SUPPLEMENTARY INFORMATION (Tables and Figures) for

Integrative Omics analyses reveal epigenetic memory in diabetic renal cells regulating genes associated with kidney dysfunction.

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This Supplementary file includes:

Supplementary Figures S1 to S9
Supplementary Tables S1 to S3

Supplementary Figure S1

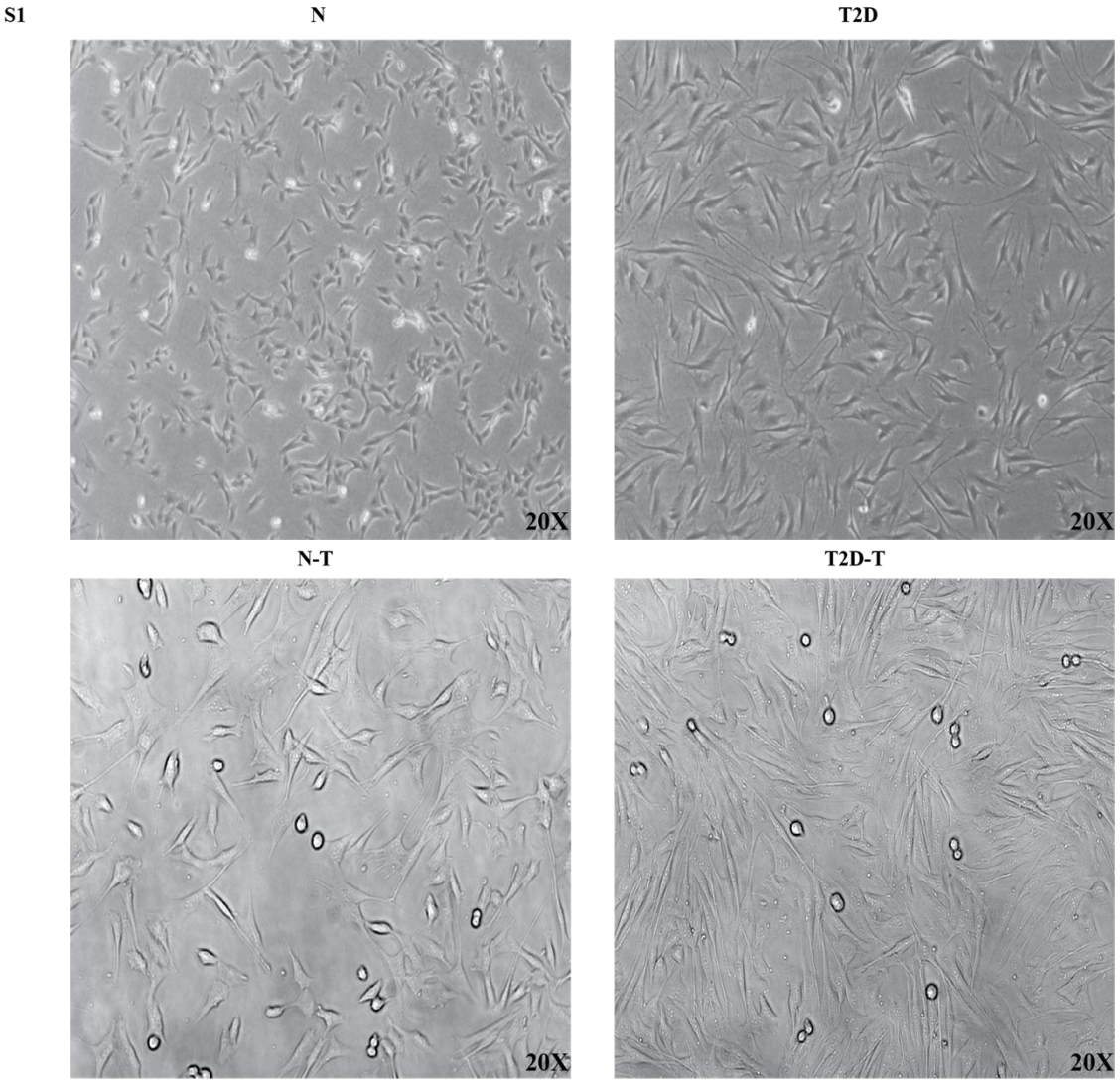

Fig. S1: Brightfield (20X) images showing morphological features of PTECs. N and T2D-PTECs images were taken 5 days after seeding (Passage 2, seeding density 50,000 cells in 10cm dish). N-T and T2D-T images were taken 24 hours after treatment with TGFβ1. The images were taken with EVOS FL Auto microscope.

