

Supplementary Material

Studies on the Role of the Transcription Factor Tcf21 in the Transdifferentiation of Parietal Epithelial Cells into Podocyte-Like Cells

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Supplementary information

Supplementary Tables

Supplementary Table 1: Antibodies used for immunostaining.

Name	Product number	Origin	Species	Dilution
anti-Tcf21	sc-32914	Santa Cruz	rabbit	1:200
anti-c-myc	sc-40	Santa Cruz	mouse	1:400
anti-YY1	sc-1703	Santa Cruz	rabbit	1:100
anti-Cyclin D1	sc-8396	Santa Cruz	mouse	1:100
anti-Cyclin D2	sc-376676	Santa Cruz	mouse	1:200
anti-MDM2	sc-965	Santa Cruz	mouse	1:100
anti-Podocin	29040	IBL	rabbit	1:150

Supplementary Table 2: Used qRT-PCR Primer.

Detected gene	Sequence / Identity
<i>Gapdh</i>	F: GGGTTCCTATAAATACGGACTGC R: ATGAAGGGGTCGTTGATGGC
<i>Rpl32</i>	F: AGTTCATCAGGCACCAGTCAG R: ATCAGGATCTGGCCCTTGAAC
<i>Tcf21</i>	F: ACACTCTCAGGCTGGCGTCC R: GGCGGTCACCACTTCCTTCA
<i>tGFP</i>	F: CTACCTGCTGAGCCACGTGATG R: TCGTAGCGGTAGCTGAAGCTCA
<i>Cav1</i>	F: GCACACCAAGGAGATTGACC R: GTGCAGGAAGGAGAGAATGG
<i>Ctnnb1</i>	F: GAACAGGGTGCTATTCCACGACTA R: AGGATGTGGAGAGCTCCAGTACAC
<i>Pax2</i>	F: CCCAAAGTGGTGGACAAGAT R: GAAAGGCTGCTGAACTTTGG
<i>Synpo</i>	F: TCCAGGCCTTCCTTCTC R: AGGGGGACATTGGTGGAG
<i>Wt1</i>	F: CCGACCATCTGAAGACCCAC R: ACACTTTCCTGCCTGGGATG
<i>Cd2ap</i>	F: AGGAAGAAGGATGGCTAGAAGG R: GTGCTGATTCGCTGTACAAGAC
<i>Yy1</i>	F: CGCAAATTGAAGTCCAGTGA R: TGAGAAAGCATCTGCACACC
<i>Ccnd1</i>	F: GGATGAGAACAAGCAGACCATC R: GGTCACTTGATGACTCTGGA
<i>Ccnd2</i>	F: CTCACGACTTCATTGAGCACAT R: CGTGAGTGTGTTCACTTCATCA
<i>Ccnb1</i>	F: GAGCCTGAACCTGAACTTGAAC R: CATCAGAGAAAGCCTGACACAG
<i>Ccne1</i>	F: TTGCCAAGATTGACAAGACTGTG R: ACATGATCCTCCAAACCTCTTCTC
<i>Mapt</i>	F: GGACCTGAGCAAAGTGACCT R: GCCAATCTTCGACTGGACTC
<i>Mdm2</i>	F: GTCTGTGTGAGCTGAGGGAGAT R: CCGAGTCCAGAGACTCAACTTC
<i>Cdc45</i>	F: CCTTCAGCCTGATGAAGACAGT R: GGAACCTCAAGGTCATCCTCTT

Supplementary Table 3: Antibodies used for Western Blot.

Name	Product number	Origin	Species	Dilution
anti-Tcf21	HPA013189	Sigma-Aldrich	rabbit	1:1000
anti-tGFP	AB513	Evrogen	rabbit	1:4000
Anti-synaptopodin	sc-21537	Santa Cruz	goat	1:500
anti-WT1	sc-192	Santa Cruz	rabbit	1:1500
anti-Pax2	ab38738	Abcam	rabbit	1:1500
anti-caveolin-1	sc-894	Santa Cruz	rabbit	1:2000
anti-beta-catenin-1	sc-7199	Santa Cruz	rabbit	1:500
anti-CD2AP	sc-9137	Santa Cruz	rabbit	1:2500
anti-YY1	sc-1703	Santa Cruz	rabbit	1:1000

Supplementary Table 4: GO enrichment genes (Tcf21 peak(s) within +/- 1kb from TSS [2791 peaks]) - Biological Process.

ToppGene was used to determine GO enrichment using a single gene list ranked by expression with a p-value cutoff of 0.05.

