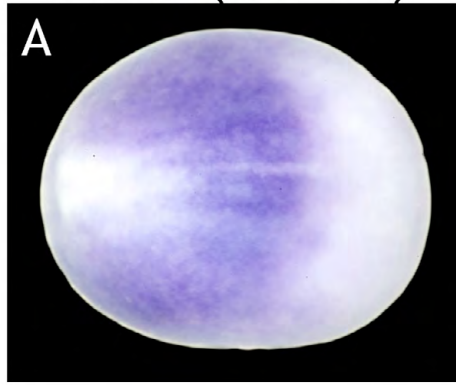
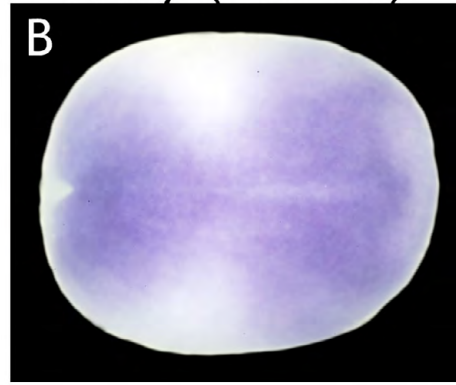


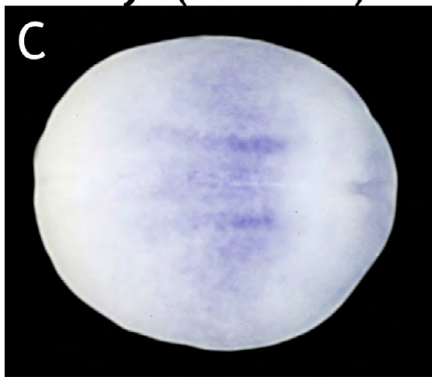
Rar α (St. 14)



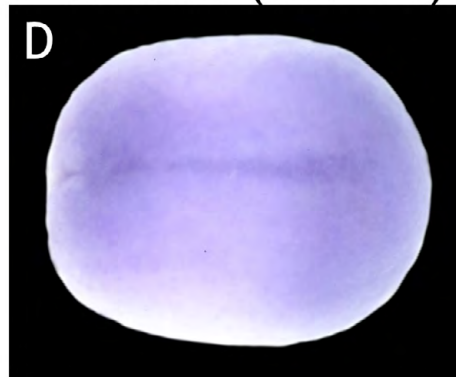
Rar γ (St. 14)



Erf (St. 14)



Etv3/3l (St. 14)



Ddx20 (St. 14)

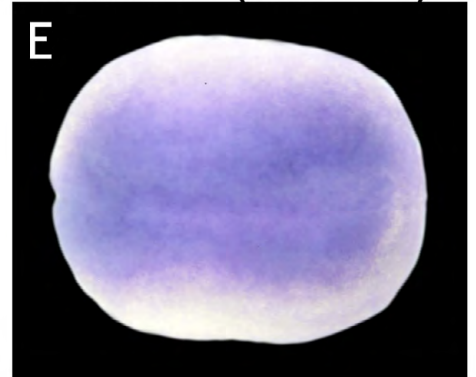


Fig. S1. Qualitative expression of *Rara*, *Rar* γ , *Erf*, *Etv3/3l* and *Ddx20* at stage 14. (A-E) Whole-mount *in situ* hybridization of *Rara* (A), *Rar* γ (B), *Erf* (C), *Etv3/3l* (D) and *Ddx20* (E) gene expression at Nieuwkoop and Faber stage 14. Dorsal views are shown with anterior towards the right.

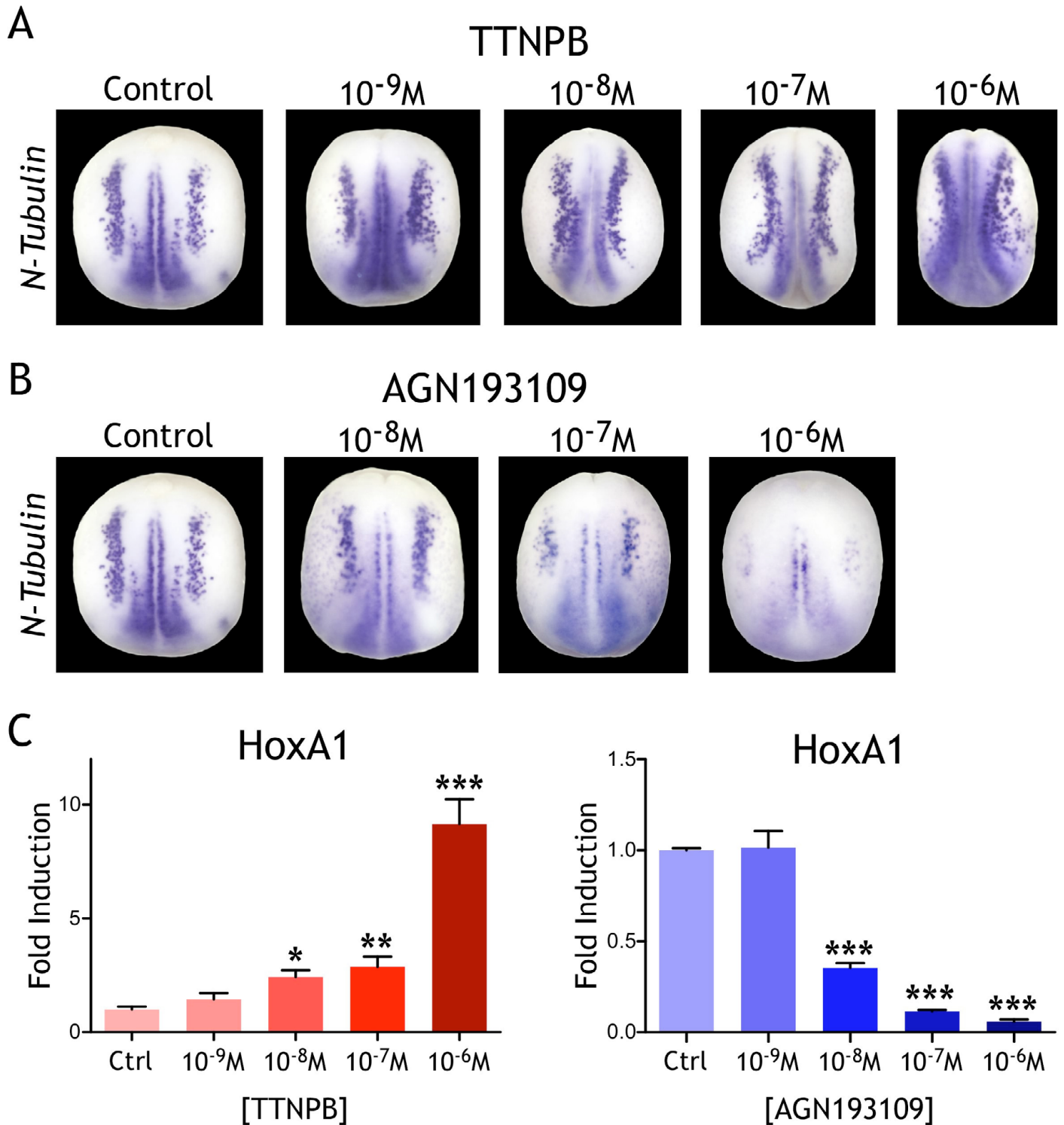


Fig. S2. Dose response of TTNPB and AGN193109. (A,B) Whole-mount *in situ* hybridization from whole embryos treated at stage 7/8 with 1 nM to 1 μ M TTNPB, 10 nM to 1 μ M AGN193109 or control vehicle (0.1% ethanol). Dorsal *N-tubulin* expression is increased with TTNPB treatment at doses as low as 10^{-9} M, compared with control. AGN193109 causes loss of *N-tubulin* expression at doses as low as 10^{-7} M. (C) QPCR showing *HoxA1* expression in embryos treated at stage 7/8 with 1 nM to 1 μ M TTNPB, 1 nM to 1 μ M AGN193109 or vehicle (0.1% ethanol). The y-axis represents $2^{-\Delta\Delta C_t}$ values normalized to *Histone H4* and expressed as fold induction relative to control. *HoxA1* is induced by TTNPB at doses as low as 10^{-9} M and repressed by AGN193109 at doses as low as 10^{-8} M. Asterisks represent statistical significance compared with control (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

