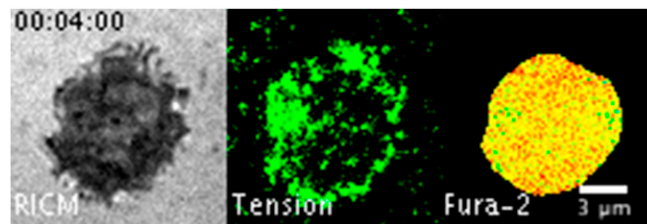


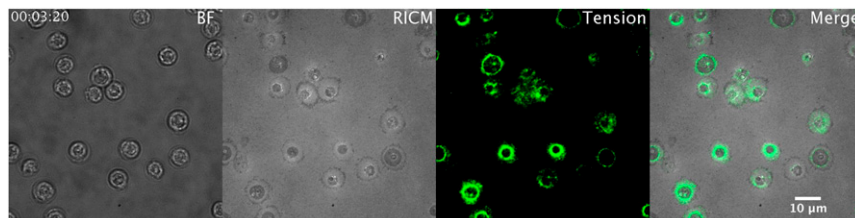
Supporting Information

Liu et al. 10.1073/pnas.1600163113



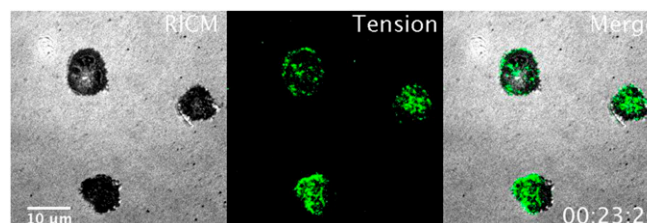
Movie S1. Simultaneous imaging of TCR tension generation and T-cell activation. Time-lapse video showing a naïve OT1 cell pretreated with fura-2 ratio-metric dye plated on a 19-pN tension probe substrate presenting α -CD3. The video is 10 min long. The RISM channel (*Left*), TCR tension channel (*Middle*), and fura-2 ratio channel (*Right*) are shown side by side.

[Movie S1](#)



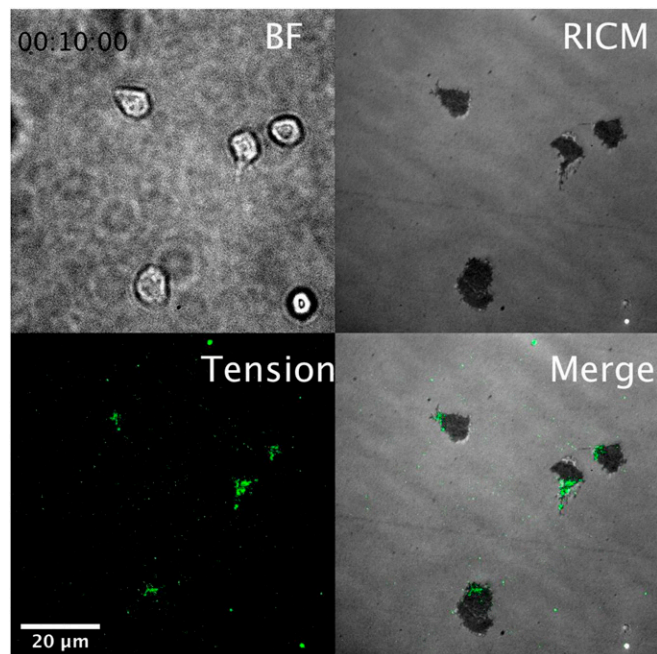
Movie S2. ML-7 leads to the shrinkage of cell-surface contact and condensation of tension. Time-lapse video showing naïve OT1 cells plated on a 12-pN tension probe substrate presenting N4 pMHC on treatment with 40 μ M of ML7. $t = 0$ min represents the time at which ML7 was added. The bright-field channel, RISM channel, TCR tension channel, and merged channel from RISM and tension are shown from left to right.

[Movie S2](#)



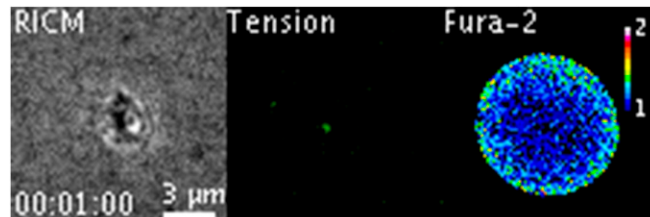
Movie S3. TCR tension dynamics in response to pMHC stimulation. Time-lapse video of naïve OT1 cells plated on a 12-pN tension probe substrate presenting N4 pMHC. The time-lapse movie was captured from $t = 0$ min to $t = 34$ min to show the spatiotemporal dynamics of TCR tension on cell-substrate contact. The RISM channel, TCR tension channel, and merged channel from RISM and tension are shown from left to right.

[Movie S3](#)



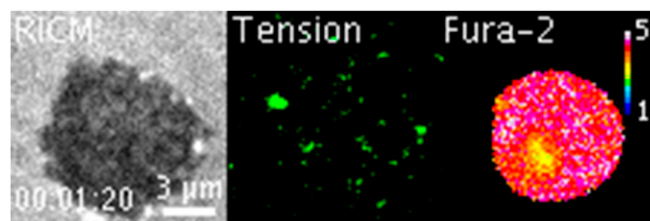
Movie S4. Cell migration and tension dynamics in response to the copresentation of pMHC/ICAM-1. Time-lapse video of naïve OT1 cells plated on a 19-pN tension probe substrate copresenting N4 pMHC and ICAM-1 ligands at 15 min after plating. The time-lapse movie was captured for 10 min to show the migratory behavior of T cells and characteristic TCR tension at the trailing edge. The interval between each frame is 30 s. The bright-field channel (*Top Left*), RICM channel (*Top Right*), TCR tension channel (*Bottom Left*), and merged channel from RICM and tension (*Bottom Right*) are shown in a quadrant.

[Movie S4](#)



Movie S5. OT-1 cell tension dynamics and $[Ca^{2+}]$ flux in response to the V4 ligand. Time-lapse video of naïve OT1 cells pretreated with fura-2 ratiometric dye plated on a 12-pN tension probe substrate presenting V4 pMHC. The time-lapse movie is ~10 min long. The RICM channel (*Left*), TCR tension channel (*Middle*), and fura-2 ratio channel (*Right*) are shown side by side. The normalized fura-2 ratio is color-coded to represent values from 1.0 to 2.0.

[Movie S5](#)



Movie S6. OT-1 cell tension dynamics and $[Ca^{2+}]$ flux in response to the N4 ligand. Time-lapse video of naïve OT1 cells pretreated with fura-2 ratiometric dye plated on a 12-pN tension probe substrate presenting N4 pMHC. The time lapse movie is ~6 min long. The RICM channel (*Left*), TCR tension channel (*Middle*), and fura-2 ratio channel (*Right*) are shown side by side. The normalized fura-2 ratio is color-coded to represent values from 1.0 to 5.0.

[Movie S6](#)

Other Supporting Information Files

[SI Appendix \(PDF\)](#)