a. Supplementary Figures and Legends

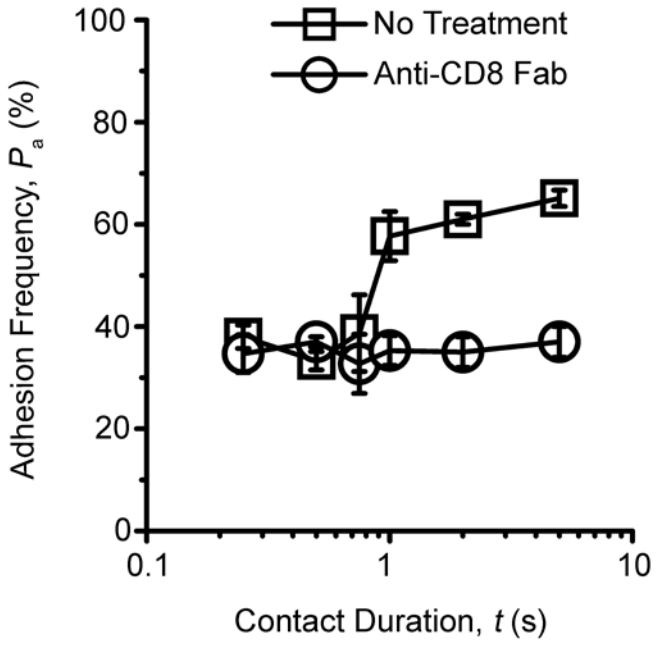

Figure S1, related to Figure 2. Anti-CD8 Fab blocked the second-stage adhesion increment. Adhesion frequency P_a vs. contact duration t data (points) of OT1 T cells interacting with RBCs bearing 4 OVA:H-2K^b/μm^2 in the absence (□) or presence (○) of anti-CD8 Fab. Each T cell-RBC pair was tested repeatedly for 50 contact-retract cycles at a given contact duration to estimate an adhesion frequency, and 2-6 cell pairs were tested for each t to calculate a mean P_a ± s.e.m.

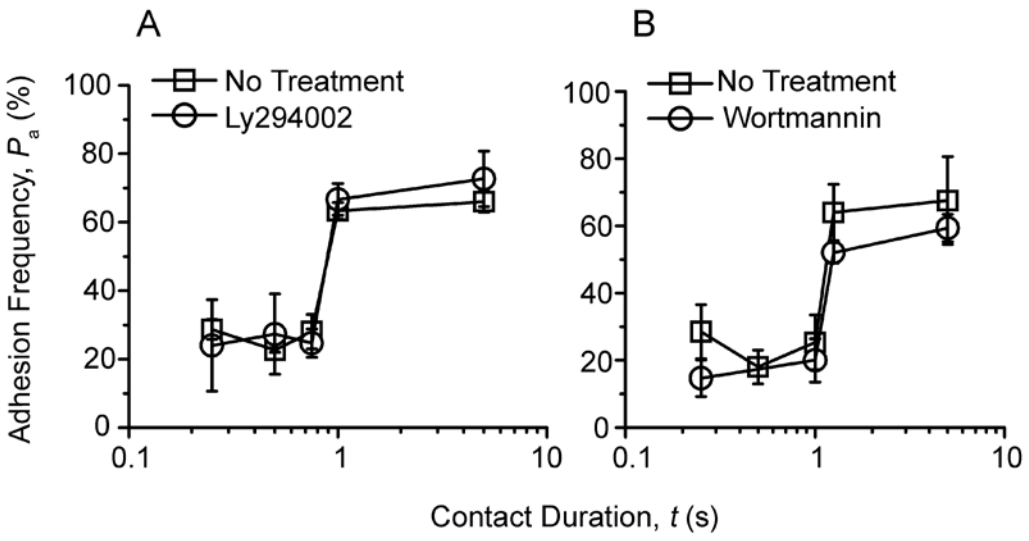

Figure S2, related to Figure 3. TCR-induced CD8-dependent increased adhesion is not affected by PI3K inhibition. Adhesion frequency P_a vs. contact duration t data of F5 T cells interacting with RBCs bearing 32 NP68:H-2D^b/μm^{-2} in the absence (□) and presence (○) of Ly294002 (**a**) and Wortmannin (**b**). Each T cell-RBC pair was tested repeatedly for 50 contact-retract cycles at a given contact duration to estimate an adhesion frequency, and 3-5 cell pairs were tested for each t to calculate a mean P_a ± s.e.m.

