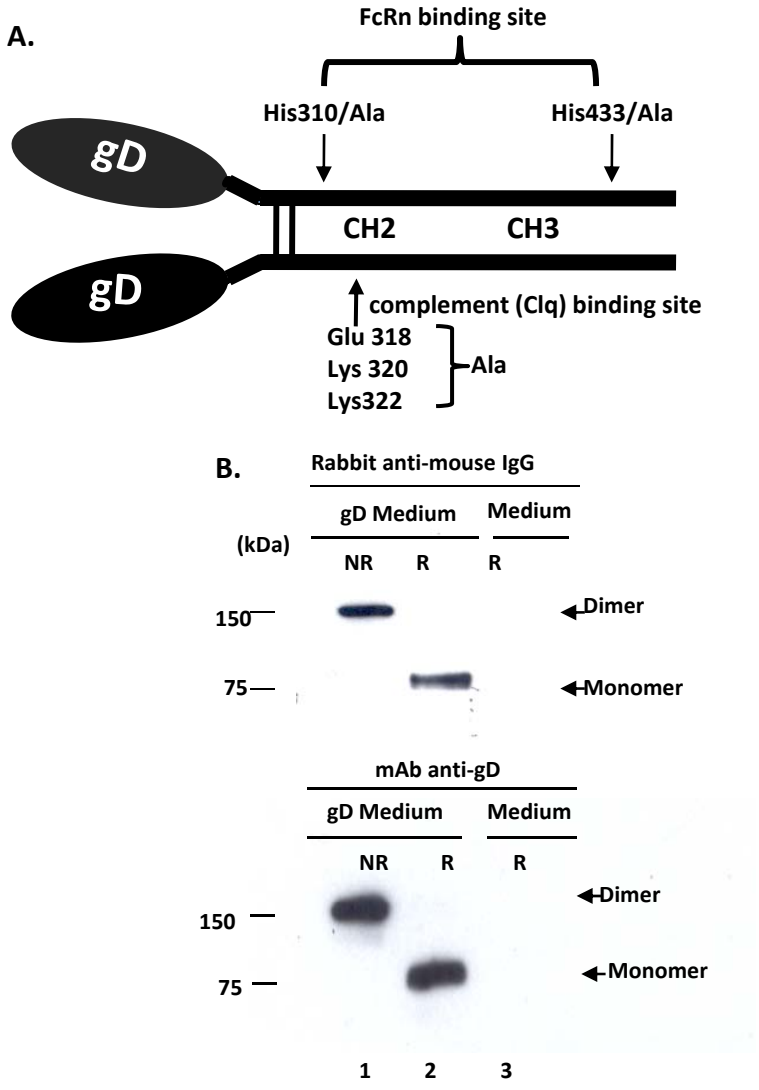


Supplementary Figure 1. Design and characterization of HSV-2 gD fused to IgG Fc fragment. (**A**). Schematic illustration for the genetic fusion of HSV-2 gD and murine Fcγ2a cDNA to create a gD-Fc fusion gene. Mutations were made in the CH2 domain of Fcγ2a fragment by using site-directed mutagenesis to replace Glu318, Lys320, and Lys322 with Ala residues to remove the complement C1q binding site, and His 310 and His 433 with Ala residues to eliminate FcRn binding sites. The fourteen codons for glycine and serine residues (GSGGGGSGGGGSGS) were inserted between the gD and Fc fragments. (**B**). The gD-Fc fusion proteins were secreted by CHO cells. The gD-Fc was recognized by either rabbit anti-mouse IgG (top panel) or a mAb anti-gD (bottom panel). The fusion protein was exhibited as a dimer under non-reducing (NR) or a monomer under reducing (R) condition.

