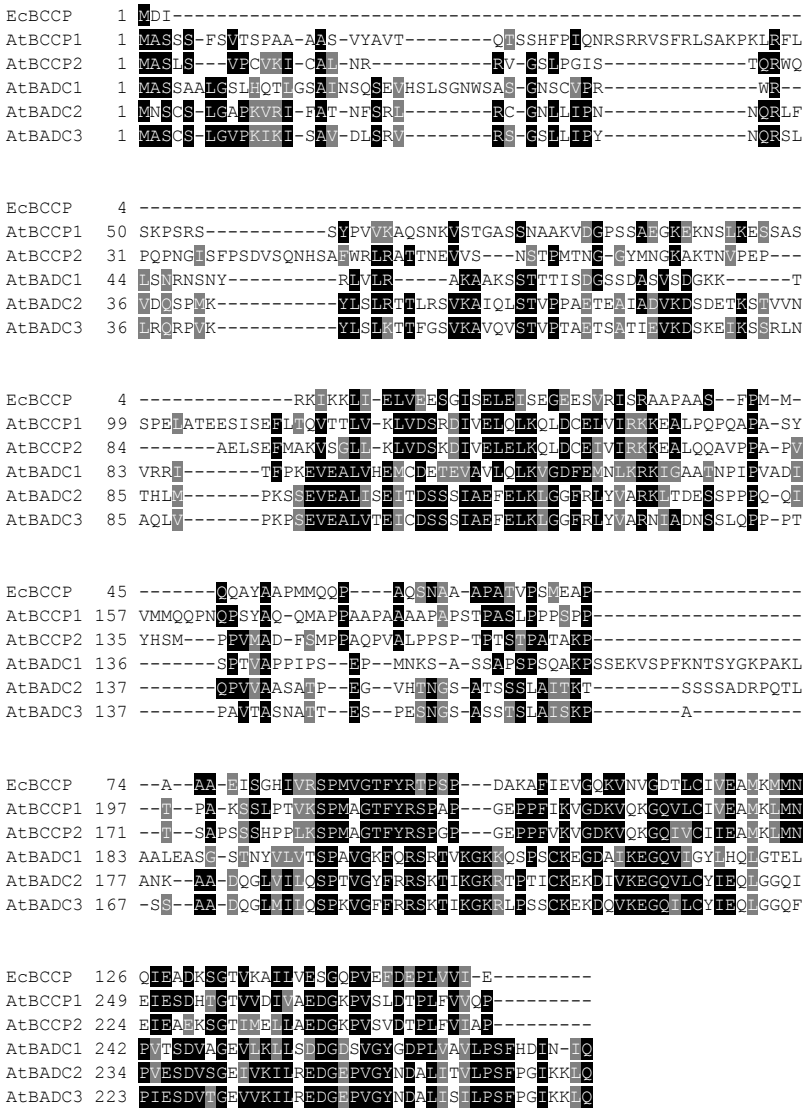
A

Protein Annotation	Protein Accession	No. of replicates identified (n=7)	Average Peptides	Average PSMs
acetyl Co-enzyme a carboxylase carboxyltransferase alpha subunit	AT2G38040	7	47.8	231.0
acetyl-CoA carboxylase carboxyl transferase subunit beta	ATCG00500	7	5.0	19.6
acetyl Co-enzyme a carboxylase biotin carboxylase subunit	AT5G35360	5	6.1	14.3
chloroplastic acetylcoenzyme A carboxylase 1	AT5G16390	3	2.4	6.4
Single hybrid motif superfamily protein	AT1G52670	2	2.5	3.5
biotin/lipoyl attachment domain-containing protein	AT3G56130	1	2.0	5.0

