Supplemental Figure 1. CNR2-selective signaling modulates HSC number in the AGM.

(A) Exposure of WT embryos to a synthetic dual CNR1-/CNR2-selective agonist (O2545) or CNR1-selective agonist (ACEA) during HSC formation (12-30hpf) indicated that only CNR2-stimulation impacted *runx1* expression in the AGM (n>85/condition; see also **Figure 1B**).

(B) *In vivo* imaging of *flk1:dsRed*;*cmyb:egfp* embryos showed the number of Flk1:dsRed^{+};cMyb:GFP^{+} HSCs (arrowheads) was increased in the AGM at 36hpf following exposure to O2545 but not ACEA (CNR1-selective) (n≥7/condition; see also **Figure 1D**).

(C) qPCR quantification of the increase in *runx1* and *cmyb* expression at 36hpf in embryos exposed to AM1241 during HSC formation (12-36hpf) (*runx1*: 1.25-fold, *cmyb*: 1.20-fold, *p≤0.05, 1-tailed t-test, n=25 pooled embryos/condition x 4 replicates).

(D) qPCR analysis of FACS-sorted populations from *flk1:dsRed*;*cmyb:egfp* embryos at 48hpf revealed *cnr2* was expressed on HSCs (Flk1:dsRed^{+};cMyb:GFP^{+}) but not on endothelium (Flk1:dsRed^{+};cMyb:GFP^{-}) (HSC: 0.015 a.u.; endothelium: not detected (ND), normalized to *18s*).

(E) Exposure to JWH015 (CNR2-selective agonist) during HSC formation (12-30hpf) increased *runx1* expression in the AGM (n≥130/condition) at 30hpf.

(F) Qualitative phenotypic distribution of embryos from panel S1E, scored with low, medium or high *runx1* expression in the AGM.

(G) AM1241-exposure failed to increase *runx1* expression in the AGM of *cnr2* morphants, confirming phenotypic receptor specificity (n≥15/condition) at 30hpf.

(H) Qualitative phenotypic distribution of embryos from panel S1G, scored with low, medium or high *runx1* expression in the AGM.

(I) Exposure to the CNR2 antagonist AM630 during HSC formation (12-30hpf) reduced *runx1* expression in the AGM (n≥50/condition) at 30hpf.

(J) Qualitative phenotypic distribution of embryos from panel S1I, scored with low, medium or high *runx1* expression in the AGM.

Scale bars: A,B,E,G,I=100μm.

Figure S2. CNR2-signaling is required for HSC production, expansion and CHT colonization.

(A) Schematic representation of the different stages of HSC development starting during somitogenesis (12hpf) and continuing until larval stages (5dpf). HSCs are specified in the hemogenic endothelium prior to 24hpf, and are actively produced between 28 and 48hpf in the AGM. HSCs bud and migrate to CHT starting around 33hpf. HSC expansion occurs in the CHT, and HSCs begin to exit to seed the kidney marrow (KM) and thymus by 72hpf.

(B) *In vivo* imaging confirmed numbers of Runx1:GFP^{+} HSPCs were increased in the AGM at 36hpf following AM1241 exposure during niche specification (18-24hpf) (n≥10/condition).

(C) The effect of JWH015 (18-24hpf) on HSPCs was quantified by FACS analysis of *cmyb:gfp*;*lmo2:dsRed2* embryos (2.48-fold; *p<0.05, 2-tailed t-test, n≥6/condition).

(D) Embryos exposed to AM1241 during HSC production (30-38hpf) exhibited no apparent increase in *runx1*;*cmyb*$^{+}$ HSPCs within the AGM region (n≥65/condition).

(E) Time course analysis (24-42hpf) of the qualitative phenotypic distribution of *runx1;cmyb* expression in the CHT, indicating increased HSPC colonization in response to AM1241-treatment as compared to controls (n≥55/condition).

(F) *In vivo* imaging of *cd41:egfp* embryos indicated the number of CD41:GFP^{+} HSCs (arrowheads) was decreased in the CHT at 48hpf following exposure to AM630 (n≥15/condition; see also **Figure 2K**).

Scale bars: B,D,E,F=100μm.

Figure S3. CNR2-signaling increases the number of *ikaros*$^{+}$ progenitors in the thymus.

(A) Time course analysis of *ikaros* expression during thymus colonization (60-96hpf) revealed increased thymic progenitors present by 72hpf in embryos exposed to AM1241 compared to controls (n≥40/condition; see also **Figure 3J**).

Scale bars: A=200μm.

Figure S4. CNR2- and PGE2-signaling interact at the level of Ptgs2.

(A) Treatment with selective cyclooxygenase (Ptgs)-1 (FR122047) and Ptgs2 (Celecoxib, CAY10404) (12-30hpf) inhibitors decreased *runx1* expression in the AGM; addition of AM1241 restored *runx1* expression to baseline in FR122047-, but not Celecoxib- or CAY10404-treated embryos (n≥15/condition).

(B) Western blot analysis confirmed CNR2-modulation altered Ptgs2 protein levels at 24hpf; β-actin shown as control (DMSO: 1 a.u., AM1241: 2.86 a.u., AM630: 0.43 a.u., normalized to β-actin, n=80 pooled embryos/condition, x 3 replicates).

(C) Qualitative phenotypic distribution of embryos injected with *ptges* MO, revealed loss of PGE2 production antagonized the effect of AM1241 on HSPC expression (n≥80/condition).

(D) Qualitative phenotypic distribution of embryos injected with *ptger2/4* MOs, confirmed PGE2-signaling was required to mediate the effect of AM1241-treatment (n≥65/condition).

(E) Qualitative phenotypic distribution of embryos exposed to AM1241 and dmPGE2 (2.5μM each) indicating a collaborative effect on *runx1* expression in the AGM (n≥40/condition).

(F) Qualitative phenotypic distribution of embryos exposed to AM1241 and dmPGE2 (5μM each) indicating a collaborative effect on *runx1* expression in the AGM (n≥130/condition).

Figure S5. AM1241 increases HSC number in the AGM in a PGE2-dependent manner, without affecting the vasculature.

(A) *In vivo* imaging of *flk1:dsRed*;*cmyb:egfp* embryos confirmed PGE2 production and signaling are required to mediate the effect of AM1241 on AGM HSCs (n≥10/condition).

(B) *In vivo* imaging of *cd41:gfp* embryos confirmed that the AM1241-mediated increase in HSCs in the CHT is independent of PGE2 production and signaling (n≥9/condition).

(C) Western blot analysis showed CNR2-modulation during niche specification altered pERK levels at 24hpf (DMSO: 1 a.u., AM1241: 1.55 a.u., AM630: 0.49 a.u., normalized to β-actin, n=50 pooled embryos/condition, x 3 replicates).

(D) Western blot analysis confirmed CNR2-modulation altered pAKT protein levels at 24hpf (DMSO: 1 a.u., AM1241: 1.79 a.u., AM630: 0.63 a.u., normalized to β-actin, n=50 pooled embryos/condition x 3 replicates).

(E) Flk1:GFP vascular patterning was not impacted by AM1241 exposure (12-30hpf) (n≥20/condition).

(F) Expression of *flt4* in the vein was not affected in AM1241-treated embryos (n≥25/condition).

(G) Arterial identity, as indicated by *ephrinB2a*, was not altered in AM1241-exposed embryos (n≥20/condition).

Scale bars: A,B,G=100μm, E,F=125μm.

Figure S6. Most chemokines, adhesion molecules and ECM proteins involved in HSC trafficking are not differentially regulated in PGE2 and AM1241-treated embryos.

(A) qPCR analysis for known eicosanoid targets in embryos treated with either AM1241 or PGE2 during HSC production (30-36hpf): *cxcr4b* (AM1241: 1.20-fold), *sdf1b* (dmPGE2: 1.49-fold, AM1241: 1.34-fold), *mmp9* (dmPGE2: 1.31-fold, AM1241: 1.19-fold) (*p≤0.05, 2-tailed t-test, n=25 pooled embryos/condition x 4 replicates).

(B) Qualitative phenotypic distribution of embryos exposed to AMD3100 (Cxcr4-selective inhibitor) and AM1241 revealed CXCR4/SDF1 activity did not account for the increase in *runx1*;*cmyb* expression in the CHT mediated by CNR2-stimulation (n≥50/condition).

(C) qPCR analysis of embryos exposed to PGE2 and AM1241 (30-36hpf) showed no significant differences in the expression level of *cadherins*: *cadh1*, *cadh2*, *cadh4*, *cadh5*, *cadh13* (2-tailed t-test, samples as describe in panel S6A).

(D) qPCR analysis, as described in panel S6A, showing no effect on *integrins*: *itga5*, *itgb1a*, *itgb1b.1*, *itgb1b.2*.

(E) qPCR analysis, as described in panel S6A, showing no significant alterations in *protocadherins*: *pcdh1a*/*b*, *pcdh7a*/*b*, *pcdh10a*/*b*, *pcdh18a*/*b*.

(F) qPCR analysis of FACS-sorted populations from *flk1:dsRed; cmyb:gfp* embryos at 48hpf revealed *selp* is expressed on endothelial cells (Flk1:dsRed^{+};cMyb:GFP^{-}: 0.0033a.u.,

Flk1:dsRed;cMyb:GFP^{+}: not detected) but *sele* is expressed on both endothelial and HSC fractions (Flk1:dsRed^{+};cMyb:GFP^{-}: 23.9 a.u., Flk1:dsRed;Cmyb:GFP^{+}: 4.83 a.u., normalized to *18s*).

Figure S7. **CNR2-signaling promotes HSPC migration to secondary niches via P-selectin.**

(A) In *psgl-1* morphant embryos, while thymic colonization is reduced, *cmyb* expression is observed to accumulate in the CHT (middle panel) and/or appear in isolated cells scatter throughout the embryo (lower panel) in the presence and absence of AM1241-treatment (30-96hpf).

(B) Qualitative phenotypic distribution of *cmyb* expression within the CHT (left side) or dispersed throughout the embryo (right side) in *psgl-1* morphants with and without AM1241-treatment compared to sibling controls (n≥35/condition).

(C) qPCR analysis of *psgl-1* morphant embryos showed no significant alterations in *cmyb* expression in the presence or absence of AM1241-treatment (5mM) (AM1241 : 1.09-fold, *psgl-1* MO/DMSO : 1.33-fold*, psgl-1* MO/AM1241 : 1.26-fold; NS, 2-tailed t-test, n=25 pooled embryos/condition x 4 replicates).

(D) Qualitative phenotypic distribution of embryos injected with *psgl-1* and *selp* MOs and subsequently exposed to AM1241 revealed *mpo* expression, indicative of myeloid commitment, was not increased following P-selectin knockdown (n≥30/condition).

(E) Absolute counts of CD41:GFP^{+} cells from embryos injected with *psgl-1* and *selp* MOs and exposed to AM1241 (30-96hpf, 5μM) revealed inhibition of P-selectin activity caused prolonged retention of HSCs in the CHT compared to controls (48hpf: Uninjected/DMSO: 15±1.5, Uninjected/AM1241:16.1±1.7, *psgl-1* MO/DMSO: 13.8±1.5, *psgl-1* MO/AM1241:12.2±1.3, *selp* MO/DMSO: 14.0±1.2, *selp* MO/AM1241: 12.6±1.5; 72hpf: Uninjected/DMSO: 35.6±2.4, Uninjected/AM1241:39.4±3.4, *psgl-1* MO/DMSO: 39.9±3.1, *psgl-1* MO/AM1241:46.4±3.2, *selp* MO/DMSO: 46.7±2.7, *selp* MO/AM1241: 46.2±2.0; 96hpf: Uninjected/DMSO: 29.2±3.1, Uninjected/AM1241:30.5±3.7, *psgl-1* MO/DMSO: 36.7±1.9, *psgl-1* MO/AM1241:37.6±3.3, *selp* MO/DMSO: 34.9±2.3, *selp* MO/AM1241: 36.1±2.6; *p≤0.05, **p≤0.01, ***p≤0.001, 2-tailed t-test, n≥10/condition).

(F) Embryos exposed to KF38789 (30-96hpf) confirmed the effects of P-selectin on normal and AM1241-enhanced colonization of Rag2:GFP^{+} lymphoid progenitors in the thymus at 4dpf (n≥10/condition).

(G) Embryos exposed to AM630 during HSC expansion and thymic colonization (48-96hpf) showed decreased Rag2:GFP intensity in the thymus (n≥10/condition).

(H) *In vivo* imaging of *cd41:gfp* embryos (48-96hpf) showed exposure to AM630 cause HSC numbers to remain elevated in the CHT at 96hpf (n≥15/condition).

(I) qPCR analysis confirmed decreased *ikaros* expression at 96hpf in embryos exposed to AM630 (30-96hpf) (0.65-fold; ***p≤0.001, 2-tailed t-test, n=25 pooled embryos/condition x 4/replicates).

Scale bars: A =800μm, F,G=175μm, H=200μm.

Figure S8. CNR2-signaling affects HSC production and migration by modulating PGE2 and P-selectin activity.

During niche specification, CNR2-signaling increases HSC production in the AGM by up-regulating the expression of *ptgs2* thus augmenting PGE2 signaling. During HSC migration and expansion, CNR2-signaling increases HSC movement to the CHT and the thymus by up-regulating *selp* and *psgl-1* expression.

Supplemental Methods

Supplemental Table 1. Chemical modifiers utilized and concentrations

Compound	Target	Concentration
2-Arachidonoylglycerol (2-AG)	Endogenous CB	5μM
Anandamide (AEA)	Endogenous CB	5μM
O2545	CNR1/2 agonist	5μM
Arachidonyl-2'-chloroethylamide (ACEA)	CNR1 agonist	5μM
AM1241	CNR2 agonist	5-10μM
JWH015	CNR2 agonist	5-10μM
AM630	CNR2 antagonist	5-10μM
16,16-dimethyl prostaglandin E2 (dmPGE2)	PGE2 agonist	5μM
Prostaglandin E2 (PGE2)	PGE2 agonist	5μM
SC-560	Ptgs1 inhibitor	10μM
FR122047	Ptgs1 inhibitor	20μM
NS-398	Ptgs2 inhibitor	10μM
Celecoxib	Ptgs2 inhibitor	20μM
CAY10404	Ptgs2 inhibitor	5μM
H89	PKA inhibitor	5μM
AMD3100	CXCR4 antagonist	10μM
KF38789	P-selectin inhibitor	0.5μM

Supplemental Table 2. Antibodies utilized and concentrations

Antibody	Company	Dilution
phospho-histone H3 (06-570)	Millipore	1 :500
phospho-histone H3 (ab14955)	Abcam	1 :500
GFP (MAB3580)	Millipore	1 :500
GFP (SC-9996)	Santa-Cruz	1:200
dsRed (632496)	Clontech	1:400
PTGS2 (10034)	Cayman Chemicals	1:200
b-actin (4967)	Cell signaling	1:1000
Goat anti-rabbit IgG	Invitrogen	1:1000
Goat anti-mouse IgG	Invitrogen	1:1000
Goat anti-rabbit IgG HRP conjugated	Cell signaling	1:1000
Bovine anti-Goat IgG HRP conjugated	Jackson ImmunoResearch	1:10,000

Supplemental Table 3. Sequences of morpholino oligonucleotides utilized

Gene	Morpholino sequence	Reference
cnr1 (E1-I1 splice)	GTGCTATCAACAACATACCTTTGTG	
cnr2 (E1-I1 splice)	GCCATGAAACAAACAGTACCTGTGG	
ptgs1 (*ptgs1* - ATG)	TCAGCAAAAAGTTACACTCTCTCAT	North et al., 2007
ptgs2a (*ptgs2a* - ATG)	AACCAGTTTATTCATTCCAGAAGTG	North et al., 2007
ptgs2b (*ptgs2b* - ATG)	AGGCTTACCTCCTGTGCAAACCACG	Yeh et al., 2009
ptges (E1-I1 splice)	GTTTTGTGCTCTTACCTCCTACAGC	North et al., 2007
ptger2a (ATG)	ACTGTCAATACAGGTCCCATTTTC	North et al., 2007
ptger4a (ATG)	CACGGTGGGCTCCATGCTGCTGCTG	North et al., 2007
selp	TTTGTAAGCCACCATCGCCGCCATC	
psgl-1	CATTGACTGATAAACACAGTGGCGT	

North TE, Goessling W, Walkley CR, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature*. 2007;447(7147):1007-1011.

Yeh JR, Munson KM, Elagib KE, et al. Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. *Nat Chem Biol.* 2009;5(4):236-243.

