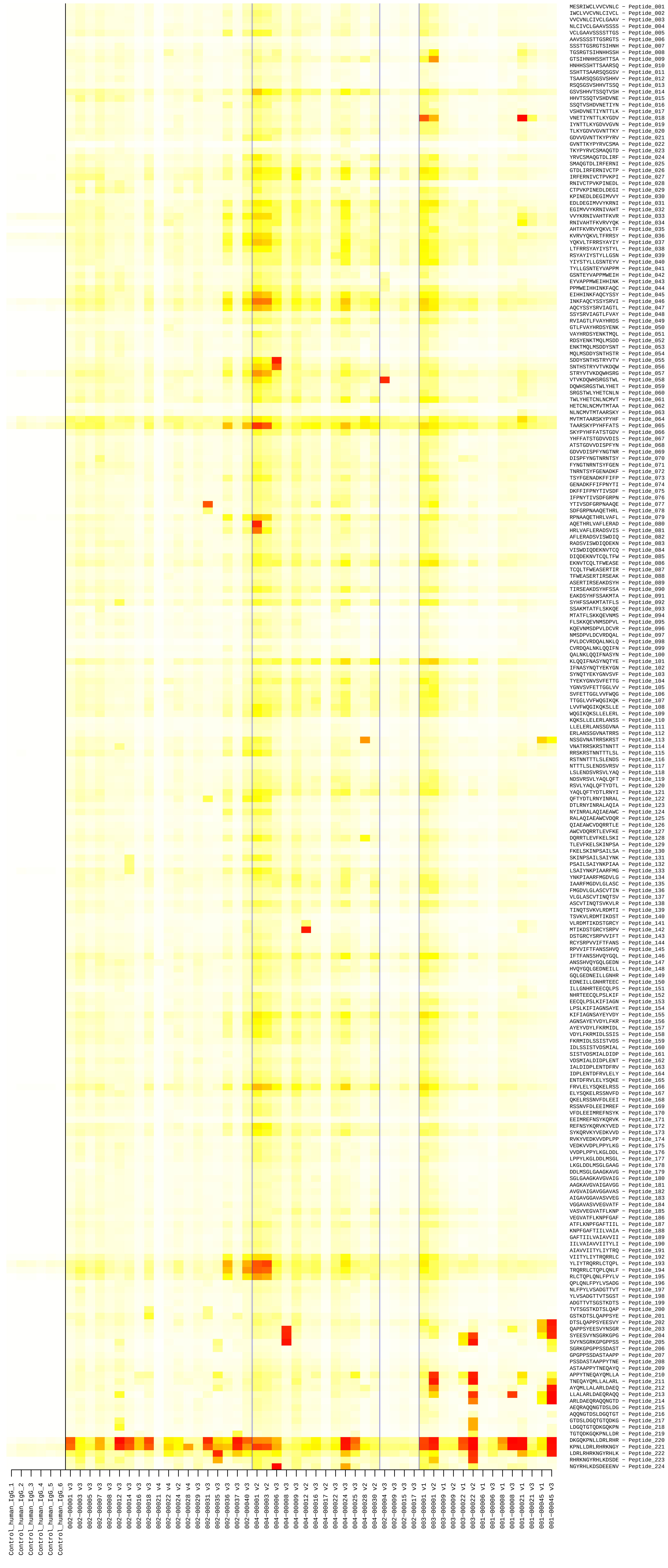
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001–00021 v3
001–00045 v1
001–00045 v3

Figure S1 Breadth of the humoral response against linear epitopes of gB in vaccinated individuals. Representative heatmap representing antibody binding across the entirety of gB protein in sera from seronegative vaccine sera and seropositive control sera. White is no binding, Yellow is medium binding and red represents high binding. AD6 is located between peptide 162 and peptide 173 inclusive. For full dataset please supplementary dataset 1. 002-00001 to 002-00040 and 004-00001 to 004-00030 represent HCMV seronegative individuals receiving 1-2 doses of gB/MF59 vaccine. 002-0004 to 002-00017 represent HCMV seronegative patients receiving placebo. 003-00001 to 001-00045 represents HCMV seropositive patients receiving gB/MF59 vaccine. V1 refers to pre-vaccine sample and v2 onwards is post vaccine.

