

Supplementary Materials

Purine Biosynthesis Pathways Are Required for Embryonic Myogenesis in *Xenopus laevis*

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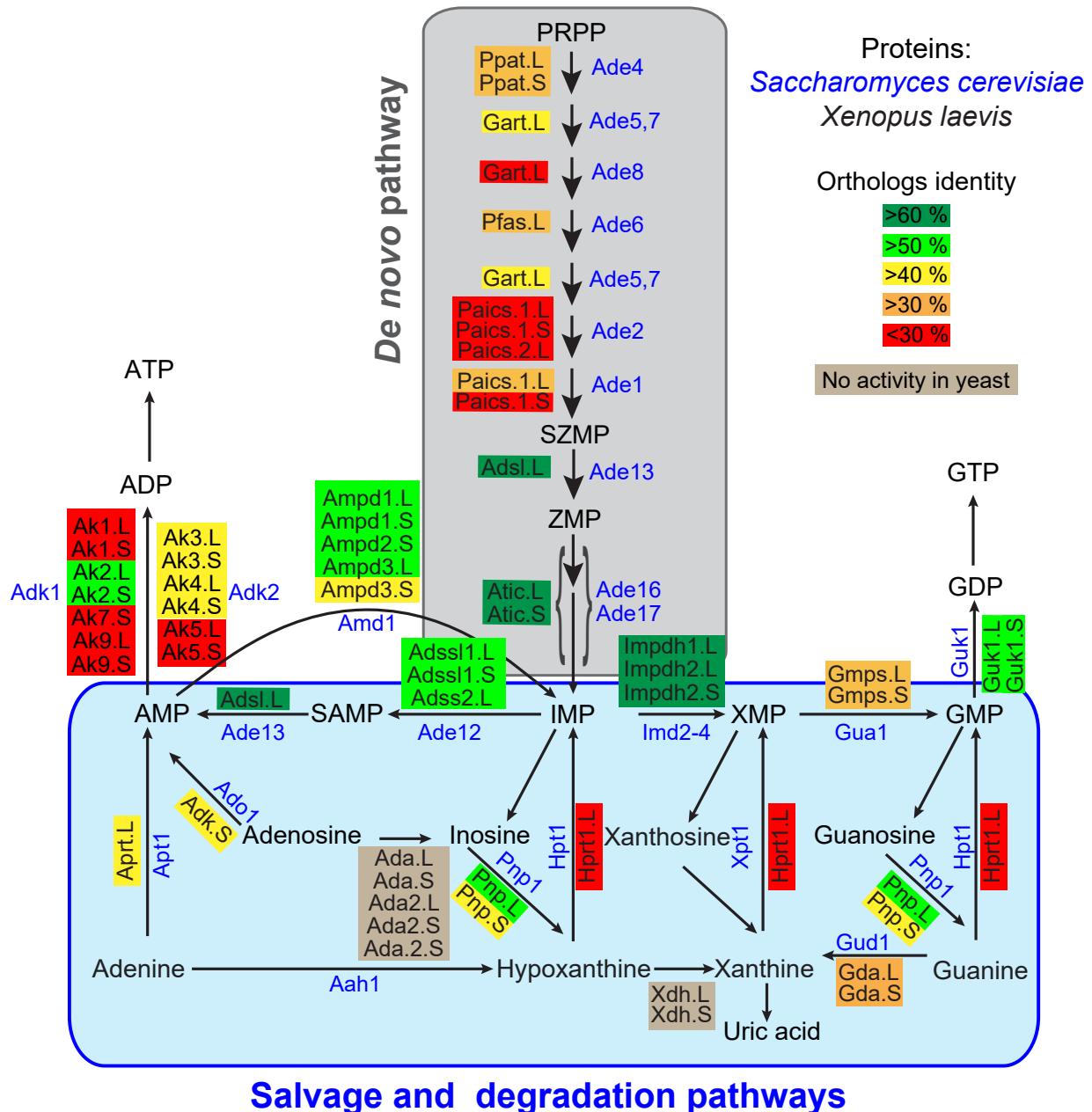


Figure S1. Conservation of purine biosynthesis pathways between the yeast *Saccharomyces cerevisiae* and *Xenopus laevis*. Features of the *X. laevis* and yeast enzymes are listed in Table S7. Abbreviations: AMP: adenosine monophosphate; GMP: guanosine monophosphate; IMP: Inosine monophosphate; PRPP: Phosphoribosyl pyrophosphate; SAMP: Succinyl-AMP; SZMP: Succinyl Amino Imidazole Carboxamide Ribonucleotide monophosphate; XMP: Xanthosine monophosphate; ZMP: Amino Imidazole CarboxAmide Ribonucleotide monophosphate.

ADSL	Hs	1-MAAGGDHGSPDS-----YRSPLASRYASPEMCVFSDRYKFRTRQLWLWLAEEAEQTILGLPITDEQIQEMKSLENI-72 1-M..G....S.DS----- YRSPL. SRYAS. EM.F. FSD ..KF.TWR.LWLWLA.AE..LGIPIT.EQIQEM..NLENI
Adsl.	Xl	1-ME-GSSGLSMDSNTRITGSVSPLTGPGPEEVMRYSPLVSRYAS SREMAFNFSDSKKFQTWRRLWLWLAQAERSLGLPITEEQIQEMEANLENI -91
ADSL	Hs	73-DFKMAAEEEKRLRHDMAHVHTFGHCCPKAAGIIHLGATSCYVGDNLDLILRNALDLLPKLARVISRLADFAKERASLPTLGFTHFCPAQ-164 DFKMAAEEEKRLRHDMAHVHTF.HCCPKAA..IHLGATSCYVGDNLDLI.LR..DLLL PKLARV..RLADFA..A..PTLGFTH.CPAQ
Adsl.	Xl	92-DFKMAAEEEKRLRHDMAHVHTFAHCCPKAAPVIHLGATSCYVGDNLDLIVLRGFDLLLPKLARVLNLRADFAEKYAEMPTLGFTHYCPAQ-183
ADSL	Hs	165-LTTVGKRCLWIQDLCMDLQNLKRVRDDLRFRGVKGTTGTQASFQLQLFEGDDHKVEQLDKMVTEKAGFKRAFIITGQTYTRKVVDIEVLSVLA-256 LTTVGKR. CLW.QDLCMDL..L.R.R..LRFRGVKGTTGTQASFQLF.GD..KVE.LD.MVT..AGFKRA.I.TGQTY.RKVD.EV.SVLA
Adsl.	Xl	184-LTTVGKRACLWLQDLCMDLRNLERARNELRFRGVKGTTGTQASFQLFLDGHDKVEELDRMVTSMAGFKRAYIVTGQTYSRKVVDVEVVSVLA-275
ADSL	Hs	257-SLGASVHKICTDIRLLANLKEMEEPFEKQQIGSSAMPYKRNEMRSERCCSLARHIMTLMVMDPLQTAQWFERTLDDSANRRICLAEAFLTA-348 SLGA.VHKICTDIRLLANKE.EEPFEK.QIGSSAMPYKRNEMRSERCCSLARHIMTL.M.PLQTAQWFERTLDDSANRRICLAEAFLTA
Adsl.	Xl	276-SLGATVHKICTDIRLLANLKELLEPFKEQIGSSAMPYKRNEMRSERCCSLARHIMTLMNPLQTAQWFERTLDDSANRRICLAEAFLTA-367
ADSL	Hs	349-DTILNTLQNISEGLVVPKVIERRIRQELPFMATENIIMAMVKAGGSRQDCHEKIRVLSQQASVVKQEGGDNDLIERIQVDAYFSPIHSQL-440 D IL.TLQNISEGLVVPKVIERRIRQELPFMATENIIMAMVK.GG.RQDCHE.IRVLSQQA..VVKQEGGDNDLI.RIQ.D.YF.P.HA.H
Adsl.	Xl	368-DIILSTLQNISEGLVVPKVIERRIRQELPFMATENIIMAMVKNGGNRQDCHERIRVLSQQAGAVVKQEGGDNDLIFRIQSDFSYFAPIHAHL-459
ADSL	Hs	441-FSPIHSQDHLLDPSSFTGRASQQVQRFLLEEVEVPLLKPYESVMKVKAELCL-484 F.PIH..L..LLDP.SF.GRA.QQV..FL.EEV.PLL.PY.S.M.VK.EL.L
Adsl.	Xl	460-FAPIHAHLEQLLDPKSFIGRAPQQVLKFLKEVIPLLSPYQSKMDVKMELEL-503

Figure S2. Sequence comparison between the *H. sapiens* (*Hs*) and *X. laevis* (*Xl*) adenylosuccinate lyase enzymes. Conserved amino acids are indicated in bold. Red and orange boxes point to residues required for catalysis and yellow and orange boxes to mutated residues found in *ADSL*-deficient patients.

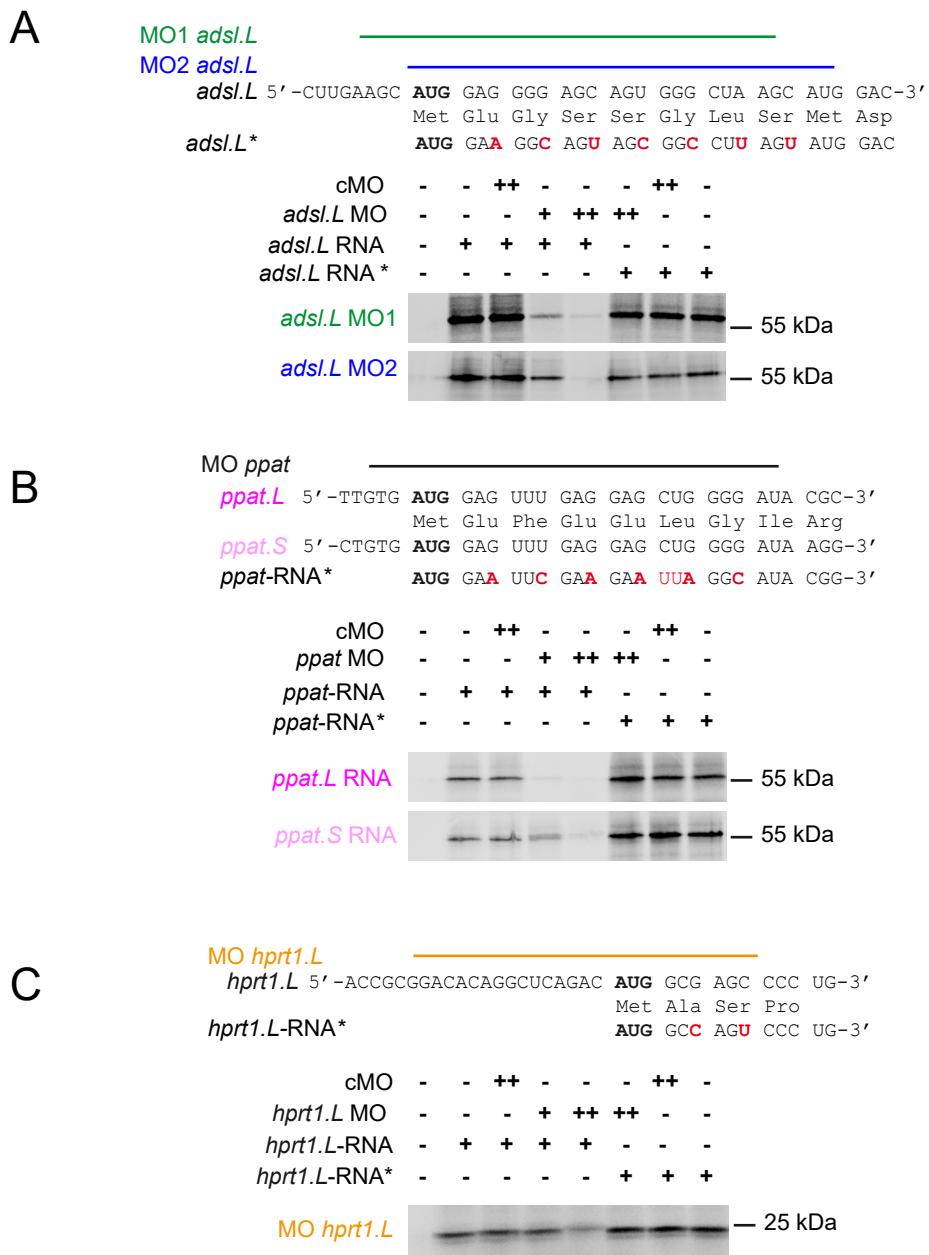


Figure S3. Validation of the morpholinos translation interference by *in vitro* translation. The *adsl.L* (**A**), *ppat.L/ppat.S* (**B**) and *hprt1.L* (**C**) RNAs were produced using the *mMessage mMachine SP6* kit (ambion) according to the supplier procedure. *In vitro* translation was then performed by using the reticulocyte rabbit lysate system kit (Promega) in 25 µl reaction mix containing 500 ng of indicated RNA, 0.5 µl of the amino acid mix w/o Met (1 mM), 2 µl of [³⁵S]-methionine (1,200 Ci/mmol; 10 mCi/ml), 17.5 µl of the reticulocytes lysate, 0.5 µL of RNase inhibitor (40 u/µl; Promega) and in the presence or the absence of 40 (+) or 400 (++) ng of indicated Morpholino (MO). Proteins were separated by SDS-PAGE and radiolabeled proteins were detected by phosphorimaging (Typhoon biomolecular imager, Amersham). RNA* refers to mutated RNAs whose translation is not affected by the gene-specific MOs.

