Supporting Information

Multi-step compositional remodeling of supported lipid membranes by interfacially active phosphatidylinositol kinases

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Table S1. QCM-D summary for anti-PI4P IgM binding to PI4P-containing supported lipid bilayers.

EXPERIMENTAL SECTION

Materials. 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (rho-PE) were obtained from Avanti Polar Lipids (Alabaster, AL). Phosphatidylinositol diC16 (PIP), Phosphatidylinositol 4-phosphate diC16 (PI4P) and Phosphatidylinositol 4,5-bisphosphate diC16 [PI(4,5)P2], Fluorescein conjugated Anti-PI(4,5)P2 IgM, Anti-PtdIns (4,5)P2 IgM and Anti-PtdIns(4)P IgM were obtained from Echelon Biosciences (Salt Lake City, UT). Phosphatidylinositol-4-Phosphate 5-Kinase, Type I, Alpha (PIP5K1A) was obtained from SignalChem (Richmond, BC, Canada). Phosphatidylinositol 4-kinase beta (PI4KB) was purchased from Thermo Fisher Scientific, Inc.

Quartz Crystal Microbalance-Dissipation. A Q-Sense E4 (Q-Sense AB, Gothenburg, Sweden) instrument was employed to monitor the adsorption kinetics of lipids onto silicon oxide- and gold-coated 5 MHz, AT-cut piezoelectric quartz crystals. Changes in frequency (ΔF) and energy dissipation (ΔD) were recorded as functions of time, as previously described[1]. The measurement data was collected at the n=3-11 overtones, and the reported values were recorded at the third overtone ($\Delta F_{n=3}/3$). All samples were introduced at a flow rate of 50 μL/min using a peristaltic pump (Ismatec Reglo Digital) under continuous flow conditions. The temperature of the flow cell was fixed at 24.00 ± 0.5°C. Before experiment, sensor surfaces were treated with oxygen plasma at 180 W for 1 min (March Plasmod Plasma Etcher, March Instruments, California) immediately before use.

To calculate the mass of antibodies deposited on QCM-D crystals, frequency and dissipation shifts at different overtones (n= 3, 5, 7, 9, 11, 13), were fitted to the viscoelastic model

implemented in QTools (Q-sense AB, Sweden), by using density as an input parameter to obtain surface mas density, shear modulus, and viscosity.

Fluorescence Microscopy. Fluorescence microscopy imaging was performed using an inverted epifluorescence Eclipse TE 2000 microscope (Nikon) equipped with a 60× oil immersion objective (NA 1.49), and an Andor iXon+ EMCCD camera (Andor Technology, Belfast, Northern Ireland). The acquired images had dimensions of 512 × 512 pixels with a pixel size of 0.267 × 0.267 μm. The samples were illuminated by a mercury lamp (Intensilight C-HGFIE; Nikon Corporation) with a TRITC (rhodamine–DHPE) filter set. All image processing was done using Image J.

Fluorescence Recovery After Photobleaching (FRAP). For FRAP measurements, a ~20 μm-wide circular spot was photobleached with a 532 nm, 100 mW laser beam, followed by time-lapsed recording. Images were collected at a rate of 0.5 images per second. Pre-bleach images and images without illumination were acquired to compensate for uneven illumination. Diffusion coefficients were determined by the Hankel transform method, which transforms the fluorescence recovery into the spatial frequency domain[2]. For all fluorescence imaging experiments, glass coverslips (Menzel Gläser, Braunschweig, Germany) were used.

PI4K Kinase Activity Solution Assay. The PI4KIIIB lipid kinase assay was performed using recombinant enzyme in a membrane capture assay described previously[3]. Phosphoinositides were purchased from Avanti Polar Lipids: L-α-Phosphatidylinositol (PI, Cat # 840024P) and DOPS:DOPC lipids (Cat # 790595P) were sonicated in water to generate 1 mg/mL PI:DOPS:DOPC. γ^{32}P-ATP was purchased from Perkin Elmer (Cat #BLU502A001MC). STF-00200211 (25 mM, 100% DMSO) was diluted in 10% DMSO. Reaction was set-up as follows 1) kinase assay buffer, PI:DOPS:DOPC, BSA and PI4KIIIβ, were combined in a total volume of 10

