Supplementary Information

Allosteric substrate switching in a voltage sensing lipid phosphatase

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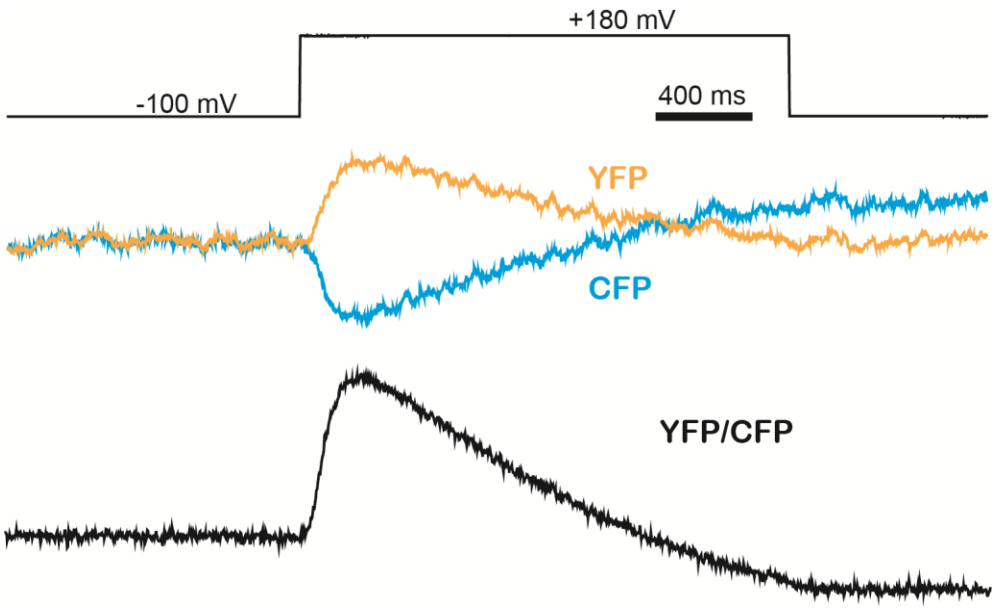
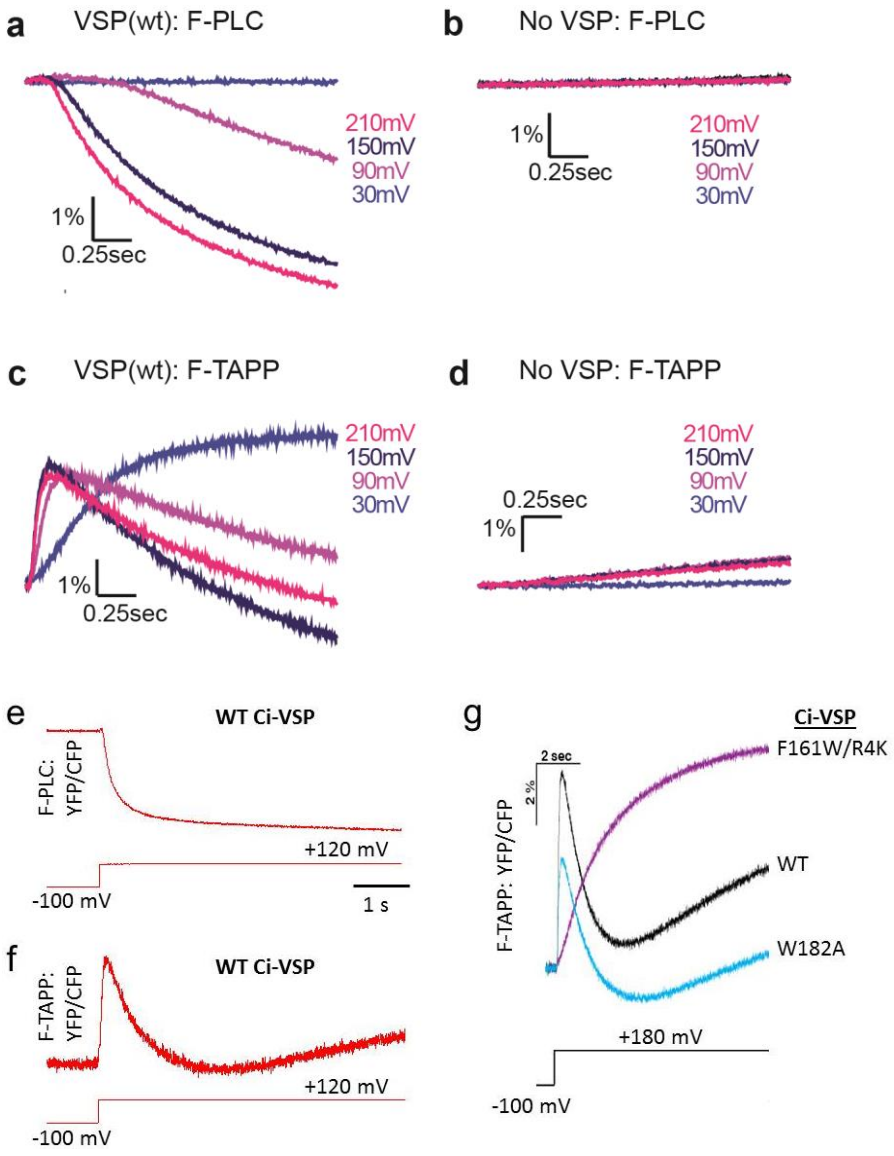
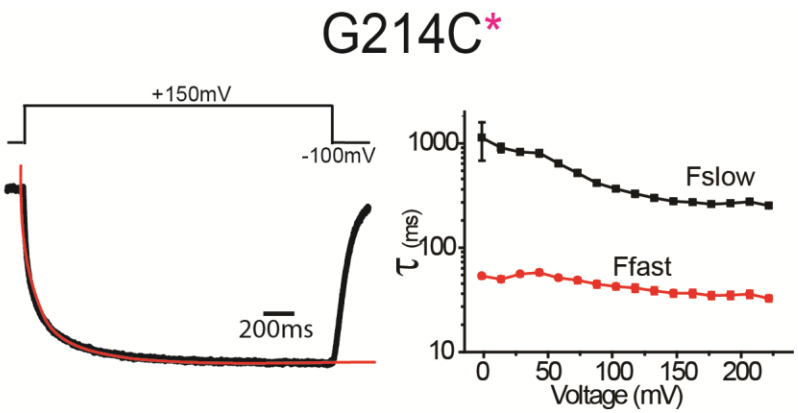
Supplementary Results