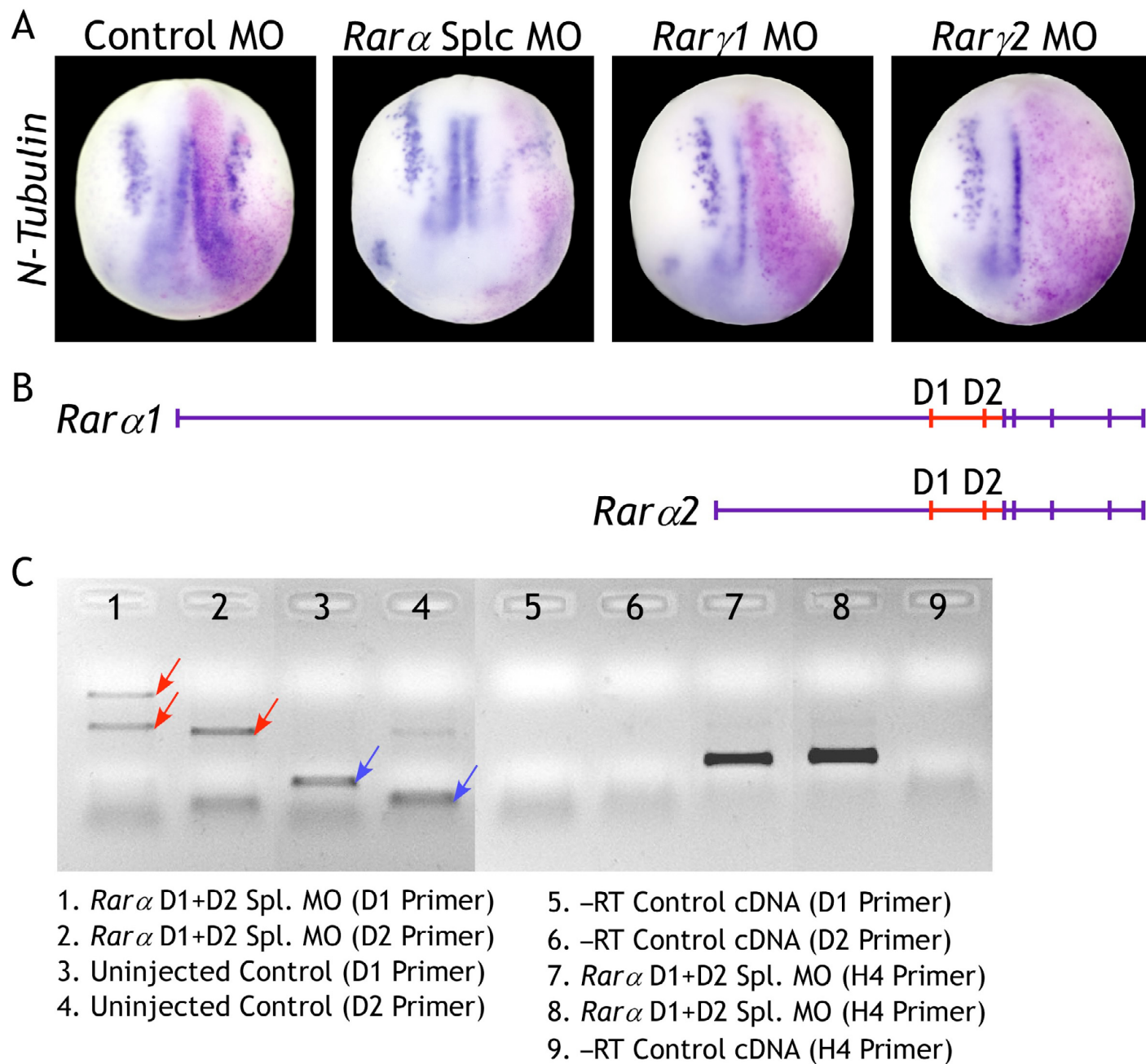


Fig. S3. Specificity of *Rara* and *Rary* MO phenotypes. All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. For *Rara*, we employed two additional splice MOs that target two exon-intron splice donor boundaries of *Rara* (D1, splice donor 1; D2, splice donor 2). For *Rary*, we used two different translation-inhibiting MOs, one targeting *Rary*1 and the other *Rary*2. (A) Knockdown of *N-tubulin* was observed in embryos injected with 10 ng *Rara* splice MO D1 + 20 ng *Rara* splice MO D2 (14/18 embryos), and in embryos injected with 3.75 ng *Rary*1 MO (17/17) or 3.75 ng *Rary*2 MO (7/11). Embryos are shown in dorsal view with anterior at the bottom, at stage 14. (B) The exon-intron borders targeted by the two *Rara* splice MOs. The splice MOs target both *Rara*1 and *Rara*2. (C) PCR and gel electrophoresis of cDNA from uninjected embryos or embryos bilaterally injected with *Rara* splice MOs. Spliced mRNAs are indicated by blue arrows. Both *Rara* splice MOs (D1 and D2) result in unspliced PCR products (indicated by red arrows), whereas spliced PCR products are diminished in these lanes. RT, minus reverse transcriptase control (cDNA synthesis of pooled RNA without reverse transcriptase enzyme); H4, *Histone H4* (reference gene).

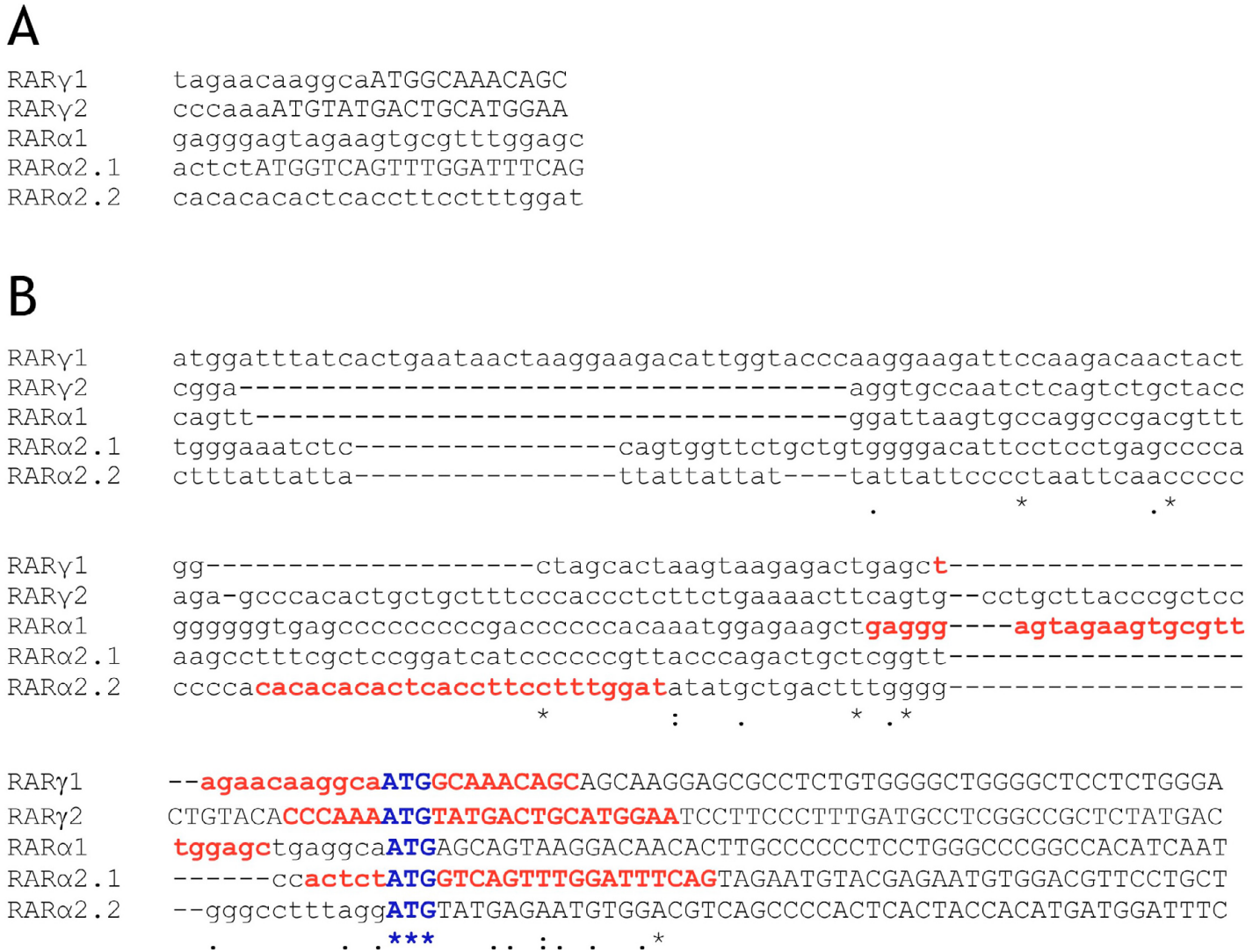


Fig. S4. Uniqueness of *Rar* MO target sequence. (A) Nucleotide sequence targeted by MOs with protein-coding sequence in capitals. (B) MAFFT alignment of relevant regions of the 5'UTR and variable 5' coding of RAR isoforms in *Xenopus laevis*, demonstrating no sequence similarity and that all MOs are specific for the receptor subtype or isoform they are directed against.

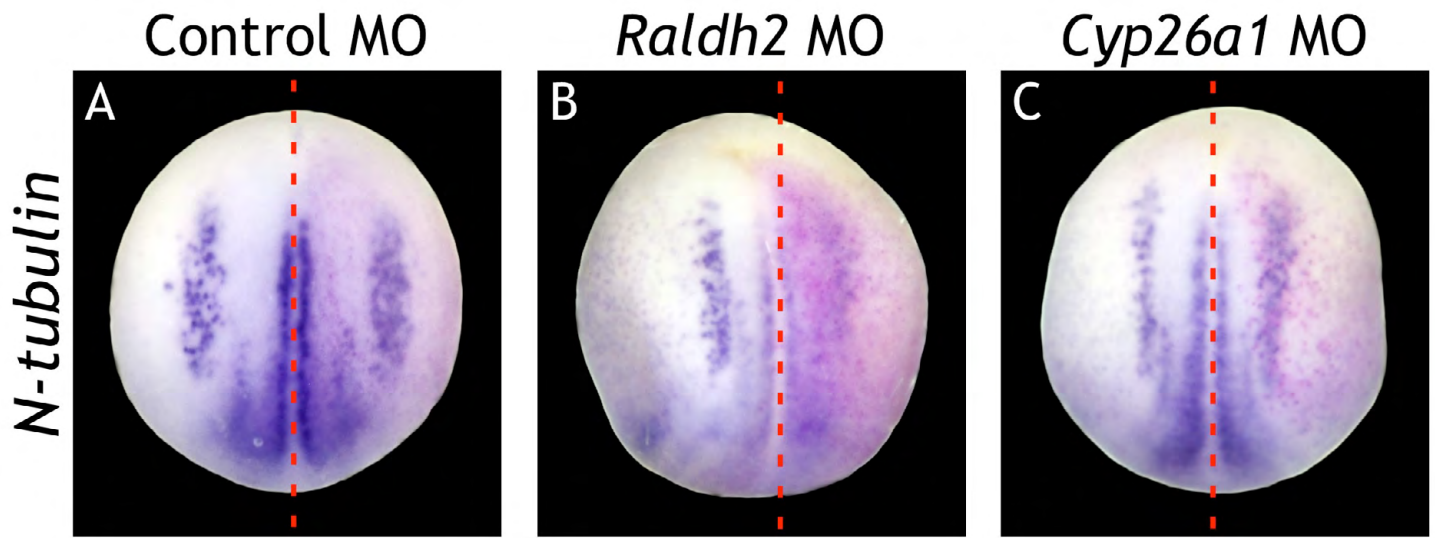


Fig. S5. *N-tubulin* is RA responsive and requires RALDH2 for its expression, whereas knockdown of *Cyp26a1* increases *N-tubulin* expression. All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. **(A)** Control expression of *N-tubulin*. **(B)** 20 ng *Raldh2* MO reduced expression of *N-tubulin* (14/31 embryos), confirming the requirement for RA signaling in primary neurogenesis. **(C)** 20 ng *Cyp26a1* MO increased expression of *N-tubulin* (12/28 embryos) presumably by increasing embryonic RA levels. All embryos are shown in dorsal view with anterior at the bottom, at stage 14.

