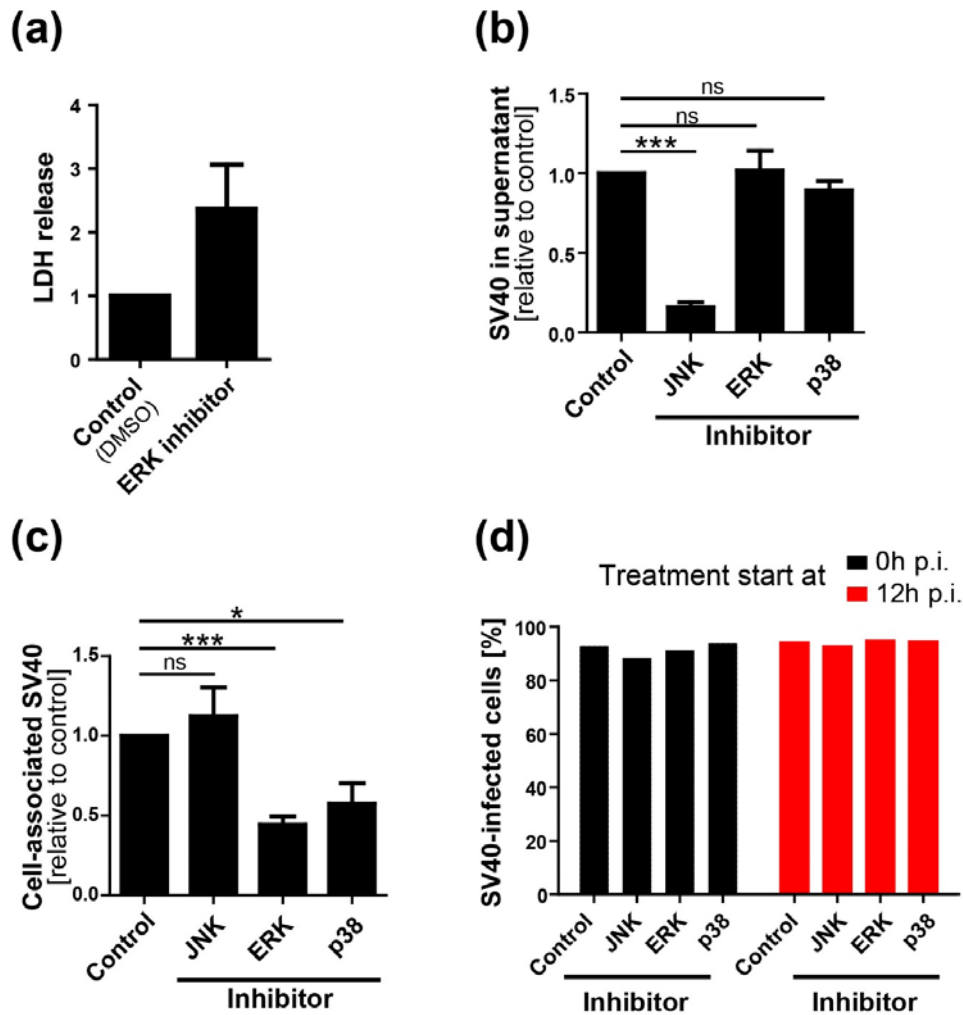
