**Supplementary Table 1.** Summary of clinical outcomes of unvaccinated control IFNAR-/- mice inoculated subcutaneously with CCHFV Turkey-200406546.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cage** | **Sex** | **CCHFV challenge dose\*** | **Euth or FD** | **DPI** | **Outcome** | **Highest clinical score** | **Total weight change at euth** |
| 11 | Male | 24 TCID50 | FD | 8 | Fatal | ≥10 | -24.9% |
|  | Male | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -27.2% |
|  | Male | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -27.1% |
|  | Male | 24 TCID50 | EUTH | 8 | Fatal | ≥10 | -26.2% |
| 12 | Female | 24 TCID50 | EUTH | 8 | Fatal | ≥10 | -26.0% |
|  | Female | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -26.6% |
|  | Female | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -26.0% |
|  | Female | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -25.7% |
| 13 | Male | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -20.2% |
|  | Male | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -23.7% |
|  | Male | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -17.5% |
|  | Male | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -20.7% |
| 14 | Female | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -21.3% |
|  | Female | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -20.9% |
|  | Female | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -25.8% |
|  | Female | 2.4 TCID50 | EUTH | 8 | Fatal | ≥10 | -32.3% |

All mice were challenged at 52 d of age. \*Target dose: 100 TCID50 or 10 TCID50; Actual dose: 24 or 2.4 TCID50, respectively. Euth, euthanized; FD, found dead. Mice with a clinical score of ≥10 met end-point criteria and were humanely euthanized. Mice found dead were allocated a score of ≥10.

**Supplementary Table 2.** Summary of clinical outcomes of VRP-vaccinated IFNAR-/- mice inoculated subcutaneously with CCHFV Turkey-200406546.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cage** | **Sex** | **Time of VRP vaccination** | **CCHFV challenge dose\*** | **Euth or FD** | **DPI** | **Outcome** | **Highest clinical score** | **Total weight change at euth** |
| 1 | Male | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +9.1% |
|  | Male | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +10.5% |
|  | Male | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +12.8% |
|  | Male | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +11.2% |
| 2 | Female | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +4.9% |
|  | Female | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +9.5% |
|  | Female | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +5.9% |
|  | Female | -14 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +9.1% |
| 3 | Male | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +6.8% |
|  | Male | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +7.4% |
|  | Male | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +3.7% |
|  | Male | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +9.5% |
| 4 | Female | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +6.4% |
|  | Female | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +7.6% |
|  | Female | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +13.9% |
|  | Female | -7 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +11.9% |
| 5 | Male | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 2 | +3.9% |
|  | Male | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +3.4% |
|  | Male | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +7.8% |
|  | Male | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +5.7% |
| 6 | Female | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 2 | +3.8% |
|  | Female | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +8.1% |
|  | Female | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +2.4% |
|  | Female | -3 dpi | 24 TCID50 | EUTH | 21 | Survivor | 0 | +3.1% |
| 7 | Male | -1 dpi | 24 TCID50 | EUTH | 6 | Fatal | ≥10 | -25.6% |
|  | Male | -1 dpi | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -23.5% |
|  | Male | -1 dpi | 24 TCID50 | EUTH | 8 | Fatal | ≥10 | -26.8% |
|  | Male | -1 dpi | 24 TCID50 | EUTH | 5 | Fatal | ≥10 | -15.4% |
| 8 | Female | -1 dpi | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -25.6% |
|  | Female | -1 dpi | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -28.6% |
|  | Female | -1 dpi | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -29.0% |
|  | Female | -1 dpi | 24 TCID50 | EUTH | 5 | Fatal | ≥10 | -19.6% |
| 9 | Male | +1 dpi | 24 TCID50 | EUTH | 8 | Fatal | ≥10 | -25.8% |
|  | Male | +1 dpi | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -26.0% |
|  | Male | +1 dpi | 24 TCID50 | EUTH | 7 | Fatal | ≥10 | -25.9% |
|  | Male | +1 dpi | 24 TCID50 | EUTH | 8 | Fatal | ≥10 | -27.0% |
| 10^ | Female | +1 dpi | 24 TCID50 | FD | 8 | Fatal | ≥10 | -22.0% |
|  | Female | +1 dpi | 24 TCID50 | EUTH | 5 | Fatal | ≥10 | -18.9% |
|  | Female | +1 dpi | 24 TCID50 | FD | 8 | Fatal | ≥10 | -24.9% |

