Supplementary material to

A role for polycystin-1 and polycystin-2 in neural progenitor cell differentiation

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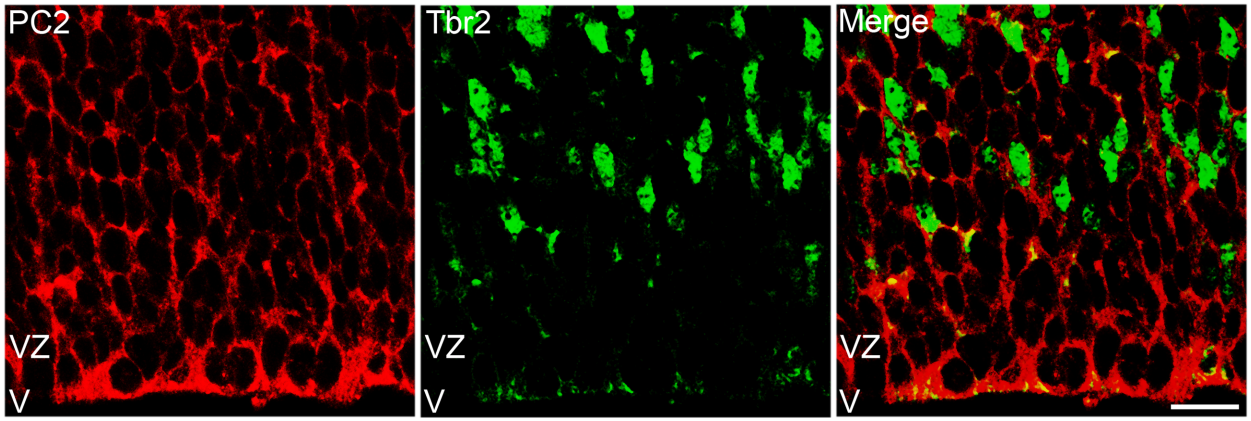
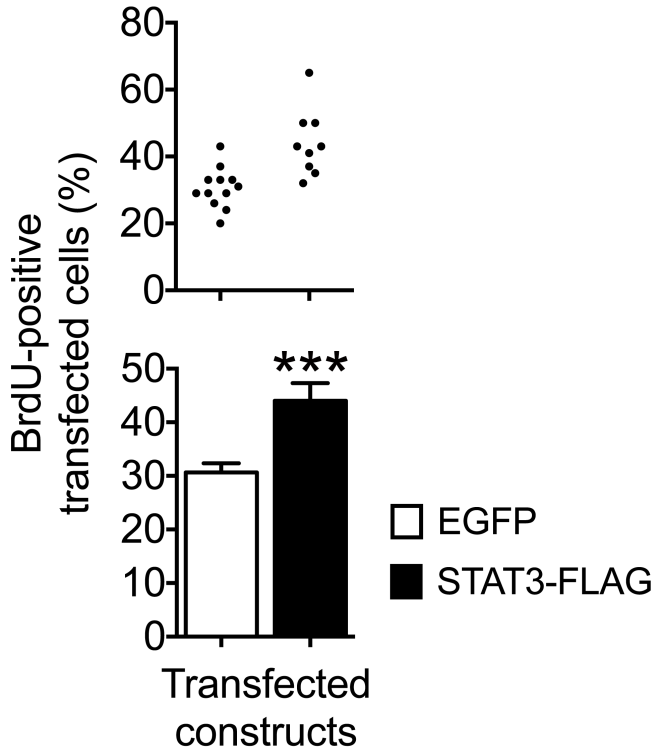


Suppl. Fig. S1 PC2 can be detected in Tbr2-positive cells during neurogenesis. Coronal sections of E14.5 embryonic mouse brains were analyzed by indirect immunofluorescence staining with a monoclonal antibody to PC2 followed by a polyclonal antibody to Tbr2. A detail picture showing the VZ including the transition to the subventricular zone is shown. PC2 can be detected in Tbr2-positive cells. Scale bar, 15 μm. V, ventricle; VZ, ventricular zone.



