1   **Full Title (200 characters):** Genetic background influences survival of infections with

2   *Salmonella enterica* serovar Typhimurium in the Collaborative Cross

3   **Short Title (70 characters):** Host genetic background and *Salmonella enterica*

4   Typhimurium

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Abstract (300)

Salmonella infections typically cause self-limiting gastroenteritis, but in some individuals these bacteria can spread systemically and cause disseminated disease. *Salmonella* Typhimurium (STm), which causes severe systemic disease in most inbred mice, has been used as a model for disseminated disease. To screen for new infection phenotypes across a range of host genetics, we orally infected 32 Collaborative Cross (CC) mouse strains with STm and monitored their disease progression for seven days by telemetry. Our data revealed a broad range of phenotypes across CC strains in many parameters including survival, bacterial colonization, tissue damage, complete blood counts (CBC), and serum cytokines. Eighteen CC strains survived to day 7, while fourteen susceptible strains succumbed to infection before day 7. Several CC strains had sex differences in survival and colonization. Surviving strains had lower pre-infection baseline temperatures and were less active during their daily active period. Core body temperature disruptions were detected earlier after STm infection than activity disruptions, making temperature a better detector of illness. All CC strains had STm in spleen and liver, but susceptible strains were more highly colonized. Tissue damage was weakly negatively correlated to survival. We identified loci associated with survival on Chromosomes (Chr) 1, 2, 4, 7. Polymorphisms in *Ncf2* and *Slc11a1*, known to reduce survival in mice after STm infections, are located in the Chr 1 interval, and the Chr 7 association overlaps with a previously identified QTL peak called *Ses2*. We identified two new genetic regions on Chr 2 and 4 associated with susceptibility to STm infection. Our data reveal the diversity of responses to STm infection across a range of host genetics and identified new candidate regions for survival of STm infection.

Author Summary (200)

Salmonella Typhimurium (STm) infections typically cause self-limiting diarrheal symptoms, but in some individuals, the bacteria can spread throughout the body and cause life-threatening infection. We used a population of genetically different mice (Collaborative Cross) to identify their range of responses to STm infection. We identified a broad range of outcomes across these different mice, including a group of mice susceptible to lethal infection and a group that survived our 7 day study. We found that mice that survived STm infection had a cooler core body temperature before infection than susceptible mice, while remaining active. Thus, body temperature, rather than activity, appears to be a better predictor of poor outcomes after STm infection. We identified several regions of the mouse genome that are associated with outcome after STm infection. One of these regions, mouse Chromosome (Chr) 1 has genes that are already known to influence susceptibility to STm infection. Two other regions that we identified to influence survival after STm infection, located on mouse Chr 2 and 4, are novel and contain numerous genes of interest that may be linked to susceptibility. Our work defines the utility of exploring how host genetic diversity influences infection outcomes with bacterial pathogens.

4

Introduction

Salmonellae are Gram negative facultative intracellular bacteria that cause self-limiting gastroenteritis, typhoid fever, or sepsis in human beings (1–3). Non-typhoidal *Salmonella* (NTS) infections are estimated to cause 93.8 million human cases per year, resulting in 155,000 deaths worldwide (4). While NTS infection is typically limited to the intestine and causes diarrheal disease, NTS can also cause more severe disseminated disease, depending on the *Salmonella* serotype and host factors such as age, immune status, and genetics (5). Worldwide, 535,000 human cases of NTS disseminated disease, either *Salmonella* bacteremia or invasive non-typhoidal salmonellosis, occur annually and cause 77,500 deaths (6).

Although antibiotics are not generally used to treat patients with NTS gastroenteritis, they are used to treat patients with NTS bacteremia and invasive non-typhoidal salmonellosis (iNTS). Thus, a concern of increasing importance is the development of multi-drug resistant strains of *Salmonella* that cause 212,500 human cases per year in the US alone, with 16% of all NTS cases resistant to at least one antibiotic as of 2017 (7). Exploitation of differences in host immunity may help identify new interventions that could become treatment alternatives to antibiotics (8–10).

Salmonella enterica serotype Typhimurium (STm) is a serotype of non-typhoidal *Salmonella* that causes murine typhoid resembling severe invasive human infections. Some mouse strains are particularly susceptible (C57BL/6J and BALB/cJ), while others are able to resist severe systemic disease (CBA/J, 129/SvJ, and A/J) (11–13). Previous studies have largely attributed differential susceptibility to a mutation in solute carrier family 11 member 1 (*Slc11a1*) encoding a metal cation pump in macrophages that

104   reduces intracellular growth of bacteria (2,14–17). Natural variation in other genes has

105   also been shown to play a role in differential survival after STm infection, including

106   neutrophil cytosolic factor 2 (*Ncf2*) and toll-like receptor 4 (*Tlr4*). *Ncf2* encodes a

107   component of the NADPH complex in neutrophils that generates superoxide and is vital

108   to neutrophil's killing ability (18–20). Toll-like receptor 4 (*Tlr4*) recognizes

109   lipopolysaccharide (LPS) and is a key component of innate immune response activation

110   and cytokine production (21,22).

111   To identify new host genetic mechanisms that could influence differential

112   susceptibility to STm infections, we used the Collaborative Cross (CC) mouse genetic

113   reference population (23–26). The CC has greater variability across all parameters than

114   traditional strains, including immune phenotypes, because of its genetic diversity (27–

115   29). This population has been used to identify novel genetic variants and discover new

116   phenotypes in mice, such as tolerance (high pathogen load with minimal disease) (30–

117   32) for infectious agents including Influenza A, Ebola, *Mycobacterium tuberculosis*,

118   Theiler's murine encephalomyelitis virus, SARS-Corona virus, and West Nile virus (33–

119   39).

120   To identify new mechanisms underlying diverse host responses to STm

121   infections in mice, we infected CC strains orally and monitored infected animals for one-

122   week post-infection using telemetry to track temperature and activity levels. At

123   necropsy, organs and blood were collected to analyze colonization, histopathology,

124   complete blood counts (CBC), and cytokine levels. Strains that survived to day 7 had

125   lower uninfected baseline body temperatures and were less active during their active

126   period pre-infection. Temperature patterns were disrupted earlier than activity patterns,

127   suggesting that temperature is a more sensitive measurement for illness. All parameters

128   were strain dependent, but in general, mice were colonized in systemic organs

129   independent of survival time, spleen damage was most correlated to survival, and

130   cytokines and CBC parameters were not predictive of survival. Several CC strains

131   showed sex differences across survival, weight change, and bacterial burden, but one

132   strain, CC027/GeniUnc, showed stark sex-dependence across all parameters.

133   Quantitative trait locus (QTL) analysis for survival time revealed three significant and

134   two suggestive associations, confirming known loci and detecting previously unknown

135   variants providing new candidate genes not previously associated with STm.

136   Furthermore, despite having susceptible alleles in both *Slc11a1* and *Ncf2* that are linked

137   to early death from *Salmonella* infection, CC045/GeniUnc mice were able to survive 7

138   days with a high bacterial load and exhibited tolerance to systemic *Salmonella* infection.

139

140   **Results**

141   Response to oral *Salmonella* Typhimurium infection is variable across CC strains

142   We infected thirty-two Collaborative Cross (CC) strains (3 male and 3 female)

143   with *S.* Typhimurium (STm) ATCC14028 and monitored them for up to one week to

144   identify variable susceptibility to STm infection. Due to their well-characterized response

145   to STm infection, C57BL/6J (B6) mice, which develop systemic murine typhoid, and

146   CBA/J (CBA) mice, which are resistant to systemic infection, were used as infection

147   controls. At the time of necropsy, sections of ileum, cecum, colon, liver, and spleen

148   were collected to determine bacterial load. We focused our analysis on the systemic

149   phase of infection. In comparison to previous studies that injected STm intravenously

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150 into CC mice, our STm oral infections produced a much wider range of colonization in

151 critical organs (Fig. 1). Using sex-combined median data, the correlation between liver

152 and spleen colonization with survival time was R = -0.62 and -0.63, respectively (Supp.

153 Fig. 1).

154   All B6 mice met the euthanasia criteria between day 3 and 6 post-infection with a

155 median at day 4 and were classified as highly susceptible (Fig. 2A). B6 mice lost a

156 median of 20.73% of their body weight during infection (Fig. 2B). CBA mice survived to

157 day 7 and were classified as resistant (Fig. 2A) with a median loss of 7.92% of their

158 body weight during infection (Fig. 2B). B6 mice were highly colonized in the liver and

159 spleen with medians of 2.56 x10^6 and 1.21 x10^7 CFU/g, respectively (Fig. 2C, D), while

160 CBA mice were colonized at least two orders of magnitude lower with medians of 5.45

161 x10^3 and 1.84 x10^4 CFU/g, respectively (Fig. 2C, D).

162   Of the 32 CC strains we infected, 14 were categorized as susceptible (3 or more

163 mice out of 6 infected met euthanasia criteria prior to day 7 post-infection) and 18

164 strains were called resistant or tolerant (Fig. 2A, susceptible in shaded region).