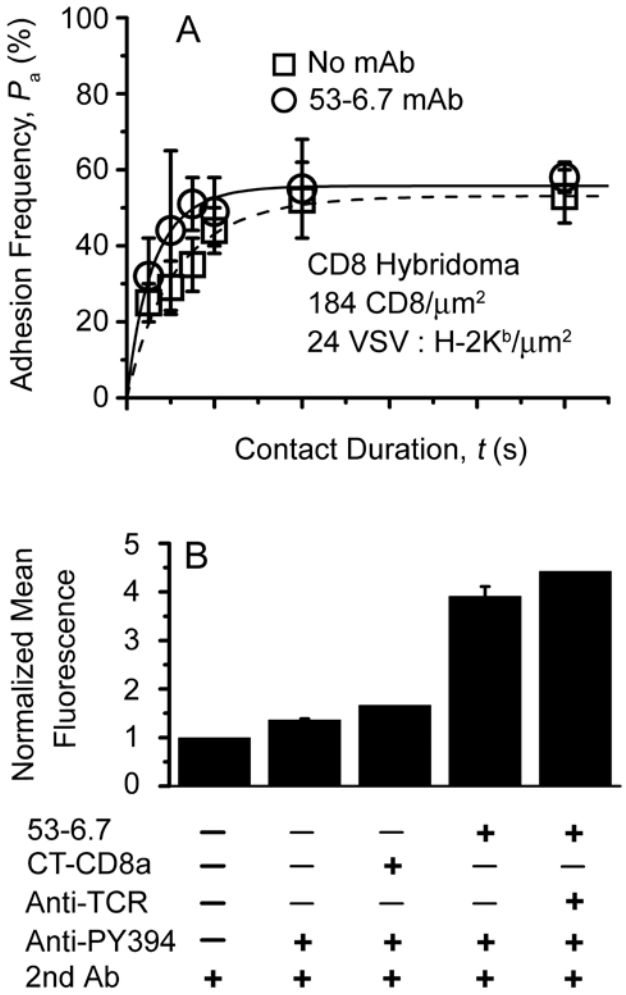

Figure S3, related to Figure 4. Effect of 53-6.7 mAb on pMHC-CD8 binding kinetics and phosphorylation of Lck Y394. **A**, P_a vs. t data of $CD8^+$-TCR^- OT1 hybridoma interacting with RBCs coated with VSV:H-2K^b pMHC in the absence (□) or presence of 53-6.7 mAb (○). Data (points) were presented as mean ± s.e.m. of 2-3 pairs of cells each making 50 contacts to estimate an adhesion frequency. Curves represent model-fit to data (Chesla et al., 1998). **B**, OTI T cells were incubated with 53-6.7 or CT-CD8a Fab in the absence or presence of anti-TCR mAb. Cells were then incubated with anti-PY394 mAb followed by staining with PE-conjugated second antibody and analyzed by flow cytometry. Data are presented as mean ± s.e.m. of 1-2 experiments.

