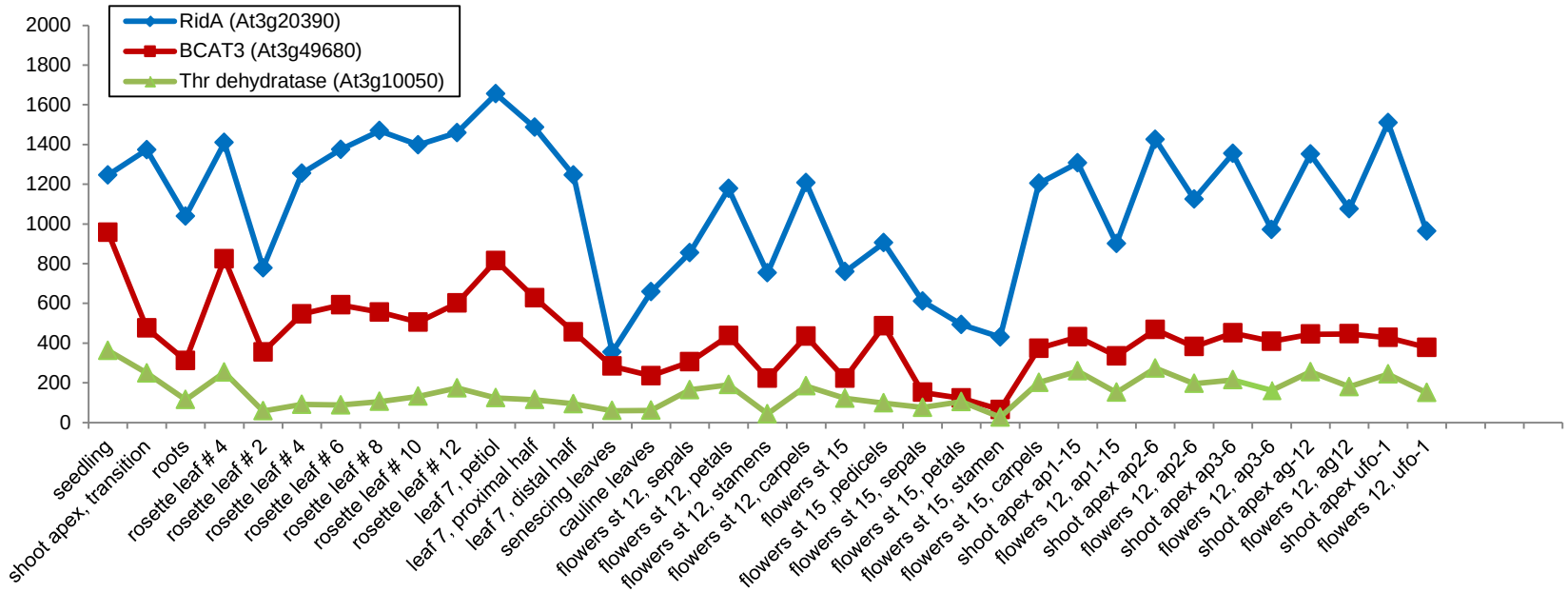
Supplemental Figure 1. Transcriptomic evidence connecting *Arabidopsis* and maize RidA with BCAT3 and Thr dehydratase.

(A) Expression profiles of *Arabidopsis* RidA (At3g20390), BCAT3 (At3g49680), and Thr dehydratase OMR1 (At3g10050) in various tissues and developmental stages. Expression data were exported from the CSB.DB *Arabidopsis* database.

(B) Expression profiles of maize RidA (GRMZM2G117642), BCAT3 homologs (GRMZM2G071208, GRMZM2G055899), and Thr dehydratase homologs (GRMZM2G155323, GRMZM2G569855).

(C) ATTED co-expressed gene network linking *Arabidopsis* RidA, BCAT3, Thr dehydratase, and other genes of branched-chain amino acid biosynthesis (marked with a red dot). Black lines are co-expression links. Protein-protein interactions (orange lines) are also shown. The network was drawn using RidA, BCAT3, and Thr dehydratase as query genes.

