Supporting Information

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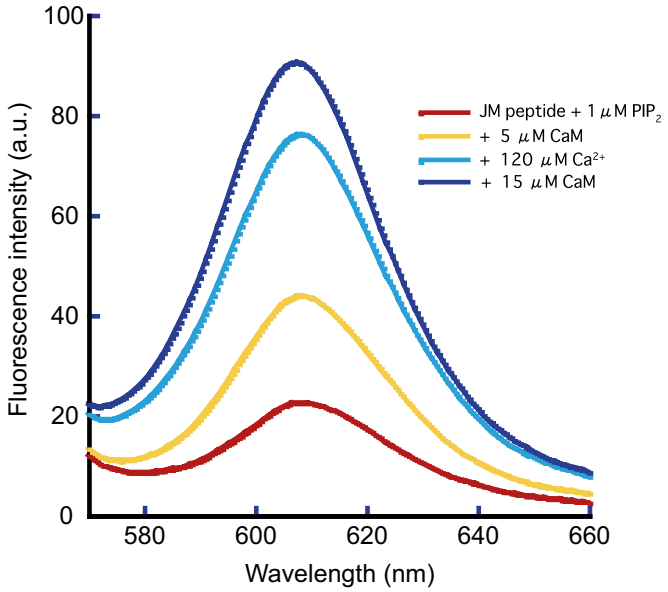

Fig. S1. The addition of the Ca^{2+} complex of calmodulin (Ca/CaM) reverses Texas Red quenching in isolated JM peptides. In Fig. 2*A* of the main text, we show that the addition of PIP_2 leads to quenching of a Texas Red fluorescent probe attached to the C-terminal end of the *Neu* JM peptide. Our interpretation of the observed quenching is that PIP_2 leads to JM–JM association on the negatively charged membrane surface. Here, we show that binding of the JM peptides can be reversed by the addition of Ca/CaM, which has been shown to bind strongly positively charged JM peptides corresponding to the ErbB1 receptor. Large unilamellar vesicles were formed by extrusion of multilamellar vesicles through 100-nm polycarbonate filters. POPC and POPS in the ratio of 10:5 were used for the lipid bilayers at a lipid concentration of 250 μM in Mops buffer (10 mM Mops and 0.1 M KCl, pH 7.0). The peptide-to-lipid molar ratio was 1:500 and PIP_2 was introduced into the membranes by addition of PIP_2 micelles to the vesicle solution. The PIP_2 concentration was 1 μM. Calmodulin was subsequently added to a final concentration of 5 μM and then Ca^{2+} was added to a final concentration of 120 μM. Finally, the calmodulin concentration was increased by 15 μM to saturate binding of the Ca/CaM complex to the isolated JM peptides. Fluorescence experiments were carried out on a Hitachi F-2500 fluorescence spectrophotometer.

