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## **A High Content Screen for Mucin-1-Reducing Compounds Identifies Fostamatinib as a Candidate for Rapid Repurposing for Acute Lung Injury during the COVID-19 pandemic**

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## 33 **Summary**

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35 Drug repurposing is the only method capable of delivering treatments on the shortened time-  
36 scale required for patients afflicted with lung disease arising from SARS-CoV-2 infection.  
37 Mucin-1 (MUC1), a membrane-bound molecule expressed on the apical surfaces of most  
38 mucosal epithelial cells, is a biochemical marker whose elevated levels predict the development  
39 of acute lung injury (ALI) and respiratory distress syndrome (ARDS), and correlate with poor  
40 clinical outcomes. In response to the pandemic spread of SARS-CoV-2, we took advantage of a  
41 high content screen of 3,713 compounds at different stages of clinical development to identify  
42 FDA-approved compounds that reduce MUC1 protein abundance. Our screen identified  
43 Fostamatinib (R788), an inhibitor of spleen tyrosine kinase (SYK) approved for the treatment of  
44 chronic immune thrombocytopenia, as a repurposing candidate for the treatment of ALI. *In vivo*,  
45 Fostamatinib reduced MUC1 abundance in lung epithelial cells in a mouse model of ALI. *In*  
46 *vitro*, SYK inhibition by Fostamatinib promoted MUC1 removal from the cell surface. Our work  
47 reveals Fostamatinib as a repurposing drug candidate for ALI and provides the rationale for  
48 rapidly standing up clinical trials to test Fostamatinib efficacy in patients with COVID-19 lung  
49 injury.

## 53 **Keywords**

54 COVID-19, SARS-CoV-2, MUC1, acute respiratory distress syndrome (ARDS), acute lung  
55 injury (ALI), Fostamatinib, drug repurposing

## 59 **Introduction**

60  
61 Drug repurposing is a strategy to identify novel uses for approved or investigational  
62 drugs outside the scope of their originally designated purposes. This approach offers several  
63 advantages over *de novo* drug development (Ashburn and Thor, 2004; Pushpakom et al., 2019).  
64 First and foremost, the risk of toxicity is much lower as repurposed approved drugs have been  
65 proven safe for human use in the original indication. Second, and of critical importance for  
66 addressing the global public health crisis attributed to SARS-CoV-2, is that drug repurposing  
67 offers the only method for delivering treatments on the shortened time-scale required to treat  
68 COVID-19 patients. Current management of COVID-19 is largely supportive and with severely  
69 limited therapeutic options. Once infection with SARS-CoV-2 is established, a subset of patients  
70 experience severe complications such as acute respiratory distress syndrome (ARDS), an  
71 extreme form of acute lung injury characterized by disruption to the alveolar epithelium (Ruan et  
72 al., 2020; Zhou et al., 2020). ARDS is a life-threatening condition with mortality rates as high as  
73 40% (Acute Respiratory Distress Syndrome et al., 2000; Determann et al., 2010; Rubenfeld et  
74 al., 2005). COVID-19-associated ARDS is often fatal, especially in the presence of several pre-  
75 existing conditions. The currently limited therapeutic interventions available for COVID-19  
76 (Cao et al., 2020; Grein, 2020) have contributed to an estimated 400,000 deaths worldwide at the  
77 time of writing (Dong et al., 2020). Identification of drugs with efficacy in treating ALI in  
78 severely affected COVID-19 patients remains an urgent need.

79  
80 ARDS patients exhibit high serum levels of mucin-1/MUC1 (KL-6) (Nakashima et al.,  
81 2008). MUC1 is a transmembrane protein expressed on the apical membrane of most mucosal  
82 epithelial cells and plays a critical role in lining the airway lumen (Kato et al., 2017). Mucins are  
83 glycoproteins that impart specific properties to mucus. In response to specific stimuli, goblet  
84 cells can rapidly secrete mucus by exocytosis to form a mucus layer that lines the airways. In  
85 healthy individuals, mucus along the lumen serves as a major protective barrier against inhaled  
86 pathogens, toxins, and other foreign particles. However, excessive mucus in the airways has been  
87 linked to increased frequency and duration of infections, decreased lung function, and increased  
88 mortality from respiratory diseases (Vestbo, 2002). Abnormalities in mucus production  
89 contribute to severe pulmonary complications and death from respiratory failure in patients with  
90 diseases such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), and acute lung  
91 injury due to viral pathogens, such as SARS-CoV2. Elevated serum KL-6/MUC1 levels are an  
92 early prognostic marker of the therapeutic effect of high-dose corticosteroids in patients with  
93 rapidly progressing idiopathic pulmonary fibrosis (Yokoyama et al., 1998). Serum KL-6/MUC1  
94 levels are also elevated in patients with interstitial pneumonitis (Ishikawa et al., 2012; Kohno,  
95 1999). Moreover, transgenic mice expressing human MUC1 and subjected to LPS-induced ALI  
96 exhibit elevated KL-6 both in alveolar pneumocytes and in serum (Sakai et al., 2013).

97  
98 Prompted by the connection between elevated MUC1 and ALI, we investigated the  
99 possibility of identifying MUC1-reducing drugs for rapid repurposing. We had originally  
100 screened the Broad Repurposing Library (comprised of 3,713 compounds at different stages of  
101 pre-clinical and clinical development (Corsello et al., 2017)) to identify compounds capable of  
102 reducing a mutant MUC1 neo-protein (MUC1-fs) causing autosomal dominant tubulo-interstitial  
103 kidney disease-*mucin1* (ADTKD-*MUC1* or *MUC1* kidney disease, MKD) (Dvela-Levitt et al.,  
104 2019). In this context wildtype MUC1 (MUC1-wt) served as a control, as we sought compounds  
105 that specifically reduced the mutant, but not the wildtype form of MUC1. As the number of  
106 COVID-19 cases increased globally, we turned our attention to identifying MUC1-reducing  
107 compounds and mined this dataset to identify approved drugs that reduce expression of MUC1-  
108 wt. We searched for MUC1-reducing compounds based on the following criteria: 1) a drug that  
109 reduces MUC1-wt protein in a dose-dependent manner; 2) a drug with a favorable toxicity  
110 profile; 3) a drug that reduces MUC1-wt by non-transcriptional mechanisms (Dvela-Levitt et al.,  
111 2019), unlike transcriptional suppressors such as vitamin D agonists that have proven ineffective  
112 in the clinic (Castro et al., 2014); and 4) a drug that is US Food and Drug Administration (FDA)-  
113 approved. Based on these criteria, our screen identified R406, the active metabolite of  
114 Fostamatinib (R788, an oral prodrug rapidly converted to R406), as a repurposing candidate for  
115 the treatment of ALI.

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## 118 **Results**

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### 120 **The FDA approved SYK inhibitor R406 depletes MUC1 from epithelial cells without** 121 **affecting cell viability**

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123 To investigate the expression pattern of MUC1 in human tissue, we took advantage of the  
124 openly available Human Protein Atlas (HPA)([www.proteinatlas.org](http://www.proteinatlas.org)) (Uhlen et al., 2015).

