Polycystin-1 maturation requires polycystin-2 in a dose-dependent manner

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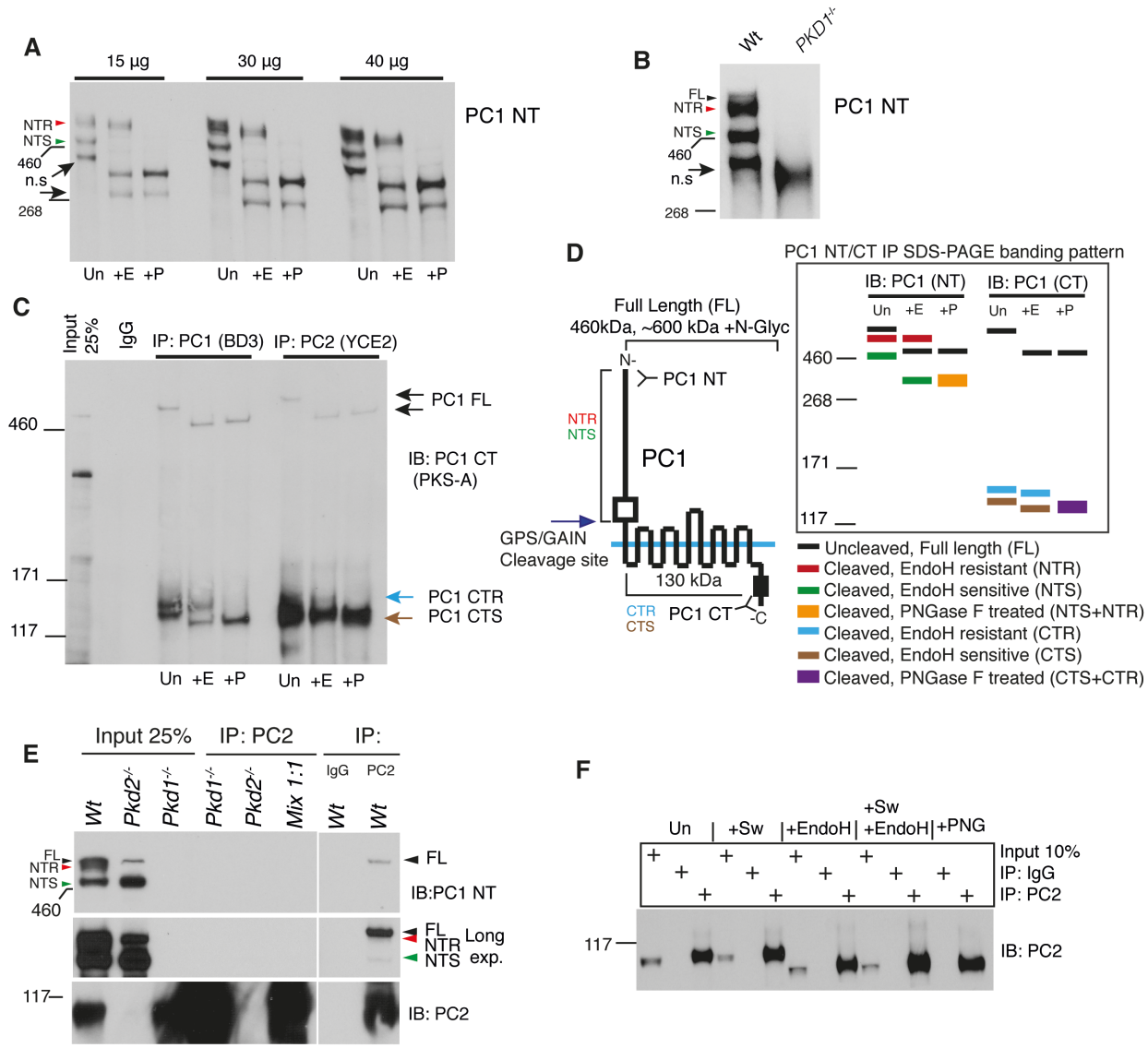

Figure S1

Supplemental Figure S1. Analysis of endogenous PC1/PC2 expression and glycosylation