Supplementary Figure S2

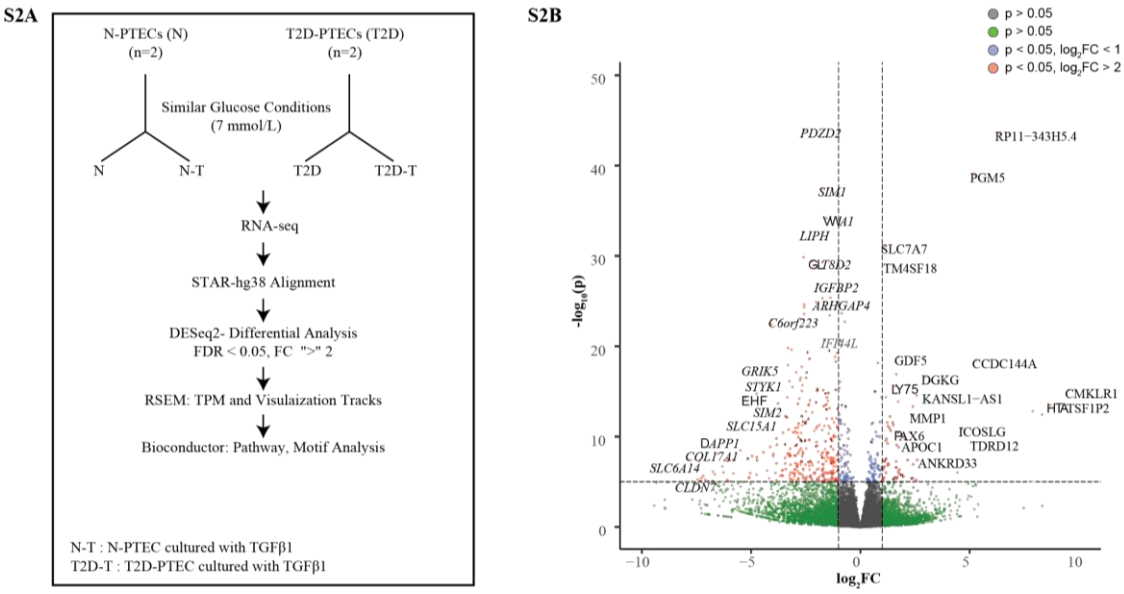

Fig. S2: A) Workflow for RNA-seq and differential expression analysis in PTECs. B) Volcano plot showing DEGs in T2D vs N-PTECs. Grey (non-significant fold change), green (fold change more than 2 and p-value greater than 0.05), blue (fold change less than 2 and p-value lesser than 0.05), and pink (fold change more than 2 and p-value less than 0.05, most significant).