165 Susceptible strains that suffered earlier mortality also had greater weight loss (Fig. 2B)

166 with a correlation of R = 0.67 (Supp. Fig. 2). Susceptible strains had a least 10-fold

167 lower bacterial burden in liver than B6 control mice, with the exception of CC013,

168 CC027, and CC042 (Fig. 2C). CC013 and CC027 had comparable colonization in liver

169 to B6, and CC042 had 100-fold higher with a median colonization of 1.02 x10^8 CFU/g

170 (Fig. 2C), consistent with previous reports (40,41). In spleen, all strains had at least a

171 10-fold lower bacterial burden than B6 mice, with the exception of CC042 (Fig. 2D),

172 which were colonized 100-fold higher than B6 mice (Fig. 2D). The high colonization of

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173 CC042 has been previously attributed to a *de novo* mutation in integrin alpha (*Itgal*)

174 (41). Susceptible strains were also colonized at least 10-fold higher than CBA control

175 mice, with the exception of CC032 (Fig. 2C, D). CC032 mice were colonized in both

176 liver and spleen similarly to CBA with a median of 4.11 x10^3 and 3.96 x10^4 CFU/g,

177 respectively, yet succumbed to infection before day 7 (Fig. 2C, 2D).

178   An additional 18 strains were categorized as resistant or tolerant. Resistance

179 was defined as surviving infection by preventing colonization or by clearing the bacteria.

180 Tolerance was defined as surviving infection while maintaining high bacterial burden.

181 Both resistant and tolerant strains had </= 2 mice meeting euthanasia criteria prior to

182 day 7. Of these 18 strains, all 6 mice lived to day 7 in 12 strains (Fig. 2A). Strains that

183 survived longer lost less weight than those that did not with a correlation of R = 0.71

184 across all strains (Fig. 2B and Supp. Fig. 1). However, CC011 lost substantially more

185 weight than expected for a surviving strain. All the resistant and tolerant strains, with

186 two exceptions (CC045 and CC051), were equally colonized across systemic organs,

187 thus making these phenotypes difficult to differentiate.

188   One strain – CC051 – had 100-fold lower bacterial burden than CBA in both liver

189 and spleen with medians of 9.30 x10^1 and 2.92 x10^2 CFU/g, respectively, and it

190 therefore met our criteria for resistance (Fig. 2C, D). Another strain – CC045 – had a

191 100-fold higher bacterial burden than CBA mice with 3.31 x10^5 and 4.83 x10^6 CFU/g,

192 respectively, making it the strongest candidate for tolerance (Fig. 2C, D). Of the

193 remaining 16 surviving strains, eight were colonized in the liver to the same level as

194 CBA mice, while eight additional strains were colonized in the liver 10-fold higher than

195 CBA mice (Fig. 2C).

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196

Sex-dependent variation in susceptibility is strain dependent

198   Sex has been shown to be an influencing factor in many phenotypes, including

199   response to infection (28,42–44). Across the CC population that we tested, sex did not

200   significantly influence survival time ($P = 0.8149$), weight change post-infection ($P =$

201   0.9764), spleen CFU ($P = 0.7289$), or liver CFU ($P = 0.5571$). However, several

202   individual strains did display sex-dependent variation. CC013 and CC030 males

203   survived longer than females, and CC042 and CC027 females survived longer than

204   males (Fig. 3A). CC015, CC027, and CC072 females lost less weight than males and

205   CC057 males lost less than females (Fig. 3B). CC027 was the only strain where weight

206   loss influenced survival in a sex-dependent manner.

207   CC013, CC019, and CC027 males had higher bacterial burdens than females,

208   while CC057 females were more highly colonized than males (Fig. 3C, D). CC013 and

209   CC019 had different liver colonization (Fig. 3C), while CC027 had different spleen

210   colonization (Fig. 3D), and CC057 had differences in both organs (Fig. 3C, D). Just as

211   with weight loss, CC027 bacterial burden influenced survival time in a sex-dependent

212   manner. CC027 females survived to day 7, had a lower bacterial burden, and lost less

213   weight than males. As such, CC027 males are susceptible to STm infection, while

214   females are tolerant or resistant.

215

Surviving mice have lower baseline body temperatures and stay on baseline circadian pattern for activity longer

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218   Implantable telemetry devices tracked body temperature and activity levels at

219   one-minute intervals both before and throughout infections. Prior to infection, each

220   strain had unique diurnal pattern of baseline temperature and activity prior to infection

221   (Supp. Fig. 3). When strains were grouped by survival outcome, differences between

222   groups became apparent (Fig. 4). Resistant/tolerant mice that survived the infection had

223   a baseline pre-infection median minimum temperature of 36.32 °C during their resting

224   period compared to susceptible non-surviving mice with a pre-infection median

225   minimum temperature of 36.67 °C at rest (Fig. 4A, P = <0.0001). The pre-infection

226   median overall temperature for surviving versus susceptible mice was 36.61 °C and

227   36.95 °C, respectively (Fig. 4A, P = <0.0001). This significant difference was also found

228   in maximum temperature reached during the active periods with medians of 37.08 °C

229   and 37.35 °C, for surviving versus non-surviving mice respectively (Fig. 4A, P =

230   0.0025). Thus, surviving strains had a statistically significantly lower pre-infection

231   baseline body temperature relative to susceptible strains.

232   Pre-infection activity measurements were analyzed by what fraction of the time

233   the mouse was active, with a higher fraction suggesting more activity. Before infection,

234   all mice had maximal activity during their active period at night. In pre-infection periods

235   where mice were minimally active (day), both susceptible strains and surviving strains

236   were active for equal amounts of time (Fig. 4B). Considering an entire 24-hour period

237   prior to infection, there was no statistically significant difference in activity between

238   susceptible and surviving strains (Fig. 4B). During the most active periods for the mice,

239   strains susceptible to STm infection spent significantly more time active than strains that

240   survived STm infection (0.83 versus 0.74, Fig. 4B, P = 0.0139). Thus, at pre-infection

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241   baseline, surviving strains appeared to spend more time at rest during the normal

242   waking period of the mouse (night).

243   Post-infection telemetry data were analyzed explicitly incorporating assumptions

244   about oscillatory behavior to model when mice deviated from their circadian pattern of

245   body temperature and activity after infection with STm (time to "off-pattern") (45). For

246   temperature measurements, surviving and susceptible strains did not differ significantly

247   for their time to circadian pattern disruption after infection (Fig. 4C, P = 0.3961).

248   Susceptible and surviving strains also did not differ significantly for time to off pattern for

249   activity (Fig. 4C, P = >0.9999). However, susceptible strains did stay on their normal

250   activity pattern significantly longer than they stayed on their normal temperature pattern

251   with medians of 1789 minutes (1.24 days) and 770.75 minutes (0.54 days) (Fig. 4C, P =

252   0.0397). Surviving strains did not stay on either activity or temperature pattern

253   significantly longer (Fig. 4C, P = 0.1057).

254

255   Tissue damage is weakly correlated to survival time and is driven by splenic damage

256   Tissue sections were stained with H&E, and a board-certified pathologist scored

257   each tissue blindly for damage using a scale of 0 to 4 (0 = normal to 4 = severe

258   damage, Supp. Table 1 and Supp. Fig. 4). Minimal damage was noted in the intestinal

259   sections examined (ileum, cecum, and colon, scores 0-1) and these were excluded from

260   further analyses (Supp. Fig. 5). To assess the relationship between survival and tissue

261   damage, linear regression was performed showing survival and combined spleen and

262   liver pathology scores with R^2= 0.1256, while the spleen pathology score alone was

263   0.1323 and the liver pathology score alone was 0.08382 (Fig. 5B, C, D, all P = <0.001).

264   Thus, damage to the spleen appears to be driving the small but significant

265   correlation between histopathology scores and survival. Organs from susceptible strains

266   were not necessarily more damaged than those from surviving strains, perhaps

267   because the mice succumbed to infection before more severe damage could occur (Fig.

268   5A).

269

270   Complete blood counts and circulating cytokines/chemokines do not explain differential

271   host response to STm infection

272   Complete blood counts (CBC) were performed on pre-infection blood and on

273   blood collected at necropsy (S1 Table and S2 Table). The differential counts for

274   circulating white blood cell (WBC), neutrophils (NEU), monocytes (MON), lymphocytes

275   (LYM) were analyzed. No significant differences were observed between surviving and

276   non-surviving strains (Supp. Fig. 6, P = 0.4415, 0.2833, 0.9031, and 0.2505). The levels

277   of 36 serum cytokines and chemokines were also determined from serum collected at

278   necropsy and compared to those of uninfected control animals from each strain (S3

279   Table and S4 Table). Susceptibility or survival was not significantly correlated to the

280   cytokine profile. The cytokine profile was influenced more by strain than infection status.

281   Highly STm susceptible CC042 mice had high levels of almost every cytokine,

282   suggesting that the mice were suffering a cytokine storm (S4 Table).

283

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284 <u>Known polymorphisms do not always predict infection outcome</u>

285   A point mutation in *Slc11a1* (also known as *Nramp1*, or *Ity*) is associated with

286 susceptibility to lethal systemic STm infection (2,14,16). B6 is the only CC founder strain

287 to carry this mutation, and B6 is susceptible to systemic infection with STm. Only 5 CC

288 strains in the panel analyzed – CC021, CC031, CC042, CC045, and CC061 – carry the

289 *Slc11a1* mutation (Fig. 2). All of these strains were highly colonized in liver and spleen,

290 and, with the exception of one strain (CC045), succumbed to infection before day 7.

291 CC045 had a high bacterial burden but survived to day 7 post-infection. This result

292 suggests that CC045 has compensatory mechanisms to overcome STm susceptibility

293 caused by the *Slc11a1* mutation.

