Figure S1.

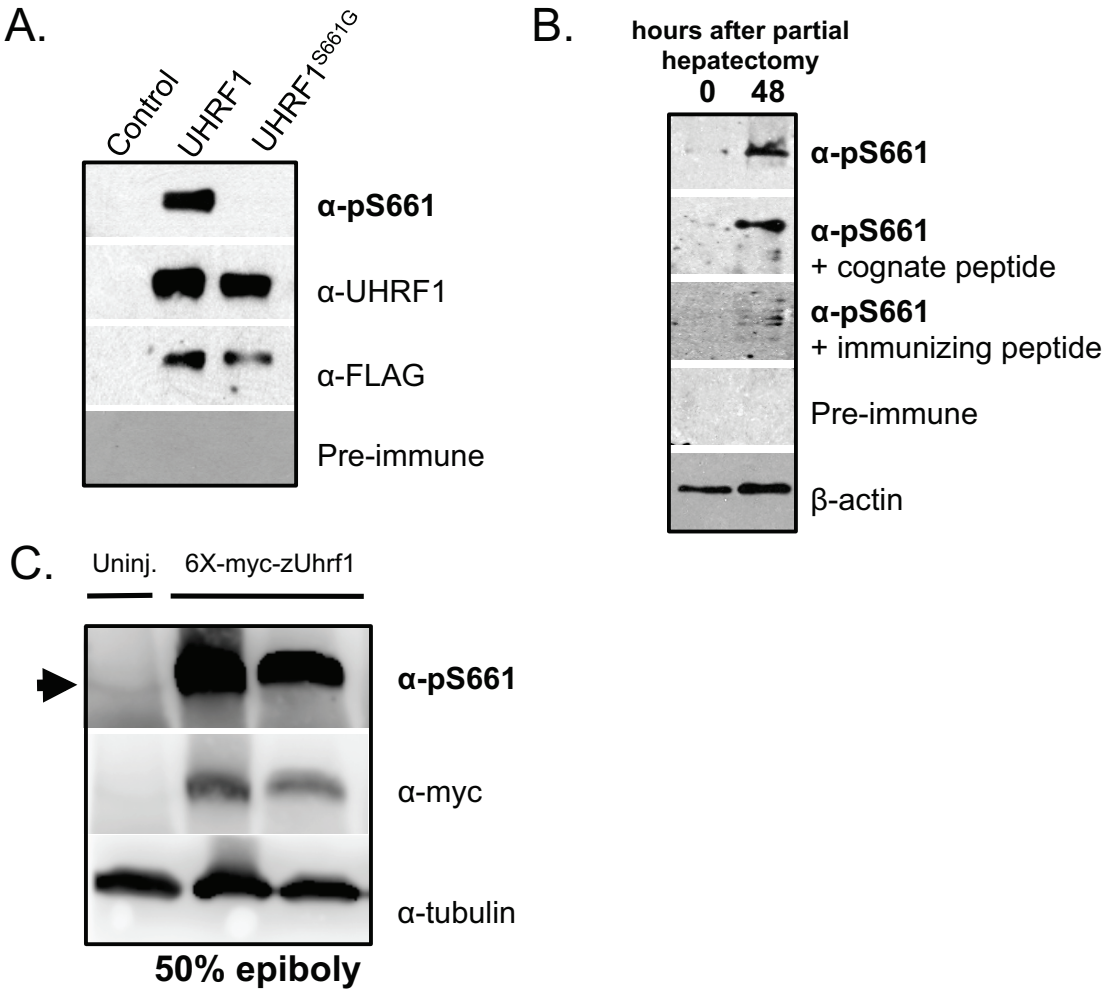

Figure S1. α-pS661 does not detect UHRF1^{S661G} and recognizes endogenous UHRF1 in multiple organisms. **(A.)** 293T cells transfected with empty plasmid (CONTROL), a plasmid with FLAG-tagged wild-type (UHRF1) or mutant with Ser-661 changed to a glycine (UHRF1S661G). α-pS661 specifically recognizes a protein of appropriate size in FLAG-UHRF1 but not in plasmid or FLAG-UHRF1^{S661G} transfected cells, whereas both FLAG-UHRF1 and FLAG-UHRF1^{S661G} are both recognized by α-UHRF1 and α-FLAG. No reactivity occurs with pre-immune (PI) serum. In this preparation of cells, endogenous UHRF1 is not detectable. **(B.)** Liver samples collected from mice following partial hepatectomy at 0 and 48 hours after surgery were blotted with α-pS661 that was either incubated with a cognate (non-phosphorylated peptide), the phosphorylated peptide (immunogen) or with anti-actin as a loading control. α-pS661 recognizes a protein enriched 48 hours after PH and addition of the immunizing peptide blocks antibody binding (panel 4) while the cognate peptide does not (panel 3). **(C.)