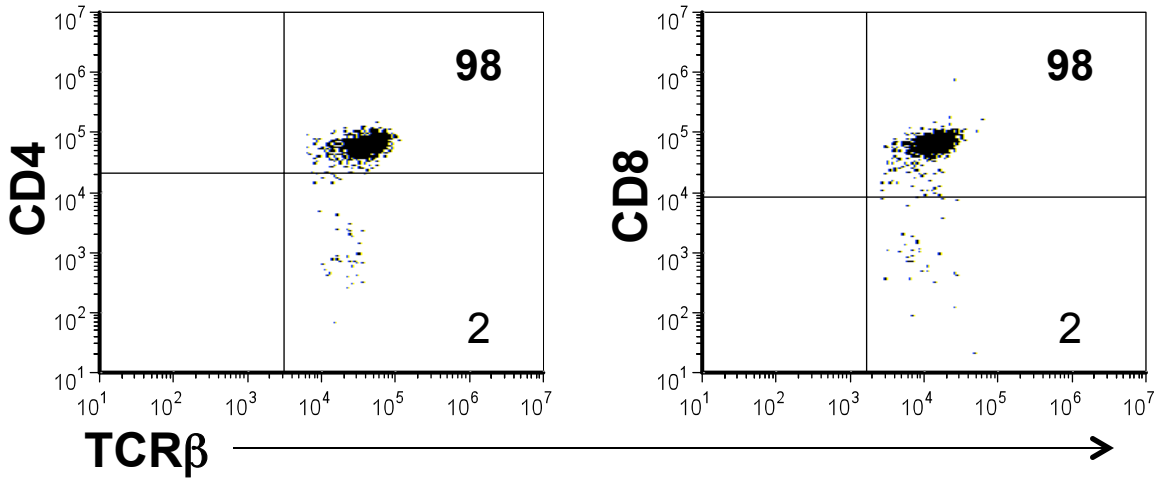
