Supplementary Information

An attachment glycoprotein nanoparticle elicits broadly neutralizing antibodies and protects against lethal Nipah virus infection

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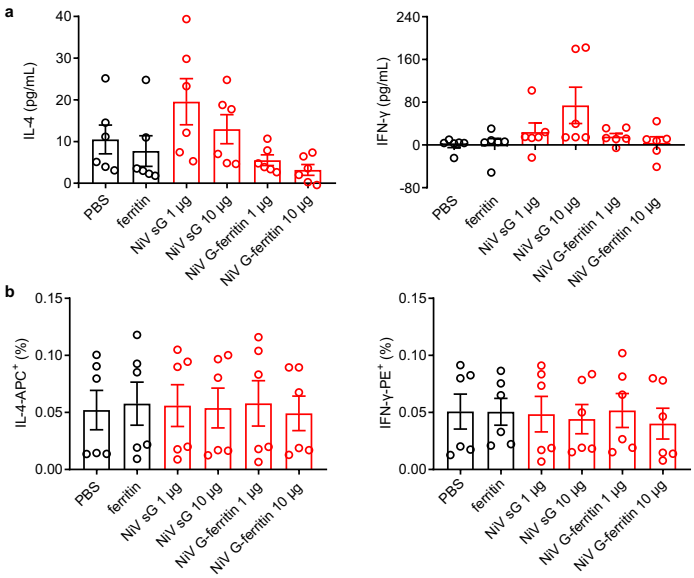
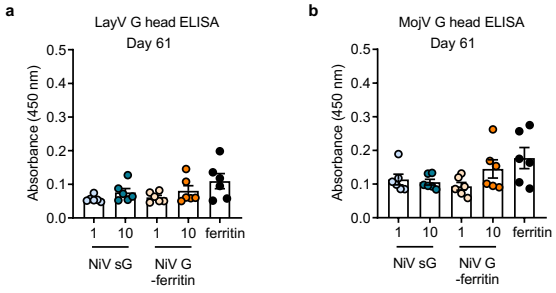
This PDF file includes:

