**On-line Supplement**

# NOTCH3 contributes to Rhinovirus-induced goblet cell hyperplasia in COPD airway epithelial cells

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## MATERIALS AND METHODS

### Cell cultures

Collection of tracheobronchial segments at the time of double lung transplantation from both COPD recipients and donor lungs for isolation of airway basal epithelial cells was approved by the University of Michigan Institutional review board. Age and clinical status of COPD subjects and healthy donors are provided in Supplemental Table 1. Airway basal epithelial cells were expanded in collagen-coated plastic plates using Bronchial Life medium (Lifeline Cell Technology, Frederick, MD)[1]. Cells at passage one were seeded in transwells, grown in bronchial life medium until confluent and then cultured at air/liquid interface for 3 weeks in differentiation medium as described previously to promote mucociliary differentiation[1,2]. Briefly, the differentiation medium consisted of 1:1 mixture of LHC8 basal medium and DMEM (high glucose), bovine serum albumin (0.5 mg/ml), bovine pituitary extract (0.8%), Insulin (5 μg/ml), Transferrin (10 μg/ml), Hydrocortisone (0.5 μg/ml), triiodothyronine (6.5 ng/ml), epinephrine (0.5 μg/ml), EGF (0.5 ng/ml), retinoic acid (5 x $10^{-8}$M), phosphorylethanolamine (0.5 μM), ethanolamine (0.5 μM), zinc sulphate (3 μM), Calcium chloride (0.11 mM), ferrous sulphate (1.5 x $10^{-6}$M), Magnesium chloride (6 x $10^{-4}$M), 1 X trace elements B (Corning™ cellgro™).

### RV and infection of airway epithelial cell cultures

RV16 and RV1B were purchased from ATCC, propagated in H1 HeLa cells, partially purified and viral titer was determined by plaque assay as described [3]. Less than 100kDa fraction from purified RV preparation was used as sham control. All the *in vitro* experiments were performed with RV16, a major group virus. *In vivo* experiments were conducted with minor group virus RV1B, because major group virus do not infect mice efficiently [3]. Moreover, RV1B has been

demonstrated to stimulate similar cytokine and chemokine responses in human airway epithelial cells *in vitro* [3].

**Infection of cell cultures**

Apical surface of mucociliary-differentiated cells was washed with 0.15% sodium bicarbonate to remove secreted mucus and rinsed once with PBS and then infected apically with 10 µl of PBS containing RV at 1 MOI or equivalent volume of sham and incubated for 15 days as described previously [4]. Medium in the basolateral chamber was changed every other day. In some experiments, cells were treated basolaterally with 0.1, 1, 2.5 or 5 µM erlotinib or 1, 5, 10 or 20 µM γ-secretase inhibitor N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT, Sigma Aldrich LLC, St. Louis, MO) or DMSO (vehicle control) every second day starting from 2 days after RV infection. In selected experiments cells were treated with neutralization antibody to IL-13 (4 µg/ml) or normal mouse IgG (both from R & D systems, Minneapolis, MN) either basolaterally or apically every second day starting from 2 days post-infection. In some experiments, cells were pretreated with erlotinib for 7 days every other day and infected with sham or RV, and cultures were maintained as above. Cytotoxicity of these agents was determined by assessing lactate dehydrogenase (LDH) levels in the basolateral medium using CytoTox96® Non-radioactive Cytotoxicity assay kit (Promega Corp., Madison, WI).

**Treatment of cell cultures with IL-13**

Normal cell culture (N43) was cultured with IL-13 (1ng/ml) during the 2nd and 3rd of culturing. Cells were maintained for additional one week without added IL-13 in the medium. Some cultures were treated with IL-13 neutralizing antibody (4 µg/ml) or isotype control from the

basolateral side every other day for 14 days.

**Transduction of primary human airway epithelial cells**

Primary human airway epithelial cells were transduced with GIPZ lentivral human NOTCH1 or NOTCH3 shRNA or control shRNA (all form Dharmacon) during the first week of culturing as described previously [4].

**Quantitation of goblet cells**

Quantitation of goblet cells in airway epithelial cell cultures was performed as described previously[1]. After relevant treatment, 0.3 ml of 0.15% sodium bicarbonate in PBS was added to the apical surface and incubated for 30 min to remove the apical mucus. The apical surface of the culture was then washed twice with PBS and then immediately fixed in ice cold methanol for 5 minutes. The cultures were then washed with PBS, blocked in normal PBS containing 5% donkey serum and 1% BSA (blocking buffer) and incubated with 1:5,000 diluted rabbit polyclonal antibody to human tracheobronchial mucins[5] overnight at 4° C. Unbound antibody was washed and the bound antibody was detected with antirabbit IgG conjugated with Alexafluor-594. Cell cultures were counterstained with DAPI to detect nuclei. Cultures were mounted, imaged using confocal microscope and the optical section just below the apical surface was used to count the goblet cells. Goblet cells were counted in 25 random 0.1 mm2 areas per culture and averaged.

**RV infection of mice**

All the protocols used for animal experiments were approved by Institutional Animal Care and User Committee at the University of Michigan. Mice with COPD phenotype were generated as described previously[6]. Briefly, 6 - 8 weeks old C57BL/6 female mice were exposed to cigarette

smoke using TE10 automated cigarette smoking machine for 2 hours a day, 5 days a week for 8 weeks. These mice were also treated with heat-killed nontypeable H. influenzae twice at the end of 2 weeks and then again at the end of 4 weeks during the 8 week exposure to cigarette smoke. These treatment conditions induced mild COPD-like lung disease encompassing both parenchyma and conductive airways. Mice with mild COPD phenotype and age matched normal C57BL/6 mice were briefly anesthetized with isoflurane and infected with 50 µl of 1 x $10^8$ PFU/ml RV1B by intranasal route [6]. Mice were then treated every other day with 50 µl of gamma secretase inhibitor L685,458 (Sigma Aldrich) (0.003, 0.03 or 0.3 mg/kg body weight corresponding to 0.1, 1 or 10 nmol) or DMSO by intranasal route[7]. At 8 days post-infection mice were sacrificed and lungs were processed for either isolation of total RNA or fixed and embedded in paraffin.

**Western blot analysis**

After relevant treatment, cells were washed with cold PBS and lysed in RIPA buffer containing protease and phosphatase inhibitors. Equal amount of protein was subjected to Western blot analysis with antibodies to NOTCH1, NOTCH3 or HEY1 (Cell signaling), or β-actin (Sigma Aldrich). Specific bands were quantified by densitometry using NIH image J and expressed as fold change over β-actin or over respective total protein.

**Histology**

**PAS-Alcian blue staining**

Primary human airway epithelial cell cultures were fixed in buffered formalin, and embedded in paraffin as agarose plugs as previously described[8]. Five micron thick sections were deparaffinized and stained with PAS/Alcian blue using Leica Alcian Blue and PAS Special Stain

Kit (Leica Biosystems Inc., Buffalo Grove, IL).

After relevant treatment, mice were sacrificed, lung were inflation fixed and embedded in paraffin. Five micron thick sections were deparaffinized and stained with periodic Schiffs reagent to detect goblet cells in the airway epithelium.

**Gene arrays and real time PCR**

Microarrays and analysis was performed in Microarray core facility and the microarray data was analyzed by a Bioinformatics specialist at the University of Michigan. Normal and COPD cells infected with sham or RV were harvested at 14 days post-infection, total RNA was isolated by using microRNeasy kit (Qiagen, Valencia, CA) involving DNAse treatement step. Quality and concentration of each RNA samples was assessed with Agilent Bioanalyzer (Agilent Technologies, Paol Alto, CA). Double stranded cDNA was synthesized from 2 µg of total RNA followed by clean up with GeneChip Sample Cleanup Module. Biotin-labeled cRNA was then synthesized by in vitro transcript reaction using GeneChip IVT labeling kit. Purified and quantified cRNA was hybridized to affymatrix Human Gene ST 2.1 test chips and the microarrays were performed following instructions from the manufacturer. Raw expression data obtained from Affymetrix microarrays were background corrected and quantile normalized across experimental conditions[9]. The LIMMA methodology (Linear Models for Microarray Data) was applied to the $\log_2$-transformed expression data to identify differentially expressed genes for each comparison[10]. Differentially expressed genes were identified based on statistical significance as well as biological significance. Differentially expressed genes from these filters were combined and a list of common genes showing greater statistical and biological significance (lower p value and up/down regulated by more than 2-fold) were identified for further bioinformatics and pathway analyses using the Ingenuity Pathway Analysis

(www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/).

