*Figure S1. Related to Figure 1.*

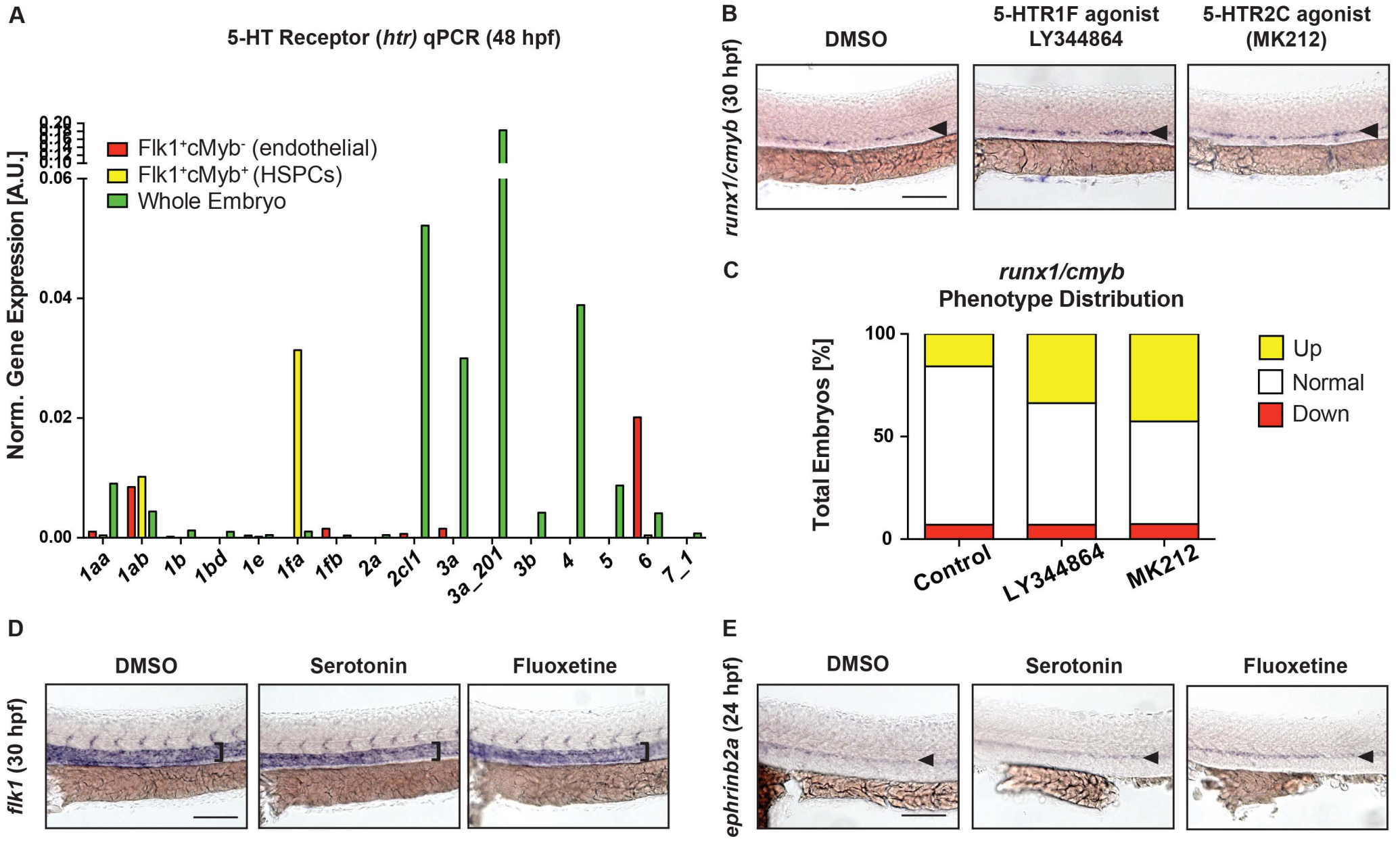


*Figure S2. Related to Figure 2.*

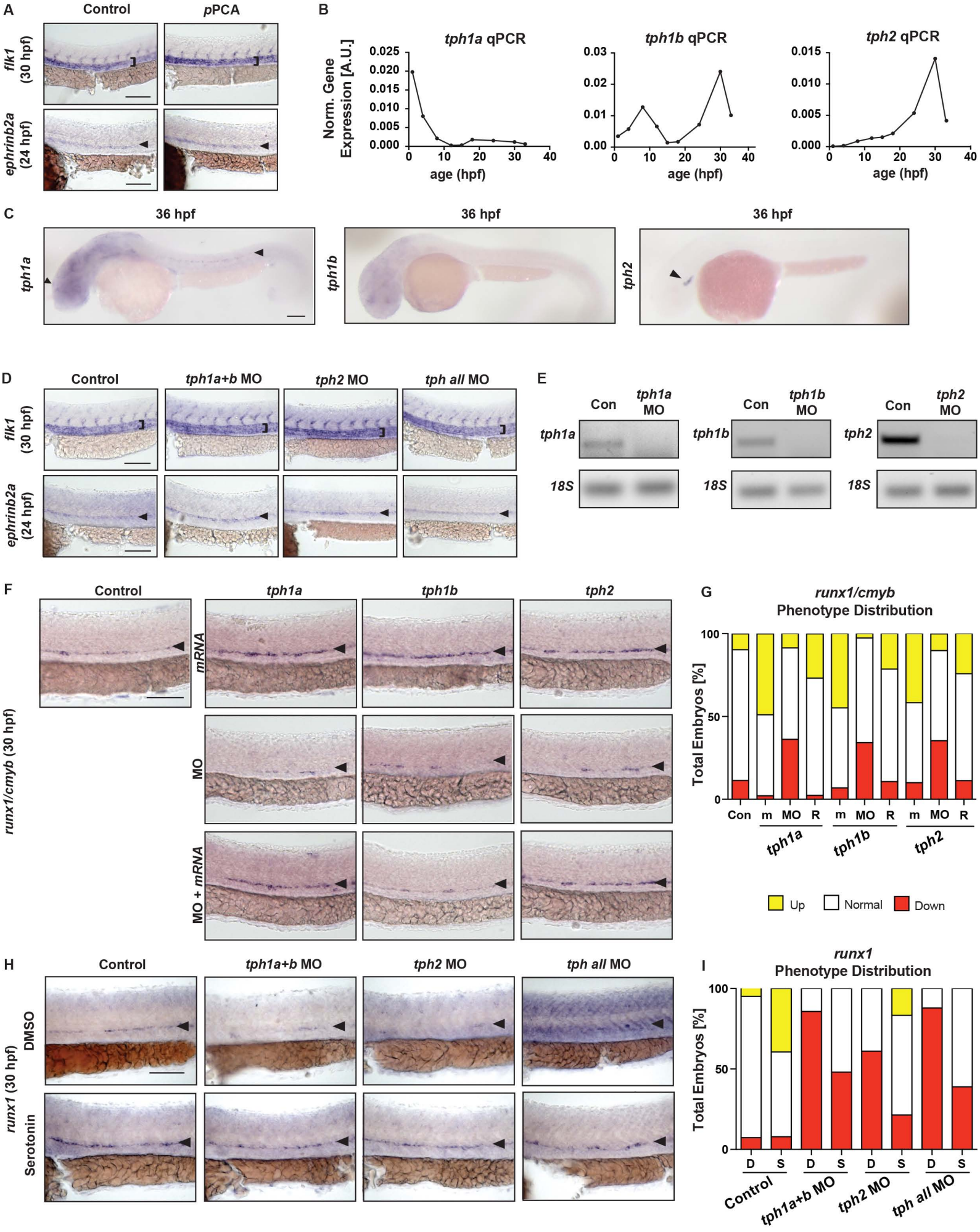