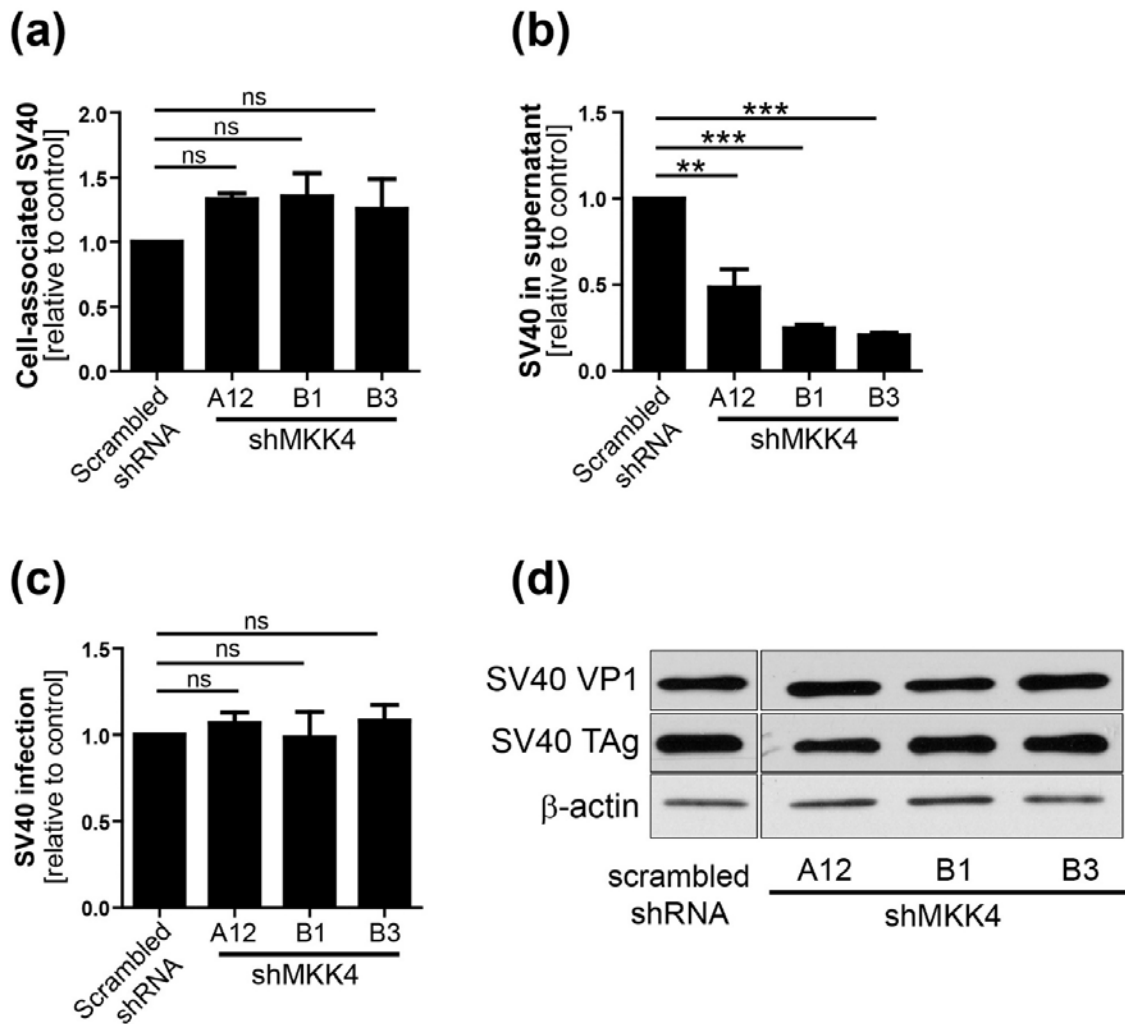