After relevant treatment, total RNA was isolated from airway epithelial cells or mouse lungs and the expression of NOTCH1, NOTCH2, NOTCH3, NOTCH4, JAG1, JAG2, DLL1, DLL2, DLL4, HEY1, HEYL, HES1, HES5, MUC5AC, MUC5B, FOXA2, FOXA3, FOXJ1, and SPDEF was determined by using gene specific primers and presented as fold change over house-keeping gene, glyceraldehyde 3-phosphate dehydrogenase (G3PDH for cells) [11] or β-actin (for mouse). All primers and probes were purchased from either Applied Biosystems or Intetrated DNA Technologies.

**Statistical analysis**

All cell culture experiments were performed 3 times in duplicate or triplicates and data was averaged for each subject and then subjected statistical analyses. Animal experiment was conducted two times with 3 animals in each group for a total of 6 groups per group. Data was analyzed by non-parametric analyses, such as the Wilcoxon rank sum test (for comparisons between two independent groups), or sign-rank test (for paired comparison between the groups or intracomparison between sham and RV-infected cultures) to assess the statistical significance. A p-value of $<0.05$ was considered statistically significant. SAS version 9.4 (SAS Institute Inc., Cary, NC) was used for all the data analyses. Percent of inhibition was calculated considering 100% for DMSO-treated or NTshRNA-transfected samples and expressed as % inhibition ± SD.

Supplemental Table 1

Characteristics of patients with COPD and healthy non-smokers

| No | Age (Yr) | Gender | FEV1 (%) | Smoking history (pack years) | Emphysema |
|---|---|---|---|---|---|
| COPD | | | | | |
| 1 | 62 | Male | 25 | 28 | Severe |
| 2 | 53 | Female | 13 | 70 | Severe |
| 4 | 55 | Female | 56 | 27 | Mild |
| 6 | 65 | Male | 15 | 120 | Severe |
| 7 | 58 | Female | 25 | 70 | Moderate |
| 9 | 59 | Male | 22 | 70 | Severe |
| 11 | 59 | Male | 22 | 25 | Moderate |
| 12 | 45 | Male | 17 | 31.25 | None |
| Healthy non-smokers | | | | | |
| 1 | 54 | Female | | | |
| 2 | 59 | Male | | | |
| 3 | 47 | Male | | | |
| 4 | 48 | Male | | | |
| 5 | 41 | Female | | | |
| 6 | 50 | Female | | | |
| 7 | 40 | Male | | | |
| 8 | 48 | Female | | | |
| 9 | 46 | Male | | | |

All tissue samples were obtained from the University of Michigan and have been previously described [8]

**Table 2: List of genes changed by 2 fold with p value of <0.05 in RV-infected COPD cell cultures**

| Transcript Cluster ID | C-RV (log2) | C-S (log2) | Fold Change (linear) (C-RV vs. C-S) | ANOVA p-value (C-RV vs. C-S) | FDR p-value (C-RV vs. C-S) | Gene Symbol |
|---|---|---|---|---|---|---|
| 16774436 | 4.33 | 5.41 | -2.11 | 0.000038 | 0.151281 | LINC00284 |
| 16734237 | 6.32 | 4.97 | 2.55 | 0.000107 | 0.157297 | RP11-532E4.2 |
| 16684080 | 7.61 | 6.36 | 2.38 | 0.000165 | 0.160371 | IFI6 |
| 16720601 | 8.66 | 7.3 | 2.57 | 0.000408 | 0.179661 | MUC5B |
| 17089003 | 5.03 | 3.39 | 3.13 | 0.000433 | 0.179661 | OLFML2A |
| 16986501 | 5.37 | 4.26 | 2.17 | 0.000777 | 0.181216 | PDE8B |
| 16825561 | 3.68 | 2.53 | 2.22 | 0.000997 | 0.181216 | SEZ6L2 |
| 16683105 | 5.95 | 4.9 | 2.08 | 0.001134 | 0.185069 | HSPG2 |
| 16902254 | 5.23 | 4.23 | 2.01 | 0.001189 | 0.188646 | TMEM185B |
| 16949062 | 3.09 | 2.03 | 2.07 | 0.001631 | 0.201113 | EPHB3 |
| 17001299 | 3.88 | 2.85 | 2.05 | 0.001794 | 0.201625 | DPYSL3 |
| 17097661 | 8.11 | 6.74 | 2.57 | 0.00194 | 0.201625 | TNC |
| 16777777 | 6.02 | 4.87 | 2.22 | 0.002022 | 0.201625 | SLC7A1 |
| 16856627 | 5.22 | 3.91 | 2.48 | 0.002049 | 0.201625 | |
| 17119118 | 5.22 | 3.91 | 2.48 | 0.002049 | 0.201625 | |
| 17090713 | 7.36 | 5.35 | 4 | 0.002444 | 0.202803 | CEL |
| 17078452 | 4.53 | 3.09 | 2.72 | 0.002479 | 0.202803 | HEY1 |
| 16752715 | 6.56 | 5.42 | 2.2 | 0.002555 | 0.203548 | LRP1 |
| 16840611 | 5.58 | 4.32 | 2.4 | 0.002947 | 0.203548 | YBX2 |
| 17115505 | 5.56 | 4.53 | 2.05 | 0.002971 | 0.203548 | FLNA |
| 16954898 | 1.76 | 2.83 | -2.1 | 0.003068 | 0.203548 | RNU6-856P |
| 16832479 | 4.65 | 3.57 | 2.11 | 0.003224 | 0.203548 | SLC13A2 |
| 16989736 | 6.03 | 4.81 | 2.33 | 0.003279 | 0.203548 | EGR1 |
| 17108816 | 5.53 | 3.79 | 3.34 | 0.003382 | 0.203548 | MXRA5 |
| 16686060 | 10.13 | 9.03 | 2.13 | 0.003496 | 0.203548 | SLC2A1 |
| 16718126 | 6.65 | 5.62 | 2.04 | 0.003619 | 0.203548 | COL17A1 |
| 16924602 | 3.46 | 2.44 | 2.03 | 0.004328 | 0.20477 | ADAMTS1 |
| 16996180 | 3.31 | 4.86 | -2.93 | 0.00458 | 0.20477 | MIR449C |
| 16657598 | 5.8 | 4.67 | 2.18 | 0.004685 | 0.20477 | AGRN |
| 16837938 | 8.54 | 7.29 | 2.37 | 0.005006 | 0.204916 | ITGB4 |
| 17117581 | 5.74 | 4.64 | 2.15 | 0.005173 | 0.205852 | LOC100128816 |
| 16836824 | 3.99 | 2.83 | 2.24 | 0.005213 | 0.206494 | MRC2 |
| 16869769 | 6.04 | 4.75 | 2.45 | 0.005706 | 0.209394 | NOTCH3 |
| 16839642 | 5.76 | 4.53 | 2.34 | 0.005873 | 0.209394 | CLUH |
| 16702175 | 6.73 | 5.67 | 2.08 | 0.005879 | 0.209394 | PFKFB3 |
| 16712277 | 3.78 | 2.43 | 2.54 | 0.005904 | 0.209394 | ST8SIA6 |
| 17067422 | 1.43 | 2.56 | -2.19 | 0.00591 | 0.209394 | RNA5SP259 |

