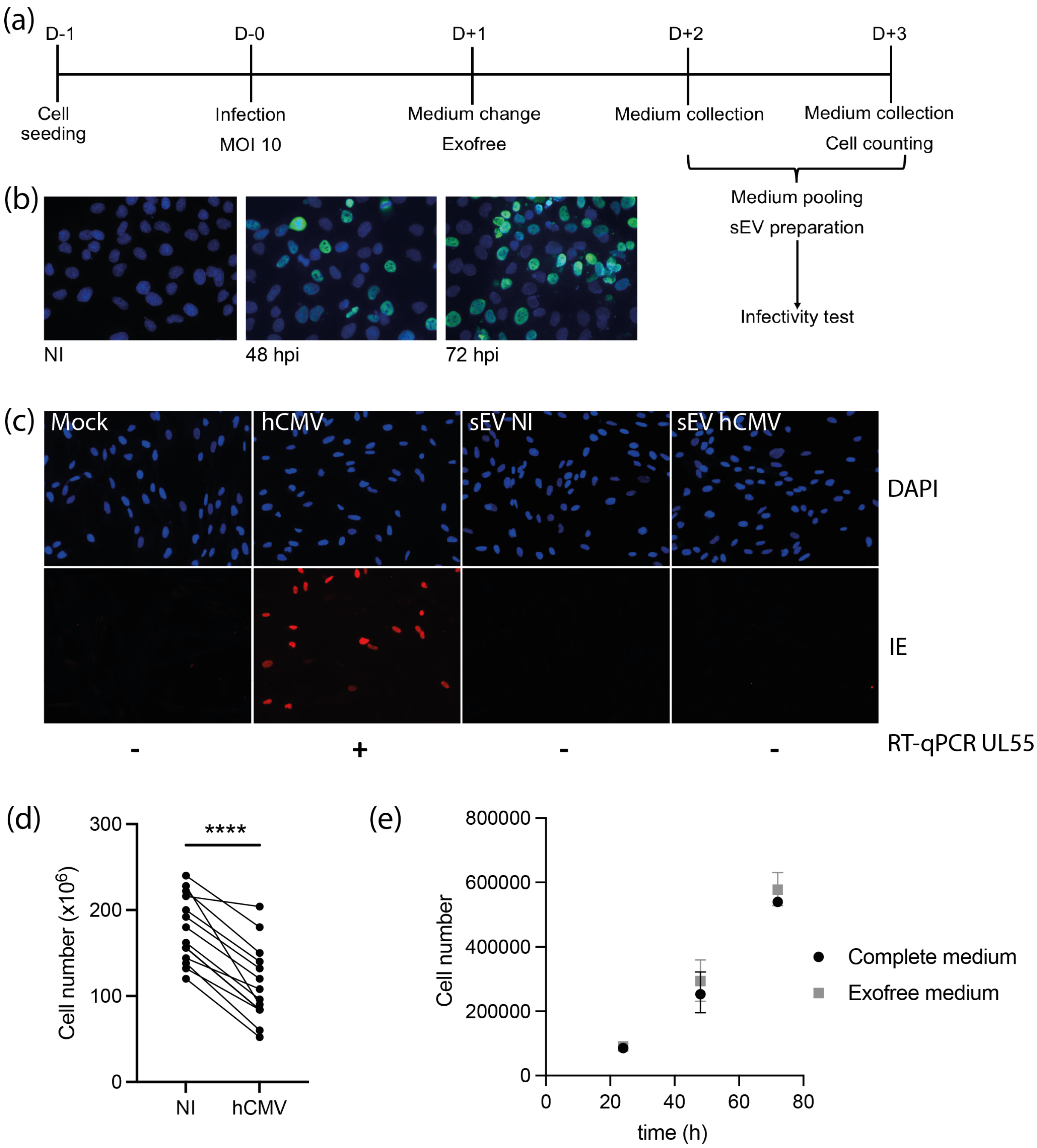
