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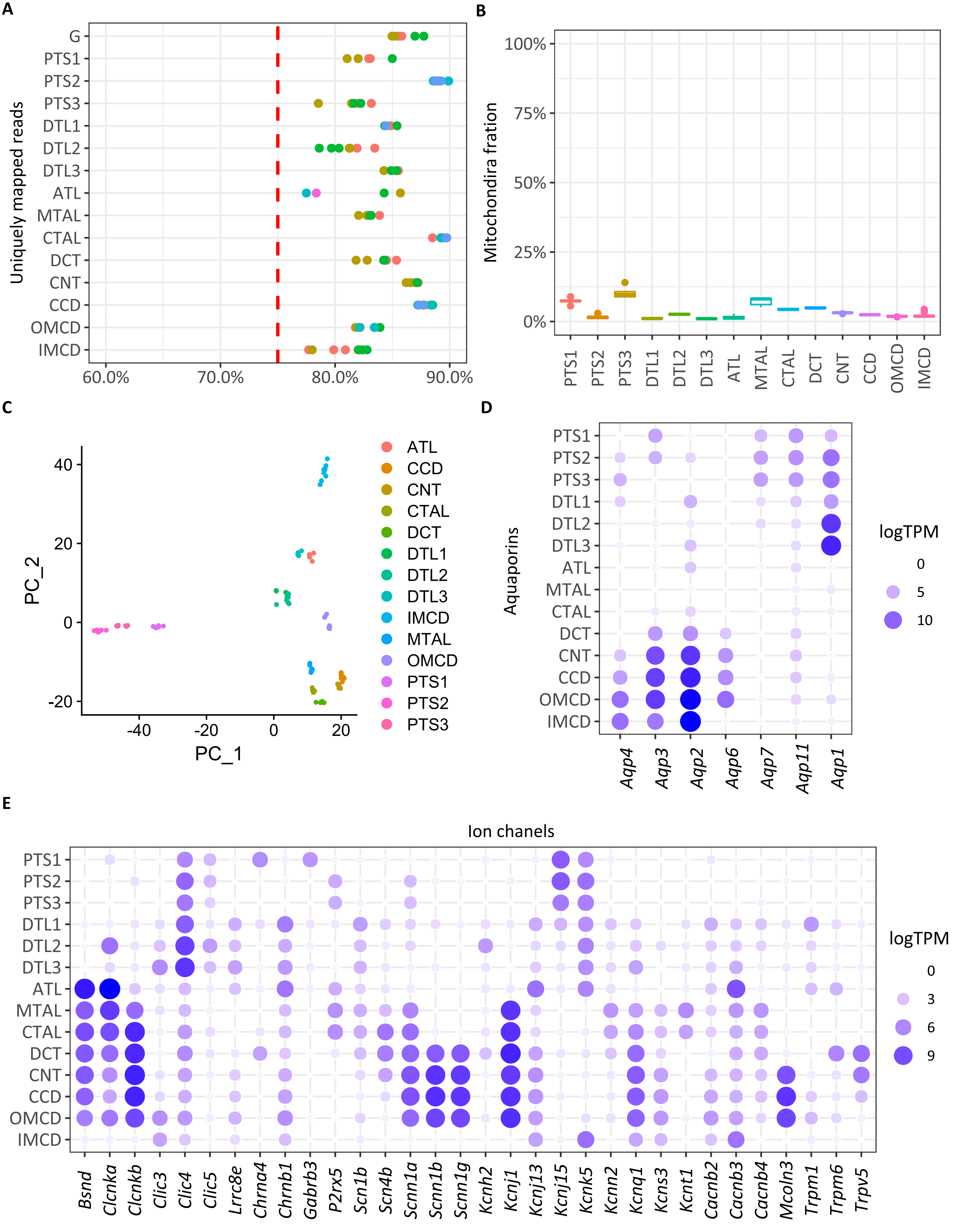
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PTS2
PTS3
DTL1
DTL2
DTL3
ATL
MTAL
CTAL
DCT
CNT
CCD
OMCD
IMCD
60.0%
70.0%
80.0%
90.0%
B
Mitochondira fration
100%
75%
50%
25%
0%
PTS1
PTS2
PTS3
DTL1
DTL2
DTL3
ATL
MTAL
CTAL
DCT
CNT
CCD
OMCD
IMCD
C
PC_2
PC_1
40
20
0
-20
-40
-20
0
20
ATL
CCD
CNT
CTAL
DCT
DTL1
DTL2
DTL3
IMCD
MTAL
OMCD
PTS1
PTS2
PTS3
D
Aquaporins
PTS1
PTS2
PTS3
DTL1
DTL2
DTL3
ATL
MTAL
CTAL
DCT
CNT
CCD
OMCD
IMCD
Aqp4
Aqp3
Aqp2
Aqp6
Aqp7
Aqp11
Aqp1
logTPM
0
5
10
E
Ion chanels
PTS1
PTS2
PTS3
DTL1
DTL2
DTL3
ATL
MTAL
CTAL
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CNT
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Scnn1g
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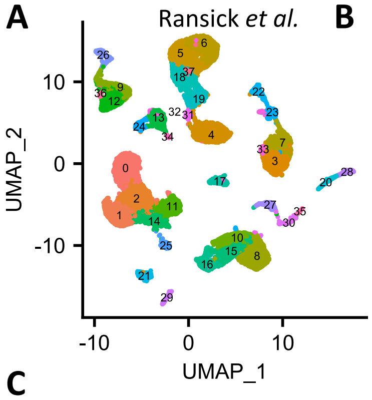
Kcnt1
Cacnb2
Cacnb3
Cacnb4
Mcoln3
Trpm1
Trpm6
Trpv5
logTPM
0
3
6
9