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|---|---|---|---|---|---|---|
| 17100036 | 5.38 | 4.31 | 2.1 | 0.005951 | 0.209394 | NOTCH1 |
| 17030426 | 7.71 | 6.59 | 2.17 | 0.006378 | 0.212632 | DDR1 |
| 17001901 | 7.39 | 6.26 | 2.18 | 0.006831 | 0.213754 | FAT2 |
| 17100675 | 8.97 | 7.95 | 2.03 | 0.00697 | 0.214023 | MT-TH |
| 16681477 | 8.61 | 7.54 | 2.09 | 0.007014 | 0.214023 | CLSTN1 |
| 16818501 | 5.57 | 4.55 | 2.03 | 0.007372 | 0.216137 | RP11-598D12.4 |
| 16824063 | 4.04 | 2.96 | 2.11 | 0.00785 | 0.219444 | LOC101927311 |
| 17033131 | 7.76 | 6.71 | 2.07 | 0.00786 | 0.219444 | DDR1 |
| 17037907 | 7.76 | 6.71 | 2.07 | 0.00786 | 0.219444 | DDR1 |
| 16828913 | 3.48 | 2.12 | 2.56 | 0.00807 | 0.219444 | RNU1-103P |
| 16908822 | 4.45 | 3.36 | 2.14 | 0.008594 | 0.219911 | CHPF |
| 16797196 | 7.45 | 6.43 | 2.03 | 0.008757 | 0.219911 | AHNAK2 |
| 16700888 | 2.96 | 1.75 | 2.3 | 0.008858 | 0.219978 | NID1 |
| 17027581 | 7.66 | 6.64 | 2.03 | 0.009095 | 0.219978 | DDR1 |
| 16802903 | 4.66 | 3.36 | 2.46 | 0.009132 | 0.219978 | LOXL1 |
| 16715847 | 1.53 | 0.45 | 2.11 | 0.00969 | 0.221789 | RNU6-1266P |
| 16958403 | 6.16 | 4.94 | 2.32 | 0.009874 | 0.222578 | HEG1 |
| 17117537 | 4.79 | 3.76 | 2.04 | 0.010177 | 0.224257 | LOC100131262 |
| 16989861 | 2.47 | 3.5 | -2.03 | 0.010598 | 0.226159 | |
| 16981694 | 2.65 | 3.82 | -2.25 | 0.010861 | 0.22704 | SPATA4 |
| 16729155 | 3.2 | 2.07 | 2.19 | 0.010982 | 0.227726 | MOGAT2 |
| 16669180 | 7.64 | 6.53 | 2.17 | 0.011394 | 0.229138 | PTGFRN |
| 17108067 | 4.12 | 2.86 | 2.4 | 0.012144 | 0.232047 | |
| 16850107 | 6.89 | 5.66 | 2.35 | 0.012167 | 0.232047 | FASN |
| 16855242 | 1.08 | 2.11 | -2.04 | 0.012764 | 0.234088 | RNA5SP457 |
| 16892073 | 2.03 | 3.31 | -2.43 | 0.014076 | 0.240425 | RNU1-93P |
| 16908197 | 5.97 | 4.71 | 2.4 | 0.015489 | 0.246436 | IGFBP5 |
| 16660976 | 6.88 | 5.84 | 2.06 | 0.016342 | 0.251089 | SEPN1 |
| 16777571 | 5.08 | 3.88 | 2.29 | 0.016587 | 0.251802 | SHISA2 |
| 16872276 | 3.54 | 2.2 | 2.52 | 0.01681 | 0.252259 | FCGBP |
| 16825055 | 2.86 | 3.87 | -2.01 | 0.016947 | 0.252828 | |
| 16770254 | 2.87 | 3.92 | -2.07 | 0.016975 | 0.252828 | |
| 16703174 | 1.45 | 2.47 | -2.03 | 0.016983 | 0.252828 | RP11-301N24.3 |
| 16885976 | 4.15 | 5.16 | -2.02 | 0.017863 | 0.256046 | |
| 17087926 | 0.88 | 2.04 | -2.24 | 0.018543 | 0.257915 | |
| 16820157 | 8.87 | 7.86 | 2.01 | 0.018719 | 0.258562 | HSD11B2 |
| 17098624 | 2.29 | 1.22 | 2.1 | 0.018896 | 0.259266 | RNA5SP296 |
| 17100685 | 8.74 | 7.56 | 2.25 | 0.019114 | 0.259953 | MT-TT |
| 16854777 | 1.79 | 2.88 | -2.12 | 0.020026 | 0.262512 | |
| 17101193 | 2.77 | 1.76 | 2.01 | 0.020976 | 0.265456 | |
| 17005072 | 4.01 | 5.04 | -2.03 | 0.02156 | 0.266673 | RNU6-522P |
| 16824810 | 5.36 | 4.22 | 2.21 | 0.02177 | 0.267481 | CRYM |

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|---|---|---|---|---|---|---|
| 17020727 | 0.58 | 2.23 | -3.14 | 0.0225 | 0.269548 | MIR30A |
| 16673654 | 3.59 | 4.78 | -2.28 | 0.023181 | 0.271908 | MROH9 |
| 17007048 | 3.16 | 2.14 | 2.02 | 0.0232 | 0.27195 | C4A |
| 16712076 | 3.58 | 2.57 | 2.01 | 0.02527 | 0.27876 | FAM171A1 |
| 16915113 | 1.96 | 0.89 | 2.1 | 0.026857 | 0.283786 | |
| 17041111 | 3.26 | 2.24 | 2.04 | 0.026891 | 0.283896 | C4B |
| 16859657 | 8.47 | 7.43 | 2.06 | 0.027874 | 0.287794 | SLC5A5 |
| 16825623 | 4.46 | 3.41 | 2.07 | 0.028346 | 0.289129 | TBX6 |
| 16947869 | 2.64 | 4.04 | -2.63 | 0.029578 | 0.294049 | RNU4-38P |
| 17031045 | 3.3 | 2.28 | 2.03 | 0.029723 | 0.294629 | C4B |
| 16912224 | 1.32 | 2.93 | -3.05 | 0.030841 | 0.299381 | RP13-401N8.1 |
| 16972961 | 2.6 | 3.78 | -2.26 | 0.031137 | 0.299962 | C4orf47 |
| 17113129 | 3.22 | 4.45 | -2.34 | 0.033299 | 0.306089 | NUP62CL |
| 16707541 | 5.3 | 4.21 | 2.14 | 0.033677 | 0.307944 | CYP26A1 |
| 16814366 | 6.03 | 4.94 | 2.14 | 0.03562 | 0.314685 | MSLN |
| 16832218 | 3.95 | 2.85 | 2.15 | 0.0379 | 0.322678 | |
| 16819484 | 8.05 | 6.94 | 2.16 | 0.037984 | 0.322734 | CX3CL1 |
| 16882536 | 2.68 | 3.87 | -2.27 | 0.038036 | 0.32291 | |
| 16961197 | 2.86 | 4.12 | -2.39 | 0.038407 | 0.323631 | |
| 16989396 | 3.95 | 2.93 | 2.03 | 0.039038 | 0.324572 | PCBD2 |
| 17007118 | 3.29 | 2.19 | 2.13 | 0.042704 | 0.334322 | C4B |
| 16816962 | 5.78 | 4.75 | 2.05 | 0.043612 | 0.336693 | SCNN1G |
| 17011555 | 2.43 | 3.51 | -2.12 | 0.043626 | 0.336693 | CCDC162P |
| 16996157 | 3.62 | 4.96 | -2.53 | 0.045998 | 0.343354 | CDC20B |
| 16908037 | 2.97 | 5.21 | -4.74 | 0.049596 | 0.351828 | FN1 |

