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Figures and figure supplements

Herpes simplex type 2 virus deleted in glycoprotein D protects against vaginal, skin and neural disease

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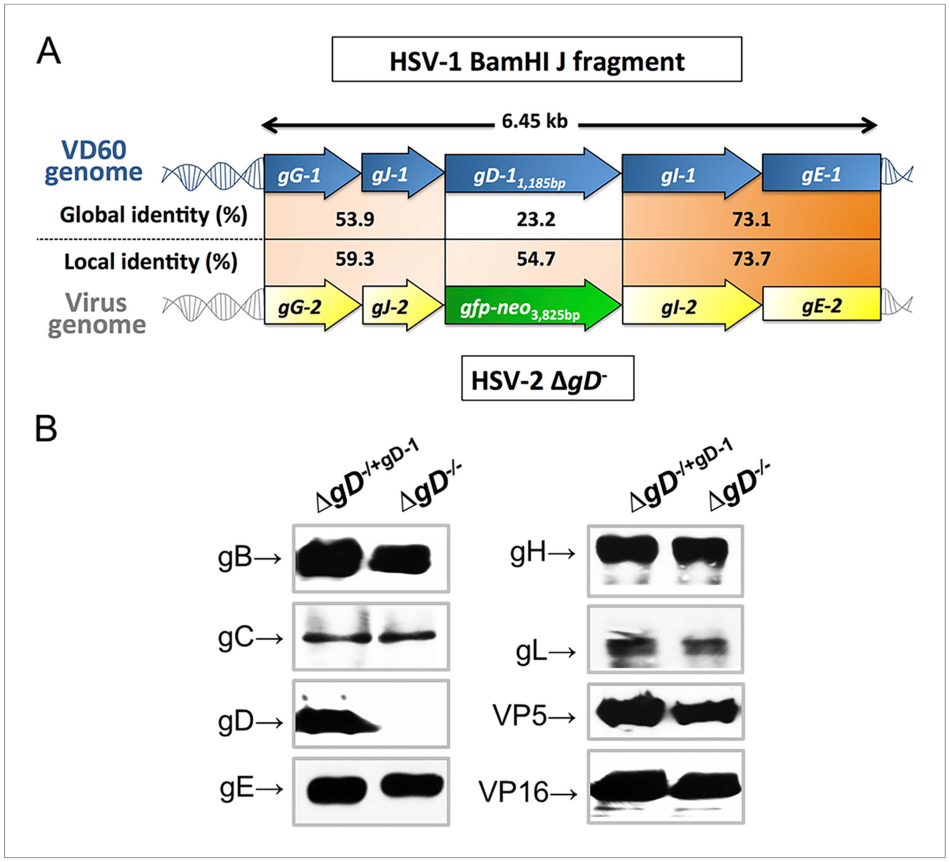

Figure 1. Characterization of the ΔgD$^{-/-}$ virus. (**A**) Alignment of the upstream and downstream regions of *gD* located within the HSV-1 BamHI J fragment encoded in VD60 cells and within the genome of Δ*gD*$^{-/-}$ using LALIGN (ExPASy) (***Myers and Miller, 1988***). Global alignments assess end-to-end sequences and local pairwise alignments search for regions with high identity. (**B**) Western blots of dextran gradient-purified virus isolated 24 hr after infection of VD60 (Δ*gD*$^{-/+gD-1}$) and Vero (Δ*gD*$^{-/-}$) cells. Protein expression was assessed for viral glycoproteins B (gB, *U*$_L$*27*), gC (*U*$_L$*44*), gD (*U*$_s$*6*), gE (*U*$_s$*8*), gH (*U*$_s$*22*), gL (*U*$_L$*1*), VP5 (*U*$_L$*19*) and VP16 (*U*$_L$*48*).

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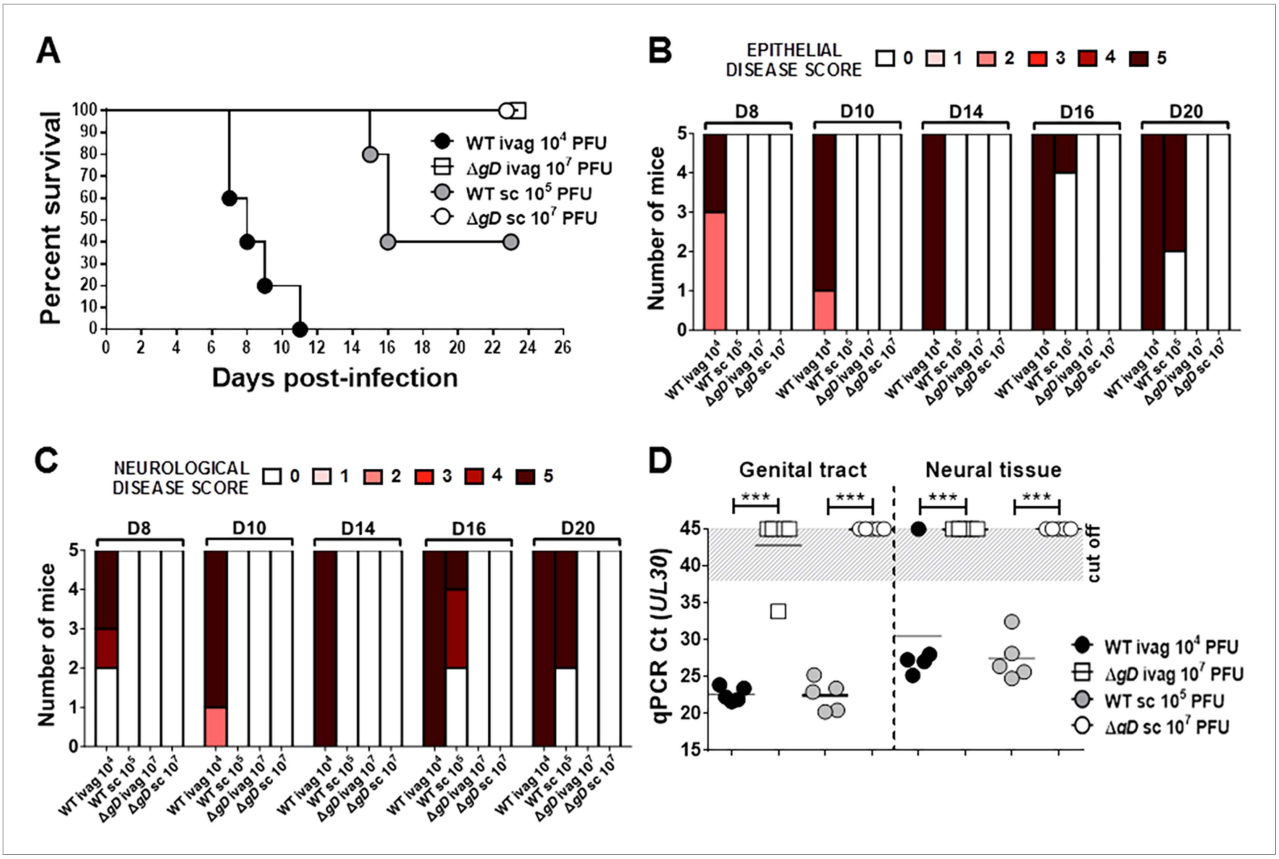

