

1 **Supporting Information for**

2
3 **A clinically authentic mouse model of enterovirus 71 (EV-A71)-induced**
4 **neurogenic pulmonary oedema**

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12 **This file includes:**

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14 **Movie S1.** Hallmark observations in EV71:TLLmv-infected mice presenting Class-IA signs
15 of disease

16 **Movie S2.** Hallmark observations in EV71:TLLmv-infected mice presenting Class-IB signs
17 of disease

18 **Figure S1.** Seroconversion in mice inoculated (I.P.) with various EV-A71 strains

19 **Figure S2.** Median humane endpoint (HD₅₀) of EV71:TLLmv in one-week-old mice

20 **Figure S3.** Seroconversion observed in animals inoculated with EV71:TLLmv

21 **Figure S4.** Reproducible distribution of EV71:TLLmv-infected mice into different classes

22 **Figure S5.** Absence of viral replication and inflammation in the lung and heart tissues of
23 Class-IA mice

24 **Figure S6.** Lesions and viral antigens observed in brain tissue sections of EV71:TLLmv-
25 infected mice

26 **Figure S7.** Viral antigen and lesion localization in NPE trigger zones in the caudal brainstem

27 **Figure S8.** Representative images of brain coronal sections from mock-inoculated healthy
28 mice

29 **Figure S9.** Comparable histopathology and viral antigen distribution between Class-IA and
30 Class-IB mice brains

31 **Figure S10.** Lesions and viral antigens observed in spinal cord sections of EV71:TLLmv-
32 infected mice

33 **Figure S11.** Viral replication in the limb muscles evident in I.M.-inoculated mice

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46 **SUPPLEMENTARY VIDEOS**

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48 **Movie S1**

49 **Hallmark observations in EV71:TLLmv-infected mice presenting Class-IA signs of disease**

50 This video is comprised of two video clips from two different Class-IA mice. Both animals were
51 unable to self-right and were in a state of coma. Severe respiratory distress presenting as tachypnea
52 with subcostal recession is evident in the first mouse. Gaspings, subcostal recession and a frothy
53 fluid emanating from the nostrils can be seen in the second mouse.

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55 **Movie S2**

56 **Hallmark observations in EV71:TLLmv-infected mice presenting Class-IB signs of disease**

57 The animal shown in the video prior to necropsy was unable to self-right and was in a state of
58 stupor. Ipsilateral paralysis of the right limbs and persistent tremor of the left hind-limb were also
59 observed.

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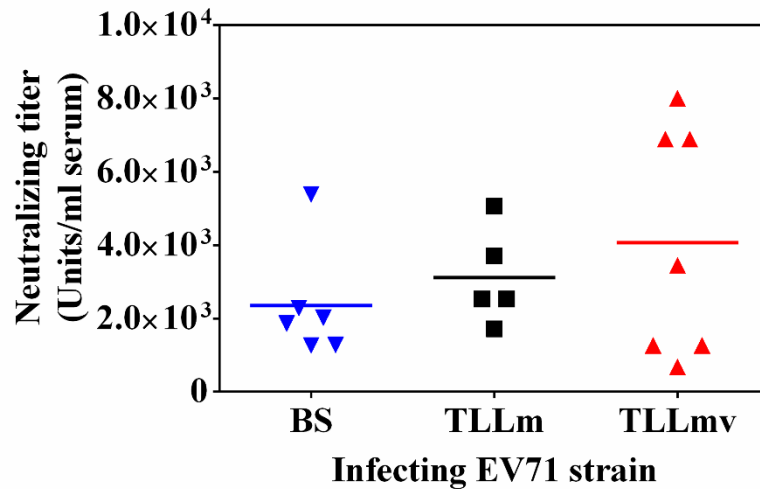
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SUPPLEMENTARY FIGURES

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69 **Figure S1**

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73 **Seroconversion in mice inoculated (I.P.) with various EV-A71 strains**

74 Neutralizing antibody titers in terminally collected sera from 1-week-old mice inoculated with

75 different virus strains. Virus neutralization tests were performed using Vero cells for assessment

76 of neutralization titers against EV71:BS; and NIH/3T3 cells for assessment of neutralization titers

77 against EV71:TLLm and EV71:TLLmv. Points represent individual titre values, while lines

78 indicate mean titre values.

