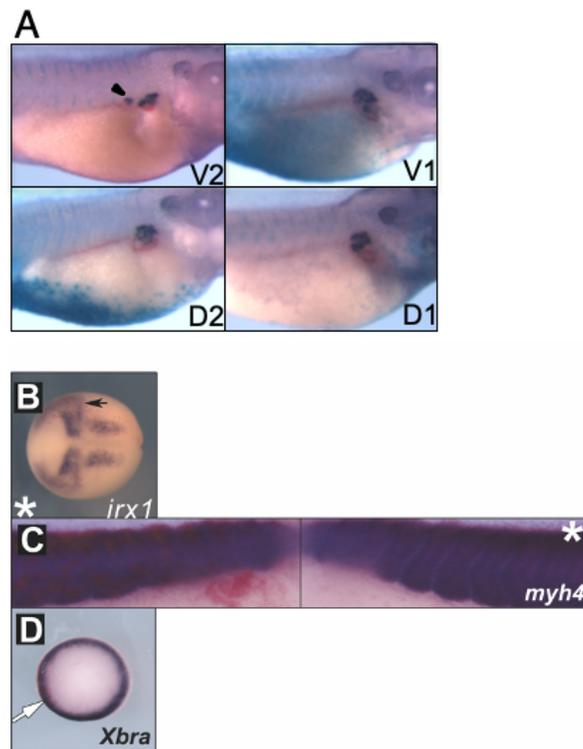


Supplementary Information

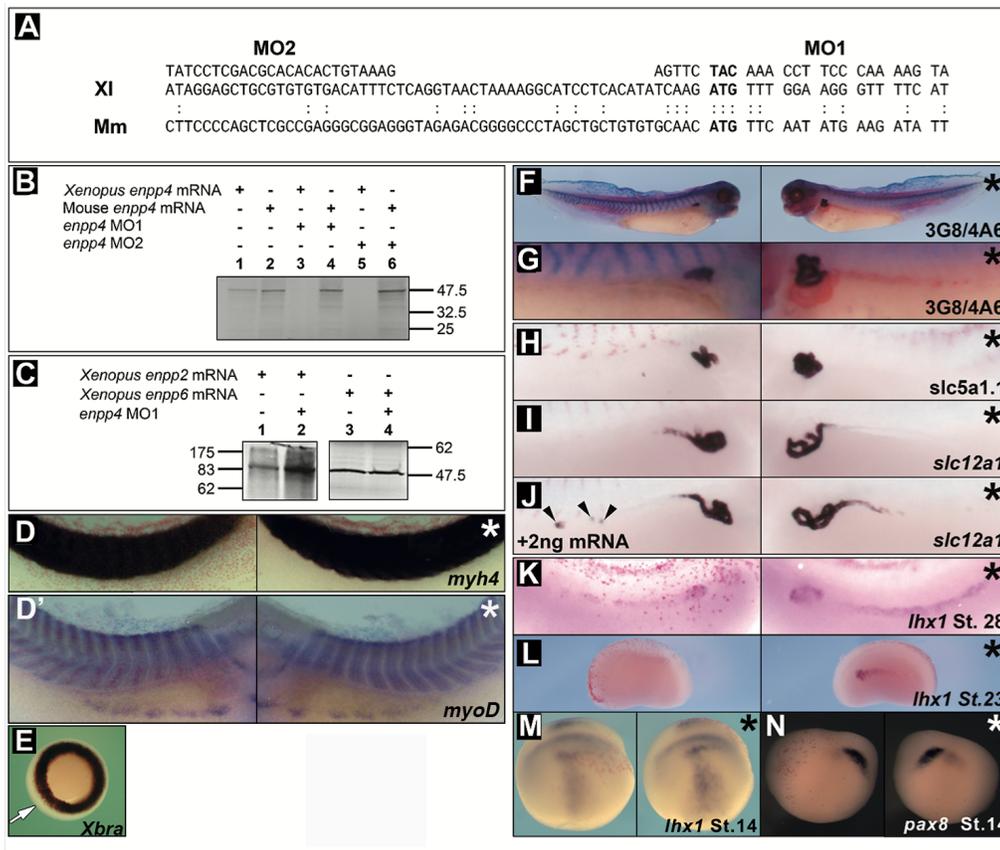
The *enpp4* ectonucleotidase regulates kidney patterning signalling networks in *Xenopus* embryos

by Karine Massé, Surinder Bhamra, Christian Paroissin, Lilly Maneta-Peyret,
Eric Boué-Grabot and Elizabeth A. Jones

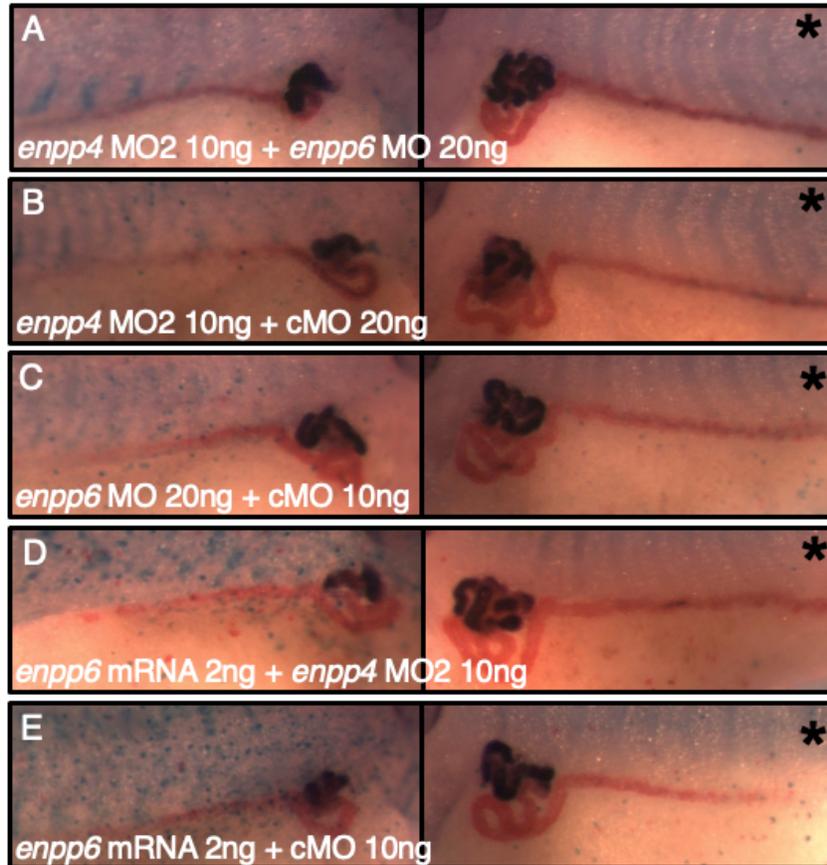
This PDF file includes: Supplementary Fig. 1 to 7, unedited gels and western blot are in Supplementary Figure 8 and 9 and Supplementary Tables 1 to 5.



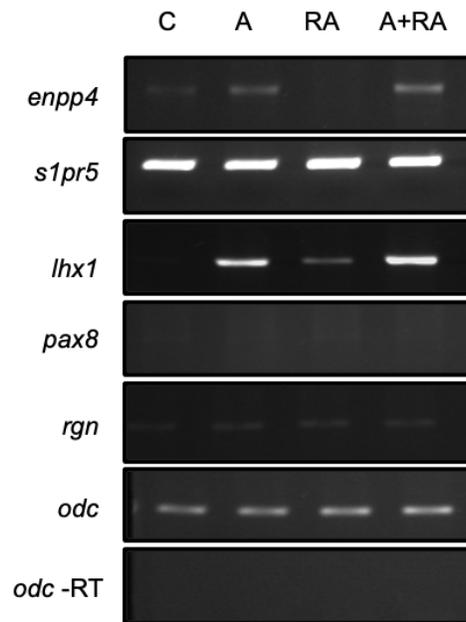
Supplementary Fig. 1. Phenotypes caused by *enpp4* mRNA injection (related to Fig. 1). (A) Ectopic pronephric tissues were only induced following *enpp4* mRNA injection into the V2 blastomere. Embryos injected with 2ng of *enpp4* and 250 pg of *LacZ* mRNAs into the V2, V1, D2 or D1 blastomere at 8-cell stage were examined by whole mount antibody staining 3G8/4A6 at stage 41. The black arrowhead indicates the ectopic 3G8 staining. (B- D) No change in *irx1* expression and in mesoderm formation caused by *enpp4* overexpression. Embryos targeted with 2ng of *enpp4* and 250 pg of *LacZ* mRNAs were examined by whole mount *in situ* hybridization with the *irx1* (B), *myf4* (C) and *xbra* (D) probes at stages 14, 32 and 10.5 respectively. The asterisk denotes the uninjected side of each embryo. The black arrow in B indicates the pronephros. The white arrow in D indicates the injection site marked by the red-gal staining. The raw data and statistical analyses are provided in Supplementary Table 1.



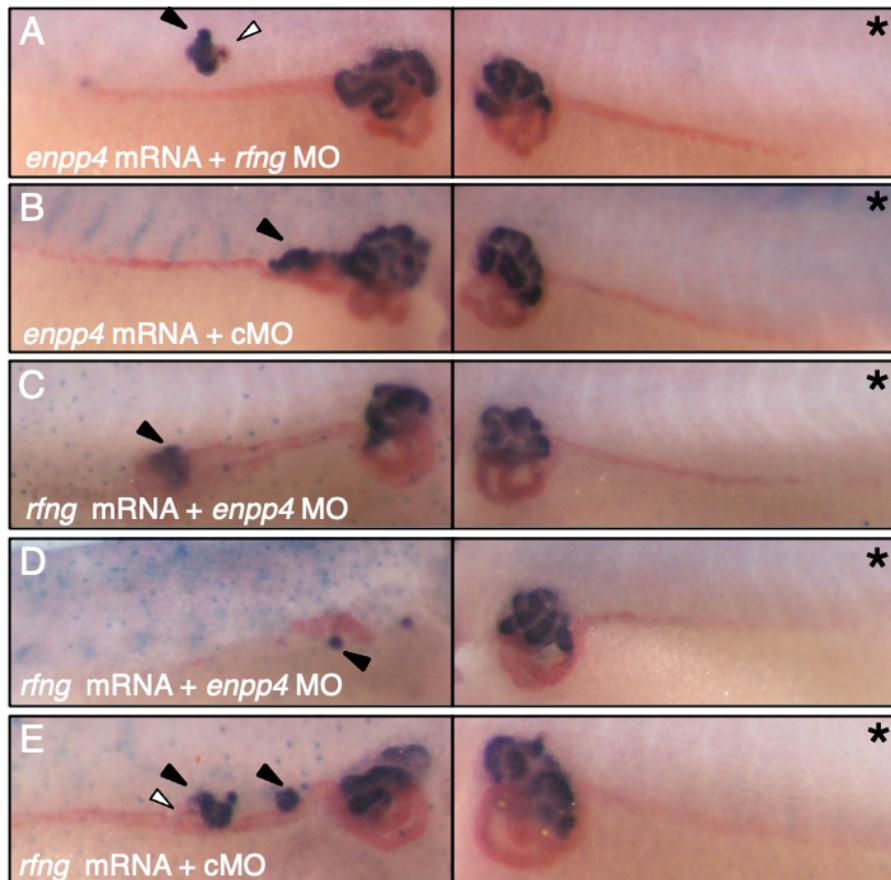
Supplementary Fig. 2. *Enpp4* MO1 and MO2 specificity and phenotypes caused by injection of *enpp4* MO2 (related to Fig. 2). (A-C) *enpp4* MO1 and 2 specifically inhibited *Xenopus enpp4* translation. (A) Alignment of the 5'UTR of *Xenopus* (XI) and mouse (Mm) *enpp4* sequences and position of *enpp4* MO1 and 2 in relation to *Xenopus enpp4* cDNA. The ATG is indicated in bold and identical nucleotides (nt) by dots. Only 12 nt of *enpp4* MO1 and 3 nt of *enpp4* MO2 (located in the 5' UTR sequence) are conserved between the two species. (B) *Xenopus enpp4* but not mouse *enpp4* translation was blocked by *enpp4* MO1 and 2. Autoradiograph of a 10% SDS-PAGE gel of *in vitro* translated ³⁵S-Methionine radiolabeled *enpp4* proteins. Capped synthetic *enpp4* RNA was translated *in vitro* in the Rabbit Reticulocyte Lysate System (Promega). Translation of *Xenopus enpp4* mRNA (0.5 µg) (Lane 1) and mouse *Enpp4* (Lane 2) produced a protein of 51 kDa. *Xenopus enpp4*, but not mouse *Enpp4* (Lane 4 and 6), was almost abolished in presence of 10 µg of *enpp4* MO1 or MO2 (Lane 3 and 5). (C) *enpp4* MO1 is specific to *enpp4* mRNA. Autoradiograph of 10% SDS-PAGE gel of *in vitro* translated ³⁵S-Methionine radiolabeled *Xenopus enpp2* and 6 proteins. *Xenopus enpp2* and 6 RNAs were translated *in vitro* in the Rabbit Reticulocyte Lysate System (Promega). Translation of *Xenopus enpp2* mRNA (0.5 µg) produced a protein of 99 kDa (Lane 1) that is unaffected by the addition of 10 µg *enpp4* MO1 (Lane 2). Translation of *Xenopus enpp6* mRNA (0.5 µg) produced a protein of 51 kDa (Lane 3) that is unaffected by the *enpp4* MO1 (Lane 4). Mm: *Mus musculus*; XI: *Xenopus laevis*. (D-E) Microinjection of *enpp4* MO1 did not alter general mesoderm formation. Embryos injected with 10 ng of *enpp4* MO1 at 8-cell stage were examined by whole mount *in situ* hybridization with the *myf4* (D), *myoD* (D') and *xbra* (E) probes at stage 32, stage 39 and at stage 10.5 respectively. Asterisk denotes uninjected sides. The arrow in (E) indicates the site of injection marked by red-gal staining. (F-N) Microinjection of *enpp4* MO2 resulted in similar phenotypes than those with *enpp4* MO1. Embryos injected with 10 ng of *enpp4* MO2 at 8-cell stage were examined by 3G8/4A6 antibody staining at stage 40 (F and G) or whole mount *in situ* hybridization with the following probes: *slc5a1.1* (H), *slc12a1* (I and J) at stage 37/38; *lhx1* at stage 28, 23 and 14 (K, L and M respectively) and *pax8* at stage 14 (N). Embryo shown in (J) was co-injected with 2 ng of mouse *Enpp4* mRNA for rescue experiment. Asterisk denotes uninjected sides. Arrowheads indicate ectopic *slc12a1* staining. The raw data and statistical analyses are provided in Supplementary Table 2.



