

Figure S1. Autophagy induced by nitrogen starvation in *Arabidopsis* roots. Wild type seedlings expressing GFP-ATG8a fusion were grown for 7 days on N-rich medium and then transferred to the same medium (+N), or to a N-deficient medium (-N) 4 additional days. The root cells were visualized by confocal fluorescence microscopy. Representative images of fluorescence, visible and overlaid of both are shown.

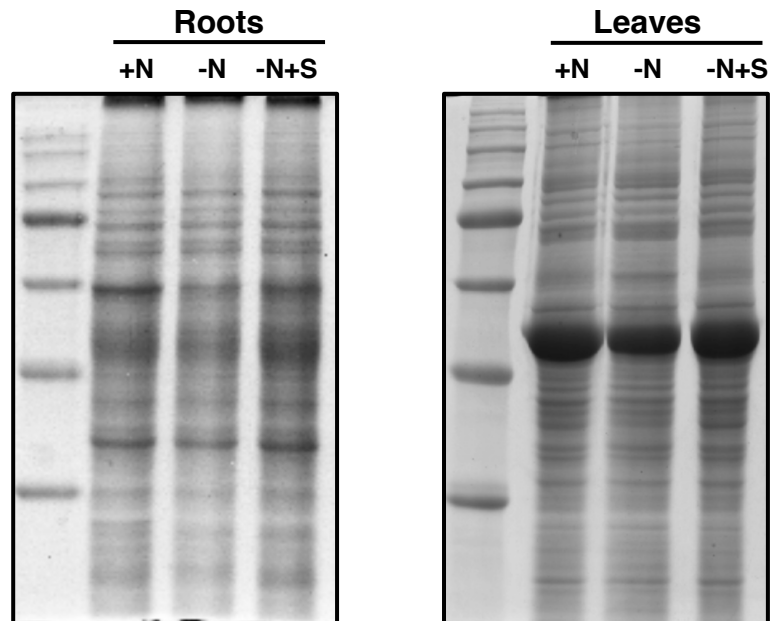


Figure S2. Representative SDS-PAGE of protein extracts. Wild type seedlings expressing the GFP-ATG8a fusion were grown for 7 days on N-rich medium and then transferred to the same medium (+N), to a N-deficient medium (-N), or to a N-deficient medium containing 200 μ M NaHS for 4 additional days. Root (20 mg) and leaf (100 mg) plant materials were ground separately in liquid nitrogen with 100 and 300 μ l of extraction buffer, respectively, as described in Materials and Methods, and 10 μ l of the final supernatant fractions were electrophoresed in 10 % acrylamide gels and stained with Coomassie Brilliant Blue.

