

Supporting Information

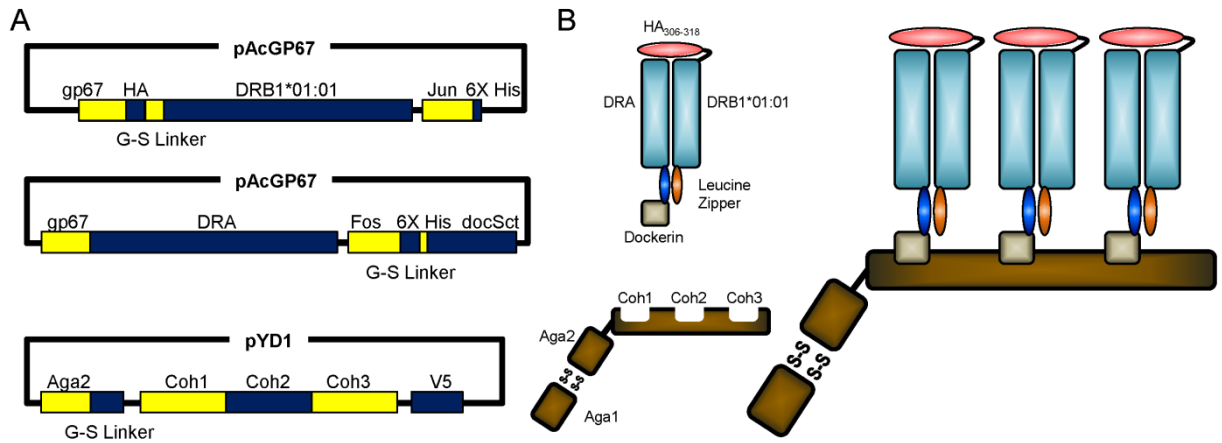


Figure S1. Schematic depicting the construction and assembly of recombinant pMHC and yeast-displayed scaffold. (A) The N-terminus of the pMHC beta-chain (DRB1*01:01) is fused to the well-studied hemagglutinin peptide (HA₃₀₆₋₃₁₈). The C-terminus of the pMHC alpha-chain (DRA) is fused to a dockerin-domain from *C. thermocellum* which specifically associates with corresponding cohesin-domains from *C. thermocellum*. (B) Yeast surface displayed Aga2-fused cohesin scaffolds direct the assembly of the recombinant dockerin-fused, multivalent pMHC complexes. A valency of three is shown as an example here.

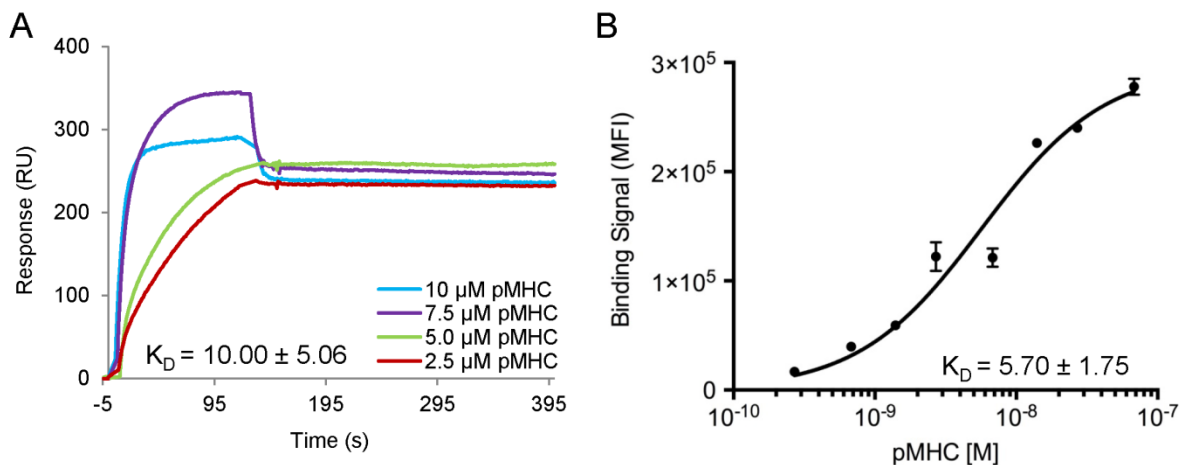


Figure S2. Dockerin-fused pMHC and cohesin binding assays. (A) Biacore 3000 response curves from immobilized dockerin-fused pMHC and flowing soluble APC1 protein (sAPC1). (B) Flow-cytometric apparent binding affinity curve between yAPC1 and dockerin-fused pMHC. The average binding affinities determined from the assays in (A) and (B) were 10.0 nM and 5.70 nM, respectively.

