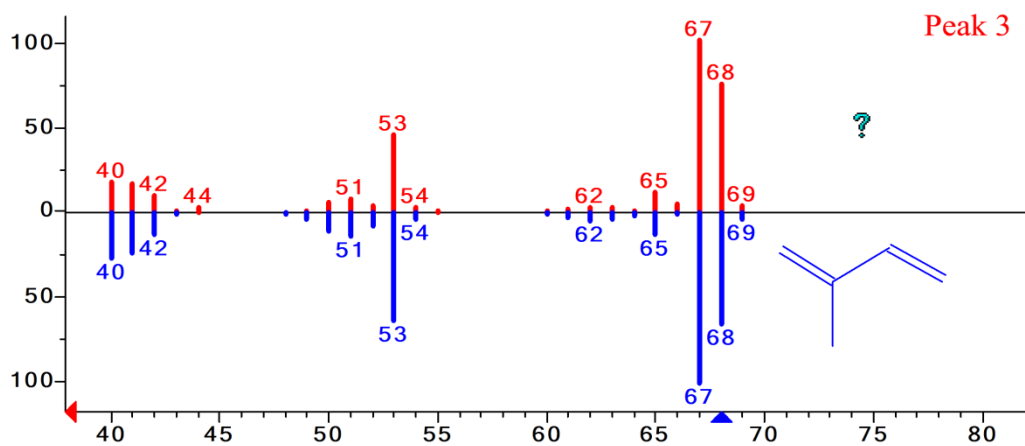
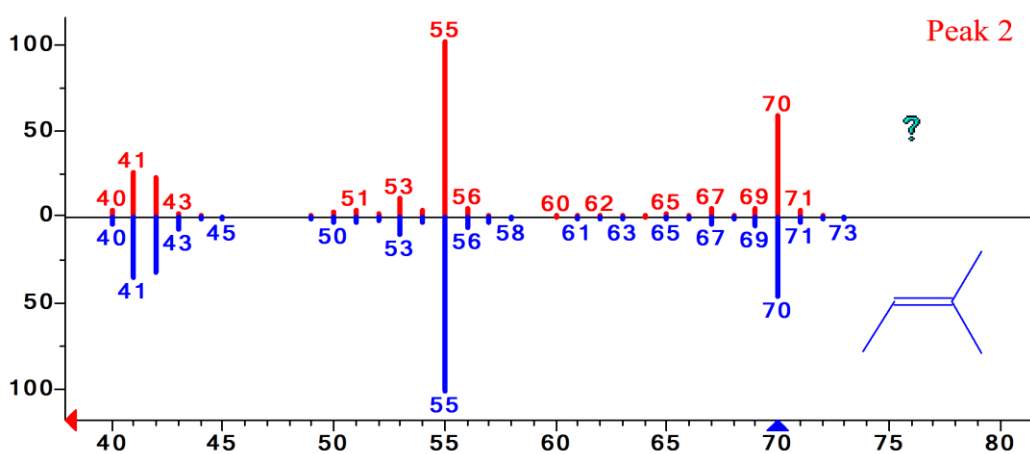
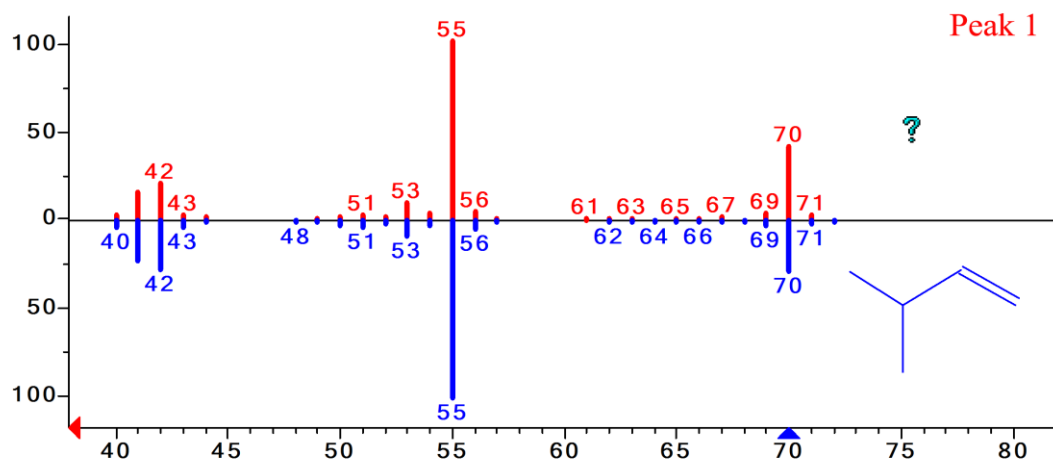


