Supplementary Figures

Supplementary Figure S1: CONSORT diagram for patient disposition.

Supplementary Figure S2: Analysis of tumor immune cells. **a,b,c,d,e,** There was no significant difference between patients with clinical benefit (n=4) (complete response, partial response, and stable disease ≥ 6 months) versus patients without clinical benefit (n=20) (stable disease < 6 months, progressive disease, and inevaluable) when comparing CD8+ cells **(a)**, PD1+ cells **(b),** CD8+PD1+ cells **(c)**, CD8:FOXP3 ratio **(d),** or PD1:PDL1 ratio **(e)** by multichannel immunofluorescence.

Supplementary Figure S3: Tumor immune microenvironment by multichannel immunofluorescence (20X magnification) for patient 7 (best response as progressive disease to pembrolizumab). Blue, DAPI; white, CD8; orange, PD1; green, PD-L1; pink, cytokeratin.

Supplementary Figure S4. Biomarkers associated with outcomes. **a,** Tumor mutational burden in patients with clinical benefit (complete response, partial response, and stable disease ≥ 6 months) versus patients without clinical benefit (stable disease < 6 months, progressive disease, and inevaluable) (median: 5.703 mut/Mb vs. 6.084 mut/Mb, P=0.3485). **b,** Combined positive score (CPS) in patients with clinical benefit versus patients with no clinical benefit (median: 15 vs. 10, P=0.6297). **c,** Peripheral blood mononuclear cells before and after exposure to pembrolizumab. PD1 mean fluorescent intensity significantly decreases from baseline after first dose of pembrolizumab. Green color indicates patient with durable complete response to pembrolizumab. **d,** HLADR+ CD38+ CD45RO+ T cells increase after first dose of therapy. Green color indicates patient with durable complete response to pembrolizumab.

Supplementary Figure S5: T cell gating strategy for flow cytometry of peripheral blood mononuclear cells.

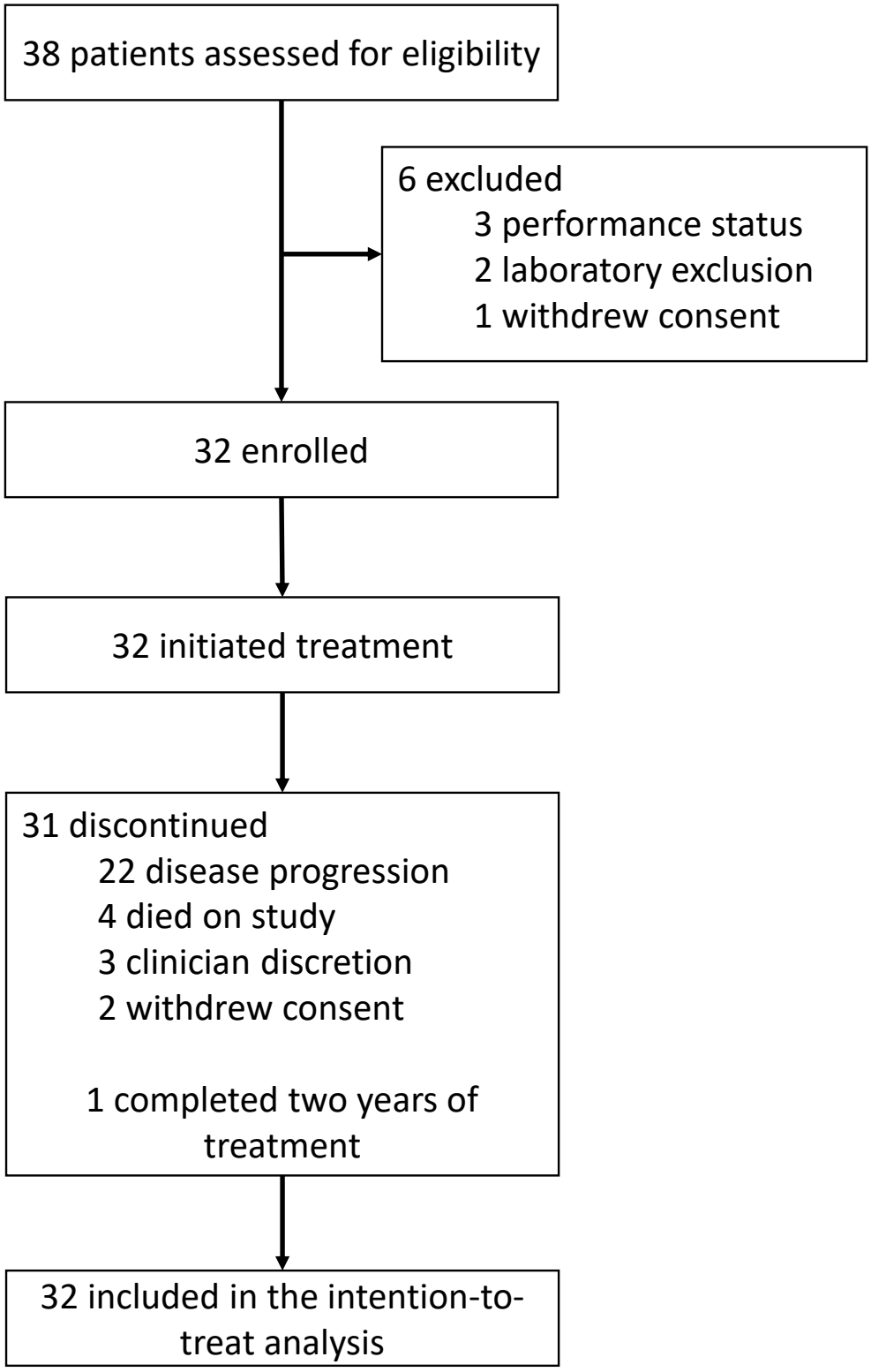
Supplementary Figure S6: B cell gating strategy for flow cytometry of peripheral blood mononuclear cells.

