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SUPPLEMENTARY MATERIAL

corresponding to:

Ectophosphodiesterase/nucleotide phosphohydrolase (Enpp) nucleotidases: cloning, conservation and developmental restriction

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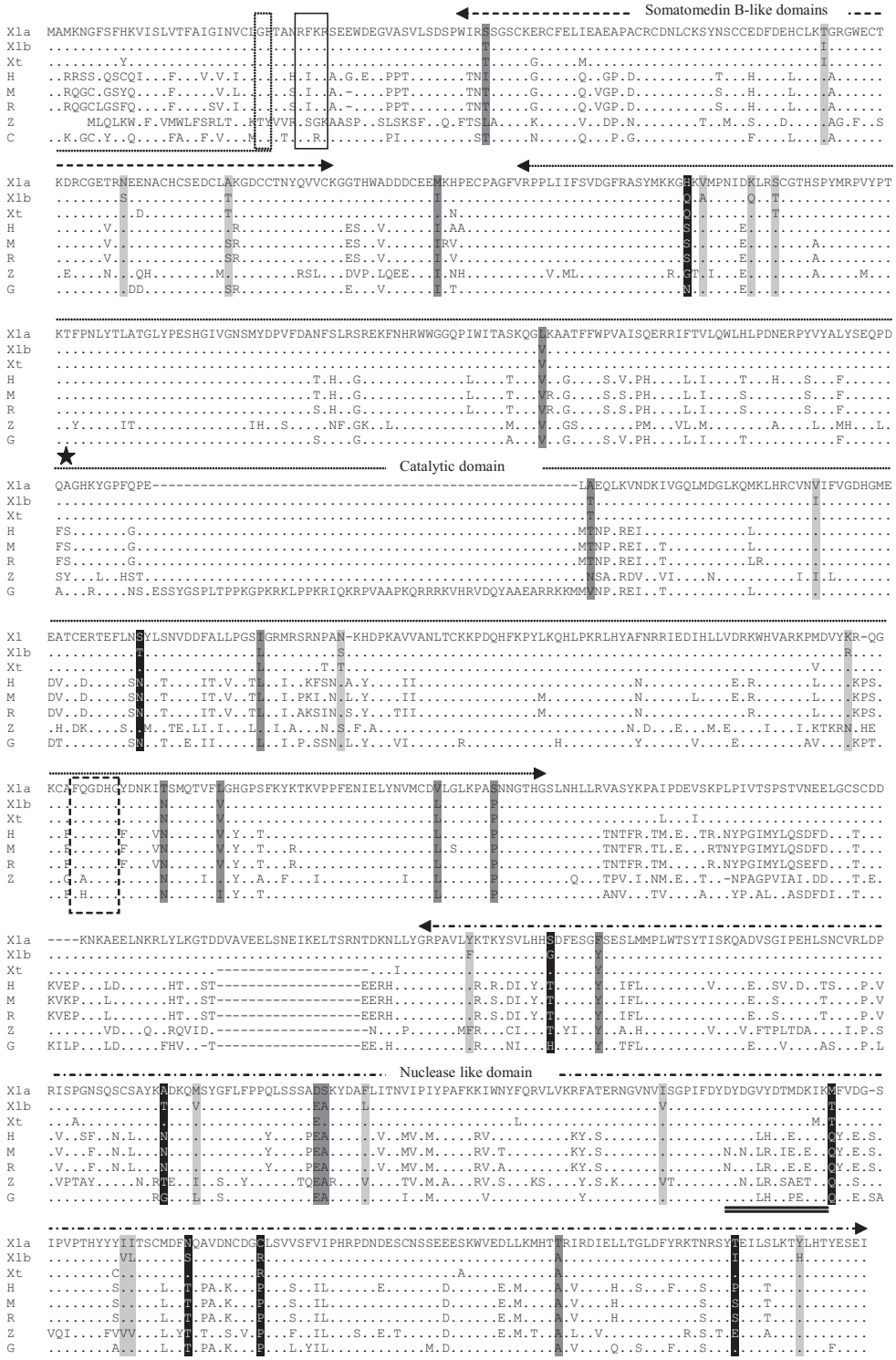

Supplementary Fig. 1. Alignment of enpp2 proteins. *The sequence of the three* Xenopus *enpp2 (Xlenpp2a (Xla), Xlenpp2b (Xlb), Xt enpp2 (Xt)) proteins were aligned against other vertebrate enpp2 proteins using the CLUSTALW software. Identical residues to* X. laevis *enpp2a sequence are indicated by (.). The 32 non-conserved residues between the two* X. laevis *sequences are highlighted. Letters highlighted in light grey are conserved between* X. laevis *enpp2a and its enpp2 ortholog but not enpp2b whereas letters highlighted in dark grey are conserved between* X. laevis *enpp2b and its enpp2 orthologs but not enpp2a. Letters highlighted in black are the least conserved between the enpp2 sequences. The different functional domains are indicated by the dashed and dotted arrows. The dotted underlined sequences indicate the signal peptide and the signal peptide cleavage site is marked by the dotted box. The consensus site for recognition by furin is marked by the black box. The FxGXXG motif is indicated by a dashed box. The double underlined sequences represent the EF hand motif. ★ : Threonine catalytic-site. Gaps are indicated by dashes. The Genbank accession numbers of the ENPP2 are given in the legend of the figure 3 except for Rat ENPP2: NP_476445. C, Chick; H, Human; M, Mouse; R, Rat; Z, Zebrafish.*