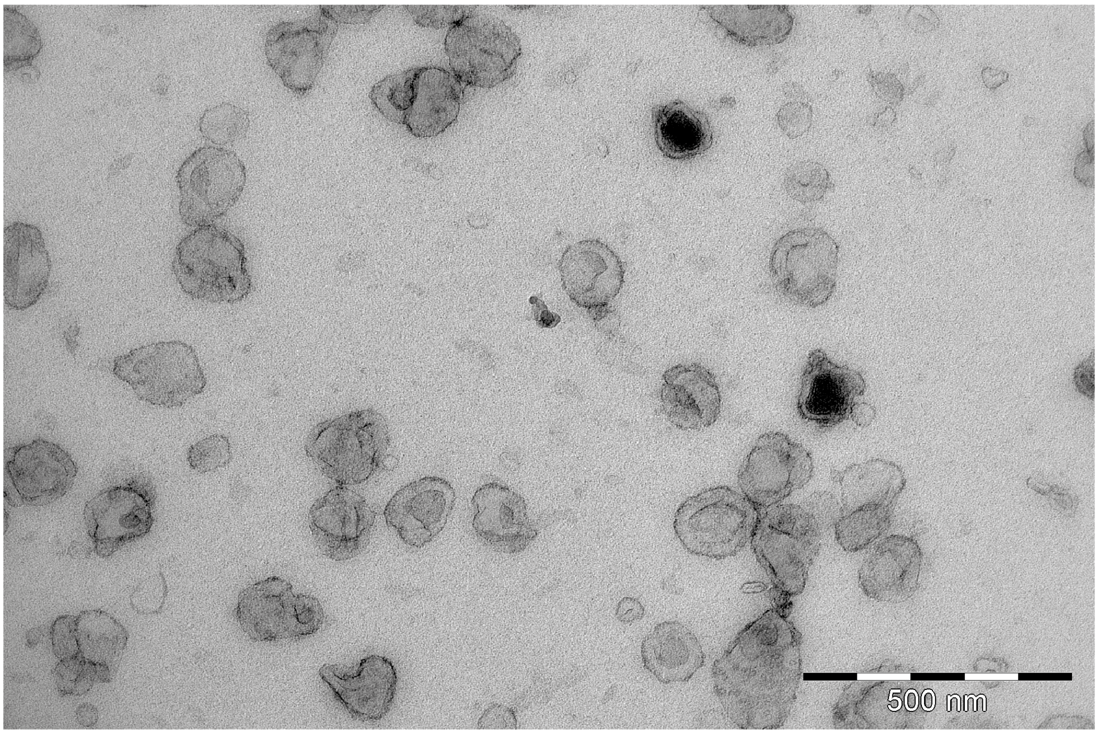
Figure S1: Model system.



