

## Electronic Supplementary Material

### Visualization of the Oncolytic Alphavirus M1 Life Cycle in Cancer Cells

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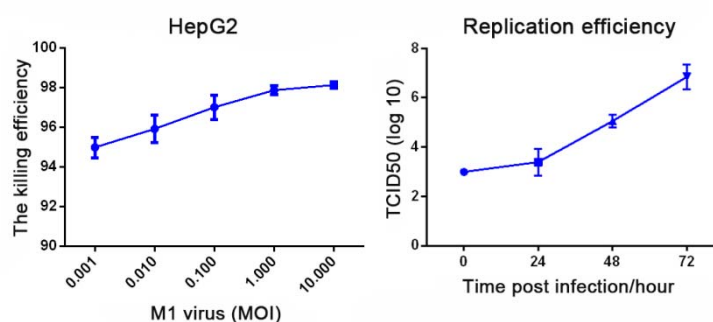
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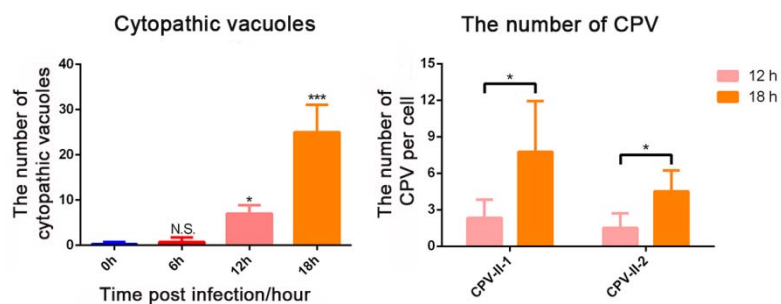
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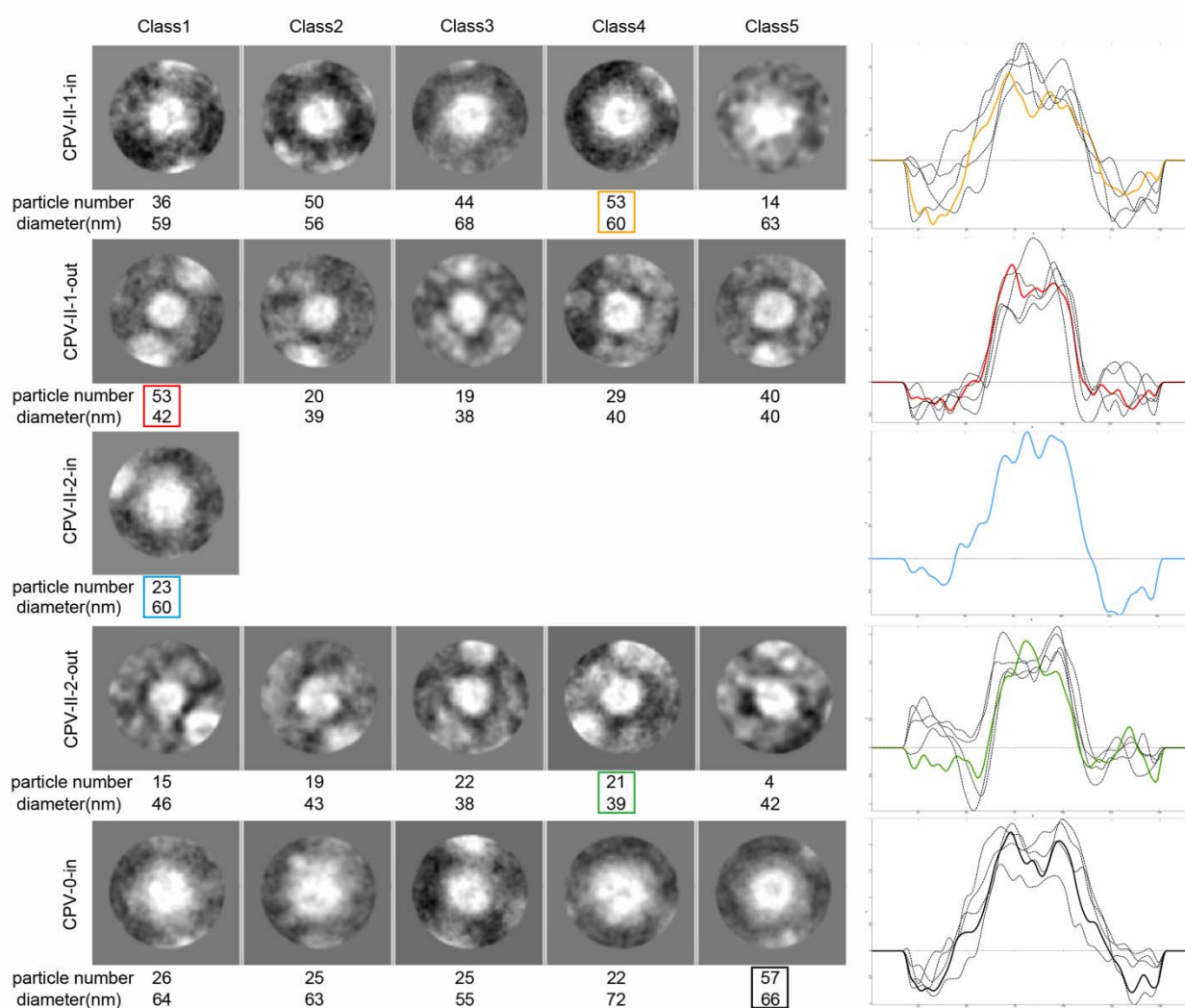
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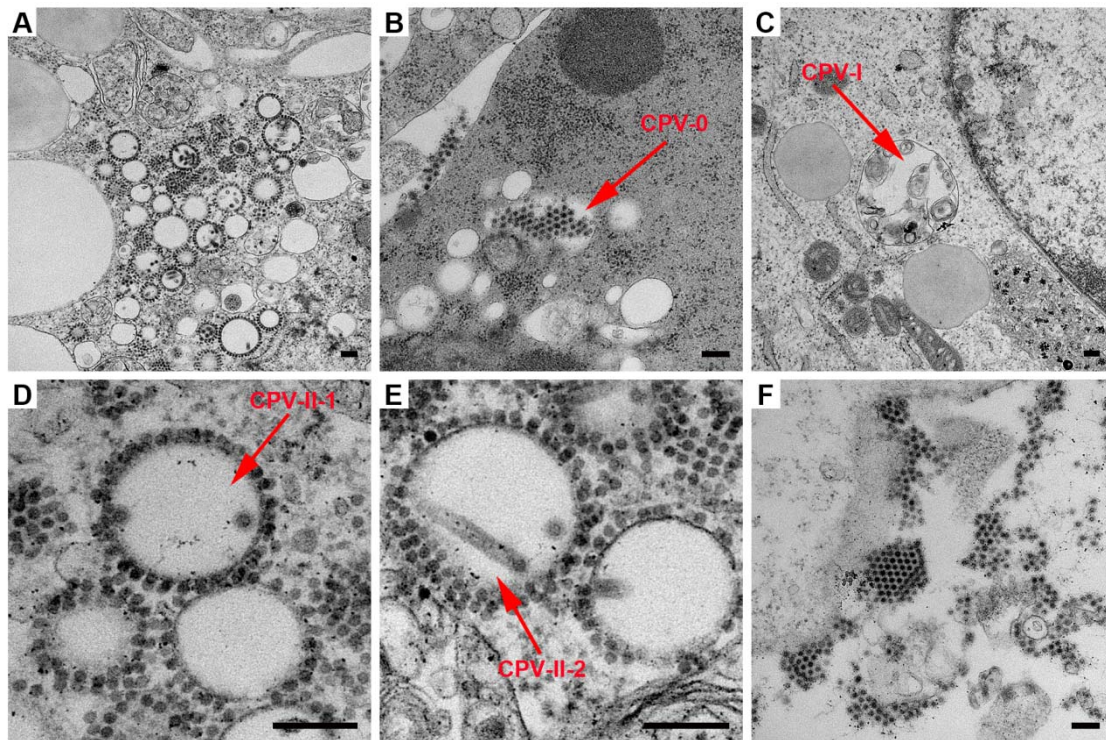
**Fig. S1** HepG2 cells constitute a tumor cell line highly sensitive to oncolytic M1 virus. **A** An MTT assay was performed on HepG2 cells exposed to different doses of M1 virus for 72 h: the killing efficiency = (1 – cell viability efficiency) × 100%. **B** An M1 virus replication efficiency assay was performed with HepG2 cells exposed to 0.001 MOI M1 virus infection, and the virus titers were determined at different infection times.



**Fig. S2** Statistical analysis of the number of vacuoles in the M1 virus-infected HepG2 cells. The number of vacuoles per cell (left) and the number of CPV-II-1 and CPV-II-2 per cell (right). Unpaired two-tailed Student's test: \* $P < 0.05$ , \*\*\* $P < 0.001$ . N.S.: non-significant.



**Fig. S3** The 2D classification of the progeny M1 virus particles. Representative 2D classifications of particles in different locations, with the number of particles, average diameters and radial density curves are shown.



**Fig. S4** Cytopathic changes in Vero cells caused by M1 infection. **A** M1 induced vacuolization of the Vero cells. **B-E** Representative morphologies of typical CPVs appeared in Vero cells. **F** Mature viral particles arranged in the form of lattice on the outside of the Vero cell membrane. Time point: 24 h. MOI: 1 pfu per cell. Bar: 200 nm.