

Supplemental Material

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Segment	Region	Full name	Short description
S1	Cortex	S1 Proximal tubule	Proximal tubule directly attached to the glomerulus.
S2	Cortex	S2 Proximal tubule	Straight part of proximal tubule obtained from medullary ray.
S3	Cortex	S3 Proximal tubule	Final portion of proximal tubule from outer medulla before transitioning into thin limb.
DTL1	Outer medulla	Short descending limb	Thin descending limb from outer medulla characterized by attachment to S3, transitioning into ascending limb within outer medulla, and smaller diameter than DTL2.
DTL2	Outer medulla	Long descending limb, outer medulla	Thin descending limb from outer medulla, wider than DTL1 and continues into inner medulla.
DTL3	Inner medulla	Long descending limb, inner medulla	Thin descending limb from inner medulla.
ATL	Inner medulla	Thin ascending limb	Thin ascending limb from inner medulla characterized by transitioning into thick ascending limb.
mTAL	Outer medulla	Medullary thick ascending limb	Thick ascending limb from outer medulla, bigger than thin limb and more granular appearance.
cTAL	Cortex	Cortical thick ascending limb	Thick ascending limb from medullary ray with smaller diameter than S2 and CCD.
DCT	Cortex	Distal convoluted tubule	Convolutated tubule segment in the cortical labyrinth, having a smaller diameter than proximal tubule. The appearance of DCT is different from adjacent CNT segment which has cobblestone appearance. Only DCTs within around 0.5 mm from macula densa were collected.
CNT	Cortex	Connecting tubule	Branching tubules in the cortical labyrinth with cobblestone appearance.
CCD	Cortex	Cortical collecting duct	Tubule segments dissected from medullary rays of the cortex with cobblestone appearance.
OMCD	Outer medulla	Outer medullary collecting duct	Tubule segments dissected from outer medulla with cobblestone appearance.
IMCD	Inner medulla	Inner medullary collecting duct	The largest tubule segment in the inner medulla. Multiple segments are merging together as they descend deeper in the medulla.

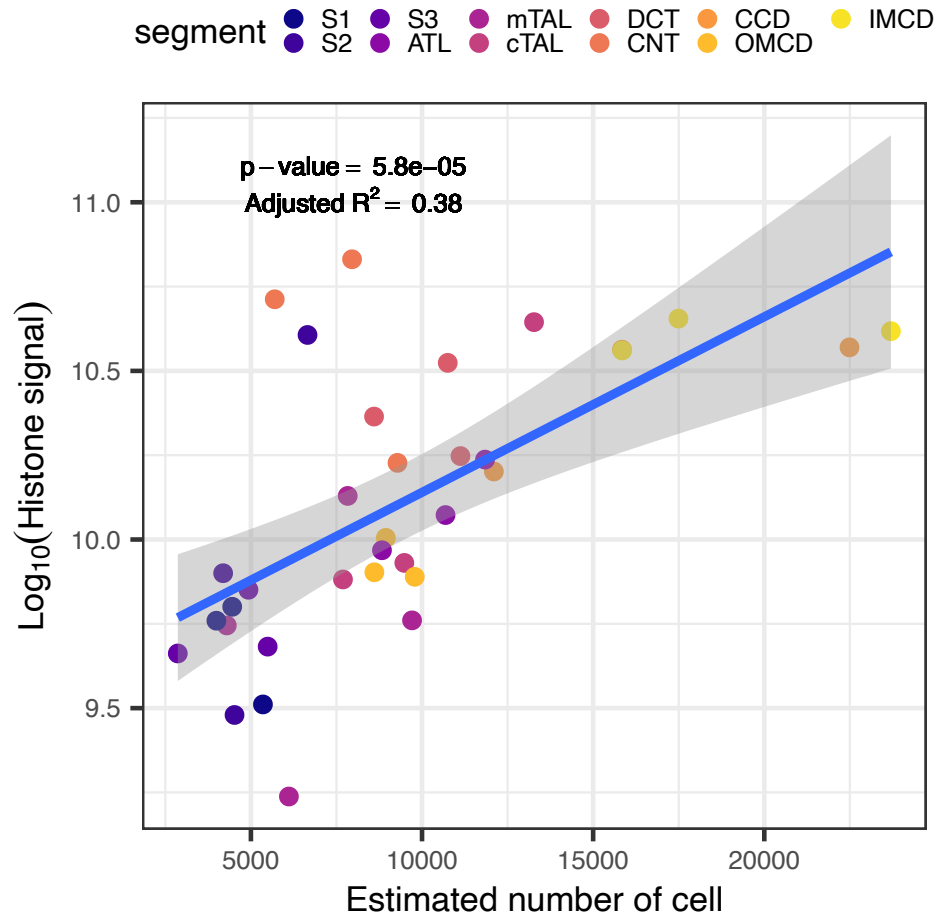
Supplemental Table 1: A short description of each renal tubule segment.

