

1 **Supplemental Figure S1.** Variant ACC2 splice site and altered transcripts in the Spro-2 accession.

2

	EXON 29 -----	Intron 29 -----	Exon 30 -----
3			
4			
5	Col-0 Genomic	AACGTGACTGGCCGTACCTGCACTGTCCAT	<u>gt</u> aaattcagctt (62x) ttcatgtttc <u>ag</u> ATATACCGAGAAGTG
6	Spro-2 Genomic:	AAC <u>GT</u> GACTGGCCGTACCTGCACT <u>GT</u> CCAT	<u>tt</u> aaattcagctt (62x) ttcatgtttc <u>ag</u> ATATACCGAGAAGTG
7	Transcript #1	AACGTGACTGGCCGTACCTGCACTGTCCAT	<u>tt</u> aaattcagctt (62x) ttcatgtttc <u>ag</u> ATATACCGAGAAGTG
8	Transcript #2	AACGTGACTGGCCGTACCT <u>A</u> CACTGTCCAT	<u>tt</u> aaattcagctt (62x) ttcatgtttc <u>ag</u> ATATACCGAGAAGTG
9	Transcript #3	AAC <u>GT</u> GACTGGCCGTACCTGCACTGTCCAT	<u>tt</u> aaattcagctt (62x) ttcatgtttc <u>ag</u> ATATACCGAGAAGTG
10	Transcript #4	AACGTGACTGGCCGTACCTGCACT <u>GT</u> CCAT	<u>tt</u> aaattcagctt (62x) ttcatgtttc <u>ag</u> ATATACCGAGAAGTG

- 11
- 12 **Col-0 Genomic** Intron 29 (87 bp) contains normal splice donor (gt) and acceptor (ag) sites. Some nucleotides (x) not specified, for clarity.
- 13 **Spro-2 Genomic** Intron 29 splice donor site changed from “gt” to “tt” (highlighted with gray background). Alternative sites noted in blue.
- 14 **Transcript #1 (2/9)** Intron 29 retained in a defective transcript (green) encoding a truncated protein (1567 residues; final 6 residues altered).
- 15 **Transcript #2 (2/9)** Same as transcript #1 except for an unexplained G>A mutation (highlighted) and C1558Y variant in the truncated protein.
- 16 **Transcript #3 (4/9)** Intron 29 removed (red), along with 27 nucleotides from exon 29, to encode a defective protein missing 9 residues (1553-61).
- 17 **Transcript #4 (1/9)** Intron 29 removed (red), along with 6 nucleotides from exon 29, to encode a defective protein missing 2 residues (1560-61).

18

19 Other variant transcripts may be present, but were not included among the nine cloned RT-PCR products specific to ACC2 that were evaluated.

20 **Supplemental Figure S2.** Short ACC2 deletion and altered transcripts in the Ip-Ber-0 accession.

21

	EXON 17	Intron 17	-----	EXON 18	-----
24 Col-0 Genomic	XXXXXXXX	<u>gt</u> xxx (72x)	gtcaat	tttggcttg	ttc <u>ag</u> AATGATCATGATCCTTCGAAGTTGATGGCTGAGACACCAT (58X) XXXAGXXXXXXXX
25 Ip-Ber-0 Genomic	XXXXXXXX	<u>gt</u> xxx (72x)	gtcaa	-----	-----TGATCCTTCGA <u>AG</u> TTGATGGCTGAGACACCAT (58X) XXX <u>AG</u> XXXXXXXX
26 Transcript #1	XXXXXXXX	<u>gt</u> xxx (72x)	gtcaa	-----	-----TGATCCTTCGAAGTTGATGGCTGAGACACCAT (58X) XXXAGXXXXXXXX
27 Transcript #2	XXXXXXXX	<u>gt</u> xxx (72x)	gtcaa	-----	-----TGATCCTTCGA <u>AG</u> TTGATGGCTGAGACACCAT (58X) XXXAGXXXXXXXX
28 Transcript #3	XXXXXXXX	<u>gt</u> xxx (72x)	gtcaa	-----	-----TGATCCTTCGAAGTTGATGGCTGAGACACCAT (58X) XXX <u>AG</u> XXXXXXXX

30 **Col-0 Genomic** Intron 17 (97 bp; lower case) contains normal splice donor (gt) and acceptor (ag) sites. Some nucleotides (X) not specified, for clarity

31 **Ip-Ber-0 Genomic** Confirmed deletion (**red dash**), not clearly reflected in 1001 Genomes data, removes final 15 bp of intron 17 and first 8 bp of exon 18.

32 **Transcript #1 (5/8)** Intron 17 retained and included in a defective transcript (**green**) encoding a truncated protein (805 residues; final 32 residues altered).

33 **Transcript #2 (2/8)** Intron 17 removed (**red**), along with 21 nucleotides from exon 18, to encode a defective protein missing 7 residues (774-780).

34 **Transcript #3 (1/8)** Intron 17 removed, along with 103 nucleotides from exon 18, to encode a truncated protein (777 residues; final 4 residues altered).

36 Other variant transcripts may be present, but were not included among the eight cloned RT-PCR products evaluated. The 23 bp deletion is not accurately
 37 reflected in 1001 Genomes data, which fills in the missing nucleotides with the adjacent, downstream sequence.

323 **Sixth row:** Protein domains; 1, Transit peptide; 2, Biotin carboxylase; 3, Biotin carboxyl carrier protein; 4, Central domain; 5,
324 Carboxyltransferase, beta subunit; 6, Carboxyltransferase, alpha subunit; *, Biotin binding site within the BCCP domain.

325 **Supplemental Table S1.** Seedling responses of 20 accessions with the ACC2 variants I404K and T1902K.

326

Accession	ABRC Seed Stock	Genetic Screen	Variant Confirmation ^a	Spectinomycin Response		
				Category	Score	Seedlings
Knox-18	CS76530	Forward	A	Hypersensitive	1.1	80
RRS-10	CS76592	Forward	A	Sensitive	1.3	81
Gifu-2	CS76494	Forward	B	Sensitive	1.3	78
Tul-0	CS76618	Forward	A	Sensitive	1.7	81
Tol-0	CS76614	Forward	A	Sensitive	2.0	73
Pna-10	CS76574	Forward	C	Sensitive	1.4	112
Buckhorn Pass	CS76733	Reverse	D	Sensitive	1.7	29
Dem-4	CS76794	Reverse	D	Sensitive	1.7	28
Gre-0	CS76497	Reverse	D	Sensitive	1.4	48
MIC-31	CS77082	Reverse	D	Sensitive	2.1	49
MNF-Jac-12	CS77097	Reverse	D	Sensitive	2.5	33
MNF-Pot-21	CS77099	Reverse	D	Hypersensitive	1.4	50
MNF-Pot-75	CS77100	Reverse	D	Sensitive	2.2	32
Mdn-1	CS77077	Reverse	E	Sensitive	2.9	43
Mv-0	CS76556	Reverse	D	Hypersensitive	1.1	56
NC-6	CS77124	Reverse	D	Sensitive	1.6	54
PT2.21	CS77191	Reverse	D	Sensitive	1.5	55
Rmx-A02	CS76589	Reverse	D	Sensitive	2.1	36
Rmx-A180	CS77218	Reverse	D	Sensitive	1.8	42
SLSP-31	CS77254	Reverse	D	Sensitive	1.4	53

^a A, Predicted variants confirmed; B, Both variants confirmed, I404K not predicted; C, Both confirmed, neither predicted; D, Not tested; E, Both confirmed, along with E1567K (not predicted).

