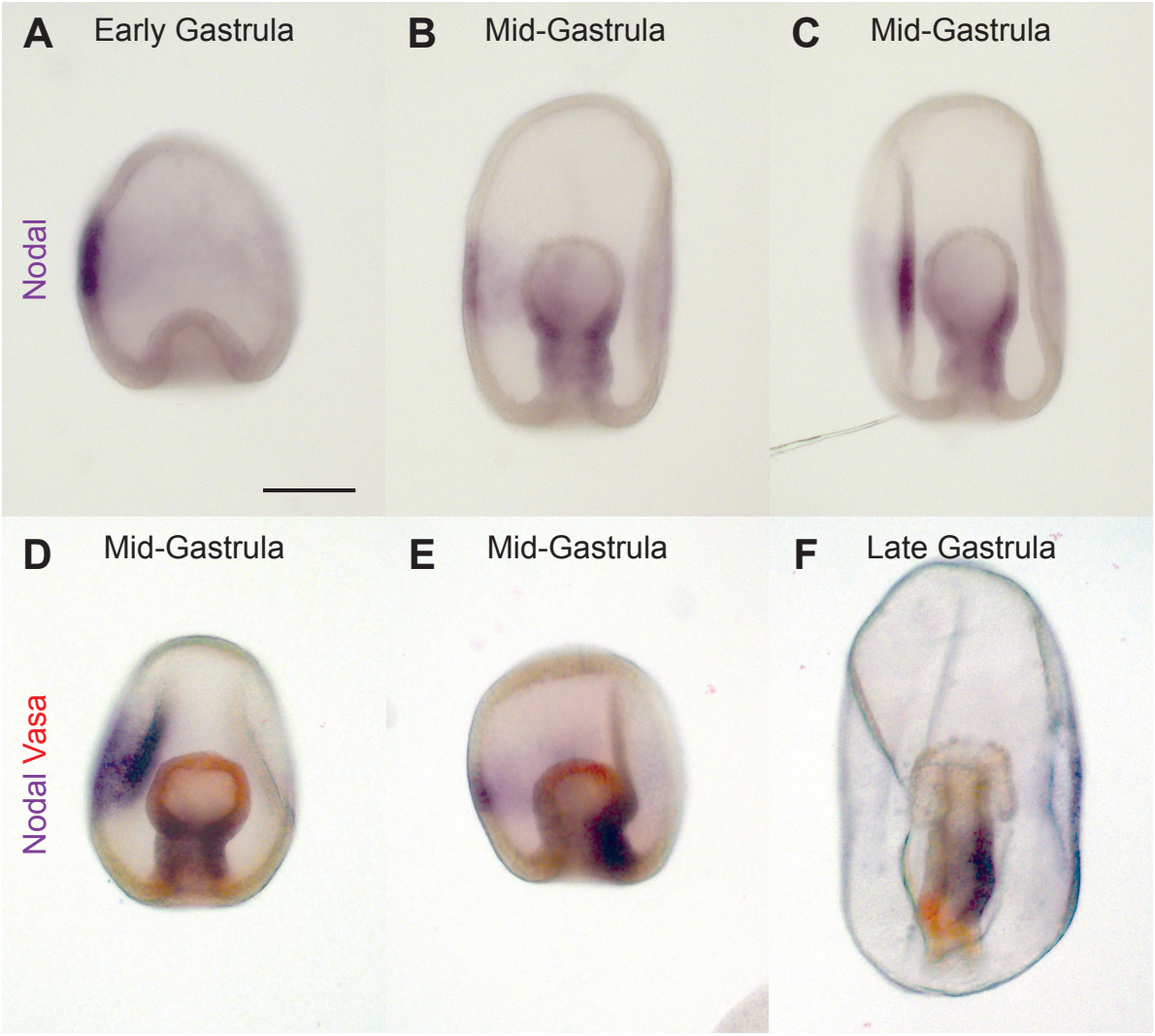