Supplemental Table 4. Primers utilized for RT-qPCR

Gene	Forward	Reverse	Reference
tbp	CGGTGGATCCTGCGAATTA	TGACAGGTTATGAAGCAAAACA ACA	McCurley et al., 2008
18s	TCGCTAGTTGGCATCGTTTAT	CGGAGGTTCGAAGACGATCA	
cmyb	TGATGCTTCCCAACACAGAG	TTCAGAGGGAATCGTCTGCT	Harris et al., 2013
runx1	CGTCTTCACAAACCCTCCTCAA	GCTTTACTGCTTCATCCGGCT	Harris et al., 2013
ikaros	AGAAGGGTAACCTGCTCCGAC AC	GGGCTTTCCAACCGAATGAGT	Harris et al., 2013
cnr2	TGGAGAACAAACTGGAACAAG A	GGTCAGCAGGACCAAAATGT	
ptgs1	AGGGTCATCGTTGAATCGAG	ATTTCCACCATGCTTTCACC	
ptgs2a	CCAGACAGATGCGCTATCAA	GAGCTCCCATTTCCACCATA	
ptgs2b	AGGGCGTAATGTAGCACCAG	CAGCATAAAGCTCCACAGCA	
psgl-1	TCCAGTGCAGACCGTTAATG	GTTGGGTGTGCAAACTAATC	Sun et al., 2012
selp	TCGGGCATACTACTGGATTG	GGTTATTCGGTTCATTTGTCG	Sun et al., 2010
sele	TCGCTCTTCAAAGCAACAGA	TCTTATAAGCCTGCGGTGGA	
cxcr4a	GCTGGAGACTGAAGGAGCTG	CCCGATAAGACCCAAAACAA	
cxcr4b	TGGAGACTTATTGCGCCTTT	CAAGCACCACAAGTCCATTG	
cxcl12a	CACAGTCCCACAGAGAAGCA	GGGCTGTCAGATTTCCTTGT	
cxcl12b	GGAGCATCCGAGAGATCAAG	CGCACATCCTCGTCTGTTTA	
mmp9	TCATGATCTCTGCGAAGTGG	TTGCCTTTTCCTCTCTGCAT	
itga5	TGGCGATTCTAGCTGGTCTT	TTCCTCCGCCGTCTACTCTA	
itgb1a	GGTGTAGTGGCAGGAATCGT	GGCCATTATTTGCCTTCGTA	
itgb1b	GTCTGCAGCAACAATGGAGA	AGAGGGCAGTCACAAGCACT	
itgb1b.2	AAAAGAGAAAACCCGGAGGA	ACATTCACAGATGCCGTGTC	
cdh1	AGCAGGGATTCCACTGTTTG	CCTCTGTGCAGGACACTCAA	
cdh2	TGCAGCAGCCTGATACTCTG	GTCAAACACCAGCAGGGAGT	
cdh4	GACATCGGGGAGTTCATCAC	GGTCTGCCAGCTTTTTGAAG	
(VE-)cdh5	ACACAAGATCCACACGCTGG	GAACATACACTCAGGAGCGTG	Harris et al., 2013
cdh13	CTCCGTATGTGTTCCCGTCT	GCAACATGATCTGGGAGTGA	
pcdh1a	GGACCACTACGAAAGGACCA	TGATAAGGCACAAACGTGGA	
pcdh1b	GCTCCAACCTCAATCACCAT	GGCTGTGGAGAAGGTCACTC	
pcdh7a	TAGTGGGGTGGAGGACTCAG	CATTCGTGTGTGCATTTTCC	
pcdh7b	TACAGCAGCCAAACCATCAA	CGGGCGTAGTCCTCTCATAG	
pcdh10a	TCCAACGGGAGCATTTTATC	GCACTCTTCAGTGCAGTTGG	
pcdh10b	GGGGGATCAGTCATTCTCAA	GGTATGTCCACTCGGAAGGA	
pcdh18a	CCAGCTTCAGAGGGAACAAA	GGCACTCCTCCGTACACAAT	
pcdh18b	TGCTTGGAGAAGGCTTCAGT	GCACTTCAGGAGTGGCTTTC	

Harris JM, Esain V, Frechette GM, et al. Glucose metabolism impacts the spatiotemporal onset and magnitude of HSC induction in vivo. *Blood*. 2013;121(13):2483-2493.

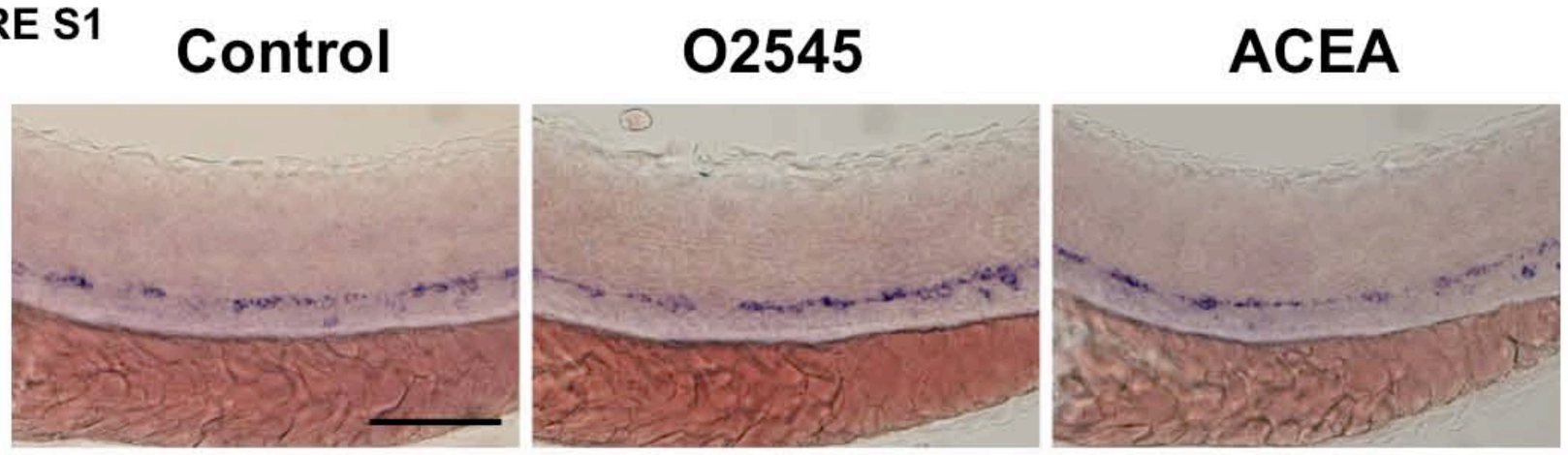
McCurley AT, Callard GV. Characterization of housekeeping genes in zebrafish: male-female differences and effects of tissue type, developmental stage and chemical treatment. *BMC Mol Biol.* 2008;9:102.

Sun G, Pan J, Liu K, Wang X, Wang S. Molecular Cloning and Expression Analysis of P-Selectin from Zebrafish (Danio rerio). *Int J Mol Sci*. 2010;11(11):4618-4630.

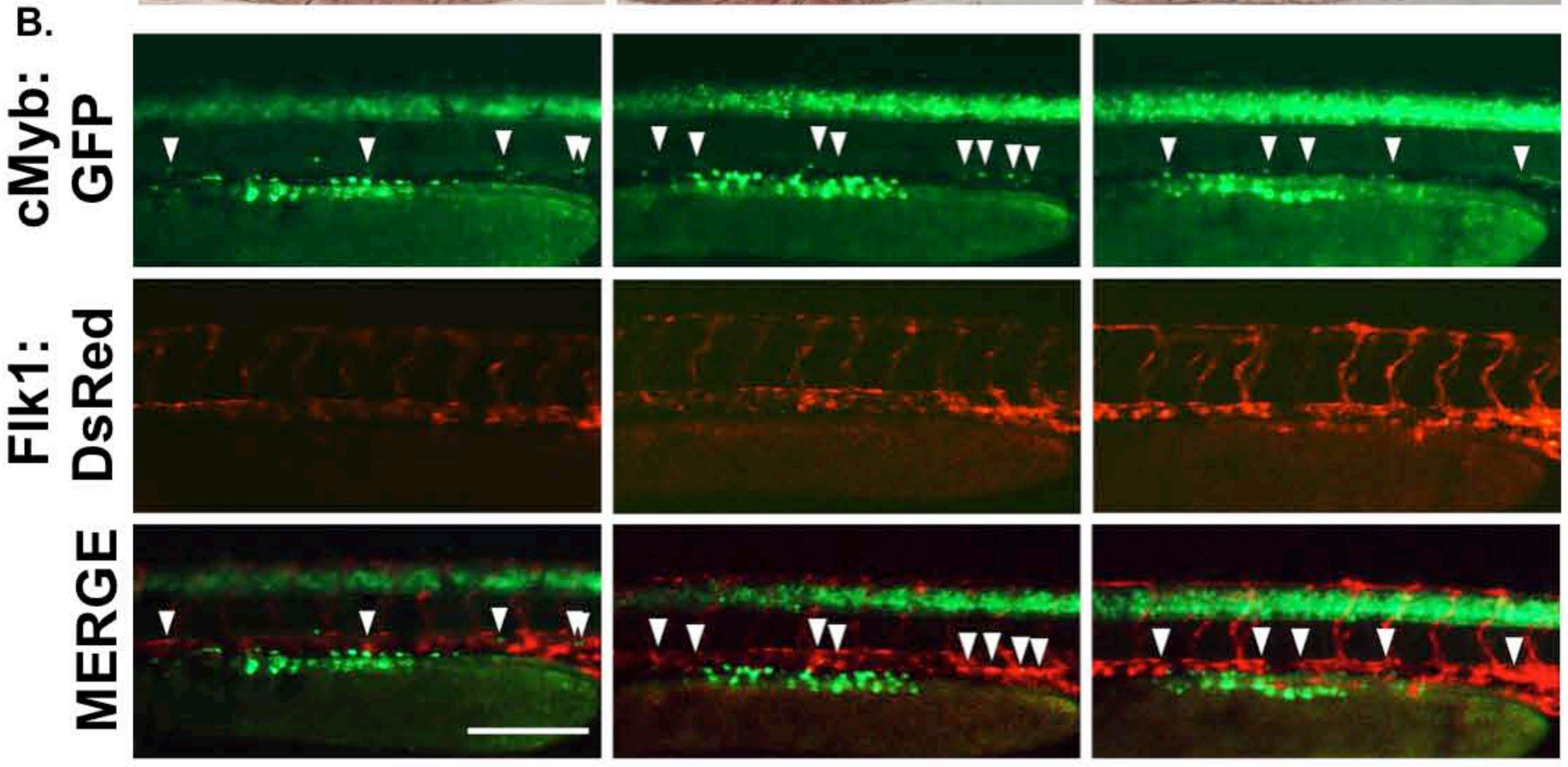
Sun G, Pan J, Liu K, Wang S, Wang X. Molecular cloning and expression analysis of P-selectin glycoprotein ligand-1 from zebrafish (Danio rerio). *Fish physiology and biochemistry*. 2012;38(2):555-564.

FIGURE S1

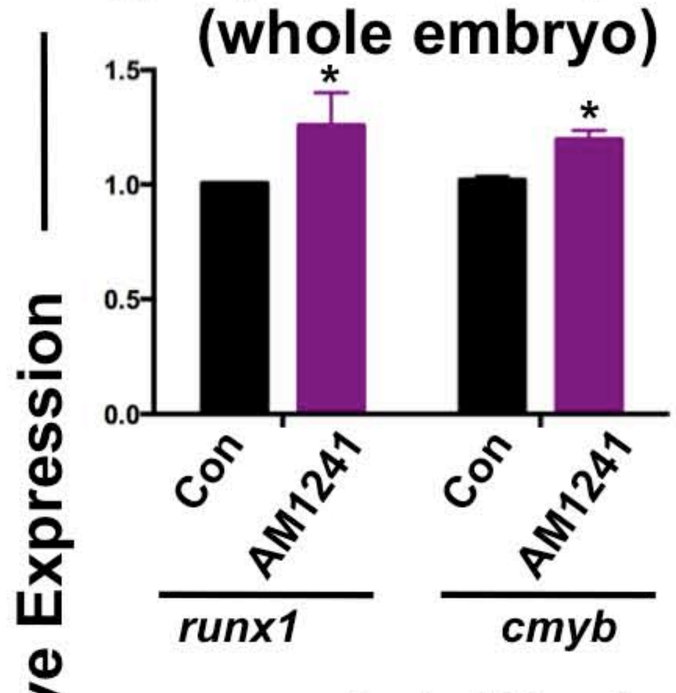
A.



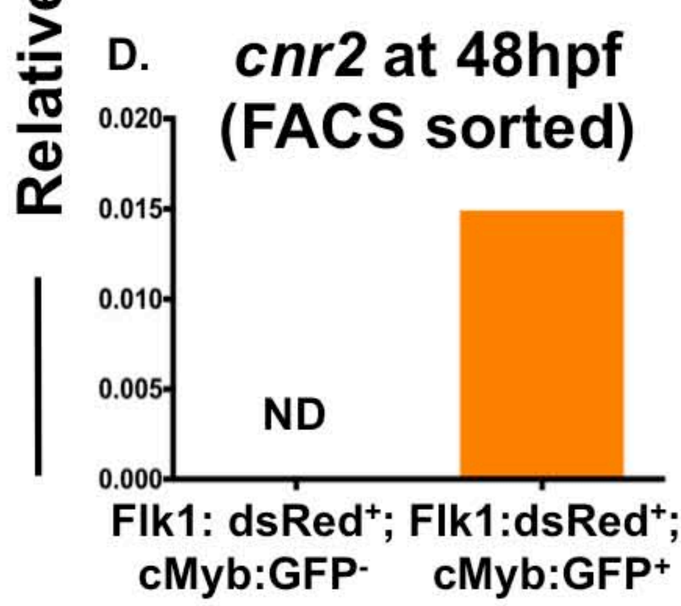
B.



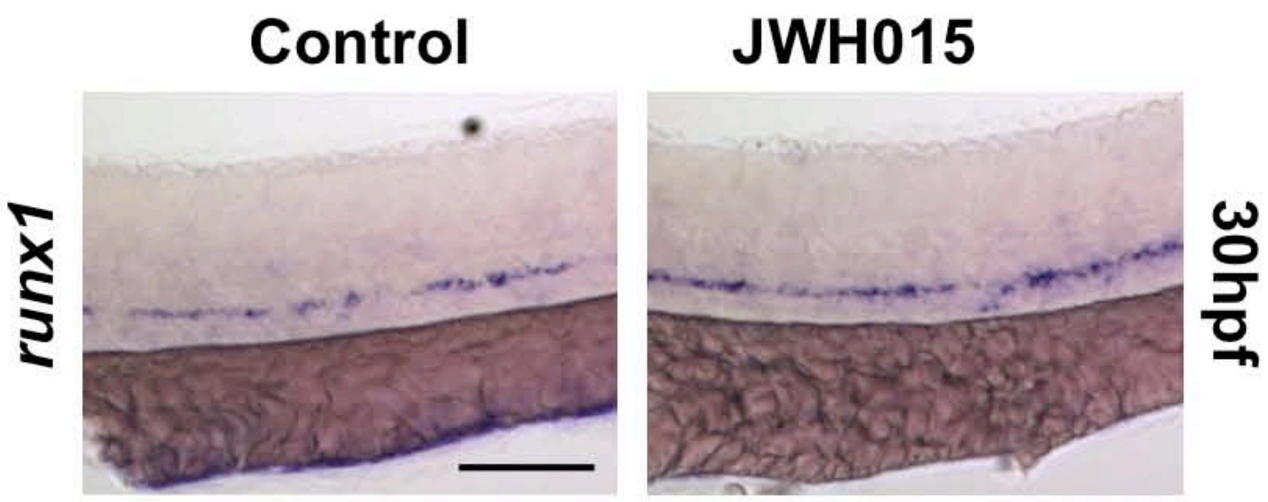
C.



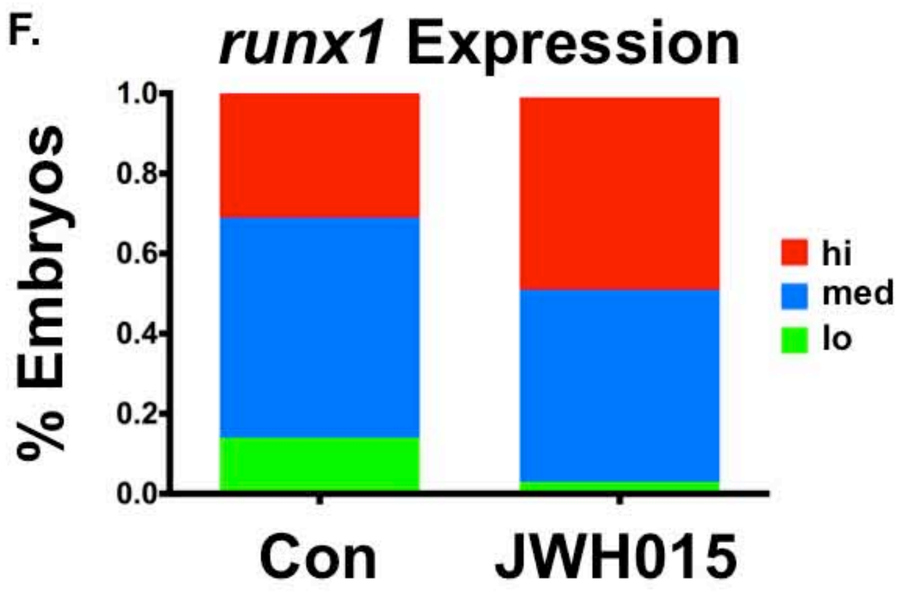
D.



