Supporting Information

Jin et al. 10.1073/pnas.0906144106

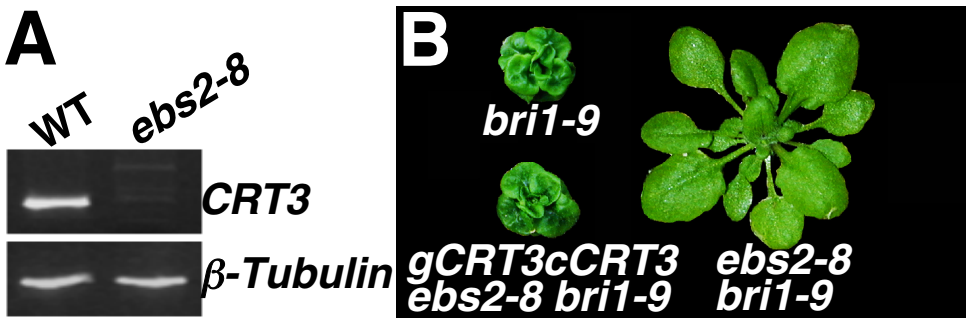

Fig. S1. A T-DNA insertional mutation in Arabidopsis CRT3 suppresses the *bri1–9* mutant phenotype. (*A*) *RT-PCR* analysis of *EBS2* gene expression in WT and *ebs2–8*. Total RNAs isolated 3-week-old seedlings were converted into cDNA by using the Invitrogen SuperScript first-strand synthesis system for RT-PCR. The first-strand cDNA (0.5 μL) was used for templates to amplify transcripts of *EBS2* and β-*TUBULIN* (as a control) using the primer set listed in Table S1. *EBS2* transcripts were amplified for 30 cycles, whereas β-*TUBULIN* transcripts were amplified for 19 cycles. (*B*) Four-week-old soil-grown plants of *bri1–9*, *ebs2–8 bri1–9*, and *pPZP222-gCRT3:cCRT3 ebs2–8 bri1–9*.

