

## Mucin 4 Protects Female Mice from Coronavirus Pathogenesis

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Running Title: *Muc4* Protects Female Mice from CoV Pathogenesis.

Keywords: coronavirus, SARS-CoV, zoonotic, SARS-CoV-2, emergence, 2019-nCoV

## Abstract

Using incipient lines of the Collaborative Cross (CC), a murine genetic reference population, we previously identified a quantitative trait loci (QTL) associated with low SARS-CoV titer. In this study, we integrated sequence information and RNA expression of genes within the QTL to identify mucin 4 (*Muc4*) as a high priority candidate for controlling SARS-CoV titer in the lung. To test this hypothesis, we infected *Muc4*<sup>-/-</sup> mice and found that female, but not male, *Muc4*<sup>-/-</sup> mice developed more weight loss and disease following infection with SARS-CoV. Female *Muc4*<sup>-/-</sup> mice also had more difficulty breathing despite reduced lung pathology; however, no change in viral titers was observed. Comparing across viral families, studies with chikungunya virus, a mosquito-borne arthralgic virus, suggests that *Muc4*'s impact on viral pathogenesis may be widespread. Although not confirming the original titer QTL, our data identifies a role for *Muc4* in the SARS-CoV disease and viral pathogenesis.

## Importance

Given the recent emergence of SARS-CoV-2, this work suggest that *Muc4* expression plays a protective role in female mice not conserved in male mice following SARS-CoV infection. With the SARS-CoV-2 outbreak continuing, treatments that modulate or enhance *Muc4* activity may provide an avenue for treatment and improved outcomes. In addition, the work highlights the importance of studying host factors including host genetics and biological sex as key parameters influencing infection and disease outcomes.

## 1 **Introduction**

2           Most viral infections present with an array of symptoms following human infection,  
3 ranging from asymptomatic to self-limiting, chronic, and sometimes fatal disease. Some of this  
4 diversity is mediated by age, sex, and other comorbidities, but these demographics do not  
5 account for all of the variability in disease outcomes. Genome-wide association studies (GWAS)  
6 and candidate gene studies have established a number of genes associated with human  
7 susceptibility to infectious diseases, but are limited by the large populations required to detect  
8 genetic effects and by existing knowledge gaps (Fellay et al., 2007; Ge et al., 2009; Lindesmith  
9 et al., 2003; López et al., 2010). Furthermore, during outbreak settings there is often limited  
10 access to clinical samples from infected humans. Thus, host susceptibility allele identification is  
11 oftentimes heavily compromised by situational expediency and new approaches are needed to  
12 identify alleles that regulate emerging virus pathogenesis.

13           Severe acute respiratory syndrome virus (SARS-CoV) is a respiratory pathogen that first  
14 emerged in the Guangdong Province of China in November 2002, and rapidly spread to 28  
15 countries, resulting in over 8,000 cases with a 10% case fatality ratio. The subsequent  
16 emergence of Middle East respiratory syndrome coronavirus (MERS-CoV) and the discovery of  
17 several other related “pre-epidemic” bat coronaviruses that seem poised for sudden emergence  
18 emphasize the need for a comprehensive understanding of how highly pathogenic human  
19 coronaviruses, such as SARS-CoV, interact with their hosts to regulate disease severity  
20 (Coleman and Frieman, 2013; Menachery et al., 2015b; Menachery et al., 2016). The  
21 importance of these early predictions was demonstrated by the ongoing outbreak of SARS-CoV-  
22 2 (Gorbalenya et al., 2020; Gralinski and Menachery, 2020), which has caused 45,171  
23 confirmed cases and 1,115 deaths as of February 12, 2020 (World Health Organization, 2020).

24           We have developed a model of CoV infection in the genetically diverse Collaborative  
25 Cross (CC) population (Churchill et al., 2004; Collaborative Cross Consortium, 2012). This  
26 model allows us to assess the impact of host genetic variation on CoV disease, such as in the

27 context of SARS-CoV pathogenesis (Gralinski et al., 2015). The CC panel contains mouse lines  
28 derived from a funnel breeding scheme of eight founder strains, including 5 traditional lab  
29 strains and 3 wild-derived strains (Churchill et al., 2004; Collaborative Cross Consortium, 2012;  
30 UNC Systems Genetics Core Facility, 2012). This population takes advantage of naturally-  
31 occurring genetic polymorphisms to capture approximately 90% of the common genetic diversity  
32 within *Mus musculus*, and that diversity is distributed evenly throughout the genome, with  
33 elevated minor allele frequencies compared to standard natural populations (Aylor et al., 2011;  
34 Yang et al., 2007; Yang et al., 2009; Yang et al., 2011). Our initial screen within an incompletely  
35 inbred set of CC mice (the preCC screen) (Gralinski et al., 2015), along with an F2 screen  
36 utilizing a highly resistant and highly susceptible CC mouse line (Gralinski et al., 2017),  
37 identified nine quantitative trait loci (QTLs) impacting SARS-CoV disease. Among these QTLs  
38 was locus *HrS2* on chromosome 16, which was identified as modulating SARS-CoV titer levels  
39 in the lung (Gralinski et al., 2015).

40 Here, we narrow the *HrS2* locus to a priority candidate gene, mucin 4 (*Muc4*), via the  
41 integration of sequence data from the CC founders (Keane et al., 2011) and RNA expression  
42 results from our preCC experiment. Low expression of *Muc4* in mice containing the PWK/PhJ  
43 allele corresponded with the low SARS-CoV titer associated with the QTL located at locus *HrS2*.  
44 We subsequently examined infection of mice lacking *Muc4*, predicting reduced viral load.  
45 However, our findings suggest that while *Muc4* does not regulate SARS-CoV titer, it does have  
46 broad activity attenuating the pathogenic impact of viral infections.

## 47 **Results**

### 48 Selection of *Muc4* as a SARS-CoV disease associated gene

49 In earlier studies, incipient CC lines were used to identify four QTLs associated with  
50 SARS-CoV phenotypes in female mice, one of which was mapped on the basis viral titer in the  
51 lung at four DPI (Gralinski et al., 2015). A subsequent study utilizing both male and female F2

52 mice derived from a highly susceptible and highly resistant CC mouse line identified five more  
53 SARS-CoV-associated QTLs, three of which were mapped at least partially on the basis of viral  
54 titer (Gralinski et al., 2017). Located on chromosome 16 from nucleotides 31,583,769-  
55 36,719,997, the pre-CC QTL accounted for 22% of the variation in viral titer, which ranged from  
56 over  $10^8$  PFU/lobe to below the limit of detection ( $10^2$  PFU/lobe). The F2 QTLs, by comparison,  
57 were located on chromosomes 18, 7, and 12 and explained 12.9%, 12.3%, and 5.4%,  
58 respectively, of the observed variation in titers from high  $10^2$  to low  $10^7$  PFU/lobe. Thus, while  
59 SARS-CoV titer in the lung is clearly influenced by multiple host genetic factors, the preCC QTL  
60 on chromosome 16 was chosen for candidate gene selection and validation. Analysis of allele  
61 effects revealed that the founder PWK/PhJ was the main driver of low SARS-CoV titer in the  
62 lung (Fig. 1A), and PWK/PhJ also had lower titers than any other founder strain at four DPI  
63 (Gralinski et al., 2015). We therefore used private SNPs or In/Dels found in PWK/PhJ to reduce  
64 the potential targets in the QTL to seven ncRNAs and 74 genes for downstream analysis.

