Supporting Information for:

Visualizing enveloping layer glycans during zebrafish early embryogenesis

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General materials and methods. All chemical reagents were of analytical grade, obtained from commercial suppliers, and used without further purification. Flash chromatography was carried out using Silicycle P60 230-400 mesh silica gel according to the procedure described by Still (1). NMR spectra (^{1}H and ^{13}C) were obtained using a 600 MHz Bruker spectrometer. High-resolution electrospray ionization (ESI) mass spectra were obtained at the UC Berkeley QB3/Chemistry Mass Spectrometry Facility. Flow cytometry analysis was performed on a BD Biosciences FACSCalibur flow cytometer and analyses were performed using FloJo software. Cell viability was ascertained by gating the samples on the basis of forward and side scatter, and 5 x 10^4 live cells were analyzed for each sample. Wide-bore Pasteur pipets (borosilicate glass disposable serological pipets with wide tip) were purchased from Fisher Scientific. Dulbecco's phosphate-buffered saline (PBS) was obtained from HyClone Laboratories, fetal bovine serum (FBS) was obtained from Omega Scientific, and ethylene diamine tetraacetic acid (EDTA) was obtained from Gibco. Rhodamine-dextran (dextran, tetramethylrhodamine, 10,000 MW, lysine fixable) and aminooxy Alexa Fluor 488 were obtained from Invitrogen. Phenol red, *N*-phenylthiourea (PTU), pronase (protease, Type XIV, from *Streptomyces griseus*), tricaine (ethyl 3-aminobenzoate methanesulfonate), tris(2-carboxyethyl)phosphine (TCEP) hydrochloride, and UDP-GalNAc were obtained from Sigma-Aldrich. UDP-GalNAz (2), Ac_4GalNAz (3), ManNAz (4), Ac_4ManNAz (5), DIFO-488 (6), DIFO-555 (7), and DIFO-647 (6) were prepared as described previously. Confocal microscopy was performed at the UC Berkeley Molecular Imaging Center.

Synthesis of GalNAz. A flame-dried round bottom flask was charged with Ac_4GalNAz (20 mg, 0.047 mmol) and an N_2 atmosphere was established. Anhydrous MeOH (1.5 mL) was added,

followed by a catalytic amount of NaOMe (0.3 mg, 0.005 mmol). After 15 min, the reaction was quenched by addition of Amberlite and then filtered, rinsing with MeOH. The filtrate was concentrated on a rotary evaporator and purified by silica gel chromatography (gradient of 9:1 to 5:1 CH_2Cl_2:MeOH). The resultant oil was dissolved in water and lyophilized to afford a white solid (11 mg, 89% yield) as a 3:1 (α:ß) mixture of anomers. Thin-layer chromatography: R_f = 0.3 in 4:1 CH_2Cl_2:MeOH, visualized with either 10% H_2SO_4 in EtOH or ceric ammonium molybdate stain. ^{1}H NMR (600 MHz, D_2O) δ 5.23 (α, d, 1H, *J* = 3.6 Hz), 4.69 (ß, d, 1H, *J* = 8.5 Hz), 4.18 (α, dd, 1H, *J* = 11.0, 3.5), 4.10 (α, t, 1H, *J* = 6.2 ppm), 4.06 (α and ß, s, 2H), 3.99 (α, m, 1H), 3.94 (α, dd, 1H, *J* = 11.0, 3.0), 3.92 (ß, m, 2H), 3.74–3.79 (ß, m, 2H), 3.73 (α, d, 2H, *J* = 6.1), 3.68 (ß, dd, 2H, *J* = 7.5, 4.5). ^{13}C NMR (150 MHz, D_2O) δ 171.2, 170.8, 95.1, 90.9, 75.2, 70.8, 70.5, 68.5, 67.8, 67.3, 61.2, 60.9, 53.8, 52.0, 51.7, 50.3 ppm. HRMS (ESI^+) calcd for $C_8H_{14}O_6N_4Na$ 285.0809, found 285.0806.

Zebrafish stocks and husbandry. Wild-type AB-derived and H2A-GFP transgenic (8) zebrafish lines were kept at 28.5 °C on a 14-h light/10-h dark cycle. Embryos were obtained from natural spawnings and were maintained in embryo medium (150 mM NaCl, 0.5 mM KCl, 1.0 mM $CaCl_2$, 0.37 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 2.0 mM $MgSO_4$, 0.71 mM $NaHCO_3$ in deionized H_2O, pH 7.4). Embryos and larvae were incubated at 28–29 °C and were developmentally staged according to Kimmel and coworkers (9).

Image acquisition and analysis. Fluorescence and brightfield images shown in Figs. 5C and S16 and Movie S2 were acquired on an Olympus FV1000 laser scanning confocal microscope equipped with 488 nm, 559 nm, and 635 nm laser lines and a 60x/1.20 UPlanSApo water

immersion objective. Fluorescence and brightfield images shown in Fig. S13 were acquired on a Zeiss 200M epifluorescence microscope with a 10x air objective. All other fluorescence and brightfield images were acquired on a Zeiss LSM 510 META laser scanning confocal microscope equipped with 488 nm, 543 nm, and 633 nm laser lines. For Figs. 1C–D, 3B, 4B, 5A, S2–9, S10 (top panels), S11, S14A, and S15A, images were acquired using a 10x/0.30 Plan-Neofluor air objective with focus step sizes ranging from 5 to 7.5 μm. For Fig. S17, images were acquired using a 20x/0.5 Achroplan water dipping objective with a 2.5 μm step size. For Fig. 5B, images were acquired using a 40x/0.80 Achroplan IR water dipping objective with a 0.5 μm step size. All other images obtained on the Zeiss LSM 510 microscope were acquired using a 63x/0.95 Achroplan IR water dipping objective with a 0.5 μm step size. All images were analyzed using Slidebook 4.2 (Intelligent Imaging Innovations), and movies were assembled using Adobe Photoshop CS4.

Flow cytometry of zebrafish embryos. Zebrafish embryos were microinjected with 1 nL of a 25 mM solution of GalNAz or no sugar and phenol red (0.1% w/v) in 0.2 M KCl. At 3 hpf, the embryos were manually cleaned and dechorionated with pronase, then allowed to develop at 28.5 ºC. At 10 hpf, the embryos were reacted with DIFO-488 (100 μL of a 100 μM solution in embryo medium) for 1 h at 28.5 ºC. Following this reaction, the embryos were rinsed six times in embryo medium, then deyolked by passing through a 200 μL pipette tip in low-calcium Ringer's solution (116 mM NaCl, 2.6 mM KCl, 5 mM HEPES, pH 7.0). After a 5 min incubation in Ringer's, the embryos were transferred to a solution of 5 mM EDTA (pH 8.0) in PBS and incubated for 15 min at 28.5 ºC to dissociate the cells. The reaction was stopped by the addition of 5% FBS and 1 mM $CaCl_2$ in PBS. The cells were rinsed once with labeling buffer (PBS, pH

7.6, 1% FBS), then reacted with DIFO-647 (100 µL of a 100 µM solution in labeling buffer) for 30 min at 22 ºC. Following this reaction, the cells were rinsed three times with suspension buffer (PBS, pH 7.6, 1% FBS, 0.8 mM $CaCl_2$). After the final wash, the cells were resuspended in 800 µL of suspension buffer and passed through a 35 µm filter before analysis by flow cytometry.

Labeling of GalNAz-containing glycans at 84 and 96 hpf. Zebrafish embryos at the one-cell or two-cell stage were microinjected into the yolk cell with 1 nL of a 25 mM solution of GalNAz or no sugar and phenol red (0.1% w/v) in 0.2 M KCl. The embryos were dechorionated using pronase and allowed to develop at 28.5 °C in embryo medium containing PTU. At 84 hpf, the embryos were transferred to a flat-bottomed 96-well plate containing DIFO-488 (100 µL of a 100 µM solution in embryo medium) and allowed to react for 1 h at 28.5 ºC. After the labeling reaction, the embryos were rinsed six times with embryo medium. The embryos were then reacted with 50 mM TCEP (pH 7.4) in embryo medium for 10 min at 28.5 °C. Following this reaction, the embryos were rinsed eight times with embryo medium and transferred to fresh PTU-containing embryo medium to develop for an additional 11 hours at 28.5 °C. At 96 hpf, the embryos were reacted with DIFO-555 (100 µL of a 100 µM solution in embryo medium) and allowed to react for 1 h at 28.5 ºC.

Dual labeling of sialic acids using Ac_4ManNAz and $NaIO_4$. Zebrafish embryos were allowed to develop to 3 hpf, at which point they were dechorionated using pronase. The embryos were then incubated for three days at 28.5 ºC in embryo medium containing 5 mM Ac_4ManNAz and PTU. At 72 hpf, the embryos were transferred to a freshly-made solution of $NaIO_4$ (500 µM) or no reagent in embryo medium and incubated for 30 min at 22 ºC. The embryos were then

transferred to a Petri dish containing 10 mM glycerol for 2 min to quench any excess $NaIO_4$, followed by another transfer to a dish containing PBS, pH 6.7. Embryos were then transferred to a flat-bottom 96-well plate containing a solution of 100 μM AO-488 and 100 μM DIFO-555 (in 100 μL PBS, pH 6.7) and allowed to react for 1 h at 28.5 ºC.