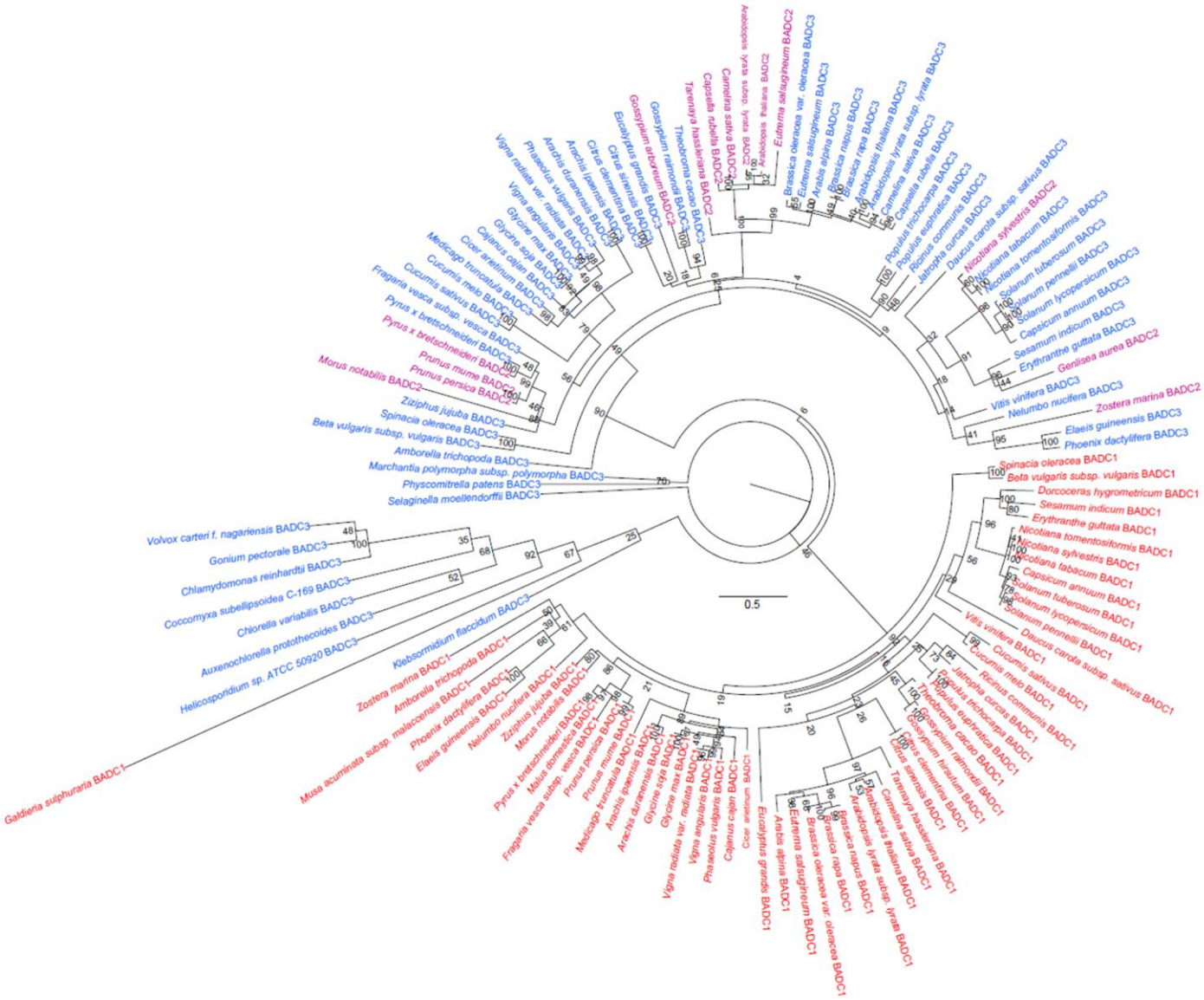
B

Protein Annotation	Protein Accession	No. of replicates identified (n=7)	Average Peptides	Average PSMs
acetyl Co-enzyme a carboxylase biotin carboxylase subunit	AT5G35360	7	14.5	67.6
biotin carboxyl carrier protein 2	AT5G15530	7	2.9	15.1
biotin/lipoyl attachment domain-containing protein	AT3G56130	7	3.7	13.7
Single hybrid motif superfamily protein	AT1G52670	6	2.6	14.4
chloroplastic acetylcoenzyme A carboxylase 1	AT5G16390	6	4.5	15.5
acetyl Co-enzyme a carboxylase carboxyltransferase alpha subunit	AT2G38040	3	4.0	5.7