*Figure S3. Related to Figure 2.*

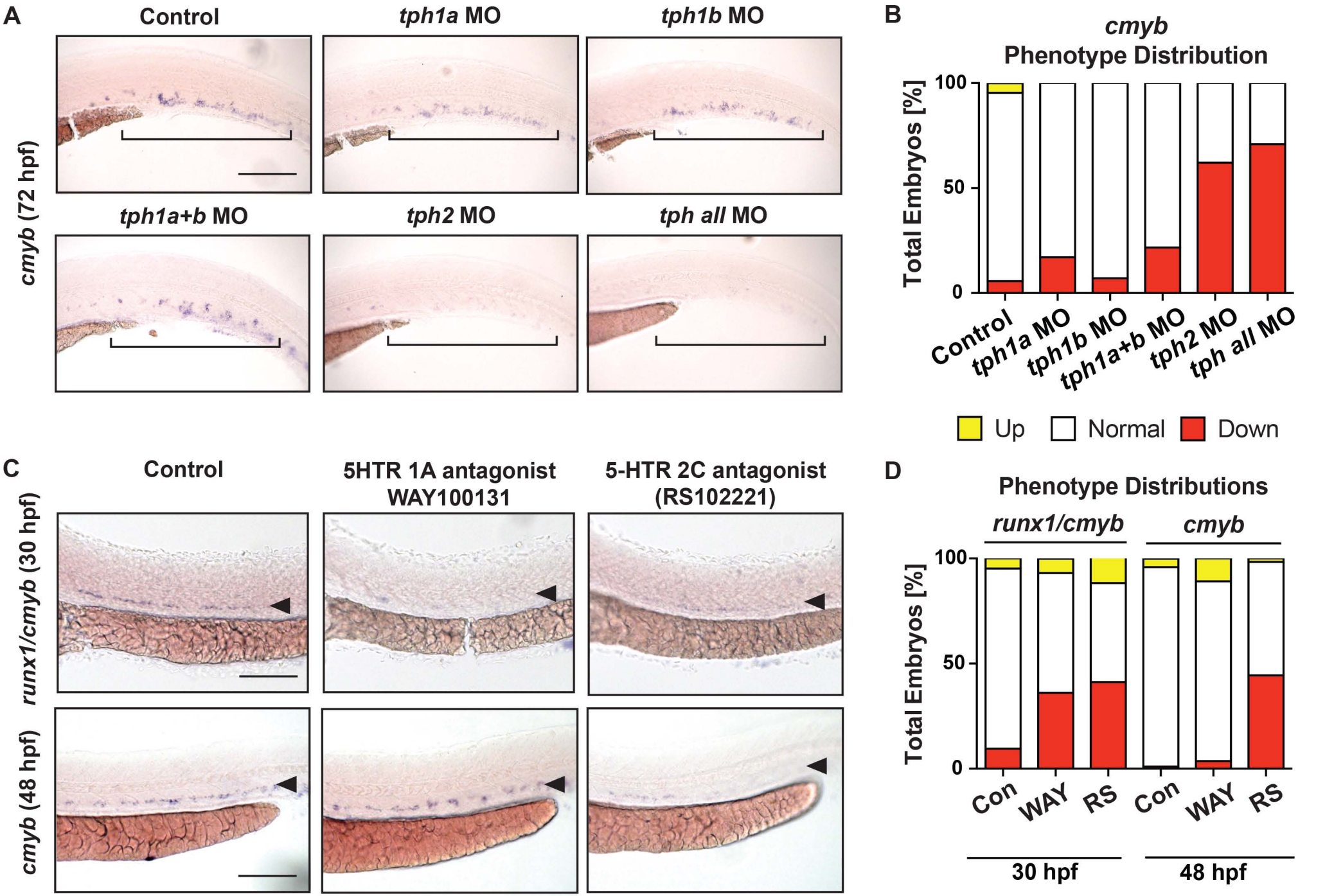


*Figure S4. Related to Figure 3.*

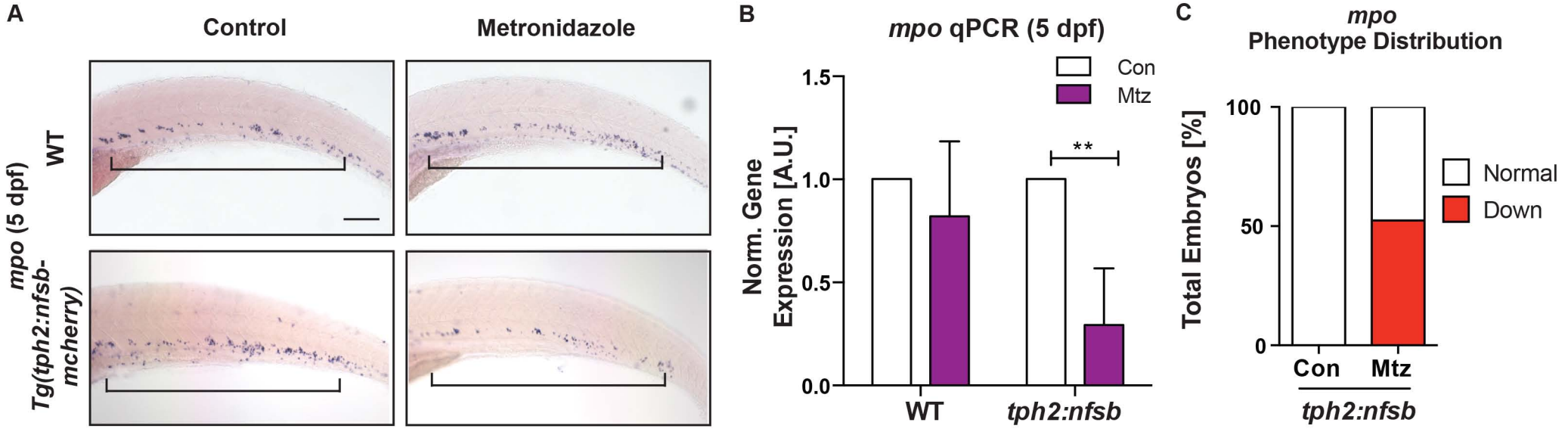


