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Supplemental Information

Soft Polydimethylsiloxane-Supported Lipid Bilayers for Studying T Cell Interactions

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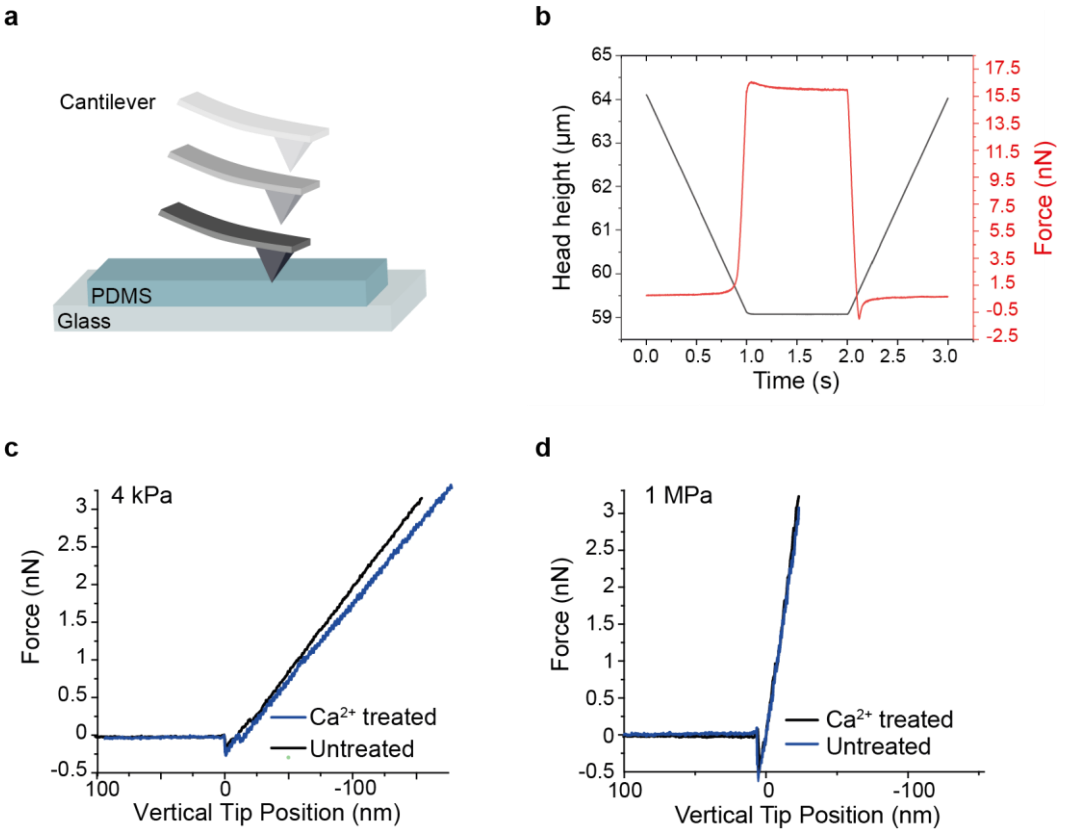
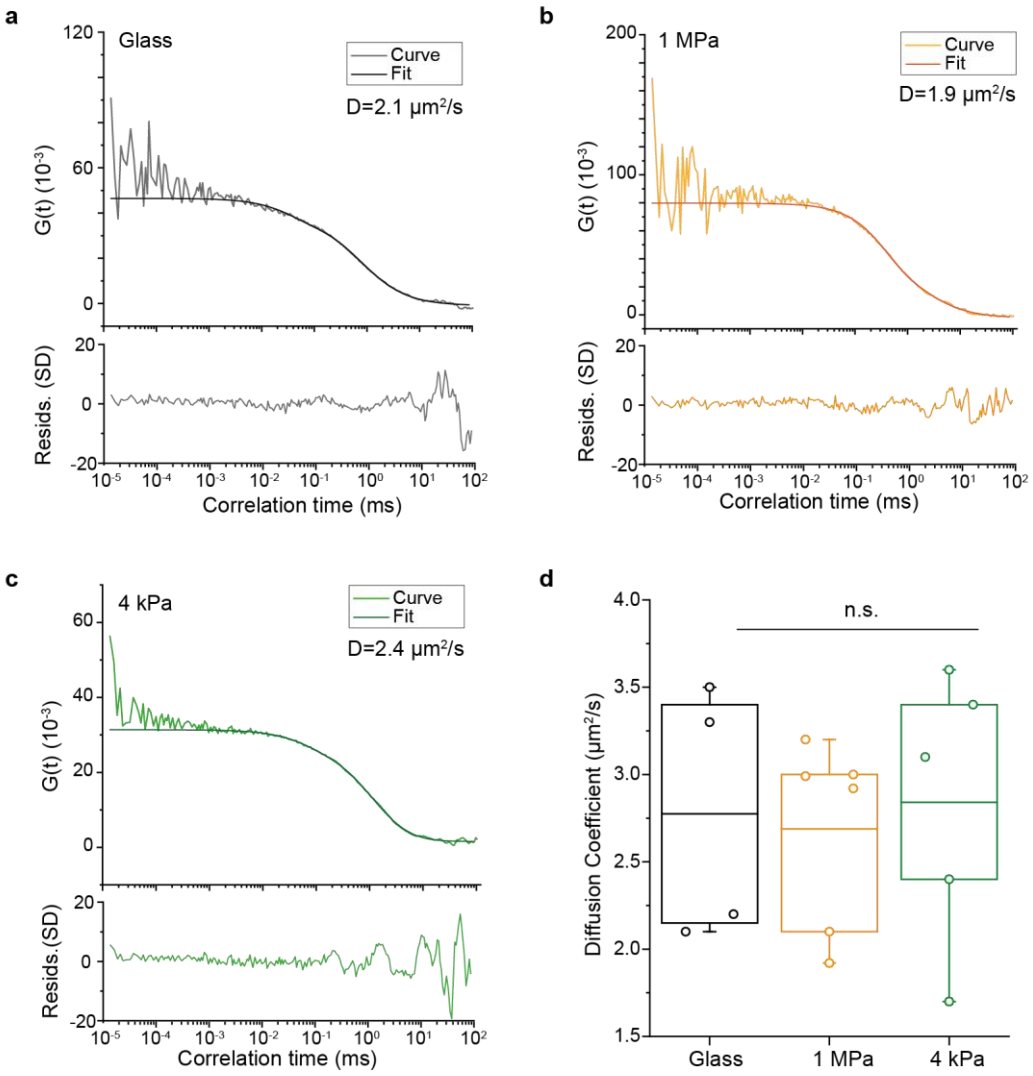
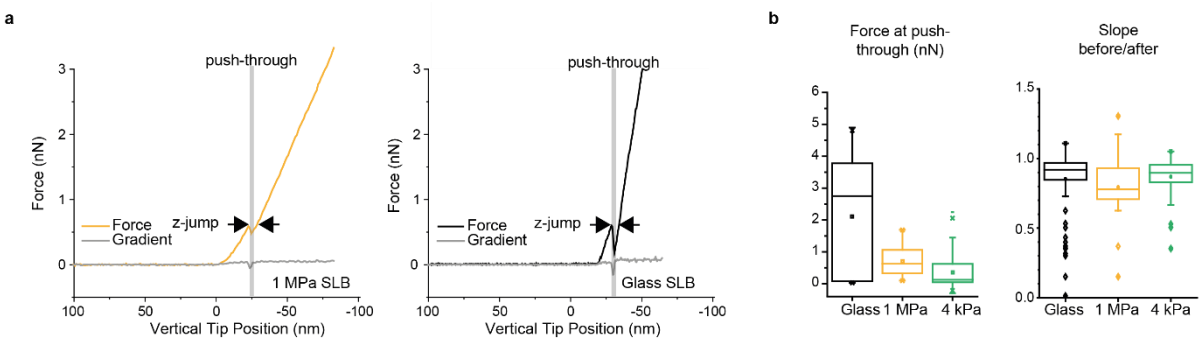


