Supplementary Information

Targeting of antigen to the herpesvirus entry mediator augments primary adaptive immune responses

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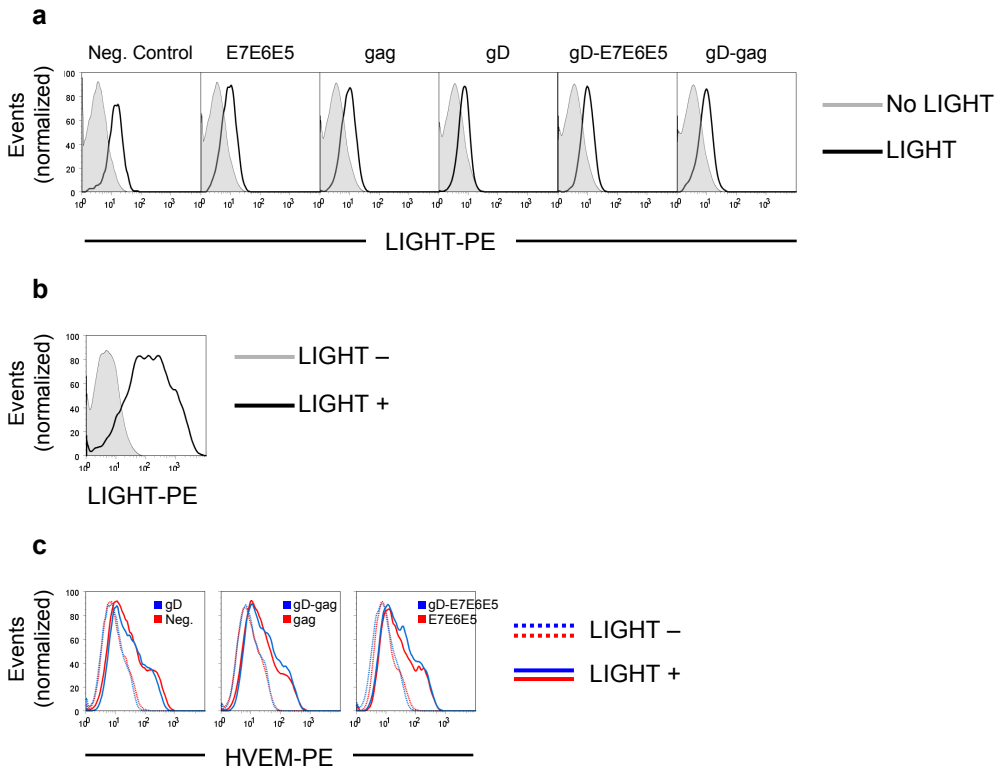
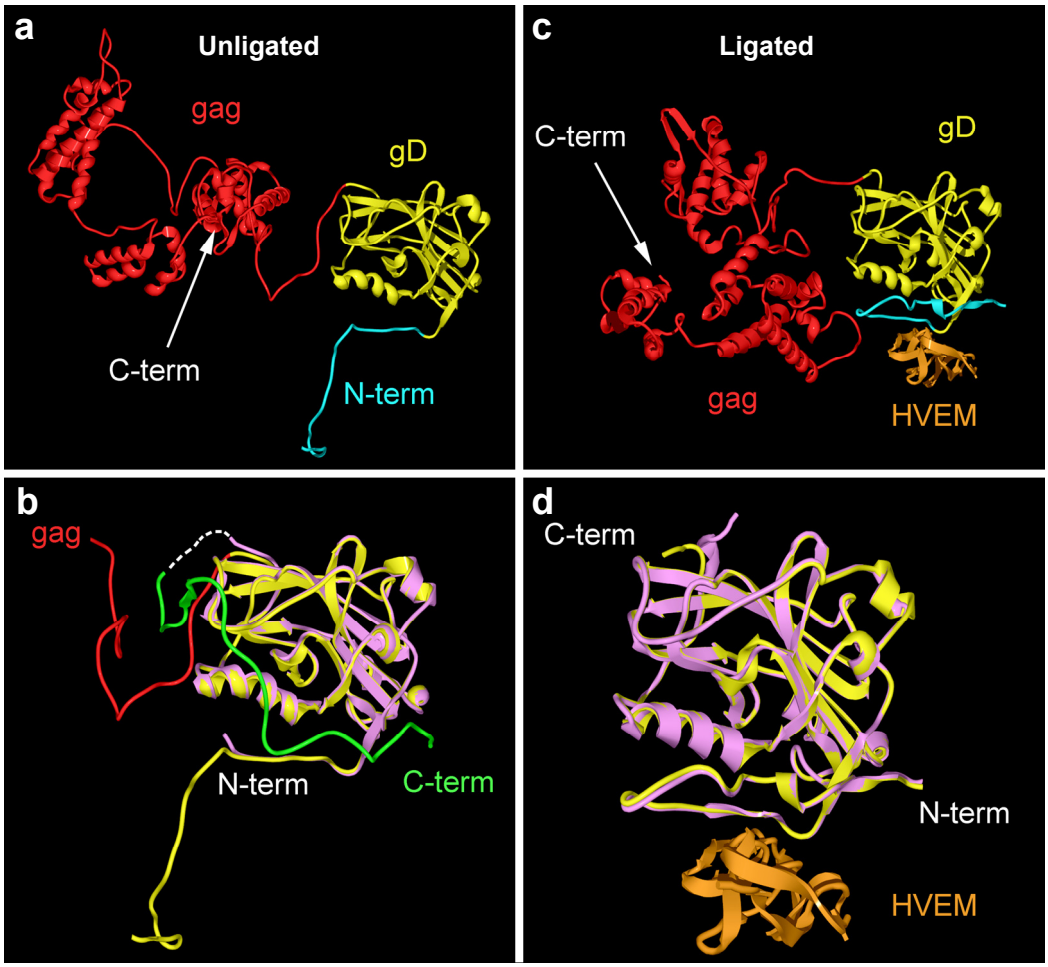
Supplementary Figure 1