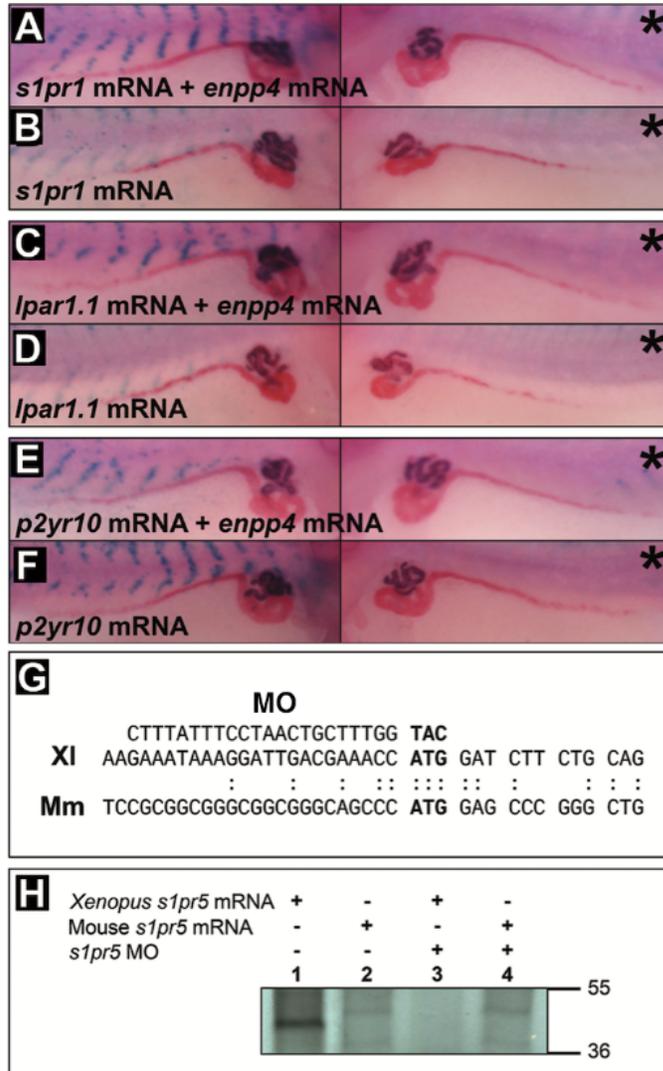
Supplementary Fig. 3. Phenotypes caused by the co-injection of the *enpp4* and *enpp6* MOs or *enpp4* MO and *enpp6* mRNA (related to Fig. 2). Injection of *enpp6* mRNA, but not *enpp6* MO, worsened the pronephric phenotypes caused by *enpp4* knock-down. Injected embryos were analysed by 3G8/4A6 antibody staining at stage 41. (A) Embryo injected with 10 ng of *enpp4* MO2 and 20 ng of *enpp6* MO. (B) Embryo injected with 10 ng of *enpp4* MO2 and 20 ng of control MO. (C) Embryo injected with 20 ng of *enpp6* MO and 10 ng of control MO. (D) Embryo injected with 2 ng of *enpp6* mRNA and 10 ng of *enpp4* MO. (E) Embryo injected with 2 ng of *enpp6* RNA and 10 ng of control MO. Asterisk denotes uninjected sides. The raw data and statistical analyses are provided in Supplementary Table 2.



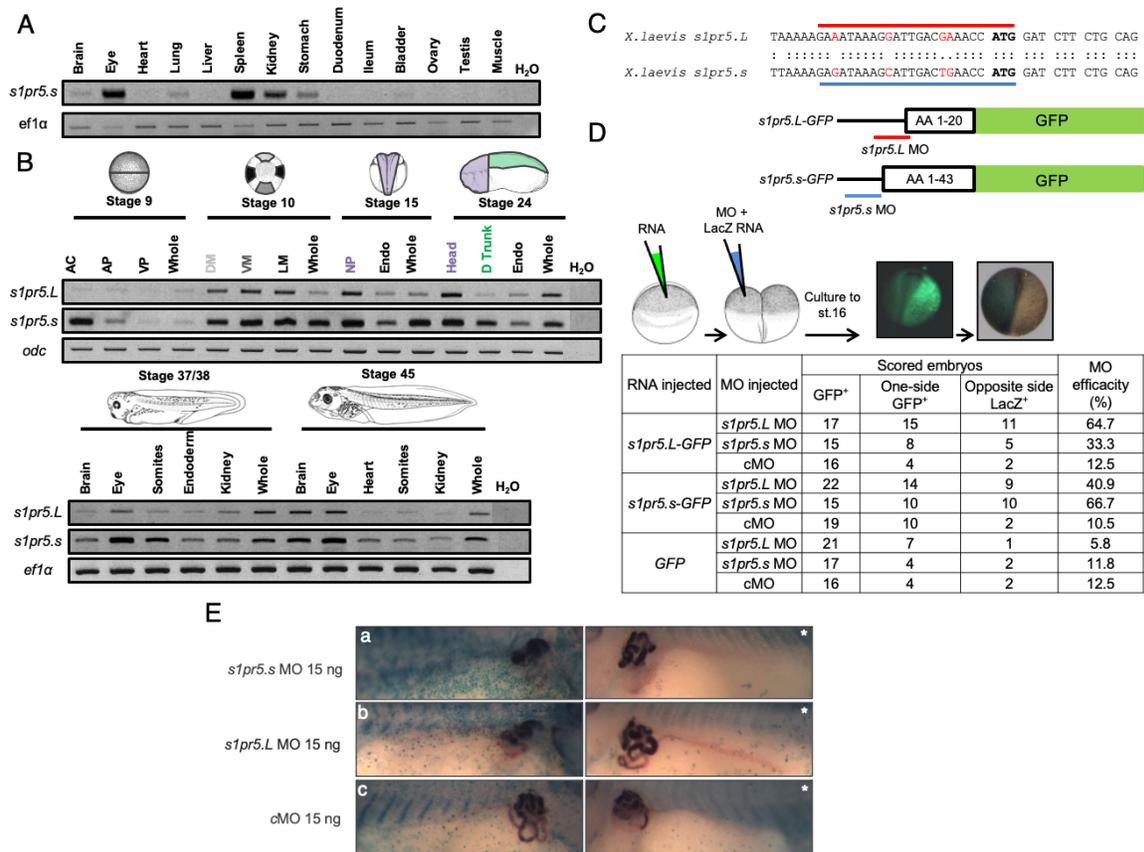
Supplementary Fig. 4. *Enpp4* expression is regulated by activin but not by retinoic acid in animal cap assay (related to Fig. 3). Animal caps were taken at blastula stage and cultured during 3 hours in the presence of activin (A), retinoic acid (RA) or activin + retinoic acid (A+RA) and total RNA extracted. RT-PCR was performed on treated and control (C) animal caps. The amplification of *odc* on samples with no reverse transcriptase (-RT) was carried out to control the purity of the RNAs.



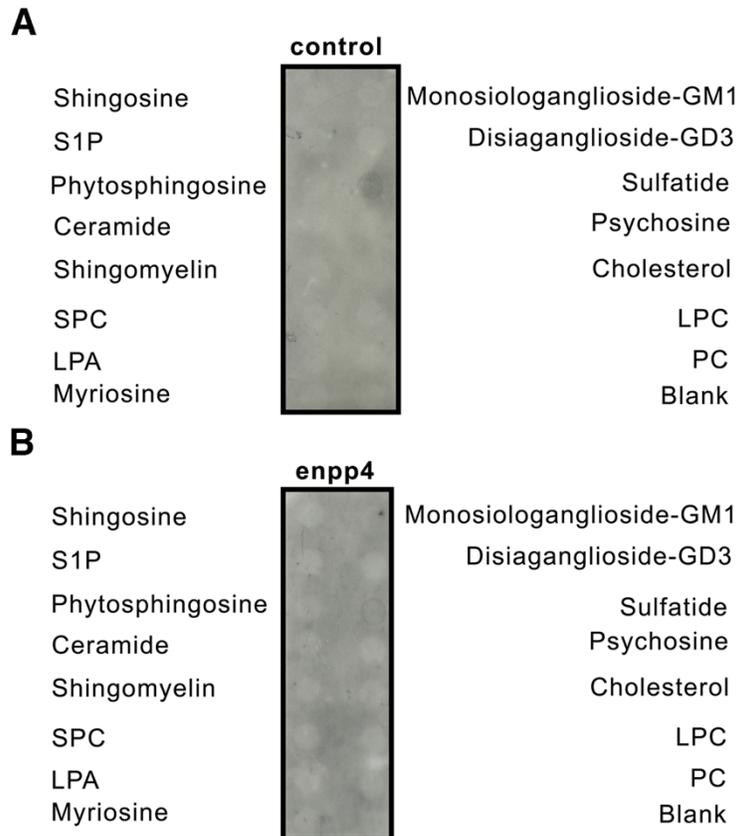
Supplementary Fig. 5. Phenotypes caused by co-injection of *enpp4* or *rfng* mRNA and MO (related to Fig. 4). Injection of *rfng* MO did not prevent ectopic pronephros formation caused by *enpp4* over-expression whereas co-injection of *enpp4* MO and *rfng* mRNA altered ectopic and endogenous pronephros formation. Injected embryos were harvested at stage 40, and analysed by 3G8/4A6 antibody staining. (A) Embryo injected with 2 ng of *enpp4* mRNA and 20 ng of *rfng* MO. (B) Embryo injected with 2 ng of *enpp4* mRNA and 20 ng of control MO. (C, D) Embryo injected with 2 ng of *rfng* mRNA and 10 ng of *enpp4* MO. (E) Embryo injected 2 ng of *rfng* mRNA and 10 ng of control MO. Asterisk denotes uninjected sides. Black arrowheads indicate 3G8 ectopic staining and white arrowheads 4A6 ectopic staining. The raw data and statistical analyses are provided in Supplementary Table 3.



Supplementary Fig. 6. Phenotypes caused by injection of *s1pr1*, *lpar1.1* and *p2yr10* mRNA and *in vitro* test of the *s1pr5.L* MO specificity (related to Fig. 6). (A-F) Microinjection of *s1pr1*, *lpar1.1* and *p2yr10* mRNA did not induce ectopic pronephros even when co-injected with *enpp4* mRNA. Injected embryos were harvested at stage 40, and analyzed by 3G8/4A6 antibody staining. (A) Embryo injected with 2 ng of *s1pr1* mRNA and 1ng of *enpp4* mRNA or (B) 2ng of *s1pr1* mRNA alone. (C) Embryo injected with 2ng of *lpar1.1* mRNA and 1ng of *enpp4* mRNA or (D) 2ng of *lpar1.1* mRNA alone. (E) Embryo injected with 2ng of *p2yr10* mRNA and 1ng of *enpp4* mRNA or (F) with 2ng of *p2yr10* mRNA alone. Asterisk denotes uninjected sides. The raw data and statistical analyses are provided in Supplementary Table 5. (G-H) *s1pr5.L* MO specifically inhibited *Xenopus s1pr5.L* mRNA translation. (G) Alignment of the 5'UTR of *Xenopus* (XI) and mouse (Mm) *s1pr5* sequences and position of *s1pr5.L* MO in relation to *Xenopus s1pr5.L* cDNA. The ATG is indicated in bold and identical nucleotides (nt) by dots. This alignment shows that only 8 nt of *s1pr5.L* MO sequence are conserved between the two species. (H) *Xenopus s1pr5.L* but not mouse *S1pr5* translation was blocked by *s1pr5.L* MO; autoradiograph of a 10% SDS-PAGE gel of *in vitro* translated ³⁵S-Methionine radiolabeled *s1pr5* proteins. Capped synthetic *s1pr5* RNAs was translated *in vitro* in the Rabbit Reticulocyte Lysate System (Promega) according to manufacturer's protocol. Lane 1, Translation of *Xenopus s1pr5.L* mRNA (1μg) produced a protein of 43 kDa. Lane 2, Translation of mouse *S1pr5* mRNA (1 μg) produced a protein of 42 kDa. Lane 3, Translation of *Xenopus s1pr5.L* mRNA was severely affected by the addition of 10 μg of *s1pr5.L* MO. Lane 4, Translation of mouse *S1pr5* was unaffected by *s1pr5.L* MO. Mm: *Mus musculus*; XI: *Xenopus laevis*.