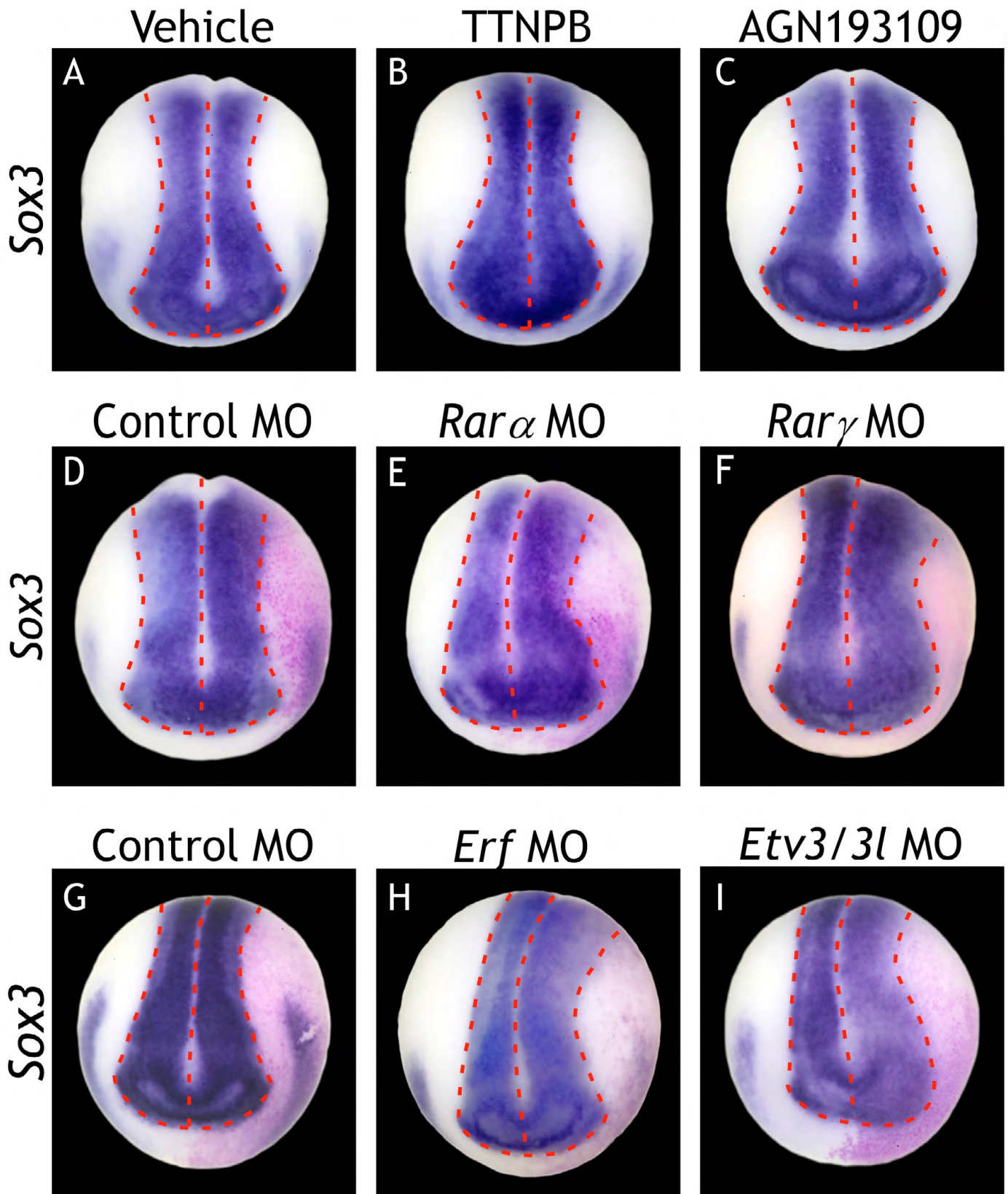


Fig. S6. *Sox3* is modulated by RAR signaling; ERF or ETV3/3L knockdown expand expression of *Sox3*. (A-C) Whole-mount *in situ* hybridization from whole embryos treated at stage 7/8 with 1 μ M TTNPB, 1 μ M AGN193109 or control vehicle (0.1% ethanol). *Sox3* expression is slightly narrowed in the anterior domain with TTNPB treatment (17/19) compared with control. AGN193109 (a RAR-specific antagonist) causes anterior expansion of *Sox3* (15/22). (D-I) All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. (D,G) Control expression of *Sox3*. (E) 3.3 ng *Rara1* MO + 3.3 ng *Rara2.1* MO + 3.3 ng *Rara2.2* MO expanded expression of *Sox3* (13/15 embryos). (F) 3.75 ng *Rary1* MO + 3.75 ng *Rary2* MO expanded expression of *Sox3* (21/22). (H,I) 10 ng Erf MO expanded *Sox3* expression (8/14) and 20 ng *Etv3/3l* MO expanded *Sox3* expression (11/17). All embryos are shown in dorsal view with anterior at the bottom, at stage 14.

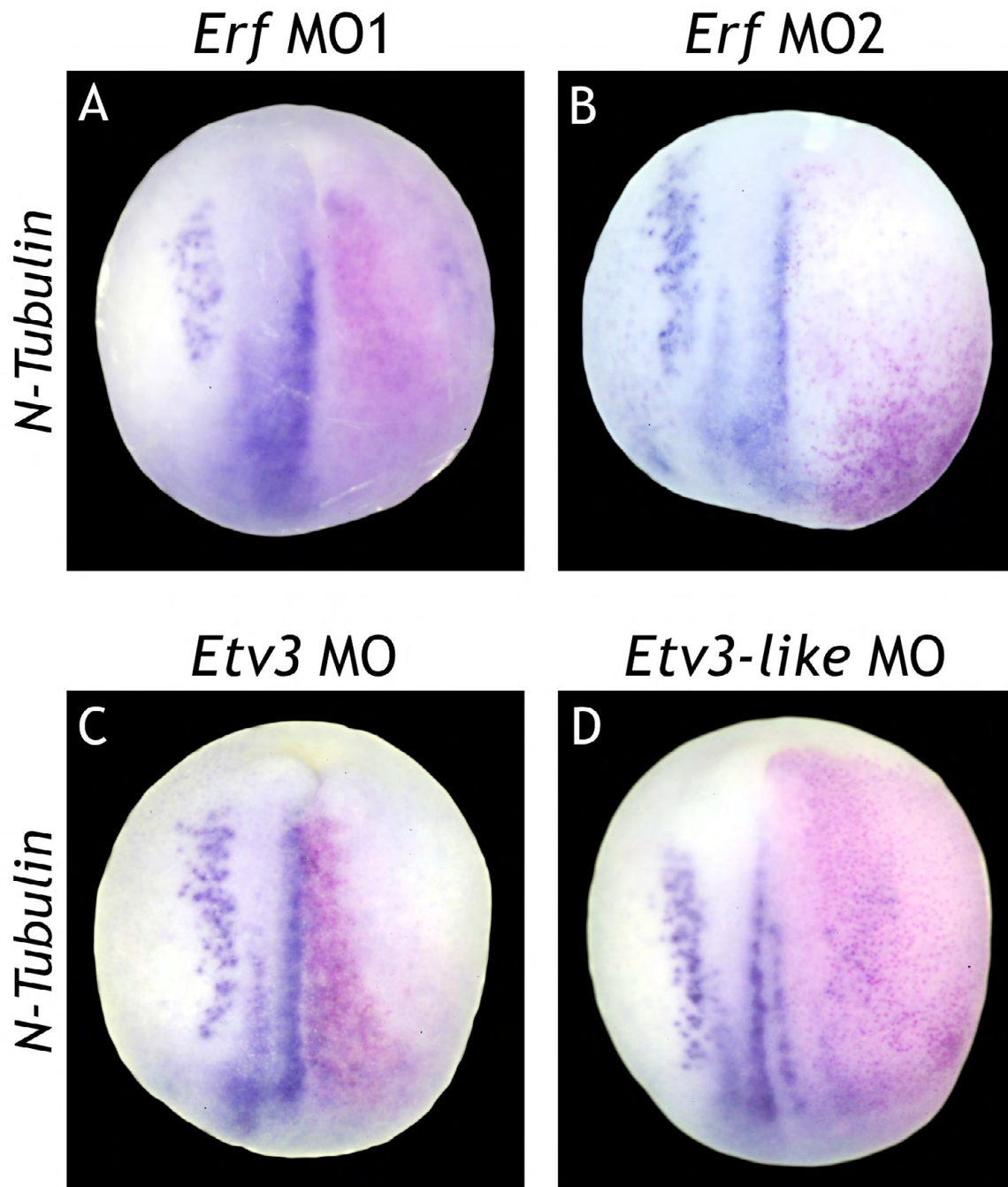
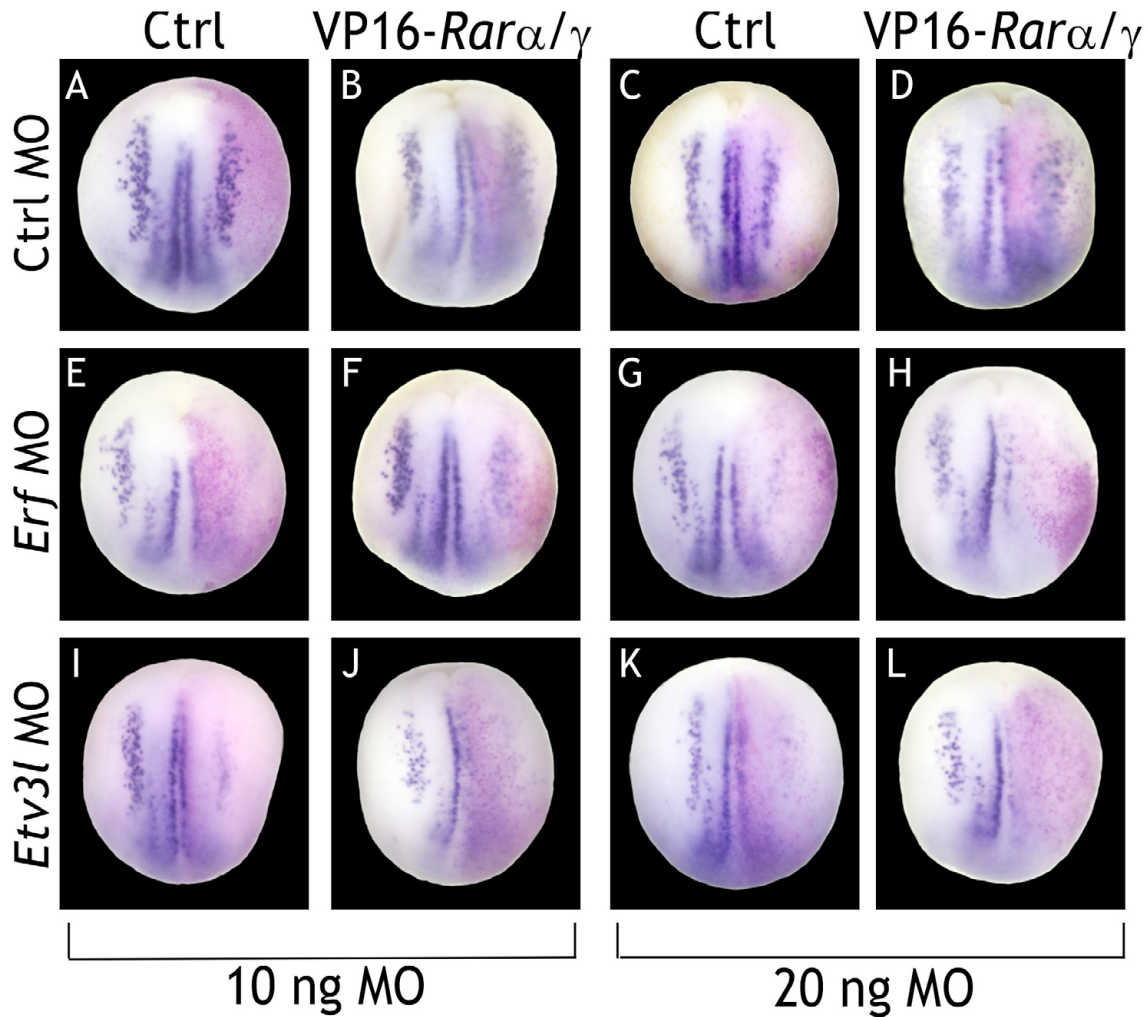