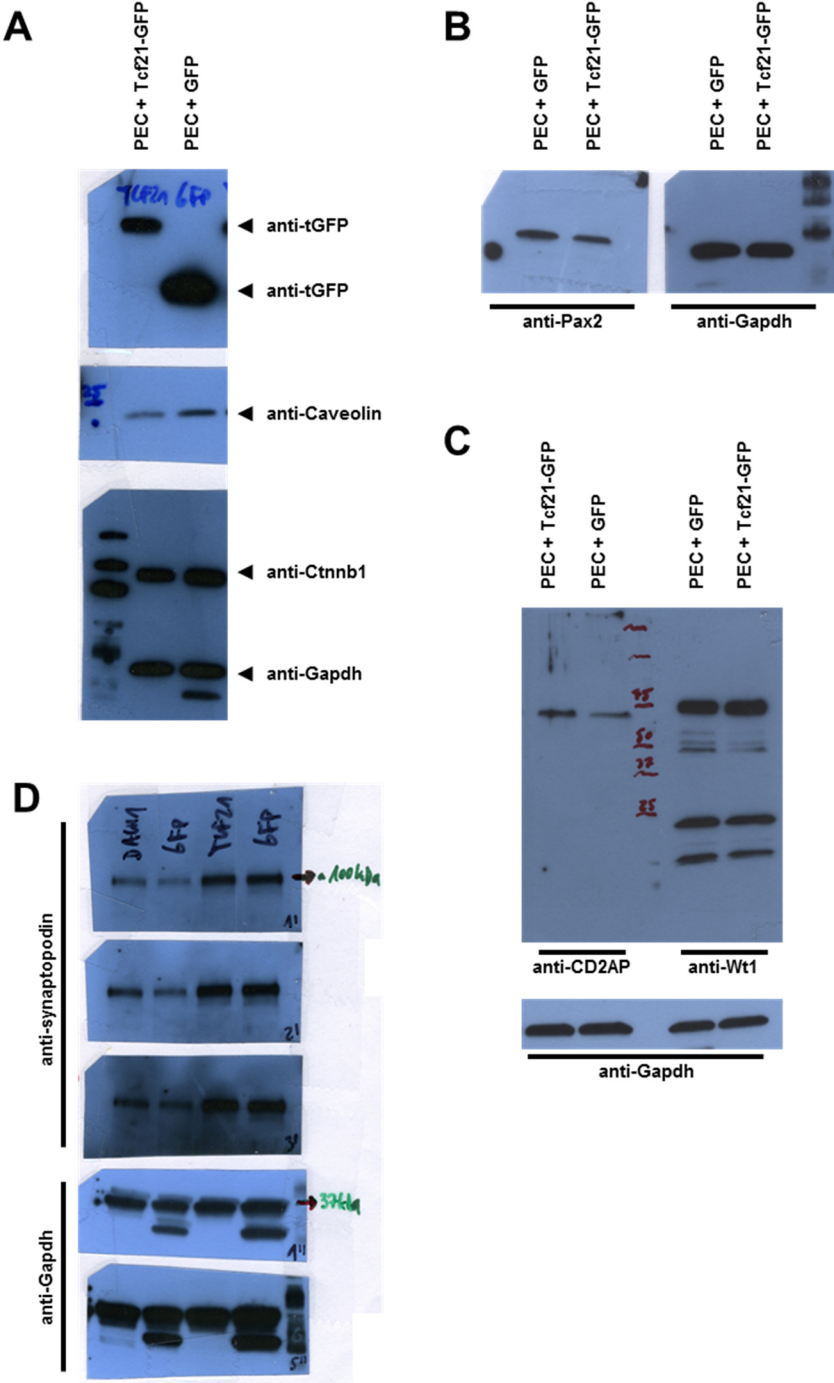
	ID	Name	pValue	FDR B&H	Genes from Input
1	GO:0007049	cell cycle	1.539E-24	1.423E-20	414
2	GO:1903047	mitotic cell cycle process	2.104E-23	9.724E-20	251
3	GO:0000278	mitotic cell cycle	1.222E-22	3.766E-19	266
4	GO:0022402	cell cycle process	3.673E-22	8.490E-19	335
5	GO:0007005	mitochondrion organization	5.049E-20	9.335E-17	209
6	GO:1902582	single-organism intracellular transport	7.677E-18	1.183E-14	403
7	GO:0070727	cellular macromolecule localization	6.486E-17	8.566E-14	368
8	GO:0034613	cellular protein localization	1.272E-16	1.470E-13	365
9	GO:0015031	protein transport	5.926E-16	6.087E-13	412
10	GO:0034660	ncRNA metabolic process	1.075E-15	9.938E-13	155
11	GO:0044248	cellular catabolic process	3.116E-15	2.619E-12	387
12	GO:0006461	protein complex assembly	3.845E-15	2.734E-12	341
13	GO:0070271	protein complex biogenesis	3.845E-15	2.734E-12	341
14	GO:0032446	protein modification by small protein conjugation	6.539E-15	4.318E-12	219
15	GO:0065003	macromolecular complex assembly	1.801E-14	1.110E-11	384
16	GO:0070647	protein modification by small protein conjugation or removal	2.335E-14	1.349E-11	240
17	GO:0051726	regulation of cell cycle	3.009E-14	1.637E-11	237
18	GO:0051276	chromosome organization	2.245E-13	1.139E-10	267
19	GO:0006886	intracellular protein transport	2.340E-13	1.139E-10	251
20	GO:0033365	protein localization to organelle	4.376E-13	2.023E-10	216
21	GO:0016567	protein ubiquitination	2.372E-12	9.981E-10	187
22	GO:0044770	cell cycle phase transition	2.375E-12	9.981E-10	144
23	GO:0044265	cellular macromolecule catabolic process	3.174E-12	1.276E-9	223
24	GO:0051301	cell division	3.939E-12	1.457E-9	165
25	GO:0044772	mitotic cell cycle phase transition	3.939E-12	1.457E-9	138
26	GO:0006396	RNA processing	1.211E-11	4.307E-9	209
27	GO:0009057	macromolecule catabolic process	2.763E-11	9.460E-9	263
28	GO:0034622	cellular macromolecular complex assembly	2.923E-11	9.650E-9	222
29	GO:1901575	organic substance catabolic process	3.420E-11	1.090E-8	381