A



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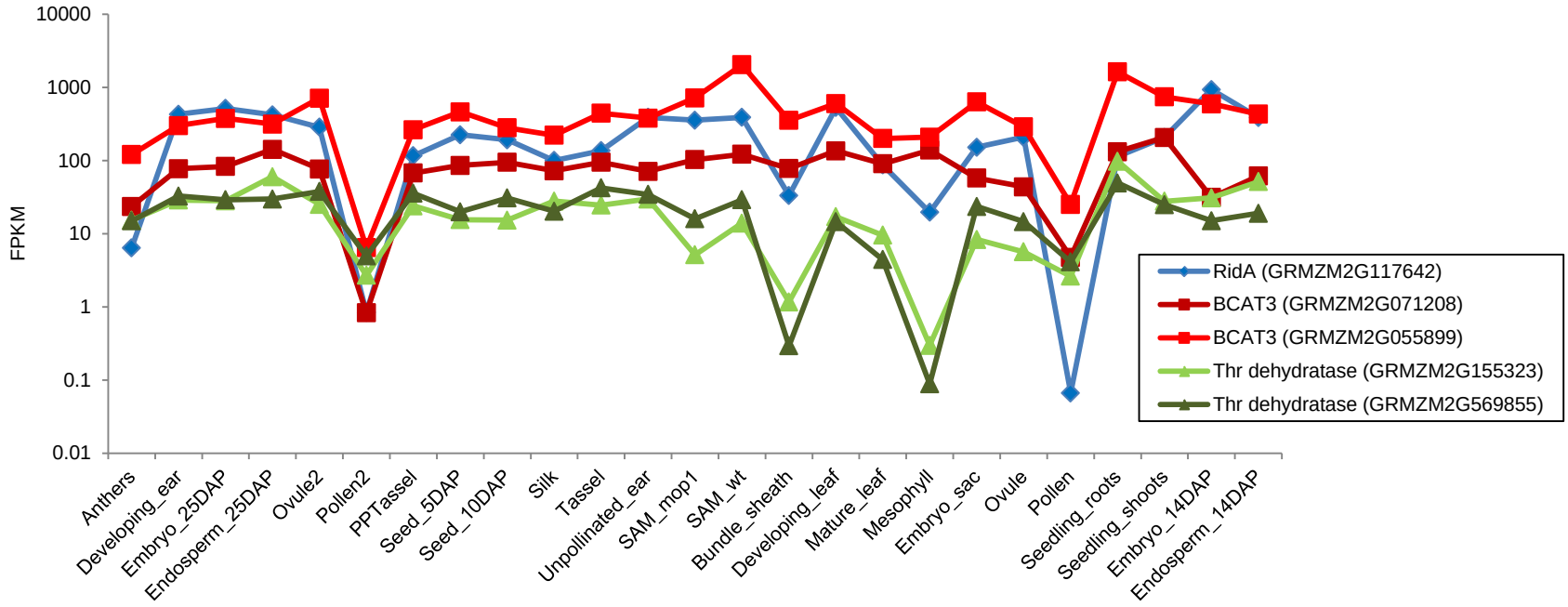
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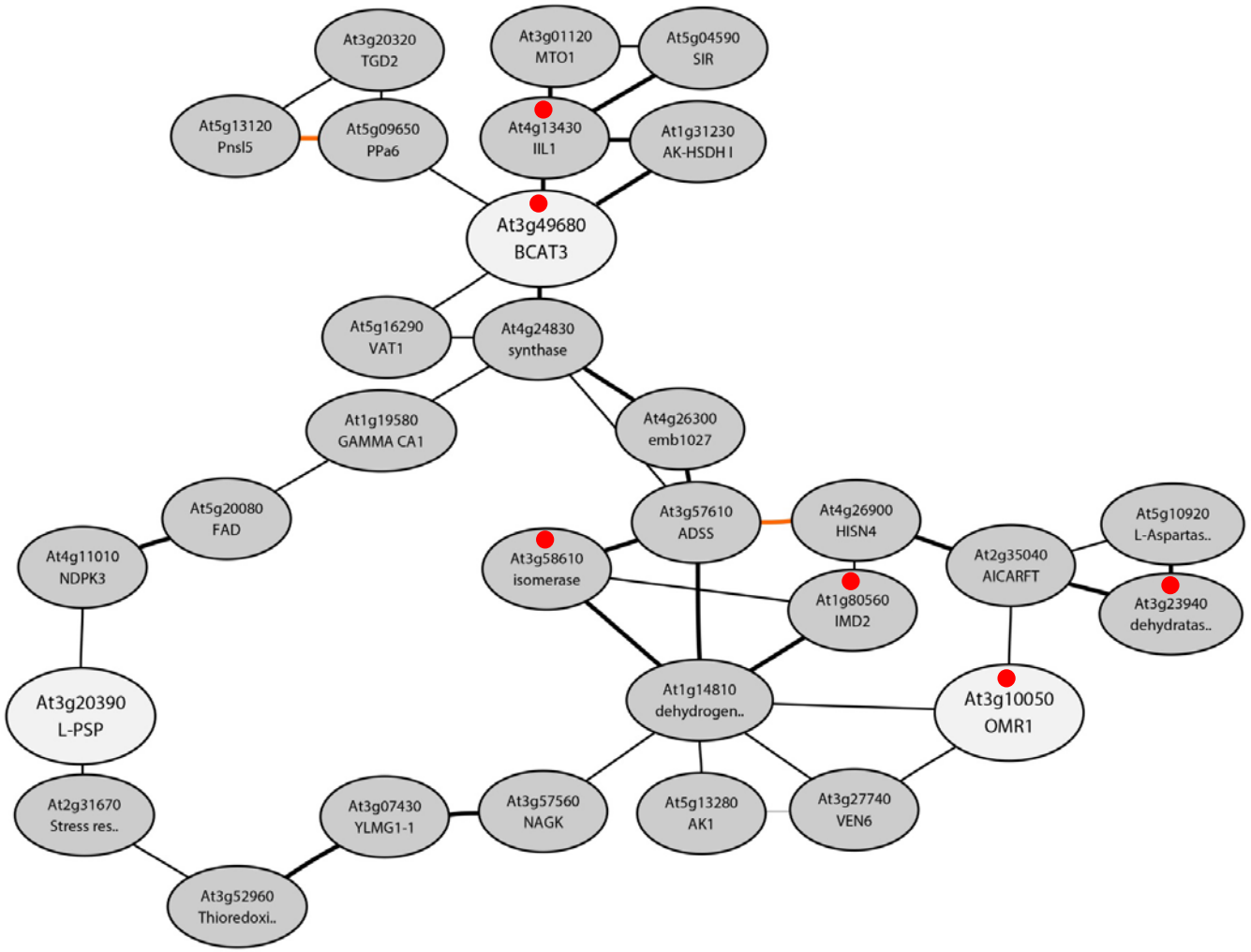
Ovule2
Pollen2
PPTassel
Seed_5DAP
Seed_10DAP
Silk
Tassel
Unpollinated_ear
SAM_mop1
SAM_wt
Bundle_sheath
Developing_leaf
Mature_leaf
Mesophyll
Embryo_sac
Ovule
Pollen
Seedling_roots
Seedling_shoots
Embryo_14DAP
Endosperm_14DAP
RidA (GRMZM2G117642)
BCAT3 (GRMZM2G071208)
BCAT3 (GRMZM2G055899)
Thr dehydratase (GRMZM2G155323)
Thr dehydratase (GRMZM2G569855)