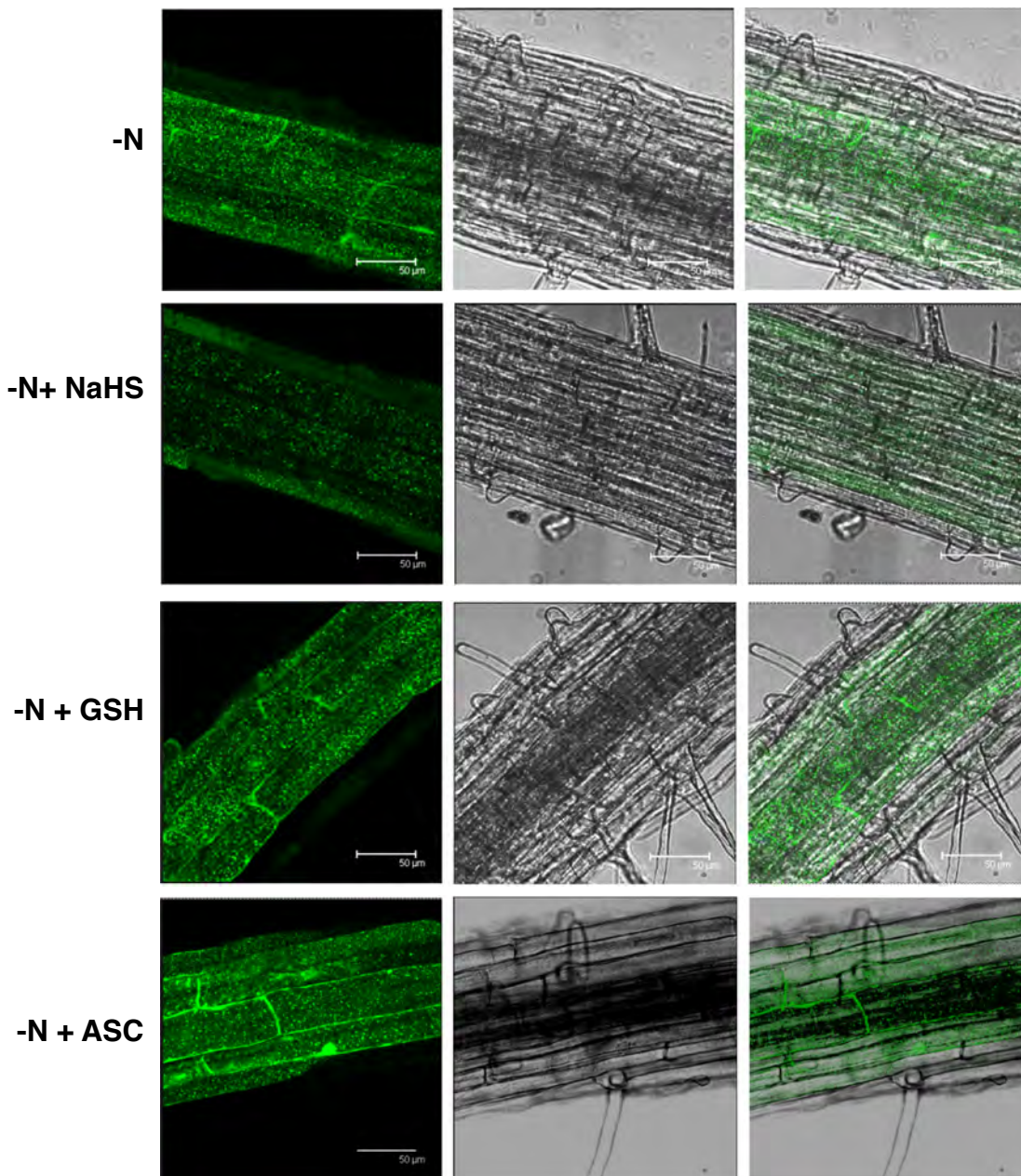


Figure S3. Representative single optical section of fluorescence, visible and overlaid images visualized by confocal microscopy of the root cells corresponding to the experiment described in Figure 5.

