SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Quantification of pH-dependent RNA editing at GluA2 sites minimally targeted by ADAR1 or ADAR2. Plotted values represent the means of three biological replicates (○) ± SD. Statistical significance between groups for each site in GluA2 and $5HT_{2C}$ transcripts was determined using the Holm-Sidak t-test for multiple comparisons; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ns, not significant.

Supplementary Figure 2. Quantification of $5HT_{2C}$ RNA editing profiles. The relative expression of $5HT_{2C}$ RNA isoforms constituting ≥1% of total $5HT_{2C}$ transcripts is presented. Permutations of editing are designated by the presence of adenosine (A) or guanosine (G) residues at sites A, B, E, C, and D within the sequenced cDNA library (AAAAA = non-edited; GGGGG = fully edited). Plotted values are the means of three biological replicates (○) ± standard deviation. Statistical significance between groups for each $5HT_{2C}$ isoform was determined using the Holm-Sidak t-test for multiple comparisons; *p ≤ 0.05; ***p ≤ 0.001; ****p ≤ 0.0001.

Supplementary Figure 3. Time course for the expression of $5HT_{2C}$ RNA editing isoforms at reduced pH. (**A**) Relative expression of $5HT_{2C}$ RNA editing isoforms (constituting ≥1% of total $5HT_{2C}$ transcripts) generated by ADAR1 or (**B**) ADAR2-mediated editing at control (pH 7.4, —●—) or acidic pH (pH 6.7, ---○---) after 1, 3, 6, 12, or 24 hours is presented. Permutations of editing are designated by the presence of adenosine (A) or guanosine (G) residues at sites A, B, E, C and D within the sequenced cDNA library (AAAAA = non-edited; GGGGG = fully edited). Plotted values are the means of three biological replicates ± standard deviation. Statistical significance between groups at a given time point was determined using Sidak's multiple comparisons test; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

Supplementary Figure 4. Time-dependent editing of Gli1 transcripts at reduced pH. (**A**) The predicted secondary structure of the Gli1 minigene-derived transcript is presented; editing sites are indicated in inverse lettering. (**B**) Quantification of ADAR1 or ADAR2-mediated Gli1 editing from HEK293T cells incubated with control media (pH 7.4) or under acidic conditions (pH 6.7) for 6 hours. (**C**) Quantification of the extent of ADAR1 or ADAR2-mediated Gli1 editing from HEK293T cells incubated with control media (pH 7.4) or under acidic conditions (pH 6.7) for 24 hours. Plotted values are the means of three biological replicates (each replicate is shown as ○) ± standard deviation. Statistical significance between groups for each Gli1 site was determined using the Holm-Sidak t-test for multiple comparisons; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001; ns, not significant.

Supplementary Figure 5. Effects of varying pH on site-selective $5HT_{2C}$ RNA editing. (**A**) The relationship between bicarbonate ($NaHCO_3$) concentration and the pH of cell culture medium after a 24-hour incubation at 37°C and 5% CO_2 is shown. Plotted values are the means of five independent pH measurements ± standard deviation. (**B**) Pairwise statistical comparisons for quantitative analyses of site-specific $5HT_{2C}$ editing at pH 6.7-7.4 (as presented in **Figure 3A-B**) are shown. Statistical significance was determined using two-way ANOVA with Tukey's multiple comparisons test.

Supplementary Figure 6. Expression of $5HT_{2C}$ RNA editing isoforms at varying pH. (**A**) Relative expression levels of $5HT_{2C}$ RNA isoforms (constituting ≥1% of total $5HT_{2C}$ transcripts) generated by ADAR1- or (**B**) ADAR2-mediated editing at varying pH (*top*); Pairwise statistical comparisons for quantitative analyses of relative $5HT_{2C}$ isoform expression. $5HT_{2C}$ isoform expression at each pH is compared to the percentage of that isoform at pH 7.4 (*bottom*).

Permutations are designated by the presence of adenosine (A) or guanosine (G) residues at sites A-E within the sequenced cDNA library (AAAAA = non-edited; GGGGG = fully edited). Plotted values on graphs are the means of three biological replicates (○) ± standard deviation. Statistical significance was determined using two-way ANOVA with Dunnett's multiple comparisons test.

Supplementary Figure 7. Changes in HeLa cell intracellular pH in response to incubation with acidic medium. Representative 20x confocal images of live HeLa cells incubated with control media (pH 7.4; *top*) or acidic media (pH 6.7; *bottom*) and double labelled with pHrodo Red AM Intracellular pH Indicator and BioTracker 488 Green Nuclear Dye.

