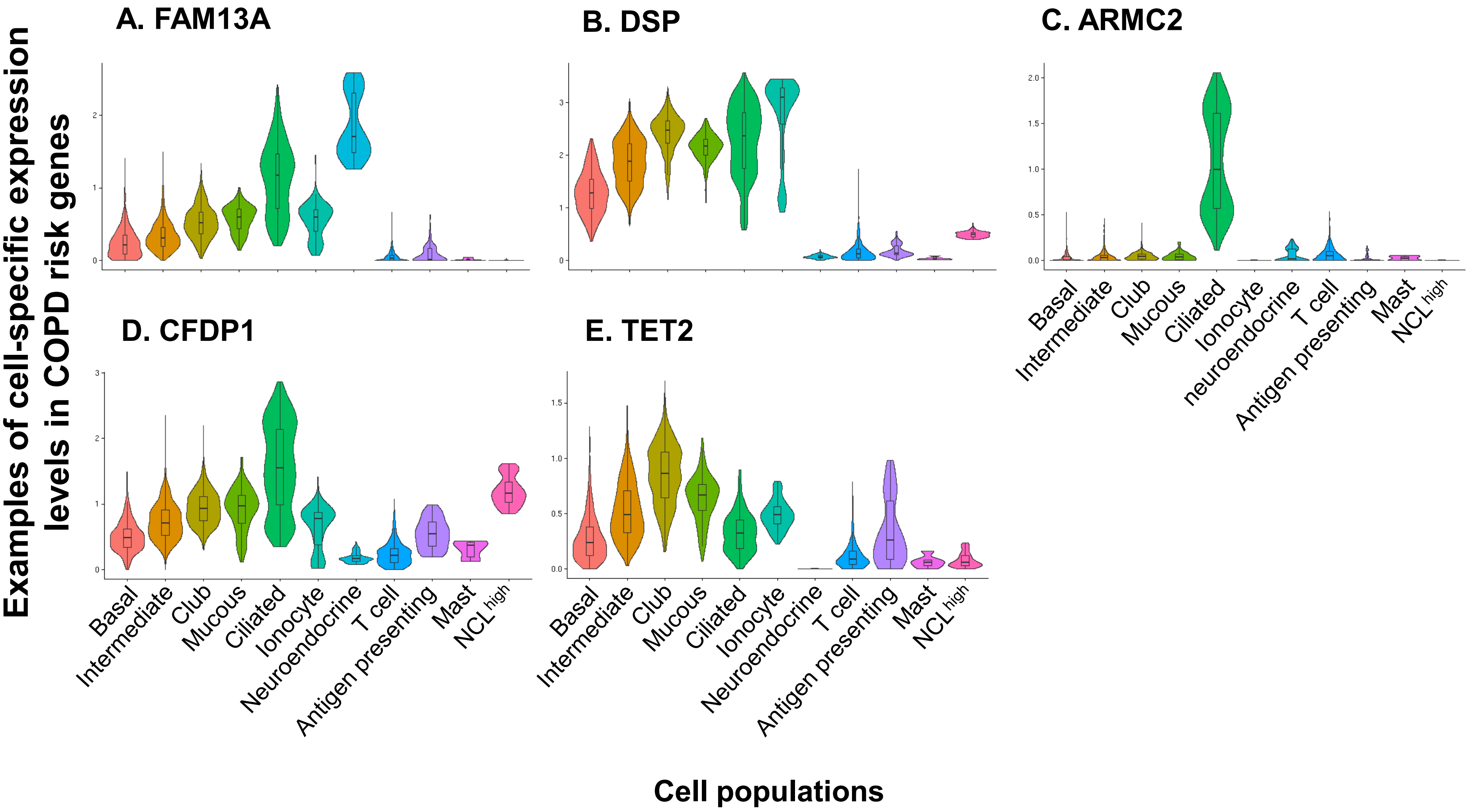
F.



