

Supplemental Figure 1. *RCI1A* expression is not regulated by NaCI, dehydration or ABA treatments.

Histochemical analysis of GUS activity in whole 2-week-old seedlings containing the $RCI1A_{PRO}$ -GUS fusion grown under control conditions (20°C), exposed 3 days to 100mM NaCl (NaCl), dehydrated until they lost 30% of their fresh weight (DH), or exposed 3 days to 100mM ABA (ABA).



Supplemental Figure 2. Phenotypic characterization of *rci1a* mutant.

(A) to (C) Morphological phenotype of Col-0 (WT), *rci1a* and *c-rci1a* plants grown one week (A), 3 weeks (B) and 8 weeks (C) under control conditions. (D) Confocal microscopy images of leaves from 3-week-old Col-0 (WT), *rci1a* and *c-rci1a* plants. The perimeter of two cells has been highlighted in each photo. Bars= 50μ m.

(E) Cell sizes, as determined by perimeter measurement, in Col-0 (WT), *rci1a* and *c-rci1a* plants. Values SD were obtained for $n \ge 25$. Asterisks indicate significant differences (p<0.05) with WT plants.



Supplemental Figure 3. RCI1A is not involved in *Arabidopsis* tolerance to drought and salt stress.

(A) Drought tolerance of 2-week-old Col-0 (WT) and *rci1a* seedlings. Tolerance was estimated as the percentage of remaining fresh weight (FW) after transferring seedlings to a wet filter paper and allowing them to dehydrate for 2 days. Data is the mean of three independent experiments, including at least 20 seedlings per genotype in each experiment. Bars indicate SD.

(B) Tolerance to NaCl of 5-day-old Col-0 (WT) and *rci1a* seedlings. Tolerance was estimated as the number of green leaves and remaining fresh weight 14 days after transferring seedlings to plates supplemented with 100mM NaCl. Values are represented as a percentage of the number of green leaves and remaining fresh weight in WT unstressed plants. Data is the mean of three independent experiments, including at least 30 seedlings per genotype in each experiment. Bars indicate SD.

(C) and (D) Representative Col-0 (WT) and *rci1a* seedlings after being dehydrated 2 days on a wet filter paper (C) or exposed 14 days to100mM NaCl (D).



Supplemental Figure 4. *rci1a* mutants display an ET overproducer phenotype.

Phenotypes of 4-day-old Col-0 (WT), *rci1a*, *crci1a* and *eto1-3* etiolated seedlings germinated in vertical plates containing MS medium (**A**), MS medium supplemented with 10mM AgNO₃ (AgNO₃) (**B**), or in MS medium supplemented with 0.1mM ACC (ACC) (**C**). Bar = 0,5 cm.



Supplemental Figure 5. RCI1A interacts with multiple ACS isoforms.

Visualization of *in vivo* interactions between *Arabidopsis* RCI1A and ACS2, ACS5, ACS6, ACS8, ACS11 and RGA1 proteins by BiFC assays. Interactions of nYFP-RCI1A/cYFP-ACSs and nYFP-RCI1A/cYFP-RGA1 protein pairs were tested by *A. tumefaciens*-mediated transformation in leaves of *N. benthamiana* plants grown at 20°C or exposed 4 hours to 4°C (4°C). Bars=75µm.



Supplemental Figure 6. *rci1a* mutants show decreased constitutive freezing tolerance and cold acclimation capacity when growing on MS in petri dishes.

(A) Freezing tolerance of 2-week-old Col-0 (WT) and *rci1a* seedlings grown in petri dishes on MS medium supplemented with 1% sucrose and exposed to the indicated freezing temperatures, before and after being cold acclimated 5 days at 4°C. In both cases, freezing tolerance was estimated as the percentage of surviving plants after 4 days of recovery under control conditions. Data are expressed as means of three independent experiments with 25 plants each. Bars indicate SD. Asterisks indicate significant differences (p<0.05) with WT plants.
(B) and (C) Representative non-acclimated (B) and cold

(B) and **(C)** Representative non-acclimated **(B)** and cold acclimated **(C)** Col-0 (WT) and *rci1a* seedlings 4 days after being exposed to -5°C and -10 C for 1 hour, respectively.

Supplemental Table 1

Oligonucleotide sequences of primers used in this study

Mutant genotyping	
Name	Sequence 5'-3'
SalK- <i>rci1a</i> 5′	TTCATCAGTCTCTTCTTATTCC
SalK- <i>rci1a</i> 3′	ATTATAAAATGACTCTTCCTC
Salk-LB	GGCAATCAGCTGTTGCCGTCTCACTGGTG
Salk-RB	TGATAGTGACCTTAGGCGACTTTTGAACGC
qRT-PCR	
Name	Sequence 5'-3'
RCI1A F	AACTTTCATGTGATAATCTGAG
RCI1A R	AGTTTTGATTTCATTAGAAAGG
GUS F	GCGCGTTACAAGAAAGCCGG
GUS R	AGTCCCGCTAGTGCCTTGTC
CBF1 F	GTC AAC ATG CGC CAA GGA TA
CBF1 R	TCG GCA TCC CAA ACA TTG TC
CBF2 F	GAA TCC CGG AAT CAA CCT GT
CBF2 R	CCC AAC ATC GCC TCT TCA TC
CBF3 F	CAA CTT GCG CTA AGG ACA
CBF3 R	TCT CAA ACA TCG CCT CAT
COR8.5 F	CCGATGCGAAAGCTCCGTG
COR8.5 R	CTCGAACTTTAGCATTCAGC
GOLS2 F	AAGAAGCAACAGACACTTCAGCAG
GOLS2 R	TGAAGAGGCGTATGCAGCAAC
OXS3 F	CTTATGTTCAAAGAGATGAATCTG
OXS3 R	ACATCATCATCATCATCCTCTG
RAV1 F	CACATTTCTGTTTTCTCCATTG
RAV1 R	TCCCATCCTGTATAAGTTACCTAC
ERF11 F	GCCGCTCGTGCCTACGACAA
ERF11 R	CCACGGTGCTGCTCTGGCTC
RAP2.1 F	CCAGAGTCGACGCTCTTCTAGC
RAP2.1 R	TCTAGATCAATGGAAAGAGAACAAGAA
ABF1 F	GGCCTGGAGAAGGTTGTTGAGAGA

