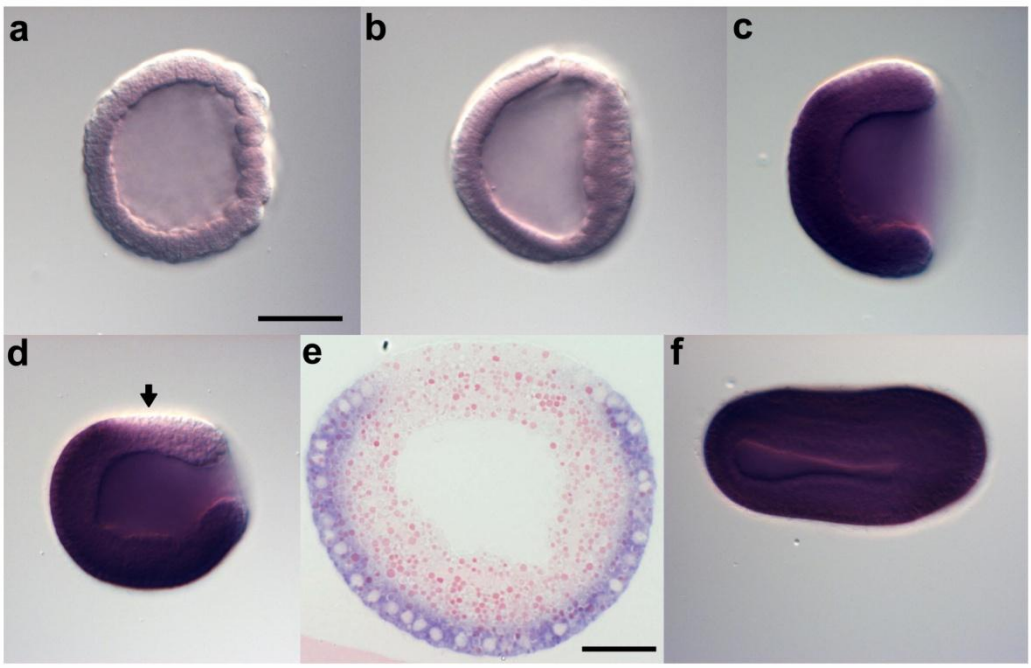
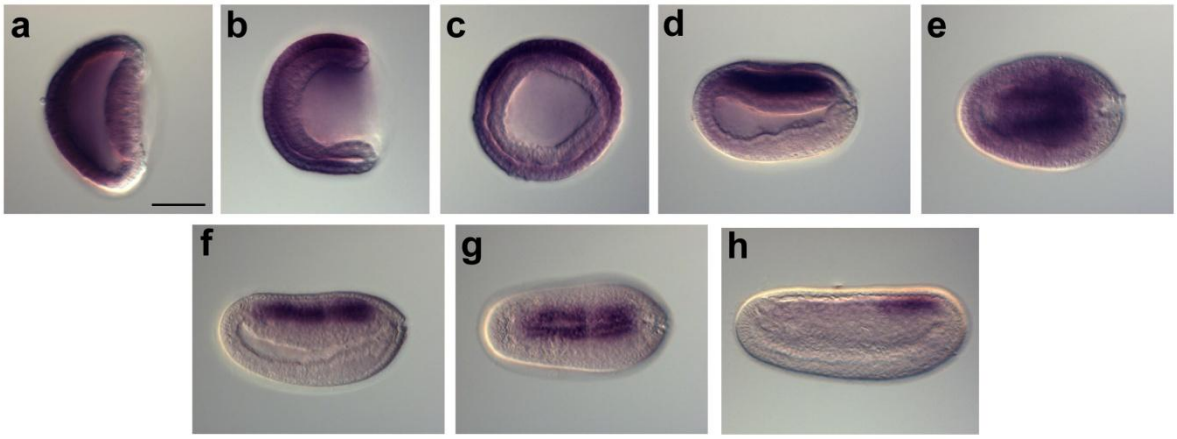
Nodal/Activin Pathway is a Conserved Neural Induction Signal in Chordates

