

Fig. S1. Specificity of the P-Smad1/5/8 immunostaining. P-Smad1/5/8 immunostaining was performed on neurulae (St. 13/14). Active BMP signaling (magenta) was detected in nuclei of the ventral epidermis midline and the underlying endomesoderm in control embryos. Activation (treatment with BMP2 protein) and inhibition (treatment with DMH1) were started at progressively later stages indicated on the figure) and terminated at the time of fixation (St. 13/14). All treatments with BMP2, even the 30 min treatment, induced ectopic nuclear P-Smad1/5/8 throughout the epidermis. While DMH1 treatment from the 8-cell stage completely abolished P-Smad1/5/8 detection, shorter treatments strongly downregulated P-Smad1/5/8 levels. Embryos are shown in ventral views with anterior to the left (DAPI in cyan to highlight the nuclei). Scale bar: 20 μ m. The experiment was performed once except the treatments from the 8-cell stage that have been analyzed in at least two independent experiments.

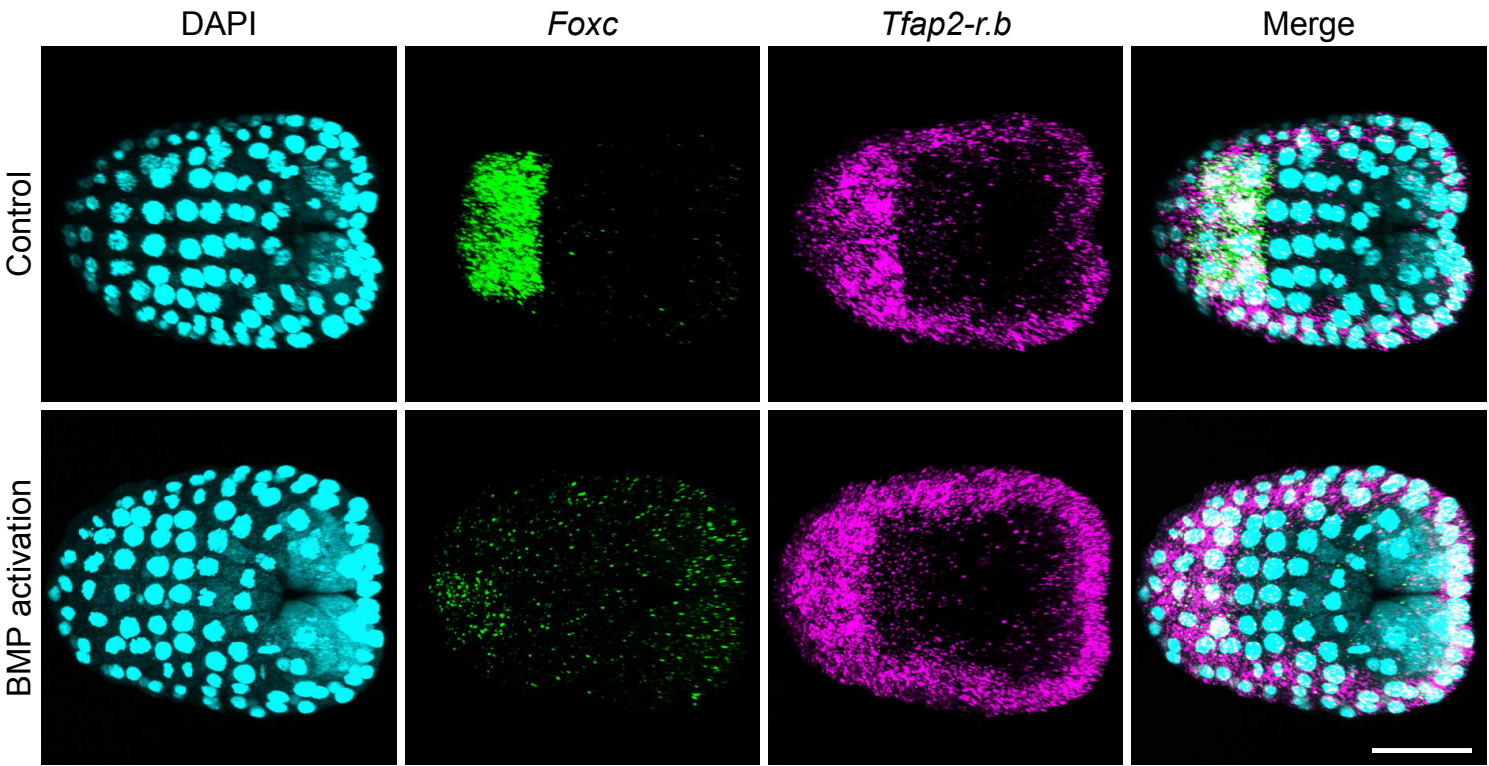


Fig. S2. Early BMP activation represses *Foxc* but not *Tfap2-r.b*. Embryos were treated with BMP2 protein and fixed at early neurula stages (St. 14) to determine *Foxc* (green) and *Tfap2-r.b* (magenta) expression by fluorescent *in situ* hybridization. In control embryos, *Foxc* was expressed in 8 cells that are also *Tfap2-r.b* positive (100%, n=6). By contrast, in treated embryos, *Foxc* was not detected while *Tfap2-r.b* expression was unchanged (100%, n=6). Embryos are shown in neural plate views with anterior to the left (DAPI in cyan to highlight the nuclei). Scale bar: 50 μ m. The experiment was performed once.

