

Supplementary Table 1. Kidney-enriched gene expression in human primary proximal tubule epithelial cells and their qPCR primer sequences

Gene name	Gene expression (FPKM) in mouse kidney	Gene expression (Ct) in human primary proximal tubular cells	Human Forward Primer	Human Reverse Primer
FXYD2	2774.65995	18.31177616	CTATGAGACCGTTCGCAATGGG	CTGCTGTTACGGCTCATCTTCAT
PTH1R	488.4913196	21.62650681	TGAAGGACGCTGTGCTCTACTC	AGTAGAGGAAGAAGGTCACGGC
MIOX	2248.91558	21.75587559	CACCAGACAGTGGACTTCGTCA	GGTCCGACTCATCCACCAGCC
CLDN16	11.27556516	21.84978771	TGCTCCTTGGTCTTGACTGCGT	CAGCATAACCACACAGAGCCAATG
CLTRN	1083.212671	23.43063259	ACCTCTTCAAAGCGATGGTAGCT	CCACAAACCAGAATGATACCCTC
KCNJ1	119.8628784	23.43682480	GGCTACCGTTTTGCTCCCATAG	CATAGCCTCTCTTCATCCTGGC
KIF12	125.0021099	24.39956474	GGCTAACAGCATCAACCGAAGC	CAGTGAGTCTGCCAGCAACTTG
SLC12A1	380.0086368	24.84384251	AGGCTCTTTCCTACGTGAGTGC	GCCACTGTTCTTGGTAAAGGCG
WDR72	19.17317882	25.14006901	CTGTGAGGATGATAAAATGGCACC	CGTGCTCTTCTCCTGTCTCATG
GCNT1	164.2010069	25.70534134	CTGGAAACGGAGAGGATGCCAT	CACGAAGTAGGCACTGCCAGAA
SLC13A3	486.9399631	26.39008904	CCATTGAGGAGTGGAACCTGCA	GTGTTGCTCAGCCACATGGACA
SLC12A3	876.7241794	27.31577682	TGGACGACCATTTCCTACCTGG	CACTCGGTGAAGTTCCAGCCAT
TMEM174	97.55403185	27.73207092	CTGCATTTGTGGTTGATGAGGGC	CTCCTCTTCCAGCTGTGTCTCT
SLC17A1	287.3676954	27.74902534	GCACAGATCCACATGGTTTGCC	CACCATAGGAGGTGGAACCTCAAG
ATP6V0A4	265.090171	27.77474499	TTGGAGAGCGTCAATGTGAGGC	CTTCTGCACCTTGATGAGCCAG
UGT1A10	7.358240458	27.77906322	GGGCAAAATCCCTCAGACAGTC	AGCATGGGTGATAAAGGCACGG
SLC5A2	629.7820805	27.89231873	GCTGGAACATCTATGCCTCCGT	TGACGAAGGTCTGTACCGTGTC
SLC34A1 (NPT2A)	2378.589173	28.48502922	TCAAGGTGCCACTGATGCTCAC	GTTGGACAGGATGGCGTTATCC
TMEM207	9.210039552	29.78539562	TCTCGGACCTACCATGCGAAGA	GAGAAGAGCTGCCACCAAAACC
EMX1	38.86996539	30.19829941	GCCTTCGAGAAGAACCACTACG	CGGTTCTGGAACCACACCTTCA
MPV17L	387.5466651	30.27896786	GAGCATTCTCCAAGGAAAGGATG	CAGGAACAAGGCTGAAGTTGGTC
BSND	27.38305111	30.33249950	TGGTCATCCACAAGGGCTCAGA	TGCTGCCTTCACTGGAGTCCAT
GSTA2	827.2496087	30.36894512	CTGCCCTTTAGTCAACCTGAGG	ACAAGGTAGTCTTGTCCGTGGC
ATP6V1B1	158.9617186	30.38901424	GTCTGCCTCTTCCTGAACCTTG	CCGTCAGTATGACCAGCACATG
CLCNKA	54.2336863	30.40070057	CCCTCTACAAGACCAGTTTCCG	CGCTGACAGAAGAGGTAAGCAC
SLC16A14	56.98146616	30.41907310	GCCATCTTTGCTGGTGGTTACC	TGGTCCAGAAAGGACAGCCATC
KL	140.1899897	30.75775719	CCTCCTTTACCTGAAAACCAGCC	CCACAGATAGACATTTCGGGTCAG
NAPSA	3463.27736	30.85197449	TCATTGGAGGGGCTCGTCAACTC	CTGGAAATACCTGCCACCATG
GCM1	2.271767189	31.31601238	AGTGAACACAGCACCTTCCTCC	TTGGACGCCTTCCTGGAAAGAC
SLC4A9	55.04520185	31.50790024	CTGGCTCACATGGACAGTCTTC	GAAGATGGAGGCTCCTGTAAGG