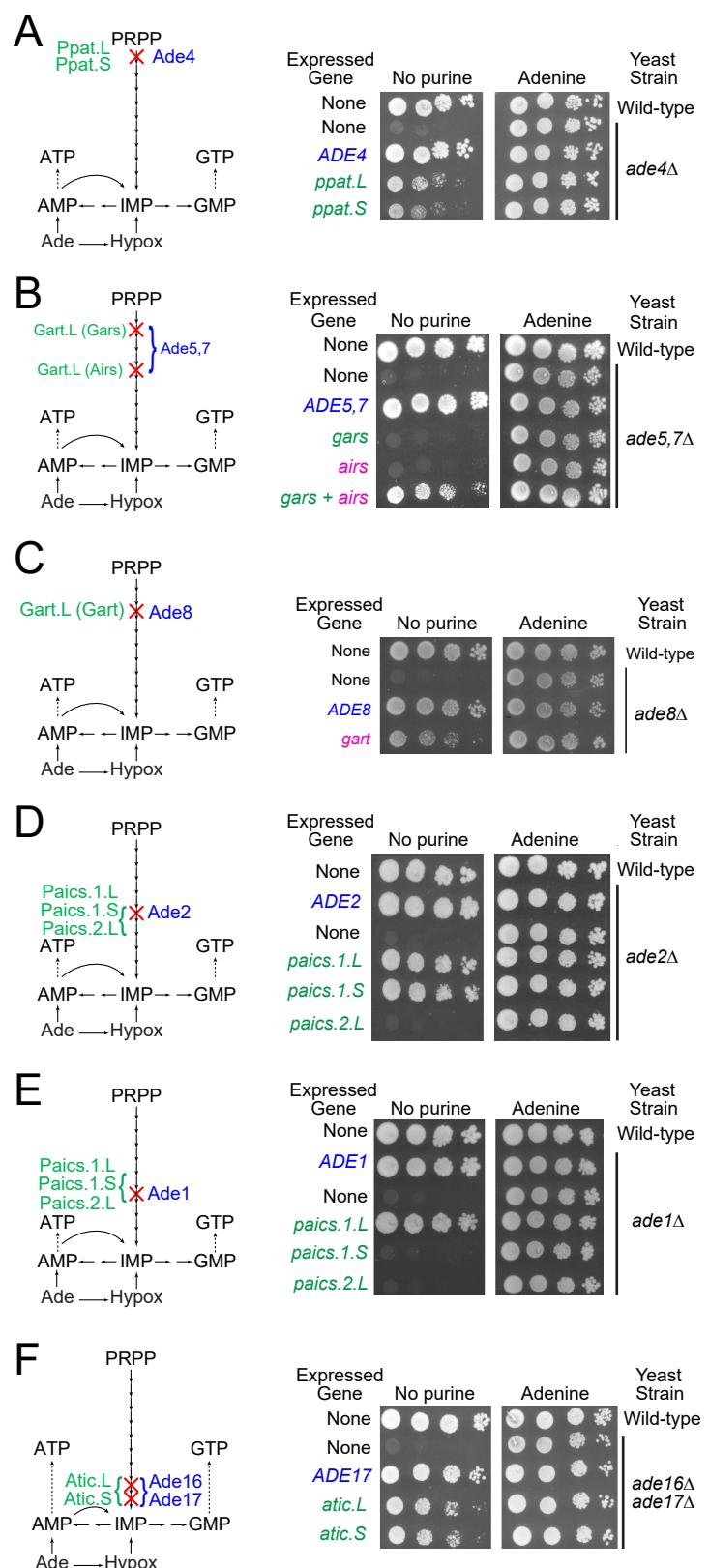


Figure S4. Functional complementation of yeast purine *de novo* pathway mutants by the *X. laevis* ortholog genes. Red crosses indicate the purine pathway steps knocked-out in each yeast mutant. *S. cerevisiae*, *X. laevis* and *X. tropicalis* protein or gene names are written in blue, green and pink, respectively. For Airs and Gart activities, functional complementation was performed by expressing the *X. tropicalis* corresponding open reading frames. Abbreviations: Ade: adenine; Airs: aminoimidazole ribonucleotide synthetase activity; Gars: glycinamide ribonucleotide synthetase activity; Gart: glycinamide ribonucleotide transformylase activity; Hypox: hypoxanthine; IMP: Inosine monophosphate; PRPP: Phosphorybosyl pyrophosphate. Yeast mutants were transformed with plasmids allowing expression of the indicated yeast (blue) or *X. laevis* (green) or *X. tropicalis* (pink) genes or with the empty vector (None). Transformants were serial (1/10) diluted and spotted on SDcasaW medium supplemented or not (no purine) with adenine as sole external purine source. Plates were imaged after 2 (**F**), 4 (**A, D-E**) or 7 days (**B-C**) at either 30°C (**A, D-F**) or 37°C (**B-C**).

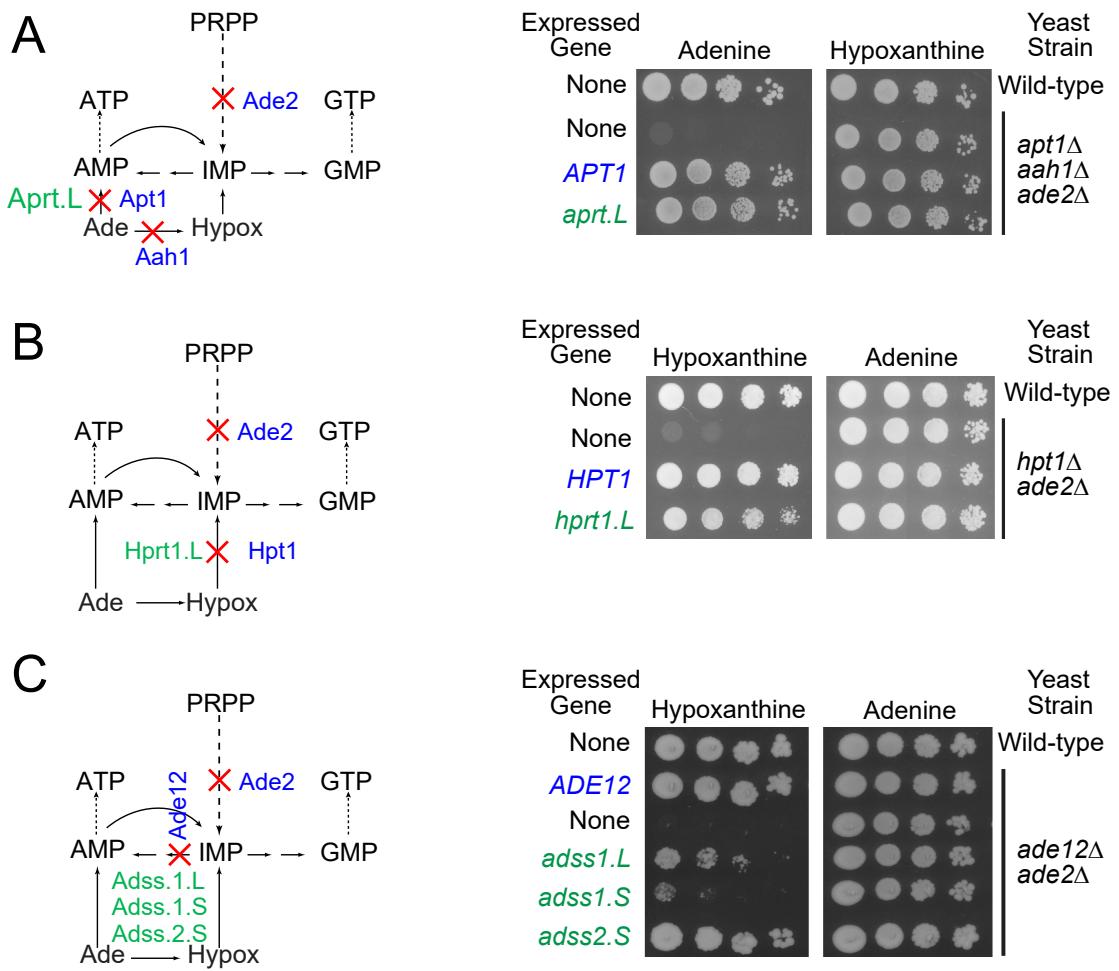


Figure S5. Functional complementation of yeast purine salvage knocked-out mutants by the *X. laevis* ortholog genes. The vertical dashed line symbolizes the *de novo* purine pathway. Red crosses are used to indicate the purine pathway steps absent in each yeast knock-out mutant. *S. cerevisiae* and *X. laevis* protein or gene names are written in blue and green, respectively. Ade: Adenine; Hypox: Hypoxanthine; IMP: Inosine monophosphate; PRPP: 5-phosphorybosyl-pyrophosphate. Wild-type and mutant yeast strains were transformed with plasmids allowing expression of the indicated *S. cerevisiae* (blue) or *X. laevis* (green) genes or with the empty vector (None). Transformants were serial diluted (1/10) and spotted on SDcasaW medium supplemented with either adenine or hypoxanthine as sole external purine source. Plates were imaged after 2 days at 30°C.

