SUPPLEMENTARY FIGURES AND FIGURE LEGENDS

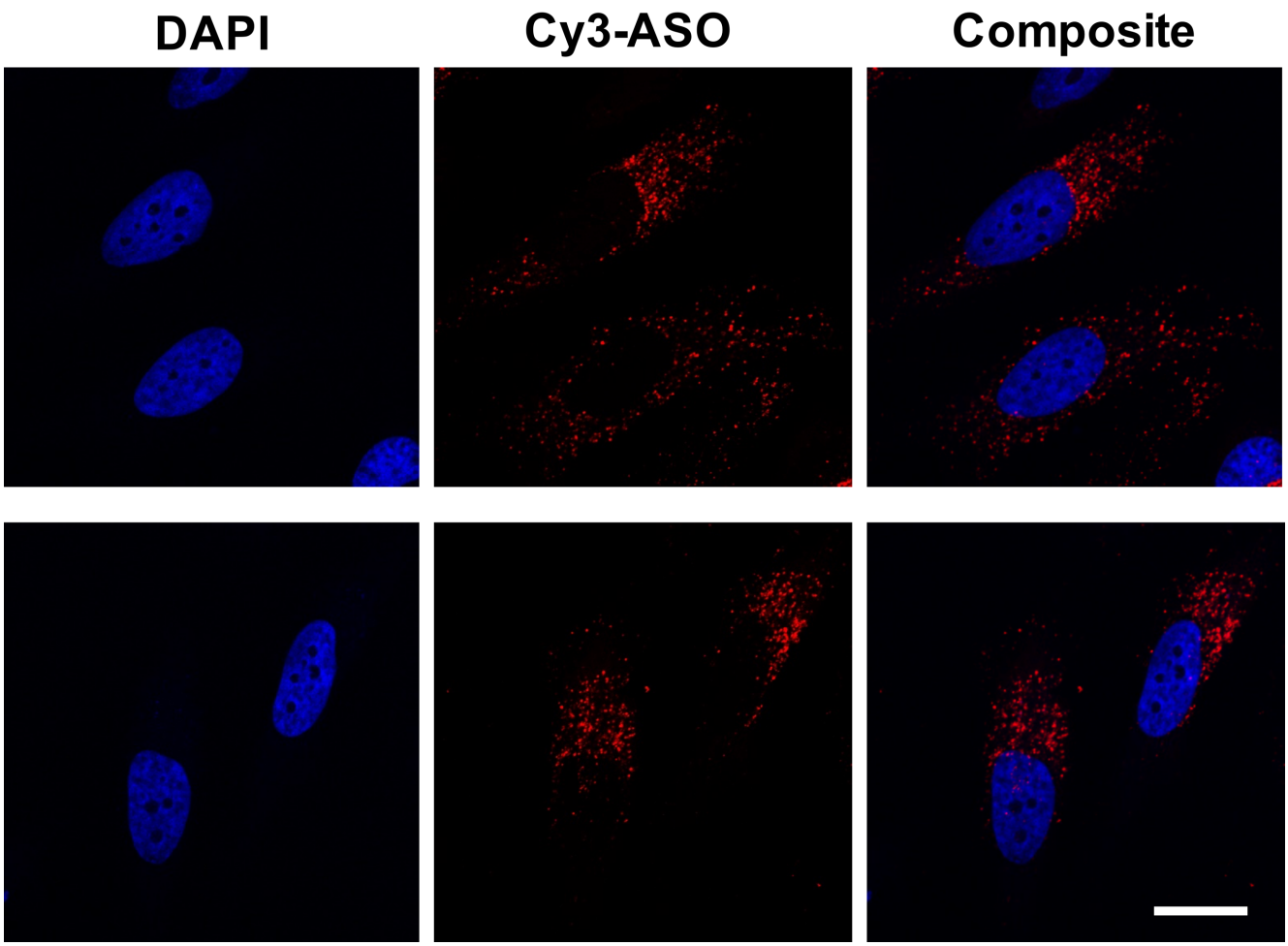


Fig. S1. Confocal fluorescence microscopy images of Cy3-ASO in cells. HeLa cells were incubated in medium containing Cy3-ASO ("free uptake") for 24 h and processed for confocal microscopy. Nuclei were stained with DAPI. Merged images reveal that most but not all of the ASO was located in foci in the cytoplasm. Scale bars, 10 μm.

