SUPPLEMENTAL MATERIAL

Figure S1

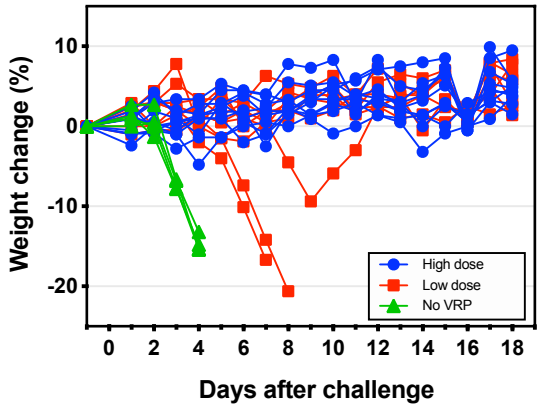

Figure S1. Individual mouse weight post-challenge. Mice were challenged SC with a lethal dose of CCHFV (100 TCID50) 32 days after high dose (n=10), low dose (n=9) or mock (n=3) VRP vaccination. Animal weights are shown as percentage weight change relative to a baseline set at 1 day before challenge.

Figure S2

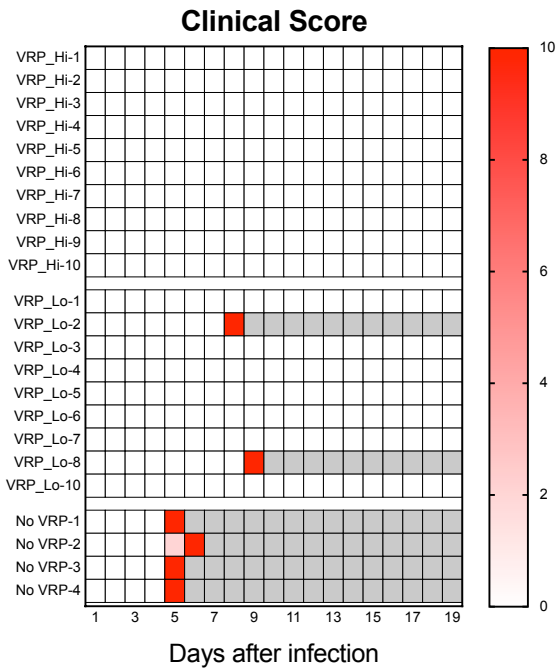

Figure S2. Clinical scores of individual mice post challenge. Mice were challenged SC with a lethal dose of CCHFV (100 TCID50) 32 days after high dose (n=10), low dose (n=9) or mock (n=3) VRP vaccination. Scoring criteria are detailed in the supplemental methods. A greater intensity of red indicates more severe disease signs. Grey shading indicates mice that are deceased.

Figure S3

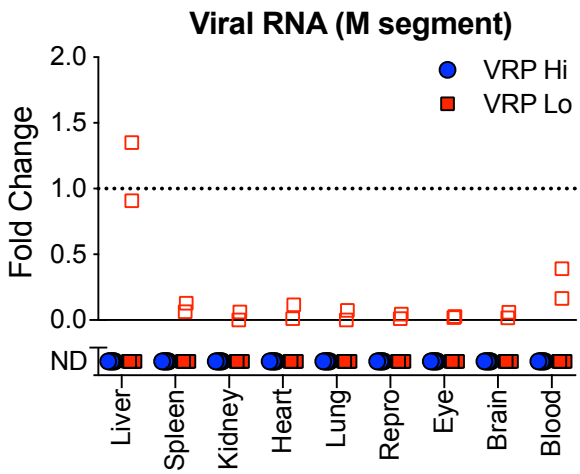

Figure S3. Detection of the CCHFV M segment in various organs by qRT-PCR. RNA was isolated from homogenized tissues either when animals reached clinical end points (open symbols) or at the end of the study (closed symbols). Datapoints indicate RNA levels relative to the unvaccinated control mice.

