Supporting Information

Ca^{2+} Binding by Labeled PIP2. A lipid monolayer containing 25 mol% C16 BODIPY-FL PI(4,5)P_2 and 75% SOPC was titrated with Ca^{2+} and the surface pressure change was used to calculate the apparent Ca2+ binding affinity of the labeled PIP2 (Fig. S1A). The total pressure drop after adding saturating [Ca^{2+}] to labeled PIP_2 is much lower than the pressure drop for unlabeled L-α-PIP_2 (~0.3 mN/m vs ~2.2 mN/m), but the apparent affinity is higher (K_D = 0.3 – 0.4 μM vs 2 - 5 μM). A monolayer containing 25 mol% C16 BODIPY-FL PI(4,5)P_2 and 39 μM Ca^{2+} was transferred onto a coverslip and imaged by tapping mode AFM (Fig. S1B). The Ca^{2+}-induced clusters were much smaller than clusters formed by natural PIP_2 under similar conditions, as shown in Figure S2D. These results rule out the possibility that the Ca^{2+}-induced surface pressure change and cluster formation in a PIP_2-containing monolayer is due to non-specific aggregation of labeled PIP_2.

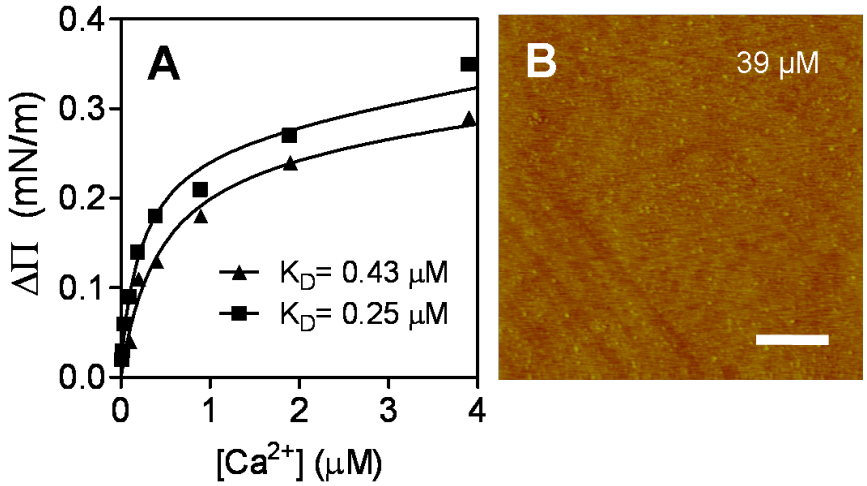

Fig. S1 (A) Two independent Ca^{2+}-binding affinity measurements using 25 mol% C16 BODIPY-FL PIP_2 in a background of SOPC. (B) A lipid monolayer of this composition transferred at 39 uM Ca^{2+} and imaged by tapping mode AFM. Scale bar: 1 μm.

Ca^{2+} Dependence of PIP_2-rich Cluster Formation. The Ca^{2+} dependence of PIP_2-rich cluster formation is studied by sequentially transferring a 25 mol% PIP_2-containing monolayer onto coverslips at different Ca^{2+} concentrations and imaging by tapping mode AFM (Fig. S2). Ca^{2+}-induced cluster formation and surface pressure changes (Fig 1) have similar [Ca^{2+}] dependence, suggesting that cluster formation and surface pressure changes are related. Stronger evidence that clustering causes the surface pressure drop would be made by comparing the time course of cluster growth with the time course of a Ca^{2+}-induced surface pressure change. However, the cluster growing dynamics is not accessible by the methods applied in this study. Preliminary evidence from fluorescence microscopy and FRET shows that the clusters form in seconds after Ca^{2+} is added, similar to the time course of the pressure drop.