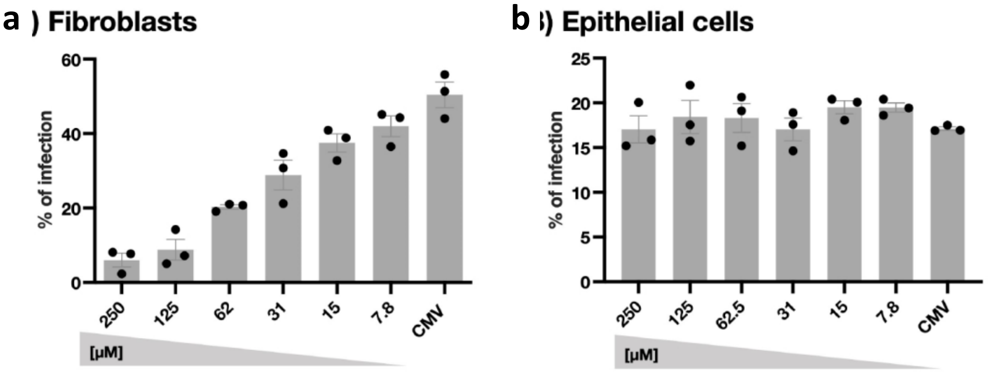

Figure S2 Full length AD-6 polypeptide blocks CMV infection in fibroblasts but not in epithelial cells. **a)** HFF cells were infected with Merlin (MOI:2) pre-incubated with AD-6 ($gB_{(648-697)}$) with concentrations ranging from 250µM to 3.9µM. **b)** ARPE-19 cells were infected with TB40e (MOI:3) pre-incubated with AD6 at concentrations ranging from 250µM to 7.8µM pre-treated with AD-6 prior to infection of ARPE-19. Infection was measured as a percentage of IE-positive 20h post infection. Bars represent mean + SEM of technical replicates and results are representative of 3 independent experiments.

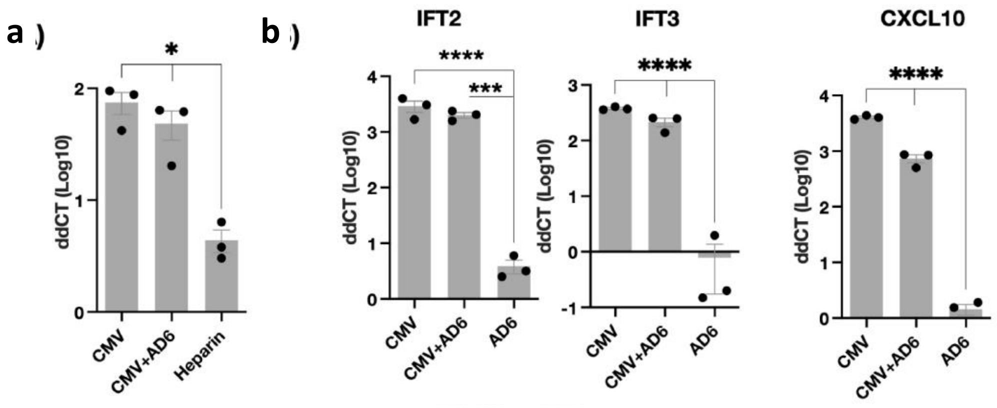

Figure S3 Impact of AD-6 polypeptide on post-attachment events during virus entry. **a)** Virus attachment to cells was measured in the presence or absence of AD-6 or Heparin. CMV Merlin strain (MOI:2), polypeptides (250µg/mL) or controls were inoculated on HFF for 90 minutes at 4^{o}C and washed to remove unbound virions. Virion attachment was measured by the amount of viral DNA in the sample. Data expressed as UL138 Ct values normalized to housekeeping gene 18s. **b)** HFF cells infected with CMV UV treated (Merlin, MOI=2), AD-6 or CMV pre-treated with AD-6. 6hpi, RNA was isolated and mRNA levels of CXCL10, IFTI2, IFIT3 were measured by RT-qPCR (mean + SEM, n=3). Data expressed as fold change normalized to housekeeping gene 18s relative to mock treated cells. *P* values were calculated by a Kruskal-Wallis test with Dunn's multiple-comparison test where appropriate. ****, $P < 0.0001$; ***$P < 0.001$; *, $P < 0.01$; ns (nonsignificant), $P > 0.05$. For a and b the error bars represent 1 standard deviation from the mean.