Detection of GalNAz-labeled glycans in dividing cells with two copper-free click chemistry reactions in rapid succession. Zebrafish embryos at the one-cell or two-cell stage were microinjected into the yolk cell with 1 nL of a 25 mM solution of GalNAz or no sugar and phenol red (0.1% w/v) in 0.2 M KCl. The embryos were dechorionated using pronase and allowed to develop at 28.5 °C in embryo medium. At 10 hpf, the embryos were transferred to a flat-bottomed 96-well plate containing DIFO-555 (100 μL of a 100 μM solution in embryo medium) and allowed to react for 1 h at 22 ºC. After the labeling reaction, the embryos were rinsed twice in embryo medium, then immediately transferred to a solution containing DIFO-488 (100 μL of a 100 μM solution in embryo medium) and allowed to react for 15 min at 22 ºC.

References.

1. Still WC, Kahn M, & Mitra A (1978) Rapid chromatographic technique for preparative separations with moderate resolution. *J Org Chem* 43:2923-2925.
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5. Laughlin ST*, et al.* (2006) Metabolic labeling of glycans with azido sugars for visualization and glycoproteomics. *Methods Enzymol* 415:230-250.
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9. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, & Schilling TF (1995) Stages of embryonic development of the zebrafish. *Dev Dyn* 203:253-310.

Supporting Figures.

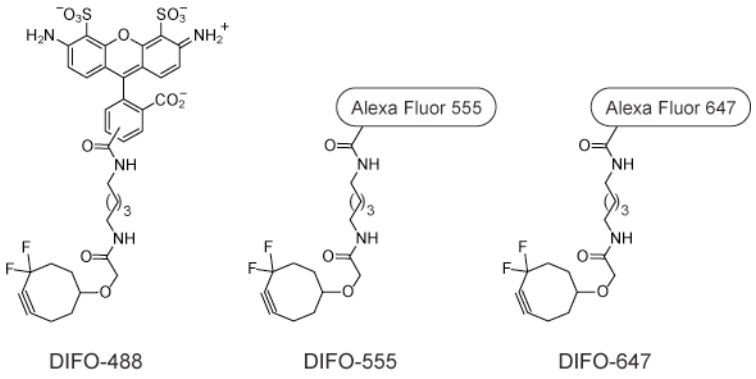


Figure S1. Structures of DIFO-Alexa Fluor conjugates used in this study.

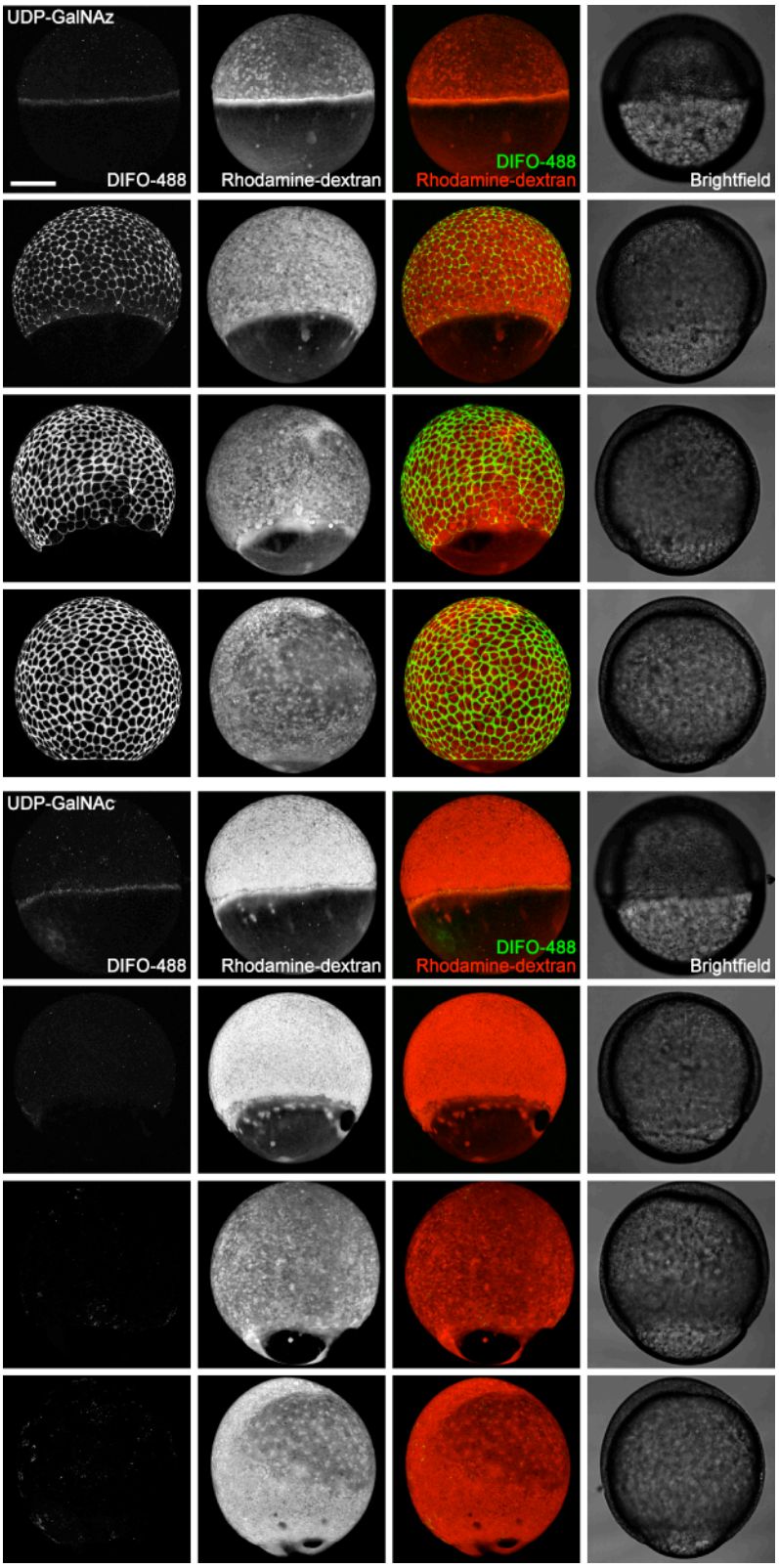

UDP-GalNAz
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield
UDP-GalNAc
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield

Figure S2. *In vivo* imaging of glycans during epiboly using microinjection of UDP-GalNAz and detection with copper-free click chemistry. Zebrafish embryos were microinjected with 25 pmol of UDP-GalNAz (top) or UDP-GalNAc (bottom), along with the tracer dye rhodamine-dextran, and allowed to develop to 50% epiboly (5.5 hpf, 1st and 5th rows), 65% epiboly (7 hpf, 2nd and 6th rows), 80% epiboly (8.5 hpf, 3rd and 7th rows), or 95% epiboly (9.5 hpf, 4th and 8th rows). Embryos were then reacted with DIFO-488 (100 μM, 1 h) and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Embryos are orientated with the animal pole up, but randomly with respect to the dorsal-ventral axis. Faint azide-independent signal at 50% epiboly is due to nonspecific uptake of DIFO-488 by rapidly endocytosing dorsal forerunner cells. Green, DIFO-488; red, rhodamine-dextran. Scale bar: 200 μm.

