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Supplementary Information for

Human cytomegalovirus glycoprotein B variants affect viral entry, cell fusion, and genome stability

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Figures S1 to S5

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Supplementary Information

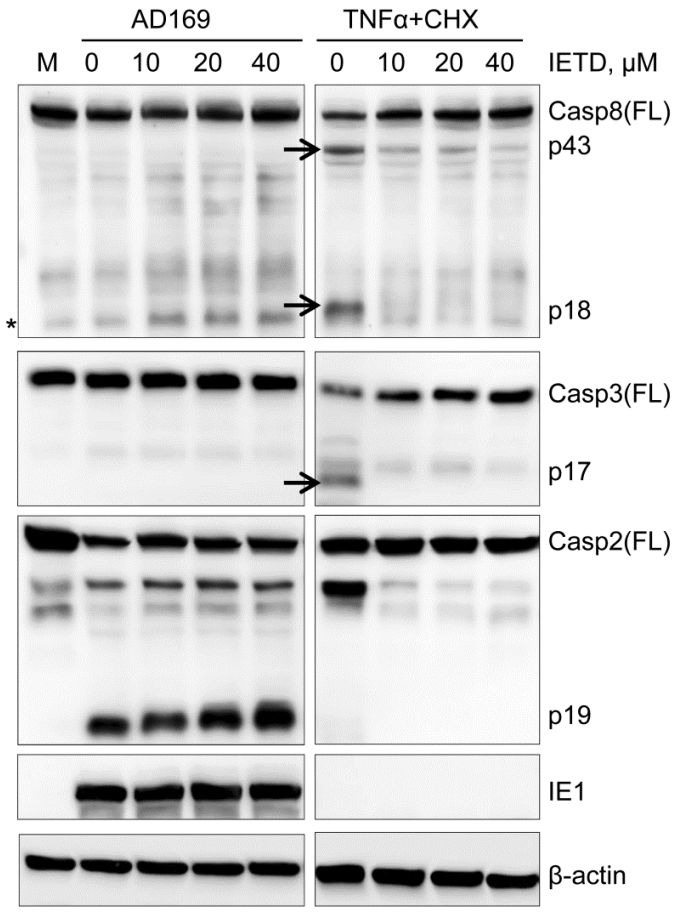


Fig. S1. Caspase-2 activation upon HCMV AD169 infection is independent of caspase-8. MRC-5 fibroblasts were pre-treated with caspase-8 inhibitor (Z-IETD-FMK) at the indicated concentrations. After 1 h, cells were either infected with AD169 (MOI 5) for 24 h or treated with 50 ng/ml TNFα + 50 μg/ml CHX for 9 h. Activation of caspase-8, caspase-3, and caspase-2 was analyzed by immunoblot analysis. IE1 and β-actin were used as infection and loading controls, respectively. Arrows show the active subunit of caspase-8 and caspase-3, * non-specific band.

A

	Main function(s)	Amino acid differences between other strains and AD169
UL32	Restricts HCMV gene expression to G0/G1	A85V
UL36	vICA, suppresses extrinsic apoptosis	C131R
UL69	Induces cells to accumulate in G1	Y61H
UL76	Induces DNA damage response	A211T, G234D, V308F, R319C
UL82	Stimulates quiescent cell to enter the cell cycle and arrests at G1/S	R464P
UL97	Disrupts APC, promotes G2/M progression, Releases E2F from Rb-E2F complex	D68N, V244I
UL117	Inhibits cellular DNA synthesis	V159A

