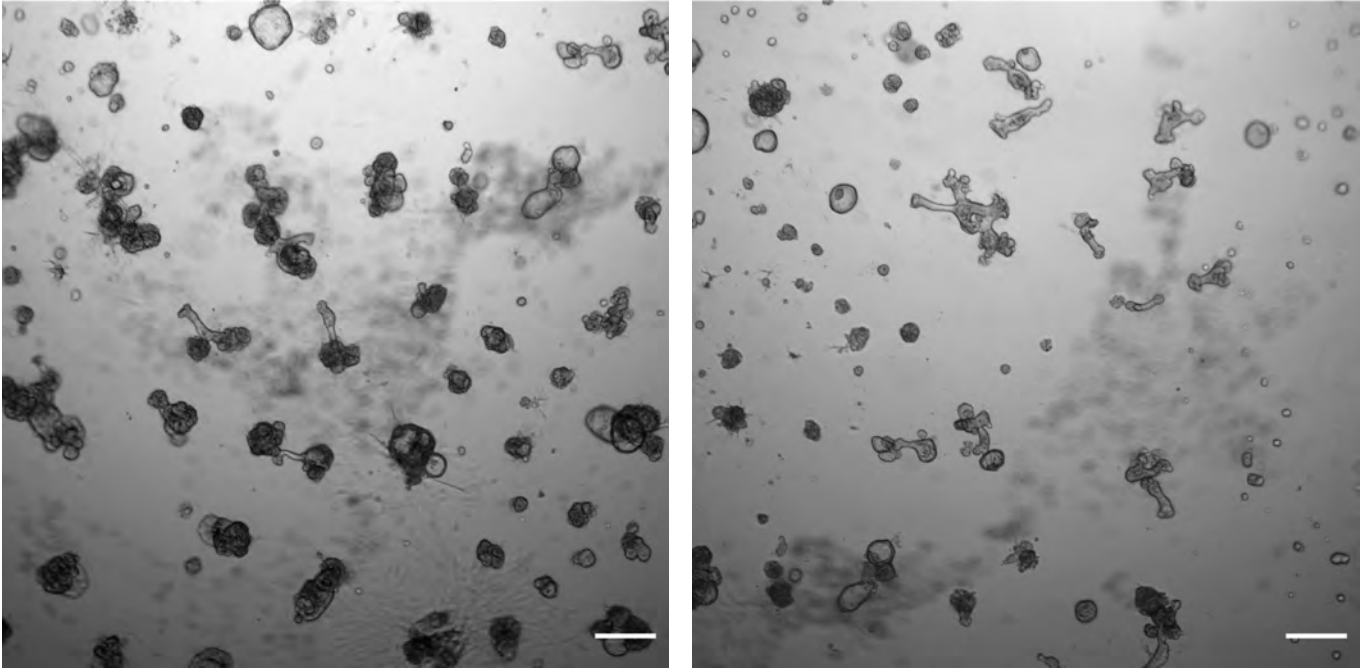


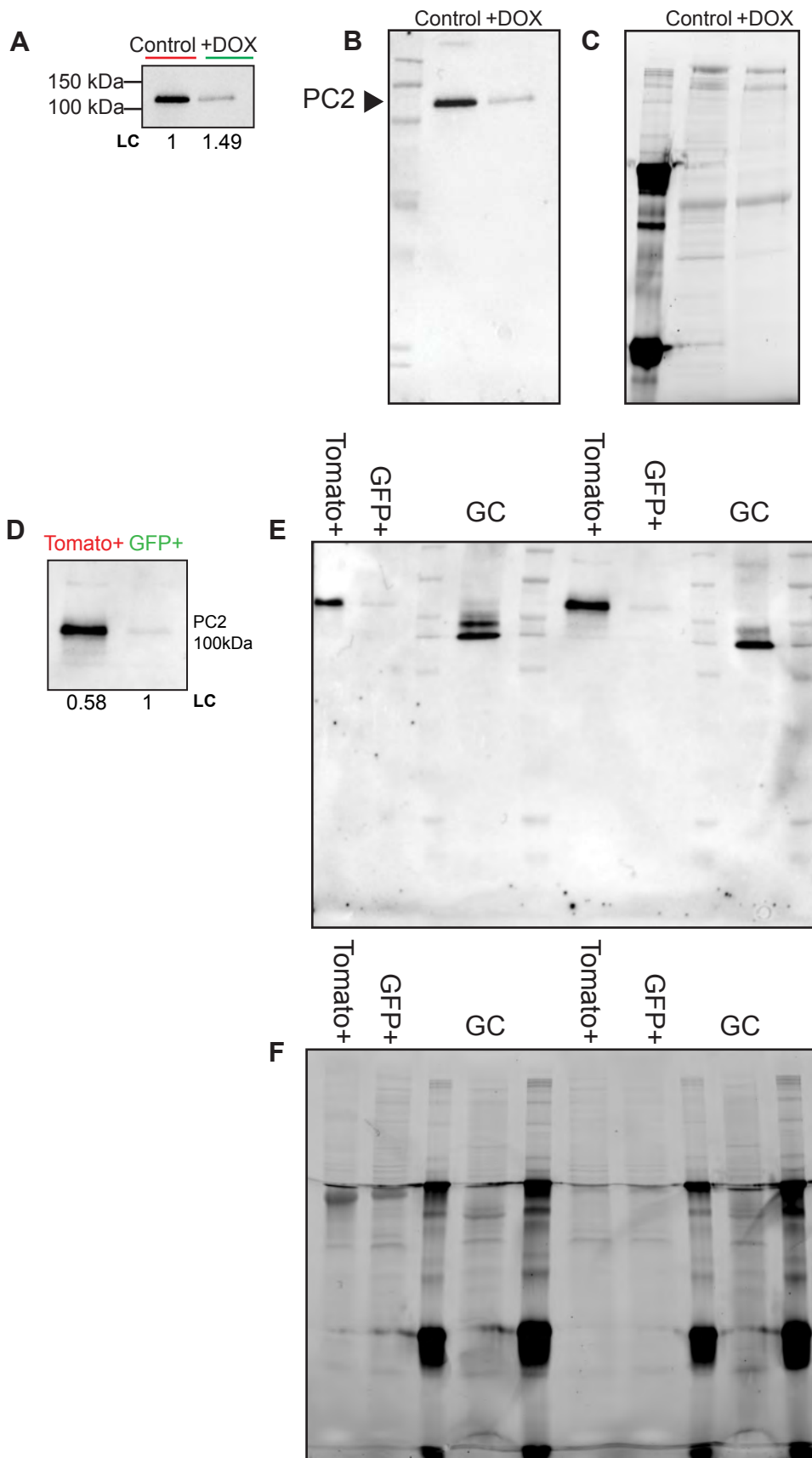
Fig. S1: Tubuloid culture with *Pkd1fl/fl Pax8rtTA TetOCre +mTmG*

A



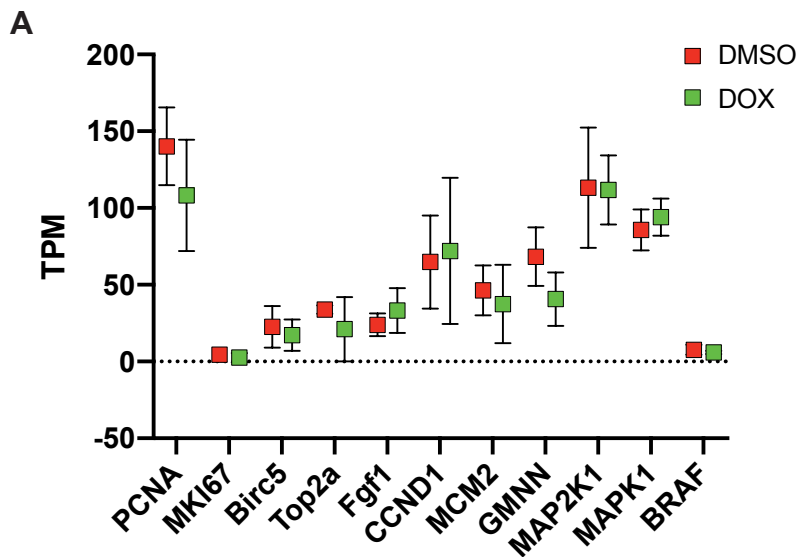
(A) Culture protocol can implement primary cells from mouse models with other genotypes. Brightfield images of tubuloids from *Pkd1fl/fl Pax8rtTA TetOCre +mTmG*. Magnification 4X, Scale Bar 250 μ m.

