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# Genetic Diversity of Collaborative Cross Mice Controls Viral Replication, Clinical Severity, and Brain Pathology Induced by Zika Virus Infection, Independently of Oas1b

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1 **Title: Genetic diversity of Collaborative Cross mice controls viral replication,**  
2 **clinical severity and brain pathology induced by Zika virus infection,**  
3 **independently of *Oas1b*.**

4 Running title: Host genetic susceptibility to Zika virus disease

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24

25 **ABSTRACT**

26 The explosive spread of Zika virus (ZIKV) has been associated with major variations in severe  
27 disease and congenital afflictions among infected populations, suggesting an influence of host  
28 genes. We investigated how genome-wide variants could impact susceptibility to ZIKV infection  
29 in mice. We first describe that the susceptibility of *Ifnar1* knockout mice is largely influenced by  
30 their genetic background. We then show that the broad genetic diversity of Collaborative Cross  
31 mice, of which receptor to type I interferon (IFNAR) was blocked by anti-IFNAR antibody,  
32 expressed phenotypes ranging from complete resistance to severe symptoms and death with large  
33 variations in the peak and rate of decrease of plasma viral load, in brain viral load, in brain  
34 histopathology and in viral replication rate in infected cells. Differences in susceptibility between  
35 CC strains were correlated between Zika, Dengue and West Nile viruses. We identified highly  
36 susceptible and resistant mouse strains as new models to investigate the mechanisms of human  
37 ZIKV disease and other flavivirus infections. Genetic analyses revealed that phenotypic  
38 variations are driven by multiple genes with small effects, reflecting the complexity of ZIKV  
39 disease susceptibility in human population. Notably, our results rule out a role of the *Oas1b* gene  
40 in the susceptibility to ZIKV. Altogether, this study emphasizes the role of host genes in the  
41 pathogeny of ZIKV infection and lays the foundation for further genetic and mechanistic studies.

42

43 **IMPORTANCE**

44 In recent outbreaks, ZIKV has infected millions of people and induced rare but potentially severe  
45 complications, including Guillain-Barré syndrome and encephalitis in adults. While several viral

46 sequence variants were proposed to enhance the pathogenicity of ZIKV, the influence of host  
47 genetic variants in mediating the clinical heterogeneity remains mostly unexplored. We have  
48 addressed this question using a mouse panel which models the genetic diversity of human  
49 population and a ZIKV strain from a recent clinical isolate. Through a combination of *in vitro*  
50 and *in vivo* approaches, we demonstrate that multiple host genetic variants determine viral  
51 replication in infected cells, and clinical severity, kinetics of blood viral load and brain pathology  
52 in mice. We describe new mouse models expressing high susceptibility or resistance to ZIKV  
53 and to other flaviviruses. These models will facilitate the identification and mechanistic  
54 characterization of host genes that influence ZIKV pathogenesis.

55

56 **KEYWORDS**

57 Zika virus, flavivirus, mouse model, host genetics, genetic diversity, Collaborative Cross

58

59

60 Zika virus (ZIKV) is a mosquito-borne flavivirus isolated in 1947 from a febrile rhesus  
61 monkey in Uganda (1). Until 2007, ZIKV had circulated in Africa and Asia causing mild flu-like  
62 syndromes, with rare reported clinical cases (2). However, during recent epidemics, ZIKV  
63 infection triggered severe complications including Guillain-Barré syndrome and encephalitis in  
64 adults (3, 4), and congenital malformations in fetuses of infected pregnant women (5, 6). Viral  
65 mutations may have contributed to ZIKV enhanced pathogenicity (7, 8) but only partly explain  
66 the variable proportion of symptomatic infections between populations (9) and the increased  
67 incidence of congenital Zika syndrome (CZS) in Polynesia (10) and Brazil (11), suggesting a  
68 role for host genetic variants. Recent evidence indicates that the regulation of innate immunity  
69 genes is driven by host genetic background in human fetal brain-derived neural stem cells  
70 (hNSCs) infected *in vitro* with ZIKV (12). Additionally, the analysis of pairs of dizygotic twins  
71 exposed to ZIKV during pregnancy and discordant for CZS suggests multigenic host  
72 susceptibility to ZIKV-induced brain malformations (13).

73 Multiple mouse models have been proposed to decipher the mechanisms of ZIKV disease  
74 pathogenesis (14, 15). These models allow the investigation of several key features of human  
75 infection, such as neuronal damage (16, 17), sexual and vertical transmission (18-21), fetal  
76 demise and CZS (22-25). However, while non-structural ZIKV proteins efficiently inhibit innate  
77 antiviral responses in humans (26, 27) allowing viral replication, ZIKV replicates poorly in wild-  
78 type mice due to the inability of its NS5 protein to antagonize STAT2 and type I interferon (IFN)  
79 response as in humans (28). Effective systemic infection in mice occurs when this response is  
80 abrogated by genetic inactivation of the *Ifnar1* gene (29) or by blocking the type I IFN receptor  
81 with the MAR1-5A3 monoclonal antibody (mAb) (30, 31). So far, host genetic factors involved  
82 in mouse susceptibility to ZIKV infection have been investigated mainly through reverse genetic  
83 approaches, by studying the consequences of genetic ablation of specific genes, such as innate or

84 adaptive immunity genes (29, 32-35). While these models have contributed to our understanding  
85 of the mechanisms of ZIKV disease, they do not model the simultaneous contribution of variants  
86 in multiple pathways, as would most likely be observed in the natural population. A recent study  
87 has reported strain-specific differences in the susceptibility to neonatal ZIKV infection across  
88 four mouse laboratory strains, affecting neuropathology and behavior in adulthood (36). More  
89 extensive studies investigating the role of genome-wide genetic variations on the susceptibility to  
90 ZIKV infection are needed, using mouse models that reflect the phenotypic and genetic diversity  
91 of the human population (37).

92         In this study, we addressed this question using two types of susceptible mouse models.  
93 First, since the phenotype of a single gene modification often varies under the influence of  
94 modifier genes (38, 39), we assessed the effect of host genetic background on the susceptibility  
95 of *Ifnar1*-deficient mice. We then investigated the impact of host genetic diversity on the  
96 susceptibility to ZIKV infection in the Collaborative Cross (CC), a panel of recombinant inbred  
97 mice produced via a funnel breeding scheme that combined eight founder inbred strains,  
98 including five classic laboratory strains and three wild-derived strains (40). As a result, every CC  
99 strains has inherited a unique and balanced contribution for each of the eight founder strains.  
100 These founder strains capture approximately 90% of the genetic variants present in the *Mus*  
101 *musculus* species (41) and the resulting CC strains, which segregate an estimated 45 million  
102 polymorphisms, have more genetic diversity than the human population (42). Extensive  
103 variations in pathogenic phenotypes have been previously reported in the CC panel after viral  
104 (43-50), bacterial (51, 52) and fungal (53) infections, demonstrating that this resource is ideally  
105 suited for investigating the role of host genetic variants in the pathophysiology of infectious  
106 diseases (54).

107        Susceptibility to ZIKV in *Ifnar1*-deficient mice was strongly influenced by the genetic  
108 background, which has practical implications for future virology studies to identify modifier  
109 genes. The challenge of 35 immunocompetent CC strains with ZIKV after MAR1-5A3 mAb  
110 treatment allowed efficient viral replication. We show that genetic diversity in the CC panel  
111 enabled large variations in the clinical severity of ZIKV disease, in the peak and kinetics of  
112 plasma viral load and in the severity of ZIKV-induced brain pathology. Genetic diversity also  
113 resulted in differences in the permissiveness of CC mouse cells to viral replication which likely  
114 contributes to the *in vivo* phenotypic range. In a subset of CC strains, we found correlated  
115 differences of susceptibility to ZIKV, dengue virus (DENV) and West Nile virus (WNV),  
116 suggesting shared underlying mechanisms. We identified highly susceptible and resistant mouse  
117 strains as new models to investigate the mechanisms of human ZIKV disease and other flavivirus  
118 infections. Finally, genetic analysis revealed that susceptibility to ZIKV in the CC is driven by  
119 multiple loci with small individual effects, and that *Oas1b*, a major determinant of mouse  
120 susceptibility to WNV, is not involved.

121

## 122 **RESULTS**

### 123 **Genetic background controls the susceptibility of *Ifnar1*-deficient mice to ZIKV**

124 Many studies have used *Ifnar1* knock-out mice on 129S2/SvPas (129) (55, 56) or C57BL/6J  
125 (B6) (23, 29, 34) inbred backgrounds, but the differences in ZIKV susceptibility between these  
126 two strains have not been reported and remain unclear due to heterogeneous experimental  
127 conditions between studies. We compared the susceptibility of age-matched 129S2/SvPas-*Ifnar1*<sup>-/-</sup>  
128 (129-*Ifnar1*) and C57BL/6J-*Ifnar1*<sup>-/-</sup> (B6-*Ifnar1*) mice infected intraperitoneally (IP) with 10<sup>7</sup>  
129 FFUs of FG15 ZIKV. B6-*Ifnar1* mice showed increasingly severe symptoms, with body weight  
130 loss, ruffled fur, ataxia and hind limb paralysis from day 4 p.i. and were all (10/10) moribund or  
131 dead by day 7 p.i. By contrast, 129-*Ifnar1* mice developed mild symptoms (ruffled fur, hunched  
132 back) starting on day 6 p.i. and declining over the second week of infection, with one mouse  
133 dying on day 9 p.i. (Fig. 1), demonstrating that the susceptibility to ZIKV infection conferred by  
134 *Ifnar1* genetic inactivation is critically influenced by the host genetic background.

### 135 **mAb blockade of IFNAR is a robust model to study ZIKV infection in CC mice**

136 *Ifnar1* genetic deficiency permanently abrogates IFN- $\alpha/\beta$ -mediated immune responses but is  
137 not currently available on diverse genetic backgrounds. We therefore tested the suitability of  
138 transient IFNAR blockade mediated by mAb treatment as a model to study ZIKV infection in  
139 genetically diverse mice like the CC. Since MAR1-5A3 mAb was generated in a laboratory  
140 strain (129-*Ifnar1* mice) (30) and since the CC strains differ in their *Ifnar1* allele (Table 1), we  
141 first compared the coding sequence of the *Ifnar1* gene across the eight founder strains. While  
142 129S1/SvImJ, NOD/ShiLtJ, NZO/HiLtJ and WSB/EiJ mice carry the same *Ifnar1* allele as  
143 C57BL/6J, A/J, PWK/PhJ and CAST/EiJ mice differ by one, three and seven amino-acids from  
144 the C57BL/6J reference sequence, respectively (Table 2). Notably, all three PWK/PhJ variants



145 were found in CAST/EiJ. Therefore, we assessed the efficacy of the MAR1-5A3 mAb by  
146 Western Blot analysis on mouse embryonic fibroblasts (MEFs) isolated from two CC strains  
147 (CC001 and CC071) both of which inherited the *Ifnar1* allele from the most divergent CAST/EiJ  
148 strain (57), by comparison with B6 MEFs. IFNAR stimulation by IFN- $\alpha/\beta$  activates the  
149 JAK1/TYK2 pathway and results in the phosphorylation of STAT1. We found that, in B6,  
150 CC001 and CC071 MEFs, STAT1 phosphorylation was equally induced by murine IFN- $\alpha$  and  
151 fully inhibited by the MAR1-5A3 mAb (Fig. 2A).

152 To assess MAR1-5A3 mAb efficacy *in vivo*, we infected CC001 and CC071 strains with  $10^7$   
153 FFUs of FG15 ZIKV IP and we measured the kinetics of plasma viral load in mice with and  
154 without 2 mg mAb treatment 24 hours prior to infection. Consistent with previous studies in B6  
155 (31, 58) and BALB/c (59) mice, viral load was consistently 4 to 5 log<sub>10</sub> units higher in mAb-  
156 treated mice of both CC001 and CC071 strains compared to untreated mice, demonstrating that  
157 MAR1-5A3 mAb treatment successfully increases CC mouse permissiveness to ZIKV  
158 replication (Fig. 2B).

159 We then measured the kinetics of plasma viral load in 129-*Ifnar1* strain as well as in four  
160 mAb-treated CC strains infected with  $10^7$  FFUs of FG15 ZIKV IP. We established that the peak  
161 plasma viral load occurred in most individuals at day 2 p.i., independently of mouse genetic  
162 background (Fig. 2C).

163 In previous studies, viral loads have been measured either by FFU titration or by RT-qPCR  
164 quantification of viral genome copies. We compared these two methods in B6-*Ifnar1*, 129-  
165 *Ifnar1*, and in ten mAb-treated CC strains (Fig. 2D). We performed Focus Forming Assays  
166 (FFA) to measure viral particles in the plasma at day 2 p.i. and confirmed the production of  
167 infectious ZIKV in the blood of all strains. Next, we compared the plasma viral load measured

168 by RT-qPCR and by FFA. We found that these two parameters were strongly correlated over a 2  
169  $\log_{10}$  range (Pearson coefficient,  $r^2=0.89$ ,  $p=9.9 \times 10^{-17}$ ), with the number of genome copies being  
170 on average 3  $\log_{10}$  units higher than the number of FFUs (Fig. 2D). We therefore validated that  
171 RT-qPCR measurement of plasma viral load could be used as a labor-efficient proxy for viremia  
172 throughout the study.

173 Finally, we compared plasma viral load at day 2 p.i. between males and females in 129-  
174 *Ifnar1* and in four mAb-treated CC strains in which both sexes had been tested. We found no  
175 significant difference between sexes across diverse genetic backgrounds (two-way ANOVA,  
176  $p=0.24$ ; Fig. 2E), validating the use of merged data from males and females in mouse ZIKV  
177 infection experiments.

#### 178 **CC genetic diversity drives ZIKV disease severity and plasma viral load**

179 To explore broad genetic variation, we assessed the susceptibility of mAb-treated mice of 35  
180 CC strains. B6-*Ifnar1*, 129-*Ifnar1* and mAb-treated B6 mice were included as reference strains.  
181 Only mice from three CC strains developed symptoms as shown on Fig. 3A which summarizes  
182 clinical observations at day 7 p.i. CC021 and CC026 mice recovered and survived, while  
183 symptoms worsened in 7/9 (78%) CC071 mice which were moribund or died between days 7 and  
184 9 p.i.

185 Plasma viral load was measured on days 2 and 6 p.i. At day 2 p.i., which corresponds to its  
186 peak, viral load was generally characterized by small within-strain heterogeneity and large inter-  
187 strain variations spread over a 2.8  $\log_{10}$  range (Fig. 3B), demonstrating a strong effect of host  
188 genes (Kruskal-Wallis,  $p=4.8 \times 10^{-15}$ ) with a broad sense heritability of 86% (60). The three  
189 symptomatic CC strains showed the highest peak viral load, close to that of B6-*Ifnar1* and 129-  
190 *Ifnar1* mice. However, other strains (such as CC005 and CC061) had similarly high viral loads  
191 but never showed any clinical signs of disease, indicating that peak viral load is unlikely the sole

192 factor controlling clinical severity. At day 6 p.i., within-strain variations were larger and more  
193 heterogeneous, but we still observed highly significant inter-strain differences (Kruskal-Wallis,  
194  $p=1.1 \times 10^{-10}$ ). Interestingly, viral load on days 2 and 6 p.i. were only moderately correlated  
195 (Pearson coefficient,  $r^2=0.46$ ;  $p=0.004$ ), indicating that viral load at day 2 p.i. was not predictive  
196 of viral load at day 6 p.i. (see for example CC018 and CC040, or CC026 and CC071).

197 We used the difference of the  $\log_{10}$  plasma viral loads between days 2 and 6 p.i. to estimate  
198 the clearance rate of the virus from the blood stream (Fig. 3C; the order of the strains sorted by  
199 increasing clearance rate was therefore different from those in Fig. 3A and 3B). This rate varied  
200 over a 3.3  $\log_{10}$  range between strains, demonstrating a strong effect of host genes (Kruskal-  
201 Wallis,  $p=2.2 \times 10^{-12}$ ) with a broad sense heritability of 76%. B6-*Ifnar1* mice showed a slower  
202 decrease in viral load than 129-*Ifnar1* mice (Wilcoxon,  $p=1.7 \times 10^{-5}$ ), despite similar peak viral  
203 load at day 2 p.i. To confirm that differences between CC strains were not due to differential  
204 rates of MAR1-5A3 antibody turnover, we treated CC012 (which showed 4.4 log decrease) and  
205 CC037 (1.9 log decrease) with 2 mg of mAb 24 hours before ZIKV infection, with or without  
206 two additional injections of 1 mg on days 2 and 4 post-infection. No differences in plasma viral  
207 load on days 2 and 6 p.i. were observed between the two conditions (Fig. 2F).

