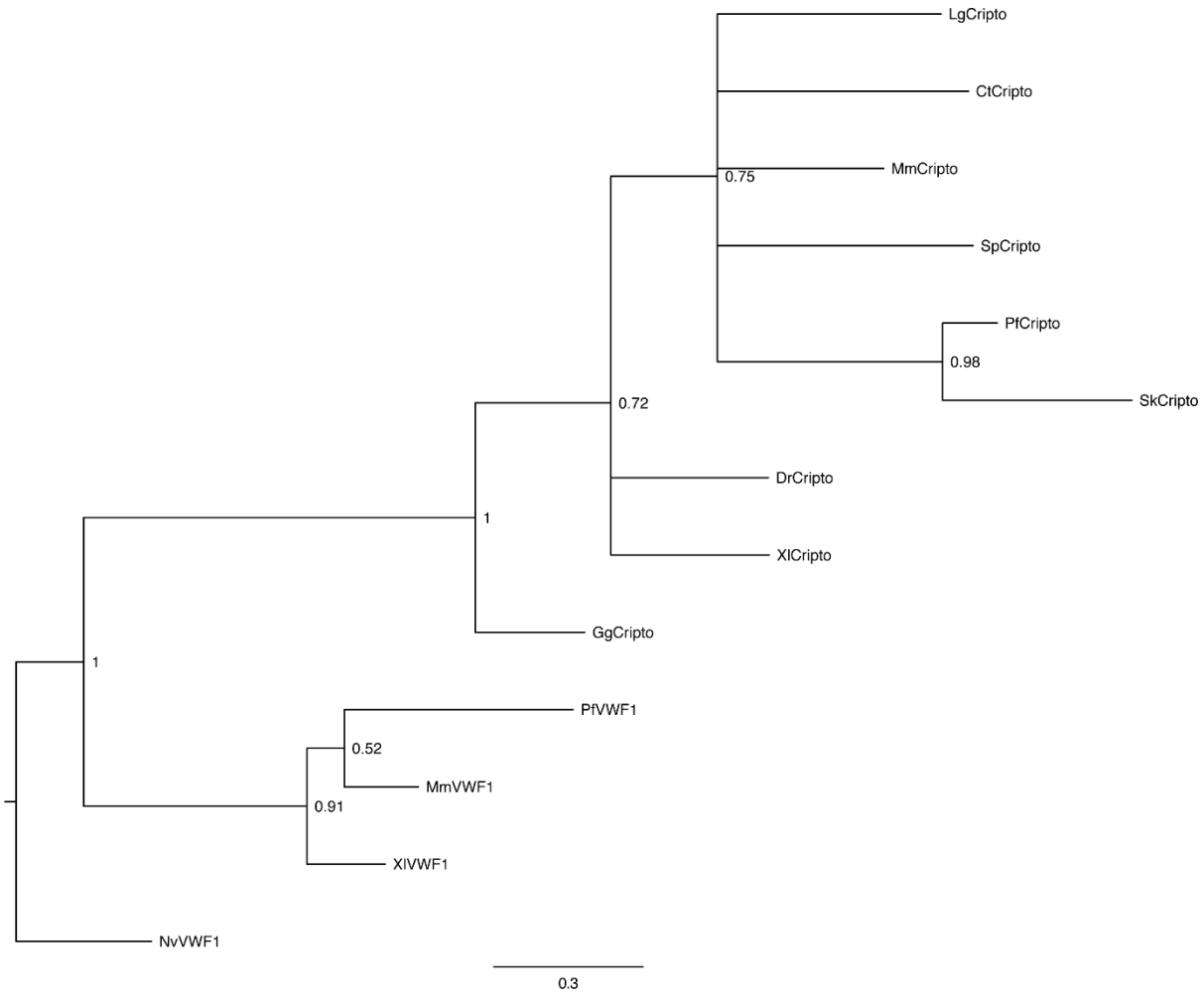
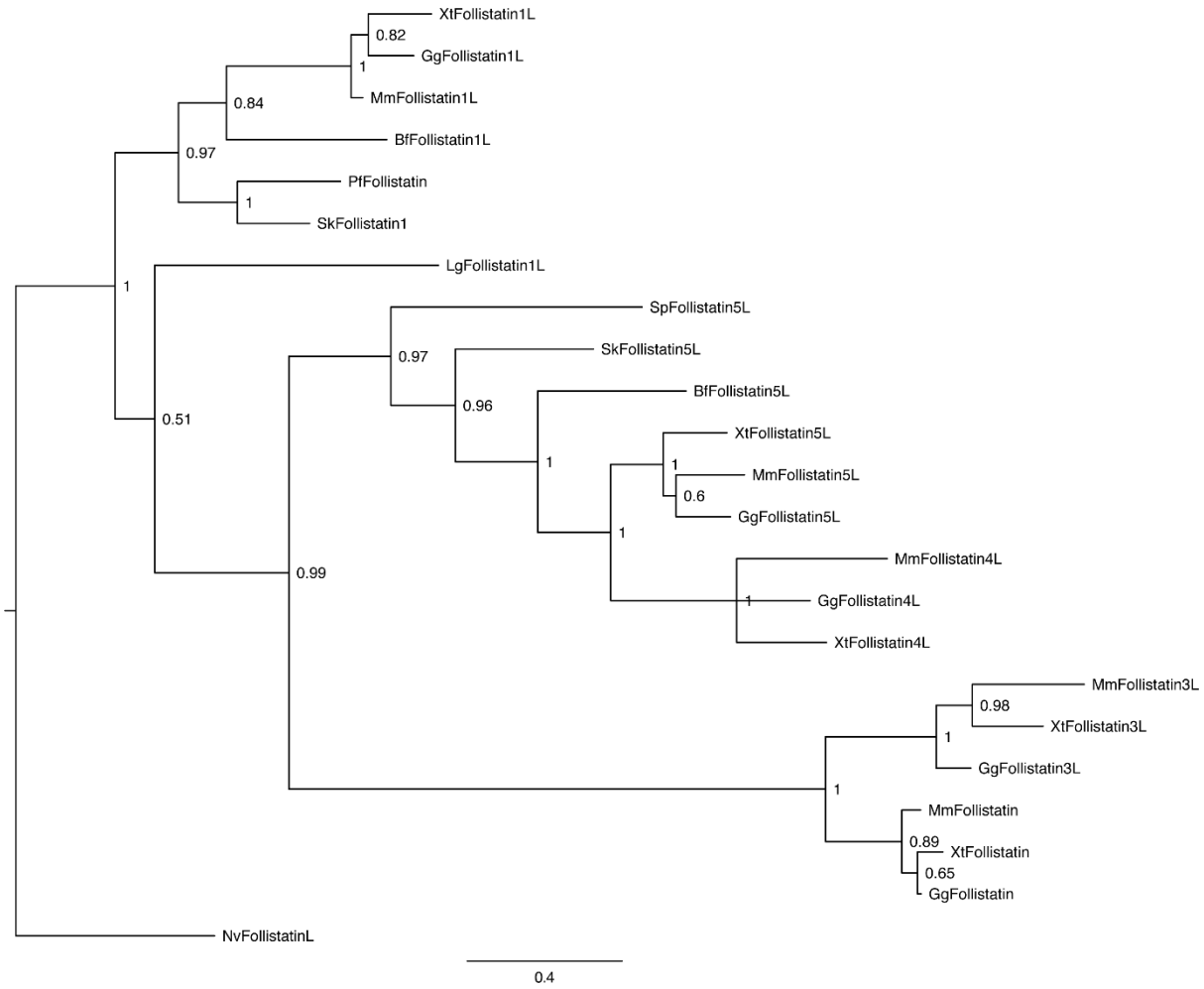
Phylogenetic trees

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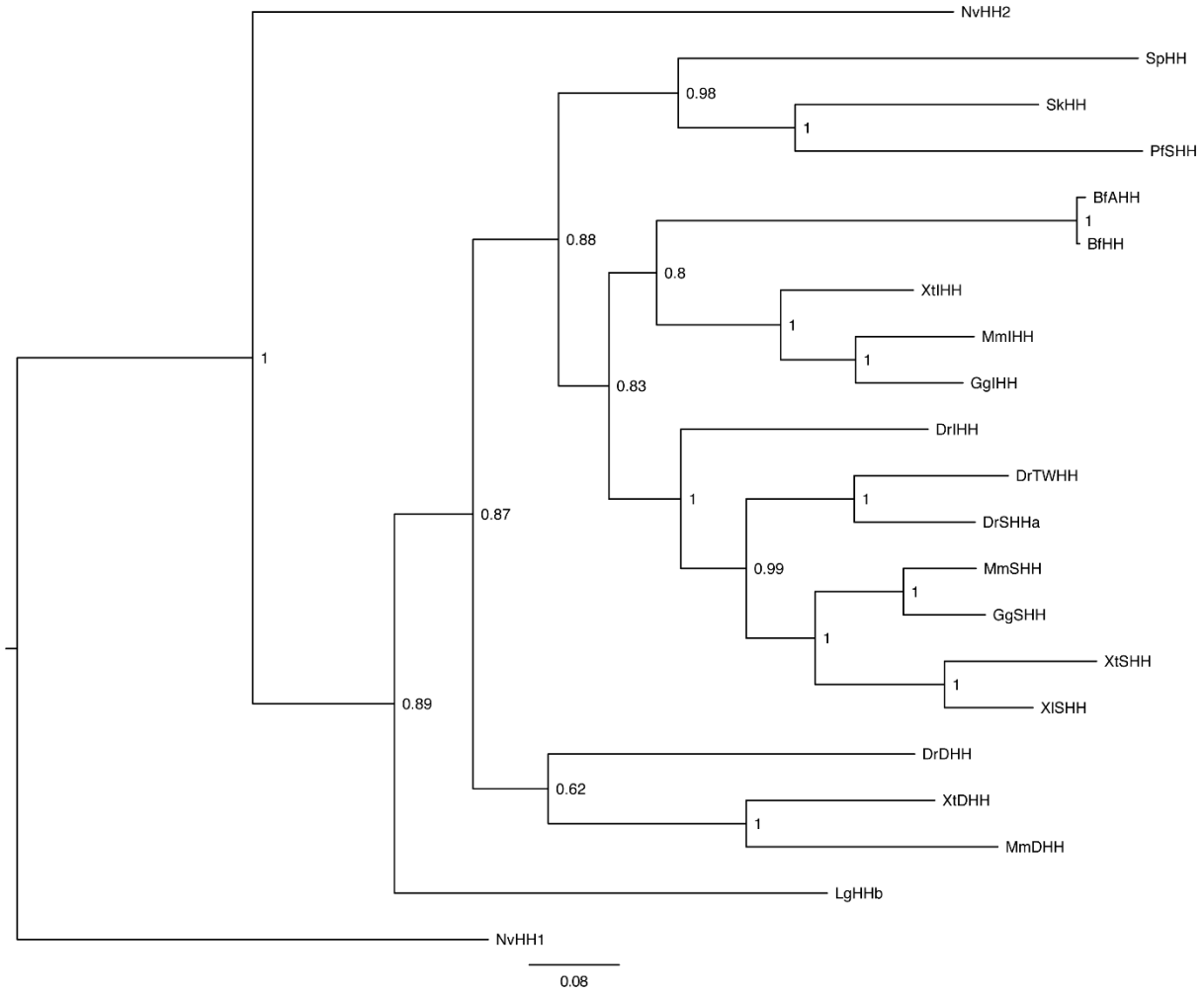
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GgSHH
XtSHH
XlSHH
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XtDHH
MmDHH
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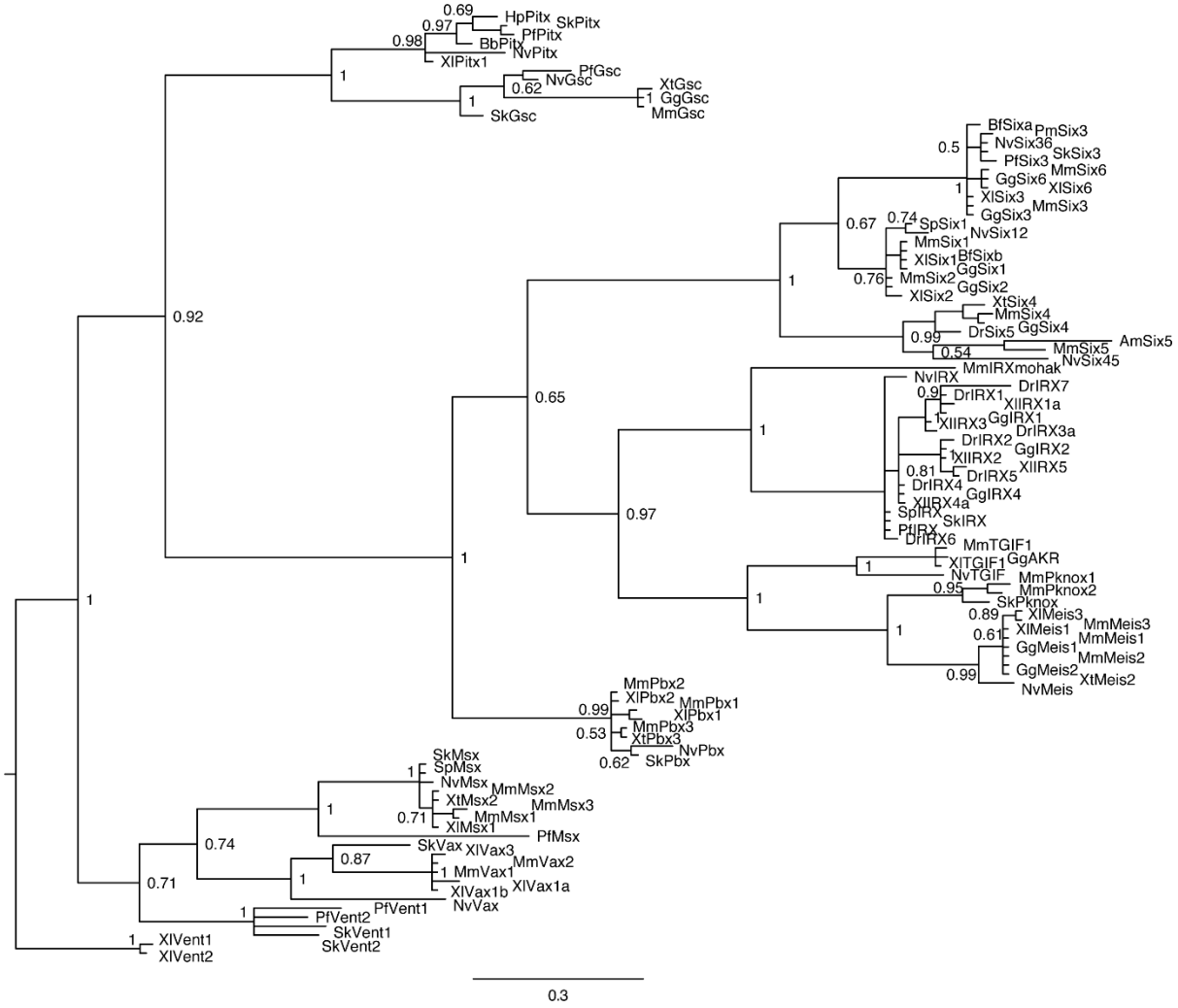
