
Supplementary information

A blastoporal organizer in a ctenophore

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Supplementary figures and legends

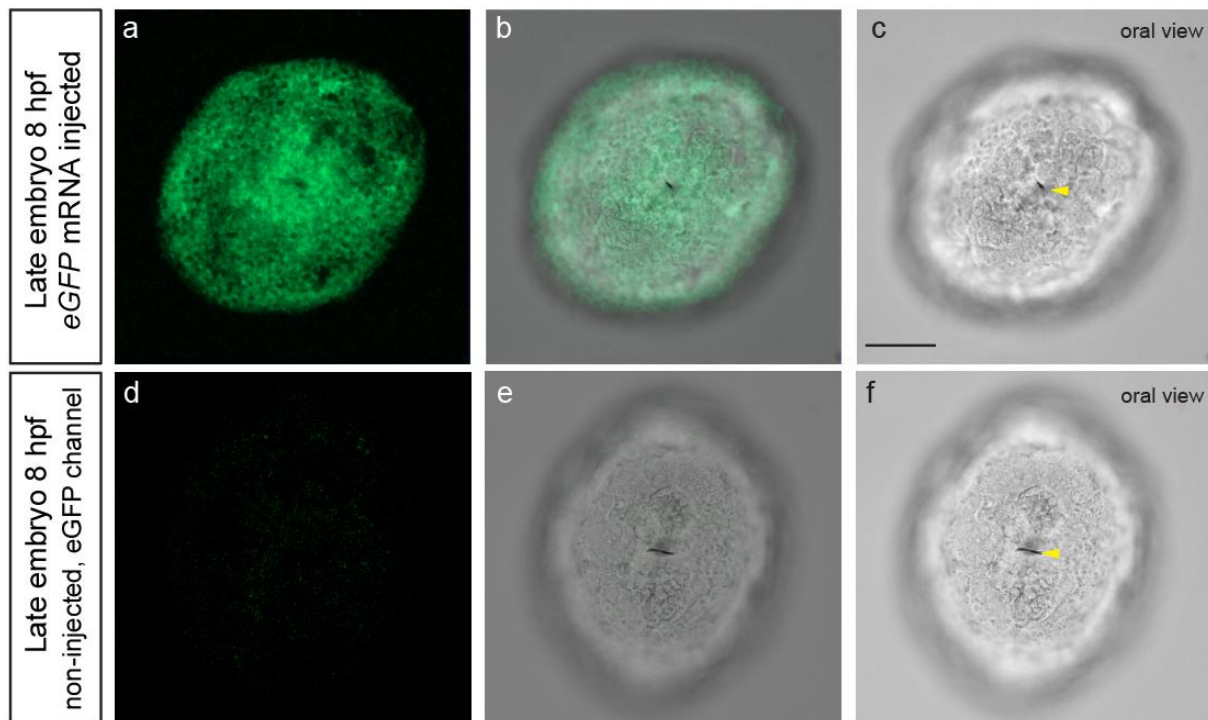


Figure S1: eGFP expression following mRNA microinjection in *Mnemiopsis leidyi* embryos. **a–c,** Embryos injected with *eGFP* mRNA at the zygote stage, imaged at ~8 hpf. **a**, eGFP fluorescence. **b**, Overlay of eGFP fluorescence and transmitted light. **c**, Transmitted light image. **d–f**, Uninjected control embryos at the same stage. **d**, eGFP channel showing no detectable signal. **e**, Overlay of eGFP channel and transmitted light. **f**, Transmitted light image. Fluorescence images in panels **a** and **d** represent confocal z-stack projections. All images were acquired using identical imaging settings (laser power, pinhole size, digital gain, and offset). For each condition (*eGFP* mRNA-injected and non-injected), imaging was performed on at least $n = 3$ individual embryos. Yellow arrowheads indicate the blastopore. Scale bar: **a–f**, 50 μm . hpf, hours post-fertilization.