Fig. S7. Specificity of *Erf*-MO and *Etv3/3l*-MO phenotypes. All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. Knockdown or knockout of *N-tubulin* was observed in embryos injected with (A) 10 ng *Erf* AUG MO #1 (30/35 embryos), (B) 10 ng *Erf* AUG MO #2 (18/19), (C) 20 ng *Etv3* AUG MO (10/11) and (D) 20 ng *Etv3l* AUG MO (16/21). Embryos are shown in dorsal view with anterior at the bottom, at stage 14.



M

	Injection Combination	NT	LT	NC	ExN	EcN	Total
A	10 ng Ctrl MO + 100 pg <i>mCherry</i> mRNA	0	4	34	0	0	38
B	10 ng Ctrl MO + 100 pg VP16- <i>Rarα/γ</i> mRNA	0	11	2	11	12	36
C	20 ng Ctrl MO + 100 pg <i>mCherry</i> mRNA	0	13	30	0	6	49
D	20 ng Ctrl MO + 100 pg VP16- <i>Rarα/γ</i> mRNA	0	10	2	5	19	36
E	10 ng <i>Erf</i> MO + 100 pg <i>mCherry</i> mRNA	15	14	0	1	0	30
F	10 ng <i>Erf</i> MO + 100 pg VP16- <i>Rarα/γ</i> mRNA	7	6	4	3	5	25
G	20 ng <i>Erf</i> MO + 100 pg <i>mCherry</i> mRNA	17	6	1	0	0	24
H	20 ng <i>Erf</i> MO + 100 pg VP16- <i>Rarα/γ</i> mRNA	29	3	0	1	0	33
I	10 ng <i>Etv3l</i> MO + 100 pg <i>mCherry</i> mRNA	4	20	9	0	0	33
J	10 ng <i>Etv3l</i> MO + 100 pg VP16- <i>Rarα/γ</i> mRNA	29	11	0	0	0	40
K	20 ng <i>Etv3l</i> MO + 100 pg <i>mCherry</i> mRNA	21	6	0	0	0	27
L	20 ng <i>Etv3l</i> MO + 100 pg VP16- <i>Rarα/γ</i> mRNA	35	5	0	0	0	40

NT = no tubulin; LT = low tubulin; NC = no change; ExN = Extra Neurons; EcN = Ectopic Neurons

Fig. S8. ERf or ETV3L knockdown rescues the extra/ectopic neuron phenotype generated by VP16-*Rarα/γ* mRNA. All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. (A,C) 10 ng or 20 ng control MO + 0.1 ng *mCherry* (control) mRNA does not change expression of *N-tubulin*. (B,D) 10 ng or 20 ng control MO + 0.1 ng VP16-*Rarα/γ* mRNA results in extra and/or ectopic neurons. (E-H) 10 ng *Erf* MO partially rescues (68%) and 20 ng *Erf* MO completely rescues (97%) VP16-*Rarα/γ* mRNA extra/ectopic neuron phenotype. (I-L) 10 ng or 20 ng *Etv3l* MO completely rescues (100%) VP16-*Rarα/γ* mRNA extra/ectopic neuron phenotype. Embryos are shown in dorsal view with anterior at the bottom, at stage 14. (M) Detailed scoring of embryos represented in A-L.

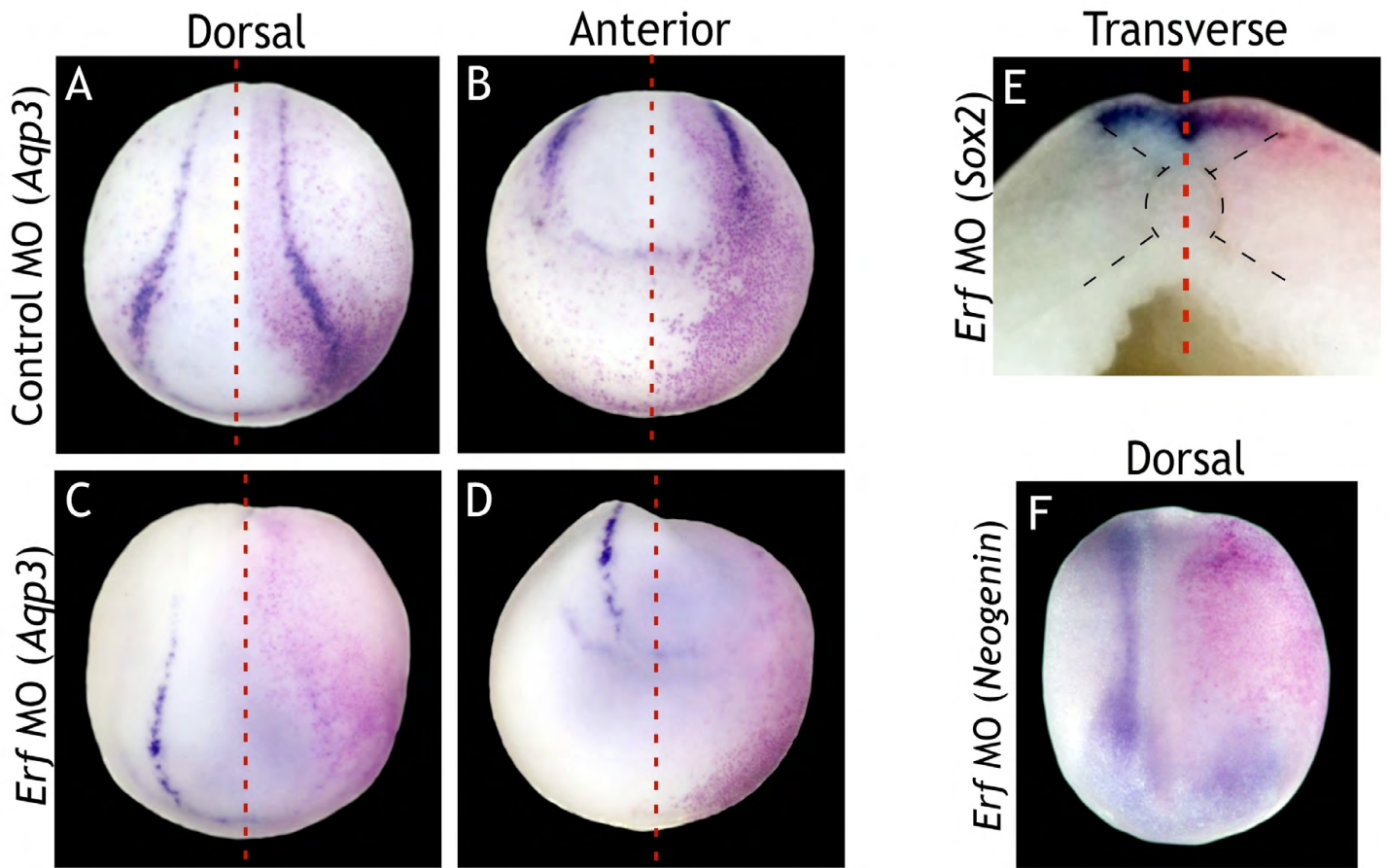
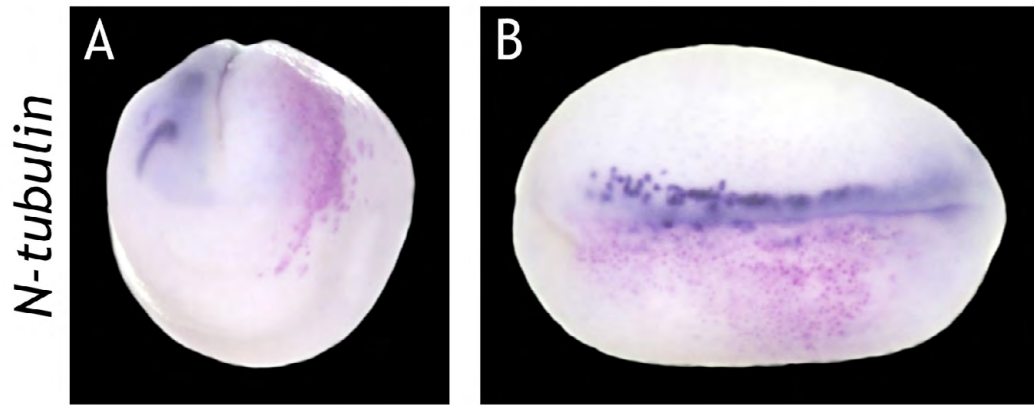


Fig. S9. ERF knockdown inhibits neural fold elevation. All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. (A,B) Dorsal (A) and anterior (B) views of 10 ng control MO, stained for *Aqp3* at stage 14. (C,D) Dorsal (C) and anterior (D) views of 10 ng *Erf* MO, which resulted in a flattening of neural folds and significant reduction of *Aqp3* at stage 14 (7/7 embryos). The flattening of the neural folds was observed in 60-70% of embryos in all experiments using *Erf* MO. (E) A transverse section of a stage 22 embryo injected with 10 ng *Erf* MO reveals relatively normal neural structure, as revealed by *Sox2* expression. (F) Dorsal view of 10 ng *Erf* MO which resulted in loss of *Neogenin* (7/9 embryos) at stage 14.

