Supplementary Methods

Molecular cloning and plasmid construction

cDNA clones for *Xenopus* ERK5 and *Xenopus* MEK5 were obtained from *Xenopus* gastrula library by plaque hybridization. The coding region of them were amplified by PCR and cloned into the expression vector. The cDNA for SoxD and Xngnr1 were isolated by PCR. The constructs of mERK5 AEF, mMEK5 D, MEF2C R24L were described elsewhere (Kato *et al,* 1997; Kamakura *et al,* 1999; Molkentin *et al*, 1996). xERK5 AEF was constructed by replacing Thr214 and Tyr216 with alanine and phenylalanine, respectively. To obtain xMEK5 D, Ser311 and Thr315 were replaced with aspartic acids. Expression plasmids were constructed as described (Kusakabe *et al,* 2001).

TUNEL staining

TUNEL staining was performed as described (Hensey *et al*, 1998). Embryos were stained with BM purple (Roche).

The sequences of primer pairs for RT-PCR

The sequences of primer pairs for RT-PCR were described elsewhere (Kusakabe *et al,* 2001; Mizuseki *et al,* 1998; Sasai *et al,* 1996; Wilson *et al,* 1997). The sequences of other primer pairs used were follows: xERK5 [forward (f), 5'-GTGCCTGACATACGGAAACC-3'; reverse (r), 5'-CCCAACCTCTATAAGAGGA-G-3'], xMEK5 [f, 5'-CATGGCCTCAAGGTGAACAC-3'; r, 5'-CACTTGTACAGGAT-TTCAAG-3']. The sequences of primer pairs for real-time PCR are follows: xFGF2 [f, 5'-GTTAAAGGATGGACCAAGCAAACT-3'; r, 5'-GCACCTGCAACCCCAACT-3'], xFGF3, [f, 5'-TGTGGGAATTGTTGCCATCA-3'; r, 5'-CTTTGGTTCATGGCCAGA-TATCT-3'], xFGF8b [f, 5'-CCTGGTGACCGACCAACTAAG-3'; r, 5'-CCGGCTGTA-CAACTGGTAGGTT-3'], xFGF9 [f, 5'-AGTGGATACCCCTGTGTTGTTAAGT-3'; r, 5'-GCCCCCCTGCTTCAGAA-3'], xFGF13 [f, 5'-TCACTTCACGATCTCACA-GAATTTT-3'; r, 5'-ACGCTTCTGCTTTTTGTTGGA-3'], xFRL1 [f, 5'-TTAA-TGCCACCCATGGAAAGA-3'; r, 5'-ATACCCAAGAAGGGCAAGGTTT-3'], eFGF [f, 5'-GTGGGCATCGGGTTTCATAT-3'; r, 5'-CGTTGTGCATGCCATTTATCC-3'], xIGF1 [f, 5'-AGCCAAATCAGCACGTTCTGT-3'; r, 5'-TCTGGGCTTTTGGCATATCAG-3'], xIGF3 [f, 5'-GCTGTGCCTGTCTCTTGTGTTT-3'; r, 5'-TCAGACAGCGAGCGT-TGGT-3'].

Immunoblotting

Embryos were lysed in a buffer consisting of 20 mM Hepes pH 7.2, 0.25 M sucrose, 0.1 M NaCl, 2.5 mM $MgCl_2$, 10 mM NaF, 10 mM EGTA, 10 mM β-glycerophosphate, 1 mM vanadate, 1 mM PMSF, 0.5% aprotinin and 1 mM dithiothreitol and then centrifuged. The supernatant was used for immunoblotting with anti-myc antibody (9E10; Santa Cruze) or anti-ERK5 antibody (Sigma).