Supplementary Figure 8. ADAR2 expression in HeLa cells. (**A**) Representative Western blot for ADAR2 (80 kDa) protein expression in HeLa cells incubated with control (pH 7.4) or acidic (pH 6.7) media for 24 hours. The migration positions of a loading control (β-actin) and a positive control (+ control) for ADAR2 protein from HEK293T cells transfected with an ADAR2 expression vector is shown. (**B**) Relative expression of ADAR2 mRNA from HeLa cells incubated with control (pH 7.4) or acidic media (pH 6.7). Plotted values represent the means of three biological replicates (○) ± SD. Statistical significance between groups was determined using unpaired t-test; **p ≤ 0.01.

Supplementary Figure 9. Changes in HeLa cell intracellular pH in response to niclosamide treatment. Representative 20x confocal images of live HeLa cells incubated with DMSO (*top*) or 5 μM niclosamide (*bottom*) and double labelled with pHrodo Red AM Intracellular pH Indicator and BioTracker 488 Green Nuclear Dye.

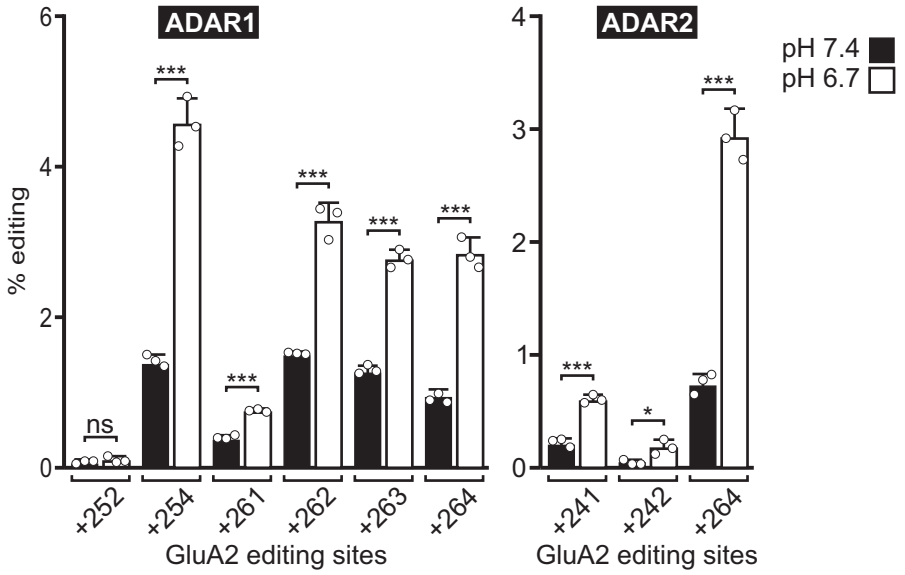
Supplementary Figure 10. Effects of niclosamide treatment in HEK292T cells. (**A**) Representative 20x confocal images of live HEK293T cells treated with 5 μM niclosamide (*bottom*) or an equivalent volume of vehicle (DMSO; *top*) for 6 hours and double labelled with pHrodo Red AM Intracellular pH Indicator and BioTracker 488 Green Nuclear Dye. (**B**) Quantification of normalized mean pHrodo Red fluorescence intensity per cell from HEK293T cells treated with 5 μM niclosamide or an equivalent volume of vehicle (DMSO); means ± SD (n = 106 DMSO-treated cells from three independent experiments and n = 109 niclosamide-treated cells from three independent experiments) were compared by unpaired t-test with Welch's correction; ****p < 0.0001. (**C**) Quantification of the extent of ADAR1 or (**D**) ADAR2-mediated $5HT_{2C}$ editing from cells treated with DMSO (vehicle) or 5 μM niclosamide. Plotted values represent the means of three biological replicates (○) ± SD. Statistical significance between groups for each $5HT_{2C}$ site was determined using the Holm-Sidak t-test for multiple comparisons; *p ≤ 0.05; **p ≤ 0.01; ns, not significant.

Supplementary Figure 11. Protonation-independent hydrogen bonding. A schematic diagram of the hydrogen bonding contact between E488 of ADAR2 and an orphan base (uridine) showing protonation-independent hydrogen bonding for wild-type ADAR2.

