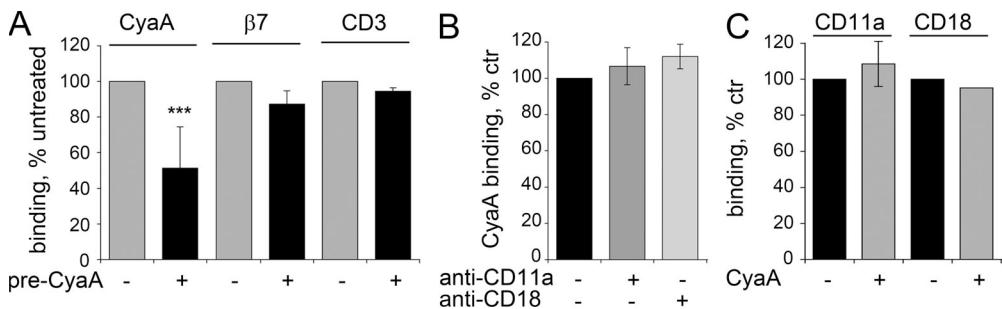
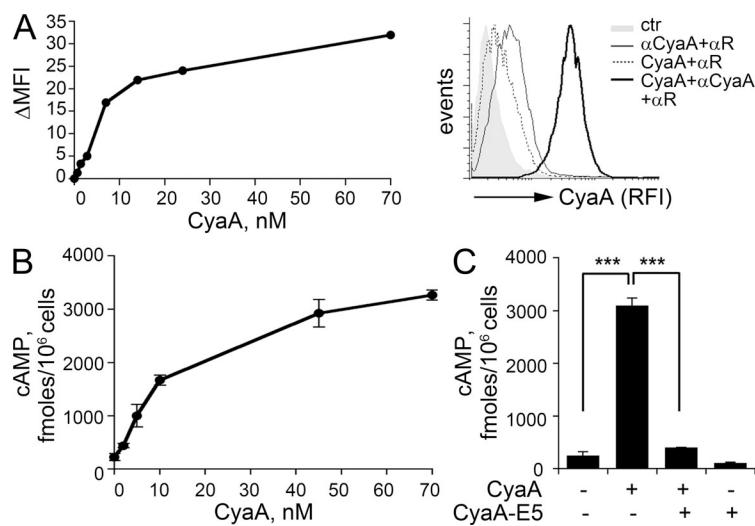


SUPPLEMENTAL MATERIAL

Rossi Paccani et al., <http://www.jem.org/cgi/content/full/jem.20101558/DC1>**Figure S1.** CyaA does not compete with either the anti-CD11a mAb HI111 or the anti-CD18 mAb CLB-LFA-1/1 for binding to LFA-1.

(A) Flow cytometric analysis of binding of CyaA or mAbs directed against $\beta 7$ integrin or CD3 to peripheral T cells previously exposed to 45 nM CyaA at 37°C for 30 min to induce internalization of the CyaA receptor ($n > 3$ for CyaA; $n = 2$ for $\beta 7$ and CD3). The results are expressed as percentage binding to CyaA-treated cells as compared with untreated cells (taken as 100%). (B) Flow cytometric analysis of CyaA (45 nM) binding to peripheral blood T cells, either untreated or preincubated for 30 min on ice with anti-CD11a (HI111) or anti-CD18 mAb (CLB-LFA-1/1) before addition of CyaA ($n > 3$). (C) Flow cytometric analysis of anti-CD11a (HI111) or anti-CD18 mAb (CLB-LFA-1/1) binding to peripheral blood T cells, either untreated or preincubated for 30 min on ice with 45 nM CyaA. The data are expressed as the percentage of binding to untreated cells (taken as 100%). Error bars represent the SD ($n > 3$). n indicates the number of independent experiments.