125 Immunoperoxidase staining of human lung showed MUC1 expression in alveolar epithelium.  
126 This finding confirmed multiple reports of MUC1 expression in normal and diseased human lung  
127 (Figure 1A) (Ishizaka et al., 2004; Ohtsuki et al., 2007). These data were corroborated by the  
128 expression of MUC1 mRNA in human lung reported by the HPA, GTEx and FANTOM5  
129 databases (Figure 1B) and by the Human Lung Atlas project (Muus, 2020)

130  
131 We screened 3,713 compounds of the Repurposing Library for their ability to reduce  
132 MUC1 protein levels (Figure 2A). The screen employed high-content immunofluorescence (IF)  
133 imaging of an immortalized kidney tubular epithelial cell line (P cells) that express endogenous  
134 MUC1 on the plasma membrane, to simultaneously assess MUC1 protein abundance and cell  
135 number as an index of cell toxicity (Dvela-Levitt et al., 2019). The bromodomain inhibitor JQ1  
136 served as positive control, as preliminary experiments demonstrated complete transcriptional  
137 suppression of MUC1 by JQ1. Each compound in the Repurposing Library was tested in a 5  
138 concentration, 10-fold dilution series with a top concentration of 35  $\mu$ M in the initial screen.  
139 Positive hits from the 5-dose screen were defined by two criteria: lack of cellular toxicity (less  
140 than 20% reduction in cell number compared to the DMSO control); and reduction of MUC1  
141 abundance by >30% (normalized to DMSO and JQ1 controls) at 2 or more consecutive non-toxic  
142 concentrations of test compound. The compounds that met these criteria included two major  
143 groups, bromodomain inhibitors (blue) and vitamin D receptor agonists (orange, Figure 2B). Our  
144 screen also identified drugs that increased MUC1 levels (Figure 2B), including glucocorticoid  
145 receptor agonists. Most compounds that increased MUC1, such as epidermal growth factor  
146 receptor (EGFR) inhibitors (green), also reduced cell number, indicating an association between  
147 cell toxicity and increased MUC1 levels (Figure 2B).

148  
149 Two hundred and three compounds were re-tested at 10 concentrations to generate more  
150 complete dose response curves for each compound. In this screen, any compound that reduced  
151 MUC1 by >30% at 2 or more consecutive concentrations without evidence of toxicity (cell  
152 numbers within 20% of the DMSO control) was considered a positive hit. Thirteen hits from this  
153 screen were analyzed further in secondary profiling assays, including quantitative PCR (qPCR)  
154 and cell viability screens. MUC1 qPCR (Figure 2C) showed that for most compounds, reduction  
155 of MUC1 protein abundance (MUC1 IF) was highly correlated with parallel reductions in  
156 mRNA abundance (MUC1 qPCR). Of interest, 4 compounds reduced MUC1 protein without  
157 affecting MUC1 mRNA. These included the SYK inhibitor R406 (red, Figure 2C); the RAF  
158 inhibitor LGX818 (violet, Figure 2C); the mannosidase inhibitor Kifunensine (green, Figure 2C),  
159 and the IKK inhibitor TPCA-1 (turquoise, Figure 2C). None of these four compounds exhibited  
160 detectable toxicity (cell death or apoptosis) at any of the 10 tested concentrations. Importantly,  
161 only the SYK inhibitor R406 is FDA-approved (Figure 2A and D). No additional compounds  
162 with known activity against SYK met our screening criteria. R406 decreased MUC1 protein  
163 abundance in cells with an EC<sub>50</sub> of approximately 200 nM. The lack of effect on MUC1 mRNA  
164 levels (as shown by qPCR) indicated that MUC1 protein reduction was not achieved via  
165 transcriptional repression (representative images of cells treated with R406 at a range of  
166 concentrations; Figure 2E). The parent molecule of R406, Fostamatinib (R788), showed no  
167 activity in the initial 5-concentration screen.

168  
169 **R406 preferentially depletes MUC1 from the plasma membrane**

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171 Image analysis from the high content screen revealed that, while MUC1 was  
172 preferentially localized to the plasma membrane in DMSO-treated control cells, treatment with  
173 200 nM of R406 reduced MUC1 from the plasma membrane and redistributed a fraction of the  
174 protein to the intracellular compartment with a perinuclear distribution pattern (Figure 3A). We  
175 quantified this relocation using a STAR morphology “Profile” Module (Star Methods) that  
176 allowed selective measurement of MUC1 signal distribution in cellular sub-compartments.  
177 Figure 3B shows an analysis sequence for the sub-compartment corresponding to the cell region  
178 closest to the plasma membrane. Within each cell, the nucleus border was identified based on  
179 Hoechst staining (blue, second panel, Figure 3B) and the plasma membrane (orange, second  
180 panel, Figure 3B) was identified based on the MUC1 signal. The calculated STAR morphology  
181 “Membrane Profile” image was generated as illustrated in Figure 3B, panel 3 (STAR Methods).  
182 The calculated STAR Membrane Profile values for MUC1 in each well were compared with total  
183 cell MUC1 intensity (Figure 3C). As shown by a local regression, while most compounds that  
184 decreased MUC1 did not affect MUC1 membrane localization, R406 produced preferential  
185 depletion of MUC1 from the plasma membrane region at all active concentrations.  
186

### 187 ***In vivo*, R406 reduces lung epithelial MUC1 in mice with ALI**

188

189 SYK inhibition has previously been shown to suppress both local and remote lung injury  
190 (Pamuk, et al., 2010). R788 (fostamatinib disodium) is a methylene phosphate prodrug of R406  
191 suitable for oral administration (McAdoo and Tam, 2011). To investigate whether administration  
192 of R788 might ameliorate ischemia-reperfusion (I/R)-induced remote lung injury by reducing  
193 MUC1 levels from the plasma membrane in the lung epithelium, C57BL/6J mice were fed a  
194 chow diet containing 3 grams/kilogram of R788 for 10 days. Immunohistochemical images  
195 obtained from formalin-fixed paraffin sections of lung tissues stained with MUC1, Phalloidin,  
196 and DAPI demonstrated that I/R-mediated ALI induced increased levels of MUC1 in lung  
197 epithelium, consistent with previous reports that excess MUC1 is injurious (Sakai et al., 2013).  
198 Importantly, MUC1 was significantly reduced by treatment with R788 (Figure 4A). Quantitative  
199 image analysis confirmed the *in vivo* efficacy of R788 in reducing MUC1 in injured lung  
200 epithelium (Figure 4B and C).

## 201 **Discussion**

202

203  
204 Our high-content screen identified R406, the active metabolite of Fostamatinib, as an  
205 FDA-approved candidate repurposing compound for the reduction of MUC1 protein levels in  
206 lung epithelium in the setting of ALI. R406 is a potent inhibitor of spleen tyrosine kinase (SYK),  
207 a cytosolic protein tyrosine kinase required for the expression of several proinflammatory  
208 cytokines (Yi et al., 2014). SYK is expressed in most leukocyte populations with roles in  
209 mediating signaling via classical immunoreceptors such as B-cell receptors and Fc receptors  
210 (Mocsai et al., 2010). SYK plays diverse roles in cellular adhesion, innate immune recognition,  
211 and platelet activation, and its central role in immune cell responses has made it a compelling  
212 target for the development of therapeutic agents. Over 70 patent filings describe small molecule  
213 inhibitors of SYK developed for treatment of diseases ranging from arthritis to asthma (Geahlen,  
214 2014).

