

Supplementary FIGURE 1. *arHIF434-Ct* displays an earlier leaf senescence than *arHIF434-Col.* **(A)** *arHIF434* is represented with horizontal bars (black for *Col-0* allele, white for *Ct-1* allele, grey for heterozygous). Dashed vertical bars represent markers delimiting the candidate interval on chromosome 4. Numbers correspond to marker position (Mb). Position of *ACL1* and *ACD6* genes are shown above the arHIF. **(B)** Rosette leaves of 5-week-old plants. Upper rows: *arHIF434-Ct*, lower rows: *arHIF434-Col*. Scale bar corresponds to 1 cm. **(C)** Percentage of senescent leaves in *arHIF434-Ct* (yellow triangle line) and *arHIF434-Col* (dark green circle line) during the reproductive phase. Flowering transition occurred in average at 23.7 DAS for *arHIF434-Col* and 23.9 DAS for *arHIF434-Ct*.

| | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 |
|---------------|----------------------------|-----------------------------|--------------------------------|--------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|--------------------------|----------------------------------------------------------|--------------------------------------------------------|-------------------------------------------|----------------------------------------------------------|-------------------------------------------------|----------------------------|--------------------|
| Col-0 Ct-1 | HDSSG HDSSG | ADLDRIEF ADLDRIEF | iqrshlyshdqi iqrshlyshdqi | RKDFSHSGGV(RKDFSHSGGV(| ATTSPTGDTEI ATTSPTGDTEI | PYPKFRTNLKI PYPKFRTNLKI | .SDLFALPGE .SDLFALPGE | DVENTPEIFGO DVENTPEIFGO | GMSNGEKECLE GMSNGEKECLE | KLRSNGTPHE Klrsngtphe | RYKSNTGDS Ryksntgds | ELHIAAKAGHL ELHIAAKAGHL | ELYKEIIFEC ELYKEIYFEC | PCLLFE PCLLFE |
| | 131 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 | 250 | 260 |
| Col-0 Ct-1 | QNS 5R QNS 5R | ROTPLHVAT Rotplhvar | THGGHTKYYEAI AHGGHTKYYEAI | LVASVTSALA LVASVTSASA | SLSTEESEGLI SLSTEESE <mark>R</mark> LI | IPHYLKDE DGI IPHY <mark>RKDE</mark> DGI | NTALYYAIEG NTALYYAIEG | RYLEMATCLYI Rylematclyi | NADKDAPF ⁻ LGH NADKDAPF ⁻ LGH | INKGISSLYEF INKGISSLYEF | aydagnkfedi Aydagnkfedi | _YKAILKTTDE _YKAILKTTDE | INVDREVRKFN INVDREVRKFN | ildsklq Ildsklq |
| | 261 | 270 | 280 | 290 | 300 | 310 | 320 | 330 | 340 | 350 | 360 | 370 | 380 | 390 |
| Col-0 Ct-1 | GNKHL GNKHL | .AHYALKAK .AHYALKAK | KSIGYLDYILDI KSIGYLDYILDI | EYPSLMD eqdi Eypslmd <mark>eqdi</mark> | DGRTCLSYGI DGRTCLSYGI | ISIGYYKGLCI Isigyykglci | NILNRSTKGV NILNRSTKGV | YV <mark>CDQ</mark> DGSFP3 YV <mark>CDQ</mark> DGSFP3 | CHSAAKNEHYE CHSAAKNDHYE | IIKEFIKRCF IIKEFIKRCF | Paskyl <mark>lnr</mark> l(Paskyl <mark>lnr</mark> l(| GONILHYAAKN GONILHYAAKN | ieasltayml) Ieasltayml) | HDKDTK ILDKDTK |
| | 391 | 400 | 410 | 420 | 430 | 440 | 450 | 460 | 470 | 480 | 490 | 500 | 510 | 520 1 |
| Col-0 Ct-1 | HL GYG HL GYG | iQDYDGNTF iQDYDGNTF | ?LHLAYMNHDFI ?LHLAYMNHDFI | DSITCLASRNI DSITCLASRNI | IEILK <mark>LRNKSI</mark> IEILK <mark>LRNKS</mark> I | ilrardiaesi ilrardiaesi | EVKPNYIFHE EVKPNYIFHE | RATLALLLYA) RATLALLLYA) | CHSSGFESVKS CHSSGFESVKS | SLT <mark>t</mark> qsypldf Sltkqsypldf | ?KKNRHYYNAI ?Knnrhyynai | LVVAALVATV LVVAALVATV | /TFAAGFTIPO /TFAAGFTIPO | iGYISDS iGYISDS |
| | 521 | 530 | 540 | 550 | 560 | 570 | 580 | 590 | 600 | 610 | 620 | 630 | 640 | 650 1 |
| Col-0 Ct-1 | KKPNL KKPNL | .GRATLATN .GRATLATN | IPTLFIFLLFD: IPTLFIFLLFD: | ELANQSSVATI Elanqssvati | ICTLINAQLGI ICTLINAQLGI |)LALILKSLH)PNLIRKSLH * | /ALPLLLFSL /ALPLLLFSL | LCMPYAFLFG\ LCMPYAFLFG\ | /ITAIAHYKAL /ITAIAHYKAL | LYTISIISGO LYTISIISGO | SFFL <mark>F</mark> AIFILO SFFL <mark>C</mark> AIFILO | 3PHYHLQRS <mark>hl</mark> 3PhyhlQrsyf — * | PPSSGIFLKT PPSAGIYLRT | FMLTID FMLTID |
| Col-0 Ct-1 | 651 ISELF ISEFF | 660 VILIKACF VRKIKTCF | 670 1 GCVACSE -GCVACE | | | | | | | | | | | |