Variant ^a	Conservation (%) ^b	Protein Domain ^c	1001 Genomes Accessions with Predicted Variant	Accessions Evaluated on Spectinomycin ^d	Variant Confirmed	Seedlings Classified	Spectinomycin Response	
							Category	Score
G135E	95.7	(BC)	0	Sav-0 ^e	Yes	275	Hypersensitive	1.2
F363L	99.3	BC	5	Sei-0	Yes	56	Intermediate	6.9
V376A	100.0	BC	12	Col-0	Yes	287	Intermediate	5.6
I404K	94.8	BC	20	Knox-18 Group	Yes	Table IV	Hypersensitive; Sensitive	Table IV
Y443C	94.0	BC	1	Etna-2	Yes	111	Sensitive	1.9
L474F	94.5	BC	1	Chi-0	Yes	75	Intermediate	5.1
P475L	99.7	BC	1	Lm-2	Yes	70	Tolerant	8.3
Q478K	97.6	BC	28	Multiple ^{d,f}	Assumed	126	Intermediate	6.0
				Uod-1	Yes	78	Tolerant	8.5
R494G	99.9	BC	1	Ip-Pal-0	Yes	51	Intermediate	6.1
F520L	94.0	BC	2	In-0	Yes	84	Intermediate	4.8
T538A	99.9	BC	1	Ip-Tor-1	Yes	46	Low Intermediate	4.1
V618I	90.9	BC	9	Ip-Cum-1 Ped-0	Yes	129 56	Sensitive	2.3 3.1
				Ip-Gua-1 Ip-Hom-4	Yes	81 94	Intermediate	6.3 4.5
N725S	96.9		44	Multiple ^{d,g}	Assumed	496	Intermediate	5.8
				Pog-0	Yes	60	Tolerant	8.6
G739E	95.2		1	Wa-1	Yes	40	Intermediate	5.3
				Mh-0	Not Tested	28	Intermediate	6.2
R762C	96.6		6	Tsu-0 Tu-0	Yes	490 84	Tolerant	8.8 9.4
				Leska-1-44 Smolj-1	Yes	52 51	Intermediate	5.4 4.5
G833R	99.3	BCCP	3	Dja-1	Yes	52	Intermediate	4.6
L847P	96.0		1	WAR	Yes	39	Low Intermediate	3.7
F1206L	96.3		1	Aitba-1	Yes	53	Sensitive	2.8
E1355G	98.7		116	Multiple ^{d,h}	Assumed	625	Intermediate	5.1
				Ema-1; Si-0	Yes	124	High Intermediate	8.2
R1405Q	96.1		1	Db-1	Yes	75	Intermediate	7.2
Y1594H	97.9		6	None	Not Tested	None	Not Tested	
E1689G	97.0	CT-Beta	1	Ts-1	Yes	70	Sensitive	2.5
S1739C	94.3	CT-Beta	12	Multiple ^{d,i}	Assumed	97	Intermediate	5.6
				CYR	Yes	76	High Intermediate	7.8

G1766D	97.6	CT-Beta	39	Multiple ^{d,j}	Assumed	380	Intermediate	5.7
				Pog-0	Yes	60	Tolerant	8.6
I1821V	98.2	CT-Beta	1	MNF-Che-2	Yes	53	Intermediate	4.3
T1834S	99.4	CT-Beta	2	Nemrut-1	Yes	75	Intermediate	4.2
S1883T	97.0	CT-Beta	8	Multiple ^{d,k}	Assumed	211	Intermediate	6.2
G1897S	99.4	CT-Alpha	2	Sch1-7 WalhaesB4	Yes	70	Intermediate	4.8
						39		5.6
P2013L	98.5	CT-Alpha	3	Balan-1	Yes	52	Intermediate	6.2
A2014E	99.0	CT-Alpha	1	Appl-16	Yes	54	Intermediate	5.6
A2059V	98.2	CT-Alpha	1	Grivo-1	Yes	73	Sensitive	2.0
P2098S	93.0	CT-Alpha	2	Hod	Yes	72	Intermediate	5.3
I2115R	98.2	CT-Alpha	1	Iasi-1	Yes	44	Intermediate	5.0
H2207Q	98.1	CT-Alpha	1	Ip-Lso-0	Yes	55	Intermediate	7.5

^a The first letter denotes the most common residue found among sequenced accessions.

^b In alignment of 667 homomeric (eukaryotic) acetyl-CoA carboxylases.

^c BC, Biotin carboxylase; BCCP, Biotin carboxyl carrier protein; CT, carboxyltransferase.

^d Accessions with the same variant but a more sensitive or problematic seedling response are excluded to highlight the most tolerant responses observed with the variant present. A full list of accession responses is presented in Supplemental Table S3. Accessions with <30 seedlings scored were not evaluated in detail.

^e The Sav-0 variant was uncovered by sequencing the ACC2 cDNA; whole genome sequence for this accession is not available.

^f Intermediate responses: Aa-0; Hsm; Kyoto; Rag1-1; Ws-2.

^g Intermediate responses: Boot-1; Col-0; Ga-0; Hi-0; Kn-0; Ler-1; NFA-8; Pi-0; Tscha-1; Van-0.

^h Intermediate responses: Ber; CON-7; Dja-1; Est; Gy-0; Nie1-2; Pla-0; Sch1-7; Wa-1; WalHaesB4.

ⁱ Intermediate responses: Fei-0; Kin-0; Seattle-0; Sq-8.

^j Intermediate responses: Boot-1; Col-0; Kn-0; Pi-0; Van-0.

^k Intermediate responses: Dra3-1; Kni-1; Pna-17; Spr1-2.

330
331**Supplemental Table S3.** Natural accessions analyzed and spectinomycin responses obtained.

Accession ^a	ABRC Stock Number	Cold Treatment ^b	Seedlings Analyzed	Reported Country of Origin	Response Score	Spectinomycin Response Category	Initial Purpose ^c
<i>acc2-1</i>	Salk-148966C		925	Col-0 Accession	1.0	Hypersensitive	KO
<i>tic20-iv-1</i>	Sail-97-F10		690	Col-0 Accession	1.0	Hypersensitive	KO
Aa-0	CS76428	G	27	Germany	7.3	Intermediate	SFS
Ag-0	CS76430	G	28	France	7.6	High Intermediate	SFS
Aitba-1	CS76649	G	53	Morocco	2.8	Sensitive	SFS
An-1	CS28015		20	Belgium	8.4	Tolerant	FFS
Ang-0	CS76436		28	Belgium	8.1	High Intermediate	SFS
App1-14	CS76668		38	Sweden	3.7	Low Intermediate	REV
App1-16	CS76669	V	54	Sweden	5.6	Intermediate	REV
ARGE-1-15	CS76672		28	France	7.2	Intermediate	SFS
Ba-1	CS76441	V	22	United Kingdom	4.8	Intermediate	REV
Baa-1	CS76442	G	28	Netherlands	7.8	High Intermediate	SFS
Balan-1	CS76687	V	52	Russia	6.2	Intermediate	REV
Bay-0	CS28056		68	Germany	3.6	Low Intermediate	FFS
Bch-4	CS28060		20	Germany	3.2	Sensitive	FFS
Bd-0	CS76445	G	79	Germany	2.2	Sensitive	TRP
Be-1	CS28063		341	Germany	9.5	Tolerant	FFS
Ber	CS76448		25	Denmark	4.4	Intermediate	SFS
Berkeley	CS28067		20	USA (CA)	3.7	Low Intermediate	FFS
Bik-1	CS76449	G	28	Lebanon	3.8	Low Intermediate	SFS
Bil-5	CS76709	V	46	Sweden	6.0	Intermediate	PNL
Bl-1	CS76450		49	Italy	2.4	Sensitive	SFS
Bla-1/12	CS28086		20	Spain	3.9	Low Intermediate	FFS
Blh-1	CS28089		71	Czech Republic	1.3	Sensitive	PNL
Blh-1(2)	CS76098		20	Czech Republic	7.5	High Intermediate	PNL
Boot-1	CS76452	G	26	United Kingdom	5.2	Intermediate	SFS
Bor-4	CS76454	G	26	Czech Republic	7.4	Intermediate	SFS
Borky*	CS76453		53	Czech Republic	6.8	Intermediate	REV
BRI-2*	CS76725		31	France	5.1	Intermediate	REV
Bs-1	CS76456		28	Switzerland	6.4	Intermediate	SFS
Bsch-0	CS76457		24	Germany	7.2	Intermediate	TRP
Buckhorn Pass	CS76733	V	29	USA (CA)	1.7	Sensitive	REV
Bur-0	CS76734		70	Ireland	2.3	Sensitive	SFS
C24*	CS28127		73	Uncertain	7.9	High Intermediate	FFS
Can-0*	CS76740		96	Spain	4.7	Intermediate	SFS
CATS-6*	CS76760	G	47	France	6.4	Intermediate	PNL
Chat-1	CS76463	G	23	France	9.7	Tolerant	SFS

