

Supplementary Material

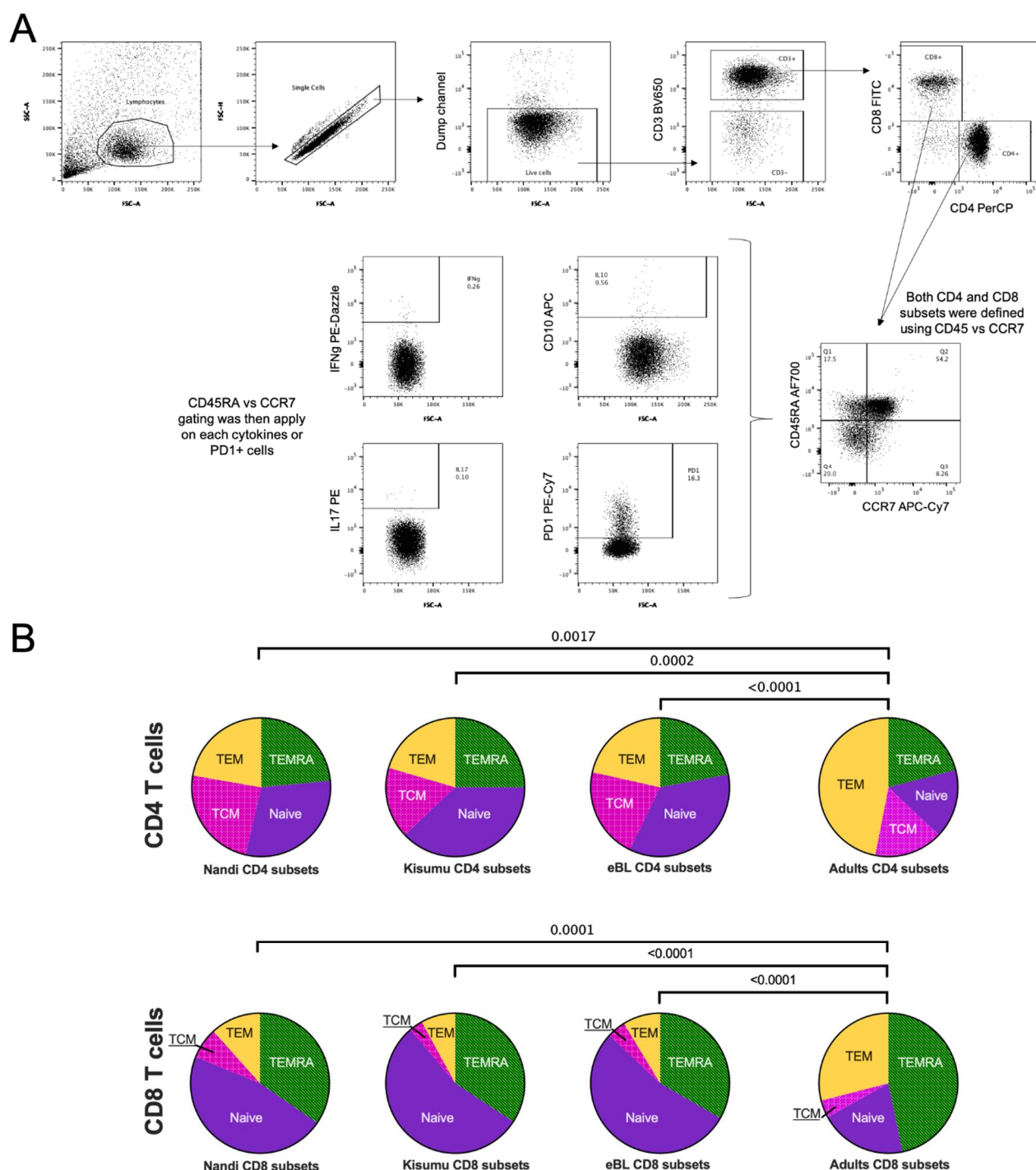
# Interplay between IL-10, IFN- $\gamma$ , IL-17A and PD-1 Expressing EBNA1-Specific CD4<sup>+</sup> and CD8<sup>+</sup> T Cell Responses in the Etiologic Pathway to Endemic Burkitt Lymphoma

