

## Supplemental Figures and Tables for:

### Renal endothelial single-cell transcriptomics reveals spatiotemporal regulation and divergent roles of differential gene transcription and alternative splicing in murine diabetic nephropathy

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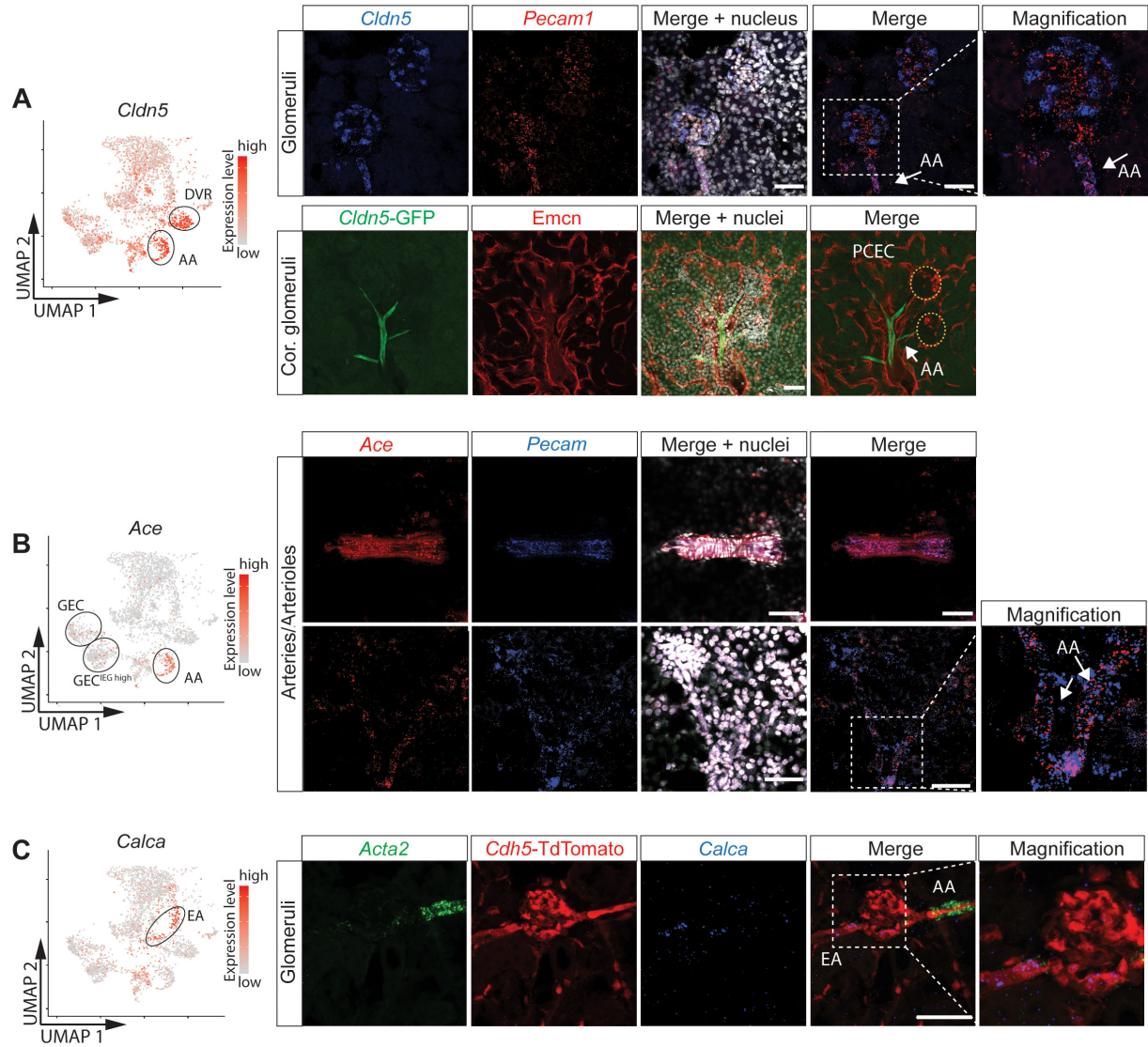
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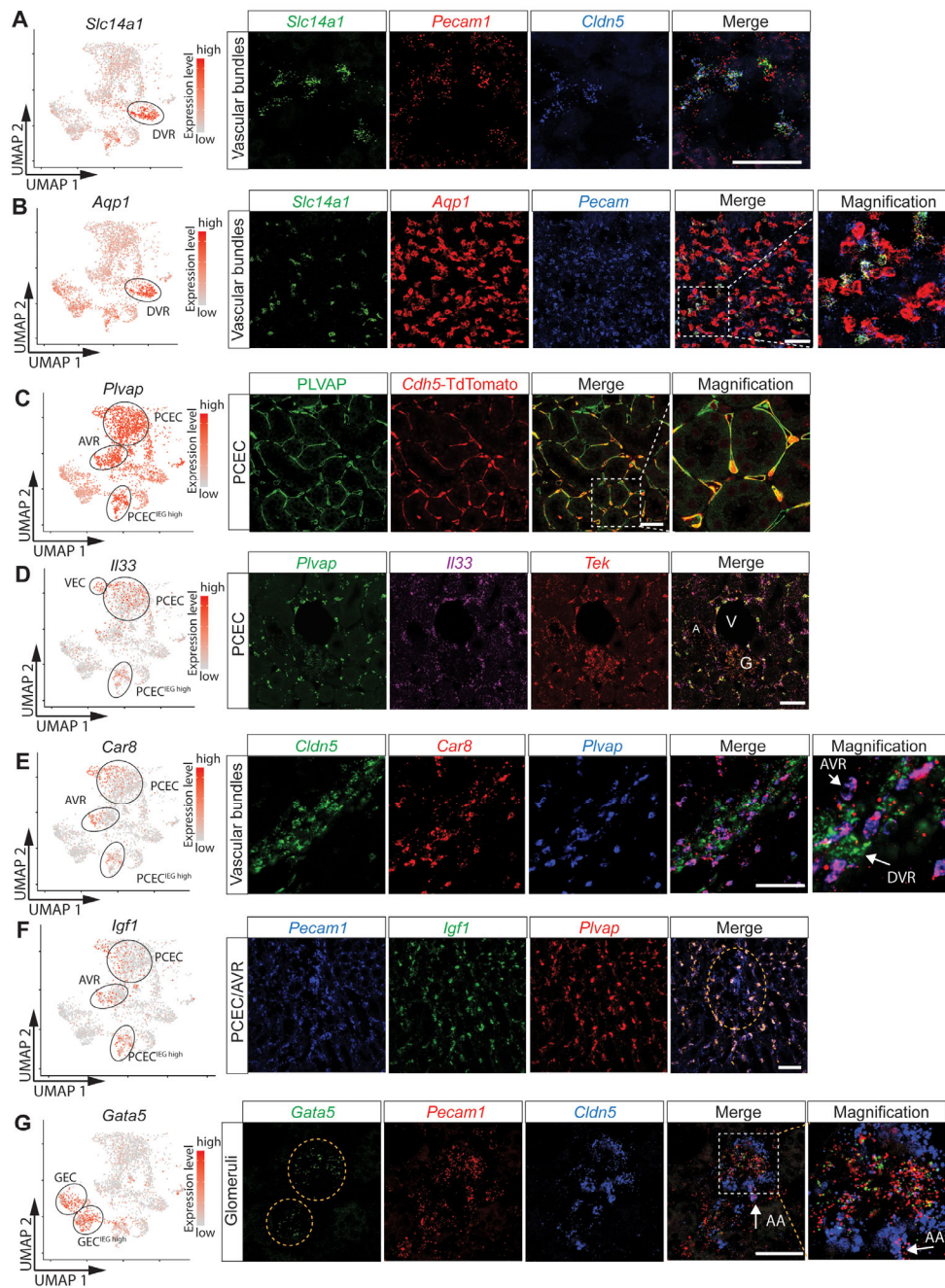
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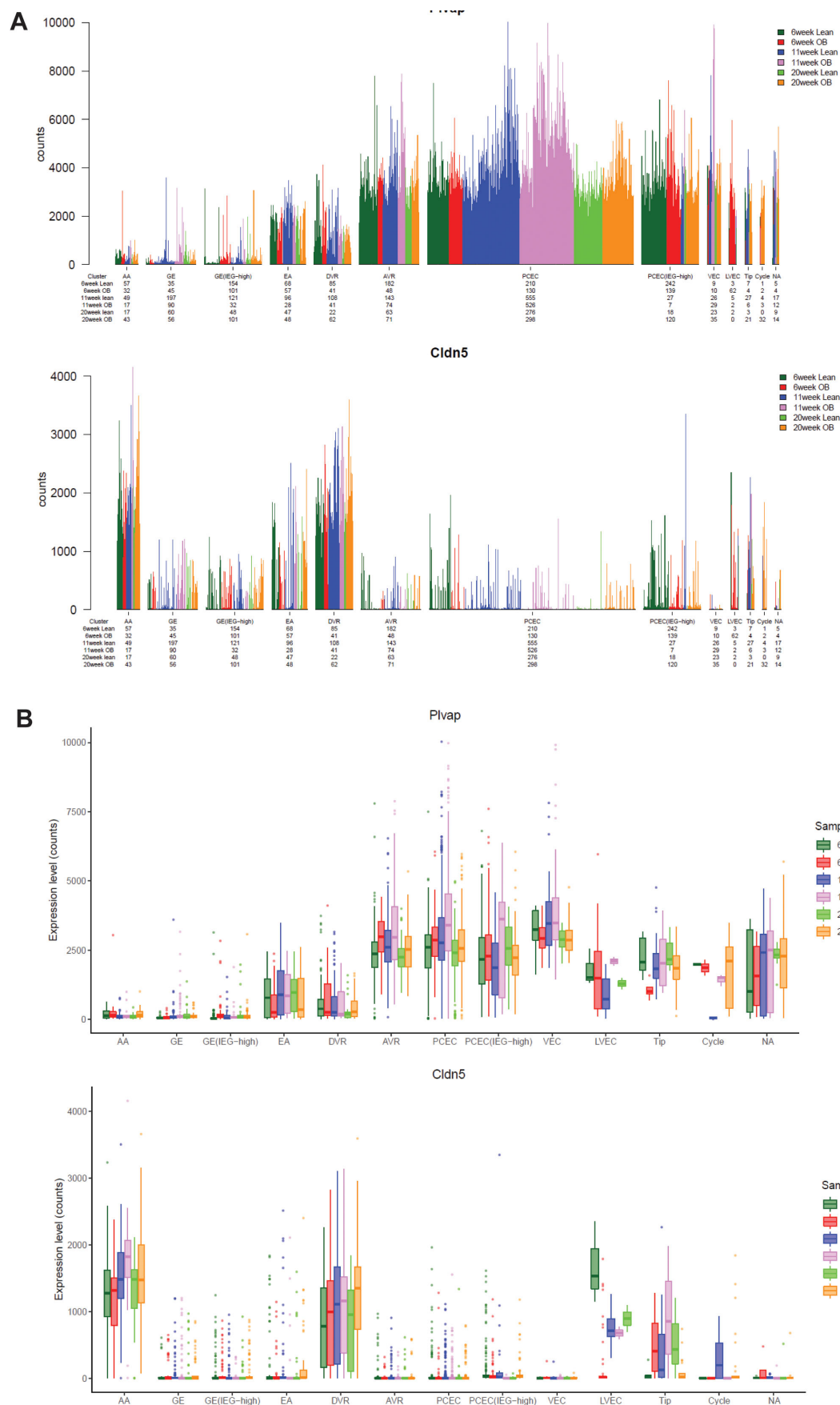
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**Figure S1.** Validation of vascular segments I. (A) UMAP of *Cldn5* and RNA-ISH for *Cldn5* in arteries and AA together with *Pecam1* expression. *Cldn5* expression can also be seen in podocytes, excluded from *Pecam1*<sup>+</sup> cells in the glomerulus, but colocalizing with *Pecam1* in the afferent arteriole. *Cldn5*-GFP reporter mice showed GFP expression in arterioles and AA but not in podocytes. Orange circles indicate glomeruli. (B) UMAP of *Ace* and RNA-ISH identified *Ace* as a novel marker for arteries and AA. Lower panel shows AA branching to two glomeruli. (C) UMAP of *Calca* and RNA-ISH identified expression of *Calca* as a marker for EC in efferent arterioles (EA). The smooth muscle layer of the afferent arteriole is labelled with *Acta2*. Scale bar = 50  $\mu$ m.

