

1 **Title:** Decreased fucosylation impacts epithelial integrity and increases risk for COPD.

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31 **Abstract (212/250 words):**

32 COPD causes significant morbidity and mortality worldwide. Epithelial damage is fundamental to
33 disease pathogenesis, although the mechanisms driving disease remain undefined. Published
34 evidence from a COPD cohort (SPIROMICS) and confirmed in a second cohort (COPDgene)
35 demonstrate a polymorphism in *Fucosyltransferase-2 (FUT2)* is a trans-pQTL for E-cadherin,
36 which is critical in COPD pathogenesis. We found by MALDI-TOF analysis that *FUT2* increased
37 terminal fucosylation of E-cadherin. Using atomic force microscopy, we found that *FUT2*-
38 dependent fucosylation enhanced E-cadherin-E-cadherin bond strength, mediating the
39 improvement in monolayer integrity. Tracheal epithelial cells from *Fut2^{-/-}* mice have reduced
40 epithelial integrity, which is recovered with reconstitution of *Fut2*. Overexpression of *FUT2* in
41 COPD derived epithelia rescues barrier function. *Fut2^{-/-}* mice show increased susceptibility in an
42 elastase model of disease developing both emphysema and fibrosis. We propose this is due to
43 the role of *FUT2* in proliferation and cell differentiation. Overexpression of *FUT2* significantly
44 increased proliferation. Loss of *Fut2* results in accumulation of Spc⁺ cells suggesting a failure of
45 alveolar type 2 cells to undergo transdifferentiation to alveolar type 1. Using a combination of
46 population data, genetically manipulated mouse models, and patient-derived cells, we present a
47 novel mechanism by which post-translational modifications modulate tissue pathology and serve
48 as a proof of concept for the development of a disease-modifying target in COPD.

49

50 **Introduction:**

51 Chronic obstructive pulmonary disease (COPD), characterized by airflow obstruction,
52 emphysema, and epithelial barrier dysfunction, kills over three million people globally per year (1,
53 2). The primary cause of COPD in the US is long-term exposure to inhaled cigarette smoke (CS
54 (3) although other chronic insults such as air pollution significantly contribute to its global
55 incidence(4). The lung epithelium is the first point of contact for inhalants and is responsible for
56 serving as a barrier to prevent access to subepithelial tissues. E-cadherin, an adherens junction
57 protein, regulates the permeability, polarization, and differentiation of the epithelium. As such, E-
58 cadherin's crucial role in the formation and maintenance of the lung epithelium is clear (5).

59 E-cadherin is most known for its role in maintaining calcium-dependent cell-cell adhesion
60 in epithelial cells (6). However, studies have shown that it is involved in a wide range of cellular
61 activities such as cell maturation, differentiation and migration, cell signaling, immune response,
62 and tumor suppression (5, 7). This versatile role makes E-cadherin a protein of interest for
63 numerous diseases, especially COPD. Decreases in E-cadherin in both the airways and the
64 alveoli have long been associated with COPD (8–14). Our lab has demonstrated that primary
65 bronchial epithelial cells derived from patients with COPD have a significant reduction in E-
66 cadherin levels compared to age- and sex-matched normal cells (15, 16). Moreover, we have
67 found that loss of E-cadherin can drive epithelial dysfunction and tissue remodeling (15) in mouse
68 models. However, mechanisms of modulating E-cadherin in COPD are unknown.

69 Post-translational modifications are covalent changes to amino acids within a protein and
70 can significantly alter protein function or stability. They are one of the last steps in protein
71 biosynthesis and are independent of their original gene transcript. The ability of proteins to
72 undergo post-translational modification at any stage allows for alterations to protein structure and
73 function, which can have far-reaching effects on cellular function (17). One of the most common
74 and highly regulated post-translational modifications is glycosylation, which plays a vital role in
75 governing protein folding, stability, and protein-protein interactions (18). Glycosylation is based

76 on enzymatic reactions that add glycans to proteins and encompass a wide selection of sugar
77 moieties to specific amino acids. Glycosylation occurs in the endoplasmic reticulum and golgi
78 apparatus (PMID 26956395, NCBI Gene 14344). Fucosylation, a specific type of glycosylation, is
79 the process of transferring of a fucose sugar to its substrates, N- and O-linked glycans that are
80 attached to a protein structure, by fucosyltransferases (FUTs) (19).

81 Fucosyltransferase-2 is an intracellular protein responsible for catalysis of α [1,2]
82 fucosylation on the terminal galactose. This primarily occurs on glycan type 1 chain precursors
83 with specificity for epithelial cells (20). Intracellularly, fucosyltransferase-2 is primarily localized in
84 the Golgi apparatus. GDP-l-fucose is transported to the Golgi by a GDP-l-fucose transporter
85 where it is then transferred to the glycan. Following their fucosylation and other modifications
86 made in the Golgi apparatus, these fucosylated proteins are then shuttled to their final
87 destinations in vesicles (21).

88 In this study, we combine clinical genomic data with purified protein analysis, genetically
89 manipulated mouse models, patient-derived differentiated epithelia, and human precision-cut lung
90 slices, to investigate the impact of terminal fucosylation of E-cadherin on epithelial integrity and
91 susceptibility to lung damage from CS. We began with the identification of an SNP that results in
92 loss of FUT2 that is associated with E-cadherin and COPD in two independent clinical cohorts.
93 Having confirmed that FUT2 could post-translationally modify E-cadherin by mass spectrometry
94 and immunoprecipitation, we studied mouse models and patient derived cells to assess its effect
95 on monolayer integrity and lung morphometry.

96 **Results**

97 *Co-localization of trans pQTL for E-cadherin and cis eQTL for FUT2.*

98 In a meta-analysis (22), including a subset of the two large cohorts of current and former
99 smokers from the SPIROMICS and COPDGene cohorts, protein-Quantitative Trait Locus (pQTL)
100 approaches were used to identify single nucleotide polymorphisms (SNPs) associated with
101 measurement of 88 blood proteins, including E-cadherin. Analysis of both SPIROMICS and
102 COPDGene suggests a locus on chromosome 19 as a key determinant of serum E-cadherin
103 regulation (**Fig 1A-B**), which our lab found strongly correlates with lung epithelial E-cadherin in a
104 group of patients at risk for COPD (**Supplementary Figure 1**). The peak SNP rs516246 (meta-
105 analysis, $p=4 \times 10^{-27}$) is the strongest locus in both cohorts with a p-value of 8.95×10^{-16} and 1.21
106 $\times 10^{-16}$ in SPIROMICS and COPDGene, respectively. This is a trans pQTL for E-cadherin as the
107 locus maps to an intronic region in *FUT2*, the gene encoding fucosyltransferase-2 (FUT2). Since
108 we did not have FUT2 protein levels in these patients, we leveraged results from the GTEx
109 consortium (23) to look for evidence of transcript regulation of FUT2 levels by these SNPs. We
110 found that rs516246 is a significant eQTL for *FUT2* transcript levels (**Fig 1C**) across numerous
111 tissues, but notably, also in lung. Individuals heterozygous for this eQTL have decreased levels
112 of *FUT2* mRNA transcript. When homozygous, transcript levels are found to be even less. This
113 revealed the peak pQTL SNP is in 100% linkage disequilibrium with an exonic SNP rs601338,
114 previously shown to result in no expression of the fucosyltransferase-2 (24).

115

116 *E-cadherin is fucosylated by fucosyltransferase-2.*

117 Given the strong genetic association between *FUT2* and *CDH1* in two independent
118 cohorts, we found it pertinent to determine whether we could establish a functional relationship.
119 Based on previous literature there are four N-glycosylation sites in E-Cadherin at residues 554,
120 566, 618, and 633 (25). We utilized MALDI-TOF mass spectrometry based glycomic
121 methodologies to characterize the N-glycans of E-Cadherin when co-expressed with FUT2. A

122 heterogeneous glycan profile is observed which is dominated by complex type N-glycans with
123 fucosylation and/or sialylation (m/z 1590-4588, NeuAc₀₋₄Gal₀₋₄Man₃GlcNAc₄₋₆Fuc₀₋₅). More minor
124 levels of high mannose glycans (m/z 1579-2396, Man₅₋₉GlcNAc₂) are also observed
125 (**Supplementary Figure 2**) by comparing the relative intensities of related fucosylated N-glycan
126 molecular ions levels of E-Cadherin fucosylation increase when co-expressed with FUT2. For
127 example, signals at m/z 2605, 2779 and 2952 which are consistent with a monosialylated bi-
128 antennary complex glycan with 1, 2 and 3 fucose residues and signals at m/z 3054, 3228, 3401
129 and 3576 which are consistent with a monosialylated tri-antennary complex glycan with 1, 2, 3
130 and 4 fucose (Fuc) residues (**Fig 1D-E**).

131 More detailed N-glycan structural analysis, in particular the assignment of the positions of
132 fucosylation, was achieved by MS/MS analysis of selected molecular ions. Exemplar data is
133 shown for the m/z 2779 molecular ion with composition of NeuAc₁Gal₂Man₃GlcNAc₄Fuc₂. Key
134 fragment ions which indicate the fucosylation of the terminal Gal residue, and therefore indicate
135 the action of FUT2, include m/z 433, 834 and 1967. All these ions increased abundance when E-
136 Cadherin was co-expressed with FUT2 (**Supplementary Figure 2**).

137

138 *Fucosylated E-cadherin has higher E-cadherin-E-cadherin bond strength.*

139 Terminal fucosylation of E-cadherin by FUT2 occurs in the extracellular domain, the region
140 of the protein that mediated bonds between E-cadherin molecules, and increased E-cadherin
141 bonds increase surface stabilization of the protein. Therefore, we sought to determine if FUT2-
142 dependent fucosylation affected bond strength. We measured the protein bond strength of
143 purified cell-free FUT2-fucosylated E-cadherin or E-cadherin control using atomic force
144 microscopy (AFM). (**Fig 2A**). We found that FUT2-fucosylation increased the bond strength of E-
145 cadherin. Plotting the probability density functions of force measurements revealed that FUT2-
146 dependent fucosylation resulted in distinct force distributions (**Fig 2B**). FUT2-dependent
147 fucosylation increased E-cadherin bond strength from ~63 nN (without terminal fucosylation) to

148 ~80 nN (with terminal fucosylation, **Fig 2C**). Of note, although the strength was higher with FUT2-
149 dependent fucosylation, we were able to detect more break-off events of E-cadherin when
150 compared to unfucosylated-E-cadherin (**Fig 2D**). A Coomassie stain confirmed purified protein
151 for each condition (**Fig 2E**). It is interesting that in the fucosylated E-cadherin, an additional N-
152 terminal fragment which was verified by mass spectrometry to be an extracellular fragment of E-
153 cadherin isolated along with the purified protein, suggesting the high adhesion of the extracellular
154 domain. It is of note that FUT2 did not change the abundance of E-cadherin (**Fig 2F**) or CDH1
155 transcript (**Supplementary Figure 3**) but did result in the presence of an additional band with a
156 slightly lower molecular weight in A549s.

157

158 *Fut2 is required to maintain epithelial barrier function.*

159 At baseline exposure to air, *Fut2* deficient mouse tracheal cells (mTEC) show decreased
160 epithelial integrity as measured by decreased transepithelial electrical resistance (TEER) and
161 increased FITC dextran flux (**Fig 3A-B**). CS induced a decrease in TEER in both WT and *Fut2*^{-/-}
162 mTEC. Interestingly, while CS induced a significantly increased permeability in WT mTEC, this
163 worsening barrier was not noted in *Fut2*^{-/-} mTEC suggesting that a loss of *Fut2* is sufficient alone
164 to cause epithelial barrier dysfunction. Lentiviral mediated restoration of *Fut2* shows increased
165 epithelial integrity, evident by increased TEER compared to empty vector controls (RFP) and
166 nearing that of WT cells (**Fig 3C**). As a proof of principle, we demonstrate that overexpression of
167 FUT2 also increases TEER in COPD derived bronchial epithelial cells (**Fig 3D**). Transduction
168 efficiency is demonstrated in **Supplementary Figure 4**.

169

170 *Lack of Fut2 results in increased susceptibility to elastase induced emphysema and fibrosis.*

171 Elastase is commonly used as an *in vivo* model for emphysema (15, 26, 27).
172 Representative 10X and 0.5X H&E images reveal that after intratracheal administration of
173 elastase in WT and *Fut2*^{-/-} mice there was marked alveolar destruction. However, *Fut2*^{-/-} mice

174 developed visibly worse emphysema (**Fig 4A**). Masson's Trichrome staining demonstrates
175 increased collagen deposition in *Fut2*^{-/-} airways and alveoli, both more apparent in mice treated
176 with elastase (**Fig 4B**). *Fut2*^{-/-} mice have increased total lung capacity and residual volume
177 compared to the WT mice (**Fig 4C-D**). Compliance increased with elastase treatment in WT mice
178 but demonstrated no significant change in *Fut2*^{-/-} mice (**Fig 4E**). However, this is consistent with
179 our finding of both *Fut2* airspace enlargement and increased fibrosis on lung histology (**Fig. 4B**).
180 Elastase induced a significant increase in mean linear intercept in *Fut2*^{-/-}, but not WT mice (**Fig**
181 **4F**). *Fut2*^{-/-} was confirmed by qPCR (**Fig 4G**).

182

183 *Fut2* deficiency increases susceptibility of precision cut lung slices to CS.

184 Using mouse precision-cut lung slices, we have demonstrated *Fut2* expression modifies
185 CS-induced alveolar destruction. CS exposure of WT PCLS transduced with a control vector
186 (RFP) increased the mean linear intercept (MLI) indicating alveolar destruction. At baseline the
187 *Fut2*^{-/-} PCLS demonstrated an increased mean linear intercept compared to the background WT
188 mice. Moreover, *Fut2*^{-/-} RFP PCLS exposed to CS showed a significantly higher MLI than WT
189 RFP PCLS exposed to CS. However, when *Fut2* is reconstituted in *Fut2*^{-/-} PCLS, it abrogates CS
190 induced injury (**Fig 4I-J**). Interestingly, unlike the mouse elastase model, the PCLS did not
191 demonstrate increased fibrosis, which could be a limitation of the model. Transduction efficiency
192 is demonstrated in **Supplementary Figure 4**.

193

194 *FUT2* is necessary for sufficient proliferation of the epithelium.

195 E-cadherin has mostly been studied in the context of contact inhibition, although there is
196 some evidence that E-cadherin can promote cell proliferation in some cancer models (7, 15, 28).
197 Previously we have shown that a loss of E-cadherin results in decreased proliferation in lung basal
198 cells (15). Overexpression of FUT2, in the lung epithelial cell line A549, results in increased
199 proliferation as measured by doubling time and Ki67+ cells (**Fig 5A-C**). The proliferation defect

200 resulting from knockdown of E-cadherin, a phenocopy of COPD, is recovered by overexpression
201 of FUT2 (**Fig 5D-E**).

202

203 *FUT2 is required for a well-differentiated airway epithelium.*

204 Given E-cadherin's central role in the maintenance of a well differentiated epithelium, we
205 examined cell type specific markers for club cells (*Scgb1a1*), goblet cells (*Muc5ac*), basal cells
206 (*Krt5*), and ciliated cells (*Foxj1*). This revealed both the club cell and ciliated cell populations are
207 dependent on *Fut2* expression *in vivo* (**Fig 6A-D**). Interestingly, this did not correlate with our
208 human *in vitro* data (**Fig 6E-H**).

