Am J Physiol Lung Cell Mol Physiol 323: L308–L328, 2022.
First published July 19, 2022; doi:10.1152/ajplung.00349.2021

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
LUNG CELLULAR AND MOLECULAR PHYSIOLOGY

RESEARCH ARTICLE

In vitro airway models from mice, rhesus macaques, and humans maintain species differences in xenobiotic metabolism and cellular responses to naphthalene

Jacklyn Kelty,[1] Nataliia Kovalchuk,[2] Eric Uwimana,[2] Lei Yin,[2] Xinxin Ding,[2] and Laura Van Winkle[1]

[1]*Department of Anatomy, Physiology and Cell Biology, Center for Comparative Respiratory Biology and Medicine, School of Veterinary Medicine and Center for Health and the Environment, University of California at Davis, Davis, California and*
[2]*Pharmacology and Toxicology Department, College of Pharmacy, University of Arizona, Tucson, Arizona*

Abstract

The translational value of high-throughput toxicity testing will depend on pharmacokinetic validation. Yet, popular in vitro airway epithelia models were optimized for structure and mucociliary function without considering the bioactivation or detoxification capabilities of lung-specific enzymes. This study evaluated xenobiotic metabolism maintenance within differentiated air-liquid interface (ALI) airway epithelial cell cultures (human bronchial; human, rhesus, and mouse tracheal), isolated airway epithelial cells (human, rhesus, and mouse tracheal; rhesus bronchial), and ex vivo microdissected airways (rhesus and mouse) by measuring gene expression, glutathione content, and naphthalene metabolism. Glutathione levels and detoxification gene transcripts were measured after 1-h exposure to 80 µM naphthalene (a bioactivated toxicant) or reactive naphthoquinone metabolites. Glutathione and glutathione-related enzyme transcript levels were maintained in ALI cultures from all species relative to source tissues, while cytochrome P450 monooxygenase gene expression declined. Notable species differences among the models included a 40-fold lower total glutathione content for mouse ALI trachea cells relative to human and rhesus; a higher rate of naphthalene metabolism in mouse ALI cultures for naphthalene-glutathione formation (100-fold over rhesus) and naphthalene-dihydrodiol production (10-fold over human); and opposite effects of 1,2-naphthoquinone exposure in some models—glutathione was depleted in rhesus tissue but rose in mouse ALI samples. The responses of an immortalized bronchial cell line to naphthalene and naphthoquinones were inconsistent with those of human ALI cultures. These findings of preserved species differences and the altered balance of phase I and phase II xenobiotic metabolism among the characterized in vitro models should be considered for future pulmonary toxicity testing.

in vitro models; naphthalene; pulmonary toxicity; species differences; xenobiotic metabolism

INTRODUCTION

Airway epithelial cells express phase I and phase II xenobiotic metabolism enzymes, which act on both endogenous and exogenous substrates delivered through inhalation or pulmonary circulation. Xenobiotic metabolism is fundamental to pharmacokinetics and toxicity testing, most convincingly demonstrated by the need for S9 fractions containing active enzymes for in vitro genotoxicity evaluations (1). When establishing mechanisms of action for pulmonary toxicants, variations in in vivo xenobiotic metabolism are often considered (2, 3). Yet, critiques about the uncertainty of interspecies extrapolation from animal studies to human risk assessment are emerging alongside movements to reduce, refine, and replace experimental animal experimentation (4, 5). As relevance to physiology and human source tissues is prioritized, established and emerging in vitro airway models need to be validated for their ability to predict in vivo xenobiotic metabolism of respiratory toxicants (6–9).

Epithelial cell lines are known to lose substantial xenobiotic metabolism enzyme activity over time in culture (10–12). As an alternative to cell lines, primary air-liquid interface (ALI) cultures widely used to study lung cell biology were designed to maintain morphological characteristics for months in culture conditions but lack validation of xenobiotic transformation compared with in vivo source tissue (13, 14). Ex vivo cultures (microdissected airways, precision-cut lung slices, and isolated/perfused lungs) preserve the in vivo structural context, yet xenobiotic metabolism enzyme activity can decline heterogeneously between cell types (15–18). Thus, more thoughtful positioning of in vitro models of pulmonary airway epithelial toxicity within emerging high-throughput toxicity testing pipelines should account for the potential bioactivation and detoxification of test compounds by considering the maintenance of xenobiotic metabolism.

Correspondence: X. Ding (xding@pharmacy.arizona.edu); L. Van Winkle (lsvanwinkle@ucdavis.edu).
Submitted 20 August 2021 / Revised 4 April 2022 / Accepted 1 July 2022

Within the complicated pulmonary structure, airway epithelial xenobiotic metabolism capacity is determined by the site-specific density of cells expressing enzymes, the cellular regulation of enzyme proteins, and the substrate-specific activity of enzyme isoforms (Table 1). Differences in metabolism capacity are influenced by airway location, sex, and age that affect the risk of toxicity for bioactivated toxicants (65). Expression of xenobiotic enzymes is even responsive to environmental stimuli like volatile organic compounds and direct-acting oxidants (64, 66–70). Species differences in enzyme isoform activity and substrate specificity also impact bioactivated chemical toxicity among human, rhesus macaque, rat, and mouse airways (65, 71). Moreover, airway donor tissue source and in vitro culture conditions will also influence xenobiotic metabolism, but the outcome is scarcely studied and remains hard to predict.

Pulmonary metabolism is sufficient to bioactivate a variety of volatile organic compounds, as was demonstrated by studies on naphthalene toxicity in isolated, perfused lungs, microdissected airways, and isolated airway cells from mice (72–75). Naphthalene forms oxidized metabolites, including potentially toxic quinones, after initial bioactivation by airway cytochrome P450 monooxygenases (CYP) (76). Naphthalene toxicity is mitigated in mice by preventing initial bioactivation through broad CYP inhibition or knockout of the *Cyp2abfgs* genes (77, 78). Next, microsomal epoxide hydrolase (mEH) can generate secondary reactive metabolites, including 1,2-naphthoquinone and 1,4-naphthoquinone (76). Naphthoquinones are cytotoxic (72, 73), form DNA adducts (79), and generate protein adducts (80–82). Glutathione (GSH) conjugation is the major route of naphthalene clearance and detoxification through nonenzymatic and glutathione S-transferase (GST) catalyzed pathways (83). Increasing the GSH detoxification capacity using GSH prodrug pretreatment diminishes naphthalene airway toxicity in mice, whereas GSH-depleting pretreatments intensify airway toxicity (78, 84). The balance of phase I (mostly bioactivating) and phase II (detoxifying) metabolism is key to the site- and cell-specific toxicity of naphthalene within the lung.

Table 1. *Review of species and airway tree location-specific effects on cell composition and xenobiotic metabolism*

	Trachea	Proximal Bronchi (Branches 2–6)	Distal Bronchi (Branches 7+)
Epithelial structure*	Ciliated pseudo-stratified columnar	Ciliated pseudo-stratified columnar	Simple columnar or cuboidal
Submucosal glands*	Present	Mouse—absent Rhesus—present Human—present	Absent
Club cell density*	Mouse (49%) Rhesus (0%) Human (0%)	Mouse (61%) Rhesus (0%)	Mouse (>50%) Rhesus (0%, RB > 90%) Human (11%–41%, RB 22%)
Goblet cell density*	Mouse (<1%) Rhesus (17%) Human (9%)	Mouse (0%) Rhesus (15%)	Mouse (0%) Rhesus (14%, RB +) Human (2%, RB 0%)
Ciliated cell density*	Mouse (39%) Rhesus (33%) Human (49%)	Mouse (36%) Rhesus (47%)	Mouse (<50%) Rhesus (49%, RB < 10%) Human (+, RB +)
Basal cell density*	Mouse (10%) Rhesus (42%) Human (33%)	Mouse (1%) Rhesus (32%)	Mouse (0%) Rhesus (29%, RB +) Human (+, RB +)
Cytochrome P450 Monooxygenase (CYP)	Human isoforms: CYP 2A6, 2A13, 2B6, 2S1 Mouse: (25, 26) Rhesus macaque: (24)	Club cells >>> Type II cells > macrophages Human isoforms: CYP 1A1, 1A2, 2A6, 2A13, 2F1 (19–24)	
Microsomal epoxide hydrolase	Higher protein expression in mouse proximal vs. terminal Human Club cells >>> Type II cells > macrophages Mouse + + (+) (27–29) Human + (+ +) (19, 26, 27, 29, 30)		
Glutathione (GSH)	GSH *S*-transferase: Mouse + + + + (26–29, 31) Rhesus + + consistent activity through airway generations (32) Human + + (+) (19, 26, 27, 29, 30, 33, 34) GSH: Mouse + + (+) (25, 35–43) Rhesus + + (35, 36, 40) Human + (+ + +) (44–48) GSH synthesis: After GSH depletion, mouse GSH synthesis was twofold Rhesus with trachea < lower airways (36)		

Epithelial structure, cell densities, and xenobiotic metabolism activities divided by the respective locations in the airway tree: trachea, proximal bronchi, distal bronchi, and respiratory bronchioles (RB). Species included when data were available are mice, rhesus macaques, and humans. For airway epithelial structure and cell densities: *mouse: (49), rhesus macaque: (50, 51), human: (52–54), and multiple species: (55–59). The cytochrome P450 monooxygenase enzyme comparisons were based on protein expression and activity, not gene expression. The microsomal epoxide hydrolase and glutathione-related enzyme comparisons were based on activity, not on gene or protein expression. For microsomal epoxide hydrolase and glutathione enzyme activities and glutathione content: + represents relative activity/abundance and may incorporate comparisons with multiple substrates from multiple studies. Airway xenobiotic metabolizing enzyme activity and expression is reviewed in Refs. 10, 60–64, and 12.

This study characterizes the naphthalene metabolism activity and detoxification capacity of ALI airway epithelial cell cultures, isolated airway cells, and microdissected airways using source tissues enriched with airway epithelial cells from mice, rhesus macaques, and humans. The epithelial differentiation and the expression of selected naphthalene metabolism enzymes were measured in these in vitro airway models at baseline. Then the early xenobiotic metabolism changes and oxidative stress responses were documented after exposures to naphthalene, 1,2-naphthoquinone, or 1,4-naphthoquinone. Although media for differentiation and isolation varied, all exposures occurred in uniform sulfhydryl amino acid deficient media to allow for direct comparisons among different tissues and various in vitro models. The models were compared with assess maintenance of species-differences and site-specific naphthalene metabolism in culture conditions.

METHODS

Study Design

In vitro models and fresh source samples from specific airway locations were included in this study to determine the change in xenobiotic metabolism after in vitro processing. Airway tissues from adult mice, rhesus macaques, and humans were compared including a variety of airway isolation and culture strategies relevant for each species (Supplemental Table S1). Samples were from individual animals or human donors to evaluate the diversity of responses in the source tissue and the in vitro models, except for the pooled mouse epithelial cells (MEC) grown at air-liquid interface that were combined from multiple mice.

All animal experiments were performed under protocols approved by the University of California Davis (UCD) Institutional Animal Care and Use Committee (IACUC) in accordance with National Institutes of Health guidelines. Experiments with deidentified human tissue were determined to be exempt from review by the UCD Institutional Review Board. Female mice and rhesus macaques were included in this study because females are more susceptible to naphthalene toxicity than males (2, 85). Both male and female human donors were included. The sample size for each tissue type and pooling strategy for the mouse samples are summarized in Supplemental Table S1. Human and rhesus tissues were not pooled. None of the media used for cell culture contained serum or GSH. Viability was >80% for all isolated airway cells as counted using trypan blue stain and hemocytometer. Cell culture incubators were maintained at 37°C, atmospheric oxygen concentrations, 95% humidity, and 5% carbon dioxide.