294   Another mutation previously linked to STm susceptibility is in *Ncf2*, although it is

295 not as detrimental to murine survival after *Salmonella* infection as the *Slc11a1* mutation

296 (18,19). Three of the CC founders (PWK/PhJ, CAST/EiJ, NZO/ HlLtJ) carry the *Ncf2*

297 mutation, and 12 of the CC strains in the current panel – CC013, CC015, CC023,

298 CC025, CC030, CC032, CC036, CC038, CC045, CC058, CC072, CC078 – also carry

299 the *Ncf2* mutation (Fig. 2). Six out of these 12 strains did not survive to day 7 post-

300 infection (CC013, CC023, CC025, CC030, CC032, CC036), while the remaining 6

301 survived.

302   *Tlr4* also has a known point mutation linked to STm susceptibility, but none of the

303 CC founder strains carry a mutation in this gene, making it impossible to verify the effect

304 of this mutation on STm infection using CC mice (21,22).

14

305   Polymorphisms in *Ncf2* and *Slc11a1* combined explained STm susceptibility in

306   10 of the 14 susceptible strains, but the phenotypes of 4 of the strains – CC005, CC019,

307   CC027, CC037 – are not explained by mutations in either of these genes. This finding

308   suggests that other genetic factors contribute to STm susceptibility in these strains.

309   CC045, the only strain to carry both *Slc11a1* and *Ncf2* mutations, is particularly unusual

310   since 4 out of 6 mice of this strain survived STm infections.

311   Genetic basis for variable response to infection with *Salmonella* Typhimurium

312   We used gQTL (46) to identify genetic regions associated with survival after oral

313   STm infection. Since CC042 has a *de novo* mutation that explains its extreme

314   susceptibility to STm infections, it was excluded from genetic analysis. CC045 was also

315   excluded, as it carries mutations in both *Slc11a1* and *Ncf2*, yet survives infection. Using

316   median survival time and percent of mice surviving to day 7 post-infection, significant

317   associations were detected on Chromosomes (Chr) 4, 6, and 7 and suggestive

318   associations were identified on Chr 1 and 2 (Fig. 6A and C, Table 1). We identified a

319   significant association on Chr 4 (*Stq1* (Survival Time QTL)) and a significant association

320   on Chr 7 (*Stq2*). The heritability of survival time was 45.55. Although the slope of

321   survival percentages over time did not return significant temporal QTL, suggestive

322   associations overlapped with those from the other survival parameters (Supp. Fig. 7).

323   Other parameters such as weight change, CFUs, and CBCs were also analyzed using

324   gQTL with no significant or suggestive associations detected, further supporting their

325   independence from STm infection survival.

326   Two suggestive associations on Chr 1 overlapped with the *Slc11a1* and *Ncf2*

327   genes, suggesting that these genes underlie these associations. The significant

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328 association on Chr 6 contains four genes, three hypothetical genes and one annotated

329 gene, *Cntn3*, which is involved in nervous system development (Supp. Fig. 8A).

330 However, no SNP differences were found in *Cntn3* that were consistent with founder

331 haplotype effects. Although genes on Chr 2 and 4 have previously been shown to be

332 involved in STm infections, the QTL regions we identified do not overlap with previously

333 detected loci. The association on Chr 7 overlaps with a previously detected QTL region

334 called *Ses2* (*S. enteritidis* susceptibility locus 1) which is linked to *Salmonella* clearance

335 in the spleen, but the causative gene in this region remains unknown (47,48).

336   Using founder effect plots (Fig. 6 and Supp. Fig. 8 and 9), haplotype effects were

337 determined for each QTL association. For median survival time, having a CC founder

338 haplotype of PWK or CAST in the Chr 2 QTL region led to poor survival (Fig. 6B, Table

339 1). *Stq1* (Chr 4) was linked to poor survival if the region was inherited from the CC

340 founders WSB and NZO (Fig. 6B, Table 1). B6, NOD, and WSB had low effect

Table 1: QTL associations for median survival time and percent survived to day 7 after STm infections. 30 CC lines were included in the gQTL analysis, excluding CC042 and CC045.

Table 1: QTL suggestive and significant peaks

QTL name	Phenotype	Chr	LOD	P-value	Proximal (Mb)	Max (Mb)	Distal (Mb)	Haplotype Effects
	survival time	1	8.24	3.09E-06	73.96	152.07	152.07	
	survival time	2	7.64	1.03E-05	151.39	157.79	158.05	low: PWK, CAST
Stq1	survival time	4	9.42	2.78E-07	16.30	19.51	19.63	low: WSB, NZO
	survival time	7	7.24	2.27E-05	78.07	79.71	90.04	low: WSB, NOD, B6
	% survived to day 7	1	6.91	4.31E-05	73.97	152.07	154.46	
	% survived to day 7	2	6.66	7.08E-05	151.01	157.79	166.40	low: PWK, CAST
	% survived to day 7	4	6.91	4.33E-05	16.12 16	17.67	19.72	low: WSB, NOD, B6
Stq2	% survived to day 7	7	9.11	5.29E-07	78.40	79.42	82.00	low: WSB, NOD, B6

haplotypes for *Stq2* (Chr 7), leading to poor survival (Supp. Fig. 8B, Table 1). For percent of mice that survived to day 7, PWK and CAST alleles on the Chr 2 region led to poor survival, (Supp. Fig. 9A, Table 1), *Stq1* (Chr 4) was linked to poor survival for B6, WSB, and NOD alleles in this region (Supp. Fig. 9B, Table 1), and the *Stq2* (Chr 7) region was linked to poor survival for B6, NOD, and WSB (Fig. 6D, Table 1). No high haplotype effects or QTL linked to enhanced survival after STM infections were found in these associations.

To narrow the number of candidate genes for each association, SNP differences in coding regions in the CC founder strains were identified using the Mouse Phenome Database (The Jackson Laboratory). Ensembl Variant Effect Predictor (VEP) was used to determine whether the SNP difference had a predicted effect at the protein level. Haplotype effects for each locus were used to filter candidate genes that had differences in their sequences. For median survival time, Chr 2 had 259 genes with 13 SNP differences in genes that matched haplotype differences (Table 2). *Stq1*, with 23 genes, and Chr 7, with 232 genes, had no

Table 2: Candidate genes from median survival QTL associations that had SNP differences corresponding to haplotype differences. The impact the SNP difference is predicted to have on the resulting protein is shown.

Table 2: Median Survival Time QTL Candidate Genes	
Gene	**Impact on protein**
6430550D23Rik	moderate
Bpifb2	moderate
Fer1l4	modifier
Myh7b	modifier
Nrsn2	modifier
Rbl1	modifier, moderate
Rpn2	modifier
Samhd1	moderate
Soga1	moderate
Sun5	modifier
Tldc2	low, modifier
Tmem74bos	moderate
Tti1	moderate

17

364   candidate genes with SNP differences. For percent of mice that survived to day 7, the

365   Chr 2 region had 503 genes, of these genes 51 had SNP differences matching

366   haplotype effects (Table 3). The Chr 4 region had 28 genes and *Stq2* had 115 genes,

367   but neither region had SNP differences corresponding to haplotype differences.

Table 3: Candidate genes from percent survival to Day 7 QTL associations that had SNP differences corresponding to haplotype differences. The impact the SNP difference is predicted to have on the resulting protein is shown.

Table 3: % Survived to Day 7 QTL Candidate Genes	
Gene	**Impact on protein**
6430550D23Rik	moderate
Arhgap40	moderate
Bpi	modifier, moderate
Bpifb2	moderate
Cd40	moderate
Dbndd2	modifier
Dhx35	modifier
Emilin3	moderate
Fer1l4	modifier
Jph2	moderate
L3mbtl1	modifier
Lbp	moderate
Lpin3	moderate
Mafb	moderate
Mmp9	moderate
Mybl2	moderate
Myh7b	low, modifier
Nrsn2	modifier
Ocstamp	moderate
Oser1	moderate
Pltp	moderate
Ptprt	moderate
R3hdml	moderate
Rbl1	modifier, moderate
Rpn2	modifier
Samhd1	moderate
Slc13a3	moderate
Snhg11	moderate
Soga1	moderate
Spata25	moderate
Sulf2	modifier
Sun5	modifier
Svs2	moderate
Sys1	modifier
Tldc2	low, modifier
Tmem74bos	moderate
Tomm34	moderate
Tox2	modifier, moderate
Trp53rka	moderate
Trp53tg5	modifier
Tti1	moderate
Wfdc15b	moderate
Wfdc5	moderate
Wfdc6b	moderate
Wfdc8	moderate
Zfp334	moderate
Zfp335	moderate
Zfp663	moderate
Zhx3	moderate
Zmynd8	moderate
Zswim1	moderate

368

369   **Discussion**

370   Although CC strains have been extensively used to identify genes influencing

371   susceptibility to viral infection (34–36,49–53), host determinants influencing the

372   outcomes of bacterial infection have only been addressed in a small number of studies

373   (37,39–41). We orally infected 32 CC strains of mice to study outcomes of STm

374   infection in genetically diverse strains of mice. Our goal in this 7 day screen of acute

375   STm infection was to identify the range of survival and colonization phenotypes and

376   putative genetic differences behind these phenotypes.