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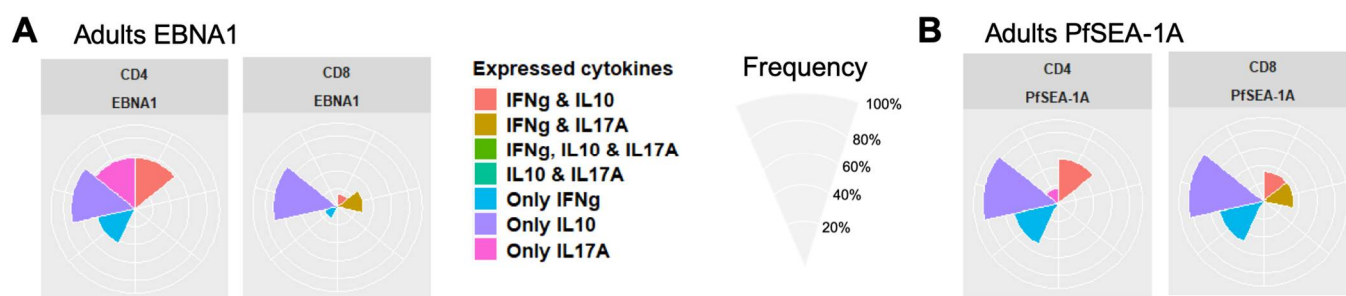
Table S1. Population characteristics.

	Nandi ( <i>n</i> = 20)	Kisumu ( <i>n</i> = 19)	eBL ( <i>n</i> = 19)	Nandi vs Kisumu ( <i>p</i> )	Nandi vs eBL ( <i>p</i> )	Kisumu vs eBL ( <i>p</i> )
Age <sup>A</sup> :	8 [2–14.6]	6.85 [1–14.8]	8.3 [3.3–11.90]	0.45 <sup>B</sup>	0.58 <sup>B</sup>	0.25 <sup>B</sup>
Sex (% of male)	57%	66%	63%	0.54 <sup>C</sup>	0.69 <sup>C</sup>	0.82 <sup>C</sup>
EBV load (copies per $\mu$ g of human DNA) <sup>A</sup>	0 [0–192]	0 [0–320]	10,773 [0–183,568]	0.87 <sup>B</sup>	<0.0001 <sup>B</sup>	<0.0001 <sup>B</sup>
EBV seropositivity (%)	100%	100%	100%	>0.99 <sup>B</sup>	>0.99 <sup>B</sup>	>0.99 <sup>B</sup>
Serology expressed in Median Fluorescence Intensity (MFI) <sup>A</sup>						
EBNA1	15,861 [53–23,657]	12,794 [8,700–26,657]	9,659 [66–18,602]	0.93 <sup>B</sup>	0.04 <sup>B</sup>	0.04 <sup>B</sup>
VCA	17,380 [72–23,877]	10,465 [7,527–23,530]	17,714 [6,289–25,711]	0.13 <sup>B</sup>	0.61 <sup>B</sup>	0.24 <sup>B</sup>
MSP-1	2,535 [330–8,592]	6,542 [2,000–15,995]	6,568 [1,205–20,057]	0.01 <sup>B</sup>	0.007 <sup>B</sup>	>0.99 <sup>B</sup>
AMA1	211 [116–10,362]	18,118 [2,251–28,423]	19,849 [644–31,689]	<0.0001 <sup>B</sup>	<0.0001 <sup>B</sup>	0.71 <sup>B</sup>
Ratio CD4 <sup>+</sup> /CD8 <sup>+</sup> T cells <sup>A</sup>	2.38 [1.14–6.19]	2.75 [1.50–9.93]	2.75 [1.03–4.56]	0.06 <sup>B</sup>	0.57 <sup>B</sup>	0.22 <sup>B</sup>
% CD14 <sup>+</sup> CD19 <sup>+</sup> CD3 <sup>+</sup> cells <sup>A</sup>	86 [59–98]	95 [88–98]	94 [73–98]	0.002 <sup>B</sup>	0.01 <sup>B</sup>	0.30 <sup>B</sup>