Chi-0	CS76464		75	Russia	5.1	Intermediate	TRP
CIBC-5	CS76465		27	United Kingdom	4.4	Intermediate	SFS
Co-1	CS76468		28	Portugal	7.3	Intermediate	SFS
Col-0	Lab		287	From George Redei	5.6	Intermediate	FFS
Com-1	CS76469		32	France	5.3	Intermediate	SFS
CON-7	CS76781		28	France	6.8	Intermediate	SFS
Cvi-0	CS28197		20	Cape Verde Islands	5.3	Intermediate	FFS
CYR	CS76790		76	France	7.8	High Intermediate	SFS
Da(1)-12	CS76470		24	Czech Republic	6.0	Intermediate	SFS
Db-1	CS28203		75	Germany	7.2	Intermediate	FFS
Del-10	CS76397	G	24	Yugoslavia	4.2	Intermediate	SFS
Dem-4	CS76794	V	28	USA	1.7	Sensitive	REV
Di-G	CS76472		82	France	2.5	Sensitive	TRP
Dja-1	CS76473	G	52	Kyrgyzstan	4.6	Intermediate	SFS
Dog-4	CS76386	V	53	Turkey	3.7	Low Intermediate	TRP
Dra3-1	CS76811	V	56	Sweden	5.3	Intermediate	REV
Draha2	CS76812		27	Czech Republic	6.7	Intermediate	SFS
Durh-1	CS76477		17	United Kingdom	4.7	Intermediate	SFS
Ema-1	CS76480	G	41	United Kingdom	8.2	Tolerant	SFS
En-1	CS28233		20	Germany	8.3	High Intermediate	FFS
En-D	CS28230		20	Ukraine	8.5	Tolerant	FFS
Erg2-6	CS76845		27	Germany	8.3	Tolerant	SFS
Eri-1	CS28240		20	Sweden	6.0	Intermediate	FFS
Est	CS76485		27	Germany	5.0	Intermediate	SFS
Est-0/1	CS28243		20	Russia	2.4	Sensitive	FFS
Etna-2	CS76487	V	111	Italy	1.9	Sensitive	SFS
Faneromnemi-3	CS76853		27	Greece	2.4	Sensitive	SFS
Fei-0	CS28250		20	Portugal	4.9	Intermediate	FFS
Fell1-10	CS76855		27	Germany	8.6	Tolerant	TRP
Filet-1	CS76858		27	Italy	7.6	Intermediate	SFS
Ga-0	CS76490		28	Germany	7.0	Intermediate	SFS
Gd-1	CS28275		20	Germany	7.6	Intermediate	FFS
Geg-14	CS76876		28	Armenia	5.5	Intermediate	SFS
Gel-1	CS76492		28	Netherlands	4.8	Intermediate	SFS
Giffo-1*	CS76878		25	Italy	8.0	High Intermediate	REV
Gifu-2*	CS76494		78	Japan	1.3	Sensitive	SFS
Gn-1	CS76880		83	Germany	1.1	Hypersensitive	PNL
Gn2-3*	CS76881		191	Germany	1.1	Hypersensitive	SFS
Go-0	CS28282		58	Germany	3.1	Sensitive	FFS
Gr-1 (Graz)	CS76496		26	Austria	4.8	Intermediate	SFS
Gradi-1.9645	CS76887		8	Croatia	4.0	Low Intermediate	PNL
Gre-0	CS76497		48	USA (MI)	1.4	Sensitive	REV

Grivo-1	CS76888	G/V	73	Bulgaria	2.0	Sensitive	REV
Gu-0	CS28331		20	Germany	4.6	Intermediate	FFS
Gy-0	CS76499		26	France	4.6	Intermediate	SFS
Ha-HBT1-2	CS76898		28	Germany	9.1	Tolerant	SFS
Ha-HBT2-10	CS76899		27	Germany	5.8	Intermediate	SFS
Ha-S-B	CS76903		28	Germany	4.6	Intermediate	SFS
Hi-0	CS28346		20	Netherlands	5.7	Intermediate	FFS
Hn-0	CS76513		28	Germany	5.7	Intermediate	TRP
Hod	CS76924		72	Czech Republic	5.3	Intermediate	PNL
Hof-1	CS76925	G	56	Germany	5.9	Intermediate	PNL
Hovdala-2	CS76937	V	56	Sweden	4.5	Intermediate	PNL
HR-10	CS28355		20	United Kingdom	2.4	Sensitive	FFS
Hs-0	CS76515		25	Germany	5.2	Intermediate	SFS
HSm	CS76941		24	Czech Republic	6.1	Intermediate	SFS
Iasi-1	CS76944		44	Romania	5.0	Intermediate	REV
In-0	CS76516		84	Austria	4.8	Intermediate	SFS
IP-Alo-0	CS76662	G	51	Portugal	1.1	Hypersensitive	PNL
IP-Ber-0	CS78887	G	49	Spain	1.3	Sensitive	PNL
IP-Cor-0	CS76782	G/V	8	Spain	2.6	Sensitive	REV
IP-Cum-1	CS76787	G	129	Spain	2.3	Sensitive	TRP
IP-Gua-1	CS76894	G	81	Spain	6.3	Intermediate	REV
Ip-Hom-4	CS76929	G/V	94	Spain	4.5	Intermediate	REV
IP-Lso-0	CS77055	G	55	Spain	7.5	Intermediate	REV
IP-Pal-0	CS77159	G	51	Spain	6.1	Intermediate	REV
IP-Ren-6	CS77212	G	42	Spain	2.8	Sensitive	PNL
IP-Tor-1	CS77378	G	46	Spain	4.1	Low Intermediate	REV
IP-Vin-0	CS78846	G	33	Spain	1.2	Hypersensitive	PNL
IP-Vis-0	CS78848	G	47	Spain	3.3	Low Intermediate	REV
IP-Voz-0	CS78849	G	72	Spain	5.5	Intermediate	PNL
Is-0	CS76517		28	Germany	7.2	Intermediate	TRP
Jl-3	CS28369		352	Czech Republic	9.4	Tolerant	FFS
Jm-0	CS76520		27	Czech Republic	5.0	Intermediate	SFS
Kar-1	CS76522	G	28	Kyrgyzstan	5.2	Intermediate	SFS
Karag-2	CS76961		28	Russia	7.1	Intermediate	SFS
Kas-2	CS76523	G	26	India	6.1	Intermediate	SFS
Kb-0	CS76524		73	Germany	1.4	Sensitive	PNL
Kil-0	CS76526		28	United Kingdom	4.0	Low Intermediate	SFS
Kin-0	CS76527		23	USA (MI)	6.0	Intermediate	SFS
Kl-1	CS28390		20	Germany	9.0	Tolerant	FFS
Kl-5	CS76528		76	Germany	1.1	Hypersensitive	PNL
Kn-0	CS28395		19	Lithuania	5.9	Intermediate	FFS
Kni-1	CS76970	V	56	Sweden	7.2	Intermediate	REV

Knjas-1	CS76971		22	Serbia	3.4	Low Intermediate	SFS
Knox-18	CS76530		80	USA (IN)	1.1	Hypersensitive	SFS
Koch-1	CS76396		25	Ukraine	6.1	Intermediate	SFS
Kolar-1	CS76974		27	Bulgaria	4.5	Intermediate	SFS
Koln	CS76976		28	Germany	6.6	Intermediate	SFS
Kolyv-6	CS76980		27	Russia	4.0	Intermediate	SFS
Kondara	CS76532	G	28	Tajikistan	6.7	Intermediate	SFS
Koren-1*	CS76983	G	0	Bulgaria		Not Tested	ACC1
K-oze-1	CS76957		28	Russia	4.8	Intermediate	SFS
Kyoto	CS76535		28	Japan	6.7	Intermediate	SFS
Kz-1	CS28427		20	Kazakhstan	4.9	Intermediate	FFS
La-0	CS76538		106	Germany	2.0	Sensitive	SFS
LDV-18	CS77013		21	France	3.7	Low Intermediate	SFS
Ler-1	CS28449		20	Germany	4.5	Intermediate	FFS
Leska-1-44	CS77030	V	52	Bulgaria	5.4	Intermediate	REV
Lip-0*	CS76542		53	Poland	6.2	Intermediate	SFS
Litva	CS76543		27	Lithuania	3.9	Intermediate	SFS
Lm-2	CS28473		70	France	8.3	Tolerant	FFS
Lo-1	CS28474		20	Germany	7.5	Intermediate	FFS
Lu3-30	CS77057		54	Germany	1.1	Hypersensitive	PNL
Lu4-2	CS77058		55	Germany	1.3	Hypersensitive	PNL
Mdn-1*	CS77077		43	USA	2.9	Sensitive	REV
Melni-2	CS77080	G	0	Bulgaria		Not Tested	ACC1
Mer-6	CS76414	G	28	Spain	4.5	Low Intermediate	SFS
Mh-0	CS76550		28	Poland	6.2	Intermediate	SFS
Mh-1	CS28493		20	Poland	9.3	Tolerant	FFS
MIC-31	CS77082		49	USA (MI)	2.1	Sensitive	REV
MNF-Che-2	CS77096		53	USA	4.3	Intermediate	REV
MNF-Jac-12	CS77097		33	USA (MI)	2.5	Sensitive	REV
MNF-Pot-21	CS77099		50	USA	1.4	Hypersensitive	REV
MNF-Pot-75	CS77100		32	USA	2.2	Sensitive	REV
Mt-0	CS28502		20	Libya	8.4	Tolerant	FFS
Mv-0	CS76556		56	USA (MA)	1.1	Hypersensitive	REV
Mz-0	CS28506		20	Germany	9.1	Tolerant	FFS
Nc-1	CS76559		20	France	7.3	High Intermediate	SFS
NC-6	CS77124		54	USA (NC)	1.6	Sensitive	REV
Nd-0/1	CS28528		19	Germany	4.6	Intermediate	FFS
Nemrut-1	CS76398	V	75	Turkey	4.2	Intermediate	TRP
Neo-6	CS76560		27	Tajikistan	4.5	Intermediate	SFS
Nfa-8	CS28532		20	United Kingdom	7.1	Intermediate	FFS
Nie 1-2	CS76402		235	Germany	5.1	Intermediate	FFS
Nok-3	CS76562		26	Netherlands	4.5	Intermediate	SFS

