Renal plasticity revealed through reversal of polycystic kidney disease in mice

Ke Dong[1], Chao Zhang[1], Xin Tian[1], Daniel Coman[2], Fahmeed Hyder[2,3], Ming Ma[1], Stefan Somlo[1,4,‡]

Departments of [1]Internal Medicine, [2]Radiology and Biomedical Imaging, [3]Biomedical Engineering and [4]Genetics, Yale University School of Medicine, New Haven, Connecticut, USA

‡Address correspondence to:
Stefan Somlo, M.D.
Section of Nephrology
Yale University School of Medicine
P.O. Box 208029
333 Cedar Street
New Haven, CT 06520-8029
Phone: (203) 737-2974
Fax: (203) 785-4904
E-mail: stefan.somlo@yale.edu

Supplementary Note

Characterization of Rosa26^{FlpoER} activity in the adult kidney

We verified that *Rosa26^{FlpoER}* is active in the adult kidney using the *REC:FRT* (*REC*) reporter mouse line which expresses EGFP in cells in which Flp recombinase is active[1]. *REC*;*Rosa26^{FlpoER}* mouse kidneys showed no EGFP protein expression at 4 weeks age in the absence of tamoxifen. Following daily tamoxifen for 7 days beginning at 3 weeks age, most nephron segments showed strong EGFP expression at 4 weeks age detected by anti-EGFP antibody (Supplementary Fig. 1a, b). FlpoER activity has rapid onset as a single dose of tamoxifen given at 3 weeks resulted in substantial albeit mosaic EGFP expression at 4 weeks (Supplementary Fig. 1c, d).

Construction of the Pkd2^{FSF} BAC

The target allele for *Pkd2* reactivation was constructed using BAC recombineering[2,3]. A strong 4-copy transcriptional stop sequence (4XSTOP) flanked by *FRT* sites[4] was introduced into the first intron of *Pkd2* at IVS1+2861 (Supplementary Fig. 2A, B). A triple HA epitope tag sequence (3XHA) was introduced immediately before the termination codon to allow specific detection of the *Pkd2^{FSF}* BAC expressed PC2-HA protein. The insertion FRT-4XSTOP-FRT (FSF) was verified by direct sequencing and by successful Flp mediated in vitro excision of the 4XSTOP in bacterial cells (Supplementary Fig. 2c, d).

Pkd2^{FSF} BAC transgenic founder identification

Transgenic mouse lines were produced following pronuclear injection into mouse zygotes of linearized *Pkd2^{FSF}* BAC DNA. Two independent founder mouse strains, denoted as *Pkd2^{FSF}*, were obtained. Each founder line integrated two copies of the transgene as determined by genomic quantitative PCR.

Characterization of Pkd2$^{Cre/Flpo}$ experimental mice

The compound heterozygous combination of *Pkd2^{fl}* and *Pkd2^{-}* alleles in *Pkd2$^{Cre/Flpo}$* mice allowed for clarity in genotyping and for somewhat faster progression of polycystic kidney disease compared to *Pkd2$^{fl/fl}$*. This model of ADPKD is completely penetrant but has variable severity. While sex dimorphism has been reported in adult models of ADPKD based on *Pkd1* [5], this has not been reported for models based on *Pkd2* and did not explain the observed variation in this model (Supplementary Fig. 4). Power calculations done prospectively based on the observed variation at 16 weeks indicated that 12 animals per group would give 80% power to detect a 40% change in kidney to body weight ratio at a significance threshold of $P<0.05$.

Apoptosis following PC2-HA re-expression

The relationship of alterations in apoptosis with ADPKD is not understood. Orthologous gene models of ADPKD typically do not show increased levels of apoptosis suggesting that apoptosis is not a central feature of cyst development in these models[6-9]. Nonetheless, apoptosis has been found to increase in late stage kidneys in both humans[10] and orthologous mouse models[11], perhaps occurring as a consequence rather than a cause of advanced ADPKD. We examined levels of cleaved caspase-3 and cleaved Parp as a function of PC2 expression in our models (Supplementary Fig. 8). At 14 weeks, 1 week after the start of re-expression, there was a small but significant increase in apoptosis in kidneys with PC2-HA re-expression compared to both non-cystic and cystic kidneys without re-expression (Supplementary Fig. 8a, b). This suggests that some programmed cell death does occur in the early stage response to re-expression of PC2-HA. At 16 weeks, there was an increase in whole kidney apoptotic rates in the cystic kidneys without PC2 re-expression, but kidneys with PC2-HA re-expression showed apoptosis levels similar to non-cystic controls (Supplementary Fig. 8c, d).

Pkd1^{FSF} BAC construction

The target allele for *Pkd1* reactivation was constructed using a BAC previously modified to contain N-terminal triple-FLAG and C-terminal triple-HA epitope tags[2,3]. We introduced the 4XSTOP sequence flanked by *FRT* sites into the first intron of *Pkd1* at IVS1+4317. The integrity *Pkd1^{FSF}* BAC construct containing the FRT-4XSTOP-FRT was verified in a manner analogous the *Pkd2^{FSF}*. Three founder mouse strains were obtained, which integrated 8, 4 and 1 copies of the *Pkd1^{FSF}* transgene. The 8 copy transgene integrated on the Y chromosome. All three founders were used in the studies.

References

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Supplementary Table 1: Kidney volume progression determined by MRI.

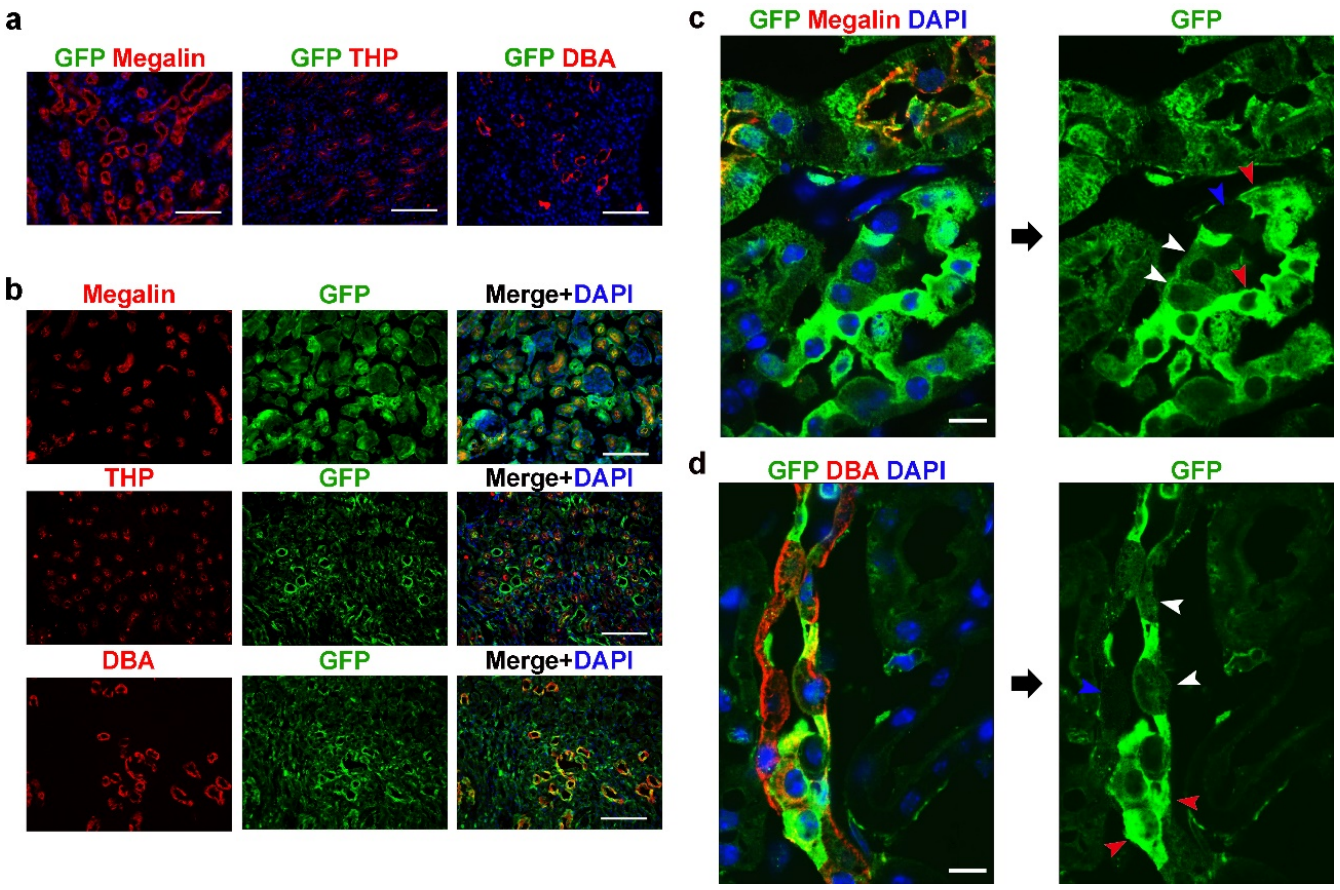
Genotype	Treatment	Sequential kidney volume by MRI (mm^3)			Kidney weight -to-body weight (%)*	Cystic index (%)*	BUN (mg/dL)*
		13 weeks	16 weeks	19 weeks	19 weeks		
Pkd2$^{fl/-}$; *Pkd2*^{FSF}; *Pax8*^{rtTA}; *TetO*^{Cre}; *Rosa*^{FlpoER}	None	456	455	441	1.7	16	27
		367	388	380	1.6	14	17
		372	380	360	1.7	12	22
		390	381	375	1.9	12	18
Pkd2$^{fl/-}$; *Pkd2*^{FSF}; *Pax8*^{rtTA}; *TetO*^{Cre}; *Rosa*^{FlpoER}	Doxycycline	963	1760	3238	20.2	73	110
		1116	2514	3261	16.6	72	na
		1420	3351	4804	20.9	67	163
Pkd2$^{fl/-}$; *Pkd2*^{FSF}; *Pax8*^{rtTA}; *TetO*^{Cre}; *Rosa*^{flpoER}	Doxycycline + Tamoxifen	1057	519	535	1.8	18	22
		583	404	308	1.4	20	20
		907	549	507	1.8	21	18
		3510	988	521	2.2	28	47

* — Kidney weight-to-body weight (%), Cystic index (%), BUN (mg/dL) were measured at 19 weeks only.
na — not available.
Bold text are column headings.
Italics are used to identify mouse genotypes.