*Figure S5. Related to Figure 4.*

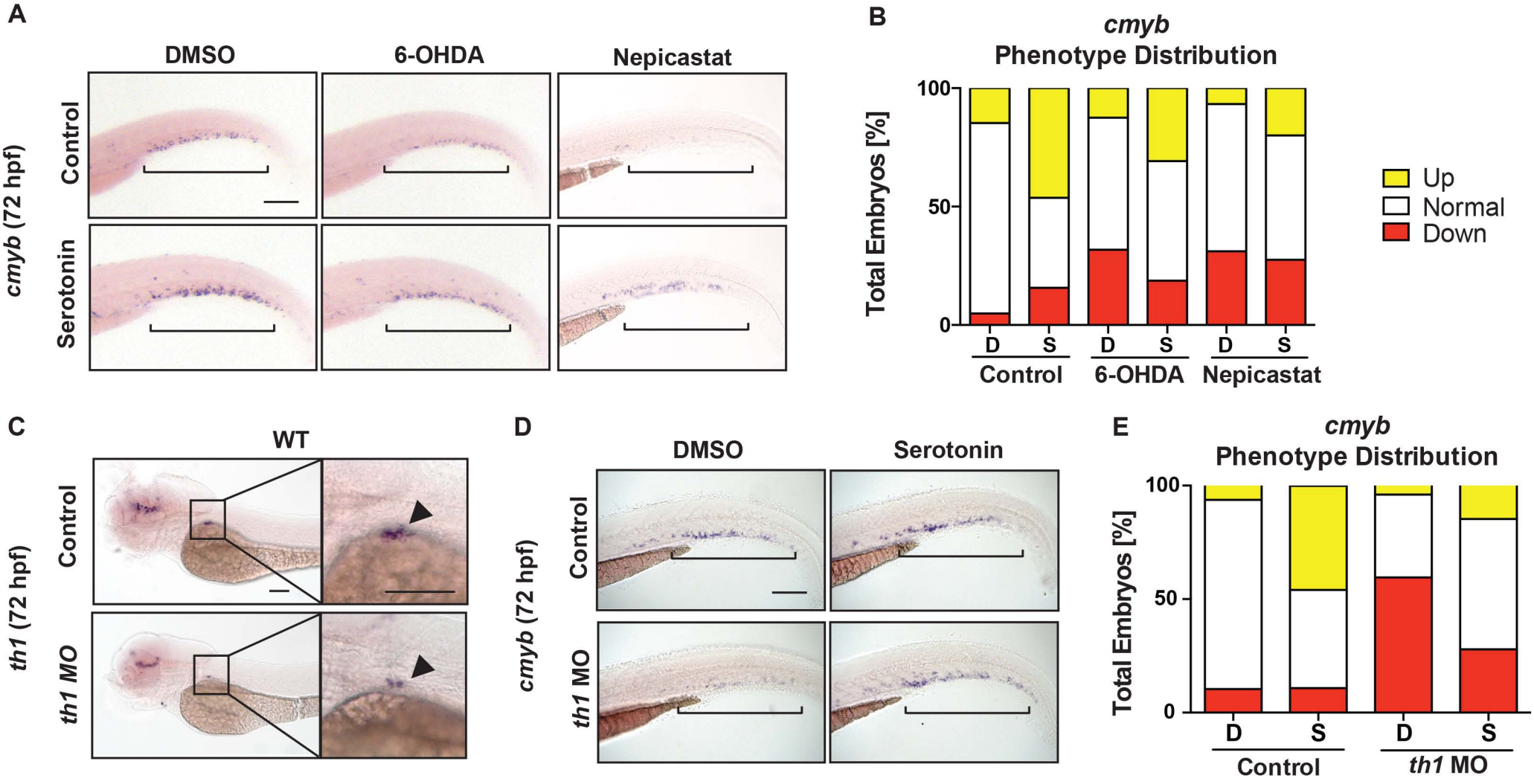


*Figure S6. Related to Figure 5.*

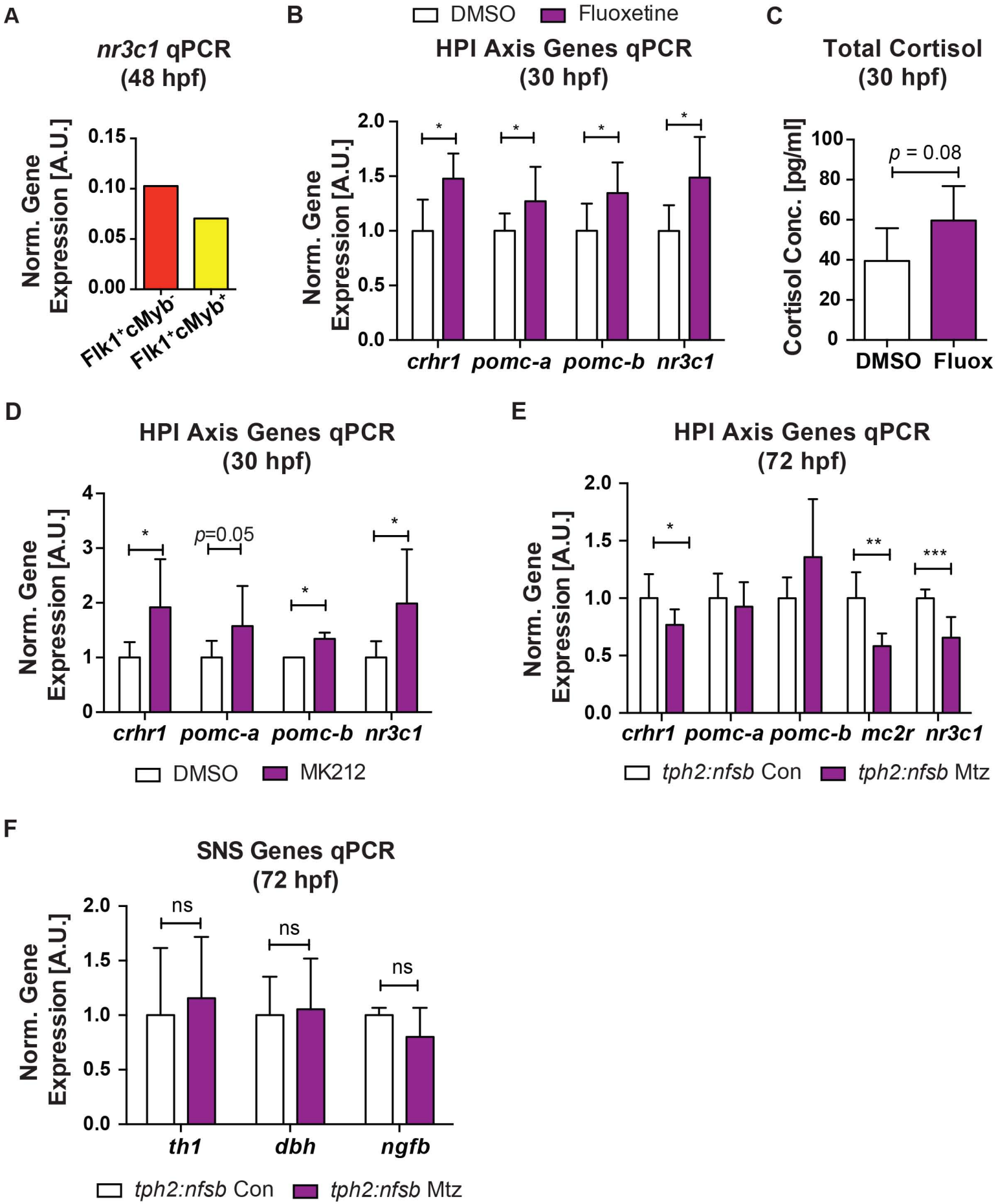
