# Supplemental Data

2	PALS1-dependent modulations of mRNA profiles
3	in MDCK II cells grown in non-confluent monolayers and
4	3-dimensional cysts.
5	by
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## 1 Table Of Content Supplemental Data

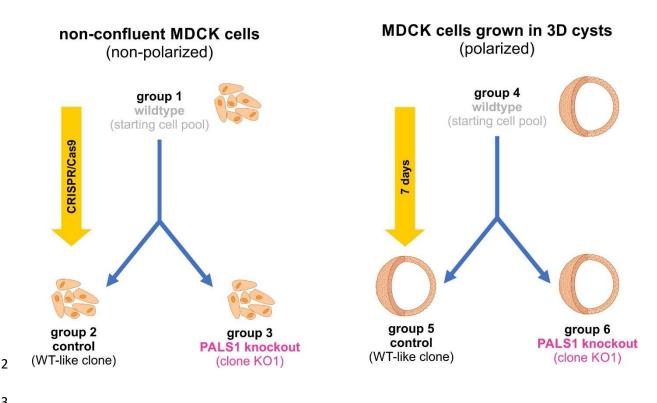
#### 2 Supplemental Figures

- 3 Fig. SF1: Preparation of mRNA samples for transcriptome analyses.
- 4

#### 5 Supplemental Tables

- 6 Table ST1 MDCK transcriptomes of wildtype, PALS1 knockout and the starting MDCK cell
   7 pool.
- 8 Table ST2A Differential expressed genes (DEGs) of subset A (WT-3D vs WT-nc)
- 9 Table ST2B DEGs of subset B (KO-3D vs KO-nc)
- 10 Table ST2C DEGs of subset C (KO-3D vs WT-3D)
- 11 Table ST2D DEGs of subset D (KO-nc vs WT-nc)
- 12 Table ST03 Shared DEGs of subset A and subset B
- 13 Table ST04Gene ontology biological processes (GO-BP) enrichment analysis for14upregulated DEGs of subset A
- 15 Table ST05 GO-BP enrichment analysis for upregulated DEGs of subset B
- 16 Table ST06 REACTOME analysis for upregulated DEGs of subset A
- 17 Table ST07 REACTOME analysis for upregulated DEGs of subset B
- 18 Table ST08 KEGG analysis for upregulated DEGs of subset A
- 19 Table ST09 KEGG analysis for upregulated DEGs of subset B
- 20 Table ST10 GO-BP enrichment analysis for downregulated DEGS of subset A
- 21 Table ST11 GO-BP enrichment analysis for downregulated DEGs of subset B
- 22 Table ST12 REACTOME analysis for downregulated DEGs of subset A
- 23 Table ST13 REACTOME analysis for downregulated DEGs of subset B
- 24 Table ST14 KEGG analysis for downregulated DEGs of subset A
- 25 Table ST15 KEGG analysis for upregulated DEGs of subset B
- 26 Table ST16 Shared DEGs of subset C and subset D
- 27 Table ST17 GO-BP enrichment analysis for upregulated DEGs of subset C
- 28 Table ST18 GO-BP enrichment analysis for downregulated DEGs of subset C.
- 29 Table ST19 GO-BP enrichment analysis for upregulated DEGs of subset D
- 30 Table ST20 GO-BP enrichment analysis for downregulated DEGs of subset D.
- 31 Table ST21 REACTOME analysis for upregulated DEGs of subset C
- 32 Table ST22 REACTOME analysis for downregulated DEGs of subset C
- 33 Table ST23 REACTOME analysis for upregulated DEGs of subset D
- 34 Table ST24 REACTOME analysis for downregulated DEGs of subset D
- 35 Table ST25 KEGG analysis for upregulated DEGs of subset C
- 36 Table ST26 KEGG analysis for upregulated DEGs of subset C
- 37 Table ST27 KEGG analysis for upregulated DEGs of subset D
- 38 Table ST28 KEGG analysis for upregulated DEGs of subset D
- 39 Table ST29 Gene lists: cell junction, cell polarity, and cell-ECM interaction
- 40

#### **Supplemental Figures**



#### Fig. SF1: Preparation of mRNA samples for transcriptome analyses.

A: Scheme of used transcriptomes. Initially the analysis included six groups of transcriptomes samples: The first three groups (1-3) are samples from cells grown under non-confluent conditions (upper panel). group 1: transcriptomes derived from the starting pool of non-confluent MDCK II cells (wildtype non-confluent, n=4). group 2: Samples derived from a clone that ran through a CRISPR/Cas9 but maintained a wildtype PALS1 gene (Ctr non-confluent, n=4). group 3: samples derived from a PALS1 knockout clone (KO1 non-confluent, n=4) (Groh et al., 2024). The next are three groups (4-6) are samples derived from cells grown for 7 days in 3D cysts. group 4: 3D-cyst from the MDCK starting pool (WT-nc, n=4).group 5: Samples derived from 3D cysts of the clone that run through a CRISPR/Cas9 but maintained a wildtype PALS1 gene (Ctr-3D, n=3). group 6: Samples derived from 3D cysts of the KO1 clone (KO1 3D, n=3). 