Supplemental Figure Legends

Supplemental Figure 1. RNA-seq quality control and gene expression patterns along the renal tubules. (A) Percentage of uniquely mapped reads for all samples. The red dashed line is set at 75%. (B) Mitochondria gene fraction in all samples. (C) Principal component analysis of all the renal tubule samples. Colors indicate different renal tubule segments. (D) Representative gene expression pattern for aquaporins. Dot size and expression are proportion to $\log_2$(TPM+1). (D) Dot plot showing the distribution of abundant ion channels across renal tubule segments.

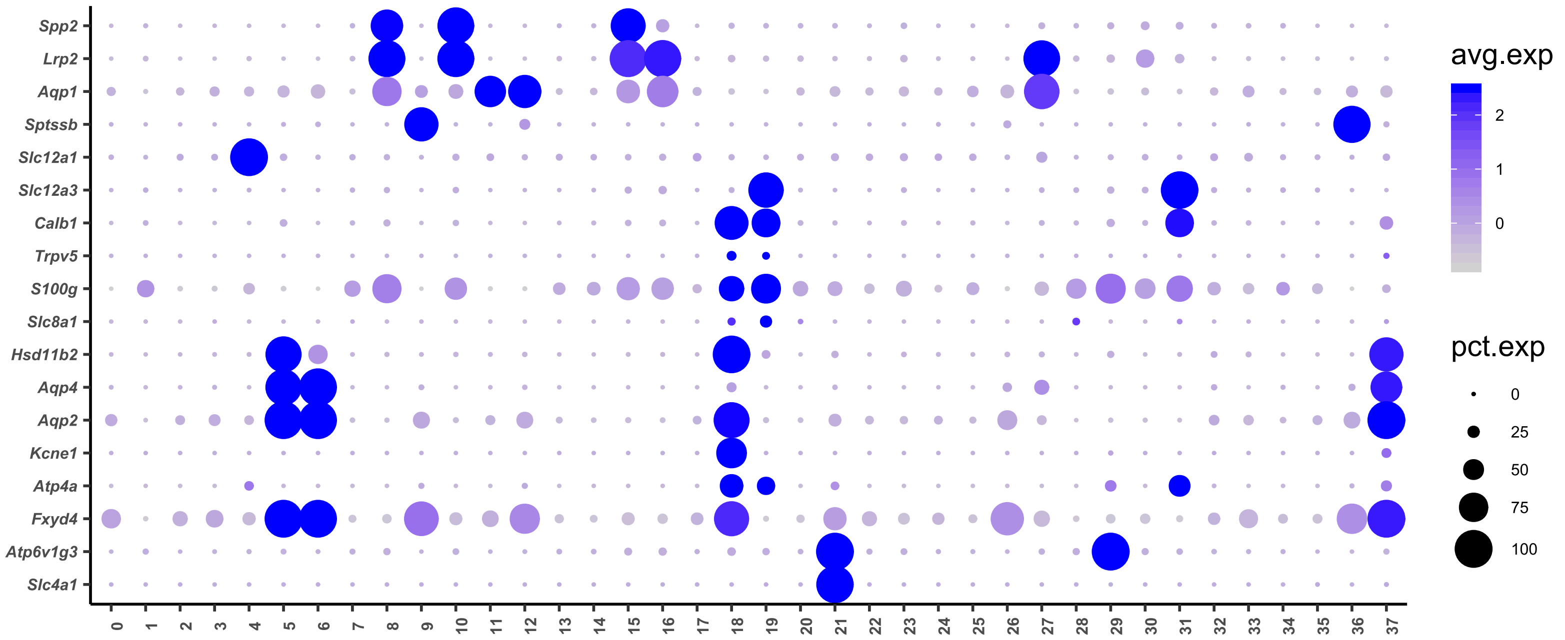
A



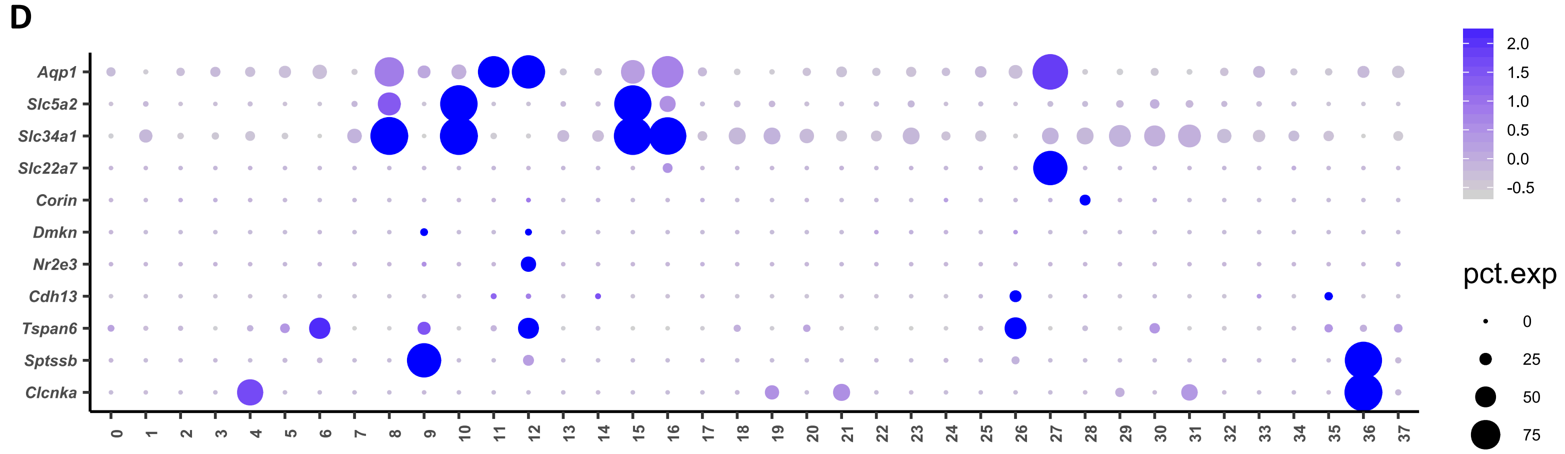
B

Cluster	Markers	Annotation	Cluster	Markers	Annotation
10	*Slc5a2,Slc34a1*	Female PTS1	4	*Slc12a1*	TAL
8	*Slc34a1*	Female PTS2	31	*Slc12a3*	DCT1
27	*Lrp2,Slc22a7*	Female PTS3	19	*Slc12a3,Trpv5*	DCT2
15	*Slc5a2,Slc34a1*	Male PTS1	18	*Aqp2,Calb1,S100g*	CNT
16	*Lrp2,Slc34a1*	Male PTS2	37	*Aqp2,Aqp4,Atp4a*	CCD
28	*Corin*	DTL1	21	*Atp6v1g3,Slc4a1*	A-IC
26	*Cdh13,Tspan6*	DTL2	29	*Atp6v1g3*	B-IC
12	*Tspan6,Aqp1,Nr2e3*	DTL3	5	*Aqp2,Aqp4,Hsd11b2*	OMCD
36	*Sptssb, Clcnka*	ATL	6	*Aqp2,Aqp4*	IMCD

