

Supplementary Information

Article

Insight into *Nephrocan* Function in Mouse Endoderm Patterning

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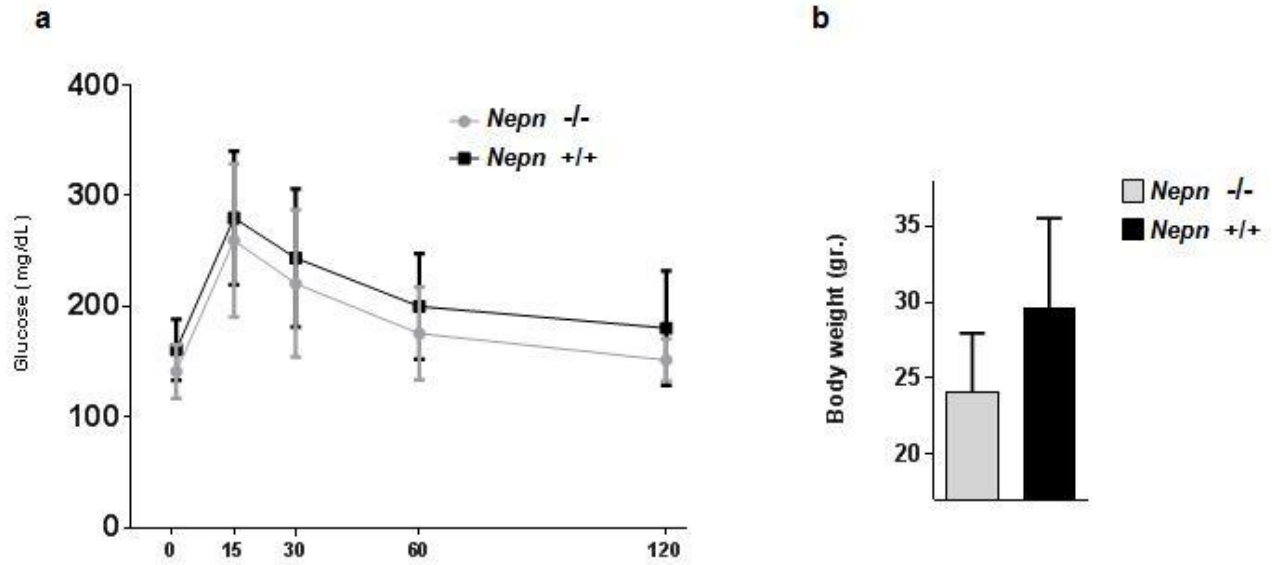
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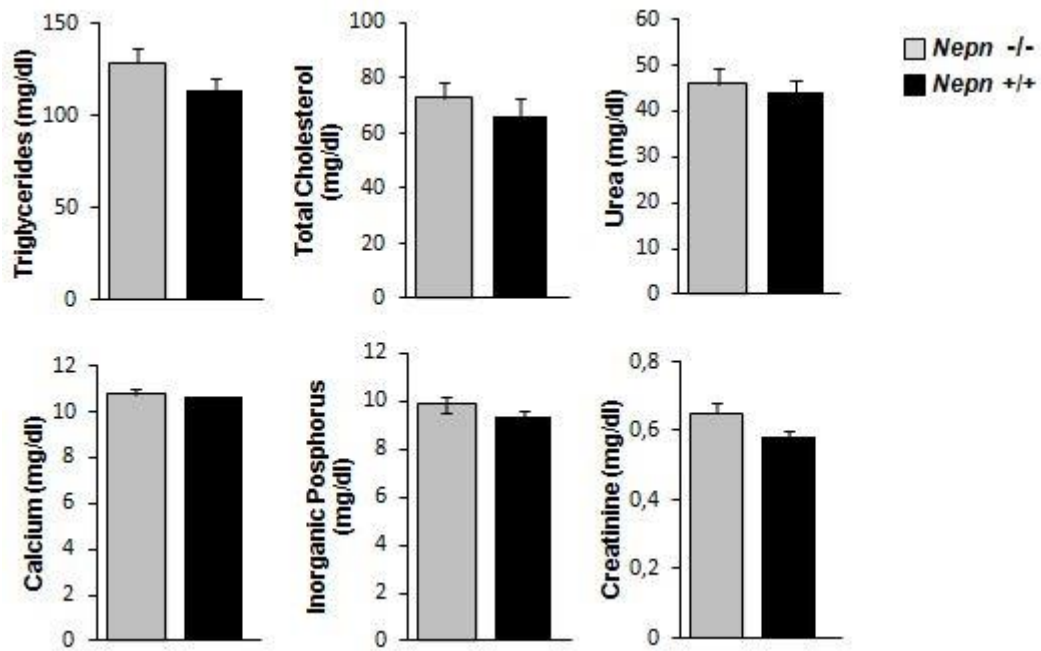
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Supplementary Figure S1: Pancreatic functionality. **a)** Intra-peritoneal Glucose Tolerance Test (IPGTT). Evaluation of blood glucose of *Nepn*^{-/-} mice compared to *Nepn*^{+/+} in a group of 7-12 months old mice. In figure, blood glucose levels are represented at 0, 15, 30, 60 and 120 minutes from the glucose administration (*Nepn*^{+/+}: 7 mice; *Nepn*^{-/-}: 7 mice). **b)** Evaluation of mouse body weight of *Nepn*^{-/-} compared to *Nepn*^{+/+} mice during time life in a group of 7-12 months old mice.