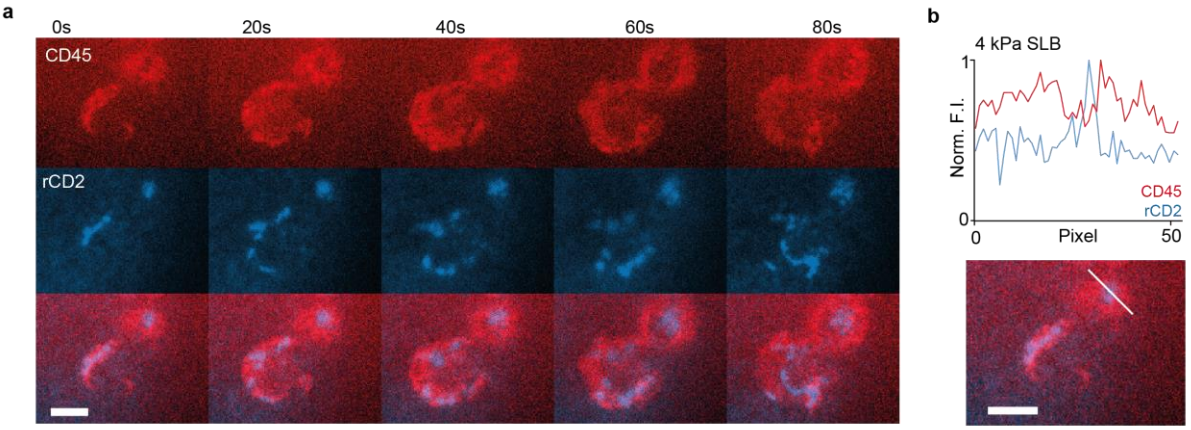
Suppl. Fig. 1: PDMS characterisation using AFM. (**a**) Schematic of the experiment. (**b**) Creep response curve of 4 kPa gel, recording the force on the cantilever (red) at constant cantilever head height (black). (**c, d**) Force response of indentation experiments plotting force over vertical tip positions comparing Ca^{2+} treated (blue) and untreated (black) 4 kPa (**c**) and 1 MPa (**d**) PDMS gels.



