Supplemental Data:

Opposing Nodal/Vg1 and BMP signals mediate axial patterning in embryos of the basal chordate amphioxus

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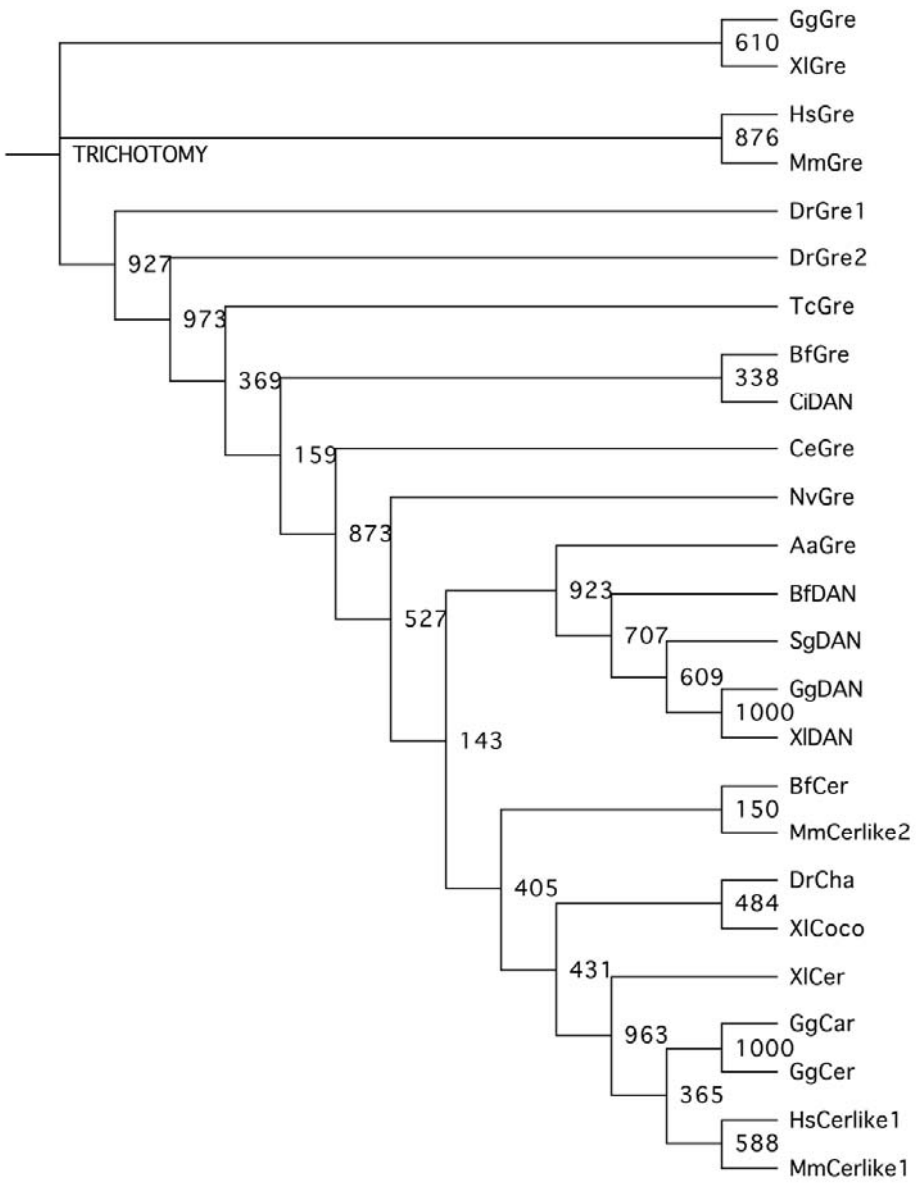

Figure S1. Unrooted tree of Dan Family proteins. Sequences were aligned with MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) using default settings for proteins. The phylogenetic tree was inferred with ClustalW http://clustalw.ddbj.nig.ac.jp/top-i.html) at the DDBJ server. Support values were with the NJ tree option with 1000 bootstrap trials. Aa, *Aedes aegypti*, Bf, *Branchiostoma floridae*, Ce, *Caenorhabditis elegans*, Ci, *Ciona intestinalis*, Dr, *Danio rerio*, Gg, *Gallus gallus*, Hs, *Homo sapiens*, Mm, *Mus musculus*, Sg, *Saccoglossus kowalevskii*, Tc, *Tribolium castenaneum*, Xl, *Xenopus laevis.*

