Table I. Primers used for RT-PCR.

Primer	Sequence
G35v1_EcoRV_for	AAGATATCATGATTCTTTCATTGGAAGAGCG
G35v2_EcoRV_for	AAGATATCATGGGAGCTTTGCAGCTAAT
G35_SmaI_rev	TCCCCCGGGTCCCTGTGGAGTTTCGTGATCT
G35_splicing_for	GTTGGCACCATGGGAGCTTTGCAGCT
G35_genot_for	TGTTTCCATGGGATTTATGCTCC
G35_genot_rev	TCGCCTGTACTTCATCACTCTC
G35V1_NotI_for	CTAGCGGCCGCATGATTCTTTCATTGGAAGAGCG
G35V1_NotI_rev	GAATGCGGCCGCTCACTGTGGAGTTTCGTGATCT
AtSag12for	ACTGGAGGAAGAAAGGAGCTGT
AtSag12rev	TGATCCGTTAGTAGATTCGCCGT
ActRTfor	ATTCAGATGCCCAGAAGTCTTGTTCC
ActRTrev	ACCACCGATCCAGACACTGTACTTCC

Supplementary figure legends:

Movie 1. *Arabidopsis* leaves agroinfiltrated with *A. tumefaciens* strain harboring the pGREAT-2x35S-G35v1::DsRed2 construct.

Movie 2. *Arabidopsis* leaves agroinfiltrated with *A. tumefaciens* strain harboring the mito-mCherry construct that target the mCherry reporter to the mitochondria.

Figure S1. The mitochondria isolated from Arabidopsis are enriched in cytochrome c.
The chloroplast preparation was not contaminated by mitochondria (no cyctochrome c was present). The mitochondria preparation contained a slight contaminationb y PsaA of Photosystem I.
The 85 kDa band in the mitochondria could not be merely due toco ntamination by the chloroplasts, given the relative intensity of the bands recognized by the anti-GLR3.5 antibody (upper panel).

Movie 3. Ten-day-old Arabidopsis WT roots stained with TMRM.

Movie 4. Ten-day-old *Arabidopsis* mutant (*atglr3.5-1*) roots stained with TMRM

Figure S2. Transcript analysis of 4 putative silenced plants. PCR were performed with primers reported in Table I. TEM micrographs of mitochondria from GLR3.5-silenced (*glr3.5 #1 and #2*) and not-silenced (*glr3.5 #3 and #4*) *Arabidopsis* plants.

Figure S3. Ultrastructure of organelles in WT, *atglr3.5-1* and silenced plants. TEM micrographs from 3 week-old (A) or 6 week-old (B) WT Col0 and knockout plants. Upper and lower rows show representative images from WT and knockout plants, respectively. C) Representative images from non-silenced (*glr3.5 #4)* and silenced plants (*glr3.5 #1* at 3 and 6 weeks).

Figure S4. $[Ca^{2+}]_{mit}$ dynamics in root tip cells of WT and *atglr3.5-1* Arabidopsis seedlings expressing the 4mt-YC3.6 (mitochondrial Cameleon) probe, challenged with 0.01 mM of NAA or 0.1 mM ATP.

