Supplementary Material

Supplementary Figure Legends

Fig. S1
The elution pattern of apo GPPMT from size exclusion chromatography using S200 10/300 column (GE healthcare, Fairfield, USA) is shown in red. Retention volumes of standard proteins are shown in blue with their molecular weights.

Fig. S2
Hydrophobic interaction and hydrogen bond formation between monomers of hexameric GPPMT related with point group 32. From (a) to (e), the apo- structures are shown. In (f), the SFG/GPP complex structure is shown. In order to clarify, it is defined that monomers A-B and monomers A-C are related with two-fold rotation, thus monomers B-C is related with three-fold rotation. (a) Hydrogen bonds between crystallographically two-fold related monomer A (green) and monomer B (magenta) are shown with dotted lines. Numbers indicate distance between two atoms. Residues from monomer B is indicated with a prime sign next to residue numbers. (b) Residues making hydrophobic interactions between monomer A (green stick with green surface) and monomer A (magenta stick) are shown. Residues from monomer B is indicated with a prime sign next to residue numbers. (c) Hydrogen bonds between crystallographically two-fold related monomer A (green) and monomer C (light blue) are shown with dotted lines. Numbers indicate distance between two atoms. Residues from monomer C is indicated with a prime sign next to residue numbers. (d) Residues making hydrophobic interactions between monomer A (green stick with green surface) and monomer C (light blue stick) are shown. (e) The monomer B-C interface related with three-fold rotation axis. Two residues at the interface which are hydrogen bonded are described with distance. Residue from monomer C is indicated with a prime sign next to residue number. (f) Hydrogen bonds and hydrophobic interaction only observed in SFG/GPP complex structure. Monomer A (green) - C (light blue) is related with NCS two-fold axis. Residues from monomer C is indicated with a prime sign next to residue numbers.

Fig. S3
Amino acid sequence alignment of GPPMT and structurally homologous proteins by CLUSTALW (Thompson *et al.*, 1994) and ESPRIPT (Gouet *et al.*, 2003). The secondary structure of GPPMT (substrate-bound form) is also shown with typically conserved motifs. Identical residues are highlighted in red, and similar residues are framed in black with red letters. Putative catalytic base of GPPMT is marked with a blue star. Residues recognizes SAM are marked with blue circles.

Fig. S4
Enzymatic activity monitored by capillary GC-MS (Shimadzu GC-MS-QP2010). (a) Reaction scheme catalyzed by GPPMT. (b) Retention time (horizontal axis) of substrate (GPP and SAM) enzyme mixture from gas chromatography of each sample as labeled in the inset. The vertical axis indicates intensity. The signals observed around 5 min are from hydrolyzed product of GPP (geraniol), the signals around 5.8 min are from hydrolyzed product of 2-methyl GPP (2-methyl geraniol). (c) GC-MS spectra of geraniol

with m/z along the horizontal axis and relative intensity of the signal for each component along the vertical axis. (d) GC-MS spectra of 2-methyl geraniol.

Fig. S5
Amino acid sequence alignment of GPPMT , IPPMT, and representative of sterol 24-C-methyltransferase (EC. 2.1.1.41), cycloartenol 24-C-methyltransferase (EC. 2.1.1.142), and 24-methylenesterol C-methyltransferase (EC. 2.1.1.143). The protein designations are as follows; GPPMT, GPPMT from *Streptomyces lasaliensis*; Lon23, IPPMT from *Streptomyces argenteolus*; EC.2.1.1.41_Pichia , sterol 24-C-methyltransferase from *Pichia pastoris*; EC.2.1.1.41_Cryptococcus, sterol 24-C-methyltransferase from *Cryptococcus gattii*; EC.2.1.1.42_Candida, 24-C-methyltransferase from *Candida albicans*; EC.2.1.1.42_Saccharomyces, sterol 24-C-methyltransferase from *Saccharomyces cerevisiae*; EC.2.1.1.43_Arabidopsis, 24-methylenesterol C-methyltransferase from *Arabidopsis thaliana*; EC.2.1.1.43_Glycine, sterol 24-C methyltransferase from *Glycine max*.

