Fig. S1

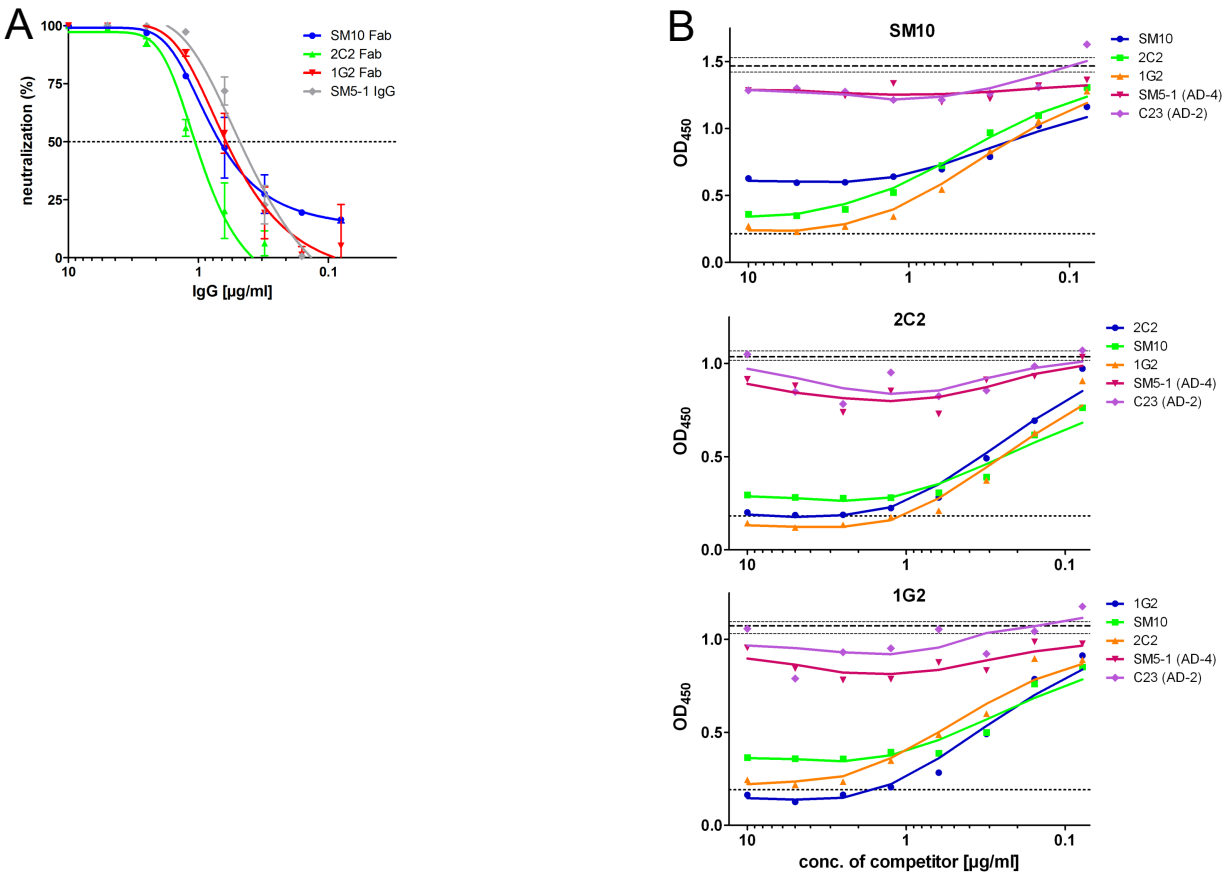

Fig S1. Biological characteristics of anti-AD-5 MAbs.
(A) Neutralizing activity of antibody Fab fragments towards HCMV strain AD169 tested on fibroblasts. Neutralizing activity was assessed in duplicate on HFF and infectivity was quantified as a measure of luciferase activity (relative light units) 48 h post infection. Percent neutralization was calculated relative to the controls. (B) Competition of anti-AD-5 MAbs for gB-binding in ELISA. An ELISA was performed with 25 ng soluble gB as antigen. Antibodies were added in serial $\log_2$ dilutions starting at 10 µg/ml followed by incubation with the respective biotinylated anti-AD-5 MAb as indicated above the graph. Binding of biotinylated antibody was detected by HRP-conjugated streptavidin. C23 (AD-2-specific) and SM5-1 (AD-4-specific) were used as control antibodies for no competition and the respective non-biotinylated anti-AD-5 MAb as a control for competition. The thick dashed lines represent the OD_{450} value and the thin dashed lines the range of the biotinylated antibody at a constant concentration (n=6) in the absence of competing antibody. The lower dotted line gives the background. Concentrations of biotinylated antibody were 0.45, 0.13 and 0.12 µg/ml for SM10, 2C2 and 1G2, respectively.