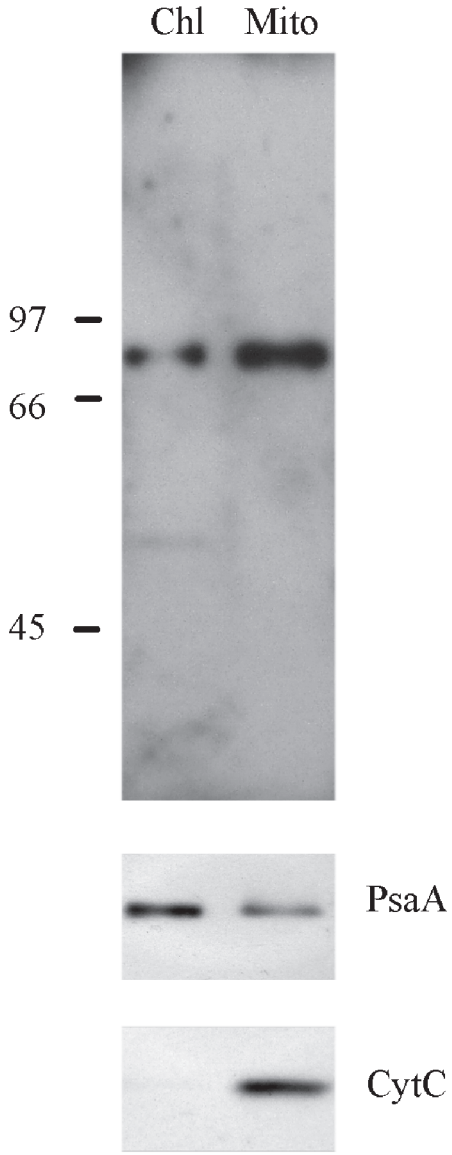
A-D, Root tip cells of seedlings were treated with 0.01 mM of NAA for the indicated time (black rectangles above traces) and the response of cells from the transition and elongation zones analyzed with a widefield fluorescence microscope. A, Depicted Ca^{2+} kinetics, of WT (black) and *atglr3.5-1* (grey), have been obtained averaging traces from 17 and 21 independent experiments respectively for each genotype. B, Statistical analysis of maximum $\Delta R/R_0$ variations, occurred in both genotypes in response to the applied stimulus. C, Statistical analysis of $\Delta R/R_0$ variations measured in both genotypes 500 sec after the sensing of the NAA. D, Statistical analysis of times at which the maximum $\Delta R/R_0$ variations are measured in both genotypes in response to NAA.

E-H, Root tip cells of seedlings were treated with 0.1 mM of ATP for the indicated time (black rectangles above traces) and the response of cells from the meristematic zone analyzed with a widefield fluorescence microscope. E, Depicted Ca^{2+} kinetics, for WT (black) and *atglr3.5-1* (grey), have been obtained averaging traces from 8 independent experiments for each genotype. F, Statistical analysis of maximum $\Delta R/R_0$ variations measured in both genotypes in response to the applied stimulus. G, Statistical analysis of $\Delta R/R_0$ variations measured in both genotypes 300 sec after the sensing of the applied stimulus. H, Statistical analysis of times at which the maximum $\Delta R/R0$ variations are measured in both genotypes in response to applied stimulus.

Data are reported as avareges ± SD (standard deviation) (p-values calculated by Student T-test.* 0.111; ** 0.193; *** 0.011; ● 0.691; ●● 0.6; ●●● 0.895).

Figure S5. $[Ca^{2+}]_{mit}$ dynamics in leaf cells of WT and *atglr3.5-1* 4-week-old Arabidopsis plants expressing the 4mt-YC3.6 (mitochondrial Cameleon) probe challenged with wounding and analyzed with a widefield fluorescence microscope. A, Depicted Ca^{2+} kinetics, for WT (black) and *atglr3.5-1* (grey), have been obtained averaging traces from 6 independent experiments for each genotype. B, Statistical analysis of maximum $\Delta R/R_0$ variations measured in both genotypes in response to wounding. C, Statistical analysis of $\Delta R/R_0$ variations measured in both genotypes 200 sec after wounding. D, Statistical analysis of times at which the maximum $\Delta R/R_0$ variations are measured in both genotypes in response to wounding. E, Series of representative Ratio Images (false colours) of leaf from WT and *atglr3.5-1* subjected to wounding. Data are reported as avareges ± SD (standard deviation) (p-values calculated by Student T-test.* 0.016; ** 0.306; *** 0.094).

Figure S1.



