Supplemental Table 1: List of gene panels used in Xenium spatial transcriptomics

1	A2M	44	C1R	87	CD80	130	CXCL2	173	FOXA3
2	ABCA3	45	CA4	88	CD84	131	CXCL5	174	FOXI1
3	ACE	46	CADM1	89	CD86	132	CXCL9	175	FOXJ1
4	ACKR1	47	CALD1	90	CD8A	133	CXCR3	176	FOXP3
5	ACOXL	48	CAMP	91	CD8B	134	CXCR5	177	FZD5
6	ACTA2	49	CARD11	92	CDH2	135	CXCR6	178	FZD6
7	ACTG2	50	CASC15	93	CDK1	136	CYP4B1	179	GATA3
8	ADAM17	51	CAV1	94	CDKN1A	137	DAPK2	180	GDF15
9	ADAM28	52	CCDC78	95	CDKN2A	138	DCDC1	181	GFPT2
10	ADAMTS1	53	CCL17	96	CEACAM6	139	DDIT3	182	GLDN
11	ADGRL2	54	CCL18	97	CEACAM8	140	DDIT4	183	GLI1
12	AFF3	55	CCL2	98	CEMIP2	141	DEFB1	184	GLIPR2
13	AGER	56	CCL20	99	CENPF	142	DES	185	GNG2
14	AGR2	57	CCL22	100	CENPW	143	DKK2	186	GPD1
15	AGRP	58	CCL26	101	CFAP299	144	DMBT1	187	GPI
16	AIF1	59	CCL3	102	CFTR	145	DNAH12	188	GPNMB
17	ALOX15	60	CCL4L2	103	CHIT1	146	DNAJB9	189	GPR171
18	ALOX15B	61	CCL5	104	CLCA1	147	EDN1	190	GPR183
19	ANK3	62	CCNB2	105	CLDN4	148	EDNRB	191	GPR34
20	ANKRD33B	63	CCR3	106	CLDN5	149	EHF	192	GPRC5A
21	ANPEP	64	CCR6	107	CLEC10A	150	EOMES	193	GPSM2
22	AOAH	65	CCR7	108	CLEC12A	151	EPCAM	194	GRP
23	APOD	66	CCR8	109	CLEC4E	152	EREG	195	GSN
24	APOE	67	CD14	110	CLIC5	153	ERN1	196	GZMA
25	AQP9	68	CD163	111	CLU	154	ERN2	197	GZMB
26	AREG	69	CD19	112	CNOT6L	155	ETV5	198	GZMK
27	ARG1	70	CD1A	113	COL1A1	156	FABP3	199	HAS1
28	ARG2	71	CD1C	114	COL4A1	157	FABP4	200	HAVCR2
29	ASPN	72	CD2	115	COL8A1	158	FABP5	201	HERPUD1
30	ATF4	73	CD24	116	COX4I2	159	FAM20C	202	HHIP
31	ATP10A	74	CD247	117	CP	160	FAS	203	HMGCS1
32	ATP1B1	75	CD27	118	CPE	161	FASLG	204	HP
33	AUTS2	76	CD274	119	CST1	162	FASN	205	HPGDS
34	BACH2	77	CD28	120	CST4	163	FCER1A	206	HSPA1B
35	BANK1	78	CD300E	121	CST7	164	FCGR1A	207	HSPA5
36	BCHE	79	CD38	122	CSTA	165	FCGR3A	208	ICOS
37	BICC1	80	CD3D	123	CTHRC1	166	FCMR	209	IFI27
38	BPIFB1	81	CD3E	124	CTLA4	167	FCN1	210	IFI6
39	BTNL9	82	CD4	125	CTSK	168	FCN3	211	IFNB1
40	C15orf48	83	CD40	126	CTSL	169	FFAR4	212	IFNG
41	C1QA	84	CD68	127	CXCL10	170	FKBP11	213	IGF1
42	C1QB	85	CD70	128	CXCL12	171	FN1	214	IGFBP3
43	C1QC	86	CD79A	129	CXCL14	172	FOXA2	215	IGFBP6

