Functionally conserved Pkd2, mutated in autosomal dominant polycystic kidney disease, localizes to the endoplasmic reticulum and regulates cytoplasmic calcium homeostasis in fission yeast

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Abstract

Mutations in *PKD1* or *PKD2* genes lead to autosomal dominant polycystic kidney disease (ADPKD) that is the most frequent family inherited renal disorder. These genes encode polycystin-1/PC-1 and polycycstin-2/PC-2, respectively. Although the genetic basis of ADPKD is well established, the crucial functions of polycystins underlying onset and development of cyst formation remain elusive. Fission yeast *Schizosaccharomyces pombe* has a single polycystin homolog, Pkd2, which is essential for cell growth. In this study, the truncation analyses of Pkd2 reveal that Pkd2 localizes to not only the plasma membrane but also the endoplasmic reticulum (ER) and regulates cytoplasmic calcium signaling in fission yeast. Internal transmembrane domains within Pkd2 are sufficient for these processes. Surprisingly, more than half of Pkd2 is not required for cell viability. Cytoplasmic calcium levels are mainly regulated through C-terminus of Pkd2. Importantly, human Pkd2 also localizes to the ER and furthermore, fully complements the loss of fission yeast Pkd2. As the functions of polycystin-2 are conserved, fission yeast provides a suitable model to study the mechanism of ADPKD as well as polycystins.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most frequent genetic inherited renal diseases in the world, with an estimated prevalence of one in 1,000 individuals (Lanktree et al., 2018). The disease responsible genes, *PKD1* and *PKD2* which encode polycystin-1 (PC-1) and polycystin-2 (PC-2) respectively, have already been identified (Hughes et al., 1995; Mochizuki et al., 1996). PC-1 consists of a large N-terminal extracellular domains, 11 transmembrane domains and a C-terminal cytoplasmic region (Ong and Harris, 2015). PC-2, a member of the transient receptor potential (TRP) superfamily, has six transmembrane regions and a C-terminal cytoplasmic region. The C-terminus contains an EF-hand motif, a coiled-coil domain, and an endoplasmic reticulum (ER) retention motif (Brill and Ehrlich, 2020; Cai et al., 1999; Vien et al., 2020), whereas the N-terminus possess cilia localization sequence (Geng et al., 2006). It has been suggested that PC-1 and PC-2 function in the same pathway, as mutations in either gene cause the similar cyst phenotypes (Harris and Torres, 2014). In agreement with this, initial studies suggest that PC-2 interacts with PC-1 via each C-terminus and localizes to cilia in a PC-1 dependent manner (Grieben et al., 2017; Hanaoka et al., 2000; Ong and Harris, 2015). The mechanism by which loss of ciliary PC-1 or PC-2 function results in cyst formation through decreasing in cytoplasmic calcium levels and increase in cAMP is thought to be central to ADPKD pathology for a long time (Cornec-Le Gall et al., 2019; Torres and Harris, 2014). In addition,

58 structural analysis revealed that human PC-1 and PC-2 form a hetero-tetramer in 1:3 ratio (Su et
59 al., 2018). However, it was also reported that PC-2 predominantly localized to the ER, forming a
60 homo tetramer, acting as a cation channel independent of PC-1 (Cai et al., 1999; Liu et al., 2018;
61 Shen et al., 2016). In addition, recent study suggested that ER localized PC-2 contributes to
62 calcium release and anti-cystogenesis (Padhy et al., 2022). Thus, ADPKD pathology associated
63 functions of polycystins remain to be established.

64 Fission yeast *Schizosaccharomyces pombe*, a unicellular model organism, has Pkd2 which is
65 essential for cell viability, as a solo homolog of polycystins. Pkd2 consists of the N-terminal
66 extracellular region, the nine transmembrane domains and the C-terminal cytoplasmic region. The
67 roles of each region, however, have not been determined yet. Pkd2 reportedly localizes to the
68 plasma membrane and cell division site (septum in fungi) (Morris et al., 2019). Mutations in *pkd2*
69 cause cell separation defect and fails to maintain cellular integrity (Morris et al., 2019; Sinha et
70 al., 2022). Furthermore, overexpression of *pkd2* causes growth arrest and increase of cytoplasmic
71 calcium levels (Ma et al., 2011; Palmer et al., 2005).

72 Here, we carried out the truncation analyses of fission yeast Pkd2 and revisited its cellular
73 localization. We show that Pkd2 localizes to the ER rather than the septum site and regulates the
74 cytoplasmic calcium homeostasis. More than a half of N-terminus is not required for cell viability.
75 C-terminus, including the latter four transmembrane regions has a central role in the regulation of

cytoplasmic calcium homeostasis. Interestingly, human *PKD2* fully complements the loss of *pkd2* in fission yeast.