Figure 2. HSV-2 ΔgD$^{-/+gD-1}$ is attenuated in severe combined immunodeficiency (SCID) mice. (**A**) Survival of SCID mice inoculated with up to 10^7 pfu of HSV-2 Δ*gD*$^{-/+gD-1}$ or up to 10^5 pfu of the parental HSV-2(G) strain either intravaginally (ivag) or subcutaneously (sc). Statistical significance was measured by log-rank Mantel–Cox test; **p < 0.01 for Δ*gD* and WT after ivag inoculation. (**B**) Epithelial and (**C**) Neurological disease scores for SCID mice inoculated with the different viruses at indicated doses. (**D**) HSV-2 DNA (qPCR, U_L30 gene) in genital tract and neural tissue samples at day 5 post-virus inoculation. The Ct cut off was determined with HSV-uninfected naïve samples. Statistical significance was measured by two-way ANOVA with Sidak's multiple comparisons test for (**B**, **C** and **D**); ***p < 0.001. HSV-2 Δ*gD*$^{-/+gD-1}$ and its parental strain are abbreviated as Δ*gD* and WT, respectively.

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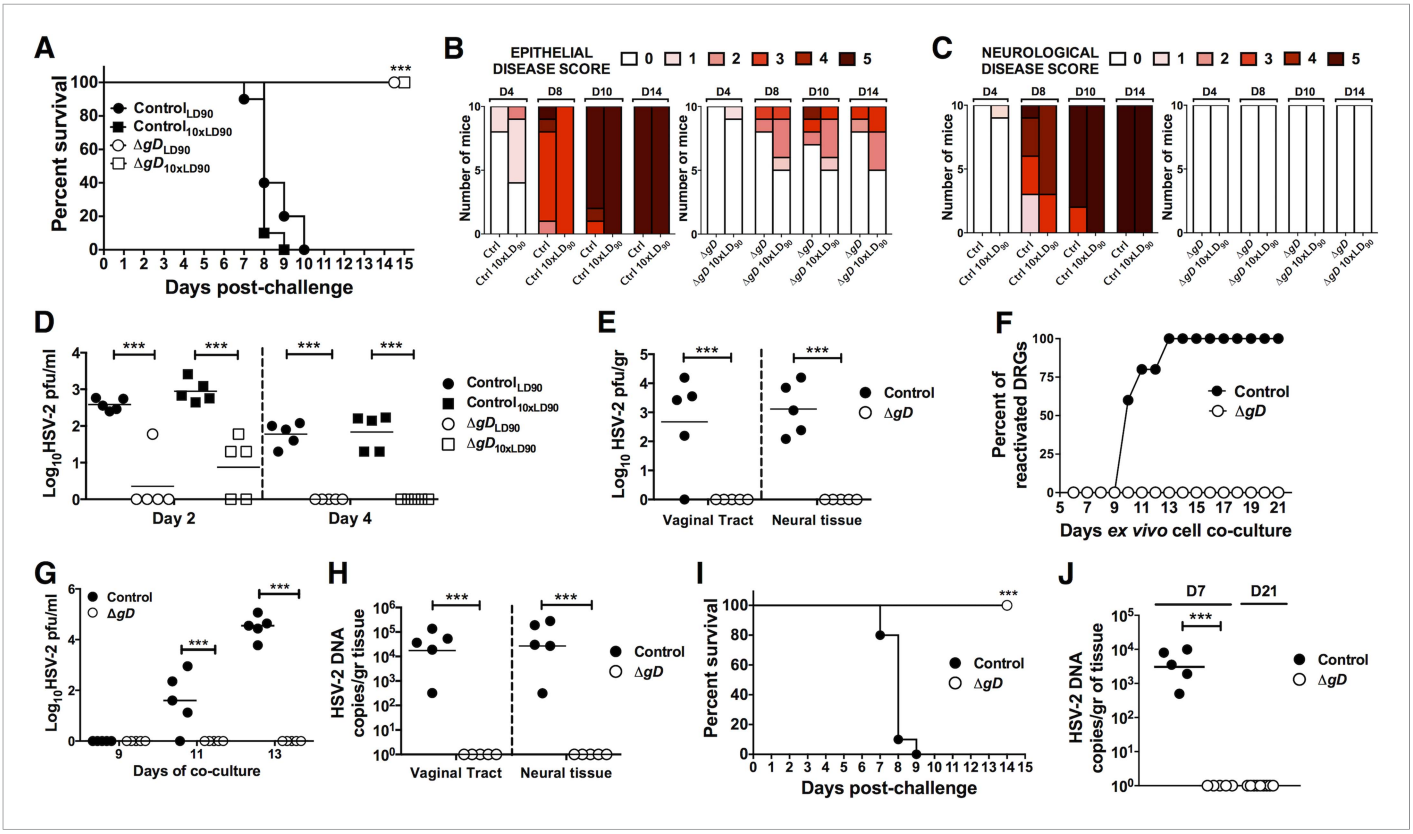