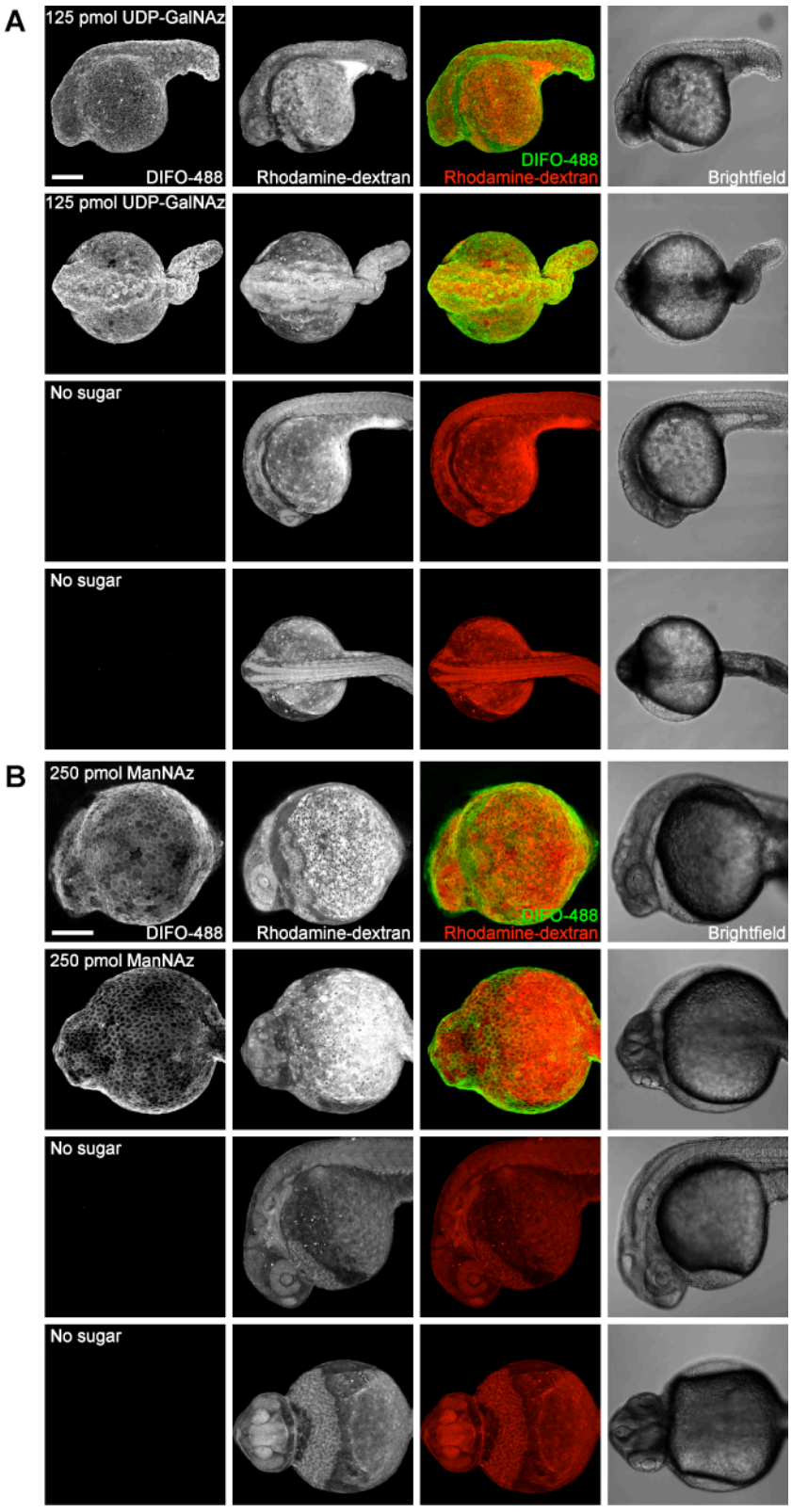

A
125 pmol UDP-GalNAz
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield
125 pmol UDP-GalNAz
No sugar
No sugar
B
250 pmol ManNAz
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield
250 pmol ManNAz
No sugar
No sugar

Figure S3. Microinjection of higher doses of azidosugars. Zebrafish embryos were microinjected with 125 pmol of UDP-GalNAz (A, top), 125 pmol of UDP-GalNAc (A, bottom), 250 pmol of ManNAz (B, top), or no sugar (B, bottom), along with the tracer dye rhodamine-dextran. The embryos were allowed to develop to 24 hpf, reacted with DIFO-488 (100 μM, 1 h), and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, DIFO-488; red, rhodamine-dextran. Scale bar: 200 μm.

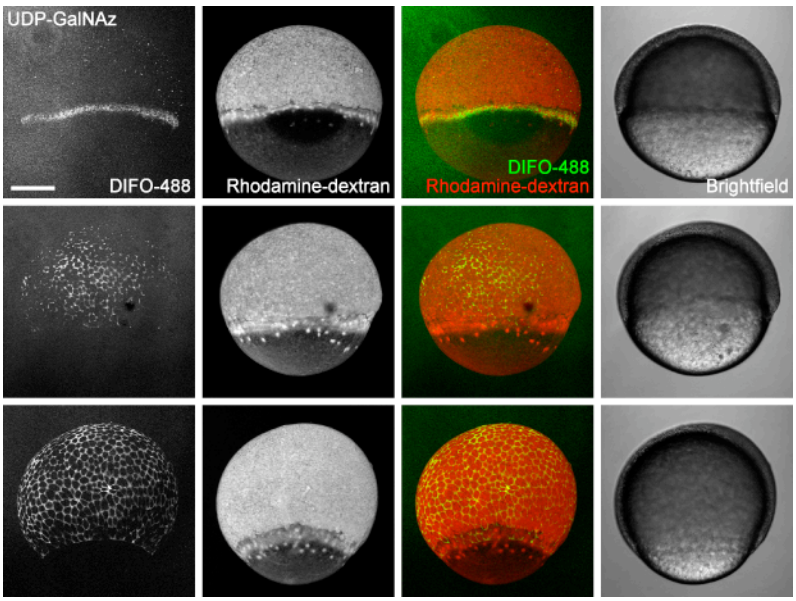


Figure S4. Manual dechorionation with forceps does not afford labeling before 65% epiboly. Zebrafish embryos were microinjected with 25 pmol of UDP-GalNAz and the tracer dye rhodamine-dextran, manually dechorionated using forceps at 3 hpf, and allowed to develop to 50% epiboly (5.5 hpf, 1st row), 65% epiboly (7 hpf, 2nd row), or 80% epiboly (8.5 hpf, 3rd row). Embryos were then reacted with DIFO-488 (100 µM, 1 h) and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, DIFO-488; red, rhodamine-dextran. Scale bar: 200 µm.

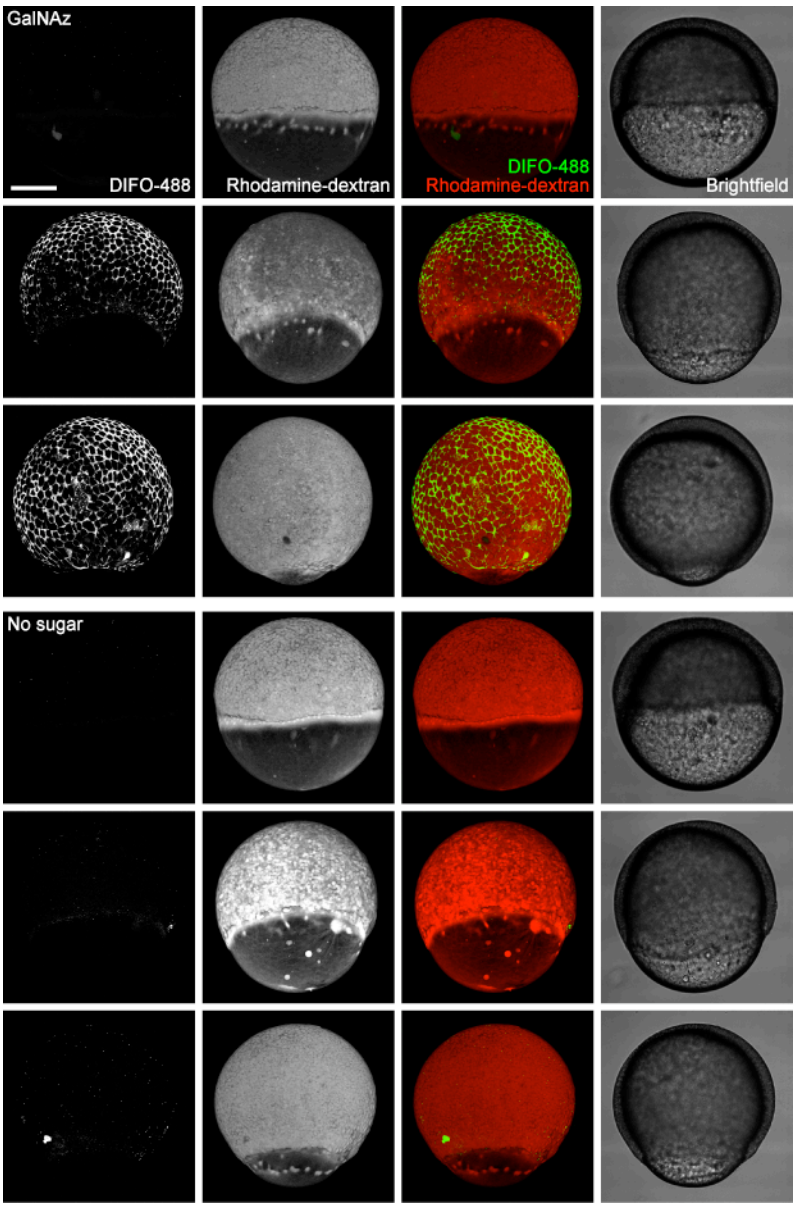

GalNAz
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield
No sugar

Figure S5. *In vivo* imaging of glycans during epiboly using microinjection of GalNAz and detection with copper-free click chemistry. Zebrafish embryos were microinjected with 25 pmol of GalNAz (top) or no sugar (bottom), along with the tracer dye rhodamine-dextran, and allowed to develop to 50% epiboly (5.5 hpf, 1st and 4th rows), 65% epiboly (7 hpf, 2nd and 5th rows), or 95% epiboly (9.5 hpf, 3rd and 6th rows). Embryos were then reacted with DIFO-488 (100 μM, 1 h) and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, DIFO-488; red, rhodamine-dextran. Scale bar: 200 μm.