Fig. S2: Western blots and total loading controls for PC2



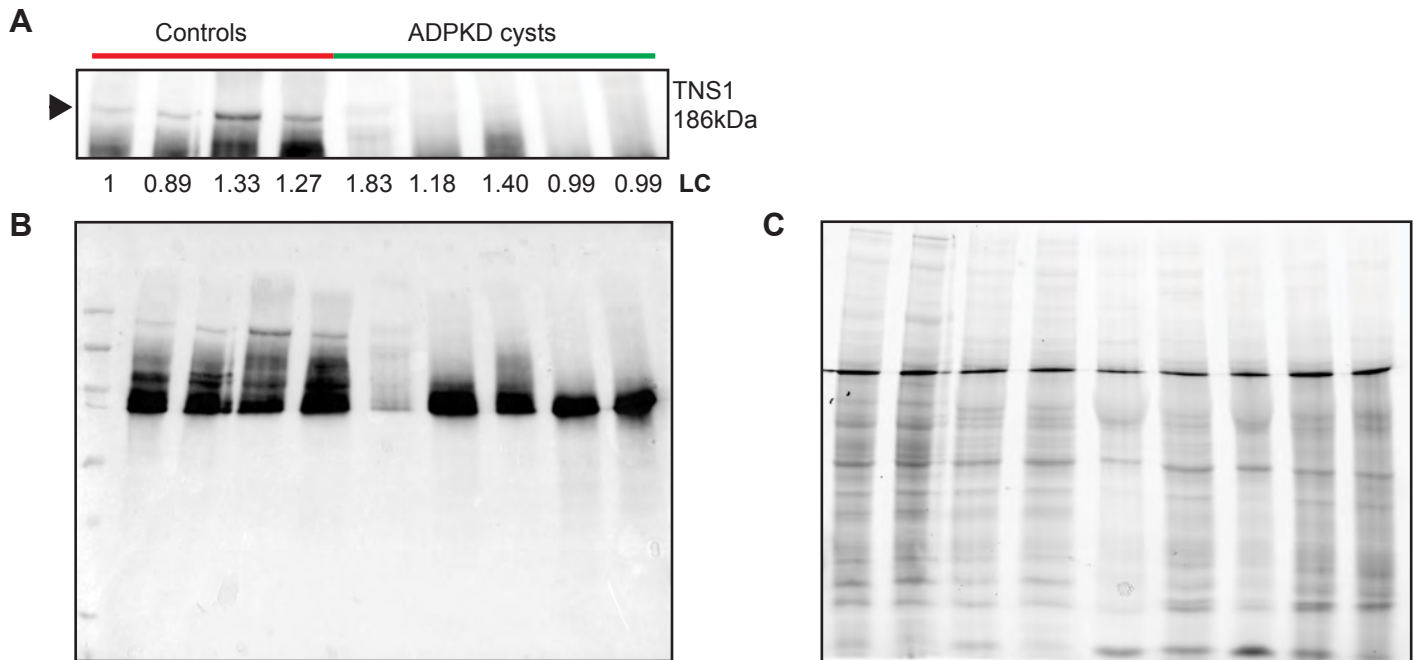
(A) Western blot for PC2 abundance, normalized to total loaded protein (LC) (n=3; two-tailed Student's t-test, p-value = 0.0420). (B) Uncropped representative western blot for PC2 in organoids as seen in Figure 4C. (C) Total loaded protein used for normalization of blot in (A). (D) Western blot to confirm significant decrease in PC2 abundance in green fluorescent cells (GFP+) when compared to controls (Tomato+), normalized to total loaded protein (LC) (two-tailed Student's t-test, p-value = 0.0336). (E) Uncropped representative western blot for PC2 in FACS cells as seen in Figure 4D, with gating control (GC). (F) Total loaded protein used for normalization of blot in (D).

