Supplementary table 1

Table. Clinical severity in cases infected with EV71 of different mutations associated with 3C sumoylation

Mutation	Clinical severity (number)	
	Mild illness	CNS involvment
K52R	0	1
V491	4	1
I47V	1	1
K45R	6	0

CNS denotes central nervous system

Supplementary Figure legend

Supplementary Figure 1. *In vitro* sumoylation of EV71 3C with SUMO-1 and SUMO-2. A, Immunoblot analysis showing *in vitro* sumoylation of HA-tagged 3C with His-tagged SUMO-1 or SUMO-2. B, Western blot analysis showing *in vitro* sumoylation of the His-tagged PML recombinant protein with SUMO at the indicated time points. The arrowheads and arrow indicate SUMO-modified and unmodified proteins, respectively.

Supplementary Figure 2. The effect of RNF4 on EV71 3C ubiquitination and protein stability. A, Immunoblot showing 3C ubiquitination in 293T cells transfected with the indicated constructs. Ubiquitin-modified and unmodified GFP-3C proteins are depicted by brackets and arrow, respectively. B, Western blot analysis showing 3C stability modulated by RNF4. 293T cells transfected with vector (HA, HA-RNF4 WT expression construct or the catalytically inactive CS mutant) were cultured for 1 day, split and transfected with either 3C WT or the K52R mutant. Cells were further treated with CHX for the indicated periods of time. The amount of 3C relative to levels in untreated cells is indicated at the bottom of each set.

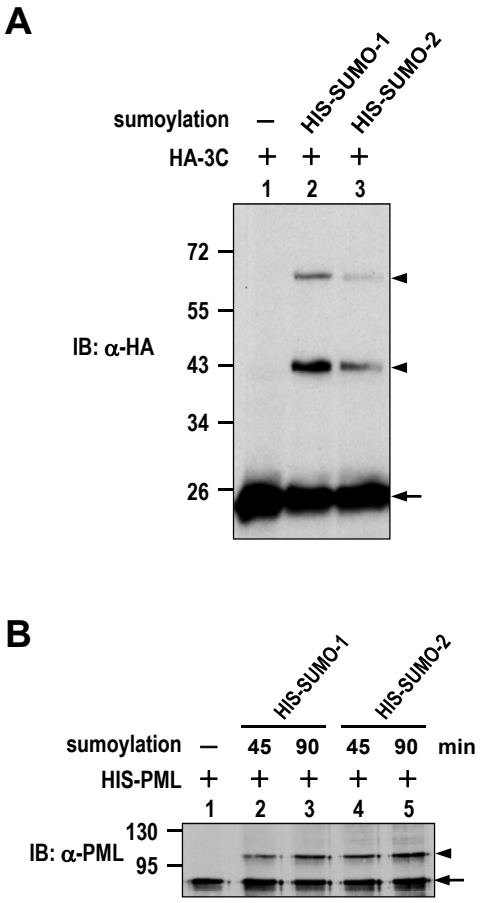
Supplementary Figure 3. The effect of EV71 viral infection on mouse body weight. Bar graph showing the body weight of 1-day-old ICR mice after oral inoculation with EV71 virus or mock inoculation. Error bars represent the standard deviation of 6 observation per group; *, $p < 0.05$ and **, $p < 0.01$ (n=3).

Supplementary Figure 4. Poliovirus 3C protease could not be sumoylated. A, Protein sequence alignment of poliovirus and EV71 adjacent to the 3C sumoylation site. B, Western blot showing GFP-tagged EV71 or poliovirus 3C WT or mutant protein modified by

