Table S1. Experimental statistics for toxin-mediated ablation of zebrafish neurons (related to Figure 1).

Panel	Transgenic line (heterozygous)	Stage	Treatment	Total number of animals	Animals with neural ablation[1]
C	*Tg(tuba1a:Gal4VP16; myl7:gfp)*	24 hpf	None	21	0
C	*Tg(tuba1a:Gal4VP16; myl7:gfp)*	24 hpf	Rimantadine	25	0
C	*Tg(tuba1a:Gal4VP16; myl7:gfp)*	48 hpf	None	20	0
C	*Tg(tuba1a:Gal4VP16; myl7:gfp)*	48 hpf	Rimantadine	24	0
C	*Tg(tuba1a:Gal4VP16; myl7:gfp)*	72 hpf	None	30	0
C	*Tg(tuba1a:Gal4VP16; myl7:gfp)*	72 hpf	Rimantadine	28	0
C	*Tg(tuba1a:Gal4VP16; UAS:M2^{H37A};myl7:gfp, mCherry)*	24 hpf	None	24	24
C	*Tg(tuba1a:Gal4VP16; UAS:M2^{H37A};myl7:gfp, mCherry)*	24 hpf	Rimantadine	22	0
C	*Tg(tuba1a:Gal4VP16; UAS:M2^{H37A};myl7:gfp, mCherry)*	48 hpf	None	20	20
C	*Tg(tuba1a:Gal4VP16; UAS:M2^{H37A};myl7:gfp, mCherry)*	48 hpf	Rimantadine	25	0
C	*Tg(tuba1a:Gal4VP16; UAS:M2^{H37A};myl7:gfp, mCherry)*	72 hpf	None	32	32
C	*Tg(tuba1a:Gal4VP16; UAS:M2^{H37A};myl7:gfp, mCherry)*	72 hpf	Rimantadine	31	0
D	Tg(*tuba1a:Gal4VP16; UAS:NTR-mCherry; myl7:GFP*)	24 hpf	DMSO	25	0
D	Tg(*tuba1a:Gal4VP16; UAS:NTR-mCherry; myl7:GFP*)	24 hpf	Metronidazole	32	0
D	Tg(*tuba1a:Gal4VP16; UAS:NTR-mCherry; myl7:GFP*)	48 hpf	DMSO	26	0
D	Tg(*tuba1a:Gal4VP16; UAS:NTR-mCherry; myl7:GFP*)	48 hpf	Metronidazole	25	3

D	Tg(*tuba1a:Gal4VP16; UAS:NTR-mCherry; myl7:GFP*)	72 hpf	DMSO	31	0
D	Tg(*tuba1a:Gal4VP16; UAS:NTR-mCherry; myl7:GFP*)	72 hpf	Metronidazole	28	28

[1]Neural ablation was scored by the lack of the midbrain-hindbrain boundary at 24 hpf and presence of dead tissue in the head at 32 and 48 hpf.

Table S2. Experimental statistics for GAVPO- and light-mediated nitroreductase expression (related to Figure 2).

Panel	Transgenic line (heterozygous)	Experimental condition	Total number of animals	Animals with mCherry expression
B	*Tg(UAS:NTR-mCherry)*	Uninjected 470-nm light	56	0
B	*Tg(UAS:NTR-mCherry)*	100 pg *GAVPO* mRNA 470-nm light	39	39
B	*Tg(UAS:NTR-mCherry)*	200 pg *GAVPO* mRNA 470-nm light	33	33
B	*Tg(UAS:NTR-mCherry)*	200 pg *GAVPO* mRNA	48	2

Table S3. Experimental statistics for GAVPO- and light-mediated toxin expression (related to Figure 3).