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SkGsc
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PmSix3
NvSix36
SkSix3
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MmSix6
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XlSix3
MmSix3
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SpSix1
NvSix12
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XlSix1
BfSixb
GgSix1
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GgIRX2
XlIRX2
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GgMeis2
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MmVax2
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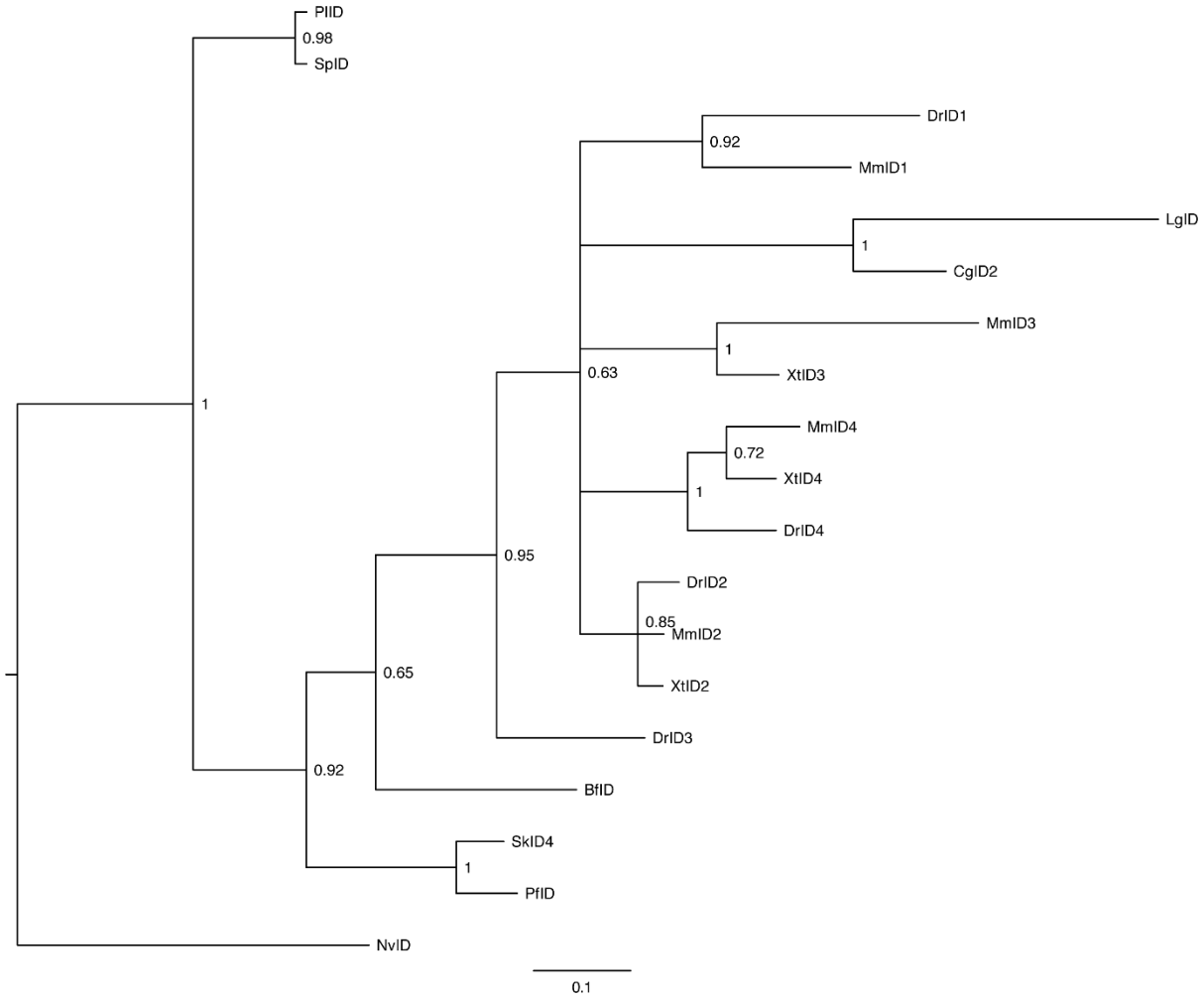
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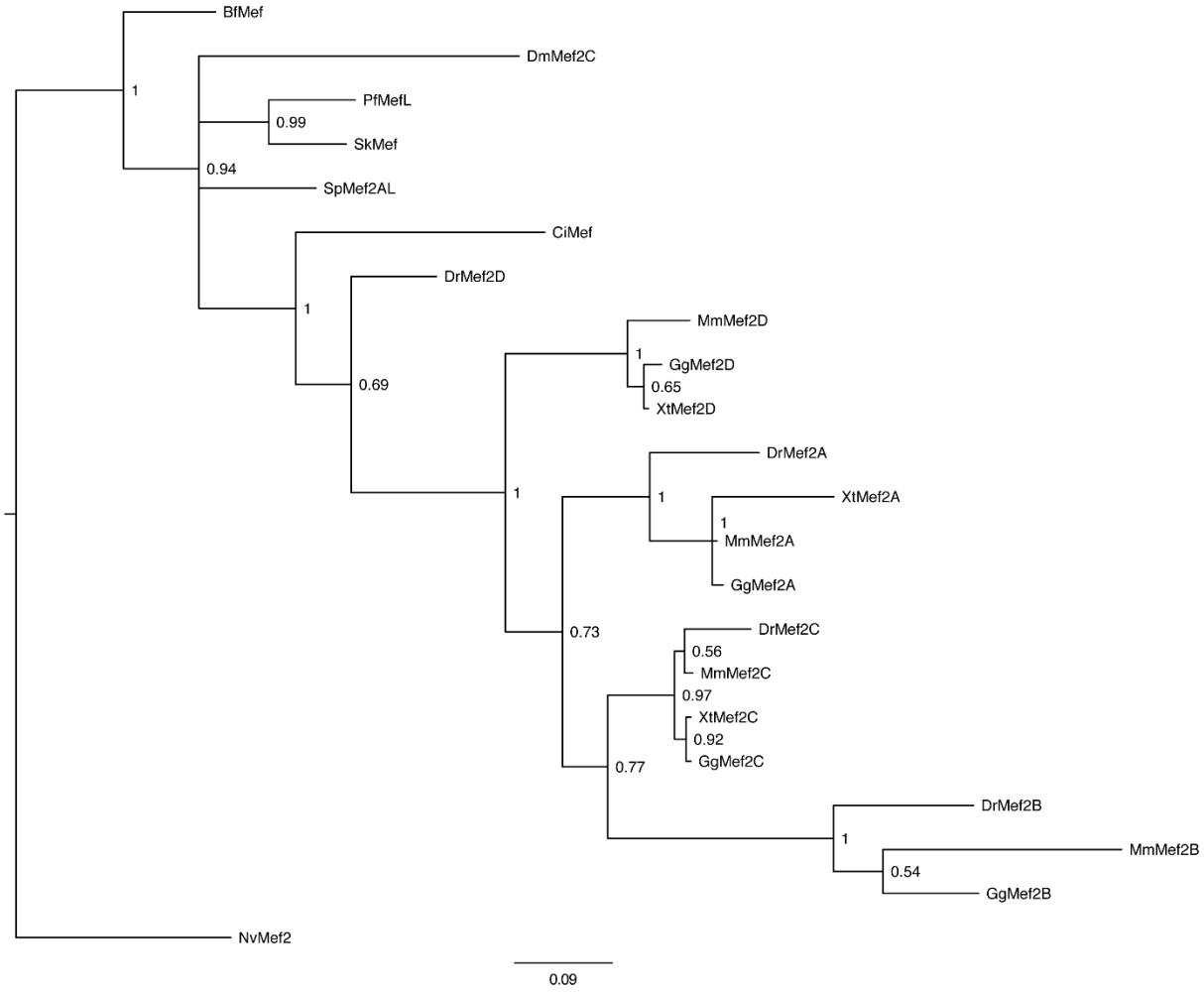