Supplementary Table 1. Serology and associated outcome post-challenge in CCHF VRP-vaccinated IFNAR$^{-/-}$ mice.

Study ID		DPI	Outcome	Gc		NP	
				Pre	Post	Pre	Post
No VRP	1	4	Fatal	-	-	-	-
	2	5	Fatal	-	-	-	-
	3†	4	Fatal	-	N/A	-	N/A
	4	4	Fatal	-	-	-	-
Low dose	1	18	Survivor	150	150	-	150
	2	7	Fatal	150	150	-	-
	3	18	Survivor	150	150	-	150
	4	18	Survivor	150	4050	50	12150
	5*	18	Survivor	-	12150	50	12150
	6	18	Survivor	150	150	-	50
	7	18	Survivor	150	-	-	50
	8	8	Fatal	150	450	50	-
	9**	18	Survivor	150	N/A	450	N/A
	10	18	Survivor	150	450	450	4050
High dose	1	18	Survivor	450	4050	450	12150
	2	18	Survivor	150	150	150	150
	3	18	Survivor	450	450	1350	1350
	4	18	Survivor	450	450	150	450
	5	18	Survivor	1350	1350	1350	1350
	6	18	Survivor	1350	4050	4050	12150
	7	18	Survivor	150	150	1350	12150
	8	18	Survivor	450	450	4050	4050
	9	18	Survivor	<250#	1350	750#	1350
	10	18	Survivor	450	1350	450	1350

Levels of IgG antibodies against CCHFV Gc and NP determined 28 days after vaccination (Pre), or at the end of the study or at time of euthanasia (Post). Values (1:x) represent the highest dilution at which a positive ELISA result was observed or the absence (-) of a positive reaction at 1:50. Animals were challenged subcutaneously with CCHFV IbAr10200 at 100 $TCID_{50}$ 32 days post VRP vaccination, or mock vaccination (No VRP). Low dose indicates subcutaneous VRP vaccination at 1×10^3 $TCID_{50}$; High dose indicates subcutaneous VRP vaccination at 1×10^5 $TCID_{50}$; †No post-challenge sample could be obtained; *Animal showed clinical signs 7 – 8 DPI; #Pre-bleed volume too small to test at 1:50, 1:250 was the lowest dilution tested; **Animal removed from study prior to challenge due to unrelated health issues.

Supplementary Material

VRP production

Six-well plates were seeded with 3.5×10^5 Huh7 cells/well 1 day prior to transfection in 3 mL of DMEM supplemented with 1% non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. 16–24 h later, cells were transfected with pT7-S (1 μg) or pT7-S-P2A-zsGreen (1 μg) to produce fluorescent reporter protein[8], together with pT7-L (1 μg), pCAGGS-L (0.33 μg), pCAGGS-N (0.66 μg), pCAGGS-GPC-Oman (1 μg), and pCAGGS-T7 (1 μg), combined with 12.5 μL of Mirus LT1 transfection reagent (Mirus Bio, Madison, WI, USA) in 250 μL of OPTI-MEM (Life Technologies, Grand Island, NY, USA) (Fig. 1a). Supernatants containing VRPs were harvested 4–5 days post transfection. VRP stocks were titrated by 50 percent tissue culture infective dose ($TCID_{50}$) on BSR/T7 cells using the Reed-Muench method. Positive wells were scored based on the detection of at least one CCHFV NP positive cells detectable by immunofluorescence using a rabbit anti-NP antibody (#04-0011, IBT Bioservices) and Alexa-488 goat anti-rabbit secondary antibody.