GCCTGTTTTCGAGCCCTTGATCTA
CATCGAGCTAGAGGATTAGAAG
GGTATCTAGCATATAGCCGGCGAG
CCCGGACCAGCTCCGACCAT
GGCGGCTGACTAACCGGAGG
CATCCCTCTTATCATAGGCGCTCAGTA
CCTCTTAAAGTGGCTTTCCACTTGTCT
GAGCTGAAGTGGCTTCAATGAC
GGTCCGACATACCCATGATCC
CTCTGCTCAAACTCATCGGCGTAA
TCGGCTTTTCTTGACGCTCATTCT
GCTAAATCGCCGTCTTCCAGAACG
TCCTACGACATCGCAGCTTGTAGA
CATGGCTAAGTTTGCTTCCA
GTTGCATGATCCATGTTTGG
ACTTTCCGCTTCTGTCGCCG
CGTCCACGTCAGCATACACATCGT
TTTTGGACCTGATGGGGATCGGTA
GCGATCTAAACGCCGATGTCACAG
GCCTTGGCCGCCTCCGTAAG
GTTCCAGGGCTCACTGCCGC
CACTCCGGACCGTTGGCACA
GGCACGTGACCATTCTCGATGCT
TGCGACGAGCTCGGGATTCG
GCAATGATCGTGGACCAGTGGGA
TCCGTTGCACGGAACCTGGA
GGATCCGTCCAAGCGCCACA
ACGCGGGTTTGTTCTGTTGGGT
CCAACCCGGTTCGGTGCAGT
GGAGGAGACTAAACCGATGGCTGC
GGCACAGGCGAATGAGGCGA
GAAGAGGTTCGTTGATGGACCGTCG
ATCGTTCCTCGGGTTCACGGTCGTG
CCAGGCTCATCGTGTCATTGCGA
GCAACCTCCATCGTTTGGTCCGA
GAAACCGCAGATACATTGGG
TAACTGATTAGGTAAGACCC

LTI78 F	CTGAAGAACGAATCTGATATCG
LTI78 R	CCAGGTCTTCCCTTCGCCAG
KIN1 F	ATTCGGGTCAAATTTGGGAG
KIN1 R	TGAATATAAGTTTGGCTCGTC
COR47 F	TCGTTGATTGCATTTGATCC
COR47 R	CACACAACTTACACAAAC
CHS F	TCGCCGAGAACAACCGTGGA
CHS R	CGGCGGCGCCATCACTGAAA
BIFC CONSTRUCTS	
Name	Sequence 5'-3'
inFRCI1A-F	CGCCACTAGTGGATCAAGATGTCGACAAGGGAAGAGAATG
inFRCI1A-R	GAGCGGTACCCTCGATTCGGGTTTCCTCGGCAC
inFACS2 F	CGCCACTAGTGGATCATGGGTCTTCCGGGAAAAAATAAAGG
inFACS2 R	GAGCGGTACCCTCGATGCTCGGAGAAGAGGTGAGTGTGG
inFACS5 F	CGCCACTAGTGGATCCAGAGAATGAAACAGCTTTCGAC
inFACS5 R	GAGCGGTACCCTCGATCGTTCATCAGGTACACG
inFACS6 F	CGCCACTAGTGGATCATGGTGGCTTTTGCAACAGAGAAG
inFACS6 R	GAGCGGACCCTCGAAAGTCTGTGCACGGACTAGCGGAGAAG
inFACS8 F	CGCCACTAGTGGATCGAAAATGGGTCTCTTGTCAAAGAAAG
inFACS8 R	GAGCGGTACCCTCGATCGTTCCTCGGGTTCACGGTCGTG
inFACS11 F	CGCCACTAGTGGATCATGTTGTCAAGCAAAGTTGTTGGCG
inFACS11 R	GAGCGGTACCCTCGAACGTTCTGATTCACAAGTAACAGAGG
inFRGA F	CGCCACTAGTGGATCAATGAAGAGAGATCATCACCAATTCC
inFRGA R	GAGCGGTACCCTCGACGCCGCCGTCGAGAG
GUS CONSTRUCTS	
Name	Sequence 5'-3'
P1AMUT	CCCTTGTCGACTTCTTC
P1ACOM	GGGGGTCGACAAGTGGCACGGAAGCTGGT
GFP CONSTRUCTS	
Name	Sequence 5'-3'
RCI1A promoter GFP	GGGGACAAGTTTGTACAAAAAGCAGGCTTCTGCCGGAATTTGAATCTGGACATGC
RCI1A 3´GFP	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCGGCACCATCGGGCTTTGATGCC