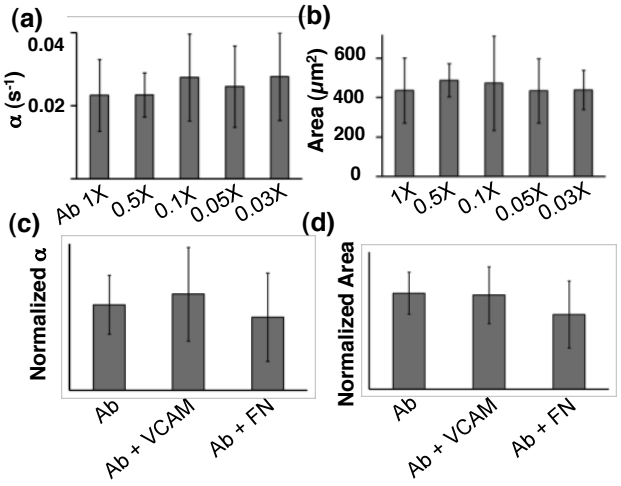
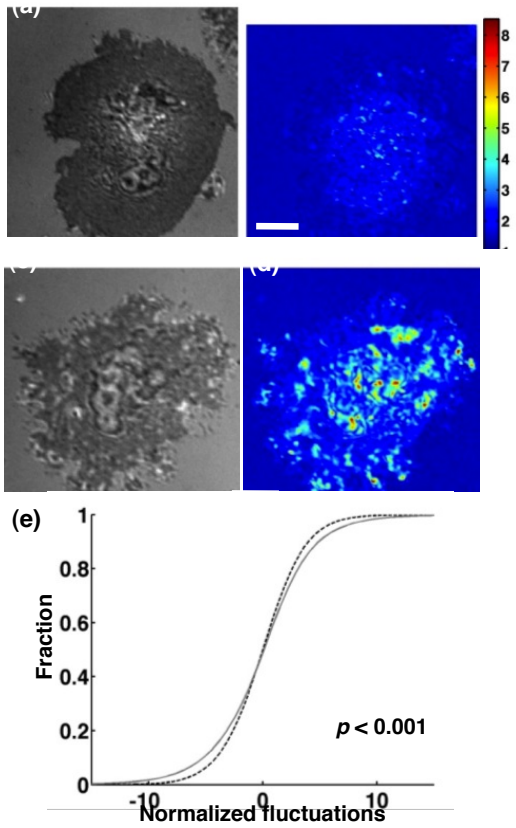
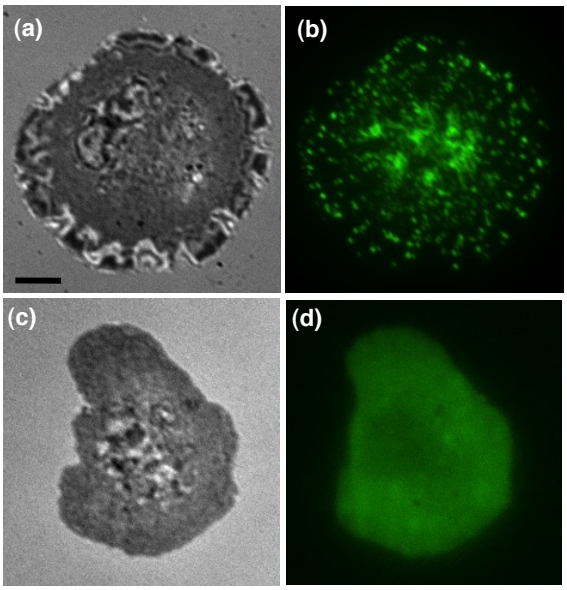
Supplementary Information for the manuscript entitled: "**Membrane dynamics correlate with formation of signaling clusters during cell spreading**"



Supplementary Figure S1: Dependence of spreading parameters on substrate properties. (a) Spreading rate (α) is unaffected by coating antibody concentration. 1X denotes the control antibody condition of 10μg/ml. (b) Spread area is unaffected by coating antibody concentration. (c) The effect of VCAM and Fibronectin on spreading rate. (d) VCAM and Fibronectin do not affect final spread area.



Supplementary Figure S2: Addition of LPA enhances membrane fluctuations. (a) IRM image of a Jurkat T cell upon spreading in serum free media. (b) Intensity fluctuations relative to shot noise for cell in (a). (c) IRM image of a spread cell in serum free media supplemented with 10 uM LPA. (d) Fluctuation map of the cell in (c), showing overall higher fluctuations in LPA. (e) Cumulative distribution of fluctuations in the serum free (dashed line) and in the presence of LPA (gray solid line). This shows that the fluctuation amplitudes are larger in LPA.



Supplementary Figure S3: Signaling clusters do not form in the absence of antibody. (a), (b) IRM and TIRF images, respectively, of a Jurkat T cell spreading on a glass substrate coated with anti-CD3 antibody. Clusters of YFP-ZAP70 are seen to robustly form in the TIRF image. (c), (d) IRM and TIRF images of a cell spreading on a substrate coated with poly-L-lysine alone. As seen in the TIRF, signaling clusters do not form on this surface, showing that engagement of the TCR with antibody is essential to the formation of signaling clusters. Most cells spread to a much smaller extent on a PLL coated surface.