Etv3l MO



Erf MO

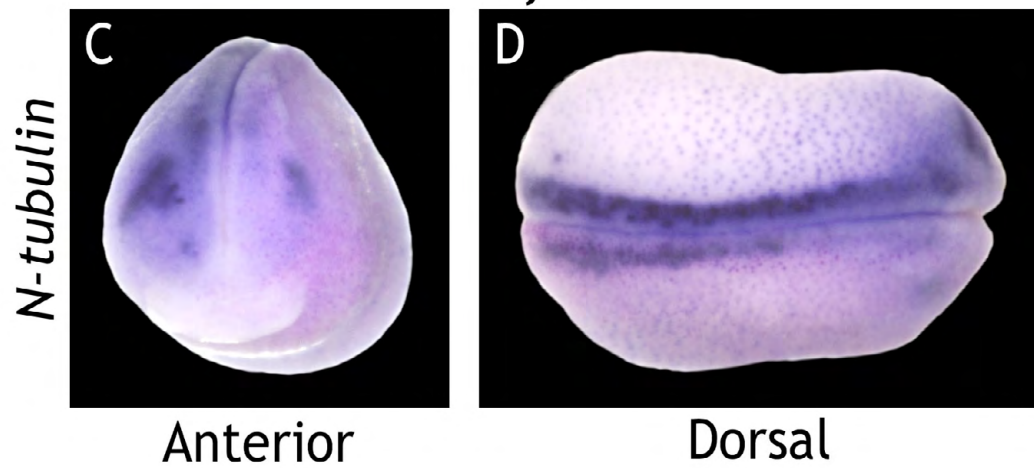


Fig. S10. ERF or ETV3L knockdown causes a decrease or loss of *N-tubulin* in stage 22 embryos. All embryos were injected unilaterally at the two- or four-cell stage. The injected side is indicated by the magenta β -gal mRNA lineage tracer. (A,B) Anterior (A) and dorsal (B) views of 20 ng *Etv3l* MO, which resulted in loss of *N-tubulin* expression at stage 22 (25/26 embryos). (C,D) Anterior (C) and dorsal (D) views of 10 ng *Erf* MO, which resulted in decreased *N-tubulin* expression at stage 22 (16/32 embryos).

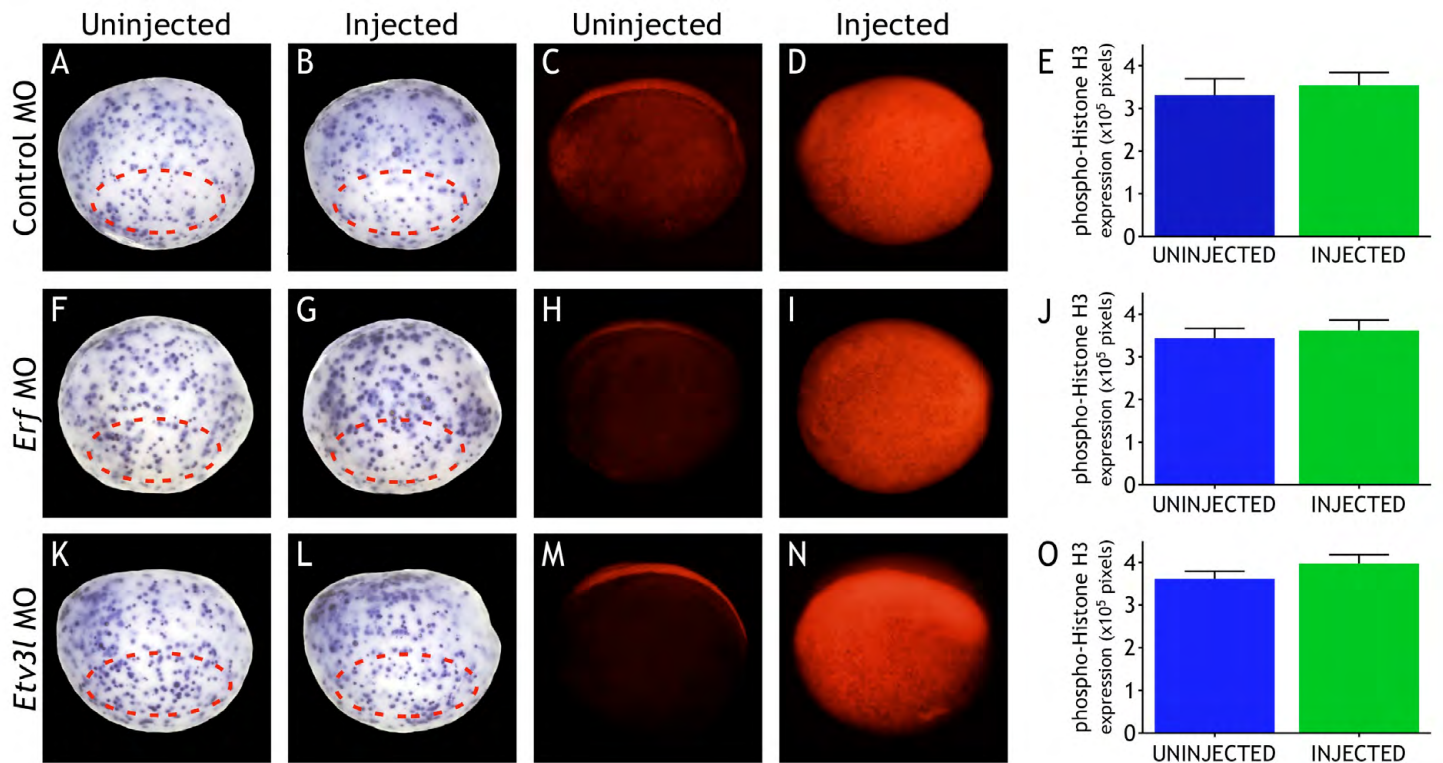


Fig. S11. ERF or ETV3L knockdown does not change proliferation outside of the neural plate. Embryos were injected unilaterally at the 2- or 4-cell stage. The injected side is indicated by the red fluorescent lineage tracer. (A-D,F-I,K-N) Representative photographs in bright-field (A,B,F,G,K,L) and fluorescence (C,D,H,I,M,N) are shown. (A-D) Photographs of the same stage 14 embryo, in lateral view, with B and D flipped horizontally from A and C, such that the anterior of the embryo is always on the left. The same is true for F-I and K-N. (E,J,O) Quantitation of phospho-*Histone H3* staining for all embryos in the experiment is provided in bar graphs. (A-O) Embryos injected with 10 ng control MO (A-E), 10 ng *Erf* MO (F-J) or 20 ng *Etv3l* MO (K-O) showed no significant difference in phospho-*Histone H3* staining on the injected versus the uninjected side (Ctrl MO, $n=21$, $P=0.4654$; *Erf* MO, $n=39$, $P=0.5329$; *Etv3l* MO, $n=47$, $P=0.2256$).

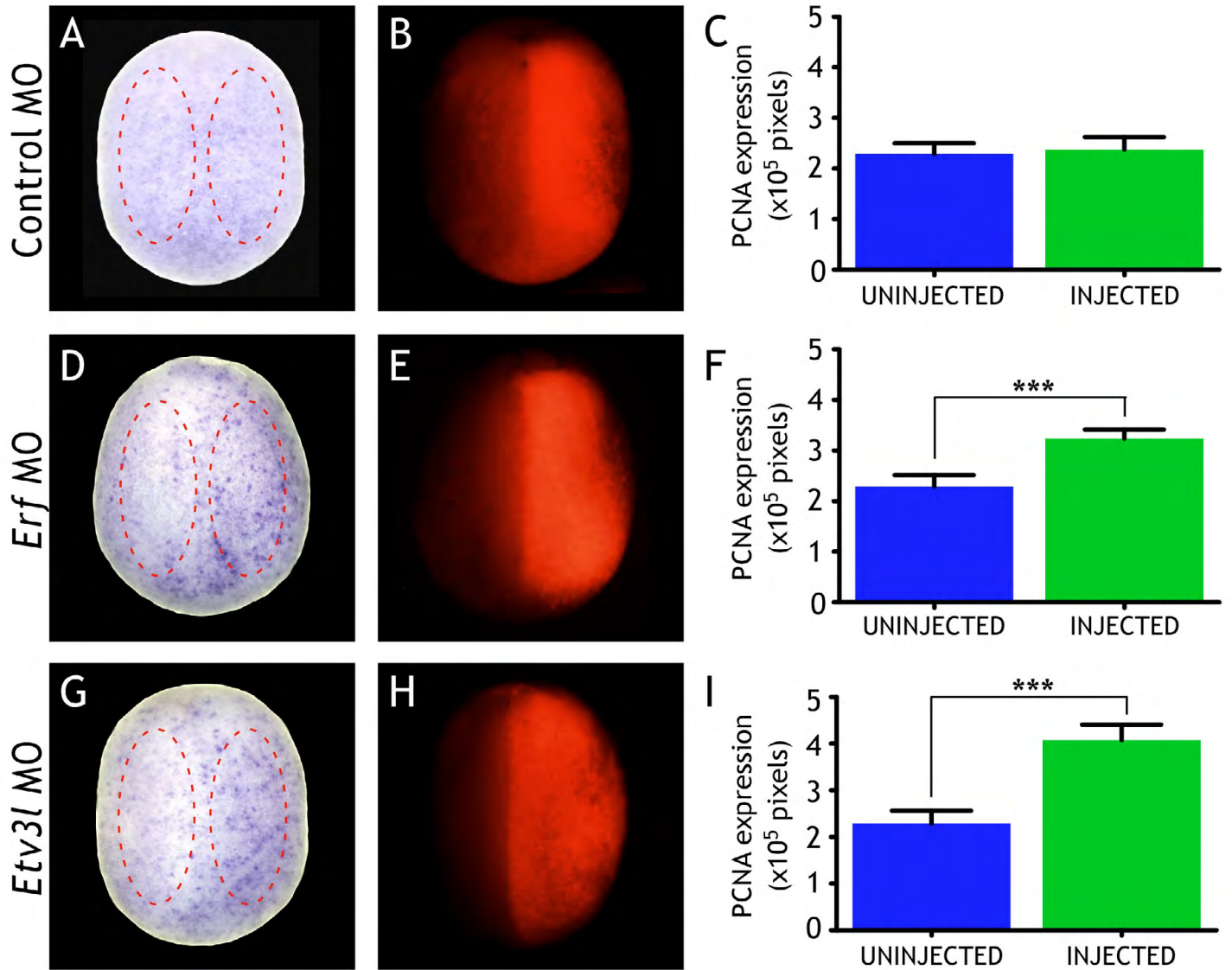


Fig. S12. ERF or ETV3L knockdown increases proliferation in the neural plate. Embryos were injected unilaterally at the 2- or 4-cell stage. (A,B,D,E,G,H) The injected side is indicated by the red fluorescent lineage tracer. Representative photographs in brightfield (A,D,G) and fluorescence (B,E,H) are shown. (C,F,I) Quantitation of PCNA staining for all embryos in the experiment is provided in bar graphs. Embryos are shown in dorsal view at stage 14; anterior is at the bottom. (A-C) Embryos injected with 10 ng control MO showed no significant difference in the number of PCNA nuclei on the injected versus the uninjected side ($n=41$; $P=0.776$). (D-F) Embryos injected with 10 ng *Erf* MO showed an increased number of PCNA nuclei on the injected side ($n=45$; $P=0.0009$). (G-I) Embryos injected with 10 ng *Etv3l* MO showed an increased number of PCNA nuclei on the injected side ($n=46$; $P=0.0009$).