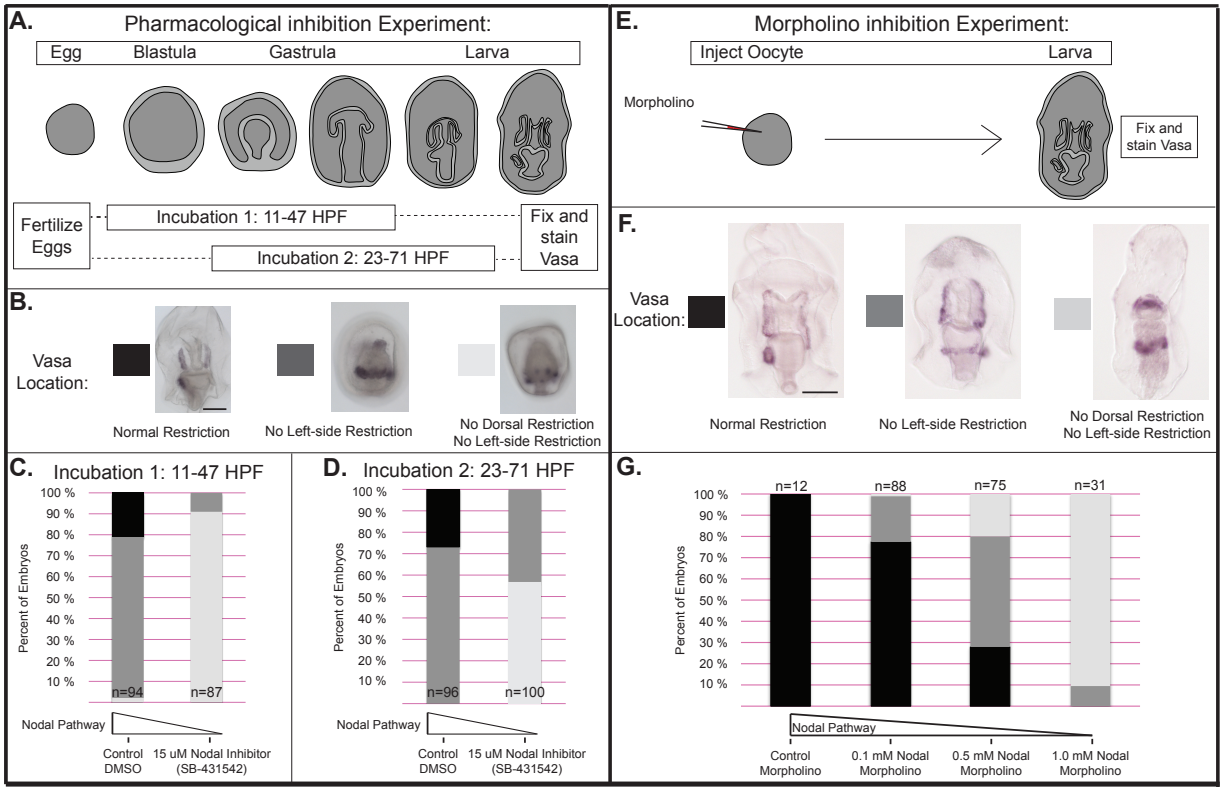



