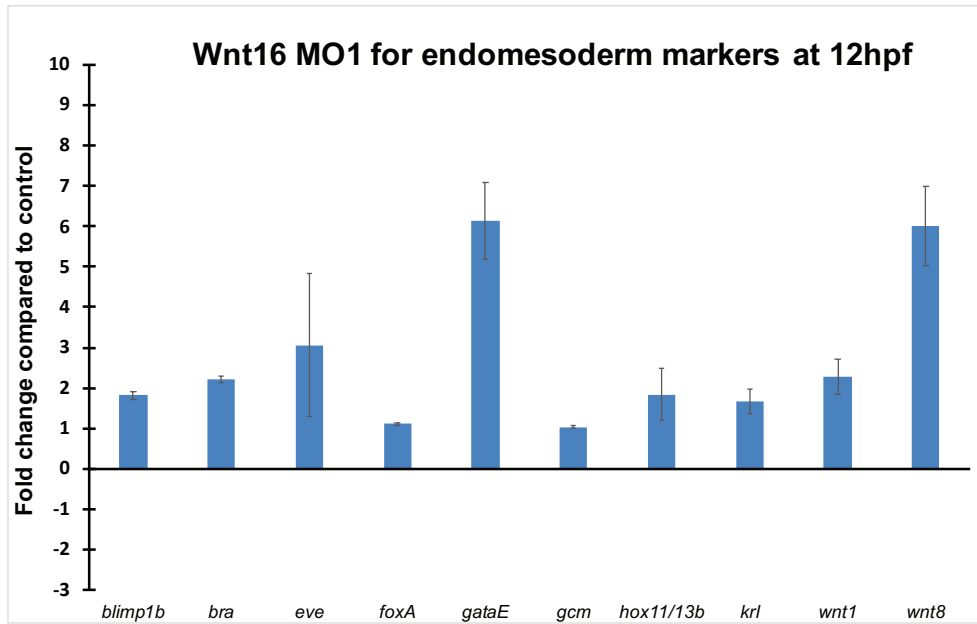


Figure S1. Wnt16 overexpression phenotype and qPCR analysis for ANE GRN members and *wnt16* expression. (A) Phenotypes for control and Wnt16 overexpression at late gastrula stage. (B) qPCR analysis from three different batches of embryos showing the downregulation of ANE regulatory factors at mesenchyme blastula stage (24 hpf) in Wnt16 knockdowns. (C) qPCR data showing that *wnt16* expression was downregulated in embryos injected with a dominant negative form of Fzl5/8 ($\Delta Fzl5/8$) at mesenchyme blastula stage (24 hpf). The y-axis shows the fold change in gene expression level in Wnt16 knockdowns and $\Delta Fzl5/8$ mRNA injected embryos compared to control embryos at mesenchyme blastula stage (24 hpf). MO, morpholino; Scale bars: 20 μm .

A



B

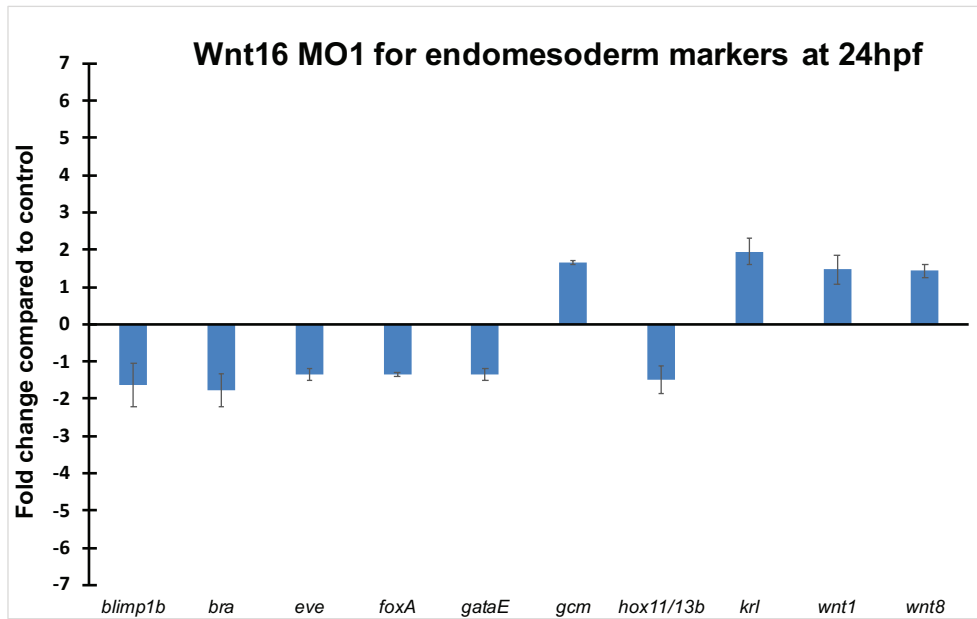


Figure S2. qPCR analysis for endomesoderm GRN factors.