30	GO:0034976	response to endoplasmic reticulum stress	8.114E-11	2.500E-8	80
31	GO:0044711	single-organism biosynthetic process	8.503E-11	2.536E-8	356
32	GO:0042254	ribosome biogenesis	8.876E-11	2.564E-8	92
33	GO:0007067	mitotic nuclear division	1.900E-10	5.324E-8	118
34	GO:0006974	cellular response to DNA damage stimulus	2.380E-10	6.472E-8	183
35	GO:0030163	protein catabolic process	4.756E-10	1.256E-7	190
36	GO:0006259	DNA metabolic process	6.278E-10	1.604E-7	217
37	GO:0071103	DNA conformation change	6.418E-10	1.604E-7	82
38	GO:0009894	regulation of catabolic process	6.687E-10	1.627E-7	177
39	GO:0006399	tRNA metabolic process	7.523E-10	1.783E-7	59
40	GO:0034470	ncRNA processing	7.938E-10	1.835E-7	105
41	GO:0006914	autophagy	9.917E-10	2.236E-7	142
42	GO:0033043	regulation of organelle organization	1.046E-9	2.303E-7	250
43	GO:0044257	cellular protein catabolic process	1.143E-9	2.458E-7	161
44	GO:0022613	ribonucleoprotein complex biogenesis	1.198E-9	2.506E-7	118
45	GO:0016197	endosomal transport	1.231E-9	2.506E-7	76
46	GO:0010256	endomembrane system organization	1.247E-9	2.506E-7	139
47	GO:0007346	regulation of mitotic cell cycle	1.470E-9	2.892E-7	126
48	GO:0044839	cell cycle G2/M phase transition	1.725E-9	3.323E-7	64
49	GO:0051603	proteolysis involved in cellular protein catabolic process	1.947E-9	3.674E-7	154
50	GO:0007017	microtubule-based process	2.541E-9	4.699E-7	153
51	GO:0007034	vacuolar transport	3.132E-9	5.678E-7	81
52	GO:0009141	nucleoside triphosphate metabolic process	3.823E-9	6.797E-7	82
53	GO:0031329	regulation of cellular catabolic process	3.944E-9	6.879E-7	153
54	GO:0010608	posttranscriptional regulation of gene expression	4.546E-9	7.783E-7	118
55	GO:0006260	DNA replication	5.067E-9	8.436E-7	92
56	GO:0043632	modification-dependent macromolecule catabolic process	5.110E-9	8.436E-7	142
57	GO:0016236	macroautophagy	5.875E-9	9.528E-7	107
58	GO:0019941	modification-dependent protein catabolic process	6.971E-9	1.111E-6	140
59	GO:0048285	organelle fission	8.004E-9	1.254E-6	147
60	GO:0010498	proteasomal protein catabolic process	1.036E-8	1.596E-6	106
61	GO:0000226	microtubule cytoskeleton organization	1.253E-8	1.899E-6	114
62	GO:0000819	sister chromatid segregation	1.273E-8	1.899E-6	66

