SUPPLEMENTAL MATERIAL

Chen et al., https://doi.org/10.1084/jem.20160620

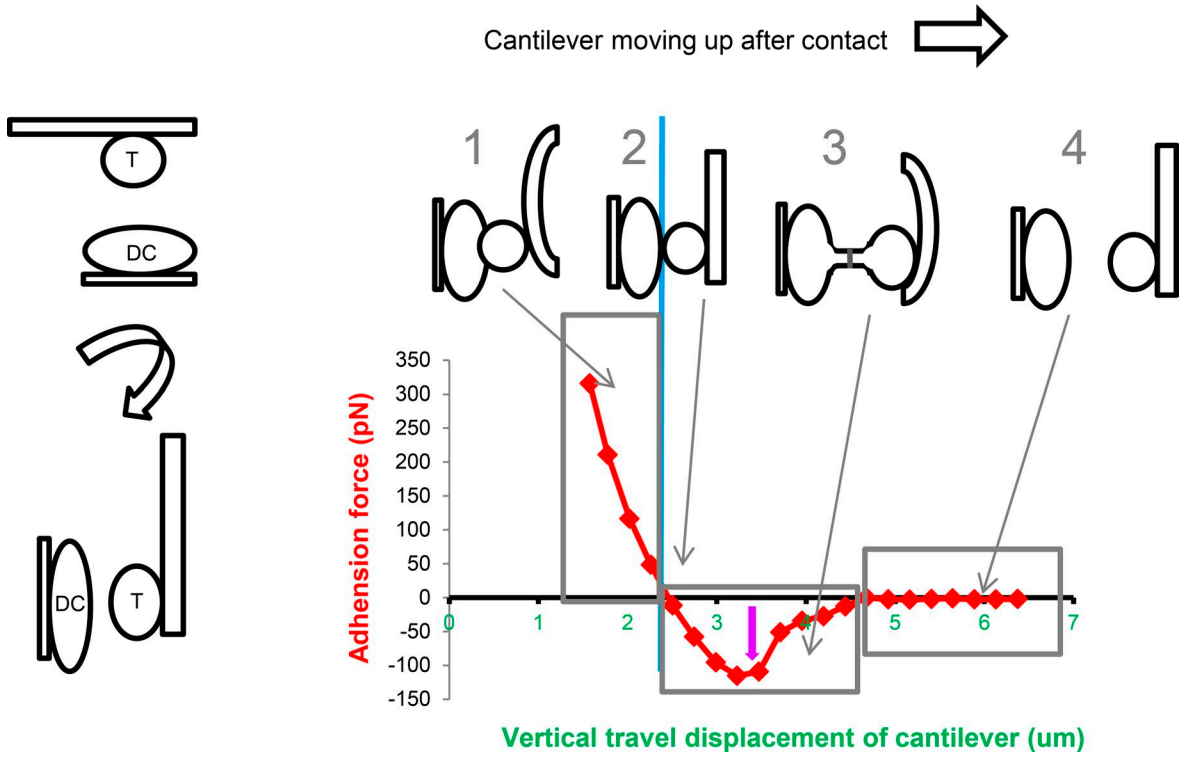


Figure S1. **For SCFS analysis of adhesion forces between T cells and DCs, a viable T cell is glued to the cantilever tip and is brought into contact with a DC grown on the glass disk.** At position 1, two cells are pressed against each other, causing inward bending of the cantilever as monitored by a laser beam focused onto its back. This inward bending is registered as a positive force reading. The cantilever is then lifted upward to separate the two cells: at the position 2, the cantilever reaches an unbent state, giving zero force reading; further upward motion of the cantilever is counteracted by the adhesion between the T cell and the DC, causing the cantilever to be bent outward (position 3). This outward bending is registered as a negative force reading that is proportional to the strength of adhesion between the two cells. The maximal reading (purple arrow) is reached right before relaxation of the cantilever due to forced relinquishment of adhesion between the two cells (position 4). Each red dot represents a point at which laser deflection from the back of the cantilever is read during the controlled contact–adhesion–separation measuring process. This process is repeated 14–30 times for each cell couple, and the maximum binding force of each measurement cycle (purple arrow) is determined by JPK software analysis. Data on each T–DC couple are presented as maximum force plotted against time or as the mean of maximum forces measured over all time points. To conduct these force measurements, each T cell must be freshly glued to a new cantilever tip and therefore requires new SCFS calibration, giving rise to an analysis throughput of approximately one T–DC couple per hour, on average. Depending on the calibration status, the absolute adhesion force reading in *Newton* for the same type of T–DC couples may vary from day to day. Care has been taken to analyze experimental and control groups on the same day.

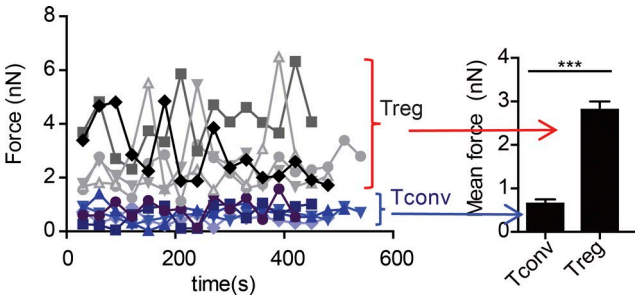
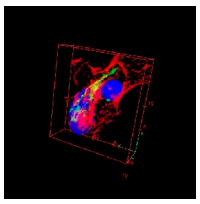
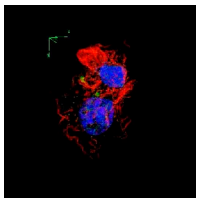


Figure S2. **To be added.** An illustration of how data points generated in force readings were used to produce the bar graphs for data analysis and presentation.



Video 1. **Heightened Fascin-1 accumulation at the site of T reg cell–BMDC contacts.** 3D rending of SIM stacks of a T reg cell–DC couple shown in Fig. 4 C. Notice the clustering of Fascin-1 at the deeply intermingled interface between the two cells. This experiment was independently performed three times.



Video 2. **Fascin-1 accumulation at the site of OT-II–BMDC contacts.** 3D rending of SIM stacks of an OT-II-DC couple shown in Fig. 4 C. This experiment was independently performed three times.