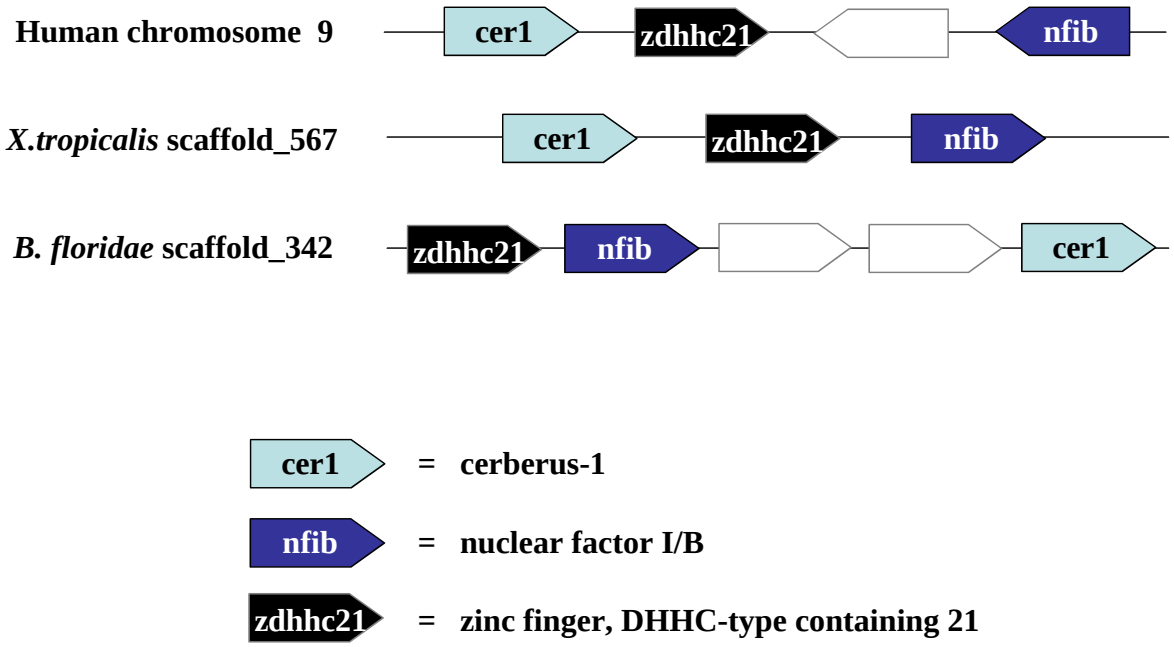

Fig. S2. Conserved synteny of *Cerberus* in amphioxus (*Branchiostoma floridae*), *Xenopus tropicalis* and human (*Homo sapiens*). In the two vertebrates, *Cerberus-1* is located on the same scaffold/chromosome as *zdhhc21* and *nfib*. Similarly in amphioxus, *Cerberus* is on the same scaffold as the other two genes, although there have been some gene rearrangements. This conserved synteny indicates that the amphioxus gene we have identified as *Cerberus* is probably a true homolog of vertebrate *Cerberus* genes even though gene sequences are sufficiently diverged that bootstrap support is low in phylogenetic analyses (Fig. S1).

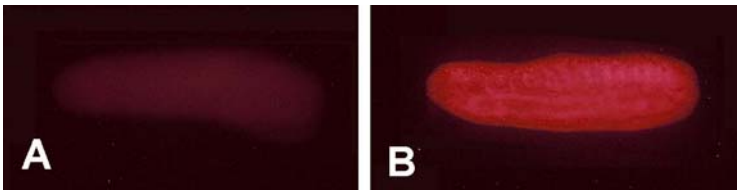

Figure S3. In vivo translation of capped mRNA. Amphioxus neurula injected with ß-gal mRNA (A) and tdtomato mRNA showing effective translation in amphioxus embryos. Anterior is at left. pCS2-td tomato was gift from Ken W Cho. pCS2-blimp-myc, pCS2-chd-myc were amplified by RT-PCR and subcloned into pCS2 vector respectively at the BamHI/XhoI site or EcoRI/XhoI site.