Results and Discussion

1. The transmembrane region of Pkd2 is required for cell survival and cytoplasmic calcium homeostasis

Previous reports suggested that overexpression of *pkd2* results in cell death accompanied with increased calcium levels in the cytoplasm (Ma et al., 2011; Palmer et al., 2005). In order to analyze the requirement of each region of Pkd2 for the growth and calcium signaling, we constructed and overexpressed a series of truncations in fission yeast cells (Fig. 1A). The CDRE (Calcineurin Dependent Response Element) reporter system was used to estimate the cytoplasmic calcium level (Kume et al., 2017; Kume et al., 2011). As expected, the CDRE-dependent transcription was increased by adding $CaCl_2$ in a Prz1-dependent manner (Fig. 1B). Neither deletion of N- nor C-terminus of Pkd2 affected the growth and CDRE activation (Fig. 1C, D). N- or C-terminus of Pkd2 alone did not affect either. Interestingly, the internal transmembrane region was sufficient to induce both growth inhibition and CDRE activation (Fig. 1C, D). These data suggest that the transmembrane region plays a critical role for Pkd2, while either N- or C-terminal region is dispensable.

To classify the roles of the transmembrane domains, we divided Pkd2 into Pkd2$^{1\text{-}424}$, which includes the first five transmembrane domains and Pkd2$^{464\text{-}710}$, which includes the latter four transmembrane domains. Overexpression of either construct inhibited the growth like full length (Fig. 1C), indicating that each of them is capable of inducing cytotoxicity upon overexpression. With regards to calcium signaling, the overexpression of *pkd2*$^{1\text{-}424}$ failed to increase cytoplasmic calcium levels, whereas the overexpression of *pkd2*$^{464\text{-}710}$ showed noticeable increase, comparable to full length (Fig. 1D). These data suggest that each of the first five or the latter four transmembrane regions has an independent role for the growth, and that C-terminus including the latter four transmembrane regions regulates the cytoplasmic calcium homeostasis.

2. Pkd2 localizes to the ER and the internal transmembrane region is sufficient for its localization

Previous work showed that Pkd2 localizes to the plasma membrane, concentrated at the cell tip and the medial cell division site/septum (Morris et al., 2019). This localization pattern was obtained using C-terminally tagged GFP (Pkd2-GFP). We checked the localization of full-length Pkd2 and its truncations that contained N-terminally tagged GFP (GFP-Pkd2) from the plasmid in the cells. Intriguingly, full length of Pkd2 (GFP-Pkd2) colocalized with Pmr1, a marker for the ER (Nakazawa et al., 2019), indicating that Pkd2 may localize to the ER (Fig. 2A). Localization

of Pkd2ΔN170, Pkd2ΔC577, or Pkd2TM (Fig. 2B) was identical to full length Pkd2 (Figs 2B, S1A); no signals were evident at the septum. N-terminus of Pkd2 (Pkd2N) localized to the cytoplasm and weakly to the nuclear periphery, whereas C-terminus of Pkd2 (Pkd2C) displayed a uniform cytoplasmic pattern (Fig. 2B, S1A). These data suggest that the transmembrane region is sufficient for ER localization. We noted that although GFP-Pkd2 and various truncations were expressed from plasmids under the *nmt41* prompter (Fig. S1B), this level of expression did not compromise the growth even under the inducible condition (Fig. S1C).

To decipher whether Pkd2's ER localization is due to overexpression, we integrated a single copy of *GFP-pkd2* or *pkd2-GFP* under the endogenous promoter in the *pkd2* deleted background. As previously reported (Morris et al., 2019), C-terminally tagged Pkd2 (Pkd2-GFP) localized to the septum and the plasma membrane, especially enriched at cell tips (Fig. 2C). It is noted that Pkd2-GFP signals were also detected as numerous cytoplasmic dots (Fig. 2C, arrowheads). On the other hand, N-terminally tagged Pkd2 (GFP-Pkd2) localized to the ER, like a plasmid-derived expression (Fig. 2A, C). The protein expression of both GFP-Pkd2 and Pkd2-GFP was examined by immunoblotting analysis (Fig. 2D). GFP-Pkd2 showed only a single band around the expected full-length size, whereas Pkd2-GFP expressing cells showed two prominent bands: expected full-length size (~110 kDa) and smaller size (~75 kDa). Overall, GFP tagging of Pkd2 to its C-terminus resulted in different localization patterns and susceptibility to proteolytic cleavage. Given the

results of GFP-Pkd2 that did not lead to the emergence of proteolysis or cytoplasmic dots, we posit that fission yeast Pkd2 localizes to the ER via the transmembrane regions.

Why C-terminally-GFP tagged Pkd2 (Pkd2-GFP) localizes to the plasma membrane is currently unknown. It is possible that C-terminal tagging affects the Pkd2 expression level and the localization, as the different sized band was included in the extract from Pkd2-GFP expressing cells. Polycycstin-2 levels are regulated by microRNAs (miRNAs) and contribute to cyst formation (Lee et al., 2019). Recent study suggests that the deletion of miR-17 binding sites within the 3'UTR increased expression levels of polycystin-2 and attenuated the cyst formation in mouse ADPKD model (Lakhia et al., 2022). In our C-terminal tagging constructs, its own 3'UTR regions are inactivated. It is interesting whether 3'UTR region of fission yeast Pkd2 also contributes to their expression and miRNA cis-inhibition system is evolutionary conserved.

