**Materials and methods**

**Cells and viruses.** A9 mouse fibroblasts (ref) and NB324K cells (ref), were routinely propagated under a minimal number of passages in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies, Carlsbad, CA) supplemented with 5 % fetal calf serum (FCS) at 37 °C in 5 % CO2 atmosphere.

Stocks of MVM were propagated on A9 cells. As soon as the cytopathic effect became evident, the supernatant was collected and pre-cleared from cell debris by low-speed centrifugation. Thereby, intracellular capsids were discarded. In order to remove low-molecular contaminants, virus containing media was pelleted through 20 % sucrose cushion. Virus titers were determined by qPCR as DNA-containing particles per microliter.

**Virus separation by CsCl.** Sucrose purified capsids were prepared as previously described. The virus pellet was resuspended in 10 mL phosphate buffered saline (PBS). In order to separate FC from EC or FC-P1 from FC-P2, CsCl (Sigma-Aldrich, St. Louis, MO) was added to a density of 1.38 g/cm3 (ηi=1.371) or 1.47 g/cm3 (ηi=1.376), respectively, adjusted by using a refractometer at 4 °C. The gradient was centrifuged to equilibrium for 24 h at 41’000 rpm and 4 °C in a swinging bucket SW-41 Ti rotor (Beckman, Brea, CA). Gradients were fractionated and tested for intact capsids by dot blot analysis using B7 mAb (ref?). CsCl was depleted from the corresponding fractions by size-exclusion chromatography through PD-10 desalting columns (GE Healthcare, Chalfont St Giles, UK) and the capsids were concentrated in Amicon® centrifugal filter devices (Merck Millipore, [Billerica](https://de.wikipedia.org/wiki/Billerica), MA) when required.

**Anion exchange chromatography (AEX).** The Mono Q HR 5/5 column (5 x 50 mm; Pharmacia, Uppsala, SW) was connected to the ÄKTAmicro chromatography system (GE Healthcare, Chalfont St Giles, UK) that was operated by the UNICORN (GE Healthcare, Chalfont St Giles, UK) control software. The Mono Q column was equilibrated with five column volumes (CV) starting buffer (20 mM Tris-HCl, 1 mM EDTA, pH 7.2). Samples (1mL) containing at least 1 × 108 virus particles in 10 mM Tris-HCl, 1 mM EDTA, pH 8 were applied to the Mono Q column trough a 2 mL injection loop. Following sample application the loop and the column were rinsed with six CV starting buffer. After elution of the protein, which did not bind to the column, a linear salt gradient (0-2 M NaCl) in 20 mM Tris-HCl, 1 mM EDTA, pH 7.2, was applied. The total elution volume of 24 CV was collected in fractions of 0.185 mL in 96-well plates. The flow rate was constantly kept at 1.5 mL/min and salt concentration was monitored by measuring the electrical conductivity. Viral genomes in each fraction were quantified by qPCR. All buffers were filtered and degassed before application to the Mono Q column.

**Antibodies, enzymes and chemicals**. Rabbit anti-VPs (polyclonal against MVM structural proteins), rabbit anti-N-VP2 (polyclonal against the N-terminus of VP2), and mouse anti-capsid (monoclonal against intact capsids; clone B7) antibodies have been previously described (ref?). Goat anti-mouse fluorescein isothiocyanate (FITC) (sc-2010), goat anti-mouse rhodamine (sc-2092), goat anti-rabbit FITC (sc-2012), and goat anti-rabbit rhodamine (sc-2091) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Goat anti-rabbit (P0448) and goat anti-mouse (P0447) horseradish peroxidase-conjugated antibody were purchased from DakoCytomation (Glostrup, DK).

Bafilomycin A1 (B1793) and chymostatin (C7268) were purchased from Sigma-Aldrich (St. Louis, MO) and reconstituted in ethanol at 0.1 mg/mL or in DMSO at 10 mM, respectively. All drugs were stored at −20°C.

Complete Mini, EDTA-free protease inhibitor cocktail tablets (1 tablet/10 mL; Roche, Basel, CH) and 1 mM sodium orthovanadate (Na3VO4; Sigma-Aldrich, St. Louis, MO) in combination with 1 mM sodium fluoride (NaF; Sigma-Aldrich, St. Louis, MO) was supplemented to each lysis buffer avoid enzymatic digestion or dephosphorylation during the processing of cell extracts.

**Cell fractionation**. Isolation of A9 and NB nuclei was performed by using the Nuclei EZ Prep Nuclei Isolation Kit (NUC-101, Sigma-Aldrich, St. Louis, MO) following the manufacturer’s instructions. In order to obtain highly pure nuclear fractions, the isolated nuclei were further processed by centrifugation through a sucrose gradient by low speed centrifugation at 500 g for 10 min. Extracted nuclei were lysed in 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1 % Triton X-100, 1 mM Na3VO4, 1 mM NaF, protease inhibitor cocktail (Roche), pH 7.2 at 4 °C for 30 min. Following vortexing thoroughly the nuclear lysate was passed through a 27 G needle 10 times. Debris was removed by centrifugation at 10’000 rpm for 10 min at 4 °C.

Cytoplasmic fractions were extracted in 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1 % NP-40, 1 mM Na3VO4, 1 mM NaF, protease inhibitor cocktail (Roche), pH 7.2 at 4 °C for 30 min. Following vortexing thoroughly, intact nuclei and cell debris was removed by centrifugation at 10’000 rpm for 10 min at 4 °C.

