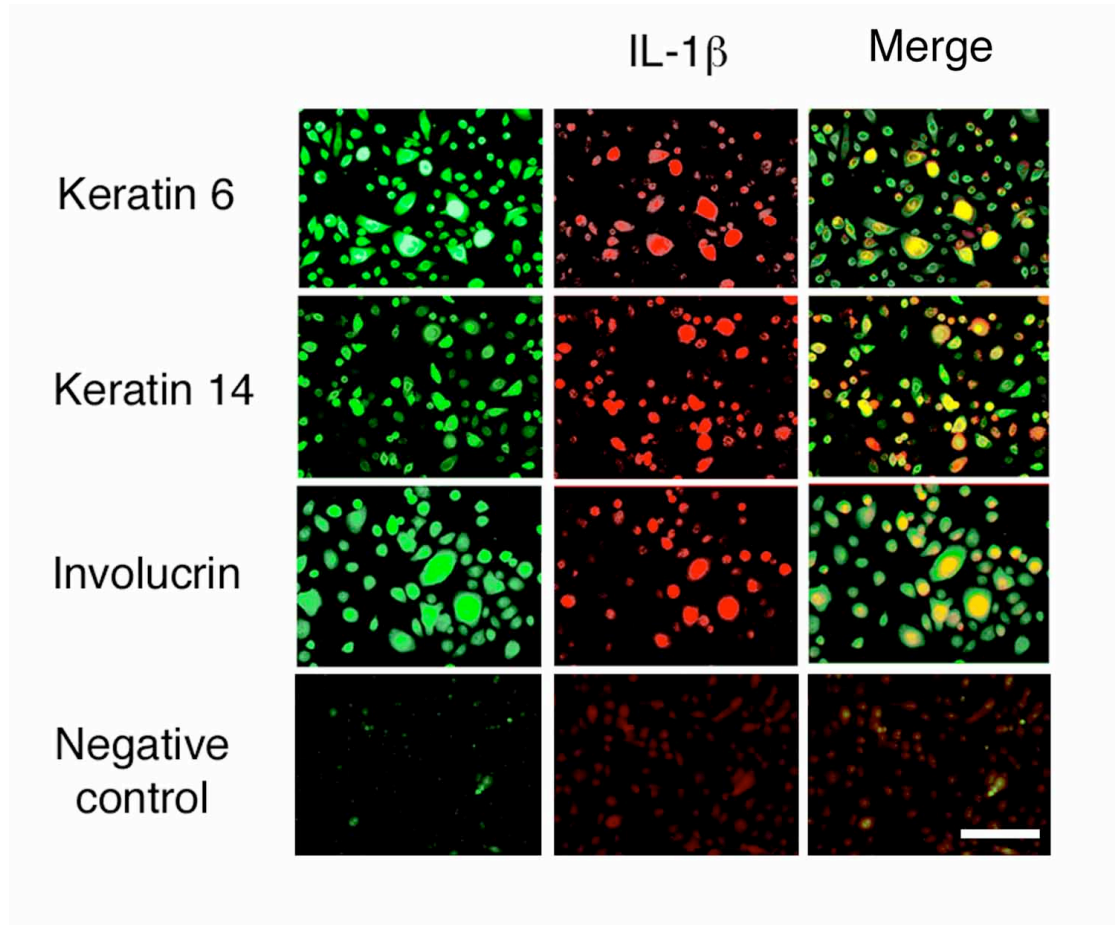


*Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD.*

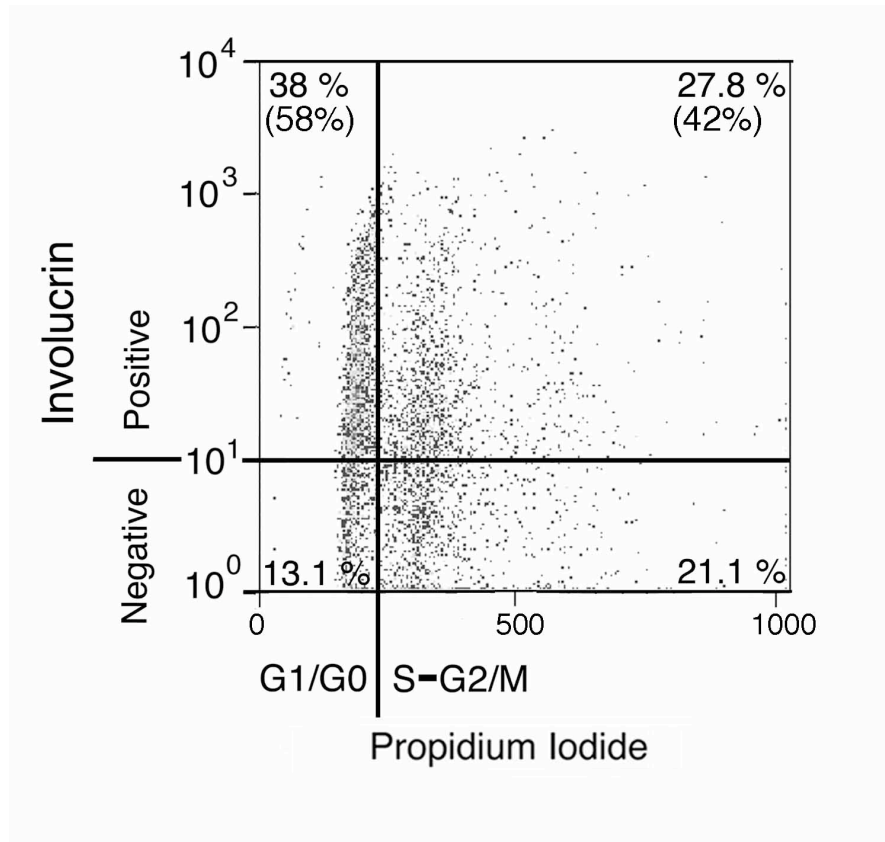
**Supplemental Figure 1:**



**Supplemental Figure 1: Colocalization of IL-1 $\beta$  with Keratin 6, Keratin 14 and Involucrin.** Passage 3 human bronchial epithelial cells were fixed, permeabilized and stained using biotinylated goat-anti-IL-1 $\beta$  (R&D Systems) and mouse anti-keratin 6, -keratin 14 and involucrin (LabVision), as indicated, and detected using FITC conjugated donkey anti-mouse (Jackson ImmunoResearch) and streptavidin Alexa Fluor 594 (Invitrogen). Pseudo colored and merged images were created (Adobe Creative Suite2) from images acquired using a digital imaging system (Spot Digital Imaging). Bar=75 $\mu$ m

*Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD.*

**Supplemental Figure 2:**



**Supplemental Figure 2: Involucrin expressing human bronchial epithelial cells are proliferating.** Passage 3 human bronchial epithelial cells were fixed and permeabilized with 70% alcohol and stained with propidium iodide. Samples were washed and stained with mouse anti-involucrin (LabVision), followed by a FITC conjugated secondary antibody and analyzed by flow cytometry, as described (1). Shown is the histogram plotting FITC vs propidium iodide staining and the gates used for determining quadrant statistics (FloJo). The percentage of cells in each quadrant are shown. The relative percentages of involucrin positive cells in G0/G1 and S/G2/M are shown in parentheses.

1. Nakatani, S., Okada, N., Okumura, H., and Yoshikawa, K. 1992. A two-colour flowcytometric study of cell kinetics and differentiation of human keratinocytes in culture. *Arch Dermatol Res* 284:349-352.

*Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD.*

**Supplemental Data**

**Table I. Gene ontology analysis of up-regulated genes using Gostat**

The set of up-regulated genes in passage 3 relative to passage 0 were subject to gene ontology analysis of biological processes using Gostat (<http://gostat.wehi.edu.au/>). Of these 278 significantly up-regulated probes, 243 are unique and annotated; among the 23044 probes represented on the arrays, 13509 are unique and annotated.