216	IKZF2	259	KLRD1	302	MMRN1	345	PROX1	388	SOSTDC1
217	IL10RA	260	KRT14	303	MPEG1	346	PTGDR2	389	SPARC
218	IL12B	261	KRT15	304	MS4A1	347	PTGS1	390	SPIB
219	IL13	262	KRT17	305	MS4A2	348	RBP4	391	SPP1
220	IL15	263	KRT19	306	MS4A4A	349	RETN	392	ST6GAL1
221	IL17A	264	KRT5	307	MSR1	350	RGS1	393	STAC
222	IL17RB	265	KRT7	308	MT1E	351	RGS16	394	STAT3
223	IL18	266	KRT8	309	MT1G	352	RGS5	395	STEAP4
224	IL18R1	267	LAD1	310	MT1M	353	RHOH	396	SYK
225	IL1B	268	LAMA3	311	MT1X	354	RNF125	397	SYNE1
226	IL1R2	269	LAMP3	312	MUC1	355	RORC	398	TAGLN
227	IL1RL1	270	LCK	313	MUC16	356	RSPH1	399	TBX21
228	IL25	271	LCN2	314	MUC5AC	357	RUNX3	400	TCF7
229	IL2RA	272	LEPR	315	MUC5B	358	S100A12	401	TCL1A
230	IL32	273	LGMN	316	MXRA8	359	S100A2	402	TFF2
231	IL33	274	LILRA4	317	MYH11	360	S100A3	403	TFF3
232	IL3RA	275	LILRA5	318	MYL9	361	S100B	404	TGFBI
233	IL4	276	LILRB2	319	MZB1	362	SAA1	405	THBS1
234	IL5	277	LILRB4	320	NEBL	363	SAMD3	406	TMEM100
235	IL5RA	278	LIPA	321	NGFR	364	SCEL	407	TMEM45A
236	IL6	279	LMNA	322	NIBAN1	365	SCGB3A2	408	TNFRSF13B
237	IL7	280	LRRIQ1	323	NKG7	366	SCN7A	409	TNFRSF13C
238	IL7R	281	LSAMP	324	NLRP3	367	SCT	410	TNFRSF17
239	IL9	282	LTF	325	NR4A1	368	SDF2L1	411	TNFRSF18
240	INHBA	283	LYVE1	326	OTOA	369	SDS	412	TNFRSF9
241	INMT	284	MAF	327	P2RX1	370	SEC61A1	413	TNFSF13B
242	IQGAP2	285	MAGI1	328	PBX4	371	SELL	414	TOP2A
243	IRF4	286	MANF	329	PCOLCE2	372	SEMA3C	415	TOX
244	IRF8	287	MARCO	330	PDCD1	373	SERPINB2	416	TP63
245	ITGA6	288	MATK	331	PENK	374	SFRP2	417	TREM2
246	ITGA8	289	MCEMP1	332	PI16	375	SFRP4	418	TSLP
247	ITGAE	290	MCTP2	333	PIM2	376	SFTPA2	419	TSPAN1
248	ITGAM	291	MEDAG	334	PLA2G2A	377	SFTPD	420	TSPAN8
249	ITGAV	292	MFAP5	335	PLA2G4F	378	SH3RF3	421	UBE2C
250	ITGB1	293	MFSD2A	336	PLA2G7	379	SHH	422	VCAN
251	ITGB4	294	MGP	337	PLCB1	380	SIGLEC8	423	VEGFD
252	ITGB6	295	MIS18BP1	338	PLD5	381	SIRPA	424	VSIG4
253	ITGB8	296	MKI67	339	PLIN2	382	SLC16A1	425	VWF
254	ITK	297	MMP1	340	PLVAP	383	SLC18A2	426	WIF1
255	KIAA1217	298	MMP11	341	POSTN	384	SLC1A3	427	XBP1
256	KIT	299	MMP12	342	POU2AF1	385	SLCO2A1	428	XCR1
257	KLRB1	300	MMP7	343	PPP1R16B	386	SMIM24	429	YES1
258	KLRC1	301	MMP9	344	PRDM1	387	SOCS1	430	ZNF385D

Supplemental Table 2: List of antibodies used for cell sorting FACS, western blot, and immunofluorescence.

Antibodies used for FACS analysis

Name	Catalog number	Company	Dilution
Live dead draq7	7406	Cell Signaling	
CD45	557833	BD Biosciences	1:200
CD31	563653	BD Biosciences	1:200
CD11b	557754	BD Biosciences	1:200
Epcam CD326	324206	BD Biosciences	1:200
HTII-280	TB-27AHT2-280	Terrace Biotech	1:100
Alexa Fluor 488 goat anti IgM	A21042	Invitrogen	1:1000
CD66c	12-0667-42	Invitrogen	1:100
CD49f	313610	Biolegend	1:100
NGFR	345108	Biolegend	1:100

Antibodies used for Immunofluorescence

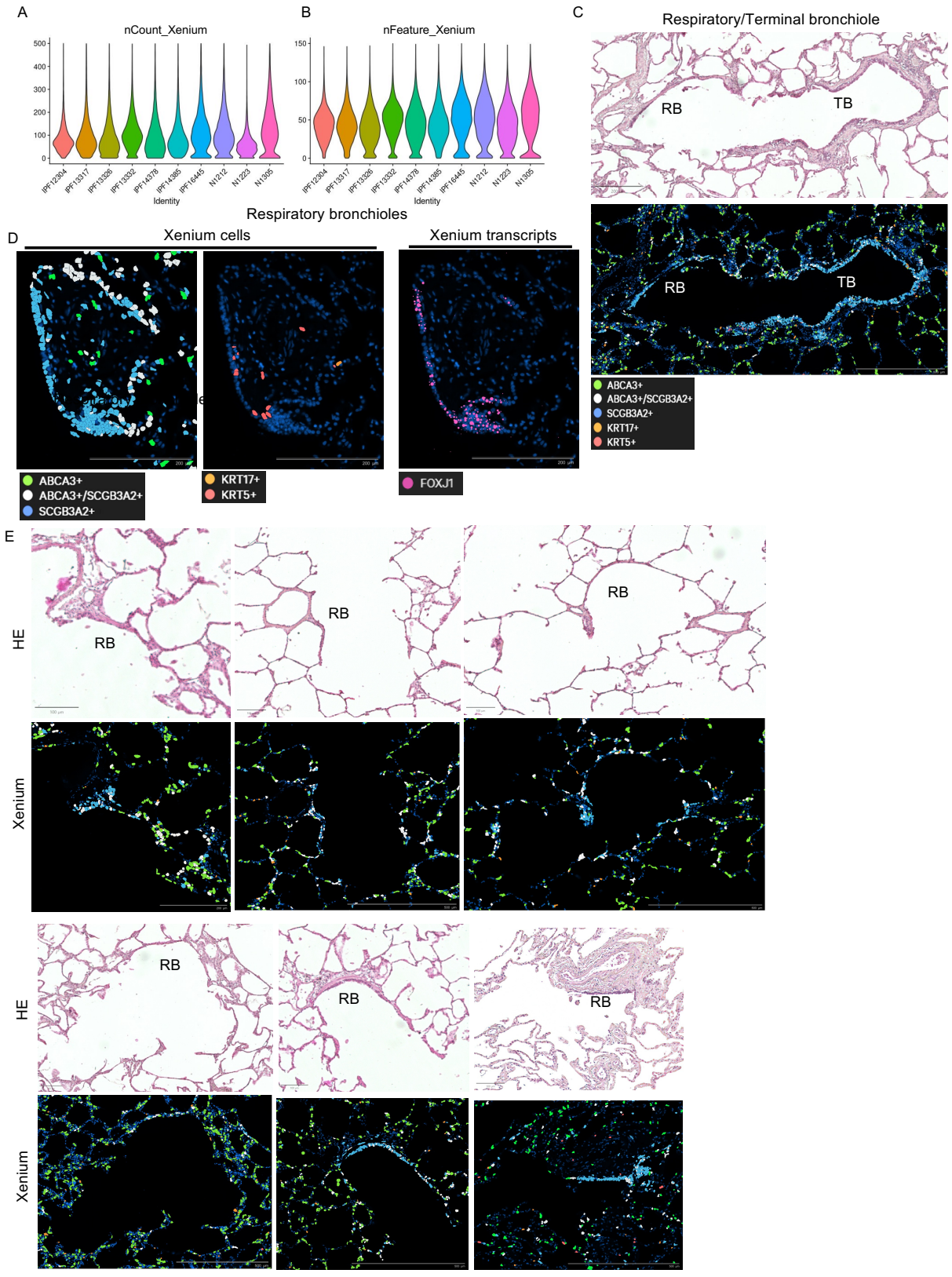
Name	Catalog number	Company	Dilution
Anti-Prosurfactant Protein C (proSP-C) Antibody	AB3786	Millipore	1:2500
Cytokeratin 17 (E-4)	sc393002	Santa Cruz Biotechnology	1:500
Cytokeratin 5	MA5-12596	Thermo Fisher	1:100
HTII-280	TB-27AHT2-280	Terrace Biotech	1:100
SCGB3A2	AF3545	R&D	1:100
Collagen I	1310-01	Southern Biotech	1:200
B-CHP	BIO60	3Helix	20uM