65 To prioritize the list of ncRNAs and genes within the QTL, the relationship between RNA  
66 transcript levels and viral titer in the lung was examined. Sixty seven preCC mice had both  
67 microarray and titer data available, of which sixty had titers above the limit of detection  
68 (Gralinski et al., 2015). Of the 74 candidate genes, 54 had one or more probes on the  
69 microarray (Table S4) and *Muc4* expression had the strongest correlation with viral titer, with  
70 five of the six *Muc4* probes yielding positive correlations of 0.34-0.45 (Fig. 1B, Table S5). Using  
71 infection data from the founder mice, *Muc4* RNA expression levels mimicked the allelic effects  
72 of the QTL with PWK/PhJ mice exhibiting low *Muc4* RNA levels (Fig. 1C). Other potential  
73 targets included *Lrrc33*, *Sec22a*, *Parp14*, and *Ildr1*; however, these genes either have little  
74 known linkage to viral replication and the immune response (e.g., *Lrrc33*, *Sec22a*, and *Ildr1*) or  
75 the directionality of the correlation did not support their relationship to viral titer in the lung  
76 (*Parp14*). For *Muc4*, high levels of expression at mucosal surfaces and in various human

77 cancers have been reported (Andrianifahanana et al., 2001; Chaturvedi et al., 2008; Kamikawa  
78 et al., 2015). In addition, *Muc4* plays a role in cell and anti-apoptotic signaling (Chaturvedi et al.,  
79 2008; Funes et al., 2006; Moniaux et al., 2007). Together, the data suggest that *Muc4* may play  
80 a role in viral titers at day 4 post SARS-CoV infection.

### 81 *Muc4* drives sex-based difference in weight loss

82 To examine the role of *Muc4* during SARS-CoV infection, we utilized *Muc4*<sup>-/-</sup> mice  
83 (Rowson-Hodel et al., 2018) which have no gross differences in behavior, overall health, size, or  
84 lung function, the latter phenotype measured by plethysmography. *Muc4*<sup>-/-</sup> and WT control mice  
85 were infected with SARS-CoV and examined over a four-day time course. Following infection, a  
86 significant sex-based difference in weight loss between *Muc4*<sup>-/-</sup> and WT mice was observed  
87 (Table S3). Female *Muc4*<sup>-/-</sup> and control were essentially indistinguishable through two DPI (Fig.  
88 2A). However, infected *Muc4*<sup>-/-</sup> females continued to lose weight through day four, with 42% (6  
89 of 14) meeting euthanasia requirements (≥20% weight loss) and another 42% (6 of 14) requiring  
90 increased observation (≥10% weight loss). In contrast, the WT mice held static between days  
91 two and three and began to recover by day four, with no mice meeting the requirements for  
92 euthanasia and only 13% (2 of 15) requiring increased observation by a margin of ≤0.1g. This  
93 *Muc4*-driven difference in weight loss was not observed in male mice following infection with  
94 SARS-CoV (Fig. 2B).

### 95 *Muc4* has minimal impact on viral load

96 Next, the impact of *Muc4*<sup>-/-</sup> on SARS-CoV viral load was measured. Lung lobes from  
97 male and female *Muc4*<sup>-/-</sup> and WT mice were harvested at two DPI, an acute timepoint previously  
98 associated with high SARS-CoV titer (Sheahan et al., 2008), and at four DPI, the timepoint at  
99 which the QTL on chromosome 16 was mapped (Gralinski et al., 2015). Surprisingly, despite  
100 trends for higher mean viral loads in *Muc4*<sup>-/-</sup> mice on day 2 and day 4, virus titers were not

101 significantly different from WT mice at either timepoint (Fig. 3A). Similarly, despite differences in  
102 weight loss, viral titer in the lung also exhibited no sex effect (Table S3). Given the distribution  
103 of Muc4 in mucosal surfaces, viral load was further interrogated with IHC to determine whether  
104 Muc4 might impact viral tropism in a way not readily determined by the measurement of  
105 infectious virus per lobe. Upon examining SARS-CoV antigen staining, however, no significant  
106 differences in antigen distribution were noted between *Muc4*<sup>-/-</sup> and WT mice in either airway or  
107 parenchymal staining (Fig. 3B and 3C). Together, the results indicated that the loss of Muc4 had  
108 minimal impact on SARS-CoV viral load or distribution.

#### 109 *Muc4*<sup>-/-</sup> mice have reduced histopathological damage

110 Differences in lung damage were assessed using histopathological scoring of H&E  
111 stained lungs harvested at either two or four DPI. Similar to weight loss, the histopathology  
112 results demonstrated a clear and consistent sex-based phenotype. Female *Muc4*<sup>-/-</sup> mice had a  
113 pattern of lower pathology scores than WT mice (Fig. 4). Airway damage (denudation, debris,  
114 and inflammation) was minimally present in female *Muc4*<sup>-/-</sup> at day two or four post-infection (Fig.  
115 4A-C). Similarly, other histopathology and inflammation measures had very low scores in  
116 female *Muc4*<sup>-/-</sup> mice as compared to female WT mice. In contrast, male *Muc4*<sup>-/-</sup> mice induced  
117 similar damage to male WT mice (Table S1).

#### 118 Lung function altered in *Muc4*<sup>-/-</sup> female mice

119 To further evaluate damage to the lung following infection, whole body plethysmography  
120 was utilized to examine changes in pulmonary function (Fig. 5). Using only females, both WT  
121 and *Muc4*<sup>-/-</sup> mice were challenged with a lower dose (10<sup>4</sup> PFU) to ensure their survival over the  
122 full six day time course required to observe the onset, peak, and recovery of disordered lung  
123 function following SARS-CoV challenge in mice (Menachery et al., 2015a). Examining airway  
124 resistance (penH), the time to peak expiratory flow relative to total expiratory time (rPEF), and

125 mid-tidal expiratory flow (EF50), all three measurements had statistically significant differences  
126 between SARS-CoV-infected and mock-infected mice within the *Muc4*<sup>-/-</sup> and WT groups  
127 (Supplementary Table S3). However, no *Muc4*-dependent differences in either penH (Fig. 5A)  
128 or rPEF (Fig. 5B) were observed following SARS-CoV infection. In contrast, while not reaching  
129 statistical significance, EF50 trended higher in the *Muc4*<sup>-/-</sup> mice as compared to WT mice,  
130 peaking at day three post-infection (Fig. 5C). These results show a shift in the early breathing  
131 curve in *Muc4*<sup>-/-</sup> mice with more rapid exhalation and more labored breathing. These findings  
132 may have been exacerbated with the original, higher dose, but that experiment was precluded  
133 by the limited survival of *Muc4*<sup>-/-</sup> mice. Overall, the whole body plethysmography data indicate  
134 more difficult breathing for female *Muc4*<sup>-/-</sup> mice relative to their WT counterparts following  
135 SARS-CoV infection.