Supplementary Figure S3

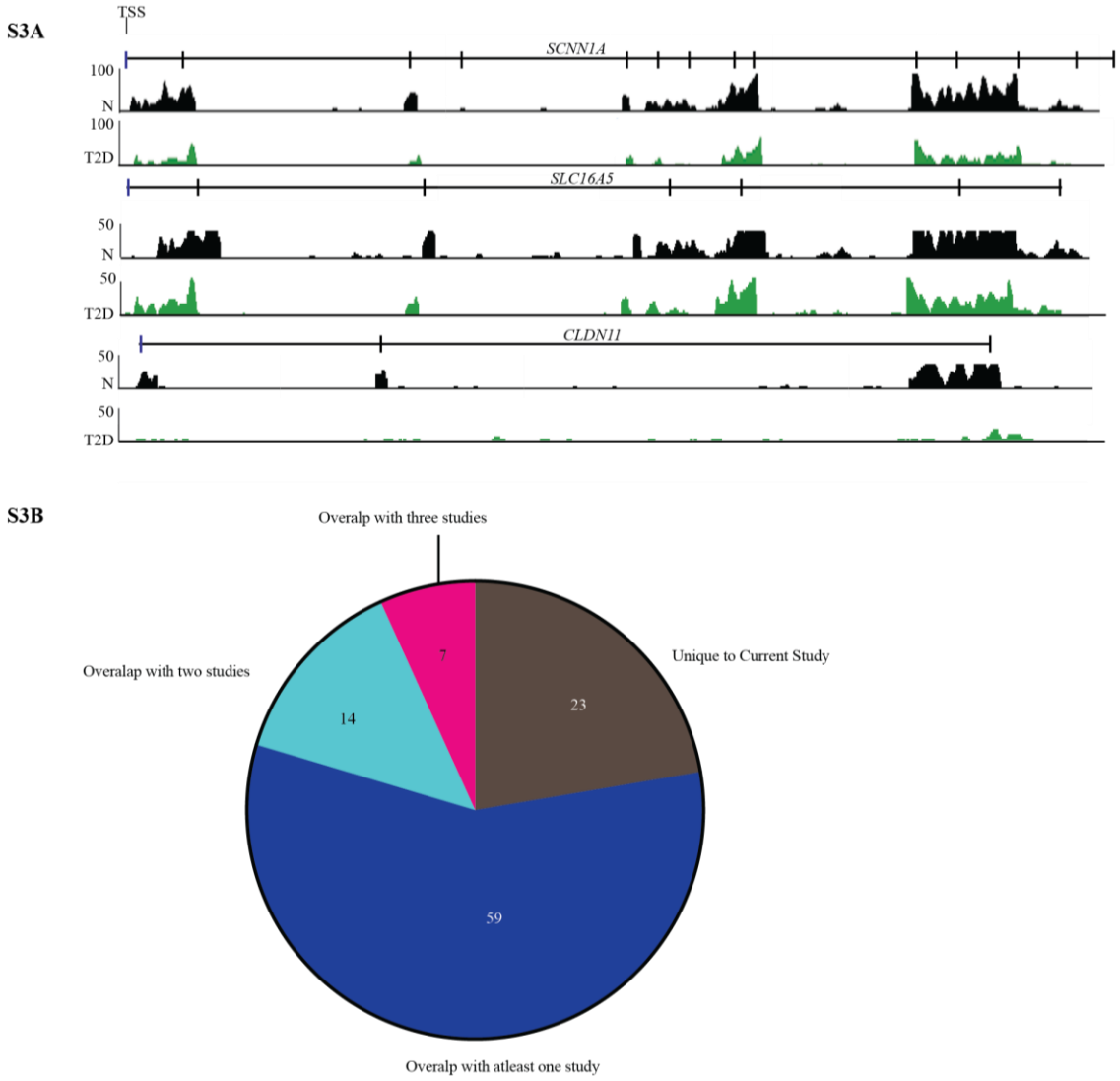

Fig. S3: A) Genome browser shots showing expression of a few TAGs (*SCCN1A*, *SLC16A5*, and *CLDN11*) in N-PTECs (black) and T2D-PTECs (green), as observed in RNA-seq. B) Pie chart showing overlap of current study with published DKD studies (1-3). Dark blue color in the pie chart shows that 59 of our differentially expressed TAGs also show differential expression in at least one of the three published studies. Light blue color shows that 14 TAGs in our study are also differentially expressed in any two of the three studies while magenta color shows that 7 TAGs are differentially expressed in all three published DKD studies and our study. 23 TAGs, highlighted in brown, show differential expression only in our study. We identified differential TAGs in published DKD studies from patient samples and identified overlapping TAGs with our study.

Supplementary Figure S4

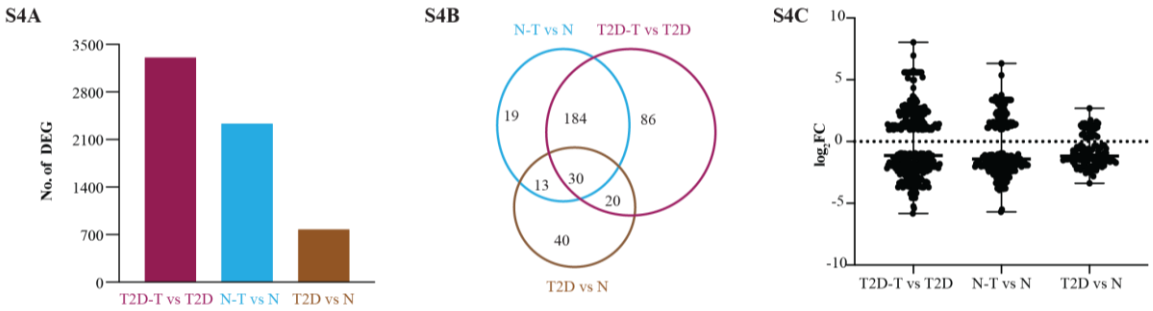

Fig. S4: Gene expression profiles of PTECs. A) Bar plot showing number of DEGs in T2D-T vs T2D (magenta), N-T vs N (blue), and T2D vs N (Brown). B) Venn diagram showing number of overlapping differential TAGs in various comparisons. in T2D-T vs T2D (magenta), N-T vs N (blue), and T2D vs N (Brown). C) Violin plots showing fold changes of TAGs in T2D-T vs T2D, N-T vs N, and T2D vs N based on RNA-seq (p-adjusted < 0.05). Each dot represent a single TAG and error bars represent minimum and maximum fold changes.

