

Supplementary Fig. 1: Comparison of CCHFV GPC-specific antibody titers in CCHFV-M₁₀₂₀₀ vaccinated mice by ELISA coated with either secreted CCHF_{VLP} or CCHFV-IbAr 10200 CCHF_{VLP} transfected cell lysates.

Groups of 10 female C57BL/6 mice were vaccinated with 50 μ g CCHFV-M₁₀₂₀₀ or empty vector on days 0, 21, and 42 by IM-EP. Sera antibody responses were analyzed 21 days post third vaccination. ELISA plates were coated with either secreted CCHF_{VLP} or CCHFV-IbAr 10200 CCHF_{VLP} transfected cell lysates as indicated. Data are the group mean averages +/- SD. ****p <0.0001. p values were determined by one-way ANOVA with Tukey's post hoc test with a 95% confidence interval.

lgG



Supplementary Fig. 2: CCHFV- M_{10200} vaccination stimulates T_h1 and T_h2 cytokine signaling.

10 female C57BL/6 mice were vaccinated with 50 μ g CCHFV-M₁₀₂₀₀ or empty vector on days 0, 21, and 42 by IM-EP, and then euthanized on day 49. Sera were collected at the time of euthanasia and cytokine signaling was quantified by Luminex assay. Shown are significant differences in cytokines after vaccination. Data are the group mean averages +/- SD. *p <0.05; **p <0.01; ****p <0.0001. p values were determined by one-way ANOVA with Tukey's post hoc test with a 95% confidence interval.



Supplementary Fig. 3: *In vitro* expression of the glycoprotein genes from the CCHFV-M_{Afg09} DNA vaccine plasmid.

a The total (permeabilized cells) of G_C was examined 48 hours after transfection of 293T cells with CCHFV-M_{Afg09} over a dose curve ranging from 10 to 200 ng of plasmid. Figure is representative of 2 independent experiments performed in duplicate. Representative gating strategy for G_C expression in 293T cells transfected with 200 ng of **b** empty vector or **c** CCHFV-M_{Afg09}. *In vitro* expression by Western blot of **d** G_C (75 kDa) and **e** G_N (37 kDa) in 293T cells 48 hours after transfection of 250 ng of CCHFV-M_{Afg09} or empty vector. Note: As G_C is nonreduced, it runs larger than 75 kDa.



Supplementary Fig. 4: Schematic representations of truncated CCHFV DNA vaccines.

Local: Smith-Waterman Pr	stein alignment [Matrix: "BLOSUM62" Gap penalty: 10 Gap extension penalty: 1]
10200 M Afg	109-2990 M Gap Gap
Alignment 1>1684 1>	1984 7:Johanning 7:Johanning Adamina Coulint Lenguin Scote Lenguin 1984 903% 933% 90.0% 1.5.20 1.581 0 0 8.163 1.684
-	
Ruler	1 10 20 30 40 50 60 70 80 90 1 10 20 30 40 50 60 70 80 90
10200 M	MHISLMYAILCLQLCGLGETHGSHNETRHNKTDTMTTHGDNPSSEPPVSTALSITLDPSTVTPTTPASGLEGSGEVYTSPPITTGSLPLSETTPELPVT
Afg09-2990 M	MHISLMYAVECLOLCGLCGLCGTNGPHNGTEHNNTHVMTTPDDSSSPEPPVSTALPVTPDPSTVTPSTPASGLEGSGEVVTSSPITTKGLSLPENTSEPPAT
Ruler	110 120 130 140 150 160 170 180 190
10200 M	GTDTLSAGDVDPSTQTAGGTSAPTVRTSLPNSPSTPSTPQDTHHPVRNLLSVTSPGPDETSTPSGTGKESSATSSPHPVSNRPPTPPATAQGPTENDSHN
Afg09-2990 M	S V VT S SA SD T D S ST Q A G D T PT PT V R SL P S S P ST PS T S Q G I H P V N SL S V T S P N P E ST P T P S S K K N LA T N S P P T A S R P T T A Q P T N S H N
Ruler	210 220 230 240 250 260 270 290 290
10200 M	ATEHPESLTQSATPGLMTSPTQIVHPQSATPITVQDTHPSPTNRSKRNLKMEIILTLSQGLKKYYGKILRLLQLTLEEDTEGLLEWCKRNLGLDCDDTFF
Afg09-2990 M	T T E QLE S LT H LA T LG SM I S PT Q V L POS Y T S I A LOD I'H N S PT N R S K K N LDMET I LT LS GG LK KY Y GK I LK LL H LT LE E D E G L E WC K R N LG L C D T F F
Ruler	310 320 330 340 350 360 370 380 390
10200 M	QKRIEEFFITGEGHFNEVLQFRTPGTLSTTESTPAGLPTAEPFKSYFAKGFLSIDSGYYSAKCYSGTSNSGLQLINITRHSTRIVDTPGPKITNLKTINC
Afg09-2990 M	QKRIEEFFITGEGHFNEVLOFRTLGTLSTTEST HAGS PT VEPFKSYFAKGFLSIDSGY FSAKCYS RTSNSGLQLINV TRHSTRIADTPGPKITNLKTINC
Ruler	410 420 430 440 450 450 470 480 490
10200 M	INLKASIFKEHREVE INVLLPQVAVNLSNCHVVIKSHVCDVSLDIDGAVRLPHIYHEGVFIPGTYKIVIDKKNKLNDRCTLFTDCVIKGREVRKGQSVLR
Afg09-2990 M	
Ruler	510
10200 M	QYKTEIRIGKASTGF
Afg09-2990 M	

Supplementary Fig. 5: Sequence alignment of MLD and GP38.

Smith-Waterman protein sequence alignment of the MLD and GP38 domains of CCHFV-IbAr

10200 and CCHFV-Afg09-2990.