A) Analysis of the non-specific band detected with the PC1 NT (7e12) antibody in human samples. IB of 15, 30, 40µg of total membrane protein from RCTE cells deglycosylated using EndoH (+E), PNGase F (+P) or untreated (Un) showing that the non-specific (n.s) protein (arrow) seen below the PC1-NTR and PC1-NTS glycoforms is EndoH sensitive. B) Comparison of RCTE cells (WT) and *PKD1* null human renal

epithelial cells (9-12; *PKD1*$^{-/-}$) showing that the non-specific protein is present in the null cells. Representative blots are shown from three independent experiments. C) IB of RCTE samples IP with PC1 CT or PC2, deglycosylated and detected with a PC1 CT antibody (note that PC1-CTS and FL are only detected in IP but not input, likely because enrichment is necessary for detection). PC1 FL (FL) and the PC1-CTS and PC1-CTR glycoforms are detected in both PC1 CT and PC2 IP samples, but PC2 seems to predominantly co-IP with PC1-CTS suggesting that the bulk of PC2 is complexed with the intracellular pool of PC1. D) Schematic of PC1 cleavage and glycosylation showing the size of the FL, the two GPS/GAIN N-terminal cleavage products, NTS and NTR and the C-terminal glycoforms, CTS and CTR. E) To test if the interaction between PC1-FL and PC2 occurs only post cell lysis and not in-vivo, cell lysates of *Pkd1*$^{-/-}$ (expressing just PC2) and *Pkd2*$^{-/-}$ (expressing just PC1) cells were mixed after cell lysis and subjected to IP with a PC2 antibody. IB of lysate or IP with PC2 in high-salt (500mM NaCl) buffer from wildtype (Wt), *Pkd1*$^{-/-}$, *Pkd2*$^{-/-}$ MEFs, or a 1:1 mixture of *Pkd1*$^{-/-}$, *Pkd2*$^{-/-}$ lysates (Mix 1:1) detected with PC1 FL or a PC2 antibody. IP with PC2 of the *Pkd1*$^{-/-}$ and *Pkd2*$^{-/-}$ mixture shows no co-IP of the proteins occurs after lysis. E) Effect of prolonged Swainsonine treatment alone or in combination with enzymatic deglycosylation was evaluated. RCTE membrane fractions are subjected to EndoH (+E) or PNGase F (PNG) treatment after incubation with Swainsonine for 72 hours (+Sw) or left untreated (Un), and IP with PC2 or control (IgG). All detectable PC2 remains fully EndoH sensitive, did not respond to Swainsonine treatment. Representative blots are shown from three independent experiments.