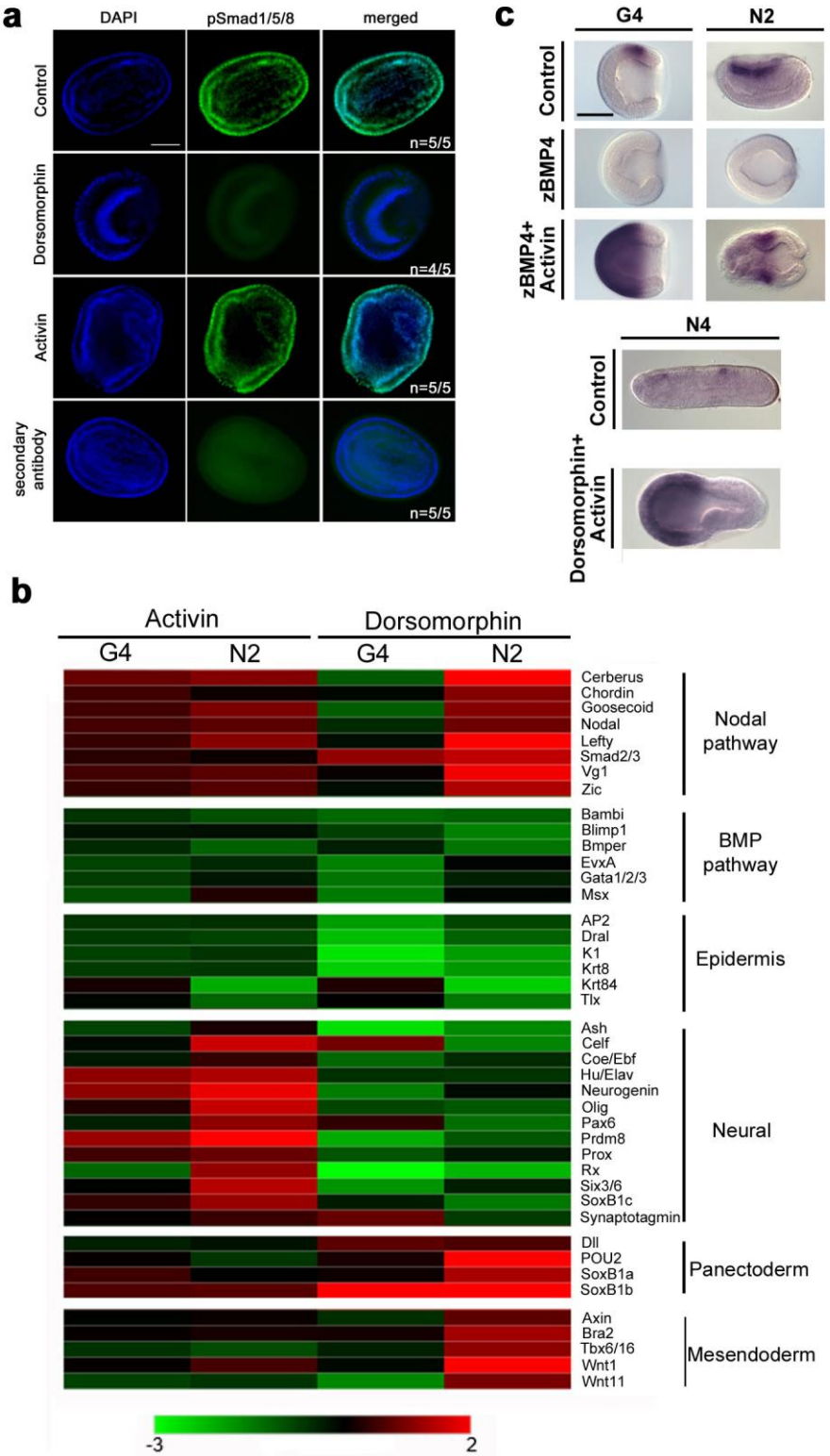
Supplementary Figures



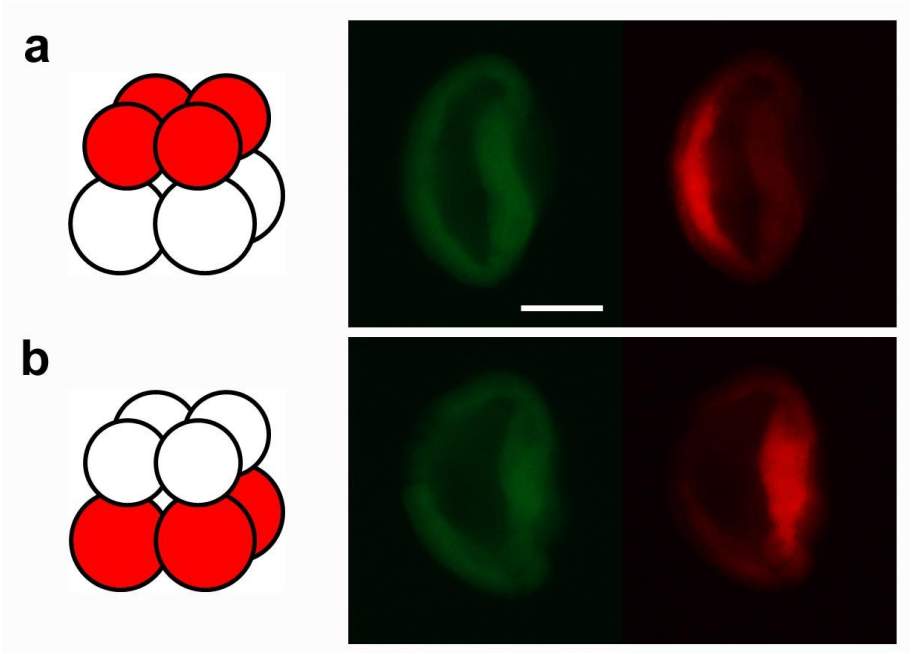
Supplementary Figure 1. *K1* expression pattern. *In situ* hybridization of *K1* at blastula (**a**), G1 (**b**), G4 (**c**), G6 (**d-e**) and N1 (**f**) stages. **e**, cross section of (**d**) at the level of the arrow. Whole mount *in situ* hybridization pictures are all side views with anterior to the left and dorsal to the top. Scale bars, 50 µm. Expression