Supplementary Figure S7: Analysis of circulating immune cells at baseline. There was no significant difference between responders (n=4) by clinical benefit (complete response [CR], partial response [PR], and stable disease [SD] ≥ 6 months) and nonresponders (stable disease < 6 months, progressive disease [PD], and inevaluable) (n=18) in CD4+ T cells **(a)**, peripheral helper T cells (Tph) (CD4+CD45RO+ICOS+PD1+) **(b)**, CD8+ T cells **(c)**, CD8+CX3CR1+ T cells **(d),** naïve B cells (CD19+IgD+CD71-) **(e)**, antibody secreting B cells (CD19+CD20−IgD−CD71+) **(f)**, or activated B cells (CD19+CD20+IgD−CD71+CD10−) **(g)**. Median baseline values with 95% confidence interval (CI) plotted.

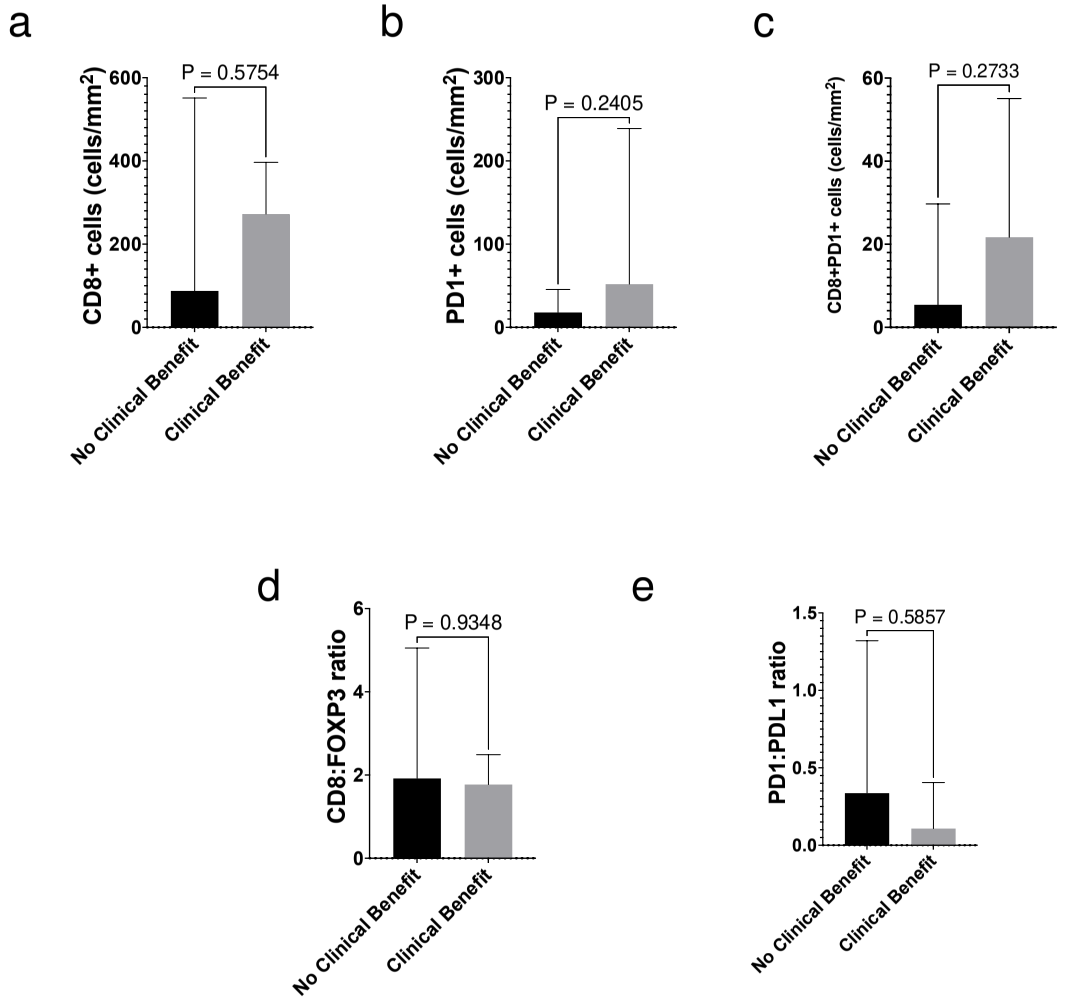
Supplementary Figure S8: Analysis of circulating antibodies to HPV antigen, E6. There were 21.7% of nonresponders (stable disease, progressive disease, or inevaluable) (n=5/18) and 33.3% of responders (complete response or partial response) (n=1/3) who had detectable E6 antibody by enzyme linked immunosorbent assay.

Supplementary Figure S9: Analysis of changes in TTMV-HPV DNA score by best objective response. **a,** TTMV-HPV DNA score percent change from baseline after cycle 1 (range: 2–4 weeks after first dose of pembrolizumab) by best objective response (complete response [CR] and partial response [PR]) versus non-responders without objective response (stable disease [SD], progressive disease [PD], and inevaluable). **b**, TTMV-HPV score percent change from baseline after cycle 2 (range: 5-7 weeks after first dose of pembrolizumab) by best objective response versus non-responders without objective response.

