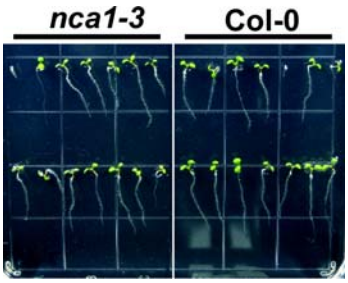
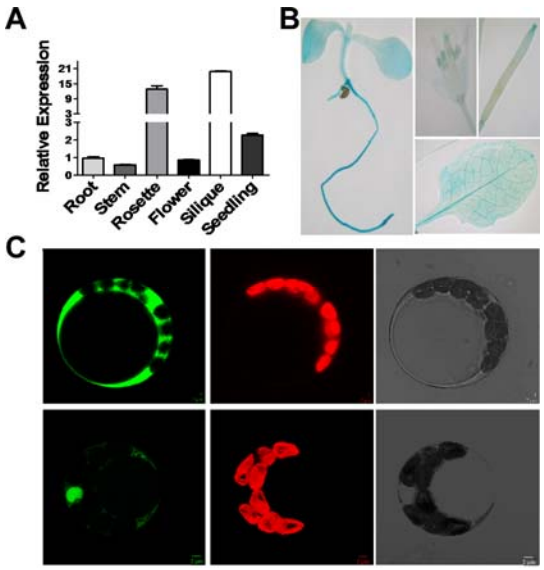


Supplemental Figure 1. Growth of five-day-old *nca1-3* seedlings is similar to that in Col-0 seedlings.

Col-0 and *nca1-3* seeds were germinated on, and seedlings grown on, MS medium at pH 5.8. Photographs were taken five days after seeds were sown.

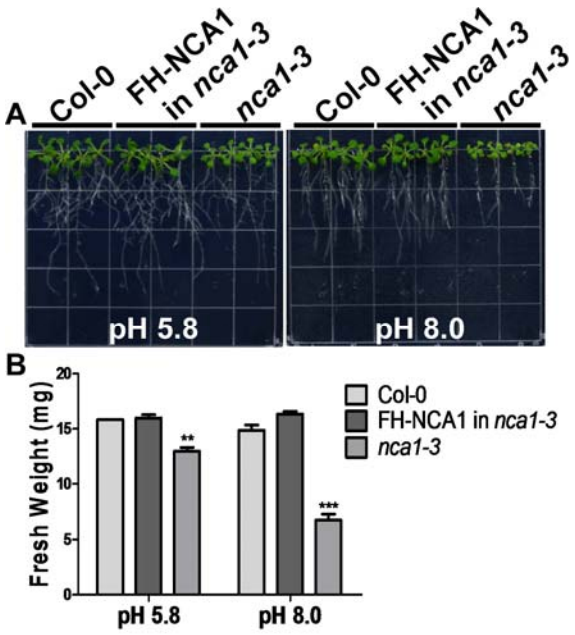


Supplemental Figure 2. *NCA1* gene expression and protein localization.

A. Analysis of *NCA1* expression in roots, stems, rosette leaves, flowers, siliques, and 10-day-old seedlings. Real-time PCR analysis with *TUBULIN* as the internal control. Error bars represent SD (n=3) for at least three replicate experiments.

B. *NCA1* promoter-*GUS* expression in Col-0 seedlings, leaves, flowers, and siliques.

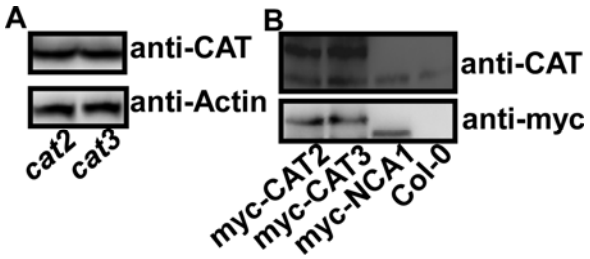
C. Subcellular localization of NCA1 in protoplasts. *Pro35S:NCA1-GFP* construct was transformed into *Arabidopsis* protoplasts and GFP fluorescence was monitored with a Zeiss confocal microscope. Top row, *Pro35S:NCA1-GFP*; bottom row, *Pro35S:GFP*. In each row, left panel, GFP; middle panel, autofluorescence; right panel, bright field image.