215

216 Fostamatinib is an effective treatment in experimental animal models of severe  
217 inflammatory diseases, including immune glomerulonephritis (McAdoo et al., 2014; Smith et al.,  
218 2010) and vasculitis (McAdoo et al., 2020). Phase II clinical trial results are expected assessing  
219 the effect of SYK inhibition in proliferative IgA nephropathy, an inflammatory kidney disease  
220 (NCT02112838). Fostamatinib was approved in April 2018 by the US Food and Drug  
221 Administration (FDA) for the treatment of chronic immune thrombocytopenia (ITP), an  
222 autoimmune disease that results in low levels of circulating platelets (Argade et al., 2015;  
223 Hilgendorf et al., 2011; Newland et al., 2018; Singh et al., 2012). Fostamatinib has also been  
224 extensively studied and found to be safe in more than 3000 patients with rheumatoid arthritis  
225 (Kunwar et al., 2016). In a Phase III clinical trial (Bussel et al., 2018), Fostamatinib was well  
226 tolerated at an oral dose of 100 mg twice daily. Mild or moderate adverse effects included  
227 diarrhea, hypertension, nausea, and an increase in alanine aminotransferase (ALT). These  
228 resolved spontaneously or with medical management, including antihypertensive or antimotility  
229 agents. This well-characterized clinical safety profile makes Fostamatinib an ideal candidate for  
230 rapid repurposing (Weinblatt et al., 2010).

231  
232 Our finding that R406 preferentially depletes MUC1 abundance in or near the plasma  
233 membrane is consistent with a previously described mechanism by which SYK inhibition results  
234 in dephosphorylation of integral membrane proteins followed by their endocytic removal from  
235 the plasma membrane. For example, SYK signaling modulates CFTR abundance in human  
236 airway epithelial cell plasma membrane (Mendes et al., 2011). Interestingly, SYK in mucin-  
237 producing human NCI-H292 cells and in primary human nasal epithelial cells also regulates  
238 MUC5AC, a gel-like mucin that promotes lung epithelial injury (Na et al., 2016). Finally, in  
239 further support of the notion that MUC1 reduction is beneficial to injured lung epithelium, *Muc1*  
240 knockout in rat airway epithelial cells and MUC1 reduction in human lung epithelium resulted in  
241 diminished mucin hypersecretion and protection from lung injury (Kato et al., 2020).

242  
243 Severe COVID-19 symptoms include viral-induced pneumonitis accompanied by  
244 prolonged, systemic cytokine release (Moore and June, 2020; Zhang et al., 2020) in which levels  
245 of interleukin-6 (IL-6) levels and other cytokines and acute phase reactants correlate with  
246 respiratory failure. Macrophage-derived IL-6 upregulates MUC1 in the human colon cancer HT-  
247 29 cell line (Li et al., 2009), suggesting a similar IL6-mediated upregulation of MUC1 may occur  
248 in SARS-CoV-2 infected lungs. A recent comparison of 15 hospitalized COVID-19 patients, 9  
249 of whom were critically ill, to 28 critically ill patients with ARDS or sepsis found no statistically  
250 significant difference in circulating levels of IL-1b, IL-1RA, IL-6, IL-8 and TNF-alpha among  
251 these conditions (Wilson, 2020). These results indicate that COVID19-related ARDS is  
252 associated with inflammatory cytokine levels no higher than in ARDS due to other critical  
253 illnesses (Wilson, 2020). Another recent study analyzing serum concentrations of KL-6/MUC1  
254 levels in hospitalized COVID19 patients suggested KL-6/MUC1 as a good prognostic biomarker  
255 of disease severity in COVID-19 patients (d'Alessandro et al., 2020). Given the roles of excess  
256 KL6/MUC1 in ALI and ARDS, we propose that Fostamatinib may confer benefit in patients with  
257 COVID-19 lung injury. In conclusion, our *in vitro* and *in vivo* data support the efficacy of  
258 Fostamatinib for the treatment of ALI. Here we provide a rationale for imminently designing and  
259 executing clinical trials to test whether repurposing this FDA-approved drug might confer  
260 clinical benefit for COVID-19 patients in the clinic.

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### **Acknowledgments**

We gratefully acknowledge stimulating discussions with Broad colleagues Deborah Hung, Ramnik Xavier, Jay Rajagopal and Aviv Regev. We thank Terry Woo (BWH Pathology) and Stephen Straub (Perkin Elmer) for excellent technical support and expertise. SYK inhibitor R788 was provided as a gift to GCT by Rigel Pharmaceuticals (South San Francisco, CA).

This work was supported by PHS NIH R01AI148161 (GCT) and by the Slim Initiative for Genomic Medicine in the Americas (SIGMA), a collaboration of the Broad Institute with the Carlos Slim Foundation. FWKT is supported by the Ken and Mary Minton Chair of Renal Medicine.

### **Author Contributions**

MA, ASat, MDL, MM, JS, JG performed experiments; MA, EHS, ASat, MDL, BC, ASub performed data analysis and provided data visualization; SMcA, FT, GCT provided tissue and expertise in rodent models of lung disease; MA, EHS, MDL, BC, JS and AG wrote the manuscript; all authors participated in discussions on the scientific rationale for this study, read and approved the manuscript; AG supervised the project.

### **Declaration of Interests**

The authors declare no competing interests. FWKT has received research project grants from Rigel Pharmaceuticals, and has consultancy agreements with Rigel Pharmaceuticals, and is the Chief Investigator of an international clinical trial of a SYK inhibitor in IgA nephropathy (ClinicalTrials.gov NCT02112838), funded by Rigel Pharmaceuticals.

### **Figure Legends**

#### **Figure 1. High relative expression of MUC1 in human lung.**

- A. Immunoperoxidase staining of three human lung samples demonstrates MUC1 protein abundance in lung tissue.
- B. mRNA expression data from three datasets (HPA, GTEx, and FANTOM5, heatmaps) combined into a CONSENSUS normalized transcript expression level (bar plot) show enhanced expression of MUC1 mRNA in human lung. NX, normalized expression; pTPM, protein-coding transcripts per million.

307 **Figure 2. High Content Screening reveals significant and dose-dependent reduction in**  
308 **MUC1 by the FDA-approved SYK inhibitor R406**

- 309 A. Screening pipeline.  
310 B. Primary screen revealed four major groups of compounds which affected MUC1  
311 levels. MUC1 immunofluorescence (IF) signal intensity per cell (normalized to positive  
312 control JQ1 minus DMSO-treated controls) plotted vs. DMSO-normalized cell number.  
313 Horizontal and vertical dashed lines delineate mean DMSO values  $\pm 3*SD$  for both  
314 MUC1 intensity and cell number. Lavender-shaded area demarcates candidate MUC1  
315 suppressors.  
316 C. qPCR and cell viability profiling screens identified four compounds that reduced  
317 MUC1 protein abundance without changing MUC1 mRNA level, and in the absence of  
318 cytotoxicity. Left: MUC1 signal intensity per cell plotted vs. MUC1 mRNA level (qPCR  
319 assay). Both parameters are normalized to JQ1 minus DMSO-treated controls. Right: JQ1  
320 minus DMSO-normalized MUC1 signal intensity per cell plotted vs. DMSO-normalized  
321 cell viability (a number of viable cells after 6 days exposure to the test compounds).  
322 Horizontal dashed lines delineate SD for DMSO treated control wells for both cell  
323 viability and MUC1 qPCR. Green-shaded areas demarcate candidate hits.  
324 D. R406 concentration response curves for MUC1 protein abundance (black), MUC1  
325 mRNA abundance (light gray), and cell viability (dark gray).  
326 E. MUC1 IF in kidney epithelial cells treated for 48 hours in the absence (DMSO) and  
327 presence of R406 at the indicated concentrations.  
328