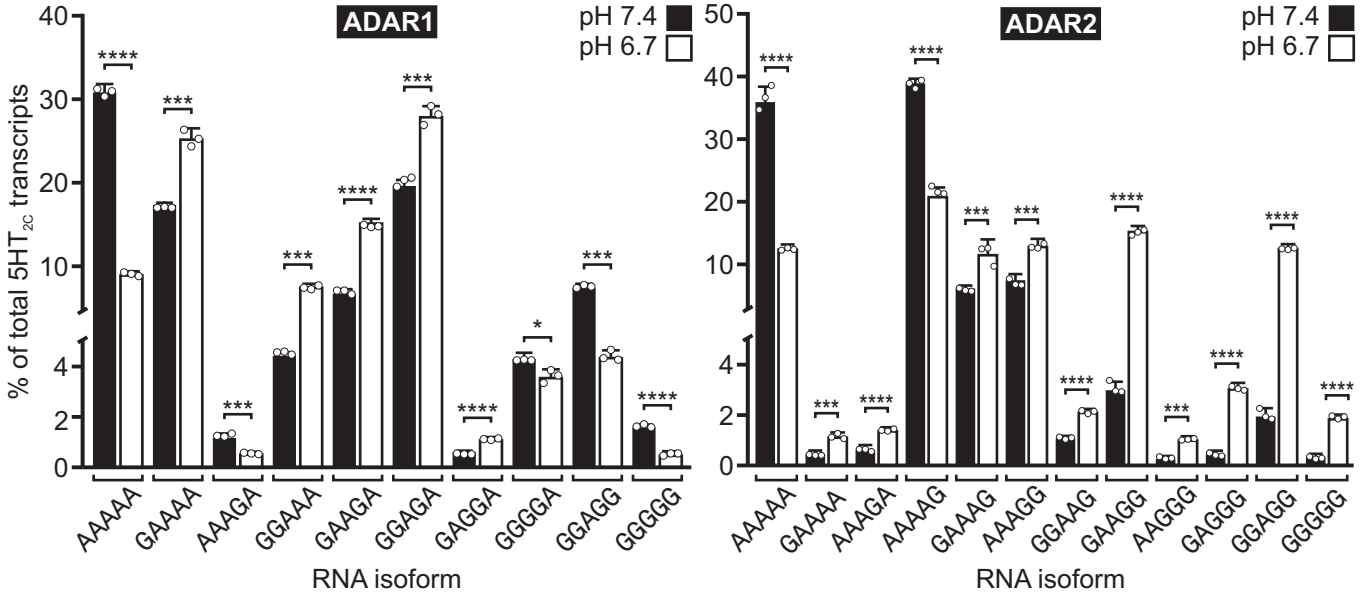
Supplementary Figure 12. Effects of hypoxia on endogenous A-to-I editing in Hela cells. (**A**) Representative electropherogram traces from Sanger sequencing of Cog3 or (**B**) Eef2k RT-PCR amplicons generated from HeLa cells incubated under normoxic (20% O_2) or hypoxic (1% O_2) conditions. The editing position is highlighted in yellow (*left*) and quantification of editing is presented (*right*). Plotted values are the means of three biological replicates (○) ± standard deviation. Statistical significance between groups was determined using the unpaired t-test with Welch's correction; ***p ≤ 0.001. (**C**) Representative Western blot for ADAR1 and ADAR2 protein expression levels from HeLa cells incubated under normoxic (20% O_2) or hypoxic (1% O_2)

conditions. ADAR2 protein expression from HEK293T cells transfected with an ADAR2 expression vector was included as a positive control (+ control) in the ADAR2 blot; β-actin (43 kDA) was used as a loading control. (**D**) Quantification of ADAR1 protein expression normalized to the β-actin loading control is presented. Plotted values are the means of three biological replicates (○) ± standard deviation. Statistical significance between groups was determined using the unpaired t-test with Welch's correction; ns, not significant.

