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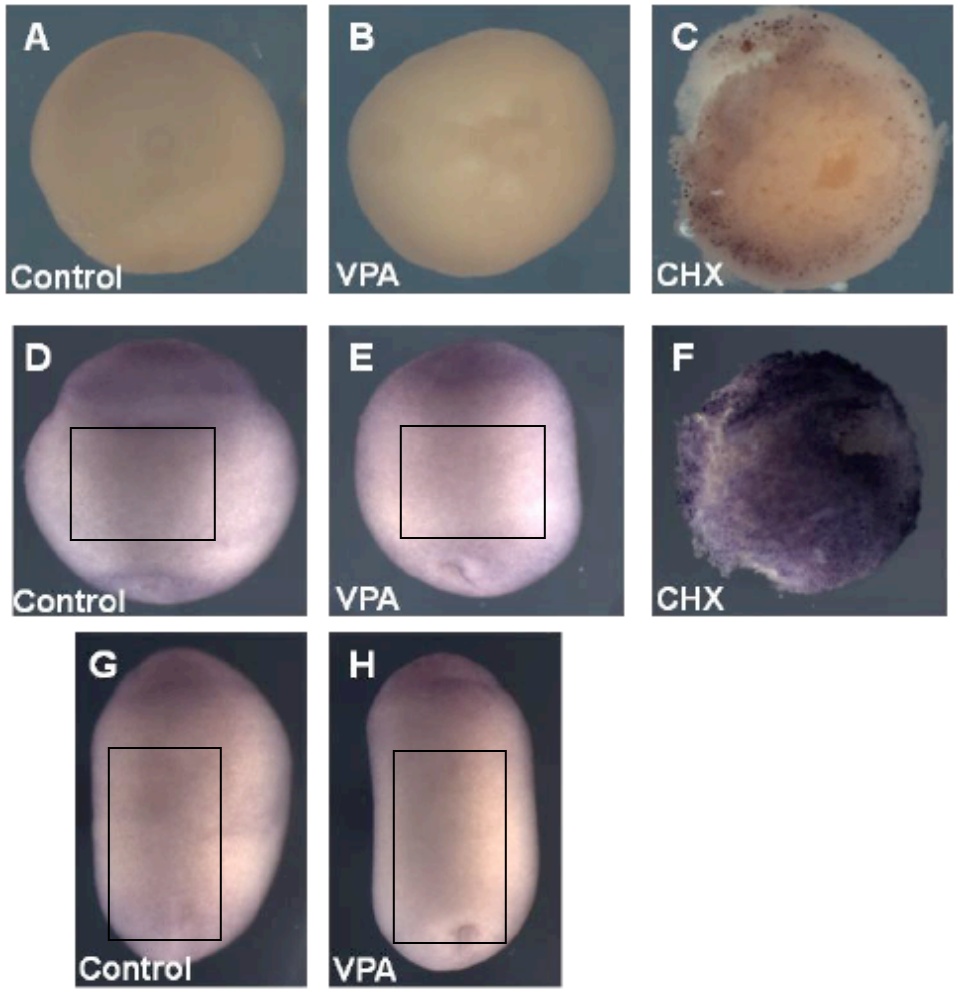
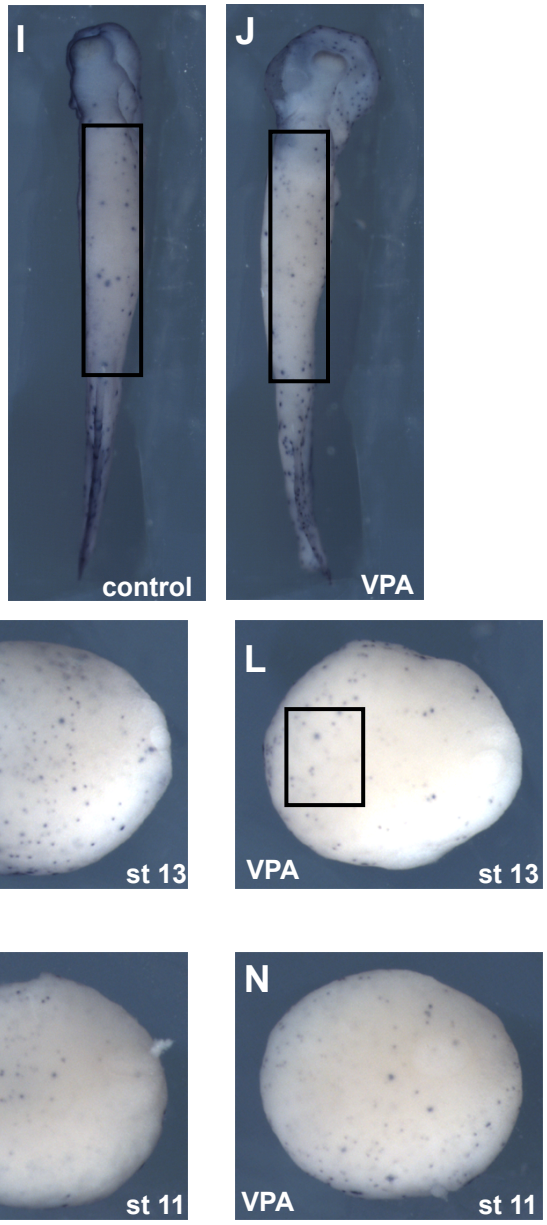
Treatment with VPA does not alter proliferation or cell death