208 Overall, genetic diversity in the CC panel controlled clinical severity of ZIKV infection,  
209 mouse survival, and the peak and clearance rate of plasma viral load. Of note, there was no  
210 association, across the 35 CC strains tested, between the peak plasma viral load and the *Ifnar1*  
211 allele inherited from the founder strain (ANOVA,  $p>0.09$ ). This analysis confirms our *in vitro*  
212 data (Fig. 2A) and indicates that the variations in peak plasma viral load do not result from  
213 differences in mAb treatment efficacy due to the mAb turnover or to the *Ifnar1* alleles.

214 From this screening, we identified several strains with extreme phenotypes, in particular  
215 CC071 which was the most susceptible to ZIKV infection, CC001, CC011, CC017 or CC060

216 with low peak plasma viral load, CC040 with slowly decreasing plasma viral load and CC045 or  
217 CC026 with high peak but fast-decreasing plasma viral loads.

### 218 **CC mice show correlated susceptibility to ZIKV, DENV and WNV**

219 We further characterized three CC strains (indicated by arrows on Fig. 3B and Fig. 3C)  
220 among those showing lowest (CC001) and highest peak viral loads with (CC071) or without  
221 (CC005) clinical symptoms. To establish whether the above differences were specific of the  
222 FG15 ZIKV strain of the Asian lineage, we first assessed the susceptibility of the three selected  
223 strains to the HD78788 ZIKV strain of the African lineage. 129-*Ifnar1* mice and mAb-treated  
224 CC mice were infected with  $10^3$  FFUs of HD78788 ZIKV IP which proved to be highly  
225 pathogenic in *Ifnar1*-deficient mice with rapid and severe symptoms and 100% mortality (Fig.  
226 4A). CC001 was fully resistant with no or mild clinical signs (Fig. 4A, left and center panels).  
227 By contrast, all CC071 mice were moribund or dead by day 10 p.i., with early and quickly  
228 aggravating symptoms, almost like 129-*Ifnar1* mice. Only one of the 5 CC005 mice developed  
229 symptoms and died. Peak viral load (day 2 p.i.) varied over a 2.4  $\log_{10}$  range and the differences  
230 between strains were similar to those observed with the FG15 ZIKV strain (Fig. 3B). Here again,  
231 plasma viral load at day 2 p.i. was the highest in the very susceptible CC071 and 129-*Ifnar1*  
232 strains and low in the resistant CC001, but it was also very high in CC005 mice which were  
233 moderately susceptible, confirming that clinical severity does not depend solely on peak plasma  
234 viral load.

235 To evaluate whether these differences in susceptibility were specific to ZIKV or extended to  
236 other flaviviruses, we assessed the phenotype of a few strains after infection with DENV and  
237 WNV, two other members of the *Flaviridae* family.

238 We measured plasma viral load after IV infection with  $2 \times 10^6$  FFUs of KDH0026A DENV  
239 (DENV 1 serotype) in mAb-treated CC001, CC071 and B6 mice and in 129-*Ifnar1* and B6-

240 *Ifnar1* mice (Fig. 4B right). Most inter-strain differences observed with ZIKV FG15 strain (Fig.  
241 4B left, data from Fig. 3B) were also observed with DENV, with the CC071 mice displaying the  
242 highest plasma viral load after mAb treatment. DENV infection was overall much less clinically  
243 severe since only B6-*Ifnar1* mice developed non-lethal symptoms including ruffled fur, hunched  
244 back and ataxia.

245 We also investigated the susceptibility of the selected CC strains to WNV. *Oas1b* was  
246 previously shown to be a major host genetic determinant of susceptibility to WNV in mice (61).  
247 Of note, the three selected CC strains carry the same truncated, non-functional allele of *Oas1b*  
248 inherited from the laboratory strain founders (Table 1), conferring them susceptibility to WNV  
249 infection. CC mice were infected IP with  $10^4$  FFUs of WNV IS-98-ST1 and monitored for 14  
250 days p.i. (efficient WNV infection does not require anti-IFNAR mAb treatment in *Oas1b*-  
251 truncated mice). All CC071 mice died 7 days p.i., significantly faster than CC001 and CC005  
252 mice (logrank,  $p < 0.01$ ; Fig. 4C, left panel), indicating that genetic diversity between CC strains  
253 also influences their susceptibility to WNV even in the context of *Oas1b* deficiency.

254 To assess whether the differences of susceptibility between these CC strains also applied to  
255 other viruses, we infected them with  $10^2$  PFUs of RVFV ZH548 IP. No significant difference  
256 was found between the three CC strains (logrank,  $p > 0.3$  for all pairwise comparisons; Fig. 4C,  
257 right panel) which succumbed late from the infection, like the commonly used BALB/cByJ mice.  
258 Only CC001 mice died significantly later than BALB/c mice (logrank,  $p = 0.04$ ).

### 259 **Genetic analysis suggests a polygenic control of susceptibility to ZIKV in CC mice**

260 To identify host genetic factors controlling the susceptibility to ZIKV in CC strains, we  
261 performed a genome-wide association study between the plasma viral loads at days 2 and 6 p.i.  
262 or the decrease rate of plasma viral load, and the genotypes of the 35 CC strains. Genetic  
263 associations were plotted as LOD scores. We did not find any genome locations at which LOD

264 scores reached the minimum 0.1 significance threshold for any of the three traits (Fig. 5), which  
265 would be expected if phenotypic variations were controlled by one or two loci with strong  
266 effects. Therefore, these results suggest that plasma viral load is controlled by multiple small-  
267 effect genetic variants.

### 268 **Genetic diversity of CC strains controls brain viral load and pathology**

269 To assess the influence of host genetics on the brain pathology caused by ZIKV infection,  
270 we further characterized the three previously selected CC strains. We measured the viral load in  
271 the brain 6 days after IP infection with FG15 ZIKV in mAb-treated CC mice and 129-*Ifnar1*  
272 mice (Fig. 6A). CC005 and CC071 which had higher peak plasma viral load also had higher  
273 brain viral load (mean=6.5 log<sub>10</sub> copies/μg RNA for CC005 and CC071, compared with 5 log<sub>10</sub>  
274 copies/μg RNA for CC001). As expected, 129-*Ifnar1* mice showed the highest viral load in the  
275 brain. These results indicate overall correlation between plasma and brain viral loads.

276 Histopathological analysis, carried out in the brain of the same mice (Fig. 6B-N), revealed  
277 different lesion profiles between the four mouse strains (Fig. 6B, F, I and L for low  
278 magnification images). 129-*Ifnar1* mice clearly displayed the most severe inflammatory lesions  
279 with subacute leptomeningo-encephalitis (*i.e.* infiltration of perivascular spaces and  
280 leptomeninges by lymphocytes, plasma cells and macrophages; Fig. 6B-E) and activation of  
281 microglial cells with microglial nodules (Fig. 7B-D). By contrast, almost no histological lesions  
282 were detected in the brain of CC001 mice (Fig. 6F-H), with normal, non-activated, microglial  
283 cells (Fig. 7E-G). Only very rare small clusters of activated microglial cells were detected.  
284 CC005 displayed moderate inflammatory lesions characterized by perivascular cuffing (Fig. 6I-  
285 K), activation of microglial cells (hyperplasia and thickening of cell processes) and microglial  
286 nodules (Fig. 7H-J). CC071 mice displayed almost no lesions in HE (Fig. 6L-N) but  
287 inflammatory lesions of intermediate severity with activation of microglial cells and microglial

288 nodules (Fig. 7K-M) similar to that of 129-*Ifnar1* mice (Fig. 7A), as revealed by Iba1  
289 immunolabeling.

290 The nature and intensity of brain histological lesions may depend on the circulating viral  
291 load, on the capacity of the virus and of the mAb to cross the blood-brain barrier and on the  
292 permissiveness of brain cells (in particular neurons and microglia). To assess the differences in  
293 susceptibility of brain cells between CC strains, we performed intra-cerebral infections to deliver  
294 the virus directly into the brain tissue. 129-*Ifnar1* and mAb-untreated CC mice received  $10^5$   
295 FFUs of FG15 ZIKV in the left ventricular region of the brain and were followed for 3 weeks.  
296 Mild and transient symptoms (ruffled fur, hunched back) were observed in a few mice of the  
297 three strains and one CC005 mouse died on day 19 p.i. A second group of CC mice were infected  
298 similarly and euthanized at day 6 p.i. for histological analysis. Differences in brain viral load  
299 between CC strains were similar to those observed after IP infection, with CC005 and CC071  
300 mice showing significantly higher brain viral load than CC001 mice (Fig. 8A). Compared with  
301 IP route of infection, lesions in 129-*Ifnar1* mice were mostly similar, with marked subacute  
302 leptomeningo-encephalitis (Fig. 8B-E) and activation of microglial cells (Fig. 9B-D). By  
303 contrast, lesion profiles were clearly different in the three CC strains. Two of the five CC001  
304 mice displayed no significant histological lesions with normal resting microglial cells, while the  
305 other three displayed minimal lesions with gliosis (Fig. 8F-H), and rare small clusters of  
306 activated microglial cells (Fig. 9E-G). By this route of infection, CC005 mice displayed  
307 heterogeneous lesion profiles with either suspected meningitis and gliosis (Fig. 8I-K, 4/5 mice)  
308 or moderate leptomeningo-encephalitis (1/5 mice). Activation of microglial cells with variable  
309 severity was detected in all animals (Fig. 9H-J). Strikingly, all CC071 mice displayed marked  
310 leptomeningo-encephalitis (Fig. 8L-O) with the strongest activation of microglial cells (Fig. 9K-  
311 M and Fig. 9A).

312           These results indicate that CC strains differ in their permissiveness to viral replication in the  
313 brain and in their susceptibility to ZIKV-induced histological brain damage. The comparison  
314 between IP and IC infection routes shows that these variations cannot be explained only by the  
315 possibility of differential dissemination of ZIKV from the circulation to the brain between CC  
316 strains.

317 **Viral replication in CC071 cells is increased *in vitro***

318           Differences in peak plasma viral load and the results from IC infections suggested that  
319 different efficiencies of viral replication could contribute to the variations in susceptibility  
320 between CC strains. To address this point, we measured the production of infectious viral  
321 particles in MEFs derived from the CC001 and CC071 strains. Cells were infected with ZIKV  
322 FG15 at a MOI of 5. While viral titers remained stable in CC001 between 24 and 72 hours, the  
323 production of viral infectious particles by CC071 MEFs increased very significantly over the  
324 same period (Fig. 10). These results suggest that increased replication efficiency in CC071 mice  
325 could contribute to their susceptible phenotype.

326



327 **DISCUSSION**

328 ZIKV is a serious public health concern considering the occurrence of severe neurological  
329 complications in adults and congenital malformations that can result from the infection of  
330 pregnant women. The variable outcomes of ZIKV infection in humans has led investigators to  
331 hypothesize a role for host genetic factors (9, 13) although this has never been demonstrated thus  
332 far. As for other infectious diseases, human genetic studies on susceptibility to ZIKV would  
333 require large cohorts of patients and would be confounded by pathogen genetics, pathogen dose,  
334 mosquito-dependent factors and multiple environmental parameters. Also, due to the randomness  
335 of virus infections, trios are not available for genetic analyses.

336 Several mouse models of human ZIKV infection have already been described and have  
337 substantially improved our understanding of viral tropism, dissemination, pathogenesis,  
338 persistence, transmission and vaccine protection. To overcome the inability of ZIKV to inhibit  
339 IFN induction and signaling pathways in mice as was observed in humans (27), most studies  
340 have been performed using *Ifnar1*-deficient mice which have become a reference model.  
341 However, high levels of viral replication can also be achieved by temporary inhibition of IFN  
342 signaling by anti-IFNAR mAb treatment (30, 31, 62) or even in immunocompetent mice by  
343 infecting neonates (36, 63-65) or using a combination of mouse-adapted ZIKV strains and  
344 human STAT2 knock-in mice (66).

345 The choice of the ZIKV strain used in an animal model is important to maximize the  
346 relevance of mouse studies to human infection. Previous mouse studies have used different  
347 ZIKV strains from the African or Asian lineages. Mouse-adapted strains of the African lineage  
348 derived from a large number of serial passages are more pathogenic in mice at lower doses (34)  
349 but carry mutations that may bias the translatability of results to humans. To avoid this  
350 limitation, mouse studies have often used different ZIKV strains from the Asian lineage derived

351 from clinical isolates. Genetic differences between these two lineages are suspected to be  
352 responsible for the emergence of symptomatic cases in human starting with the Yap Island  
353 epidemics in 2007 (7, 8). Therefore, while the ZIKV strain needs to be standardized in  
354 experimental studies, generalization of the results obtained with one viral strain require  
355 confirmation using another strain. Because of the incidence of neurological complications  
356 associated with infections by Asian lineage ZIKV, we used for our genetic screening a low-  
357 passage strain derived from a 2015 case of French Guyana, at an early stage of the South-  
358 American epidemics. Since this strain had not been adapted to the mouse, high doses were  
359 required to achieve high circulating viral loads.

360 Most mouse studies have used either B6-*Ifnar1* or 129-*Ifnar1* strains without specific  
361 rationale and their results cannot be directly compared due to many experimental differences  
362 such as ZIKV strain, dose and route of inoculation (67). Under strictly identical conditions, we  
363 found that B6-*Ifnar1* mice developed more rapid and severe clinical symptoms and higher  
364 mortality than 129-*Ifnar1* mice, despite similar levels of plasma viral RNA at day 2 p.i. We also  
365 found that viral load persisted longer in B6-*Ifnar1* mice. These results show that, under our  
366 experimental conditions, these two *Ifnar1*-deficient strains have clearly distinct susceptibility to  
367 ZIKV. To our knowledge, these two strains have been compared in only one study which found  
368 no difference in survival after WNV infection (68). However, their extreme susceptibility might  
369 have prevented the identification of any difference. Our results have practical implications for  
370 many studies based on *Ifnar1*-deficient mice and motivate further genetic studies to identify the  
371 determinants and mechanisms controlling differences of susceptibility between B6 and 129  
372 inbred backgrounds.

373 To further investigate the role of host natural genetic variants on ZIKV susceptibility, we  
374 leveraged the genetic diversity across CC strains. The CC has been developed as a collection of

375 inbred strains that more accurately reproduce the genetic diversity and phenotypic range seen in  
376 human population (69). To enable systemic ZIKV replication after parenteral inoculation in  
377 diverse genetic backgrounds, we blocked type I IFN response using MAR1-5A3 mAb (23, 31).

378 Several lines of evidence demonstrate that the mAb treatment was effective in all CC strains.  
379 First, we showed similar abrogation of IFN $\alpha$ -induced STAT1 phosphorylation in MEFs from B6  
380 and two CC strains carrying the most divergent *Ifnar1* haplotypes (Fig. 2A). Moreover, the  
381 differences in peak plasma viral load across 35 CC strains were not associated with the *Ifnar1*  
382 allele each CC has received from the founder strains. Secondly, the peak viral load in all mAb-  
383 treated CC mice was at least 2.5 logs higher than that of untreated CC071 mice (Fig. 2B and 3B).  
384 Finally, we showed, in two CC strains, that increasing the dose of mAb to 4 mg did not modify  
385 plasma viral loads at days 2 and 6 p.i. (Fig. 2F), indicating that the 2 mg dose was not limiting.  
386 This is consistent with the 5.2 days half-life of the MAR1-5A3 antibody previously reported  
387 (30). These results validate that the MAR1-5A3 mAb is effective across a broad range of mouse  
388 genetic backgrounds, which will be useful to develop new models of viral infections.

389 A single injection of MAR1-5A3 mAb 24 hours before ZIKV infection resulted in moderate  
390 to very high levels of viral RNA in the blood and brain. ZIKV infection was symptomatic in a  
391 minority of CC strains (3/35), as observed in infected humans (9, 70), and mortality was  
392 observed only in CC071. These results confirm that ZIKV can replicate and establish viremia  
393 without inducing symptoms (29). Moreover, while all symptomatic strains had high peak viral  
394 loads, other strains with similar viral loads (like CC005 or CC061) never developed any signs of  
395 acute illness, indicating that other pathogenic mechanisms are required to result in symptomatic  
396 infection and that viral load alone does not reliably predict clinical outcome of ZIKV infection in  
397 a genetically diverse mouse population.

398 Since all experimental parameters were carefully standardized between strains (in particular,  
399 the microbiological environment in which they were bred), which resulted in small intra-strain  
400 variations, and since the MAR1-5A3 mAb treatment was effective across all CC strains,  
401 differences in peak viral load between strains can be confidently attributed to host genetic  
402 variants. The 86% broad sense heritability further indicates that genetic background is the  
403 principal factor driving peak viral load across CC strains.

