Supporting Information

Liu et al. 10.1073/pnas.0909112107

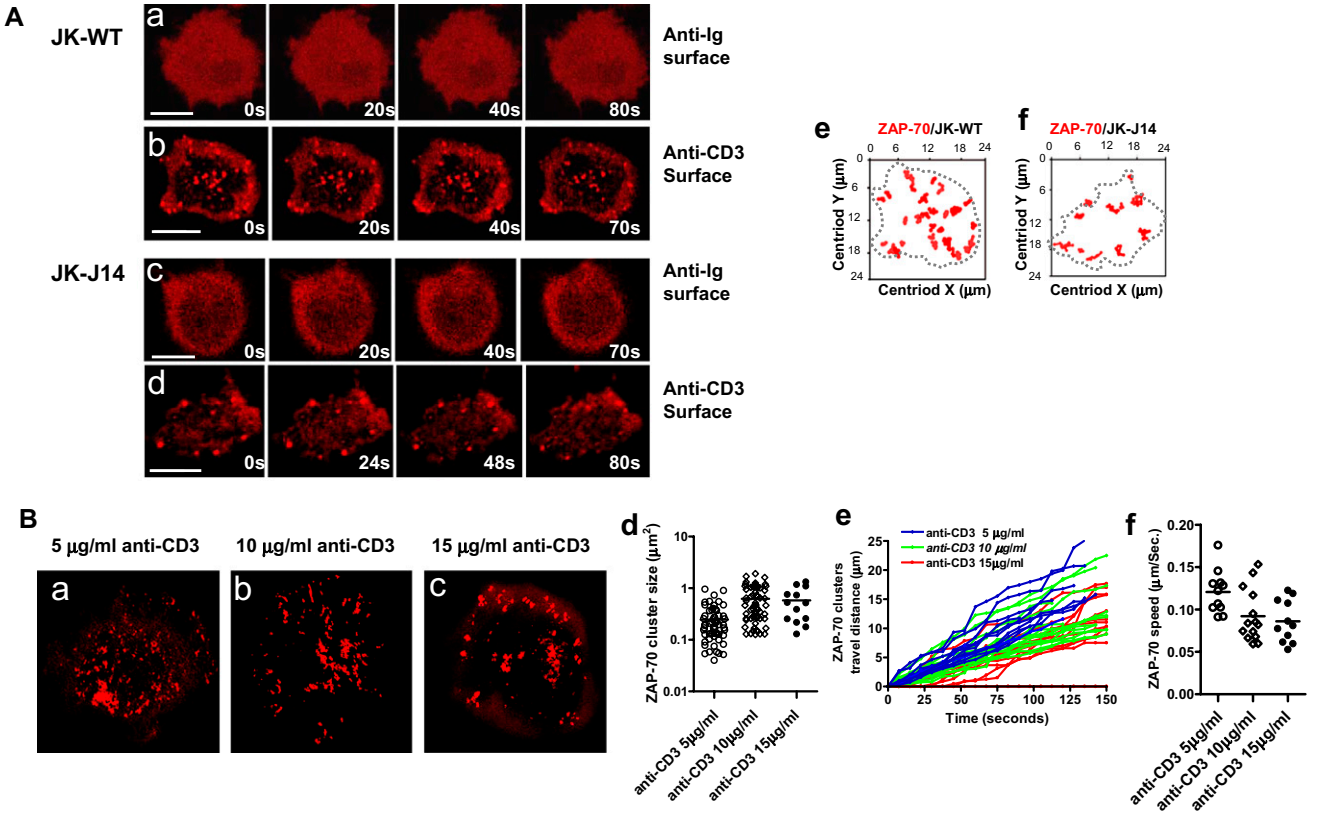


Fig. S1. (*A*) Time-lapse confocal images of ZAP-70-mRFP cluster formation and distribution at the interface of transfected Jurkat WT or J14 cells and anti-CD3–coated slides over a time course about 80 s. Jurkat WT cells expressing ZAP-70-mRFP on anti-CD3–coated slides (*b*) or on control anti-Ig–coated slides (*a*); SLP-76 deficient J14 cells expressing ZAP-70-mRFP on anti-CD3–coated slides (*d*) or on control anti-Ig–coated slides (*c*). The movement of ZAP-70 individual cluster expressed in Jurkat WT cells (*e*) or J14 cells (*f*) over the time course was tracked. The dotted line indicates boundary of T cell/coverslip interface. (Scale bars, 10 μm.) (*B*) Titration of anti-CD3 signaling on ZAP-70 microcluster distribution, size and motility. Jurkat J14 cells were cotransfected with ZAP-70-mCFP and SLP-76-EYFP whereas images of microclusters were sequentially acquired in time lapse at the interface between cells and antigenic surface with a Zeiss LSM 510 confocal microscopy at 458 nm for mCFP and 514 nm for EYFP. (*a*–*c*) examples of images of ZAP-70-mCFP microclusters at the interface between T cells on a glass surface coated with different concentrations (5, 10, and 15 μg/mL) of human anti-CD3 OKT3. (*c*) histograms showing the size (*d*); travel distance with time (*e*) and speed of movement (*f*) of individual ZAP-70-mCFP clusters at different concentration of anti-CD3 stimulation.