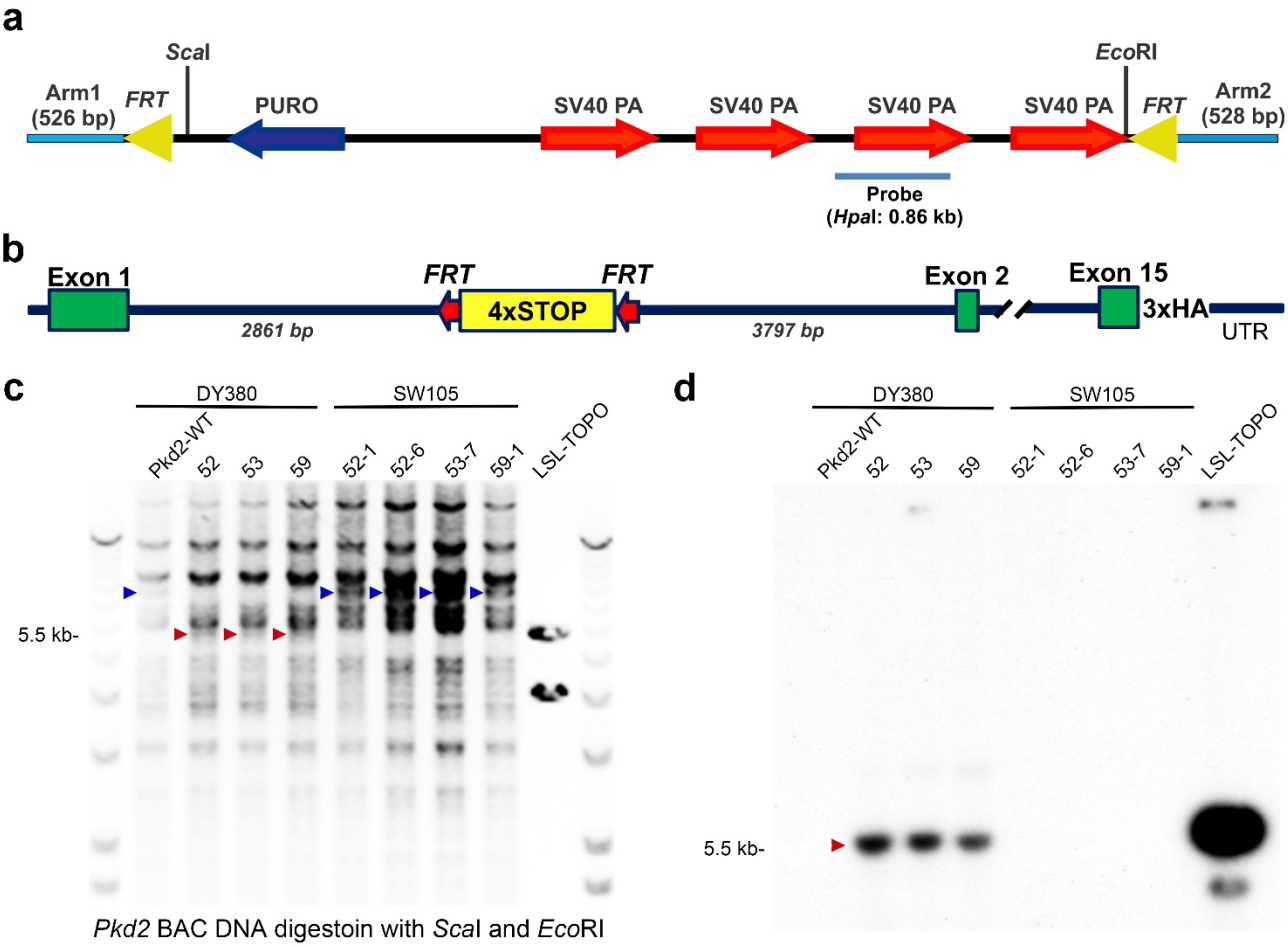
Supplementary Table 2: RT-PCR primer sequences.

Gene	Forward (5'→3')	Reverse (5'→3')
F4/80	AGTACGATGTGGGGCTTTTG	ACTCCTGGGCCTTGAAAGTT
IL-6	CTTCCTACCCCAATTTCCAATG	ATTGGATGGTCTTGGTCCTTAGC
Mcp1	CTTCTGGGCCTGCTGTTCA	CCAGCCTACTCATTGGGATCA
Col1A1	GCCAAGAAGACATCCCTGAA	GTTTCCACGTCTCACCATTG
Col3A1	ACAGCTGGTGAACCTGGAAG	ACCAGGAGATCCATCTCGAC
Fibronectin	ACAAGGTTCGGGAAGAGGTT	CCGTGTAAGGGTCAAAGCAT
Fsp1	GAAGCTGCATTCCAGAAGGTGA	CATCATGGCAATGCAGGACA
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Bold text are column headings.
Italics are used to identify genes.



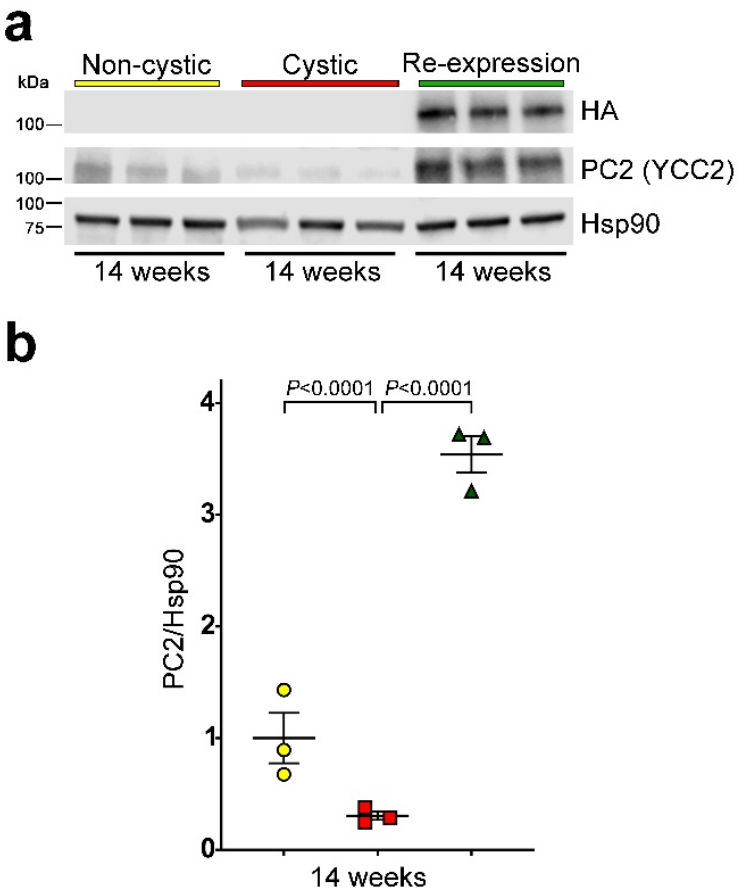
Supplementary Figure 1: Reporter analysis for *Rosa*^{FlpoER} activity in adult kidney. *REC*;*Rosa*^{FlpoER} mice were treated with (**a**) vehicle injections or (**b**) daily tamoxifen from P21-28 and examined at P28 by double indirect immunofluorescence staining with anti-GFP (green), segment specific markers (all red): megalin for proximal tubule (PT); Tamm Horsfall protein, THP, for medullary thick ascending limb (mTAL); Dolichos biflorus agglutinin, DBA, for collecting duct (CD). Nuclei are marked with DAPI (blue). (**a**) Mice that did not receive tamoxifen did not have detectable anti-GFP immunoreactivity. (**b**) Mice treated with tamoxifen had diffuse anti-GFP immunoreactivity in the cortex and medulla in PT, mTAL and CD segments. **a, b**, scale bars, 100 μm. (**c, d**) *REC*;*Rosa*^{FlpoER} mice were treated with a single dose of tamoxifen at P21 and examined at P28 resulting in a mosaic expression of anti-GFP immunoreactive protein with some renal tubule cells with weak (white arrowhead) or absent (blue arrowhead) immunoreactivity while other tubule cells showed strong expression of GFP protein (red arrowhead). Each image is representative of 1-3 sections examined from each of 3 mice. **c, d**, scale bars, 10 μm.



Pkd2 BAC DNA digestoin with *Sca*I and *Eco*RI

Supplementary Figure 2: Structure and in vitro validation of the *Pkd2* BAC re-expression allele. (**a**) Schematic of the 6801 bp 4X STOP "FSF" cassette inclusive of the homology arms showing relative location of the *FRT* sites, puromycin cassette, four SV40 polyadenylation signal cassettes (SV40 PA), the *Sca*I and *Eco*RI sites and the Southern blot hybridization probe (Probe). (**b**) Schematic of the $Pkd2^{FSF}$ BAC construct showing the integration site of the FSF cassette (5748 bp exclusive of homology arms) into IVS1 of the *Pkd2* BAC and also indicating the presence of triple HA epitope tag sequence before the termination codon in exon 15. (**c**) Restriction enzyme fingerprint for three independent $Pkd2^{FSF}$ BAC clones (#52, #53, #59) originally in the DY380 *E*. coli strain that were electroporated into the SW105 strain which contains l-arabinose inducible Flp recombinase. Cultures were induced with l-arabinose and DNA from both cells were digested with *Sca*I and *Eco*RI. $Pkd2^{FSF}$ BAC DNA in DY380 cells lacking the Flp recombinase contain a 5.5 kb band (red arrowheads, left panel) introduced by the FSF cassette (**a**) and not present in wild type *Pkd2*. Following l-arabinose induction in SW105 cells, this band disappears, and a 7-8 kb band also present in wild type *Pkd2* appears (blue arrowheads, *left panel*) indicating excision of FSF by Flp recombinase. (**d**) The presence of the FSF cassette in DY380 cells is verified by Southern

blot (red arrowhead) using an internal probe (**a**) and this cassette disappears after Flp induction in SW108 cells and is also absent from wild type *Pkd2* BAC DNA. LSL-TOPO is a plasmid positive control.



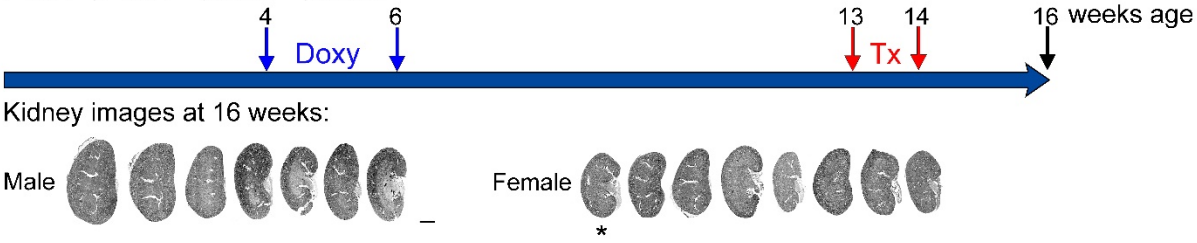
Supplementary Figure 3: Relative expression of PC2-HA from the *Pkd2*^{FSF} BAC transgene. (**a**) Immunoblots and (**b**) quantitation using densitometric ratios of kidney tissue lysates from *Pkd2*$^{Cre/Flpo}$ at 14 weeks age. *Pkd2*$^{Cre/Flpo}$ mice include the *Pkd2*$^{fl/—}$ allele combination, so PC2 expression occurs from a single allele, which produces ~50% of the total protein expressed from the wild type diploid genome. Treatment groups: non-cystic (yellow), no treatment; cystic (red), doxycycline from P28-42; re-expression (green), doxycycline from P28-42 and tamoxifen for 7 days beginning at 13 weeks age. (**a**) Top panel detected with anti-HA detects PC2-HA only in the re-expression group. Middle panel detected with anti-PC2 antisera (YCC2). (**b**) Densitometric ratios of PC2 detected with YCC2 to Hsp90 for each lane relative to the mean of the ratio in the single copy, haploinsufficient non-cystic samples which is set a value of 1.0. Multiple group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test and are presented as the mean ± SEM.

The densitometric quantitation data show that re-expression kidneys have about ~3.5-fold greater expression detected by anti-native PC2 antisera than the true heterozygous kidneys (compare green triangles vs. yellow circles). The quantitative data on the knockout kidneys (red) suggests that there is ~30% residual expression of native PC2, presumably from cells where *Pax8*^{rtTA} is not active. The *Rosa26*^{FlpoER} is active much more broadly active than *Pax8*^{rtTA} so while knockout is incomplete, activation of the *Pkd2*^{FSF}-BAC is likely to be nearly ubiquitous (see Supplementary Figure 1). Therefore, the net

over-expression from the two-copy *Pkd2*^{FSF}-BAC, after discounting the residual native PC2, of ~3 fold, or not more that ~1.5-fold the normal diploid PC2 expression level.