377   STm colonization and survival of CC mice after oral STm infection were highly

378   variable by strain, but were quite consistent within a strain. The 32 CC strains we tested

379   differed in their range of colonization in spleen >20,000 fold (2.92×10^2 to 7.8×10^8

380   CFU/g) and >1×10^6 fold (9.3×10^1 to 1.02×10^8 CFU/g) in liver. This wide range of

381   colonization phenotypes reflects the genetic diversity of the CC, and is a wider range

382   than represented in previous studies of the CC with STm (40,41). We identified 14 CC

383   strains that were very susceptible to STm infection that did not survive the 7 day

384   screening period. Several of these strains (CC025, CC021, CC013, and CC031)

385   developed signs of systemic infection in the same time frame as B6 (susceptible

386   control), but with lower bacterial colonization of both liver and spleen, suggesting

387   increased sensitivity to STm infection. One of the susceptible strains, CC019, is also

388   susceptible to intravenous infection with *M. tuberculosis* (37). Mice in the CC042 strain

389   developed very high bacterial loads in liver and spleen in our studies, consistent with

390   previous reports on intravenous infection with STm and infection with *M. tuberculosis*

391   (40,41). The phenotype of CC042 mice has been linked to a mutation in the integrin

20

392   alpha L gene (*Itgal*, also known as *Cd11a*) resulting in a defect in T cell recruitment

393   (39,41). Finally, the remaining 18 CC strains we screened survived the full study period.

394   Two of these strains, CC001 and CC002, were previously shown to display resistance

395   to *M. tuberculosis* infection (37).

396   The range of survival for CC strains in our experiments was four to seven days

397   (median), similar to those observed for B6 and CBA mice in our acute infection

398   paradigm. The limited survival range is likely due to technical parameters of our

399   experiment. Four days appears to be the fastest mice display symptoms of serious

400   systemic disease after STm infection using our current infection paradigm. Seven days,

401   the length of our screening period, is not long enough to capture the variability of longer

402   survival after acute infection. Previous studies using CC mice and STm infection have

403   not captured variability in survival after acute infection, because all mice were

404   euthanized at day 4 post-infection regardless of disease state (40).

405   A few CC strains had unique infection profiles relative to those of traditional

406   inbred mouse strains. CC051 mice were very poorly colonized and appeared healthier

407   than CBA mice (resistant control). Thus, CC051 mice are more resistant to STm

408   infection than previously studied strains, results consistent with low level colonization

409   after intravenous STm infection (40). CC045 mice have mutations in both *Slc11a1* and

410   *Ncf2*, both typically leading to poor survival after STm infection (14–16,18,19). While

411   CC045 mice had higher bacterial loads of STm similar to susceptible mice, they

412   survived the full study period and lost only ~7% of their body weight. Thus, CC045 is a

413   candidate "tolerant" strain, as it survives STm infection in the context of a bacterial

414   colonization load that would be lethal to an otherwise susceptible mouse. CC045 mice

21

415 must have compensatory mechanisms for survival despite the *Slc11a1* and *Ncf2*

416 mutations that remain to be discovered.

417   Sex is well known to influence both innate and adaptive immune responses (54)

418 as well as infection outcome (28,42,43,55,56) . We identified several CC strains that

419 exhibited sex differences in survival after STm infection (CC013, CC042, CC030, and

420 CC027, Figure 2). In keeping with the general hypothesis that females have stronger

421 innate and adaptive immune responses than males and are less susceptible to many

422 infections (56), CC027 and CC042 females survived longer than males. CC027

423 exhibited the most dramatic sex difference as males were susceptible and all became

424 moribund during the study period, while females survived. Consistent with the survival

425 data, CC027 males were more heavily colonized in spleen and liver than females:

426 (males: 4.80×10^6 and 1.63×10^6 CFU/g, females: 9.33×10^5 and 8.87×10^4 CFU/g,

427 respectively). However, for the remaining two strains that exhibited sex differences in

428 survival, CC013 and CC030, males survived longer than females. This finding is not

429 without precedent however, as female mice lacking leucine-rich repeat kinase-2 protein

430 (*Lrrk2*) are more susceptible to S. *Typhimurium* infection than males (57). Future work

431 focused on the sex differences we describe should uncover additional genes linked to

432 sexual dimorphism in susceptibility to bacterial infection.

433   Telemetry allows tracking of each mouse's body temperature and activity minute

434 by minute (58). These readings allowed us to define each mouse's unique diurnal

435 pattern of body temperature and activity, and to pinpoint the minute that each mouse

436 started to develop disease. On average, a change in diurnal rhythm of temperature

437 patterns were observable by telemetry 4,101 minutes (2.85 days) before infected mice

22

438 developed clinical signs observable to the eye (Supp. Fig. 10), similar to previous work

439 (58). Furthermore, our experiments mice that survived STm infection had a lower

440 baseline core body temperature prior to infection, a novel and unexpected finding. As

441 fever is generally thought to be disease fighting (59–62), we expected mice with higher

442 baseline temperatures to be able to mount a quicker fever response. Although female

443 B6 mice reportedly have a higher baseline body temperature than males, it is not known

444 whether this difference influences susceptibility to infection (63). Finally, lower ambient

445 temperature can lower the temperature and some parts of the body and impair antiviral

446 immune responses (64), but the effect of lower core temperature on susceptibility to

447 bacterial infections is unexplored.

448   When evaluating pre-infection baseline activity, surviving mice spent more time

449 resting than susceptible mice during 'active' periods. Increased rest in surviving strains

450 is unlikely to be the cause of lower core body temperature in these strains because it

451 only occurred during the active period, while the temperature difference was maintained

452 across all periods of the day. Furthermore, there was no significant difference in how

453 rapidly surviving and susceptible infected mice deviated from their normal circadian

454 pattern of activity after infection. However, susceptible mice deviated from their

455 temperature pattern significantly earlier than their activity pattern, suggesting that core

456 temperature is a more sensitive measurement to detect early disease.

457   Tissue damage had a small but significant correlation with survival time. Spleen

458 and liver exhibited severe damage across most strains. Splenic damage drove the small

459 correlation suggesting that liver damage, while more severe, is better tolerated than

460 splenic damage. Interestingly, there were some strains that exhibited quite severe

23

461 damage in both organs yet survived (CC011, CC017, CC045, CC072, and CC078) and

462 some strains that had milder damage and did not survive (CC005, CC025, and CC030).

463 While mice are typically categorized into resistant and susceptible, this variable damage

464 data suggests that there is more than one way to respond to infection. Discovery of

465 these phenotypes highlights the usefulness of the CC as a way to assay responses that

466 may be influenced by genetic diversity.

467   While we studied the response to an oral STm infection, others have examined

468 STm colonization after intravenous injection (40). Intravenous injection bypasses the

469 intestine and defenses in this niche. Eighteen CC strains were shared between our

470 study and previous work (40). When median colonization in liver and spleen was

471 compared, the oral infection route (current infection) produced higher bacterial burdens

472 and a wider range of colonization in general (liver: 10^4 to 10^6 CFU/g vs 10^3 to 10^4 CFU/g

473 and spleen:10^3 to 10^7 CFU/g vs 10^4 vs 10^5 CFU/g, excluding CC042) (Fig. 2). Possible

474 reasons for the differences in colonization levels include different route of delivery,

475 different time to necropsy and bacterial strain differences (40). CC051 was an

476 exception, with lower bacterial counts in the spleen after the oral infection versus IV

477 infection. This finding suggests that CC051 mice are better at clearing intestinal STm,

478 are better able to contain STm in the intestine, or are better at clearing STm prior to

479 sustained systemic infection. CC051 is a model to explore true resistance to STm

480 infection as it was the only strain that appeared to control systemic spread and systemic

481 growth of STm after oral infection (1,65).

482   We analyzed all of the parameters we collected to identify putative linked

483 genomic regions using gQTL (46). Only survival time yielded significant, 0.85 or higher,

24

484   associations. Intervals on Chr 1 contained *Slc11a1* and *Ncf2*, known genes that have

485   mutations linked to poor survival after STm infection (2,14–16,18–20). One strain,

486   CC045, with *Slc11a1* and Ncf2 mutations survived the entire study period, as did six of

487   twelve strains with *Ncf2* mutations. These strains must have alternative/compensatory

488   mechanisms that allow them to overcome *Slc11a1* and *Ncf2* mutations. CC045 was

489   highly colonized in both spleen and liver, making it a candidate for 'tolerance' to STm

490   infection. The genetic basis for the ability of CC045 to survive STm infection in the face

491   of these two mutations requires further exploration.

492   In addition to the intervals on Chr 1, suggestive and significant intervals on Chr 2,

493   4, and 7 were also identified by gQTL analysis. The intervals on Chr 2 and 4 do not

494   contain any genes previously linked to STm infection, but the interval on Chr 7 overlaps

495   with a previously discovered QTL region, *Ses2* (47,48). Genes linked to STm

496   susceptibility underlying *Ses2* interval have not been identified. These regions were

497   narrowed further as described above into a candidate gene list.

498   The suggestive QTL interval on Chr 2 had 13 candidate genes linked to median

499   survival time after considering haplotypes (Table 2). The suggestive interval on Chr 2

500   for percent of mice surviving to day seven had 51 candidate genes remaining (Table 3).

501   Promising genes in this region include: BPI fold containing family B, member 2 (*Bpifb2*),

502   and SAM domain and HD domain, 1 (*Samhd1*). *Bpifb2* is a member of the lipid-transfer

503   protein family and is predicted to have bactericidal and permeability-increasing

504   properties, making it a promising candidate for further investigation (66). *Samhd1*

505   regulates interferon pathways in response to viral infections through NF-KB (67,68).

506   While *Samhd1* is linked to the immune response in viral infections, it may play a

25

507 previously unknown role in immunity against bacterial pathogens. Further investigation

508 is needed to prove such a link. In both median survival and percent survived to day

509 seven QTL intervals for Chr 4 and 7, haplotype differences did not help us narrow the

510 gene list. RNA expression analysis, currently underway, should identify candidate genes

511 for these intervals.

