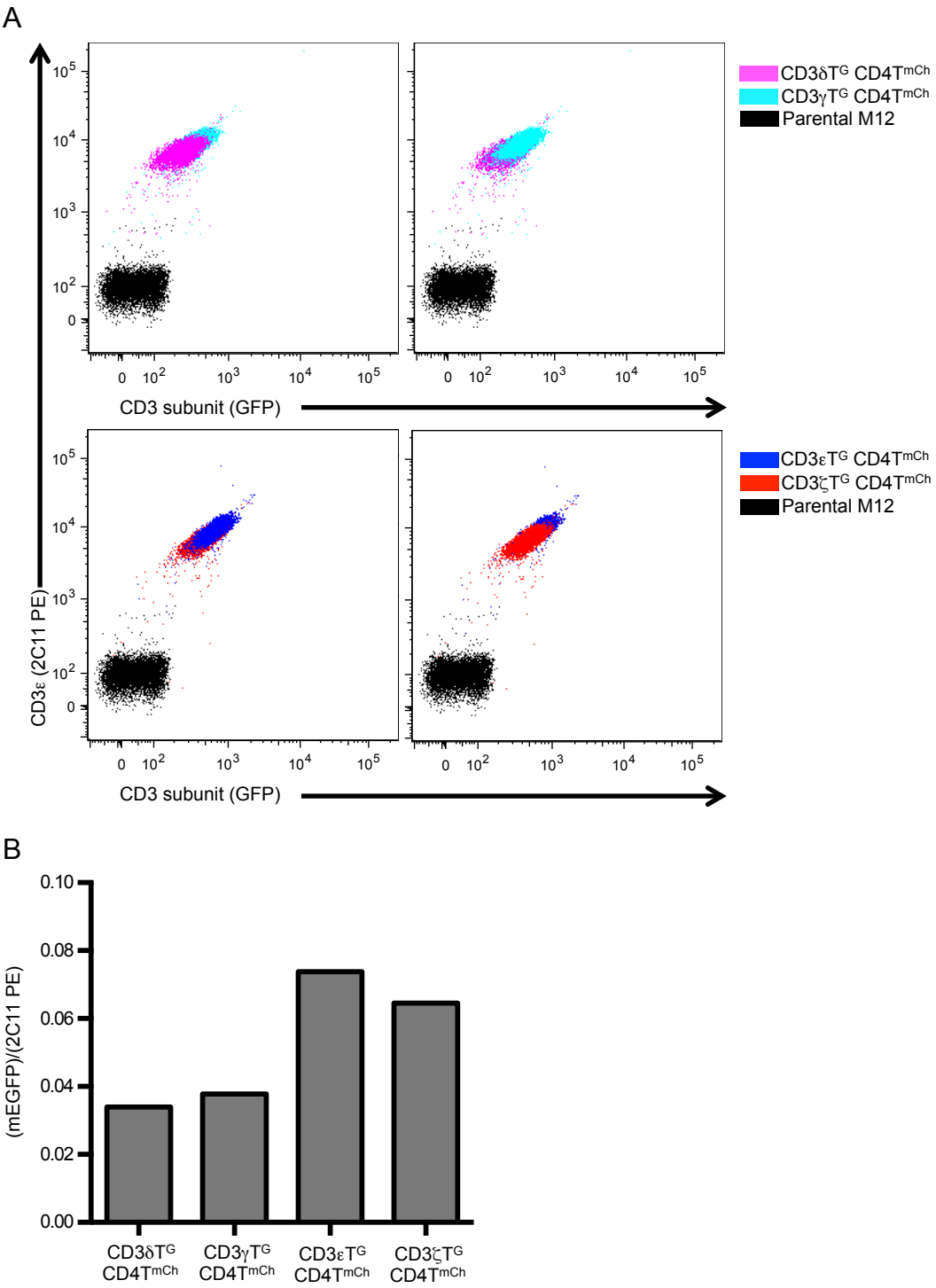
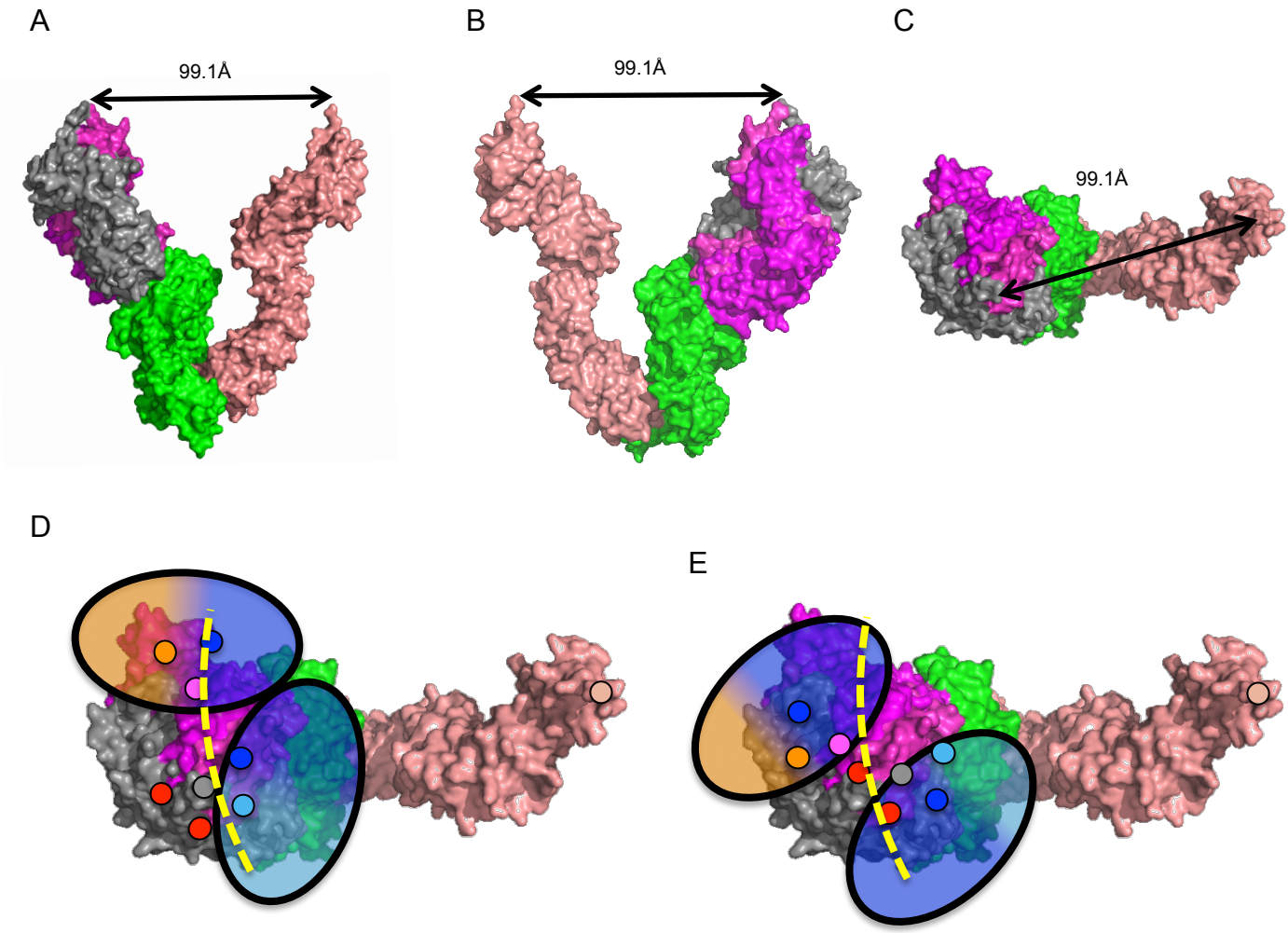


Supplement 1. TCR-CD3 complex containing mEGFP tagged CD3 subunits assemble with appropriate stoichiometry. Whole cell lysates from M12 cell lines were analyzed using a flow-based flourophore-linked immunosorbant assay (FFLISA). Cell lysates were immunoprecipitated (IP'd) with anti-TCR (anti-TCRβ mAb H57)-coated latex beads as described in the methods. Detection of TCR (anti-TCRα mAb APC), CD3ε (2C11 PE) and CD3xT-mEGFP subunits (by GFP intensity) on the beads was used to assess complex assembly. (**A**) Proportional relationship between CD3ε and CD3xTG. Dot plots of flow cytometry analysis of beads are shown as labeled. The same populations are shown in the left and right plots but with different populations in the foreground. This was done to emphasize the equivalent slope of the diagonal, which reflects the proportional distribution of the IP'd subunits. (**B**) Appropriate stoichiometry of TCR-CD3. Bars represent the ratio of mEGFP (gMFI) to CD3ε (gMFI) for beads with matched TCRα intensity (i.e. equivalent TCR IP), and reflect the proportion of CD3 subunits per complex. Results are representative of three experiments.