Suppl. Fig. S2 STAT3 overexpression leads to an increase in the number of proliferating NPCs. Primary cells prepared from the neocortices of E13.5 mice were cultured in the presence of 20 ng/ml bFGF, transfected on days in vitro 1 (DIV1) with the indicated constructs, and subjected to BrdU labeling 3 days later (DIV4). The percentage of BrdU-positive transfected cells was assessed. Overexpression of STAT3-FLAG increases the percentage of BrdU-positive proliferating cells (to 144%) when compared to the EGFP control. Data are presented as means ± SEM in a histogram (means are 30.6 ± 1.7 for EGFP and 44 ± 3.3 for STAT3-FLAG; ***$p < 0.005$; Mann-Whitney test; 3 replicates) and in a scatter plot (one dot represents the percentage of BrdU-positive transfected cells of a group of 100 analyzed transfected cells).

Supplementary table 1

Figure	Experimental schedule	Number of independent experiments (replicates)	Number of data points measured (number of cells analyzed, number of WB bands analyzed)	Parameter analyzed Constructs: mean ± SEM	Statistical test
2a	Co-transfection of HEK 293 cells with the indicated (figure legend) constructs; analysis of expression of recombinant FLAG-PC1 in HEK 293 cells via SDS-PAGE and WB; equal total protein amounts were applied to the SDS-PAGE and WB for each experimental condition; the intensity of WB bands (anti FLAG immunoreactivity, normalized to anti β-Actin immunoreactivity) was determined.	3	FLAG-PC1 + kdcontrol: 6 WB band pairs (FLAG-PC1/β-Actin) FLAG-PC1 + Pkd1-kd1: 6 WB band pairs (FLAG-PC1/β-Actin) FLAG-PC1 + Pkd1-kd2: 6 WB band pairs (FLAG-PC1/β-Actin)	Signal intensity of WB bands (normalized) FLAG-PC1 + kdcontrol: 0.74 ± 0.1 FLAG-PC1 + Pkd1-kd1: 0.24 ± 0.1 FLAG-PC1 + Pkd1-kd2: 0.3 ± 0.1	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
2a	Co-transfection of HEK 293 cells with the indicated (figure legend) constructs; analysis of expression of recombinant MYC-PC2 in HEK 293 cells via SDS-PAGE and WB; equal total protein amounts were applied to the SDS-PAGE and WB for each experimental condition; the intensity of WB bands (anti FLAG immunoreactivity, normalized to anti β-Actin immunoreactivity) was determined.	3	MYC-PC2 + kdcontrol: 6 WB band pairs (MYC-PC2/β-Actin) MYC-PC2 + Pkd2-kd1: 6 WB band pairs (MYC-PC2/β-Actin)	Signal intensity of WB bands (normalized) MYC-PC2 + kdcontrol: 0.84 ± 0.03 MYC-PC2 + Pkd2-kd1: 0.51 ± 0.05	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
2b	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; analysis of expression of recombinant FLAG-PC1 via anti FLAG immunofluorescence analysis; the fluorescence background was subtracted.	3	FLAG-PC1 + kdcontrol: 98 transfected cells analyzed FLAG-PC1 + Pkd1-kd1: 103 transfected cells analyzed FLAG-PC1 + Pkd1-kd2: 104 transfected cells analyzed	Relative fluorescence intensity (AU) FLAG-PC1 + kdcontrol: 95.7 ± 8.7 FLAG-PC1 + Pkd1-kd1: 12.7 ± 2.4 FLAG-PC1 + Pkd1-kd2: 31.4 ± 2.4	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
2b	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; analysis of expression of recombinant FLAG-PC2/UTR via anti FLAG immunofluorescence analysis; the fluorescence background was subtracted.	3	FLAG-PC2/UTR + kdcontrol: 156 transfected cells analyzed FLAG-PC2/UTR + Pkd1-kd1: 153 transfected cells analyzed FLAG-PC2/UTR + Pkd1-kd2:	Relative fluorescence intensity (AU) FLAG-PC2/UTR + kdcontrol: 80.0 ± 8.8	Kruskal-Wallis test followed by post-hoc multiple