C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IIL1
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AK-HSDH I
At3g49680
BCAT3
At5g16290
VAT1
At4g24830
synthase
At1g19580
GAMMA CA1
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L-Aspartas..
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isomerase
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IMD2
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dehydrogen..
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Stress res..
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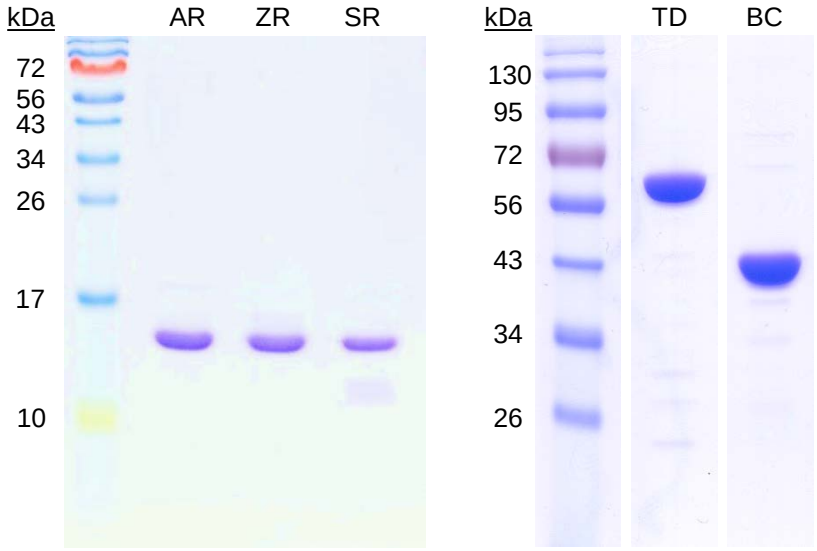
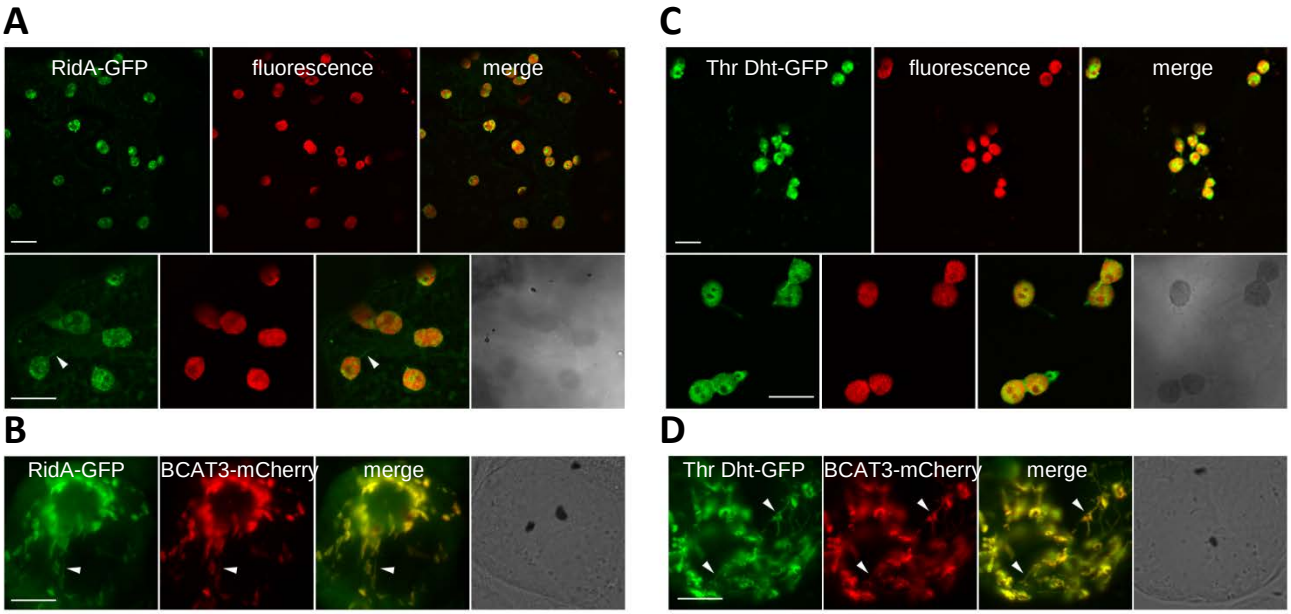