Panel	Transgenic line (heterozygous)	Experimental condition	Total number of animals	Animals with axial mCherry expression
A	*Tg(UAS:NTR-mCherry)*	Uninjected DMSO 470-nm light	14	0
A	*Tg(UAS:NTR-mCherry)*	50 pg *GAVPO* mRNA 470-nm light DMSO	11	8
A	*Tg(UAS:NTR-mCherry)*	50 pg *GAVPO* mRNA 470-nm light Metronidazole	10	7
A	*Tg(UAS:NTR-mCherry)*	50 pg *GAVPO* mRNA DMSO	19	0
Panel	**Transgenic line (heterozygous)**	**Experimental condition**	**Total number of animals**	**Animals with axial defects**
D	*Tg(UAS:M2^{H37A}; myl7:mCherry)*	Uninjected 470-nm light	12	1
D	*Tg(UAS:M2^{H37A}; myl7:mCherry)*	Uninjected 470-nm light Rimantadine	12	1
D	*Tg(UAS:M2^{H37A}; myl7:mCherry)*	30 pg *GAVPO* mRNA 470-nm light	25	20
D	*Tg(UAS:M2^{H37A}; myl7:mCherry)*	30 pg *GAVPO* mRNA 470-nm light Rimantadine	25	3
D	*Tg(UAS:M2^{H37A}; myl7:mCherry))*	30 pg *GAVPO* mRNA	50	6
D	*Tg(UAS:M2^{H37A}; myl7:mCherry)*	30 pg *GAVPO* mRNA Rimantadine	51	7

Table S4. Experimental statistics for light-inducible, neuron-specific gene expression (related to Figure 4).

Panel	Transgenic line (heterozygous)	Experimental condition	Total number of animals	Animals with mCherry expression
A	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Global irradiation 30 min	28	28
A	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Global irradiation 60 min	25	25
A	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Global irradiation 120 min	30	30
A	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Global irradiation 240 min	24	24
B	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Head irradiation 20x objective 2.5 min	12	11
B	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Head irradiation 20x objective 5 min	24	22
C	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Head irradiation 63x objective 1 min	12	10
C	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Head irradiation 63x objective 2.5 min	12	11
C	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Head irradiation 63x objective 5 min	12	12
D	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Global irradiation 30 min	30	30
F	*Tg(elavl3:GAVPO; UAS:NTR-mCherry)*	Spot irradiation 5 min	24	24

