Supplemental Figure 1: Phylogenetic Tree for Wbp2 and Wbp2nl orthologues. Tree analysis shows that the Wbp/Wbp2nl proteins separate into two clades across several vertebrate taxa (Homo=human; mus=mouse; anolis and alligator = reptiles; Falco = falcon (bird); Danio = zebrafish; X. Laev and X. trop = amphibian). Mammalian WBP2NL (human, mouse) do not tightly cluster with the homologous proteins of the other vertebrates. Numbers on branches are Bootstrap Values. Protein identifiers are: Human_Wbp2nl - AAH22546.1, Mus_Wbp2nl - NP_083342.1, Alligator_Wbp2 - XP_014372734.1, Falco_Wbp2 - XP_014142228.1, Anolis_Wbp2 - XP_014142228.1, Mus_Wbp2 - AAH55058.1, Homo_Wbp2 - AAH10616.1, X.laev._Wbp2 - NP_001083140.1, X.trop._Wbp2 - AAH88817.1, Danio_Wbp2 - AAH95106.1, X.laev._Wbp2nl - NP_001088037.1, X.trop._Wbp2nl = NP_001107397.1, Anolis_Wbp2nl - XP_008108972.1, Falco_Wbp2nl - XP_014137125.1, Alligator_Wbp2nl - XP_006027702.1, Danio_Wbp2nl - NP_956004.1.

Supplemental Figure 2: Wbp2nl MO sequences and their alignment to mRNAs. Top: Sequence of the endogenous *wbp2nl* mRNA including 5'UTR (green) and ORF (black). Translational start site is underlined. Middle: Sequences of the two MOs used in the described studies (MO#1 = blue; MO#2 = red). They are perfectly complementary to the endogenous mRNA. Bottom: *wbp2nl* ORF is preceded by 6 in-frame copies of the *myc* epitope sequence. Thus, in the rescue mRNA, the *wbp2nl* ORF is precede by a long myc sequence (part of which in shown in lavender), the translational start site of which is 350 base pairs upstream of the binding site of MO#2. In addition, MO#1 is not complementary to this mRNA. Thus, the rescue mRNA is resistant to MO-mediated

knock-down, as demonstrated in Supplemental Figure 3.

Supplemental Figure 3: Wbp2nl MOs are effective and specific. **(A)** *wbp2nl* mRNA consisting of the endogenous 5'UTR and ORF followed by a 3' HA tag is abundantly expressed in the absence of MO#1 + MO#2 (left two lanes), but is not detected in the presence of the MOs (middle two lanes). Uninj = lysate from uninjected oocytes. **(B)** the rescue *wbp2nl* mRNA, consisting of 6 copies of the myc epitope in-frame with the *wbp2nl* ORF is abundantly expressed in both absence (left two lanes) and presence (middle two lanes) of MO#1 + MO#2. See Supplemental Figure 1 for sequence of this "rescue" mRNA. **(C)** Left: Two examples of the rescue of the diminished *foxd3* domain and expanded neural plate domain (red and black bars) after co-expression of an MO-resistant *wbp2nl* mRNA (rescue) with the Wbp2nl MOs (10/10 embryos showed rescue). Right: Two examples of the rescue of the expanded *sox2* neural plate domain after co-expression of an MO-resistant *wbp2nl* mRNA (rescue) with the Wbp2nl MOs (11/15 embryos showed rescue).

Supplemental Figure 4: *Xenopus* Wbp2nl (colored amino acids) contains several predicted sites of phosphorylation (underlined) many of which are contained within motifs (black letters with phosphorylation site in bold) characteristic of a number of kinases (listed on left margin).