Supplemental Figure 2. Recombinant proteins used in this study were purified to near homogeneity. Two to five µg of Ni^{2+}-affinity purified *Arabidopsis* RidA (AR), maize RidA (ZR), *Salmonella* RidA (SR), *Arabidopsis* Thr dehydratase (TD), and *Arabidopsis* BCAT3 (BC) were analyzed by SDS-PAGE (RidA, 15% gel; Thr dehydratase and BCAT3, 10% gel) with Coomassie staining. Each protein preparation was judged to be ≥90% pure.

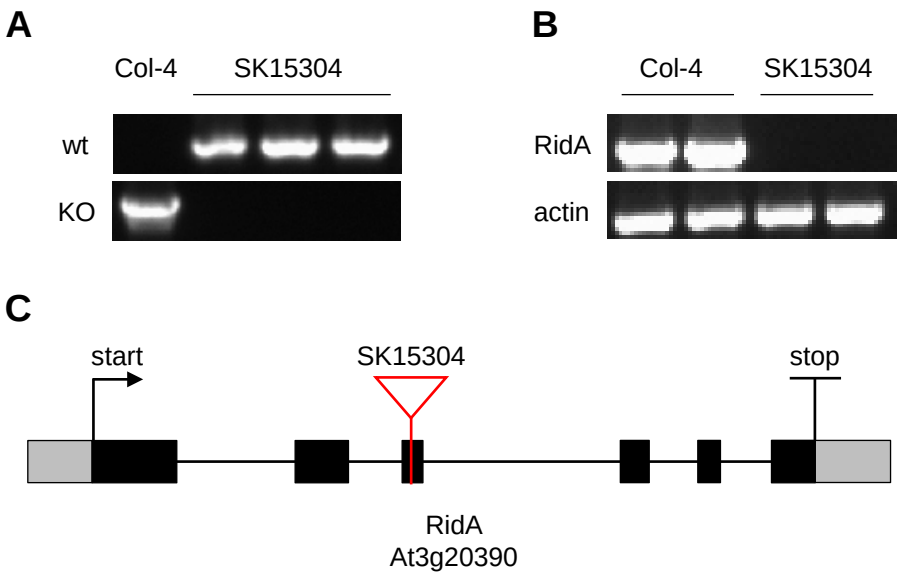


Supplemental Figure 3. *Arabidopsis* RidA and Thr dehydratase GFP fusions are targeted to chloroplasts in *N. benthamiana* leaf mesophyll cells and to leucoplasts in *N. tabacum* BY-2 cells.

(A) and (**C)** Representative micrographs of *N. benthamiana* mesophyll cells stably-expressing (via *Agrobacterium* transformation) *Arabidopsis* RidA-GFP or Thr dehydratase (Thr Dht)-GFP. GFP and chlorophyll fluorescence (as indicated by panel labels) were observed by confocal microscopy. The bottom row of images in A and C shows a portion of a RidA-GFP- or Thr Dht-GFP-transformed mesophyll cell at higher magnification. Arrowheads in A (bottom row) indicate examples of RidA-GFP localized to stromules. Shown also in the bottom row in A and C is the corresponding DIC micrograph. Bars = 10 μm.
(B) and **(D)** Representative micrographs of dark-grown *N. tabacum* BY-2 cells transiently co-expressing (via biolistic bombardment) *Arabidopsis* RidA-GFP or Thr Dht-GFP and BCAT3-mCherry. GFP and mCherry fluorescence (as indicated by panel labels) were observed by epifluorescence microscopy. Note that both sets of fusion proteins co-localized exclusively in plastids (i.e., leucoplasts), including stromules (refer to arrowheads in B and D), which are commonly observed in BY-2 cells (Simpson et al., 2008; Schattat et al., 2012). DIC micrographs are shown for each set of images. Bars = 10 μm.