Chemical Sources

We used the following reagent grade or better chemicals for exposures: Naphthalene (Fisher 134-500), 1,2-naphthoquinione (Aldrich 346616), 1,4-naphthoquinione (Aldrich 15257), and piperonyl butoxide (Sigma 45626).

Mice for In Vitro Experiments

C57BL/6 mice (Envigo) were acclimated for 7 days before use. All mice were maintained in a barrier facility with filtered air in AAALAC-approved conditions on a 12-h light/dark cycle with food and water ad libitum. Female mice were housed 4–5 per cage and male mice were housed 1–4 per cage. Sentinel mice tested negative for respiratory virus for the duration of the study. Mice were euthanized with an overdose of pentobarbital (Fatal Plus at 0.25 mL ip per mouse, MWI Animal Health Amerisource Bergen 015199).

Mouse microdissected airways.

Mouse lungs were inflated either with RNALater (Qiagen) or 1% (wt/vol) low melting temperature agarose in Waymouth's media, then stored on ice until microdissection as previously described (86). Lung microdissection occurred within 4 h of agarose inflation or 1 wk after RNALater inflation. For gene expression, the cleaned trachea from RNALater inflation served as "trachea" samples and intrapulmonary airways from the left lobe were split into proximal ("proximal bronch") and distal ("distal bronch") segments. The agarose-inflated, whole lung bronchi ("bronch") were used for GSH characterization and naphthalene/naphthoquinone exposures. Tracheal cell isolation was completed as described in Ref. 87. Briefly, the cleaned tracheas from agarose inflated lungs were incubated in 1.5 mg/mL pronase media overnight then cells were isolated as "trachea-EC." The mean mouse trachea-EC yield was 3.6×10^5 (SD 2.3×10^5) cells/trachea.

Mouse primary air-liquid interface trachea cells.

Each MEC sample represents the trachea-EC pooled from a batch of 10–11 mice as in Ref. 87. Briefly for MEC, the trachea-EC were plated onto Primaria Plates (Corning C353803) to selectively attach fibroblasts. Then the suspended epithelial cells were collected, washed with media, and plated at an average of 3.6×10^5 (SD 2.3×10^5) cells per Corning Transwell insert (Corning 3460) coated with 66 ng/mm^2 of human placenta collagen IV (Sigma C7521) (88). Experiments were conducted after 3 to 4 wk at ALI in rodent differentiation media reaching 5,444 cells/mm^2 (SD 623) with 95% (SD 3%) viability (87).

Rhesus Macaques for In Vitro Experiments

Rhesus macaques included in this study were euthanized as a part of normal UCD National Primate Center colony maintenance in response to nonrespiratory disease as indicated in Supplemental Table S1 and following IACUC-approved protocols. The colony is housed in outdoor social groups and is closely monitored by veterinary staff (89). Rhesus macaques were sedated with between 5 and 30 mg/kg of ketamine and euthanized with 120 mg/kg sodium pentobarbital. Tracheas and uninflated whole lungs were placed in media on ice and microdissected within 6 h of necropsy.

Rhesus macaque microdissected airways.

Trachea microdissection consisted of *1*) removing interstitial tissue (Fig. 1), *2*) removing two collagen rings of the proximal trachea for use in whole trachea ("trachea") gene expression and GSH content, and *3*) pronase digestion of the trachea as described earlier (87). After pronase digestion, isolated trachea cells were used for characterization and exposure endpoints as "trachea-EC." Rhesus macaque trachea-EC viability was >80% as counted using trypan blue stain and hemocytometer with a mean yield of 1×10^6

