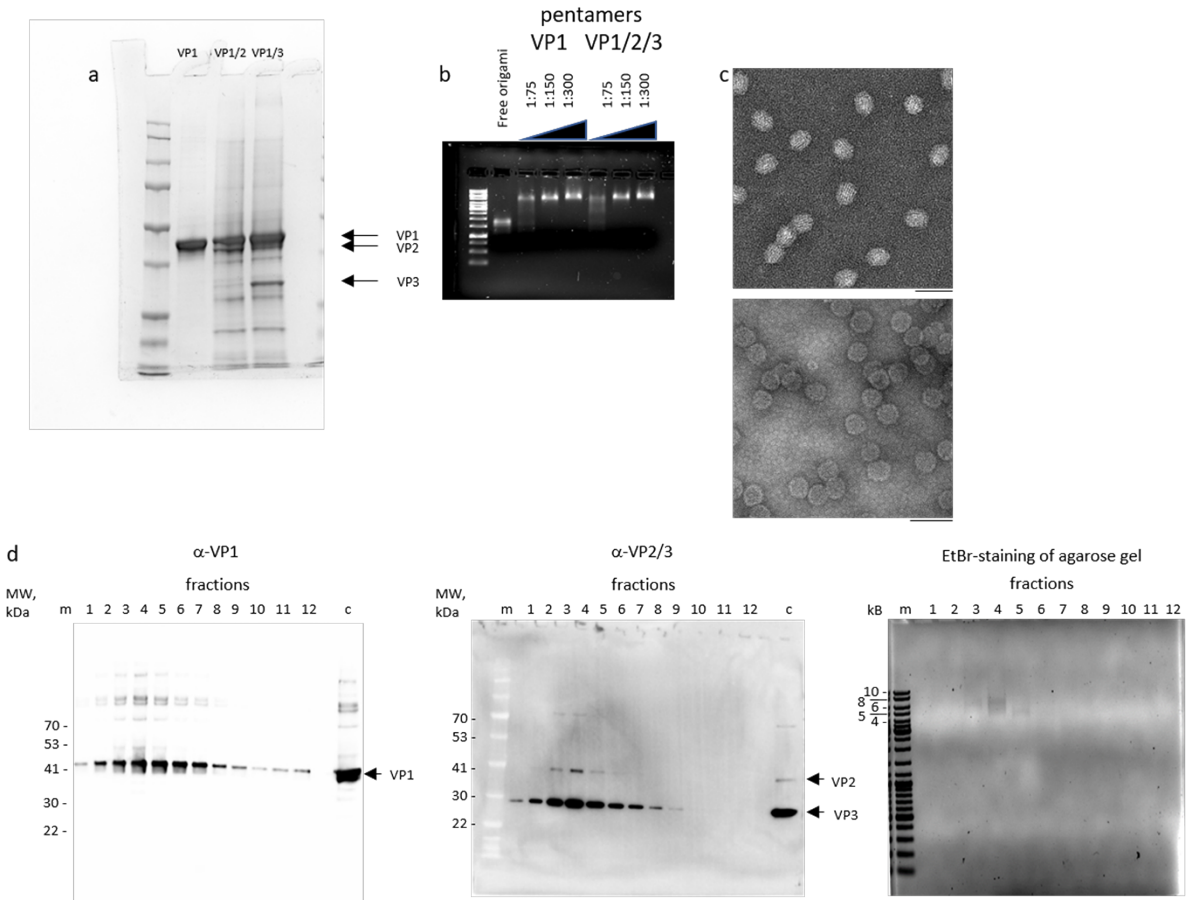

Fig. S1 VP1, VP2 and VP3 assembly on DNA origami. (a) VLPs composed of either VP1 alone or VP1 associated with either VP2 or VP3 were purified from insect cells and were visualized by Coomassie blue staining. (b) Electrophoretic Mobility Shift Assay (EMSA) analysis showing formation of VP1/DNA origami or VP1/2/3/DNA origami complexes at increasing pentamer/origami ratios. (c) Negative stain TEM images of 35 nm DNA origami (upper panel, scale bar 50 nm) and 50 nm VP1/2/3 particles formed on DNA origami at a 150:1 pentamer/origami ratio (lower panel, scale bar 100 nm). (d) VP1/2/3/DNA origami assembly products were then fractionated by sedimentation velocity ultracentrifugation through 2.5-ml 20-60% sucrose gradient. 200 µl fractions were collected and analyzed for the presence of VP1 by anti-VP1 antibody (left), VP2 and VP3 by anti-VP2/3 antibody (middle) and for the presence of DNA by agarose gel (right).