** α-pS661 detects endogenous (arrow) and exogenous phosphorylated UHRF1 in early embryos. Lane 1: uninjected embryos. Lane 2 and 3: embryos injected with 100 and 200 pg of 6X-myc-zUhrf1 mRNA, respectively. α-myc detects only exogenous mRNA. Tubulin as loading control.

Fig S2.

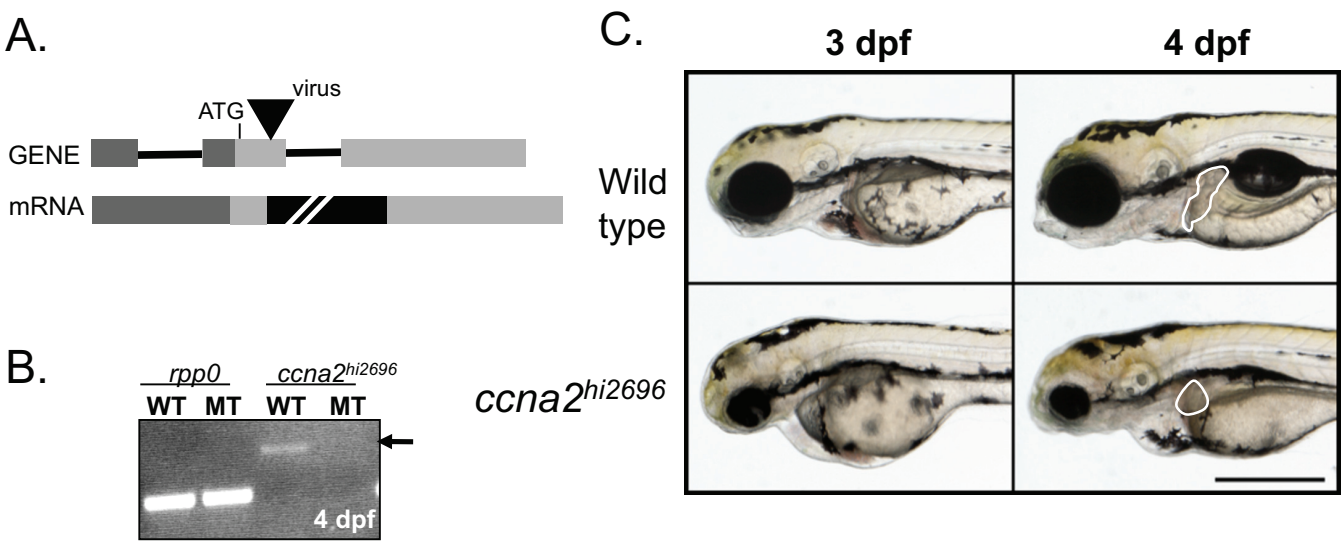

Figure S2. *ccna2* mutation causes developmental defects. (A.) A retroviral insertion located in the 2nd exon (1st coding) of the *ccna2* gene is predicted to cause an interrupted mRNA. Solid boxes depict exons, with the light grey indicating protein coding sequence. Not to scale. **(B.)** On 4 dpf, PCR detects *rpp0* transcript in phenotypic wild-type (WT) siblings as well as in *ccna2*^{hi2696} mutants (MT) but the *ccna2* transcript is only detected in WT larvae (arrow). **(C.)** The phenotype of *ccna2*^{hi2696} mutants on 3 and 4 dpf includes small eye, small jaw, and underdeveloped brain and a small liver (outlined). Scale bar = 500 μm.

Figure S3.