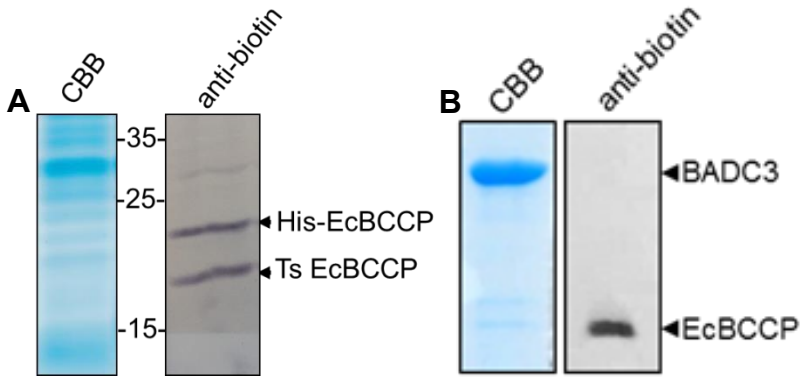
Supplemental Figure 1. Mass spectrometry analysis of hetACCase co-immunoprecipations. Shown are the details of the identification of hetACCase and BADC proteins via mass spectrometry from co-immunoprecipitations performed with anti-α-CT **(A)** or anti-BCCP2 **(B)** antibody. Column descriptions: Protein annotation, the given annotation based on the TAIR10 protein database; Protein accession, accession number assigned to the protein in the TAIR10 protein database; No. of replicates identified, indicates the number of biological replicates in which the specific protein was identified out of seven total; Average peptides, average number of peptides identified (when identified); Average PSMs, average number of peptide spectral matches (when identified).



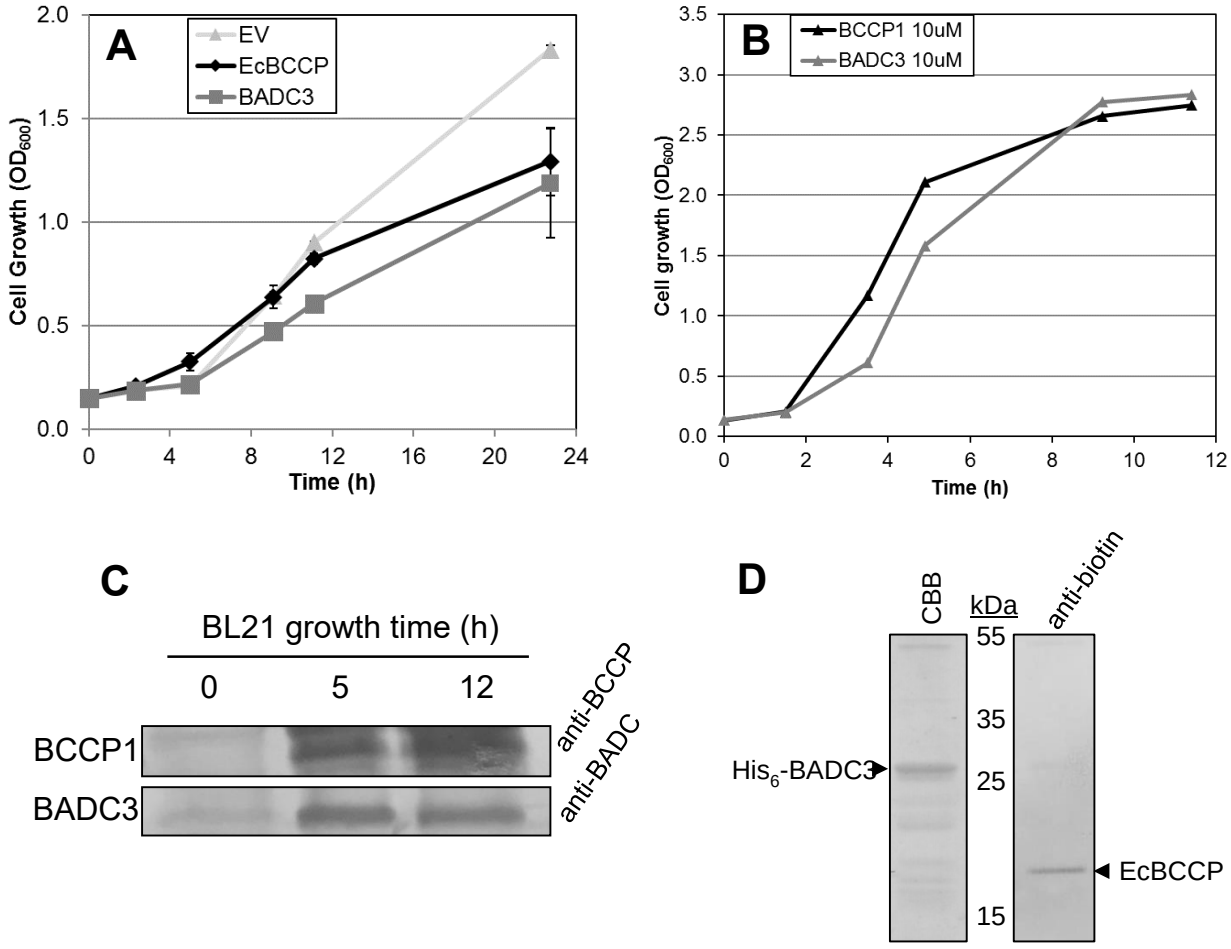
Supplemental Figure 2. Multiple sequence alignment of BADC and BCCP primary sequences. The full primary sequence of *E. coli* (Ec) BCCP and *A. thaliana* (At) BCCP and BADC proteins is shown. Conserved and identical residues are shown in grey and black, respectively.