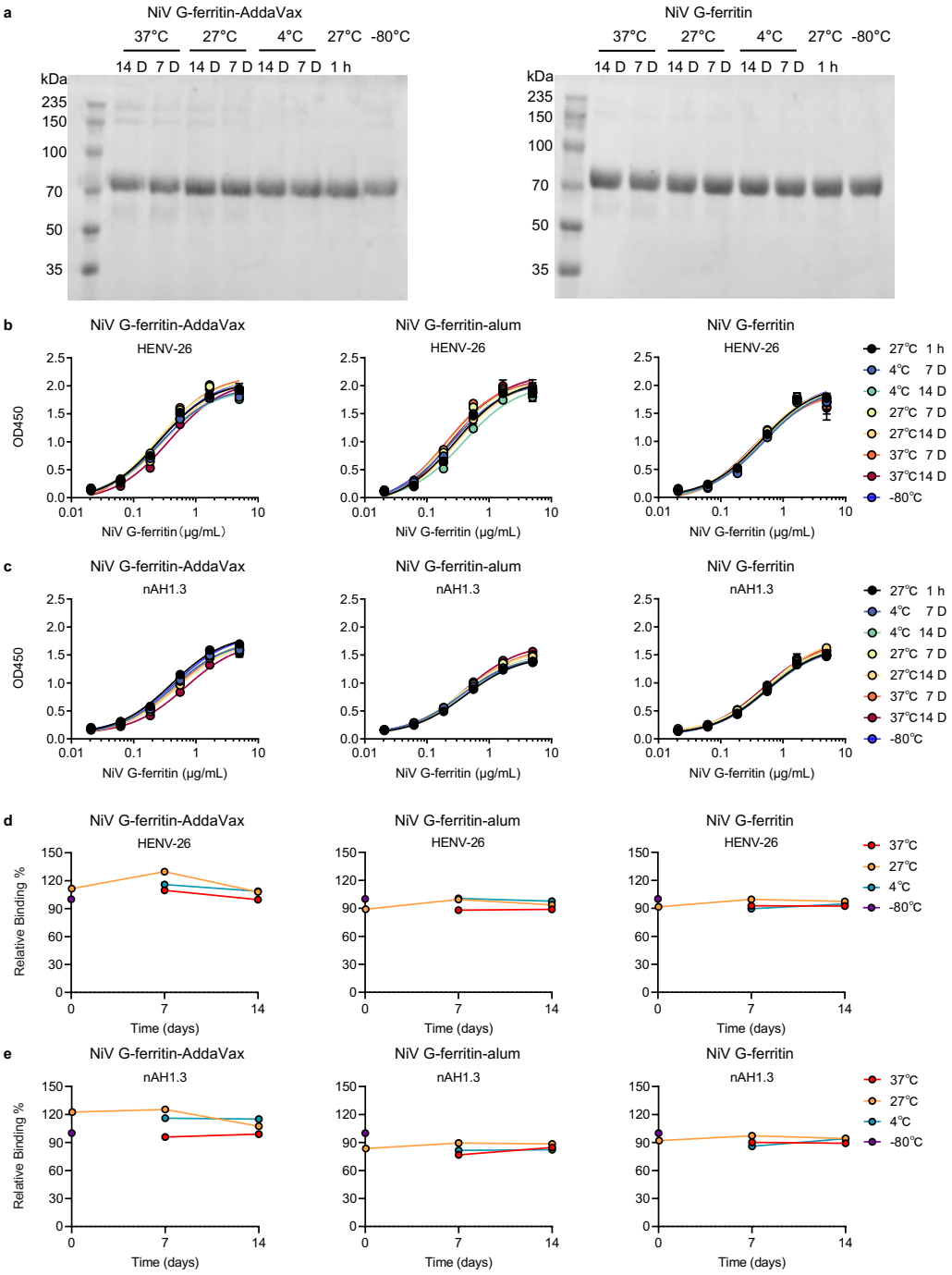
Supplementary Fig. 1 to 4



Supplementary Fig. 1 | Cellular immune response induced by NiV G-ferritin and NiV G immunogens. The mice were immunized as shown in Fig. 2a, and the mice's spleens were harvested ~three weeks after the third immunization for cellular immune response analysis. **a** Detection of IFN-γ and IL-4 in cell culture supernatant by ELISA. **b** Intracellular cytokine staining assay to measure the percentage of IFN-γ^+/IL4$^+$ cells stimulated with recombinant NiV sG protein. The experiments were performed in duplicate, and data in each group are shown as mean ± SEM.