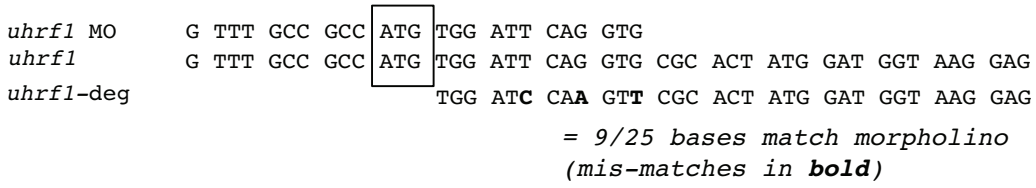

Figure S3. ***uhrf1*** **morpholino targets endogenous zebrafish uhrf1 but not 6X-myc-zUHRF1 mRNA.** Sequence data surrounding start site of translation for *uhrf1* MO, zUhrf1, and 6X-myc-zUhrf1 mRNA (zUHRF1deg). Degenerate base pairs are in bold.

Figure S4

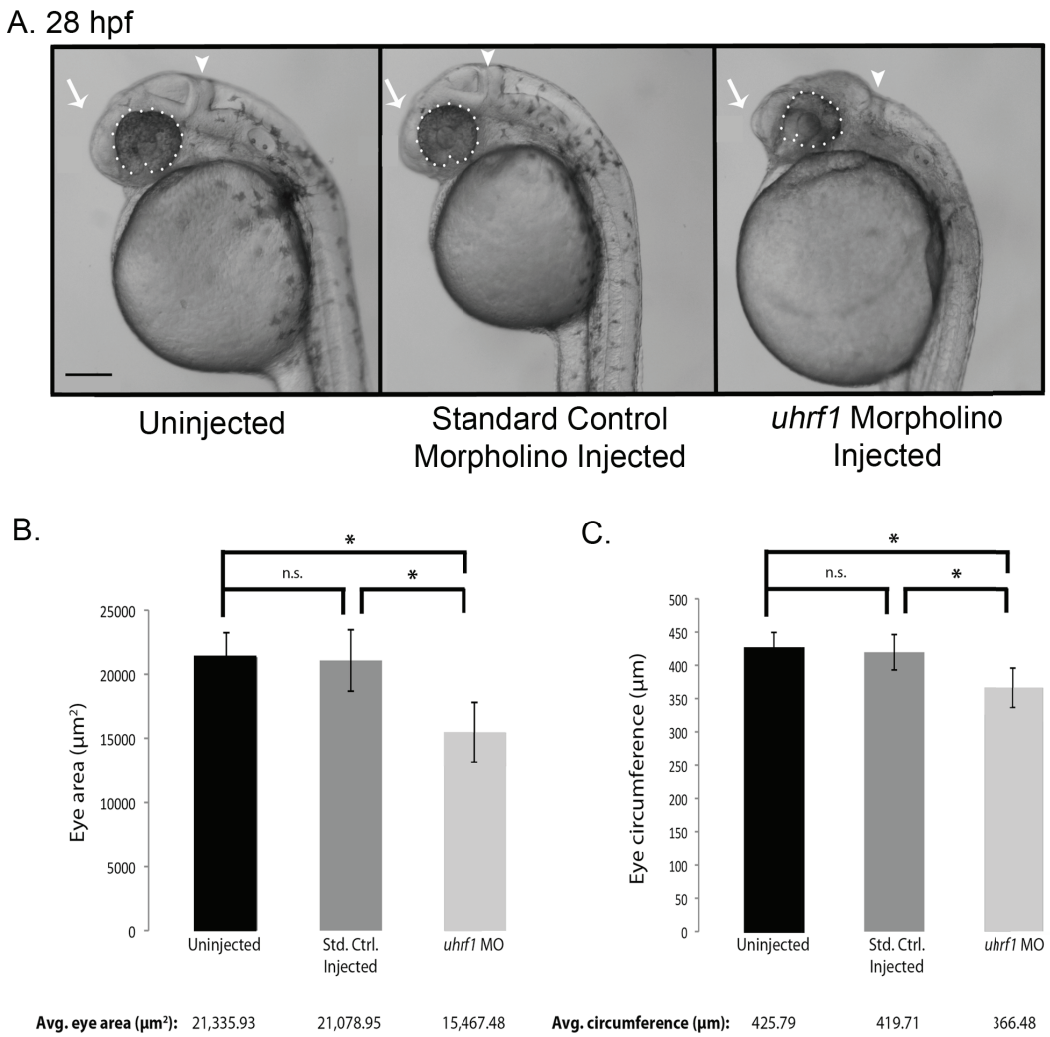

Figure S4. Knockdown of *uhrf1* in early embryos results in small eye size and disrupted midbrain/hindbrain barrier. (A.) At 28 hpf, uninjected, standard control morpholino injected, and *uhrf1* morpholino injected fish are shown. Arrowheads designate the midbrain/hindbrain barrier. Arrows point out the shape of the head of each. Dotted lines outline the shape of each respective eye. There are no evident differences between uninjected and standard control injected fish. *uhrf1* morphants have smaller eyes, misshapen heads, and a disrupted midbrain/hindbrain barrier. Scale bar = 100 µm. **(B.)