Anti-EBOV GP IgG ELISA. Anti-EBOV GP IgG ELISA titers were measured as described previously[8]. ELISA titers are expressed as EC_{90}, reciprocal serum dilution values, which represent the dilution at which there is a 90% decrease in antigen binding.

Intracellular cytokine staining. Whole-blood samples were collected and peripheral blood mononuclear cells (PBMCs) were isolated as described previously[8]. PBMCs were stimulated for 6 h at 37 °C with or without co-stimulation (CD28 and CD49d) plus Brefeldin A (Sigma-Aldrich) and either dimethyl sulfoxide (DMSO) or a pool of peptides spanning the entire EBOV GP open reading frame. The peptides were 15-mers overlapping by 11 amino acids reconstituted in fresh sterile DMSO. Following peptide stimulation, PBMCs were stained with a mixture of antibodies against lineage markers using one of the two following antibody panels (given as protein the antibody is directed to followed by the fluorescent label). Panel 1 consisted of primary staining with CD4 Alexa 700 (clone RPA-T4), CD8 PerCP-Cy5.5 (clone SK1), CD28 PE-Cy7 (clone 28.2), CD95 Cy5PE (clone DX2), CD14 PB (M5E2), CD20 PB (clone L27), and the viability dye ViVid to allow discrimination between live and dead cells, followed by 2 washes. The cells were then fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) and stained with CD3 APC (clone SP34-2), TNF-α FITC (MAb11), IFN-γ APC (clone B27), IL-2 PE (clone MQ14H12). Panel 2 consisted of primary staining with CD4 QD605 (clone M-T477) or CD4 BV421 (clone OKT4), CD45RA CY7PE (clone L48), CD28 Cy5PE (clone CD28.2), and AquaViD a viability dye to allow for discrimination between live and dead cells, followed by 2 washes. The cells were then fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) and stained with CD8 PB (clone RPA-T8) or CD8 BV570 (clone RPA-T8), CD69 ECD (clone TP1.55.3), CD3 Cy7APC (clone SP34-2), IFN-γ APC (clone B27), IL-2 PE (clone MQ1-17H12), TNF-α FITC (clone Mab11). Cytokine-positive cells were defined as a percentage within $CD4^+$ and $CD8^+$ T cell memory subsets. Memory subsets were defined as either $CD45RA^+CD95^+$, $CD28^+CD95^+$ or $CD45RA^+CD28^+$. Samples were acquired on an LSR II cytometer (BD Biosciences), collecting up to 1,000,000 events and analyzed using FlowJo (Tree Star, Inc.) and SPICE software[25] (http://www.niaid.nih.gov/LABSANDRESOURCES/RESOURCES/BIOINFORMATICS/SOFTWAREAPPLICATIONS/Pages/spice.aspx#niaid_inlineNav_Anchor). Boolean gating was used to define subsets of T cells expressing all combinations of IFN-γ, IL-2, and/or TNF-α.

Detection of EBOV. RNA was isolated from plasma of EBOV-exposed NHP by real-time qPCR as described previously[26]. Primers and probe were specific for the EBOV GP gene (GenBank accession no. AF086833) (Forward, 1,000 nM: TTT TCA ATC CTC AAC CgT AAg gC; Reverse, 1,000 nM: CAg TCC ggT CCC AgA ATg Tg (Oligos Etc.); Probe, 100 nM: 6FAM - CAT gTg CCg CCC CAT CgC TgC - TAMRA (Applied Biosystems)). The absolute quantification was compared to a viral RNA standard curve using LC480 software (version 1.5.0.39) and a standard calibrator on each plate. The viral standard curve was created through serial dilution and extraction of virus stock with known PFU/mL to yield PFU equivalents (relative PFU).

Statistical analyses. Comparison of anti-GP ELISA IgG titers and intracellular cytokine production by T cell memory subsets were calculated using the Mann-Whitney test (GraphPad Prism software).

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