Supplemental Figure 3C



Supplemental Figure 3D


A. FAM13A

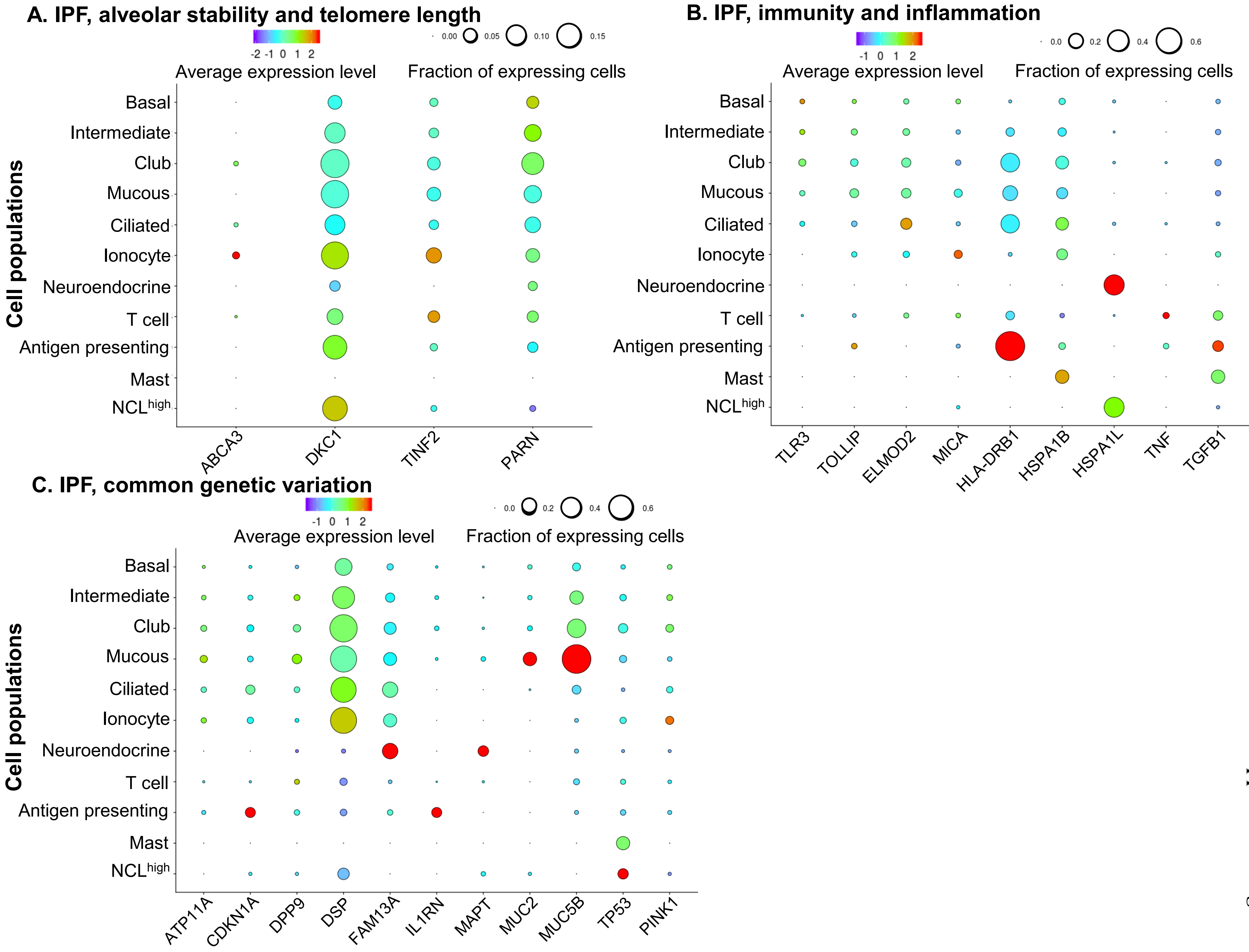
B. DSP

C. ARMC2