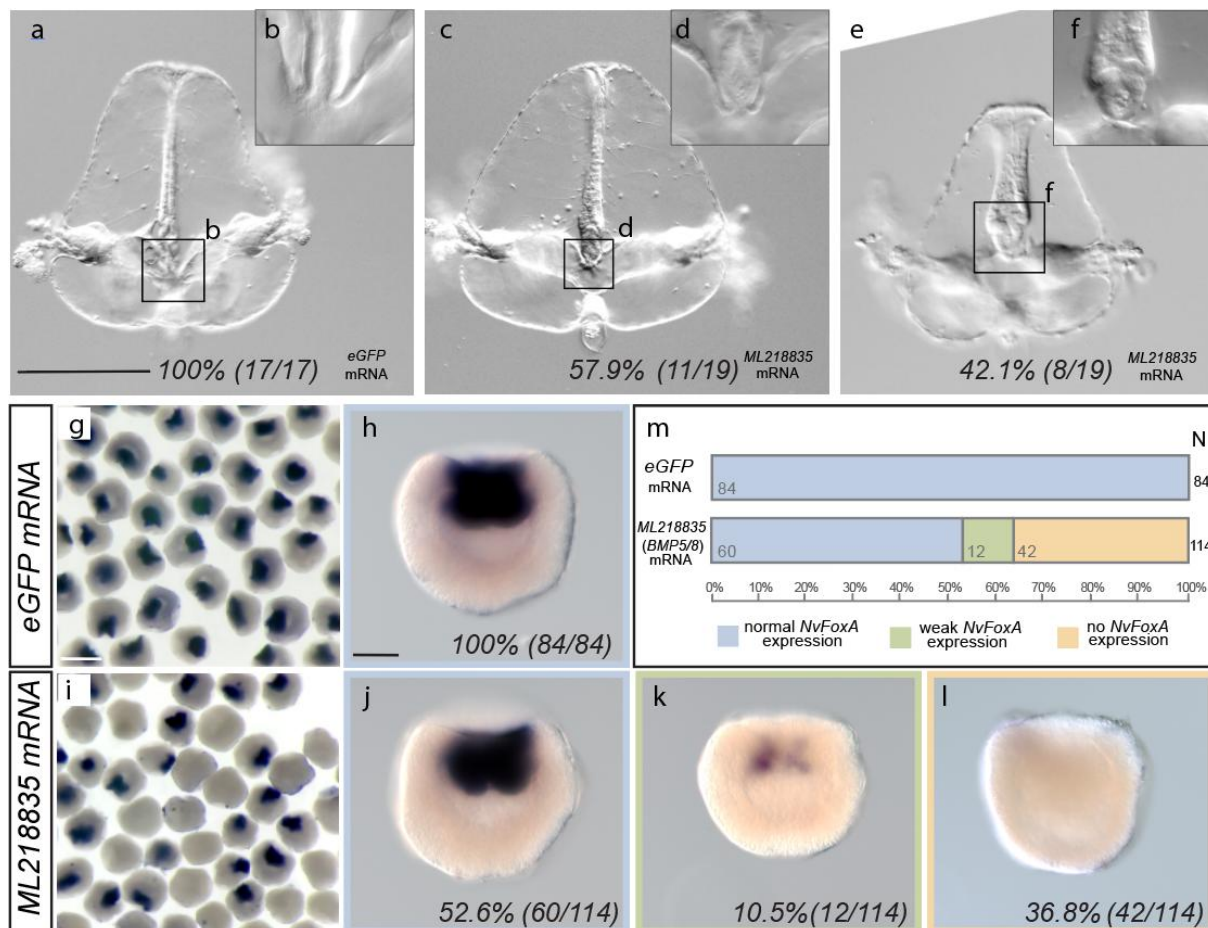


Figure S2: Overexpression of the *M. leidy* TGFβ ligand *TGFβ_ML218835*, which is expressed aborally during gastrulation, does not induce ectopic oral structure formation in either *M. leidy* or *N. vectensis*. **a–f**, Microinjection of *M. leidy* *TGFβ_ML218835* mRNA into *M. leidy* zygotes disrupts pharynx–infundibulum fusion in cydippid larvae (**e**, **f**). Control embryos were injected with *eGFP* mRNA (**a**). **g–m**, Ectopic expression of *M. leidy* *TGFβ_ML218835* in a single *N. vectensis* blastomere at the 8-cell stage suppresses *NvFoxA* expression (**i**, **k–m**). Control embryos were injected with *eGFP* mRNA (**g**, **h**). (**m**) Stacked bars show pooled proportions. Scale bar: **a**, **c**, **e**, 250 μm; **g**, **i**, 200 μm; **h**, **j–l**, 50 μm. Experiments shown in panels **a–f** and **g–m** were independently repeated three times (n=3). The number of samples and the proportion of each phenotype are shown in the panels.