

SUPPLEMENTARY MATERIAL

corresponding to:

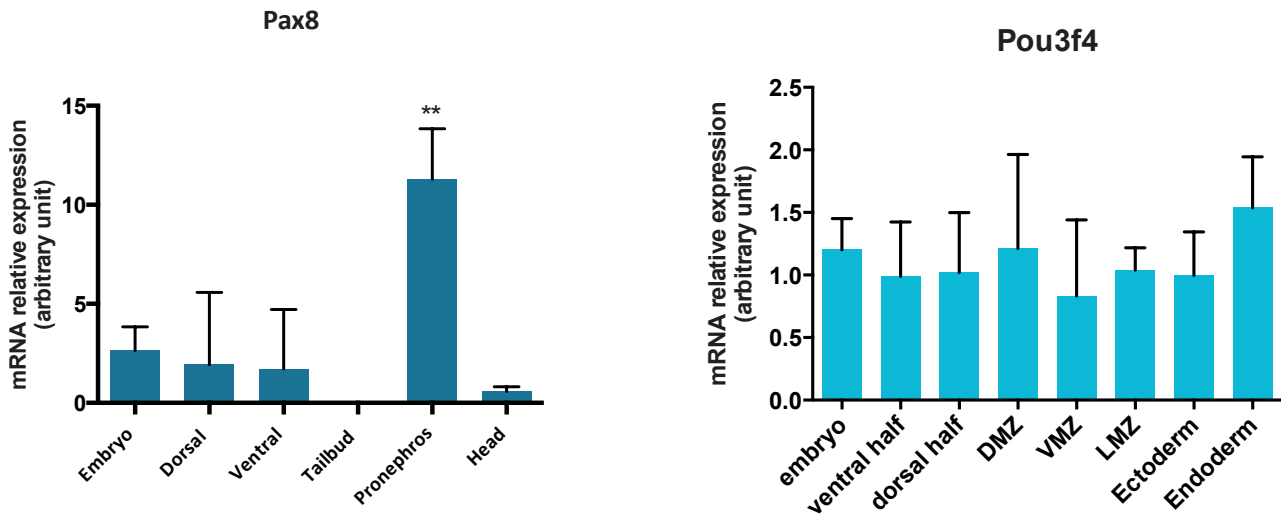
***Pou3f* transcription factor expression during embryonic development highlights distinct *pou3f3* and *pou3f4* localization in the *Xenopus laevis* kidney**

CAMILLE COSSE-ETCHEPARE, ISABELLE GERVI, ISABELLE BUISSON, LAURENT FORMERY, MICHAEL SCHUBERT, JEAN-FRANÇOIS RIOU, MURIEL UMBHAUER* and RONAN LE BOUFFANT*

*Address correspondence to: Muriel Umbhauer -  <https://orcid.org/0000-0001-7419-8070> and Ronan Le Bouffant -  <https://orcid.org/0000-0002-0995-7373>
Sorbonne Université, CNRS-Biologie du Développement, Institut de Biologie Paris-Seine (IBPS), 9 quai Saint-Bernard, 75005 Paris, France. Tel: +33 1 44 27 29 01.
E-mails: ronan.le_bouffant@upmc.fr - muriel.umbhauer@upmc.fr

See Suppl. Fig. 1 at <https://dx.doi.org/10.1387/ijdb.170260rl>

Supplementary Fig. S1. Sequence alignment used for establishing sequence identity scores and performing phylogenetic tree analyses. Species abbreviations are as follows: *Bf*, Branchiostoma floridae (Florida amphioxus); *Dr*, Danio rerio (zebrafish); *Gg*, Gallus gallus (chicken); *Hs*, Homo sapiens (human); *Lo*, Lepisosteus oculatus (spotted gar); *Mm*, Mus musculus (mouse); *Oi*, Oryzias latipes (medaka); *Sp*, Strongylocentrotus purpuratus (purple sea urchin); *Tr*, Takifugu rubripes (Japanese puffer); *Xl*, Xenopus laevis (African clawed frog).



Supplementary Fig. S2 (left). Validation of tailbud-stage embryo dissection. Expression of the kidney marker *pax8* analyzed by real-time quantitative polymerase chain reaction (RT-qPCR) on dissected explants from stage 25 *Xenopus laevis* embryos. Embryos were dissected either into ventral and dorsal halves, or into tailbud, pronephric anlage and head. The *pax8* gene is significantly enriched in the pronephric anlage in comparison to other embryonic tissues. *: $P < 0,05$, relative to the whole embryo.

