

Figure S1. Phenotypes of the Arabidopsis *thf1* line, *ALC1*-silenced *N. benthamiana*, and *ALC1* silenced tomato share similarities. (a) Leaves of 28C12 (ALC1)-silenced *N. benthamiana* plants showed a normal phenotype similar to the leaves of mock-inoculated (TRV::GFP) control plants until 4 weeks post-silencing (middle panel). After 5 weeks of silencing, some leaves showed a variegated chlorotic phenotype (right panel). Photographs were taken 5 weeks post silencing. (b) Leaves of *ALC1*-silenced tomato plants also showed a normal phenotype similar to the leaves of mock-inoculated (TRV::GFP) control plants until 4 weeks of *ALC1*-silenced tomato plants also showed a normal phenotype similar to the leaves of mock-inoculated (TRV::GFP) control plants until 4 weeks post silencing (middle panel). After 5 weeks of silencing, some leaves showed variegated chlorotic phenotype (right panel). Photographs were taken 5 weeks post silencing. (c) The Arabidopsis *thf1* mutant grew slightly slower than the wild-type and showed variegation on the leaves (inset). Photographs were taken when plants were 4-weeks old.



Figure S2. Determination of silencing efficiency of *ALC1*. Total RNA was extracted from the leaves of *N. benthamiana* and tomato silenced with *ALC1* and *SlALC1* respectively, and cDNAs were synthesized for qRT-PCR analyses. The transcript levels were normalized against *Actin* that was used as endogenous control as described by Pfaffl (2001).

A. qRT-PCR result showing the reduction of transcript levels of *ALC1* silenced *N. benthamiana* and *SlALC1* silenced tomato line using VIGS. Samples were collected three weeks post agro-inoculation.

B. qRT-PCR result showing reduction in transcript level of *ALC1* in tomato RNAi line *3-2*. Leaf samples were collected from four week old plants.

All the data shown here represent the average of three biological replicates and three technical replicates with the standard deviation values shown as the error bars.