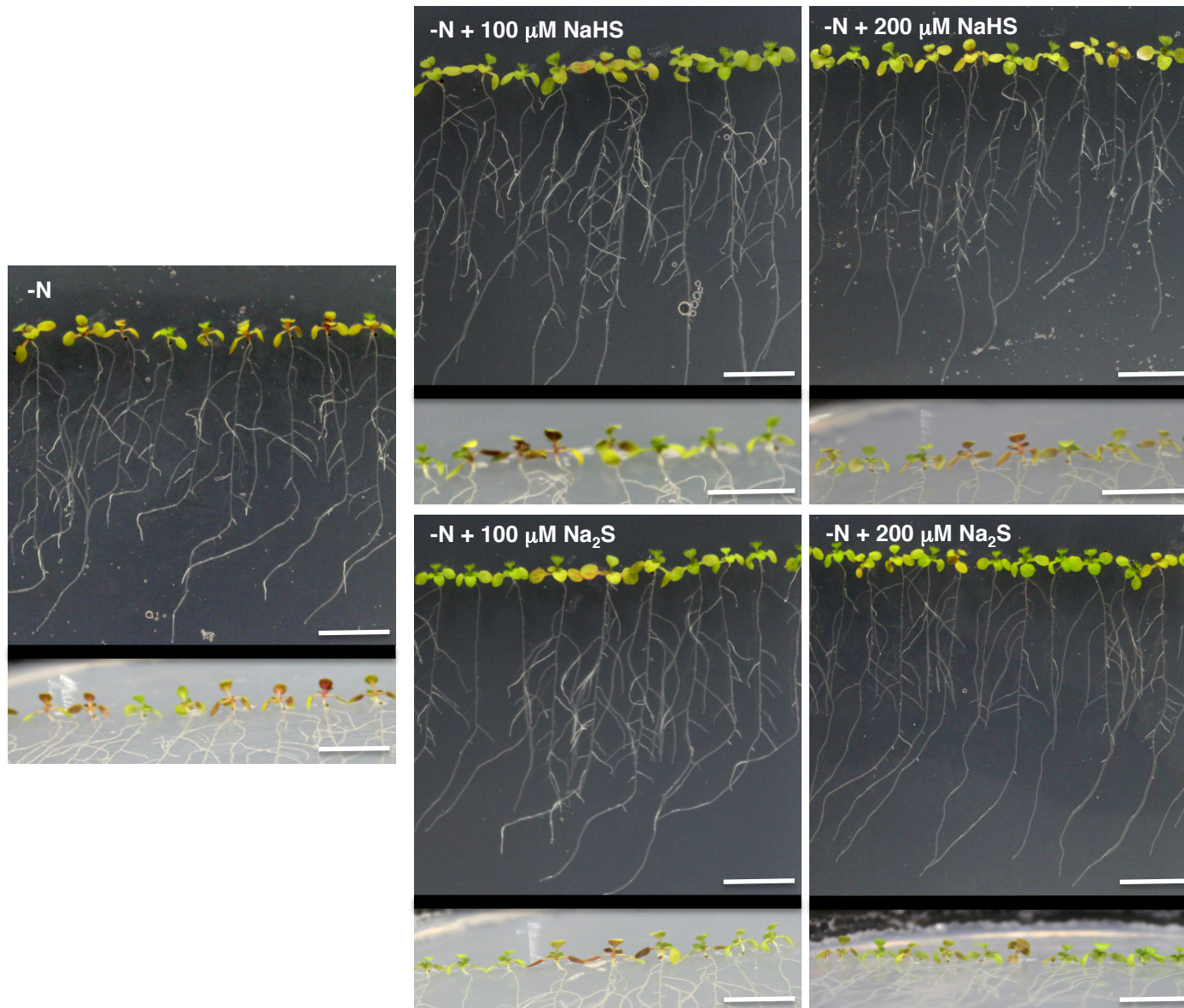


Figure S4. Phenotypes of the wild type seedlings expressing GFP-ATG8a under different conditions. Representative bright field images of 7-day-old wild-type grown on N-rich medium and then transferred to a N-deficient medium (-N) or to the N-deficient medium containing NaHS or Na₂S at 100 or 200 μM for 6 additional days. Lower panels show images of the abaxial part of the leaves. Bars = 1 cm