μL (2.5x solution); 2) 5 μL of inhibitor solution was added (5x solution) and incubated with enzyme mixture for 15 minutes; 3) 10 μL cold ATP and γ^{32}P-ATP were added (2.5x solution) to initiate the reaction which ran for 20 minutes. Final conditions were as follows: 20 mM Bis-Tris Propane pH 7.5, 10 mM $MgCl_2$, 0.075 mM Triton X-100, 0.5 mM EGTA, 1 mM DTT, 100 μM PI, 500 ng/μL BSA, 2.5 nM PI4KIIIβ, 2% DMSO, 100 μM ATP and 1uCi γ^{32}P-ATP. Upon completion of the reaction, 4 μL was spotted onto 0.2 μm nitrocellulose (Bio-Rad Catalog #162-0112). The membrane was dried for 5 minutes under a heat lamp followed by 1 x 30second wash and 6 x 5min washes in 1 M NaCl / 1% Phosphoric Acid. The membrane was dried for 20 minutes under a heat lamp followed by overnight exposure to a phosphor screen and phosphorimaging followed on a Typhoon 9500. Intensities were quantified using SPOT.

Protein Preparation. The DNA segment encoding full length human PI4KIIIβ was inserted into pFastBac. Baculovirus expression vector by BamHI and NotI sites with an in frame His8-TEV tag at the N-terminus. Baculovirus produced by SF9 cells was used to infect 1L of Hi5 cells at a density of 1.5-2.0x10^6 cells/mL at 27 °C for 66 hours. The cell pellets were collected by centrifugation and resuspended in 25 mM Tris-HCl pH 8.0, 150 mM NaCl, 5 mM imidazole, 10% (v/v) glycerol, and 2 mM β-mercaptoethanol, 0.1% Triton X100 with protease inhibitors cocktail. Cells were lysed by 30 strokes using a Dounce tissue grinder set (D0189, Sigma) on ice and cleared by centrifugation for one hour at 16,000 rpm, 4°C. The crude protein was purified by Ni column (GE Healthcare Bio-Sciences Corp) followed by the removal of His8 tag by TEV protease digestion overnight. The protein was further purified by Source 15Q column (GE Healthcare Bio-Sciences Corp) with 0-0.5M NaCl gradient. The pure fractions were then concentrated to 1.0 mL using an Amicon 50k centrifugal filter (Millipore) and injected on a Superdex200 column (GE Healthcare Bio-Sciences Corp) equilibrated with 10 mM Tris pH 8,

300 mM NaCl, and 10 mM DTT. Pure protein from gel filtration was collected, aliquoted and stored at -80 °C. All protein samples were examined by SDS-PAGE gel followed by Coomassie Brilliant Blue staining and the final protein concentration was determined by Bradford method.