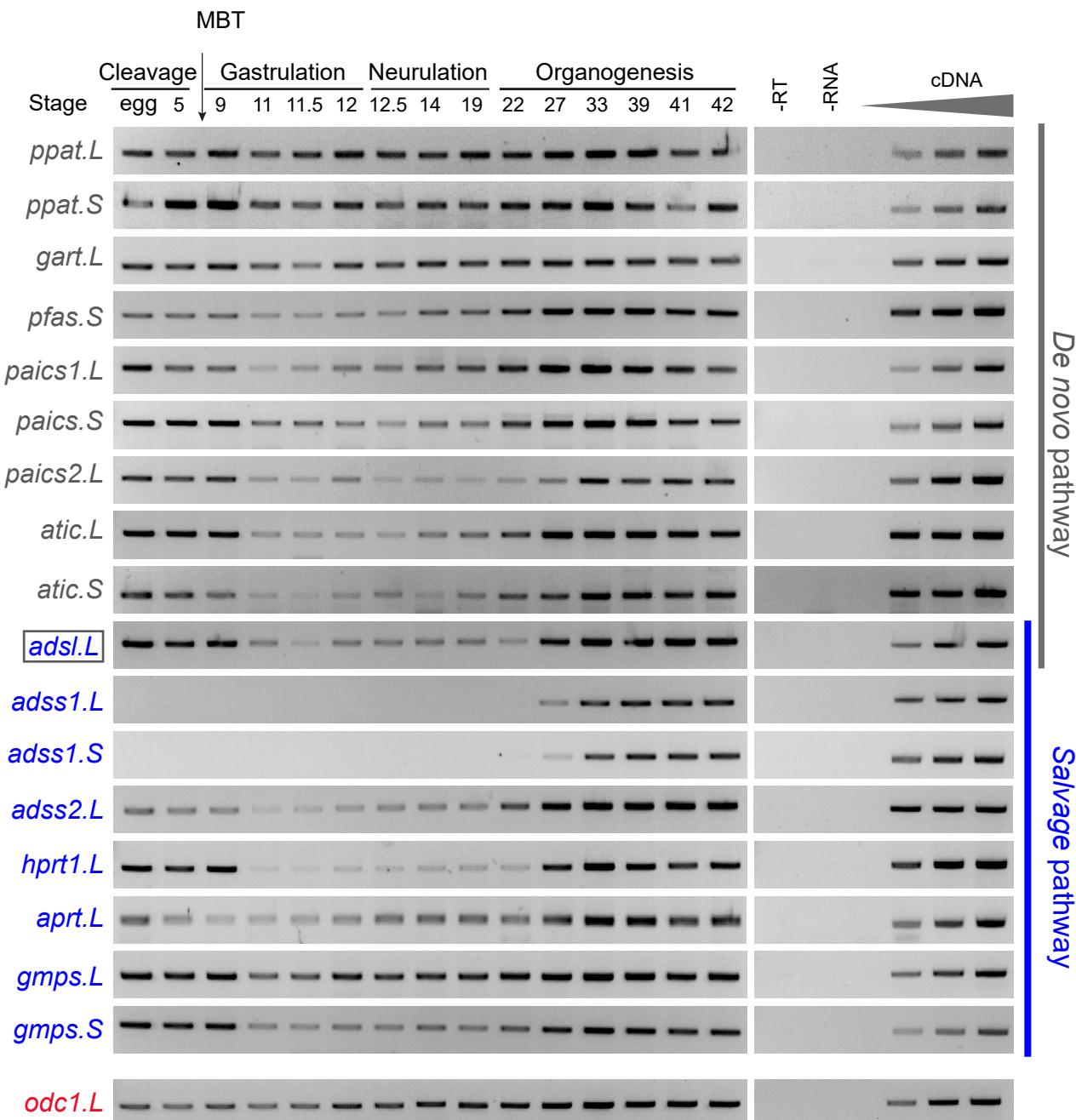


Figure S6. Temporal expression profiles of purine pathway genes during embryogenesis. The expression profile was determined for each gene by RT-PCR from cDNA of fertilized egg and whole embryo at different stages covering the different phases of *X. laevis* embryogenesis. Negative controls were performed in the absence of either reverse transcriptase (-RT) or total RNA extract (-RNA). The linearity was performed with doubling dilutions of cDNA. A loading control was done using *odc1.L* gene (in red). Names corresponding to genes encoding enzymes of the purine *de novo* and salvage pathways are written in grey and blue, respectively. The *adsl.L* gene encoding the adenylosuccinate lyase involved in both purine pathways is boxed in grey. Experiments were done at least twice ($n \geq 2$) on embryos obtained from *in vitro* fertilization of oocytes from two different females ($N=2$). Sense and antisense primers are described in Table S5. MBT: mid blastula transition.

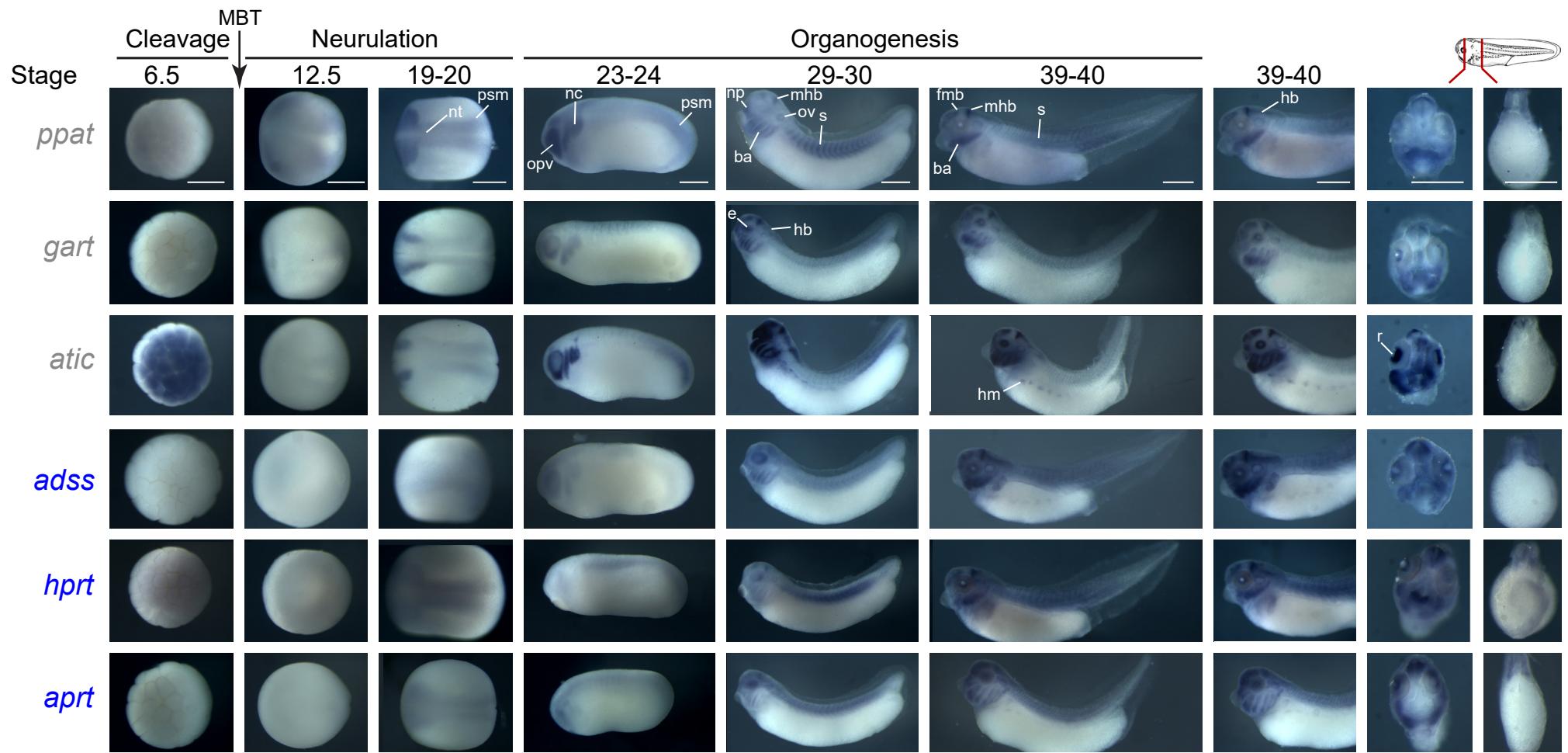


Figure S7. The expression profile was determined for each gene by *in situ* hybridization using antisense riboprobes (Table S4). No unspecific staining was detected with the control sense probes (Figure S8). MBT: Mid blastula transition. Grey and blue gene name colors refer to *de novo* and salvage purine pathways, respectively. Representative embryos were photographed. Stage 6.5: animal pole view, later stages: lateral views, with dorsal is up and anterior is left. Transverse section: dorsal is up. Abbreviations: ba, branchial arches; e, eye; fmb, forebrain-midbrain boundary; hb, hindbrain; hm, hypaxial muscles; l, lens; mhcb, midbrain-hindbrain boundary; n: nasal placode; nc, neural crest; np, neural plate; nt, neural tube; opv, optical vesicle; ov, otic vesicle; psm, presomitic mesoderm; r, retina; s, somites. Bars: 0.5 mm.

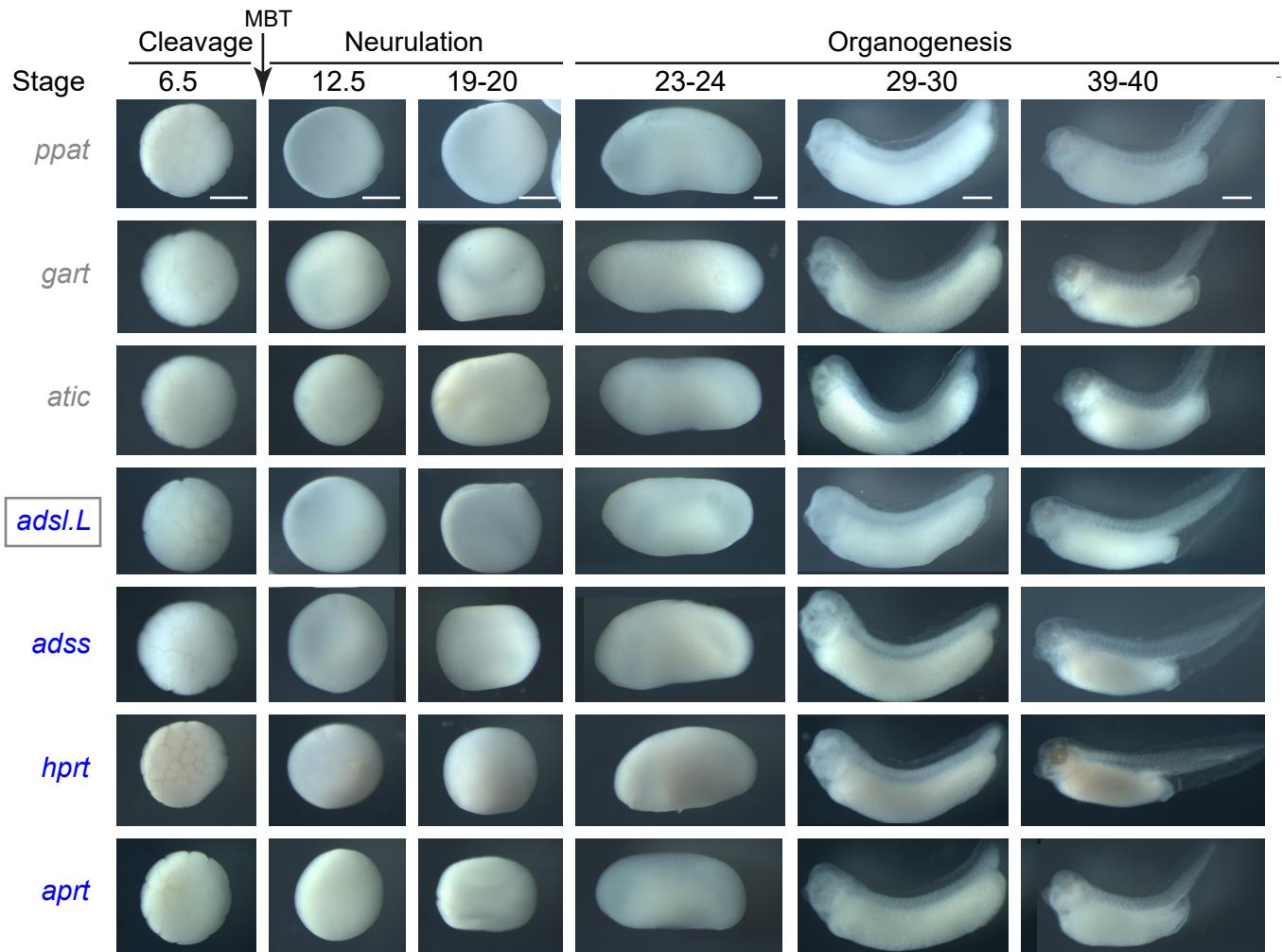


Figure S8. *In situ* hybridization using control sense riboprobes. *In situ* hybridization sense probes are described in Table S4. MBT: Mid blastula transition. Grey and blue gene name colors refer to *de novo* and salvage purine pathways, respectively. Representative embryos were photographed. Stage 6.5: animal pole view, later stages: lateral views, with dorsal is up and anterior is left. Bars: 0.5 mm.

