

**Supplemental Figure 1**. Rough map position on the Arabidopsis genome of the mutated genes rendering C17 tolerance. Using 24 general simple sequence length polymorphism (SSLP) markers, the mutated genes in C17-tolerant mutants were mapped to Arabidopsis genome. The mutated genes are divided into two groups based on the linkage with SSLP markers, which were designated by corresponding markers, CH4-14494 and CH5-512, respectively.



Supplemental Figure 2. Sequence alignment of CESA1 (A) and CESA3 (B) of several plant species. Sequences were aligned with a multiple sequence alignment programme (http://www.genome.jp/tools/clustalw/) using CLUSTALW algorithms. Protein database accession numbers are: CESA1<sub>A.thaliana</sub>-NP 194967; CESA1<sub>G.max</sub>-XP 003522623; CESA1<sub>Evesca</sub>-CESA1<sub>V.vinifera</sub>-XP\_002282575; CESA1<sub>S.lycopersicum</sub>-XP 004291468; XP 004245031; CESA1<sub>Z.mavs</sub>-NP 001104954; CESA1<sub>O.sativ</sub>a-NP 001054788; CESA3<sub>A.thaliana</sub>-NP 196136; CESA3<sub>G.max</sub>-XP 003540527; CESA3<sub>F.vesca</sub>-XP 004306536; CESA3<sub>V.vinifera</sub>-XP 002278997; CESA3<sub>S./vcopersicum</sub>-XP 004229630; CESA3<sub>Z.mays</sub>-NP 001105621; CESA3<sub>O.sativa</sub>-NP 001059162. The amino acid regions harbouring amino acid replacements in C17-tolerant mutants were selected. Arrowheads indicate the positions of the mutated amino acids. The blue lines indicate the predicted transmembrane domains (TM).



**Supplemental Figure 3.** C17 does not trigger microtubule polymerization. GFP-CESA3 localization at the plasma membrane and cortical microtubule arrays were observed in roots in absence (mock) or presence of C17 (200 nM) for 30 min. Transgenic plants harboring both GFP-CESA3 and Cherry-MBD were used. Scale bars=5  $\mu$ m.



**Supplemental Figure 4.** C17 results in the depletion of CSCs from the root plasma membrane. Representative spinning confocal microscopy images of 4-day-old GFP-CESA3 roots treated with C17 (200 nM). The images were taken at indicated time points (0 min, 5 min, 10 min, 15 min and 20 min). Depletion of GFP-CESA3 is indicated by loss of GFP fluorescence from the plasma membrane (indicated by the arrows).



**Supplemental Figure 5.** Isolation of *cwm1* and *cwm2* mutants. (**A**) Intron-exon organization of the *CWM1* gene. The black box represents the exon. The position of T-DNA insertion sites (*cwm1-1, cwm1-2,* and *cwm1-3*) are indicated. Scale bar = 200 bp. (**B**) The C17 sensitivity of two independent *AT5G44570* knockout mutants lines (SALK\_020569C and SAIL\_699\_C11). Three-day-old seedlings grown on half-strength MS medium were transferred to medium without (-) or with (+) 200 nM C17 for two days. Arrowheads indicate the root tip position at the moment of transfer. Scale bar = 5 mm. (**C**) Mapping and gene structure of *CWM2*. The *cwm2-1* locus was mapped into the 171.8-kb region between SSLP markers CH1\_11687 and CH1\_11589. Using PCR, a second T-DNA insert was found and positioned at the coding region of AT1G32415. The black box represents the exon. The position of the T-DNA insertion sites (*cwm2-1* and *cwm2-2*) are indicated.



**Supplemental Figure 6.** Both *cwm1-1* and *cwm2-1* mutations counteract the growth inhibition induced by cellulose deficiency. Three-day-old seedlings grown on half-strength MS medium were transferred to medium without (mock, left panel) or with isoxaben (4 nM panel) or indaziflam (0.2 nM, right panel). Arrowheads indicate the root tip position at the moment of transfer. Scale bar = 5 mm.