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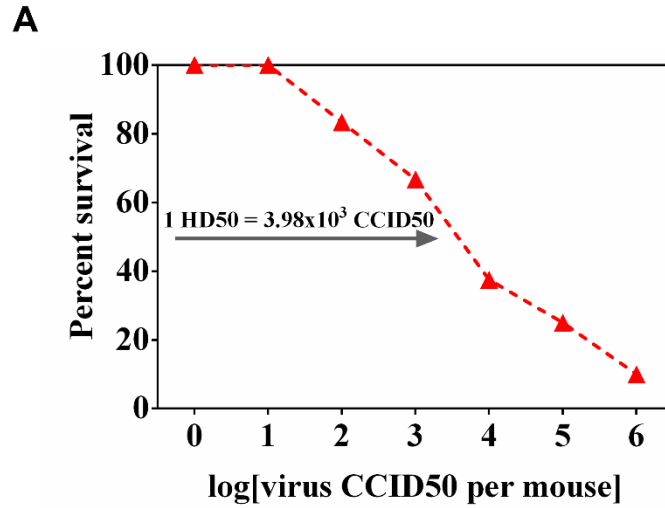
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84 **Figure S2**

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88 **Median humane endpoint (HD₅₀) of EV71:TLLmv in one-week-old mice**

89 The HD₅₀ value, which refers to the virus dose that induces signs requiring euthanasia in half of
90 the inoculated animals, was determined by inoculating (I.P.) mice with varying doses of
91 EV71:TLLmv. The calculated HD₅₀ value was equivalent to a virus dose of 3.98×10^3 CCID₅₀

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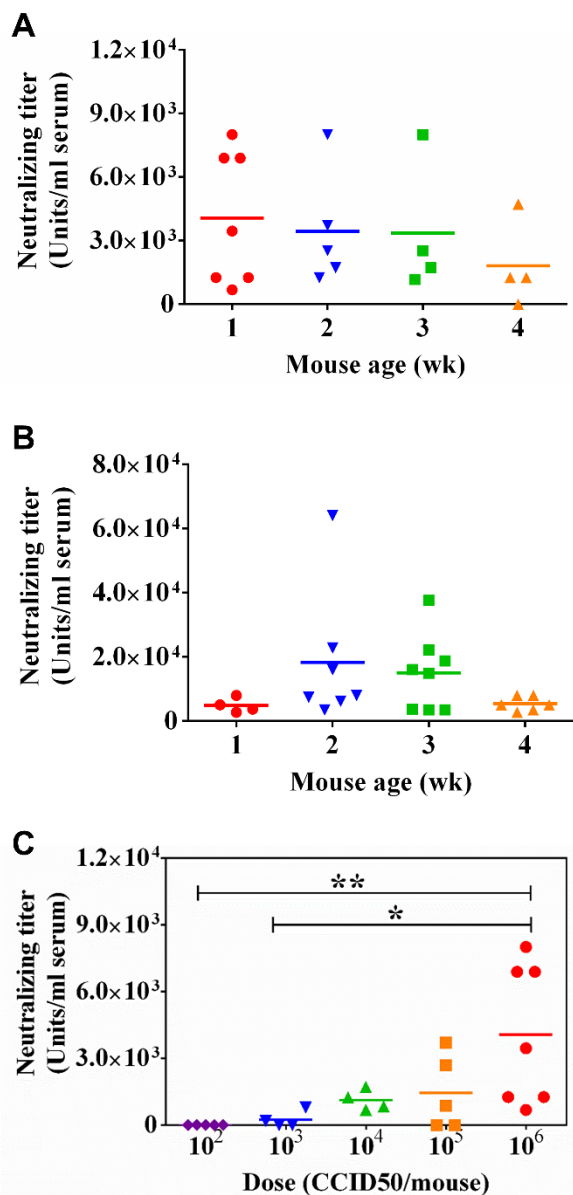
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104 **Seroconversion observed in animals inoculated with EV71:TLLmv**

105 (A-B) Neutralizing antibody titres in terminally collected sera from mice of various ages (1-4

106 weeks) inoculated with 10^6 CCID₅₀ virus either via I.P. (A) or I.M. (B) injection. (C) Neutralizing