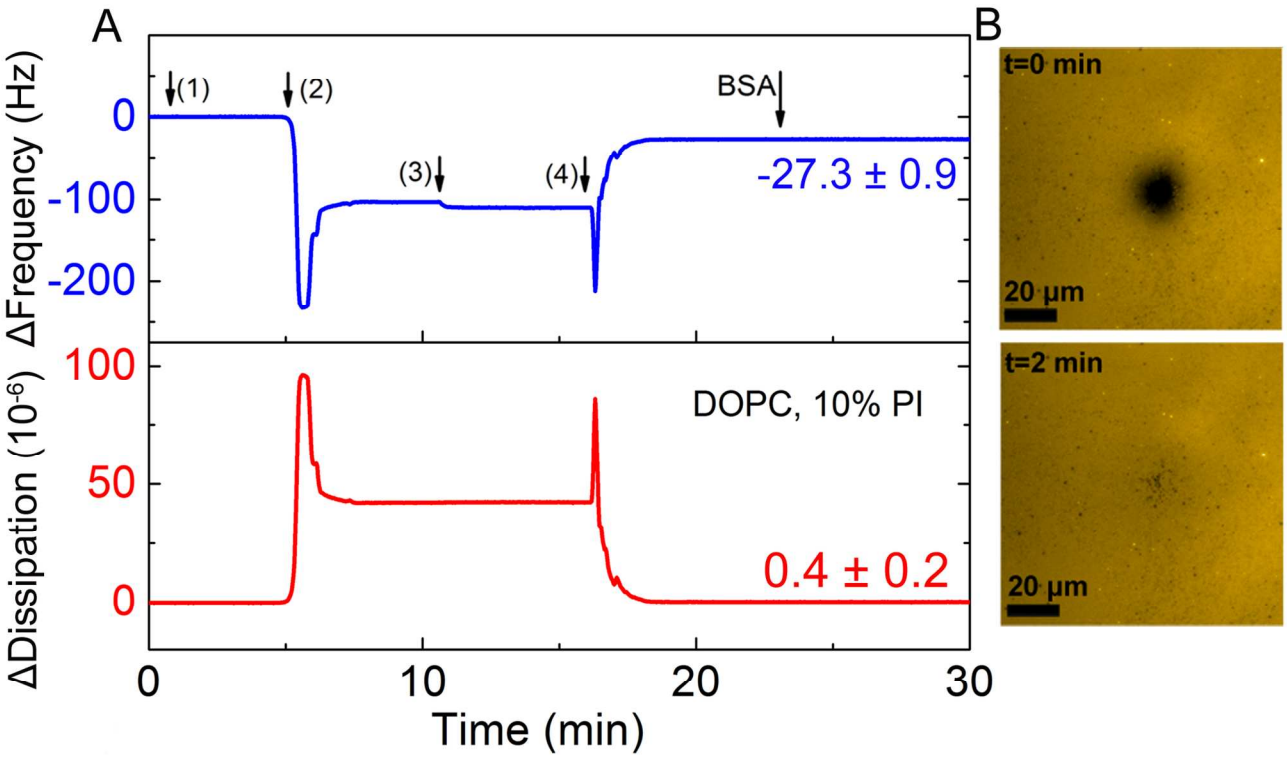

Figure S1. (A) QCM-D frequency shift (ΔF, top panel) and dissipation (ΔD, bottom panel) for the third overtone were measured as a function of time during formation of PI containing bilayers on silicon oxide using SALB method. Arrows indicate the injection of (1) Tris buffer (10 mM Tris, 150 mM NaCl, pH 7.5), (2) isopropanol, (3) lipid mixture [0.5 mg/ml of DOPC in isopropanol with 10% PI (dissolved in ethanol)], (4) tris buffer solution leading to a final ΔF and ΔD of -27.3 ± 0.9 Hz and 0.4 ± 0.2 × 10^{-6}, respectively, which correspond to a planar bilayer. Subsequently BSA protein (0.1 mg/ml) was injected which led to a frequency increase of less than 2 Hz. (B) FRAP snapshots of PI doped bilayer containing 0.5 wt % Rho-PE lipid. Top and bellow images were taken immediately and 2 min after photobleaching, respectively, giving a lateral diffusion coefficient of 1.6 μm^2/sec.