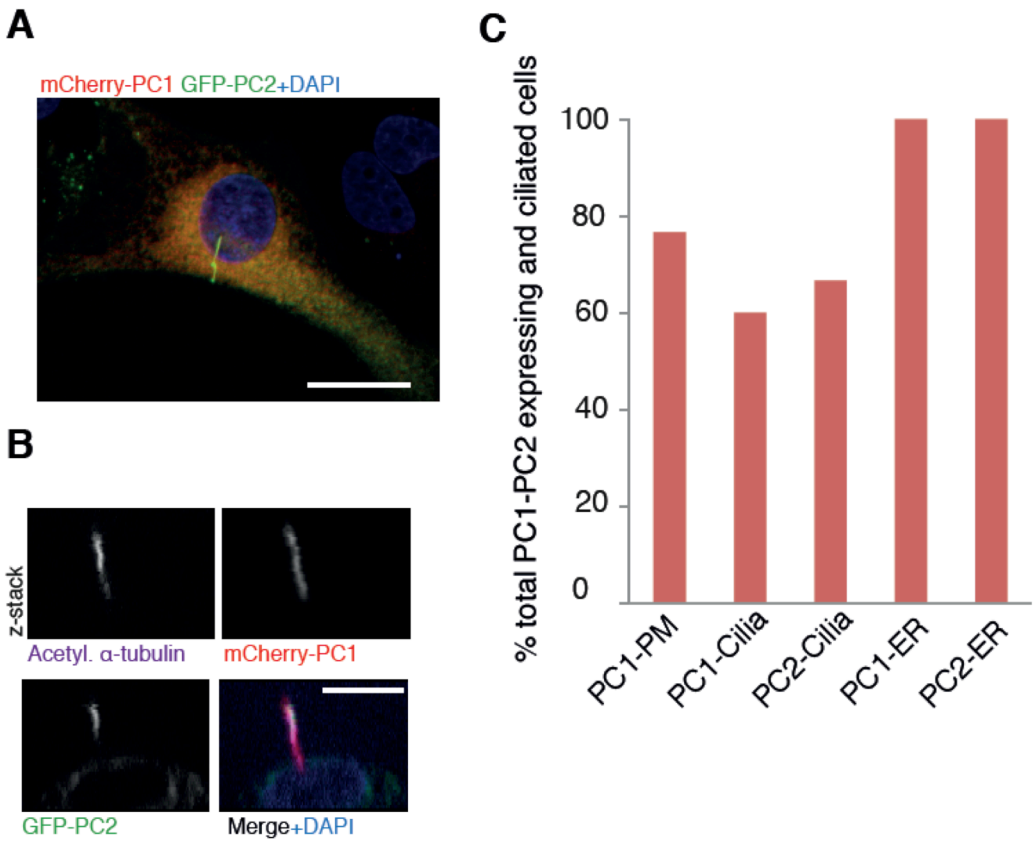

Figure S2

Supplemental Figure 2. Primary cilia localization of the polycystins

A-B) Ciliated RCTE cells co-transfected with mCherry-PC1 and GFP-PC2 showing overlap of PC1 and PC2 signals on the primary cilia. Bar=20μm and 10μm respectively. C) Quantification of ciliated RCTE cells co-transfected with mCherry-PC1 and GFP-PC2 and surface labeled with a mCherry antibody. Fifty ciliated cells are scored for PC1 and PC2 localization in the ER, PM (surface mCherry) or primary cilia (acetylated alpha tubulin) and displayed as percent total co-transfected and ciliated cells.

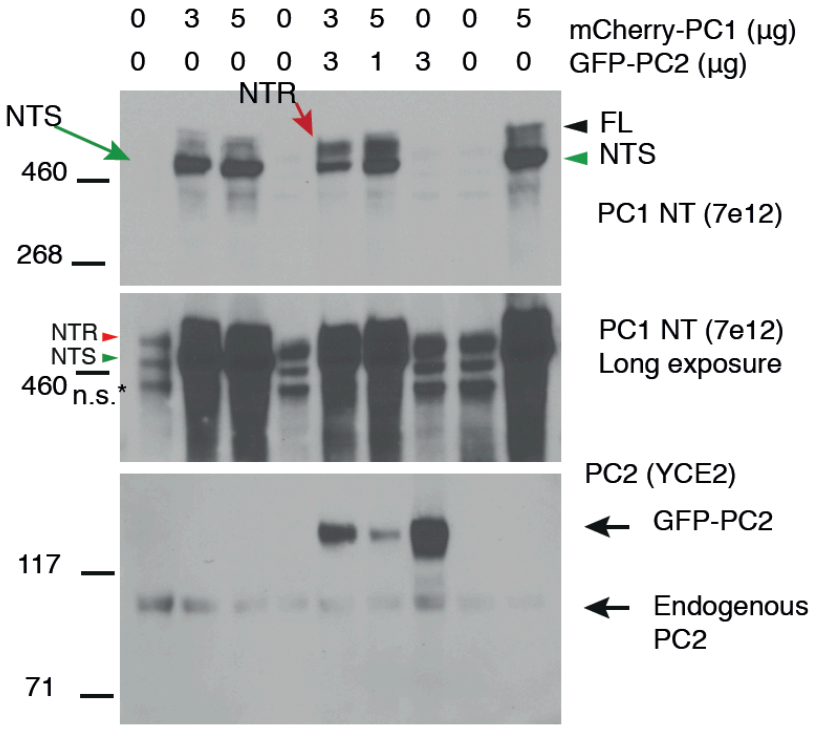

Figure S3

Supplemental Figure 3. IBs comparing levels of endogenous PC1 and PC2 to the exogenously expressed proteins

mCherry-PC1 and GFP-PC2 transfected into RCTE cells at the indicated amounts of plasmid (top) and detected with PC1 NT or PC2. Note that on a short exposure only exogenous PC1 is detected but after a longer exposure endogenous PC1 becomes evident. Representative blots from two independent experiments.