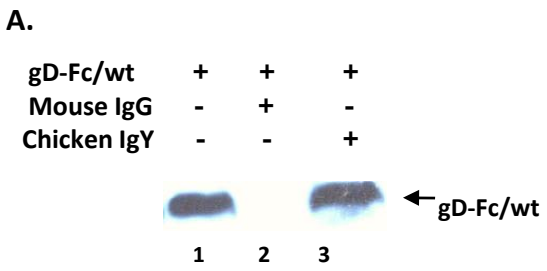
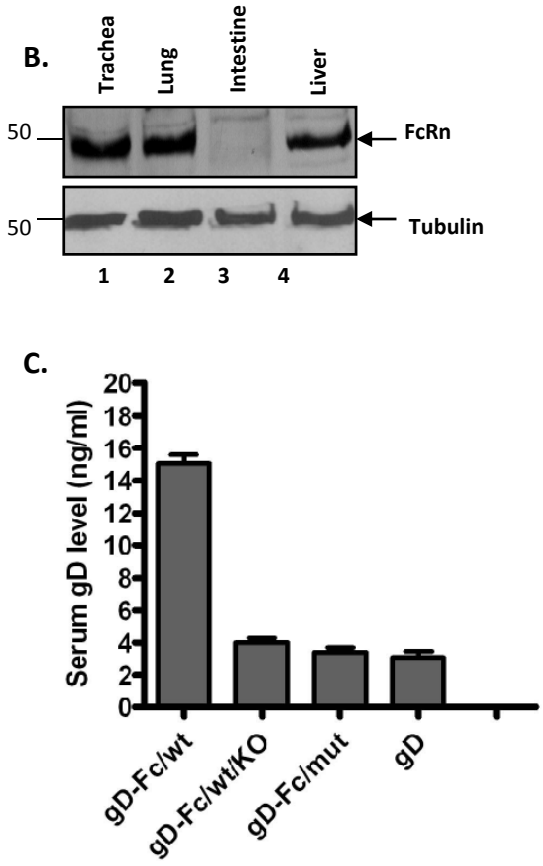


Fig. S2: FcRn-dependent transcytosis of the gD-Fc *in vitro and in vivo*.

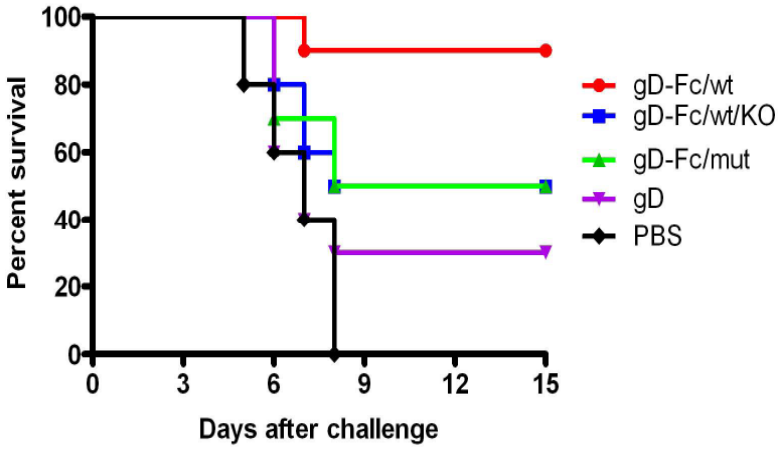
(**A**). Transport of gD-Fc/wt fusion proteins in rat IMCD-FcRn cell lines. IMCD-FcRn cells were plated onto 24-mm transwells and grown for 3-6 days to develop a polarized monolayer with resistance greater than 300 Ω cm^2. The purified gD-Fc/wt (50 μg/ml) was applied to the apical reservoir and allowed for transcytosis for 2 hr. The proteins were collected from the basolateral reservoir and blotted with anti-gD antibody under reducing condition. The gD-Fc/wt fusion protein (lane 3) was transported from the apical to basolateral surface. The transport was inhibited by an excessive amount of mouse IgG (1 mg/ml) (lane 2), but not by chicken IgY (1 mg/ml) (lane 3). Lane 1, representing gD-Fc/wt protein, was used as a positive control. Wt: wild-type.



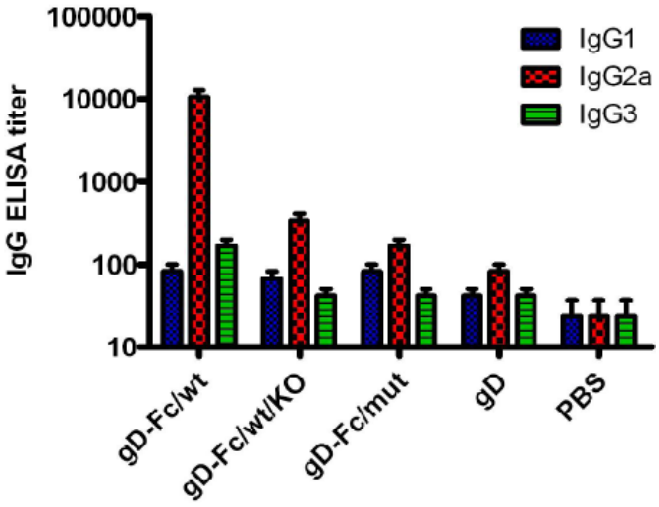
Supplementary Figure 2 (Continued). FcRn-dependent transcytosis of the gD-Fc *in vitro and in vivo* .