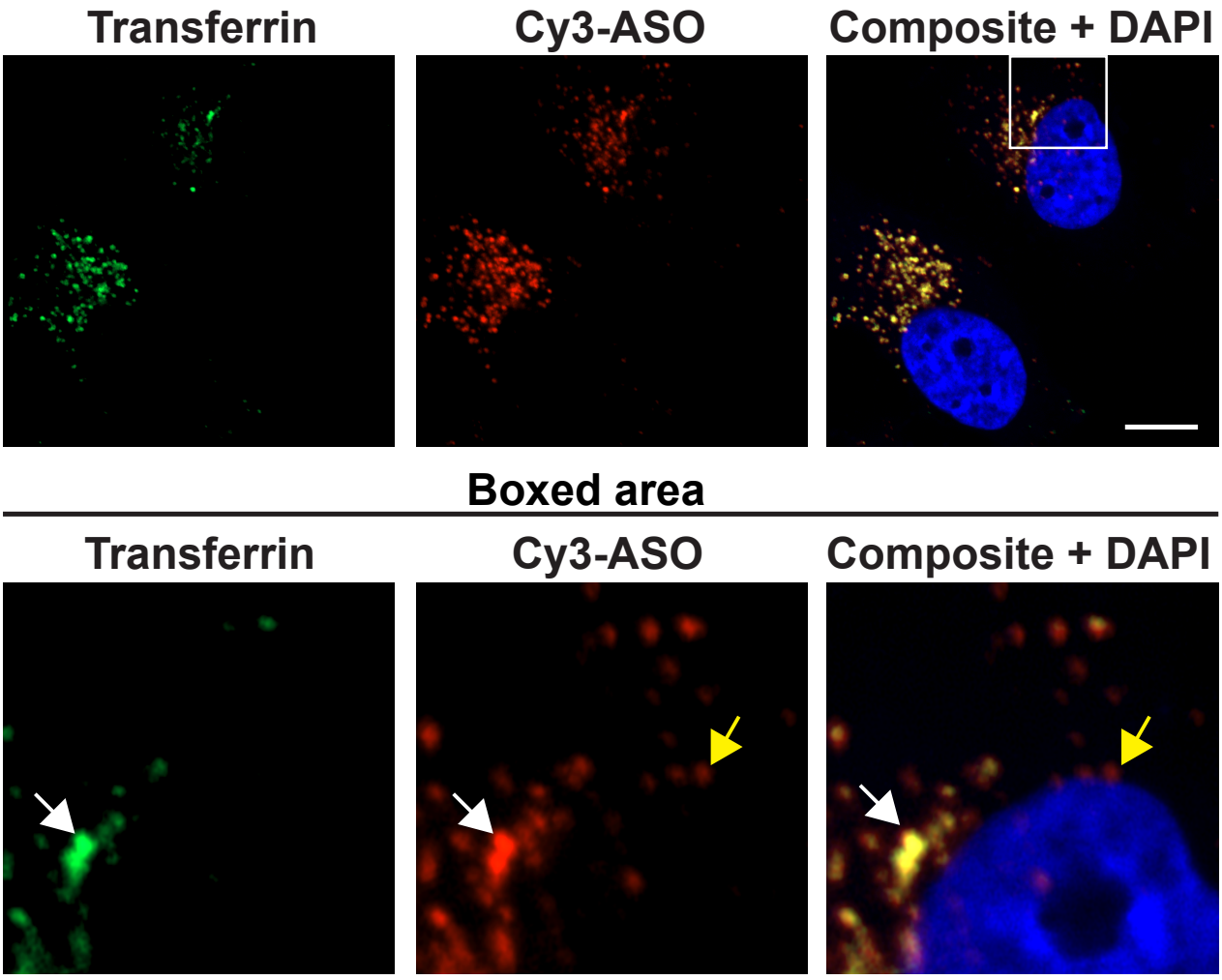


Fig. S2. Confocal fluorescence microscopy images of Cy3-ASO and Alexa 488–transferrin in cells. HeLa cells were incubated in medium containing Cy3-ASO ("free uptake") for 24 h and then incubated with Alexa 488–transferrin for 20 min. The cells were then processed for confocal microscopy. Nuclei were stained with DAPI. The composite image in the top row reveals strong colocalization of Cy3-ASO and transferrin-labeled endosomes. The boxed area in the upper panel is shown at higher magnification in the lower row. The higher-magnification composite image reveals that the majority of the ASO is located in transferrin-positive endosomes (*white* arrow) but that some of the ASO does not colocalize with transferrin-positive endosomes (*yellow* arrow). Scale bar, 10 μm.

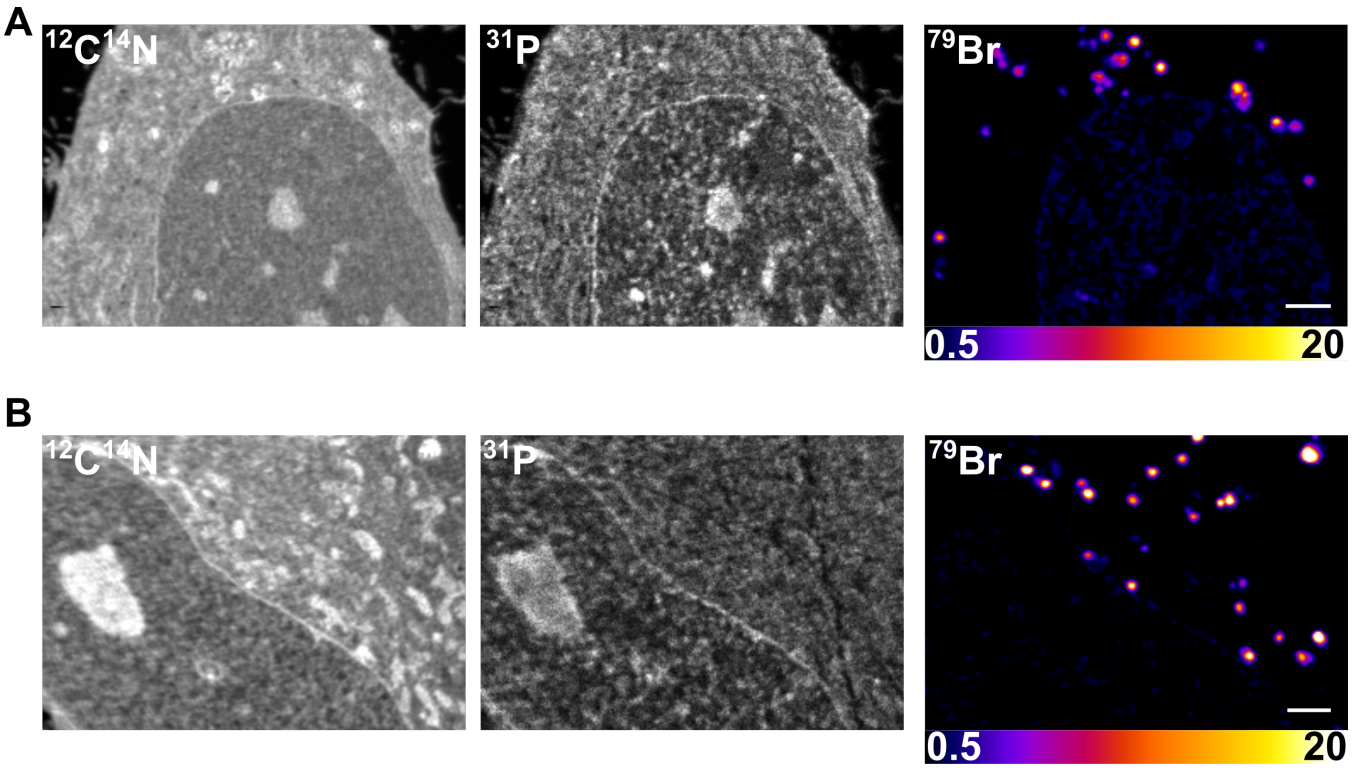


Fig. S3. Additional NanoSIMS images of the two cells shown in Panels A and B of Fig. 2. $^{12}C^{14}N$ and ^{31}P NanoSIMS images are useful for cell morphology; ^{79}Br NanoSIMS images (at a different scale than in the images shown in Fig. 2) show an accumulation of Br-ASO in endosomes within the cytoplasm. Scale bars, 2 μm.