Fig. S6
Proposed biosynthesis pathway of KS-505a (longestin). IPP methylation with IPPMT (Lon23) to produce *Z*-3-methyl IPP (mIPP) was experimentally confirmed. Methyl groups introduced are shown in pink circle.

Supplementary Table S1

Primer sequences used for generating mutants are listed on the table below. Bases changed for mutagenesis are underlined.

mutants	Forward primer	Reverse primer
Y59F	CTCTACCACCACCACTTCGGCATCGGTGCCGTG	CACGGCACCGATGCCGAAGTGGTGGTGGTAGAG
E181D	CCTCGTGGAACAACGATTCGAGCATGTACGTCG	CGACGTACATGCTCGAATCGTTGTTCCACGAGG
E181A	CTCGTGGAACAACGCGTCGAGCATGTACGTCGAC	GTCGACGTACATGCTCGACGCGTTGTTCCACGAG

References

Gouet, P., Robert, X. & Courcelle, E. (2003). *Nucleic Acids Res.* **31**, 3320-3323.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). *Nucleic Acids Res.* **22**, 4673-4680.

Fig. S1

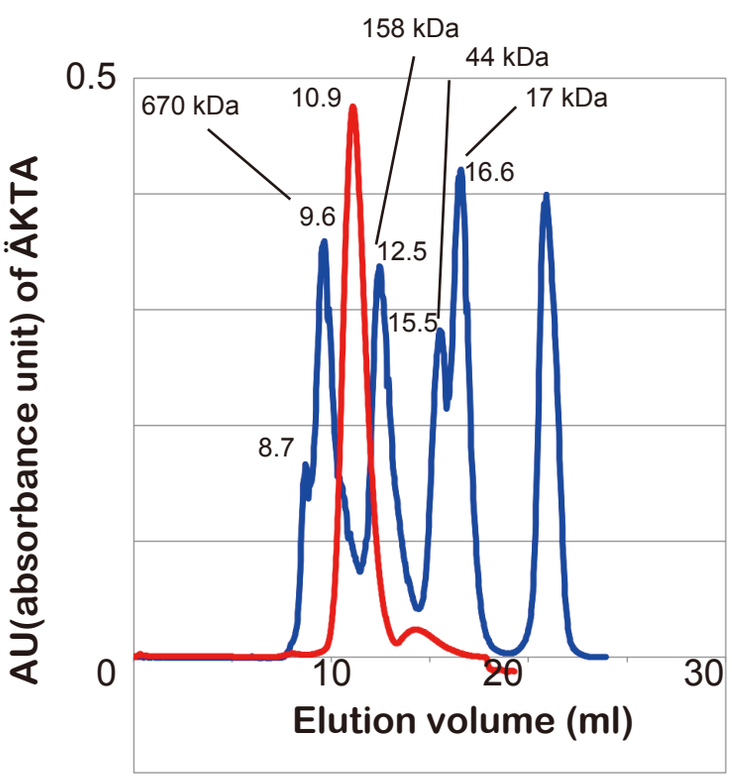
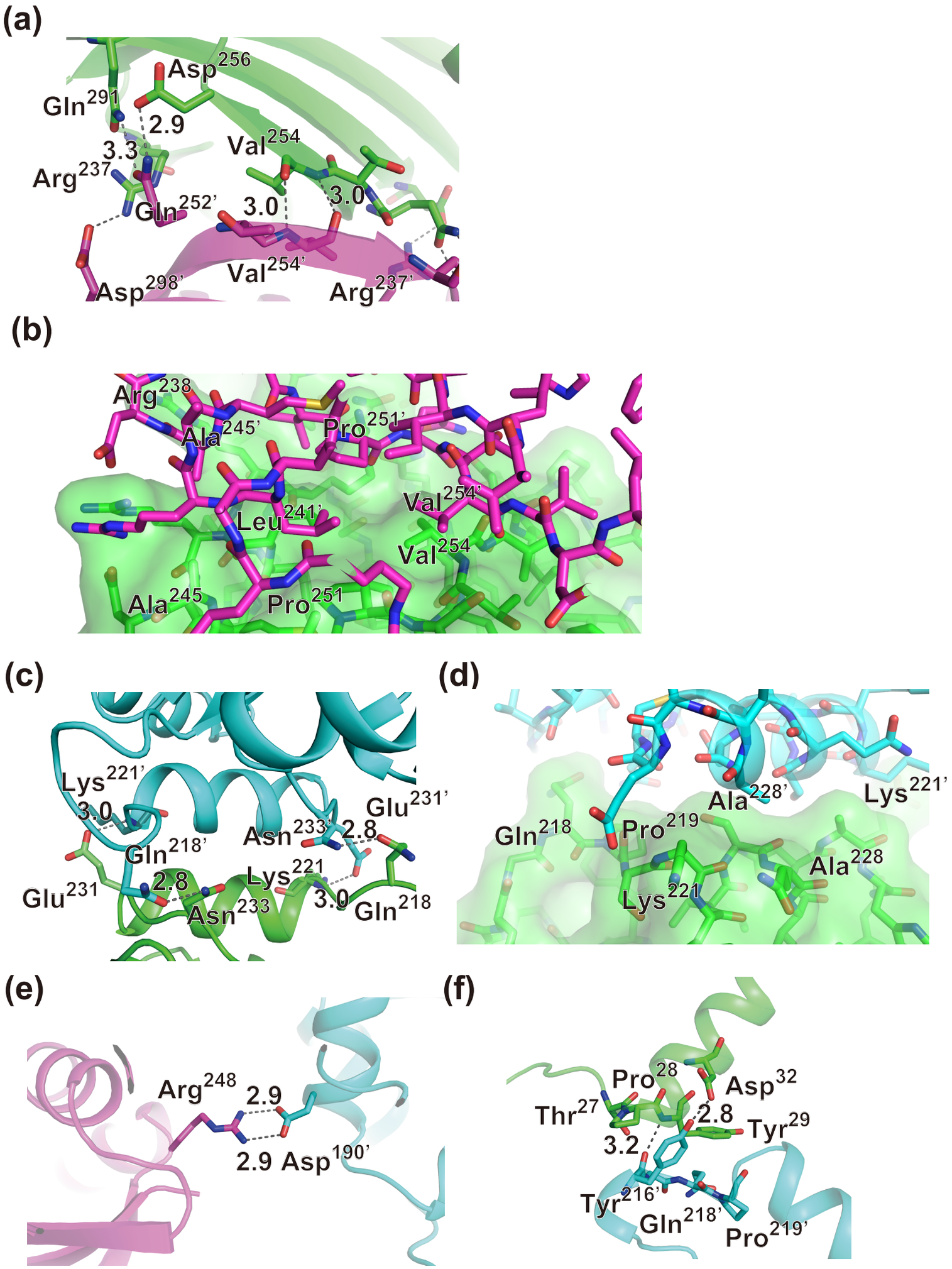