#### 136 *Muc4*<sup>-/-</sup> mice have augmented inflammation

137 Changes in the cytokine and chemokine responses following infection of *Muc4*<sup>-/-</sup> and WT  
138 mice were evaluated at two days post infection. While not significant when adjusted for multiple  
139 comparisons (Table S3), key inflammatory cytokines including IL-1 $\beta$ , TNF- $\alpha$ , and IL6 had  
140 increased expression in *Muc4*<sup>-/-</sup> mice as compared to WT mice (Fig. 6A-C). Similarly, MIP-1 $\alpha$ ,  
141 MCP-1, and KC also had augmented expression in *Muc4*<sup>-/-</sup> mice (Fig. 6D-F). Notably, the  
142 increased values in *Muc4*<sup>-/-</sup> mice were maintained across both males and females. Together, the  
143 results indicate that *Muc4*<sup>-/-</sup> mice have augmented inflammatory cytokine responses relative to  
144 WT mice, which may contribute to observed differences in pathogenesis.

#### 145 *Muc4* impacts pathogenesis for an unrelated virus

146 Because *Muc4* modulated SARS-CoV susceptibility independent of viral replication, a  
147 cross-platform validation study was undertaken to begin assessing whether the *Muc4* had  
148 widespread importance in viral pathogenesis. Chikungunya virus (CHIKV), an alphavirus,

149 causes inflammatory arthritis and swelling within the joints in human patients. Importantly,  
150 *Muc4* has been detected in synovial sarcomas in humans, thus presenting a novel tissue  
151 environment and unrelated virus to test the impact of *Muc4* (Doyle et al., 2011). Male and  
152 female WT and *Muc4*<sup>-/-</sup> mice were infected with CHIKV and monitored for swelling of the footpad  
153 for seven days post infection (Fig. 7). The WT mice have minimal early stage swelling, as was  
154 expected with the C57BL/6N model. In contrast, *Muc4*<sup>-/-</sup> mice have augmented disease early  
155 during infection, displaying a biphasic swelling pattern. At days 2-4, both male and female mice  
156 have increased footpad swelling compared to control mice. This *Muc4*-specific difference was  
157 not observed during the second swelling event that took place between days six and seven  
158 post-infection. Notably, while sex based differences were observed at days two and four post  
159 infection, disease was more robust in male mice, contrasting observations seen with SARS-CoV  
160 infection. In either sex, however, the loss of *Muc4* resulted in augmented disease during early  
161 time points and indicates a broad role for *Muc4* in viral pathogenesis.

## 162 Discussion

163 Screening genetically diverse mouse models provides an opportunity to identify natural  
164 variation in novel factors which drive viral disease responses. These studies can also provide  
165 therapeutic, prophylactic and molecular insights into emerging pathogens, which are difficult to  
166 study during the context of an outbreak. Here, prior phenotypic QTL analysis, bioinformatics,  
167 and RNA expression analysis were leveraged to identify *Muc4* as a high priority candidate gene  
168 driving differences in SARS-CoV titer. Utilizing a *Muc4* knockout mouse, a role was confirmed  
169 for the gene in augmented SARS-CoV pathogenesis. While virus titers trended higher in *Muc4*<sup>-/-</sup>  
170 mice at day 2 and 4, there was no statistically significant change as compared to WT control  
171 mice. However, in absolute terms the *Muc4*<sup>-/-</sup> mice did have a modest 62% higher titer on day  
172 two and a 51% higher titer on day four as compared to WT, and it is possible that this difference  
173 is biologically significant despite the lack of statistical significance as determined by p-value

174 (Lytsy et al., 2018; Vyas et al., 2015). In addition, the loss of *Muc4* was linked to enhanced  
175 CHIKV disease *in vivo*. Together, this study highlights both the utility and the challenges in  
176 transitioning from QTL hits to single-molecule studies. Our initial hypothesis for *Muc4* to play a  
177 role in controlling virus replication proved incorrect, or, at the very least, substantially more  
178 complex than a simple, direct correlate; however, our exploration found a disease-interaction  
179 that played a role in pathogenesis across multiple viruses.

180 Outside of human GWAS studies, where recombination rates are high, it has long  
181 proven a challenge to go from a QTL to individual candidate genes (Brown et al., 1997). Even  
182 with novel bioinformatic approaches, in the absence of large genomic deletions (Ferris et al.,  
183 2013), fortuitously gene-poor regions (Gralinski et al., 2015), or previously identified functions  
184 (Gralinski et al., 2017) candidate gene identification continues to be difficult. In this manuscript,  
185 we built a framework to integrate knowledge about the preCC genome sequences and a partner  
186 microarray dataset to narrow a QTL region down to a likely candidate based on expression  
187 correlations. *Muc4* exhibited strong correlation data and low expression in the relevant founder  
188 strain. Additionally, *Muc4* is known to play a role in anti-apoptotic signaling (Chaturvedi et al.,  
189 2008; Funes et al., 2006; Moniaux et al., 2007), analysis of published microarray data  
190 (Jonckheere N et al., 2012) reveals that cells lacking *Muc4* express lower levels of interferon-  
191 stimulated genes (Menachery VD, 2014), and the related mucin *Muc1* is both anti-apoptotic and  
192 anti-inflammatory (Kato K et al., 2014; Li Y et al., 2010; Ueno K et al., 2008). We chose *Muc4*  
193 as our priority candidate gene for follow up, with the initial hypothesis that *Muc4* suppressed  
194 apoptosis and possible the interferon response, and that its absence in a *Muc4*<sup>-/-</sup> mouse would  
195 therefore lead to increased apoptosis and inflammation, thereby inhibiting SARS-CoV  
196 replication.