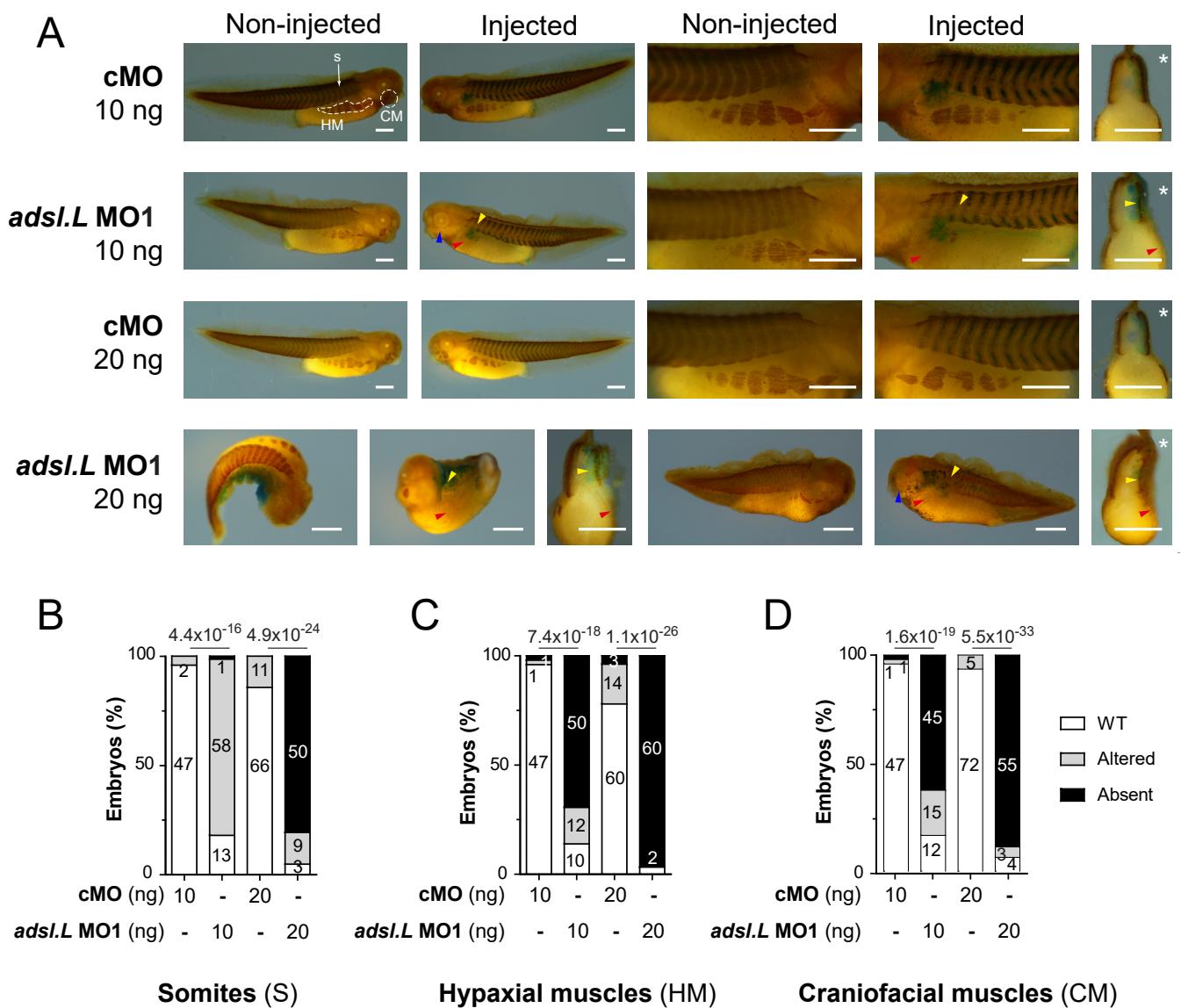
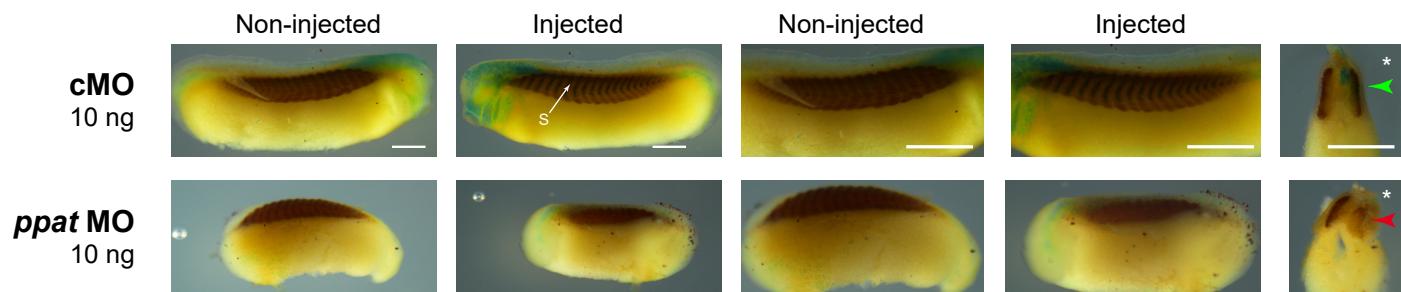
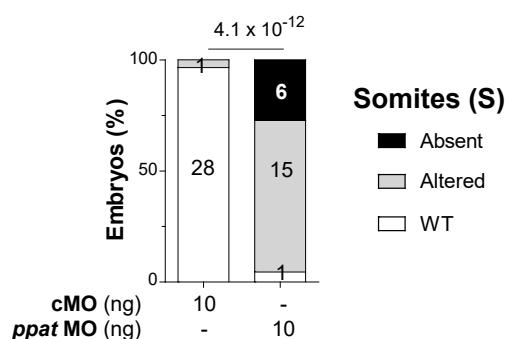


Figure S9. Severity of *adsL*. knock-down-associated phenotypes is morpholino dose-dependent. 12-101 immunolabelling revealed a strong alteration of myogenesis and somitogenesis (**A-B**), hypaxial (**A, C**) and craniofacial (**A, D**) muscle formation in *adsL*. morphant embryos. Representative images (**A**), quantification (embryo numbers in bars) and statistics of somite (**B**), hypaxial muscle (**C**) and craniofacial muscle (quadratoangularis + levator mandibularis longus) (**D**) phenotypes at tadpole stage. Yellow, blue and red arrowheads points to regions of somitic (S), craniofacial (CM) and hypaxial (HM) muscle defaults, respectively. Asterisks show injected side. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.

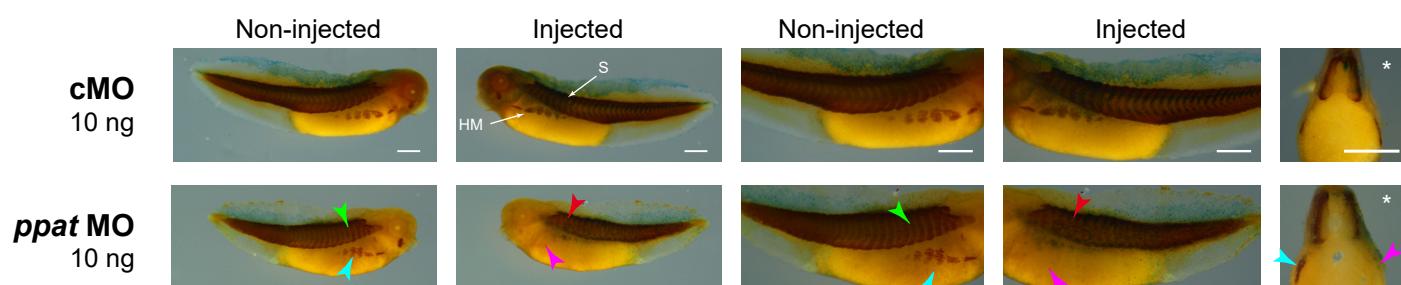
A

Tailbud Stage

B



C

Tadpole Stage

D

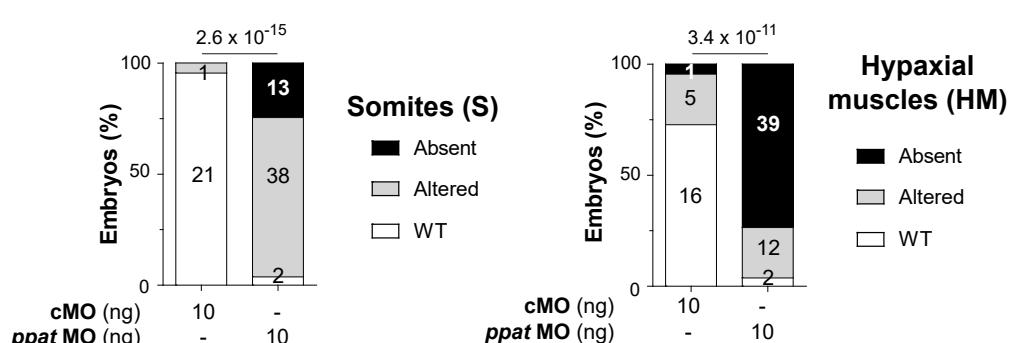
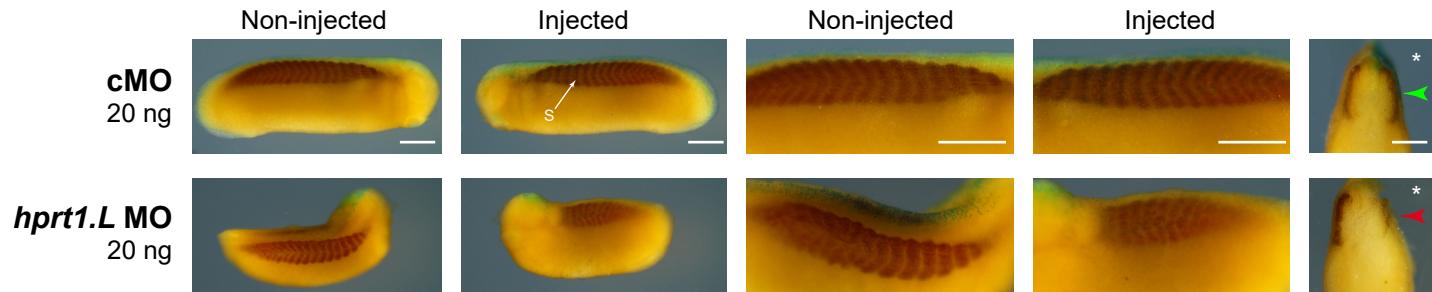
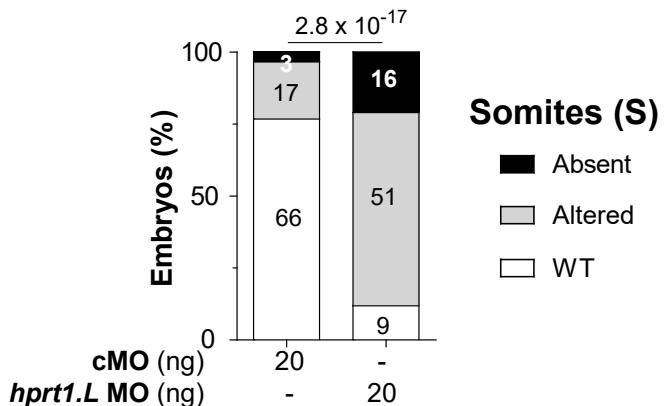


Figure S10. The *ppat.L* and *ppat.S* genes are required for somite, myotome and hypaxial muscle formation in *Xenopus laevis*. (A-D) A strong muscle alteration in *ppat* knock-down embryos is revealed by 12-101 immunolabelling. Representative images (A, C), quantification (embryo numbers in bars) and statistics (B, D) of somite and hypaxial muscle phenotypes at tailbud (A-B) and tadpole (C-D) stages. Green and red arrowheads point to normal v-shaped and altered somites, respectively; blue and pink arrowheads show normal and reduced 12/101 positive hypaxial muscle area, respectively. Injected side is indicated by asterisks. S: somites; HM: Hypaxial muscles. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.