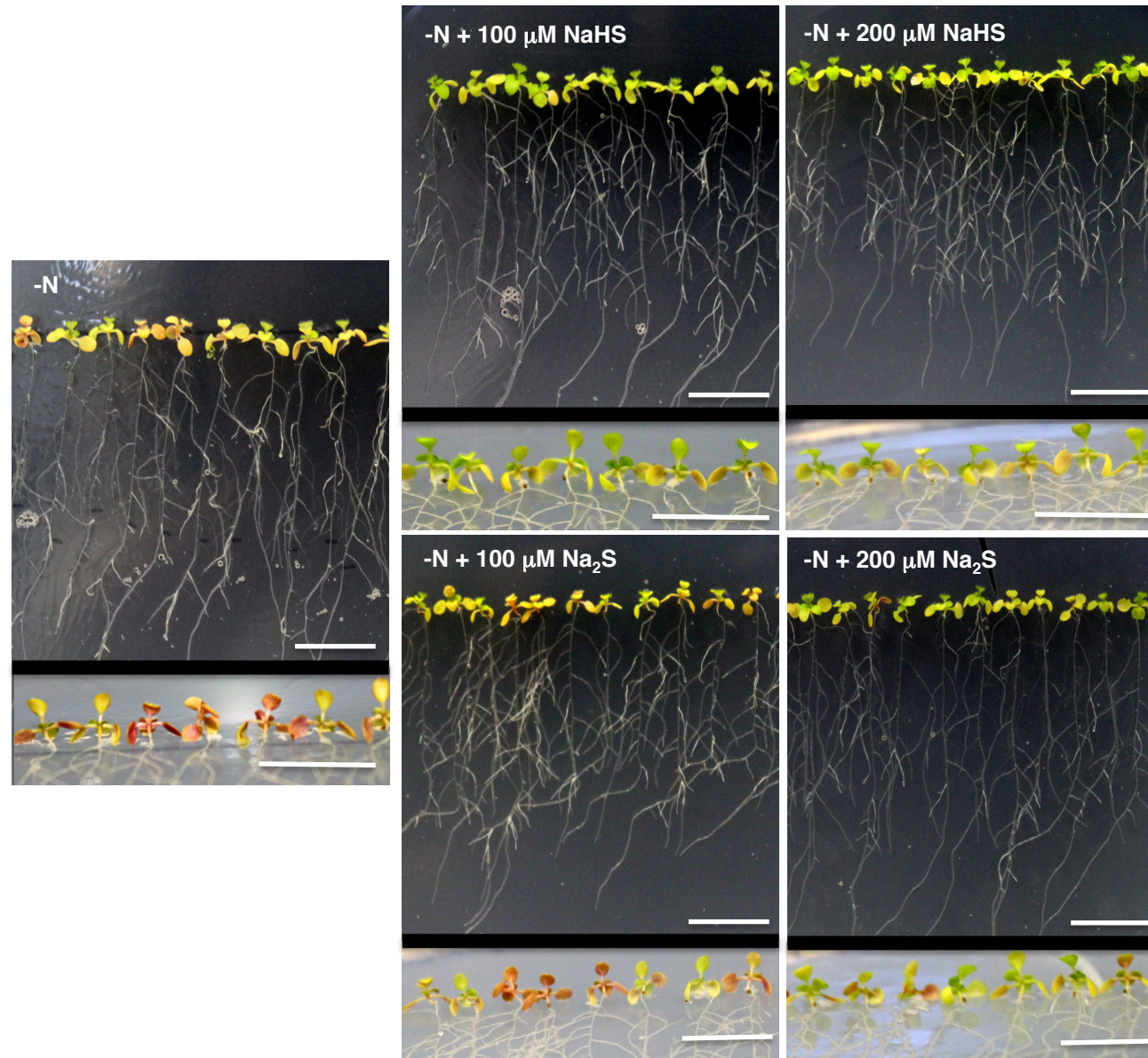


Figure S5. Phenotypes of the wild type seedlings expressing GFP-ATG8a under different conditions. Representative bright field images of 7-day-old wild-type grown on N-rich medium and then transferred to a N-deficient medium (-N) or to the N-deficient medium containing NaHS or Na₂S at 100 or 200 μM for 8 additional days. Lower panels show images of the abaxial part of the leaves. Bars = 1 cm

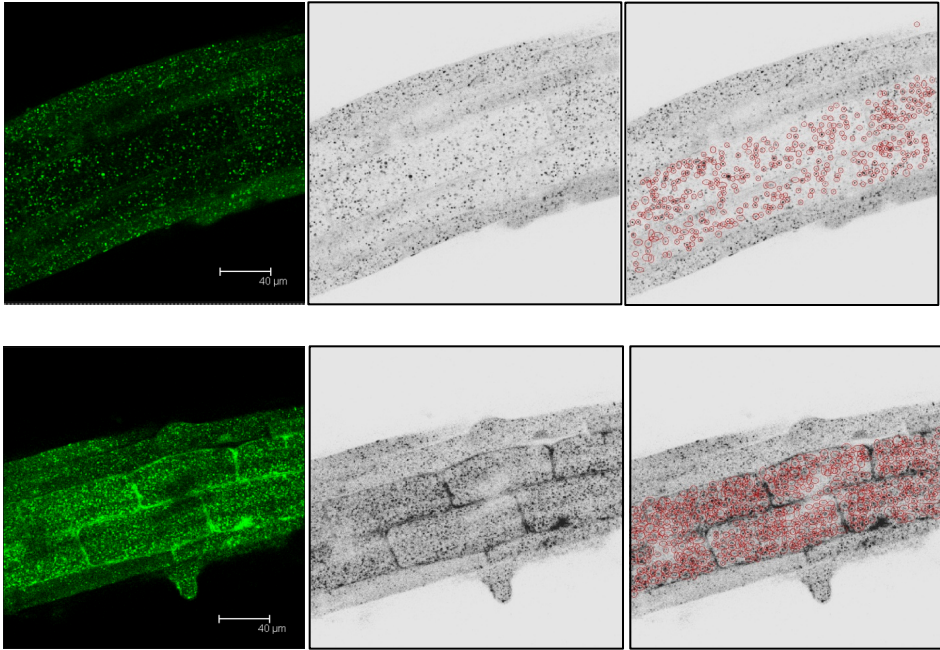


Figure S6. Image processing with PDQuest software (Bio-Rad). Two representative fluorescence confocal images were subjected to PDQuest software. The black and white images were automatically generated, and the images with selected spots are shown.

Table S1. Protein concentration of root extracts. Wild type seedlings expressing the GFP-ATG8a fusion were grown for 7 days on N-rich medium and then transferred to the same medium (+N), to a N-deficient medium (-N), or to a N-deficient medium containing 200 μ M NaHS for 4 additional days. 20 mg of plant root materials were ground in liquid nitrogen with 100 μ l of extraction buffer as described in Materials and Methods. The total amount of protein in the resulting supernatant was determined using a previously described method (Bradford, 1976). Individual experiments and the average data together with the percentages relatives to the +N sample protein concentration in parentheses are shown.

Experiment	Protein Extract (mg/ml)		
	+N	-N	-N + NaHS
1	2.32 (100)	1.59 (68)	2.57 (111)
2	1.19 (100)	0.96 (80)	1.28 (107)
3	2.26 (100)	1.68 (74)	2.02 (89)
4	2.11 (100)	1.08 (51)	1.27 (60)
5	1.39 (100)	0.59 (42)	0.95 (68)
6	2.23 (100)	1.44 (64)	1.69 (76)
Average	1.92 \pm 0.49 (100)	1.22 \pm 0.42 (64)	1.63 \pm 0.59 (85)