**Supplemental Figure 7.** Staining of respiratory protein complexes from wild type, *cwm1* and *cwm2*. Mitochondrial proteins were isolated from two-week old seedlings and solubilized by digitonin extraction buffer, separated by BN-PAGE and either visualized by Coomassie staining or by in-gel activity staining for complex I and complex IV. The identities of protein complexes are indicated on the left or right of the blots: I – complex I; IV – complex IV; V – complex V; III<sub>2</sub> – dimeric complex III; I+III<sub>2</sub> supercomplex composed of complex I and dimeric complex III; I<sub>2</sub>+III<sub>4</sub> – a dimer of supercomplex I+III<sub>2</sub>.



**Supplemental Figure 8.** Respiration rates of *cwm1* and *cwm2* mutants. (A) Oxygen consumption of whole seedlings of Col-0, *cwm1* and *cwm2* mutants. Data represent mean  $\pm$  SE (n = 4). (B) Relative KCN-resistant respiration of whole seedlings of Col-0, *cwm1* and *cwm2*. Data represent mean  $\pm$  SE (n = 4). Statistically significant differences compared with wild-type plants are indicated with P-value (two-tailed Student's *t*-test).



**Supplemental Figure 9.** C17 sensitivity of the mutants with defective mitochondrial complex I. Root growth of wild-type (Col-0) and mitochondrial complex I mutants (*ndsf4*, *bir6-2* and *otp439*). Three-day-old seedlings grown on half-strength MS medium were transferred to medium without (-) or with (+) of 200 nM C17 for two days. Arrowheads indicate the root tip position at the moment of transfer. Scale bar = 5 mm.



**Supplemental Figure 10.** Inhibition of mitochondrial activity did not restore CSC activity. (**A-F**) Representative spinning confocal microscopy images of 4day-old GFP-CESA3 plants treated with 0.1% DMSO (A and D), 200 nM C17 (B and E), or a combination of 1  $\mu$ M antimycin A with 200 nM C17 (C and F). The images were taken at 0 min (A, B and C) and 20 min (D, E and F). Depletion of GFP-CESA3 is indicated by loss of GFP fluorescence from the plasma membrane, such as the domains indicated by the arrows. Scale bars = 25  $\mu$ m.



**Supplemental Figure 11.** C17 does not directly inhibit isolated mitochondrial respiration. The oxygen uptake of isolated mitochondria was measured to evaluate the effect of C17 on mitochondria. Data represent mean  $\pm$  SE (n = 4). C17 concentrations ranging from 1 to 8 µM were added to mitochondria using succinate (succ) and NADH as substrates. A cytochrome pathway inhibitor, KCN (100 µM) was used as positive control.