197 Our validation utilized a *Muc4*<sup>-/-</sup> mouse and confirmed a role for *Muc4* in protection from  
198 SARS-CoV- and CHIKV-induced disease and pathogenesis. However, we found essentially no

199 differences in SARS-CoV viral load in the *Muc4*<sup>-/-</sup> mouse strain compared to WT mice. This may  
200 be due to sex (discussed more below), differences between naturally occurring SNPs impacting  
201 gene structure and expression and ablative knockouts, or the effects of overall genetic  
202 background on given gene variants (Leist et al., 2016). The SARS-CoV titer QTL on  
203 chromosome 16 was driven by the PWK/PhJ allele, which was strongly associated with lower  
204 viral titer and had low expression of *Muc4*. In contrast, mice with the C56BL/6J allele were  
205 associated with high titer and high expression of *Muc4*. Furthermore, PWK/PhJ mice encode  
206 functional variants in *Muc4* that are not present in the other seven founder strains. It is  
207 therefore possible that C57BL/6 genetic background for the *Muc4*<sup>-/-</sup> mice, as opposed to a  
208 PWK/PhJ background, obfuscated any role for *Muc4* in the regulation of viral titer; this possibility  
209 is especially relevant given that the hypothesized role of *Muc4* is in the context of complex  
210 signaling pathways requiring multiple molecular partners, rather than a direct role such as the  
211 physical barrier function of mucins. Alternatively, *Muc4* may simply not impact viral titer following  
212 SARS-CoV infection; this leaves the possibility that other genetic targets within the chromosome  
213 16 QTL are responsible for limiting SARS-CoV titers at later time points. Furthermore, the  
214 existence of multiple titer-driven QTLs reaffirms that no single gene is expected to serve as the  
215 sole driver of SARS-CoV titer. Yet, despite the lack of QTL validation, these studies still  
216 identified a role for *Muc4* in viral pathogenesis.

217         A surprising outcome from these studies was the demonstration of sex-specific and  
218 increased disease severity in *Muc4*<sup>-/-</sup> female mice infected with SARS-CoV. Females lacking  
219 *Muc4* consistently demonstrated increased susceptibility to SARS-CoV-induced disease, while  
220 males did not. These results affirm the National Institute of Health emphasis on conducting  
221 animal studies in both male and female subjects (Clayton and Collins, 2014; National Institutes  
222 of Health, 2015). Interestingly, SARS-CoV has been reported to cause more severe disease in  
223 male mice, with the female mice deriving their resistance at least in part from estrogen signaling

224 (Channappanavar et al., 2017). Furthermore, epidemiological data from both SARS-CoV and  
225 SARS-CoV-2 indicate that human females may be more resistant than human males (Chen et  
226 al., 2020; Huang et al., 2020; Karlberg et al., 2004; Leong et al., 2006). Estrogen increases  
227 *Muc4* transcription in a tissue-specific manner (Lange et al., 2003), offering a potential  
228 mechanism as to the greater impact of *Muc4*'s absence in female mice. Sex effects in the  
229 context of *Muc4* have also been reported in a murine model of colitis and colitis-associated  
230 colorectal cancer (Das et al., 2016). These results hint that the role of *Muc4* may differ in males  
231 and females based upon factors inherent to the host, such as localization, expression levels, or  
232 interactions with hormone signaling pathways, and may be less dependent on factors that are  
233 unique to a particular disease model.

234 Notably, a role for *Muc4* extended across two different viral pathogens. SARS-CoV  
235 infection of female *Muc4*<sup>-/-</sup> mice, and CHIKV infection of both male and female *Muc4*<sup>-/-</sup> mice,  
236 resulted in more severe disease following infection. For SARS-CoV, loss of *Muc4* exacerbated  
237 weight loss and increased lethality; surprisingly, less histopathologic damage was observed in  
238 the *Muc4*<sup>-/-</sup> lungs following infection despite augmented inflammatory cytokines. The results  
239 suggest that the loss of *Muc4* may exacerbate systemic disease and delay the repair/recovery  
240 processing in the lung following SARS-CoV infection. A similar reduction in histopathology  
241 damage was observed with MERS-CoV infection of immunocompromised rhesus macaques  
242 providing evidence for this theory (Prescott et al., 2018). Notably, footpad swelling following  
243 CHIKV infection is representative of systemic disease and is exacerbated in the absence of  
244 *Muc4*. Although speculative, it is possible that *Muc4* functions not to modulate local viral  
245 replication, but rather to limit disseminated disease. Clearly, more studies are needed to  
246 unravel these complex interactions.

247 Overall, this research demonstrates that *Muc4* is a novel regulator of viral pathogenesis.  
248 This study represents the first investigation of *Muc4* in the context of any viral infection, and the

249 absence of Muc4 exacerbates disease in two unrelated viruses. The SARS-CoV and CHIKV  
250 results indicate that Muc4 has a role in regulating susceptibility and may be widespread across  
251 viral pathogens. The study of mucin-driven cell signaling and its role in viral pathogenesis  
252 represents a novel avenue of research with the potential to identify new anti-viral therapeutic  
253 targets.

## 254 **Materials and Methods**

### 255 Infection with SARS-CoV or Chikungunya Virus

256 The generation of C57BL/6NTac *Muc4*<sup>tm1Unc</sup> mice, kindly provided by Dr. Scott Randell of  
257 the University of North Carolina at Chapel Hill and hereafter referred to as *Muc4*<sup>-/-</sup> mice, was  
258 described by Rowson-Hodel *et al* (Rowson-Hodel et al., 2018). The C57BL/6NTac genetic  
259 background of these mice was confirmed in our hands via utilization of the MiniMUGA  
260 genotyping array (Neogen Inc, Lincoln, NE), a new genotyping array which includes diagnostic  
261 markers for the substrain origin of many inbred mouse strains. Age- and sex-matched wild-type  
262 control C57BL/6NTac mice (hereafter referred to as WT mice) were obtained from Taconic  
263 (Germantown, NY). Ten- to eleven-week-old mice were anesthetized with a mixture of  
264 ketamine (Zoetis, Kalamazoo, MI) and xylazine (Akorn Animal Health, Lake Forest, IL) and  
265 inoculated intranasally with either PBS (Gibco, Grand Island, NY), 1x10<sup>4</sup> PFU, or 1x10<sup>5</sup> PFU of  
266 recombinant mouse adapted SARS-CoV (rMA15) in a 50µl volume (Roberts et al.). Mice  
267 receiving the 1x10<sup>5</sup> PFU dose and their mock-infected counterparts were weighed daily and  
268 were euthanized at either two or four days post-infection (DPI). The inferior lobe was harvested  
269 and stored intact at -80°C in 1ml PBS with glass beads for downstream titration, and the post-  
270 caval lobe was similarly stored for downstream cytokine and chemokine analysis. The left lobe  
271 was stored at 4°C in 10% buffered paraformaldehyde (Fisher Scientific, Fair Lawn, NJ) for at  
272 least seven days for downstream histopathology and immunohistochemistry. Mice receiving the  
273 1x10<sup>4</sup> PFU dose and their mock-infected counterparts were weighed daily and their lung

274 function was assessed via whole body plethysmography with in Buxco FinePoint system (Data  
275 Sciences International, New Brighton, MN) from one day prior to infection through six DPI. All  
276 data from SARS-CoV infections is summarized in Table S1.