Data not shown Figure 1 Legend

Treatment with VPA does not alter proliferation or cell death:

A-H) TUNEL staining on reveals no evidence of apoptosis in control embryos (A,D,G) or in embryos treated with VPA beginning at stage 10 (B,E,H). A-B) Stage 10, D-E) early neurula (stage 14) embryos, ventral view G-H) late neurula (stage 22) embryos, ventral view. Cyclohexamide (CHX) (C) and (F) treatment induces apoptosis and serves as a positive control. Box outlines indicate regions of hematopoietic progenitors in the VBI. I-N) Phospho-H3 antibody staining demonstrates no change in proliferation with VPA treatment (J, L, N) at stage 34 (I, J), stage 13 (K, L), and stage 11 (M, N). Ventral views; boxes outline the VBI, or hematopoietic precursor population. Phospho-H3 immunostaining was performed as described in Wu J, O'Donnell M, Gitler AD, and PS Klein. "Kermit-2/XGIPC, an IGF1R interacting protein, is required for IGF signaling in Xenopus eye development." *Development* **133**:3651-3660, 2006.

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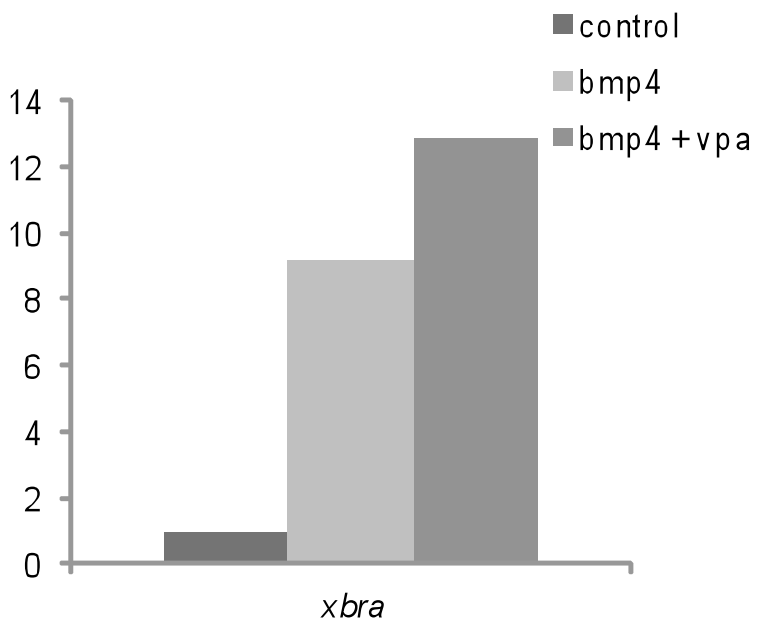

BMP4-induced expression of mesodermal marker *xbra* is not reduced by VPA: *bmp4* mRNA (1ng) was injected into the animal pole of fertilized eggs. Ectodermal explants were dissected at stage 8 and cultured in 2mM VPA from stage 10 until stage 12. Expression of *xbra* was assessed by qRT-PCR and normalized to expression of ODC. The induction of *xbra* is not reduced by exposure to VPA.