| Chr. | SSLP marker     | Forward primer  | Reverse primer           |
|------|-----------------|---|--------------------------|
| 1    | nga63           | AACCAAGGCACAGAAGCG                                    | ACCCAAGTGATCGCCACC       |
| 1    | F8K7            | GAACAATCAAGCCACCTCTAG                                 | TCAGTGTCAGTTGAAAGTTTAAGC |
| 1    | F19K23          | GAATTCTGTAACATCCCATTTCC                               | GGTCTAATTGCCGTTGTTGC     |
| 1    | T17F3           | GGACCGACGGTTACGAGAGT                                  | TAACGGGCCGTTGCAAGA       |
| 2    | T23K3           | CGTGTTTACCGGGTCGGA                                    | AAAACCCTTGAAGAATACG      |
| 2    | F3P11           | ATGTATTTGTTGCAAAATAA                                  | ТGCACAGAAGAAAAACTA       |
| 2    | T20P8           | TCCGATTCGATTAAACTC                                    | TTATTTCCTATTTCAAGACT     |
| 2    | T16B24          | ATGAACGGAGTAGCTATC                                    | CGCGTAGAACATAATCTGTA     |
| 3    | F20H23          | CAATGGGAAGAAGGTGTGAG                                  | CGCATTTCCATAAGTTTGTT     |
| 3    | MGL6            | ACCTGTTCAGTCTATGTTAC                                  | GGGAATTATTAACATTATCA     |
| 3    | T32N15          | CAAAAGAAATGCAACGAGAC                                  | TTTGATCATGAATGGTAGTG     |
| 3    | F28P10          | GAATAAACCATGTTGCCAAACATC                              | CATTTGATGCCCTGATAATTTCTC |
| 4    | T4I9            | TTATAGCAAACGTACAAGTC                                  | CTGCATACACGTCGTCTC       |
| 4    | F17A8           | CTGGACCCTAGTGGATGT                                    | GACGGTTCTCCATTAATTAT     |
| 4    | FCA8            | TTCGGAGAAAGAAACGACAT                                  | ATGGAACTATTCAGGCATTA     |
| 4    | T15N24          | GCAACCGCTGCTGCTTTA                                    | AATATTTGGCTTTGCGTAGA     |
| 4    | T4L20           | ACCCTAAAACAATGTCTCTT                                  | TGCTAACATGGAAATTTGTC     |
| 5    | MBK20           | CTCTGTTGGGGCAAAACC                                    | GATGCTGGAGAGTAGCTTAG     |
| 5    | nga139          | AGAGCTACCAGATCCGATGG                                  | GGTTTCGTTTCACTATCCAGG    |
| 5    | T26D22          | CACAGGCCATTGGATGTA                                    | TGTTAGAACCCACCATTTG      |
| 5    | K6M13           | CCTGTTCCAATGAATATG                                    | TGTAGCTGCTGAGTTGTC       |
| 5    | MGI19           | GCTTGACATGAAGTGCTAAAC                                 | TCTGTGTGATTCTCTCCAAGG    |
| 5    | CH5_512         | GAATAAATGGGCCTTATAAC                                  | TATTATTGTGCCAGATATTG     |
| 5    | CH5_1037        | GGCACAACTATATTTCCCTCCAT                               | TTGTTAGTGGTTGAATTGTGTCG  |
| 5    | CH5_1316        | ACCAGGTGAGAATAAAACTGCAA                               | ATAACATCCACCAATGCTACGTT  |
| 5    | CH5_1531        | AACACAACATGGTTTCAGT                                   | GCCGTTTGTCTCTTCAC        |
| 5    | CH5_1566        | TGGTTAGATTTGCTGTT                                     | ATTCTGCATTATTAGTTGTC     |
| 5    | CH5_1779        | CGCGATTAAAGATCCGGTA                                   | TCGAGCAATAAGAGTGATTCC    |
| 5    | CH5_2174        | CATGATCCATCGTCTTAGT                                   | AATATCGCTTGTTTTTGC       |
| 5    | CH5_3177        | AGGCACCAAAGAAACAAG                                    | CGTTGTGTTCATTTATGAC      |
| 4    | CH4_14494       | TACCTTCATGCCAGGTGCT                                   | GATGCTGAAATGGACAAGAT     |
| 4    | CH4_15294       | CATGGTGGTCTTGGAAGAT                                   | GTACGTTAAATGGCAAAGAT     |
| 4    | CH4_15593       | GGCTGTGAATCTCACAAAC                                   | CTTTGGATATCTATCTGTGTA    |
| 4    | CH4_15659       | CTGTTCCTCGTTTCGTGTTA                                  | CAGGAAGGAGCACTCAATAG     |
| 4    | CH4_15955       | GTAATTGCTAGCGTCAAAC                                   | AAGAAGCAAACCCCAAAAC      |
| 4    | CH4_16853       | AATTCAAGTTTGATGTTCTAC                                 | AAAGTTCAAAGCATGCATTA     |
|      | Cloning primers |   |                          |
|      | ANAC017_attB1   | GGGGACAAGTTTGTACAAAAAGCAGGCTTCATGGCGGATTCTTCACCCGATTC |                          |
|      | ANAC017_attB2   | GGGGACCACTTTGTACAAGAAAGCTGGGTGCTAGTCTTTCAAGAGAAGACTTC |                          |

## Supplemental Table 1. Primer sequences used for mapping and cloning