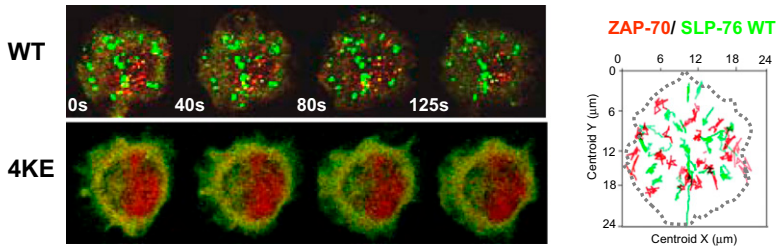


Fig. S2. Confocal microscopy of time-course of ZAP-70-mRFP clustering at the interface between transfected Jurkat T cells and antigenic coverslips. Jurkat J14 cells were coexpressed with ZAP-70-mRFP and SLP-76-EYFP and time course of ZAP-70 cluster formation and distribution was tracked over 0–125 s. ZAP-70-mRFP coexpressed with SLP-76-EYFP wt (*Upper Left*) or coexpressed with SLP-76-EYFP 4KE mutant (*Lower Left*). The movement of ZAP-70 and SLP-76 WT clusters in the cells over the time course was tracked (*Right*). ZAP-70-mRFP tracks are shown in shades of red and overlaid with tracks of SLP-76-EYFP in shades of green. The dark/black color regions indicate where the red and green was overlapped. Dotted line indicates boundary of T cell/coverslip interface.