158 kDa
44 kDa
0.5
670 kDa
10.9
17 kDa
16.6
9.6
12.5
15.5
8.7
AU(absorbance unit) of ÄKTA
0
10
20
30
Elution volume (ml)

Fig. S2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3.3
Val254
Arg237
Gln252'
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3.0
Asp298'
Val254'
Arg237'
(b)
Arg238
Ala245'
Pro251'
Leu241'
Val254'
Val254
Ala245
Pro251
(c)
Lys221'
3.0
Glu231'
Asn233'
2.8
Gln218'
2.8
Lys221
Glu231
3.0
Asn233
Gln218
(d)
Ala228'
Lys221'
Gln218
Pro219
Ala228
Lys221
(e)
Arg248
2.9
2.9
Asp190'
(f)
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Tyr29
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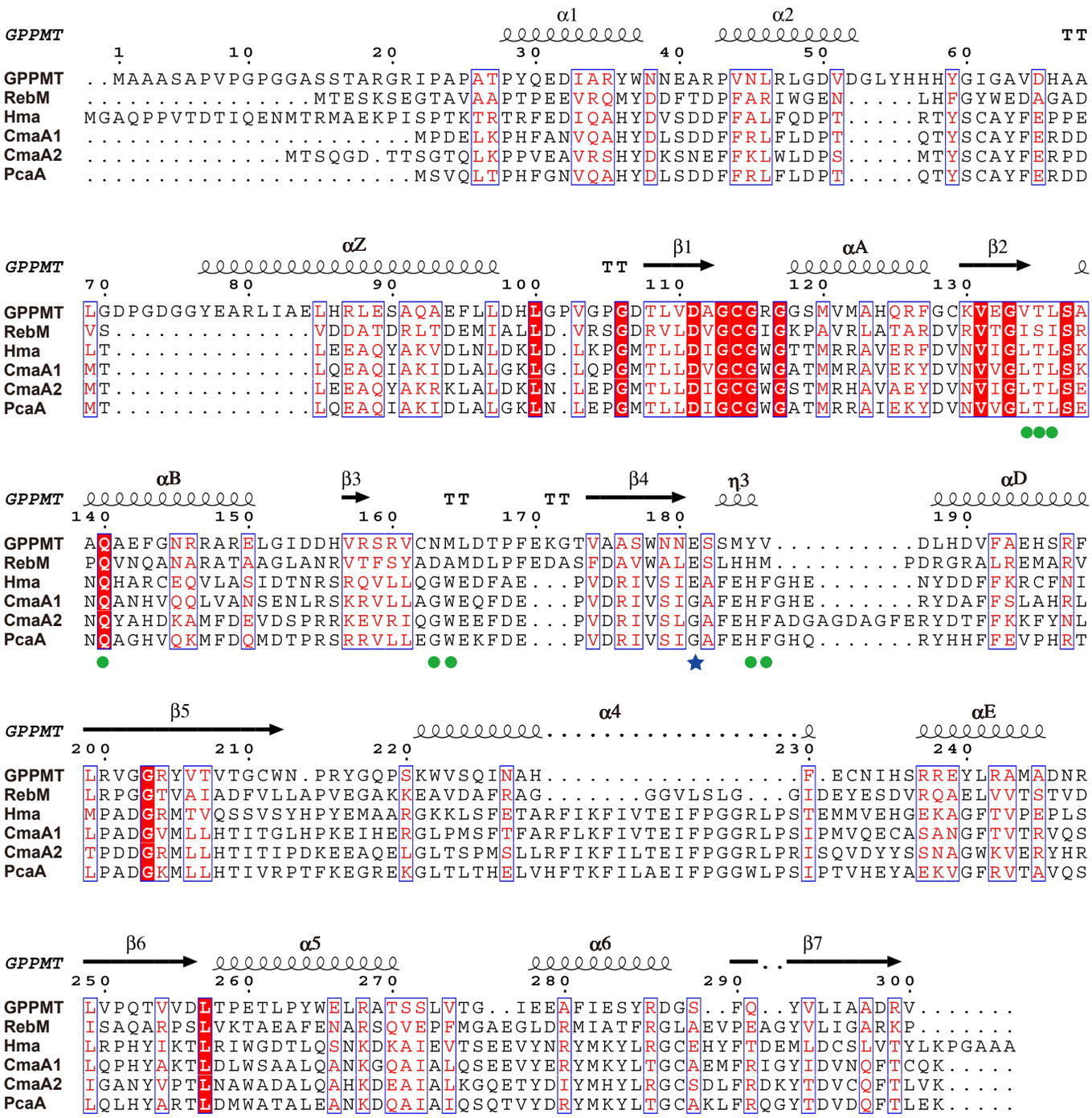
Tyr216'
Gln218'
Pro219'