Fig. S2

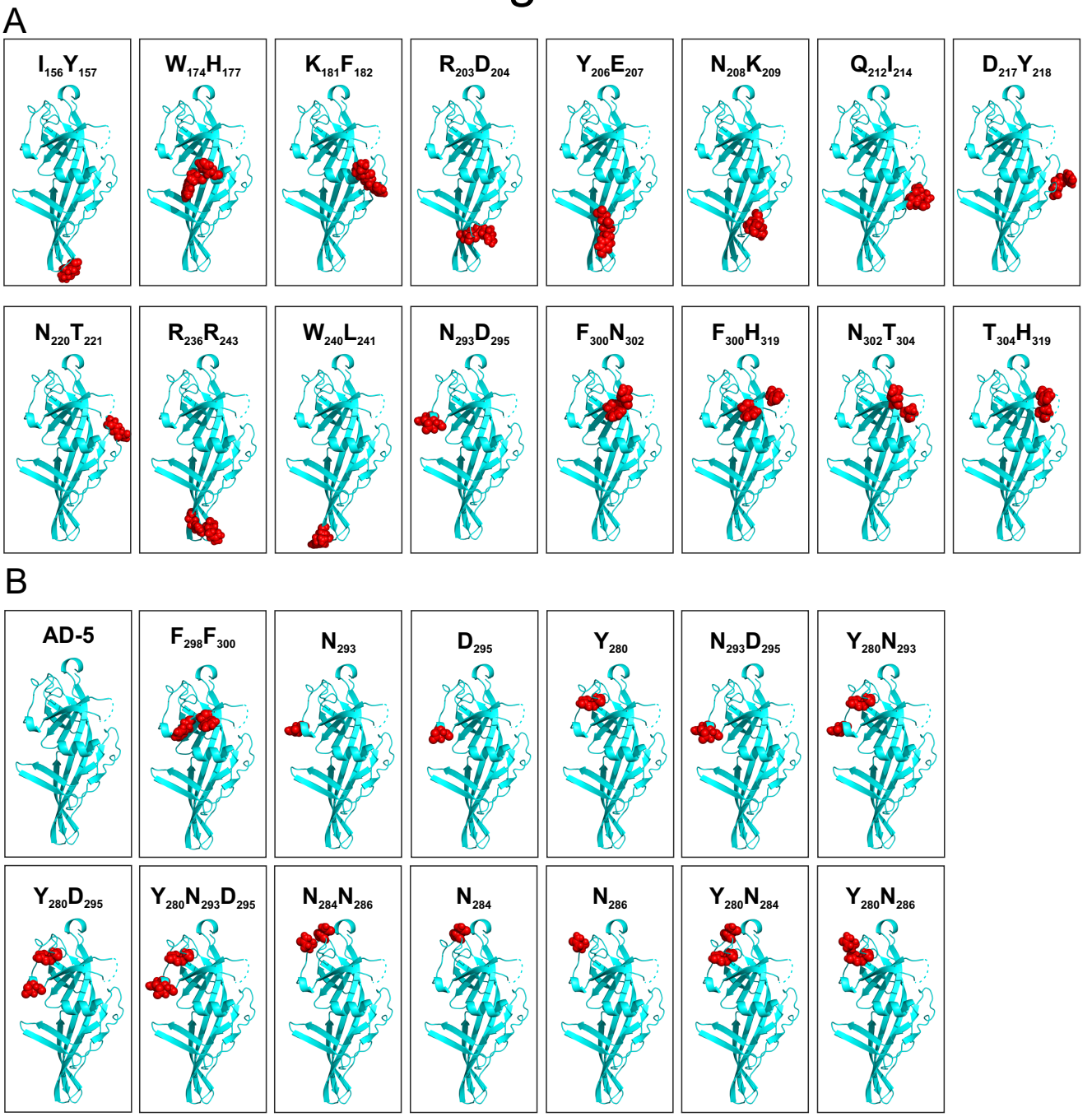

Fig S2. Structural models of the AD-5 wt and mutant proteins.
AD-5 is displayed as a ribbon diagram and residues exchanged to alanine are highlighted as red spheres. (A) In set 1 of AD-5 mutants two surface exposed residues in close proximity were exchanged to alanine per construct. All mutants have an N-terminal HA tag and a C-terminal myc tag connected to the protein by a 5×GS-spacer. (B) Set 2 of mutants was constructed in analogy to set 1 but additionally including residues P_{116} to V_{440} of gB (AD-4 of HCMV gB) for protein stability (only AD-5 depicted for simplicity). Residues exchanged to alanine were selected based on the results of the first set.