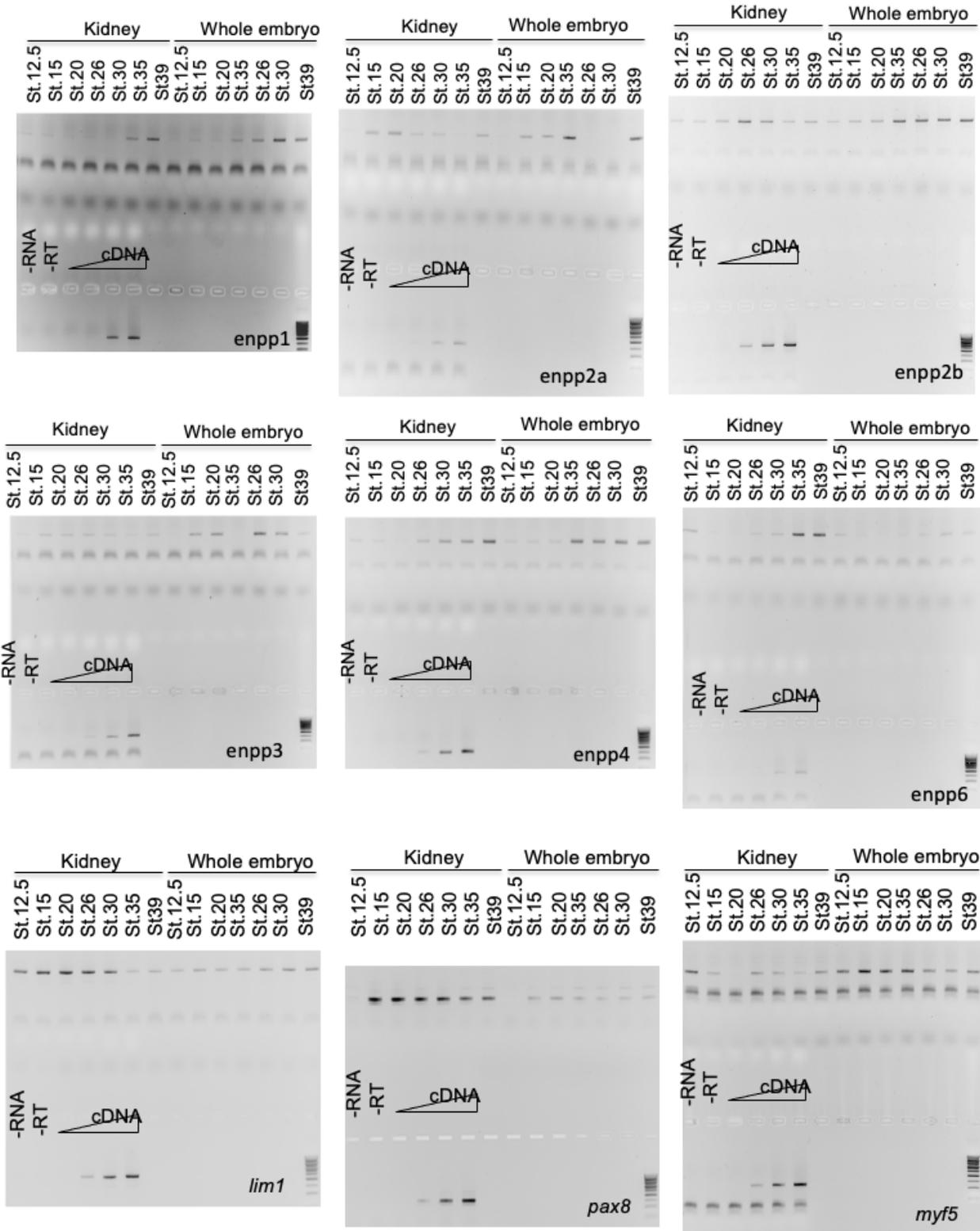


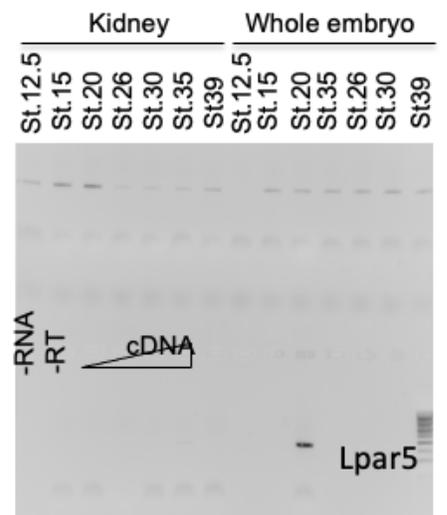
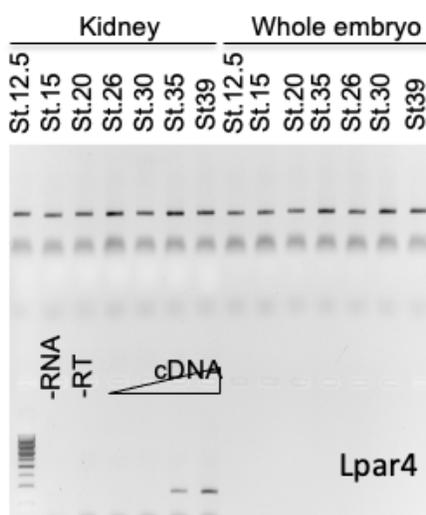
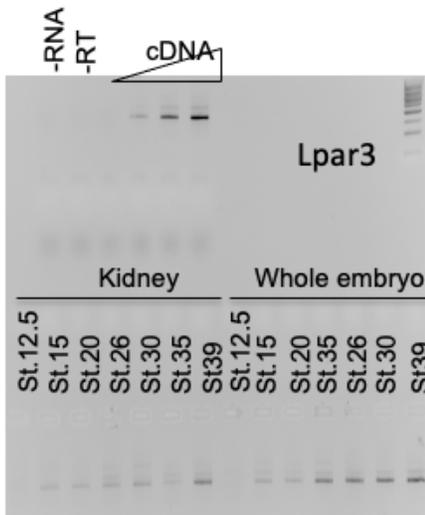
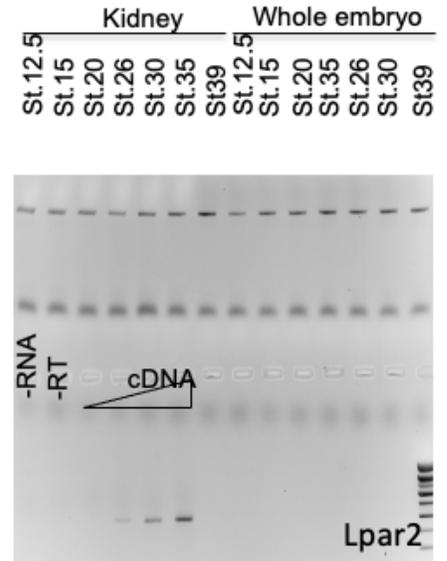
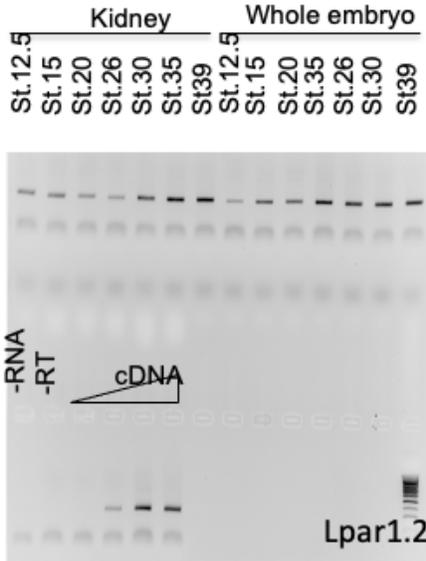
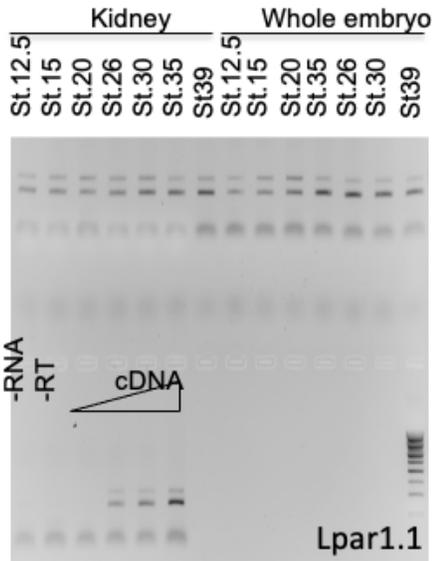
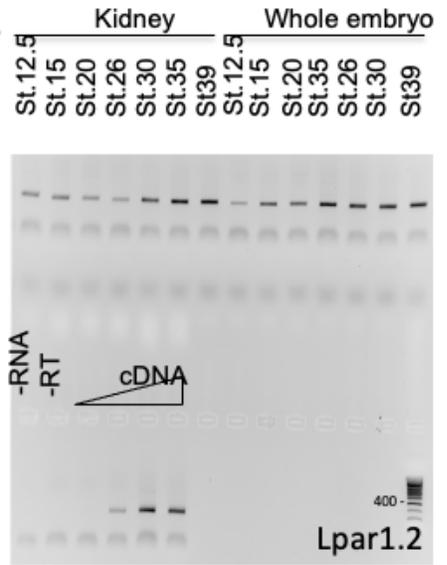
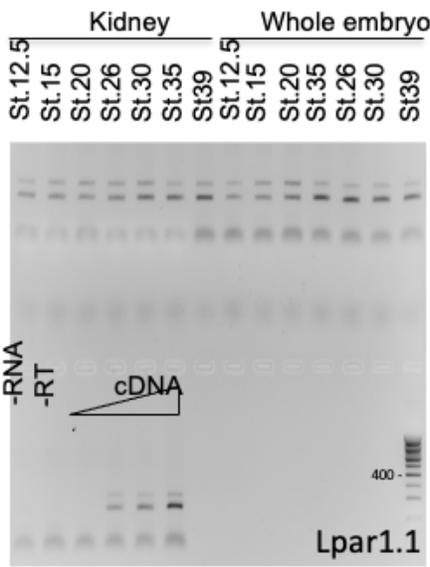
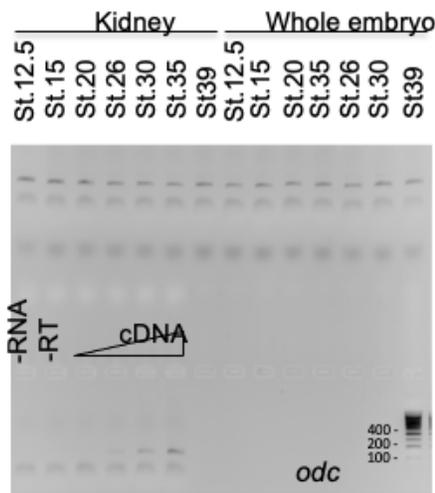
Supplementary Fig. 7. Expression profile of the *s1pr5.S* receptor gene, *in vivo* test of MO specificity and phenotypes caused by its knock-down (related to Fig. 6). (A) The *s1pr5.S* receptor displays a restricted expression profile in the adult frog and is expressed in the mesonephric tissue. Adult tissues were dissected and total RNAs from adult tissues were extracted using Trizol (Invitrogen) following the manufacturer's protocol. RT-PCR was performed along with negative control. (B) The novel *s1pr5.S* receptor is expressed in the kidney with an expression profile similar to its homolog, the *s1pr5.L* receptor. *X.laevis* embryos were dissected and total RNA extracted. RT-PCR was performed on dissected tissues and control whole embryos along with negative control. *S1pr5.S* was amplified using the following primers (forward primer, 5'- ggaggtctcgtgtgctc -3' and the reverse primer, 5'- cgtagtctcctcgtgaacc -3') with the annealing temperature of 53°C and 32 cycles, Each RT-PCR product was sequenced to confirm the specificity of the amplifications. (C) Alignment of the 5'UTR of *X.laevis* *s1pr5* homeologs sequences and position of *s1pr5.L* and *s1pr5.S* MOs. The ATG is indicated in red and identical nucleotides by dots. Only 4 nucleotides over the whole MOs sequences differ between the two genes. (D) Efficacy of the *s1pr5.L* and *s1pr5.L* MOs. Schematic representation of the GFP fusion proteins containing the 5'UTR and part of the coding region of *s1pr5.L* and *s1pr5.S*. The position of the *s1pr5.L* and *s1pr5.s* MO are indicated. *Xenopus* embryos were injected with the *s1pr5.L*GFP, *s1pr5.S* -GFP and GFP mRNAs at one cell stage followed by unilateral injections of *s1pr5.L*, *s1pr5.S* and control MOs in presence of *LacZ* mRNA at 2-cell stage. GFP⁺ positive embryos were sorted at stage 16 and stained for β-galactosidase activity. The numbers of embryos GFP⁺ fluorescent on one side and LacZ⁺ on the opposite side and the percentage of efficiency of the different MOs to inhibit *in vivo* the translation of the different mRNAs are indicated in the table. (E) Microinjection of *s1pr5.S* MO induces a similar kidney phenotype than the microinjection of the *s1pr5.L* MO. Injected embryos analysed by 3G8/4A6 antibody staining at stage 41. (a) Embryo injected with 15ng of *s1pr5.S* MO (b) Embryo injected with 15ng of *s1pr5.L* MO. (c) Embryo injected with 15ng of control MO. Asterisk denotes uninjected sides. The raw data and statistical analyses are provided in Supplementary Table 5.

