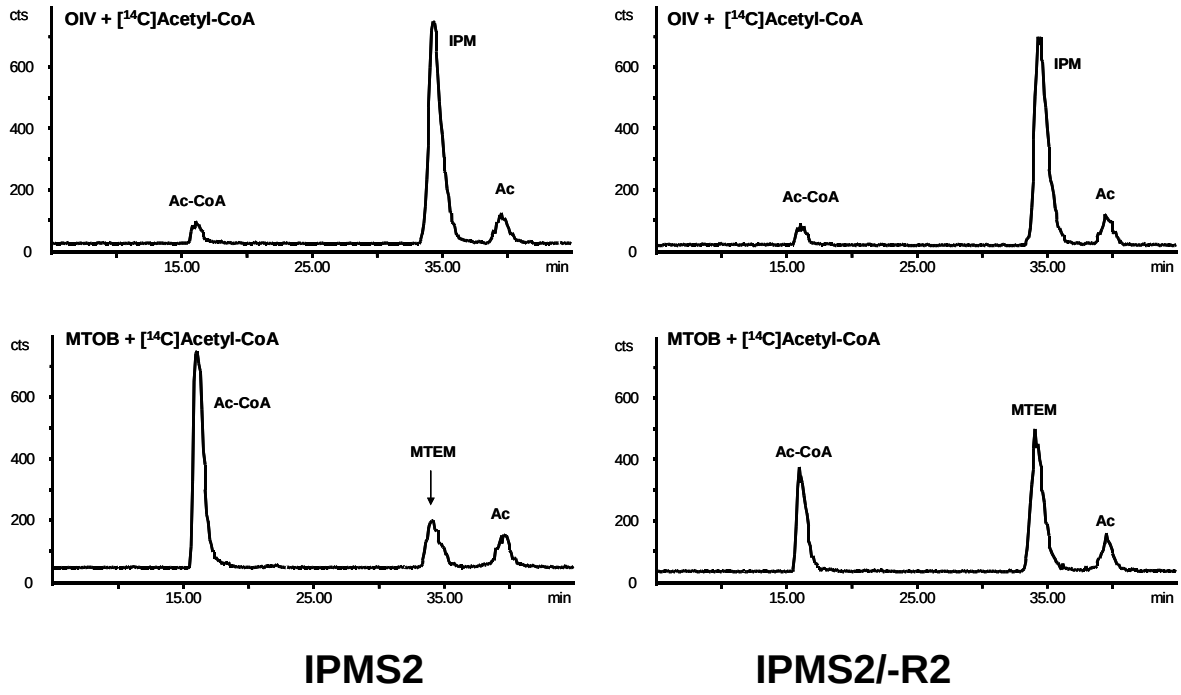

Supplemental Figure 1. Radio-HPLC traces of assays of *A. thaliana* IPMS2 and IPMS2 modified to remove the C-terminal regulatory domain (IPMS2/-R2). The modified enzyme shows increased activity for the MAM substrate in the condensation reaction with [^{14}C]acetyl-CoA. Both enzymes catalyse the conversion of the IPMS substrate, 2-oxoisovalerate (OIV) to [^{14}C]-isopropylmalate (IPM) in good yield, but IPMS2/-R2 has an increased product peak for reaction with 4-methylthio-2-oxobutyrate (MTOB) to give [^{14}C]2-(2'-methylthio)ethylmalate (MTEM). Assays were performed with 1 mM [^{14}C]acetyl-CoA (Ac-CoA), 6 mM 2-oxoalkanoate and 27.5 µg enzyme for 1 h at 30°C.