D. CFDP1

E. TET2

Supplemental Figure 4

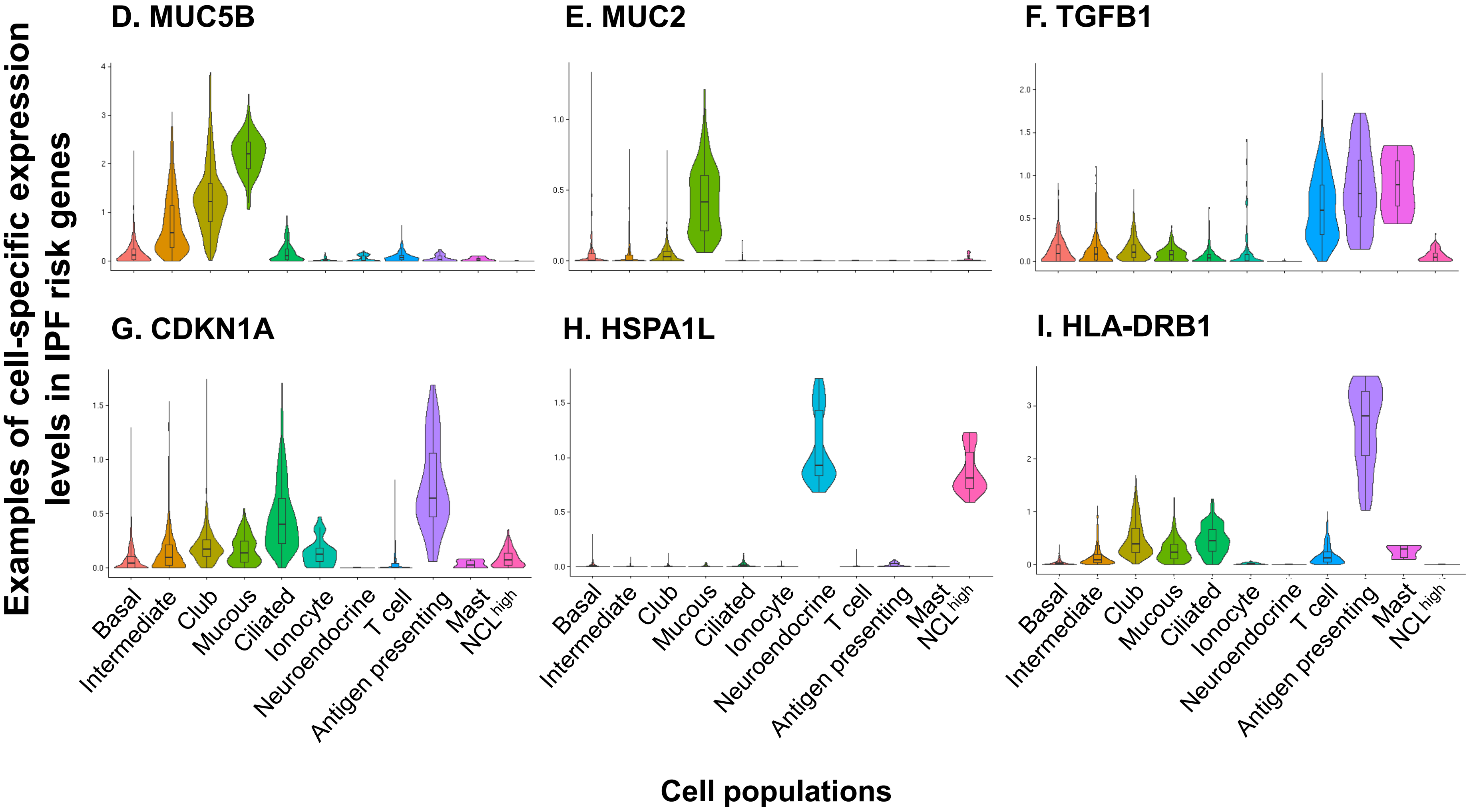


A. IPF, alveolar stability and telomere length