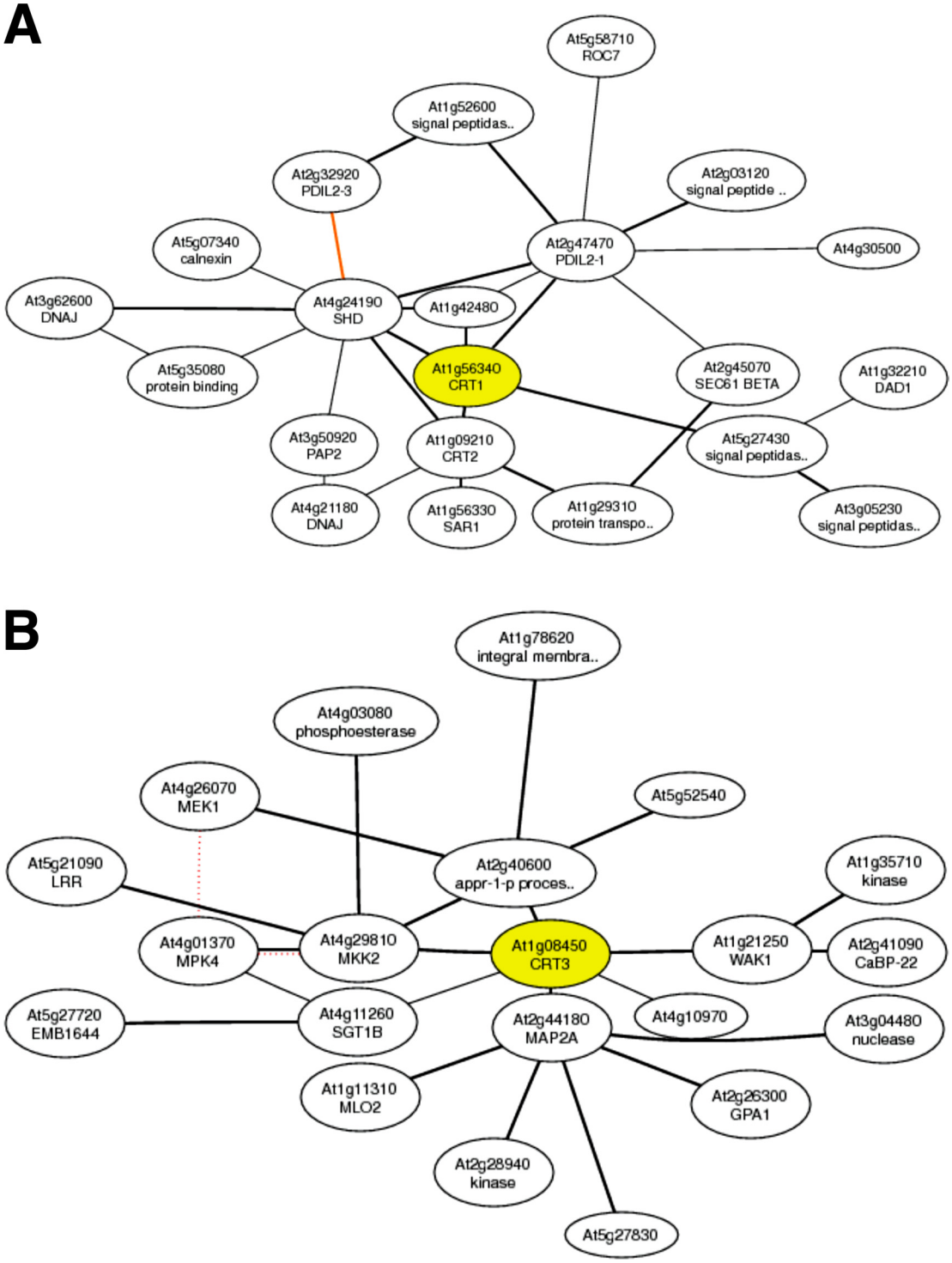

Fig. S2. Genes co-expressed with CRT1 (*A*) or CRT3 (*B*). Both figures were generated by the *Arabidopsis thaliana* trans-factor and cis-element prediction database, version 5.2 (http://atted.jp/), through its search tool. Black lines link co-expressed genes. Orange line indicates genes that are also co-expressed in at least one of 3 other organisms: human, mouse, and rat; dotted red lines indicate direct protein-protein interaction.