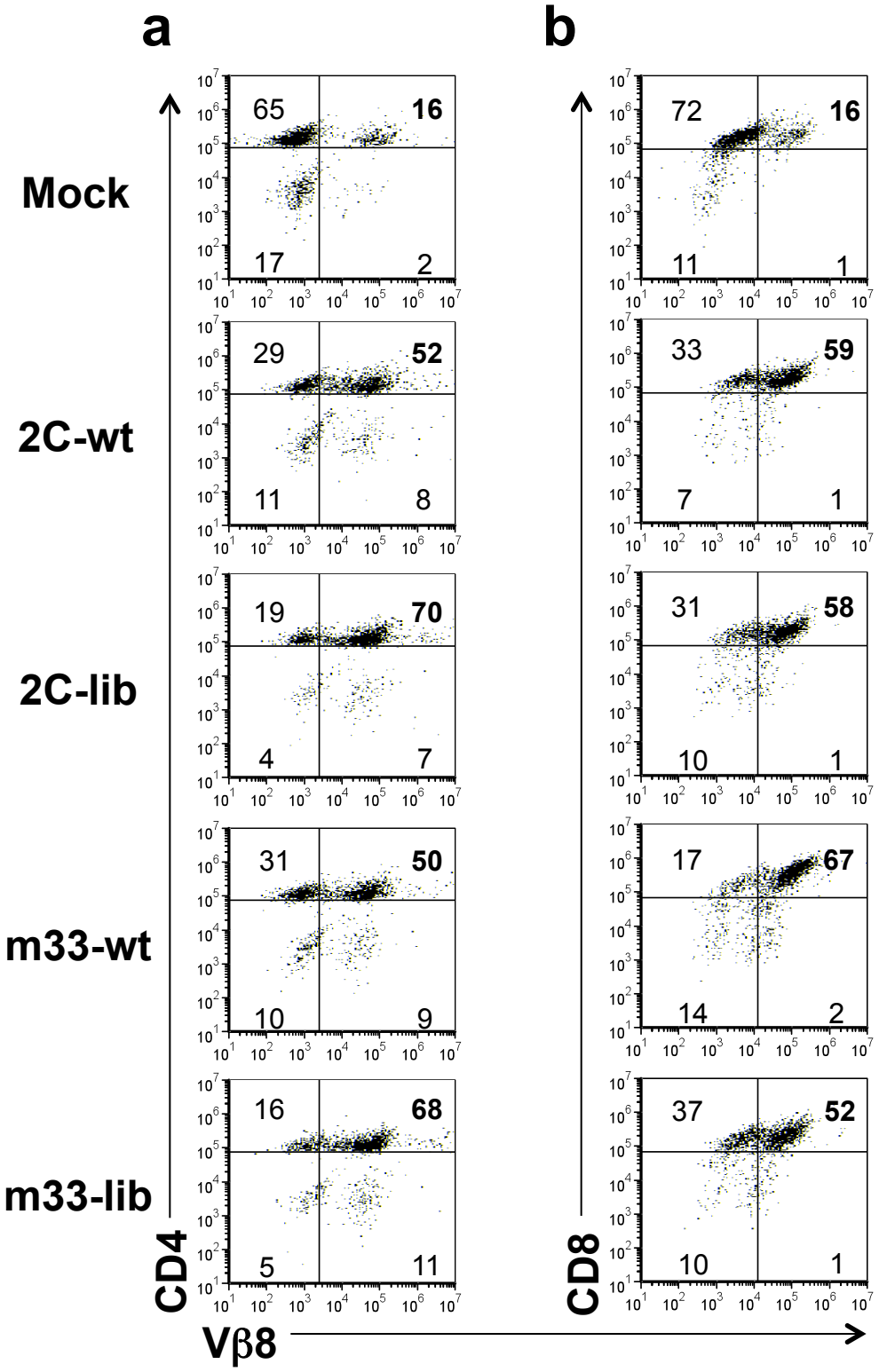
Supplementary Figure 1