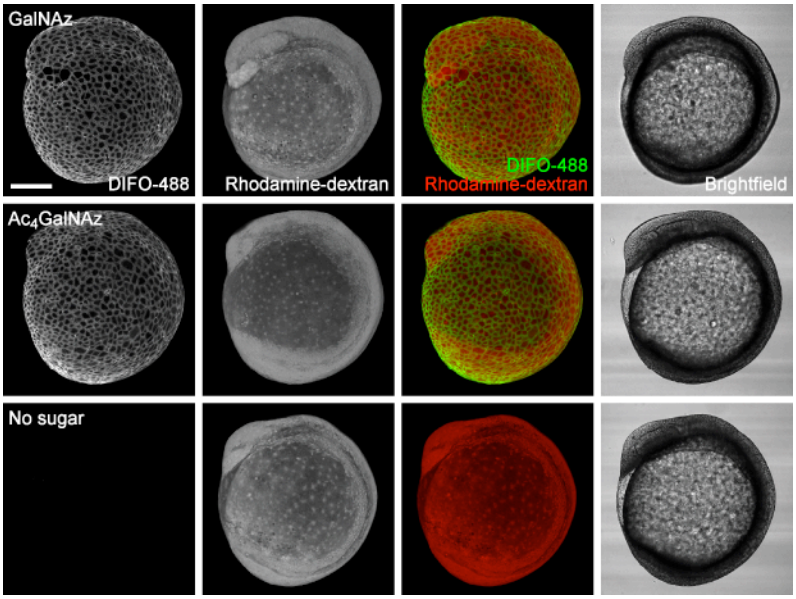
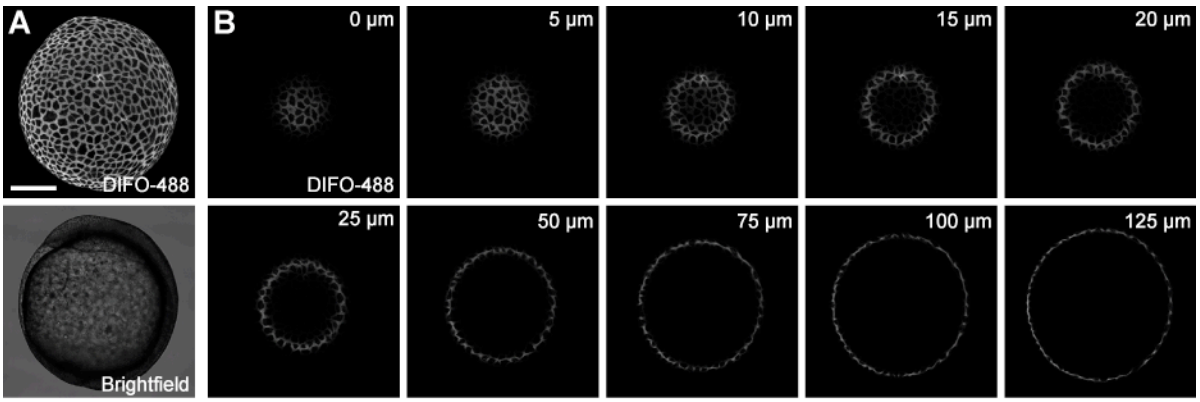
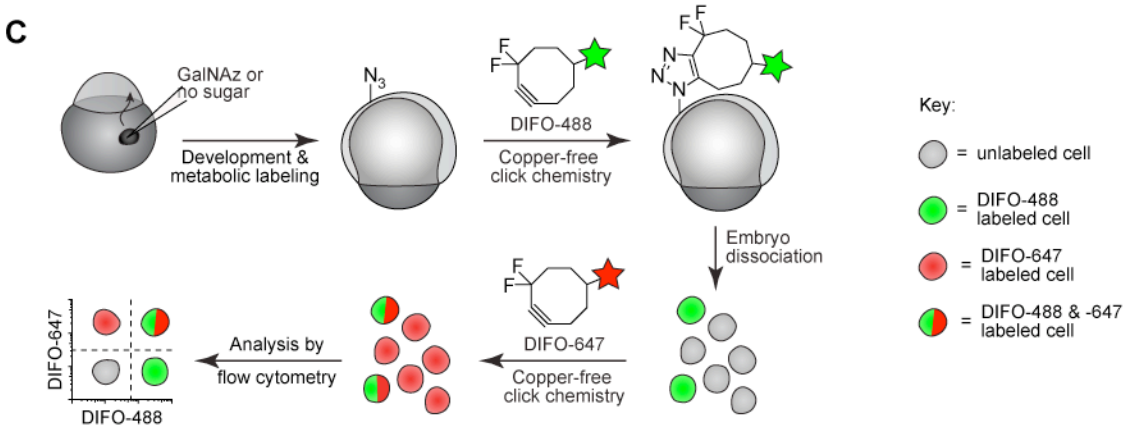


Figure S6. Microinjection of Ac_4GalNAz and GalNAz afford comparable labeling before 24 hpf. Zebrafish embryos were microinjected with 25 pmol of GalNAz, 25 pmol of Ac_4GalNAz, or no sugar, along with the tracer dye rhodamine-dextran, and allowed to develop. At 10 hpf, the embryos were reacted with DIFO-488 (100 μM, 1 h) and then imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, DIFO-488; red, rhodamine-dextran. Scale bar: 200 μm.


A
DIFO-488
Brightfield
B
0 µm
5 µm
10 µm
15 µm
20 µm
DIFO-488
25 µm
50 µm
75 µm
100 µm
125 µm


C
GalNAz or no sugar
Development & metabolic labeling
N3
DIFO-488
Copper-free click chemistry
Embryo dissociation
DIFO-647
Copper-free click chemistry
Analysis by flow cytometry
DIFO-647
DIFO-488
Key:
= unlabeled cell
= DIFO-488 labeled cell
= DIFO-647 labeled cell
= DIFO-488 & -647 labeled cell

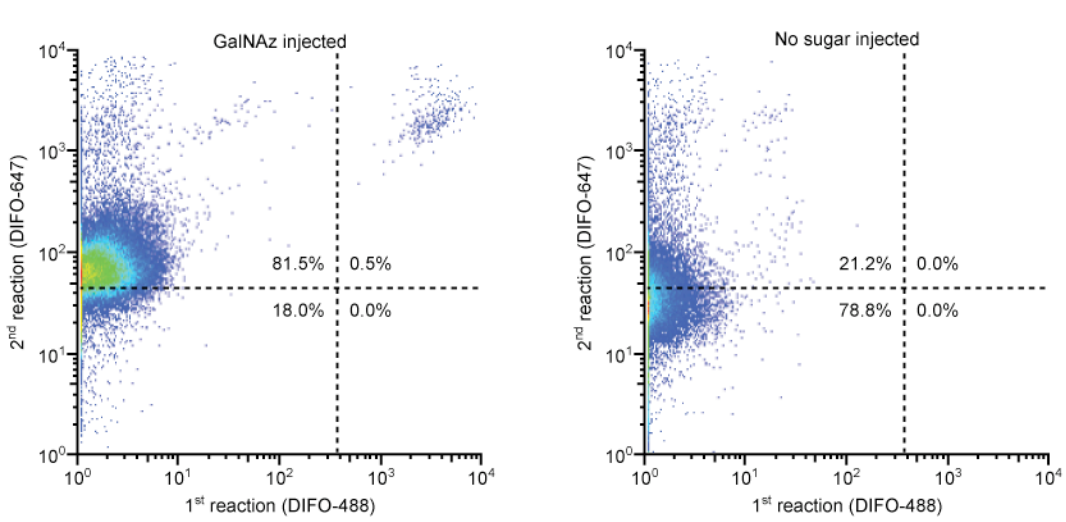

D
GalNAz injected
No sugar injected
2nd reaction (DIFO-647)
1st reaction (DIFO-488)
81.5%
0.5%
18.0%
0.0%
21.2%
0.0%
78.8%
0.0%

Figure S7. Distribution of GalNAz- and DIFO-488-labeled cells from 10 hpf embryos. Zebrafish embryos were microinjected with 25 pmol of GalNAz, allowed to develop to 10 hpf, and then reacted with DIFO-488 (100 μM, 1 h). (A) Maximum intensity z-projection fluorescence image and corresponding brightfield image. (B) Several individual z-planes from the z-projection shown in (A), demonstrating labeling of the surface cells. Distances indicated are in the z direction from the top of the embryo. (C) Schematic of dual-labeling flow experiment. Following microinjection with GalNAz and reaction with DIFO-488, embryos were dissociated, and the resulting cell suspension was labeled with DIFO-647 (100 μM, 30 min) and then analyzed by flow cytometry. (D) Representative dot plots of cells from embryos injected with GalNAz (left) or no sugar (right). In GalNAz-injected embryos, most of the cells displayed DIFO-647-derived signal, indicating that most cells of the embryo were metabolically labeled with GalNAz. Approximately 0.5% of cells were also labeled with DIFO-488, indicating that these cells were accessible to this reagent while part of the intact embryo. Scale bar: 200 μm.