### **1** Supplemental Tables (titles and descriptions)

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2
3
                     MDCK transcriptomes of wildtype, PALS1 knockout and the starting MDCK cell pool.
     Table ST1
 4
     File name: ST01 S1 sample description 030424.xlsx
 5
     Description of data: List of samples with sample ID from GEO and their description.
6
 7
                     Differential expressed genes (DEGs) of subset A (WT-3D vs WT-nc)
     Table ST2A
8
     File name: ST02A_sbst7_DESeq2_DEG_Ctr_3D_7d_vs_Ctr_2D_nconf_011223
9
     Description of data: see name of table
10
11
     Table ST2B
                    DEGs of subset B (KO-3D vs KO-nc)
12
     File name: ST02B_sbst7_DESeq2_DEG_KO_3D_7d_vs_KO_2D_nconf_011223
     Description of data: see name of table
13
14
15
     Table ST2C
                    DEGs of subset C (KO-3D vs WT-3D)
16
     File name: ST02C_sbst7_DESeq2_DEG_KO_3D_7d_vs_Ctr_3D_7d_011223
17
     Description of data: see name of table
18
19
     Table ST2D
                    DEGs of subset D (KO-nc vs WT-nc)
20
     File name: ST02D_sbst7_DESeq2_DEG_KO_2D_nconf_vs_Ctr_2D_nconf_140824
21
     Description of data: see name of table
22
23
     Table ST03
                    Shared DEGs of subset A and subset B
24
     File name: ST03 Shared DEGs of Subset A and B
25
     Description of data: Analyses of common and different DEGs of total, up-, and downregulated DEGs of
     subset A and B, including TOP 20 up- and downregulated DEGS.
26
27
28
     Table ST04
                     Gene ontology biological processes (GO-BP) enrichment analysis for upregulated DEGs
29
                     of subset A
30
     File name:
31
     ST04 UP EnrichR GO Biological Process 2021 sbst7 Ctr 3D 7d vs Ctr 2D nconf 191223
32
     Description of data: see name of table
33
34
     Table ST05
                    GO-BP enrichment analysis for upregulated DEGs of subset B
35
     File name:
36
     ST05 UP EnrichR GO Biological Process 2021 sbst7 KO 3D 7d vs KO 2D nconf 191223
     Description of data: see name of table
37
38
39
     Table ST06
                     REACTOME analysis for upregulated DEGs of subset A
40
     File name: ST06_UP_EnrichR_Reactome_2016_sbst7_Ctr_3D_7d_vs_Ctr_2D_nconf_191223
41
     Description of data: see name of table
42
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43

1	
2	Table ST07REACTOME analysis for upregulated DEGs of subset B
3	File name: ST07_UP_EnrichR_Reactome_2016_sbst7_KO_3D_7d_vs_KO_2D_nconf_191223
4	Description of data: see name of table
5	
6	Table ST08KEGG analysis for upregulated DEGs of subset A
7	ST08_UP_EnrichR_KEGG_2021_Human_sbst7_Ctr_3D_7d_vs_Ctr_2D_nconf_191223
8	Description of data: see name of table
9	Table ST09 KEGG analysis for upregulated DEGs of subset B
10	ST09_UP_EnrichR_KEGG_2021_Human_sbst7_KO_3D_7d_vs_KO_2D_nconf_191223
11	Description of data: see name of table
12	
13	Table ST10 GO-BP enrichment analysis for downregulated DEGS of subset A
14	File name:
15	ST10_DN_EnrichR_GO_Biological_Process_2021_sbst7_Ctr_3D_7d_vs_Ctr_2D_nconf_191223
16	Description of data: see name of table
17	
18	Table ST11GO-BP enrichment analysis for downregulated DEGs of subset B
19	File name:
20	ST11_DN_EnrichR_GO_Biological_Process_2021_sbst7_KO_3D_7d_vs_KO_2D_nconf_191223
21	Description of data: see name of table
22	
23	Table ST12 REACTOME analysis for downregulated DEGs of subset A
24	File name: ST12_DN_EnrichR_Reactome_2016_sbst7_Ctr_3D_7d_vs_Ctr_2D_nconf_191223
25	Description of data: see name of table
26	
27	Table ST13 REACTOME analysis for downregulated DEGs of subset B
28	File name: ST13_DN_EnrichR_Reactome_2016_sbst7_KO_3D_7d_vs_KO_2D_nconf_191223
29	Description of data: see name of table
30	
31	Table ST14 KEGG analysis for downregulated DEGs of subset A
32	ST14_DN_Enrich_KEGG_2021_Human_sbst7_Ctr_3D_7d_vs_Ctr_nconf_191223
33	Description of data: see name of table
34	
35	Table ST15 KEGG analysis for downregulated DEGs of subset B
36	File name: ST15_DN_EnrichR_KEGG_2021_Human_sbst7_KO_3D_7d_vs_KO_2D_nconf_191223
37	ST15_DN_EnrichR_KEGG_2021_Human_sbst7_KO_3D_7d_vs_KO_2D_nconf_191223
38	Description of data: see name of table
39	
40	Table ST16Shared DEGs of subset C and subset D
41	File name: ST16_Shared_DEGs_of_subset_C_and_D
42	Description of data: Analyses of common and different DEGs of total, up-, and downregulated DEGs of
43	subset C and D, including TOP 20 up- and downregulated DEGS.
ΔΔ	