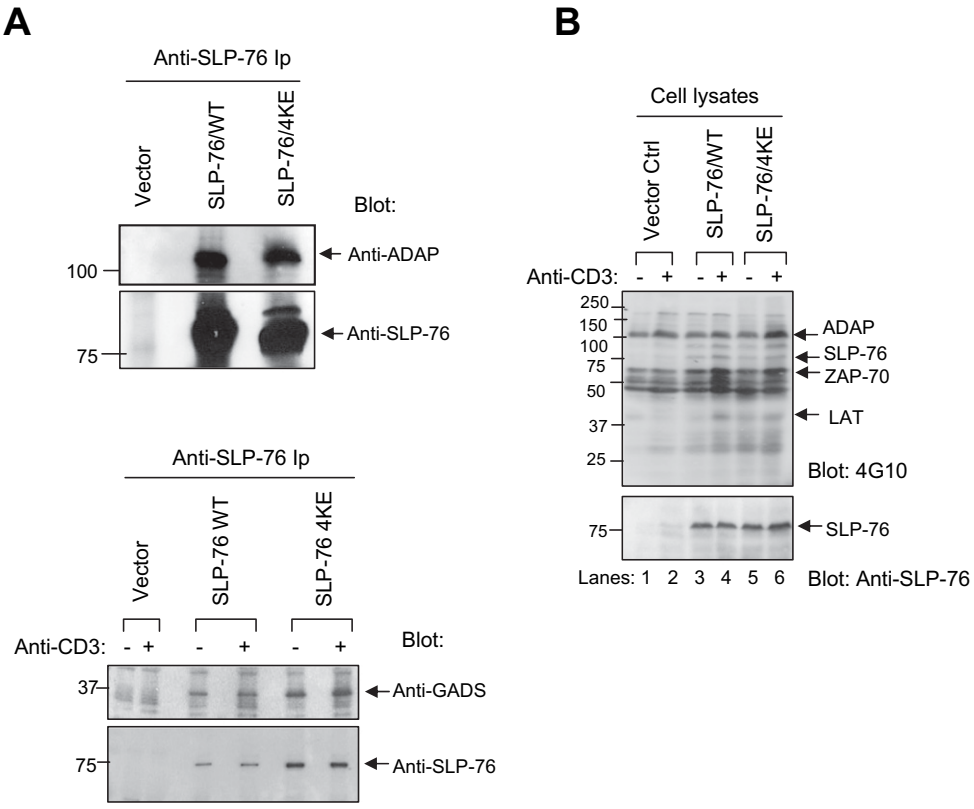


Fig. S3. (*A*) Interaction of SLP-76 WT and 4KE mutant with its binding partners ADAP and GADS. SLP-76 deficient J14 cells were transfected with either SRα empty vector or SRα HA tagged SLP-76 WT or 4KE mutant. Where indicated, the cells were left unstimulated or stimulated with 5 μg/mL human anti-CD3 OKT3 with cross-linking for 10 min before immunopreciptitation with anti-SLP-76 monoclonal antibody. The SLP-76 associated endogenous ADAP or GADS was detected with anti-ADAP (*Upper*) or anti-GADS (*Lower*). Anti-SLP-76 blot included as a positive control in both panels. (*B*) Comparison of tyrosine phosphorylation in J14 Jurkat cells transfected SLP-76 vs. SLP-76 4KE mutant. SLP-76 deficient J14 cells were transfected with the control SRα empty vector (lanes 1 and 2), SRα HA tagged SLP-76 WT (lanes 3 and 4) or SRα HA tagged SLP-76 4KE mutant (lanes 5 and 6). Anti-CD3 was used to ligated T cells for 8 min followed by blotting with 4G10 (*Upper*) and subsequently with anti-SLP-76 (*Lower*). The bands corresponding to phosphorylated ADAP, SLP-76, ZAP-70, and LAT are indicated by arrows.

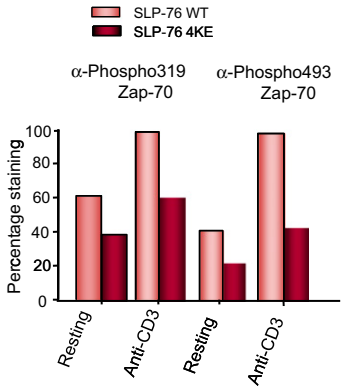


Fig. S4. FACS analysis of phosphorylation of ZAP-70 at 319 and 493 sites in Jurkat cells expressing SLP-76-EYFP WT or 4KE mutant. Jurkat J14 cells lacking SLP-76 were transfected with either WT SLP-76 or the 4KE mutant and subjected to anti-CD3 ligation for 8 min followed by intracellular staining with anti-phospho-ZAP-70 493 and 319. Histogram shows the relative percentage values of staining. The histogram showing the percentage of cells labeled with anti-pTy319 and pTy493.