Figure 3. Vaccination with HSV-2 ΔgD$^{-/+gD-1}$ protects mice against intravaginal lethal challenge. C57BL/6 mice were subcutaneously primed and boosted 3 weeks apart either with HSV-2 Δ*gD*$^{-/+gD-1}$ or VD60 cell lysate (Control). 21 days after boost, mice were challenged with an LD_{90} of wild-type HSV-2(4674) or 10 × LD_{90}. (**A**) Survival, (**B**) Epithelial and (**C**) Neurological disease scores were followed daily after challenge. (**D**) Viral titers in vaginal washes at days 2 and 4 after challenge (n = 10 mice pooled two per group, lines indicate means). (**E**) Viral titers in vaginal and neural tissue (including the dorsal root ganglia, DRG) at day 5 after challenge (n = 5, lines indicate means). (**F**) Ex vivo reactivation of neural tissue obtained from challenged mice (n = 5 per group). (**G**) HSV-2 pfu in the media of ex vivo reactivated neural tissue obtained from challenged mice (n = 5 mice per group, lines indicate means). (**H**) HSV-2 DNA (qPCR, *U_S6* gene) of genital tissue and neural tissue at day 5 after challenge (n = 5, lines indicate means). (**I**) Survival of BALB/c mice that were primed, boosted and challenged as the C57BL/6 mice described above. (**J**) HSV-2 DNA (qPCR, *U_S6* gene) in Control- and Δ*gD*-vaccinated BALB/c neural tissue at day 7 (n = 5, lines indicate means) and Δ*gD*-vaccinated BALB/c neural tissue at day 21 (n = 9, lines indicate means) after challenge. HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated group vs Control-vaccinated group were compared by log-rank Mantel–Cox test (**A**, **I**), two-way ANOVA with Sidak's multiple comparisons test (**D**, **E**, **G**, **H**) or unpaired *t*-test (**J**); ***p < 0.001. HSV-2 Δ*gD*$^{-/+gD-1}$ and Control are abbreviated as Δ*gD* and Ctrl, respectively.

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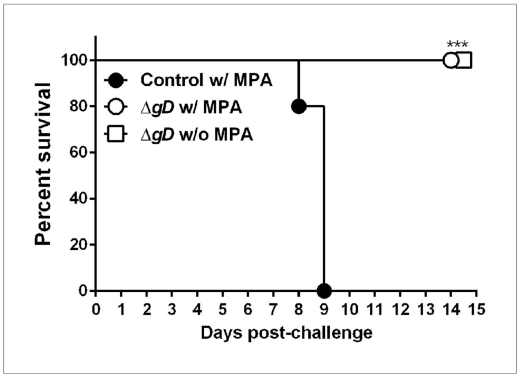

Figure 3—figure supplement 1. $\Delta gD^{-/+gD-1}$-vaccinated cycling mice are protected against intravaginal HSV-2 challenge. C57BL/6 mice were treated (w/MPA) or not (w/o MPA) with medroxyprogesterone (MPA) 5 days previous to subcutaneous prime and boost 3 weeks apart either with HSV-2 $\Delta gD^{-/+gD-1}$ (Δ*gD*) or VD60 cell lysate (Control). 16 days after boost, all mice were treated with MPA and 5 days later challenged intravaginally with an LD_{90} of wild-type HSV-2(4674) (n = 5 mice per group). Statistical significance was measured by log-rank Mantel–Cox test; ***$p < 0.001$, treatments vs Control.

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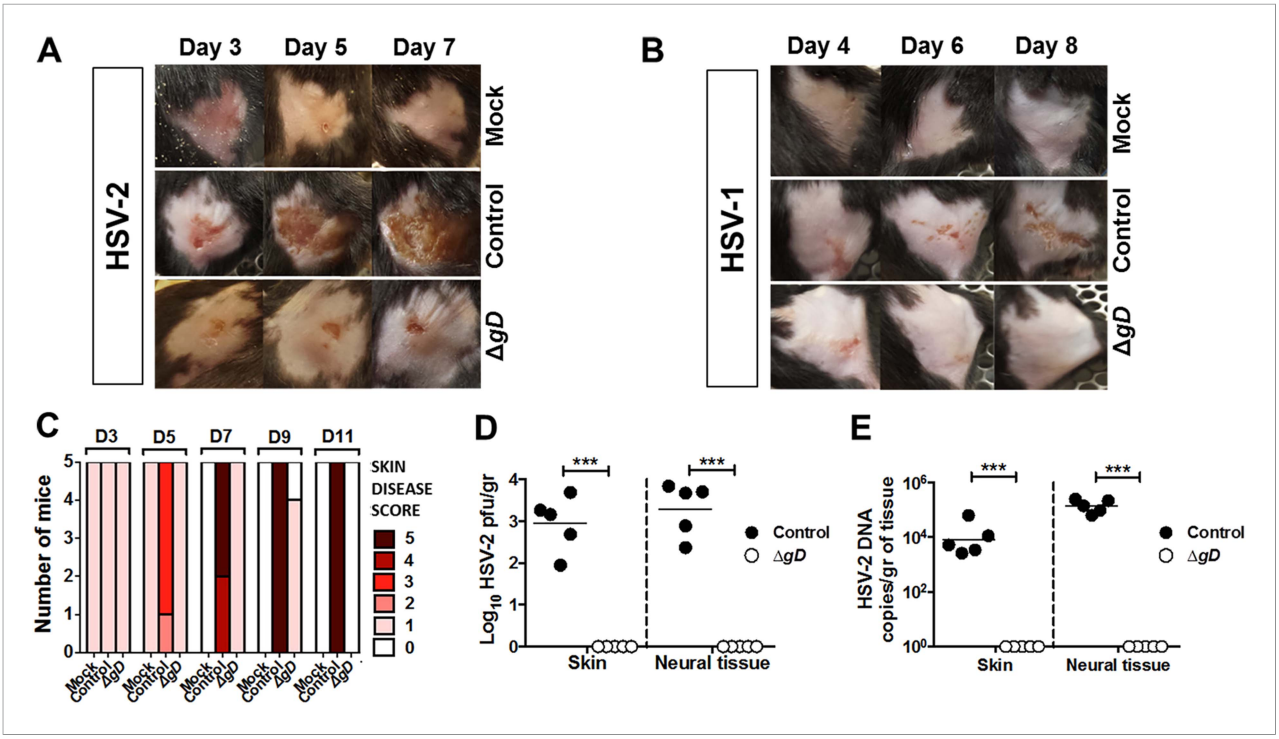

