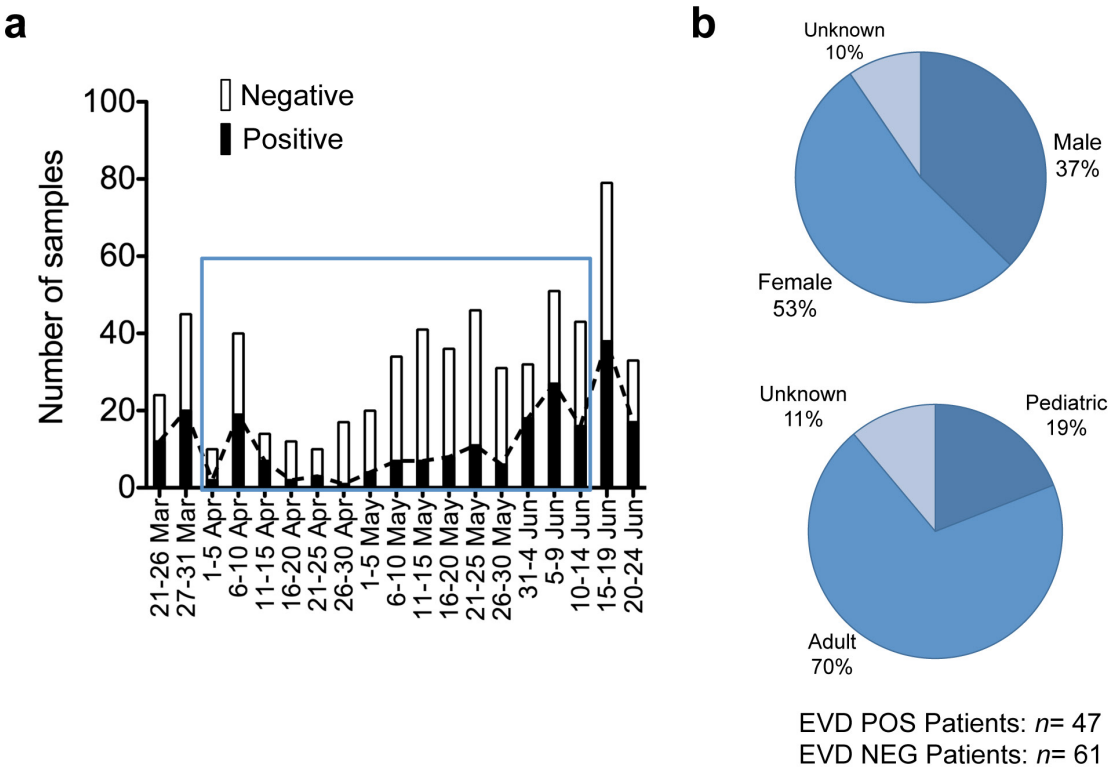
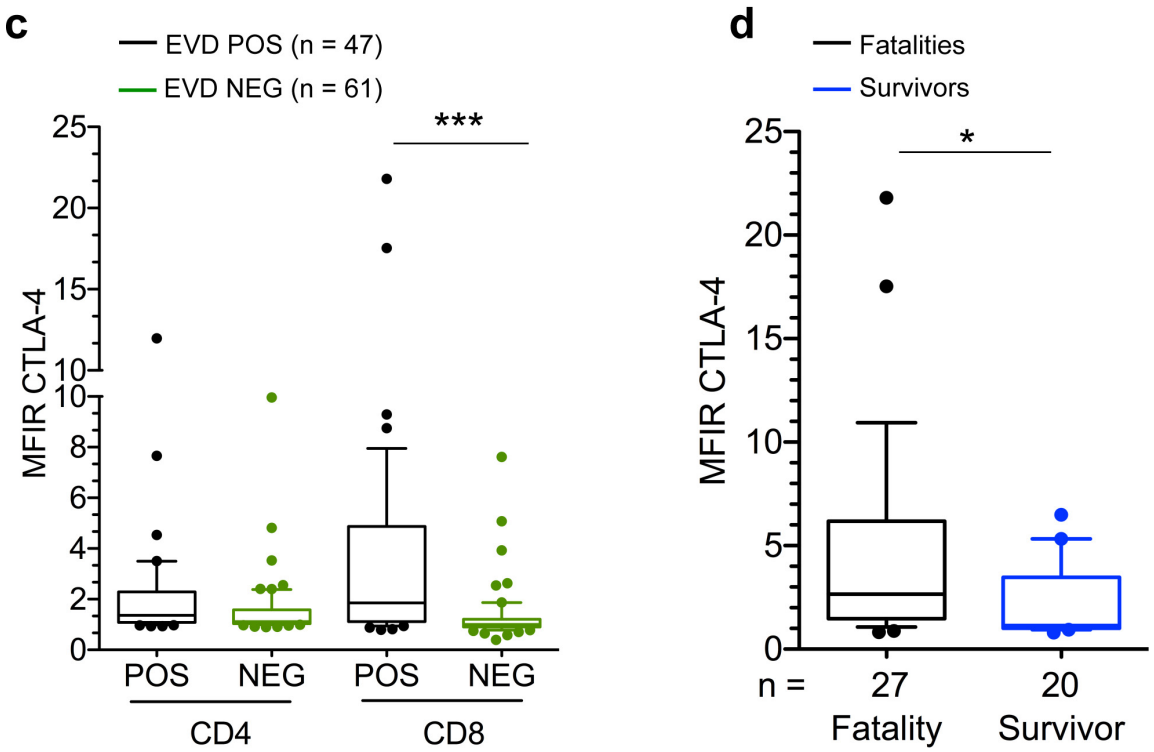
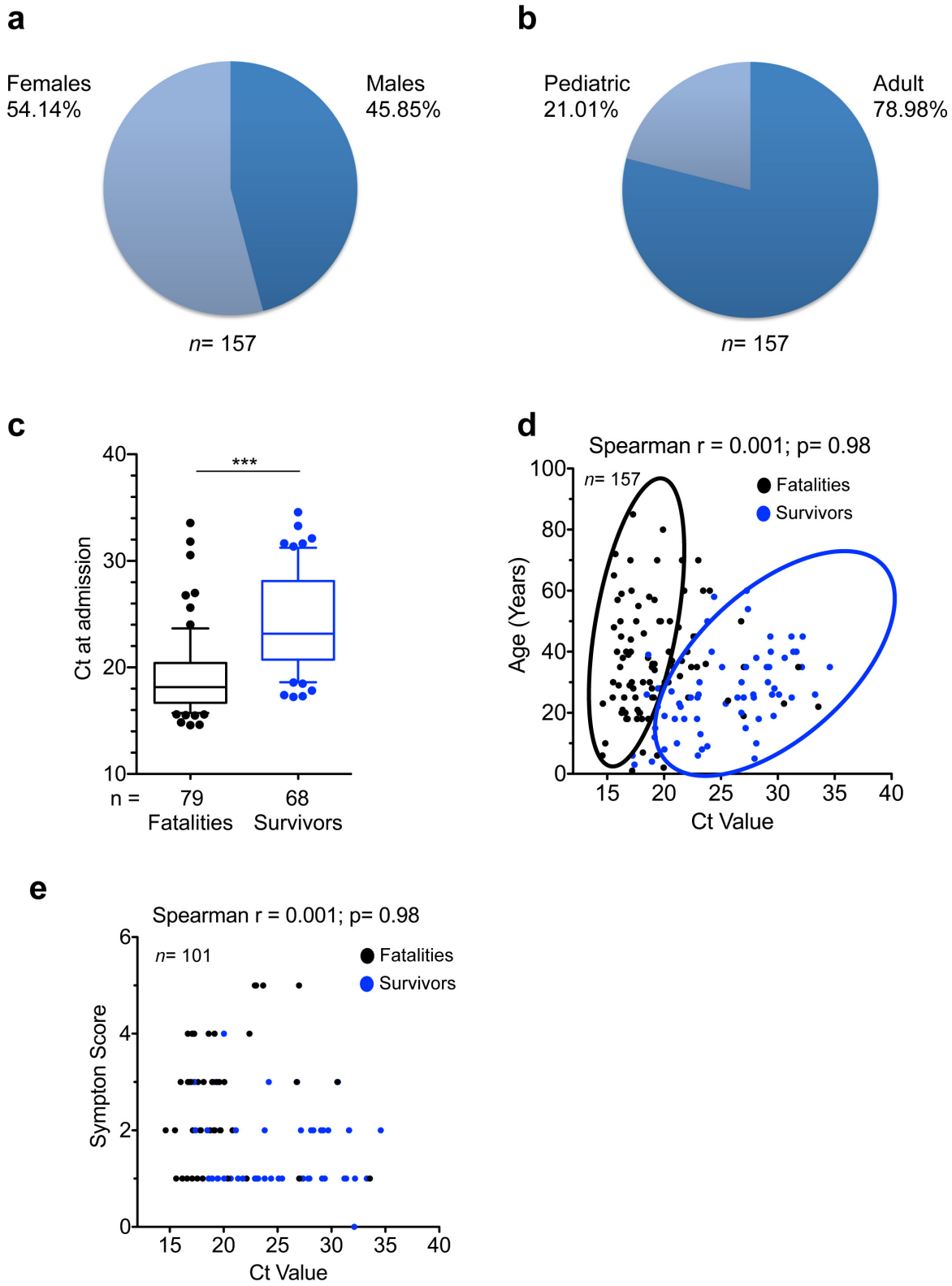
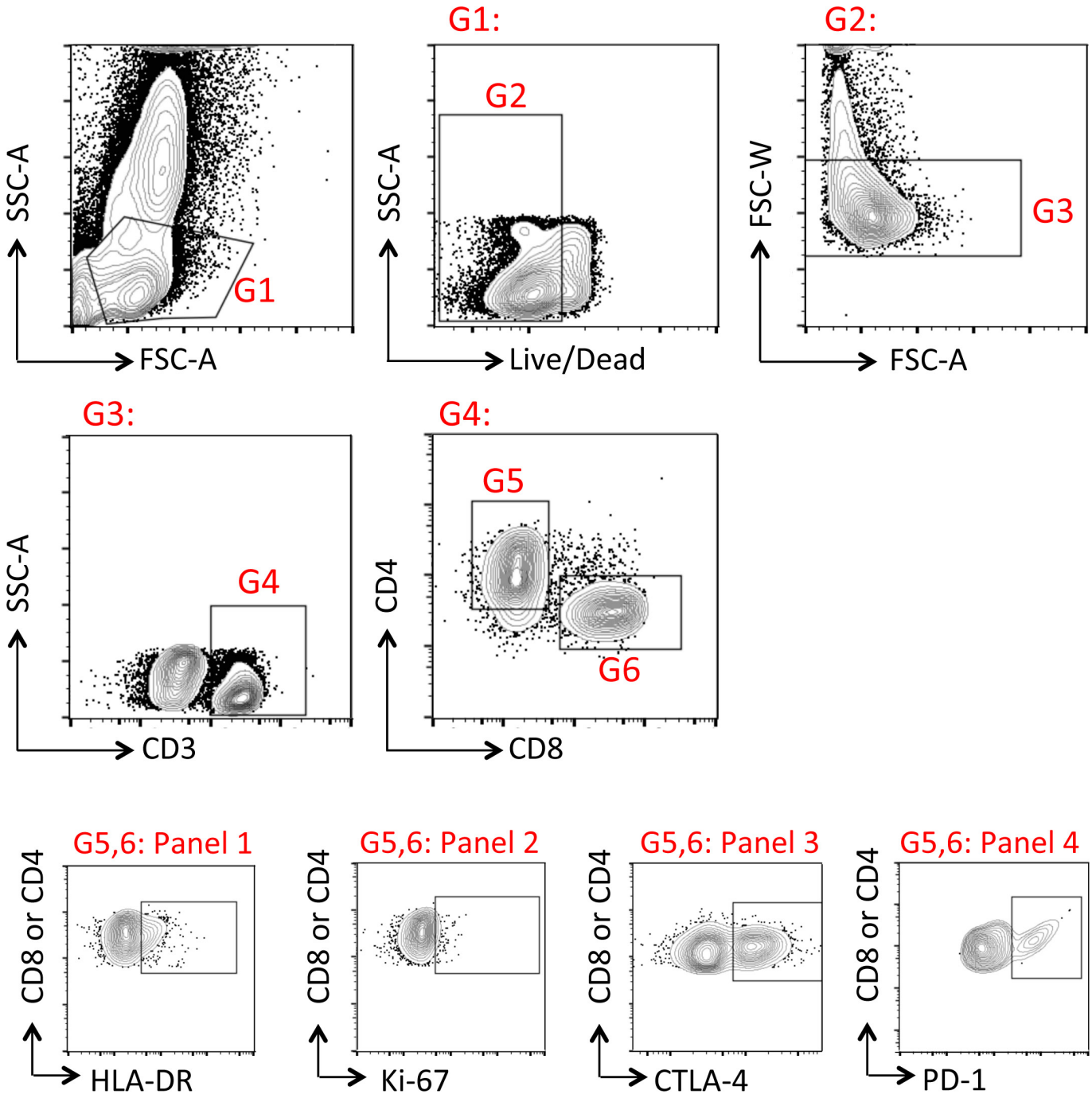




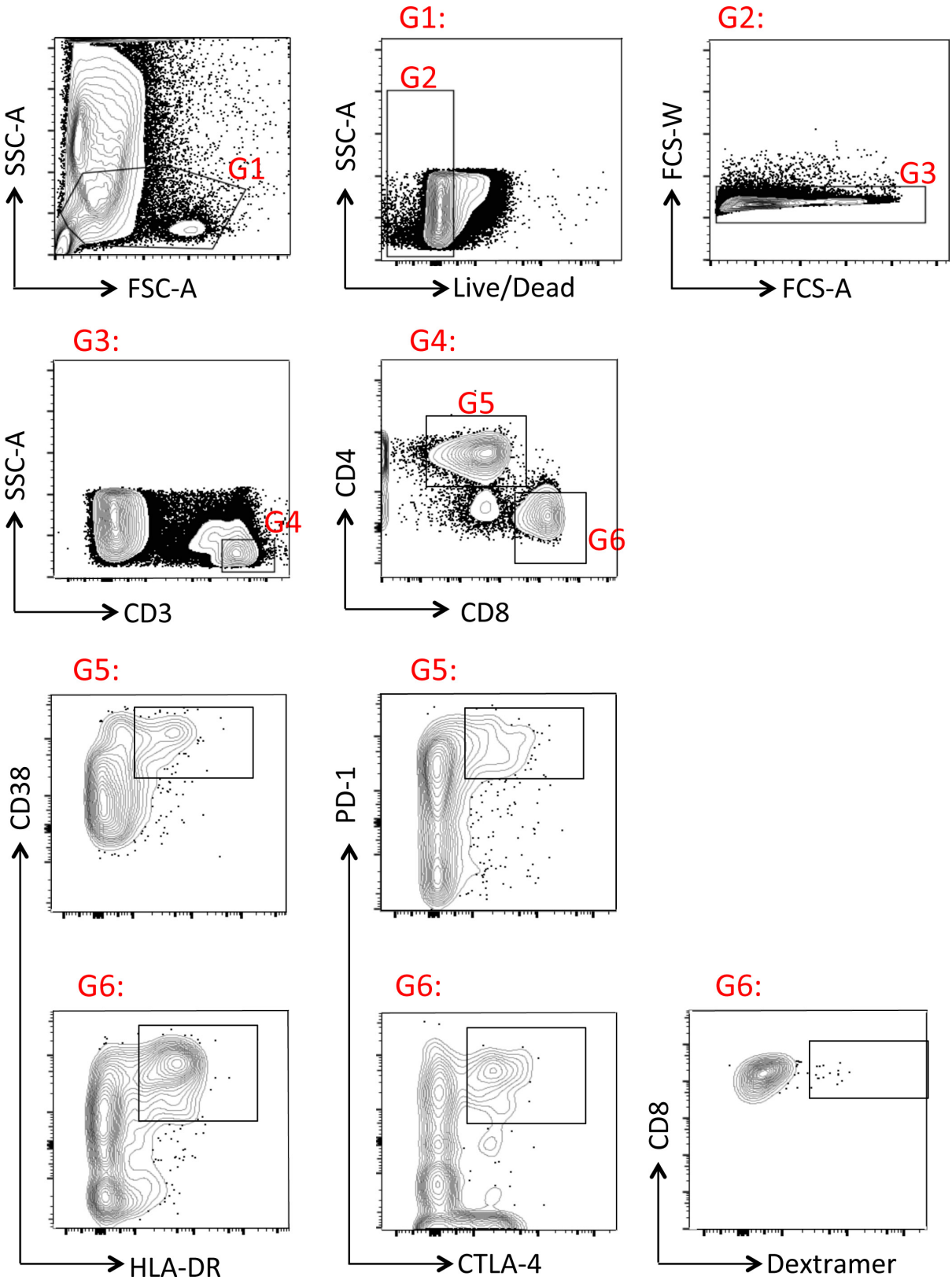
Extended Data Figure 1 | Initial immunophenotyping data from Guéckédou. a, Graph depicting the number of samples tested by the EMLab unit in Guéckédou by function of time since the beginning of the outbreak. The blue square indicates the period in which leftover whole blood samples from the diagnostic activities were shipped to the BSL-4 laboratory in Hamburg for initial immunophenotyping. **b**, Demographic data of the Guéckédou EVD patient