Supplementary Figure S2: Serum levels parameters in a group of 4-6 months old mice. Triglycerides, total cholesterol, urea, calcium and phosphorus were measured - no significant differences between *Nepn*^{+/+} and *Nepn*^{-/-} mice can be observed.

MHPLWAFLLG LSLTNGLSAN CPGRCSDDSM QSVQCYRLME FLPPPRDSTL ATAESSTFSY LTSLDCWLWK ILFCWPVEQS 80
 LLKMTPSKL* VH*RL*NFGK IN*DKSPVLS QPTLRC*N*M IMPYVLYVDQ SLKD*RT*KS LNLKTT*SRP CLPACSPHLP 160
 VCRV*WWMVT T*SPWLDL*A FRISST*AWR TTNSTLYQGM SSLPYRICSS SVSVVIF*LK FP*IYPNPCI L*RWRETSSK 240
 WSGFAI*NTW RTCPIFTCQR TSCLPLMGHS S*PI*QLWRF PKTSFKCCLP GYHRGYRSLI VAVTSFRE*Q HQNSRTSET* 320
 NICF*TTMLS ACSRLGPFRG VLSCPTWPWS RICCCLSL*G SLRPWPGWTS RAMPSTRWLK GSSGTSNNCR SSTSGETTGSR 400
 P*ISRHWRC LGSGISTWVG IPGTALAVSY EPGKS*RPRA RT*KGDSAQP QQSDRGRAGC PRRS*GNVS ITYSRVRKAK 480
 RPRRNPNPKT LPASDSTWMT TMTIMK*I 508

Supplementary Figure S3: In *Neprn*^{-/-} mESCs a frameshift mutation generates several stop codons (indicated as *) thus inhibiting protein synthesis.

Supplementary Table S1: Experimental study of renal functionality. Evaluation of urinary parameters in 24h urine output of *Neprn*^{-/-} mice compared to *Neprn*^{+/+} in a group of 7-12 months old mice.

