

1 RESEARCH ARTICLE

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3 **CX3CR1 Engagement by Respiratory Syncytial Virus Leads to Induction of Nucleolin and**
4 **Dysregulation of Cilia-related Genes**

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41 Running title: RSV CX3C Transcriptomics

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46 **Abstract (250 words)**

47 Respiratory syncytial virus (RSV) contains a conserved CX3C motif on the ectodomain of the G-protein.
48 The motif has been indicated as facilitating attachment of the virus to the host initiating infection via the
49 human CX3CR1 receptor. The natural CX3CR1 ligand, CX3CL1, has been shown to induce signaling
50 pathways resulting in transcriptional changes in the host cells. We hypothesize that binding of RSV to
51 CX3CR1 via CX3C leads to transcriptional changes in host epithelial cells. Using transcriptomic analysis,
52 the effect of CX3CR1 engagement by RSV was investigated. Normal human bronchial epithelial (NHBE)
53 cells were infected with RSV virus containing either wildtype G-protein, or a mutant virus containing a
54 CX4C mutation in the G-protein. RNA sequencing was performed on mock and 4-days-post-infected
55 cultures. NHBE cultures were also treated with purified recombinant wild-type A2 G-protein. Here we
56 report that RSV infection resulted in significant changes in the levels 766 transcripts. Many nuclear
57 associated proteins were upregulated in the WT group, including Nucleolin. Alternatively, cilia-associated
58 genes, including CC2D2A and CFAP221 (PCDP1), were downregulated. The addition of recombinant G-
59 protein to the culture lead to the suppression of cilia-related genes while also inducing Nucleolin.
60 Mutation of the CX3C motif (CX4C) reversed these effects on transcription decreasing nucleolin
61 induction and lessening the suppression of cilia-related transcripts in culture. Furthermore,
62 immunohistochemical staining demonstrated decreases in in ciliated cells and altered morphology.
63 Therefore, it appears that engagement of CX3CR1 leads to induction of genes necessary for RSV entry as
64 well as dysregulation of genes associated with cilia function.

65

66 **Importance (150 words)**

67 Respiratory Syncytial Virus (RSV) has an enormous impact on infants and the elderly including increased
68 fatality rates and potential for causing lifelong lung problems. Humans become infected with RSV
69 through the inhalation of viral particles exhaled from an infected individual. These virus particles contain
70 specific proteins that the virus uses to attach to human ciliated lung epithelial cells, initiating infection.
71 Two viral proteins, G-protein and F-protein, have been shown to bind to human CX3CR1 and Nucleolin,

72 respectively. Here we show that the G-protein induces Nucleolin and suppresses gene transcripts specific
73 to ciliated cells. Furthermore, we show that mutation of the CX3C-motif on the G-protein, CX4C,
74 reverses these transcriptional changes.

75

76 Keywords: RSV, CX3CR1, CX3CL1, Cilia, Interferon, Leukotrienes, Cytokines, Virus, Respiratory

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79 **Introduction**

80 Respiratory Syncytial Virus causes respiratory disease in humans(1). Initial infections occur
81 usually within the first year of life and continually throughout childhood and adulthood(2, 3), (4, 5). RSV
82 infection initiates in the upper airways and can be found in the lower airways during severe disease(6).
83 Symptoms are generally mild, manifesting in stages similar to the common cold (e.g. rhinorrhea, cough,
84 sneezing, fever, wheezing) but can manifest as viral pneumonia during severe disease which occurs
85 mostly in young children and elderly adults (7). The economic impact of RSV in the United States is
86 estimated at over a half a billion dollars and the World Health Organization has listed RSV as a public
87 health priority(8, 9).

88 RSV contains a negative-sense, single-stranded, genome. The RSV genome encodes 11 separate
89 proteins using host translation machinery(1). Two proteins, G and F, facilitate attachment and viral
90 penetration into the host cell(10). The RSV G-protein contains a CX3C motif that has been implicated as
91 an attachment motif of RSV(11-15). The CX3C motif is similar to that found in fractalkine (CX3CL1)
92 and has been shown to bind the host fractalkine receptor, CX3CR1, although perhaps in a manner unique
93 from fractalkine(16). RSV F-protein has been implicated in both attachment and entry into the host
94 epithelial cell. The attachment motif of F is still unknown, but attachment to the host protein via
95 Nucleolin, has been well reported(17).

96 RSV primarily targets host epithelial cells in the lungs(18), although many cell types are
97 infectible(19-21). Multiple host proteins have been indicated in RSV attachment including CX3CR1,
98 Nucleolin, ICAM-1, Heparan sulfate, chondroitin sulfate(22). The physiological location of some these
99 proteins on the apical lung epithelium has not been completely explored, although CX3CR1 have been
100 shown to exist on apical surface of human lung tissue(12, 15). CX3CR1 has been shown as a co-receptor
101 for other viruses, including HIV(23), and variations in the CX3CR1 allele has been shown to increase
102 susceptibility to HIV-1 infection and progression to AIDS(24). Although the exact mechanism behind
103 attachment and fusion of RSV to the host is still being investigated, host CX3CR1 appears to play a role
104 in RSV infection.

105 CX3CR1 engagement by its ligand CX3CL1 (fractalkine) has been shown to regulate cellular
106 transcription via its heterotrimeric G α i protein. Specifically, the binding of CX3CR1 by CX3CL1 has
107 been shown to increase numerous signaling molecules including several different secondary messengers
108 and transcription factors(25). If attachment of RSV to CX3CR1 results in host epithelial cell
109 transcriptional changes is unknown. Here we use primary human differentiated epithelial cell cultures to
110 evaluate the effect of CX3CR1 engagement by RSV.