(A) qPCR measurements from three different batches of embryos showing the upregulation of several endomesoderm factors at 120-cell stage (12 hpf) in Wnt16 knockdowns. (B) qPCR measurements from three different batches of embryos showing that the expression of endomesoderm makers was not affected in Wnt16 morphants at mesenchyme blastula stage (24 hpf). The y-axis shows the fold change in gene expression level in Wnt16 knockdowns compared to control embryos at mesenchyme blastula (24 hpf) and 120-cell (12 hpf) stages. MO, morpholino; Scale bars: 20 μm .

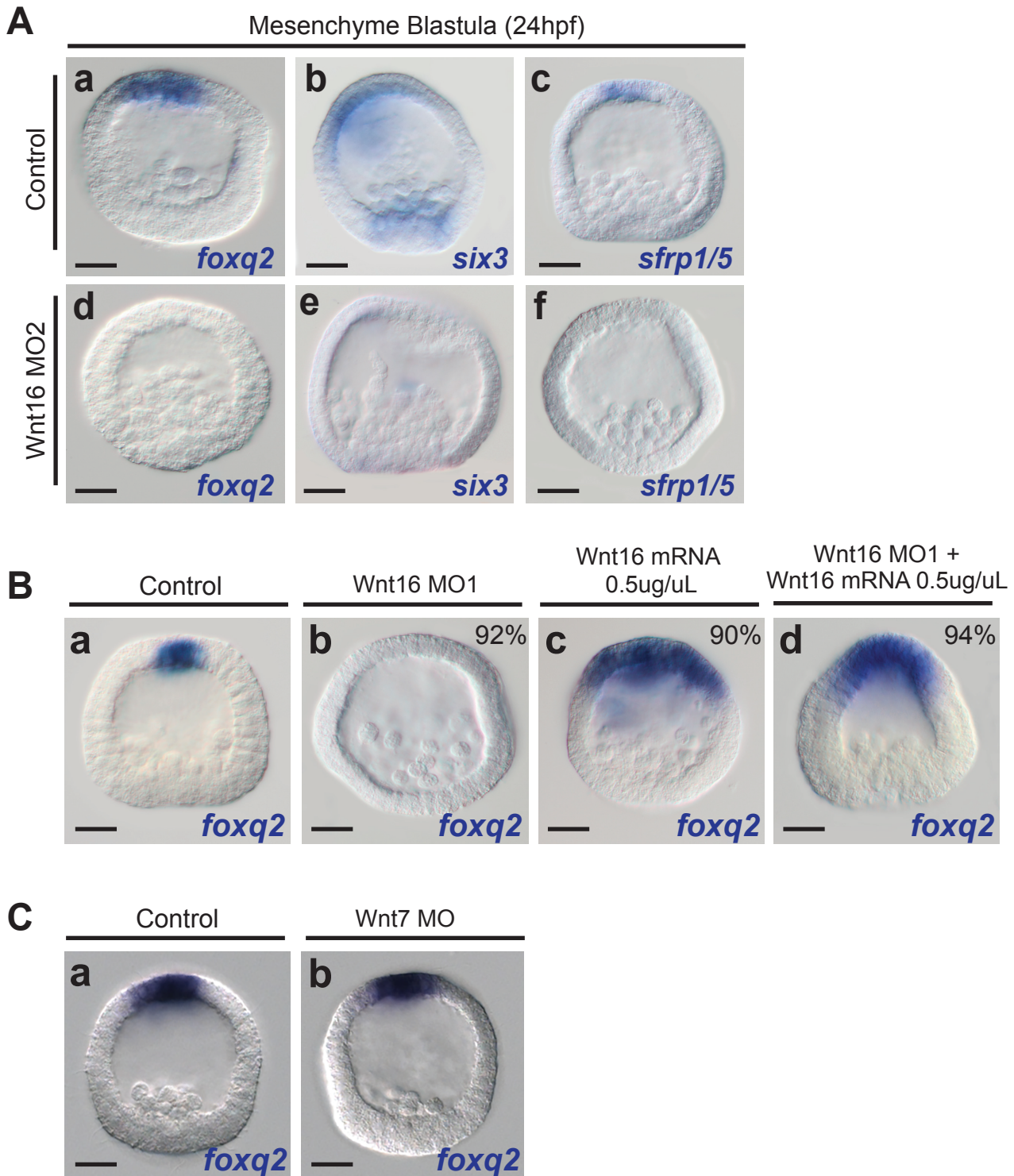


Figure S3. Additional morpholino phenotypes and controls. (A) The ANE makers are downregulated in embryos injected with Wnt16 MO2 (Ad-f) compared with control embryos (Aa-c). (B) Expression of the ANE marker *foxq2* in control (Ba), Wnt16 MO1-injected (92%; $n=68/74$; Bb), and *wnt16* mRNA-injected embryos (90%; $n=54/60$; Bc) at mesenchyme blastula stage. Wnt16 overexpression rescues *foxq2* expression in embryos injected with Wnt16 MO1 (94%; $n=64/68$; Bd), which cannot bind *wnt16* mRNA. (C) *foxq2* expression was unperturbed in embryos injected with Wnt7 morpholino. MO, morpholino; Scale bars: 20 μm .

