Supporting Information

McElroy et al. 10.1073/pnas.1502619112

SI Materials and Methods

ELISA. MaxiSorp plates (Nalgene-Nunc) were coated with 30 ng per well of Ebola virus NP (His-tagged; GenScript) or coated with 30 ng per well of Lassa virus G1 protein (negative control; GST-tagged; GenScript) diluted in PBS; the protein was allowed to adsorb overnight at 4 °C. Plates were blocked in 2% (vol/vol) FBS in PBS with 0.05% Tween-20 (PBST) for 1 h at 37 °C. Patient plasma samples were serially diluted in blocking buffer and then incubated on blocked plates for 2 h at 37 °C. After three washes in PBST, plates were incubated for 1 h at 37 °C with anti-human IgG HRP (Accurate Chemical and Scientific) diluted 1:4,000, or with anti-human IgM (Jackson ImmunoResearch) diluted 1:5,000 in blocking solution. Following three PBST washes, the plates were incubated in ABTS [2, 2′-azino-di(3-ethylbenzthiazoline-6-sulfonate)] substrate (KPL) for 5 min; reactions were stopped with the addition of 1% SDS, and plates were read at 405 nm. Data were analyzed using Excel (Microsoft) and Prism (GraphPad) software.

Antigen-Specific B-Cell ELISpot. Ninety-six–well filter plates (Millipore) were coated with whole-cell lysate from Vero-E6 cells infected with Ebola virus (1:200 lysate dilution), whole-cell lysate of uninfected Vero-E6 cells (1:2,000 dilution), 100 ng per well of Ebola virus NP (His-tagged; GenScript), 100 ng per well of Lassa virus G1 protein (GST-tagged; GenScript), or 50 ng per well of anti-human IgG (MT91/45; Mabtech) diluted in PBS. The plates were left to adsorb overnight at 4 °C. Plates were then washed four times in PBS and incubated in RPMI medium with 10% (vol/vol) FBS for 30 min at 37 °C. RPMI was removed, and freshly prepared PBMCs suspended in RPMI with 10% FBS were placed in each well, with threefold dilutions down the plate. Plates were incubated at 37 °C overnight. The following day, plates were washed four times in PBST and incubated for 2 h at room temperature with biotinylated anti-human IgG (MT78/145; Mabtech) at 1 μg/mL diluted in PBST with 1% FBS. Plates were washed four times in PBST and incubated for 1 h at room temperature with streptavidin-HRP (Mabtech) diluted 1:1,000 in PBST with 1% FBS. Plates were washed three times each in PBST and PBS, and then incubated with TMB (3,3′,5,5′-tetramethylbenzidine) substrate for 15 min until spot development. Plates were washed with water and allowed to dry, and images were obtained using a CTL ELISpot plate reader.

Antigen-Specific T-Cell Assays. Cryopreserved PBMCs (5×10^5 to 1×10^6) were rested overnight and then incubated for 6 h at 37 °C with pooled virus peptides at a final concentration of 2 μg/mL of each peptide in the presence of CD28 and CD49d (diluted 1:100; BD Biosciences) and GolgiPlug (1:1,000 dilution; BD Biosciences). Peptide pools were constructed as 15-mers with 10-amino acid overlaps from the following viral proteins: NP (pool 1, 75 peptides; pool 2, 74 peptides), VP24 (49 peptides), VP30 (56 peptides), VP35 (66 peptides), VP40 (64 peptides), and GP (pool 1, 76 peptides; pool 2, 79 peptides). Negative-control samples were left unstimulated, and positive-control samples were treated with Staphylococcal enterotoxin B at a final concentration of 3 μg/mL. Following experimental and control stimulation, PBMCs were stained for the surface markers PB CD3-558117, Ax700 CD4-557922, and APC Cy7 CD8-560179 (all from BD Biosciences) and Aqua live/dead stain L34947 (Life Technologies). After fixation/permeabilization with Cytofix/Cytoperm, cells were stained for the intracellular markers FITC IL-2 340448, PE Cy7 TNF-α 557647, and APC IFN-γ 554702. After the cells were washed, data were collected on an LSRII (BD Biosciences) and analyzed using FlowJo software (Tree Star).