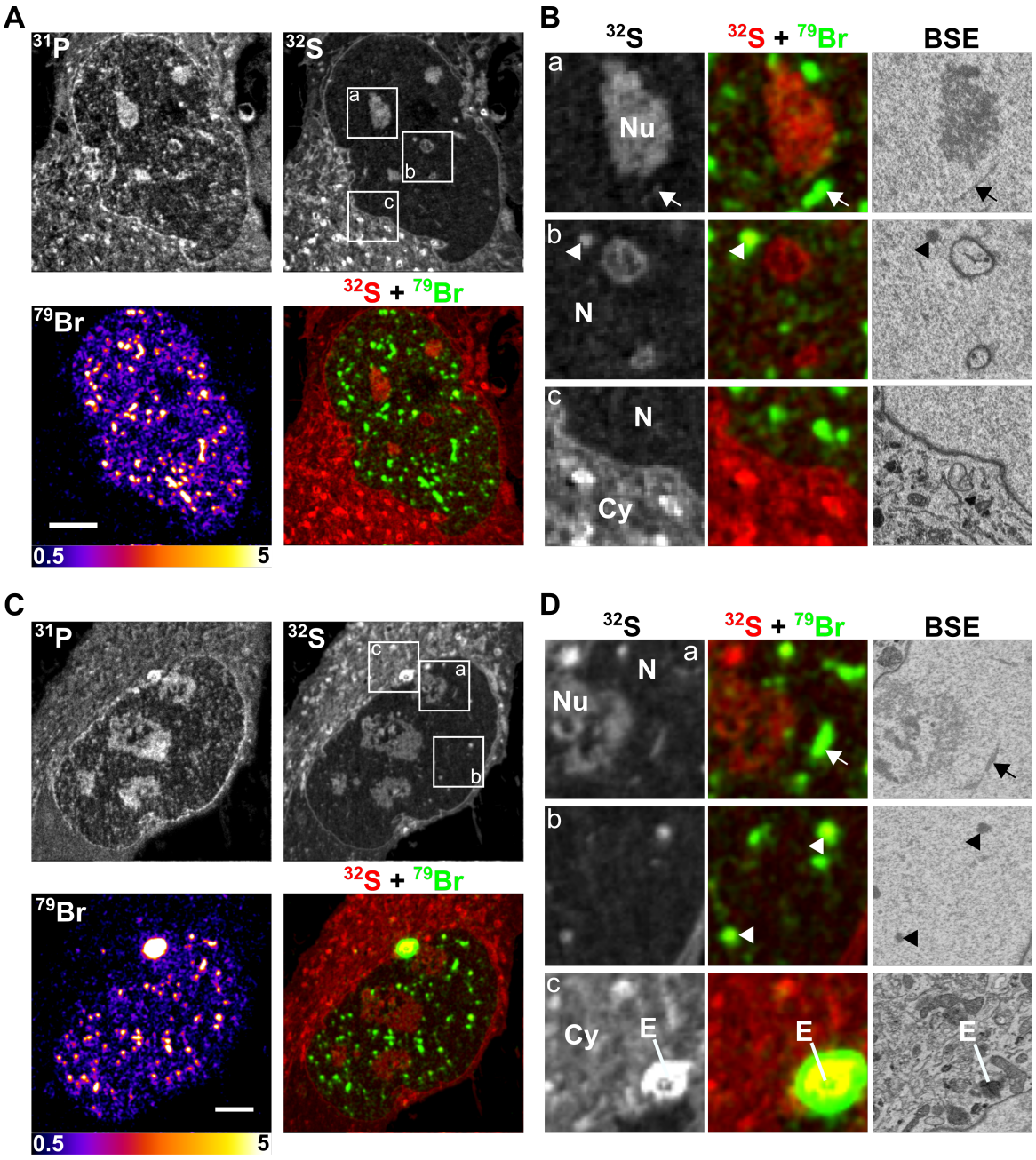


Fig. S4. NanoSIMS images of HeLa cells that had been transfected with a Br-labeled ASO. HeLa cells were transfected with 50 nM Br-ASO with lipofectamine. After 4 h, the cells were processed for correlative NanoSIMS and BSE imaging. (A, C) NanoSIMS images of two cells. ^{31}P and ^{32}S NanoSIMS images are useful for defining cell morphology; ^{79}Br and composite ^{32}S (*red*) + ^{79}Br (*green*) NanoSIMS images show the distribution of Br-ASO. Boxed regions in panels A and C (*a*, *b*, *c*) are shown at higher magnification in panels B and D (both NanoSIMS and backscattered electron (BSE) images). The higher-magnification NanoSIMS images along with the BSE images reveal an accumulation of Br-ASO in filamentous features (arrows) and granular features (arrowheads) in the nucleus (N), nucleolus (Nu), and endosomes (E) in the cytoplasm (Cy). Scale bars, 4 μm.

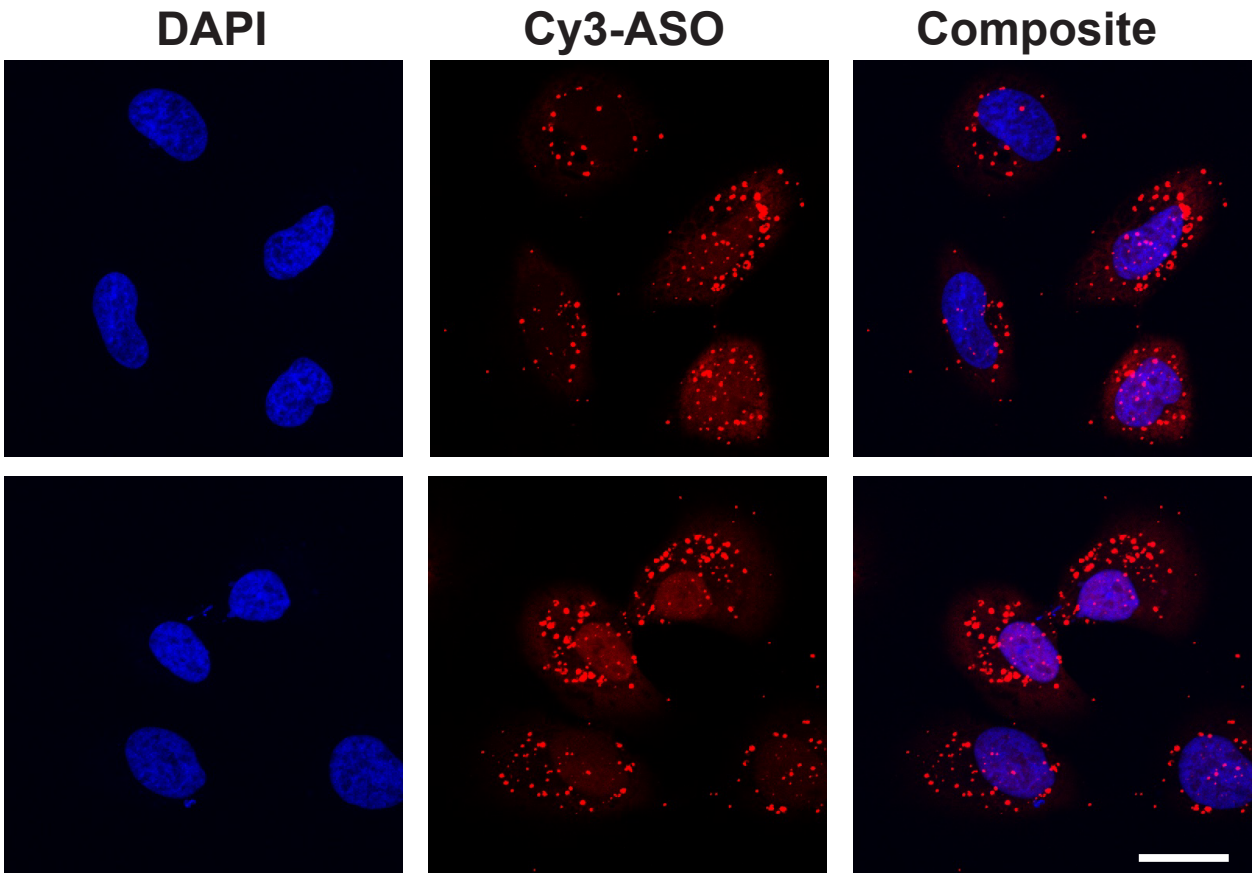
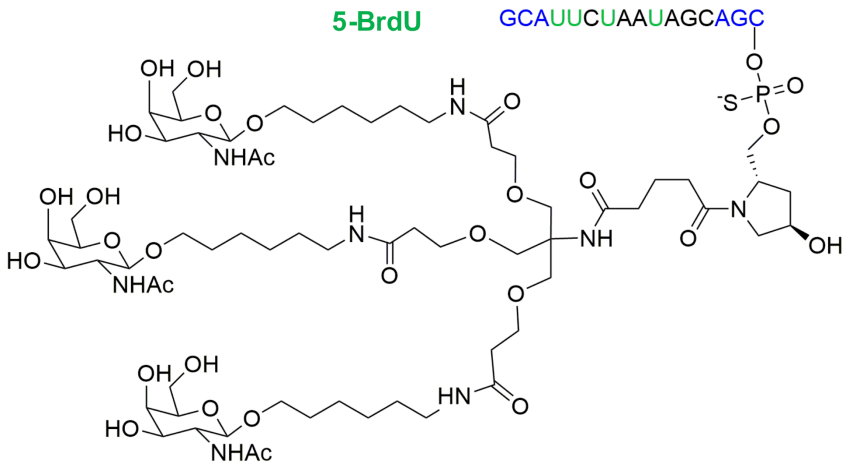
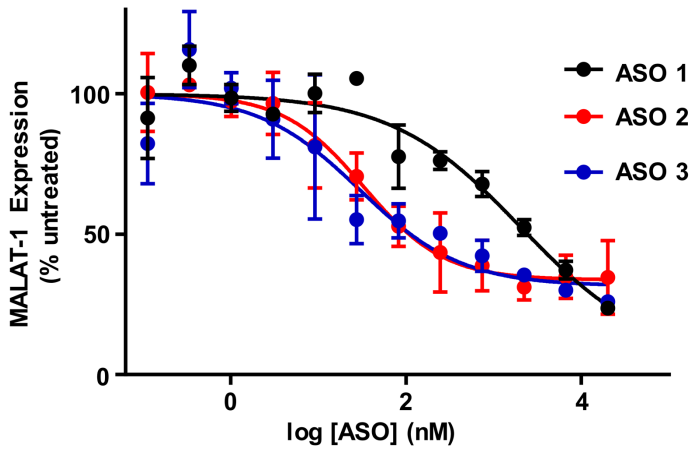


