Supplementary Materials

Molecular Biology of the Cell

Poddar *et al*.

Supplemental Information

Membrane stretching activates calcium-permeability of a putative channel Pkd2 during fission yeast cytokinesis

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#: Equal contributions

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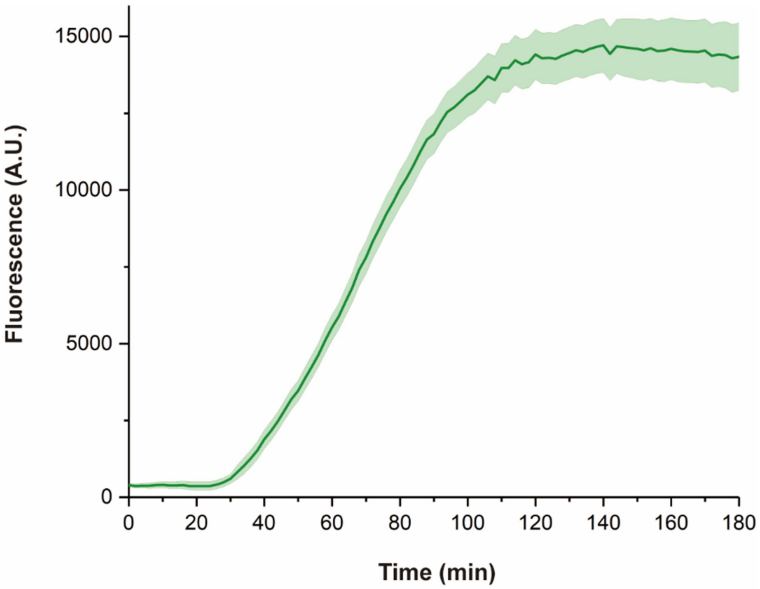
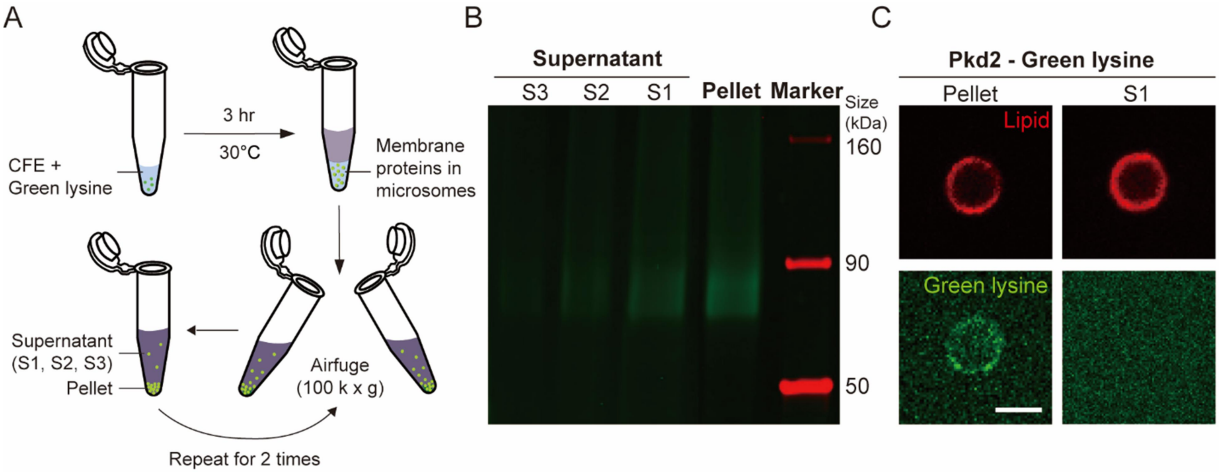
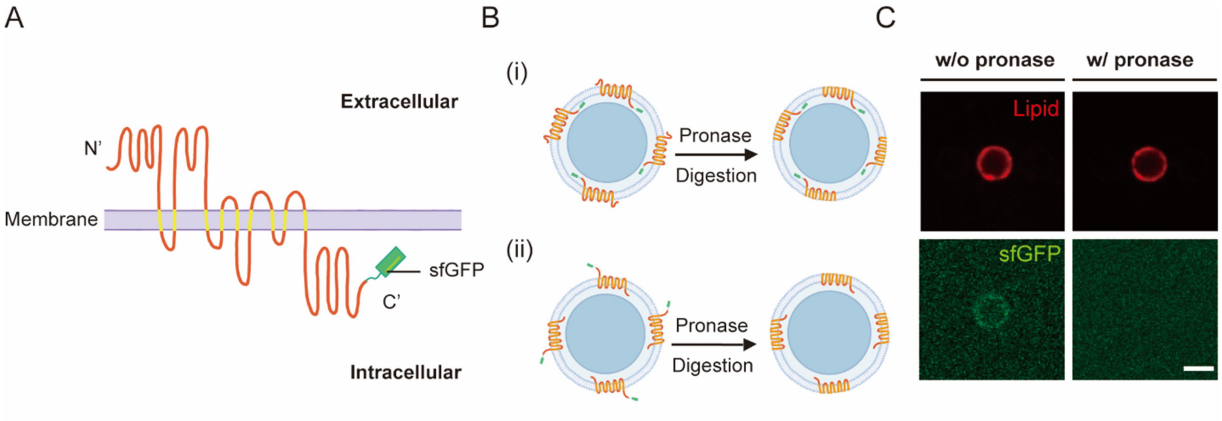