Supplementary Figure S5

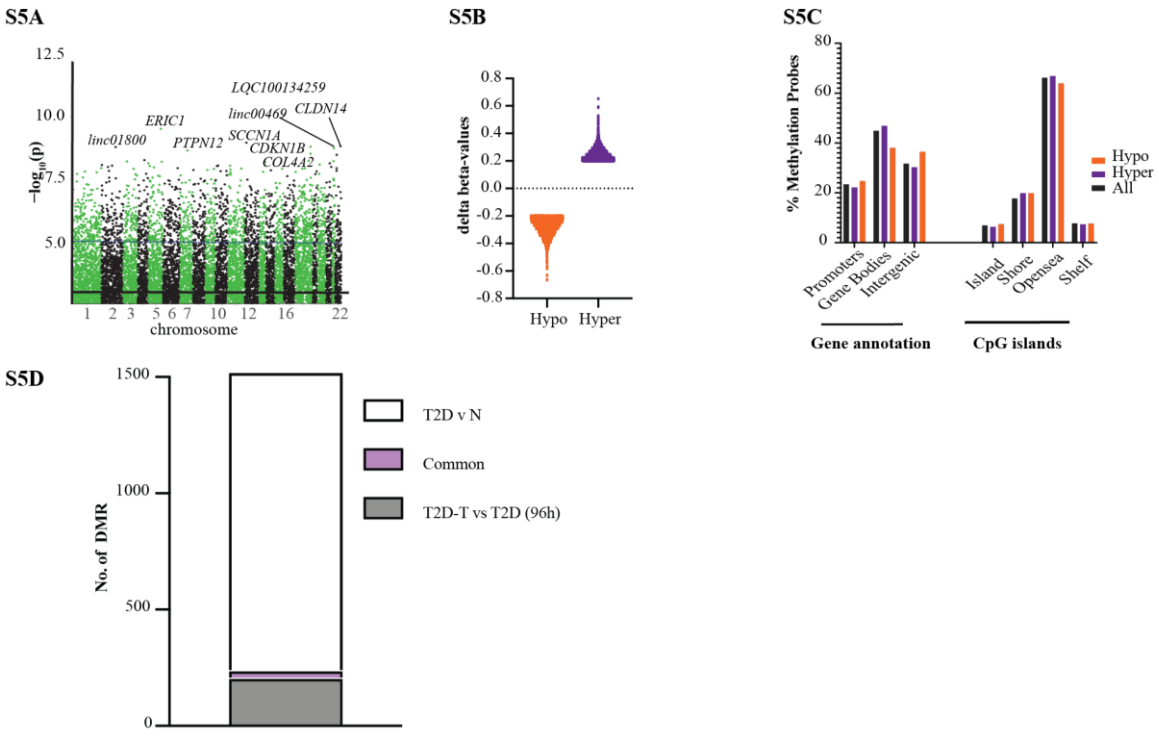

Fig. S5: DNA methylation profiles of PTECs. A) Manhattan plot showing differentially methylated probes (DMPs, FDR <0.05) in T2D-PTECs as compared to N-PTECs. A few top hits are highlighted. B) Violin plot showing distribution of DMPs with at least 20% fold change. C) Bar plot showing genomic distribution of DMPs in T2D. D) Stacked bar plot with number of differentially methylated regions (DMRs) in T2D-T (96 hours) v T (grey), T2D vs N (clear) and overlapping between T2D-T (96 hours) vs T2D vs N (pink).