404 Viremia decreased between days 2 and 6 p.i., as previously reported in several studies (55,  
405 71, 72) but not in others (29, 56) for reasons that have not been discussed and remain unclear. In  
406 our study, the rate of decrease, which was estimated as the difference of the  $\log_{10}$  plasma viral  
407 load between day 2 and day 6 p.i., showed remarkable homogeneity between individuals of the  
408 same CC strain and very large variations across CC strains. Injecting additional doses of mAb  
409 during the course of infection did not modify the kinetics of viral load (Fig. 2F), ruling out the  
410 potential role of differential rates of antibody turnover. These results demonstrate a strong  
411 influence of host genes on the clearance rate of ZIKV from the blood stream. The decrease of  
412 circulating viral load is the net result of ZIKV production in infected tissues, dissemination to the  
413 blood stream and elimination from the circulation. Therefore, host genes could control the  
414 kinetics of viral load through multiple mechanisms.

415 After exploring the range of susceptibility to ZIKV across broad genetic diversity, we  
416 focused our study on a few CC strains exhibiting contrasted phenotypes with the aim of  
417 characterizing new models (54). CC001 is one of the least permissive to ZIKV, with low peak  
418 viral load. At the other extreme of the distribution, CC005 and CC071 have similarly high  
419 plasma viral loads while only CC071 shows symptoms and high mortality. These differences  
420 between CC strains were strikingly conserved with the African, mouse-adapted, HD78788 strain  
421 (Fig. 4A). The use of lower infectious doses with HD78788 virus was supported by its higher

422 pathogenicity resulting from mouse adaptation. The consistency between these two experiments  
423 suggest that the large phenotypic diversity we have reported should apply to most ZIKV strains.

424 Overall, brain viral load and brain pathology after IP infection were consistent with peak  
425 plasma viral load. In CC mice, the most notable microscopic lesions included signs of  
426 neuroinflammation evidenced by Iba1 immunohistochemistry. Neuroinflammation was similar in  
427 129-*Ifnar1*, CC005 and CC071 mice. These changes were less pronounced than in a previous  
428 study which reported more severe CNS lesions in MAR1-5A3-treated B6 mice (31), infected  
429 with a more virulent African lineage ZIKV strain. The variable severity of lesions observed in  
430 CC mice, ranging from very mild abnormalities in CC001, to inflammatory lesions with  
431 perivascular cuffing, activation of microglial cells and microglial nodules in CC005, indicates  
432 that the genetic background also controls ZIKV neuropathogenesis.

433 The less severe histological lesions observed in CC mice compared with 129-*Ifnar1* mice  
434 could be due to the limited access to the brain of the virus or of the mAb which does not  
435 appreciably cross the blood-brain barrier (29). Therefore, intracerebral infection aimed at  
436 comparing brain lesions between strains while controlling the amount of virus effectively  
437 delivered. Surprisingly, CC001 and CC005 mice showed similar types and severity of lesions  
438 (although not all CC001 mice showed lesions) while CC071 mice developed much more severe  
439 signs of leptomeningo-encephalitis with massive neuroinflammation, similar to those of 129-  
440 *Ifnar1* mice. This last result suggests that the milder lesions observed in CC071 compared with  
441 129-*Ifnar1* mice after IP infection were likely due to reduced viral dissemination to the brain.  
442 Importantly, mice did not receive prior mAb treatment, allowing for the development of local  
443 and systemic antiviral responses. These results emphasize the complex interplay between  
444 infected cells and effectors of the immune response, which likely differs between CC strains  
445 under the control of host genes.

446 Viral replication rate between resistant CC001 and highly susceptible CC071 mice was  
447 investigated as a plausible mechanism for the differences in susceptibility between these two  
448 strains. MEFs are a semi-permanent source of cells which have been extensively used to assess  
449 viral replication (73, 74) including with ZIKV (75, 76). In CC001 MEFs, the production of  
450 infectious viral particles was stable between 24 and 48h and decreased between 48 and 72h,  
451 while it steadily increased over time in CC071 MEFs, leading to significantly higher viral titers  
452 starting at 48h. Our data is consistent with the observation by Caires-Junior et al. who reported  
453 increased ZIKV replication rate in iPS-derived neuroprogenitor cells from CZS-affected babies  
454 compared with their unaffected dizygotic twin (13). Therefore, our results strongly suggest that  
455 increased replication rate in CC071 compared with CC001 likely contributes to its higher plasma  
456 and brain viral loads and to its higher overall susceptibility to ZIKV.

457 Investigating the genetic diversity of a large number of CC strains has significantly extended  
458 the range of phenotypes induced by ZIKV infection in mice and better model the heterogeneity  
459 of the human population. It has allowed testing important factors such as mouse gender and the  
460 method of viral load measurement across multiple host genetic background, providing robust  
461 conclusions (37). Importantly, we found no differences between male and female mice in their  
462 susceptibility to ZIKV disease, nor in the peak viral load (Fig. 2E). We also found a high  
463 correlation between viral loads measured by titration and by qRT-PCR over a 2 log<sub>10</sub> range (Fig.  
464 2D). This is in contrast with a study on Ebola virus which showed that, in spleen and liver, the  
465 susceptible mice produced similar amounts of viral genomes but 1 to 2 log<sub>10</sub> more infectious  
466 virions than the resistant mice (43).

467 Genetic diversity also allowed us to assess correlations between traits, which cannot be  
468 achieved in a single strain. We showed that brain viral load at day 6 after IP was consistent with  
469 plasma viral load at day 2 p.i., but that plasma viral loads at days 2 and 6 p.i. were only

470 moderately correlated. Likewise, we found that clinical severity did not correlate with the  
471 intensity of brain histological lesions and neuroinflammation, as summarized in Table 3. These  
472 dissociations between phenotypes provide evidence for partly distinct mechanisms and genetic  
473 control (37) and lead to distinct mouse models.

474 A recent study has reported strain-dependent variations in the long-term neuropathological  
475 and behavioral consequences of ZIKV infection after neonatal infection between four mouse  
476 inbred strains known to differ in their susceptibility to pathogens (36). Since they are all  
477 laboratory strains, they do not cover the same genetic variation as in our study and it is likely that  
478 even more diverse phenotypes would be observed in this model with the CC panel.

479 Genetic analysis of our results strongly suggests that, by contrast with other viruses for  
480 which major host genetic determinants have been identified (e.g. *Oas1b* for WNV (61) or *Mx1*  
481 for influenza virus (77)), susceptibility to ZIKV in CC strains is under polygenic control. This is  
482 supported by the continuous distributions, across the CC strains, of the values of peak plasma  
483 viral load (Fig. 3B) and of the rate of viral decrease (Fig. 3C), and by the absence of any regions  
484 of the genome significantly associated with variations in viral load (Fig. 5). Calculations based  
485 on CC genotypes show that, with 35 strains and an average of 5 mice per strain, we had 80%  
486 power of detecting a bi-allelic QTL explaining 30% or more of the phenotypic variance (78).  
487 This clearly rules out the possibility that the phenotypic variations measured across CC strains  
488 were controlled by one or few genes with major effects, as observed with *Oas1b* for WNV (47).  
489 Dissecting the genetic architecture of resistance and susceptibility to ZIKV in these strains will  
490 require combining complementary strategies. Intercrosses between contrasted strains such as  
491 CC001 and CC071 will reduce genetic complexity and may result in increased power for QTL  
492 mapping due to larger sample size (54). However, the mapping resolution achieved in an F2 or a  
493 backcross leads to large genetic intervals. Other approaches, such as gene expression studies on

494 various cell types (such as immune cells, neurons or glial cells) will be used to identify  
495 differentially activated host response pathways and reduce the number of candidate genes in  
496 these intervals.

497 *Oas1b* is an interferon-stimulated gene and a major determinant of mouse susceptibility to  
498 WNV (45). A variant in OAS3, a member of the human homologous gene family, has been  
499 associated with increased severity of dengue (79). Most laboratory strains, including five of the  
500 eight CC founders, carry the same non-functional allele of *Oas1b* which renders them susceptible  
501 to WNV infection (61), while the three wild-derived CC founders carry polymorphic but  
502 functional alleles and are resistant (45). CC strains therefore carry either functional or non-  
503 functional *Oas1b* alleles. Our results provide multiple lines of evidence to rule out a significant  
504 role of *Oas1b* in the variations of susceptibility to ZIKV across CC strains. First, since mAb-  
505 mediated blockade of type I IFN response likely inhibits temporarily *Oas1b* induction, *Oas1b*  
506 allele is unlikely to explain differences in peak viral load at day 2 p.i. Moreover, our QTL  
507 mapping analysis showed that mouse genotype at *Oas1b* (located on distal chromosome 5) did  
508 not significantly contribute to variations in viral load at day 2 or day 6 p.i., or in the rate of viral  
509 load decrease (Fig. 5). Finally, since both CC001, CC005 and CC071 strains carry the *Oas1b*  
510 truncated allele (Table 1), their differences in clinical severity, brain pathology and replication  
511 rate in infected cells must be controlled by other genetic variants. Interestingly, the large  
512 difference of survival time after WNV infection between CC071 and CC001 or CC005 provides  
513 ideal strain combinations to identify novel genes controlling susceptibility to this virus.

514 Out of this large series of CC strains, we identified several new mouse models of ZIKV  
515 disease. CC071 mice were the most susceptible to ZIKV infection, more than mAb-treated B6  
516 mice (Fig. 3A,B). mAb treatment was required to achieve high circulating viral load (Fig. 2B),  
517 showing that CC071 has functional type I IFN response. CC071 mice were also very susceptible



518 to DENV and WNV, two flaviviruses related to ZIKV. However, they are not uniformly  
519 susceptible to infectious agents since they showed susceptibility to RVFV similar to that of  
520 BALB/c, CC001 and CC005 mice, and intermediate susceptibility to *Salmonella* Typhimurium  
521 (52). Together with other susceptible strains like CC021 and CC026 which developed symptoms,  
522 or CC005 which developed severe brain lesions, CC071 will help identifying mechanisms of  
523 severe ZIKV infection and their genetic control. By contrast, CC001 mice were highly resistant,  
524 even to a strongly pathogenic African ZIKV strain, despite blockade of type I IFN signaling.  
525 Extensive analysis of these CC strains with extreme phenotypes may elucidate how genetic  
526 variants affect susceptibility as well as innate and adaptive immune responses to flaviviral  
527 infection (54) and provide deeper understanding of the pathophysiology of severe complications  
528 of human ZIKV disease.

529

530 **MATERIALS AND METHODS**

531

532 **Mice**

533 All Collaborative Cross (CC) mice (purchased from the Systems Genetics Core Facility,  
534 University of North Carolina and bred at the Institut Pasteur) (80), C57BL/6J mice (purchased  
535 from Charles River Laboratories France), BALB/cByJ and *Ifnar1* knock-out mice (*Ifnar1*<sup>tm1Agt</sup>  
536 allele on 129S2/SvPas or C57BL/6J background, designated 129-*Ifnar1* and B6-*Ifnar1*,  
537 respectively, and bred at the Institut Pasteur) were maintained under SPF conditions with 14:10  
538 light-dark cycle and *ad libitum* food and water in the Institut Pasteur animal facility. All CC  
539 strains bred at the Institut Pasteur were included in this study. In all experiments, mice were  
540 killed by cervical dislocation. All experimental protocols were approved by the Institut Pasteur  
541 Ethics Committee (projects #2013-0071, #2014-0070, #2016-0013, #2016-0018 and dap190107)  
542 and authorized by the French Ministry of Research (decisions #00762.02, #7822, #6463, #6466  
543 and #19469, respectively), in compliance with French and European regulations.

544

545 **Cell lines**

546 Vero cells (ATCC CRL-1586) were cultured at 37°C in Dulbecco's Modified Eagle Medium  
547 (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Eurobio). C6/36 cells  
548 (ATCC CRL-1660) were cultured at 28°C in Leibovitz Medium (L-15 Medium, Gibco)  
549 supplemented with 10% FBS, 1% Non-Essential Amino Acids (Life Technologies) and 1%  
550 Tryptose Phosphate Broth (Life Technologies).

551

552 **Viruses**

553 The FG15 Asian Zika virus (ZIKV) strain, isolated from a patient during ZIKV outbreak in  
554 French Guiana in December 2015, was obtained from the Virology Laboratory of the Institut  
555 Pasteur of French Guiana. The HD78788 African ZIKV strain, isolated from a human case in  
556 Senegal in 1991, was obtained from the Institut Pasteur collection. The KDH0026A DENV  
557 serotype 1 (DENV-1) strain, isolated from a patient in Thailand in 2010, was previously  
558 described (81). Viral stocks were prepared from supernatant of infected C6/36 cells, clarified by  
559 centrifugation at 800g and titrated on Vero cells by focus-forming assay (FFA). Stocks were kept  
560 at -80°C. The West Nile virus (WNV) strain IS-98-ST1 (or Stork/98) was obtained, cultured and  
561 used as described in Mashimo *et al.* (61). The Rift Valley Fever virus (RVFV) strain ZH548 was  
562 obtained, cultured and used as described in Tokuda *et al.* (82).

563

564 **Mouse experiments**

565 All infection experiments were performed in a biosafety level 3 animal facility. Mice were  
566 maintained in isolators.

567 ZIKV and DENV systemic infection. CC mice received 2 mg of IFNAR-blocking mouse  
568 mAb (MAR1-5A3, BioXCell) by intraperitoneal (IP) injection one day before ZIKV or DENV  
569 infection (83). Groups of 6-8 week-old mice were inoculated IP with  $10^7$  focus-forming units  
570 (FFUs) of ZIKV FG15 or  $10^3$  FFUs of ZIKV HD78788, in 200 $\mu$ L PBS. For DENV infection,  
571 mice were anesthetized by IP injection with a solution of Xylazine (5 mg/kg) and Ketamine (80  
572 mg/kg) and afterwards inoculated by intravenous (IV) injection in the retro-orbital sinus with  
573  $2.10^6$  FFUs of DENV-1 KDH0026A, in 100 $\mu$ L PBS. Survival and clinical signs were monitored  
574 daily for up to 14 days. Clinical signs were scored as follows: 0, no symptom; 1, ruffled fur; 2,  
575 emaciation, hunched posture and/or hypo activity; 3, hind limb weakness, prostration and/or

576 closed eyes; 4, moribund or dead. Blood samples were collected at several time points from the  
577 retromandibular vein for plasma viral load assessment.

578 ZIKV intracerebral infection. Mice were anesthetized by IP injection with a solution of  
579 Xylazine (5 mg/kg), Ketamine (75 mg/kg) and Buprenorphine (0.03 mg/kg). Groups of 5-6  
580 week-old mice were then inoculated by intracerebral (IC) injection in the right brain hemisphere  
581 with a 26-gauge needle affixed to a Hamilton syringe sheathed by a wire guard allowing no more  
582 than a 4-mm penetrance into the skull cavity, as described in (84). Mice received either  $10^5$  FFUs  
583 of ZIKV FG15 in PBS or PBS alone, in a volume of 10 $\mu$ L. Survival and clinical signs were  
584 monitored daily for 6 days and euthanized for brain collection. Another cohort of mice (n=7-8  
585 per strain) was infected similarly and monitored daily for 21 days to assess symptoms and  
586 survival.

587 WNV and RVFV infection. Groups of 8-12 week-old mice were inoculated IP with  $10^3$   
588 FFUs of WNV strain IS-98-ST1 or  $10^2$  PFUs of RVFV strain ZH548. Survival and clinical signs  
589 were monitored daily for up to 14 days (RVFV) or 21 days (WNV).

590

### 591 **Mouse embryonic fibroblasts (MEFs) isolation and infection**

592 MEFs were isolated from individual fetuses from one or more genetically identical females  
593 at day 13.5-14.5 of gestation, and cultured in DMEM supplemented with 10% FBS (Eurobio)  
594 and 1% penicillin/streptomycin (Gibco) at 37°C. MEFs were used until passage 2.

595 MEFs were plated at identical densities in culture dishes 24 hours before infection. MEFs  
596 were infected with ZIKV FG15 strain at a MOI of 5. After 2 hours of incubation at 37°C, the  
597 inoculum was replaced with fresh medium. Supernatants were collected at 24, 48 and 72 hours  
598 post-infection. Titration was performed by FFA in Vero cells.