329 **Figure 3. R406 preferentially depletes MUC1 from the plasma membrane.**

- 330 A. R406 (at EC50 concentration) substantially reduced MUC1 abundance in or near the  
331 plasma membrane, with a portion of MUC1 retained in cytosolic and perinuclear cell  
332 compartments.  
333 B. Image analysis for cell compartmentation using STAR morphology “Membrane -  
334 Profile” calculation (see Star Methods). Image 1: cells with MUC1 preferentially  
335 localized at plasma membrane; Image 2: Harmony software identification of nucleus  
336 (blue) and plasma membrane (gold) in each cell; Image 3: STAR morphology  
337 “Membrane Profile” for the MUC1 predominant localization within membrane  
338 compartment  
339 C. STAR morphology” Membrane Profile” analysis of 203 compounds screened at 10  
340 doses. R406 at most active concentrations reduced plasma membrane MUC1 abundance  
341 to a greater degree than most other compounds, as shown by deviation from the local  
342 regression. □ MUC1 IF signal intensity per cell (normalized to JQ1 minus DMSO-treated  
343 controls) plotted vs. DMSO-normalized MUC1 predominance in plasma membrane  
344 region as calculated using the STAR morphology “Membrane Profile” module.  
345 Horizontal and vertical dashed lines delineate mean DMSO values  $\pm 2*SD$  for both  
346 plotted parameters. Local regression was calculated by locally estimated scatterplot  
347 smoothing (loess) method  $\pm 95\%$  confidence interval (gray-shaded).  
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350 **Figure 4. *In vivo*, R788 reduces excess MUC1 from lung epithelia of mice with ALI**  
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- 352 A. Immunofluorescence images from lung tissue sections stained with MUC1 (green),  
353 Phalloidin (yellow), and DAPI (grey) demonstrate that ischemia-reperfusion (I/R)-  
354 induced remote ALI resulted in increased MUC1 in lung epithelium. Treatment with  
355 Fostamatinib over the course of 10 days suppressed MUC1 levels in mouse lung  
356 epithelium.
- 357 B. Single cell tissue analysis, based on immunofluorescence of MUC1 and phalloidin (panel  
358 1). In each image (panel 1) nuclei were identified based on DAPI staining (rainbow  
359 colors represent different cell nuclei in panel 2). The cell bodies were identified based on  
360 phalloidin staining surrounding each nucleus (orange cell borders, panel 3). Lastly,  
361 MUC1 IF intensity (green on panel 3) and Phalloidin intensities were calculated within  
362 each cell body.
- 363 C. Bar graph ratio of MUC1/Phalloidin intensities in all cells of tissue sections from sham-  
364 treated mice and mice subjected to I/R-induced ALI, treated either with or without  
365 Fostamatinib. Average MUC1 intensity values per cell were normalized to the average  
366 phalloidin levels. Mean  $\pm$  SD (n = 3 mice/condition/dose). ns, \*p < 0.05 \*\*p < 0.01 \*\*\*p <  
367 0.001.

## 368 **STAR Methods**

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### 370 **Broad Repurposing Library**

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372  
373 Quality control of The Broad Repurposing Library is performed at the time of plating by LCMS  
374 analysis using a Waters Acquity LC System with UV PDA detector and single quad (SQ) mass  
375 spectrometer R406 was confirmed present (MS (ESI/SQ) m/z: [M + H]<sup>+</sup> Calculated for  
376 C22H23FN6O5+H: 471.2; Found: 470.9) in > 97% purity (PDA integration).

377

### 378 **Human Protein Atlas**

379

380 MUC1 immunoperoxidase images were obtained from the Human Protein Atlas with the original  
381 source available at the following link: ([https://www.proteinatlas.org/ENSG00000185499-](https://www.proteinatlas.org/ENSG00000185499-MUC1/tissue/lung)  
382 [MUC1/tissue/lung](https://www.proteinatlas.org/ENSG00000185499-MUC1/tissue/lung)). mRNA expression data ([https://www.proteinatlas.org/ENSG00000185499-](https://www.proteinatlas.org/ENSG00000185499-MUC1/tissue)  
383 [MUC1/tissue](https://www.proteinatlas.org/ENSG00000185499-MUC1/tissue)) were downloaded from the Human Protein Atlas:  
384 (<http://www.proteinatlas.org/ENSG00000185499.xml>) (Uhlen et al., 2015). All graphs were  
385 visualized using ggplot2.

386

### 387 **Experimental Models**

388

#### 389 **Cell Lines**

390 Human P kidney epithelial cells (female) were previously generated from a patient with MUC1  
391 kidney disease (Dvela-Levitt et al., 2019). The cells were maintained at 37°C with 5% CO<sub>2</sub> in  
392 RenaLife Renal Basal Medium supplemented with RenaLife LifeFactors® (Lifeline Cell  
393 Technology), with the exclusion of Gentamycin and Amphotericin B. For all experiments, P cells  
394 were maintained below passage 12. The cells were generated with informed consent under  
395 WFUHS IRB00014033.

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397

## 398 **Fluorescence image acquisition and analysis**

399

400 All fluorescence imaging performed in this study was done using the Opera Phenix High-  
401 Content Screening System (PerkinElmer). For fluorescence imaging of cells (live cell or fixed  
402 cell imaging), CellCarrier 384-well Ultra microplates (Perkin Elmer) were used, and a minimum  
403 of nine fields was acquired per well using 20x water immersion objectives in a confocal mode.

404

405 Image analysis for all imaging experiments was performed using the Harmony software  
406 (PerkinElmer). Cell nuclei were first identified using Hoechst staining, and cell number was  
407 calculated. Cytoplasmic regions were then detected around each nucleus based on MUC1  
408 channel. The cells from the edge of the field were eliminated from the analysis. For the  
409 quantification of MUC1 abundance, the total signal intensity value was calculated in the cell  
410 cytoplasm and the average signal per cell was calculated for each well.

411

412 For live cell image analysis, caspase 3/7 activation and/or DRAQ7 signal were used to detect  
413 cells going through apoptosis and/or cell death, respectively. Single cells were first identified  
414 using the digital phase contrast channel and cell number was calculated. Fluorescence intensities  
415 were then measured and the threshold for caspase 3/7 and DRAQ7 positive signal was  
416 determined. As an output, the number of live (neither caspase3/7 nor DRAQ7 signal detected)  
417 cells was calculated in each well at a particular time point.

418

419 For the MUC1 membrane prevalence, the images acquired during 10-dose screening were  
420 analyzed using the Harmony software STAR morphology feature, which calculates the signal  
421 distribution across different cell compartments. The inner side of plasma membrane compartment  
422 was analyzed by generating a “Membrane Profile Image” (profile 1/5 in Harmony software) (fig  
423 3B). This function measures the closest distance of a given pixel to a cell border within a width  
424 of 4 pixels to preferentially weigh and quantify signal intensity (MUC1) closest to the plasma  
425 membrane signal.