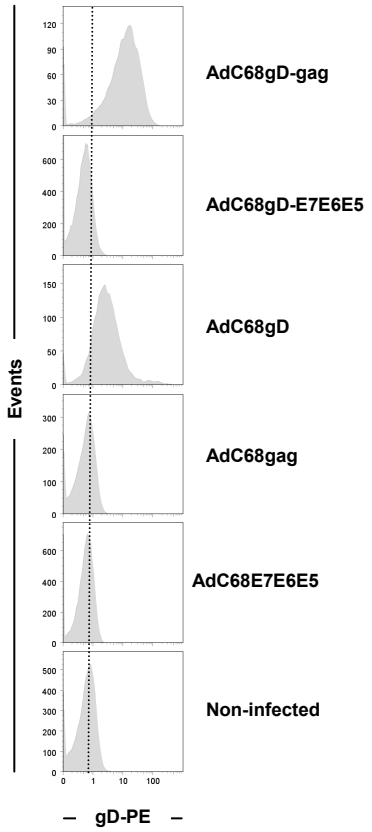
Supplementary Figure 1. Chimeric gD proteins do not affect LIGHT binding to HVEM. (**a**). HVEM$^+$ B78-H1/3E5 cells were pre-treated with protein extracts from CHO/CAR cells infected with AdC68 vectors as described in legend to **Fig 1d**. Cells were washed and purified hLIGHT was added (Black lines), followed by hLIGHT-specifc MAb and mouse-specific Ig conjugated to PE. Gray filled lines represent cells non-treated with hLIGHT. Extracts from uninfected cells were used as negative controls. (**b**) Expression of hLIGHT on membrane of 293T cells transfected with either a pcDNA3.1(+) vector carrying hLIGHT or an empty vector was assess by indirect immunofluorescence with an antibody to hLIGHT. (**c**) The effect of binding of HVEM to gD or gD chimeric proteins on its ability to bind to cell-bound LIGHT was tested on the same batch of LIGHT transfected (solid lines) and sham-transfected (dashed lines) 293T cells shown in **Supplementary Fig. 1b**. LIGHT transfected cells or sham-transfected cells were treated with mixtures of HVEM pre-treated with normalized extracts containing gD or gD chimeric proteins (blue lines) or the appropriate control extracts (red lines). The amount of bound HVEM was determined by indirect immunofluorescence with a polyclonal antibody to hHVEM.