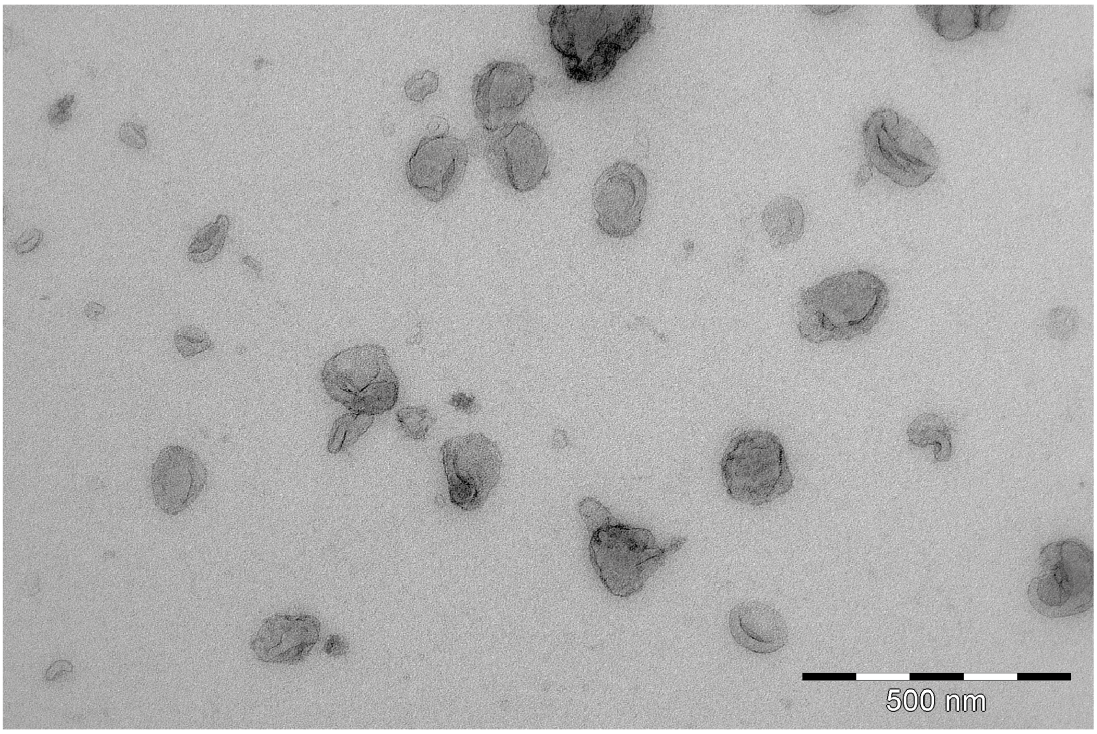
(**a**) Timeline representing the HIPEC seeding, infection, medium renewal and collection before sEV preparation. D: days; MOI: Multiplicity of infection. (**b**) HIPECs are permissive to hCMV infection. Immunofluorescence against Immediate Early 1/2 (IE) antigens was done on HIPECs either non-infected (NI) or at 48 h or 72 h post-infection (hpi). Green: IE; Blue: DAPI; Magnification: 20 x. (**c**) Immunofluorescence realized against hCMV IE1/2 antigens in non-treated MRC5 cells (Mock), or upon incubation during 24 h with either hCMV at MOI 3 (hCMV), sEVs isolated from non-infected (sEV NI) or from hCMV-infected HIPECs (sEV hCMV). Magnification = 20 x. Blue (upper panel): DAPI; red (lower panel): IE. Below images are indicated the results of RT-qPCR realized against hCMV UL55 mRNAs on RNA extracted from MRC5 cells at 48 h post-incubation with hCMV or sEV preparations (+: amplification; -: no amplification). Data are representative for at least three independent experiments. (**d**) Total cell number counted at the time of sEV preparation, at 72 hpi, for non-infected (NI) or hCMV infected HIPECs. Results are representative of 15 independent experiments. $p<0.0001$ by paired *t*-test. (**e**) Comparison of cell growth between complete and Exofree culture medium, by counting of HIPEC number in 12-well plates in which 10^5 cells were initially seeded. Symbols represent mean ± SEM for three independent experiments. A 2-way ANOVA statistical test indicated no significant difference between complete and Exofree medium culture conditions ($p=0.4414$).