3. The C-terminal region of Pkd2 plays a central role in cytoplasmic calcium regulation

We then constructed several truncations within GFP-Pkd2 that were expressed from its own promoter in the absence of endogenous *pkd2* (Fig. 3A). N-terminal deletions up to 170 amino acid residues did not affect either viability or ER localization (Fig. 3B). Remarkably, even a further truncation construct, GFP-Pkd2ΔN424 expressing cells, in which the first five transmembrane regions are deleted, was viable and localized to the ER like full length GFP-Pkd2 (Fig. 3B).

148 Although Pkd2 has a signal sequence in N-terminus (Morris et al., 2019), the N-terminal deletions
149 did not affect to the ER localization, suggesting that Pkd2 localizes to the ER via multiple signals.
150 On the other hand, a C-terminal deletion, GFP-Pkd2ΔC577 was viable, but displayed noticeable
151 defects: localization to the cortical ER was weakened and cells showed abnormal morphologies
152 including multiseptation (Fig. 3B). The protein levels were examined by immunoblotting analyses,
153 and we found that the band signals of GFP-Pkd2ΔN170 and GFP-Pkd2ΔC577 were somewhat
154 weakened (Figure 3C). In addition, the C-terminal deletion strain was hypersensitive to $CaCl_2$
155 (Figure 3D), indicating that the C-terminal region is required for cytoplasmic calcium
156 homeostasis. Taking these data together, we concluded that each of the first five and the latter four
157 transmembrane regions has a mutually complementing activity for cell viability and that C-
158 terminus plays a major role in ER localization and calcium homeostasis. Other groups studies also
159 suggest that fission yeast Pkd2 appears to regulate the cytoplasmic calcium levels (Ma et al.,
160 2011). We envisage that the C-terminal region including the latter four transmembrane domains
161 has a crucial role in calcium regulation, as the deletion of the C-terminal region caused more
162 severe defects and C-terminus overexpression was sufficient to increase the cytoplasmic calcium
163 levels. N-terminus is not required for the cell viability, however, the overexpression of N-terminus
164 leads to the cell death as well as C-terminus. It appears that Pkd2 has two independent functions,
165 but somehow compensates with each other. Further analysis, such as the identification of the

166 molecules which genetically and/or physically interact with Pkd2 will uncover the importance of
167 each region.
168
169 **4. Human *PKD2* complements fission yeast *pkd2***
170 To examine functional complementation of Pkd2 between humans and fission yeast, we
171 expressed human *PKD2* (*hpkd2*) in the absence of fission yeast Pkd2. Cells expressing GFP-
172 hPkd2 or hPkd2-GFP under the *pkd2* promoter in Δ*pkd2* background were viable even under the
173 high $CaCl_2$ condition (Fig. 4A), suggesting that human *PKD2* can effectively complement the loss
174 of fission yeast *pkd2*. Both GFP-hPkd2 and hPkd2-GFP localized to the ER (Fig. 4B), and the
175 expected sized proteins (~140 kDa) were expressed in fission yeast (Fig. 4C), indicating that the
176 functional proteins were produced. We noted that the existence of cleavage products (~75 kDa)
177 in hPkd2-GFP-expressing cells, which is similar to that produced from fission yeast Pkd2-GFP,
178 though its amount was substantially lower. We conclude that *PKD2* genes are evolutionary
179 conserved between humans and fission yeast.
180 We next overexpressed *hpkd2* in fission yeast. Overexpression of *hpkd2* also activated
181 cytoplasmic calcium and caused growth arrest in fission yeast (Fig. 4D, E); therefore, human Pkd2
182 recapitulated the physiological impact caused by fission yeast Pkd2. These data reinforce the
183 notion that human polycystin-2 is functional in fission yeast and has similar functions to Pkd2.

184 The level of calcineurin activation, however, was not as much as that of fission yeast *pkd2*
185 overexpression (Figure 4E), indicating that hPkd2 might not fully substitute for cellular
186 physiologies derived from fission yeast Pkd2.

187 Recent work suggests that polycystin-2 is a non-selective cation channel and involved in
188 permeability of other monovalent cations (Liu et al., 2018; Shen et al., 2016). Consistent with this,
189 the effect of *hpkd2* overexpression on cytoplasmic calcium levels was limited in this study.
190 Interestingly, the overexpression effect of N-terminal region ($pkd2^{1\text{-}424}$) on calcium homeostasis
191 is also minor. One possibility is that the N-terminal region is conserved in human and fission yeast,
192 and C-terminus of fission yeast Pkd2 is an additional, divergent region. Indeed, human Pkd2
193 consists of six transmembrane domains, whereas fission yeast has nine. Furthermore, human Pkd2
194 have a large extracellular polycystin domain (~200 amino acid long; also referred TOP domain),
195 in which ADPKD pathogenic gene mutations are accumulated, and it contributes to channel
196 assembly and function (Douguet et al., 2019; Grieben et al., 2017; Shen et al., 2016; Wilkes et al.,
197 2017). This polycystin domain exists like a physical 'lid' of the channel (Douguet et al., 2019;
198 Shen et al., 2016). Fission yeast Pkd2 has a large extracellular region in N-terminus (1-170 amino
199 acid). So far, what N-terminus does in the context of cellular physiology is unclear, but at least
200 the overexpression causes cell death. Conservation of the functional and structural polycystin
201 domain is of great interest and its roles should be investigated in the future.