277 Seven-week-old mice were anesthetized with isoflurane (Primal Enterprises, Andhra  
278 Pradesh, India) and inoculated subcutaneously in the left rear footpad with either diluent (PBS  
279 supplemented with 1% FBS, 1mM CaCl<sub>2</sub>, and 0.5mM MgCl<sub>2</sub>) or 100 PFU of recombinant  
280 chikungunya virus (CHIKV) SL15649 in a 10µl volume (Morrison et al., 2011). Footpad size was  
281 measured and recorded daily using a caliper to measure the vertical height of the ball of the left  
282 rear foot. Mice were humanely euthanized at seven DPI. All data from CHIKV infections is  
283 summarized in Table S2.

#### 284 Lung Titration

285 Lung samples were thawed at 37° and lysed for 60 seconds at 6,000 rpm in a MagNA  
286 Lyser (Roche, Mannheim, Germany). Debris was pelleted, and lung homogenate was serially  
287 diluted 10-fold in PBS. Vero E6 cell monolayers in 6-well plates were infected with 200µl of  
288 diluted lung homogenate (10<sup>-1</sup>-10<sup>-6</sup>) for one hour at 37°C with 5% CO<sub>2</sub>. Monolayers were  
289 overlaid with a semi-solid overlay containing MEM supplemented with 2% FBS (HyClone,  
290 Logan, UT), pencicillin/streptomycin (Gibco, Grand Island, NY), and 0.8% agar (Lonza,  
291 Rockland, ME). At two DPI plates were stained with neutral red (Fisher Scientific, Fair Lawn,  
292 NJ) for approximately four hours and plaques were visualized with a light box.

#### 293 Cytokine Analysis

294 Cytokine production was measured using the Bio-Plex Pro Mouse Cytokine 23-plex Assay (Bio-  
295 Rad, Hercules, CA) on the MAGPIX Multiplex Reader (Bio-Rad, Hercules, CA) according to the  
296 manufacturer's instructions. Lung samples were homogenized and clarified as described for the  
297 lung titration assay prior to analysis.

298 Histopathology and Immunohistochemistry

299           Formalin-fixed lobes were embedded in paraffin and 4µm sections were mounted on  
300 either Superfrost slides (Fisher Scientific, Waltham, MA) for hematoxylin and eosin staining or  
301 on ProbeOn slides (Fisher Scientific, Waltham, MA) for immunohistochemistry (IHC).  
302 Embedding, mounting, and hematoxylin and eosin staining was performed by the LCCC Animal  
303 Histopathology Core Facility at the University of North Carolina at Chapel Hill.

304           For IHC, sections were deparaffinized with xylene and rehydrated, then placed in near-  
305 boiling antigen retrieval buffer (10mM Tris base, 1mM EDTA, 0.05% Tween-20, pH 9.0) for 20  
306 minutes. Non-specific binding was blocked with 10% normal goat serum (Sigma, St. Louis, MO)  
307 and 1% nonfat milk (Lab Scientific, Highlands, NJ) in TBS for two hours at room temperature.  
308 Rabbit anti-SARS-CoV nucleocapsid polyclonal antibody (PA1-41098, Thermo Scientific,  
309 Rockford, IL) diluted 1:2,000 in blocking buffer was allowed to bind overnight at 4°C. Slides  
310 were washed three times in TBS with 0.025% Triton X-100 (TBST) (Sigma, St. Louis, MO), then  
311 incubated in 0.3% H<sub>2</sub>O<sub>2</sub> in TBS for 15 minutes to prevent endogenous peroxidases from  
312 generating a background signal. Slides were incubated with horseradish peroxidase-conjugated  
313 goat anti-rabbit IgG secondary antibody (ab97051, Abcam, Cambridge, MA) diluted 1:1,000 in  
314 blocking buffer for one hour in a humidity box, then washed three times in TBST. Slides were  
315 developed using the Metal Enhanced DAB Substrate Kit (Thermo Scientific, Rockford, IL) and  
316 counterstained with Richard-Allan Scientific Modified Mayer's Hematoxylin (Thermo Scientific,  
317 Rockford, IL) and 0.2M lithium carbonate (Sigma, St. Louis, MO) prior to rehydration and  
318 mounting. All scoring was conducted in a blinded fashion on a severity scale of 0 (none) to 3  
319 (severe).

320 Statistical Analysis

321 RNA expression levels were analyzed by one-way ANOVA with Dunnett's multiple  
322 comparisons test comparing PWK/PhJ to the other seven CC founder lines using Prism 7 for  
323 Windows version 7.01 (GraphPad Software, La Jolla, CA). Buxco data was transformed as  
324 previously described (Menachery et al., 2015a) Daily results for all titer-related data types  
325 ( $\log_{10}$ -transformed viral load and immunohistochemistry) and cytokine levels were analyzed by  
326 two-way ANOVA using RStudio version 1.1.383 with R version 3.2.0 (R Core Team, 2017;  
327 RStudio Team, 2015) to determine the impact of mouse strain and sex. Transformed daily  
328 Buxco results were similarly analyzed by two-way ANOVA to determine the impact of infection  
329 and mouse strain. Daily results for all pathology-related data types (histopathology, weight  
330 change, and footpad swelling) was analyzed by three-way ANOVA to determine the impact of  
331 mouse strain, sex, and infection status. When three-way ANOVA results indicated significant  
332 differences on the basis of sex (weight loss, histopathology, and footpad swelling), the strain-  
333 based differences between infected animals of a single sex were analyzed using multiple t-tests  
334 with the Benjamini and Hochberg correction for false discoveries in Prism. All graphing was  
335 done in Prism. A significance threshold of  $>0.05$  after correction for multiple comparisons was  
336 considered significant throughout all studies. The results of all statistical analyses are  
337 summarized in Table S3.

### 338 Data Availability

339 All phenotypic data associated with the infection of *Muc4*<sup>-/-</sup> mice and their WT  
340 counterparts are summarized in Table S1 and S2. *Muc4*<sup>-/-</sup> mice are available upon request.

### 341 **Funding**

342 JAP, LEG, MTF, MTH and RSB were funded by NIH NIAID (U19 AI 100625, U19 AI 109761,  
343 and U54 AI 081680). KSP was funded by NIH NIAID (T32 AI 007151-36A1 and F32 AI  
344 126730). AB was funded by NIH NIAID (T32 AI 7419-22). VDM was funded by the NIH NIA

345 (K99 AG 049092). The funders had no role in study design, data collection and interpretation,  
346 or the decision to submit the work for publication.