Fig. S3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α2
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CmaA2 MTSQGD.TTSGTQLKPPVEAVRSHYDKSNEFFKLWLDPSMTYSCAYFERPD
PcaA MSVQLTPHFGNVQAHYDLSDDFFRLFLDPTQTYSCAYFERDD
αZ
β1
αA
β2
GPPMT
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VS VDDATDRLTDEMIALLD.VRSGDRVLDVGCGIGKPAVRLATARDVRVTGISISR
LT LEEAQYAKVDLNLDKLD.LKPGMTLLDIGCGWGTTMRRAVERFDVNVIGLTLSK
MT LQEAQIAKIDLALGKLG.LQPGMTLLDVGCGWGATMMRAVEKYDVNVVGLTLSK
MT LEEAQYAKRKLALDKLN.LEPGMTLLDIGCGWGSTMRHAVAEYDVNVIGLTLSE
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αB
β3
β4
η3
αD
GPPMT
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β5
α4
αE
GPPMT
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α5
α6
β7
GPPMT
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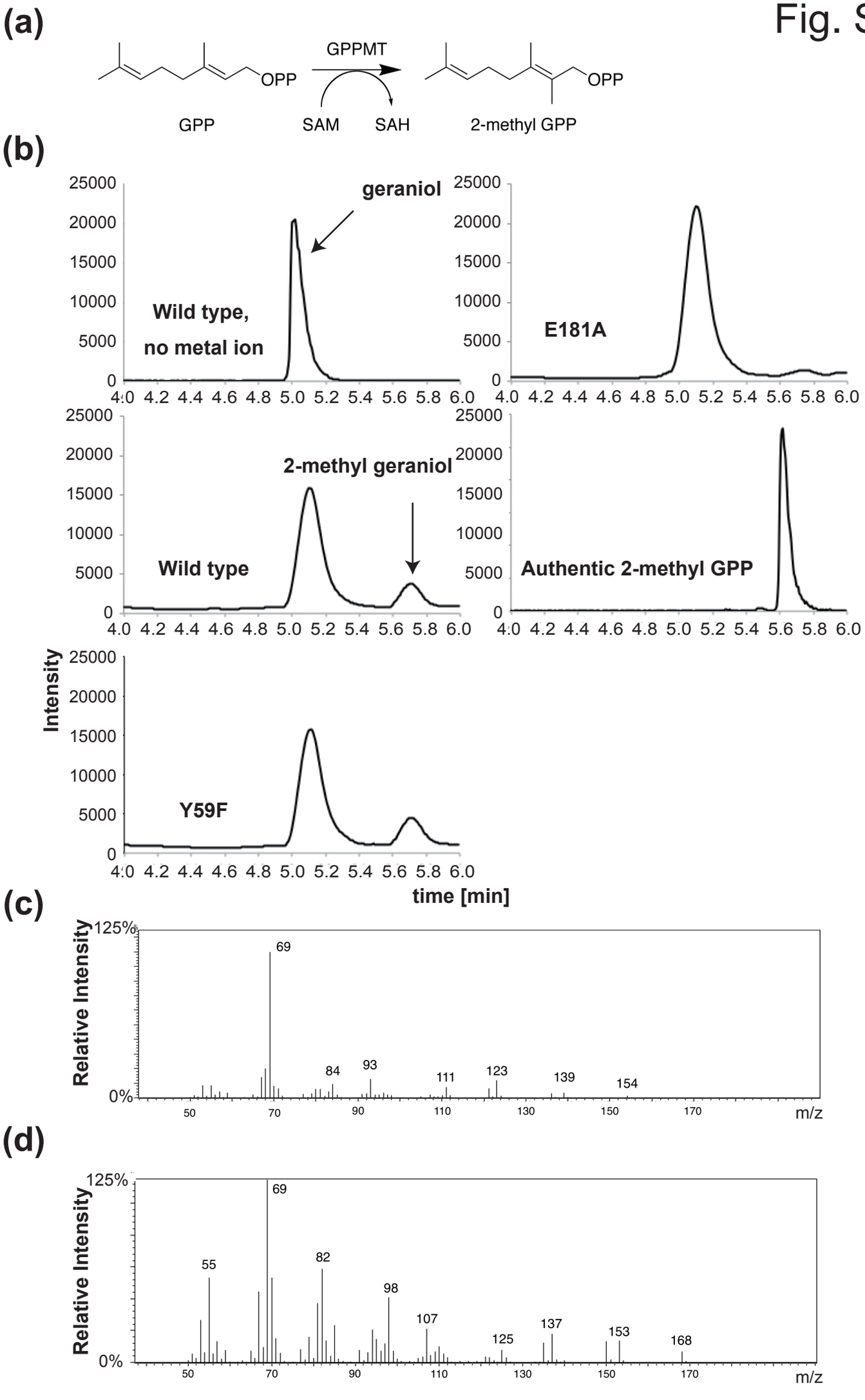
Fig. S4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OPP
OPP
GPP
SAM
SAH
2-methyl GPP
(b)
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Wild type,
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2-methyl geraniol
Wild type
Authentic 2-methyl GPP
Y59F
Intensity
time [min]
(c)
Relative Intensity
125%
0%
69
84
93
111
123
139
154
m/z
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Relative Intensity
125%
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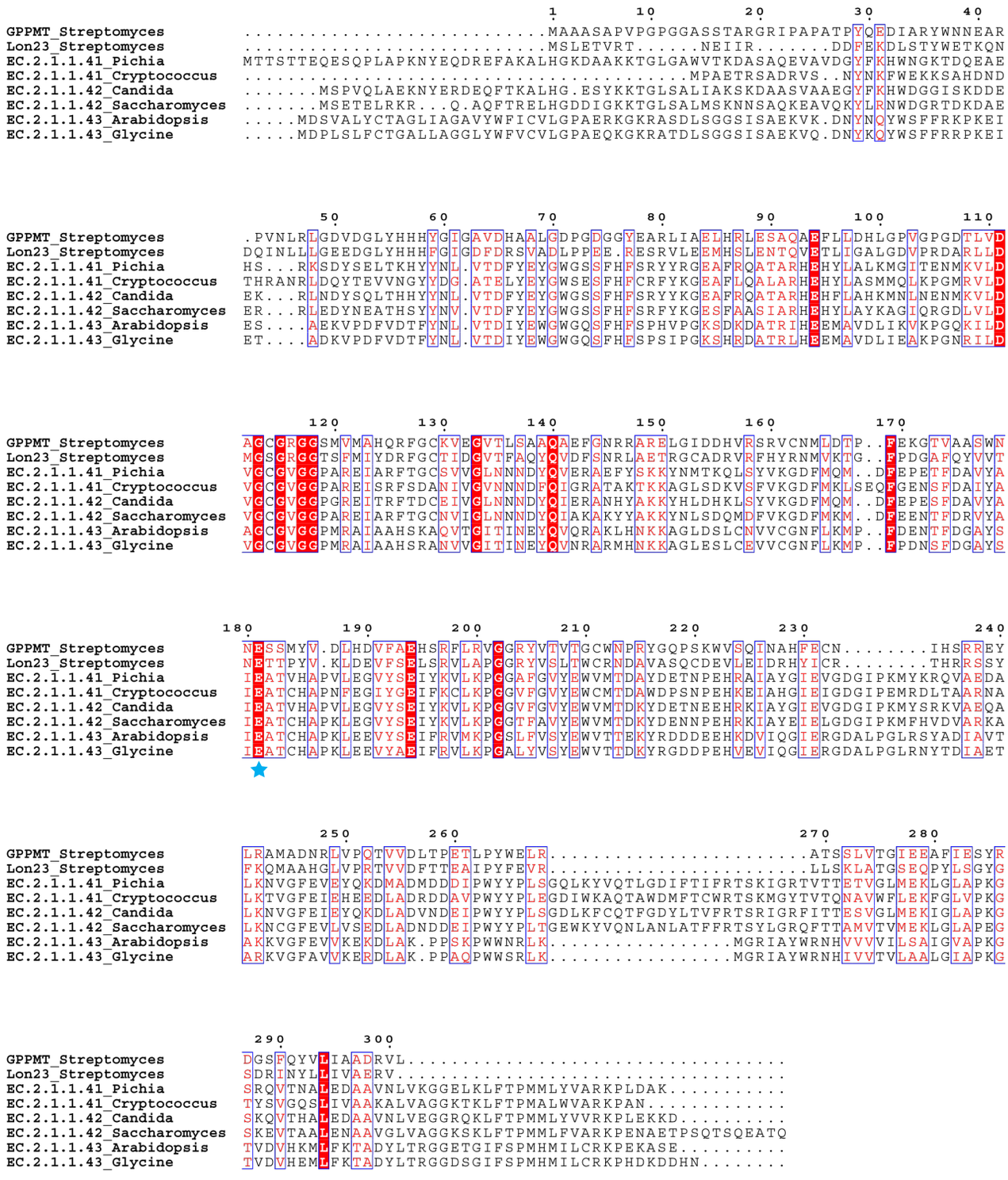
69
55
82
98
107
125
137
153
168
m/z