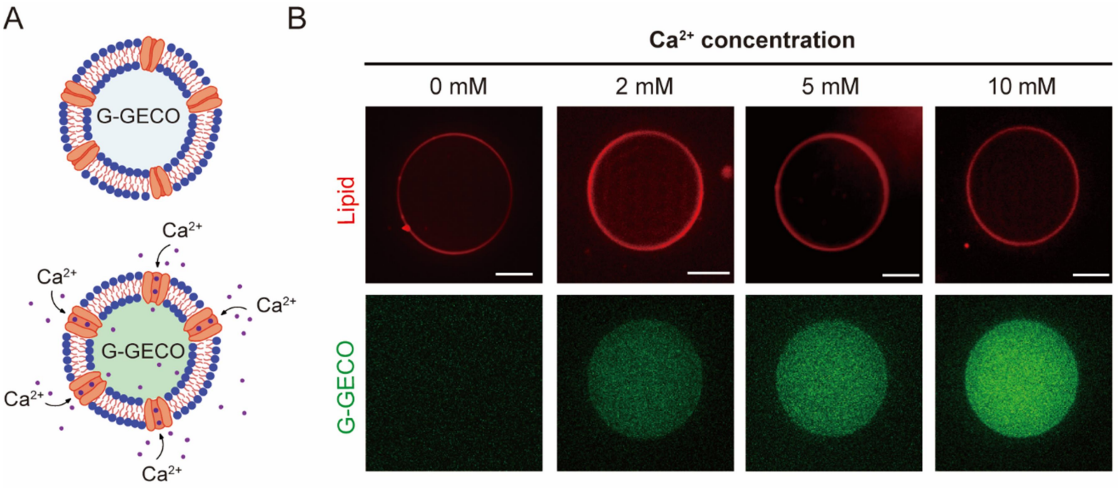
Supplemental Figure 1: Synthesis of Pkd2 using a mammalian CFE system. Time course of the expression of Pkd2-sfGFP using HeLa-based CFE reaction by monitoring sfGFP fluorescence. Error bands represent the standard deviation calculated from three independent reactions.



