

## Supplemental figures:

**S1:** Phylogenetic tree of *Mesp* genes in mammals, fish, avians and amphibia. Clustal W alignment of full-length protein sequences. Genes aligned are: chimp (*Pan troglodytes*) *Mesp1*: XP\_523151 and *Mesp2*: XP\_523152; *Homo sapiens* *Mesp1*: NP\_061140 and *Mesp2*: NP\_001035047; *Macaca mulatta* *Mesp1*: XP\_001093487 and *Mesp2*: XP\_001093605; *Rattus norvegicus* *Mesp1*: XP\_218826 and *Mesp2*: XP\_001065650; *Mus musculus* *Mesp1*: BAA12041 and *Mesp2*: AAB51199; opossum (*Monodelphis domestica*) *Mesp1* (predicted) and *Mesp2*: XP\_001369514; amphioxus (*Branchiostoma floridae*) *Mesp*: ABD57444, fugu (*Takifugu rubripes*) *mespa*: (predicted) and *mespb*: SINFRUP00000180356; Medaka (*Orizyas latipes*) *mespa*: 31681832 and *mespb*: 31675801; zebrafish (*Danio rerio*) *mespa*: NP\_571626 and *mespb*: NP\_571627; *X. tropicalis* *mespa* ENSXETG00000027628 and *mespb* ENSXETG0000001772; *X. laevis* (each gene has two pseudotetraploid alleles) *Mespa*: XL218m17 and XL194g13; *Mespb*: CAA74798 (*Thylacine1*) and CAA74799 (*Thylacine2*); chick (*Gallus gallus*) *meso-1*: Y17043 and *meso-2*: AJ421070.

**S2: (A)** Live image of an *Xl-mespb-GFP* transgenic embryo at approximately 20 hpf. **(B)** GFP fluorescence in an *Xl-mespb-GFP* transgenic embryo, with strong expression seen in the anterior portion of newly formed somites (arrowhead).

**S3: (A)** Zebrafish embryos either mock-treated or treated with RA for in the indicated times, and then fixed and stained for *Dr-mespa* RNA. Shown is a dorsal view of the PSM, with anterior oriented up. **(B)** Zebrafish embryos were either mock-treated, treated with RA, CHX or both for the indicated times and then stained for *Dr-mespb* RNA. Shown is a side view of the PSM with anterior oriented up. **(C)** Zebrafish embryos transgenic for *Xl-mespb-GFP* were treated for one hour with RA, and then stained for *GFP* expression. The expression of *Xl-mespb-GFP* continues to be upregulated after longer time periods of RA treatment (data not shown). **(D)** The 3.5 kb *Xl-mespb-GFP* (FL), or the 1.7 kb *Xl-mespb-GFP*(Stu) transgenes were introduced into a batch of embryos. Each batch was subdivided, either mock-treated or treated with RA for 1.5 hrs, and then fixed and stained for *GFP* expression in parallel. About half of the embryos prepared with *Xl-mespb-GFP*-FL (Blue) show transgenic expression and this frequency does not change upon

RA treatment. By contrast, the frequency of transgene expression in embryos prepared with *Xl-mespb-GFP-Stu* (Red) drops significantly upon RA treatment, indicating that RA is not activating but repressing expression from *Xl-mespb-GFP-Stu*. At least 100 embryos were scored under each experimental condition.