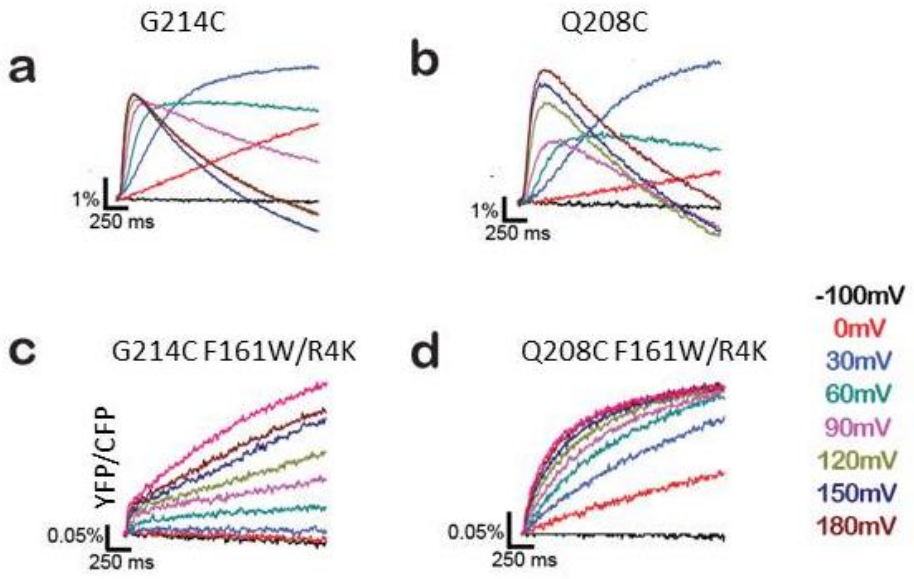
Supplementary Figure 1. Membrane-Associated F-TAPP Reporter fluorescence signals. A depolarizing voltage step to an oocyte co-expressing WT Ci-VSP and the F-TAPP reporter elicits a transient rise in YFP fluorescence and a parallel decrease in CFP fluorescence due to FRET, which is expressed as the YFP/CFP signal.