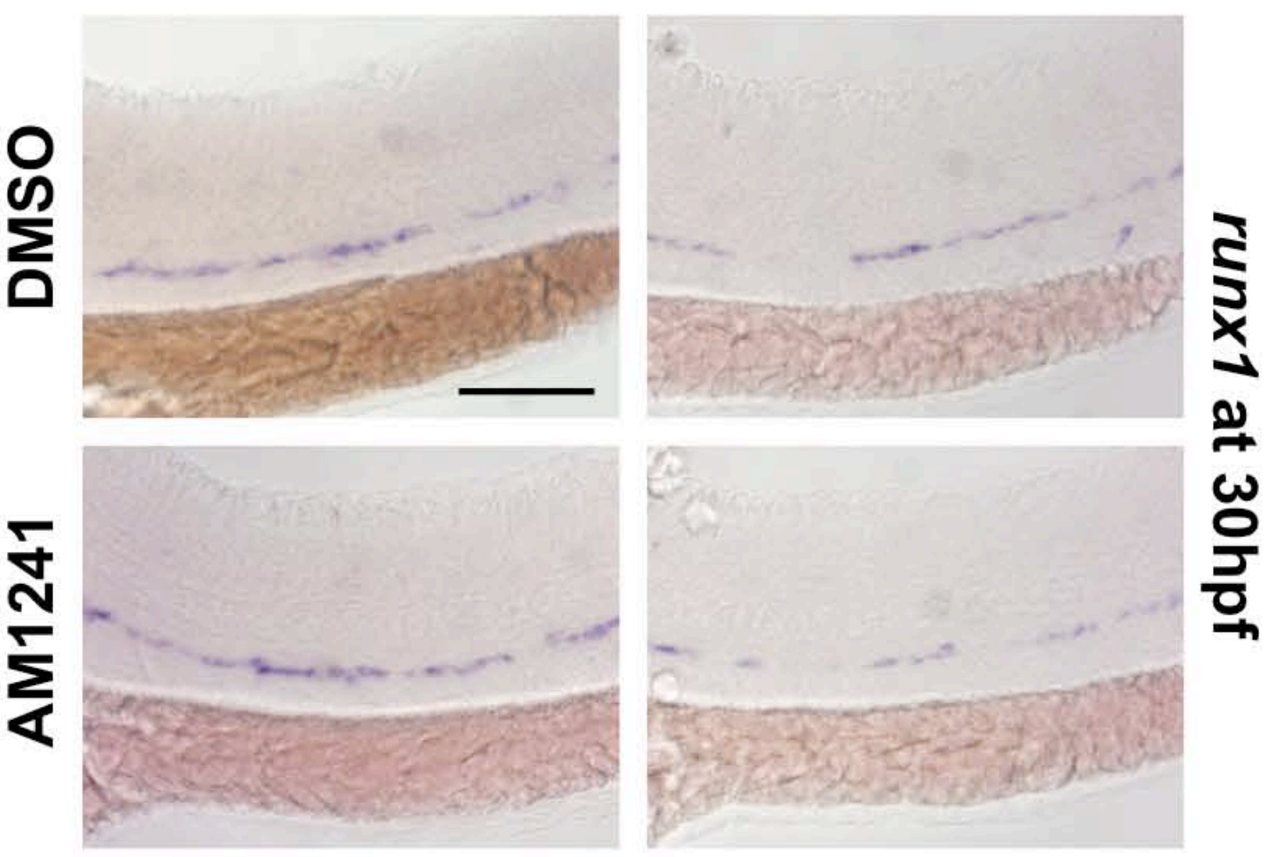
E.



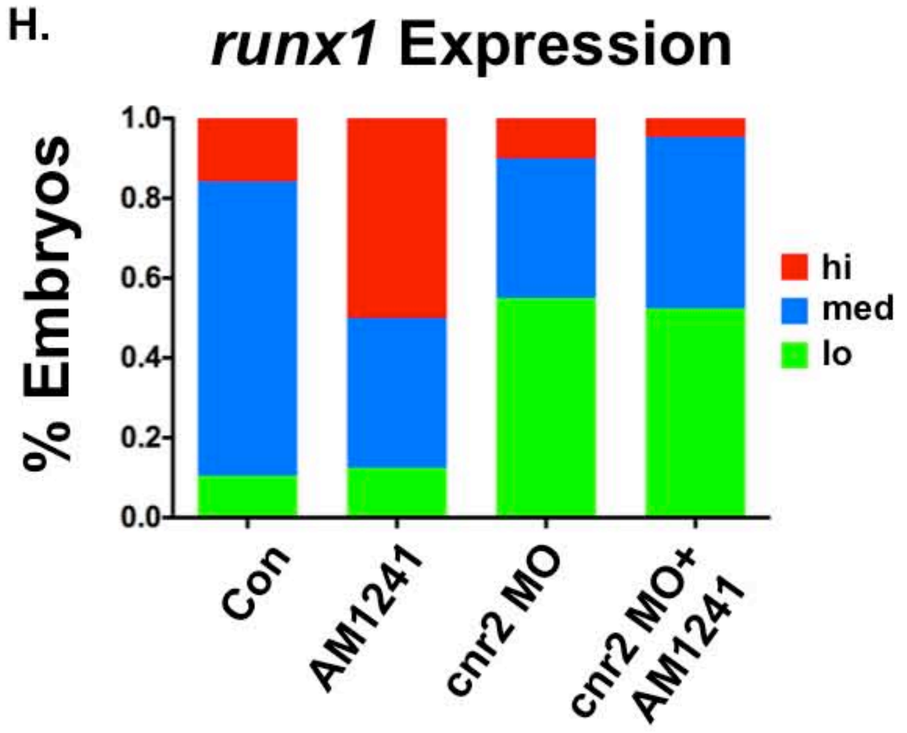
F.



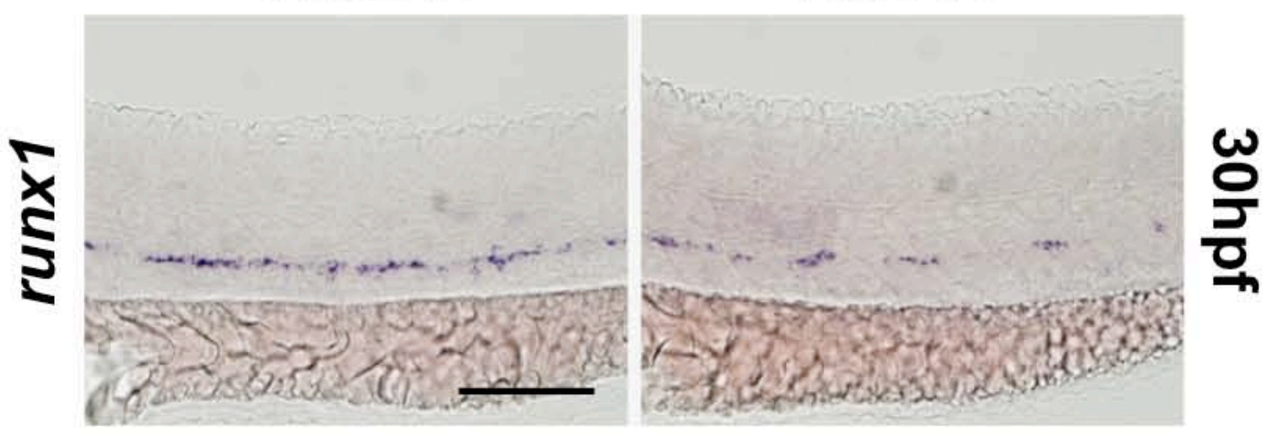
G.



H.



I.



J.

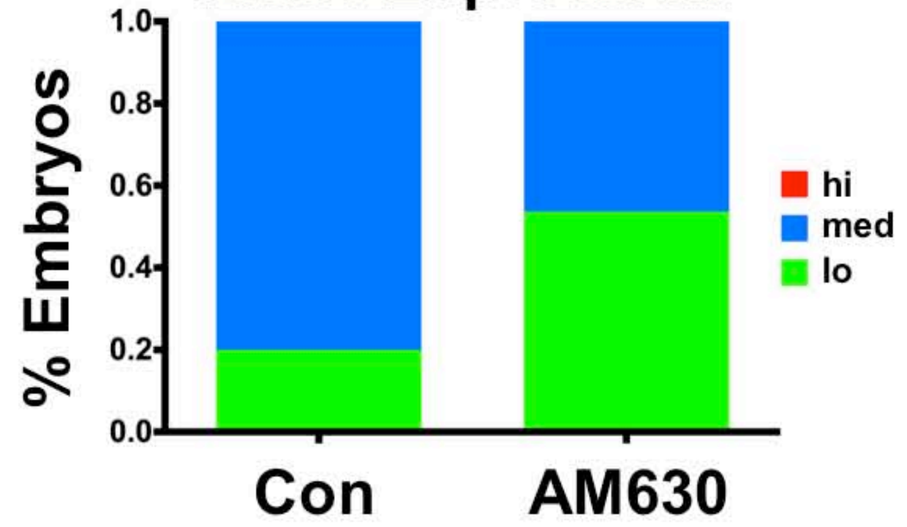
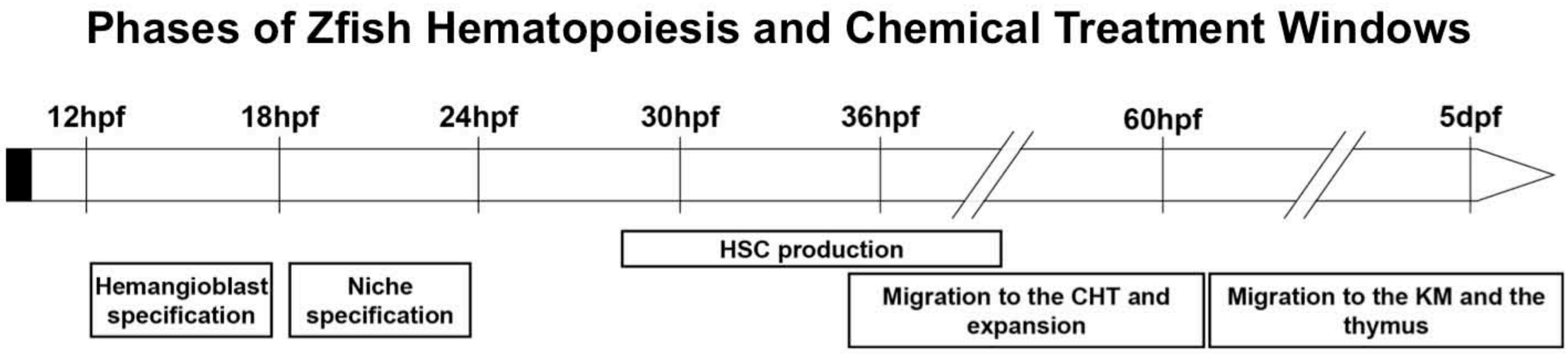
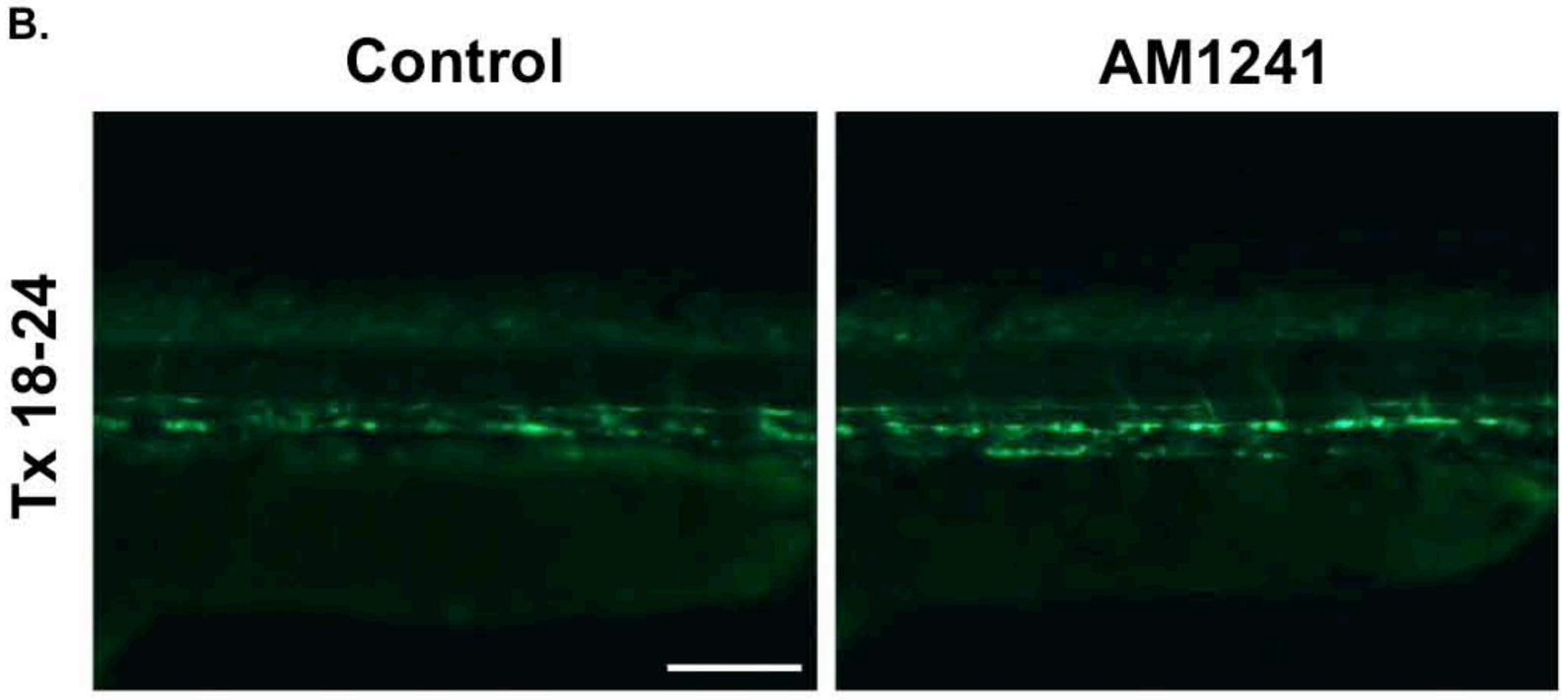


FIGURE S2

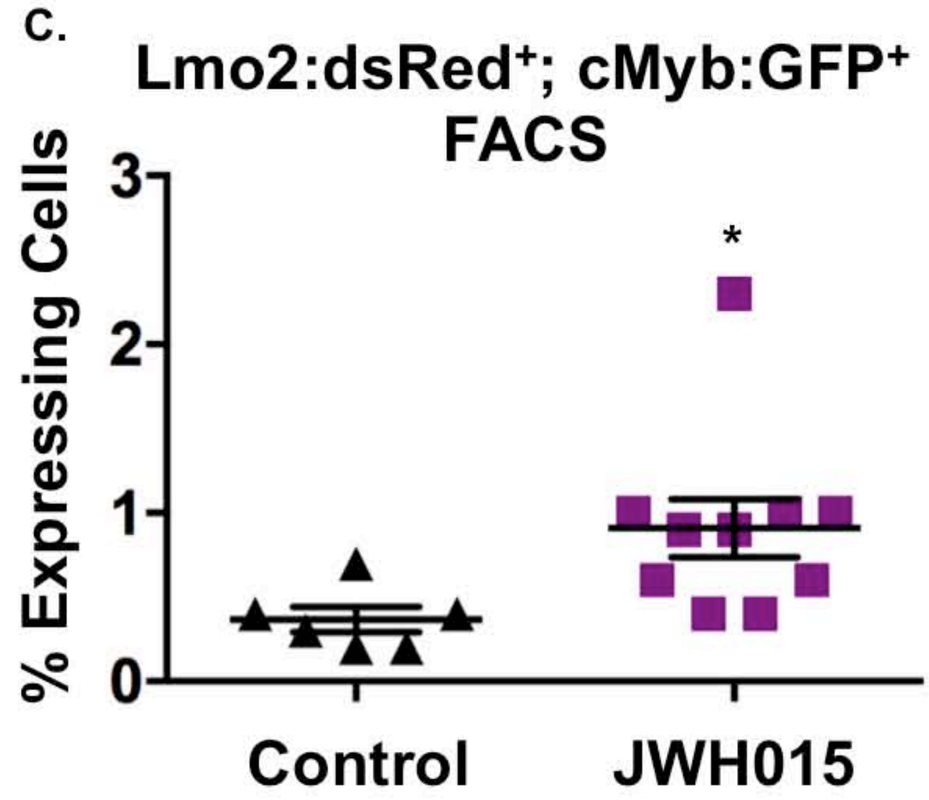
A.



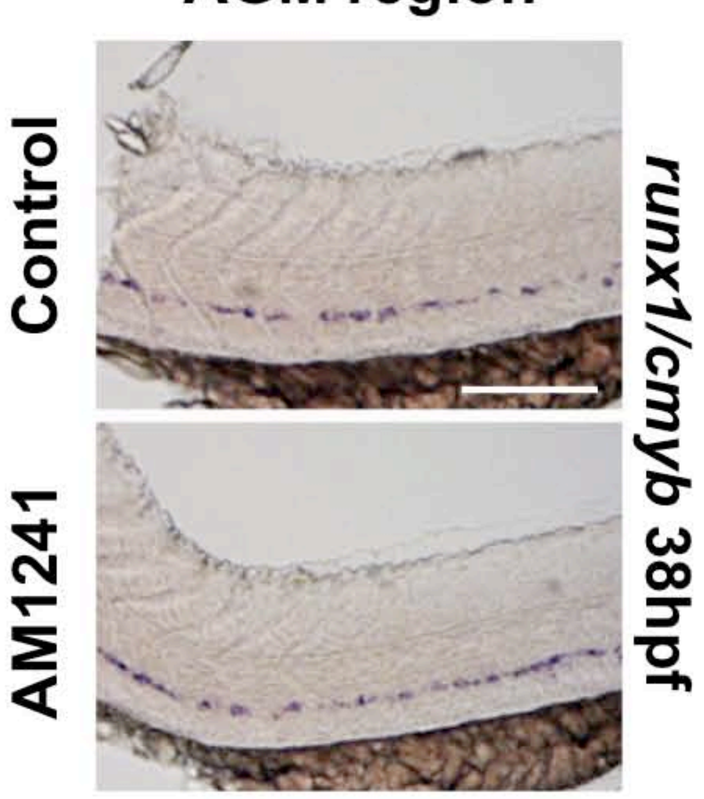
B.