209

210 *FUT2 is required for ATII to AT1 transdifferentiation.*

211 Analysis of RNA from whole lung homogenate from WT and *Fut2*^{-/-} mice instilled with PBS
212 or elastase indicates no significant change in *Ager* (AT1), *Sftpc* (Spc, AT2), or *Cldn4* (PATS)
213 expression (**Fig 6I-K**). However, overexpression of *FUT2* resulted in increased the transcriptional
214 expression of *AGER*, *SFTPC*, and *CLDN4* compared to the empty vector control (RFP) (**Fig 6L-**
215 **N**) suggesting increased activation of the AT2 to AT1 transdifferentiation pathway. Analysis of
216 protein via immunofluorescence revealed a stark difference in the abundance of various cell types
217 (**Fig 6O**). In *Fut2*^{-/-} mice there is a distinct increase in Spc⁺ cells indicating a persistence of
218 alveolar type II (AT2) cells when compared to WT. Elastase resulted in increased *Cldn4*
219 expression (PATS cells) in both WT and *Fut2*^{-/-} mice, but there is notably more present in *Fut2*^{-/-}
220 mice. Hopx⁺ (AT1) cells remained nearly constant in WT vs *Fut2*^{-/-} mice in both PBS and elastase
221 conditions.

222

223 Discussion

224 Genome-wide association studies have associated lower *CDH1* levels in COPD patients
225 with a worsened prognosis (9, 29–31) and our lab has demonstrated that genetic loss of E-
226 cadherin alone is sufficient to cause both airspace enlargement and airway disease (15).
227 However, to date there has not a body of literature exploring the molecular mechanisms by which
228 E-cadherin is regulated in the context of COPD. This is difficult due to the large clinical variability,
229 however dissecting mechanisms mediating increased susceptibility and identifying strategies to
230 reduce it accordingly can provide the necessary base of knowledge for both risk stratification and
231 therapeutic intervention. While the obvious answer may be to increase E-cadherin abundance,
232 this can be technically challenging and regulation of protein abundance is a very small aspect of
233 maintenance of the proteome. We demonstrate that improving function of E-cadherin is also
234 sufficient providing another potential therapeutic avenue for a multipronged approach targeting
235 COPD.

236 We have identified a novel post-translational modification of E-cadherin namely terminal
237 fucosylation catalyzed by fucosyltransferase 2. This significantly increases bond strength
238 between E-cadherin molecules, which is critical given E-cadherin bond strength is correlated with
239 protein stability (32, 33). Due to experimental limitations, it is unclear whether this increased bond
240 strength is due to increased aggregation of E-cadherin or because single molecules of E-cadherin
241 have strong trans-bond strength. As the AFM probe, a SiO₂ sphere, allows for multiple E-cadherin
242 molecules to attach meaning both possibilities would result in increased observed bond strength.
243 Although future studies with a smaller probe could differentiate between these possibilities, both
244 mechanisms result in the desired result of increased adhesion. In addition to increased bond
245 strength, it is interesting that the fucosylated E-cadherin had several breaks before the full bonds
246 separated. As there were several identified fucosylation sites, with the potential of differential
247 fucosylation of E-cadherin with the overexpression of FUT2, it is possible that these breaks reflect

248 the disruption of E-cadherin with fewer fucosylated sites. Future studies may be able to dissect
249 this possibility further.

250 Our data, both *in vitro* and *in vivo*, clearly demonstrate a significant impact on regeneration
251 and repair of the airway and alveolar epithelia. We show that epithelial barrier integrity requires
252 FUT2 and propose that defects in proliferation and differentiation are the cause. We found there
253 is a clear dependence of proliferation on FUT2 expression, with proliferation increasing with FUT2
254 expression. In addition to having a significant effect on proliferation, we also demonstrate FUT2
255 is required for a well-differentiated airway and alveolar epithelium. Of note, loss of *Fut2* results in
256 accumulation of *Spc*⁺ (AT2) cells, which is particularly interesting considering we have previously
257 shown knockout of *Cdh1* in *Spc*⁺ cells alone is sufficient to cause emphysema (15). This begs
258 the question of the fate of *Spc*⁺ cells that lack *Cdh1* and whether they can effectively
259 transdifferentiate into alveolar type 1 cells.

260 Using large clinical datasets, we demonstrate a significant genetic association
261 between *CDH1* and *FUT2* prevalent in smokers, former smokers, and COPD patients, confirming
262 previous literature (22). Here we extend this genetic association with an arsenal of molecular
263 approaches, ultimately determining this genetic association extends to a molecular interaction
264 with far reaching impacts holding significant therapeutic potential.

265

266 **Methods**

267 *Animals and study design*

268 This study was approved by the Institutional Animal Care and Use Committee of the Johns
269 Hopkins University Animal Use and Care Committee and compiled within the Guidelines for Care
270 and Use of Laboratory Animals issued by the National Institutes of Health. The study used both
271 C57BL/6 (Jackson Laboratory, Bar Harbor ME) and *Fut2*^{-/-} (kindly donated by Dr. Christopher
272 Evans, University of Colorado Anschutz) strains. All mice were bred and maintained in a specific
273 pathogen-free environment.

274

275 *pQTL and eQTL Results*

276 To identify pQTLs for E-Cadherin protein levels, we leveraged previously published QTL
277 genome-wide scans from two large cohorts of current and former smokers with and without COPD
278 [SPIROMICS (N = 750); COPDGene (N = 590)]. As previously described, the study aimed to
279 identify single nucleotide polymorphisms (SNPs) associated with measurement of 88 blood
280 proteins (protein quantitative trait loci; pQTLs) including E-Cadherin. Here, we extracted the
281 genome-wide QTL analysis for E-Cadherin alone. To identify what gene expression the identified
282 pQTLs for E-Cadherin were associated with, we leveraged the GTEx portal
283 (<https://www.gtexportal.org/home/>).

284

285 *FUT2 treatment on E-cadherin*

286 E-cadherin with 6x His-tag and native FUT2 enzyme synthetic genes were created. After plasmid
287 DNA was purified, E-cadherin DNA was transfected either with or without FUT2 DNA in EXPI293
288 cells. E-cadherin was then purified from both conditions with nickel capture and size exclusion
289 chromatography.

290

291 *Mass Spectrometry*

292 N-linked glycan analysis was performed according to Jang-Lee et al (34). The E-Cadherin
293 glycoprotein samples were reduced, carboxymethylated, and digested with trypsin. N-Glycans
294 were enzymatically released by peptide N-glycosidase F (E.C. 3.5.1.52; Roche Applied Science)
295 digestion then purified by C18-Sep-Pak (Waters Corp., Hertfordshire, UK). The purified N-glycans
296 were permethylated using the sodium hydroxide procedure and purified by C18-Sep-Pak. The
297 permethylated N-glycans were then dissolved in methanol before an aliquot was mixed at a 1:1
298 ratio (v/v) with 10 mg/ml 3,4-diaminobenzophenone in 75% acetonitrile. The glycan-matrix mixture
299 was spotted on a stainless-steel target plate and dried in vacuum. MALDI-TOF MS and MALDI-
300 TOF/TOF MS/MS data were obtained using a 4800 MALDI-TOF/TOF mass spectrometer (AB
301 Sciex UK Limited) in the positive-ion mode. For MS/MS, the collision energy was set at 1 kV, and
302 argon was used as the collision gas. The obtained MS and MS/MS data were viewed and
303 processed using Data Explorer 4.9 (AB Sciex UK Ltd). All N-glycans were assumed to have a
304 core of Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc based on known biosynthetic pathways
305 and susceptibility to peptide N-glycosidase F digestion. Monosaccharide compositions in terms
306 of numbers of Hex, HexNAc, etc. derived from MALDI-MS. MALDI-TOF/TOF MS/MS fragment
307 ions were identified manually and with the assistance of the Glycoworkbench tool (35).

308

309 *Immunoprecipitation (IP)*

310 1000 ug of protein, determined by BCA assay were precleared for 1 hour with Protein G
311 Sepharose 4 Fast Flow beads (GE17-0168-01, Millipore Sigma) at 4°C. Following preclearance,
312 protein lysate was removed and coupled with a polyclonal E-Cadherin antibody (20874-1-AP,
313 Proteintech Group, IL, USA) overnight at 4°C. Antibody coupled lysate was then incubated with
314 Protein G Sepharose 4 Fast Flow beads for 1 hour at 4°C. Supernatant was removed for further
315 analysis and RIPA, 4X Bolt LDS Sample Buffer (B0007, ThermoFisher Scientific), and 10X Bolt
316 Sample Reducing Agent (B0009, ThermoFisher Scientific) were added to the Sepharose beads.

317 Samples were then boiled at 95°C for 15 minutes with agitation. The supernatant, which contained
318 the immunoprecipitated protein, was then removed to be analyzed via western blot.

319

320 *Western blot assay*

321 Western blot analysis was carried out as previously described (15, 36). Briefly, proteins were
322 separated on Bolt 4 – 12%, Bis-Tris gradient gel (ThermoFisher Scientific, NY, USA) and then
323 transferred to a Immobilon-P PVDF membrane (Millipore Sigma, MA, USA). Following transfer,
324 the PVDF membrane was blocked in 5% w/v BSA in 1X PBS with 0.1% Tween® 20 Detergent,
325 (1X PBST, Millipore Sigma, MA, USA). The membrane was then probed for E-cadherin, GAPDH,
326 and UEA1 (E-cadherin (24E10) Rabbit mAb, 135 kDa and GAPDH (14C10) Rabbit mAb, 37 kDa
327 antibodies from Cell Signaling Technology, MA, USA), Ulex Europaeus Agglutinin I Biotinylated
328 (B-1065-2) from Vector Laboratories) according to manufacturers' instructions. The blot was then
329 probed with secondary antibody (IRDye® 800CW Streptavidin and IRDye® 680RD Goat anti-
330 Rabbit IgG Secondary Antibody, LI-COR) and imaged. Blots were quantified using ImageStudio
331 (LI-COR).

332

333 *Quantitative Polymerase Chain Reaction (qPCR)*

334 RNA was extracted from human bronchial epithelial cells / mice lung tissues and purified Trizol
335 (ThermoFisher). RNA was then converted to cDNA after addition of dNTP mix, 10X RT Random
336 Primers, Reverse Transcriptase, and nuclease free water and the following PCR cycle: 25°C for
337 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes (High Capacity cDNA Reverse Transcription
338 Kit, ThermoFisher). Following conversion, equal amounts of cDNA from each sample were added
339 to previously designed primers and SYBR Green mix in duplicate. The following qPCR cycle was
340 run: 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Relative expression
341 ratio was determined using the method. Based on comparative Ct method, gene expression levels

342 were calculated utilizing GAPDH as the housekeeping gene. Primers are listed in **Supplementary**
343 **Table 2.**

344

345 *Atomic Force Microscopy (AFM)*

346 E-cadherin was conjugated directly over a substrate of $15\text{mm} \times 15\text{mm}$ n-type silicon wafer
347 fragment and a gold coated AFM colloidal probe (HQ:CSC38/Cr-Au, MikroMasch). A 4" silicon
348 wafer was coated with silicon nitride (Si_3N_4) by a PECVD (Plasmatherm 790) at 250°C for 5min
349 to achieve a 100nm thick Si_3N_4 thin film. The wafer was then diced into a series of $15\text{mm} \times 15\text{mm}$
350 fragments for experimentation.

351 ***Probe and surface preparation:*** Before functionalization, both substrate and probe were
352 cleaned with piranha etching solution ($\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4$ at 1:2 w/w). While a $15\text{mm} \times 15\text{mm}$ substrate
353 was cleaned in freshly prepared solution for 30min, the gold coated AFM probes, held in an in-
354 house made HDPE probe holder, were cleaned with one-day old cold piranha solution for 10
355 seconds. Note that probes remained in the holder for cleaning and later functionalization
356 procedure. After cleaning, the testing substrate was subsequently rinsed with DI water, acetone,
357 methanol, isopropanol, and then DI water again, while probes were soaked in abovementioned
358 solutions for >10 minutes each with gentle shaking. The testing substrate was then dried with N_2
359 and baked at 120°C for 1 min. The AFM probe was air dried in a glove box (EW-34788-10, Cole-
360 Parmer) with N_2 purging overnight.

361 ***Probe and surface functionalization:*** Illustrated with **Fig. 4A**, to attach E-cadherin to the
362 surfaces of substrates and probe, we applied amino functionalization by APTES (3-
363 aminopropyltriethoxysilane) to the probe and wafer. Before amino functionalization, the stock
364 APTES (CAS 919-30-2, Sigma-Aldrich) was purified at the distillation temperature of 103°C under
365 a vacuum of 20mmHg. The purified APTES was then dispensed into 1-2ml screw cap vials in an
366 Ag filled glove box. Shelf-life for the distilled APTES was over 6 months when stored at -20°C .

367 **Aminosilanization:** Probe and wafer were functionalized by a gas phase aminosilanization
368 procedure. 30 μ l of APTES and 10 μ l of triethylamine were placed in two separate trays together
369 with probe and testing wafer into a desiccator. The desiccator was pumped down to 200 mTorr
370 and then filled to 75 Torr with Argon. Vapor deposition continued for 4 hours. The APTES on
371 probe and wafer was cured at room temperature in an Argon filled glove box for 2 days.

372 **APTES conjugation:** Probe and wafer surfaces was further functionalized by a biotinylated
373 polyethylene glycol (PEG). PEGylation solution of 80mg of m-PEG-SVA (5 kDa, M-SVA-5K,
374 Laysan Bio, Inc) and 4 mg of Biotin-PEG-SVA (Laysan Bio, Inc.) in 320 μ l PEGylation buffer (0.1M
375 sodium bicarbonate) was freshly prepared. A make-shift reaction chamber made from a 5" petri
376 dish was used to perform PEGylation functionalized as following: A small tray containing 2 ml of
377 DI water was placed in the chamber to maintain the humidity. The wafer fragment was placed in
378 the reaction chamber with the functionalized surface facing up. A 70 μ l of the PEGylation mixture
379 was deposited over each testing wafer fragment (e.g. 15 mm \times 15mm). To prevent the cantilever
380 from being destroyed, the probe functionalization was completed by dipping the probe in the
381 mixture mounted on an in-house developed mini-manipulator. The apparatus including probe,
382 manipulator, and PEGylation container was sufficiently small to be enclosed in a 5" petri dish.
383 The chamber was then sealed with parafilm and placed in dark overnight. After functionalization,
384 the testing wafer fragments were then rinsed with DI water and dried with N₂; while the probe
385 mounted on manipulator was soaked in DI water bath for 20 minutes and then air dried in N₂ filled
386 glovebox. The PEGylated cantilever and testing wafer surfaces can be stored in desiccator for
387 ~2 weeks. Note that most of abovementioned procedures were performed in a laminar hood.

388 **Streptavidin attachment:** The PEG-functionalized testing surface and probe were incubated in
389 0.1 mg/ml BSA in TB buffer (i.e. 10 mM Tris, 100 mM NaCl, 10 mM KCl, and 2.5 mM CaCl₂) for
390 12 hours to minimize nonspecific protein binding, followed by the incubation with 0.1 mg/mL
391 streptavidin (Sigma-Aldrich) in TB buffer for 30 min.

392 ***E-Cadherin immobilization:*** The surface and probe were incubated in 200 nM biotinylated E-
393 cadherin (Sinobiological, Inc) in TB buffer for 45 min. After E-cadherin immobilization, the surface
394 and probe was further incubated in 2 μ M biotin in TB buffer for 10 min and the free biotin was
395 washed away using TB buffer.