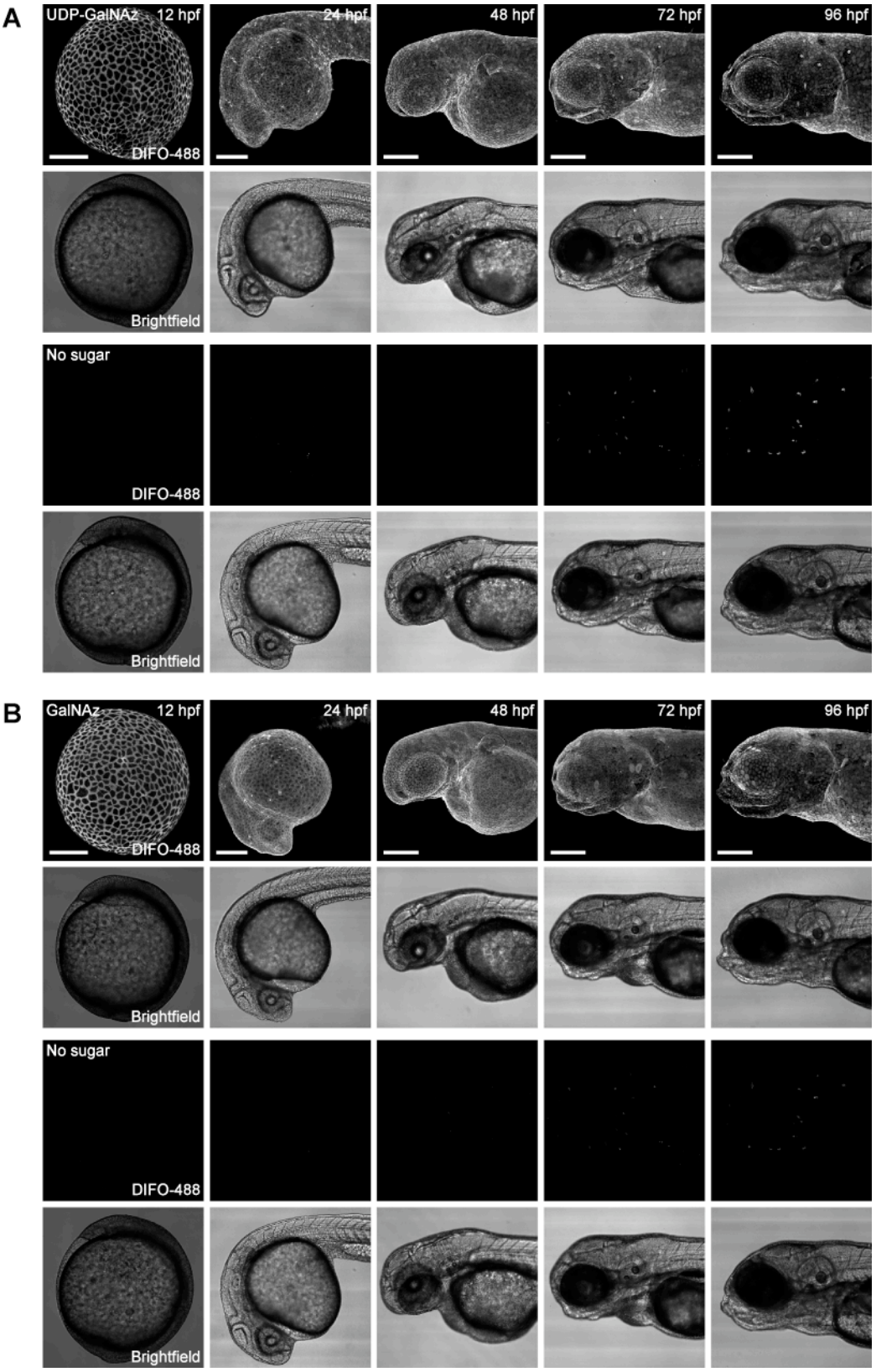

A
UDP-GalNAz
12 hpf
24 hpf
48 hpf
72 hpf
96 hpf
DIFO-488
Brightfield
No sugar
DIFO-488
Brightfield
B
GalNAz
12 hpf
24 hpf
48 hpf
72 hpf
96 hpf
DIFO-488
Brightfield
No sugar
DIFO-488
Brightfield

Figure S8. Labeling from a single bolus of GalNAz or UDP-GalNAz persists for 96 hpf. Zebrafish embryos were microinjected with 25 pmol of UDP-GalNAz (A, top), 25 pmol of GalNAz (B, top), or no sugar (A and B, bottom), and allowed to develop normally. At each timepoint shown, embryos were reacted with DIFO-488 (100 μM, 1 h) and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Scale bar: 200 μm.

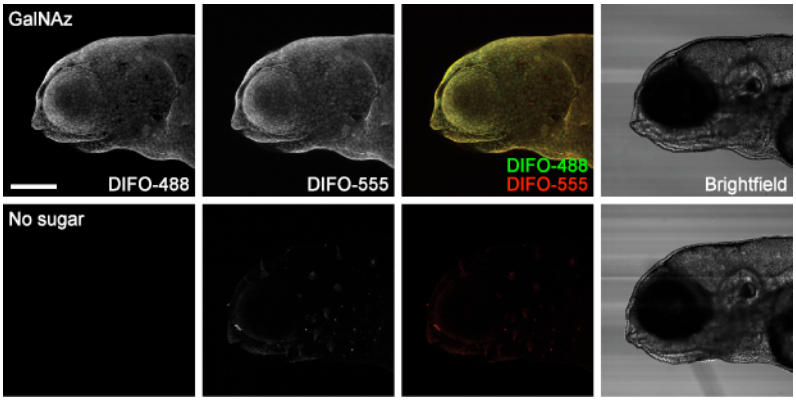


Figure S9. New azide-containing glycans are presented on the surface of cells out to 96 hpf. Zebrafish embryos were microinjected with 25 pmol of GalNAz (top) or no sugar (bottom) and allowed to develop to 84 hpf, at which point they were reacted with DIFO-488 (100 μM, 1 h). Unreacted azides were immediately quenched by using tris(2-carboxyethyl)phosphine (TCEP) (50 mM, 10 min), and the embryos were allowed to develop further. At 96 hpf, the embryos were reacted with DIFO-555 (100 μM, 1 h) and then imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images of surface epithelial cells and corresponding brightfield images. Green, DIFO-488; red, DIFO-555. Scale bar: 200 μm.

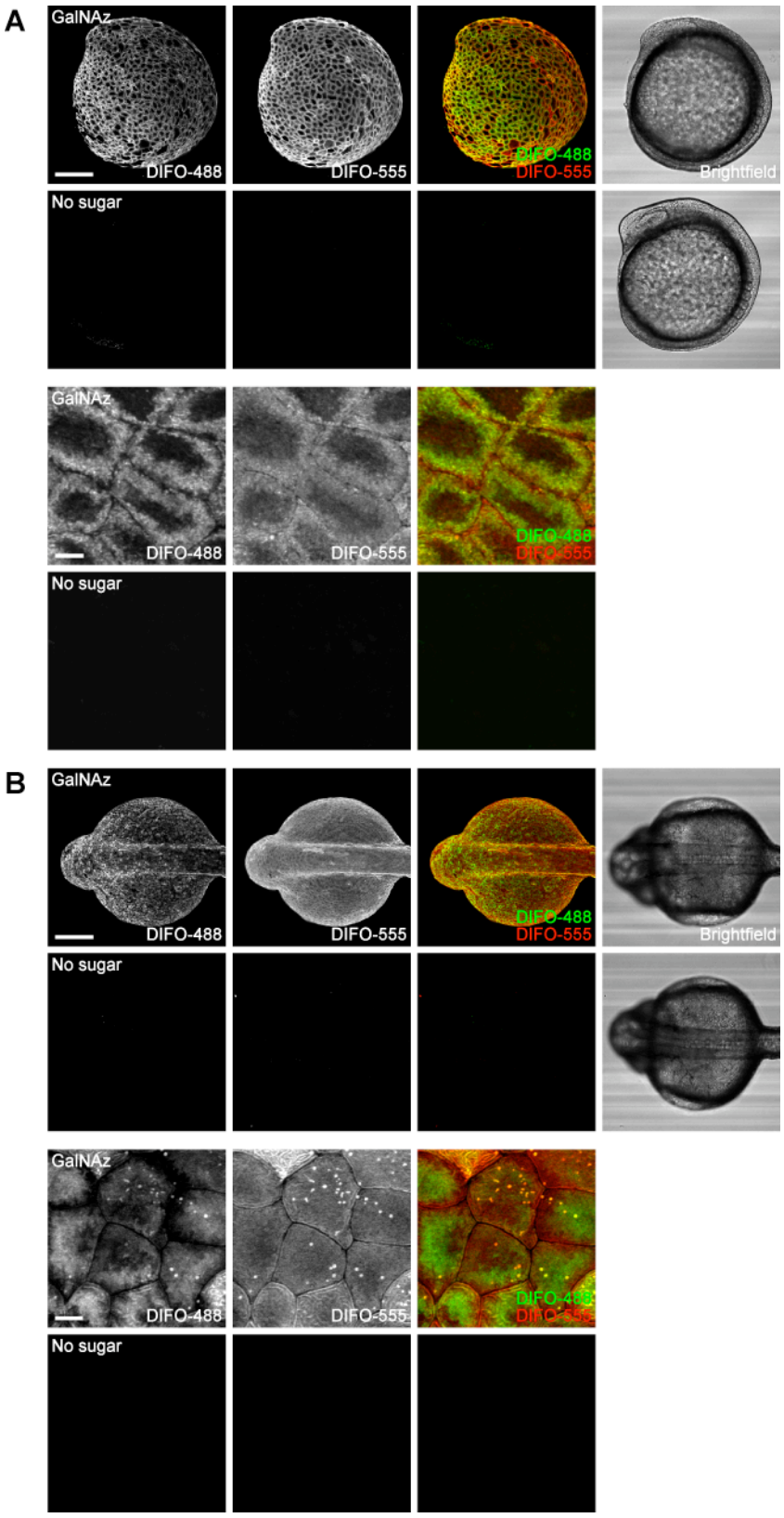

A
GalNAz
DIFO-488
DIFO-555
DIFO-488
DIFO-555
Brightfield
No sugar
GalNAz
DIFO-488
DIFO-555
DIFO-488
DIFO-555
No sugar
B
GalNAz
DIFO-488
DIFO-555
DIFO-488
DIFO-555
Brightfield
No sugar
GalNAz
DIFO-488
DIFO-555
No sugar

