Supporting Information

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SI Materials and Methods

DNA Constructs. Maps and annotated sequences of all constructs are available upon request; construct descriptions below include a unique identifying number used in the Weidinger laboratory. Constructs were created using standard PCR-based methods.

RNA expression constructs. To test the efficiency and leakiness of TetActivator and Gal4-EcR variants in zebrafish embryos, the following constructs were created in the expression vector "goblin," which allows for capped sense RNA synthesis using the T3 promoter in vitro. The RNA contains 5′ and 3′ UTR sequences of the *Xenopus* globin gene to increase mRNA stability and translational efficiency.

goblin irtTA(3F) (no. 384)
goblin irtTA(VP16) (no. 383)
goblin irtTAM2(3F) (no. 873)
goblin irtTA(VP16)-GBD* (no. 385, short name goblin TetA-GBD)
goblin irtTA(VP16)-GBD* p2a Cherry (no. 878, short name goblin TetA-GBD Cherry): STOP codon removed from irtTA(VP16)-GBD* and fused to the viral p2a sequence (1) and Cherry (2) for translation of irtTA(VP16)-GBD* and Cherry as individual peptides from one ORF.
goblin irtTA(VP16)-EcR' (no. 761, short name goblin TetA-EcR): GBD* in goblin irtTA(VP16)-GBD* replaced with a mutated variant (V454I Y474E) of the *Bombyx mori* ecdysone receptor hormone binding domain (3).
goblin Gal4-VP16-EcR' (no. 1000): irtTA in goblin irtTA(VP16)-EcR' replaced with the Gal4 DNA binding domain.
goblin Gal4-VP16-F-EcR' (no. 1001): GV-EcR-F' created by Esengil et al. (3) moved into the goblin vector.

Transgenic activator constructs.

myl7:TetA-GBD-p2a-mCherry (no. 441): 5.1-kb zebrafish *myosin light chain regulatory polypeptide 7 (myl7, cmlc2)* genomic sequence (4) upstream of irtTA(VP16)-GBD*-p2a-mCherry in a vector containing MiniTol2 inverted repeats for transposon-based transgenesis (5).

her4.1:TetA-GBD-p2a-mCherry (no. 898): 3.4-kb zebrafish her4.1 genomic sequence (6) upstream of irtTA(VP16)-GBD*-p2a-mCherry in a vector containing MiniTol2 inverted repeats (5).

Transgenic responder constructs.

TetRE:Axin1-YFP (no. 404): Tet response element "tight" (Clontech) plus CMV minimal promoter (unidirectional) upstream of mouse *axin1* fused to YFP at the C terminus in a vector containing MiniTol2 inverted repeats (5). The *axin1* ORF used lacks the RGS domain at the N terminus (the first 355 amino acids) and represents a splice variant that lacks exon 9.

TetRE:Dkk1-GFP (no. 657): same as TetRE:Axin1-YFP (no. 404), but Axin1-YFP replaced with a fusion protein of zebrafish Dickkopf1 with mmGFP5 at the C terminus (7). Cassette flanked by two copies of the 5′HS4 chicken globin insulator sequences on each side (8).

Eukaryotic expression vectors.

The tetracycline activators irtTA(VP16), irtTAM2(3F), irtTA(VP16)-GBD*, and irtTA(VP16)-EcR′ were cloned under the control of the CAGGs promoter followed by IRES-puromycin. For the tetracycline regulated Luciferase reporter, we used the pBI-L plasmid (Clontech).

Sense RNA Synthesis and Microinjection. Capped sense RNAs were synthesized from goblin expression vectors using the Message Machine kit (Ambion) and injected into one-cell–stage embryos at the quantities indicated in the figure legends according to standard procedures.

Whole-Mount in Situ Hybridization. mRNA in situ hybridization was performed as described by Jowett and Lettice (9). A probe against EGFP was used to detect Axin1-YFP expression.

Drug Injections into Adult Fish. Adult zebrafish were anesthetized using tricaine (0.02%), and intraperitoneally injected using a 30-gauge 1/2″ canula with 20 μL drugs or solvent diluted in Hanks' buffer (0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 4.2 mM $NaHCO_3$).

