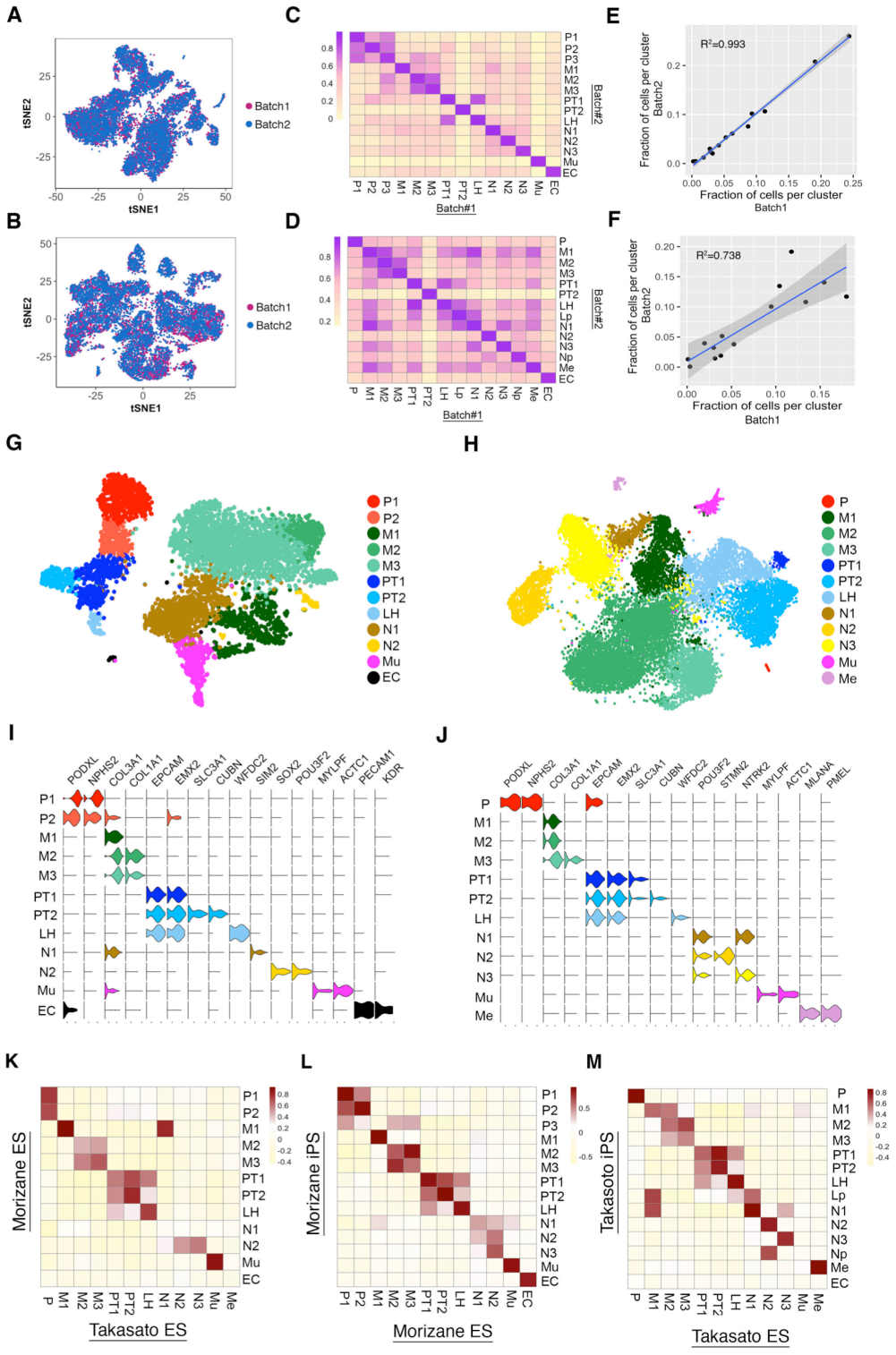

Figure S1, Related to Figure 1 and Figure 2. Batch effects and comparison of kidney cell types and differentiation state in hESC-derived kidney organoids generated with both protocols. (A, B) Cells in tSNE are visualized according to their batch of origin. Nearly all clusters in organoids from either the Morizane **(A)** or Takasato protocol **(B)** contain cells from both batches. **(C, D)** Correlation heatmaps of cell expression profiles between batches. Cells in the same cluster from both batches show highly correlated gene expression (Pearson's r > 0.9 for all clusters) in the Morizane protocol **(C)** and Takasato protocol **(D)**. **(E, F)** The overall cell fraction for each cluster between batches in the Morizane protocol **(E)** and Takasato protocol **(F)** is similar. The higher proportional representation between batches

from the Morizane protocol likely reflects the fact that 8 organoids were combined for each batch, whereas the larger Takasato protocol organoids consisted of one single organoid per batch. (**G,H**) tSNE of all cells clustered by Seurat, showing clusters and their identity for the Morizane protocol (**G**) and Takasato protocol (**H**). (**I,J**) Violin plots of marker gene expression for each cell type across clusters for Morizane (**I**) and Takasato (**J**) organoids. Heatmap indicating Pearson's correlations on the averaged profiles among common cell types for Morizane and Takasato organoids for hESC-derived organoids (**K**) and iPS vs. hESC-derived according to protocol (**L and M**).