cohort. Adults were ≥ 18 years of age and paediatric patients were <18 years of age. **c**, Comparison of the expression of CTLA-4 assessed by median fluorescence intensity ratio (MFIR) in $CD4^+$ and $CD8^+$ T cells of EVD patients (POS, black boxes) and non-EVD controls (NEG, green boxes). MFIR represents the ratio between the CTLA-4-specific signal divided by the fluorescence minus one (FMO) signal of the same cell population. **d**, Comparison between CTLA-4 MFIR values in $CD8^+$ T cells from fatal (black) versus surviving (blue) EVD cases. In all panels, the ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentile, respectively. Statistical analysis was performed by non-parametric Mann–Whitney test; $*P \leq 0.05$; $***P \leq 0.0001$.



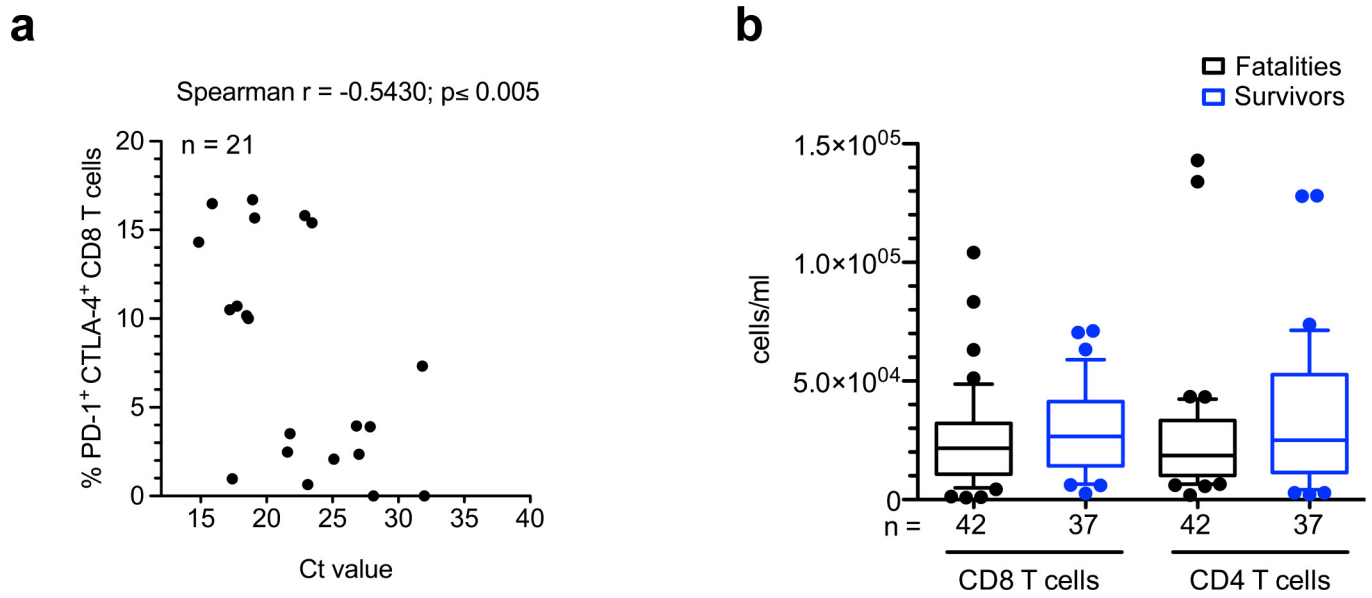
Extended Data Figure 2 | Epidemiological data of patients tested by EMLab unit in Coyah. a, **b**, Demographic data of the Coyah EVD patient cohort. Adults were ≤18 years of age and paediatric patients were <18 years of age. The median age of the 157 patients in the study was 26 years (interquartile range (IQR) 20–38 years). Percentages of males and females were comparable within all groups, with adults accounting for 79% of patients. **c**, Box-and-whisker plots depicting statistical association