396 ***AFM measurement:*** After functionalization, the testing surface and probe were mounted in
397 environmental AFM (AFMWorkshop, LLC). The testing surface was placed in a flow cell
398 containing TB buffer. Each wet nano-indentation measurement was performed by a procedure.
399 To promote the interaction of E-cadherins between surface and probe, the probe was allowed to
400 extend 200nm into the surface at the rate of 500nm/s. At the end of the extension, the probe and
401 surface maintained in contact for 10min with nominal contact force of \sim 5nN. After 10min “binding”
402 period, the probe was allowed to retract 13 μ m away from the surface at the rate of 500nm/s. The
403 large retraction distance was specifically selected to ensure the separation of the probe and
404 surface. The above procedure was repeated at different locations (e.g. totaling \sim 100 per
405 experiments). Note that these locations were randomly selected over the entire 15mm \times 15mm
406 surface.

407 ***AFM data analysis:*** The measurements were processed with in-house developed Matlab
408 software.

409

410 *Lentivirus Construction and Generation*

411 *E. coli* strains producing plasmids pEF.CMV.RFP (Addgene #17619, pEF.CMV.RFP was a gift
412 from Linzhao Cheng), psPAX2 (Addgene #12260, psPAX2 was a gift from Didier Trono), and
413 pMD2.G (Addgene #12259, pMD2.G was a gift from Didier Trono) were inoculated in LB Broth
414 supplemented with ampicillin. Plasmid DNA was miniprepmed (Qiagen). Human and mouse
415 reference RNA were converted to cDNA as described above. Sequence for the gene of interest
416 was PCR amplified from the reference cDNA using primers and cycles in **Supplementary Table**
417 **3**. Subsequent PCR product and pEF.CMV.RFP was digested with EcoRV according to the

418 manufacturer's protocol (NEB). These were then ligated together using T4 ligase (NEB) and
419 transformed into STBL3 bacteria. Colonies were screened with PCR and positive clones were
420 confirmed with sequencing yielding pLVmFut2 and pLVhFUT2.

421

422 *Intratracheal Elastase Administration and Pulmonary Function Tests*

423 4.5U of elastase were intratracheally administered as previously described (15, 26). Briefly, mice
424 were anesthetized with xylazine-ketamine mixture. The trachea was visualized, and tracheas
425 were cannulated. 4.5U (50 uL) of elastase was administered and then mice were ventilated for 20
426 seconds. Mice were harvest 21 days following elastase administration. After 21 days mice were
427 again anesthetized, and pulmonary function tests were performed with a FlexiVent(26). Lungs
428 were then inflated with formalin and sent to Oncology Tissue Services (SKCCC, Baltimore MD)
429 for paraffin embedding and mounting on slides. Mean linear intercept was quantified as previously
430 described (37).

431

432 *Precision Cut Lung Slices (PCLS)*

433 PCLS were prepared as previously described (38). PCLS were cut to be 250 microns thickness
434 with a vibrating blade vibratome (Microm HM650V) and allowed to acclimate for 48 hours in
435 DMEM F12 supplemented with 1% insulin-transferrin-selenium, and 1% antibiotic-antimycotic.
436 Slice viability was assayed with Alamar Blue (ThermoFisher). Slices were transduced with fresh
437 viral supernatant supplemented with 20 ug/mL of polybrene for 48 hours and then switched to
438 fresh media for 24 hours before CS exposure. PCLS were exposed to CS or humidified air using
439 the Vitrocell Systems GmbH smoking chamber with a previously described exposure protocol.
440 PCLS were exposed to 8 cigarettes, with 1 cigarette every 8 minutes. After the last CS exposure,
441 PCLS were returned to fresh media and incubated overnight. Following fixation, PCLS were
442 processed by Oncology Tissue Services. MLI was quantified as above.

443

444 *Immunofluorescence*

445 Deparaffinization, antigen retrieval, and immunofluorescence were performed as described
446 previously (21). Slides were deparaffinized with xylenes followed by ethanol rehydration. Antigen
447 retrieval was performed using Citrate Buffer (pH:6.0, ThermoFisher) for paraffin embedded
448 tissues. Ki67 primary antibody (MA5-14520, ThermoFisher) and HOPX (11419, ProteinTech)
449 were diluted 1:100 and SPC (518029, Santa Cruz Biotechnology (SCBT)) and
450 CLDN4:AlexFluor488 (376643, SCBT) were diluted 1:50 and incubated overnight. AlexaFluor 555
451 and AlexaFluor 647 were diluted 1:200 and incubated for 1 hour. After secondary antibody
452 incubation, the slides were incubated for 30 minutes in NucBlue™ Live ReadyProbes™ Reagent
453 (ThermoFisher #R37605). After 30 minutes the slides were washed three times with PBS and
454 mounted using the ProLong™ Glass Antifade Mountant (ThermoFisher #P36980). All
455 immunofluorescent images were taken using the Johns Hopkins School of Medicine Microscope
456 Facility Zeiss LSM700 Confocal.

457

458 *Cell Culture*

459 Primary non-diseased human bronchial epithelial and COPD human bronchial epithelial cells
460 were purchased from the Marsico Lung Institute (University of North Carolina Chapel Hill)
461 expanded on collagen coated T75 flasks. Growth and differentiation conditions utilized were
462 previously described (15, 16, 31, 38). A549 cells were a gift from Dr. Joseph Bressler (Johns
463 Hopkins Bloomberg School of Public Health) and maintained in F12K media supplemented with
464 10% fetal bovine serum and 1% penicillin streptomycin. Cells were transduced with lentiviral
465 supernatant for 48 hours supplemented with 20 ug/mL of polybrene. A549s that were transduced
466 were then cell sorted at the Ross Flow Cytometry Core on a FACS Aria IIu Sorter as previously
467 described (38) for RFP (and eGFP).

468

469 *Isolation of mice tracheal epithelial cells (mTECs)*

470 Mouse tracheal epithelial cells were isolated as previously described (15, 38). Briefly, mice were
471 euthanized by following carbon dioxide narcosis followed by cervical dislocation. The trachea
472 were dissected out and added to 1X-Phosphate Buffer Saline (1X-PBS, ThermoFisher Scientific,
473 New York, USA) supplemented with Penicillin-Streptomycin (ThermoFisher Scientific, NY, USA).
474 Following incubation, the trachea were transferred to 0.15% Pronase solution and incubated
475 overnight at 4°C. The solution was agitated and passed through a 70 µm cell strainer (Corning
476 Life Sciences, MA, USA). Cells were pelleted from solution and resuspended in DMEM
477 supplemented with FBS and Penicillin-Streptomycin. Cells were allowed to incubated in a flask
478 for 4 hours to allow fibroblasts and mononuclear cells to attach. The remaining epithelial cells
479 were transferred to a rat tail collagen I coated T75 flask and expanded in PneumaCult™-Ex Plus
480 Basal Medium Supplemented with 10 mL PneumaCult™-Ex Plus 50X Supplement, 0.5 mL
481 Hydrocortisone stock solution and 5 mL of 1% Penicillin-Streptomycin: StemCell Technologies
482 Inc., Vancouver, Canada). At subconfluency cells were transferred to Transwell® (Corning)
483 inserts and when confluent were allowed to differentiate at ALI for two weeks.

484

485 *Cigarette-smoke (CS) exposure to mTECs*

486 The mTECs at 2 weeks ALI were exposed to either exposed to CS smoke or humidified air for 4
487 days as we previously described (15, 38). One CS exposure consisted of 2 cigarettes which
488 burned for ~ 8 minutes using the ISO puff regimen.

489

490

491 *Barrier Function Analysis*

492 To determine monolayer integrity of the human and murine bronchial and tracheal epithelium at
493 ALI, TEER was measured using epithelial voltohmeter (EVOM, World Precision Instruments Inc,
494 FL, USA) with the STX2 electrodes as previously described (15). Values were corrected for fluid
495 resistance and surface area. The paracellular permeability of the epithelium at ALI was

496 determined using fluorescein isothiocyanate-dextran (FITC-Dextran) flux assay as described
497 previously (15, 36).

498

499

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502 tissues were processed by Oncology Tissue Services at the Sidney Kimmel Comprehensive
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508

509 Figure Captions

510 **Figure 1:** Fucosyltransferase-2 fucosylated E-cadherin. **A.** Protein quantitative trait loci (pQTL)
511 analysis of E-cadherin variants in the SPIROMICS database. **B.** Protein quantitative trait loci
512 (pQTL) analysis of E-cadherin variants in the COPDGene database. **C.** Measured expression
513 quantitative trait loci (eQTL) transcript levels of FUT-2 by rs516246 genotype in lung tissue from
514 Genotype-Tissue Expression (GTEx). **D.** *FUT2* mRNA transcript is significantly decreased in
515 COPD derived bronchial epithelia ($p = 0.0079$). **E.** Partial MALDI-TOF mass spectra (m/z 2500-
516 3800) of E-cadherin N-glycans. **F.** Partial MALDI-TOF mass spectra (m/z 2500-3800) of E-
517 cadherin co-expressed with FUT2 N-glycans, demonstrating an increase in fucosylation. **G.** An
518 immunoprecipitation of E-cadherin indicates less UEA-1+ E-cadherin in bronchial epithelial cells
519 derived from COPD patients.

520

521 **Figure 2:** FUT2-fucosylated E-cadherin has higher bond strength than non-FUT2-fucosylated E-
522 cadherin. **A.** Schematic of the experimental setup for AFM based nano-indentation measurements
523 of e-cadherin with or without fucosylation. **B.** Probability Density Function (PDF) of maximum
524 adhesion forces between e-cadherin filaments. Square: non-fucosylated e-cadherin. Delta:
525 fucosylated e-cadherin. Lines: normal distribution fits for non-fucosylated (solid: $\bar{F} =$
526 $63.47\text{ nN}, \sigma = 2.8585\text{ nN}$) and fucosylated e-cadherin (dashed: $\bar{F} = 79.801\text{ nN}, \sigma =$
527 2.9432 nN) **C.** Bar graph showing mean maximum adhesion force (nN) of non-fucosylated (dark)
528 and fucosylated (light) e-cadherin. Error bar: one standard deviation. Total five pairs of
529 experimental runs are presented. (Each run contains ~100 measurements, **Supplementary**
530 **Table 1**) **D.** Sample adhesion curve showing the maximum binding force and break-offs (cliffs,
531 blue circle). Single (Row 1) and two (Row 2) break-offs of the bonded non-fucosylated e-cadherin
532 filaments, while Row 3 & 4 show maximum & partial break-offs of the bonded fucosylated e-
533 cadherin filaments. Inset: schematics of adhesion measurement using AFM. **E.** Immunoblot
534 depicting the presence of N-terminal fragment of E-cadherin when co-expressed with *FUT2*. **F.**
535 E-cadherin expression does not change with FUT2 overexpression.

536

537 **Figure 3:** FUT2 is necessary to maintain integrity of the airway epithelium through its regulation
538 of E-cadherin. **A.** *Fut2*^{-/-} mTEC have lower TEER than WT mTEC, which is worsened by CS-
539 induced injury (n = 6). **B.** *Fut2*^{-/-} mTEC have higher permeability than WT mTEC, but it is not
540 further worsened by CS exposure (n = 6). **C.** Reconstitution of *Fut2* in *Fut2*^{-/-} mTEC recovers
541 TEER (n = 6). **D.** Overexpression of FUT2 improves TEER in COPD derived epithelia (n = 3).

542

543 **Figure 4:** *Fut2*^{-/-} mice develop fibrosis and emphysema with elastase administration. **A.**
544 Hematoxylin and eosin staining of WT and *Fut2*^{-/-} mice administered PBS or elastase
545 intratracheally (n = 3). **B.** Masson's Trichrome staining indicates increased fibrosis of the alveoli
546 and airways in *Fut2*^{-/-} mice (n = 3). **C.** *Fut2*^{-/-} mice have increased total lung capacity and **D.**
547 residual volume (n = 4 to 9). **E.** Compliance does not significantly change in *Fut2*^{-/-} mice (n = 4 to
548 9). **F.** Elastase induces alveolar destruction, measured by mean linear intercept (n = 4 to 6). **G.**
549 *Fut2*^{-/-} mice have undetectable *Fut2* transcript in the lung (n = 4-8). **H.** Hematoxylin and eosin
550 staining of WT and *Fut2*^{-/-} PCLS exposed to air or CS. **I.** *Fut2*^{-/-} PCLS show increased
551 susceptibility to CS as measured by mean linear intercept, which is rescued by reconstitution of
552 *Fut2*.

553

554 **Figure 5:** FUT2 is required for sufficient proliferation of the lung epithelium. **A.** Overexpression of
555 FUT2 decreases doubling time of A549s (n = 8 to 12). **B and C.** Overexpression of FUT2
556 increases the number of KI67+ cells (n = 12); scale bar (upper left) = 250 microns. **D and E.**
557 Knockdown of CDH1 decreases the number of KI67+ cells, overexpression of FUT2 in CDH1
558 knockdown A549s has a limited effect (n = 6); scale bar (upper left) = 125 microns.
559

560 **Figure 6:** FUT2 is required for regeneration of the alveolar and airway epithelia. **A.** *Scgb1a1*
561 transcripts are decreased in *Fut2*^{-/-} mice instilled with elastase when compared to WT. **B and C.**
562 *Muc5ac* and *Krt5* transcripts are unchanged with loss of *Fut2* or elastase. **D.** *Foxj1* expression
563 trends to be lower with loss of *Fut2*. **E.** *SCGB1A1* expression slightly decreases with FUT2
564 overexpression. **F.** *MUC5AC* expression slightly increases with FUT2 overexpression. **G and H.**
565 *KRT5* and *FOXJ1* expression are relatively unchanged with FUT2 overexpression. **I, J, and K.**
566 There is no change in *Ager*, *Sftpc*, or *Cldn4* expression with loss of *Fut2* or elastase. **L, M, and**
567 **N.** *AGER*, *SFTPC*, and *CLDN4* expression increase with FUT2 overexpression. **O.** *Fut2*^{-/-} mice
568 have increased Spc+ cells in both PBS and elastase. Elastase induces increased *Cldn4*
569 expression (n = 2); scale bar (upper left) = 50 microns.

