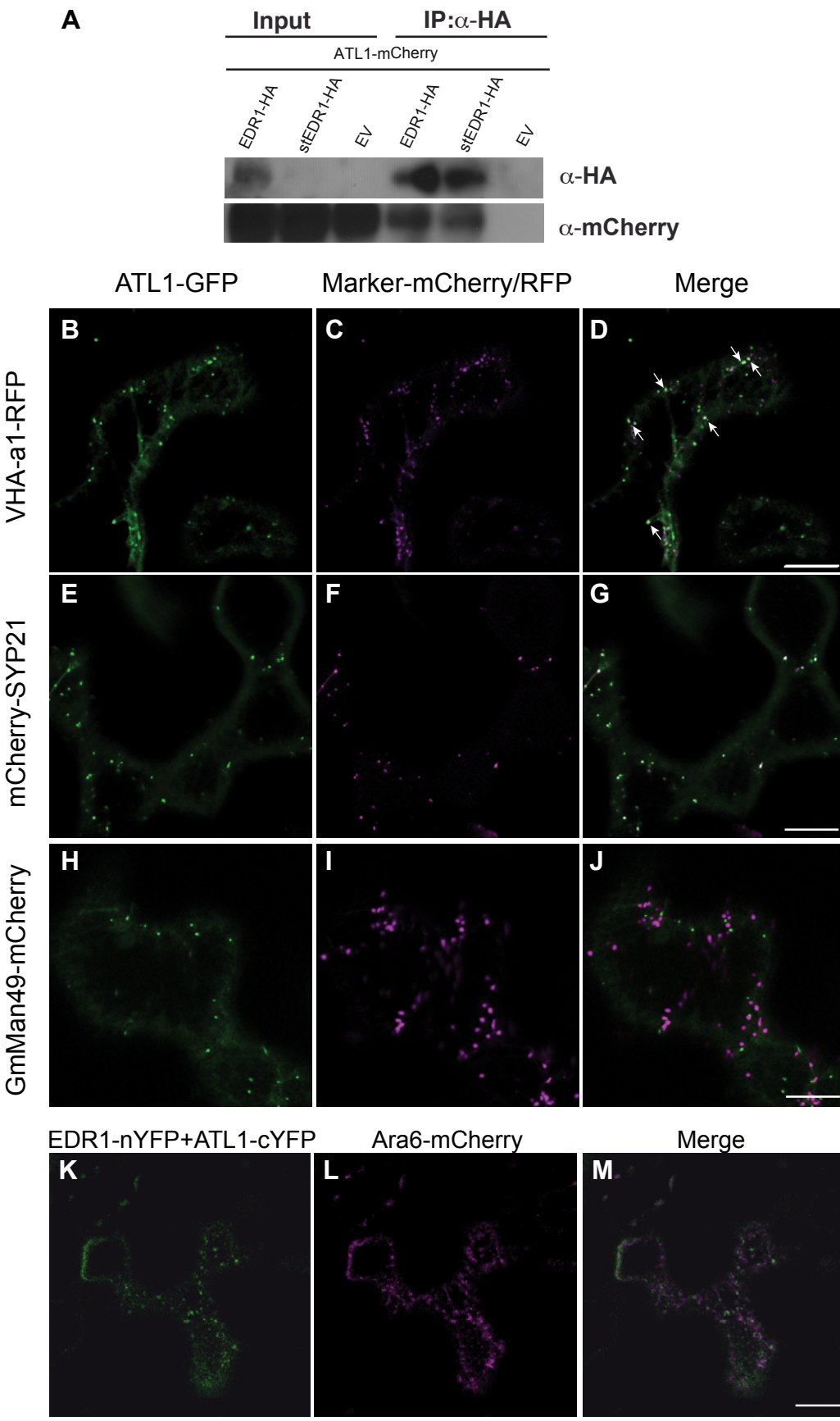
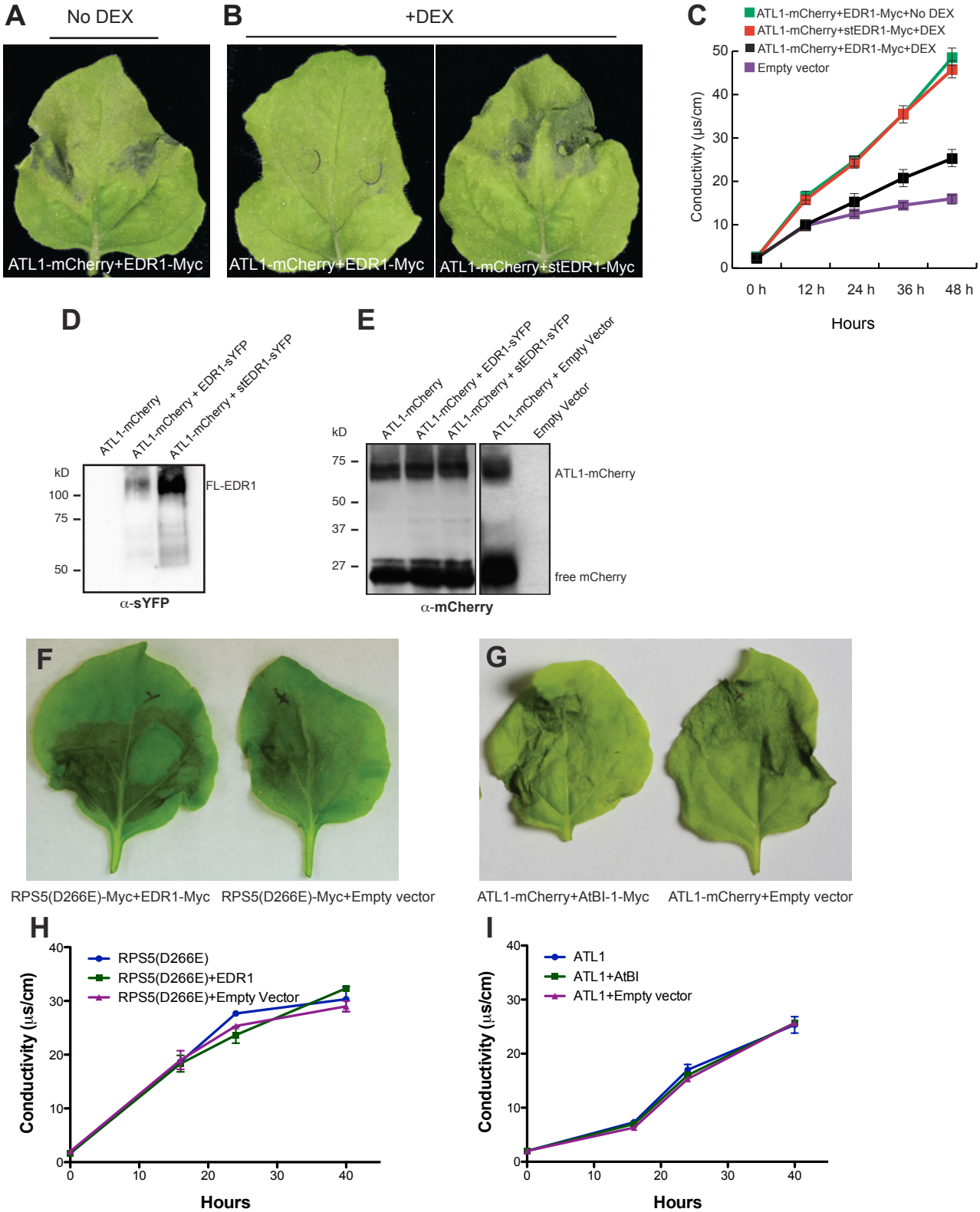