Immunofluorescence of Heart Sections. Adult fish were killed using high doses of tricaine. Hearts were extracted, fixed in 4% PFA (in phosphate buffer) and cryosectioned into 14-μm thin sections. Sections were stained with anti-GFP 1:500 (rabbit, Invitrogen; A11122) and with secondary antibody Alexa Fluor 488 goat antirabbit IgG 1:500 (Invitrogen; A11034). PEMT buffer (80 mM Na–Pipes, 5 mM EGTA, 1 mM $MgCl_2$, pH 7.4, and 0.2% Tween-20) was used for immunohistochemistry. TetA-GBD p2a Cherry expression was detected using Cherry fluorescence. Nuclei are shown by DAPI staining. Confocal images were acquired using a Leica Sp5 confocal microscope.

Axin1-YFP Expression Analysis by Semiquantitative PCR. Total RNA was isolated using TRIzol (Invitrogen) from 10 embryos derived from an incross of TetRE:Axin1-YFP at 78 hpf or from an outcross of *myl7*:TetA-GBD Cherry; TetRE:Axin1YFP double transgenics with WT fish, sorted for presence of the *myl7*:TetA-GBD Cherry transgene by fluorescence at 48 hpf and treated with EtOH vehicle or 25 μg/mL Dox plus 100 μM Dex until 72 hpf. RNA was DNase digested and cDNA synthesized with Thermoscript (Invitrogen) using a 1:1 mixture of oligodT and random primers, a reverse transcriptase negative control was included using the Dox/Dex-treated sample. Axin1-YFP was detected using primers axin1(S) TTGACTGTGGTGTGGTATTTGAG and YFP(AS) AGATGAACTTCAGGGTCAGCTTG.

Axin1-YFP and GFP Expression Analysis Using Quantitative PCR. For each sample, total RNA was isolated from eight to 15 embryos using TRIzol (Invitrogen). RNA was DNase digested and cDNA synthesized with Thermoscript (Invitrogen) using a 1:1 mixture of oligodT and random primers. Relative expression of *axin-YFP* or *GFP* was determined with a Stratagene MX 3000P QPCR machine and the Brilliant SYBR Green QPCR Master Mix kit (Stratagene). Rox served as reference dye. Primers used to amplify a 236-bp product from *axin-YFP* cDNA were as follows: axinYFP Q(S) CTTGCCTGTCTTTGAAGAAAAGAT and axinYFP Q(AS) AGATGAACTTCAGGGTCAGCTTG; primers for amplification of *GFP* were ACGACGGCAACTACAAGACC and ACCTTGATGCCGTTCTTCTG. *axin1-YFP* or *GFP* levels were normalized to *β-actin*, a 194-bp fragment of which was amplified using primers bactin1 Q (S) GAAGGAGATCACCTCTCTTGCTC and bactin1 Q (AS) GTTCTGTTTAGAAGCACTTCCTGTG. For each sample triplicates were run and two separate runs were done. Error bars shown are the SEM of the obtained six values. In each reaction with *axin-YFP* primers 1 μL undiluted cDNA was used whereas the cDNA used for *β-actin* amplification was diluted 1:20. Relative expression of *axin-YFP* was calculated with the $2^{(-\Delta\Delta CT)}$ method (10).

Cell Culture and Transfections. HEK293 cells were cultured in DMEM supplemented with 10% FCS, 2 mM L-glutamine, and 1% penicillin/streptomycin. Cells were transfected in triplicates using the Ca-phosphate method, and after 48 h Luciferase was measured as described elsewhere (11).