426 For in vivo lung imaging, 20x water immersion objective was used, with 5% overlap for entire  
427 lung sections. Tissue Image Region for every tissue section was identified based on Gaussian  
428 Smoothed filtered global DAPI channel. Every Tissue Image Region was resized to exclude the  
429 very peripheral area of the sections. Sliding parabola filtered DAPI channel within the resized  
430 tissue area was used to find nuclei; and Phalloidin was used to identify a cytoplasm (see fig 4B).  
431 Mean intensity of MUC1 and Phalloidin was calculated in each cell, and averaged per cell for the  
432 entire tissue sample. The average MUC1 signal was then normalized to Phalloidin signal to take  
433 in account variability in slide staining conditions.

## 434 **High Content Screening**

435 The automated high content screening system consisted of robotic arms; plate stackers; a  
436 HighRes Pin Tool; Liconic incubators; Biotek plate washers; dedicated Thermo Fisher Combi  
437 Multidrop dispensers for each assay reagent; and PerkinElmer High Content Imaging Instrument  
438 Opera Phenix, all choreographed by Cellario software. Cell fixation and immunostaining were all  
439 performed in a custom-designed light-protected hood (HighRes Biosolutions). Data analysis and  
440 representation was performed using Genedata Screener (Genedata AG) and Spotfire (TIBCO).

441 For the immunofluorescence screen, P cells were seeded 24 h prior to compound treatment at a  
442 density of 12,000 cells/well in 384 well CellCarrier Ultra plates (Perkin Elmer), pre-coated with  
443 0.25 mg/mL Synthemax II SC Substrate (Corning). Compounds of the repurposing library set  
444 (Corsello et al., 2017) were used at either 5 doses (35, 3.5, 0.35, 0.035 and 0.0035  $\mu$ M) or 10  
445 doses (16, 5.6, 1.8, 0.6, 0.21, 0.07, 0.02, 0.008, 0.002 and 0.0008  $\mu$ M) as indicated. The  
446 compounds were transferred in replicate from compound source plates to the cell plates using the  
447 HighRes Pin Tool. DMSO was used as a negative control and JQ1 (250 nM) (a bromodomain  
448 inhibitor) as positive control, based on earlier studies showing potent reduction of total MUC1  
449 mRNA levels (data not shown). After 48 h incubation, cells were fixed 20 min in 4% PFA  
450 (Electron Microscopy Sciences) in PBS, washed twice, then permeabilized (10 min) with 0.5%  
451 Triton X-100 (Sigma-Aldrich) in PBS and washed once more. Cells were blocked for 10 min at  
452 RT with Blocking solution (100mM Tris HCL pH8; 150mM NaCl; 5g/L Blocking Reagent  
453 [Roche]), then incubated 90 min at RT with 1:2000, monoclonal mouse anti-MUC1 (214D4)  
454 antibody (Millipore) in Roche Blocking solution, followed by four PBS wash cycles. Then the  
455 secondary antibody Alexa Fluor® 546 Goat anti-mouse IgG, Thermo Fisher Scientific and  
456 Hoechst 33342 stain, Thermo Fisher Scientific, were applied at a 1:1000 dilution in Roche  
457 blocking solution and incubated at RT for 45 min, followed by four PBS wash cycles. Plates  
458 were then sealed with a Plate Loc plate and stored in a Liconic incubator at 10°C until imaging.  
459

460 Image acquisition and analysis was as described in the Fluorescence image acquisition and  
461 analysis section. Upon image analysis, two parameters were selected, i) total MUC1 cytoplasmic  
462 intensity and ii) cell number as was detected by Hoechst 33342 stained nuclei. MUC1 levels in  
463 the presence of DMSO or of JQ1 were defined, respectively as 0 and -100% activity. Values for  
464 test compounds were normalized accordingly. Cell number was normalized to DMSO control.  
465 All compound concentrations showing  $> -20\%$  reduction in cell number were masked out. Based  
466 on  $\pm 3$  median absolute deviation value, hit calling criteria for the initial 5 doses screen were  
467 chosen as MUC1 reduction  $> 30\%$  in 2 or more consecutive concentrations for both replicates.  
468 For the initial 10 doses screen, dose response curves were generated for each parameter using  
469 Genedata Screener (Genedata AG), and positive hits for the profiling screens were selected based  
470 on the compound's activity in reducing MUC1 abundance without cell toxicity.

471 For the RT-PCR-based screen (Bittker, 2012), P cells seeded at 2000 cells/well in 384-well, clear  
472 bottom, white wall plates were grown for 24 h, then treated with profiling compounds transferred  
473 by pinning to duplicate plates. JQ1 (250 nM) and DMSO were used for controls as above. After  
474 24 h, cells were washed and cDNAs generated using ABI Cells-to-Ct kit (Thermo Fisher  
475 Scientific, Waltham, MA). MUC1 and HMBS delta Cp values were determined using a Roche  
476 LightCycler 480 Instrument in 5 $\mu$ L reactions using TaqMan probes for MUC1 FAM (4351368  
477 assay ID Hs00159357\_m1) and HMBS VIC (4448486- assay ID Hs00609297\_m1) (Thermo  
478 Fisher Scientific). The fold change effect of the compounds on total MUC1 mRNA was  
479 normalized to JQ1 and DMSO controls, as described above.  
480

481 For the viability profiling screen, P cells were seeded 12 h prior to profiling compound treatment  
482 at a density of 12,000 cells/well in 384 well Cell Carrier Ultra plates (Perkin Elmer), pre-coated  
483 with 0.25 mg/mL Synthemax II SC Substrate (Corning). After 24 h, Cellevent Caspase-3/7  
484 Green Detection Reagent (Thermo Fisher Scientific) and DRAQ7 (Biostatus) were added at

485 1:5000 final dilution. Cells were imaged daily during 7 days to monitor viability. Image  
486 acquisition was done as described below and viability was assessed as number of live cells at the  
487 day 6, when most of DMSO treated wells reached about 95% of confluence.

488

## 489 **Mice**

490

491 Adult, 7-week-old male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor,  
492 ME) and maintained in specific pathogen-free conditions at the Beth Israel Deaconess Medical  
493 Center (BIDMC) and allowed to acclimate for 1 week before use in experiments. All mice used  
494 in this study were 8–12 weeks old.

495

## 496 **Administration of SYK inhibitor R788**

497

498 SYK inhibitor R788 was provided by Rigel Pharmaceuticals (South San Francisco, CA). Mice  
499 chow was prepared by Research Diets (New Brunswick, NJ). C57BL/6J mice were fed chow  
500 containing 3 g/kg R788 ad libitum for 10 days before experimentation. Control mice were fed  
501 normal chow.