Figure S2: Wide-field transmission electron microscopy.

NI

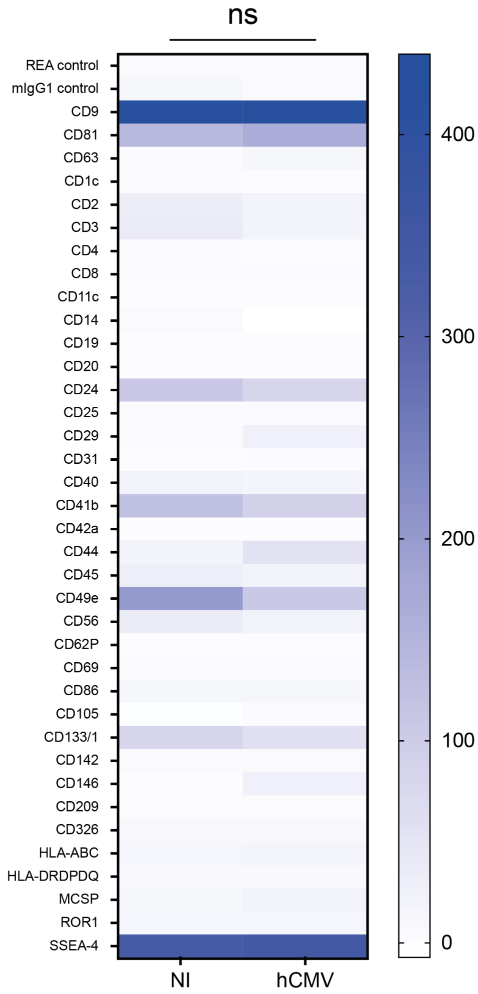


hCMV



TEM micrographs showing sEV from HIPEC either non-infected (NI; upper panel) or infected (hCMV; lower panel). Scale bar: 500 nm.

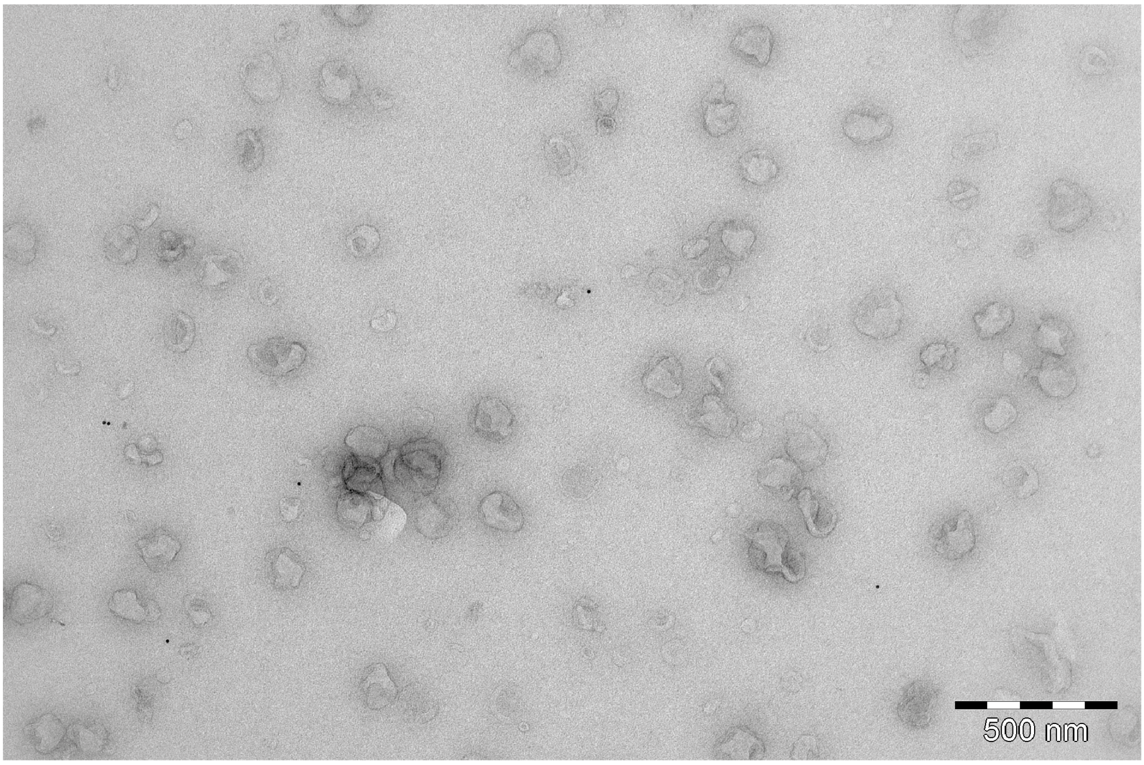
Figure S3: Multiplex bead-based flow cytometry comparison of sEV prepared from non-infected of hCMV-infected HIPECs.