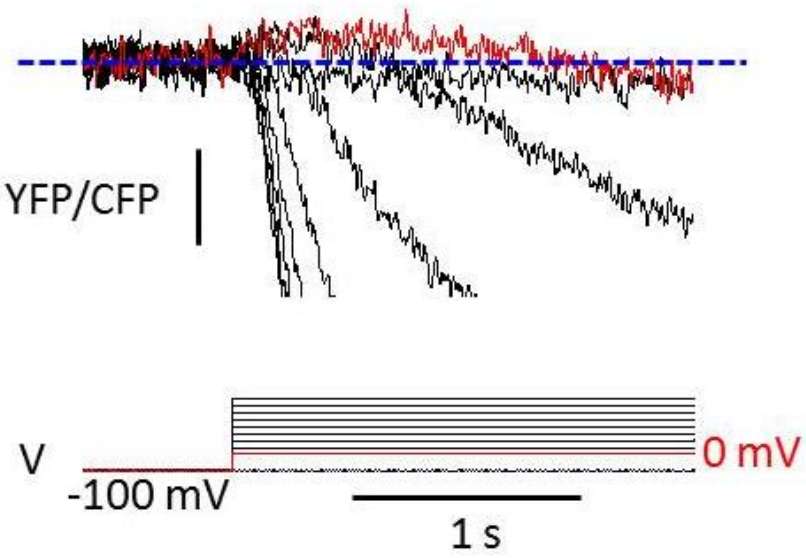
Supplementary Figure 2. Specific detection of Ci-VSP activity in Xenopus oocytes with new FRET PIP_2 reporters. F-PLC (a,b) and F-TAPP (c,d) YFP/CFP fluorescence responses to depolarizing steps in oocytes co-expressing WT Ci-VSP (a,c) and without Ci-VSP (b,d). No background activity detected by F-PLC (b). Small background activity detected by F-TAPP (d) (that is likely due to a native Xenopus oocyte VSP) is slow, only seen at high depolarizations and is expected to blunt the late phase of decrease in YFP/CFP signal and so lead to a small underestimate. e-g) Activity elicited by long depolarizing voltage steps to +120 mV (e,f,) or +180 mV (g), using F-PLC to track PI(4,5)P2 (e) or F-TAPP to track PI(3,4)P2 (f and g, black trace). Drop in PI(4,5)P2 triggered in oocytes expressing WT Ci-VSP is sustained (e), whereas drop in PI(3,4)P is not. This difference can be attributed to presence of background PI(3,4,5)P3→PI(3,4)P2 activity at strong depolarizations of a VSP that is native to Xenopus oocytes (d). No such background activity contaminates the F-PLC signal (e), as seen when F-PLC is expressed alone (b). The background VSP activity is greater at +180 mV (g, black trace) than at +120 mV (f).



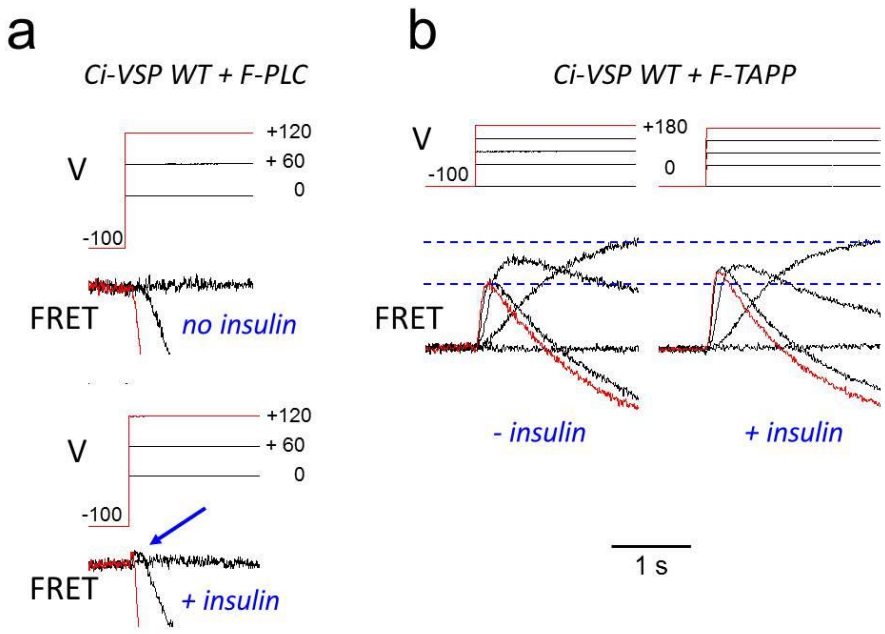
Supplementary Figure 3. Two exponential fluorescence decrease of G214C-TMRM (G214C*) in response to depolarization step. Left) Fluorescence trace evoked by step to +150 mV is well fit by double exponential (τ_{fast} = 36.5 $\pm$ 7.5 s, τ_{slow} = 278.2 $\pm$ 12.8 s; n=8), this fast component was earlier associated with gating charge movement and slow component with a relaxation step[37]. **Right)** Voltage dependence of fast and slow components of G214C-TMRM fluorescence show that fast component is always ~10-fold faster than the slow component. This indicates that the slow component is too slow to account for the fast transition to the A1 active state seen by F-TAPP.