1

Pkd2$^{fl/-}$;Pkd2^{FSF};TetO^{Cre};Rosa^{FlpoER}



Kidney images at 16 weeks:

Male

Female

*

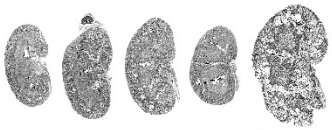
Pkd2$^{fl/-}$;Pkd2^{FSF};Pax8^{rtTA};TetO^{Cre};Rosa^{FlpoER}

Kidney images at 13 weeks:

Male

*

Female

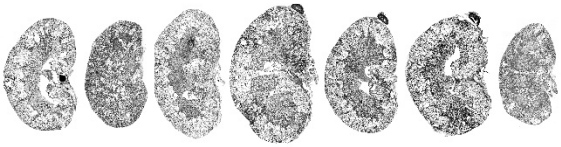


Pkd2$^{fl/-}$;Pkd2^{FSF};Pax8^{rtTA};TetO^{Cre};Rosa^{FlpoER}

Kidney images at 16 weeks:

Male

Female



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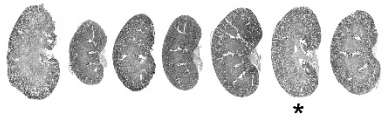
Pkd2$^{fl/-}$;Pkd2^{FSF};Pax8^{rtTA};TetO^{Cre};Rosa^{FlpoER}

Kidney images at 14 weeks:

Male



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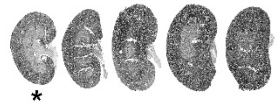
Female

Pkd2$^{fl/-}$;Pkd2^{FSF};Pax8^{rtTA};TetO^{Cre};Rosa^{FlpoER}

Kidney images at 16 weeks:

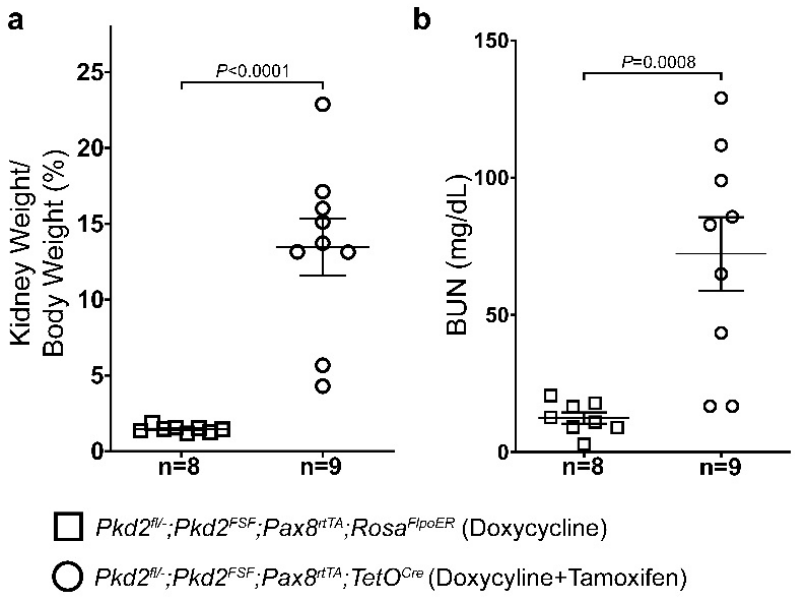
Male



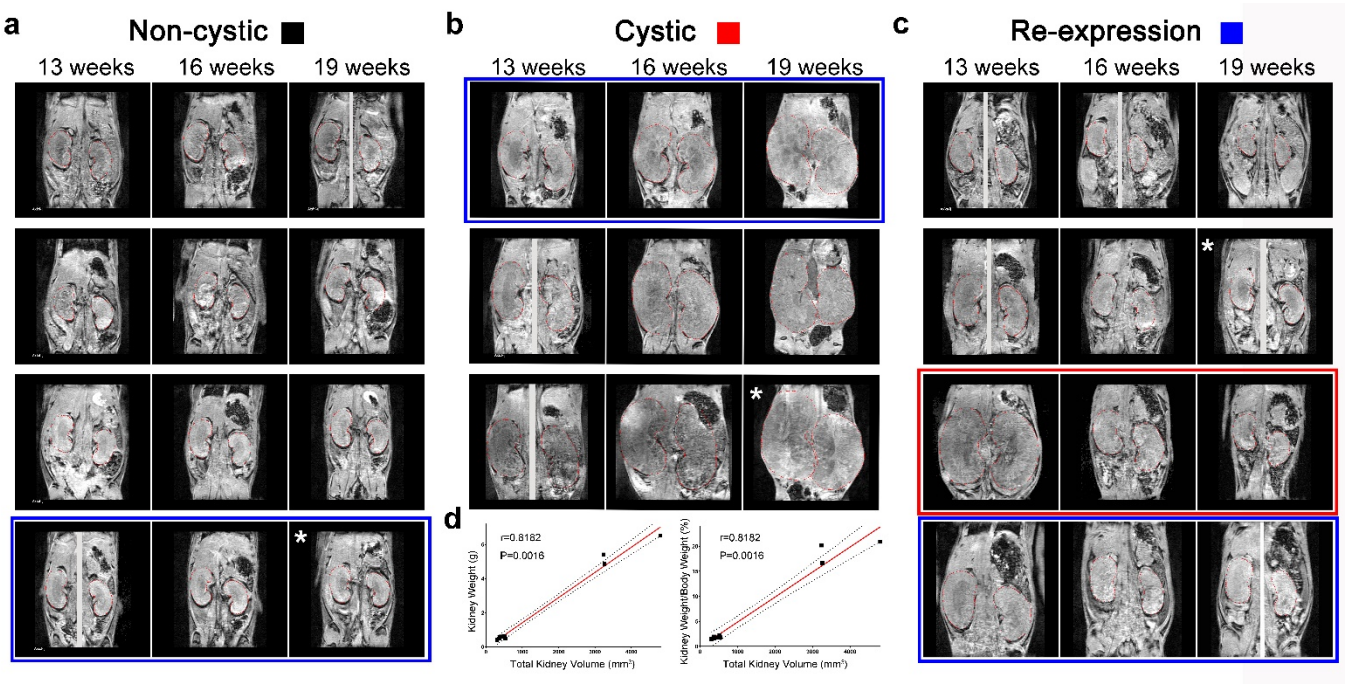
*

Female

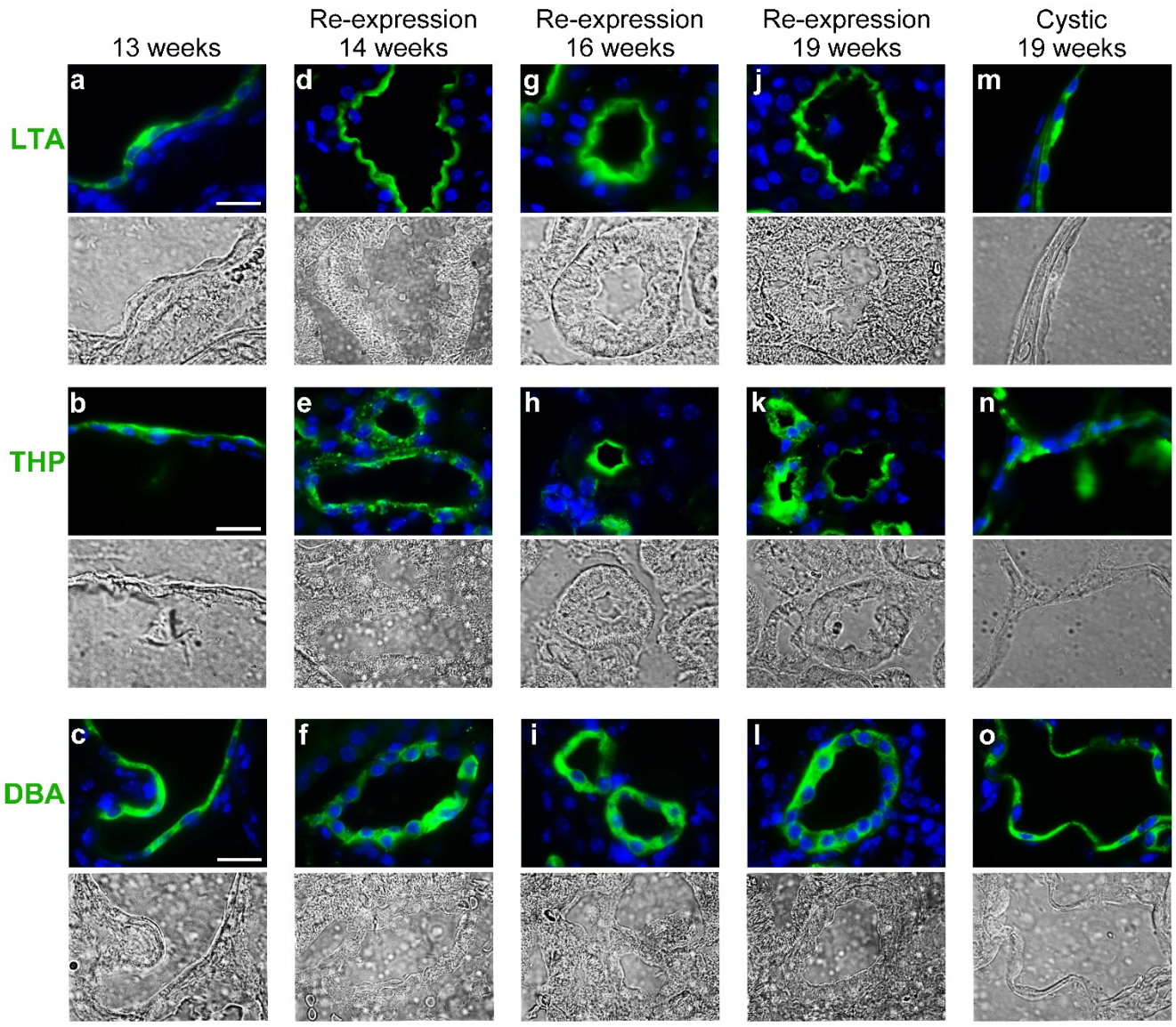
Supplementary Figure 4: Primary data used for *Pkd2* models Figure 1e, g. The five genotypes are indicated, and the colors of the timelines correspond to the colors used for the genotype groups in Fig. 1e-i. The timing of oral doxycycline (Doxy) administration from weeks 4-6 and of administration of intraperitoneal tamoxifen (Tx) was for 7 days beginning at week 13 are indicated as is the time of examination of the kidneys at the end of the timeline arrow. Each image used for the cystic index calculation is provided, separated by sex. The asterisk in each group marks the images used in Fig. 1e from each genotype group. Scale bar, 2 mm.