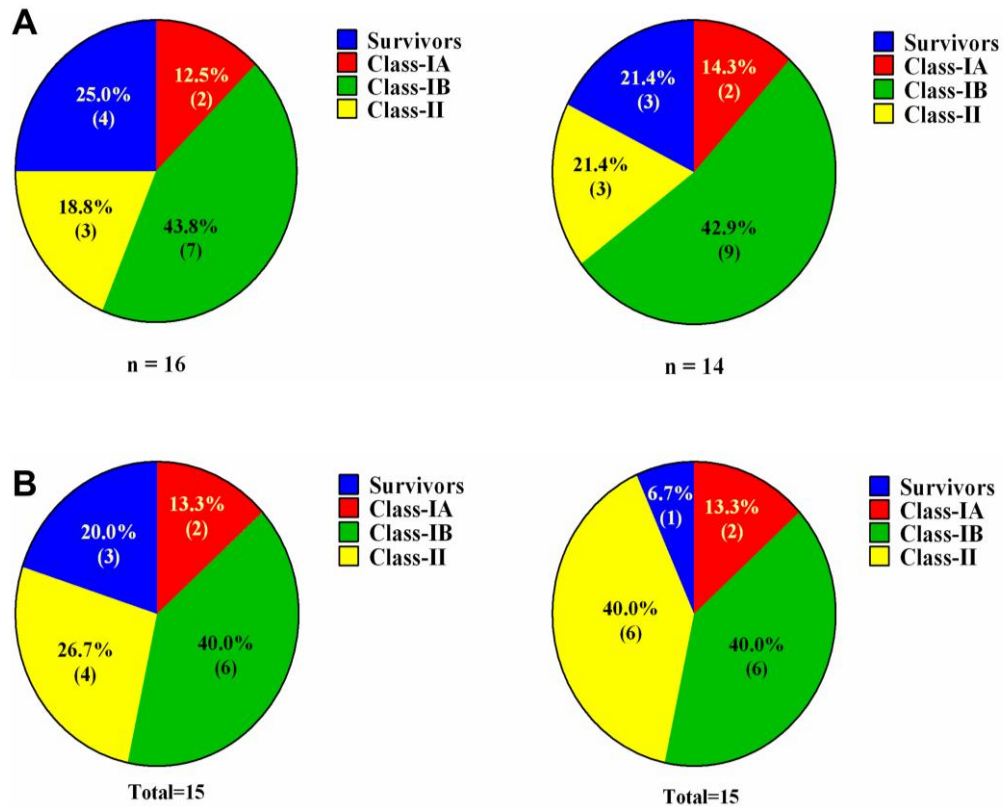
107 antibody titres in terminally collected sera from 1 week-old-mice inoculated (I.P.) with various

108 doses of virus. Mean titre values were compared using *t*-test. * $p < 0.05$; ** $p < 0.005$.

109 **Figure S4**

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115 **Reproducible distribution of EV71:TLLmv-infected mice into different classes**

116 Distribution of mice inoculated either (A) I.P. or (B) I.M. with EV71:TLLmv into various classes

117 of clinical presentation derived from two independent experiments. Each experiment was

118 comprised of mice collected from two separate litters.

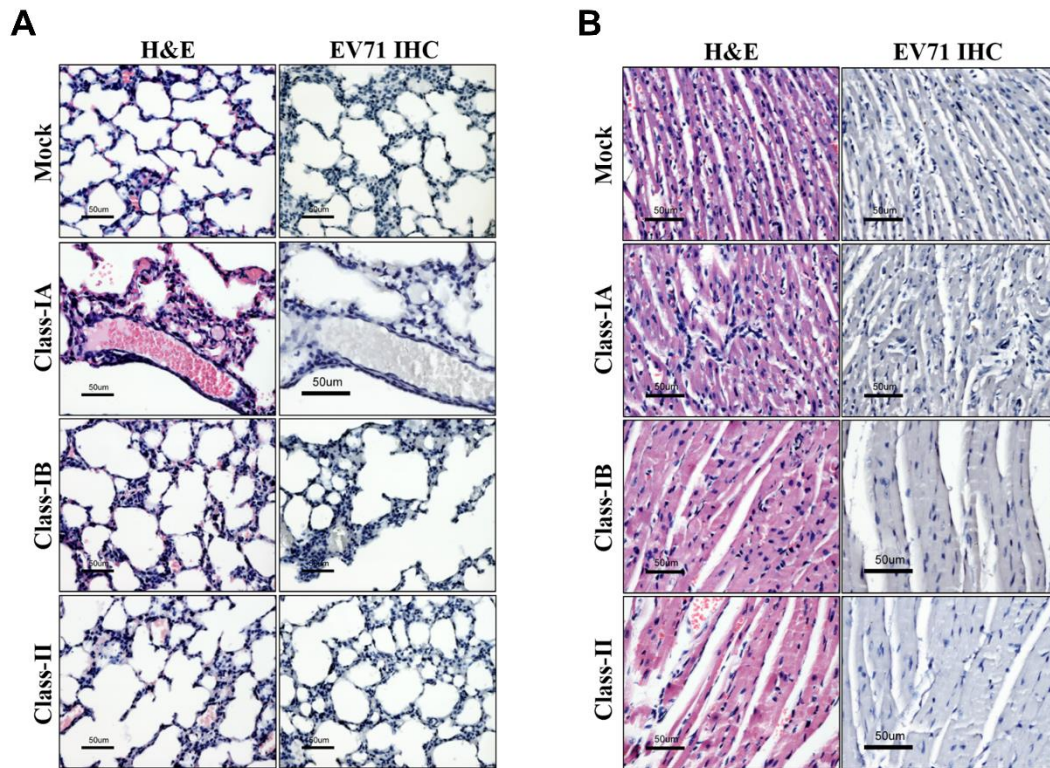
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122 **Figure S5**