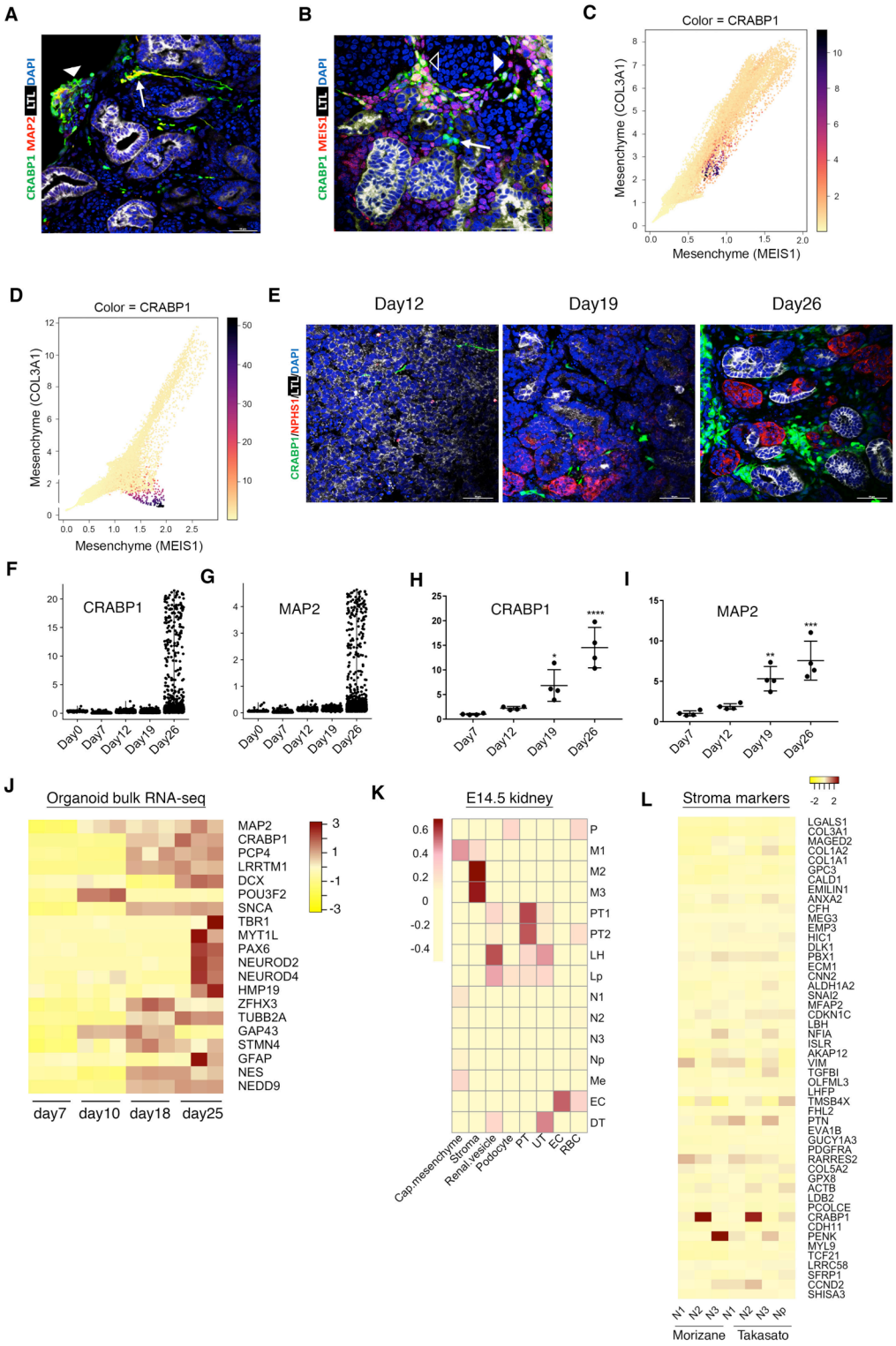

Figure S2, Related to Figure 2. Characterizating off target neuron neuronal differentiation in kidney organoids. (A-B) Immunofluorescence analysis of day 26 organoids differentiated using the Takasato protocol. Scattered interstitial cells coexpress both neuronal markers CRABP1 and MAP2 in the interstitium. Some of these extend long processes resembling neurites (arrow). Clusters of neuron-like cells can also be seen, usually on the periphery of the organoid (arrowhead). LTL, lotus tetraglobonus lectin. Scale bar 50µm. **(B)** CRABP1 immunostaining of day 26 Takasato protocol kidney organoids shows that CRABP1 is coexpressed in a subset of MEIS1 positive renal stroma. Merged image shows CRABP1 and MEIS1 copositive cells (open arrowhead), cells positive for CRABP1 only (arrow) and cells positive for MEIS1 only (filled arrowhead). Scale bar 50µm.

(C) Gene imputation analysis using MAGIC to visualize gene-gene interactions. CRABP1 is expressed in a minority of COL3A1/MEIS1 positive mesenchyme in day 26 Morizane protocol organoids, supporting the immunofluorescence data. **(D)** Similar analysis from Takasato protocol day 26 organoids, where CRABP1 expression is more correlated with MEIS1 expression than with COL3A1 expression. **(E)** Co-staining of CRABP1, podocyte marker NPHS1 and proximal tubule marker LTL during organoid development (Takasato protocol). Very few CRABP1 cells are detected at day 19 with many more at day 26. **(F,G)** Analysis of CRABP1 and MAP2 expression in DropSeq dataset reveals an abrubt rise of expression in a subset of organoid cells at day 26. **(H,I)** Analysis of CRABP1 and MAP2 mRNA by qPCR of bulk extracted RNA over time (Takasato protocol). **(J)** Reanalysis of organoid RNA-seq expression from Takasato *et al*. showing that a panel of genes found in neural clusters by scRNA-seq in this paper were expressed at later timepoints in the original dataset. (**K**) Pearson's correlation for all Takasato protocol organoid cell clusters compared to e14.5 kidney. There is poor correlation of off target clusters (N1, N2, N3, Np, Me) with any mouse embryonic kidney cluster. (**L**) Very few genes from embryonic mouse stroma are expressed in off target cell types from either protocol. Mouse data is from (Magella et al., 2018).