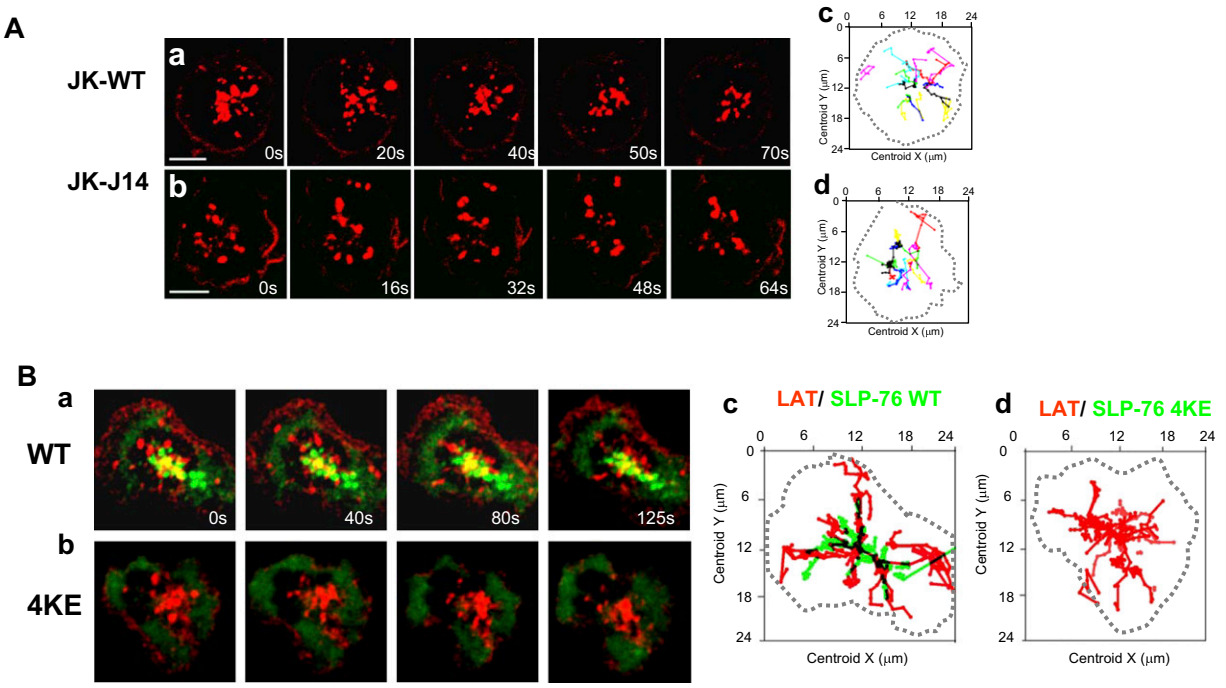
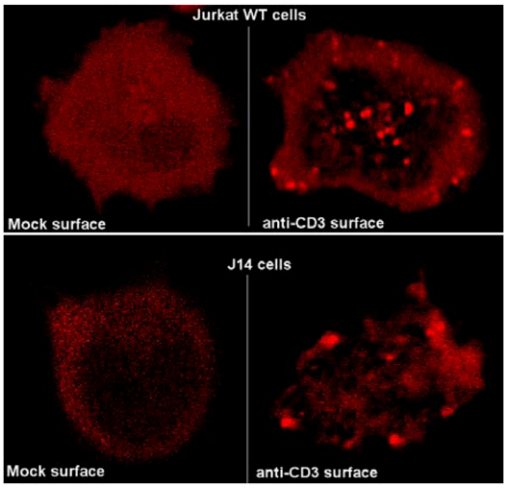
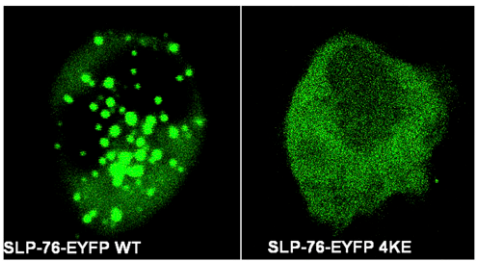


Fig. S5. (*A*) Time-lapse confocal images of LAT-mCherry cluster formation and distribution at the interface of transfected cells and anti-CD3–coated slides. LAT-mCherry microcluster formation in Jurkat WT cells over a time course of 70 s (*a*) or in SLP-76 deficient J14 cells over a time course of 64 s (*b*). The movement of LAT-mCherry individual cluster expressed in Jurkat WT cells (*c*) or J14 cells (*d*) over the time course was tracked. The dotted line indicates boundary of T cell/coverslip interface. (Scale bars, 8 μm.) (*B*) Confocal microscopy of time-course of LAT-mCherry clustering at the interface between transfected cells and antigenic coverslips. LAT-mCherry and SLP-76-EYFP were coexpressed in Jurkat J14 cells followed by a time course of LAT cluster formation over 0–125 s. LAT-mCherry coexpressed with SLP-76-EYFP WT (*a*) or coexpressed with SLP-76-EYFP 4KE mutant (*b*). The movement of LAT and SLP-76 clusters was tracked in the cells over the time course. LAT-mCherry tracks are shown in shades of red and overlaid with tracks of SLP-76 in shades of green. The dark/black color regions indicate where the red and green was overlapped. Tracks of LAT-mCherry (red) and SLP-76-EYFP WT (green) (*c*); Tracks of LAT-mCherry (red) and SLP-76-EYFP 4KE (few green tracks) (*d*). Dotted line indicates boundary of T cell/cover-slip interface.



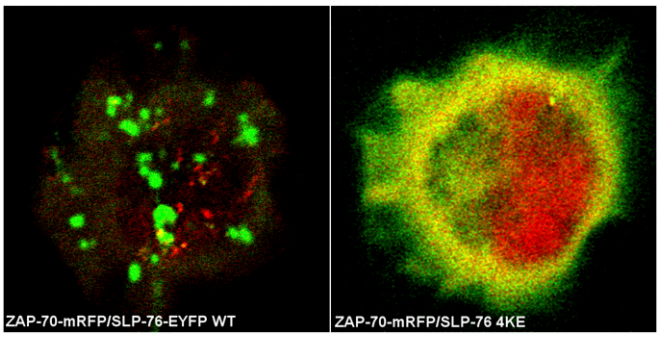
Movie S1. Time-lapse images of ZAP-70 clusters formation at the interface between ZAP-70-mRFP transfected Jurkat T cells and OKT3-coated coverslips or mock anti-Ig–treated coverslips (*Upper*), or between ZAP-70-mRFP–transfected SLP-76–deficient J14 cells and OKT3-coated coverslips or anti-Ig mock treated coverslips (*Lower*).