between C_t values and outcome. The case–fatality ratio (CFR) was 51.6%. Fatalities and survivors were compared via non-parametric Mann–Whitney test; ***$P < 0.001$. **d**, Correlation between C_t value and age of the patients. The C_t value did not correlate with age. However, survivors clustered in a group characterized by C_t value higher than 18 and by age less than 40 years (cluster encircled in blue). Statistical significance was tested by non-parametric Spearman correlation analysis. **e**, C_t values correlated negatively with symptom scoring, so that low C_t values were associated with severe disease symptoms. Symptom score was calculated as the summation of individual symptoms (bleeding, liver dysfunction, respiratory distress, kidney failure, neurological symptoms and anorexia) from '0' (no symptoms) to '6' (all symptoms present). In the box-and-whisker plot the ends of the whiskers represent the 10th and 90th percentile, respectively. Statistical analysis was performed by non-parametric Mann–Whitney test; ***$P \leq 0.0001$.



Extended Data Figure 3 | Gating strategy for flow cytometry studies in Guinea. All samples evaluated in the field were aliquoted for four panels. All panels had the following common gating: G1, lymphocyte gate; G2, live cells; G3, singlets; G4, T cells; G5, $CD4^+$ T cells; G6, $CD8^+$ T cells. Panels 1, 2, 3 and 4 evaluated expression of HLA-DR, Ki-67, CTLA-4 and PD-1, respectively, in either $CD4^+$ or $CD8^+$ T cells.



Extended Data Figure 4 | Gating strategy for flow cytometry in Hamburg. Cryopreserved PBMC samples from Coyah were thawed as indicated in the Methods. The following gates were used for sample analysis: G1, lymphocyte gate; G2, live cells; G3, singlets; G4, T cells; G5, $CD4^+$ T cells; G6, $CD8^+$ T cells. In G5 or G6, samples were evaluated for co-expression of the indicated cell markers. Dextramer staining was evaluated in G6 following protocols described in the Methods.