Urinary Parameters	<i>Neprn</i> ^{+/+}	<i>Neprn</i> ^{-/-}
Urinary Volume (ml)	2.14±0.78	2.36±0.69
Creatinine excretion (µmol/g body weight)	0.26±0.02	0.26±0.03
Na ⁺ / creatinine	27.42±2.78	24.01±3.15
K ⁺ / creatinine	27.92±2.91	34.66±4.40
Cl ⁻ / creatinine	59.53±6.24	51.50±5.63
Creatinine clearance	120.59±8.83	113.65±15.73

Supplementary Table S2: RT-PCR and qRT-PCR primers (5'-3')

Gene Name	Forward Primer 5'-3'	Reverse Primer 5'-3'
<i>Cxcr4</i>	gtaaccaccacggctgtaga	agtagatggtggcaggaag
<i>Gapdh</i>	cggagtcacggatttgctgat	gaagatggtgatgggcttc
<i>Gata2</i>	agctcatgactatggcagca	ccggttctgtccattcatct
<i>Nanog</i>	aaccagtgggtgaagactagcaatggtc	ttccagatgcgttcaccagatagc
<i>NeuroD</i>	gctccagggttatgagatcg	ctctgattcatggcttcaa
<i>Oct4</i>	ccgtgtgaggtggagtctggagac	cgccggttacagaaccatactcg
<i>Foxa2</i>	ctgggagccgtgaagatggaag	tccagcgcacataggatg
<i>Gata4</i>	tcaaacagaaaacggaagc	ctgctgtgccatagtgaga
<i>Gata6</i>	caccatcaccgacctactc	gcatgattgcacaggtaat
<i>Sox9</i>	ggtctgcctggactgtatgtggatg	ctgtccgatgtctctctgcaggag
<i>Hnf6</i>	caaagagggtggcagcgtatc	gctcttccgtttgcaggctg
<i>Nkx6.1</i>	gacagcaaatcttcgacctgg	tttccgaagtccccttgagcc
<i>Rex1</i>	cagaagaagcaggatcgctc	gccactgtctttgccgtttc
<i>Neprn</i> Iso B	gatgcagttgtgattctatgc	tagactctgttccactggc
<i>Neprn</i> Iso B (2)	aacctgtgttggacaatgc	gtgctgcaaggcgattaagt
<i>Neprn</i> Iso A	agatccttctctgcaatgtgaac	tcttccagaccaacagctcc
<i>Neprn</i> Iso A (2)	agatccttctctgcaatgtgaac	gtgctgcaaggcgattaagt
<i>Neprn_b</i> FR	tgtgattctatgagctgtgaca	ggattctgtctggctaatg
<i>Neprn</i> Crispr F3-R2	aaaagattctgtatcagactccg	cgagggtcctctggataggaagc
<i>Neprn</i> Crispr Del	tcatggaactcccatcaggc	tagactctgttccactggc
<i>Neprn</i> Crispr Ko	ctcatggaattccttccacc	tagactctgttccactggc
Cds-Neo	gggatctcatgctggagtctctcg	agaattccccttccatctgctcc
Bax1b-5'	tggtagacattttagatt	agaattccccttccatctgctcc
<i>lacZ</i>	gggatctcatgctggagtctctcg	agaattccccttccatctgctcc

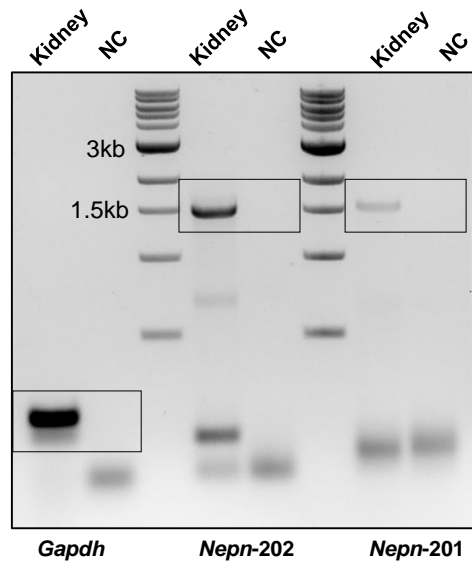
<i>lacZ</i> -(Cre)	cgtcagtatcggcggaattcc	tccccttcacatctgectcc
Int1	gaaactttcacagtatgg	tccccttcacatctgectcc