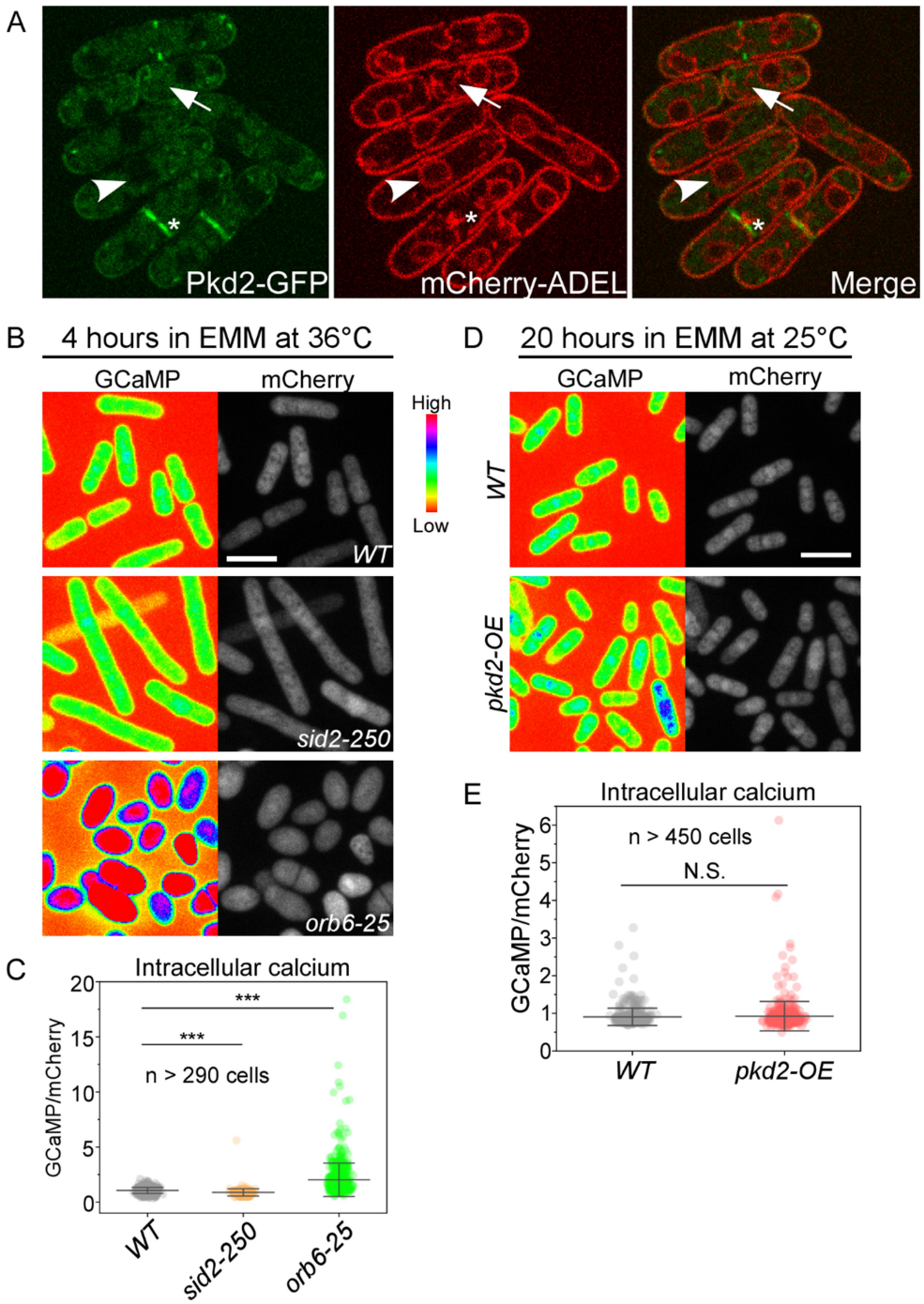
Supplemental Figure 2: Application of a fractionation assay for reconstitution of Pkd2 into SUPER templates. **(A)** Schematics illustrating the fractionation assay. Cell-free expressed membrane proteins, including Pkd2, were translocated into microsomes after three hours of incubation. The CFE reaction was then centrifuged by using an airfuge three times. Membrane proteins incorporated into microsomes become concentrated as pellets while the remaining CFE reaction remains as supernatant. 10 nM green lysine was added to the CFE reaction before incubation to label Pkd2 for observation. **(B)** Fluorescent gel image of pellet and supernatant fractions of a CFE reaction. S1-S3 represent the supernatant with increasing number of washing cycles using an airfuge. **(C)** Representative confocal images of pellet fractions incorporating into SUPER templates using the same approach mentioned in Fig. 1A. Pkd2 did not localize in the supernatant fraction of SUPER templates. Scale bar: 5 µm.



Supplemental Figure 3. Application of a pronase digestion assay to determine the orientation of CFE-synthesized Pkd2 in SUPER templates. (A) Illustration of the putative topology of Pkd2 channel with sfGFP fused to its C-terminus. **(B)** Schematic illustrating the principle of using pronase digestion to determine the orientation of inserted cell-free expressed Pkd2 into SUPER templates. If Pkd2 proteins were oriented with their N-termini exposed to the solution, sfGFP would be protected from degradation (i). On the other hand, if Pkd2 proteins were oriented with their C-termini protruding away from the lipid-coated bead, then sfGFP would be degraded by pronase (ii). **(C)** Confocal fluorescence images of SUPER templates (red) incorporating cell-free expressed Pkd2-sfGFP (green) with and without the addition of pronase. Scale bar: 5 µm.