599

600 **Focus-forming assay**

601 Vero cells were seeded at  $3 \cdot 10^4$  per well in 100  $\mu$ l complete medium (DMEM, FBS 10%) in  
602 96-well plates. After overnight incubation at 37°C, medium was replaced with 40  $\mu$ L of serial  
603 10-fold dilutions of the samples, and 115  $\mu$ L of methylcellulose overlay was added 2 hours later.  
604 After 40 hours incubation, culture medium was removed and cells were fixed with 100  $\mu$ L/well  
605 of 4% paraformaldehyde for 20 minutes and permeabilized with a solution of 0.3% Triton and  
606 5% FBS in PBS for 20 minutes. Cells were washed, incubated with a mouse mAb directed  
607 against ZIKV envelop protein (4G2, purified from the ATCC hybridoma) for 1 hour at 37°C  
608 (1/250° in blocking buffer). Cells were further washed, incubated with secondary antibody  
609 (AlexaFluor-488-conjugated anti-mouse IgG, Invitrogen) for 45 minutes at 37°C and washed.  
610 Infected cell foci were counted using an ImmunoSpot CTL analyzer and viral titers were  
611 calculated from the average number of foci.

612

613 **Viral genome quantification by RT-qPCR**

614 Blood samples were centrifuged to recover plasma from which viral RNA was extracted  
615 with the QIAamp Viral RNA Mini Kit (Qiagen). Brain samples were homogenized at 4°C in 1  
616 mL of TRIzol reagent (Life Technologies) using ceramic beads and automated homogenizer  
617 (PreCellys). Total RNA was extracted according to manufacturer's instructions. cDNA synthesis  
618 was performed using MMLV reverse transcriptase (Life Technologies) in a Bio-Rad *Mycycler*  
619 thermocycler. ZIKV and DENV cDNA were quantified by TaqMan quantitative PCR (qPCR) in  
620 a ViiA7 Instrument (Life Technologies) using standard cycling conditions. Primer sets adapted  
621 from previous works (85-87) were used to detect ZIKV and DENV RNA. ZIKV FG15: forward,  
622 5'-CCG CTG CCC AAC ACA AG- 3'; reverse, 5'-CCA CTA ACG TTC TTT TGC AGA CAT-  
623 3'; probe, 5'-6FAM-AGC CTA CCT TGA CAA GCA ATC AGA CAC TCA A-MGB- 3' (Life

624 Technologies). ZIKV HD78788: forward, 5'-AAA TAC ACA TAC CAA AAC AAA GTG GT-  
625 3'; reverse, 5' -TCC ACT CCC TCT CTG GTC TTG- 3'; probe, 5'-6FAM-CTC AGA CCA  
626 GCT GAA G-MGB- 3' (Life Technologies). DENV-1 KDH0026A: forward, 5' –GGA AGG  
627 AGA AGG ACT CCA CA- 3'; reverse, 5'- ATC CTT GTA TCC CAT CCG GCT- 3'; probe, 5'  
628 -6FAM CTC AGA GAC ATA TCA AAG ATT CCA GGG-MGB- 3' (Life Technologies). Viral  
629 load is expressed on a Log<sub>10</sub> scale as viral genome copies per milliliter (plasma samples) or per  
630 total RNA microgram (brain samples) after comparison with a standard curve produced using  
631 serial 10-fold dilutions of a plasmid containing the corresponding fragment of ZIKV genome.

632

### 633 **Western Blot analysis**

634 MEFs ( $5 \cdot 10^6$ ) were pre-incubated with 100 µg IFNAR1-blocking antibody (MAR1-5A3,  
635 BioXCell) for 7 hours and then stimulated, or not, with 300 IU/mL mouse IFN-α (Miltenyi  
636 Biotec) for 15 minutes. MEFs were detached and centrifuged at 300xg for 5 minutes and cell  
637 pellet was re-suspended in cold PBS. MEFs were then lysed into extraction buffer (10 mM Tris–  
638 HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% NP40, 10% glycerol, 30 mM NaP, 50 mM  
639 NaFluoride) completed with protease inhibitor (Complete, EDTA free, Roche) and phosphatase  
640 inhibitors (phosStop easy pack, Roche) with 2.5 UI of benzonase nuclease (Sigma). Lysates were  
641 incubated on ice for 30 minutes and non-soluble fraction was separated by centrifugation. Protein  
642 concentrations were determined by Bradford assay, and equal amounts of protein were further  
643 used. Protein denaturation was performed in Laemmli buffer at 95°C for 5 minutes. After  
644 separation on a 12% polyacrylamide gel (Biorad), proteins were transferred on Immun-Blot  
645 polyvinylidene difluoride (PVDF) membrane (Biorad) and incubated overnight with the  
646 following antibodies: Anti-Phospho-Stat1 Tyr701 (1/1,000<sup>e</sup>, #9167, Cell Signaling), Anti-Total  
647 Stat1 N-terminus (1/500<sup>e</sup>, # 610115, BD Biosciences), Anti-αTubulin (1/8,000<sup>e</sup>, # T5168,

648 Merck). Membranes were incubated for 1.5 hour at room temperature with an anti-mouse or an  
649 anti-rabbit IgG horseradish peroxidase-linked secondary antibody (1/10,000<sup>e</sup>, NA931 and  
650 NA934V, Amersham) and signals were visualized using autoradiography.

651

## 652 **Histopathology**

653 After necropsy, brain was removed, fixed for 48-72 hours in 10% neutral-buffered formalin  
654 and embedded in paraffin; 4µm-thick sections were stained in hematoxylin-eosin. Morphology  
655 of microglial cells was assessed by immunohistochemistry using rabbit anti-Iba1 primary  
656 antibody (# 01919741, Wako chemical, dilution 1:50) as previously described (88). Sections  
657 were analyzed by a trained veterinary pathologist in a blind study on coded slides. Quantification  
658 of the microglia reaction was carried out using automated detection of the Iba1 labeling signal  
659 and measurement of the labelled area on a low magnification (x2) picture of a brain section of  
660 each mouse, using ImageJ software (<https://imagej.nih.gov/ij/>).

661

## 662 **Genetic analysis**

663 Broad sense heritability was calculated as previously described (60).

664 Plasma viral load at days 2 and 6 p.i. and plasma viral load decrease, measured on 159 mice  
665 from 35 CC strains (average of 4.5 mice per strain), were used in quantitative trait locus (QTL)  
666 mapping using the *rqtl2* R package (89) and GigaMUGA genotypes of CC founders and CC  
667 strains available from <http://csbio.unc.edu/CCstatus/CCGenomes/#genotypes>. Genome scan was  
668 performed using the *scan1* function with a linear mixed model using a kinship matrix. Statistical  
669 significance levels were calculated from 1,000 permutations.

670 Genotype-phenotype associations for specific genes (*Ifnar1*, *Oas1b*) were tested by Kruskal-  
671 Wallis test using the founder haplotype as the genotype.

672

### 673 **Statistical analysis**

674 Statistical analyses were performed using R software (v3.5.2). Kaplan-Meier survival curves  
675 were compared by logrank test. Two-way ANOVA was used for testing mouse strain and sex  
676 effects on plasma viral load at day 2 p.i. (Fig. 2E). Student's *t*-test was used to compare viral  
677 loads in tissues, except when data showed heterogeneous variance between groups, in which case  
678 we used Kruskal-Wallis and Wilcoxon non-parametric tests. These tests were also used for  
679 assessing mouse strain effect on plasma viral load and on plasma viral load decrease (Fig. 3).  
680 Pearson's coefficient was used for the correlation between plasma viral load at days 2 and 6 p.i.  
681 (Fig. 3B) and for the correlation between measurements of plasma viral load by FFA and RT-  
682 qPCR (Fig. 2D). Student's *t*-test was used to compare viral titers between strains in *in vitro*  
683 experiments. P-values < 0.05 were considered statistically significant.

684

685

### 686 **NOTES**

687 We are grateful to the Virology Laboratory of the Institut Pasteur of French Guyana  
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1024

1025 **FIGURE LEGENDS**

1026 **FIG 1** ZIKV disease severity in *Ifnar1*-deficient mice is driven by the genetic background.

1027 6-7 week-old 129-*Ifnar1* (n = 7) and B6-*Ifnar1* (n = 10) mice were infected IP with  $10^7$  FFUs of  
1028 ZIKV FG15 and monitored for 14 days. (A) Average clinical score, with numerical values given  
1029 as follows: 0, no symptom; 1, ruffled fur; 2, emaciation, hunched posture and/or hypo activity; 3,  
1030 hind limb weakness, prostration and/or closed eyes; and 4, moribund or dead. (B) Kaplan-Meier  
1031 survival curves showing 100% lethality in B6-*Ifnar1* mice at day 7 p.i. and survival of 6/7 129-  
1032 *Ifnar1* mice (logrank test, \*\*\*: p = 0.0002). B6-*Ifnar1* mice developed early symptoms which  
1033 rapidly evolved to death, while 129-*Ifnar1* mice developed symptoms two days later which  
1034 eventually resolved in most mice.

1035 **FIG 2** Establishment and validation of the experimental conditions for assessing susceptibility to  
1036 ZIKV in CC strains. (A) The efficacy of the MAR1-5A3 mAb (100 $\mu$ g for  $5 \cdot 10^6$  cells) to block  
1037 the IFNAR receptor in diverse mouse genetic backgrounds was determined by assessing STAT1  
1038 phosphorylation by Western blotting on mouse embryonic fibroblasts (MEFs) derived from  
1039 C57BL/6J, CC001, CC071 and CD-1 strains. (B) Plasma viral load measured on days 2, 3 and 6  
1040 p.i. by RT-qPCR on 6-8 week-old mice of the CC001 and CC071 strains treated with MAR1-  
1041 5A3 24h prior to ZIKV infection (filled circles; n = 9 and 8, respectively) or untreated (open  
1042 circles; n = 3 and 2, respectively). "x" denotes a sample below the detection level. (C) Kinetics of  
1043 plasma viral load in 129-*Ifnar1* and 4 CC strains measured by RT-qPCR. Each circle represents a  
1044 6-8 week-old mouse analyzed on days 1, 2, 3 and 6. (D) Correlation between plasma viral load  
1045 determined by FFA (x-axis) and RT-qPCR (y-axis) on 46 blood samples from 129-*Ifnar1*, B6-  
1046 *Ifnar1* and 10 CC strains (circles show the mean of each strain; the number of 6-8 week-old mice



1047 per strain is shown in parentheses). (E) Plasma viral load measured by RT-qPCR at day 2 p.i. in  
1048 6-8 week-old males and females of 129-*Ifnar1* and 4 CC strains for which both sexes had been  
1049 tested (with  $n \geq 4$  mice per group). (F) Effect of the dose of MAR1-5A3 antibody treatment on  
1050 the rate of decrease of plasma viral load. 7-9 week-old mice of the CC012 and CC037 strains (4  
1051 mice per group) received 2mg MAR1-5A3 mAb one day prior to being inoculated IP with  $10^7$   
1052 FFUs of ZIKV FG15. The 4mg groups received additional IP injections of 1mg of mAb on days  
1053 2 and 4 p.i. Plasma viral load was measured on days 2 and 6 p.i. (above and below by the dashed  
1054 line, respectively).

1055 **FIG 3** CC genetic diversity strongly impacts clinical severity and plasma viral load. Thirty-five  
1056 CC strains ( $n = 2$  to 9, 6-8 week-old, mice per strain) were infected IP with  $10^7$  FFUs of ZIKV  
1057 FG15, 24hr after IP injection of 2 mg of MAR1-5A3 mAb. 129-*Ifnar1* ( $n = 24$ ) and B6-*Ifnar1* ( $n$   
1058  $= 5$ ) mice were similarly infected without mAb treatment. (A) Clinical scores at day 7 p.i. as the  
1059 percentage of mice in the five levels of severity (same as in Fig. 1). (B) Plasma viral load at days  
1060 2 (upper values) and 6 p.i. (lower values) quantified by RT-qPCR, shown as box-whisker plot  
1061 with outliers as dots (strains are shown in the same order as in A). (C) Difference between  
1062 plasma viral loads at days 2 and 6 p.i. Strains are sorted by increasing absolute difference,  
1063 therefore in a different order from A and B. (B and C) Arrows indicate the subset of CC mouse  
1064 strains selected for detailed study.

1065 **FIG 4** The differences in susceptibility to ZIKV between CC strains are conserved with other  
1066 flaviviruses. (A) Six to 8 week-old mice from three selected CC strains treated with MAR1-5A3  
1067 mAb and 129-*Ifnar1* mice were infected intraperitoneally with  $10^3$  FFUs of ZIKV HD78788.  
1068 Left: average clinical score, with numerical values given as in Fig. 1. Center: Kaplan-Meier  
1069 survival curves (logrank test). Right: plasma viral load at day 2 p.i., measured by RT-qPCR

1070 (Wilcoxon). (B) Viral load after ZIKV infection (left, data extracted from Fig. 3) and DENV  
1071 infection (right, IV infection with  $2 \cdot 10^6$  FFUs of DENV KDH0026A) was compared in mAb-  
1072 treated CC001, CC071 and B6 6-8 week-old mice and in 129-*Ifnar1* and B6-*Ifnar1* (*t* test). (C)  
1073 Left: Kaplan-Meier survival curves of four, 8-12 week-old, male mice of each of the three  
1074 selected CC strains infected IP with 1000 FFU of WNV strain IS-98-ST1 and monitored for 14  
1075 days (logrank test). Right: Kaplan-Meier survival curves of four to five, 8-12 week-old, male  
1076 mice of BALB/cByJ and each of the three selected CC strains infected IP with 100 PFUs of  
1077 RVFV strain ZH548 and monitored for 14 days (logrank test,  $p > 0.05$ ). \*  $p < 0.05$ ; \*\*  $p < 0.01$ ;  
1078 \*\*\*  $p < 0.001$ .

1079 **FIG 5** Genetic analysis of susceptibility to ZIKV fails to identify simple genetic control.  
1080 Genome-wide linkage analysis for the plasma viral load at day 2 p.i.(A), the plasma viral load at  
1081 day 6 p.i. (B) and the decrease rate of plasma viral load (C) on the 35 CC strains shown on FIG  
1082 3. The x-axis represents genomic location; the y-axis is the LOD score, representing the  
1083 statistical association between the phenotype and the genomic location. Genome-wide thresholds  
1084  $p = 0.1$ ,  $p=0.05$  and  $p = 0.01$ , computed from 1000 permutations, are represented by dashed  
1085 black, dashed red and plain red lines, respectively. No genome location reached the  $p = 0.05$   
1086 threshold.

1087 **FIG 6** Genetic variations between CC strains control brain viral load and histological profile in  
1088 infected mice. Four to five, 6-8 week-old, mice of 129-*Ifnar1* and three selected CC strains were  
1089 infected IP with  $10^7$  FFUs of ZIKV FG15 24hr after IP injection of 2 mg of MAR1-5A3 mAb.  
1090 (A) Brain viral load measured by RT-qPCR at day 6 p.i. (Wilcoxon, \*:  $p < 0.05$ ). (B-N)  
1091 Representative HE-stained brain sections at three different magnifications. (B-E) 129-*Ifnar1*  
1092 mice (n=3). Black rectangle: encephalitis with perivascular lymphocyte cuffs; arrows: lesions of

1093 subacute leptomeningo-encephalitis. (F-H) CC001 mice (n=5). (I-K) CC005 mice (n=5). Black  
1094 rectangle: encephalitis with perivascular lymphocyte cuffs; arrow: perivascular cuffing. (L-N)  
1095 CC071 mice (n=4).

1096 **FIG 7** Genetic variations between CC strains control brain neuroinflammation in infected mice.  
1097 Microglial reactivity was assessed on brain sections from the same mice as in Fig. 6 by anti-Iba1  
1098 immunohistochemistry. (A) Quantification of Iba1 labeling signal on the brain sections (t tests; \*  
1099  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). (B-M) Representative anti-Iba1 immunohistochemistry of  
1100 brain sections at three different magnifications. (B-D) 129-*Ifnar1* mice (n=3), (E-G) CC001 mice  
1101 (n=5), (H-J) CC005 mice (n=5), (K-M) CC071 mice (n=4). Arrowheads: nodules of activated  
1102 microglial cells.

1103 **FIG 8** Intracranial ZIKV FG15 infection results in strain-dependent viral load and brain  
1104 histological lesions. Groups of 5-6 week-old mice of 129-*Ifnar1* and three selected CC strains (3-  
1105 5 mice per strain) were infected IC with  $10^5$  FFUs of ZIKV FG15 in the absence of prior anti-  
1106 IFNAR treatment. (A) Brain viral load measured by RT-qPCR at day 6 p.i. (Wilcoxon, \*\*:  $p <$   
1107 0.01). (B-O) Representative HE-stained brain sections at three different magnifications. (B-E)  
1108 129-*Ifnar1* mice (n=4). Black rectangles: encephalitis with perivascular lymphocyte cuffs,  
1109 arrows: leptomeningitis. (F-H) CC001 (n=5). (I-K) CC005 (n=4). (L-O) CC071 mice (n=5).  
1110 Black rectangles: encephalitis with perivascular lymphocyte cuffs, arrows: leptomeningitis.