** *uhrf1* morphant eyes display a significant reduction in area (μm^2) compared to uninjected and standard control eyes. Average eye area for each is indicated. Error bars correspond to the standard deviation. * indicates $p < .00002$ by Student's t-test. **(C.)** *uhrf1* morphant eyes display a significant reduction in eye circumference (µm) compared to uninjected and standard control eyes. Average eye circumference for each is indicated. Error bars correspond to the standard deviation. * indicates $p < .00023$ by Student's t-test.

Figure S5

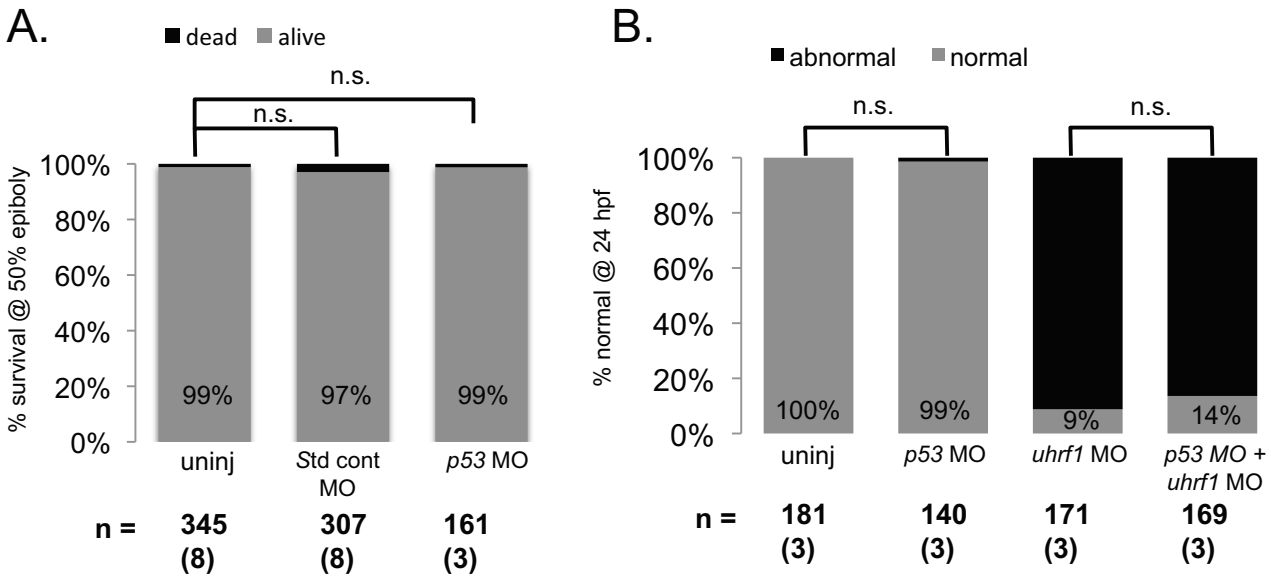

Figure S5. *p53* morpholino co-injection does not affect embryo survival at 50% epiboly or decrease *uhrf1* morphant phenotype at 24 hpf. (A.) *p53* morpholino (MO) injection does not affect survival at 50% epiboly. 1.68 pg of MO injected. p = 0.159 comparing uninjected to standard control MO. p = 0.670 comparing uninjected to *p53* MO by Fisher's exact test. **(B.)** There is no effect on *uhrf1* morphant phenotype at 24 hpf when co-injected with *p53* MO. p = 0.1717. 1.68 pg of all morpholinos were injected.

Figure S6.