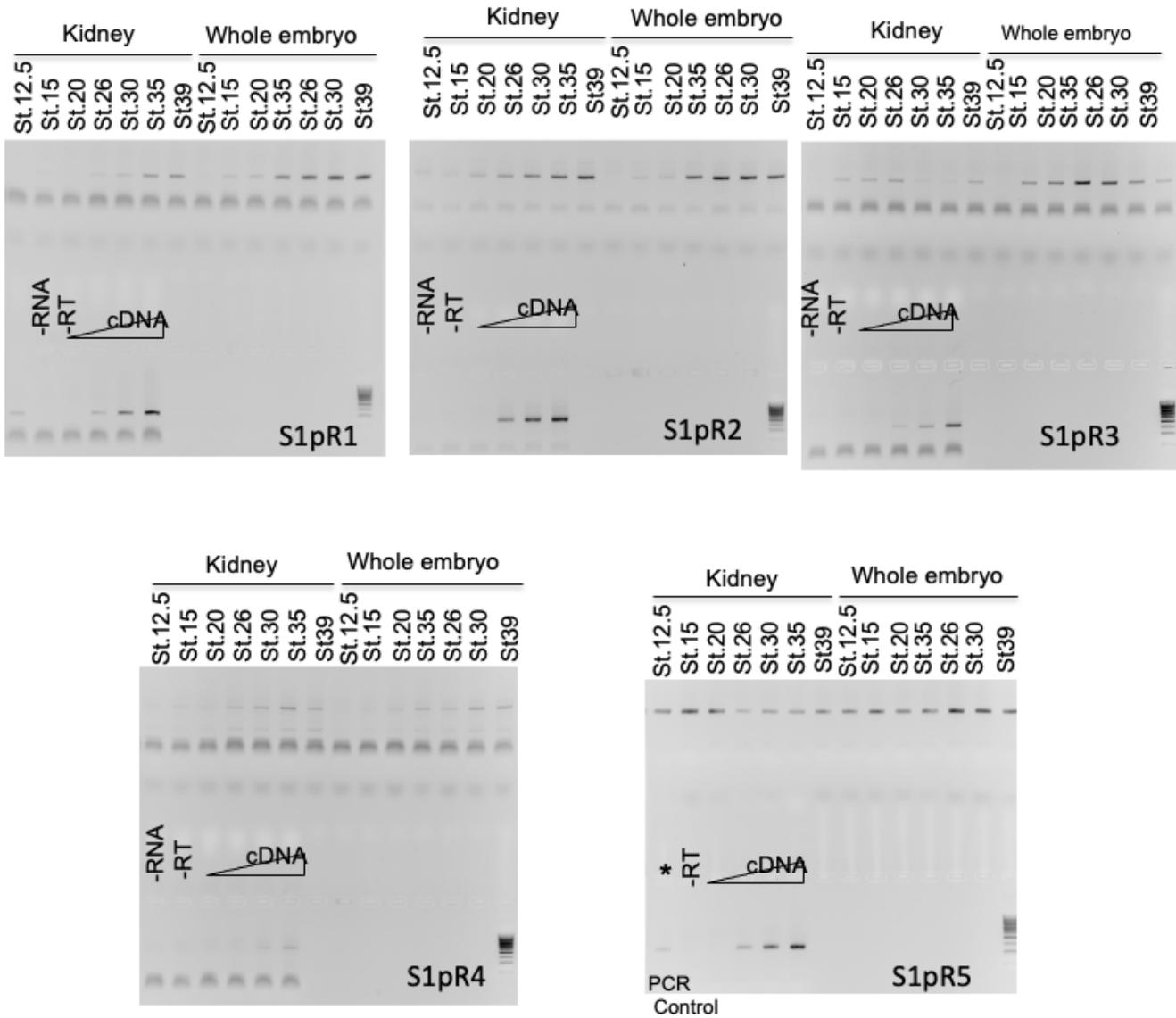


Supplementary Fig. 8. Enpp4 does not interact with bioactive lipids metabolized by enpp2, enpp6 and enpp7 (related to Fig. 7). ShingoStrips™ was incubated with membrane protein extracts from (A) control pcDNA3.1 transfected CHO cells or (B) from enpp4 over-expressing CHO cells and the bound enpp4 protein detected with anti-Xlenpp4 serum. LPA, lysophosphatidic acid; LPC, lysophosphocholine; PC, phosphatidylcholine; S1P, shingosine-1-phosphate; SPC, Shingosylphosphorylcholine.

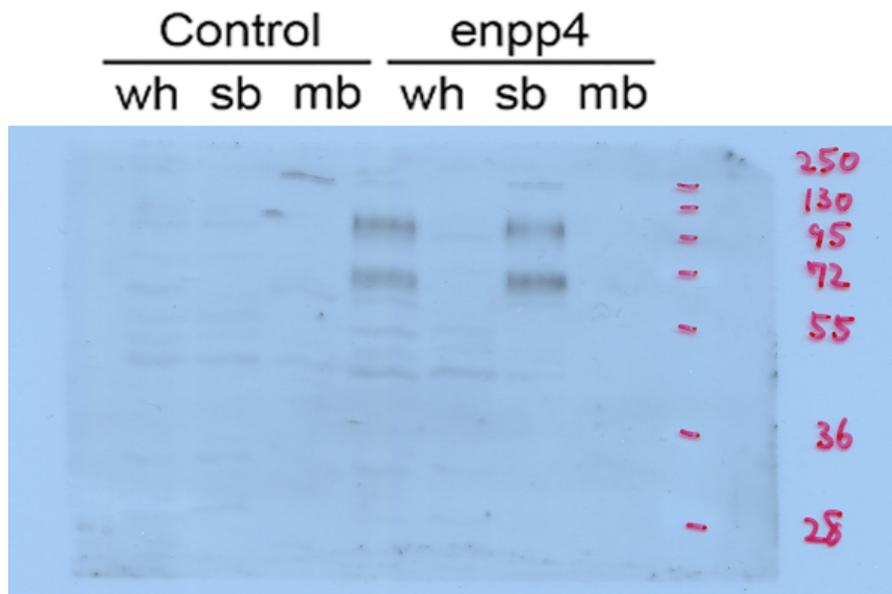
Unedited gels and western blots







Supplementary Fig. 8. Unedited gels corresponding to markers (in the same order) illustrated in Figure 3. Smartladder 100-1000 (eurogentec) was used in all gels. Size are indicated on the first gel.



Supplementary Fig. 9. Unedited western blot corresponding to Figure 5a. Size indicated are indicated in kDa.

Supplementary Table 1a

Histological Analysis	Figure	Injection	Marker analysed	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
1	1B-E	<i>enpp4</i> mRNA 2ng	3G8	47 (52%)	16 (18%)	7 (8%)	0 (0%)	21 (23%)	91
2	1B-E	<i>enpp4</i> mRNA 2ng	4A6	63 (69%)	18 (20%)	8 (9%)	0 (0%)	2 (2%)	91
3	S1A	<i>enpp4</i> mRNA 2ng V2	3G8	19 (47.5%)	8 (20%)	5 (12.5%)	0 (0%)	8 (20%)	40
4	S1A	<i>enpp4</i> mRNA 2ng V2	4A6	29 (72.5%)	6 (15%)	4 (10%)	0 (0%)	1 (2.5%)	40
5	S1A	<i>enpp4</i> mRNA 2ng V1	3G8	30 (88%)	0 (0%)	4 (12%)	0 (0%)	0 (0%)	34
6	S1A	<i>enpp4</i> mRNA 2ng V1	4A6	30 (88%)	0 (0%)	4 (12%)	0 (0%)	0 (0%)	34
7	S1A	<i>enpp4</i> mRNA 2ng D2	3G8	25 (96%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	26
8	S1A	<i>enpp4</i> mRNA 2ng D2	4A6	24 (92%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	26
9	S1A	<i>enpp4</i> mRNA 2ng D1	3G8	22 (92%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)	24
10	S1A	<i>enpp4</i> mRNA 2ng D1	4A6	23 (96%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	24
11	1M	Mouse <i>enpp4</i> mRNA 2ng	3G8	19 (30%)	0 (0%)	8 (13%)	1 (2%)	35 (56%)	63
12	1M	Mouse <i>enpp4</i> mRNA 2ng	4A6	32 (51%)	12 (19%)	11 (17.5%)	1 (2%)	7 (11%)	63
13	1N	<i>enpp4</i> T72S mRNA 2ng	3G8	50 (76%)	1 (1.5%)	15 (23%)	0 (0%)	0 (0%)	66
14	1N	<i>enpp4</i> T72S mRNA 2ng	4A6	52 (79%)	0 (0%)	14 (21%)	0 (0%)	0 (0%)	66
15		<i>enpp4</i> T72A mRNA 2ng	3G8	42 (68%)	1 (2%)	18 (29%)	0 (0%)	1 (1%)	62
16		<i>enpp4</i> T72A mRNA 2ng	4A6	51 (82%)	0 (0%)	11 (18%)	0 (0%)	0 (0%)	62
17	1O	<i>enpp4</i> D36N mRNA 2ng	3G8	53 (77%)	6 (9%)	9 (13%)	0 (0%)	1 (1%)	69
18	1O	<i>enpp4</i> D36N mRNA 2ng	4A6	62 (90%)	1 (1%)	6 (9%)	0 (0%)	0 (0%)	69
19		<i>enpp4</i> D189N mRNA 2ng	3G8	57 (75%)	2 (3%)	8 (10.5%)	4 (5%)	5 (7%)	76
20		<i>enpp4</i> D189N mRNA 2ng	4A6	68 (89.5%)	1 (1%)	4 (5%)	3 (4%)	0 (0%)	76
21		<i>LacZ</i> mRNA 250pg	3G8	52 (91%)	0 (0%)	5 (9%)	0 (0%)	0 (0%)	57
22		<i>LacZ</i> mRNA 250pg	4A6	53 (93%)	0 (0%)	4 (7%)	0 (0%)	0 (0%)	57
23	1P	<i>enpp4</i> mRNA 2ng	<i>slc5a1.1</i>	27 (47%)	8 (14%)	5 (9%)	0 (0%)	17 (30%)	57
24		<i>LacZ</i> mRNA 250pg	<i>slc5a1.1</i>	60 (94%)	0 (0%)	3 (5%)	1 (2%)	0 (0%)	64
25	1Q	<i>enpp4</i> mRNA 2ng	<i>slc12a1</i>	30 (57%)	14 (25%)	3 (5%)	0 (0%)	10 (17.5%)	57
26		<i>LacZ</i> mRNA 250pg	<i>slc12a1</i>	63 (98%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	64
27	1R	<i>enpp4</i> mRNA 2ng	<i>clcnkb</i>	40 (69%)	11 (19%)	7 (12%)	0 (0%)	0 (0%)	58
28		<i>LacZ</i> mRNA 250pg	<i>clcnkb</i>	60 (94%)	0 (0%)	2 (3%)	2 (3%)	0 (0%)	64
29	1S	<i>enpp4</i> mRNA 2ng	<i>gata3</i>	17 (39.5%)	1 (2%)	25 (58%)	0 (0%)	0 (0%)	43
30		<i>LacZ</i> mRNA 250pg	<i>gata3</i>	33 (89%)	0 (0%)	4 (11%)	0 (0%)	0 (0%)	37
31	1T	<i>enpp4</i> mRNA 2ng	<i>wt1</i>	26 (44%)	20 (34%)	13 (22%)	0 (0%)	0 (0%)	59
32		<i>LacZ</i> mRNA 250pg	<i>wt1</i>	84 (95.5%)	1 (1%)	3 (3%)	0 (0%)	0 (0%)	88
33	1U	<i>enpp4</i> mRNA 2ng	<i>nphs1</i>	32 (36%)	22 (25%)	30 (34%)	1 (1%)	3 (3%)	88
34		<i>LacZ</i> mRNA 250pg	<i>nphs1</i>	52 (96%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	54
35	1V	<i>enpp4</i> mRNA 2ng	<i>lhx1</i>	20 (39%)	31 (61%)	0 (0%)	0 (0%)	0 (0%)	51
36		<i>LacZ</i> mRNA 250pg	<i>lhx1</i>	76 (96%)	1 (1%)	2 (2%)	0 (0%)	0 (0%)	79
37	1W	<i>enpp4</i> mRNA 2ng	<i>pax8</i>	14 (28%)	35 (70%)	0 (0%)	0 (0%)	1 (2%)	50
38		<i>LacZ</i> mRNA 250pg	<i>pax8</i>	72 (97%)	0 (0%)	2 (3%)	0 (0%)	0 (0%)	74
39	1X	<i>enpp4</i> mRNA 2ng	<i>lhx1</i>	30 (65%)	9 (20%)	7 (15%)	0 (0%)	0 (0%)	46
40		<i>LacZ</i> mRNA 250pg	<i>lhx1</i>	28 (67%)	4 (9%)	10 (24%)	0 (0%)	0 (0%)	42
41	1Y	<i>enpp4</i> mRNA 2ng	<i>pax8</i>	20 (29%)	12 (17%)	24 (34%)	0 (0%)	15 (21%)	70
42		<i>LacZ</i> mRNA 250pg	<i>pax8</i>	34 (60%)	2 (3%)	21 (37%)	0 (0%)	0 (0%)	57
43	S1C	<i>enpp4</i> mRNA 2ng	<i>irx1</i>	24 (73%)	2 (6%)	7 (21%)	0 (0%)	0 (0%)	33