Supplementary Figure S6

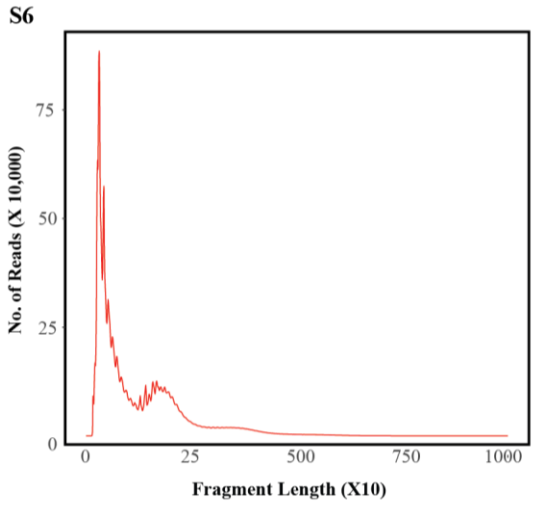

Fig. S6: Representative line chart showing fragment distribution of ATAC-seq reads.

Supplementary Figure S7

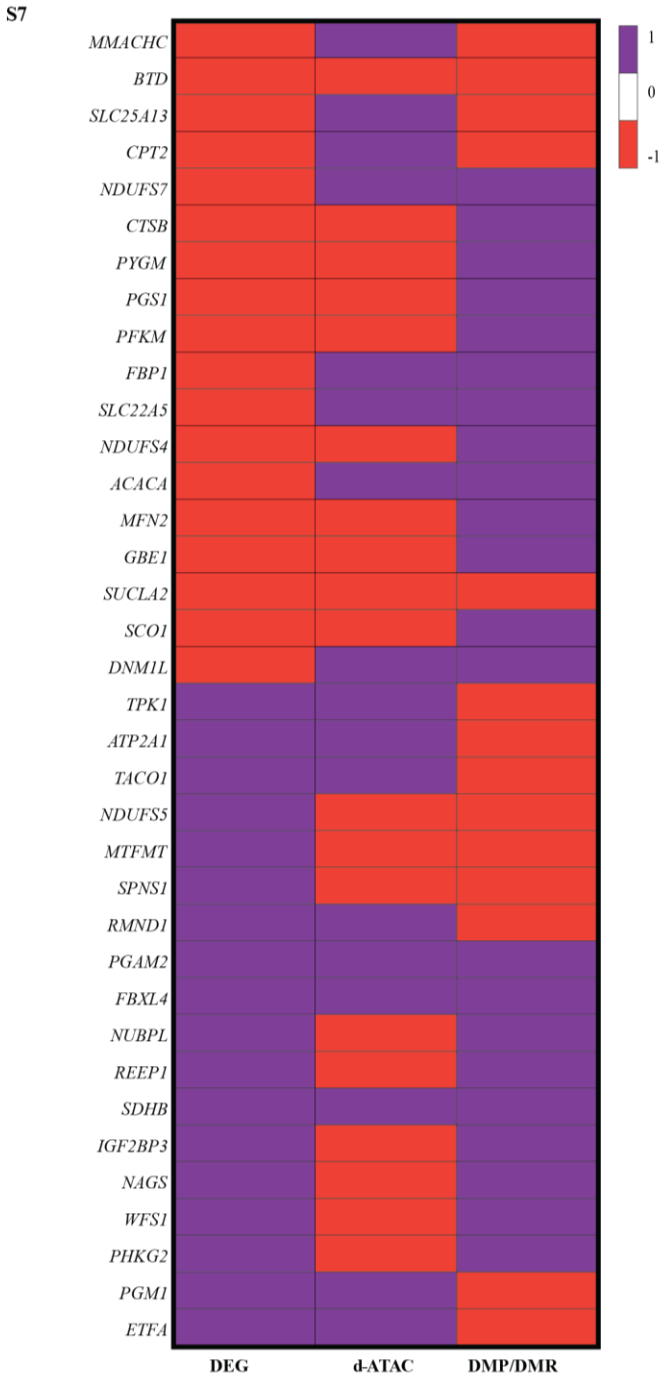

Fig. S7: Pseudo-color heat map showing mitochondrial function associated DEGs and corresponding d-ATAC (differential ATAC peaks) and DMP/DMR within 50kb of their TSS. Mitochondrial function associated genes are identified as described in the mitochondria associated database (4). Orange color represents down regulation of gene expression, hypomethylation, and decreased ATAC peaks while purple color represents up regulation of gene expression, hypermethylation, and increased ATAC peaks.