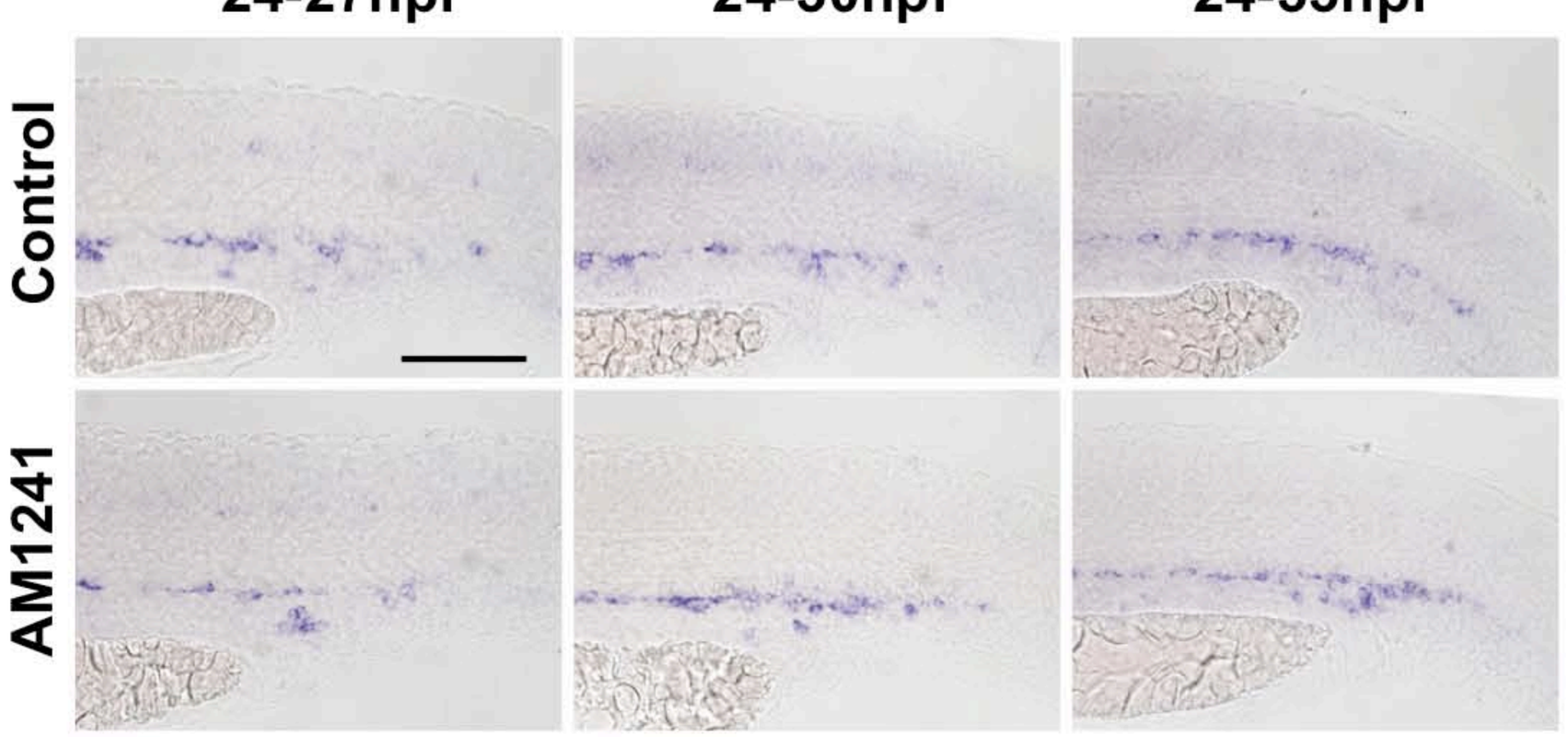
C.



D.



E.



F.

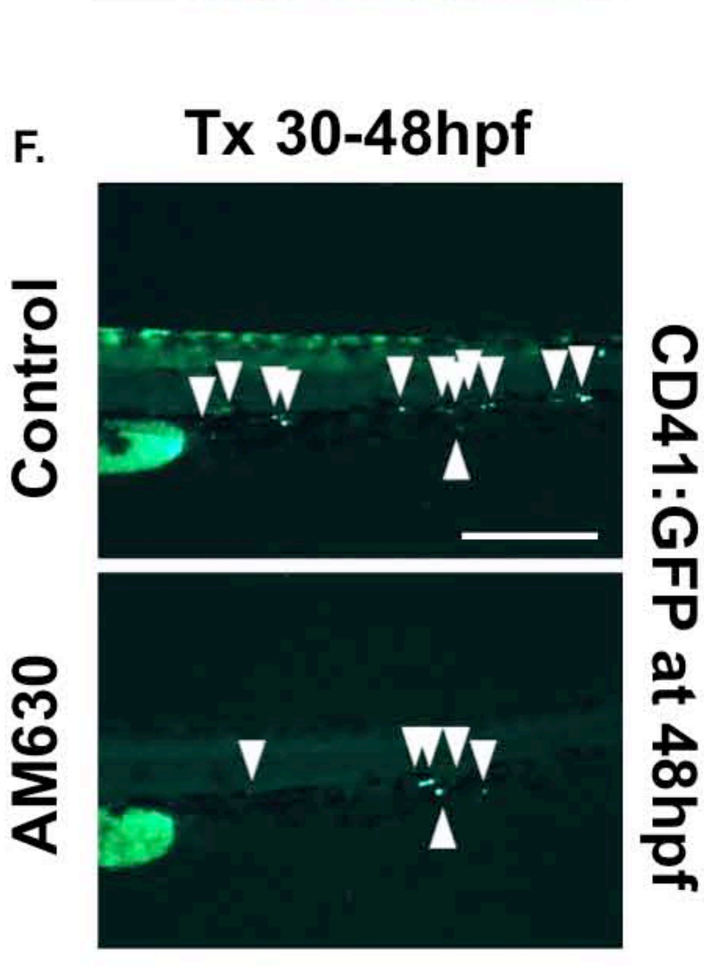


FIGURE S3

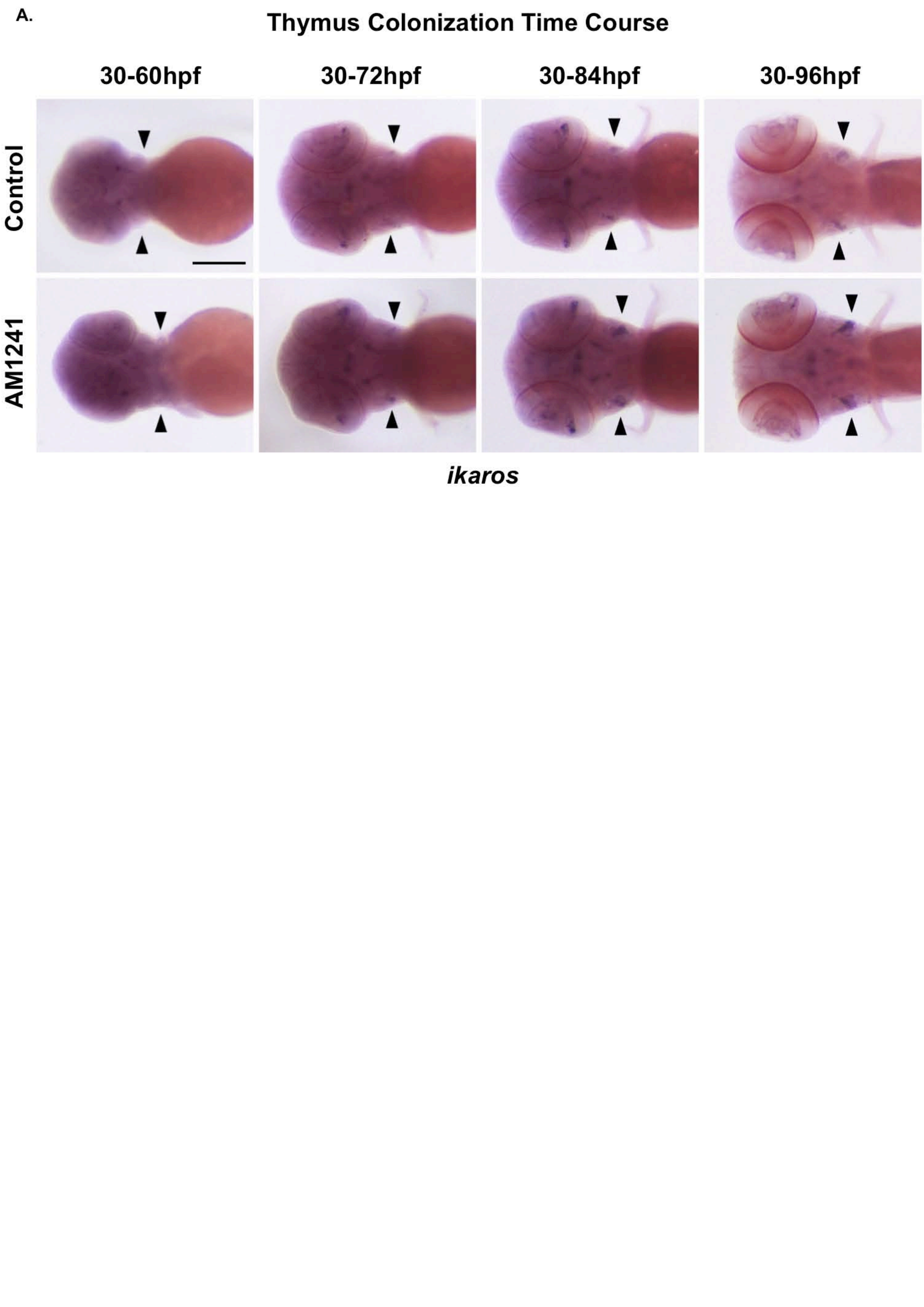
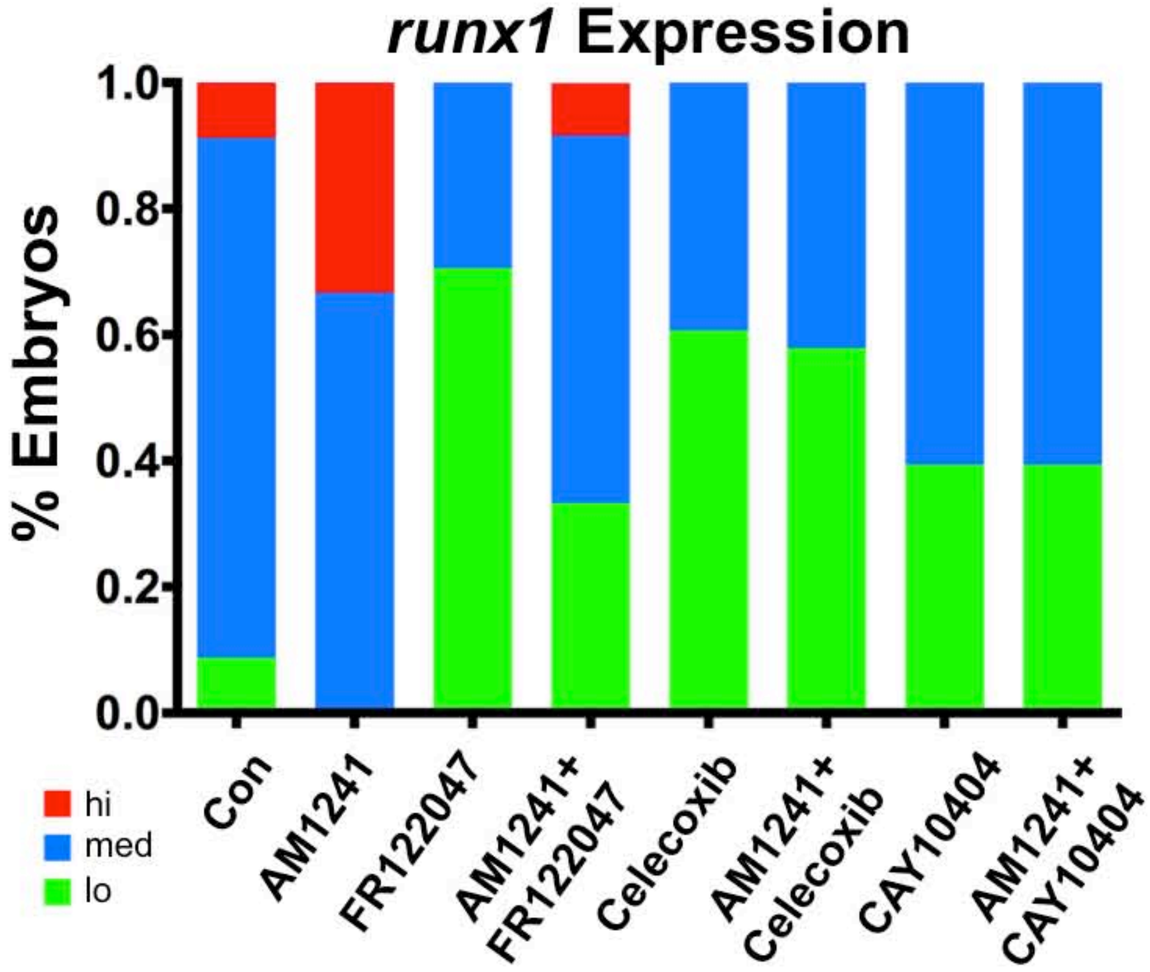
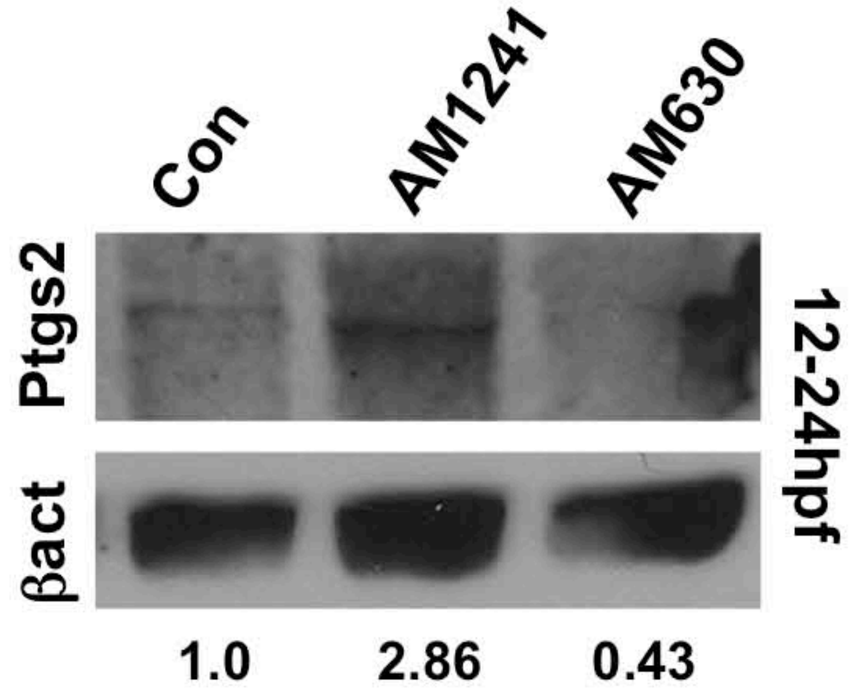

A.
Thymus Colonization Time Course
30-60hpf
30-72hpf
30-84hpf
30-96hpf
Control
AM1241
ikaros

FIGURE S4

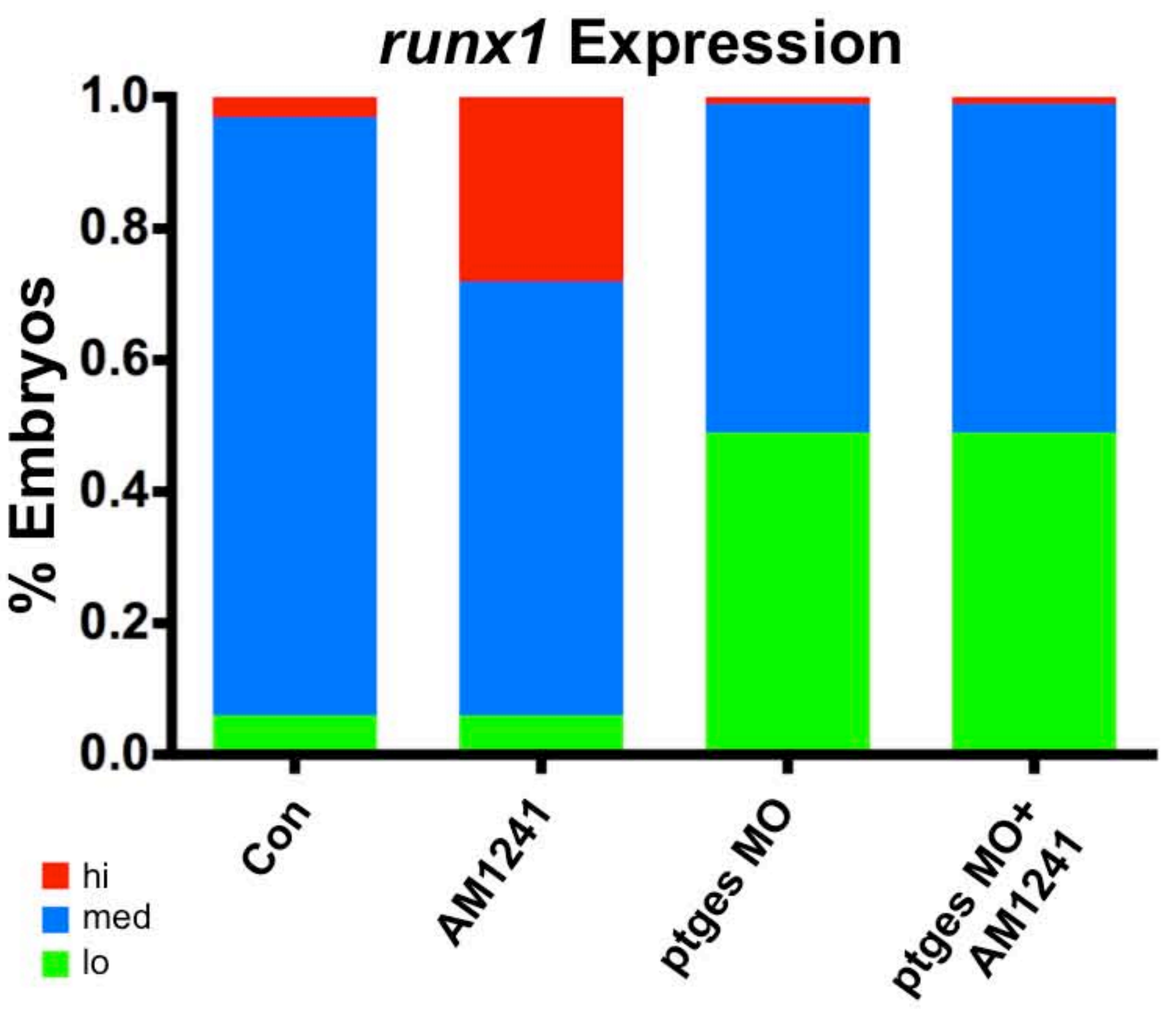
A.