Nonetheless, human *PKD2* complements the loss of *pkd2* in fission yeast and its gene protein like that of fission yeast localizes to the ER; hPkd2 appears to be functionally conserved. Thus, our data provide a potential that fission yeast is an excellent model organism in which to study the molecular basis of ADPKD as well as the cellular function(s) of Pkd2.

Materials and Methods

Yeast general method

Standard media and methods for fission yeast were used. C-terminal tagging and gene deletion were carried out with the PCR-based method using homologous recombination (Bahler et al., 1998; Sato et al., 2005). The full length of *pkd2* gene or *pkd2-GFP-hphMX*, including 5'UTR region (324 bases) and 3'UTR region (569 bases) were amplified from the genomic DNA of wild type or *pkd2-GFP-hphMX* strain, respectively. The genomic DNA were prepared with GenTLE (Takara Bio, Japan). The fragments were subcloned into the integrated plasmid pJK148 carrying the *leu1*$^{+}$ gene, the resulting plasmids were linearized by *Nru*I within the *leu1*$^{+}$ gene and integrated to the *leu1-32* locus. For truncations and N-terminal GFP tagged strains construction, the fragments were amplified with PrimeSTAR MAX (Takara Bio, Japan, R045) with the appropriate primer pairs having overlapping sequence. The purified fragments were integrated into digested vectors by using In-fusion Snap Assembly Master mix (Takara Bio, Japan, 638947). Our lab stock plasmids, pREP1, pREP41-GFP(N), and pJK148 were used as the vector. Human

221 *PKD2* cDNA was purchased (Horizon Discovery, MHS6278-211688893). Multiple tagging
222 strains were constructed by random spore. Strains used in this study were listed in supplementary
223 table S1. Strains were grown in rich YE5S media and incubated at 27 °C unless otherwise stated.
224 PMG or EMM media (Sunrise Science Products, TN, U.S.A., 2060 or 2005) were used to induce
225 overexpression. Appropriate supplements (leucine, lysine, histidine, uracil, and adenine) were
226 added at concentration of 50 mg/L each if required.
227
228 **Microscope**
229 Fluorescent microscope images were obtained by the Olympus IX83 inverted microscope
230 system with UPLXAPO 60x objective lens (NA 1.42, immersion oil) and a DP80 digital camera.
231 Cells were collected by the centrifuge at 5,000rpm for 1 min, and spotted onto glass slide. The
232 cells were observed immediately after coverslip. Images were processed and analyzed by using
233 CellSens Dimension (OLYMPUS, Japan) and Adobe PhotoShop.
234
235 **Western blotting**
236 Whole cell extracts were prepared according to the alkaline method (Matsuo et al., 2006).
237 10-20 ml of cell cultures (OD_{600}: 0.4-0.7) were centrifuge at 3,500 rpm for 1 min. The cells were
238 washed with 1 ml of distilled water, centrifuged for 1 min. After resuspending in 0.3 M NaOH,

239 the cells were kept at room temperature for 10 min with shaking. Discarding the supernatant,
240 sample buffer (60 mM Tris-HCl (pH6.8), 4% β-Mercaptoethanol, 4% SDS, 0.01% BPB, 5%
241 glycerol) was added, and the samples stayed at room temperature for at least 1 h. The samples
242 were separated by 10% of SDS-PAGE gel (Bio-rad, CA, U.S.A., 4561035) and transfer to
243 polyvinylidene difluoride membrane. The membranes were blocked with 5 % of skimmed milk
244 in TBS-tween20 (TBST) for 30 min at room temperature, subsequently incubated with primary
245 antibodies diluted in TBST at 4ºC for overnight. Anti-GFP (MBL, Japan, 598) and Anti-GFP
246 (Roche, U.S.A., 11814460001) were used for CDRE-GFP, GFP tagged Pkd2, respectively. After
247 washing, the membranes were incubated with appropriate secondary antibodies at room
248 temperature for 60 min; anti-Rabbit (Thermo Fisher Scientific, G-21234), anti-Mouse (Thermo
249 fisher Scientific, G-21040). Then the membranes were incubated with Clarity (Bio-Rad, 1705061)
250 or Clarity Max (Bio-Rad, 1705062) western ECL substrate. For the control, the membranes were
251 re-incubated with anti-Cdc2 (SantaCruz Biotechnology, Texas, U.S.A., SC-53217) in TBST with
252 0.1% of sodium azide at room temperature for 3 h. Amersham Image Quant 800 (Cytiva, Japan)
253 was used for detection of chemiluminescence. The band intensities were measured by ImageJ.
254 The CDRE-GFP intensities were normalized by dividing by Cdc2 intensities. The bar graphs
255 showed the average of three experiments, with exception of Figure 1B. Error bars indicated
256 standard deviation (S.D.).

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Competing interests

The authors declare no competing interests.