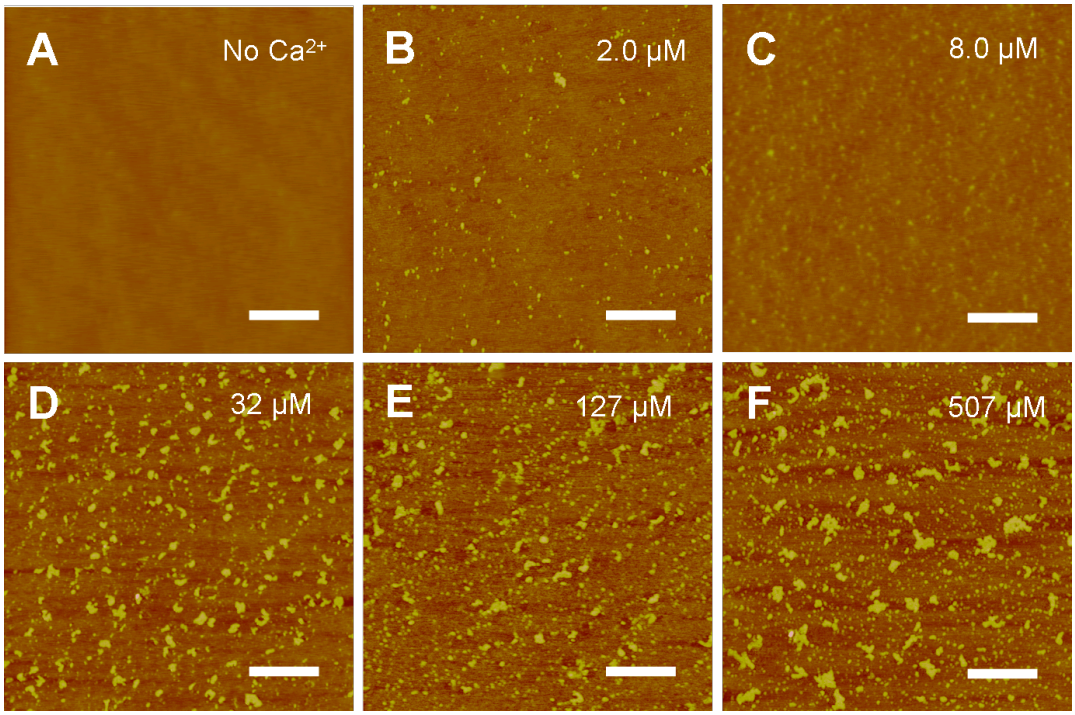

Fig. S2 The Ca^{2+} dependence of PIP2-rich cluster formation. 25 mol% L-α-PIP2 lipid monolayers in a background of SOPC are transferred at (A) 0 μM (B) 2.0 μM (C) 8.0 μM (D) 32 μM (E) 127 μM (F) 507 μM Ca^{2+} and imaged by tapping mode AFM. Scale bar: 1 μm.

Decreased Apparent K_D obtained from FRET Measurements as a Result of Reduced Surface Potential. The apparent Ca^{2+} binding affinities determined by SP-FRET measurements using LUVs (Fig. 6A) were lower than those calculated from surface pressure measurements using a lipid monolayer. This difference is rationalized by the differences in PIP_2 fraction and therefore the surface potential of the membranes. In order to make an effective comparison, both sets of data were normalized to their maximum values and plotted as the surface coverage ratio θ as a function of Ca^{2+} concentration. These normalized data were fit by a Langmuir adsorption model. The first several data points of the FRET study were omitted from the fitting since they fail to reflect the change in average fluorophore distances as a result of too high a labeling density (0.3 mol% each fluorophore). The apparent K_D obtained by fitting the Ca^{2+} titration data for the FRET measurement (5 mol% total PIP_2) is approximately 220 μM, and the apparent K_D for a 25 mol% PIP_2-containing monolayer is about 5 μM. Assuming the intrinsic binding affinities are the same in both systems, a 47 mV difference in surface potential is estimated to account for a 44-fold difference in apparent K_D based on Eq. 7 at $[Mg^{2+}] = 0$. The estimated difference in surface potential is in consistent with the PIP2 fraction-dependent zeta potential change as reported by Toner et al.[38]

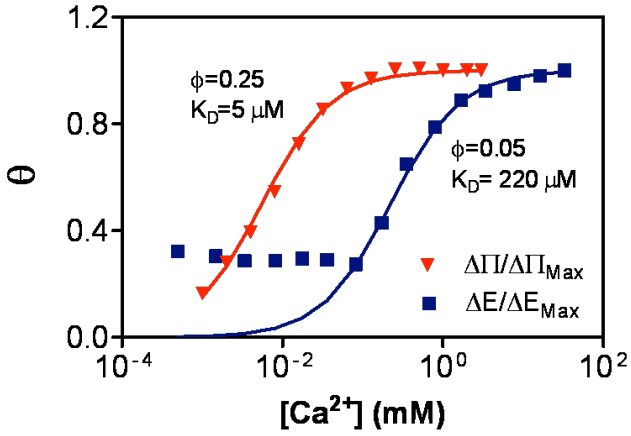

Fig. S3 Two sets of Ca^{2+} titration data from surface pressure and SP-FRET measurements were normalized to their maximum values and plotted as a function of Ca^{2+} concentration. The solid lines are the fitting result based on a Langmuir adsorption model.