Fig. S3: Expression of proliferation genes in *Pkd2*- tubuloids



(A) A profile of ten proliferation genes reveals no significant change in expression between doxycycline treated (green square) and DMSO control (red square) tubuloids (n=4). Significance was defined in a two-tailed Student's t-test as a p-value of less than or equal to 0.05. Bars represent +/-SEM and those not seen are hidden behind boxes.

Fig. S4: Western blots and total loading controls for tensin-1



(A) Western blot exhibits significant decrease in tensin-1 (TNS1) in human male ADPKD cysts (n=5) compared to normal human male kidneys (n=4) and normalized to total loaded protein (LC) (two-tailed Student's t-test, p-value = 0.0340). (B) Uncropped western blot for TNS1 in human tissue as seen in Figure 6D. (C) Total loaded protein used for normalization of blot in (A).

Table S1: GDNF pulse differentially expressed genes

A list of genes with reported fold change, p-value, and adjusted p-value (≤ 0.05) resulting from differential expression (DESeq) analysis of GDNF pulsed (n=4 cultures) and control (n=4 cultures) tubuloids.

[Click here to Download Table S1](#)

Table S2: GDNF pulse differential pathway analysis

A list of significantly altered gene ontology (GO) terms from pathway analysis of GDNF pulsed (n=4 cultures) and control (n=4 cultures), determined by nominal p-value (≤ 0.05) along with reported values for size, es, nes, fdrQVal, fwerPVal, and rankAtMax. From significantly altered GO terms, non-neuro specific GO terms and MGI GO number with nominal p-value were included in REViGO representation.

[Click here to Download Table S2](#)

Table S3: GDNF pulse PC TF analysis

GENE	GDNF1	GDNF2	GDNF3	GDNF4	CTL1	CTL2	CTL3	CTL4	One-tailed Student's t-test
<i>Gata3</i>	32.26604	15.72488	35.3853	40.9055	24.96285	9.642504	24.51733	13.17762	0.049949809
<i>Tfap2b</i>	3.052619	1.859329	4.514373	7.2772	2.650732	0.593037	3.272445	3.129981	0.115260295
<i>Ehf</i>	33.49256	13.52809	37.78405	40.72878	21.69552	5.139788	22.66092	17.01686	0.04549041
<i>Irf1</i>	41.69357	27.52123	43.46952	37.97642	31.63945	24.41116	29.73884	19.68488	0.022550114
<i>Irf9</i>	24.43075	14.76461	23.20745	22.94073	13.58074	14.81047	16.50369	10.28917	0.013168292
<i>Stat1</i>	10.12961	7.084977	6.76742	9.216172	7.086282	5.745113	5.906681	4.200367	0.021996706
<i>Tsc22d3</i>	22.12605	18.84984	17.24039	13.46117	14.82216	29.72123	17.12784	17.69298	0.315742822
<i>Nfia</i>	9.771741	8.088848	6.936182	5.848866	4.677708	6.133078	4.613625	1.950422	0.016812074
<i>Maf</i>	9.315971	7.586065	9.245634	7.061692	5.883636	4.943121	7.791981	2.774088	0.023882416
<i>Nfix</i>	8.523388	7.49054	10.18001	8.829175	6.379807	6.788277	7.339321	3.597376	0.017148745
<i>Glis2</i>	23.42146	20.22029	36.06616	29.29902	18.2061	14.47908	20.82394	10.46062	0.01755182
<i>Grl2</i>	13.71164	7.388358	15.9763	11.19042	11.66951	1.548885	9.833759	6.625391	0.078952635

Table S3: GDNF pulse PC TF analysis

Transcripts per million (TPM) are reported for each of the 12 principal cell specific transcription factors (Fig. 3) from GDNF pulsed (n=4 cultures) and control (n=4 cultures) tubuloids with p-values, determined by one-tailed Student's t-test.