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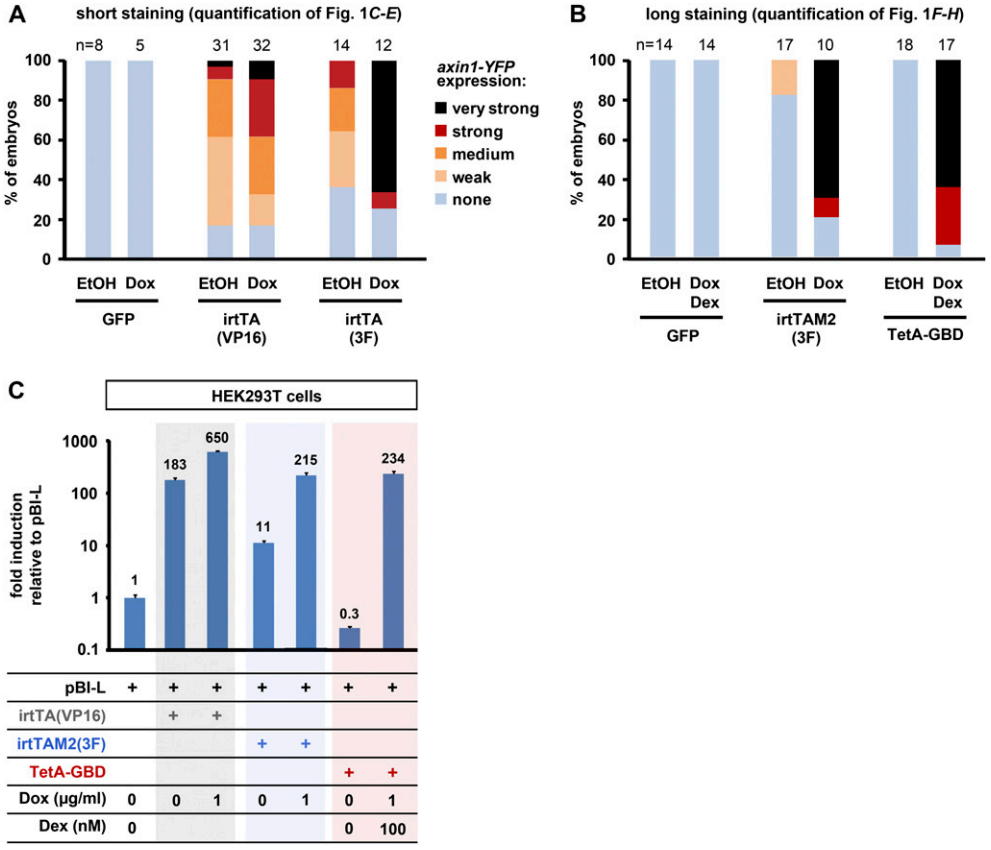

Fig. S1. Comparison of TetActivator constructs leakiness. (*A* and *B*) Quantification of data presented in Fig. 1 *C–E*. Axin1-YFP RNA expression levels in TetRE: Axin1-YFP transgenic embryos injected with the indicated RNAs and treated with the indicated drugs were classified into five classes, and the number of embryos in each class was counted. (*B*) Quantification of data shown in Fig. 1 *F–H*. Note that data in *A* and *B* are not directly comparable, as the whole mount in situ hybridizations quantified in *A* were processed for a shorter length of time than those in *B*. (*C*) TetA-GBD is less leaky than irtTAM2(3F) in mammalian cells. HEK293 cells were transiently transfected with the Tet-responsive firefly luciferase reporter pBI-L, a constitutively expressed Renilla luciferase, and the indicated activators, luciferase levels, were measured after treatment with the indicated doses of drugs and firefly luciferase levels normalized to renilla levels. Levels are shown relative to cells transfected with pBI-L only. Error bars indicate SEM.