502

## 503 **Mesenteric Ischemia-Reperfusion (I/R)**

504

505 All animal procedures were performed in accordance with the guidelines and approval of the  
506 Institutional Animal Care and Use Committee (IACUC) of the BIDMC. Mice were randomly  
507 assigned to sham or I/R groups and were anesthetized by intraperitoneal injection of 72 mg/kg  
508 pentobarbital. Animals were subjected to I/R, as previously described (Pamuk et al., 2010). Mice  
509 were anesthetized with 72 mg/kg nembutal (Butler Schein Animal Health) given i.p.  
510 Additionally, 36 mg/kg nembutal was given s.c. during the experiment as needed to maintain  
511 anesthesia. All procedures were performed on anesthetized, spontaneously breathing animals  
512 with body temperature maintained at 37°C with a controlled heating pad. A midline laparotomy  
513 was performed, and the superior mesenteric artery was identified and isolated. Ischemia was  
514 induced by application of ~85 g of pressure for 30 min via a small nontraumatic vascular clamp  
515 (Roboz Surgical Instruments, Gaithersburg, MD). After 30 min of ischemia, the clamp was  
516 removed, the laparotomy incision was repaired with 4-0 Sofsilks (Covidien, Mansfield, MA), the  
517 mice were resuscitated with 1.0 ml of prewarmed sterile PBS s.c., and the intestine was allowed  
518 to reperfuse for 180 min. Sham-operated mice were subjected to the same operative procedure as  
519 the experimental group except that clamping of the superior mesenteric artery was not  
520 performed. At the conclusion of the reperfusion period, mice were euthanized by carbon dioxide  
521 asphyxiation, following the IACUC Guidelines of the BIDMC. Lung removal consisted of intact  
522 extraction of the bronchial tree after expansion with tracheal administration of 200–300 of ice-  
523 cold 10% phosphate-buffered formalin and fixed overnight in 10% phosphate-buffered formalin  
524 at 4°C. Formalin-fixed lung tissues were washed extensively in PBS, processed, and embedded  
525 in paraffin for immunohistochemical analysis.

526

## 527 **Immunohistochemistry**

528

529 Immunohistochemical staining was performed on formalin-fixed paraffin sections of lung  
530 tissues. The samples were subjected to rehydration and antigen retrieval by overnight immersion  
531 in 10mM citric acid buffer (pH 6), for overnight at 60° C. Following antigen retrieval,  
532 endogenous peroxidase was blocked with 0.3% H<sub>2</sub>O<sub>2</sub> for 15 min followed by blocking with  
533 2.5% fetal bovine serum (FBS) in PBS for 30 min. Sections were then incubated at 4 C overnight  
534 with primary antibodies in 2.5% BSA in PBS ((1:500, monoclonal Armenian hamster anti-  
535 MUC1, Abcam; 1:400, Rhodamine Phalloidin, Cytoskeleton Inc). Following washing, the  
536 sections were incubated for 1 h at 37° C with secondary antibody diluted in 2.5% BSA in PBS  
537 (1:500, Alexa Fluor® 488-conjugated AffiniPure Goat anti-Armenian hamster IgG, Jackson  
538 Immunoresearch; 1:500). Slides were washed and mounted in ProLong Gold Antifade Mountant  
539 with DAPI (Thermo Fischer Scientific).

540  
541

## 542 **Statistical analysis**

543 Statistical analysis was performed and presented using Graphpad Prism version 7.0 software. All  
544 data are presented as means ± standard deviation for ‘n’ experiments unless otherwise specified  
545 in the figure legends. The exact value of ‘n’ for each experiment can be found in the figure  
546 legends. Statistical comparisons of two groups for a single variable with normal distributions  
547 were analyzed by unpaired t test. Statistical comparisons of two or more groups with one  
548 independent variable were analyzed by One-way ANOVA with Tukey post-tests. Statistical  
549 comparisons of two or more groups with two independent variables were analyzed by Two-way  
550 ANOVA with Tukey post-tests. \*p < 0.05 \*\*p < 0.01 \*\*\*p < 0.001 \*\*\*\*p < 0.0001

