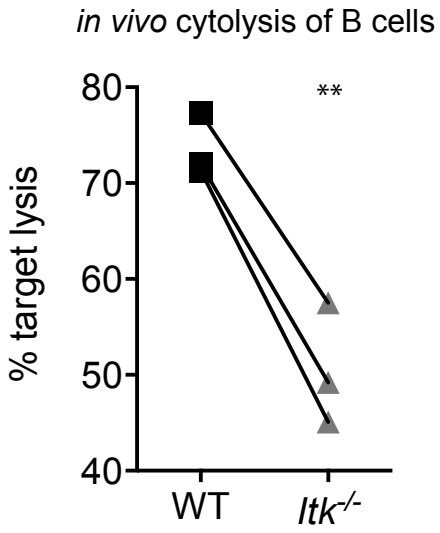
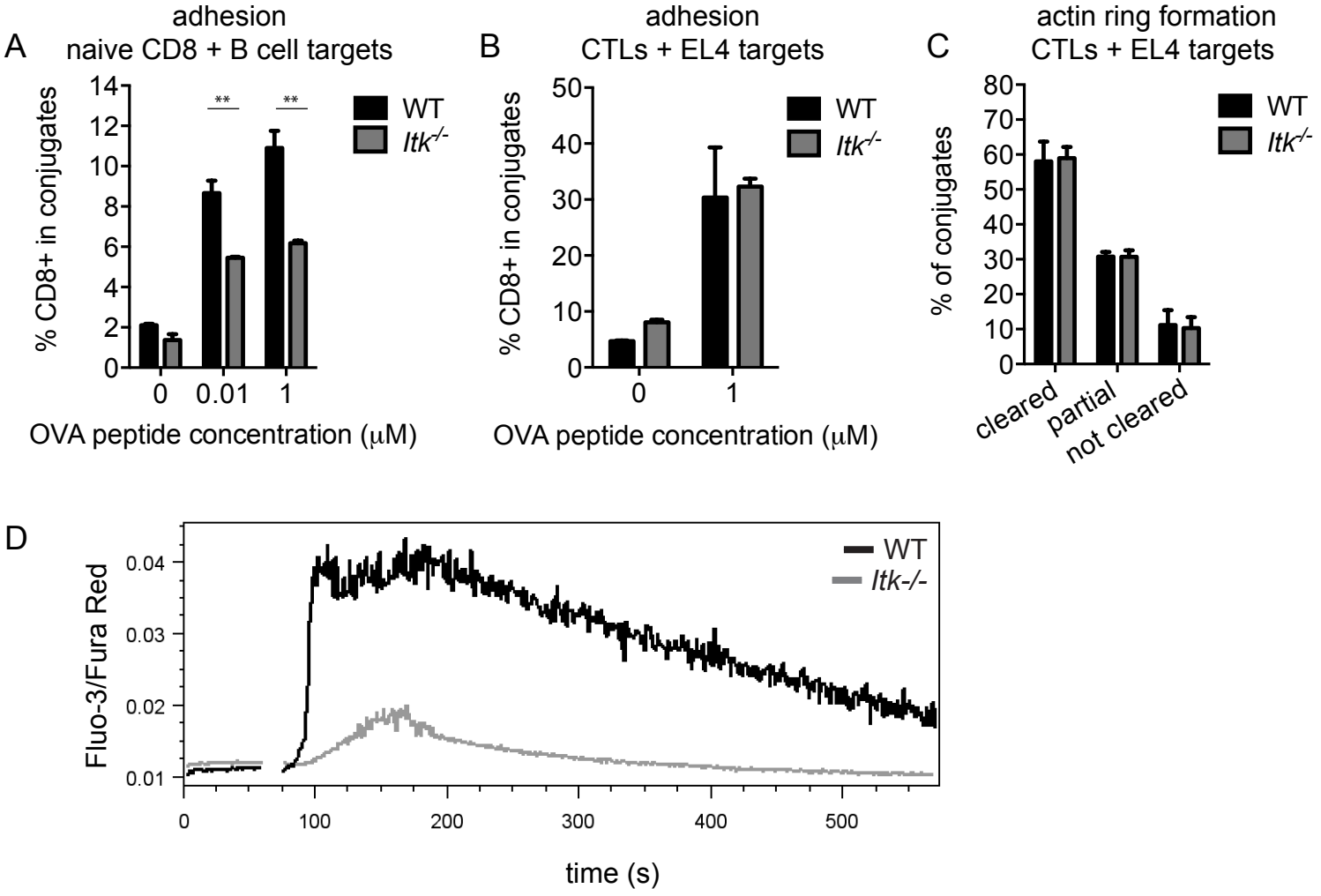
Supplemental figure 1