of *K1* starts at the G4 stage and is restricted to the future epidermis.



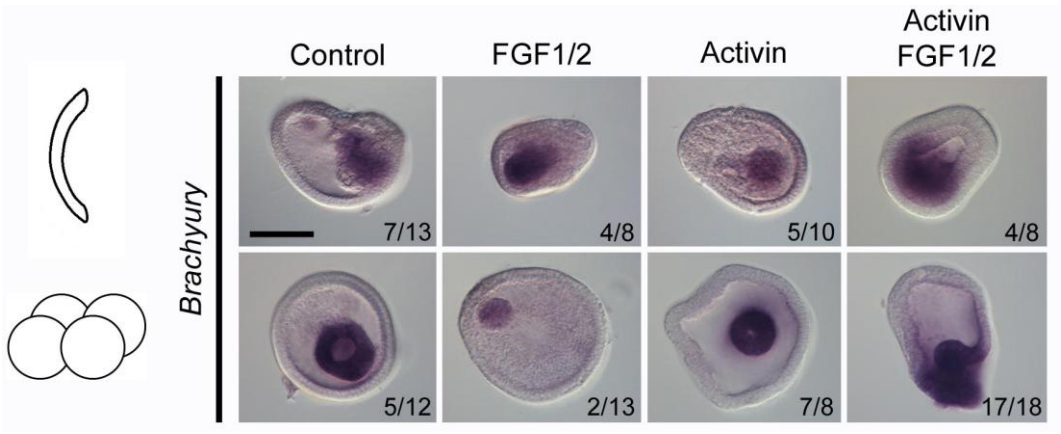
Supplementary Figure 2. *SoxB1a* expression pattern. *In situ* hybridization at different developmental stages. (**a**), (**b**), (**d**), (**f**) and (**h**) are side views. (**c**) is a blastoporal view of the embryo shown in (**b**). (**e**) and (**g**) are dorsal views of the embryos shown in (**d**) and (**f**), respectively. Scale bar, 50µm.