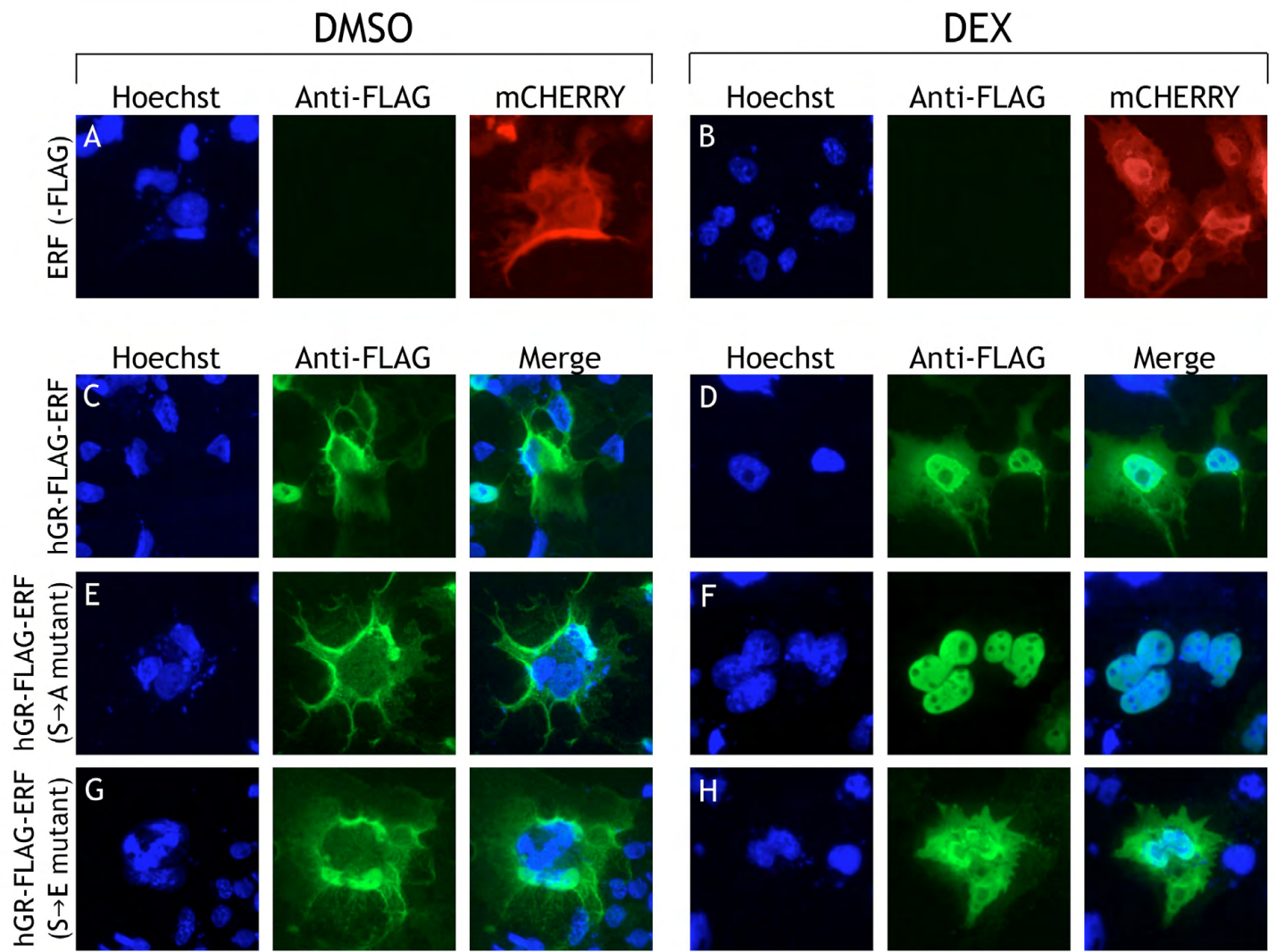


Fig. S13. Subcellular localization of hGR-ERF. COS-7 cells were co-transfected with *mCherry* and either *Erf* (no FLAG), FLAG-*hGR-Erf* (WT), FLAG-*hGR-Erf* ($S^{246,251} \rightarrow A^{246,251}$) or FLAG-*hGR-Erf* ($S^{246,251} \rightarrow E^{246,251}$), then treated with 1 μ M dexamethasone (DEX) or 0.01% DMSO. (A,B) Negative control with *Erf* (no FLAG); transfected cells are indicated by mCHERRY fluorescence. (C,E,G) All FLAG-hGR-ERF proteins are found mostly in the cytoplasm when cells were treated with DMSO. (D) Dexamethasone-treated FLAG-hGR-ERF (WT) is located in the cytoplasm and the nucleus. (F) Dexamethasone-treated FLAG-hGR-ERF ($S^{246,251} \rightarrow A^{246,251}$) is located exclusively in the nucleus (H) Dexamethasone-treated FLAG-hGR-ERF ($S^{246,251} \rightarrow E^{246,251}$) is located in the cytoplasm and the nucleus.

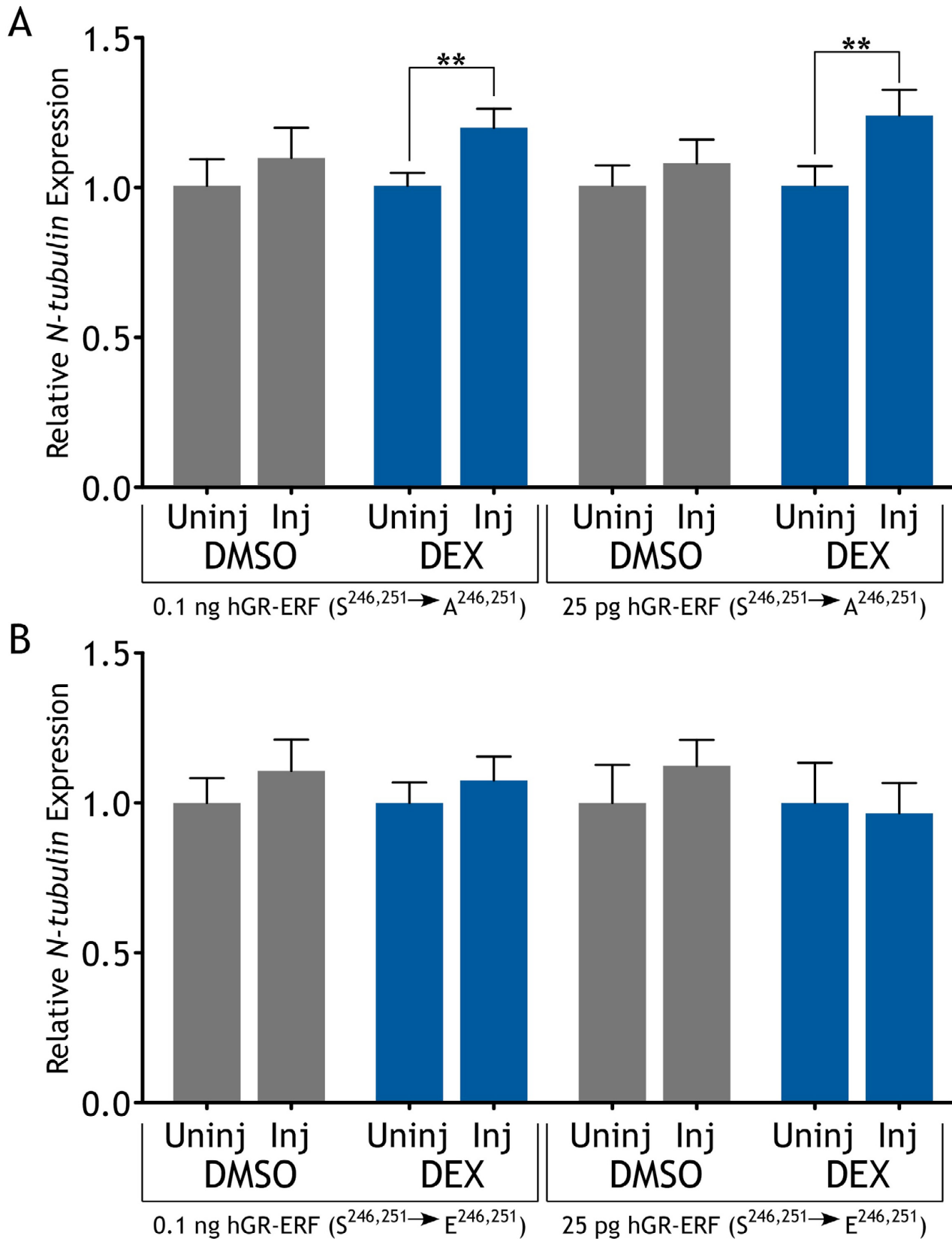


Fig. S14. ERF gain of function increases primary neurons. Embryos were injected unilaterally at the 2- or 4-cell stage, then treated with 10 μ M dexamethasone (DEX) or 0.1% DMSO vehicle at stage 11. Quantitation of *N-tubulin* expression at stage 14 is shown. (A) Embryos injected with 0.1 ng or 25 pg *hGR-Erf* (S^{246,251}→A^{246,251}) mRNA and treated with dexamethasone showed a significant increase in neurons on the injected side (0.1 ng, $n=29$, $P=0.0073$; 25 pg, $n=23$, $P=0.0049$). DMSO-treated embryos showed no significant difference in neurons (0.1 ng, $n=22$, $P=0.4170$; 25 pg, $n=22$, $P=0.2558$) (B) Embryos injected with 0.1 ng or 25 pg *hGR-Erf* (S^{246,251}→E^{246,251}) mRNA showed no significant difference in neurons on the injected versus the uninjected side in dexamethasone-treated (0.1 ng, $n=22$, $P=0.2055$; 25 pg, $n=13$, $P=0.7354$) or DMSO-treated embryos (0.1 ng, $n=16$, $P=0.6233$; 25 pg, $n=10$, $P=0.1309$).