Table S4: Proliferation genes in *Pkd2*- tubuloids

GENE	DOX1	DOX2	DOX3	DOX4	DMSO1	DMSO2	DMSO3	DMSO4	Two-tailed Student's t-test
<i>Pcna</i>	132.0346	72.45922	146.0151	82.279	120.8519	121.7592	174.7354	143.3277	0.198243095
<i>Mki67</i>	1.84064	0.514181	6.985879	0.887624	2.314329	4.406596	7.160676	4.035646	0.328269372
<i>Birc5</i>	15.05231	14.21884	31.59758	7.910873	3.513497	32.02806	32.26265	22.77518	0.542083124
<i>Top2a</i>	17.04601	2.512155	50.96887	13.71334	34.49851	30.01402	34.47899	36.5376	0.269189562
<i>Fgf1</i>	28.08196	54.17641	30.51607	20.29025	24.06256	13.53972	29.65386	28.47016	0.296875262
<i>Ccnd1</i>	27.52501	34.37395	110.4346	115.9078	51.53557	34.25258	105.1313	68.45452	0.806612205
<i>Mcm2</i>	32.82517	5.989377	67.66323	43.3237	70.25122	34.48768	38.81866	41.99352	0.576219144
<i>Gmnn</i>	32.78538	51.02927	58.67824	20.23719	63.26325	63.86299	95.36338	50.55107	0.076769
<i>Map2k1</i>	90.28978	103.3186	110.5618	143.1013	100.5623	153.0988	64.42082	134.8364	0.952167396
<i>Mapk1</i>	90.18806	79.34079	107.6615	99.08689	104.5303	76.477	75.97767	85.87185	0.389986305
<i>Braf</i>	5.33684	5.976084	7.261508	4.97417	11.38655	5.905678	9.284145	4.080653	0.341010592

Table S4: Proliferation genes in *Pkd2*- tubuloids

Transcripts per million (TPM) are reported for each of the 11 proliferation genes from DOX treated (*Pkd2*-) (n=4 cultures) and DMSO treated (n=4 cultures) tubuloids with p-values, determined by two-tailed Student's t-test.

Table S5: *Pkd2*- differentially expressed genes

GENE	Key	Fold Change (log2)	P-value	P-adj
<i>Tns1</i>	21817	-0.886637	1.54E-09	2.13E-05
<i>Fhod3</i>	7436	-2.2331063	6.24E-08	0.000246
<i>Plscr2</i>	16979	-0.551824	4.80E-08	0.000246
<i>Rp2h</i>	18538	-0.7319563	7.13E-08	0.000246
<i>Ttc7b</i>	22225	0.96903559	1.01E-07	0.000278
<i>Lurap1l</i>	11828	-0.8584363	2.04E-07	0.000469
<i>Cyp11a1</i>	5398	-3.95454	2.50E-07	0.000493
<i>Uap111</i>	22345	1.07427609	5.74E-07	0.000991
<i>Cnpy3</i>	4867	0.74646905	7.22E-07	0.001109
<i>Renbp</i>	18194	0.9932179	1.81E-06	0.002502
<i>Cdsn</i>	4356	4.45575235	5.54E-06	0.006956
<i>Grhl3</i>	9482	5.2921039	1.05E-05	0.012112
<i>Zhx2</i>	23940	0.70640915	1.35E-05	0.014314
<i>Ankrd37</i>	2440	-3.1662621	1.48E-05	0.014521
<i>Hdhd2</i>	9818	0.34203803	1.58E-05	0.014521
<i>B630005N14Rik (Bmt2)</i>	3116	-0.8049442	1.84E-05	0.015883
<i>Heatr2</i>	9823	0.70116212	2.06E-05	0.016785
<i>Alg6</i>	2292	-0.8873579	2.24E-05	0.017181
<i>Eprs</i>	6736	1.02868284	2.61E-05	0.018066
<i>Slc3a2</i>	19719	0.83796655	2.49E-05	0.018066
<i>Nfkbiz</i>	14381	-1.2770209	2.76E-05	0.018139
<i>Tph1</i>	21873	-5.1697975	3.05E-05	0.019149
<i>Psen2</i>	17578	1.45523883	3.7E-05	0.02223
<i>Lars</i>	11396	0.94356128	5.13E-05	0.029549
<i>Cox7a1</i>	5027	-3.5191817	5.72E-05	0.030392
<i>Slc1a3</i>	19515	2.13120498	5.66E-05	0.030392
<i>Krt5</i>	11219	9.00795606	7.85E-05	0.040196
<i>Ccnd2</i>	4084	0.63569798	9.14E-05	0.042103
<i>Cd38</i>	4188	1.03533186	9.09E-05	0.042103
<i>Mapkap1</i>	12046	0.53194512	9.02E-05	0.042103
<i>Slc6a9</i>	19790	1.57457384	9.58E-05	0.042698
<i>Adamts5</i>	2030	-1.623841	0.00011	0.047624
<i>Gaa</i>	7711	0.88069113	0.000117	0.047624
<i>Mfsd7c</i>	12332	1.89261777	0.000117	0.047624
<i>Etv1</i>	6850	0.83819896	0.000123	0.048598

