Developmental Cell *15*

Supplemental Data

Crossveinless-2 Is a BMP Feedback Inhibitor that Binds Chordin/BMP to Regulate *Xenopus* Embryonic Patterning

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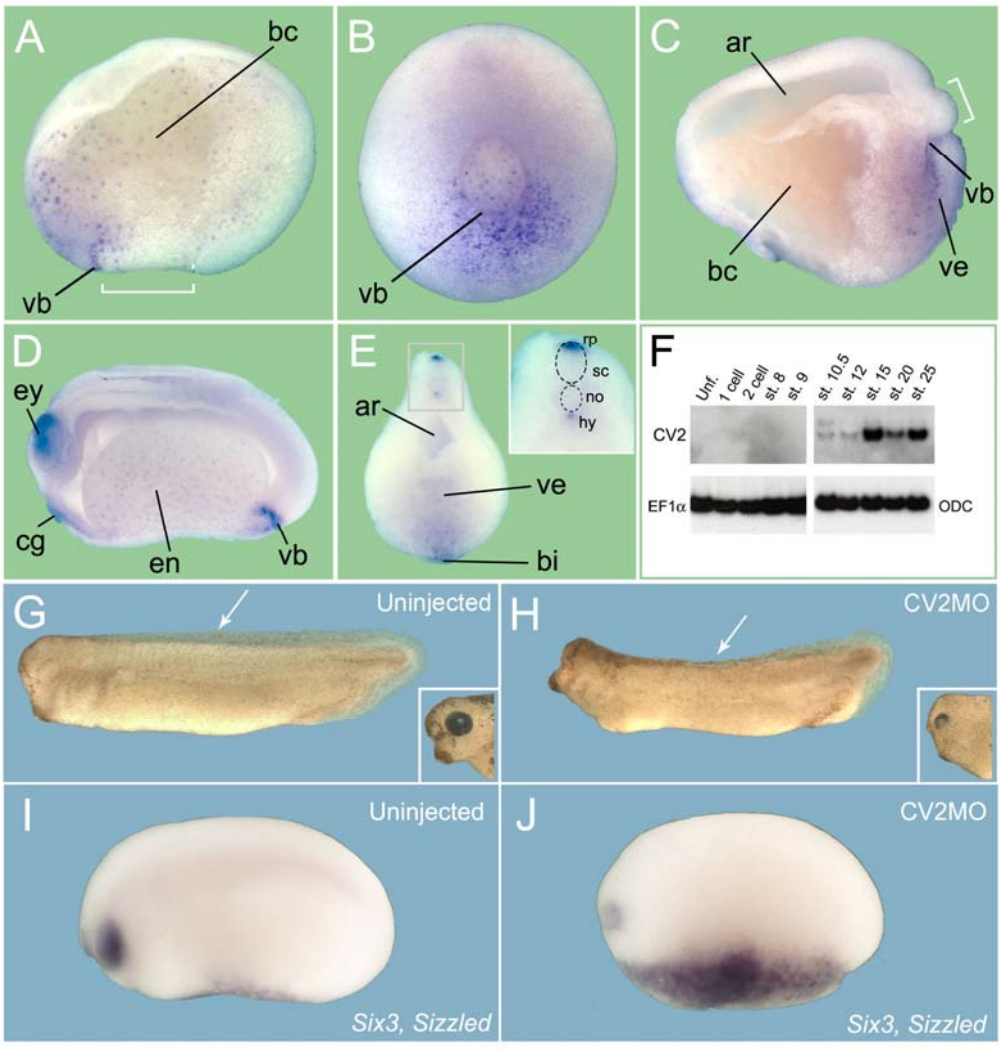

Figure S1. CV2 mRNA Is Expressed in Regions of High BMP and Its Depletion Ventralizes *Xenopus* Embryos

The expression pattern of CV2 in Xenopus embryos has not been previously reported. CV2 was expressed in all three embryonic layers, ectoderm, mesoderm, and endoderm in domains overlapping with those of BMP4 expression [Fainsod, A, Steinbeisser, H., and De Robertis, E.M. (1994). EMBO J. *13*, 5015-5025]. At later stages, CV2 expression was detected in other regions of high BMP signaling such as the dorsal region of the eye and the cement gland, the roof plate, the hypochord, and blood islands. Before gastrulation, CV2 transcripts were undetectable by in situ hybridization or RT-PCR.

(A) In a hemisected mid-gastrula stage embryo, CV2 is expressed in broadly in ventral regions. Bracket marks the blastopore.

(B) At late gastrula, CV2 expression concentrates around the ventral blastopore.

(C) In a neurula stage hemisected embryo, CV2 expression is seen in the ventral endoderm around the blastopore marked by bracket.