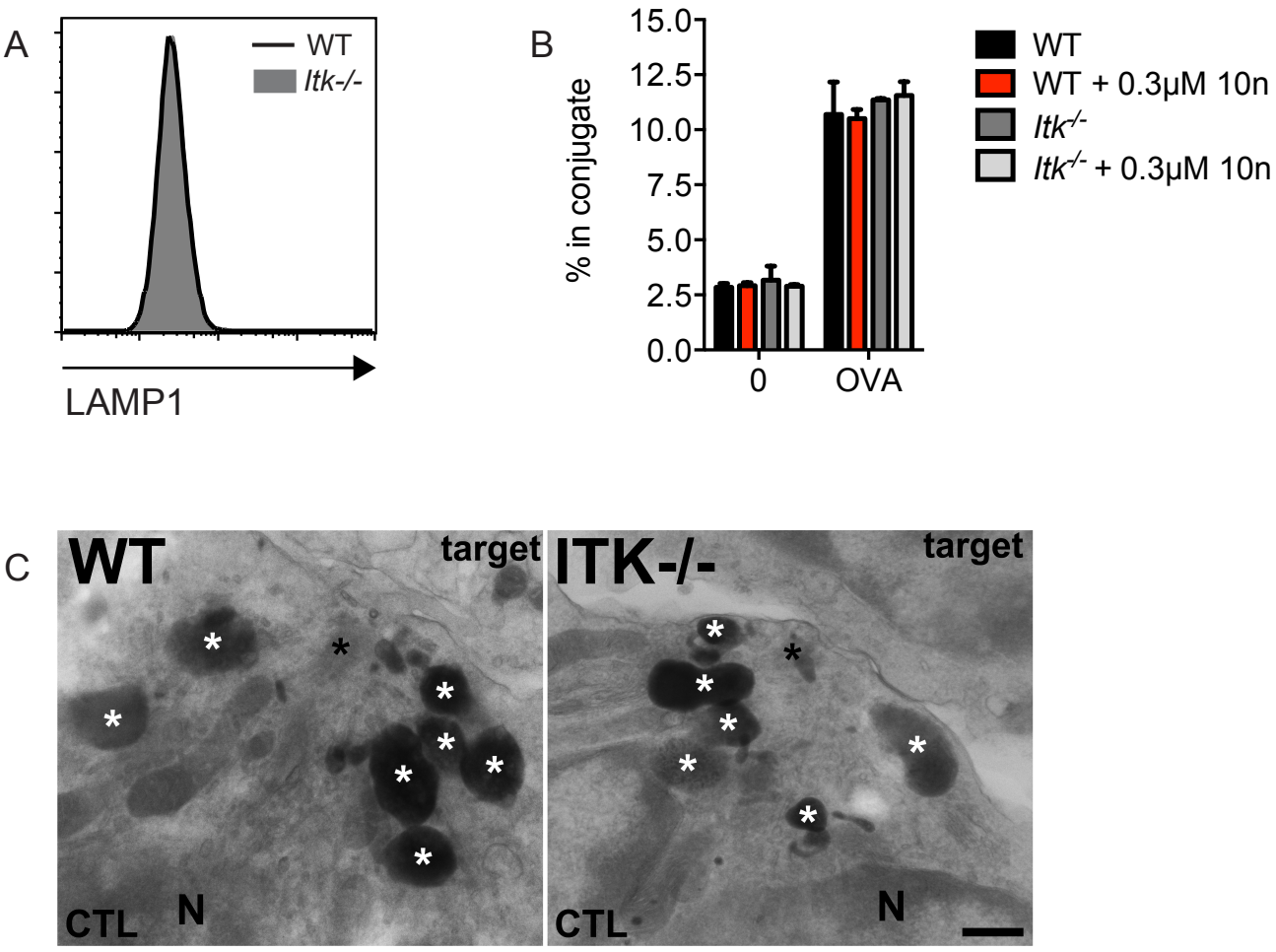
Supplemental figure 1: *In vivo* cytolysis of 1 μM OVA peptide-pulsed LPS-activated B cell targets. Graph shows percent target lysis, where each line represents paired mice from three individual experiments. **P<0.01 calculated by paired sample t-tests.