Fig. S5. Confocal fluorescence microscopy images of Cy3-ASO in transfected HeLa cells. HeLa cells were transfected with 50 nM Br-ASO with lipofectamine. After 4 h, the cells were processed for confocal microscopy. Nuclei were stained with DAPI. Merged DAPI (*blue*) and Cy3-ASOs (*red*) images reveal the presence of Cy3-ASO in both nuclei and endosomes. Scale bars, 10 μm.

A



B



ASO #	Sequence (5′ to 3′)	Labelling	IC_{50} (nM)
1	GsCsAsTsTsCsTsAsAsTsAsGsCsAsGsC	none	>2000
2	GsCsAsTsTsCsTsAsAsTsAsGsCsAsGsC-GalNAc3	none	33
3	GsCsAsUsUsCsUsAsAsUsAsGsCsAsGsC-GalNAc3	5-BrdU	30

Fig. S6. GalNAc-conjugated and Br-labeled phosphorothioate ASO (GalNAc-Br-ASO) for NanoSIMS imaging. (A) Schematic of GalNAc-Br-ASO. (B) Design, chemistry, and antisense activities of unlabeled ASO (ASO 1), GalNAc-conjugated ASO (GalNAc-ASO; ASO 2), and GalNAc-Br-ASO (ASO 3). Effect of the ASOs on *MALAT1* transcript levels was measured in ASGR1-expressing HEK293 cells (1A4 cells). Transcript levels were measured after treating cells overnight with different ASO concentrations without transfection reagents; data were normalized to *MALAT1* transcript levels in untreated cells. *Green* shows the 5-bromo-2′-deoxyuridine (5-BrdU) residues; *blue* shows nucleic acids with constrained ethyl modifications.

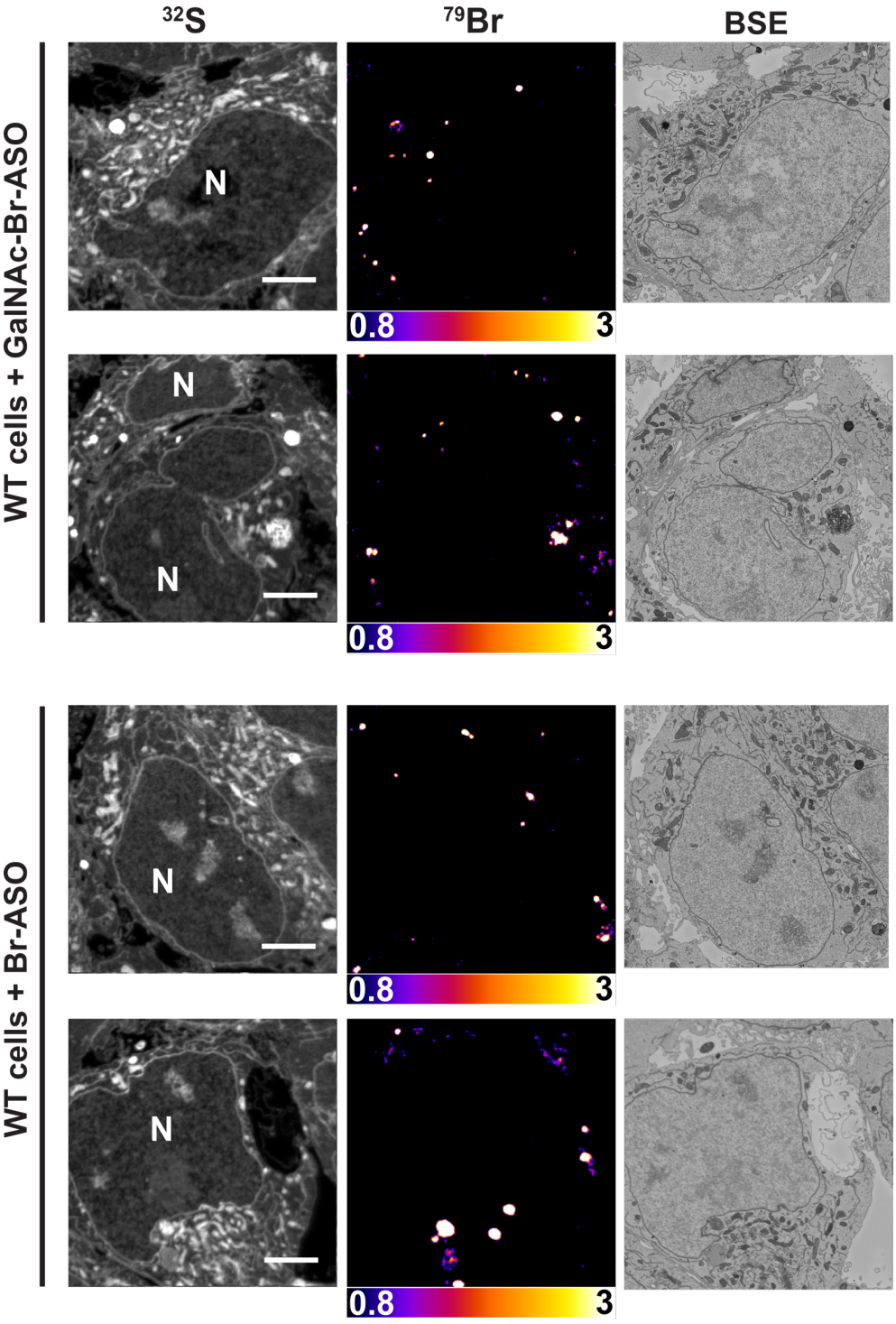


Fig. S7. NanoSIMS images and backscattered electron (BSE) images of wild-type (WT) HEK293 cells that had been incubated with Br-ASO or GalNAc-Br-ASO (from the experiment shown in Fig. 3). The ^{32}S NanoSIMS images are useful for defining cell morphology; the ^{79}Br images show the distribution of Br-ASO (largely in endosomes). N, nucleus. Scale bar, 4 µm.