Table S5: *Pkd2*- differentially expressed genes

A list of genes with reported fold change, p-value, and adjusted p-value (≤ 0.05) resulting from differential expression (DESeq) analysis of DOX treated (*Pkd2*-) (n=4 cultures) and DMSO treated (n=4 cultures) tubuloids, represented in volcano plots and Heatmapper in Fig. 6.

Table S6: Annotated *Pkd2* - differentially expressed genes

Down- and upregulated differentially expressed genes in *Pkd2* - (DOX treated) tubuloids annotated with protein, function, and corresponding fold change. Functions were summarized described from National Library of Medicine (US), National Center for Biotechnology Information.

GENE (DOWN)	Protein	Function [Human]	Fold Change (log2)
<i>Tph1</i>	Tryptophan hydroxylase 1	encodes protein that catalyzes the rate-limiting step of serotonin biosynthesis	-5.1697975
<i>Cyp11a1</i>	Cholesterol side-chain cleavage enzyme, mitochondrial	encodes protein that localizes to the mitochondrial inner membrane and catalyzes the conversion of cholesterol to pregnenolone, the rate-limiting step in the synthesis of the steroid hormones	-3.95454
<i>Cox7a1</i>	Cytochrome c oxidase subunit 7A1, mitochondrial	encodes component of heteromeric complex involved in electron transfer	-3.5191817
<i>Ankrd37</i>	Ankyrin repeat domain 37	undefined	-3.1662621
<i>Fhod3</i>	FH1/FH2 domain-containing protein 3	encodes protein that is part of the diaphonous-related formins and has a predicted role in actin filament polymerization	-2.2331063
<i>Adamts5</i>	A disintegrin and metalloproteinase with thrombospondin motifs 5	encodes a protein in the ADAMTS family that is involved in proteoglycan cleavage	-1.623841
<i>Nfkbiz</i>	NF-kappa-B inhibitor zeta	encodes a member of the ankyrin-repeat family and is involved in inflammatory responses	-1.2770209
<i>Alg6</i>	Alpha-1,3-glucosyltransferase	encodes a protein of the ALG6/8 glucosyltransferase family that catalyzes the addition of glucose residue for N-linked glycosylation	-0.8873579
<i>Tns1</i>	Tensin-1	encodes a protein that localizes to the focal adhesions and crosslinks with the actin cytoskeleton; involved in signal transduction	-0.886637
<i>Lurap11</i>	Leucine rich adaptor protein 1-like	undefined	-0.8584363
<i>B630005N1</i> <i>4Rik (Bmt2)</i>	base methyltransferase of 25S rRNA 2	undefined	-0.8049442
<i>Rp2h</i>	retinitis pigmentosa 2 homolog or RP2 activator of ARL3 GTPase	predicted gene product may be involved in beta-tubulin folding	-0.7319563
<i>Plscr2</i>	Phospholipid scramblase 2	encodes a membrane protein that mediates plasma membrane exposure to phospholipids and phosphatidylserines	-0.551824

Down- and upregulated differentially expressed genes in *Pkd2*- (DOX treated) tubuloids annotated with protein, function, and corresponding fold change. Functions were summarized described from National Library of Medicine (US), National Center for Biotechnology Information.