Supplementary Figure 2



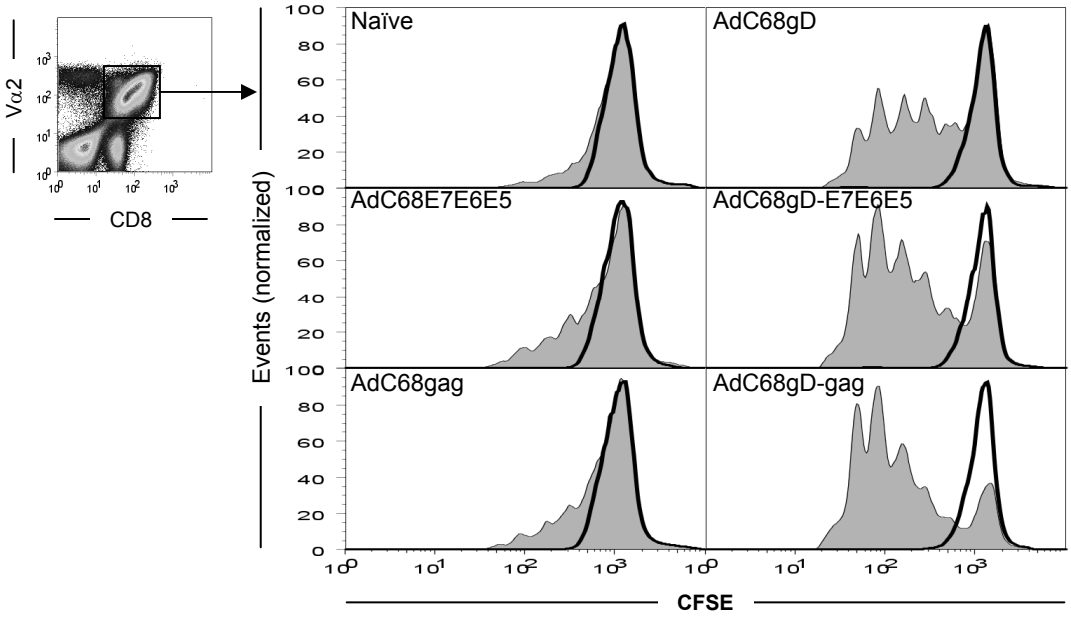
Supplementary Figure 2. Molecular modeling of the gD-gag chimeric protein. Ribbon representations of gD-gag in the unligated (**a, b**) and HVEM ligated (**c, d**) conformations. (**a**) The gag insertion (red) is connected to the gD ectodomain core (yellow) by a long flexible linker. (**b**) A superposition of the native gD X-ray structure (2C36) (purple) and that of the gD-gag chimera model (yellow) shows that the gag insert (red) repositions the C-terminus of native gD (green) away from the HVEM binding pocket. The dashed line indicates an 11 residue gD loop segment that like the first 22 N-terminal residues (not shown) is unresolved in the X-ray structure and presumed to be highly flexible[13]. (**c**) gD-gag chimera model in the HVEM ligated conformation (colored as in **a**) with HVEM (orange) positioned as observed in the gD-HVEM complex X-ray structure (1JMA). The gD N-terminus (blue) changes conformation upon formation of the HVEM complex. (**d**) A superposition of the native gD X-ray structure (2C36) (purple) with the gD-gag chimera model (yellow) in the HVEM (orange) bound conformation shows that the gag insert does not disrupt the gD core domain.