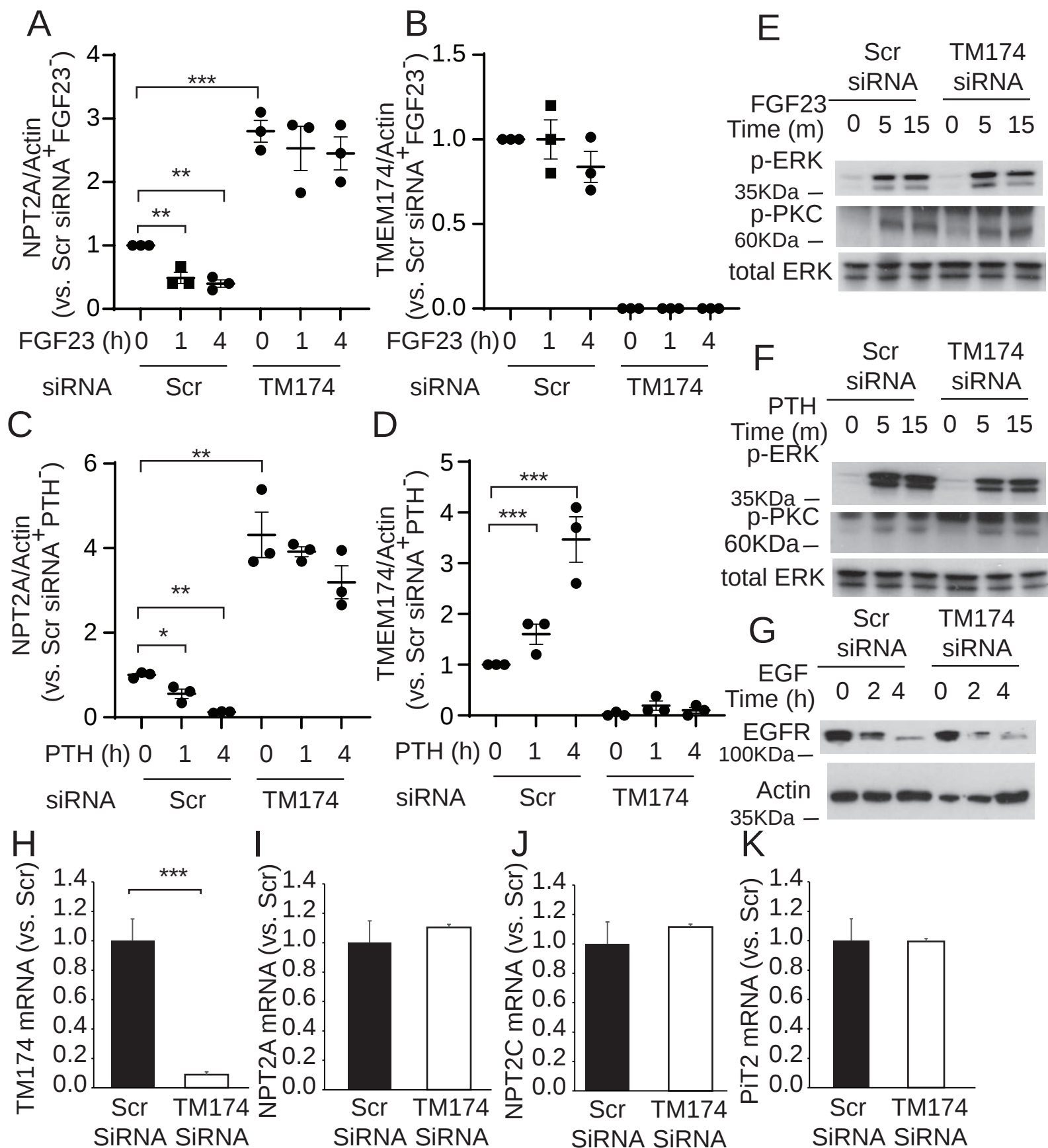
EGF	1188.888438	31.79271221	TGCGATGCCAAGCAGTCTGTGA	GCATAGCCCAATCTGAGAACCAC
CLCNKB	202.3008855	31.85879993	CCCTCTACAAGACCAGTTTCCG	GCTGACAGAAGAGGTAAGCGCT
CYP24A1	61.69707751	32.46832943	GCTTCTCCAGAAGAATGCAGGG	CAGACCTTGGTGTGAGGCTCT
SLC22A13	78.44515706	32.61600399	TTTCCGCAACTGGAGGCTCCTT	GTTGTATCGCCTCGTCCATCCT
SLC16A4	94.9982799	33.21296406	GCCATCTTTGCTGGTGGTTACC	TGGTCCAGAAAGGACAGCCATC
SLC18A1	91.23480697	33.28619766	CAGCCTTCCAAAGTCTCTCCTG	GCACATGGTCTGCATCATCCAG
TMEM72	16.63745416	33.30992413	GGCTGCTTCCAGAAGTTCTCTGG	GCTTGCTCAGAAGGAAGTAGGC
TMEM213	108.8226162	33.73869705	CTATCGCAGCTCTGCACTGTGT	GCTCAGAGCTACAGCAAGTCCA
ADRA2B	40.89529407	35.05657387	TCTTCGGCAACGCTCTGGTCAT	CCAGCGAGAAAGGGATGATGAG
APELA	27.73218891	35.26553917	CGAGTGCCCTTCCCATGA	TTGCTTTCACCCTTCTTCTGGTA
ABCA13	32.69779907	35.39467621	GTACGTGTGCTCACCATCGTTG	AAGTGGTCCTGCGCCAAAGTGA