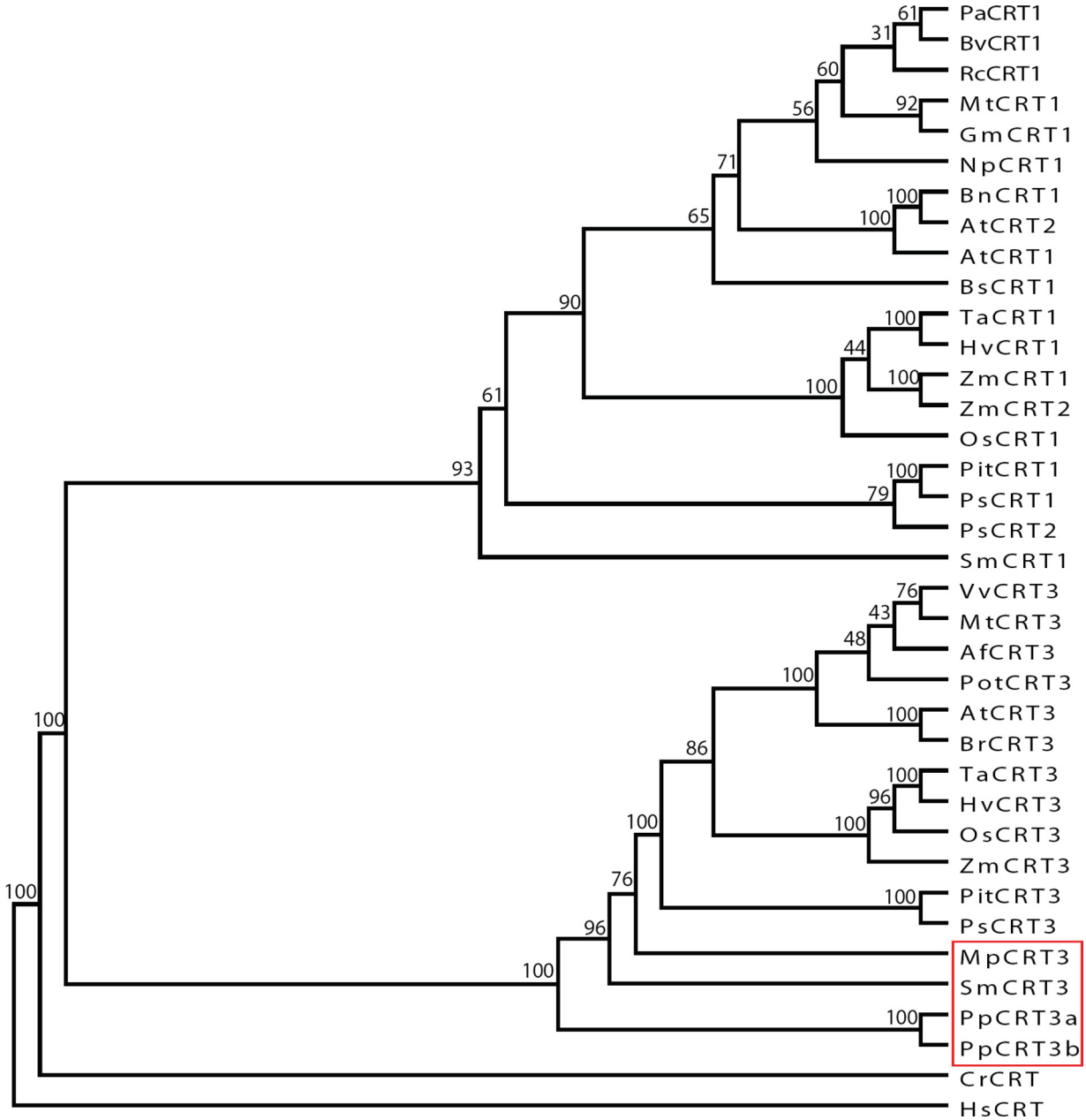

Fig. S3. Phylogeny analysis of CRT homologues in plants. Plant CRT protein sequences and the human CRT sequence were aligned with ClustalW. The neighbor-joining method of the Phylogeny Inference Package (version 3.68) was used to construct the tree with 100 bootstrap replicates. The numbers above the branches are bootstrap values derived from neighbor-joining analysis. The HsCRT sequence was used as the out group to root the tree, and 4 CRT3 homologues from lower plant species were boxed in red. Names and accession numbers of the analyzed CRT proteins used for analyses are as follows: PaCRT1 (AAD32207, *Pa*: *Prunus armeniaca*), BvCRT1 (O81919.1, *Bv*: *Beta vulgaris*), RcCRT1 (AAB71419, *Rc*: *Ricinus communis*), GmCRT1 (BAF36056, *Gm*: *Glycine max*), MtCRT1 [obtained from Legume genome scanner (http://www.kazusa.or.jp/LGS/index.html), AC149080.6, *Mt*: *Medicago truncatula*], NpCRT1 (CAA95999, *Np*: *Nicotiana plumbaginifolia*), AtCRT2 (NP_172392, *At*: *Arabidopsis thaliana*), BnCRT1 (AF019376.1, *Bn*: *Brassica napus*), AtCRT1 (NP_176030), HvCRT1 (AAA32949, *Hv*: *Hordeum vulgare*), TaCRT1 (AAW02798, *Ta*: *Triticum aestivum*), ZmCRT2 (AAF01470, *Zm*: *Zea mays*), ZmCRT1 (CAA86728), OsCRT1 (BAC82933, *Os*: *Oryza sativa*), BsCRT1 (AAD17490, *Bs*: *Berberis stolonifera*), PitCRT1 (AAG01147, *Pit*: *Pinus taeda*), PsCRT1 (ABK24327, *Ps*: *Picea sitchensis*), PsCRT2 (ABK23433), SmCRT1 (translated from nucleotide sequence FE455037.1, FE466672.1, and FE453208.1, *Sm*: *Selaginella moellendorffii*), VvCRT3 (translated from nucleotide sequence XM_002276397.1, *Vv*: *Vitis vinifera*), MtCRT3 (translated from nucleotide sequence BT052978.1), AfCRT3 (translated from nucleotide sequences DR940519.1 and DT750168.1, *Af*: *Aquilegia formosa*× *Aquilegia pubescens*), PotCRT3 (EEE90236.1, *Pot*: *Populus trichocarpa*), BrCRT3 (translated from nucleotide sequences EX116073.1, EX026998.1, and EX117522.1, *Br*: *Brassica rapa*), AtCRT3 (NP_563816), HvCRT3 (translated from nucleotide sequence AK248906.1), TaCRT3 (EF452301.1), OsCRT3 (BAC06263), ZmCRT3 (translated from nucleotide sequence AY105822), PitCRT3 (translated from nucleotide sequences BF777977.1, CO198952.1, and CO158387.1), PsCRT3 (translated from nucleotide sequence EF678532.1), MpCRT3 (translated from nucleotide sequence BJ858135.1 and BJ850397.1, *Mp*: *Marchantia polymorpha*), SmCRT3 (translated from nucleotide sequence FE490612.1 and FE490611.1), PpCRT3a and PpCRT3b [deduced from contig sequence scaffolds 65 and 34, respectively, from the Joint Genome Institute (http://genome.jgi-psf.org) using *Physcomitrella patens* subsp. *patens* v. 1.1, *Pp*: *Physcomitrella patens*], CrCRT (EDP09399.1, *Cr*: *Chlamydomonas reinhardtii*), and HsCRT (NP_004334.1, *Hs*: *Homo sapiens*).