551  
552  
553

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555

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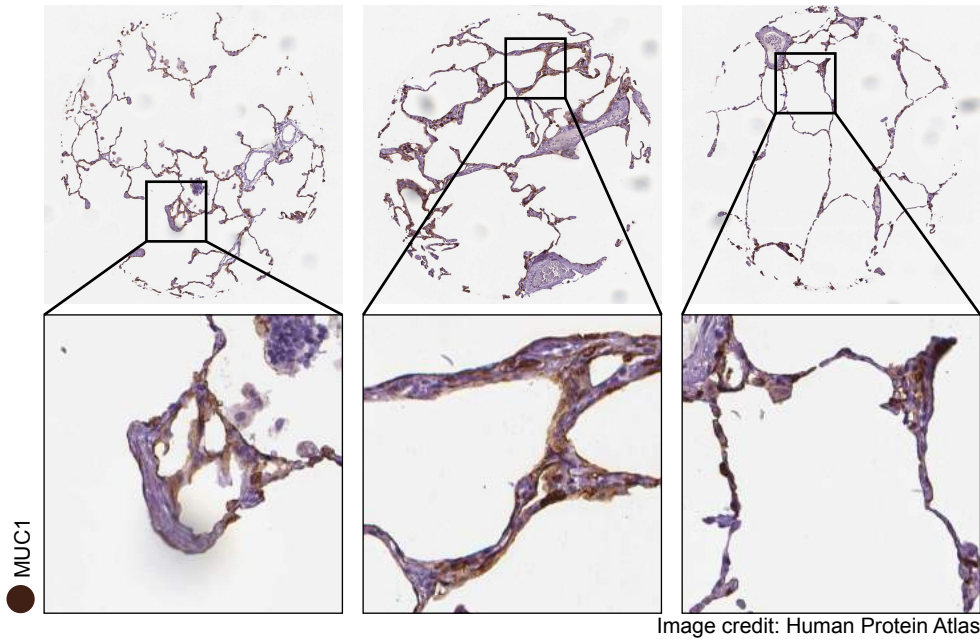
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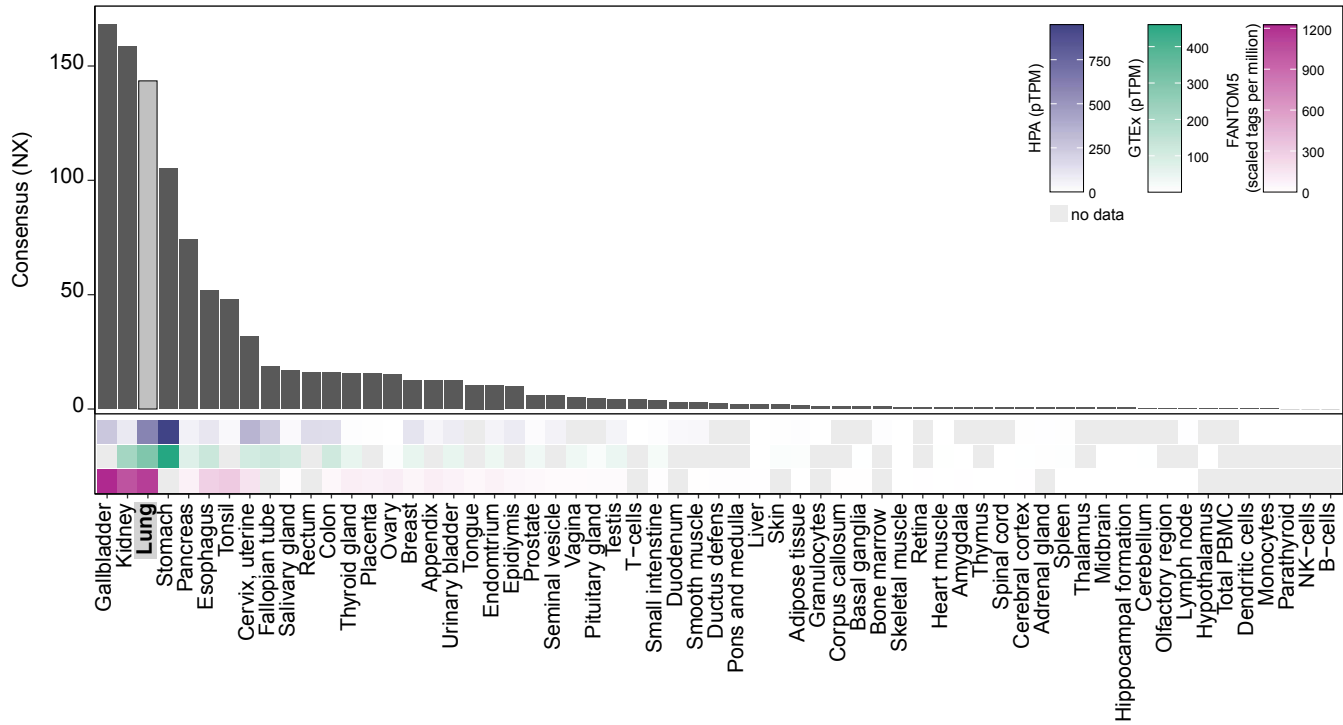
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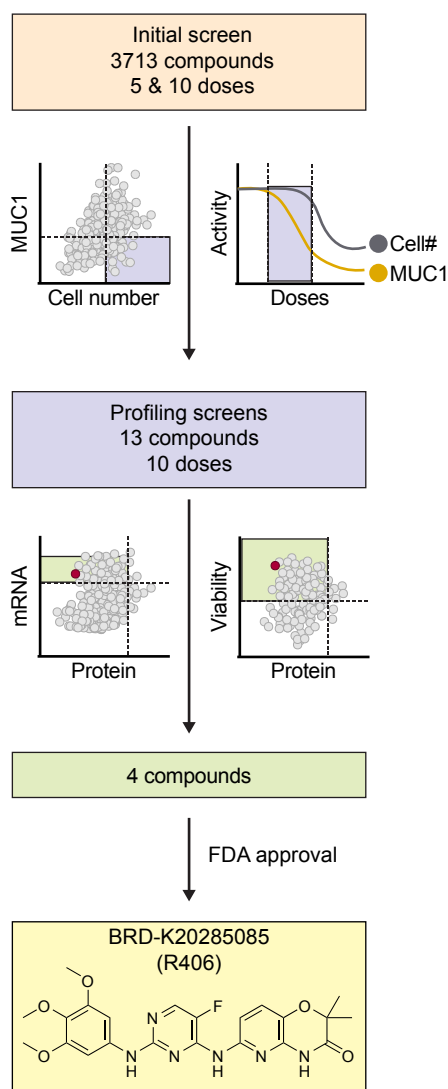
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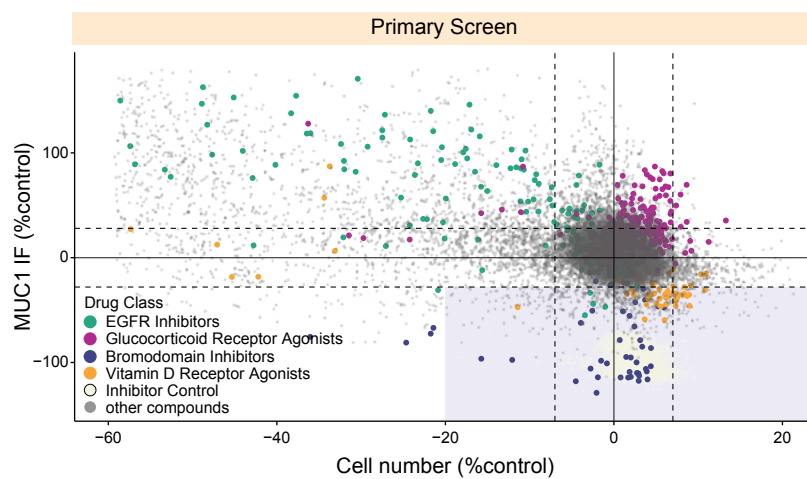
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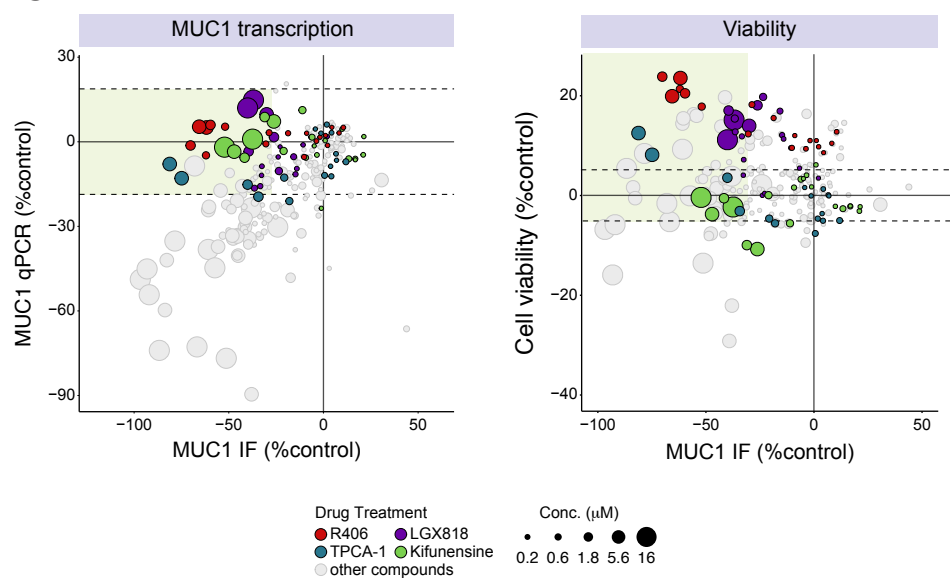
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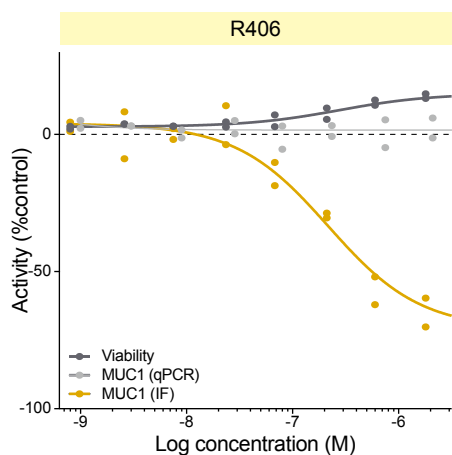