63	GO:0006511	ubiquitin-dependent protein catabolic process	1.736E-8	2.547E-6	137
64	GO:0048193	Golgi vesicle transport	2.010E-8	2.903E-6	89
65	GO:0009144	purine nucleoside triphosphate metabolic process	2.139E-8	3.013E-6	75
66	GO:0035967	cellular response to topologically incorrect protein	2.151E-8	3.013E-6	47
67	GO:0043604	amide biosynthetic process	2.447E-8	3.377E-6	171
68	GO:0022411	cellular component disassembly	3.028E-8	4.117E-6	150
69	GO:0000086	G2/M transition of mitotic cell cycle	3.260E-8	4.368E-6	59
70	GO:0035966	response to topologically incorrect protein	3.909E-8	5.163E-6	56
71	GO:0009199	ribonucleoside triphosphate metabolic process	4.114E-8	5.357E-6	74
72	GO:0051186	cofactor metabolic process	4.309E-8	5.533E-6	102
73	GO:0043623	cellular protein complex assembly	4.462E-8	5.651E-6	133
74	GO:0006520	cellular amino acid metabolic process	5.169E-8	6.458E-6	100
75	GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	5.426E-8	6.689E-6	98
76	GO:0009116	nucleoside metabolic process	5.588E-8	6.797E-6	102
77	GO:0044403	symbiosis, encompassing mutualism through parasitism	6.825E-8	8.026E-6	174
78	GO:0044419	interspecies interaction between organisms	6.825E-8	8.026E-6	174
79	GO:0006839	mitochondrial transport	6.858E-8	8.026E-6	76
80	GO:0061024	membrane organization	6.959E-8	8.042E-6	224
81	GO:0080135	regulation of cellular response to stress	7.541E-8	8.606E-6	164
82	GO:0034620	cellular response to unfolded protein	7.690E-8	8.606E-6	44
83	GO:0007059	chromosome segregation	7.726E-8	8.606E-6	86
84	GO:0006986	response to unfolded protein	8.681E-8	9.555E-6	53
85	GO:0009119	ribonucleoside metabolic process	9.424E-8	1.025E-5	97
86	GO:1901657	glycosyl compound metabolic process	1.226E-7	1.318E-5	105
87	GO:0070925	organelle assembly	1.421E-7	1.510E-5	145
88	GO:0009205	purine ribonucleoside triphosphate metabolic process	1.467E-7	1.541E-5	71
89	GO:0000280	nuclear division	1.586E-7	1.629E-5	135
90	GO:0010564	regulation of cell cycle process	1.586E-7	1.629E-5	135
91	GO:1901990	regulation of mitotic cell cycle phase transition	1.662E-7	1.684E-5	82
92	GO:0000209	protein polyubiquitination	1.688E-7	1.684E-5	69
93	GO:0006412	translation	1.709E-7	1.684E-5	147

94	GO:0009123	nucleoside monophosphate metabolic process	1.713E-7	1.684E-5	79
95	GO:1901987	regulation of cell cycle phase transition	1.800E-7	1.751E-5	86
96	GO:0043043	peptide biosynthetic process	1.908E-7	1.822E-5	152
97	GO:0044802	single-organism membrane organization	1.912E-7	1.822E-5	202
98	GO:1901566	organonitrogen compound biosynthetic process	2.068E-7	1.951E-5	281
99	GO:0016032	viral process	2.151E-7	2.008E-5	163
100	GO:0097193	intrinsic apoptotic signaling pathway	2.316E-7	2.141E-5	78

Supplementary Figures



Supplementary Figure 1: Representative Western blots of Tcf21 transfected PECs used for Figure 2.

(A) WB with anti-tGFP, anti-Cav1, anti-Ctnnb1 and anti-Gapdh. (B) WB with anti-Pax2 and Gapdh as loading control. (C) WB with anti-CD2AP and anti-Wt1. (D) WB with anti-synaptopodin antibody showed only in Dach1 transfected PECs an increased expression of synaptopodin (shown in Endlich *et al.*; *J Cell Mol Med* 2018 May; 22(5):2656-2669; doi: 10.1111/jcmm.13544). In contrast, Tcf21 transfected PECs did not show increased expression of synaptopodin. Different exposure times of the x-ray film can be seen. Used protein amounts: tGFP, Cav1 and Ctnnb1 lanes loaded with 10 μ g protein, Pax2 (15 μ g),

synaptopodin (80 μ g), CD2AP (80 μ g), Wt1 (60 μ g). Loading control was usually performed after stripping and reprobing Western blots with anti-Gapdh antibody.