Supplemental Figure S1. Expression of dominant-negative Ras does not affect infection levels of CV-1 cells. CV-1 cells were transfected with wild-type mEGFP-HRas (WT) or dominant-negative mEGFP-HRas S17N (DN), respectively. Twelve hours p.i., cells were infected with SV40 at MOI of 10. At 24 h.p.i., cells were harvested and stained for large T antigen. Cells were analyzed by flow cytometry for GFP and T antigen fluorescence. The fraction of infected cells in the GFP⁺ population is shown.

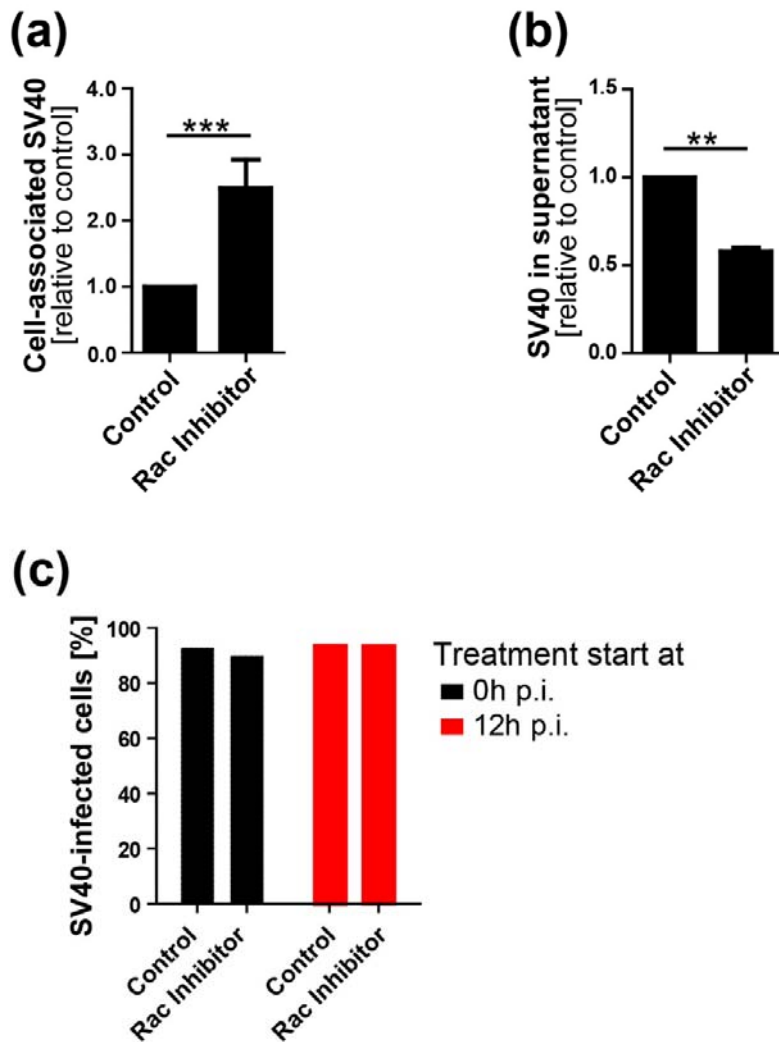


Supplemental Figure S2. Effect of MAPK inhibitors on SV40 replication and cell viability.

(a) ERK inhibitor reduces cell viability as assessed by LDH release. Mock-infected CV-1 cells were treated with ERK inhibitor or vehicle alone for 36 h and read out for LDH activity in the cell supernatant. Depicted is the mean of three independent experiments \pm SEM. **(b)** Treatment with JNK Inhibitor SP600125 reduces release of SV40 from infected cells. CV-1 cells were infected at MOI of 10. Inhibitor treatment was started at 12 h.p.i. and infectious units in the supernatant were quantified at 48 h.p.i. **(c)** Treatment with JNK inhibitor does not affect virus production. CV-1 cells were treated as in (b). Infectious units were quantified in cells at 48 h.p.i. **(d)** Inhibitor treatment of CV-1 cells does not influence SV40 infectivity. CV-1 cells were infected at MOI of 10 and treated with inhibitors against JNK, ERK, and p38 or DMSO vehicle at the time of infection (0 h.p.i. – black bars, 12 h.p.i.- red bars). At 24 h.p.i., cells were harvested, stained for expression of large T antigen and read out by flow cytometry.



Supplemental Figure S3. MKK4 knock-down reduces SV40 release while maintaining infection levels and virus replication. (a) MKK4 knockdown does not affect virus production. CV-1 cells expressing shRNAs to MKK4 or scrambled shRNA were infected with SV40 at MOI of 10. At 48 h.p.i., cells were harvested for quantitation of infectious units. (b) MKK4 is required for efficient virus release. Experiments were set up as in (a). At 48 h.p.i., infectious units were quantified in the supernatant. (c, d) MKK4 knockdown does not influence SV40 infectivity or replication. Experiments were set up as described in (a). (c) At 24 h.p.i. infected cells were harvested and subjected to large T antigen staining for determination of infection levels. Depicted is the mean of three experiments +/-SEM. (d) At 48 h.p.i., expression levels of the early protein large T antigen and the late protein VP1 were determined by immunoblotting.



Supplemental Figure S4. Effect of Rac1 inhibitor EHT1864 on SV40 replication. (a) Rac1 inhibition increases levels of cell-associated virus. CV-1 cells were infected at MOI of 10. Inhibitor treatment was started at 12 h.p.i. and cell-associated infectious units were quantified at 48 h.p.i. **(b)** Treatment with Rac1 Inhibitor EHT1864 reduces release of SV40 from infected cells. CV-1 cells were treated as in (a). Infectious units in the supernatant were quantified at 48 h.p.i.. **(c)** Inhibitor treatment of CV-1 cells does not influence SV40 infectivity. CV-1 cells were infected at MOI of 10 and treated with Rac1 inhibitor EHT1864 or DMSO vehicle at the time of infection (0 h.p.i. – black bars) or 12 h.p.i (red bars). At 24 h.p.i., cells were harvested, stained for expression of large T antigen and read out by flow cytometry.