Figure 4. Vaccination with HSV-2 ΔgD$^{-/+gD-1}$ protects mice infected with HSV-2 and HSV-1 in a skin scarification model. Mice were subcutaneously primed and boosted 3 weeks apart either with HSV-2 Δ*gD*$^{-/+gD-1}$, Control VD60 cell lysate or PBS. 3 weeks later, mice were depilated and challenged in the flank skin with PBS (mock), (**A**) 5 × 10^4 pfu HSV-2(4674) or (**B**), 1 × 10^7 pfu HSV-1(17). Representative images are shown. (**C**) Skin disease scores for HSV-2(4674)-challenged mice at days 3–11. (**D**) Viral titers from biopsies of skin or neural tissue obtained on day 6–7 (Control mice) and day 14 (HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice) (n = 5 mice per group, lines indicate means). (**E**) HSV-2 DNA (qPCR, U_S6 gene) in skin biopsies and neural tissue of Control mice (day 6–7) and HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice (day 14) challenged with virus (5 mice per group, lines indicate means). Statistical significance was measured by two-way ANOVA with Sidak's multiple comparisons test (**D** and **E**); ***p < 0.001, Δ*gD*$^{-/+gD-1}$-vaccinated group vs control group. HSV-2 Δ*gD*$^{-/+gD-1}$ is abbreviated as Δ*gD*.

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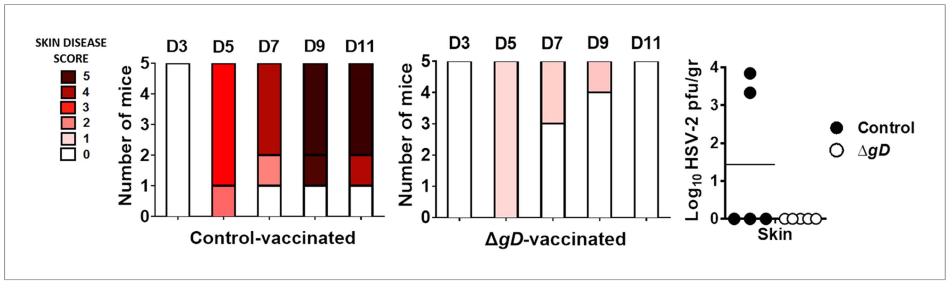

Figure 4—figure supplement 1. Vaccination with HSV-2 ΔgD$^{-/+gD-1}$ protects mice infected with HSV-1 in a skin scarification model. Mice were subcutaneously primed and boosted 3 weeks apart either with HSV-2 Δ*gD*$^{-/+gD-1}$ or Control VD60 cell lysate. 3 weeks later, mice were depilated and challenged in the flank skin with1 × 10^7 pfu HSV-1 (17). Skin disease scores shown for challenged mice at days 3–11. Viral titers from biopsies of skin obtained on day 7–8 (Control mice) and day 14 (HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice) (n = 5 mice per group, lines indicate means). Statistical significance was measured by unpaired *t*-test.

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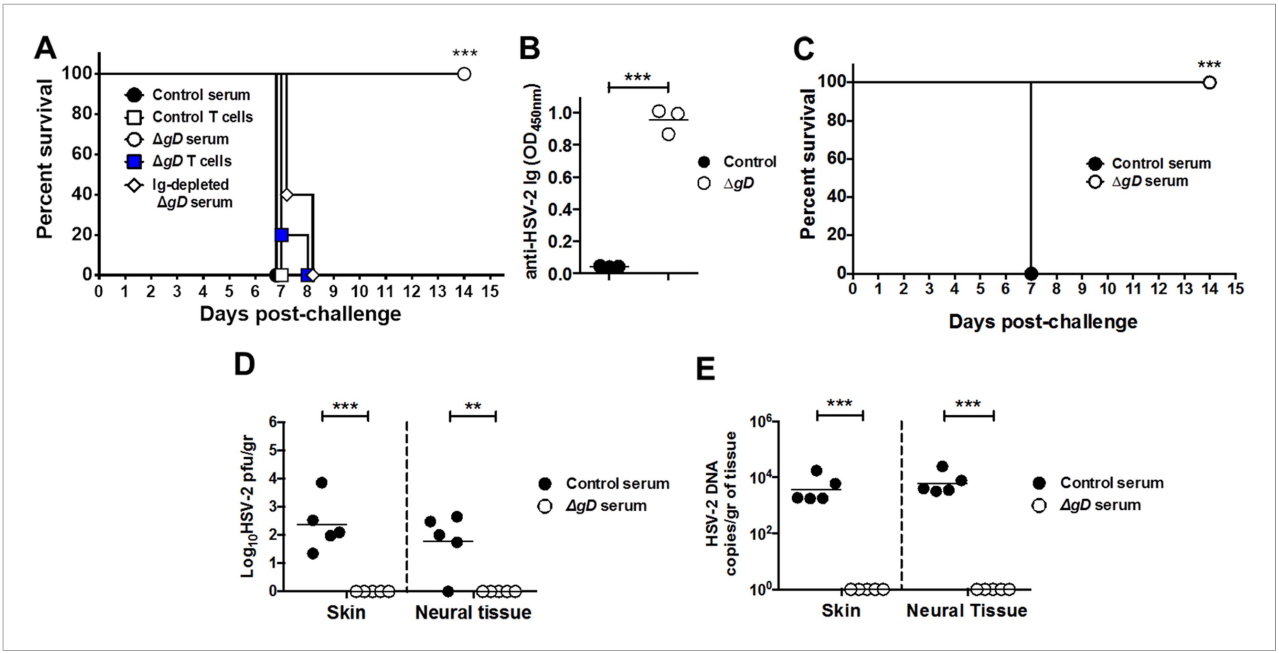