A

GSIFDNIMVTDDAAVAKKFAEDTWGKTKAGEKAMMDA

EEEEERKKREEEMKRMDEERAKLGEDEDEEDEDEDED

FDDEEEDEEEDYNAKDEL

B	R	K	H	D	E	Total	Net Charge
MvCRT	4	6	0	12	24	62	-26

Fig. S4. The *Mesostigma viride* CRT C-terminal fragment is acidic. (*A*) Amino acid sequence of the C-terminal fragment of a *M. viride* CRT translated from an EST (EC729890) identified by tBLASTn using AtCRT1 as query against the EST collection of *M. viride* in GenBank. The sequence underlined in blue is a part of the globular domain of the CRT, and the red open box denotes the C-terminal domain. (*B*) Numbers of basic and acidic residues and the net charge in the C terminus of the *M. viride* CRT.

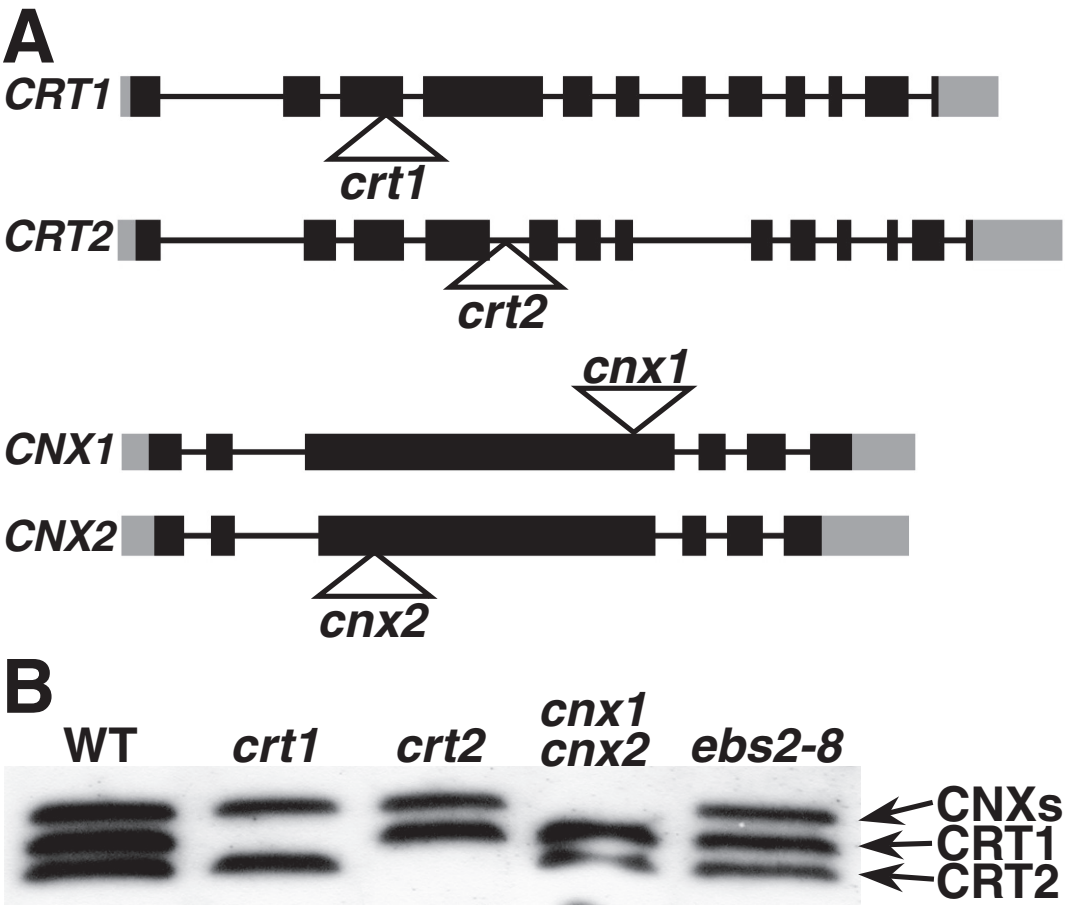

Fig. S5. The anti-maize CRT antibody fails to detect Arabidopsis CRT3. (*A*) Schematic illustration of *crt1*, *crt2*, *cnx1*, and *cnx2* T-DNA insertional lines. Exons, introns, and untranslated regions are shown as black boxes, lines, and gray boxes, respectively. Triangles represent T-DNA insertion sites. (*B*) Western blot analysis of crude protein extract from 4-week-old seedlings of WT, *crt1*, *crt2*, and *cnx1 cnx2* and *ebs2–8* T-DNA insertional mutants using anti-maize CRT antibody.