(**B**). Expression of mouse FcRn in the epithelial cells of mouse trachea and lung. The epithelial cell lysates from trachea, lung, intestine, and liver of 6 week C57B6/L mice was blotted with anti-mouse FcRn and anti-tubulin antibody. While murine FcRn is highly expressed in the intestinal epithelium of weaning mouse, its intestinal expression is minimal after weaning (21 days) [7, 10]. Molecular weight markers are indicated in kDa.

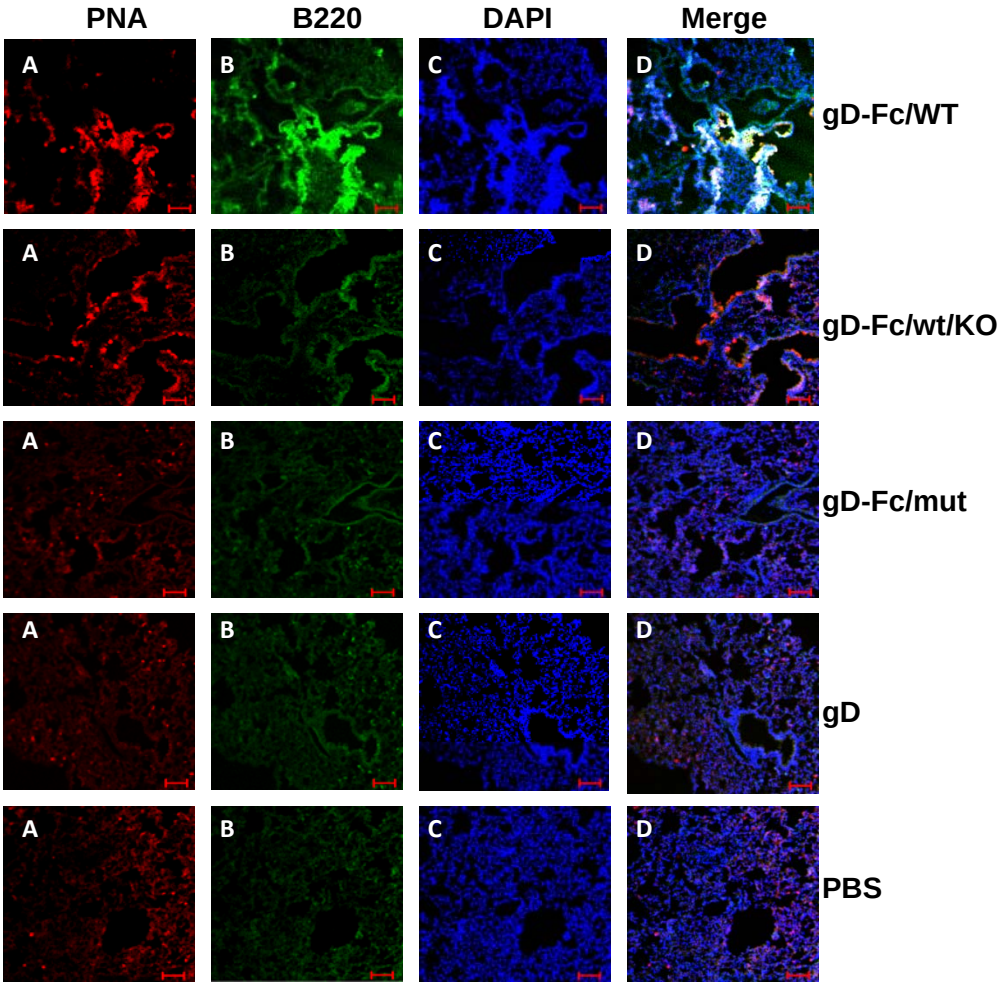
(**C**). The biotin-labeled gD, gD-Fc/wt and gD-Fc/mut proteins (20 μg) were intranasally (i.n.) inoculated into wild-type and FcRn KO mice as indicated. 8 hr later, the mouse sera were collected and the gD or gD-Fc protein concentration was measured by ELISA. Inoculation conditions are displayed at the bottom of the panel. Values marked with asterisk in this and subsequent Figures: * , $P < 0.05$; **, $P < 0.01$.