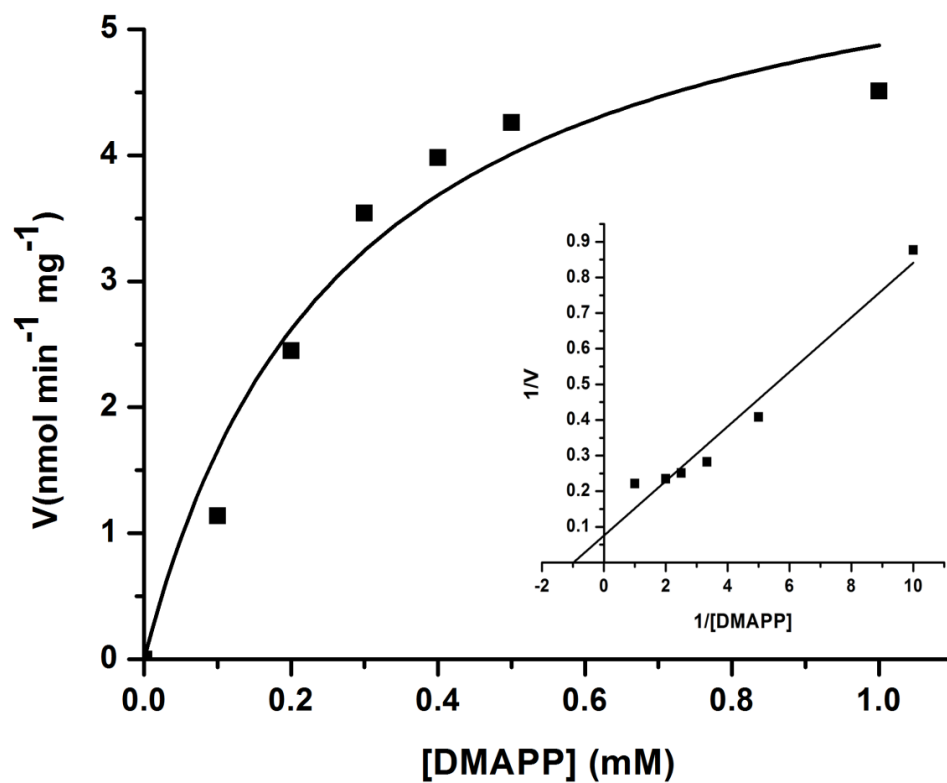
**Fig. S1 GC profile.** (A) GC profile of the headspace of the *E.coli* trans109 strain containing pSTV165HF after incubation for 10 h at 37 °C. (B) GC profile of isoamylene and isoprene standard. (a) 3-methyl-1-butene; (b) 2-methyl-2-butene; (c) 2-methyl-1-butene; (d) isoprene.