1111 **FIG 9** Intracranial ZIKV FG15 infection results in strain-dependent neuroinflammation of the  
1112 brain. Microglial reactivity was assessed on brain sections from the same mice as in Fig. 8 by  
1113 anti-Iba1 immunohistochemistry. (A) Quantification of Iba1 labeling signal on the brain sections  
1114 (t tests; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). (B-M) Representative anti-Iba1  
1115 immunohistochemistry of brain sections at three different magnifications. (B-D) 129-*Ifnar1* mice



1116 (n=4), (E-G) CC001 mice (n=5), (H-J) CC005 mice (n=4), (K-M) CC071 mice (n=5).





1117 Arrowheads: nodules of activated microglial cells.

1118 **FIG 10** Enhanced ZIKV replication in CC071 MEFs compared with CC001. MEFs derived from  
1119 CC001 (blue) and CC071 (red) embryos were infected with ZIKV FG15 at MOI 5. ZIKV titer in  
1120 the supernatant was quantified by focus-forming assay at 24, 48 and 72 hours p.i. Mean +/- SEM  
1121 from 3 biological replicates. Results were replicated in 3 independent experiments. (t tests; \*\*\*  
1122  $p < 0.001$ ).

1123

Strain	<i>Oas1b</i>	<i>Ifnar1</i>
CC001	B	F
CC002	A	B
CC003	H	B
CC004	G	B
CC005	B	E
CC006	E	D
CC007	D	B
CC009	E	D
CC011	B	G
CC012	H	A
CC013	E	E
CC017	H	D
CC018	D	C
CC019	H	C
CC021	C	D
CC024	E	A
CC025	H	A
CC026	A	H
CC027	B	G
CC032	B C *	B
CC037	B	C
CC039	H	B
CC040	B D *	D
CC041	F	D
CC042	H	F
CC043	C	C
CC045	C	H
CC049	B D *	D
CC051	E	B
CC059	E	B
CC060	D	F
CC061	E	A
CC068	B	E G *
CC071	C	F
CC072	C	D

functional   
 not functional 

 A/J allele  
 B6 allele or identical  
 CAST allele  
 PWK allele

1124 \* heterozygous strain

1125 A: A/J; B: C57BL/6J; C: 129S1/SvImJ; D: NOD/ShiLtJ; E: NZO/HILtJ; F: CAST/EiJ; G: PWK/PhJ; H: WSB/EiJ

1126 Data from <http://csbio.unc.edu/CCstatus/CCGenomes/#genotypes>.

1127 **Table 1. Origin of *Oas1b* and *Ifnar1* alleles in the 35 CC strains tested.**

1128

Position*	dbSNP	Reference (B6)	I29S1/SvImJ	A/J	CAST/EiJ	NOD/ShiLtJ	NZO/HILtJ	PWK/PhJ	WSB/EiJ	Amino acid	AA change**
91,485,366	rs235809125	C	-	-	T	-	-	-	-	8	A -> V
91,485,368	rs255866699	G	-	-	A	-	-	-	-	9	A -> T
91,499,433	rs31418313	G	-	A	-	-	-	-	-	274	R -> H
91,499,468	rs223171563	A	-	-	G	-	-	-	-	286	N -> D
91,499,532	rs240163845	A	-	-	G	-	-	G	-	307	H -> R
91,501,623	rs263194450	T	-	-	G	-	-	G	-	376	I -> R
91,501,631	rs246470801	G	-	-	A	-	-	A	-	379	E -> K
91,505,284	rs219805193	C	-	-	T	-	-	-	-	549	A -> V

1129 \* positions 16:91,485,344-91,505,411 retrieved from the Sanger Institute Mouse Genomes Project  
1130 ([https://www.sanger.ac.uk/sanger/Mouse\\_SnpViewer/rel-1505](https://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1505))

1131 \*\* amino-acid changes compared with the reference (B6) sequence (CCDS database :  
1132 <https://www.ncbi.nlm.nih.gov/CCDS/>)

1133

1134 **Table 2. Variants in the coding sequence of the *Ifnar1* gene.**

1135

1136

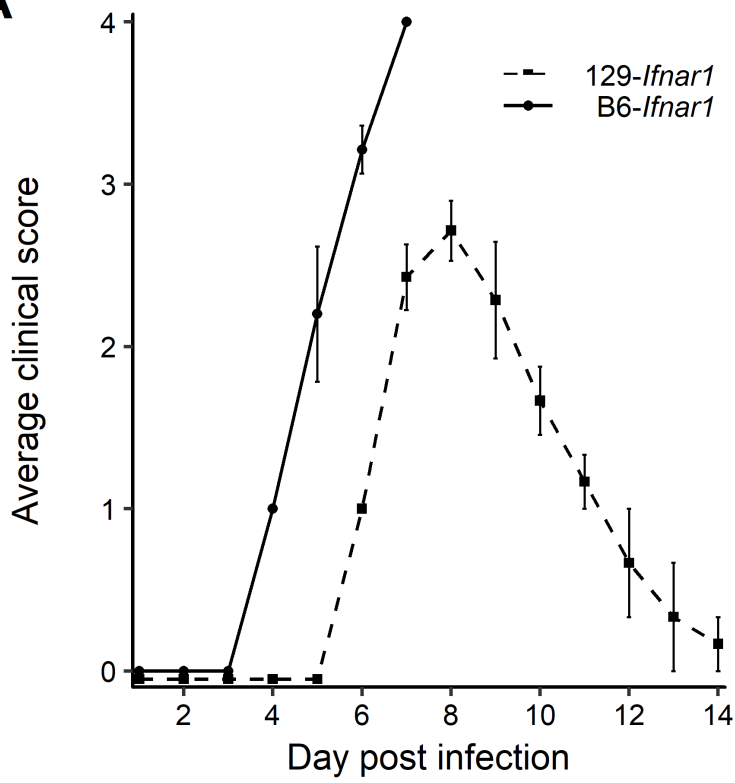
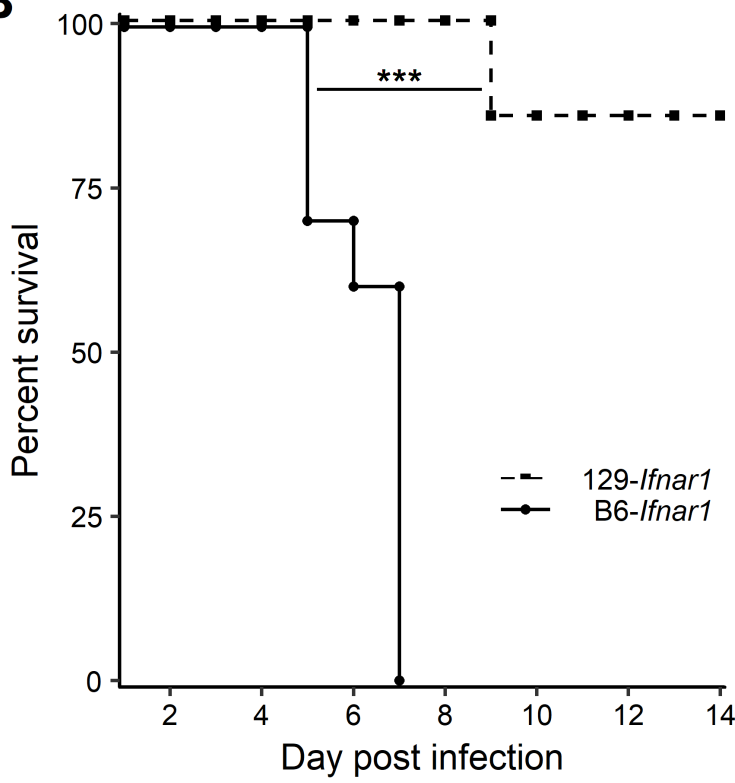
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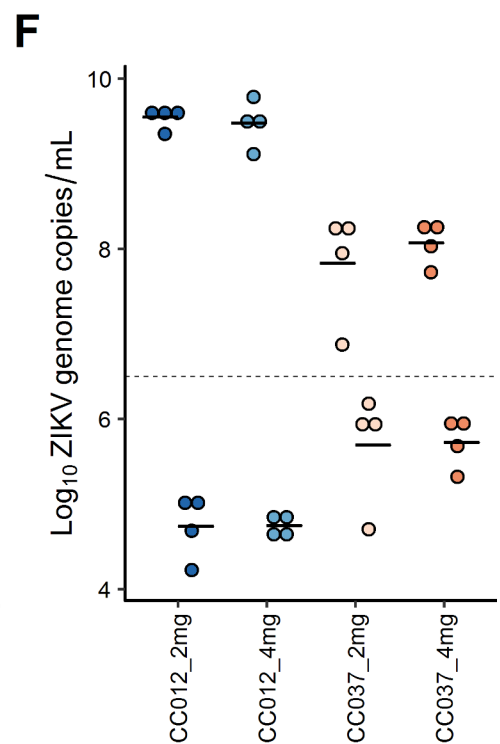
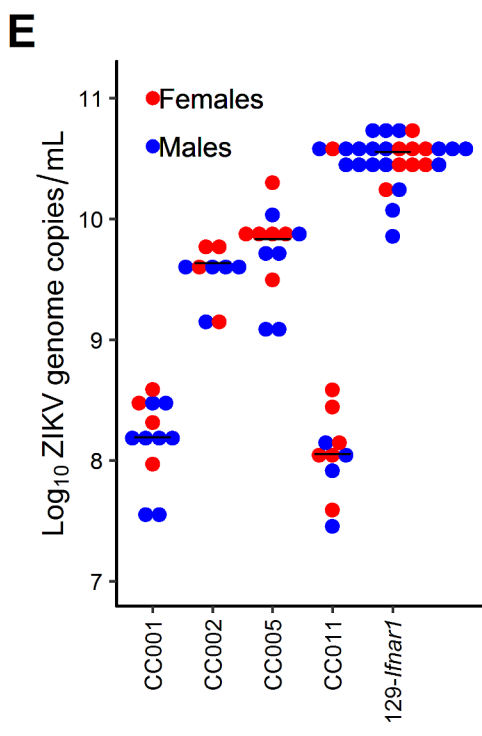
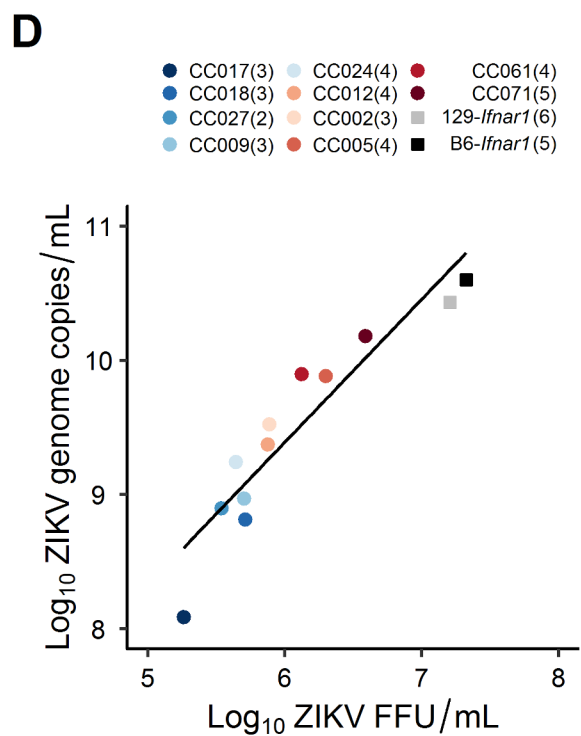
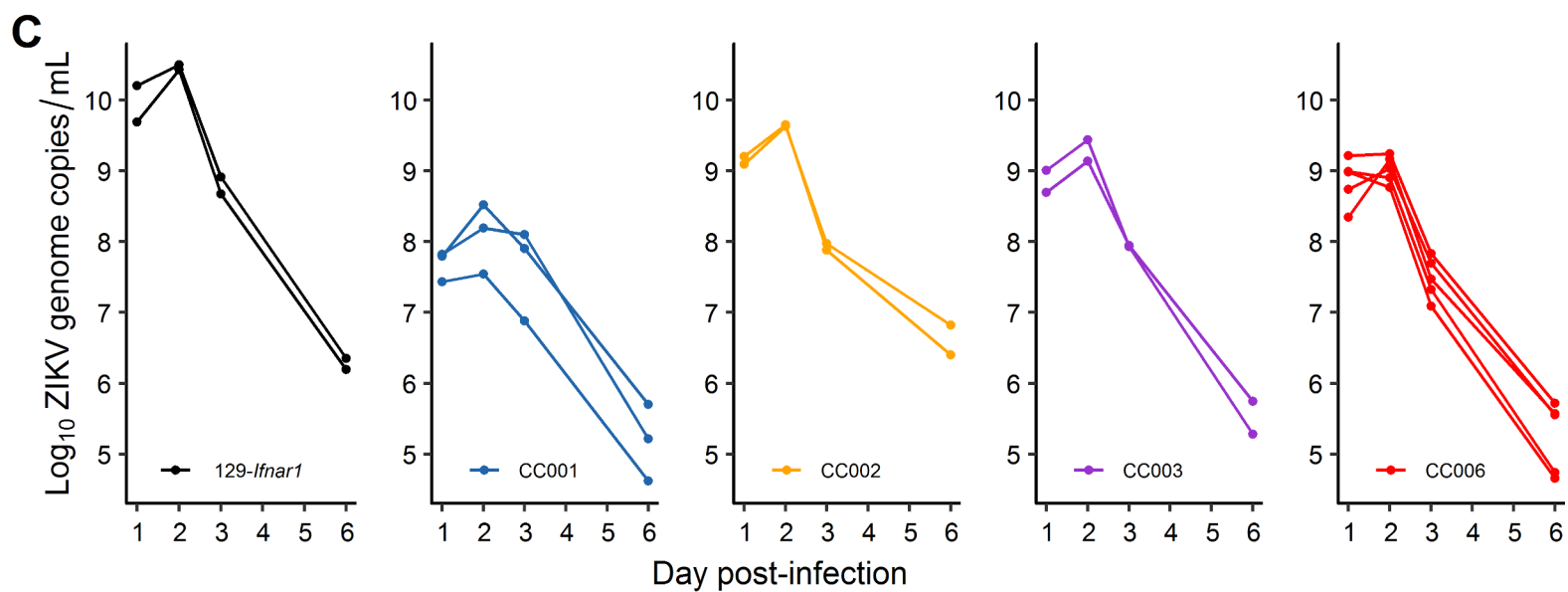
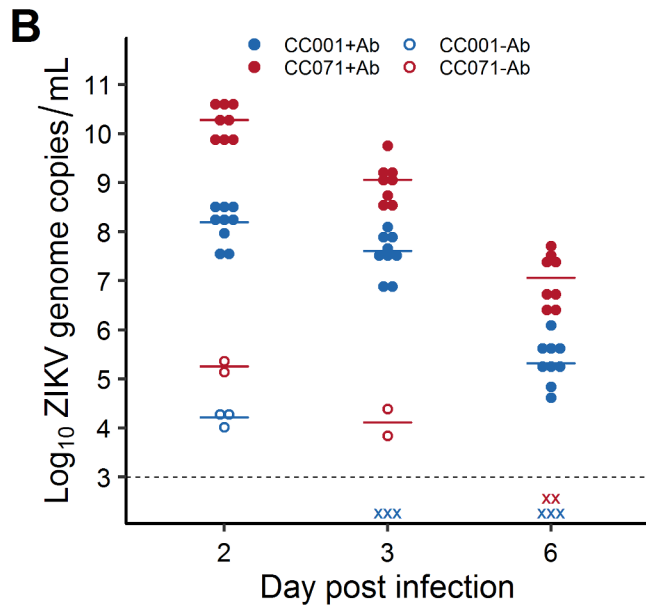
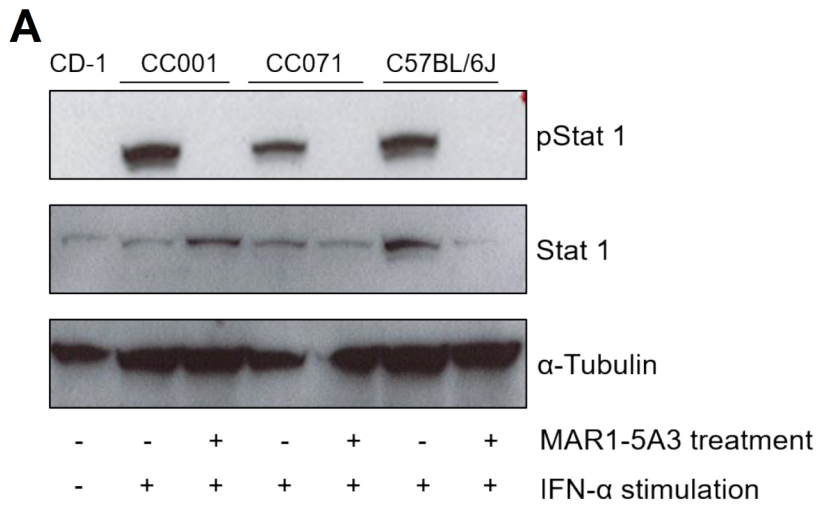
	CC001	CC005	CC071	129- <i>Ifnar1</i>
Symptoms	—	—	+	+
Mortality	0%	0%	78%	12.5%
Peak plasma viral load	+	+	+	+
Rate of decrease of plasma viral load	↘	↓	↘	↓
Brain viral load (systemic infection)	+	+	+	+
Brain pathology (systemic infection)	—	+	+	+
Brain viral load (intracerebral infection)	+	+	+	+
Brain pathology (intracerebral infection)	+	+	+	+
Viral replication in vitro (24-72 hours)	↘	-	↗	-