Antibodies used for Western Blot

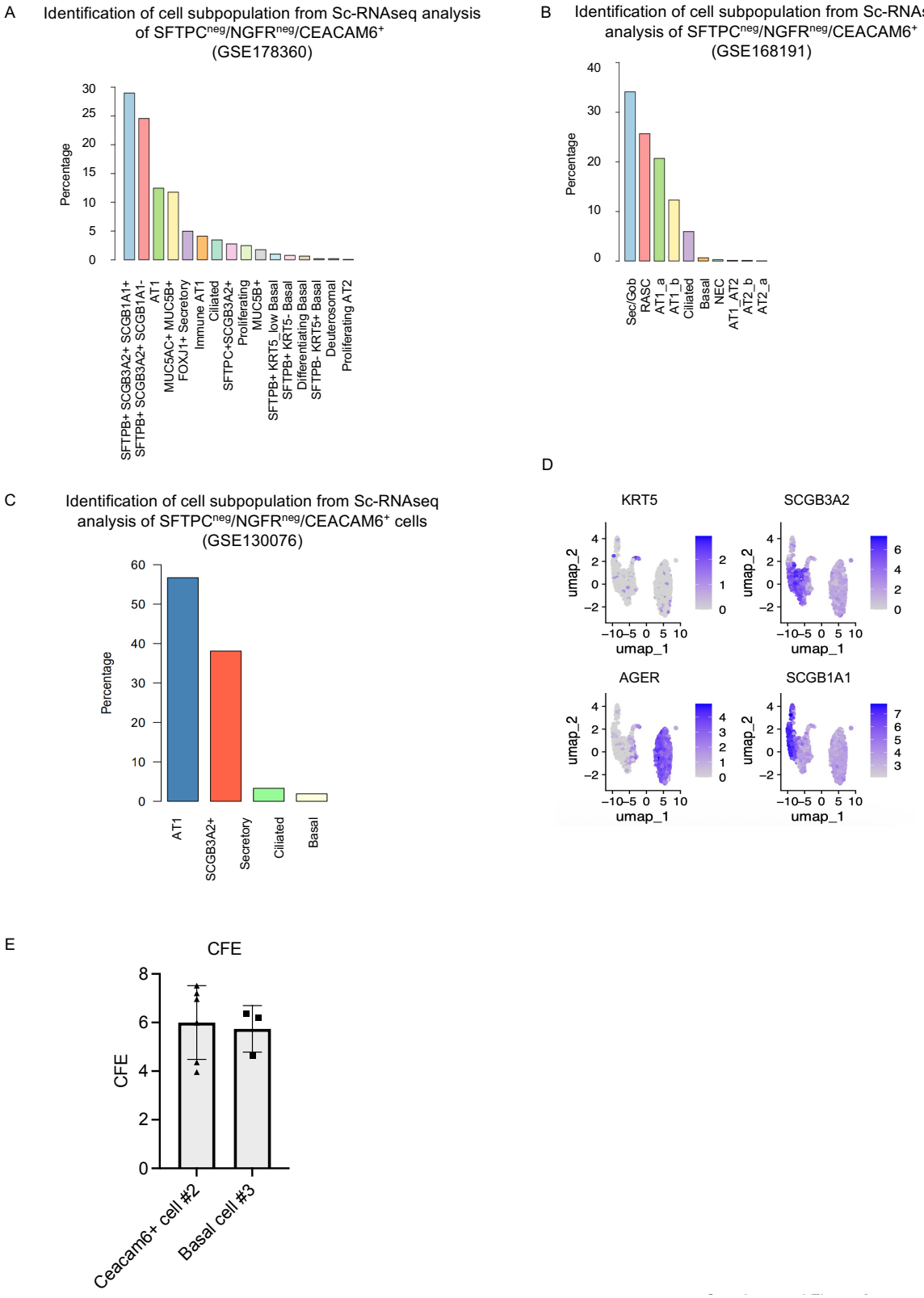
Name	Catalog number	Company	Dilution
β-Actin	A5441	Sigma-Aldrich	1:5000
FZD5	MA5-17080	ThermoFisher	1:500
NFATC3	sc-8405	Santa Cruz Biotechnology	1:200
NUP62	13916-1-AP	ThermoFisher	1:1000
Collagen I	PA1-26204	ThermoFisher	1:1000
GAPDH (0411)	sc-47724	Santa Cruz	1:200