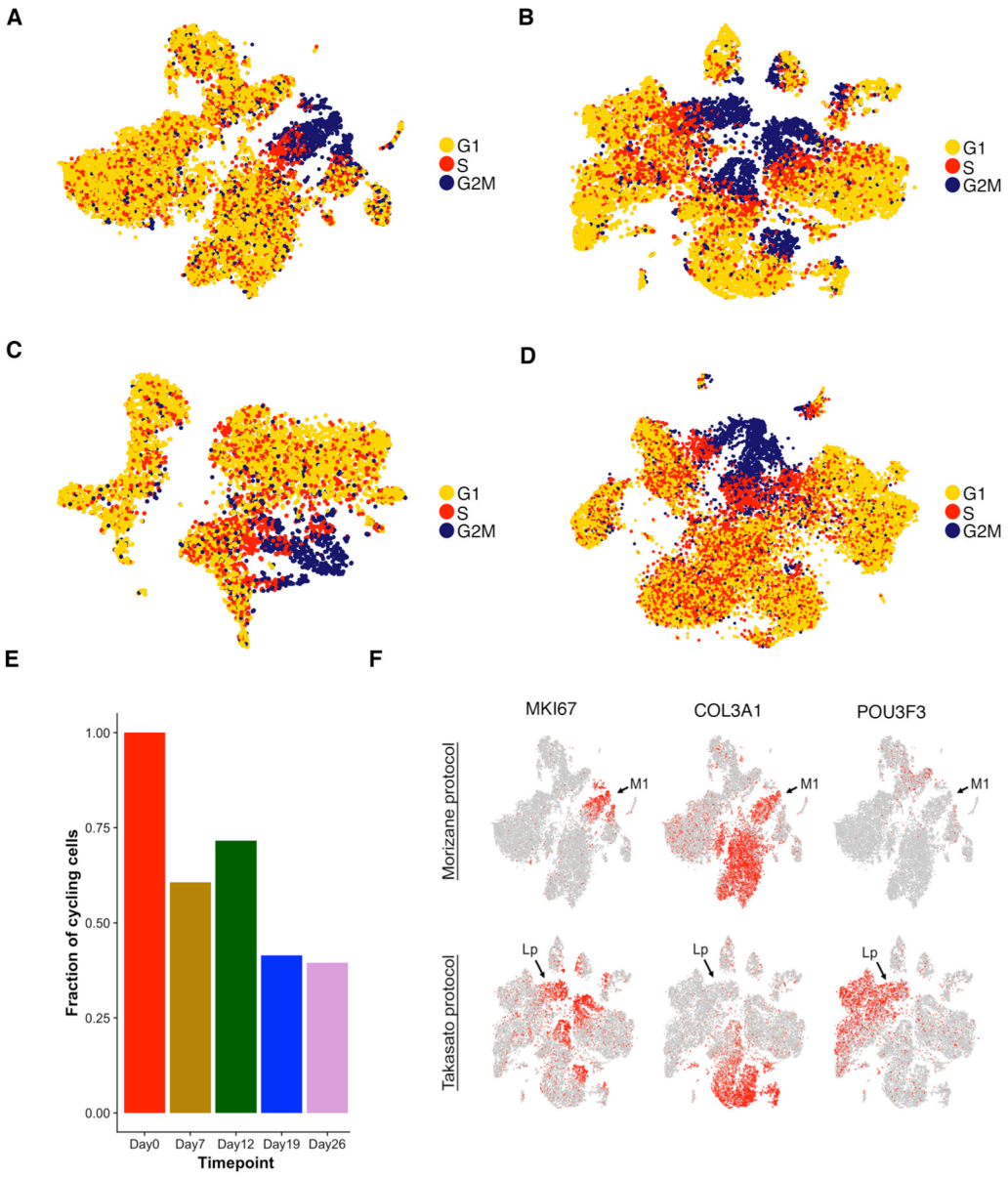

Figure S3, Related to Figure 2. Comparison of cell cycle status across protocols. iPSC-derived organoids using the Morizane (**A**) and Takasato protocols (**B**). hESC-derived organoids from the Morizane (**C**) and Takasato (**D**) protocols. (**E**) Fraction of total organoid cells in the G2M, S or G1 cell cycle across time in iPSC differentiated using the Takasato protocol. **(F)** Both the Morizane M1 and the Takasato Lp (loop progenitor) cluster expressed high levels of the cell cycle gene MKI67, but divergent expression of COL3A1 and POU3F3.