SLC13A1	182.6038581	35.74983025	GCCTACTCTTCTACCATTGGTGG	AGTTGAGGCAACGACAGTCAGG
LRP2	548.9856901	36.35147858	GTCTAACCGCACTGTGATAGCC	CGGAAGTTTCTCCCAATGTGG
SLC22A8	255.5372826	37.21566391	CAACAGCACCAAGGACTCCATTG	CTGTCAGACAGGTCTCCAAGCA
AQP2	126.4735203	ND	TCTGCTCCATGAGATCACGCCA	ATCGGTGGAGGCGAAGATGCAG
HIGD1C	3.764063045	ND	TCCTGTGGTCTTTACAAGCTAAAG	ACTCACTGAAGAATCGTGGTCTAA
HMX2	6.893029422	ND	GCTTCTGCCCAGACCAGCACG	AGTGCGAAGGAGAGAGGAAAGG
SLC22A6	149.7679749	ND	GGCTTCCTTGTCATCAACTCCC	CACAGCAAGAGAGGTTCCGGACA
SLC34A3 (NPT2C)	84.41642797	ND	GTAGGCACATCCATCACCAGCA	TGTGAGCCAGTTGAAGATCCCG
SLC5A10	155.5076537	ND	GACCTCCATCTTCAACAGCAGC	TCACGCCGATGAGTGCCACTAT
SLC6A18	558.466737	ND	TCCATCGCTGTCTTCTCTGTCC	TAGTCGTCCCTGGAGATGCTCT
TMEM52B	261.6886831	ND	ATCACGACAGCACTCTCCAGAG	TGTCCAAAGAGGAGGGCAGCTG
TRPV5	10.96289847	ND	GGTCATCTTGGGATTTGCCTCC	CAAGTCCACGTCGTAGTTGGCA
UMOD	2926.586609	ND	TGACCAACTGCTATGCCACACC	GAACATCTGGACGGAAAATCGGC