Supplemental Table 3: List of human primers used for qPCR

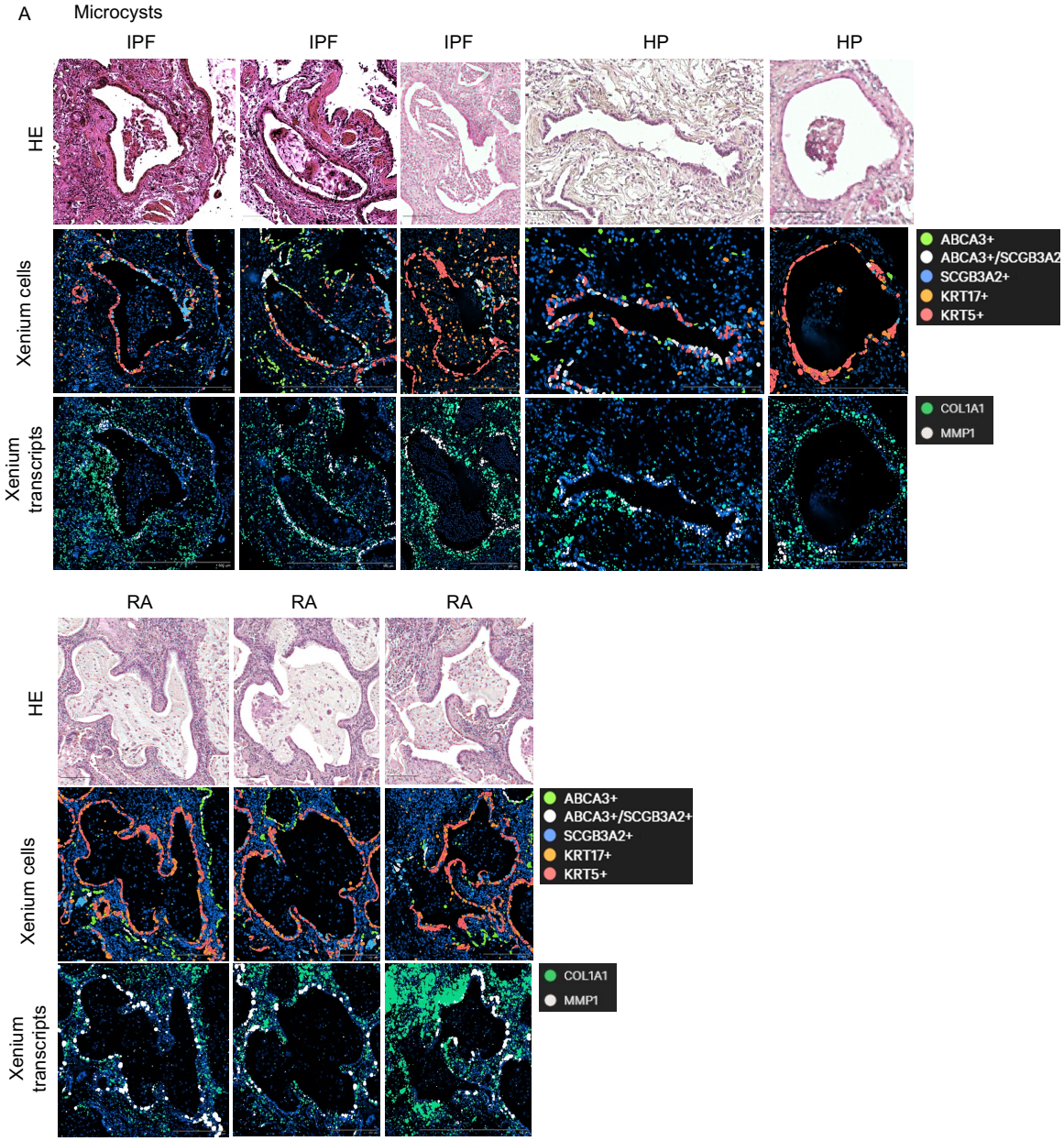
Target gene	**Forward (5'–3')**	**Reverse (5'–3')**
PPIA	GCA TAC GGG TCC TGG CAT CT	ACT TTG CCA AAC ACC ACA TGC T
SFTPC	GTG CCT ACG TCT AAG CTG GG	TTC CAC TGA CCC TGC TCA CC
ABCA3	CAT TAG GTA CGA CAA CTG CTC	TTT CTC CGT GTG TAA CTG AAC C
AXIN2	TAC ACT CCT TAT TGG GCG ATC A	TTG GCT ACT CGT AAA GTT TTG GT
ETV5	GTG TTG TGC CTG AGA GAC TGG A	CGA CCT GTC CAG GCA ATG AAG T
KRT5	GGA ATG CAG ACT CAG TGG AGA	GCT GCT GGA GTA GTA GCT TCC
KRT17	GGT GGG TGG TGA GAT CAA TGT	CGC GGT TCA GTT CCT CTG TC
TP63	GCC ATG CCC AGT ATG TAG AAG	TCA TCC CTC CAA CAC AAC TG
NGFR	CCT CAT CCC TGT CTA TTG CTC C	GTT GGC TCC TTG CTT GTT CTG C
MMP1	ATG AAG CAG CCC AGA TGT GGA G	TGG TCC ACA TCT GCT CTT GGC A
SCGB3A2	GGC TAA GGA AGT GTG TAA ATG AGC	CCA TCC ACC TCC GCT CTT TAT C