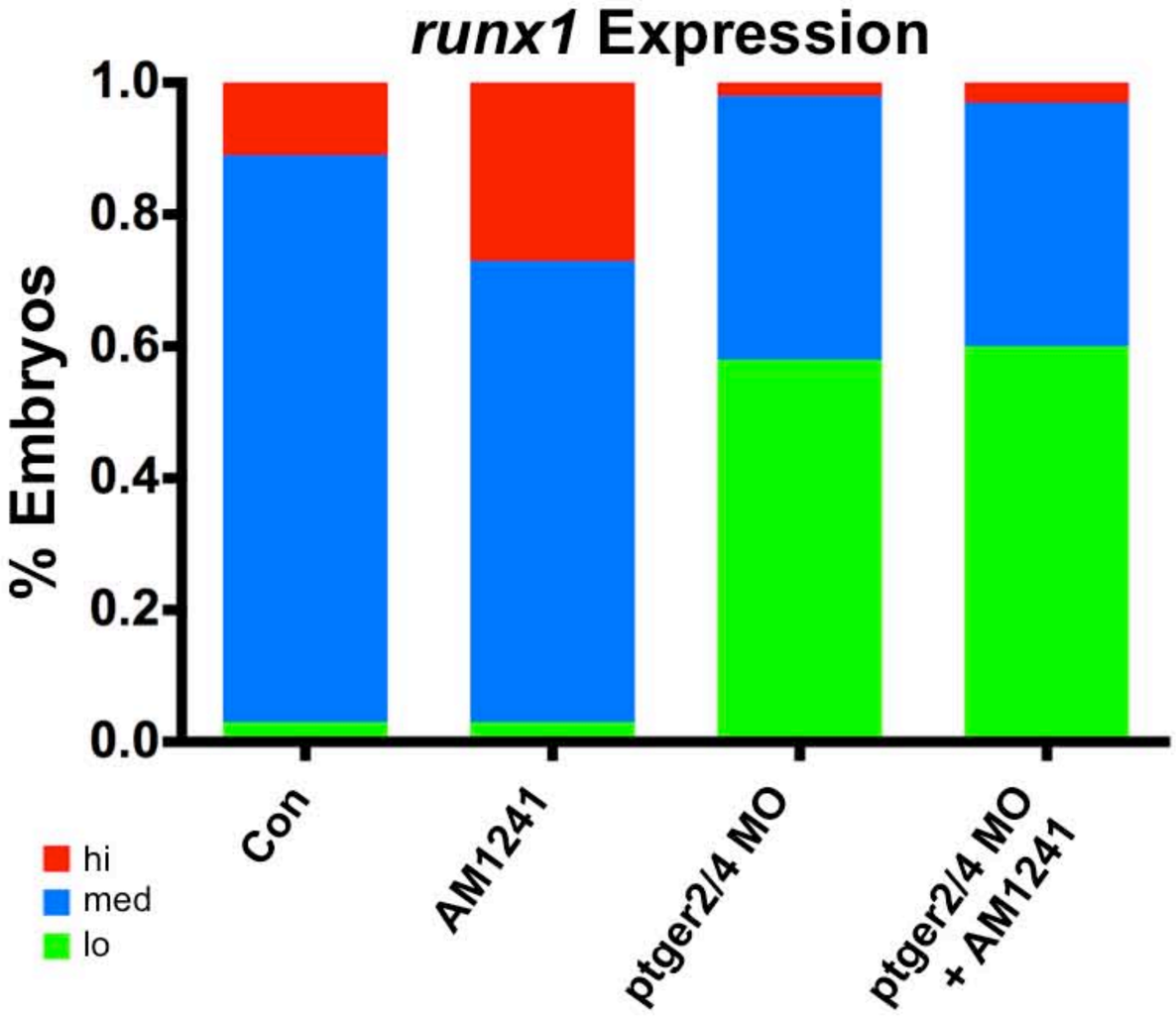
B.



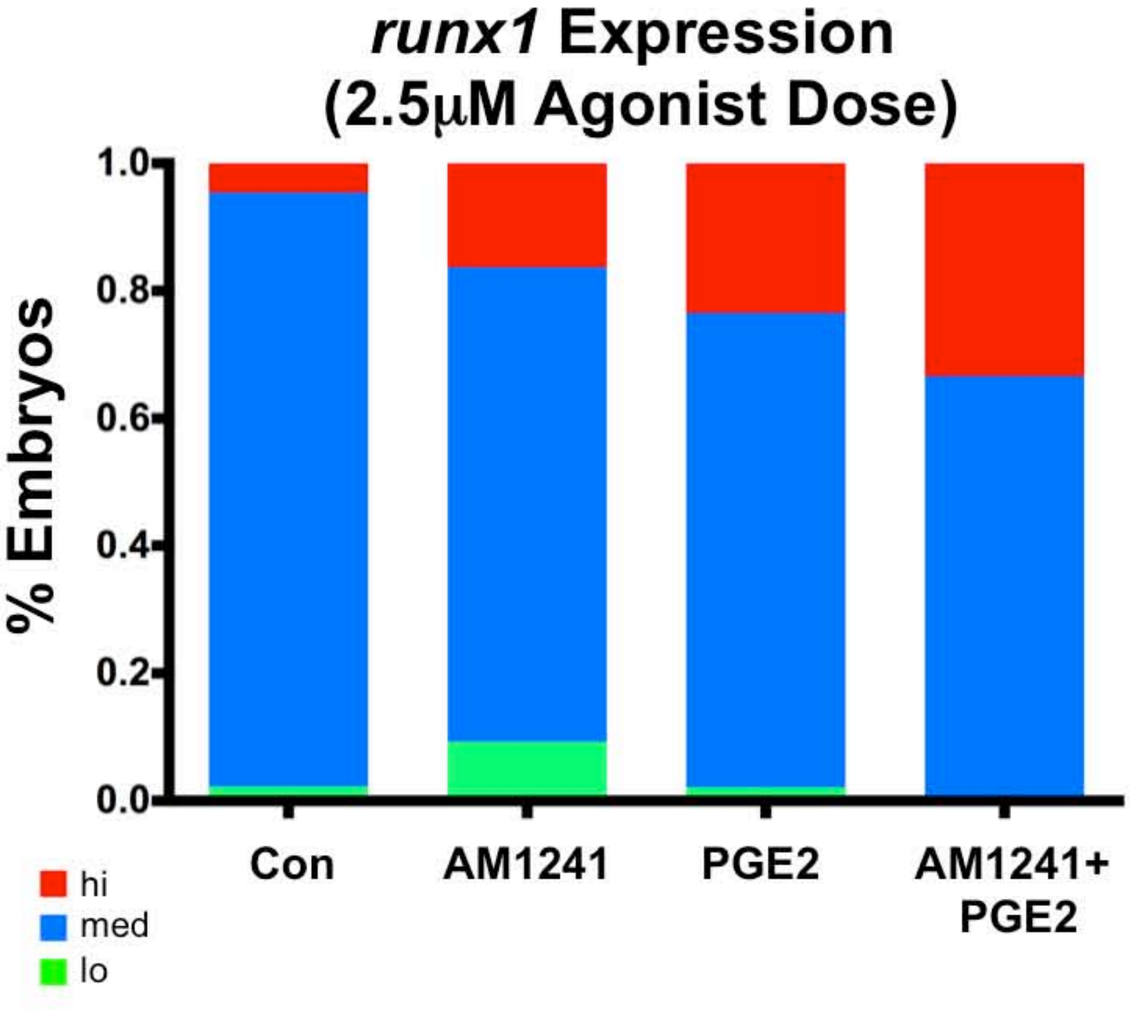
C.



D.



E.



F.

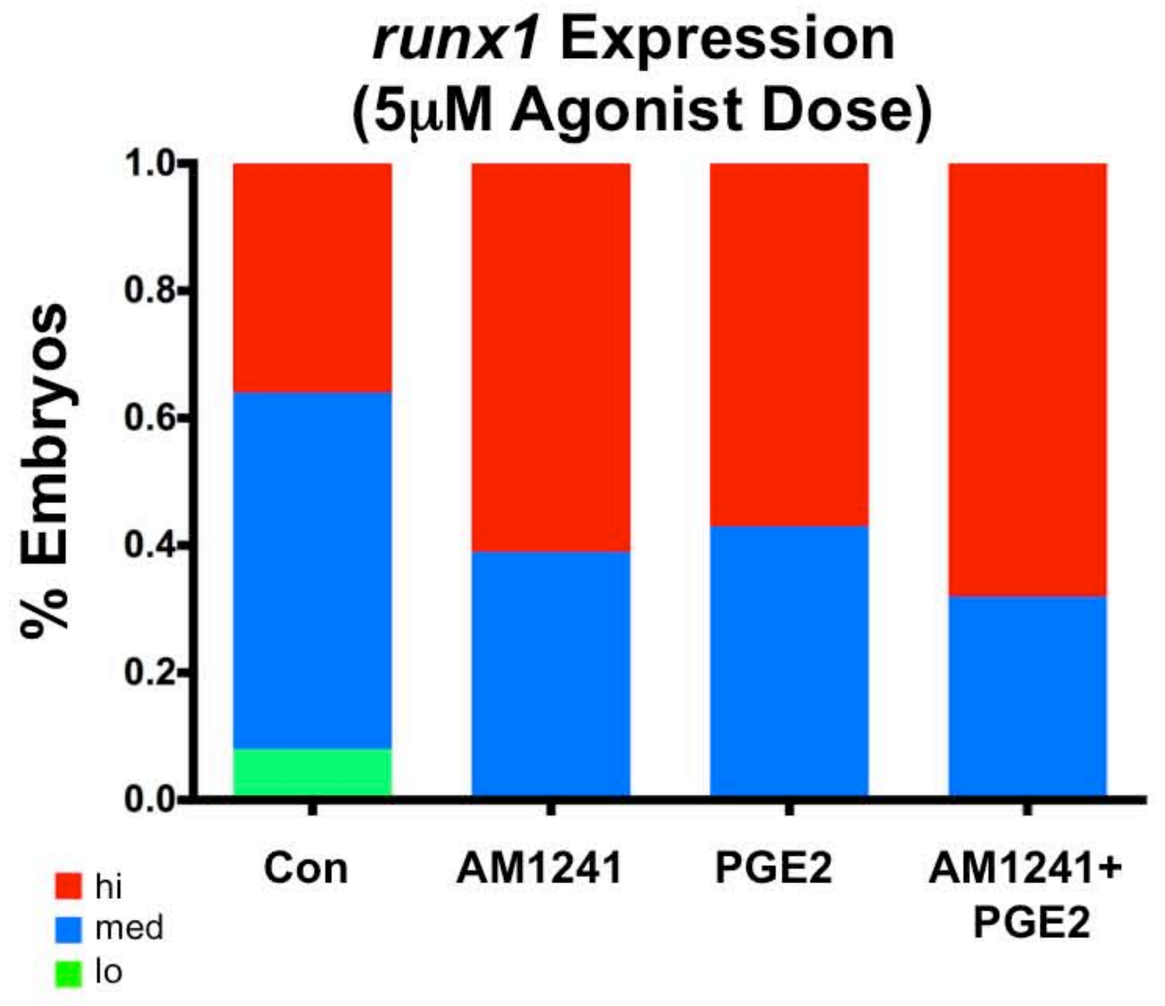
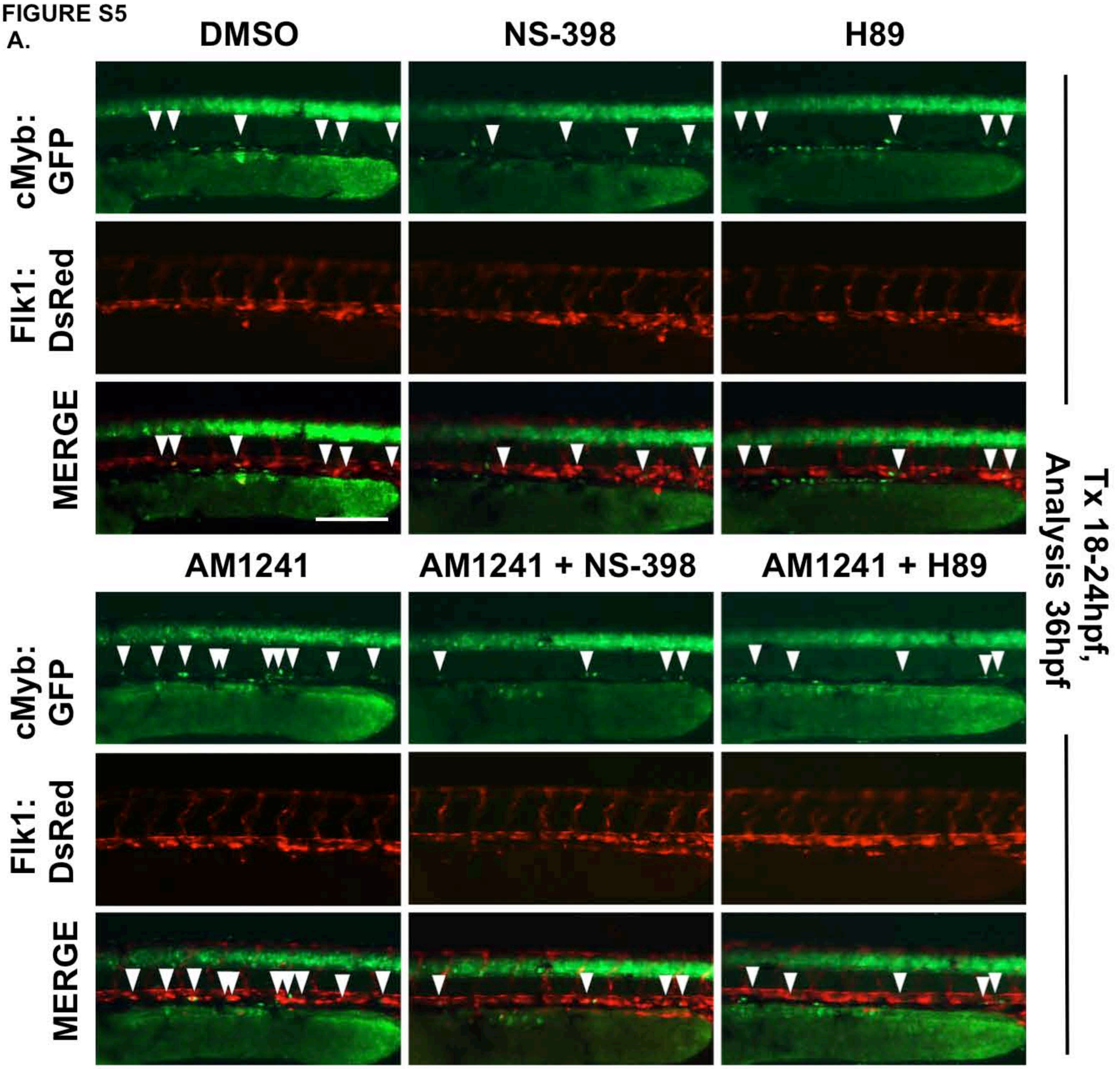
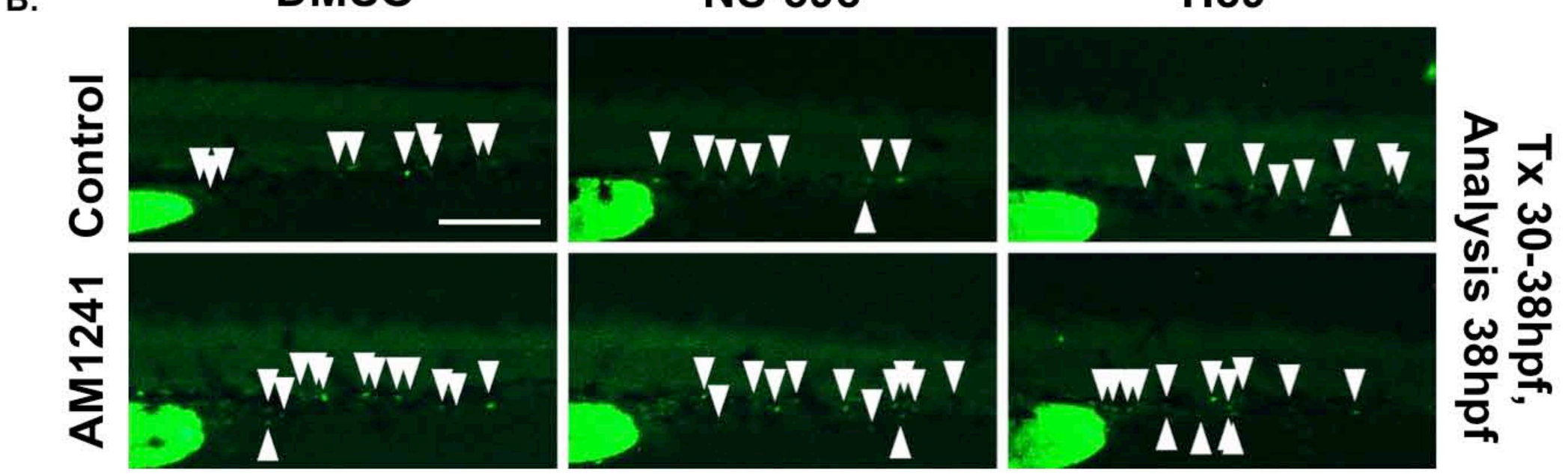


FIGURE S5

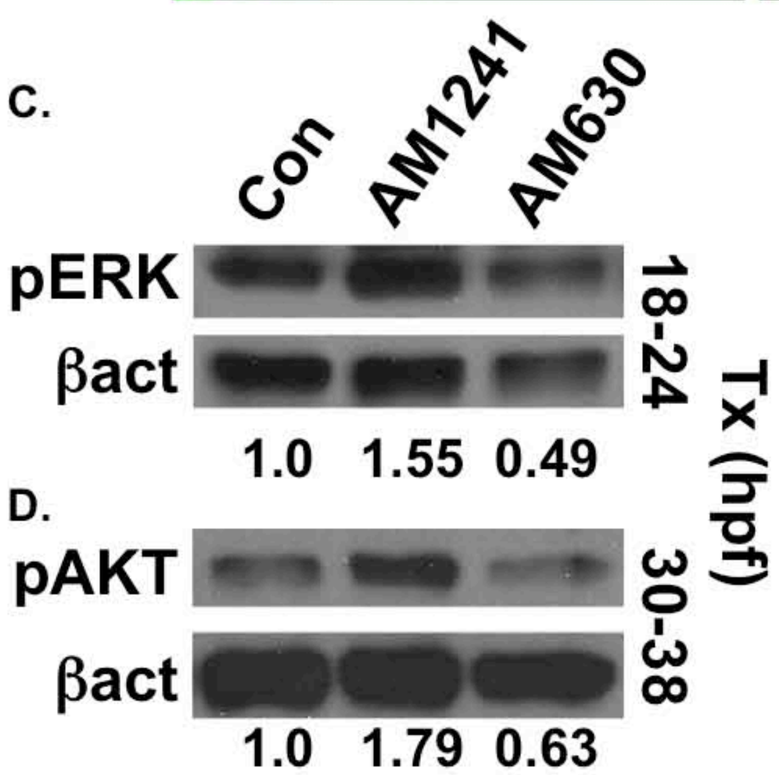
A.