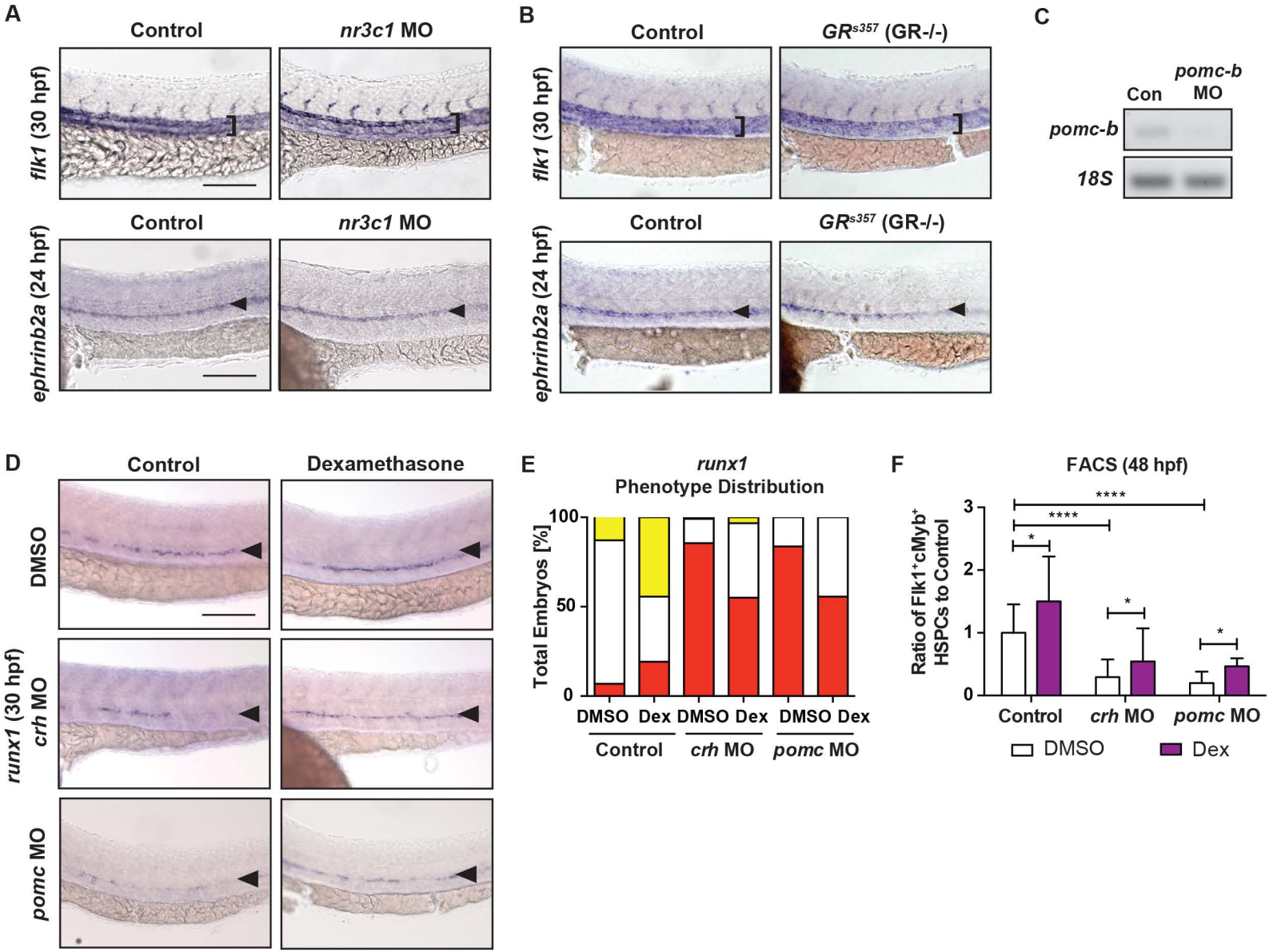


*Figure S7. Related to Figure 6.*



**Supplemental Figure Legends**

**Figure S1. Related to Figure 1.** Serotonin and Fluoxetine Treated Embryos Have Normal Hematopoietic Niche Development, and 5-HTR Agonists Alter HSPC Formation.