Figure S10. Multicolor imaging of time-resolved populations of GalNAz-labeled glycans. Zebrafish embryos were microinjected with 25 pmol of GalNAz or no sugar and allowed to develop to 9 hpf, at which point they were reacted with DIFO-488 (100 μM, 1 h). The embryos were then allowed to further develop for 2 h (A) or 12 h (B), at which point they were reacted with DIFO-555 (100 μM, 1 h) and then imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images of surface epithelial cells and corresponding brightfield images. Green, DIFO-488; red, DIFO-555. Scale bars: 200 μm (1st, 2nd, 5th, and 6th rows), 10 μm (3rd, 4th, 7th, and 8th rows).

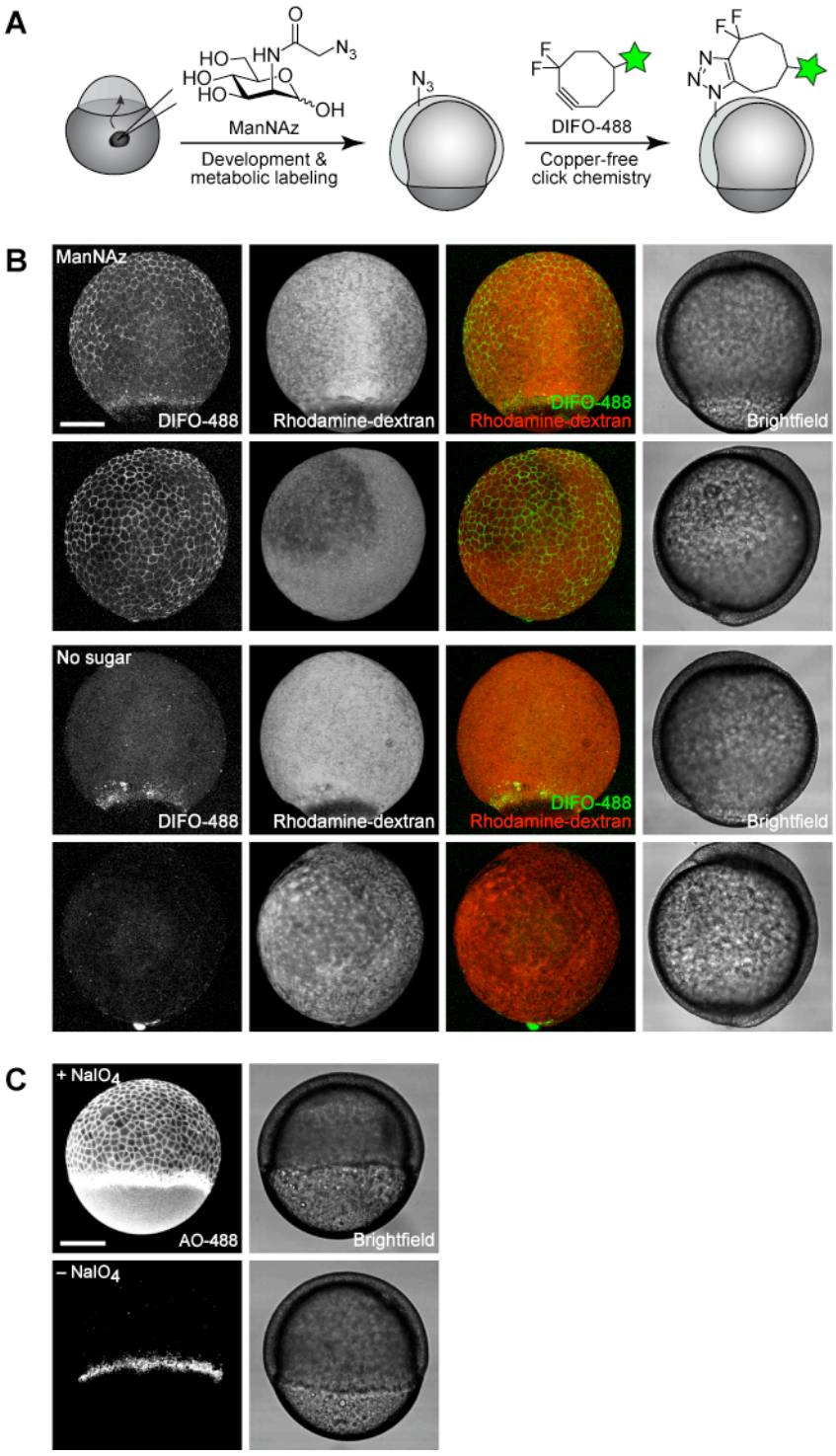

A
ManNAz
Development &
metabolic labeling
DIFO-488
Copper-free
click chemistry
B
ManNAz
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield
No sugar
DIFO-488
Rhodamine-dextran
DIFO-488
Rhodamine-dextran
Brightfield
C
+ NaIO4
AO-488
Brightfield
– NaIO4

Figure S11. Imaging of sialylated glycans via either microinjection of ManNAz or treatment with sodium periodate. (A) Schematic depicting metabolic approach for imaging of sialylated glycans. (B) Zebrafish embryos were microinjected with 25 pmol of ManNAz or no sugar, allowed to develop to 8 hpf, reacted with DIFO-488 (100 μM, 1 h), and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, DIFO-488; red, rhodamine-dextran. (C) Zebrafish embryos (6 hpf) were bathed in $NaIO_4$ (500 μM, 30 min, top row) or no reagent (bottom row), reacted with AO-488 (100 μM, 1 h, pH 6.7), and imaged by confocal microscopy. Shown are maximum intensity z-projection images of AO-488 fluorescence and corresponding brightfield images. Scale bars: 200 μm.

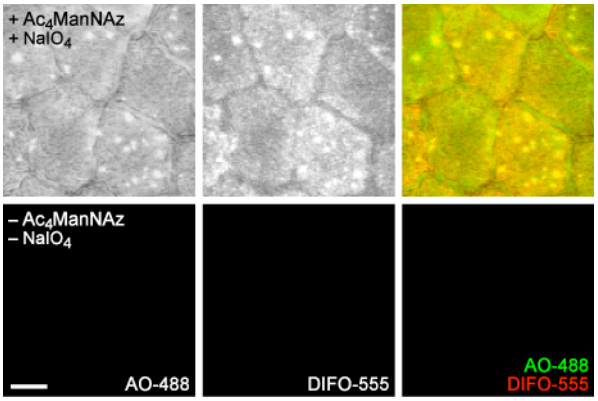


Figure S12. Dual labeling of sialic acids with ManNAz and $NaIO_4$ followed by reaction with DIFO- and aminooxy-fluorophores shows substantial colocalization. Zebrafish embryos were bathed in 5 mM Ac_4ManNAz or no sugar for three days, then treated with $NaIO_4$ (500 μM, 30 min) or no reagent. Embryos were then reacted in a mixture of DIFO-555 (100 μM) and AO-488 (100 μM) in PBS (pH 6.7) for 1 h and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images. Green, AO-488; red, DIFO-555. Scale bar: 10 μm.

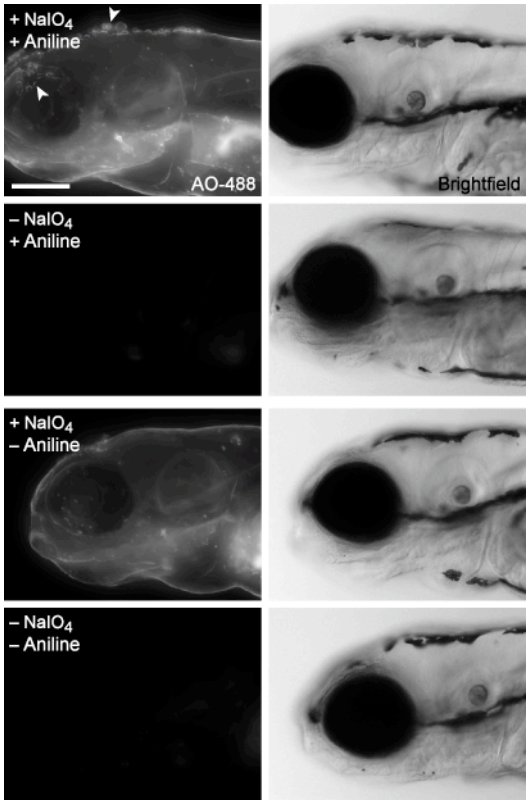


Figure S13. An aniline catalyst provides a modest increase in labeling of periodate-reacted glycans with aminooxy probes. Zebrafish embryos (120 hpf) were bathed in $NaIO_4$ (1 mM, 30 min, 1st and 3rd rows) or no reagent (2nd and 4th rows), then reacted with AO-488 (100 μM, 1 h, pH 6.7) in the presence (1st and 2nd rows) or absence (3rd and 4th rows) of aniline (10 mM). Shown are maximum intensity z-projection images of AO-488 fluorescence and corresponding brightfield images. Arrowheads indicate damage to the epidermis caused by aniline. Scale bar: 200 μm.