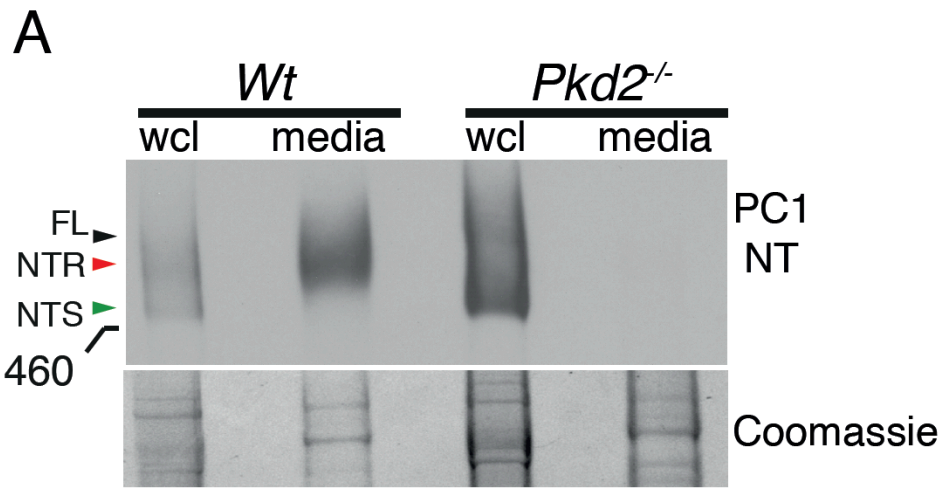

Figure S4

Supplemental Figure 4. IB of secreted PC1 media of *Pkd2*$^{-/-}$ cells

A) Media was collected after 48h from Wt and *Pkd2*$^{-/-}$ MEFs, subjected to ultracentrifugation to remove dead cells/cell membranes and concentrated (see Experimental Procedures for details). Equal quantities of whole cell lysate (wcl) and total media protein (media) were detected with the PC1 NT antibody. No PC1-NTR is detected in the *Pkd2*$^{-/-}$ media. Representative blots are shown from three independent experiments.

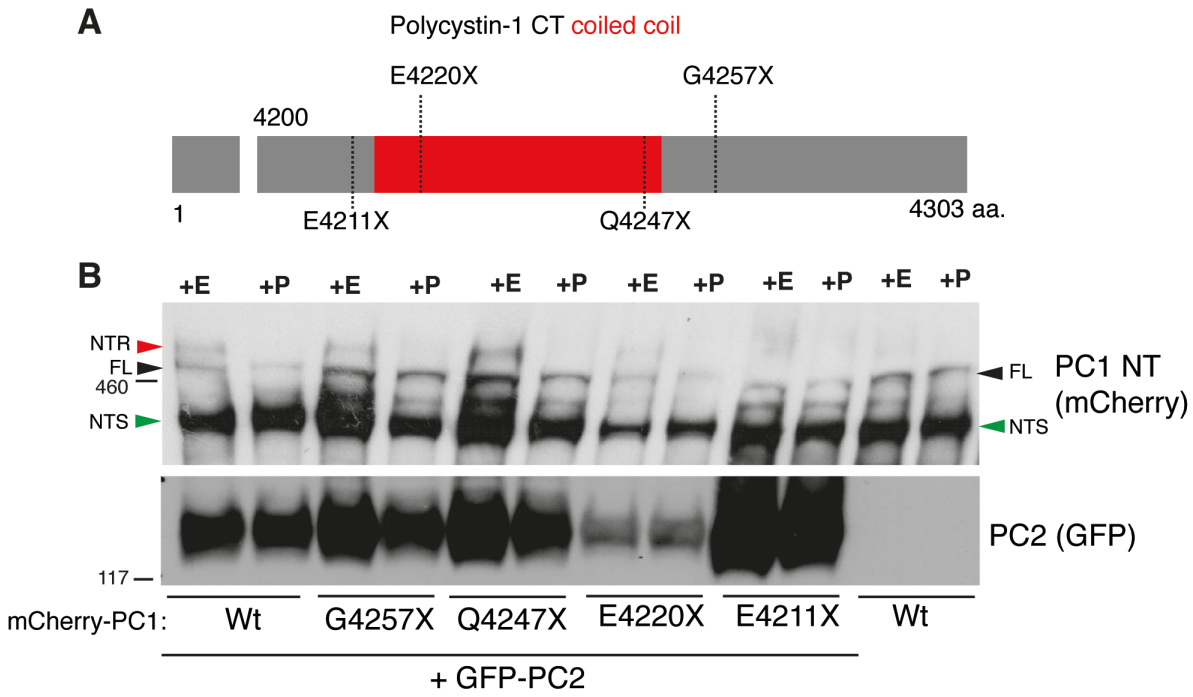

Figure S5

Supplemental Figure 5. The PC1 coiled coil domain is required for PC1 glycosylation