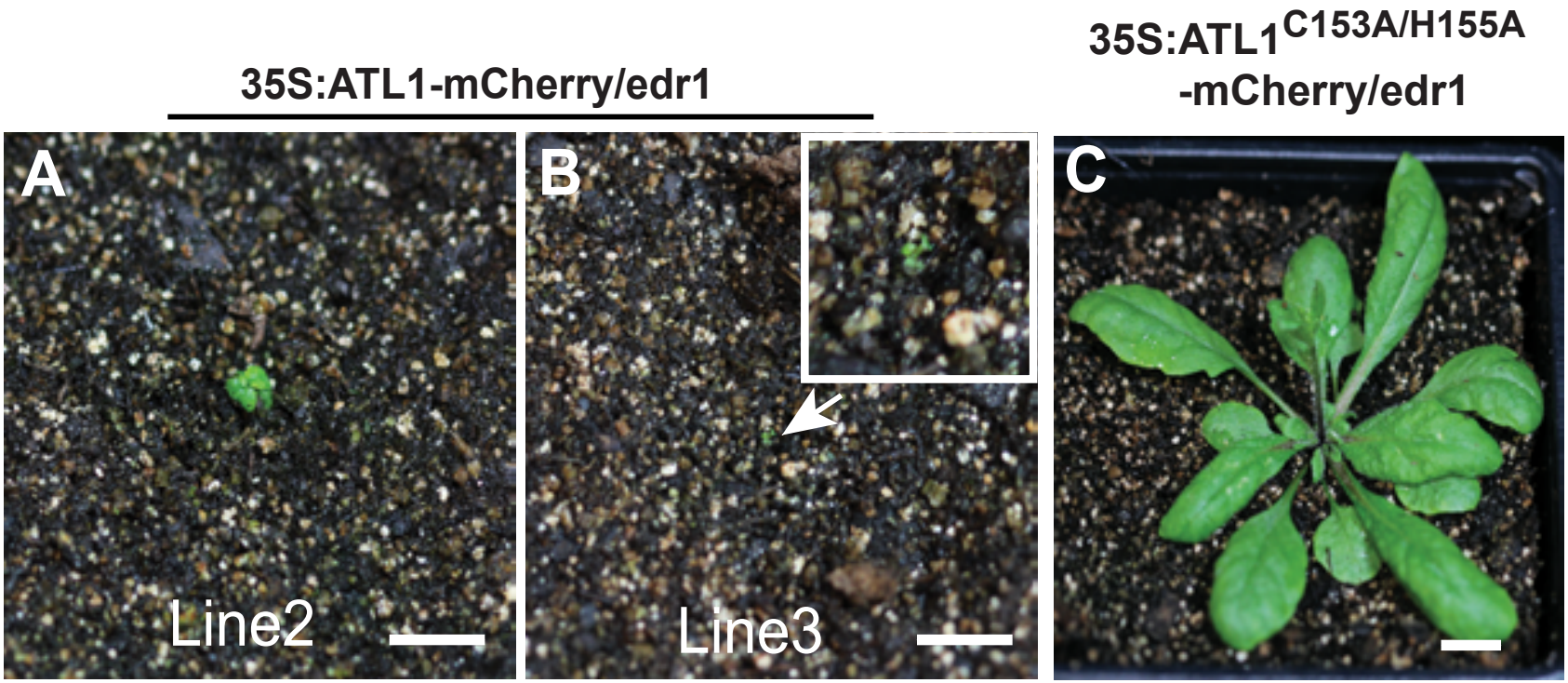
Supplemental Figure 1. ATL1 Co-Immunoprecipitates with EDR1 and Localizes to Endomembrane Structures.

