Supplemental Experimental Procedures

Immunoprecipitation and Western blot analysis. Embryos were homogenized in lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 0.1% SDS, 50 mM NaF and 1mM Na_3VO_4) containing of 1 mM PMSF (phenylmethanesulphonylfluoride), 15 mM β-glycerophosphate, and 1 X proteinase inhibitor cocktail (Calbiochem, Darmstadt, Germany) and then used for Western blotting and immunoprecipitation. Immunoprecipitation experiments were performed by incubating extracts overnight with monoclonal anti-HA (Covance, Princeton, NJ). For Western blotting, monoclonal anti-Flag M2-peroxidase conjugate (Sigma, St. Louis, MO), anti-HA-peroxidase conjugate (Roche, Indianapolis, IN) and monoclonal anti-actin (Sigma) were used. Proteins were visualized using ECL Western blotting detection reagents (Amersham, Pittsburgh, PA).

Morpholino experiment. The antisense morpholino oligonucleotides (MOs) were obtained from Gene Tools (Philomath, OR). The c-Jun morpholino sequences were as follows: *MO-Jun54*, 5'-CTGGAGCTTATGTCAGTGTGA-3'; *MO-Jun55*, 5'-GTAGTTTCCATCTTTGCGTTCATAC-3'. *MO-Jun54* and *MO-Jun55* were designed to bind to complementary sequences found in 2 types of *Xenopus c-jun* mRNAs. Oligos were re-suspended in sterile water and injected in doses of 20 ng per embryo. Animal caps, explanted from embryos injected with *BMP-4* (2 ng) or *MO-*

Jun either alone or together, were incubated until stage 24. Animal caps derived from *MO-Jun*-injected embryos were incubated in the presence or absence of activin (40 ng/ml) until stage 24. Gene expression was analyzed by RT-PCR.

Semi-quantitative RT-PCR. Animal cap explants isolated at the blastula stage were subjected to RT-PCR analysis as described in Materials and Methods.

Real Time quantitative RT-PCR (qRT-PCR). Animal cap explants isolated at the blastula stage were subjected to reverse transcription as described in Material and Methods. PCR reactions were carried out with SYBR Premix (Qiagen, Valencia, CA) and a thermal cycler Real Time System (Qiagen Rotor-Gene-Q, Valencia, CA). Primers used in the semi-quantitative RT-PCR were as described in Material and Methods. All values were normalized to the level of ODC or EF1α in each sample. *XSCL (5'-TGATTGAGCTGCTCAGAAG-3'; 5'-CTGGAGTCAATGATGCTCTG-3'); XLMO2 (5'-GGGAAGTCGGAAGGAGAC-3'; 5'-CGGTCACCCACGCAGAAG-3') and Xneptune (5'-GGATCCTACACTGCCAATCAGA-3'; 5'-CATCTGCATGATACCAGCCTTC-3').*

Supplemental Figure Legends

Supplemental Figure 1. Co-expression of JunD and c-Fos induces expression of globin in a

dose-dependent manner. Animal caps isolated from embryos injected with the indicated mRNAs were used for qRT-PCR. The relative level of expression (fold induction) normalized to ODC expression is shown for *globin*. * P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001.

Supplemental Figure 2. ***Control-MO (Cont-MO)*** **does not change the expression of the JunD protein or the transcription of hematopoietic markers, including the dorsal mesoderm marker,** ***actin*****.** Western blot analysis of embryonic extracts probed with anti-HA show that Cont-MO had no effect on the expression of XJunD. Actin serves as a control to confirm equal protein loading. RT-PCR analysis of animal cap explants. *EF1*α, loading control; -RT, control reaction without reverse transcriptase.

Supplemental Figure 3. ***Control-MO*** **does not change BMP-4 induction of hematopoietic genes or** ***globin*****.** RT-PCR analysis of animal cap explants. *EF1*α, loading control; w.e., whole embryo used as a positive control for PCR; -RT, control reaction without reverse transcriptase.