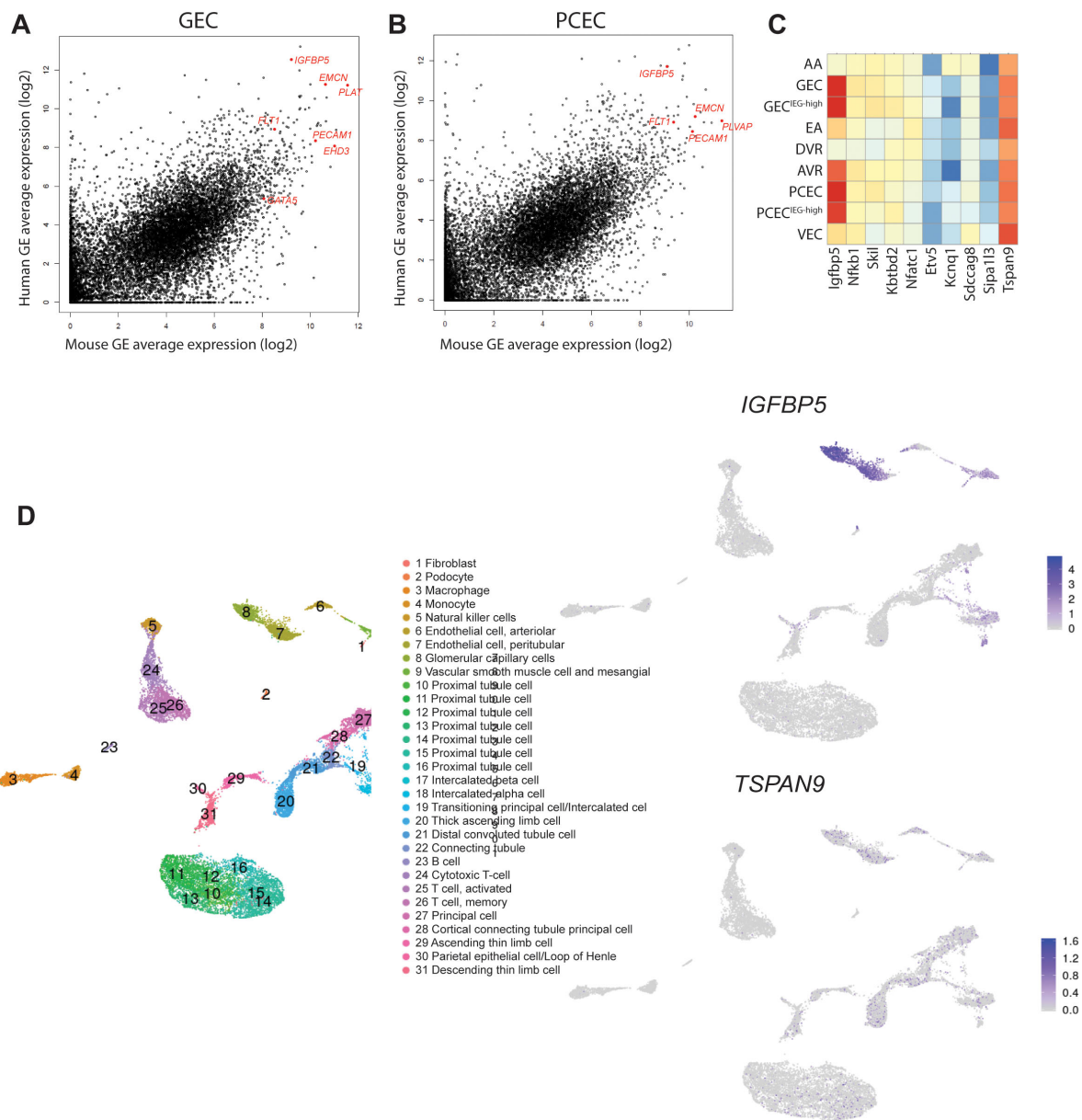


**Figure S2.** Validation of vascular segments II. (A) UMAP of the known DVR marker *Slc14a1* and *Slc14a1* RNA-ISH show colocalization of *Slc14a1* with *Cldn5* in DVR. (B) UMAP of *Aqp1* and RNA-ISH for *Aqp1* show *Aqp1* expression in DVR, but at much lower levels than in epithelial cells of the medulla. (C) UMAP of *Plvap* and immunohistochemistry staining of PLVAP show its expression in PCEC together with *Cdh5*-driven TdTomato. (D) UMAP of *Il33* and RNA-ISH for *Il33* show its expression in PCEC, VEC (V, veins) but not in glomeruli (G) or arteries (A). (E) UMAP of *Car8* and RNA-ISH for *Car8* show its expression in *Plvap*<sup>+</sup> AVR in vascular bundles. (F) UMAP of *Igf1* and RNA-ISH for *Igf1* show its expression in *Plvap*<sup>+</sup> AVR in vascular bundles indicated by tallow circle. (G) UMAP of *Gata5* and RNA-ISH for *Gata5* show specific expression in GEC, glomeruli marked with orange circle. Scale bar = 50  $\mu\text{m}$ .

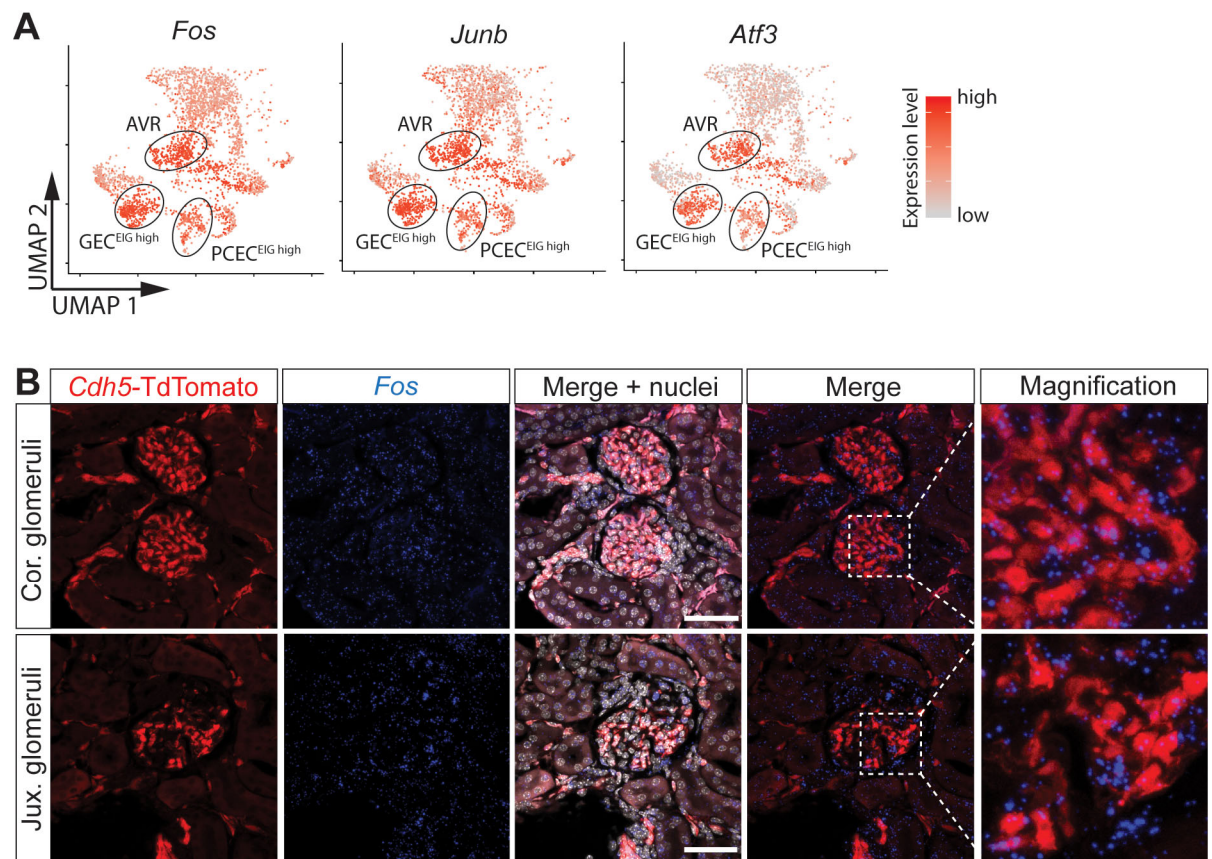


**Figure S3.** Searchable database. The searchable database show counts for individual cells in a bar plot illustrated by one line (A) or as median (B) for each group and EC population.

