Supporting Information

Live-cell imaging of the chloroplast outer envelope membrane using fluorescent dyes

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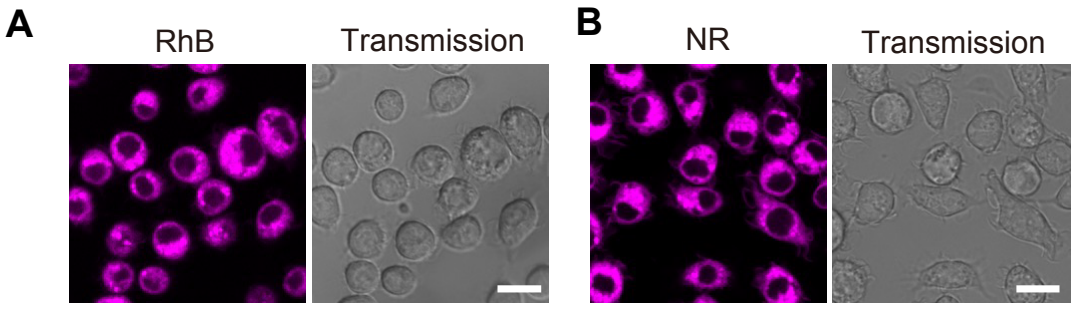

Figure S1. Membrane permeability of RhB and NR.
Drosophila melanogaster S2 cells treated with RhB (A) or NR (B) to confirm membrane permeability. Scale bars, 10 μm.

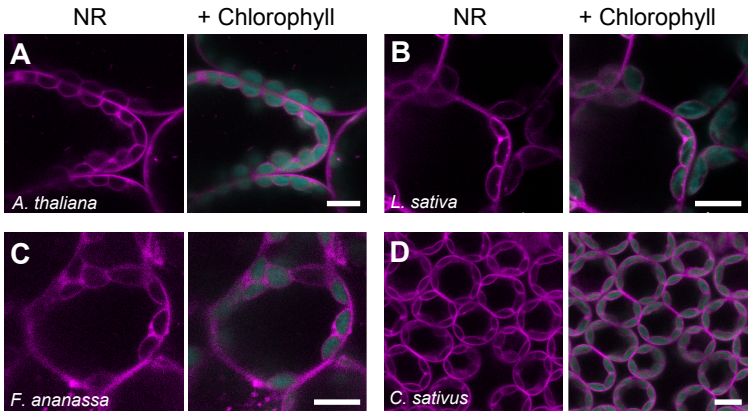

Figure S2. NR staining of the chloroplast envelope in several plant species.
Leaf cells of (A) thale cress (*A. thaliana*), (B) lettuce (*L. sativa*), (C) strawberry (*F.* × *ananassa*), and (D) cucumber (*C. sativus*) stained with 1 μM NR. Scale bars, 10 μm.

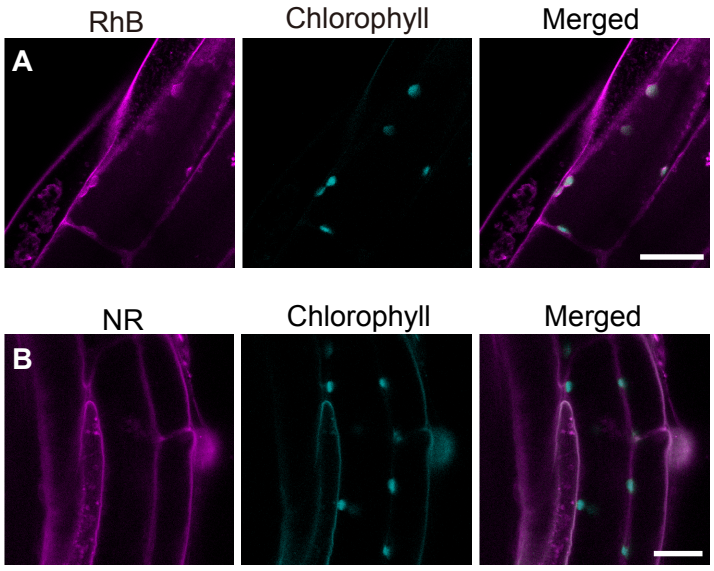

Figure S3. RhB and NR staining in *Arabidopsis thaliana* root.
Arabidopsis thaliana roots were treated with 1 μM RhB (A) or 1 μM NR (B). Scale bars, 20 μm.

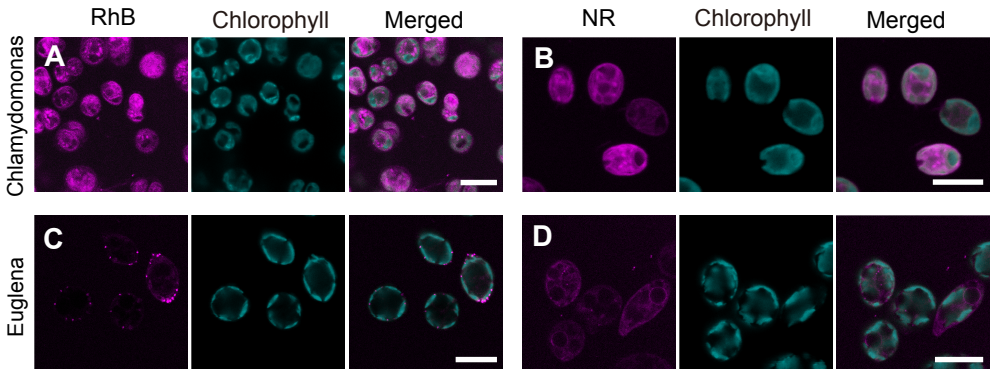

Figure S4. RhB and NR staining in algae
Chlamydomonas reinhardtii (A and B) and *Euglena gracilis* (C and D) were treated with 1 μM RhB or NR. Scale bars, 10 μm (A and B) or 20 μm (C and D).

Table S1. Primers used in this study.

Primer name	Primer sequence (5′ to 3′)
TIC21-SalI-F	AACCAATTCAGTCGACATGCAATCACTACTCTTG
TIC21-EcoRV-R	AAGCTGGGTCTAGATATCCAGCAACCTTAGGAACTAC
mVenus-SalI-F	AACCAATTCAGTCGACATGGTGAGCAAGGGCGAG
mVenus-EcoRV-R	AAGCTGGGTCTAGATATCTTACTTGTACAGCTCGTC
OEP7-SalI-F	AACCAATTCAGTCGACATGGGAAAAACTTCGGGA
OEP7-linker-R	ACCGCCGCTACCGCCGTCATCGGGGTCTTTGGT
linker-mVenus-F	GGCGGTAGCGGCGGTATGGTGAGCAAGGGCGAG