(A) Co-immunoprecipitation of ATL1 with EDR1. ATL1-mCherry was transiently co-expressed with wild-type EDR1-HA, the substrate trap form of EDR1-HA or empty vector in Arabidopsis protoplasts. Proteins were immunoprecipitated with anti-HA affinity matrix (IP). A portion of each sample was taken prior to immuno-precipitation as the input control. Immunoblotting with the indicated antibodies was used to detect the epitope-tagged proteins.
(B) to **(D)** ATL1-eGFP partially co-localizes with the TGN/EE marker VHA-a1-RFP (arrows).
(E) to **(G)** The majority of ATL1-eGFP co-localizes with the multivesicular body/late endosome (MVB/LE) marker mCherry-SYP21.
(H) to **(J)** ATL1-GFP localization is distinct from that of cis-Golgi cisternae marker GmManI49-mCherry.
(K) to **(M)** EDR1 and ATL1 BiFC signal (green) does not overlap with the MVB/LE marker ARA6-mCherry (magenta). All images were obtained from transiently transformed epidermal cells of *N. benthamiana*. Scale bars = 10 μm.



Supplemental Figure 2. EDR1 Specifically Antagonizes ATL1-induced Cell Death in *N. benthamiana*.

(A) to **(C)** ATL1-mCherry-induced cell death in *N. benthamiana* is suppressed by co-expression with EDR1-Myc, but not with the substrate trap mutant version, stEDR1-Myc, or when EDR1 expression is not induced.
(D) and **(E)** Immunoblots of total protein extracts from leaves co-infiltrated with ATL1-mCherry and EDR1-sYFP or stEDR1-sYFP.
(F) EDR1-Myc does not suppress the cell death induced by an auto-active RPS5 mutant (D266E).
(G) AtBI-1-Myc does not suppress the cell death induced by ATL1-mCherry.
(H) and **(I)** Ion leakage measurements for RPS5(D266) and ATL1-induced cell death.
The ATL1-mCherry and AtBI-Myc constructs were expressed under control of a 35S promoter, while the RPS5(D266E)-Myc and EDR1-Myc constructs were under control of a dexamethasone inducible promoter. Dexamethasone was applied 16 h after infiltration and photographs were taken 48 h after infiltration. Error bars indicate standard deviation, n=3. Experiments were repeated twice with similar results.



Supplemental Figure 3. ATL1 Causes Severe Dwarfing When Overexpressed in the Arabidopsis *edr1* mutant.