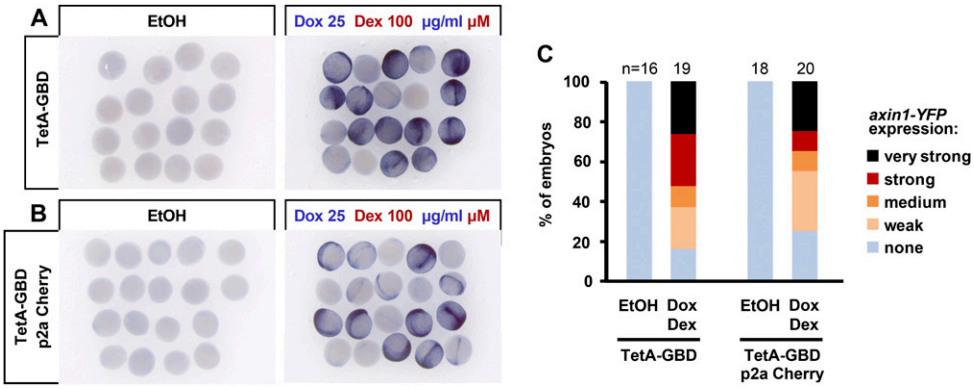

Fig. S2. TetA-GBD tagged with p2a Cherry is functional. (*A* and *B*) YFP RNA expression detected by whole-mount in situ hybridization in TetRE:Axin1-YFP transgenic embryos injected with equimolar amounts of TetA-GBD (50 pg) or TetA-GBD p2a Cherry (65 pg), and treated with EtOH vehicle or 25 µg/mL Dox plus 100 µM Dex from 5 h postfertilization (hpf) for 3.5 h. (*C*) Quantification of data presented in *A* and *B*. Axin1-YFP RNA expression levels were classified into five classes, and the number of embryos in each class was counted.