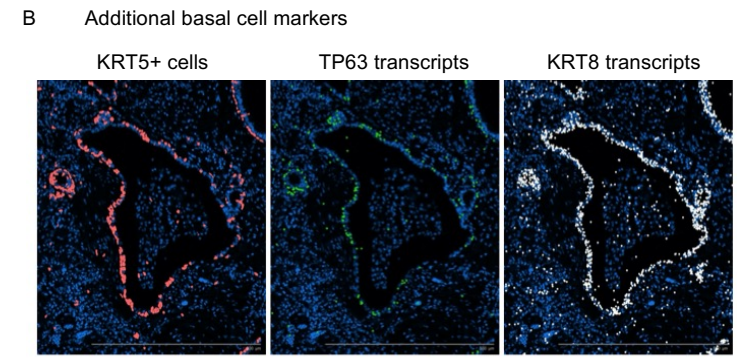


Supplemental Figure 1. (A) Number of transcripts per cell (nCount) and (B) number of genes per cell (nFeature) after quality filtering from Xenium assay. (C) Representative HE image of a respiratory bronchiole (RB) and terminal bronchiole (TB) in normal human lungs and with annotated cells from Xenium assay. (D) Respiratory bronchiole with annotated cells from Xenium assay with corresponding cell type annotations from Xenium assay with ABCA3^{pos}, ABCA3^{pos}/SCGB3A2^{pos}, SCGB3A2^{pos} cells (left panel), KRT17^{pos}, KRT5^{pos} cells (middle panel), and *FOXJ1* transcripts (right panel). (E) Selection of HE staining of six RBs (top panels) and with annotated cells from Xenium assay with corresponding cell type annotations from Xenium assay (bottom panels).