Expression of the kidney-enriched genes (blue) is more than 10 times higher in the kidney than in the 20 other tissues.

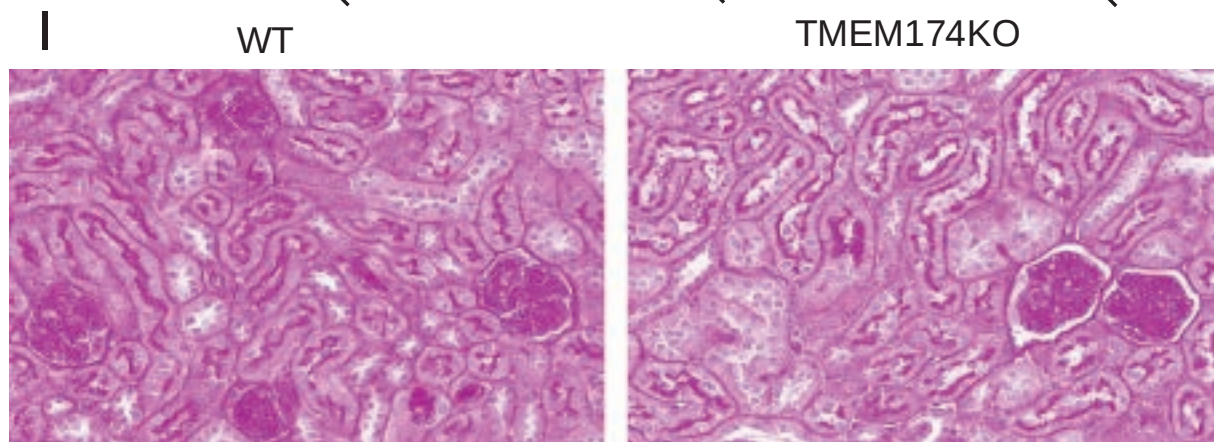
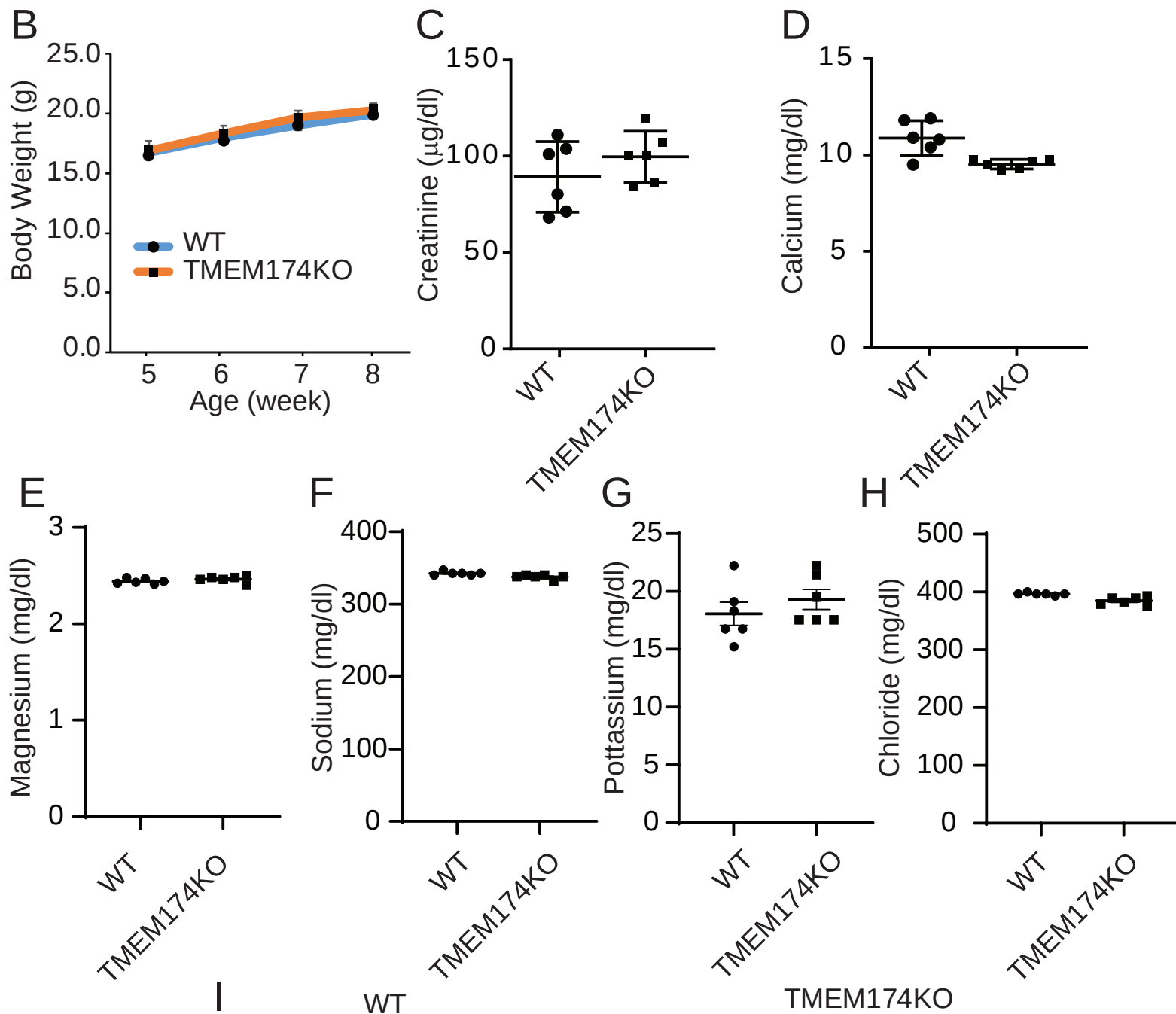
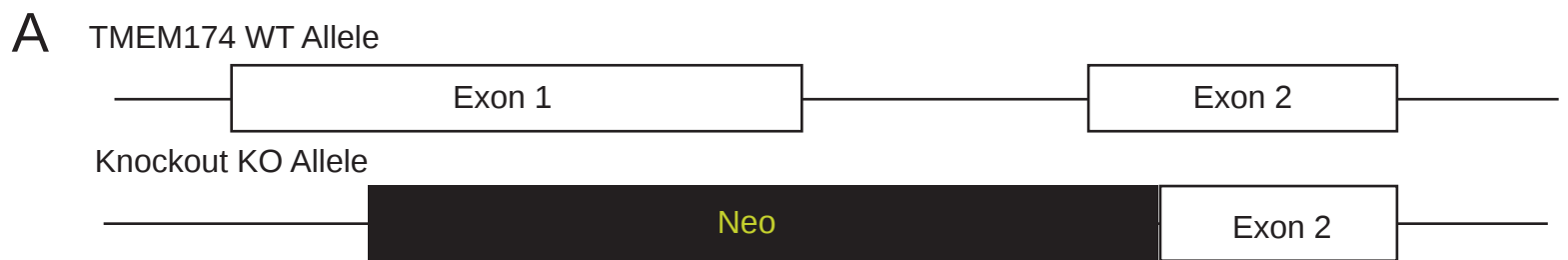
The proximal tubular-enriched genes (red) have a Ct value under 30.

Total RNA isolated from the kidneys of 12 week-old male C57B6/J mice (N=3).