111

112 **Methods**

113 **Virus Propagation.** Four RSV strains were used for the NHBE infection: A2, A2CX4C mutant with the
114 an alanine A¹⁸⁶ insertion in the CX3C motif (¹⁸²CWAIAC¹⁸⁷) of G protein, recombinant rA2 line 19F
115 (r19F), and r19FCX4C mutant (provided by Dr. M.L. Moore, formerly at Emory University and presently
116 with Meissa Vaccines, Inc., South San Francisco, CA). The prototype A2 strain of RSV (ATCC,
117 Manassas VA) was propagated in HEp-2 cells after serial plaque purification to reduce defective-
118 interfering particles. A stock virus designated as RSV/A2 Lot# 092215 SSM containing approximately
119 5.0×10^8 PFU/mL in sucrose-stabilizing media and stored at -80 °C for inoculation. This stock of virus
120 has been characterized and validated for upper and lower respiratory tract replication in the cotton rat
121 model. The A2CX4C, r19F and r19FCX4C viruses were grown in HEp-2 cells at 0.1 or 0.01 multiplicity
122 of infection (MOI) to reduce defective-interfering particles, purified through a sucrose cushion, and stored
123 at -80 °C. For inoculation, the virus was thawed, diluted to a final concentration with PBS (pH 7.4 w/o
124 Ca²⁺ or Mg²⁺), kept on ice, and used within an hour.

125

126 **NHBE Cells.** Normal human bronchial epithelial cells healthy adult patients were kindly provided by Dr.
127 C.U. Cotton (Case Western Reserve University), expanded, and cultured as described (26). Briefly, cells
128 were plated on a layer of mitomycin C (Sigma) treated 3T3 mouse fibroblast feeder cells and grown until
129 70% confluency in the 3:1 mixture of Ham's F12/DMEM media (HyClone) supplemented with 5% FBS
130 (Sigma), 24 μ g/mL adenine (Sigma), 0.4 μ g/mL hydrocortisone (Sigma), 5 μ g/mL Insulin (Sigma), 10

131 ng/mL EGF (Sigma), 8.4 ng/mL Cholera toxin, and 10 μ M ROCK1 inhibitor Y-2763 (Selleck Chemical
132 LLC). After expansion, cells (passage 3 or 4) were plated on Costar Transwell inserts (Corning Inc.,
133 Corning, NY), grown until confluency, then transferred to air-liquid interface where cells were
134 maintained in 1:1 mixture of Ham's F12/DMEM media supplemented with 2% Ultrosor G (Pall Biosepra,
135 SA, Cergy-Sainte-Christophe, France) for 4 weeks until they were differentiated. Differentiation was
136 confirmed by transepithelial resistance measurements >500 ohms, and flow cytometry staining for FoxJ1
137 (eBioscience) and acetylated α -tubulin (Life Technologies).

138
139 **NHBE Inoculation.** The differentiated NHBE cultures were inoculated with RSV MOI of 0.1 or 0.3 as
140 determined by infectivity titration in HEP-2 cells. The NHBE were washed with PBS and virus in PBS or
141 PBS alone was added to the apical surface of the cells and incubated for 1 h at 37 °C, the virus inoculum
142 aspirated, the apical surface washed with PBS and fresh media added to the basolateral compartment. The
143 RSV-infected NHBE were incubated for 4 days at 37 °C and 5% CO₂, and then cell lysates were collected
144 by washing cells twice with PBS and adding 100ul of RNA lysis buffer (Qiagen) to each well.

145
146 **RNA Isolation.** RNA was extracted, DNase treated, and purified using a Qiagen RNeasy kit (QIAGEN).
147 The RNA was reverse transcribed into cDNA using an iScript cDNA synthesis kit (Bio-Rad) following
148 the manufacturer's instruction.

149
150 **Real-Time PCR.** Semi-quantitative PCR was carried out on a 7500 Fast Real-time PCR system (Applied
151 Biosystems) using Power SYBR Green PCR master mix (Applied Biosystems). The CT values were
152 normalized using control PPIA CT values from the same samples. Top genes were validated using qPCR
153 performed on samples from a separate experiment. Primer sequences: NCL Primer FWD
154 GAACCGACTACGGCTTTCAAT, NCL Primer REV AGCAAAAACATCGCTGATACCA; CFAP221
155 Primer FWD GGGTTGTTTCGCAATCAAGAAGA, CFAP221 Primer REV

156 GTTGAAACCGTTTTTGTGCC; CC2D2A Primer FWD CAAGCAGCGAGGTCCAAAG, CC2D2A
157 Primer REV GGCTCTGTGCCAAATTCAGTC.

158

159 **RNA Sequencing.** Purified RNA was sequenced using the HiSeq 2500. Gene with low reads counts were
160 filtered as previously described(27). Filtered reads were aligned to the GRCh38 reference genome. The
161 RSV A2 virus genome (GenBank: KT992094.1) was used for virus transcript alignment. Gene counts
162 were normalized and log transformed (R 3.4.4, rLog package). Multiple comparisons were corrected
163 using the Benjamini-Hochberg method (R 3.4.4, “p.adjust()” command).

164

165 **Statistical Models**

166 Significant association between transcript levels and condition, both marginal association (mock infection
167 vs RSV infection) and viral-transcript-level adjusted models (wildtype and CX4C mutant), was
168 determined by linear regression (R 3.4.4. *lm* function). The wildtype and CX4C mutant comparisons were
169 adjusted in the statistical model by using the eigenvalue for each sample derived using principal
170 component analysis (FractalMineR) of 11 RSV gene transcript levels. Resulting p-values were adjusted
171 for multiple testing using Benjamini-Hochberg method (R 3.4.4. *p.adjust* function). P values less than
172 0.05 was considered significant.