**Quantitatice PCR (qPCR).** Amplification and real-time detection of PCR products was performed using the CFX96TM Real-Time System (Biorad, Hercules, CA). Specificity of the amplification was determined by melting curve analysis. Primers for MVM DNA amplification were as follows: forward (5’-GACGCACAGAAAGAGAGTAACCAA-3’ from nucleotides 231 to 254) and reverse (5’-CCAACCATCTGCTCCAGTAAACAT-3’ from nucleotides 709 to 732). Template DNA was extracted by using the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, DE) following the manufacturer’s guidelines. Amplification and real-time detection of PCR products was performed by using the iTaqTM Universal SYBR® Green Supermix (Biorad, Hercules, CA) following the manufacturer’s instructions. As external standard, an infectious clone of MVM (ref) was used in 10-fold serial dilutions.

**Virus infection.** A9 or NB324K cells (105 for qPCR or 3 × 106 for AEX) were infected with MVM (5’000 DNA-containing particles per cell, corresponding to approximately 10 PFU/cell) for 1 h at 4 °C for binding. Unbound virus was removed by washings and incubated at 37 °C to initiate infection. At progressive times post-internalization total cellular DNA was extracted for qPCR analysis or cells were fractionated and subjected to AEX.

**Western and dot blot.** Viruses (108 DNA-containing particles) were dissolved in 20 μL protein loading buffer containing 2 % SDS and 10 % glycerol. Viral proteins were separated through a NuPAGE® 10 % Bis-Tris Gel (Invitrogen, Carlsbad, CA). Following separation, the proteins were blotted on a 0.2 μm polyvinylidene fluoride (PVDF) Immobilon® Transfer Membrane (Merck Millipore, [Billerica](https://de.wikipedia.org/wiki/Billerica), MA). Blotting was carried out using XCell II™ Blot Module (Invitrogen, Carlsbad, CA). The membrane was blocked in TBS-T buffer supplemented with 5 % milk overnight at 4 °C. Subsequently, the membrane was probed with a polyclonal rabbit antibody against linear MVM-VP epitopes (ref) that was diluted 1:2000 in 3 mL TBS-T containing 1 % milk. The first antibody was incubated for 1 h at RT. The PVDF membrane was washed in TBS-T for a total 90 min with many buffer replacements. Subsequently, the horseradish peroxidise conjugated secondary antibody (goat α-rabbit-HRP, ref) was added for 1 h at RT. To deplete remaining antibodies, the membrane was washed in the same way as described above except for a final wash step with TBS (ref). VP1, VP2, and possibly VP3 were visualized by a chemiluminescence system (SuperSignalTM West Femto Maximum Sensitivity Substrate; Life Technologies, Carlsbad, CA) following the manufacturer’s instructions. Following this treatment, the PVDF membrane was exposed to a film (Amersham Hyperfilm™ ECL, GE Healthcare, Amersham, UK). Finally, the film was developed using Anatomix Developer Replenisher Solution and Fixer and Replenisher Solution (Sigma-Aldrich, St. Louis, MO).

**Indirect immunofluorescence staining.** A9 cells (3 × 105) were seeded onto coverslips within 12-well plates. After 24 h, the cells were infected with 2’500 DNA-containing particles, per cell, corresponding to approximately 5 PFU/cell ([40](http://jvi.asm.org/content/80/2/1015.long#ref-40)), for 1 h at 4 °C. Subsequently, the cells were washed to remove unbound virus, and incubated at 37 °C. At different times, cells were washed and processed for immunofluorescence as previously described (ref?) with secondary antibodies coupled to rhodamine or FITC. Cells were mounted with Mowiol (Calbiochem, Billerica, MA) containing 30 mg/ml of DABCO (Sigma-Aldrich, St. Louis, MO) as an anti-fading agent and examined by confocal fluorescence microscopy (LSM 512 Meta or Axiovert 200 M, Carl Zeiss, Oberkochen, DE). Immunostained samples were visualized by a 100x oil immersion objective using the according filter sets. Images were processed by LSM Image Browser and BioImageXD software (Ref).

**Immunoprecipitation.** *In vitro* treated viruses or viruses from cell extracts were transferred to LoBind eppendorf tubes that were pre-blocked with PBS containing 1 % bovine serum albumin (PBSA 1 %). The volume was adjusted to 200 μL with PBSA 1 %. The antibody was added in excess and incubated with the viral capsids for 1 h at 4 °C on a shaker. Subsequently, 20 μL protein G-agarose beads were added. Following overnight incubation at 4 °C and centrifugation at 2500 rpm for 5 min the supernatant was discarded. The beads were washed with PBSA 1 %. To remove residual BSA an additional washing step was carried out with PBS. Finally, the beads were frozen at -20 °C until further use or immediately processed.

**Enzymatic reactions**. All enzymatic reactions were performed with 1 × 108 virus particles in a reaction volume of 50 uL. Viruses were incubated in PBS for 1.5 h at 37 °C with 0.5 mg/mL chymotrypsin (C3142, Sigma-Aldrich). The reaction was blocked by adding 100 μM chymostatin (C7268, Sigma-Aldrich).

Phosphatase lambda treatment (2000 Units; 539514, Merck Millipore) was performed in 50 mM Tris-HCl, 100 mM NaCl, 2 mM MnCl2, 5 mM DTT, pH 7.8 for 3 h at 37 °C in PBSA 1% pre-blocked Protein LoBind eppendorf tubes. Phosphatase lambda was inactivated by supplementing the enzymatic reaction with 1 mM Na3VO4 and 1 mM NaF.

Free DNA was digested using 50 Units DNaseI (04716728001; Roche) in 1x incubation buffer according to the manufacturer’s protocol. DNaseI was inhibited by incubation at 75 °C for 15 min. Negative controls were incubated in the same buffer for the same time.