(A) *Tg(flk1:dsred;cmyb:gfp)* embryos were disaggregated and FACS sorted into endothelial niche (Flk1:dsRed$^{+}$cMyb:GFP$^{-}$) and HSPC (Flk1:dsRed$^{+}$cMyb:GFP$^{+}$) populations at 48hpf, then used to evaluate relative expression of 5-HT receptors (*htr*) in comparison to whole embryos.

(B) Exposure to serotonin receptor agonists demonstrated that stimulation of representative 5-HTRs expressed on HSPCs (5-HTR1F) and neural-associated (5-HTR2C) both impacted *runx1/cmyb*$^{+}$ HSPCs by WISH at 30hpf (LY344864, 10μM; MK212, 7.5μM).

(C) Qualitative phenotype distribution graph of (B) (≥20 embryos per condition, ≥3 independent experiments). Up=yellow bar; Normal=white bar; Down=red bar.

(D,E) WISH showing normal expression of *flk1* (30hpf) and *ephrinb2a* (24hpf) in serotonin- and fluoxetine-treated embryos (n≥20, no difference observed).

Scale bars=100μM.

**Figure S2. Related to Figure 2.** mRNA and Exogenous Serotonin Can Rescue the Reduction in HSPCs Caused by Morpholino Knockdown of *tryptophan hydroxylase 1* and *2*.

(A) WISH showing normal expression of *flk1* (30hpf) and *ephrinb2a* (24hpf) in *para*-Chlorophenylalanine (*p*CPA)-treated embryos (n≥20, no difference observed).

(B) Time-course analysis of whole embryo *tph1a*, *tph1b*, and *tph2* expression from 0 to 30hpf by qPCR.

(C) WISH showing spatial-temporal expression of *tph1a*, *tph1b*, and *tph2* at 30hpf.

(D) WISH indicating normal expression of *flk1* (30hpf) and *ephrinb2a* (24hpf) in *tph1a+b*, *tph2*, and *tph all* morphants (n≥20, no difference observed).

(E) RT-PCR showing decreased gene expression following successful MO targeting in the respective morphants at 24hpf for *tph1a*, and 30hpf for *tph1b* and *tph2* (pool of 30 embryos/band).

(F) Administration of *tph1a*, *tph1b*, and *tph2* mRNA restored *runx1/cmyb* expression in each respective morphant at 30hpf.

(G) Qualitative phenotype distribution graph of (F). Con=control; m=mRNA; MO=morphant; R=rescue.

(H) Serotonin (7μM) exposure restored *runx1* expression in *tph* morphants at 30hpf.

(I) Qualitative phenotype distribution graph of (I). D=DMSO; S=serotonin.

Scale bars=100μM.

**Figure S3. Related to Figure 2.** Sustained HSPC Effects Are Present in Embryos with *tph2*-Morpholino Knockdown and 5HT2C Antagonist Treatment.

(A) WISH showing decreased *cmyb* expression at 72hpf in the caudal hematopoietic tissue (CHT) in *tph2* and *tph all* (combined *tph1a, tph1b, tph2*) morphants but not for *tph1a*, *tph1b*, or *tph1a+b*.

(B) Qualitative phenotype distribution graph of (A).

(C) WISH showing decreased HSPC formation (*cmyb*) is sustained in embryos treated with a 5-HTR2C antagonist (RS100121, 10μM), but not 5-HTR1A antagonist (WAY100131, 2.5μM) at 48hpf, despite negative effects on *runx1* expression for both compounds at 30hpf.

Scale bars=100μM.

**Figure S4. Related to Figure 3.** Selective Ablation of Serotonergic Neurons Decreases the Myeloid Lineage Population**.**

(A) Representative images of *mpo*+ myeloid cells in wildtype (WT) and *tph2:nfsb-mcherry* embryos at 5dpf after metronidazole (Mtz) treatment.

(B) Expression of *mpo* by qPCR at 5dpf was significantly decreased in *tph2:nfsb-mcherry* embryos with metronidazole (Mtz) treatment, compared to controls (n≥5 replicates of 30 embryos pooled; mean±SD; two-way ANOVA, Holm-Sidak *post hoc*: $^{**}p<0.01$).

(C) Qualitative phenotype distribution of *tph2:nfsb*-mcherry embryos from (A).

Scale bar=100μM.

**Figure S5. Related to Figure 4.** Serotonin Exposure Increases HSPC Formation Despite Inhibition of the Sympathetic Nervous System *by tyrosine hydroxylase 1* Morpholino Knockdown.

(A) Representative images of *cmyb*$^{+}$ HSPCs in the CHT of embryos treated with serotonin in the absence or presence of either 6-hydroxydopamine (6-OHDA, 200μM) or dopamine beta hydroxylase inhibitor nepicastat (30μM) at 72hpf.

(B) Qualitative phenotype distribution of (B).

(C) Representative images of *tyrosine hydroxylase* (*th1*) WISH demonstrating sympathetic ganglion inhibition (boxed) in *th1* morphants at 72hpf.

(D) Representative images of *cmyb*$^{+}$ HSPCs in the CHT of embryos treated with serotonin in the absence or presence of *th1*-MO knockdown at 72hpf.

(E) Qualitative phenotype distribution of (D).

Scale bars=100μM.

