

Figure S1 Plasma membrane sheet (PMS) preparation and rupture force

**measurements.** (**A**) A schematic illustration of the preparation of the plasma membrane sheet. (**B**) Typical force-extension traces when the tip pressed on the PMS at a set-point force of 100 pN. Red line represented the fitting of the contact region in the "extend" trace using the Hertz model. (**C**) Histogram of Young's modulus from

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individual single molecule events. The Gaussian fitting showed an average ~ 0.7 MPa. n = 929. (**D**) The representative force-extension curves with specific rupture force events and only thermal noise in the force spectroscopy with hCD3 $\epsilon_{CD}$  WT-Cys peptide. Approaching and retraction force curves are labeled in gray and blue colors, respectively. Black lines are worm-like chain (WLC) fitting to the curves. (**E**) Histogram of force values of specific rupture force events (red open bar, n = 707) and thermal noise (black open bar, n = 2270). Blue solid bar represents the force detection limit of AFM in the measurements.