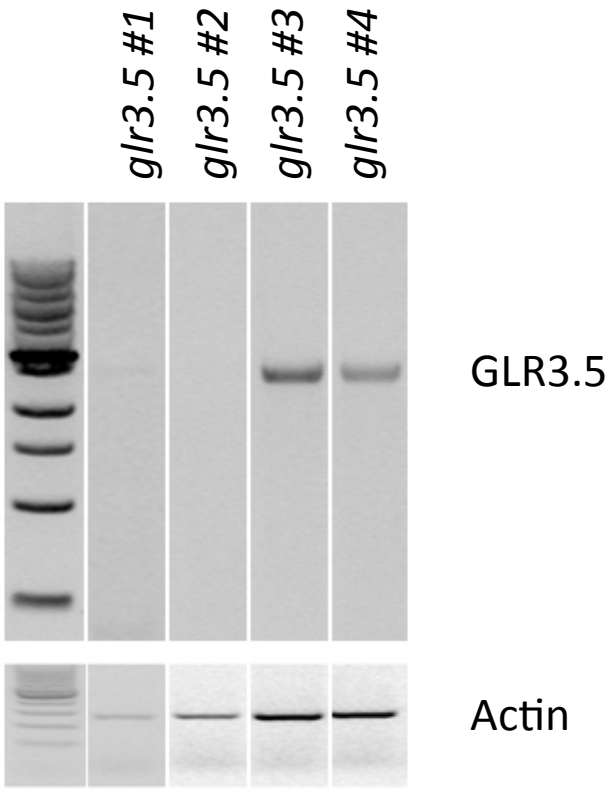


Figure SH.

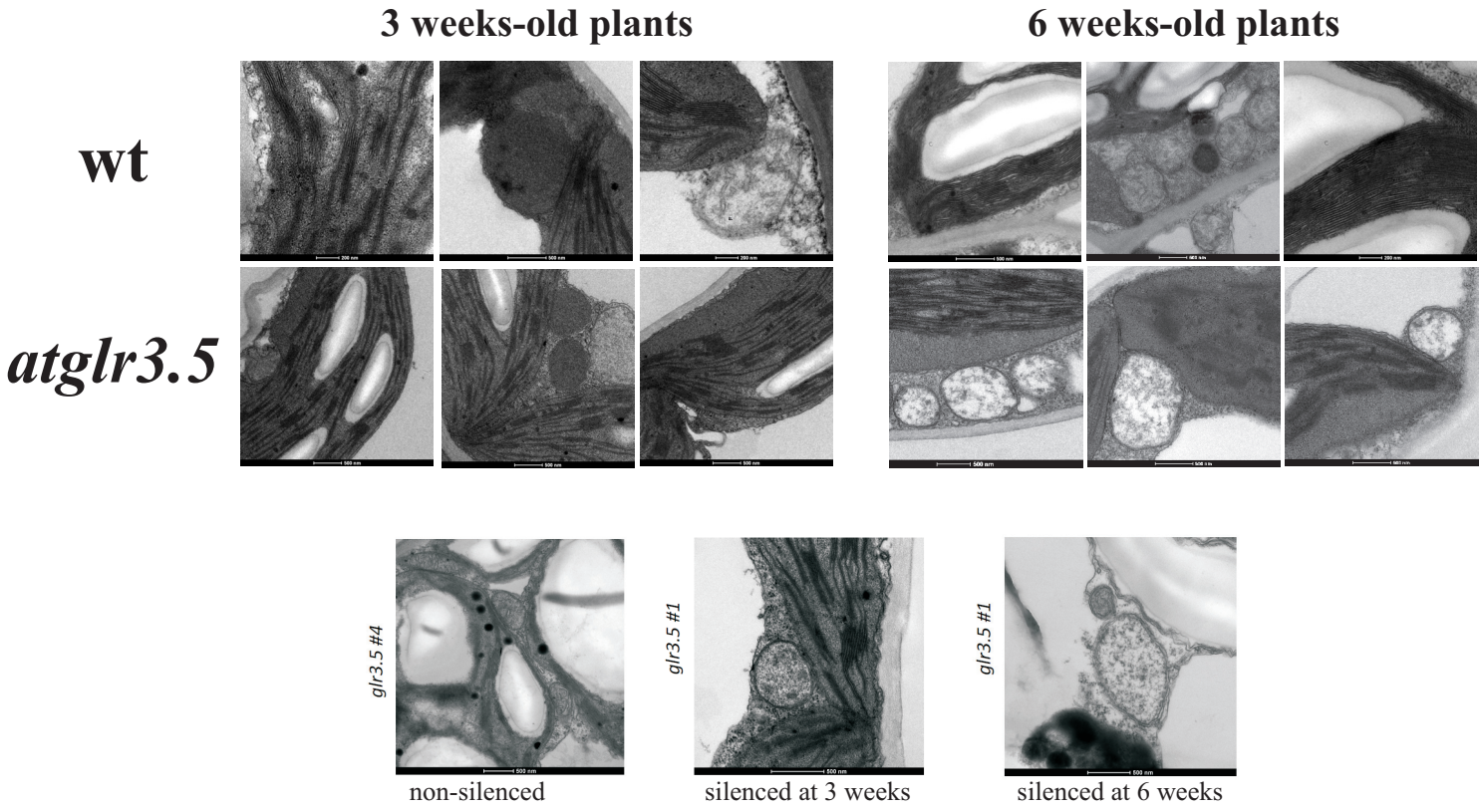

3 weeks-old plants
6 weeks-old plants
wt
atglr3.5
glr3.5 #4
glr3.5 #1
glr3.5 #1
non-silenced
silenced at 3 weeks
silenced at 6 weeks