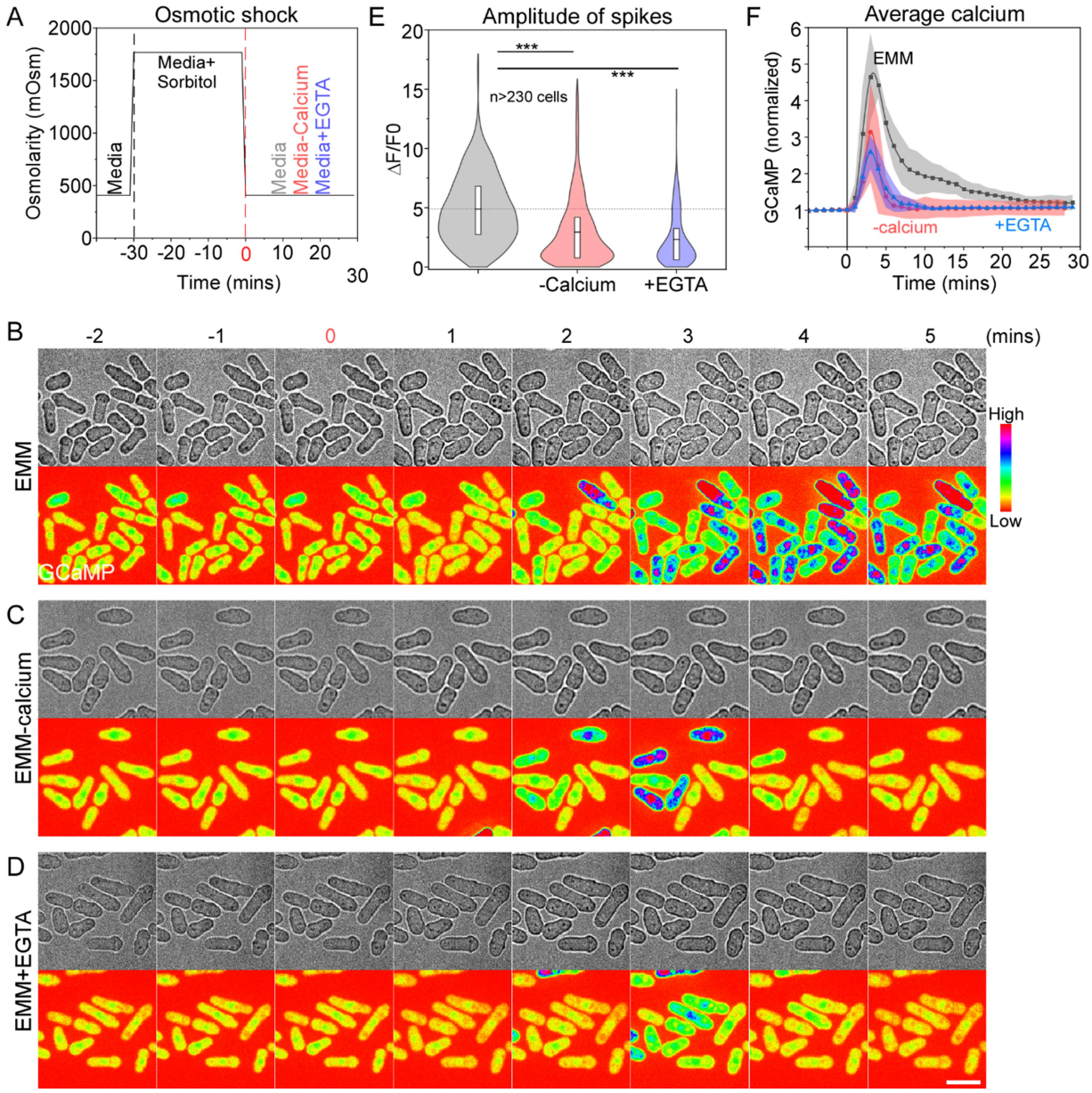


Supplemental Figure 4: Pkd2 mediates calcium influx in response to osmotic pressure in GUVs. (A) Schematic illustrating the function of G-GECO in a Pkd2-expressing GUV. **(B)** Representative confocal fluorescence images of GUVs encapsulating G-GECO in a hypo-osmotic solution with different external calcium concentrations as indicated. Plasmid concentration of G-GECO was fixed at 1 nM. GUVs were subjected to a hypo-osmotic medium after encapsulation following 3 hours of incubation. The osmolarity difference between internal CFE reactions and external hypo-osmotic solutions was 100 mOsm. Scale bars: 20 µm.



Supplemental Figure 5: Intracellular calcium level of other fission yeast mutants besides the *pkd2* mutants. (A) Pkd2 primarily localizes to the plasma membrane. Fluorescence micrograph of the cells expressing both Pkd2-GFP (green) and the ER marker mCherry-ADEL (red). Only the center slice of the Z-series is shown. Asterisk: Equatorial plane localization, Arrowhead: Perinuclear ER, Arrow: Membrane tethers. **(B-C)** The intracellular calcium level of two other temperature-sensitive mutants: *sid2-250* and *orb6-25*. **(B)** Fluorescence micrograph of *wild-type (WT), sid2-250,* and *orb6-25* cells expressing both GCaMP-mCherry at the restrictive temperature. Cells were imaged after incubation at 36°C for 4 hours (restrictive). **(C)** Scatter

interval plot of the intracellular calcium level. **(D-E)** Calcium levels of *pkd2* overexpression cells *(pkd2-OE)*. **(D)** Fluorescence micrograph of *WT* and *pkd2-OE* cells expressing both GCaMP-mCherry after 20 hours of induction to over-express *pkd2*. **(E)** Scatter interval plot of intracellular calcium levels. ***: $P < 0.001$. Two-tailed student t-tests with unequal variants were used. Scale bars: 10 μm.