**Figure S6. Related to Figure 5.** Endogenous Serotonin Activates the HPA/I axis.

(A) FACS-sorted endothelial niche cells (Flk1:dsRed$^{+}$cMyb:GFP$^{-}$) and HSPCs (Flk1:dsRed$^{+}$cMyb:GFP$^{+}$) expressed *nr3c1* as evaluated by qPCR at 48hpf.

(B) Fluoxetine (30μM) significantly increased expression of HPA/I axis genes at 30hpf by qPCR (n≥4 replicates of 30 embryos pooled; mean±SD; two-tailed *t*-test: *$p$<0.05).

(C) Fluoxetine elevated whole embryo cortisol levels by ELISA assay at 30hpf (n≥5 replicates of 30 embryos pooled; mean±SD; $p$=0.08).

(D) MK212 (7.5 $\mu$ M) significantly increased expression of HPA/I axis genes at 30hpf by qPCR (n≥3 replicates of 30 embryos pooled; mean±SD; two-tailed *t*-test: *$p$<0.05).

(E,F) *tph2:nfsb-mcherry* embryos with metronidazole (Mtz, 10 $\mu$ M) treatment significantly decreased expression of HPA/I axis genes (E), but not SNS genes (F) at 72hpf by qPCR (n≥6 replicates of 30 embryos pooled; mean±SD; two-tailed *t*-test: *$p$<0.05, **$p$<0.01, *$p$<0.001).

**Figure S7. Related to Figure 6.** *nr3c1* Morphants and *GR* Mutants Have Normal Hemogenic Endothelium Development.

(A,B) WISH showing normal expression of *flk1* (30 hpf) and *ephrinb2a* (24hpf) in *nr3c1* MO-injected (A) and *GR*$^{s357}$ embryos (B). (n≥20, no difference observed).

(C) RT-PCR showing decreased gene expression of *pomc-b* in *pomc-b* MO-injected embryos at 30hpf (pool of 30 embryos/band).

(D) Dexamethasone (1μM) partially rescued HSPC defects seen in *crh* and *pomc* MO-injected embryos at 30hpf by *runx1* WISH.

(E) Qualitative phenotype distribution of (E).

(F) Dexamethasone (1μM) partially rescued Flk1:dsRed$^{+}$cMyb:GFP$^{+}$ HSPCs in *crh* and *pomc* MO-injected embryos at 48hpf by FACS (n≥5 replicates of 5 embryos pooled; mean±SD; two-way ANOVA, Holm-Sidak *post hoc*: *$p$<0.05, ***$p$<0.001).

Scale bars=100μM.

**Supplemental Experimental Procedures**

**Transgenic and Mutant Zebrafish Lines** (Refers to Zebrafish Husbandry)

| **Official Line Name** | **Common Name** | **Reference** |
|---|---|---|
| *Tg(runx1P1:egfp)* | *runx1:gfp* | (Lam et al., 2010) |
| *Tg(cmyb:egfp)* | *cmyb:gfp* | (North et al., 2010) |
| *Tg(kdrl:dsred2)* | *flk1:dsred* | (Kikuchi et al., 2011) |
| *Tg(tph2:nfsb-mcherry)y226* | *tph2:nfsb* | (Yokogawa et al., 2012) |
| *nr3c1*$^{s357}$ | *GR*$^{s357}$ | (Ziv et al., 2013) |
| *Tg(-6.0itga2b:egfp)* | *cd41:gfp* | (Bertrand et al., 2008) |
| *Tg(phd3:gfp)* | *phd3:gfp* | (Santhakumar et al., 2012) |

**Morpholino Sequences** (Refers to Morpholino, mRNA, and Plasmid Injections)

| **Gene** | **Morpholino** | **Type** | **Reference** |
|---|---|---|---|
| *tph1a* | CGACTCCTAAAAGTGCTTACTTCAT | splice | unpublished |
| *tph1b* | ATGCTTGTAAAAGCTCGTACCTCAT | splice | unpublished |
| *tph2* | CAATGGGTTCAGCACTCACCATGGA | splice | unpublished |
| *nr3c1* | CTCCAGTCCTCCTTGATCCATTTTG | splice | (Nesan and Vijayan, 2013) |
| *crh* | TGGTGACGAGAAAATTGAGCTTCAT | splice | (Wagle et al., 2011) |
| *pomc-a* | ACAACATCCTCACTCCCCTCACCAT | splice | (Wagle et al., 2011) |
| *pomc-b* | CACTGCTGTGGAGTCAGGATAGAGA | splice | unpublished |
| *th1* | CAGGTTAACAGACTTACATTTGACC | splice | (Formella et al., 2012) |