**Figure S2.** CyaA binds to and intoxicates Jurkat cells. (A) Flow cytometric analysis of CyaA binding to Jurkat cells. The data are expressed as the difference between the MFI of cells incubated with CyaA, anti-CyaA antibodies (α -CyaA), and anti-rabbit Ig FITC-labeled secondary antibodies (α -R), and the MFI of cells incubated with primary and secondary antibodies in the absence of CyaA (ΔMFI). A representative concentration-response curve ($n > 3$) and FACS profile (45 nM CyaA) are shown. RFI, relative fluorescence intensity. (B) Quantification of cAMP production in lysates of Jurkat cells treated with different concentrations of CyaA for 30 min at 37°C. The results are expressed as fmol/ 10^6 cells (corresponding to 100 μ g total protein). A representative concentration-response curve (duplicate samples) is shown ($n \geq 2$). (C) Quantification of cAMP production in lysates of Jurkat cells treated with 45 nM CyaA or CyaA-E5. Where indicated, cells were preincubated for 5 min at 37°C with CyaA-E5 before addition of CyaA ($n = 2$). Error bars represent the SD. ***, $P \leq 0.001$. n indicates the number of independent experiments.

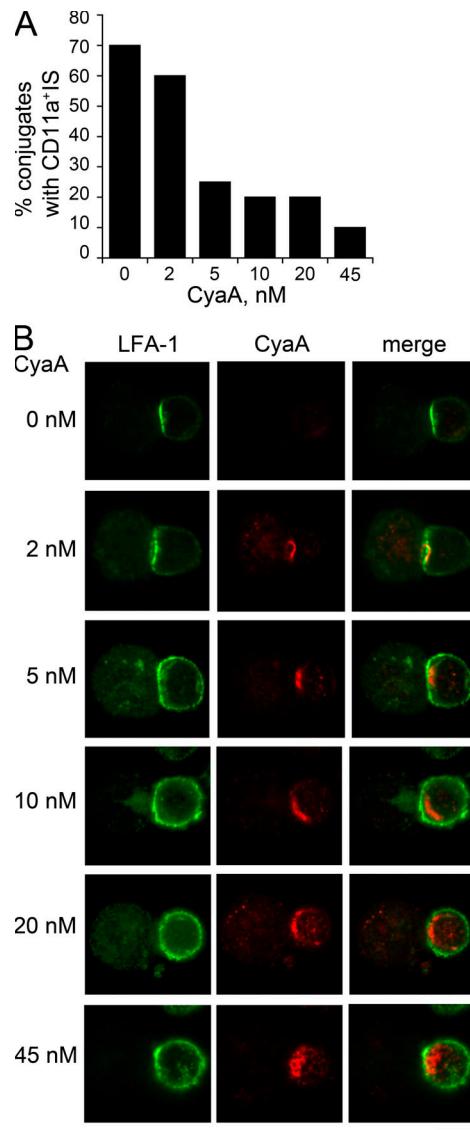


Figure S3. Dose-dependent disengagement of LFA-1 from the IS by CyaA. (A) Results of an immunofluorescence analysis of CD11a in conjugates of peripheral T cells and antigen-pulsed APC (SAg), incubated at 37°C for 15 min. Where indicated, T cells were incubated for 15 min on ice with different concentrations of CyaA before washing and mixing with the APC. The data are expressed as percentage of antigen-specific T cell–APC conjugates harboring CD11a clustering at the IS (n conjugates ≥ 20). Representative images (medial optical sections) for each CyaA concentration are shown (B). Bar, 5 μ m.

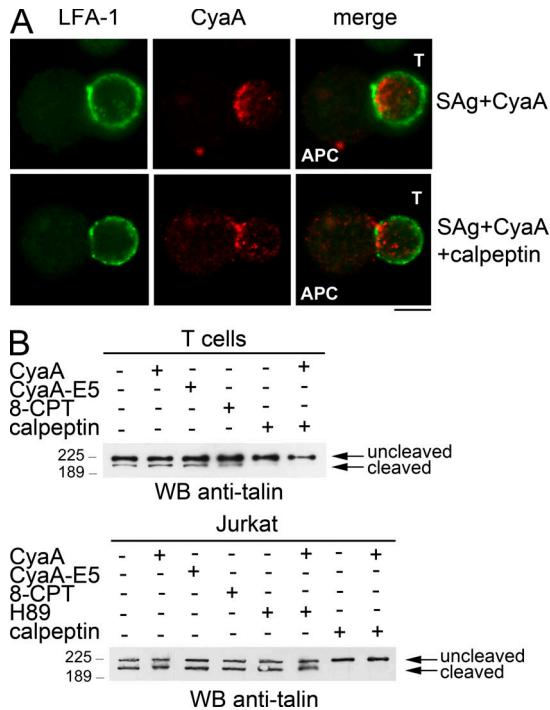


Figure S4. The calpain inhibitor calpeptin does not affect the capacity of CyaA to induce the premature release of LFA-1 from the IS.