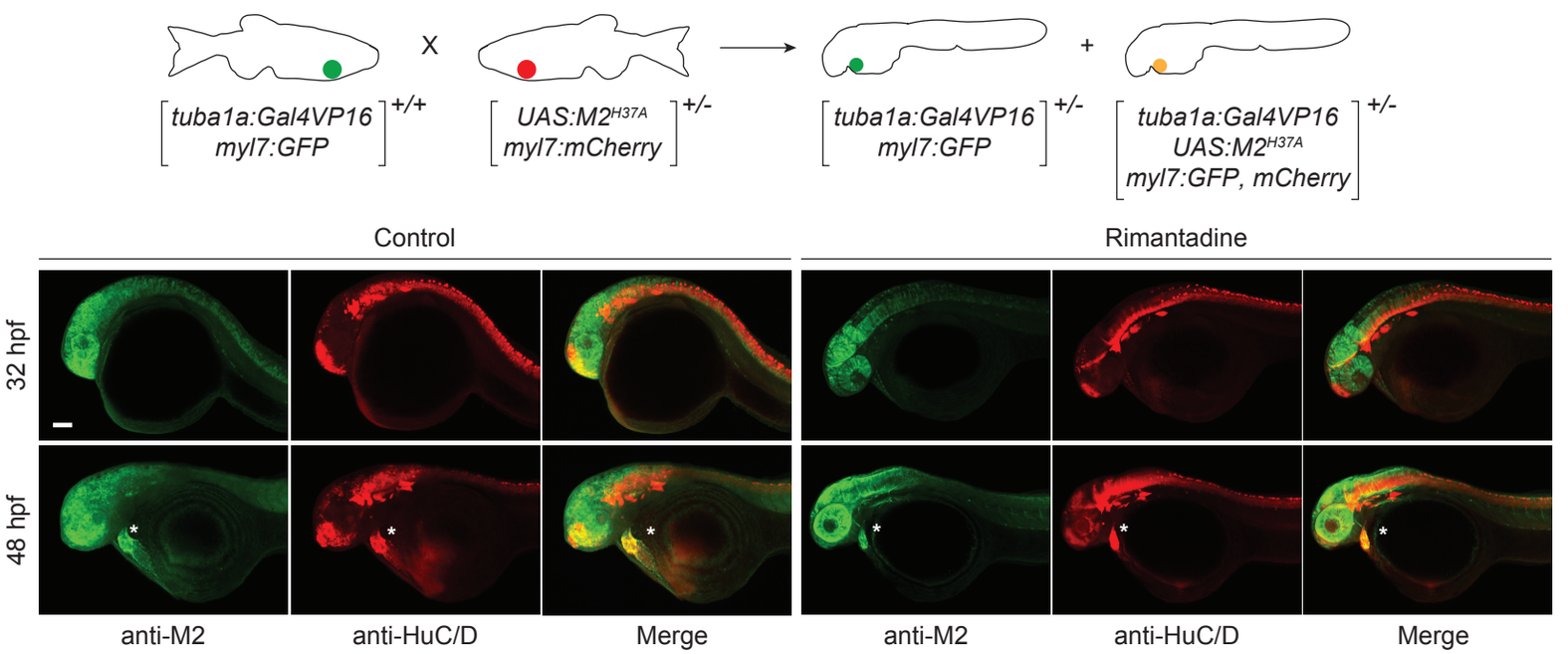

Figure S1. Neuron-specific expression of M2^{H37A}. Representative maximum intensity projection micrographs of heterozygous *Tg(tuba1a:Gal4VP16;UAS:M2^{H37A};myl7:GFP, mCherry)* embryos immunostained for the M2-derived channel (green) or HuC/D (red), an early neuronal marker. Asterisks denote residual fluorescence from reporter genes expressed in the heart. Embryo orientations: lateral view, anterior left. Scale bar: 100 µm.