SUPPLEMENTARY TABLE 1A

CLONING STRATEGY USED TO IDENTIFY THE 7 *enpp* GENES

marker	*Xenopus laevis*	*Xenopus tropicalis*
enpp1	RT-PCR (primers based on *X. tropicalis* sequence)	BLAST search (EST clones; genomic sequence) RT-PCR
enpp2a	BLAST search (FL clone)	BLAST search (FL clone)
enpp2b	BLAST search (FL clone)	n/a
enpp3	BLAST search (EST clones) RT-PCR	BLAST search (EST clones; genomic sequence)
enpp4	BLAST search (FL clone)	BLAST search (FL clone)
enpp6	BLAST search (FL clone)	BLAST search (EST clones alignment)
enpp7	BLAST search (EST clones alignment)	BLAST search (EST clones alignment)

FL: full length

n/a: not applicable

SUPPLEMENTARY TABLE 1B

ACCESSION NUMBERS OF THE DIFFERENT CLONES USED IN THIS STUDY

	Xenopus Laevis			*Xenopus tropicalis*		
Marker	**EST used: Accession number Genbank**	**Clone I.M.A.G.E ordered: ID**	**DNA and (protein) Accession number Genbank**	**EST Accession number JGI and Genbank**	**DNA and (protein) Accession number Genbank**	**Scaffold JGI site**
enpp1	none	none	FJ603316	Unigene Str.33465	FJ603321	200
enpp2a	BC044675	5570505	NM_001087057 (NP_001080526)		NM_001015936 (NP_001015936)	330
enpp2b	BU912868	6639131	BC089138 (NP_001087397)			
enpp3	EG575107 EB473478 EB468830 EG570319 EB468830	none	FJ603317	Unigene St.53243	FJ603320	200
enpp4	BF611703	3557085	BC079717 (AAH79717)		(AAI21206)	328
enpp6	CB558973	4031206	BC077499 (AAH77499)	Unigene Str.37100	FJ603322	
enpp7	Unigene Xl.60172 Xl.74867 EB481135	none	FJ603318	Unigene Str.76870	FJ603319	1094

SUPPLEMENTARY TABLE 2

PRIMER SEQUENCES AND PCR CONDITIONS FOR THE REQUIRED MARKERS

Marker	Sequence (5'-3')	Annealing Temp. °C	Cycles	References
enpp1	U-CTTGCGTTGGTGGATTCCAT D-AAGAGCAGCCTAAGCTGTCA	59	28	This work
enpp2a	U-ACAACAGTCGTCGCGAGGAC D-CGCGGAATCCATCAACTGAG	57	31	This work
enpp2b	U-CATTGTCTAAAGATCGGC D-ATGTTGCTGCCTTCACC	53	26	This work
enpp3	U-CGTTGCCTCCTACACTAACA D-TCCACTGATCACGTTGACTC	59	29	This work
enpp4	U-TCCCGCCATGGTCACTCA D-AGGCATCGGGAATCTCGTTAG	59	27	This work
enpp6	U-CTGCCCCCGTCCTCTGCT D-TCGGCTCCATCCCACCATT	59	27	This work
enpp7	U-CAGGCTGCACTATTCCAACA D-CACCAGCATGTTCCGTGTCA	59	29	This work
ODC	U-GGAGCTGCAATTTGGAGA D-TCAGTTGCCAGTGTGGTC	55	20-22	Bassez et al.,1990
EF1 α	U-CAGATTGGTGCTGGATATGC D-CACTGCCTTGATGACTCCTA	55	19 -21	Mohun et al.,1989

T. Bassez, J. Paris, F. Omilli, C. Dorel, H.B. Osborne, Post-transcriptional regulation of ornithine decarboxylase in *Xenopus laevis* oocytes. Development 110 (1990) 955-62.

T.J. Mohun, M.V. Taylor, N. Garrett, J.B. Gurdon, The CArG promoter sequence is necessary for muscle-specific transcription of the cardiac actin gene in *Xenopus* embryos.EMBO J. 8 (1989) 1153-61.

SUPPLEMENTARY TABLE 3

IN VITRO TRANSCRIPTION CONDITIONS FOR THE *IN SITU* PROBES UTILISED IN THIS STUDY

Marker Gene	RNA polymerase	Linearization	Plasmid Details
enpp1 (antisense)	Sp6	*Sac*I	enpp1:pGEMT (2 separate clones)
enpp1 (sense)	Sp6	*Sac*I	(coding region; 0.66kb;1098-1757)
enpp2a (antisense)	T3	*Sac*I	enpp2a :pBSKS
enpp2a (sense)	T7	*Kpn*I	(3'UTR ;0.57 kb ; 2706-3283)
enpp2b (antisense)	T3	*Sac*I	enpp2b :pBSKS
enpp2b (sense)	T7	*Kpn*I	(3'UTR ; 0.42 kb ; 2727-3154)
enpp3 (antisense)	Sp6	*SacII*	enpp3:pGEMT (2 separate clones)
enpp3 (sense)	Sp6	*SacII*	(coding region;0.58kb;1998-2585)
enpp4 (antisense)	Sp6	*Nco*I	enpp4:pGEMT
enpp4 (sense)	T7	*Sac*I	(5'UTR+coding region; 1.25kb; 96-1350)
enpp6 (antisense)	T7	*KpnI*	enpp6 :pBSKS (2 separate clones)
enpp6 (sense)	T7	*KpnI*	(3'UTR; 1.8 kb; 1066-2800)
enpp7 (antisense)	Sp6	*Sac*I	enpp7:pGEMT (2 separate clones)
enpp7 (sense)	Sp6	*Sac*I	(coding region; 0.58kb;598-1180)