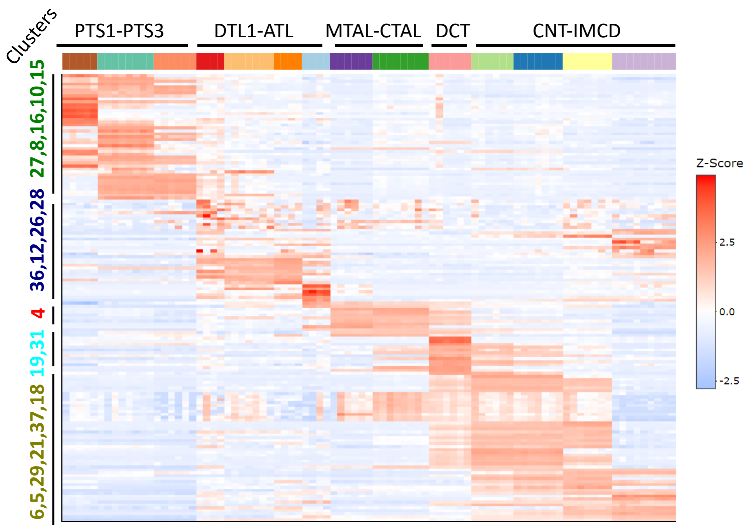
C



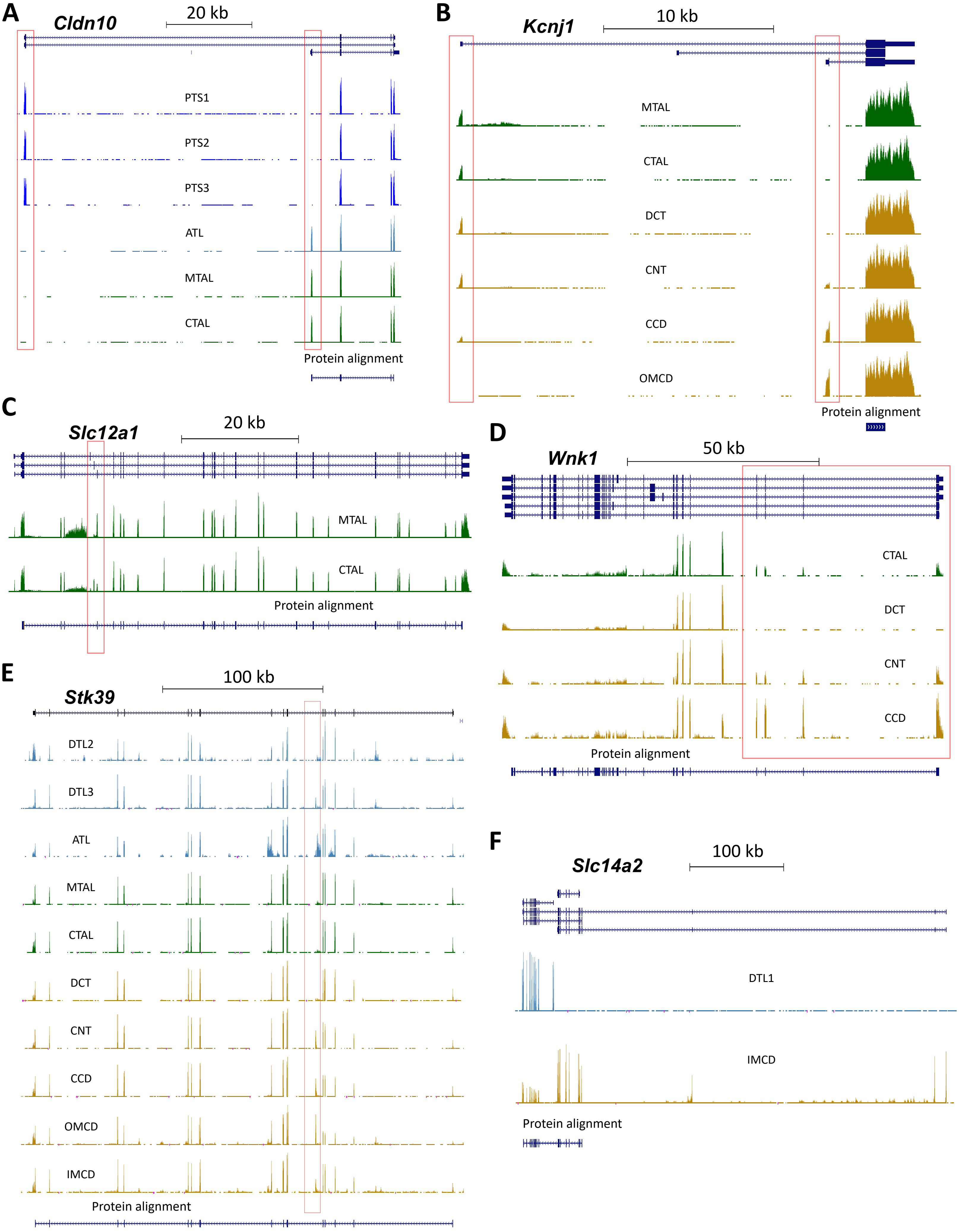
D



E



Supplemental Figure 2. Comparison of small-sample RNA-seq and published single-cell RNA-seq data. (A) UMAP projection of published mouse kidney single-cell RNA-seq data (GSE129798). (B) Annotation of epithelial cell clusters based on cell type-specific markers proposed in GSE129798. (C) and (D) Dot plots showing the distribution of cell type-specific marker in each cell cluster. (E) Distribution of top 10 epithelial cell cluster markers along the mouse renal tubule data. The data related to this figure are provided in Supplemental Table 2.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MTAL