Supplementary Figure S8

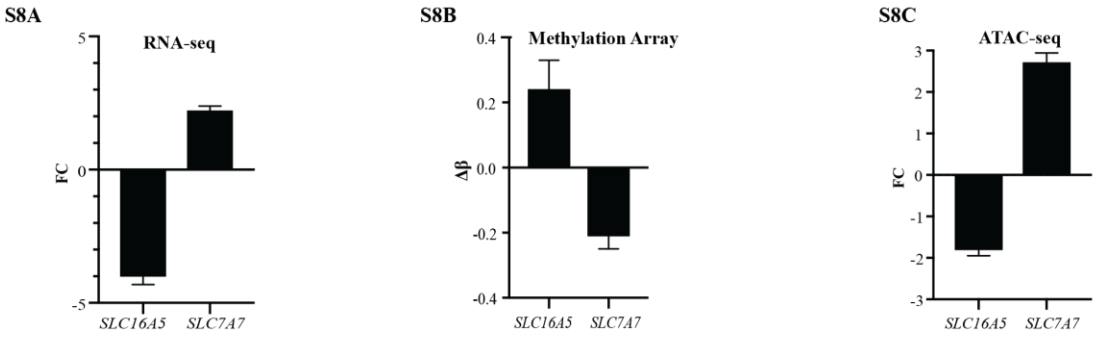

Fig. S8: Potential regulation of TAGs via epigenetic memory. A-C) Altered gene expression (A), methylation (B), and chromatin accessibility using ATAC (C) of candidate TAGs *SLC16A5* and *SLC7A7*. Gene expression is measured by RNA-seq, fold change (FC) calculated by DESeq2, delta-beta (Δβ) values for methylation are calculated using ChAMP package, and differential ATAC-seq fold change (FC) is calculated using MACS2. All the displayed data showed p-values <0.05 in respective analyses.

Supplementary Figure S9

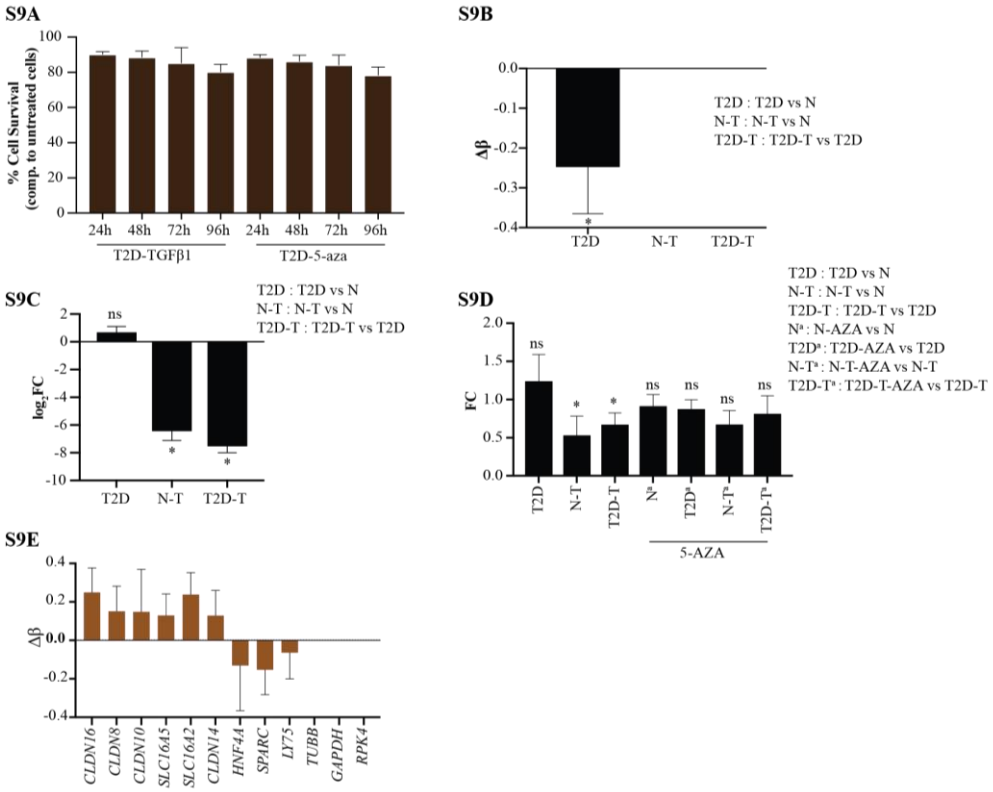

Fig. S9: HNF4A as a potential regulator for candidate TAGs. A) Bar plot showing cell viability after 5-azacytidine (5-aza) treatment in N and T2D-PTECs. B) Altered methylation levels of *HNF4A* promoter in T2D, N-T, and T2D-T cells. C) Variation in *HNF4A* gene expression in T2D, N-T, and T2D-T cells. D) Variation in HNF4A protein expression in T2D, N-T, and T2D-T cells before and after 5-aza treatment. E) Variation in methylation levels of representative TAGs based on EPIC array analysis. All the data shown has p-value <0.05 based on EPIC-array analysis. Bars represent average variation in gene expression or methylation and error bars represent standard deviation, *= p-value <0.05.