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Figure legends

Figure 1 Transmembrane region of Pkd2 is required for the functions

(A) Schematic structures of Pkd2 truncations. Dark blue boxes indicate transmembrane (TM) region. (B) Calcineurin activity determined by CDRE-GFP reporter system. Indicated strains were cultured in YE5S at 27ºC with or without 0.1 M $CaCl_2$ for 2 h. Calcineurin activity was estimated by immunoblotting with anti-GFP antibodies. (C) Spot tests. Indicated strains were serially diluted and spotted onto PMG with or without thiamine. The plates were kept at 27ºC for 4 days. (D) Calcineurin activity determined by CDRE-GFP reporter system. Indicated strains were cultured in PMG without thiamine for 18 h to induce overexpression. Calcineurin activity was estimated by immunoblotting with anti-GFP antibodies.

Figure 2 N-terminally tagged Pkd2 localizes to the ER

(A, B) Localization of GFP-Pkd2 (A) or GFP-Pkd2 truncations (B) expressed from plasmids in Pmr1-tdTomato expressing cells. For truncations, only GFP images were shown. Pmr1-tdTomato and merged images were shown in supplementary Figure S1A. Bars; 10 µm. (C) Localization of Pkd2. Pkd2-GFP or GFP-Pkd2 expressed from its own promoter in *Δpkd2* background. (D) Whole cell extracts were prepared from the indicated strains and immunoblotting carried out with anti-GFP and anti-Cdc2 (as a control) antibodies. The positions of size markers are shown on the right.

Figure 3 The C-terminus of Pkd2 is required for the viability and cytoplasmic calcium homeostasis

(A) Schematics of truncations. Gray-dashed lines indicated deleted regions. (B) Localization of GFP-Pkd2 truncations expressed from its own promoter in *Δpkd2* background. Bar; 10 µm. (C) Whole cell extracts were prepared from the indicated strains and immunoblotting carried out with anti-GFP and anti-Cdc2 (as a control) antibodies. Arrowheads indicate the position of the expected bands. The positions of size markers are shown on the right. (D) Spot tests. Serially diluted strains were spotted onto YE5S with or without 0.2 M $CaCl_2$ and incubated at 27ºC for 3days.

Figure 4 Human *PKD2* complements fission yeast *pkd2*

(A) Spot tests. Serially diluted strains were spotted onto YE5S with or without 0.2 M $CaCl_2$ and incubated at 27ºC for 3days. (B) Localization of hPkd2-GFP or GFP-hPkd2 expressed from *pkd2* promoter in *Δpkd2* cells. Bar; 10 µm. (C) Protein levels of hPkd2. Whole cell extracts were prepared from the indicated strains and immunoblotting carried out with anti-GFP and anti-Cdc2

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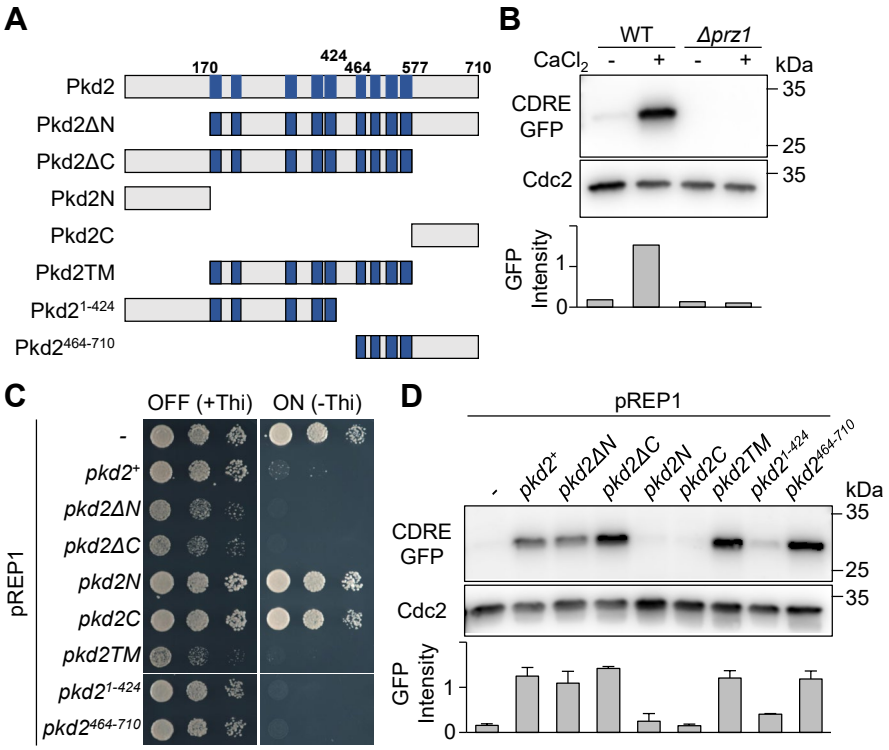