a
DAPI
pSmad1/5/8
merged
Control
Dorsomorphin
Activin
secondary antibody
n=5/5
n=4/5
n=5/5
n=5/5
c
G4
N2
Control
zBMP4
zBMP4+ Activin
N4
Control
Dorsomorphin+ Activin
b
Activin
Dorsomorphin
G4
N2
G4
N2
Cerberus
Chordin
Goosecoid
Nodal
Lefty
Smad2/3
Vg1
Zic
Nodal pathway
Bambi
Blimp1
Bmper
EvxA
Gata1/2/3
Msx
BMP pathway
AP2
Dral
K1
Krt8
Krt84
Tlx
Epidermis
Ash
Celf
Coe/Ebf
Hu/Elav
Neurogenin
Olig
Pax6
Prdm8
Prox
Rx
Six3/6
SoxB1c
Synaptotagmin
Neural
Dll
POU2
SoxB1a
SoxB1b
Panectoderm
Axin
Bra2
Tbx6/16
Wnt1
Wnt11
Mesendoderm
-3
2

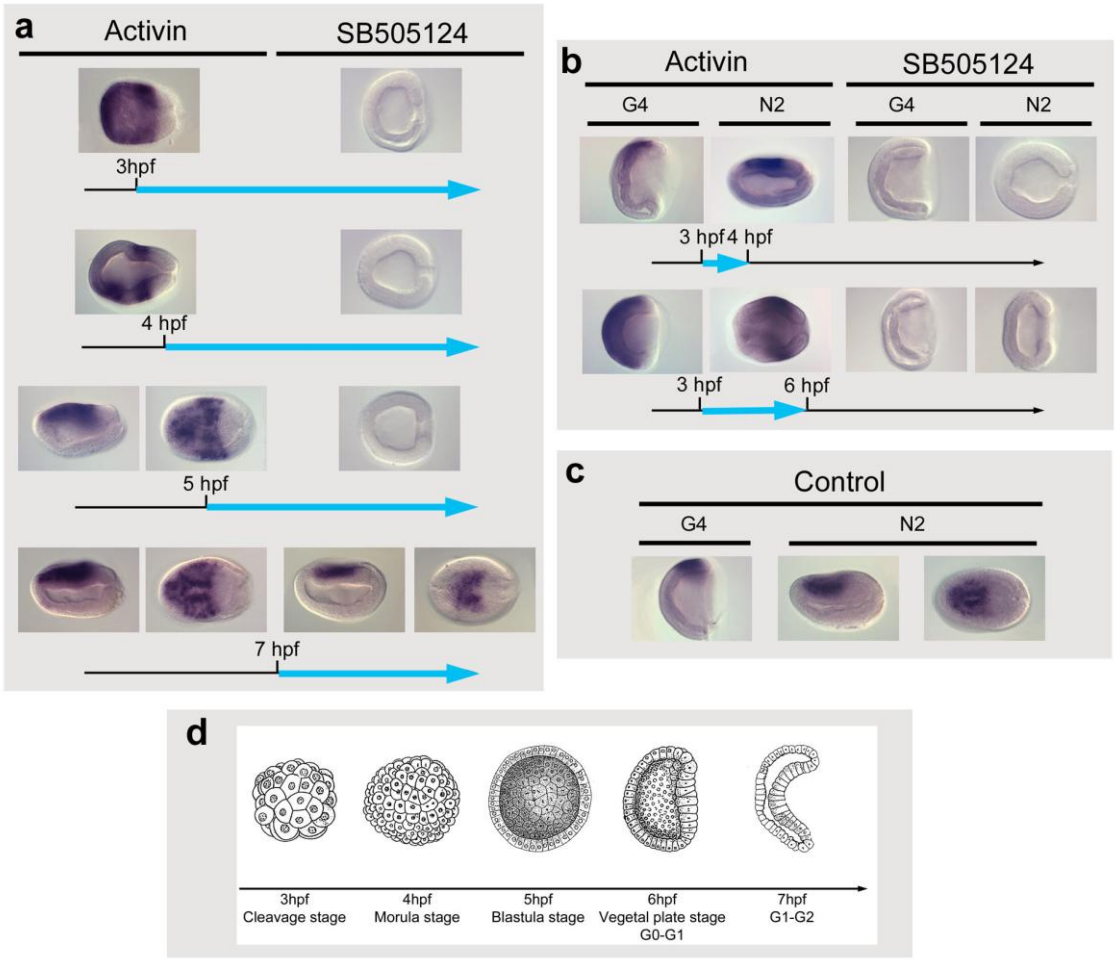
Supplementary Figure 3. Interaction between Nodal/Activin and BMP pathways. a, Immunostaining against pSmad1/5/8 and nuclear DAPI staining in control, dorsomorphin-treated and Activin-treated N2 stage amphioxus embryos. **b**, Heat map of log2 fold changes for selected genes that are up- (red) or down-regulated (green) in whole embryos at the G4 and N2 stages after Activin and dorsomorphin treatments. For both treatments the expression of genes orthologous to classical Nodal and BMP targets in vertebrates was up and down-regulated, respectively, confirming that BMP and Nodal are globally opposing signals. Concerning ectoderm cell fate commitment, the expression of epidermal genes was down-regulated after both treatments whereas neural genes were overexpressed only in Activin-treated embryos. In dorsomorphin-treated embryos the expression of pan-ectoderm genes, such as *SoxB1a*, *POU2* or *Dll*, was up-regulated. Expression of dorsal mesendoderm genes was up-regulated mainly in dorsomorphin-treated embryos. **c**, Expression of *Neurogenin* at the G4 and N2 stages in control embryos and after zBMP4 and Activin + zBMP4 treatments, and at the N4 stage in control embryos and embryos treated with both Activin and dorsomorphin. *In situ* hybridization images are side views with anterior towards the left. Scale bar, 50 µm.