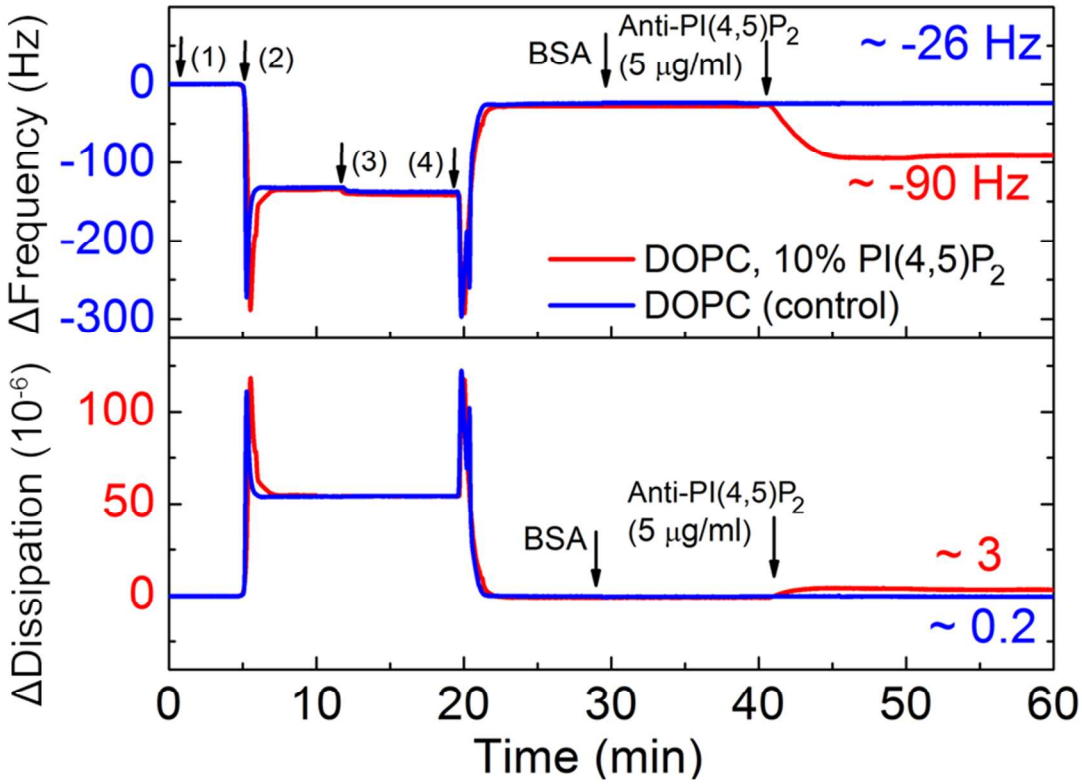

Figure S2. QCM-D monitoring of formation of $PI(4,5)P_2$-containing supported lipid bilayer using the SALB method. QCM-D frequency shift (ΔF, top panel) and dissipation (ΔD, bottom panel) for the third overtone were measured as functions of time during formation of $PI(4,5)P_2$-containing bilayers on silicon oxide. Arrows indicate the injection of (1) Tris buffer (10 mM Tris, 150 mM NaCl, pH 7.5), (2) isopropanol, (3) lipid mixture [0.5 mg/ml of DOPC in isopropanol with (red curves) and without 10% PI (blue curves)], (4) Tris buffer solution leading to a final ΔF and ΔD of ~ -26 Hz and ~ 0.2×10^{-6}, respectively, which correspond to a planar bilayer. Subsequently BSA protein (0.1 mg/ml) was injected followed by addition of anti-PI4P and anti-$PI(4,5)P_2$ (5 μg/ml), leading to a further decrease in ΔF of PI-containing bilayers. No changes in the ΔF of pure DOPC bilayers were observed upon antibody injection, indicating specific binding of antibodies to the PIP lipids.

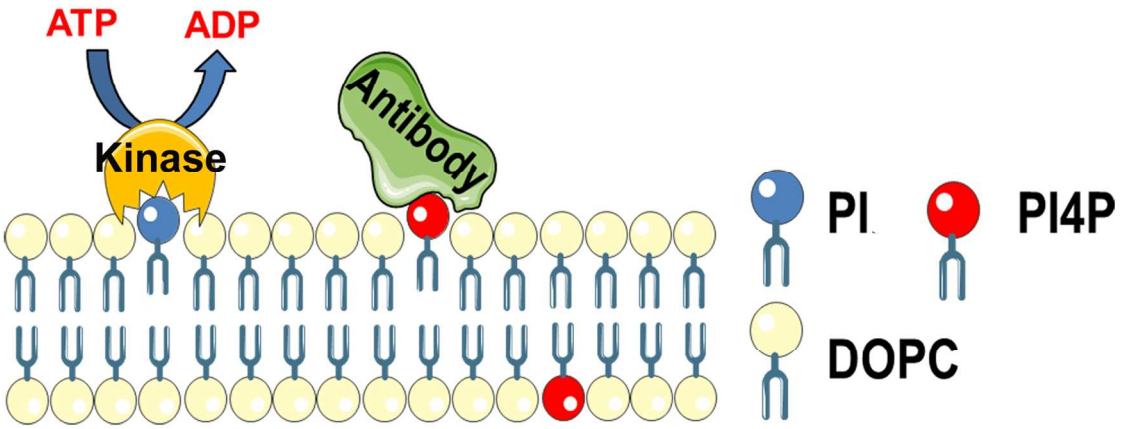

Figure S3. Schematic illustration of PI4K action on PI-containing supported bilayer and subsequent *in situ* detection of PI4P generation using anti-PI4P antibody.

