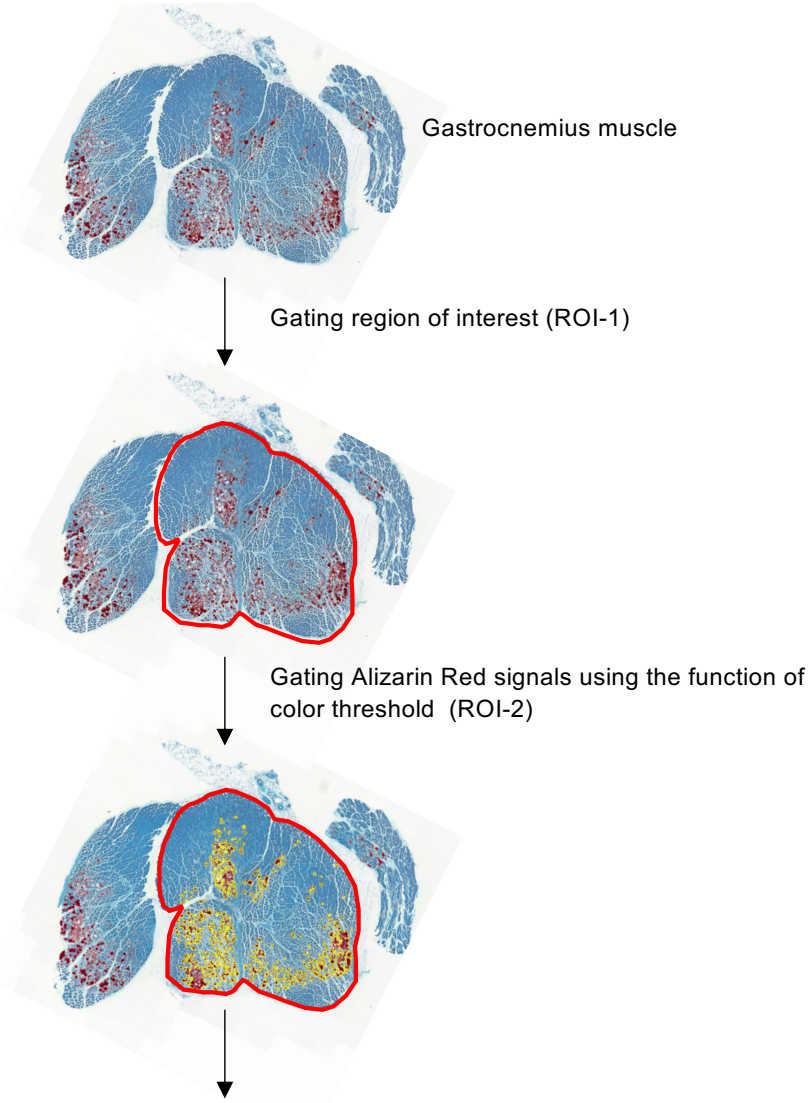