Supplementary Table S1: Characteristics of human renal PTECs used in the study

Donor	Age	Gender	Ethnicity	Characteristics	Anti-diabetic Drugs
N	57	M	Caucasian	No diabetes or renal disease	None
N	60	F	African-American	No diabetes or renal disease	None
T2D	69	M	Caucasian	diabetes	Metformin, Furosemide
T2D	61	F	Caucasian	diabetes	Metformin

N: = Healthy control; T2D: Type 2 Diabetes

PTECs from non-diabetic (control, N) and diabetic (T2D) individuals were cultured in renal epithelial growth media (REGM, Lonza, NJ, USA), which contains 7 mmol/L glucose as per manufacturer's formulation. These PTECs were from two donors each for N [Lonza, CC-2553, lot nos. 0000530068 (Male) and 0000442212 (Female)] and T2D [Lonza, CC-2925, 0000231627 (Male), 0000238893 (Female)].

Supplementary Table S2. List of primers used in the study for gene expression, methylation and ChIP-qPCR.

Gene Expression qPCR		
Symbol	**Primer**	**Sequence**
CLDN3	Forward Primer	CCAAGGCCAAGATCACCAT
CLDN3	Reverse Primer	GGTTGTAGAAGTCCCGGATAATG
CLDN4	Forward Primer	GCCTTACTCCGCCAAGTATT
CLDN4	Reverse Primer	AGGGAAGAACAAAGCAGAGAG
CLDN8	Forward Primer	GCTTCCGTGATGTCCTTCTT
CLDN8	Reverse Primer	GAGCCTTCACCTTCTCATTGT
CLDN10	Forward Primer	AGACACAGGCTTCTTCCTAGA
CLDN10	Reverse Primer	GAGTGAGACAGGACATGAAAGG
CLDN11	Forward Primer	CGAACTCCTGGACTCAAAGTATC
CLDN11	Reverse Primer	GGCTCCCATTGTCATCTGTATC
CLDN14	Forward Primer	GCTCCGGAAGAACTTTCAGATA
CLDN14	Reverse Primer	GTTCTTGACTTCTTGGCTTGTG
CLDN16	Forward Primer	CCCTGATGAGCCGTACATTAAA
CLDN16	Reverse Primer	CATACCACACAGAGCCAATGA
COL6A2	Forward Primer	CAACAACTGCCCAGAGAAGA
COL6A2	Reverse Primer	CATGTGGAAGAGCAGGATGT
CTCF	Forward Primer	TACAAACACACCCACGAGAAG
CTCF	Reverse Primer	CTCCAGTATGAGAGCGAATGTG
GAPDH	Forward Primer	GGTGTGAACCATGAGAAGTATGA
GAPDH	Reverse Primer	GAGTCCTTCCACGATACCAAAG
HNF4A	Forward Primer	GGAGAGGACAAGATGGGTAAAC