"Nossen"	RIKEN		571	Uncertain	2.3	Sensitive	FFS
Np-0	CS76563		28	Germany	4.8	Intermediate	SFS
Nw-0	CS76564		28	Germany	7.0	Intermediate	TRP
Nz-1	CS28578		20	New Zealand	6.4	High Intermediate	FFS
Ob-0	CS76566		74	Germany	1.2	Hypersensitive	PNL
Old-1	CS76567		75	Germany	1.2	Hypersensitive	PNL
Olympia-2*	CS77144		33	Greece	1.9	Sensitive	PNL
Oy-0	CS28591		229	Norway	2.1	Sensitive	FFS
Pa-2	CS28595		20	Italy	2.5	Sensitive	FFS
Ped-0	CS76415	V	56	Spain	3.1	Sensitive	REV
Per-1	CS76571		27	Russia	2.4	Sensitive	SFS
Petergof	CS77170	G	0	Russia		Not Tested	ACC1
Pi-0	CS76572		28	Austria	6.9	Intermediate	SFS
Pi-2	CS28639		40	Austria	9.0	Tolerant	FFS
Pla-0	CS76573		27	Spain	4.3	Intermediate	SFS
Pna-10*	CS76574		112	USA (MI)	1.4	Sensitive	SFS
Pna-17	CS76575	V	52	USA (MI)	6.4	Intermediate	REV
Pog-0	CS76576	G	60	Canada	8.6	Tolerant	SFS
Pro-0	CS76577	G	28	Spain	6.0	Intermediate	SFS
PT2.21	CS77191		55	USA (PT)	1.5	Sensitive	REV
Pu2-23	CS76579	G	23	Czech Republic	5.4	Intermediate	SFS
Qar-8a	CS76581	V	100	Lebanon	2.4	Sensitive	PNL
Qui-0	CS76417		18	North Africa/Spain	2.5	Sensitive	FFS
Ra-0	CS28665		20	France	3.2	Low Intermediate	FFS
Ragl-1	CS76583		27	United Kingdom	4.3	Intermediate	SFS
Rennes-1	CS76586		44	France	3.9	Low Intermediate	SFS
Rev-2	CS77215	V	53	Sweden	6.6	Intermediate	PNL
RLD-2	CS28688		19	Russia	4.1	Intermediate	FFS
Rmx-A02	CS76589		36	USA (MI)	2.1	Sensitive	REV
Rmx-A180	CS77218		42	USA (MI)	1.8	Sensitive	REV
RRS-10	CS76592		81	USA (IN)	1.3	Sensitive	SFS
RRS-7	CS76593		28	USA (IN)	4.7	Intermediate	TRP
Rubexhnoe-1	CS76594	G	27	Ukraine	6.0	Intermediate	SFS
Sapporo-0	CS28724		16	Japan	3.2	Low Intermediate	FFS
Sav-0	CS28725		275	Czech Republic	1.2	Hypersensitive	FFS
Schl-7	CS77240		70	Germany	4.8	Intermediate	REV
Se-0	CS76597	G	28	Spain	7.3	Intermediate	SFS
Seattle-0	CS76598		28	USA (WA)	6.1	Intermediate	SFS
Sei-0	CS76599		56	Italy	6.9	Intermediate	SFS
Sha*	CS28736		74	Tajikistan	8.1	High Intermediate	FFS
Sha(2)	CS76382		20	Tajikistan	7.6	Intermediate	FFS
Si-0	CS76601		83	Germany	8.2	High Intermediate	SFS

Slavi-1	CS76419	G	27	Bulgaria	7.6	Intermediate	SFS
SLSP-31	CS77254		53	USA (MI)	1.4	Sensitive	REV
SLSP-35*	CS77255		50	USA (MI)	6.2	Intermediate	REV
Smolj-1	CS77256	G	51	Bulgaria	4.5	Intermediate	REV
Sorbo	CS76602		28	Tajikistan	8.1	Tolerant	SFS
Spr1-2	CS77261	V	47	Sweden	6.0	Intermediate	REV
Spro-1	CS77263	V	52	Sweden	5.1	Low Intermediate	PNL
Spro-2	CS77264	G/V	80	Sweden	1.3	Sensitive	PNL
Sq-8	CS76604		26	United Kingdom	6.7	Intermediate	SFS
Star-8	CS76400		27	Germany	4.7	Intermediate	TRP
Ste-2	CS77274	G/V	83	Sweden	1.1	Hypersensitive	PNL
Ste-3	CS77275	G/V	82	Sweden	1.0	Hypersensitive	PNL
Stw-0	CS76605		28	Russia	7.2	Intermediate	SFS
T1020	CS77289	V	49	Sweden	6.4	Intermediate	PNL
Ta-0	CS76608		28	Czech Republic	7.0	Intermediate	SFS
TAMM-2	CS76610	V	54	Finland	2.8	Sensitive	PNL
Tha-1	CS76611		84	Netherlands	3.4	Low Intermediate	SFS
Tol-0	CS76614		73	USA (OH)	2.0	Sensitive	SFS
Ts-1	CS76615		70	Spain	2.5	Sensitive	REV
Tscha-1	CS76616		28	Austria	5.4	Intermediate	SFS
Tsu-0	CS28780		490	Japan	8.8	Tolerant	FFS
Tu-0	CS76617		84	Italy	9.4	Tolerant	SFS
Tul-0	CS76618		81	USA (MI)	1.7	Sensitive	SFS
Ty-0	CS76619		28	United Kingdom	3.2	Sensitive	SFS
Uk-1	CS76620	G	27	Germany	8.3	High Intermediate	SFS
UKSWO6-333*	CS78813		15	United Kingdom	5.1	Intermediate	REV
Ulies-1*	CS78815		109	Romania	3.9	Low Intermediate	REV
Ullapool-8*	CS78821	G	51	United Kingdom	6.3	Intermediate	PNL
Uod-1	CS76621		78	Austria	8.5	Tolerant	SFS
Utrecht	CS76622		28	Netherlands	3.6	Low Intermediate	SFS
Vaar2-6	CS78831	V	54	Sweden	6.7	Intermediate	PNL
Van-0	CS28796		20	Canada	5.4	Intermediate	FFS
Vimmerby	CS78845	G/V	67	Sweden	1.0	Hypersensitive	PNL
Wa-1	CS76626		40	Poland	5.3	Intermediate	REV
WalHaesB4	CS76408	G	39	Germany	5.6	Intermediate	REV
WAR	CS78853		39	USA (RI)	3.7	Low Intermediate	REV
Wei-0	CS28816		79	Switzerland	9.0	Tolerant	FFS
Wil-1	CS28819		20	Russia	2.4	Sensitive	FFS
WI-0	CS76630	G	79	Germany	1.4	Sensitive	PNL
Ws-2	CS28828		20	Russia	5.3	Intermediate	FFS
Yeg-1	CS76394		20	Armenia	7.8	Intermediate	FFS
Yo-0	CS76633	V	51	USA (CA)	4.5	Low Intermediate	REV

Zal-1	CS76634	G	28	Kyrgyzstan	6.5	Intermediate	SFS
Zdr-1	CS76635		28	Czech Republic	5.8	Intermediate	SFS
Zu-1	CS28847		20	Switzerland	6.6	Intermediate	FFS

^a Asterisk: Variants of interest in these accessions were not confirmed by Sanger sequencing (Refer to Supplemental Table S8).