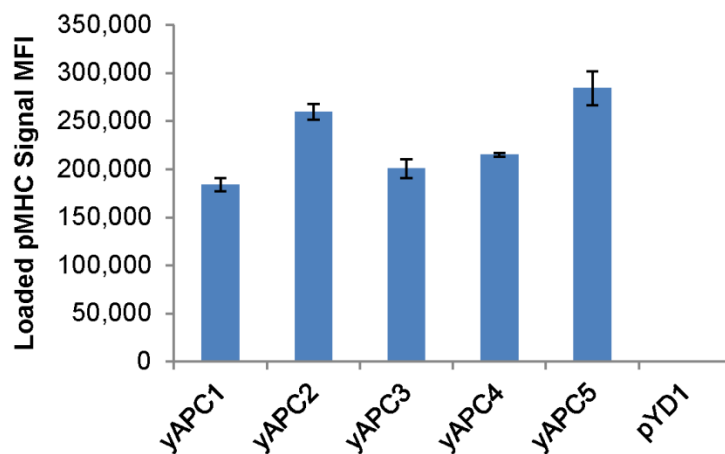


Figure S3. Loaded pMHC signal on unsorted yAPC. Data represent mean $\pm$ SD (n = 3).

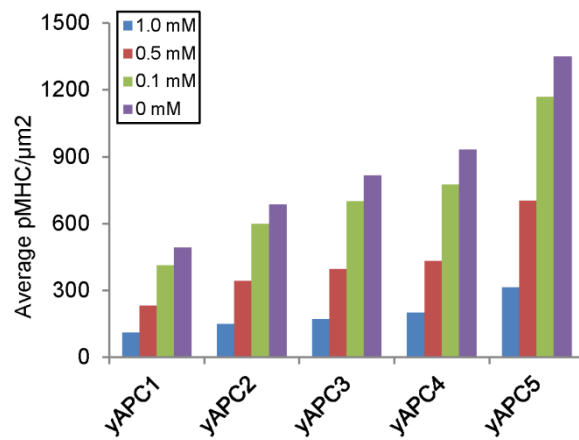


Figure S4. Average pMHC surface density after treating with TCEP to remove a fraction of total scaffold.

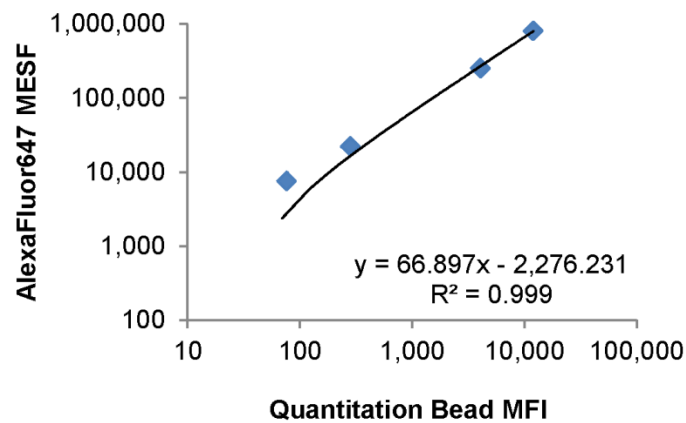


Figure S5. An example standard curve used to define relationship between MESF and quantitation bead MFI, which allows calculation of pMHC surface density.