Supplementary Figure 4. Fate of animal and vegetal blastomeres at the 8-cell stage. Schematic representation of the four labeled animal cells (micromeres) or vegetal cells (macromeres) at the 8-cell stage. Cells were labeled by contact with DiI (Invitrogen). Green auto-fluorescence of the labeled embryo at the G2 stage is shown. Red fluorescence is observed in the ectoderm in embryos in which animal cells were labeled (**a**) whereas red fluorescence is observed in the mesendoderm when vegetal cells were labeled (**b**). Scale bar, 50 µm.

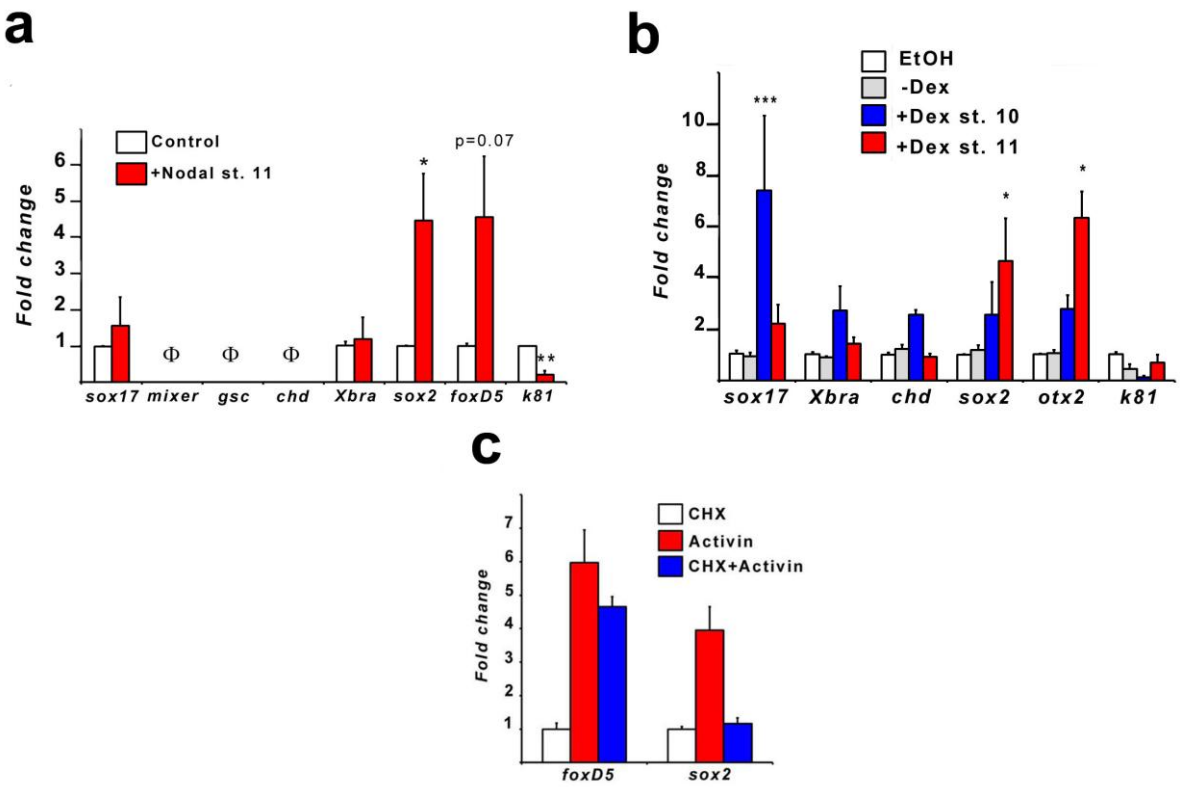


Supplementary Figure 5. *Brachyury* expression in explants. *Brachyury* expression in FGF1/2 (**a**, **d**), Activin (**b**, **e**) and Activin-FGF1/2 (**c**, **f**) treated GE and BE, respectively. In GE or BE that show inner cell mass, these cells are expressing *Brachyury*, in wild-type as in treated explants. Scale bar, 50 µm.