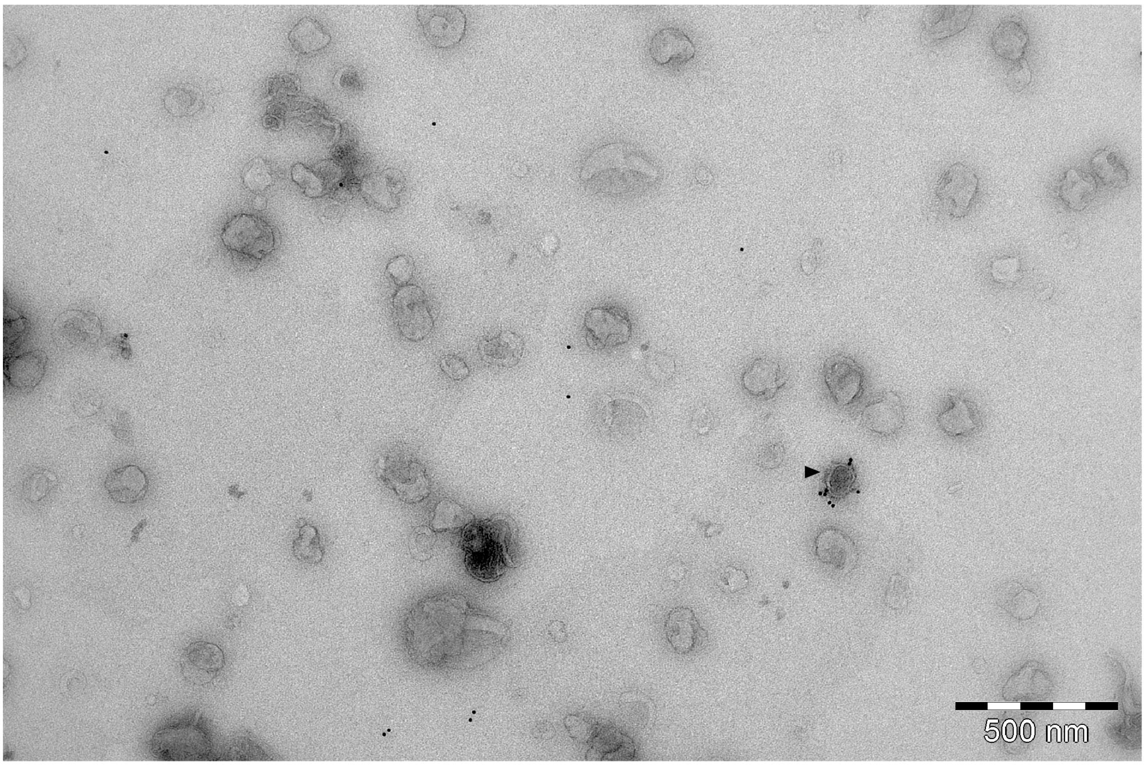
Bead-based multiplex analysis using the MACSPlex Exosome Kit, human (Miltenyi Biotec) were realized on sEV preparations by flow cytometry according to the manufacturer's instructions. This allowed the quantification of 39 different EV markers, distinguishable by flow cytometry by a specific PE and FITC labeling. The MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec) was used for analysis. The tool MACSQuantify was used to analyze data (v2.11.1746.19438). GraphPad Prism (v8) software was used to perform statistical analysis of the data. The heat-map represents the mean of 3 independent experiments, for different sEV markers indicated on the left column, for sEV prepared from non-infected of hCMV-infected HIPECs. Blue intensity is proportional to the level of expression calculated in Median Fluorescence Intensity, indicated on the right of the heat-map. ns, non-significant by two-way ANOVA.