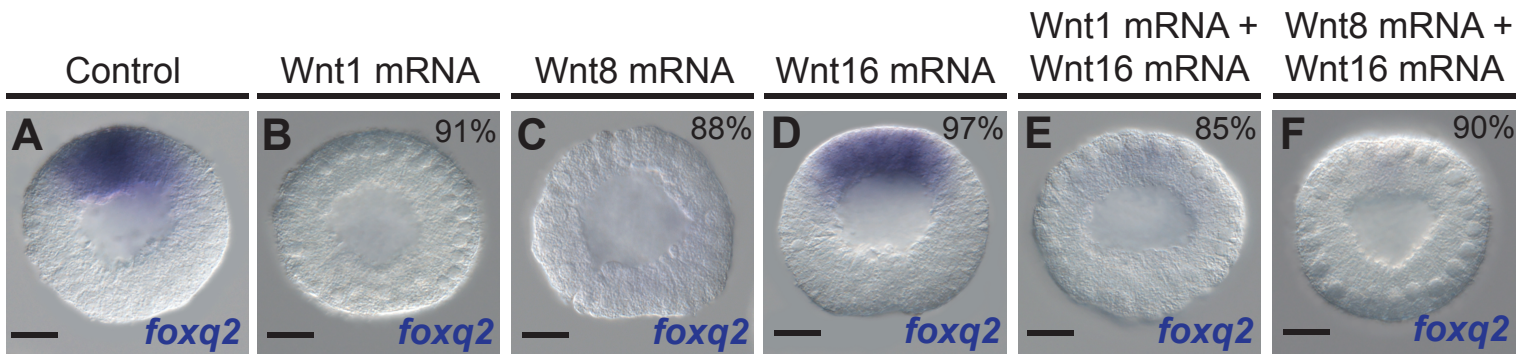


Figure S4. Expression of the ANE marker *foxq2* in embryos co-injected with *wnt16* mRNA and *wnt1* or *wnt8* mRNA at 120-cell stage. The expression of the ANE marker *foxq2* was downregulated in embryos overexpressing *wnt1* (91%) and *wnt8* mRNA (88%) at 120-cell stage (B and C). *foxq2* was expressed in embryos injected with *wnt16* mRNA (97%) (D). In addition, double injected embryos with *wnt16* mRNA and either *wnt1* (85%) (E) or *wnt8* mRNA (90%) (F) downregulated the expression of *foxq2*. Scale bars: 20 μ m.

Table S1. qPCR primers pairs used for expression analysis.

<i>Sp-mitochondrial 12s rRNA</i>	Forward 5'-ACTCTCTCCTCGGAGCTATA-3' Reverse 5'-GTATAATTTTTGCGTATTTCGGC-3'
<i>Sp-zic2</i>	Forward 5'-GAGGGATGTGATCGTCGTTT-3' Reverse 5'-ACTGCTGTCGTTGGCTTCTT-3'
<i>Sp-blimp1b</i>	Forward 5'-TCGCTATGCGGGATCTCTAC-3' Reverse 5'-GGGGTCCTTGACCTCGTAA-3'
<i>Sp-bra</i>	Forward 5'-ACACATCGACCCATCATCAA-3' Reverse 5'-CATGGTGTGATATCTTGAAAG-3'
<i>Sp-eve</i>	Forward 5'-CACAGACCCTGGACTTTCGT-3' Reverse 5'-GACAAACGGTCATCCCACTT-3'
<i>Sp-foxA</i>	Forward 5'-CCAACCGACTCCGTATCATC-3' Reverse 5'-CGTAGCTGCTCATGCTGTGT-3'
<i>Sp-gataE</i>	Forward 5'-CTGGCTCAAGACGAGAAGGA-3' Reverse 5'-CCTCTTCCGAGTCTGAATGC-3'
<i>Sp-gcm</i>	Forward 5'-CGACTGATAACCACGCTCAA-3' Reverse 5'-TTAACGACGTCGGTCGATTC-3'
<i>Sp-hox11/13b</i>	Forward 5'-CACAGGCTCTCGACCTAACC-3' Reverse 5'-GGTGGATGAGGTGGTAGATGA-3'
<i>Sp-krl</i>	Forward 5'-CACGAACTCTTCGCAATCAA-3' Reverse 5'-CCAAGGGACAGGAGTGAAGA-3'
<i>Sp-wnt1</i>	Forward 5'-ACCTTCAGAACGGTGGGTGA-3' Reverse 5'-AGCTTGATTGGTTTCGCG-3'
<i>Sp-wnt8</i>	Forward 5'-TGTCGTTTCATTCAAGCCATC-3' Reverse 5'-TATCACTCGCCATTCGTTCA-3'
<i>Sp-wnt16</i>	Forward 5'-CTTACCGAATGTGGATGTGC-3' Reverse 5'-TATCGCCAGAGTCGACAAAC-3'