Supplementary References

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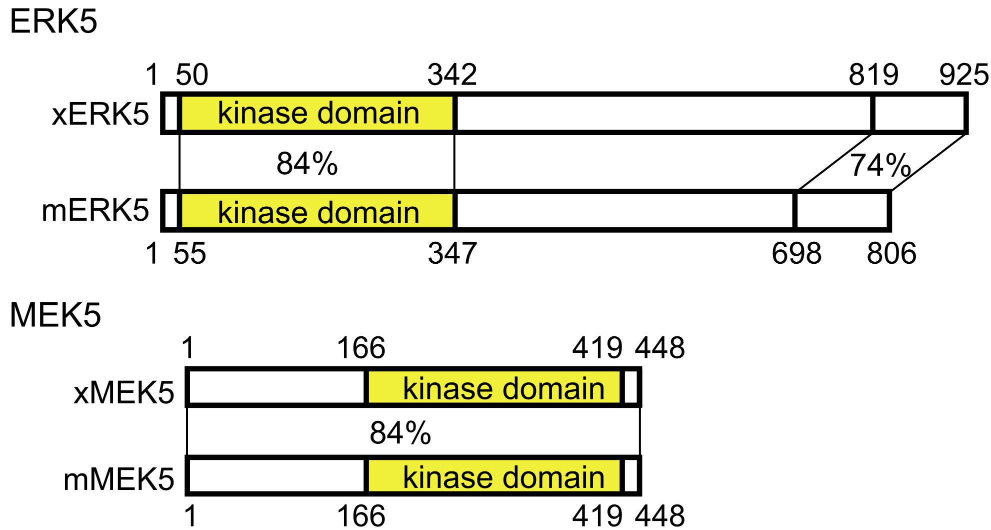
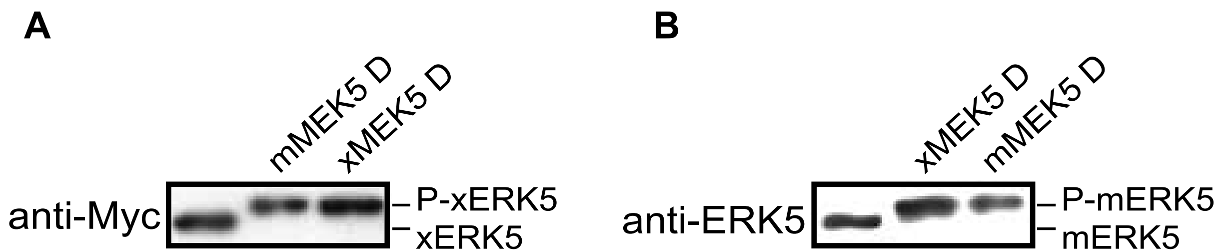


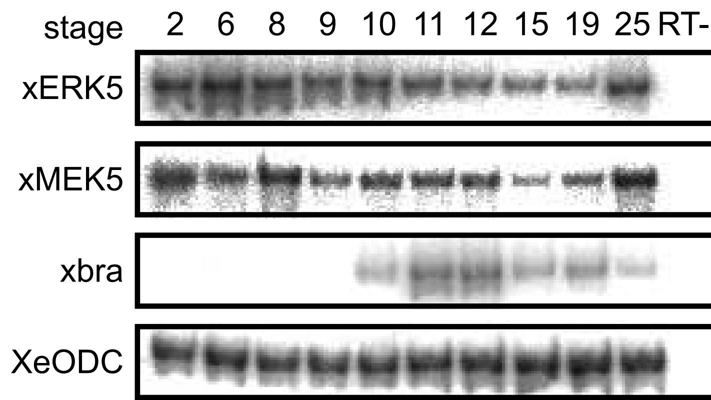
Supplementary Fig 1

The schematic structures of xERK5 and xMEK5 with their mouse orthologs. The identified xERK5 and xMEK5 consist of 925 amino acids and 448 amino acids, respectively, and show 59% and 84% identity to mouse ERK5 (mERK5) and mouse MEK5 (mMEK5), respectively. The percentage of identical amino acids between xERK5 and mERK5 in their whole sequence is relatively low, as xERK5 has a larger C-terminal sequence than mERK5. We conclude, however, that this is a *Xenopus* ortholog of ERK5 because of following reasons. It shows 84% identity in amino acid sequence to mERK5 in the kinase domain including the identical activating phosphorylation site sequence (TEY), and has a large C-terminal regulatory domain, which is unique to ERK5.

