Figure Legends additional figures

1.

**Kinetics of nuclear export depends on input virus.** (A) A9 cells were infected with WT or 5SG MVM using 10 PFU per cell. Following binding at 4 °C unbound virus was removed by washings. Cells were incubated at 37 °C for the indicated times. Phase contrast pictures of the infected cells were taken using a 20× magnification objective built in a Zeiss Axiovert 35 microscope. Cell viability was accessed via trypan blue exclusion using the TC10TM automated cell counter (BioRad). The average of three independent measurements is indicated. (B) A9 cells (8 × 103) were infected with the indicated PFU of WT or 5SG virus. Following binding at 4 °C unbound viruses were removed. Viral DNA was extracted and quantified at the indicated times post-infection. (C) A9 cells (3 × 106) were infected using the indicated viruses and PFU. Binding was performed at 4 °C. Unbound viruses were removed prior to incubation at 37 °C for the indicated times. Cytosolic fractions were isolated and applied for AEX-qPCR. Exported FC-P2 virions were quantified using qPCR. All infections were performed in the presence of α-capsid mAb (B7) and neuraminidase in order to prevent re-infections.

2.

**Purification and analysis of FC and EC.** (A) FC and EC were separated by differential centrifugation through a CsCl gradient as described in section xy... Fractions (500 uL each) are labeled from top to bottom of the gradient. 2 uL of each fraction were spotted on a nitrocellulose membrane and probed with an α-capsid mAB (B7). A HRP-coupled secondary antibody was used and the membrane was developed by exposure to a photo film. (B) EC and FC fractions were pooled. qPCR analysis was performed to quantify DNA-containing particles. (C) N-VP2 accessibility of ECs and FCs was tested by IP using an antibody raised against the N-VP2 region. The total amount of applied viral particles was verified using an α-capsid antibody (B7 mAb). (D) ECs and FCs (108 particles each) were treated with 0.5 mg/mL chymotrypsin (CHT) or not. Proteolytic N-VP2 processing was analyzed by 10 % SDS-PAGE. After transfer to a polyvinylidene difluoride membrane, the blot was probed with a rabbit α-VP pAb, followed by a HRP conjugated secondary antibody. The membrane was developed by exposure to a photo film. (E) Following treatment with 50 U/mL neuraminidase (Neur) or not, A9 cells were infected at 4 °C using the indicated PFU of FCs or ECs. Following washings to remove unbound viruses the cells were lysed in protein loading buffer and proteins were separated by 10 % SDS-PAGE. Membranes were probed as outlined above. (F) Estimation of the effective dose of Neur in order to completely deplete MVM binding on A9 fibroblasts. 3 × 105 cells were infected at 5 PFU.

3.

**FCs are preferentially bound to the SA residues on the cell surface**. (A) A9 cells (3 × 105) were grown on cover slips and infected independently or combined with FC and EC (5 PFU per cell) at 4 °C. In the 4th row ECs were incubated with the cells 30 min prior to the addition of FCs. Following removal of unbound viruses the cells were fixed and stained for IF using an antibody (mAb B7) raised against assembled capsids (green) and an antibody recognizing N-VP2 (red). Percentage of capsids showing N-VP2 signal was calculated for the indicated areas of interest. (B) Scatter plots analysis showing the indicated areas of interest were used to calculate the corresponding correlation coefficient as a measurement for the degree of co-localization.

4.

**N-VP2 alone is not responsible for the better binding to SA moieties.** (A) A9 cells (3 × 105) were incubated with MVM at increasing PFU. Following binding at 4 °C unbound virus was removed and total amount of bound virus was quantified. (B) ECs or FCs were bound to A9 cells at the PFUs indicated in brackets (black bars). In order to compete the bound FCs (5 PFU) increasing amounts of ECs were added (5-80 PFU, white bars). (C) FCCHT or FCs were bound to A9 cells at the PFUs indicated in brackets (black bars). In order to compete the bound FCs (5 PFU) increasing amounts of FCCHT were added (5-40 PFU, white bars). (D) ECs or FCCHT were bound to A9 cells at the PFUs indicated in brackets (black bars). In order to compete the bound FCCHT (5 PFU) increasing amounts of ECs were added (5-80 PFU, white bars). Grey bar: As a negative control experiment, FCs (5 PFU) were simultaneously incubated with an excess of ECs (80 PFU). (E) A9 cells (8 × 103) were infected at the PFUs indicated in brackets. Increasing amount of ECs were added to FCs. Inputs are represented by white bars.

5.

**Anion-exchange chromatography can be applied to study other parvoviruses.** (A) B19V is produced in the bone marrow of infected individuals. Only mature particles accumulate in the blood plasm where they productively infect erythroid progenitor cells. (B) CPV was produced in tissue cell culture. Pre-mature and mature particles were not efficiently separated. (C) AEX profile of B19V derived from blood plasm. (D) AEX profile of B19V following binding to UT-7/EPO-S1 cells at 4 °C for 1h. (E) AEX profile of B19V internalized in UT-7/EPO-S1 cells for 30 min at 37 °C. (F) AEX profile of CPV produced in canine A72 cells. Virus was recovered from infected cells by repeated freeze and thaw cycles to lyse the cells.