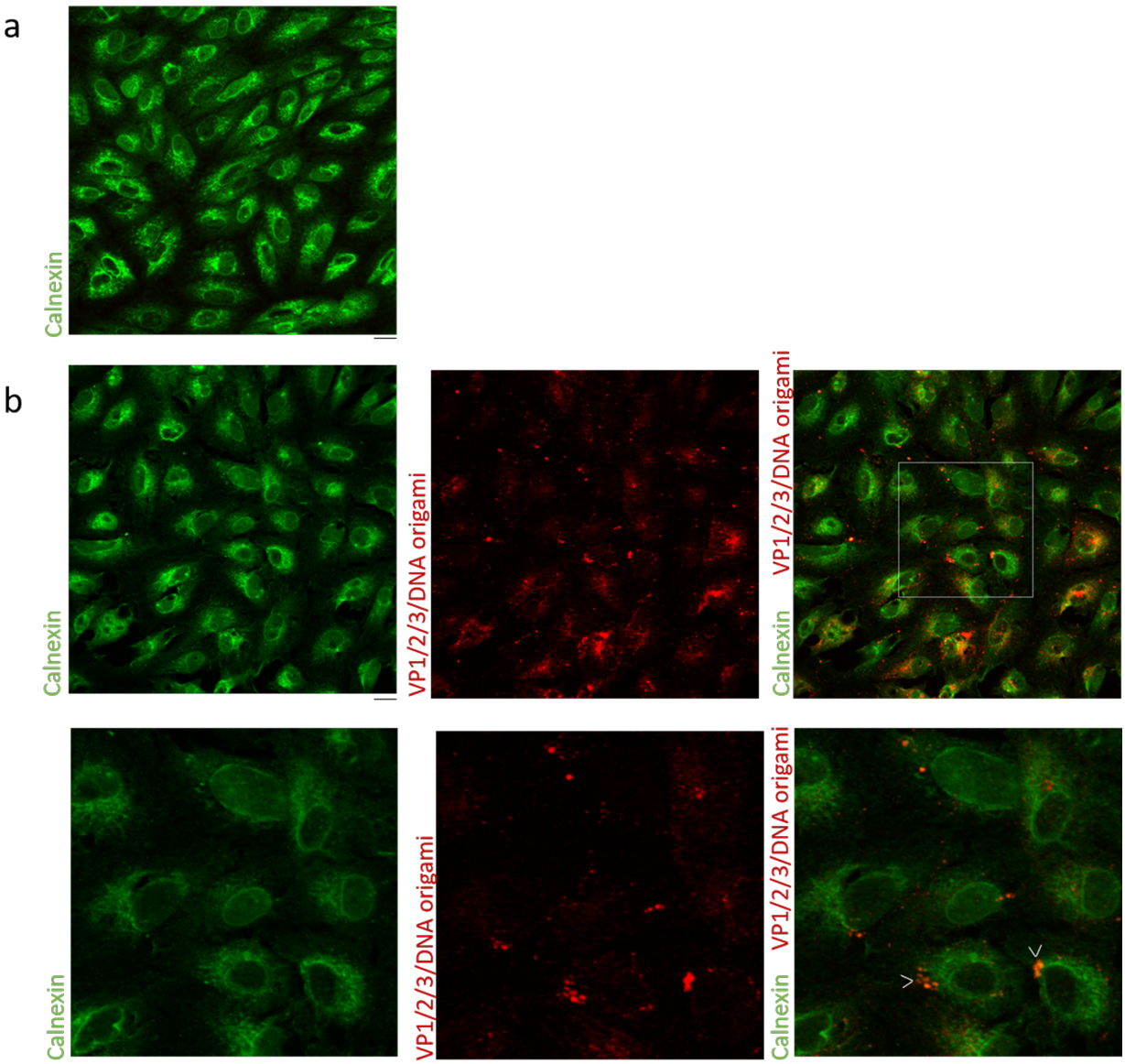

Fig. S2 Fluorescence analysis of CV-1 cells transfected with Cy5 labeled VP1/2/3/DNA origami particle. (a) Calnexin staining (green) in untreated CV--1 cells (scale bar 14 μm). (b) Top: CV-1 cells transfected for 18 h with VP1/2/3/DNA origami particle (red), at a ratio of 15,000 particles per cell, and stained with Calnexin antibodies showing no major reorganization in Calnexin staining pattern. Bottom: an enlargement of the square indicated in the merged image in the upper row. Some VP1/2/3/DNA origami stain faintly for calnexin (arrowheads in right panel). Images are a merge of 4 internal sections (scale bar 14 μm).