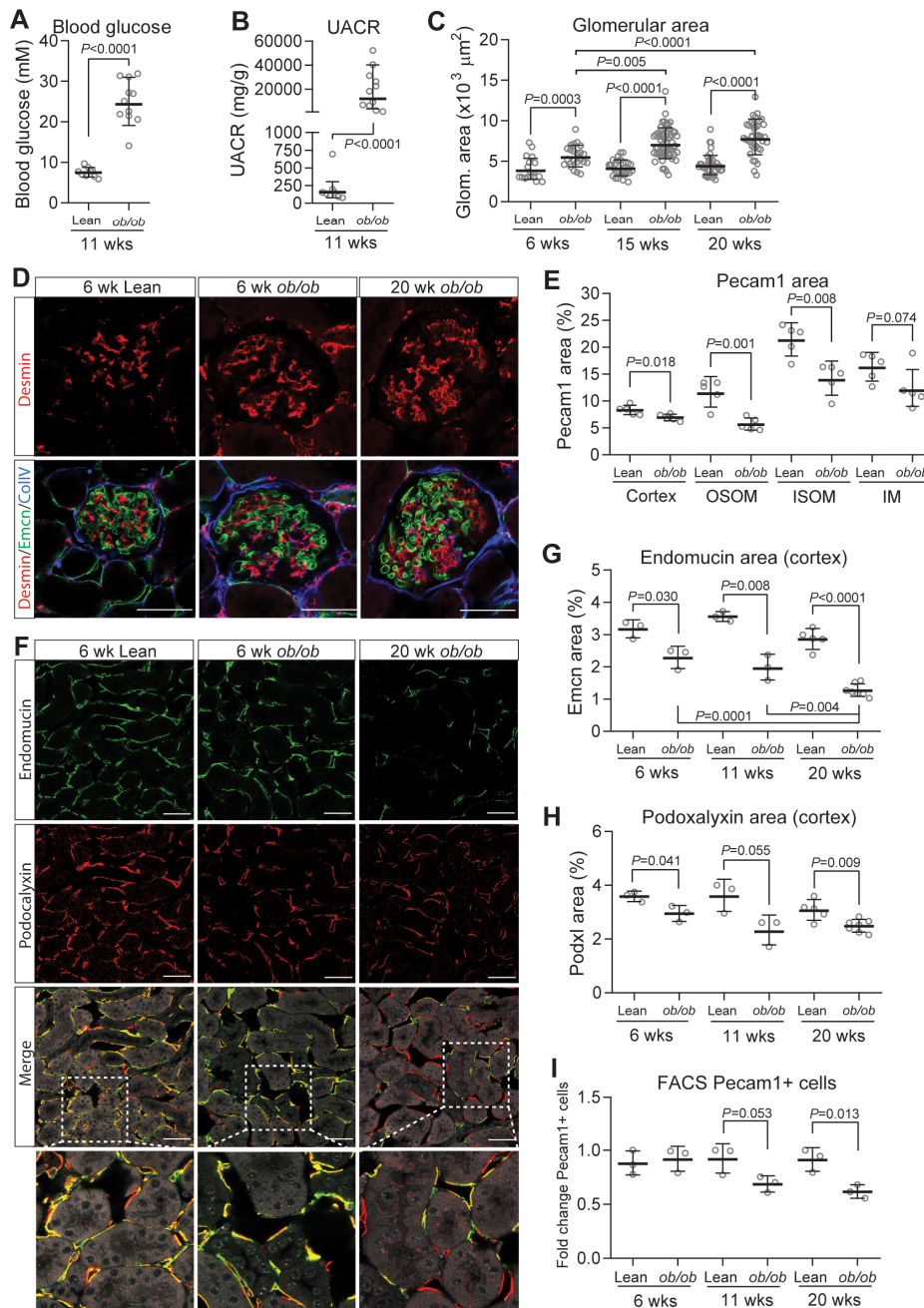




**Figure S4.** Mouse and human expression correlation. (A, B) Comparison of human GEC (A) and PCEC (B) to the same EC population in the current dataset. (C) 10 of 53 genes associated with human DKD by GWAS could be mapped to EC expression in the current dataset. (D) *IGFBP5* and *TSPAN9* in human EC show similar expression pattern as in mouse.

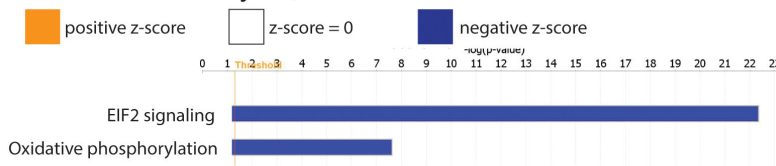


**Figure S5.** Immediate early gene (IEG) expression. (A) UMAP's for the IEG's *Fos*, *Junb*, and *Atf3*. (B) RNA-ISH for *Fos* in cortical and juxtamedullary glomeruli.

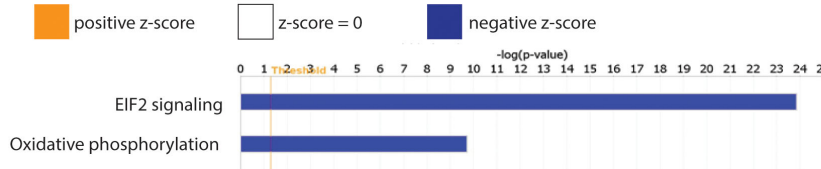


**Figure S6.** Validation of DKD. (A) Blood glucose levels and (B) urinary albumin creatinine ratio (UACR) in 11-week-old non-diabetic (Lean) and diabetic (*ob/ob*) mice. (C, D) Immunohistochemistry of glomeruli for desmin, collagen IV, and endomucin to quantify glomerular area at indicated timepoints in non-diabetic (Lean) and diabetic (*ob/ob*) mice. (E) Quantification of PECAM1 stained area in different regions of the kidney; cortex, outer stripe of medulla (OSOM), inner stripe of medulla (ISOM), and inner medulla (IM) in 11-week-old non-diabetic (Lean) and diabetic (*ob/ob*) mice. (F) Immunohistochemistry and quantification (G, H) for endomucin and podocalyxin in renal cortex of non-diabetic (Lean) and diabetic (*ob/ob*) mice at indicated ages. (I) Cell counts of PECAM1+ cells sorted with FACS from kidneys of non-diabetic (Lean) and diabetic (*ob/ob*) mice at indicated timepoints. Data expressed as geometric mean  $\pm$  geometric SD. Scale bar = 50  $\mu m$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ .