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126 **Absence of viral replication and inflammation in the lung and heart tissues of Class-IA mice**

127 Representative images of lung tissue cross sections (5µm) (A) and heart tissue longitudinal

128 sections (5µm) (B) stained with Hematoxylin & Eosin (H&E) for histopathological examination;

129 or labelled with anti-EV-A71 serum (EV71 IHC) for virus antigen localization. *Mock*, control

130 tissues from sham-inoculated mice.

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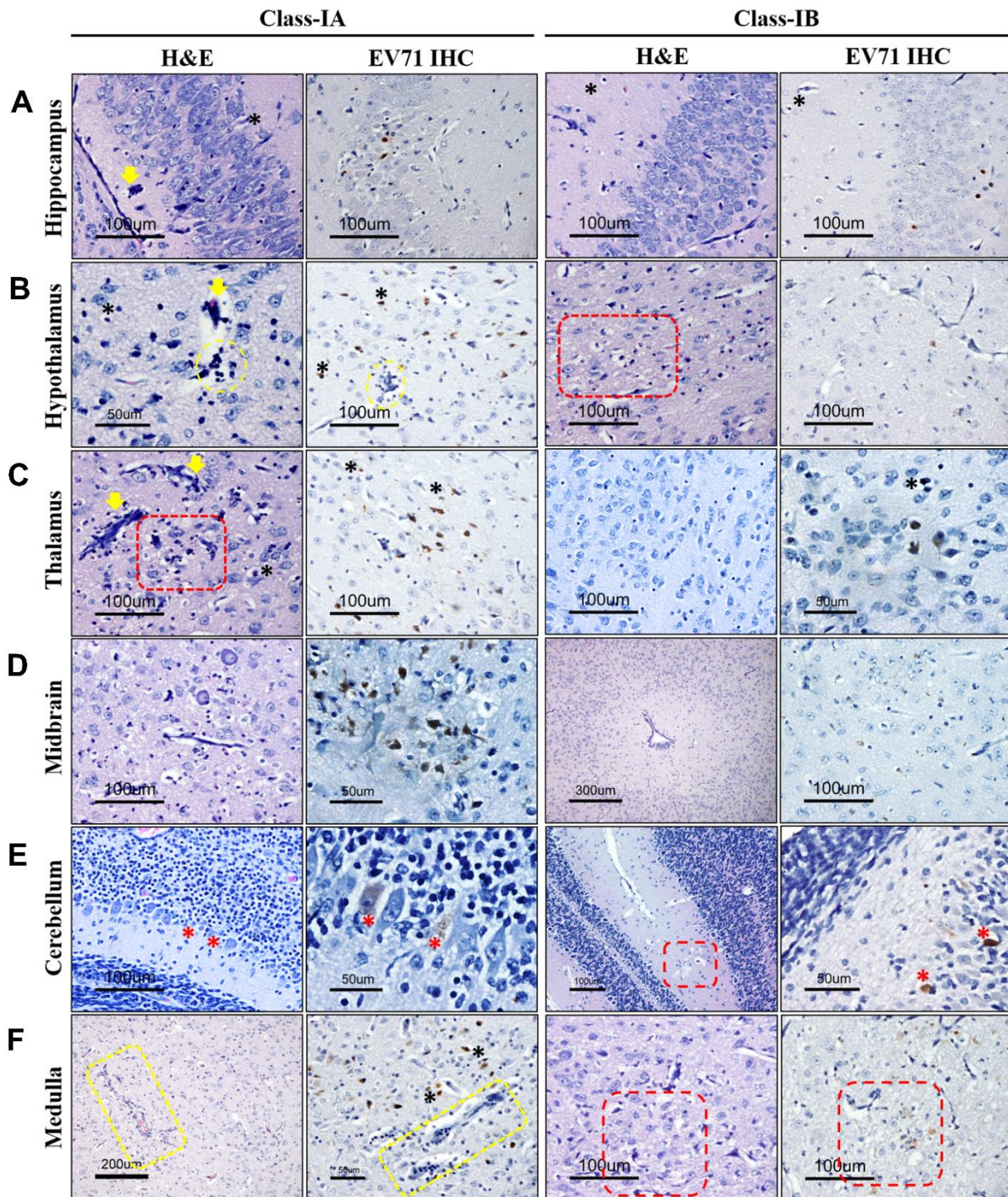
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135 **Figure S6**