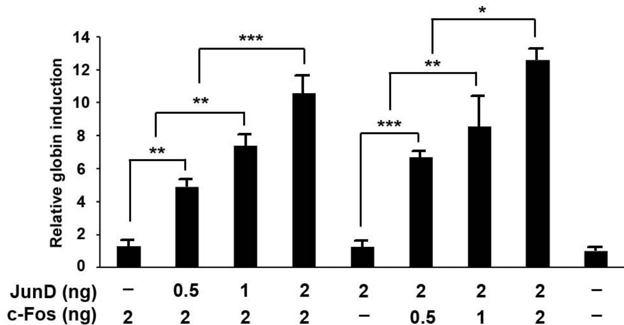
Supplemental Figure 4. Schematic model illustrating the relationship between BMP-4 and AP-1$^{JunD/c\text{-}Fos}$ during hematopoetic specification in ***Xenopus laevis*****.** First, BMP-4 enhances the transcription of *junD* (1). Second, BMP-4 signaling is involved in the phosphorylation of serine

66 (S66) of JunD through an as yet unidentified kinase (X) (2).

Supplemental Figure 5. c-Jun is not required for BMP-4-induced hematopoiesis. RT-PCR analysis of animal cap explants is described in Supplemental Experimental Procedures. The neural marker, *NCAM*, and dorsal mesoderm marker, *actin*, were used as positive controls of activin. *EF1α*, loading control; w.e., whole embryo was used as a positive control for PCR; -RT, control reaction without reverse transcriptase.

Supplemental Figure 6. JunD is not required for activin-induced dorsal mesoderm and endoderm. qRT-PCR analysis of animal cap explants is described in Supplemental Experimental Procedures. The relative level of expression (fold induction) normalized to *EF1α* expression is shown for each gene. *Chordin* and *Goosecoid*, dorsal mesoderm marker; *Mixer*, endoderm marker. ** P-value < 0.01, *** P-value < 0.001.

S1



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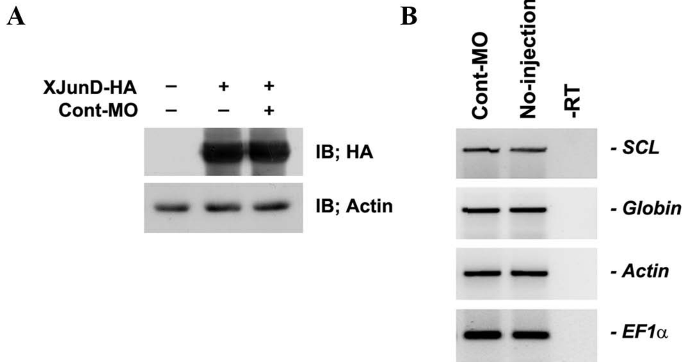

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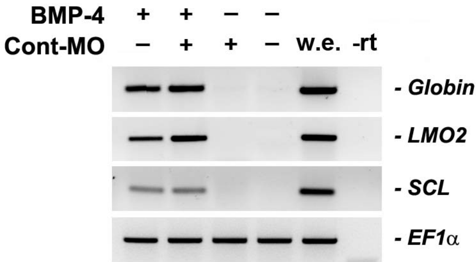

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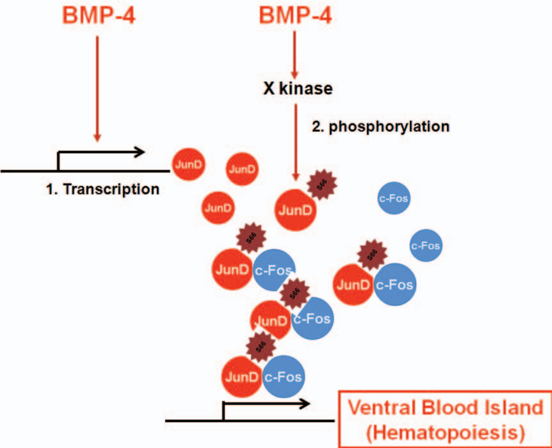

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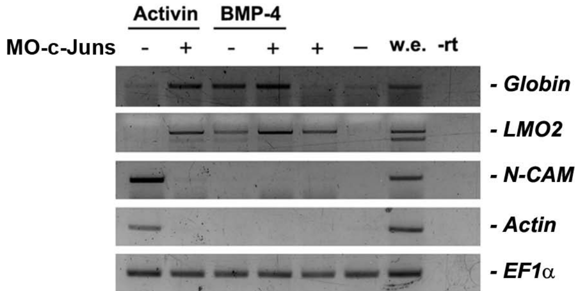

S6