(A) Immunofluorescence analysis of CD11a and CyaA localization in conjugates of peripheral T cells and antigen-pulsed APC (SAg). Where indicated, T cells were preincubated at 37°C for 1 h in the presence of 280 μM of the calpain inhibitor, calpeptin, and then washed, transferred to ice, added with CyaA and further incubated on ice for 15 min before washing and mixing with the APC. Median optical sections are shown. Bar, 5 μm. (B) Immunoblot analysis of Jurkat and peripheral T cell lysates either untreated or treated with CyaA/CyaA-E5 (45 nM), or 8-CPT (100 μM), or H89 (20 μM) in the presence or absence of calpeptin (280 μM). Representative blots out of two independent experiments are shown.

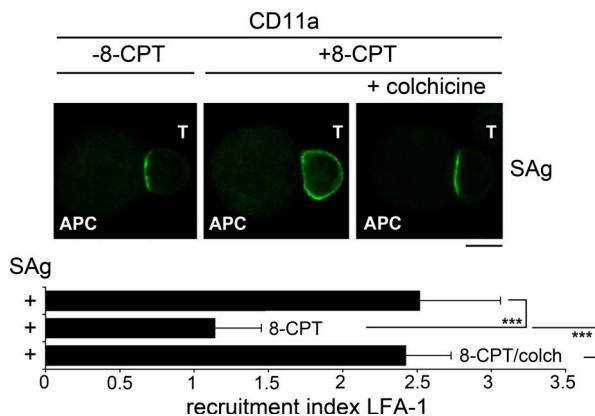


Figure S5. The cAMP-dependent redistribution of LFA-1 from the IS requires an intact microtubule cytoskeleton. Immunofluorescence analysis of LFA-1 in conjugates of peripheral T cells and antigen-pulsed APC (SAg). Where indicated, 100 μM 8-CPT, either alone or in combination with 20 μM colchicine, was added 5 min after mixing T cells and APC to allow formation of conjugates and LFA-1 clustering at the IS. Conjugates were incubated at 37°C for further 10 min. Median optical sections are shown. Bar, 5 μm. The histogram shows the relative LFA-1 fluorescence at the T cell-APC contact site compared with the remaining T cell area (relative recruitment index; n conjugates \geq 20) are shown. Error bars are represented by SD. ***, P < 0.001.

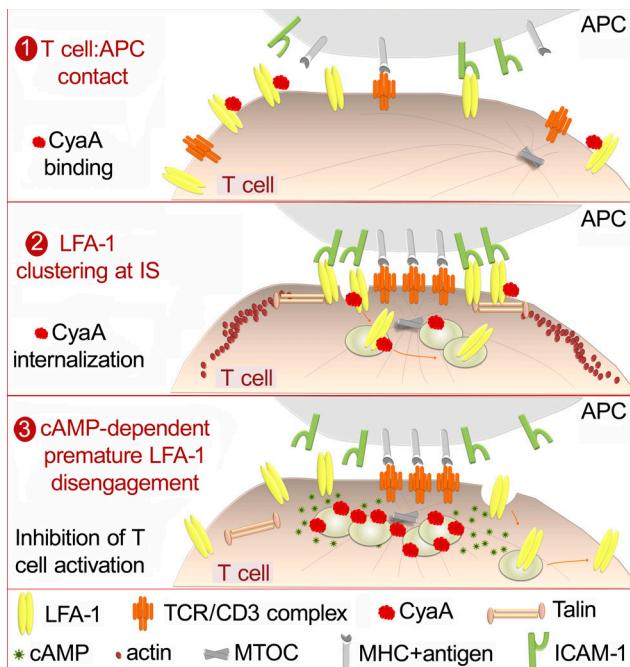


Figure S6. Model of LFA-1-mediated targeting of the IS by CyaA. (1) CyaA binds to LFA-1 and clusters at the forming IS. (2) CyaA is internalized with LFA-1, which then dissociates and recycles back to the plasma membrane, while CyaA remains in the vicinity of the IS associated to a vesicular compartment. (3) cAMP production by CyaA triggers LFA-1 disengagement from the IS.