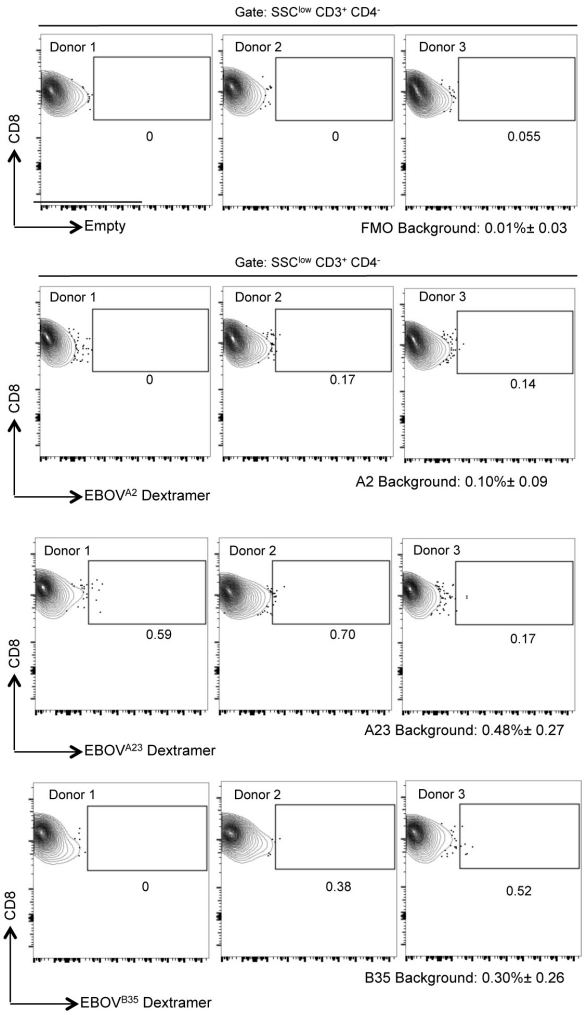
Extended Data Figure 5 | Correlation of double positive PD-1$^+$/CTLA-4$^+$ CD8$^+$ T cells with C_t values and lymphopenia. a, Graph showing correlation between the frequency of CD8$^+$ T cells co-expressing PD-1 and CTLA-4 and the C_t value. Correlation analysis was done via non-parametric Spearman correlation test. **b**, Box-and-whisker plots depicting the concentration of CD4$^+$ and CD8$^+$ T cells in blood of fatal and surviving EVD patients. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentile, respectively.

a

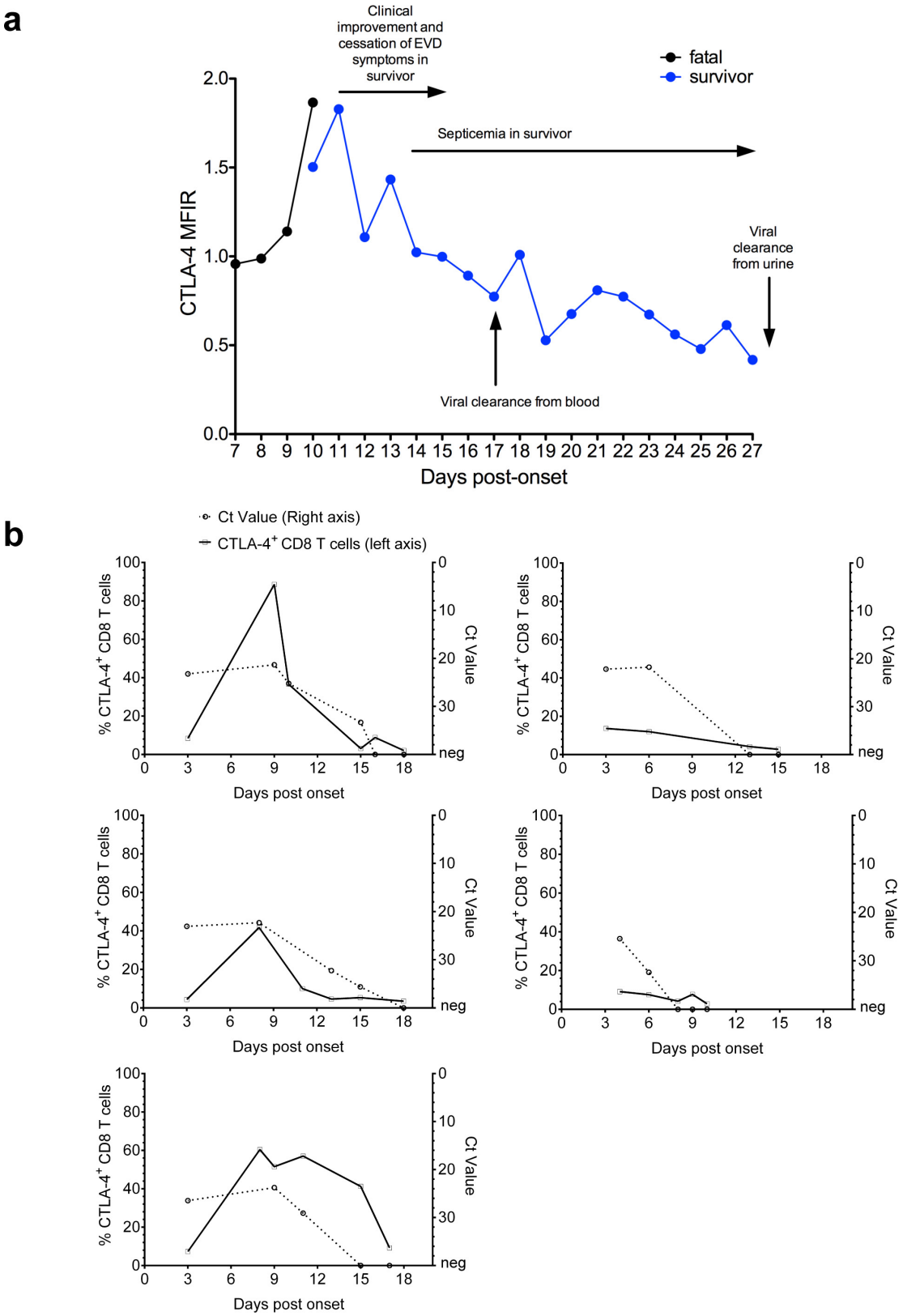
	Aminoacid position in NP		Sequence	IC_{50}
HLA-A*02.01	74	82	LLMLCLHHA	15.6
	83	91	YQGDYKLFL	26.68
	116	124	RLEELLPAV	15.31
	150	158	FLSFASLFL	11.48
	202	210	RLMRTNFLI	11.88
	311	319	GLFPQLSAI	35.09
	404	412	KLTEAITAA	23.86
	669	677	HMMKDEPVV	40.46
HLA-A*23.01	82	90	AYQGDYKLF	40
	313	321	FPQLSAIAL	7
	688	696	YPDSLEEEY	7
HLA-B*35:01	179	187	HAEQGLIQY	12
	660	668	GPFDAVLYY	19
	58	66	QAFEAGVDF	21
	603	611	TVAPPAPVY	23
	421	429	YDDDDDIPF	27

Red: Previously published peptides with affinity for the indicated HLAs. IC_{50}= Half maximal inhibitory concentration (nM). Only peptides with predicted IC_{50}< 50nM were selected. Blue squares= Peptides chosen for dextramer design.

b