<i>Segment</i>	<i>Sum copy per cell</i>	<i>Detection threshold</i>	<i>Number of quantified proteins</i>	<i>Number of undetected proteins</i>	<i>Estimated undetected copy per cell</i>	<i>Percent detected</i>
<i>S1</i>	2.13E+09	3150	2952	5048	1.59E+07	99.26
<i>S2</i>	4.47E+09	444	5410	2590	1.15E+06	99.97
<i>S3</i>	4.49E+09	1378	3881	4119	5.67E+06	99.87
<i>DTL1</i>	7.20E+08	2560	4369	3631	9.29E+06	98.73
<i>DTL2</i>	9.01E+08	41	4089	3911	1.60E+05	99.98
<i>DTL3</i>	8.09E+08	1969	4596	3404	6.70E+06	99.18
<i>ATL</i>	8.80E+08	1870	3513	4487	8.39E+06	99.06
<i>MTAL</i>	1.26E+09	148	4229	3771	5.59E+05	99.96
<i>CTAL</i>	1.85E+09	211	5865	2135	4.50E+05	99.98
<i>DCT</i>	1.75E+09	259	5688	2312	5.98E+05	99.97
<i>CNT</i>	1.59E+09	627	5647	2353	1.48E+06	99.91
<i>CCD</i>	1.91E+09	12	6550	1450	1.67E+04	100
<i>OMCD</i>	1.14E+09	643	4395	3605	2.32E+06	99.8
<i>IMCD</i>	1.35E+09	2086	5621	2379	4.96E+06	99.63

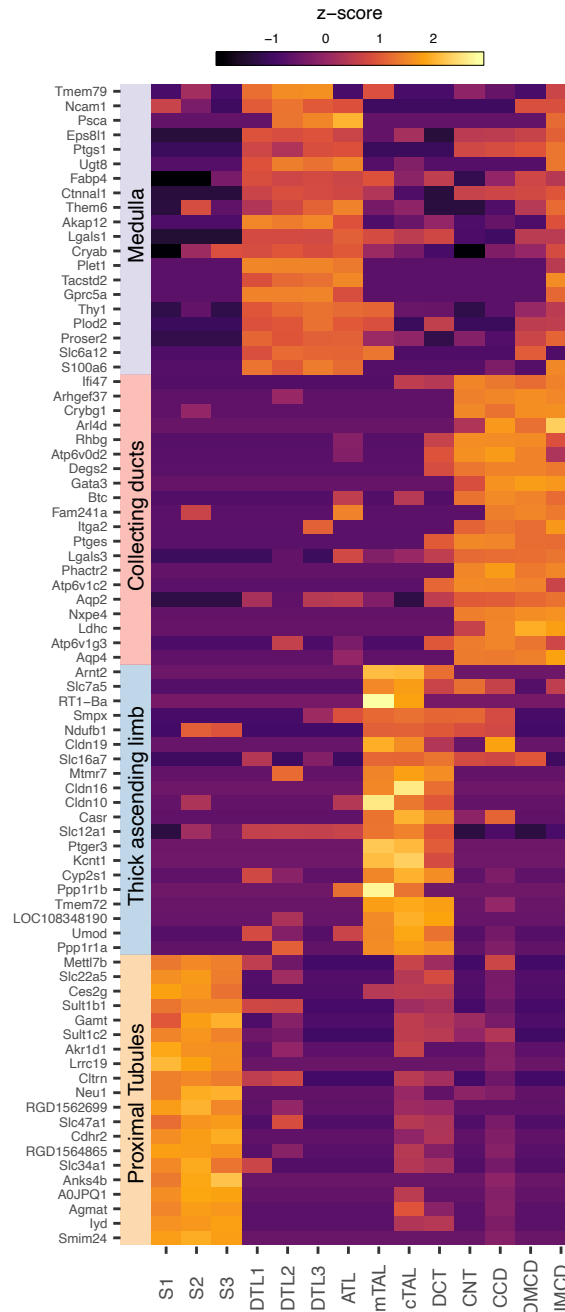
Supplemental Table 2. Estimation of percent of total protein detected. For each tubule segment, the sum of average protein copy per cell was calculated. Because undetected proteins are likely to have low copy number, we used 20th percentile of copy number in each segment as estimate copy number for the missing proteins. Assuming that 8000 different proteins are expressed in each segment, the numbers of undetected proteins are calculated by subtracting number of quantified proteins from 8000. The total estimated copy per cell of all undetected proteins are then simply multiplication of number of undetected proteins and the corresponding detection threshold.