Α

| | | | – Arabidopsis – Rice |
|--------------|--------------------|---|--|
| | | L-Potato | A |
| | | Tomato | |
| | | Topacco | |
| Б | | ſ N. benthamiana | |
| D | | | |
| WIIE | al | оо – – – – – – – – – – – – – – – – – – | |
| Ric | e at | MRH 68 | |
| Ara | bidopsis | NPSFLVERKS 67 | |
| Pot | ato | AGR 89 | |
| Tom | ato | AGR 89 | |
| Tob | acco | AGR 96 | |
| Nb | enthamiana | AGR Alignment scores | |
| Whe | at | I <mark>N</mark> K <mark>K</mark> SVDRDLDVYRN <mark>L</mark> LSKLVQA <mark>K</mark> ELLKEY <mark>I</mark> EREKKK <mark>R</mark> EERLE <mark>T</mark> PK <mark>P1</mark> | <mark>ieavak</mark> fd <mark>gs</mark> ayp |
| Ric | e | I <mark>NKR</mark> SVDRDLDVYRN <mark>I</mark> LSKLVQA <mark>K</mark> ELLKEY <mark>V</mark> EREKKK <mark>R</mark> EERSETPK <mark>SI</mark> | <mark>JEAVTK</mark> FD <mark>GS</mark> LNS |
| Ara | bidopsis | I <mark>NKK</mark> SVDRDLDVYRN <mark>L</mark> LSKLVQA <mark>N</mark> ELLKEY <mark>V</mark> EREKKK <mark>Q</mark> EERAQ <mark>S</mark> QK <mark>A</mark> I | VETISKCLGDTLY |
| Pota | ato | VNKKSVDRDLDVIRNLISKLVQAKELLKEYVEREKKKRGERE-TOKA | VETVTKCLGDYOY |
| | ato | VNKKSVDRDLDVYTRIUDSKUVQAREDDKETVEREKKKRGERE-SQKA2 | |
| N.b | enthamiana | INKKSVDRDLDVYRNULSKLVQAKELLKEYVEREKKKRGERE-SQKAI | EAVTKCLGDYQY |
| · · · | and based as | | |
| Whe | at | VEFSS <mark>RDGEIESIL</mark> KDI <mark>S</mark> ERAQGKG <mark>NFS</mark> YSRFFAVGLFRLLEL <mark>SNA</mark> T | EPT <mark>V</mark> LDKLCAALN |
| Ric | e | VEFSSKDGEIEAILKDISERAQGKG <mark>SFSYSRF</mark> FAVGLFRLLEL <mark>ANA</mark> T | PTILDKLCAALN |
| Ara | bidopsis | VDFSSKEGDIEAVLKDIAGRAGSKEGESYSREFAVGLERLLELASATI | PTVLDKLCASLN |
| Pot | alu ato | VDFSSKEGETENTEKDIAORAGIKUGFCISKEFAVGEFKLEELANVI VDFSSKEGETENTEKDIAORAGIKUCFCVSRLEAVGEFRLIELANVI | |
| Tob: | acco | VDFSSKDGEVENILKDIAQKAGTKNSFCYSKLFAVGLFRLLELANVT | DETTLEKLCASLN |
| N.b | enthamiana | VDFSSRDGEVENILKDIAQRAGTKD <mark>SFC</mark> YSRLFAVGLFRLLELANVT | OPTILEKLCASLN |
| | . . | | |
| Whe | - at | VFALGFVTVYDQLMEGYPSTEDRDAIFK <mark>S</mark> YVTAL <mark>N</mark> EDPEOYRADAO <mark>RI</mark> | EEWAR <mark>SONG</mark> NLL |
| Ric | 6 | VFALGFVTVYDOLMEGYPSNEDRDAIFKAYTTALNEDPEOYRADAOKI | EEWARSONGNSL |
| Ara | bidopsis | VFALGEVTVYDQLMEGTE GEEDKNALFAATTEALNEDFEQIKADAQKI VFALGEVTVYDQLMEGYPSDODRDATFKAYTEALNEDPKOVRTDAOKI | EEWARSOTSASL |
| Pot | alu | VFALGEVIVIDUMEGIFSEEDKNAIFKAIVEALKEDPEQYRADAQKI | |
| TOD | acco | VFALGEVIVIQLMEGYPSEBURDAIFKAYIEALNEUPVQYRADAQKI | EEWARTQNANTL |
| N.b | enthamiana | VFALGFVTVYDQLMEGYPSEEDRDAIFKAYIEALNEDPVQYRADAQKI | EEWARTQNANTL |
| | _ | | |
| Whe | at | <mark>R</mark> S <mark>VVR</mark> CVATAGDIPP <mark>TVAD</mark> TKM <mark>NFLKSYKRPI</mark> P <mark>SIYS</mark> TVLQELLVQQI | HLM <mark>RYK<mark>ST</mark>YQYDP</mark> |
| Ric | e | RS <mark>VVRCVA</mark> TAGDVPP <mark>TVAETKMN</mark> FLKSYKRPILSIYSTVLQELLVQQ | ILMRYKTTYQYDA |
| Ara | bidopsis | KSLIHCMSNVTADVPPVSETKSKFLKAYKRPIPSIYNTVLOELTVOOF | ILMRYKKTYRYDP |
| Pot | ato | REVVICTSSSADDETVADTKLKELTAYKRETETVINTVLOELTVOO | ILTRYKKSYOYDP |
| TOD | ato | REVUNCTOSSADD. DTVALIKMINE DAAIKREIPIVINIVLOEDIVLOI REVUNCTOSSADD. DTVADTKMIKET. TAYKRDIDTVVNTVLOEDIVLOI | ILTRYKKSYOYDP |
| | entnamiana acco | | ILIKIKKSIRIDP |
| | | | |
| Whe | at | M <mark>AAISSLP</mark> FAALRRAADWRPSTATAAVSV- <mark>S</mark> GGVMLNARAR | RG <mark>S</mark> |
| Ric | e | MAAISSLPFAALRRAADCRPSTAAAAAGAGAGAGAVVLSVRPR | RG <mark>S</mark> |
| Ara | bidopsis | MAATAISSLSFPALGOSDKISNFASSRPLASAIRICTKFSRLSLNSR- | ST <mark>S</mark> |
| Pot | ato | = -MAAVISVSFSALIQSALASSVSSSSSIDIFRFROMFSFDSVNVR= -MAAVISVSFSALIQSALASSVSSSSSIDIFRFROMFSFDSVNVR | SSN = = = = = = STS |
| 'I'OD | acco | | SSNSSFSSNSST <mark>S</mark> |
| N.b | enthamiana | MAAVTSVSFSALAQSAERKSSVSPSRSVDTFRFRSNVSIDCFNVR | SSNSSFSSNPSTS |
| NT 1. | | | |