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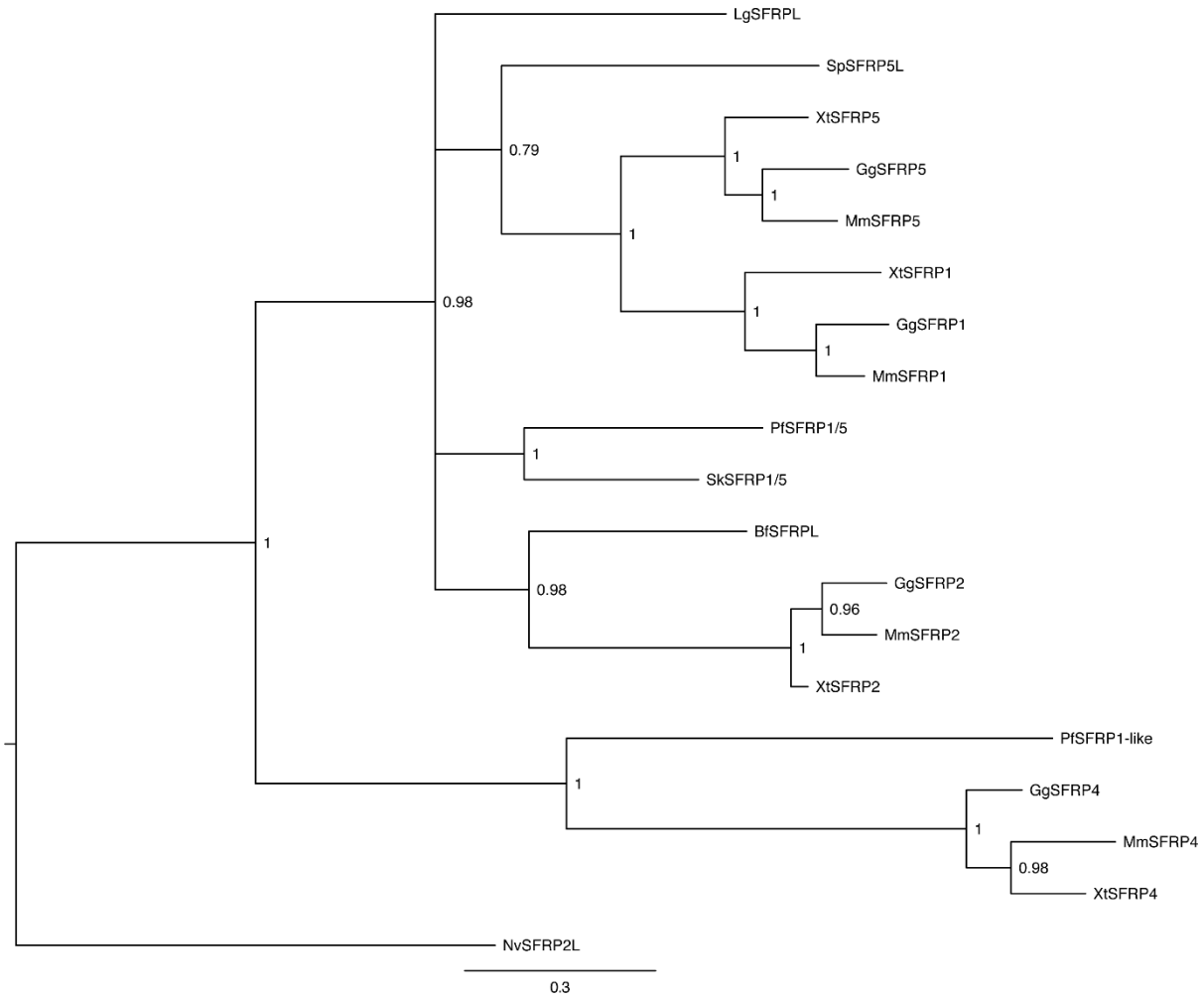
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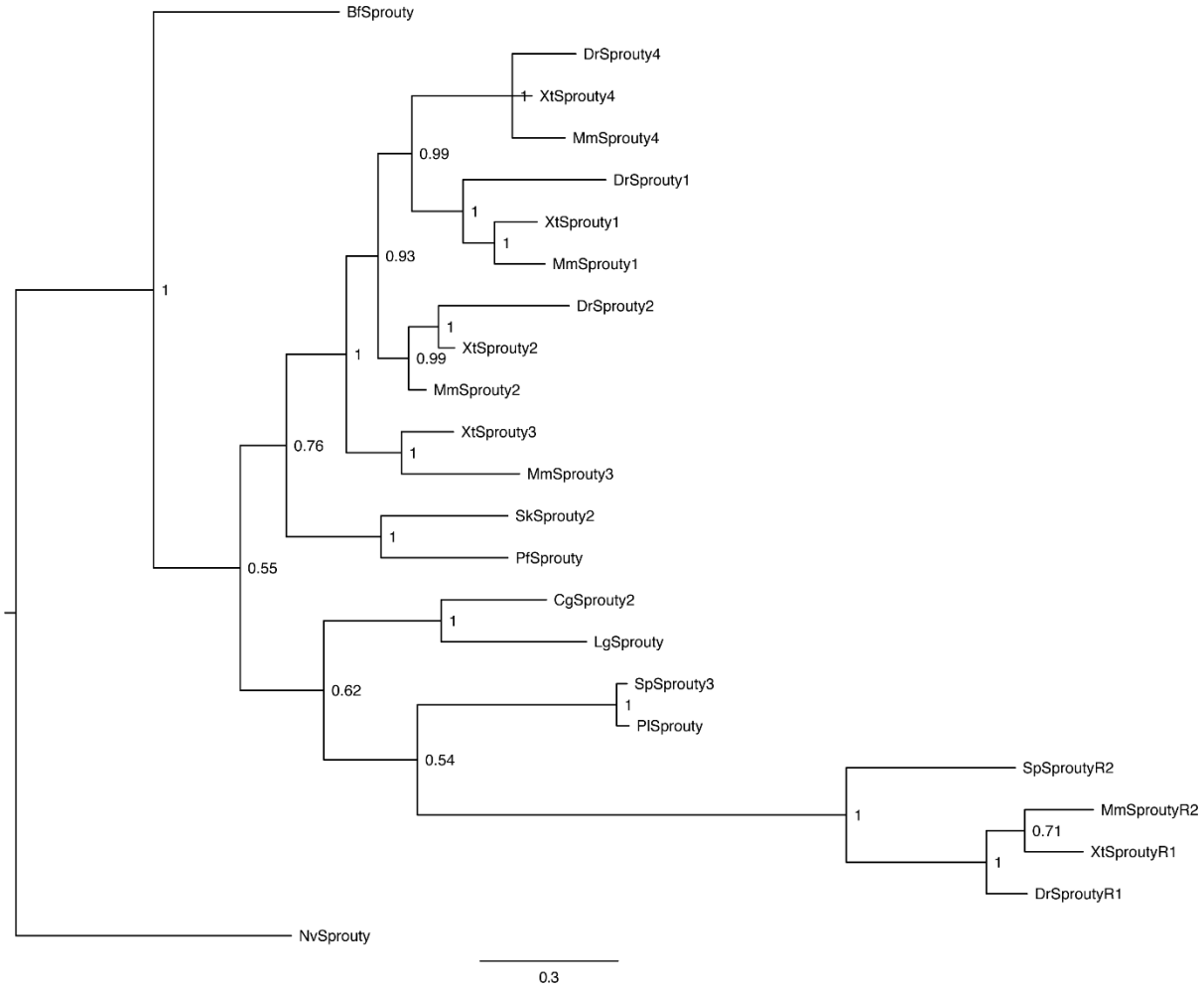
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XtSprouty2
0.99
MmSprouty2
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SkSprouty2
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PfSprouty
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CgSprouty2
