Supplemental material

Rayaprolu et al., https://doi.org/10.1085/jgp.201812064

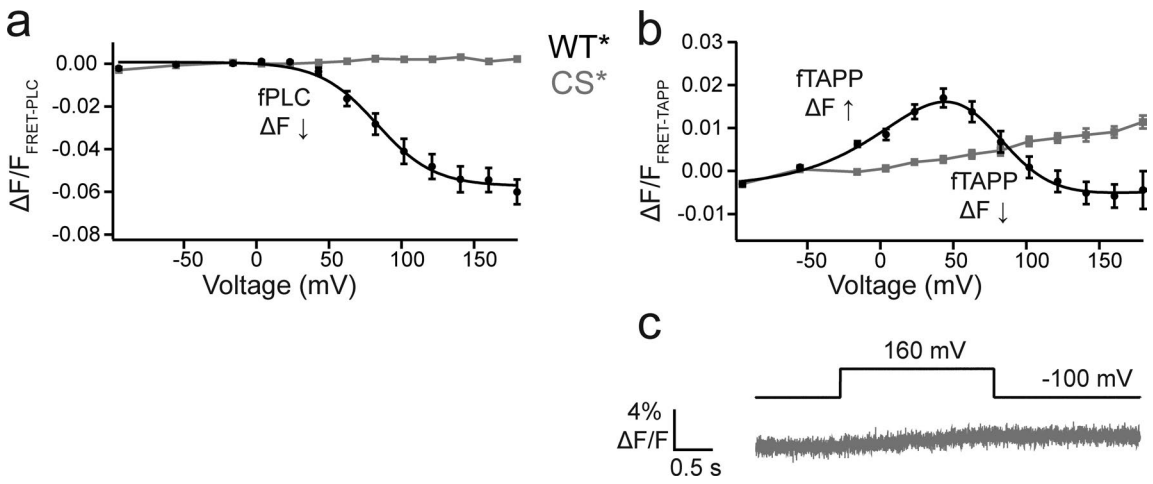

Figure S1. **Catalytically inactive Ci-VSP does not alter PIP concentrations in oocytes.** (**a**) ΔF/F fPLC FRET ratio versus voltage for WT* and catalytically inactive C363S (CS*) Ci-VSP. WT* shows a fluorescence decrease (net 5-phosphatase reaction), whereas CS* does not. Error bars are ±SEM; $n = 16$. WT* data fit with a single Boltzmann equation. (**b**) ΔF/F fTAPP FRET ratio versus voltage for WT* and CS*. WT* shows a fluorescence increase (net 5-phosphatase reaction) predominating at lower voltages and the fluorescence decrease (net 3-phosphatase reaction) predominating at higher voltages. CS* shows a small increase in fluorescence at higher voltages indicative of the endogenous VSP found in *X. laevis* oocytes. Error bars are ±SEM; $n \geq 20$. WT* data fit with a double Boltzmann equation. (**c**) Averaged fTAPP FRET trace over time during a voltage step from a holding potential of −100 to 160 mV for fTAPP coexpressed with CS*. Endogenous VSP causes a small and very slow increase in FRET over time. $n = 11$.

