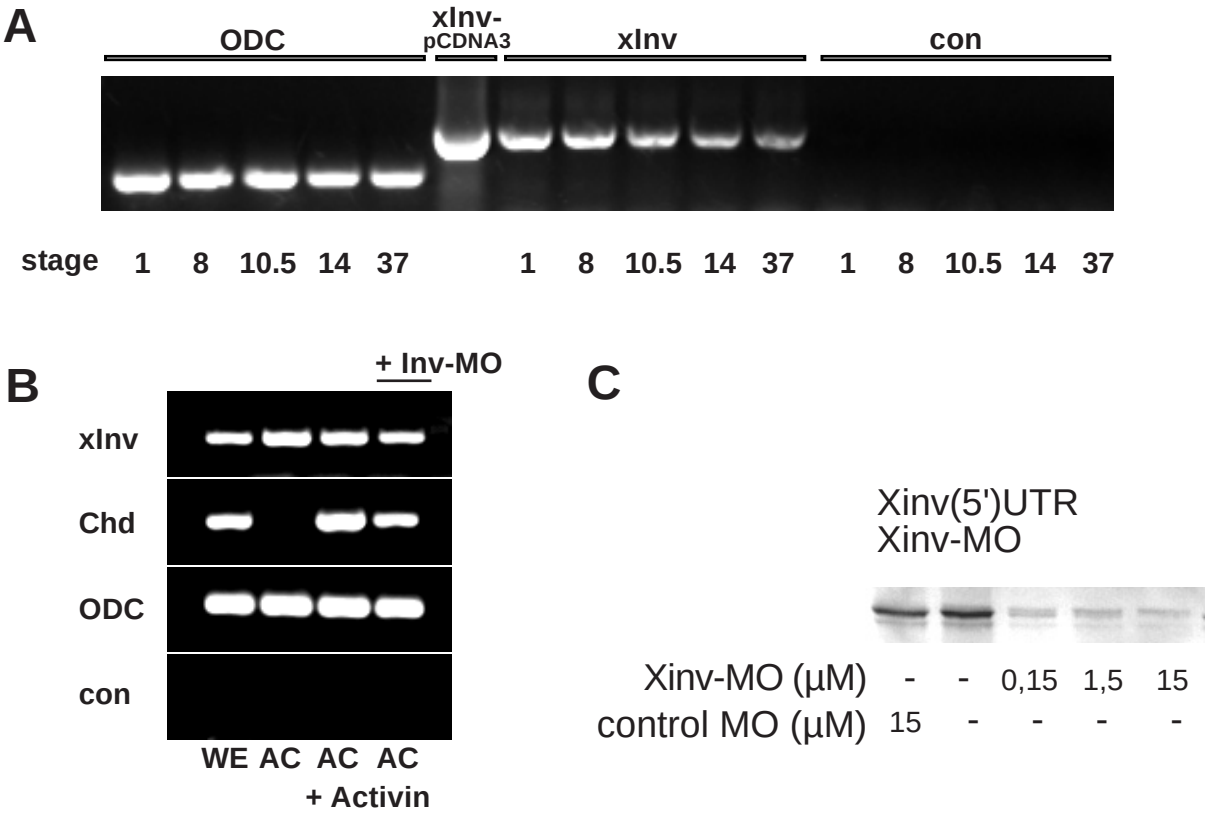
Supplementary Figure 3



Supplementary Figure 3: Inversin transcripts during early Xenopus development. (**a**) mRNA was isolated from Xenopus embryos at indicated developmental stages using the RNeasy kit (Qiagen). RT-PCR was performed with SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturers instructions. RT-PCR of ornithine decarboxylase (ODC, forward primer: 5´-aatggatttcagagacca-3´, backward primer: 5´-ccaaggctaaagttgcag-3´) and a partial xInv DNA sequence in pCDNA3 (Inv-pCDNA3) were used as positive controls. DNA-free RNA extracted from embryos served as negative control (con). Primer for xInv were: forward primer: 5´-gtgcaaaggtgcaccttgtagac-3´, backward primer: 5´-gaggctgcaatgtcctgaatggc-3´. Developmental stages of embryos are as follows: 1 cell stage (1), mid blastula (8), early gastrula (10.5), early neurula (14), early tadpole (37). Note that Inv transcripts are present at early blastula and gastrula stages and at lower levels in later stages. (**b**) Xenopus inversin transcripts were detectable in whole embryos (WE) and animal cap explants (AC). Injection of inversin MO (32ng) did not abrogate the expression of inversin or the dorsal mesodermal marker chordin (Chd). Similar results were obtained for goosecoid (data not shown). (**c**) Inhibition of Xenopus inversin translation in vitro by Xenopus inversin antisense morpholinos (Inv-MO). A plasmid encoding Xenopus inversin including the 5'-UTR (50ng) was used as a template in a cell-free coupled transcription/translation reactions. Reactions were performed in the presence of [^{35}S]methionine, and analyzed by SDS-PAGE and autoradiography. An unrelated antisense sequence (control MO) was used as a control morpholino to exclude unspecific effects.