Supplementary Figure 1



Supplementary Figure 2



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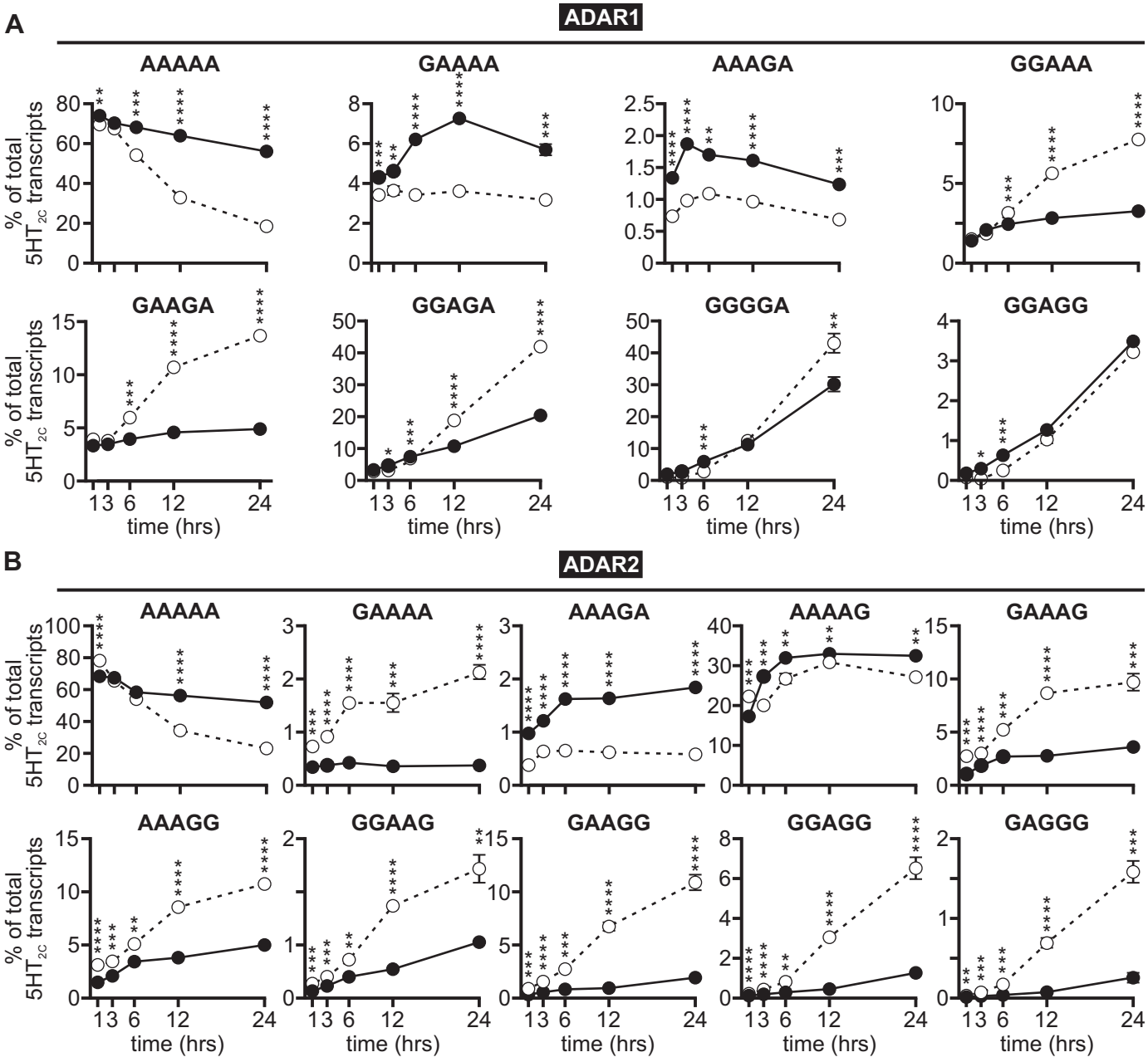

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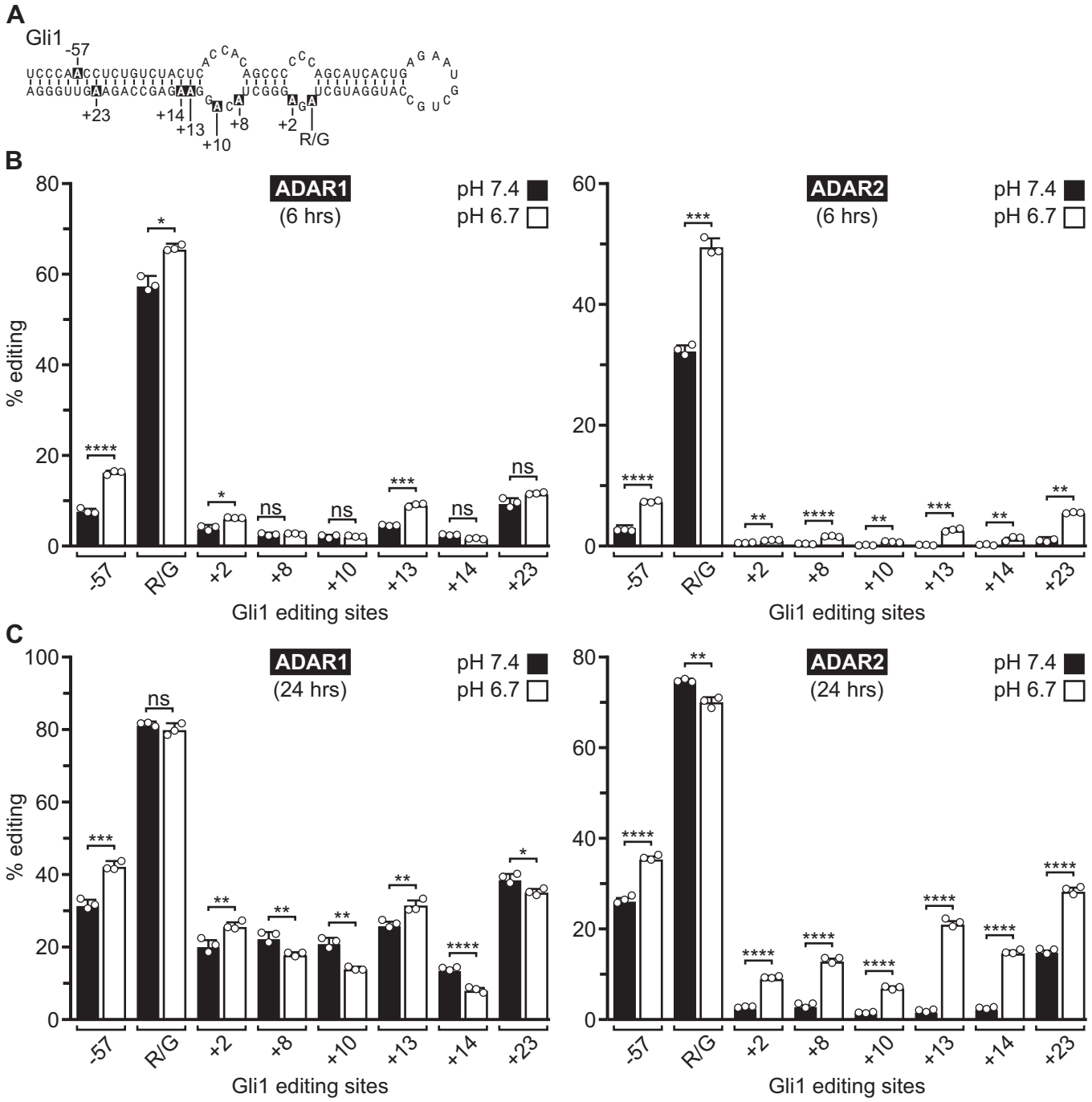