B. IPF, immunity and inflammation

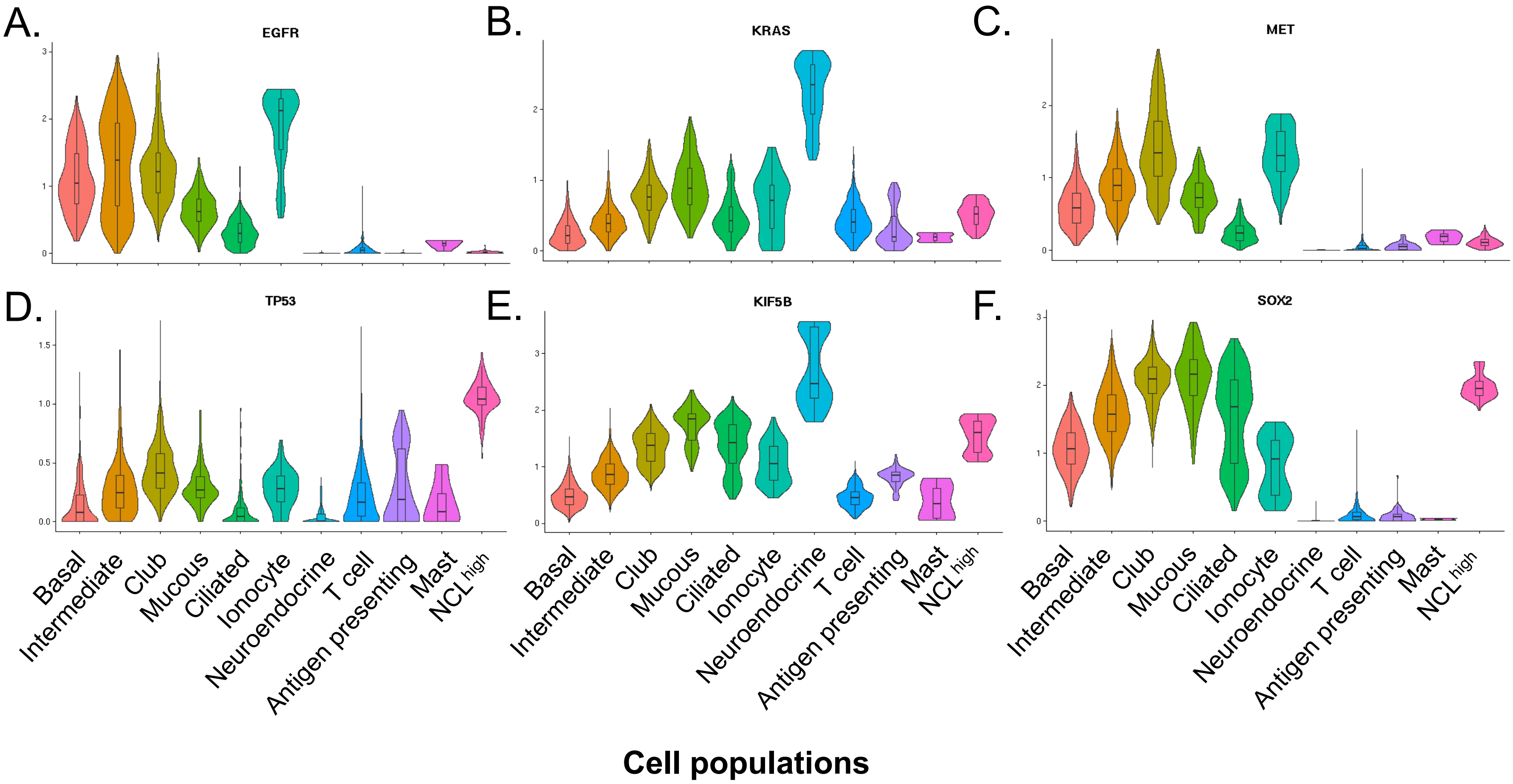
C. IPF, common genetic variation