			155 transfected cells analyzed	FLAG-PC2/UTR + Pkd2-kd1: 23.6 ± 2.8 FLAG-PC2/UTR + Pkd2-kd2: 23.8 ± 4.6	comparison analysis (Dunn)
2d	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; in addition, the constructs mPkd1-kd2 and mPkd2-kd2 were co-transfected; BrdU labeling 3 days after transfection; counting the number of BrdU-positive transfected cells.	3	kdcontrol: 9 coverslips with 4,200 transfected cells (42 groups of cells including 100 transfected cells each) analyzed Pkd1-kd1: 8 coverslips with 2,400 transfected cells (24 groups of cells including 100 transfected cells each) analyzed Pkd1-kd2: 9 coverslips with 4,200 transfected cells (42 groups of cells including 100 transfected cells each) analyzed Pkd2-kd1: 6 coverslips with 2,700 transfected cells (27 groups of cells including 100 transfected cells each) analyzed Pkd2-kd2: 10 coverslips with 4,500 transfected cells (45 groups of cells including 100 transfected cells each) analyzed Pkd1-kd2 + Pkd2-kd2: 8 coverslips with 3,300 transfected cells (33 groups of cells including 100 transfected cells each) analyzed	BrdU-positive transfected cells (%) kdcontrol + kdcontrol: 32.5 ± 1.5 Pkd1-kd1 + kdcontrol: 43.0 ± 2.5 Pkd1-kd2 + kdcontrol: 48.7 ± 2.1 Pkd2-kd1 + kdcontrol: 44.3 ± 1.8 Pkd2-kd2 + kdcontrol: 46.5 ± 1.5 Pkd1-kd2 + Pkd2-kd2: 48.0 ± 1.8	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
2e	Transfection of cortical NPCs with the indicated (figure legend) constructs; immunostaining of cleaved caspase 3 on day 3 after transfection;	3	kdcontrol: 10 coverslips with 9,200 transfected cells analyzed	Cleaved caspase 3-positive transfected cells (%)	Kruskal-Wallis test followed by

	counting the number of cleaved caspase 3-positive transfected cells.		Pkd1-kd1: 7 coverslips with 4,600 transfected cells analyzed Pkd1-kd2: 7 coverslips with 5,900 transfected cells analyzed Pkd2-kd1: 8 coverslips with 4,800 transfected cells analyzed Pkd2-kd2: 7 coverslips with 4,300 transfected cells analyzed	kdcontrol: 1.56 ± 0.3 Pkd1-kd1: 1.76 ± 0.4 Pkd1-kd2: 1.35 ± 0.2 Pkd2-kd1: 1.85 ± 0.4 Pkd2-kd2: 1.69 ± 0.4	post-hoc multiple comparison analysis (Dunn)
2g	Transfection of cortical NPCs with the indicated (figure legend) constructs; BrdU labeling 3 days after transfection; counting the number of BrdU-positive transfected cells.	3	EGFP: 9 coverslips with 2,700 transfected cells (9 groups of cells including 300 transfected cells each) analyzed MYC-PC2: 9 coverslips with 2,700 transfected cells (9 groups of cells including 300 transfected cells each) analyzed	BrdU-positive transfected cells (%) EGFP: 31.7 ± 1.5 MYC-PC2: 33.7 ± 1.5	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
3b	Transfection of cortical NPCs with the indicated (figure legend) constructs; immunostaining of MAP2 on day 3 following transfection; counting the number of MAP2-positive transfected cells.	4	kdcontrol: 5 coverslips with 3,000 transfected cells (5 groups of cells including 600 transfected cells each) analyzed Pkd1-kd1: 4 coverslips with 2,400 transfected cells (4 groups of cells including 600 transfected cells each) analyzed Pkd1-kd2: 4 coverslips with 2,400 transfected cells (4 groups of cells including 600 transfected cells each) analyzed	MAP2-positive transfected cells (%) kdcontrol: 54.7 ± 1.8 Pkd1-kd1: 35.0 ± 2.9 Pkd1-kd2: 35.8 ± 2.3 Pkd2-kd1: 35.9 ± 2.2 Pkd2-kd2: 37.6 ± 2.4	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)