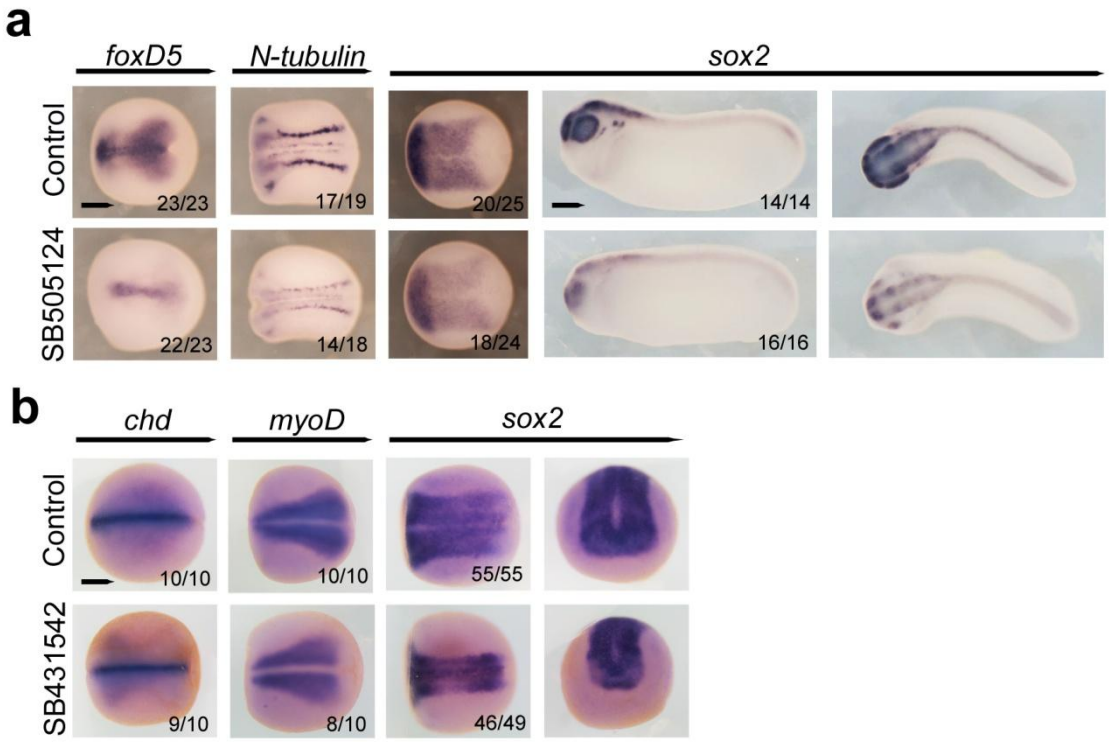


Supplementary Figure 6. Timing of Nodal/Activin signal requirement for neural induction. a, Expression of *Neurogenin* at the N2 stage in embryos after continuous treatment with Activin or SB505124 from 3 hpf, 4 hpf, 5 hpf or 7 hpf at 19°C. **b**, Expression of *Neurogenin* in G4 and N2 stage embryos after treatment with Activin or SB505124 between 3 hpf and 4 hpf or between 3 hpf and 6 hpf at 19°C. **c**, Expression of *Neurogenin* in G4 and N2 control embryos. **d**, Drawing of amphioxus embryos at the stages at which treatments were performed, adapted from Conklin. When two pictures are presented, the left one is side view with anterior to the left and dorsal to the top whereas the right picture is dorsal view with anterior to the left. Blue arrows correspond to the period of treatment. Our results show that inhibiting the Nodal/Activin pathway *via* the application of SB505124 at any time between cleavage stage and G1 stage impeded the formation of *Neurogenin* positive neural tissue (**a**, **c-d**). Moreover, one-hour treatment between cleavage stage and morula stage was also

sufficient to preclude neural tissue formation (**b**, **c-d**). Conversely, treatment with Activin between cleavage stage and the beginning of gastrulation (G0-G1 stage) induced neural commitment in the whole ectoderm at G4, although neural fate was lost in the anterior region at N2 (**b**, **c-d**). In contrast, activating the Nodal/Activin pathway after the morula stage was not sufficient to neuralize all ectodermal cells (**a**, **c-d**). Altogether these results suggest that Nodal/Activin signal is required very early for neural induction to occur, and that the maintenance of the neural fate in the anterior region is also dependent upon this signal.



Supplementary Figure 7. Nodal/Activin induces neural tissue in *Xenopus* animal cap assays. a, RT-qPCR from animal caps treated from stage 11 to 15 with Nodal. Bars represent SEM of 5 independent experiments, Φ represents undetectable (background) gene expression levels. Statistical analysis has been performed using Paired t-test, * $p<0.05$; ** $p<0.005$. **b**, RT-qPCR from animal caps injected with GR-t-Smad2, explanted at early gastrula stage and induced or not with dexamethasone at stage 10 or 11. Uninjected animal caps were treated with ethanol and served as reference. Bars represent SEM of 4 independent experiments, except for *chd* and *otx2* for which they represent 2 independent experiments. Statistical analysis has been performed using Two-way ANOVA followed by Bonferroni post-test; * $p<0.05$; ** $p<0.005$; ***$p<0.001$. **c**, RT-qPCR analyses of *sox2* and *foxD5* expression in animal caps treated with cycloheximide, Activin, or both. Error bars indicate SEM.