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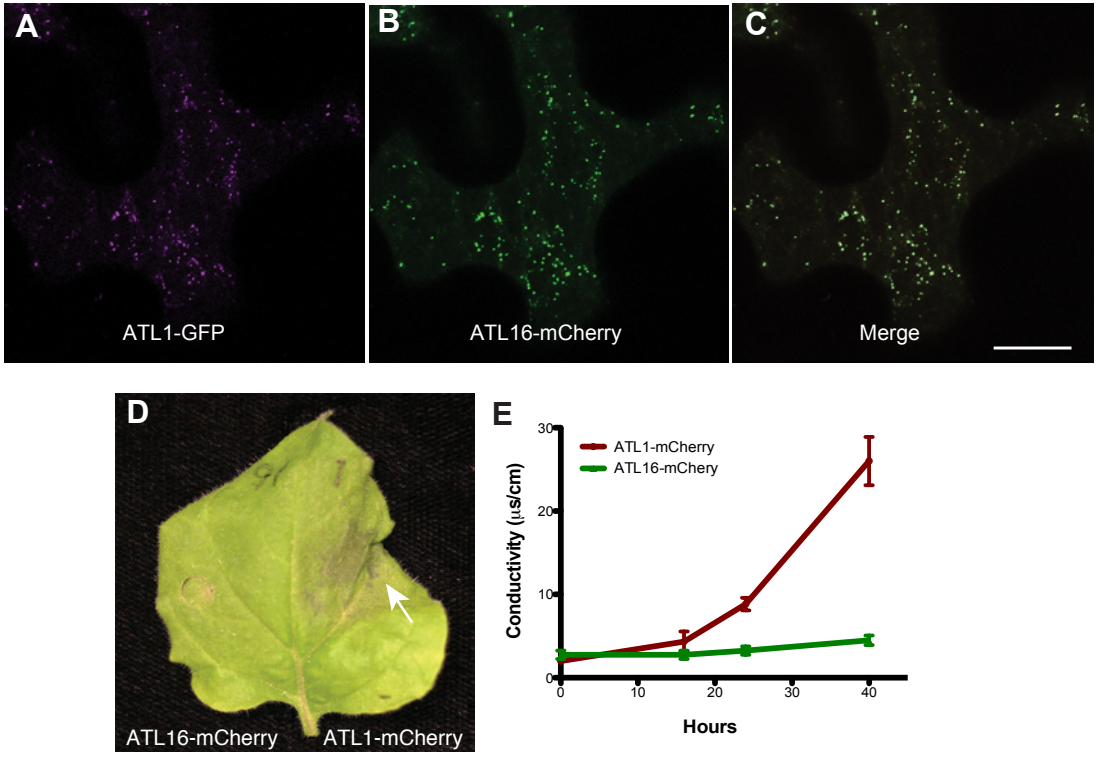
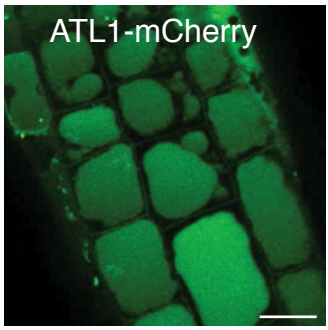
(A) and **(B)** Four-week old plants from two independent 35S:ATL1-mCherry lines in an *edr*1 mutant background.
(C) Mutation of the ATL1 RING domain blocks ATL1-induced dwarfing. Image shows a four-week old transgenic plant expressing 35S:ATL1$^{C153A/H155A}$-mCherry in an *edr1* mutant background.
Scale bars = 1 cm.

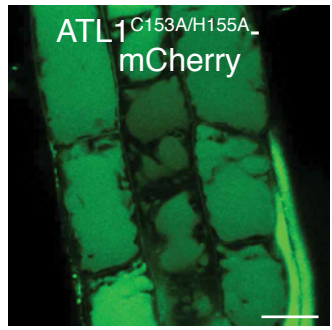


Supplemental Figure 4. ATL16 Does Not Induce Cell Death When Overexpressed in *N. benthamiana.*

(A) to **(C)** Transient co-expression of ATL1-GFP and ATL16-mCherry in *N. benthamiana.* Images were obtained from transiently transformed epidermal cells of *N. benthamiana.* Scale bars = 10 µm.
(D) and **(E)** ATL1-mCherry, but not ATL16-mCherry induces cell death upon transient overexpression in *N. benthamiana.* Genes were expressed using a 35S promoter and leaf photographed 48 hrs after infiltration. Panel E shows electrolyte leakage data. Error bars indicate standard deviation, n=3. This experiment was repeated twice with similar results.

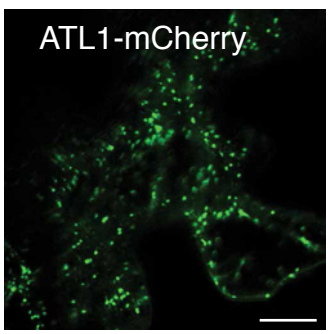
A

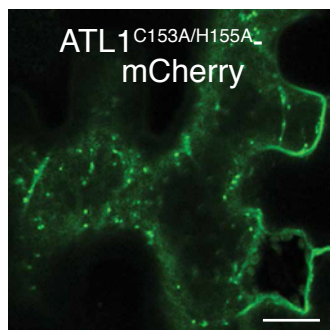




	N	Mean	Std.Dev	t statistic
ATL1-mCherry	8	224585	26674	p=0.643
ATL1$^{C153A/H155A}$-mCherry	8	229436	31697	