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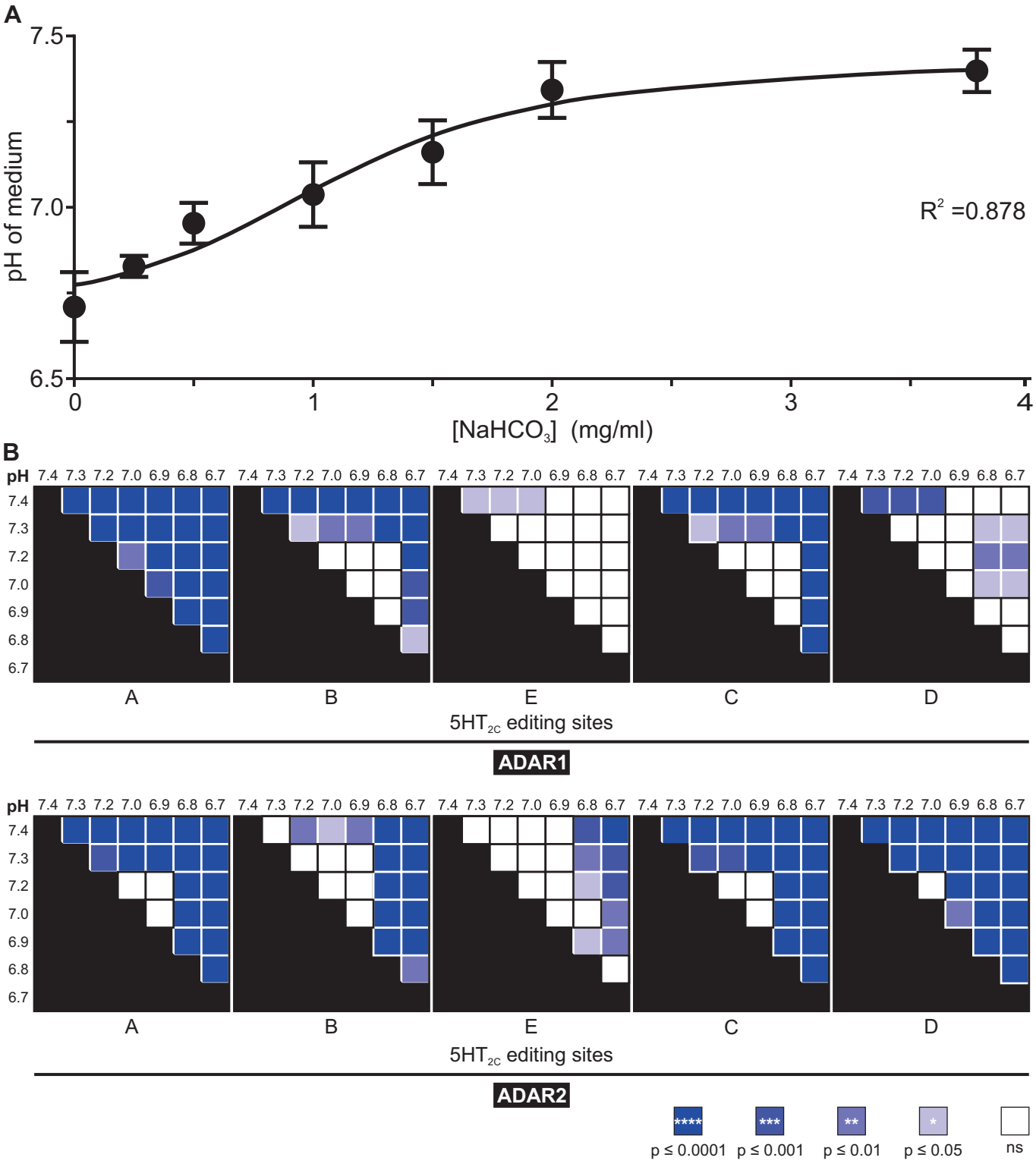