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139 **Lesions and viral antigens observed in brain tissue sections of EV71:TLLmv-infected mice**
140 Representative images of brain coronal sections (5µm) of hippocampus (**A**), hypothalamus (**B**),
141 thalamus (**C**), midbrain (**D**), cerebellum (**E**), and medulla (**F**) derived from Class-IA (left panels)
142 and Class-IB (right panels) mice. Sections were stained with Hematoxylin & Eosin (H&E) for
143 histopathological examination; or labelled with anti-EV-A71 sera (EV71 IHC) for virus antigen
144 localization. Pathological lesions observed include edema (*red box*), infiltrating immune cells
145 (*yellow box*), neuronophagia (*yellow arrows*), neurodegeneration (*black asterisks*), and
146 degeneration of Purkinje cells (*red asterisks*).

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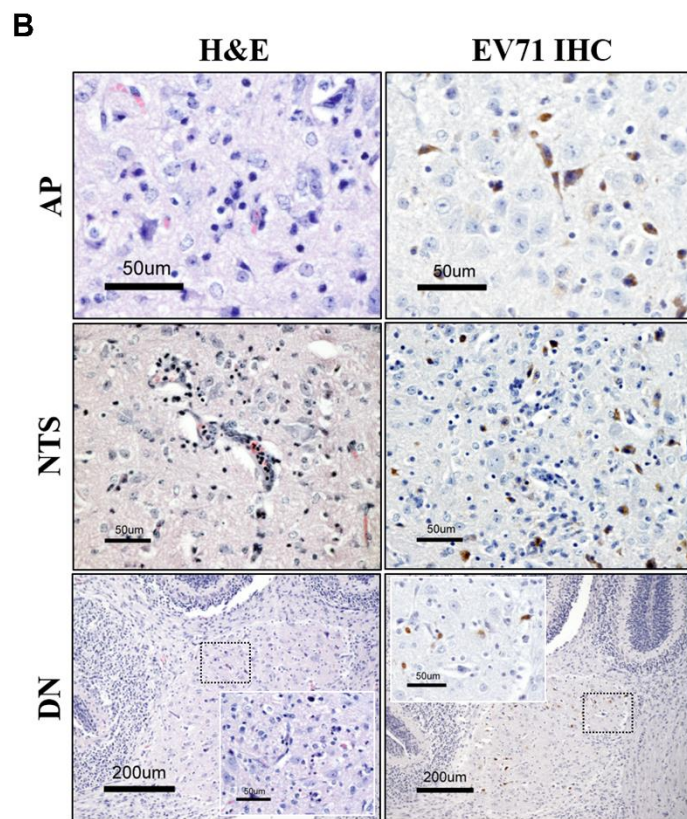
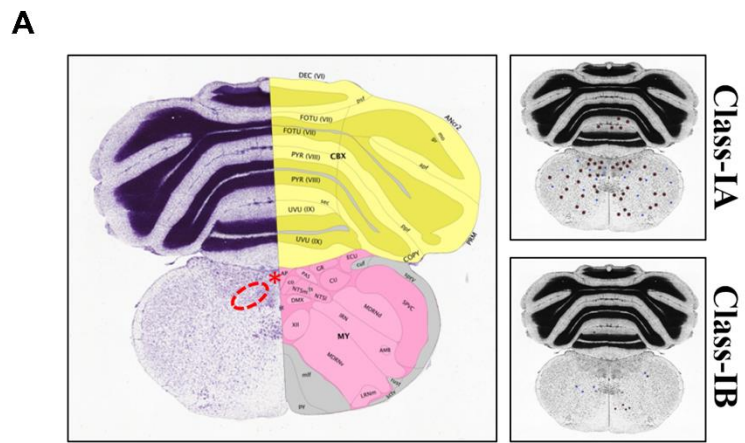
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162 **Figure S7**