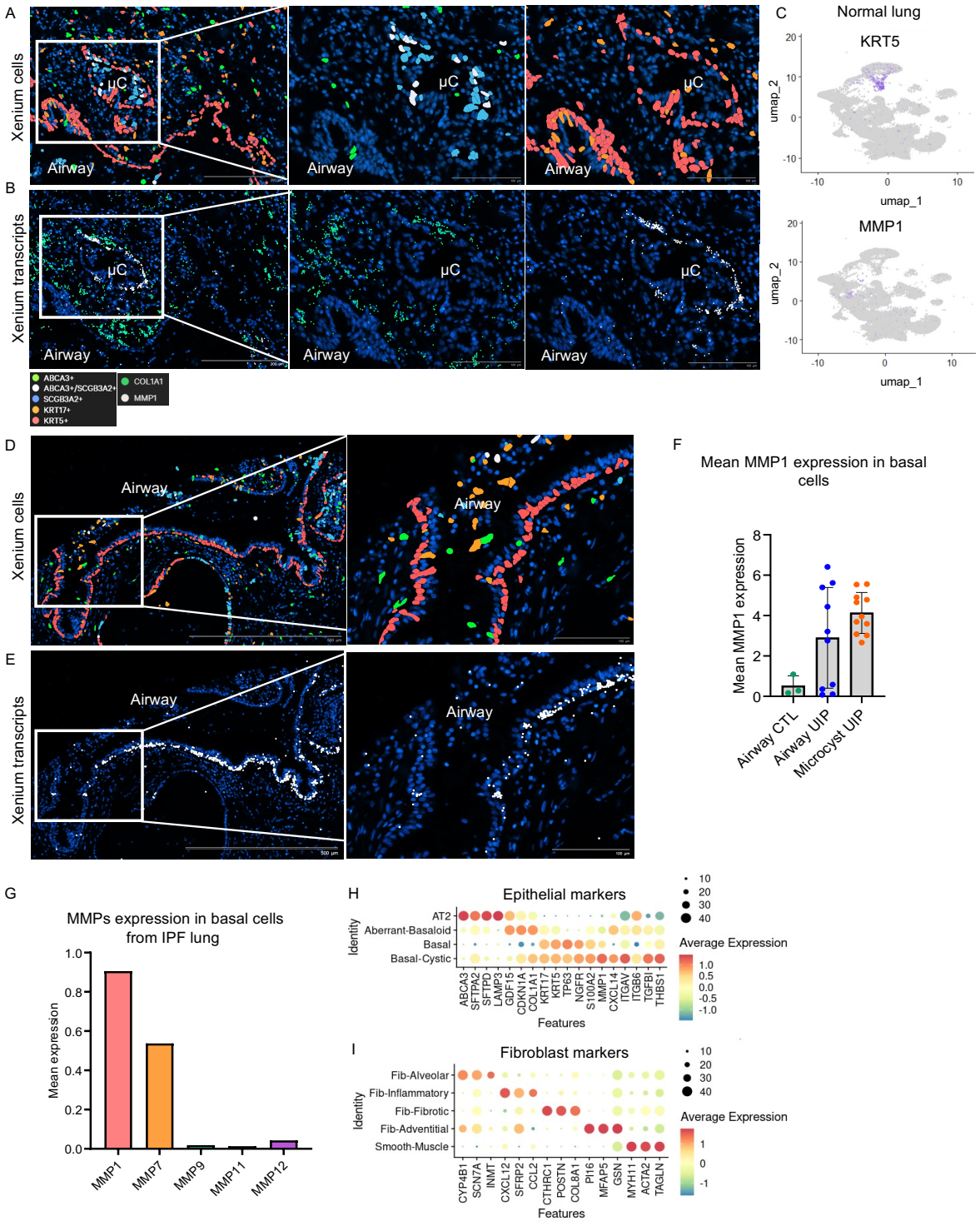


Supplemental Figure 2. (A-C) Percentage of cellular subpopulations by scRNA-seq analysis of HTII-280^{neg}/NGFR^{neg}/CEACAM6^{pos} cells (RB cells) from three independent public datasets: (A) GSE178360, (B) GSE167191, (C) GSE130076. (D) UMAP representation of scRNA-seq analysis of HTII-280^{neg}/NGFR^{neg}/CEACAM6^{pos} cells, highlighting the expression levels of *KRT5, SCGB3A2, AGER,* and *SCGB1A1* (GSE130076). (E) Percentage of Colony Forming Efficiency (CFE) of 9-day organoids prepared with RB cells (subpopulation #2, n = 5) or basal cells (#3, n = 3) co-cultured with human mesenchymal cells.