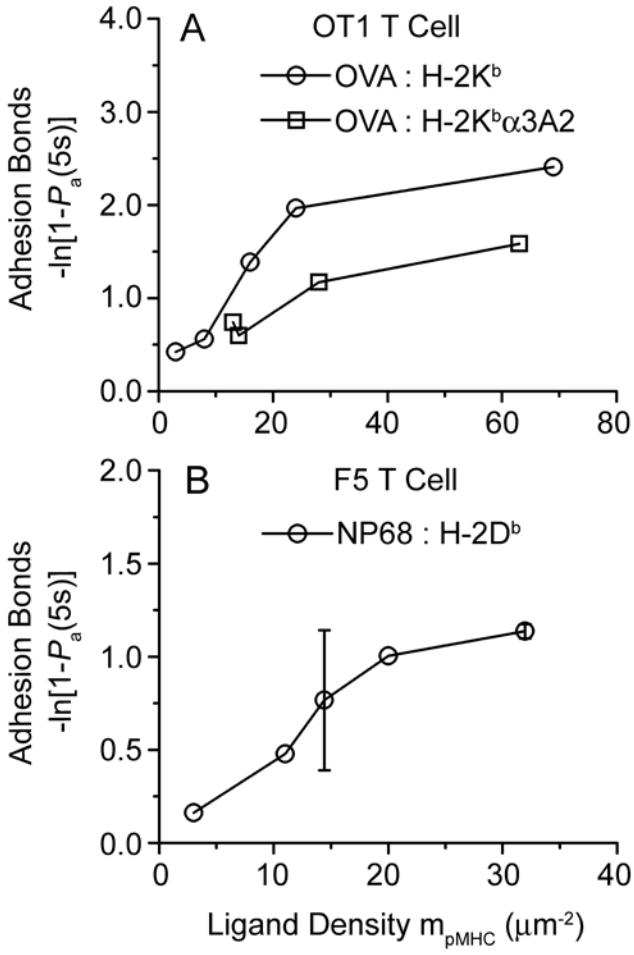

Figure S4, related to Figure 5. The adhesion bonds as a function of ligand density. Adhesion bonds calculated from adhesion frequency P_a measured at 5 s contact time are converted to adhesion bonds <*n*> by inverting Eq. 1 and plotted vs. ligand density for all OT1 (**A**) and F5 (**B**) T cells tested in this work. Each point is the average value of 3-5 pairs of cells from the same batch except for the two with error bars, which are averages of 13-23 cells from2-3 batches T cells. Error bars in this figure indicate variations in cells from different batches. Error bars indicating variations in cells from the same batch are shown in other figures of this paper.