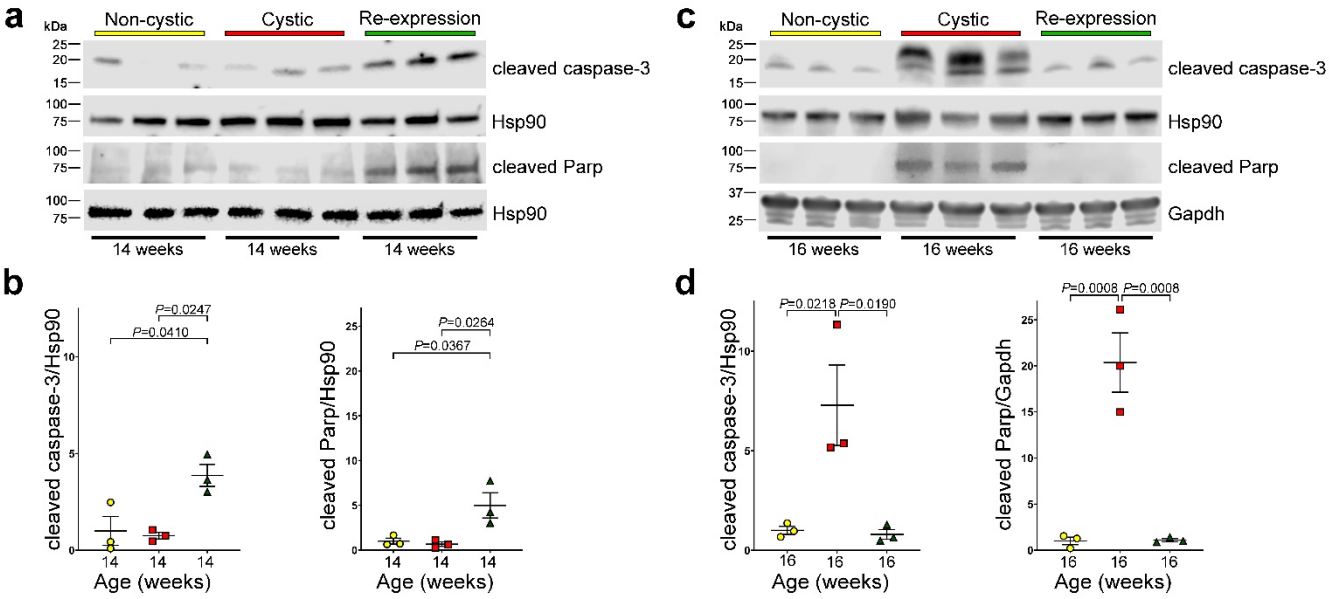
Supplementary Figure 5: No effect of tamoxifen in the absence of *Rosa*^{FlpoER}. Mice with the indicated genotypes received doxycycline from P28-42. Mice with the *Pkd2*^{FSF} BAC transgene but lacking *Rosa*^{FlpoER} (open circles) also received tamoxifen for one week beginning at 13 weeks age. All mice were examined at 16 weeks. Mice lacking *TetO*^{Cre} (open squares) did not develop polycystic disease as indicated by normal kidney weight-to-body weight ratio and BUN. Mice that lacked *Rosa*^{FlpoER} (open circles) developed progressive PKD and renal failure despite tamoxifen. Two groups comparisons were performed using the two-tailed unpaired t-test and are presented as mean ± SEM.



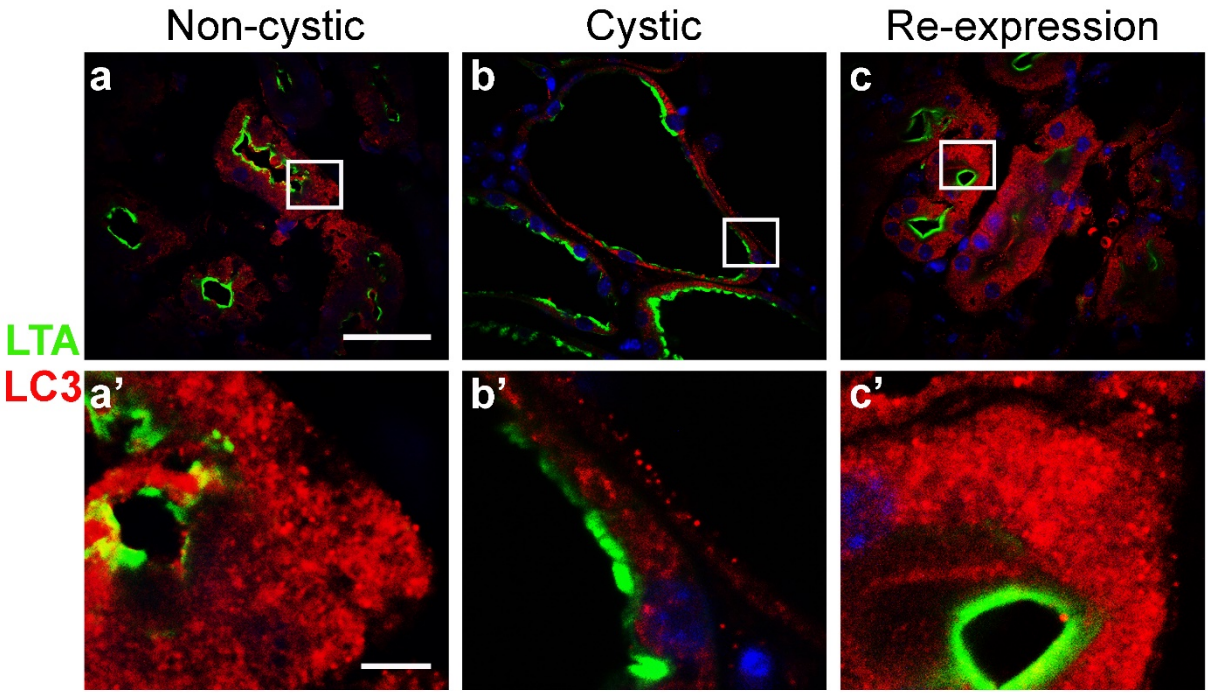
Supplementary Figure 6: Representative frontal plane images for all mice in serial MRI study. All mice have the *Pkd2*$^{fl/—}$; *Pkd2*^{FSF}; *Pax8*^{rtTA}; *TetO*^{Cre}; *Rosa*^{FlpoER} genotype. (**a**) No treatment ("non-cystic"). (**b**) Doxycycline only from P28-42 to inactivate *Pkd2* ("cystic"). (**c**) Doxycycline from P28-42 followed by tamoxifen for 7 days beginning at 13 weeks age to first inactivate and then re-express *Pkd2* ("re-expression"). The descriptions and colors of the symbols correspond to the treatment groups from Fig. 2; data are provided in Supplementary Table 1. The blue rectangles highlight the images that also appear in Fig. 2a. White asterisks mark the kidneys from which histological sections are shown in Fig. 2d. The red rectangle highlights the mouse with very large (3510 mm^3) kidneys at 13 weeks described in the text and Supplementary Table 1 and for which histology the histology at 19 weeks is shown in Fig. 2e. Kidneys are outlined with dashed red lines. Some mice were rotated during imaging so frontal planes for each kidney were obtained from different slices of the same MRI acquisition for purposes of presentation. These are indicated by the gray bar between left and right kidneys in the images. (**d**) One tailed Spearman non-parametric correlation of total kidney volume determined by MRI with total kidney weight and with kidney weight body weight ratio at 19 weeks (r=0.8182, *P*=0.0016 for both). 95% confidence interval (0.4119 to 0.9531) indicated by the dashed line.



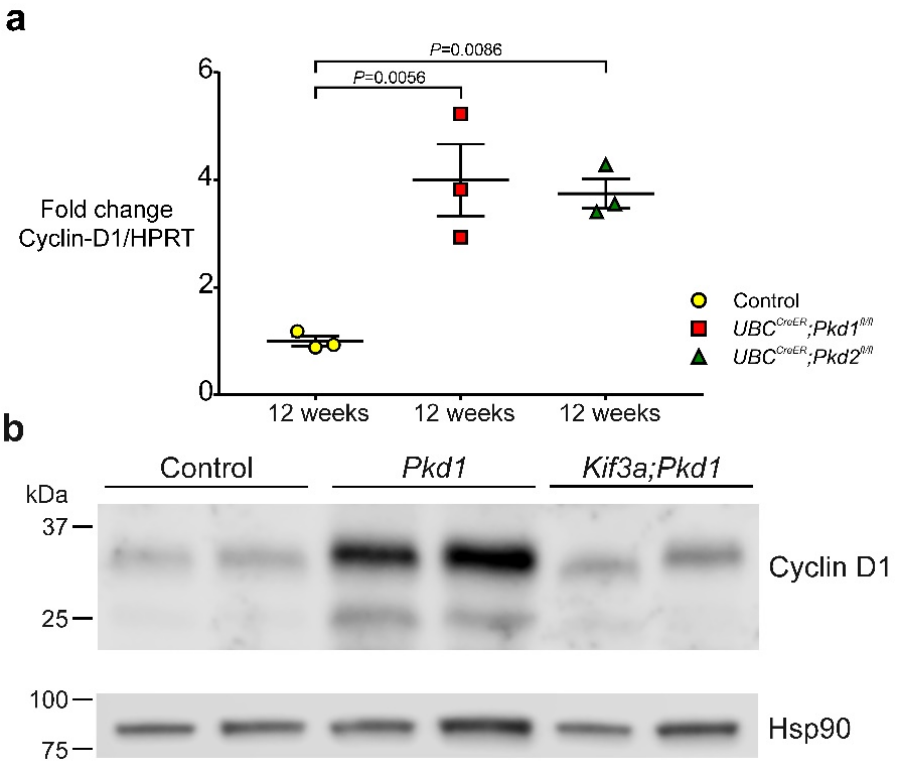
Supplementary Figure 7: Changes of tubule cell shapes following re-expression of PC2. Immunohistochemistry at the indicated stages in the absence (**a-c**, **m-o**) or presence (**d-l**) of PC2 re-expression. Proximal tubule are marked by Lotus tetragonolobus agglutinin (LTA; **a, d, g, j, m**), thick ascending limb of the Loop of Henle are marked by Tamm Horsfall protein (THP; **b, e, h, k, n**) and collecting ducts are marked by Dolichos biflorus agglutinin (DBA; **c, f, i, l, o**), all shown in green in the upper panels. Nuclei are stained with Hoechst stain (blue). Lower panels are the same field as the respective upper panels imaged with differential interference contrast (DIC) light microscopy. At least one section from 3 kidneys for each treatment and age group were examined with each nephron segment marker. Scale bars, 20 μm.