RANK	S1	S2	S3	DTL1	DTL2	DTL3	ATL	MTAL	CTAL	DCT	CNT	CCD	OMCD	IMCD
1	Gprc5c (5.04)	Gprc5c (5.69)	Gprc5c (5.62)	Gprc5a (5.14)	Gprc5a (5.1)	Gprc5c (5.22)	Gprc5c (4.5)	Ptger3 (4.36)	Casr (5.4)	Gprc5c (4.61)	Gprc5c (4.76)	Gprc5c (4.68)	Gprc5c (4.49)	Gprc5c (4.96)
2	Adgrg1 (4.32)	Adgrg1 (4.15)	Adgrg1 (1.18)	Gprc5c (4.88)	Gprc5c (4.95)	Gprc5a (5.15)	Adgrg1 (3.87)	Gprc5c (4.15)	Gprc5c (4.45)	Casr (4.38)	Adgrf5 (4.18)	Adgrf5 (4.51)	Adgrf5 (4.34)	Avpr2 (4.46)
3	Lpar3 (3.9)	Pth1r (3.84)		Adgrg1 (3.54)	Adgrg1 (3.67)	Ackr3 (4.18)	Gprc5a (3.68)	Casr (3.91)	Ptger3 (4.12)	Gpr39 (3.63)	Ackr3 (3.8)	Ackr3 (4.17)	Ackr3 (3.97)	Gprc5a (4.42)
4		Adgrl2 (2.04)		Adgrf5 (2.88)	Ackr3 (1.4)	Adgrf5 (3.77)	Adgrf5 (3.47)	Adgrg1 (3.66)	Gcgr (3.98)	Celsr2 (3.22)	Adgrg1 (3.56)	Gpr39 (4)	Gpr39 (3.67)	Gprc5b (4.21)
5		Adgrf5 (2)		Celsr2 (2.82)	Adgrl2 (0.97)	Adgrg1 (3.74)		Adgrf5 (3.17)	Calcr (2.84)	Adgrl2 (2.69)	Celsr2 (3.55)	Adgrg1 (3.85)	Celsr2 (3.35)	Ackr3 (4.03)
6						Adgrl2 (2.68)		Adgrl2 (2.26)	Adgrl2 (2.79)	Ackr3 (2.51)	Gpr183 (2.62)	Casr (3.57)	Gprc5b (2.45)	Fzd6 (3.89)
7						Celsr2 (2.45)			Adgrg1 (2.69)	Ptger3 (2.43)	Gpr39 (2.59)	Celsr2 (3.35)	Avpr2 (1.4)	Adgrg1 (3.79)
8						Calcr1 (2.41)			Pth1r (2.43)	Adgrg1 (2.35)	Calcr (2.54)	Celsr1 (3.06)	Celsr1 (0.84)	Gpr39 (3.3)
9						Gpr39 (2.26)			Oxtr (2.33)	Adgrf5 (2.07)	Fzd1 (2.06)	Fzd6 (2.75)		Celsr1 (3.17)
10									Celsr2 (2.24)	Celsr1 (1.95)	Celsr1 (2.01)	Avpr2 (1.13)		Fzd1 (2.82)
11									Adgrf5 (2.11)	Calcr1 (1.12)	Grm6 (2.01)	Lpar1 (0.98)		Grm6 (2.42)
12									Celsr1 (1.87)		Gprc5b (1.27)	Fzd4 (0.96)		Celsr2 (2.42)
13									Calcr1 (1.17)		Casr (1.1)	Fzd1 (0.83)		Lpar5 (1.13)
14												Calcr (0.82)		
15												Grm6 (0.76)		