Histological Analysis	Figure	Injection	Marker analysed	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
44		<i>LacZ</i> RNA 250 pg	<i>irx1</i>	20 (95%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)	21
45	S1D	<i>enpp4</i> mRNA 2ng	<i>myh4</i>	55 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	55
46		<i>LacZ</i> mRNA 250pg	<i>myh4</i>	53 (98%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	54
47	S1E	<i>enpp4</i> mRNA 2ng	<i>xbra</i>	39 (85%)	0 (0%)	7 (15%)	0 (0%)	0 (0%)	46
48		<i>LacZ</i> mRNA 250pg	<i>xbra</i>	46 (88.5%)	0 (0%)	6 (11.5%)	0 (0%)	0 (0%)	52

Supplementary Table 1b

Histological analysis compared	Figures compared	Injections compared	Marker	Calculated p-value	Calculated p-value (with Bonferroni correction)	Standardized p-value (with Bonferroni correction)
1/21	1B	<i>enpp4/LacZ</i>	3G8	9.53E-09	1.19E-06	(0;0.001]
2/22	1B	<i>enpp4/LacZ</i>	4A6	1.57E-04	1.96E-02	(0.01;0.05)
11/21	1M	<i>Enpp4(mouse)/LacZ</i>	3G8	1.53E-14	1.91E-12	(0;0.001]
12/22	1M	<i>Enpp4(mouse)/LacZ</i>	4A6	3.15E-07	3.94E-05	(0;0.001]
5/1	1N/1C	<i>enpp4T72S/enpp4</i>	3G8	6.04E-09	7.55E-07	(0;0.001]
6/2	1N/1C	<i>enpp4T72S/enpp4</i>	4A6	2.10E-05	2.62E-03	(0.001;0.01)
7/1		<i>enpp4T72A/ enpp4</i>	3G8	5.89E-08	7.36E-06	(0;0.001]
8/2		<i>enpp4T72A/ enpp4</i>	4A6	7.79E-05	9.74E-03	(0.001;0.01)
9/1	1O/1C	<i>enpp4D36N/ enpp4</i>	3G8	3.00E-05	3.76E-03	(0.001;0.01)
10/2	1O/1C	<i>enpp4D36N/ enpp4</i>	4A6	4.83E-04	6.04E-02	NS
11/1		<i>enpp4D189N/ enpp4</i>	3G8	2.01E-05	2.51E-03	(0.001;0.01)
12/2		<i>enpp4D189N/ enpp4</i>	4A6	4.10E-05	5.13E-03	(0.001;0.01)
15/16	1P	<i>enpp4/LacZ</i>	<i>slc5a1.1</i>	6.36E-11	7.95E-09	(0;0.001]
17/18	1Q	<i>enpp4/LacZ</i>	<i>sclc12a1</i>	6.24E-11	7.81E-09	(0;0.001]
19/20	1R	<i>enpp4/LacZ</i>	<i>clcnkb</i>	1.06E-05	1.33E-03	(0.001;0.01)
21/22	1S	<i>enpp4/LacZ</i>	<i>gata3</i>	6.04E-06	7.55E-04	(0;0.001]
23/24	1T	<i>enpp4/LacZ</i>	<i>wt1</i>	3.89E-13	4.87E-11	(0;0.001]
25/26	1U	<i>enpp4/LacZ</i>	<i>nphs1</i>	9.66E-13	1.21E-10	(0;0.001]
27/28	1V	<i>enpp4/LacZ</i>	<i>lhx1 (st28)</i>	2.37E-15	2.96E-13	(0;0.001]
29/30	1W	<i>enpp4/LacZ</i>	<i>pax8 (st28)</i>	4.88E-20	6.10E-18	(0;0.001]
31/32	1X	<i>enpp4/LacZ</i>	<i>lhx1 (neurula)</i>	3.37E-01	1.00E+00	NS
33/34	1Y	<i>enpp4/LacZ</i>	<i>pax8 (neurula)</i>	3.13E-06	3.91E-04	(0;0.001]
43/41	S1A	<i>enpp4V1/enpp4V2</i>	3G8	6.57E-05	8.21E-03	(0.001;0.01)
44/42	S1A	<i>enpp4V1/enpp4V2</i>	4A6	5.79E-02	1.00E+00	NS
45/41	S1A	<i>enpp4D2/enpp4V2</i>	3G8	1.47E-04	1.84E-02	(0.01;0.05)
46/42	S1A	<i>enpp4D2/enpp4V2</i>	4A6	1.31E-01	1.00E+00	NS
47/41	S1A	<i>enpp4D1/enpp4V2</i>	3G8	2.31E-03	2.89E-01	NS
48/42	S1A	<i>enpp4D1/enpp4V2</i>	4A6	9.21E-02	1.00E+00	NS
35/36	S1C	<i>enpp4/LacZ</i>	<i>irx1</i>	1.00E-01	1.00E+00	NS
37/38	S1D	<i>enpp4/LacZ</i>	<i>myh4</i>	4.95E-01	1.00E+00	NS
39/40	S1E	<i>enpp4/LacZ</i>	<i>xbra</i>	7.67E-01	1.00E+00	NS

Supplementary Table 1. Scoring analysis of kidney phenotypes in embryos over-expressing *enpp4* (related to Fig.1 and Supplementary Fig. 1). (1a) Results from immunohistochemistry and *in situ* hybridization of injected embryos. Embryos were injected with *enpp4* wild type or mutant RNA. Embryos were scored for differences between the injected side (identified by Blue or Red Gal staining) and uninjected side acting as contralateral control side. The numbers of embryos displaying renal phenotype are indicated. The corresponding percentages are indicated in bracket. Each histological analysis was numbered as reference for the statistical comparisons in Supplementary Table 1B. Only the pronephros phenotype on the injected side is indicated. **(1b) Statistical analyses.** Statistical comparisons between pairs of histological analysis listed in Supplementary Table 1A were carried out as indicated in the table. The Bonferroni multiple testing correction was applied to all Fisher's Exact Test. The calculated p value before and after correction and a standardized corrected p-value are given. NS: not significant.

Supplementary Table 2a

Histological analysis	Figure	Injection	Marker analysed	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
1	2A. B	<i>enpp4</i> MO1 10ng	3G8	37 (35%)	0 (0%)	70 (65%)	0 (0%)	0 (0%)	107
2	2A. B	<i>enpp4</i> MO1 10ng	4A6	67 (63%)	0 (0%)	30 (28%)	10 (9%)	0 (0%)	107
3		cMO 10ng	3G8	27 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	27
4		cMO 10ng	4A6	27 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	27
5	2C	<i>enpp4</i> MO1 10ng+MO2 10ng	3G8	2 (10%)	0 (0%)	18 (90%)	0 (0%)	0 (0%)	20
6	2C	<i>enpp4</i> MO1 10ng+MO2 10ng	4A6	0 (0%)	0 (0%)	15 (75%)	5 (25%)	0 (0%)	20
7		<i>enpp4</i> MO1 10ng + cMO 10ng	3G8	5 (33%)	0 (0%)	10 (67%)	0 (0%)	0 (0%)	15
8		<i>enpp4</i> MO1 10ng + cMO 10ng	4A6	7 (47%)	0 (0%)	8 (53%)	0 (0%)	0 (0%)	15
9		<i>enpp4</i> MO2 10ng + cMO 10ng	3G8	9 (56%)	0 (0%)	7 (44%)	0 (0%)	0 (0%)	16
10		<i>enpp4</i> MO2 10ng + cMO 10ng	4A6	7 (44%)	0 (0%)	9 (56%)	0 (0%)	0 (0%)	16
11		cMO 20ng	3G8	10 (59%)	0 (0%)	7 (41%)	0 (0%)	0 (0%)	17
12		cMO 20ng	4A6	10 (53%)	0 (0%)	7 (47%)	0 (0%)	0 (0%)	17
13	2D	<i>enpp4</i> MO1 10ng	<i>slc5a1.1</i>	27 (42%)	0 (0%)	37 (58%)	0 (0%)	0 (0%)	64
14		cMO 10ng	<i>slc5a1.1</i>	34 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	34
15	2E	<i>enpp4</i> MO1 10ng	<i>slc12a1</i>	33 (44%)	0 (0%)	42 (56%)	0 (0%)	0 (0%)	75
16	2F	<i>enpp4</i> MO1 10ng + <i>enpp4</i> mRNA 2ng	<i>slc12a1</i>	47 (65%)	0 (0%)	17 (24%)	0 (0%)	8 (11%)	72
17		cMO 10ng	<i>slc12a1</i>	39 (83%)	0 (0%)	8 (17%)	0 (0%)	0 (0%)	47
18	2G	<i>enpp4</i> MO1 10ng	<i>clcnkb</i>	19(47.5%)	0 (0%)	21 (53.5%)	0 (0%)	0 (0%)	40
19		cMO 10ng	<i>clcnkb</i>	42 (76%)	0 (0%)	13 (24%)	0 (0%)	0 (0%)	55
20	2H	<i>enpp4</i> MO1 10ng	<i>gata3</i>	29 (69%)	0 (0%)	13 (31%)	0 (0%)	0 (0%)	42
21		cMO 10ng	<i>gata3</i>	33 (89%)	0 (0%)	4 (11%)	0 (0%)	0 (0%)	37
22	2I	<i>enpp4</i> MO1 10ng	<i>wt1</i>	32 (94%)	1 (3%)	1 (3%)	0 (0%)	0 (0%)	34
23		cMO 10ng	<i>wt1</i>	32 (94%)	0 (0%)	2 (6%)	0 (0%)	0 (0%)	34
24	2J	<i>enpp4</i> MO1 10ng	<i>nphs1</i>	34 (83%)	1 (2%)	6 (15%)	0 (0%)	0 (0%)	41
25		cMO 10ng	<i>nphs1</i>	45 (92%)	1 (2%)	3 (6%)	0 (0%)	0 (0%)	49
26	2K	<i>enpp4</i> MO1 10ng	<i>lhx1</i>	15 (65%)	0 (0%)	8 (35%)	0 (0%)	0 (0%)	23
27		cMO 10ng	<i>lhx1</i>	21 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	21
28	2L	<i>enpp4</i> MO1 10ng	<i>lhx1</i>	5 (25%)	0 (0%)	15 (75%)	0 (0%)	0 (0%)	20
29		cMO 10ng	<i>lhx1</i>	14 (70%)	0 (0%)	6 (30%)	0 (0%)	0 (0%)	20
30	2M	<i>enpp4</i> MO1 10ng + cMO 10ng	<i>lhx1</i>	14 (31%)	0 (0%)	17 (37%)	15 (31%)	0 (0%)	46
31	2N	<i>enpp4</i> MO1 10ng+MO2 10ng	<i>lhx1</i>	5 (16%)	2 (6%)	18 (58%)	6 (19%)	0 (0%)	31
32		cMO 20ng	<i>lhx1</i>	30 (77%)	4 (10%)	5 (13%)	0 (0%)	0 (0%)	39
33	2O	<i>enpp4</i> MO1 10ng + cMO 10ng	<i>pax8</i>	20 (29%)	1 (1%)	49 (70%)	0 (0%)	0 (0%)	70
34		cMO 20ng	<i>pax8</i>	17 (63%)	4 (15%)	6 (22%)	0 (0%)	0 (0%)	27
35	S2D	<i>enpp4</i> MO1 10ng	<i>myh4</i>	47 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	47
36		cMO 10ng	<i>myh4</i>	59 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	59
36		<i>enpp4</i> MO1 10ng	<i>myoD (Som)</i>	35 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	35
36		<i>enpp4</i> MO1 10ng	<i>myoD (HypM)</i>	14 (40%)	0 (0%)	21 (60%)	0 (0%)	0 (0%)	35
36		cMO 10ng	<i>myoD (Som)</i>	42 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	42
36		cMO 10ng	<i>myoD(HypM)</i>	39 (93%)	0 (0%)	3 (7%)	0 (0%)	0 (0%)	42