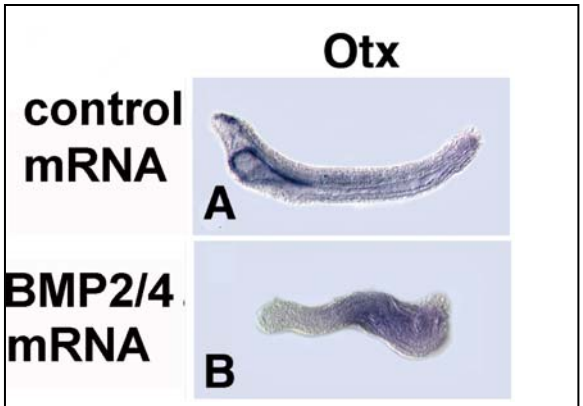

Figure S4. Overexpression of BMP2/4 mRNA suppresses dorsal-anterior development. A). 36 hr larva injected with the control mRNA. Anterior at left. B). Embryos that are injected with amphiBMP2/4 mRNA do not express the forebrain and pharynx marker Otx and are strongly posteriorized.

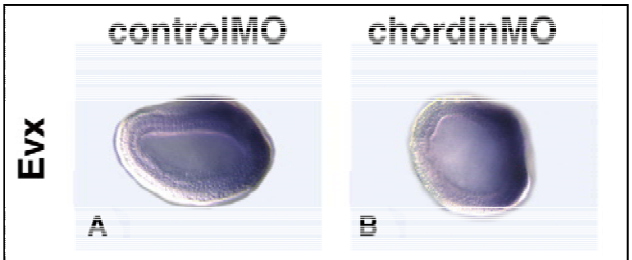

Figure S5. Attenuation of chordin has little effect on Evx expression.
A). Evx is expressed in the posterior CNS and around the blastpore at the very early neurula stage B). Although the Chordin MO delays development, the expression pattern of Evx is normal .

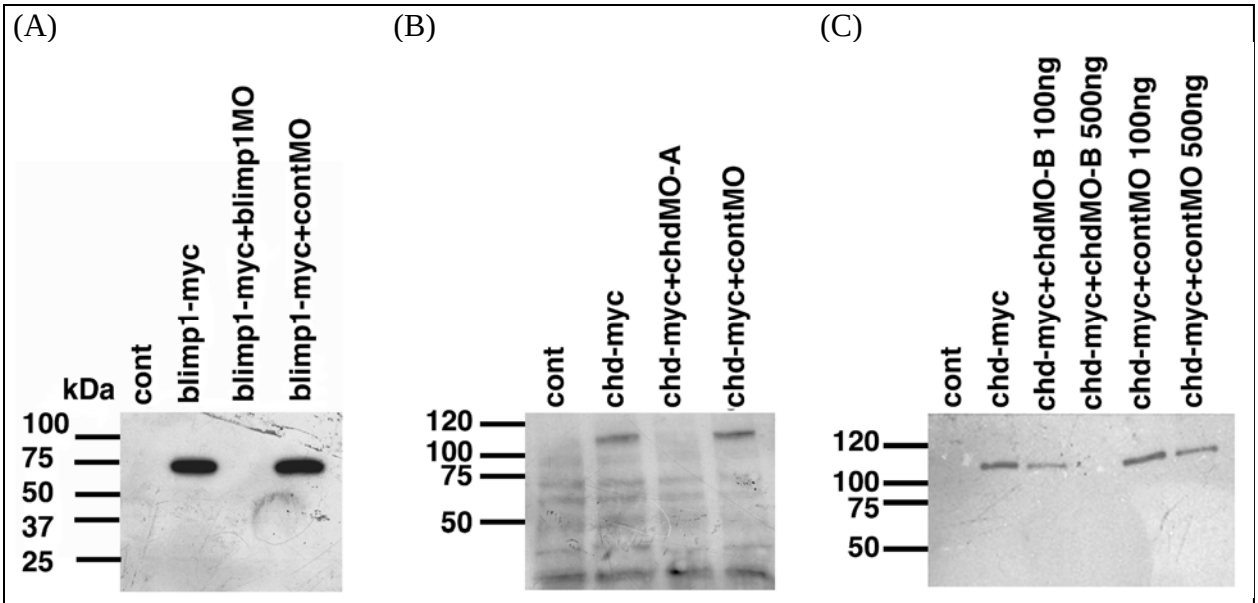

Figure S6. **In vitro translation demonstrates that MOs effectively block translation.** Control lanes at left included the pCS2 vector, no MO. Second lanes from left, plasmid but no MO. Third lanes from left plasmid plus gene-specific MO. Fourth lanes from left, plasmid plus control MO. A) Amphioxus Blimp1 MO. B) Amphioxus *Chordin* morpholino-A. C) Amphioxus *Chordin* MO-B. morpholino . In vitro tanslation for the *pCS2Amphichd-myc* and *pCS2Amphiblimp-myc* plasmids was with the TNT SP6 Quick Coupled Transcription/Translation System (Promega Inc., Fitchburg, WI, USA). Samples separated on SDS-polyacrylamide gels were subjected to western blotting and probed with an anti-myc antibody (Roche Molecular Biochemicals, Indianapolis, IN, USA at a 1:1000 dilution. Detection was with the Amersham ECL PLUS™ Western Blotting Detection Reagents (GE Healthcare, Piscataway, NJ, USA).