			Pkd2-kd1: 5 coverslips with 3,000 transfected cells (5 groups of cells including 600 transfected cells each) analyzed Pkd2-kd2: 6 coverslips with 3,600 transfected cells (6 groups of cells including 600 transfected cells each) analyzed		
3d	Transfection of cortical NPCs with the indicated (figure legend) constructs; immunostaining of DsRed (derived from the expression of the Tα1p-DsRed2 sensor) on day 3 following transfection; assessment of the fluorescence derived from this sensor; the fluorescence background was subtracted.	4	kdcontrol: 198 transfected cells analyzed Pkd1-kd1: 101 transfected cells analyzed Pkd1-kd2: 101 transfected cells analyzed Pkd2-kd1: 201 transfected cells analyzed Pkd2-kd2: 254 transfected cells analyzed	Tα sensor fluorescence of transfected cells (AU) kdcontrol: 1315 ± 75.4 Pkd1-kd1: 403 ± 80.8 Pkd1-kd2: 343 ± 81.7 Pkd2-kd1: 626 ± 66.4 Pkd2-kd2: 739 ± 61.1	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
4b	Transfection of cortical NPCs with the indicated (figure legend) constructs; immunostaining of GFP (derived from the expression of the CBFRE-EGFP sensor) on day 2 following transfection; assessment of the fluorescence derived from this sensor; the fluorescence background was subtracted.	3	kdcontrol: 400 transfected cells analyzed Pkd1-kd1: 338 transfected cells analyzed Pkd1-kd2: 388 transfected cells analyzed Pkd2-kd1: 388 transfected cells analyzed Pkd2-kd2: 201 transfected cells analyzed	CBFRE sensor fluorescence of transfected cells (AU) kdcontrol: 251 ± 14.7 Pkd1-kd1: 420 ± 18.0 Pkd1-kd2: 557 ± 23.6 Pkd2-kd1: 619 ± 30.0 Pkd2-kd2: 525 ± 32.8	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
5b	Transfection of cortical NPCs with the indicated (figure legend) constructs; immunostaining of nestin on day 3 following transfection; analysis of $GFP^+/Nestin^+$-positive clusters.	3	kdcontrol: 8 coverslips with 945 clusters (27 groups of 35 clusters each) analyzed Pkd1-kd1: 8 coverslips with 665 clusters (19 groups of 35 clusters each) analyzed	Number of $GFP^+/Nestin^+$-positive clusters (%) kdcontrol: 57.9 ± 2.9 Pkd1-kd1: 77.1 ± 2.3 Pkd1-kd2: 80.0 ± 1.6 Pkd2-kd1: 71.2 ± 1.7 Pkd2-kd2: 73.6 ± 1.7	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)

			Pkd1-kd2: 8 coverslips with 735 clusters (21 groups of 35 clusters each) analyzed Pkd2-kd1: 8 coverslips with 735 clusters (21 groups of 35 clusters each) analyzed Pkd2-kd2: 8 coverslips with 770 clusters (22 groups of 35 clusters each) analyzed		
5c	Transfection of cortical NPCs with the indicated (figure legend) constructs; immunostaining of nestin on day 3 following transfection; analysis of mixed clusters comprising GFP^+/$Nestin^+$-positive and GFP^+-positive but $Nestin^-$-negative cells.	3	kdcontrol: 8 coverslips with 945 clusters (27 groups of 35 clusters each) analyzed Pkd1-kd1: 8 coverslips with 665 clusters (19 groups of 35 clusters each) analyzed Pkd1-kd2: 8 coverslips with 735 clusters (21 groups of 35 clusters each) analyzed Pkd2-kd1: 8 coverslips with 735 clusters (21 groups of 35 clusters each) analyzed Pkd2-kd2: 8 coverslips with 770 clusters (22 groups of 35 clusters each) analyzed	Number of mixed clusters (%) kdcontrol: 36.4 ± 2.6 Pkd1-kd1: 18.0 ± 1.8 Pkd1-kd2: 15.4 ± 1.4 Pkd2-kd1: 21.6 ± 1.4 Pkd2-kd2: 21.5 ± 1.8	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
5e	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; the BrdU labeling was performed on day 2 after transfection; a triple immunostaining of RFP (driven by the polycystin shRNA constructs), GFP (driven by the CBFRE-EGFP sensor construct), and BrdU was performed on day 3 following transfection. Cell pairs to be	3	kdcontrol: 65 cell pairs analyzed Pkd1-kd2: 38 cell pairs analyzed Pkd2-kd2: 28 cell pairs analyzed	Ratio of CBFRE sensor (GFP) fluorescence in cell pairs kdcontrol: 1.2 ± 0.03 Pkd1-kd2:1.1 ± 0.03 Pkd2-kd2: 1.1 ± 0.02	Kruskal-Wallis test followed by post-hoc multiple comparison