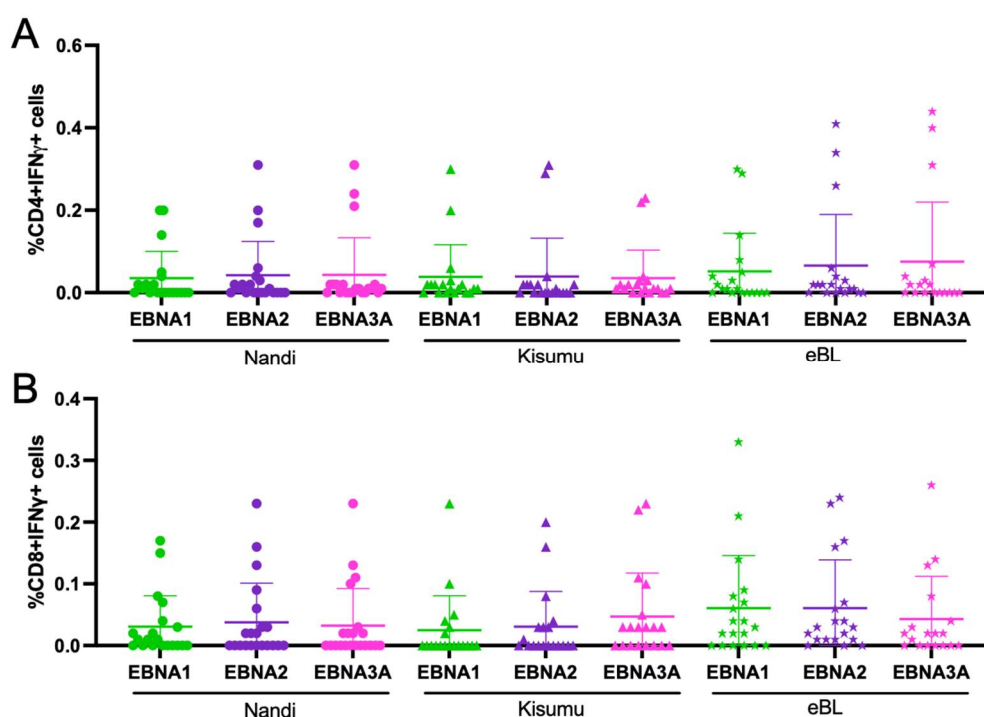
<sup>A</sup> Median [min–max] are represented; <sup>B</sup> *p* value from Mann Whitney statistical test; <sup>C</sup> *p* value from  $\chi^2$  statistical test