Supplemental Figure 5



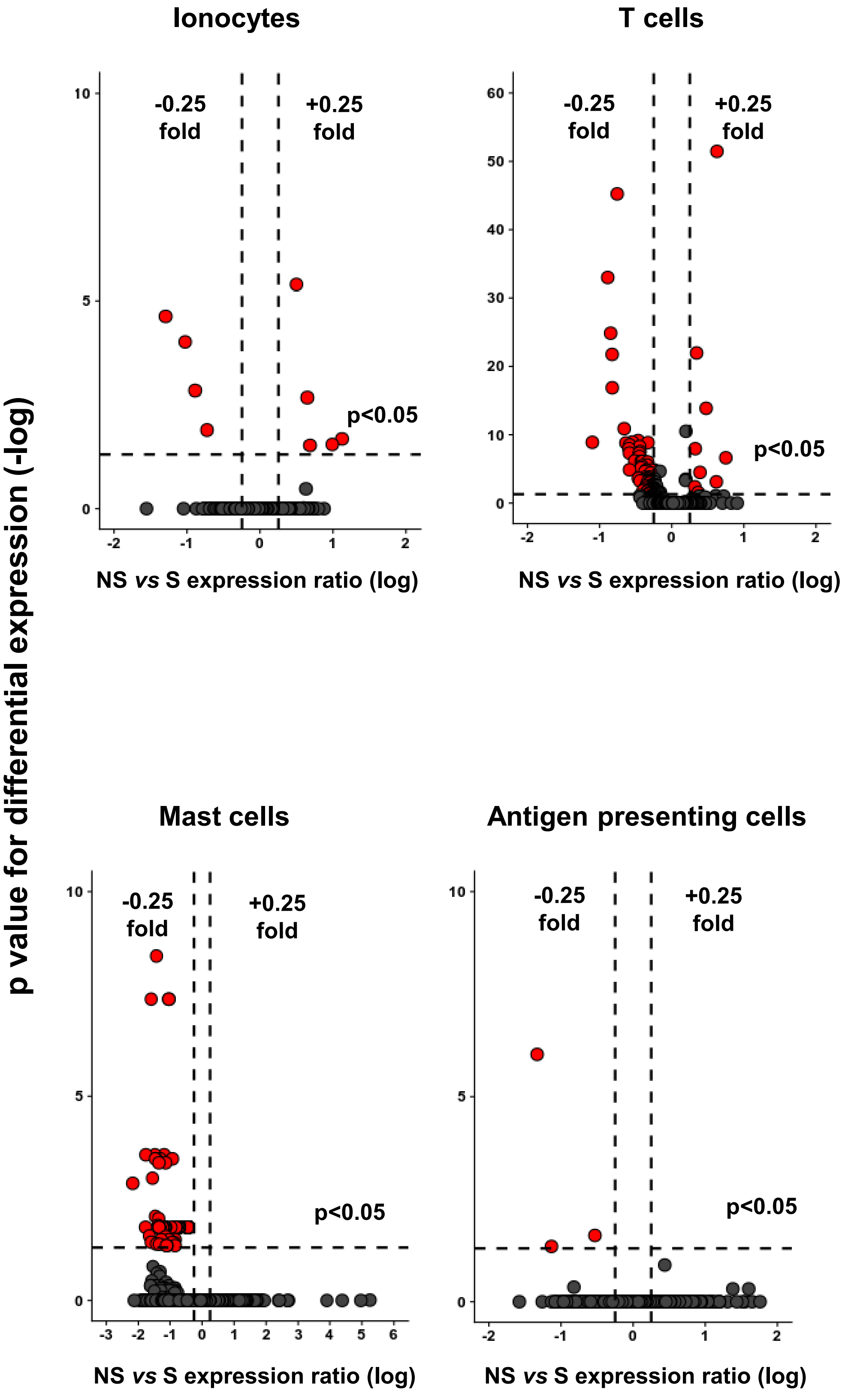
Supplemental Figure 5

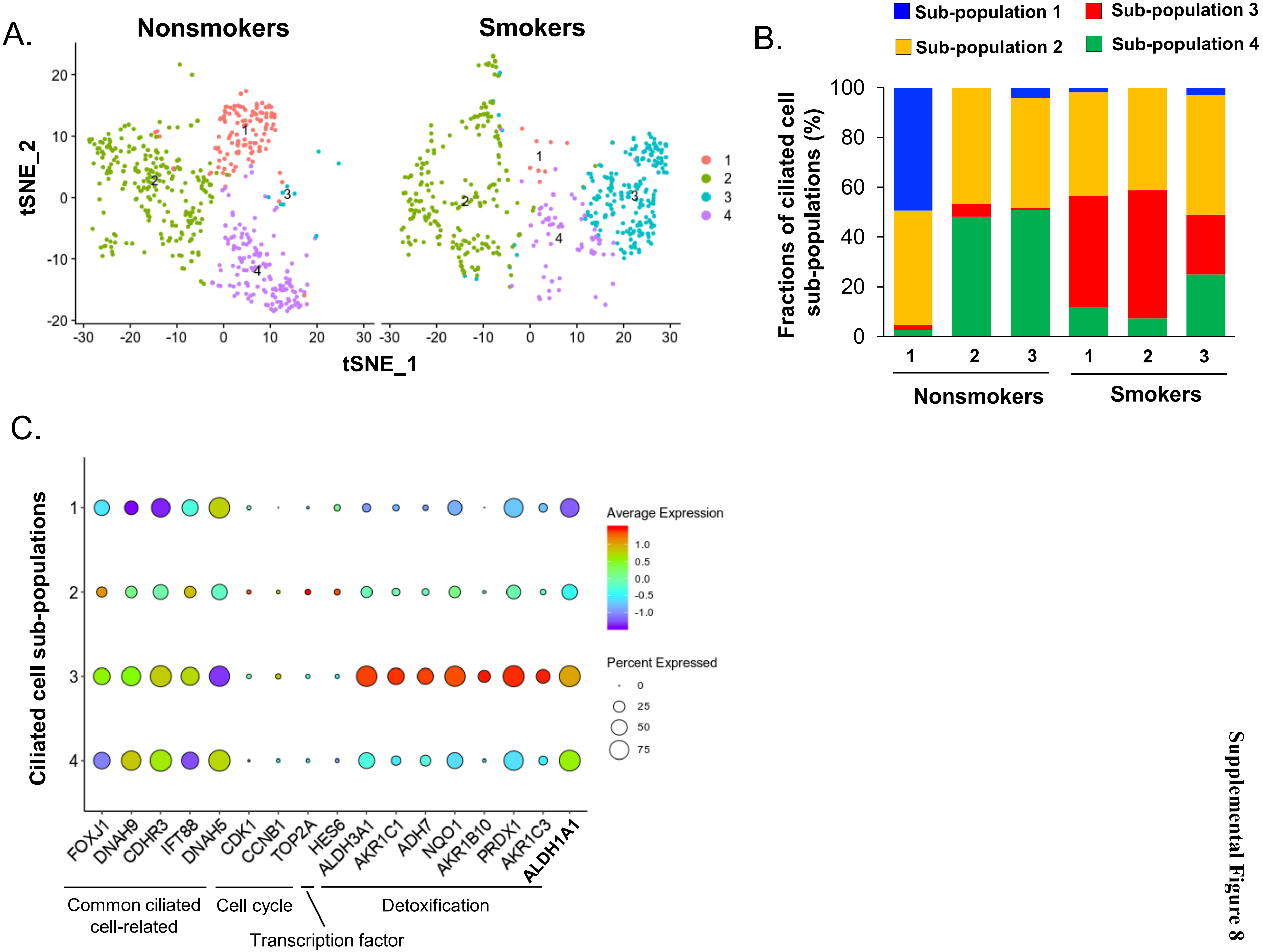
Examples of cell-specific expression levels of “driver” genes that if mutated participate in the development of lung cancer