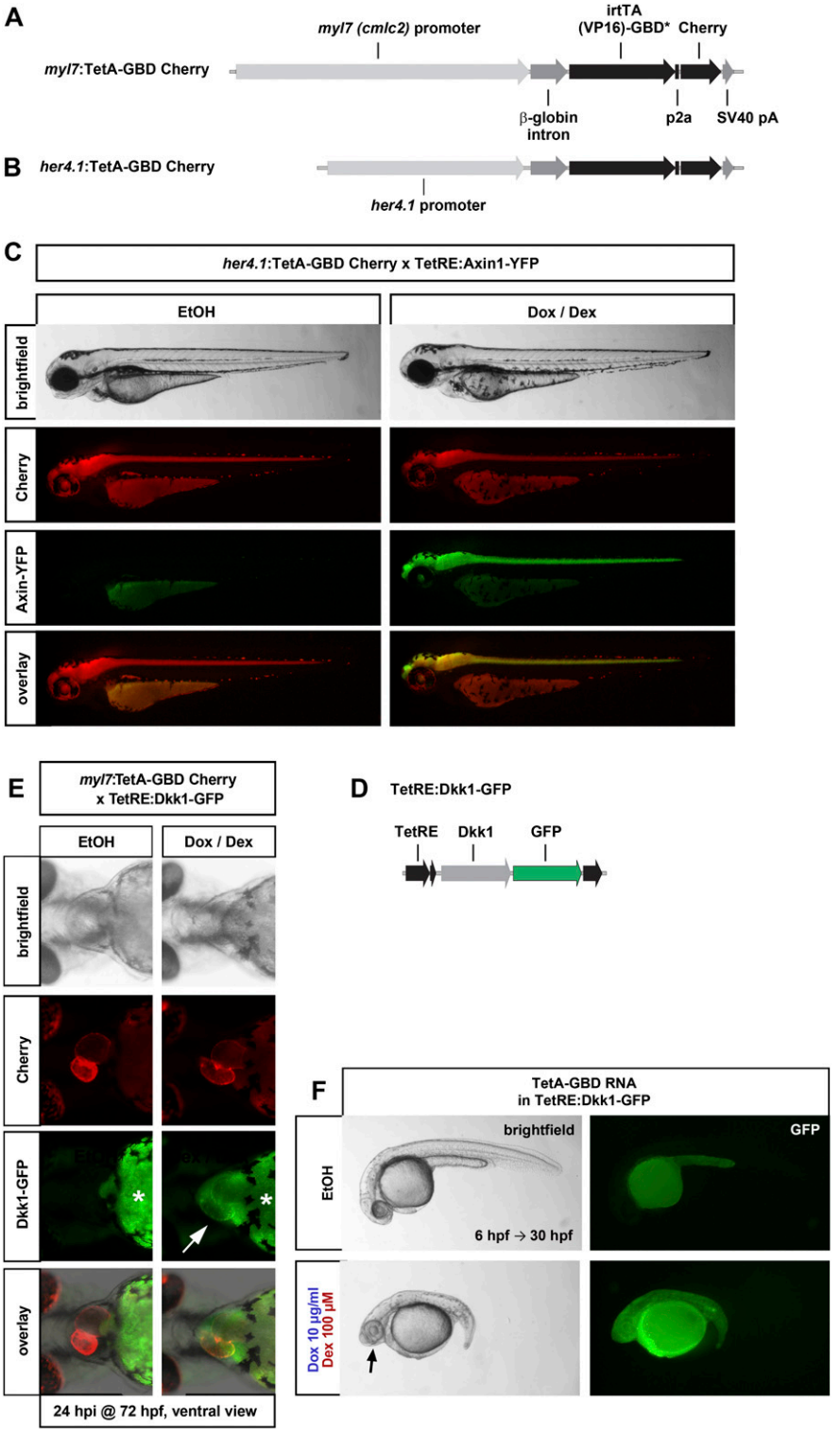

Fig. S3. Conditional, spatially controlled induction using additional TetA-GBD drivers and TetRE responders. (*A*) Transgenic driver construct for expression of TetA-GBD Cherry specifically in the myocardium. (*B*) Transgenic driver construct for expression of TetA-GBD Cherry specifically in *her4.1* expression domains, primarily in the CNS. (*C*) *her4.1*:TetA-GBD Cherry drives efficient induction of Axin1-YFP in embryos doubly transgenic with TetRE:Axin1-YFP. Embryos were treated with EtOH or 25 μg/mL Dox plus 100 μM Dex from 24 h postfertilization (hpf) and photographed at 48 hpf. (*D*) Transgenic responder line construct for expression of Dkk1-GFP. (*E*) Heart-specific expression of Dkk1-GFP in *myl7*:TetA-GBD Cherry; TetRE:Dkk1-GFP double transgenic fish. Images of double transgenic embryos at 72 hpf, treated with EtOH vehicle or 25 μg/mL Dox plus 100 μM Dex from 48 hpf. Note that secreted Dkk1-GFP protein accumulates in the pericardial sac (arrow) in Dox/Dex-treated embryos. *Yolk background fluorescence. *n* = 20 EtOH, 24 Dox/Dex. (*F*) Wnt/β-catenin loss-of-function phenotypes as evidenced by posterior truncations and expanded eyes (arrow) in TetRE:Dkk1-GFP embryos injected with 95 pg TetA-GBD RNA and treated with 10 μg/mL Dox plus 100 μM Dex from 4 hpf until 24 hpf. *n* = 7 EtOH, 8 Dox/Dex.