Movie S1



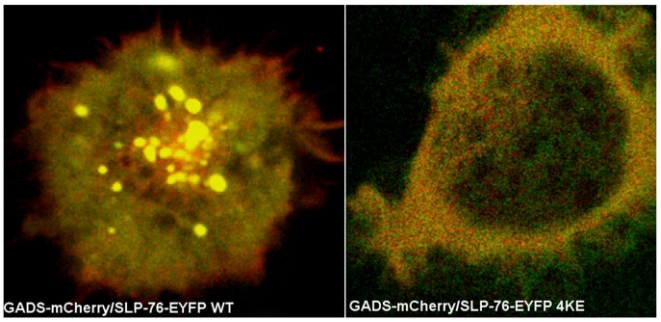
Movie S2. Time-lapse images of SLP-76 WT or SLP-76 4KE mutant cluster formation at the interface between SLP-76 WT-EYFP–transfected Jurkat T cells (*Left*) or SLP-76/4KE-EYFP–transfected Jurkat cells (*Right*) and OKT3-coated coverslips.

Movie S2



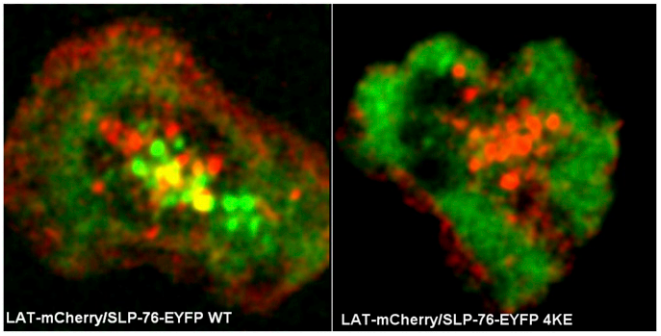
Movie S3. Time-lapse images of ZAP-70 cluster formation at the interface between transfected Jurkat J14 T cells and OKT3-coated coverslips. Jurkat J14 T cells were cotransfected with ZAP-70-mRFP (red) and SLP-76/WT-EYFP (green) (*Left*) or with ZAP-70-mRFP (red) and SLP-76/4KE-EYFP (green) (*Right*).

Movie S3



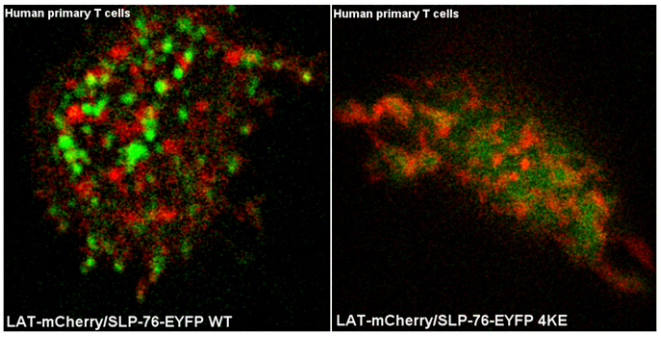
Movie S4. Time-lapse images of GADS cluster formation at the interface between transfected Jurkat T cells and OKT3-coated coverslips. Jurkat T cells were cotransfected with GADS-mCherry (red) and SLP-76/WT-EYFP (green) (*Left*) or with GADS-mCherry (red) and SLP-76/4KE-EYFP (green) (*Right*).

Movie S4



Movie S5. Time-lapse images of LAT clusters formation at the interface between transfected Jurkat J14 T cells and OKT3-coated coverslips. J14 T cells were cotransfected with LAT-mCherry (red) and SLP-76/WT-EYFP (green) (*Left*) or with LAT-mCherry (red) and SLP-76/4KE-EYFP (green) (*Right*).

Movie S5



Movie S6. Time-lapse images of LAT clusters formation in at the interface between transfected human primary T cells and OKT3-coated coverslips. Human primary T cells were cotransfected with LAT-mCherry (red) and SLP-76/WT-EYFP (green) (*Left*) or with LAT-mCherry (red) and SLP-76/4KE-EYFP (green) (*Right*).

Movie S6