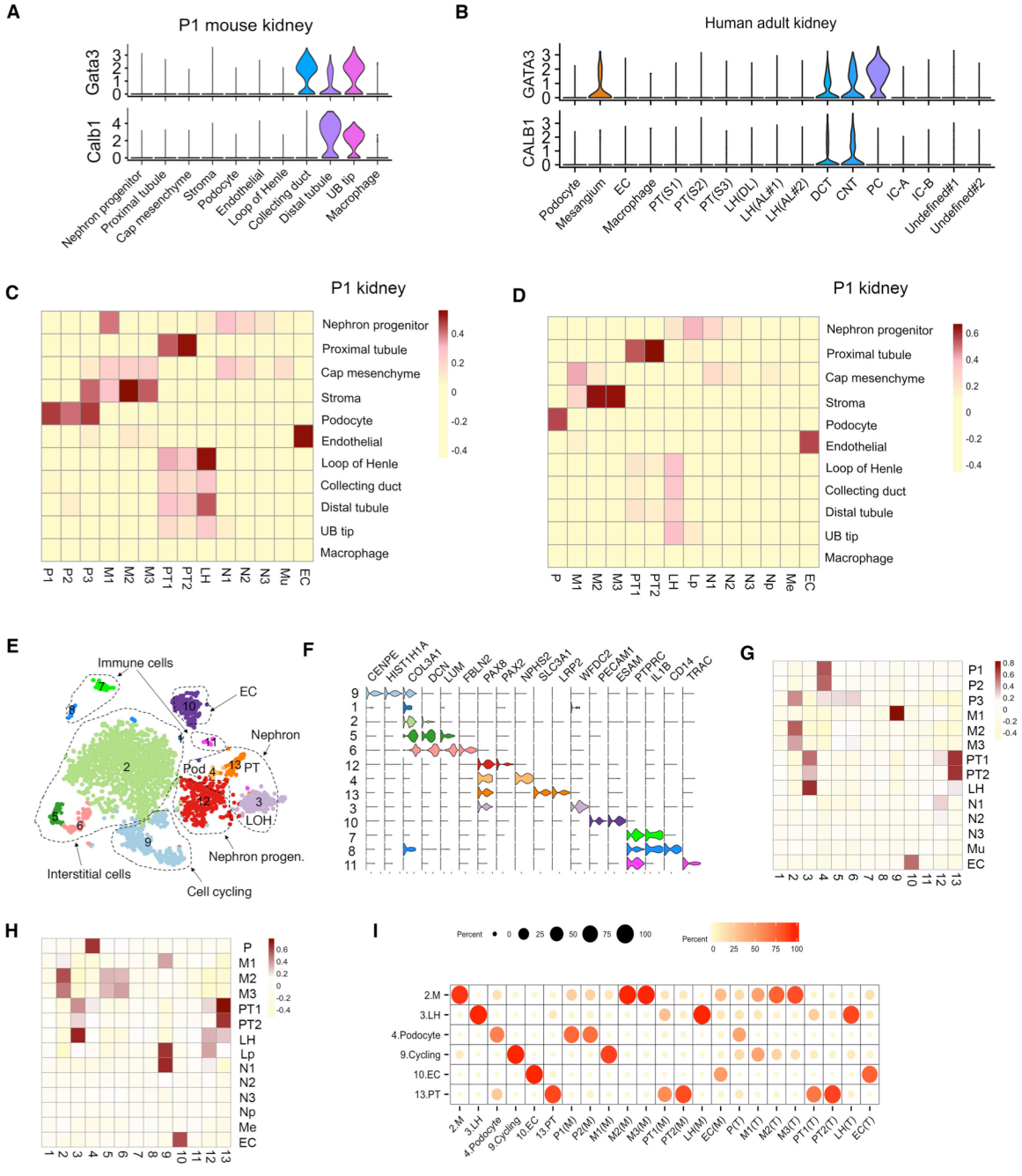

Figure S4, Related to Figure 3. Expression of GATA3 and CALB1 in distal nephron segments and comparison of organoids to P1 mouse kidney and human fetal kidney (week 16). (**A**) Violin plot showing expression of GATA3 in collecting duct, distal tubule and ureteric bud tip in P1 mouse kidney. In the same dataset, CALB1 is expressed in distal tubule and UB tip. P1 mouse kidney data is from (Adam et al., 2017). (**B**) GATA3 expression in adult human kidney (data from this manuscript) is limited to mesangium, distal connecting tubule, connecting segment and principal cells. CALB1 expression is observed only in distal connecting tubule and connecting segment but not in principal cells. **(C,D)** Kidney organoids compared to mouse p1 kidney. Pearson's correlation for all organoid cell clusters from Morizane protocol (**C**) or Takasato protocol (**D**). P1 mouse kidney data is from (Adam et al., 2017). (**E**) Reanalysis of a recently published scRNA-seq dataset from human week 16 fetal kidney. tSNE showing major cell clusters as reported originally (Lindstrom et al., 2018). (**F**)

Violin plot showing marker gene expression for all 13 clusters. (**G,H**) Pearson's correlation between organoid cell types from each protocol and human fetal kidney from Morizane protocol (**G**) or Takasato protocol (**H**). (**I**) Dot plot showing the proportion of cells in each cluster that were classified to each organoid cluster using a multiclass random forest classifier as in (Habib et al., 2017). 60% of the human fetal dataset was used as a training dataset (first six columns on left side of dotplot).