Supplemental Figure 3. *Flag-HA-NCA1* rescues the *nca1-3* phenotype.

A. The phenotype of the *nca1-3* mutant is rescued in *Pro35S:3×Flag-HA-NCA1* (*FH-NCA1*) transgenic plants in the *nca1-3* background. Five-day-old seedlings of Col-0, *nca1-3*, and the *nca1-3* mutant expressing FH-NCA1 from seeds sown on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or pH 8.0. Photographs were taken 10 days after transfer.

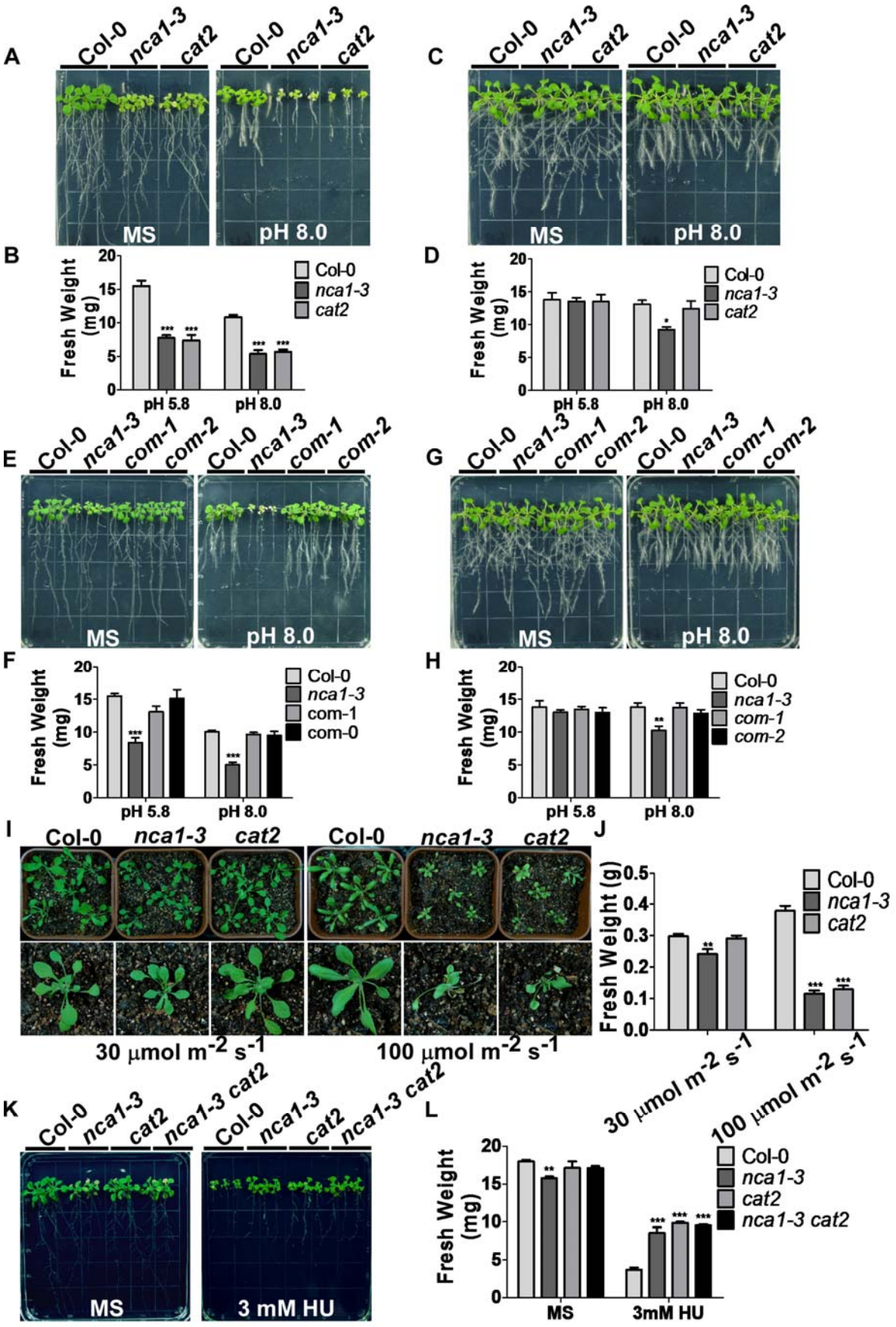
B. Fresh weight of Col-0, *nca1-3* and *nca1-3* seedlings expressing FH-NCA1. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test in comparison to Col-0 values. (**P<0.01 and ***P<0.001).