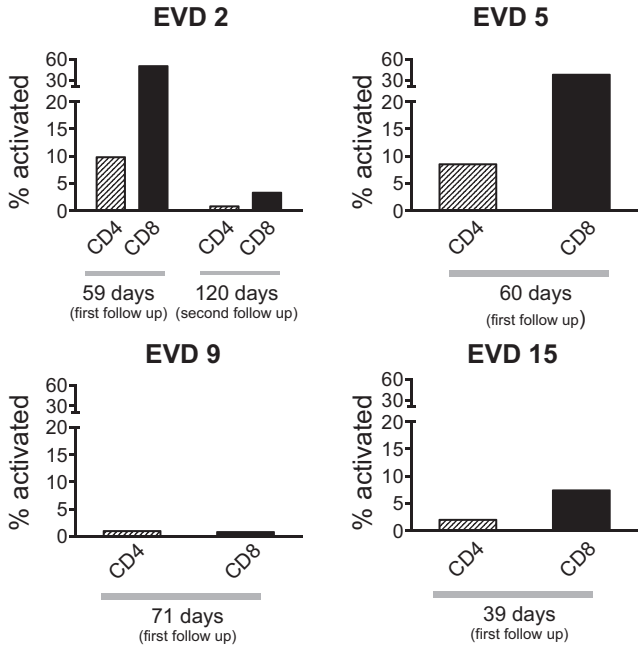

Fig. S1. CD4 and CD8 T-cell responses in convalescent EVD patients. Graphical representation of the frequency of activated CD4 and CD8 T-cell populations when measured during follow-up visits for all four patients. EVD5, EVD9, and EVD15 samples were from the first follow-up visit. Days noted in all panels are days post symptom onset.

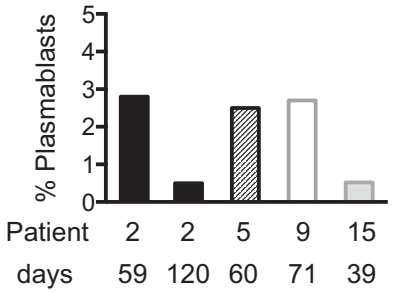

Fig. S2. Convalescent plasmablasts. Plasmablasts ($CD27^{+}CD38^{+}$ $CD19^{+}$ cells) were identified in convalescent samples by flow cytometric staining. Days indicate the days after symptom onset that the samples were collected.

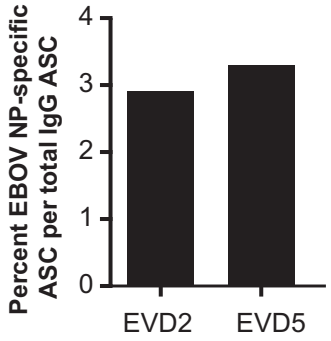

Fig. S3. B-cell responses during convalescent EVD. Graphical representation of Ebola NP-specific ASCs, obtained from EVD2 (59 d post symptom onset) and EVD5 (60 d post symptom onset) at their first follow-up visit, as a percentage of total IgG-secreting cells.

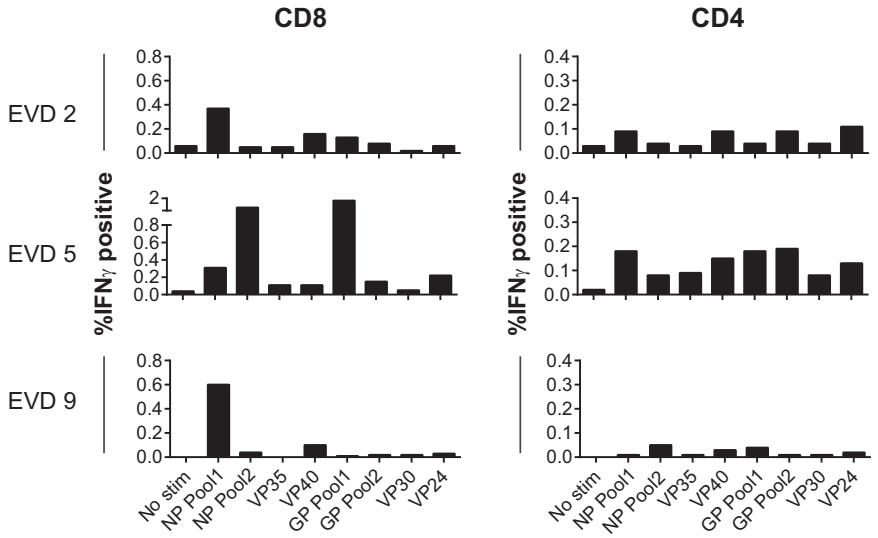

Fig. S4. Total IFN-γ–producing CD4 or CD8 T cells. Graphical representation of the percent of IFN-γ–positive CD8 T cells or CD4 T cells in EVD2, 5, and 9 after incubation with pools of peptides from various viral proteins as indicated.