1138

1139 **Table 3. Summary of the main features of ZIKV infection in mAb-treated CC strains and**  
1140 **129-*Ifnar1* mice.**

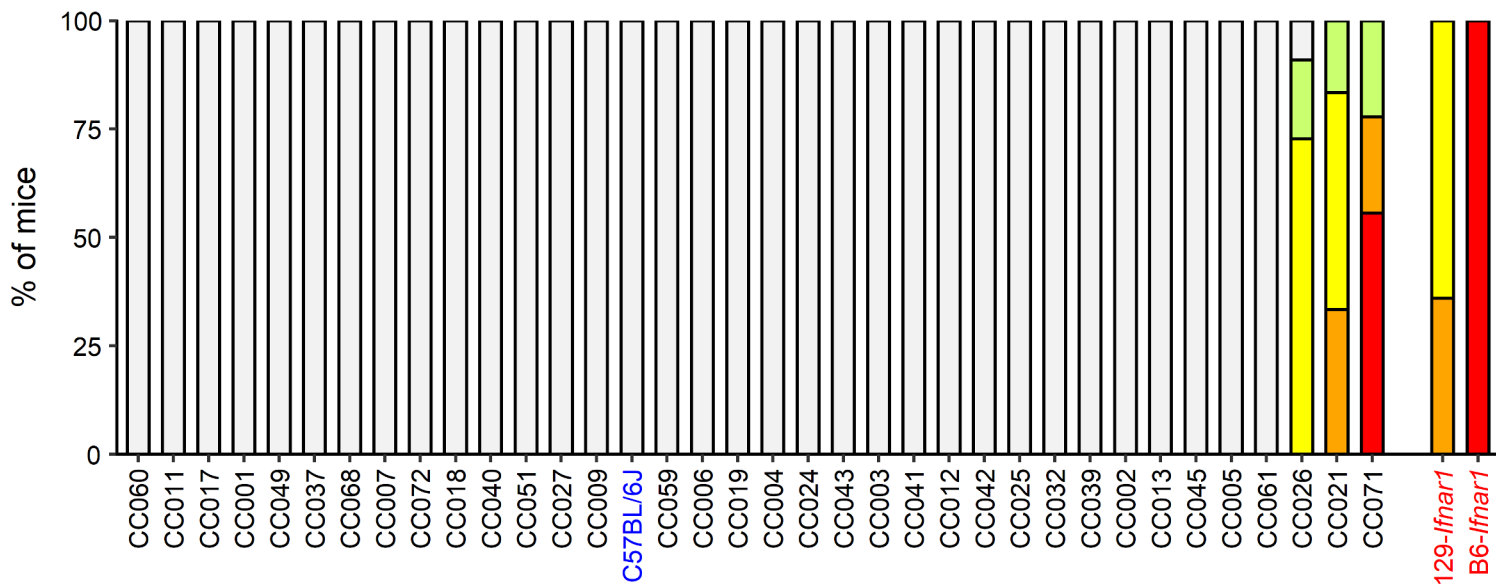
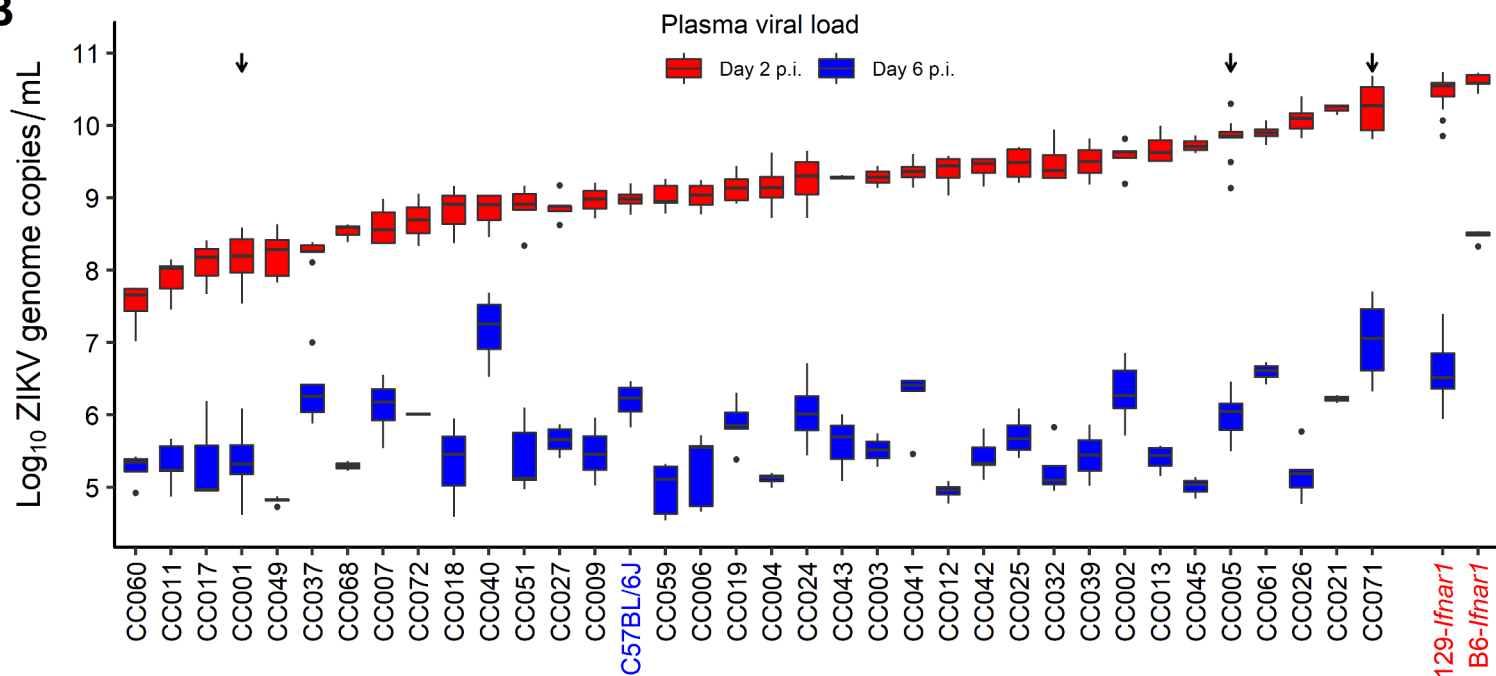
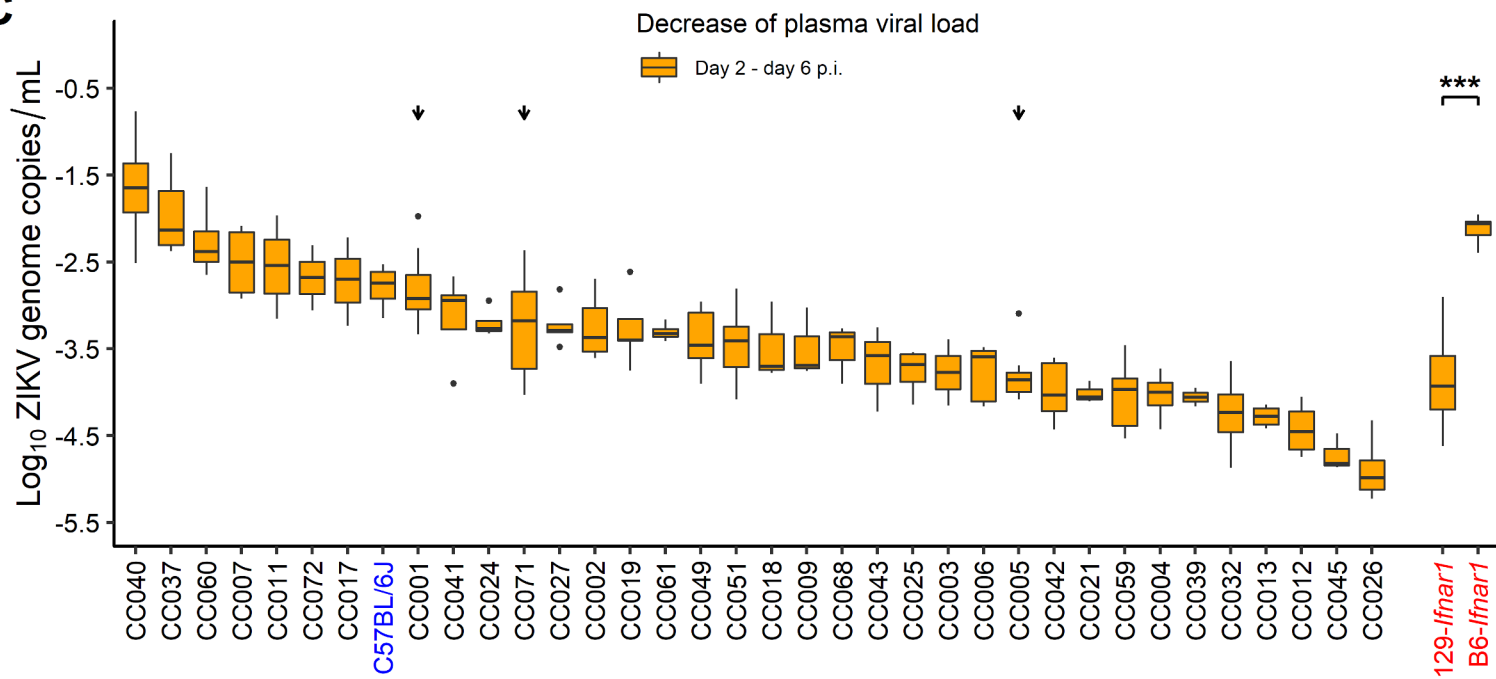