### 347 **Author Contributions**

348 JAP was involved with the design of all experiments, performed all SARS-CoV mouse  
349 experiments, and prepared the manuscript. KSP and MTH designed and performed the CHIKV  
350 mouse experiments. LEG provided feedback regarding experimental design and performed  
351 SARS-CoV mouse experiments and BioPlex analysis. AB performed immunohistochemistry  
352 staining. VDM provided feedback regarding experimental design, assisted with SARS-CoV  
353 mouse experiments, and assisted substantially with the editing of the manuscript. MTF  
354 provided guidance regarding statistical analysis. MTF, DB, and SKM performed array analysis  
355 and assisted with the selection of *Muc4* from the list of potential target genes. RSB scored  
356 gross pathology and immunohistochemistry and contributed to all aspects of study design,  
357 experimental design, and manuscript design and editing.

### 358 **Acknowledgements**

359 The authors thank Dr. Scott H. Randell of the Department of Cell Biology and Physiology at the  
360 University of North Carolina at Chapel Hill for contributing the *Muc4*<sup>-/-</sup> mice, without which these  
361 studies would not have been possible.

362

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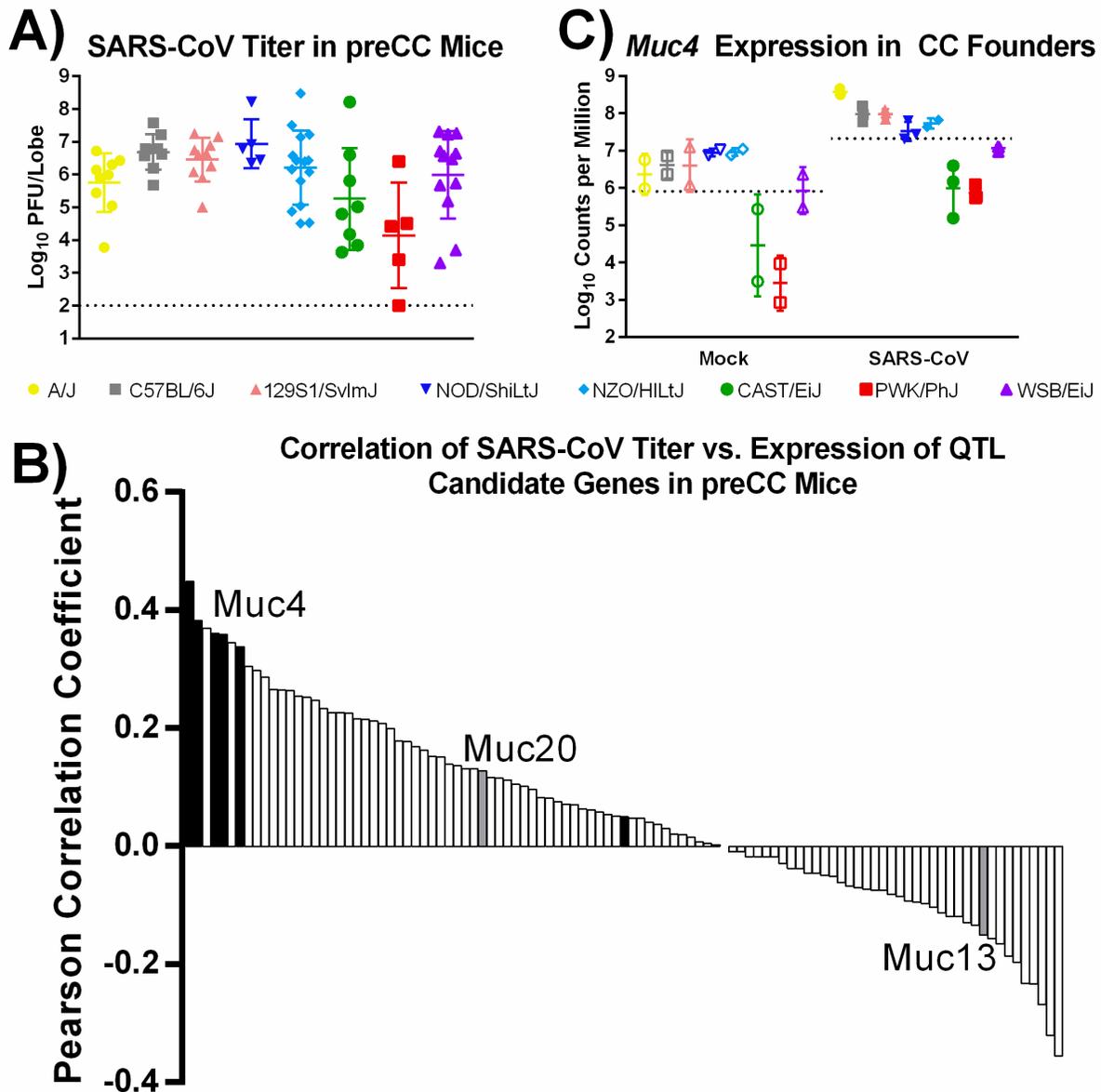
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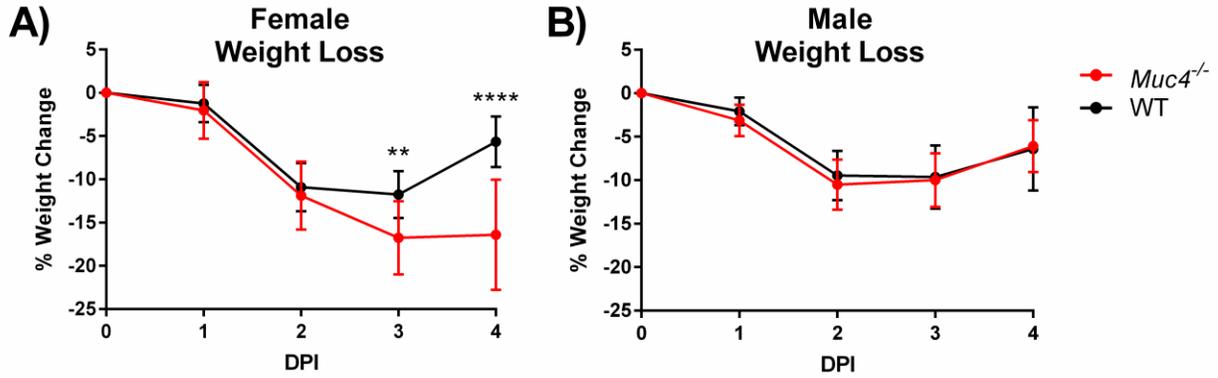
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593  
 594 **Fig. 1.** Selection of *Muc4* as a candidate gene from the preCC screen of SARS-CoV lung titer. (A) SARS-  
 595 CoV lung titer at four DPI in preCC mice that were homozygous for one of the eight indicated founder  
 596 strains at genotype marker nearest to the *Muc4* gene. Dashed line represents the lower limit of  
 597 detection for the assay. (B) Correlation values for  $\text{Log}_{10}$ -transformed viral titers and host RNA expression  
 598 values as determined by microarray in the lungs of preCC mice at four DPI with SARS-CoV. Bars  
 599 correspond to individual microarray probes representing genes in the titer QTL region. Black bars  
 600 indicate probes for *Muc4*. Grey bars indicate probes for other mucins (*Muc13* and *Muc20*) within the  
 601 QTL. Open bars represent non-mucin genes within the QTL. (C) Expression of *Muc4* RNA in the CC  
 602 founder lines at four DPI following either mock (open symbols) or SARS-CoV (closed symbols). Dashed  
 603 lines represent the mean expression level for all founder mice.