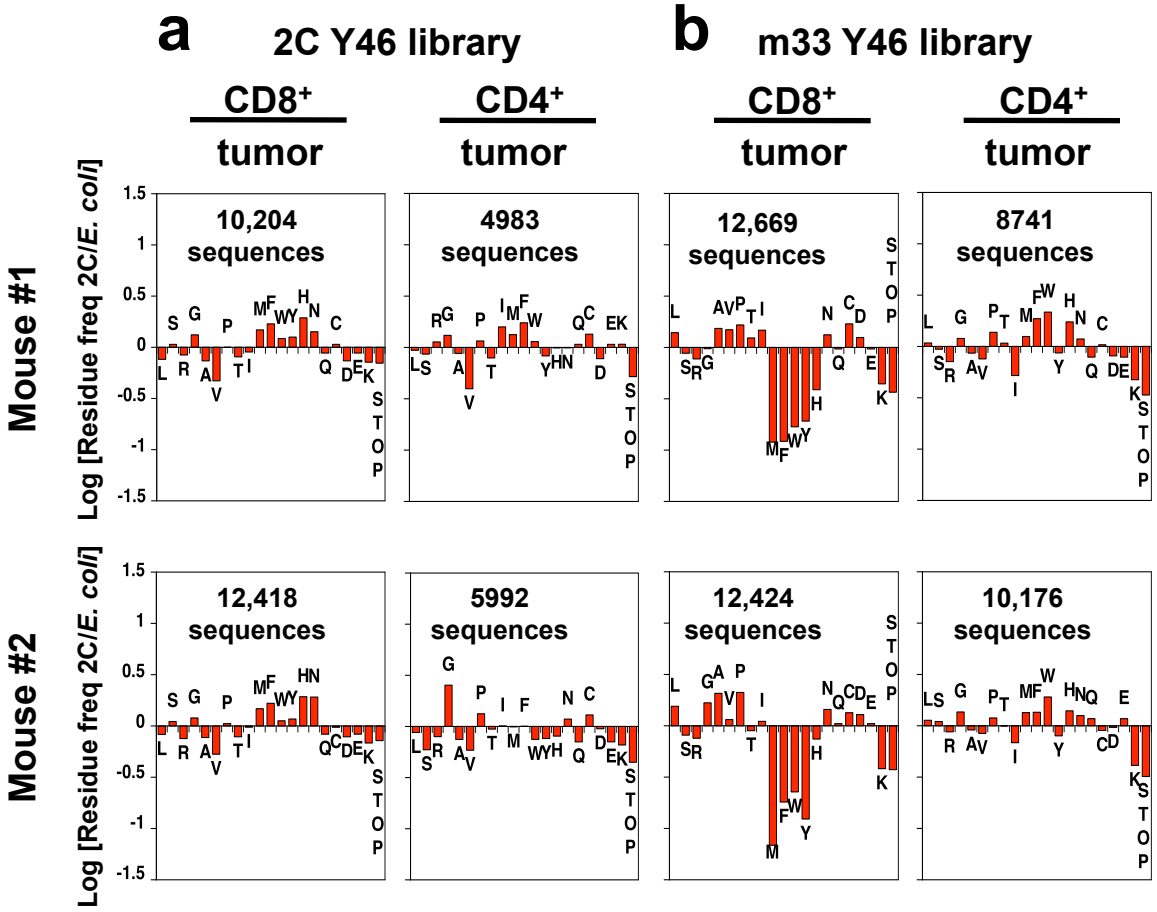
Supplementary Figure 1. Isolation of CD4⁺ or CD8⁺ C57BL/6 T cells. Splenocytes from a C57BL/6 mouse were harvested and either CD4⁺ or CD8⁺ T cells were isolated using the CD4⁺ T Cell Isolation Kit II or CD8α⁺ T Cell Isolation Kit II from Miltenyi. Isolated T cells were then stained with anti-TCRβ and anti-CD4 or anti-CD8α antibodies. Dot plots are representative of all isolations (>4) performed through the course of these experiments.