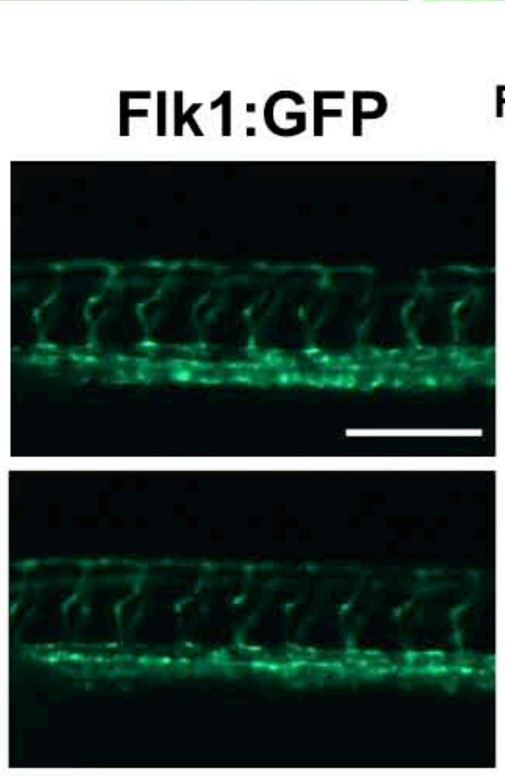
B.



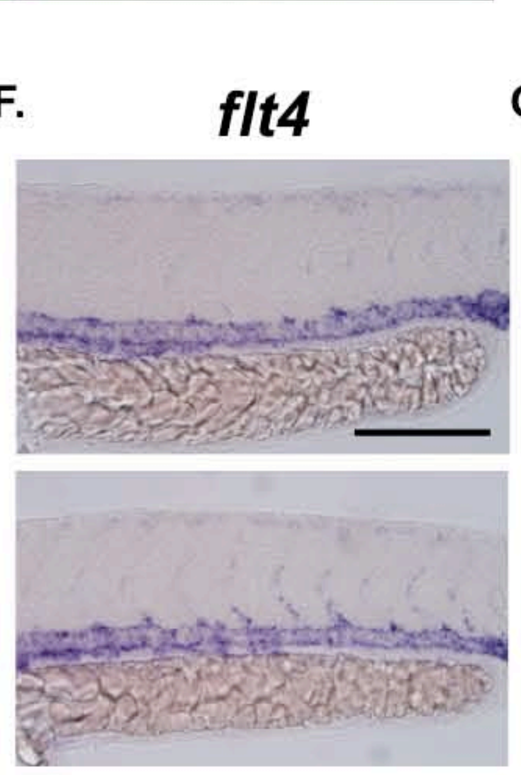
C.



E.



F.



G.

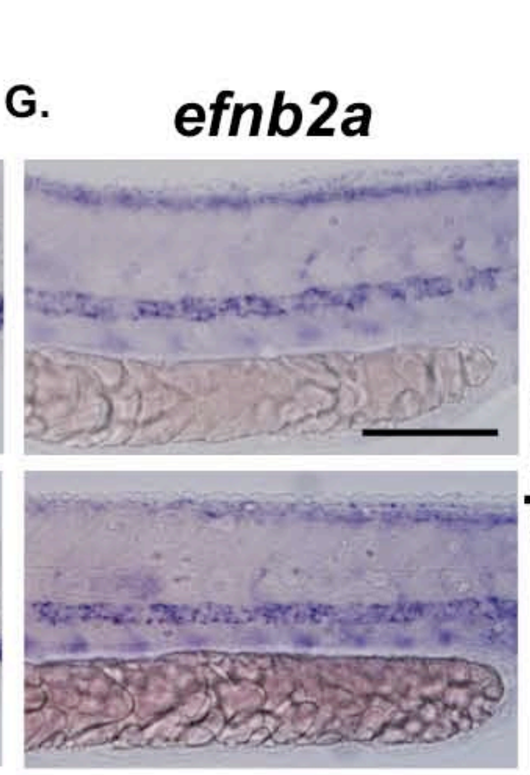
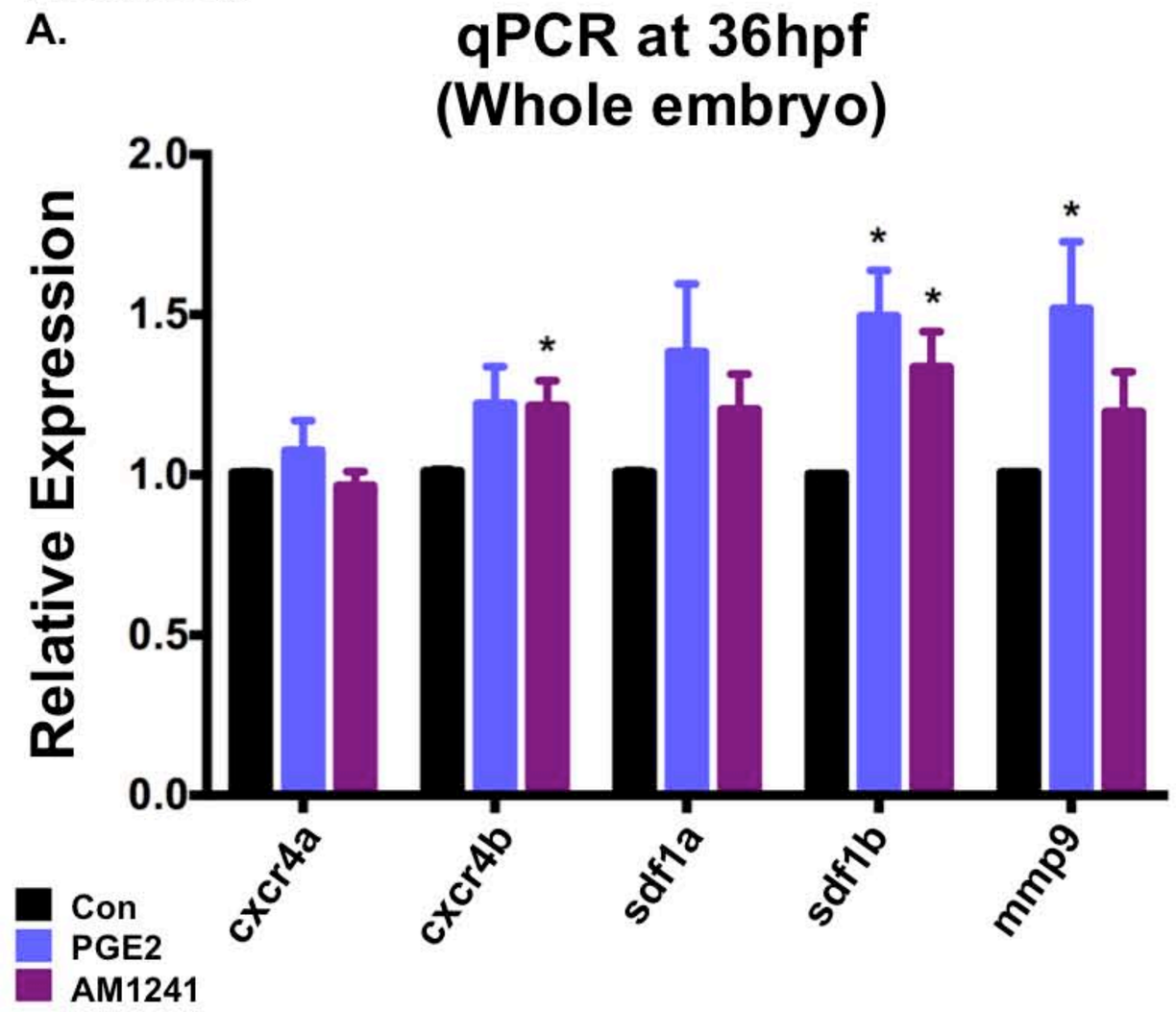
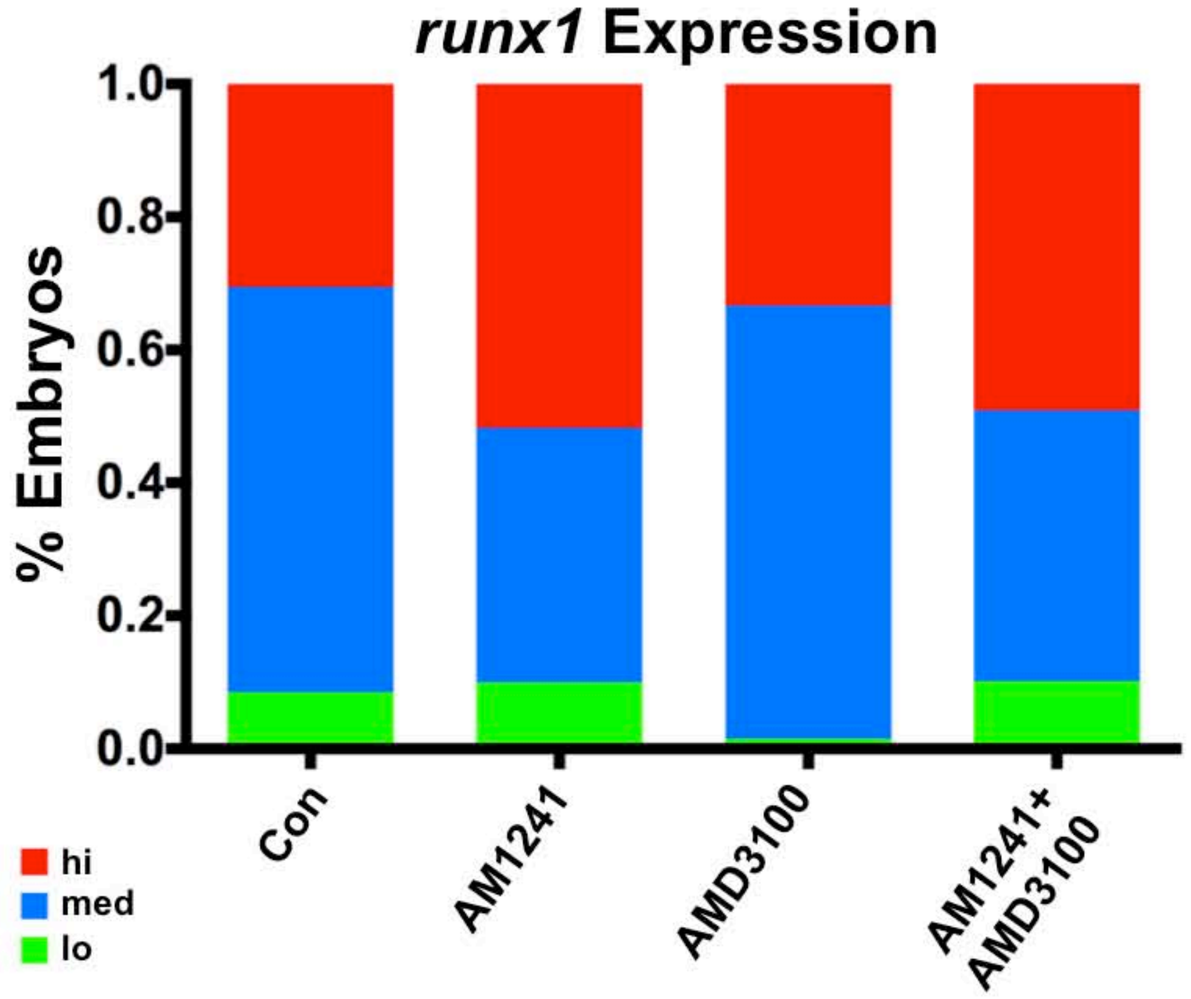


FIGURE S6

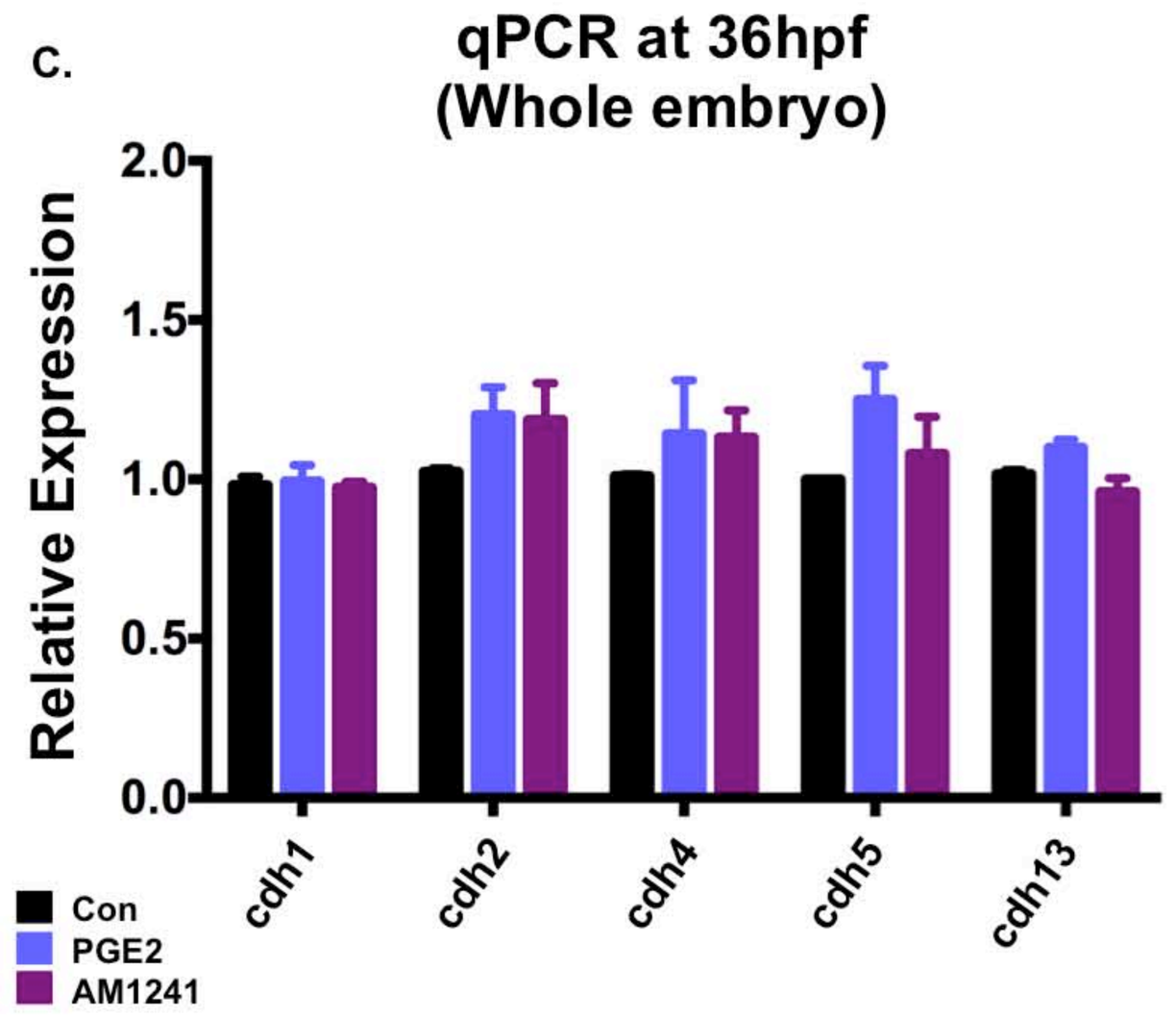
A.