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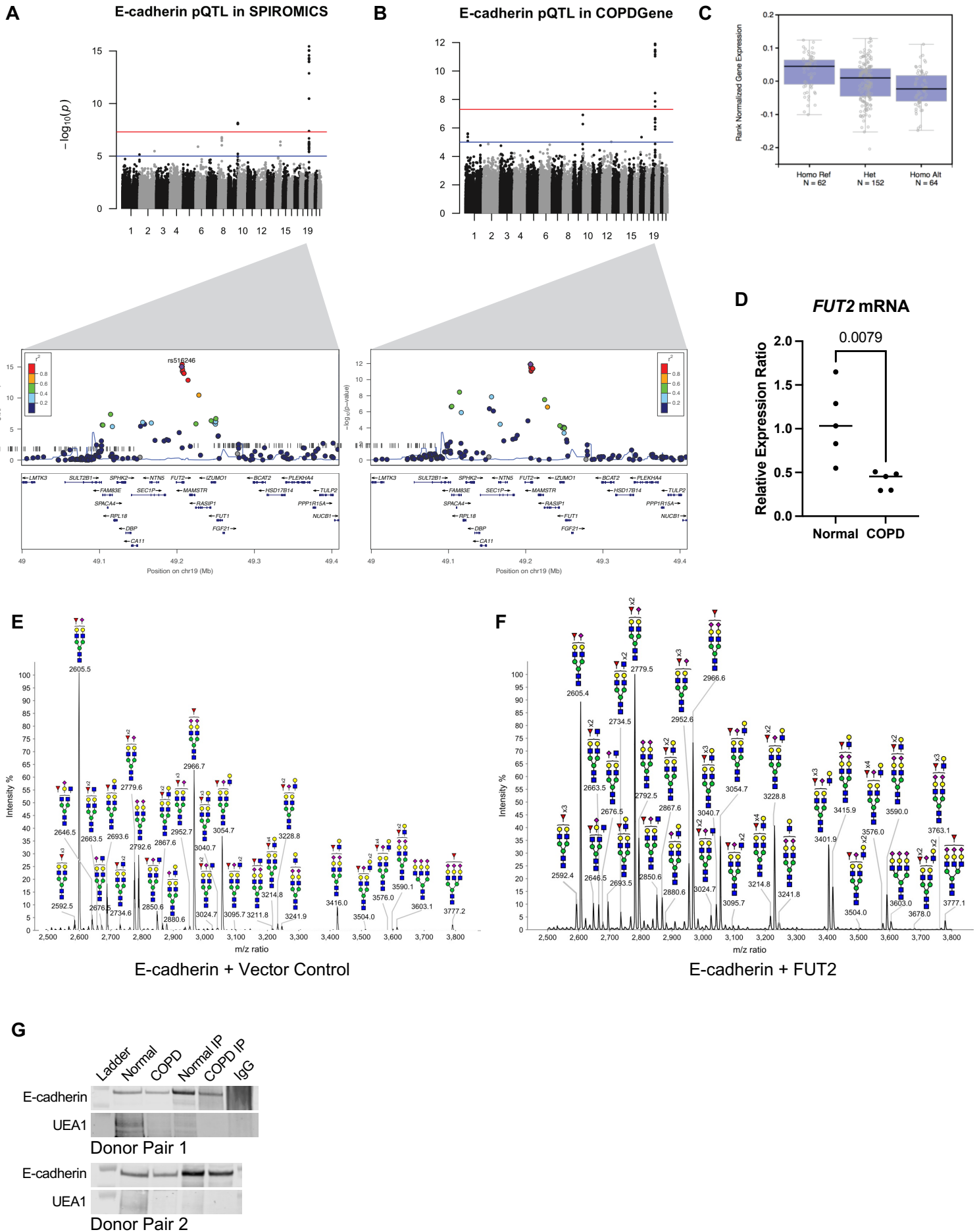


Figure 1: Fucosyltransferase-2 fucosylates E-cadherin

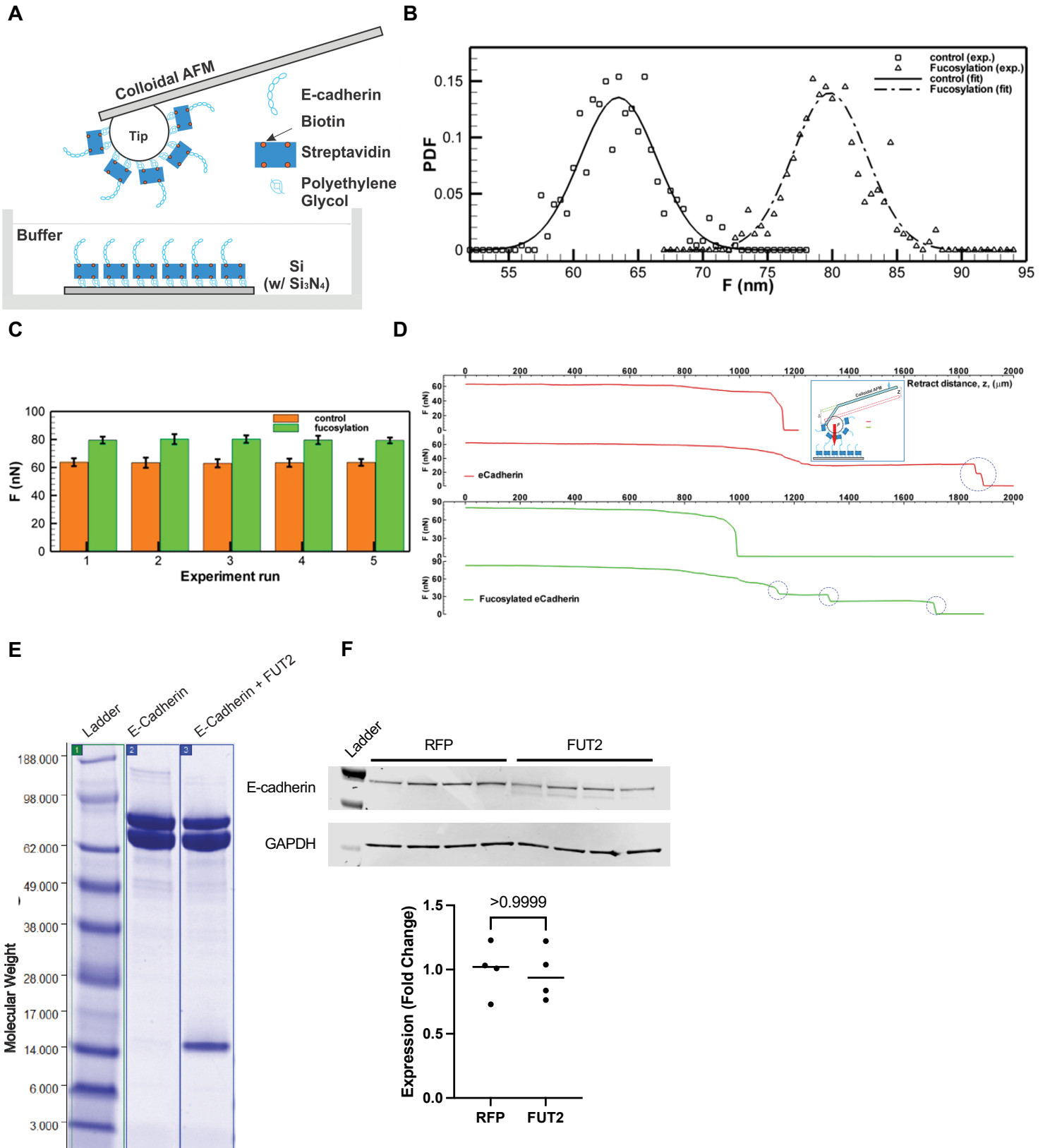


Figure 2: Fucosyltransferase-2 fucosylated E-cadherin has higher bond strength

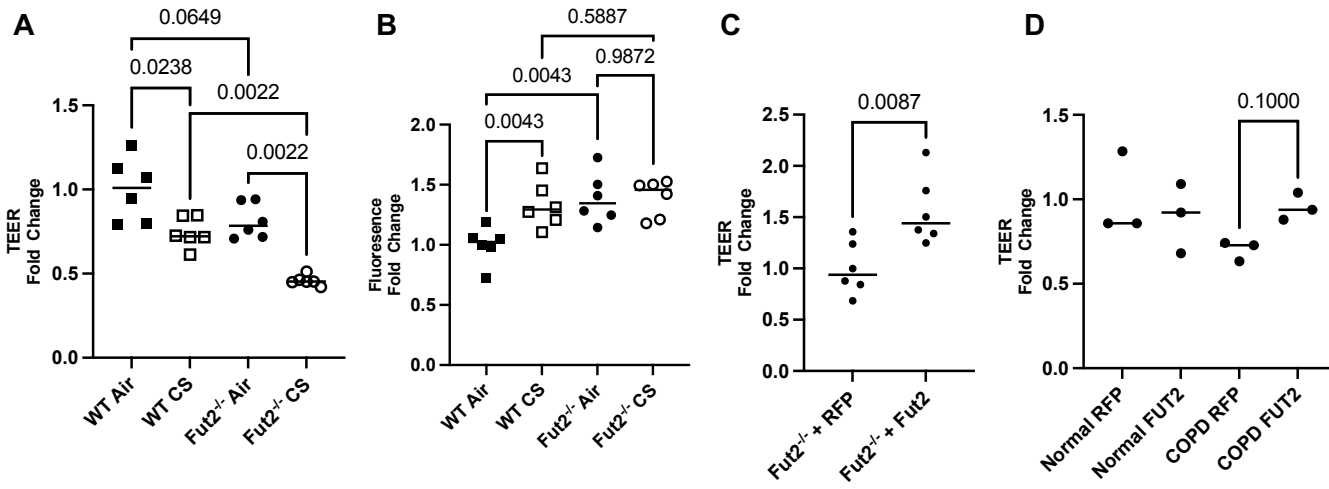
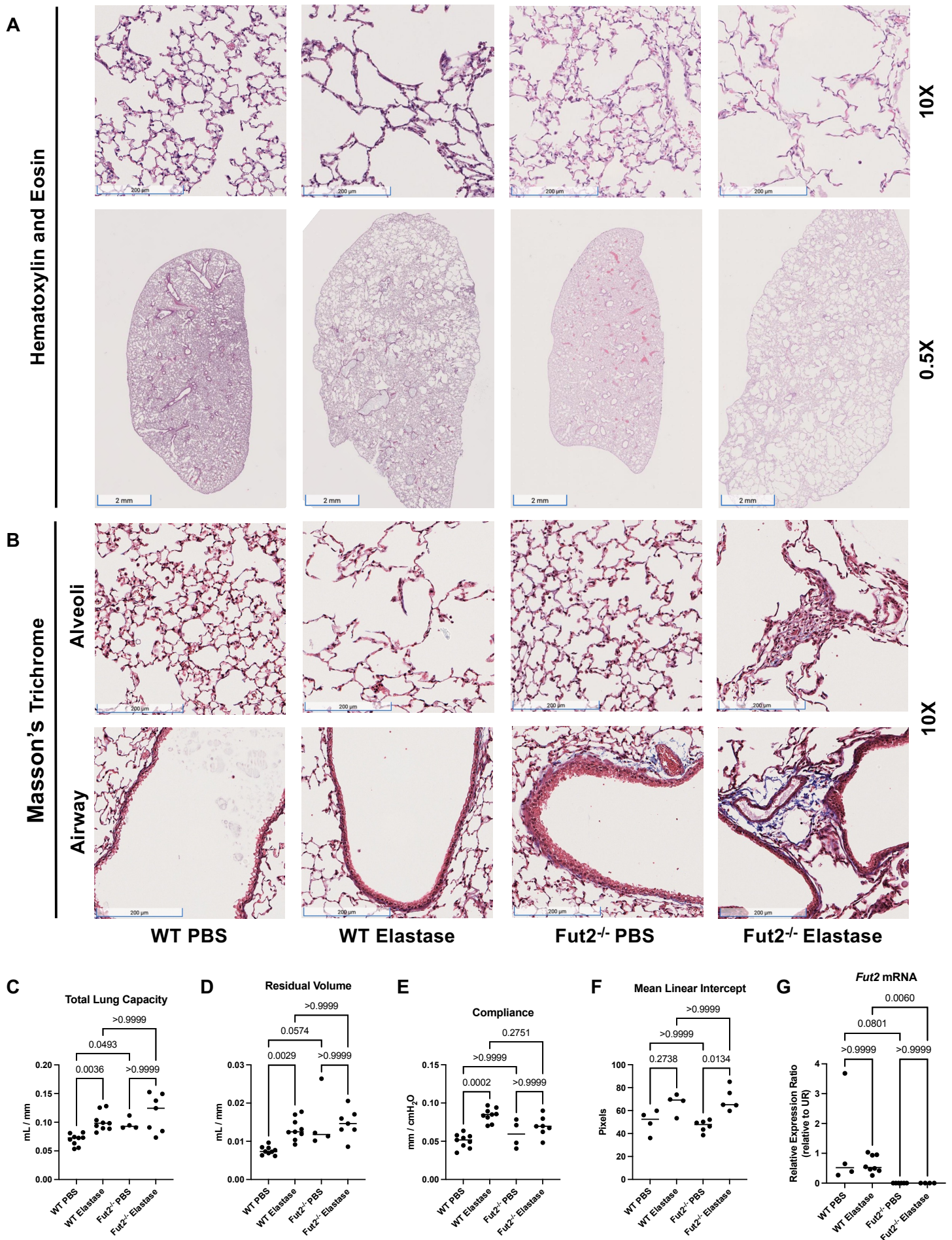


Figure 3: FUT2 is necessary to maintain integrity of the airway epithelium through its regulation of E-cadherin



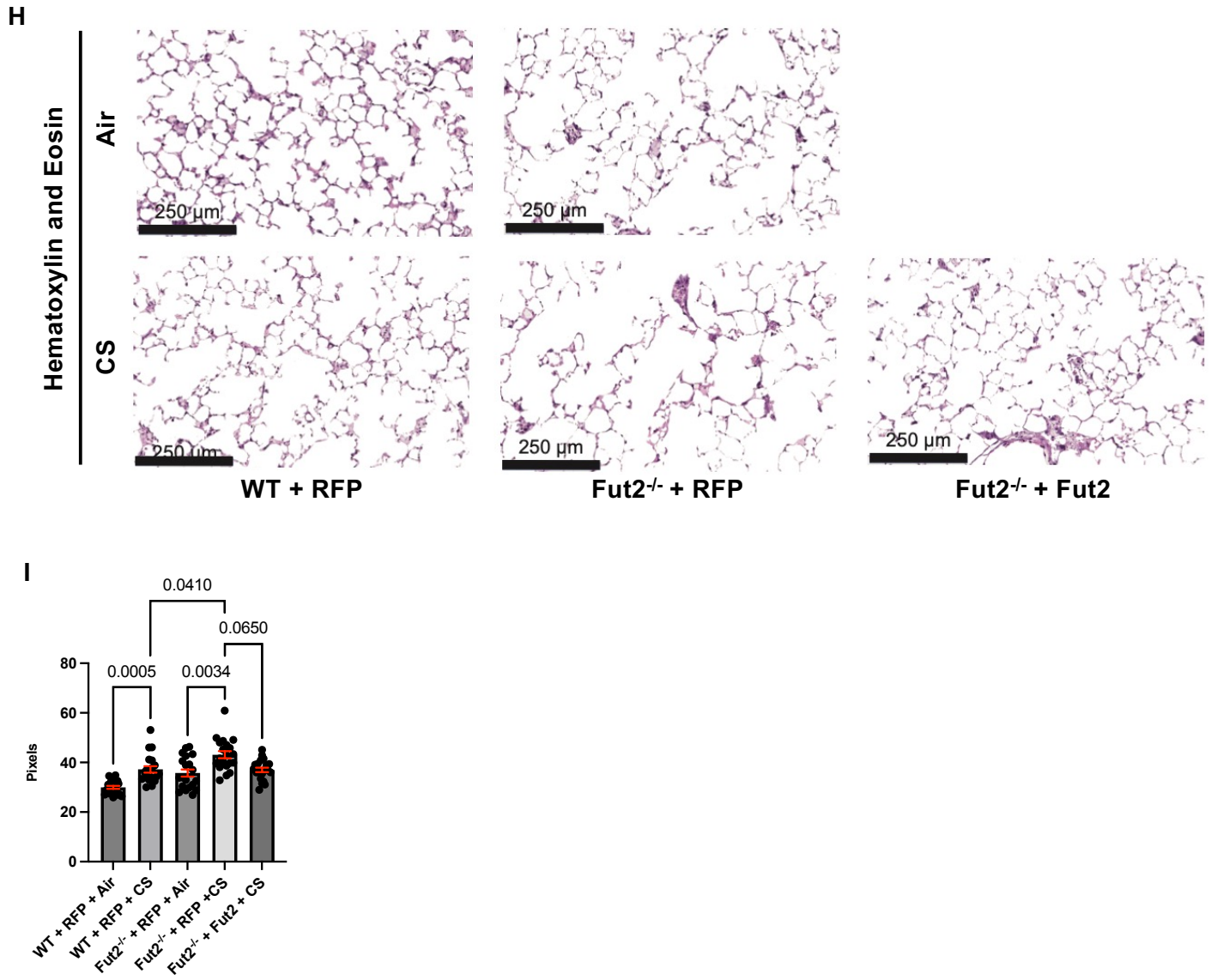


Figure 4: Fut2^{-/-} mice develop fibrosis and emphysema with elastase administration