Supplementary Figure 3



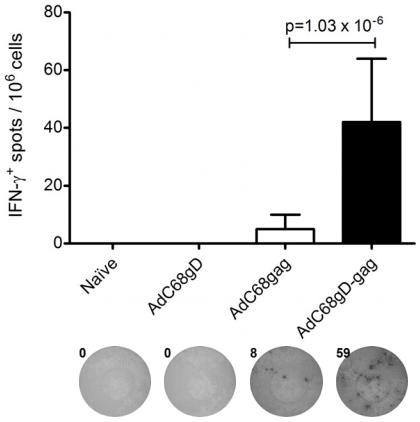
Supplementary Figure 3. gD protein binds to HVEM in trans. Presence of gD on the surface of non-infected HVEM^{+} cells co-cultivated with HVEM^{-} cells infected with AdC68gD-gag, AdC68gD-E7E6E5, AdC68gD, AdC68E7E6E5 or AdC68gag was evaluated. Infected CHO/CAR (HVEM^{-}) cells were cultured with B78H1/3E5 (HVEM^{+}) cells for 48 hours. Presence of gD on surface of B78H1/3E5 cells was tested by indirect immunofluorescence. Non-infected CHO/CAR cells were used as control. The dotted line shows the peak immunofluorescence of the non-infected control cells.

Supplementary Figure 4



Supplementary Figure 4. Antigen-presenting cells transduced with AdC68 vectors expressing gD induce enhanced antigen-driven proliferation of $CD8^+$ T cells *in vitro*. Cells isolated from lymph nodes of naïve mice and mice immunized with AdC68E7E6E5, AdC68gag, AdC68gD, AdC68gD-E7E6E5 or AdC68gD-gag were irradiated and incubated with CFSE-labeled $CD8^+V\alpha2^+$ cells from OT-1 mice for 72 hours. Total live cells for each combination were analyzed for expression of $CD8^+V\alpha2^+$ (an example for gating is shown on the left). CSFE expression by double positive cells (highlighted by the square in the left graph) is shown in the graphs on the right. SIINFEKL (filled gray line) peptide or an unspecific peptide (black line) were used for stimulation. The degree of CFSE-labeled $CD8^+$ OT-1 cell proliferation was defined as reduction of CFSE staining below that of 99.5% of double positive cells stimulated with the unspecific peptide. Percentages of the populations that had 1 or more cycles of replications are as follows for cells incubated with antigen-presenting cells from naïve mice: 8%; AdC68E7E6E5-immune mice: 19%; AdC68gag-immune mice: 16%; AdC68gD-immune mice: 47%; AdC68gD-E7E6E5-immune mice: 65%; AdC68gD-gag-immune mice: 76%.

Supplementary Figure 5



Supplementary Figure 5. Gag-specific $CD4^+$ T cell responses to AdC68gD-gag. ELISpot assay for IFN-γ was carried out with splenocytes from BALB/c mice immunized with AdC68gD, AdC68gag or AdC68gD-gag re-stimulated *in vitro* with a $H\text{-}2^d$ MHC class II binding gag peptide (NPPIPVGELIY). Naive mice were used as controls. Graphs show numbers of IFN-γ spots per 10^6 splenocytes. Differences between numbers of spots obtained with cells from AdC68gag and AdC68gD-gag immune mice are statistically significant (p=1.03 x 10^{-6}). Bottom panel shows one representative well of the ELISpot assay from each group.

Supplementary Table 1. List of oligonucleotides used in this work.