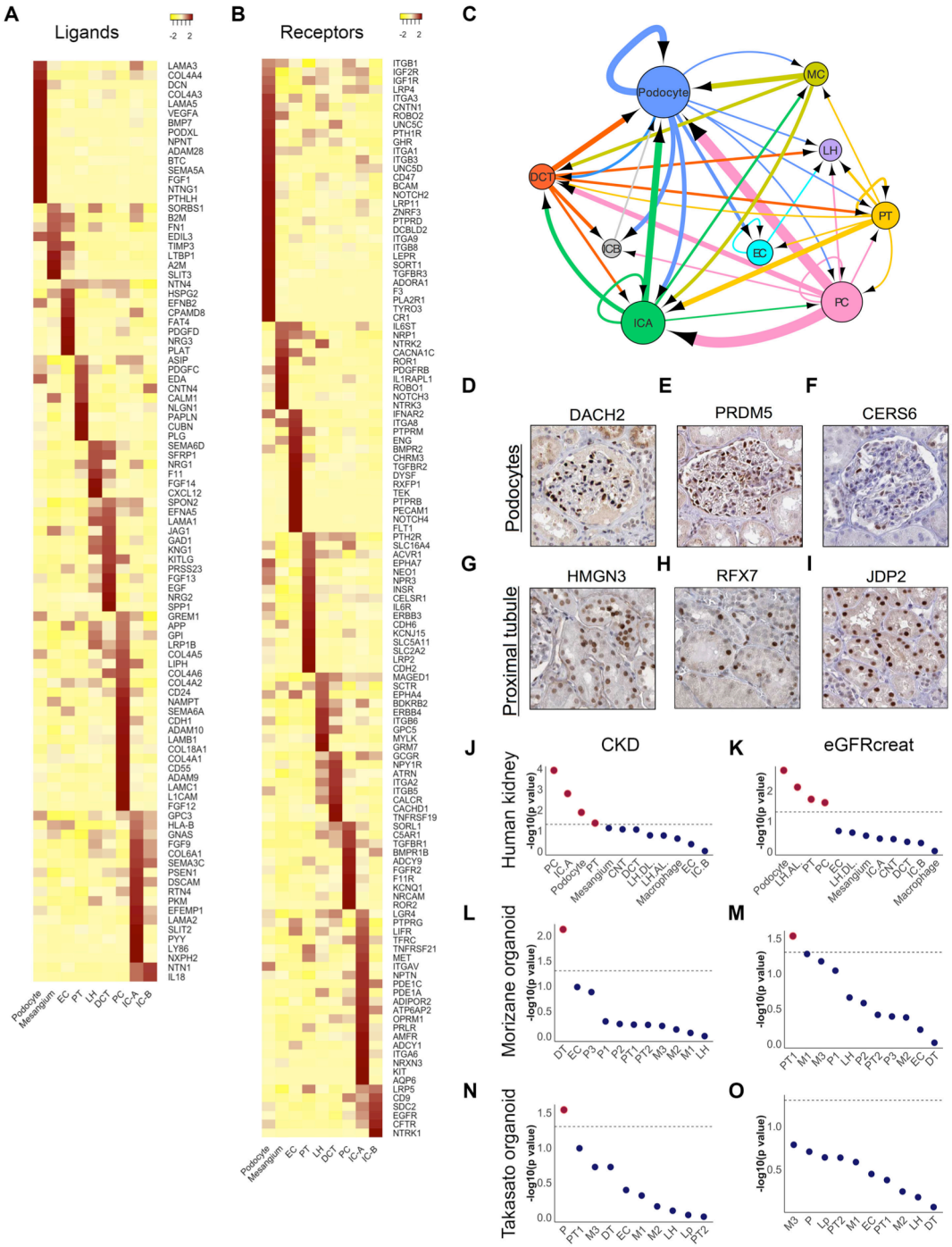

Figure S5, Related to Figure 4 and Figure 5. Receptor – ligand and transcription factor expression with CKD and eGFR GWAS associations in healthy adult kidney cell types. (**A**) Ligands detected in adult kidney and cell type specific expression. (**B**) Receptors for which ligands could also be detected, and cell specific expression. **(C)** Receptor and ligand connectome in healthy adult kidney based on cell-type specific expression of ligand-receptor pairs. **(D-F)** Novel transcription factors expressed in podocytes and **(G-I)** proximal tubule. Immunohistochemistry data from the Human Protein Atlas (https://www.proteinatlas.org/). **(J-O)** CKD and eGFR GWAS associations with single cell types from adult kidney vs. organoids. RolyPoly-inferred trait relevant cell types in CKD or eGFRfor adult kidney (**J,K**), Morizane organoids (**L,M**) and Takasato organoids (**N,O**). Cell types are ranked by p value, which represents the strength of the association with CKD or eGFR. RolyPoly analysis was based on (Calderon et al., 2017).