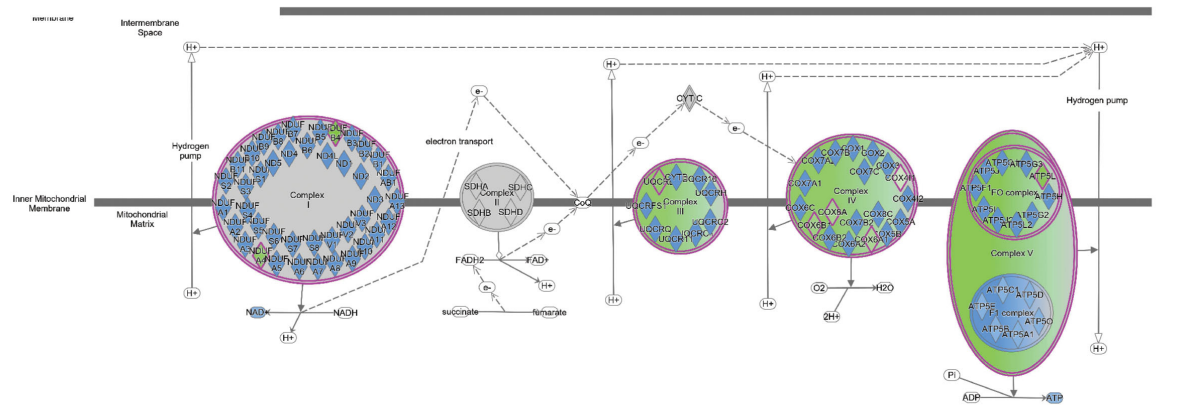
### A Statistical analysis, GEC 20 weeks



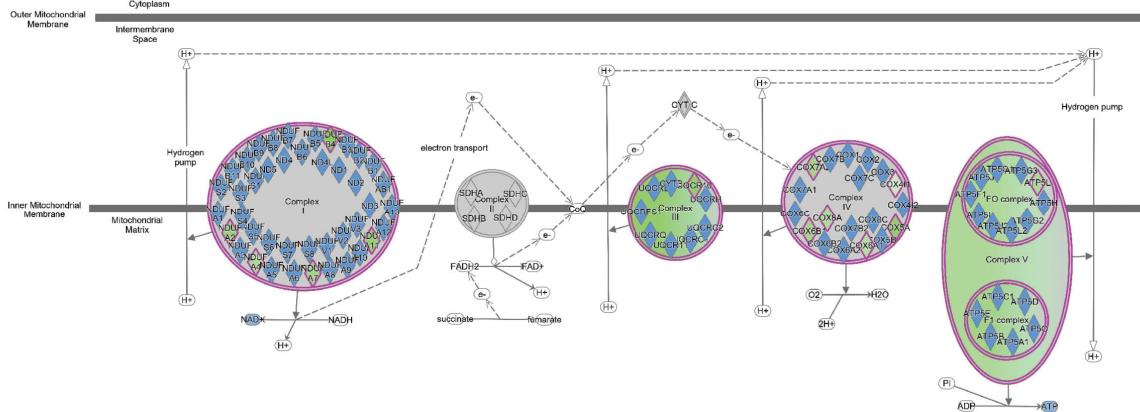
### B Statistical analysis, PCEC 20 weeks



### C Oxidative phosphorylation, GEC 20 weeks



### D Oxidative phosphorylation, PCEC 20 weeks

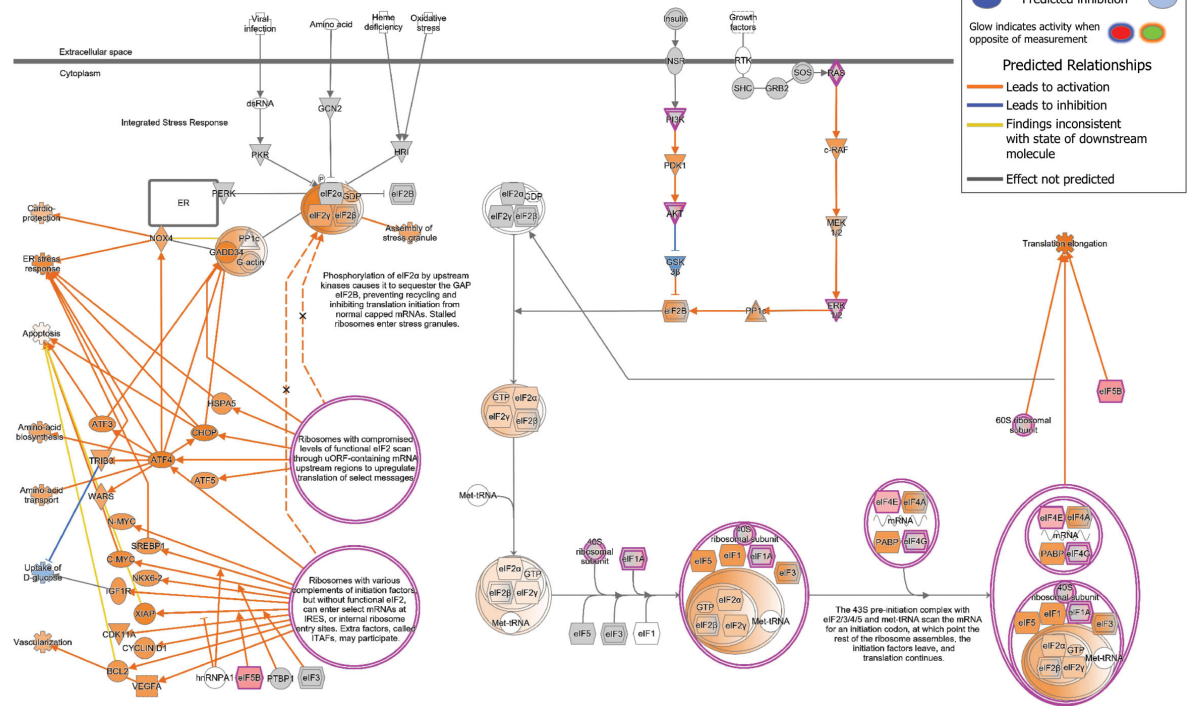


**Figure S7.** Statistics of the top enriched pathways and oxidative phosphorylation in diabetic GEC and PCEC at 20 weeks. Statistical analysis of EIF2 signaling and oxidative phosphorylation in GEC (A) and PCEC (B) at 20 weeks based on DEGs comparing non-diabetic (Lean) and diabetic (*ob/ob*) mice. Schematic diagram of oxidative phosphorylation in GEC (C) and PCEC (D) at 20 weeks.