CTAL
Protein alignment
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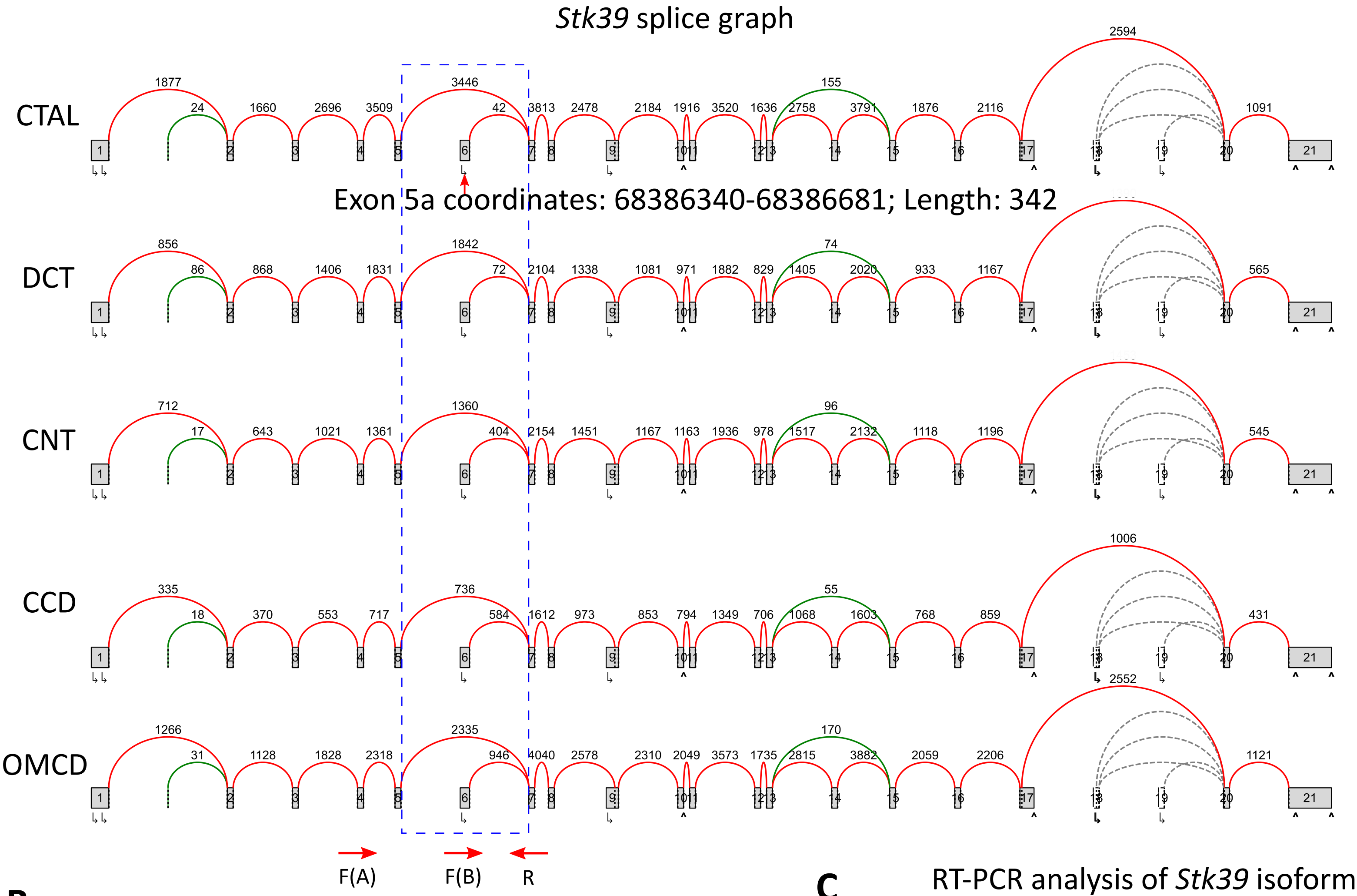
OMCD
Protein alignment
C
Slc12a1
20 kb
MTAL
CTAL
Protein alignment
D
Wnk1
50 kb
CTAL
DCT
CNT
CCD
Protein alignment
E
Stk39
100 kb
DTL2
DTL3
ATL
MTAL
CTAL
DCT
CNT
CCD
OMCD
IMCD
Protein alignment
F
Slc14a2
100 kb
DTL1
IMCD
Protein alignment

Supplemental Figure 3. Known alternative splicing examples in kidney. Isoform distribution of (A) *Cldn10*, (B) *Kcnj1* (ROMK), (C) *Slc12a1* (NKCC2), (D) *Wnk1*, (E) *Stk39* (SPAK), and (F) *Slc14a2* (Urea transporter A) along renal tubule segments. Red rectangles indicate the alternative exons.