**mRNA Cloning** (Refers to Morpholino, mRNA, and Plasmid Injections)

For mRNA rescue: *tph1a*, *tph1b* and *tph2* Coding Data Sequences were amplified by PCR (see primers below) from IMAGE clones 4789933, 6792324 and 36hpf cDNA, respectively, prior to cloning into pCS2+ (BamHI/EcoRI). After linearization with NotI, the CDS of *tph1a*, *tph1b* and *tph2* was transcribed using mMessage mMachine SP6 transcription kit (ThermoFisher Scientific), according to manufacturer's protocol. Rescue experiments were performed by co-injection of the respective targeting MO either with either 50ng/µL (*tph1b*) or 200ng/µL (*tph1a*, *tph2*) of the mRNA. For GR overexpression study: *nr3c1* was amplified from 36hpf cDNA using primers indicated below, and cloned into pME-MCS. pDest-*runx1-nr3c1* was generated using the Multigateway Tol2 system with p5E-*runx1*, pME-*nr3c1*, p3EpolyA, and pDesTol2AB2 as previously described (Kwan et al., 2007). pDest-*runx1-nr3c1* and Tol2-transposase mRNA were co-injected into one-cell stage embryos. For Hif1α loss: *dnhif1* mRNA was prepared as previously described *(Elks et al., 2011)*.

| Gene | Forward | Reverse |
|---|---|---|
| *tph1a* | ACCATGTACTCGAGTAAAAGCGACG | TCAGACTCCTAGTTGTTTGTTTAGC |
| *tph1b* | ACCATGCTCTCCAACAAGCTTGAC | TCAGACACCGAGGTGTGTG |
| *tph2* | ACCATGTATGACCAACAGCACCTTG | GGGACTGTGTTTGTTTAGATACCG |
| *nr3c1* | GCAAAATGGATCAAGGAGGA | CTGCTGTTGGGAGGAGATTC |

**qPCR Primers** (Refers to RNA Extraction and Quantitative Reverse Transcriptase Polymerase Chain Reaction)

| **Gene** | **Forward** | **Reverse** |
|---|---|---|
| *18s* | TCGCTAGTTGGCATCGTTTAT | CGGAGGTTCGAAGACGATCA |
| *runx1* | CGTCTTCACAAACCCTCCTCAA | GCTTTACTGCTTCATCCGGCT |
| *cmyb* | TGATGCTTCCCAACACAGA | TTCAGAGGGAATCGTCTGCT |
| *tph1a* | TGACTTGCAAACAGGAGTGC | CACTCTGCGTGTACGGGTTA |
| *tph1b* | TGCTAAAATCCTGCCCTTTG | GAGAATGGACGCTGGATTGT |
| *tph2* | GAGACTTCCTGGCTGGACTG | ACTGAGCAAATTTGGGATCG |
| *rag2* | ACGCTCATGTCCAACTGGGATA | CTCTGCTGTCTACGCTCAACATGTA |
| *mpo* | TGATGTTTGGTTAGGAGGTG | GAGCTGTTTTCTGTTTGGTG |
| *th1* | GCTCTAAAAGCCCTGCGCT | TTTGGTGACAAGATGATGGCA |
| *dbh* | TTGGTGATTCTGTGGGATGA | CAACGATAGGATGGGATGCT |
| *ngfb* | GCCCGCCATTGGAACTC | TGAAGTCAGCGCACGTACAAA |
| *crhr1* | CTTGGGGTCCGATACAACAC | AGTGGCCCAGGTAGTTGATG |
| *pomc-a* | AGGTCGACTATCCGCAAGAA | TCCTCGGTTGGTCTTTATGC |
| *pomc-b* | TGTGTTTTCACAGCCCACAT | GCAAACCCAAGCTCAGACTC |
| *nr3c1* | AGACCTTGGTCCCCTTCACT | CCCAATGTGTCCAAAGGAAT |
| *htr1aa* | GACCTTATGGTGTCGGTGCT | GTCTATGGGATCGGTGATGG |
| *htr1ab* | ACATTAAAACGCGCTGCTCT | TGTAAAATGCGCAAAAGGTG |
| *htr1b* | GGTCTCTGGGCAGTGACAAT | GACGAACAGAGGGGAATCAA |
| *htr1bd* | TTGAAGACTCGCTCGTGATG | GATGGCTGGTTTTGCAGTTT |
| *htr1e* | ACGTGGGCTACACCATCTTC | GACACAGAAGGCATGCTTGA |
| *htr1fa* | AGATCTACCGAGCAGCGAAG | ATTCGAGATGCGATGTCTCC |
| *htr1fb* | CCATGTGGCTTTTACCGTCT | AGACTTCTCGATTGGGCAGA |
| *htr2a* | GTCACTTGCGGTTGCAGATA | ATTGCACACAGGTGCATGAT |
| *htr2cl1* | ACAGACCCCCTCCGAATC | CTCCAGCAGGCAGGAATG |
| *htr3a* | TGGGATCCTGAGGAATTTGA | GGCAGTCACTACTTGGATAGGC |
| *htr3a_201* | TGGGATCCTGAGGAATTTGA | GGCTTATAGTTGCTGACAAGTCC |
| *htr3b* | TGTGGACGGACAGACTCAAA | TTGCCAACATCAACAAATTCA |
| *htr4* | TGCTCAACCCCATCCTCTAC | GGAGCAGCCGTTCAGTACAT |
| *htr5* | TTCCAAATGCTGTTGCAGTC | ACGCTGAACACTGTCTGTGG |
| *htr6* | TCCGCTGTCGGTGAACAG | TGGTACACACTCGCACACC |
| *htr7_1* | TGGAGAGGTCTTCTGCAACA | TTCCAAGGTATCTATCCACACTGA |
| *mc2r* | CTCCGTTCTCCCTTCATCTG | ATTGCCGGATCAATAACAGC |

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