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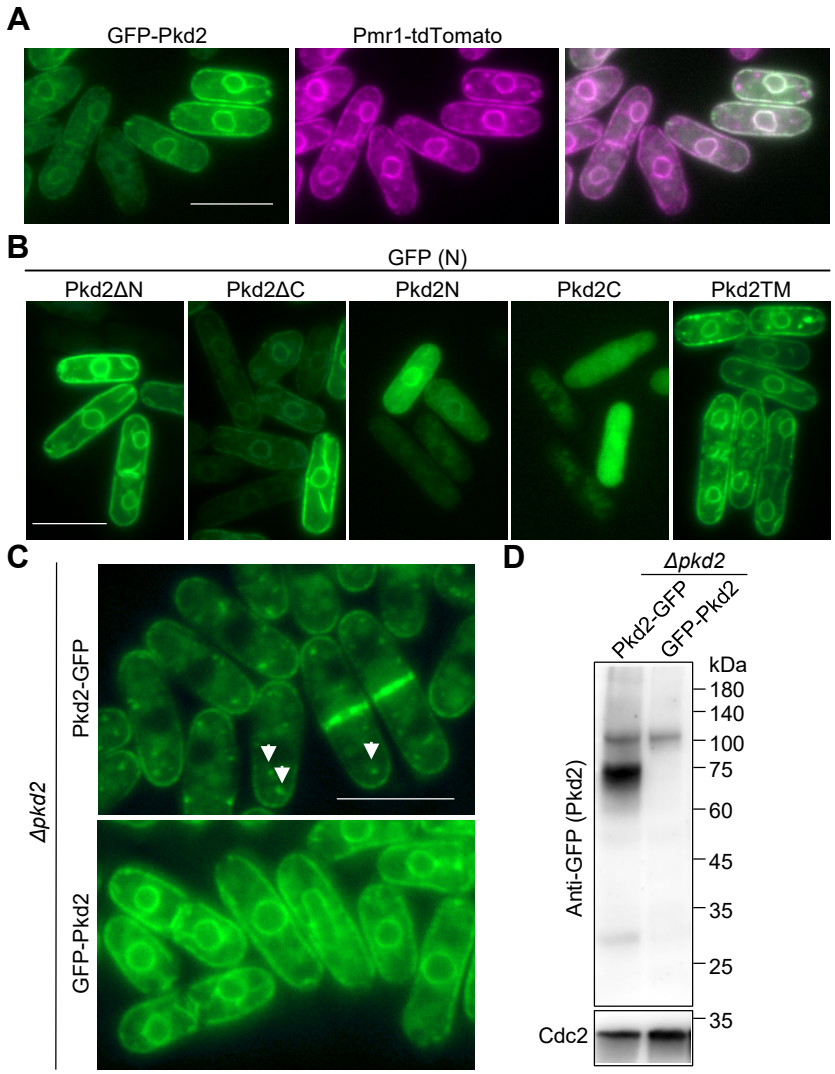

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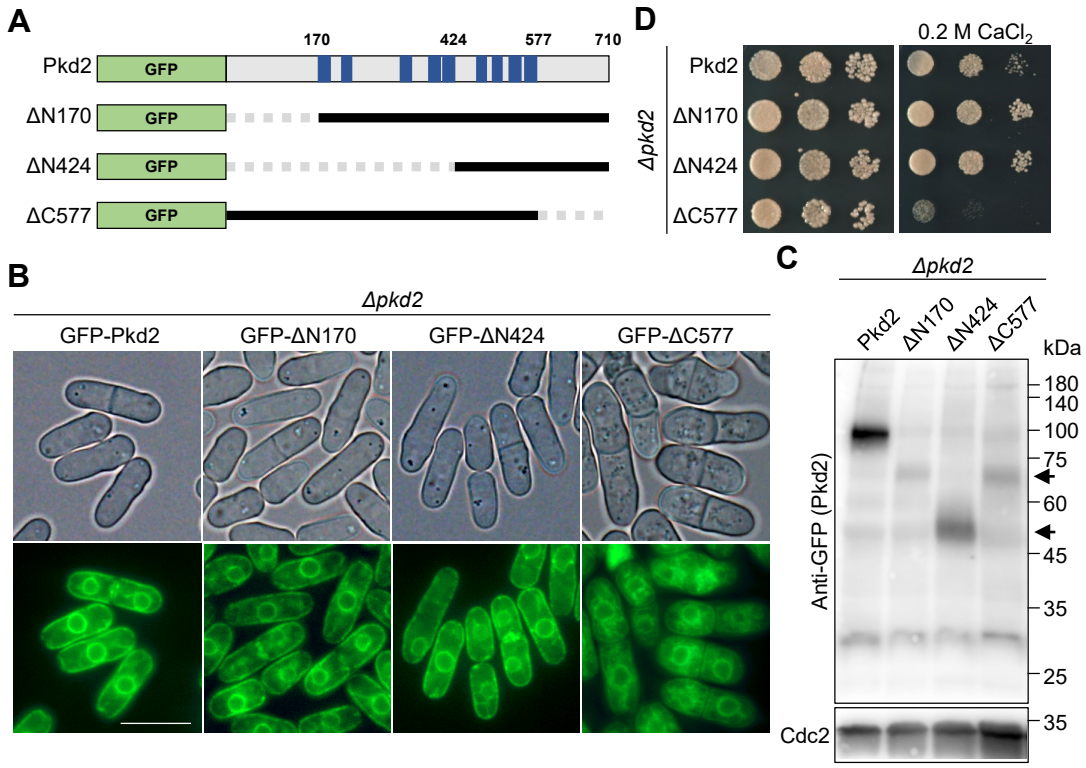