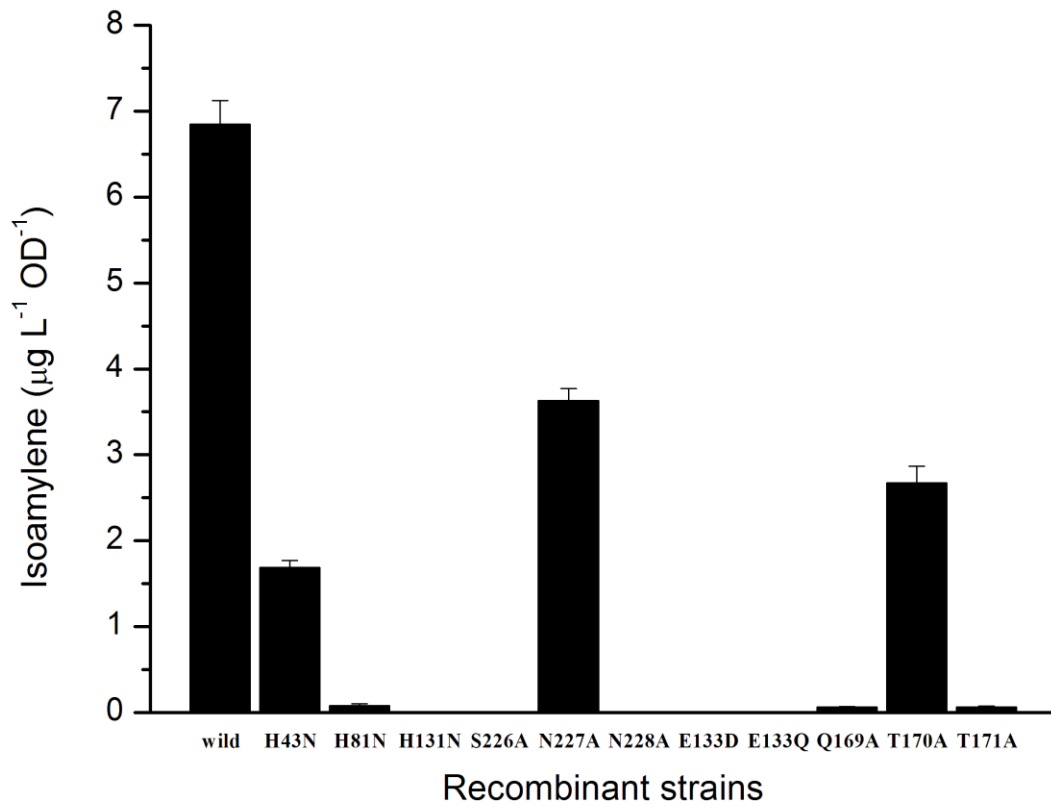
**Fig. S2.** Identification of the unknown substances in the headspace of the liquid cultures by GC-MS analysis. Red is the mass spectrum of the sample, Blue is the mass spectrum of the standard. Peak 1, Peak 2 and Peak 3 are identified to be 3-methyl-1-butene, 2-methyl-2-butene and isoprene respectively.

Table S1. Isoamylene and isoprene reaction system production under various conditions