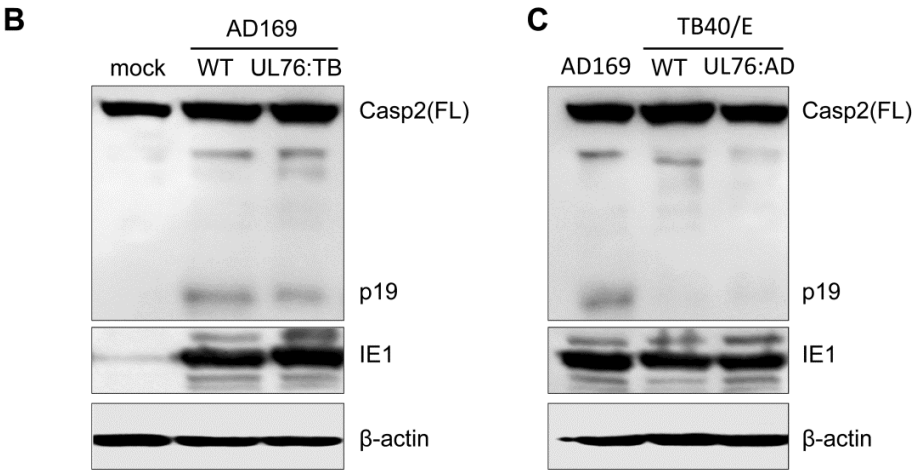
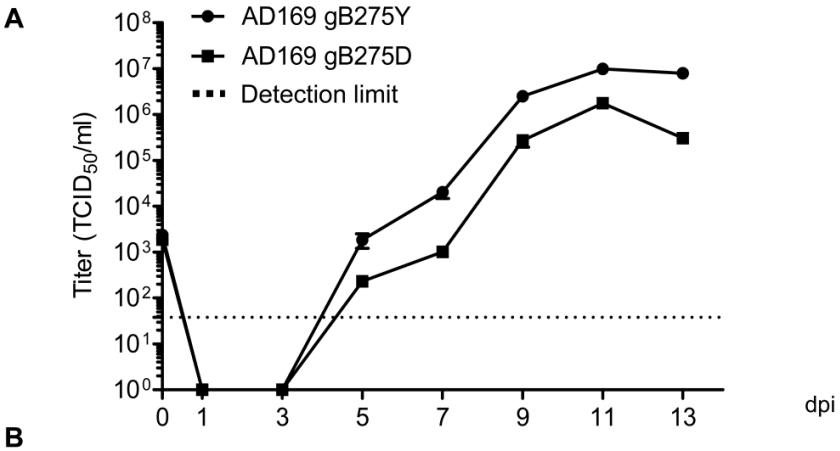


Fig. S2. Analysis of candidate genes. (A) Viral genes known to be involved in apoptosis, cell cycle regulation, or DDR were considered as candidate genes involved in caspase-2 activation if an AD169-specific variant existed. Candidate gene *UL76* was mutated at target region in both, AD169 **(B)** and TB40/E **(C)**. Their ability to activate caspase-2 was determined by immunoblot analysis.