Figure 5. Serum from HSV-2 $\Delta gD^{-/+gD-1}$-vaccinated mice protects naïve mice against HSV-2 intravaginal and skin challenge. Mice were subcutaneously primed and boosted 3-weeks apart either with HSV-2 $\Delta gD^{-/+gD-1}$ or VD60 cell lysate (Control). 21 days later, blood and spleen were collected for serum and T cell purification and transferred intraperitoneally and intravenously, respectively, into naïve wild-type C57BL/6 mice. 24 hr and 48 hr after serum and T cell transfer, respectively, mice were challenged intravaginally with LD_{90} of HSV-2(4674) and followed for survival (n = 5 mice per group). Serum immunoglobulins were depleted using a Protein L column (**A**). (**B**) Transferred anti-HSV-2-antibodies were assessed by ELISA in vaginal washes of recipient mice (washes pooled from five mice in three independent experiments). (**C**) Pooled serum from Control- or HSV-2 $\Delta gD^{-/+gD-1}$-vaccinated mice was transferred into naïve wild-type C57BL/6 mice. 24 hr after serum transfer, mice were depilated in the flank skin and challenged with HSV-2(4674) and followed for survival (n = 5 mice per group). (**D**) Viral titers in skin biopsies and neural tissue of mice receiving Control-serum (day 7) and $\Delta gD^{-/+gD-1}$-serum (day 14) (n = 5 mice per group). (**E**) HSV-2 DNA (qPCR, U_S6 gene) in skin biopsies and neural tissue of mice receiving Control-serum (day 7) and $\Delta gD^{-/+gD-1}$-serum (day 14) (n = 5 mice per group). Statistical significance was measured by log-rank Mantel–Cox test (**A** and **C**), *t*-test (**B**) and two-way ANOVA with Sidak's multiple comparisons test (**D**); **p < 0.01, ***p < 0.001, treatment vs control. HSV-2 $\Delta gD^{-/+gD-1}$ is abbreviated as ΔgD.

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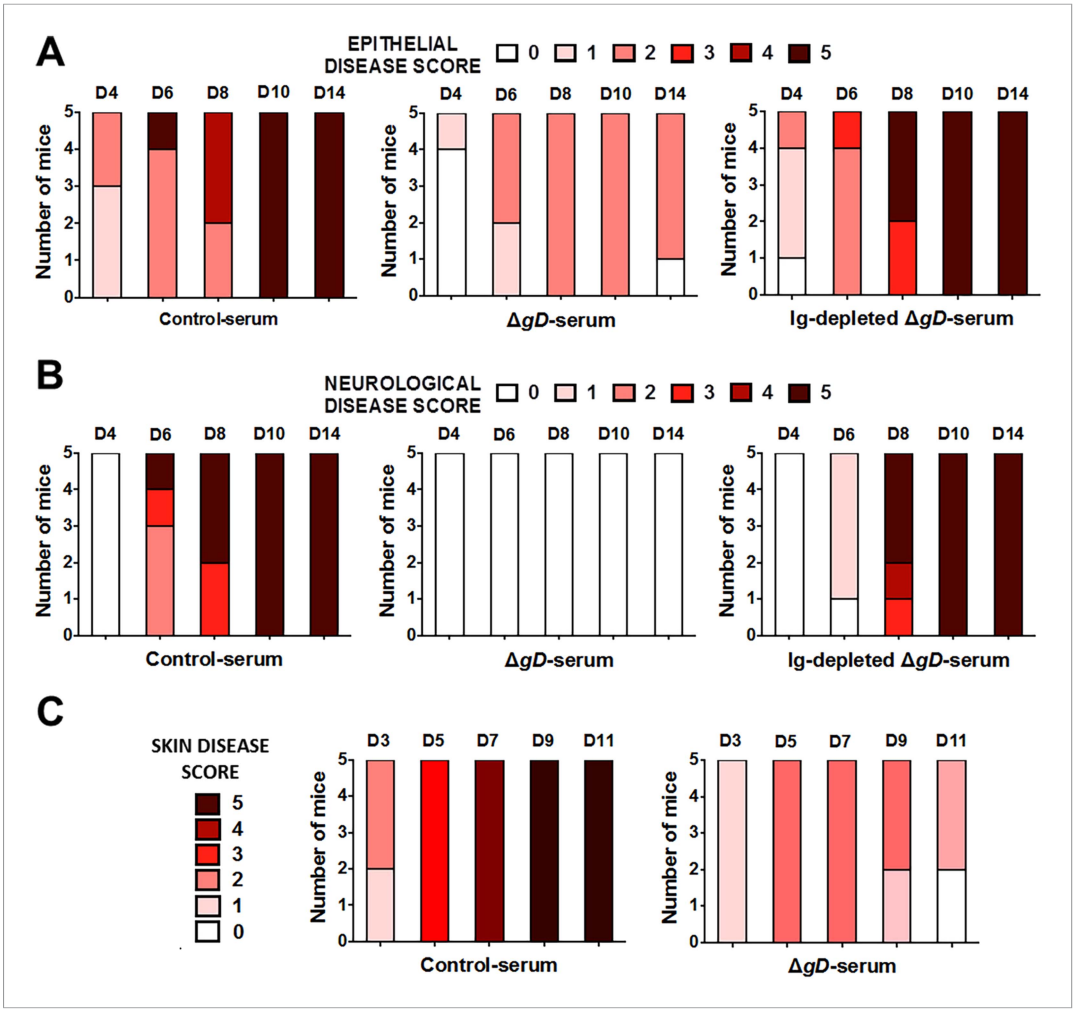

Figure 5—figure supplement 1. Serum from HSV-2 ΔgD$^{-/+gD-1}$-vaccinated mice protects naïve mice against epithelial and neurological disease after HSV-2 intravaginal and skin challenge. Serum from Control- (VD60 cell lysate), Δ*gD*$^{-/+gD-1}$-vaccinated mice, or Δ*gD*$^{-/+gD-1}$ serum depleted of immunoglobulins using a Protein L column was transferred intraperitoneally into naïve wild-type C57BL/6 mice. 24 hr later, mice were challenged intravaginally with LD_{90} of HSV-2(4674) and followed for (**A**) epithelial and (**B**) neurological disease (n = 5 mice per group). (**C**) Serum from Control- or HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice was transferred intraperitoneally into naïve wild-type C57BL/6 mice. 24 hr later, mice were depilated and challenged in the flank skin with HSV-2(4674) and followed for epithelial disease.