<b>GO Term</b>	<b>Genes</b>	<b># in inquiry</b>	<b># in background</b>	<b>p-value</b>
<b><u>GO:0006928</u></b> cell mobility	<u>SPHK1</u> <u>ACTB</u> <u>BDNF</u> <u>HIF1A</u> <u>LAMA3</u> <u>MSN</u> <u>ALOX15B</u> <u>ACTN1</u> <u>KLF7</u> <u>ACTR2</u>	19	329	0.001
<b><u>GO:0051674</u></b> localization of cell	<u>GAPDHS</u> <u>CALD1</u> <u>ANXA1</u> <u>SEMA3E</u> <u>PDPN</u> <u>TGFBR1</u> <u>ARPC2</u> <u>IL1B</u> <u>MTSS1</u>			
<b><u>GO:0044421</u></b> extracellular region part	<u>SEMA3C</u> <u>EREG</u> <u>COL4A5</u> <u>FJX1</u> <u>LAD1</u> <u>LAMA3</u> <u>PTHLH</u> <u>GRN</u> <u>COL5A3</u> <u>FLRT2</u> <u>AREG</u> <u>PCSK1N</u> <u>APOE</u> <u>IL1F9</u> <u>TGFBI</u> <u>SMOC1</u> <u>SEMA3E</u> <u>IL1F5</u> <u>RS1</u> <u>S100A8</u> <u>IL1A</u> <u>WNT7A</u> <u>SFRP1</u> <u>IL1B</u> <u>COL17A1</u> <u>CSPG2</u> <u>GPX3</u>	27	643	0.003
<b><u>GO:0005856</u></b> cytoskeleton	<u>KRT6B</u> <u>ACTB</u> <u>FRMD4B</u> <u>DRD2</u> <u>KRIT1</u> <u>FSCN1</u> <u>PKP2</u> <u>MSN</u> <u>MAP4</u> <u>DSC2</u> <u>ACTN1</u> <u>COTL1</u> <u>KRT14</u> <u>ACTR2</u> <u>BASP1</u> <u>CALD1</u> <u>ANXA1</u> <u>ACTR1A</u> <u>LIMA1</u> <u>IVL</u> <u>FHL3</u> <u>PERP</u> <u>ARPC2</u> <u>MYO1E</u> <u>KRT75</u> <u>TUBB6</u> <u>MTSS1</u> <u>MYC</u> <u>PSTPIP2</u> <u>SPRR3</u> <u>DSG3</u>	31	802	0.004
<b><u>GO:0007398</u></b> ectoderm development	<u>KRT6B</u> <u>IVL</u> <u>STX2</u> <u>PTHLH</u> <u>LAMA3</u> <u>BARX2</u> <u>MCOLN3</u> <u>KRT14</u> <u>COL17A1</u> <u>PLOD1</u> <u>SPRR3</u>	11	130	0.007
<b><u>GO:0005149</u></b> interleukin-1 receptor binding	<u>IL1F9</u> <u>IL1B</u> <u>IL1F5</u> <u>IL1A</u>	4	12	0.01
<b><u>GO:0042127</u></b> regulation of cell proliferation	<u>CAV1</u> <u>SHC1</u> <u>POLD4</u> <u>ANXA1</u> <u>PGF</u> <u>MXI1</u> <u>SPHK1</u> <u>EREG</u> <u>MNT</u> <u>BDNF</u> <u>TGFBR1</u> <u>IL1A</u> <u>PTHLH</u> <u>GRN</u> <u>IL1B</u> <u>ALOX15B</u> <u>CDKN2B</u> <u>MYC</u>	18	396	0.01