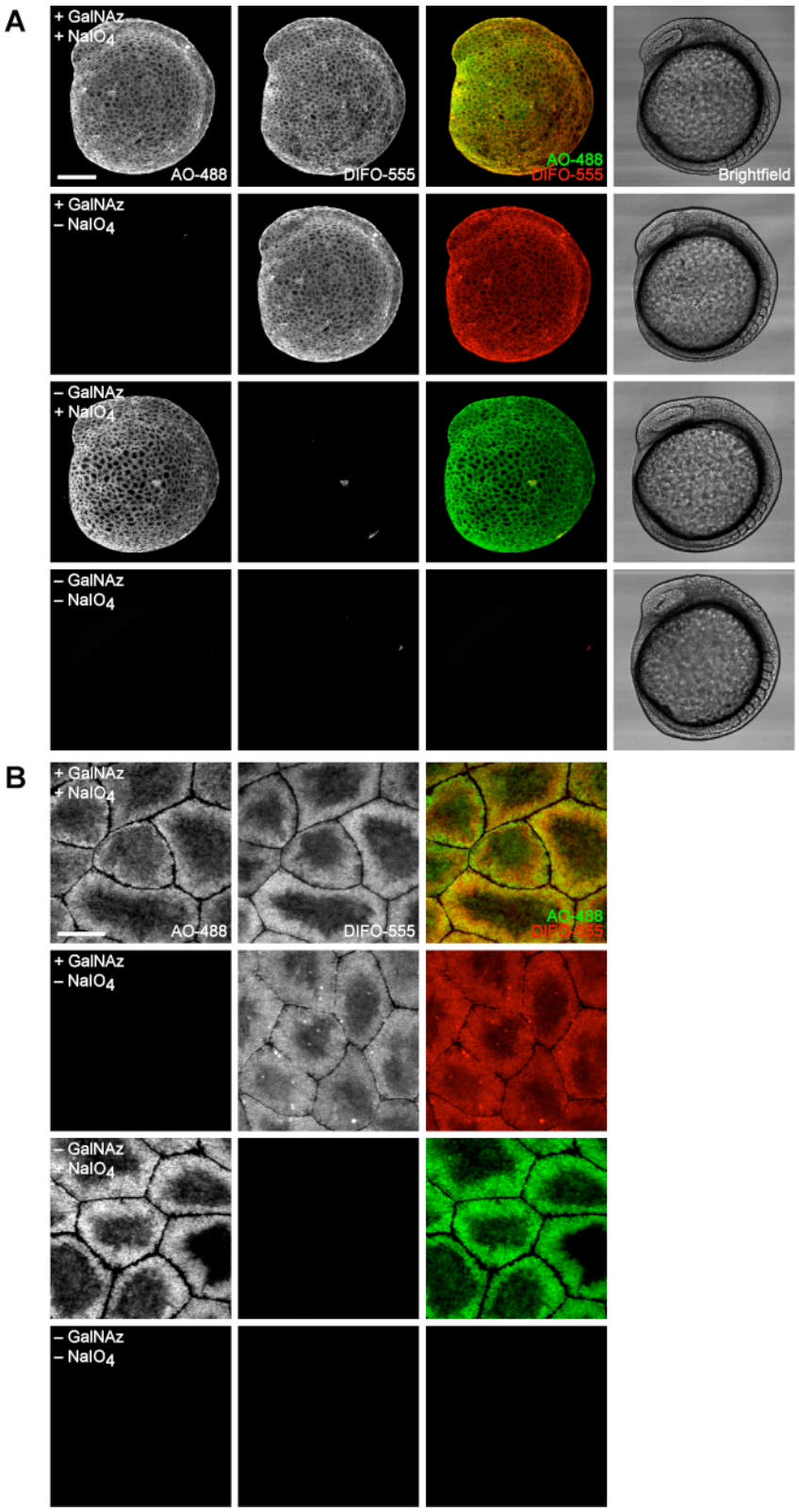

A
+ GalNAz
+ NaIO4
AO-488
DIFO-555
AO-488
DIFO-555
Brightfield
+ GalNAz
– NaIO4
– GalNAz
+ NaIO4
– GalNAz
– NaIO4
B
+ GalNAz
+ NaIO4
AO-488
DIFO-555
AO-488
DIFO-555
+ GalNAz
– NaIO4
– GalNAz
+ NaIO4
– GalNAz
– NaIO4

Figure S14. Simultaneous visualization of O-glycans and sialylated glycans during the early segmentation period. Zebrafish embryos were microinjected with GalNAz or no sugar, allowed to develop to 10 hpf, and then bathed in $NaIO_4$ (500 μM, 30 min) or no reagent. Embryos were then reacted in a mixture of DIFO-555 (100 μM) and AO-488 (100 μM) in PBS (pH 6.7) for 1 h and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, AO-488; red, DIFO-555. Scale bars: 200 μm (A), 20 μm (B).

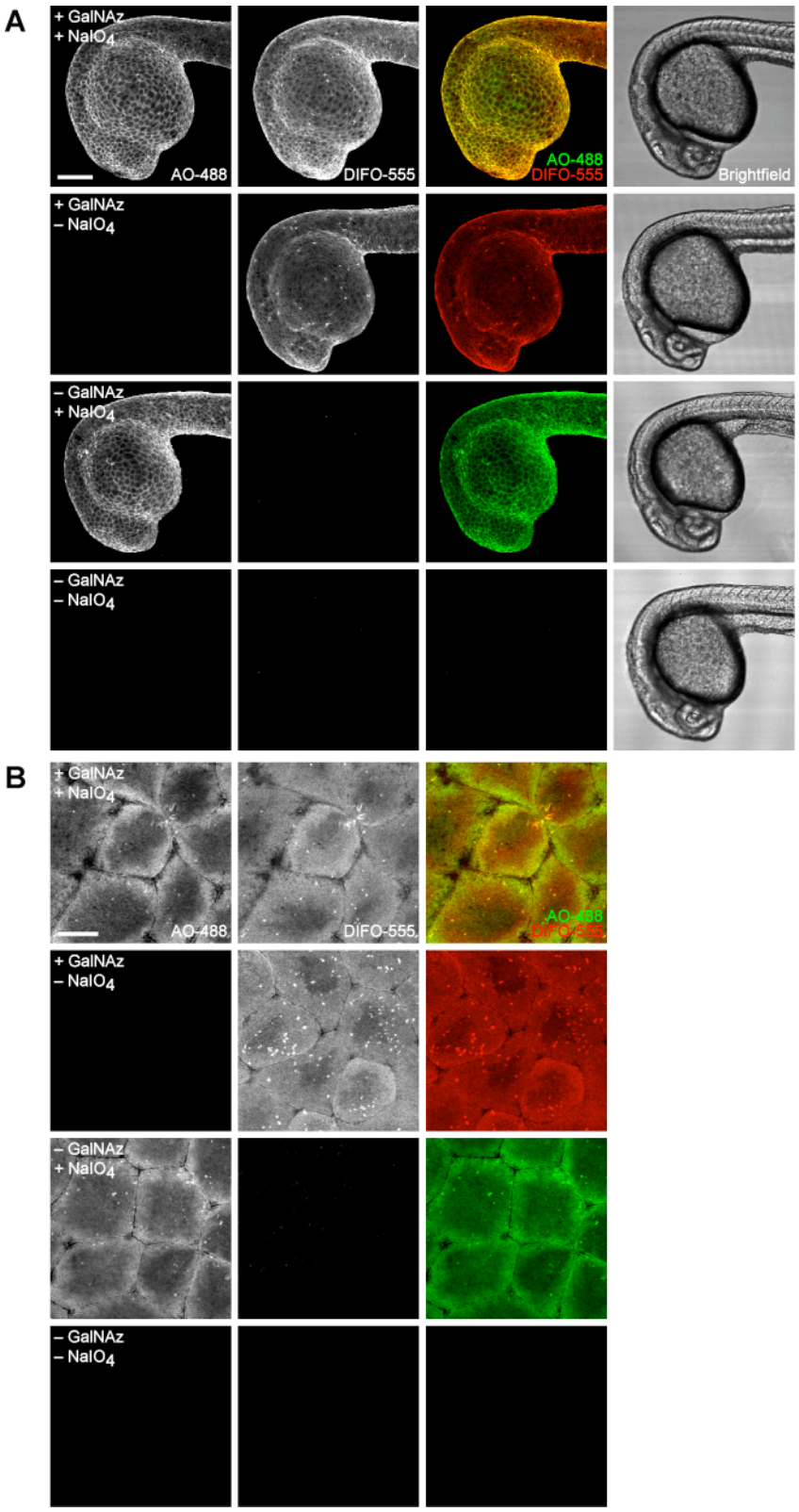

A
+ GalNAz
+ NaIO4
AO-488
DIFO-555
AO-488
DIFO-555
Brightfield
+ GalNAz
– NaIO4
– GalNAz
+ NaIO4
– GalNAz
– NaIO4
B
+ GalNAz
+ NaIO4
AO-488
DIFO-555
+ GalNAz
– NaIO4
– GalNAz
+ NaIO4
– GalNAz
– NaIO4

Figure S15. Simultaneous visualization of O-glycans and sialylated glycans at 24 hpf. Zebrafish embryos were microinjected with GalNAz or no sugar, allowed to develop to 24 hpf, and then bathed in $NaIO_4$ (500 μM, 30 min) or no reagent. Embryos were then reacted in a mixture of DIFO-555 (100 μM) and AO-488 (100 μM) in PBS (pH 6.7) for 1 h and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images and corresponding brightfield images. Green, AO-488; red, DIFO-555. Scale bars: 200 μm (A), 20 μm (B).