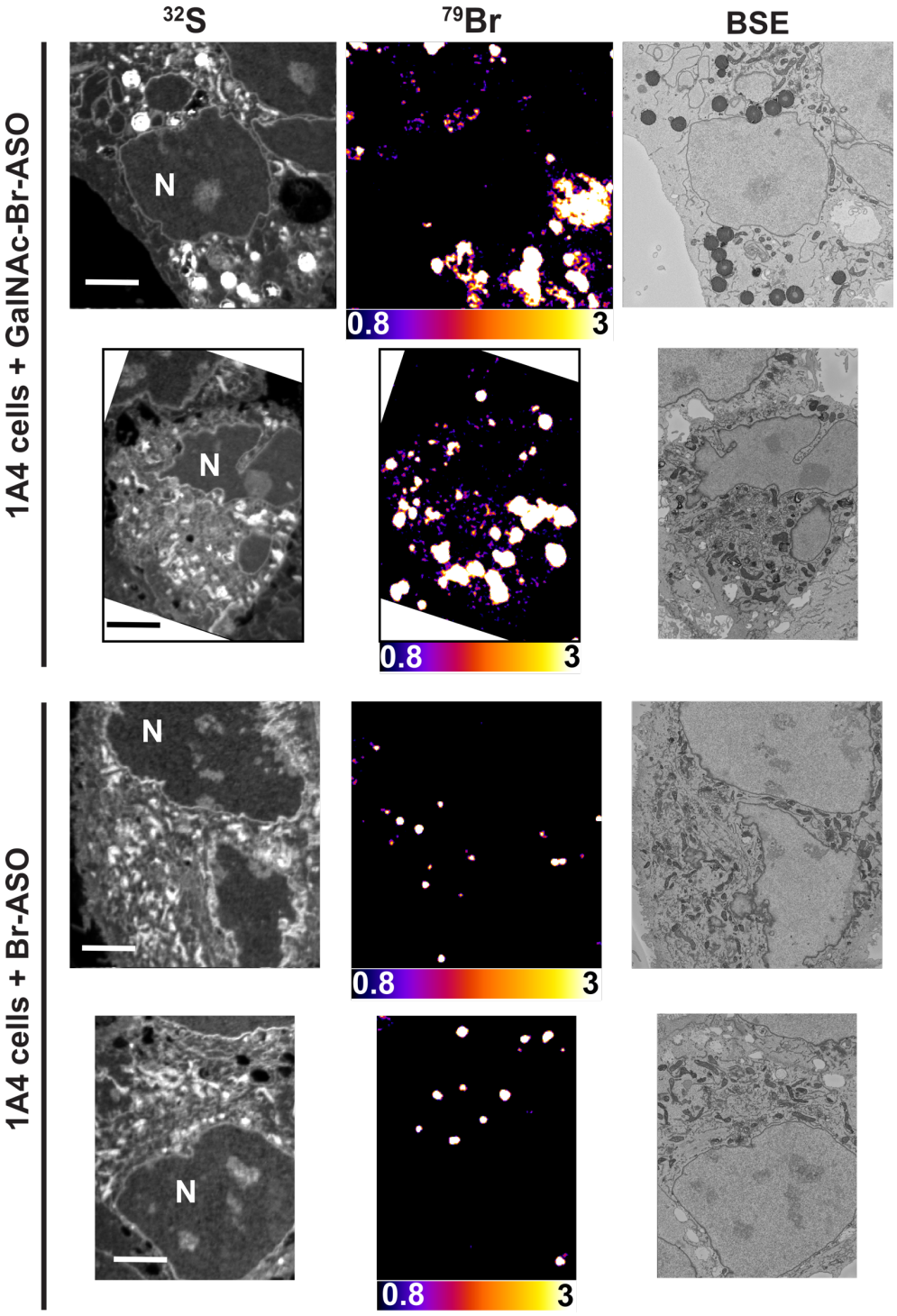


Fig. S8. NanoSIMS images and backscattered electron (BSE) images of asialoglycoprotein receptor 1 (AGSR1)–expressing HEK293 cells (1A4 cells) that had been incubated with Br-ASO or GalNAc-Br-ASO (from the experiment shown in Fig. 3). The ^{32}S NanoSIMS images are useful for defining cell morphology; the ^{79}Br images show the distribution of Br-ASO (largely in endosomes). N, nucleus. Scale bar, 4 μm.

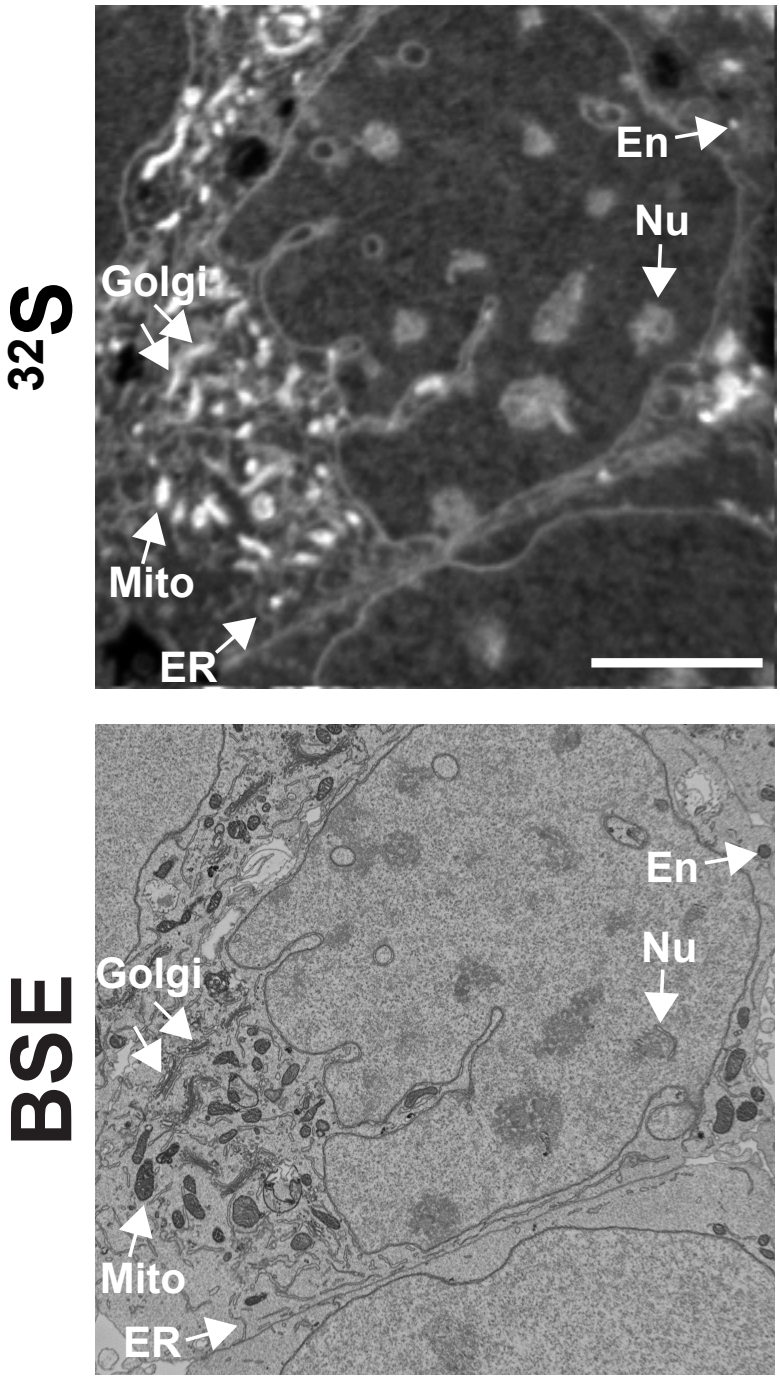


Fig. S9. Identification of subcellular compartments by NanoSIMS and backscattered electron (BSE) imaging. Shown here are ^{32}S NanoSIMS and BSE images from the same section, revealing organelles in a wild-type HEK293 cell that had been incubated in medium containing 150 nM Br-ASO ("free uptake"). Nucleolus (Nu), mitochondria (Mito), endoplasmic reticulum (ER), Golgi apparatus (Golgi). Scale bar, 5 μm.