^b Extended treatment at 4°C used for germination (G) of seeds on plates, or vernalization (V) of plants at the rosette stage.

^c KO, Knockout mutant; FFS, First forward genetic screen; SFS, Second forward genetic screen; PNL, Putative null allele; REV, Reverse genetic screen; TRP, Transit peptide variant.

333 **Supplemental Table S4.** Terminal phenotypes and allele strengths of *ACC1* nonsense and
 334 missense mutants.

335

Feature Evaluated	<i>emb22</i>	<i>pas3-1</i>	<i>pas3-2</i>
Mutation	Nonsense	Missense	Missense
Terminal Embryo Phenotype	Green Blimp	Green Blimp	Cotyledon Initials; Expanded Hypocotyl
Seeds Screened ^a	444	507	435
Mutant Seeds	22.7 %	21.3 %	23.5 %
Mutant Embryos Measured	101	108	102
Embryo Length ^b	300 ± 50 µm	325 ± 40 µm	375 ± 45 µm
Mutant Seedlings Screened ^c	33	59	Not Tested
Seedling Length ^b	0.7 ± 0.2 mm	3.7 ± 2.0 mm	Not Tested
Dark Green Seedlings	3.0 %	76.3 %	Not Tested
Brown Seedlings	72.8 %	0.0 %	Not Tested
Terminal Seedling Phenotype	Minimal Growth	Extensive Growth	Not Tested
Allele Strength	Null	Strong	Moderate
^a Seeds were examined in siliques of selfed heterozygotes. ^b Mean length ± S.D. ^c Seedlings were evaluated after 5 weeks of growth in culture.			

336

337

338 **Supplemental Table S5.** Spectinomycin responses of F2 seedlings from crosses between sensitive accessions and
 339 informative knockout mutants.

340

Accession Parent	Knockout Parent	Seedlings Classified	Phenotype Score	Distribution of F2 Seedling Phenotypes (%)						
				Sensitive			Intermediate			Tolerant
				1	2	3	5	6	7	9
"Gn2-3"	<i>acc2</i>	82	1.0	97.6	2.4					
Sav-0	<i>acc2</i>	163	1.0	99.4	0.6					
Sav-0	"Nossen"	184	1.2	87.5	4.9	7.1		0.5		
Knox-18 ^b	<i>acc2</i>	127	1.0	96.1	3.9					
Tul-0 ^b	<i>acc2</i>	135	1.1	90.4	8.9	0.7				
Pna-10 ^b	<i>acc2</i>	165	1.0	97.6	2.4					
"Gn2-3"	<i>tic20-iv</i>	156	3.6	39.1	5.1	5.1	30.8	1.9	16.7	1.3
Sav-0	<i>tic20-iv</i>	152	3.2	31.6	7.9	25.7	26.3	0.7	7.8	
Knox-18 ^b	<i>tic20-iv</i>	124	4.0	46.0	4.8		12.1	4.0	21.8	11.3
Tul-0 ^b	<i>tic20-iv</i>	138	3.8	38.5	10.9		15.2	10.1	21.0	4.3
Aitba-1	<i>acc2</i>	325	2.2	42.5	28.6	17.2	5.5	3.1	3.1	
Aitba-1	<i>tic20-iv</i>	124	3.7	29.9	11.3	15.3	21.0	4.8	13.7	4.0
La-0	<i>acc2</i>	152	2.6	23.7	27.6	35.5	5.9	6.6	0.7	
La-0	<i>tic20-iv</i>	154	2.5	25.3	29.2	35.1	5.9	4.5		

^a Refer to Figure 1 for examples of sensitive, intermediate, and tolerant seedling phenotypes. Red, Most common phenotypes.

^b Part of the Knox-18 group of sensitive accessions with shared variants of interest.

341 **Supplemental Table S6.** ACC2 variants in the Sav-0 accession that differ from the consensus among sequenced accessions.
 342

Variant ^a	Conservation (%) ^b	Protein Domain ^c	1001 Genomes Accessions with Predicted Variant	Accessions Evaluated on Spectinomycin ^d	Variant Confirmed	Seedlings Classified	Spectinomycin Response	
							Category	Score
A18T	Low	TP	39	Multiple ^e	Assumed	231	Intermediate	5.0
S66F	Low	TP	57	Giffo-1	Not Needed	25	High Intermediate	8.0
				Fell1-10; JI-3		379	Tolerant	9.3
G135E	95.7	(BC)	0	Sav-0 ^f	Yes	275	Hypersensitive	1.2
M445T	Low	BC	189	Multiple ^g	Assumed	1063	Tolerant	9.0
V472I	83.7	BC	51	Nz-1; Uk-1	Not Tested	47	High Intermediate	7.5
				Mt-0; Mz-0		40	Tolerant	8.8
D521N	Low	BC	51	Nz-1; Uk-1	Not Needed	47	High Intermediate	7.5
				Mt-0; Mz-0		40	Tolerant	8.8
S1758L	Low	CT	192	Multiple ^g	Assumed	1063	Tolerant	9.0
S2230L	Low		48	Nz-1; Uk-1	Not Needed	47	High Intermediate	7.5
				Mt-0		20	Tolerant	8.4
T2284R	Low		60	Nz-1; Uk-1	Not Needed	47	High Intermediate	7.5
				Lm-2; Mt-0; Mz-0		110	Tolerant	8.5

^a The first letter denotes the most common residue found among 855 sequenced accessions. Six variants (E87D; A376V; S725N; M800I; S1384T; D1766G) where the Sav-0 sequence differs from the Col-0 reference but not the accession consensus are excluded.

^b In alignment of 667 homomeric (eukaryotic) acetyl-CoA carboxylases.

^c TP, Transit Peptide; BC, Biotin carboxylase; CT, carboxyltransferase.

^d Accessions with the same variant but a more sensitive or problematic seedling response are excluded to highlight the most tolerant responses observed with the variant present. A full list of accession responses is presented in Supplemental Table S3. Accessions with <30 seedlings scored were not evaluated in detail.

^e Intermediate responses: Durh-1; Hn-0; Hovdala-2; Ler-1; Litva; Nw-0; RRS-7; Star-8.

^f The Sav-0 variant was uncovered by sequencing the ACC2 cDNA; whole genome sequence for this accession is not available.

^g Tolerant responses: Fell1-10; JI-3; Lm-2; Mt-0; Mz-0; Tsu-0; Tu-0.

343 **Supplemental Table S7.** Informative variants that alter conserved residues or disrupt protein structure in eukaryotic, homomeric acetyl-CoA carboxylases.

344

Residue (ACC2)	Variant (ACC2) ^a	Variant Type	Organism	Variant Impact ^b	Allele Strength	Locus	Domain	Conservation (%)			Genotype	Source AA Residue	Reference	Additional Notes
								MUSCLE (667)	Plant (139)	Original (20)				
135	G135E	Missense	Arabidopsis	LD	Strong	ACC2	(BC)	95.7	100.0	95	Sav-0	Same	This Report	Unique to Sav-0 Accession
153	K153E	Site Directed	Yeast	D	Strong	ACC1	Dimer Interface	96.9	100.0	100	K73E	73	Wei and Tong (2015)	Loss of Enzyme Activity <i>in vitro</i>
156	R156E	Site Directed	Yeast	D	Strong	ACC1	Dimer Interface	93.6	99.3	100	R76E	76	Wei and Tong (2015)	Loss of Enzyme Activity <i>in vitro</i> ; Soraphen A Interaction Site
188	E188K	Missense	Arabidopsis	D	Weak	ACC1	BC	78.0	99.3	90	<i>gsd1</i>	86	Lü et al. (2011)	Vegetative Phenotype
193	A193V	Missense	Arabidopsis	ND	Normal	ACC1	BC	98.4	99.3	100	Meln1-2	91	This Report	Maintained in Natural Populations
219	219	Splicing	Arabidopsis	D	Strong	ACC1	BC	NA	NA	NA	<i>gk-U413; gk-sc</i>	114	Kajiwara et al. (2004)	Embryo Defective; Updated Location
333	G333D	Missense	Arabidopsis	D	Strong	ACC1	BC	99.4	97.1	100	<i>acc1-3</i>	231	Kajiwara et al. (2004)	Embryo Defective; Seeds Unavailable
363	F363L	Missense	Arabidopsis	PD	Some Function	ACC2	BC	99.3	97.1	100	Sei-0	Same	This Report	
376	V376A	Missense	Arabidopsis	PD	Some Function	ACC2	BC	100.0	100.0	100	Col-0	Same	This Report	
383	Y383H	Missense	Arabidopsis	ND	Normal	ACC1	BC	90.6	71.9	80	Consensus		This Report	ACC1 Consensus Differs from ACC2
397	Q397X	Nonsense	Drosophila	D	Strong	ACC	BC	NA	NA	NA	<i>Acc¹</i>	359	Sasamura et al. (2013)	Lethal
402-3	x	x	Arabidopsis	x	x	x	Large Intron	x	x	x	x	x	x	Large Intron
404	I404K	Missense	Arabidopsis	LD	Strong	ACC2	BC	94.8	100.0	95	Knox-18 Group	Same	This Report	See Others in Group
406	E406K	Missense	Arabidopsis	D	Weak	ACC1	BC	100.0	100.0	100	<i>sfr1</i>	304	Amid et al. (2012)	Vegetative Phenotype
443	Y443C	Missense	Arabidopsis	PD	Strong	ACC2	BC	94.0	92.8	95	Etna-2	Same	This Report	Crosses Remain to be Analyzed