(D) CV2 expression is turned on in the dorsal eye, the cement gland and is still present in the ventral endoderm in hemisected tadpoles.

(E) CV2 is expressed in high BMP regions both in the ventral and the dorsal side of transversely sectioned embryos. Inset shows CV2 expressed in the roof plate of the spinal cord and in the hypochord.

(F) RT-PCR analysis indicates CV2 expression is zygotic, starts at gastrula, and reaches its maximum at neurula stage.

(G) Stage 32 uninjected embryo (inset shows stage 40).

(H) The CV2MO injected embryo shows a reduced head and dorsal (white arrow) and ventral fins and a strikingly smaller eye (inset).

(I) Uninjected embryo stained with eye marker *Six3* and the ventral BMP signaling marker *Sizzled*.

(J) CV2 depletion reduces the eye marker *Six3* and increases ventral BMP signaling marked by *Sizzled*.

bc, blastocoele; vb, ventral blastopore; ve, ventral endoderm; ar, archenteron; ey, eye; cg, cement gland; bi, blood island; rp, roof plate; sc, spinal cord; no, notochord; hy, hypochord

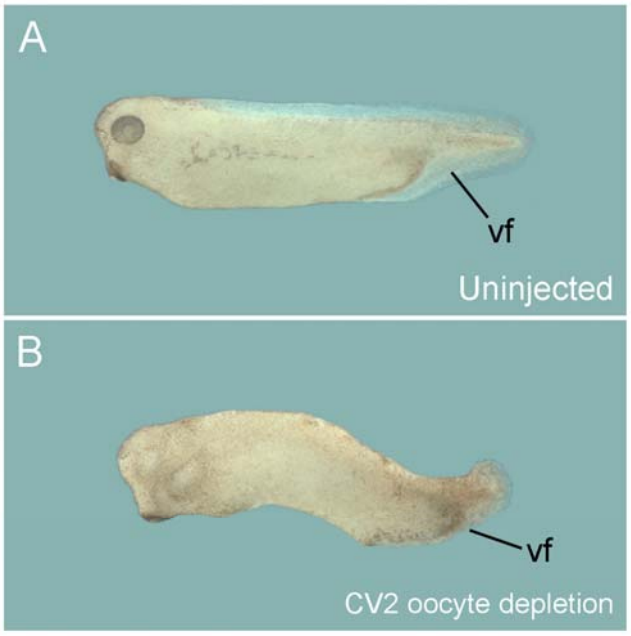

Figure S2. Maternal Injection of CV2 MO into *Xenopus tropicalis* Oocytes

(A) Uninjected embryo at stage 33.

(B) The CV2 MO oocyte injection phenotype does not differ from the one obtained when embryos are injected at 2-cell stage. Note the reduction in the ventral fin (vf), which indicates a pro-BMP function for endogenous CV2. This experiment indicates that there is no significant maternal contribution to the CV2 phenotype in Xenopus.

Figure S3. CV2 Knockdown Affects D-V Patterning in the Mesoderm and the Endoderm

(A-B) Embryos stained with the blood island marker *Scl* (Stem cell leukemia). CV2 depletion increases staining in the blood island indicating an increase in BMP signaling.

(C-D) In situ hybridization using the endodermal marker *Cad2*. CV2 depletion severely affects endoderm formation. Note that the ventral fin (vf) is decreased in size.