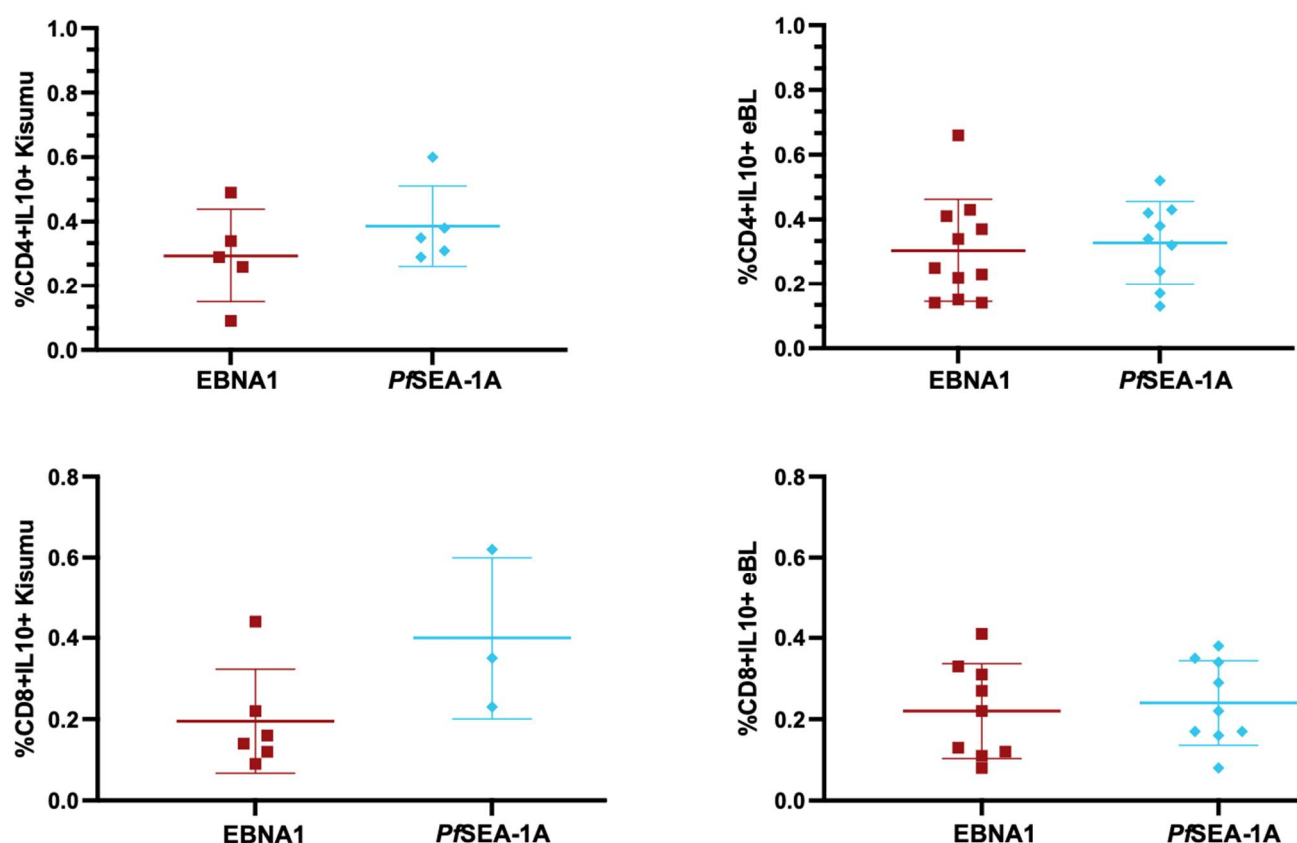
**Figure S1.** Flow cytometry gating strategy and T cell subsets. **(A)** Compensation and FMO control tubes were made for each experiment and used to design the compensation matrix and to determine our gating. First, lymphocytes were identified by SSC-A vs. FSC-A cytoplots. After selecting single cells (FSC-A vs. FSC-H), a dump channel allowed us to select only CD14<sup>+</sup>CD19<sup>+</sup> live cells. Then, a clear CD3<sup>+</sup> gate isolated T-cells that were differentiated by CD8 vs. CD4 expression. CD45RA and CCR7 expression defined the following CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets: TEMRA (CD45RA<sup>+</sup>CCR7<sup>+</sup>), T<sub>Naive-like</sub> (CD45RA<sup>+</sup>CCR7<sup>+</sup>), T<sub>CM</sub> (CD45RA<sup>+</sup>CCR7<sup>+</sup>) and T<sub>EM</sub> (CD45RA<sup>+</sup>CCR7<sup>+</sup>). We also evaluated the percentage of cytokines and/or PD-1 expressing CD4<sup>+</sup> and CD8<sup>+</sup> T cells and identified their subsets of origin based on CD45RA/CCR7 expression. **(B)** CD4 and CD8 T cell subsets were defined by CD45RA and CCR7 expression: TEMRA (green, CD45RA<sup>+</sup>CCR7<sup>+</sup>), T<sub>Naive-like</sub> (purple, CD45RA<sup>+</sup>CCR7<sup>+</sup>), T<sub>CM</sub> (pink, CD45RA<sup>+</sup>CCR7<sup>+</sup>) and T<sub>EM</sub> (yellow, CD45RA<sup>+</sup>CCR7<sup>+</sup>). The mean proportion of each subset is represented (pie slice).  $\chi^2$  test was applied and *p* values are indicated.



