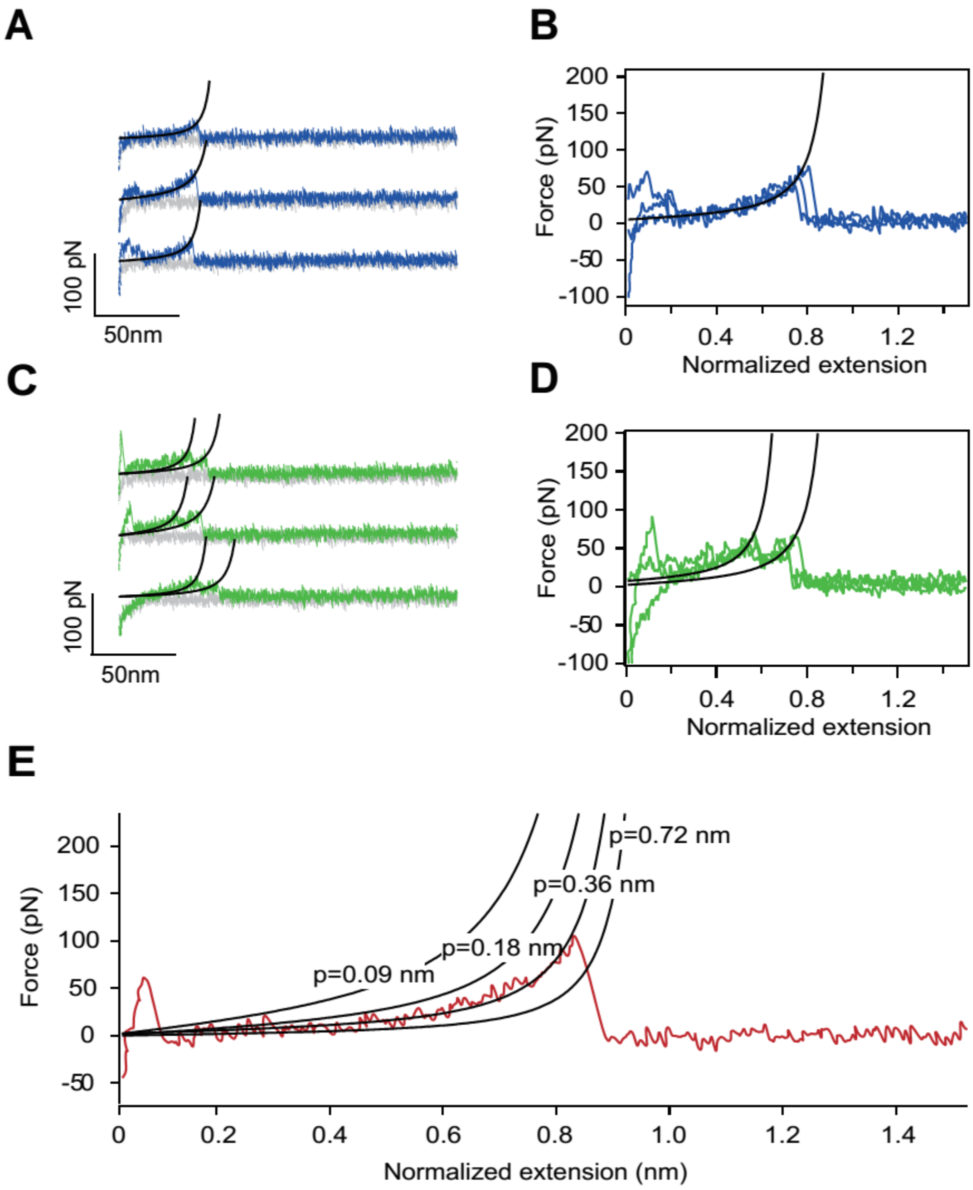

Figure S2 The mechanical signature of the PEG linker and the polypeptide. Before the disruption of the membrane binding, the PEG linker and the polypeptide were being stretched. The mechanical feature of this region can be used to estimate whether the events are indeed at the single molecule level. (**A**) Three representative force-extension curves with single rupture peak. Black lines are worm-like chain (WLC) fitting to the curves. The persistence lengths are all around 0.36 nm, in good agreement of the value reported in literature for PEG and unstructured polypeptide. (**B**) The three curves shown in **A** are normalized by their contour length (Normalized extension equals the extension divided by the total contour length of the linker region.) The normalized traces are superimposable, indicating that they share the same mechanical features, regardless of the rupture forces and fitting models. (**C**) Three representative force-extension curves with two rupture peaks. Black lines are WLC fitting to the individual peaks. (**D**) Superpositioning of the normalized traces shown in **C**. The normalized traces are superimposable, indicating that they share the same mechanical features, regardless of the rupture forces and fitting models. (**E**) The representative single molecule trace (red) and the simulated traces (black) using WLC model with the same contour length and different persistence lengths. Clearly, the mechanical feature of the PEG + polypeptide can be used to identify single molecule events.