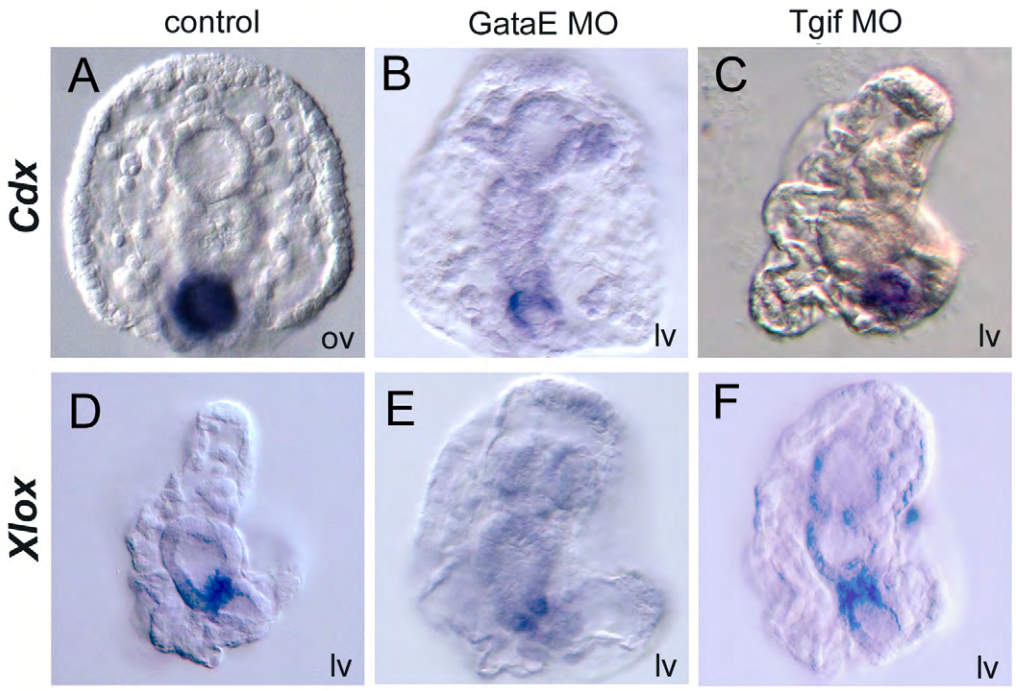
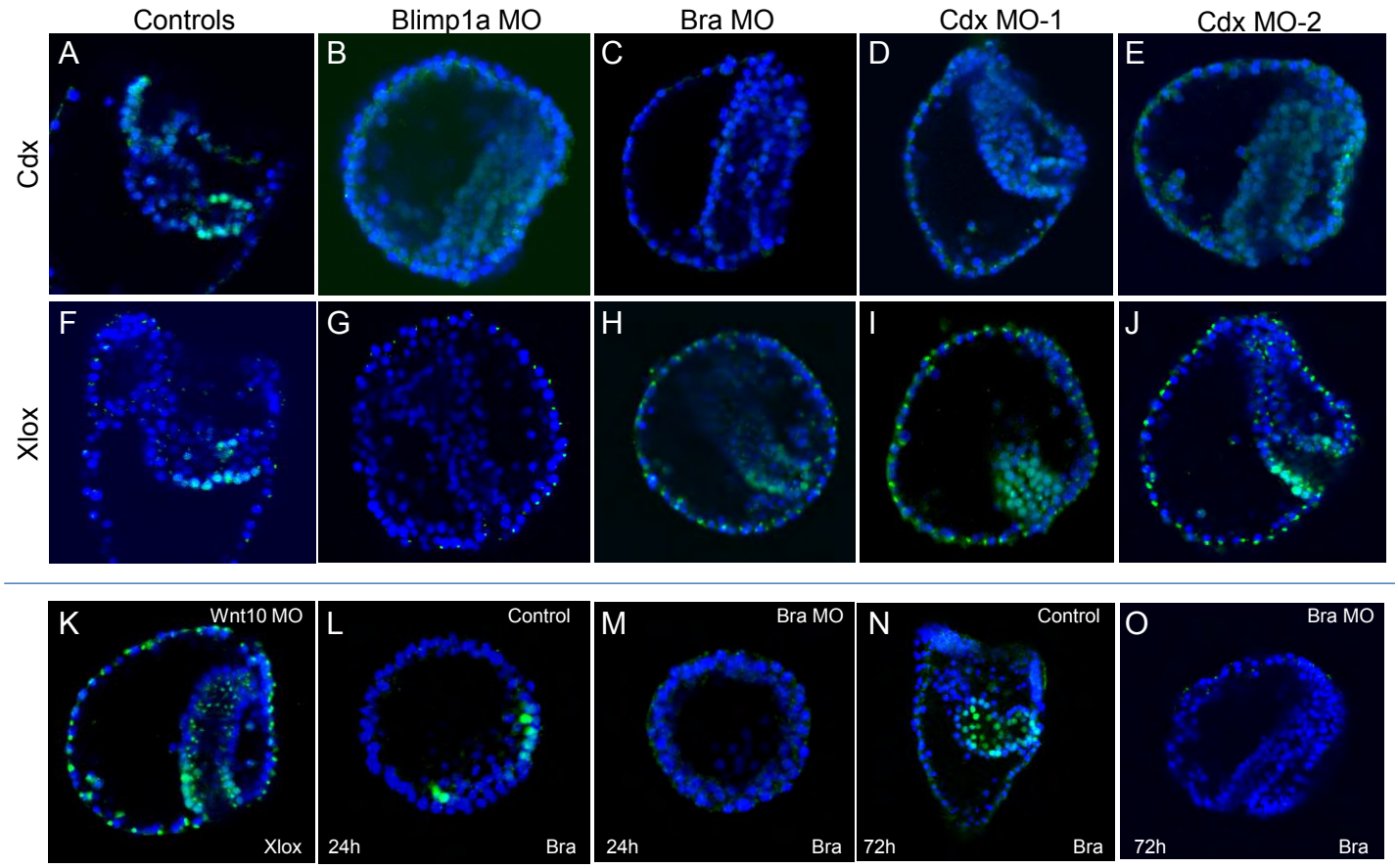