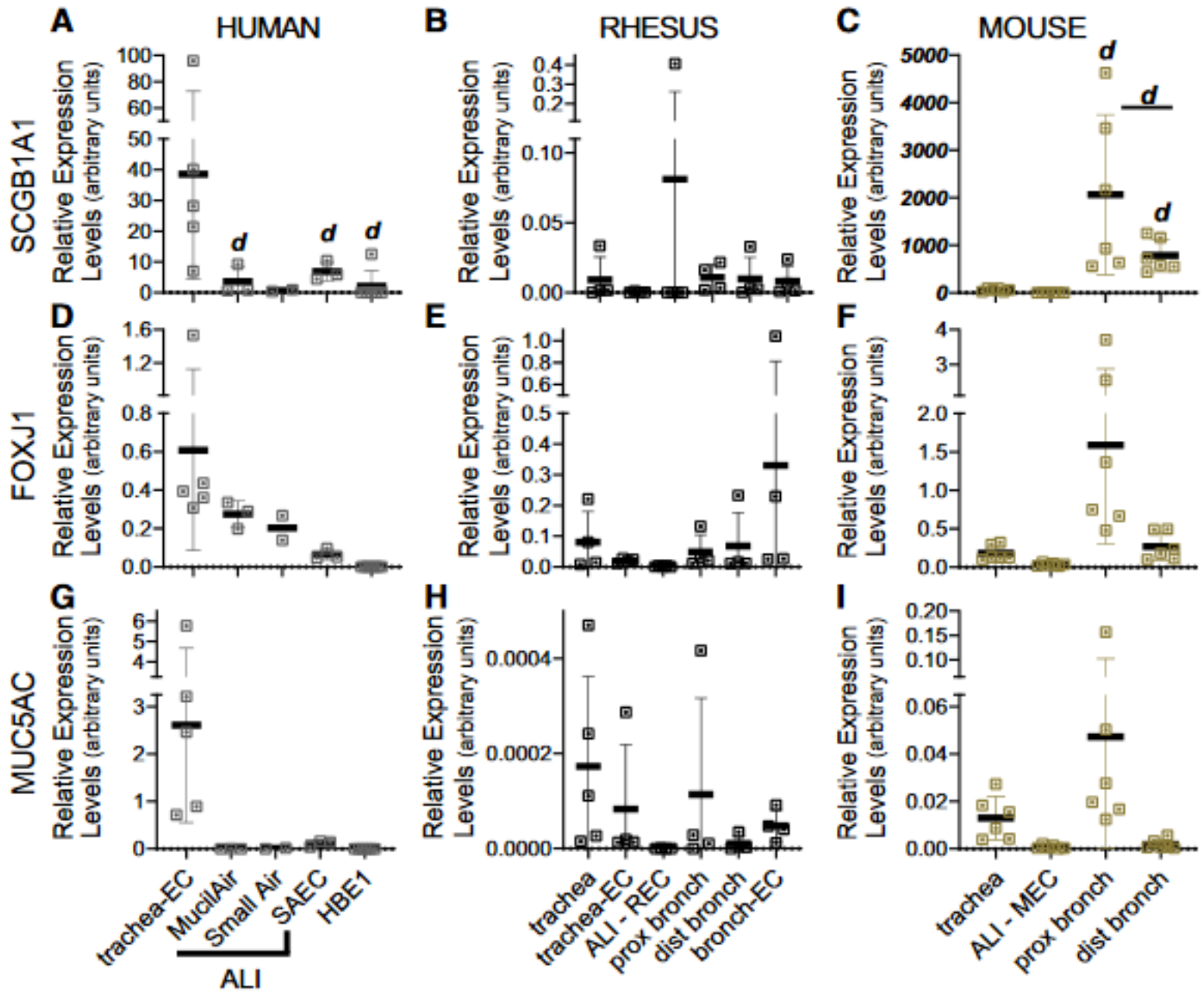

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

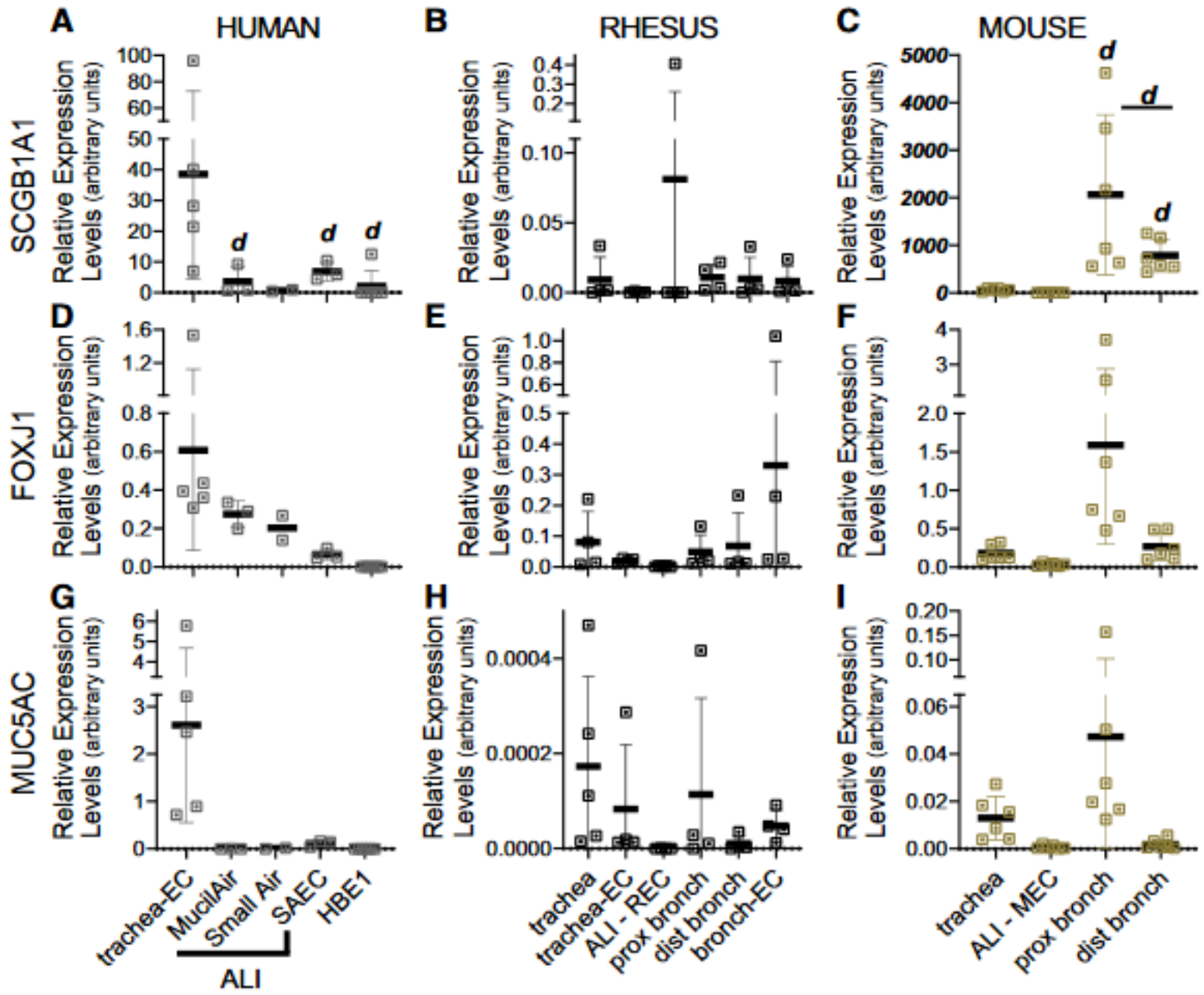

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

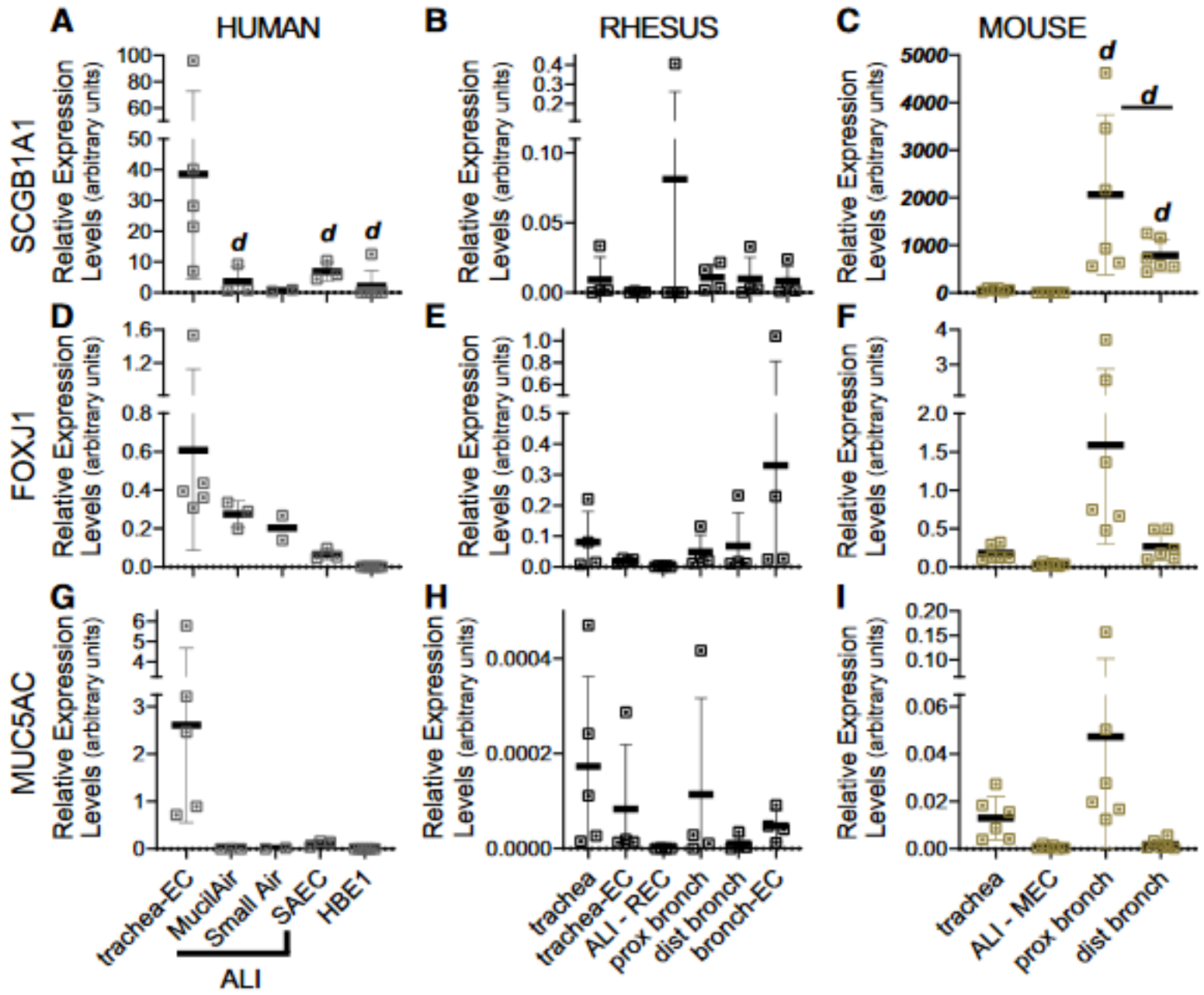

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

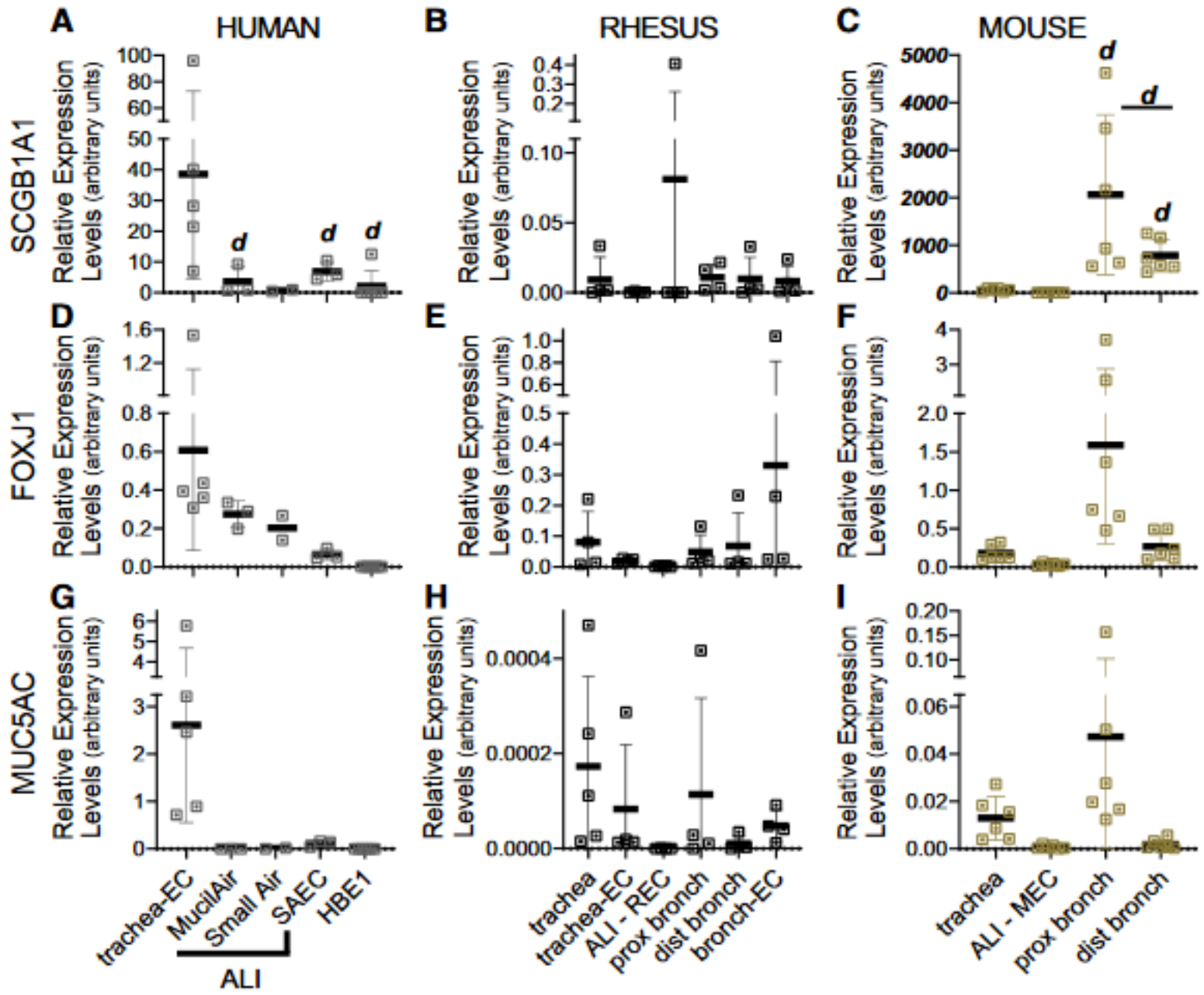

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

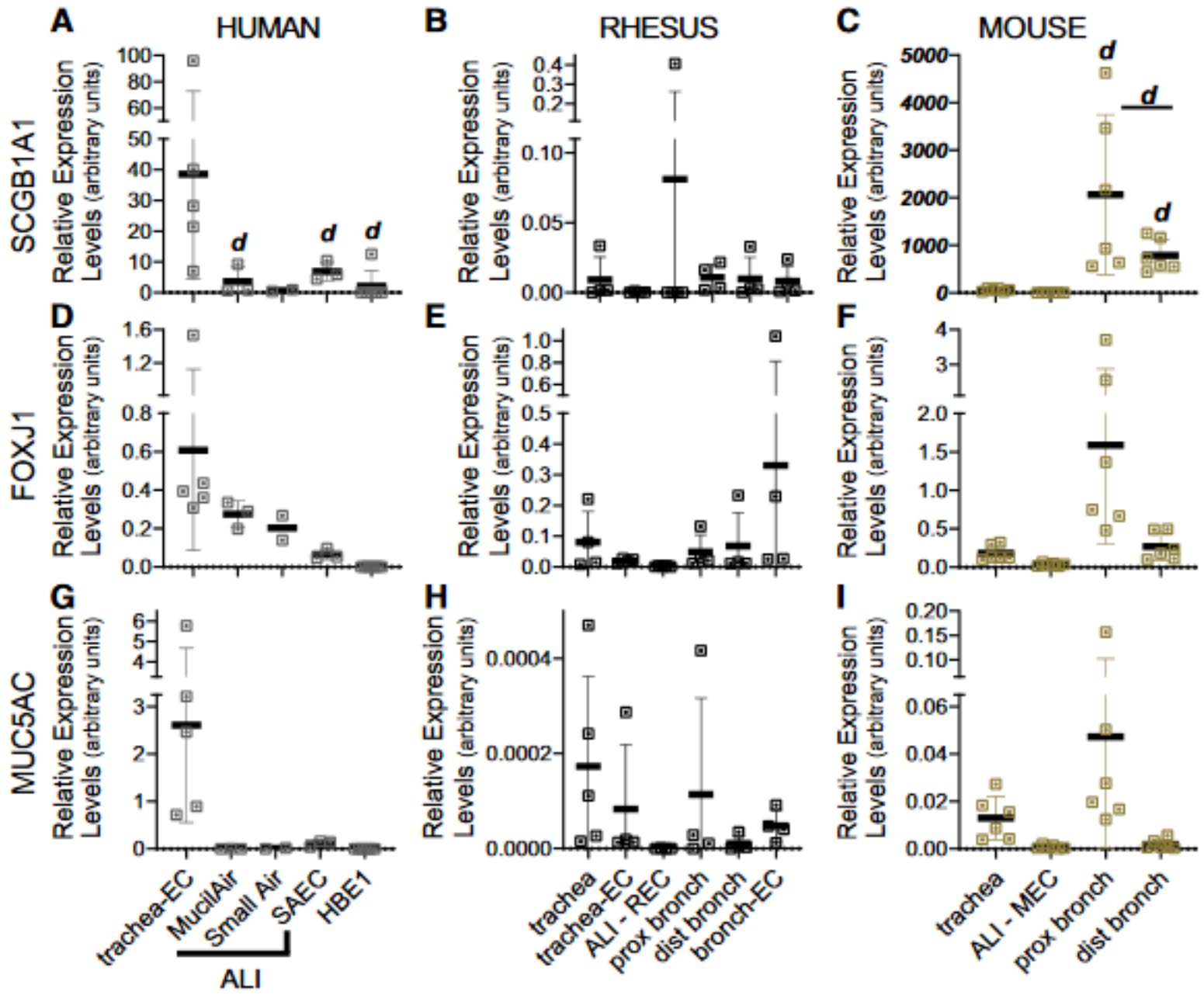

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

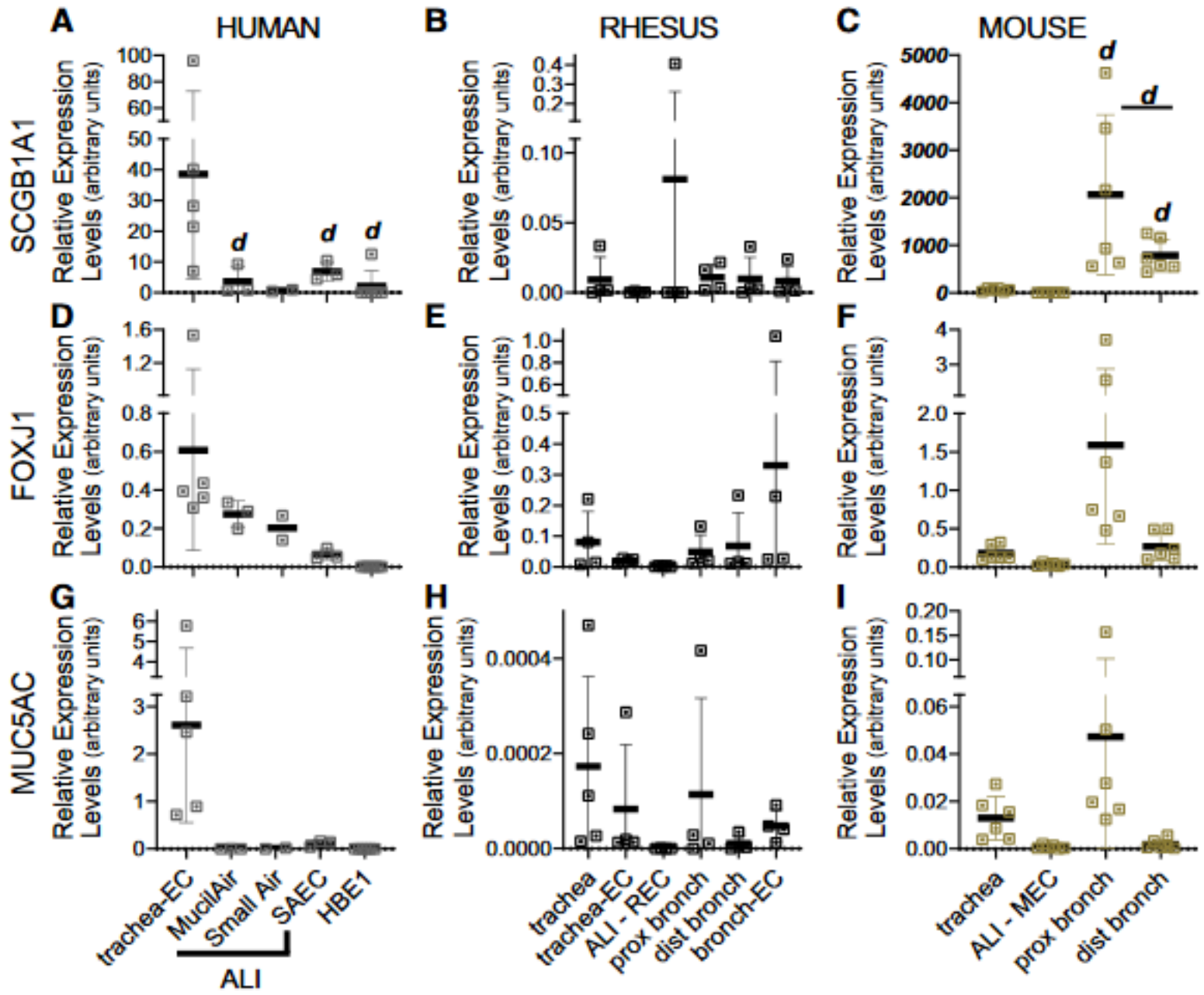

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

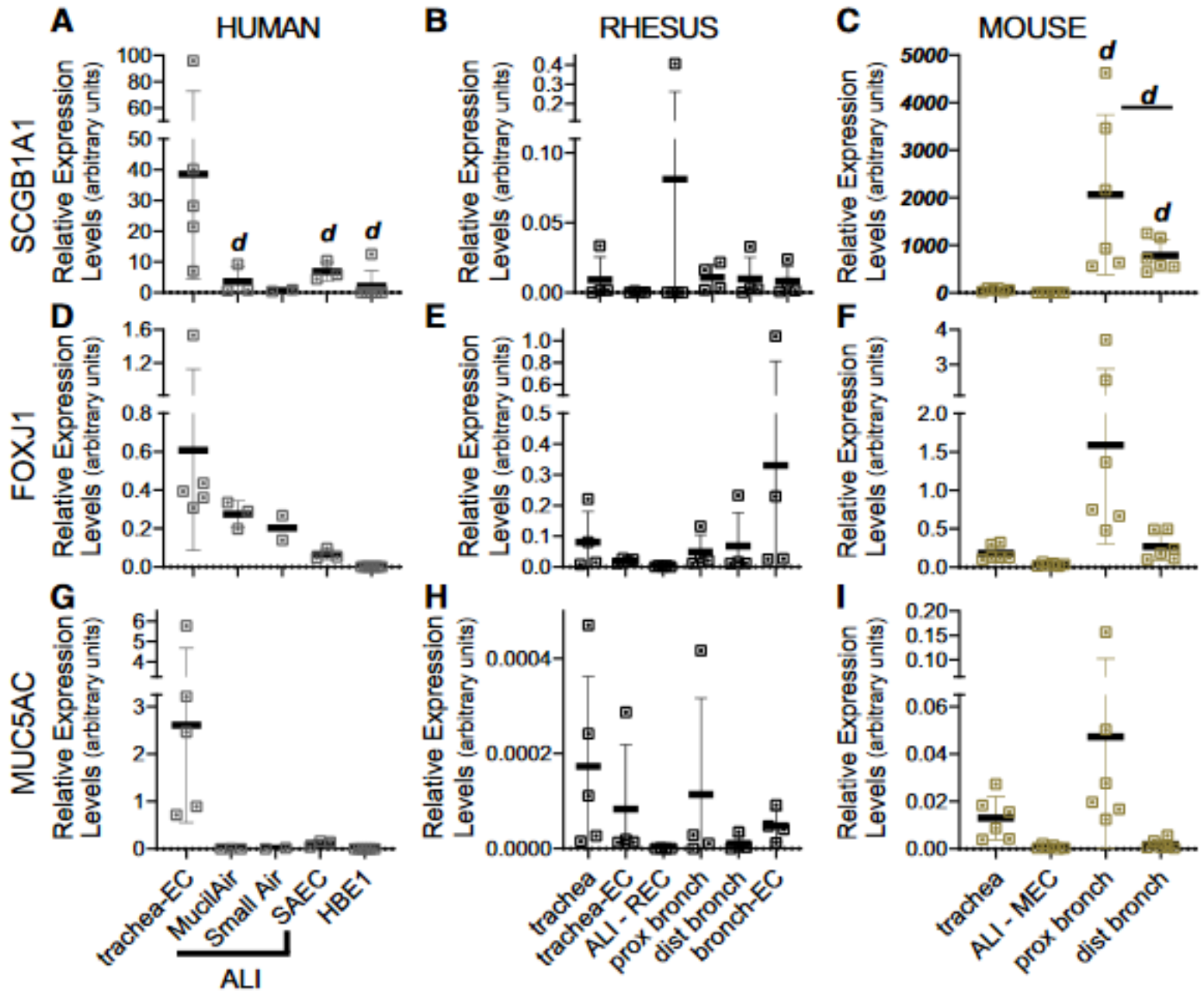

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

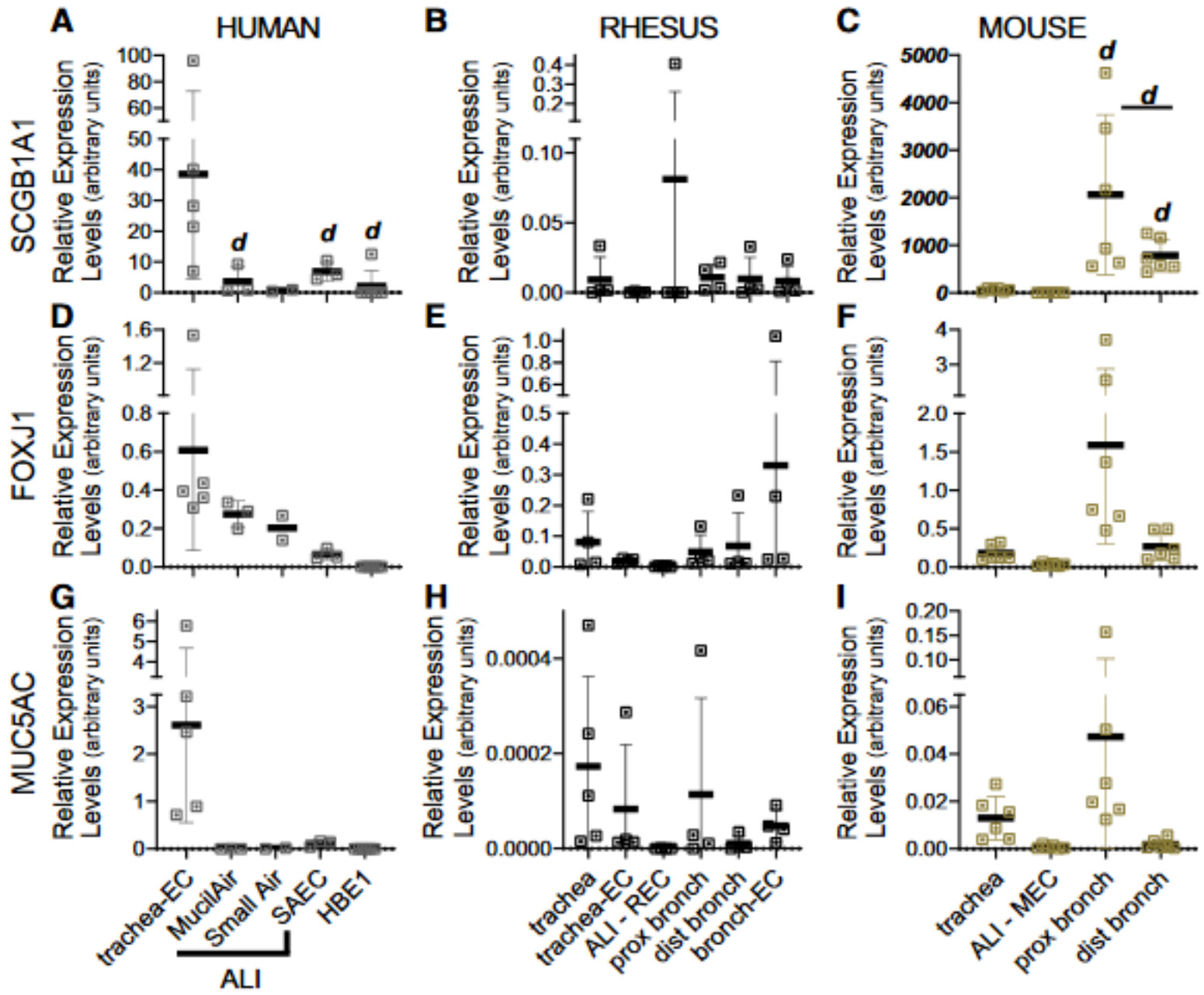

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

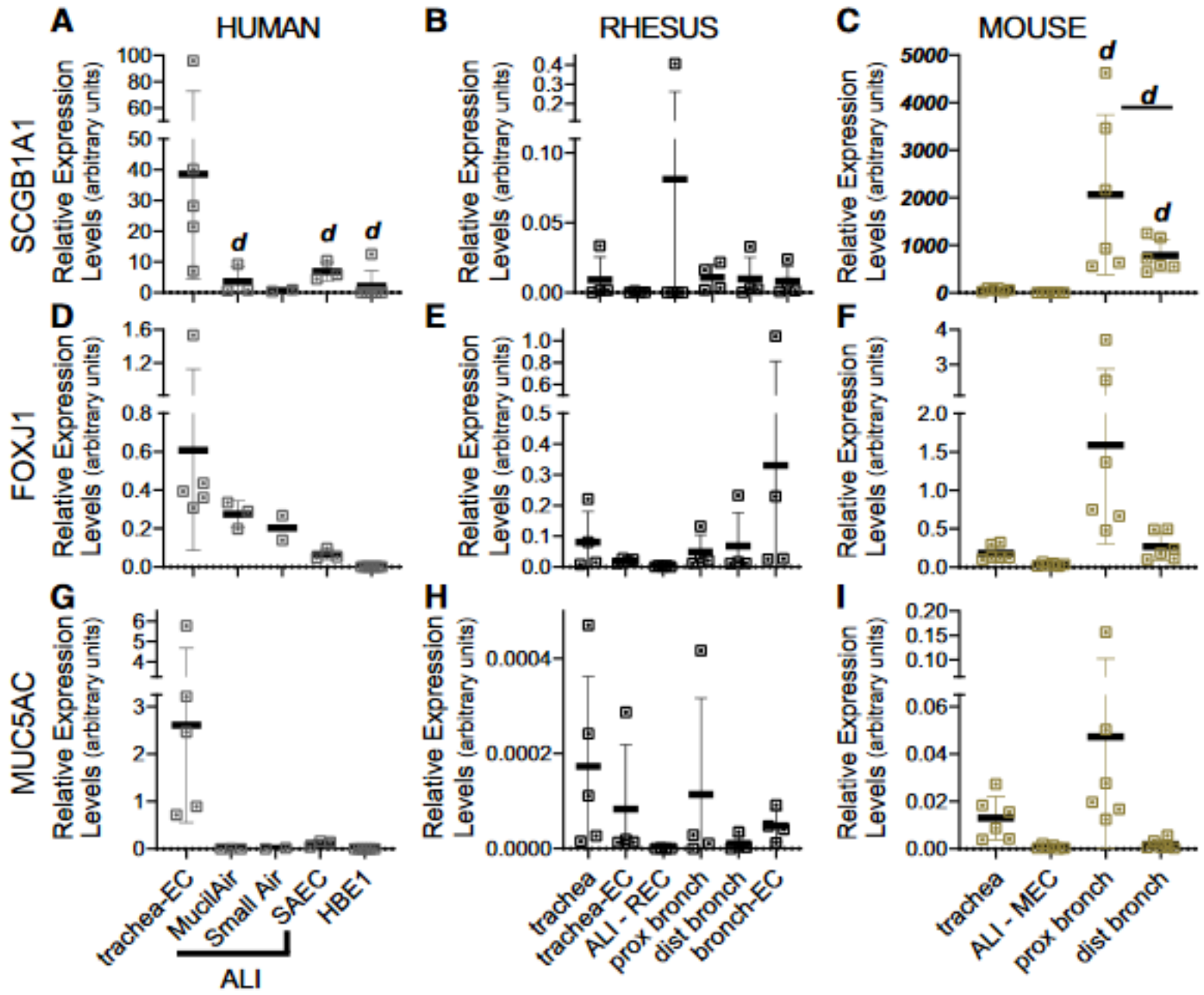

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