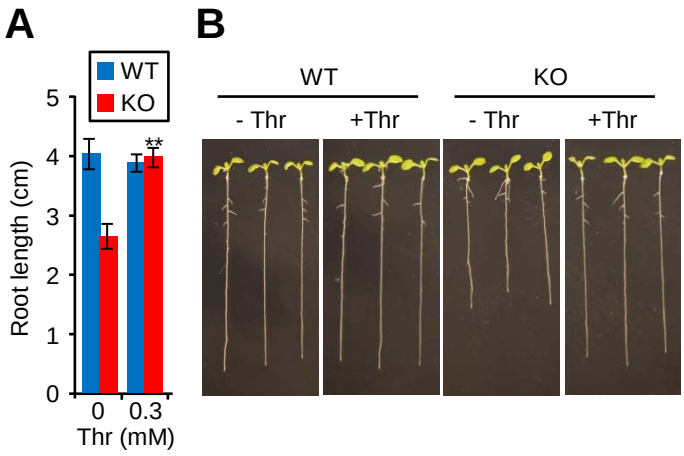
Schattat, M.H., Griffiths, S., Mathur, N., Barton, K., Wozny, M.R., Dunn, N., Greenwood, J.S., and Mathur, J. (2012). Differential coloring reveals that plastids do not form networks for exchanging macromolecules. Plant Cell **24:** 1465–1477.

Simpson, J.P., Di Leo, R., Dhanoa, P.K., Allan, W.L., Makhmoudova, A., Clark, S.M., Hoover, G.J., Mullen, R.T., and Shelp, B.J. (2008). Identification and characterization of a plastid-localized *Arabidopsis* glyoxylate reductase isoform: comparison with a cytosolic isoform and implications for cellular redox homeostasis and aldehyde detoxification. J. Exp. Bot. **59:** 2545–2554.



Supplemental Figure 4. Confirmation That the *Arabidopsis RidA* T-DNA Insertion Line Is a Knockout.

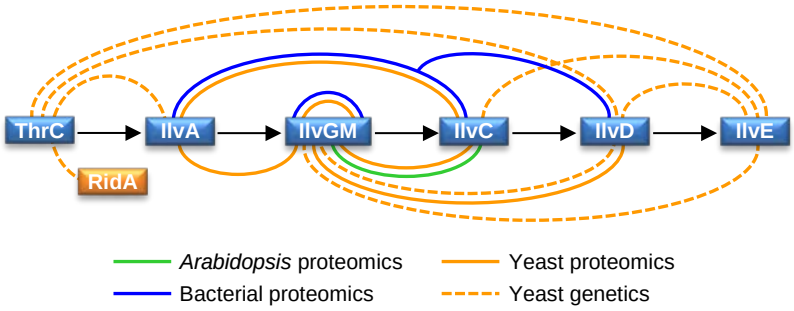
(A) Genotyping the SK15304 line. Genomic DNA from SK15304 plants or the corresponding wild-type, Columbia-4 (Col-4) was used as template for PCR reactions with wild-type allele primers (wt, primers 37 and 38) or T-DNA insertion allele primers (KO, primers 38 and 39). Amplicons were analyzed by agarose gel electrophoresis.
(B) Testing for the presence of the *RidA* transcript. Total RNA was used for first-strand cDNA synthesis, followed by PCR with primers designed to amplify the coding sequence of the *RidA* transcript (primers 40 and 41) or a fragment of the actin-7 transcript (primers 42 and 43). Amplicons were analyzed by agarose gel electrophoresis.
(C) T-DNA insertion site in the *RidA* gene. The PCR amplicon obtained with the T-DNA insertion allele primers in A above was cloned and sequenced. The gene model is drawn to scale with exons as black boxes, introns as black lines, and 5′- and 3′-UTRs as gray boxes. The T-DNA insertion site in exon 3 is indicated in red. The start and stop codons are indicated.



Supplemental Figure 5. Restoration by Thr of Root Growth of the *Arabidopsis* RidA Knockout.

(**A**) Root growth of Col-0 (WT) and the RidA knockout (KO) with or without 0.3 mM Thr after seven days on half-strength MS medium containing 1% sucrose. Data are means and SE for 6-12 replicate plants. For each strain, significant differences (Student's *t* test) from growth without Thr are asterisked: **, P<0.01.

(**B**) Images of WT and KO plants grown for seven days with or without 0.3 mM Thr.



Supplemental Figure 6. Evidence for protein complex formation among enzymes of branched chain amino acid biosynthesis.