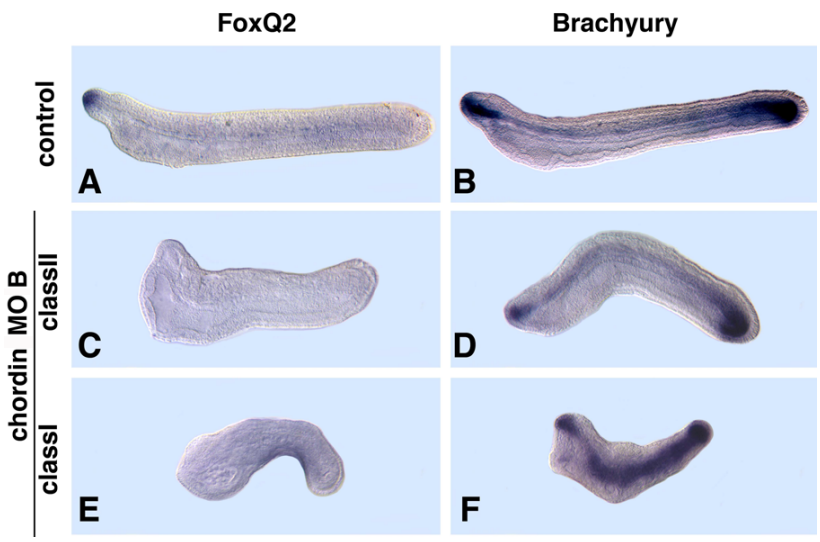

Figure S7. The Chordin MO-B suppresses head formation as does Chordin MO-A. Microinjection of chdMO-B into amphioxus eggs suppresses expression of the anterior marker *FoxQ2* in early larvae (A, C, E). The notocordal marker *Brachyury* is normally expressed (B, D,F). ChdMO-B injected embryos have two classes of phenotypes. In both, head structures are lost. Class I shows severe effects on elongation, while class II shows less severe effects on elongation.

The numbers of embryos injected and the percentage expressing a particular phenotype for each treatment are listed below for each text figure.

Fig2. Human activin protein was added to about 200 embryos for each of 3 egg batches. More than 80% had the expanded head expanded phenotype at the neurula stage. For gastrula stage embryos, the phenotypes are representative ones. Ten or more embryos per probe were assayed and more than 80% showed the representative phenotype.

Fig3. For gastrula phenotypes, more than 100 embryos per batch were treated with SB505124. At least 10 embryos for each probe were analyzed. The phenotypes showed in the figures are representative of more than 90% of the embryos. For neurula stage phenotypes, about 200-300 embryos per batch were treated with SB505124. Over 90% showed the representative phenotype.

Fig4. We injected more than 100 embryos per batch for each of 4 egg batches with 1.0µg/µl BMP2/4 mRNA. Only strongly fluorescence embryos were assayed. The neurula phenotypes shown are characteristic of more than 60% of the embryos assayed. For the figure 4 I-K the numbers of embryos are I (100% n=10), J (100% n=15), K (66% n=21)

Fig5. We injected 1.0 mM chdMOA into more than 100 embryos/each of 10 batches and isolated only strongly fluorescent embryos. The phenotypes shown for neurula to larva are representative of more than 60% of the embryos assayed. For gastrula fixed embryos, we analyzed the embryos

10-15 embryos/probes and the phenotypes are representative of more than 80% of the embryos assayed.

Fig6. We injected 1.0μg/μl Cerberus mRNA into more than 100 embryos/batch for 2 times and isolated only strongly fluorescence embryos. The neurula phenotypes are representative of more than 60% of embryos assayed. We injected 1.0mM blimp1 MO into more than 100 embryos/batch for 4 times and isolated only strongly fluorescence embryos. The phenotypes are representative of more than 60% of embryos assayed.

Fig7 For *Xenopus* injections, the numbers of the embryos are A (100% n=10), B (37% n=8), C (100% n=12), D (100% n=8), E (100% n=12), F (100% n=14)