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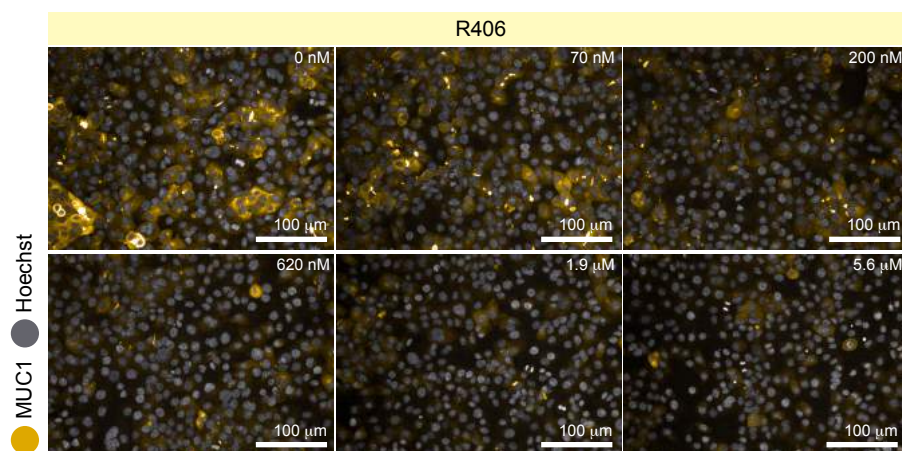
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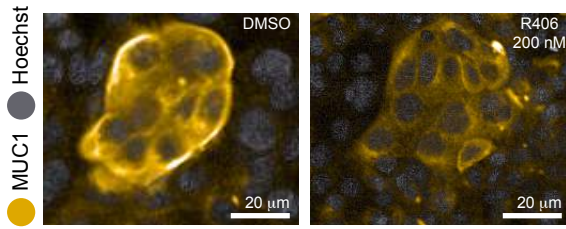
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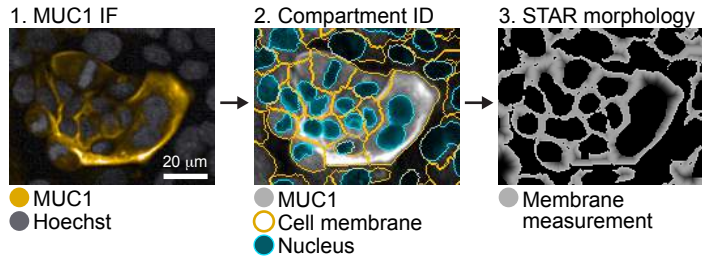
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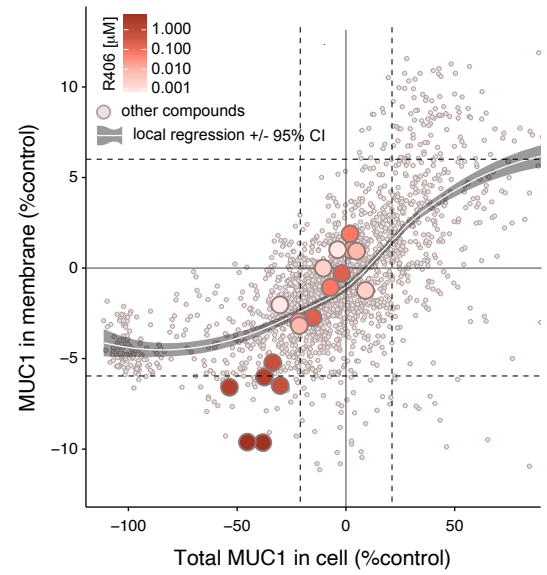
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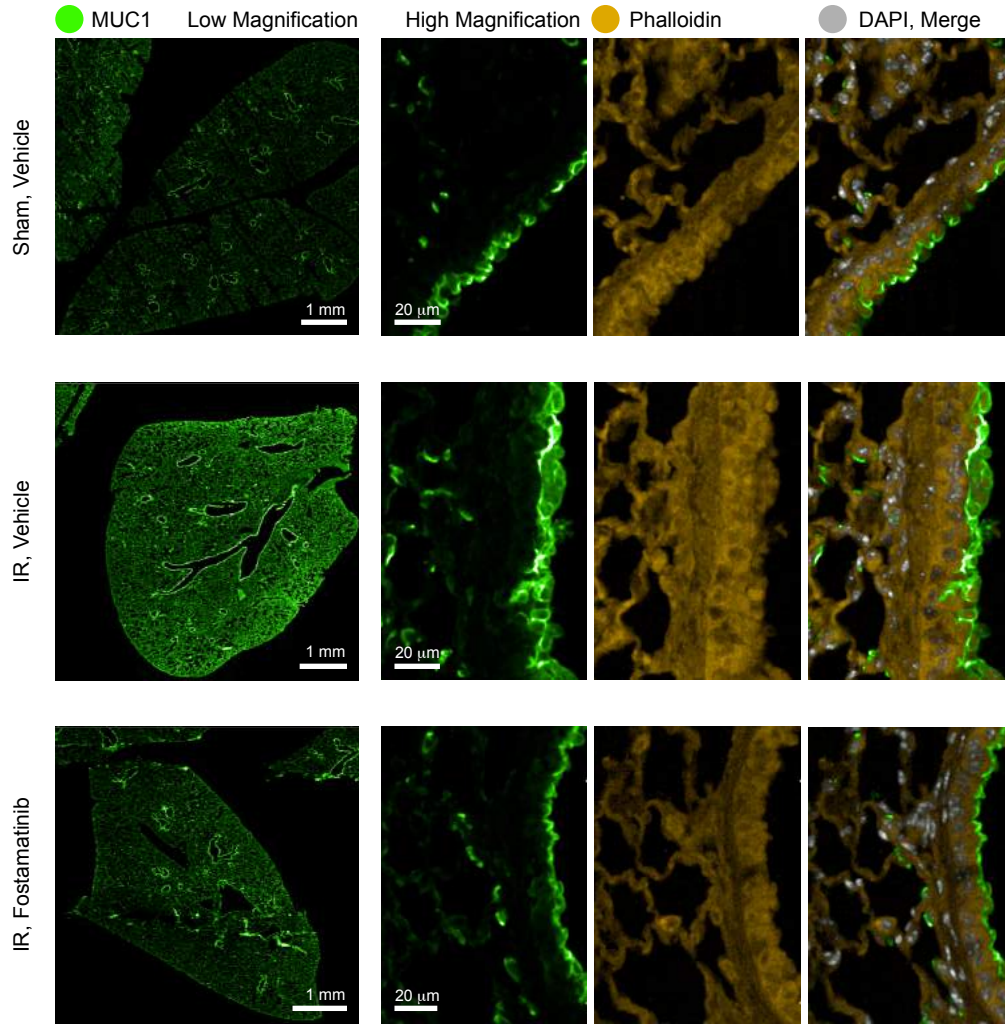
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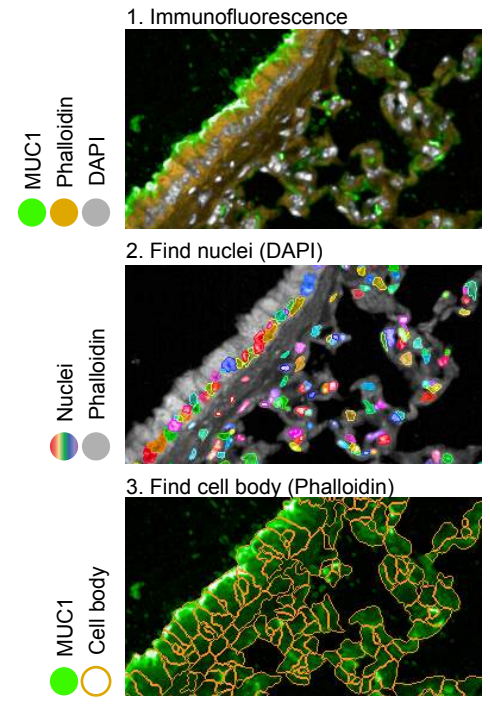
**C**



**A**



**B**



**C**