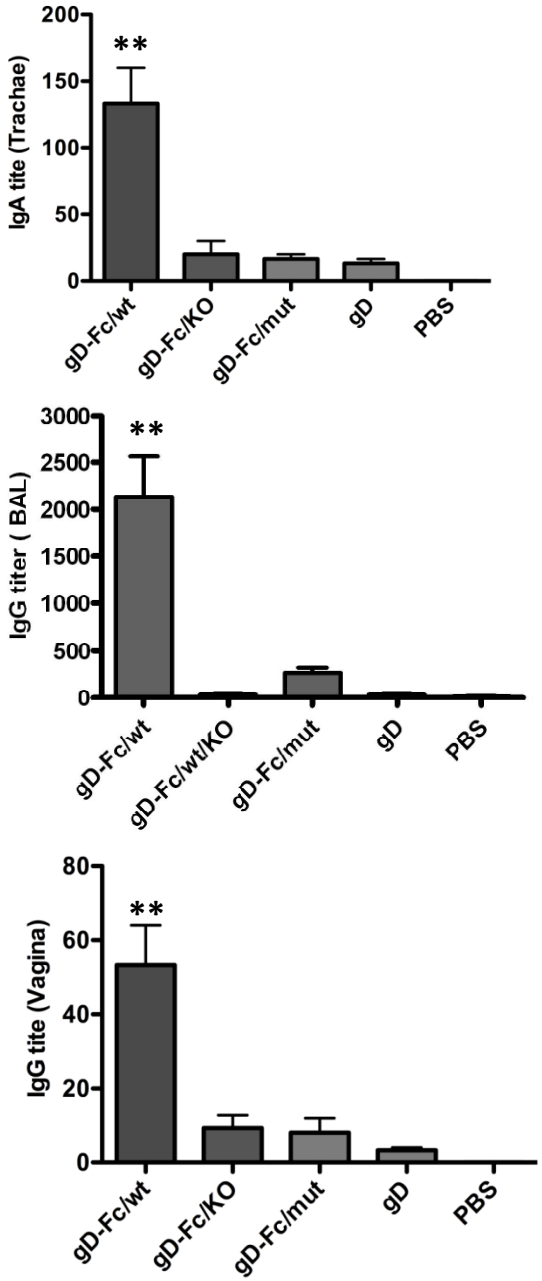
Supplementary Figure 3. Survival of mice following passive transfer of immunized mouse sera. Groups of five mice received 0.3 ml of immunized sera prior to ivag inoculation with 1 X 10^4 PFU HSV-2. Mean survival following adoptive serum transfer and genital HSV-2 challenge. 24 hr following the transfer, mice were challenged intravaginally with 1×10^4 pfu of HSV-2. The percentage of mice from protection on the indicated days is presented.



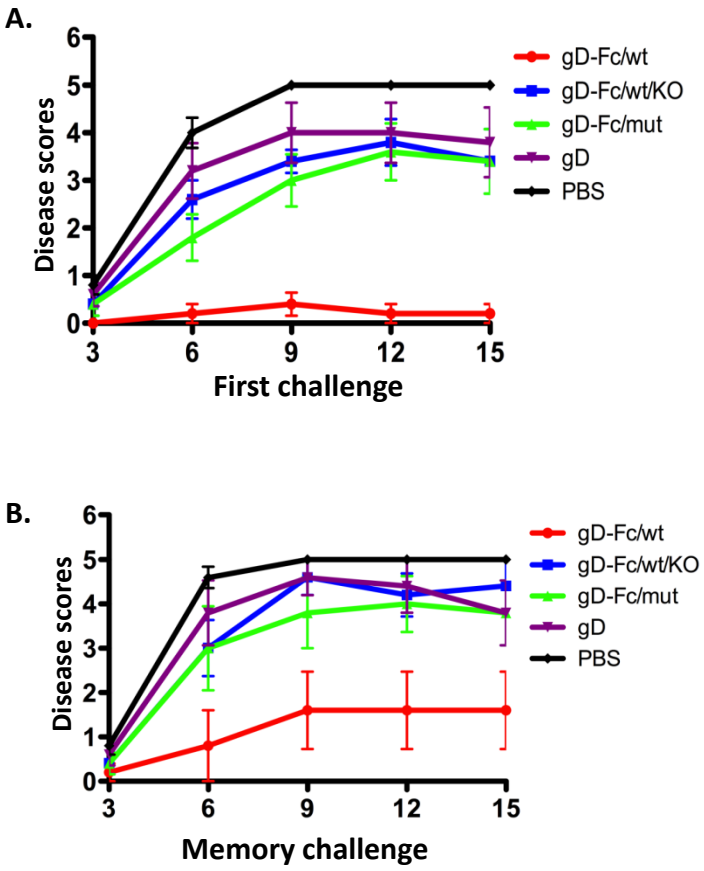
Supplementary Figure 4. Measurement of HSV-2 gD-specific IgG isotype titers in the serum. Blood samples were taken from mice by tail bleeding. HSV-2 gD-specific IgG isotype at 56 days was measured in serum by ELISA. Immunization conditions are displayed at the bottom. The bars represent mean values for each group (±S.E.M.).