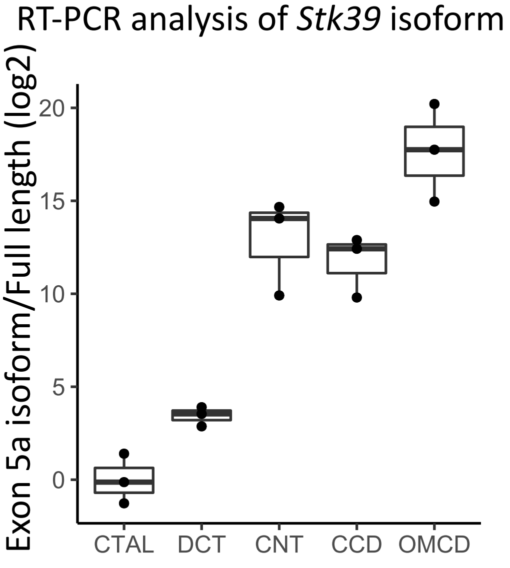
A



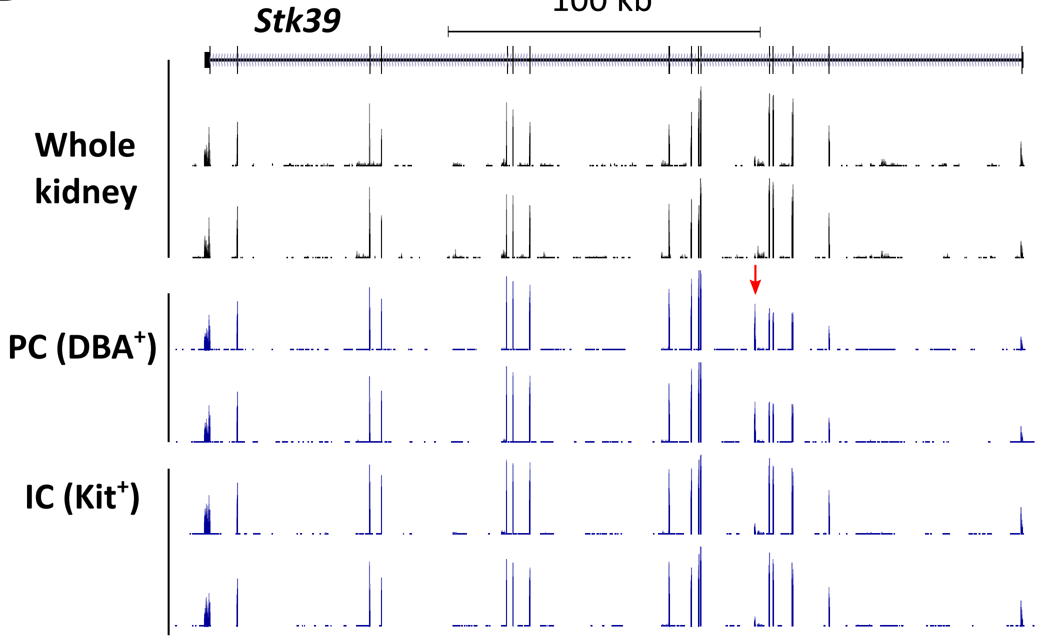
B

	Sequence (5'->3')	Length	Tm
Forward primer A	GCACAGAAACGGTCAGATCC	20	58.9
Reverse primer	CATCACCCCCTGTGGCTAAG	20	60.1
Product length	119	Detect **full length**	
%			
Forward primer B	CCAACTCAAGCTGTTGCTATGG	22	59.8
Reverse primer	CATCACCCCCTGTGGCTAAG	20	60.1
Product length	196	Detect **Exon 5a** isoform	