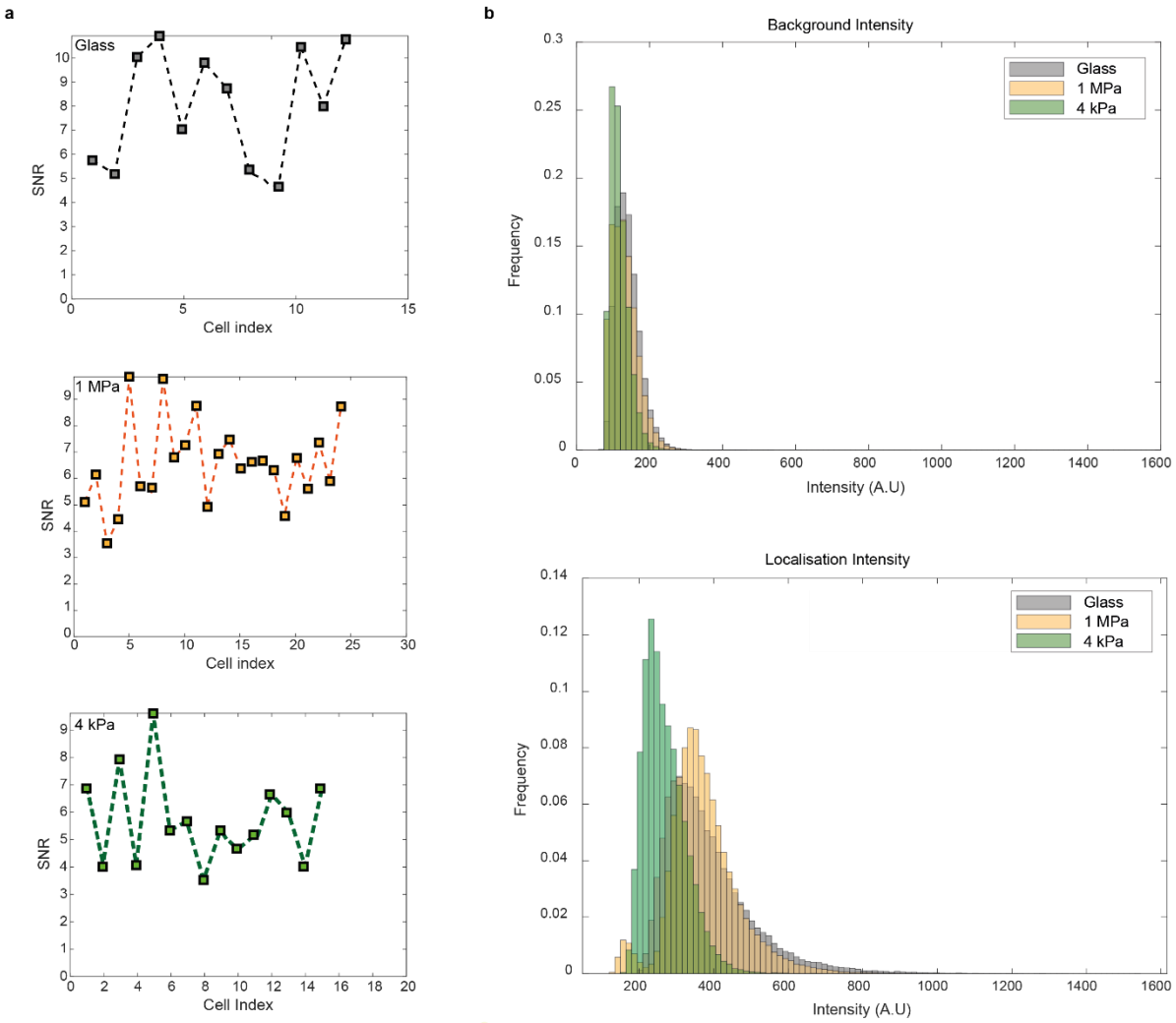
Suppl. Fig. 2: FCS measurements of glass- (**a**), 1MPa (**b**) and 4 kPa (**c**) PDMS-supported bilayers. Shown are representative autocorrelation curves G(t) and their respective fits as well as a boxplot comparing diffusion coefficients (**d**). Each data point represents the diffusion coefficient obtained from one bilayer at RT. Boxes indicate the 25% and 75% quartile, the horizontal line the mean, and whiskers the 1.5 IQR. A Mann-Whitney-U test was performed to test for significant differences.



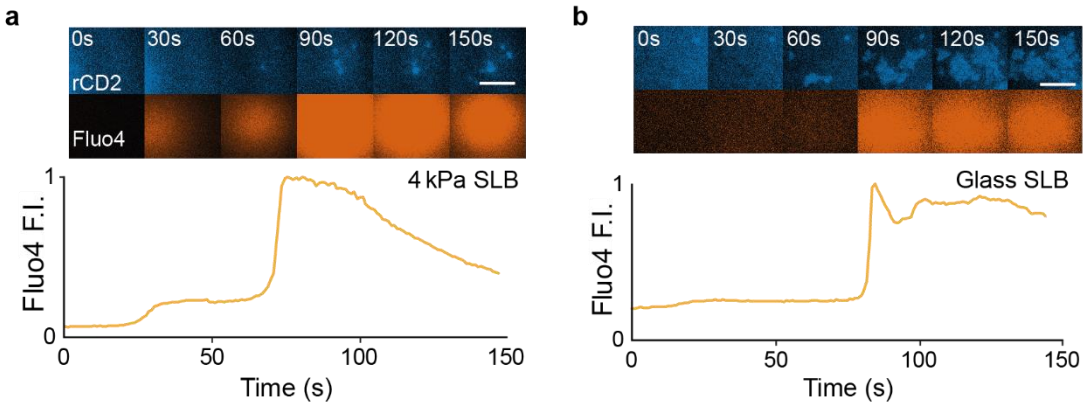
Suppl. Fig. 3: AFM-based characterisation of glass-supported and 1 MPa and 4 kPa PDMS-supported lipid bilayers. (**a**) Representative force trace of a cantilever pushing through a 1 MPa (force: orange) and glass supported bilayer (force: black) and gradient of the force (grey). The push-through event is indicated as a "z-jump" (grey area with indicating arrows). (**b**) Boxplots comparing push-through force (left) and slope before/after the push-through (right) (number of force curves analysed: 52 (4 kPa PDMS), 30 (1 MPa PDMS), 31 (glass) from 3 independent experiments). Boxes indicate the 25% and 75% quartile, the square is the mean, the horizontal line the median, the diamonds the outliers and whiskers the 1.5 IQR.