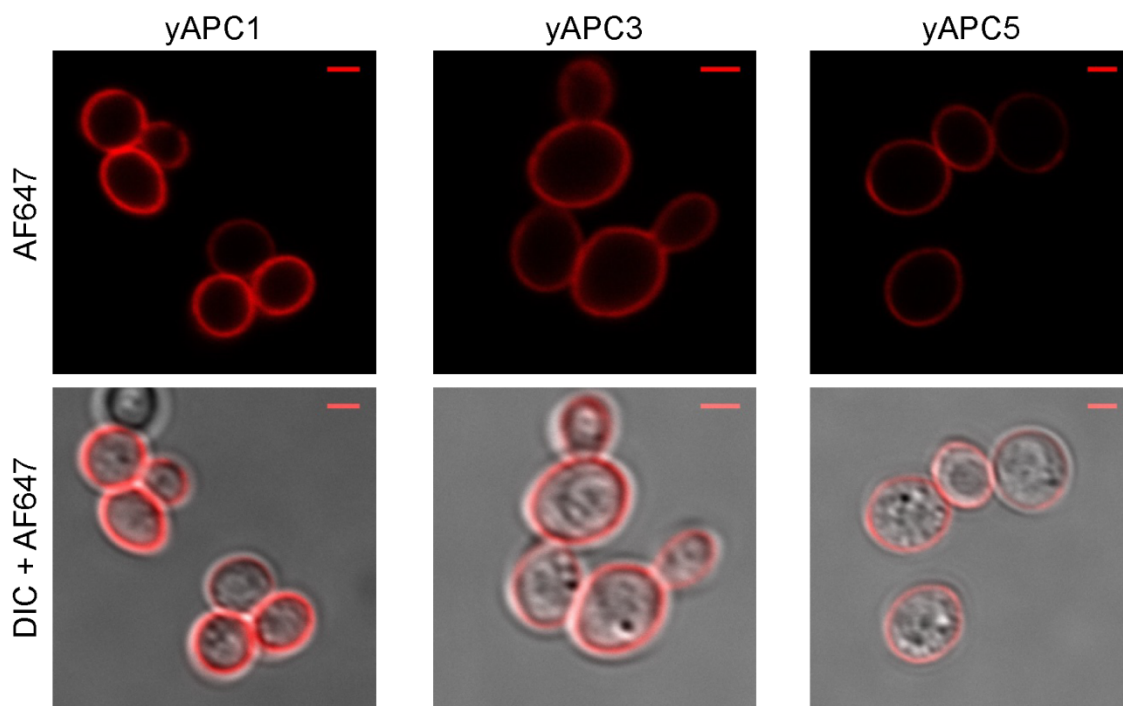


Figure S6. Confocal microscopy of yAPC1, yAPC3, yAPC5. Scaffold protein is stained with Alexa Fluor 647 (AF647) (top) and its expression is approximately uniform across yAPC surface. Fluorescent and DIC overlay shown in bottom. The scale bar in each figure is 2 μm .