173

174 **Pathway Analysis**

175 Gene symbols with unadjusted p-values of less than 0.05 was used for pathway analysis. Pathway
176 analysis of gene sets were performed using ToppGene functional analysis software(28) and genes were
177 referenced against the Gene Ontology (GO) Biological Process GO Term.

178

179 **Results**

180 **Transcriptional Changes after RSV Infection**

181 The transcriptional profile of normal human bronchial epithelial cells after infection with RSV
182 was determined. Infection by RSV both increased and decreased gene transcripts relative to mock
183 infected (Table 1, Supplemental Document 1). RSV infection significantly increased 558 gene transcripts.
184 Infection resulted in the induction of transcripts encoding antiviral enzymes (OAS1, OAS2, CMPK2,
185 HERC5, HERC6), antiviral peptides (RSAD2), interferon response genes (RIG-I, STAT1, STAT2, MX1,
186 IFIT1, IFIT2, IFI44L, IRF1, OASL), mucin genes (MUC5AC, MUC5B, MUC15, MUC21, MUC13), and
187 stat inhibitor genes (SOCS1, SOCS53), chemokines/cytokines (CX3CL1, CCL5, CXCL9, CXCL8,
188 CXCL10, TGFA), interleukins and their receptors (IL1A, IL15, IL15R IL22RA1, IL2RG, IL18R1),
189 Major Histocompatibility Complex genes (HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-DMA, HLA-
190 DRB1), Type-II helper T cell associated genes (PSENEN), toll-like receptors (TLR2), and innate immune
191 cell differentiation/survival genes (BATF2, JAGN1). Both pro-apoptotic (CASP1, CASP4, CASP7,
192 CASP8) and anti-apoptotic (Birc3, BCL2L13, BCL2L14, BCL2L15) transcripts were increased during
193 infection. The 2'-O-ribose cap methyltransferase (CMTR1), a protein required for viral RNA cap
194 snatching and shown to decrease interferon type I, was also increased after infection. Taken together,
195 these results demonstrate many genes were upregulated in bronchial epithelial cells in response to RSV.

196 Pathway analysis of significantly upregulated genes (Mock vs RSV) demonstrated 1033
197 significantly enriched biological processes (Figure 1A, Supplemental Document 2). The most
198 significantly enriched biological processes included defense processes, responses to foreign objects,
199 innate immune responses, and cytokine-associated responses. Innate immune responses were also found
200 to be enriched. Taken together, we found that cellular defense and immune system biological processes
201 were associated with increased viral transcript after RSV infection.

202 RSV infection also resulted in the reduction of 208 transcripts relative to mock infection with
203 many transcripts associated with cell morphology (Table 1, Supplemental Document 1). This included
204 microtubule-associated protein transcript levels (MAP6, MAPT, MAST1) and myosin transcript levels

205 (MYO6, MYO9A, MYO3A, MYO5A) were decreased after infection. We found many cilia associated
206 genes (CC2D2A, PCDP1), lysosomal genes (LAMP1), leukotriene B4 hydrolase (LTA4H), WNT
207 signaling transcripts (MCC, WNT2B), and RNA helicases (DHX40) to also be decreased during
208 infection. Interestingly, the heparan sulfate sulfotransferase (HS3ST4) was among one of the most
209 significantly associated down-regulated transcripts.

210 Pathway analysis of differentially expressed down-regulated genes identified 110 enriched
211 biological processes (Figure 1B, Supplemental Document 3). Down-regulated genes were associated with
212 biological process related to cellular morphology, including cell projection. Microtubule and cellular
213 transplant related biological processes were also enriched for down-regulated genes including
214 microtubule bundle formation, cytoskeletal transport, and protein localization.

215

216 **CX3C Specific Transcriptional Changes**

217 Infection with an A2 RSV virus containing a mutation in the CX3C motif (CX4C) resulted in a
218 number of significant transcriptional changes relative to the wildtype virus (Table 2, Supplemental
219 document 2). Five host transcripts were significantly decreased after mutant virus infection compared to
220 wild-type virus infections. NCL (Nucleolin), the eukaryotic nucleolar phosphoprotein, was the most
221 significantly decreased gene in the mutant virus infection compared to wildtype. The adenylate kinase,
222 AK2, an apoptotic activator; the Golgi homeostasis protein, TMEM199, recently identified to be essential
223 in influenza A virus infection; and the transcriptional coactivator (SNW1) which can bind the vitamin D
224 receptor binding domain and retinoid receptors to enhance many gene expression pathways were also
225 decreased after infection with the mutated compared to the wildtype virus.

226 There were 354 biological processes enriched for gene transcripts that were decreased in the
227 mutant CX4C virus compared to mock infection (Figure 2A, Supplemental Document 5). Biological
228 processes enriched for decreased genes included response to cytokines, response to type I interferon, and
229 RNA processing. Innate immune response biological processes including Nucleolin and many virus-

230 signaling genes (e.g. BCL6, BATF and IRF2) were also decreased after mutant compared to wildtype
231 infection.

232 After multiple comparison correction, two gene transcripts were significantly increased after
233 mutant CX4C RSV infection compared to wildtype virus (Table 2, Supplemental Document 4). The most
234 significantly associated transcript, C2orf81, has no known function and the other transcript, CC2D2A,
235 encodes a protein that forms a complex localized at the transition zone of primary cilia.

236 The 1067 transcripts increased (uncorrected p-value < 0.05) after mutant virus infection relative
237 to wildtype infection resulted in 78 biological pathways that were significantly enriched (Figure 2B,
238 Supplemental Doc 6). The biological processes were all associated with cilia and microtubule function
239 and formation. These included transport, organization, and assembly. The genes associated with these
240 pathways included many cilia-related genes (CC2D2A, CFAP221, CFAP43, CFAP52). Dynein-related
241 genes (DNAH3, DNAH6, DNAH9, DNAH10, DNAH12) were also associated with enriched pathways.
242 Taken together, mutating the CX3C domain resulted in a significant increase in cilia-related genes and
243 biological processes.