**Supplemental Figure 1: Nodal and Vasa localization during sea star development.**
**A-C)** Nodal in situ hybridization during the gastrula stage.
**D-F)** Nodal and Vasa double in situ hybridization during the gastrula stage.
All views are left side views except for F which is a dorsal view.
Scale bar is 100 microns.




**Supplemental Figure 2: Nodal inhibition causes Vasa to fail to restrict in the dorsal and left sides of the developing gut.**

**A)** Pharmacological experimental design: Embryos were incubated with 15 uM Nodal inhibitor (SB-431542) from either 11-47 hours post-fertilization or from 23-71 hours post fertilization. Embryos were then fixed at the larva stage and Vasa mRNA was localized via an in situ hybridization.

**B)** When embryos were treated with inhibitor Vasa was localized in 3 different patterns. If there was normal restriction then Vasa was localized in the left side of the gut (black). If there was no left-side restriction then Vasa was localized in the dorsal side of the gut (dark gray). If there was no dorsal or left side restriction then Vasa was localized in a ring around the gut (light gray). Scale bar is 100 microns.

**C)** When embryos were treated with inhibitor at an earlier time point then there was a shift in Vasa localization such that there is less restriction in embryos treated with inhibitor relative to embryos incubated with DMSO as a control.

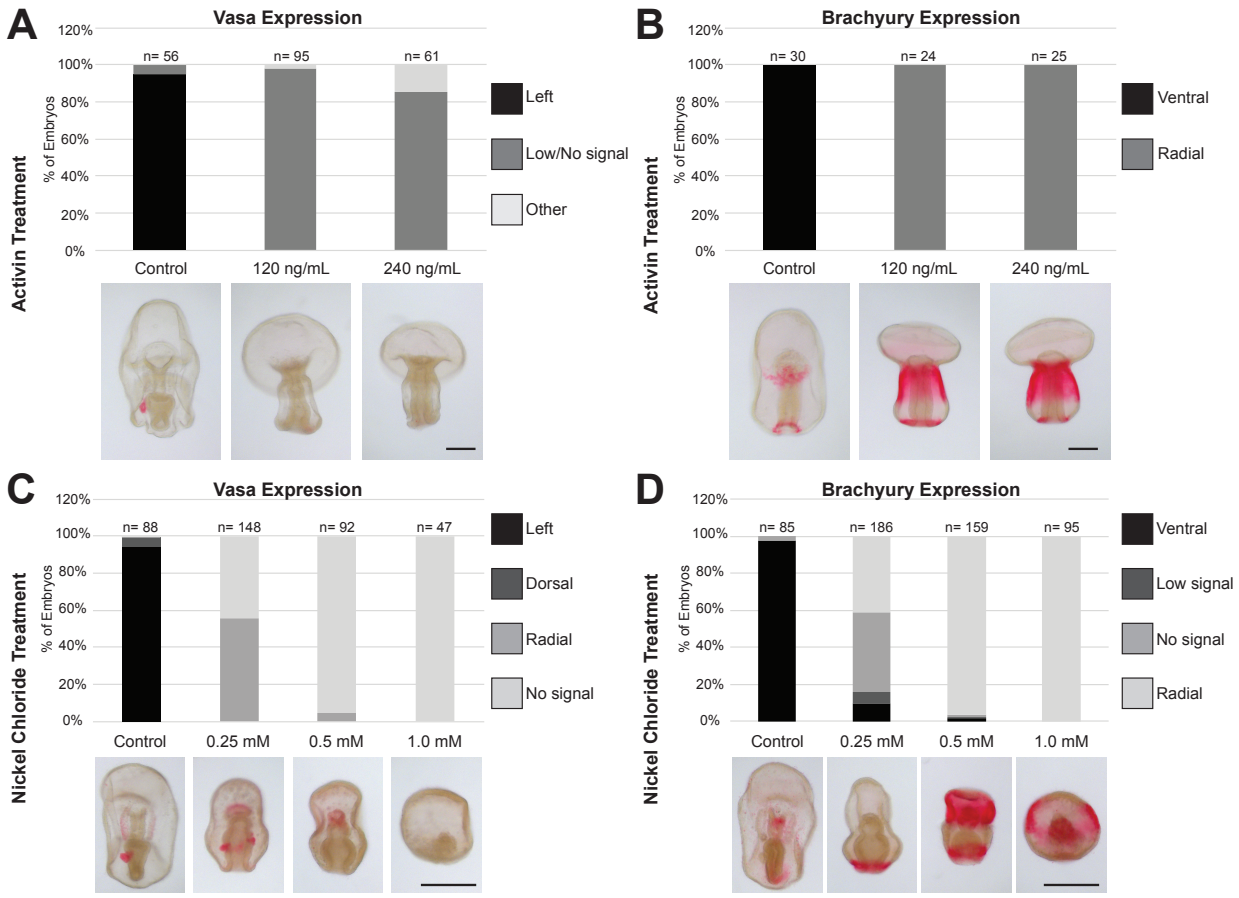
**D)** When embryos were treated with inhibitor at a later time point then there was a shift in Vasa localization such that there is less restriction in embryos treated with inhibitor relative to embryos incubated with DMSO as a control.