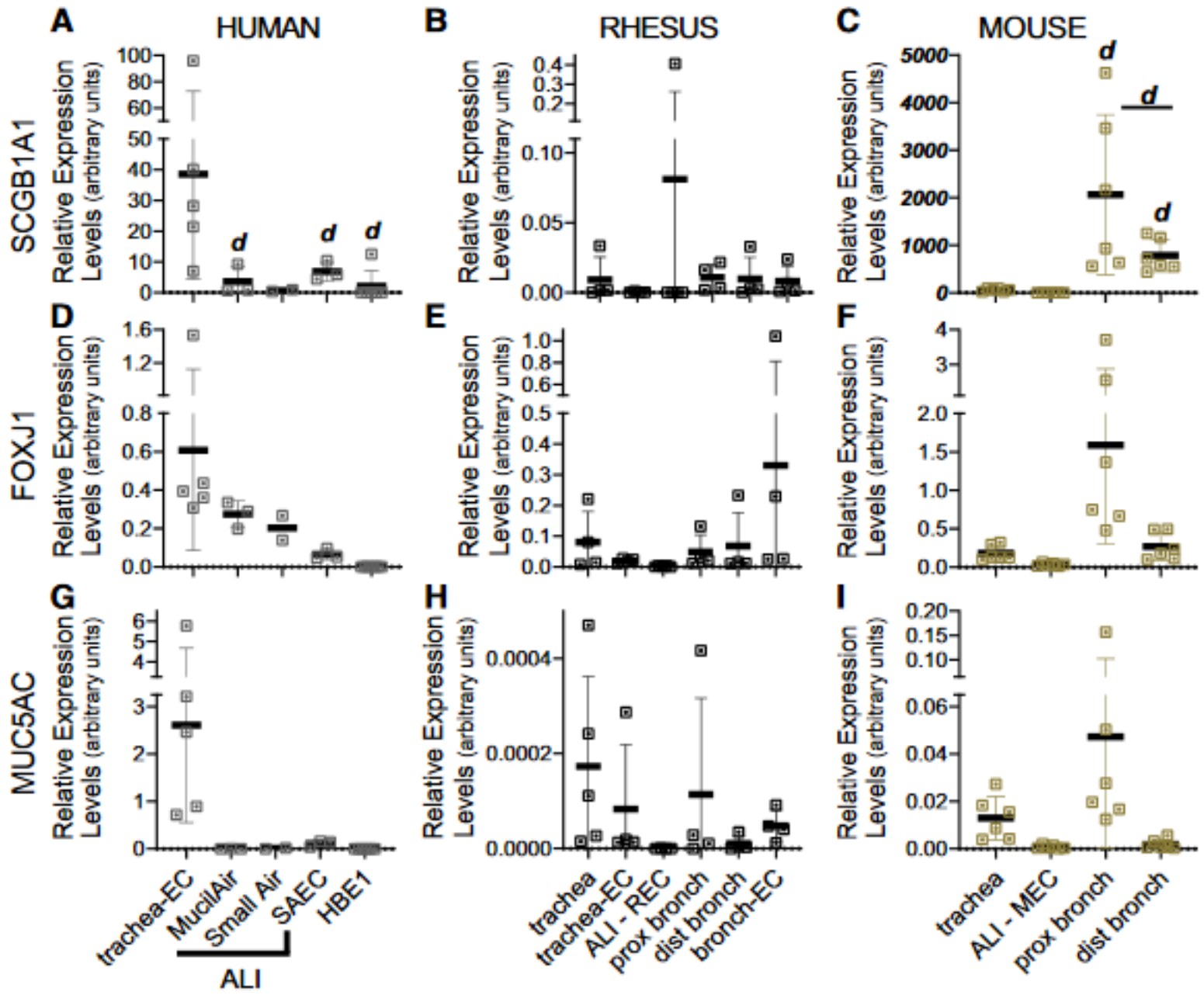

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

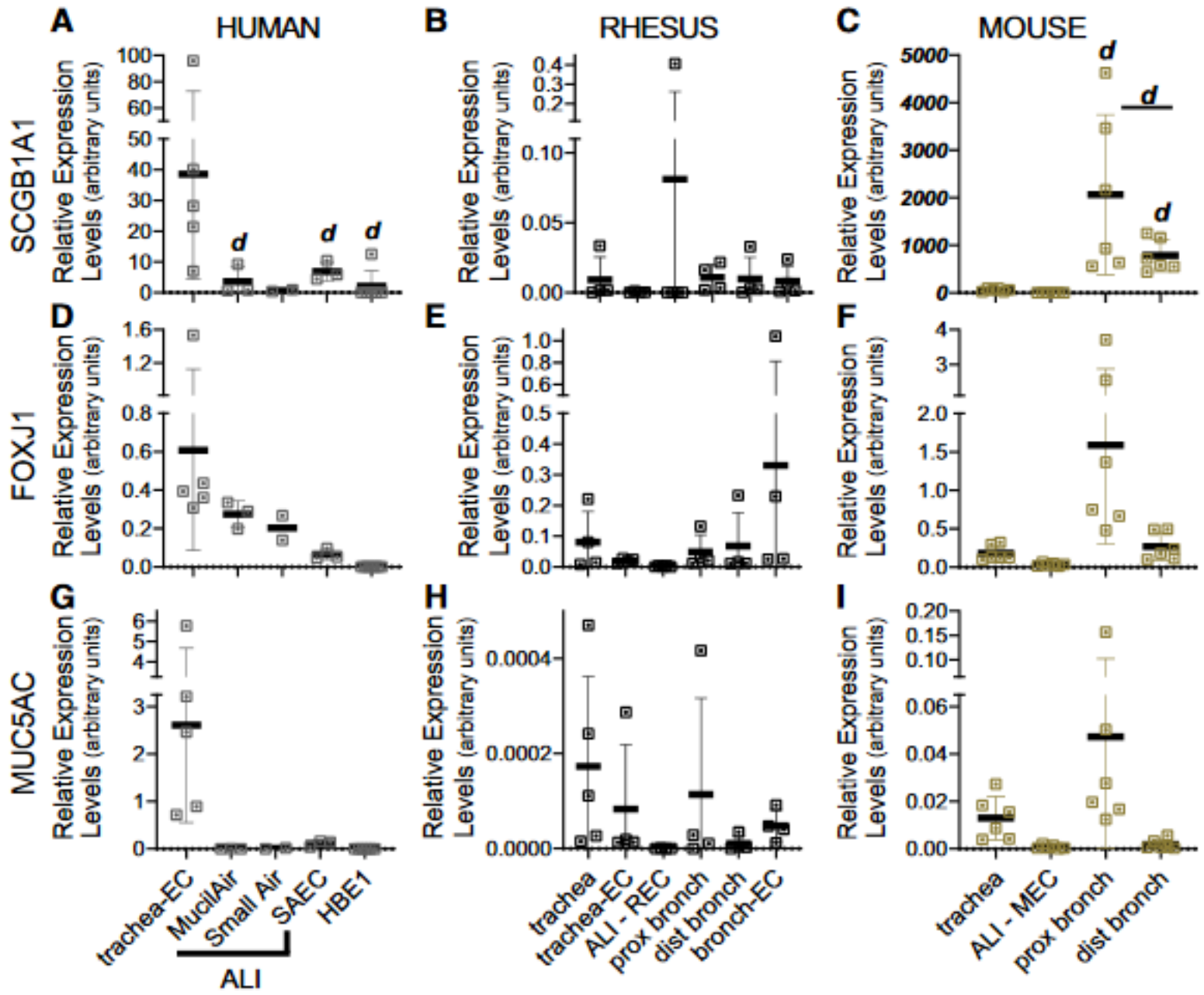

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

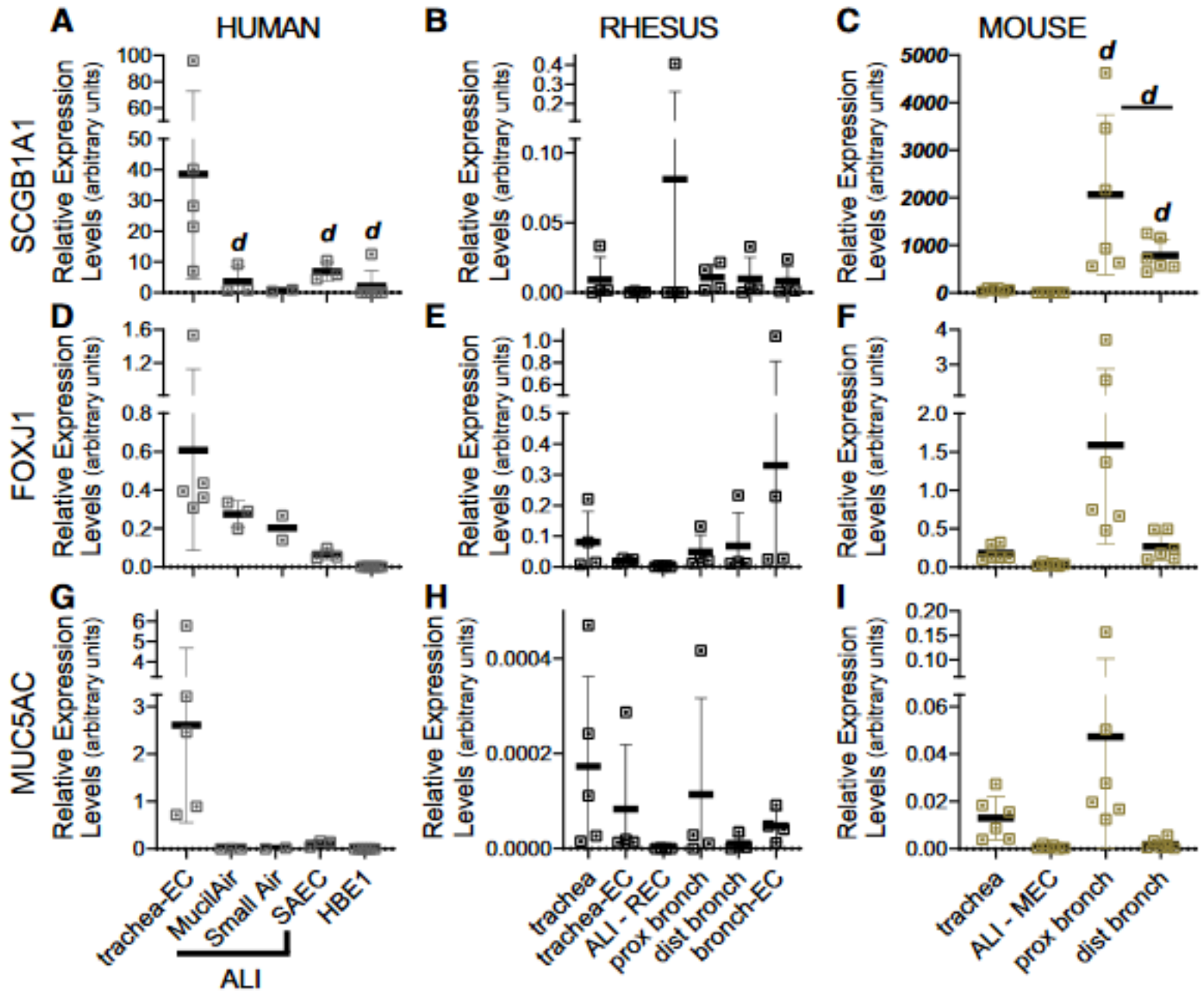

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

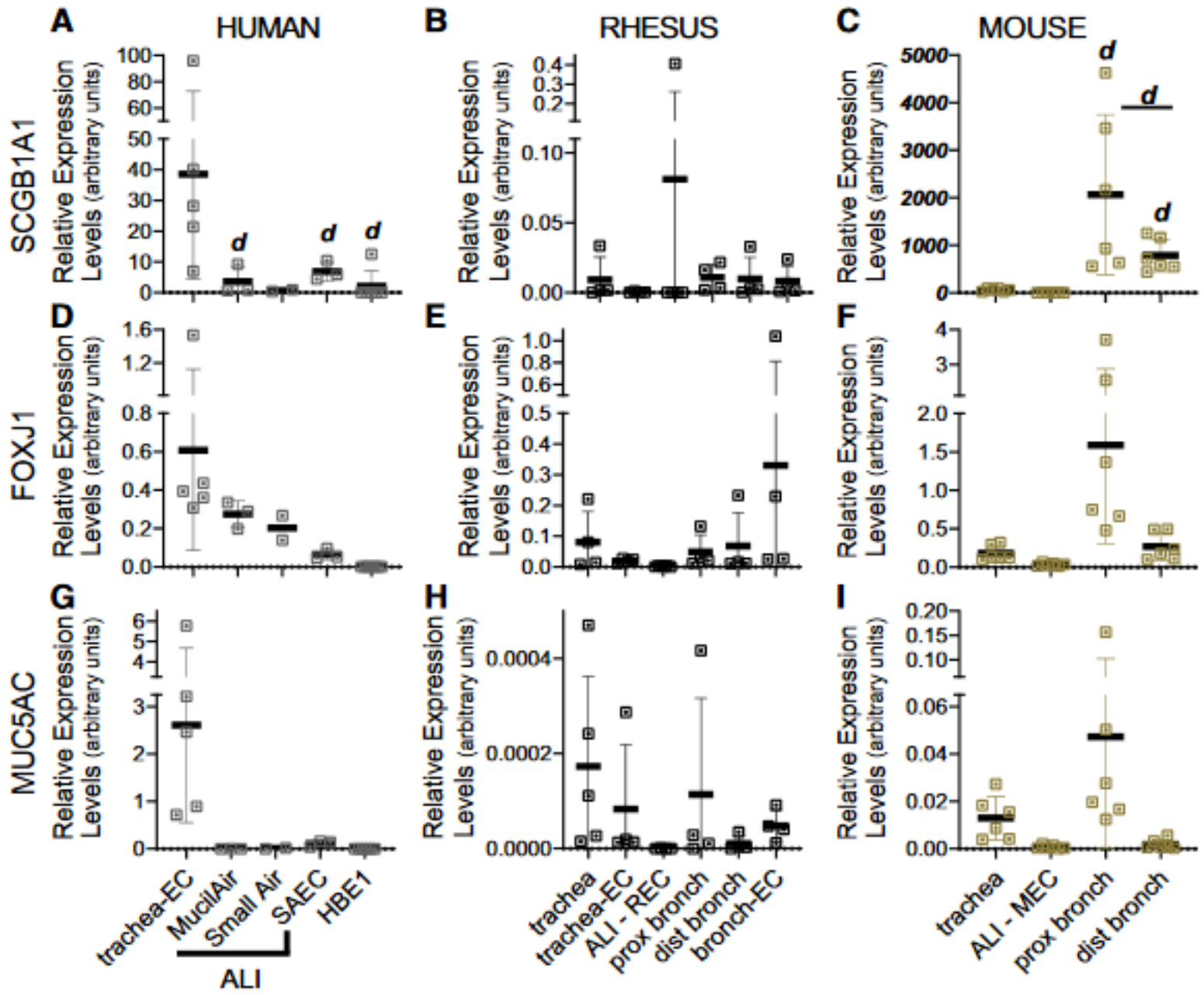

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

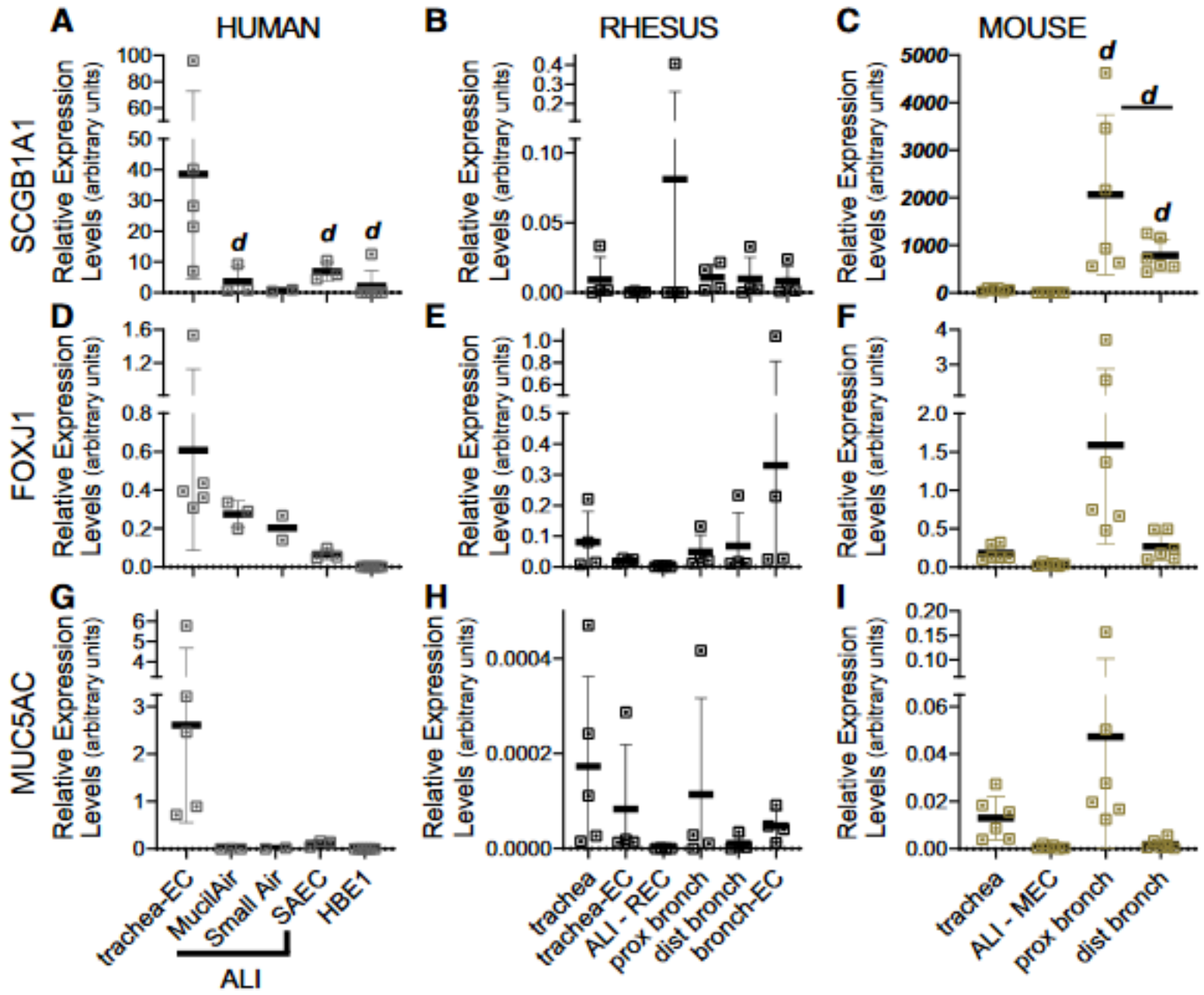

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

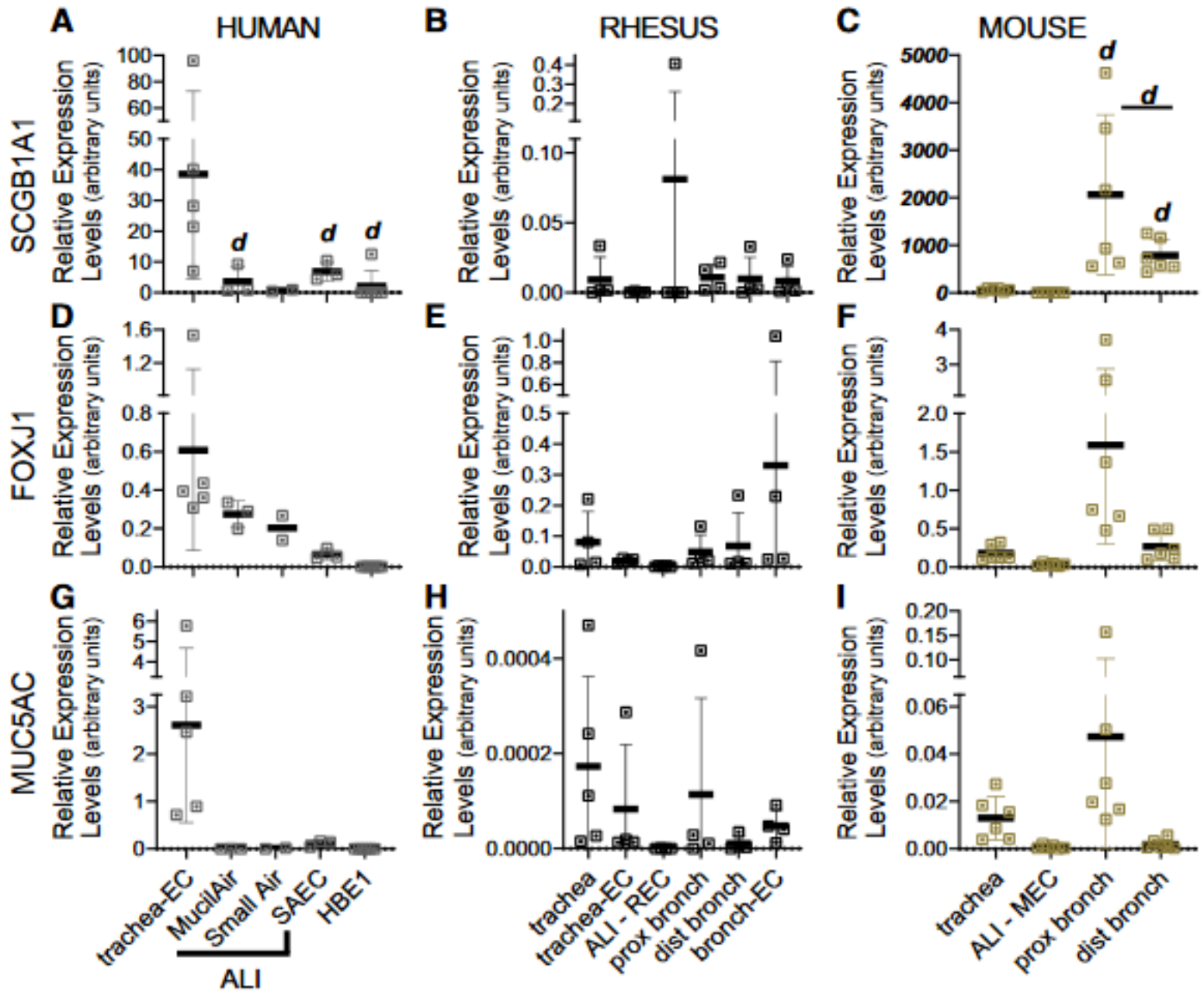

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

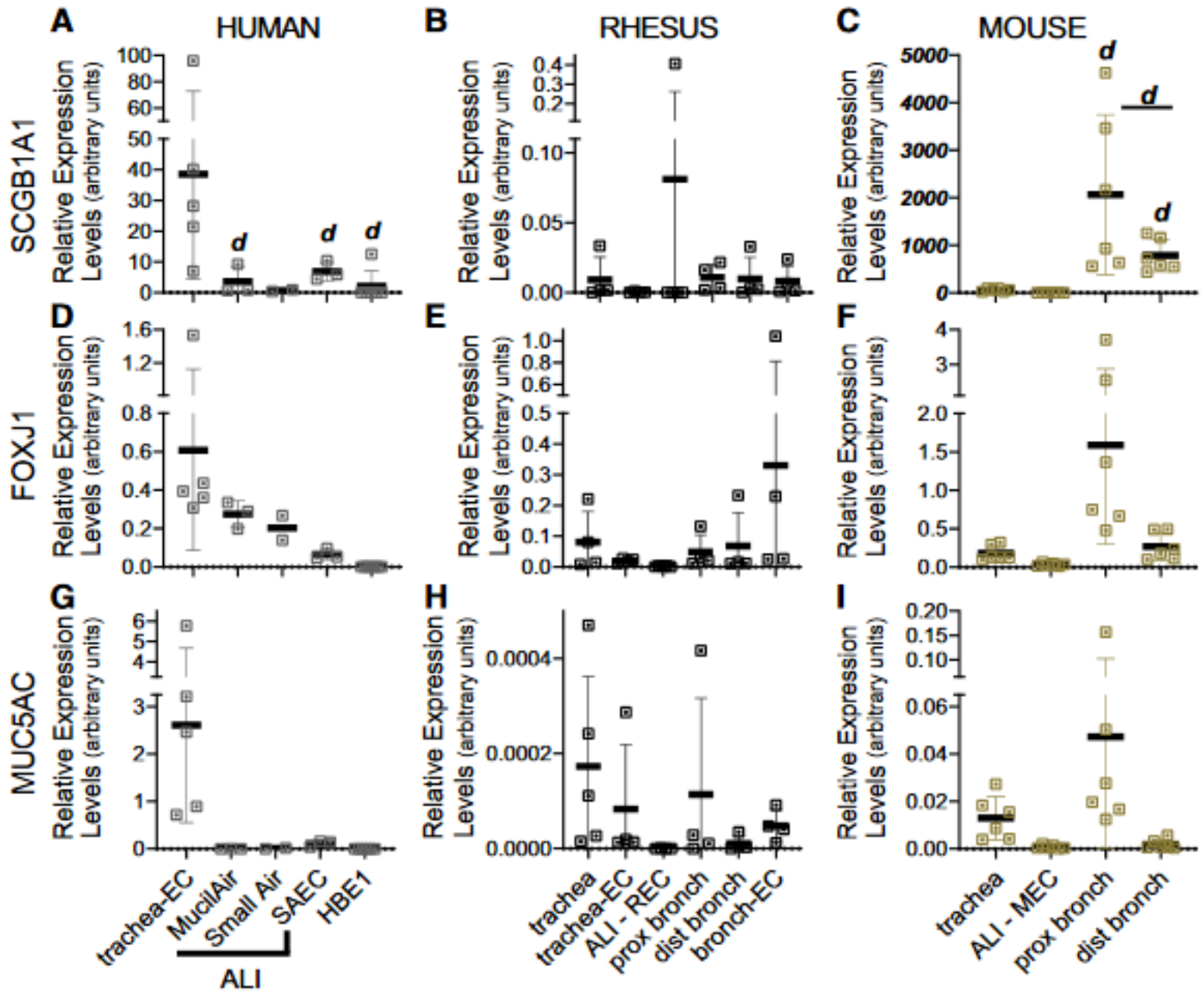

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

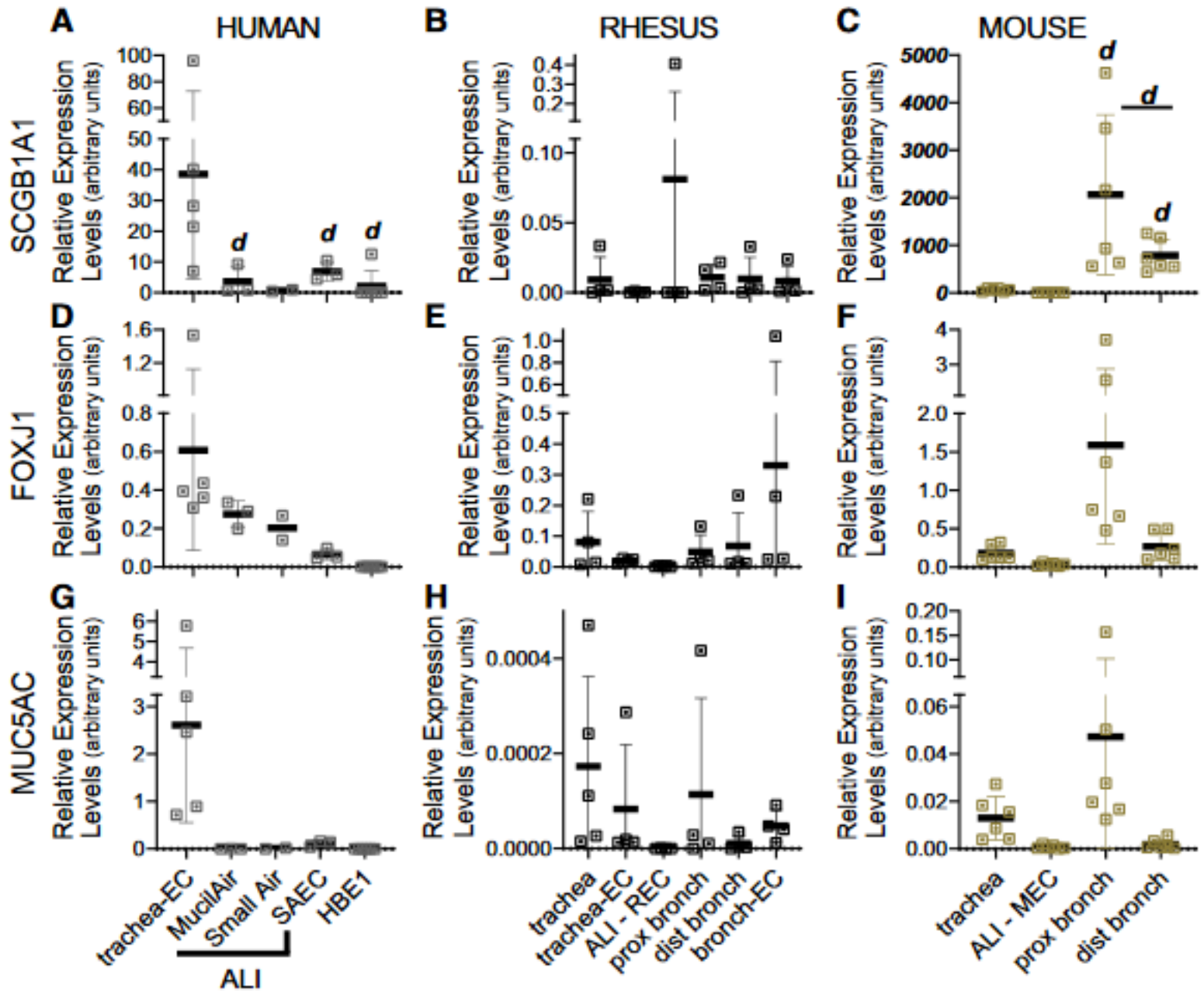

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

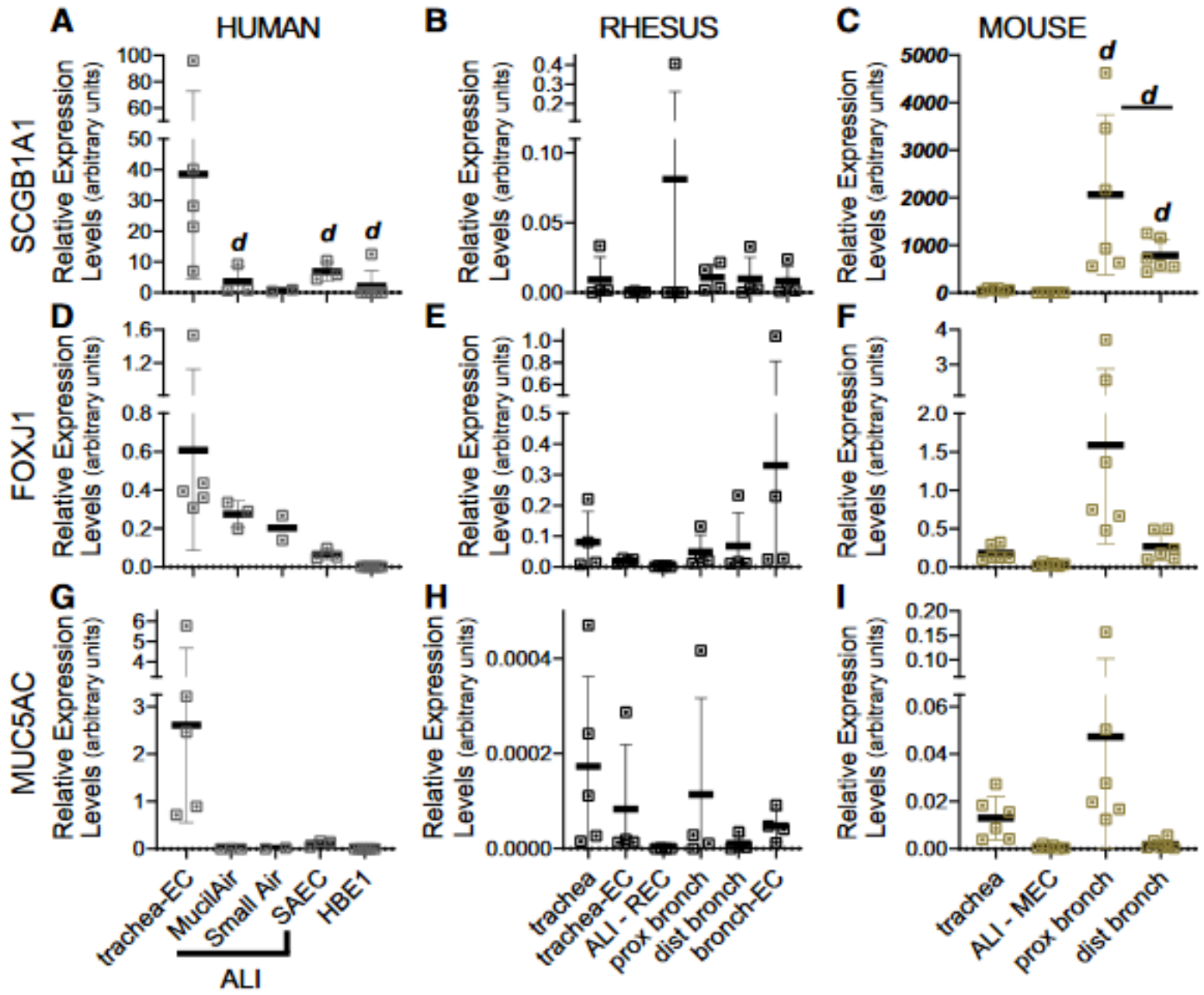

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.

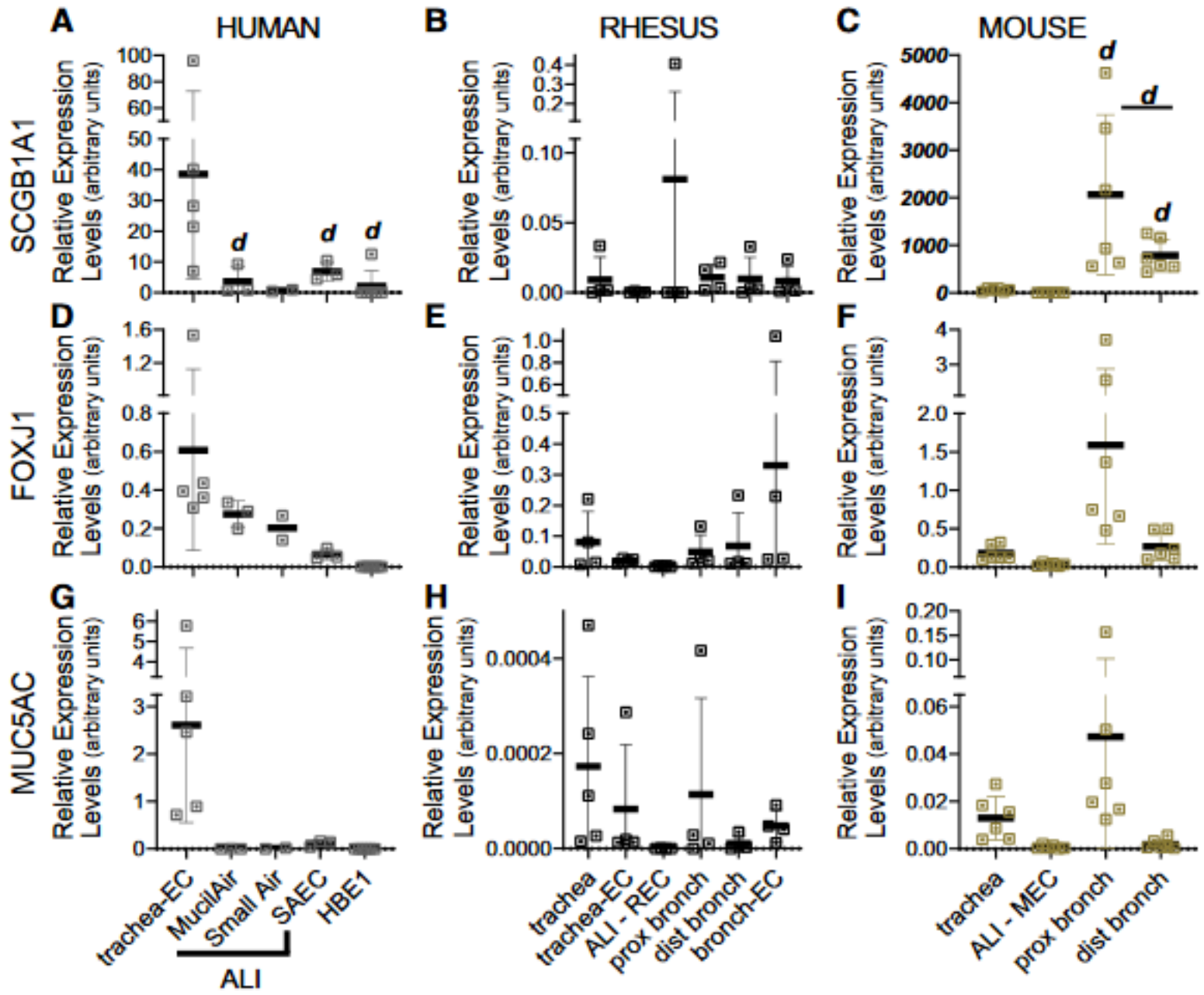