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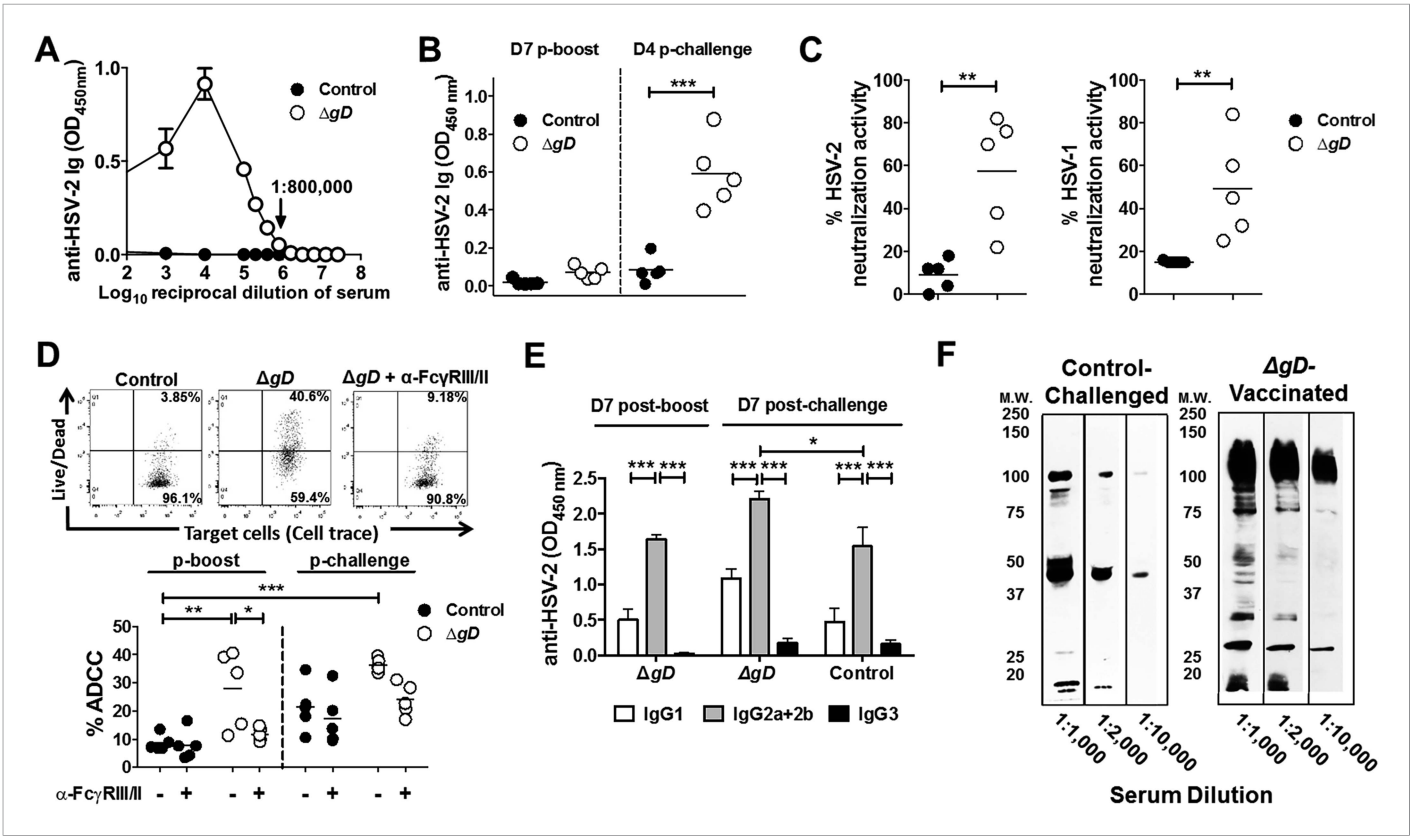

Figure 6. Vaccination with HSV-2 ΔgD$^{-/+gD-1}$ induces protective mucosal antibodies targeting multiple HSV proteins with ADCC activity. (**A**) Anti-HSV-2 antibodies detected by ELISA in serum samples at day 7 post-boost in mice subcutaneously primed and boosted 3-weeks apart with Δ*gD*$^{-/+gD-1}$ or VD60 lysate (Control) (4 independent pools of serum from 5–10 mice each, results shown as means ± SD). (**B**) Anti-HSV-2 antibodies detected by ELISA in vaginal washes at day 7 post-boost and day 4 post–challenge with HSV-2(4674) (n = 5 mice per group, lines indicate means). (**C**) In vitro neutralizing activity of serum antibodies (1:5 dilution) obtained from HSV-2 Δ*gD*$^{-/+gD-1}$- or Control-vaccinated mice against HSV-2 (left) and HSV-1 (right) (n = 5 mice per group, lines indicate means). (**D**) Antibody-dependent cell-mediated cytotoxicity (ADCC) using mouse splenocytes, HSV-2-infected Vero cells and serum obtained either from Control- (VD60 cell lysate) or HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice conducted in the absence or presence of anti-CD16/CD32 Ab to FcγRIII and FcγRII. % ADCC is defined as the percentage of dead (Live/Dead^{+}) target cells within HSV-2 GFP^{High} positive cells. A representative dot blot is shown in the upper panel and lower panel shows results for five mice per group (lines indicate means). (**E**) Isotype of anti-HSV-2 serum antibodies obtained from five mice each that were either HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated and HSV-2(4674)-challenged or Control-vaccinated and HSV-2(4674)-challenged (results shown as means ± SD). (**F**) Western blots of cellular lysates infected with HSV-2(4674) and probed with dilutions of sera obtained from VD60 lysate-vaccinated and then subsequently infected mice (Control-Challenged) or dilutions of sera from HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice 7 days post boost (Δ*gD*-Vaccinated); blots are representative of five independent experiments. HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated groups were compared to control-vaccinated mice by two-way ANOVA with Sidak's multiple comparisons test (**A**, **B**, **D** and **E**) and unpaired *t*-test (**C**); *p < 0.05; **p < 0.01, ***p < 0.001. HSV-2 Δ*gD*$^{-/+gD-1}$ is abbreviated as Δ*gD*.