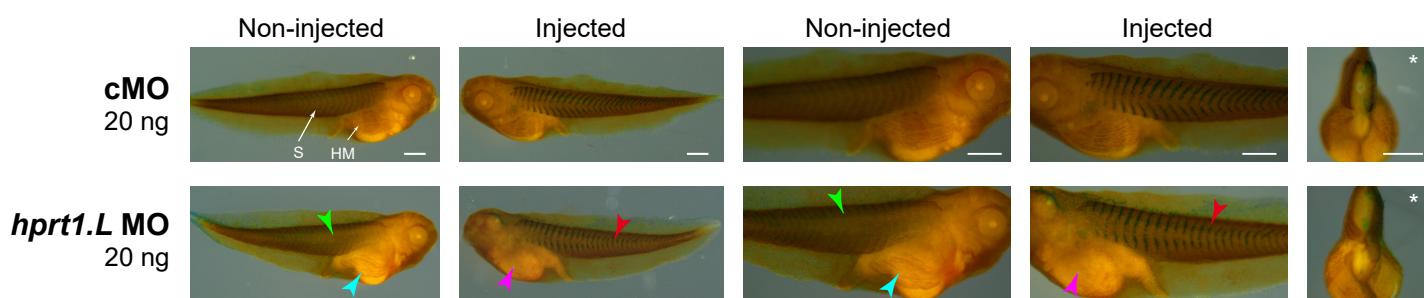
A

Tailbud stage

B



C

Tadpole Stage

D

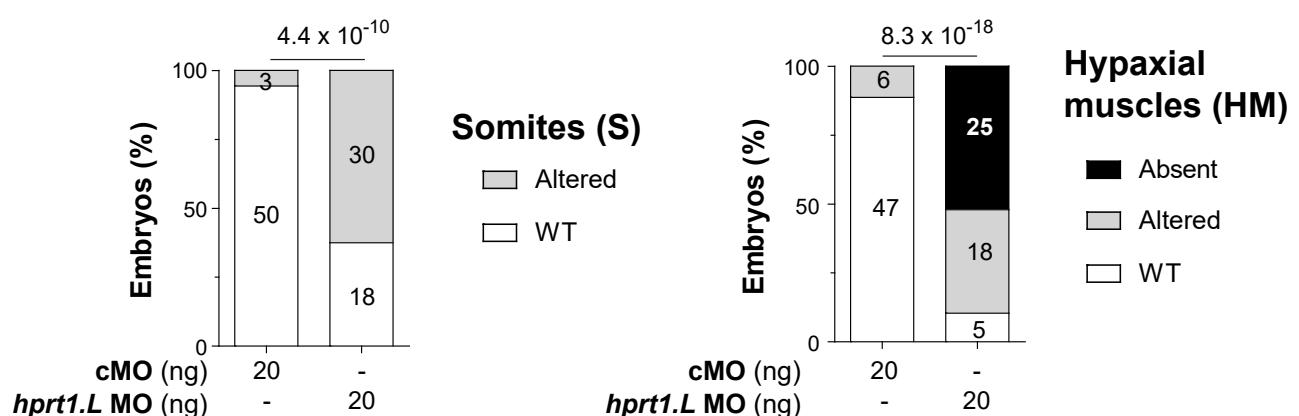


Figure S11. The *hprt1.L* gene is required for somite, myotome and hypaxial muscle formation in *Xenopus laevis*. (A-D) A strong muscle alteration is revealed by 12-101 immunolabelling in *hprt1.L* knock-down embryos. Representative images (A, C), quantification (embryo numbers in bars) and statistics (B, D) of somite and hypaxial muscle phenotypes at tailbud (A-B) and tadpole (C-D) stages. Green and red arrowheads point to normal v-shaped and altered somites, respectively; blue and pink arrowheads show normal and reduced 12/101 positive hypaxial muscle area, respectively. Injected side is indicated by asterisks. S: somites; HM: Hypaxial muscles. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.

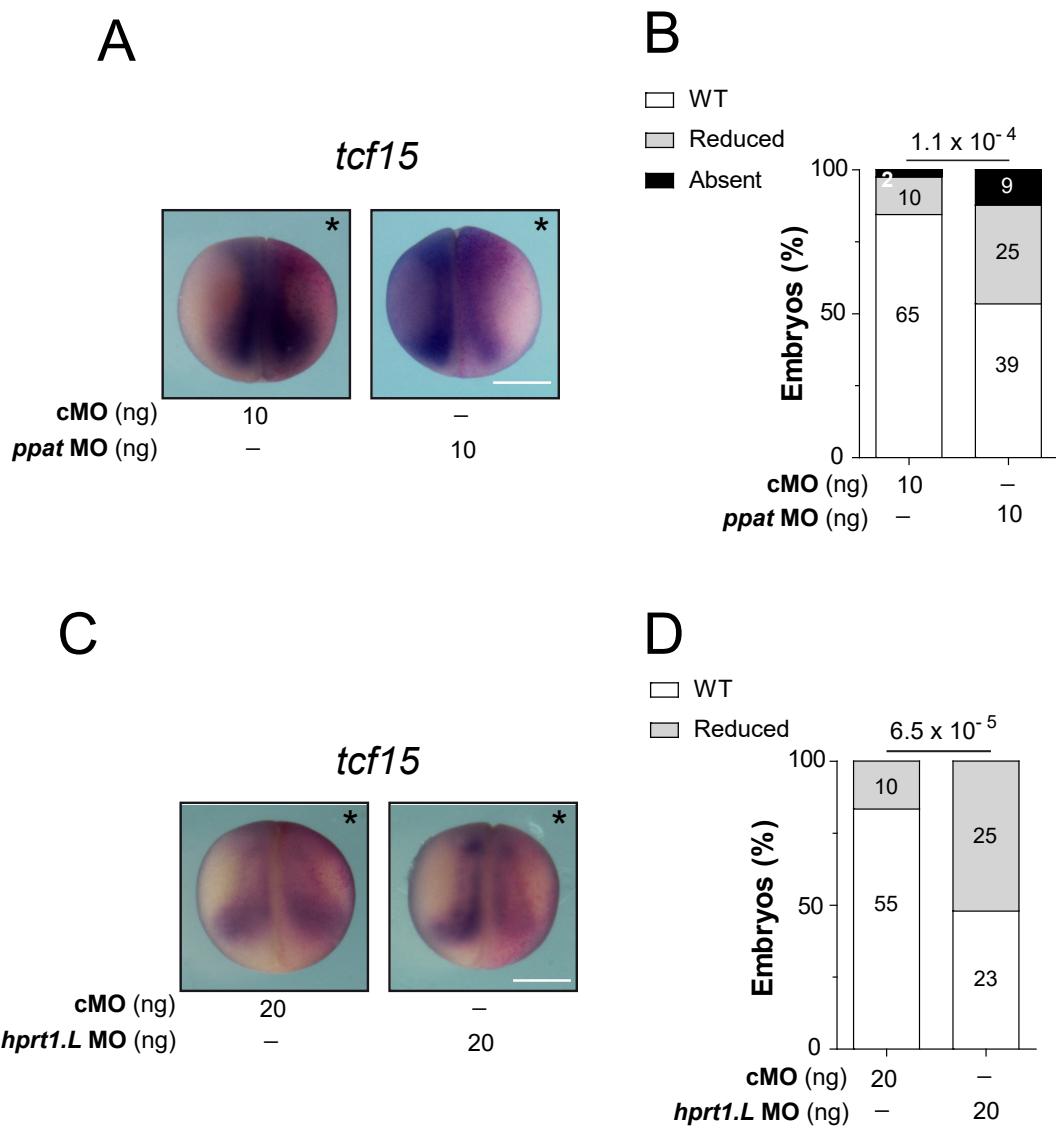


Figure S12. The *ppat.L/ppat.S* and *hprt1.L* genes are required for expression of *tcf15* gene. Expression of *tcf15* RNA was monitored by *in situ* hybridization at late neurula stage (stage 17-19) on embryos injected with the control morpholino (cMO) or the specific MO targeting either *ppat* or *hprt1.L* genes. Representatives images are presented for *ppat* (**A**) and *hprt1.L* (**C**) genes. Quantification (numbers in bars) and statistics of the *tcf15* expression phenotypes are presented in (**B**, **D**). Injected side is indicated by asterisks. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.

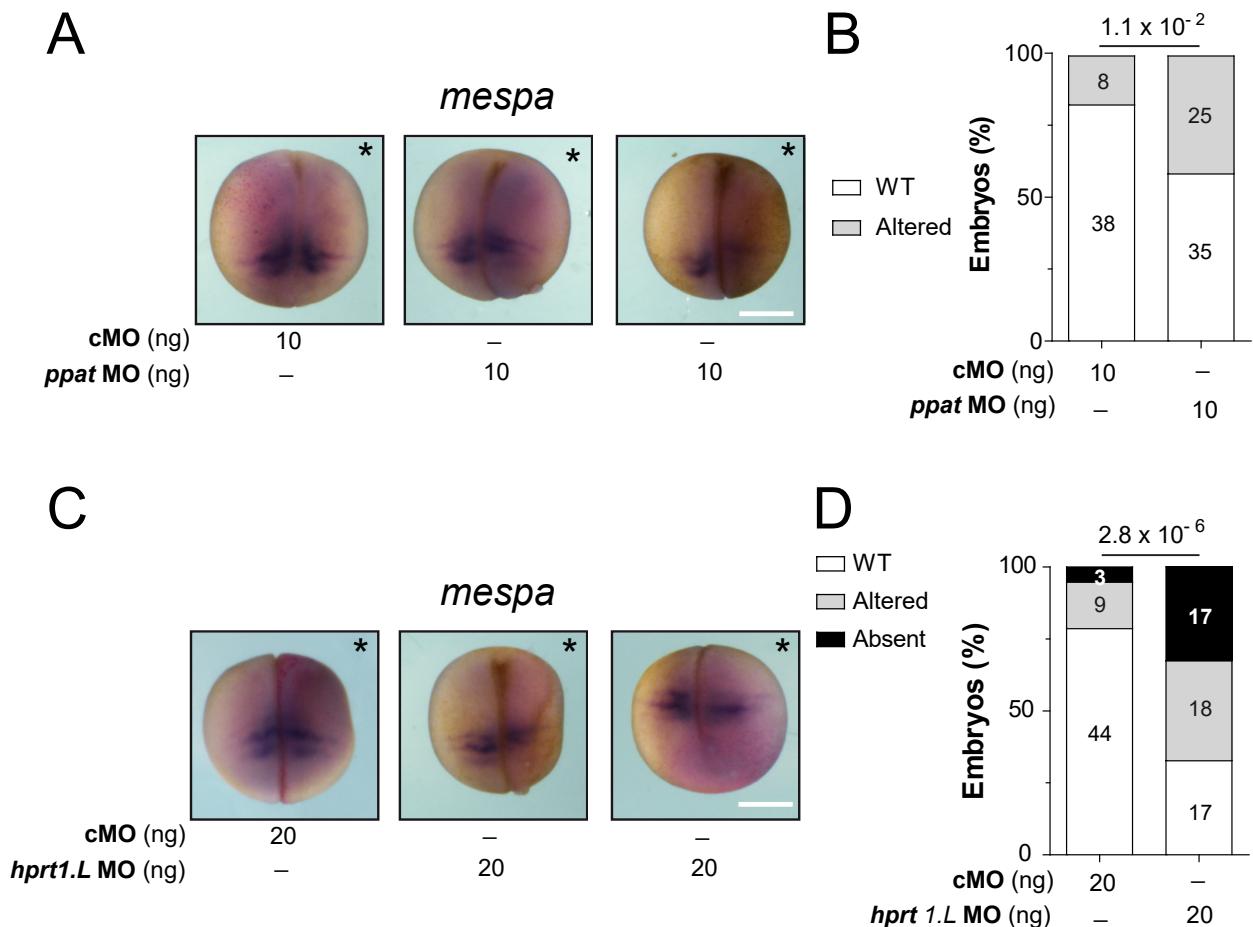


Figure S13. The *ppat.L/ppat.S* and *hpprt1.L* genes are required for *mespa* gene expression. Expression of *mespa* RNA was monitored by *in situ* hybridization at late neurula stage (stage 17-19) on embryos injected with the control morpholino (cMO) or the specific MO targeting either *ppat* or *hpprt1.L* genes. Representatives images are presented for *ppat* (**A**) and *hpprt1.L* (**C**) genes, respectively. Quantification (numbers in bars) and statistics of the *mespa* expression phenotypes are presented in (**B**, **D**). Injected side is indicated by asterisks. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.