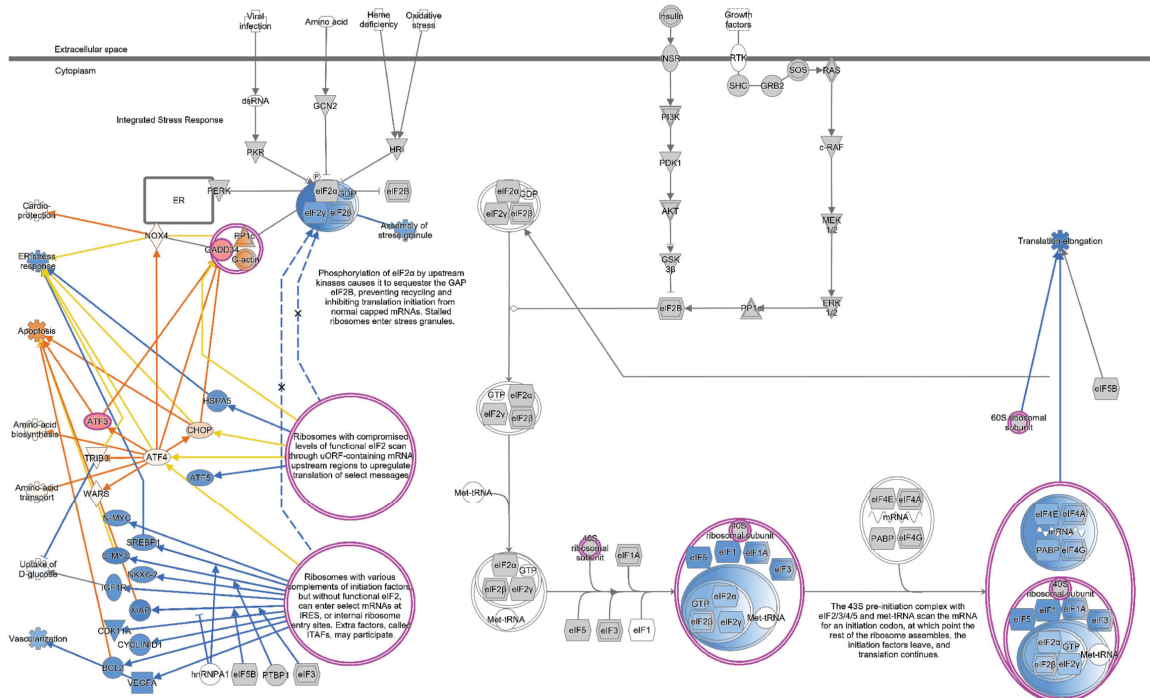




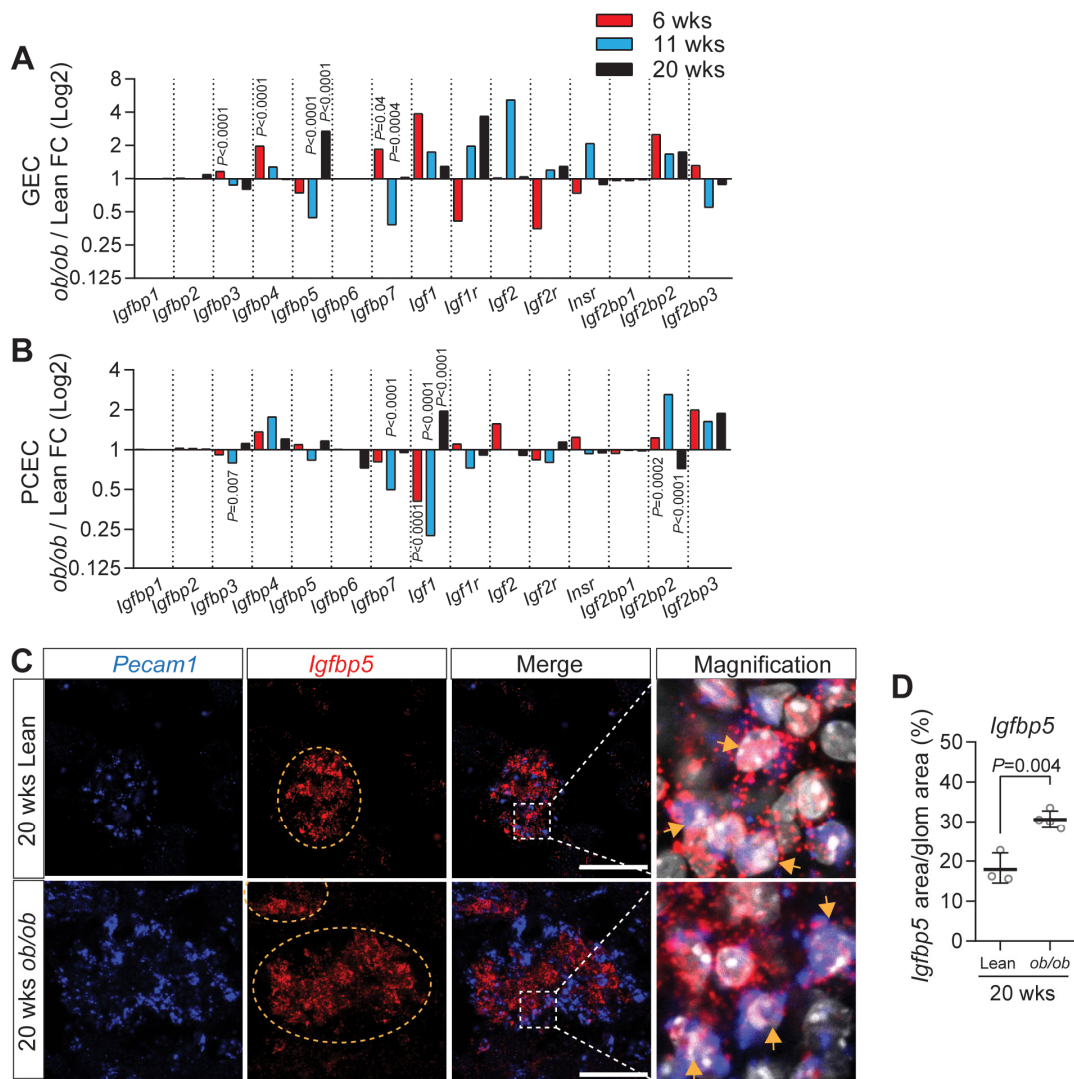
## A EIF2 signaling, GEC 6 weeks



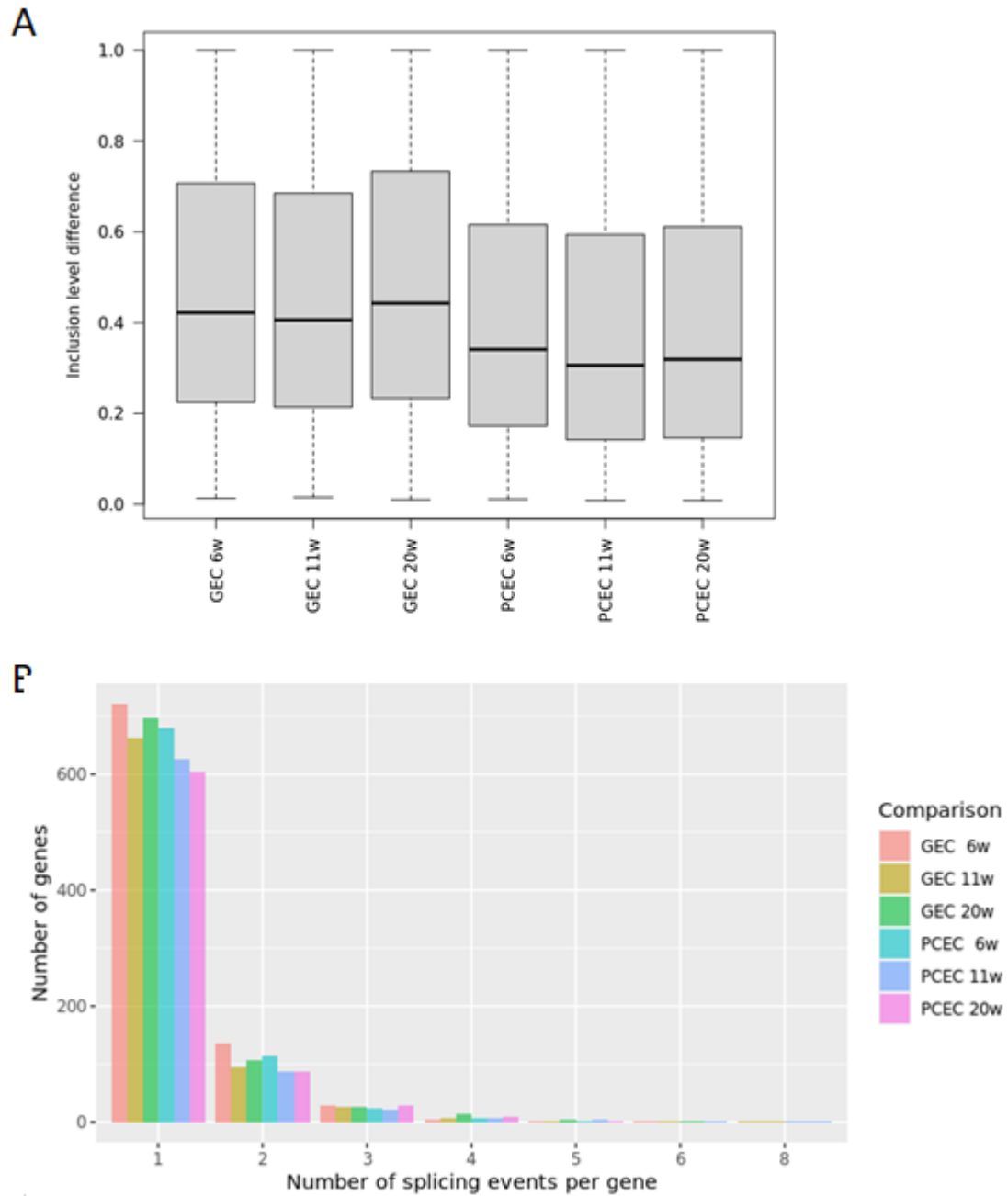
## B EIF2 signaling, GEC 20 weeks



**Figure S9.** EIF2 signaling in diabetic GEC at 6 and 20 weeks. Schematic diagram of EIF2 signaling in GEC at 6 weeks (A) and 20 weeks (B).

