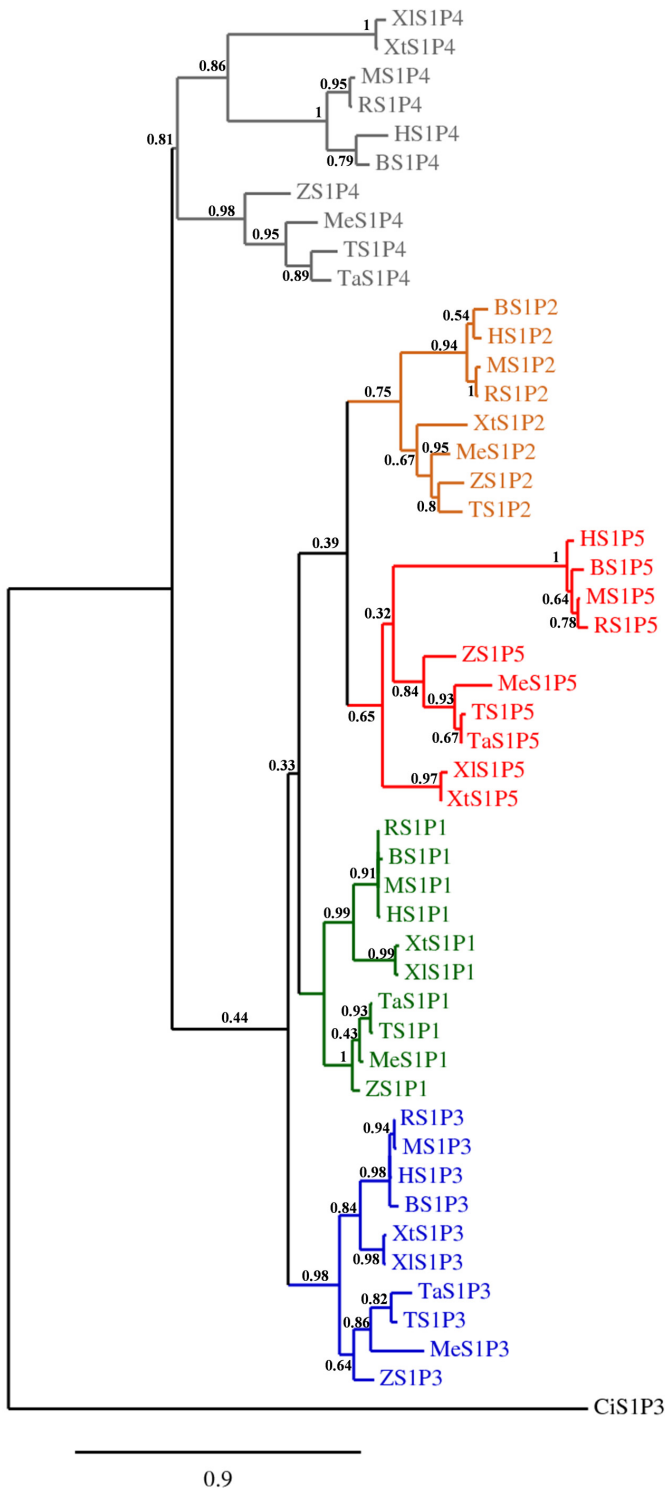## SUPPLEMENTARY MATERIAL

corresponding to:

The lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) receptor gene families: cloning and comparative expression analysis in *Xenopus laevis*

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Supplementary Fig. 1. Relatedness of the sphingosine-1-phosphate (S1P) receptors. *A phylogenetic tree was created on the phylogeny.fr platform (Dereeper* et al.*, 2008). S1P receptor protein sequences were aligned using Muscle program. The phylogeny was reconstructed using the maximum likelyhood method (PhyML program) and reliability for internal branch was assessed using the Bootstrapping procedure (100). The tree rendering was performed using TreeDyn software. A rectangular configuration is shown here and bootstrap values are indicated in black. Protein sequences for S1P 1-5 receptors were identified on the NCBI and Ensembl websites. The Genbank (G) or Ensembl (E) accession numbers of the different receptors are as follows:*