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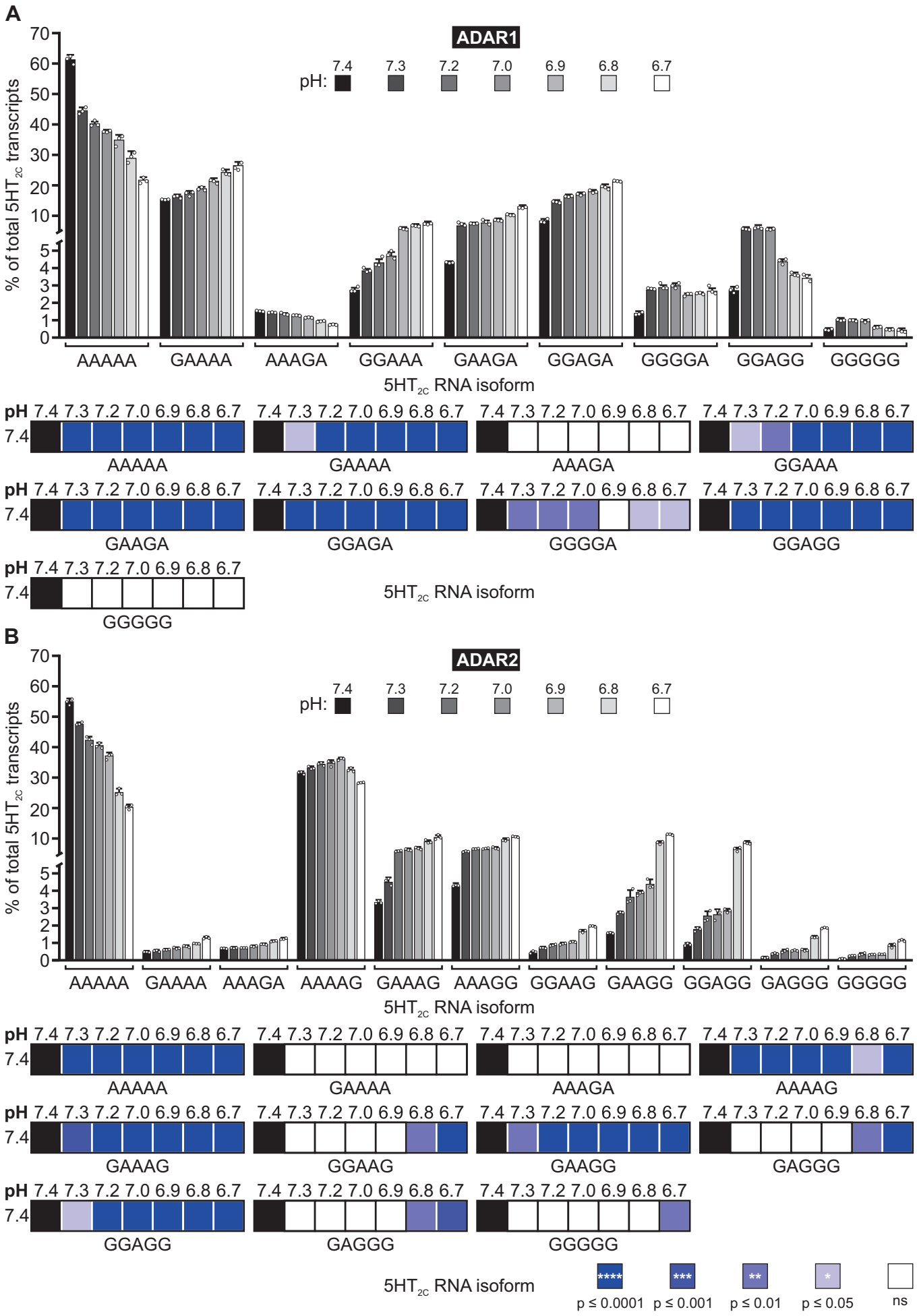