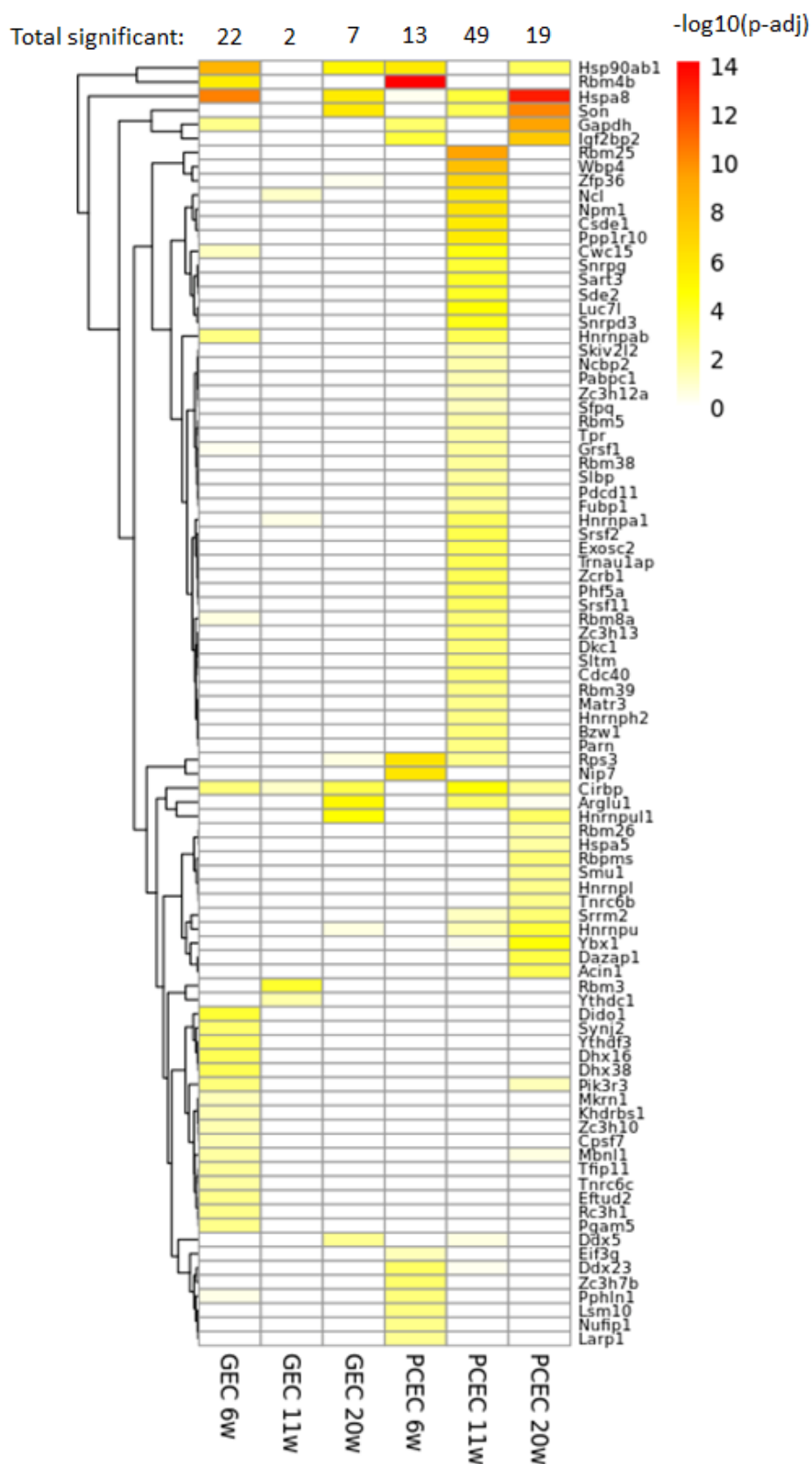


**Figure S10.** The IGF system in DKD. (A, B) Fold change expression of genes in the IGF system comparing non-diabetic (Lean) and diabetic (*ob/ob*) mice in GEC and PCEC, respectively. (C) RNA-ISH for glomerular expression of *Igfbp5* and *Pecam1* in 20-week-old non-diabetic (Lean) and diabetic (*ob/ob*) mice, glomeruli marked with orange circle. Orange arrows indicate *Pecam*<sup>+</sup>/*Igfbp5*<sup>+</sup> cells. Scale bar = 50  $\mu$ m. (D) Quantification of *Igfbp5* expression per glomerular area in non-diabetic (Lean) and diabetic mice (*ob/ob*) at 20 weeks. Data shown as mean  $\pm$  SD.



**Figure S11.** Characterization of DSEs. (A) Absolute inclusion level difference of DSEs (FDR<0.05) between BTBRLean and BTBR*ob/ob* GEC and PCEC at different timepoints. (B) Number of significant DSEs (FDR<0.05 and absolute inclusion level difference > 0.4) per gene.





**Figure S12.** Overlapping DEGs with a compiled list of mouse RBPs from 3 databases: rMAPS (<http://rmaps.cecsresearch.org>), RBPDB (<http://rbpdb.ccbr.utoronto.ca>), and Splicesome (<http://spliceosomedb.ucsc.edu>).

## SUPPLEMENTAL TABLES

**Table S1.** Number of sequenced cells per cluster and timepoint

	6 wks		11 wks		20 wks	
Population	Lean	<i>ob/ob</i>	Lean	<i>ob/ob</i>	Lean	<i>ob/ob</i>
GEC	35	45	197	90	60	56
GEC <sup>IEG-high</sup>	154	101	121	32	48	101
PCEC	210	130	555	526	276	298
PCEC <sup>IEG-high</sup>	242	139	27	7	18	120
AA	57	32	49	17	17	43
EA	68	57	96	28	47	48
DVR	85	41	108	41	22	62
AVR	182	48	143	74	63	71
VEC	9	10	26	29	23	35
LVEC	3	62	5	2	2	0
Tip	7	4	27	6	3	21
Cycling	1	2	4	3	0	32
Unknown	5	4	17	12	9	14

GEC glomerular endothelial cells; IEG immediate early gene; PCEC peritubular capillary cells; AA arterioles/afferent arteriole; EA efferent arteriole; DVR descending vasa recta; AVR ascending vasa recta; VEC venous endothelial cells; LVEC lymphatic vascular endothelial cells; Tip cells

**Table S2.** Differentially expressed genes comparing Lean and *ob/ob* mice

	6 wks			11 wks			20 wks		
Population	down	up	total	down	up	total	down	up	total
GEC	121	618	739	62	39	101	151	34	185
PCEC	205	277	482	786	106	892	351	133	484
AA	0	2	2	0	0	0	0	2	2
EA	12	28	40	2	0	2	34	5	39
DVR	2	9	11	0	5	5	1	2	3
AVR	16	33	49	6	5	11	43	2	45
VEC	0	0	0	0	0	0	3	2	5

*GEC* glomerular endothelial cells; *PCEC* peritubular capillary cells; *AA* arterioles/afferent arteriole; *EA* efferent arteriole; *DVR* descending vasa recta; *AVR* ascending vasa recta; *VEC* venous endothelial cells

**Table 3S.** RNAscope probes and reagents

<b>Probe (mouse gene)</b>	<b>Type</b>	<b>Lot</b>	<b>Reference</b>
<i>Mm-Calca</i>	C1	18012B	420361
<i>Mm-Gata5</i>	C1	18205A	549061
<i>Mm-Slc14a1</i>	C1		448001
<i>Mm-Cldn5</i>	C3	19297B	491611-C3
<i>Mm-Aqp1</i>	C2	19179I	504741-C2
<i>Mm-Igf1</i>	C2		443901-C2
<i>Mm-Sele</i>	C1	19042A	438621
<i>Mm-Car8</i>	C2	19179I	514171-C2
<i>Mm-Gper1</i>	C3	19179I	475251-C3
<i>Mm-Ace</i>	C1	18218A	442731
<i>Mm-Il33</i>	C1		400591
<i>Mm-Fos</i>	C2	19176C	316921-C2
<i>Mm-Junb</i>	C1	18278B	556651
<i>Mm-Atf3</i>	C3	19179I	426891-C3
<i>Mm-Pecam1</i>	C3	18355C	316721-C3
<i>Mm-Plvap</i>	C1	19323A	440221
<i>Mm-Plvap</i>	C3	20072C	440221-C3
<i>Mm-Acta2</i>	C2	19316C	319531-C2
<i>Mm-Acta2</i>	C1	20281B	319531
<i>Mm-Igfbp5</i>	C1		425731
<b>Reagents</b>	<b>Type</b>	<b>Lot</b>	<b>Reference</b>
Protease III		2007018, 2002759	322340
3-plex Positive Control Probe			320881
3-plex Negative Control Probe			320871
Multiplex Fluorescent Reagent Kit v2		2007959, 2010572	323100
Multiplex Fluorescent Reagent		2007507	320851
Wash Buffer			310091
Opal520 Reagent		200106003	FP1487001KT
Opal570 Reagent		200106008	FP1488001KT
Opal690 Reagent		200106010	FP1497001KT



**Table S4. Antibodies**

<b>Name (protein/antigen)</b>	<b>Company</b>	<b>Reference</b>	<b>Host</b>	<b>Lot</b>	<b>Dilution</b>
Endomucin	Abcam	AB106100	rat		1:200
Plvap	BD Bioscience	550563	rat	9324203	1:200
Podocalyxin	R&D Systems	AF1556	goat		1:200
Pecam1-FITC	BD Bioscience	AF553370	rat	56813	1:200
Pecam1	Abcam	Ab28364	rabbit	GR3247742-11	1:200
Desmin	Abcam	Ab15200	rabbit		1:200
Collagen IV	BioRad	134001	goat		1:200
anti goat IgG-Alexa633	Thermo Fisher Scientific	A21082	donkey	1889311	1:200
anti rabbit IgG-Alexa680	Thermo Fisher Scientific	A10043	donkey	1917929	1:200
Anti rat IgG-Alexa633	Thermo Fisher Scientific	A21094		2002975	1:200
Hoechst33342	Thermo Fisher Scientific	H3570		1664690	1:1000