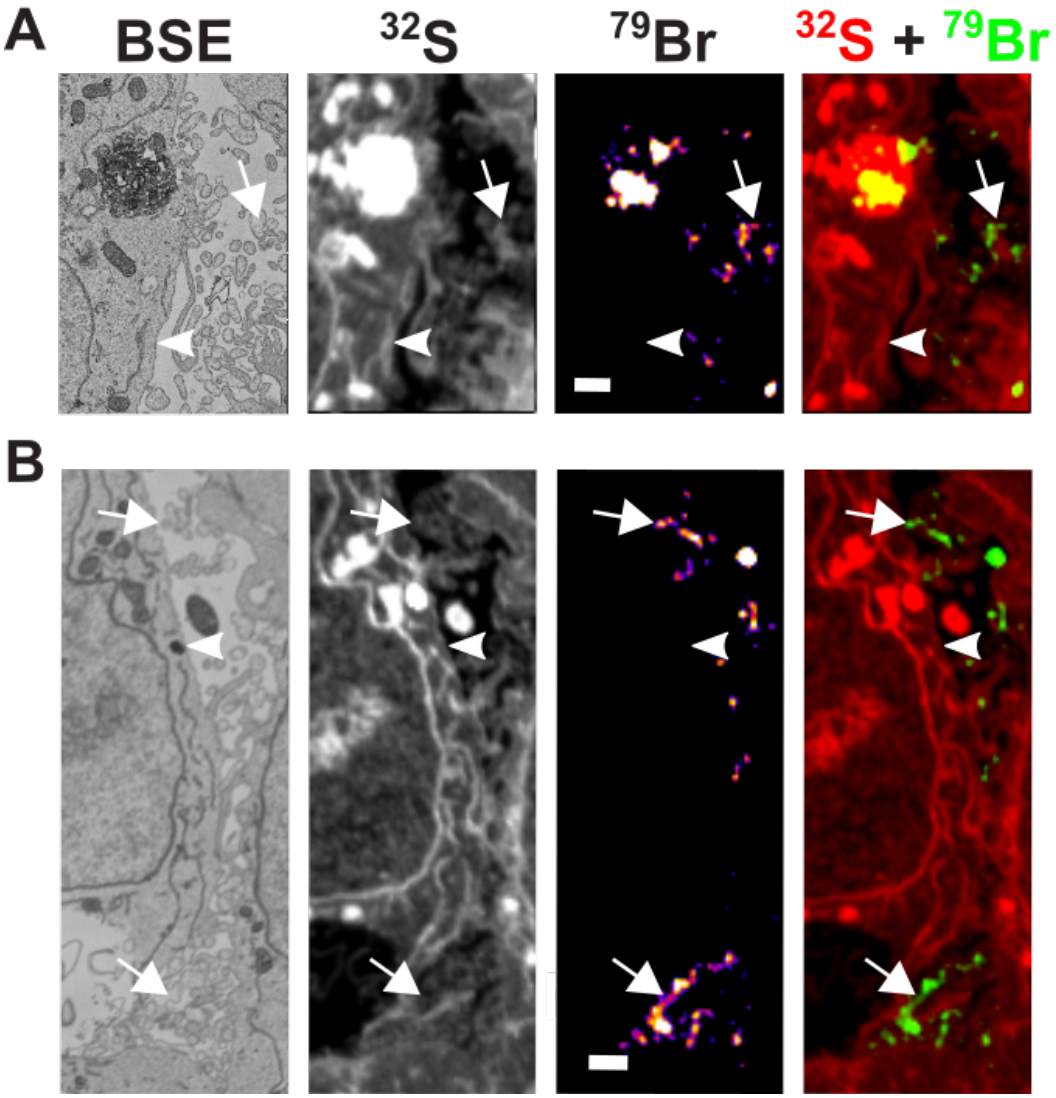


Fig. S10. ^{79}Br enrichment in filopodia of HEK293 cells. High-magnification NanoSIMS images [^{32}S, ^{79}Br, and composite ^{32}S (*red*) + ^{79}Br (*green*)] and backscattered electron (BSE) images of wild-type HEK293 cells that had been incubated in medium containing GalNAc-Br-ASO (A) or Br-ASO (B), revealing ^{79}Br enrichment in regions containing filopodia (arrows). ^{79}Br enrichment in adjacent areas of the plasma membrane was negligible (arrowheads). Scale bar, 1 µm.