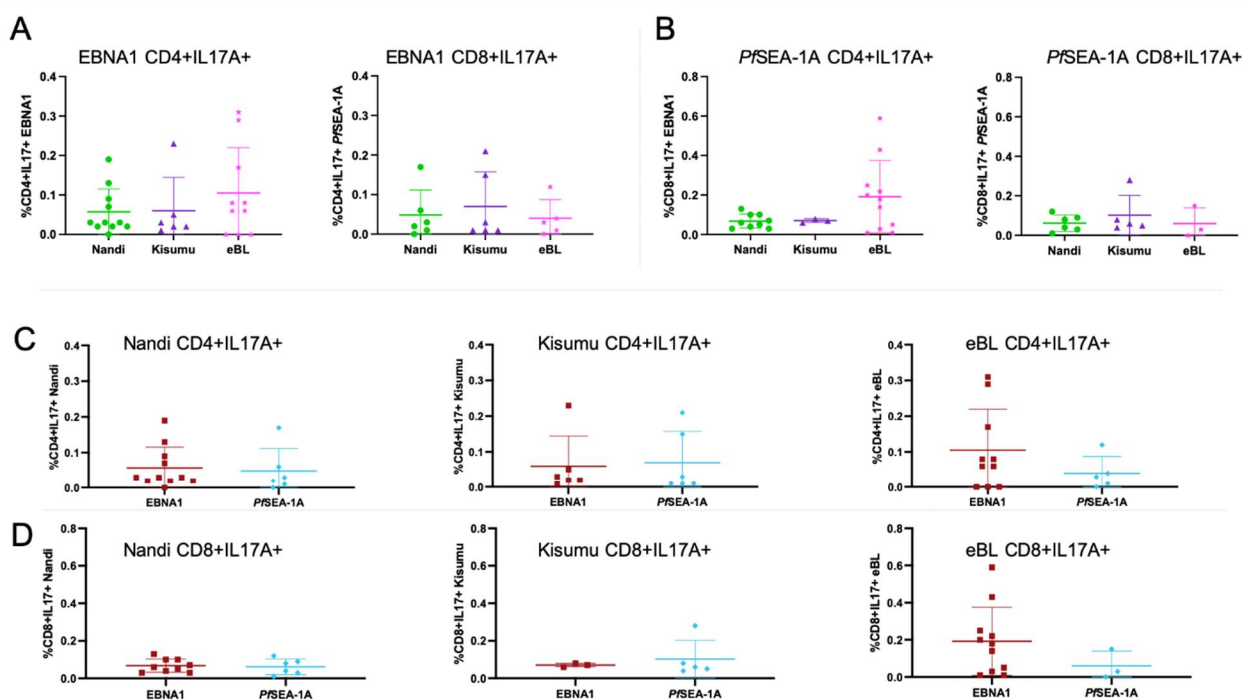
**Figure S2.** Adult T cell cytokine profiles in response to EBNA1 and *PfSEA-1A*. Radar/flower plots represent the frequency (from 0 to 100%) of Kenyan adults who express cytokines from CD4<sup>+</sup> and/or CD8<sup>+</sup> after (A) EBNA1 or (B) *PfSEA-1A* stimulation. Each petal represents a different combination of cytokine (IFN- $\gamma$ , IL-10, IL-17A) expression



**Figure S3.** Comparison of IFN- $\gamma$ <sup>+</sup> T cell responses between EBNA1, EBNA2 and EBNA3A stimulation for each group of children. Dot plots of mean and standard deviation (SD) for (A) CD4<sup>+</sup> and (B) CD8<sup>+</sup> T cells expressing IFN- $\gamma$ <sup>+</sup> after EBNA1 (green), EBNA2 (purple), and EBNA3A (pink) stimulation across our groups of children Nandi (round), Kisumu (triangle), and eBL (star). No *p* values were significant (*p* < 0.05).