All mice were challenged at 52 d of age. \*Target dose: 100 TCID50; actual dose: 24 TCID50. Euth, euthanized; FD, found dead. ^One mouse removed from +1 dpi VRP vaccination group prior to challenge due to severe malocclusion resulting in failure to maintain weight. Clinical score values range from 2–10. Mice with a clinical score of ≥10 met end-point criteria. Mice found dead were allocated a score of ≥10.

**Supplementary Methods:**

**Virus**

CCHFV Turkey-200406546 (GenBank: KY362517, KY362519, KY362515), isolated from a hospitalized patient with unknown clinical outcome, was passaged once in suckling mouse brain and once in SW13 cells. The virus stock was verified by next-generation sequencing and confirmed mycoplasma free. Viral stock titers and inoculum back-titers (calculated as TCID50) were determined by a method based on that of Reed and Muench on BSR-T7/5 cells fixed and stained at 5 dpi (rabbit anti-CCHFV NP pAb, IBT Bioservices 04-0011; 1:2500) and Alexa-488 goat anti-rabbit secondary antibody.

**VRP production and titration**

As previously described, 6-well plates were seeded with 3.5 × 105 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), pT7-L (1 μg), pCAGGS-L (0.33 μg), pCAGGS-NP (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. Supernatants containing VRPs were harvested 4–5 days post transfection. VRP stocks were titrated by TCID50 (Reed and Muench, 1938) on BSR-T7/5 cells, a generous gift from K.K. Conzelmann (Ludwig-Maximilians-Universität, Munich, Germany). Positive wells were scored based on the detection of at least one CCHFV NP-positive cell, visualized by immunofluorescence using a rabbit anti-NP antibody (IBT Bioservices 04-0011) and Alexa-488 goat anti-rabbit secondary antibody.

**VRP vaccination and CCHFV challenge in mice**

Groups of 8 (4 female and 4 male) B6.129S2-*Ifnar1tm1Agt*/Mmjax mice (MMRRC Stock No: 32045-JAX) were vaccinated SC in the interscapular region with 200 µL of CCHFV VRPs under isoflurane anesthesia at the indicated timepoints before or after challenge (target dose: 1 × 105 TCID50; back-titer dose: 2 × 104 TCID50). All mice, both vaccinated and unvaccinated, were challenged at 52 days of age SC as above with 100 µL of CCHFV Turkey-200406546 under isoflurane anesthesia. Unvaccinated mice challenged with a low dose of virus were inoculated in parallel with a 10-fold dilution of the same inoculum preparation given to the other mice.

**Viral RNA quantification**

To assess viral levels in tissues of vaccinated animals that survived or succumbed to infection, RNA was extracted from blood and homogenized tissue samples using the MagMAX-96 Total RNA Isolation Kit (Thermo-Fisher Scientific) on a 96-well ABI MagMAX extraction platform with a DNaseI treatment step according to manufacturer’s instructions. CCHFV S segment copy number was quantified using a one-step qRT-PCR targeting the Turkey-200406546 N gene sequence (Spengler et al., 2019). Values were standardized for each tissue type using 18S with a SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific) according to manufacturer’s instructions. Specifically, for each tissue type, 18S Ct values were averaged for all animals in the study to generate a tissue-specific Ct constant to which the individual tissue Ct values were normalized. Viral S genome copy numbers were calculated using standards prepared from in vitro-transcribed S genomic RNA and expressed per μL of eluted RNA.

**Statistical analysis**

Statistical significance of survival was determined using a log-rank (Mantel-Cox) test using GraphPad Prism v9.1.0.

**References for supplementary methods:**

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