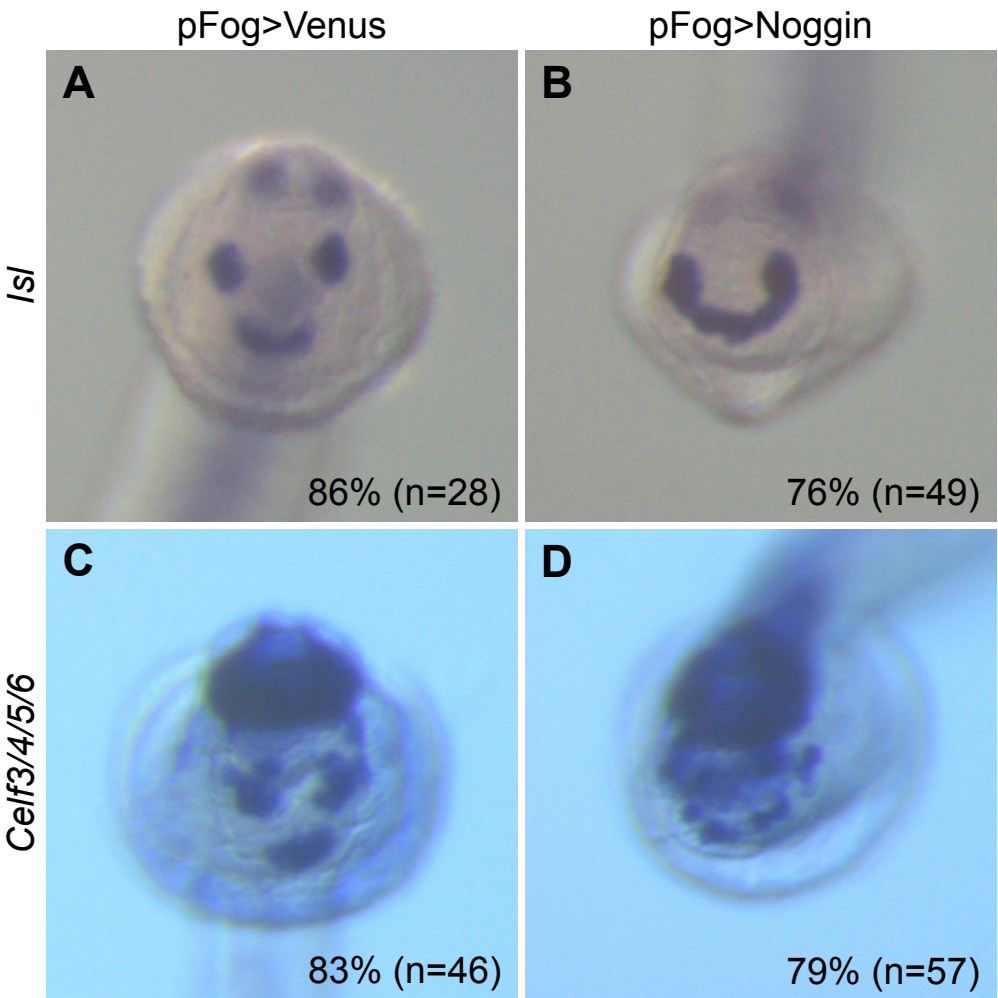


Fig. S3. BMP inhibition by electroporation of pFog>Noggin alters palp formation. Embryos were electroporated with pFog>Venus (A,C) or pFog>Noggin (B,D) and fixed for *in situ* hybridization against *Is/* at mid tailbud stages (St.22) (A,B) and *Celf3/4/5/6* at late tailbud stages (St. 23) (C,D). For each panel, n indicates the number of embryos examined. The percentages indicate the frequency of the phenotype depicted in the picture. Each experiment has been performed once.