Supplement 2. CD3γ and CD3ζ are likely to be >100Å from CD4 in a V-like arch.
(A–C) Surface rendered representations of the TCR-pMHC-CD4 ternary complex showing CD4 (salmon), class II MHC (green), TCRα (gray) and TCRβ (magenta) from the **(A-B)** side and **(C)** a view as if looking out from the T-cell surface. The ternary TCR-pMHC-CD4 structure was used as a staring point (PDB ID 3T0E). Our experiments were performed using the 5c.c7 TCR engaging MCC:I-E^k, so we aligned the structure of the highly related 226 TCR bound to MCC:I-E^k (PDB ID 3QIU) via the MHC to consider the docking of the cytochrome c related TCRs on their cognate ligand. Finally, we aligned the 2C TCR structure (PDB ID 1TCR) to the 226 TCR to take advantage of the well refined constant regions, with an intact inter-chain disulfide bond, to better appreciate the mouse TCR constant regions. The net result shown is the 2C TCR shown with the MHC and CD4 from the original ternary structure. The distance shown (99.1Å) is the distance measured from the most C-terminal residue of the TCRα subunit to the most C-terminal residue of CD4 using PyMol (PyMOL Molecular Graphics System, Version 1.7.6.2. Schrödinger, LLC).
(D-E) Models of the TCR-CD3 complex that are consistent with the FRET data presented here would likely place CD3γ and CD3ζ >100Å from CD4 in a V-like arch. Shaded spheres represent general positioning of the ectodomains of the CD3 heterodimers **(D)** clustered on the face of the TCR inside the arch with CD4 in the order δ:ε:ε:γ or **(E)** placed on opposite sides of the TCR. The CD3γε (PDB ID 1SY6) and CD3δε (PDB ID 1XIW) structures were used to approximate their placement on opposite side of the TCR as depicted in (32), and used to adjust our model (1) in consideration of the recent EM structure (20) such that the CD3 heterodimers are nested further below the TCR. Solid spheres represent rough approximation of where the transmembrane domains would emerge from the membrane into the cytoplasm (CD3δ: cyan; CD3γ: orange; CD3ζ: red; CD3ε: marine). For these placements, we used the C-terminal end of the CD3 subunit G-strands and the most C-terminal residue of CD4. To place the CD3 heterodimers in relationship to the TCR subunits we used the transmembrane domain heterotrimer of the NKG2C receptor as a guide (PDB ID 2L35). This was also used as a guide to place the CD3ζζ subunits ~140º offset from CD3δε based on previous work (21). Yellow dashed line represents the boundary of a circle centered at CD4 (salmon dot) with a radius of ~100Å.