Histological analysis	Figure	Injection	Marker analysed	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
37	S2E	<i>enpp4</i> MO1 10ng	<i>xbra</i>	16 (84%)	0 (0%)	3 (16%)	0 (0%)	0 (0%)	19
38		cMO 10ng	<i>xbra</i>	20 (95%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)	21
39	S2F.G	<i>enpp4</i> MO2 10ng	3G8	44 (51%)	0 (0%)	43 (49%)	0 (0%)	0 (0%)	87
40	S2F.G	<i>enpp4</i> MO2 10ng	4A6	66 (76%)	0 (0%)	21 (24%)	0 (0%)	0 (0%)	87
41	S2H	<i>enpp4</i> MO2 10ng	<i>slc5a1.1</i>	26 (68%)	0 (0%)	12 (32%)	0 (0%)	0 (0%)	38
42	S2I	<i>enpp4</i> MO2 10ng <i>enpp4</i> MO2 10ng	<i>slc5a12a1</i>	24 (86%)	0 (0%)	4 (14%)	0 (0%)	0 (0%)	28
43	S2J	+ <i>enpp4</i> mRNA 2ng	<i>slc5a12a1</i>	14 (50%)	0 (0%)	0 (0%)	0 (0%)	14 (50%)	28
44	S2K	<i>enpp4</i> MO2 10ng	<i>lhx1</i>	11 (55%)	3 (15%)	6 (30%)	0 (0%)	0 (0%)	20
45	S2L	<i>enpp4</i> MO2 10ng <i>enpp4</i> MO2 10ng + cMO 10ng	<i>lhx1</i>	9 (45%)	0 (0%)	11 (55%)	0 (0%)	0 (0%)	20
46	S2M	<i>enpp4</i> MO2 10ng + cMO 10ng	<i>lhx1</i>	19 (31%)	3 (5%)	39 (64%)	0 (0%)	0 (0%)	61
47	S2N	<i>enpp4</i> MO2 10ng + cMO 10ng	<i>pax8</i>	4 (21%)	0 (0%)	11 (58%)	4 (21%)	0 (0%)	19
48	S3A	<i>enpp6</i> MO 20ng <i>enpp4</i> MO2 10ng + <i>enpp6</i> MO 20ng	3G8	25 (33%)	0 (0%)	49 (64%)	2 (3%)	0 (0%)	76
49		<i>enpp6</i> MO 20ng	4A6	28 (37%)	0 (0%)	47 (62%)	1 (1%)	0 (0%)	76
50	S3B	<i>enpp4</i> MO2 10ng + cMO 20ng	3G8	27 (40%)	0 (0%)	39 (62%)	2 (3%)	0 (0%)	68
51		<i>enpp4</i> MO2 10ng + cMO 20ng	4A6	22 (32%)	0 (0%)	42 (62%)	4 (6%)	0 (0%)	68
52	S3C	<i>enpp6</i> MO 20ng + cMO 10ng	3G8	31 (47%)	0 (0%)	34 (51%)	1 (2%)	0 (0%)	66
53		<i>enpp6</i> MO2 20ng + cMO 10ng	4A6	23 (35%)	0 (0%)	42 (63%)	1 (2%)	0 (0%)	66
54		cMO 30ng	3G8	44 (70%)	0 (0%)	19 (30%)	0 (0%)	0 (0%)	63
55		cMO 30ng	4A6	54 (86%)	0 (0%)	9 (14%)	0 (0%)	0 (0%)	63
56	S3D	<i>enpp6</i> mRNA 2ng + <i>enpp4</i> MO2 10ng	3G8	14 (20%)	0 (0%)	45 (63%)	12 (17%)	0 (0%)	71
57		<i>enpp6</i> mRNA 2ng + <i>enpp4</i> MO2 10ng	4A6	24 (34%)	0 (0%)	28 (39%)	19 (27%)	0 (0%)	71
58		<i>enpp4</i> MO2 10ng	3G8	23 (47%)	0 (0%)	25 (51%)	1 (2%)	0 (0%)	49
59		<i>enpp4</i> MO2 10ng <i>enpp6</i> mRNA 2ng + cMO 10ng	4A6	28 (57%)	0 (0%)	21 (43%)	0 (0%)	0 (0%)	49
60	S3E	<i>enpp6</i> mRNA 2ng + cMO 10ng	3G8	1 (3%)	0 (0%)	27 (79%)	6 (18%)	0 (0%)	34
61		<i>enpp6</i> mRNA 2ng + cMO 10ng	4A6	4 (12%)	0 (0%)	22 (65%)	8 (23%)	0 (0%)	34

Supplementary Table 2b

Histological analysis compared	Figures compared	Injections compared	Marker	Calculated p-value	Calculated p-value (with Bonferroni correction)	Standardized p-value (with Bonferroni correction)
1/3	2B	<i>enpp4</i> MO1/cMO	3G8	5.67E-11	7.09E-09	(0;0.001]
2/4	2B	<i>enpp4</i> MO1/ cMO	4A6	1.54E-04	1.92E-02	(0.01;0.05]
39/3	S2G	<i>enpp4</i> MO2/ cMO	3G8	3.98E-07	4.97E-05	(0;0.001]
40/4	S2G	<i>enpp4</i> MO2/ cMO	4A6	3.21E-03	4.01E-01	NS
5/7	2C/2B	<i>enpp4</i> MO1+2/MO1	3G8	1.12E-01	1.00E+00	NS
6/8	2C/2B	<i>enpp4</i> MO1+2/MO1	4A6	4.39E-04	5.48E-02	NS
5/9	2C/S2G	<i>enpp4</i> MO1+2/MO2	3G8	4.17E-03	5.21E-01	NS
6/10	2C/S2G	<i>enpp4</i> MO1+2/MO2	4A6	3.51E-04	4.39E-02	(0.01;0.05]
48/50	S3A/S3B	<i>enpp6</i> MO+ <i>enpp4</i> MO2/ <i>enpp4</i> MO2	3G8	7.29E-01	1.00E+00	NS
49/51	S3A/S3B	<i>enpp6</i> MO+ <i>enpp4</i> MO2/ <i>enpp4</i> MO2	4A6	3.21E-01	1.00E+00	NS
48/52	S3A/3C	<i>enpp6</i> MO+ <i>enpp4</i> MO2/ <i>enpp6</i> MO	3G8	2.46E-01	1.00E+00	NS
49/53	S3A/3C	<i>enpp6</i> MO+ <i>enpp4</i> MO2/ <i>enpp6</i> MO	4A6	9.30E-01	1.00E+00	NS
52/50	S3C	<i>enpp6</i> MO/ <i>enpp4</i> MO2	3G8	6.10E-01	1.00E+00	NS
53/51	S3C	<i>enpp6</i> MO/ <i>enpp4</i> MO2	4A6	5.13E-01	1.00E+00	NS
56/58	S3D/S3B	<i>enpp6</i> RNA+ <i>enpp4</i> MO2/ <i>enpp4</i> MO2	3G8	8.35E-04	1.04E-01	NS
57/59	S3D/S3B	<i>enpp6</i> RNA+ <i>enpp4</i> MO2/ <i>enpp4</i> MO2	4A6	3.32E-05	4.16E-03	(0.001;0.01]
56/60	S3D/S3E	<i>enpp6</i> RNA+ <i>enpp4</i> MO2/ <i>enpp6</i> RNA+cMO	3G8	5.58E-02	1.00E+00	NS
57/61	S3D/S3E	<i>enpp6</i> RNA+ <i>enpp4</i> MO2/ <i>enpp6</i> RNA+cMO	4A6	2.37E-02	1.00E+00	(NS
13/14	2D	<i>enpp4</i> MO1/cMO	<i>slc5a1.1</i>	1.26E-09	1.57E-07	(0;0.001]
41/14	S2H	<i>enpp4</i> MO2/cMO	<i>slc5a1.1</i>	2.12E-04	2.65E-02	(0.01;0.05]
15/17	2E	<i>enpp4</i> MO1/cMO	<i>slc12a1</i>	2.39E-05	2.98E-03	(0.001;0.01]
42/17	S2I	<i>enpp4</i> MO2/cMO	<i>slc12a1</i>	1.00E+00	1.00E+00	NS
16/15	2F/2E	<i>enpp4</i> MO1+RNA/ <i>enpp4</i> MO1	<i>slc12a1</i>	9.47E-06	1.18E-03	(0.001;0.01]
43/42	S2J/S2H	<i>enpp4</i> MO2+RNA/ <i>enpp4</i> MO2	<i>slc12a1</i>	3.91E-06	4.89E-04	(0;0.001]
18/19	2G	<i>enpp4</i> MO1/cMO	<i>clcnkb</i>	4.96E-03	6.19E-01	NS
20/21	2H	<i>enpp4</i> MO1/cMO	<i>gata3</i>	5.28E-02	1.00E+00	NS
22/23	2I	<i>enpp4</i> MO1/cMO	<i>wt1</i>	1.00E+00	1.00E+00	NS
24/25	2J	<i>enpp4</i> MO1/cMO	<i>nphs1</i>	4.36E-01	1.00E+00	NS
26/27	2K	<i>enpp4</i> MO1/cMO	<i>lhx1</i> (st28)	3.91E-03	4.89E-01	NS
44/27	S2K	<i>enpp4</i> MO2/cMO	<i>lhx1</i> (st28)	4.79E-04	5.99E-02	NS
28/29	2L	<i>enpp4</i> MO1/cMO	<i>lhx1</i> (st22)	1.04E-02	1.00E+00	(NS
45/29	S2L	<i>enpp4</i> MO2/cMO	<i>lhx1</i> (st22)	2.00E-01	1.00E+00	NS
30/32	2M	<i>enpp4</i> MO1/cMO	<i>lhx1</i> (neurula)	5.95E-08	7.44E-06	(0;0.001]
46/32	S2M	<i>enpp4</i> MO2/cMO	<i>lhx1</i> (neurula)	6.92E-07	8.65E-05	(0;0.001]
31/30	2N/2M	<i>enpp4</i> MO1+2/MO1	<i>lhx1</i> (neurula)	5.47E-02	1.00E+00	NS
31/46	2N/S2M	<i>enpp4</i> MO1+2/MO2	<i>lhx1</i> (neurula)	2.40E-03	3.00E-01	NS
33/34	2O	<i>enpp4</i> MO1/cMO	<i>pax8</i> (neurula)	2.23E-05	2.79E-03	(0.001;0.01]
47/34	S2N	<i>enpp4</i> MO2/cMO	<i>pax8</i> (neurula)	3.31E-04	4.14E-02	(0.01;0.05]
35/36	S2D	<i>enpp4</i> MO1/cMO	<i>myh4</i>	1.00E+00	1.00E+00	NS
37/38	S2E	<i>enpp4</i> MO1/cMO	<i>xbra</i>	3.31E-01	1.00E+00	NS

Supplementary Table 2. Scoring analysis of kidney phenotypes in *enpp4* morphants (related to Fig.2, supplementary Fig. 2 and 3). (2a) Results from immunohistochemistry and in situ hybridization of injected embryos. Embryos were injected with *enpp4* MO1 or MO2 alone or in combination. Embryos were scored for differences between the injected side (identified by Blue or Red Gal staining) and uninjected side acting as contralateral control side. The numbers of embryos displaying renal phenotype are indicated. The corresponding percentages are indicated in bracket. Each histological analysis was numbered as reference for the statistical comparisons in Supplementary Table 2B. Only the pronephros phenotype on the injected side is indicated. (Som: somites; HypM: hypaxial muscles). **(2b) Statistical analyses.** Statistical comparisons between pairs of histological analysis listed in Supplementary Table 2A were carried out as indicated in the table. The Bonferroni multiple testing correction was applied to all Fisher's Exact Test. The calculated p value before and after correction and a standardized corrected p-value are given. NS: not significant.

Supplementary Table 3a

Histological Analysis	Figure	Injection	Marker stained	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
1	3A	<i>enpp4</i> mRNA 2ng	<i>raldh1a2</i>	12 (32%)	8 (22%)	4 (11%)	0 (0%)	13 (35%)	37
2	3B	<i>enpp4</i> mRNA 2ng	<i>rdh10</i>	18 (53%)	12 (35%)	0 (0%)	0 (0%)	4 (12%)	34
3	3C	<i>enpp4</i> mRNA 2ng	<i>cyp26a1</i>	43 (98%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	44
4	3D	<i>enpp4</i> mRNA 2ng	<i>notch1</i>	17 (23%)	40 (53%)	13 (17%)	0 (0%)	5 (7%)	75
5	3E	<i>enpp4</i> mRNA 2ng	<i>dll1</i>	19 (23%)	19 (23%)	7 (9%)	3 (4%)	32 (40%)	81
6	3F	<i>enpp4</i> mRNA 2ng	<i>jag1</i>	21 (26%)	35 (44%)	8 (10%)	0 (0%)	16 (20%)	81
7	3G	<i>enpp4</i> mRNA 2ng	<i>wnt4</i>	10 (24%)	13 (32%)	11 (27%)	0 (0%)	7 (17%)	41
8	3H	<i>enpp4</i> MO1 10ng	<i>raldh1a2</i>	25 (75%)	0 (0%)	8 (25%)	0 (0%)	0 (0%)	33
9		cMO 10ng	<i>raldh1a2</i>	34 (97%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	35
10	3I	<i>enpp4</i> MO1 10ng	<i>rdh10</i>	26 (79%)	0 (0%)	7 (21%)	0 (0%)	0 (0%)	33
11		cMO 10ng	<i>rdh10</i>	47(100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	47
12	3J	<i>enpp4</i> MO1 10ng	<i>cyp26a1</i>	37 (88%)	0 (0%)	5 (12%)	0 (0%)	0 (0%)	42
13		cMO 10ng	<i>cyp26a1</i>	43 (93%)	0 (0%)	3 (7%)	0 (0%)	0 (0%)	46
14	3K	<i>enpp4</i> MO1 10ng	<i>notch1</i>	33 (80%)	4 (10%)	4 (10%)	0 (0%)	0 (0%)	41
15		cMO 10ng	<i>notch1</i>	39 (97%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	40
16	3L	<i>enpp4</i> MO1 10ng	<i>dll1</i>	31 (67%)	0 (0%)	15 (33%)	0 (0%)	0 (0%)	46
17		cMO 10ng	<i>dll1</i>	34 (85%)	1 (3%)	5 (12%)	0 (0%)	0 (0%)	40
18	3M	<i>enpp4</i> MO1 10ng	<i>jag1</i>	26 (62%)	0 (0%)	16 (38%)	0 (0%)	0 (0%)	42
19		cMO 10ng	<i>jag1</i>	39 (95%)	0 (0%)	2 (5%)	0 (0%)	0 (0%)	41
20	3N	<i>enpp4</i> MO1 10ng	<i>wnt4</i>	4 (10%)	0 (0%)	32 (82%)	3 (8%)	0 (0%)	39
21		cMO 10ng	<i>wnt4</i>	28 (85%)	0 (0%)	5 (15%)	0 (0%)	0 (0%)	33
22	S4A	<i>enpp4</i> mRNA 2ng + <i>rfng</i> MO 20ng	3G8	9 (24%)	1 (3%)	12 (32%)	0 (0%)	15 (41%)	37
23		<i>enpp4</i> mRNA 2ng + <i>rfng</i> MO 20ng	4A6	20 (54%)	0 (0%)	12 (32%)	0 (0%)	5 (14%)	37
24	S4B	<i>enpp4</i> mRNA 2ng + cMO 20ng	3G8	15 (48%)	2 (6%)	7 (23%)	0 (0%)	7 (23%)	31
25		<i>enpp4</i> mRNA 2ng + cMO 20ng	4A6	21 (68%)	0 (0%)	7 (23%)	0 (0%)	3 (9%)	31
26	S4C.D	<i>rfng</i> mRNA 2ng + <i>enpp4</i> MO2 10ng	3G8	3 (20%)	0 (0%)	6 (40%)	2(13%)	4 (27%)	15
27		<i>rfng</i> mRNA 2ng + <i>enpp4</i> MO2 10ng	4A6	4 (27%)	0 (0%)	8 (53%)	1 (7%)	2 (13%)	15
28	S4E	<i>rfng</i> mRNA 2ng + cMO 10ng	3G8	8 (38%)	0 (0%)	4 (19%)	1 (5%)	8 (38%)	21
29		<i>rfng</i> mRNA 2ng + cMO 10ng	4A6	9 (43%)	0 (0%)	8 (38%)	0 (0%)	4 (19%)	21
30		<i>enpp4</i> MO2 10ng	3G8	5 (28%)	0 (0%)	13 (72%)	0 (0%)	0 (0%)	18
31		<i>enpp4</i> MO2 10ng	4A6	6 (33%)	0 (0%)	12 (67%)	0 (0%)	0 (0%)	18
32		<i>rfng</i> MO 20ng	3G8	10 (53%)	0 (0%)	9 (48%)	0 (0%)	0 (0%)	19
33		<i>rfng</i> MO 20ng	4A6	16 (84%)	0 (0%)	3 (16%)	0 (0%)	0 (0%)	19
34		cMO 20ng	3G8	24 (78%)	0 (0%)	7 (22%)	0 (0%)	0 (0%)	31
35		cMO 20ng	4A6	24 (78%)	0 (0%)	7 (22%)	0 (0%)	0 (0%)	31