Supplemental Figure 4. Analysis of anti-CAT antibody specificity.

A. Analysis of anti-CAT antibody specificity in the *cat2* and *cat3* mutants. Total protein was extracted from 10-day-old seedlings and analyzed by immunoblot analysis with anti-CAT and anti-Actin (used as a loading control) antibodies.

B. Analysis of anti-CAT antibody specificity in Col-0 expressing *Pro35S:6Myc-NCA1*, *Pro35S:6Myc-CAT2*, or *Pro35S:6Myc-CAT3*. Total protein was extracted from 10-day-old seedlings and analyzed with anti-CAT and anti-Myc (used to mark Myc-labled protein) antibodies.


A
Col-0
nca1-3
cat2
MS
pH 8.0
B
Fresh Weight (mg)
pH 5.8
pH 8.0
C
D
E
com-1
com-2
F
com-0
G
H
I
30 μmol m-2 s-1
100 μmol m-2 s-1
J
K
nca1-3 cat2
3 mM HU
L
3mM HU

Supplemental Figure 5. NCA1 genetically interacts with CAT2.

A. Analysis of alkaline sensitivity in air under an irradiance of 90 μmol m^{-2} s^{-1} in Col-0, *nca1-3*, and *cat2* seedlings. Five-day-old seedlings of Col-0, *nca1-3*, and *cat2* from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or pH 8.0. Photographs were taken 10 days after transfer.

B. Fresh weight of Col-0, *nca1-3*, and *cat2* seedlings in **A**. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (***P<0.001).

C. Analysis of alkaline sensitivity in the presence of 3,000 ppm CO_2 under an irradiance of 90 μmol m^{-2} s^{-1} in Col-0, *nca1-3*, and *cat2* seedlings. Five-day-old seedlings of Col-0, *nca1-3*, and *cat2* from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or pH 8.0. Photographs were taken 10 days after transfer.

D. Fresh weight of Col-0, *nca1-3*, and *cat2* seedlings in **C**. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (*P<0.05).

E. Analysis of alkaline sensitivity in air under an irradiance of 90 μmol m^{-2} s^{-1} in Col-0, *nca1-3, com-1*, and *com-2* seedlings. Five-day-old seedlings of Col-0, *nca1-3*, *com-1*, and *com-2* from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or pH 8.0. Photographs were taken 10 d after transfer.

F. Fresh weight of Col-0, *nca1-3*, *com-1*, and *com-2* seedlings in **E**. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (***P<0.001).

G. Analysis of alkaline sensitivity in 3,000 ppm CO_2 under an irradiance of 90 μmol m^{-2} s^{-1} in Col-0, *nca1-3, com-1*, and *com-2* seedlings. Five-day-old seedlings of Col-0, *nca1-3 com-1*, and *com-2* from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or pH 8.0. Photographs were taken 10 days after transfer.

H. Fresh weight of Col-0, *nca1-3*, *com-1*, and *com-2* seedlings in **G**. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (**P<0.01).

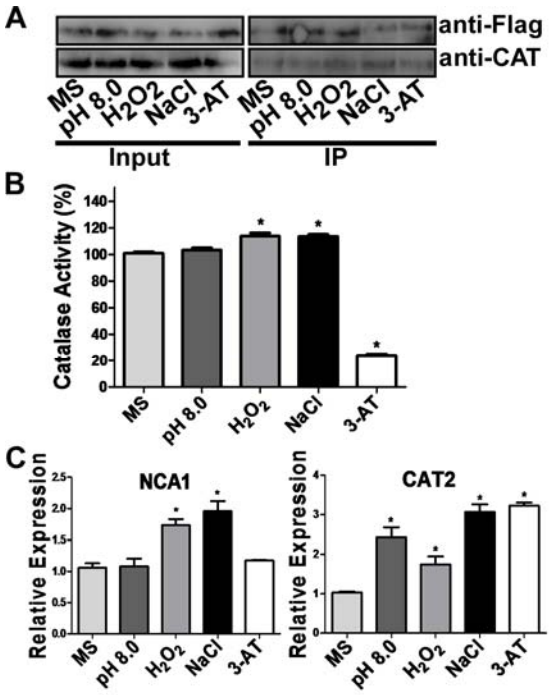
I. Analysis of the effect of different irradiances on *nca1-3* in soil. Seeds of Col-0, *cat2*, and *nca1-3* were sown in soil. Germination and growth were carried out in chambers with irradiances of 30 μmol m^{-2} s^{-1} or 100 μmol m^{-2} s^{-1}, respectively and with a 16 h photoperiod. Photographs were taken 4 weeks after seeds were sown.