Data is based on the cells obtained from 8 COPD subjects

Supplemental Table 3. List of genes changed by 2 fold following RV infection in normal cell cultures

| Transcript Cluster ID | N-RV Bi-weight Avg Signal (log2) | N-S Bi-weight Avg Signal (log2) | Fold Change (linear) (N-RV vs. N-S) | ANOVA p-value (N-RV vs. N-S) | FDR p-value (N-RV vs. N-S) | Gene Symbol |
|---|---|---|---|---|---|---|
| 17121346 | 3.89 | 4.94 | -2.07 | 0.000137 | 0.255565 | PKD1; |
| 16711413 | 5.21 | 4.19 | 2.03 | 0.000167 | 0.255565 | RP13-463N16.6 |
| 17061129 | 3.23 | 4.39 | -2.24 | 0.00032 | 0.255565 | RASA4; |
| 17038014 | 7.61 | 8.68 | -2.1 | 0.000557 | 0.255565 | MUC21 |
| 17006532 | 6.55 | 7.73 | -2.26 | 0.000885 | 0.264285 | MUC21 |
| 17026232 | 6.55 | 7.73 | -2.26 | 0.000885 | 0.264285 | MUC21 |
| 17030532 | 6.55 | 7.73 | -2.26 | 0.000885 | 0.264285 | MUC21 |
| 17035325 | 6.88 | 8.01 | -2.19 | 0.000888 | 0.264285 | MUC21 |
| 16666485 | 6.27 | 4.47 | 3.48 | 0.001505 | 0.27582 | IFI44L |
| 17033237 | 6.59 | 7.79 | -2.29 | 0.001544 | 0.27582 | MUC21 |
| 17073368 | 1.48 | 2.61 | -2.18 | 0.001623 | 0.27582 | RP11-661A12.4 |
| 17100036 | 4.2 | 5.25 | -2.08 | 0.002239 | 0.27582 | NOTCH1 |
| 16707196 | 2.55 | 1.43 | 2.17 | 0.002349 | 0.27582 | IFIT1 |
| 17080648 | 7.21 | 5.89 | 2.5 | 0.002479 | 0.27582 | HAS2 |
| 17115505 | 4.43 | 5.44 | -2.02 | 0.002648 | 0.27582 | FLNA |
| 17108816 | 3.63 | 5.26 | -3.11 | 0.002749 | 0.27582 | MXRA5 |
| 16862721 | 2.81 | 4.12 | -2.47 | 0.003169 | 0.27582 | MEGF8 |
| 16717272 | 4.55 | 5.64 | -2.12 | 0.00362 | 0.280537 | LOXL4 |
| 16929631 | 4.44 | 5.55 | -2.16 | 0.003681 | 0.281221 | |
| 17121498 | 3.76 | 4.88 | -2.18 | 0.00458 | 0.281221 | ARHGAP23P1 |
| 16867432 | 5.55 | 6.59 | -2.06 | 0.005691 | 0.281221 | PTPRS |
| 16991948 | 3.39 | 4.76 | -2.58 | 0.005979 | 0.281221 | TENM2 |
| 16963127 | 7.29 | 8.58 | -2.46 | 0.006061 | 0.281221 | MUC4 |
| 16787814 | 3.01 | 1.99 | 2.03 | 0.006093 | 0.281221 | IFI27 |
| 17100649 | 2.48 | 3.78 | -2.47 | 0.006158 | 0.281221 | MT-TI |
| 16913303 | 2.68 | 3.88 | -2.29 | 0.006709 | 0.281221 | |
| 17097034 | 1.77 | 2.89 | -2.18 | 0.006712 | 0.281221 | |
| 17022610 | 0.68 | 1.82 | -2.2 | 0.007146 | 0.281221 | RNU6-957P |
| 17112349 | 5.12 | 3.86 | 2.4 | 0.007463 | 0.281221 | CYSLTR1 |
| 16707180 | 4 | 2.95 | 2.07 | 0.007531 | 0.281221 | IFIT2 |
| 16752715 | 5.1 | 6.19 | -2.12 | 0.007854 | 0.281221 | LRP1 |
| 17098624 | 2.82 | 1.6 | 2.33 | 0.008083 | 0.281221 | RNA5SP296 |
| 16773581 | 3.29 | 4.35 | -2.1 | 0.008425 | 0.281221 | RNU6-82P |
| 17064020 | 2.79 | 4.12 | -2.51 | 0.008594 | 0.281221 | OR2A7 |
| 16683105 | 4.75 | 5.76 | -2.01 | 0.00918 | 0.281221 | HSPG2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16797196 | 6.28 | 7.57 | -2.44 | 0.009574 | 0.281221 | AHNAK2 |
| 16763966 | 4.34 | 3.17 | 2.25 | 0.009658 | 0.281221 | SNORA2A |
| 16837938 | 7.01 | 8.14 | -2.19 | 0.00993 | 0.281221 | ITGB4 |
| 17061125 | 2.28 | 3.5 | -2.33 | 0.010077 | 0.281221 | RASA4 |
| 17096166 | 4.08 | 2.95 | 2.2 | 0.010123 | 0.281221 | HSD17B3 |
| 16681477 | 7.32 | 8.38 | -2.08 | 0.010519 | 0.281221 | CLSTN1 |
| 16800536 | 4.73 | 5.75 | -2.03 | 0.01092 | 0.281221 | DUOX1 |
| 16682487 | 4.27 | 5.32 | -2.06 | 0.011604 | 0.281391 | IFFO2 |
| 16846193 | 0.67 | 2.25 | -3 | 0.011674 | 0.281391 | RNU6-1201P |
| 16775844 | 6.32 | 5.28 | 2.05 | 0.011742 | 0.281391 | CLDN10 |
| 16812344 | 3.75 | 2.07 | 3.2 | 0.012213 | 0.281867 | BCL2A1 |
| 17120724 | 1.57 | 0.5 | 2.11 | 0.012793 | 0.283184 | PABPC1P4 |
| 16668170 | 4.57 | 5.78 | -2.32 | 0.012901 | 0.283184 | CELSR2 |
| 17009316 | 7.43 | 6.29 | 2.2 | 0.013854 | 0.283184 | ENPP4 |
| 16945870 | 8.5 | 7.38 | 2.17 | 0.015061 | 0.284666 | TF |
| 17054755 | 3.33 | 4.38 | -2.08 | 0.015094 | 0.284683 | TNRC18 |
| 17089003 | 3.35 | 4.5 | -2.22 | 0.015732 | 0.287193 | OLFML2A |
| 17008651 | 1.09 | 2.17 | -2.12 | 0.015736 | 0.287193 | RNU6-890P |
| 16836824 | 2.69 | 3.87 | -2.28 | 0.016755 | 0.292885 | MRC2 |
| 16738258 | 1.33 | 2.34 | -2.02 | 0.016927 | 0.293482 | |
| 16676661 | 3.8 | 2.79 | 2.02 | 0.016972 | 0.293521 | IL19 |
| 16854780 | 2.8 | 1.28 | 2.86 | 0.017093 | 0.293521 | |
| 16681095 | 3.29 | 4.44 | -2.22 | 0.018257 | 0.297321 | GPR153 |
| 16803754 | 2.54 | 3.71 | -2.25 | 0.018361 | 0.297321 | CEMIP |
| 17067326 | 2.19 | 3.41 | -2.34 | 0.018757 | 0.297321 | MIR3622A |
| 16815812 | 2.75 | 3.93 | -2.26 | 0.019151 | 0.297321 | RP11-27M24.1 |
| 16846218 | 4.64 | 3.47 | 2.24 | 0.019423 | 0.297321 | HOXB2 |
| 16912742 | 6.17 | 4.98 | 2.28 | 0.019886 | 0.297321 | BPIFA2 |
| 16863074 | 5 | 6.19 | -2.29 | 0.020789 | 0.297321 | BCAM |
| 16855944 | 3.3 | 1.95 | 2.55 | 0.020885 | 0.297321 | |
| 16700147 | 3.83 | 4.85 | -2.03 | 0.021183 | 0.297321 | WNT9A |
| 16734299 | 1.6 | 2.84 | -2.36 | 0.021408 | 0.297904 | FAM99B |
| 16923145 | 3.66 | 4.71 | -2.07 | 0.021437 | 0.297904 | ABCG1 |
| 17125892 | 2.98 | 4.07 | -2.13 | 0.021621 | 0.297917 | |
| 17125914 | 2.98 | 4.07 | -2.13 | 0.021621 | 0.297917 | |
| 16904278 | 6.92 | 5.66 | 2.39 | 0.022553 | 0.300285 | DPP4 |
| 17121500 | 3.42 | 4.47 | -2.08 | 0.022771 | 0.300285 | ARHGAP23 |
| 16919703 | 4.91 | 5.96 | -2.07 | 0.02327 | 0.300865 | PLTP |
| 16908037 | 2.72 | 4.68 | -3.88 | 0.023724 | 0.300865 | FN1 |
| 17097432 | 3.95 | 2.92 | 2.04 | 0.024256 | 0.302081 | RNF183 |
| 17119708 | 3.18 | 2.07 | 2.15 | 0.025455 | 0.302081 | ERVK-7 |