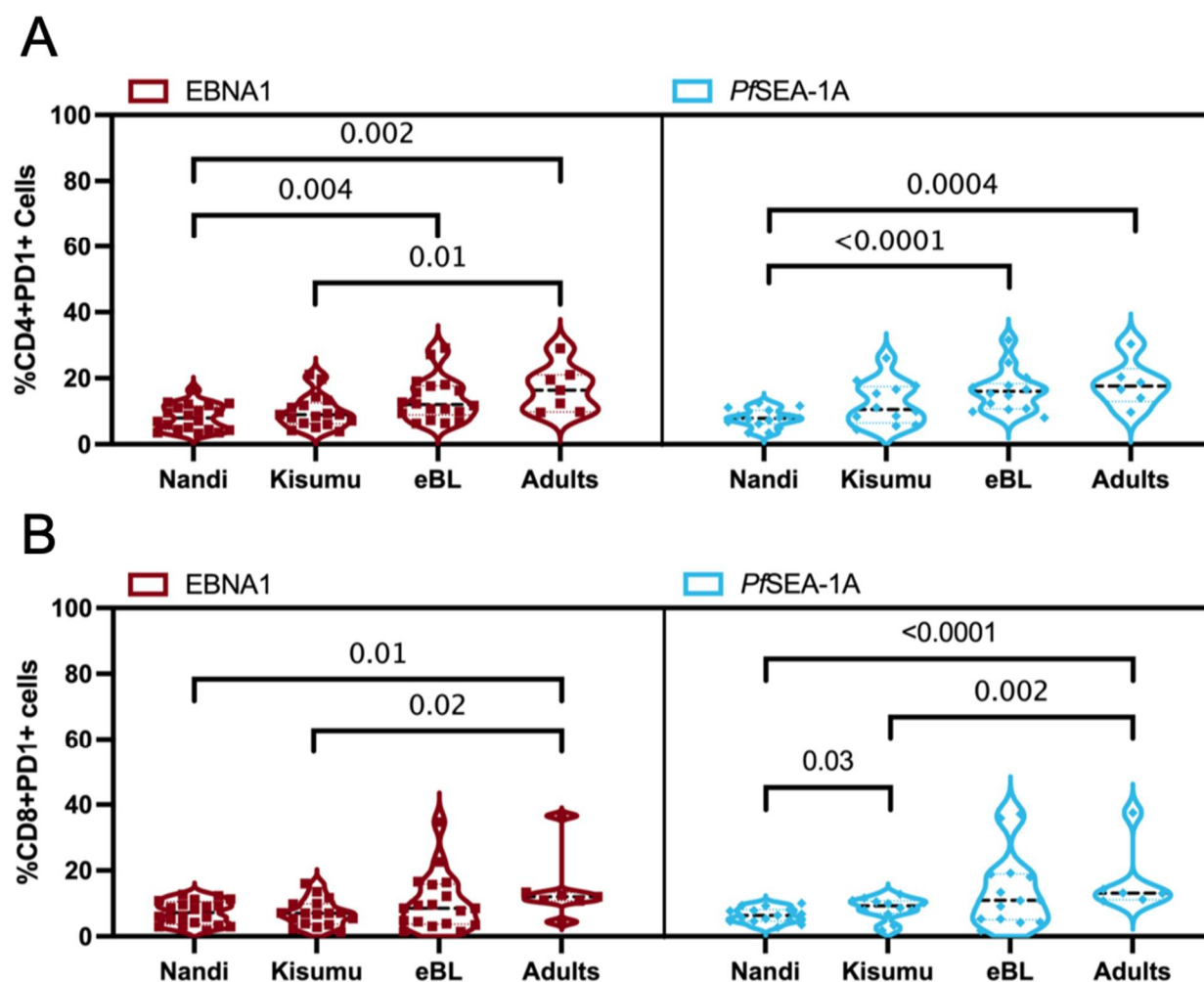


**Figure S4.** No differences were observed in IL-10 expression between EBNA1 and *Pf*SEA-1A for Kisumu or eBL children. IL-10<sup>+</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells responses after EBNA1 (red squares) and *Pf*SEA-1A (blue lozenges) stimulation, mean and SD are represented. No *p* values were significant (*p* < 0.05).

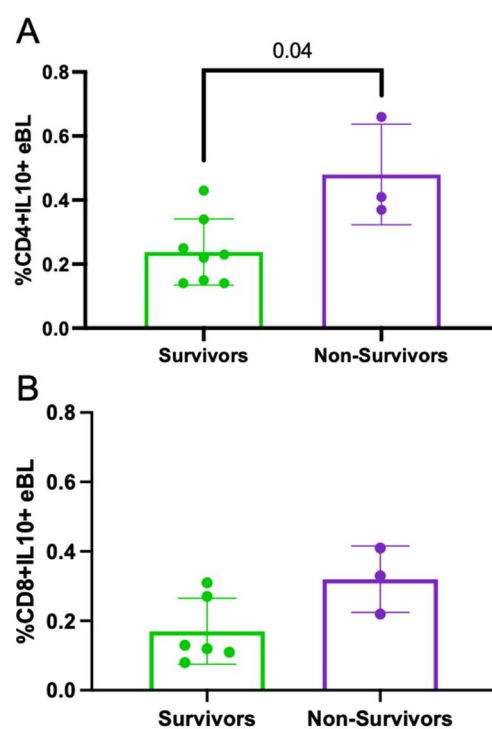


**Figure S5.** IL-17A responses to EBNA1 and *Pf*SEA-1A from CD4<sup>+</sup> and CD8<sup>+</sup> T cells within each group of children. Total CD4<sup>+</sup> and total CD8<sup>+</sup> T cells expressing IL-17A across the different groups of children: Nandi (green round), Kisumu (purple triangles) and eBL (pink stars) after (A) EBNA1 and (B) *Pf*SEA-1A stimulation. (C) Comparative dot plot of IL-

17A<sup>+</sup>CD4<sup>+</sup> T cells response between EBNA1 (red squares) and *Pf*SEA-1A (blue lozenges) within Nandi (left), Kisumu (middle), and eBL (right) children. (D) Comparative dot plot of IL-17A<sup>+</sup>CD8<sup>+</sup> T cells response between EBNA1 (red squares) and *Pf*SEA-1A (blue lozenges) within Nandi (left), Kisumu (middle), and eBL (right) children. Mean and SD are represented. No *p* values were significant (*p*<0.05).



**Figure S6.** Adults had significantly higher CD4<sup>+</sup>PD-1<sup>+</sup> T cells compared to Nandi and Kisumu children but not compared to eBL children. (A) Percentage of CD4<sup>+</sup>PD-1<sup>+</sup> cells after EBNA1 (red squares) and *Pf*SEA-1A (blue lozenges) stimulation across groups of individuals. (B) Percentage of CD8<sup>+</sup>PD-1<sup>+</sup> cells after EBNA1 and *Pf*SEA-1A stimulation across groups of individuals. *p* values were calculated using Mann-Whitney test.



**Figure S7.** EBNA1-specific IL-10<sup>+</sup>CD4<sup>+</sup> and CD8<sup>+</sup> T cell frequencies for eBL survivors compared to non-survivors. **(A)** The percentage of IL-10-expressing CD4<sup>+</sup> T cells was significantly higher for eBL non-survivors compared to survivors. **(B)** The percentage of IL-10-expressing CD8<sup>+</sup> T cells did not differ by eBL outcome. *p* values were calculated using Mann-Whitney test, mean and SD are represented.