The enzymes are arranged in their order in the pathway and designated by their names in *E. coli*. IlvA, Thr dehydratase; IlvGM (representing also the homologs IlvBN, IlvIH), acetolactate synthase; IlvC, ketol-acid reductoisomerase; IlvD, dihydroxy-acid dehydratase; IlvE, branched-chain aminotransferase. Protein-protein interactions detected by proteomics approaches in *Saccharomyces cerevisiae*, various bacteria, or *Arabidopsis* are represented by solid arcs. Protein-protein interactions in *S. cerevisiae* that are inferred genetically (from synthetic lethality, phenotypic enhancement, or phenotypic suppression assays) are represented by dashed arcs. Intersecting arcs denote three-way interactions. No interactions were detected between the enzymes shown and those specific to Leu biosynthesis (LeuA, LeuB, LeuC, and LeuD). Data were collated from ATTED-II, STRING, and BioGRID databases. For simplicity, the two acetolactate synthase subunits (IvG, IlvM) are treated as one unit.

Supplemental Table 1. RidA Family Proteins Used as Query Sequences to Search for Homologs in *Arabidopsis* and Maize

Species	Gene name / Locus tag	GenBank accession
Salmonella enterica	*ridA* (*yjgF*)	AF095578.1
Lawsonia intracellularis	LIC078	NC_008014.1
Roseiflexus castenholzii	Rcas_2625	CP000804.1
Polaribacter sp. MED152	MED152_12764	CP004349.1
Burkholderia xenovorans	Bxe_A1831	CP000270.1
Saccharomyces cerevisiae	*HMF1* / YER057C	NM_001178948.3
Meyerozyma guilliermondii	PGUG_03922	XM_001484491.1
Debaryomyces hansenii	DEHA2G19558g	XM_462393.1
Kluyveromyces lactis	KLLA0B14817g	XM_452191.1
Kluyveromyces lactis	KLLA0E04005g	XM_454124.1
Botryotinia fuckeliana	BC1G_10083	XM_001551293.1

Supplemental Table 2. Predicted Localization of *Arabidopsis* and Maize RidA, Thr dehydratase, and BCAT Proteins

Targeting of full length *Arabidopsis* (At) and maize (GRMZM) RidA, Thr dehydratase (Thr DHT), and BCAT sequences was predicted using TargetP (http://www.cbs.dtu.dk/services/TargetP/), Predotar (http://urgi.versailles.inra.fr/predotar/predotar.html) and WoLF PSORT (http://wolfpsort.seq.cbrc.jp/). Predicted locations: P, plastids; M, mitochondria. Weak predictions are in parentheses; dashes indicate that the algorithm predicted no organellar targeting. The N-terminal region of the GRMZM2G569855 protein, which was missing from the sequence at MaizeSequence.org, was deduced from ESTs.

Protein	TargetP	Predotar	WoLF PSORT
At3g20390 (RidA)	P	P	P (M)
At3g10050 (Thr DHT)	P	(P)	P
At3g49680 (BCAT3)	P	P	P (M)
GRMZM2G117642 (RidA)	P	P	P
GRMZM2G155323 (Thr DHT)	P	P	P
GRMZM2G569855 (Thr DHT)	P	P	P
GRMZM2G071208 (BCAT)	P	--	P (M)
GRMZM2G055899 (BCAT)	M (P)	--	P (M)

Supplemental Table 3. Targeted Metabolomics Analysis of Relative Levels of Thr, Ser, Branched-chain Amino Acids, and 2-Oxo Acids in RidA Knockout and Wild Type *Arabidopsis* Leaves

Metabolite	-Fold change (Knockout/wild type)	P value
Thr	1.1	0.45
2-Oxobutanoate	1.3	0.001***
Ser	1.0	0.75
Pyruvate	1.0	0.88
Ile	1.1	0.34
3-Methyl-2-oxopentanoate	1.0	0.44
Leu	1.1	0.37
4-Methyl-2-oxopentanoate	1.0	0.88
Val	1.0	0.78
2-Oxoisovalerate	1.0	0.88

Leaves were from three week-old plants. Data are means of six biological replicates, normalized to C10 (oxo acids) or C14 (amino acids) fatty acid methyl esters.