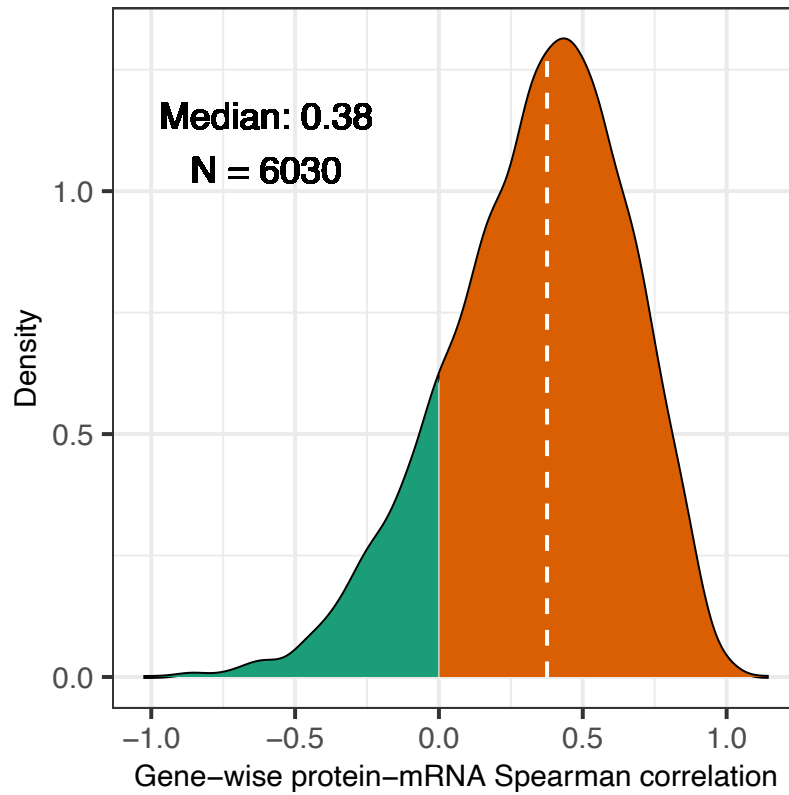
Supplemental Table 3. 7-membrane spanning receptors expressed along the renal tubule. Numbers in parentheses represent average log₁₀ copy number per cell in each tubule segment.



Supplemental figure 1. Scatter plot between estimated number of cell and mass spectrometry signal intensity of histone proteins. The number of cells in each sample was calculated by multiplying total tubule length per sample with estimated number of cells per length in the literature.¹



Supplemental figure 2. Heatmap of the most differentially expressed proteins in selected regions of the kidney. Closely related tubule segments were grouped together, namely proximal tubule (S1, S2, S3), thick ascending limbs (mTAL, cTAL), collecting ducts (CCD, OMCD, IMCD), and renal medulla (DTL1, DTL2, DTL3, ATL, mTAL, OMCD, IMCD). The average values of log₂-transformed protein copy numbers in each group were compared with the average of all other segments. Top twenty proteins of each group with at least 4 times more abundance (log₂ ratio > 2) and adjusted p-value < 0.01 are shown in the heatmap. Copy numbers were standardized with z-score to help visualized multiple proteins in the heatmap.



Supplemental figure 3. Distribution of gene-wise Spearman correlations between protein (log₂ copy number) and transcript (log₂ TPM) abundance along renal tubule. Around 85% of genes have a positive correlation with a median of 0.38. The distribution of Spearman correlations is almost identical to the distribution of Pearson correlation (See Figure 5).

Reference:

1. Clark JZ, Chen L, Chou CL, Jung HJ, Lee JW, Knepper MA: Representation and relative abundance of cell-type selective markers in whole-kidney RNA-Seq data. *Kidney Int*, 95: 787-796, 2019