Fig. S3

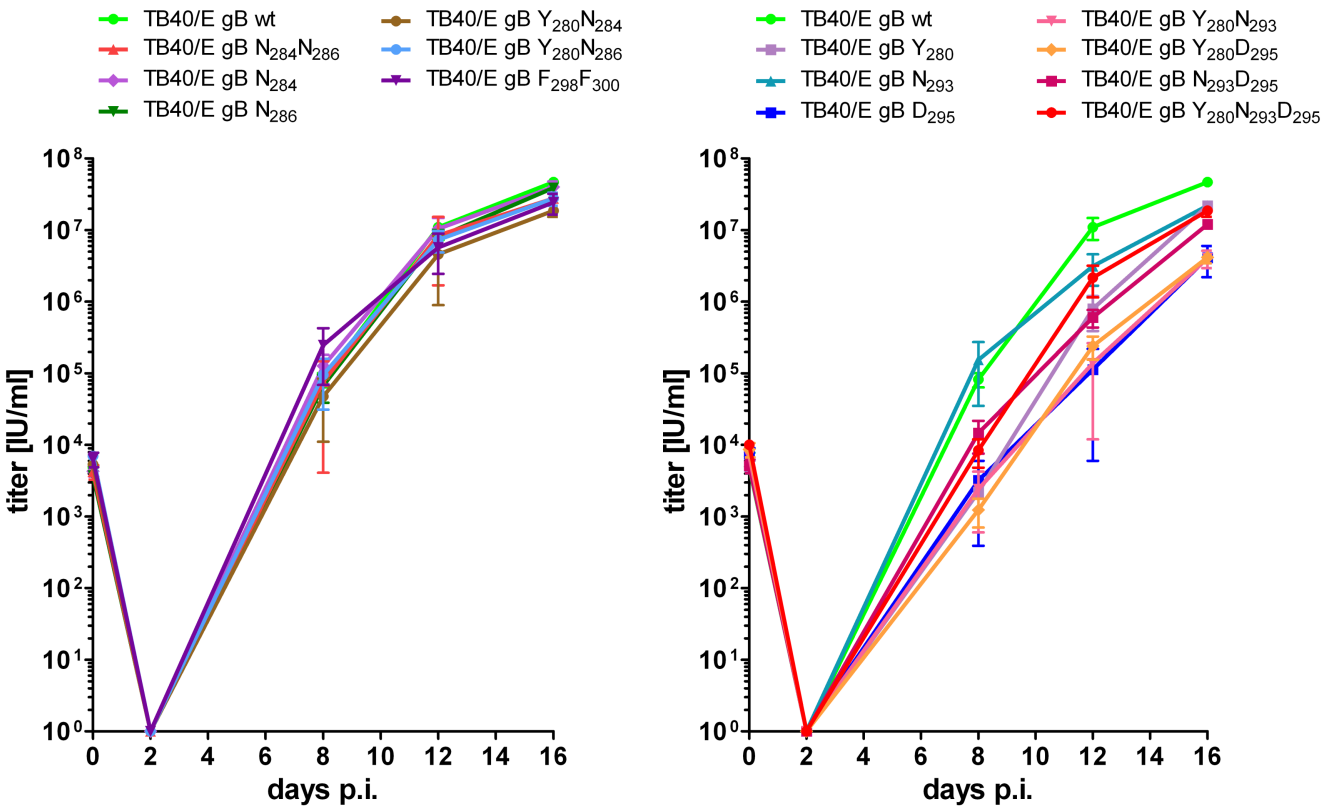

Fig S3. Growth kinetics of AD-5-mutated recombinant viruses.
HFF monolayers were spin inoculated for 30 min at 1,900×g with AD-5-mutated recombinant viruses at a MOI of 0.3-0.04. The inoculum was removed 4 h later, cells were washed with PBS and cultured in fresh medium. Cell culture supernatants were harvested on day 2, 8, 12 and 16 post infection (p.i.). To determine virus titers, HFF monolayers were infected in duplicate with $\log_2$ dilution titrations of the cell culture supernatants or a reference virus stock and 48 h later luciferase activity was measured. The titer of the reference virus stock was determined prior to the experiment by immunostaining of IE1 as described elsewhere (1) and confirmed twice. Titers in infectious units per ml (IU/ml) were calculated as a function of the reference virus stock. The mean (±SD) of two independent experiments is given. Replication curves of recombinant viruses are seperated in two panels for clarity.

Reference:
(1) Andreoni M, Faircloth M, Vugler L, Britt WJ. 1989. A rapid microneutralization assay for the measurement of neutralizing antibody reactive with human cytomegalovirus. J. Virol. Methods 23:157–167.