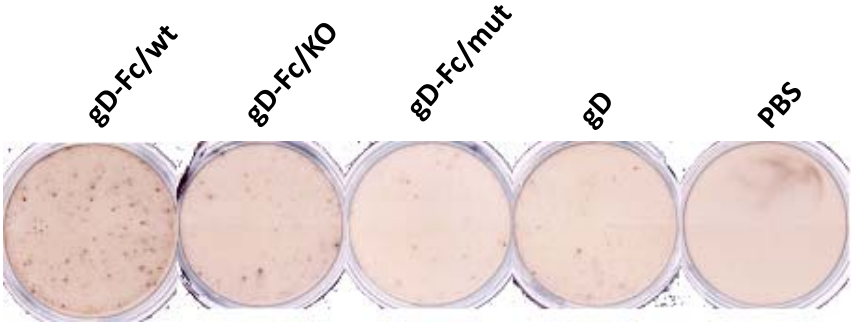
Supplementary Figure 5. Inducible bronchus-associated lymphoid tissue (iBALT) is formed in the gD-Fc/wt immunized mice. Frozen serial sections of the lung were stained with biotin-PNA (germinal centers, red) and anti-B220 (B cells, green), followed by Alexa 555-Avidin or Alexa 488 Fluro-conjugated IgG of the corresponding species. The nucleus is stained with DAPI (blue). Colocalization of all three red, green, and blue appears in white. The germinal center-like structure is shown in the merged panel that appears in white color. The data are representative of sections from at least three independent mice. Images were originally obtained at 10× magnification. Scale bars represent 100 μm. Immunization conditions are displayed at the right.



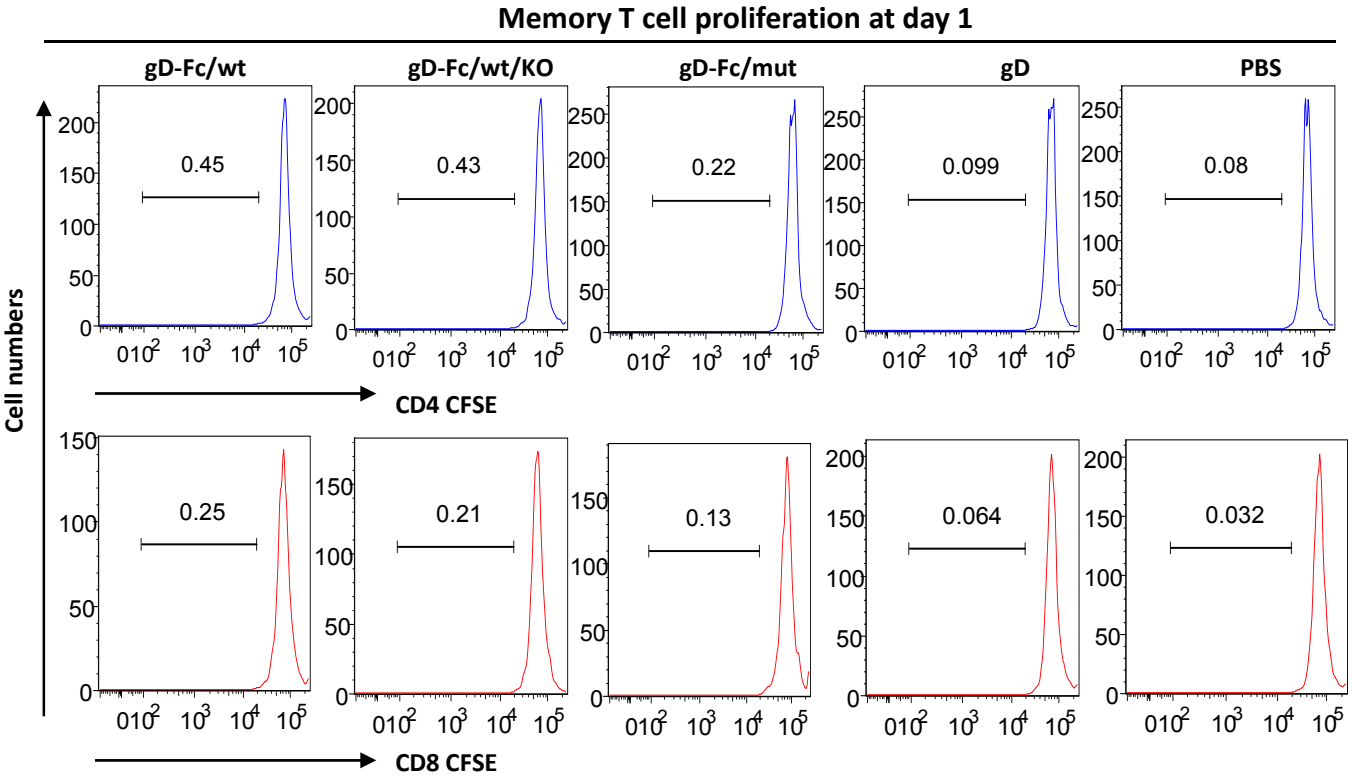
Supplementary Figure 6. **HSV-2 gD-specific antibody response in nasal washings, bronchial lavage, and vaginal secretions following the immunization**. The nasal washings (left panel), bronchial lavage (middle panel) and vaginal washes (right panel) were obtained from mice on the 10 days after the boost and gD-specific IgG and IgA titers were determined by ELISA. Antibody titers for 5 mice from a representative experiment performed at three times were quantified by endpoint titer. Titers of HSV2 gD-specific IgG antibody from serum and vaginal washes of naive mice always fell below the limits of detection and are omitted from the figure for clarity. The data shown are representative of three independent experiments. **, $P < 0.01$.