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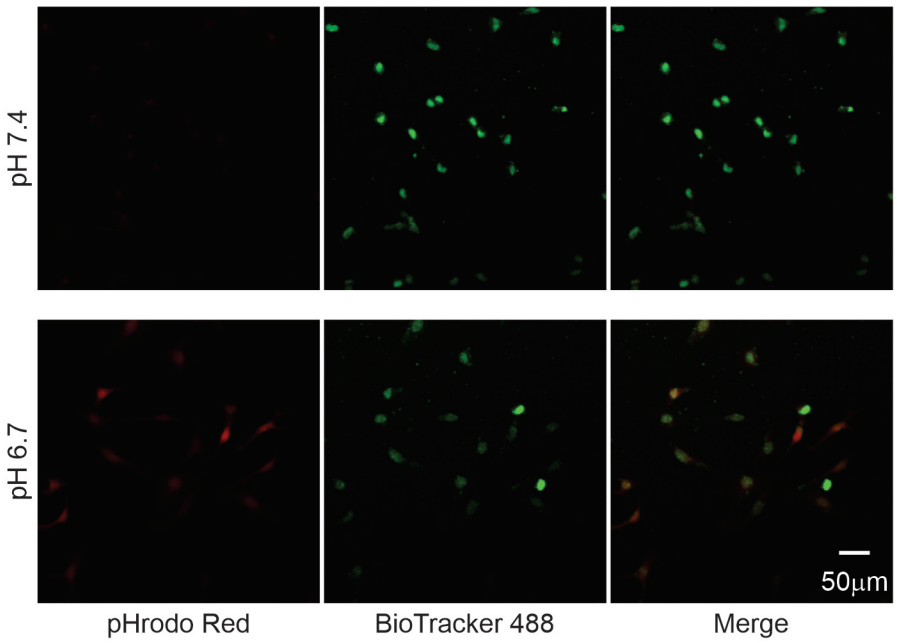

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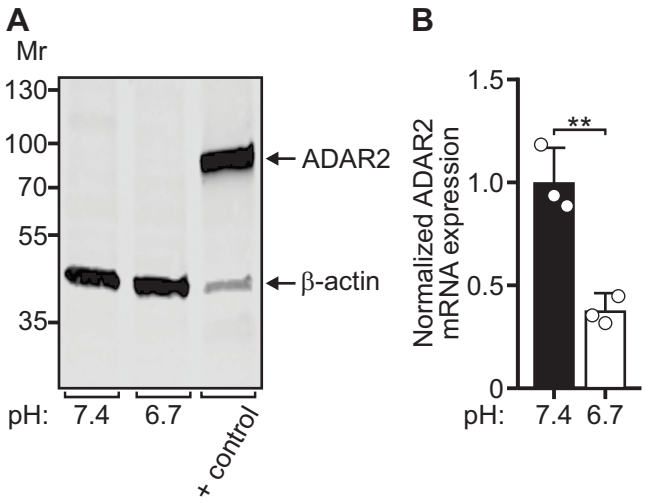

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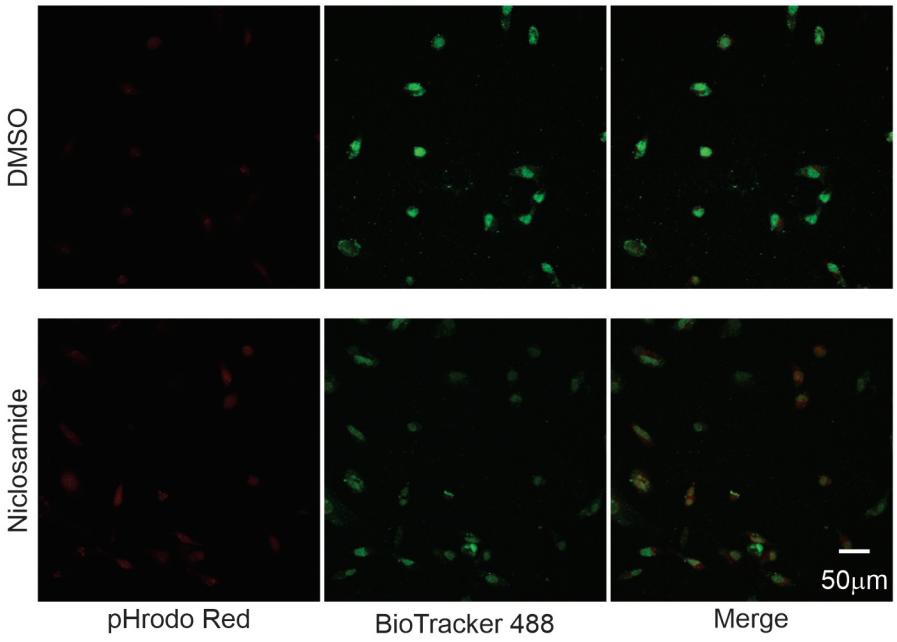