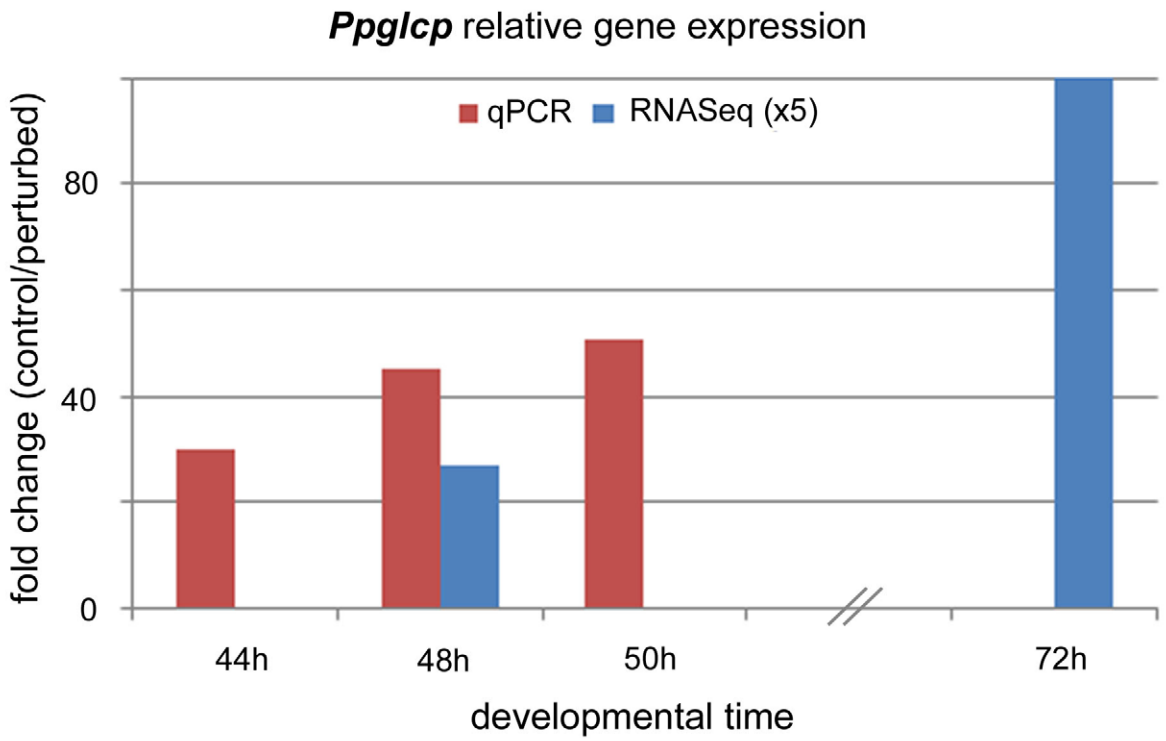