Supplementary Figure S1

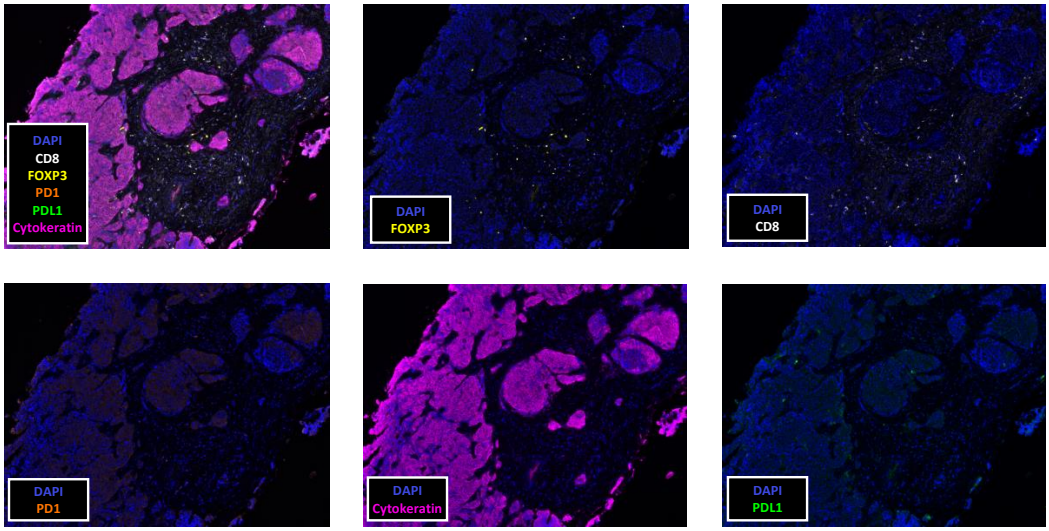


Supplementary Figure S2

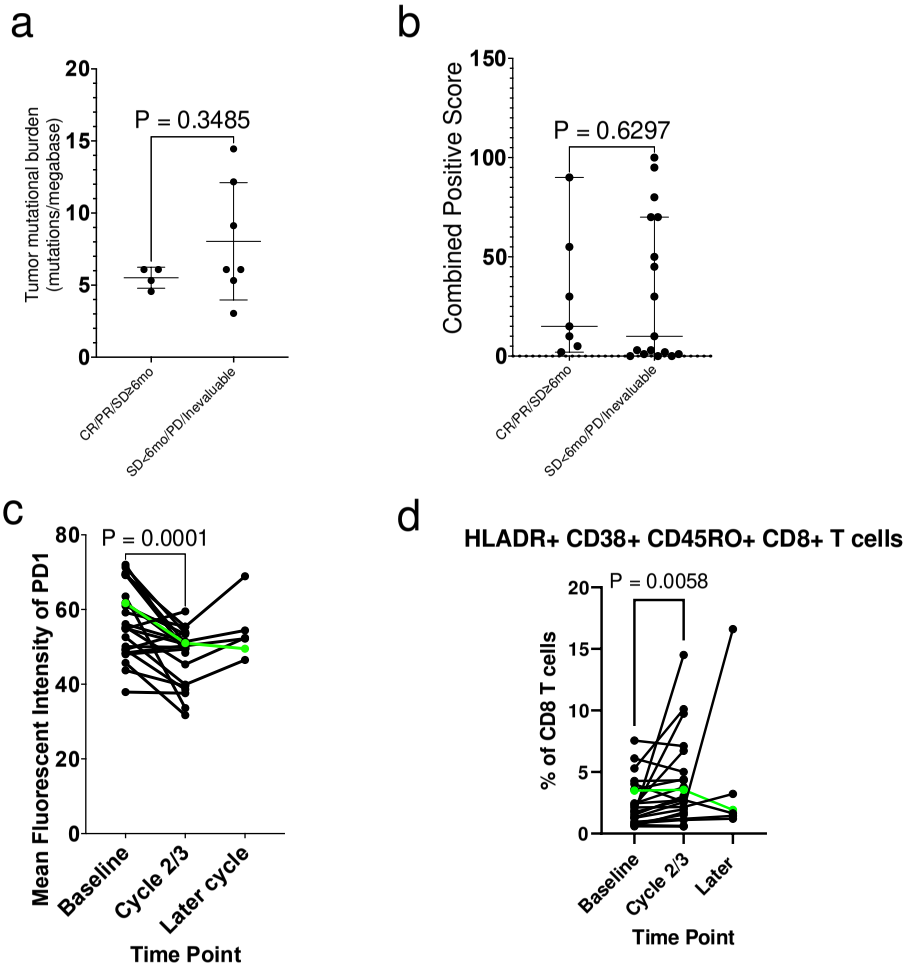


Huffman BM, *et al. J Immunother Cancer* 2024; 12:e008436. doi: 10.1136/jitc-2023-008436

Supplementary Figure S3



Supplementary Figure S4

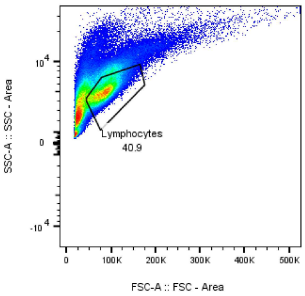


Huffman BM, *et al. J Immunother Cancer* 2024; 12:e008436. doi: 10.1136/jitc-2023-008436