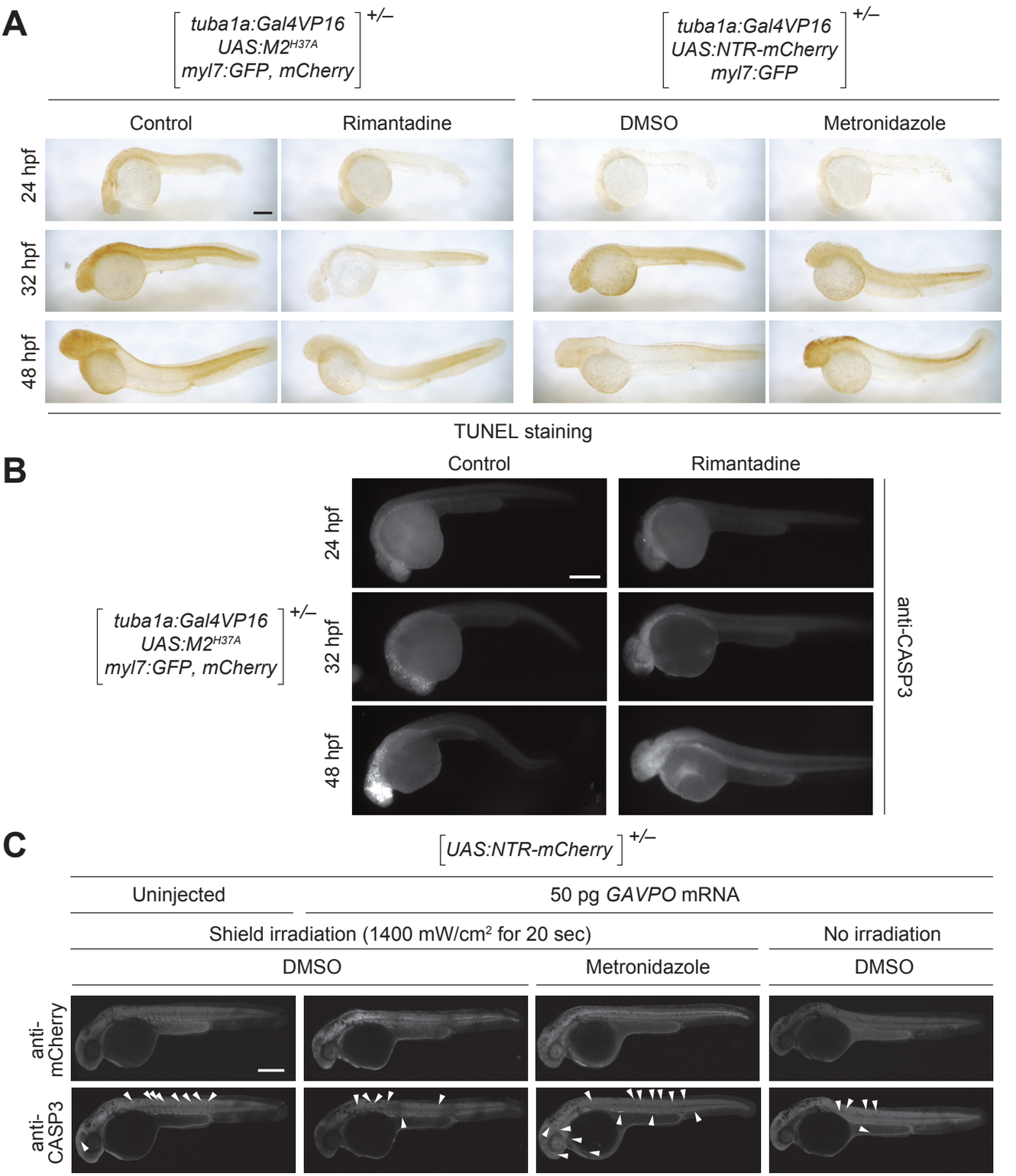

Figure S2. *M2*^{H37A}- and NTR-induced cell death in zebrafish embryos. (A) Heterozygous *Tg(tuba1a:Gal4VP16; UAS:M2*^{H37A}*;myl7:GFP,mCherry)* and *Tg(tuba1a:Gal4VP16;UAS:NTR-mCherry;myl7:GFP)* embryos were cultured in medium supplemented with DMSO, 100 µg/mL rimantadine, or 5 mM metronidazole at 10 hpf. At 48 hpf, the embryos were fixed, and apoptotic cells visualized by TUNEL staining. TUNEL-positive cells were limited to the CNS at 48 hpf in NTR-expressing embryos treated with metronidazole, but they were observed outside the CNS in M2^{H37A}-expressing embryos cultured without rimantadine. (B) Heterozygous *Tg(tuba1a:Gal4VP16; UAS:M2*^{H37A}*;myl7:GFP,mCherry)* embryos cultured in the presence or absence of 100 µg/mL rimatidine, fixed, and immunostained for activaterd caspase-3 (CASP3). (C) Heterozygous *Tg(UAS:NTR-mCherry)* zygotes were injected with the designated amount of *GAVPO* mRNA, and their embryonic shields were irradiated at 6 hpf. The embryos were then cultured in the absence or presence of 5 mM metronidazole from 10 to 32 hpf, fixed, and immunostained for mCherry and activated CASP3 (arrowheads). GAVPO-, light-, and metronidazole-dependent caspase-3 activation was observed. Embryo orientations: lateral view, anterior left. Scale bars: 300 µm.