**E)** Morpholino experimental design: Oocytes were injected with Nodal morpholino or control morpholino at varying concentrations. The resulting embryos were fixed at the larva stage and Vasa was localized via an RNA in situ hybridization.

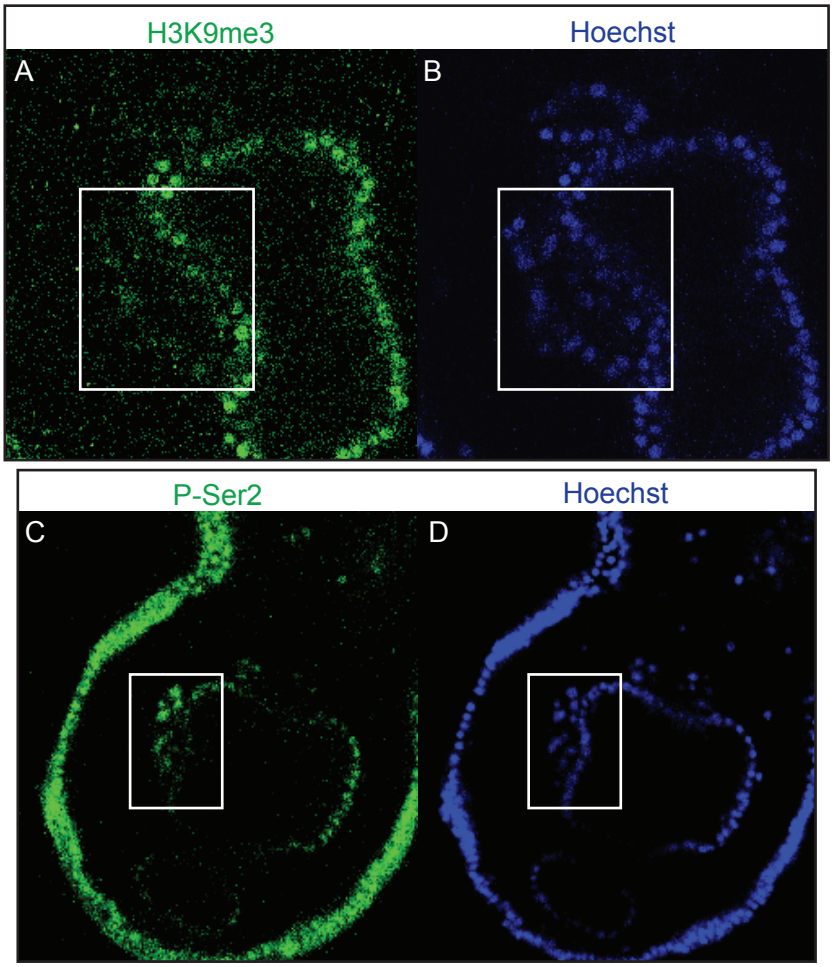
**F)** When embryos were injected with Nodal morpholino at different concentrations Vasa was localized in 3 different patterns. If there was normal restriction then Vasa was localized in the left side of the gut (black). If there was no left-side restriction then Vasa was localized in the dorsal side of the gut (dark gray). If there was no dorsal or left side restriction then Vasa was localized in a ring around the gut (light gray). Scale bar is 100 microns.

**G)** There is a dose dependent effect of Nodal morpholino upon Vasa restriction. Oocytes injected with morpholino at 0.1 mM result in 22% of embryos with a defect in left side restriction. Oocytes injected with 0.5 mM morpholino result in 52% of embryos with a defect in left side restriction and 20% of embryos with a defect in both left and dorsal side restriction. Oocytes injected with 1.0 mM Nodal morpholino result in 10% of embryos with a defect in left side restriction and 90% of embryos with a defect in both left and dorsal side restriction.