244

245 **Transcriptional Changes with CX3CR1 Ligand**

246 Recombinant G-protein soluble ligand representing the wildtype virus resulted in significant
247 changes in transcript levels (Figure 3). Cilia-related genes (CC2D2A, CFAP221) were both significantly
248 decreased after addition of G-protein ligand compared to mock treated cultures. Alternatively, Nucleolin
249 was increased after treatment with G-protein ligand, although the relative fold-change was minor. Taken
250 together, soluble G-protein effected transcript levels similarly to the differences seen between wildtype
251 and CX4C mutant infections.

252

253 **Ciliated Cells after RSV Infection**

254 Immunohistochemistry of mock or RSV infected cells was performed in order to further
255 understand the effect of RSV infection on ciliated cells (Figure 4A). Ciliated cells were found in both

256 infected and non-infected cultures. Epithelial cells infected with wildtype RSV showed a significant
257 decrease in the number of ciliated cells compared to mock infection (Figure 4B). Taken together, both
258 mock and infected cells contained ciliated cells, but RSV infection decreased the percentage of ciliated
259 cells relative to mock infection.

260

261 **Discussion**

262 Respiratory epithelial cells act as both the primary target for RSV and the primary defense against
263 the virus. Therefore, it is unsurprising that so many genes and biological processes were altered during
264 RSV infection. For successful replication the virus must first attach to the epithelium, enter the cell,
265 dispense genetic material, take over host translation machinery, and use host nutrients create new
266 genomes and protein, assemble, and release from the cell(1). During this process, the host epithelium
267 recognizes the foreign object, produces anti-viral peptides, and signals to neighboring cells (including
268 immune cells) of the infection(29). Therefore, the virus must both avoid host responses and hijack cellular
269 processes in order to successfully reproduce.

270 Here we demonstrate that these underlying biological processes occurring during virus infection
271 of adult primary epithelial cells grown at a physiological air-liquid interface. Given our experimental
272 design, it is impossible to distinguish the transcriptional effects due to the host reaction to the virus and
273 those caused directly by the virus. Both viral proteins and viral genetic material can interfere with
274 biological processes by directly acting on host cellular machinery resulting in cellular signaling cascades
275 that lead to changes in transcriptional regulation. It is of no surprise that these changes have to do with
276 mechanistic ciliary function that removes foreign objects from the respiratory tract and the induction of
277 Nucleolin, an RSV F-protein receptor(17), which would presumably provide a fitness advantage for the
278 virus.

279 These introductory studies demonstrate a unique relationship between two host genes, CX3CR1
280 and Nucleolin, and two virus genes, G-protein and F-protein. These studies suggest that CX3CR1
281 engagement by the RSV G-protein CX3C motif results in intercellular signaling impacting Nucleolin

282 expression. Although our experimental design did not allow us to discern any specifics in the signaling
283 cascade, manipulating the cytokine pathway in order to provoke Nucleolin production would likely prove
284 advantageous for the virus. Furthermore, these findings are consistent with the other viruses that
285 manipulate host transcription in order to increase host proteins necessary to the virus(30).

286 Disruption of ciliary function has been described for many pathogens, although no morphology
287 changes were seen after 24hrs post infection(31). A primary function of host immunity is the physical
288 removal of foreign objects, including dead/infected cells, during an infection. It is important to note that
289 the decrease in ciliated cells may be due to instability of the cilia and may have been disrupted during
290 processing of the cultures. Regardless, it is clear that cilia, or the cells that express cilia, are affected by
291 RSV infection. Given the role of cilia in the removal of foreign bodies, future studies will be needed to
292 further characterize cilia function after RSV infection.

293 The CX4C mutant virus does not replicate at the level of the wildtype virus. Therefore, our
294 statistical analysis required correction for variable levels of viral transcript. Although mathematical
295 correction for viral transcripts levels using linear modeling have been continually shown to be accurate
296 for these types of studies, it is not possible to completely distinguish Nucleolin induction or cilia
297 dysregulation between wildtype and mutant based on CX3CR1 engagement from virus replication
298 differences.

299 Furthermore, what effect disruption of the CX3CL1/CX3CR1 axis is having is unknown. It is
300 entirely possible that the effect we see here is due to interference with autocrine signaling of CX3CL1
301 on CX3CR1. Crystal structures(32) suggest that engagement of CX3CR1 by RSV G-protein may be
302 fundamentally different than fractalkine. Our studies cannot distinguish blocking of CX3CL1 binding
303 from receptor activation. Moreover, our results do not rule out a general immune response to viral
304 proteins. Future studies will be needed to establish the mechanism by which the G protein binding to
305 CX3CR1 increases Nucleolin and decreases cilia-related transcripts.

306 It is important to note that these studies involved only a single strain of the A-subtype (A2).
307 Given the large differences between subtype A and subtype B G-protein genes, and the fact that G protein

308 has undergone significant genetic changes since the isolation of A2, future studies will need to determine
309 if these transcriptional changes are consistent with RSV strains that have circulated recently and subtypes.

310 Taken together, RSV infection changes host gene expression and CX3CR1 appears to play a role
311 in facilitating these changes. Our results suggest that RSV disrupts defense mechanisms while also
312 increasing the expression of pro-viral proteins.