J. Fresh weight of Col-0, *cat2*, and *nca1-3* in **I**. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (*P<0.05, and **P<0.01).

K. Analysis of hydroxyurea (HU) tolerance in Col-0, *nca1-3*, *cat2*, and the *nca1-3 cat2* double mutant. Five-day-old seedlings of Col-0, *nca1-3, cat2*, and *nca1-3 cat2* from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 without or with 3 mM HU. Photographs were taken 10 d after

transfer.

L. Fresh weight of Col-0, *nca1-3*, *cat2*, and *nca1-3 cat2* seedlings in **K**. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (**P<0.01 and ***P<0.001).

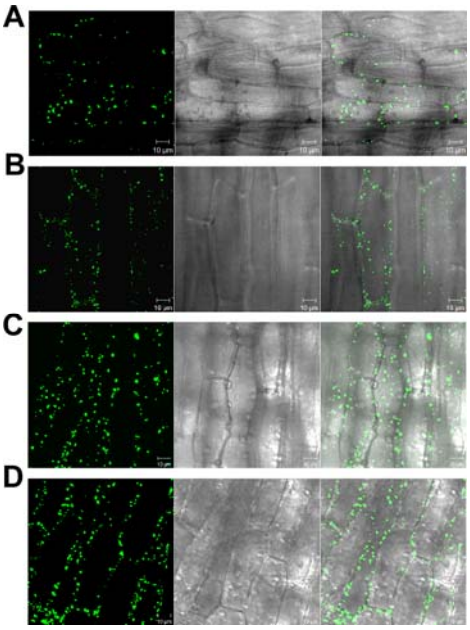


Supplemental Figure 6. Increase of catalase activity is not affected by alkaline stress.

A. Analysis of the interaction between NCA1 and catalases during stresses. *Pro35S:3×Flag-HA-NCA1* plasmids were introduced into Col-0 and transgenic plants were generated. Ten-day-old transgenic plants were treated with liquid MS medium at pH 5.8, pH 8.0, or liquid MS medium at pH 5.8 containing 20 mM H_2O_2, 150 mM NaCl, or 20 mM 3-AT for 3 hours. Flag-HA-NCA1 was immunoprecipitated with anti-Flag agarose and analyzed by immunoblot analysis with anti-Flag antibody to detect Flag-HA-NCA1 and with anti-CAT antibody to detect catalases in the precipitates.

B. Catalase activity assay of seedlings treated with liquid MS medium at pH 5.8, pH 8.0, or liquid MS medium at pH 5.8 containing 20 mM H_2O_2, 150 mM NaCl or 20 mM 3-AT for 3 hours. Catalase activity is indicated in units (U)/mg protein and all activities were calculated relative to the activity of seedlings treated with MS medium at pH 5.8 (80 U/mg). Data represent means ±SD of at least three replicate experiments.

C. Analysis of *NCA1* and *CAT2* expression in 10-d-old Col-0 seedlings treated with liquid MS medium at pH 5.8, pH 8.0, or liquid MS medium at pH 5.8 containing 20 mM H_2O_2, 150 mM NaCl, or 20 mM 3-AT for 3 h. Real-time PCR analysis was performed with *TUBULIN* as an internal control. Error bars represent SD (n=3) for at least three replicate experiments.