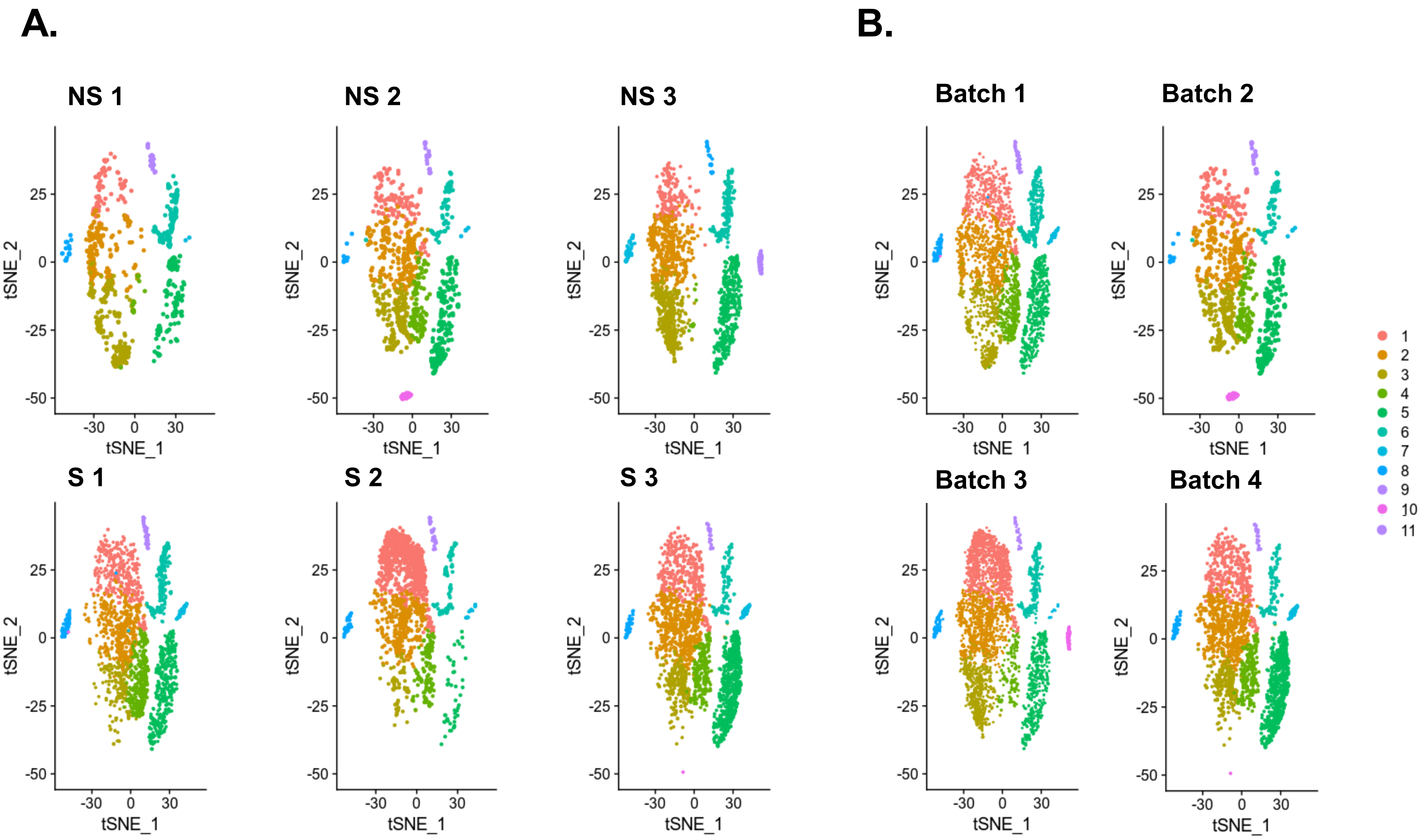
Supplemental Figure 6

Supplemental Figure 7





Supplemental Figure 8



Supplemental Figure 9