Animal experiments

Animal work was approved by the Centers for Disease Control and Prevention IACUC and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals, 8th Edition*, at an AAALAC-accredited facility. Female B6.129S2-*Ifnar1*^{tm1Agt}/Mmjax mice (MMRRC 032045-JAX; Jackson Laboratories), 8 weeks old and 17 – 21g at vaccination, were housed in a climate-controlled laboratory with a 12 h day/12 h night cycle. All animals were given sterile water and food *ad libitum*, and group housed (4 – 5/cage) on autoclaved corn cob bedding (Bed-o'Cobs® ¼", Anderson Lab Bedding), in an isolator-caging system (Thoren

Caging, Inc., Hazleton, PA, USA) with a HEPA-filtered inlet and exhaust air supply. The cage environment was enriched with shredded paper, cotton nestlets, and a hide-away structure. Mice were vaccinated subcutaneously (SC) under isoflurane anesthesia with either a high VRP dose (target dose: $1x10^5$, actual dose: $4.39x10^5$) or a low dose (target dose: $1x10^3$, actual dose: $4.64x10^3$). Thirty-two days post vaccination, animals were challenged SC under isoflurane anesthesia with a uniformly lethal dose (target dose: 100 $TCID_{50}$, actual dose: 37 $TCID_{50}$) of recombinant CCHFV-IbAr10200 (GenBank KJ648914, KJ648915, and KJ648913). Post vaccination blood collection was performed via the submandibular vein using a lancet (Monolet^{TM}). Clinical signs were scored based on 14 parameters: 2 points each for QDR (quiet, dull, responsive) disposition, hunched back, or ruffled coat; 3 points each for dehydration or abnormal huddling/hypoactivity; 5 points each for presence of neurological signs (ataxia, circling, tremors, or paresis), abnormal breathing, or anemia; 7 points for weight loss of >20% from baseline (d-1); 10 points each for inability to bear weight, paralysis, frank hemorrhage/bleeding, moribund state, or weight loss of >25% from baseline. Animals were humanely euthanized when end-point criteria were reached (clinical score ≥ 10), or at study completion (18 days post challenge).

CCHFV-Gc Construct Design

The construct of the ectodomain of the CCHFV Glycoprotein (Gc) was synthesized by cloning the Oman M segment coding region corresponding to the N-terminal signal peptide (aa. 1-29) followed by residues (999-1598) (ALT31693.1) and a C-terminal HisTag into a pcDNA3.1(+) vector.

CCHFV-Gc Expression and Purification

The Gc ectodomain expression plasmid was transformed into HEK Expi293 cells. Cells were grown in 300mL cultures at 37°C in Expi293 Expression Medium (Gibco A14351) for 72 hr until culture viability dropped below 50%. Cells were harvested by centrifugation at 2500*g* for 30 min and stored at -80°C. Frozen cell pellets were thawed at room temperature for 30 min and treated with 30mL M-PER (Thermo 78501) for 10 min with shaking. Cell lysates were separated into soluble and insoluble fractions via centrifugation at 14,000*g* for 15 min. The soluble fractions were syringe-filtered through 0.22 μm membranes. Filtered samples were loaded over a charged 5mL HisTrap HP column, washed with wash buffer [20 mM $NaPO_4$ (pH 7.6), 500 mM NaCl, and 20 mM Imidizole] until UV baseline, and eluted over a 3CV gradient from 0-100% elution buffer [20 mM $NaPO_4$ (pH 7.6), 500 mM NaCl, and 500 mM Imidizole] collecting 2 mL fractions. Pure fractions were pooled and samples were dialyzed twice in 5L of PBS for 2 hr at room temperature using the 3.5K Pur-A-Lyzer dialysis kit (Sigma PURG35020).

ELISA

To determine antibody titers, plasma was separated from whole blood collected in lithium heparin tubes by centrifuging 3 min at 8000 rpm. Samples were inactivated using gamma irradiation (5 million rads from a ^{60}Co source). CCHFV NP IgG was detected using a commercial ELISA kit (Alpha Diagnostics International AE-320400-1); CCHFV Gc IgG levels were determined using an in-house ELISA assay using purified Gc ectodomain bound to nickel-coated 96-well plates (1 μg per well)