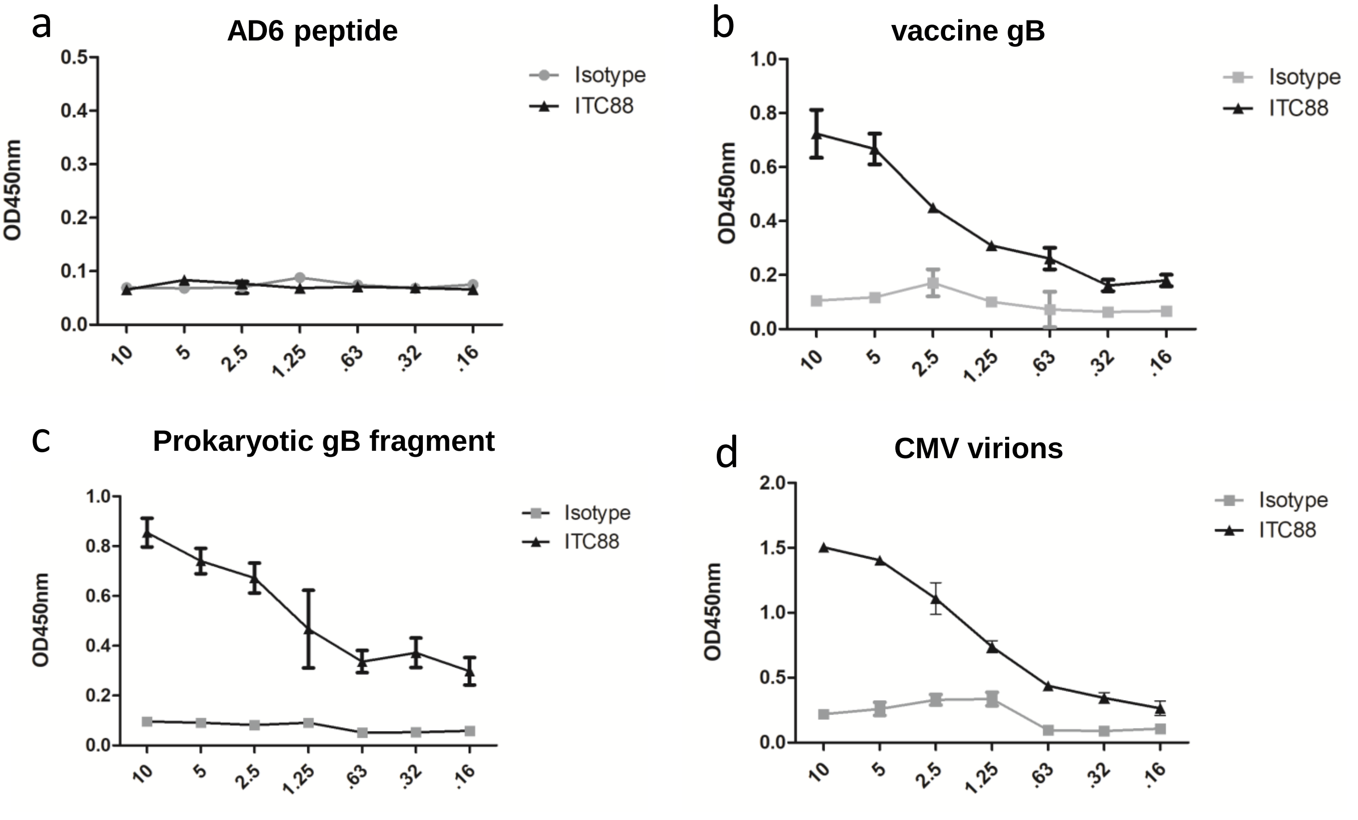

Figure S4 AD2 binding antibody, ITC88, binds recombinant gB, vaccine gB and virion gB a-d) An anti-AD2 monoclonal antibody (ITC88) or mouse isotype control were assayed by ELISA for binding to AD-6 polypeptide (a), vaccine gB (b), a commercial recombinant gB fragment produced in *E. coli (*c) or CMV virions (d). Antibody levels represented in function of absorbance (450nm) per concentration of primary antibody in mg/mL and is representative of 2 independent experiments. Each data point represents mean + SEM.