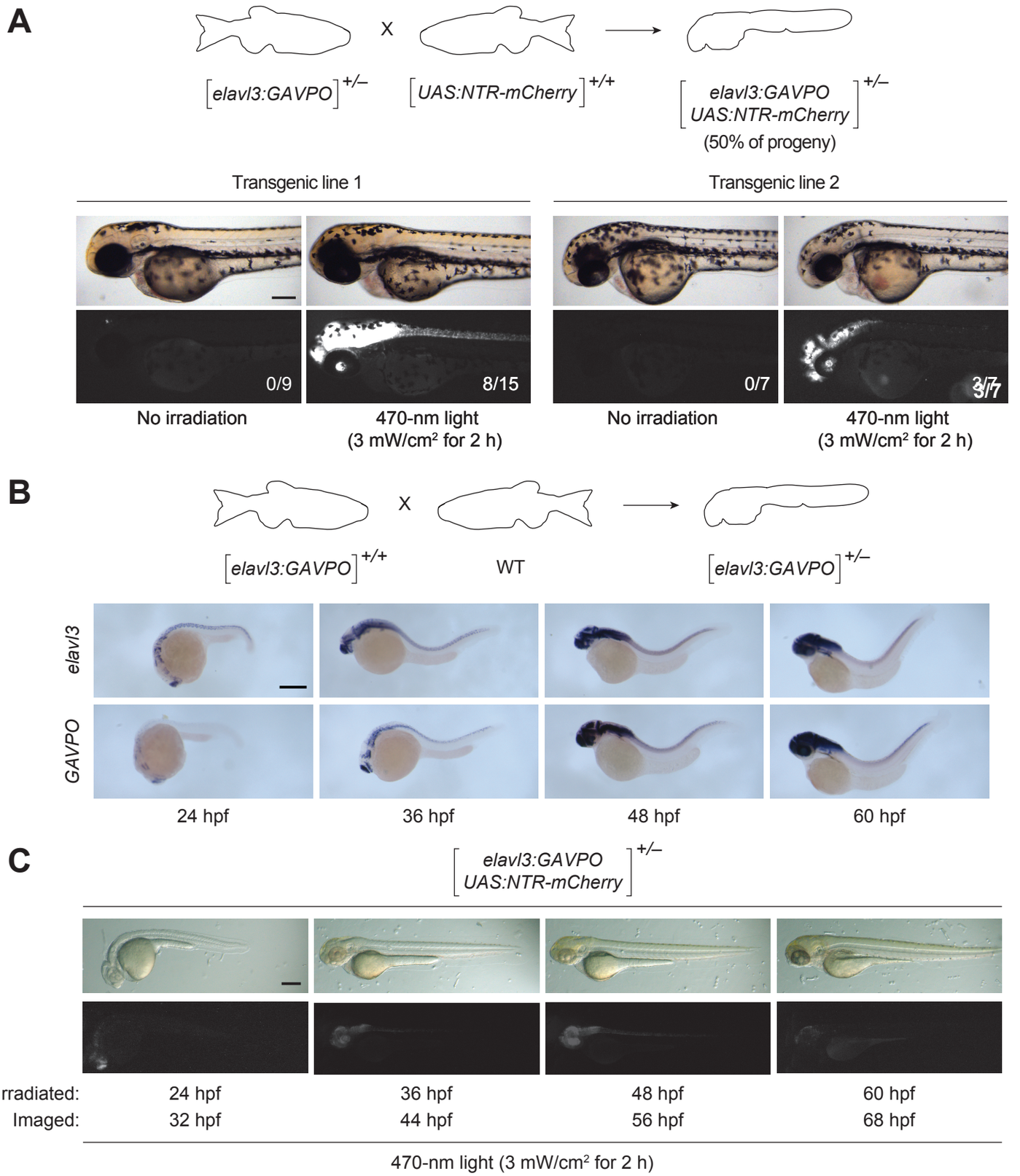

Figure S3. Generation and characterization of *Tg(elavl3:GAVPO)*zebrafish. (A) Light-inducible reporter gene expression in two distinct heterozygous *Tg(elavl3:GAVPO; UAS:NTR-mCherry)* lines. The embryos were either raised in the dark or globally irradiated with blue LED light at 32 hpf. Representative brightfield and epifluorescence micrographs from two independent experiments and the number of fluorescent embryos per condition are shown. (B) GAVPO and *elavl3* expression in *Tg(elavl3:GAVPO)* embryos at the designated developmental stages. (C) GAVPO- and light-dependent NTR-mCherry expression in the CNS of *Tg(elavl3:GAVPO;UAS:NTR-mCherry)* zebrafish. The embryos were globally irradiated with blue LED light starting at the designated times and imaged 8 hours later. Representative brightfield and epifluorescence micrographs are shown. Embryo orientations: lateral view, anterior left. Scale bars: 300 µm.

