SUPPLEMENTAL METHODS

Analysis of T-cell response. The set of MARV GP-specific peptides was purchased from JPT Peptide Technologies (PepMix Marburgvirus (GP/Angola-05) and dissolved in DMSO according to the manufacturer's instructions. Mouse splenocytes were stimulated with MARV GP peptide mix (1 µg/ml) and CD28 monoclonal antibody (1 µg/ml) (Invitrogen, #14028182) for 6 h. The protein transport inhibitor brefeldin-A was added during the last 2 h of incubation (BD Biosciences, #555029). Following stimulation, cells were stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (ThermoFisher) and surface markers for CD19, CD11b, CD4 and CD8a (Biolegend Inc, PerCP/Cyanine5.5 anti-mouse CD19, #115534, PerCP/Cyanine5.5 anti-mouse/human CD11b, #101228, FITC anti-mouse CD4, #100510, APC/Cyanine7 anti-mouse CD8a, #100714) and then fixed with BD Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences). Subsequently, intracellular staining (ICS) was performed with IFN-γ, TNF-α, and IL-2 (Biolegend Inc, Brilliant Violet 605 anti-mouse IFN-γ, #505840, PE anti-mouse TNF-α, #506306, APC anti-mouse IL-2, #503810) and analyzed using LSRFortessa (BD Biosciences). The data were analyzed using FlowJo v10.9.0.

MARV GP and VP40 IgG ELISA. Enzyme-linked immunosorbent assays (ELISAs) were conducted as described previously (1). Briefly, 8 ng/well MARV GPΔTM (IBT Bioservices, #0506-015) or 50 ng/well MARV VP40 (IBT Bioservices, #0568-001) were coated on 96-well plates (Greiner, #655061). Serum samples were tested in four-fold dilutions starting from 1:10 or 1:16 to 11 dilutions for GP antibody detection in duplicates. For VP40 antibody detection, serum samples were diluted four-fold, starting from 1:10 to 10 dilutions in duplicates. The remaining steps were carried out as described previously (1).

Assessment of MARV neutralizing antibodies and viremia. Both assays were performed as previously described (2). Briefly, serum samples were diluted twofold, starting from 1:10 to 11 dilutions in duplicates. The diluted serum was mixed with 200 PFU of MARV and incubated for 1 h at 37°C. The mixtures were then added to Vero E6 cell monolayers and incubated for 1 h at 37°C. Then, the virus/serum mixture was removed and incubated for 4 days in methylcellulose and minimal essential medium mixture. Finally, the cells were fixed in formalin and immunostained to visualize plaques. Viremia in serum samples was determined by incubating diluted serum samples in Vero E6 cell monolayers for 1 h at 37°C and then formalin fixation and

immunostaining. Viremia in tissue samples was also determined in Vero E6 cells by plaque assay using tissue homogenates.

REFERENCES

1. Kimble JB, Malherbe DC, Meyer M, Gunn BM, Karim MM, Ilinykh PA, et al. Antibody-Mediated Protective Mechanisms Induced by a Trivalent Parainfluenza Virus-Vectored Ebolavirus Vaccine. *J Virol.* 2019;93(4): e01845-18.
2. Meyer M, Garron T, Lubaki NM, Mire CE, Fenton KA, Klages C, et al. Aerosolized Ebola vaccine protects primates and elicits lung-resident T cell responses. *J Clin Invest.* 2015;125(8):3241-55.