human S1P1: NP_001391 (G); Human S1P2: NP_004221 (G); Human S1P3: NP_005217 (G); Human S1P4: AAH14940 (G); Human S1P5: NP_110387 (G); mouse S1P1: NP_031927†(G); mouse S1P2: AAD16976 (G); mouse S1P3: NP_034231 (G); mouse S1P4: NP_034232 (G); mouse S1P5: NP_444420 (G); rat S1P1: NP_058997 (G); rat S1P2: NP_058888 (G); rat S1P3: XP_225216 (G); rat S1P4: NP_001101545 (G); rat S1P5: NP_068543 (G); bovin S1P1: NP_001013603 (G); bovin S1P2: AAI33423 (G); bovin S1P3: AAI50077 (G); bovin S1P4: XP_587940 (G); bovin S1P5: XP_595705 (G); zebrafish S1P1: NP_571766 (G); Tetraodon S1P1: CAG90490 (G); Tetraodon S1P2: ENSTNIP00000002390 (E); Tetraodon S1P3: CAG12442 (G); Tetraodon S1P4: ENSTNIP00000000128†(E); Tetraodon S1P5: CAF92590 (G); Medaka S1P1: ENSORLP00000017674 (E); Medaka S1P2: ENSORLP00000006996†(E); Medaka S1P3: ENSORLP00000007246†(E); Medaka S1P4: ENSORLP00000021851†(E); Medaka S1P5: ENSORLG00000003793†(E); Takifugu S1P1: ENSTRUP00000024703†(E); Takifugu S1P3: ENSTRUP00000023201†(E); Takifugu S1P4: ENSTRUP00000030129†(E); Takifugu S1P5: ENSTRUP00000010450†(E); zebrafish s1p2: XP_682912 (G); zebrafish s1p3: XP_001345724 (G); zebrafish s1p4: AAH66587 (G); zebrafish s1p5: NP_001007317 (G); Ciona S1P3: AAP91719 (G). B: Bovin; Ci: Ciona; H: Human; M: Mouse; Me: Medaka; R: Rat; Ta: Takifugu; T: Tetraodon; Xl: X. laevis; Xt: X. tropicalis; Z: Zebrafish.
DEREEPER A*, GUIGNON V*, BLANC G, AUDIC S, BUFFET S, CHEVENET F, DUFAYARD JF, GUINDON S, LEFORT V, LESCOT M, CLAVERIE JM, GASCUEL O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res. 36 (Web Server issue): W465-9 * joint first authors.*

SUPPLEMENTARY TABLE 1A

CLONING STRATEGY USED TO IDENTIFY THE *LPA/S1P* GENES

Gene name	*Xenopus laevis*	*Xenopus tropicalis*
LPA1 (Edg2)	Published	BLAST search (FL clone)
LPA1.2 (Edg2)	Published	n/a
LPA2 (Edg4)	BLAST search (FL clone)	Published
LPA3 (Edg7)	BLAST search (EST clones) RT-PCR	BLAST search (genomic sequence)
LPA4 (p2y9; gpr23)	BLAST search (FL clone)	BLAST search (FL clone)
LPA5 (gpr92)	BLAST search (EST clones)	BLAST search (FL clone)
S1P1 (Edg1)	BLAST search (FL clone)	BLAST search (FL clone)
S1P2 (Edg5)	BLAST search (EST clone)	BLAST search (EST clone; genomic sequence)
S1P3 (Edg3)	BLAST search (EST clones) RT-PCR	BLAST search (EST clones; genomic sequence)
S1P4 (Edg6)	BLAST search (FL clone)	BLAST search (genomic sequence)
S1P5 (Edg8)	BLAST search (FL clone)	BLAST search (FL clone)

FL: full length

n/a: not applicable

SUPPLEMENTARY TABLE 1B

ACCESSION NUMBERS OF THE DIFFERENT CLONES USED IN THIS STUDY

Marker	*Xenopus laevis*			*Xenopus tropicalis*		
	EST used: Accession number Genbank	Clone ordered: ID	DNA and (protein) Accession number Genbank	EST Accession number JGI and Genbank	DNA and (protein) Accession number Genbank	Scaffold JGI site
LPA1.1	n/a	BC049389	AJ249843 (CAB62282)	Str.67218	NM_001102843 (NP_001096313)	590
LPA2	Xl.16757	BC081023	NM_001094167 (NP_001087636)	Str.25552	AY652941 (AAT94556)	1235
LPA3	EB473280	EB473280	Submitted	none	Submitted	6
LPA4	Xl.25260	BC123269	NM_001097025 (NP_001090494)	Str.44796	NM_001079379 (NP_001072847)	258
LPA5	BU913691	BU913691	Submitted	Str.36863	BC168435 (AAI68435)	877
S1P1	Xl.18157	BC074356	NM_001092761 (NP_001086230)	Str.18760	NM_001079425 (NP_001072893)	85
S1P2	DT076716	DT076716	Submitted	ES675275.1|	Submitted	92
S1P3	BJ031045 BJ045989	XL005o11	Submitted	CX480666 CX311738	Submitted	256
S1P4	Xl.48354	BC078064	Submitted	none	Submitted	202
S1P5	Xl.9971	XL173p21 XL257i10	Submitted	Str.2177	NM_001127068 (NP_001120540)	520