METHODS

Production of recombinant SV40 capsid proteins

Three Recombinant flashBACULTRA baculoviruses, expressing either VP1, VP2 or VP3 were prepared by Oxford Expression Technologies (Oxford, UK) and propagated in Spodoptera frugipedra (Sf9) insect cells as described in[1]. SV40 VLPs comprising of either VP1 alone, or VP1 and VP2 (VP1/2) or VP1 and VP3 (VP1/3) were produced in Trichoplusia ni (Tni) insect cells by infection of VP1 baculovirus alone at multiplicity of infection 10 or co-infection of VP1 baculovirus and either VP2 or VP3 baculoviruses at multiplicity of infection 8.75 and 1.25 for VP1 and VP2/3 respectively. Nuclear extracts were isolated as described in[2] and VLPs were purified using two successive preparative cesium chloride density gradient ultracentrifugation at 200,000g, 4°c for 40 hrs. VLPs were collected, dialyzed against 0.5M NaCl and used for further assembly experiments. VLPs protein composition was confirmed by Western Blotting using anti-SV40 VP1 and VP2/3 antibodies (ab53983 and ab53977, Abcam).

DNA origami

The 35 nm spherical DNA origami structure was designed and produced by tilibit nanosystems GmbH, Munich, Germany. All relevant details have been previously described.[3, 4] The structure was assembled as 108 parallel dsDNA helices of varying length arranged in a honeycomb-type lattice, with a 7560 nt-long scaffold DNA strand based on a *M13mp18* bacteriophage derivative and 227 shorter staple strands. Assembly was achieved through a cooperative self-assembly process in a one-pot reaction. A fluorescently labeled DNA origami was produced similarly, by introducing 1 Cy5 molecule dye into 25 staple strands (25 Cy5 molecules per one origami structure).

SV40 VP1, VP1/2 and VP1/3 assembly on DNA origami

VP1, VP1/2 and VP1/3 pentamers were produced, each from its respective empty VLPs, using our disassembly protocol.[5] For assembly reactions, 10 nM DNA origami structure was incubated with either VP1 pentamers or VP1/2 and VP1/3 pentamers mixed at the ratio of 1:10 (representing VP1/2:VP1/3 ratio in the native SV40 virion), at increasing pentamer/origami ratios , in 150 mM NaCl, 6 mM $MgCl_2$, 50 mM MOPS pH 7.2. The reaction was allowed to proceed for 1 hour at room temperature, followed by the addition of $CaCl_2$ to the final concentration of 5 mM and overnight incubation at 4°c.

Analysis of SV40 assembly on DNA origami by electrophoretic mobility shift assay

Assembly reaction or free origami was mixed with a loading buffer supplemented with $MgCl_2$ to the final concentration of 6 mM free $MgCl_2$. The samples were run at 4°c on 2% agarose gel supplemented with 6 mM $MgCl_2$ in 0.5x TBE buffer. The bands of DNA origami were visualized using ethidium bromide.

Sucrose Density Gradient Ultracentrifugation

Step gradient of sucrose was made using 60-, 40- and 20% sucrose solutions supplemented with 10 mM MOPS pH 7.2 and 150 mM NaCl. 200 µl of assembly reaction mixture was layered on the top of 2.5 ml gradient and centrifuged in TLS-55 swinging bucket rotor in Optima Max TL ultracentrifuge at 93,000 g, 4°c for 3 hrs. After completion of the run, 200 µl fractions were collected from the bottom of the tube and analyzed for VP1 and VP2/3 by Western Blot using anti-SV40 VP1 and VP2/3 antibodies (ab53983 and ab53977, Abcam), and for DNA by 1% agarose gel supplemented with ethidium bromide.