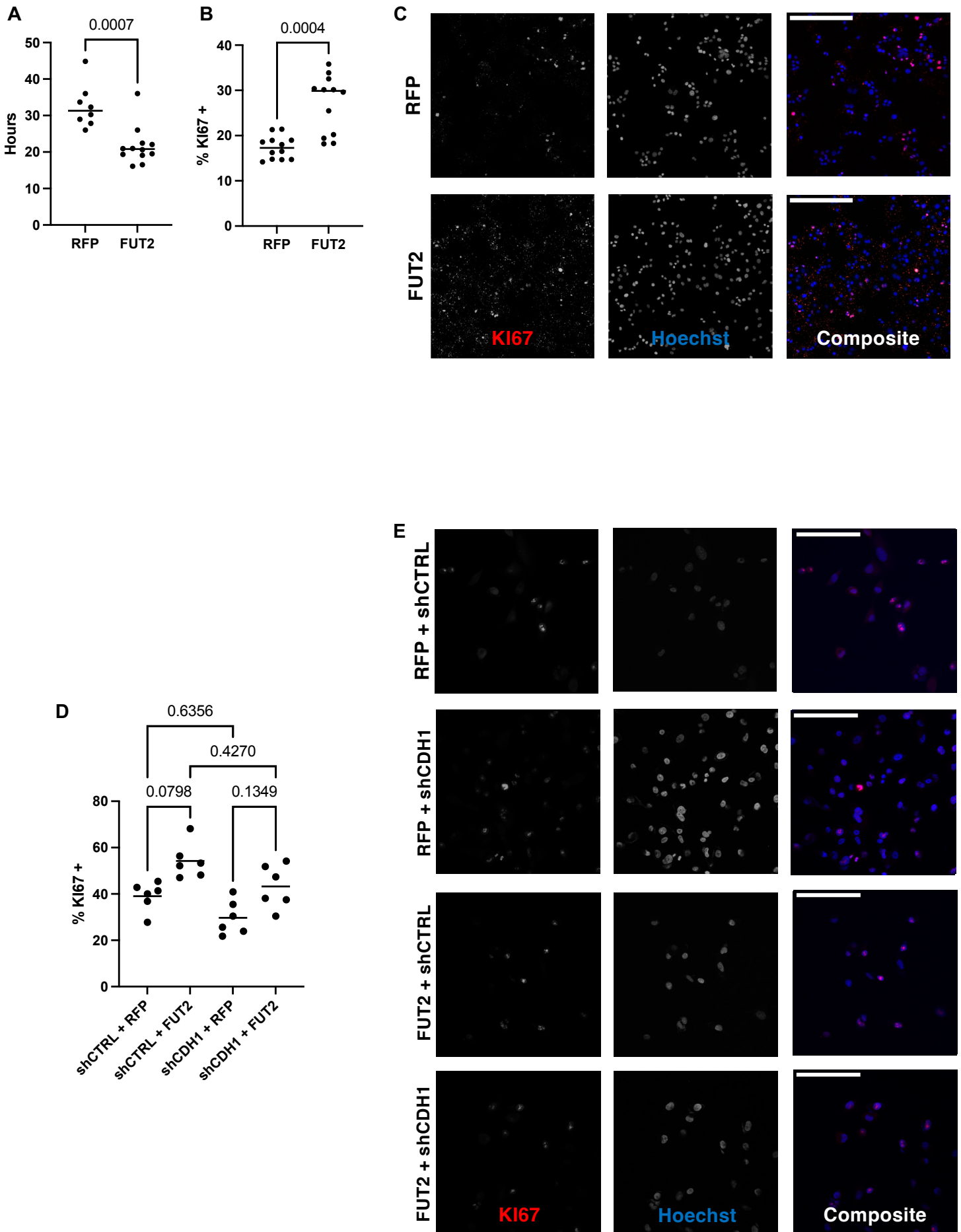


Figure 5: FUT2 is required for sufficient proliferation of the lung epithelium

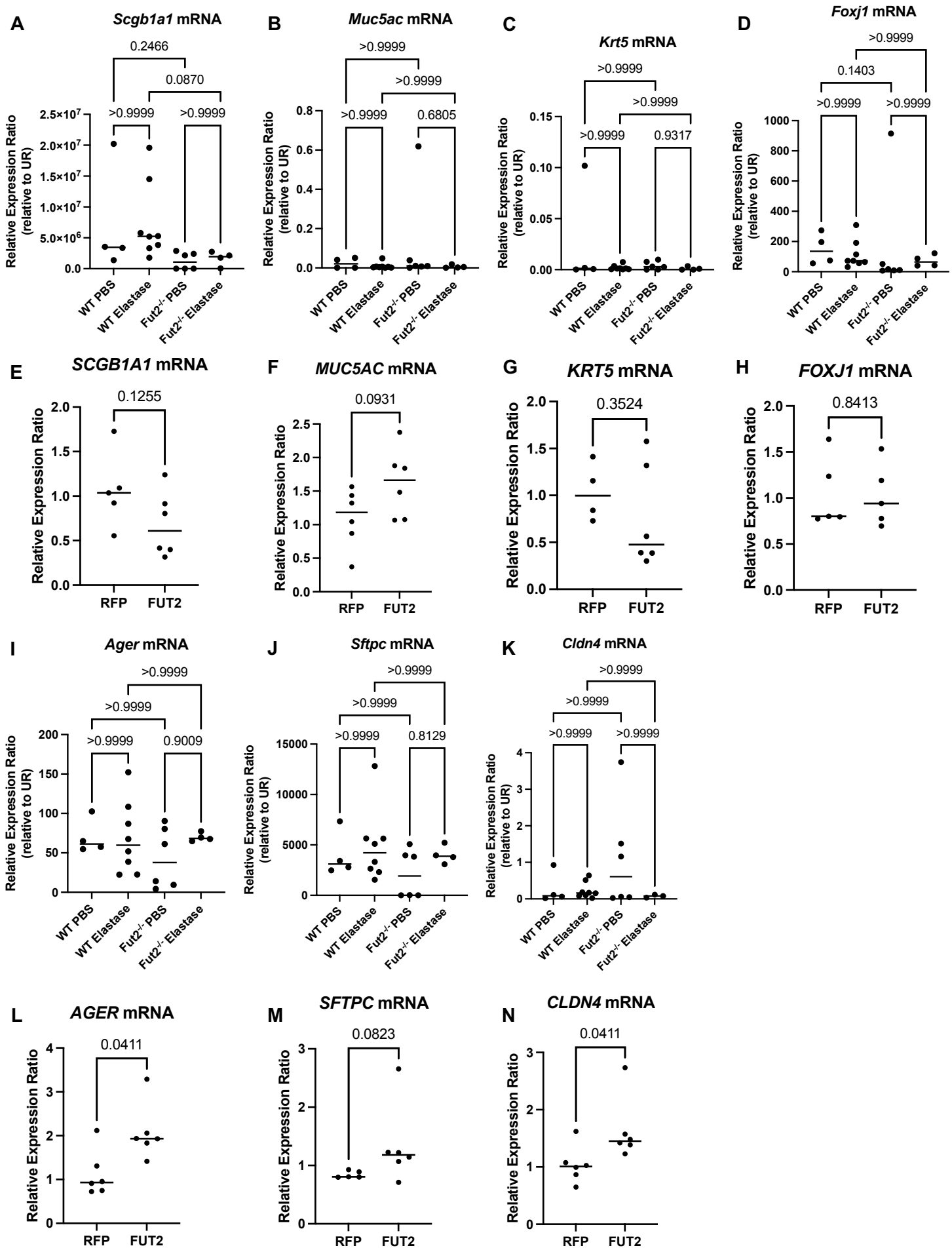


Figure 6: FUT2 is required for regeneration alveolar and airway epithelia

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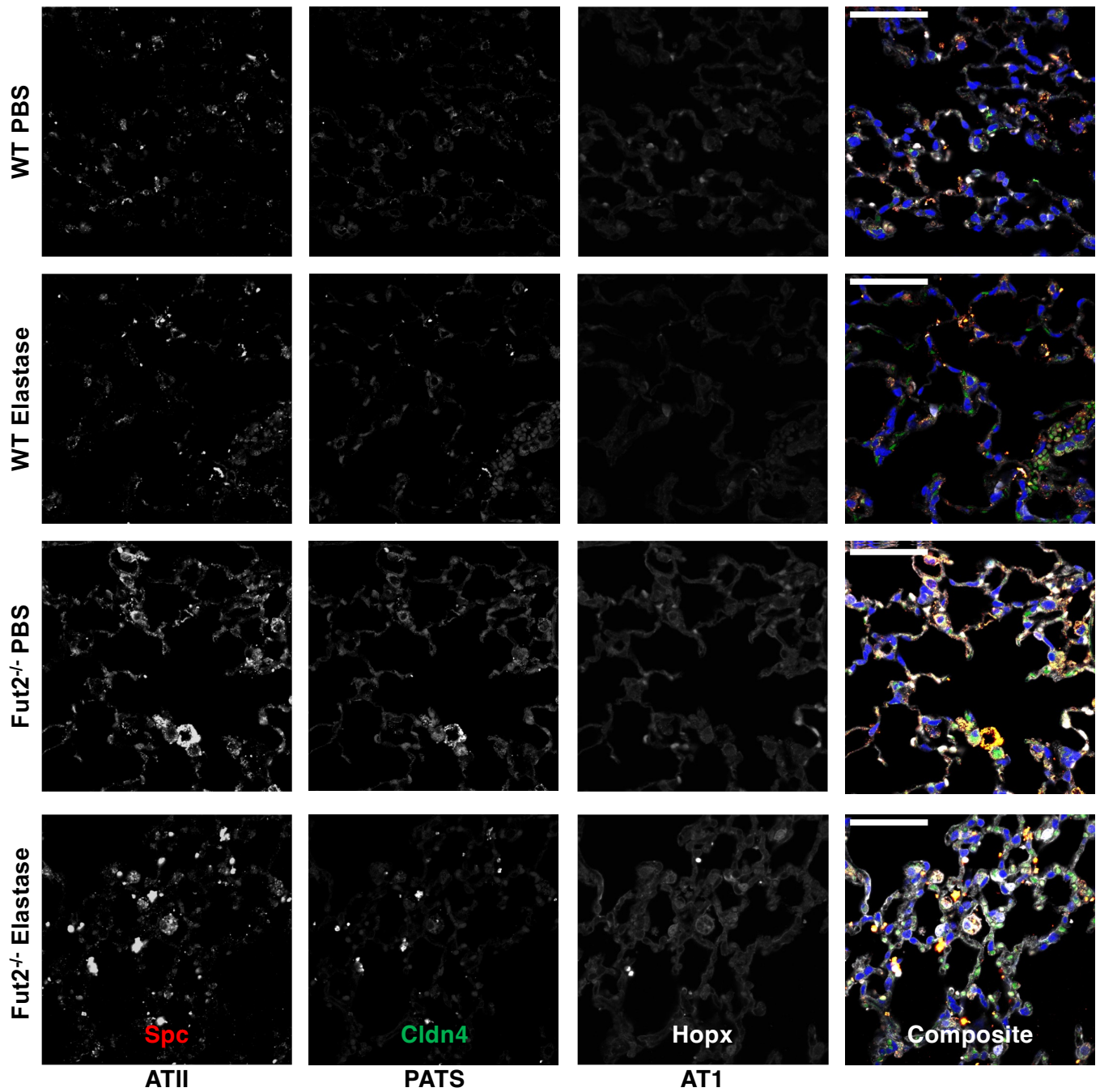
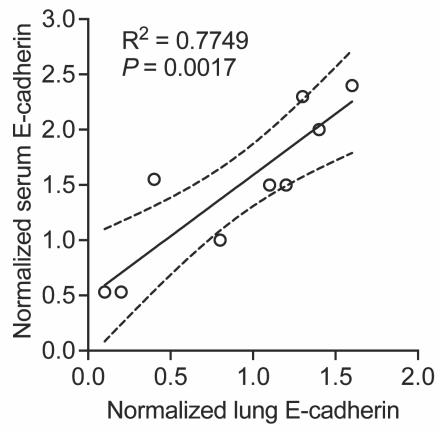
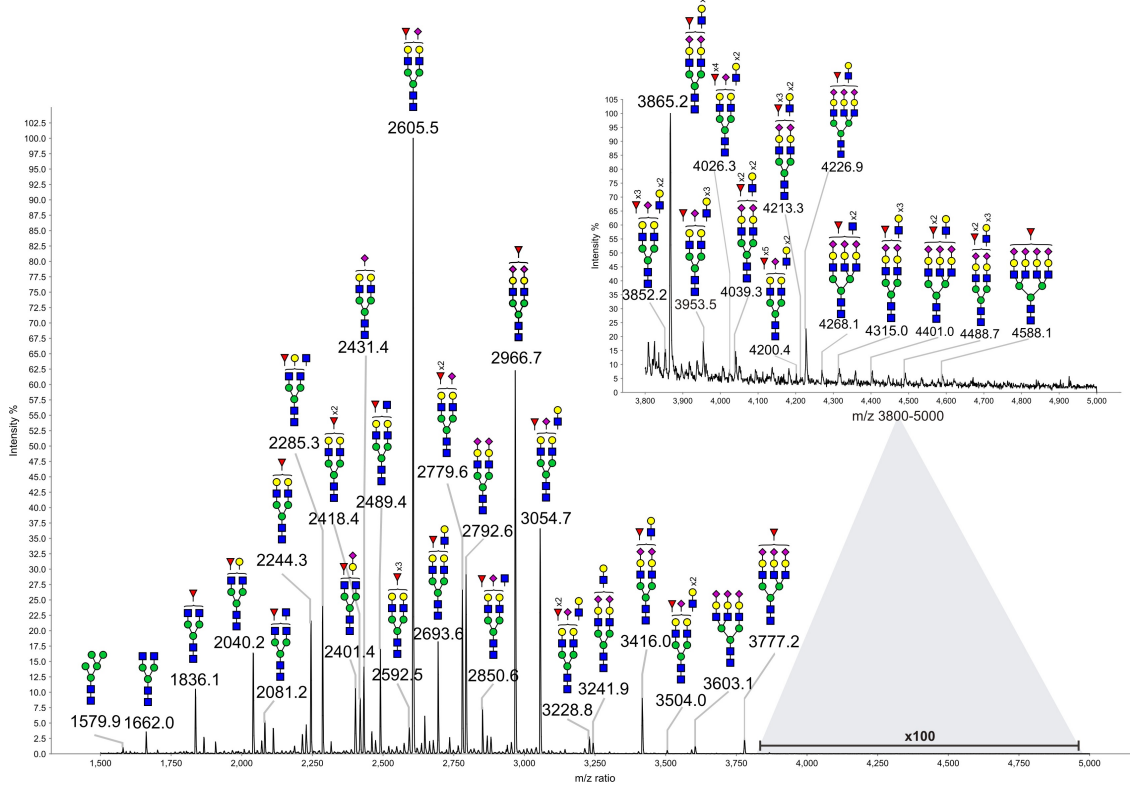