456	N456I	Missense	Drosophila	D	Strong	ACC	BC	100.0	100.0	100	Acc ²	417	Sasamura et al. (2013)	Lethal
474	L474F	Missense	Arabidopsis	PD	Some Function	ACC2	BC	94.5	97.8	100	Chi-0	Same	This Report	
475	P475L	Missense	Arabidopsis	LND	Normal	ACC2	BC	99.7	99.3	100	Lm-2	Same	This Report	
478	Q478K	Missense	Arabidopsis	LND	Normal	ACC2	BC	97.6	99.3	100	Uod-1	Same	This Report	
493	I493L	Missense	Arabidopsis	ND	Normal	ACC1	BC	94.6	99.3	100	Multiple	391	This Report	Maintained in Natural Populations
494	R494G	Missense	Arabidopsis	PD	Some Function	ACC2	BC	99.9	99.3	100	Ip-Pal-0	Same	This Report	
520	F520L	Missense	Arabidopsis	PD	Some Function	ACC2	BC	94.0	82.7	40	In-0	Same	This Report	
528	P528S	Missense	Arabidopsis	ND	Normal	ACC1	BC	90.1	99.3	90	Multiple	426	This Report	Maintained in Natural Populations
538	T538A	Missense	Arabidopsis	PD	Some Function	ACC2	BC	99.9	99.3	100	Ip-Tor-1	Same	This Report	
564	M564V	Missense	<i>C. elegans</i>	(D)	Weak		BC	93.3 (V)	93.5 (V)	95 (V)	ye60 (A471V)	471	Rappleye et al. (2003)	Temperature Sensitive
565	W565A	Site Directed	Yeast	D	Strong	ACC1	Dimer Interface	99.3	97.1	100	W487A	487	Wei and Tong (2015)	Loss of Enzyme Activity <i>in vitro</i>
565	W565X	Nonsense	Arabidopsis	D	Strong	ACC1	BC	NA	NA	NA	emb22	463	Kajiwara et al. (2004)	Embryo Defective
572	See Text	Splicing	Arabidopsis	D	Strong	ACC2	Intron 10	NA	NA	NA	Gn-1; "Gn2-3"	Same	This Report	Results in Frameshift
668	S668S	Missense	Yeast	(D)	Weak	ACC1		33.0 (H)	68.4 (S)	35 (S)	acc1 ^{ts} (F>S)		Schneiter et al. (2000)	
686	Q686R	Site Directed	Yeast	LND	Normal	ACC1		96.7	99.3	95	Q608R	608	Wei and Tong (2015)	Functional Enzyme <i>in vitro</i>
725	N725S	Missense	Arabidopsis	LND	Normal	ACC2		96.9	95.7	100	Pog-0	Same	This Report	
734	H734E	Site Directed	Yeast	LND	Normal	ACC1		44.2 (R)	69.8 (H)	70 (H)	R656E	656	Wei and Tong (2015)	Functional Enzyme <i>in vitro</i>
739	G739E	Missense	Arabidopsis	PD	Some Function	ACC2		95.2	97.8	95	Wa-1	Same	This Report	
753	Y753X	Nonsense	Arabidopsis	D	Strong	ACC2		NA	NA	NA	Kb-0; Kl-5	Same	This Report	

762	R762C	Missense	Arabidopsis	ND	Normal	ACC2		96.6	97.1	100	Tsu-0; Tu-0	Same	This Report	
774	See Fig. S2	Deletion	Arabidopsis	D	Strong	ACC2	Intron 17; Exon 18	NA	NA	NA	Ip-Ber-0	Same	This Report	23 bp Deletion; Defective Transcripts
777	D777N	Missense	Arabidopsis	PD	Some Function	ACC2	(BCCP)	97.2	100.0	95	Leska-1-44; Smolj-1	Same	This Report	
794	V794I	Missense	Arabidopsis	ND	Normal	ACC1	BCCP	85.6	84.9	95	Multiple	692	This Report	Maintained in Natural Populations
813	K813R	Site Directed	Yeast	D	Strong	ACC1	Biotin Binding	100.0	100.0	100	Biotin Binding	735	Schneiter et al. (1996)	Site-Directed Mutagenesis
833	G833R	Missense	Arabidopsis	PD	Some Function	ACC2	BCCP	99.3	98.6	100	Dja-1	Same	This Report	
847	L847P	Missense	Arabidopsis	PD	Some Function	ACC2	Central	96.0	100.0	100	WAR	Same	This Report	
865	R865X	Nonsense	Arabidopsis	D	Strong	ACC2	Central	NA	NA	NA	"Nossen"	Same	Parker et al. (2014)	
901	See Text	Splicing	Arabidopsis	D	Strong	ACC2	Intron 19	NA	NA	NA	WI-0	Same	This Report	Results in Frameshift
955	[955]	Insertion	Arabidopsis	D	Strong	ACC2	Exon 21	NA	NA	NA	acc2-2	Same	Salk Insertion	T-DNA Insertion Mutant
1171	1171fs	Frameshift	Arabidopsis	D	Strong	ACC2	Central	NA	NA	NA	Ip-Alo-0; Ip-Vin-0	Same	This Report	
1206	F1206L	Missense	Arabidopsis	LD	Moderate	ACC2	Central	96.3	100	85	Aitba-1	Same	This Report	
1225	K1225X	Nonsense	Arabidopsis	D	Strong	ACC2	Central	NA	NA	NA	Blh-1	Same	This Report	
1229	[1229]	Insertion	Arabidopsis	D	Strong	ACC2	Exon 27	NA	NA	NA	acc2-1	Same	Salk Insertion	T-DNA Insertion Mutant
1355	E1355G	Missense	Arabidopsis	VUS; LND	Uncertain; (Normal)	ACC2	Central	98.7	100.0	100	Knox-18 Group; (Si-0; Ema-1)	Same	This Report	
1376; 1377	K1376R; Δ1377	Deletion	Arabidopsis	LD	Strong	ACC2	Central	Low Low	80.6 43.9	35 20	Qar-8a	Same	This Report	Crosses Remain to be Analyzed
1405	R1405Q	Missense	Arabidopsis	PD	Some Function	ACC2	Central	96.1	100.0	100	Db-1	Same	This Report	
1479	Δ1479	Deletion	Arabidopsis	PD	Some Function	ACC2	Central	19.3 (E)	92.1 (E)	45 (E)	Ip-Voz-0	Same	This Report	Arabidopsis ACC2: Glu

