



**Fig. S1. Transient ERT2-GAL4 expression under the control of the *ubiquitin* promoter drives widespread, 4-OHT dependent activity.** One-cell stage embryos were co-injected with the Ubiquitin::ERT2-GAL4 and UAS::H2B-citrine plasmids, and were treated overnight with ethanol (control, A,B,E,F) or 0.75  $\mu$ M 4-OHT (C,D,G,H) from 50% epiboly to 21 hpf. Embryo-wide GAL4 activity is revealed by the expression of Citrine in a 4-OHT-dependent manner (Citrine in green channel, dark field in grey channel). The non-uniform pattern of Citrine expression reflects the mosaic inheritance of plasmid DNA in transient transgenics. A small number of Citrine-positive cells are seen in untreated embryos (A,B,E,F), but not in stable transgenics bearing the same constructs (see Fig. 4). E-H are higher magnification images from embryos in A-D, respectively.