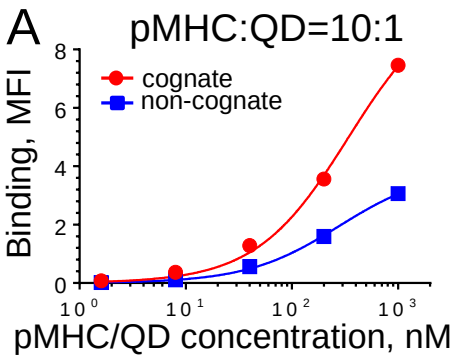
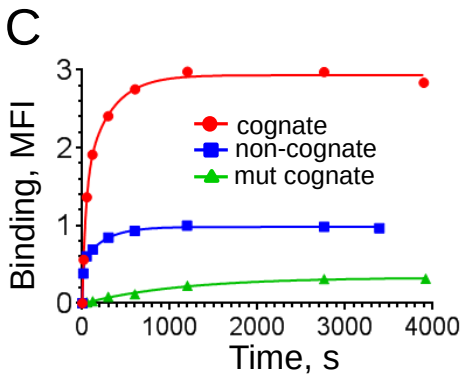
Supplemental Information

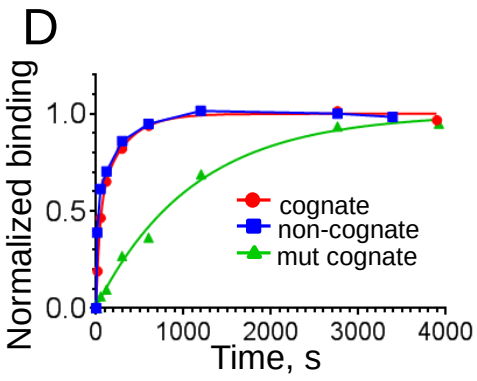
Supplemental Figure 1



B

pMHC	Kapp, nM	Normalized plateau
cognate	338±43	1
noncognate	277±22	0.392





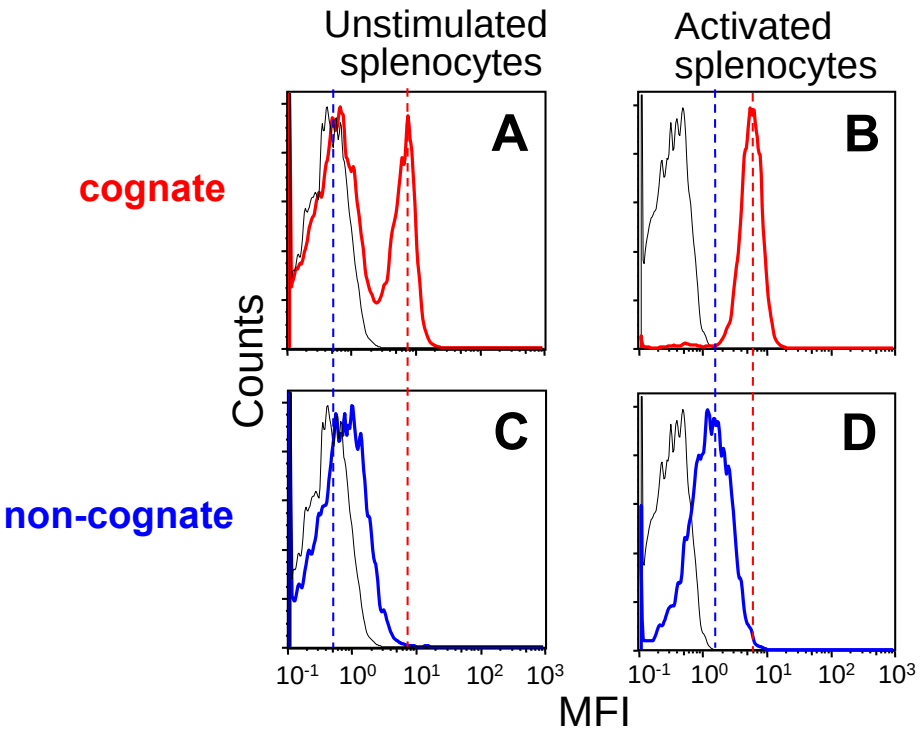
E

pMHC	Association curve	t_{fast} [1], s	t_{slow}, s	% fast binding component
Cognate	Two phase	25.9±5.10	282.5±71.0	56.6±10.0
Non-cognate	Two phase	8.41±1.40	177.7±59.1	54.7±14.4
Cognate, mut	One phase	947±240	NA	NA

[1] t is a time constant in equation (2); mean±SD are shown

Supplemental Figure 1. Equilibrium and kinetics analysis of pHLA-A2/QDs interaction with CTL surface. Representative measurements of pHLA-A2/QD equilibrium binding to CER43 cell surface at room temperature (**A**) and corresponding equilibrium binding parameters from 3 independent experiments (**B**) are shown. Representative kinetics measurements for pHLA-A2/QDs binding to 68A62 CTL surface at 18°C (**C**) and corresponding normalized binding data and fitted curves (**D**) are presented. Association kinetics parameters were derived from analysis of 3 independent experiments (**E**).

Supplemental Figure 2



Supplemental Figure 2. **pMHC/NiNLP binding to the surface of naïve and activated 2C cells.** Splenocytes from 2C transgenic mice were activated with SIYRYGL (SIY) peptide at 1 μM for 24 hr at 37^0C. The cells were washed to remove free peptide and incubated for an additional 48 hours with 100 u/ml of IL-2. Unstimulated splenocytes (**A** and **C**) and activated splenocytes mostly containing activated 2C cells (**B** and **D**) were incubated with 10 nM of NiNLPs bearing 30 molecules of either cognate SIY-K^b protein (**A** and **B**) or non-cognate pOV8-K^b protein (**C** and **D**) for 30 min at room temperature. The cells were washed and analyzed by flow cytometry.

Supplemental Table 1

Supplemental Table 1. Expression level of TCR and CD8 molecules on CTL surface

CTL	TCR	CD8
68A62	77,718±14,170	297,426±30,547
CER-43	94,509±12,836	190,561±22,850

Cytotoxic lymphocytes were stained with saturation amounts of Alexa 488 labelled with mouse antibodies against human CD3 (OKT-3, purified on protein A) or CD8 alpha (BD Pharmingen, RPA-T8) in DPBS with 2% FBS for 1 hour at 4^{o}C. The cells were briefly washed with chilled buffer and were immediately analyzed by flow cytometry. Alexa 488 calibrated beads (Quantum™ Alexa Fluor® 488 MESF, Bangs Laboratories) were suspended in the same FACS buffer and analyzed in line with fluorescently labelled cells. Cell-bound fluorescence were quantify by Bangs Laboratories quantitative template QuickCal. Number of TCR molecules calculated with assumption that two molecules of CD3 epsilon chains surrounded one α,β-TCR molecule. Mean±SD is indicated (N=3).