| 16712277 | 3.9 | 2.67 | 2.35 | 0.0256 | 0.302081 | ST8SIA6 |
|---|---|---|---|---|---|---|
| 16990294 | 3.19 | 4.22 | -2.04 | 0.026426 | 0.303146 | PCDHB10; PCDHB9 |
| 16936097 | 5.37 | 6.41 | -2.07 | 0.030474 | 0.310853 | CELSR1 |
| 16839642 | 4.55 | 5.68 | -2.18 | 0.031959 | 0.313263 | CLUH |
| 16975417 | 1.6 | 2.67 | -2.11 | 0.032953 | 0.316136 | MIR4802 |
| 17029847 | 3.11 | 1.76 | 2.56 | 0.033373 | 0.317657 | HLA-DPA1 |
| 17118030 | 1.35 | 2.44 | -2.13 | 0.035521 | 0.321374 | LOC100131826 |
| 16824810 | 4.74 | 3.69 | 2.08 | 0.035533 | 0.321374 | CRYM |
| 16694611 | 2.5 | 3.64 | -2.21 | 0.035615 | 0.321374 | |
| 16956792 | 2.91 | 3.97 | -2.08 | 0.035623 | 0.321374 | ABI3BP |
| 16862427 | 4.71 | 5.78 | -2.1 | 0.035729 | 0.321504 | CYP2S1 |
| 16858573 | 3.24 | 2.17 | 2.11 | 0.03717 | 0.323767 | |
| 17120814 | 1.05 | 2.16 | -2.15 | 0.037347 | 0.32392 | |
| 17126022 | 3.09 | 4.22 | -2.19 | 0.038553 | 0.32527 | |
| 17096328 | 2.8 | 1.58 | 2.33 | 0.038897 | 0.326287 | |
| 16908817 | 1.16 | 2.22 | -2.08 | 0.040016 | 0.328088 | AC053503.6 |
| 16929197 | 3.84 | 2.7 | 2.21 | 0.040711 | 0.330269 | RNA5SP496 |
| 17093227 | 1.14 | 2.4 | -2.38 | 0.045345 | 0.335868 | |
| 17125866 | 1.38 | 2.54 | -2.23 | 0.046011 | 0.337703 | MIR3135A |
| 16913176 | 2.81 | 1.8 | 2.01 | 0.046242 | 0.33816 | SPAG4 |
| 16758730 | 2.61 | 3.74 | -2.2 | 0.047372 | 0.340469 | DNAH10 |

The data is based on microarray analysis carried out on cells obtained from 9 healthy non-smokers

**Supplemental Figure Legends**

Supplemental Figure 1. Mucin gene expression correlates with percent predicted FEV1 in COPD subjects. Mucociliary-differentiated cell cultures was established from airway basal cells obtained from 8 COPD subjects. A and B. Total RNA was isolated and expression of *MUC5AC* and *MUC5B* was determined by qPCR and plotted against percent of predicted FEV1 for individual patient. $R^2$ was calculated from linear regression analysis.

Supplemental Figure 2. Effect of RV on the expression levels of FOXA2, SPDEF, FOXJ1 or SCGB1A1 in either normal or COPD cell cultures. Mucociliary-differentiated normal or COPD airway epithelial cell cultures established from airway basal cells obtained from 9 healthy non-smokers and 8 COPD subjects were infected with sham or RV. At 15 days post-infection, total RNA was isolated and mRNA expression of FOXA2, FOXJ1, SPDEF and SCGB1A1, was determined by qPCR. Expression of these genes was normalized to house-keeping gene G3PDH and average from 3 independent experiments was calculated and presented as range with median. A, C, E and G, comparison of sham-infected normal COPD cultures (the Wilcoxon rank sum test). B, D, F and H, intracomparison of gene expression between sham- and RV-infected cultures (the sign-rank test).

Supplemental Figure 3. IL-13 does not contribute to RV-induced GCH in COPD cell cultures. A. Mucociliary-differentiated airway epithelial cell cultures established from 8 healthy and 8 COPD subjects were infected with RV or sham and expression of *IL-13* was determined by qPCR after 15 days). Data represent average from 3 independent experiment for each subject (intracomparison, the sign-rank test). B to D. Cell cultures established from 3 COPD subjects were infected with sham or RV, treated with either neutralizing antibody to IL-13 or isotype control for 14 days on every second day starting from 2 days post-infection and number of goblet

cells and expression of mucin genes were determined. Data represent average calculated from 3 independent experiments for each subject. E to H, Normal cell culture (N43) was cultured with IL-13 to promote GCH. Cultures were maintained for additional one week with no IL-13. I to K, Normal cell culture (N43) cultured with IL-13 were treated with neutralizing antibody to IL-13 or isotype control (IgG). E. Paraffin sections of control and IL-13-treated cell cultures were stained with PAS to detect goblet cells (Arrows indicate goblet cells). Image is representative of three independent experiments conducted in duplicates. F. From identical experiments, cells were fixed with methanol and immuno-stained with antibody to tracheobronchial mucins to quantify goblet cells. G - K. Total RNA was isolated and the expression of mucin genes and *SPDEF* were determined by qPCR. Data in F – K represent mean calculated from three independent experiments performed in duplicates.

Supplemental Figure 4. Erlotinib inhibits EGFR phosphorylation in COPD airway epithelial cells. Mucociliay-differentiated COPD airway epithelial cell culture (cells obtained from one subject, C7) was infected with sham or RV and maintained for 15 days. Cultures were treated basolaterally with DMSO (control) or erlotinib at 0.5, 1, 2.5 or 5 μM every other day starting from 2 days post-infection. A. Total protein isolated from DMSO or erlotinib (0.5 and 1 μM) treated cultures was subjected to Western blot analysis to assess p-EGFR and total EGFR. Image is representative of three independent experiments carried out in triplicates. B. Spent medium was collected at 6, 10, 12 and 14 days post-infection for measurement of LDH activity. Data represent mean calculated from 3 independent experiments in triplicates.

Supplemental Figure 5: Pretreatment with erlotinib does not reduce RV-induced GCH in COPD cell cultures. Mucociliay-differentiated COPD airway epithelial cell culture (cells obtained from one subject, C7) was pretreated with 1 μM for 7 days and then infected with RV or sham. A and

B. At 15 days post-infection, total RNA was isolated and expression of MUC5B and MUC5AC was determined. C. From identical experiments, cells were fixed with methanol and immuno-stained with antibody to tracheobronchial mucins to quantify goblet cells. Data represent mean calculated from 3 independent experiments in triplicate.