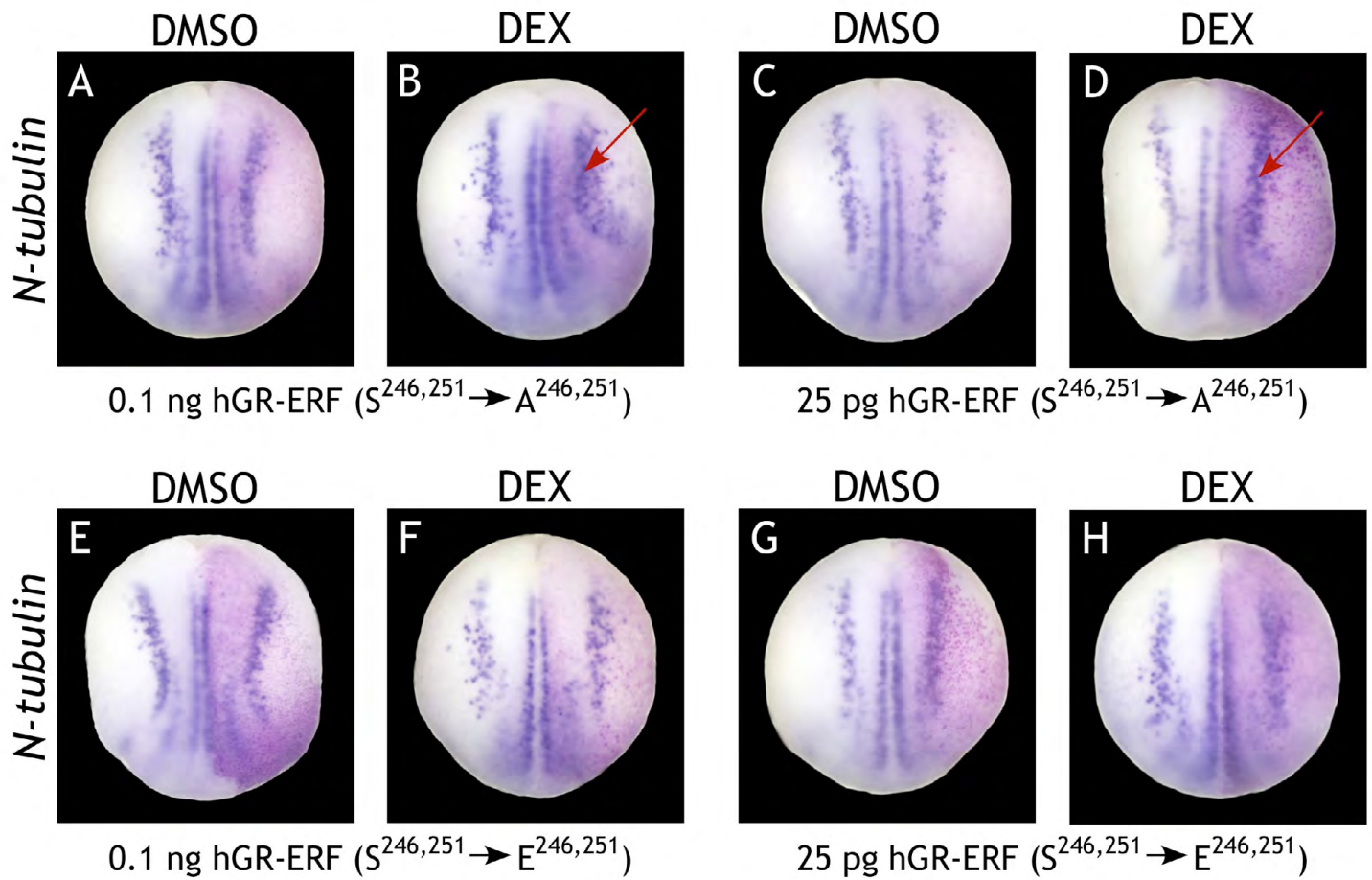


Fig. S15. ERF gain of function increases primary neurons. Embryos corresponding to data provided in Fig. 9. (A) DMSO-treated or (B) dexamethasone-treated embryos injected with 0.1 ng *hGR-Erf* ($S^{246,251} \rightarrow A^{246,251}$) mRNA. (C) DMSO-treated or (D) dexamethasone-treated embryos injected with 25 pg *hGR-Erf* ($S^{246,251} \rightarrow A^{246,251}$) mRNA. (E) DMSO-treated or (F) dexamethasone-treated embryos injected with 0.1 ng *hGR-Erf* ($S^{246,251} \rightarrow E^{246,251}$) mRNA. (G) DMSO-treated or (H) dexamethasone-treated embryos injected with 25 pg *hGR-Erf* ($S^{246,251} \rightarrow E^{246,251}$) mRNA. The red arrows indicate the increase in *N-tubulin* expression. All embryos are shown in dorsal view with anterior at the bottom, at stage 14.

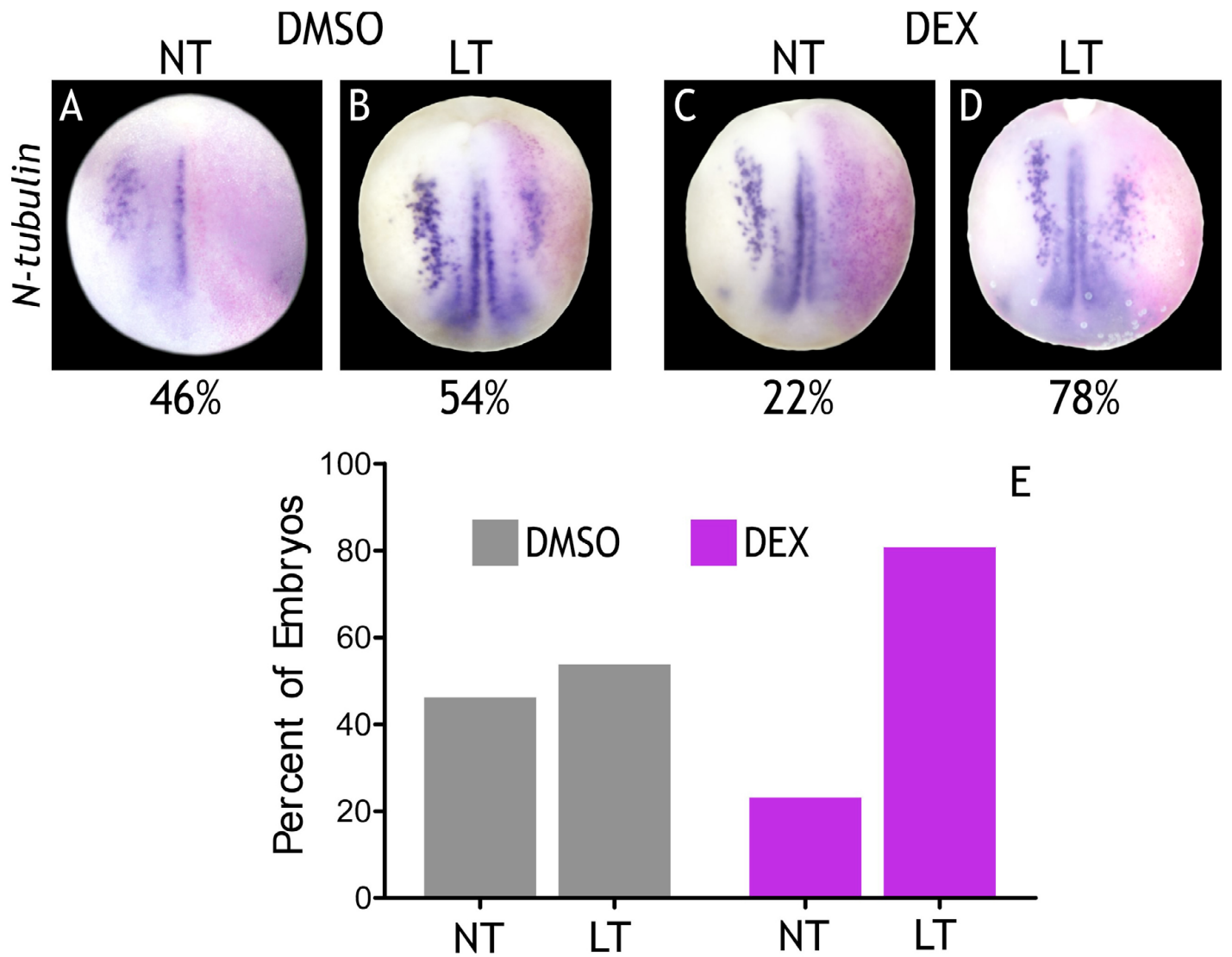


Fig. S16. ERF gain-of-function partially rescues *N-tubulin* expression in *RARγ*-MO embryos. All embryos were injected unilaterally with 3.75 ng *Rary1* MO + 3.75 *Rary2* MO + 0.1 ng *hGR-Erf* ($S^{246,251} \rightarrow A^{246,251}$) mRNA at the two- or four-cell stage, then treated with 10 μ M dexamethasone (DEX) or 0.1% DMSO vehicle at stage 11. The injected side is indicated by the magenta β -gal mRNA lineage tracer. (A,B) Embryos treated with DMSO exhibited no tubulin (NT) or low tubulin (LT) of *N-tubulin* in 46% and 54% of embryos, respectively. (C,D) Embryos treated with dexamethasone exhibited no tubulin (NT) or low tubulin (LT) of *N-tubulin* in 23% and 81% of embryos, respectively. All embryos are shown in dorsal view with anterior at the bottom, at stage 13/13.5. (E) Scoring of embryos.