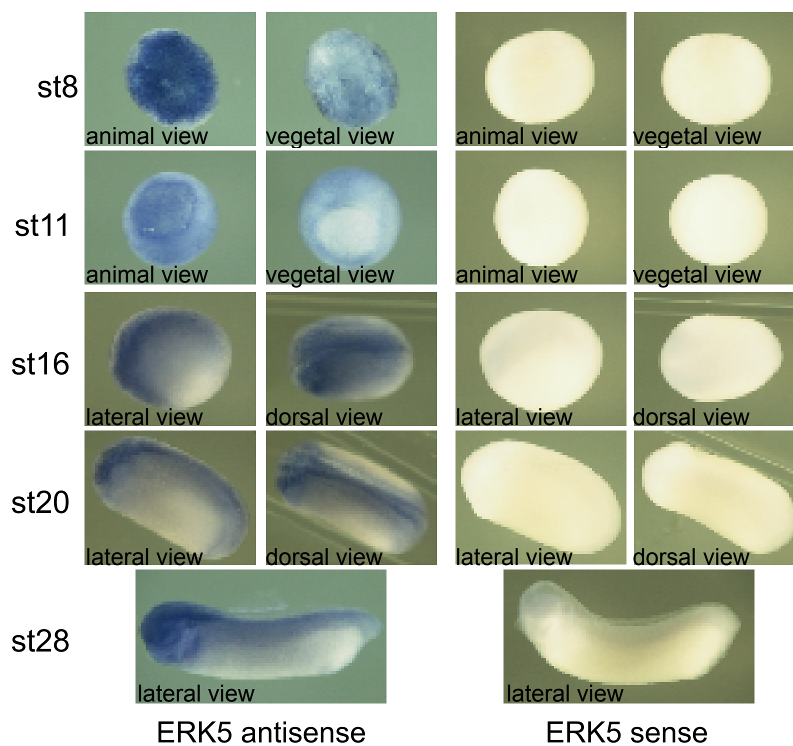


Supplementary Fig 2

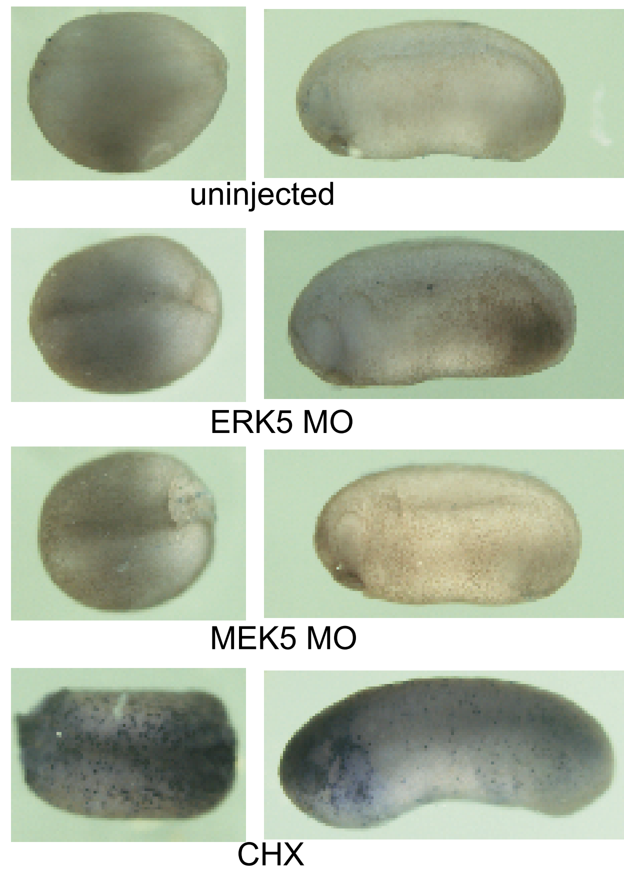
(A) Myc-xERK5 and constitutively active mMEK5 (mMEK5 D) or xMEK5 (xMEK5 D) were expressed in animal caps, and phosphorylation of xERK5 was analyzed. xERK5 was able to be phosphorylated by constitutively active xMEK5 (xMEK5 D) or mMEK5 D. (B) mERK5 and xMEK5 D or mMEK5 D were expressed in animal caps, and phosphorylation of mERK5 was analyzed. mERK5 was phosphorylated by mMEK5 D or xMEK5 D. These results indicate that xERK5 is a downstream target of xMEK5 and that the MEK5-ERK5 pathway is conserved among vertebrates.



Supplementary Fig 3
Temporal expression patterns of xERK5 and xMEK5. The total RNA isolated from embryos at indicated stages was subjected to RT-PCR analysis. *Xenopus* embryonic ornithine decarboxylase (XeODC) was used as an RNA loading control. Xbra was also examined. Both xERK5 and xMEK5 were expressed maternally and throughout early embryogenesis.



Supplementary Fig 4
Whole-mount in situ hybridization against xERK5 at the indicated stages. xERK5 was widely expressed in presumptive mesoderm and ectoderm during blastula (stage 8) and gastrula stages (stage 11). The strong staining was detected in head and dorsal structures during neurula (stage 16, 20) and tailbud stages (stage 28).



Supplementary Fig 5

Embryos were injected with ERK5 MO (20 ng) or MEK5 MO (200 ng) into the dorsal marginal zone, or treated with cycloheximide (0.2 mg/ml) and subjected to whole-mount TUNEL staining. Dorsal views of neurula stage embryos are shown in left panels and lateral views of tailbud stage embryos are shown in right panels with the anterior side at the left. Any increase of TUNEL positive cells was not detected in embryos injected with ERK5 MO or MEK5 MO, suggesting that the defects in ERK5 MO- or MEK5 MO-injected embryos are not caused by increased apoptosis.