Figure 6: FUT2 is required for regeneration alveolar and airway epithelia

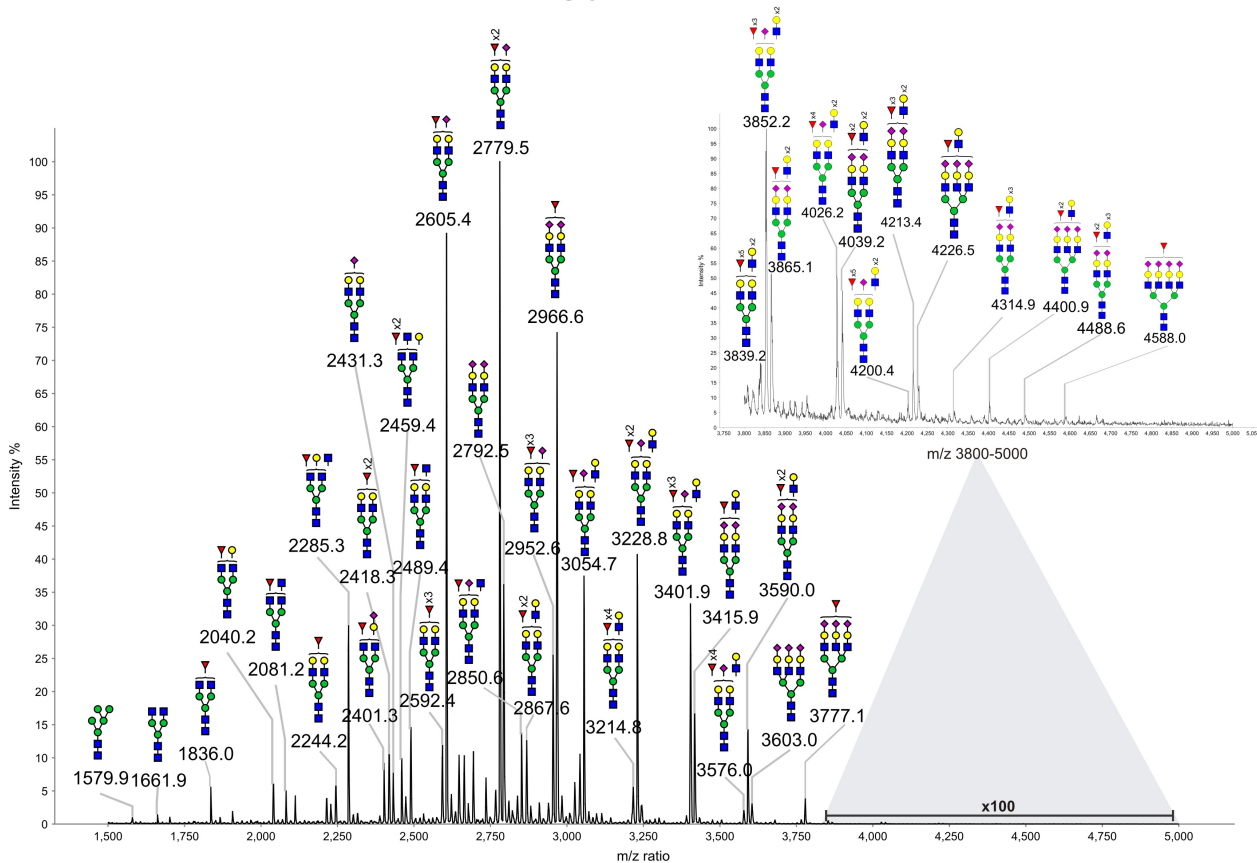


SF1: Serum levels of E-cadherin are highly correlated with lung levels of E-cadherin

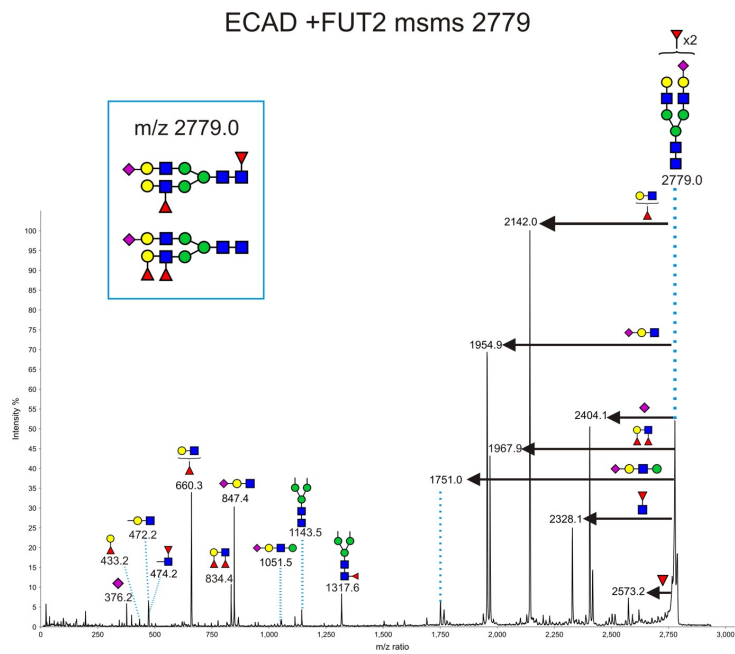
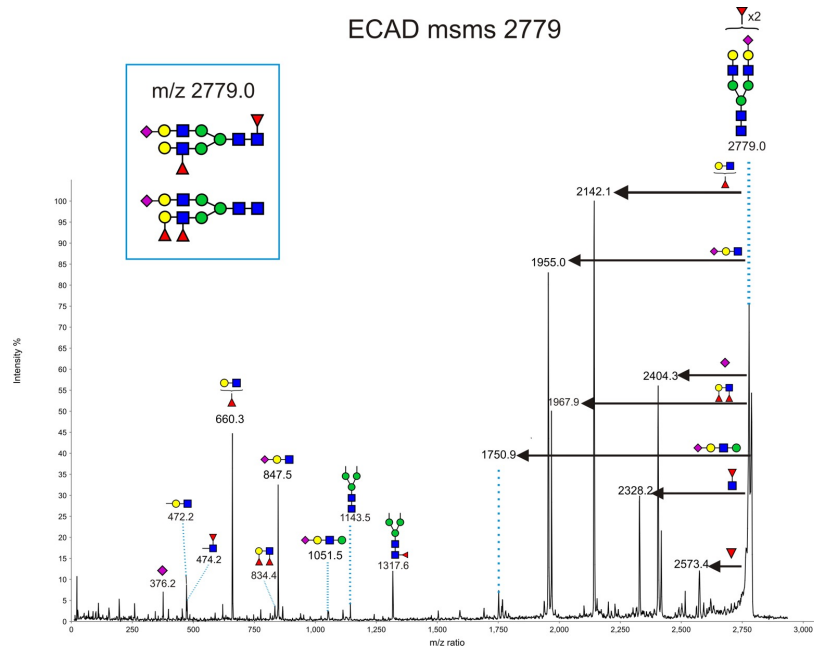
ECAD N-glycans m/z 1500-5000

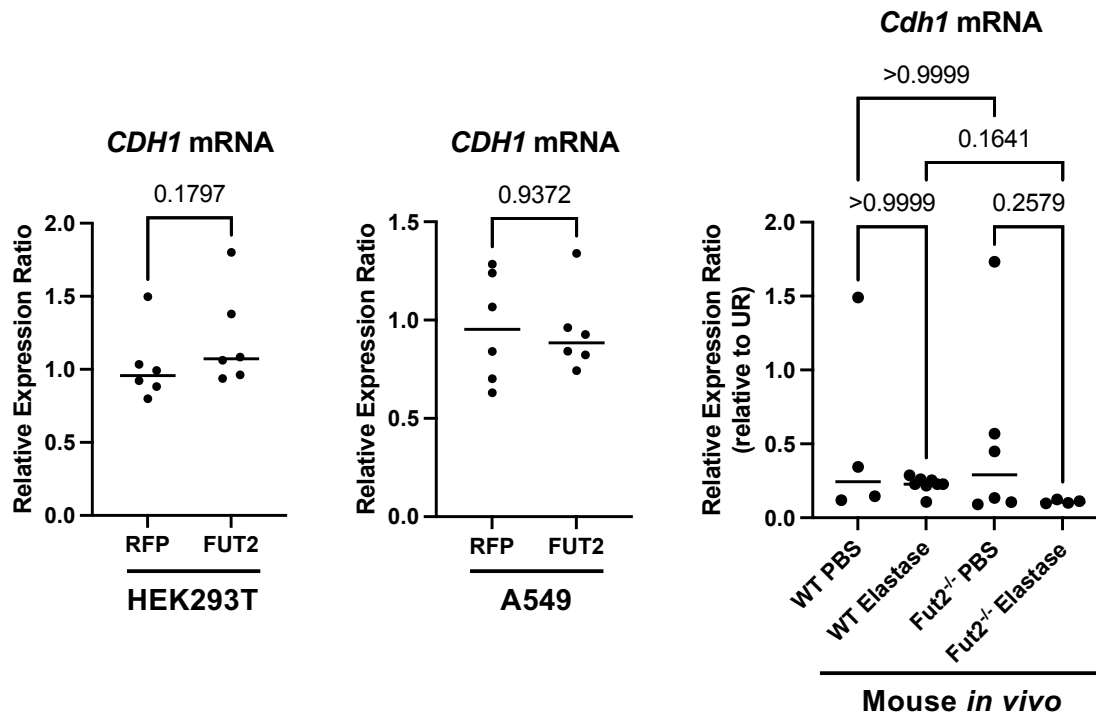


ECAD FUT2 N-glycans m/z 1500-5000

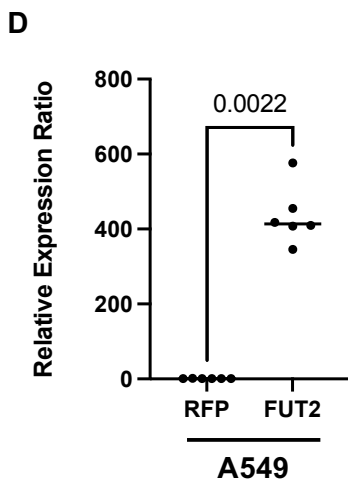
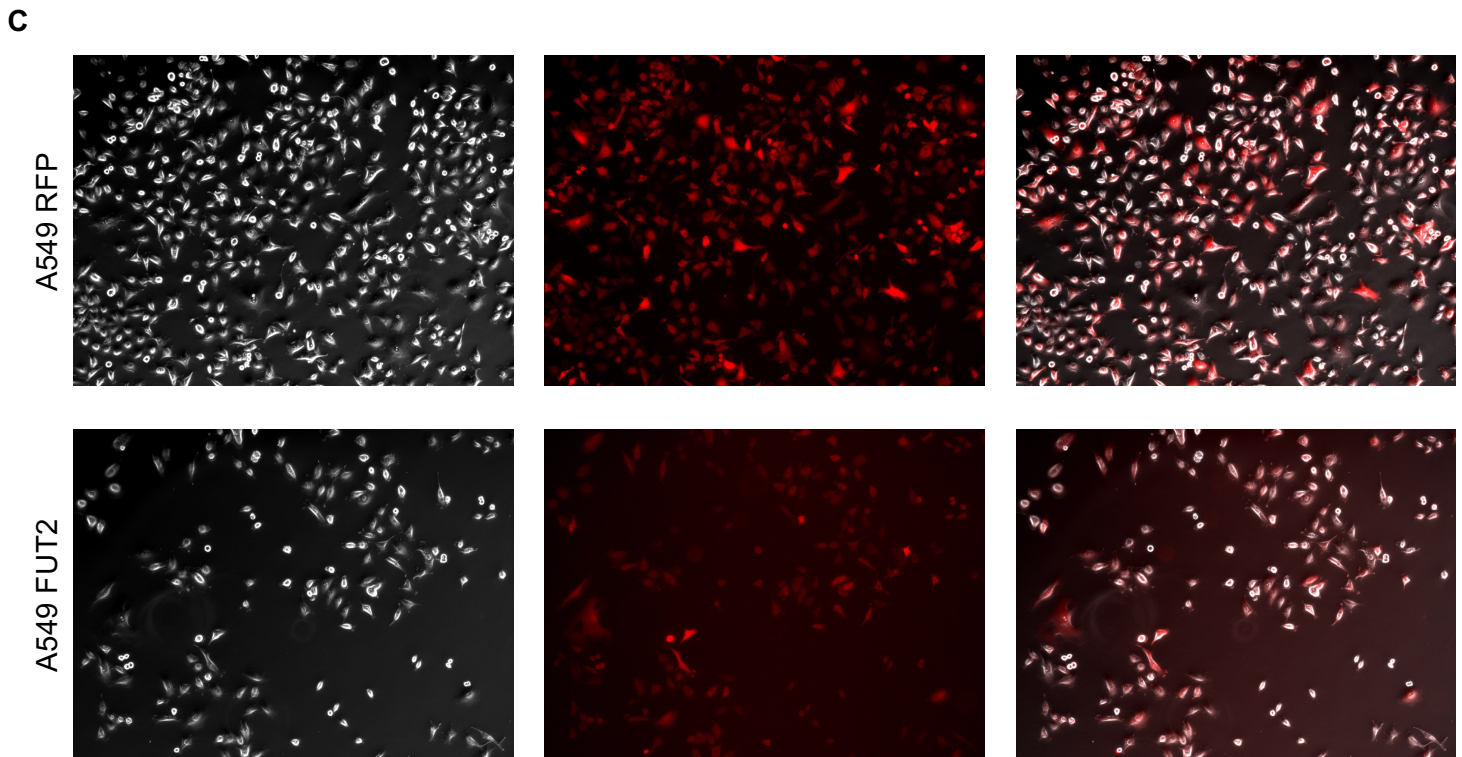
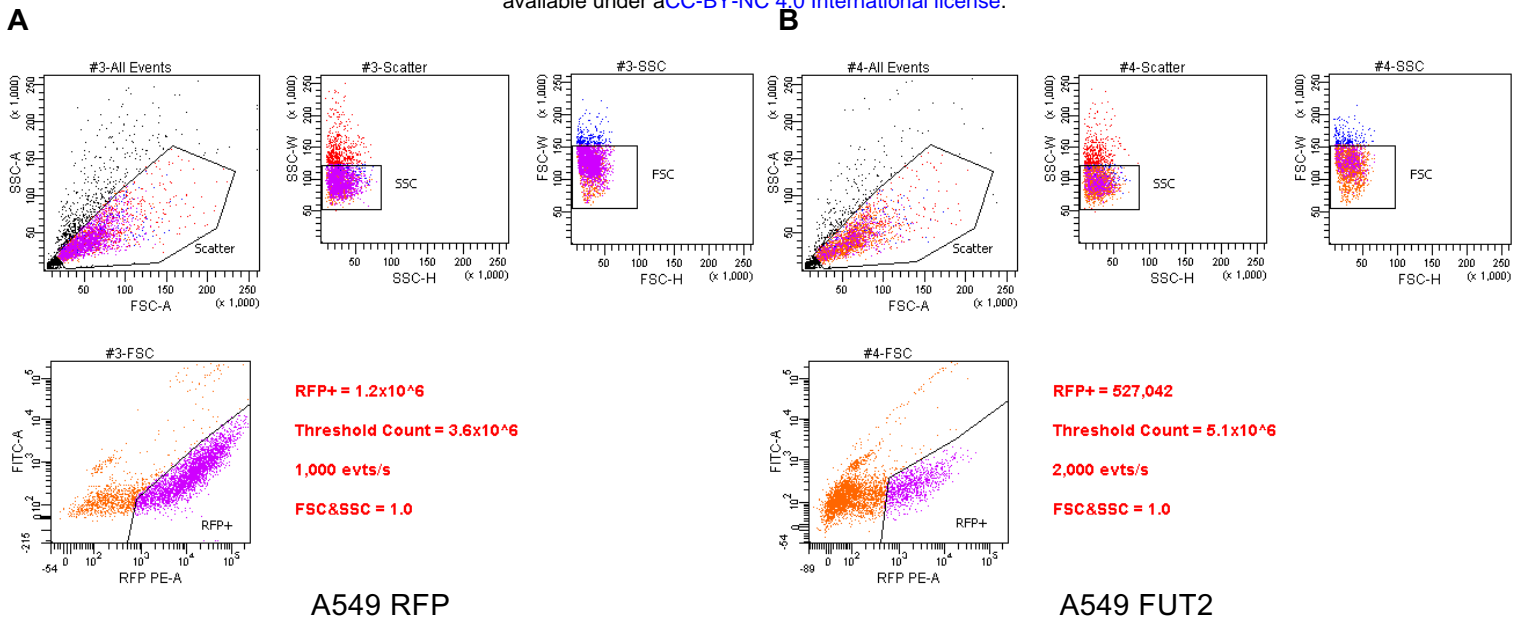