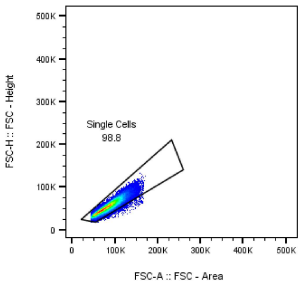
Supplementary Figure S5

T cell Gating Strategy

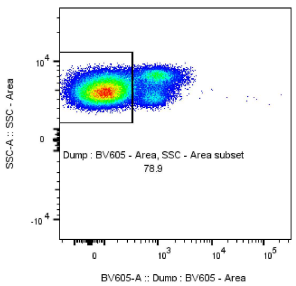
All cells



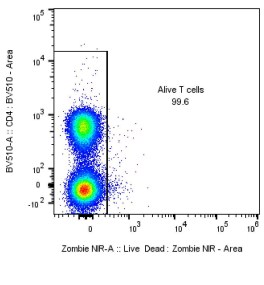
Lymphocytes



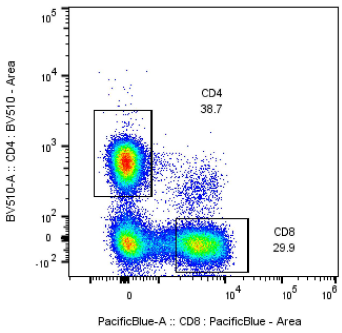
Single cells