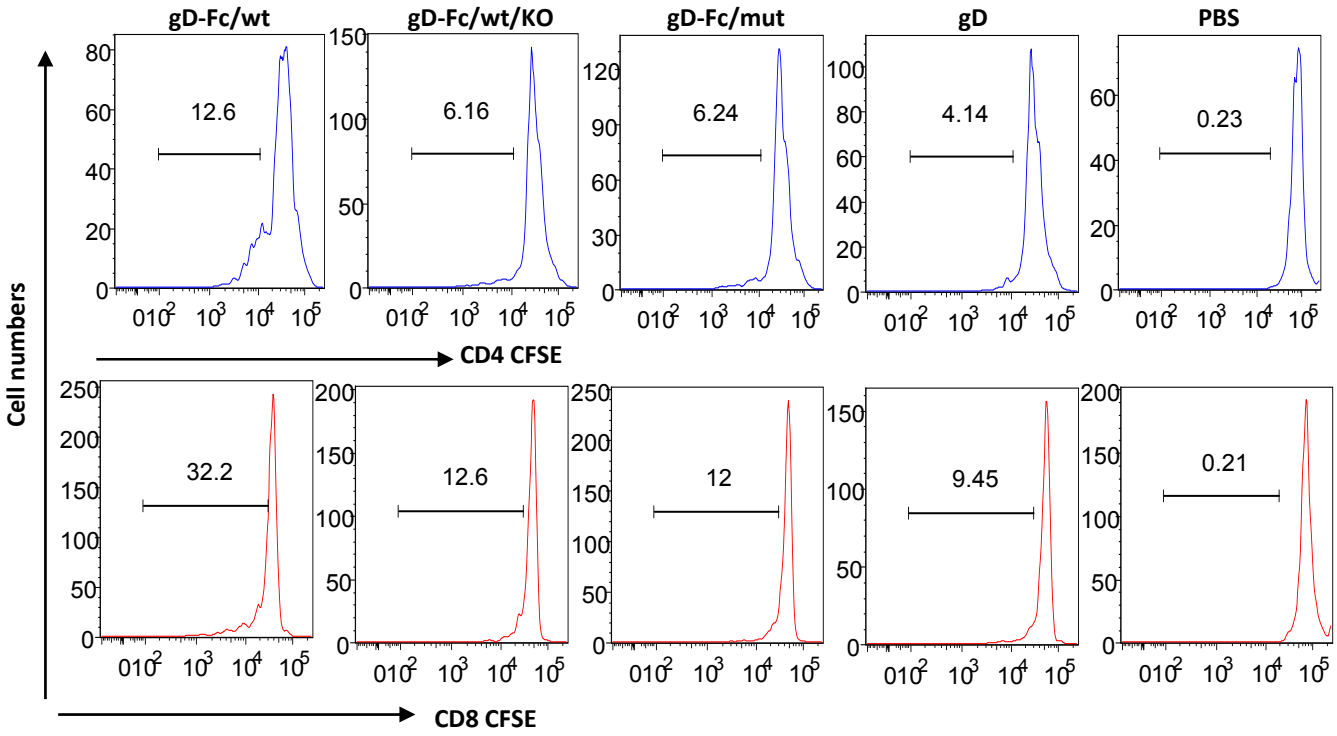


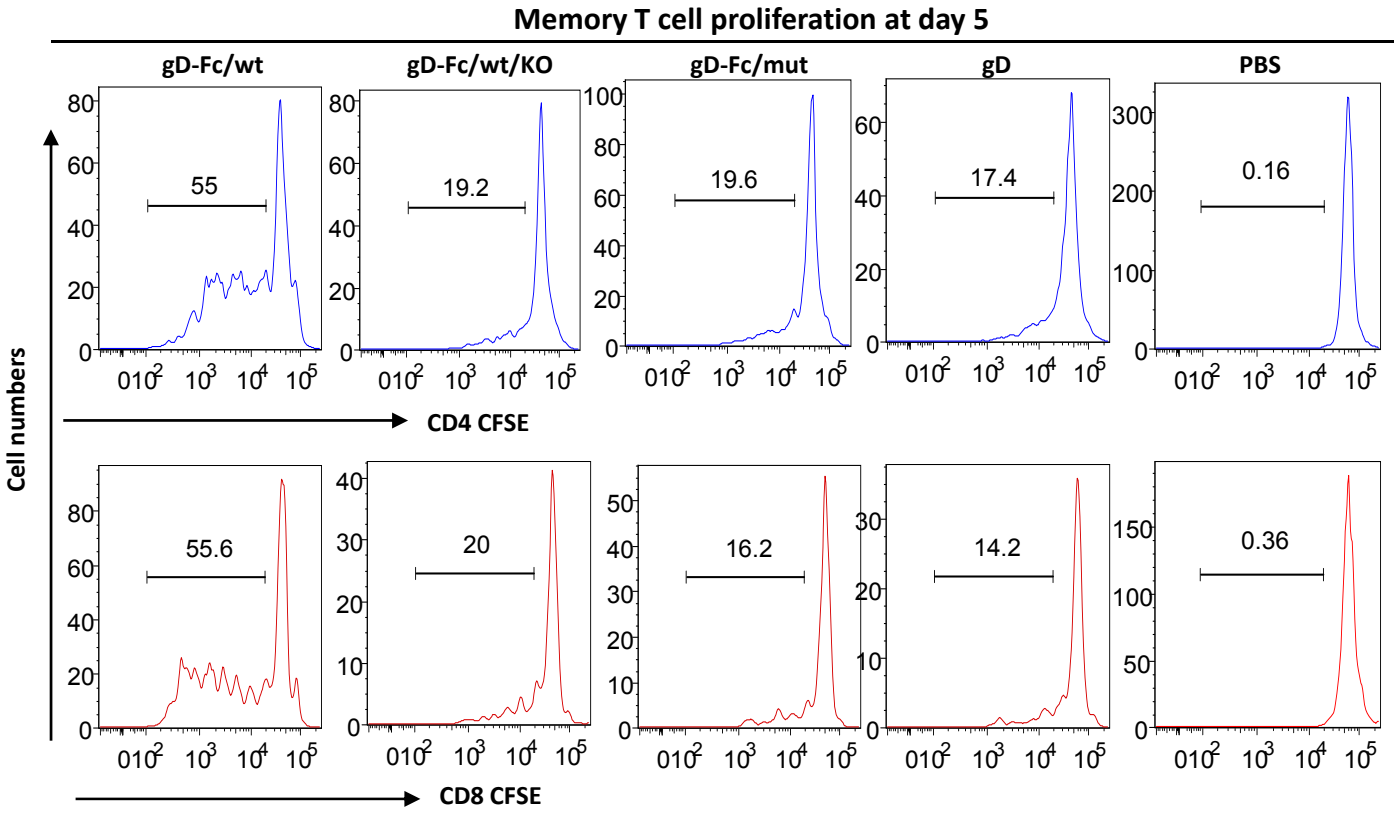
Supplementary Figure 7. Disease scores in primary HSV-2 infections in mice.
After challenge with wild-type HSV-2 186, individual mice described in the legends of Fig. 3A and Fig. 4E were observed during a 15-day follow-up period for the incidence of genital and disseminated HSV-2 diseases using the following score: 0, no apparent infection; 1, slight redness of external vagina; 2, redness and swelling of external vagina; 3, severe redness and swelling of external vaginal and surrounding tissue; 4, genital ulceration with severe redness, swelling and hair loss of genital and surrounding tissue; 5, severe genital ulceration.