1562	See Fig. S1	Splicing	Arabidopsis	D	Strong	ACC2	Intron 29	NA	NA	NA	Spro-2; Ste-2; Ste-3; Vimmerby	Same	This Report	Variety of Defective Transcripts
1603	K1603Q	Missense	Arabidopsis	ND	Normal	ACC1		91.0	88.5	80	Consensus		This Report	ACC1 Consensus Differs from ACC2
1621	x	x	Arabidopsis	x	x	x	Start of Large Exon	x	x	x	x	x	TAIR	Start of Large Exon (# 31 of 32)
1623	E1623E	Missense	<i>C. elegans</i>		Weak		CT-Beta	61.3 (R)	87.1 (E)	35 (E)	ye162 (G1351E)	1351	Rappleye et al. (2003)	
1689	E1689K;G	Missense	Arabidopsis	D	Strong	ACC1; ACC2	CT-Beta	97.0	99.3	100	pas3-1 (E>K); Ts-1 (E>G)	1588; Same	Baud et al. (2004); This Report	Embryo Defective (<i>acc1</i>); Spectinomycin Sensitive (<i>acc2</i>)
1739	S1739C	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Beta	94.3	86.3	90	CYR	Same	This Report	Conserved Residue in CoA Binding Pocket
1766	G1766D	Missense	Arabidopsis	LND	Normal	ACC2	CT-Beta	97.6	99.3	100	Pog-0	Same	This Report	
1794	G1794A	Missense	Arabidopsis	ND	Normal	ACC1	CT-Beta	94.8	100.0	95	Multiple	1693	This Report	Maintained in Natural Populations
1815	I > L,V,A,T	Missense	Resistant Grasses	ND	Normal	ACC2	CT-Beta	94.3 (L)	74.8 (L)	90 (L)	Plastid ACCase	1781	Kaundun (2014) GenBank: AJ310767	Herbicide Resistant Grasses; Arabidopsis ACC2: Leu
1821	I1821V	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Beta	98.2	100.0	100	MNF-Che-2	Same	This Report	
1834	T1834S	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Beta	99.4	100.0	100	Nemrut-1	Same	This Report	
1854	[1854]	Insertion	Arabidopsis	D	Strong	ACC1	CT-Beta	NA	NA	NA	<i>acc1-1</i>	1753	Baud et al. (2004)	
1878	R1878X	Nonsense	Yeast	D		ACC1	CT-Beta	NA	NA	NA	<i>Acc1^{C-term}</i>		Schneiter et al. (2000)	
1883	S1883T	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Beta	97.0	100.0	100	Several	Same	This Report	
1888	G1888S	Missense	Arabidopsis	D	Strong	ACC1	CT-Beta	99.4	100	100	pas3-2	1787	Baud et al. (2004)	Embryo Defective
1889	G1889A	Missense	Yeast	(D)	Weak	ACC1	CT-Beta	99.4	100	100	<i>Acc1^{GS}</i>		Schneiter et al. (2000)	
1890	P1890C	Missense	<i>C. elegans</i>	(D)	Weak		CT-Beta	42.1 (T)	97.1 (P)	45 (P)	ye180		Rappleye et al. (2003)	
1897	G1897S	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Alpha	99.4	100.0	100	Sch1-7; WalHaesB4	Same	This Report	

1902	T1902K	Missense	Arabidopsis	LD	Strong	ACC2	CT-Alpha	87.6	100.0	95	Knox-18 Group	Same	This Report	See Others in Group
1968	G1968E	Missense	Arabidopsis	D	Moderate	ACC1	CT-Alpha	99.0	99.3	100	<i>gk-101</i>	1867	Kajiwara et al. (2004)	Embryo Defective; Seeds Unavailable
2013	P2013L	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Alpha	98.5	97.1	95	Balan-1	Same	This Report	
2014	A2014E	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Alpha	99.0	97.8	100	App1-16	Same	This Report	
2020	2020fs	Frameshift	Arabidopsis	D	Strong	ACC2	CT-Alpha	NA	NA	NA	Lu4-2; Lu3-30	Same	This Report	
2033	W > C,L,S	Missense	Resistant Grasses	ND	Normal	ACC2	CT-Alpha	98.5	98.6	100	Plastid ACCase	1999	Kaundun (2014) GenBank: AJ310767	Herbicide Resistant Grasses; Arabidopsis ACC2: Trp
2059	A2059V	Missense	Arabidopsis	VUS	Uncertain	ACC2	CT-Alpha	98.2	98.6	100	Grivo-1	Same	This Report	Crosses Suggest ACC2 is Functional
2061	W > C	Missense	Resistant Grasses	ND	Normal	ACC2	CT-Alpha	97.3	98.6	100	Plastid ACCase	2027	Kaundun (2014) GenBank: AJ310767	Herbicide Resistant Grasses; Arabidopsis ACC2: Trp
2098	P2098S	Missense	Arabidopsis	VUS	Some Function	ACC2	CT-Alpha	93.0	97.8	100	Hod	Same	This Report	Nonsense Mutation Also Present
2112	D > G	Missense	Resistant Grasses	ND	Normal	ACC2	CT-Alpha	98.5	97.8	100	Plastid ACCase	2078	Kaundun (2014) GenBank: AJ310767	Herbicide Resistant Grasses; Arabidopsis ACC2: Asp
2115	I2115R	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Alpha	98.2	97.8	100	lasi-1	Same	This Report	
2122	C > R	Missense	Resistant Grasses	ND	Normal	ACC2	CT-Alpha	81.9 (M)	70.5 (M)	80 (M)	Plastid ACCase	2088	Kaundun (2014) GenBank: AJ310767	Herbicide Resistant Grasses; Arabidopsis ACC2: Met
2130	G > A,S	Missense	Resistant Grasses	ND	Normal	ACC2	CT-Alpha	80.1	96.4	90	Plastid ACCase	2096	Kaundun (2014) GenBank: AJ310767	Herbicide Resistant Grasses; Arabidopsis ACC2: Gly
2207	H2207Q	Missense	Arabidopsis	PD	Some Function	ACC2	CT-Alpha	98.1	98.6	100	Ip-Lso-0	Same	This Report	
2208	[2208]	Insertion	Arabidopsis	D	Strong	ACC1	CT-Alpha	NA	NA	NA	<i>acc1-2</i>	2107	Baud et al. (2004)	
2325	Q2325X	Nonsense	Arabidopsis	VUS	Some Function	ACC2		NA	NA	NA	Hod	Same	This Report	
2337	x	x	Arabidopsis	x	x	x	End of Large Exon	x	x	x	x	x	TAIR	End of Large Exon (# 31 of 32)
2355	x	x	Arabidopsis	x	x	x	End of Protein	x	x	x	x	x	TAIR	End of ACC2 Protein

^a The first letter denotes the most common residue found among 855 sequenced accessions.

^b D, Deleterious to protein function; LD, Likely deleterious; PD, Potentially deleterious; VUS, Variant of unknown significance; LND, Likely not deleterious; ND, Not deleterious to protein function.

345

Variant Confirmed ^a	Accession	ABRC Stock	Residue	1001 Genomes Predicted Variant	Notes
No	Borky	CS76453	788	K788M	ACC2 Reverse
No	BRI-2	CS76725	1677	G1677R	ACC2 Reverse
No	C24	CS28127	725	N725S	ACC2 Reverse
No	Can-0	CS76740	777	D777N	ACC2 Reverse
No	CATS-6	CS76760	Intron 4	Splice Acceptor	Putative Splicing
No	Giffo-1	CS76878	2082; 2083	I2082M; V2083G	ACC2 Reverse
No	Gn2-3	CS76881	FL cDNA	Multiple	Identical to Gn-1
No	Koren-1	CS76983	271*; 272*	A271D*; Q272R*	* ACC2 Variant # (ACC1 Reverse)
No	Lip-0	CS76542	833	G833R	ACC2 Reverse
No	Olympia-2	CS77144	230	TTG > T-G	Putative Frameshift
No	Pna-10	CS76574	1883	S1883T Not Confirmed; I404K; T1902K Not Predicted	Belongs Instead to Knox-18 Group
No	Sha	CS28736	376; 725	V376A; N725S	Accession Name Duplicate
No	SLSP-35	CS77255	404; 1902	I404K; T1902K	Not Part of Knox-18 Group
No	UKSW06-333	CS78813	404; 1902	I404K; T1902K	Not Part of Knox-18 Group
No	Ulies-1	CS78815	742*	L742S	* ACC2 Variant # (ACC1 Reverse)
No	Ullapool-8	CS78821	Intron 4	Splice Acceptor	Putative Splicing
Yes	Aitba-1	CS76649	1206	F1206L	Forward Screen
Yes	App1-16	CS76669	2014	A2014E	ACC2 Reverse
Yes	Balan-1	CS76687	2013	P2013L	ACC2 Reverse
Yes	Blh-1	CS28089	1225	K1225X	Putative Null
Yes	Chi-0	CS76464	474	L474F	ACC2 Reverse
Yes	Col-0	Lab	376	V376A	ACC2 Reverse
Yes	CYR	CS76790	1739	S1739C	ACC2 Reverse
Yes	Db-1	CS28203	1405	R1405Q	ACC2 Reverse
Yes	Dja-1	CS76473	833	G833R	ACC2 Reverse
Yes	Ema-1	CS76480	1355	E1355G	ACC2 Reverse
Yes	Etna-2	CS76487	443	Y443C	Forward Screen
Yes	Gn-1	CS76880	572	Splice Defect	Putative Null
Yes	Grivo-1	CS76888	2059	A2059V	ACC2 Reverse
Yes	Hod	CS76924	2098	P2098S	ACC2 Reverse
Yes	Hod	CS76924	2325	Q2325X	Putative Null