SF2: Glycan profiles of E-cadherin change with FUT2 overexpression



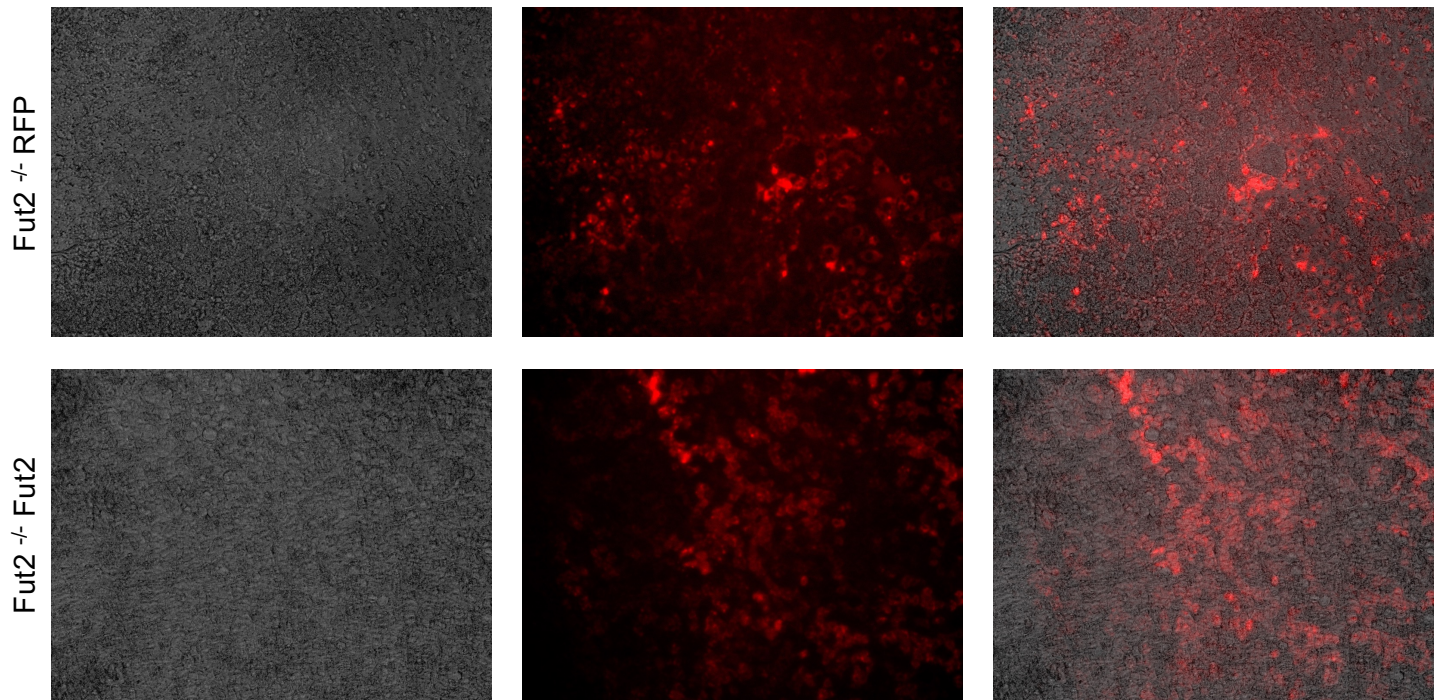


SF3: *CDH1* transcript levels are not dependent on *FUT2* expression.



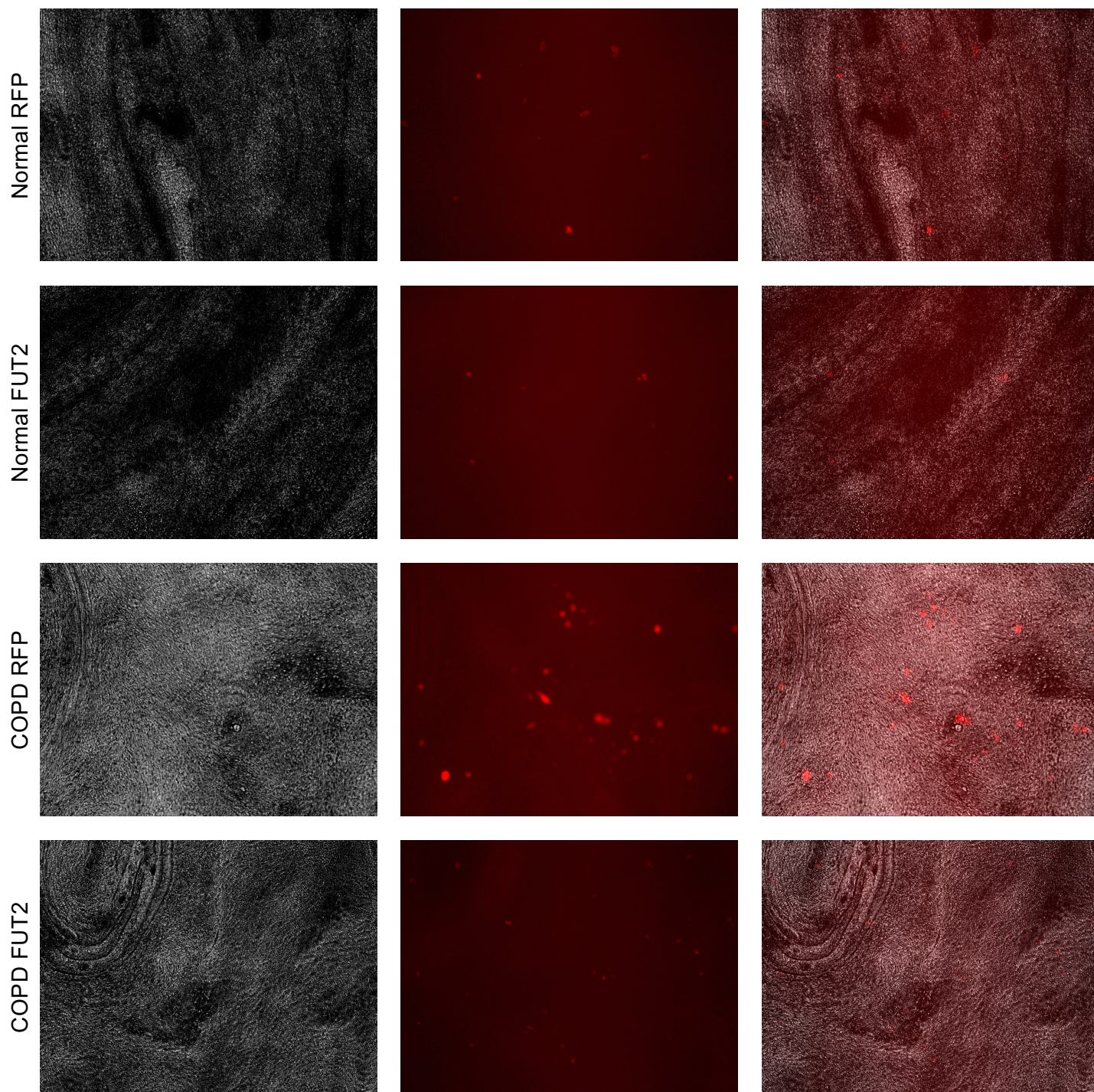
SF4: Transduction efficiency of RFP, FUT2, Fut2, shCTRL, shCDH1.

E

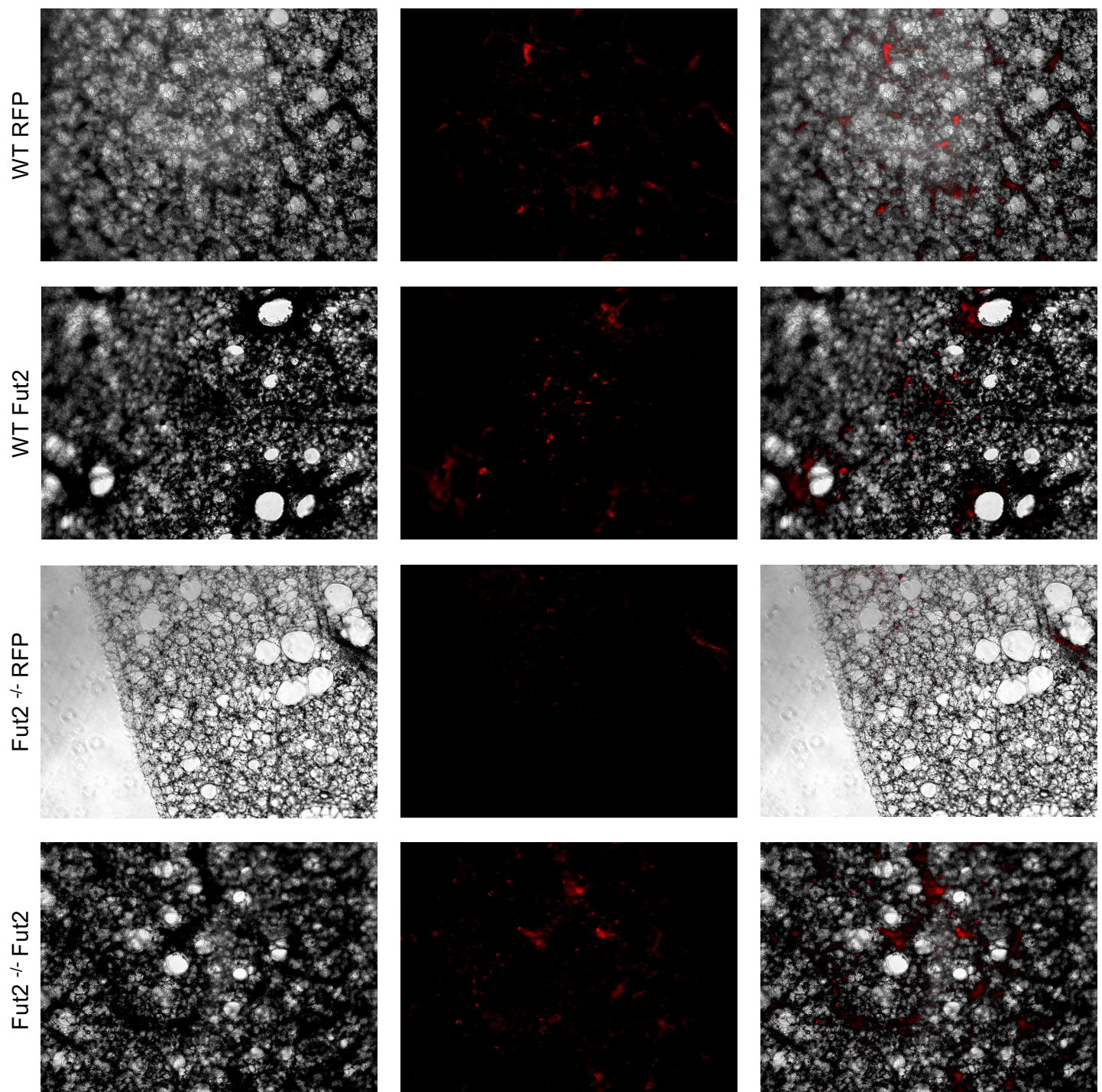


SF4: Transduction efficiency of RFP, FUT2, Fut2, shCTRL, shCDH1.