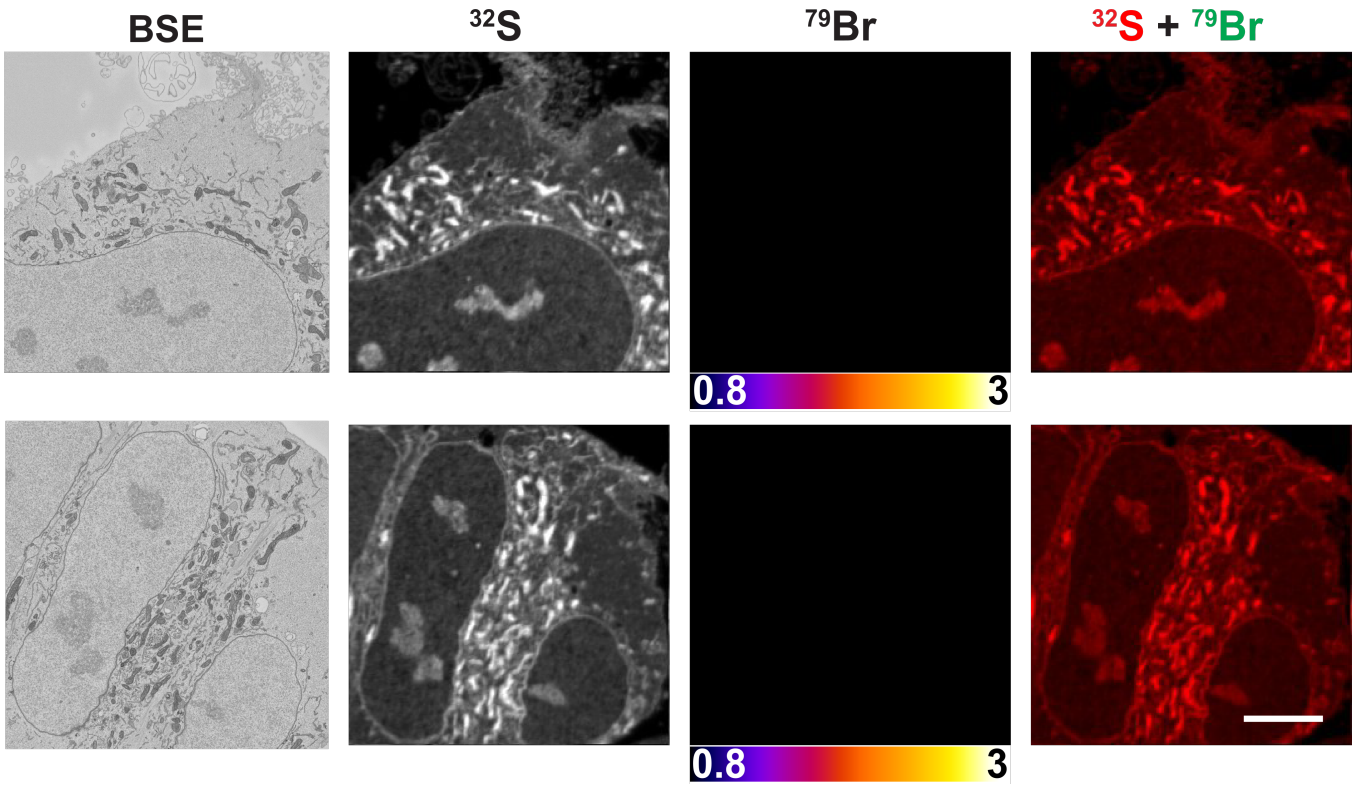


Fig. S11. Backscattered electron (BSE) images and NanoSIMS images of HEK293 cells that were not treated with an ASO. Shown here are BSE images along with ^{32}S, ^{79}Br, and composite ^{32}S (*red*) + ^{79}Br (*green*) NanoSIMS images of untreated HEK293 cells. Scale bar, 5 µm.

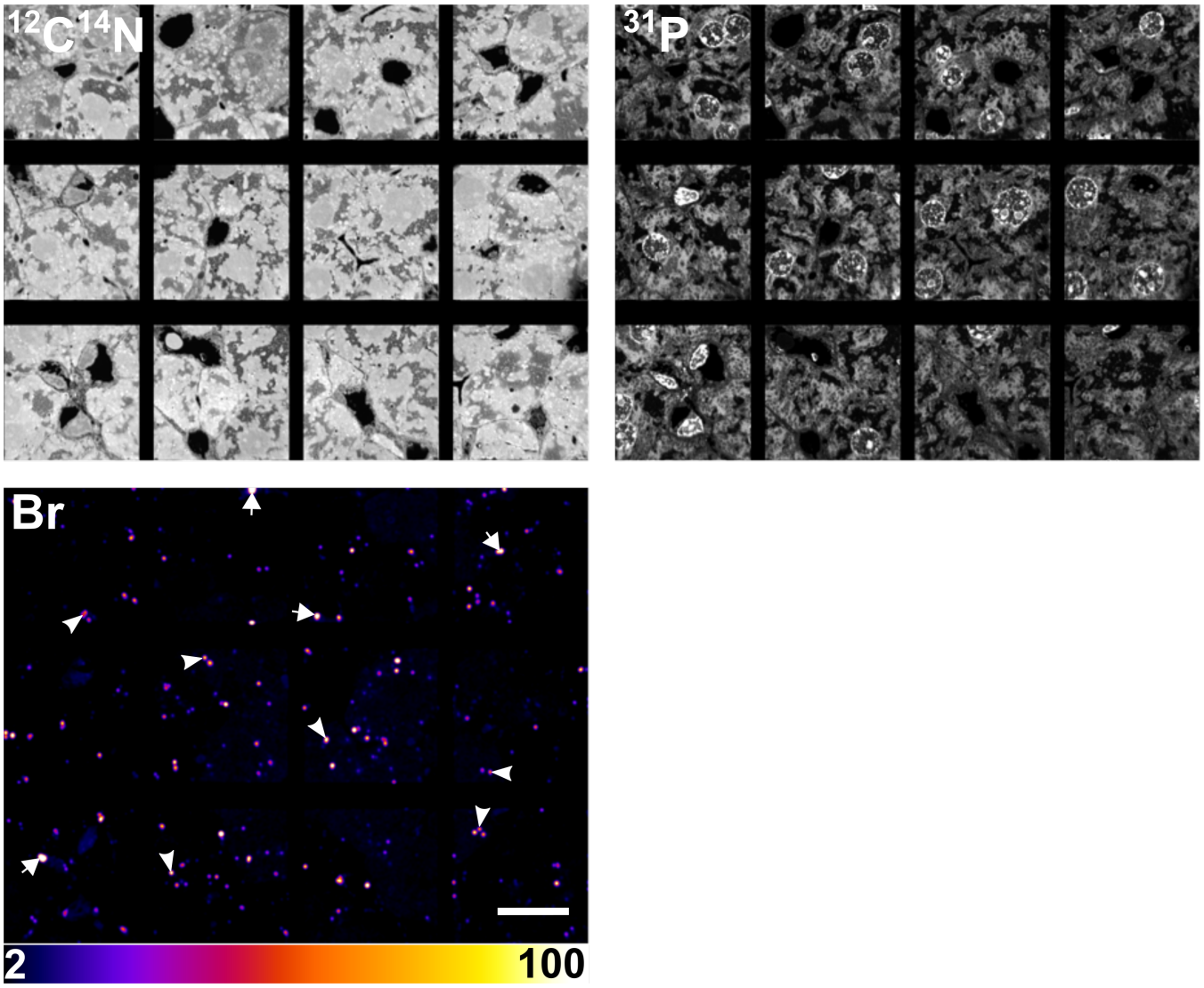


Fig. S12. Additional NanoSIMS images of the mouse liver mosaic shown in Fig. 5, with bromine (Br) NanoSIMS images (^{79}Br + ^{81}Br) shown on a different scale. The ^{12}C^{14}N and ^{31}P NanoSIMS images are useful for morphology, with the 31P images particularly useful for visualizing cell nuclei. The bromine image (^{79}Br + ^{81}Br) at a scale of 2 to 100 show intense bromine enrichment (endolysosomes) within hepatocytes (arrowheads) and Kupffer cells (arrows). Scale bar, 20 μm.