313

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317

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414

415 **Table 1. Mock vs Wildtype RSV Infection Differentially Expressed Genes**

Relative to Mock	Gene Symbol	Gene Description	NCBI_GeneID	<i>B</i>	<i>P</i> value
Up	BATF2	Basic Leucine Zipper ATF-Like Transcription Factor 2	116071	-2.219807223	0.00000841
Up	STAT1	Signal transducer and activator of transcription 1	6772	-1.932894088	0.00000841
Up	MX1	MX dynamin like GTPase 1	4599	-3.026455262	0.00000841
Up	TRIM14	Tripartite motif containing 14	9830	-1.373708618	0.00000887
Up	MX2	MX dynamin like GTPase 2	4600	-3.352521625	0.00000887
Up	OASL	2'-5'-oligoadenylate synthetase like	8638	-3.527345499	0.00000887
Up	HSH2D	Hematopoietic SH2 domain containing	84941	-1.924851569	0.00000887
Up	IFI27	Interferon alpha inducible protein 27	3429	-2.936030535	0.00000887
Up	HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6	55008	-2.27262453	0.00000887
Up	HEL2Z	Helicase with zinc finger 2	85441	-2.022967637	0.00000887
Down	MTURN	maturin, neural progenitor differentiation regulator homolog	222166	0.663532629	0.000362109
Down	EOGT	EGF domain specific O-linked N-acetylglucosamine transferase	285203	0.199675192	0.001184083
Down	GXYLT2	Glucoside xylosyltransferase 2	727936	0.494404491	0.001387687
Down	MYLK	Myosin light chain kinase	4638	0.637636967	0.001431977
Down	DHX40	DEAH-box helicase 40	79665	0.31391556	0.001549972
Down	RP11-410E4.1	lincRNA	-	0.139630264	0.001792911
Down	ACO1	Aconitase 1	48	1.139317636	0.001839785
Down	GALNT11	Polypeptide N-acetylgalactosaminyltransferase 11	63917	0.273330797	0.002265838
Down	PDE5A	Phosphodiesterase 5A	8654	0.743763137	0.00297151
Down	HS3ST4	HS3ST4 – heparan sulfate-glucosamine 3-sulfotransferase 4	9951	0.225923605	0.003275347

416

417 **Table 2. Wildtype vs CX4C-Mutant Differentially Expressed Genes**

Relative to WT	Gene Symbol	Gene Description	NCBI_GeneID	<i>B</i>	<i>P</i> value	<i>P</i> value (BH)
Down	NCL	Nucleolin	4691	-0.1118883	5.84E-06	0.032346124
Down	AK2	Adenylate kinase 2	204	-0.1604704	7.28E-06	0.032346124
Down	TMEM199	Transmembrane protein 199	147007	-0.1412261	8.51E-06	0.032346124
Down	SNW1	SNW domain containing 1	22938	-0.1587004	1.13E-05	0.035780973
Down	CDKAL1	CDK5 regulatory subunit associated protein 1 like 1	54901	-0.2689998	1.81E-05	0.049244299
Down	GPKOW	G-patch domain and KOW motifs	27238	-0.2037808	3.54E-05	0.070297745
Down	TRAFD1	TRAF-type zinc finger domain containing 1	10906	-0.1964691	3.76E-05	0.070297745
Down	PELI3	Pellino E3 ubiquitin protein ligase family member 3	246330	-0.1745717	3.77E-05	0.070297745
Down	CALCOCO2	Calcium binding and coiled-coil domain 2	10241	-0.2155026	4.07E-05	0.070297745
Down	ZMYND8	Zinc finger MYND-type containing 8	23613	-0.2766429	4.65E-05	0.073595815
Up	C2orf81	Chromosome 2 open reading frame 81	388963	0.34626864	7.19E-06	0.032346124
Up	CC2D2A	Coiled-coil and C2 domain containing 2A	57545	0.59449472	7.39E-06	0.032346124
Up	CFAP221	Cilia and flagella associated protein 221	200373	0.77283464	6.46E-05	0.076430535
Up	CERKL	Ceramide kinase like	375298	0.52666645	7.28E-05	0.076780874
Up	BBOF1	Basal body orientation factor 1	80127	0.48543271	0.00016933	0.104057284
Up	PPIL6	Peptidylprolyl isomerase like 6	285755	0.73103569	0.0001922	0.104057284
Up	CCDC60	Coiled-coil domain containing 60	160777	0.33676074	0.00020953	0.104057284
Up	AKAP14	A-kinase anchoring protein 14	158798	0.78099765	0.00021646	0.104057284
Up	PSENE1	Presenilin enhancer, gamma-secretase subunit	55851	0.24667032	0.00023446	0.104057284
Up	DNAH6	Dynein axonemal heavy chain 6	1768	0.5313297	0.00023723	0.104057284

418

419

420 **Figure 1. Changes in Biological Processes After RSV Infected.** (A) Top ten biological processes, and
 421 associated genes, significantly enriched in transcripts increased after RSV infection. (B) Top ten
 422 biological processes, and associated genes, significantly enriched in transcript decreased after RSV
 423 infection. Colors represent the biological processes distinguished in the legend.

424

425 **Figure 2. Changes in Biological Processes between wild-type and mutant RSV Infection.** (A) Top ten
426 biological processes, and associated genes, significantly enriched in transcripts increased after wild-type
427 RSV infection compared to mutant (CX4C) infection. (B) Top ten biological processes, and associated
428 genes, significantly enriched in transcript decreased after wild-type RSV infection compared to mutant
429 (CX4C) infection. Colors represent the biological processes distinguished in the legend.

430

431 **Figure 3. Effect of G-protein on Epithelial Cell Transcripts.** Transcript levels of expression after
432 treatment of epithelial cell cultures with recombinant G-protein for (A) CC2D2A, (B) CFAP331, (C) and
433 Nucleolin.

434

435 **Figure 4. Ciliated Cells after RSV Infection.** Quantification of the number of ciliated cells staining
436 positive for acetylated tubulin per nucleus (dapi) in mock or RSV infected cultures.