B

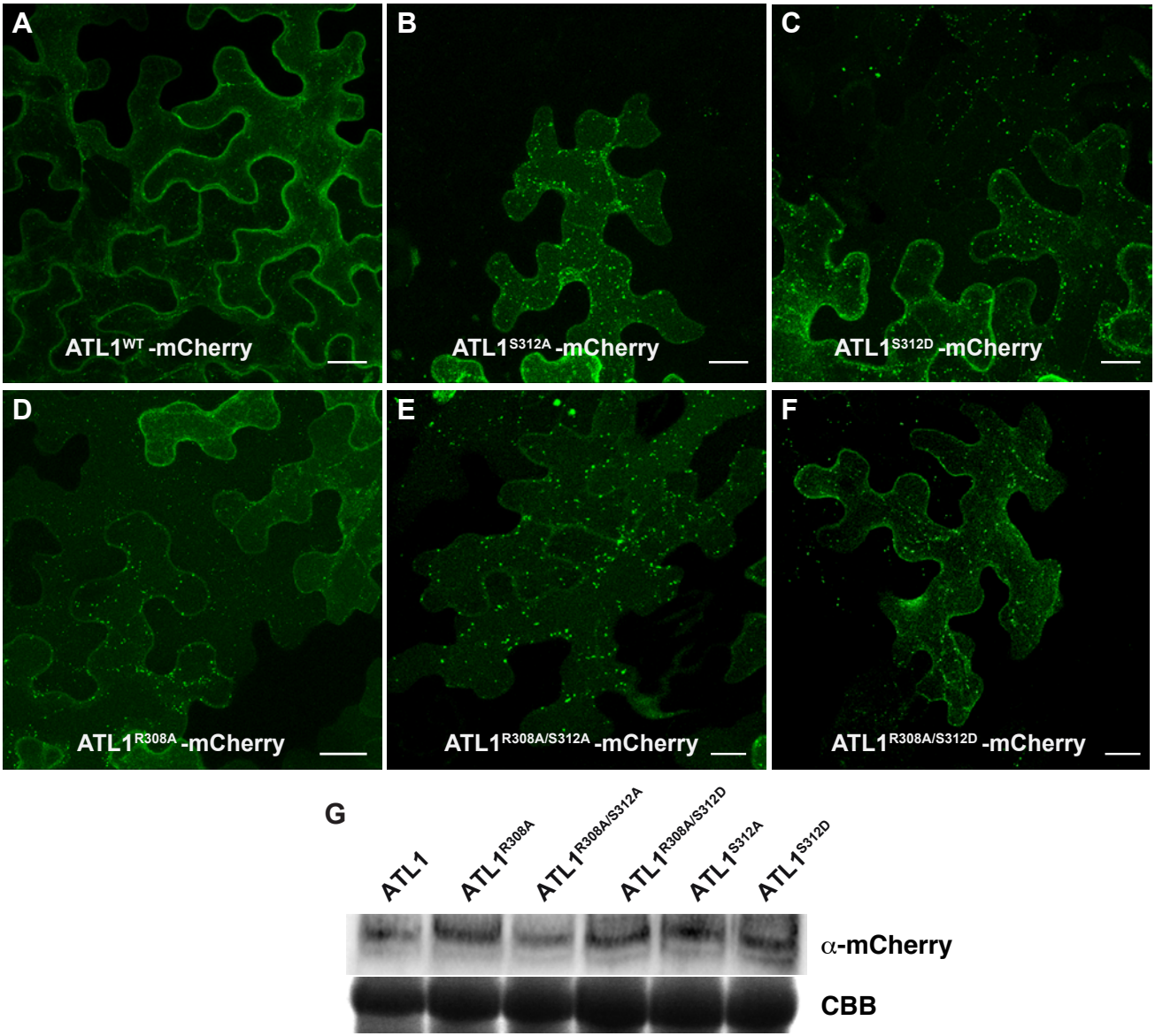




	N	Mean	Std.Dev	t statistic
ATL1-mCherry	20	364689	13116	p=0.619
ATL1$^{C153A/H155A}$-mCherry	20	367287	7920	