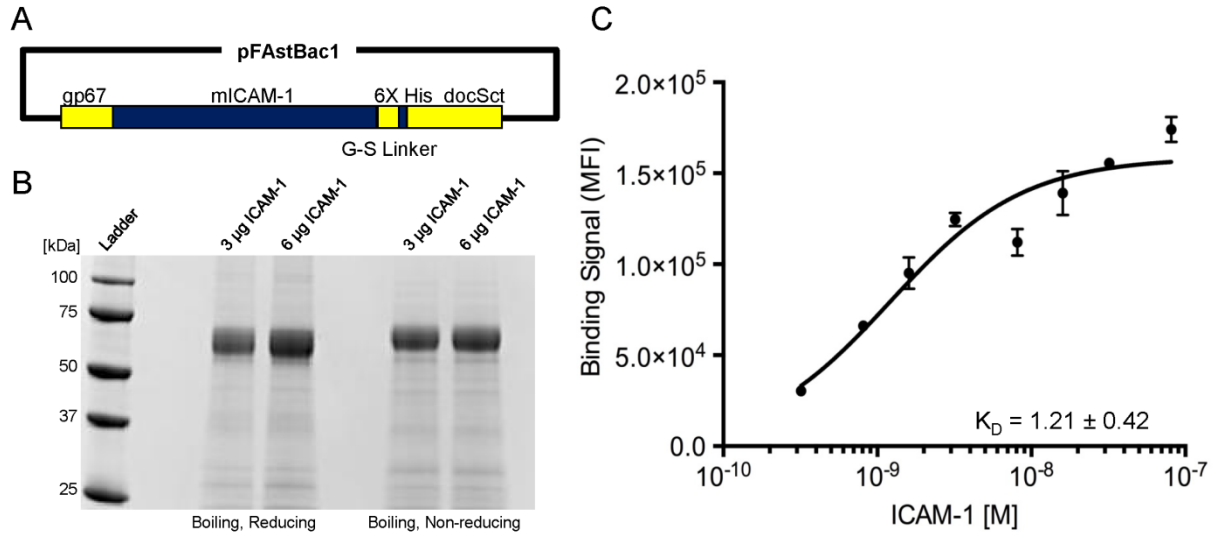


Figure S7. Construction and characterization of dockerin-fused ICAM-1. (A) Schematic showing the design of recombinant ICAM-1. (B) SDS-PAGE analysis of purified dockerin-fused ICAM-1. (C) Flow-cytometry apparent binding affinity curve between yAPC1 and dockerin-fused ICAM-1. The average binding affinity determined from the assay in (C) was 1.21 nM.

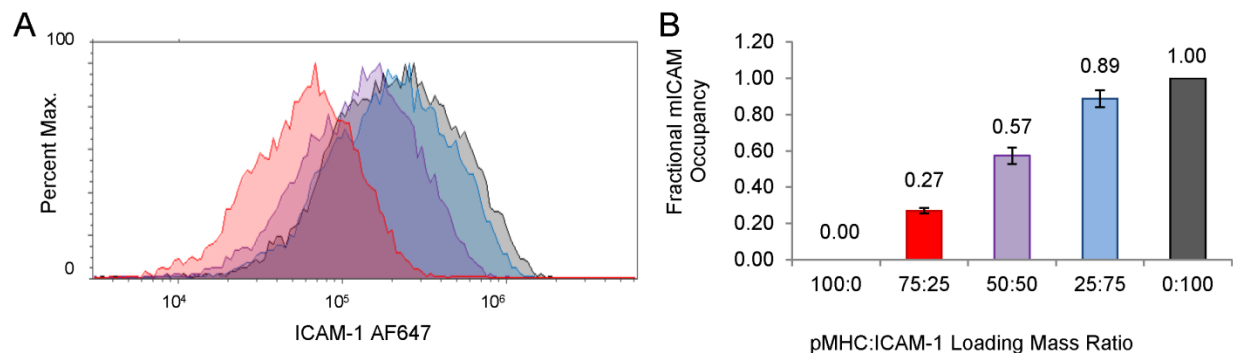


Figure S8. Determining fractional ICAM-1 display ratio using flow cytometry. (A) Flow cytometer histogram corresponding to ICAM-1 signal observed for yAPC1 loaded with various pMHC:ICAM-1 mass ratios (red: 75:25; purple: 50:50; blue: 25:75; gray: 0:100). (B) The actual fractional ICAM-1 occupancy was found by dividing the MFI for each mass-loading ratio by the MFI observed when only ICAM-1 was loaded (0:100).

