Supplemental Data

Supplemental Figure S1. SDS-PAGE and immunoblot analysis of chloroplast stromal-targeted GFP. Equal amounts (10 μg for gel stain, 4 μg for immunoblotting) of total soluble proteins extracted from fresh leaves expressing chloroplast stromal-targeted GFP (no incubation) and from the leaves incubated at 23°C for 20 h in darkness in 10 mM Mes-NaOH (pH 5.5) with 1% (v/v) dimethyl sulfoxide (- Conc. A) or with 1 μM concanamycin (+ Conc. A) were separated by SDS-PAGE, and either stained with Coomassie Brilliant Blue R250 (gel stain) or analyzed by immunoblotting with anti-GFP antibodies (anti-GFP). Black arrowheads indicate the mature form of CT-GFP after cleavage of the transit peptide. Sizes of molecular mass markers (M. M.; kD) are indicated on the left of the stained gel.

Supplemental Figure S2. Separation of GFP fluorescence and chlorophyll autofluorescence by a laser scanning confocal microscopy. A, Chlorophyll-free plastids of a transgenic Arabidopsis root expressing chloroplast-targeted GFP. B, Chloroplasts of a wild-type Arabidopsis leaf. Both GFP and chlorophyll were excited with the 488 nm line of a multi-Argon ion laser and emission of GFP and chlorophyll was detected between 500 and 530 nm, and over 650 nm by a multi channel detector with filters, respectively. The same laser power and gain of each detector were used in both samples. Bars = 50 μm.

Supplemental Video S1. Movement of GFP-labeled spherical bodies in living cells of leaves of CT-GFP plants treated with concanamycin A and incubated in nutrient-free medium for 20 hours in darkness. The movie runs at 5 times normal speed.

Supplemental Video S2. Movement of autophagic bodies in living cells of concanamycin A-treated leaves from transgenic plants expressing GFP-ATG. The movie runs at 5 times normal speed.

Supplemental Video S3. Visualization of stroma-targeted DsRed and GFP-ATG8 in living cells of concanamycin A-treated leaves. The movie runs at 5 times normal speed.