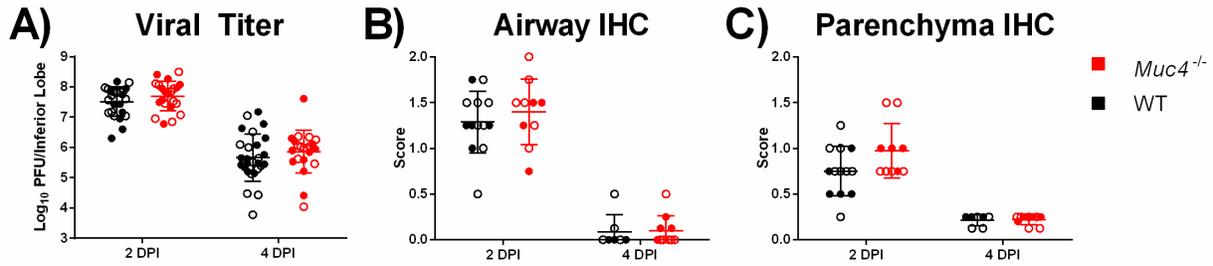


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605 **Fig. 2.** Weight loss following SARS-CoV infection. Weight loss was monitored in (A) female and (B) male  
606 *Muc4*<sup>-/-</sup> and WT mice. Black indicates WT mice and red indicates *Muc4*<sup>-/-</sup> mice. Points represent the  
607 mean, and error bars represent standard deviation. Asterisks indicate statistical significance (\*\* =  
608  $q < 0.01$ , \*\*\*\* =  $q < 0.0001$ ).

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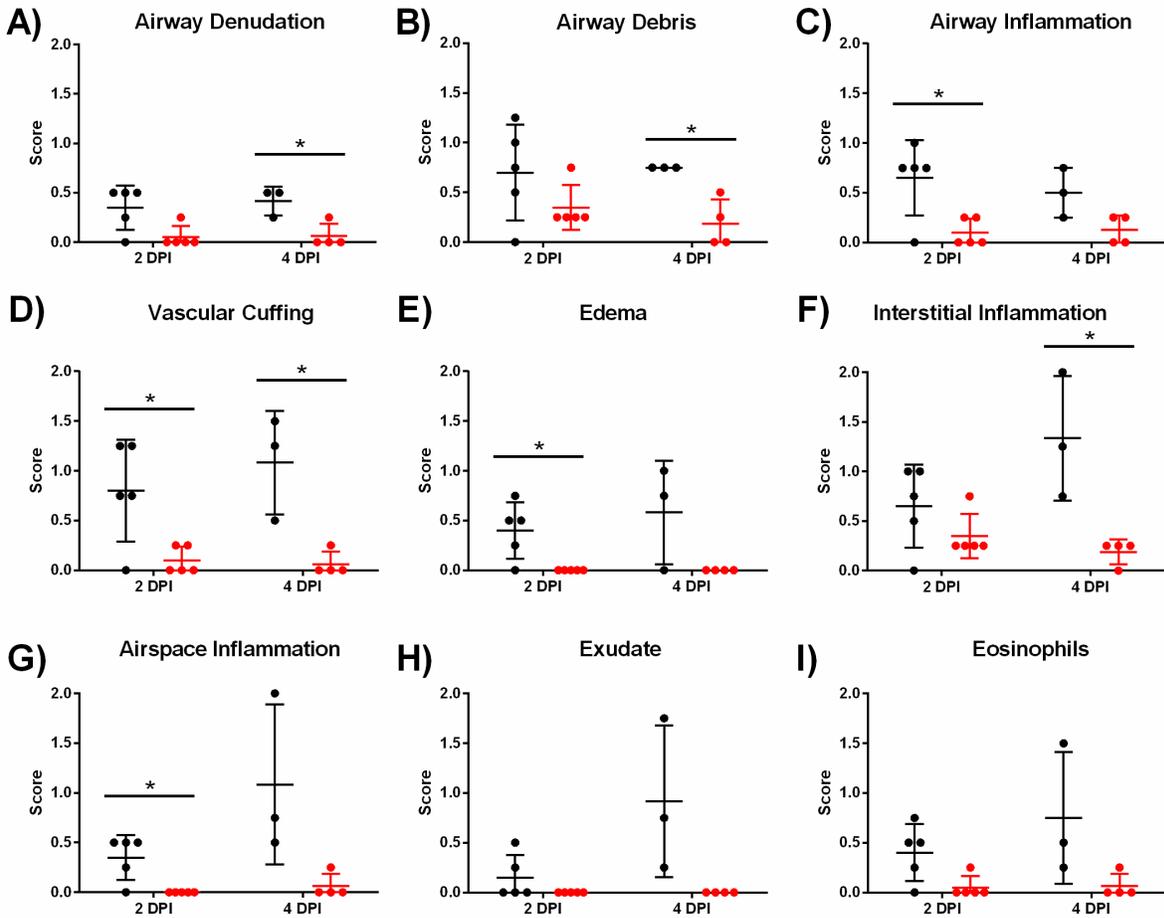
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612 **Fig. 3.** Viral titer and tropism following SARS-CoV infection. (A) viral titer in the inferior lobes of SARS-  
613 CoV infected mice at two DPI and four DPI was measured via plaque assay. SARS-CoV tropism in the (B)  
614 airway and (C) parenchyma of the left lobe at two and four DPI was measured via blind scoring following  
615 IHC staining. Black indicates WT mice and red indicates *Muc4*<sup>-/-</sup> mice. Points represent individual mice  
616 (close = female, open = male), the midline represents the mean, and error bars represent standard  
617 deviation. No comparisons of WT versus *Muc4*<sup>-/-</sup> mice achieved statistical significance after correction  
618 for multiple comparisons ( $q \geq 0.05$ )