Supplementary Table 3b

Histological analysis compared	Figures compared	Injections compared	marker	Calculated p-value	Calculated p-value (with Bonferroni correction)	Standardized p-value (with Bonferroni correction)
1/9	3A	<i>enpp4/cMO+LacZ</i>	<i>raldh1a2</i>	1.04E-09	1.30E-07	(0;0.001]
2/11	3B	<i>enpp4/cMO+LacZ</i>	<i>rdh10</i>	6.56E-08	8.20E-06	(0;0.001]
3/13	3C	<i>enpp4/cMO+LacZ</i>	<i>cyp26a1</i>	2.42E-01	1.00E+00	NS
4/15	3D	<i>enpp4/cMO+LacZ</i>	<i>notch1</i>	7.17E-15	8.96E-13	(0;0.001]
5/17	3E	<i>enpp4/cMO+LacZ</i>	<i>dll1</i>	7.66E-12	9.57E-10	(0;0.001]
6/19	3F	<i>enpp4/cMO+LacZ</i>	<i>jag1</i>	5.93E-14	7.41E-12	(0;0.001]
7/21	3G	<i>enpp4/cMO+LacZ</i>	<i>wnt4</i>	8.93E-08	1.12E-05	(0;0.001]
8/9	3H	<i>enpp4MO/cMO</i>	<i>raldh1a2</i>	1.21E-02	1.00E+00	NS
10/11	3I	<i>enpp4 MO/cMO</i>	<i>rdh10</i>	1.34E-03	1.68E-01	NS
12/13	3J	<i>enpp4 MO/cMO</i>	<i>cyp26a1</i>	4.71E-01	1.00E+00	NS
14/15	3K	<i>enpp4 MO/cMO</i>	<i>notch1</i>	4.88E-02	1.00E+00	NS
16/17	3L	<i>enpp4 MO/cMO</i>	<i>dll1</i>	4.00E-02	1.00E+00	NS
18/19	3M	<i>enpp4 MO/cMO</i>	<i>jag1</i>	3.25E-04	4.06E-02	(0.01;0.05]
20/21	3N	<i>enpp4 MO/cMO</i>	<i>wnt4</i>	1.29E-10	1.61E-08	(0;0.001]
22/24	S4A/S4B	<i>rfgMO+enpp4RNA/cMO+ enpp4 RNA</i>	3G8	1.16E-01	1.00E+00	NS
23/25	S4A/S4B	<i>rfgMO+enpp4RNA/cMO+ enpp4 RNA</i>	4A6	5.61E-01	1.00E+00	NS
26/28	S4C.D/S4E	<i>enpp4MO2+rfgRNA/cMO+rfg RNA</i>	3G8	3.52E-01	1.00E+00	NS
27/29	S4C.D/S4E	<i>enpp4MO2+rfgRNA/cMO+rfg RNA</i>	4A6	5.05E-01	1.00E+00	NS

Supplementary Table 3. Scoring analysis of *enpp4* mis-expression on RA. Notch and Wnt signalling pathways (related to Fig. 3 and Supplementary Fig. 4). (3a) Results from immunohistochemistry and *in situ* hybridization of *enpp4* mRNAs or MOs injected embryos. Embryos were injected with *enpp4* RNA or MO alone or in combination with *rfg* RNA or MO. Embryos were scored for differences between the injected side (identified by Blue or Red Gal staining) and uninjected side acting as contra-lateral control side. The numbers of embryos displaying renal phenotype are indicated. The corresponding percentages are indicated in bracket. Each histological analysis was numbered as reference for the statistical comparisons in Supplementary Table 3B. Only the pronephros phenotype on the injected side is indicated. **(3b) Statistical analyses.** Statistical comparisons between pairs of histological analysis listed in Supplementary Table 3A were carried out as indicated in the table. The Bonferroni multiple testing correction was applied to all Fisher's Exact Test. The calculated p value before and after correction and a standardized corrected p-value are given. NS: not significant

Supplementary Table 4a

Accession number	Full Protein name	Mw
Q9CQN1	Heat shock protein 75 kDa. mitochondrial OS= <i>M.musculus</i> GN= Trap1 PE=1 SV=1	80158.65
Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1 OS= <i>M.musculus</i> GN= Slc25a12 PE=1 SV=1	74522.94
P38647	Stress-70 protein. mitochondrial OS= <i>M.musculus</i> GN= Hspa9 PE=1 SV=2	73482.8
Q62167	ATP-dependent RNA helicase DDX3X OS= <i>M.musculus</i> GN= Ddx3x PE=1 SV=3	73056.16
P63017	Heat shock cognate 71 kDa protein OS= <i>M.musculus</i> GN= Hspa8 PE= 1 SV=1	70827.34
P29341	Polyadenylate-binding protein OS= <i>M.musculus</i> GN= Pabpc1 PE=1 SV=1	70598
Q7TMK9	Heterogeneous nuclear ribonucleoprotein OS= <i>M.musculus</i> GN= Syncrip PE=1 SV=2	69589.6
Q8BGD9	Eukaryotic translation initiation factor 4B OS= <i>M.musculus</i> GN= Eif4b PE=1 SV=1	68799.19
Q91YQ5	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit OS= <i>M.musculus</i> GN= Rpn1 PE=2 SV=1	68485.86
P61222	ATP-binding cassette sub-family E member OS= <i>M.musculus</i> GN= Abce1 PE=2 SV=1	67271.14
Q9Z0X1	Apoptosis-inducing factor 1 OS= <i>M.musculus</i> GN= Aifm1 PE= 1 SV=1	66723.96
O70194	Eukaryotic translation initiation factor 3 subunit D OS= <i>M.musculus</i> GN= Eif3d PE= 1 SV=2	63948.5
Q9Z247	Peptidyl-prolyl cis-trans isomerase FKBP9 OS= <i>M.musculus</i> GN= Fkbp9 PE=1 SV=1	62955.57
Q7TMK9-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein Q OS= <i>M.musculus</i> GN= Syncrip	62633.38
P14685	26S proteasome non-ATPase regulatory subunit 3 OS= <i>M.musculus</i> GN= Psm3 PE= 2 SV=2	60661.43
P80317	T-complex protein 1 subunit zeta OS= <i>M.musculus</i> GN= Cct6a PE=1 SV=3	57967.8
Q6AX80	Ectonucleotide pyrophosphatase/phosphodiesterase family member 4 OS= <i>X.laevis</i> GN= enpp4 PE= 2 SV=1	51278.25
P61979	Isoform 3 of Heterogeneous nuclear ribonucleoprotein K OS= <i>M.musculus</i> GN= Hnrnpk PE= 1 SV=1	50944.43
Q99LC5	Electron transfer flavoprotein subunit alpha. mitochondrial OS= <i>M.musculus</i> GN= EtfA PE=1 SV=2	34987.51

Supplementary Table 4b

Accession number	Full Protein name	Mw
Q9CQN1	Heat shock protein 75 kDa. mitochondrial OS= <i>M.musculus</i> GN= Trap1 PE=1 SV=1	80158.65
Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1 OS= <i>M.musculus</i> GN= Slc25a12 PE=1 SV=1	74522.94
P38647	Stress-70 protein. mitochondrial OS= <i>M.musculus</i> GN= Hspa9 PE=1 SV=2	73484.8
Q62167	ATP-dependent RNA helicase DDX3X OS= <i>M.musculus</i> GN= Ddx3x PE=1 SV=3	73056.16
Q8K297	Procollagen galactosyltransferase 1 OS= <i>M.musculus</i> GN= Glt25d1 PE=1 SV=3	71015.15
P63017	Heat shock cognate 71 kDa protein OS= <i>M.musculus</i> GN= Hspa8 PE=1 SV=2	70827.34
Q99K51	Plastin-3 OS= <i>M.musculus</i> GN= Pls3 PE=1 SV=3	70697.33
P29341	Polyadenylate-binding protein OS= <i>M.musculus</i> GN= Pabpc1 PE=1 SV=1	70598
Q7TMK9	Heterogeneous nuclear ribonucleoprotein OS= <i>M.musculus</i> GN= Syncrip PE=1 SV=2	69589.6
Q91YQ5	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit OS= <i>M.musculus</i> GN= Rpn1 PE=2 SV=1	68485.86
P61222	ATP-binding cassette sub-family E member OS= <i>M.musculus</i> GN= Abce1 PE=2 SV=1	67271.14
Q9Z0X1	Apoptosis-inducing factor 1 OS= <i>M.musculus</i> GN= Aifm1 PE= 1 SV=1	66723.96
O70194	Eukaryotic translation initiation factor 3 subunit D OS= <i>M.musculus</i> GN= Eif3d PE= 1 SV=2	63948.5
Q9Z247	Peptidyl-prolyl cis-trans isomerase FKBP9 OS= <i>M.musculus</i> GN= Fkbp9 PE=1 SV=1	62955.57
Q7TMK9-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein Q OS= <i>M.musculus</i> GN= Syncrip	62633.38
P14685	26S proteasome non-ATPase regulatory subunit 3 OS= <i>M.musculus</i> GN= Psm3 PE= 2 SV=2	60661.43
P80317	T-complex protein 1 subunit zeta OS= <i>M.musculus</i> GN= Cct6a PE=1 SV=3	57967.8
P27773	Protein disulfide-isomerase A3 OS= <i>M.musculus</i> GN= Pdia3 PE=1 SV=2	56642.78
O70475	UDP-glucose 6-dehydrogenase OS= <i>M.musculus</i> GN= Ugdh PE=1 SV=1	54797.35
Q8R180	ERO1-like protein alpha OS= <i>M.musculus</i> GN= Ero1l PE=1 SV=2	54050.26
P97855	Ras GTPase-activating protein-binding protein 1 member 4 OS= <i>M.musculus</i> GN= G3bp1 PE=1 SV=1	51796.96
P61979	Isoform 3 of Heterogeneous nuclear ribonucleoprotein K OS= <i>M.musculus</i> GN= Hnrnpk PE= 1 SV=1	50944.43
Q99LC5	Electron transfer flavoprotein subunit alpha. mitochondrial OS= <i>M.musculus</i> GN= EtfA PE=1 SV=2	34987.51

Supplementary Table 4. Mass spectrometry analysis of membrane fractions (related to Fig. 4). Proteins of over-expressing *X.laevis* enpp4 protein (a) or control (b) CHO cells membrane fraction were extracted and separated on a polyacrylamide gel. The band labelled by the Xlenpp4 antibody from the transfected Xlenpp4-pCDNA3 lane and the molecular weight equivalent band from the control lane were cut and analysed by mass spectrometry.

