Supplementary Materials for

T Cell Activation Results in Conformational Changes in the Src Family Kinase Lck to Induce Its Activation

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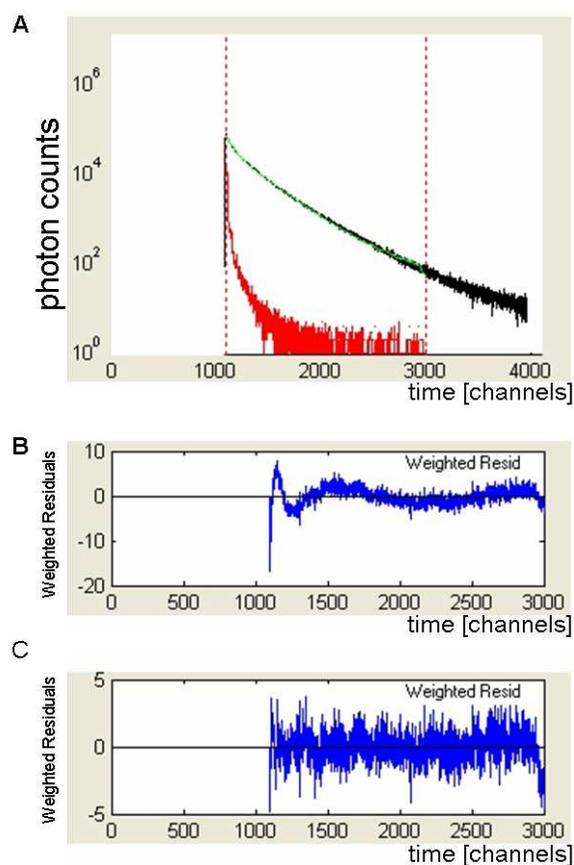
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Fig. S1. Two versus three lifetimes are needed to describe the fluorescence decay of an ECFP-EYFP FRET pair. (**A** to **C**) JCam1.6 cells expressing CLckY-1 were measured with the QA detector. (A) The measured data (black line) were deconvolved with the measured IRF (red line) and fitted with three (green line) lifetimes. In the time axis one channel corresponds to 12.5 ps. To demonstrate the quality of the fit, the weighted residuals between the experimental data and calculated curves are shown with (B) two or (C) three individual lifetimes. As visualized in (C) by the flat line of the weighted residuals, three lifetimes were necessary to describe the measured date with high precision. This analysis resulted in the following lifetimes and their fractional distributions: 3.2 ns (40%), 1.4 ns (35%), and 0.8 ns (25%). The graph shows a typical result of 10 independent experiments.
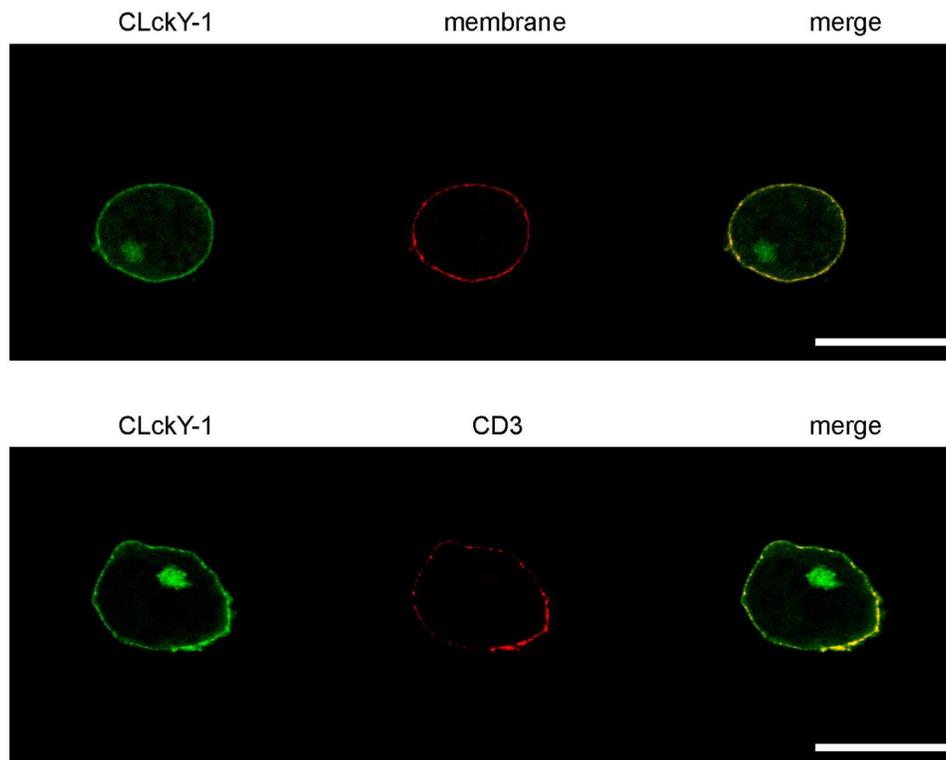
Fig. S2. Localization of the biosensor CLckY-1 at the plasma membrane. Confocal images show the distribution of the biosensor CLckY-1 (green) and the plasma membrane (red) or anti-CD3 staining (red). Merged images reveal localization at the plasma membrane in transfected JCam1.6 cells expressing CLckY-1. Lck-deficient JCam1.6 cells expressing CLckY-1were plated on slides and immediately fixed. After permeabilization and blocking, cells were incubated with monoclonal antibody against CD3 (C305). After washing and a second blocking step, specimens were treated with a secondary antibody (DyLight 549). For membrane staining, Cell Tracker (CM-Dil, Molecular Probes) was added to the plated JCam1.6 cells expressing CLckY-1, incubated for 10 min, washed with PBS, and fixed. Samples were scanned with a confocal microscope. The data are representative of three independent experiments. Scale bar: 10 µm.
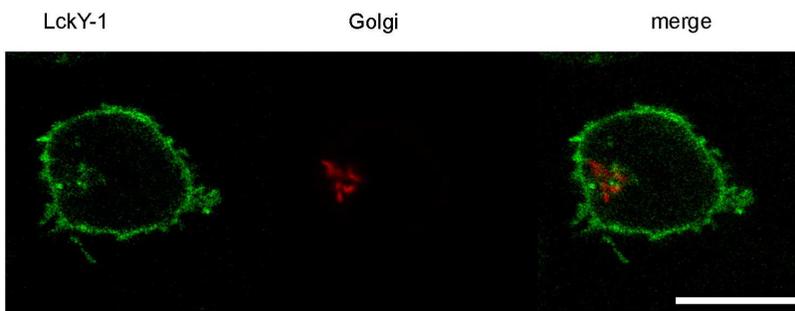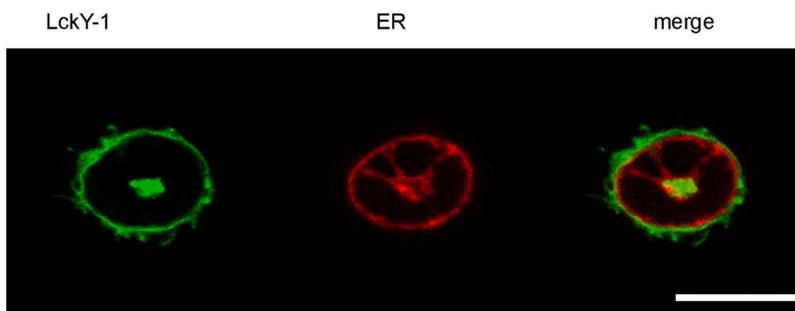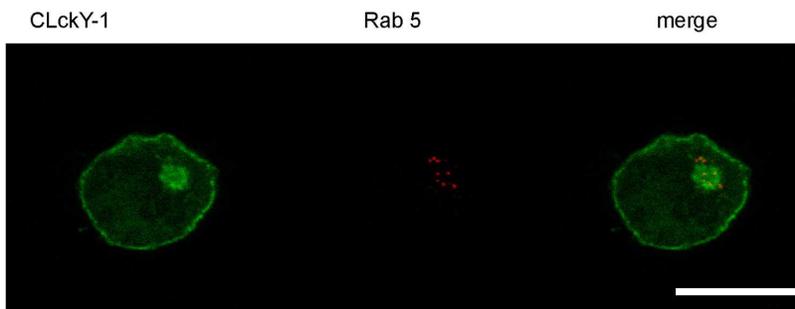
CLckY-1	Rab 11	merge