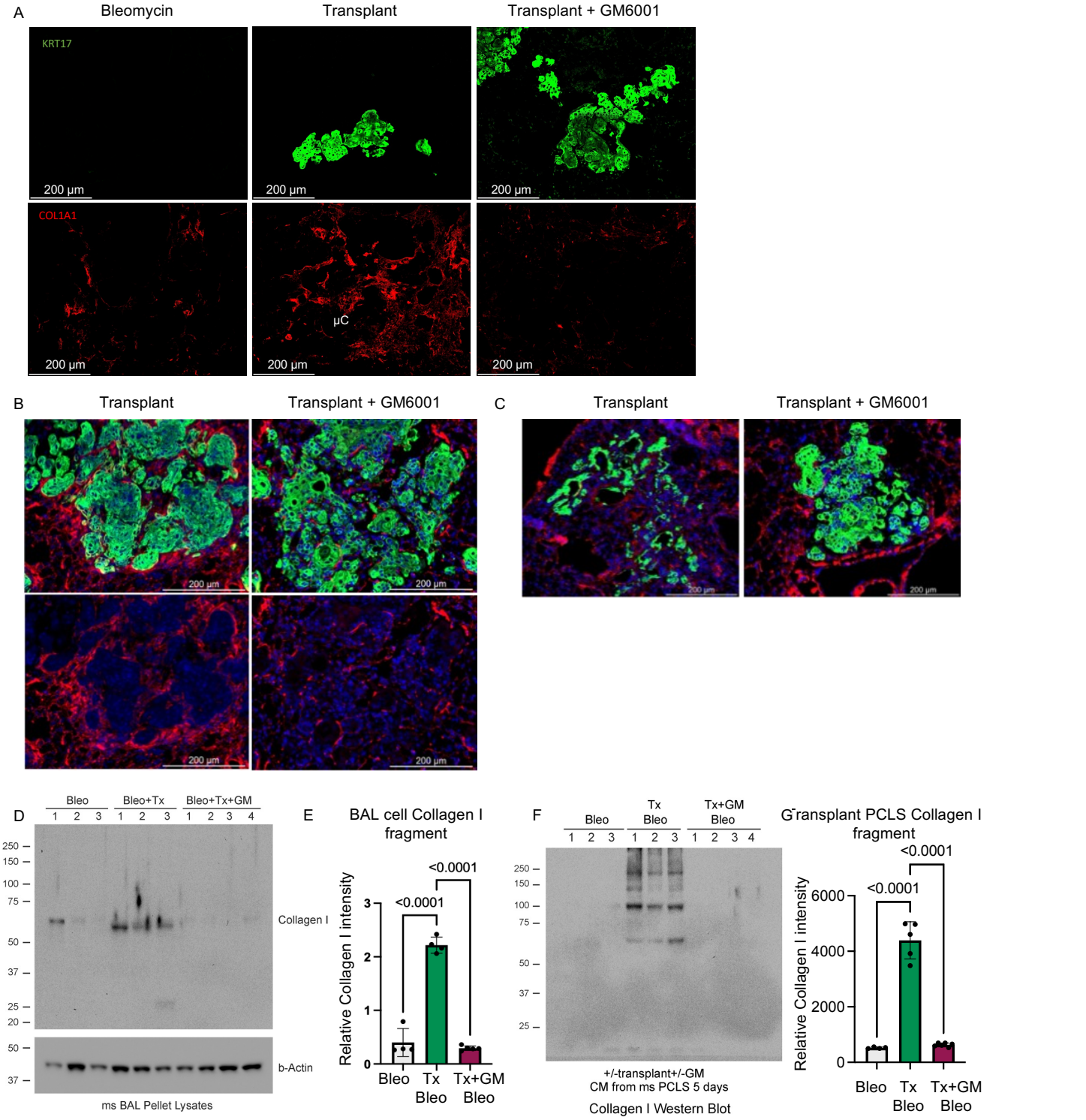




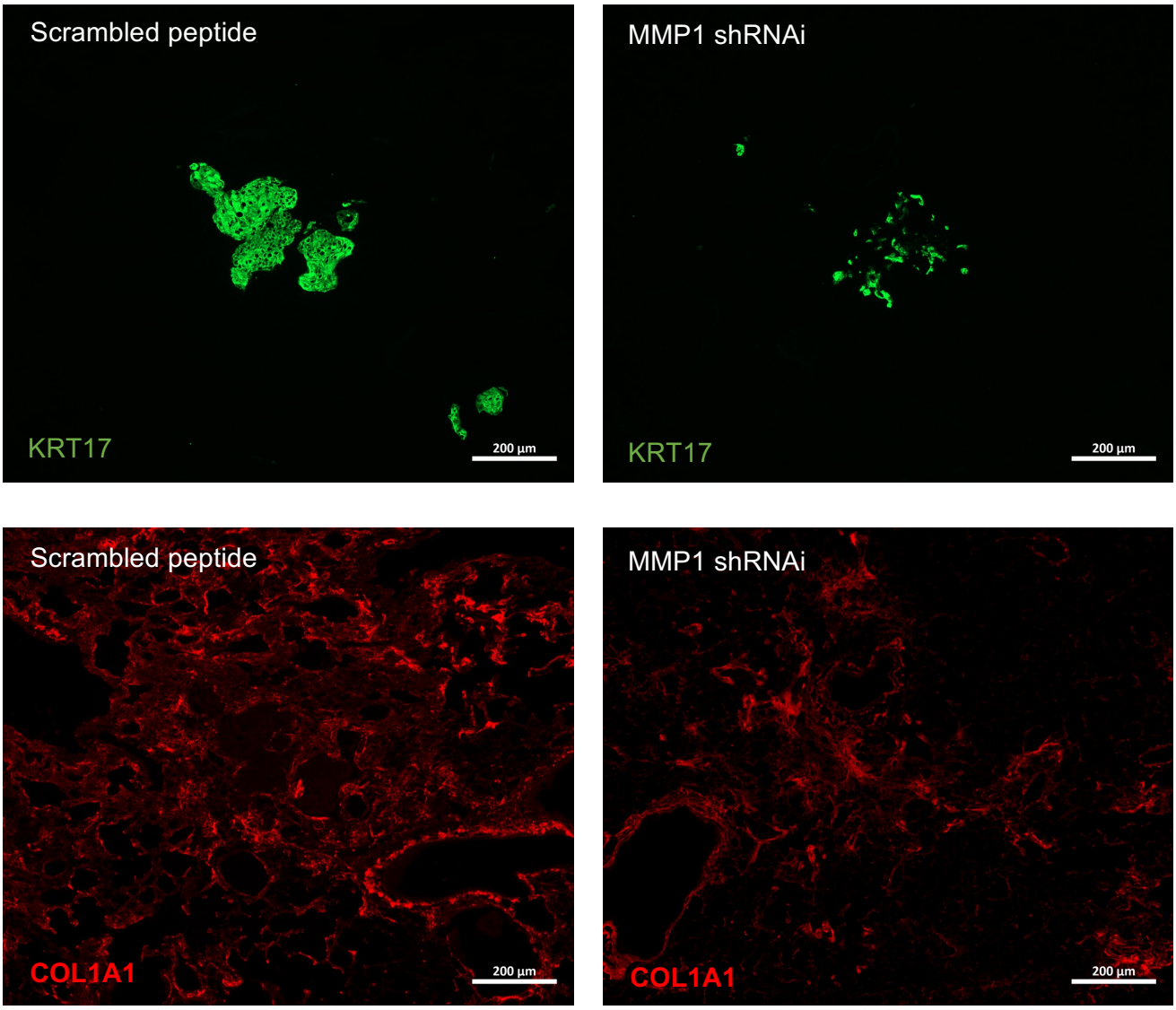
Supplemental Figure 3. (A) Selection of eight microcysts from lungs with a UIP pattern, including IPF (n = 3 microcysts from 3 patients), hypersensitivity pneumonitis (HP, n = 2 microcysts from 3 patients), and rheumatoid arthritis (RA, n = 3 microcysts from 3 patients) diseases shown in HE (top panels) and with corresponding cell type annotations from Xenium assay (bottom panels). (B) Additional basal cell markers from spatial transcriptomic Xenium assay of the IPF microcyst from Figure 2: KRT5^{+} cells (red), TP63 transcripts (green), and KRT8 transcripts (white).