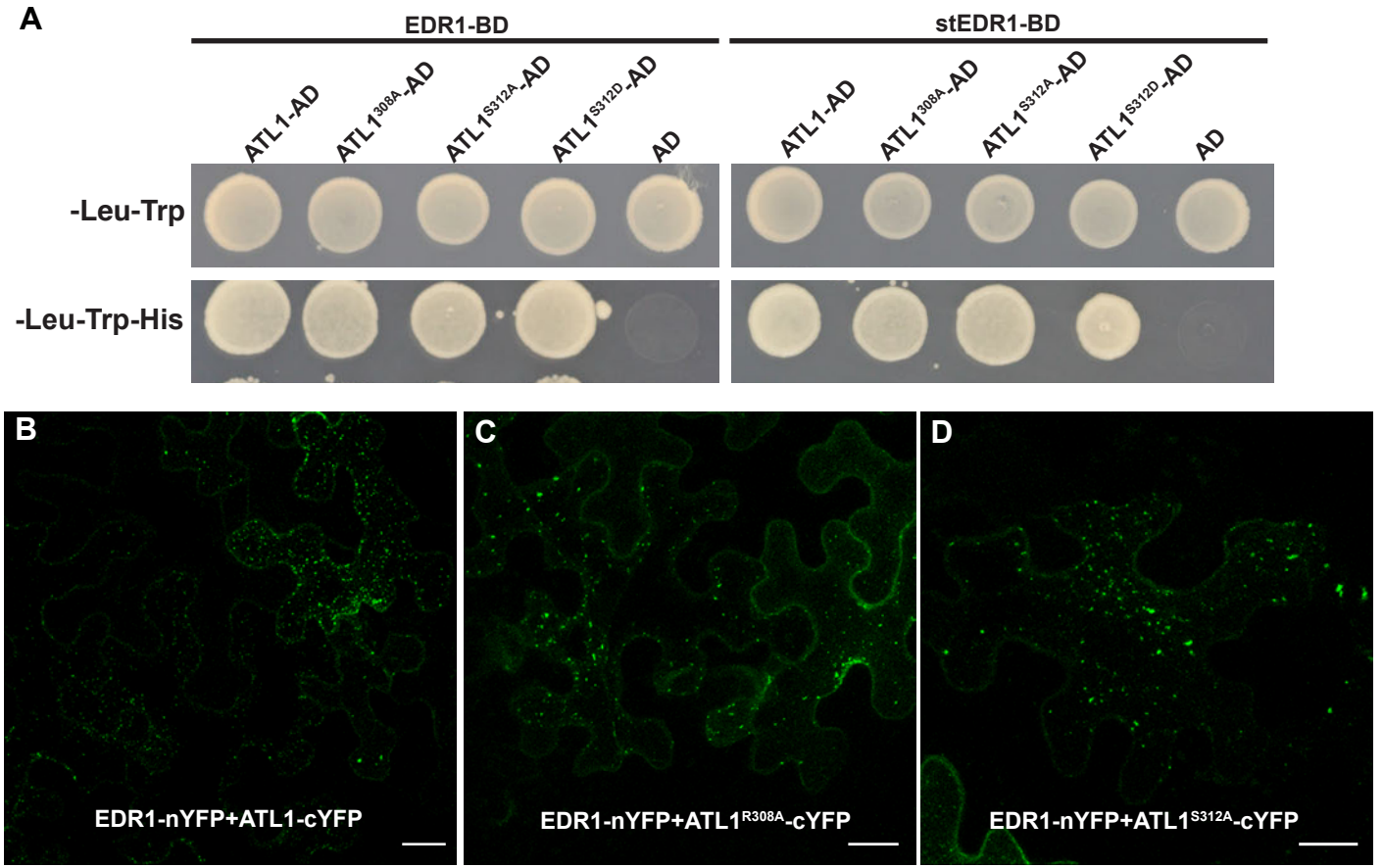
Supplemental Figure 5. Comparison of ATL1-mCherry and ATL1$^{C153A/H155A}$-mCherry Expression Levels Using Fluorescence Quantification.

(A) Single section images of root cells from 35S:ATL1-mCherry line 1 and 35S:ATL1$^{C153A/H155A}$-mCherry line 1 in a Col-0 wild-type background. Fluorescence quantification was performed using ImageJ software. Eight seedlings from each line were randomly sampled and florescence of each sample was determined by the mean fluorescence of two different areas within the sample.
(B) Single section images of *N. benthamiana* epidermal cells expressing 35S:ATL1-mCherry and 35S:ATL1$^{C153A/H155A}$-mCherry constructs. Twenty cells expressing each construct were sampled and florescence of each cell was determined by the mean fluorescence of two different areas within the sample.
Scale bars = 10 μm.