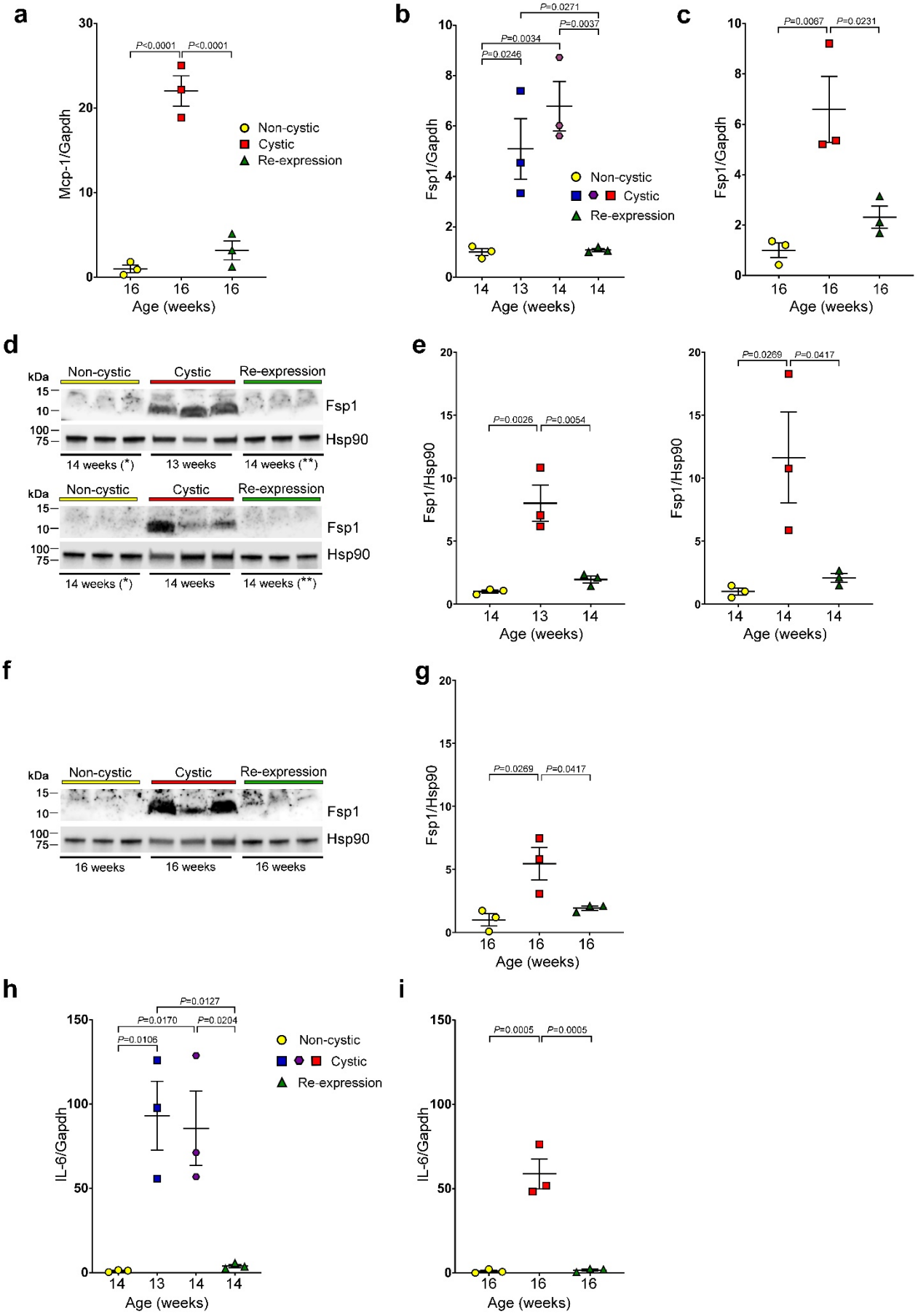
Supplementary Figure 8: Apoptosis is a marker of cyst progression. (**a, c**) Immunoblots and (**b, d**) quantitation using densitometric ratios of markers of apoptosis cleaved caspase-3 and cleaved Parp from kidney lysates from 3 *Pkd2*$^{Cre/Flpo}$ mice at 14 weeks age (**a, b**) and 16 weeks age (**c, d**) for each treatment indicated by the colors: yellow, non-cystic; red, cystic (doxycycline from P28-42); green, re-expression (doxycycline from P28-42 and tamoxifen for 7 days beginning at 13 weeks). Hsp90 and Gapdh serve as loading controls. (**b, d**) Densitometric ratios for each lane are shown relative to the mean of the ratio in the non-cystic samples which is set a value of 1.0. There is a relative increase in apoptosis in 14-week re-expression and in 16-week cystic kidney lysates (see Supplementary Note for details). *n*=3 for each group. Multiple group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test and are presented as the mean ± SEM.



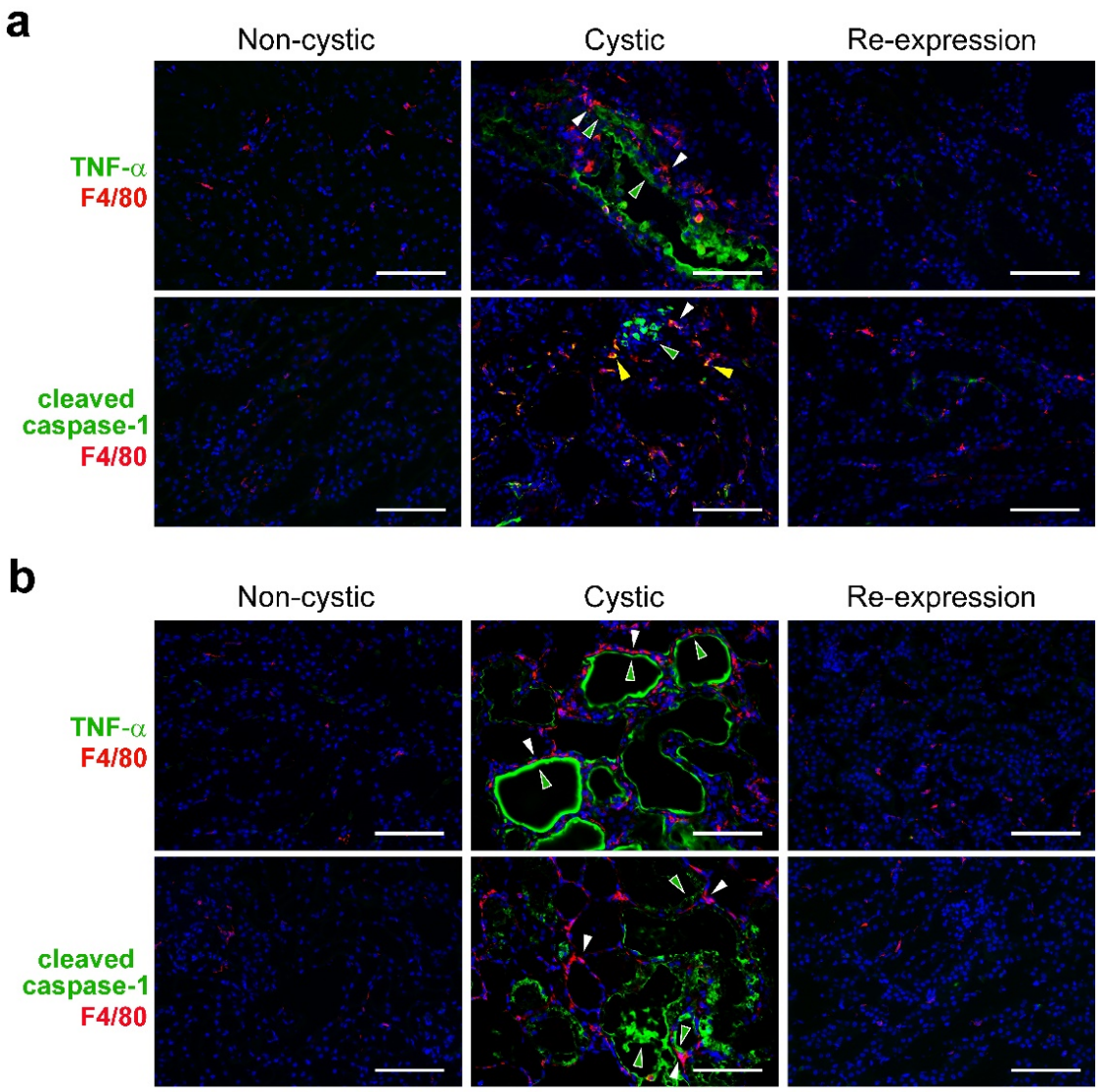
Supplementary Figure 9: Increased LC3 puncta formation in the kidneys following PC2 re-expression. LC3 puncta (red) show differences in endogenous expression in mouse kidney sections at 14 weeks. (**a, a'**) "Non-cystic" mice received neither doxycycline nor tamoxifen; (**b, b'**) "Cystic" mice received only doxycycline from P28-42; (**c, c'**) "Re-expression" mice received doxycycline from P28-42 and tamoxifen for 1 week beginning at 13 weeks age. All mice were fasted for 20 hours and treated with bafilomycin A_1 2 hours before euthanasia. Representative images show that LC3 positive puncta are decreased in the cytosol of LTA positive (green) proximal tubule cells in cystic kidneys (**b, b'**), but increased in kidneys with PC2 re-expression (**c, c'**). LTA, Lotus tetrogonolobus agglutinin; nuclei are stained with DAPI (blue). **a'**, **b'**, **c'** are enlarged views of the white box regions in **a**, **b**, **c**, respectively. At least 3 tissue sections from 3 different mice for each treatment condition were examined. Scale bars: 40 μm (**a**, **b**, **c**); 5 μm (**a'**, **b'**, **c'**).



Supplementary Figure 10: Cyclin-D1 expression is increased following inactivation of *Pkd1* and *Pkd2*. (**a**) Cyclin-D1 mRNA expression in whole kidney from mice with the indicated genotypes ("control" is non-cystic). *Pkd1* or *Pkd2* knockout was induced by treatment with tamoxifen from P28-35 and mice were examined at 12 weeks age when the kidneys are cystic. Fold-change in relative expression is shown relative to the mean for non-cystic controls kidneys which is set to 1.0. *n*=3 for each group. Multiple group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test and are presented as the mean ± SEM. (**b**) Immunoblotting for cyclin-D1 in $Pkd1^{fl/fl}$; $Pax8^{rtTA}$; $TetO^{Cre}$ (labelled *Pkd1*) and $Pkd1^{fl/fl}$; $Kif3a^{fl/fl}$; $Pax8^{rtTA}$; $TetO^{Cre}$ (labelled *Kif3a;Pkd1*) induced with oral doxycycline from P28-42 and examine at 10 weeks when there is mild tubule dilation but no cyst formation in the *Pkd1* model. The *Kif3a;Pkd1* model is protected from cyst formation and does not show elevation of cyclin-D1 expression. Hsp90 was used as a loading control. "Control" mice are wild type.

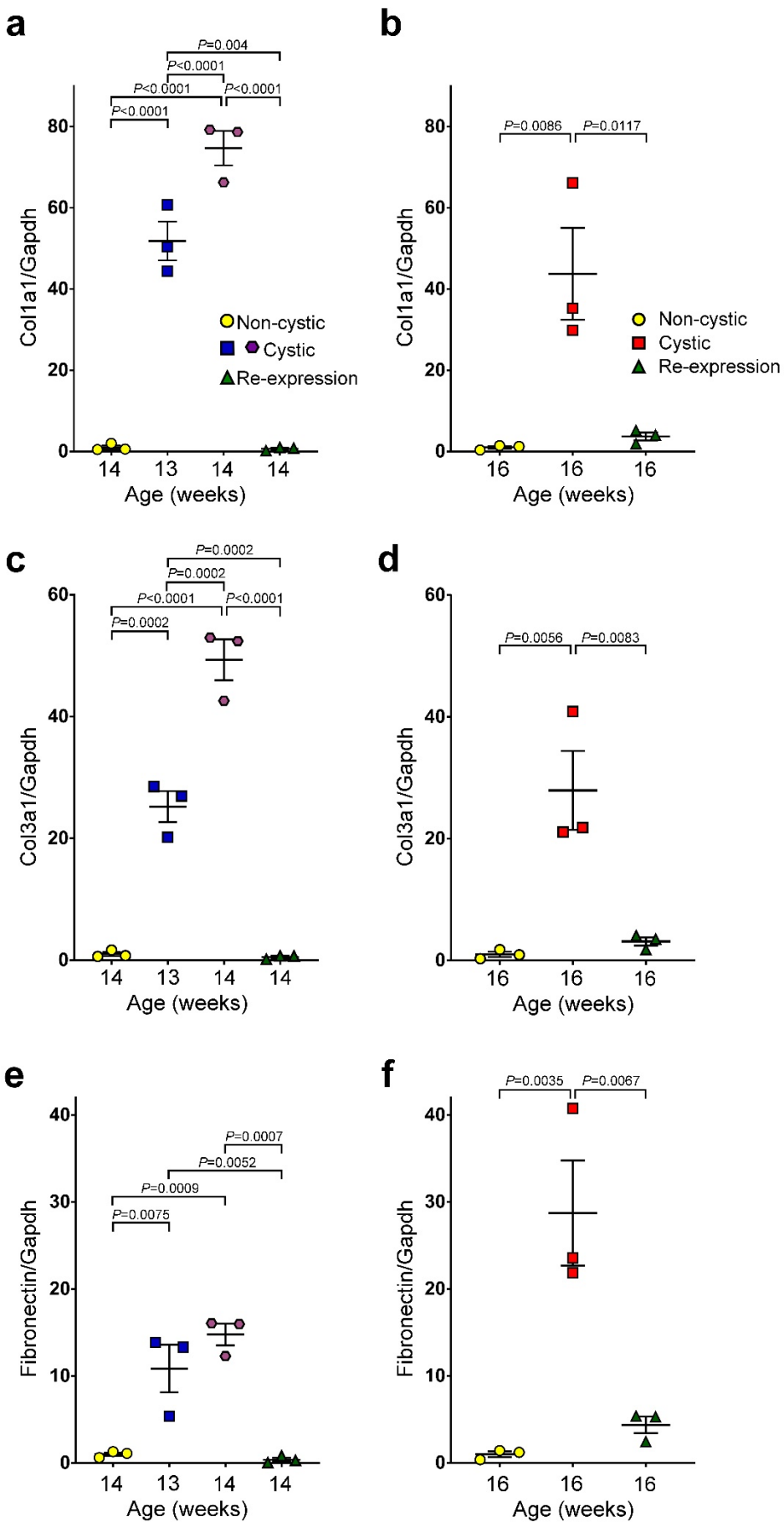

a
Mcp-1/Gapdh
P<0.0001
P<0.0001
Non-cystic
Cystic
Re-expression
Age (weeks)
16 16 16
b
Fsp1/Gapdh
P=0.0271
P=0.0037
P=0.0034
P=0.0246
Non-cystic
Cystic
Re-expression
Age (weeks)
14 13 14 14
c
Fsp1/Gapdh
P=0.0067
P=0.0231
Age (weeks)
16 16 16
d
kDa
Non-cystic
Cystic
Re-expression
Fsp1
Hsp90
14 weeks (*)
13 weeks
14 weeks (**)
14 weeks
e
Fsp1/Hsp90
P=0.0026
P=0.0054
Age (weeks)
14 13 14
P=0.0269
P=0.0417
14 14 14
f
16 weeks
g
P=0.0269
P=0.0417
16 16 16
h
IL-6/Gapdh
P=0.0127
P=0.0170
P=0.0204
P=0.0106
14 13 14 14
i
P=0.0005
P=0.0005
16 16 16