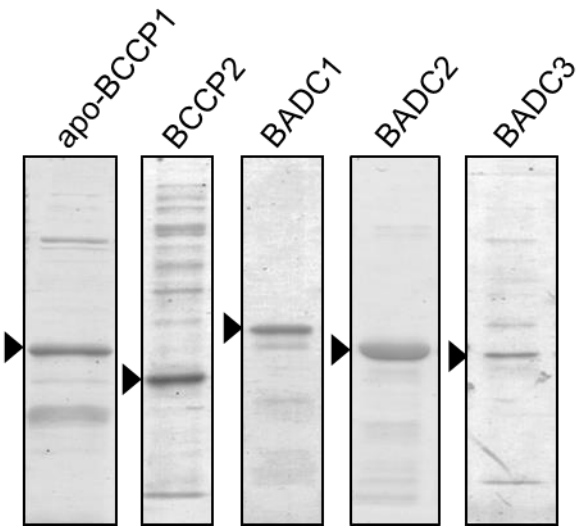
Supplemental Figure 3. A maximum-likelihood phylogenetic tree of BADC full-length proteins. BADC1, BADC2, and BADC3 clades are highlighted in red, purple, and blue, respectively. The confidence support for each branch from 1000 bootstrapping iterations is noted. The unit length of branches represented 0.5 substitutions per amino acid site.



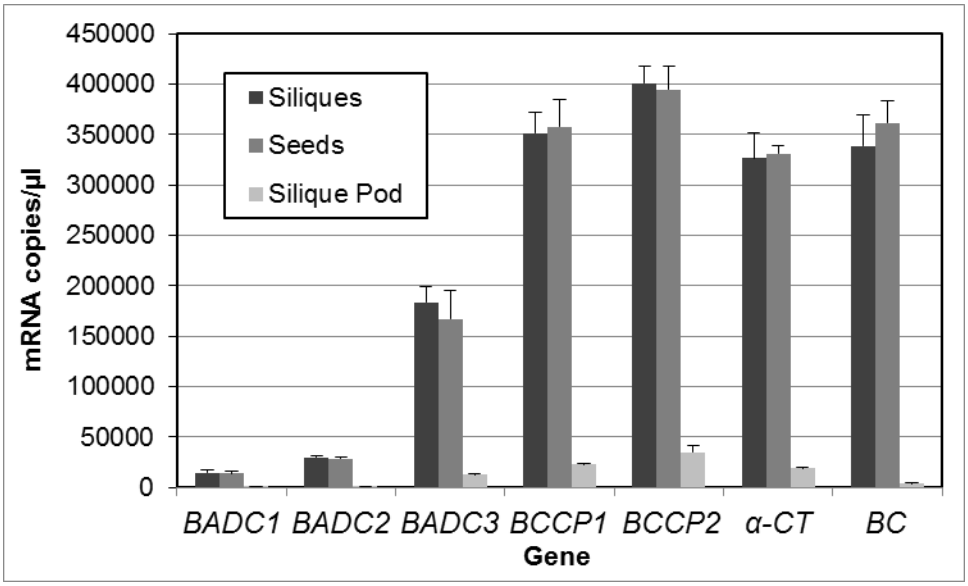
Supplemental Figure 4. Recombinant protein expression in L8 *E. coli* cells. (A) Confirmation of protein expression in L8 *E. coli* cells. Cells were lysed in 6x SDS sample buffer and lysate was resolved by SDS-PAGE. Anti-biotin blot shows the presence of both the temperature sensitive (Ts) BCCP and the introduced His_6-BCCP. **(B)** Coomassie-stained gel (left) and anti-biotin protein blot (right) of Ni^{2+}-NTA purified His_6-BADC3 from *E. coli*. Blot shows the presence of EcBCCP in the purification, suggesting that BADC3 interacts with EcBCCP.