SUPPLEMENTARY TABLE 2

**PRIMER SEQUENCES AND PCR CONDITIONS
FOR THE REQUIRED MARKERS**

Marker	Sequence (5'-3')	Annealing Temp. °C	Cycles	References
S1P1	U-GGCTGAGATCGGATCAGACT D-GGACTACGTGCTGGCTATTG	59	30	This work
S1P2	U-AAGTCCAGCCACGGAGAAGT D-GGATGTAGGCAGGTCGTGTT	57	29	This work
S1P3	U-GGAACTTGCTGGCTCATCTC D-ACTCTGCGGCTACTGGACTT	57	31	This work
S1P4	U-GAACTGTCTGCTGTCCTGTA D-GAGTCTGATGGAGGTGTGAT	57	29-30	This work
S1P5	U-GCCTTGGTTCCTTCCTGTAA D-ACCATACATCAGGCTCTAGG	59	29	This work
LPA1.1	U-ACTGCCGTAGAGGCCTAGGT D-AGGCAGTGCAGCTACACAAG	57	30	This work
LPA1.2	U-CCGGCTTCTCTGCATAAGAT D-AAGGCAGTGCAGCTACACAA	57	30	This work
LPA2	U-AGCCAATGGTGCTGCCTTAT D-AATGGCATGTGGTGGTCAGA	57	29	This work
LPA3	U-GCATCGTCCGTCAAGAATGT D-AGCCAAGCGCTGATCACCAT	57	30-31	This work
LPA4	U-AGGAGCTGACTGAACAGATG D-TACTGACCAGGCTGAATACC	57	30	This work
LPA5	U-TGAGCAGGATGAGTGGTCAG D-CGACTTGGACCATGGCAGTA	59	30	This work
ODC	U-GGAGCTGCAATTTGGAGA D-TCAGTTGCCAGTGTGGTC	55	20	Bassez et al.,1990
EF1a	U-CAGATTGGTGCTGGATATGC D-CACTGCCTTGATGACTCCTA	55	19	Mohun et al.,1989

T. BASSEZ, J. PARIS, F. OMILLI, C. DOREL, H.B. OSBORNE (1990). Post-transcriptional regulation of ornithine decarboxylase in *Xenopus laevis* oocytes. *Development* 110: 955-962.

T.J. Mohun, M.V. Taylor, N. Garrett, J.B. Gurdon (1989). The CArG promoter sequence is necessary for muscle-specific transcription of the cardiac actin gene in *Xenopus* embryos. *EMBO J.* 8: 1153-1161.

SUPPLEMENTARY TABLE 3

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

Gene	RNA polymerase	Linearization	Plasmid
S1P1 (antisense) *S1P1* (sense)	T3 T7	*Sac*II *Kpn*I	*S1P1*:pBSKS (3'UTR; 1kb; 3145-4106)
S1P2 (antisense) *S1P2* (sense)	T3 T7	*Sac*II *Kpn*I	*S1P2*:pBSKS (2 separate clones)(coding region+3'UTR 1.4kb;1-1300)
S1P3 (antisense) *S1P3* (sense)	T7 T3	*Eco*RI *Xho*I	XL005o11 (3kb; full length clone)
S1P5 (antisense) *S1P5* (sense)	T7 T3	*Eco*RI *Xho*I	XL173p21 (3kb; full length clone)
LPA1.1 (antisense) *LPA1.1* (sense)	T7 T3	*Kpn*I *Sac*I	*LPA1.1*:pBSKS (3'UTR; 0.88kb;3620-4500)
LPA2 (antisense) *LPA2* (sense)	T7 T7	*Sac*I *Sac*I	*LPA2*:pGEMT (2 separate clones) (3'UTR; 0.85kb; 1286-2140)
LPA3 (antisense) *LPA3* (sense)	T7 SP6	*Eco*RI *Not*I	EB473280 (1.8 kb; full length clone)
LPA4 (antisense) *LPA4* (sense)	T7 T7	*Sac*I *Sac*I	*LPA4*:pGEMT (2 separate clones) (3'UTR; 0.5kb;1399-1899)
LPA5 (antisense) *LPA5* (sense)	T7 Sp6	*Eco*RI *Not*I	BU913691(6640087) (2.5 kb; full length clone)