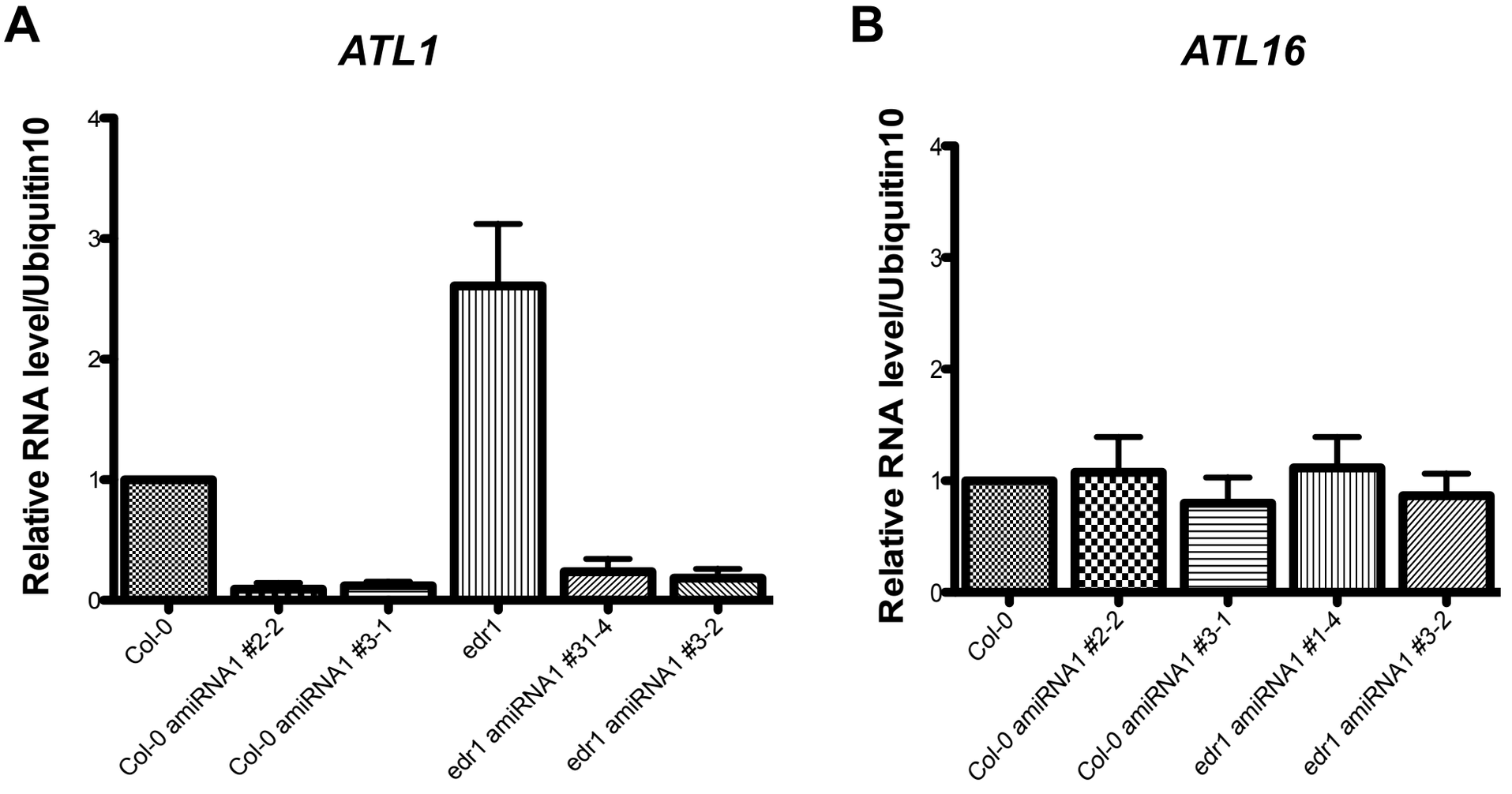
Supplemental Figure 6. Mutations in the ATL1 RxxxS Motif Do Not Alter ATL1 Localization.

(A) to **(F)** ATL1 and mutated derivatives tagged with mCherry transiently expressed in *N. benthamiana*. Scale bars = 10 µm.
(G) Immunoblot of total protein extracts from leaves transiently expresssing 35S-ATL1-mCherry WT or RxxxS mutants. Protein was isolated 40 h after infiltration of Agrobacterium.



Supplemental Figure 7. Mutations in the RxxxS Motif of ATL1 Do Not Alter Its Interaction With EDR1.

(A) EDR1 and ATL1 RxxxS mutants interact in a yeast two-hybrid assay. Yeast strains expressing the indicated constructs were plated on double drop out (-Leu-Trp) media to select for the bait and prey plasmids and triple drop out (-Leu-Trp-His) media to select for interaction between the indicated proteins. Empty vectors containing the activating- or the binding-domain were used as negative controls for interaction.
(B) to **(D)** EDR1 and ATL1 RxxxS mutants interact in a BiFC assay. EDR1-nYFP and ATL1-cYFP were transiently co-expressed in *N. benthamiana*. Scale bars = 10 μm.



Supplemental Figure 8. ATL1 amiRNAi Lines Have Reduced Levels of ATL1 mRNA.