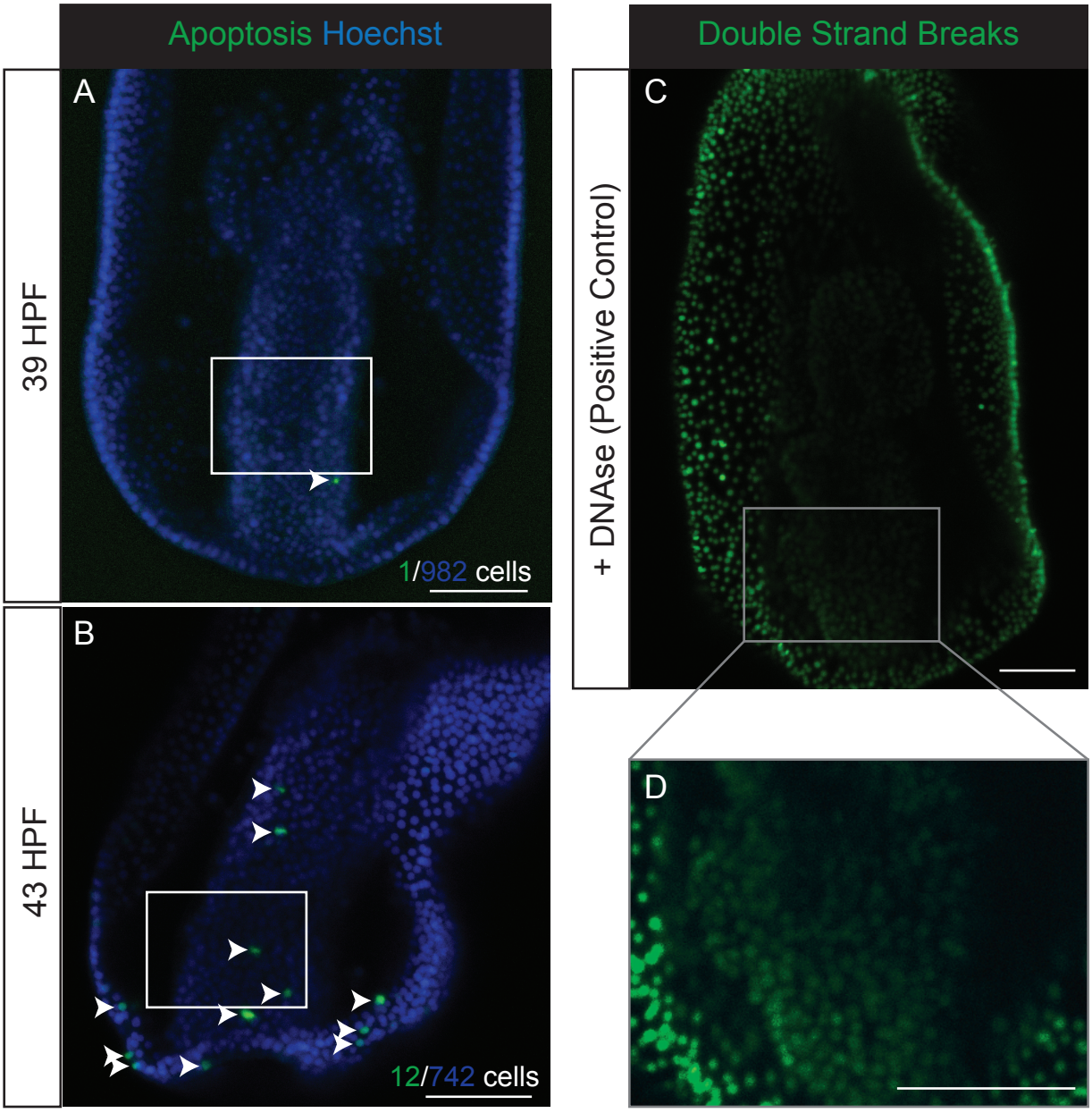
(All images are dorsal views of embryos.)