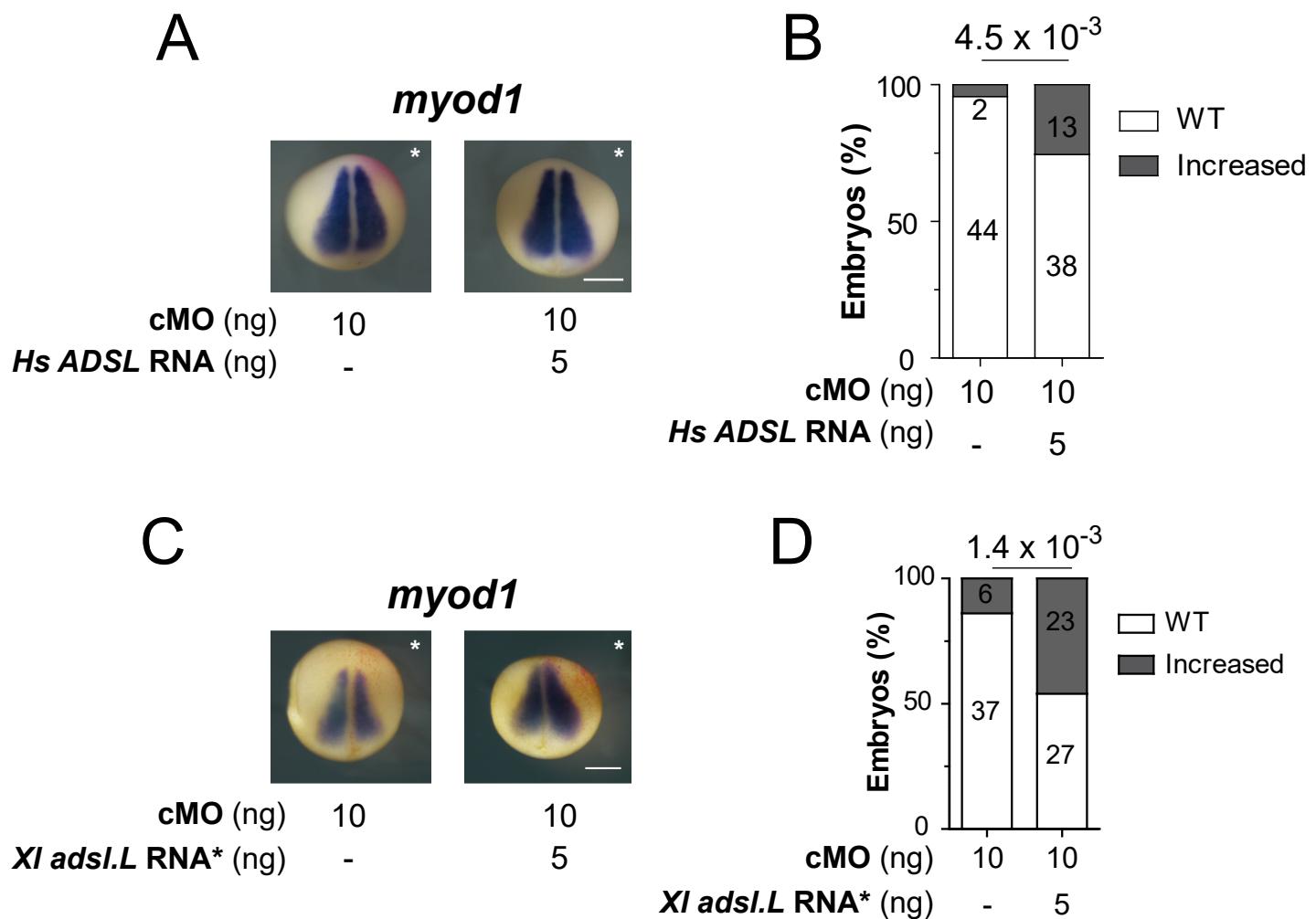


Figure S14. Effect of *H. sapiens* *ADSL* and *X. laevis* *adsl.L* RNA* on *myod1* expression at stage 12.5. (A, C) Expression of *myod1* was monitored by *in situ* hybridization at stage 12.5 on embryos injected with the control morpholino (cMO) and co-injected or not (-) with either the *H. sapiens* *ADSL* RNA (A) or the *X. laevis* MO non-targeted *adsl.L* RNA* (C). (B, D) Quantification (numbers in bars) and statistics of the *myod1* expression phenotypes are presented in (A, C). Injected side is indicated by asterisks. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.

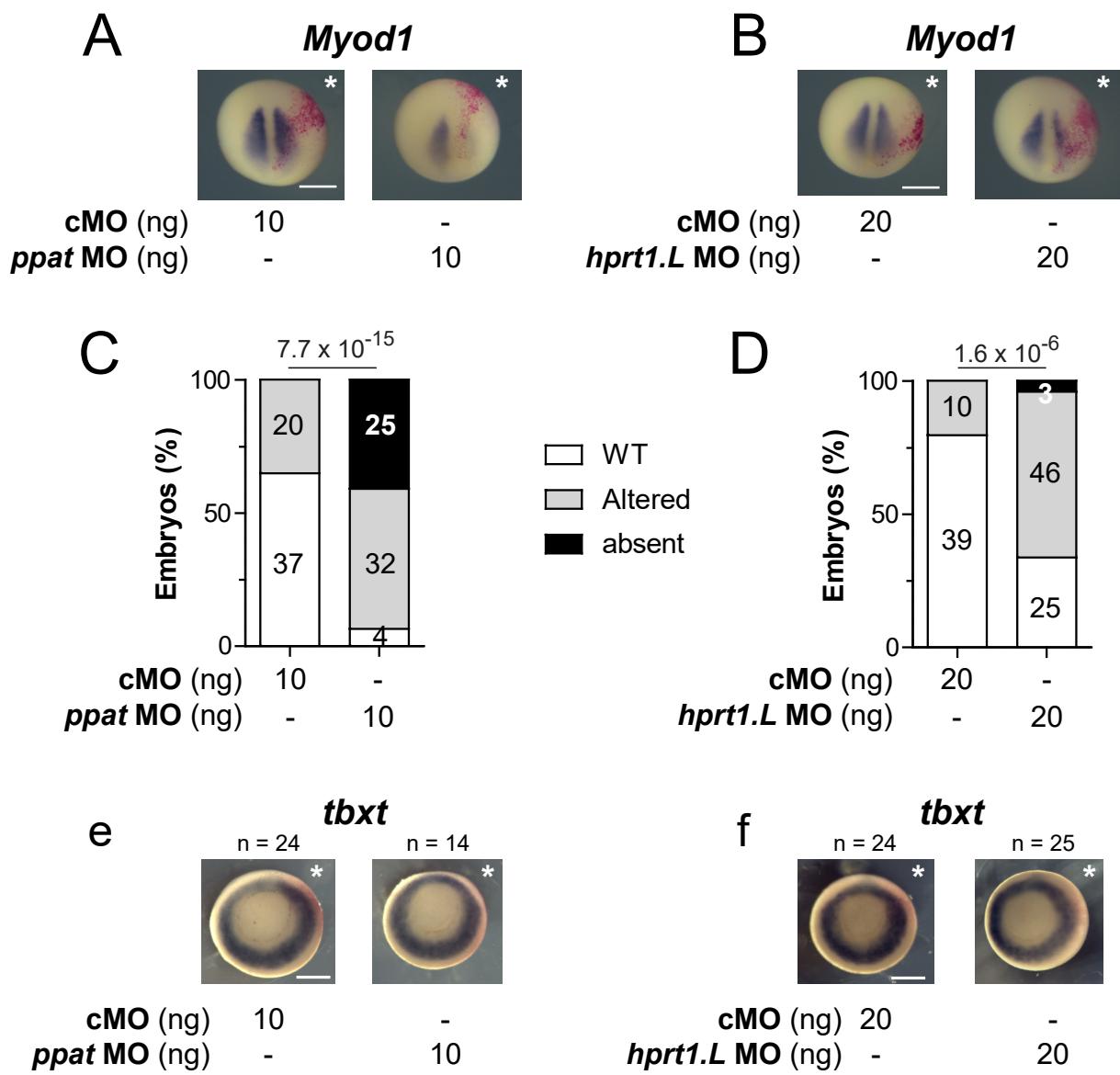
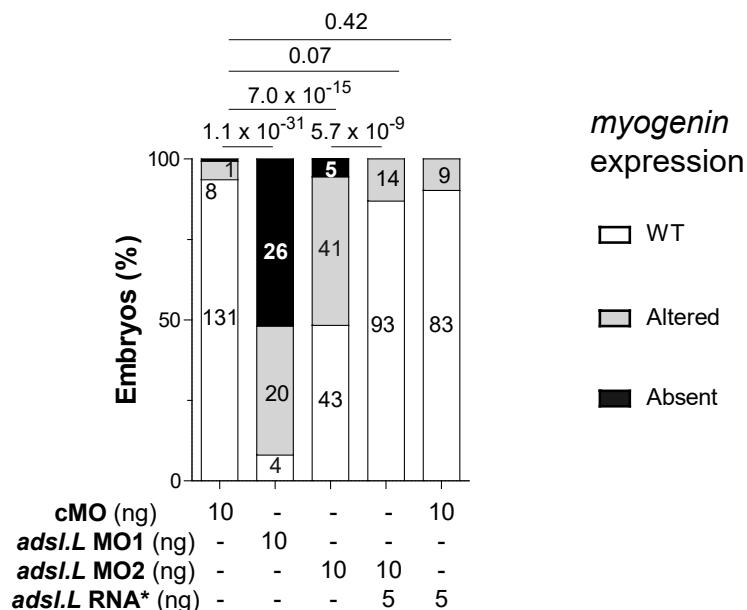
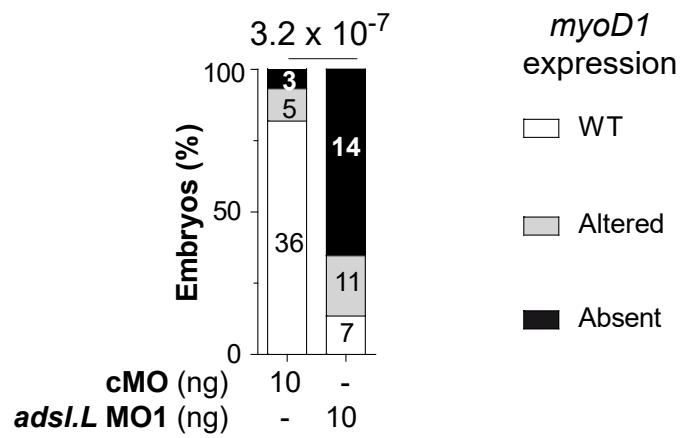


Figure S15. Expression of the myogenic regulatory factor *myod1* gene in the paraxial mesoderm is strongly affected by knock-down of *ppat* and *hprt1.L* genes. **(A-B)** Representative images of *myod1* expression alteration by the knock-down of either *ppat.L/ppat.S* (**A**) or *hprt1.L* (**B**) genes at stage 12.5, **(C-D)** Quantification (embryo numbers in bars) and statistics of the *myod1* expression phenotypes presented in **(A-B)**. **(E-F)** Knock-down of both *ppat.L/ppat.S* (**E**) or *hprt1.L* (**F**) genes has no significant effect on general mesoderm formation, as revealed by the pan-mesoderm *tbxt* (*xbra*) expression pattern at stage 11. Injected side is indicated by asterisks. Bars: 0.5 mm. Numbers above the bars in the histograms correspond to p-values.



Craniofacial muscles (CM)

Figure S16. Statistical analysis of the effects associated with *adsL.L* knock-down on *myogenin* expression in craniofacial muscles in late tailbud stage embryos. Quantification (numbers in bars) and statistics of the *myogenin* expression phenotypes presented in Figure 6A. Numbers above the bars in the histograms correspond to p-values.



Hypaxial muscles (HM)

Figure S17. Statistical analysis of the effects consecutive to *adsL.L* knock-down on *myoD1* expression in hypaxial muscles in late tailbud stage embryos. Quantification (numbers in bars) and statistics of the *myoD1* expression phenotypes presented in Figure 5C. Numbers above the bars in the histograms correspond to p-values.

Table S1: Yeast strains

Strain	Genotype	Reference
BY4741	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ</i>	Euroscarf
BY4742	<i>Mata ura3Δ leu2Δ his3Δ met15Δ</i>	Euroscarf
DS1-2B/1	<i>Matα ura3Δ ade2 apt1 aah1</i>	R. Woods
Y1036	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ ade1::kanMX4</i>	Lab collection
Y1057	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ ade4::kanMX4</i>	Lab collection
Y1059	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ ade5,7::kanMX4</i>	Lab collection
Y1063	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ ade8::kanMX4</i>	Lab collection
Y1095	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ ade16::kanMX4 ade17::kanMX4</i>	Lab collection
Y1133	<i>Mata ura3Δ leu2Δ his3Δ lys2Δ ade8::kanMX4</i>	Lab collection
Y3574	<i>Matα ura3Δ leu2Δ his3Δ ade1::kanMX4 ade13::kanMX4</i>	Lab collection
Y8093	<i>Matα ura3Δ leu2Δ hisΔ ade2::kanMX4 hpt1::kanMX4</i>	Lab collection
Y11114	<i>Matα ura3Δ leu2Δ his3Δ lys2Δ ade2::KanMX4 ade12::HIS3</i>	Lab collection

Table S2: Plasmids used for functional complementation in *S. cerevisiae*

Plasmid	I.M.A.G.E Clone used for amplification	Characteristics	Reference
pCM189	N/A	<i>CEN ARS URA3 tet-OFF promoter</i>	Gari 1997 ^a
p4930	IRBH990G1167D	<i>CEN ARS URA3 tet-atic.L. (X. laevis)</i>	This study
p4933	IRBH990H0610D	<i>CEN ARS URA3 tet-adsl.L. (X. laevis)</i>	This study
p5153	IRAKp961G14156Q	<i>CEN ARS URA3 tet-atic.S. (X. laevis)</i>	This study
p5255	IRBH990F0459D	<i>CEN ARS URA3 tet-paics.1.L. (X. laevis)</i>	This study
p5257	IRBH990G071D	<i>CEN ARS URA3 tet-paics.1.S. (X. laevis)</i>	This study
p5303	IRBH990C0135D	<i>CEN ARS URA3 tet-adss.1.S. (X. laevis)</i>	This study
p5304	IRBH990B1280D	<i>CEN ARS URA3 tet-adss.1.L. (X. laevis)</i>	This study
P5318	IRBH990B02030D	<i>CEN ARS LEU2 tet-hprt.L (X. laevis)</i>	This study
p5321	IRAKp961E17253Q	<i>CEN ARS URA3 tet-aprt.L (X. laevis)</i>	This study
p5551	IRBH990H1017D	<i>CEN ARS URA3 tet-ppat.L.(X. laevis)</i>	This study
p5400	IMAGp998O0914583Q	<i>CEN ARS URA3 tet-ppat.S (X. laevis)</i>	This study
p5550	IRBH990B02030D	<i>CEN ARS LEU2 tet-hprt.S (X. laevis)</i>	This study
p5697	IRAKp961P06157Q	<i>CEN ARS URA3 tet-paics.2 (X. laevis)</i>	This study
p5740	IMAGp998B1011965Q	<i>CEN ARS URA3 tet-gart (X. tropicalis)</i>	This study
P5744	IMAGp998B1011965Q	<i>CEN ARS URA3 tet-gars (X. tropicalis)</i>	This study
P5269	IRAKp961C16299Q	<i>CEN ARS LEU2 tet-airs (X. laevis)</i>	This study

^a E Garí, L Piedrafita, M Aldea, E Herrero A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in *Saccharomyces cerevisiae* **1997 Yeast** 13(9):837-48. DOI: 10.1002/(SICI)1097-0061(199707)13:9<837::AID-YEA145>3.0.CO;2-T

Table S3: Plasmids used for capped mRNA synthesis.