Supplementary Figure 2



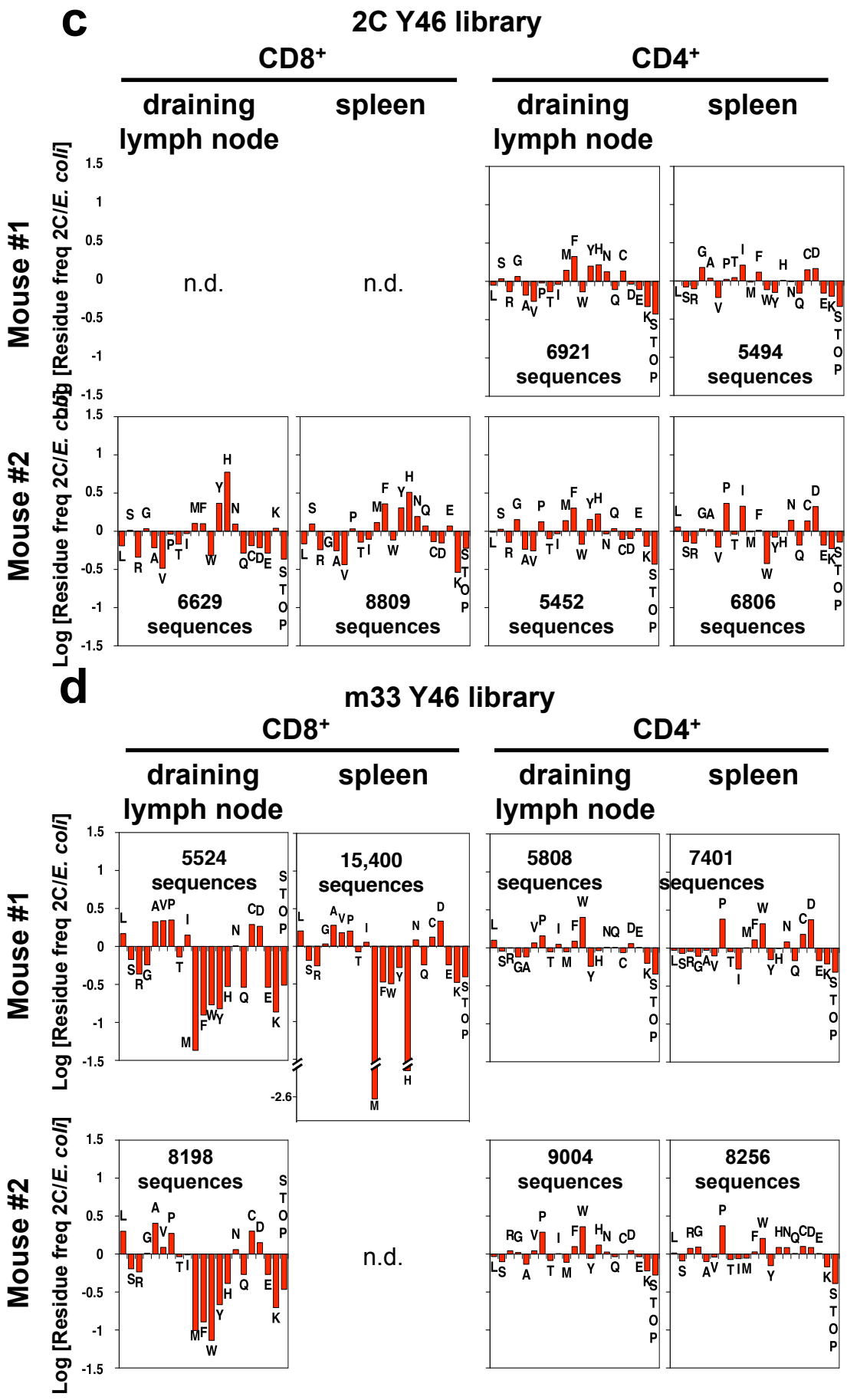
Supplementary Figure 2. Characterization of transduced CD4⁺ or CD8⁺ T cells before adoptive transfer into tumor-bearing mice. (a) CD4⁺ or **(b)** CD8⁺ T cells 3 days post-transduction were stained with anti-Vβ8.1/8.2:PE and anti-CD4:647 or anti-CD8α:647. Dot plots are representative of transductions (>4) performed throughout the course of experiments. Transduced T cells were also stained with the 2C clonotypic antibody 1B2 or SIY/K^b Ig dimer to confirm transgene expression.