A) Schematic representation of PC1-CT coiled-coil (red) with sites of truncating mutations indicated (p.E4211X, p.E4220X, p.Q4247X and p.G4257X). B) IB of MDCK membrane protein, deglycosylated with EndoH or PNGase and detected with a mCherry antibody, in cells co-transfected with wildtype (Wt) or mutant PC1, with or without wildtype PC2 (GFP). The EndoH resistant PC1-NTR is not seen without PC2 co-transfection. In PC2 (GFP) co-transfected cells, PC1-NTR is strongly detected with Wt PC1 p.G4257X and p.Q4247X and is not seen with p.G4211X. Representative blots from two independent experiments.

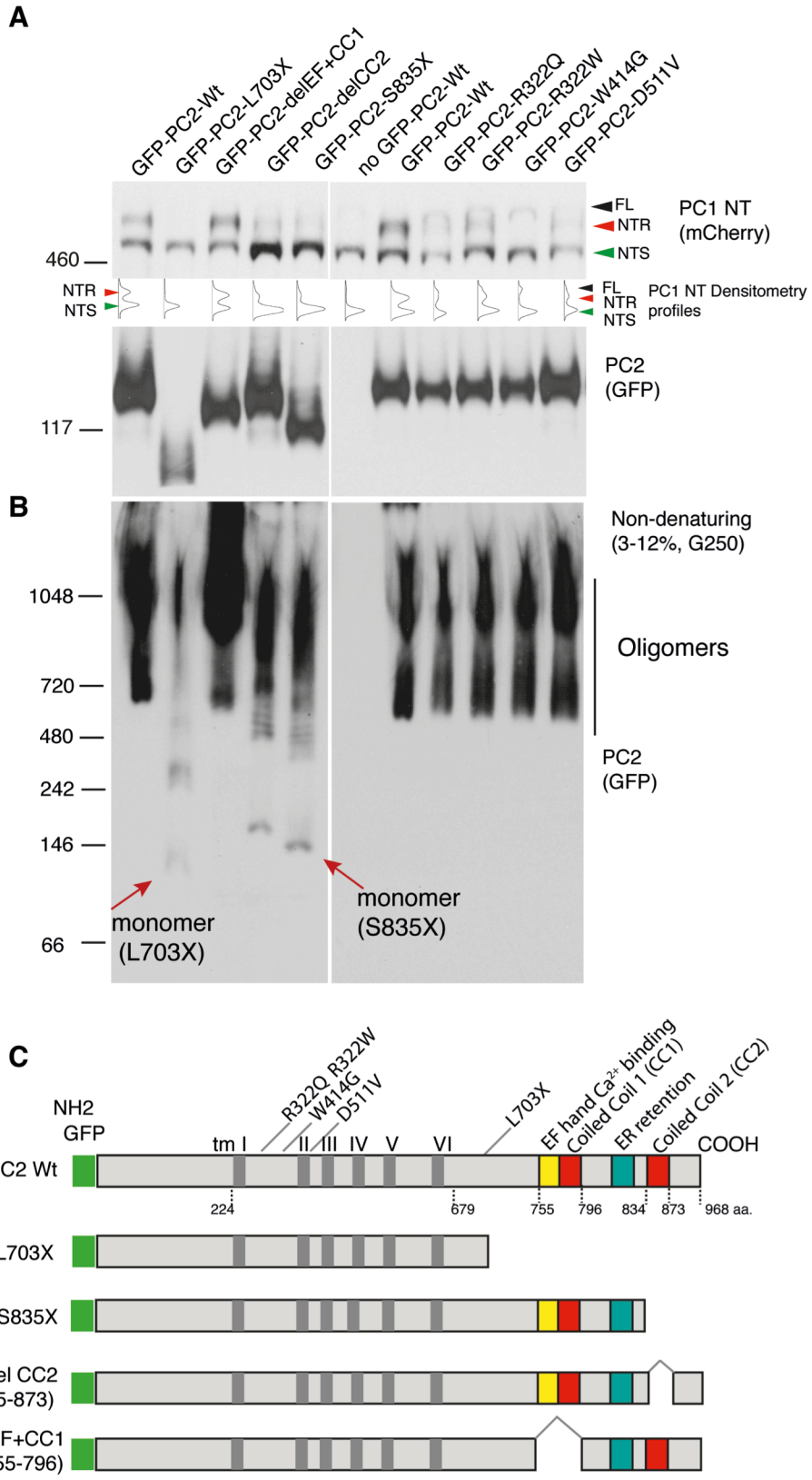
A
GFP-PC2-Wt
GFP-PC2-L703X
GFP-PC2-delEF+CC1
GFP-PC2-delCC2
GFP-PC2-S835X
no GFP-PC2-Wt
GFP-PC2-Wt
GFP-PC2-R322Q
GFP-PC2-R322W
GFP-PC2-W414G
GFP-PC2-D511V
460
FL
NTR
NTS
PC1 NT
(mCherry)
NTR
NTS
PC1 NT Densitometry
profiles
117
PC2
(GFP)
B
Non-denaturing
(3-12%, G250)
1048
720
480
242
146
66
Oligomers
PC2
(GFP)
monomer
(L703X)
monomer
(S835X)
C
NH2
GFP
tm I
II
III
IV
V
VI
COOH
R322Q
R322W
W414G
D511V
L703X
EF hand Ca2+ binding
Coiled Coil 1 (CC1)
ER retention
Coiled Coil 2 (CC2)
PC2 Wt
224
679
755
796
834
873
968 aa.
L703X
S835X
PC2 del CC2
(del835-873)
PC2 del EF+CC1
(del755-796)