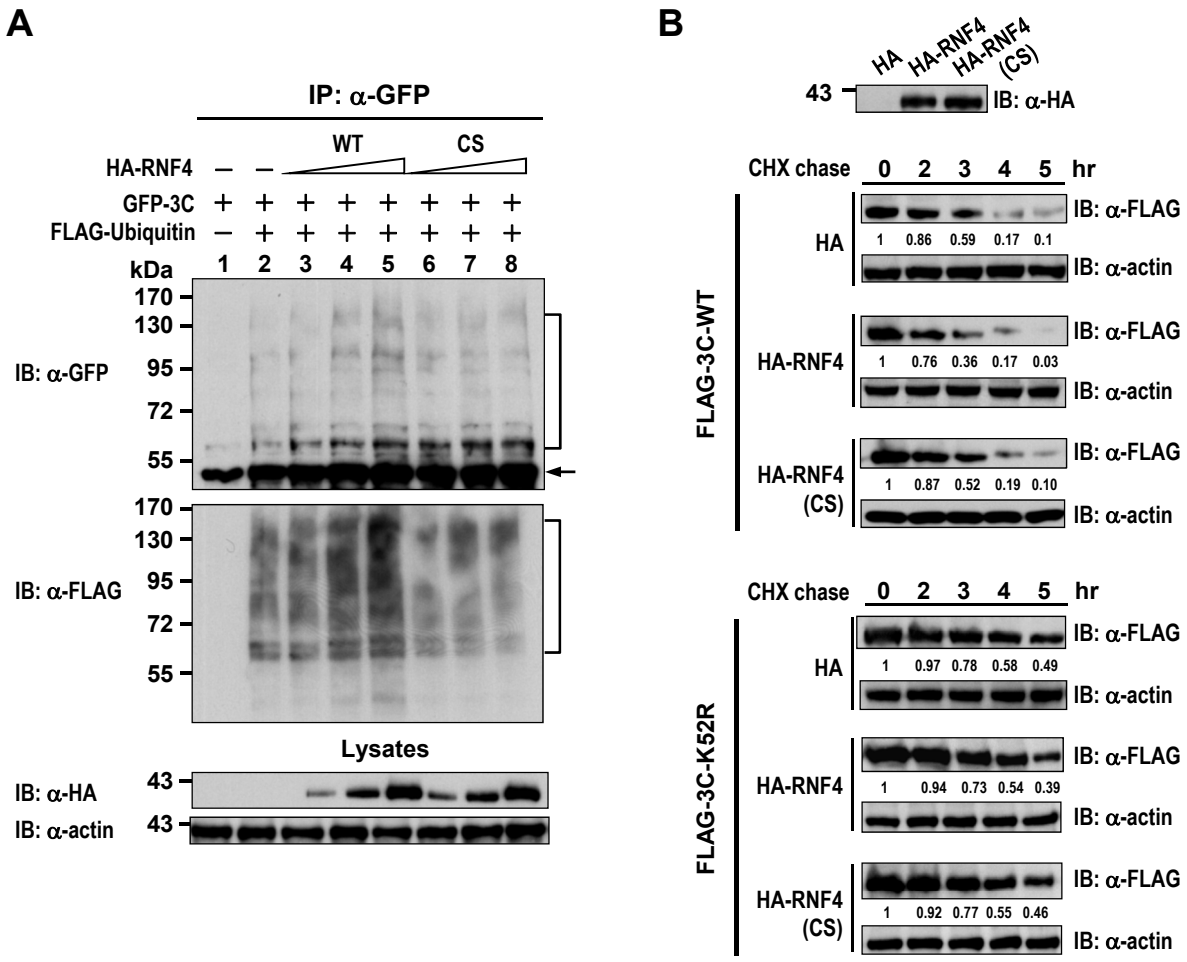
His-tagged SUMO-1 in HeLa cells. Polio (45-55EV71) and Polio (45-55EV71-K52R) represent chimeric proteins in which poliovirus 3C residues 45-55 were replaced by EV71 3C residues 45-55 containing WT or K52R, respectively. The SUMO-1-modified and unmodified GFP-3C are depicted by bracket and arrow, respectively.

Supplementary Figure 5. CREB cleavage potential and protein stability of EV71 3C mutants. A, Western blot analysis showing HA-tagged CREB in HeLa cells transfected with the indicated GFP-tagged 3C expression constructs. The intact and cleaved products are indicated by an arrow and arrowhead, respectively. The quantification of HA-CREB cleaved by each 3C mutant is summarized in Fig. 6D. B, Western blot analysis showing the expression of EV71 3C WT or mutants in 293T cells treated with 50 μg/ml CHX for the indicated periods of time. The plot shows the quantification of the WT and mutant 3C protein half-life from 3 independent experiments by non-linear regression analysis.

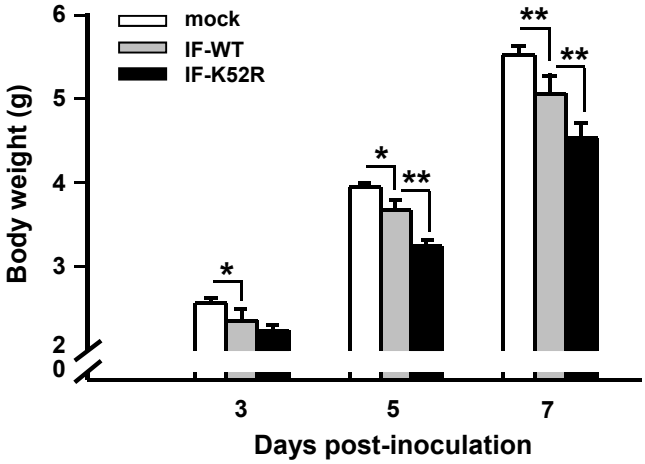
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

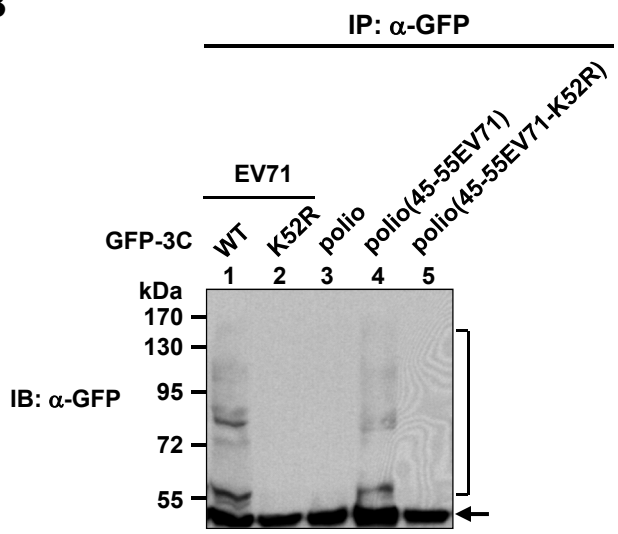


Supplementary Figure 4

A

EV71	^{45}K	T	I	W	V	E	H	**K**	L	I	N^{55}
polio	^{45}E	S	I	V	I	D	G	**K**	E	V	E^{55}

B



Supplementary Figure 5