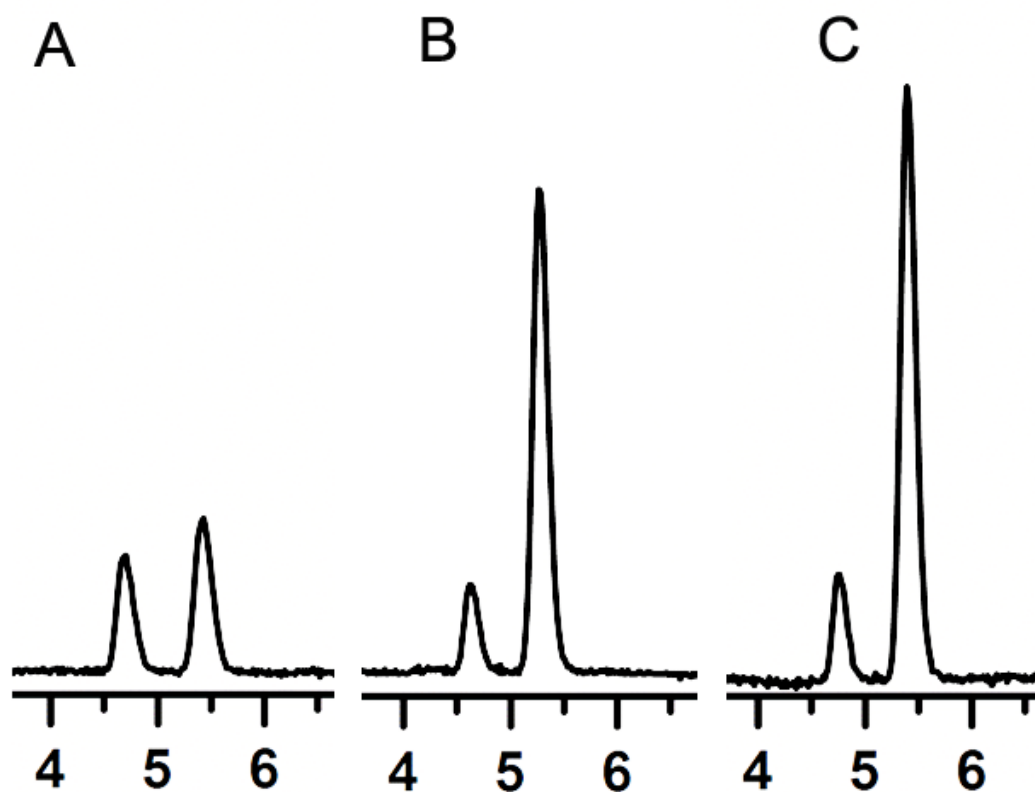
Supplements						Isoamylene/Isoprene
IspH	FNR	Fdx	Fld	NADPH	Mg <sup>2+</sup>	production
-	+	+	-	+	+	-
+	-	+	-	+	+	-
+	+	-	+	+	+	-
+	+	+	-	-	+	-
+	+	+	-	+	+	+
+	+	+	-	+	-	+



**Fig. S3.** Michaelis-Menten plot of the activity of the recombinant protein IspH toward DMAPP. DMAPP concentrations were varied from 0 to 1 mM. The  $K_m$  value for DMAPP was approximately 275  $\mu\text{M}$ . The inset shows the Lineweaver-Burk plot.



**Fig. S4.** Isoamylene production of the recombinant strains in which wild-type IspH and the IspH mutants were expressed. Error bars represent the standard deviations of three replicates.



**Fig. S5.** GC profile of isoamylene (2M2B and 3M1B) produced by the recombinant *E. coli* strains. A. The production of *E. coli* trans109 strain containing pSTV12HF, the ratio of 2M2B (2.1  $\mu\text{g/L}$ ) and 3M1B (1.4  $\mu\text{g/L}$ ) is approximately 1.5:1. B. The production of of *E. coli* trans109 strain containing pSTV168HF, the ratio of 2M2B (6.1  $\mu\text{g/L}$ ) and 3M1B (1.2  $\mu\text{g/L}$ ) is approximately 5:1. C. The production of the *E. coli* trans109 strain containing pSTV165HF, the ratio of 2M2B (7.3  $\mu\text{g/L}$ ) and 3M1B (1.4  $\mu\text{g/L}$ ) is approximately 5:1.