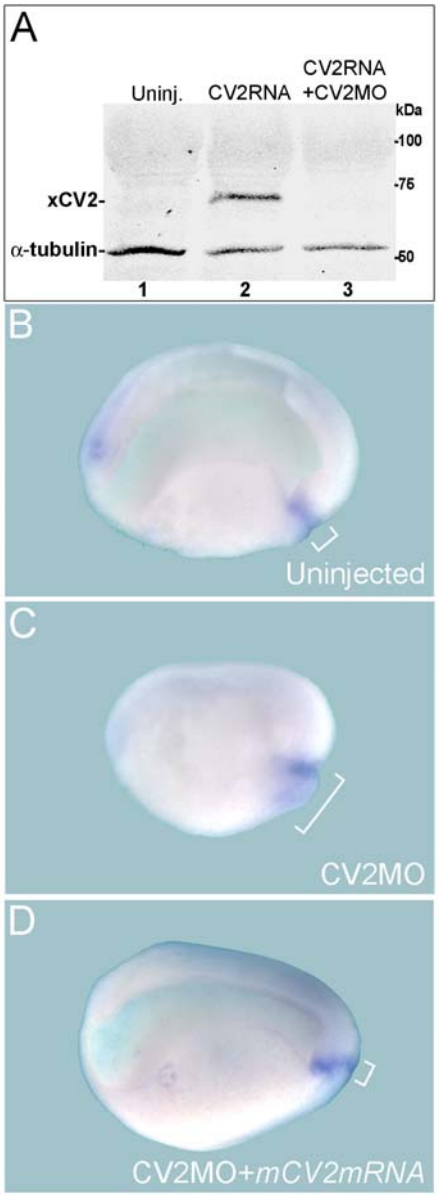

Figure S4. The CV2 MO Is Effective and Can Be Rescued by mCV2 mRNA

(A) CV2 MO inhibits the translation of microinjected *Xenopus CV2* mRNA. Embryos were injected four times with 250 pg of mRNA with or without CV2 MO, harvested at stage 13 and analyzed by Western blot using α-tubulin (1:2000, Calbiochem) as loading control. The novel anti-xCV2 antibody used here (1:2000 dilution) was generated in rabbit (Covance) from a

synthetic peptide encoding the amino-terminal sequence of *Xenopus* CV2 (SSFLTGSIAKAENEGEALQIPFITDNPC).

(B) Hemisected embryo stained with CV2.

(C) Expansion of the ventral CV2 domain after CV2 MO radial injection.

(D) Mouse CV2 mRNA (Coffinier et al., 2002) prevents the effect of CV2 MO.

The white brackets indicate the width of the CV2 domain around the ventral blastopore.

