

**Title: Supplementary Movie 1.**

**Description:** Realtime Fura-2 340/380 ratiometric pseudo-color movie of an activated OT1 T cell stimulated with SIINFEKL:H2-K<sup>b</sup>-coated RBC and in contact with a BSA coated bead using the fMAF setup.

**Title: Supplementary Movie 2.**

**Description:** Realtime RICM and TIRF images of a PD-1 CHO cell spreading on PD-L2-coupled tension probe of 4.7 pN threshold force.

**Title: Supplementary Movie 3.**

**Description:** SMD simulated trajectory for pulling the mPD-1–mPD-L2 complex. The C-terminal residue of mPD-1 was harmonically constrained and the C terminus of mPD-L2 was pulled by a dummy spring moving at  $\sim 0.1$  nm/ns with a spring constant of  $\sim 70$  pN/nm.