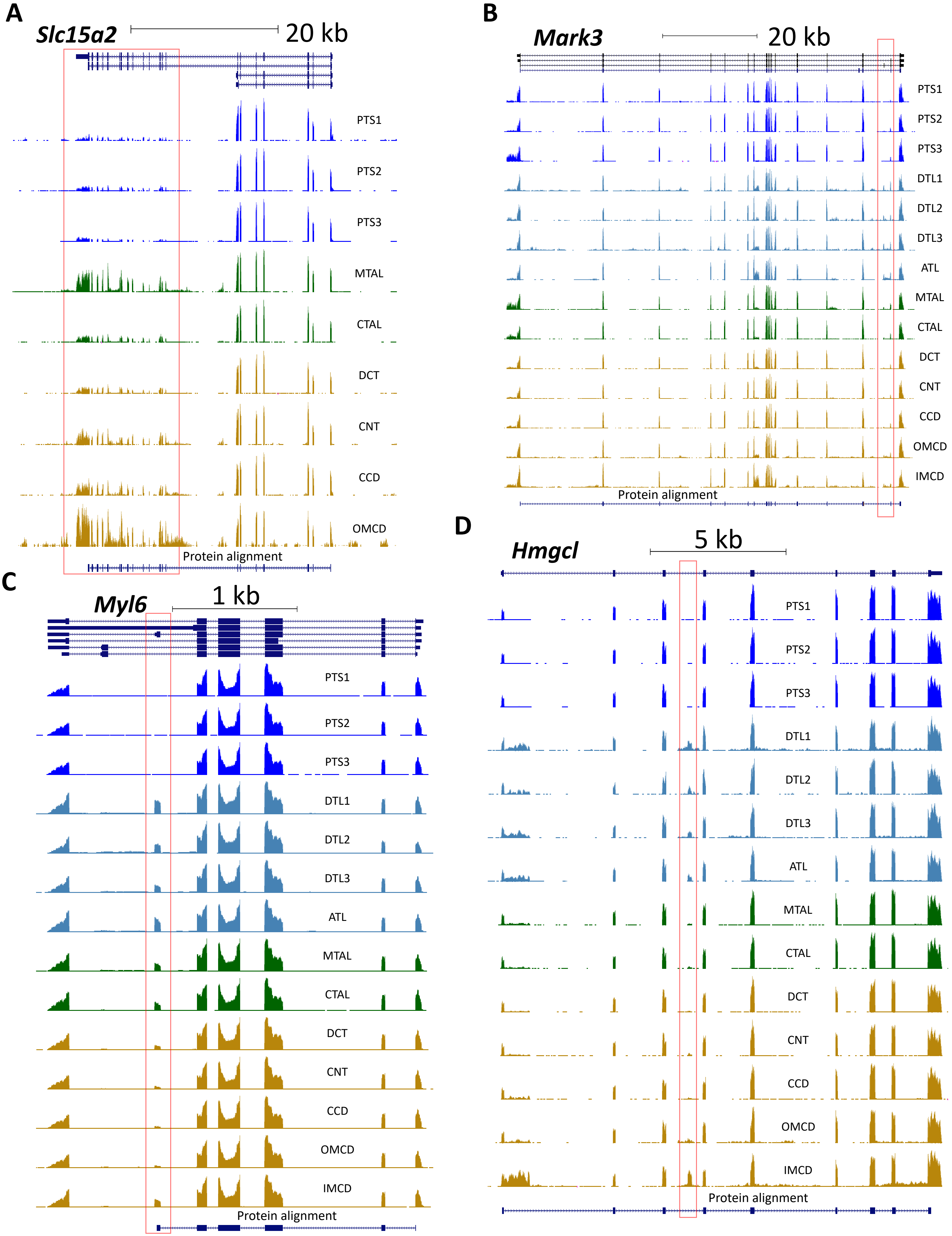
C



D



Supplemental Figure 4. Distribution of *Stk39* isoforms along renal tubule segments. (A) Splice graphs of *Stk39* isoforms. Exon 6, highlight in blue rectangle, is generally referred to as exon 5a. A short isoform, termed KS-SPAK, starts with exon 5a, showing an increased junctional reads ratio of exon 5a/exon 6 to exon 4/exon 6 from CTAL to OMCD. The junctional reads on the splice graph are a combination of replicate reads of each segment. The red arrows at the bottom indicate the primer targeting sites in (B). (B) Primers used to quantify exon 5a *Stk39* isoform and full length *Stk39*. (C) RT-PCR showing exon 5a isoform increased from CTAL to OMCD. The data were log2 transformed. (D) Genome browser tracks showing the distribution of *Stk39* isoform in whole kidney RNA-seq (GSE111837) and FACS-enriched principal cells (DBA$^+$) and IC cells (Kit$^+$) (GSE99701). Red arrow points to exon 5a.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CNT
CCD
OMCD
Protein alignment
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Mark3
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PTS2
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DTL3
ATL
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CTAL
DCT
CNT
CCD
OMCD
IMCD