Name[a]	*Sequence (5'-3')*
E7FwApaI	GCTGTAGGGCCCCATGGAGATACACCTAC
E7RvNarI	CATGGTGGCGCCTGGTTTCTGAGAACAG
E6FwNarI	AGACATGGCGCCCACCAAAAGAGAACTGC
E6RvNotI	CTCCATGCGGCCGCCCAGCTGGGTTTCTCTACG
E5FwNotI	GACAAAGCGGCCGCCTGCATCCACAACATTAC
E5RvApaI	ACATATGGGCCCTGTAATTAAAAAGCGTGC
E7FwHindIII	GGGTGGAAGCTTATGGGAGATACACCTAC
E5RvHindIII	TGGGGCAAGCTTTTAAATTAAAAAGCGTGC
gDFwXbaI	CCCTAGTCTAGAATGGGGGGGGCTGCCGCC
gDRvXbaI	CCCTAGTCTAGACTAGTAAAACAAGGGCTGGTG
gag37FwNarI	AAGAAGGGCGCCGGTGCGAGAGCGTCAG
gag37RvNarI	AAGGGTGGCGCCCAAAACTCTTGCCTTATGGC
gDFwHindIII	AAGCCCAAGCTTATGGGGGGGGCTGCCGCC
gDRvHindIII	AAGCCCAAGCTTCTAGTAAAACAAGGGCTGGTG
NBEFgDRv	GACCGGAAGGTCTTTGCCGCGAAAGCGAGCGGGGTCGGCCGCCTTGAG
NBEFgDFw	CGCTTTCGCGGCAAAGACCTTCCGGTCGCGGACGCGGCGGCCGCCCC
SgDFw	CAAATCCAACAAAACGCGCACATAGGCTCGATCC
SgDRv	GATCGACGGTATGTGCGCGTTTGGTGGGATTTGC
LIGHTFwBamHI	CGCGGATCCATGGAGGAGAGTGTCGTA
LIGHTRvEcoRI	CGCGAATTCACACCATGAAAGCCCCGAAGT

a. Fw, forward. Rv, reverse.

Supplementary Table 2. List of vectors used.

Vector name	Gene encoded	Vaccine carrier
pgD[a]	gD	DNA vaccine
pE7E6E5	E7, E6, and E5[e]	DNA vaccine
pgD-E7E6E5	gD, E7, E6, and E5	DNA vaccine
pNBEFgD-E7E6E5	NBEFgD[f], E7, E6, and E5	DNA vaccine
pSgD-E7E6E5	gDW294A[g], E7, E6, and E5	DNA vaccine
pgD-E7[b]	gD, and E7	DNA vaccine
pSgD-E7	gDW294A[g], and E7	DNA vaccine
pgag[c]	gag37[h]	DNA vaccine
pgD-gag	gD, and gag37	DNA vaccine
AdC68E7	E7	E1-deleted adenovirus vector, chimpanzee serotype 68
AdC68gD	gD	E1-deleted adenovirus vector, chimpanzee serotype 68
AdC68E7E6E5	E7, E6, and E5	E1-deleted adenovirus vector, chimpanzee serotype 68
AdC68gD-E7E6E5	gD, E7, E6, and E5	E1-deleted adenovirus vector, chimpanzee serotype 68
AdC68gag[d]	gag37	E1-deleted adenovirus vector, chimpanzee serotype 68
AdC68gD-gag	gD, and gag37	E1-deleted adenovirus vector, chimpanzee serotype 68

a. pgD vector was previously described as pRE4[31].
b. pgD-E7 vector was described previously[22].
c. pgag vector is pcDNA3.1(+) carrying gag37.
d. AdC68gag was described before[26].
e. E7, E6, and E5 genes are from HPV-16 expressed as fusion polypeptides.
f. NBEFgD, gD gene was genetically modified by replacing 7 amino acids at the N-terminus i.e., M11, N15, L25, Q27, L28, T29 and D30, replaced alanine residues; this modified gD does not bind HVEM.
g. SgD, gD was genetically altered by replacing tryptophan (W) in position 294 to alanine (A). This modified form of gD shows increased binding to HVEM[13].
h. gag37 is a truncated form of gag of HIV-1[19].