Supplemental Figure 5. *E. coli* growth during BADC3 expression. (A) Growth curve of L8 cells at 30 °C in M63 minimal media. Under these conditions L8 cells are not temperature-sensitive, as exhibited by EV control. Cells expressing BADC3 also show substantial growth, however at a slower rate than cells expressing EcBCCP. Average of three replicates is shown. Error bars denote SD. **(B)** Growth curve of BL21 *E. coli* in M63 minimal media at 37 °C. Recombinant expression of BCCP1 and BADC3 was induced with 10 μM IPTG at T = 0 h. Cells expressing BADC3 are capable of growth, however at a slower rate than the control cells expressing BCCP1. **(C)** Protein blot of BL21 cell extracts showing protein expression. Aliquots of cells expressing BCCP1 or BADC3 were taken at various time points during cell growth. Protein was resolved by SDS-PAGE and blotted with the antibody shown. **(D)** His_6-BADC3 protein was purified from the BL21 cells in **(B)** and resolved by SDS-PAGE. At left, a Coomassie Blue stain of the SDS gel showing the purified BADC3 protein; at right, a protein blot performed using anti-biotin antibody.



Supplemental Figure 6. Purity of recombinant protein purifications for ACCase activity assays. Coomassie-stained SDS gels show the purity of Ni^{2+}-NTA purified recombinant proteins that were used in the ACCase activity assays. Arrows indicate the recombinant protein.



Supplemental Figure 7. hetACCase and BADC gene expression in whole silique, seed, and silique pod/septa. Graph shows the absolute transcript quantitation from each tissue by qPCR. Whole siliques were collected 10 days after flowering and after 6 h light exposure. Prior to RNA extraction, some of these siliques were dissected to isolate developing seeds and silique pod. RNA extracted from these tissues was used in the qPCR analysis. Results shown are the average of four biological replicates, each consisting of tissue from at least 10 siliques. Three technical replicates were performed per biological replicate. Error bars denote standard deviation. Y-axis represents number of mRNA copies per µL cDNA.

A

Name	Accession	Sequence
BCCP1 sense	AT5G16390	5'-CATATGTCAGCTGAAGGAAAGGAGAAAAACTCATTG-3'
BCCP1 anti-sense		5'-GGATCCCTACGGTTGAACCACAAACAGAGGAG-3'
BCCP2 sense	AT5G15530	5'-CATATGGAATTTATGGCTAAAGTCTCTGGTCTT-3'
BCCP2 anti-sense		5'-GGATCCTCAAGGTGCGATGACAAAAAGAG-3'
BADC1 sense	AT3G56130	5'-GAATTCGCTAAGGCCGCTAAATCTTCGAC-3'
BADC1 anti-sense		5'-CTCGAGTCACTGGATGTTGATGTCGTG-3'
BADC2 sense	AT1G52670	5'-CATATGACGACTCTGCGATCTGTGAAAGCT-3'
BADC2 anti-sense		5'-GGATCCTTACTGAAGCTTCTTGATGCCAGGA-3'
BADC3 sense	AT3G15690	5'-CATATGGCTGTCCAAGTGTCTACTGTCCC-3'
BADC3 anti-sense		5'-GGATCCTTACTGAAGCTTCTTGATCCCAGGG-3'
apo-BCCP1 mutant sense	AT5G16390	5'-GCATTGTTGAAGCCATGAGGTTAATGAATGAAATA-3'
apo-BCCP1 mutant antisense		5'-TATTTCATTCATTAACCTCATGGCTTCAACAATGC-3'