	analyzed were selected according to the criteria specified in *Materials and methods*.				analysis (Dunn)
6d	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; BrdU labeling 3 days after transfection; counting the number of BrdU-positive transfected cells.	5	kdcontrol: 9 coverslips with 3,600 transfected cells (12 groups of cells including 300 transfected cells each) analyzed kdcontrol + STAT3-kd1: 9 coverslips with 4,500 transfected cells (15 groups of cells including 300 transfected cells each) analyzed kdcontrol + STAT3-kd2: 9 coverslips with 2,700 transfected cells (9 groups of cells including 300 transfected cells each) analyzed	BrdU-positive transfected cells (%) kdcontrol: 23.5 ± 1.7 kdcontrol + STAT3-kd1: 15.8 ± 1.6 kdcontrol + STAT3-kd2: 15.0 ± 2.2	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
6e	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; BrdU labeling 3 days after transfection; counting the number of BrdU-positive transfected cells.	5	Pkd1-kd2 + kdcontrol: 9 coverslips with 4,200 transfected cells (14 groups of cells including 300 transfected cells each) analyzed Pkd1-kd2 + STAT3-kd1: 9 coverslips with 4,500 transfected cells (15 groups of cells including 300 transfected cells each) analyzed Pkd1-kd2 + STAT3-kd2: 9 coverslips with 2,700 transfected cells (9 groups of cells including 300 transfected cells each) analyzed	BrdU-positive transfected cells (%) Pkd1-kd2 + kdcontrol: 28.4 ± 2.0 Pkd1-kd2 + STAT3-kd1: 20.8 ± 2.0 Pkd1-kd2 + STAT3-kd2: 18.5 ± 2.8	Kruskal-Wallis test followed by post-hoc multiple comparison analysis (Dunn)
6f	Co-transfection of cortical NPCs with the indicated (figure legend) constructs; BrdU labeling 3 days after transfection; counting the number of BrdU-positive transfected cells.	5	Pkd2-kd2 + kdcontrol: 9 coverslips with 3,000 transfected cells (10 groups of	BrdU-positive transfected cells (%)	Kruskal-Wallis test followed by

			cells including 300 transfected cells each) analyzed Pkd2-kd2 + STAT3-kd1: 9 coverslips with 4,200 transfected cells (14 groups of cells including 300 transfected cells each) analyzed Pkd2-kd2 + STAT3-kd2: 9 coverslips with 3,000 transfected cells (10 groups of cells including 300 transfected cells each) analyzed	Pkd2-kd2 + kdcontrol: 28.5 ± 2.2 Pkd2-kd2 + STAT3-kd1: 17.3 ± 2.3 Pkd2-kd2 + STAT3-kd2: 18.5 ± 2.0	post-hoc multiple comparison analysis (Dunn)
7b	Transfection of cortical NPCs with the indicated (figure legend) constructs; addition of the STAT3 inhibitor S3I-201 (or DMSO) on day 2 after plating. BrdU labeling 3 days after transfection; counting the number of BrdU-positive transfected cells.	4	kdcontrol + DMSO: 8 coverslips with 4,800 transfected cells (16 groups of cells including 300 transfected cells each) analyzed kdcontrol + S3I: 8 coverslips with 4,800 transfected cells (16 groups of cells including 300 transfected cells each) analyzed Pkd1-kd2 + DMSO: 8 coverslips with 5,100 transfected cells (17 groups of cells including 300 transfected cells each) analyzed Pkd1-kd2 + S3I: 8 coverslips with 5,400 transfected cells (18 groups of cells including 300 transfected cells each) analyzed	BrdU-positive transfected cells (%) kdcontrol + DMSO: 20.2 ± 1.2 kdcontrol + S3I: 15.2 ± 1.2 Pkd1-kd2 + DMSO: 24.4 ± 1.2 Pkd1-kd2 + S3I: 16.0 ± 1.0	Mann-Whitney test