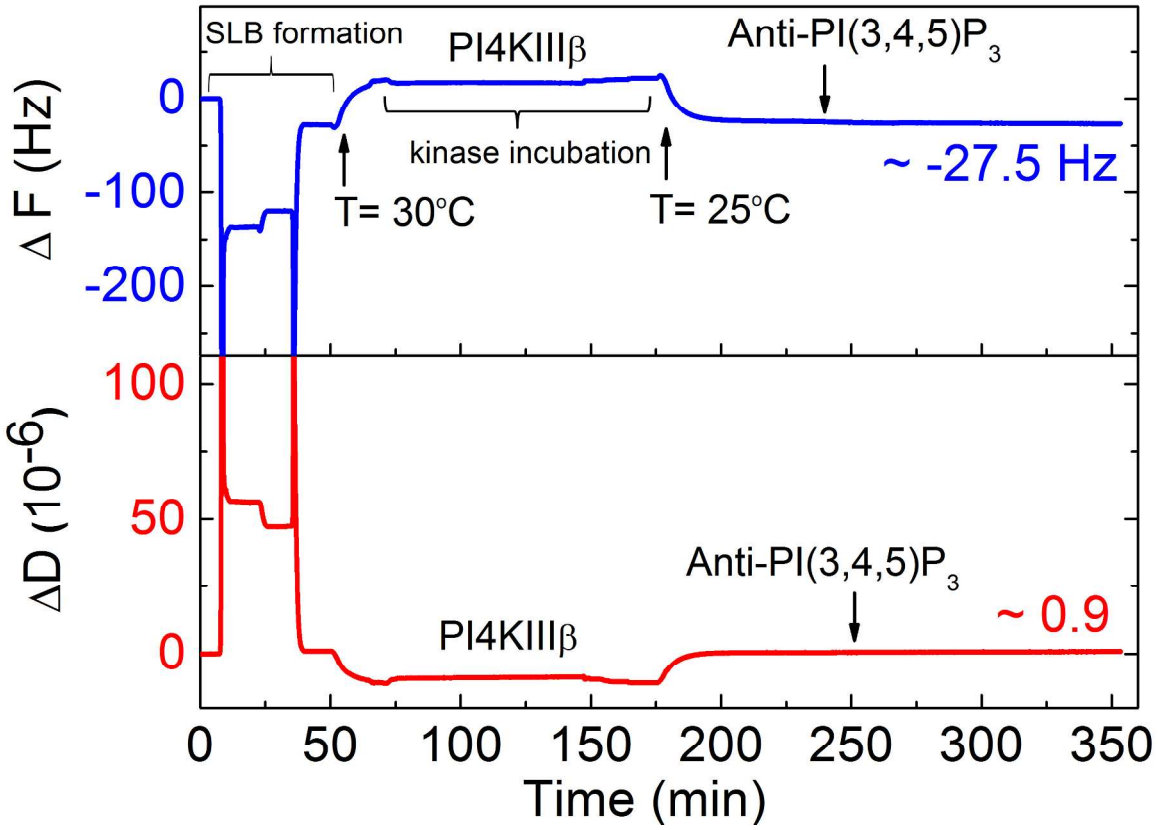

Figure S4. QCM-D analysis to test the specificity of the kinase assay. Bilayer containing 10% PI lipids was prepared by SALB method and exposed to PI4K kinase to generate PI4P. Next a nonspecific antibody (anti-PI(3,4,5)P3 IgM) was added. No binding was observed indicating the specificity of the assay.

