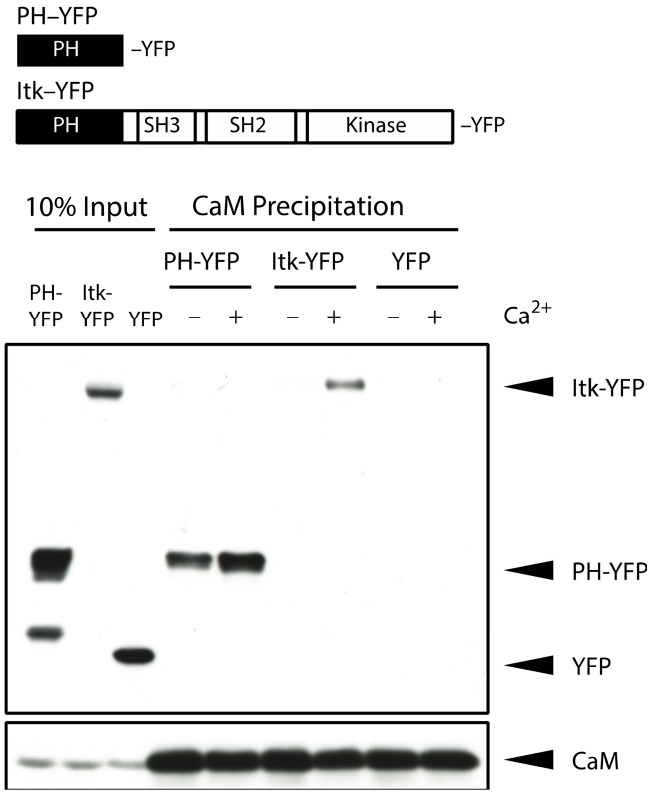

Fig. S1. CaM binding maps to the Itk PH domain. Full-length Itk and the Itk PH domain were tagged with YFP and expressed in 293 epithelial cells. CaM association was assessed by coprecipitation with CaM-coated beads in the presence or absence of 1 µM Ca^{2+}. Interacting proteins were detected by Western blot using antibodies recognizing GFP and YFP (JL-8). Data are representative of 3 experiments.

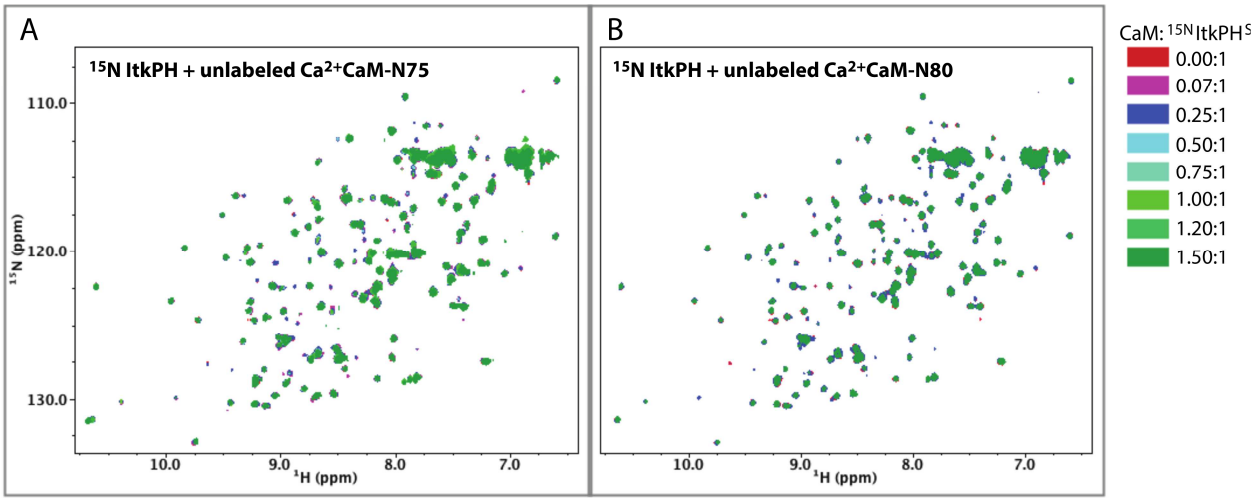

Fig. S2. The N-domain of CaM does not bind to the Itk PH domain. ^{1}H-^{15}N HSQC NMR titrations of ^{15}N-Itk PH were performed with two different constructs of the N-terminal subdomain of CaM: (**A**) Ca^{2+}/CaM-N75 (amino acids 1-75), (**B**) Ca^{2+}/CaM-N80 (amino acids 1-80); CaM-N preps were first saturated with Ca^{2+} and then dialyzed into NMR buffer with 1 mM $CaCl_2$, as described in the main text Materials and Methods.

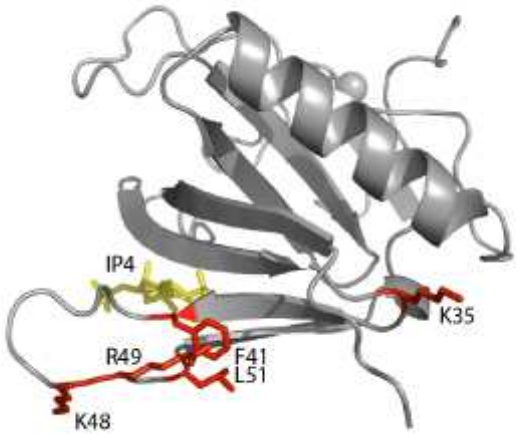

Fig. S3. Structural model of the Itk PH domain showing the location of the mutated residues tested. See table S1 for a list of the mutations. IP_4 is yellow; the indicated residues are labeled in red.