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DrLefty2
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SkLefty
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HsMIS
XlGDNF
1
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HsGDNF
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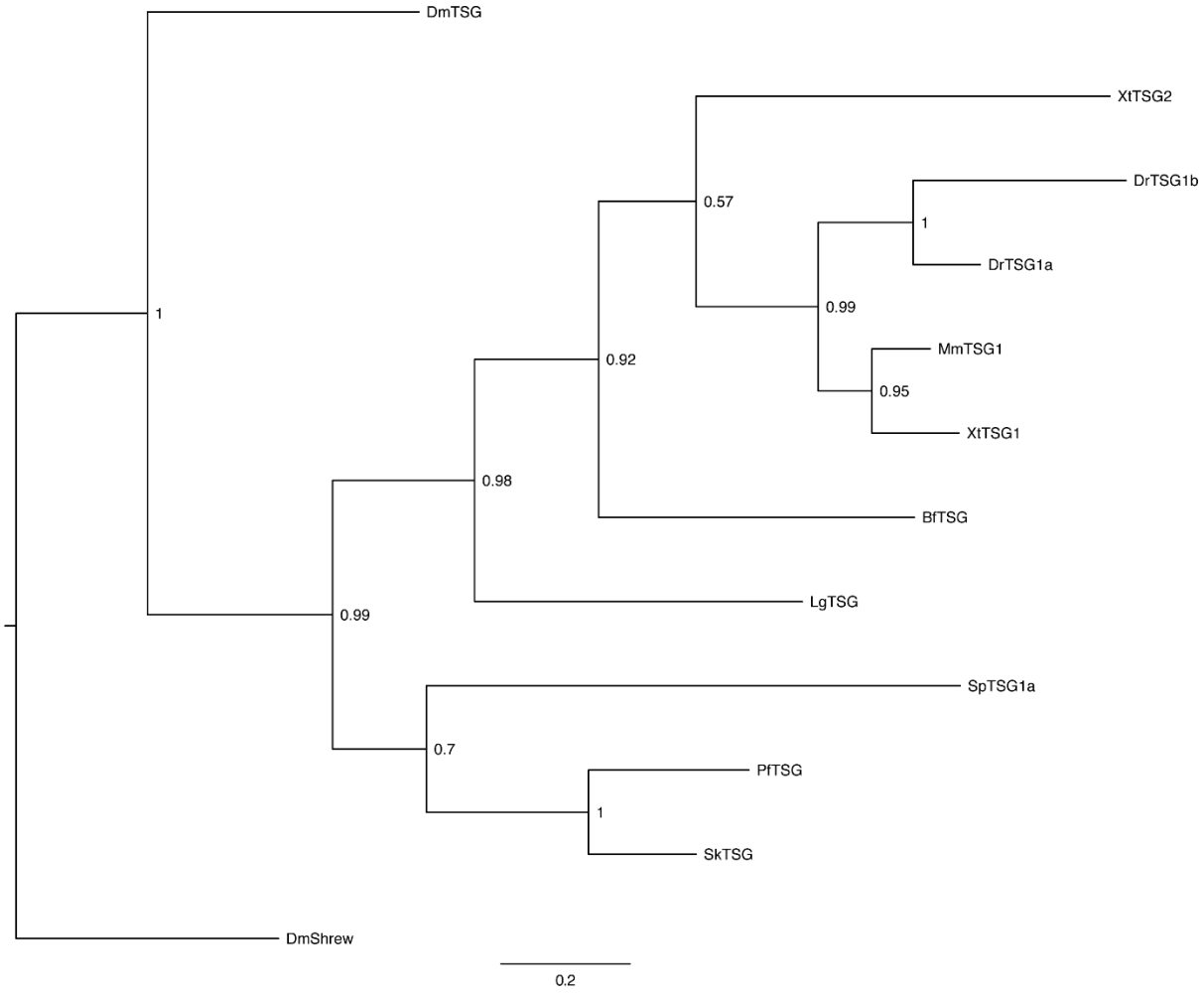
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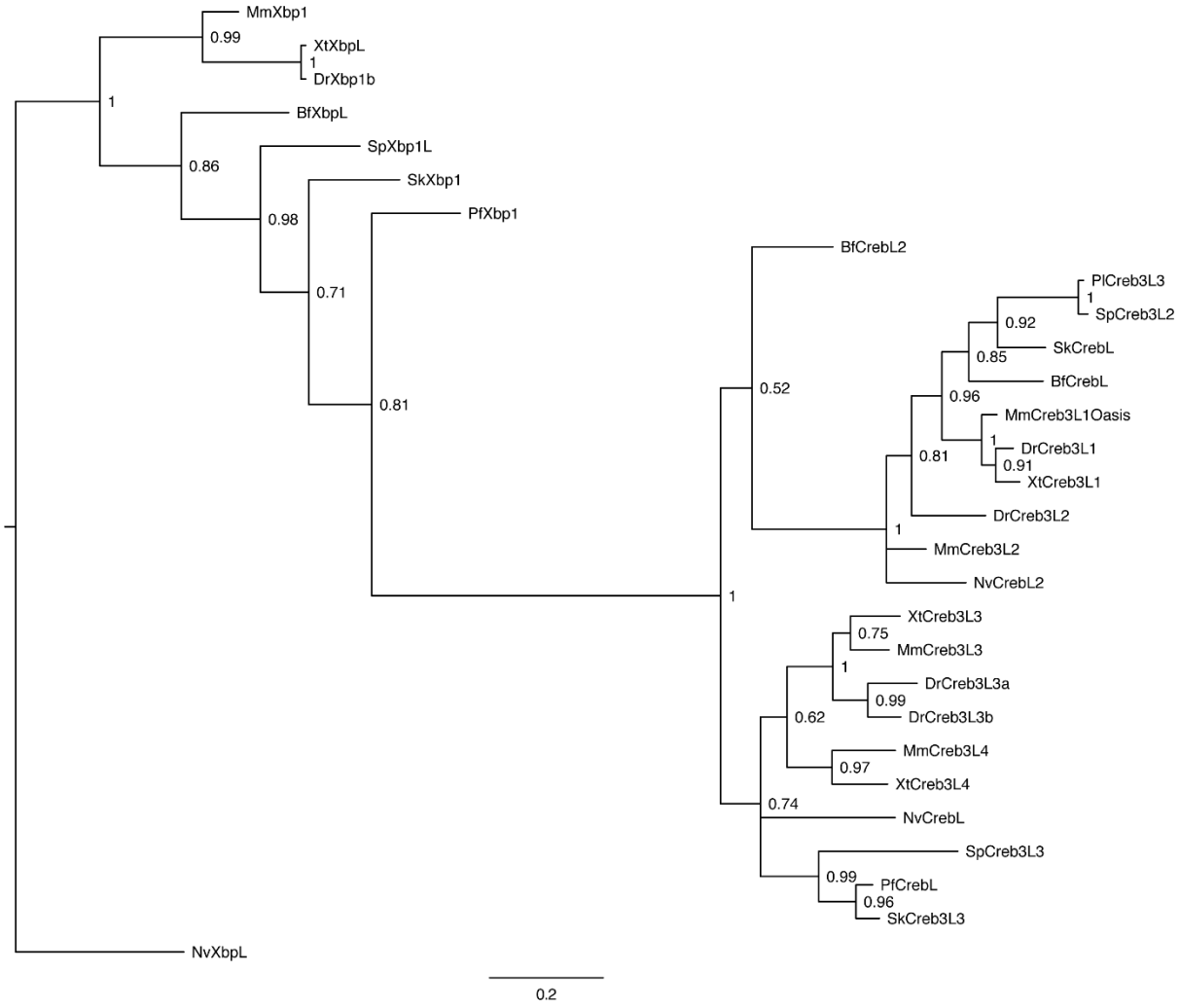