Figure S6

Supplemental Figure 6. Analysis of wildtype and mutant PC2 oligomerization

A) IBs of RCTE cells co-transfected with Wt PC1 alone or with Wt or mutant PC2 constructs separated by (A) SDS-PAGE and detected with PC1 NT (mCherry) or PC2 (GFP), or (B) resolved on a non-denaturing (native) gel and detected with PC2 (GFP). A) As shown in Figure 8, co-transfection of PC2 promotes PC1 maturation but this is disrupted if the PC2 coiled coil 2 (CC2) is deleted and significantly reduced in various PC2 missense mutations. Profiles showing the relative levels of the PC1-NTR, PC1-NTS and PC1-FL forms are shown. Representative blots are shown from three independent experiments. B) Larger oligomers of PC2 are detected on the non-denaturing gel and deletion of the PC2-CC2 affects this oligomerization, with monomers detected in the p.L703X, p.S835X and delCC2 mutants. PC2 missense mutations did not cause an apparent oligomerization defect. C) Schematic representation of WT and mutant PC2 with various conserved domains and sites of mutations highlighted.

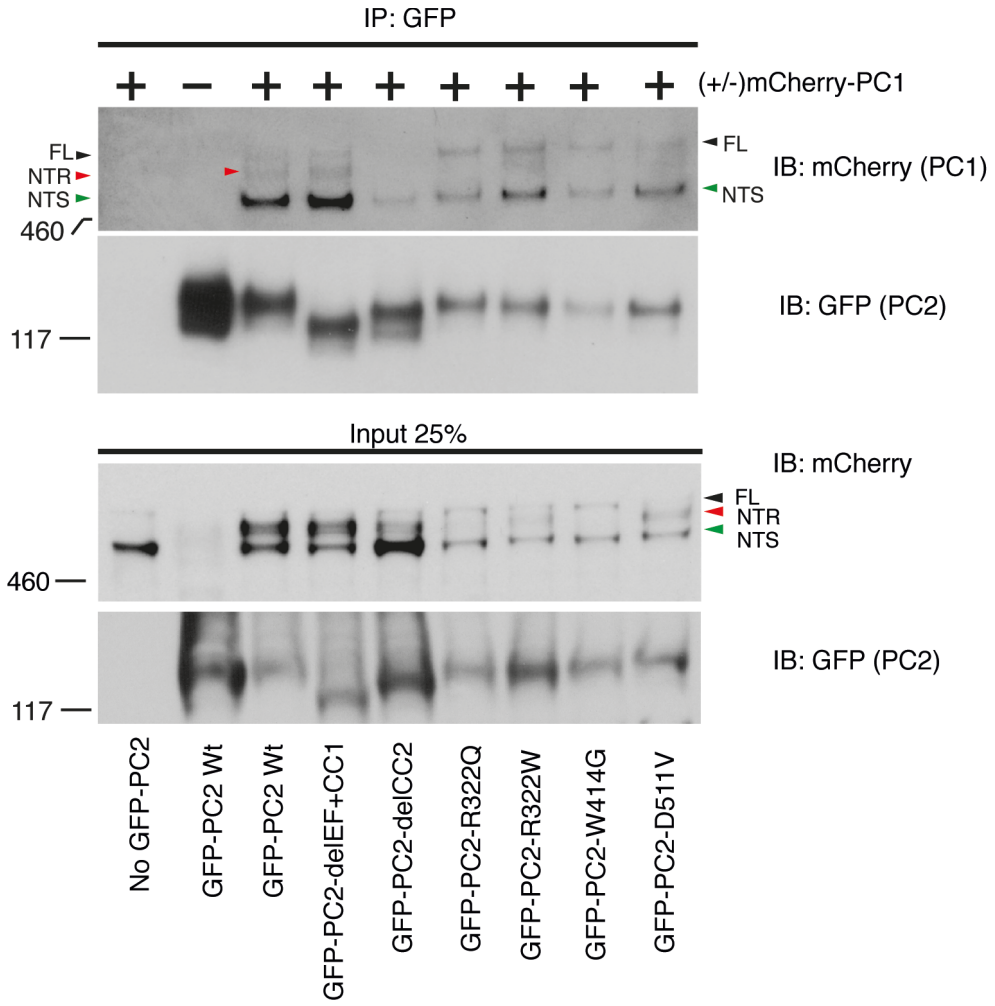

Figure S7

Supplemental Figure 7. Co-IP of PC1 and various PC2 mutants

A, B) IB of RCTE cells lysates (bottom) co-transfected with mCherry-PC1 and various GFP-PC2 wildtype (Wt), or deletion or missense mutants and detected with mCherry (PC1) or GFP (PC2), or IP with PC2 (GFP) before detection (top). As shown in Figure 7, PC2 mutant constructs deleting the PC2 coiled-coil 2 (CC2), or the missense mutants, disrupt