Supplementary Figure 1
GataE and TgiF knockdown does not have any effect on *Xlox* and *Cdx* transcription. Chromogenic ISHs in control embryos (A, D) and in embryos injected with MOs directed against the translation of: GataE (B, E), and TgiF (C, F) proteins. All the embryos have been fixed at 72 h. ov, view from the oral side; lv, lateral view.

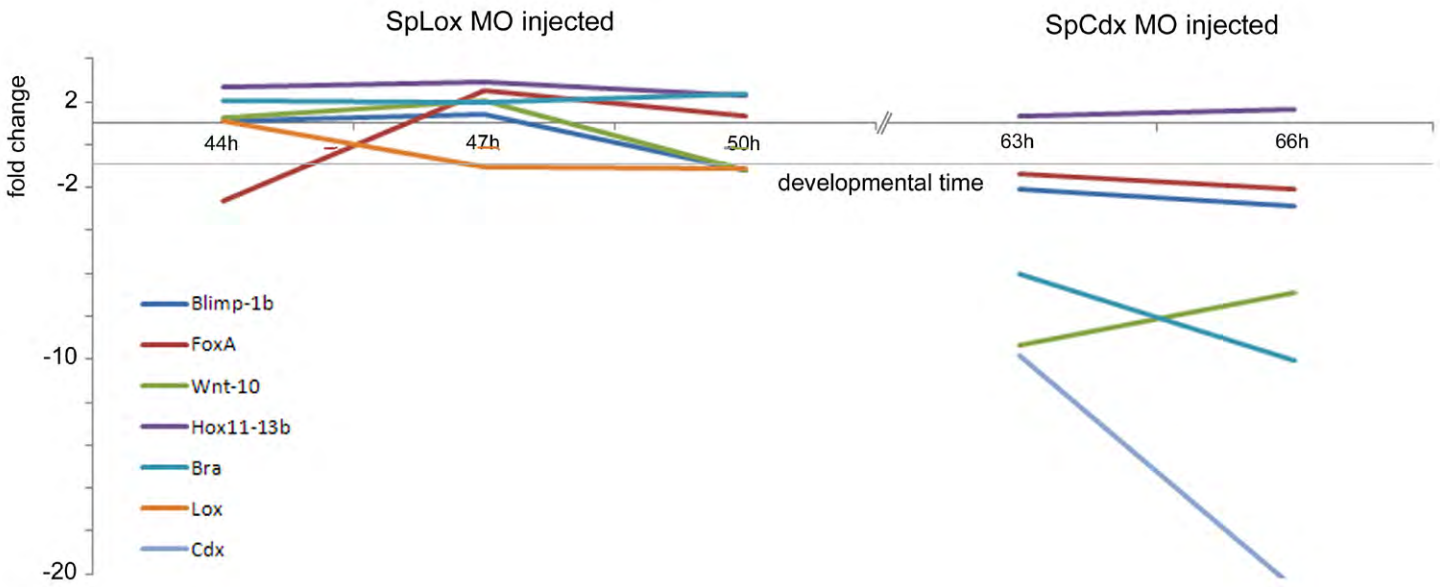


Supplementary Figure 2

Control immunolocalization experiments. Cdx (A-E), Xlox (F-K) and Bra (L-O) protein accumulation visualized by immunostaining (green) in control (A, F, L, N) and knockdown embryos and larvae (B-E, G-K, M,O). In all the panels nuclei are stained with DAPI and depicted in blue and a single slice of a z-stack is shown. With the exception of the blastulas in (L, M) all the embryos have been fixed 72 h after fertilization. Embryos and larvae are all in lateral view, with the exception of the larva in G that is seen from the oral side. CdxMO-1 is the morpholino targeting the donor splice site between the first and second *SpCdx* exons, CdxMO-2 is a second morpholino designed to block the translation of Cdx protein. Both MOs were able to impair Cdx protein formation.



Supplementary Figure 3
Validation of RNAseq data by qPCR. The graph shows reduction of *Ppglcp* gene expression detected by both qPCR (red) and RNAseq (blue) techniques. For details on the RNAseq technique see the Materials and Methods section. The qPCR experiments were performed following the protocol described in (Cole et al., 2009). In particular, total RNA was collected from control and Xlox-MO injected larvae at three time points 44, 47 and 50 hours post fertilization. Data are expressed as a fold difference from control larvae, all as positive values. In order to compare the results obtained with the two techniques (RNAseq and qPCR), the RNAseq data are visualized in a 5x scale. The reduction of *Ppglcp* gene expression measured by qPCR is strong already in the 44 h embryos and become stronger with time, confirming the effect discovered through the RNAseq experiment.



Supplementary Figure 4

Time course qPCR in SpLox and SpCdx knockdown embryos. 3 hour time resolution qPCR analysis of the gut GRN transcription factor and signaling molecule in Xlox and Cdx knockdown embryos and larvae. The first analyzed time points for both Xlox and Cdx knockdown are 44 and 63 hours respectively, which represent the times of development when the transcript accumulation of each endogenous gene is half of its maximum level (Arnone et al., 2006)Amino Acid</keyword><keyword>Sea Urchins/classification/*embryology/*genetics/growth & development</keyword><keyword>Sequence Alignment</keyword><keyword>Sequence Homology, Amino Acid</keyword></keywords><dates><year>2006</year><pub-dates><date>Dec 1</date></pub-dates></dates><accession-num>16959236</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16959236 </url></related-urls></urls></record></Cite></EndNote>. The qPCR experiments were performed following the protocol described in (Cole et al., 2009). Data are expressed as a fold difference from control larvae. The results of the qPCR experiments are coherent with the results obtained by ISHs (see Fig. 2) and unexpectedly revealed an unknown effect of Cdx knockdown on *Bra* transcription that resulted strongly reduced at both analyzed stages (60 and 63 h).