**Table S2 Strains and plasmids in this study**

Strain or plasmid	Genotype or relevant characteristic	Source or reference
<b><i>E. coli</i></b>		
BL21(DE3)	<i>F ompT gal dcm lon hsdS<sub>B</sub>(r<sub>B</sub><sup>-</sup> m<sub>B</sub><sup>-</sup>) λ(DE3 [lacI lacUV5-T7gene1 ind1 sam7 nin5])</i>	TransGen Biotech
<i>Trans DMT</i>	<i>F φ80 lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(r<sub>K</sub><sup>-</sup>, m<sub>K</sub><sup>+</sup>) phoA supE44 thi-1 gyrA96 relA1 tonA</i>	TransGen Biotech
<i>Trans109</i>	<i>endA1 recA1 gyrA96 thi-1 hsdR17 (r<sub>k</sub><sup>-</sup>m<sub>k</sub><sup>+</sup>)relA1 supE44 D (lac-proAB) [F'traD36 proAB laqI qZΔM15]</i>	TransGen Biotech
<b><i>Bacillus</i></b>		
<i>Bacillus</i> sp. N16-5	A facultative alkaliphilic strain, producing isoprene	Laboratory stock
N165D1	Δ <i>fer</i>	This study
N165D2	Δ <i>fld</i>	This study
N165H	<i>Bacillus</i> sp. N16-5 containing pMH	This study
N165D1H	N165D1 containing pMH	This study
N165D2H	N165D2 containing pMH	This study
<i>Bacillus subtilis</i> 168	used for <i>ispH</i> and <i>fer</i> gene amplification	Laboratory stock
<b>Plasmid</b>		
pSTV28	<i>E. coli</i> cloning vector; Cm <sup>r</sup>	TaKaRa
pSTV165H	pSTV28 containing the <i>ispH</i> gene from <i>Bacillus</i> sp. N16-5	This study
pSTV165HF	pSTV28 containing the <i>fer</i> gene and the <i>ispH</i> gene from <i>Bacillus</i> sp. N16-5	This study
pSTV168HF	pSTV28 containing the <i>fer</i> gene and the <i>ispH</i> gene from <i>Bacillus subtilis</i> 168	This study
pSTV12HF	pSTV28 containing the <i>fld</i> gene and the <i>ispH</i> gene from <i>Escherichia coli</i>	This study
pNNB194	Shuttle vector used for gene disruption; Amp <sup>r</sup> Erm <sup>r</sup>	Laboratory stock
pND1	pNNB194 containing a 1.6 kb fragment fused by two 800 bp homologous regions flanking the <i>fer</i> gene	This study
pND2	pNNB194 containing a 1.6 kb fragment fused by two 800 bp homologous regions flanking the <i>fld</i> gene	This study
pMK4	Shuttle vector used for gene expression; Amp <sup>r</sup> Cm <sup>r</sup>	Laboratory stock
pMH	pMK4 containing the <i>ispH</i> gene under the control of the P <sub><i>ldh</i></sub> promoter	This study
pET-28a(+)	used for expression of IspH, FNR and ferredoxin proteins from <i>Bacillus</i> sp. N16-5	Novagen

**Table S3 Primers used in this study**

<b>Primer</b>	<b>Sequence (5'→3')</b> <sup>a</sup>	<b>Purpose</b>
QFLAupF	<u>CGGGATCC</u> ATCACGTGATCATATTATTGC	pND1 construction
QFLAupR	TCGTTATTTTAAGGTAGTAGCGCTTGCAAATACGAGTA ATAC	pND1 construction
QFLAdownF	GTATTACTCGTATTTGCAAGCGCTACTACCTTAAAATA ACGA	pND1 construction
QFLAdownR	<u>CGGAATTC</u> GATCAAGTTATGAAGCTTTCACG	pND1 construction excision
QFLAF	CTATTCCTTGCTGTTCCGATGGC	confirmation excision
QFLAR	ACATGAGGAGGTAGTTGTTAATGG	confirmation pND1
QFERupF	<u>CGGAATTC</u> CTGTAGCCTGTGCTGAATGATG ACCGGTGGCCGATTATTTACACAAAAGCCTCCCATTA	construction pND1
QFERupR	AG CTTTAATGGGAGGCTTTTGTGTAATAATCGGCCACCG	construction pND1
QFERdownF	GT	construction pND1
QFERdownR	<u>CGGGATCC</u> TCAATCAAATGTCATATAAACG	construction excision
QFERF	CCATAATCGATACATTACCGTG	confirmation excision
QFERR	CATGGTCCATCTCCTAACGTGC	confirmation pMH
MPldhupF	<u>CCGGAATTC</u> CTGATGGTAGGACGCTTGTA GGAAATTTTCAAAACCTCCATGTTTAAACATCTACCTT	construction pMH
MPldh275F	TCCC GGGAAAGGTAGATGTTTAAACATGGAGGTTTTGAAAA	construction pMH
M275PldhR	TTTCC	construction pMH
M275downR	<u>CGGGATCC</u> TTAGGCCCGCTCCGCTTTTTTCTTTCTC	construction

<sup>a</sup> Underlined, restriction enzyme recognition sites.