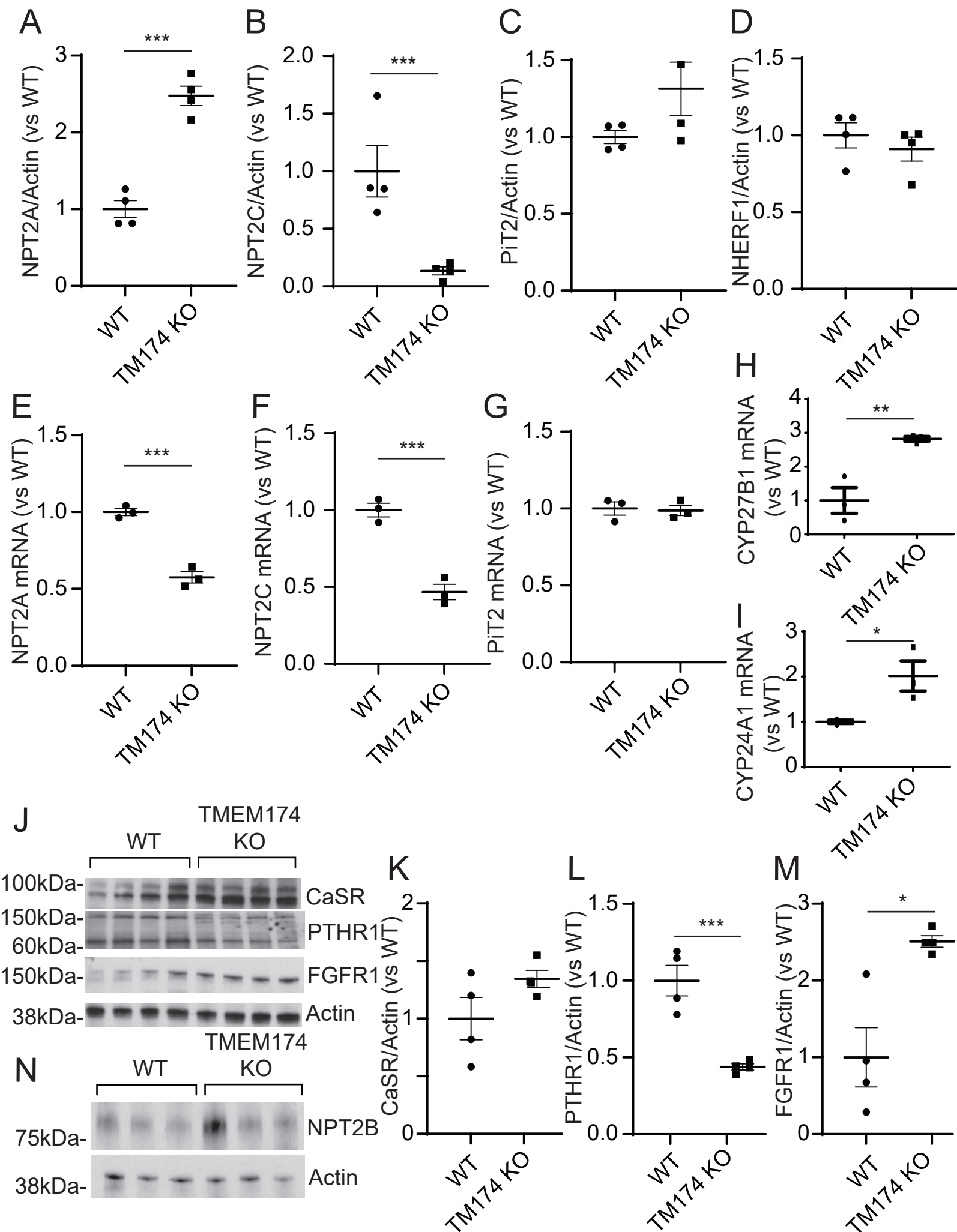
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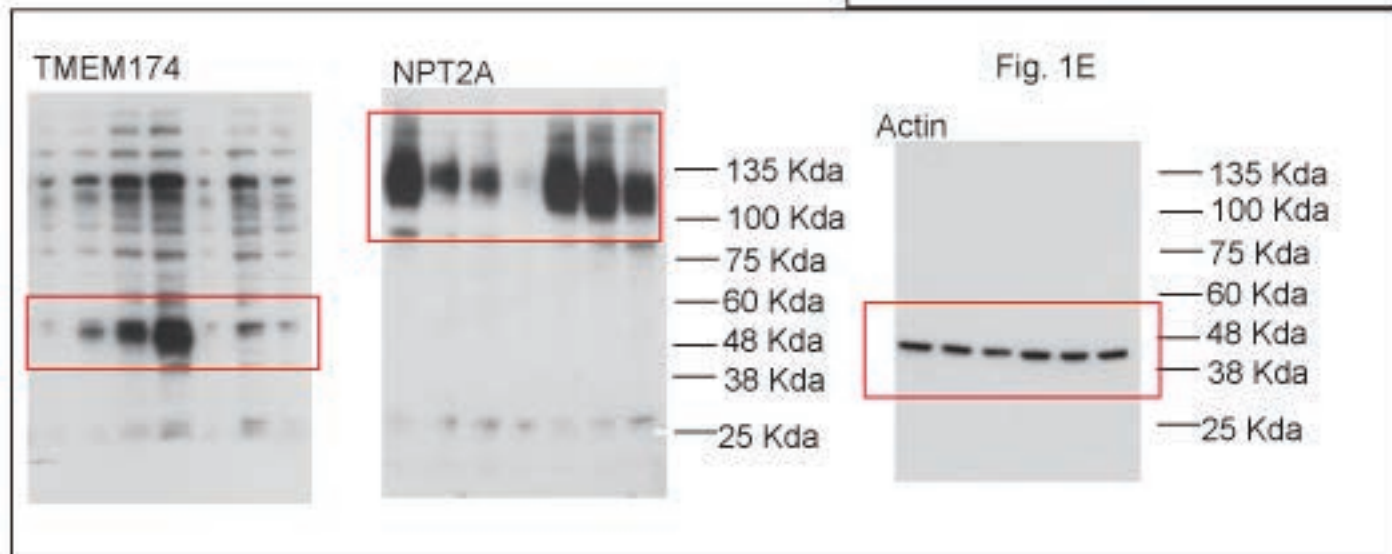
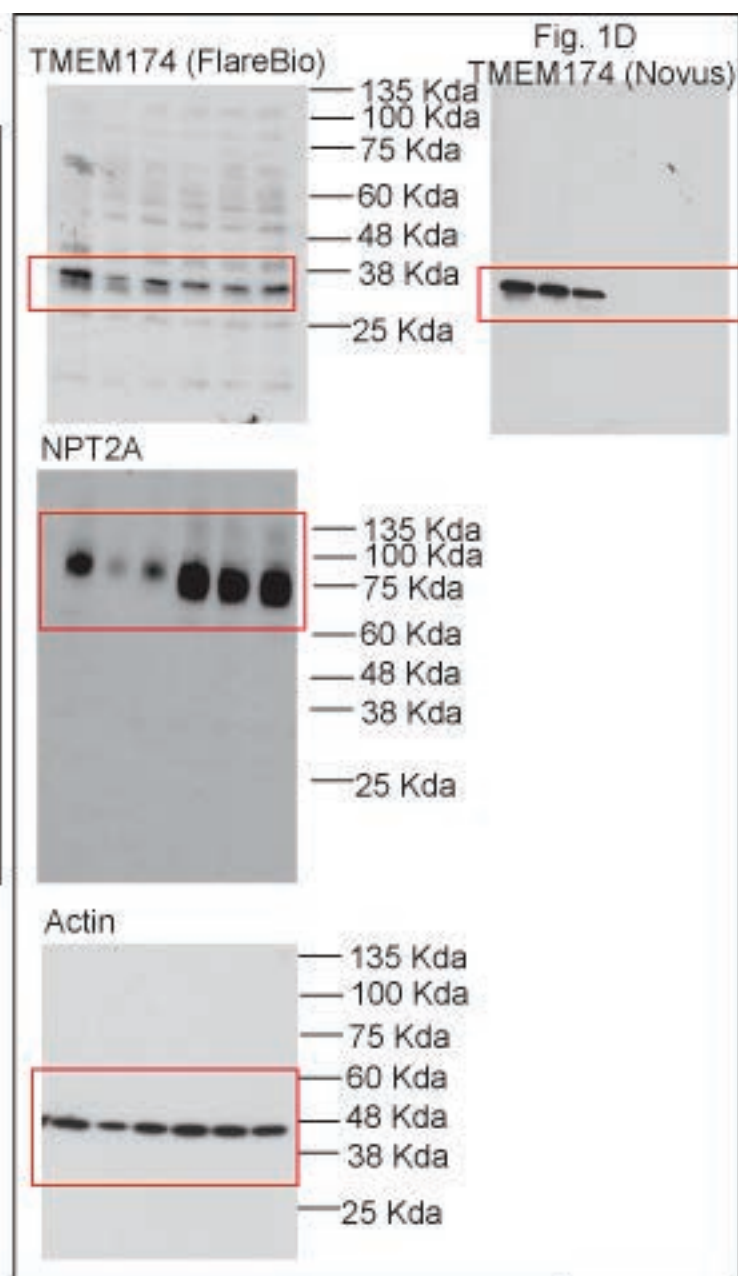
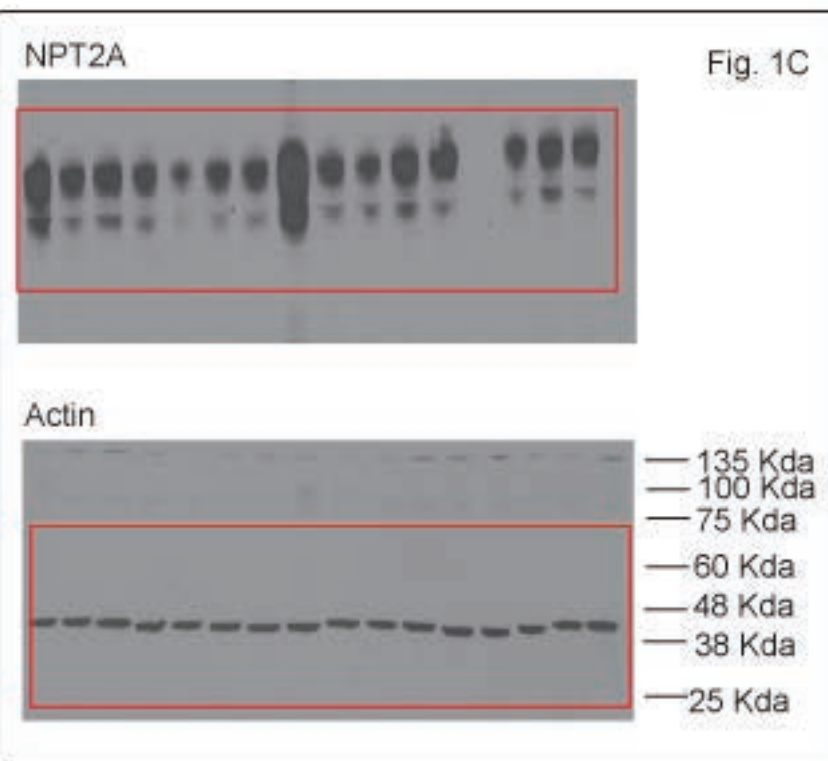
Supplementary Fig. 1. TMEM174 is involved in FGF23- and PTH-mediated NPT2A degradation. A, B) The densitometry analysis (A, NPT2A and B, TMEM174) of the immunoblot analysis shown in Figure 1C. C, D) The densitometry analysis (C, NPT2A and D, TMEM174) of the immunoblot analysis shown in Figure 1D. E, F) Levels of phospho (p)-ERK and p-PKC in E) human proximal tubule cells treated with 10 nM FGF23 and F) OK-P cells treated with 10nM PTH for 5-15 minutes. G) Levels of EGFR in OK-P cells treated with 10nM EGF for 2-4 hours. H) TMEM174, I) NPT2A, J) NPT2C and K) PiT2 mRNA in OK-P cells treated with TMEM174 siRNA for 24 hours. OK-P cells were treated with 20 nM TMEM174 siRNA for 24 hours. N=3, **P<0.01, ***P<0.001