Supplementary Figure 11: Cytokine and the monocyte marker Fsp1 expression are correlated with PC2 inactivation. (**a**) Mcp-1 mRNA expression in whole kidney from 16-week-old *Pkd2*$^{Cre/Flpo}$ mice for each treatment indicated by the color key. (**b, c**) Fsp1 mRNA expression in whole kidney from *Pkd2*$^{Cre/Flpo}$ mice at the indicated ages for each treatment indicated by the color key. “Non-cystic”, no treatment; “Cystic”, doxycycline from P28-42; “Re-expression”, doxycycline from P28-42 and tamoxifen for 7 days beginning at 13 weeks. Fold-change of expression normalized to Gapdh is shown relative to mean for non-cystic control kidneys which is set to 1.0. (**d-g**) Changes in Fsp1 protein expression with *Pkd2* inactivation and re-expression. (**d**) Immunoblots and (**e**) corresponding quantitation using densitometric ratios of three biological samples for each treatment indicated by the colors noted above and at the indicated ages. Kidneys at 14 weeks age with re-expression beginning from 13 weeks show decreased Fsp1 compared to both 13 week and 14 week old kidneys without re-expression. (**f**) Immunoblots and (**g**) corresponding quantitation using densitometric ratios of three biological samples for each treatment in 16-week-old mice. Hsp90 serves as loading control. Densitometric ratios of Fsp1 to Hsp90 for each lane are shown relative to the mean of the ratio in the non-cystic samples which is set a value of 1.0. (**h, i**) IL-6 mRNA expression in whole kidney lysates from *Pkd2*$^{Cre/Flpo}$ mice at the indicated ages and with the treatments indicated by the color key. IL-6 expression after re-expression at 14 weeks is lower than before re-expression at 13 weeks indicating reversal of inflammation. *n*=3 for each group. Multiple group comparisons were performed using one-way ANOVA followed by Tukey’s multiple comparison test and are presented as the mean ± SEM.

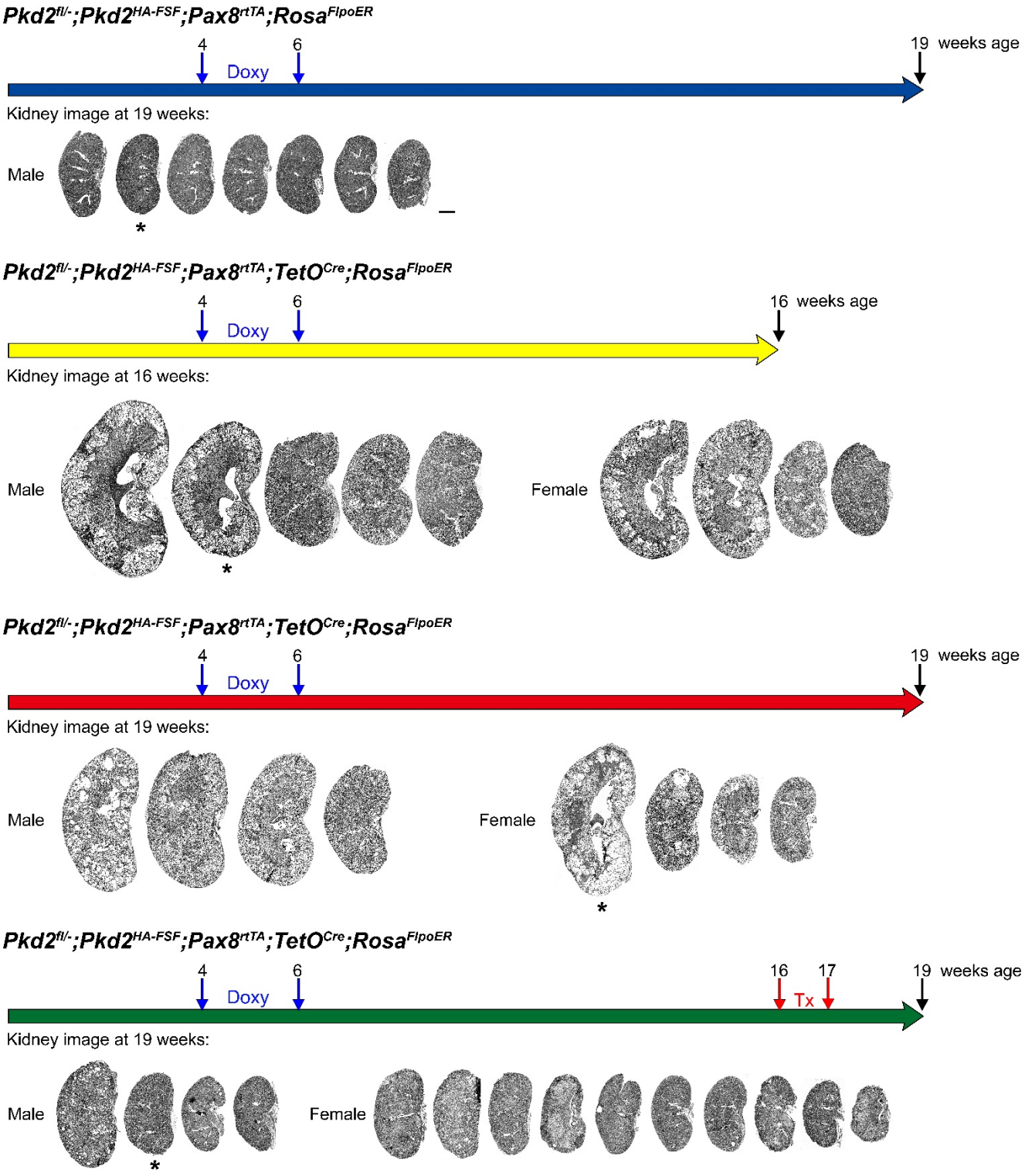


Supplementary Figure 12: Cytokine expression occurs in areas of macrophage infiltration. F4/80 co-staining with TNF-α and cleaved caspase-1 at (**a**) 14 weeks and (**b**) 16 weeks age in *Pkd2*$^{Cre/Flpo}$ mice undergoing the indicated treatments. Non-cystic, no treatment; Cystic, doxycycline from P28-42; Re-expression, doxycycline from P28-42 and tamoxifen for 1 week beginning at 13 weeks. Cysts with infiltrating F4/80 positive macrophages on the basal aspect of the cyst lining (white arrowheads) often show TNF-α and cleaved caspase-1 expression on the apical cyst luminal aspect as well as in the cyst lumens (green arrowheads). Occasional macrophages expressing cleaved caspase-1 are also seen (yellow arrowheads, **a**). There is absence of staining for macrophages, TNF-α and cleaved caspase-1 in kidney after re-expression of PC2 at both (**a**) 14 weeks and (**b**) 16 weeks. At least one section from 3 kidneys for each treatment and age group were examined for each image shown. Scale bar, 100 μm.

1

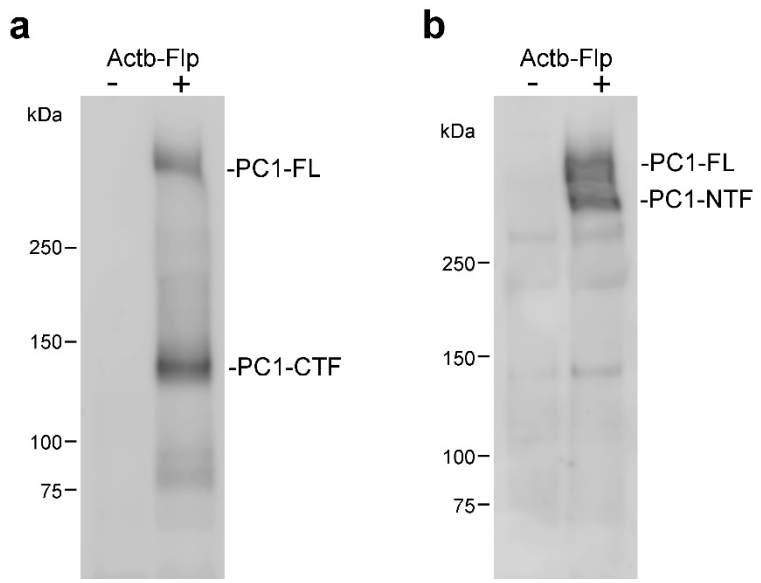

a
b
c
d
e
f
Col1a1/Gapdh
Col3a1/Gapdh
Fibronectin/Gapdh
Age (weeks)
Non-cystic
Cystic
Re-expression
P=0.004
P<0.0001
P<0.0001
P<0.0001
P<0.0001
P=0.0086
P=0.0117
P=0.0002
P=0.0002
P<0.0001
P<0.0001
P=0.0002
P=0.0056
P=0.0083
P=0.0007
P=0.0052
P=0.0009
P=0.0075
P=0.0035
P=0.0067

Supplementary Figure 13: Extracellular matrix expression and myofibroblast activation reverses with PC2 re-expression. *Col1a1* (**a, b**), *Col3a1* (**c, d**) and fibronectin (**e, f**) mRNA expression in whole kidney from *Pkd2*$^{Cre/Flpo}$ mice at the indicated ages for each treatment indicated by the color key in **a, b**. "Non-cystic", no treatment; "Cystic", doxycycline from P28-42; "Re-expression", doxycycline from P28-42 and tamoxifen for 1 week beginning at 13 weeks age. Fold-change of expression normalized to Gapdh is shown relative to mean for non-cystic control kidneys which is set to 1.0. *n*=3 for each group. Multiple group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test and are presented as the mean ± SEM.