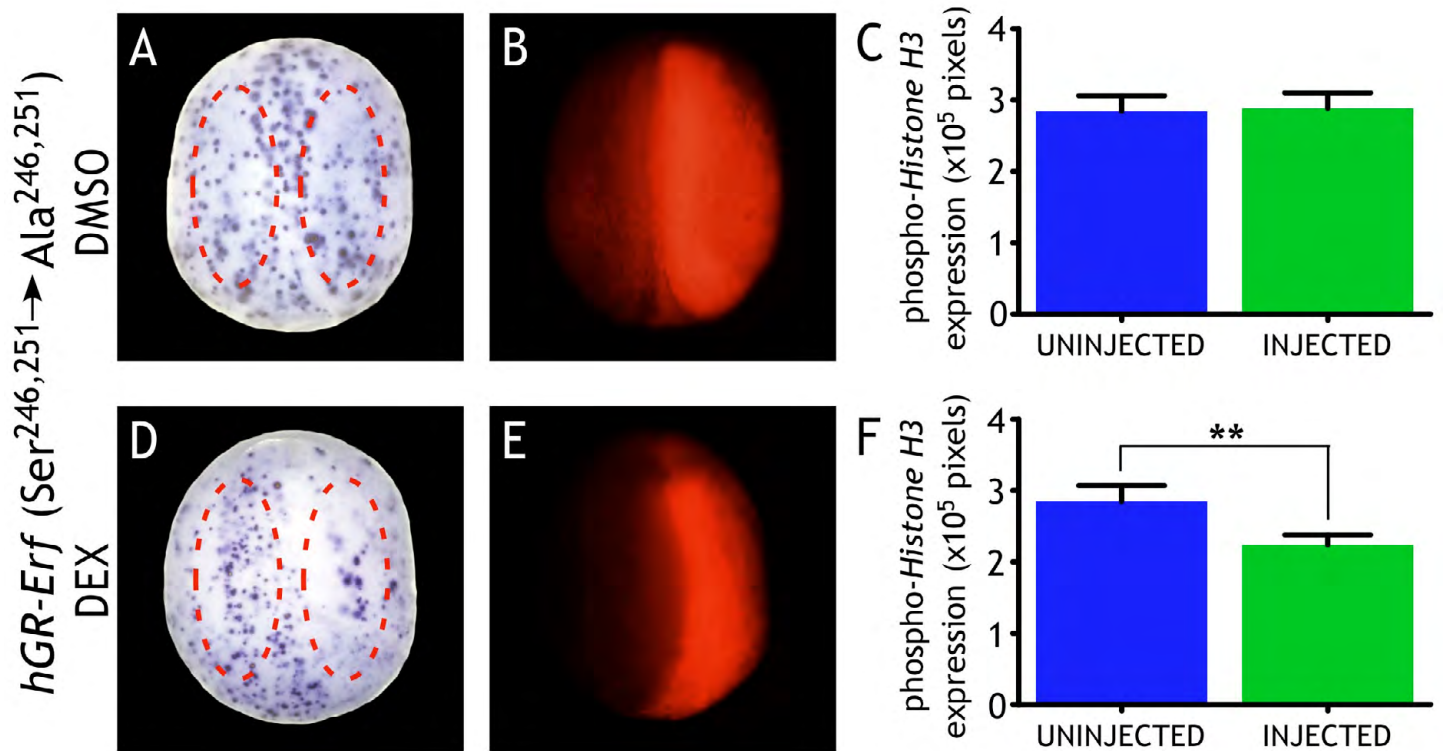


Fig. S17. ERF gain of function decreases proliferation in the neural plate. Embryos were injected unilaterally at the 2- or 4-cell stage, then treated with 10 μ M dexamethasone (DEX) or 0.1% DMSO vehicle at stage 11. The injected side is indicated by the red fluorescent lineage tracer. (A,B,D,E) Representative photographs in bright field (A,D) and fluorescence (B,E) are shown. (C,F) Quantitation of phospho-*Histone H3* staining for all embryos in the experiment. Embryos are shown in dorsal view at stage 14; anterior is at the bottom. (A-C) DMSO-treated embryos injected with 0.1 ng *hGR-Erf*(S^{246,251}→A^{246,251}) mRNA showed no significant difference in the number of phospho-*Histone H3* nuclei on the injected versus the uninjected side ($n=30$; $P=0.758$). (D-F) Dexamethasone-treated embryos injected with 0.1 ng *hGR-Erf*(S^{246,251}→A^{246,251}) mRNA showed a decreased number of phospho-*Histone H3* nuclei on the injected versus the uninjected side ($n=37$; $P=0.0028$).

Table S1. Morpholinos

MO	Type	Sequence (5'→3')
<i>Rary1</i>	AUG	GCT GTT TGC CAT TGC CTT GTT CTA
<i>Rary2</i>	AUG	TTC CAT GCA GTC ATA CAT TTT GGG
<i>Rara1:</i>	AUG	GCT CCA AAC GCA CTT CTA CTC CCT C
<i>Rara2.1:</i>	AUG	CTG AAA TCC AAA CTG ACC ATA GAG T
<i>Rara2.2:</i>	AUG	ATC CAA AGG AAG GTG AGT GTG TGT G
<i>Rara</i> (D1)	Splice	GGG TAA CAC TTA CCT TGC AAC CTT C
<i>Rara</i> (D2)	Splice	GCG CCC GTT ACT CAC ATT CTT TAG A
<i>Raldh2</i>	AUG	TCT CTA TTT TAC TGG AAG TCA TGT C
<i>Cyp26a1</i>	AUG	TAG TGA GCA GAG TAT ACA GAT CCA T
<i>Etv3l</i>	AUG	CCT TCT CTT CTT GCT TAG TAA CAT C
<i>Etv3</i>	AUG	GTT TCC TTC TTG CTG ACG GGA TCG A
<i>Erf #1</i>	AUG	CCA CTA GCG CTG CTC TCC CCT CGG T
<i>Erf #2</i>	AUG	GGT CTG TGC TGC TTC TCC TCC TCC A

Table S2. Probes with T7 adapters

Primer	Sequence (5'→3')
F (<i>Geminin</i>):	TAC CAA CAA GAA GCA GAG ATT GGA
R (<i>Geminin</i>):	taa tac gac tca cta tag ggA TTC TGA TCT GAA TTA GAG GGC CG
F (<i>Foxd41l</i>):	ATG CAG GAC TTT CTG ATG AGG A
R (<i>Foxd41l</i>):	taa tac gac tca cta tag ggT AAG CAC AGC TGG GAG AAG G
F (<i>Neogenin</i>):	AGC CCG ACT TCA CTG GAT CA
R (<i>Neogenin</i>):	taa tac gac tca cta tag ggC TGT GGT TAT GGC ATT TAG ATC
F (<i>Sox3</i>):	GTT GGA CAC CGA CAT CAA GAG
R (<i>Sox3</i>):	taa tac gac tca cta tag ggG TAC CGT GCC ATT GAC TCC A
F (<i>Zic1</i>):	GTG ACG ACT TTC GGT TCC TC
R (<i>Zic1</i>):	taa tac gac tca cta tag ggG TGA TTG GAC GTG TGA TGT ACT G
F (<i>Zic3</i>):	ACA ATG CTA TTA GAT GGA GGA CCG
R (<i>Zic3</i>):	taa tac gac tca cta tag ggT GTT GTT AGT CTG ATG TGT TGC TG

Table S3. Probe plasmids

Gene	Restriction enzyme	Polymerase
<i>Dll</i>	<i>XhoI</i>	T7
<i>Myt1</i>	<i>ClaI</i>	T7
<i>Ngnr1</i>	<i>BamHI</i>	T3
<i>Zic2</i>	<i>BamHI</i>	SP6

Table S4. QPCR

Primer	Sequence (5'→3')
F (<i>Etv3</i>)	GGA AGT GGG ATT AAT AAG GCG G
R (<i>Etv3</i>)	CCG TCA GCA AGA AGG AAA CAT G
F (<i>Etv3l</i>)	GCG ACC AAT TCC TAC GTG TG
R (<i>Etv3l</i>)	GCT GTT CTT CAG GTT CAA ACT TCC
F (<i>Erf</i>)	TTC GGA AAT GCA AAC CGC AG
R (<i>Erf</i>)	GGT AAA GCG TTT GCC TTT GGT
F (<i>HoxA1</i>)	AAG TTT GTG GTT CTC CTG CC
R (<i>HoxA1</i>)	TTT GTG GTG AAG TTG GTC CTG
F (<i>Histone H4</i>)	GAT AAC ATC CAG GGC ATC AC
R (<i>Histone H4</i>)	TAA CCT CCG AAT CCG TAC AG

Table S5. Cloning pCDG1-FLAG-*Erf-hGR*⁵¹²⁻⁷⁷⁷

Primer	Sequence (5'→3')
A	CAG ATA CCA TGG ATT ATA AAG ATG ATG ATG ATA AGC TTA TGA AAA CCC CGG CAG AG
B	GGA TTT TCA GAT CTG GAA TCG CGG TTT TCC AGG
C	CCG CGA TTC CAG ATC TGA AAA TCC TGG TAA CAA AAC AAT AG
D	ACT AGT GGA TCC TTA CTA TCA CTT TTG ATG AAA CAG AAG TTT TTT G

Table S6. Cloning pCDG1-FLAG-Erf-hGR⁵¹²⁻⁷⁷⁷ mutants

Primer	Sequence (5'→3')
A	CAG ATA CCA TGG ATT ATA AAG ATG ATG ATG ATA AGC TTA TGA AAA CCC CGG CAG AG
B (Ala ²³⁷ , Ala ²⁴²)	<u>TGC</u> CAC TGG GAA TGG <u>TGC</u> GAG AGG CTC TGG CAC CCG
B (Glu ²³⁷ , Glu ²⁴²)	<u>TTC</u> CAC TGG GAA TGG <u>TTC</u> GAG AGG CTC TGG CAC CCG
C (Ala ²³⁷ , Ala ²⁴²)	<u>GCA</u> CCA TTC CCA GTG <u>GCA</u> CCC ATG GGT GCA CCA GC
C (Glu ²³⁷ , Glu ²⁴²)	<u>GAA</u> CCA TTC CCA GTG <u>GAA</u> CCC ATG GGT GCA CCA GC
D	ACT AGT GGA TCC TTA CTA TCA CTT TTG ATG AAA CAG AAG TTT TTT G