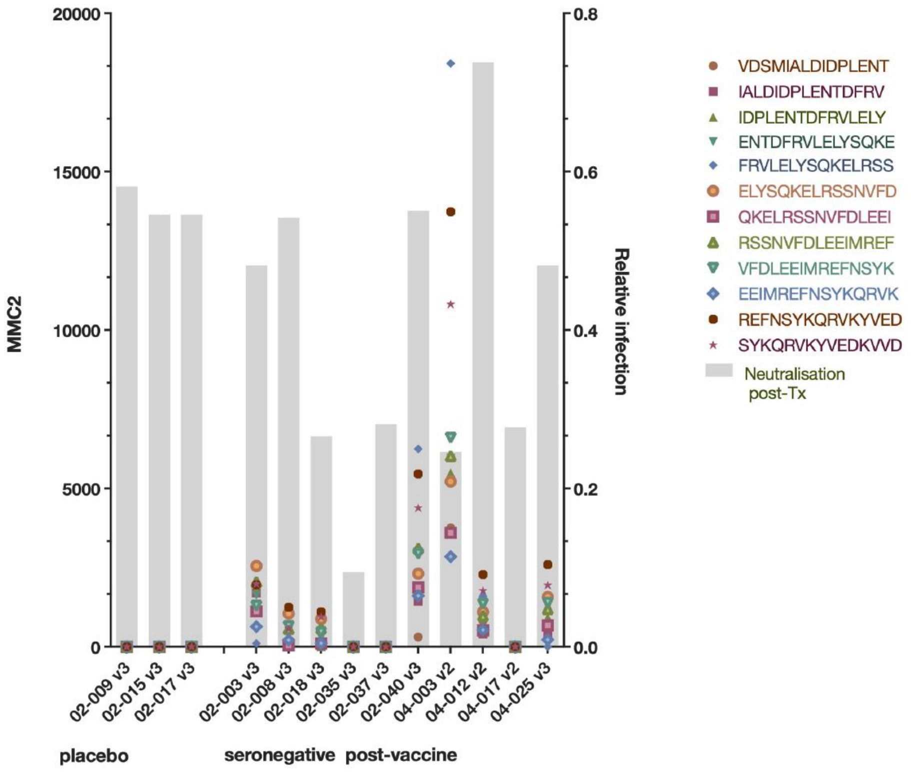

Figure S5 Presence of neutralising gB antibodies post-transplant does not correlate with AD-6 antibody responses pre-transplant. Anti-AD-6 IgG levels post-immunisation in seronegative vaccine recipients by array (left Y-axis – symbols) and capacity of post-transplant sera to neutralise CMV infection represented as reduction in the percentage of infected cells relative to mock-treated virus (right Y-axis; grey bars) were plotted together to compare neutralising activity with presence of antibody responses to epitopes within AD-6.

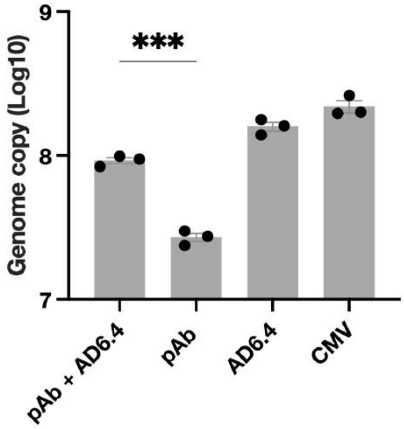

Figure S6 Anti-AD-6 antibodies reduce cell-associated spread in fibroblasts. HFFs were infected with BAC derived Merlin-IE2-GFP (MOI = 0.01). Then, at 1 dpi and at 5 dpi, anti-AD-6 antibody was added at the indicated concentrations except for some conditions the anti-AD-6 antibody was pre-adsorbed with shorter peptide spanning the C-terminus of AD-6, herein named AD-6.4 ($gB_{(685\text{-}699),}$ Towne) at a 10x molar excess prior to addition to infected cells 1 dpi and at 5 dpi. Then, at 10 dpi. total DNA was harvested, and CMV genome copies per 10^6 cells assessed by qPCR ($n = 2$). *P* values were calculated by a two tailed unpaired t test. ***, $P < 0.000194$. The error bars represent 1 standard error from the mean.