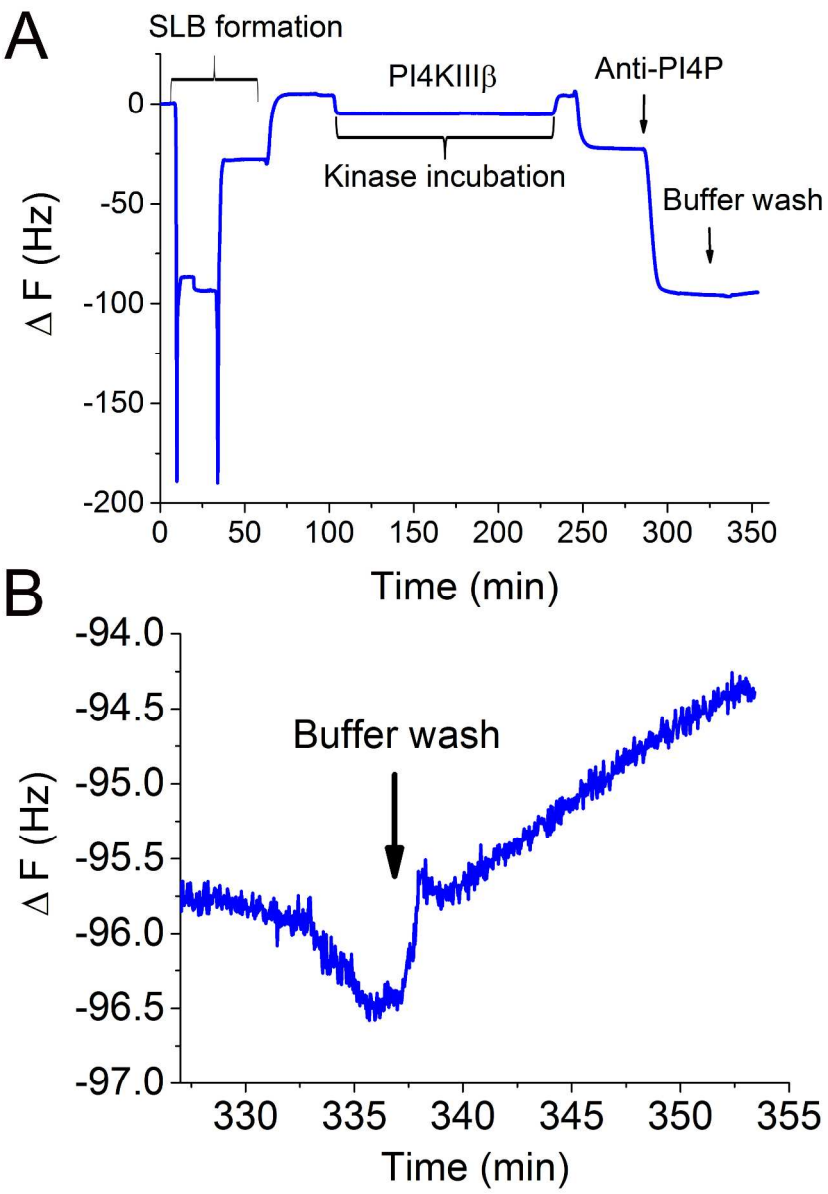

Figure S5. QCM-D analysis to test the specificity of the kinase assay. (A) Bilayer containing 10% PI lipids was prepared by SALB method and exposed to PI4K kinase to generate PI4P. A washing step included after antibody binding. (B) The magnified view of frequency changes during buffer wash step. A positive frequency shift of < 2 Hz in the course of ~ 20 min was observed, indicating a minute nonspecific binding.

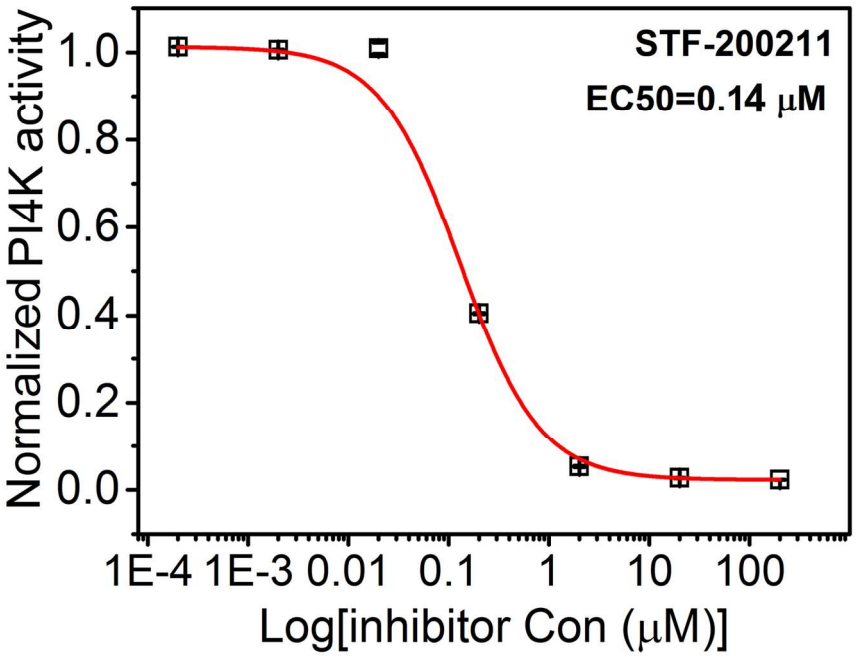

Figure S6. Enzyme inhibition curve of PI4K in the presence of various concentrations of inhibitor (STF-200211) obtained using a solution-based assay. Calculated EC_{50} = 0.14 μM.

PI4P	Frequency & Dissipation Shifts	Surface Mass Density (ng/cm^2)	Number of Antibodies/μm^2
Included in the bilayer	ΔF=67± 12.6 Hz $\Delta D \sim 4 \times 10^{-6}$	2356 ± 77	14621 ± 477
Generated by PI4K	ΔF= 61 ± 9 Hz $\Delta D \sim 3 \times 10^{-6}$	2076 ± 121.5	12885 ± 754

Table S1. QCM-D summary for anti-PI4P IgM binding to PI4P-containing supported lipid bilayers. A molecular weight of 970 kDa was assumed for anti-PI4P IgM.

REFERENCES

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(3) Knight, Z. A.; Feldman, M. E.; Balla, A.; Balla, T.; Shokat, K. M. *Nat. Protoc* **2007**, *2*, 2459-2466.