Fig. S4

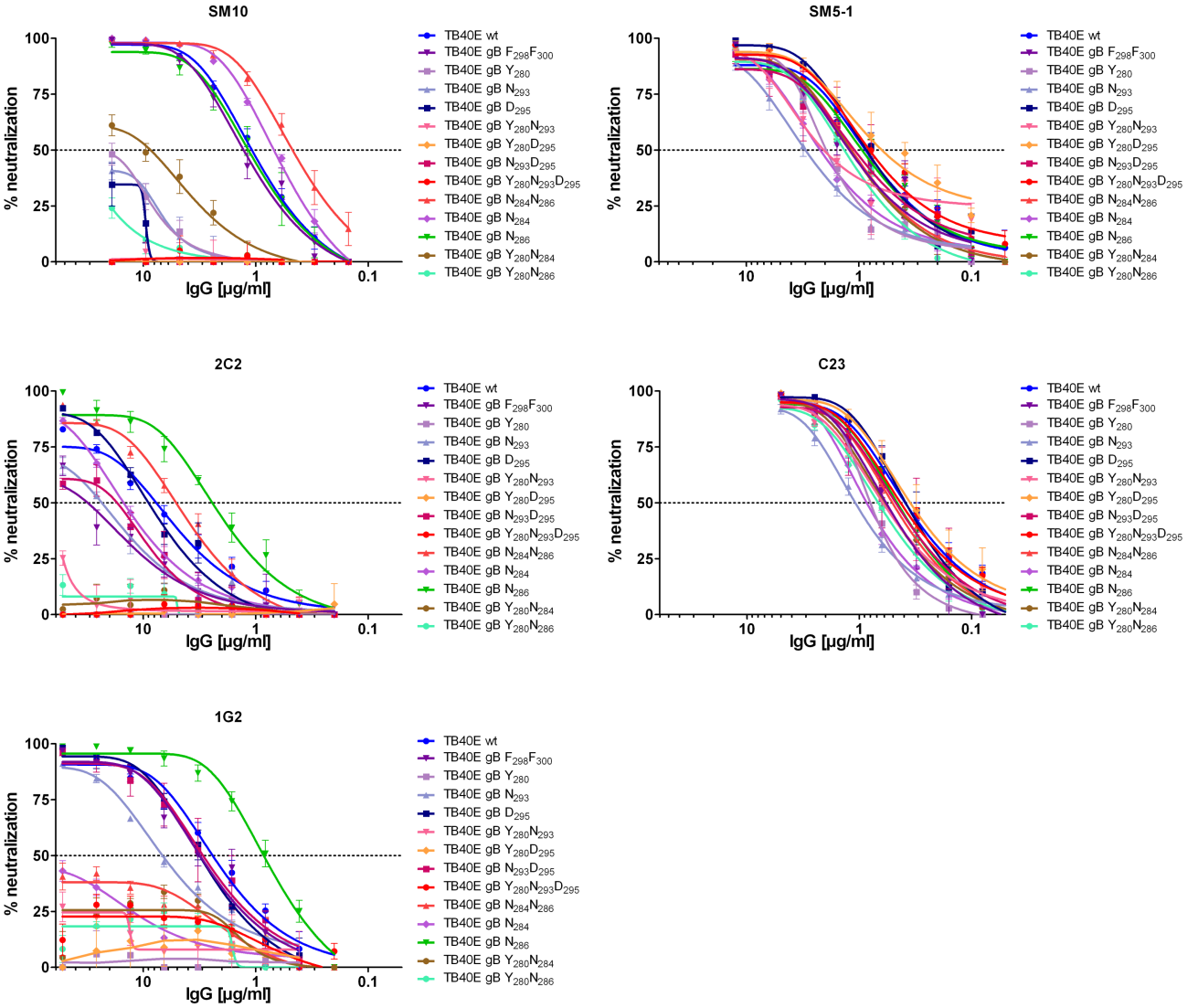

Fig S4. Neutralization capacity of anti-AD-5 MAbs against viral mutants.
Recombinant viruses harboring mutations in AD-5 were incubated with the indicated AD-5-specific MAbs for 1 h and HFF were infected with the virus-antibody mixture. Medium was changed 4 h after infection and 48 h later the extent of infection was quantified as a measure of luciferase activity. Percent neutralization was calculated as a function of the control wells treated with no virus or no antibody.

