Hu and Butte, http://www.jcb.org/cgi/content/full/jcb.201511053/DC1

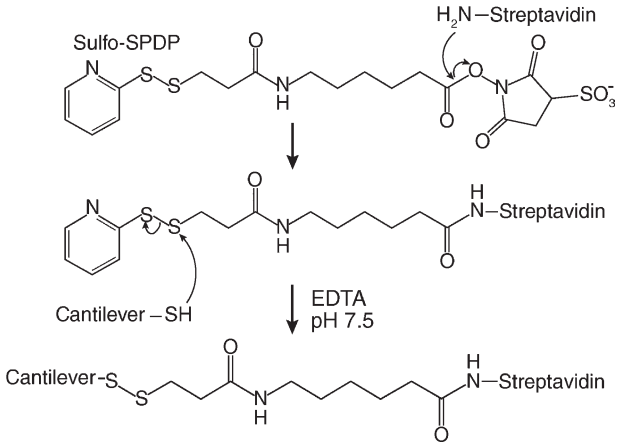

Figure S1. **Cross-linking strategy.** Chemical strategy of covalently attaching streptavidin to the cantilever using a sulfo-LC-SPDP cross-linker.

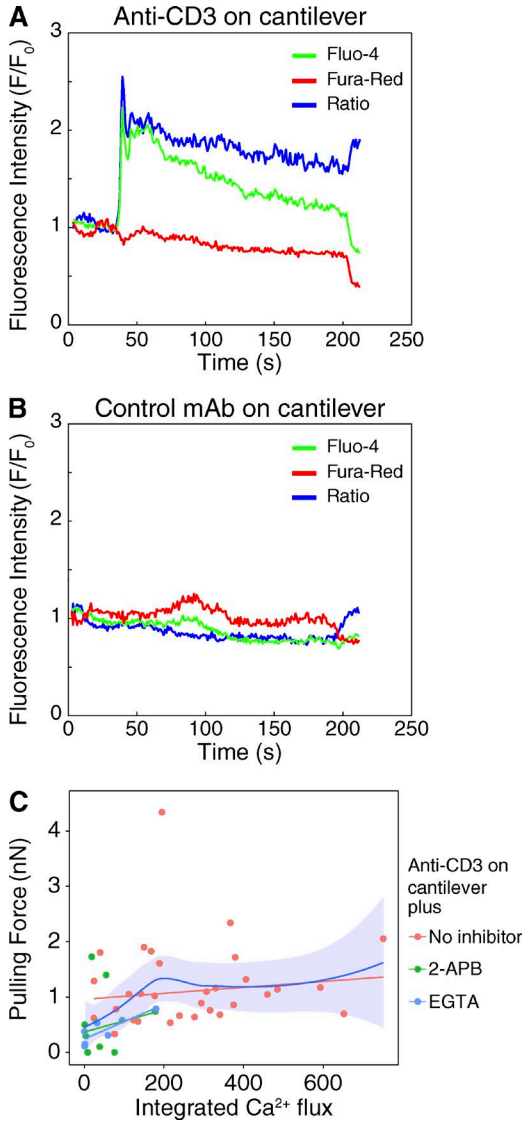

Figure S2. **Pseudoratiometric imaging of calcium influx shows that Fluo-4 intensity rise is caused by Ca^{2+} concentration increase, not shape change of the cell.** Time course of Fluo-4 intensity (green) and Fura-Red intensity (red) and ratio of Fluo-4 to Fura-Red intensity (blue) for T cells continuously contacted with anti-CD3–coated tip (A) and control mAb–coated tip (hCD25; B). (C) Pulling forces become maximal at a low integrated Ca^{2+} flux. All touches shown use an anti-CD3–coated cantilever and also appear elsewhere in this article. A linear fit is given for each condition: T cells treated with the CRAC inhibitor 2-APB (green), the calcium chelator EGTA (blue), or control treatment (red). A local regression scatterplot smoothing (LOESS) curve is shown (blue) spanning all the data points with CI of fit (light blue). At an integrated Ca^{2+} higher than ~200, the pulling force becomes near-maximal.