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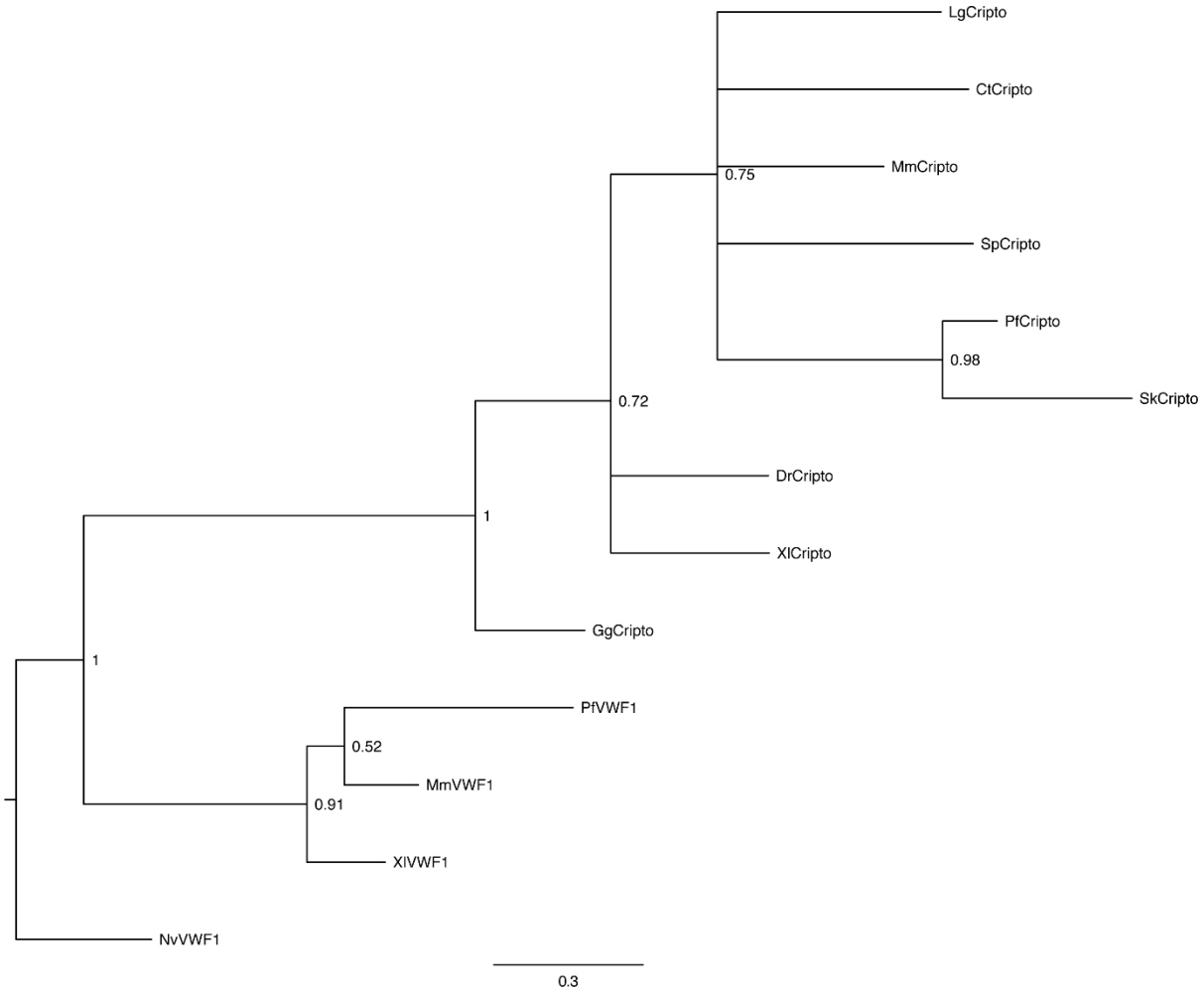

Figure S1. Phylogenetic analysis of newly identified hemichordate genes. Phylogenetic trees were analysed using Bayesian inference of conserved protein domains aligned using MUSCLE. Trees were run through 5 million generations and are based on a “mixed” model of evolution. Domains