Figure S4: Wide field immuno-electron microscopy anti CD63.

NI

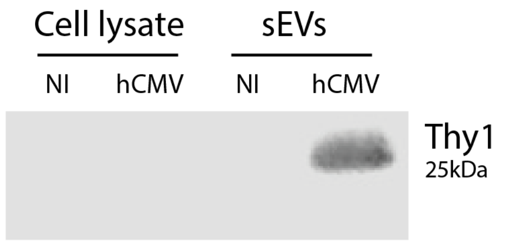


hCMV



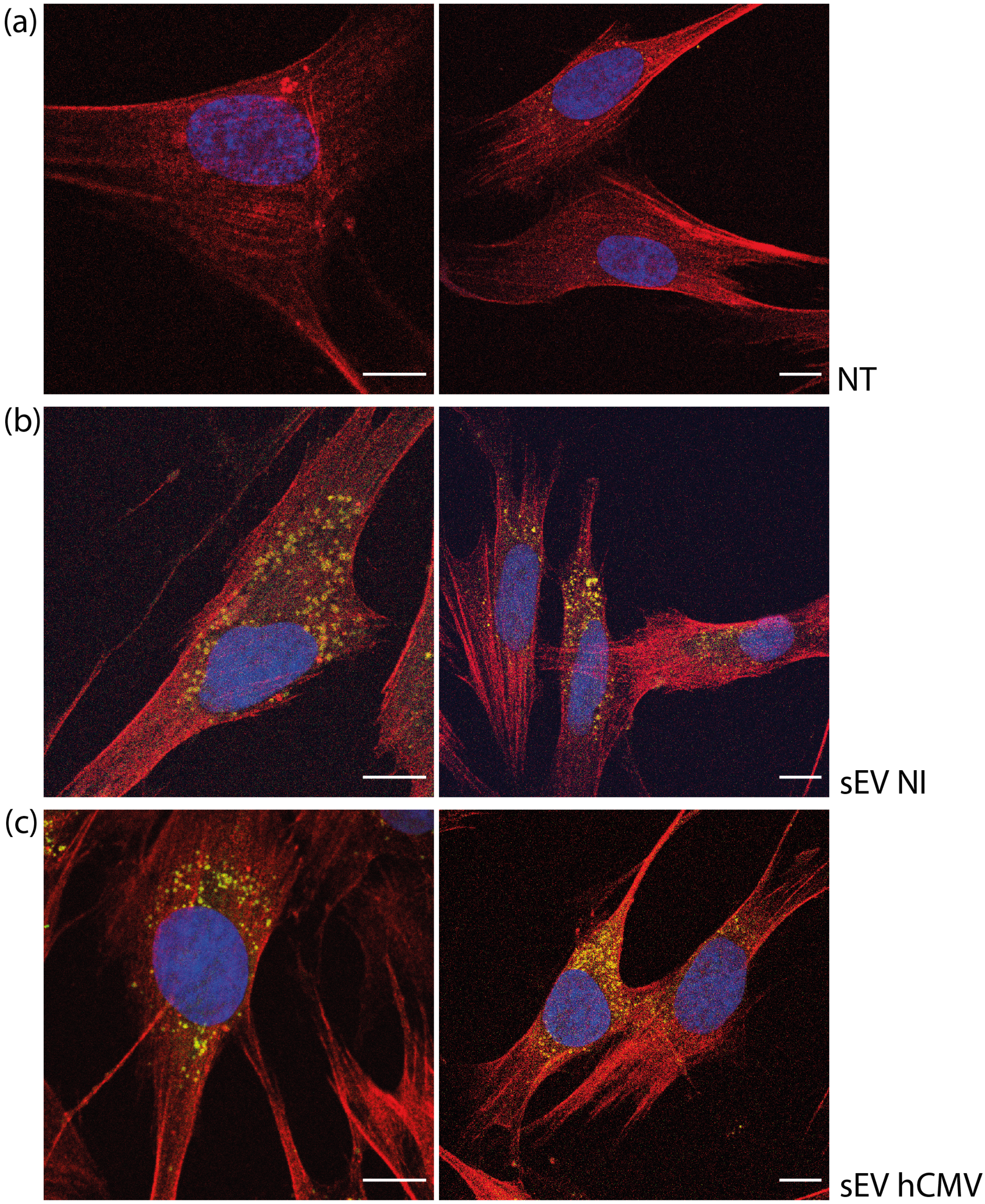
TEM micrographs showing sEV from HIPECs either non-infected (NI; upper panel) or infected (hCMV; lower panel), after immunogold labelling against CD63. Arrow in the lower panel indicates a CD63 positive sEV. Scale bar: 500 nm.

