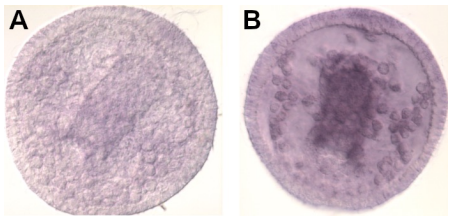
Figure S7. Whole mount in situ hybridization (WMISH) with *Pl-foxo* probe on *P. lividus* embryos at the late gastrula stage.



(A) Sense and (B) anti-sense *Pl-foxo* mRNA probes. WMISH was performed as previously described by Chiaramonte et al. [23] with some modifications. Hybridizations were carried out at 58 °C for 68 h and, after extensive washing, embryos were incubated with an anti-DIG alkaline phosphatase conjugated antibody (Roche Applied Science, Germany) for 3 h at room temperature. The DIG-labeled probes were detected by staining with the chromogenic 5-bromo-4-chloro-3-indolyl-phosphate and 4-toluidine/4-nitro blue tetrazolium chloride substrates (Roche). After staining, embryos were observed under a Zeiss Axioskop 2 Plus microscope and images were recorded.