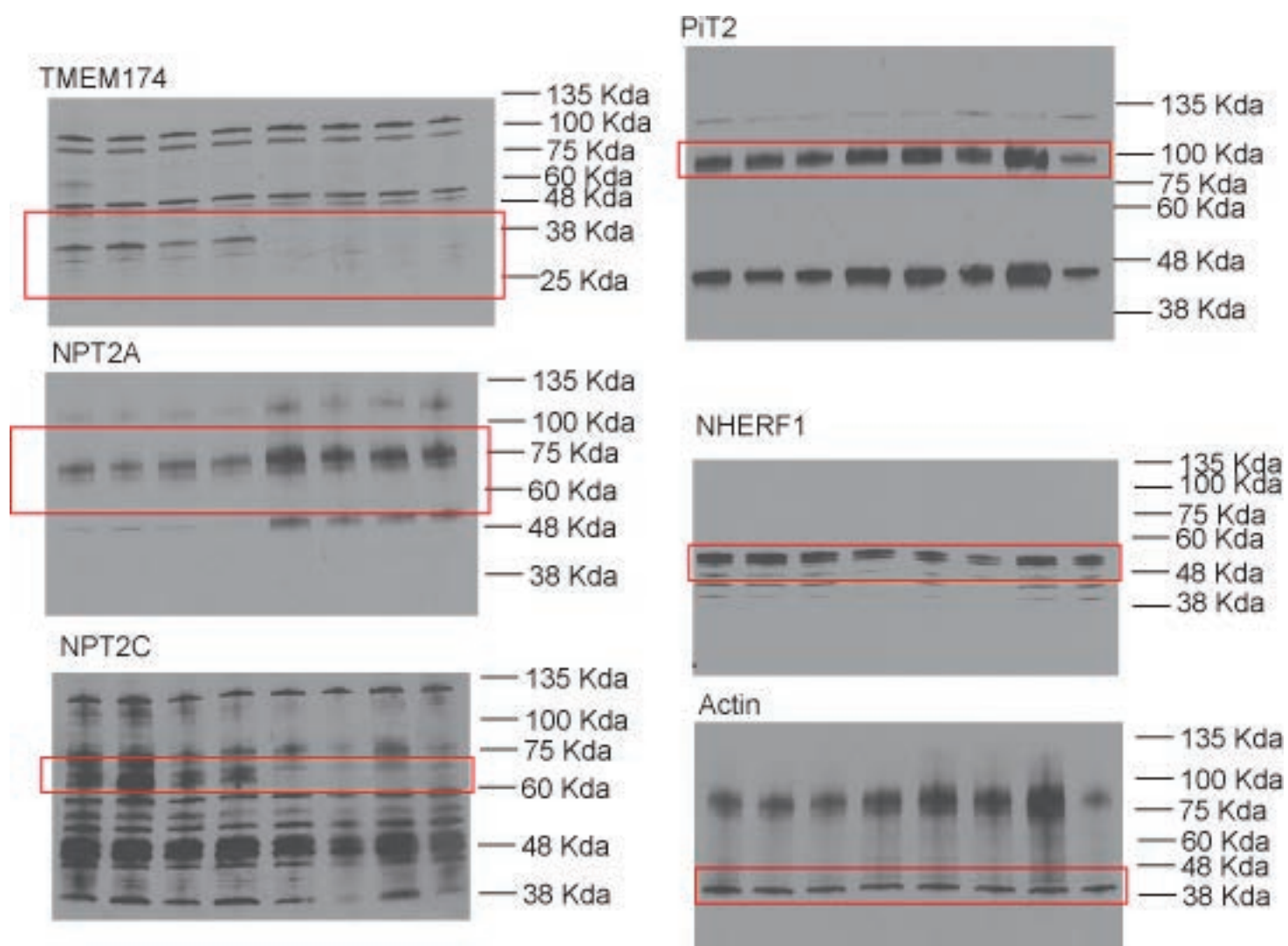


Supplementary Fig. 2. Generation of TMEM174 KO mice. A) The strategy that was used for generating TMEM174 KO mice. B) Body weights of TMEM174 KO mice were measured weekly and mice were sacrificed at 8 weeks old. C) Serum creatinine was analyzed by LC-MS/MS. D) Ca, E) magnesium, F) sodium, G) potassium and H) chloride levels were analyzed with a dry-chemistry autoanalyzer. I) PAS stain of a TMEM174 KO mouse kidney.



Supplementary Fig. 3. Sodium-dependent Pi co-transporter expression in the renal BBM of TMEM174 KO mice. A-D) The densitometry analysis (A, NPT2A; B, NPT2C; C, PiT2; D) NHERF1) of the immunoblot analysis shown in Figure 3E. Renal E) NPT2A, F) NPT2C, G) PiT2, H) CYP27B1 and