437

Mock \updownarrow Infection

Mock \updownarrow Infection

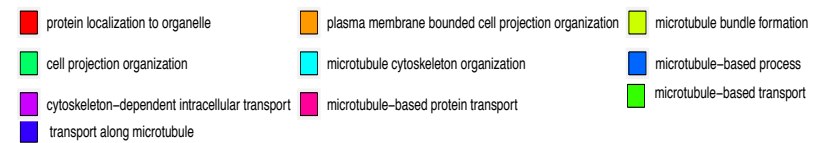
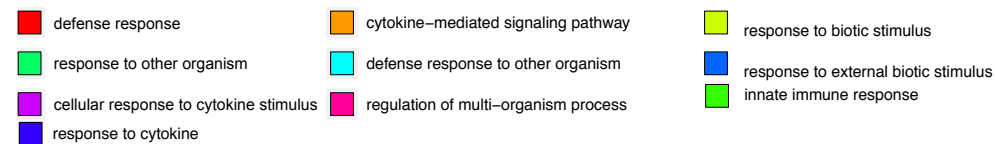
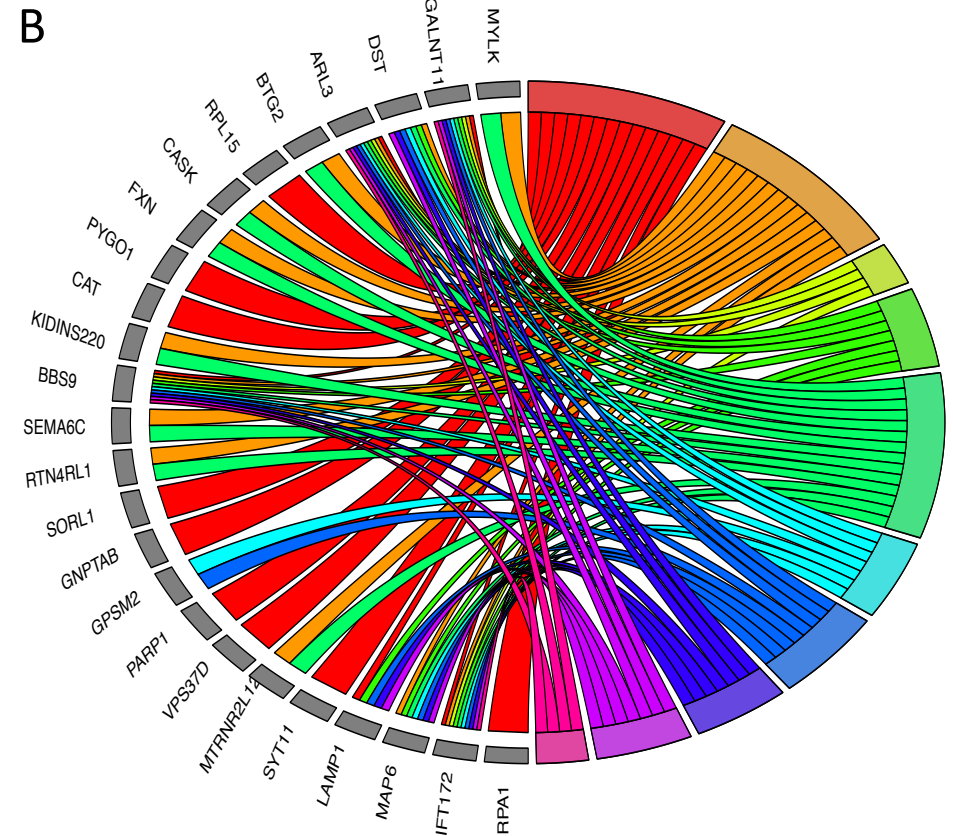
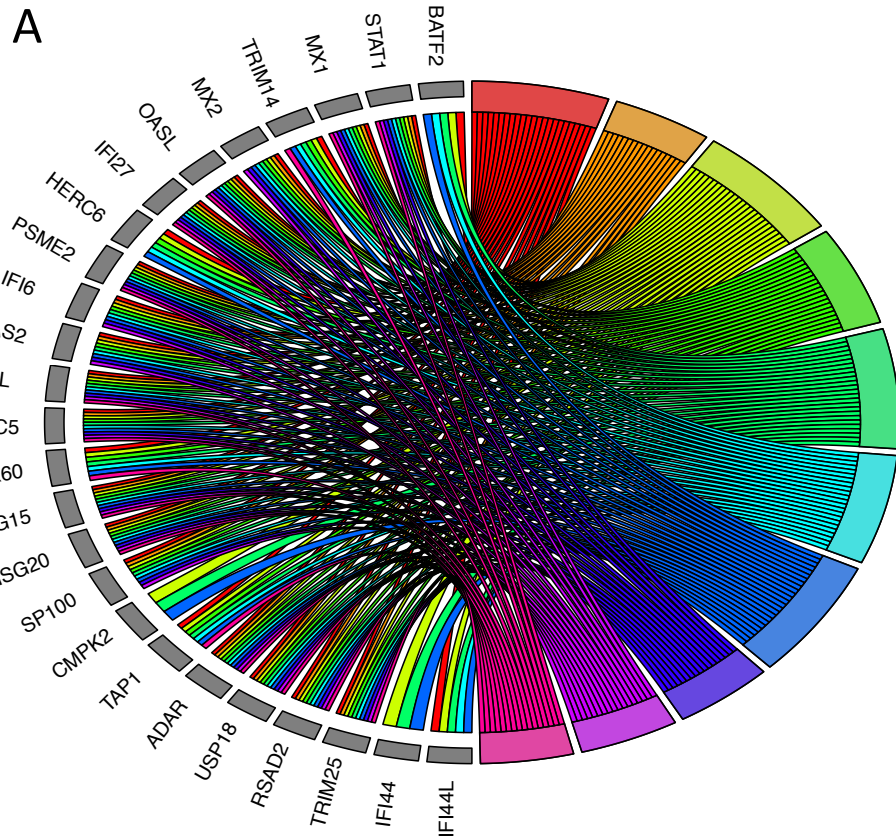
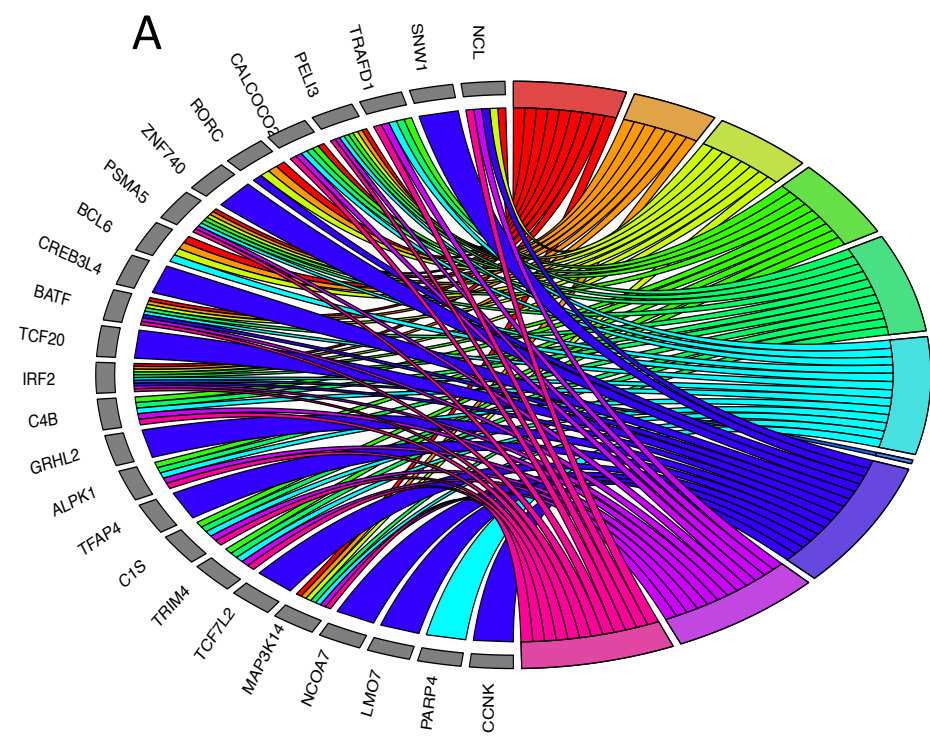