Supplementary FIGURE 2. Protein sequence alignment of ACD6 from *Col-0* and *Ct-1* accessions. Numbers indicate amino acid position from the first Methionine. Grey box correspond to ankyrin repeats and black lines to transmembrane domains as predicted using SMART website (<u>http://smart.embl-heidelberg.de/</u>). * Amino acids 566 and 634.



Supplementary FIGURE 3. Expression of *ACD6* in *arHIF434-Ct* and *arHIF434-Col*. Plants were grown under long days (8 h light/16 h dark) for 35 d after sowing and then harvested. Transcript levels of *ACD6* **(A)**; *SAG12* **(B)** and *RBCS1A* **(C)** marker genes of leaf senescence, and *PR1* **(D)** involved in SA signaling process, were monitored using RT– qPCR and specific primers (<u>Supplementary Table S1</u>). Expression of *ACD6* was normalized using *PP2AA3* and *APC2*. Expression of *PR1*, *SAG12* and *RBCS1A* were normalized using *PP2AA3*.



Supplementary FIGURE 4. Effect of *ACD6* on N and C percentages in the different parts of the plants. N and C percentages in rosette **(A,B)**, stem **(C,D)**, and seeds **(E,F)**. N and C percentages for the four genotypes (*arHIF434-Ct*, *arHIF434-Col*, *Col-O* and *acd6-2*) are shown. Least-square means from 3 independent experiments \pm s.e. are shown (n \geq 18 for each genotype). Different letters indicate significant difference (Tukey's test, p-value \leq 0.05).



Supplementary FIGURE 5. ACD6 does not affect N translocation (T1) and remobilization (T2) from old leaves to young leaves during the vegetative phase. The four genotypes (*arHIF434-Ct*, *arHIF434-Col*, *Col-O* and *acd6-2*) were grown on sand in short day conditions (8 hours). After 48h of labelling with ¹⁵NO3, lower (ranks 1 to 10) and upper (ranks >10) leaves were harvested and grouped. Proportion of total ¹⁵N is measured in the two groups of leaves, just after the labelling period (T1) to estimate the N translocation, and 7 days after (T2) to estimate the N remobilization from old leaves to young leaves during vegetative phase.