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167 **Viral antigen and lesion localization in NPE trigger zones in the caudal brainstem**

168 (A) Schematic diagram of a coronal section through the caudal brainstem (*left panel*) marked to
169 exhibit the localization of viral antigens (*brown dots*) and lesions (*blue dots*) in Class-IA and Class-
170 IB brains (*right panels*). Template images were downloaded from The Mouse Brain Atlas and
171 depict the location of the AP (*red asterisk*) and NTS (*red circle*). (B) Representative images of
172 coronal sections through the caudal brainstem of Class-IA mice depicting the AP (area postrema),
173 NTS (nucleus of the solitary tract), and DN (dentate nucleus). Tissue sections (5 μ m) were stained
174 with Hematoxylin & Eosin (H&E) for histopathological examination, or labelled with anti-EV-
175 A71 sera (EV71 IHC) for virus antigen localization. Boxed areas are shown magnified in the inset.
176 *CBX*, cerebellar cortex; *MY*, medulla oblongata.

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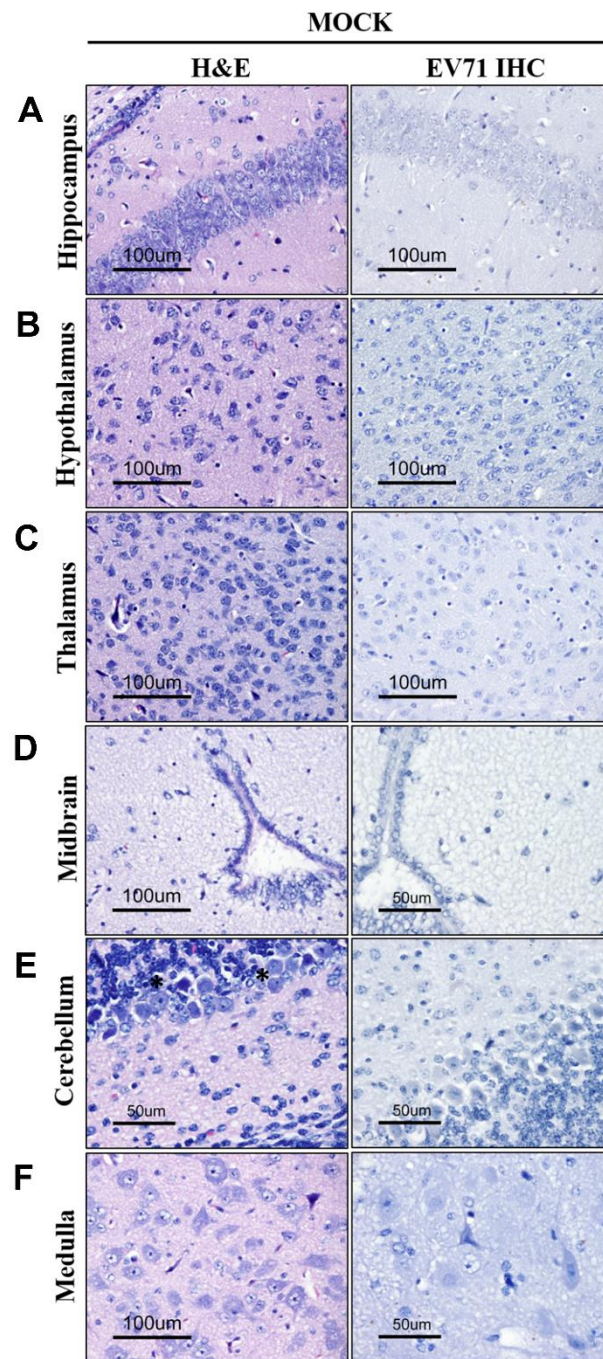
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190 **Figure S8**

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195 **Representative images of brain coronal sections from mock-inoculated healthy mice**

196 Coronal sections (5µm) of hippocampus (A), hypothalamus (B), thalamus (C), midbrain (D),
197 cerebellum (E), and medulla (F) derived from healthy control mice sacrificed at the same day as
198 Class-IA and Class-IB mice. Tissues were stained with Hematoxylin & Eosin (H&E) for
199 histopathological examination; or labelled with anti-EV-A71 sera (EV71 IHC) for virus antigen
200 localization. Note the regular morphology of Purkinje cells (*black asterisks*).