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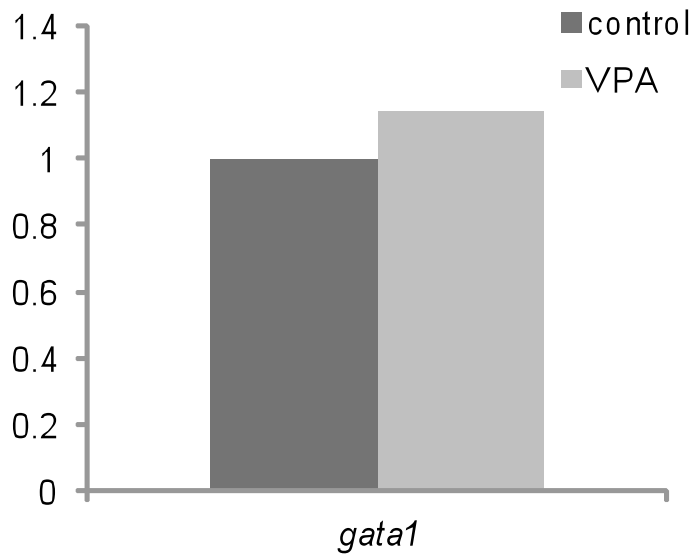

VPA treatment does not reduce expression of gata1 at late neurula stages. Embryos were treated with VPA (2 mM) or buffer control during the gastrula stage (see text) and then transferred to 0.1X MMR until stage 20. Expression of *gata1* in whole embryos was assessed by qRT-PCR (see text for methods). VPA treated embryos do not show significant change in *gata1* expression.

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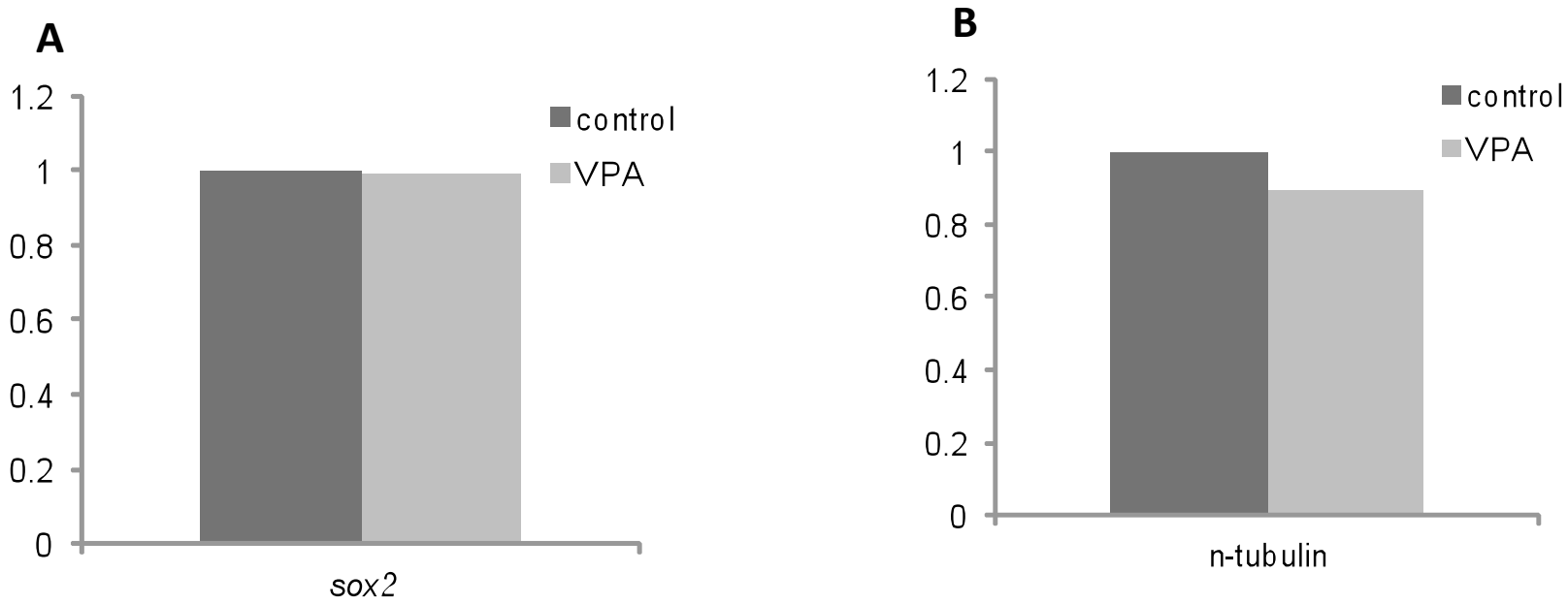

VPA treament of ectodermal explants does not induce the neural markers *sox2* and *n-tubulin*. Ectodermal explants (animal caps) were dissected at stage 8 blastulae and cultured in 0.5X MMR until siblings reached stage 28. Light gray bars indicate explants that were exposed to VPA during the gastrula stage (sibling stage 10 thru 12). *Sox2* and *n-tubulin* expression (assessed by qRT-PCR) did not increase with VPA treatment.