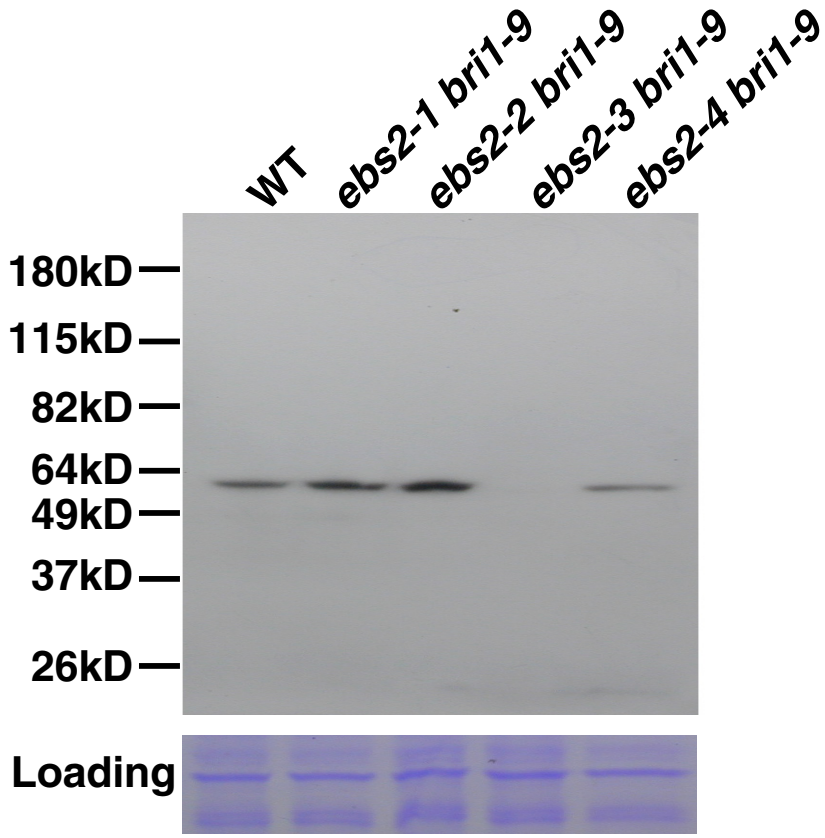

Fig. S6. The anti-CRT3 antibody specifically detects CRT3. Total protein extracts from WT, *ebs2–1 bri1–9*, *ebs2–2 bri1–9*, *ebs2–3 bri1–9*, and *ebs2–4 bri1–9* were separated by 10% SDS/PAGE and analyzed by immunoblotting with anti-CRT3 antibody. Coomassie blue-stained SDS/PAGE gel is shown to control equal loading.