PC1 maturation. PC2 mutants not influencing PC1 maturation (delEF+CC1) and the missense mutations are able to co-IP with PC1, while the PC2-delCC2 mutation IP PC1 at only a low level. Representative blots are from two experiments.

Supplemental Table 1

Summary of measurements for the *Pkd1*^{RC} and *Pkd2*^{WS25} crosses

Measurements (mean±SD)[1]	**Genotypes (n)**					
	Pkd1$^{RC/RC}$**; *Pkd2***$^{+/+}$ **(n=6F, 6M)**	***Pkd1***$^{RC/RC}$**; *Pkd2***$^{WS25/+}$ **(n=6F, 6M)**	***Pkd1***$^{RC/RC}$**; *Pkd2***$^{+/-}$ **(n=7F, 6M)**	***Pkd1***$^{RC/RC}$**; *Pkd2***$^{WS25/-}$ **(n=2F, 3M)**[2]	***Pkd1***$^{RC/+}$**; *Pkd2***$^{WS25/-}$ **(n=6F, 6M)**	***Pkd1***$^{+/+}$**; *Pkd2***$^{WS25/-}$ **(n=5F, 7M)**
%KW/BW	1.82±0.16	2.28±0.61	3.20±1.19	7.57±2.84	2.66±1.06	1.90±0.59
p-value vs. *Pkd1*$^{RC/RC}$; *Pkd2*$^{+/+}$	N/A	0.28	*1.05E-03*	*6.06E-15*	0.052	0.88
Cystic Index (%)	8.77±2.57	16.14±9.34	27.10±12.07	60.32±14.31	25.51±15.35	13.54±15.80
p-value vs. *Pkd1*$^{RC/RC}$; *Pkd2*$^{+/+}$	N/A	0.15	*3.87E-04*	*1.93E-10*	*0.0016*	0.39
BUN (mg/dl)	20.17±4.67	19.75±3.86	23.38±4.07	39.00±5.09	22.00±4.05	20.50±5.62
p-value vs. *Pkd1*$^{RC/RC}$; *Pkd2*$^{+/+}$	N/A	0.82	0.076	*2.98E-10*	0.33	0.98
Plasma Creatinine (mg/dl)	0.265±0.051	0.258±0.049	0.271±0.040	0.404±0.108	0.258±0.062	0.289±0.055
p-value vs. *Pkd1*$^{RC/RC}$; *Pkd2*$^{+/+}$	N/A	0.86	0.74	*1.08E-04*	0.78	0.33

[1]p-values in italics are significant <0.05
[2]Five animals with this genotyped died before 4m (F: P42, P74; M: P38, P51, P79