

<u>Supplemental Figure 1.</u> Confocal imaging of both ATI-ER bodies and ATI-PS bodies.

Panels A and B of this figure show a number green ATI1-GFP labeled bodies either in the cytosol (yellow arrowheads) or attached to a chlorophyll auto-fluorescence emitting (magenta signal; Chl) plastid (white arrowheads). Panel C illustrates that the two bodies marked by yellow arrowheads are ATI1-ER bodies as they are visible in the transmission image, while the two bodies marked by white arrowheads are ATI1-PS bodies as they are not detected in the transmission image. Scale bars indicate 2 μ m



Supplemental Figure 2. Confocal imaging of ATI-PS bodies located within plastids. The plastid-associated ATI1-GFP labeled bodies (ATI-PS bodies; white arrowheads) are found also within the plastids as indicated by their colocalization with the magenta labeled chlorophyll autofluorescence (ChI), resulting in white labeled bodies in the right panel.



<u>Supplemental Figure 3.</u> A comparison between stromules visualized by either, the CT-GFP or the ATI1-GFP, fusion proteins. Confocal imaging of stroma filled tubules (stromules) labeled by either (A) chloroplast-targeted GFP (CT-GFP; see Methods) or (B) ATI1-GFP that is seen concentrated mainly in ATI-PS bodies on the plastid and within a stromule protruding out of the plastid (white arrowheads). The magenta signal in the right panel is the result of chlorophyll auto-fluorescence (Chl). The scale bar indicates 2 μ m

Supplemental Figure 4. Increasing ATI1-**GFP** expression along the progression of C starvation stress in seedling hypocotyl cells. Confocal imaging of hypocotyl cells of transgenic seedlings expressing ATI1-GFP and grown under either C rich conditions (control), or under C starvation for 24 and 72 h (See Methods). Bottom panels display enlargements of the area delimited by the red dashed rectangle (showing cortex cells enriched with plastids) in the corresponding panel above.

<u>Supplemental Figure 5:</u> ATI1-GFP expression in cotyledon epidermis cells of a 7-dold seedling grown under favorable growth conditions. Enlargemnt of the area marked by white dashed rectangel is presented in the right panel. The green signal is seen labeling the ER network, the plastid periphery and bodies within plastids (highlighted by yellow arrows in the right panel).

<u>Supplemental Figure 6:</u> Vital and senescing mesophyll cells of a mature stem leaf. Confocal imaging of a ConA treated adult stem leaf taken from a 4-week-old transgenic plant stably expressing chloroplast-targeted GFP (CT-GFP; green signal) and ATI1-mCherry (red). Chlorophyll auto-fluorescence is also shown (magenta). Co-localization of green and red signals results in a yellow to orange labeling while green and magenta results in white labeling.

Supplemental Figure 7: ATI1-GFP expression and localization when driven by the

ATI1 native promoter. Confocal imaging of plastids (chlorophyll autofluorescence is shown as magenta) of hypocotyl cortex (A) and cotyledon mesophyll cells (B-D) of a proATI1:ATI1-GFP seedling imaged following 24 h C starvation (A, B and D) or 72 h C starvation (C). White arrows in B and D denote ATI-PS bodies and the yellow arrowhead in D points towards a labeled stromule.

<u>Supplemental Figure 8:</u> C starvation induces the expression of ATI1-GFP in plastids also when driven by the ATI1 native promoter. Confocal imaging of rosette-leaf mesophyll cell plastids (chlorophyll autofluorescence is shown as magenta in the right panels) of a four weeks old proATI1:ATI1-GFP plant imaged without any treatment (top panels) or following 24 h of IDL treatment (See Methods; bottom panels). In both treatments, the GFP signal was imaged with identical laser intensity and high voltage (HV) parameters.

ATI1pro::ATI1-GFP; darkened rosette leaf

transmittance

transmittance + GFP

Supplemental Figure 9: ATI1-GFP labels ATI-ER bodies also when driven by the ATI1 native promoter following 24 h C starvation stress. Confocal imaging of a proATI1:ATI1-GFP hypocotyl epidermal cell showing bodies in the cytosolic focal plane that display the ATI-ER bodies characteristic visualization in the transmittance channel (left panel; white arrowheads). These bodies are labeled by ATI1-GFP also when driven by the endogenous ATI1 promoter (right panel). Scale bars indicate 10 μ m