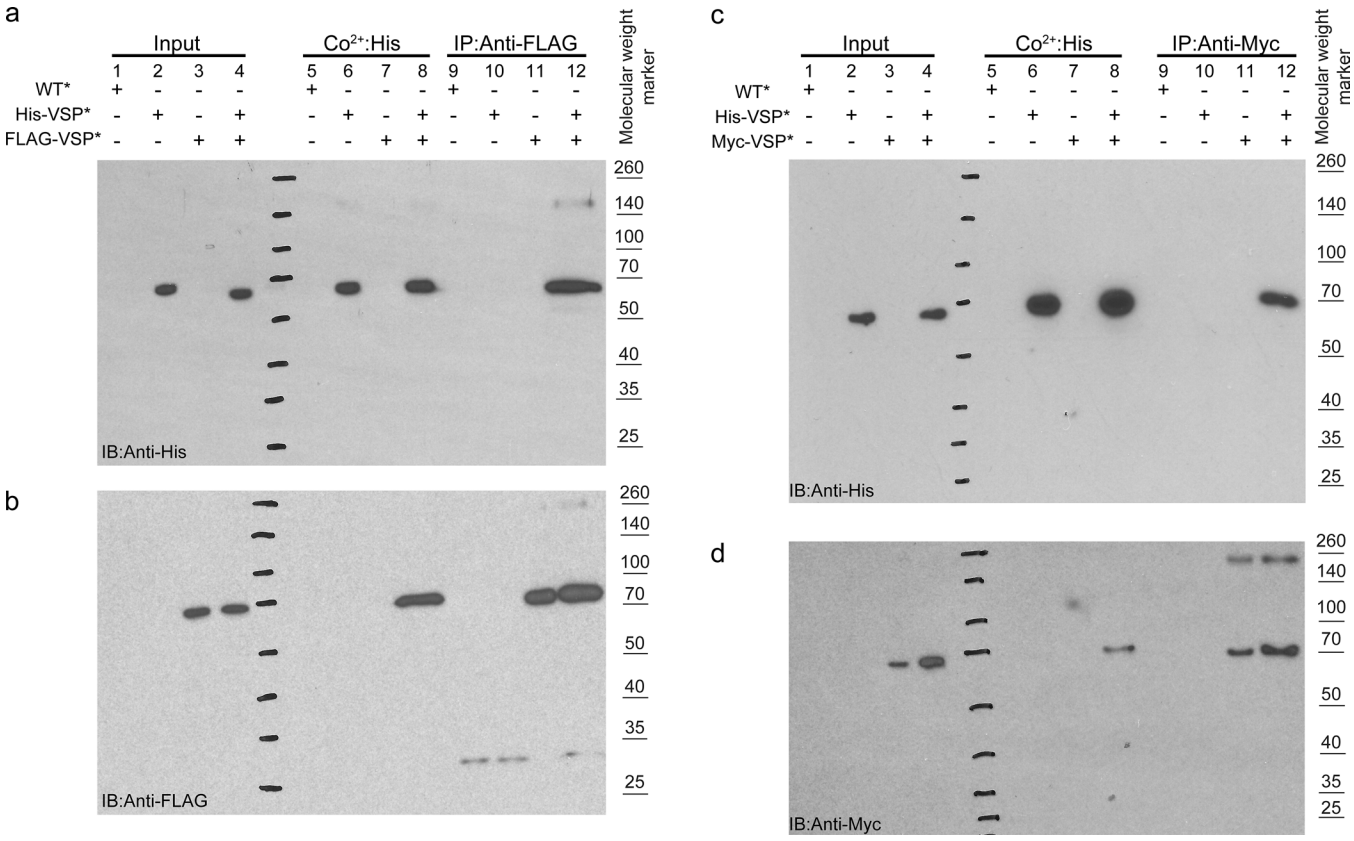

Figure S2. **Raw images of Western blots shown in Fig. 2.** The data are arranged as in Fig. 2. IB, immunoblotting; IP, immunoprecipitation.

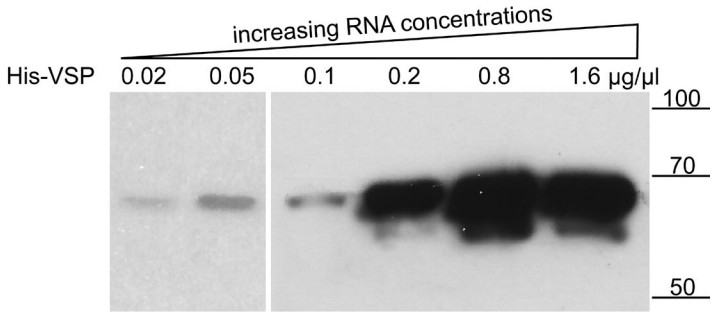

Figure S3. **Increasing cRNA concentrations lead to increasing protein expression.** Individual oocytes were injected with concentrations of His-VSP cRNA ranging from 0.02 to 1.6 µg/µl. Each cell lysate was probed for protein expression by Western blot. The higher concentrations of cRNA led to higher protein expression.

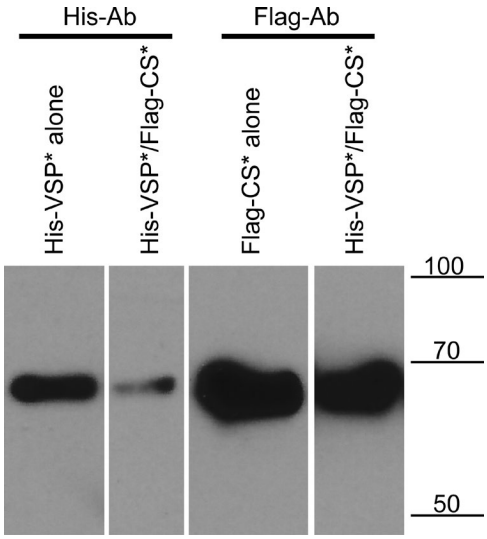

Figure S4. **His-VSP*/FLAG-CS* mixtures had significantly higher FLAG-CS* concentrations.** Cells were injected with a 1:10 ratio of His-VSP to FLAG-CS cRNA to induce a bias toward heterodimerization of an active and an inactive subunit for testing with fTAPP. Western blots confirm the presence of the induced bias. His-VSP* and FLAG-CS* alone were used as positive controls.

Table S1. **Voltage dependence of VSD motions**

VSP	*n*	$V_{1/2}$	Slope
G214C	14	55.7 ± 0.5	26.5 ± 0.4
FLAG-G214C	13	59.3 ± 0.9	24.5 ± 0.8
His-G214C	11	58.8 ± 0.5	24.6 ± 0.5
Myc-G214C	11	58.8 ± 0.7	23.9 ± 0.7
HA-G214C	12	60.5 ± 0.4	27.0 ± 0.4
G214C D331A	11	21.8 ± 0.3	19.9 ± 0.2
G214C/G214C D331A	13	30.6 ± 0.4	23.7 ± 0.4
WT/G214C D331A	12	16.5 ± 0.2	18.8 ± 0.2