FIGURE S3

FIGURE S4

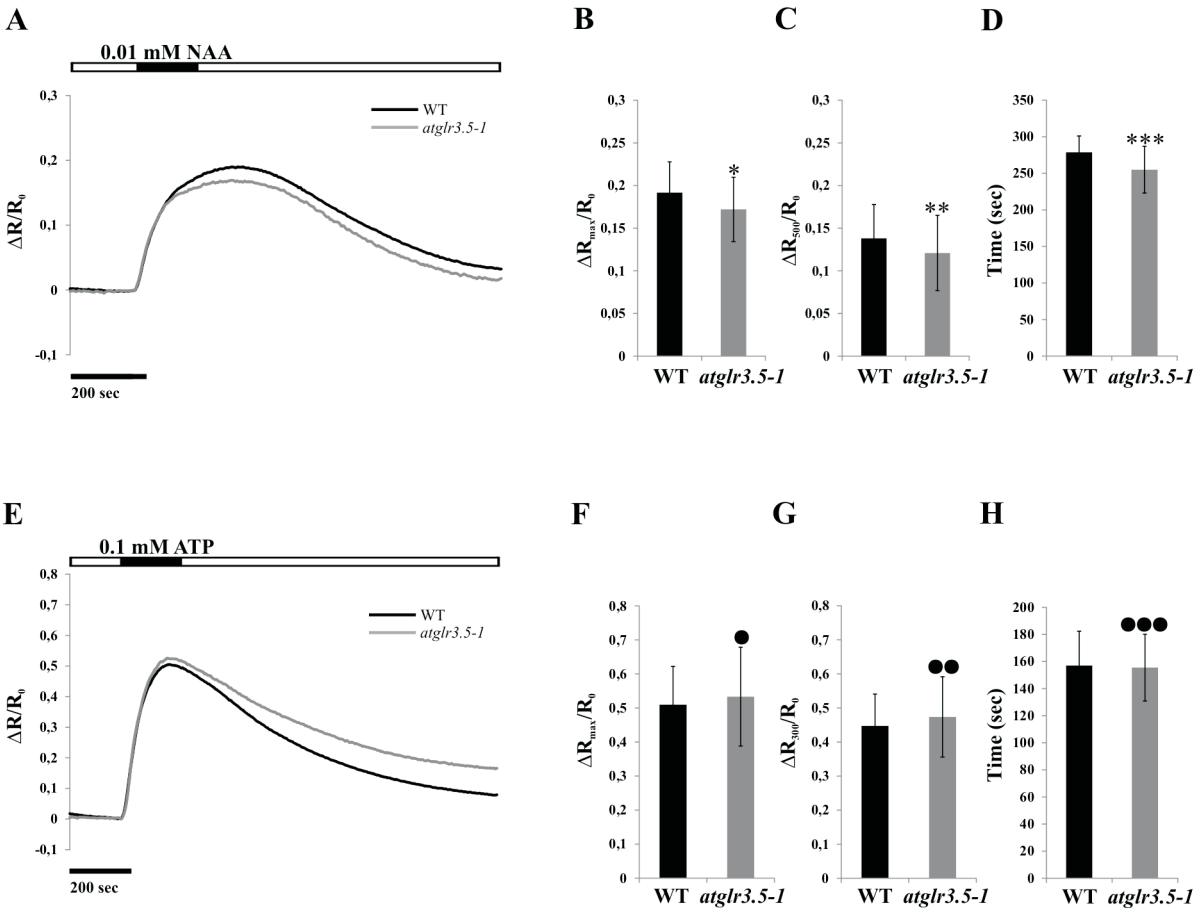


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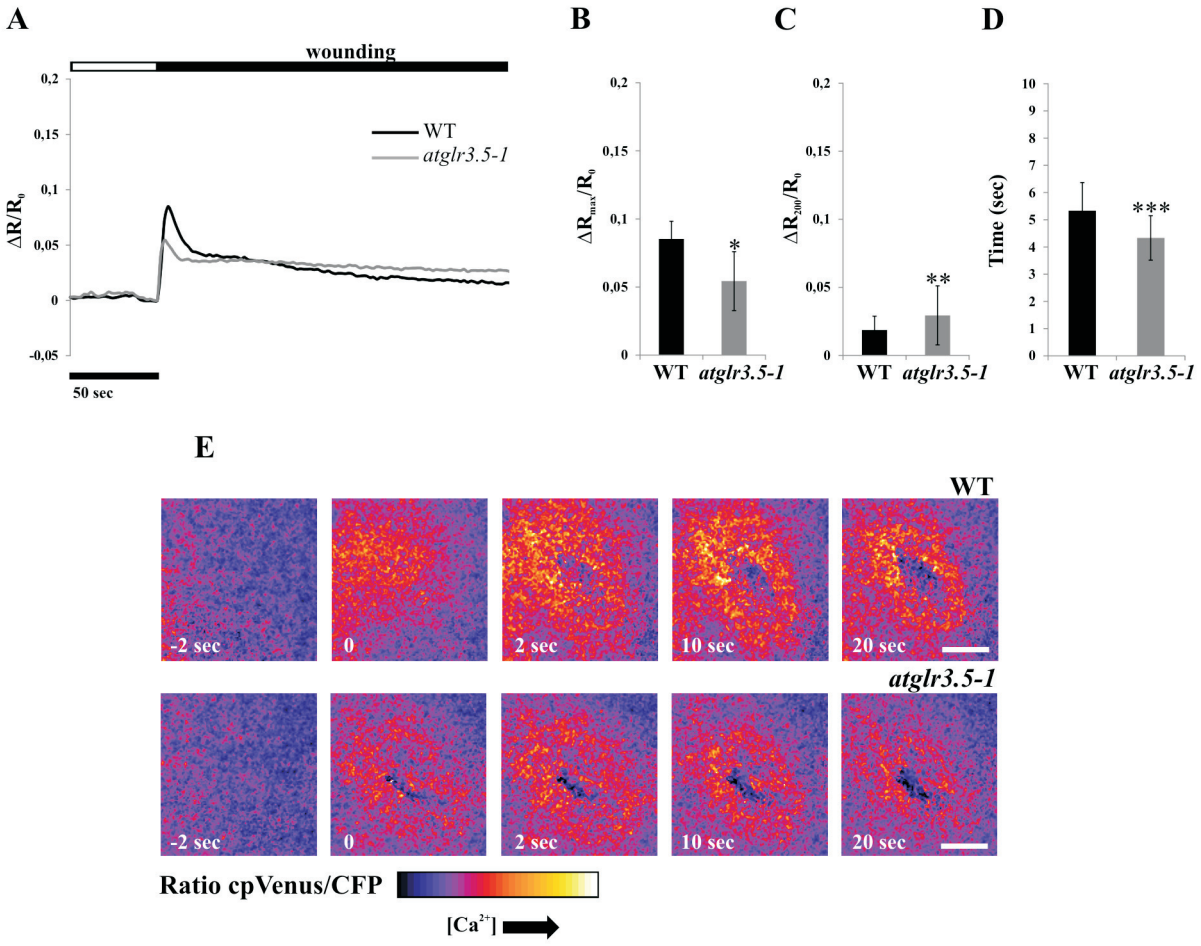
Figure S5.



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name	Assay ID	Assay Mix Œ	Forward Primer Seq.	Reverse Primer Seq.	Reporter Dye	Reporter Sequence
G3.5v1	AIY9ZX4	20x	CAGGACACTAATTGCAGTGGATTT	TTATGTGACCAATTCCTGAAGATTG	FAM	TGGGAGCTAATGGAGAACA
G3.5v2	AI0IX4C	20x	CAGGACACTAATTGCAGTGGATTT	TTATGTGACCAATTCCTGAAGATTG	FAM	TTTGCAGCTAATGGAGAACA
actin 2	At0232991	20x	nd	nd	FAM	TCGGTGGTTCCATTCTTGCTTCCC

TABLE II