512 We have carefully characterized the clinical course of STm infection using a

513 population of genetically diverse Collaborative Cross mice and novel tools including

514 telemetry analysis. We have identified novel responses to STm infection, sex

515 differences in survival after STm infection, and determined that lower baseline body

516 temperature is correlated with survival of STm infection. We defined CC strains bearing

517 *Slc11a1* or *Ncf2* mutations that unexpectedly survive acute STm infection. Finally, we

518 identified both known and novel regions of the mouse genome associated with survival

519 time after STm infection. Overall, this research opens a rich area for discovery into the

520 host genetics underlying the complex host response to bacterial infections.

521

528

Materials and Methods

Bacterial strains and media

The *Salmonella enterica* ser. Typhimurium strain (HA420) used for this study was derived from ATCC14028. HA420 is a fully virulent, spontaneous nalidixic acid resistant derivative of ATCC14028 (69). Strains were routinely cultured in Luria-Bertani (LB) broth and plates, supplemented with antibiotics when needed at 50 mg/L nalidixic acid (Nal).

For murine infections, strains were grown aerobically at 37°C to stationary phase in LB broth with nalidixic acid and diluted to generate an inoculum of 2-5 x10^7 organisms in 100 microliters. Bacterial cultures used as inocula were serially diluted and plated to enumerate colony forming units (CFU) and determine the exact titer.

Murine Strains

Both conventional mice (C57BL/6J and CBA/J) and Collaborative Cross (CC) mice were utilized in these experiments. All Collaborative Cross strains were obtained from UNC's Systems Genetics Core Facility (SGCF) and either used directly for experiments or subsequently bred independently at Division of Comparative Medicine at Texas A&M University prior to these experiments (Supp. Table 2). Our experiments utilized 32 strains of CC mice, 3 females and 3 males per strain for a total of 228 mice (CC045 only had 2 females due to a surgical complication) (Supp. Table 2). Mice were fed Envigo Teklad Global 19% Protein Extruded Rodent Diet (2919) or Envigo Teklad Rodent Diet (8604) based on strain need.

Ethics Statement

All animal experiments were conducted in accordance with the Guide for the

Care and Use of Laboratory Animals, were reviewed by the TAMU IACUC and under

AUP#s 2018-0488 D and 2015-0315 D.

Placement of Telemetry Devices

5 to 9-week-old mice (C57BL/6, CBA, CC strains) were anesthetized with an

isoflurane vaporizer for surgical telemetry device placement. The abdomen was opened

with a midline abdominal incision (up to 2 cm). Starr life science G2 E-mitter devices

were loosely sutured to the ventral abdominal wall. The abdominal muscle layer was

closed with 5-0 vicryl, and the skin layer was closed using stainless steel wound clips

(FST 9mm). Animals were given an intraperitoneal injection of Buprenorphine (0.0001

mg/g) for pain prior to recovery from anesthesia, and every 8 hours thereafter as

necessary for pain control. After recovery from anesthesia, implanted mice were group-

housed and monitored twice daily for pain and wound closure for 7 days post-surgery,

when the clips were removed. Any animals found to have serious complications after

surgery were humanely euthanized.

Infection with *Salmonella* Typhimurium

After 4-7 days of acclimation in the BSL-2 facility, 8 to 12-week-old implanted

mice were weighed and infected by gavage with a dose of 2-5 x 10^7 CFU of *S.*

28

572   Typhimurium HA420 in 100 microliters of LB broth. Infected mice were monitored twice

573   daily for signs of disease and activity by visual inspection. When telemetry and health

574   condition data suggested the development of clinical disease from infection, mice were

575   humanely euthanized. Animals that remained clinically healthy throughout the duration

576   of the experiment were humanely euthanized at 7 days post-infection.

577

578   Bacterial load determination

579   Mice were humanely euthanized by CO_2 asphyxiation, and the spleen, liver,

580   ileum, cecum, and colon were collected. A third of each organ was collected in 3 mL of

581   ice-cold PBS, weighed, homogenized, serially diluted in PBS, and plated on Nal plates

582   for enumeration of S. Typhimurium in each organ. Data are expressed as CFU/g of

583   tissue.

584

585   Telemetry Monitoring

586   Prior to placing implanted mice on telemetry platforms, mice were moved into

587   individual cages and provided with a cardboard hut and bedding material. Individual

588   cages containing implanted mice were placed onto ER4000 receiver platforms, and

589   body temperature (once per minute) and gross motor activity (continuous measurement

590   summed each minute) data was collected. Body temperature and gross motor activity

591   data were collected for 4-7 days pre-infection. Mice were removed briefly from the

592   receiver platforms for infection and then were placed back on the platforms and data

593　collection by telemetry was resumed. Infected animals were continuously monitored by

594　telemetry in additional to twice daily visual assessment.

595

Identification of deviation from circadian pattern of body temperature

597　　　　Additional clinical information, such as the time of inoculation relative to the start

598　of the experiment (denoted T), was reported and used in centering time series for

599　comparison between mice (typically, seven days after the beginning of monitoring).

600　Quantitative detection of deviation from the baseline "off-pattern," using temperature

601　data was calculated on an individual basis. A temperature time series was filtered, a

602　definition of healthy variation was defined, then the time of first "off-pattern" was

603　calculated using that definition on post-inoculation data.

604　　　　Each mouse time series was preprocessed using a moving median filter with a

605　one-day window. For a specific minute t, the median collection of temperature values

606　from [t-720, t+720] was used in calculating a median for the value t. After this

607　processing, healthy variation was defined as any temperature falling within the range of

608　minimum to maximum values during the pre-inoculation phase [T-5760,T] (5760

609　minutes = 4 days). This choice allowed for enough data to account for natural inter-day

610　variation due to potential factors such as inter-strain variation, epigenetic differences,

611　and sex differences, while avoiding bias due to observed acclimation time after transfer

612　to a new facility in some mice in the first few days of observation (Supp. Fig. 11A).

613　　　　Identifying post-inoculation off-pattern behavior was done by identifying

614　temperature values that fall outside the interval of healthy variation. The post-inoculation

615   interval ranges from [T+60,T+10080] (7 days), where the one hour gap was used to

616   avoid false positive detection due to the physical disturbance associated with

617   inoculation (Supp. Fig. 11A).

618

619   Detection of deviation from circadian pattern of activity

620   While activity data does exhibit circadian patterns, this data necessitated a

621   different approach to preprocessing compared to temperature data because activity

622   values are inherently non-negative and have a modal value of zero. Two approaches

623   were taken. One was from the perspective of parameter estimation of iid sampling from

624   a statistical distribution. The second approach was based on plainly calculating the

625   fraction of non-zero activity values measured in a moving window. Hence, we

626   approached the analysis of activity data from the perspective of determining the

627   parameters of a stochastic process. In the first approach, for a given time interval t, we

628   worked from the assumption that the number of activity values observed to be i obeys

629   the following distribution:

$$ln(p(A(t) = i)) = \alpha + \beta i$$

630   where the coefficient β, expected to be negative, corresponds to the modeling

631   assumption that the relative drop in observed activity counts ought to decay (note

632   0<e^β<1 if β is negative). For instance, if the value of β= -0.693, so that $e^{\beta} \approx 1\sqrt{2}$, this

633   would represent an assumption that there are half as many activity values observed to

634   be 1 (one movement per minute) than 2 (two movements per minute). In actuality, this

31

635   decay coefficient is typically seen to be β≈-0.025 to β≈-0.015, representing that

636   observed activity values decay by half around every 27-46 values.

637   This theory is implemented in practice by windowing a mouse's activity time

638   series, creating a binning (empirical distribution) $(i, c(i))$,, the performing a log-linear fit

639   by transforming $c(i) \rightarrow ln(1 + c(i))$ and applying linear least-squares regression to

640   calculate α and β. Statistical models with simpler assumptions stemming from a

641   "memoryless" assumption were attempted but did not see any feasible agreement with

642   observed activity data (Supp. Fig. 11B).

643   The second approach mentioned above produced a qualitatively cleaner signal,

644   which again is based on windowing a mouse's activity time series, and calculating the

645   fraction of activity values in a window for which A(t)>0. One might expect not weighting

646   by intensity of activity loses information, but we saw this approach to filtering activity

647   data resulted in a time series for which we could apply the same methods as with the

648   temperature time series (Supp. Fig 11B)

649

650   Histopathology

651   After euthanasia, samples of liver, spleen, ileum, cecum, and colon from each

652   mouse were collected and fixed in 10% neutral buffered formalin at room temperature

653   for 24 hours and stored in 70% ethanol before embedding in paraffin, sectioning at 5 μm

654   and staining with hematoxylin and eosin (H&E). Histologic sections of all tissues were

655   evaluated in a blinded manner by brightfield microscopy and scored on a scale from 0 to

656   4 for tissue damage by a board-certified veterinary pathologist (Supp. Table 1 and

32

657 Supp. Fig. 4). The combined scores of spleen and liver from individual mice were used

658 to calculate the median and interquartile range for each group. Whole slide images of

659 liver and spleen H&E-stained sections were captured as digital files by scanning at 40x

660 using a 3DHistech Pannoramic SCAN II FL™ scanner (Epredia, Kalamazoo, MI). Digital

661 files were processed by Aiforia Hub™ (Aiforia, Cambridge, MA) software for generating

662 the images with 100 µm scale bars.

663

664 Complete Blood Count

665   For some mice, whole blood was collected by submandibular bleed for complete

666 blood count (CBC) one week prior to surgery for the pre-infection sample to determine

667 each mouse's baseline. Blood was also collected at necropsy by cardiac puncture.