Supplemental Figure 6: RV increases *JAG1* and *JAG2* expression in COPD cell cultures. Mucociliary-differentiated normal or COPD airway epithelial cell cultures established from airway basal cells obtained from 8 healthy non-smokers and 8 COPD subjects were infected with sham or RV. At 15 dyas post-infection, total RNA was isolated and mRNA expression JAG1, JAG2, DLL1, DLL2, DLL4, NOTCH2, NOTCH4, HES1, HES5, and HEYL was determined by qPCR. Expression of all these genes was normalized to house-keeping gene G3PDH. Intracomparison of gene expression between sham- and RV-infected cultures in normal and COPD groups (n=3, the sign rank test).

Supplemental Figure 7. Treatment with 10 μM DAPT reduces RV-induced *HEY1* in COPD cell cultures. Mucociliay-differentiated COPD airway epithelial cell culture (cells obtained from one subject, C7) was infected with sham or RV and maintained for 15 days. Cultures were treated basolaterally with DMSO (control) or DAPT at 1, 5, 10 and 20 μM every other day starting from 2 days post-infection. A. Total protein isolated at 15 days post-infection from DMSO and DAPT treated cells was subjected to Western blot analysis to assess HEY1 expression. Image is representative of three independent experiments carried out in triplicates. B. Spent medium was collected at 14 days post-infection for measurement of LDH activity. Data represent mean calculated from 3 independent experiments in triplicates. C. COPD cell cultures (8 subjects) were infected with sham or RV and then treated with DAPT every second day for 15 days.

Expression of SCGB1A1 was determined by qPCR using total RNA. Data represent intracomparison between sham and RV infected cultures (n=3, the sign-rank test).

Supplemental Figure 8. Neutralization of IL-13 does not inhibit RV-induced GCH *in vivo*. Mice with COPD phenotype were infected with sham or RV1B. A. At 8 days post infection, total RNA was isolated and lung mRNA levels of IL-13 was assessed by qPCR. B and C. RV- or sham-infected mice were treated with neutralizing antibody to IL-13 or isotype control by intranasal route every alternate day for 8 days. mRNA levels of Muc5AC and Foxa3 were determined by qPCR. Data represent range with median and data was compared between sham and RV within the groups (the Wilcoxon rank sum test).

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# Supplemental Figure 1

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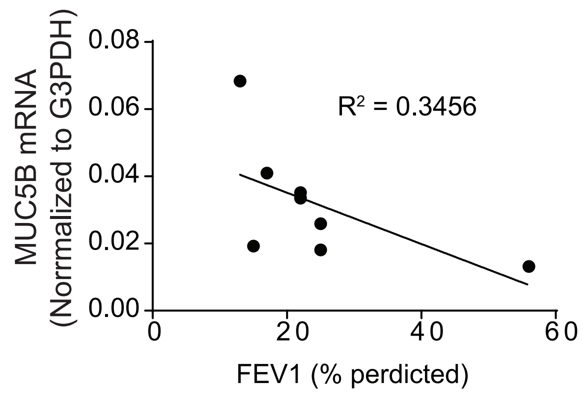


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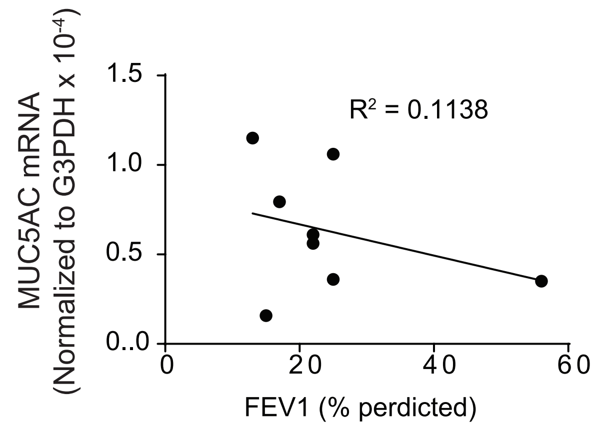
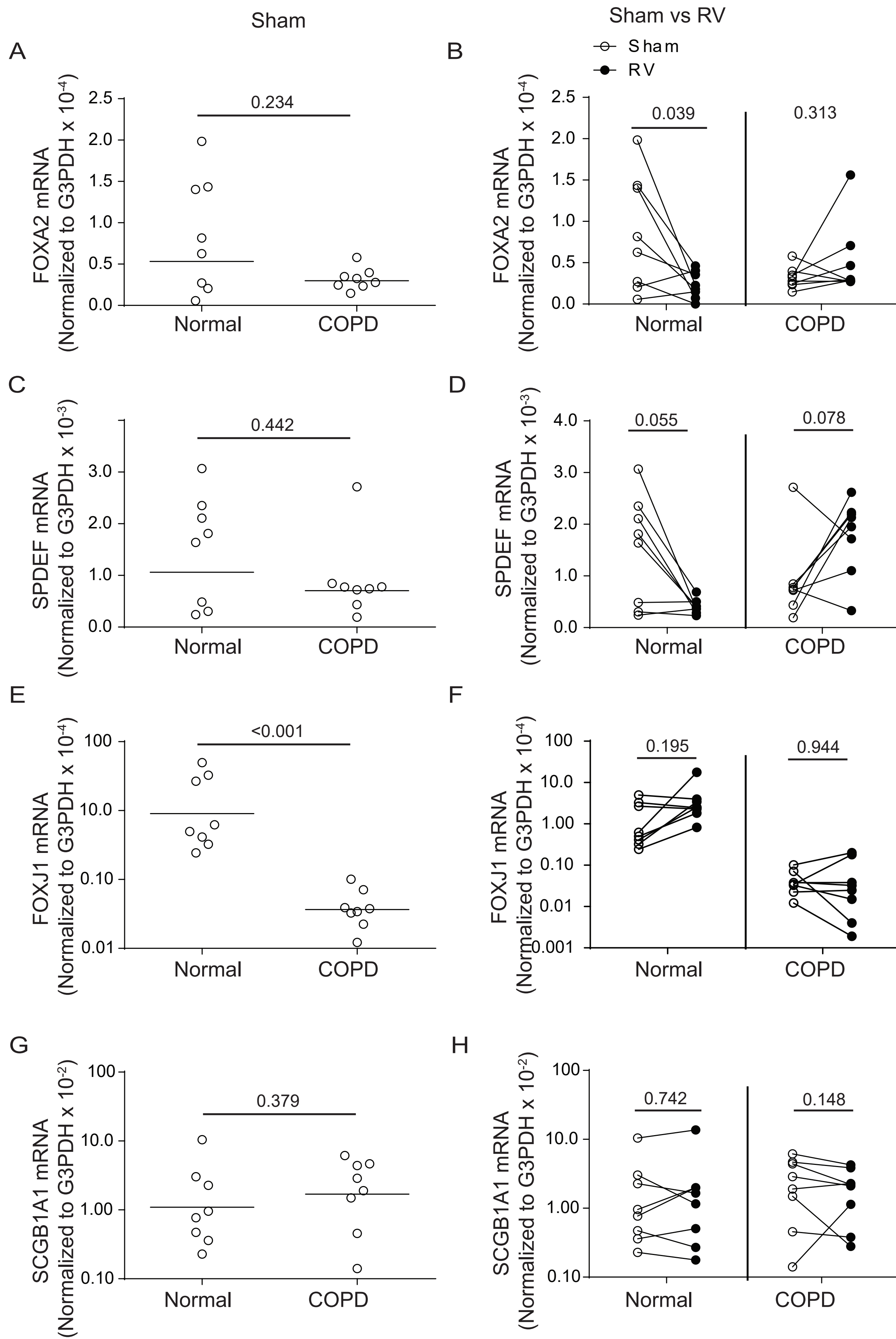