Supplementary Table S3: PCR condition used in the study

Genes	Denaturation (temp./duration)	Cyclic Denaturation, Annealing and Elongation (temp./duration)	No. of Cycles	Final Elongation and Cooling (temp./duration)
<i>Nepn</i> -202 <i>Nepn</i> -201 <i>Gapdh</i> (Fig. 1b)	95°C/5 minutes	95 °C/30 seconds 58 °C/20 seconds 72 °C/45 seconds	35	72 °C/5 minutes 4 °C/∞
<i>Nepn</i> isoform a <i>Nepn</i> isoform b <i>Gapdh</i> (Fig. 2b, 3d, 6b)	95°C/5 minutes	95 °C/15 seconds 55 °C/15 seconds 72 °C/20 seconds	38	72 °C/5 minutes 4 °C/∞
Recombinant allele Wild-type allele (Fig. 3b)	95°C/5 minutes	95 °C/15 seconds 60 °C/30 seconds 72 °C/45 seconds 95 °C/15 seconds 50 °C/30 seconds 72 °C/45 seconds	10 (-1°C/cycles) 30	72 °C/5 minutes 4 °C/∞ 72 °C/5 minutes 4 °C/∞
<i>Nepn</i> isoform b <i>Nepn</i> isoform b (Fig. 6b)	95°C/5 minutes	95°C/30 seconds 58°C/15 seconds 72°C/15 seconds	38	72 °C/5 minutes 4 °C/∞

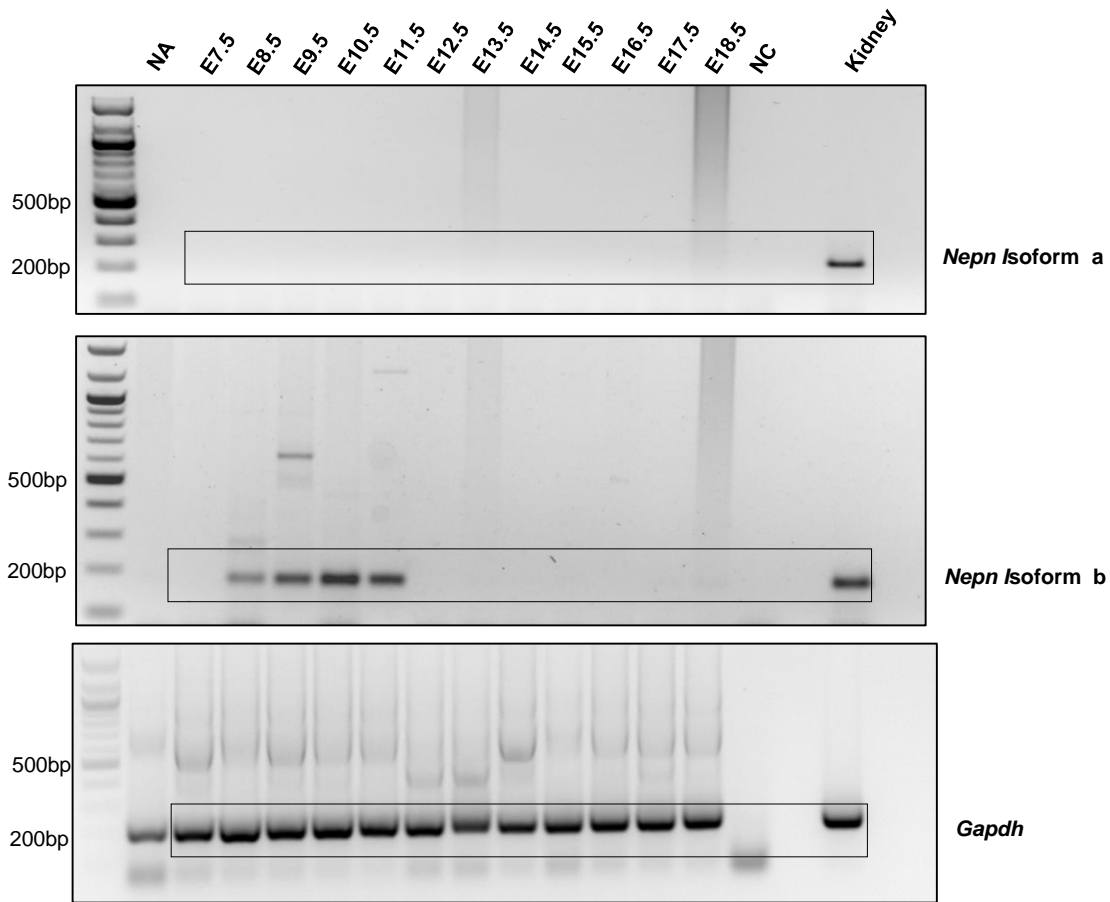
Supplementary Table S2: RT-PCR and qRT-PCR primers (5'-3')