668 Additional blood was collected from uninfected control animals by cardiac puncture after

669 euthanasia and median values were used as the baseline for mice that were not their

670 own pre-infection control. All blood was collected into EDTA tubes and analyzed on an

671 Abaxis VetScan HM5.

672

673 Cytokine and Chemokine Analysis

674   Serum was stored at $-80^{o}C$ and thawed on ice immediately prior to cytokine

675 assays. Serum cytokine levels were evaluated using an Invitrogen ProcaratPlex

676 Cytokine/Chemokine Convenience Panel 1A 36 plex kit as per manufacturer

677 instructions (ThermoFisher). Briefly, magnetic beads were added to each well of the 96-

678 well plate and washed on the Bio-Plex Pro Wash Station. 25uL of samples and

33

679   standards were then added to the plate along with 25uL of universal assay buffer.

680   Plates were then shaken at room temperature for 1 hour and washed. 25uL of detection

681   antibody was added an incubated for 30 minutes and washed away, followed by 50uL of

682   SAPE for 30 minutes and washed away. 120uL of reading buffer was added and plates

683   were evaluated using a Bio-Plex 200 (BioRad). Samples and standards were run in

684   duplicate, and samples were diluted as needed to get 25ul of serum per duplicate. The

685   kit screens for the following 36 cytokines and chemokines: IFN gamma; IL-12p70; IL-13;

686   IL-1 beta; IL-2; IL-4; IL-5; IL-6; TNF alpha; GM-CSF; IL-18; IL-10; IL-17A; IL-22; IL-23;

687   IL-27; IL-9; GRO alpha; IP-10; MCP-1; MCP-3; MIP-1 alpha; MIP-1 beta; MIP-2;

688   RANTES; Eotaxin; IFN alpha; IL-15/IL-15R; IL-28; IL-31; IL-1 alpha; IL-3; G-CSF; LIF;

689   ENA-78/CXCL5; M-CSF. Median values of uninfected animals for each strain were used

690   as the baseline for that strain.

691

692   QTL analysis

693   gQTL, an online resource designed specifically to run CC QTLs, was used to

694   identify putative QTLs (46). Briefly, median values of each strain for various parameters

695   were uploaded to the website and QTLs were run using 1000 permutations with

696   "automatic" transformation. Automatic picks either log or square root transformations,

697   whichever normalizes the data best.

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Figure 1: CC strains infected orally with STm are more highly and variably colonized than after IV infection. A. Liver and B. spleen colonization (median) for 18 CC strains orally infected in this project and infected intravenously in the previous work by Zhang, J. et al. 2018. Strains are connected by lines, and the red line represents CC051.

Figure 2: CC strains have variable responses to STm infection. A. Survival time and B. percent weight change after infection with STm. Strains are in ascending order of survival and weight change if survival was equal between strains. Bacterial burden in C. liver and D. spleen. Dots represent individual mice; black dots represent males and grey dots represent females. Median and interquartile range are shown by lines for each strain. *Slc11a1* and *Ncf2* status shown by +/-. 32 CC strains and 2 controls (B6 and CBA) represented.

Figure 3: Sex differences in survival after STm infection. A. Survival time and B. percent weight change after infection with STm. Strains are in ascending order of survival and weight change if survival was equal between strains. Bacterial burden in C. liver and D. spleen. Dots represent individual mice; black dots represent males and grey dots represent females. Median and interquartile range are shown by lines for each strain. Eight CC strains are represented. (* P <0.05).

Figure 4: At pre-infection baseline, susceptible strains have higher body temperatures, are more active during normal rest period, and maintain activity

45

patterns longer than temperature patterns than surviving strains. Resting period, 24-hour period, and active period strain medians shown for A. temperature and B. percent of time active. C. Time to deviation from baseline activity and temperature post-infection (days). Strains are grouped by survival status. Median overall values and interquartile range are indicated. (* P <0.05, ** P <0.01, *** P <0.001).

Figure 5: Spleen and liver damage correlate weakly with survival. A. Median Liver (black) and spleen (grey) histopathology scores for each strain. Scored on a scale of 0 to 4, with 0 being normal tissue and 4 being severely damaged tissue. B. Spleen (R^2 = 0.1323, *P* = <0.0001) and C. liver (R^2 = 0.08382, *P* = <0.0001) individual linear regression of histopathology scores. D. Linear regression of CC strains on combined spleen and liver histopathology scores (R^2 = 0.1256, *P* = <0.0001). 95% confidence interval of regressions line is shown by dotted line. Medians and interquartile ranges are indicated.

Figure 6: QTLs for survival and percent of mice surviving identified using 30 CC strains. A. QTL of median time survived after infection and B. allele effect plots focused on Chr 2 and Chr 4. C. QTL of percent of mice surviving to day 7 and D. allele effect plots focused on Chr 7. Green line designates 85% significance, blue line designates 90% significance. Results obtained using gQTL.

Table 1: QTL associations for median survival time and percent survived to day 7 after STm infections. 30 CC strains were included in the gQTL analysis, excluding CC042 and CC045.

Table 2: Candidate genes from median survival QTL associations that had SNP differences corresponding to haplotype differences. The impact the SNP difference is predicted to have on the resulting protein is shown.

Table 3: Candidate genes from percent survived to day 7 QTL associations that had SNP differences corresponding to haplotype differences. The impact the SNP difference is predicted to have on the resulting protein is shown.

Supplemental Figure 1: Survival is correlated to CFU and strongly correlated to % weight change. Strain medians for 32 CC strains were analyzed using Spearman correlation. Blue detonates positive correlations and red detonates negative correlations.

Supplemental Figure 2: Survival for susceptible strains is weakly correlated to CFU and strongly correlated to % weight change. Strain medians for 14 CC strains that are susceptible were analyzed using Spearman correlation. Blue detonates positive correlations and red detonates negative correlations.

Supplemental Figure 3: Each strain has a unique circadian pattern before infection. Purple lines represent the average temperature and black lines represent the average fraction of time the mouse is active pre-infection for 7 days. The gray area represents +/- 2 standard deviations. A-N are susceptible strains, O-AF are surviving strains, and AG-AH are control strains.

Supplemental Table 1: Scoring matrix with descriptions. Final score per organ corresponds to the highest score assigned to a single category. Scoring matrix from Dr. L. Garry Adams.

Supplemental Figure 4: Representative images of spleen and liver histology scoring scheme. Tissues were sectioned and stained with H&E before being analyzed. Images from Dr. L. Garry Adams illustrating scoring matrix in Supp. Table 1.

Supplemental Figure 5: Intestinal organs were minimally damaged after STm infections. Median ileum (gray) and cecum/colon (black) histopathology scores. Scored on a logarithmic scale of 0 to 4, with 0 being normal tissue and 4 being severely damaged tissue.

Supplemental Figure 6: **White blood cell numbers are not significantly different between surviving and susceptible strains.** Strain medians for change in blood count (infected-uninfected) for A. total white blood cells, B. neutrophils, C. monocytes, and D. lymphocytes, grouped by survival status.

Supplemental Figure 7: **QTL of slope of survival curve.** This value defines how rapidly each strain succumbed to infection. QTL analysis included 30 CC strains, excluding CC042 and CC045.

Supplemental Figure 8: **Founder effects plots for QTL of median time survived**. 30 CC strains, included, after removal of CC042 and CC045. Founder effect plots for A. Chr 6 and B. 7 focused on the QTL peak, genes within the region are also shown. Results obtained using gQTL analysis.

Supplemental Figure 9: Founder effect plots of QTL of percent of mice surviving to day 7. Analysis included 30 CC strains, after removal of CC042 and CC045. Founder effect plots for A. Chr 2 and B. 4 focused on the QTL peak, genes within the region are also shown. Results obtained using gQTL analysis.

Supplemental Figure 10: 32 CC strains have earlier disruption of circadian pattern than clinical signs of disease. Time from first change in temperature pattern subtracted from time that mouse developed visually observable clinical signs of disease. Mean and standard deviation shown by strains and individual mice shown by dots.

Supplemental Table 2: Number of mice used and facility of origin for 2 control strains and 32 CC strains.

Supplemental Figure 11: **Telemetry calculations images.** Examples of how A. temperature and B. activity calculations were determined. Specifically maximum, median, minimum pre-infection temperature and activity as well "off-pattern" for post-infection.