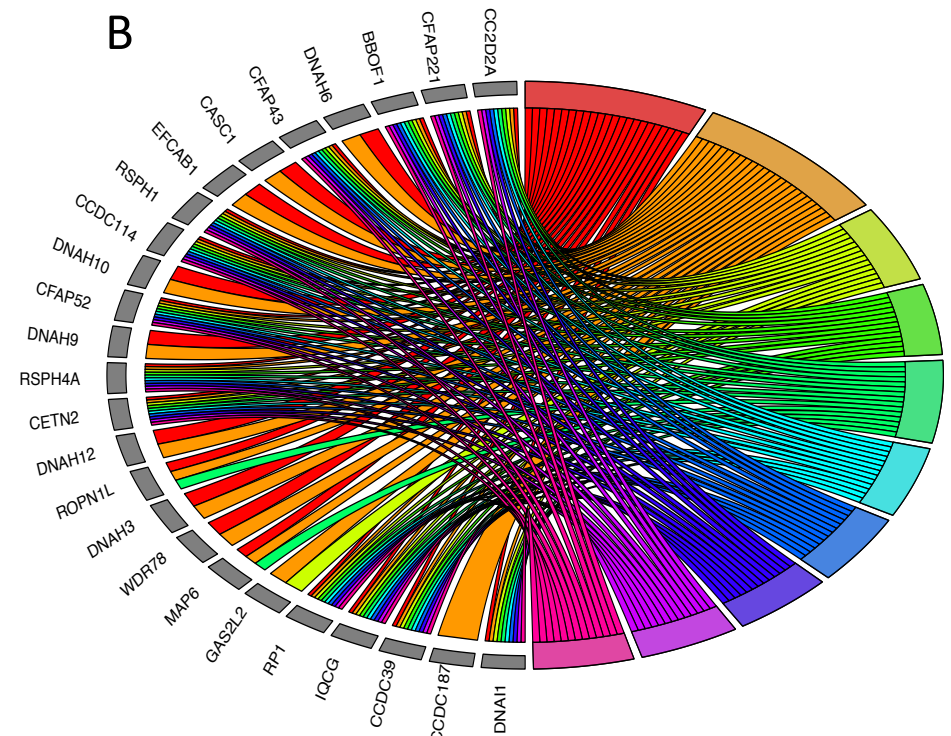
Figure 1

WT ↑ ↓ MUT

WT ↓ ↑ MUT



- response to cytokine
- cytokine-mediated signaling pathway
- cellular response to cytokine stimulus
- innate immune response
- defense response to other organism
- defense response
- response to type I interferon
- positive regulation of RNA metabolic process
- response to biotic stimulus
- response to other organism



- microtubule-based movement
- microtubule-based process
- microtubule bundle formation
- cilium organization
- microtubule-based transport
- microtubule-based protein transport
- protein transport along microtubule
- intraciliary transport
- ciliary transition zone assembly
- intraciliary transport involved in cilium assembly