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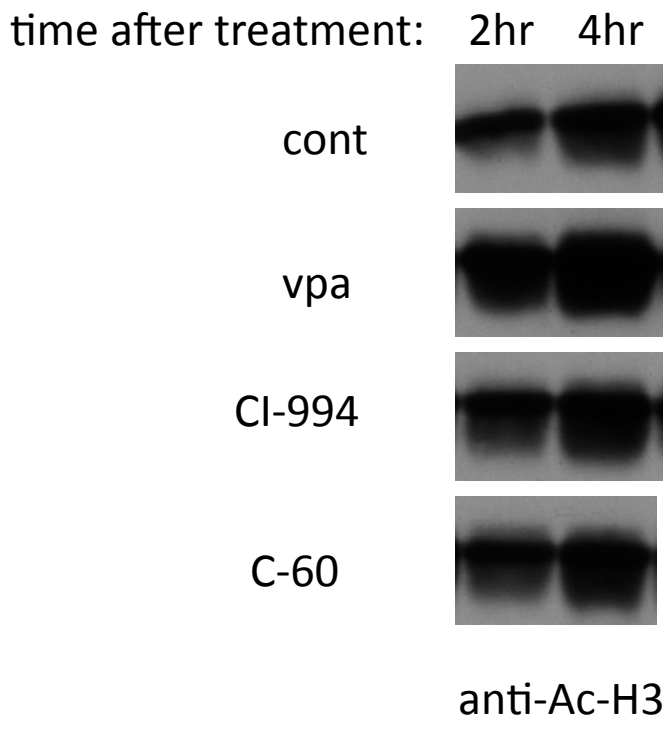

CI-994 and C60 penetrate slowly and require approximately 4.5 hours of exposure to increase global histone acetylation: Embryos were treated with VPA or with the selective HDAC inhibitors CI-994 or C60 at stage 10 and harvested 2 or 4 hours after treatment, as indicated. Proteins were extracted and acetylated H3 was detected by western blot with an antibody to acetylated H3K9/K14. (Equal loading of histones was determined by Ponceau red staining, not shown). Treatment with CI-994 or C60 for 4 hours causes a robust incease in acetylated-H3, similar to levels observed with 2 hours of treatment with VPA. “Cont” indicates buffer control. Concentrations used: VPA: 2mM, CI-994: 250 uM, C60: 100uM.