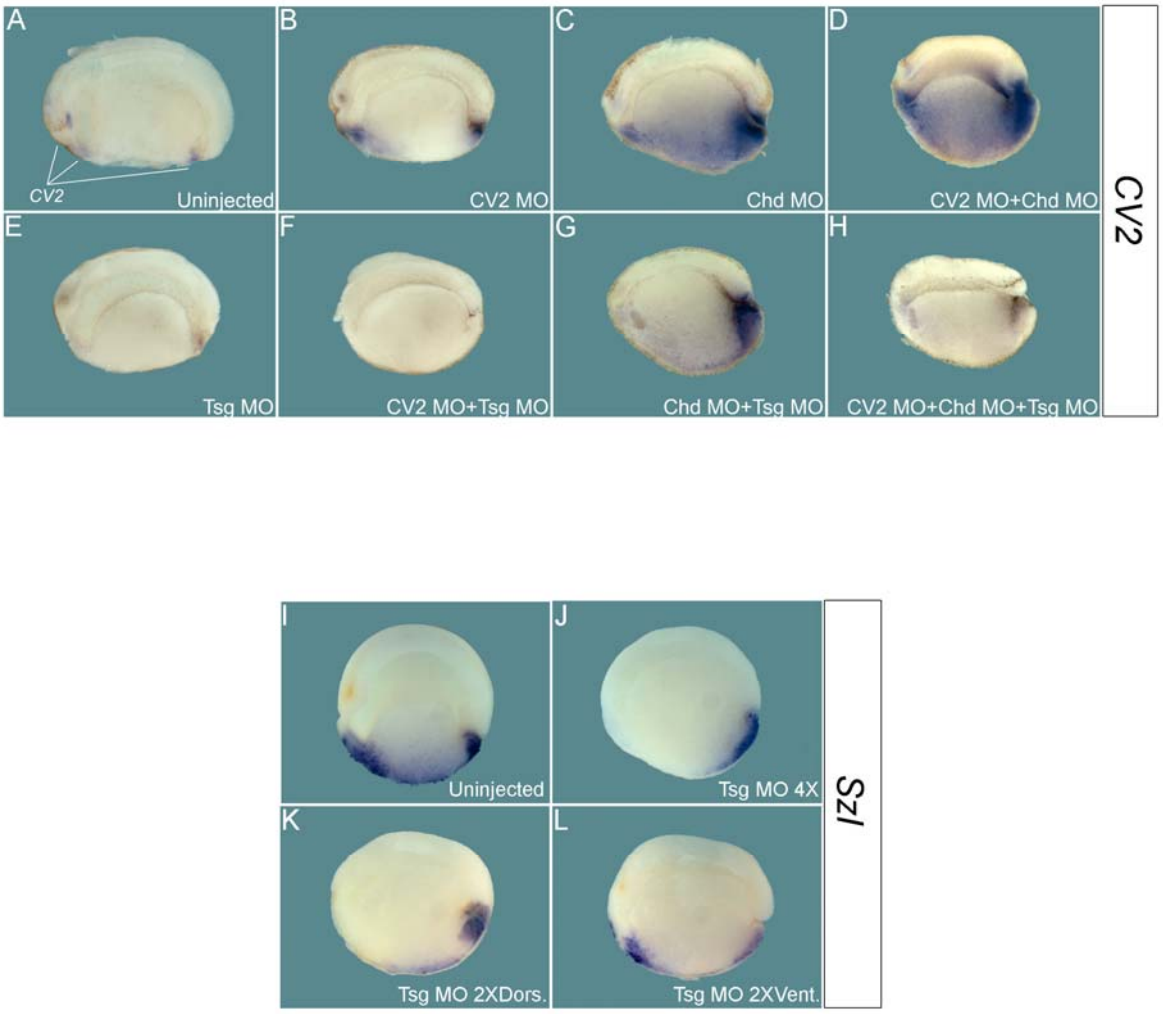

Figure S5. Tsg Functions as a Potent Pro-BMP Agent in the Absence of CV2 and/or Chordin

(A) In situ hybridization of hemisected embryo stained with *CV2*; uninjected stage 22 embryo. CV2 transcripts are used in this experiment as a reporter for BMP activity.

(B) Upregulation of *CV2* expression after CV2 MO injection.

(C) Chordin depletion results in an expansion of *CV2* expression domain.

(D) A cooperative effect is observed after co-injection of CV2 MO and Chd MO.

(E) Depletion of Tsg alone resembles wild-type embryos or slightly decreases BMP signaling. The same Tsg oligomer as in Blitz et al. (2003) was used in these experiments.

(F) Tsg depletion completely erased the expression of CV2 in CV2 MO injected embryos.

(G) Tsg knockdown reduced the *CV2* expression domain in the Chd MO injected embryos.

(H) Tsg is required (pro-BMP effect) for the striking increase in the expression of *CV2* observed in CV2 MO and Chd MO injected embryos.

(I-L) Effects of Tsg MO alone using *Sizzled* as the BMP signaling readout. Injection of Tsg MO into each cell at 4-cell stage consistently decreased *Szl* (n=25) when compared to uninjected stage 19 embryos (n=23). This is in agreement with findings in zebrafish with Tsg MO (Little and Mullins, 2004; Xie and Fisher, 2005). Microinjection of Tsg MO into the two dorsal blastomeres of embryos with clear D-V polarity inhibited dorsal *Szl* but increased its expression on the ventral side (n=24). This phenotype is probably caused by the inhibition of Chd protein function, which requires Tsg, and is consistent with phenotypes reported by Blitz et al. (2003). The depletion of Tsg in the two ventral blastomeres decreased *Szl* expression on the ventro-posterior region, but did not affect *Szl* expression in the anterior domain, which derives from non-injected cells (n=21). Differences in the phenotypes of dorsal and ventral injections of Tsg MO in *Xenopus* have been reported previously (Blitz et al., 2003). We propose that these D-V differences can be explained if dorsal depletion of Tsg caused predominantly defects in the

function of Chordin, and ventral depletion reflected predominantly the requirement of Tsg for proper CV2 and BMP function.

The most striking effect in this figure is seen by comparing the embryos in panels D and H, which are reproduced in the main text (Figure 3A and 3B). When both CV2 and Chordin are depleted, BMP signaling strongly requires Tsg. It is known that Tsg binds to BMP on its own right (Oelgeschläger et al., 2000) and that its presence does not affect significantly the binding of BMP to its receptors (Larraín et al., 2001). Tsg may have a permissive function in BMP signaling, perhaps helping maintain BMP ligands in a soluble state in the extracellular space.

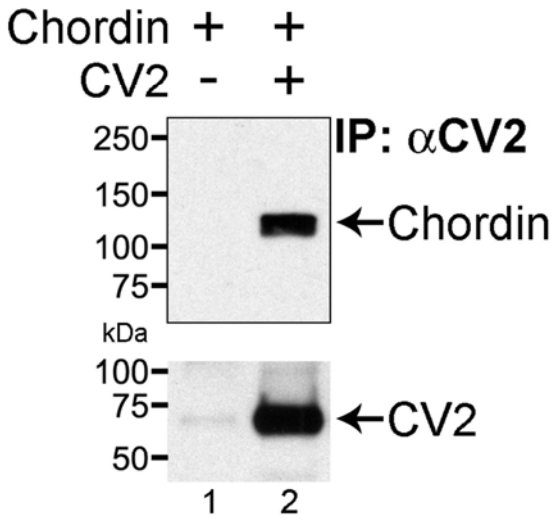

Figure S6. Chordin Binds CV2 in Solution

Co-immunoprecipitation using anti-mouse CV2 antibody bound to protein G agarose beads. Lane1; control without CV2. Lane 2; Chordin is pulled down only in the presence of CV2. This experiment provides independent support for the surface plasmon resonance experiments in Figure 5A.