Suppl. Table 1. Comparing two with three mRNA immunizations* when challenged at 1 month

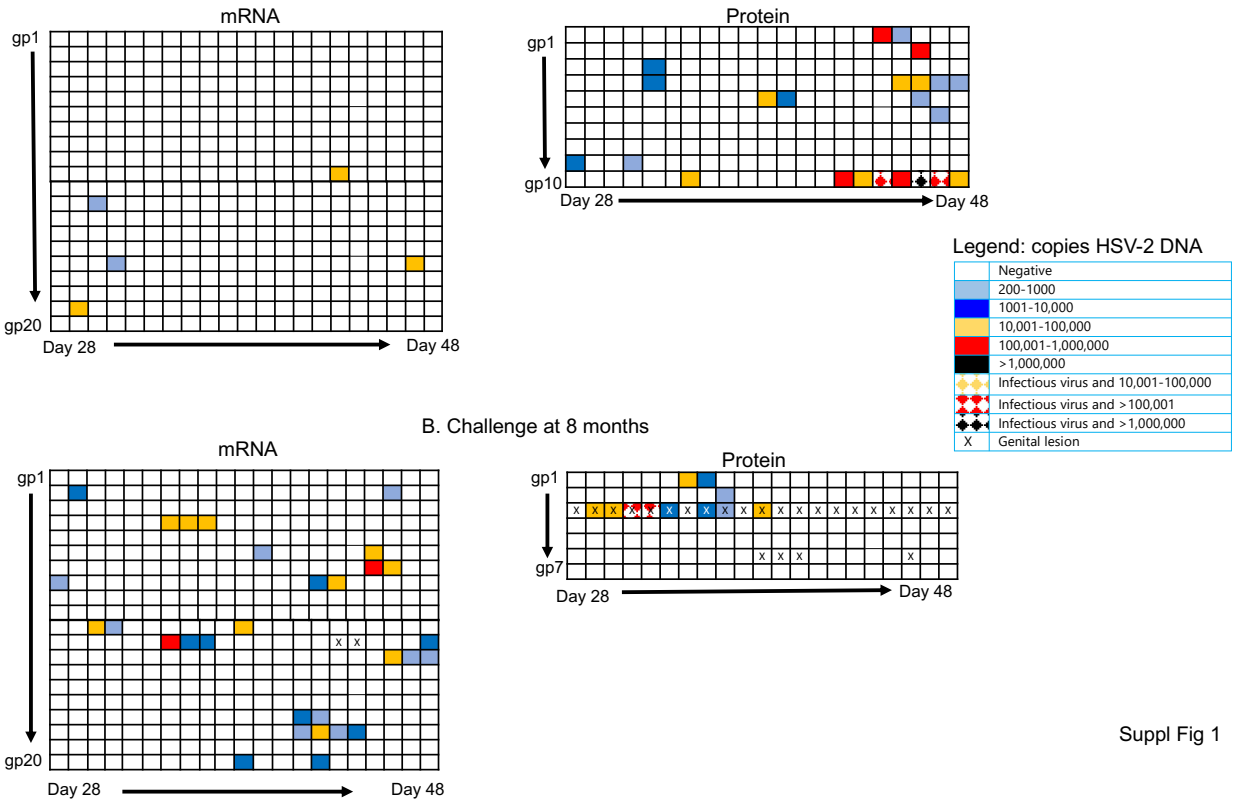
Outcome	2 immunizations	3 immunizations	P value
Serum neutralizing antibody titers ± SD	1:5,632 ± 1619	1:6,144 ± 2159	>0.9999
Vaginal neutralizing antibody titers ± SD	1:60 ± 39	1:58 ± 25	0.8250
Death	0/10 (0%)	0/10 (0%)	>0.9999
Genital lesions d1-48	0/10 (0%)	0/10 (0%)	>0.9999
Days with genital lesions	0/440 (0%)	0/440 (0%)	>0.9999
Urinary retention	0/10 (0%)	0/10 (0%)	>0.9999
Weight loss >5%	0/10 (0%)	0/10 (0%)	>0.9999
No. with positive vaginal swabs day 2	4/10 (40%)	6/10 (60%)	0.6563
Mean day 2 vaginal virus titers $\log_{10}$ ± SD	0.67 ± 1	1.36 ± 1.37	0.2783
No. with positive vaginal swabs day 4	1/10 (10%)	2/10 (20%)	>0.9999
Mean day 4 vaginal virus titers $\log_{10}$ ± SD	0.08 ± 0.25	0.32 ± 0.77	0.4737
No. with vaginal shedding HSV-2 DNA d28-48	1/10 (10%)	3/10 (30%)	0.5820
Days with vaginal shedding HSV-2 DNA d28-48	1/210 (0.5%)	4/210 (1.9%)	0.3720
Days with vaginal shedding HSV-2 replication competent virus d28-48	0/210 (0%)	0/210 (0%)	>0.9999

*Guinea pigs were immunized twice at months 0 ID and 3 IM, or three times at months 0 and 1 ID and 4 IM. P values for neutralizing titers and vaginal virus titers were calculated using the two-tailed Mann-Whitney test, while the other P values were calculated using the two-tailed Fisher's exact test.