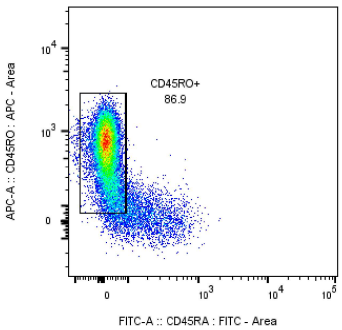
T cells



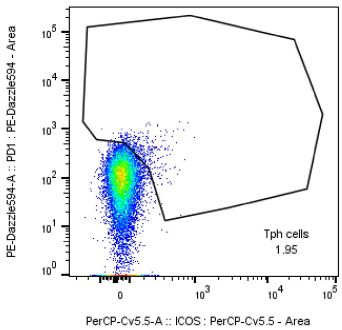
Alive T cells



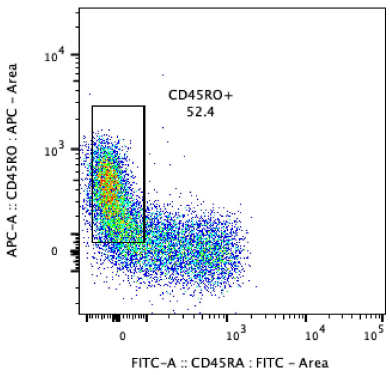
CD4+ T cells



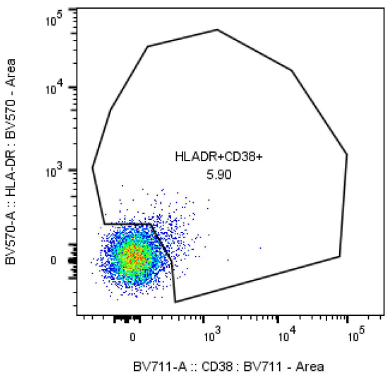
CD45RO+ T cells



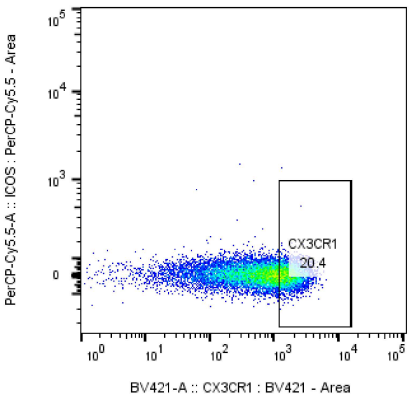
CD8+ T cells



