Supplemental Figure Legends

Supplemental Figure 1. RAR-specific pharmacological reagents affect expression of *Tg(12XRARE-ef1a:gfp)*, while pharmacological reagents specific for other nuclear hormone receptors do not. (A) Untreated control embryo. (B) Ro41-5253, a RARα-specific antagonist, inhibits reporter expression. (C) AM580, a RARα-specific agonist, activates ectopic reporter expression. Arrows (ventral eye) and arrowheads (anterior spinal cord) in B and C indicate where restricted expression is normally found in an untreated embryo (A). (D) The pan-RXR agonist methoprene acid (Biomol.com) does not affect reporter expression. Agonists of (E) PPARα (GW7647), (F) PPARδ (GW0742), and (G) PPARγ (GW1929) do not activate the reporter. The pan-PPAR antagonist LY171883(H) and the PPARα specific antagonist GW6471 (I) do not inhibit reporter expression. All PPAR-related reagents were purchased from Sigma. All images are lateral views, with anterior up and dorsal to the right.

Supplemental Figure 2. Myc-tagged zebrafish RARs are expressed in zebrafish embryos. (A) Western blot of myc-tagged Hs dnRAR and zebrafish WT RARs and dnRARs at 70-90% epiboly in zebrafish embryos that were injected with the indicated mRNAs. The decreasing relative expression from left to right reflects the timing of execution for this representative experiment, in which constructs on the left were injected earlier than those on the right. Approximate sizes of the myc-tagged proteins are: Hs myc-dnRARa, 55kDa; Dr myc-RARab, 61kDa; Dr myc-dnRARab, 55kDa; Dr myc-RARga, 67kDa; Dr myc-dnRARga, 52kDa. The approximate size of α-tubulin is 50kDa. (B-D) Wholemount immunofluorescence at 70-90% epiboly of zebrafish embryos that were injected with mRNAs for myc-tagged human and zebrafish RARs. Exogenous protein expression was analyzed at mid- to late-gastrulation stages because this is when RA signaling is required to pattern the early zebrafish embryo (Hernandez et al., 2007; Hernandez et al., 2004; Linville et al., 2008; Maves and Kimmel, 2005; Stafford et al., 2006).

Both analyses indicate that, while there may be differences in precise expression levels of different exogenous RARs, all of the exogenous RARs are expressed broadly and robustly in zebrafish embryos at these stages. Unfortunately, there are no commercially available anti-RAR antibodies that allow evaluation of the endogenous levels of zebrafish RARs. If we presume that endogenous α-tubulin is likely to be present at higher levels than endogenous RARs, the qualitative comparison of exogenous RARs and endogenous α-tubulin suggests that the exogenous RARs are present in excess of their endogenous counterparts.

Supplemental Figure 3. RARabvps and RARgavps differentially activate reporter expression in RARE transgenic reporter lines. (A-D) Ectopic expression of RAR-vps can induce ectopic expression of *Tg(12XRARE-ef1a:gfp)* at 24 hpf. (E-H) Ectopic expression of RAR-vps can induce ectopic expression of *Tg(12XRARE-tk:gfp)* at 24 hpf. Induction of expression by RARabvps (center in A-H) is typically stronger than that induced by RARgavps (right in A-H). Uninjected sibling embryos are at the left in A-H. All images are lateral views, with anterior up and dorsal to the right.

Supplemental Figure 4. Hyperactive RAR-vps induce regional activation of the RARE reporter. *Tg(12XRARE-ef1a:gfp)* embryos overexpressing RAR-vps. (A) Control uninjected embryos and embryos injected with (B) *rarab-Nvp*, (C) *rarab-*Δ*Avp*, (D) *rarab-Cvp*, (E) *rarab-*Δ*Fvp*, (F) *rarga-Nvp*, (G) *rarga*Δ*Avp*, and (H) *rarga*Δ*Fvp* mRNAs. Reporter expression is enhanced in regions of the embryo near a source of RA. Arrow in C indicates expanded ventral eye expression. Arrowhead in C indicates reporter expression within the trunk. All images are lateral views, with anterior up and dorsal to the right.

Supplemental Figure 5. Phenotypes induced by RA treatment. (A-C) RA treatment eliminates anterior neural gene expression. Cktl of probes is the same as in Figure 6. (A,D,G) Untreated control embryos. (B) Treatment with 0.5 μM RA can strongly posteriorize embryos, resulting in loss of all anterior neural markers. (C) Treatment with 0.2 μM RA can yield moderately posteriorized embryos in which the MHB is lost and the expression of *krox20* in rhombomere 3 is reduced. This more modest posteriorization is remniscent of *rar-vp* mRNA-injected embryos (Figure 6), though the eyes of *rar-vp* mRNA-injected embryos are never lost. (D-I) RA signaling positively regulates expression of *dhrs3a* and *hoxb5b*. (E, H) Treatment with DEAB inhibits *dhrs3a* and *hoxb5b* expression. (F, I) Treatment with 0.5 μM RA induces ectopic *dhrs3a* and *hoxb5b* expression. Images in A-C are lateral views with dorsal to the right. All other images are dorsal views with anterior up.

Supplemental Figure 6. Ectopic expression of hyperactive RAR-vps does not phenocopy all aspects of RA treatment. (A) Uninjected control embryo. (B) Treatment with 0.5 μM RA causes loss of the anterior CNS (arrow). (C) Treatment with 0.2 μM RA causes dysmorphic, reduced eyes (arrow and dashed yellow outline) and loss of the MHB. Injection of (D) *rarab-Nvp*, (E) *rarab-Cvp*, (F) *rarga-Nvp*, (G) *rarga-Cvp*, (H) *rarab-*Δ*Avp*, (I) *rarab-*Δ*Fvp*, and (K) *rarga-*Δ*Fvp* mRNA can eliminate the MHB (arrowheads), but the eyes are not affected (arrows and dashed yellow outline). *rarab-Nvp* and *rarab-Cvp* mRNAs are particularly effective at eliminating the MHB. (J) Injection of *rarga-*Δ*Avp* mRNA does not affect the MHB (arrowhead).

Supplemental Figure 7. Zebrafish dominant negative RARs and Enr fusion proteins do not inhibit expression of RA-responsive target genes. (A, F) Uninjected control embryos. (B-E, G-J) Injection of mRNAs for zebrafish *dnrarga* (B, G), *dnraraa* (C, H), *rarab*-Δ*Aenr* (D, I) and *rarab*-Δ*Fenr* (E, J) do not significantly affect expression of RA-responsive target genes. All images are dorsal views with anterior up.

Supplemental Figure 8. Chimeric human/zebrafish dominant negative RARs can inhibit expression of RA-responsive target genes. (A-D) Uninjected control embryos. (E-H) Embryos injected with mRNA encoding a hs-dr RAR fusion protein, in which the human A-C domains are fused to the zebrafish D-ΔF domains. (I-L) Embryos injected with mRNA encoding a dr-hs RAR fusion protein, in which the zebrafish A-C domains are fused to the human D-ΔF domains. Although neither chimeric protein was as effective as the human dnRARa (Fig. 8O-R), either can inhibit expression of RA-responsive genes.