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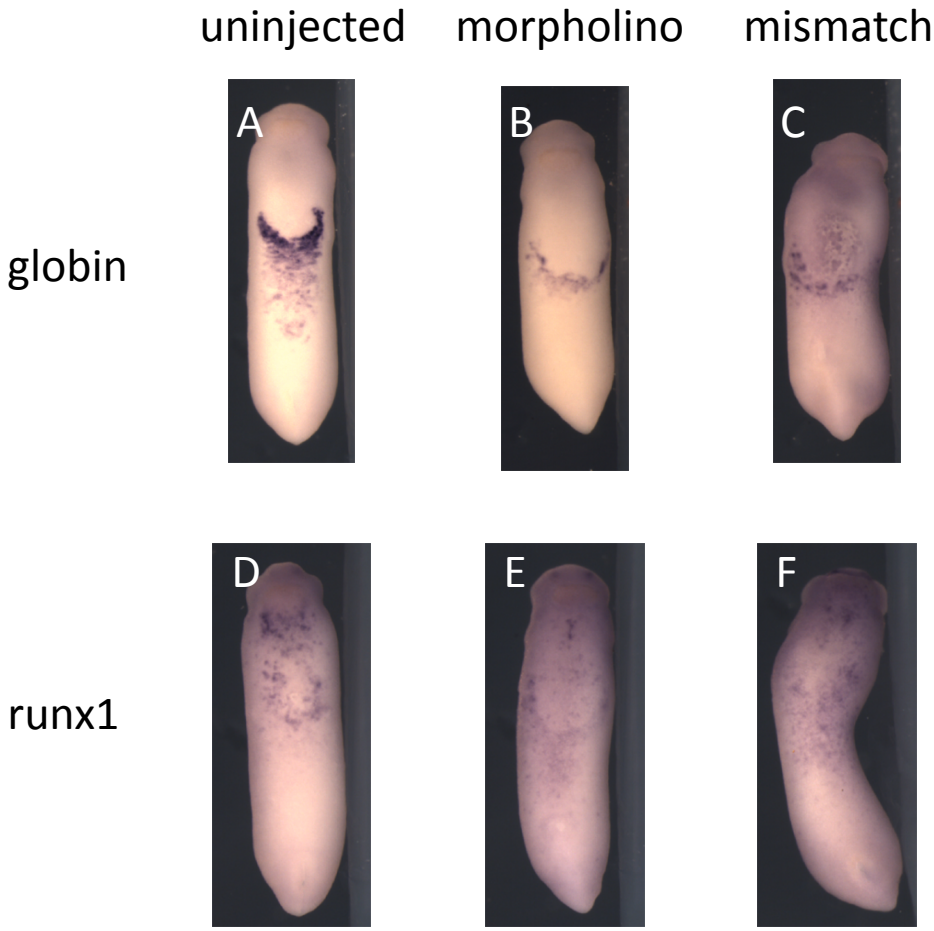

***Hdac3* specific morpholino (MO) and mismatch morpholino (MM) reduce expression of *globin* and *gata1* in the VBI at early tailbud stages.** Embryos were injected at the 2-cell stage with MO (B, E) or MM (C, F), and collected for whole mount in situ hybridaization at tailbud stage (ventral views are shown). Injection of either MO or MM reduced expression of globin(A-C) and runx1 (D-F). Only the MO reduced HDAC3 protein abundance.

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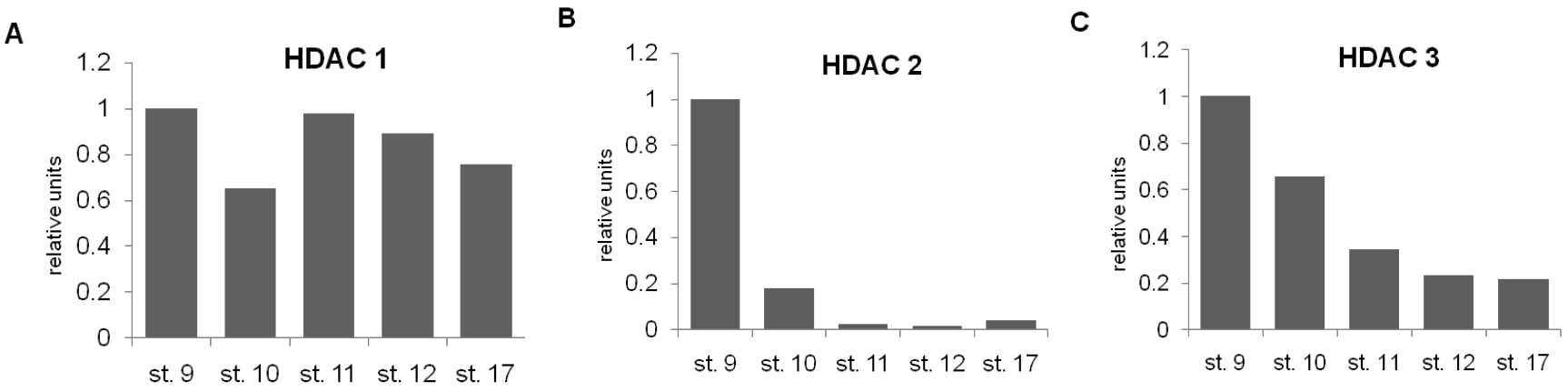

Expression of *Hdacs 1, 2*, and *3* in *Xenopus laevis*. Embryos at blastula (stage 9), gastrula (stages 10-12), and neurula (stage17) were harvested and RNA extracted for qRT-PCR using primers to detect class I *Hdacs*: *Hdac1, Hdac2*, and *Hdac3*. Expression of *Hdac1* and *Hdac3* persist through gastrula stages whereas *Hdac2* expression drops precipitously at the onset of gastrulation (stage 10).