- 1 Table ST17 GO-BP enrichment analysis for upregulated DEGs of subset C File name: ST17\_UP\_EnrichR\_GO\_Biological\_Process\_2021\_sbst7\_KO\_3D\_7d\_vs\_Ctr\_3D\_7d\_191223 2 3 Description of data: see name of table 4 5 Table ST18 GO-BP enrichment analysis for downregulated DEGs of subset C 6 File name: ST18\_DN\_EnrichR\_GO\_Biological\_Process\_2021\_sbst7\_KO\_3D\_7d\_vs\_Ctr\_3D\_7d\_191223 7 Description of data: see name of table 8 9 Table ST19 GO-BP enrichment analysis for upregulated DEGs of subset D 10 File name: 11 ST19\_UP\_EnrichR\_GO\_Biological\_Process\_2021\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt 12 Description of data: see name of table 13 14 Table ST20 GO-BP enrichment analysis for downregulated DEGs of subset D 15 File name: ST20\_DN\_EnrichR\_GO\_Biological\_Process\_2021\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt 16 17 Description of data: see name of table 18 19 Table ST21 REACTOME analysis for upregulated DEGs of subset C 20 File name: ST21\_UP\_EnrichR\_Reactome\_2016\_sbst7\_KO\_3D\_7d\_vs\_Ctr\_3D\_7d\_191223 21 Description of data: see name of table 22 23 Table ST22 REACTOME analysis for downregulated DEGs of subset C 24 File name: ST22\_DN\_EnrichR\_Reactome\_2016\_sbst7\_KO\_3D\_7d\_vs\_Ctr\_3D\_7d\_191223 25 Description of data: see name of table 26 27 Table ST23 REACTOME analysis for upregulated DEGs of subset D 28 File name: ST23\_UP\_EnrichR\_Reactome\_2016\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt 29 Description of data: see name of table 30 31 Table ST24 REACTOME analysis for downregulated DEGs of subset D 32 File name: ST24\_DN\_EnrichR\_Reactome\_2016\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt 33 Description of data: see name of table 34 35 Table ST25 KEGG analysis for upregulated DEGs of subset C 36 File name: ST25 UP EnrichR KEGG 2021 Human sbst7 KO 3D 7d vs Ctr 3D 7d 191223 37 Description of data: see name of table 38 39 Table ST26 KEGG analysis for upregulated DEGs of subset C 40 File name: ST26\_DN\_EnrichR\_KEGG\_2021\_Human\_sbst7\_KO\_3D\_7d\_vs\_Ctr\_3D\_7d\_191223 41 Description of data: see name of table 42 43 Table ST27 KEGG analysis for upregulated DEGs of subset D 44 File name: 45 ST27\_UP\_EnrichR\_KEGG\_2021\_Human\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt
- 46 Description of data: see name of table

- 1 Table ST28 KEGG analysis for upregulated DEGs of subset D
- 2 File name:
- 3 ST27\_UP\_EnrichR\_KEGG\_2021\_Human\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt
- 4 Description of data: see name of table
- 5
- 6 Table ST29 Gene lists: cell junction, cell polarity, and cell-ECM interaction
- 7 File name:
- 8 ST27\_UP\_EnrichR\_KEGG\_2021\_Human\_sbst7\_KO\_2D\_nconf\_vs\_Ctr\_2D\_nconf\_140824.txt
- 9 Description of data: Basis for the three gene lists are gene included by known GO-terms.
- 10 For the gene list cell junctions, we used the following GO terms: GO:0043296 (apical junction complex,
- 11 Cellular Component), GO:0070160 (tight junction, Cellular Component), and GO:0005912 (adherens
- 12 junction, Cellular Component). The gene list of cell polarity genes based on the GO-term GO:0007163
- 13 (establishment or maintenance of cell polarity, Biological Process). For cell substrate interactions, cell
- 14 adhesion or cell adhesion we used GO terms GO:0031589 (cell-substrate adhesion, Biological Process),
- 15 GO:0007160 (cell-matrix adhesion, Biological Process) and the GO:0033627 (cell adhesion mediated
- 16 by integrin, Biological Process).
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