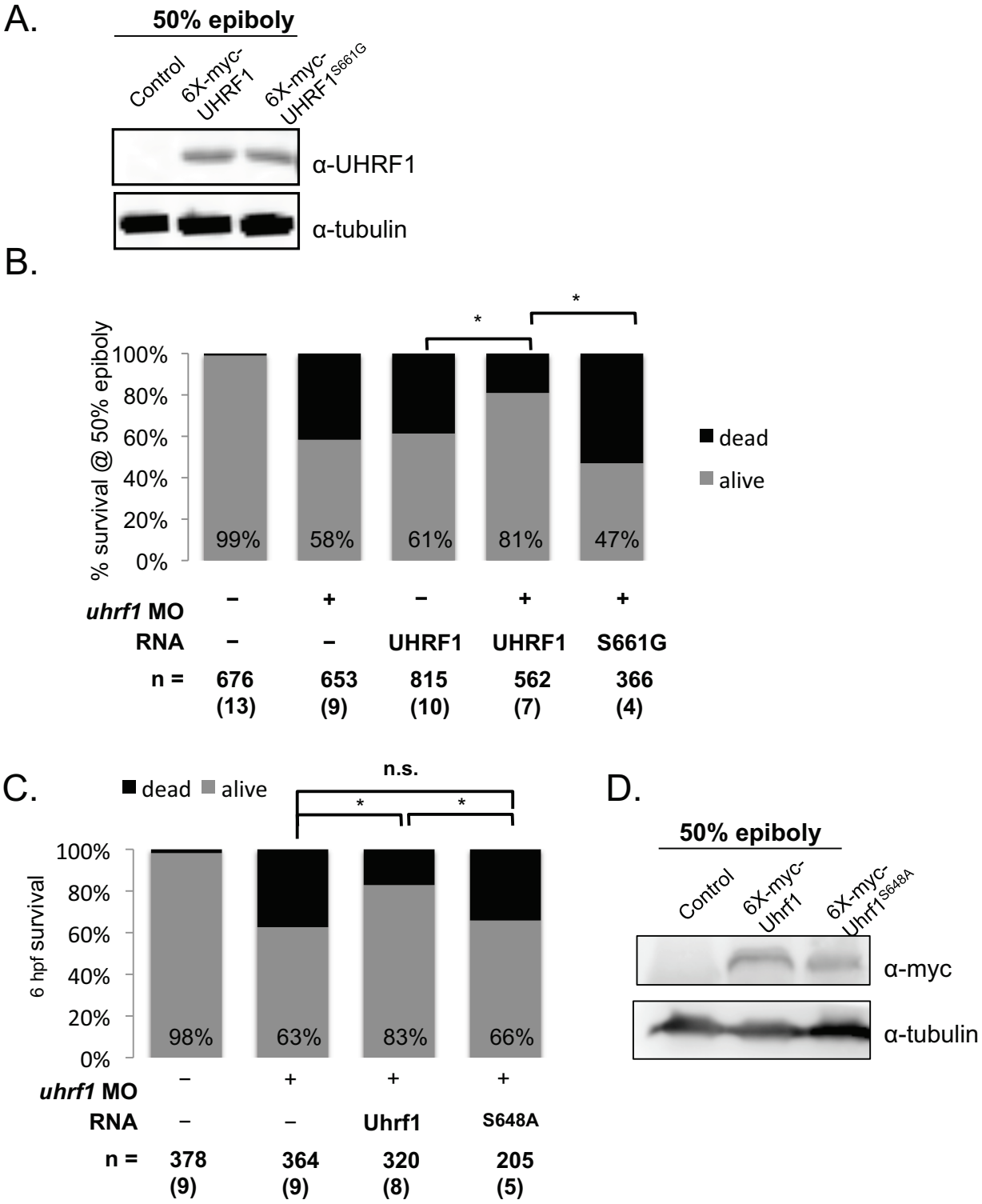
A.
50% epiboly
Control
6X-myc-UHRF1
6X-myc-UHRF1S661G
α-UHRF1
α-tubulin
B.
% survival @ 50% epiboly
100%
80%
60%
40%
20%
0%
99%
58%
61%
81%
47%
*
*
dead
alive
uhrf1 MO
RNA
n =
676 (13)
653 (9)
815 (10)
562 (7)
366 (4)
UHRF1
UHRF1
S661G
C.
dead
alive
n.s.
*
*
6 hpf survival
98%
63%
83%
66%
uhrf1 MO
RNA
n =
378 (9)
364 (9)
320 (8)
205 (5)
Uhrf1
S648A
D.
50% epiboly
Control
6X-myc-Uhrf1
6X-myc-Uhrf1S648A
α-myc
α-tubulin