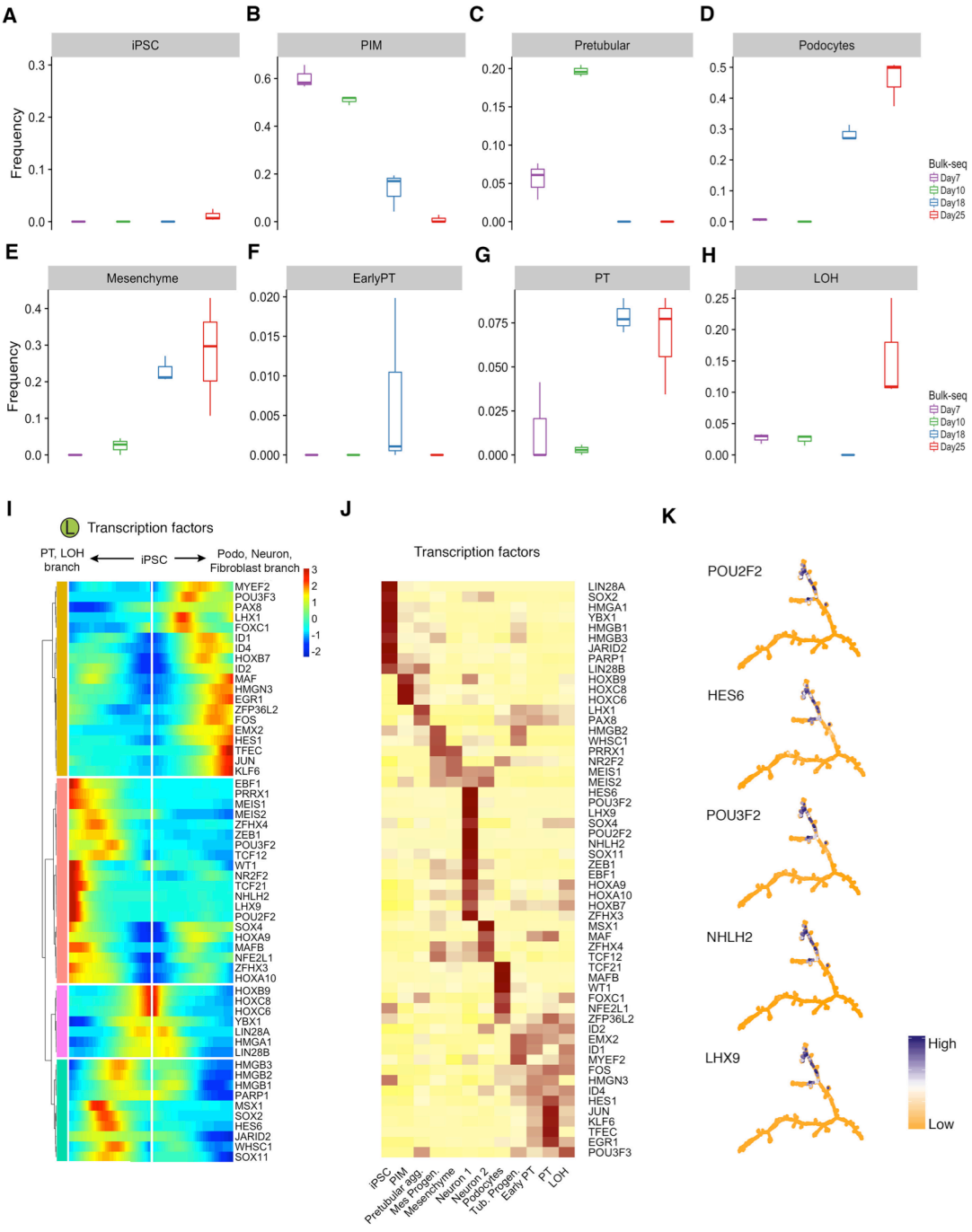

Figure S6, Related to Figure 6. Estimation of cell type proportions during differentiation of Takasato organoids and differential transcription factor expression during organoid differentiation. We used our own scRNA-seq time course data to deconvolve bulk RNA-seq data (Takasato et al., 2015). The relative proportions of iPSC (**A**), posterior intermediate mesoderm (**B**), pretubular aggregate (**C**), podocytes (**D**), mesenchyme (**E**), early proximal tubule (**F**), proximal tubule (**G**) and loop of henle (**H**) over time is shown. (**I**) Heat map showing kinetics of branch-dependent transcription factor expression identified by BEAM (Monocle2) and differential gene analysis over the full organoid time course. (**J**) The same TFs were mapped to each cell type from the time-course dataset. (**K**) Neuron specific TF expression was restricted to neural lineage cells as shown by the cell trajectory gene plot.