Supplementary Figure 8. Activin/Nodal signaling is required for proper neural tissue formation in *Xenopus*.

a, Expression of *foxD5* at stage 13, *N-tubulin* at stage 15 and *sox2* at stage 13 (left) and stage 25 (right) in control embryos and in embryos treated with 200µM SB505124 from mid-gastrula stage 11. Note the decreased intensity of neural marker gene expression in SB505124-treated embryos. Dorsal view for *foxD5*, *N-tubulin* and *sox2* at stage 13 and stage 25 (right) and lateral view for *sox2* at stage 25 (left). **b**, Expression of *chd*, *myoD* and *sox2* at early neurula stage 14 in control embryos and in embryos treated with 800µM SB431542 from stage 11. Note the reduced neural plate in SB431542-treated embryos despite normal axial and paraxial mesoderm formation. Dorsal view for *chd*, *myoD* and *sox2* (left), and anterior view for *sox2* (right). Scale bar, 200µm.

Supplementary Tables

Gene name	Genbank accession number	Size (bp)	Sequence used for probe synthesis
EvxA	KP284102	446	1-446
Zic	KP284103	1407	1-1407
K1	KP284104	1092	1-1092
SoxB1a	KP284105	729	1-729
Vg1	KP284106	918	1-918
Otx	EU685295	960	1-960
Nodal	EU685293	948	1-948
Neurogenin	EU685292	762	1-762
Brachyury2	EU685284	444	1-444
Netrin	HM359127	974	1-974
Hu/Elav	HM359133	495	1-495
FoxD5	AF162782	1786	1-1786
Sox2	AF005476	1190	1-1190
N-tubulin	NM_001086064	1768	1-1768
Gsc	NM_001087809	1140	1-1140
Xbra	M77243	2210	1-2210
Xnot2	NM_001087722	2095	1-2095

Supplementary Table 1. Sequences used for *in situ* hybridization probe synthesis.

Gene	Forward Primer	Reverse Primer	Reference
Sox17	GCAAGATGCTTGGCAAGTCG	GCTGAAGTTCTCTAGACACA	Xanthos JB, Kofron M, Wylie C, Heasman J. Maternal VegT is the initiator of a molecular network specifying endoderm in Xenopus laevis. Development. 2001 Jan;128(2):167-80.
Xbra	TTCTGAAGGTGAGCATGTCG	GTTTGACTTTGCTAAAAGAGACAGG	Sun BI, Bush SM, Collins-Racie LA, LaVallie ER, DiBlasio-Smith EA, Wolfman NM, McCoy JM, Sive HL. Derrière: a TGF-beta family member required for posterior development in Xenopus. Development. 1999 Apr;126(7):1467-82.
Sox2	TCTGCACATGAAGGAGCATC	CGTTCATGTGGGCATAAGTG	Mir A, Kofron M, Zorn AM, Bajzer M, Haque M, Heasman J, Wylie CC. Foxl1e activates ectoderm formation and controls cell position in the Xenopus blastula. Development. 2007 Feb;134(4):779-88.
FoxD5	CCAGCTGTGCTTAACTTATC	TGAGACCCAAAGTCACTTACT	
Otx2	GGATGGATTTGTTACATCCGTC	CACTCTCCGAGCTCACTTCCC	Sander V, Reversade B, De Robertis EM. The opposing homeobox genes Goosecoid and Vent1/2 self-regulate Xenopus patterning. EMBO J. 2007 Jun 20;26(12):2955-65.
K81	CACCAGAACACAGAGTAC	CAACCTTCCCATCAACCA	http://www.xenbase.org/common/redirectedResource.do
Odc	GCCATTGTGAAGACTCTCTCCATTC	TTCGGGTGATTCCTTGCCAC	Heasman J, Kofron M, Wylie C. Beta-catenin signaling activity dissected in the early Xenopus embryo: a novel antisense approach. Dev Biol. 2000 Jun 1;222(1):124-34.

Supplementary Table 2. RT-qPCR primer sequences.