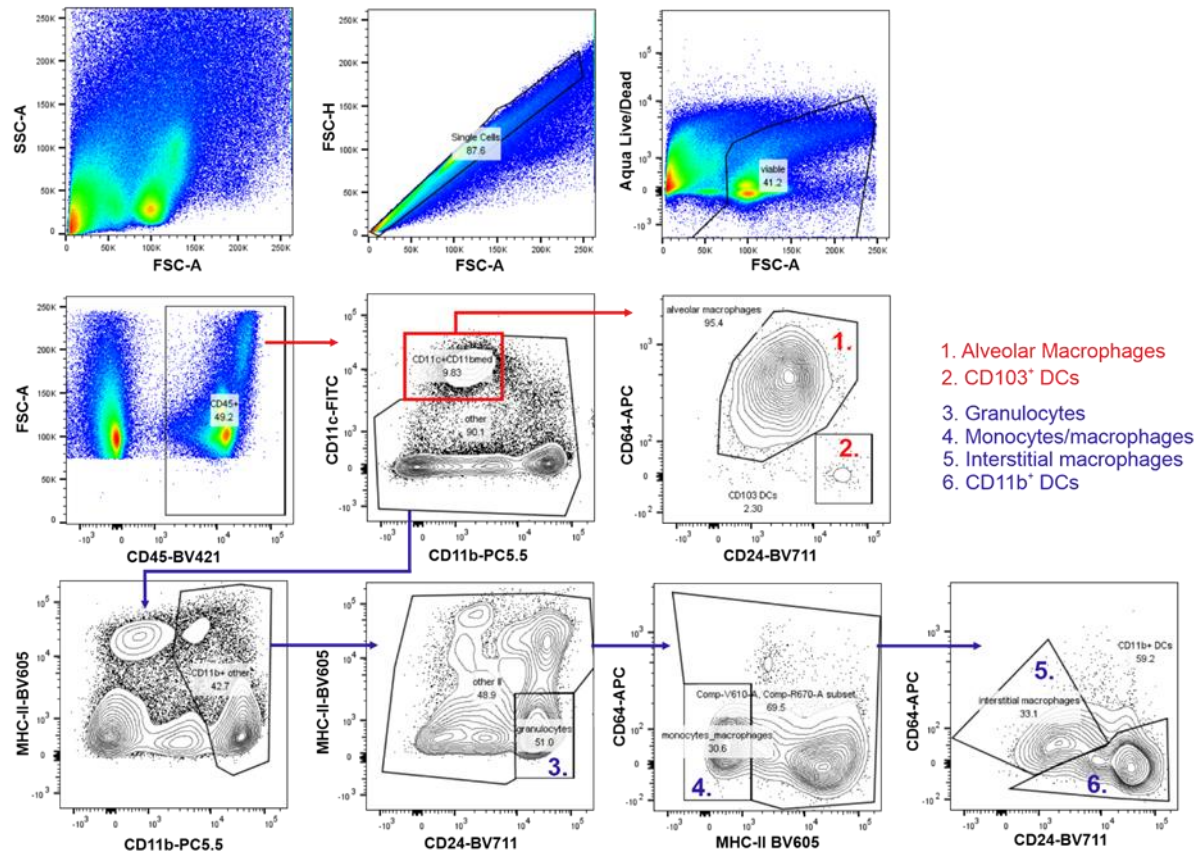# Supplemental Information

# mRNA Encoding a Bispecific Single

Domain Antibody Construct Protects

against Influenza A Virus Infection in Mice

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Supplementary information

Figure S1. Gating strategy for flow cytometric analysis of the nanoparticle uptake (DiR signal) and mCherry mRNA expression in pulmonary innate immune cells after intratracheal instillation of DOTAP/cholesterol nanoparticles containing 5 µg mCherry mRNA and fluorescent DiR dye. The gating strategy was based on a report from Knight *et al*[27]. Cells were selected on singlets (FSC-A, FSC-H) and viability (Aqua Live/Dead stain). Within the immune cell population (CD45-BV421), cell subsets were subsequently identified based on