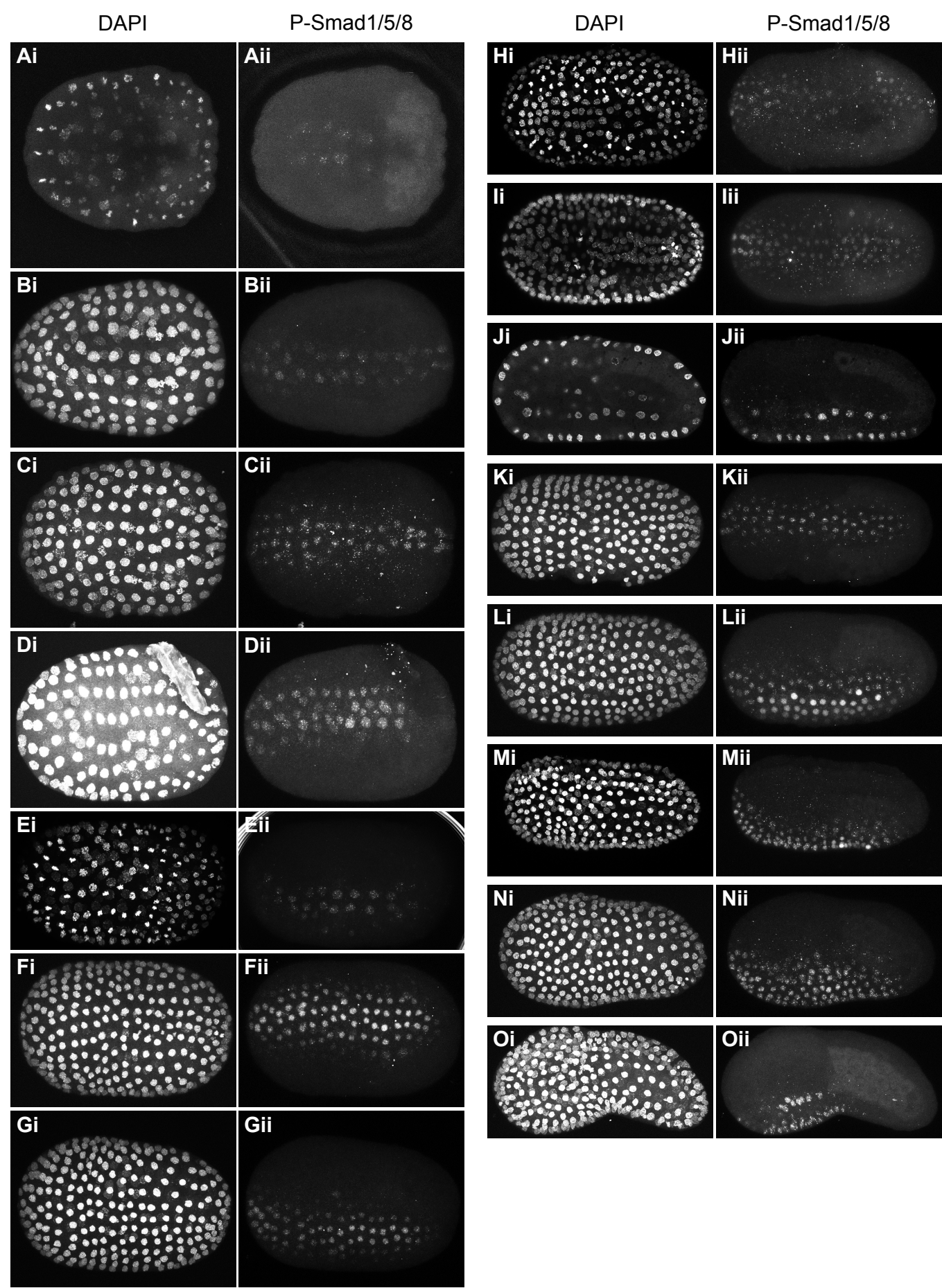


Fig. S4. P-Smad1/5 immunostaining in *P. mammillata*. Control embryos at various developmental stages were stained with DAPI (i column) and for P-Smad1/5/8 using the #9516 antibody (ii column). The images are maximum intensity projections of confocal z-stacks except J where a confocal section is shown. All embryos are shown with anterior to the left. (A-C) Progressively older gastrulae are shown in ventral views. Note that in Aii only endodermal staining is visible. For the other stages, mostly staining of ventral epidermal midlines is visible. (D-L) Progressively older neurulae are shown in ventral views (D,F,H,I,K) and more or less tilted lateral views with dorsal to the top (E,G,J,L). Ventral epidermis and endoderm staining is visible in Jii. (M-O) Progressively older tailbud stages are shown in lateral views with dorsal to the top. Note that the P-Smad1/5/8 staining was gradually lost from the tail.

Table S1. List of the 1098 genes repressed by BMP signaling in *P. mammillata*. Processing of the *P. mammillata* RNA-seq data from the BioProject PRJNA779382 has been described in (Chowdhury et al., 2022). Here are shown the genes with a negative log2 fold change as calculated by DESeq2 with a p-value<0.05. BvC: BMP4 treatment vs control. BvDorso: BMP4 treatment vs Dorsomorphin treatment. B+DvC: BMP4+DAPT treatment vs control. DvC: DAPT treatment vs control.

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Table S2. Expression patterns for a list of the 55 genes regulated by BMP signaling in *P. mammillata*.