Figure 1. Expression of genes associated with airway epithelial cell differentiation in air-liquid interface (ALI) cultured cells and airway-enriched source tissues from human, rhesus, and mouse. Transcript levels were determined for Club Cell Secretory Protein (SCGB1A1—*A*–*C*), a ciliated cell transcription factor (FOXJ1—*D*–*F*), and a secreted mucin protein (MUC5AC—*G*–*I*). A ribosomal protein housekeeping gene, RPL13A, was used to determine the relative expression between genes within each sample. For human tissues, trachea brush biopsies (trachea-EC) source tissue control group was compared with the air-liquid interface (ALI) groups of primary trachea epithelial cells (MucilAir), primary small airway epithelial cells [SmallAir and small bronchial epithelial cells (SAEC)], and the HBE1 bronchial epithelial cell line. For rhesus macaque, whole trachea source tissue was compared with isolated trachea cells (trachea-EC), microdissected intrapulmonary bronchial source tissue split into proximal (prox) and distal (dist) samples, isolated bronchial cells (bronch-EC), and ALI trachea cell cultures (REC). For mouse, whole trachea source tissue was compared with microdissected intrapulmonary bronchial source tissue split into proximal and distal samples, and isolated trachea epithelial cells grown at ALI (mouse epithelial cells, MEC). Final sample sizes represent for between 3 and 6 unique donors or animals except for the following two instances: *1*) the HBE1 groups that sampled between 3 and 6 cultures from different passages of a cell line and *2*) the SmallAir groups with sample size of 2 unique donors were presented on graphs without error bars and excluded from statistical assessment. Individual samples were measured in triplicate and mean values