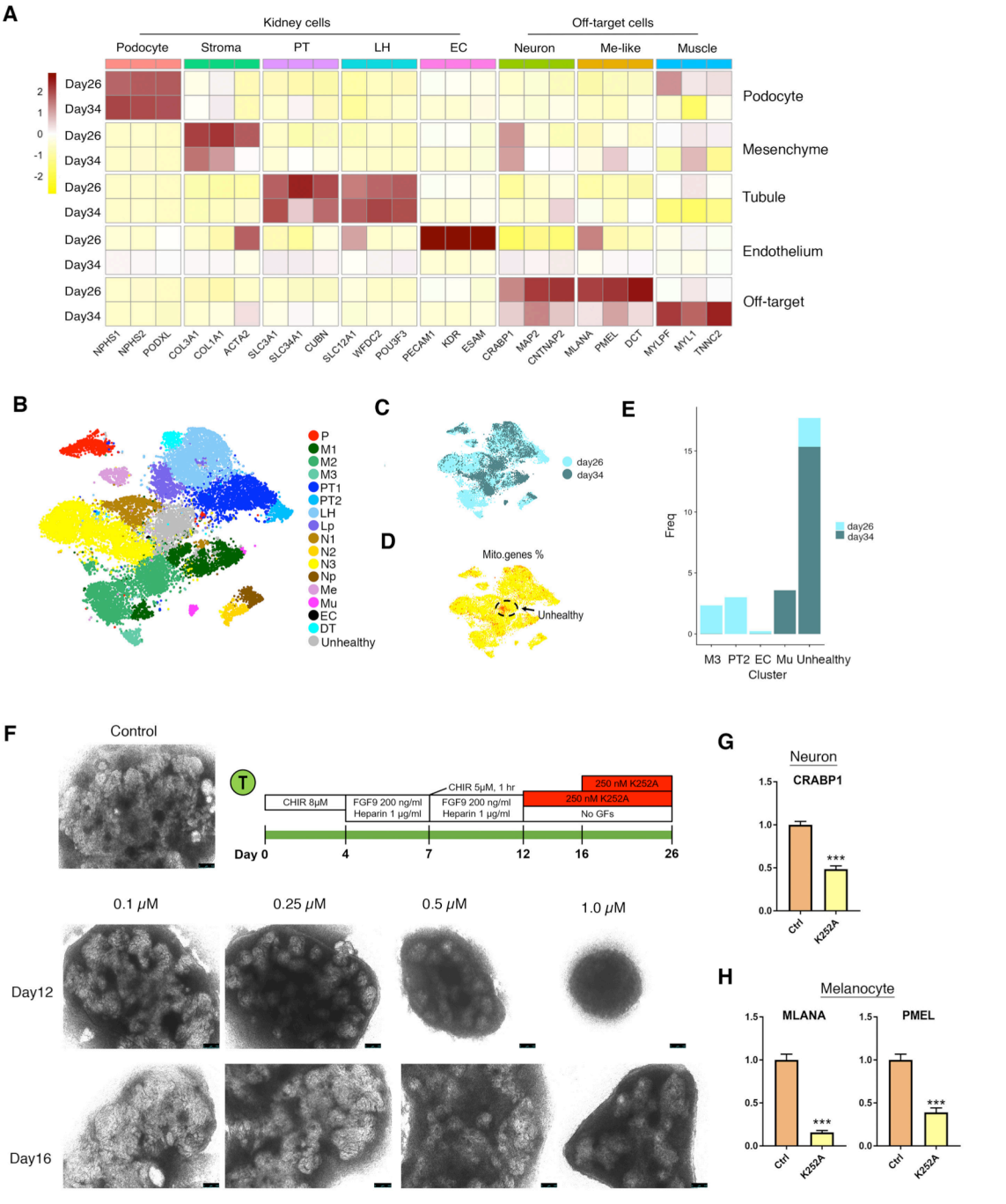

Figure S7, Related to Figure 7. Increased differentiation time does not improve differentiation state but NTRK2 inhibition reduces off-target cell types. (**A**) scRNA-seq profiles of day 26 vs. day 34 organoids generated with the Takasato protocol were compared. Average expression of selected marker genes for, major cell types are shown. In general, there is reduction in differentiated marker expression at day 34 compared to day 26, with complete loss of endothelial cells and the appearance of a muscle cluster. PT: proximal tubule; LH: Loop of Henle; EC: endothelial cells. (**B**) tSNE of all day 26 and day 34 data. Note new muscle and unhealthy cell clusters. (**C**) Contributions of day 26 vs. day 34 cells to the tSNE. (**D**) A new cluster has a high percentage of mitochondrial genes. (**E**) Relative contributions of day 24 vs. day 34 in differentiated kidney cell types: mature stroma (M3), Proximal tubule (PT2) and endothelial cells (EC). There is also an increased contribution of muscle (Mu) and unhealthy cells at day 34. **(F)** Dose and timing of NTRK2 inhibitor K252a during organoid maturation. Four doses of K252a were tested with administration starting either at day 12 or day 16. A dose of 250 nM was chosen because it did not alter organoid

size or gross tubular morphology. Higher doses caused reduced organoid size. Scale bar, 100 μm. **(G,H)** Reduction in off-target marker expression with K252a treatment during days 12 – 26 of organoid differentiation. qPCR measurements from day 26 total organoid mRNA. ***, $p < 0.001$.

Supplementary table S1, Related to STAR Methods. Count statistics

	Organoid #	Total cell #	nUMI/cell	nGene/cell	%mito/cell
Takasato iPS #1	1	7,379	2,689	1,495	5.35
Takasato iPS #2	1	7,352	2,675	1,470	7.02
Takasato iPS #3	1	5,269	1,805	1,040	3.79
Takasato ES #1	2	8,857	1,756	1,012	2.29
Takasato ES #2	2	10,471	1,687	999	2.77
Takasato BDNF #1	2	2,149	3,203	1,594	12.9
Takasato BDNF #2	2	1,165	2,775	1,385	14.3
Morizane iPS #1	12	8,471	1,756	1,049	9.33
Morizane iPS #2	12	6,720	1,764	1,082	7.61
Morizane iPS #3	12	2,911	1,574	929	2.03
Morizane ES	12	7,081	2,220	1,176	2.19
Takasato Day 0	1	218	3,865	1,798	2.99
Takasato Day 7	1	1,741	1,948	1,091	6.28
Takasato Day 12	1	1,169	2,098	1,142	6.88
Takasato Day 19	1	1,097	1,848	1,087	5.25
Takasato Day 26	1	4,965	1,544	955	6.48
Takasato Day 34	1	6,115	2,298	1,245	8.85
Human kidney #1*	NA	1,684	3,766	1,749	0.18
Human kidney #2*	NA	2,840	4,034	1,834	0.33

*single nucleus sequencing: batch number denotes technical replicate from the same kidney

Supplementary table S2, Related to Figure 4. Top transcription factors in human proximal tubule