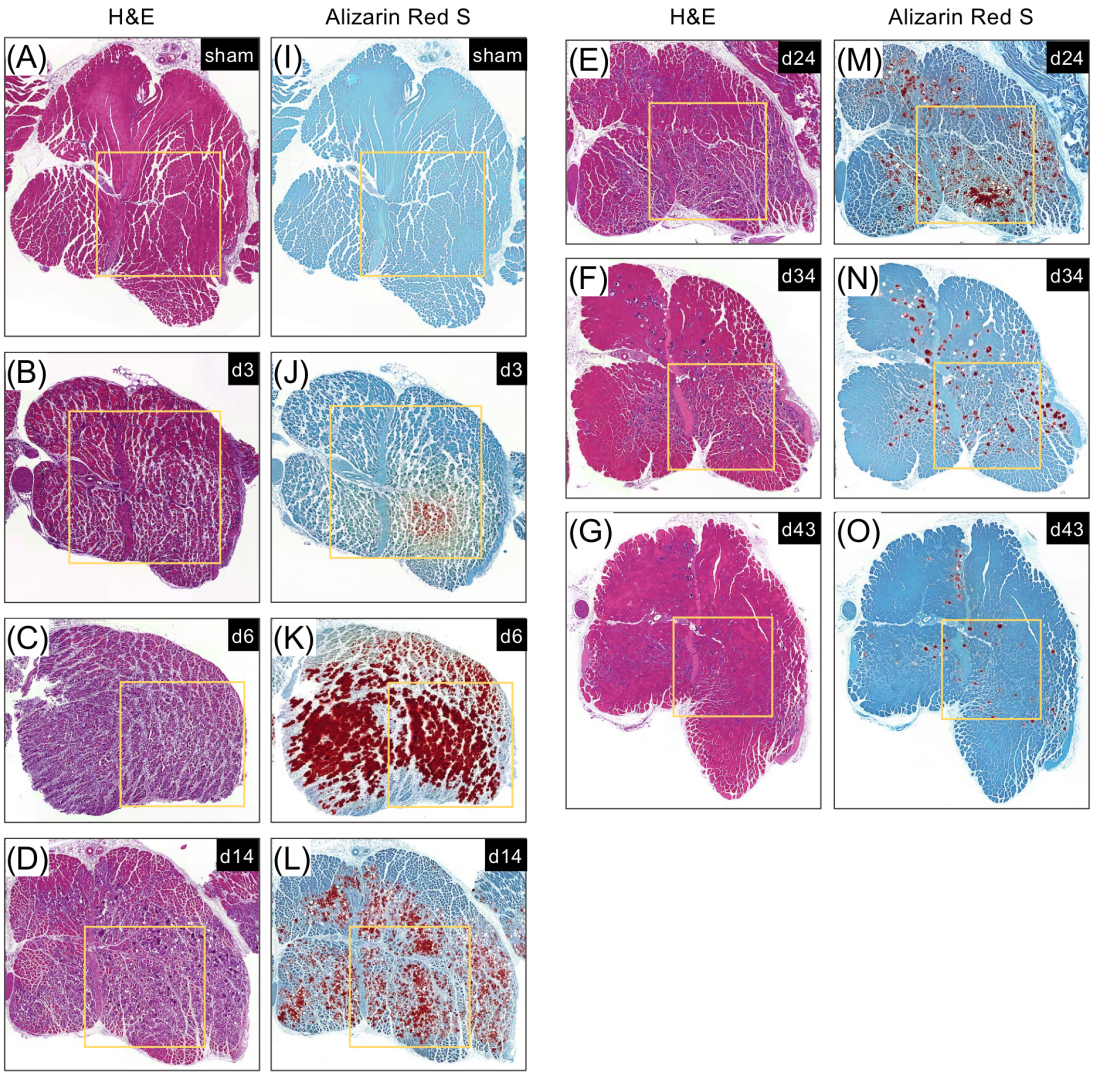