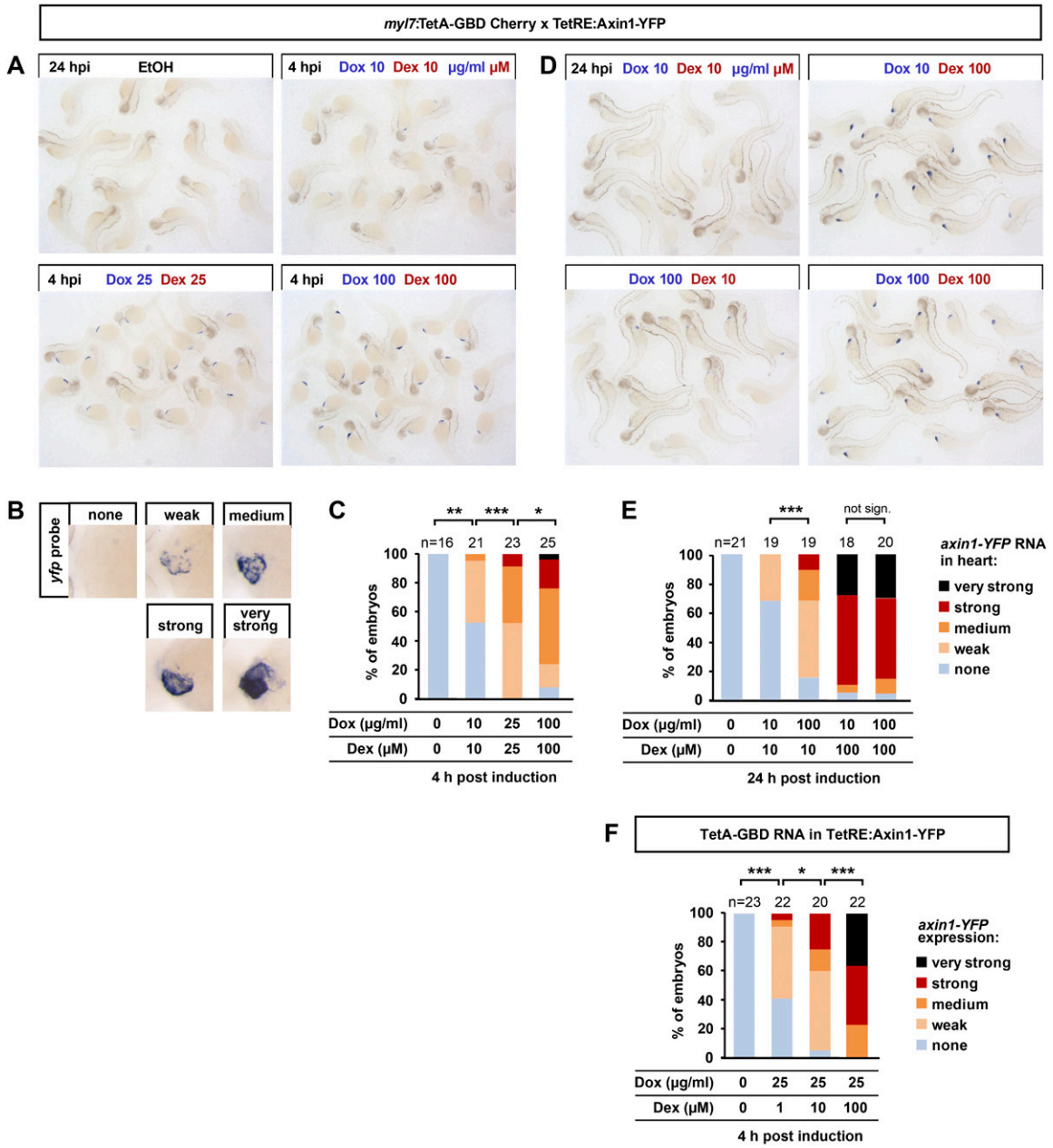

Fig. S4. Drug dose dependency of Axin1-YFP induction in *myl7*:TetA-GBD Cherry; TetRE:Axin1-YFP double transgenic embryos. (*A*) Dose–response to indicated doses of drugs applied at 48 h postfertilization (hpf). YFP RNA was detected by whole-mount in situ hybridization 4 h postinduction, EtOH control-treated embryos are shown at 24 h postinduction. (*B*) Classes of YFP expression in heart used for quantification. (*C*) Quantification of data shown in *A*. $^{**}P = 0.002$ (Fisher exact test), $^{***}P < 0.001$ (χ^2 test), $^{*}P = 0.034$ (χ^2 test). (*D*) Dex is limiting for induction in *myl7*:TetA-GBD Cherry; TetRE:Axin1-YFP double transgenic embryos. The indicated doses of drugs were applied at 48 hpf, YFP RNA was detected by whole-mount in situ hybridization 24 h postinduction. (*E*) Quantification of data shown in *D*). $^{***}P < 0.001$ (χ^2 test). (*F*) Dex is limiting for induction in TetRE:Axin1-YFP embryos injected with 90 pg TetA-GBD RNA. Embryos were treated with indicated doses of drugs at 7 hpf for 4.5 h. Axin1-YFP expression was detected by in situ hybridization and classified as in Fig S1. $^{***}P < 0.001$, $^{*}P = 0.019$ (χ^2 test).