<b><u>GO:0022610</u></b> <b>biological adhesion</b>	<u>EDIL3</u> <u>PPFIBP1</u> <u>CD44</u> <u>CDH3</u> <u>FAT</u> <u>PKP2</u> <u>LAMA3</u> <u>FLRT2</u> <u>COL5A3</u> <u>ITGB6</u> <u>DSC2</u> <u>ACTN1</u> <u>DCBLD1</u> <u>ITGB4</u> <u>TGFBI</u> <u>RS1</u> <u>VEZT</u> <u>PERP</u> <u>ITGAV</u> <u>AMIGO2</u> <u>MTSS1</u> <u>CSPG2</u> <u>COL17A1</u> <u>PALLD</u> <u>DSG3</u>	25	640	0.01
<b><u>GO:0007155</u></b> <b>cell adhesion</b>				
<b><u>GO:0006096</u></b> <b>glycolysis</b>	<u>PKM2</u> <u>PGAM1</u> <u>LDHA</u> <u>TPI1</u> <u>PGK1</u> <u>GAPDHS</u>	6	43	0.01
<b><u>GO:0005198</u></b> <b>structural molecule activity</b>	<u>CAV1</u> <u>KRT6B</u> <u>ACTB</u> <u>COL4A5</u> <u>LAD1</u> <u>PKP2</u> <u>MSN</u> <u>LAMA3</u> <u>COL5A3</u> <u>MAP4</u> <u>KRT14</u> <u>ACTR2</u> <u>ANXA1</u> <u>MAL</u> <u>ACTR1A</u> <u>IVL</u> <u>RPL5</u> <u>PERP</u> <u>ARPC2</u> <u>KRT75</u> <u>TUBB6</u> <u>COL17A1</u> <u>SPRR3</u>	23	579	0.01
<b><u>GO:0005102</u></b> <b>receptor binding</b>	<u>SEMA3C</u> <u>SHC1</u> <u>EDIL3</u> <u>PGF</u> <u>EREG</u> <u>BDNF</u> <u>PTHLH</u> <u>MSN</u> <u>LAMA3</u> <u>GRN</u> <u>ITGB6</u> <u>AREG</u> <u>ACTN1</u> <u>PCSK1N</u> <u>APOE</u> <u>ANXA1</u> <u>IL1F9</u> <u>TGFBI</u> <u>IL1F5</u> <u>IL1A</u> <u>GALP</u> <u>WNT7A</u> <u>IL1B</u> <u>MTSS1</u>	24	624	0.01
<b><u>GO:0005515</u></b> <b>Protein binding</b>	<u>SHC1</u> <u>DNAJB4</u> <u>EREG</u> <u>ACTB</u> <u>MAPK12</u> <u>CD44</u> <u>STK25</u> <u>BDNF</u> <u>CDH3</u> <u>LAMA3</u> <u>USP5</u> <u>LDHA</u> <u>CCND2</u> <u>KLF7</u> <u>MPDZ</u> <u>RGS2</u> <u>ABCA1</u> <u>EIF4EBP1</u> <u>VPS26B</u> <u>ACTR1A</u> <u>CST3</u> <u>AMIGO2</u> <u>SERPINE1</u> <u>WNT7A</u> <u>EEA1</u> <u>PALLD</u> <u>BNIP3</u> <u>RALA</u> <u>PGF</u> <u>NOMO1</u> <u>CARD6</u> <u>SPHK1</u> <u>PKM2</u> <u>HIF1A</u> <u>FAT</u> <u>PTHLH</u> <u>RABAC1</u> <u>PKP2</u> <u>CLIC3</u> <u>GRN</u> <u>PAM</u> <u>TP73</u> <u>LRRC8C</u> <u>DSC2</u> <u>ACTN1</u> <u>KRT14</u> <u>CDK6</u> <u>ANXA1</u> <u>LIMA1</u> <u>MNT</u> <u>TRIM21</u> <u>EGLN3</u> <u>CTSB</u> <u>LOC148709</u> <u>P4HA2</u> <u>CDKN2B</u> <u>TNKS1BP1</u> <u>GPX3</u> <u>TFG</u> <u>EDIL3</u> <u>POLD4</u> <u>FBXL2</u> <u>SEMA3C</u> <u>PPFIBP1</u> <u>SIAH2</u> <u>KCNMB4</u> <u>TNFRSF25</u> <u>AP1S1</u> <u>C10ORF11</u> <u>FLRT2</u> <u>BARX2</u> <u>AREG</u> <u>SLC2A1</u> <u>COTL1</u> <u>ITGB4</u> <u>IL1F9</u> <u>LDB1</u> <u>TGFBR1</u> <u>TRIM6</u> <u>IL1A</u> <u>FHL3</u> <u>RPL5</u> <u>PERP</u> <u>IL1B</u> <u>PGRMC2</u> <u>MTSS1</u> <u>MYC</u> <u>ABAT</u> <u>DSG3</u> <u>CAV1</u> <u>MXI1</u> <u>RSU1</u> <u>FRMD4B</u> <u>KRIT1</u> <u>FSCN1</u> <u>MSN</u> <u>VLDLR</u> <u>SEC14L2</u> <u>PPME1</u> <u>C1ORF160</u> <u>ITGB6</u> <u>LTBP1</u> <u>PCSK1N</u> <u>ACTR2</u> <u>CDA</u> <u>CALD1</u> <u>APOE</u> <u>PRKCQ</u> <u>SH3PXD2A</u> <u>TGFBI</u> <u>IL1F5</u> <u>FMNL1</u> <u>RS1</u> <u>DNAJB2</u> <u>GALP</u> <u>IVL</u> <u>UBE2E3</u> <u>GJA1</u> <u>ITGAV</u> <u>MYO1E</u> <u>PLOD1</u> <u>SPRR3</u>	122	5216	0.02