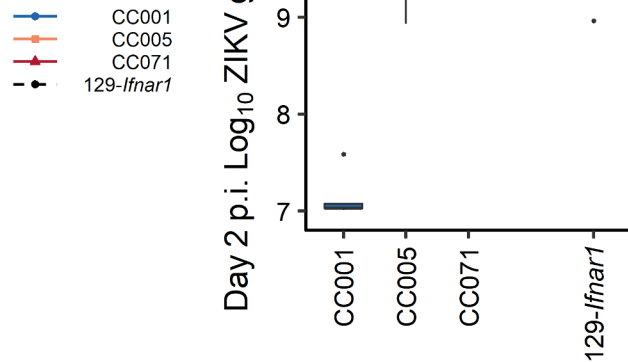
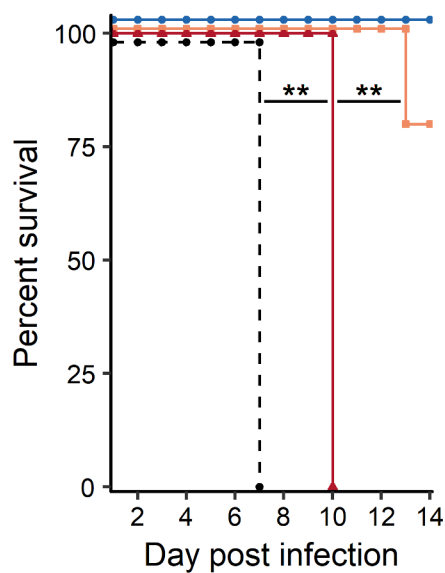
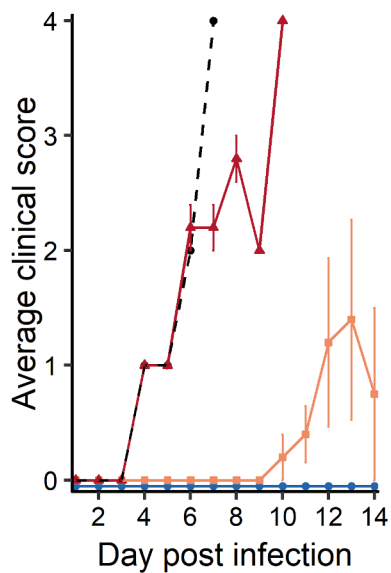
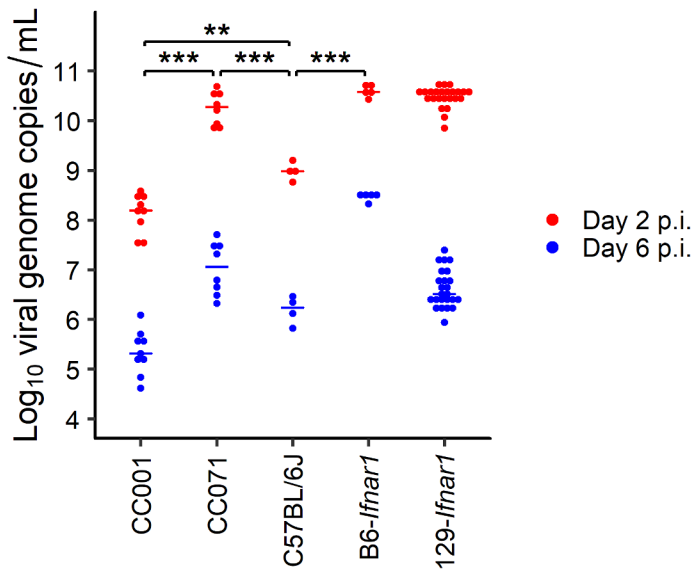
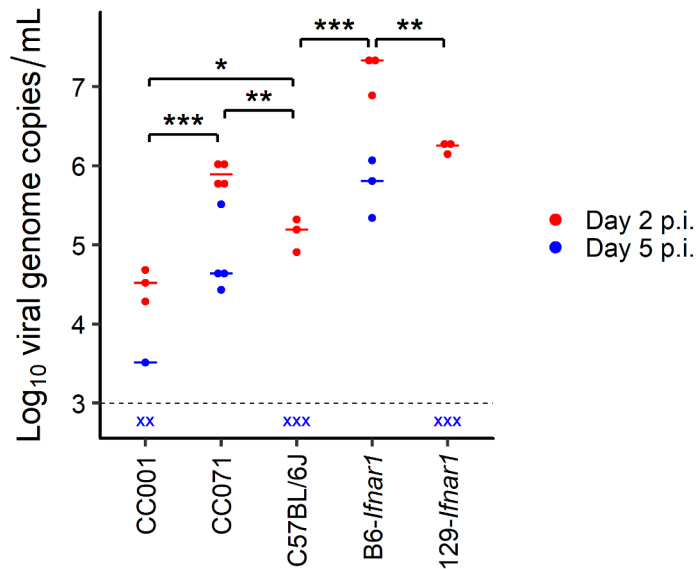
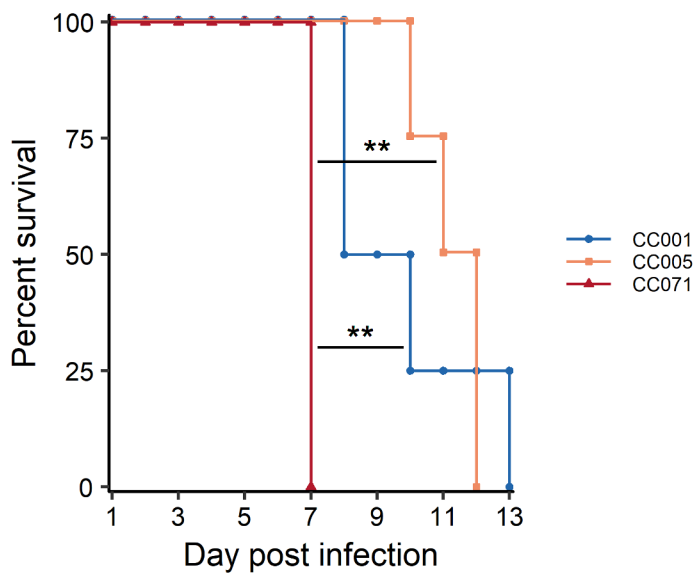
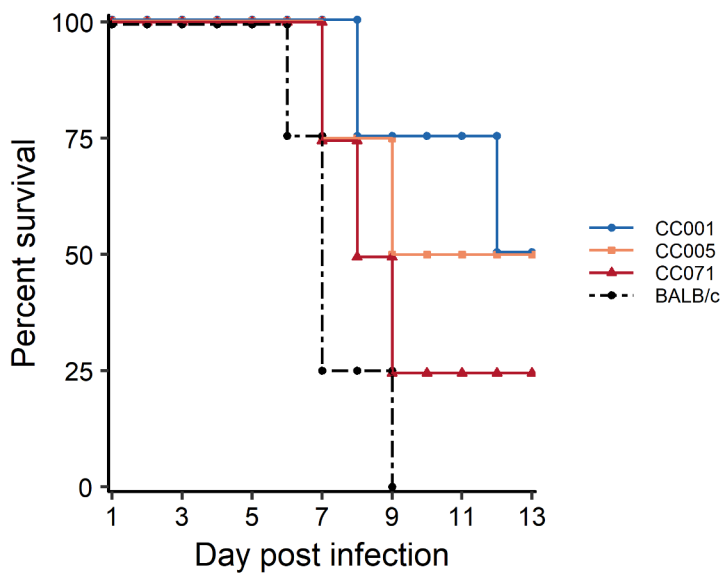
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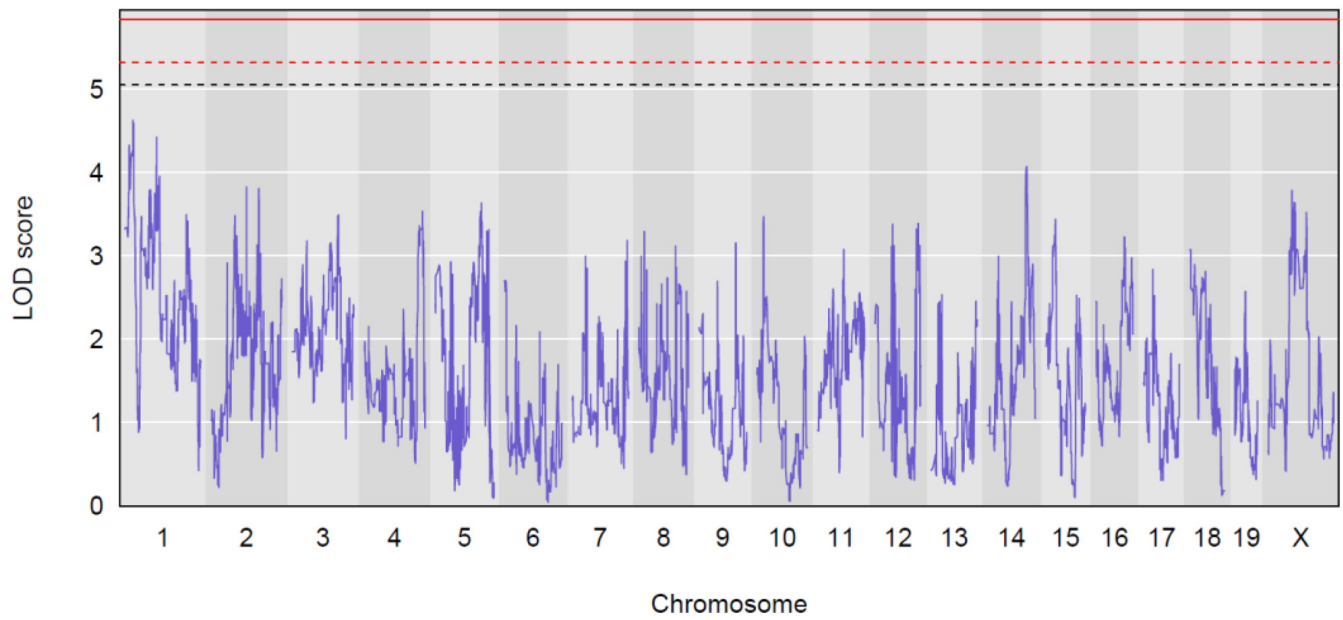
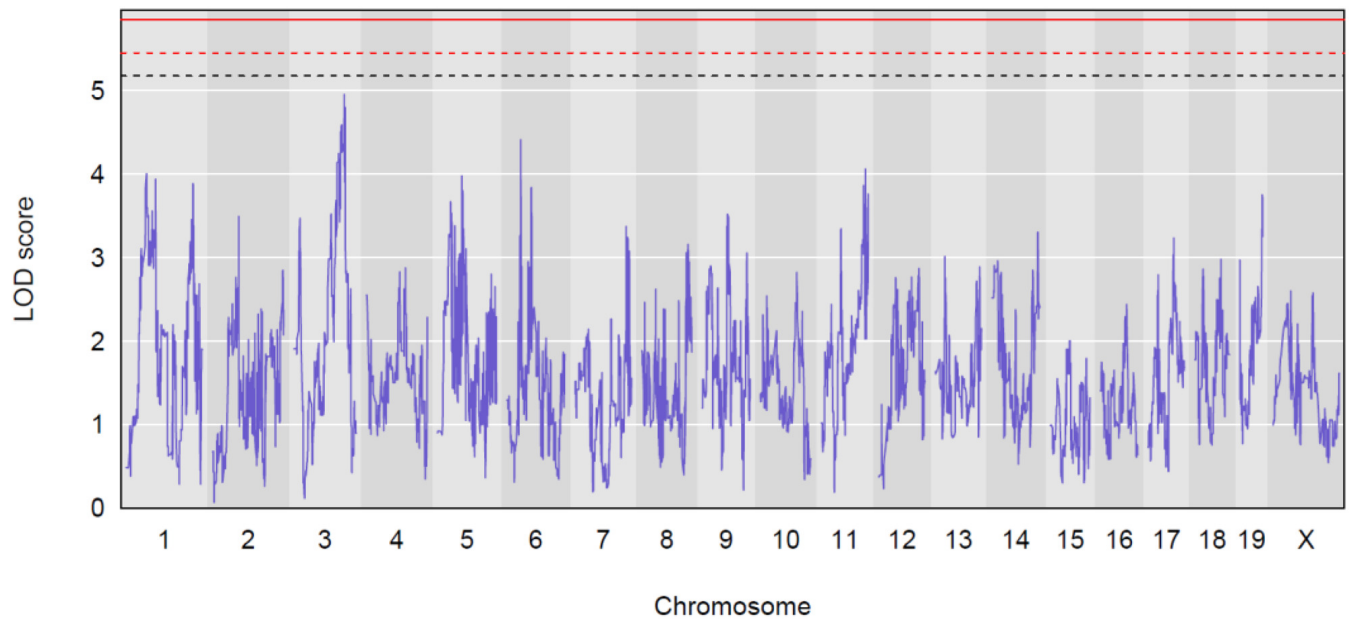
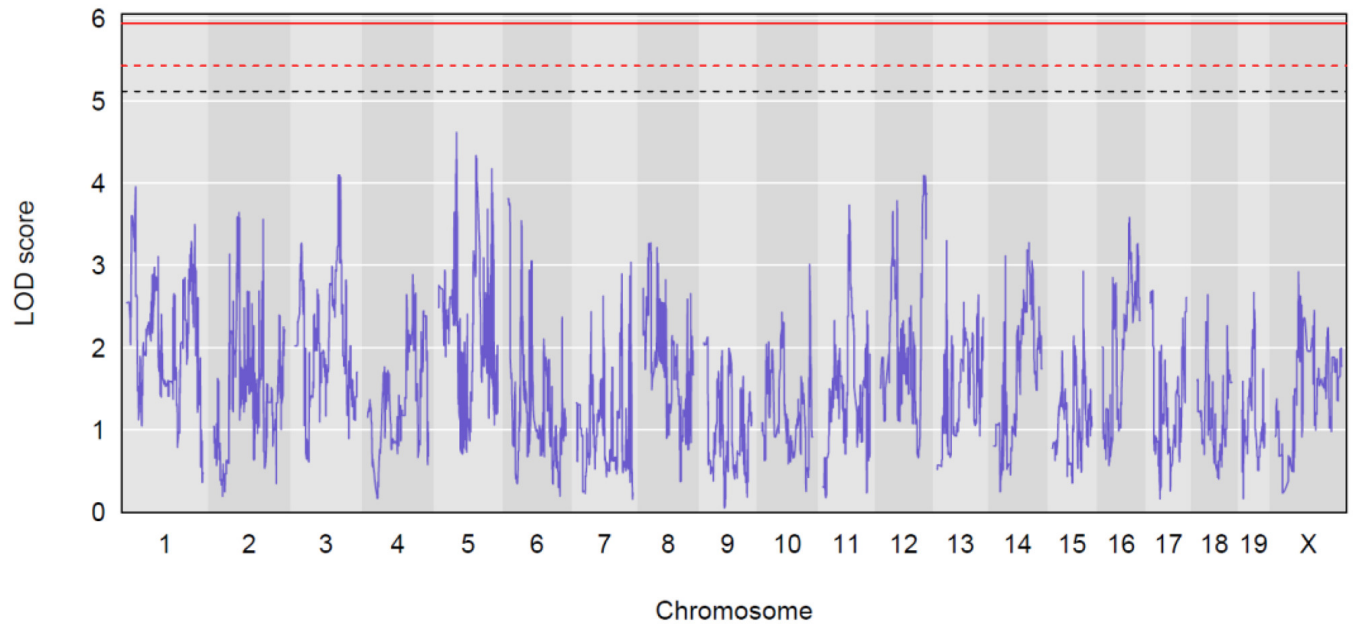


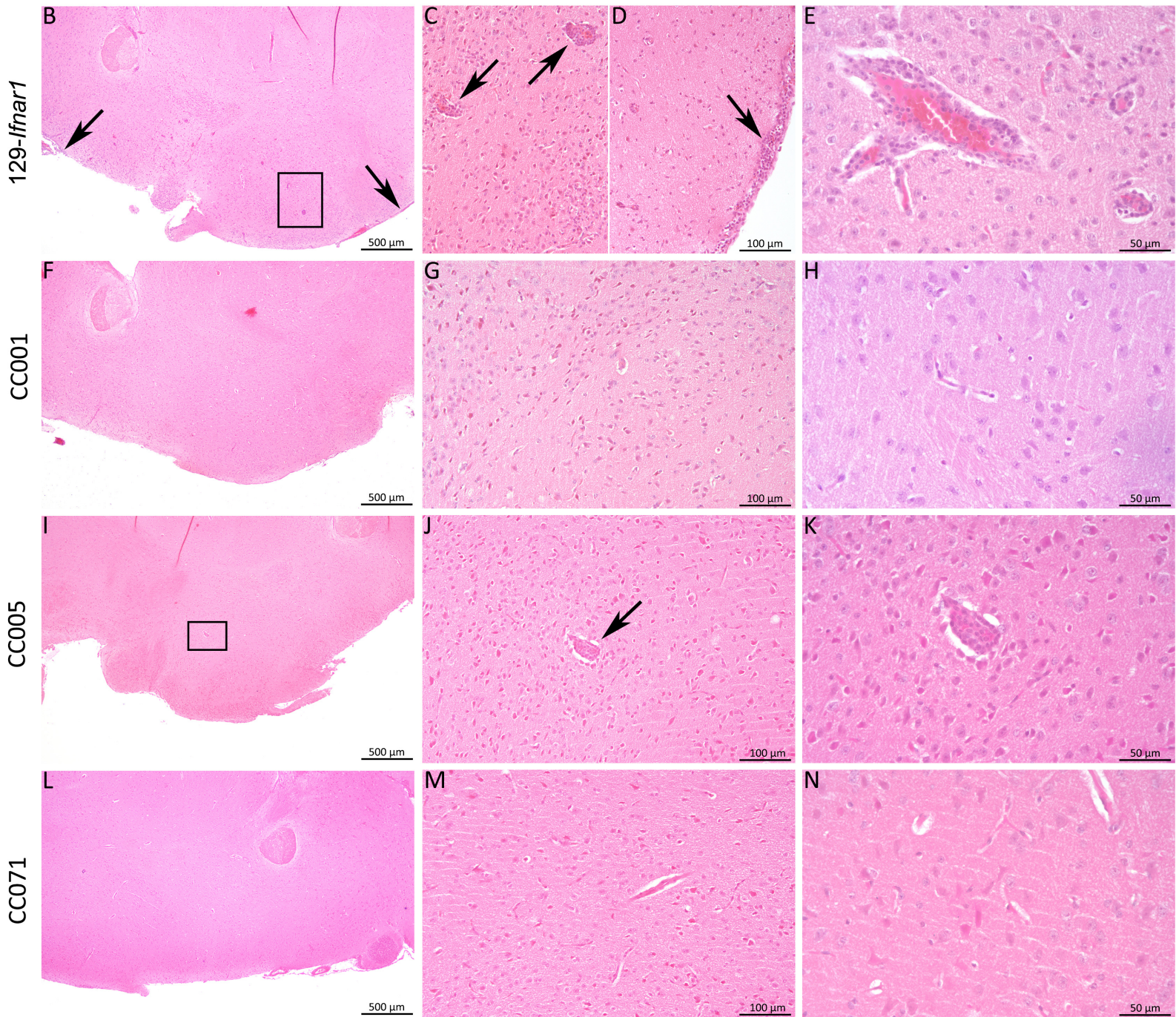
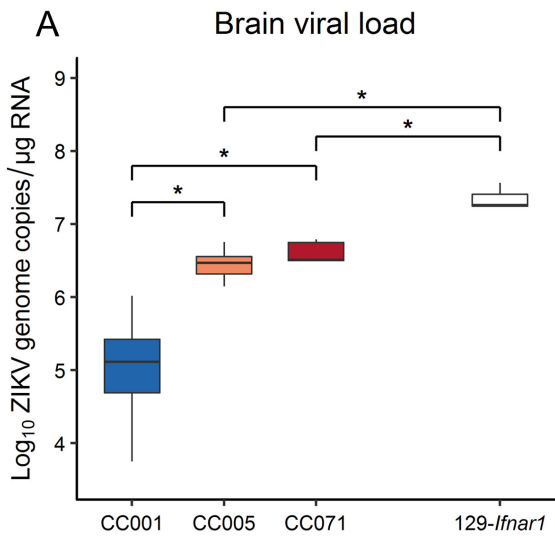
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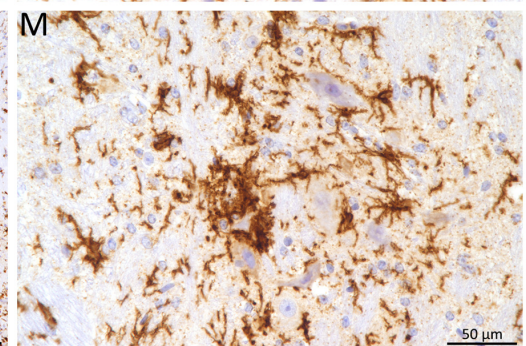
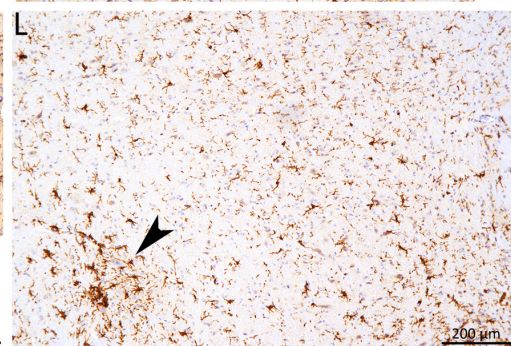
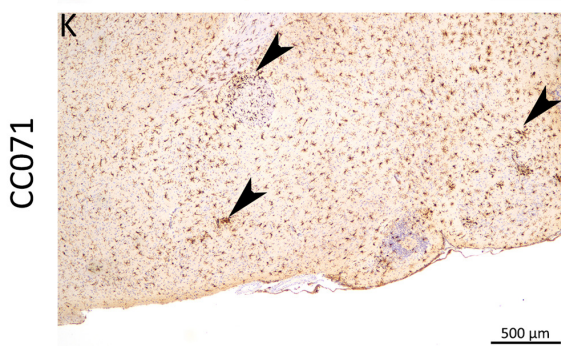
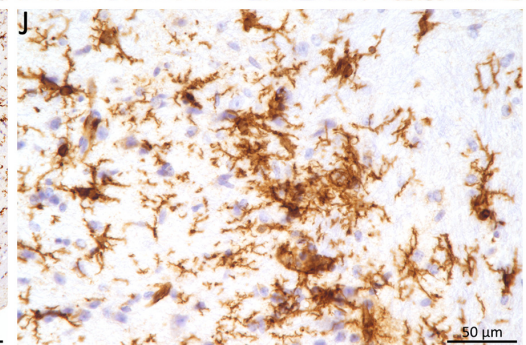
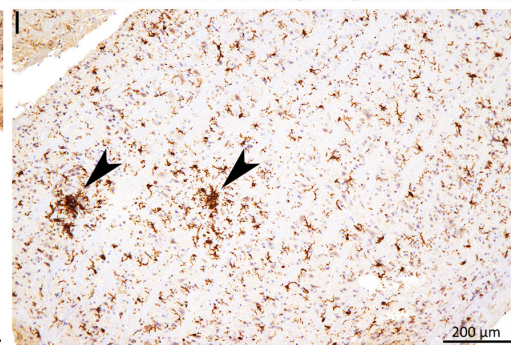
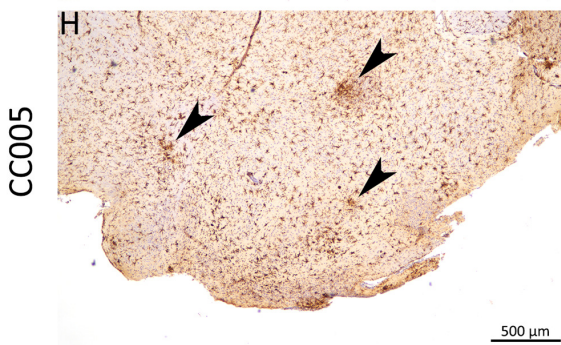
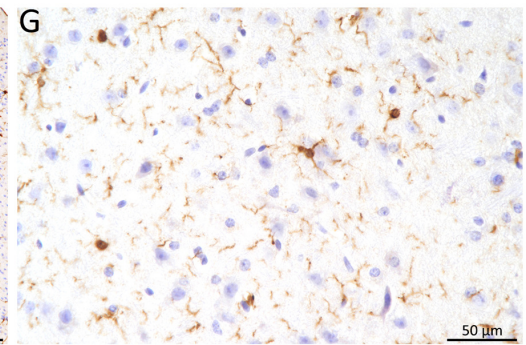
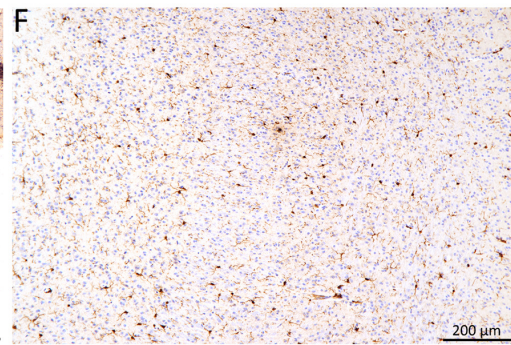
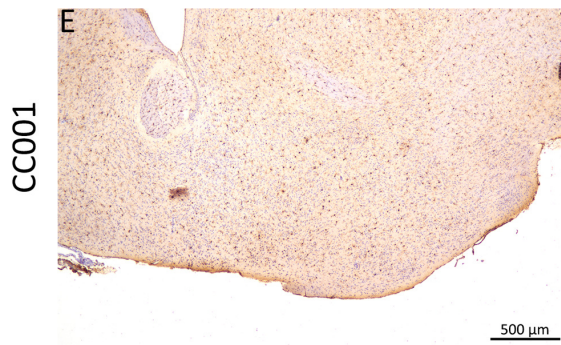
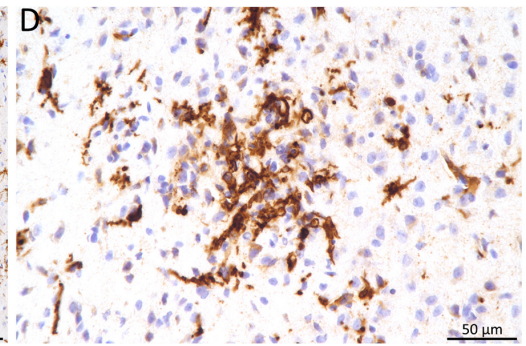
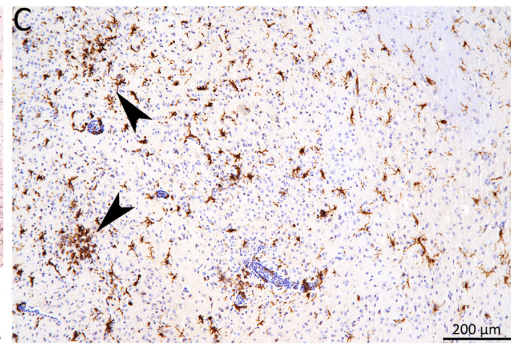
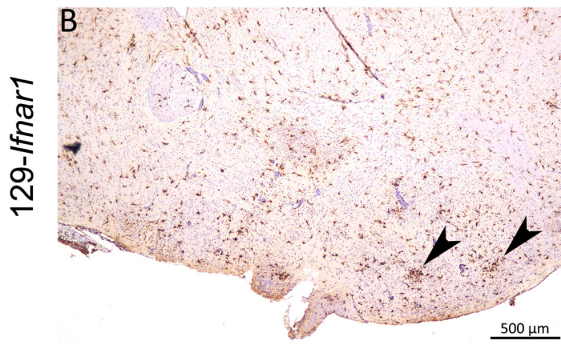
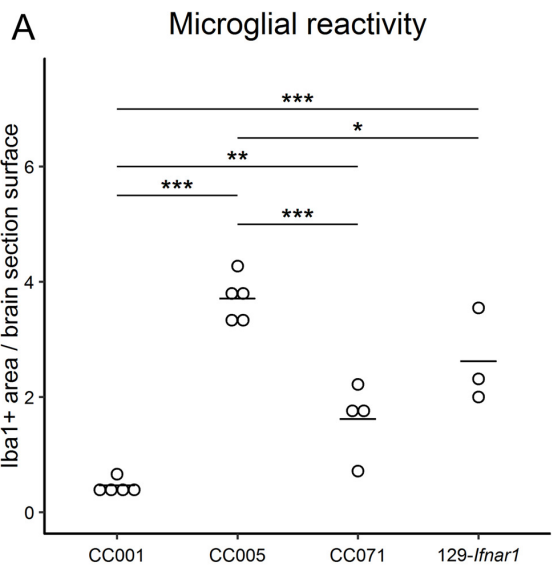
Clinical score at day 7 p.i. 0 1 2 3 4