for technical replicates are represented in graphs. Two-way ANOVA with Tukey's multiple comparison post hoc test ($\alpha = 0.05$) was used to identify significant differences between the groups from the same species. $^{d}P < 0.0001$.

cells/trachea (SD 8.2×10^5). Airway microdissection consisted of *1*) cutting the left caudal and cranial lobes through the parenchyma along the bronchial branches starting at the lobar bronchi (86), *2*) removing parenchyma and vascular tissue to enrich for airways, *3*) splitting the remaining enriched bronchial sample into an intrapulmonary proximal and distal region (Supplemental Fig. S4), *4*) cutting the proximal and distal enriched bronchi in half along the proximal to distal axis, *5*) using one half of the proximal bronchial airways ("proximal bronch") and one half of the distal bronchial airways ("distal bronch") for characterization of gene expression and GSH content, and *6*) placing the other half of the proximal and distal bronchi into one combined tube for pronase digestion as described earlier (87). After pronase digestion, isolated bronchial cells were designated "bronch-EC" for characterization and exposure endpoints. Rhesus macaque bronchi-EC yields were widely variable with a mean of 1.9×10^6 cells/left lung (SD 8.1×10^5).

Rhesus macaque primary air-liquid interface trachea cells.

For REC, trachea-EC were prepared as described earlier for MEC and plated at 10×10^5 (SD 8.1×10^5) cells/insert. REC experiments occurred after 1 wk submerged in growth media and 3–4 wk at ALI in differentiation media reaching 5,323 cells/mm^2 (SD 463) with 99% (SD 1%) viability (90, 91). Rhesus macaque tissue was limited to animals going to necropsy for colony maintenance; therefore, REC is missing from some endpoints.