**Supplemental Figure 3: Nodal overexpression causes a decrease in Vasa mRNA**
**A)** Embryos were treated with varying concentrations of human Activin AB from 30 minutes post-fertilization to the larva stage. In control embryos, Vasa localized in the left posterior enterocoel in the majority of embryos. When embryos were treated with Activin AB Vasa expression was decreased or absent in the majority of embryos.
**B)** Embryos were treated with varying concentrations of human Activin AB from 30 minutes post-fertilization to the late gastrula stage. In control embryos, Brachyury localized in a ventral patch in the ectoderm. When embryos were treated with Activin AB brachyury expression was radialized.
**C)** Embryos were treated with varying concentrations of Nickel Chloride from 30 minutes post-fertilization to the larva stage. In control embryos, Vasa localized in the left posterior enterocoel in the majority of embryos. When embryos were treated with 0.5-1.0 mM Nickel Chloride Vasa expression was absent in the majority of embryos.
**D)** Embryos were treated with varying concentrations of Nickel Chloride from 30 minutes post-fertilization to the larva stage. In control embryos, Brachyury localized in a ventral patch in the ectoderm. When embryos were treated with 0.5-1.0 mM Nickel Chloride, Brachyury expression was radialized.
Scale bars represent 100 microns.




**Supplemental Figure 4: H3k9me3 and RNA Polymerase II antibody staining in sea star development.**
**A)** H3K9me3 antibody staining in the sea star larva. The white box outlines the posterior enterocoel.
**B)** Hoechst staining of the same embryo in A.
**C)** RNA Polymerase II, Phopho-Serine II antibody staining in the sea star larva. The white box outlines the posterior enterocoel.
**D)** Hoechst staining of the same embryo in C.




**Supplemental Figure 5: Apoptosis during sea star development.**
**A)** During the ventral clearance of Nanos and Vasa RNA (~39HPF) only a few cells are fluorescently labelled with a marker of apoptosis (arrowhead) and they are not concentrated in a specific embryonic domain.
**B)** During the right clearance of Nanos and Vasa RNA (~43HPF) only a few cells are fluorescently labelled with a marker of apoptosis (arrowheads) and they are not concentrated in a specific embryonic domain.
**C)** Positive control, nuclei of embryos treated with DNAse.
**D)** Inset of C. All scale bars are 50 microns.

| Gene name | Primers | Accession |
|---|---|---|
| Lefty | F- ATGGAGTCTCGCGTAGCTGT<br>R- CATGTTTGTTGACGGGTCTG | N/A |
| Nanos | F- ATATGAGCTGGCTGACAACG<br>R- TGATATTCAATGCTAGGCCTAATAGA | KU594505 |
| Nodal | F- CGGTGGATCGTCTACCCTAA<br>R- CCCGATCAAATTGTAAAAATGC | KC669538 |
| Tbx2/3 | F- GGCCAACGACATTTTGAAGT<br>R- GCTTAACGCTGAAGGGTCTG | N/A |
| Vasa | F- CGGTCCAGAAGTACGGGATA<br>R- GTAGAAGCTGGTTGCCTTGC | FJ605737 |

**Supplemental Table 1- Primers used for in situ probe synthesis**

| Gene name | Primers |
|---|---|
| Lefty | F- GACCTGACCTCAATCCCTGC<br>R- CTGATGCTGATGGGTGGTGT |
| Nanos | F- CCGAAGAGTTGACGAGGAAG<br>R- CAACTCCAAGCACCCACAG |
| Ubiquitin | F- TTCGGTGAAAGCCAAGATTC<br>R- CCCACCTCTCATGGCTAGAA |
| Vasa | F- TGGCTGATGCTCAACAAGAC<br>R- AAAGTTTCCGCCTCCGTAAT |
| 18S | F- CGCGAGATTGAGCAATAACA<br>R- GTACAAAGGGCAGGGACGTA |

**Supplemental Table 2- Primers used for quantitative PCR**