Figure S5: Complement to proteomic analysis.



Western-blot showing the enrichment of the cellular Thy-1 protein in sEVs from infected HIPECs (NI: non-infected). This result is representative of three independent experiments.

Figure S6: sEV internalization by MRC5 at 2 h post-incubation.



MCR5 cells, observed by confocal fluorescence microcopy, upon 2h of incubation with PKH67 stained sEVs (200 sEVs/cell) prepared from non-infected (panels b), hCMV-infected (panels c) or without sEV incubation (panels a). Green: PKH67 staining of sEV; red: actin staining by phalloidin; blue: DAPI. Scale bar: 10 μm.

Table S1: Excel file.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD029146.

Table S2: Bibliography references for establishment of Figure 2d

Protein name	Gene name	Reference	Link
Capsid scaffolding protein	UL80	Loveland *et al.* J. Virol. 2007	https://doi.org/10.1128/JVI.01903-06
Capside smallest protein	UL48/49	Yu *et al.* J. Virol 2005	https://doi.org/10.1128/JVI.79.2.1327-1332.2005
DNA polymerase processivity factor	UL44	Silva *et al.* Virology 2011	https://doi.org/10.1016/j.virol.2011.06.008
Early phosphoprotein p84	UL112	Schommartz *et al.* J. Virol. 2017	https://doi.org/10.1128/JVI.00254-17
Glycoprotein B	GB / UL55	Isaacson *et al.* J. Virol 2009 Halary *et al.* Immunity 2002	https://doi.org/10.1128/JVI.01251-08 https://doi.org/10.1016/S1074-7613(02)00447-8
Glycoprotein H	UL75	Yurochko et al. J.Virol 1997	https://doi.org/10.1128/jvi.71.7.5051-5059.1997
Glycoprotein M	GM	Mach *et al.* J.Virol. 2020 Liu *et al.* Sci. Adv. 2021	https://doi.org/10.1128/JVI.79.4.2160-2170.2005 https://doi.org/10.1126/sciadv.abf3178
Glycoprotein N	UL73	Mach *et al.* J. Virol. 2005 Shimamura *et al.* J. Virol. 2006	https://doi.org/10.1128/JVI.79.4.2160-2170.2005 https://doi.org/10.1128/JVI.80.9.4591-4600.2006
Glycoprotein UL16	UL16	Dunn *et al.* JEM 2003 Müller *et al.* PloS Pathog. 2010	https://dx.doi.org/10.1084%2Fjem.20022059 https://doi.org/10.1371/journal.ppat.1000723
IE1	UL123	Mocarski *et al.* PNAS 1993 Nevels et al. PNAS 2004	https://dx.doi.org/10.1073%2Fpnas.93.21.11321 https://doi.org/10.1073/pnas.0407933101
IE2	UL122	Castillo *et al.* J. Virol. 2000 Paulus *et al.* Viruses 2009	https://doi.org/10.1128/JVI.74.17.8028-8037.2000 https://doi.org/10.3390/v1030760
IRS1	IRS1/US22	Child *et al.* J. Virol 2004 Marshall *et al.* J. Virol 2009	https://doi.org/10.1128/JVI.78.1.197-205.2004 https://doi.org/10.1128/JVI.02489-08