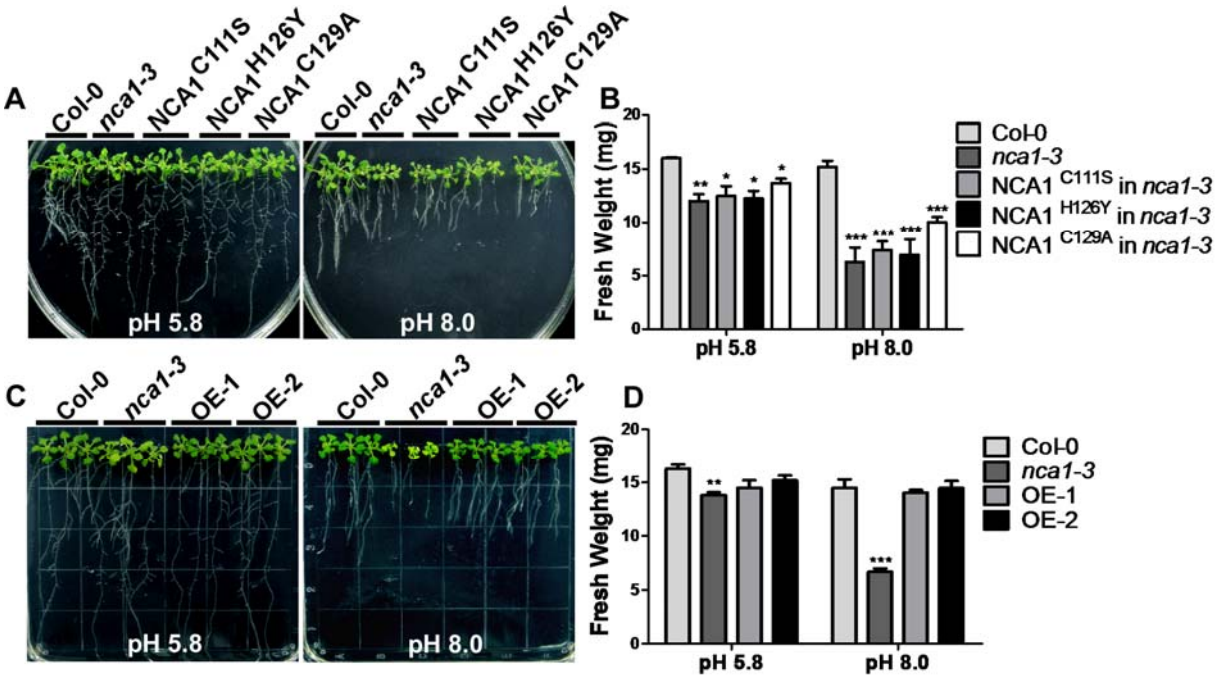
Supplemental Figure 7. NCA1 does not affect the subcellular localization of catalase.

A. Subcellular localization of CAT2 in the hypocotyl of Col-0 seedlings. *Pro35S:GFP-CAT2* plasmids were introduced into Col-0 and transgenic plants were generated. Images were collected with a Zeiss confocal microscope. Left panels, GFP; middle panels, bright field image; right panels, merged images.

B. Subcellular localization of CAT2 in the hypocotyl of *nca1-3* seedlings. *Pro35S:GFP-CAT2* plasmids were introduced into *nca1-3* and transgenic plants were generated. Images were collected with a Zeiss confocal microscope. Left panels, GFP; middle panels, bright field image; right panels, merged images.

C. Subcellular localization of CAT3 in the hypocotyl of Col-0 seedlings.

D. Subcellular localization of CAT3 in the hypocotyl of *nca1-3* seedlings.



Supplemental Figure 8. The RING-finger domain of NCA1 is required for seedling tolerance of high pH.

A. Analysis of tolerance of high pH in transgenic T_3 *nca1-3* plants harboring *Pro35S:myc-NCA1*^{C111S}, *Pro35S:myc-NCA1*^{H126Y}, or *Pro35S:myc-NCA1*^{C129A}. Five-day-old seedlings from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or 8.0. Photographs were taken 10 d after transfer.

B. Fresh weight of Col-0, *nca1-3* and *nca1-3* seedlings harboring *Pro35S:myc-NCA1*^{C111S}, *Pro35S:myc-NCA1*^{H126Y}, or *Pro35S:myc-NCA1*^{C129A}. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (*P<0.05, **P<0.01 and ***P<0.001).