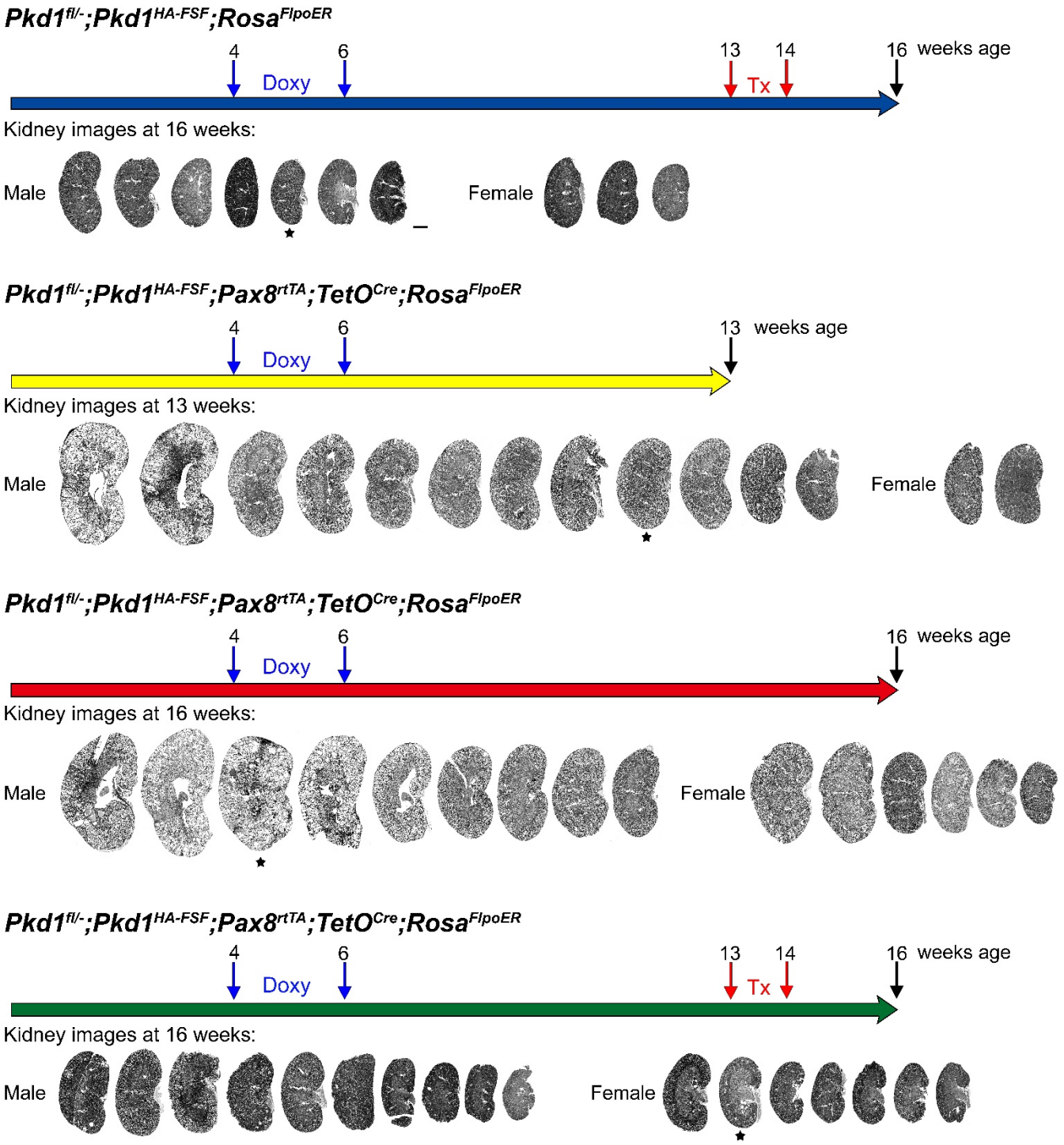


Supplementary Figure 14: Primary data used for 16-week *Pkd2* reactivation models Figure 7a, c. The four genotypes are indicated, and the colors of the timelines correspond to the colors used for the corresponding genotype groups in Fig. 7. The timing of oral doxycycline (Doxy) administration from weeks 4-6 and of administration of intraperitoneal tamoxifen (Tx) for 7 days beginning at week 16 are

indicated as is the time of examination of the kidneys at the end of the timeline arrow. Each image used for the cystic index calculation is provided, separated by sex. The asterisk in each group marks the images also used in Fig. 7a from each genotype group. Scale bar, 2 mm.



Supplementary Figure 15: PC1-HA expression in vivo following Flp recombinase activation. FLAG-PC1-HA expression after the immunoprecipitation with anti-HA antibody detected by (**a**) anti-HA and (**b**) anti-FLAG immunoblotting of the lung tissue samples from *Pkd1*$^{fl/—}$;*Pkd1*^{FSF};*Rosa*^{FlpoER} with (+) or without (-) Actb-Flp (germline Flp deleter) at 3 weeks age. Actb-Flp (+) induces expression of PC1. PC1-FL, PC1 full length protein; PC1-CTF, PC1 C-terminal fragment; PC1-NTF, PC1 N-terminal fragment. One of two independent biological repeat experiments is shown.

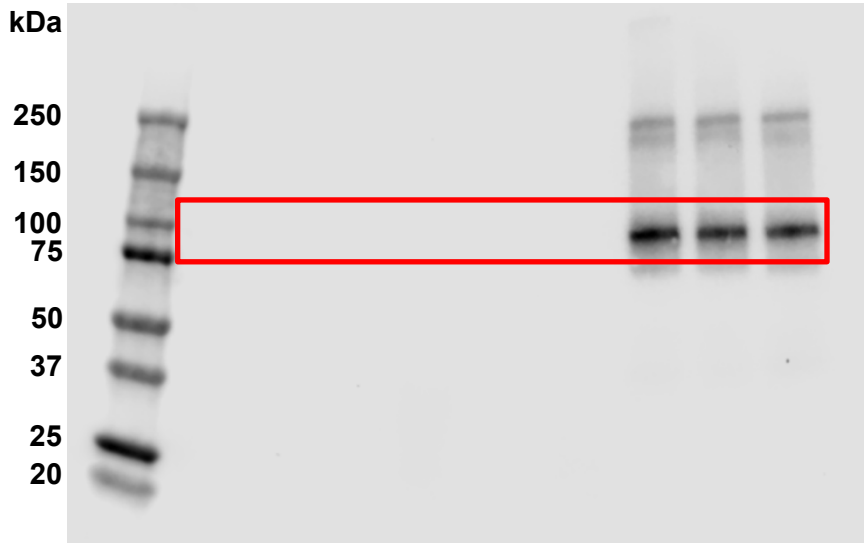


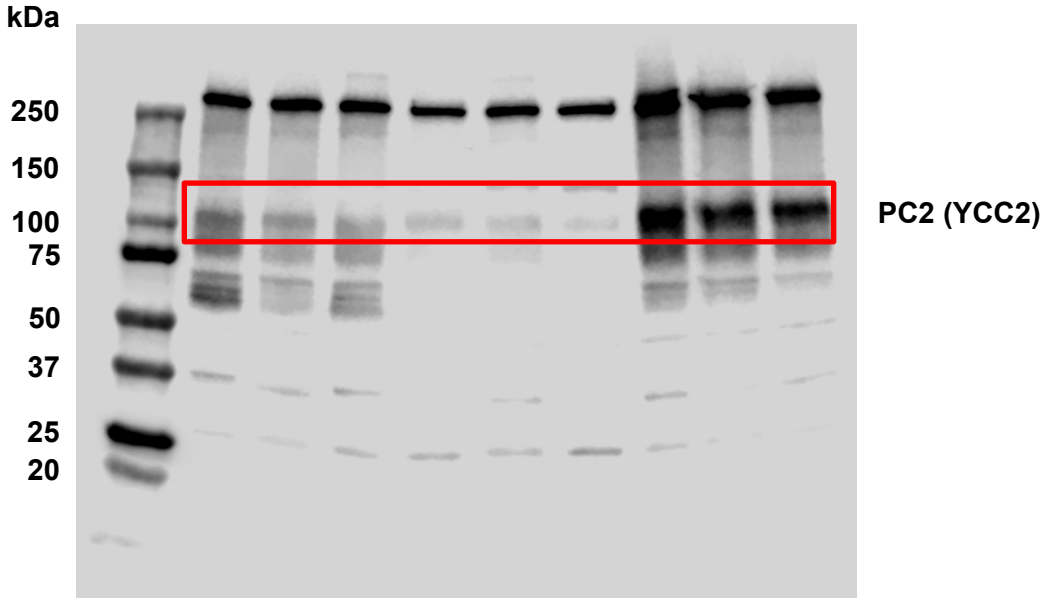
Supplementary Figure 16: Primary data used for *Pkd1* models Figure 8a, c. The four genotypes are indicated, and the colors of the timelines correspond to the colors used for the corresponding genotype groups in Fig. 8. The timing of oral doxycycline (Doxy) administration from weeks 4-6 and of administration of intraperitoneal tamoxifen (Tx) for 7 days beginning at week 13 are indicated as is the time of examination of the kidneys at the end of the timeline arrow. Each image used for the cystic

index calculation is provided, separated by sex. The asterisk in each group marks the images also used in Fig. 8a from each genotype group. Scale bar, 2 mm.

Data Sources for Supplementary Figures:

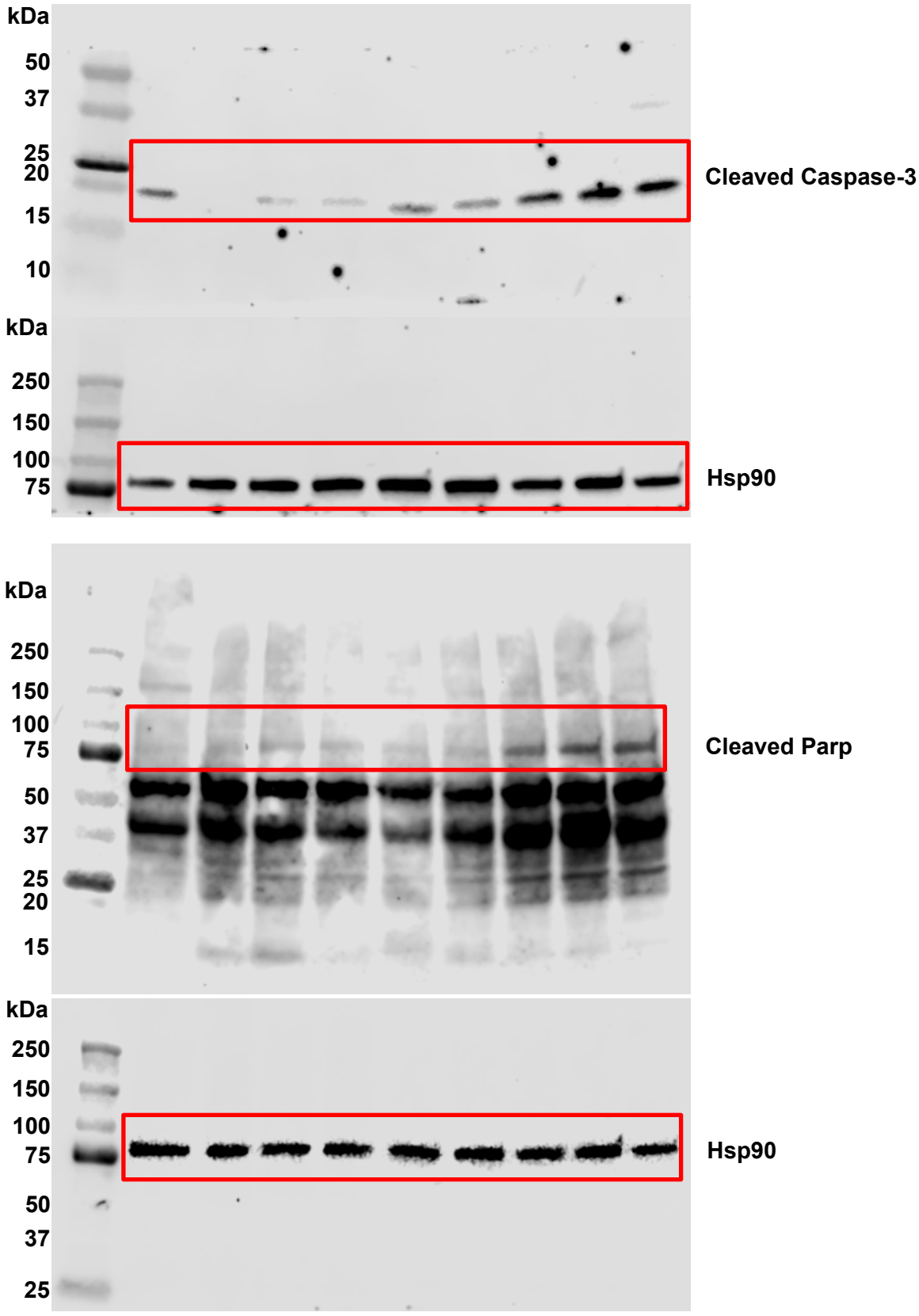
Supplemental Figure 3a



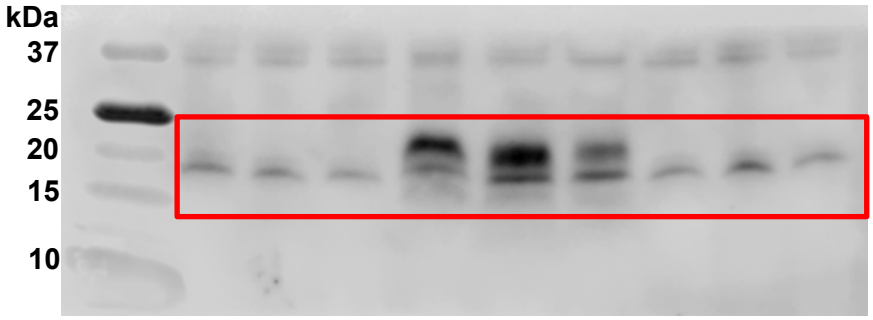


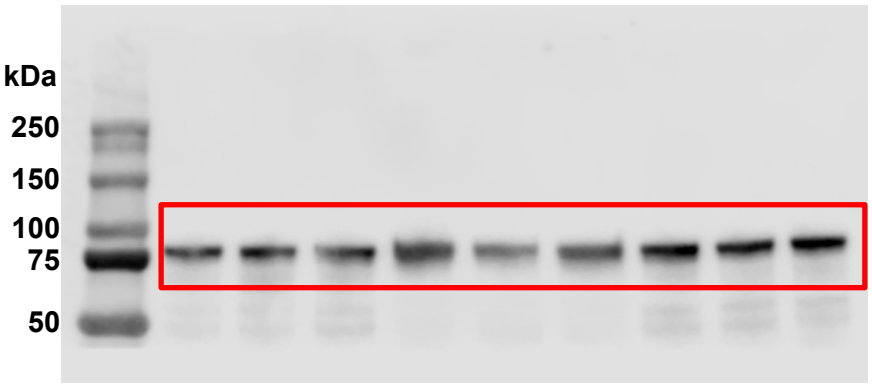

kDa
250
150
100
75
50
37
25
20
Hsp90

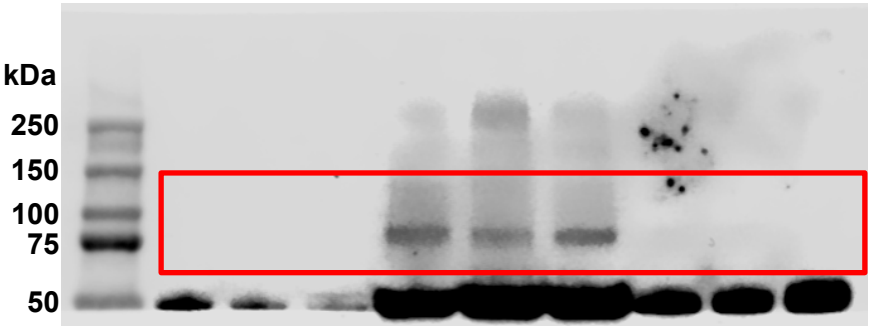
Supplementary Figure 8a

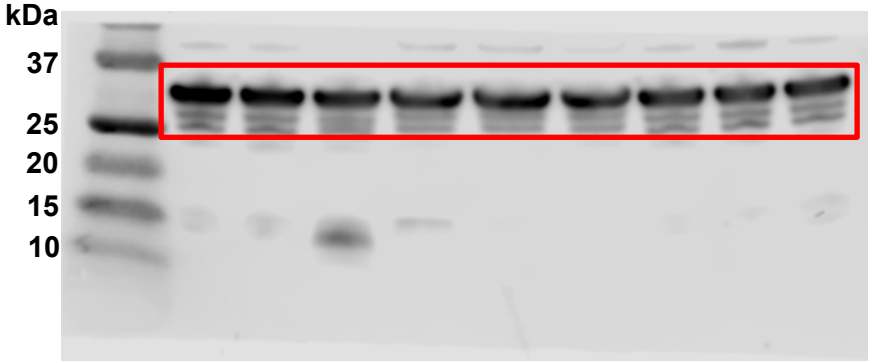


Supplementary Figure 8c

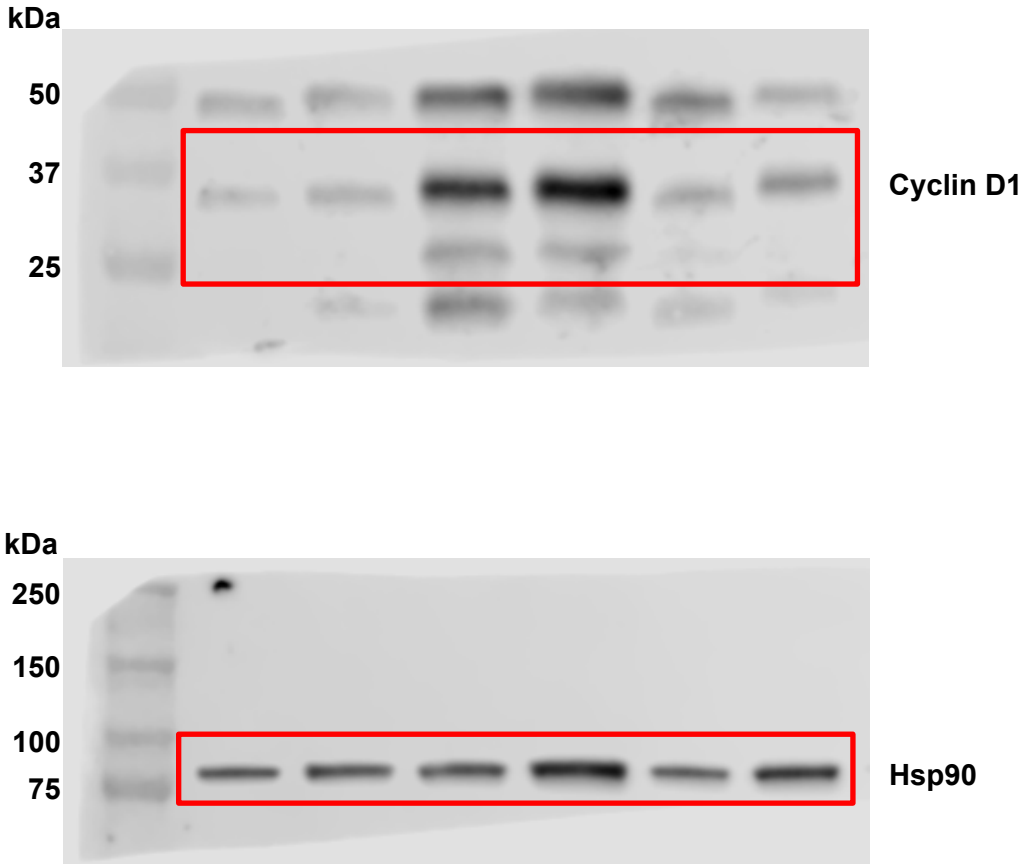




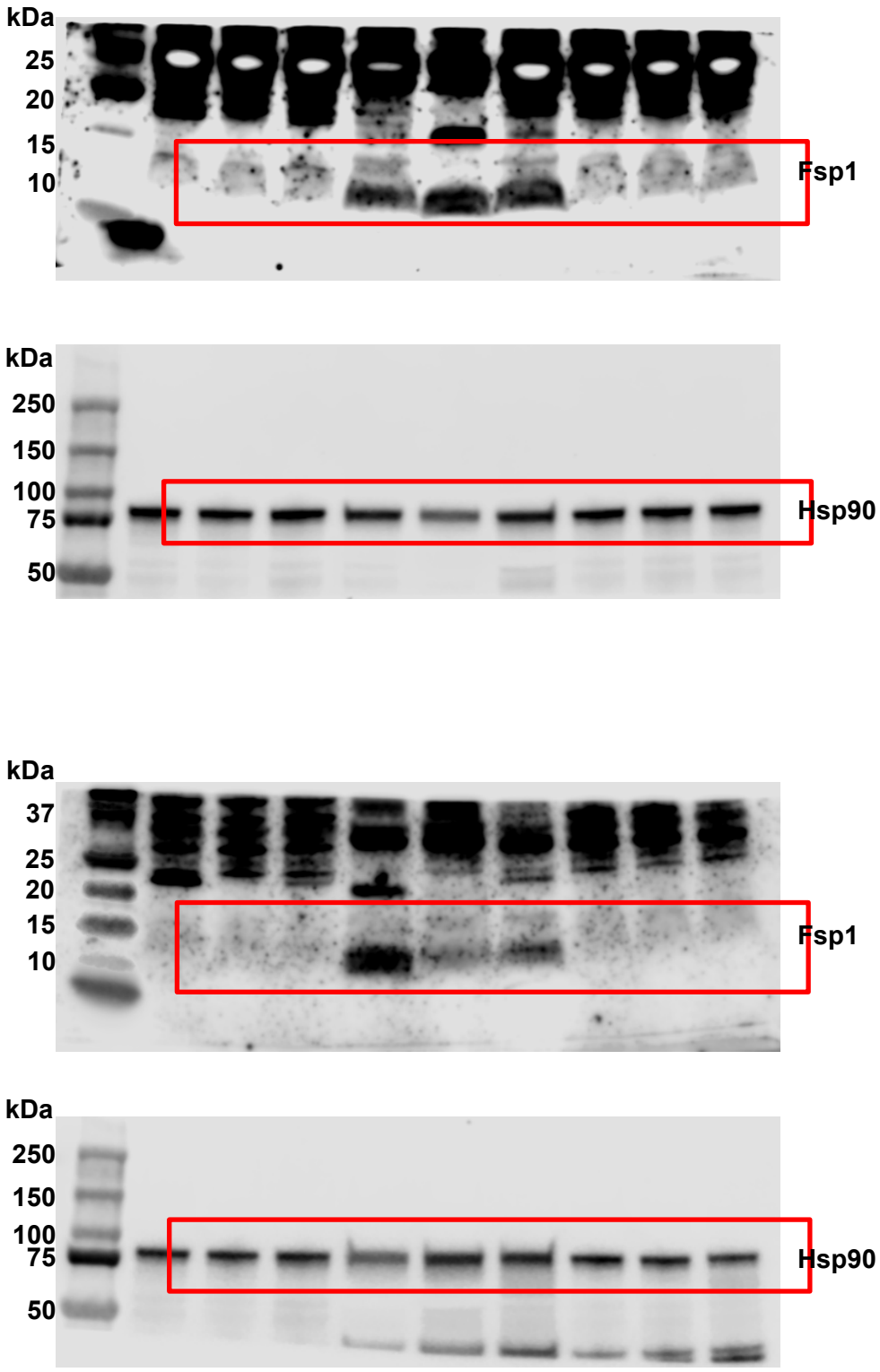




Supplementary Figure 10b



Supplementary Figure 11d



Supplementary Figure 11f