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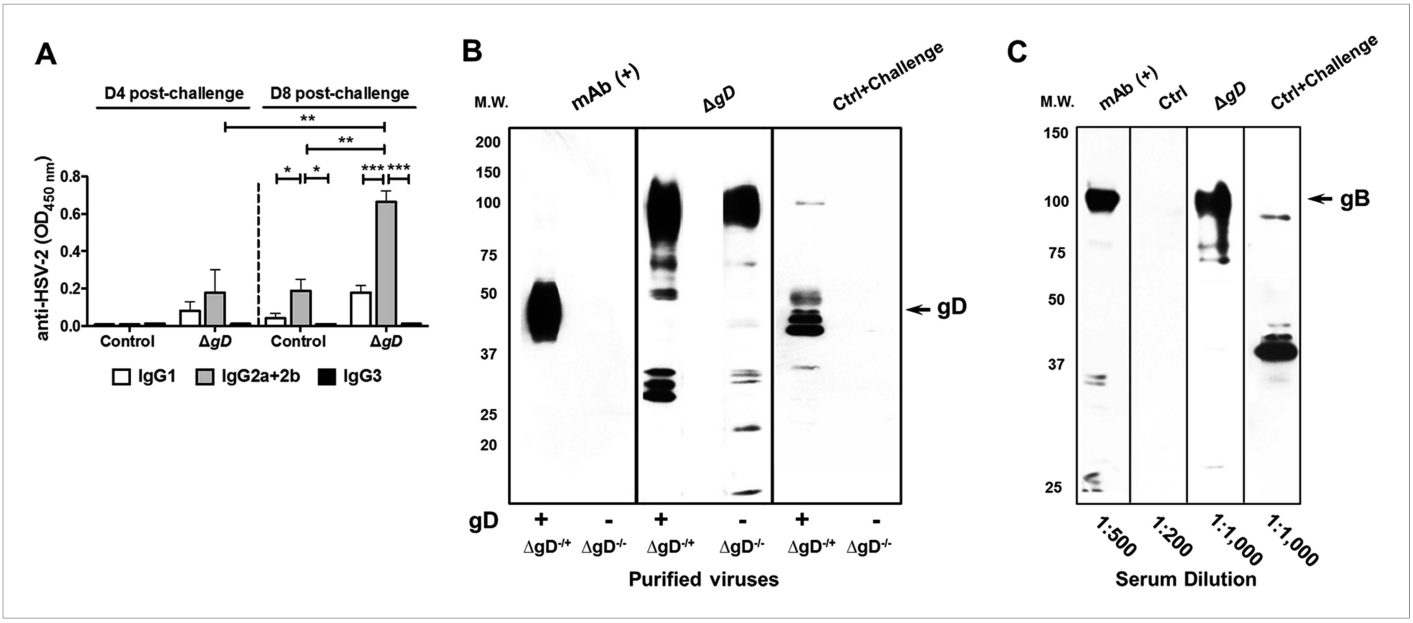

Figure 6—figure supplement 1. Characterization of vaginal wash and serum antibodies. (**A**) Isotypes of anti-HSV-2 antibodies in vaginal washes obtained at day 4 and day 8 post intravaginal challenge in mice that were immunized with HSV-2 Δ*gD*$^{-/+gD-1}$ (Δ*gD*) or VD60 cell lysate (Control). Antibody responses are shown as means ± SD (n = 5 per group) and were compared by two-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001. (**B**) Western blots with sucrose gradient-purified Δ*gD*$^{-/+gD-1}$ and Δ*gD*$^{-/-}$ viruses (equivalent particle numbers based on a Western blot for VP5) probed with an anti-gD mAb (mAb (+)), sera from HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice day 7 post-boost (Δ*gD*) or sera from VD60 lysate-vaccinated mice day 7 post-intravaginal challenge with HSV-2(4674) (Ctrl + Challenge). (**C**) Western blots with purified recombinant glycoprotein B-1 (2 μg per lane) probed with an anti-gB mAb (mAb (+)), sera from VD60 lysate -vaccinated mice (Ctrl), HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated mice day 7 post boost (Δ*gD*) or VD60 lysate-vaccinated mice day 7 post-intravaginal challenge with HSV-2(4674) (Ctrl + Challenge).