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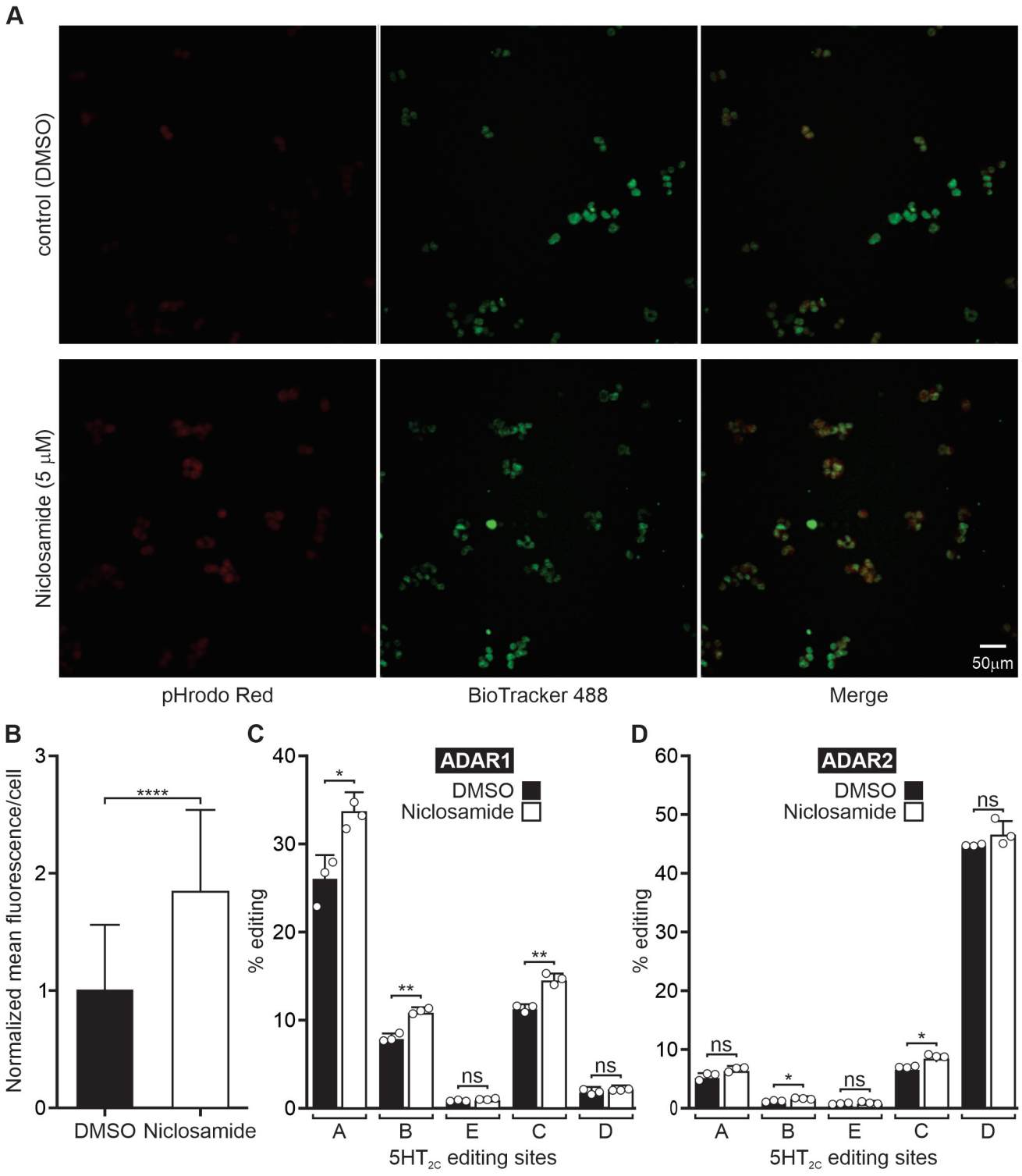

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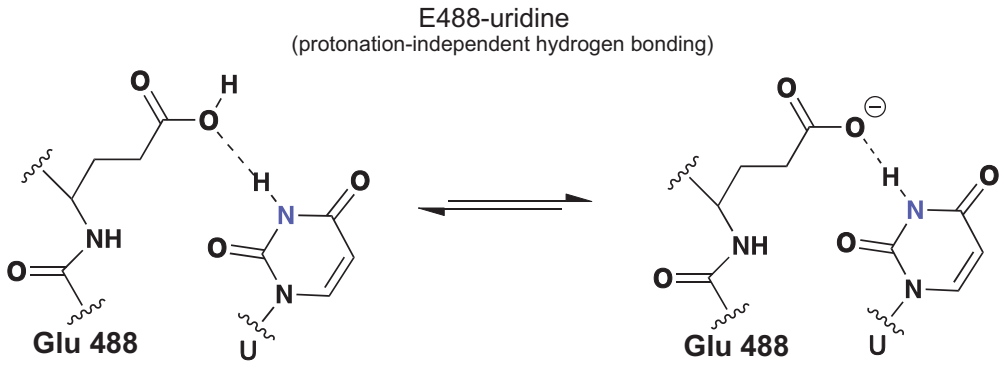

Supplementary Figure 12