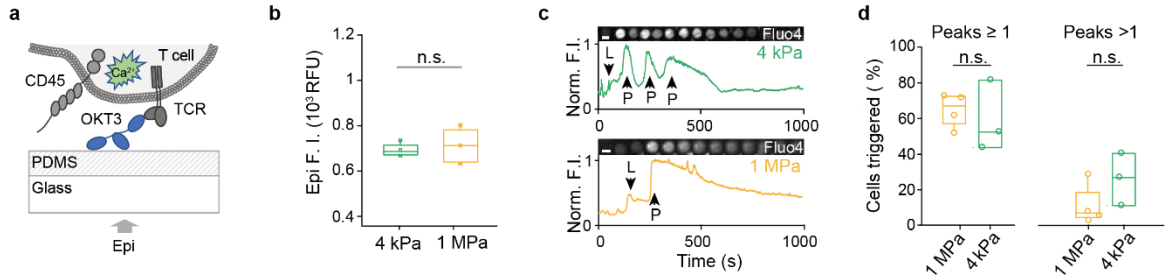
Suppl. Fig. 4: Small contacts exclude CD45. (**a**) Representative false-colour two-colour time-lapse TIRF imaging of a T cell recruiting rCD2-Alexa647 (blue) into contacts, alongside exclusion of CD45 labelled with Gap8.3 anti-CD45 antibody Fab tagged with Alexa 488 (red). (**b**) Line profile of CD45 and rCD2 fluorescence at the 0s timepoint. Scale bars 1µm.



Suppl. Fig. 5: Signal-to-noise (SNR) ratios of UCHT-1 Alexa488 diffusion data shown as SNR cell by cell (**a**) and as histograms of overall background (**b**, top) and localisation intensities (**b**, bottom).



Suppl. Fig. 6: Small rCD2 contacts exclude CD45 and produce calcium responses on 4 kPa PDMS-supported bilayers. Jurkat cells expressing rCD48 were loaded with Fluo-4 and allowed to settle onto Alexa647-tagged rCD2-presenting 4 kPa PDMS (**a**) and glass (**b**) supported bilayers. Two colour illumination with a 488 laser set to epi illumination and a 647 laser set to TIRF illumination allowed simultaneous detection of rCD2 accumulation and calcium signaling. The Fluo4 signal, as raw images and traces (orange) are shown under the time-lapse TIRF images. Scale bars 5 µm.



Suppl. Fig. 7: Calcium responses of T cells on PDMS coated with activating OKT3 anti-CD3ε antibody. (**a**) Schematic of the experiment. T cells were preloaded with Fluo-4 prior to imaging their calcium responses on OKT3-coated PDMS. (**b**) PDMS gel stiffness did not influence antibody loading at the concentration used (10 µg/ml). Gels were incubated with fluorescently labelled antibody overnight, washed and the fluorescence intensity measured using epi-illumination. A two-sided t-test was used for statistical analysis. (**c**) Example calcium signaling traces for cells contacting OKT3 antibody-coated 4 kPa and 1 MPa PDMS gels; L, cell landing, P, calcium peaks. Fluo4 fluorescence is shown above the trace. Scale bar 10 µm. (**d**) Fraction of cells exhibiting a calcium response (peak ≥ 1) and fraction of responding cells that displayed multiple peaks (peaks > 1). Each data point in the boxplot represents a single experiment with n_{cells}>50. All experiments were performed at 37°C. The Mann-Whitney U test was used for statistical analysis.