HNF4A	Reverse Primer	TAAGACAGTGCCTGGGAGTA
KCNQ1	Forward Primer	GAAGCCCTCACTGTTCATCTC
KCNQ1	Reverse Primer	GCGTCACCTTGTCTTCTACTC
LY75	Forward Primer	GAAAGGAGGCTCAGAGGAAAG
LY75	Reverse Primer	GCCAGGTCCCATCAATTAAGA
RIPK4	Forward Primer	TATGTGTGGCCAGCTCTTAAC
RIPK4	Reverse Primer	GAAACGAACGGCAGCTAGTA
SCNN1A	Forward Primer	GGCTGTGCCTACATCTTCTATC
SCNN1A	Reverse Primer	GAGAAGTCAACCTGGAGCTTATAG
SLC2A1	Forward Primer	GGACAGGCTCAAAGAGGTTATG
SLC2A1	Reverse Primer	AGGAGGTGGGTGGAGTTAAT
SLC5A2	Forward Primer	CGGTACAGACCTTCGTCATTC
SLC5A2	Reverse Primer	CTCCCAGGTATTTGTCGAAGAG
SLC14A1	Forward Primer	CACTTTGGGAGATGGAGGTAAA
SLC14A1	Reverse Primer	GCTACACCTGGCTAATGTTACT
SLC14A2	Forward Primer	GCCCTTTGACTCCATCTACTT
SLC14A2	Reverse Primer	GACGTAGAACATGCCTCCTATC
SLC16A2	Forward Primer	CTACCATGTGGCCTTCTACTTT
SLC16A2	Reverse Primer	GCTGGAATCTCTCTGCTCTTT
SLC16A5	Forward Primer	CTACATGTCCAGCTTCTTCCTC
SLC16A5	Reverse Primer	CTTGCCTTGCTCCTTCTTCT
SLC34A1	Forward Primer	ACTAGGATAGGCAGGAGTAAGG
SLC34A1	Reverse Primer	GCAAGGAGGTGTGCAAATTC
SLC47A1	Forward Primer	ACCGTTTCCCTGCTGATTAC
SLC47A1	Reverse Primer	ATGATGTCTCGGTCGGTAGTA

SLCO1A2	Forward Primer	GTCTACTCCTTACTCTCCTTCCT
SLCO1A2	Reverse Primer	CACCCTTCCTTACTCCATTCTC
SPARC	Forward Primer	GGGAGTGTATGGTTCCTGTAAG
SPARC	Reverse Primer	GCTCAGTCTGGTGCTGATAAT
TGFB1	Forward Primer	CCTGCCTGTCTGCACTATTC
TGFB1	Reverse Primer	TGCCCAAGGTGCTCAATAAA
TUBB	Forward Primer	GGGAGGTGTCAGCAGTATTATC
TUBB	Reverse Primer	GAGGTAGAGTTGGAAAGGGAAG
ChIP qPCR		
CLDN10	Forward Primer	CCGTGTCCTTGTTCTCTCATT
CLDN10	Reverse Primer	TCATCCCTTCATTGCTGTCTC
CLDN14	Forward Primer	TCCCAAAGTGCAGGGATTAC
CLDN14	Reverse Primer	GAACAGGGAAGTGGTGAATGA
CLDN16	Forward Primer	CAAGGGCTCTCAGACCATAAAT
CLDN16	Reverse Primer	CCTAACCTTTCAGCATCGTAGAG
LY75	Forward Primer	GGCCACCTCAAGTACACATTA
LY75	Reverse Primer	CTTCCTGTGGACACTGCTATC
SLC16A2	Forward Primer	AGGGCAGGAGAACAGGATA
SLC16A2	Reverse Primer	CTCCCTTCCCAAACTACTGAAC
SCL16A5	Forward Primer	CTGAGTATACAGGGTGTGGTTG
SCL16A5	Reverse Primer	GATCTGTGGACGATGGAGTTT
***Methylation* qPCR**		
CLDN10	Forward Primer	Qiagen, GPH1003703
SCL16A5	Reverse Primer	Qiagen, GPH1006016

Supplementary Table S3: Number of overlapping ATAC-seq peaks

PTEC	No. of overlapping peaks	% Overlap
N	120,412	56
T2D	118,872	48
N-T	135,309	52
T2D-T	183,368	54

References:

1. Woroniecka KI, Park AS, Mohtat D, Thomas DB, Pullman JM, Susztak K: Transcriptome analysis of human diabetic kidney disease. Diabetes 2011;60:2354-2369

2. Beckerman P, Qiu C, Park J, Ledo N, Ko YA, Park AD, Han SY, Choi P, Palmer M, Susztak K: Human Kidney Tubule-Specific Gene Expression Based Dissection of Chronic Kidney Disease Traits. EBioMedicine 2017;24:267-276

3. Wilson PC, Wu H, Kirita Y, Uchimura K, Ledru N, Rennke HG, Welling PA, Waikar SS, Humphreys BD: The single-cell transcriptomic landscape of early human diabetic nephropathy. Proc Natl Acad Sci U S A 2019;116:19619-19625

4. Lott MT, Leipzig JN, Derbeneva O, Xie HM, Chalkia D, Sarmady M, Procaccio V, Wallace DC: mtDNA Variation and Analysis Using Mitomap and Mitomaster. Curr Protoc Bioinformatics 2013;44:1 23 21-26