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Table S3. List of 68 genes expressed in the palp lineage in *Ciona or/and Phallusia*.

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Table S4. Gene identifiers and references. Unique gene identifiers have been retrieved from Aniseed.

Overexpression					Reference
Gene name	Gene ID				
<i>Admp</i>	Cirobu.g00004618				Pasini et al., 2006
<i>Noggin</i>	Cirobu.g00003129				Pasini et al., 2006
<i>Ciona intestinalis</i> cDNAs					
Gene name	Gene ID	Clone used for ISH	Forward primer	Reverse primer	Reference
<i>Sp6/7/8/9</i>	Cirobu.g00003422	cien70197			Gilchrist et al., 2015
<i>Isl</i>	Cirobu.g00011396				Giuliano et al., 1998
<i>Foxg</i>	Cirobu.g00009441	RT-PCR	AAGACCAGAAACCGTCAACA	GCATCCAATAGTTACCCTTCC	This study.
<i>Foxc</i>	Cirobu.g00012813	cilv050a24			Satou et al., 2002
<i>Celf3/4/5/6</i>	Cirobu.g00007645	citb028e11			Satou et al., 2002
<i>Otx</i>	Cirobu.g00006940				Hudson and Lemaire, 2001
<i>Tfap2-r.b</i>	Cirobu.g00008275	cien223529			Gilchrist et al., 2015
<i>Emx</i>	Cirobu.g00011241	RT-PCR	ATGAATCTGAATAGCCGTTTCGCG	TTACGTCATAGACGCTTGCGTT	This study.
<i>Pou4</i>	Cirobu.g00004616	RT-PCR	ATGTTTACTAACATGCTTGCTCC	TCGATCTCGGATAGGGCAGT	This study.
<i>Msx</i>	Cirobu.g00005203	cign067118			Satou et al., 2002
<i>Sox14/15/21</i>	Cirobu.g00013989	ciad018118			Satou et al., 2002
<i>Phallusia mammillata</i> cDNAs					
<i>Pou4</i>	Phmamm.g00008914	AHC0AAA33YA19			Coulcher et al., 2020
<i>Celf3/4/5/6</i>	Phmamm.g00007762	AHC0AAA212YP22			Coulcher et al., 2020
<i>Isl</i>	Phmamm.g00000576	McDougall lab			Dardaillon et al., 2020