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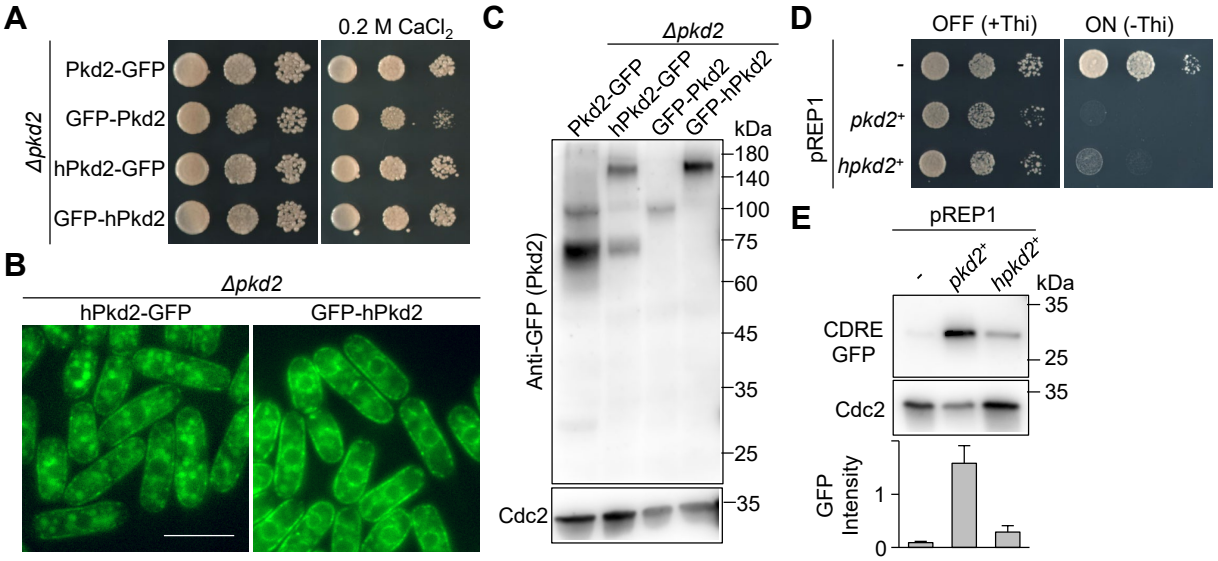

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Supplementary data

Functionally conserved Pkd2, mutated in autosomal dominant polycystic kidney disease, localizes to the endoplasmic reticulum and regulates cytoplasmic calcium homeostasis in fission yeast

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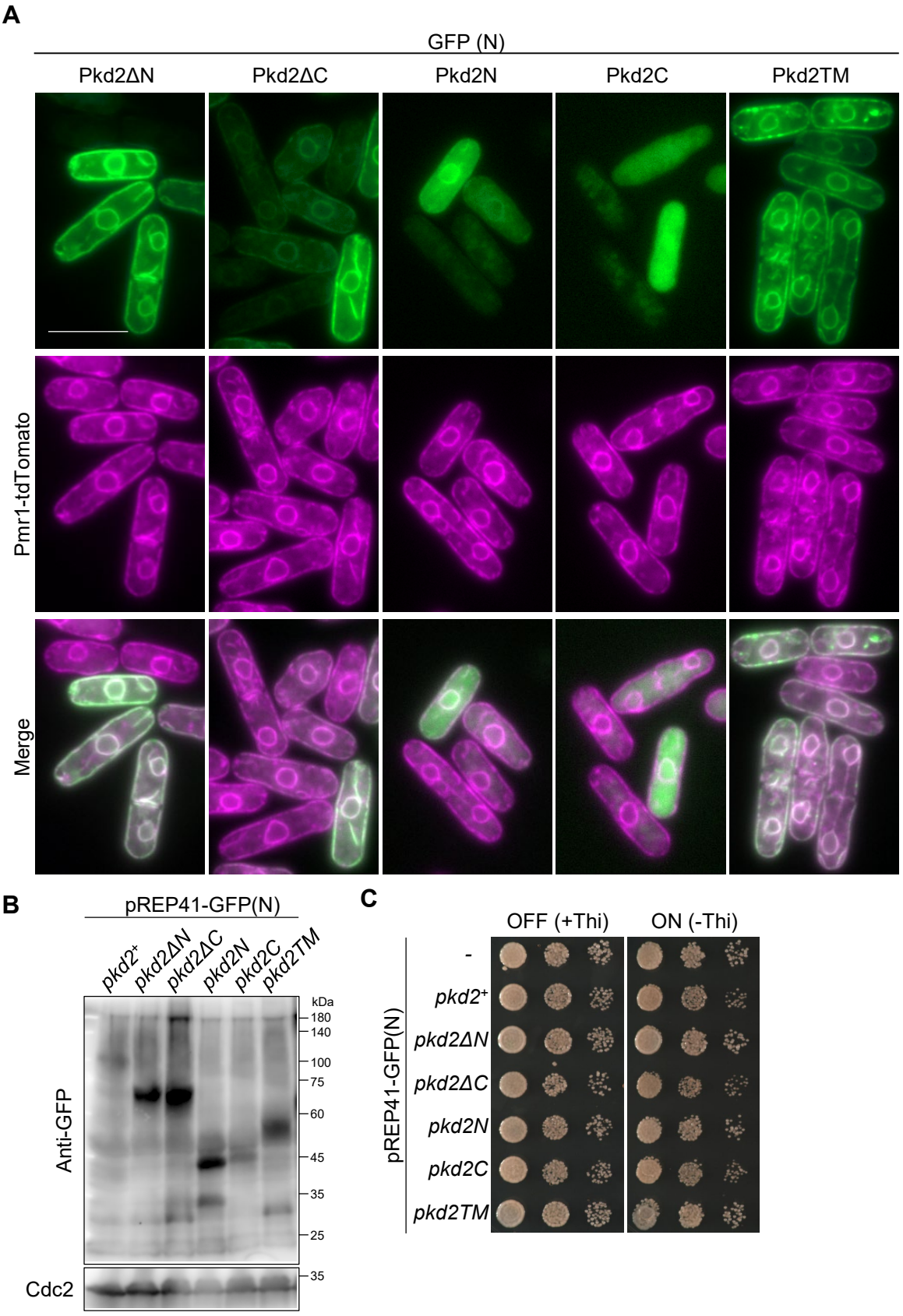
Supplementary table S1: Strains in this study