Suppl. Table 2. Comparing ID with IM mRNA immunizations* when challenged at 8 months

Outcome	ID immunization	IM immunization	P value
Serum neutralizing antibody titers ± SD	1:2,688 ± 1409	1:2,560 ± 1045	>0.9999
Vaginal neutralizing antibody titers ± SD	1:13.5 ± 10.8	1:19.5 ± 14.6	0.3159
Death	0/10 (0%)	0/10 (0%)	>0.9999
Genital lesions d1-48	1/10 (10%)	2/10 (20%)	>0.9999
Days with genital lesions	1/440 (0.2%)	3/440 (0.7%)	0.6241
Urinary retention	1/10 (10%)	0/10 (0%)	>0.9999
Weight loss >5%	0/10 (0%)	0/10 (0%)	>0.9999
No. with positive vaginal swabs day 2	8/10 (80%)	7/10 (70%)	>0.9999
Mean day 2 vaginal virus titers $\log_{10}$ ± SD	2.79 ± 1.66	1.90 ± 1.63	0.2669
No. with positive vaginal swabs day 4	4/10 (40%)	4/10 (40%)	>0.9999
Mean day 4 vaginal virus titers $\log_{10}$ ± SD	0.99 ± 1.42	0.91 ± 1.42	0.9600
No. with vaginal shedding HSV-2 DNA d28-48	5/10 (50%)	6/10 (60%)	>0.9999
Days with vaginal shedding HSV-2 DNA d28-48	12/210 (5.7%)	18/210 (8.6%)	0.3436
Days with vaginal shedding HSV-2 replication competent virus d28-48	0/210 (0%)	0/210 (0%)	>0.9999

*Guinea pigs were immunized three times ID or IM at months 0, 1, and 2. P values for neutralizing titers and vaginal virus titers were calculated using the two-tailed Mann-Whitney test, while the other P values were calculated using the two-tailed Fisher's exact test.