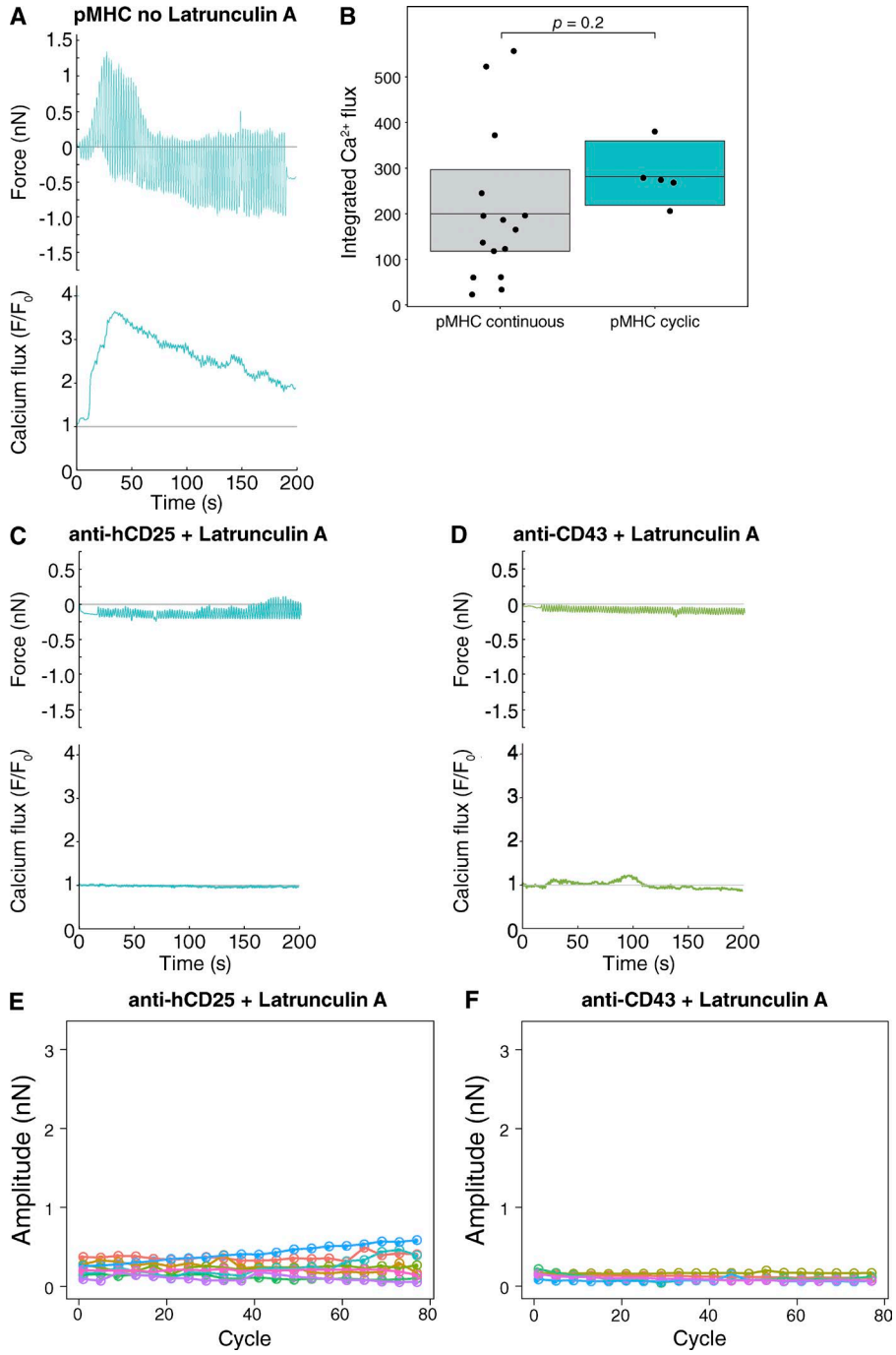

Figure S3. **Application of cyclic force in the absence of LatA does not significantly increase signaling strength compared with continuous contact.** (A) Time courses of both force on the cantilever and normalized Fluo-4 intensity during application of cyclical forces using pMHC-coated cantilevers in the absence of LatA. (B) Time-integrated calcium flux for T cells stimulated using pMHC-coated tips, using either continuous contact or cyclic force. Each dot is one T cell contacted. Data for the continuous contacts were the same LatA-treated cells in Fig. 4. Application of cyclic force with control-coated cantilevers does not rescue force or calcium flux in LatA-treated T cells. (C–F) Time courses of both force on the cantilever and normalized Fluo-4 intensity during application of cyclical forces for LatA-treated cells and control-coated cantilevers, either anti-hCD25 coated (C) or anti-CD43 coated (D). Amplitude of force oscillations over time during application of cyclical forces for LatA-treated cells and control-coated cantilevers, either anti-hCD25 coated (E) or anti-CD43 coated (F).