Supplementary Table 5a

Histologic analysis	Figure	Injection	Marker stained	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
1	6A.B	<i>s1pr5</i> mRNA 2ng + <i>enpp4</i> mRNA 1ng	3G8	29 (33%)	2 (2%)	23 (26%)	1 (1%)	34 (38%)	89
2	6A.B	<i>s1pr5</i> mRNA 2ng + <i>enpp4</i> mRNA 1ng	4A6	39 (44%)	11 (12%)	30 (34%)	1 (1%)	8 (9%)	89
3	6C	<i>s1pr5</i> mRNA 2ng	3G8	38 (73%)	1 (2%)	12 (23%)	1 (2%)	0 (0%)	52
4	6C	<i>s1pr5</i> mRNA 2ng	4A6	42 (81%)	2 (4%)	7 (13.5%)	1 (2%)	0 (0%)	52
5	6D	<i>enpp4</i> mRNA 1ng	3G8	64 (68%)	9 (10%)	14 (15%)	0 (0%)	7 (7%)	94
6	6D	<i>enpp4</i> mRNA 1ng <i>s1pr1</i> mRNA 2ng	4A6	68 (72%)	7 (7%)	14 (15%)	0 (0%)	5 (5%)	94
7	S5A	+ <i>enpp4</i> mRNA 1ng <i>s1pr1</i> mRNA 2ng	3G8	57 (64%)	12 (13.5%)	13 (15%)	0 (0%)	7 (8%)	89
8	S5A	+ <i>enpp4</i> mRNA 1ng	4A6	56 (63%)	12 (13.5%)	20 (22.5%)	0 (0%)	1 (1%)	89
9	S5B	<i>s1pr1</i> mRNA 2ng	3G8	52 (90%)	3 (5%)	2 (3%)	1 (2%)	0 (0%)	58
10	S5B	<i>s1pr1</i> mRNA 2ng <i>lpar1.1</i> mRNA 2ng	4A6	53 (91%)	0 (0%)	5 (9%)	0 (0%)	0 (0%)	58
11	S5C	+ <i>enpp4</i> mRNA 1ng <i>lpar1.1</i> mRNA 2ng	3G8	69 (65%)	15 (14%)	7 (7%)	2 (2%)	13(12%)	106
12	S5C	+ <i>enpp4</i> mRNA 1ng	4A6	81 (76%)	12 (11%)	7 (7%)	2 (2%)	4 (4%)	106
13	S5D	<i>lpar1.1</i> mRNA 2ng	3G8	53 (96%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	55
14	S5D	<i>lpar1.1</i> mRNA 2ng <i>p2yr10</i> mRNA 2ng	4A6	53 (96%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	55
15	S5E	+ <i>enpp4</i> mRNA 1ng <i>p2yr10</i> mRNA 2ng	3G8	40 (78%)	6 (12%)	2 (4%)	1 (2%)	2 (4%)	51
16	S5E	+ <i>enpp4</i> mRNA 1ng	4A6	45 (88%)	3 (6%)	1 (2%)	2 (4%)	0 (0%)	51
17	S5F	<i>p2yr10</i> mRNA 2ng	3G8	49 (91%)	2 (4%)	3 (5%)	0 (0%)	0 (0%)	54
18	S5F	<i>p2yr10</i> mRNA 2ng	4A6	53 (98%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	54
19		<i>LacZ</i> mRNA 250pg	3G8	54 (93%)	0 (0%)	4 (7%)	0 (0%)	0 (0%)	58
20		<i>LacZ</i> mRNA 250pg	4A6	55 (95%)	0 (0%)	3 (5%)	0 (0%)	0 (0%)	58
21	6E.F	<i>s1pr5.L</i> MO 15ng	3G8	24 (56%)	2 (5%)	16 (37%)	0 (0%)	1 (2%)	43
22	6E.F	<i>s1pr5.L</i> MO 15ng	4A6	17(39.5%)	0 (0%)	26(60.5%)	0 (0%)	0 (0%)	43
23		cMO 15ng	3G8	55 (90%)	0 (0%)	6 (10%)	0 (0%)	0 (0%)	61
24		cMO 15ng	4A6	53 (87%)	0 (0%)	8 (13%)	0 (0%)	0 (0%)	61
25	6G.H	<i>s1pr5.L</i> MO 7.5ng + <i>enpp4</i> MO 5ng	3G8	11 (26%)	0 (0%)	31 (74%)	0 (0%)	0 (0%)	42
26	6G.H	<i>s1pr5.L</i> MO 7.5ng + <i>enpp4</i> MO 5ng	4A6	8 (19%)	0 (0%)	34 (81%)	0 (0%)	0 (0%)	42
27	6I	<i>s1pr5.L</i> MO 7.5ng + Control MO 5ng	3G8	40 (78%)	0 (0%)	11 (22%)	0 (0%)	0 (0%)	51
28	6I	<i>s1pr5.L</i> MO 7.5ng + Control MO 5ng	4A6	36 (71%)	0 (0%)	15 (29%)	0 (0%)	0 (0%)	51
29	6J	+ <i>enpp4</i> MO 5ng Control MO 7.5ng	3G8	14 (35%)	0 (0%)	26 (65%)	0 (0%)	0 (0%)	40
30	6J	+ <i>enpp4</i> MO 5ng	4A6	16 (40%)	0 (0%)	24 (60%)	0 (0%)	0 (0%)	40
31		cMO 12.5ng	3G8	41(93.5%)	2 (4.5%)	1 (2%)	0 (0%)	0 (0%)	44
32		cMO 12.5ng <i>enpp4</i> 2ng mRNA	4A6	42 (96%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	44
33	6K	+ <i>s1pr5.L</i> MO 15ng <i>enpp4</i> 2ng mRNA	3G8	18(47.5%)	2 (7.5%)	13(42.5%)	0 (0%)	7(17.5%)	40
34		+ <i>s1pr5.L</i> MO 15ng	4A6	22 (55%)	6 (15%)	12 (30%)	0 (0%)	0 (0%)	40
35		<i>s1pr5.L</i> MO 15ng	3G8	25 (71%)	2 (6%)	6 (26%)	0 (0%)	0 (0%)	35
36		<i>s1pr5.L</i> MO 15ng <i>enpp4</i> 2ng mRNA	4A6	19 (57%)	1 (3%)	15 (45%)	0 (0%)	0 (0%)	35
37	6L	+ cMO 15ng <i>enpp4</i> 2ng mRNA	3G8	13 (50%)	6 (24%)	7 (25%)	0 (0%)	24 (48%)	50
38		+ cMO 15ng	4A6	26 (64%)	7 (14%)	12 (24%)	0 (0%)	5 (10%)	50
39		cMO 15ng	3G8	22 (76%)	1 (3%)	6 (21%)	0 (0%)	0 (0%)	29

Histological analysis	Figure	Injection	Marker stained	Phenotypes					Total number of scored embryos
				Normal	Enlarged	Reduced	Absent	Ectopic	
40		<i>cMO 15ng</i>	4A6	26 (90%)	1 (3%)	2 (7%)	0 (0%)	0 (0%)	29
41	S6E	<i>s1pr5.S MO 15ng</i>	3G8	25 (38%)	0 (0%)	34 (51%)	7 (11%)	0 (0%)	66
42		<i>s1pr5.S MO 15ng</i>	4A6	26 (39%)	0 (0%)	19 (29%)	21(32%)	0 (0%)	66
43		<i>s1pr5.L MO 15ng</i>	3G8	12 (21%)	5 (9%)	28 (49%)	12(21%)	0 (0%)	57
44		<i>s1pr5.L MO 15ng</i>	4A6	22 (39%)	0 (0%)	9 (16%)	26(45%)	0 (0%)	57
45		<i>cMO 15ng</i>	3G8	48 (72%)	2 (3%)	14 (21%)	3 (4%)	0 (0%)	67
46		<i>cMO 15ng</i>	4A6	45 (67%)	1 (1%)	12 (18%)	9 (14%)	0 (0%)	67

Supplementary Table 5b

Histological analysis compared	Figure compared	Injection compared	Marker	Calculated p-value	Calculated p-value (with Bonferroni correction)	Standardized p-value (with Bonferroni correction)
1/5	6A,B/6D	<i>s1pr5 + enpp4/enpp4</i>	3G8	1.08E-08	1.35E-06	(0;0.001]
2/6	6A,B/6D	<i>s1pr5 + enpp4/enpp4</i>	4A6	1.50E-03	1.87E-01	NS
1/3	6A,B/6C	<i>s1pr5 + enpp4/s1pr5</i>	3G8	8.80E-09	1.10E-06	(0;0.001]
2/4	6A,B/6C	<i>s1pr5.a + enpp4/s1pr5</i>	4A6	1.15E-04	1.44E-02	(0.01;0.05]
3/19	6C	<i>s1pr5/ LacZ</i>	3G8	7.66E-03	9.58E-01	NS
4/20	6C	<i>s1pr5/ LacZ</i>	4A6	7.89E-02	1.00E+00	NS
7/5	S5A/6D	<i>s1pr1 + enpp4/enpp4</i>	3G8	8.64E-01	1.00E+00	NS
8/6	S5A/6D	<i>s1pr1 + enpp4/enpp4</i>	4A6	1.17E-01	1.00E+00	NS
7/9	S5A/S5B	<i>s1pr1 + enpp4/s1pr1</i>	3G8	1.32E-03	1.65E-01	NS
8/10	S5A/S5B	<i>s1pr1 + enpp4/s1pr1</i>	4A6	1.62E-04	2.03E-02	(0.01;0.05]
9/19	S5B	<i>s1pr1/ LacZ</i>	3G8	1.55E-01	1.00E+00	NS
10/20	S5B	<i>s1pr1/LacZ</i>	4A6	7.17E-01	1.00E+00	NS
11/5	S5C/6D	<i>lpar1.1 + enpp4/enpp4</i>	3G8	1.32E-01	1.00E+00	NS
12/6	S5C/6D	<i>lpar1.1 + enpp4/enpp4</i>	4A6	1.89E-01	1.00E+00	NS
11/13	S5C/S6D	<i>lpar1.1 + enpp4/ lpar1.1</i>	3G8	2.77E-05	3.47E-03	(0.001;0.01]
12/14	S5C/S6D	<i>lpar1.1 + enpp4/ lpar1.1</i>	4A6	8.39E-03	1.00E+00	NS
13/19	S5D	<i>lpar1.1/LacZ</i>	3G8	6.80E-01	1.00E+00	NS
14/20	S5D	<i>lpar1.1/LacZ</i>	4A6	1.00E+00	1.00E+00	NS
15/5	S5E/6D	<i>p2y10 + enpp4/enpp4</i>	3G8	1.28E-01	1.00E+00	NS
16/6	S5E/6D	<i>p2y10 + enpp4/enpp4</i>	4A6	6.73E-03	8.42E-01	NS
15/17	S5E/S5F	<i>p2y10 + enpp4/p2y10</i>	3G8	1.66E-01	1.00E+00	NS
16/18	S5E/S5F	<i>p2y10 + enpp4/p2y10</i>	4A6	8.43E-02	1.00E+00	NS
17/19	S5F	<i>p2y10/ LacZ</i>	3G8	4.05E-01	1.00E+00	NS
18/20	S5F	<i>p2y10/ LacZ</i>	4A6	6.19E-01	1.00E+00	NS
21/23	6E.F	<i>s1pr5.LMO/cMO</i>	3G8	1.22E-04	1.52E-02	(0.01;0.05]
22/24	6E.F	<i>s1pr5.LMO/ cMO</i>	4A6	5.39E-07	6.74E-05	(0;0.001]
41/45	S6E	<i>s1pr5.SMO/ cMO</i>	3G8	8.61E-05	1.08E-02	(0.01;0.05]
42/46	S6E	<i>s1pr5.SMO/ cMO</i>	4A6	3.22E-03	4.02E-01	NS
41/43	S6E/6F	<i>s1pr5.SMO/ s1pr5.LMO</i>	3G8	1.08E-02	1.00E+00	NS
42/44	S6E/6F	<i>s1pr5.SMO/ s1pr5.LMO</i>	4A6	1.57E-01	1.00E+00	NS
25/27	6G.H/6I	<i>s1pr5.LMO + enpp4MO/ s1pr5.L MO + cMO</i>	3G8	8.21E-07	1.03E-04	(0;0.001]
26/28	6G.H/6I	<i>s1pr5.LMO + enpp4MO/ s1pr5.L MO + cMO</i>	4A6	9.37E-07	1.17E-04	(0;0.001]
25/29	6G.H/6J	<i>s1pr5.LMO + enpp4MO/enpp4 MO + cMO</i>	3G8	4.74E-01	1.00E+00	NS
26/30	6G.H/6J	<i>s1pr5.LMO + enpp4MO/enpp4 MO + cMO</i>	4A6	5.21E-02	1.00E+00	NS
33/37	6K/6L	<i>enpp4RNA + s1pr5.LMO /enpp4RNA + cMO</i>	3G8	4.18E-03	5.23E-01	NS
34/38	6K/6L	<i>enpp4RNA + s1pr5.LMO /enpp4RNA + cMO</i>	4A6	2.37E-01	1.00E+00	NS

Supplementary Table 5. Scoring analyses of enpp4 and lipidic receptors mis-expression (related to Fig. 6. And Supplementary Fig. 6). (a) Results from immunohistochemistry of enpp4 and lipidic receptors mRNA and MO injected embryos. Embryos were either injected with *s1pr1*, *s1pr5*, *lpar1.1*, *p2y10* and *enpp4* mRNA alone or in combination or with *s1pr5.L* *s1pr5.s* and *enpp4* MO alone or in combination. Embryos were scored for differences between the injected side (identified by Blue Gal staining) and uninjected side acting as contra-lateral control side. The numbers of embryos displaying renal phenotype are indicated. The corresponding percentages

are indicated in bracket. Each histological analysis was numbered as reference for the statistical comparisons in Supplementary Table 5b. Only the pronephros phenotype on the injected side is indicated. **(b) Statistical analyses.** Statistical comparisons between pairs of histological analysis listed in Supplementary Table 5A were carried out as indicated in the table. The Bonferroni multiple testing correction was applied to all Fisher's Exact Test. The calculated p value before and after correction and a standardized corrected p-value are given. NS: not significant.