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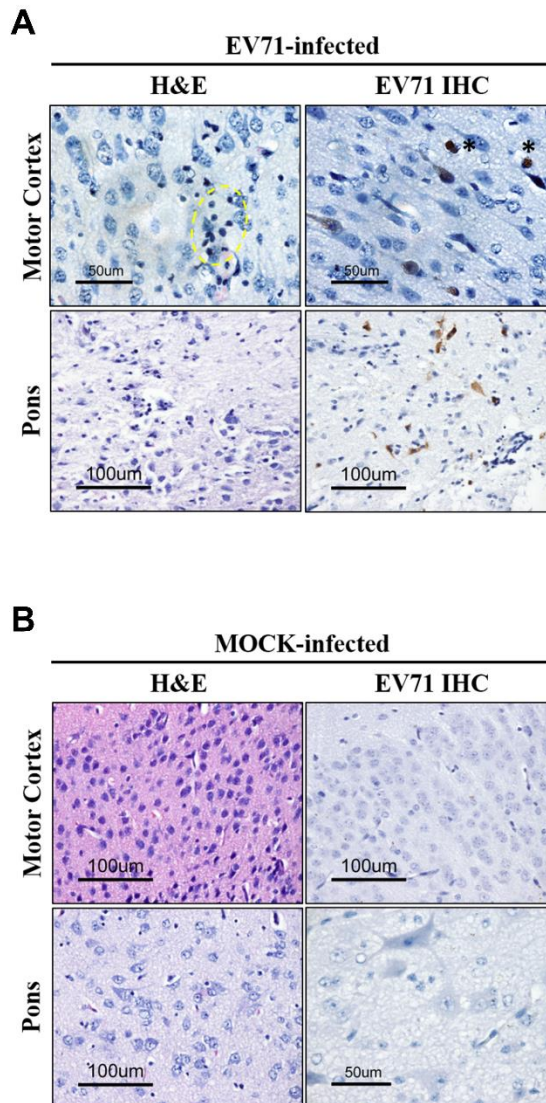
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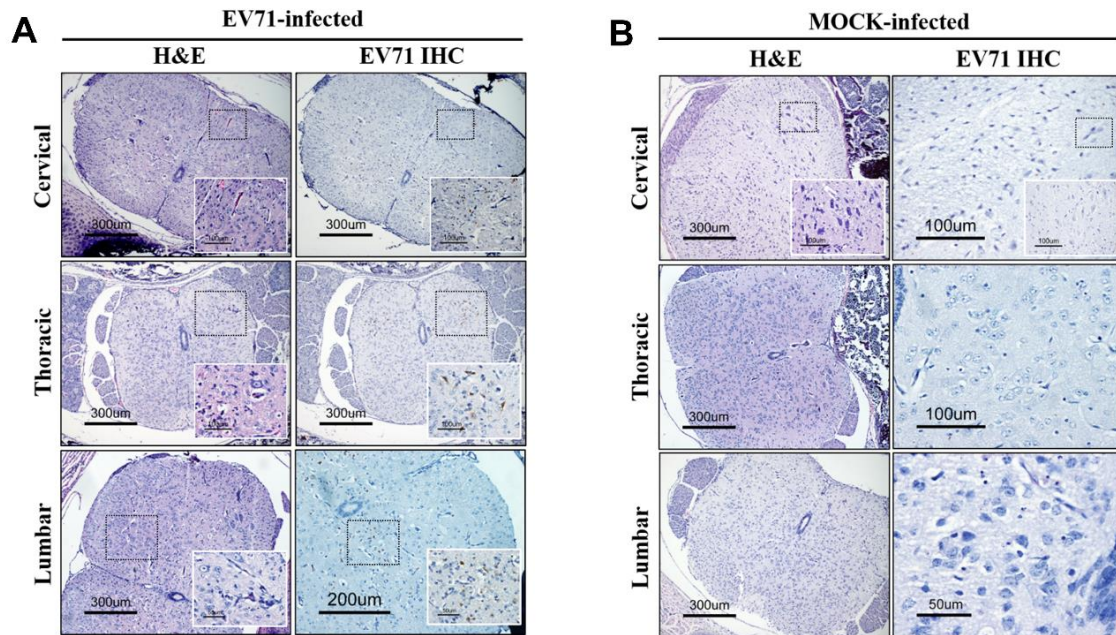