Full gel image for Fig. 1b

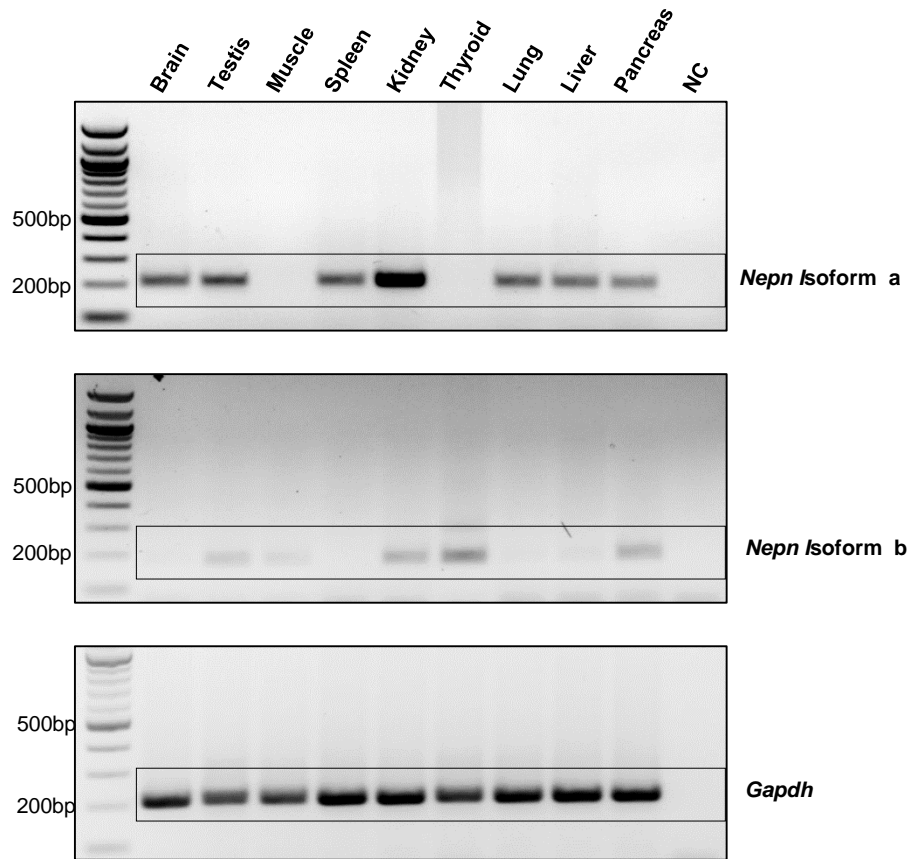


Full gel images for Fig. 2a

NA=Not Applicable

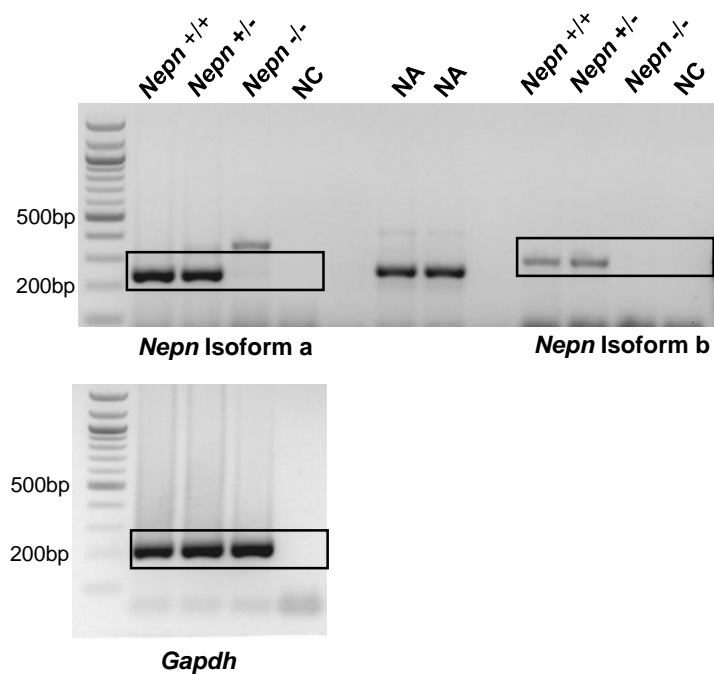


Full gel images for Fig. 2b

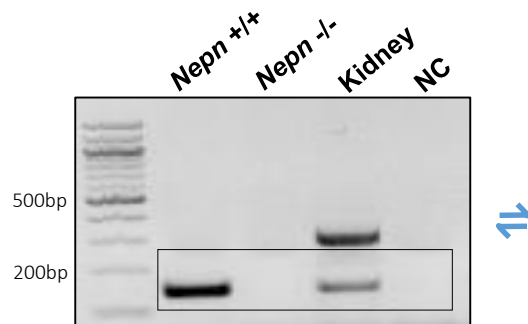