Linear. Enzyme: restriction enzyme used for linearization. *Hs*: *Homo sapiens*. * refers to morpholinos non-targeted sequences.

mRNA	Linear. Enzyme	Plasmids
<i>ads1.L</i>	<i>XhoI</i>	IRBH990H0610D
<i>ads1.L*</i>	<i>XhoI</i>	pBF- <i>adsl.L*</i> from IRBH990H0610D
<i>Hs ADSL</i>	<i>NotI</i>	pCS2 ⁺ - <i>HsADSL</i>
<i>ppat.L</i>	<i>XbaI</i>	IRBH990H1017D
<i>ppat.L*</i>	<i>SalI</i>	pBF- <i>ppat.L*</i> from IRBH990H1017D
<i>ppat.S</i>	<i>HindIII</i>	IMAGp998O0914583Q
<i>ppat.S*</i>	<i>XhoI</i>	pBF- <i>ppat.S*</i> from IMAGp998O0914583Q
<i>hprt1.L</i>	<i>NotI</i>	IRBH990B02030D
<i>hprt1.L*</i>	<i>SacI</i>	pBF- <i>hprt1.L*</i> from IRBH990B02030D
<i>LacZ</i>	<i>XhoI</i>	pSP6nucβgal

Table S4: Ribonucleotides probes used for *in situ* hybridization.

CDS: coding sequence. Linear. Enzyme : restriction enzyme used for linearization. pSK: p-BlueScript plasmid. RNA Pol.: RNA polymerase. UTR: untranslated region

Targeted genes	Probe	Linear. Enzyme	RNA Pol.	Plasmids
<i>adsl.L</i>	sense	<i>KpnI</i>	T7	p5265 <i>adsl.L</i> -pSK (CDS +3'UTR; 480 bp) from IRBHp990H0610D
	antisense	<i>SacI</i>	T3	p5265 <i>adsl.L</i> -pSK (CDS +3'UTR; 480 bp) from IRBHp990H0610D
<i>adss1.L</i> <i>adss1.S</i>	sense	<i>SacI</i>	T3	p5634 <i>adss1.L</i> -pSK (5'UTR + CDS; 444 bp) from IRBHp990C0135D
	antisense	<i>SalI</i>	T7	p5634 <i>adss1.L</i> -pSK (5'UTR + CDS; 444 bp) from IRBHp990C0135D
<i>adss2.L</i>	sense	<i>SacI</i>	T3	p5428 <i>adss2.L</i> -pKS (5'UTR + CDS; 444 bp) from IRBHp990E047D
	antisense	<i>SalI</i>	T7	p5428 <i>adss2.L</i> -pKS (5'UTR + CDS; 444 bp) from IRBHp990E047D
<i>aprt.L</i>	sense	<i>KpnI</i>	T7	p5435 <i>aprt.L</i> -pKS (CDS +3'UTR; 779 bp) from IRAKp961E17253Q
	antisense	<i>EcoRV</i>	T3	p5435 <i>aprt.L</i> -pKS (CDS +3'UTR; 779 bp) from IRAKp961E17253Q
<i>atic.L</i> <i>atic.S</i>	sense	<i>XhoI</i>	SP6	P4915 <i>atic.L</i> -pExpress1 (CDS +3'UTR; 1854 bp) from IRBHp990G1167D
	antisense	<i>EcoRI</i>	T7	P4915 <i>atic.L</i> -pExpress1 (CDS +3'UTR; 1854 bp) from IRBHp990G1167D
<i>hprt.L</i>	sense	<i>AseI</i>	T7	p5454 <i>hprt.L</i> -pKS (5'UTR + CDS; 592 bp) from IRB990B02030D
	antisense	<i>SacI</i>	T3	p5454 <i>hprt.L</i> -pKS (5'UTR + CDS; 592 bp) from IRB990B02030D
<i>gart.L</i>	sense	<i>BamHI</i>	T3	p5426 <i>gart.L</i> -pKS (CDS +3'UTR ; 530 bp) from IRAKp961C16299Q
	antisense	<i>EcoRV</i>	T7	p5426 <i>gart.L</i> -pKS (CDS +3'UTR ; 530 bp) from IRAKp961C16299Q
<i>ppat.L</i> <i>ppat.S</i>	sense	<i>NotI</i>	T3	p5527 <i>ppat.L</i> -pSK (CDS ; 481 bp) from XL.29008
	antisense	<i>EcoRV</i>	T7	p5527 <i>ppat.L</i> -pSK (CDS ; 481 bp) from XL.29008

Table S5: Oligonucleotides used for RT-PCR analyses. Amplification temperature and number of RT-PCR cycles were determined to obtain a single PCR product.

Amplified gene	Sense oligonucleotide (5'-3')	Antisense oligonucleotide (5'-3')	Amplification Temperature (°C)	Nb of cycles
<i>ppat.L</i>	GTTAGTCCCTGTGGCCGCT	GCCCCATGCCCTGTGCATT	54	28
<i>ppat.S</i>	GAAGCGCGAGGTGTGTGTG	GCCCCATGCCCTGTGCATT	58	29
<i>gart.L</i>	CAGAGACAGTTCTAGTGATTGG	GATAATCCCTGCTGCCAGAG	56	29
<i>pfas.S</i>	CTGACAGAACGTGCAGGG	CTAGTCTGGAACTTATGAG	53	29
<i>paics.1.S</i>	CAGAACACCACGTGGTACTGCC	GATCCCAGCCTCCTGCAGC	56	30
<i>paics.1.L</i>	GAAATGGAGTCTTACGCAGAAC	GATCCCAGCCTCCTGCAGC	53	27
<i>paics.2.L</i>	GCATTGTTAAGAGGTGCAG	CCAAACTTAATCCTCATGTCC	55	28
<i>adsl.L</i>	ATGGCCTTCAACTTCAGCGA	AACGTTGGCATCTCTGCGTA	55	28
<i>atic.L</i>	GCTGCTAGCGACTTATCCAG	GGGTACAGGTTACACACAAC	57	31
<i>atic.S</i>	GCTGTTGCGGAGATGGAG	GGGTACAGGTTACACACAAC	53	30
<i>adss1.L</i>	CAAGTGGCATTATCAACCCCC	CTTTGGATGAATATGTTGGTC	51	30
<i>adss1.S</i>	GTGGCATTATAAATCCTAAAGC	CTTTGGATGAATATGTTGGTC	53	29
<i>Adss2.L</i>	CCGCTACAGTAAGCGTAAC	GCTTCATAGGAAATGGTGTG	51	28
<i>hpri1.L</i>	CTAACATTATGCAGCCGATC	CATTCTGCCTGTCAAGGTGG	54	26
<i>hpri1.S</i>	CTAAACACTACGCCGCCAGC	CATTCTGCCTGTCAAGGTGG	52	29
<i>aprt.L</i>	CAGATTATGTCCGATCAGGAG	GCTAACAGATTCTGTGGGAC	57	29
<i>gmps.L</i>	GAGCGATGGCAGAGATC	CTGTAAGAGCGACAGTCC	56	28
<i>gmps.S</i>	GAGGGTTGGGTAGAGAAC	CTGTAAGAGCGACAGTCC	53	29
<i>odc1.L</i>	GTCAATGATGGAGTGTATGGATC	TCCATTCCGCTCCTGACCAC	55	23

Table S6: Comparison of the purine biosynthesis pathways encoding genes and proteins between, *X. laevis*, *H. sapiens* and *X. tropicalis*. Alignments were performed using <https://blast.ncbi.nlm.nih.gov>. * XB: xenbase: <https://www.xenbase.org/entry/>

<i>Xenopus laevis</i>			<i>Homo sapiens</i>				<i>Xenopus tropicalis</i>					
Protein name	Accession Number	XB* Gene ID	Protein name	Accession Number	Identity %	Coverage %	Protein name	Accession Number	Identity %	Coverage %	Enzymatic activity	
Ada.L	XP_018090401.1	17336388	ADA	NP_000013.2	74	97	Ada	NP_001011025.1	97	100	Adenosine deaminase	
Ada.S	NP_001085740.1	950506			70	98						
Ada2.L	NP_001090531.1	6254244	ADA2	NP_001269154.1	57	96	Ada2	XP_031754454.1	90	99	Adenosine deaminase	
Ada2.S	NP_001089165	6251688	ADA2		59	97	Ada2		88	100		
Ada.2.S	NP_001087740.1	5929096	ADA.2	No significant homolog			Ada.2	NP_001107369.1	90	100	Adenosine deaminase	
Adk.S	NP_001086357.1	997231	ADK	NP_001114.2	82	95	Adk	NP_001016698	98	99	Adenosine kinase	
Adsl.L	NP_001080593	380285	ADSL	NP_000017.1	83	93	Adsl	NP_001005457.1	96	100	Adenylosuccinate lyase	
Adssl1.L	NP_001090012.1	5758296	ADSS1	NP_689541.1	88	100	Adssl1	NP_001004939.1	97	100	Adenylosuccinate synthase	
Adssl1.S	NP_001087505.1	6254009	ADSS1		87	100	Adssl1		96	100		
Adss2.L	NP_001080088.1	944129	ADSS2	NP_001117.2	86	94	Adss2	NP_989047.1	95	100	Adenylosuccinate synthase	
Ak1.L	NP_001087683.1	6253770	AK1	NP_001305051.1	74	98	Ak1	NP_001006817.1	91	97	Adenylate kinase	
Ak1.S	NP_001085451.1	6251725	AK1		81	98	Ak1		100	100		
Ak2.L	XP_018102289.1	17344313	AK2	NP_001616.1	82	100	Ak2	XP_012812152.1	96	100	Adenylate kinase	
Ak2.S	NP_001080232.1	6254479	AK2		79	100	Ak2		97	100		
Ak3.L	NP_001089446.1	977031	AK3	NP_057366.2	74	97	Ak3	XP_012812420.1	90	100	Adenylate kinase	
Ak3.S	NP_001084561.1	17332462	AK3		75	97	Ak3		92	100		
Ak4.L	XP_018113718.1	17341782	AK4	NP_001005353.1	77	96	Ak4	XP_002931643.1	96	100	Adenylate kinase	
Ak4.S	XP_018116248.1	959957			74	94	Ak4		97	100		
Ak5.L	XP_018113675.1	6487811	AK5	AAH36666.1	72	100	Ak5	XP_012815974.2	93	100	Adenylate kinase	
Ak5.S	XP_018116212.1	17345311	AK5	NP_777283.1	71	100	Ak5		92	100		
Ak6.L	NP_001089528.1	972297	AK6	NP_057367.1	76	100	Ak6	NP_001017167.1	95	100	Adenylate kinase	
Ak6.S	NP_001087040.1	17335043	AK6		78	100	Ak6		97	100		