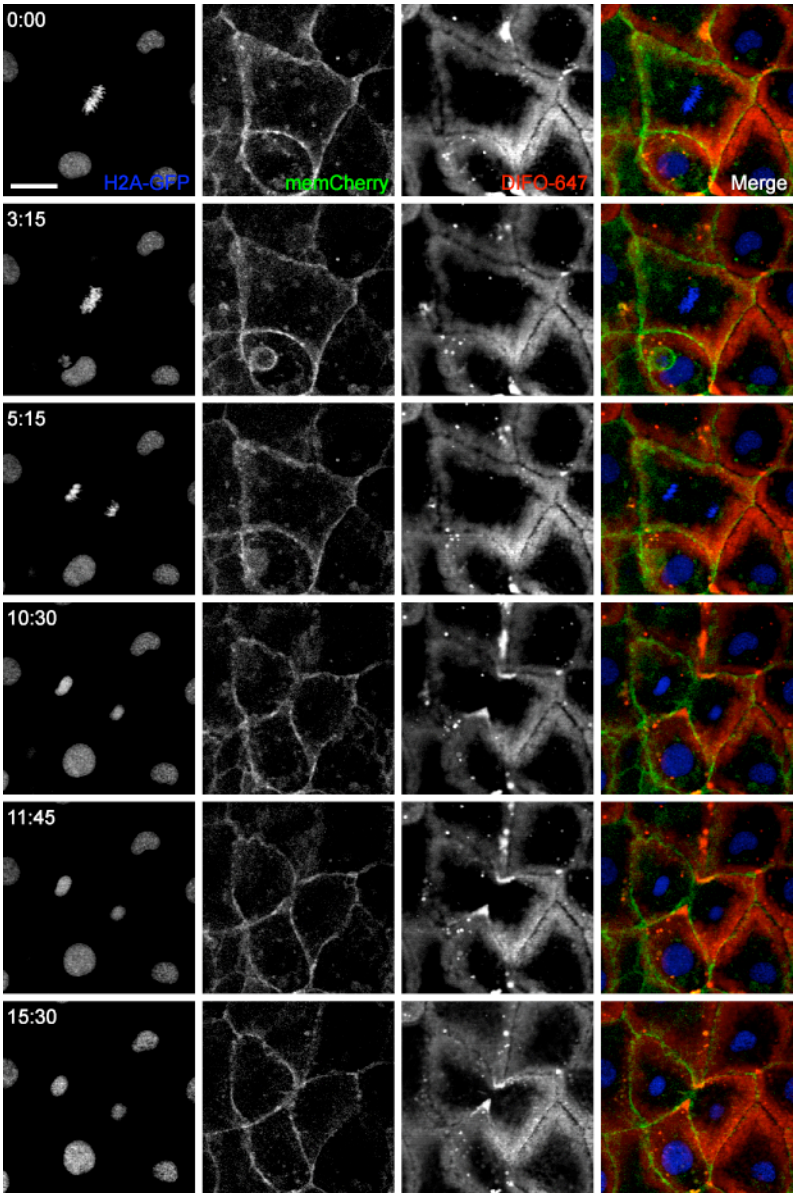


Figure S16. Time-lapse monitoring of membrane and glycan movement during mitosis. H2A-GFP transgenic zebrafish were microinjected into the blastomere cell at the one-cell stage with GalNAz and mRNA for memCherry. Embryos were allowed to develop to 10 hpf, at which point they were reacted with DIFO-647 (100 μM, 1 h) and imaged by confocal microscopy. Blue, H2A-GFP; green, memCherry; red, DIFO-647. A single z-plane fluorescence image is shown at each time indicated (min:s). Scale bar: 20 μm.

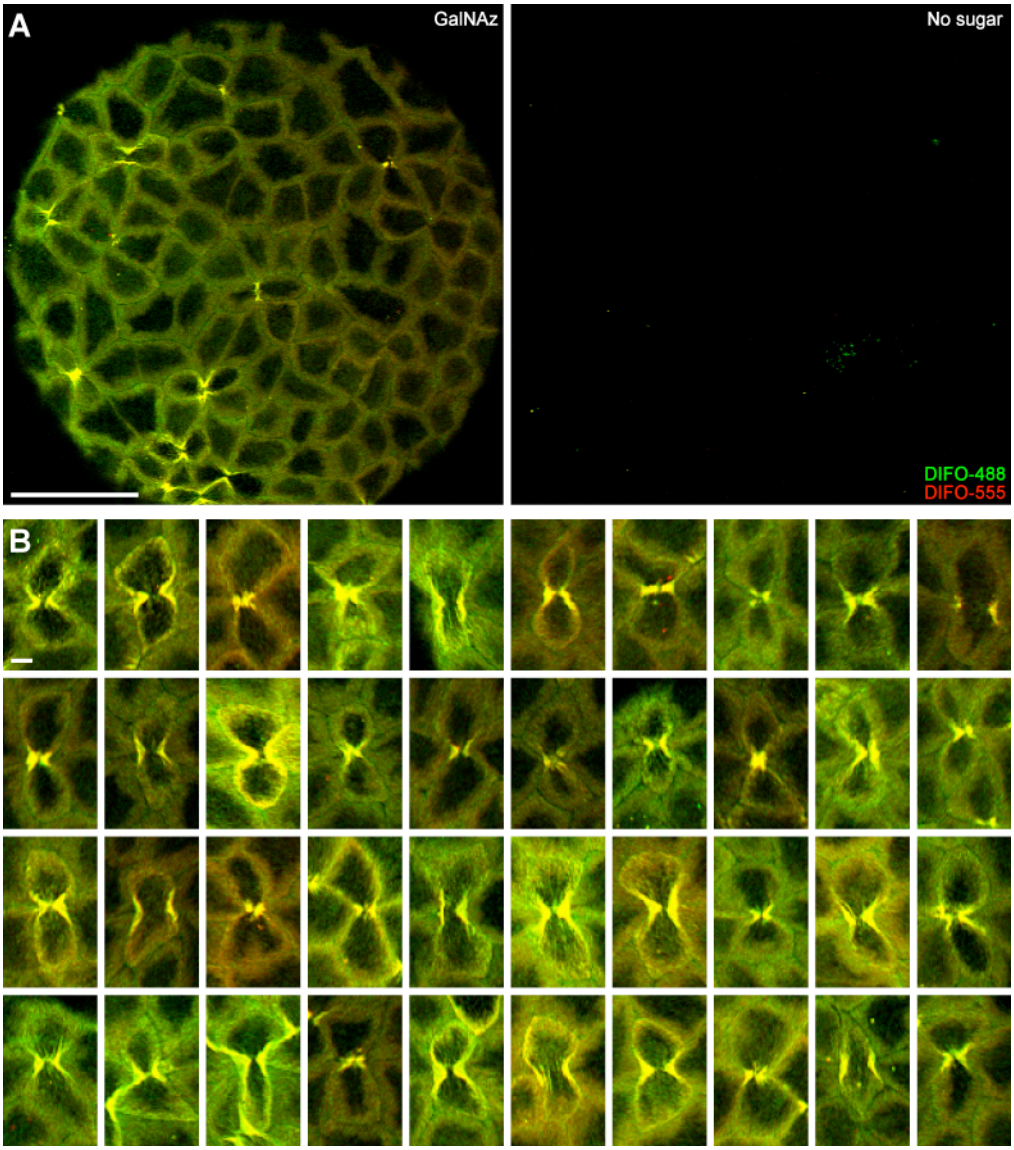


Figure S17. Multicolor imaging of GalNAz-labeled glycans at the cleavage furrow of dividing cells. Zebrafish embryos were microinjected with GalNAz or no sugar and allowed to develop to 10 hpf, at which point they were reacted with DIFO-555 (100 µM, 1 h). Immediately following this reaction, the embryos were reacted with DIFO-488 (100 µM, 15 min) and imaged by confocal microscopy. Shown are maximum intensity z-projection fluorescence images. Green, DIFO-488; red, DIFO-555. Scale bars: 100 µm (A), 10 µm (B).