GENE (UP)	Protein	Function [Human]	Fold Change (log2)
<i>Krt5</i>	Keratin 5	encodes a member of the keratin gene family coexpressed during differentiation of epithelial tissues	9.00795606
<i>Grhl3</i>	Grainyhead-like transcription factor 3	encodes for a protein that may act as a transcription factor during development to stimulate cell migration	5.2921039
<i>Cdsn</i>	Corneodesmosin	encodes a protein involved in comeocyte maturation and localizes to cornified epithelia and epidermis	4.45575235
<i>Slc1a3</i>	Solute carrier family 1 member 3	encodes a high affinity glutamate transporter	2.13120498
<i>Mfsd7c</i>	FLVCR heme transporter 2	encodes a transmembrane calcium transporter that may be involved in development	1.89261777
<i>Slc6a9</i>	solute carrier family 6 member 9	encodes a transporter involved in inhibition of glycine signaling	1.57457384
<i>Psen2</i>	Presenilin-2	encodes a component of multi-subunit gamma secretase complex that play roles in cellular processes and calcium homeostasis	1.45523883
<i>Uap111</i>	UDP-N-acetylglucosamine pyrophosphorylase 1 like 1	undefined	1.07427609
<i>Cd38</i>	CD38 molecule	encodes a transmembrane glycoprotein that synthesizes an intracellular calcium ion mobilizing messenger	1.03533186
<i>Eprs</i>	glutamyl-prolyl-tRNA synthetase	encodes a multifunctional aminoacyl-tRNA synthetase that catalyzes aminoacylation of glutamic acid and proline tRNA species	1.02868284
<i>Renbp</i>	Renin binding protein	encodes a product that inhibits renin and is a GlcNAc 2-epimerase	0.9932179
<i>Ttc7b</i>	Tetratricopeptide repeat protein 7B	undefined	0.96903559
<i>Lars</i>	Leucyl-tRNA synthetase	encodes a cytosolic leucine-tRNA synthetase that catalyzes the ATP-dependent ligation of L-leucine to tRNA	0.94356128
<i>Gaa</i>	Glucosidase alpha, acid	encodes lysosomal alpha-glucosidase	0.88069113

Down- and upregulated differentially expressed genes in *Pkd2*- (DOX treated) tubuloids annotated with protein, function, and corresponding fold change. Functions were summarized described from National Library of Medicine (US), National Center for Biotechnology Information.

GENE (UP)	Protein	Function [Human]	Fold Change (log2)
<i>Etv1</i>	ETS variant transcription factor 1	encodes a protein that plays a role in cell growth, angiogenesis, migration, proliferation, and differentiation	0.83819896
<i>Slc3a2</i>	Solute carrier family 3 member 2	encodes a transporter that regulates intracellular calcium levels and transports L-type amino acids	0.83796655
<i>Cnpy3</i>	Canopy FGF signaling regulator 3	encodes a protein that aids in toll-like receptor maturation and acts as a chaperone for protein export	0.74646905
<i>Zhx2</i>	Zinc fingers and homeoboxes 2	encodes for nuclear homodimeric transcriptional repressors that interact with the A subunit of nuclear factor-Y	0.70640915
<i>Heatr2</i>	Dynein axonemal assembly factor 5	encodes a protein needed for axonemal dynein arms in organisms with motile cilia and flagella	0.70116212
<i>Ccnd2</i>	Cyclin D2	encodes a protein in the cyclin family and functions as a regulator of CDK kinases	0.63569798
<i>Mapkap1</i>	Mitogen-activated protein kinase associated protein 1	encodes a protein similar to stress activated protein kinases	0.53194512
<i>Hdhd2</i>	Haloacid dehalogenase-like hydrolase domain containing 2	undefined	0.34203803