Supplemental Table 4. Oligonucleotide Primers Used in This Study

Primer	Name	Sequence
	Functional complementation	
1	At RidA NcoI F	catgccatggctgtctctgcttcttctgtga
2	At RidA XbaI R	ctagtctagactagagtgttgcaatacattcaa
3	Zm RidA NcoI F	catgccatggcgacctctgccaccgctg
4	Zmt RidA XbaI R	ctagtctagattagagagcagcgatgcact
5	At R165A F	cccagctccttctccagcagcatcgacgtatcaagttgc
6	At R165A R	gcaacttgatacgtcgatgctgctggagaaggagctggg
7	Zm R157A F	cccggtgccagcacctgcagcatcaacctaccaagtggcg
8	Zm R157A R	cgccacttggtaggttgatgctgcaggtgctggcaccggg
	Protein expression	
9	At RidA NcoI F	actgacccatggctgtctctgcttct
10	At RidA XhoI R	gtcagtctcgaggagtgttgcaatacattcaatct
11	Zm RidA NcoI F	actgacccatggcgacctctgccac
12	Zm RidA XhoI R	gtcagtctcgaggagagcagcgatgcactcaa
13	Se RidA NcoI F	catgccatggctatgagcaaaactattgcgacgg
14	Se RidA XhoI R	tccgctcgaggcgacgaacagcgatcgcttcaatc
15	Thr DHT NcoI F	catgccatggctcttcctttaccacgtcttaaggtc
16	Thr DHT NotI R	ataagaatgcggccgcgtgcatcagaagcttaaaatagtcg
17	BCAT3 NdeI F	gggaattccatatggctgtttcgtccaattcttccagtacg
18	BCAT3 XhoI R	ccgctcgagttaactaagattcacagtccatttc
	Chloroplast import	
19	At RidA-FL EcoRI F	ggaattcaccatgacttggtcggttttcagatcc
20	At RidA-FL HindIII R	gcccaagcttctagagtgttgcaatacattcaatc
21	At RidA-T EcoRI F	ggaattcaccatggctgtctctgcttcttctg
22	Zm RidA-FL EcoRI F	ggaattcaccatggcgtggagcgccgccgccgtc
23	Zm RidA-FL HindIII R	gcccaagcttttagagagcagcgatgcactcaatc
24	Zm RidA-T EcoRI F	ggaattcaccatggcgacctctgccaccgc
25	Thr DHT-FL XmaI F	tccccccgggaccatgaattccgttcagcttccgacg
26	Thr DHT-FL XbaI R	gctctagatcagtgcatcagaagcttaaaatag
27	Thr DHT-T EcoRI F	ggaattcaccatggagtatttgacgaatatac
	GFP fusions	
28	At RidA XmaI F	tccccccggggaaggagatagaaccatgacttggtcggttttcagatcc
29	At RidA NheI R	ttctagctagcgagtgttgcaatacattcaatctcg
30	Thr DHT XmaI F	tccccccggggaaggagatagaaccatgaattccgttcagcttccgacg
31	Thr DHT NheI R	ttctagctagcgtgcatcagaagcttaaaatagtcg
32	BCAT3 XmaI F	tccccccggggaaggagatagaaccatggagagagcagcaattctcc
33	BCAT3 NheI R	ttctagctagcactaagattcacagtccatttcatg
34	At RidA Gw F	ggggacaagtttgtacaaaaaagcaggcttcgaaggagatagaaccatgacttggtcggttttcagatcc
35	Thr DHT Gw F	ggggacaagtttgtacaaaaaagcaggcttcgaaggagatagaaccatgaattccgttcagcttccgacg
36	mGFP Gw R	ggggaccactttgtacaagaaagctgggtcttacttgtacagctcgtccatgccg
	Arabidopsis knockouts	
37	SK15304 LP	ggcaaagctgcaacttgatac
38	SK15304 RP	aattttctccaatttggaccg
39	SK LB1	ttctcatctaagcccccatttgg
40	RidA F	atgacttggtcggttttcagatcc
41	RidA R	ctagagtgttgcaatacattcaatc
42	actin-7 F	atggccgatggtgaggat
43	actin-7 R	gtctcaaacatgatctgagtc