Yes	Hof-1	CS76925	Intron 4	Small Insertion	Putative Null
Yes	lasi-1	CS76944	2115	I2115R	ACC2 Reverse
Yes	In-0	CS76516	520	F520L	ACC2 Reverse
Yes	Ip-Alo-0	CS76662	1171	R1171fs	Putative Null
(Yes)	Ip-Ber-0	CS78887	774	Small Deletion	Putative Null
Yes	Ip-Cum-1	CS76787	4; 618; 1165	R4T; V618I; S1165C	Transit Peptide Reverse
Yes	Ip-Gua-1	CS76894	4; 618; 1165	R4T; V618I; S1165C	Transit Peptide Reverse
Yes	Ip-Hom-4	CS76929	618	V618I	Compare with Ip-Cum-1
Yes	Ip-Lso-0	CS77055	2207	H2207Q	ACC2 Reverse
Yes	Ip-Pal-0	CS77159	494	R494G	ACC2 Reverse
Yes	Ip-Ren-6	CS77212	1479	Δ1479	Putative Null
Yes	Ip-Tor-1	CS77378	538	T538A	ACC2 Reverse
Yes	Ip-Vin-0	CS78846	1171	R1171fs	Putative Null
Yes	Ip-Vis-0	CS78848	618	V618I	Compare with Ip-Cum-1
(Yes)	Ip-Voz-0	CS78849	1479	Δ1479	Putative Null
Yes	Kb-0	CS76524	753	Y753X	Putative Null
Yes	KI-5	CS76528	753	Y753X	Putative Null
Yes	Knox-18	CS76530	1404; 1902	I404K; T1902K	Forward Screen
Yes	Knox-18	CS76530	FL cDNA	Multiple	FL cDNA Same as 1001 Genomes
Yes	La-0	CS76538	FL cDNA	Multiple	FL cDNA Same as 1001 Genomes
Yes	Leska-1-44	CS77030	777	D777N	ACC2 Reverse
Yes	Lm-2	CS28473	475	P475L	ACC2 Reverse
(Yes)	Lu3-30	CS77057	2020	D2020fs	Putative Null
Yes	Lu4-2	CS77058	2020	D2020fs	Putative Null
Yes	Melni-2	CS77080	193*	A193V*	* ACC2 Variant # (ACC1 Reverse)
Yes	MNF-Che-2	CS77096	1821	I1821V	ACC2 Reverse
Yes	Nemrut-1	CS76398	1834	T1834S	ACC2 Reverse
Yes	Petergof	CS77170	809*	V809A*	* ACC2 Variant # (ACC1 Reverse)
Yes	Pog-0	CS76576	725; 1766	N725S; G1766D	ACC2 Reverse
Yes	Qar-8a	CS76581	1376	K1376R; Δ1377	Putative Null
Yes	RRS-10	CS76592	1404; 1902	I404K; T1902K	Forward Screen
Yes	Sch1-7	CS77240	1897	G1897S	ACC2 Reverse
Yes	Sei-0	CS76599	363	F363L	ACC2 Reverse
Yes	Si-0	CS76601	1355	E1355G	ACC2 Reverse
Yes	Smolj-1	CS77256	777	D777N	ACC2 Reverse
Yes	Spro-2	CS77264	1562	Splice Defect	Putative Null
Yes	Ste-2	CS77274	1562	Splice Defect	Putative Null

Yes	Ste-3	CS77275	1562	Splice Defect	Putative Null
Yes	Tol-0	CS76614	1404; 1902	I404K; T1902K	Forward Screen
Yes	Ts-1	CS76615	1689	E1689G	ACC2 Reverse
Yes	Tsu-0	CS28780	762	R762C	ACC2 Reverse
Yes	Tsu-0	CS28780	FL cDNA	Multiple	FL cDNA Same as 1001 Genomes
Yes	Tu-0	CS76617	762	R762C	ACC2 Reverse
Yes	Tul-0	CS76618	1404; 1902	I404K; T1902K	Forward Screen
Yes	Uod-1	CS76621	478	Q478K	ACC2 Reverse
Yes	Vimmerby	CS78845	1562	Splice Defect	Putative Null
Yes	Wa-1	CS76626	739	G739E	ACC2 Reverse
Yes	WalHaesB4	CS76408	1897	G1897S	ACC2 Reverse
Yes	WAR	CS78853	847	L847P	ACC2 Reverse
Yes	WAR	CS78853	7**	$\Delta 7$ (Tic20-IV)	** TIC20-IV Transit Peptide Residue
Yes	WI-0	CS76630	901	Splice Defect	Putative Null
Mixed	Gifu-2	CS76494	404; 1902	I404K Not Predicted; T1902K Confirmed	Knox-18 Group
Mixed	Mdn-1	CS77077	404; 1567	I404K; T1902K Confirmed; E1567K Not Predicted	Knox-18 Group
^a Variants were analyzed by Sanger sequencing.					

348

Name	Primer Sequence	Position
F-01	ATCCGATCTCCTTCTTGGATAATG	Exon 1
F-02	TCAGCTTATTGTGGAGGTGATTAG	Exon 2 / Intron 2
F-03	GGAAGGTCTGCTTCTGTAGATATG	Exon 4 / Intron 4
F-04	GCACATTCGCTACACAACATATC	Intron 6
F-05	GGACGGGTTTGGATCGATATTA	Intron 6
F-06	CAGAGACGAAGAAGACGAAGAAC	Intron 6
F-07	ATGGAACATGGTGGAGGATATG	Exon 9
F-08	CTCTACGTGATGGAGGTCTATTG	Exon 16
F-09	AAGGCATCCTTGAGGTGATTAG	Exon 21 / Intron 21
F-10	GACTTATGGAGCAGCTTGTTTATC	Exon 24
F-11	CTGCAGTTTCTGCCGAGTATAA	Exon 26
F-12	CGACATGTTGAACCTCCTCTATC	Exon 27
F-13	CATAGGTCTGTTGGAGTGAGAATG	Exon 29
F-14	CTCGGTGTAGCTGAAGAAGTAAA	Exon 31
F-15	GGTAAATGGCTTGGTGGTATCT	Exon 31
F-16	GCCTGAAGGGATGATAGAGATAAA	Exon 31
R-01	CTGCAGGAGGACCAAGAAATATAA	Exon 3
R-02	GTCTTCTCTGGACGCTACAATC	Exon 6
R-03	CCAAACCCGTCCTACCATATAAT	Intron 6
R-04	GGTGATTGGACCTTCTCTATG	Exon 7
R-05	GACAGAGAAGTACGACCACATATT	Exon 10
R-06	AGTCCTAATATCTCCACGGATTG	Exon 12
R-07	CTCCATCACGTAGAGTGTGTATTT	Exon 16
R-08	GAAATGGGAGTTCAGGGCTATC	Exon 20
R-09	CTCTGTATGCAGCAGGATTAGG	Exon 24
R-10	GATAAAGGAGCTCGGGCATATT	Exon 26
R-11	CTATCTGCTGCTCACGTAGTATG	Exon 28
R-12	CTGGCCTGTCTGTAACACTATAC	Exon 28
R-13	CGGTTGGTCTAGTCTCTGTATG	Exon 31
R-14	CGGTTTCATCAGCGTACATTTT	Exon 31
R-65	ATTGTGCGCGCTAACAAATAC	3'-UTR
F-109	ATCCTTGAGACTATCTGGCTTGG	5'-UTR
R-63	GCCATTGCCACCAACTTTAC	Exon 2 ^b

^a To prevent amplification of the adjacent *ACC1* locus, the first half of *ACC2* was amplified using F-109 and one of the reverse primers listed above; the second half utilized R-65 and one of the forward primers. The R-63 primer was used infrequently.

^b Specific to *ACC2* N-terminus

351
352