C. Analysis of tolerance of high pH in transgenic T_3 *nca1-3* plants harboring *Pro35S:myc-NCA1* (OE-1 and OE-2). Five-day-old seedlings from seeds germinated on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 or 8.0. Photographs were taken 10 d after transfer.

D. Fresh weight of Col-0, *nca1-3*, and transgenic T_3 *nca1-3* seedlings harboring *Pro35S:myc-NCA1*. Data represent means ±SD of at least three replicate experiments. Statistical significance is based on a Student's t test (**P<0.01 and ***P<0.001).

Supplemental Table 1. Primers for plasmid construction

Genomic-F	GGAATTCGAGCTCGGGTTCAACTGGTTATGAC
Genomic-R	CGGGATCCGATTACATGGGAGGATAGTAC
NCA1-gF	GACGAAGATCTACAAGCTG
NCA1-gR	GTAGCCAGTTCAGCTAACC
Promoter-F	GGAATTCGAGCTCGGGTTCAACTGGTTATGAC
Promoter-R	CGGGATCCTCGTGAAGGTAGCAACAAC
NCA1-F	TTGGCGCGCCATGACGACGACTTCTGTTTGCC
NCA1-R	CCTTAATTAATTAGAGTGCAGTTTCAGCATCGG
NCA1-BamHI-F	CGGGATCCATGACGACGACTTCTGTTTGCC
NCA1-BamHI-R	CGGGATCCTTAGAGTGCAGTTTCAGCATC
NCA1-AscI-F	TTGGCGCGCCATGACGACGACTTCTGTTTGCC
NCA1-PacI-R	CCTTAATTAATTAGAGTGCAGTTTCAGCATCGG
NCA1-SpeI-F	GGACTAGTATGACGACGACTTCTGTTTGCC
NCA1-SpeI-R	GGACTAGTTTAGAGTGCAGTTTCAGCATC
$NCA1^{C111S}$-F	GCTGTATGTTAAGTCAAGCAC
$NCA1^{C111S}$-R	GTGCTTGACTTAACATACAG
$NCA1^{H126Y}$-F	CCTTGCACATATGTGTTCTG
$NCA1^{H126Y}$-R	GCAGAACACATATGTGCAAG
$NCA1^{C129A}$-F	GCCTTGCACACATGTGTTCGGCAAAGTATGC
$NCA1^{C129A}$-R	GTGTGCAAGGCACACATCTACTGCTC
$NCA1^{27\text{-}405}$-NdeI-F	ATTCATATGACCGCTTCTGGTTGTCCT
$NCA1^{158\text{-}405}$-NdeI-F	ATTCATATGGTTGATCAATTTATTGAAGGC
$NCA1^{185\text{-}405}$-NdeI-F	ATTCATATGGATAACAAAAAAGTGATTTAC
$NCA1^{405}$-AccI-R	ATTGTCGACTTAGAGTGCAGTTTCAGC
CAT2-AscI-F	TTGGCGCGCCATGGATCCTTACAAGTATCG
CAT2-PacI-R	CCTTAATTAATTAGATGCTTGGTCTCACG
CAT3-AscI-F	TTGGCGCGCCATGGATCCTTACAAGTATCG
CAT3-PacI-R	CCTTAATTAACTAGATGCTTGGCCTCA

CAT2-XbaI-F	GCTCTAGAATGGATCCTTACAAGTATCG
CAT2-SalI-R	GCGTCGACTTAGATGCTTGGTCTCACG
CAT3-XbaI-F	GCTCTAGAATGGATCCTTACAAGTATCG
CAT3-SalI-R	GCGTCGACCTAGATGCTTGGCCTCA

Supplemental Table 2. Primers for RT-PCR

NCA1 RT-F	GACGAAGATCTACAAGCTG
NCA1 RT-R	CTATATCCGCAACTTTGGC
CAT2 RT-F	CCTTCTTGAGGATTACCATC
CAT2 RT-R	GTCCAACAGGTTGAAGAG
CAT3 RT-F	GGATCTCAACACTCTCAC
CAT3 RT-R	TTAGTGGCGTGGCTGTGATTGG
ACTIN-F	GTCGTACAACCGGTATTGTG
ACTIN-R	GAGCTGGTCTTTGAGGTTTC