Supporting Information

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SI Text

Single-Cell Ca^{2+} and Fluorescence Video Imaging. L625.7 fibroblasts (3.5×10^5/mL) were pulsed with 1 μg/mL Ttox (830–843) peptide at 37 °C overnight. T8.1 cells (5×10^5) were incubated for 20 min at 37 °C with 1 μM Fura-2/AM (Molecular Probes) in 1 mL of a 10 mM Hepes buffer, (140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1 mM Na_2HPO_4, and 1 mg/mL glucose, pH 7.5). After washing twice, T8.1 cells were added to APCs and Fura-2 fluorescence were followed with a TE300 inverted microscope using Metafluor imaging system (Universal Imaging). Transmission light images were taken every 10 s in turn with intracellular calcium measurement (340 nm and 380 nm excitation; 510 nm emission). Ca^{2+} response from more than 30 individual conjugates of each group was calculated. To avoid a filter effect on the averaged Ca^{2+} trace due to the asynchrony of the individual responses, the peak Ca^{2+} responses of all cells were synchronized before averaging.

Tyrosine Phosphorylation and Immunoprecipitation Assay. GTP-T8.1, ADAP-T8.1, or M12-T8.1 cells were either left unstimulated or incubated with anti-CD3 at 4 °C followed by cross-linking with anti-hamster at 37 °C for 15 min. Alternatively, cells were incubated with Ttox peptide pulsed L625.7 cells as described in ref. 21. Cells were then harvested for immunoblotting with anti-4G10 or immunoprecipitation with anti-ADAP.

ELISA. GTP-T8.1, ADAP-T8.1, or M12-T8.1 cells (2×10^5) were cultured in various concentrations of anti-CD3- or anti-CD11a-coated plates for 40 h. The supernatants were collected, and IL-2 was measured using ELISA with rat anti-mouse IL-2 monoclonal antibody (ELISA Capture) and biotinylated rat anti-mouse IL-2 monoclonal antibody (ELISA Detection).

Statistical Analysis. Results are given as the mean ± SD. The difference between means was tested using unpaired student *t* test using GraphPad Prism version 3.02. $P < 0.05$ was considered significant.