Fig. S5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Lon23_Streptomyces
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EC.2.1.1.42_Saccharomyces
EC.2.1.1.43_Arabidopsis
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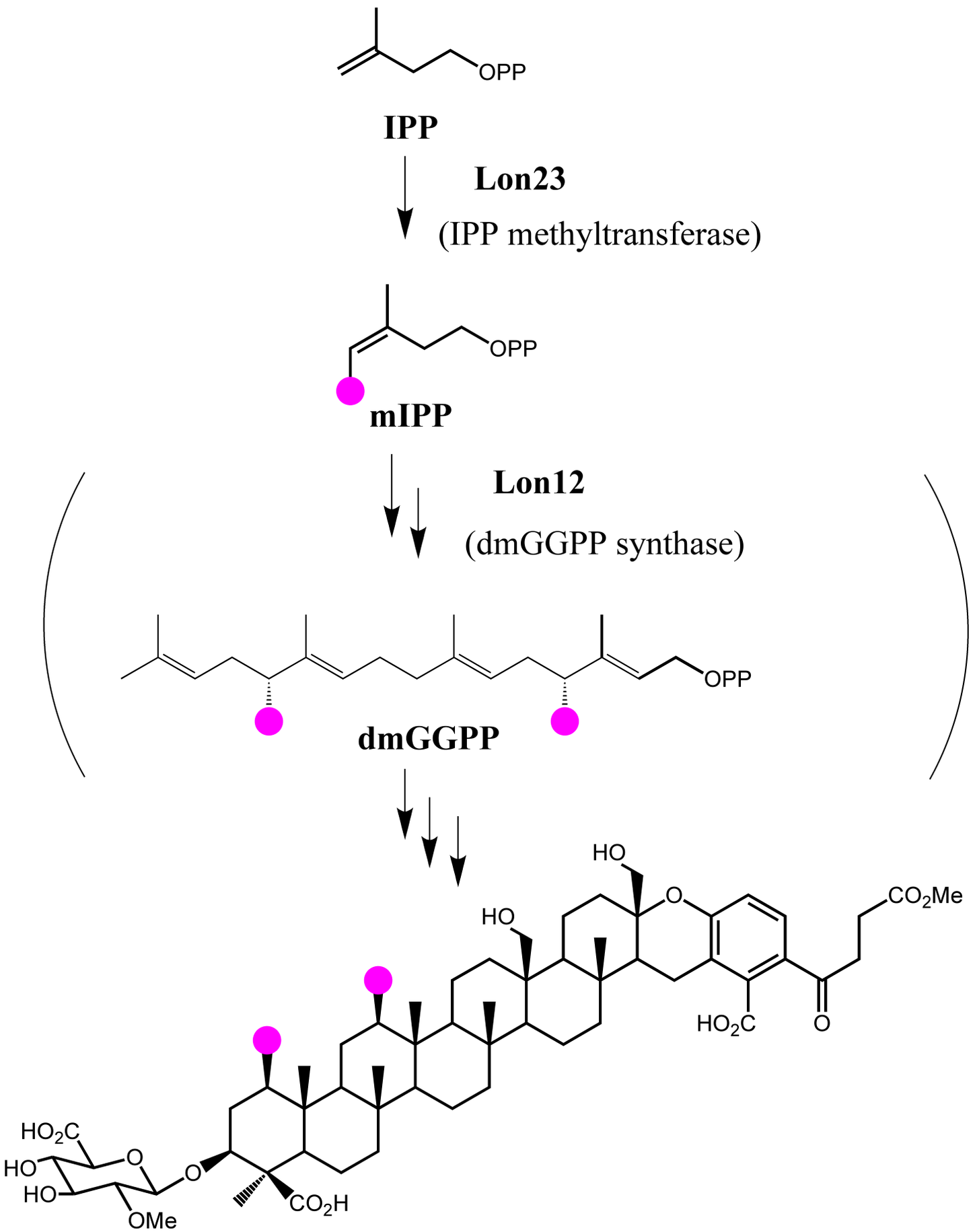
Fig. S6



KS-505a (longestin)