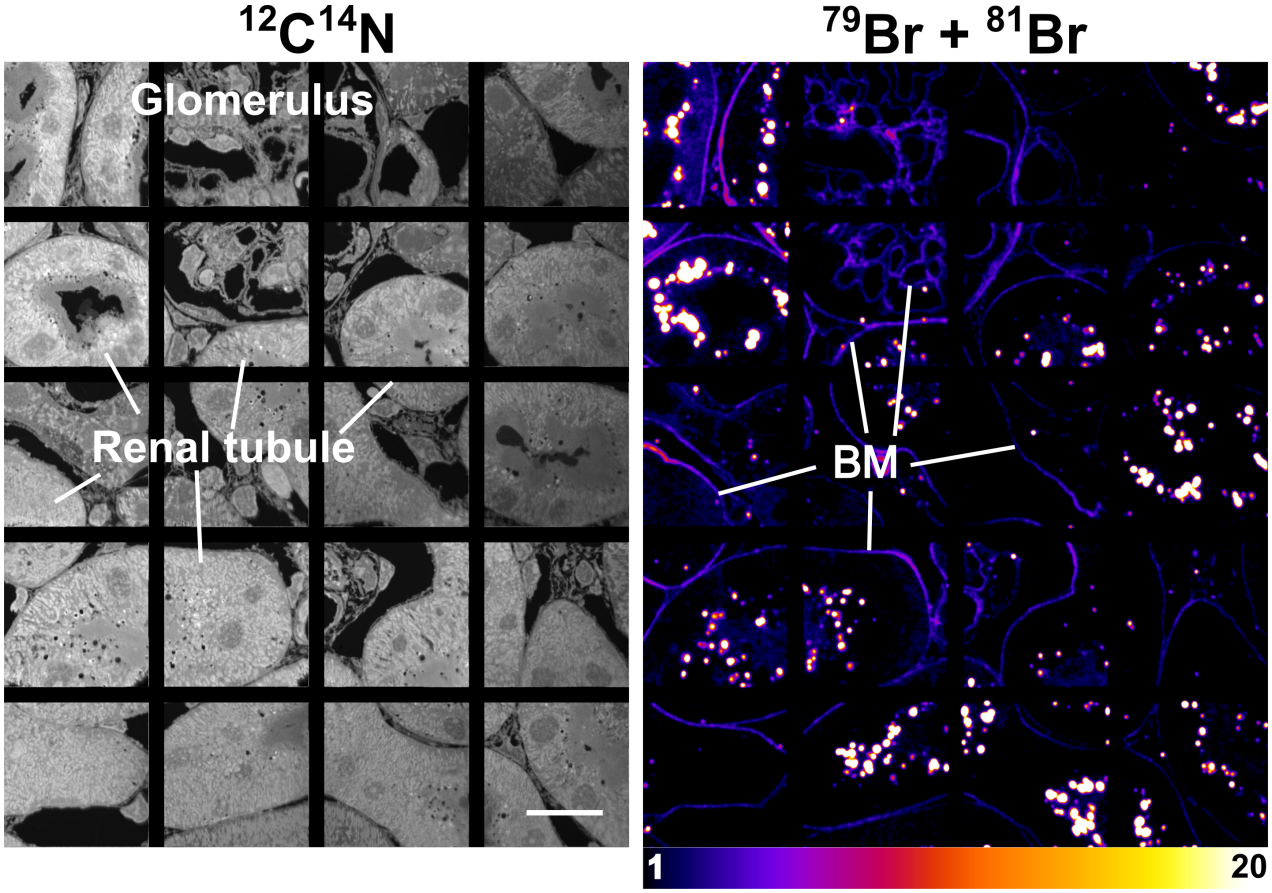


Fig. S13. Additional images of the mouse kidney NanoSIMS mosaic images shown in Fig. S15, with ^{79}Br depicted on a different scale. $^{12}C^{14}N$ NanoSIMS images are useful for defining cell morphology; the bromine (^{79}Br + ^{81}Br) images, shown here at a scale of 1 to 20, show localized regions of intense bromine enrichment in endolysosomes of renal tubule cells; they also show lower levels of bromine enrichment in basement membranes around renal tubules and in the basement membrane lying between capillary endothelial cells and podocyte foot processes in the glomerulus. Bromine enrichment in the basement membranes results from protein bromination by hypobromous acid (HOBr) generated by peroxidasin. Peroxidasin is an peroxidase of the extracellular matrix (38). BM, basement membrane. Scale bars, 20 μm.

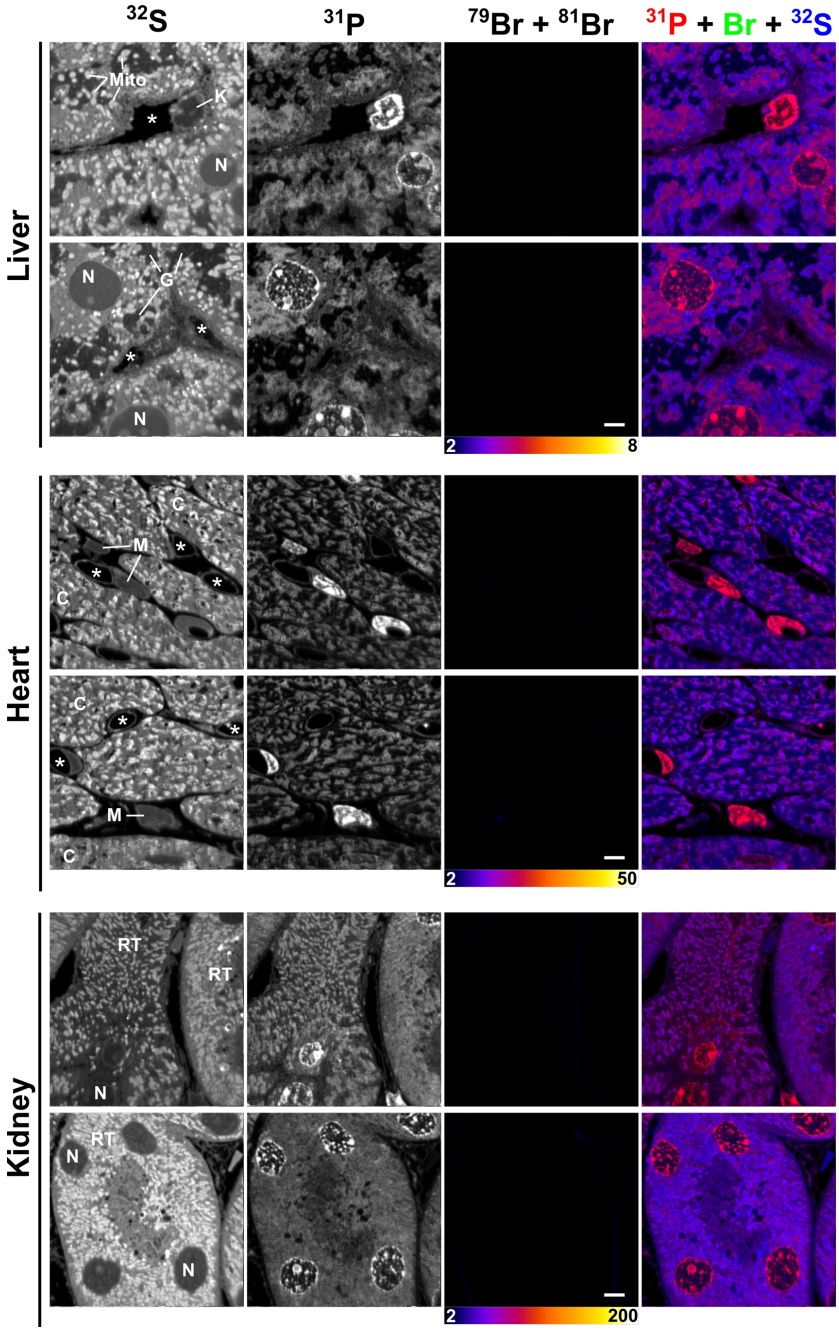


Fig. S14. NanoSIMS images of tissues from a wild-type mouse that was not treated with an ASO. A mouse was given a subcutaneous injection of 1 ml of normal saline but no ASO. After 72 h, tissues were harvested and processed for NanoSIMS analyses. ^{32}S and ^{31}P NanoSIMS images were useful for defining cell morphology; bromine (^{79}Br + ^{81}Br) images and composite ^{31}P (red) + Br (green) + ^{32}S (blue) NanoSIMS images were generated to visualize bromine distribution. The scale in these images is the same as the NanoSIMS images shown in Fig. 5, Fig. S14, and Fig. S15 (which show bromine distribution in liver, heart, and kidney, respectively, after an injection of Br-ASO). *capillary lumen; Mito, mitochondria; K, Kupffer cell; N, nucleus; G, glycogen; M, macrophages; C, cardiomyocytes; RT, renal tubule. Scale bars, 4 μm.