А LKOKVSOYYG CBV3 2B ТЗ В anti-HA anti-GM130 DAPI Merge С EV71-2B-HA EV71-2B-HA 48 kDa 48 kDa 🗕 24 kDa 24 kDa 12 kDa 12 kDa IB: anti-HA HRP IP: anti-HA IB: anti-HA HRP

Supplementary information, Figure S1

Figure S1 The EV71 2B protein has many similarities with the CBV3 2B protein. (A) Sequence alignment between the EV71 and CBV3 2B proteins. Yellow highlighting shows identical amino acid residues, and green highlighting indicates residues with high homology. The two red boxes indicate the two trans-membrane domains based on the reported structure of CBV3 2B [1]. (B) Subcellular localization of the EV71 2B protein in transfected RD cells. The cells were fixed and permeabilized 24 hours after transfection with pCAGGS-EV71-2B-HA.The 2B protein was stained with an anti-HA antibody (green), the Golgi complex was stained with an anti-GM130 antibody (red), and the nuclei were counterstained with DAPI (blue). (C) EV71 2B forms putative dimers and tetramers in transfected 293T cells. The cells were transfected with pCAGGS-EV71-2B-HA and collected 48 hours after transfection. Cell lysates were directly subjected to western blotting and immunoblotted (IB) with an anti-HA HRP-conjugated antibody (left panel). Alternatively, cell lysates were immunoprecipitated (IP) with an anti-HA antibody and blotted (IB) using an anti-HA HRP-conjugated antibody (right panel). The samples were treated or untreated with β-mercaptoethanol (β-ME). The arrows indicate bands corresponding to the monomer and putative dimer and tetramer. NC, non-transfected control cells.

Reference

1 van Kuppeveld FJ, Melchers WJ, Kirkegaard K, Doedens JR. Structure-function analysis of coxsackie B3 virus protein 2B. *Virology* 1997; **227**:111-118.