Extended Data Figure 6 | *In silico* peptide analysis and dextramer design. a, Selection of peptides consisting of nine amino acid residues corresponding to the EBOV nucleoprotein sequence predicted to bind the indicated HLA alleles. IC_{50} values for peptide binding to HLA were predicted by the artificial neural network (ANN) at the Immune Epitope Database and Analysis Resource (IEDB) (http://www.iedb.org). **b**, Dextramer background was determined by staining of HLA-matched healthy donor peripheral blood leukocyte samples. T cells were gated as indicated in Extended Data Fig. 4. Plots in the upper row represent staining of a FMO (fluorescent minus one) sample in which the APC channel was left empty. Lower rows show background dextramer staining as indicated. The mean background staining plus minus standard deviation is indicated for each dextramer and the FMO.



Extended Data Figure 7 | Longitudinal analysis of CTLA-4 expression in the CD8$^+$ T cell compartment during the course of EVD in two patients. a, Graph depicts the levels of expression of CTLA-4 in CD8$^+$ T cells of a fatal versus a surviving EVD case over the course of the disease. Both patients were treated in Europe. Samples were taken at consecutive days starting immediately after patient admission as indicated. MFIR represents the ratio between the CTLA-4-specific signal divided by the fluorescence minus one (FMO) signal of the same cell population. **b**, Longitudinal analysis of CTLA-4 expression in CD8$^+$ T cells and C_t values in survivors from Coyah.