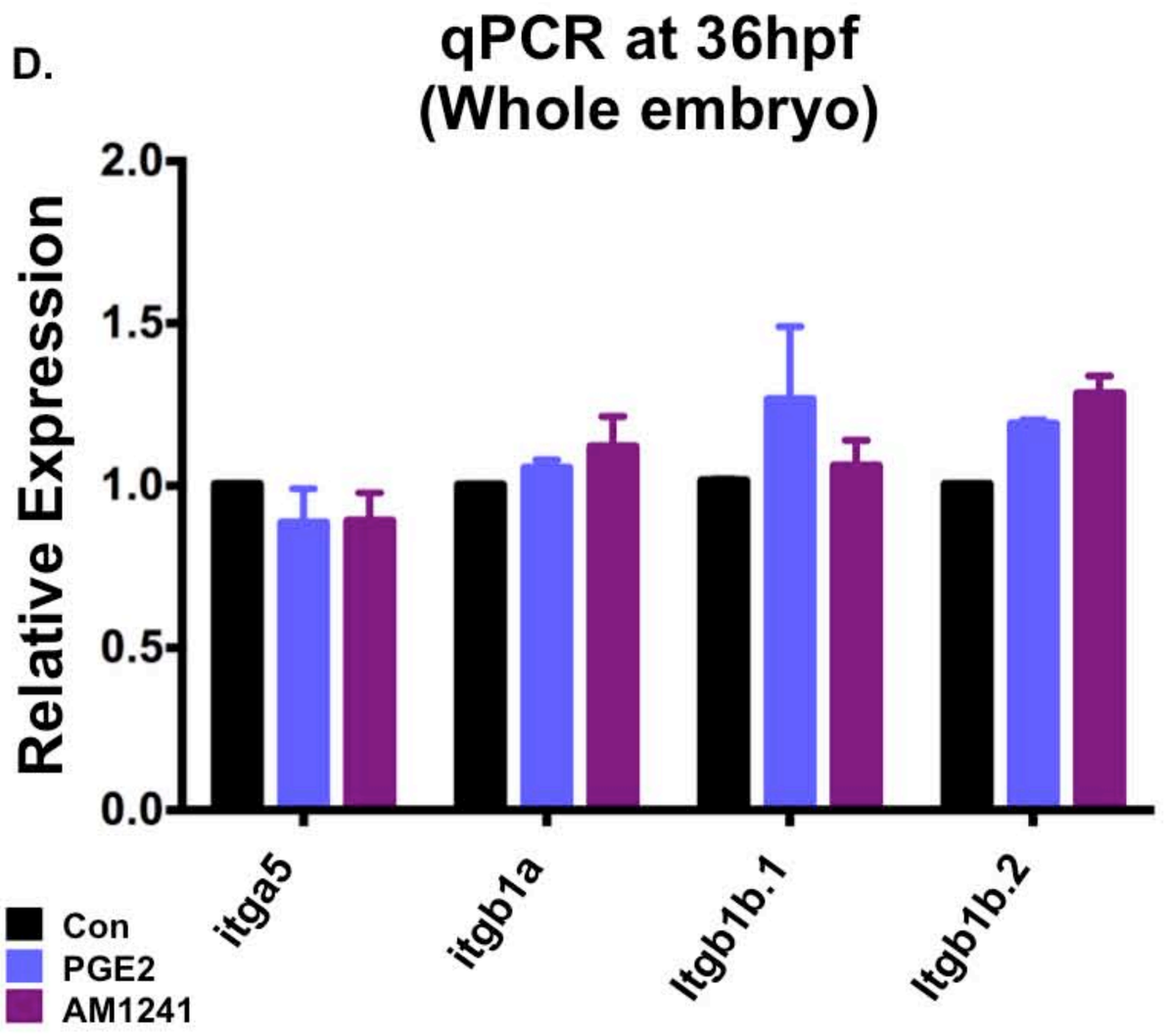
B.



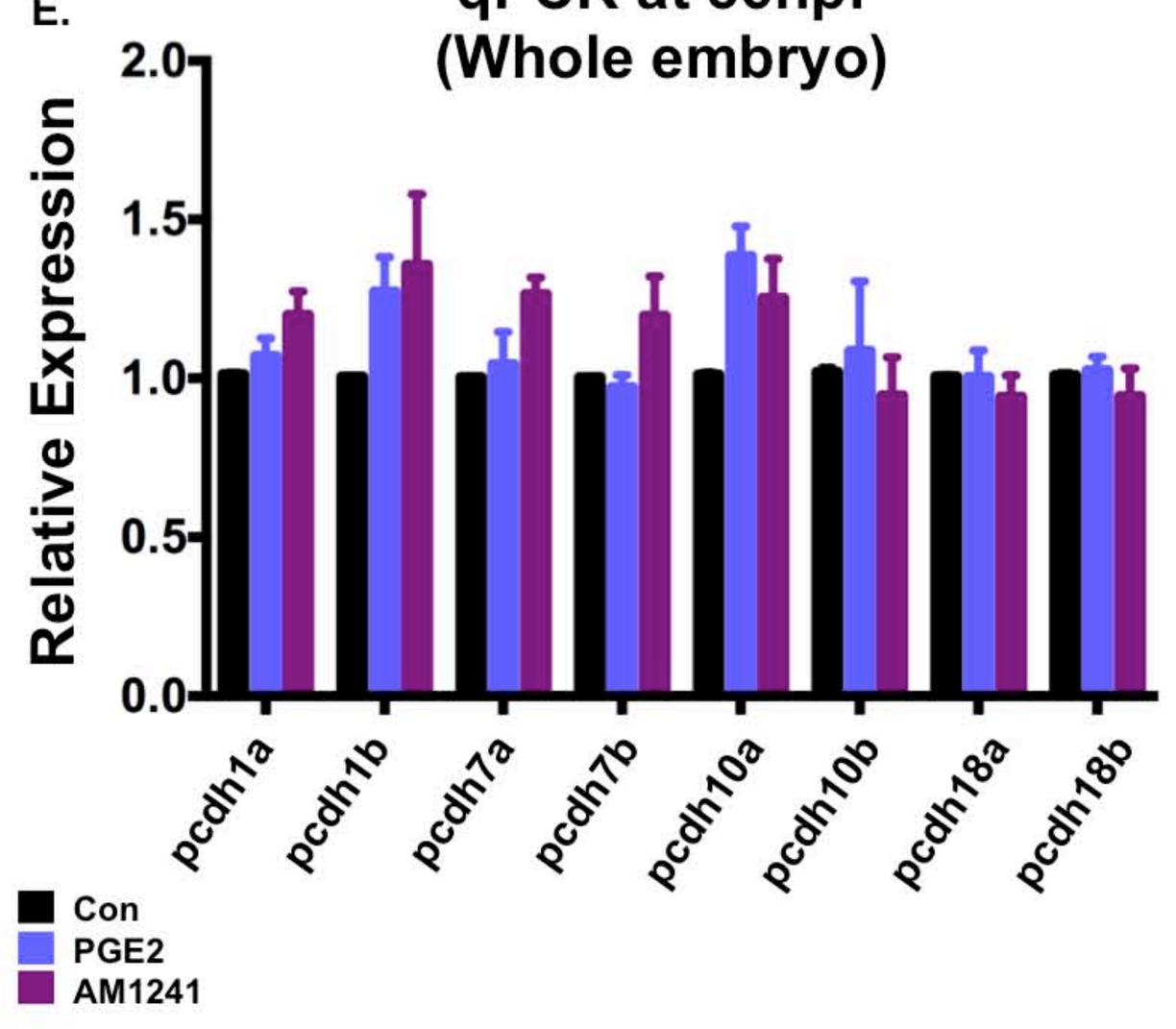
C.



D.



E.



F.

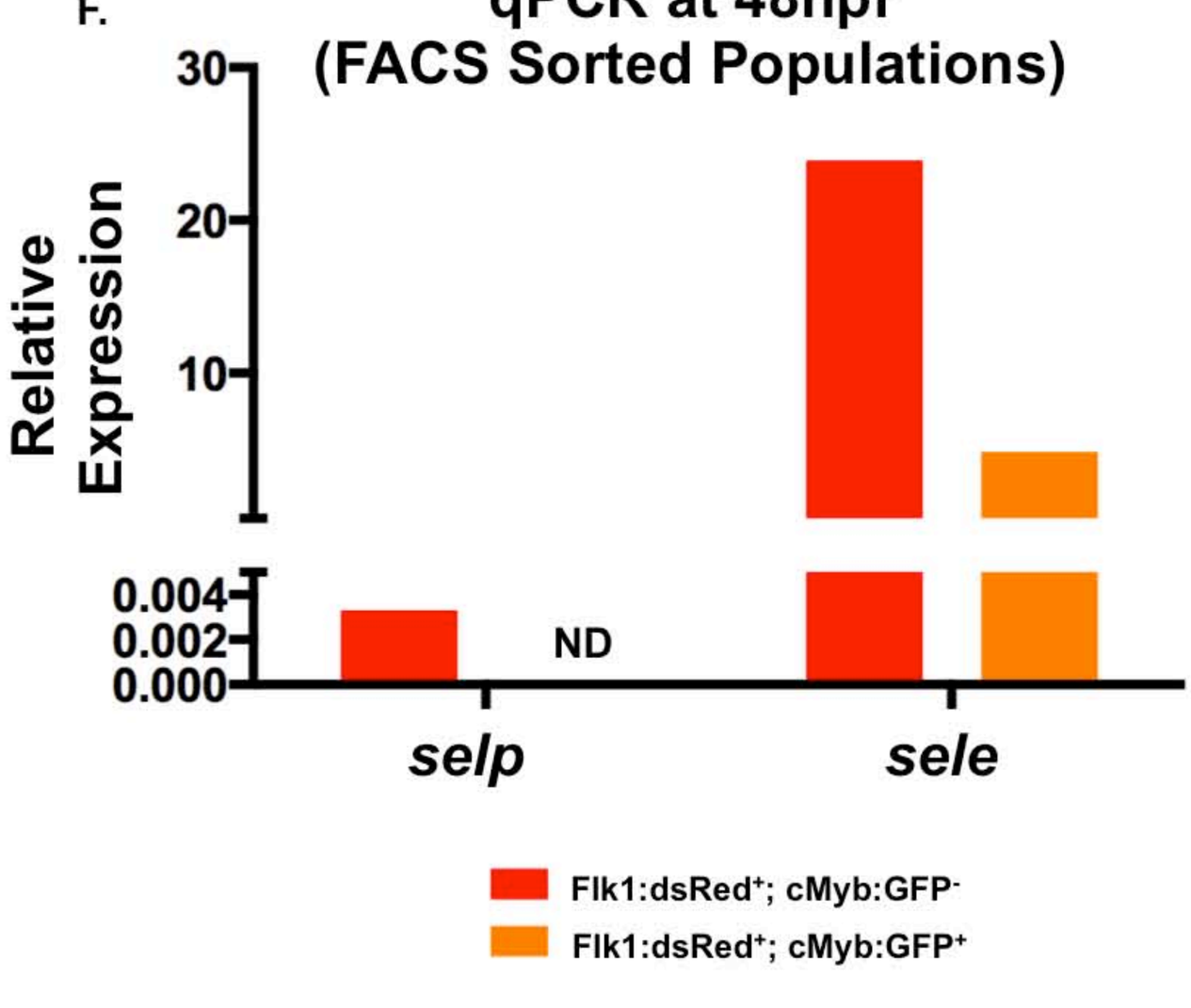
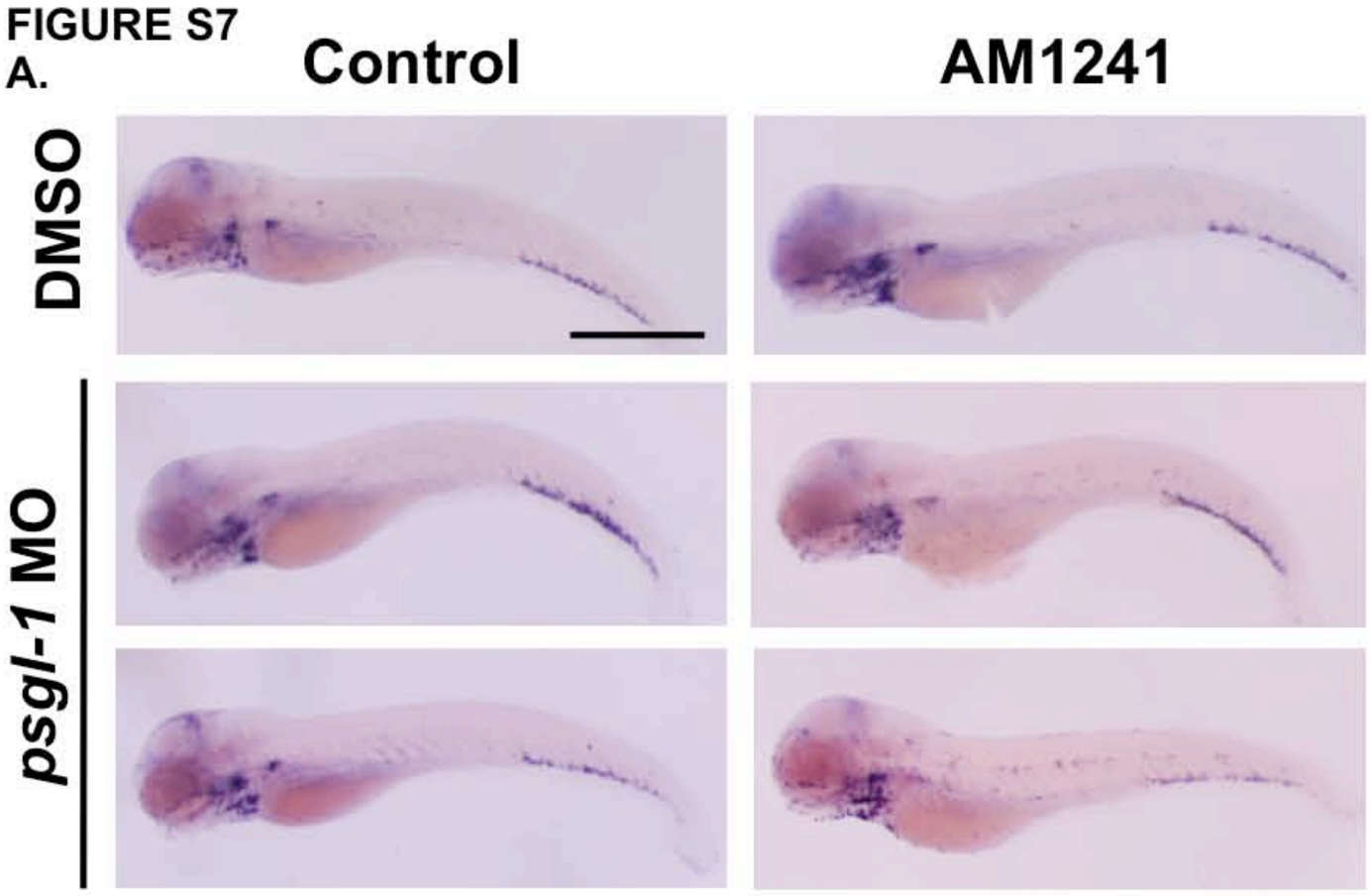


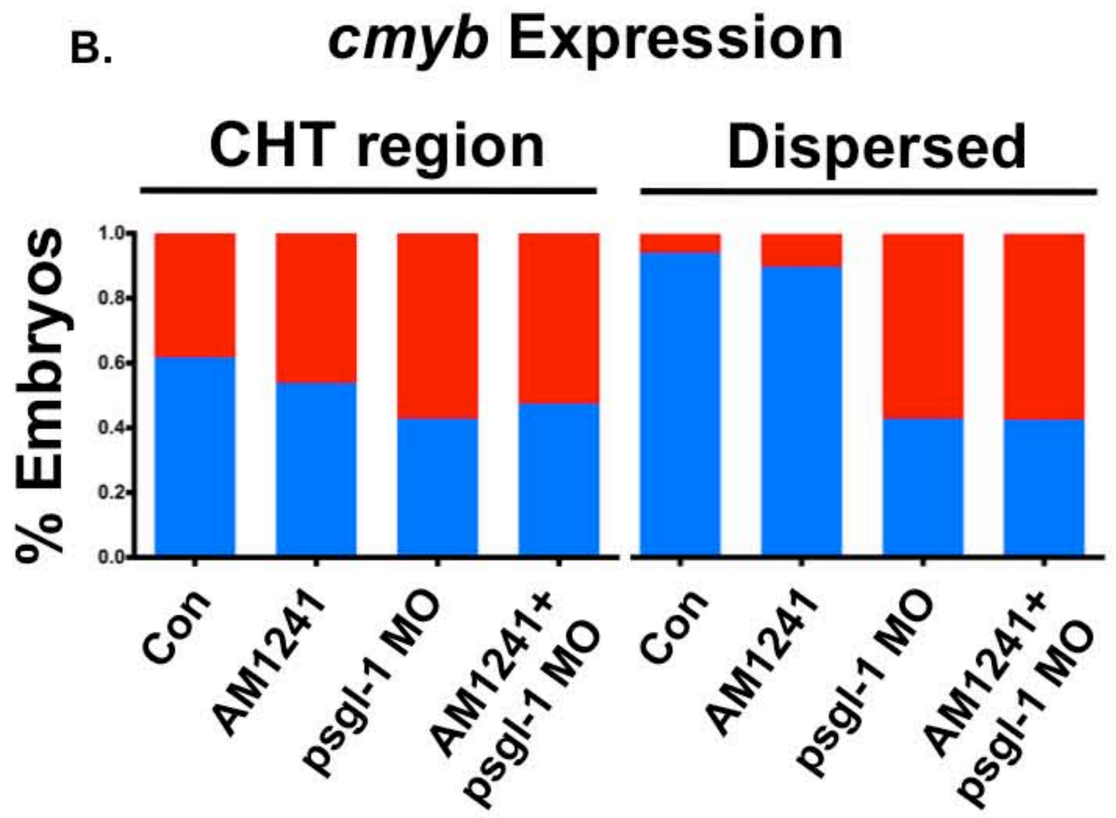
FIGURE S7

A.