Fig. S5

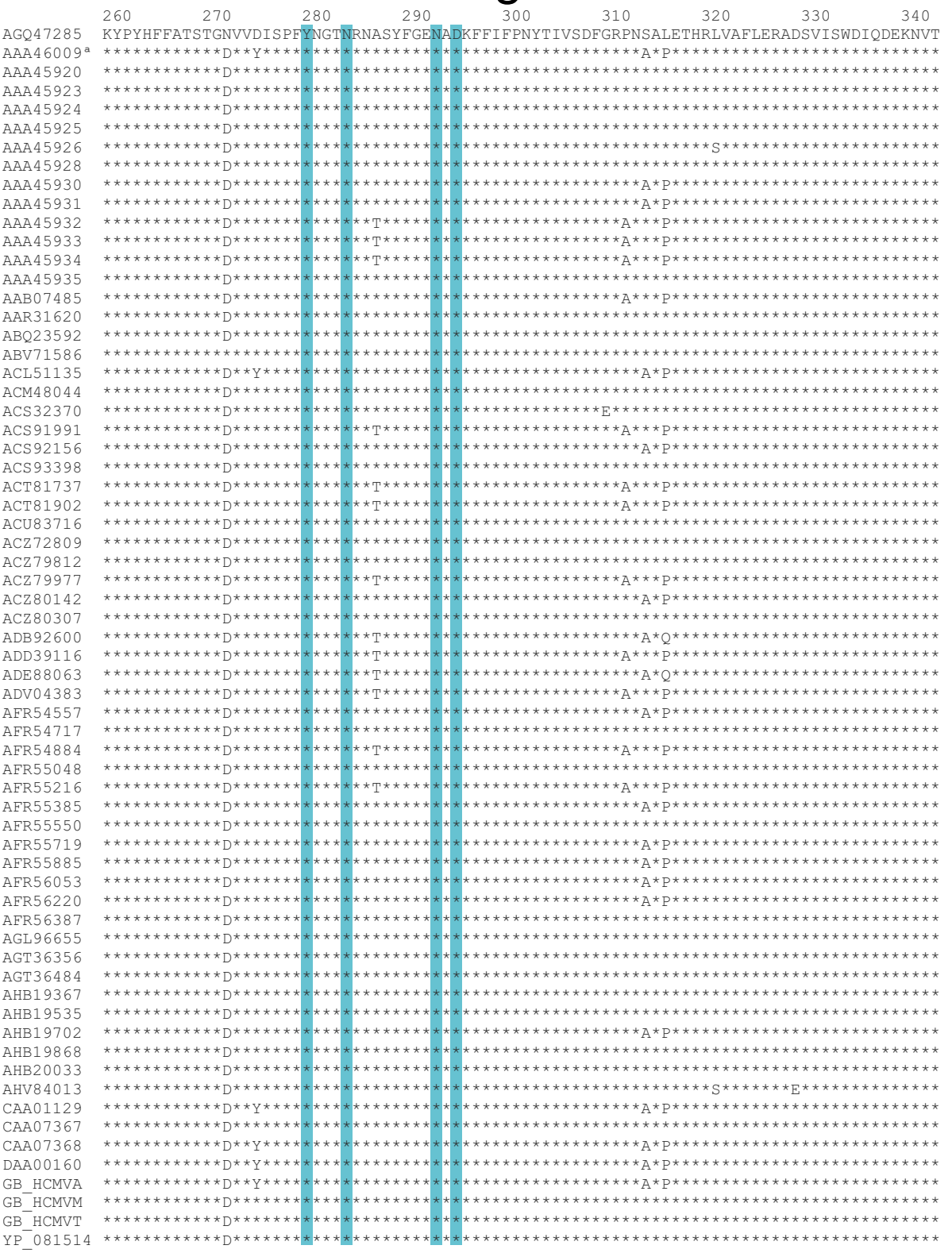

Fig S5. Alignment of HCMV gB sequences.

The amino acid sequence of the carboxyterminal part of AD-5 from residues E_{260} to T_{343} of HCMV gB were retrieved from databases and aligned to TB40/E as reference (accession numbers are given on the left). The residues Y_{280}, N_{284}, N_{293} and D_{295} are highlighted in blue. [a]HCMV strain AD169