Figure S6. UHRF1 must be phosphorylated to rescue embryonic survival. (A.) Western blot with α-UHRF1 of embryos at 50% epiboly injected with 400pg of mRNA encoding either full-length WT-UHRF1 or UHRF1^{S661G} . Tubulin is loading control. **(B.)** Co-injection of MO and mRNA encoding UHRF1^{S661G} mRNA fails to rescue mortality at 50% epiboly as compared to significantly improved survival with co-injection of MO and mRNA encoding UHRF1 ($p < 0.0001$ by Fisher's exact test comparing uhrf1 MO and co-injected with 6X-myc-UHRF1 mRNA). mRNA = 400 pg. The total number of embryos and experiments are noted under each bar. **(C.)** Co-injection of MO and mRNA encoding full-length zUhrf1, but not zUhrf1^{S648A}, significantly improves survival at 50% epiboly compared to MO alone. * represents $p<0.0001$ by Fisher's exact test, n.s. p value = 0.4681. 200pg mRNA injected. The total number of embryos noted under each bar from 8 experiments for zUhrf1 and 5 experiments for zUhrf1^{S648A}. **(D.)** Full-length zUhrf1 and zUhrf1^{S648A} are equally expressed at 50% epiboly after mRNA injection. 10 embryos were blotted with antibodies to myc and tubulin. mRNA = 200 pg.

Figure S7.

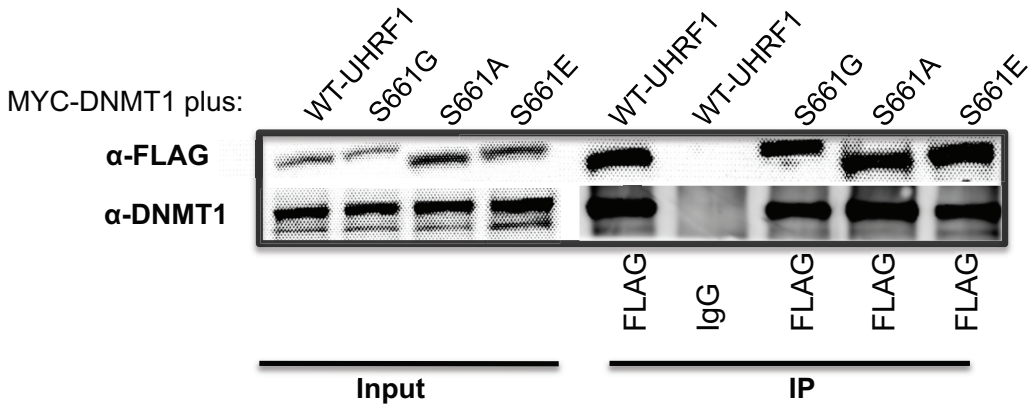

Figure S7. Phosphorylation of Ser-661 is not required for DNMT1 interaction. Coimmunoprecipitation of FLAG-UHRF1 and DNMT1. Control cells were not transfected. Input = 10% of the amount used for immunoprecipitation.

Figure S8.