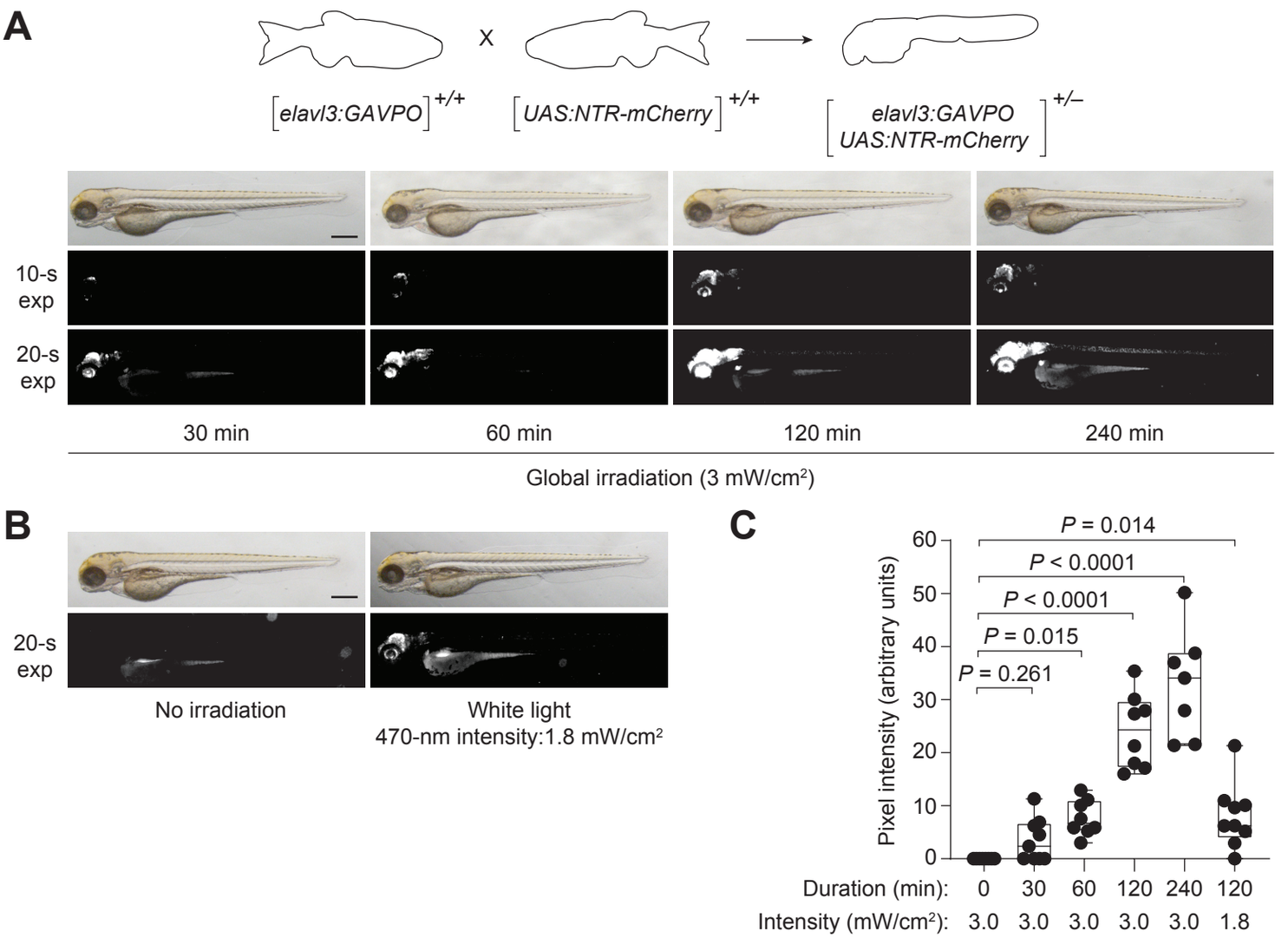

Figure S4. Optimization of light-inducible, neuron-specific gene expression using the GAVPO system. (A-B) *Tg(elavl3:GAVPO; UAS:NTR-mCherry)* embryos were irradiated globally for varying durations, starting at 48 hpf and using a (A) blue LED lamp or (B) white LED lamp. The embryos were imaged at 56 hpf, and representative brightfield and epifluorescence micrographs are shown. (C) Quantification of mCherry fluorescence in *Tg(elavl3:GAVPO;UAS:NTR-mCherry)* embryos irradiated as described in A-B. Embryo orientations:lateral view and anterior left. Scale bars: 500 μm.