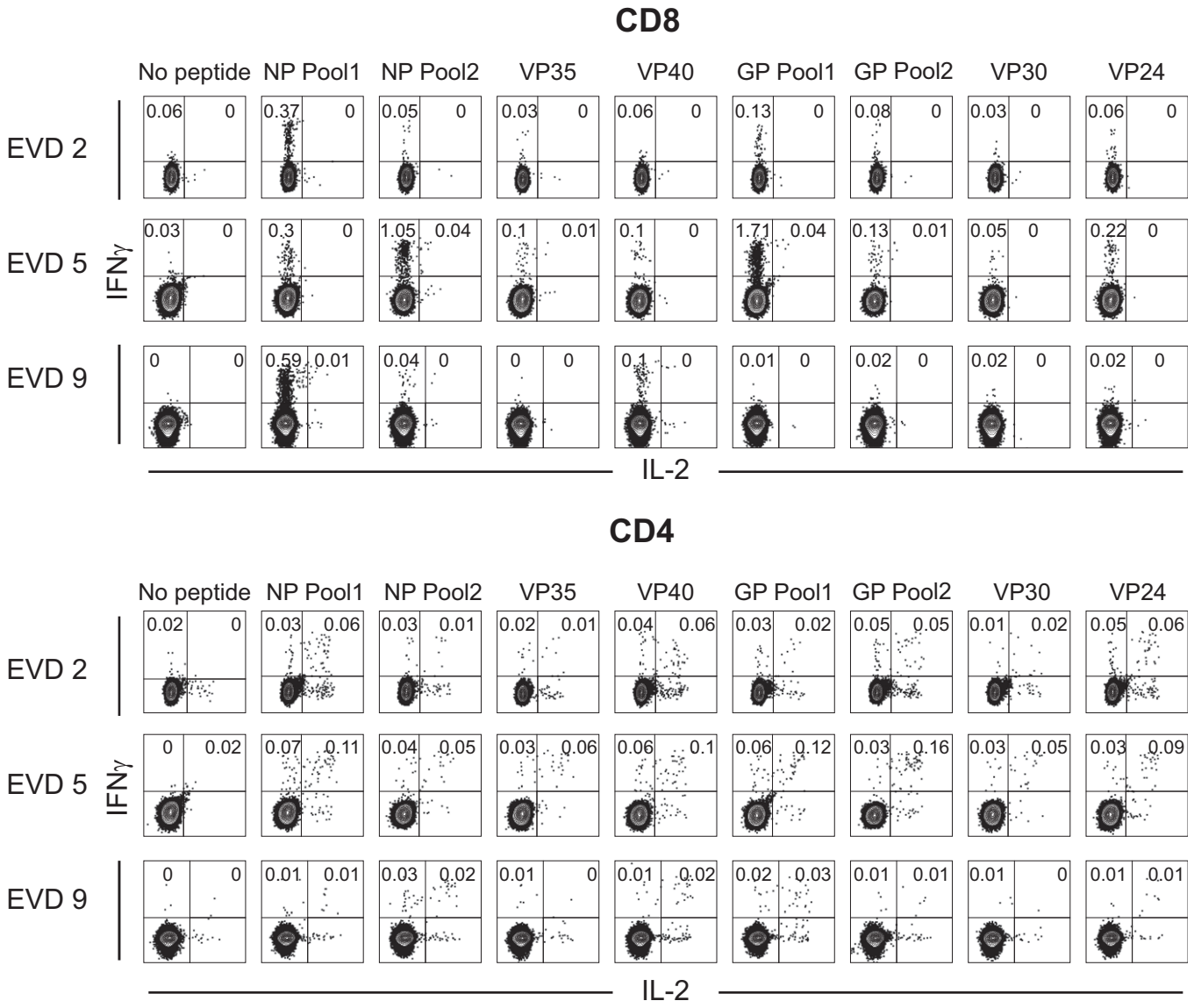

Fig. S5. Antigen specificity of CD4 and CD8 T cells in EVD patients. Flow cytometry plots showing IFN-γ and IL-2 expression in response to stimulation with the indicated peptide pools from Ebola viral proteins. Assays were performed on PBMCs obtained at 28, 144, and 71 d post onset of symptoms.

Table S1. Treatment of Ebola patients

Patient	Therapies administered beyond supportive care, d post onset of symptoms	Severity of disease
EVD2	ZMapp on days 9, 12, and 15	Moderate
EVD5	ZMapp on days 10, 13, and 17	Moderate
EVD9	TKM-100802 on days 3–8; convalescent plasma on days 8, 9, 11, 12, 14, and 15	Severe
EVD15	Brincidofovir on days 1 and 4; convalescent plasma on days 2 and 3	Mild

The patients were admitted to the Emory University Hospital at various times post onset of symptoms; EVD2 on day 12, EVD5 on day 15, EVD9 on day 5, and EVD15 on day 2.