the surface markers: CD11c-FITC, CD11b-PC5.5., CD64-APC, CD24-BV711 and MHC-II-BV605. In each cell subset, uptake of nanoparticles (DiR-APC750) and mCherry mRNA expression (mCherry-Y610) were evaluated.
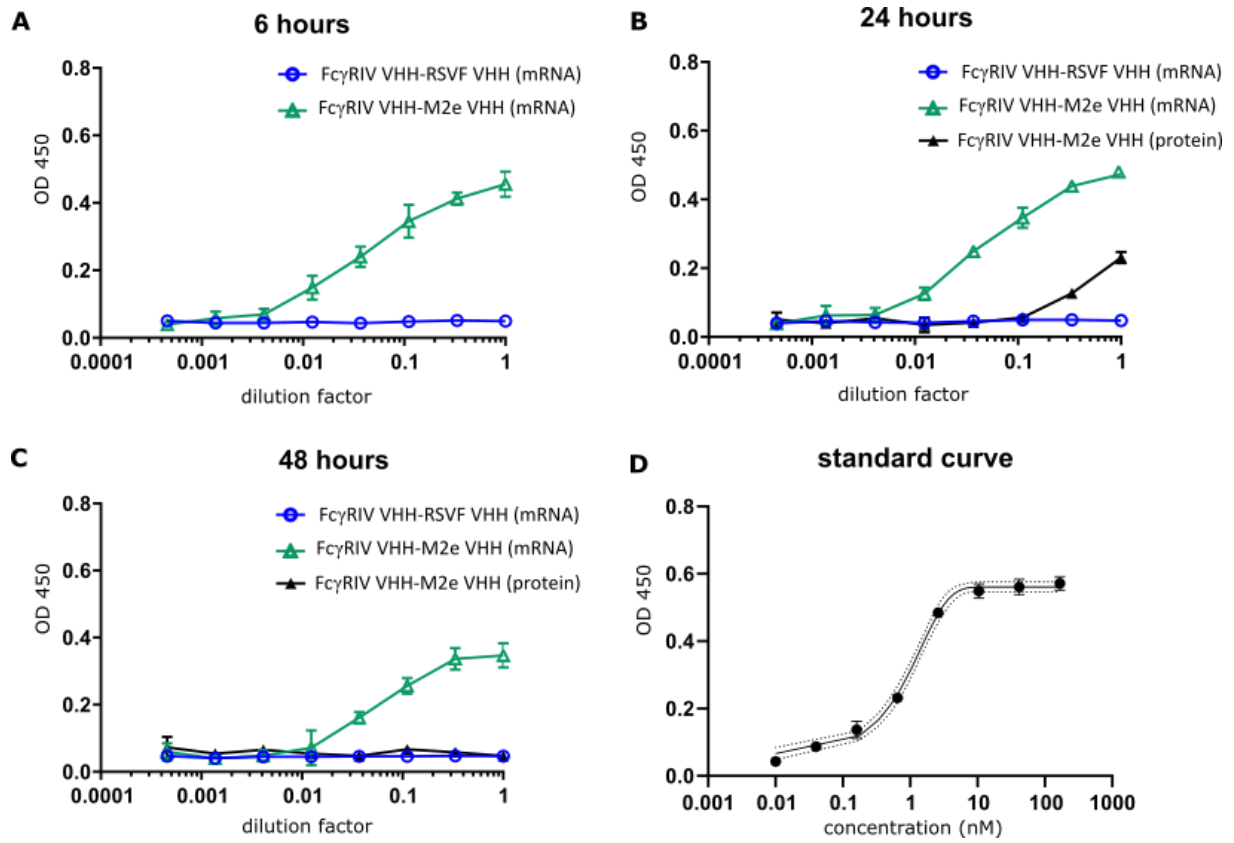
Figure S2. A, B and C. Five μg of FcγRIV VHH-M2e VHH or FcγRIV VHH-RSVF VHH (irrelevant mRNA) formulated in DOTAP/cholesterol particles or 50 μg FcγRIV VHH-M2e VHH protein was instilled i.t. in BALB/c mice. Six, 24 or 48 hours after instillation, BALF was isolated and cells were removed from the BALF and the ability of His$_6$-tagged proteins to bind to M2e was investigated in a peptide ELISA. **D.** Standard curve of binding between M2e and the M2e VHH. This standard curve was used to calculate absolute concentration of the M2e VHH in the BALF.