Figure 2

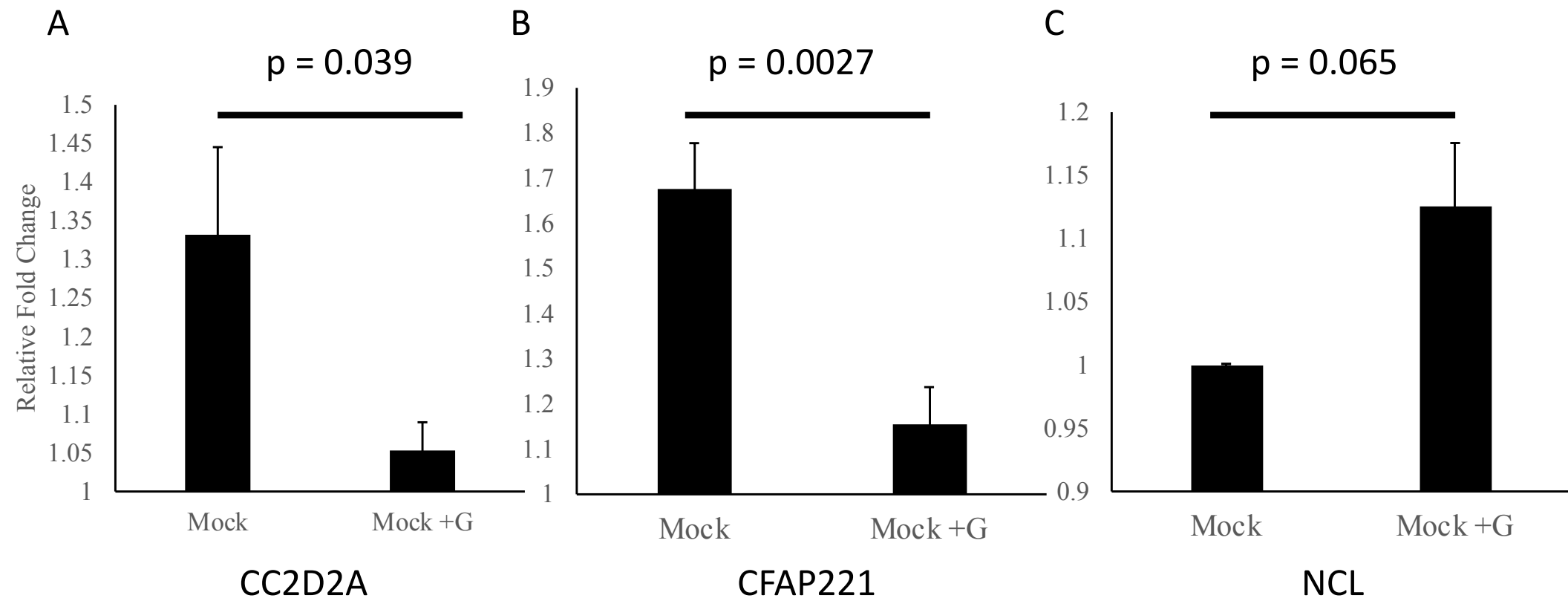


Figure 3

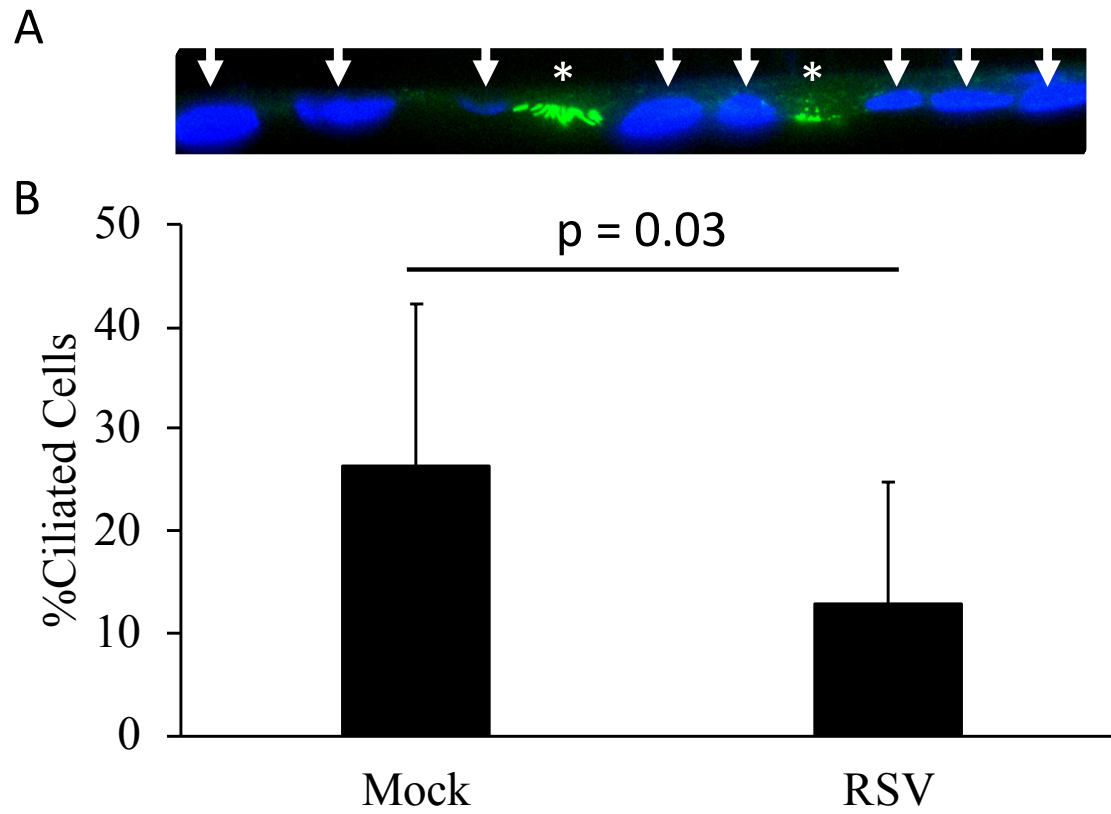


Figure 4