Supplemental figure 2



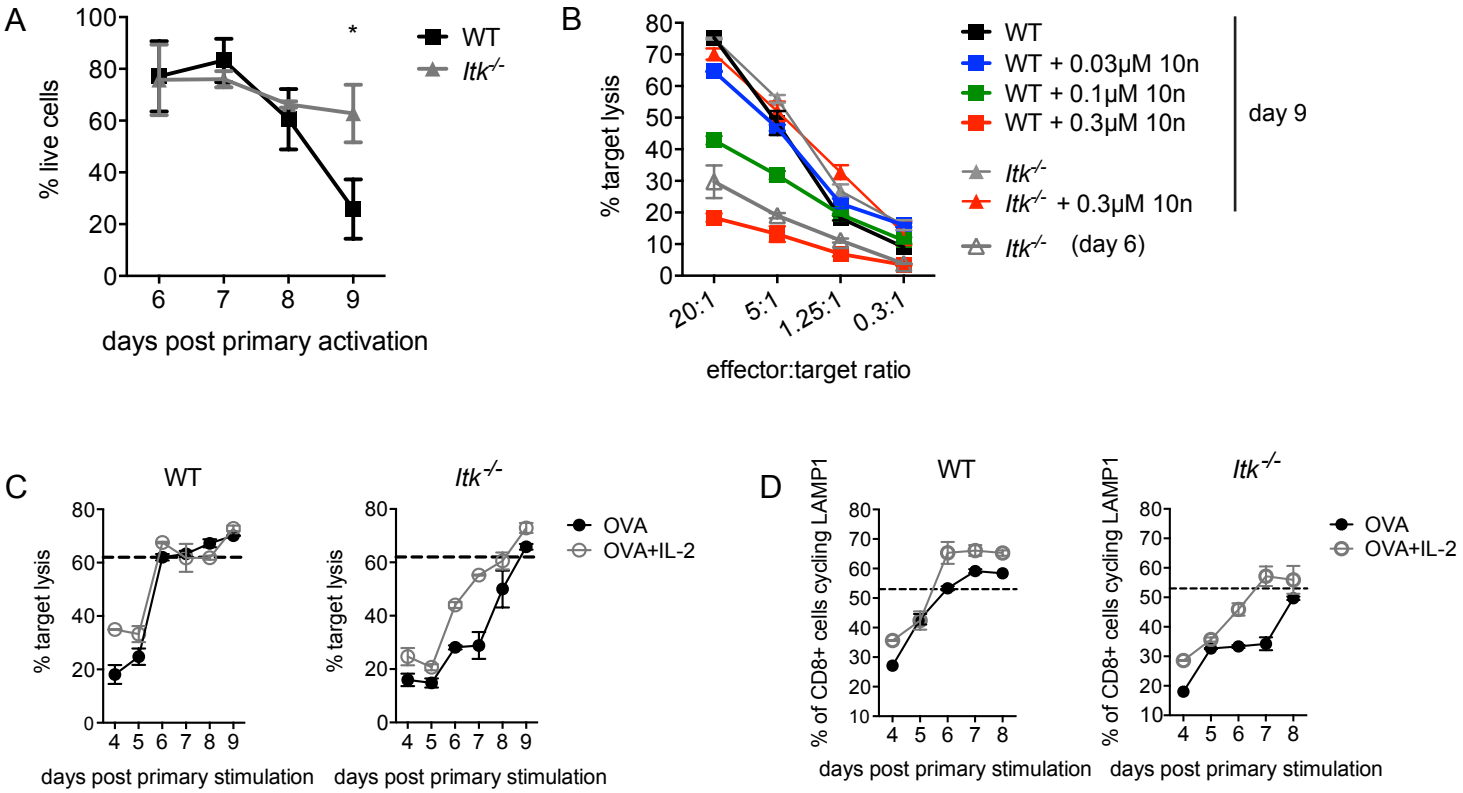
Supplemental figure 2: Adhesion, actin organization, and calcium flux in ITK-deficient and WT CTLs. (A) Adhesion of naïve CD8+ cells after 20 minutes conjugation of *ex vivo* WT (black) or *Itk*$^{-/-}$ (grey) CD8+ T cells to LPS-activated WT B cells pulsed with OVA peptide at indicated concentrations, or not pulsed as a control. Graph represents mean of triplicates ± SD of CD8+ target+ cells in total CD8+ events, and is representative of two independent experiments. **P<0.01 calculated by Student's T-test. (B) Adhesion of previously activated WT (black) or *Itk*$^{-/-}$ (grey) CTLs after 20 minutes conjugation to EL4 targets pulsed with 1μM OVA or not pulsed as a control. Graph represents mean ± SD of CD8+ target+ cells in total CD8+ events, and is representative of more than five experiments. (C) Quantification of actin ring localization at the immunological synapse between WT (black) and *Itk-/-* (grey) CTLs and EL4 targets pulsed with 1μM OVA peptide. WT (total n=185) and *Itk*$^{-/-}$ (total n=194). Bars represent mean ± SEM from more than three independent experiments. (D) Intracellular Ca2+ flux in WT (black) or *Itk*$^{-/-}$ (grey) following 5μg/mL anti-CD3 crosslinking, plotted as the ratio of Fluo-3 (FL-1)/Fura Red (FL-3) over time. Histograms are representative of two independent experiments.