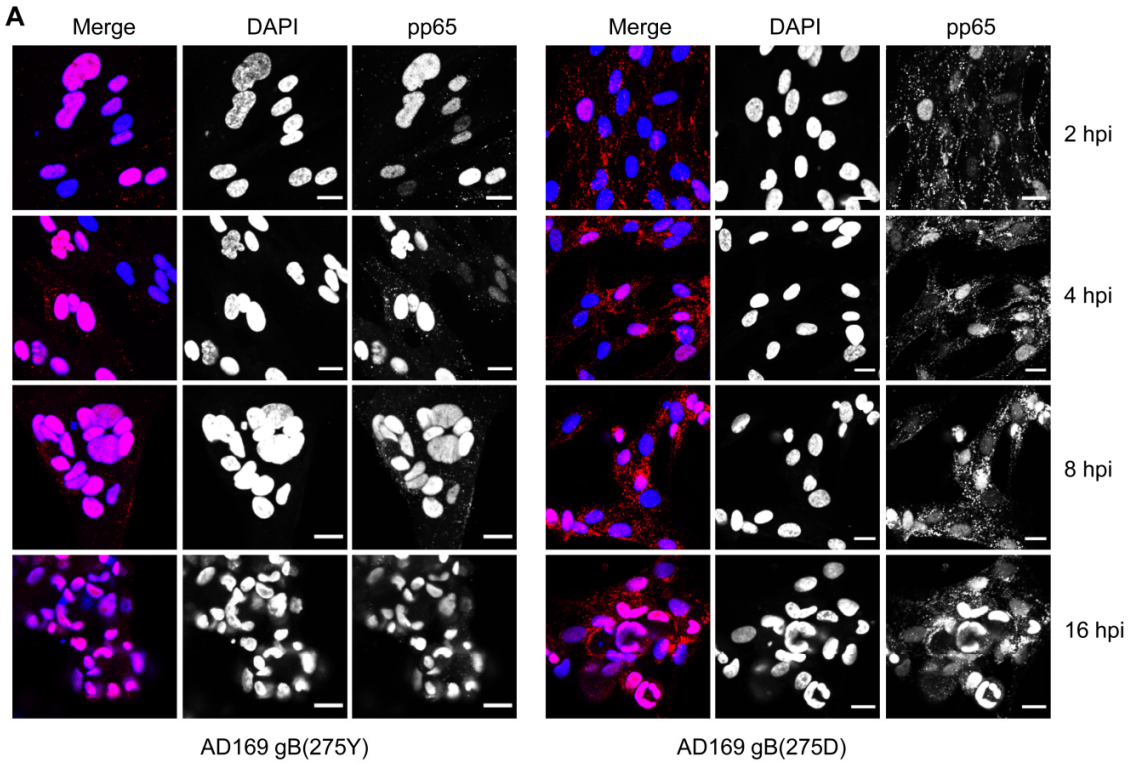
dpi	3	5	7	9	11	13
AD169 gB(275Y)	–	7	99	45	169	121
AD169 gB(275D)	–	51	365	443	696	4923

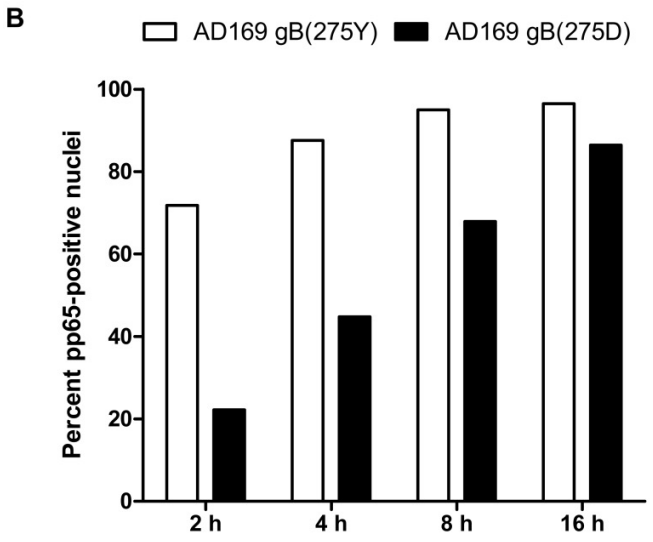
genomes / IU

C

	AD169 gB(275Y)	AD169 gB(275D)
Stock #1	82	1709
Stock #2	19	383
Stock #3	17	257
Average	39	783
Stock#4 (gradient purified)	56	5843

Fig. S3. Infectivity of viral particles. (A, B) To determine replication kinetics, MRC-5 cells were infected with AD169 gB(275Y) or AD169 gB(275D) at an MOI of 0.03 $TCID_{50}$/cell. Release of infectious virus into the supernatant was determined by titration **(A).** In parallel, viral genomes in the supernatant were quantified by qPCR **(B)**, Means ± SEM of triplicates are shown. Infectivity of viral particles was calculated as viral genomes per infectious unit (IU). **(C)** For each virus, genomes/IU ratios of 3 sucrose cushion-purified viral stocks and one glycerol-tartrate gradient-purified stock were determined by titration and qPCR. The average values of stocks #1 to 3 are shown.