## Supplemental Figure 2



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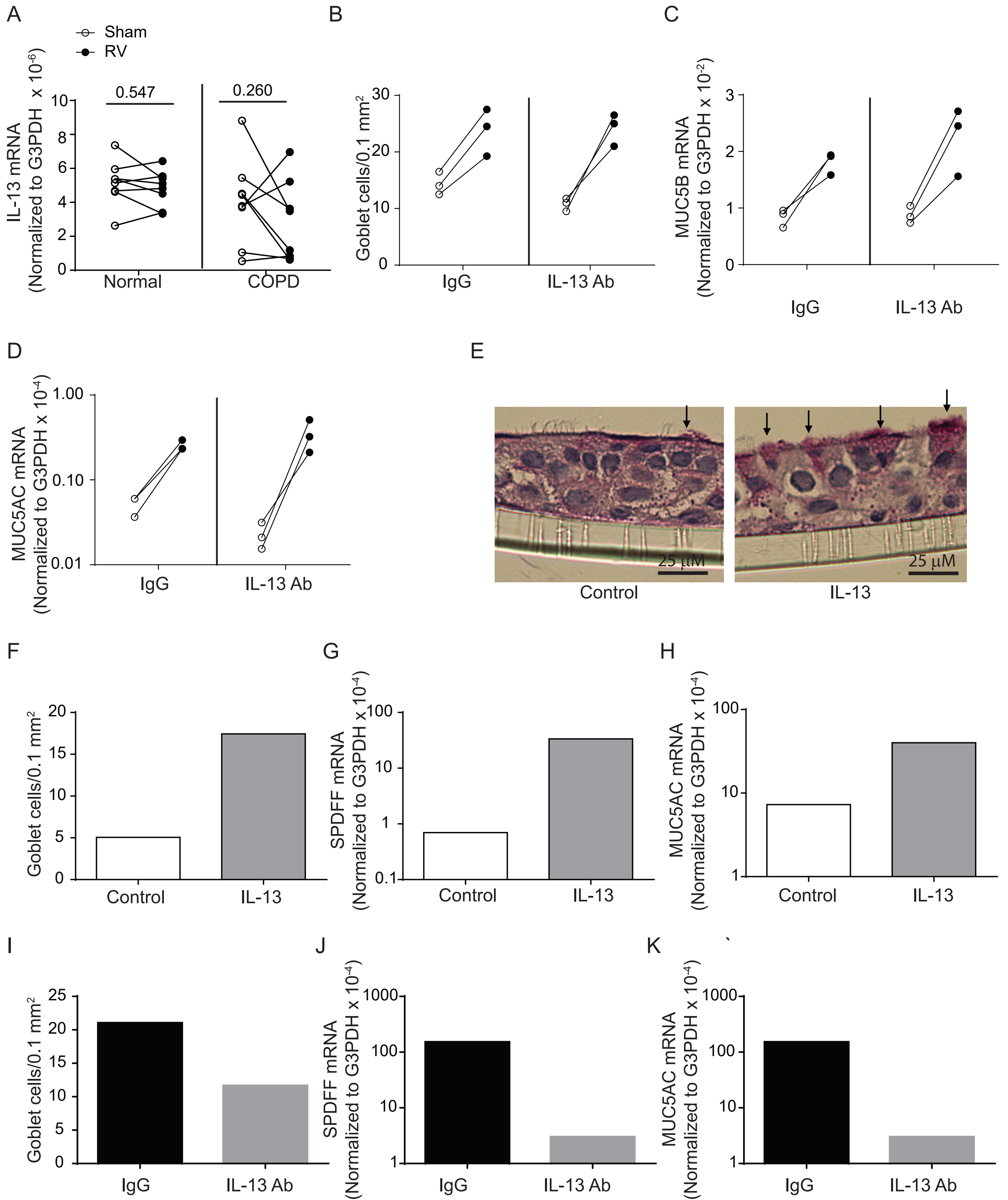

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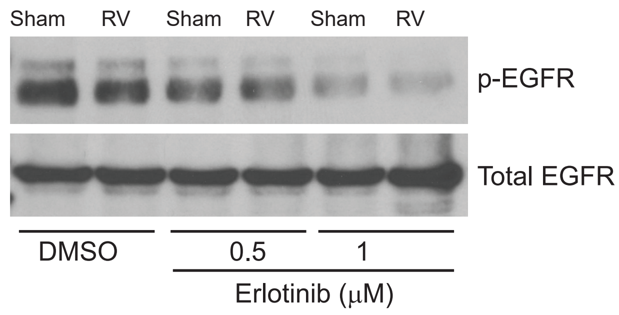


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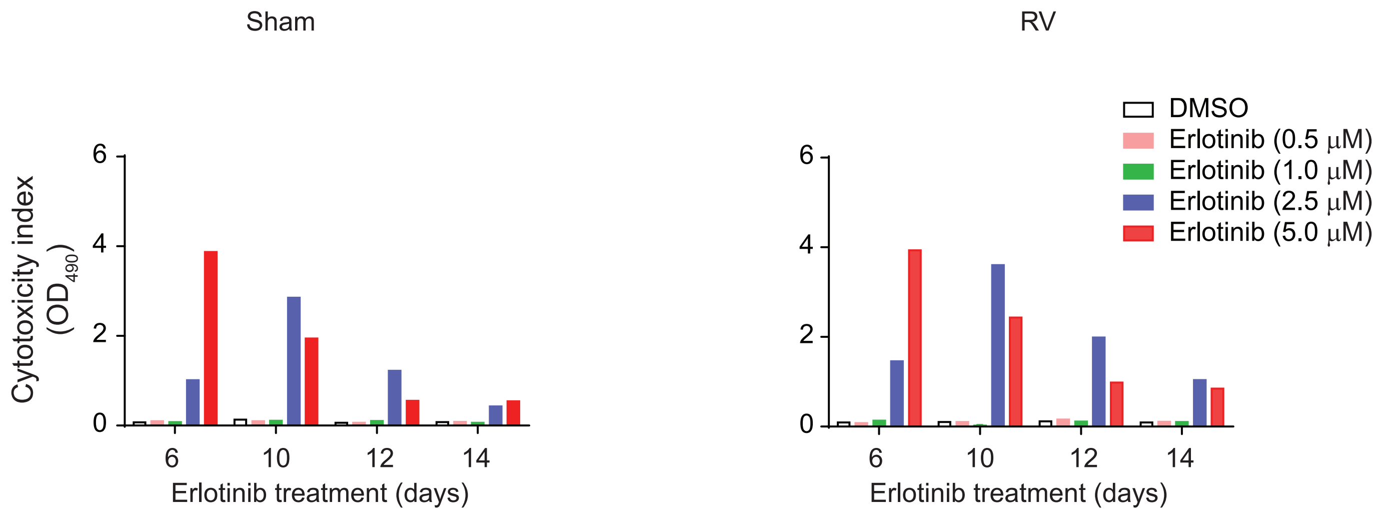

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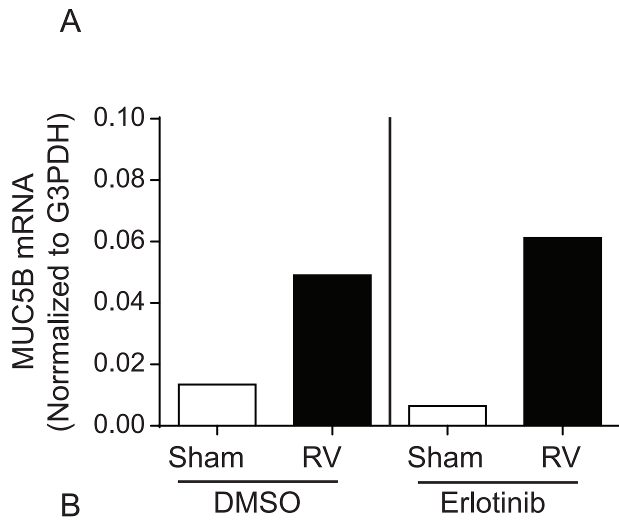