Supplemental figure 3



Supplemental figure 3: LAMP1 content and cell adhesion in the absence of ITK activity. (A) Representative histogram of total LAMP1 (CD107a) content in permeabilized WT (black) and *Itk*$^{-/-}$ (grey) CTLs from cells used in degranulation assays. (B) Adhesion of previously activated WT OT-I CTLs (black), *Itk*$^{-/-}$ OT-I CTLs (grey), or WT or *Itk*$^{-/-}$ OT-I CTLs treated with 0.3 μM 10n (red or dark grey, respectively) to EL4 cells pulsed with 1μM OVA peptide or non-pulsed as a control after 20 minutes. Bars represent mean of triplicates ± SD of CD8+ target+ cells in the CD8+ gate, from one of two independent experiments. (C) Semi-thick (200-300 nm) sections showing polarized elec-tron-dense HRP-loaded granules in both WT and ITK-deficient CTLs conjugated to target cells for 60 minutes. Centrosomes = black aster-isks. Granules = white asterisks. N=nucleus. Scale bars = 500nm.

Supplemental figure 4



Supplemental figure 4: (A) WT (black) or *Itk*$^{-/-}$ OT-I CTLs (grey) were cultured in complete RPMI plus 10% FBS and stained with Live/Dead green dye to evaluate viability. Graph depicts percent of total CD8+ cells with dye excluded at indicated time points. Points represent mean of triplicates ± SD from one of two independent experiments. *P<0.05, calculated by Student's T-test. (B) *In vitro* cytolysis of EL4 targets pulsed with 1μM OVA peptide by day 9 WT OT-I CTLs treated with indicated concentrations of the ITK inhibitor, 10n (blue, green, and red), immediately before cytolysis assays, or left untreated as a control (black), or ITK-deficient OT-I CTLs untreated (grey, closed triangles) or treated with 10n (red, closed triangles). Day 6 *Itk*$^{-/-}$ OT-I CTLs are included (grey, open triangles). Graph shows mean of triplicates ± SD, and is representative of two independent experiments. (C) *In vitro* cytolysis of 1μM OVA peptide-pulsed EL4 target cells by WT (left panel) or *Itk*$^{-/-}$ OT-I CTLs (right panel) activated with 10nM OVA peptide only and then cultured in IL-2 for 3 days (OVA, black circles) or 10nM OVA peptide plus 10 IU IL-2 before continued culture in IL-2 for 3 days (OVA+IL-2, grey circles). Graphs show percent cytotoxicity ± SD on indicated days post primary stimulation and are representative of two independent experiments. (D) Degranulation measured in a flow-based LAMP1 cycling assay in response to plate-bound anti-CD3 in WT (left panel) or *Itk-/-* (right panel) OT-I CTLs activated with 10nM OVA peptide only and then cultured in IL-2 for 3 days (OVA, black circles) or 10nM OVA peptide plus 10 IU IL-2 before continued culture in IL-2 for 3 days (OVA+IL-2, grey circles). Graphs show percent CD8+ T cells cycling LAMP1 ± SD, where CD107a-PE positive cells in the CD8+ gate were determined to be positive for degranulation on indicated days post primary stimulation and are representative of two independent experiments.