used in tree construction were identified using SMART protein prediction analysis.

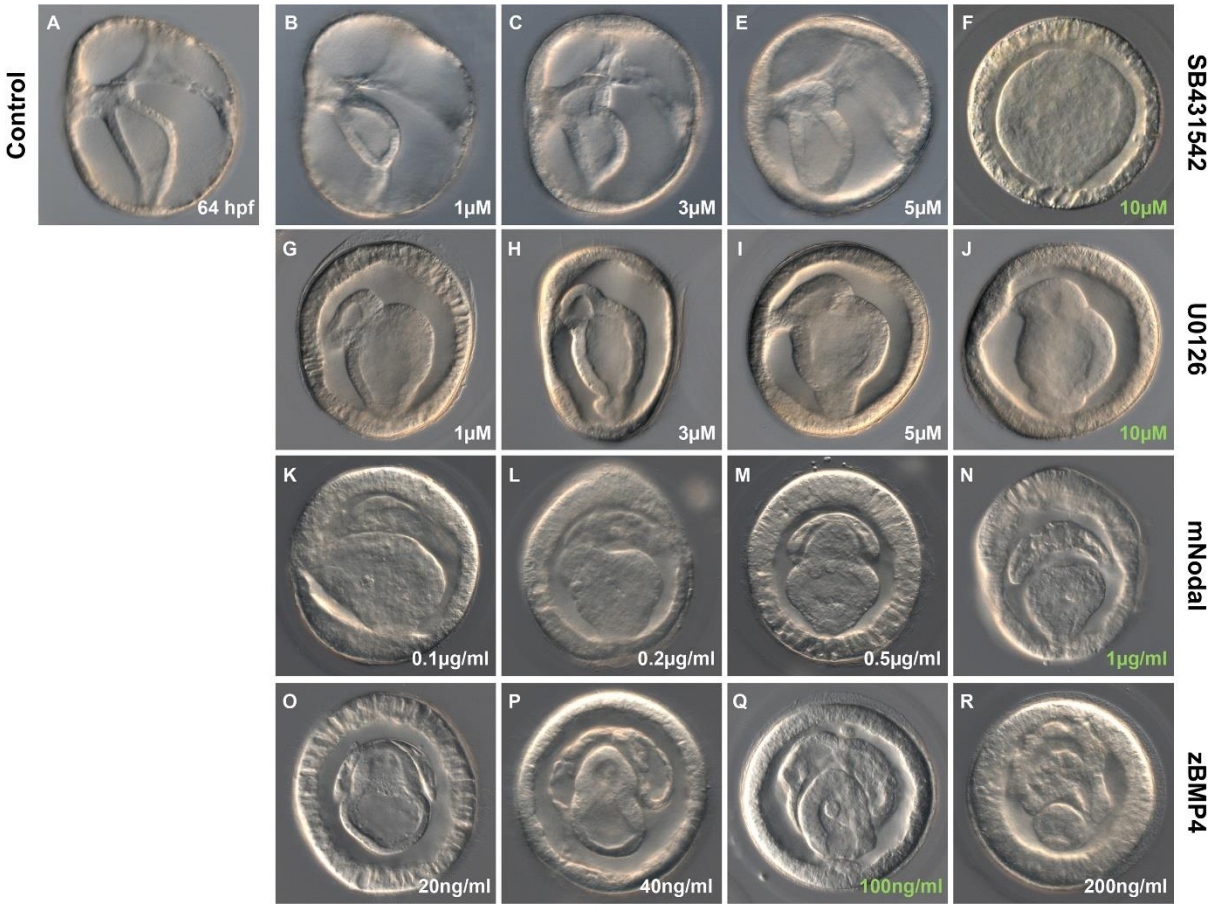

Figure S2. Dose dependent effects of SB431542, U0126, mNodal and zBmp4 treatments. Treatments were initiated at the 2-cell stage at various concentrations to determine the most reliable concentration of a given substance. Green indicates the concentration used for the present study.