A
Merge
DAPI
pp65
Merge
DAPI
pp65
2 hpi
4 hpi
8 hpi
16 hpi
AD169 gB(275Y)
AD169 gB(275D)


B
AD169 gB(275Y)
AD169 gB(275D)
Percent pp65-positive nuclei
100
80
60
40
20
0
2 h
4 h
8 h
16 h

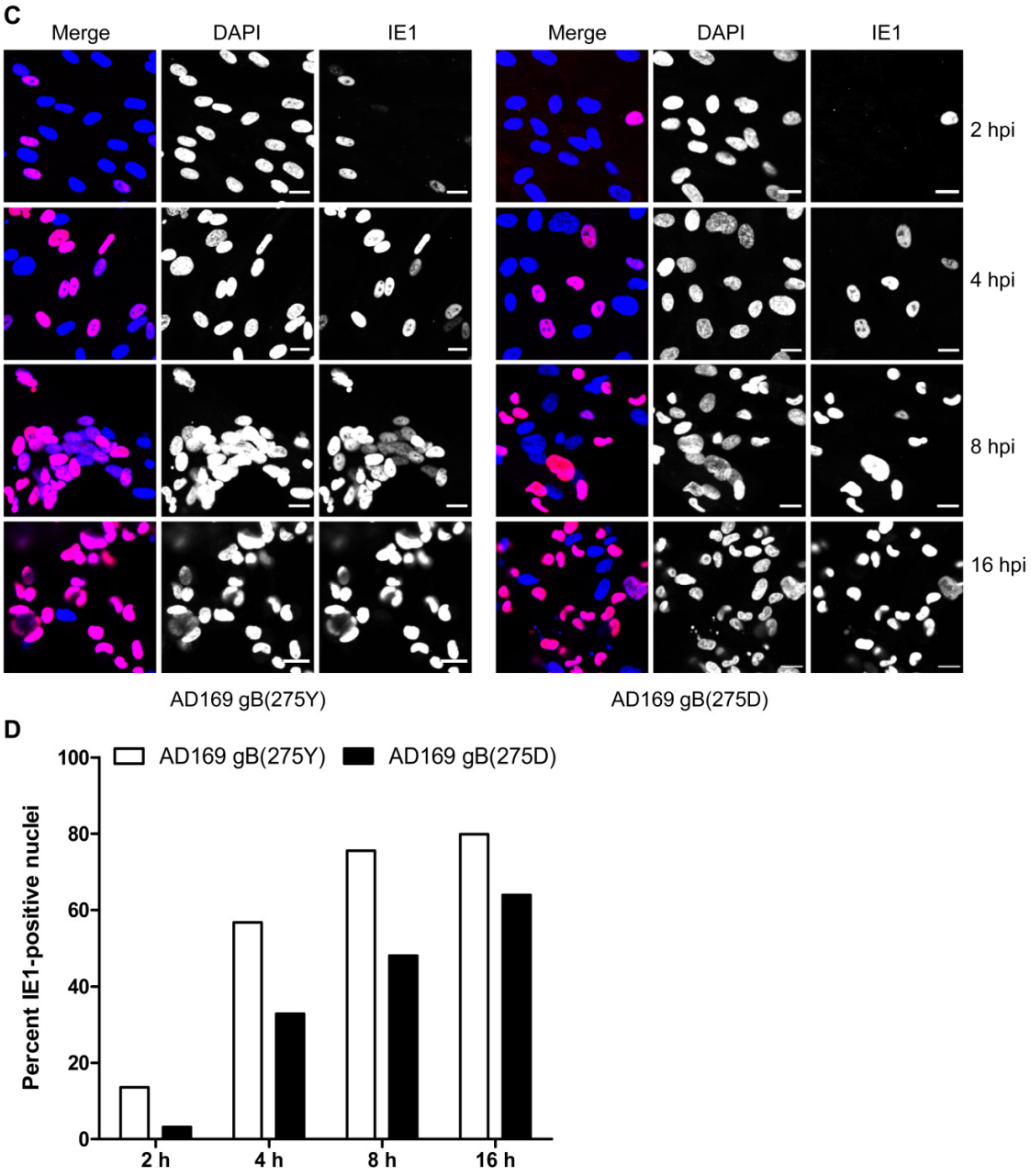


Fig. S4. Kinetics of pp65 nuclear translocation and IE1 expression. (A, C) MRC-5 fibroblasts were infected with AD169 gB(275Y) and gB(275D) at an MOI of 3. At the indicated times, cells were fixed and the viral tegument protein pp65 **(A)** and the viral IE1 protein **(C)** were detected by indirect immunofluorescence. Nuclei were counterstained with DAPI. Bar, 20 µm. **(B, D)** At least 300 nuclei were evaluated, and the percentages of pp65-positive **(B)** and IE1-positive **(D)** nuclei are shown.