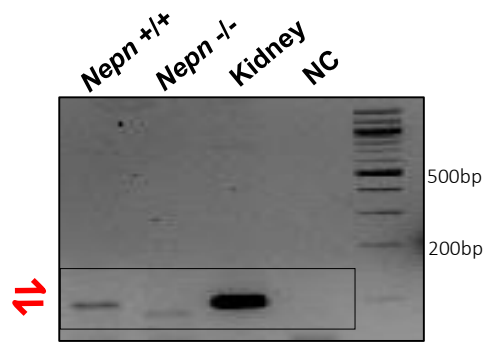


Full gel images for Fig. 3d

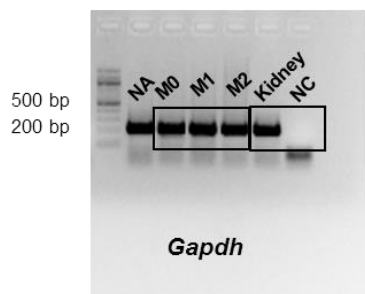
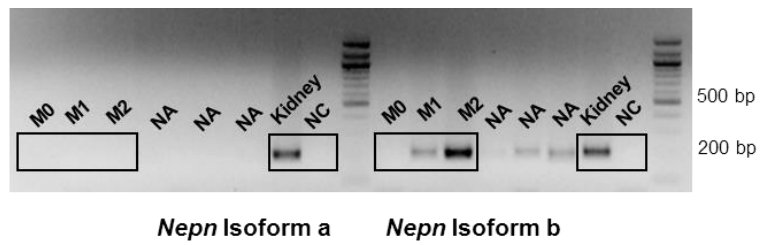
NA=Not Applicable



Full gel images for Fig. 5c



Full gel images for Fig. 6b



NA=Not Applicable