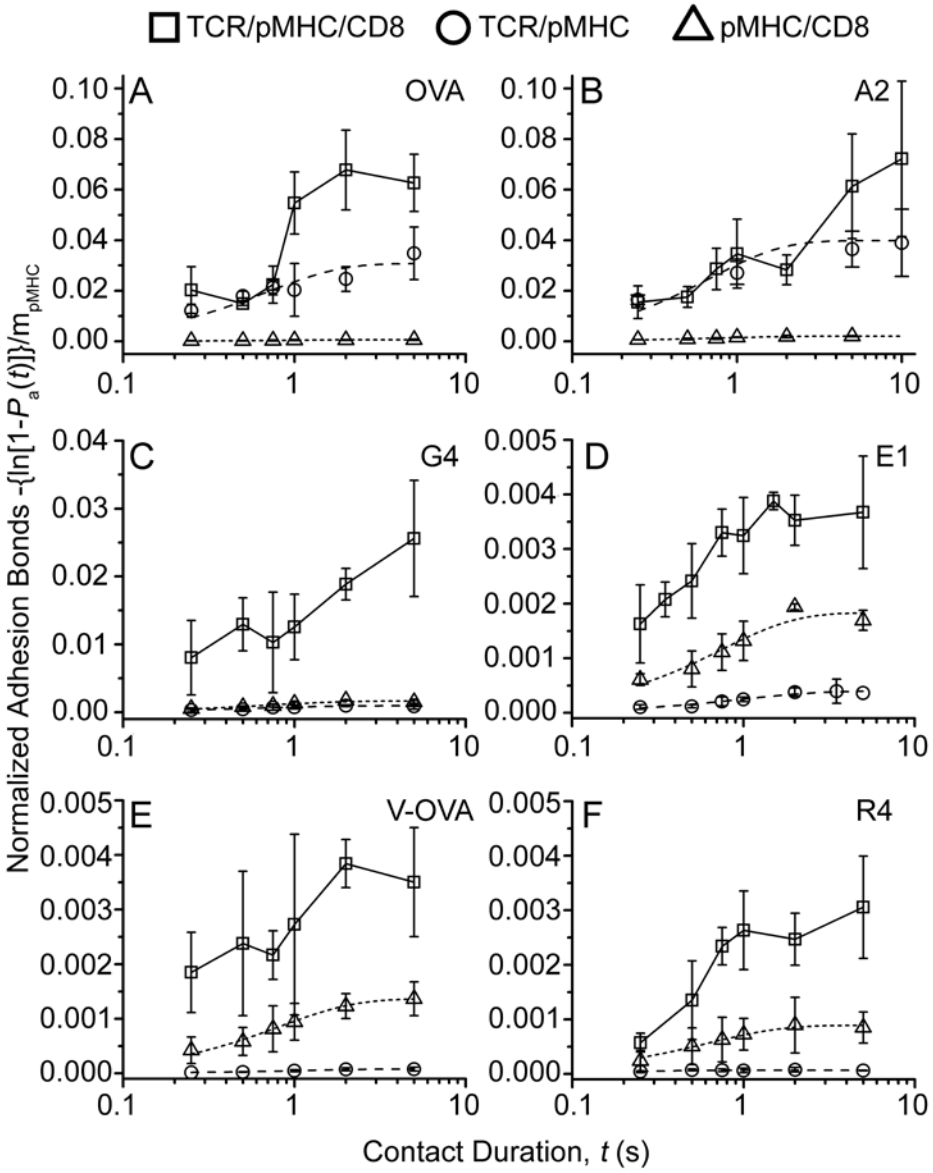

Figure S5, related to Figure 7. Synergy between TCR and CD8 amplifies T cell discrimination. Normalized adhesion bonds vs. contact duration data for OT1 T cells interacting with RBCs bearing H-2K^b (□) or H-2K^{b}α3A2 (○) complexed with OVA (**A**), A2 (**B**), G4 (**C**), E1 (**D**), V-OVA (**E**), and R4 (**F**). Also presented are data measured using RBCs bearing H-2K^b in the presence of the anti-TCR blocking mAb B20.1 (Δ). Dashed curves represent trendlines to the sum of TCR/pMHC (○) and pMHC/CD8 (Δ) data. These are the same data as Fig. 7A-F but the *y*-axis scale is shown as linear instead of logarithmic. Note that unlike Fig. 7A-F, different *y*-axis

scales are used here for different panels to show different $<n>/m_{pMHC}$ ranges for different peptides. The pMHC densities m_{pMHC} used for each peptide were listed in Table S1.

b. Supplemental Table and Legend

Table S1, related to Figure 7 and Figure S5. The pMHC densities used for binding measurements of TCR-CD8-pMHC, TCR-pMHC and CD8-pMHC interactions.

pMHC Density (μm^{-2})	**OVA**	**A2**	**G4**	**E1**	**V-OVA**	**R4**
TCR-CD8-pMHC	**22**	**20**	**117**	**246**	**158**	**184**
TCR-pMHC	**86**	**47**	**1354**	**1229**	**1471**	**1908**
CD8-pMHC	**844**	**529**	**579**	**541**	**1529**	**761**

c. Supplementary Movie Legends

Movie S1, related to Figure 1. Micropipette adhesion frequency assay. Real-time movie illustrating the micropipette adhesion frequency assay. An OTI T cell (left) was being driven by micromanipulation to test in repeated contact-retract cycles against a red blood cell (RBC, right). The RBC serves as an surrogate antigen-presenting cell by coating OVA:H-$2K^b$ onto its surface (right). It also acts as an adhesion sensor by stretching its membrane in response to the bond force when the T cell retracts, enabling visualization of adhesion. Two contact-retract cycles are shown. The first cycle results in no adhesion and the second cycle results in adhesion.

Movie S2, related to Figure 6. Alternating experiment. The left panel shows a three-time fast-motion movie of an alternating experiment. A F5 T cell (left) was repeatedly contacting a RBC (right) bearing NP68:H-2D^{b} with durations alternating between 0.25 and 5 s. The upper right panel shows the outcomes of the sequential contacts as adhesion scores (circles, right ordinate; 1 = adhesion, 0 = no adhesion) and their running frequency (triangles, left ordinate). The lower right panel shows the running frequencies for the two contact durations as a bar graph. The data plots are synchronized with the movie.

d. Supplemental Reference

Chesla, S.E., Selvaraj, P., and Zhu, C. (1998). Measuring two-dimensional receptor-ligand binding kinetics by micropipette. Biophys J *75*, 1553-1572.