Supplemental Figure 6: Hypo-osmotic stress-induced calcium spikes were reduced by removal of external calcium. (A) Time course of osmolarity change of extracellular environment during the experiments. Time zero: application of hypoosmotic shock. **(B-D)** Time lapse micrographs of *wild type* cells expressing GCaMP stimulated with hypo-osmotic shock in a microfluidics chamber. Time zero: replacement of EMM plus 1.2M sorbitol with either EMM **(B),** EMM minus calcium **(C)**, or EMM plus 2mM EGTA **(D)**. The data is pooled from two biological repeats (n > 230). **(E)** Violin plots of peak amplitude of calcium spikes in the cells treated with hypo-osmotic shock. **(F)** Time courses of normalized GCaMP fluorescence in *wild type* cells in EMM (control), EMM minus calcium (-calcium), or EMM plus EGTA (+EGTA) following the hypo-osmotic shock. ***: $P < 0.0001$. Two-tailed student t-tests with unequal variants were used. Scale bar: 10 µm.

Table S1: 2X homemade Gibson master mix for 20 reactions. The mixture is split in 5 µl aliquots for each reaction and should be froze immediately with liquid nitrogen. The reactions can be stored at -80°C for up to 3 months.

5X isothermal reaction buffer	40 µl
10U/µl T5 exonuclease	0.1µl
2U/µl Phusion polymerase	2.5 µl
40U/µl Taq DNA ligase	20 µl
UltraPure DNase/RNase-Free Distilled Water	37.4µl
Total	100 µl

Table S2. List of fission yeast strains

Strains	**Genotype**	**Source**
QC-Y941	*h- leu1::kanMX6-Padh1-GCaMP6s-TAdh1 ura4-D18 his3-D1 ade6-M216*	Lab Stock
QC-Y949	*h- kanMX6-81xnmt1-pkd2 leu1::kanMX6-Padh1-GCaMP6s-TAdh1 ura4-D18 his3-D1 ade6-M216*	This study
QC-Y1004	*h+ rlc1-tdTomato-natMX6 leu1::kanMX6-Padh1-GCaMP6s-TAdh1 ura4-D18 ade6-M216*	Lab Stock
QC-Y1027	*h? kanMX6-81xnmt1-pkd2 rlc1-tdTomato-natMX6 leu1::kanMX6-Padh1-GCaMP6s-TAdh1 ura4-D18 his3-D1 ade6-M216*	This study
QC-Y1064	*h- leu1::kanMX6-Padh1-GCaMP6s-mCherry-ura4+ his3-D1 ade6-M216*	Lab Stock
QC-Y1182	*h? pkd2::pkd2-B42-ura4+-his5+ leu1:: kanMX6-Padh1-GCaMP6s-Tadh1 his3-D1 ade6-M216*	This study
QC-Y1343	*h? leu1::kanMX6-Padh1-GCaMP6s-mCherry-ura4+ kanMX6-81xnmt1-pkd2 his3-D1*	This study
QC-Y1365	*h? pkd2::pkd2-B42-ura4+-his5+ leu1::kanMX6-Padh1-GCaMP6s-mCherry-ura4+ his3-D1*	This study
QC-Y1425	*h? sid2-250 ura4-D18 ade-M21X leu1-32 leu1::kanMX6-Padh1-GCaMP6s-mCherry-ura4+ his3-D1 ade6-M216*	This study
QC-Y1426	*h? orb6-25 ade-M21X leu1::kanMX6-Padh1-GCaMP6s-mCherry-ura4+ his3-D1*	This study
QC-Y1436	*h? pkd2::pkd2-B42-ura4+-his5+ rlc1-tdTomato-NatMX6 leu1::kanMX6-Padh1-GCaMP6s-TAdh1 ade6-M216*	This study
QC-Y1457	*h? kanMX6-P3nmt1-pkd2 leu1::kanMX6-Padh1-GCaMP6s-mCherry-ura4+ his3-D1 ade6-M216*	This study
QC-Y1586	*h? leu1::Pbip1-mCherry-ADEL leu1+ his+ ade6-M216 ura4-D18*	This study