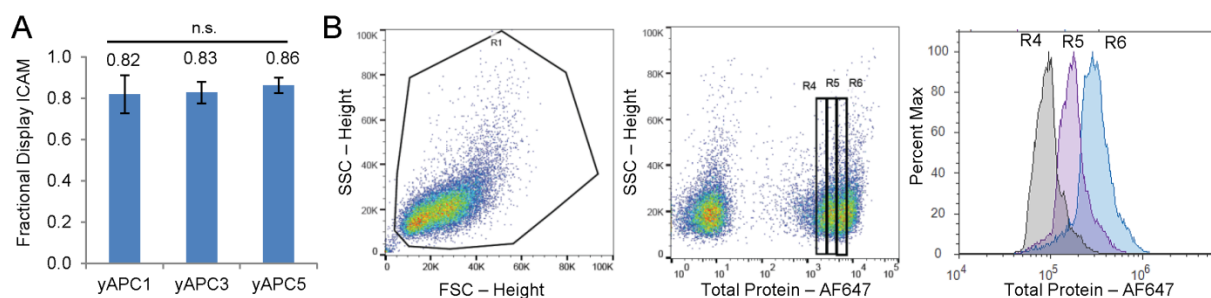


Figure S9. ICAM-pMHC co-display characterization. (A) Optimum surface occupancy of 85% ICAM-1 can be reproducibly obtained for yAPC1, yAPC3, and yAPC5. (B) yAPC codisplaying ICAM-1 and pMHC were sorted to isolate populations with defined total protein display levels (Middle: R4, R5, R6). Uniformity of sorted yAPC total protein display level was verified by flow cytometric analysis (right). Plots shown are of yAPC1 for simplicity. For (A), data represent mean $\pm$ SD ($n = 3$).

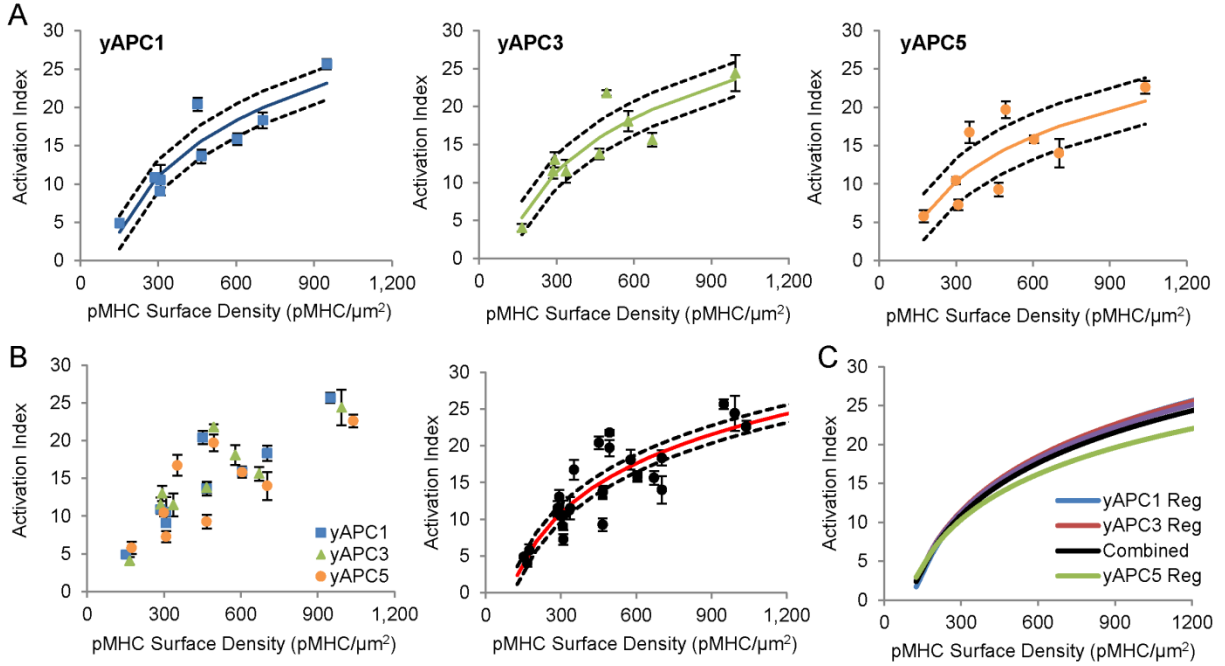


Figure S10. Activation index plotted with respect to pMHC surface density for yAPC codisplaying 85% ICAM-1 and 15% pMHC. (A) T cell activation index plotted with respect to pMHC surface density when stimulated by yAPC1, yAPC3, and yAPC5 displaying 85% ICAM-1. Solid lines represent logarithmic regression lines and dashed lines correspond to 95% confidence intervals for the regressions. (B) Total (left) and valency-independent (right) T cell activation index plotted with respect to pMHC surface density. Solid line represents valency-independent logarithmic regression line and dashed lines correspond to 95% confidence intervals for the regression. (C) Logarithmic regression lines overlay for T cell activation index vs. pMHC surface density for yAPC1 (blue) yAPC3 (red) yAPC5 (green) and all yAPC combined (black) displaying 85% ICAM-1.