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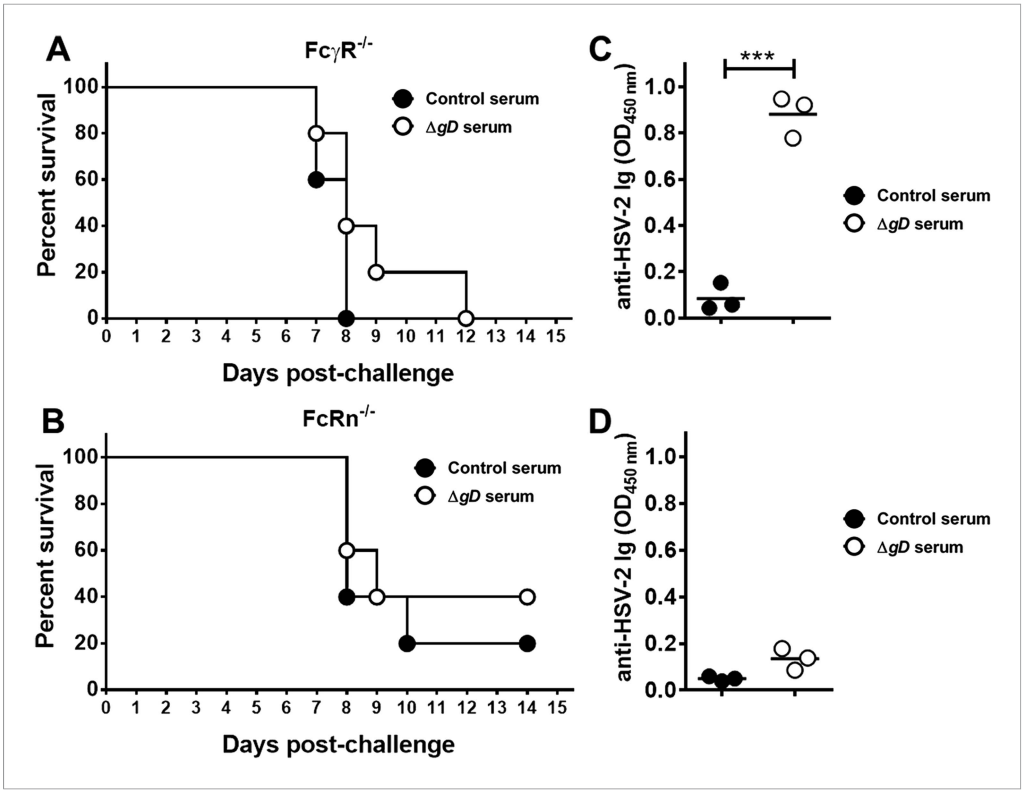

Figure 7. Antibody-mediated protection requires FcγR and FcRn expression. Survival of (**A**) FcγR$^{-/-}$ and (**B**) FcRn$^{-/-}$ mice that were either transferred serum obtained from Control- (VD60 cell lysate) or HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated wild-type mice and then challenged intravaginally with HSV-2(4674) (n = 5 mice per group). Detection of HSV-specific Abs by ELISA in pooled vaginal washes (n = 3 pools) of (**C**) FcγR$^{-/-}$ and (**D**) FcRn$^{-/-}$ mice receiving serum (intraperitoneally) from control- (VD60 cell lysate) or HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated wild-type mice. Survival curves were compared using log-rank Mantel–Cox test (**A** and **B**) and antibody titers using unpaired *t*-test (**C** and **D**); ***p < 0.001. HSV-2 Δ*gD*$^{-/+gD-1}$ is abbreviated as Δ*gD*.

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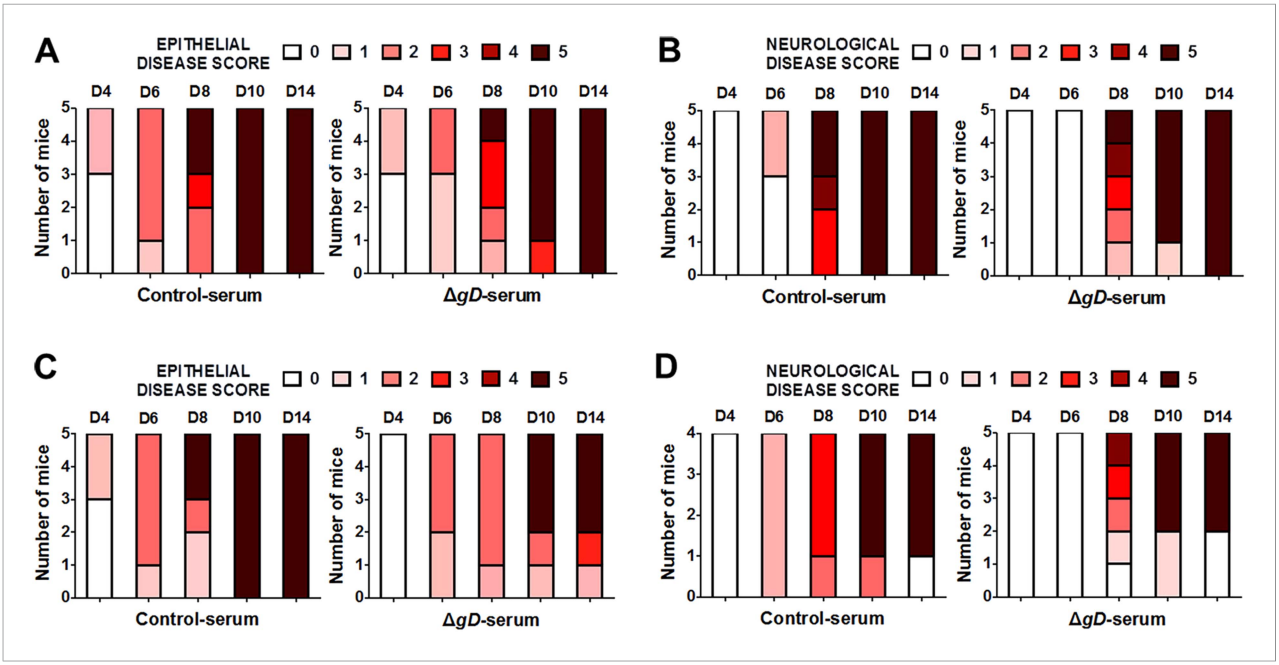

Figure 7—figure supplement 1. Antibody-mediated protection requires FcγR and FcRn expression. Epithelial and neurological disease scores in (**A**, **B**) FcγR$^{-/-}$ and (**C**, **D**) FcRn$^{-/-}$ mice receiving serum from control- or HSV-2 Δ*gD*$^{-/+gD-1}$-vaccinated wild-type mice and then challenged intravaginally with HSV-2(4674) (n = 5 mice per group).

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