Supplementary Figure 3

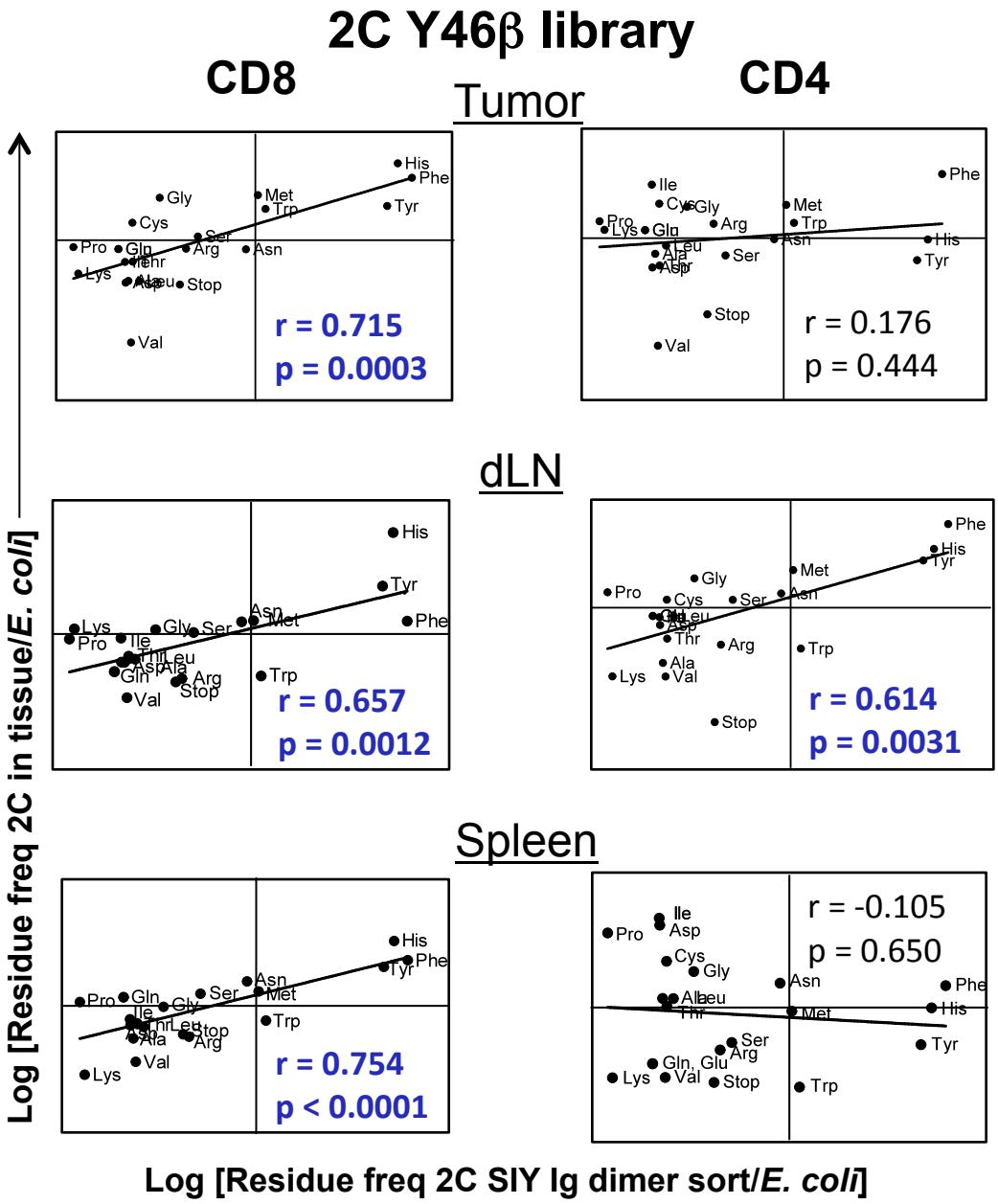


Supplementary Figure 3. Frequencies of TCR 46β residues in TCR library-transduced T cells isolated from tumor and other tissues (*in vivo* selection). **(a-b)** Relative frequencies of TCR 46β residues isolated from B16 SIY$^+$ tumors in mice treated with CD8$^+$ (left panels) or CD4$^+$ (right panels) T cells transduced with the **(a)** 2C or **(b)** m33 libraries. Individual mice are shown, and the number of sequences analyzed for each sample is indicated. **(c-d)** Relative frequencies of TCR 46β residues for T cells isolated from the draining lymph node (first and third columns) or spleen (second and fourth columns) of B16 SIY$^+$ tumor-bearing mice treated with CD8$^+$ (left two columns) or CD4$^+$ (right two columns) T cells transduced with **(c)** the 2C TCR library, or **(d)** the m33 TCR library. Individual mice are shown, and the number of sequences analyzed for each sample is indicated.