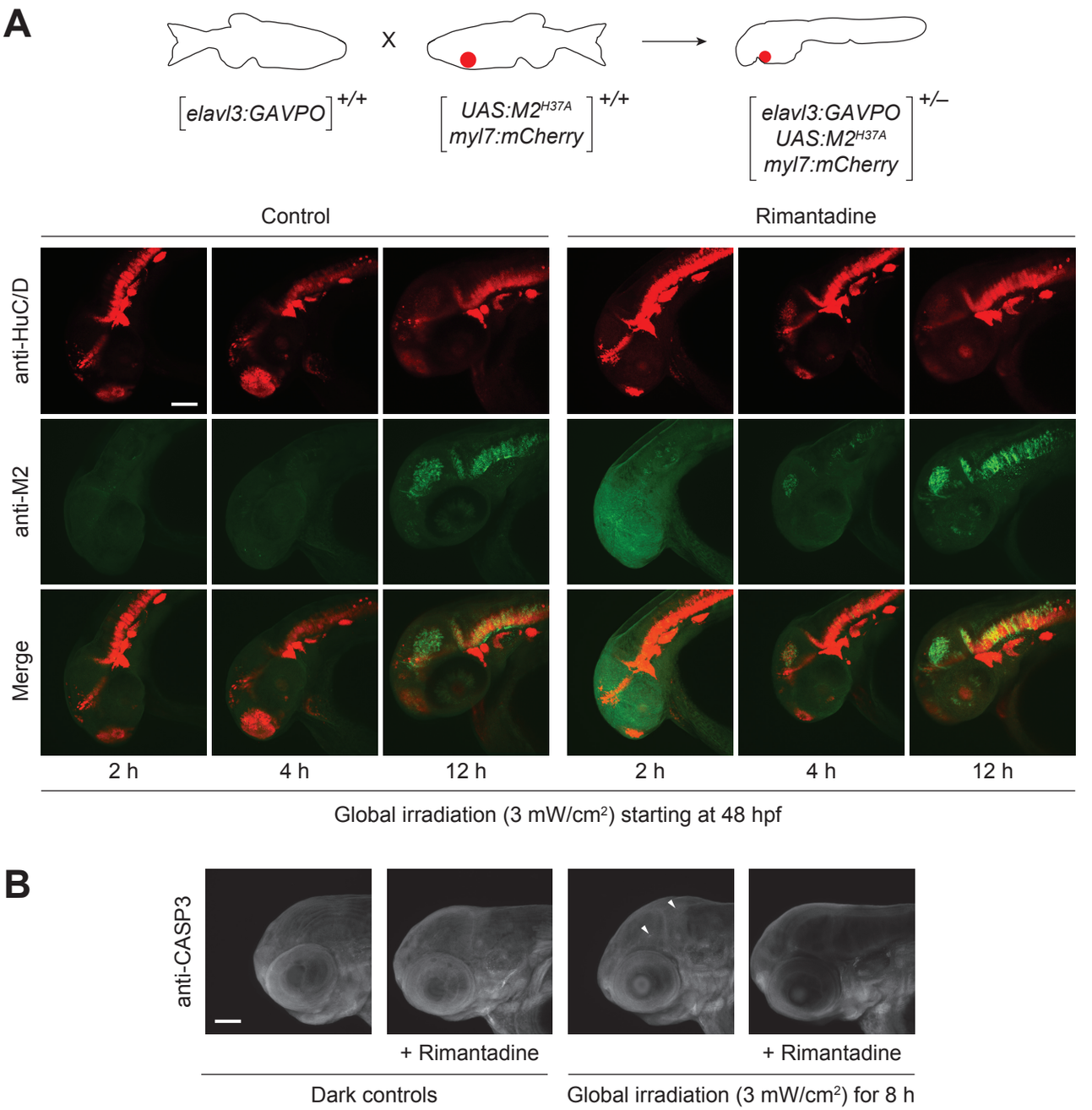

Figure S5. Light-inducible, neuron-specific M2^{H37A} expression using the GAVPO system. (A) *Tg(elavl3:GAVPO; UAS:M2^{H37A};myl7:mCherry)* embryos were globally irradiated with blue LED light at 48 hpf for the designated durations and cultured in the presence or absence of rimantadine. The embryos were then fixed, and immunostained for M2 and HuC/D expression. Representative confocal micrographs are shown as maximum intensity projections of 39 to 51 z-stack images (5-µm optical sections), demonstrating overlaying domains of M2^{H37A} and HuC/D expression in the anterior CNS. (B) *Tg(elavl3:GAVPO;UAS:M2^{H37A};myl7:mCherry)* embryos were globally irradiated with blue LED light at 48 hpf for 8 h and cultured in the presence or absence of rimantadine. The embryos were fixed 12 h later, and immunostained for activated caspase-3. Representative confocal micrographs are shown as maximum intensity projections of 30 to 50 z-stack images (7-µm optical sections), demonstrating limited non-cell autonomous apoptosis. Arrowheads: cells with activated caspase-3. Embryo orientations: lateral view, anterior left. Scale bars: 1-0 µm.