Large tegument protein deneddylase	UL48	Kim *et al.* J. Virol. 2016	https://doi.org/10.1128/JVI.02766-15
Major capsid protein	UL86	Furhmann *et al. J.* Infect. Dis. 2008	https://doi.org/10.1086/587692
Nuclear egress protein 1	UL53	Salm *et al.* J. Virol. 2009	https://doi.org/10.1128/JVI.02441-08
pp150	UL32	Bogdanow *et al.* PNAS 2013 Tandon *et al.* J. Virol 2008	https://dx.doi.org/10.1073%2Fpnas.1312235110 https://doi.org/10.1128/JVI.00533-08
pp28	UL99	Silva *et al.* J. Virol. 2003	https://doi.org/10.1128/JVI.77.19.10594-10605.2003
pp65	UL83	Cristea *et al.* J. Virol. 2010 Tomtishen *et al.* Virol. J. 2012 Arcangeletti *et al.* J. Cell Biochem. 2011	https://doi.org/10.1128/JVI.00139-10 https://dx.doi.org/10.1186%2F1743-422X-9-22 https://doi.org/10.1002/jcb.22928
pp71	UL82	Kalejta *et al.* Mol. Cell Biol. 2003 Tomtishen *et al.* Virol. J. 2012	https://dx.doi.org/10.1128%2FMCB.23.6.1885-1895.2003 https://dx.doi.org/10.1186%2F1743-422X-9-22
pp85	UL25	Nobre *et al.* eLife 2019	https://doi.org/10.7554/eLife.49894
Protein UL88	UL88	Kumar *et al* . J. Virol. 2020 Kalejta MMBR 2008	https://doi.org/10.1128/JVI.00474-20 https://doi.org/10.1128/MMBR.00040-07
RIR1	UL45	Patrone *et al.* J. Gen. Virol. 2003	https://doi.org/10.1099/vir.0.19452-0
Tegument protein UL23	UL23	Feng *et al.* PloS Pathog. 2018 Fenge *et al.* Front. Microbiol. 2021	https://doi.org/10.1371/journal.ppat.1006867 https://dx.doi.org/10.3389%2Ffmicb.2021.692515
Protein UL51	UL51	Borst *et al.* J. Virol 2013	https://doi.org/10.1128/JVI.01955-12
Tegument protein US22	US22	Kalejta MMBR 2008	https://doi.org/10.1128/MMBR.00040-07
Tegument serine/threonine kinase	UL97	Krosky *et al.* J. Virol 2003 Hertel *et al.* PloS Pathog. 2007	https://doi.org/10.1128/JVI.77.2.905-914.2003 https://doi.org/10.1371/journal.ppat.0030006
Tegument UL35	UL35	Salsman *et al.* J. Virol. 2012 Fabits *et al.* Microorganisms 2020	https://doi.org/10.1128/JVI.05442-11 https://doi.org/10.3390/microorganisms8060790
Triplex capsid protein 1	UL46	Varnum *et al.* J. Virol 2004	https://doi.org/10.1128/JVI.78.20.10960-10966.2004
Triplex capsid protein 2	UL85	Varnum *et al.* J. Virol 2004	https://doi.org/10.1128/JVI.78.20.10960-10966.2004
TRS1	TRS1	Child *et al.* J. Virol 2004 Adamo *et al.* J. Virol 2004	https://doi.org/10.1128/JVI.78.1.197-205.2004 https://doi.org/10.1128/JVI.78.19.10221-10229.2004
UL37 immediate early glycoprotein	UL37	McCormick *et al.* J. Virol. 2003	https://doi.org/10.1128/JVI.77.1.631-641.2003