S1 Table: Baseline Complete Blood Counts from uninfected mice for 32 CC strains and 2 control strains (B6 and CBA). Mean and standard deviation are shown. See excel sheet.
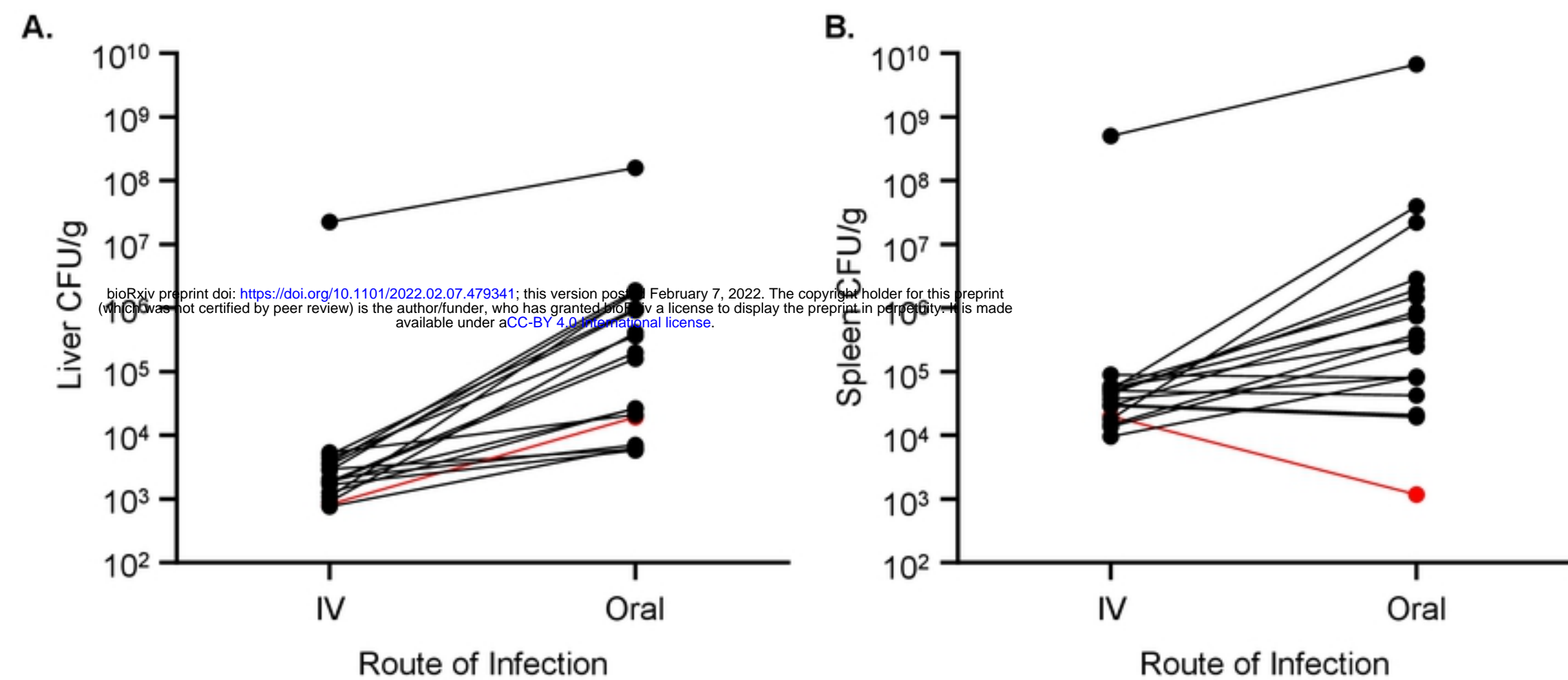
S2 Table: Change in Complete Blood Counts pre- to post-infection. The difference between white blood cell counts after infection versus pre-infection was determined for 32 CC strains and 2 control strains (B6 and CBA). Mean and standard deviation are shown. See excel sheet.

S3 Table: Baseline level for 36 cytokines prior to infection. Mean and standard deviation are shown for 28 CC strains as well as two controls (B6 and CBA). See excel sheet.

S4 Table: Change in cytokine amount for each CC strain across infection. The differences between cytokine amounts from the serum of infected animals and the cytokine amount from uninfected animals was determined for 28 CC strains and 2 control strains (B6 and CBA). Mean and standard deviation are shown. See excel sheet.