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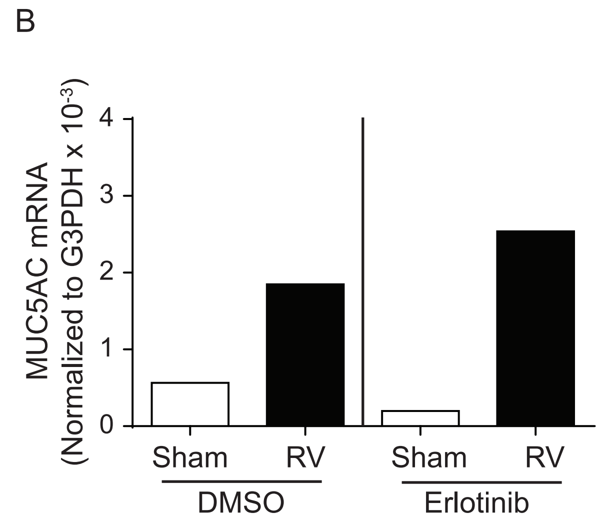


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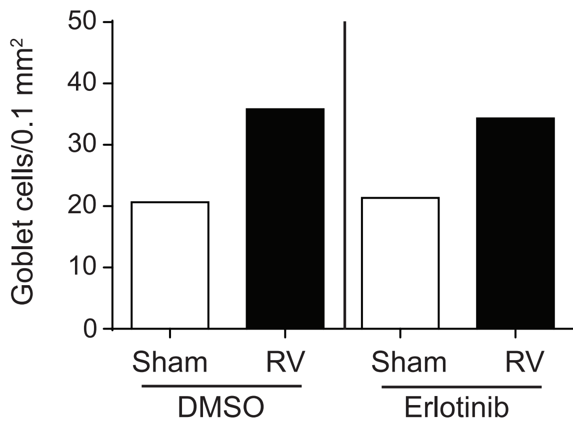

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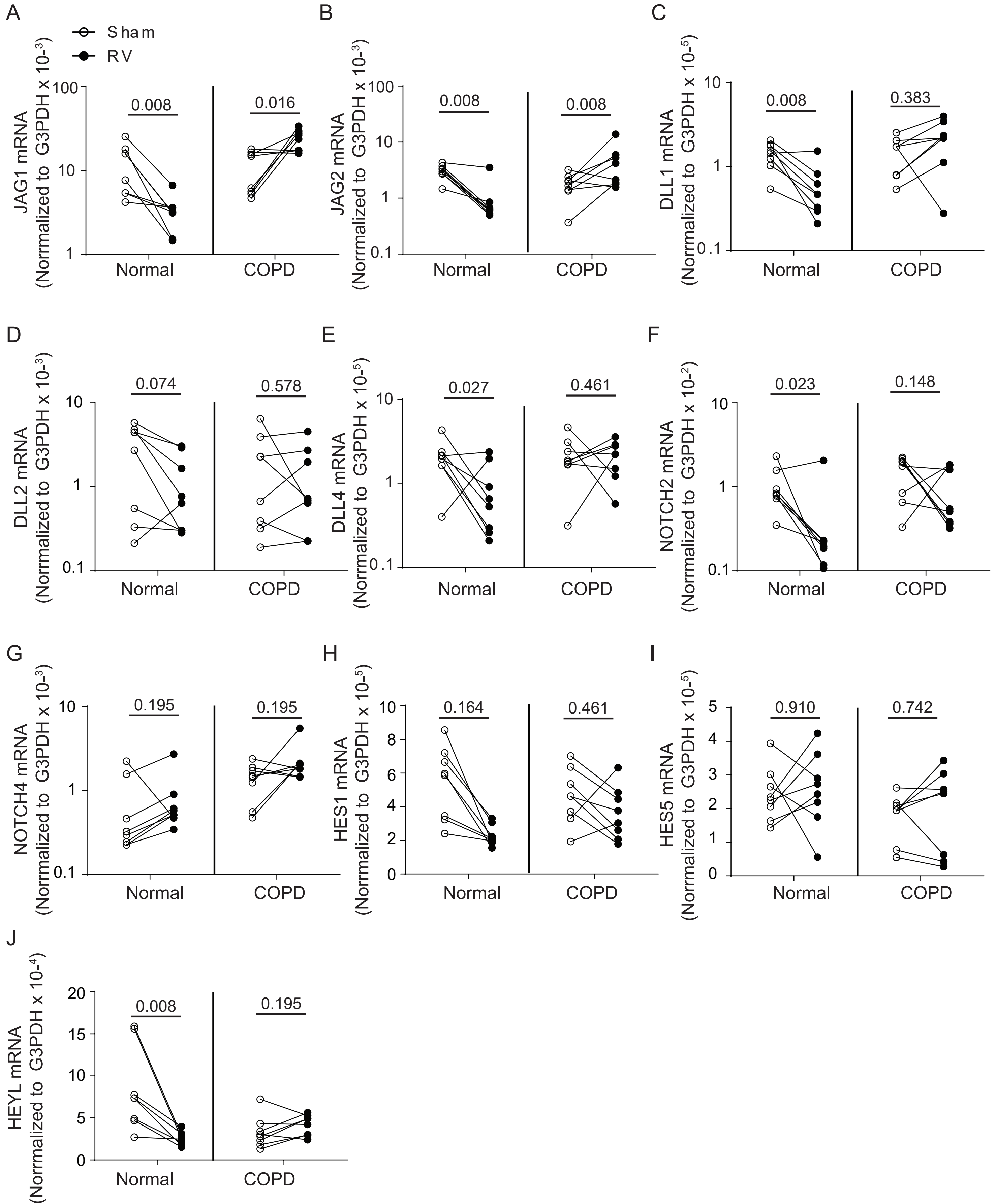

## Supplemental Figure 7

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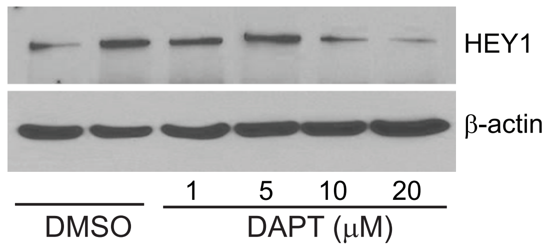


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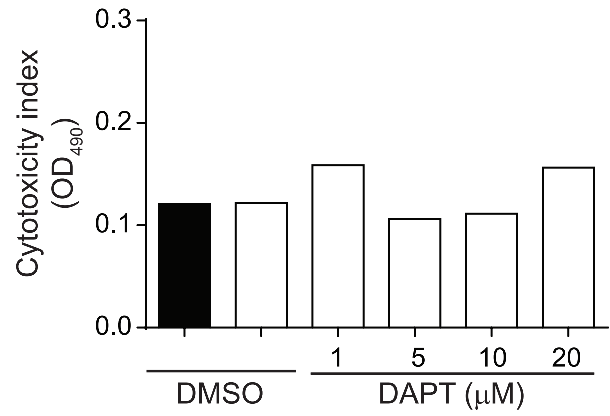


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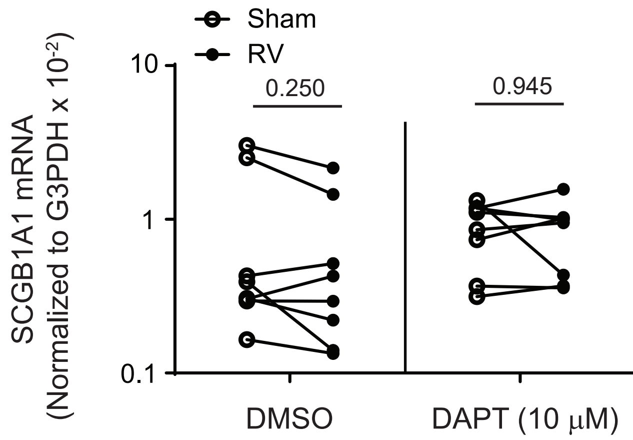

## Supplemental Figure 8