Protein alignment
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PTS3
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DTL2
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OMCD
IMCD
Protein alignment
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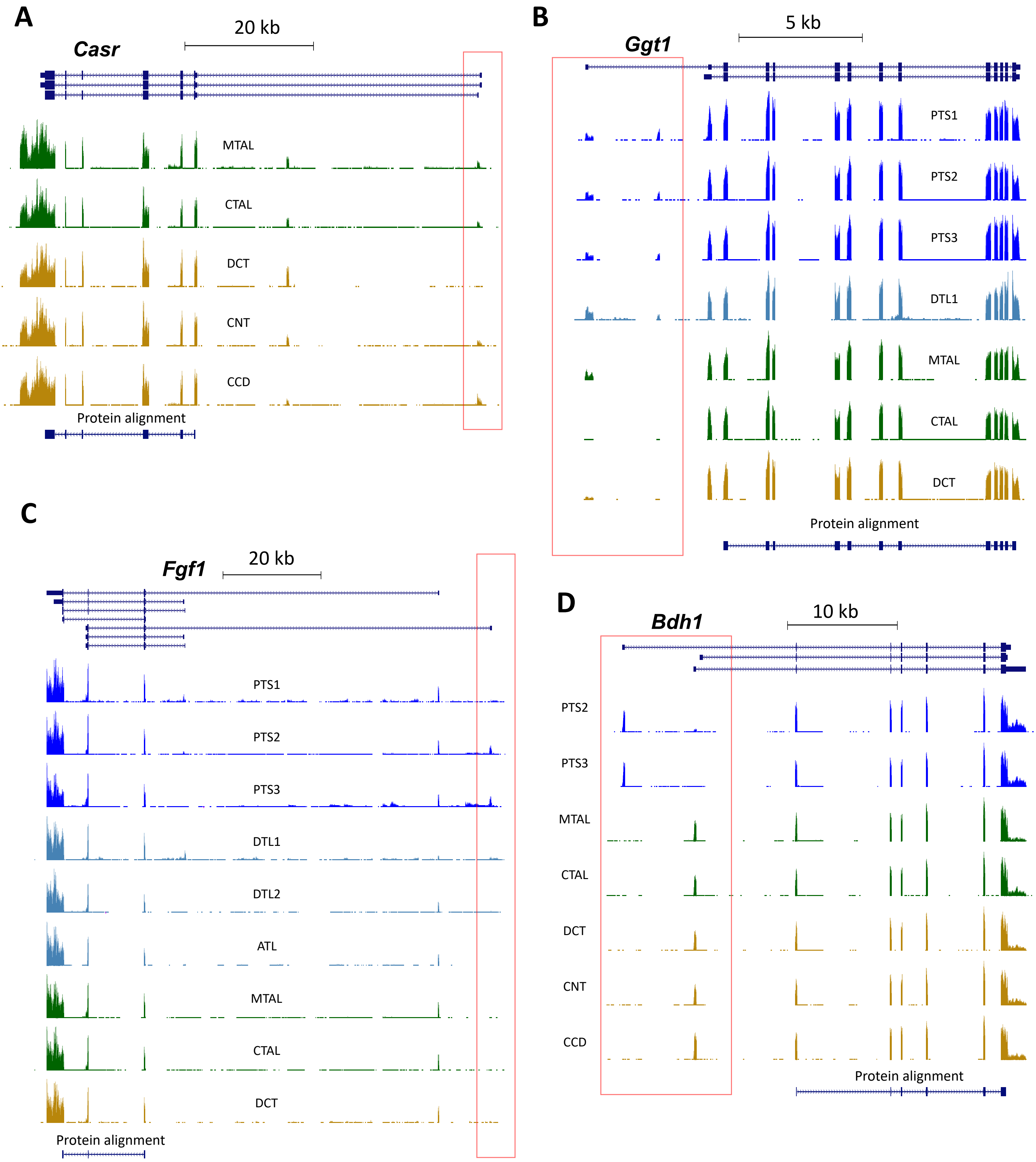
PTS2
PTS3
DTL1
DTL2
DTL3
ATL
MTAL
CTAL
DCT
CNT
CCD
OMCD
IMCD
Protein alignment

Supplemental Figure 5. Additional alternative splicing examples that have effects on protein structures. Isoform distribution of *Slc15a2* (A), *Mark3* (B), *Myl6* (C), and *Hmgcl* (D) along the renal tubule. Red rectangles indicate the alternative exons.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CTAL
DCT
CNT
CCD
Protein alignment
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PTS2
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MTAL
CTAL
DCT
Protein alignment
C
Fgf1
20 kb
PTS1
PTS2
PTS3
DTL1
DTL2
ATL
MTAL
CTAL
DCT
Protein alignment
D
Bdh1
10 kb
PTS2
PTS3
MTAL
CTAL
DCT
CNT
CCD
Protein alignment

Supplemental Figure 6. Additional alternative splicing examples that have effects on mRNA properties. Isoform distribution of *Casr* (A), *Ggt1* (B), *Fgf1* (C), and *Bdh1* (D) along the renal tubule. Red rectangles indicate the alternative exons.

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