Figure 1

Figure 2
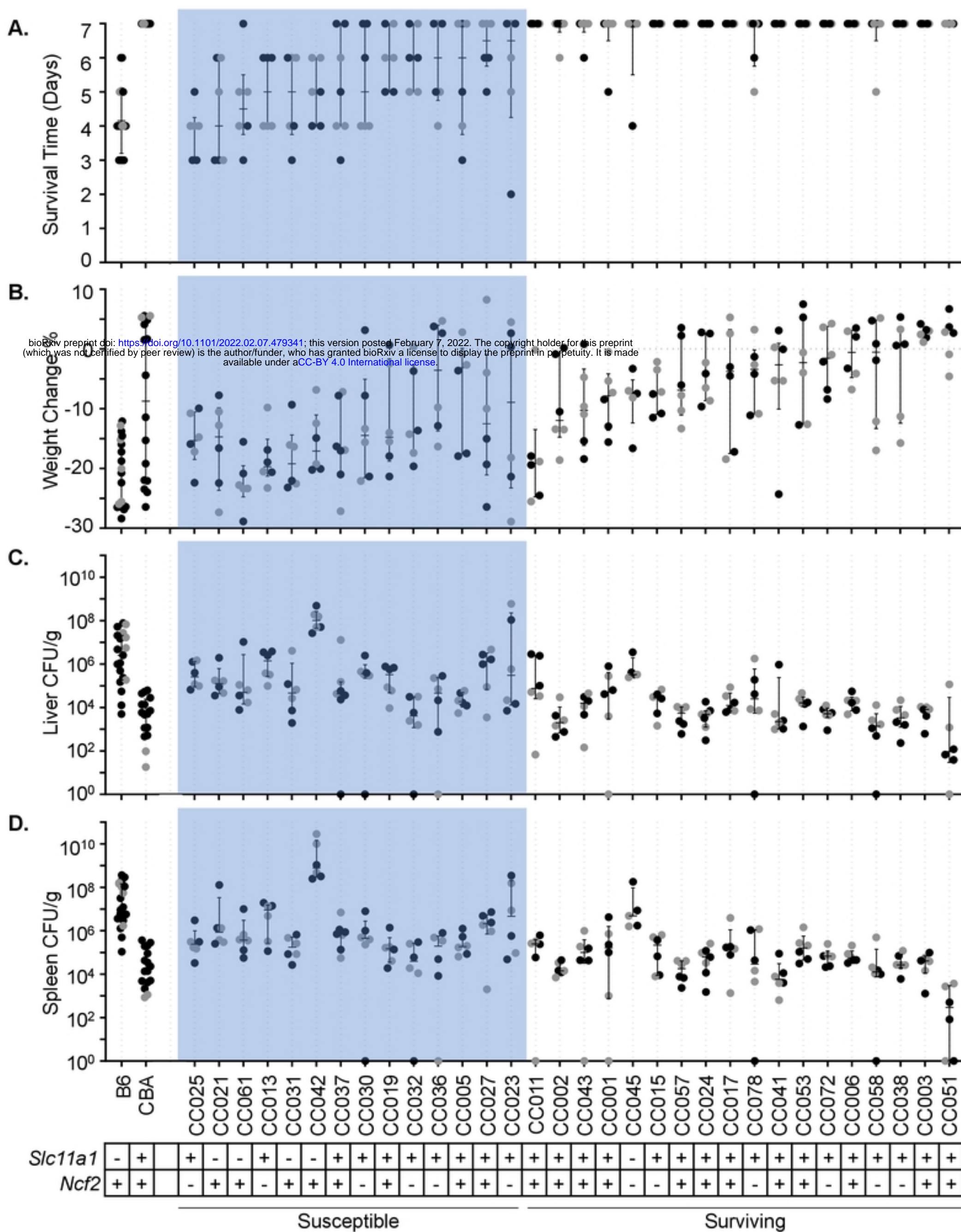
Figure 3
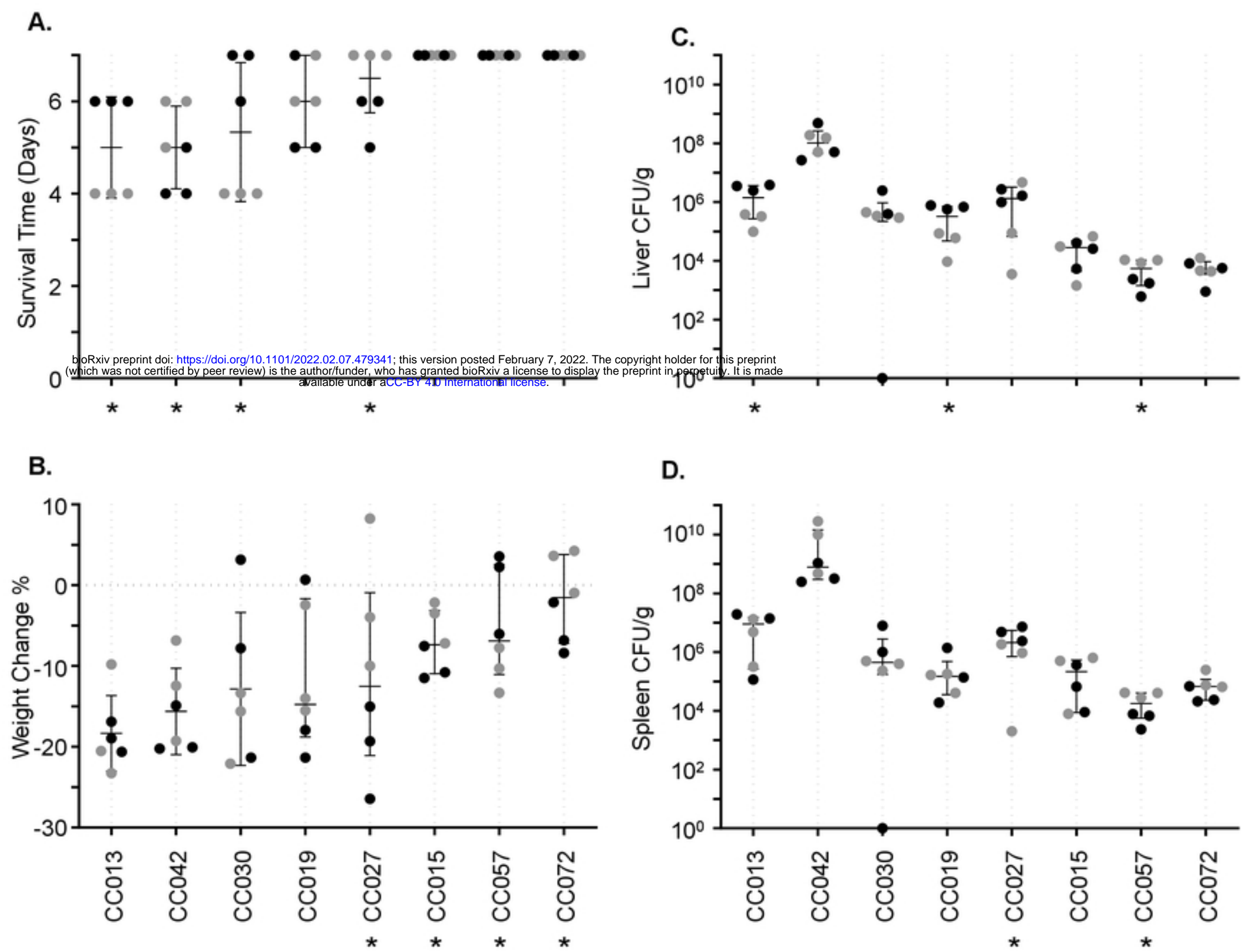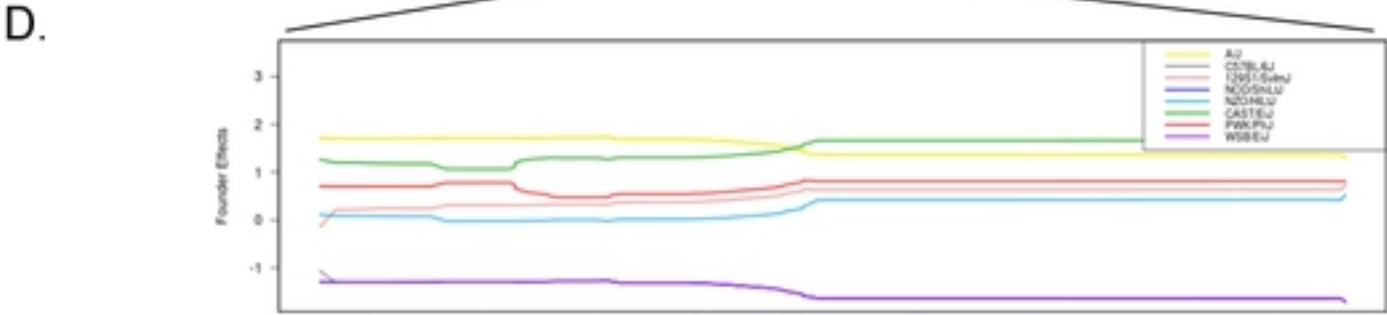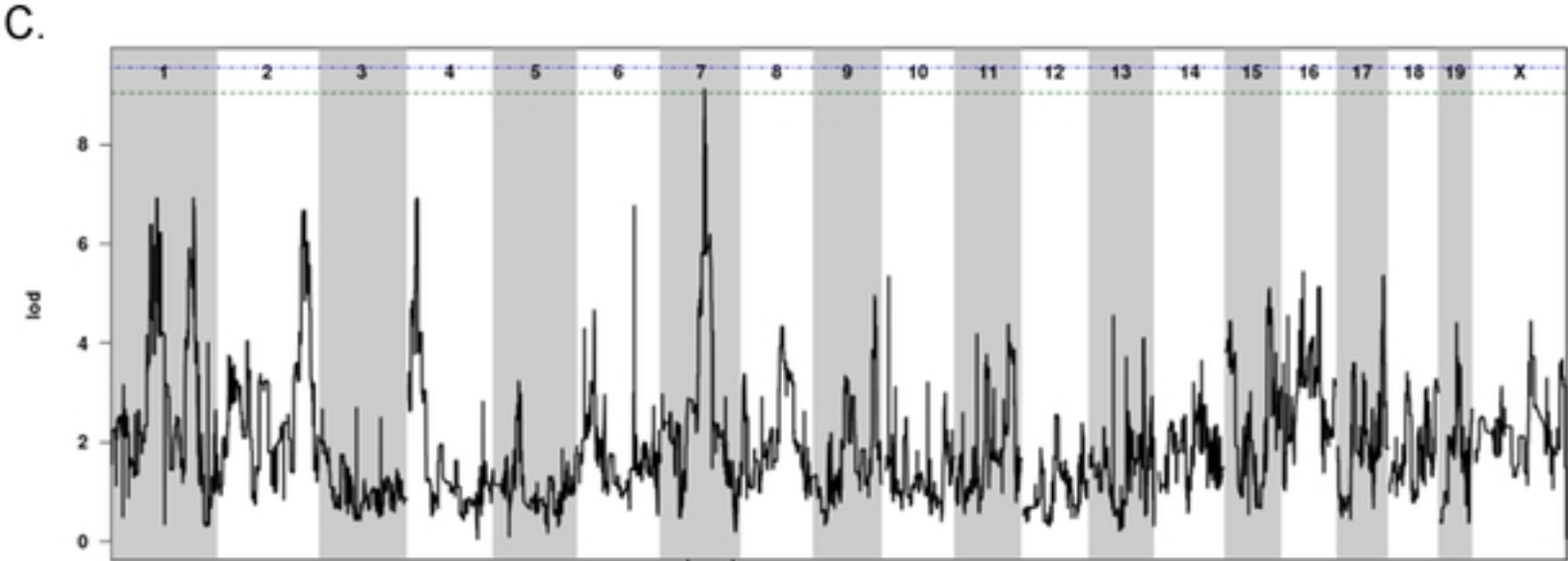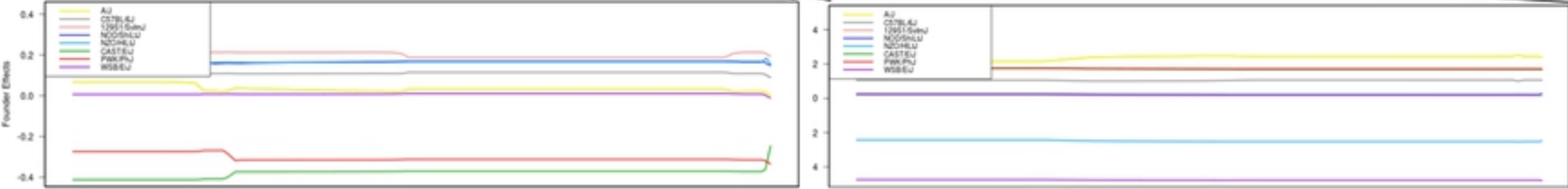
Figure 4

Figure 5

Figure 6

Table 1: QTL suggestive and significant peaks

QTL name	Phenotype	Chr	LOD	P-value	Proximal (Mb)	Max (Mb)	Distal (Mb)	Haplotype Effects
	survival time	1	8.24	3.09×10^{-6}	73.96	152.07	152.07	
	survival time	2		$\times 10^{-5}$	151.39	157.79	158.05	low: PWK, CAST
Stq1	survival time	4	9.42	2.78×10^{-7}	16.30	19.51	19.63	low: WSB, NZO
	survival time	7	7.24	2.27×10^{-5}	78.07	79.71	90.04	low: WSB, NOD, B6
	% survived to day 7	1	6.91	4.31×10^{-5}	73.97	152.07	154.46	
	% survived to day 7	2	6.66	7.08×10^{-5}	151.01	157.79	166.40	low: PWK, CAST
	% survived to day 7	4	6.91	4.33×10^{-5}	16.12	17.67	19.72	low: WSB, NOD, B6
Stq2	% survived to day 7	7	9.11	5.29×10^{-7}	78.40	79.42	82.00	low: WSB, NOD, B6

Table 2: Median Survival Time QTL Candidate Genes

Gene	Impact on protein
6430550D23Rik	moderate
Bpifb2	moderate
Fer1l4	modifier
Myh7b	modifier
Nrsn2	modifier
Rbl1	modifier, moderate
Rpn2	modifier
Samhd1	moderate
Soga1	moderate
Sun5	modifier
Tldc2	low, modifier
Tmem74bos	moderate
Tti1	moderate

Table 3: % Survived to Day 7 QTL Candidate Genes

Gene	Impact on protein
6430550D23Rik	moderate
Arhgap40	moderate
Bpi	modifier, moderate
Bpifb2	moderate
Cd40	moderate
Dbndd2	modifier
Dhx35	modifier
Emilin3	moderate
Fer1l4	modifier
Jph2	moderate
L3mbtl1	modifier
Lbp	moderate
Lpin3	moderate
Mafb	moderate
Mmp9	moderate
Mybl2	moderate
Myh7b	low, modifier
Nrsn2	modifier
Ocstamp	moderate
Oser1	moderate
Pltp	moderate
Ptprt	moderate
R3hdml	moderate
Rbl1	modifier, moderate
Rpn2	modifier
Samhd1	moderate
Slc13a3	moderate
Snhg11	moderate
Soga1	moderate
Spata25	moderate
Sulf2	modifier
Sun5	modifier
Svs2	moderate
Sys1	modifier
Tldc2	low, modifier
Tmem74bos	moderate
Tomm34	moderate
Tox2	modifier, moderate
Trp53rka	moderate
Trp53tg5	modifier
Tti1	moderate
Wfdc15b	moderate
Wfdc5	moderate
Wfdc6b	moderate
Wfdc8	moderate
Zfp334	moderate
Zfp335	moderate
Zfp663	moderate
Zhx3	moderate
Zmynd8	moderate
Zswim1	moderate