Supplementary Figure 4. Comparison of F-TAPP readout of activity in WT and mutant Ci-VSP which contains the cysteine labeling site (for TMRM) at position G214C *versus* Q208C. a-d) Enzyme activity read out by the F-TAPP reporter [rise and fall of PI(3,4)P2] is similar in WT Ci-VSP carrying the cysteine labeling site at G214C* (a) (as in Fig. 5) and Q208C* (b) (as in Fig 4). A minor difference is detected in voltage dependence and there is a better kinetic separation of the FRET increase in F161W/R4K into two components when the cysteine labeling site is at G214C* (c) as opposed to Q208C* (d). However, the major effect of the mutation is the same: the F161W/R4K mutant, which lacks the F3 component of VSD motion (Fig. 2h), shows a pure FRET increase, consistent with an entry into the A1 state and suppression of transition into the A2 state.



Supplementary Figure 5. F-PLC FRET response to activity of the R217E mutant of Ci-VSP. Activity elicited by a depolarizing voltage step to 0 mV (red trace) results in a small increase in FRET that is sustained for hundreds of milliseconds.



Supplementary Figure 6. Effect of activation of PI3 kinase by insulin on activity readout by F-PLC and F-TAPP. Oocytes pre-treated with insulin to activate native oocyte insulin receptors, which, in turn, activate PI(3) kinase. This activity leads to an increase of PIP3 due to phospholylation of PI(4,5)P2. a, b) Pretreatment with insulin boosts the amplitude of the FRET increase component seen with F-PLC [accumulation of PI(4,5)P2] (a, arrow) and F-TAPP [accumulation of PI(3,4)P2] (b, dashed lines).

Supplementary Figure 7. F-PLC FRET Reporter Sequence

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  1  MVSKGEELFT GVVPILVELD GDVNGHRFSV SGEGEGDATY GKLTLKFICT TGKLPVPWPT LVTTLTWGVQ CFSRYPDHMK QHDFFKSAMP
 91  EGYVQERTIF FKDDGNYKTR AEVKFEGDTL VNRIELKGID FKEDGNILGH KLEYNYISHN VYITADKQKN GIKAHFKIRH NIEDGGVQLA
181  DHYQQNTPIG DGPVLLPDNH YLSTQSALSK DPNEKRDHMV LLEFVTAAGI TLGMDELYLE EAAAREAAAR EAAAREAAAR EAAAREAAAR
271  SGEDLQALLK GSQLLKVKSS SWRRERFYKL QEDCKTIWQE SRKVMRTPES QLFSIEDIQE VRMGHRTEGL EKFARDVPED RCFSIVFKDQ
361  RNTLDLIAPS PADAQHWVLG LHKIGTEAAA REAAAREAAA RGGEAAAREA AAREAAARGG RMVSKGEELF TGVVPILVEL DGDVNGHKFS
451  VSGEGEGDAT YGKLTLKLIC TTGKLPVPWP TLVTTLGYGL QCFARYPDHM KQHDFFKSAM PEGYVQERTI FFKDDGNYKT RAEVKFEGDT
541  LVNRIELKGI DFKEDGNILG HKLEYNYNSH NVYITADKQK NGIKANFKIR HNIEDGGVQL ADHYQQNTPI GDGPVLLPDN HYLSYQSALS
631  KDPNEKRDHM VLLEFVTAAS SEAAAREAAA REAAAREAAA REAAAREAAA REAAAR
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Supplementary Figure 8. F-TAPP FRET Reporter Sequence

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161  GIKAHFKIRH NIEDGGVQLA DHYQQNTPIG DGPVLLPDNH YLSTQSALSK DPNEKRDHMV LLEFVTAAGI TLGMDELYLE
241  EAAAREAAAR EAAAREAAAR EAAAREAAAR SGYFTPKPPQ DSAVIKAGYC VKQGAVMKNW KRRYFQLDEN TIGYFKSELE
321  KEPLRVIPLK EVHKVQECKQ SDIMMRDNLF EIVTTSRTFY VQADSPEEMH SWIKAGTEAA AREAAAREAA ARGGEAAARE
401  AAAREAAARG GRMVSKGEEL FTGVVPILVE LDGDVNGHKF SVSGEGEGDA TYGKLTLKLI CTTGKLPVPW PTLVTTLGYG
481  LQCFARYPDH MKQHDFFKSA MPEGYVQERT IFFKDDGNYK TRAEVKFEGD TLVNRIELKG IDFKEDGNIL GHKLEYNYNS
561  HNVYITADKQ KNGIKANFKI RHNIEDGGVQ LADHYQQNTP IGDGPVLLPD NHYLSYQSAL SKDPNEKRDH MVLLEFVTAA
641  SSEAAAREAA AREAAAREAA AREAAAREAA AREAAAR
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