<b><u>GO:0008544</u></b> <b>epidermis development</b>	<u>IVL</u> <u>PTHLH</u> <u>LAMA3</u> <u>BARX2</u> <u>KRT14</u> <u>MCOLN3</u> <u>PLOD1</u> <u>COL17A1</u> <u>SPRR3</u>	9	118	0.02
<b><u>GO:0005576</u></b> <b>extracellular region</b>	<u>PROS1</u> <u>SEMA3C</u> <u>EREG</u> <u>GLIPR1</u> <u>COL4A5</u> <u>FJX1</u> <u>LAD1</u> <u>PTHLH</u> <u>LAMA3</u> <u>GRN</u> <u>COL5A3</u> <u>FLRT2</u> <u>AREG</u> <u>PCSK1N</u> <u>CDA</u> <u>APOE</u> <u>IL1F9</u> <u>TGFBI</u> <u>SMOC1</u> <u>SEMA3E</u> <u>IL1F5</u> <u>RS1</u> <u>S100A8</u> <u>GALP</u> <u>IL1A</u> <u>SFRP1</u> <u>IL1B</u> <u>WNT7A</u> <u>SERPINE1</u> <u>COL17A1</u> <u>CSPG2</u> <u>GPX3</u>	32	973	0.03
<b><u>GO:0030054</u></b> <b>cell junction</b>	<u>LIMA1</u> <u>PKP2</u> <u>GJA1</u> <u>ARPC2</u> <u>DSC2</u> <u>ACTN1</u> <u>PANX1</u> <u>MPDZ</u> <u>COL17A1</u> <u>DSG3</u>	10	154	0.03
<b><u>GO:0048523</u></b> <b>negative regulator of cellular process</b>	<u>CAV1</u> <u>MXI1</u> <u>SPHK1</u> <u>EREG</u> <u>MAPK12</u> <u>BDNF</u> <u>PTHLH</u> <u>BARX2</u> <u>MN1</u> <u>TP73</u> <u>MAP4</u> <u>ALOX15B</u> <u>ACTN1</u> <u>CDA</u> <u>RGS2</u> <u>CUTL2</u> <u>ANXA1</u> <u>EIF4EBP1</u> <u>LDB1</u> <u>TGFBI</u> <u>LIMA1</u> <u>MNT</u> <u>IL1A</u> <u>SNAI2</u> <u>AMIGO2</u> <u>SFRP1</u> <u>IL1B</u> <u>MTSS1</u> <u>CDKN2B</u> <u>MYC</u> <u>BNIP3</u>	31	935	0.03
<b><u>GO:0012501</u></b> <b>programmed cell death</b>	<u>SPHK1</u> <u>CARD6</u> <u>SIAH2</u> <u>TNFRSF25</u> <u>BDNF</u> <u>HIF1A</u> <u>TP73</u> <u>ACTN1</u> <u>ALOX15B</u> <u>SLK</u> <u>APOE</u> <u>ANXA1</u> <u>MAL</u> <u>MNT</u> <u>IL1A</u> <u>EGLN3</u> <u>CTSB</u> <u>PERP</u> <u>AMIGO2</u> <u>IL1B</u> <u>SFRP1</u> <u>MYC</u> <u>BNIP3</u>	23	643	0.04

*Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD.*

**Supplemental Data**

**Table II. Gene ontology analysis of down-regulated genes using GOstat**

The set of down-regulated genes in passage 3 relative to passage 0 were subject to gene ontology analysis using of biological processes GOstat (<http://gostat.wehi.edu.au/>). Of these 225 significantly down-regulated probes, 197 are unique and annotated; among the 23044 probes represented on the arrays, 13509 are unique and annotated.

<i>GO Term</i>	<i>Genes</i>	<i># in inquiry</i>	<i># in background</i>	<i>p-value</i>
<a href="#">GO:0002376</a> immune system process	<a href="#">APOBEC3G</a> <a href="#">VAV1</a> <a href="#">PSMB8</a> <a href="#">HLA-F</a> <a href="#">IL8RB</a> <a href="#">MX2</a> <a href="#">BCL2</a> <a href="#">BMI1</a> <a href="#">PSME2</a> <a href="#">CXCL16</a> <a href="#">IFIT3</a> <a href="#">CX3CL1</a> <a href="#">IFITM1</a> <a href="#">CLU</a> <a href="#">IFIT1</a> <a href="#">TMEM142A</a> <a href="#">SP3</a> <a href="#">FCGRT</a> <a href="#">PLUNC</a> <a href="#">CFD</a> <a href="#">CXCL6</a> <a href="#">CRIP1</a> <a href="#">DNASE2</a> <a href="#">SCIN</a> <a href="#">MX1</a>	25	745	0.03

*Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD.*

**Supplemental Data:**

**Table III. Expression of IL-1 $\beta$ , epidermal differentiation genes, integrin  $\beta$ 6 and  $\beta$ 8 subunits, Ki-67 and p63 in squamous metaplasia and normal airway mucosa, oral mucosa, squamous metaplasia of the cervix, wounded and normal adult epidermis.**

	Sq. Met Airway			Normal Airway			Oral Mucosa			Sq. Met. Cervix			Normal Cervix			Wound Epidermis			Normal Epidermis		
	B <sup>A</sup>	SB <sup>B</sup>	S <sup>C</sup>	B	SC <sup>D</sup>	C <sup>E</sup>	B	SB	S	B	SB	S	B	SB	S	B	SB	S	B	SB	S
IL-1 $\beta$	1 <sup>F</sup>	2	1	2	1	2	0	0	0	1	2	0	1	0	0	0	1	0	1	0	0
Involucrin	1	2	1	2	1	2	0	3	1	1	3	0	1	3	0	3	3	2	0	1	3
Keratin 6	1	2	1	2	1	2	0	3	2	0	3	2	0	3	1	2	3	0	1	0	0
Keratin 14	3	2	3	2	3	2	3	3	1	3	3	0	1	1	0	3	3	2	3	3	0
Integrin $\beta$ 6	2	1	2	1	2	1	2	0	0	2	2	0	1	0	0	3	2	0	1	0	0
Integrin $\beta$ 8	2	2	2	2	2	2	2	2	0	1	3	0	1	2	0	0	0	0	0	0	0
Ki-67	2	2	2	2	2	2	1	3	0	2	2	0	1	3	0	3	1	0	2	1	0
p63	3	2	3	2	3	2	3	3	0	3	3	0	3	1	0	3	3	0	3	3	0

