## **Supplemental Data**

## The Strength of T Cell Receptor Signal Controls the Polarization of Cytotoxic Machinery

## to the Immunological Synapse

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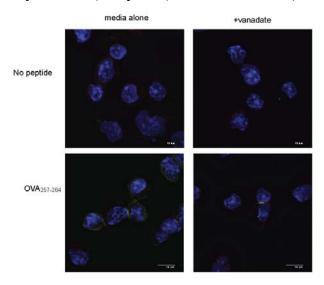


Figure S1. Orthovanadate Treatment Does Not Affect pY416 Staining in OT-1 Cells in the Absence of Peptide

Target EL4 cells were left untreated (no peptide) or pulsed with  $1\mu M$  of  $OVA_{257-264}$  peptide and conjugated to *in vitro* activated OT-I cells with or without  $100\mu M$  sodium orthovanadate. Cells were labeled with anti-Lck (488, green), pY416 (546, red) and nuclei stained with Hoechst (blue). Confocal images of a field of conjugates shown as a projection through the xy plane. Scale bars,  $10\mu M$ .

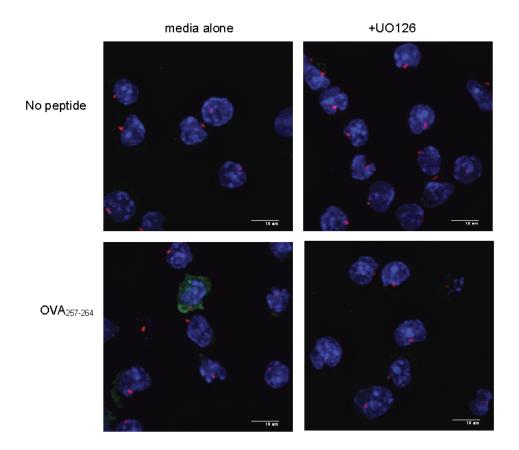


Figure S2. Preincubation of OT-1 Cells with the MEK Inhibitor, U0126, Abolishes Staining with Anti-pERK

Target EL4 cells were left untreated (no peptide) or pulsed with  $1\mu M$  of  $OVA_{257-264}$  peptide and conjugated to *in vitro* activated OT-I cells with or without  $10\mu M$  of the MEK inhibitor, UO126. Cells were labeled with anti-pERK (488, green),  $\gamma$ -tubulin (546, red) and nuclei stained with Hoechst (blue). Confocal images of a field of conjugates shown as a projection through the xy plane. Scale bars,  $10\mu M$ .