Fig. S6

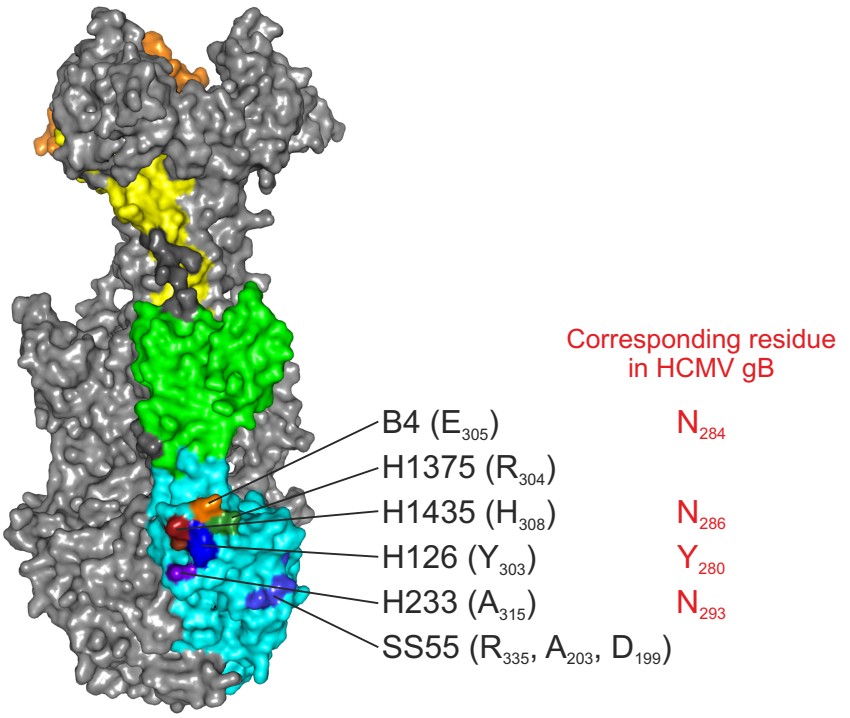

Fig S6. Epitopes of murine neutralizing MAbs in HSV-1 gB.
The HSV-1 gB trimer is depicted as accessible surface area representation (1). The epitopes of MAbs B4, H1375, H1435, H126, H233 (2) and SS55 (3) are located within structural domain I and the residues critical for virus neutralizing activity as determined by monoclonal antibody resistant (mar) mutant viruses are given in brackets.

References:
(2) Heldwein EE, Lou H, Bender FC, Cohen GH, Eisenberg RJ, Harrison SC. 2006. Crystal structure of glycoprotein B from herpes simplex virus 1. Science 313:217–220.

(3) Cairns TM, Fontana J, Huang ZY, Whitbeck JC, Atanasiu D, Rao S, Shelly SS, Lou H, Ponce de Leon M, Steven AC, Eisenberg RJ, Cohen GH. 2014. Mechanism of neutralization of herpes simplex virus by antibodies directed at the fusion domain of glycoprotein B. J Virol 88:2677-89.

Fig. S7

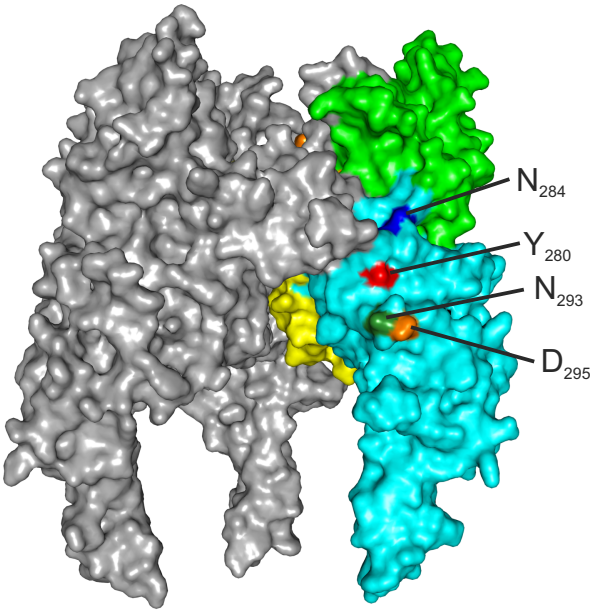

Fig S7. Hypothetical model of the prefusion conformation of HCMV gB based on the crystal structure of VSV G.
The critical residues of the YNND epitope are indicated in accordance with Fig. 7.