Supplementary Information

Small Molecules Targeting Coxsackievirus A16 Capsid Inactivate Viral Particles and Prevent Virus Binding

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Supplementary Figure

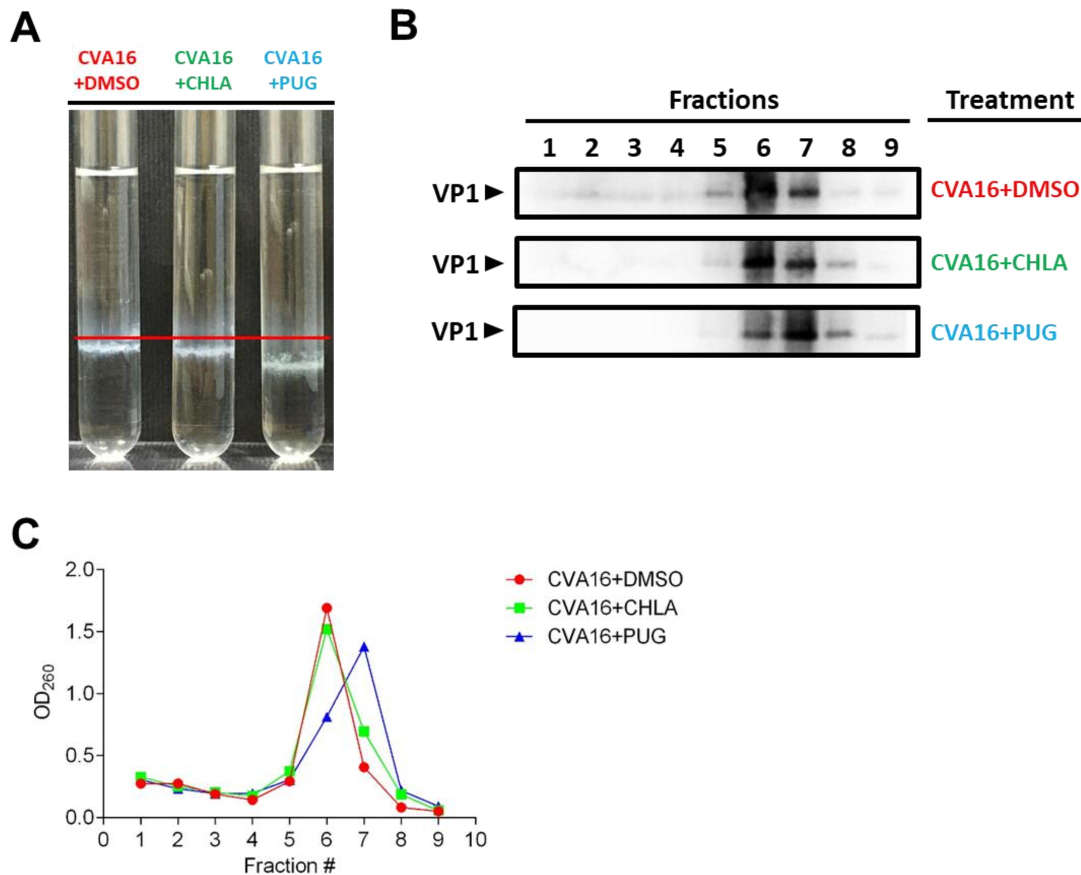

Fig. S1. Ultracentrifugation analysis of CVA16 particles treated with the tannins. Polyethylene glycol-8000 (PEG8000)-concentrated CVA16 virions were treated with DMSO (0.25%), CHLA (20 µM) or PUG (25 µM) for 1 h at 37 °C. The drug-treated virus samples were layered onto a 20% to 60% sucrose gradient in 50 mM Tris-HCl (pH = 7), and then subjected to ultracentrifugation in a Beckman SW40 Ti rotor (Beckman Coulter; Brea, CA, USA) at 35,000 rpm for 3 h at 4 °C. Sedimentation of the virions at the end of the ultracentrifugation run is shown (**a**). Nine fractions (1 ml/fraction) from the ultracentrifugation were subsequently taken from top to bottom and subjected to Western blot analysis (**b**) and optical density assessment (**c**). For Western blot analysis, 18 µl of the fractions were subjected to SDS-PAGE and then transferred to PVDF membrane, which was blocked with 5% BSA for 1 h; the membrane was subsequently probed using anti-VP1 primary antibody (1:2000; Millipore) and anti-mouse HRP-conjugated secondary antibody (1:2000; Invitrogen) before detecting and imaging immunoreactive proteins using enhanced chemiluminescence reagent (Western Lightning ECL Pro; PerkinElmer, Waltham, MA, USA) and a Bio-Rad ChemiDoc Imager (Bio-Rad; Hercules, CA, USA). For measurement of optical density, individual fractions from the ultracentrifugation were diluted 5 times using ddH_2O and then analyzed using a spectrophotometer (Eppendorf; Hamburg, Germany) at 260 nm.