



**Figure S1.** Responses of RAF-MEK-ERK kinase cascade to vemurafenib treatment at earlier time points of EV-A71 infection. **(A-C)** RD cells were infected without or with EV-A71 at an MOI of 5 and concurrently treated with 4  $\mu$ M vemurafenib or 0.02% DMSO for 1h. Cells were then washed once with DMEM and replenished DMEM. Cell lysates were collected at 15min, 30 min, and 60min post infection. Activation of RAF-MEK-ERK kinase cascade was analyzed by western blot. Phosphorylated MEK1/2 and ERK1/2 were detected using anti-phospho-MEK1/2 (Ser217/221) antibody and anti-phospho-ERK1/2 (Thr185/187) antibody. Unphosphorylated ERK and  $\gamma$ -tubulin were detected as controls. Vem: cells were mock infected and treated with 4  $\mu$ M vemurafenib. Mock: cells were mock infected and treated with 0.02% DMSO. EV71: cells were infected with EV71 at an MOI of 5 and treated with 0.02% DMSO. EV71 + vem: cells were infected with EV71 at an MOI of 5 and treated with 4  $\mu$ M vemurafenib. Blots are representative data of three independent experiments. **(B+C)** Quantification of relative amount of phosphorylated MEK 1/2 **(B)** or phosphorylated ERK 1/2 **(C)** to unphosphorylated ERK and normalized to mock for each time point. Results from three independent experiments are displayed as means  $\pm$  standard deviation.