Supplementary Fig.S3 (right). *Pou3f4* expression at the gastrula stage. Expression of *pou3f4* analyzed by real-time quantitative polymerase chain reaction (RT-qPCR) on dissected explants from early gastrula stage embryos (stage 10.5). Embryos were dissected either into ventral and dorsal halves, or dorsal (DMZ), ventral (VMZ) or lateral (LMZ) marginal zones, ectoderm and endoderm. No significant variation of *pou3f4* expression levels is observed.

SUPPLEMENTARY TABLE S1

**ACCESSION NUMBERS OF SEQUENCES USED FOR SEQUENCE
COMPARISONS AND PHYLOGENETIC ANALYSES**

Species	Gene name	Accession number
<i>Homo sapiens</i> (Hs)	Pou3f1	ENSG00000185668
	Pou3f2	ENSG00000184486
	Pou3f3	ENSG00000198914
	Pou3f4	ENSG00000196767
	Pou4f1	ENSG00000152192
<i>Mus musculus</i> (Mm)	Pou3f1	ENSMUSG00000090125
	Pou3f2	ENSMUSG00000095139
	Pou3f3	ENSMUSG00000045515
	Pou3f4	ENSMUSG00000056854
<i>Gallus gallus</i> (Gg)	Pou3f1	NP_001026755.1
	Pou3f2	XP_015140157.1
	Pou3f3	XP_015133414.1
	Pou3f4	XP_003641125.3
<i>Xenopus laevis</i> (Xl)	Pou3f1	NP_001096655.1
	Pou3f2	NP_001095225.1
	Pou3f3	XP_018102574.1
	Pou3f4	NP_001094393.1
	Pou4f1	XP_018102745.1
<i>Danio rerio</i> (Dr)	Pou3f1	ENSNDARG00000009823
	Pou3f2a	ENSNDARG00000070220
	Pou3f2b	ENSNDARG00000076262
	Pou3f3a	ENSNDARG00000042032
	Pou3f3b	ENSNDARG00000095896
Pou4f1	ENSNDARG00000005559	
<i>Oryzias latipes</i> (Ol)	Pou3f1	ENSORLGG00000014708
	Pou3f2b	ENSORLGG00000002223
	Pou3f3a	XP_020568920.1
	Pou3f3b	XP_020563964.1
	Pou3f4	ENSORLGG00000010578
<i>Takifugu rubripes</i> (Tr)	Pou3f1	ENSTRUG00000009356
	Pou3f2a	ENSTRUG00000015061
	Pou3f2b	ENSTRUG00000000239
	Pou3f3a	ENSTRUG00000010582
	Pou3f3b	ENSTRUG00000010582
	Pou3f4	ENSTRUG00000008957
<i>Lepisosteus oculatus</i> (Lo)	Pou3f1	ENSLOCG00000018292
	Pou3f2	ENSLOCG00000017875
	Pou3f3	ENSLOCG00000008712
	Pou3f4	ENSLOCG00000017491
	Pou4f1	ENSLOCG00000004613
<i>Branchiostoma floridae</i> (Bf)	Brn1-2-4	AAL85498.1
<i>Strongylocentrotus purpuratus</i> (Sp)	Brn1-2-4	XP_003729178.1

SUPPLEMENTARY TABLE S2

**REAL-TIME QUANTITATIVE POLYMERASE CHAIN
REACTION (RT-QPCR) PRIMER SEQUENCES**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>pou3f1</i>	CCTCAGGCTTCCTCTTTGCA	TGAGTGTGCATGAACTCCGGG
<i>pou3f2</i>	GGGAAGGAGCTGTCAAATGC	CGCGACTGATATGTCTGCTCAA
<i>pou3f3</i>	CGCTTCGAGCTAAGGAAACATT	GTCCCTCCCTCACTGAGCTG
<i>pou3f4</i>	GCCCAGCTTTACTGTCAGTGG	GGTGGTGTGCAGCTCCCAT
<i>pax8</i>	CAGCAATTTCAATATAGTGCACGG	TCCATTACAAAAGCCCCAC
<i>odc</i>	GGGCAAAGGAGCTTAATGTGG	TGCCAACATGGAACCTCACAC
<i>actin</i>	TCTATTGTGGGTCGCCCAAG	TTGTCCCATTCCAACCATGAC

TABLE S3

**SEQUENCES OF PRIMERS USED FOR CLONING
OF *IN SITU* HYBRIDIZATION PROBES**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>pou3f1</i>	CAGGTCCCACCTGGTTCATC	TGGGAAAGGTTTTTTTGTGTTT
<i>pou3f2</i>	CTCGGAGAGTCATGGCGACT	CTGTGTAATCCAGCTTGATCC
<i>pou3f3</i>	CAGAGGCAGGCTCTCACTGG	TGGTCTGGGAGAAGACATTG
<i>pou3f4</i>	CAGACACCTCCTGCAATGAGC	GGAGCCGTAGATATGGAAGGC