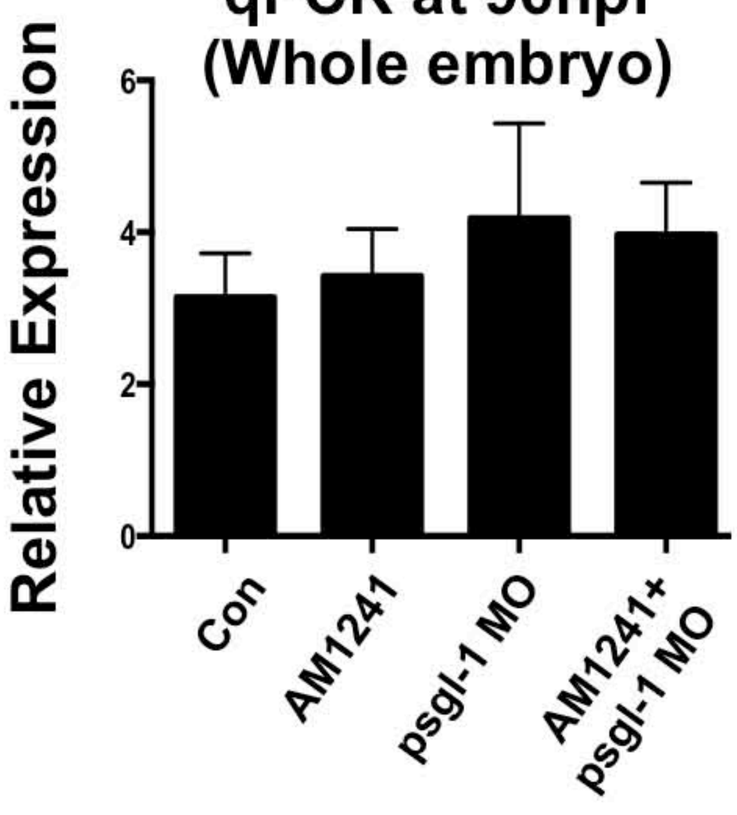


cmyb at 4dpf

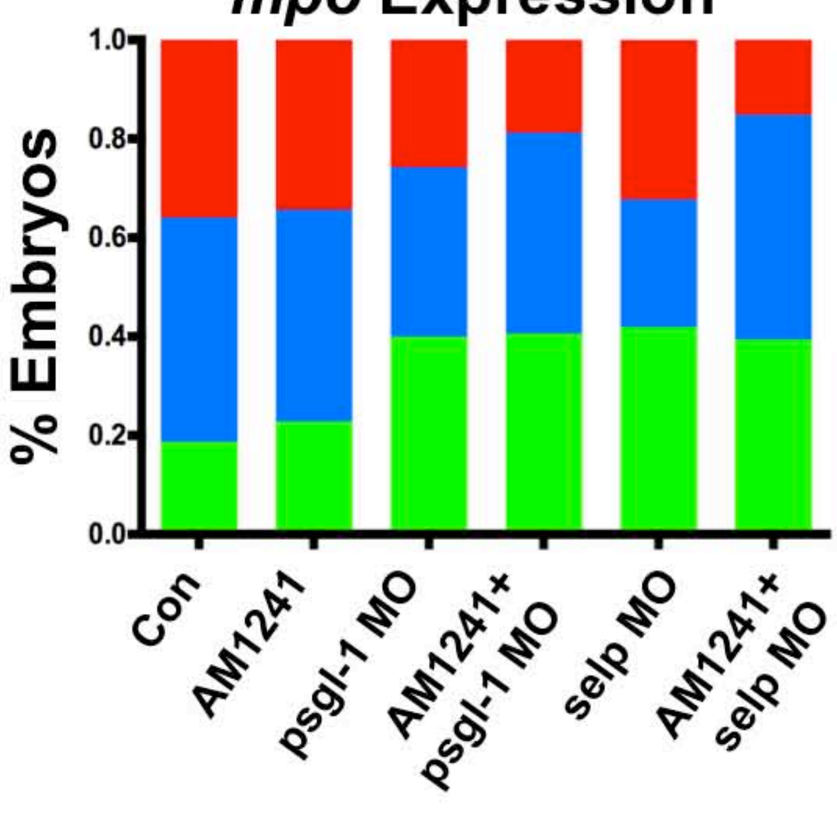
B.



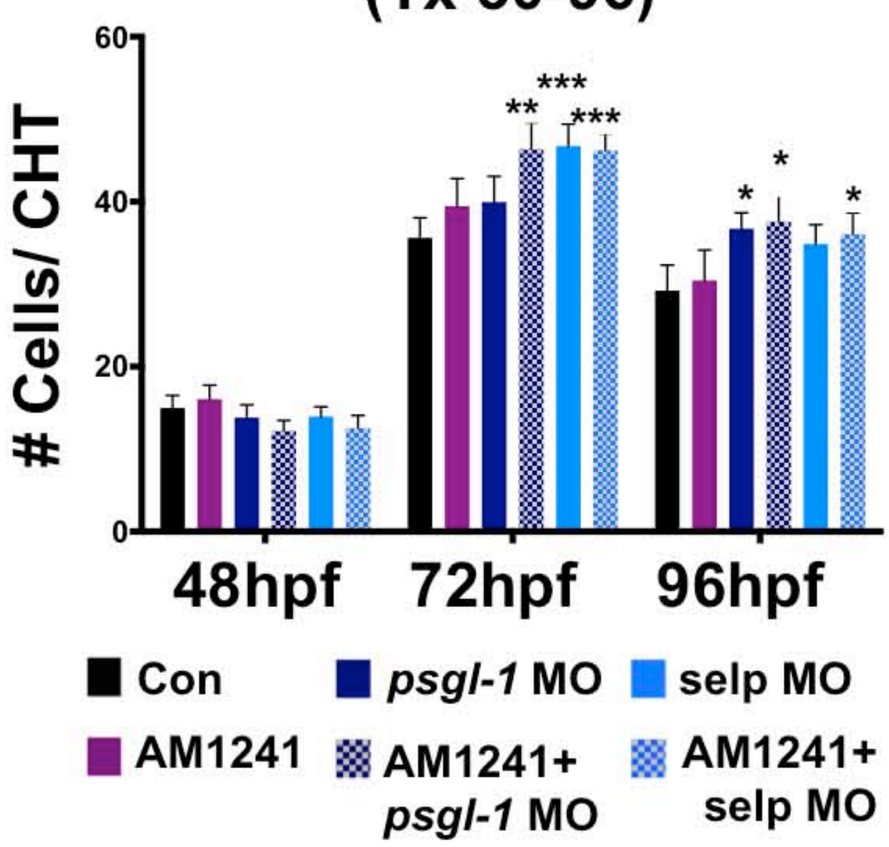
C.



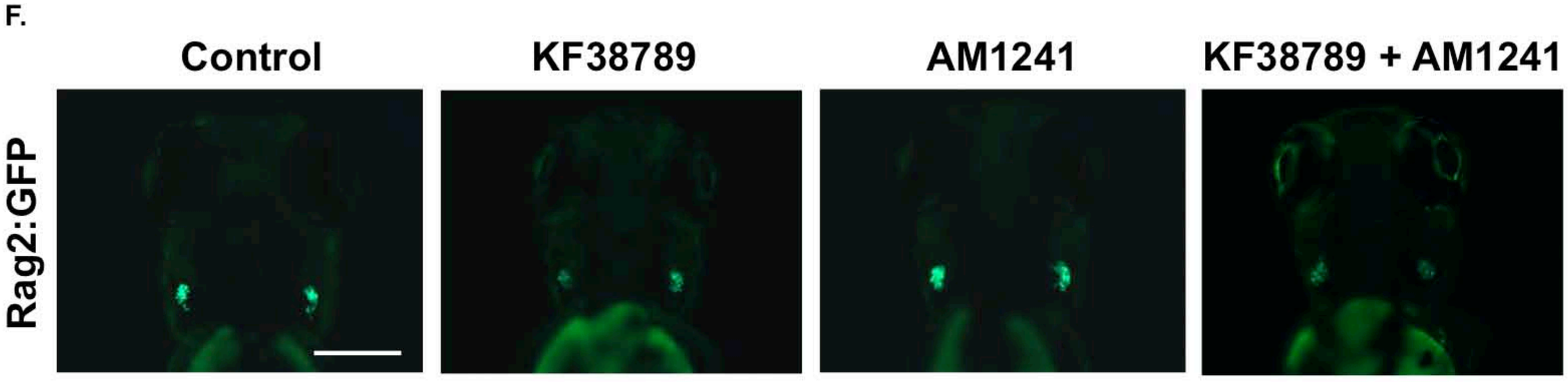
D.



E

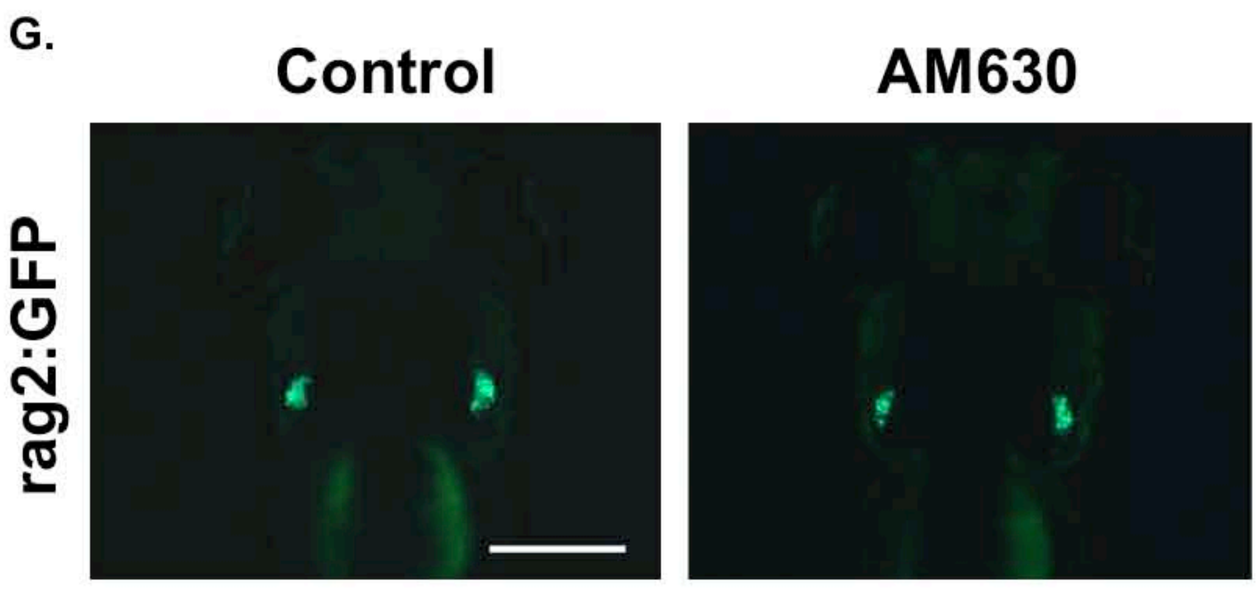


F.



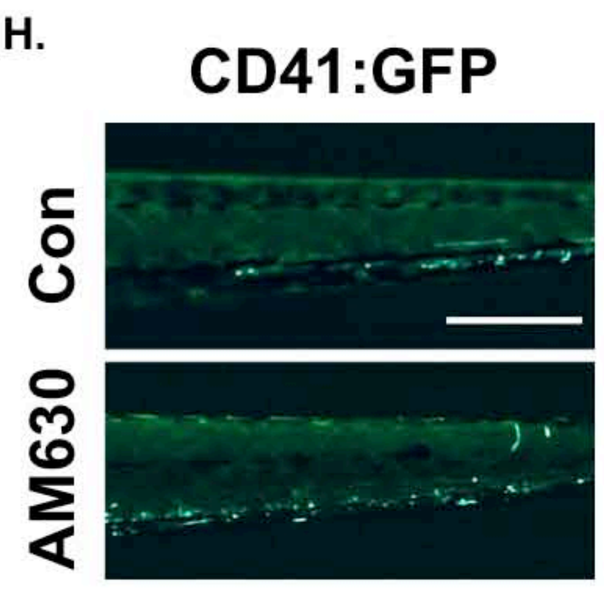
4dpf

G.



4dpf

H.



4dpf

I.

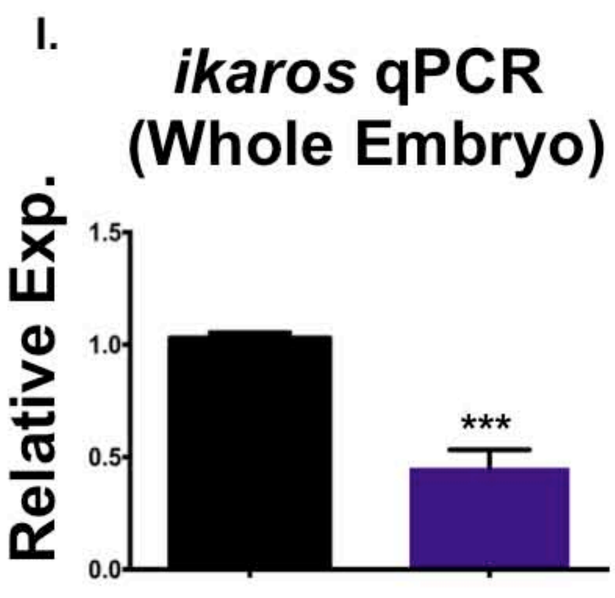
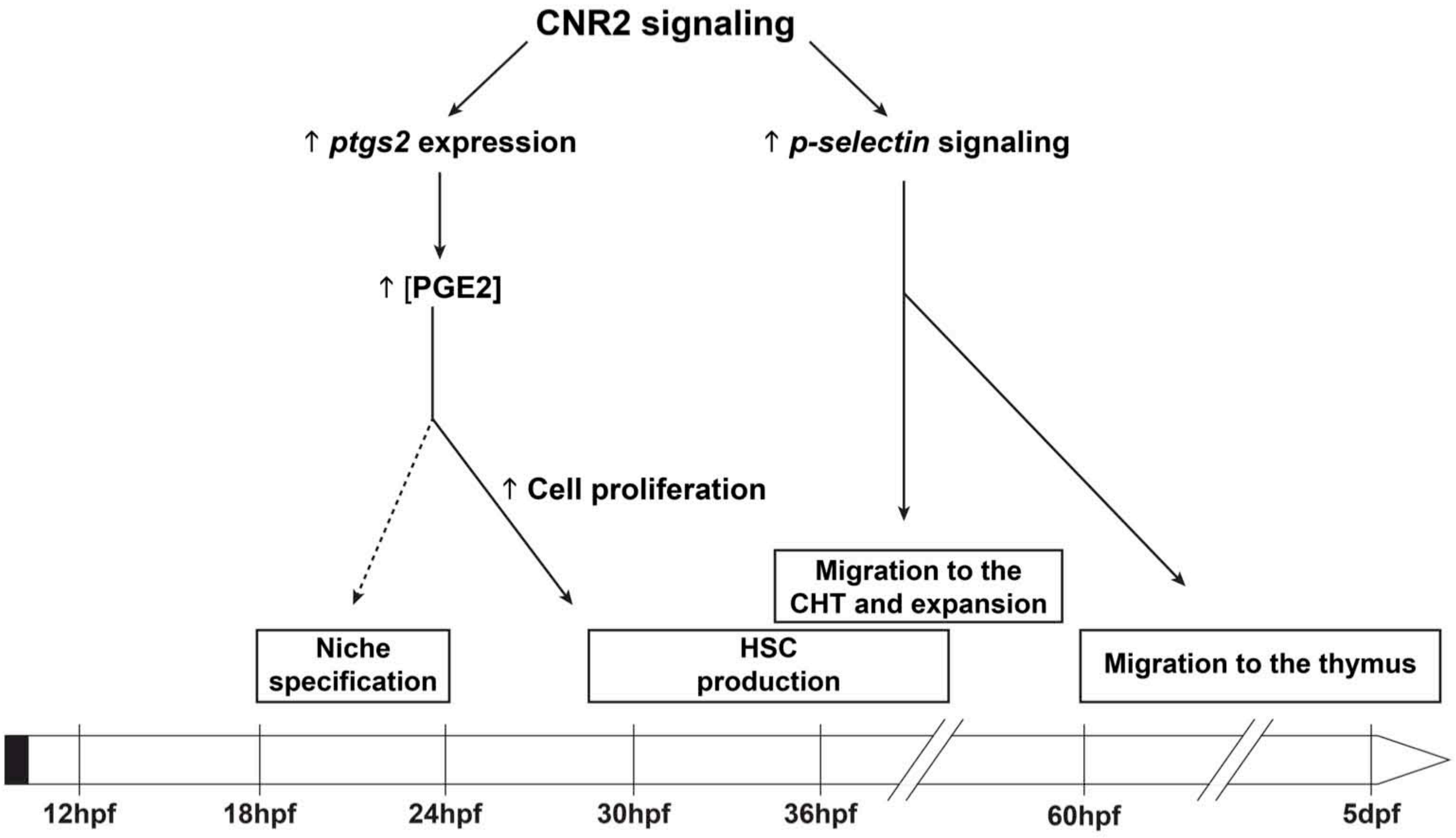


FIGURE S8

A.


CNR2 signaling
↑ ptgs2 expression
↑ p-selectin signaling
↑ [PGE2]
↑ Cell proliferation
Migration to the CHT and expansion
Niche specification
HSC production
Migration to the thymus
12hpf
18hpf
24hpf
30hpf
36hpf
60hpf
5dpf