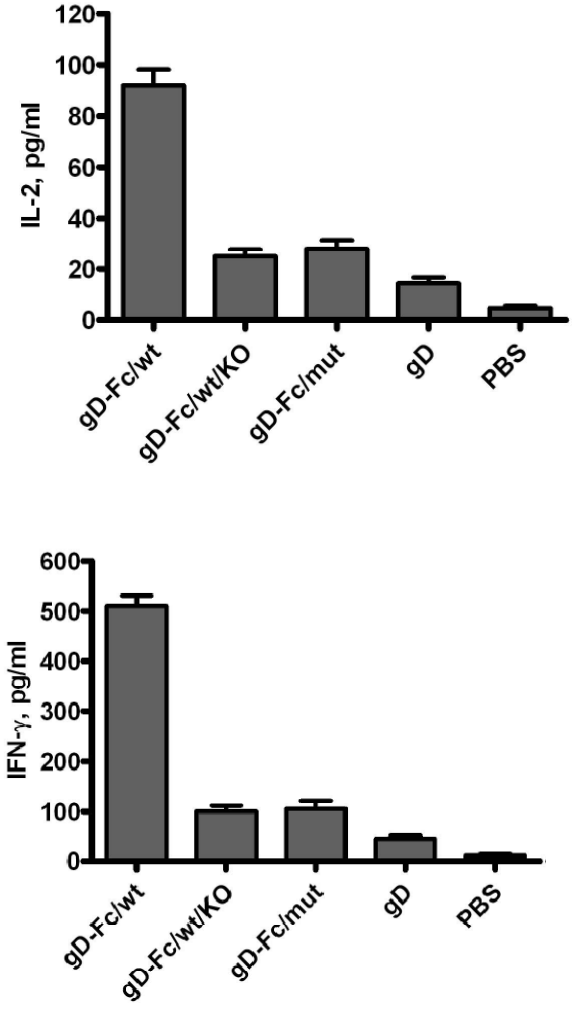
Supplementary Figure 8. Long-lived HSV gD-specific antibody-secreting cells in the bone marrow. Bone marrow cells removed 6 months after the boost were placed on gD-coated plates and quantified by ELISPOT analysis of IgG-secreting plasma cells. Data are representative of three separate experiments.


Memory T cell proliferation at day 1
gD-Fc/wt
gD-Fc/wt/KO
gD-Fc/mut
gD
PBS
Cell numbers
0.45
0.43
0.22
0.099
0.08
CD4 CFSE
0.25
0.21
0.13
0.064
0.032
CD8 CFSE


Memory T cell proliferation at day 3
gD-Fc/wt
gD-Fc/wt/KO
gD-Fc/mut
gD
PBS
12.6
6.16
6.24
4.14
0.23
CD4 CFSE
32.2
12.6
12
9.45
0.21
CD8 CFSE
Cell numbers



Supplementary Figure 9. Long-lived gD-specific T cell memory to FcRn-targeted mucosal vaccination. Spleen cells were isolated from the immunized mice two months after the boost, stained with CFSE, and stimulated *in vitro* with 20 μg/ml of purified gD for 1 (A), 3 (B), and 5 (C) days, respectively. Data are expressed in CFSE histograms of fluorescence intensity versus the number of fluorescing cells, indicating the percentage of the cell population positive for CD4 and CD8 antigen. Numbers in the quadrants are the percentage of $CD4^+$ and $CD8^+$ proliferating T cells. Representative flow cytometry profiles of two similar experiments with three mice per group are shown. Immunization conditions are displayed on the top.



Supplementary Figure 10. Cytokine secretion from re-stimulated spleen T cells. Spleen T cells were collected and pooled from three mice per group 6 months after the boost. Cells were restimulated in vitro with purified gD protein. Cytokines secreted into the culture supernatant were detected by ELISA and are presented as picograms/ml of culture supernatant. Data are representative of 3 experiments with 3 mice pooled in each experiment.