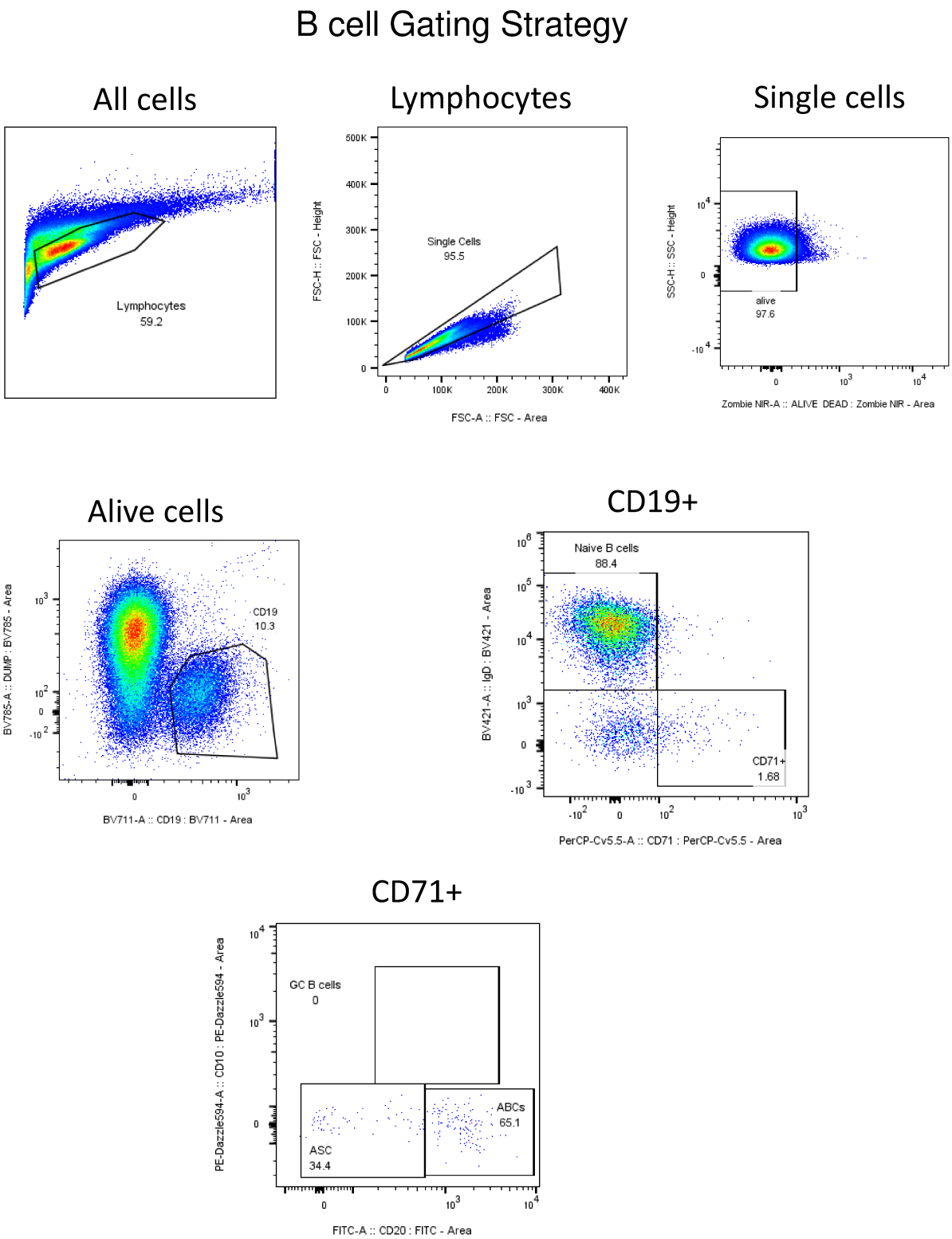
CD8+CD45RO+ T cells



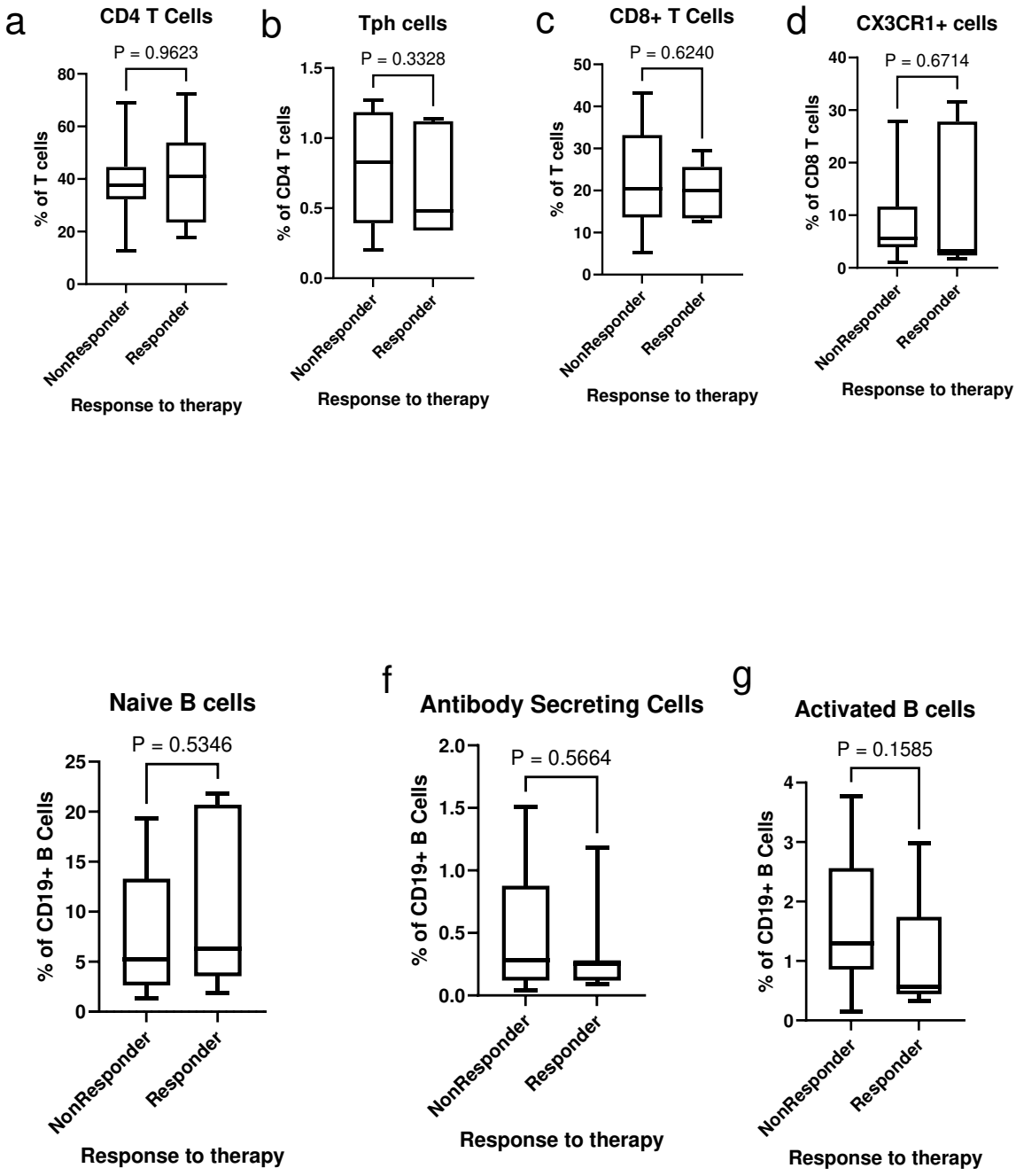
CD8+ T cells



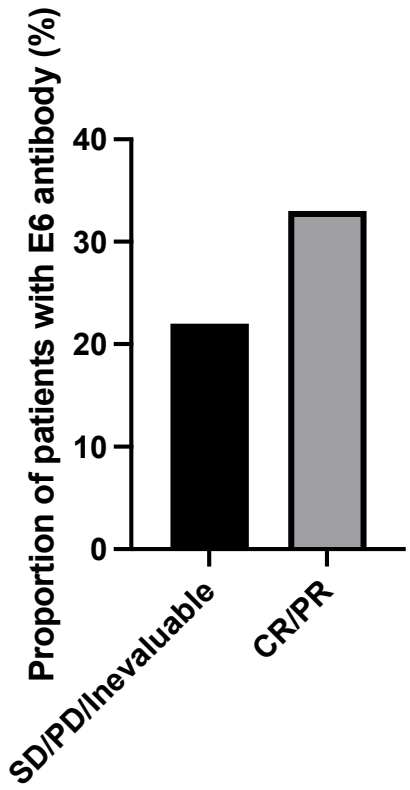
Supplementary Figure S6



Supplementary Figure S7



Supplementary Figure S8



Supplementary Figure S9