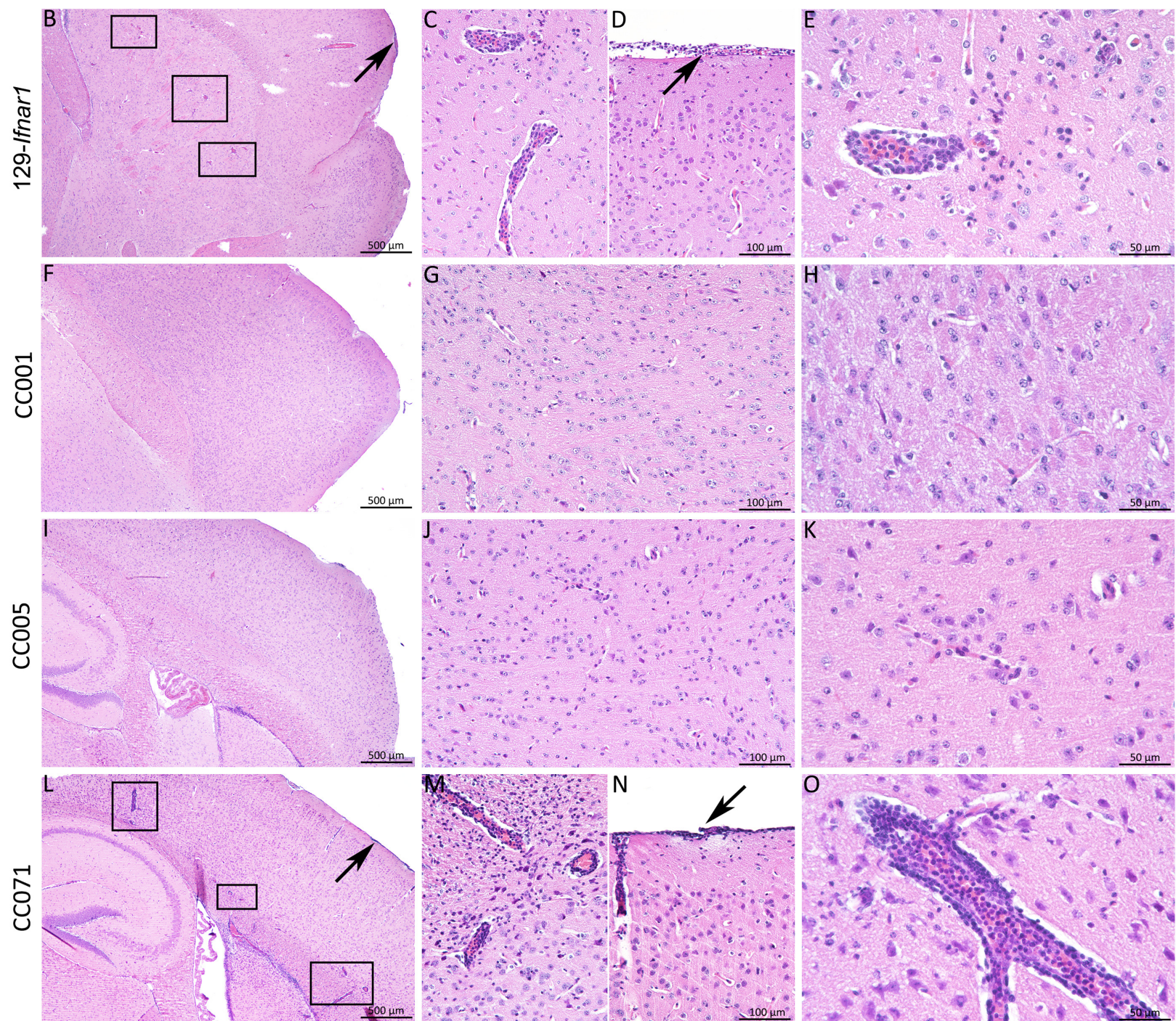
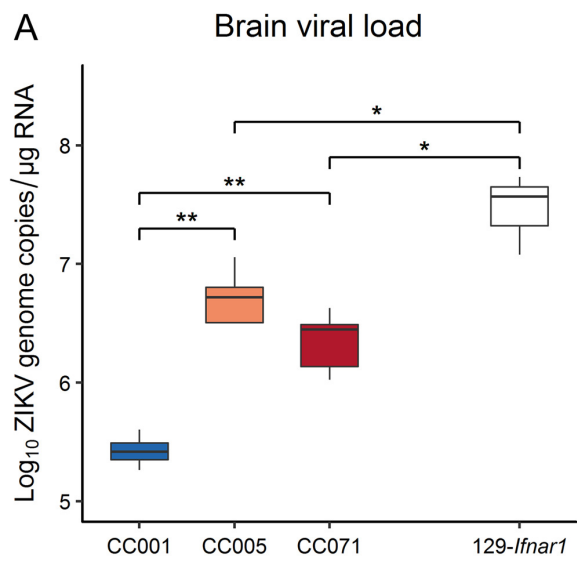
**B****C**

**A****ZIKV HD78788****B****ZIKV****DENV****C****WNV****RVFV**

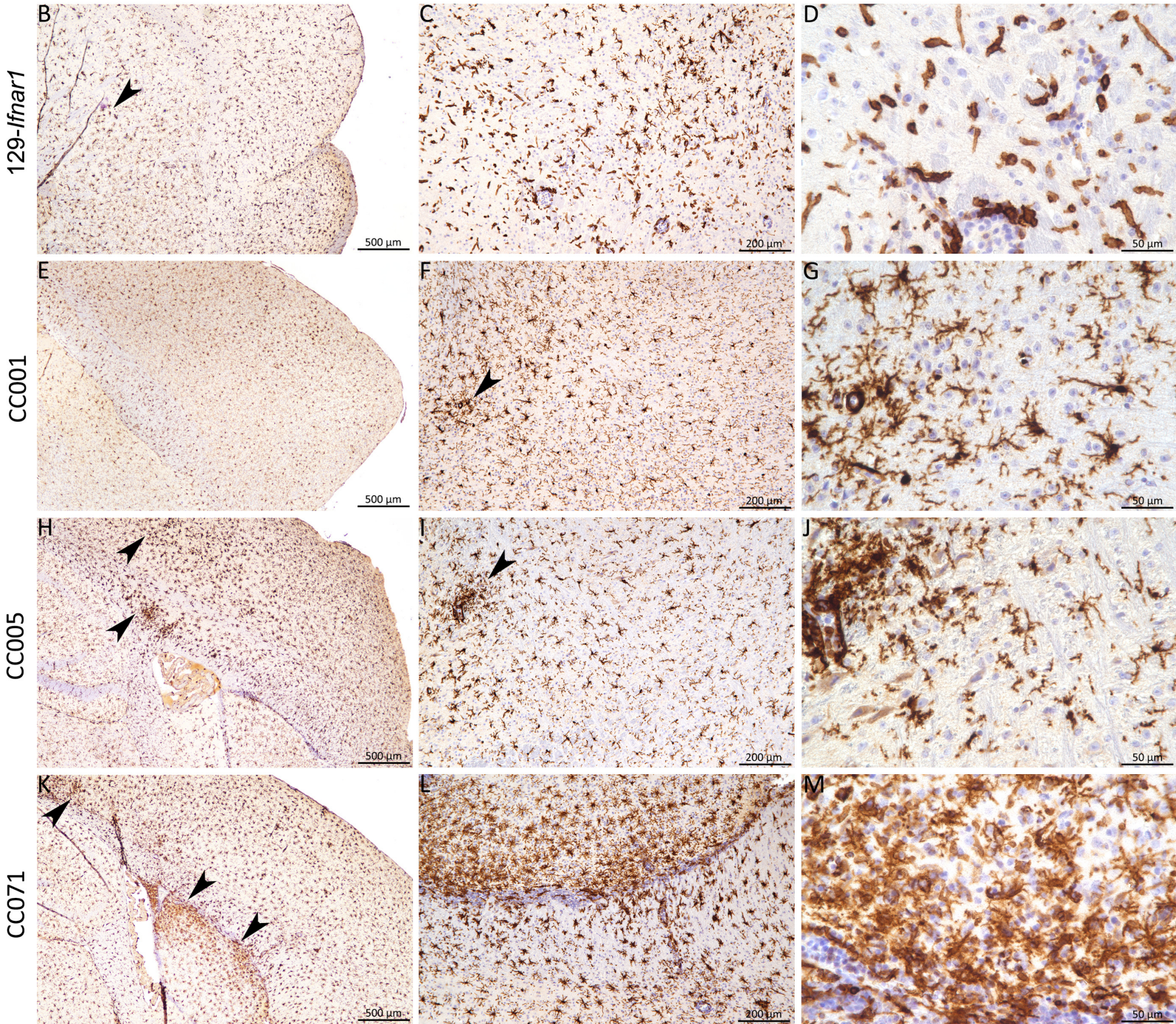
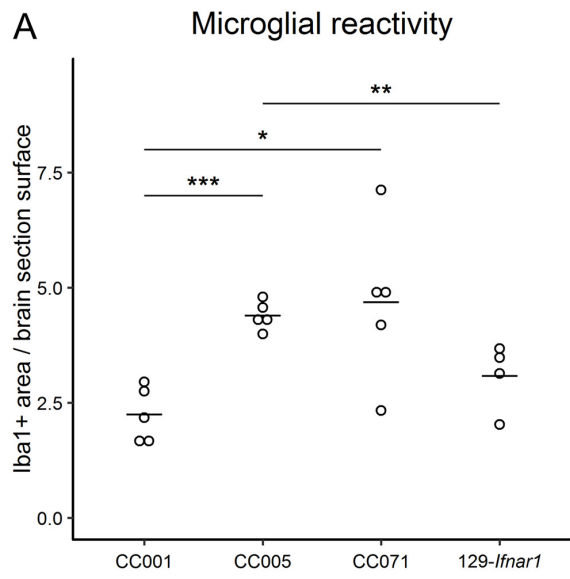
**A****Day 2 : Plasma viral load****B****Day 6 : Plasma viral load****C****Day 2 - 6 : Decrease rate of plasma viral load**











# Embryonic fibroblasts

CC001 CC071