B

Name	Predicted target peptide length
BCCP1	82
BCCP2	87
BADC1	56
BADC2	47
BADC3	54

Supplemental Figure 8. Primers used in cloning experiments. (A) Primer sequences used to create constructs for recombinant protein expression and yeast two hybrid experiments. Primers were designed to remove the predicted transit peptide coding sequence. **(B)** Predicted transit peptide length, as calculated by TargetP.

Supplemental Methods

BADC ortholog identification and maximum-likelihood tree construction

Representative splicing forms of BCCP and BADC proteins in *Arabidopsis thaliana* were used to search against KEGG SSDB (Sequence Similarity DataBase) (Kanehisa et al., 2016) for their potential orthologous proteins, respectively. SSDB contains pre-built all-vs-all Smith-Waterman (Smith and Waterman, 1981) alignments among all protein-coding genes in species with the whole genome information. The following criteria were applied to obtain orthologs with high confidence: 1) reciprocal best hits only; 2) Smith-Waterman alignment score greater than 300; 3) fragment sequences (less than 100 amino acid residues) were removed. All predicted ortholog relationships were further confirmed by independent reciprocal BLAST searches. For BCCP orthologs, the search was limited to Eukaryotes belonging to the KEGG ORTHOLOGY group K02160, which is a KEGG-produced grouping of BCCP proteins. Full-length protein sequences of orthologs were retrieved from the KEGG or NCBI database. Biotin/lipoyl attachment domains were extracted using HMMER (3.0) (Eddy, 1998) and Pfam (Finn et al., 2016) annotations (PF00364). Multiple sequence alignments were performed using MUSCLE (3.8.31) (Edgar, 2004) for both full-length and domain sequences. Maximum likelihood phylogenetic tree of BADC full-length sequences was constructed with 1000 rapid bootstrapping iterations implemented in RAxML (8.2.4) (Stamatakis, 2014) and CIPRES Science Gateway. We applied CAT for evolutionary rate heterogeneity approximation and general time reversible (GTR) amino acid substitution model (using the “RAxML -m PROTCATGTR” option).

Growth assays in L8 strain *E. coli* cells

The temperature-sensitive (Ts) L8 strain *E. coli* was obtained from the Coli Genetic Stock Center (Yale, New Haven, CT). The *accb* (*BCCP*) gene was sequenced and found to contain point mutations that changed Ile83 to Val and Gly133 to Ser. Chemically competent L8 cells were transformed with the vectors described in the text using the heat shock method. Cells were then plated on Kan, Amp, or Kan+Amp plates to select for transformants containing pET28a, pET11a or both, respectively. Plates were incubated at 30°C for two days. PCR positive colonies were added to 2 mL LB medium (1% tryptone (w/v), 0.5% NaCl (w/v), 0.5% yeast extract (w/v)) culture and grown overnight at 30°C, 250 rpm. Glycerol stocks were made from these cultures and stored at -80°C. To prevent loss of the Ts phenotype, all experiments were performed using the original glycerol stock for each transformant. Experiments were begun by inoculating 5 mL LB medium with a stab of glycerol stock and incubating at 30°C overnight, 250 rpm. Then overnight cultures were centrifuged at 3,000 *g* and resuspended in 5 mL sterile deionized water. Cultures were centrifuged again and resuspended in M63 minimal media to make OD_{600} = 3.75. Then a 200 µL cell suspension aliquot was added to 7 mL M63 media (100 mM KH_2PO_4-KOH pH 7.0, 15 mM $(NH_4)_2SO_4$, 2 µM $FeSO_4$-7 H_2O, 1.5 mM $MgSO_4$, 0.2% glycerol, 0.2% glucose, 0.2% casamino acids, 0.5 µg/mL thiamine, 1 µM IPTG) plus antibiotics in sterile culture tubes. Cultures contained Kan, and Amp if necessary, at 50 µg/mL. Cultures were then incubated at 37 °C, 250 rpm. Three separate cultures tubes were grown per transformant per experiment. At the time points shown in Fig. 4, 500 µL aliquots were removed from the culture tubes and used for absorbance analysis.