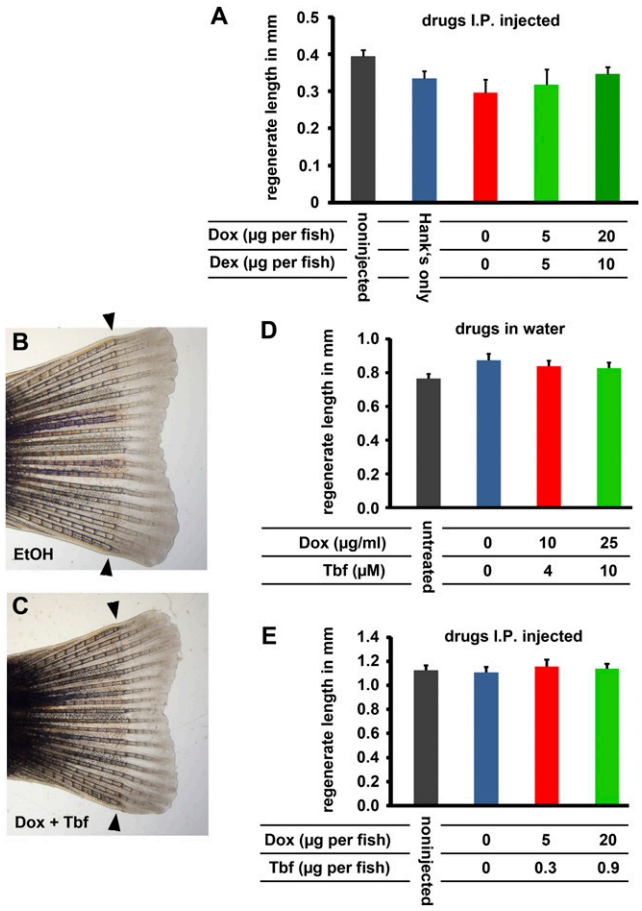

Fig. S5. Combined treatment with Dox and Dex or with Dox and Tebufenozide is not toxic for adult zebrafish. (*A*) Average regenerate length at 8 d post-amputation (dpa) of fish intraperitoneally injected with the indicated doses of drugs at 1 dpa, 3 dpa, and 6 dpa. Zero indicates EtOH vehicle only. Regenerate length is not significantly different (Student *t* test). Error bars indicate SEM. *n* = 6/group. (*B* and *C*) Representative regenerating fins at 7 dpa of fish housed in water containing vehicle or 25 µg/mL Dox plus 10 µM Tebufenozide. Note robust regeneration in both. Amputation plane is indicated by arrowheads. (*D*) Average regenerate length measured at 7 dpa of fish incubated with the indicated doses of drugs from 0.5 dpa. Zero indicates vehicle only. Regenerate length is not significantly different between vehicle control and drug-treated fins (Student *t* test). Error bars are SEM. *n* = 6/Group. (*E*) Average regenerate length at 8 dpa of fish intraperitoneally injected with the indicated doses of drugs at 1 dpa, 3 dpa, and 6 dpa. Zero indicates vehicle only. Regenerate length is not significantly different (Student *t* test). Error bars indicate SEM. *n* = 6/Group.

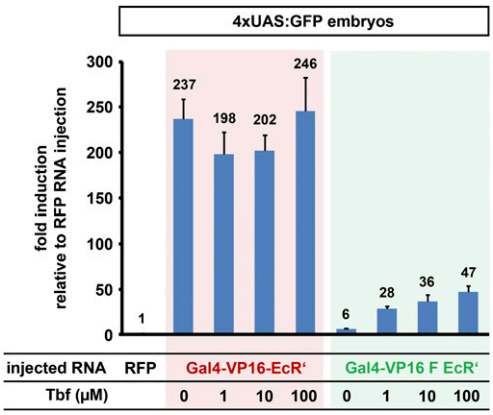

Fig. S6. Activity of ecdysone inducible Gal4-VP16 constructs in 4xUAS:GFP transgenic zebrafish. Embryos were injected with equimolar amounts of the indicated RNAs (TurboRFP 25 pg, Gal4-VP16-EcR′ 65 pg, Gal4-VP16 F-EcR′ 55 pg), treated with EtOH or the indicated doses of Tbf from 5 h postfertilization (hpf) for 4 h, and GFP expression was determined using quantitative PCR. Levels are shown relative to basal expression determined in the RFP-injected control. Error bars indicate SEM.