Total RNA was isolated from Arabidopsis wild-type Col-0, *edr1* mutant, and two transgenic lines in each background expressing a 35S-ATL1-amiRNAi construct. qRT-PCR was performed to quantify **(A)** ATL1 or **(B)** ATL16 mRNA levels. Values were normalized relative to UBIQUITIN10 and are graphed relative to wild-type Col-0. Error bars indicate standard deviation of three biological replicates.

Supplemental Table 1. List of Primers Used in This Study

ATL1attb1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGATCTAACAGAC
ATL1attb4	GGGGACAACTTTGTATAGAAAAGTTGGGTGGGGTTCCAAATAAAC
ATL1attb2	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAGGGTTCCAAATA
EDR1attb1	GGGGACAAGTTTGTACAAAAAAGCAGCTGCATGAGCATATTTTCAAGA
EDR1attb4	GGGGACAACTTTGTATAGAAAAGTTGGGTGTTGTGGTGTAGGAAGTACA
BRI-1attb1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGAAGACTTT TTCAAGC
BRI-1attb4	GGGGACAACTTTGTATAGAAAAGTTGGGTGTAATTTTCCTTC AGGAAC
AtBI-1attb1	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGATGCGTTCTCTTCC
AtBI-1attb4	GGGGACAACTTTGTATAGAAAAGTTGGGTGGTTCTCCTTTCTTCTT
mCherryattb4r	GGGGACAACTTTTCTATACAAAGTTGCTATGGTGAGCAAGGGCGAG
mCherryattb2	GGGGACAACTTTGTATACAAAAGTTGCTCTTGTACAGCTCGTCCA
nYFPattb4r	GGACAACTTTTCTATACAAAGTTGGTATGGGTAAAGGAGAAGAAC
nYFPattb2	GGACCACTTTGTACAAGAAAGCTGGGTATTAGTCCTCGATGTTGTGGCGGATC
cYFPattb4r	GGACAACTTTTCTATACAAAGTTGGAGGCGGCGTGCAGCTCGCCGACCA
cYFPattb2	GGACCACTTTGTACAAGAAAGCTGGGTTTATTTGTATAGTTCATCCATGCC
FwdATL1-NdeI	CGCGCATATGATGGATCTAACAGACCGT
RevATL1-XhoI	CGCGCTCGAGTTAGGGTTCCAAATAAAC
FwdEDR1-XmaI	ACCCGGGGATGAAGCATATTTTCAAGAAGCT
RevEDR1-SmaI	GGTCGACCTATTGTGGTGTAGGAAGTACAA
ATL1R308A-F	GAAATTCAGCCCATAGCGAGATCGATCTCGATGGATTCATCAGTGG
ATL1R308A-R	CCACTGATGAATCCATCGAGATCGATCTCGCTATGGGCTGAATTTC
ATL1R308A/S312A	GAAATTCAGCCCATAGCGAGATCGATCGCGATGGATTCATCAGTG
ATL1R308A/S312A	CACTGATGAATCCATCGCGATCGATCTCGCTATGGGCTGAATTTC
ATL1R308A/S312D	GAAATTCAGCCCATAGCGAGATCGATCGACATGGATTCATCAGTGG
ATL1R308A/S312A	CCACTGATGAATCCATGTCGATCGATCTCGCTATGGGCTGAATTTC
ATL1S312A-F	GAAATTCAGCCCATAAGAAGATCGATCGCGATGGATTCATCAGTGG
ATL1S312A-R	CCACTGATGAATCCATCGCGATCGATCTTCTTATGGGCTGAATTTC
ATL1S312D-F	GAAATTCAGCCCATAAGAAGATCGATCGACATGGATTCATCAGTGG
ATL1S312D-R	CCACTGATGAATCCATGTCGATCGATCTTCTTATGGGCTGAATTTC
amiRNA1-Aattb1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGCTGCAAGGCGATTAAGTTGGGTAAC
amiRNA1-Battb4	GGGGACCACTTTGTACAAGAAAGCTGGGTAGCGGATAACAATTTCACACAGGAAG
ATL1-amiRNA1I	GATAATCTAAATGAAAAGCTCCGTCTCTCTTTTGTATTCC
ATL1-amiRNA1II	GACGGAGCTTTTCATTTAGATTATCAAAGAGAATCAATGA
ATL1-amiRNA1III	GACGAAGCTTTTCATTATGATTTTCACAGGTCGTGATATG
ATL1-amiRNA1IV	GAAAATCTATATGAAAAGCTTCGTCTACATATATATTCCT
Ubq10fw	AGATCCAGGACAAGGAGGTATTC
Ubq10rv	CGCAGGACCAAGTGAAGAGTAG
ATL1RTF	CACAAGCCCTATATTCCCACG
ATL1RTR	AGTAAGAACGCAGTGGCTAAG
ATL16RTF	CGATTCTCGTTATCTCGAAGG
ATL16RTR	GCCGTCGTTTTGGTCGTAT