<sup>A</sup> Basal Cells; <sup>B</sup> Suprabasal Cells; <sup>C</sup> Superficial Cells; <sup>D</sup> Serous Cells; <sup>E</sup> Ciliated Cells

<sup>F</sup> Staining intensity determined on a 0-3 scale: 0 = no staining, 1=faint, 2=moderate, 3= dark

*Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD.*

**Supplemental Data**

**Table IV. PCR Primer sequences**

Gene symbol	Forward Primer	Reverse Primer	Product Size	Ref.
<i>ITGB8</i>	5'-CATCTGAAAAACAACGTCTACG-3'	5'-ATCTGGACAGATGGCGGTAAT-3'	306	(1)
<i>ITGB6</i>	5'-GACATGCTGAGCACAGATTCTG-3'	5'-GTTACAGGGGTCACCACAGGTAG-3'	292	(1)
<i>IL1A</i>	5'-CCACTCCATGAAGGCTGCATG-3'	5'-GGTGCTGACCTAGGCTTGATG-3'	236	(2)
<i>IL1B</i>	5'-CCTGTGGCCTTGGGCTCAA-3'	5'-GGTGCTGATGTACCAGTTGGG-3'	204	(2)
<i>IVL</i>	5'-GACTGCTGTAAAGGGACTGCC-3'	5'-CATTCCCAGTTGCTCATCTCTC-3'	250	(1)
<i>DSC2</i>	5'-TCGATGCTAGAAAACCTCTGGG-3'	5'-ACGATCTACAGGACGAGTACAA-3'	183	(1)
<i>SPRR1A</i>	5'-ACACAGCCCATTCTGCTCCG-3'	5'-TGCAAAGGAGCGATTATGATT-3'	453	(1)
<i>SPRR1B</i>	5'-AGACCAAGCAGAAGTAATGTG-3'	5'-AGACCTTCAGCTTCATTCAGAG-3'	192	(1)
<i>SPRR3</i>	5'-ATGAGTTCTTACCAGCAGAAGC-3'	5'-CTCCTTGTTGTGGGAACAAATA-3'	117	(1)
<i>S100A7</i>	5'-ACGTGATGACAAGATTGACAAGC-3'	5'-GCGAGGTAATTTGTGCCCTTT-3'	102	(1)
<i>HGF</i>	5'-CAGAGGGACAAAGGAAAAGAA-3'	5'-GCAAGTGAATGGAAGTCCTTTA-3'	167	(3)
<i>ACTB</i>	5'-TGACGGGGTCACCCACACTGTGCC-3'	5'-CTAGAAGCATTGCGGTGGACGATGGAGGG-3'	662	(1)
<i>PPIA(CyA)</i>	5'-CAGCGGAAAACCTTCGTGCTC-3'	5'-CCAGAACCTGTATGCTTTCGGA-3'	193	(1)
<i>GAPDH</i>	5'-ATGGGGGAAGGTGAAGGTCG-3'	5'-GGGGTCATTGATGGCAACAATA-3'	108	(1)

References:

1. Primer Bank. <http://pga.mgh.harvard.edu/primerbank>.
2. Arnush, M., Heitmeier, M.R., Scarim, A.L., Marino, M.H., Manning, P.T., and Corbett, J.A. 1998. IL-1 produced and released endogenously within human islets inhibits beta cell function. *J Clin Invest* 102:516-526.
3. Plantier, L., Marchand-Adam, S., Marchal-Somme, J., Leseche, G., Fournier, M., Dehoux, M., Aubier, M., and Crestani, B. 2005. Defect of hepatocyte growth factor production by fibroblasts in human pulmonary emphysema. *Am J Physiol Lung Cell Mol Physiol* 288:L641-647.