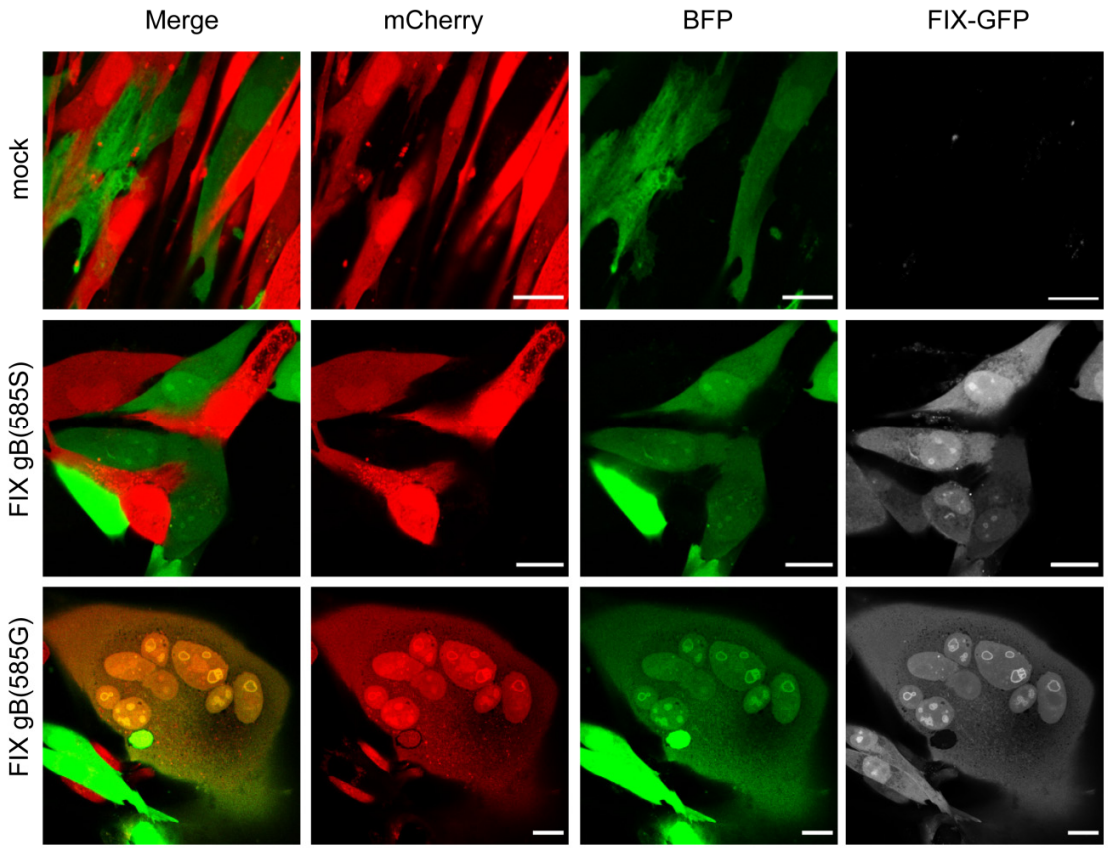


Fig S5. FIX gB(585G) promotes cells fusion. MRC-5 cells were transduced with retroviral vectors expressing BFP or mCherry, mixed equally, and infected with FIX-GFP gB(585S) or FIX-GFP gB(585G) (MOI 3). Cells were fixed 48 hpi and images were acquired using a Nikon A1+ LSM confocal microscope. For better visualization, BFP fluorescence is shown in green. Bar, 20 µm.