Negative staining TEM

The samples were prepared in the following way: 300 mesh copper grids (Ted Pella, Prod No. 01813-F) were glow discharged to make their surface hydrophilic. Next, 2.5 µL of the sample was applied on to the grid and the excess liquid was blotted with filter paper after 1 min. The grid was dried in air for 1 minute, following by applying 5 µL of uranyl acetate 2% (SPI CAS# 6159-44-0) for negative staining to increase the sample contrast. Next, the grid was blotted ones more to remove the excess uranyl acetate. Finally, the grid was dried in air before insertion into the microscope. The samples were imaged on a ThermoFisher Scientific (FEI) Tecnai T12 G^2 TWIN transmission electron microscope operating at 120 kV equipped with Gatan 794 MultiScan CCD camera and a ThermoFisher Scientific (FEI) Talos F200C transmission electron microscope operating at 200 kV equipped with Ceta 16M CMOS camera.

Immunofluorescence Microscopy

Fluorescent images of CV-1 cells transfected with SV40-encapsulated DNA origami were obtained with a confocal laser scanning microscope (FV-1200, Olympus, Japan). ImageJ software (NIH) was used for image processing, analysis, and assembly. To view the internalization of the nanoparticles, CV-1 cells were plated 1 day before transfection on u-Slide 4 well chambered coverslips (ibidi GmbH, Munich, Germany). Cells were transfected with SV40-encapsulated Cy5-labeled DNA origami, at a ratio of 15000 particles per cell, using our standard SV40 infection protocol,[6] by dilution of the nanoparticles into 100 µl serum-free medium for 60 min at 37^{o}c, with occasional agitation. This was followed by addition of fresh serum-containing medium. At 6 hours post infection cells were counterstained with Hoechst 33342 and viewed alive, directly on the u-slide dishes, at room temperature. For double staining of the nanoparticles with ER proteins, CV-1 cells were grown overnight in 24 well plates and were transfected with SV40-encapsulated Cy5-labeled DNA origami, at a ratio of 15000 particles per cell. At 18 hours post infection, cells were washed with PBS followed by fixation with 4% formaldehyde at room temperature. Cells were then permeabilized for 10 min with 0.25% saponin and blocked with 5% BSA. Primary antibodies, either BAP31 antibody (CC-4), a rat monoclonal IgG2a, directed against amino acids 123-229 of BAP31 of human origin (SC-56008, Santa Cruz Biotechnology, Inc.) or Rabbit polyclonal calnexin antibody (ab13505, Abcam), were incubated for 1 h at room temperature, followed by the appropriate fluorescent conjugated secondary antibodies (Alexa Fluor® 488 Goat Anti-Rat IgG and Alexa Fluor® 488- Goat Anti-Rabbit IgG, respectively (Jackson ImmunoResearch Inc.) for 1 h at room temperature. Coverslips were mounted with Fluoromount-G® (Southern Biotechnology, Birmingham, AL USA).

Cryo-EM Sample preparation

3 µl VLP samples were applied to holey carbon grids (Quantifoil R 1.2/1.3, Micro Tools GmbH, Germany) after 30 seconds glow discharge treatment. Grids were blotted and vitrified by rapidly plunging into liquid ethane using a home-built plunging apparatus.[7, 8]

Data acquisition

Samples were imaged under low-dose conditions on a FEI Tecnai F30 Polara microscope (FEI, Eindhoven) operating at 300 kV. Datasets were automatically collected using SerialEM[9] on a K2 Summit direct electron detector camera fitted behind an energy filter (Gatan Quantum GIF) with a calibrated pixel size of 2.3 Å. The energy filter was set to remove electrons > ±10 eV from the zero-loss peak energy. The K2 summit

camera was operated in counting mode at a dose rate of 8 electrons/pixel/second on the camera. Each movie was dose fractionated into 50 image frames, with total electron dose of 80 ē/Å^2.

Single particle reconstruction

Dose-fractionated image stacks were aligned using MotionCorr2,[10] and their defocus values estimated by Gctf.[11] The sum of the aligned frames was used for further processing and the rest of the processing was done in RELION3.1.[12] 55748 particles were autopicked and subjected to 2D classification using RELION 3.1 with 50 classes. Initial 3D references were prepared from the distinct empty capsid particles and of distinct origami-filled particles that were manually selected from the 2D class averages. We performed 3D classification and refinement of the empty capsids particles imposing icosahedral symmetry (I1), and with no assumed symmetry (C1) for the origami-filled particles.

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