Construct design for BADC1 RNAi silencing in *Arabidopsis thaliana*

Inverse repeats of the targeting sequence for At-*BADC1* were incorporated on opposite sides of the Rice Waxy-A intron in the pMU103 vector (Flores et al., 2008) using restriction enzymes. The 5' repeat was inserted using the restriction enzymes AscI and AvrII. The 3' repeat was inserted using the restriction enzymes SacI and XmaI. Expression of the mRNA in transformed *A. thaliana* plants was driven by the seed-specific glycinin promoter.

Mass spectrometry data search parameters

Search parameter settings of SEQUEST were: static modification of carboxyamidomethylation, variable modification of methionine-oxidation, two missed tryptic cleavage sites, absolute threshold: 1000, minimum ion count: 10, mass range: 650-3500, and a parent and fragment ion tolerance of 1 Da and 1000 ppm, respectively. Search result files were loaded into Proteome Discoverer 1.3 (Thermo Fisher Scientific). Identified peptides were filtered to <1% false discovery rate using the following criteria: 10 ppm peptide mass deviation, 'Xcorr versus charge state', protein grouping enabled, and 2 peptide minimum.

ACCase activity assays

HetACCase activity was directly monitored in ten-d-old siliques through the incorporation of $^{14}CO_2$ into acid-stable products. *A. thaliana* WT Col-0 plants were grown in long day conditions (16 h light, 50% humidity, 100 µmol m^{-2} s^{-1}) and 10 d old siliques were harvested after six hours of light exposure. In each trial, four biological replicates of three siliques or leaves were assayed. Tissue was collected in 1.5 mL Eppendorf tubes and pulverized immediately in 150 µL ice-cold homogenization buffer (20 mM TES, pH 7.5, 10% glycerol, 5 mM EDTA, 2 mM DTT, 2 mM benzamidine, 2 mM PMSF, 1% Triton X-100) using a plastic mortar fit for a 1.5 mL tube. After 30 s pulverizing, lysates were centrifuged at 10 k *g* for 15 s, 22 °C. Prior to tissue harvest, 1.5 mL Eppendorf tubes containing 30 uL reaction mixture were made. The reaction mixture consisted of 100 mM Tricine pH 8.2, 100 mM KCl, 4 mM $MgCl_2$, 1 mM ATP, 5 mM $NaH^{14}CO_3$ (~4000 dpm), 0.5 mM acetyl-CoA, 10 µM haloxyfop, and/or 10 µM of the specified recombinant protein. Immediately after lysate centrifugation, 20 µL of the supernatant was added to the tubes containing reaction mixture, bringing the total volume to 50 µL, and incubated at 25 °C for 20 min. Protein content of lysates was quantified after ACCase assay initiation via Bradford assay. In this way, assays were begun within 5 min of tissue harvest to minimize loss of hetACCase activity due to lysis. Assays were quenched with 50 µL concentrated HCl, spotted onto 1 cm^2 Whatman filter paper in a scintillation vial and heated in an 80 °C oven to remove excess $^{14}CO_2$. Then 3 mL scintillation cocktail was added to the vial and the radioactivity was measured via scintillation counting. Minus acetyl-CoA controls were subtracted from plus acetyl-CoA trials to determine the true hetACCase activity.

Antibody production

A. thaliana BADC1 antibody was raised against recombinant BADC1 protein in rabbits (Cocalico Biologicals, Reamstown, PA). Protein was expressed in BL21 *E. coli* cells using

the pET28a clone described above and purified by Ni^{2+}-NTA affinity chromatography. Antibody from the fourth bleed was used in this study.

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