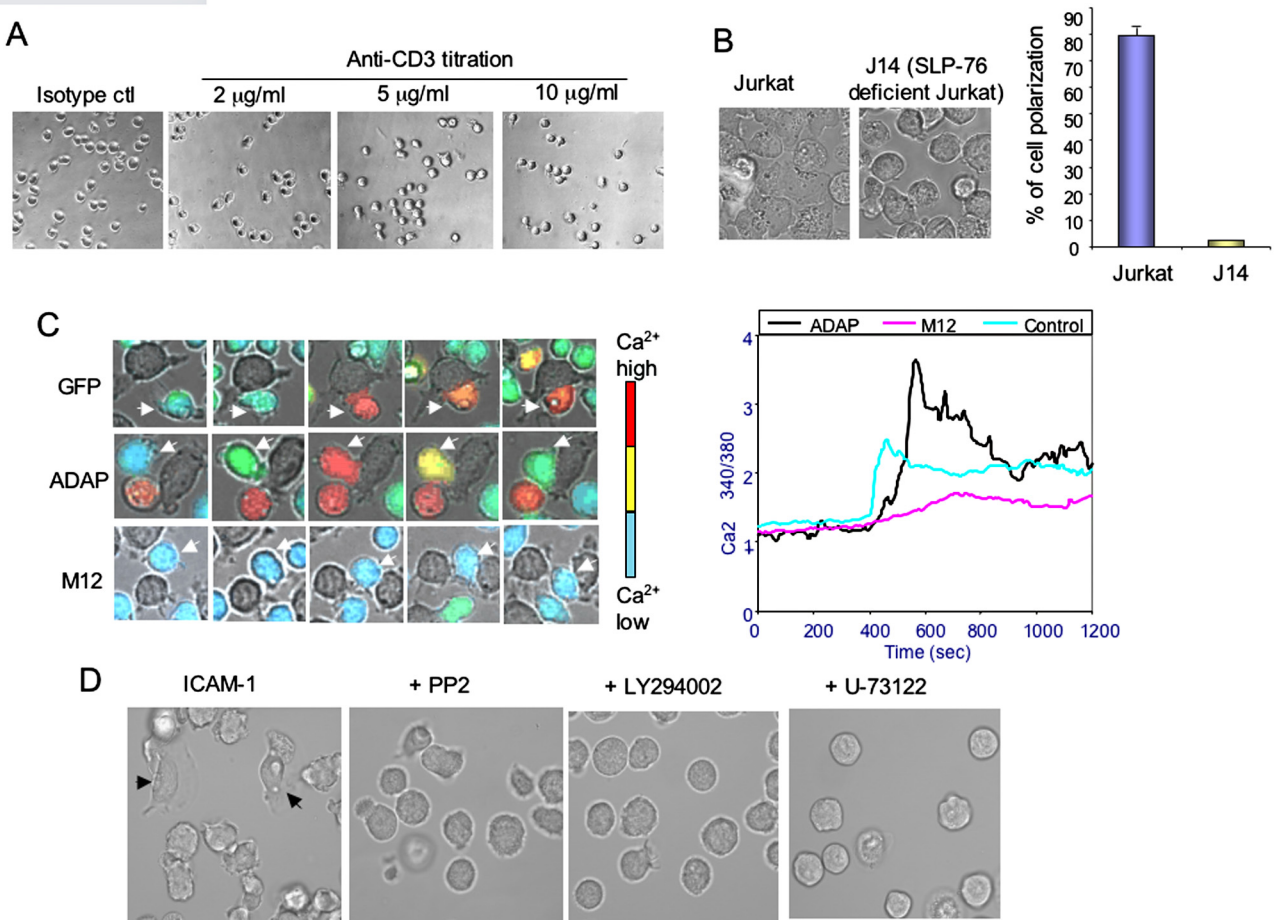
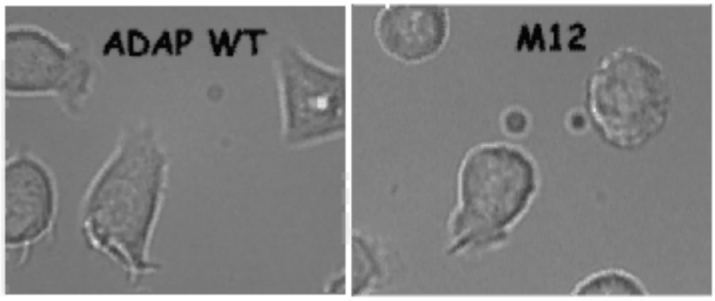
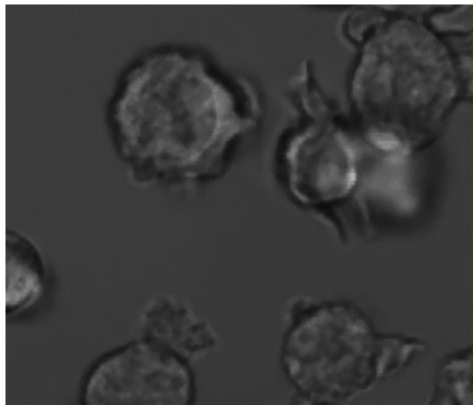


Fig. S1. SLP-76-ADAP regulates LFA-1 induced cell polarization and peptide-APC induced Ca2+ fluxes. T8.1 cells (*A*) or SLP-76 deficient Jurkat cells (J14) (*B*) were loaded to anti-CD3 alone or anti-CD3- and ICAM-1-coated plates for 60 min. (*C*) Fura-2 labeled GFP-T8.1, ADFAP-T8.1, and M12-T8.1 cells were incubated with peptide pulsed APCs and Ca^{2+} signals were recorded. (*D*) ADAP-T8.1 cells were pretreated with various inhibitors and loaded to ICAM-1-coated plates. Images were taken at 60 min and are representative of 2 independent experiments.



Movie S1. Time lapse was used to record cell morphology changes when ADAP-T8.1 or M12-T8.1 cells were seeded to anti-CD3- and ICAM-1-coated plates.

Movie S1 (AVI)



Movie S2. Time lapse was used to record cell morphology changes and cell migration when ADAP-T8.1 cells were loaded to ICAM-1 alone-coated plates.

Movie S2 (AVI)