Supplemental figure captions

Supplemental Figure 1. Radio-HPLC analyses of the products of *E. coli* IPMS. A, Incubation with 500 µM [^{14}C]acetyl-CoA and 3 mM 2-oxoisovalerate shows [^{14}C]-2-isopropylmalate (IPM) as the only radioactive labelled product. B, Incubation with 4-methylthio-2-oxobutyrate and [^{14}C]acetyl-CoA yields a small amount of [^{14}C]-2-(2'-methylthio)ethylmalate (MTEM), whereas most of the [^{14}C]acetyl-CoA (Ac-CoA) remains unconverted. C, In the absence of 2-oxo-acid substrate, a small amount of [^{14}C]acetate (Ac) is formed, whereas most of the [^{14}C]acetyl-CoA (Ac-CoA) remains intact.

Supplemental Figure 2. Predicted positions of the T-DNA inserts (vertical arrows) in the Salk insertional mutant lines for *IPMS1* (Salk_101771) and *IPMS2* (Salk_051060 and Salk_000074). The genes are shown with 5'-UTR and 3'-UTR (grey boxes), exons (black boxes) and introns (lines in between boxes).

Supplemental Figure 3. Analyses of the glucosinolate content (3MSOP = 3-methyl-sulfinylpropyl; 4MSOB = 4-methylsulfinylbutyl; 7-MSOH = 7-methylsulfinylheptyl; 4MTB = 4-methylthiobutyl; 8MSOO = 8-methylsulfinyloctyl; I3M = indol-3-ylmethyl; 4MOI3M = 4-methoxyindol-3-ylmethyl; 1MOI3M =1-methoxyindol-3-ylmethyl) of homozygous T-DNA insertion lines for *IPMS1* (A, Salk_101771 [mm]) and *IPMS2* (B, Salk_051060 [mm] and Salk_000074 [mm]). The *IPMS* mutants show no significant changes in glucosinolate content in comparison with the corresponding outsegregants (ww) and Col-0 wildtype. Error bars indicate standard deviation.

Supplemental Figure 4.

Semi-quantitative RT-PCR analysis of *IPMS1* and *IPMS2* gene expression in various plant tissues of *A. thaliana*. RNA was extracted from organs of 6-week old plants (rt, root; ml mature leaf; el, expanding leaf; cl, cauline leaf; s1, primary stem; s2, secondary stem; fl, inflorescence; si, silique) and converted to cDNA using RT and oligo dT as a primer. PCR using the cDNA as a template were done in triplicate with the primers listed in Supplemental Table 1. Average fluorescence intensities of the ethidium- bromide stained gel bands were normalized relative to that of *ACT8* (At1g49240).

Supplemental Figure 5. A, Expression levels of *IPMS1* and *IPMS2* in different plant tissues according to the Gene Atlas tool of GENEVESTIGATOR. Anatomical descriptions on the x-axis are based on standard terms (http://www.plantontology.org) with the numbers indicating a classification into six main groups and the corresponding subgroups. The numbers in parentheses represent the number of micro-arrays for each tissue or growth stage. B, Expression levels of *IPMS1* and *IPMS2* at various growth stages according the Gene Chronologer tool. The numbers on the x-axis indicate the age in days. The symbols represent the following nine successive growth stages: seed germination, development of first 2 rosette leaves, development of 3-5 rosette leaves, rosette growth to 20% of final size, rosette growth to 50% final size, inflorescence emergence, opening of 10% of flower buds to be produced, opening of 50% of flower buds, flowering completed and first silique shattered. The error bars depict standard errors. A signal intensity of 1000 is the target value used for normalization of all chip data in the GENEVESTIGATOR-program and signal intensities higher than 200 are considered to be above background level.

Supplemental Table I. Oligonucleotide primers used in this study

Name	Sequence (5'→3')	Purpose
LBa1[a]	TGGTTCACGTAGTGGGCCATCG	Screening Salk lines for T-DNA insertion
ipms1for[a]	GCCTAACGGTCCTCTTTTCTTCAT	Genomic screening of S101771
ipms1rev[a]	TTTTTCTGCTCAGCCACGGTTTT	Genomic screening of S101771
2ipms2d[c]	TTGATCTCTGAGATTTGCAGGTA	Genomic Screening of S000074
1ipms2w[b]	GGGTTCTAATCCGCGGAGA	Genomic screening of S000074
1ipms2t[b]	TGCCTAGTGAGTTTGGTCAGT	Genomic screening of S051060 Transcript level of *IPMS2*
2ipms2u[b]	GGGGCTGCGTTTGCATAC	Genomic screening of S051060 Transcript level of *IPMS2*
1ipms1g	AACTTGCTGACGCTGATGG	Transcript level of *IPMS1*
2ipms1r	AAAAGAACCTAACTTCTGTCTGAC	Transcript level of *IPMS1*
act8R[c]	GTTTTTATCCGAGTTTGAAGAGGC	Transcript level of actin 8
act8F[c]	ATGAAGATTAAGGTCGTGGCAC	Transcript level of actin 8
1ipms1k[c]	ATGGCGTCTTCGCTTCTGAG	Cloning *IPMS1* into pCR4-Topo
2ipms1j[c]	GGCAGCGACTCTGTTTTTTTG	Cloning *IPMS1* into pCR4-Topo; Subloning *IPMS1* into pCR-T7/CT-TOPO;
1ipms2m[c]	CTTACCACCGCCGGAAAATT	Cloning *IPMS2* into pBAD-Topo;
2ipms2n[c]	GGCAGGGACTTCGTTGGTC	Cloning *IPMS2* into pBAD-Topo; Subloning *IPMS2* into pCR-T7/CT-TOPO
1ipms2m+atg[c]	ATGCTTACCACCGCCGGAAAATT	Subloning *IPMS2* into pCR-T7/CT-TOPO
1ipms1i+atg[c]	ATGTGCTCAATCTCAGATCCTTCTC	Subcloning *IPMS1* into pCR-T7/CT-TOPO
mut1-for[d]	CTTCCAAGGAAAAACTTGACATCGCTCGTCAGC TAG	Restoring point mutation in cDNA of *IPMS1*
mut1-for-r[d]	CTAGCTGACGAGCGATGTCAAGTTTTTCCTTGG AAG	Restoring point mutation in cDNA of *IPMS1*
IPMEff[e]	CGCGGATCCAGCCAGCAAGTCATTATTTTCG	Cloning *leuA* of *E. coli* into pET28a
IPMErv[e]	CCGCTCGAGCACGGTTTCCTTCTTGTTTTCG	Cloning *leuA* of *E. coli* into pET28a

[a]Standard salt-free primers from Operon (Huntsville, AL)
[b]Desalted and purified by gel filtration from Invitrogen (Carlsbad, CA)
[c]Standard purified primers from Jenabioscience (Jena, Germany)
[d]SDS-gel purified primers synthesized by Qiagen (Köln, Germany).
[e]HPLC purified primers synthesized by MWG (Ebersberg, Germany)