FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. EV71(FY) -EGFP replicates and spreads in RD cells efficiently.
A, RD cells and RD cells treated with an unrelated shRNA (RD/shNC) supported robust EV71(FY)-EGFP infection; stable SCARB2 knockdown RD cell lines (RD/SCARB2 kd) lost susceptibility to EV71 infection.
B, RD or RD/SCARB2 kd cells were infected with EV71(FY) virus or EV71(FY) -EGFP stock virus at a M.O.I of 0.1. Both EV71(FY) and EV71(FY) -EGFP viruses replicated and spread in RD cells efficiently, most cells had CPE within 24h after infection, while RD/SCARB2 kd cells infected by EV71(FY) -EGFP virus showed no cytopathic effect. The figure shown is photos taken at 72h post infection, CPE is indicated by white arrows.

SUPPLEMENTAL FIGURE 2. Sequence alignment of SCARB2 from different species. Deduced amino acid sequences of SCARB2 from human, mouse, hamster and horseshoe bat were aligned. Four regions with high sequence variations were designated as HVR1 (residues 144-151), HVR2(residues 188-198), HVR3(residues 278-288) and HVR4 (residues 354-365), and highlighted with red boxes. Gene Bank accession number for human and mouse SCARB2 is NP_005497 and NP_031670, respectively. ; SCARB2s of hamster and horseshoe bat were cloned, sequenced and deposited in Gene Bank with accession numbers of JF965373 and JF965374, respectively.

SUPPLEMENTAL FIGURE 3. The N-terminal 300 residues of SCARB2 are involved in EV71 viral infection.
A, Schematic diagram of human-, mouse-, and chimeras SCARB2 proteins. The HM and MH swap mutants were generated by replacing the human SCARB2 sequence (black) with the murine counterpart (white) as indicated.
B, RD/SCARB2 kd cells transfected with plasmids encoding human-, mouse-, HM, or MH SCARB2 were infected with EV71(FY)-GFP and the result was recorded 36 hrs post initial infection.
C, BHK21 cells transfected with plasmids encoding human, mouse, HM, or MH SCARB2 were infected with the single round pseudotype virus EV71(FY)-Luc. Firefly luciferase activity in infected cells was examined 24 hrs after infection and the infectivity on different cells was normalized to that of human SCARB2 (left panel). The surface expression level of the swap mutants were also determined (right panel). Untransfected BHK21 cell was used as a control.

SUPPLEMENTAL FIGURE 4. Sequence alignment of VP1 from different EV71 isolates.
Deduced amino acid sequences of VP1 from some circulating EV71 virus and the prototype strain of EV71 (BrCr) were aligned. The secondary structure of VP1 was shown on bottom of the sequence: E: beta-sheet, H: helix, C: coil-coil. The effect of mutations examined was marked as follows: filled star indicates those most effective residues for viral binding and infection; open star indicates partial effective residues; triangle indicates no effect. Two residues Q145 and Q172 were highlighted in boxes.

SUPPLEMENTAL FIGURE 5. Cellular entry of EV71(FY)-GFP and EV71(FY)-Luc is pH dependent.
A, PSGL-1 pull-down assay of EV71. Left panel, SDS-PAGE of purified PSGL-1-mFc (human PSGL-1extracellular domain fused with mouse IgG2a Fc (mFc) and HVR1-mFc (seven amino acids IEWSQVHF

from HVR1 of human SCARB2 fused with mFc). Right panel, purified proteins (1 μg) shown in left panel were incubated with 1μl purified EV71(FY) wild type virus (~5 X 10^8 virions) at 4℃ over night together with protein A beads. After extensive wash with PBS for 5 times, the bound virions were quantified by RT-qPCR.

B, Cellular entry of EV71(FY)-GFP and EV71(FY)-Luc can be blocked by endosome acidification inhibitor bafilomycin A1 and ammonium chloride. RD cells were pretreated with Bafilomycin A1 or NH_4Cl at indicated concentration at 37℃for 1h. The cells were infected with EV71(FY)-GFP or EV71(FY)-Luc at 37℃for 1h in presence of the chemicals and then were changed to fresh culture medium after washing with PBS once. Cell viability was not affected significantly by these inhibitors at tested concentrations. GFP photos were taken at 12 hrs post infection (upper panel), firefly luciferase activity was examined at 24 hrs post infection and was normalized to the infection on untreated cell control (lower panel).