I) CYP24A1 mRNA in TMEM174 KO mice. J) Immunoblot analysis of CaSR, PTHR1 and FGFR1 in the kidneys of TMEM174 KO mice. K-M) The densitometry analysis (K, CaSR; L, PTHR1; M, FGFR1) of the immunoblot analysis shown in Supplementary Figure 3H. Eight-week-old male mice were sacrificed after a 4 hour fasting. N) Levels of NPT2B protein in the intestines of TMEM174 KO mice. *P<0.05, **P<0.01, ***P<0.001

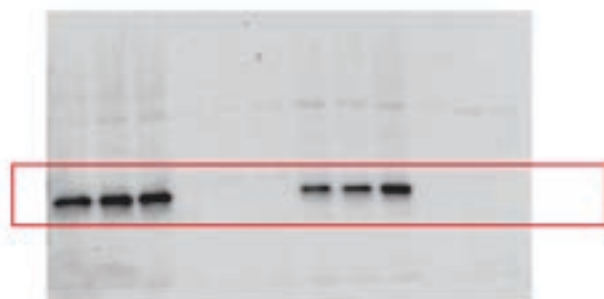




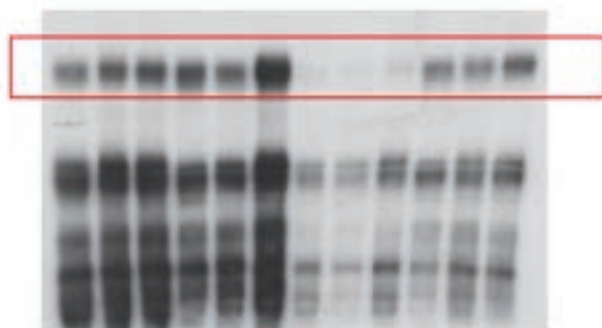
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Fig. 3E

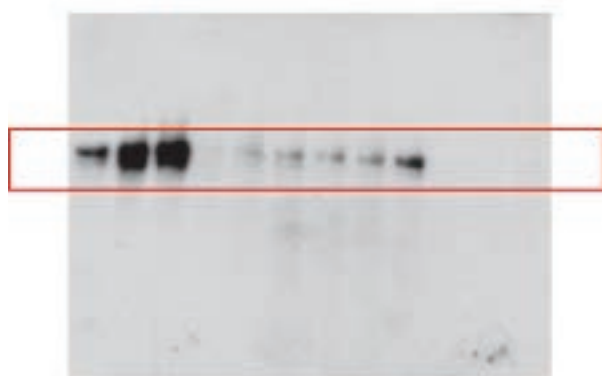
TMEM174



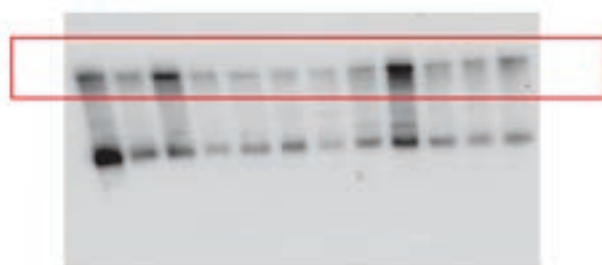
NPT2A



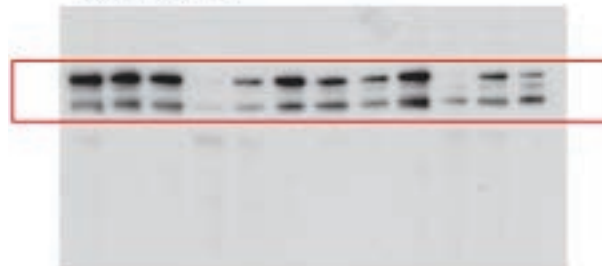
NPT2C



PiT2



NHERF1



Actin



Fig. 4B