CLckY-1	EEA1	merge

CLckY-1	Rab 5	merge

LckY-1	ER	merge

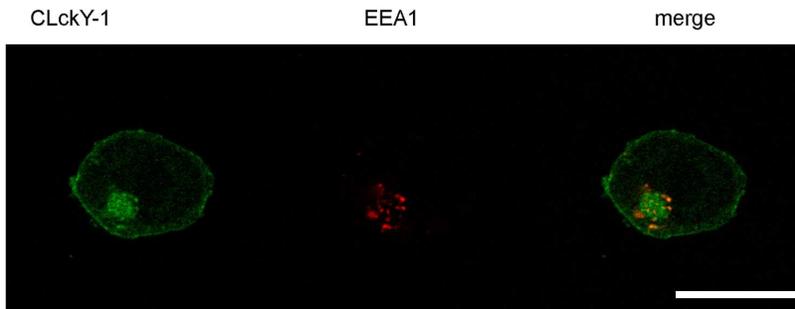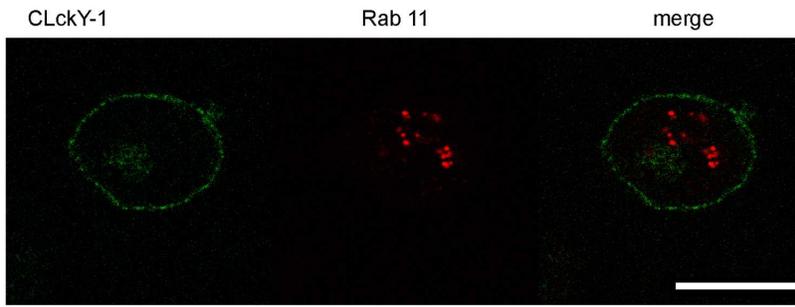| LckY-1	Golgi	merge