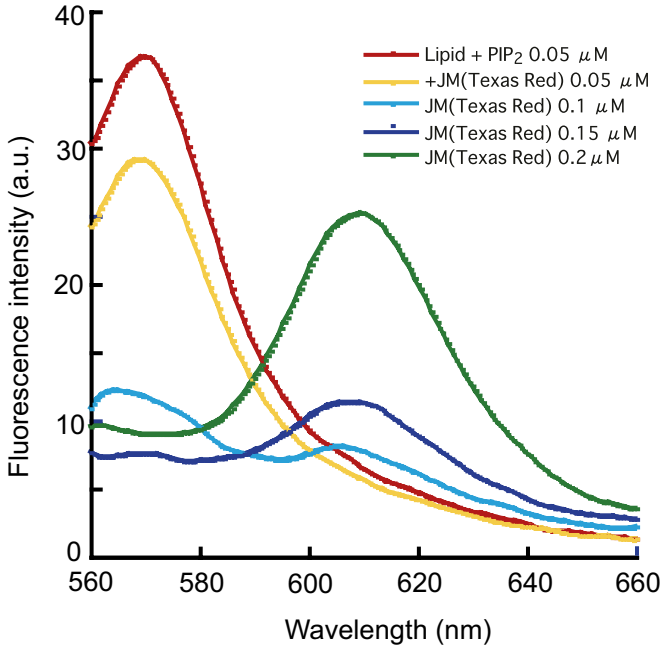

Fig. S2. Direct interaction of PIP_2 with isolated JM peptides. Fluorescence energy transfer (FRET) measurements were undertaken between Bodipy TMR-labeled PIP_2 (emission wavelength = 574 nm) and peptides corresponding to the *Neu* JM sequence labeled at the C terminus with Texas Red (emission wavelength = 615 nm). Large unilamellar vesicles were prepared by extrusion of POPC and POPS (10:5 ratio) through a 100-nm polycarbonate filter. The lipid concentration was 250 μM in Mops buffer (10 mM Mops and 0.1 M KCl, pH 7.0). Bodipy TMR–PIP_2 was incorporated into the vesicles by the addition of Bodipy TMR–PIP_2 micelles to the vesicle solution in a concentration of 0.5 μM. The JM peptide was added to the vesicle solution in an initial peptide concentration of 0.05 μM. The amount of the labeled peptide was increased in increments of 0.05 μM as shown in the figure. With a low concentration of peptide (red trace) and using an excitation wavelength of 450 nm, the fluorescence emission is predominantly from Bodipy TMR–PIP_2 at 574 nm. As the amount of the peptide is increased, the maximum of the fluorescence emission shifts to 615 nm, corresponding to the emission maximum for the Texas Red probe on the JM peptide. The observation of FRET between Bodipy TMR–PIP_2 and Texas Red suggests there is a direct interaction between the negatively charged PIP_2 and the positively charged JM peptide. Fluorescence experiments were carried out on a Hitachi F-2500 fluorescence spectrophotometer.

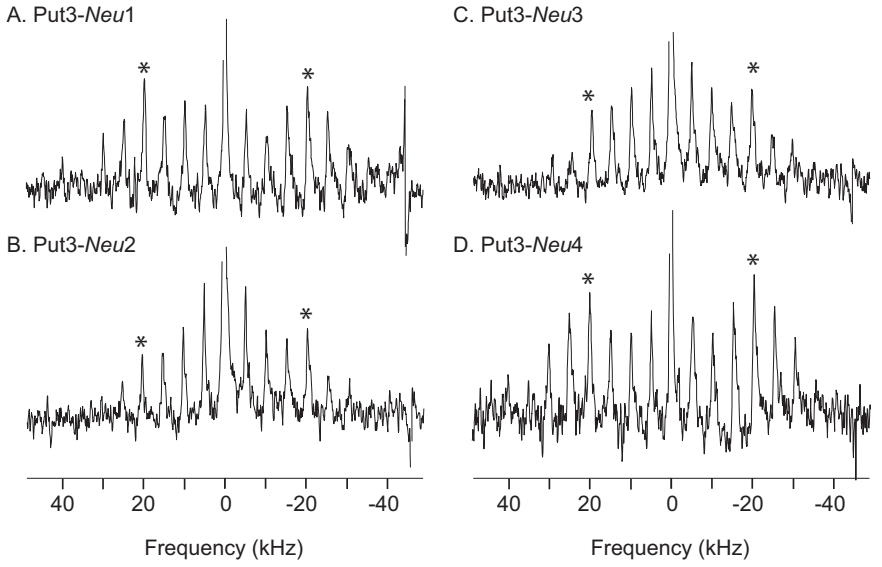

Fig. S3. Deuterium NMR MAS spectroscopy of Put3–*Neu* TM dimers. The Put3–*Neu* TM dimers are designed to place the *Neu* TM helices in four symmetric rotational orientations. The predicted interfacial residues are shown in the helical wheel plots in Fig. 4. To verify that the fusion of the Put3 domain to the *Neu* TM helix induces the predicted orientations, deuterium MAS NMR measurements were undertaken of the Put3–*Neu* TM dimers containing deuterium-labeled leucine at position 668. These peptides were reconstituted into DMPC:DMPG vesicles using detergent dialysis as described in *Materials and Methods*. MAS NMR spectra were obtained at 25 °C and a MAS frequency of 5 kHz. The intensities of the rotational side bands in the MAS spectra are sensitive to motion of the deuterated Leu668 side chain with an interfacial position predicted to have the most restricted motion. Of the four constructs, Put3–*Neu*1 and Put3–*Neu*4 are both predicted to place Leu668 in the TM interface. The observed intensities of the rotational side bands are consistent with Leu668 being more restricted in Put3–*Neu*1 and Put3–*Neu*4 than in Put3–*Neu*2 and Put3–*Neu*3. This conclusion is reflected in the relative intensity of the +4 and −4 side bands (indicated by asterisks) relative to the +1 and −1 side bands. Magic angle spinning (MAS) experiments were performed at a deuterium frequency of 76.8 MHz using 4.0-mm MAS rotors. Single-pulse excitation was used with a 5-μs 90° pulse length, followed by a 10-μs delay before data acquisition.