Supplemental Table 1. Oligonucleotide primers used in this study

Name	Sequence (5'→3')[a]	Purpose[b]
1ipms1i+atg[c]	ATGTGCTCAATCTCAGATCCTTCTC	F, Construction of IPMS1/-R1.
ipms1-short[d]	TTCATCAGATACTAAAGCTATTATGTCCGC	R, Construction of IPMS1/-R1.
1ipms2m+atg[c]	ATGCTTACCACCGCCGGAAAATT	F, Construction of IPMS2/-R2; SOEing first part of IPMS2/PS00816$_{MAM}$ and IPMS2/-R2/PS00816$_{MAM}$; Third PCR in SOEing IPMS2/PS00816$_{MAM}$ and IPMS2/-R2/ PS00816$_{MAM.}$
ipms2-short[d]	ATCAGATACTAAAGCTATTAAGTCAGCATCG	R, Construction of IPMS2/-R2; SOEing second half of IPMS2/-R2/PS00816$_{MAM}$ Third PCR in SOEing of IPMS2/-R2/PS00816$_{MAM}$
mam1xff[d]	ATGGGTTGCTCTTCTGTGTCC	F, SOEing MAM1 part of MAM1/+R2; Third PCR in SOEing of MAM1/+R2.
mam1+sttipms2[d]	cagacagcctctggctgaaaTTCATCGCTAGATGTTACTAATGCC	R, SOEing MAM1 part of MAM1/+R2.
ipms2+sttmam1[d]	attagtaacatctagcgatgaaTTTCAGCCAGAGGCTGTCTG	F, SOEing R2-part of MAM1/+R2.
2ipms2n[c]	GGCAGGGACTTCGTTGGTC	R, SOEing R2-part of MAM1/+R2 and MAM3/+R2; Third PCR of SOEing MAM1/+R2 and MAM3/+R3; SOEing second part IPMS2/ PS00816$_{MAM}$; Third PCR in SOEing IPMS2/PS00816$_{MAM.}$
mam3exff[d]	ATGGCTGAGTCCAAAAAGGTGGC	F, SOEing MAM3 part of MAM3/+R2; Third PCR in SOEing of MAM3/+R2.
mam3+sttipms2[d]	cagacagcctctggctgaaaTTCAGCACCGTTCACCACT	R, SOEing MAM3 part of MAM3/+R2.
imps2+sttmam3[d]	agtggtgaacggtgctgaaTTTCAGCCAGAGGCTGTCTG	F, SOEing R2-part of MAM3/+R2.
ipms2-mn-rv[d]	caagaccaaggtcgttgtgacaatgaacagcGATTATAACATTTTGGATC	R, SOEing first part IPMS2/PS00816$_{MAM}$ and IPMS2/-R2/PS00816$_{MAM}$.
ipms2_mn_for[d]	gctgttcattgtcacaacgaccttggtcttgCCACCGCCAACACTTTATC	F,SOEing second part IPMS2/PS00816$_{MAM}$ and IPMS2/-R2/PS00816$_{MAM}$
H167L-for[d]	GCTAAGCGGCCAAGAATCCTTACGTTTATTGCCACTAG	F, Point mutation primer.
H167L-rev[d]	CTAGTGGCAATAAACGTAAGGATTCTTGGCCGCTTAGC	R, Point mutation primer.
S216G-for[d]	GTGAAGATGTTGAATTTGGTCCAGAAGATGCCGGAAGATCG	F, Point mutation primer.
S216G-rev[d]	CGATCTTCCGGCATCTTCTGGACCAAATTCAACATCTTCAC	R, Point mutation primer.
L143I-for[d]	GTCCCTGTAATCTGTGGTATCTCGAGATGTAACAAG	F, Point mutation primer.

L143I-rev[d]	CTTGTTACATCTCGAGATACCACAGATTAC AGGGAC	R, Point mutation primer.
P252G-for[d]	GCTGGAGCAACCACTCTTAACATAGGTGA CACTGTTGGTATAACC	F, Point mutation primer.
P252G-rev[d]	GGTTATACCAACAGTGTCACCTATGTTAAG AGTGGTTGCTCCAGC	R, Point mutation primer.
N250G/P252Gfor[d]	GCTGGAGCAACCACTCTTGGCATAGGTGA CACTGTTGGTATAACC	F, Point mutation primer.
N250G/P252Grev[d]	GGTTATACCAACAGTGTCACCTATGCCAAG AGTGGTTGCTCCAGC	R, Point mutation primer.

[a]Lower case letters indicate the overhangs of primers used for gene SOEing
[b]R = reverse primer, F = forward primer
[c]Standard purified primers from JenaBioscience (Jena, Germany)
[d]HPLC purified primers synthesized by MWG (Ebersberg, Germany)