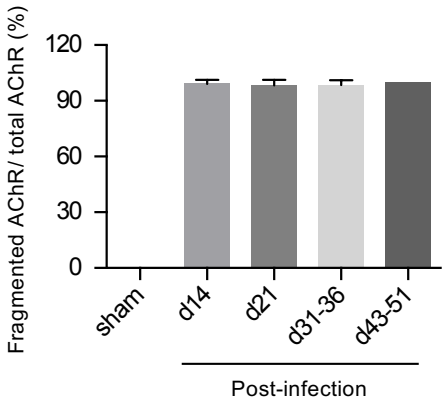
Supplementary Figure 1. The histological image acquisition and analysis in gastrocnemius muscles.

The gastrocnemius muscle of the left leg was collected, fixed and sectioned. The region of interest (ROI-1) was selected (red-circled area), which region was most close to the infection site and showed good tissue integrity. The function of image adjustment with the color threshold was used to measure the Alizarin Red signals (ROI-2, yellow area), representing the levels of calcification. The areas of ROI-1 and ROI-2 were quantified, and the ratio of ROI-2/ROI-1 was calculated as the percentage of calcification area.



Supplementary Figure 2. The histological staining of the gastrocnemius muscle from sham-infected and EV71-infected mice.

The representative images of H&E staining (A-G) and Alizarin Red S staining (I-O) of gastrocnemius muscle sections from sham-infected or EV71-infected mice at day 3 (score = 1), day 6 (score = 3.5), day 14 (score = 1), day 24 (score = 0.5), day 34 (score = 0) and day 43 (score = 0) post-infection were shown. The square areas were magnified and presented as the Figure 2 in the manuscript. The scale of each square is 1 mm × 1 mm.



Supplementary Figure 3. The quantification of fragmented AChR clusters in the gastrocnemius muscles of sham-infected and EV71-infected mice.

The Z-stack images of gastrocnemius muscle sections stained with tetramethylrhodamine α-BTX were obtained and illustrated with maximum intensity projection. The number of discrete fragments per AChR cluster equals to or more than 5 was defined as a fragmented AChR cluster (30). The percentage of fragmented AChR clusters was measured and calculated from sham-infected (Total 44 AChR clusters from 2 mice were counted) or EV71-infected mice at day 14 (65 AChR clusters from 5 mice), day 21 (59 AChR clusters from 3 mice), day 31-36 (48 AChR clusters from 3 mice) and day 43-51 (35 AChR clusters from 2 mice) post-infection.

Supplementary Table 1. Primer sequences used for quantitative PCR.

Murine gene	Forward	Reverse
VP1	GTGGCAGATGTGATTGAGAG	GTTATGTCTATGTCCCAGTT
IRF7	TCCAGCGAGTGCTGTTTGGA	CGAGCCTCGTTCAGCCA
TNFα	TTCTCATTCCTGCTTGTGGCA	TGATGAGAGGGAGGCCATTTG
IL-1β	TGTAATGAAAGACGGCACACC	TCTTCTTTGGGTATTGCTTGG
IL-6	GATGGATGCTACCAAACTGGAT	CCAGGTAGCTATGGTACTCCAGA
IFN-α	ARSYTGTSTGATGCARCAGGT	GGWACACAGTGATCCTGTGG
IFN-β	AGGGCGGACTTCAAGATC	CTCATTCCACCCAGTGCT
ISG15	ACTCCATGACGGTGTCAGAAC	TTCGTTCCTCACCAGGATGC
Mx1	CCAACTGGAATCCTCCTGGAAAA	CCTTCTCCTCATAGTGCCTGC
CXCL10	TGAGCAGAGATGTCTGAATC	TCGCACCTCCACATAGCTTACAG
CCL2	CAGTTAACGCCCCACTCACC	ATTCCTTCTTGGGGTCAGCA
TGF-b	AAGGCTCGCCAGTCCCCCAA	TAGATGGCGTTGTTGCGGTCCAC
IGF-1	ACCTCTTCCCACGTAGCTCA	TGCCACAGATGGAGTCAGGT
OPN	GATGATGATGACGATGGAGACC	CGACTGTAGGGACGATTGGAG
Pax7	AGGCCTTCGAGAGGACCCAC	CTGAACCAGACCTGGACGCG
MyoD	CAGCATCACGGTGGAGGATA	CAGTTGGGCATGGTTTCGT
Myogenin	GGCTGCCTAAAGTGGAGATCCT	AGGCCTGTAGGCGCTCAAT
GAPDH	TACTTGGCAGGTTTCTCCAG	GTCGTGGAGTCTACTGGTGT

Supplementary Table 2. Antibodies used in immunofluorescence staining and staining procedures.

(A) Staining for F4/80 and CD206

Step	Antibody	Manufacturer
1	Rat anti-F4/80 (clone: BM8)	BioLegend
2	Alexa 555 goat anti-rat IgG	Molecular Probes
3	Alexa 488 rat anti-CD206 (clone: C068C2)	BioLegend

(B) Staining for EV71 VP1

Step	Antibody	Manufacturer
1	Rabbit anti-EV71 VP1	GeneTex
2	Cy3 goat anti-rabbit IgG	Molecular Probes