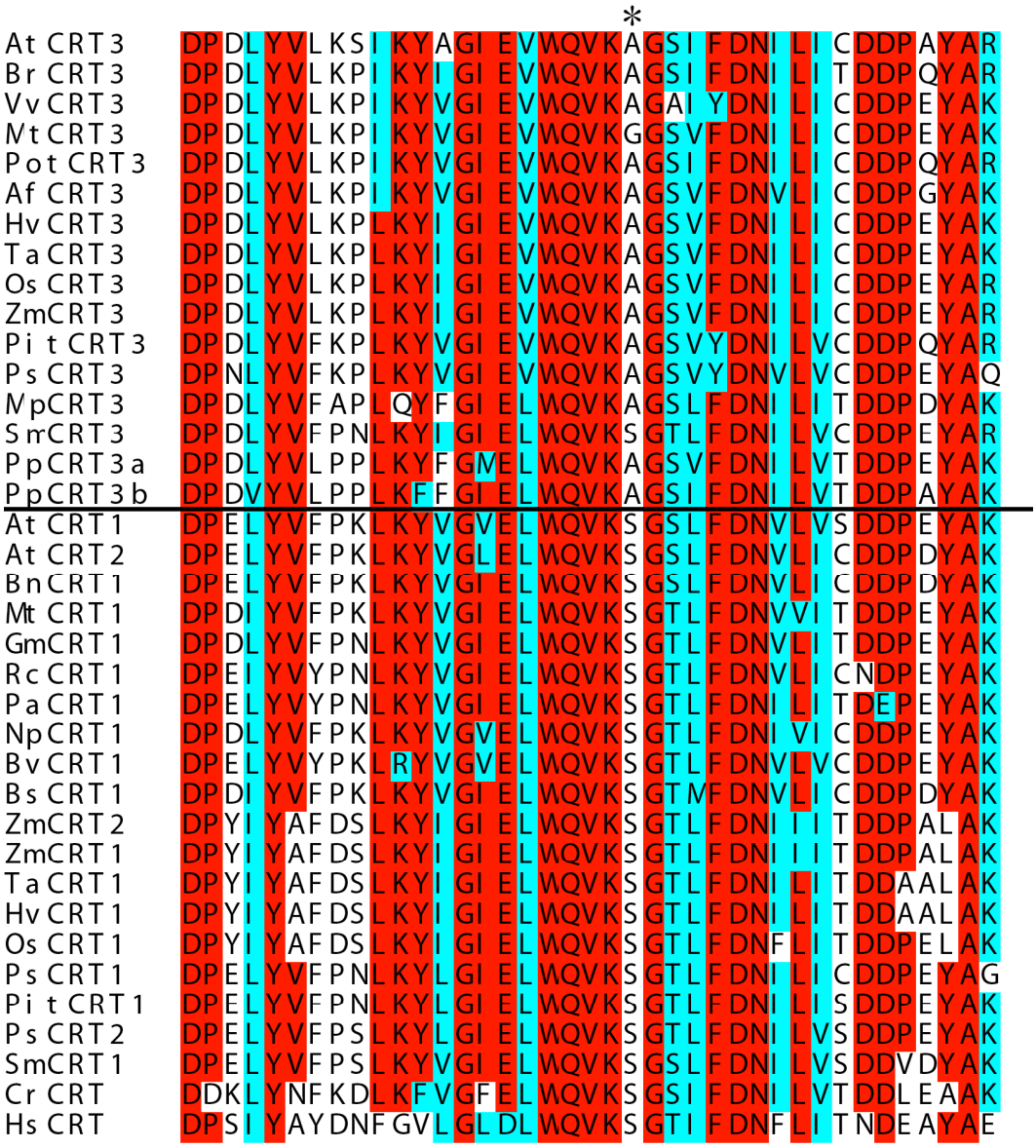

Fig. S7. Most CRT3 contains alanine instead of serine at position 333. Thirty-eight sequences from Fig. S3 that correspond to aa 312 through 350 of AtCRT3 were aligned with ClustalW and shaded with BoxShade 3.21 (http://www.ch.embnet.org/software/BOX_form.html). Residues that are identical in 80% of sequences are shaded red and similar residues are shaded cyan. A black line in the middle separates CRT3 group (*Top*) and CRT1/CRT2 group (*Bottom*). Star indicates the position of Ala-333 in AtCRT3. Most of CRT3 contain Ala at position 333, whereas all CRT1/CRT2 contain Ser at this position.

Table S1. Oligonucleotides used in this study

Name	Sequence	Comment
CER464189[a]	GATCCAAAACAAGTCTCCTGC	CAPS
	CTCTGTAAACTCGGTGAGCACG	HindIII cuts Col
T23G18_1[b]	GGATGTTGTGATTGCCAAACC	CAPs
	GTGATCTTCAGCATCCCTAAC	DpnI cuts Ws
T23G18_2[b]	GCACTGCCAAACACAGTCAA	dCAPs
	ATCCGCCACGTGGCTCACGT	HinCII cuts Ws
T27G7_1[b]	GACTTCACCTTGTCATGACCG	dCAPs
	TCCTGACCTGAATGCAGATAAAGTAC	SnaBI cuts Ws
F22O13_1[b]	CACACTTTTCGTAATAGATCAAC	CAPs
	GAGACTCTTTCAAGAAAGGAATC	AluI cuts Ws
F22O13_2[b]	GCATTCTTTGAGAGGCTCAAGAT	dCAPs
	CAACTATTTAATCGTCTCCTTAG	BglII cuts Col
JV28/29[c]	CACTTGTTCTGAAACGAAATTGA	SSLP
	GCTTCTCATTGCACTCCTTTG	Ws < Col
CRT3_RT	GTCTCTGTACTAACTCTTGC	On exon 1
	CACCAGAACGCTGTAAGAAG	On exon 4
TUB2RT	TTCCAGGTTTGTCACTCGTTG	Control for RT-PCR
	ATGAAGAAGTGAAGACGGG	
CRT3N	GACTCGAGAATCGAAAGCATGTTC	Make CRT3N-CRT1C chimeric gene
	ATCTTCTCTAGCTTTTCTTTC	
CRT1N	GAGTCGACTCCTCTTCCGTATTGGGCA	Make CRT1N-CRT3C chimeric gene
	TTCCTCTTCCTCTCTCTTCTTCTC	
CRT3C	GCCCGGATAGCACGGGAAGAAGGTGAA	Make CRT1N-CRT3C chimeric gene
	ATGGATCCAAATGCCCACCACTC	
CRT1C	GAGGAATCAAAGGATGCTCCT	Make CRT3N-CRT1C chimeric gene
	AGGGATCCTTTTTAATCCTCCACCTTTGC	

[a], This primer set was designed according to the Monsanto Arabidopsis SNP/INDEL database (1).
[b], These primers were designed using the dCAPS Finder program (2) (http://helix.wustl.edu/dcaps/dcaps.html) based on the sequences of PCR products amplified from the *bri1–9* (Ws) mutant and the published genomic sequences of the WT Col-0.
[c], The primer sequences were obtained from the Arabidopsis Information Resource database (http://www.arabidopsis.org/).

1. Jander G, *et al*. (2002) Arabidopsis map-based cloning in the post-genome era. *Plant Physiol* 129:440–450.
2. Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in Arabidopsis thaliana genetics. *Plant J* 14:387–392.