Supplemental Figure 3

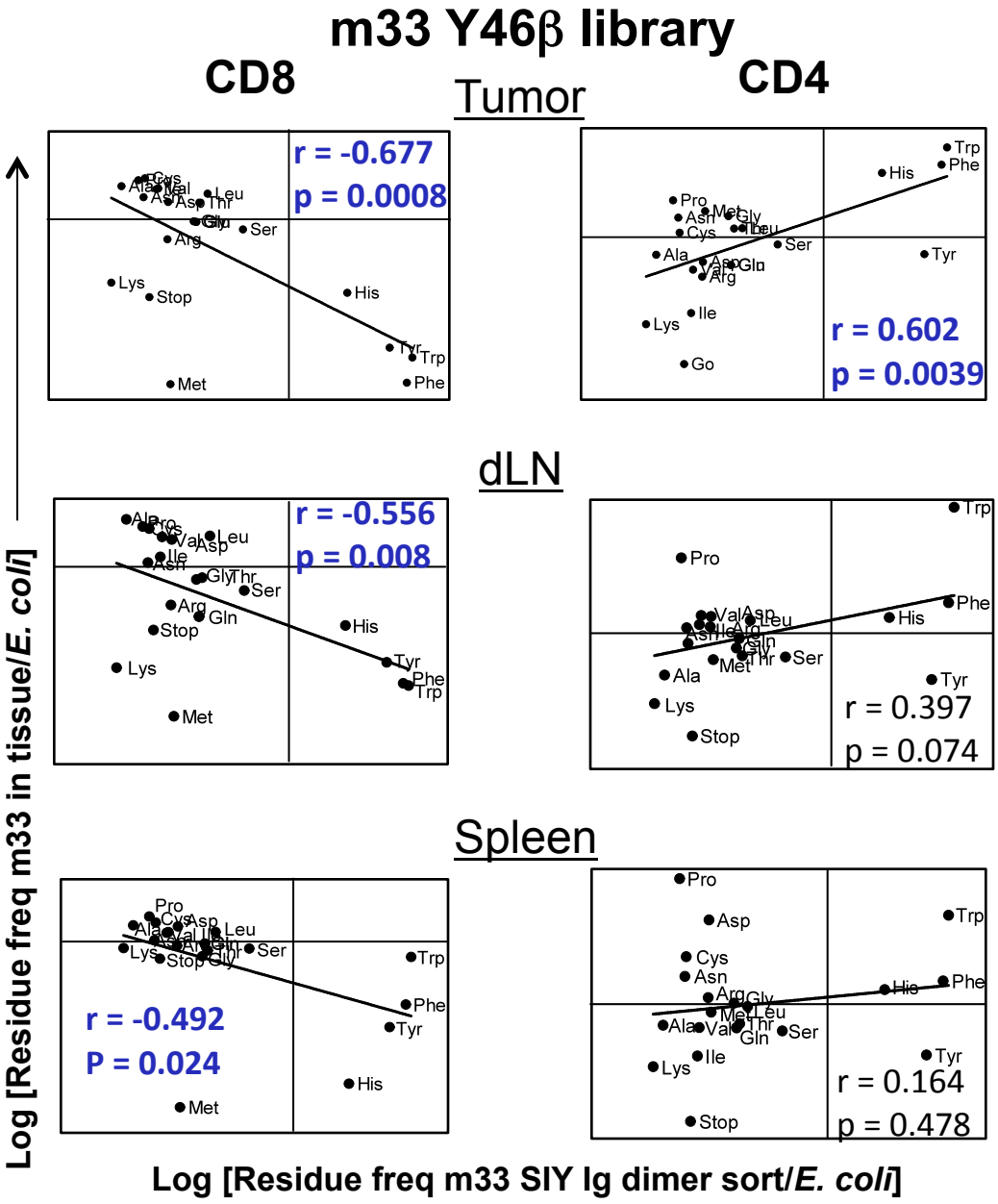


Supplementary Figure 4



Supplementary Figure 4. Correlation between 46β residues isolated *in vitro* and *in vivo* from 2C-library. Residue frequency (taken from Figure 2c) at 46β from $58^{-/-}$ T cells sorted in *vitro* with SIY/K^b-Ig dimer (x-axis) plotted against residue frequency (taken from Figure 4a,b) at 46β from primary T cells obtained from SIY^+ tumors, spleens or draining lymph nodes (y-axis). Frequencies were plotted in GraphPad Prism 5 and analyzed by linear regression to calculate probability of the slope being significantly different from zero. Plots with bolded blue font represent significant p values.

Supplementary Figure 5



Supplementary Figure 5. Correlation between 46β residues isolated *in vitro* and *in vivo* from m33-library. Residue frequency (taken from Figure 2c) at 46β from $58^{-/-}$ T cells sorted in *vitro* with SIY/K^b-Ig dimer plotted against residue frequency (taken from Figure 4a,b) at 46β from primary T cells obtained from SIY^+ tumors, spleens or draining lymph nodes. Frequencies were plotted in GraphPad Prism 5 and analyzed by linear regression to calculate probability of the slope being significantly different from zero. Plots with bolded blue font represent significant p values.