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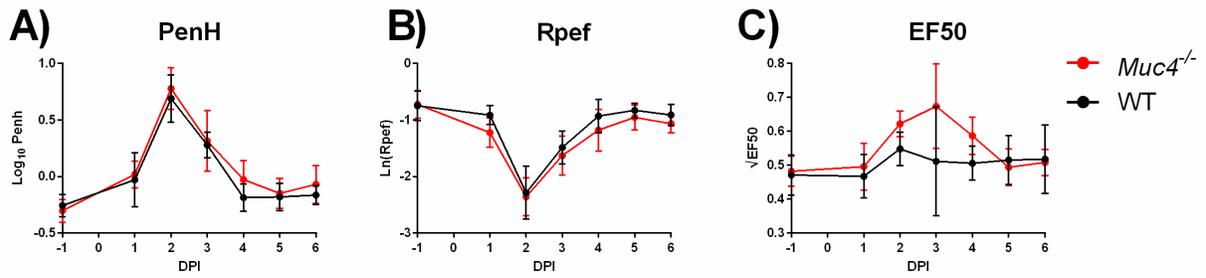


620

621 **Fig. 4.** Female WT and *Muc4*<sup>-/-</sup> mice were infected with SARS-CoV, and their left lung lobes were  
622 harvested at either two or four DPI for histopathological analysis following H&E staining. (A) Airway  
623 denudation, (B) Airway debris, (C) Airway inflammation, (D) Vascular Cuffing, (E) Edema, (F) Interstitial  
624 Inflammation, (G) Airspace Inflammation, (H) Exudate, and (I) Eosinophilia were all scored. Black  
625 indicates WT mice and red indicates *Muc4*<sup>-/-</sup> mice. Points represent individual mice, the midline  
626 represents the mean, and error bars represent the standard deviation. Asterisks indicate statistical  
627 significance (\* = q<0.05).

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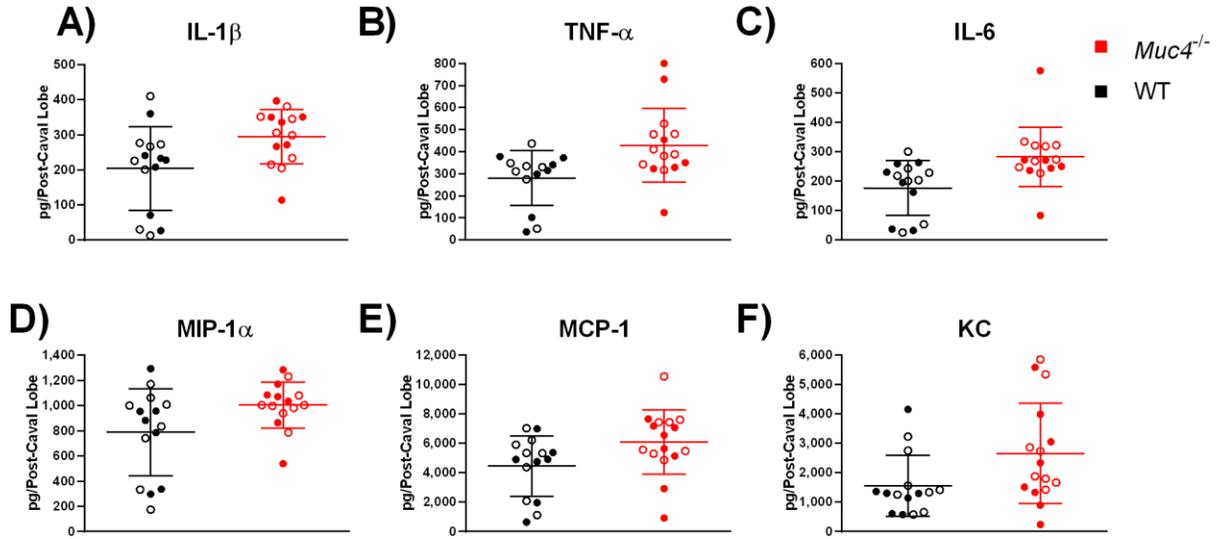
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631 **Fig. 5.** Pulmonary function as measured by normalized (A) PenH, (B) Rpef, and (C) EF50 readings was  
632 recorded in SARS-CoV-infected female mice. Black indicates WT mice and red indicates *Muc4*<sup>-/-</sup> mice.  
633 Points represent the mean and error bars represent the standard deviation. Strain-based differences  
634 did not achieve statistical significance after correction for multiple comparisons ( $q \geq 0.05$ ).

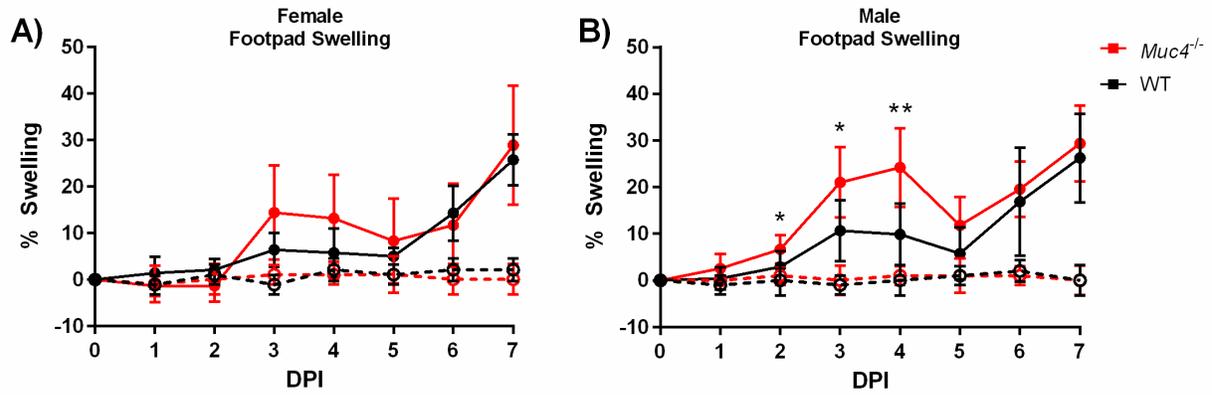
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637 **Fig. 6.** Cytokine levels, including (A) IL-1 $\beta$ , (B) TNF- $\alpha$ , (C) IL-6, (D) MIP-1 $\alpha$ , (E) MCP-1, and (F) KC, at 2 DPI  
638 in post-caval lobes of SARS-CoV-challenged mice were measured using a BioPlex assay. Black indicates  
639 WT mice and red indicates *Muc4*<sup>-/-</sup> mice. Points represent individual mice (close = female, open = male),  
640 the midline represents the mean, and error bars represent standard deviation. Strain-based differences  
641 did not achieve statistical significance after correction for multiple comparisons ( $q \geq 0.05$ ).

642



643

644 **Fig. 7.** Footpad swelling in (A) female and (B) male mice that have been either mock-infected or infected  
645 with CHIKV. Black indicates WT mice and red indicates *Muc4*<sup>-/-</sup> mice. Dashed lines with open symbols  
646 indicate mock infection, and solid lines with solid symbols indicate CHIKV infection. Points represent the  
647 mean and error bars represent the standard deviation. Asterisks indicate statistical significance (\* =  
648  $q < 0.05$ , \*\* =  $q < 0.01$ ).

649