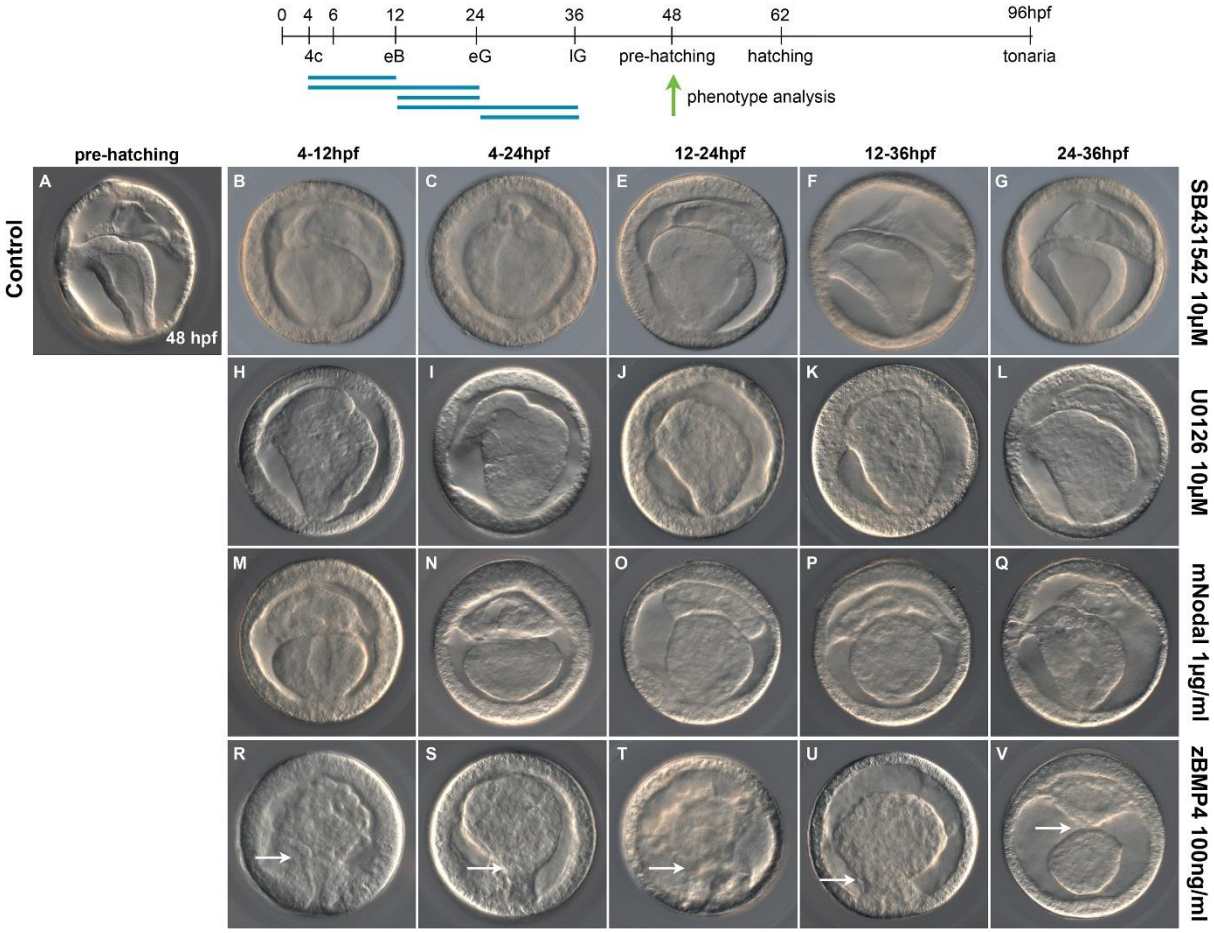

Figure S3. Drug sensitivity-periods of the various treatments in *P. flava*. Embryos were incubated with the indicated drug or recombinant protein during the indicated periods of times and scored for phenotypes at pre-hatching embryos. White arrows in R-V indicate the boundary between the endoderm and mesoderm.

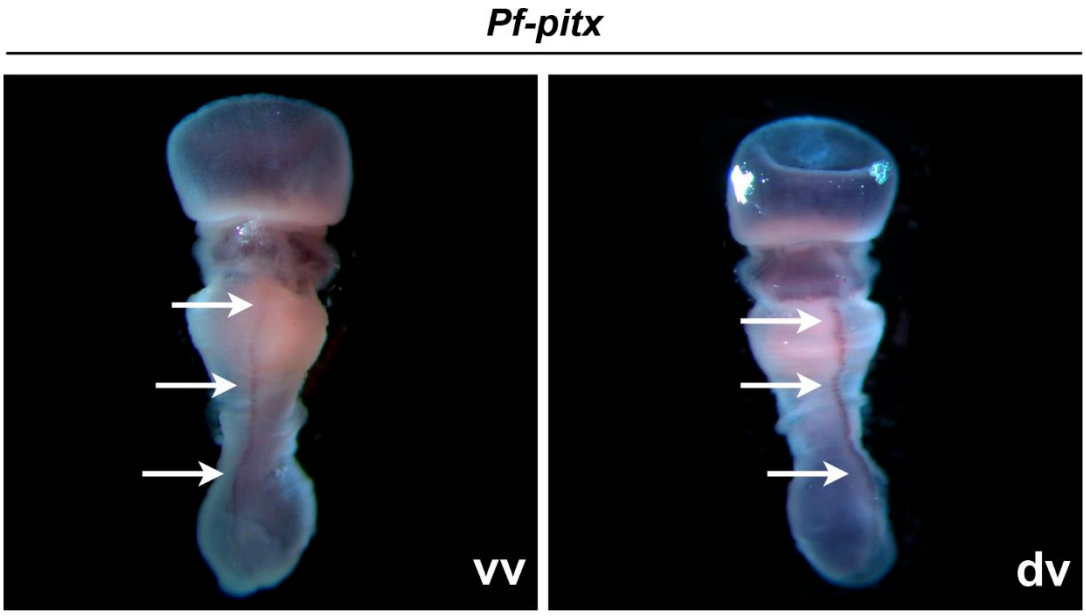

Figure S4. *Pf-pitx* is expressed in the ventral and dorsal midlines. WISH using a probe targeted against endogenous *Pf-pitx* transcripts shows that this gene is expressed in the ventral and dorsal midlines in newly metamorphosed juveniles. (dv) dorsal view, (vv) ventral view.

Table S1. Primers used to clone the full-length *Pf-nodal* gene

degenerated NODAL primers:
Nodal degF1
5'-GTCGACTTCRANMARATHGGNTGG-3'
Nodal degF2
5'-TAYAAYGCNTAYCGNTGYGARGG-3'
Nodal degR1
5'-RCANCCRCAYTCNTCNACDATCAT-3'
Nodal degR2
5'-TTCATRTANGCR TGRTTNGTNGG-3'

NODAL Race primers:
PfNodal_ 3'RACE-Fwd1
5’-GTCCCTGAACCGTGGAGATTTAGTG-3’
PfNodal_3'RACE-Fwd2
5’-AGGTTCAGAGAGACCGCCATCATTC-3’
PfNodal_5'RACE-Rev1
5’-TTTCCCGCCCTTGACCTTGGTGAAG-3’
PfNodal_5'RACE Rev2
5’-GTTGCCGTTTCTTCGTTTCTTTCCCC-3’

Full length NODAL primers:
PfNodal_FL-FWD
5’-ATGGTCCTCTGCCCTCTTTGTGTCG-3’
PfNodal_FL REV
5’-TTATCTGCAACCACATTCTTCCAC-3’