TF	DC	CC	BC	DFC	DCC	DDC	AVG. RANK	PT (T)	PT (M)	Known
BNC2	1	1	1	1	1	1	1	No	No	Yes
HNF4A	2	2	2	2	2	2	2	No	Yes	Yes
L3MBTL4	3	3	3	3	3	3	3	No	No	No
PPARA	4	4	4	6	6	5	4	Yes	Yes	Yes
GLIS1	8	6	8	4	4	4	5	No	No	Yes
NFIA	6	5	6	6	6	6	6	No	No	Yes
ZBTB20	5	7	5	6	6	7	7	No	No	Yes
CUX1	7	8	7	6	6	9	8	No	No	Yes
MAF	9	9	9	6	6	10	9	Yes	Yes	Yes
HNF4G	10	10	10	6	6	11	10	Yes	Yes	Yes
NFATC3	11	11	11	6	6	12	11	No	No	Yes
MLXIPL	12	12	12	6	6	13	12	No	No	No
ZHX3	13	13	13	6	6	14	13	No	No	Yes
ZFAT	14	14	14	6	6	15	14	No	No	No
FOXP2	15	15	15	6	6	16	15	No	No	Yes
HMGN3	16	16	16	6	6	17	16	Yes	No	No
NFIB	19	17	18	6	6	18	17	Yes	No	Yes
NR1H4	18	20	17	6	6	19	18	Yes	Yes	Yes
ZEB2	21	18	23	6	6	20	19	No	No	Yes
SMARCC1	17	25	20	6	6	22	20	No	No	Yes
PAX8	20	22	21	6	6	23	21	Yes	Yes	Yes
ESR1	22	19	25	6	6	21	22	No	No	No
CREB5	30	34	19	5	5	8	23	Yes	No	No
RFX7	23	23	27	6	6	24	24	No	No	No
SATB2	24	27	22	6	6	26	25	No	No	Yes
ZNF521	26	24	28	6	6	27	26	No	No	No
HNF1A	27	21	34	6	6	25	27	No	No	Yes
ST18	25	31	24	6	6	30	28	No	No	No
TFEC	29	25	33	6	6	28	29	Yes	Yes	Yes
HSFY2	28	32	26	6	6	29	29	No	No	No
ZBTB16	33	29	31	6	6	31	31	No	No	No
MEIS1	31	32	29	6	6	34	32	No	No	No
ZNF69	33	30	38	6	6	32	33	No	No	No
AR	32	37	30	6	6	35	34	No	No	Yes
ZKSCAN1	39	28	42	6	6	33	35	Yes	No	No
HSFY1	38	36	35	6	6	36	36	No	No	No
TSHZ1	35	38	36	6	6	38	37	No	No	No
ARID4A	36	38	39	6	6	39	38	No	No	No
NRL	40	35	41	6	6	37	39	No	No	No
RUNX1	41	41	37	6	6	41	40	No	No	No
GLI3	41	40	40	6	6	40	41	No	No	No
RORB	37	48	32	6	6	48	42	No	No	No
TUB	43	45	43	6	6	45	43	No	No	No
CSDC2	44	46	44	6	6	46	44	No	No	No
JDP2	50	42	50	6	6	42	45	No	No	No
ZNF75D	45	47	46	6	6	47	46	No	No	No
ZNF697	52	43	51	6	6	43	47	No	No	No
SLC2A4RG	51	44	51	6	6	44	48	No	Yes	No
KLF13	46	50	47	6	6	49	49	No	No	No
EMX2	47	49	48	6	6	50	50	Yes	Yes	Yes
E2F3	48	50	49	6	6	51	51	No	No	Yes
NR1I3	48	54	45	6	6	54	52	No	No	No
NFE2L1	52	52	51	6	6	52	53	No	No	No
CAPN15	52	53	51	6	6	53	54	No	No	No

TF: transcription factor; **DC**: Degree Centrality; **CC**: Closeness Centrality; **BC**: Betweenness Centrality; **DFC**: Disruptive Fragmentation Centrality; **DCC**: Disruptive Connection Centrality; **DDC**: Disruptive Distance Centrality; **AVG.RANK**: Average Ranking. **T**: Takasato protocol; **M**: Morizane protocol

Supplementary table S3, Related to Figure 4. Top transcription factors in human podocyte

TF	DC	CC	BC	DFC	DCC	DDC	AVG. RANK	Podo (T)	Podo (M)	Known
DACH1	1	1	1	2	2	1	1	No	Yes	Yes
RERE	2	2	2	1	1	2	2	No	No	No
DACH2	5	7	5	4	4	4	3	No	Yes	No
WT1	8	9	6	3	3	3	4	Yes	Yes	Yes
TCF7L1	7	3	3	7	7	5	4	No	No	No
BACH1	11	5	7	7	7	9	6	Yes	No	No
CERS6	6	13	9	6	6	7	7	No	No	No
SOX6	3	11	4	12	12	11	8	No	No	No
ARID1B	4	15	10	7	7	10	8	No	No	No
TEAD1	9	4	8	12	12	13	10	No	No	Yes
NFATC3	14	17	11	7	7	8	11	No	No	Yes
ZBTB7C	10	12	14	12	12	14	12	Yes	Yes	Yes
SMAD6	29	22	12	4	4	6	13	No	Yes	Yes
JARID2	19	16	17	7	7	12	14	No	No	No
BAZ2B	13	10	13	18	18	19	15	No	No	No
CASZ1	15	6	16	18	18	20	16	No	No	Yes
LMX1B	12	14	15	18	18	17	17	No	No	Yes
HMGN3	18	30	19	12	12	15	18	Yes	No	No
TULP4	16	18	18	18	18	21	19	Yes	Yes	No
ZNF124	21	8	23	18	18	22	20	No	No	No
PRDM5	17	19	22	18	18	23	21	No	No	No
TSHZ2	31	33	20	12	12	16	22	No	No	No
KLF7	21	20	26	18	18	24	23	No	No	No
TCF7L2	20	21	27	18	18	25	24	Yes	No	Yes
TSHZ3	33	37	25	12	12	18	25	No	No	No
ETV6	25	24	28	18	18	27	26	No	No	No
ZEB2	24	32	21	18	18	28	27	No	No	Yes
NFIC	26	23	31	18	18	26	28	No	No	No
ZNF521	23	31	24	18	18	31	29	No	No	No
ELF1	27	25	30	18	18	29	30	No	No	No
SMAD2	30	27	33	18	18	30	31	No	No	Yes
HIVEP3	36	26	29	18	18	32	32	No	No	No
ZBTB1	32	28	34	18	18	33	33	No	No	No
MAFB	27	34	32	18	18	35	34	Yes	Yes	Yes
SMAD1	37	29	37	18	18	34	35	No	No	Yes
ZNF846	34	35	35	18	18	36	36	No	No	No
ZNF250	35	36	36	18	18	37	37	No	No	No
IKZF2	37	38	37	18	18	38	38	No	No	No

TF: transcription factor; **DC**: Degree Centrality; **CC**: Closeness Centrality; **BC**: Betweenness Centrality; **DFC**: Disruptive Fragmentation Centrality; **DCC**: Disruptive Connection Centrality; **DDC**: Disruptive Distance Centrality; **AVG.RANK**: Average Ranking; **Podo**: Podocyte; **T**: Takasato protocol; **M**: Morizane protocol.