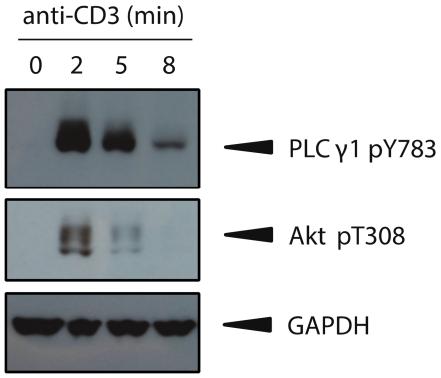

Fig. S4. TCR stimulation promotes Akt activation and PLCγ1 phosphorylation in Jurkat E6.1 cells. Jurkat E6.1 cells were rested overnight and stimulated with an antibody against CD3 (anti-CD3) for the indicated times. TCR-mediated activation of Akt (detected with antibodies recognizing the Thr^{308}-phosphorylated form) and PLCγ1 (detected with antibodies recognizing the Tyr783-phosphorylated form) were assessed by Western blot. Data are representative of 2 experiments.

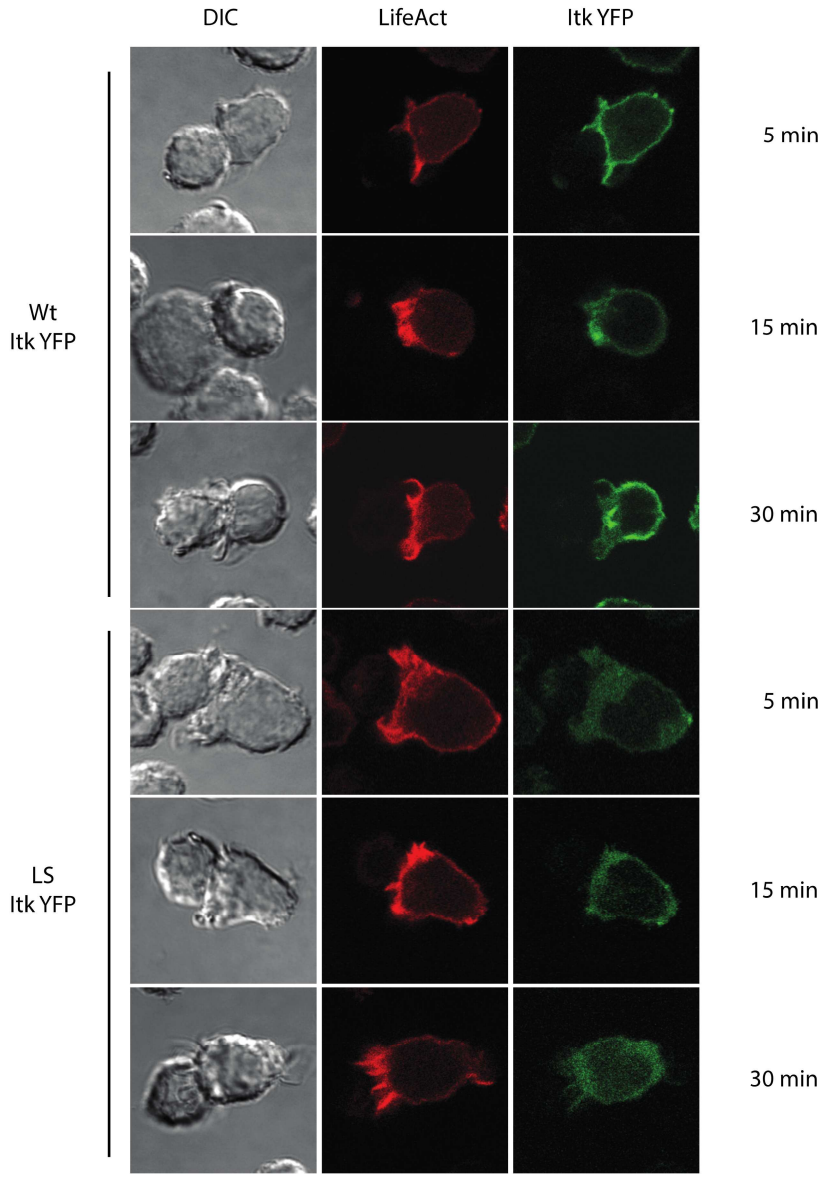

Fig. S5. Kinetic analysis of recruitment of wild-type Itk or LS-Itk to the immune synapse. Jurkat cells expressing WT-Itk-YFP or LS-Itk-YFP were transfected with pRuby-LifeAct. Jurkat-Daudi conjugates were made as previously described {Lin, 2003 #377}. Conjugates were fixed at the indicated times after copelleting and visualized by confocal microscopy. For each time point, colocalization of LifeAct-Ruby (red) and WT-Itk-YFP or LS-Itk-YFP fusion proteins (green) was assessed for 40 randomly selected cells (20 each for WT-Itk-YFP or LS-Itk-YFP). At least 80 cells were analyzed per time point in 2 independent experiments.