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220 **Comparable histopathology and viral antigen distribution between Class-IA and Class-IB**
 221 **mice brains**

222 Coronal sections (5µm) of motor cortex and pons derived from either Class-IA or Class-IB (A) or
 223 mock-infected control mice (B). Sections were stained with Hematoxylin & Eosin (H&E) for
 224 histopathological examination; or labelled with anti-EV-A71 sera (EV71 IHC) for virus antigen
 225 localization. Note the presence of cellular infiltrate (*yellow circle*) and neuronal necrosis (*black*
 226 *asterisks*).

227 **Figure S10**



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229 **Lesions and viral antigens observed in spinal cord sections of EV71:TLLmv-infected mice**

230 Representative images of coronal sections (5 μ m) of various spinal cord regions collected from
231 either Class-IA or Class-IB mice (**A**) or mock-infected animals (**B**). Sections were stained with
232 Hematoxylin & Eosin (H&E) for histopathological examination; or labelled with anti-EV-A71
233 sera (EV71 IHC) for virus antigen localization. Boxed areas are shown magnified in the respective
234 insets.

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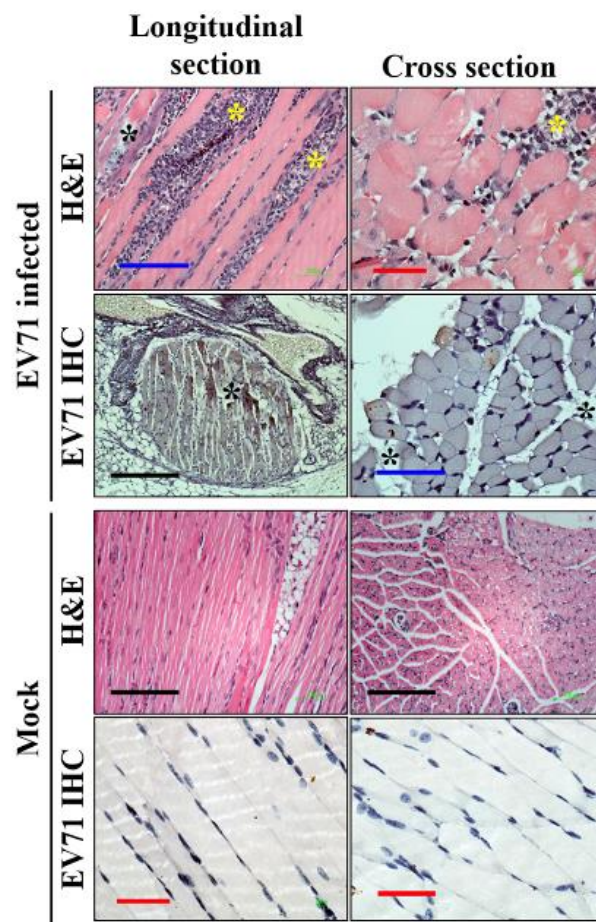
242 **Figure S11**

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245 **Viral replication in the limb muscles evident in I.M.-inoculated mice**

246 (A) Representative image of hind limb (HL) gross pathology at necropsy revealing muscle necrosis
247 (*red arrow*) in the right thigh above the inoculation site (*black arrow*) in comparison to normal
248 muscle tissue (*yellow arrow*). (B) Representative images of transverse and longitudinal tissue
249 sections (5µm) of the right HL of infected and sham-infected (Mock) control mice. Sections were
250 stained with Hematoxylin & Eosin (H&E) for histopathological examination, or labelled with anti-
251 EV-A71 sera (EV71 IHC) for virus antigen localization. Note evidence of severe inflammation
252 with massive leukocyte infiltration (*yellow asterisks*) and muscle necrosis (*black asterisks*).
253 Images are shown at different magnifications; Black bar=200µm; Blue bar=100µm; Red
254 bar=50µm.