Fig. S3. Localization of the biosensor in transfected Lck-deficient JCam1.6 cells. Confocal images show the localization of the biosensor LckY-1 in a cytoplasmic compartment (green) and Rab11 and the endosomal markers EEA1 and Rab5 (red). Images of the biosensor LckY-1 are shown with the endoplasmic reticulum (ER) and the Golgi (both in red). Rab11, Rab5, and EEA1 were detected with specific antibodies as described in fig. S2, whereas the ER and Golgi were detected in cells cotransfected with plasmids encoding a Golgi marker (GalT-CFP, pseudocolored in red) and an ER marker (Calreticulin-CFP, pseudocolored in red) The data are representative of five independent experiments. Scale bar: 10 µm.
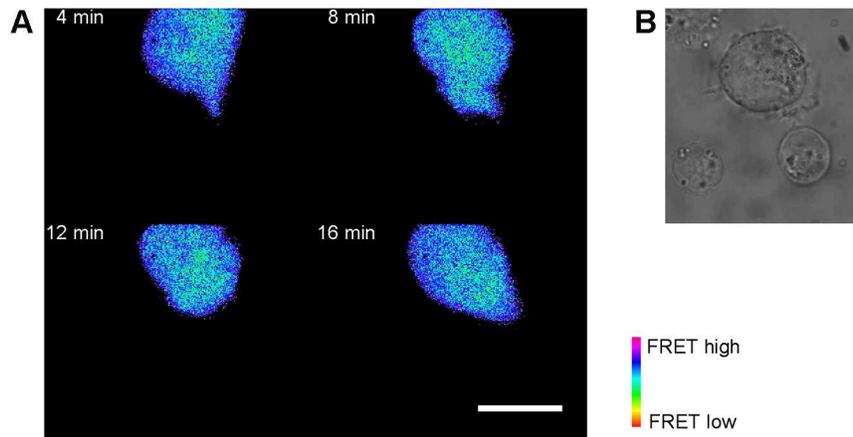
Fig. S4. Conjugate formation between transfected JCam1.6 cells expressing CLckY-1 and mock-treated Raji B cells as measured with the QA detector in the FLIM setup. (**A**) The pre-exponential factors of τ_3 were analyzed and converted to pseudocolored images. (**B**) The transmitted light image show CLckY-1-expressing JCam1.6 cells before contact with the mock-treated Raji B cells. The data are representative of five independent experiments. Scale bar: 10 µm.
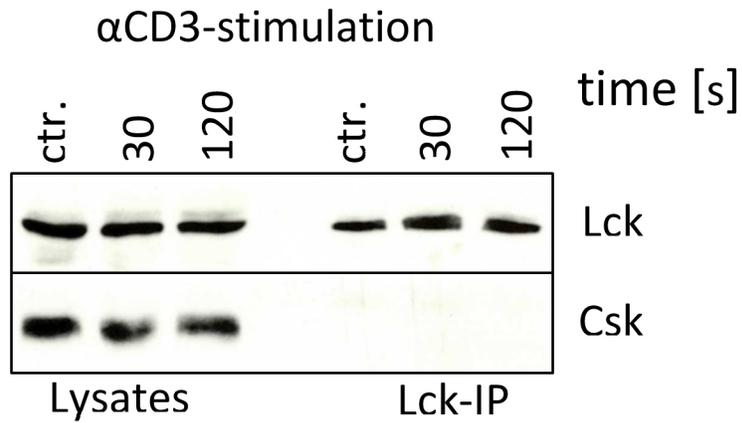
Fig. S5. Lck does not coimmunoprecipitate with Csk. Primary human T lymphocytes were either left unstimulated as a control (ctr.) or were stimulated with αCD3 for the indicated times. Cells were lysed in buffer containing NP-40 and LM and then subjected to immunoprecipitation (IP) with antibody against Lck. A fraction of the detergent lysates (left side) and the Lck immunoprecipitates (right side) were subsequently subjected to SDS-PAGE, which was followed by Western blotting analysis with antibodies against Lck and Csk. Data are representative of three independent experiments.