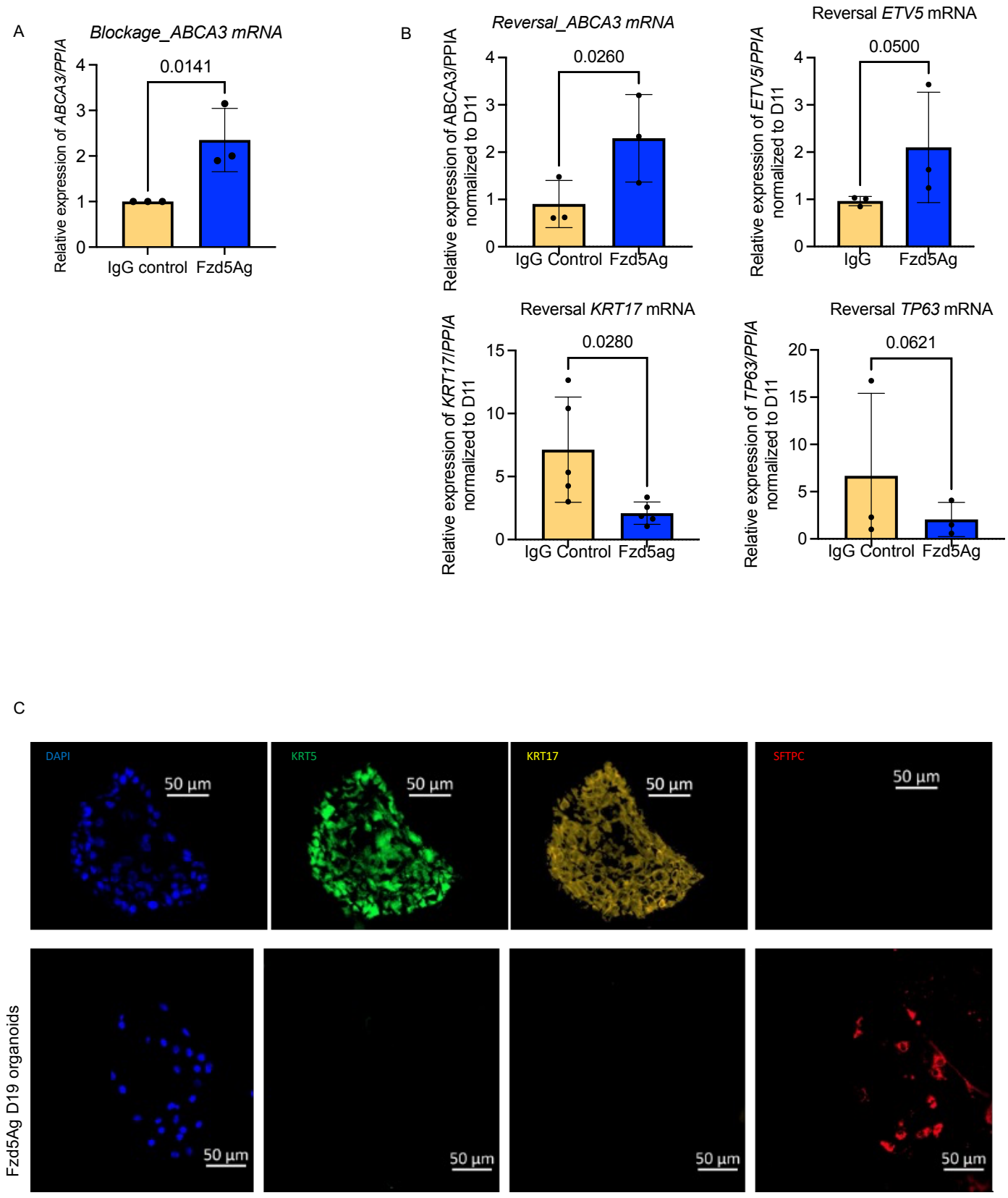
Supplemental Figure 4. (A, B) Representative image of microcyst and airway from IPF lung with annotated cells from Xenium assay with ABCA3^{+}, ABCA3^{+}/SCGB3A2^{+}, SCGB3A2^{+} cells (middle panel), KRT17^{+}, KRT5^{+} cells (right panel). (B) Same region showing *MMP1* (white), and *COL1A1* (green) transcripts. (C) UMAP display of Xenium spatial transcriptomics data from control human lung, with *KRT5* and *MMP1* expression highlighted in blue (n = 3 normal, same samples as in Figure 2). (D, E) Representative image of airway from IPF lung with (D) annotated cells from Xenium assay and (E) *MMP1* transcripts (white). (F) Mean *MMP1* expression in basal cells from normal airway, airway and microcyst from UIP lungs (n = 3 normal airways from 3 patients, n = 10 UIP airways from 5 patients, and n = 11 microcysts from 4 patients). (G) Mean expression level of MMPs (*MMP1, MMP7, MMP9, MMP11,* and *MMP12)* in basal cells from IPF lungs calculated from Xenium dataset (n = 7 IPF lungs, same samples as in Figure 2). (H, I) Dot plot supporting cell annotations for AT2, aberrant-basaloid, basal, and basal-cystic cells (G) and mesenchymal cells (I) (n = 3 normal and n = 7 IPF lungs, same samples as in Figure 2).



Supplemental Figure 5. (A) Single channel images of Figure 3 for KRT17 (green) and COL1A1 (red) in transplanted mice treated with or without GM6001 (µC = Microcyst). (B) Enlargement of images of region of engraftment showing collagen degradation area under IPF basal cells with or without GM6001 treatment. (C) Representative lung section images of microcyst-like formation of bleomycin NSG mice transplanted with IPF BCs that is abrogated by GM6001 treatment. Sections were stained by IF for human-specific KRT17 (green) and COL1A1 (red). (D) Western blot of degraded type I collagen in BAL cell lysates of bleomycin NSG mice transplanted with IPF BCs, treated with or without GM6001 (n = 3 – 4 biological replicates, 2 independent cohorts) with (E) relative type I collagen intensity. (F) Western blot of degraded type I collagen in concentrated conditioned media (CM) of PCLS from lungs of bleomycin NSG mice transplanted with IPF BCs, treated with or without GM6001 (n = 3 – 4 biological replicates, 2 independent cohorts) with (G) relative type I collagen intensity. Statistical significance was determined by ordinary one-way ANOVA (E, G).



Supplemental Figure 6. Single channel images of Figure 4 for KRT17 (green) and COL1A1 (red) in transplanted mice that received IPF basal cells transduced with Scrambled or MMP1 sh-RNAi.



Supplemental Figure 7. (A) *ABCA3* mRNA relative expression of epithelial cells (EPCAM⁺) sorted from organoids treated with IgG control or Frizzled-5 agonist (Fzd5Ag) from day 1 to day 14 of culture (n = 3). (B) *ABCA3, ETV5, KRT17,* and *TP63* mRNA relative expression of epithelial cells (EPCAM⁺) sorted from organoids treated with IgG control or Fzd5Ag from day 11 to day 19 of culture (n = 3). (C) Single channel images of organoids treated with IgG control or Fzd5Ag from day 11 to day 19 of culture stained by IF for KRT5 (green), KRT17 (yellow), and SFTPC (red). Statistical significance was determined by unpaired t-test (A) and paired t-test (B).