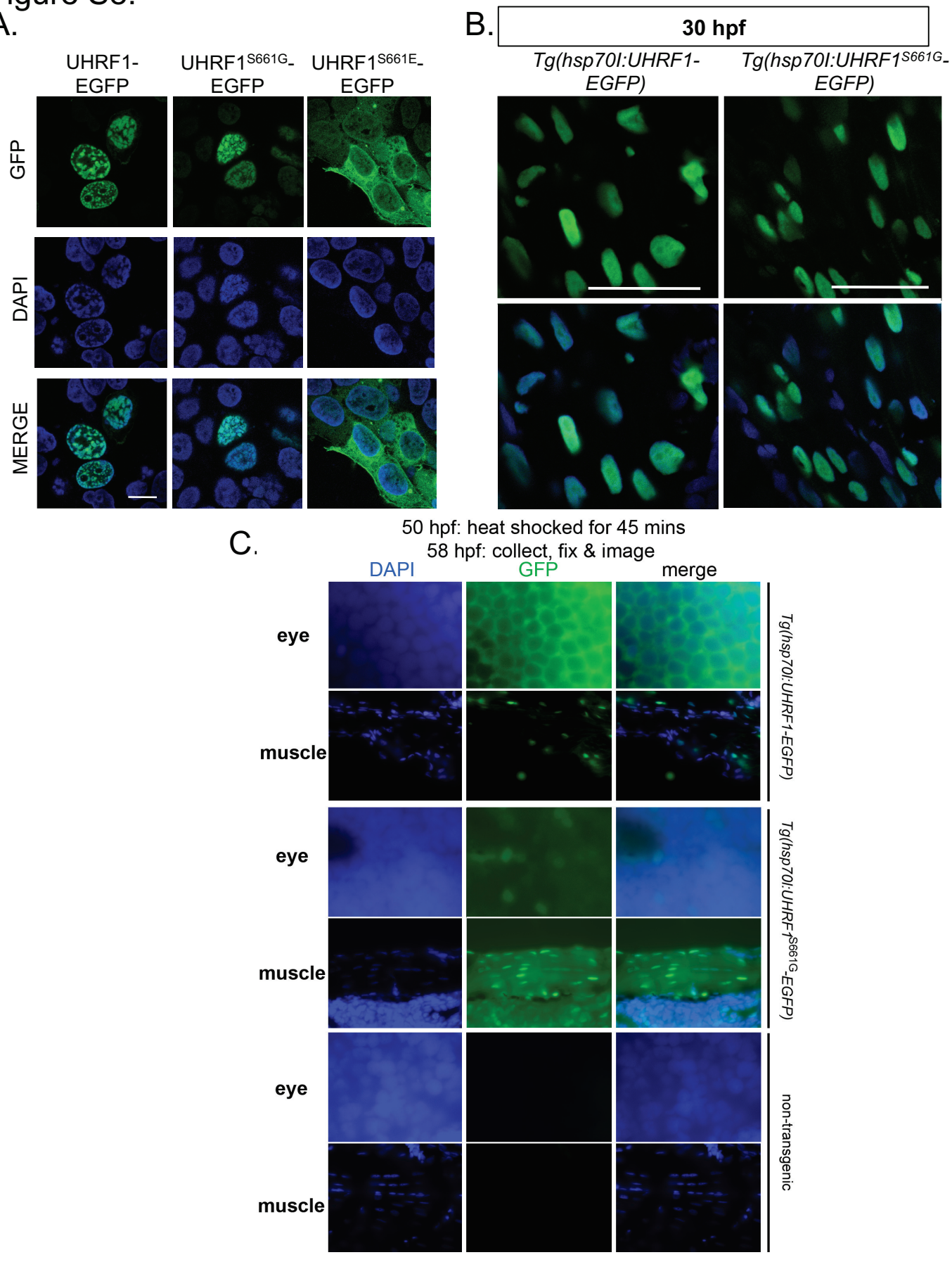
A.
UHRF1-EGFP
UHRF1S661G-EGFP
UHRF1S661E-EGFP
GFP
DAPI
MERGE
B.
30 hpf
Tg(hsp70l:UHRF1-EGFP)
Tg(hsp70l:UHRF1S661G-EGFP)
C.
50 hpf: heat shocked for 45 mins
58 hpf: collect, fix & image
DAPI
GFP
merge
eye
muscle
eye
muscle
eye
muscle
Tg(hsp70l:UHRF1-EGFP)
Tg(hsp70l:UHRF1S661G-EGFP)
non-transgenic

Figure S8. Phosphorylation is not required to localize UHRF1 to the nucleus. (A.) Top panel: GFP signal in transfected 293T cells. Middle panel: DAPI signal only. Bottom panel: Merge of top two panels. 63X magnification. Scale bar = 15 μm. (B.) Cryosections of 30 hpf *Tg(hsp70I:UHRF1-EGFP)* and *Tg(hsp70I: UHRF1^{S661G} -EGFP)* embryos visualized by confocal microscopy. Nuclear localization of both forms of UHRF1 is evident as they completely overlap with DAPI staining. Scale bar = 25 μm**.** (C.) *Tg(hsp70I:UHRF1-EGFP)* and *Tg(hsp70I: UHRF1^{S661G} -EGFP)* were heat shocked at 50 hpf and then collected at 58 hpf for sectioning and imaging using epifluorescence microscopy. Cells in the eye of *Tg(hsp70I:UHRF1-EGFP)* have predominantly cytoplasmic GFP while, in the same fish, the GFP in skeletal muscle cells of the trunk are nuclear. GFP in both the eye and tail cells of the *Tg(hsp70I: UHRF1^{S661G} -EGFP)* is nuclear.