Supplementary figure S1: N-terminally GFP tagged Pkd2 expressing from plasmids

Supplementary table S1

	Genotype
TK1042-1	*h- ura4-294::CDRE:GFP leu1-32*
TK1230-1	*h- Δprz1::kanMX ura4-294::CDRE:GFP leu1-32*
TK1223	*h- ura4-294::CDRE:GFP leu1-32/pREP1 (Transformat)*
TK1224	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2 (Transformat)*
TK1242	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2ΔN170 (Transformat)*
TK1255	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2Δ577 (Transformat)*
TK1265	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2N (Transformat)*
TK1286	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2C (Transformat)*
TK1266	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2TM (Transformat)*
TK1445	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2$^{1-424}$ (Transformat)*
TK1446	*h- ura4-294::CDRE:GFP leu1-32/pREP1-pkd2$^{465-710}$ (Transformat)*
TK1225	*h- ura4-294::CDRE:GFP leu1-32/pREP1-hpkd2 (Transformat)*
TK1396	*h- pmr1^{+}:tdTomato:natMX leu1-32/pREP41-GFP(N)-pkd2 ura4-D18 (Transformat)*
TK1397	*h- pmr1^{+}:tdTomato:natMX leu1-32/pREP41-GFP(N)-pkd2ΔC577 ura4-D18 (Transformat)*
TK1398	*h- pmr1^{+}:tdTomato:natMX leu1-32/pREP41-GFP(N)-pkd2ΔN170 ura4-D18 (Transformat)*
TK1399	*h- pmr1^{+}:tdTomato:natMX leu1-32/pREP41-GFP(N)-pkd2N ura4-D18 (Transformat)*
TK1400	*h- pmr1^{+}:tdTomato:natMX leu1-32/pREP41-GFP(N)-pkd2TM ura4-D18 (Transformat)*
TK1390	*h- pmr1^{+}:tdTomato:natMX leu1-32/pREP41-GFP(N)-pkd2C ura4-D18 (Transformat)*
TK1129-1	*h- Δpkd2::kanMX leu1-32::pkd2^{+}:GFP:hphMX (integrated)*
TK1323-1	*h- Δpkd2::kanMX leu1-32::P_{pkd2}-GFP-pkd2^{+}-T_{pkd2} (integrated)*
TK1408-1	*h+ Δpkd2::kanMX leu1-32::P_{pkd2}-GFP-pkd2ΔN170-T_{pkd2} (integrated) his2*
TK1388-1	*h- Δpkd2::kanMX leu1-32::P_{pkd2}-GFP-pkd2ΔN424-T_{pkd2} (integrated) ura4-D18*
TK1455-1	*h- Δpkd2::kanMX leu1-32::P_{pkd2}-GFP-pkd2Δ577-T_{pkd2} (integrated)*
TK1375-1	*h- Δpkd2::kanMX leu1-32::hpkd2^{+}:GFP:hphMX (integrated)*
TK1401-1	*h- Δpkd2::kanMX leu1-32::P_{pkd2}-GFP-hpkd2^{+}-T_{pkd2} (integrated)*



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(A) Localization of GFP-Pkd2 or GFP- Pkd2 truncations. Identical GFP images are shown in Fig. 2B. (B) Whole cell extracts were prepared from the indicated strains and immunoblotting carried out with anti-GFP and anti-Cdc2 antibodies. (C) Spot test. Spot-test. Indicated strains were serially diluted and spotted onto EMM with or without thiamine. The plates were kept at 27ºC for 3 days.