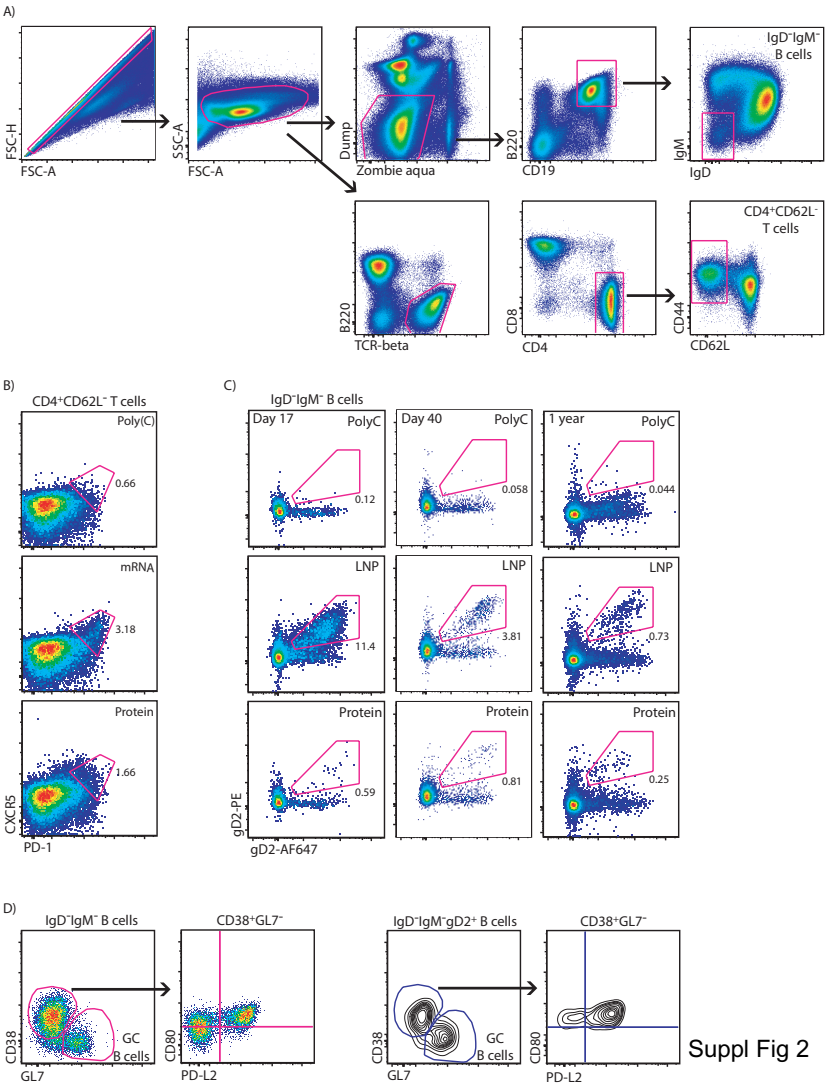


Supplementary Figure 1. HSV-2 DNA shedding and virus isolation on days 28 to 48 for each animal in the mRNA and protein groups. A. Guinea pigs in the mRNA (n=20) and protein (n=10) groups were infected one month after the last immunization. **B.** Animals in the mRNA (n=20) and protein (n=10) groups were infected eight months after the last immunization. Vaginal swabs were evaluated for HSV-2 DNA on days 28 to 48. Three of 10 animals in the protein group required humane euthanasia before day 28, leaving only 7 for HSV-2 DNA shedding studies. White boxes indicate no HSV-2 DNA detected. Color boxes indicate HSV-2 DNA was detected. The legend identifies the copy number based on the color. A stippled symbol was used to designate recovery of replication competent virus. Days with genital lesions are marked with an "x". Each row represents a separate guinea pig (gp) and each column a separate day.

Suppl. Table 3. Animals with latent infection[^]

Condition	mRNA		Protein	
	1 mo.	8 mo.	1 mo.	8 mo.[#]
Vaginal shedding HSV-2 DNA d28-48	4/20 (20%)	11/20 (55%)	8/10 (80%)	3/7 (43%)
DRG positive HSV-2 DNA	4/20 (20%)	5/20 (25%)	5/10 (50%)	3/7 (43%)
Spinal cord positive HSV-2 DNA	4/20 (20%)	6/20 (30%)	3/10 (30%)	5/7 (71%)
HSV-2 DNA detected at one or more site	7/20 (35%)[*]	12/20 (60 %)	8/10 (80%)[*]	6/7 (86%)

[^]Latent infection defined as positive HSV-2 DNA or replication competent virus detected in vaginal swabs days 28-48, or HSV-2 DNA detected in DRG or spinal cord at the end of the experiment. [#]The three sickest animals in the protein group at eight months required humane euthanasia prior to day 28; therefore, vaginal shedding, DRG and spinal cords were not evaluated for HSV-2 DNA in these three animals. [*]P=0.0502 comparing mRNA with protein at one month by two-tailed Fisher's exact test.



Supplementary Figure 2: Murine splenocyte phenotyping and gating strategy. A. Gating strategy to identify switched (IgD^-IgM^-) B cells and $CD4^+CD62L^-$ T cells within splenocytes. **B**. Representative gating of T_{FH} cells ($CXCR5^+PD\text{-}1^+$) within the $CD4^+CD62L^-$ T cell population for each treatment group at day 17 post immunization. **C**. Identification of gD2+ B cells within the IgD^-IgM^- B cell population for each treatment group at day 17, day 40 and one-year post immunization. **D**. Gating of germinal center (GC; $GL7^+CD38^-$) and memory ($GL7^-CD38^+$ and $CD80^+$ or $PD\text{-}L2^+$) subsets within total IgD^-IgM^- and $IgD^-IgM^-gD2^+$ B cells.

Suppl. Table 4. Summary of key murine results comparing mRNA and protein T_{FH} and $gD2^+$ B cells

Condition	Day 17, 1 immunization	Day 40, 1 immunization	1 year, 2 mRNA or 3 protein immunizations
Number T_{FH} cells	mRNA 2.7-fold higher	mRNA 2-fold higher	ND*
Number $gD2^+$ B cells	mRNA 118-fold higher	mRNA 7-fold higher	mRNA 3.4-fold higher
Phenotype $gD2^+$ B cells	mRNA 95% GC, protein 25% GC	mRNA memory 5.1-fold higher	mRNA memory 3.4-fold higher

*ND, not done