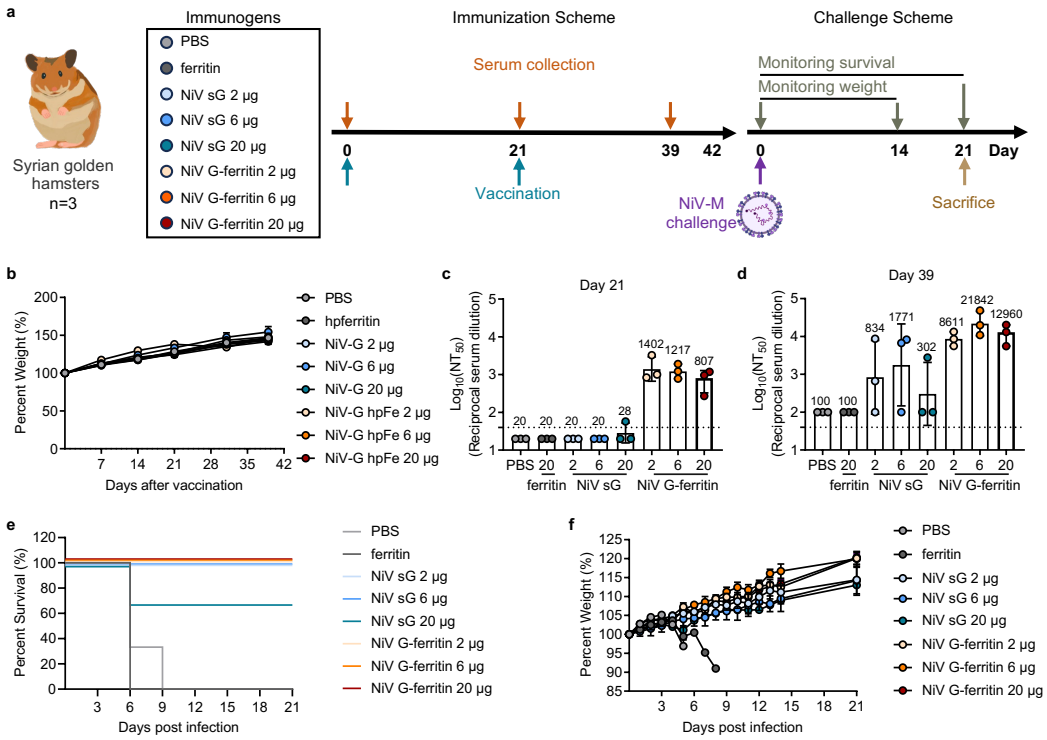


Supplementary Fig. 2 | NiV G nanoparticle barely induces cross-reactive antibodies to LayV G and MojV G proteins. a, b An ELISA measured anti-LayV G head domain **(a)** and anti-MojV G head domain **(b)** IgG levels in mice sera at Day 61 (~three weeks after the third immunization). Two independent experiments were performed in duplicate, and representative results are shown. Data in each group are shown as mean ± SEM. The data were related to Fig. 3.



Supplementary Fig. 3 | Antigenic stability of the NiV G-ferritin. a Reduced SDS-PAGE of NiV G-ferritin mixed with AddaVax adjuvant (left) or NiV G-ferritin alone (right) stored at 4°C, 27°C, and 37°C for 7 or 14 days, respectively. Samples stored at 27°C for 1 h and -80°C were included as positive controls. The gels were derived from the same experiment and processed in parallel. **b, c** An ELISA measuring the binding capacity of NiV G-ferritin or NiV G-ferritin adjuvanted with AddaVax (NiV G-ferritin-AddaVax) or Imject Alum (NiV G-ferritin-alum) to HENV-26 (**b**) or nAH1.3 (**c**) under different storage conditions. **d, e** Relative maximum binding to HENV-26 (**d**) or nAH1.3 (**e**) of analytes (NiV G-ferritin, NiV G-ferritin-AddaVax, and NiV G-ferritin-alum). The relative binding percentage of each sample to HENV-26 or nAH1.3 was

calculated to corresponding samples stored at -80℃, and the maximum binding signal of samples stored at -80℃ was defined as 100%. The data were related to Fig. 4.



Supplementary Fig. 4 | Two-dose NiV G-ferritin protected Syrian golden hamsters from the lethal NiV Malaysia challenge. a The two-dose experimental scheme of Syrian hamsters. The animals (n=3) were intramuscularly vaccinated twice with 2 μg, 6 μg, or 20 μg NiV sG, or equimolar NiV G-ferritin mixed with AddaVax adjuvant at a three-week interval, and then vaccinated hamster were challenged with the NiV Malaysia strains at 1000 LD_{50} via the i.p. route. Control group hamsters were immunized with PBS or equimolar (compared to 20 μg NiV G-ferritin) ferritin. **b** Weight change of Syrian hamsters during the immunization scheme. **c, d** NiV-M pseudovirus neutralization titers at Day 21 (three weeks after the first immunization) **(c)** and Day 39 (~three weeks after the second immunization) **(d)** for antisera from individual hamsters. Two independent experiments were performed in duplicate, and representative results are shown. **e, f** Survival **(e),** and weight change **(f)** of Syrian hamsters challenged with the NiV Malaysia strain. Data in each group are presented as geometric mean with geometric SD in **(c)** and **(d)** and mean ± SEM in **(b)** and **(f)**. Dashed lines indicate the limit of detection (LOD) of the assays. The values lower than LOD were calculated as half of LOD.