F

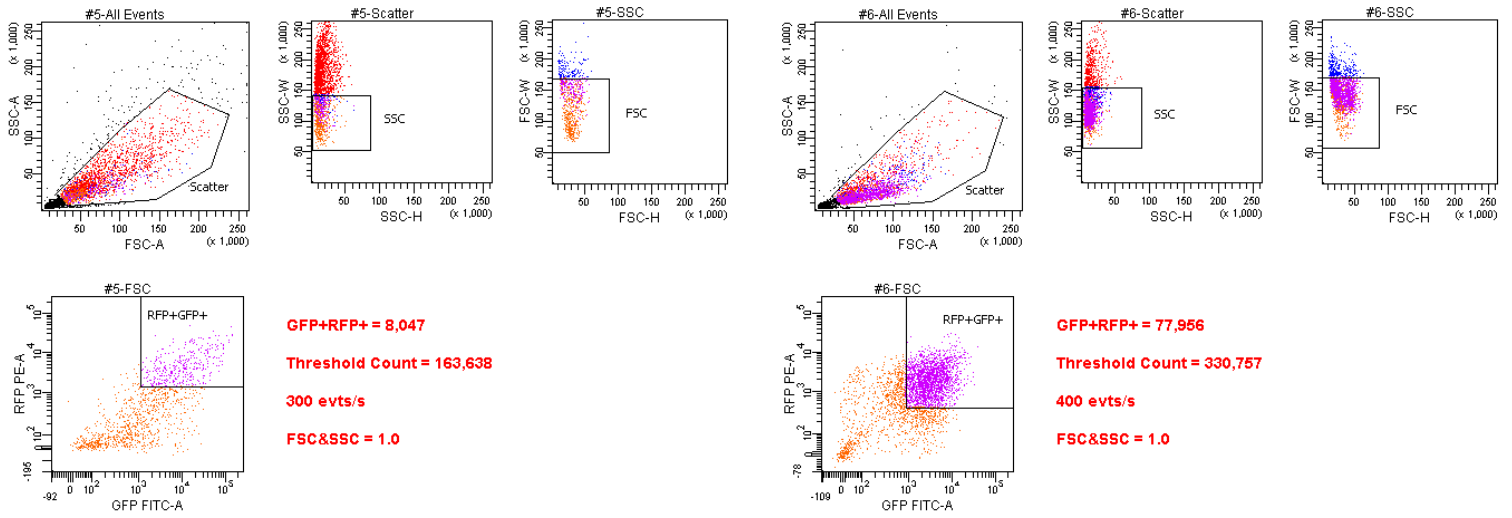


F



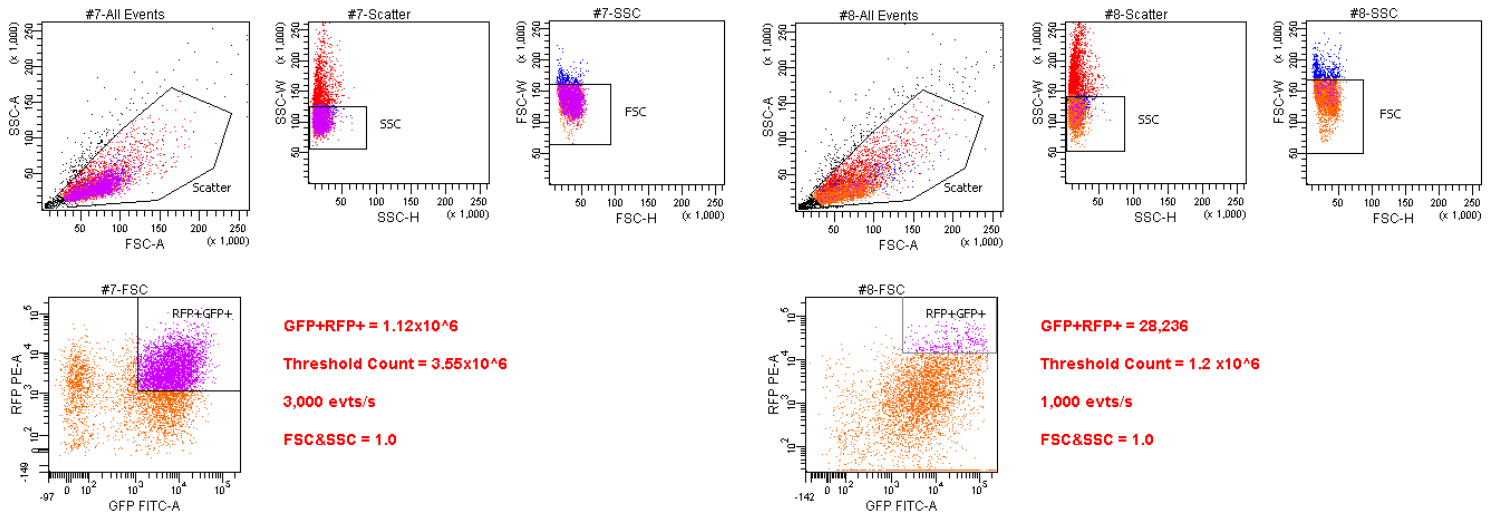
SF4: Transduction efficiency of RFP, FUT2, Fut2, shCTRL, shCDH1.

G



A549 RFP + shCTRL

A549 RFP + shCDH1

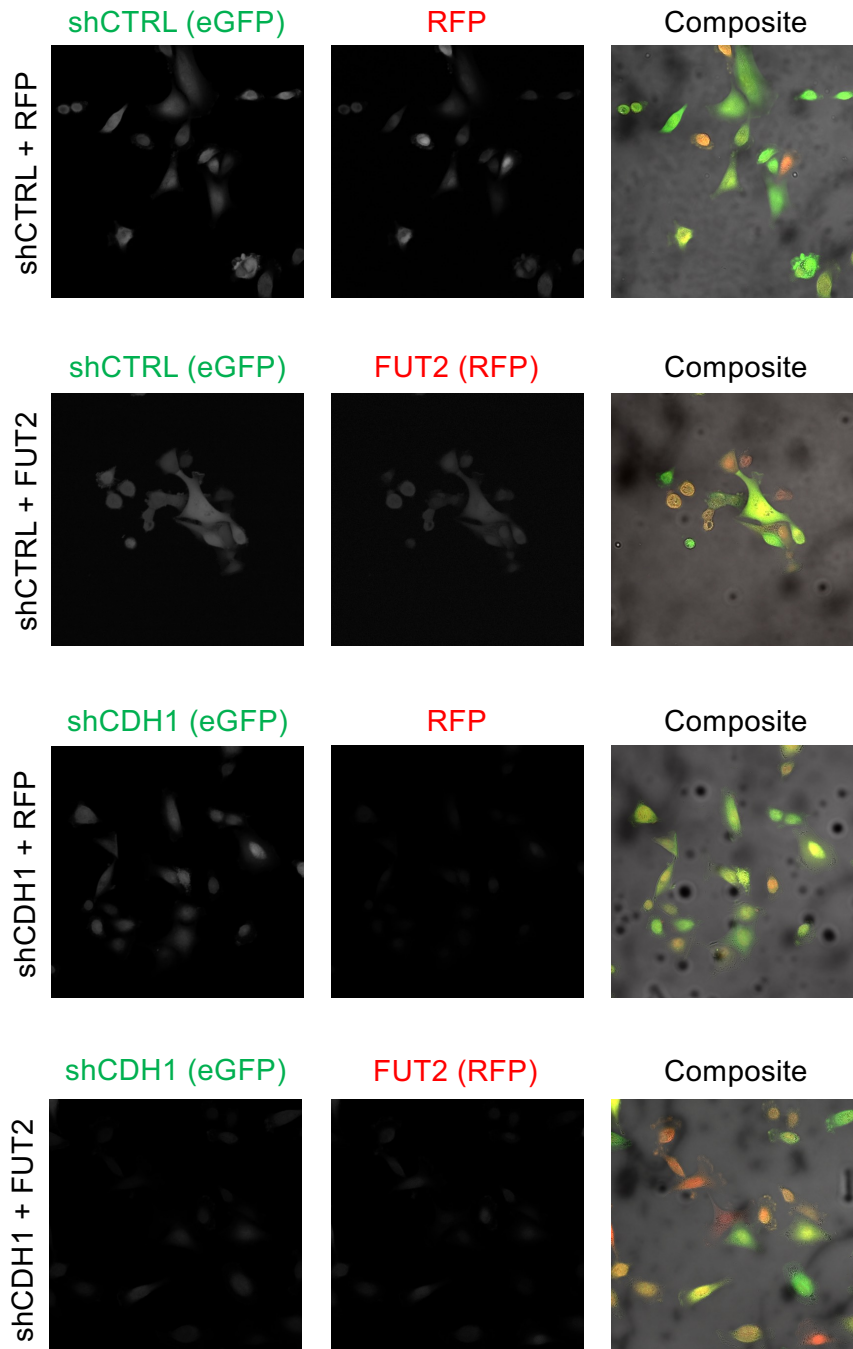


A549 FUT2 + shCTRL

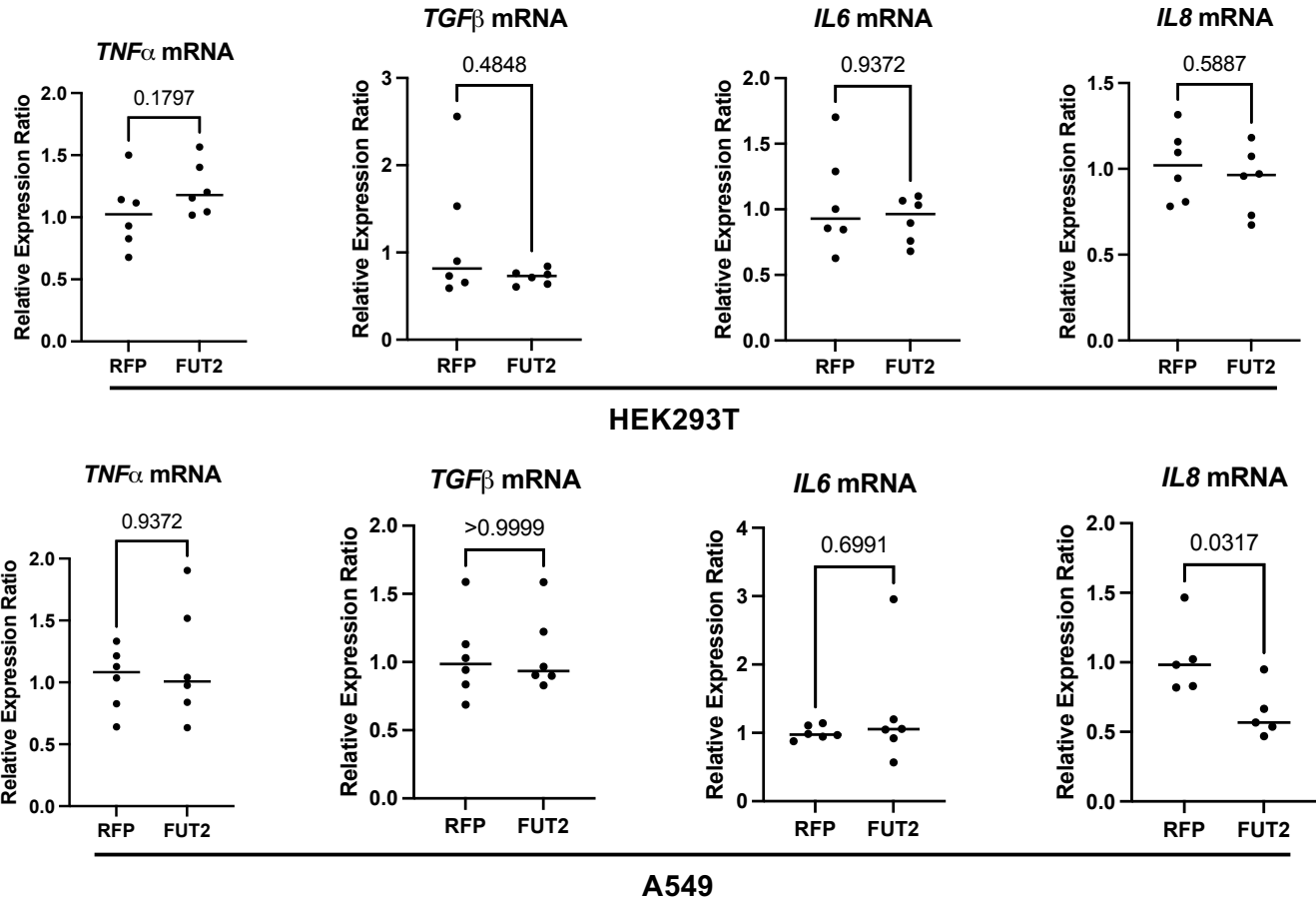
A549 FUT2 + shCDH1

SF4: Transduction efficiency of RFP, FUT2, Fut2, shCTRL, shCDH1.

H



SF4: Transduction efficiency of RFP, FUT2, Fut2, shCTRL, shCDH1.



SF4: Markers of key inflammatory genes are not changed with varying FUT2 expression

Supplementary Table 1: Cohesion measurements of E-cadherin with and without FUT2 dependent fucosylation		
E-cadherin without FUT2 fucosylation		
Run	Adhesive Force (nN)	n
1	63.759 ± 2.8563	96
2	63.428 ± 3.5936	97
3	63.0181 ± 2.9007	101
4	63.4037 ± 2.9181	102
5	63.6881 ± 2.3771	101
E-cadherin with FUT2 fucosylation		
Run	Adhesive Force (nN)	n
1	79.5199 ± 2.5149	105
2	80.2063 ± 3.5990	121
3	80.2640 ± 2.6532	117
4	79.6411 ± 3.0411	116
5	79.3105 ± 2.0907	107

Supplementary Table 2: Quantitative polymerase chain reaction primers used to determine abundance of mRNA transcripts.		
Gene	Direction	Sequence (5' – 3')
Human		
Human <i>CDH1</i>	Forward	GCCTCCTGAAAAGAGAGTGGGAAG
	Reverse	TGGCAGTGTCTCTCCAAATCCG
Human <i>KRT5</i>	Forward	TTGGACCAGTCAACATCTCTGT
	Reverse	CTGCTACCTCCGGCAAGAC
Human <i>FOXJ1</i>	Forward	AGATCCCACCTGGCAGAATTCAA
	Reverse	CCGAGGCACCTTTGATGAAGC
Human <i>SCGB1A1</i>	Forward	TGAAACTCGCTGTCACCCTC
	Reverse	CAGATCTCTGCAGAAGCGGA
Human <i>MUC5AC</i>	Forward	GAATGCCAGTCTGCCTTT
	Reverse	AGCGTGTCCATTGTAGGTG
Human <i>CLDN4</i>	Forward	ATCGGCAGCAACATTGTAC
	Reverse	GCGAGTCGTACACCTTGCAC
Human <i>AGER</i>	Forward	CACCTTCTCCTGTAGCTTCAGC
	Reverse	AGGAGTACTGCTCCAGTCT
Human <i>SFTPC</i>	Forward	GTCCTCATCGTCTGGTGATTG
	Reverse	AGAAGGTGGCAGTGGTAACCAG
Human <i>FUT2</i>	Forward	CTACCACCTGAACGACTGGATG
	Reverse	AGGAGTACTCCTGGAGGATCT
Human <i>GAPDH</i>	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCTGTTGCTGTAGCCAA
Human <i>TNFα</i>	Forward	TGGGATCATTGCCTGTGAG
	Reverse	GGTGTCTGAAGGAGGGGTA
Human <i>TGFβ</i>	Forward	GTGGAAACCCACAACGAAATC
	Reverse	GAGAGCAACACGGGTTCCAGG
Human <i>IL6</i>	Forward	CCACCGGGAACGAAAGAGAA
	Reverse	CTTGTACATGTTTGTGAGAGGA
Human <i>IL8</i>	Forward	TGGACCCCAAGGAAAAGTGG
	Reverse	ATTTGCTTGAAGTTTCACTGGCA
Mouse		
Mouse <i>Cdh1</i>	Forward	GGTCATCAGTGTGCTCACCTCT
	Reverse	GCTGTTGTGCTCAAGCCTTCC
Mouse <i>Krt5</i>	Forward	AGATGTTCTTTGATCGGGAGC
	Reverse	TGTCCATGAAAAGGACCACAG
Mouse <i>Foxj1</i>	Forward	TCCCTGAAGCCTTTCAACCC
	Reverse	CGAATGTGAGGCCTGGCT
Mouse <i>Scgb1a1</i>	Forward	TTACTCTACTGACTGCACCAACACA
	Reverse	CCCATGTAAGTGTACTGCC
Mouse <i>Muc5ac</i>	Forward	CGAGCCCTTATGGTCATCAGCA
	Reverse	ATGCTTGCCACGATGAACACGG
Mouse <i>Cldn4</i>	Forward	GCCACTGGAATTGTCGATGAGG
	Reverse	GCTGTGAGTTCCAGAGGCAGGAT
Mouse <i>Ager</i>	Forward	GTCCTCGTTGTCGTGGTGATTG
	Reverse	AAGGTAGCGATGGTGTCTGCTC
Mouse <i>Sftpc</i>	Forward	AGGCGGTTCAAATGTCTCACC
	Reverse	GCATATTCGCCATCTGGTTCC
Mouse <i>Fut2</i>	Forward	CATCACTGCCACCCAGAAGACTG
	Reverse	ATGCCAGTGAGCTTCCCCTTCAG

Supplementary Table 3: Primers used to amplify genes with polymerase chain reaction from genomic cDNA.		
Gene	Direction	Sequence (5' – 3')
Human <i>FUT2</i>	Forward	GTACGATATCATGTACCCATACGATGTTCCAGATTACGCTCTGGTCGTTCA GATGCC
	Reverse	GTACGATATCTTAGTGCTTGAGTAAGGGGGACAGGTCTGC
Mouse <i>Fut2</i>	Forward	TATAGATATCATGTACCCATACGATGTTCCAGATTACGCTGCGAGTGCCC AGGTA
	Reverse	AAAAGATATCTTAGTGCTTAAAGGAGTG