Ak7.S	NP_001081046.1	953552	AK7	NP_689540.2	63	99	Ak7	NP_001011352.1	93	100	Adenylate kinase
Ak8.L	NP_001088862.1	5831351	AK8	NP_689785.1	55	98	Ak8	NP_989104.1	90	100	Adenylate kinase
Ak9.L	XP_018118807	17335922	AK9	NP_001316531.1	52	34	Ak9	XP_031757751.1	85	96	
Ak9.S	XP_018118808	17335923	AK9		52	34	Ak9		85	96	Adenylate kinase
Ampd1.L	XP_018101841.1	17335636	AMPD1	NP_000027.2	74	99	Ampd1	XP_002935821.2	95	100	
Ampd1.S	XP_018104675.1	17335637	AMPD1		72	99	Ampd1		92	100	Adenosine monophosphate deaminase
Ampd2.S	XP_018104829	6489067	AMPD2	NP_001355738.1	80	96	Ampd2	XP_031752724.1	96	100	Adenosine monophosphate deaminase
Ampd3.L	XP_018112762.1	6489056	AMPD3	NP_000471.1	80	99	Ampd3	NP_001025687.1	94	100	
Ampd3.S	XP_018115800.1	17344991	AMPD3		80	99	Ampd3		95	100	Adenosine monophosphate deaminase
Aprt.L	XP_018096991.1	6253243	APRT	NP_000476.1	65	100	Aprt	NP_001007941.1	93	100	Adenine phosphoribosyltransferase
Atic.L	NP_001090100.1	998812	ATIC	NP_004035.2	78	99	Atic	NP_001005460.1	95	100	
Atic.S	XP_018094436.1	17331460	ATIC		79	99	Atic		96	100	Amino-Imidazole CarboxAmide Ribonucleotide transformylase and IMP cyclohydrolase
Gda.L	NP_001083074.1	987047	GAH	NP_004284.1	61	93	Gda	XP_004910825.1	93	96	
Gda.S	XP_018099342.1	17337222	GAH		60	99	Gda		83	99	Guanine deaminase
Gart.L	NP_001093352.1	6251947	GART	NP_000810.1	75	98	Gart	XP_012813454.1	92	98	Phosphoribosylglycinamide-formyltransferase, phosphoribosylglycinamide synthetase and phosphoribosylaminoimidazole synthetase
Gmps.L	XP_018119259.1	950699	GMPS	NP_003866.1	92	98	Gmps	XP_002933290.3	97	98	
Gmps.S	XP_018121231.1	17340569	GMPS		93	100	Gmps		98	100	Guanine monophosphate synthase
Guk1.L	NP_001087146.1	6254574	GUK	NP_000849.1	70	97	Guk1	NP_001034818.1	89	100	
Guk1.S	NP_001086807.1	17334557	GUK		69	97	Guk1		90	100	Guanylate kinase
Hprt1.L	NP_001090235.1	6078713	HGPRT	NP_000185.1	89	99	Hprt1	NP_989312.1	100	100	Hypoxanthine-Guanine phosphorybosyl transferase
Impdh1.L	NP_001080792	957455	IMPDH1	NP_000874.2	92	100	Impdh1	NP_001017283.1	98	100	Inosine monophosphate dehydrogenase
Impdh2.L	NP_001083990.1	17345296	IMPDH2	NP_000875.2	93	100	Impdh2	NP_001008066.1	98	100	
Impdh2.S	NP_001082410.1	478732	IMPDH2		93	100	Impdh2		97	100	Inosine monophosphate dehydrogenase
Paics.1.L	NP_001080163	17334793	PAICS	NP_001072992.1	81	100	Paics.1	XP_012811124.2	96	100	
Paics.1.S	NP_001086248.1	965998	PAICS		82	98	Paics.1		95	100	Phosphoribosylaminoimidazole carboxylase Phosphoribosylaminoimidazolesuccinocarboxamide synthetase

Paics.2.L	XP_018085590.1	5872998	PAICS	NP_001072992.1	57	37	Paics.2	NP_001090685.1	86	97	Phosphoribosylaminoimidazole carboxylase Phosphoribosylaminoimidazolesuccinocarboxamide synthetase
Pfas.S	XP_018094887.1	6485675	PFAS	NP_036525.1	65	99	Pfas	XP_012825646.2	93	99	Phosphoribosylformylglycinamide synthase
Pnp.L	NP_001079809.1	1011603	PNP	NP_000261.2	68	95	Pnp	NP_001006720.1	88	98	Purine nucleoside phosphorylase
Pnp.S	XP_018099448.1	17337633	PNP	NP_002694.3	66	93	Pnp	NP_001006720.1	90	100	
Ppat.L	NP_001083491.1	972209	GPAT	NP_002694.3	80	100	Ppat	NP_989313.1	96	100	Phosphoribosylpyrophosphate amidotransferase
Ppat.S	XP_041435416	17340761	GPAT	NP_002694.3	79	99	Ppat	NP_989313.1	96	100	
Xdh.L	XP_018117710.1	6485774	XDH	NP_000370.2	70	99	Xdh	XP_031758260.1	89	100	Xanthine dehydrogenase/oxidase
Xdh.S	XP_018120131.1	17331587	XDH	NP_000370.2	70	92	Xdh	XP_031758260.1	90	100	

Table S7: Comparison of the purine biosynthesis pathways encoding genes and proteins between *X. laevis* and *S. cerevisiae*. Alignments were performed using <https://blast.ncbi.nlm.nih.gov>. * XB: xenbase: <https://www.xenbase.org/entry/>.

^a No significant alignment between the yeast and *X. Laevis* Hprt1.L entire sequences.

ada: adenine deaminase; xdh: xanthine dehydrogenase/oxidase

<i>Xenopus laevis</i>			<i>Saccharomyces cerevisiae</i>						
Protein name	Accession Number	XB* Gene ID	Protein name	Accession Number	Identity %	SGD Locus ID	Coverage %	Enzymatic activity	
Ada.L Ada.S	XP_018090401.1 NP_001085740.1	17336388 950506		No Ada activity in S.c.				Adenosine deaminase	
Ada2.L Ada2.S	NP_001090531.1 NP_001089165	6254244 6251688		No Ada activity in S.c.				Adenosine deaminase	
Ada.2.S	NP_001087740.1	5929096	ADA.2	No Ada activity in S.c.				Adenosine deaminase	
Adk.S	NP_001086357.1	997231	Ado1	NP_012639.1	40	YJR105W	91	Adenosine kinase	
Adsl.L	NP_001080593	380285	Ade13	NP_013463.1	64	YLR359W	92	Adenylosuccinate lyase	
Adssl1.L Adssl1.S	NP_001090012.1 NP_001087505.1	5758296 6254009	Ade12	NP_014179.1	57 57	YNL220W	92 92	Adenylosuccinate synthase	
Adss2.L	NP_001080088.1	944129	Ade12	NP_014179.1	56	YNL220W	92	Adenylosuccinate synthase	
Ak1.L Ak1.S	NP_001087683.1 NP_001085451.1	6253770 6251725	Adk1	NP_010512.1	28 28	YDR226W	84 92	Adenylate kinase	
Ak2.L Ak2.S	XP_018102289.1 NP_001080232.1	17344313 6254479	Adk1	NP_010512.1	55 59	YDR226W	94 90	Adenylate kinase	
Ak3.L Ak3.S	NP_001089446.1 NP_001084561.1	977031 17332462	Adk2	NP_011097.3	46 45	YER170W	86 86	Adenylate kinase	
Ak4.L	XP_018113718.1	17341782	Adk2	NP_011097.3	43	YER170W	83	Adenylate kinase	

Ak4.S	XP_018116248.1	959957			46		83	
Ak5.L	XP_018113675.1	6487811	Adk2	NP_011097.3	27	YER170W	61	Adenylate kinase
Ak5.S	XP_018116212.1	17345311			25		58	
Ak6.L	NP_001089528.1	972297		none				Adenylate kinase
Ak6.S	NP_001087040.1	17335043						
Ak7.S	NP_001081046.1	953552	Adk1	NP_010512.1	29	YDR226W	16	Adenylate kinase
Ak8.L	NP_001088862.1	5831351		none				Adenylate kinase
Ak9.L	XP_018118807	17335922	Adk1	NP_010512.1	24	YDR226W	14	Adenylate kinase
Ak9.S	XP_018118808	17335923			24		14	
Ampd1.L	XP_018101841.1	17335636	Amd1	NP_013677.1	51	YML035C	68	Adenosine monophosphate deaminase
Ampd1.S	XP_018104675.1	17335637			51		68	
Ampd2.S	XP_018104829	6489067	Amd1	NP_013677.1	53	YML035C	82	Adenosine monophosphate deaminase
Ampd3.L	XP_018112762.1	6489056	Amd1	NP_013677.1	55	YML035C	64	Adenosine monophosphate deaminase
Ampd3.S	XP_018115800.1	17344991			48		80	
Aprt.L	XP_018096991.1	6253243	Apt1	NP_013690.1	47	YML022W	93	Adenine phosphoribosyltransferase
Atic.L	NP_001090100.1	998812	Ade17	NP_013839.1	62	YMR120C	99	Amino-Imidazole CarboxAmide
Atic.S	XP_018094436.1	17331460	Ade16	NP_013128.1	62	YLR028C	99	Ribonucleotide transformylase
			Ade17	NP_013839.1	61	YMR120C	99	and IMP cyclohydrolase
Gda.L	NP_001083074.1	987047	Gud1	NP_010043.1	39	YDL238C	96	Guanine dearnnase
Gda.S	XP_018099342.1	17337222			39		91	
Gart.L	NP_001093352.1	6251947	Ade5,7	NP_011280.1	46	YGL234W	96	Phosphoribosylglycinamide formyltransferase,
			Ade8	NP_010696.3	29	YDR408C	31	phosphoribosylglycinamide synthetase
								and phosphoribosylaminoimidazole synthetase
Gmps.L	XP_018119259.1	950699	Gua1	NP_013944.1	37	YMR217W	93	Guanine monophosphate synthase
Gmps.S	XP_018121231.1	17340569		NP_013944.1	36		94	

Guk1.L	NP_001087146.1	6254574	Guk1	NP_010742.1	55 55	YDR454C	92 92	Guanylate kinase
Hprt1.L	NP_001090235.1	6078713	Hpt1	NP_010687.3	34 ^a	YDR399W	20 ^a	Hypoxanthine-Guanine phosphorybosyl transferase
Impdh1.L	NP_001080792	957455	Imd2 Imd3 Imd4	NP_012088.3 NP_013536.3 NP_013656.1	60 63 63	YHR216W YLR432W YML056C	97 97 97	Inosine monophosphate dehydrogenase
Ilmpdh2.L	NP_001083990.1	17345296	Imd2 Imd3 Imd4	NP_012088.3 NP_013536.3 NP_013656.1	61 64 63	YHR216W YLR432W YML056C	96 96 98	Inosine monophosphate dehydrogenase
Ilmpdh2.S	NP_001082410.1	478732	Imd2 Imd3 Imd4	NP_012088.3 NP_013536.3 NP_013656.1	62 64 63	YHR216W YLR432W YML056C	96 96 97	Inosine monophosphate dehydrogenase
Paics.1.L	NP_001080163	17334793	Ade1 Ade2	NP_009409.1 NP_014771.3	25 31	YAR015W YOR128C	51 29	Phosphoribosylaminoimidazole carboxylase
Paics.1.S	NP_001086248.1	965998	Ade1	NP_009409.1	26	YAR015W	51	phosphoribosylaminoimidazolesuccinocarboxamide synthetase
Paics.2.L	XP_018085590.1	5872998	Ade2	NP_014771.3	31	YOR128C	14	Phosphoribosylaminoimidazole carboxylase phosphoribosylaminoimidazolesuccinocarboxamide synthetase
Pfas.S	XP_018094887.1	6485675	Ade6	NP_011575.1	37	YGR061C	93	Phosphoribosylformylglycinamidine synthase
Pnp.L	NP_001079809.1X	1011603	Pnp1	NP_013310.1	52	YLR209C	87	Purine nucleoside phosphorylase
Pnp.S	P_018099448.1	17337633			45		91	
Ppat.L	NP_001083491.1	972209	Ade4	NP_014029.1	36	YMR300C	91	Phosphoribosylpyrophosphate amidotransferase
Ppat.S	XP_041435416	17340761			36		92	
Xdh.L	XP_018117710.1	6485774		No Xdh activity in <i>S.cerevisiae</i> .				Xanthine dehydrogenase/oxidase
Xdh.S	XP_018120131.1	17331587						