Figure S3. *ALC1* has homologs in several plants. A. Comparative analysis of the translated sequence of the *N. benthamiana* gene, *NbALC1* (GenBank EU106046) with homologs in tobacco (*N. tabacum*, TIGR TC10126), tomato (*S. lycopersicon*, TIGR TC178313), potato (*S. tuberosum*, GenBank AY342161), Arabidopsis (*THF1*, GenBank AY899908), rice (*Oryza sativa*, inositol phosphatase-like protein; GenBank AY224446) and wheat (*Triticum aestivum*, Ptr ToxA binding protein (GenBank AY377991). A multiple sequence alignment program (ClustalW) was used to align the sequences. Identical amino acid residues are shaded in blue. Residues with conserved or semiconserved substitutions are highlighted in green and yellow, respectively. White letters on the Arabidopsis sequence indicate the two putative transmembrane domains predicted for THF1 previously (Huang *et al.*, 2006). B. A phylogram of the sequences was generated using PHYLIP TREE and shows the evolutionary distance of all the homologs in panel (a) relative to *ALC1*.

Wheat



Figure S4. Determination of silencing efficiency of *SlALC1* in COR-treated mock and *jai1* tomato mutants. Total RNA was extracted from the leaves of tomato silenced with *GFP* and *SlALC1* respectively, and cDNAs were synthesized for qRT-PCR analyses. The transcript levels were normalized against *tubulin (Tub)*.



Figure S5. Response of the Arabidopsis *thf1* mutant line to pathogens (*P. syringae* pv. *maculicola* and *Erwinia carotovora* subsp. *carotovora*) and nonhost pathogens (*P. syringae* pvs. *tabaci* and *glycinea*). A. Arabidopsis wild-type (Col-0) and *thf1* were syringe-infiltrated with pathogens (5 X 10⁵ CFU/ml) and non-pathogens (106 CFU/ml), and photos were taken 5 dpi. B. Growth of *P. syringae* pvs. *maculicola, tabaci* and *glycinea* on Co1-0 and the *thf1* mutant line 0, 1, 3 and 5 dpi. Inoculated samples were collected at various dpi, homogenized in water and serial dilutions were plated on KB medium supplemented with antibiotics when necessary.



Figure S6: Effect of coronatine on localization of ALC1 near and away from inoculation zone in *N. benthamiana* leaf samples.

Agrobacterium containing ALC1-GFP infiltrated into *N. benthamiana* leaves, treated with coronatine and observed by fluorescence microscopy. The panels denote zone away (24^1 and 48^1) from the coronatine application and near (24^2 and 48^2) the coronatine application visualized at the same time points. RED indicates autofluorescence of the chloroplast by excitation at 647 nm and emission in cyan/far red channel. GFP indicates GFP fluorescence of the tagged protein by excitation at 488nm and emission at green channel. All images magnified using 63X water immersion objective. The left series indicates the time of imaging after coronatine treatment. Scale bars – 5 μ M.



Figure S7: Effect of coronatine on localization of a chloroplast localized RecA protein in *N. benthamiana* leaf samples.

Agrobacterium containing 35S:: RecA-GFP infiltrated into N. benthamiana leaves, treated with COR. RED, autofluorescence of the chloroplast by excitation at 647 nm and emission in Cyan/Far red channel; GFP, GFPfluorescence of the tagged protein by excitation at 488nm and emission at Green channel. All images were magnified using 63X water immersion objective. The left series indicates the time of imaging after COR treatment. Scale bars–5 μ M.



Figure S8: Effect of coil silencing on expression and localization of ALC1 in *N. benthamiana* leaf samples.

Agrobacterium containing ALC1-GFP infiltrated into COI1 (TRV::*COI1*) silenced *N. benthamiana* leaves and observed by fluorescence microscopy. RED indicates autofluorescence of the chloroplast by excitation at 647 nm and emission in cyan/far red channel. GFP indicates GFP fluorescence of the tagged protein by excitation at 488nm and emission at green channel. All images magnified using 63X water immersion objective. Scale bars – 5 μ M.



Figure S9: COR-induced chlorosis and *NbALC1***-mediated necrotic phenotype are** *coi1***-dependent.** Response of silenced lines of *N. benthamiana* leaves to 2 nmol COR. COR was applied three weeks post Agro-inoculation to silenced lines of *N. benthamiana*.