Supplementary Information

SI Fig. 1. The structures of three monolignols (A), and the schema for the proposed one of electron dehydrogenation and the subsequent oxidative coupling of phenoxy radicals (B).

SI Fig 2. Site-directed mutation of the amino-acid residues of COMT (A), and IEMT (B), and the relative activity of the mutant variants on 4-*O*-methyaltion of coniferyl alcohol. The 100% in A represents the specific activity of 315 pmole mg^{-1} min^{-1} and in B of 660 pmole mg^{-1} min^{-1}. The reactions were conducted by using 15 μg purified protein at 30 ˚C for 30 mins.

SI Fig. 3. The procedure of constructing and screening IEMT mutant libraries.

SI Fig. 4. MS^n analysis on the methylated product from the reaction of IEMT mutant incubated with coniferyl alcohol and SAM. (A) The acquired mass spectra of the molecule and the major ions during tandem MS. (B) the proposed fragmentation path of the 4-methoxyconiferyl alcohol.

SI Fig. 5. The relationship of the catalytic activity of IEMT mutant on 4-*O*-methylation of monolignol with the hydrophobicity of the substituted amino-acid residues.

(A) Histogram of the catalytic efficiency of the individual single mutants at the sites of 165 and 133, and the hydrophobic values of these substitutions.
*The hydrophobicity values of the amino acid residues are referred from "Black, S. D., and Mould, D. R. (1991) Anal. Biochem. 193, 72-82".

(B) The active site-binding surface of the IEMT wild-type, single- and double-mutants. 18 amino acid residues constituting the binding surface area in the active site were extracted and calculated using the program GRASP. The areas constituted by the hydrophobic and the acidic amino acids are shown in green and red, respectively. Note the increase of the hydrophobic binding surface of the mutant variants.

SI Fig. 6. The mass- and UV-spectra of major oligolignols yielded from the *in vitro* polymerization of coniferyl alcohol.

SI Table 1. Primers used for site-directed and site-saturation mutagenesis

Name	Sequences (5`→3`)
IEMT-130F	CTTGCTCCTNNKTTGCTCACGGCTACCGACAA
IEMT-130R	CGTGAGCAAMNNAGGAGCAAGAGAAACTCCA
IEMT_131F	GCTCCTTTTNNKCTCACGGCTACCGACAA
IEMT_131R	GCCGTGAGMNNAAAAGGAGCAAGAGAAACT
IEMT-133F	TTGCTCNKKGCTACCGACAAGGTCCTTTTGGA
IEMT-133R	GTCGGTAGCMNNGAGCAAAAAAGGAGCAAGAGA
IEMT-134F	GCTCACGNNKACCGACAAGGTCCTTTTGGA
IEMT-134R	GTCGGTMNNCGTGAGCAAAAAAGGAGCAAGA
IEMT-139F	GCT ACC GAC AAG GTC NNK TTG GAG CCC TGG TTT TAC TTG A
IEMT-139R	CAA GTA AAA CCA GGG CTC CAA MNN GAC CTT GTC GGT AGC
IEMT-165F	TATGGAATGAATNNKTTCGATTACCATGGAACAGAC CAC
IEMT-165R	TGTTCCATGGTAATCGAAMNNATTCATTCCATACGC
IEMT-175F	CAT GGA ACA GAC CAC AGA NNK AAC AAG GTG TTC AAC A
IEMT-175R	TCC CTT GTT GAA CAC CTT GTT MNN TCT GTG GTC TGT
IEMT-186F	C AAG GGA ATG TCC AGC NNK TCT ACC ATC ACC ATG AA
IEMT-186R	CAT GGT GAT GGT AGA MNN GCT GGA CAT TCC CTT GTT G
IEMT-319F	ACC AAG GTA GTC ATC CAT NNK GAC GCC CTC ATG TTG
IEMT-319R	GGC CAA CAT GAG GGC GTC MNN ATG GAT GAC TAC CTT
IEMT-326F	GCC CTC ATG TTG GCC NNK AAC CCA GGC GGC AAA GAA
IEMT-326R	CCT TTC TTT GCC GCC TGG GTT MNN GGC CAA CAT GAG
IEMT-327F	GCC CTC ATG TTG GCC TAC NNK CCA GGC GGC AAA GAA
IEMT-327R	CCT TTC TTT GCC GCC TGG MNN GTA GGC CAA CAT GAG
IEMT-F130L-L131C-T133L-F	CT CTT GCT CCT TTA TGT CTC CTG GCT ACC GAC AAG GT
IEMT-F130L-L131C-T133L-R	GTC GGT AGC CAG GAG ACA TAA AGG AGC AAG AGA AAC T
IEMT-T133L-A134N-T135Q-F	GCT CCT TTT TTG CTC CTG AAT CAG GAC AAG GTC CTT TTG GA
IEMT-T133L-A134N-T135Q-R	CTC CAA AAG GAC CTT GTC CTG ATT CAG GAG CAA AAA AGG A
IEMT-F130L-L131C-T133L-A134N-T135Q-F	GGA GTT TCT CTT GCT CCT TTA TGT CTC CTG AAT CAG GAC AAG GTC CTT
IEMT-F130L-L131C-T133L-A134N-T135Q-R	CTC CAA AAG GAC CTT GTC CTG ATT CAG GAG ACA TAA AGG AGC AAG AGA
IEMT-E165F-N164T-F	C AAT AAA GCG TAT GGA ATG ACT TTT TTC GAT TAC CAT GGA
IEMT-E165F-N164T-R	CC ATG GTA ATC GAA AAA AGT CAT TCC ATA CGC TTT A

SI Table 2. The codon mutations of the selected mutant variants of IEMT

Mutant	Represented codon
L131A	TTG>GCG
L131M	TTG>ATG
L131V	TTG>GTG
T133A	ACG>GCG
T133M	ACG>ATG
T133L	ACG>CTG
E165Y	GAA>TAT
E165S	GAA>AGT, TCT
E165M	GAA>ATG
E165A	GAA>GCG/GCT
E165T	GAA>ACT
E165P	GAA>CCG
E165V	GAA>GAG
E165C	GAA>TGT
E165F	GAA>TTT
E165D	GAA>GAT
E165I	GAA>ATA
E165L	GAA>CTA
T133L-E165F	ACG>CTG, GAA>TTT
T133L-E165S	ACG>CTG, GAA>AGT
T133L-E165L	ACG>CTG, GAA>TTG/CTT
T133L-E165Y	ACG>CTG, GAA>TAT
T133L-E165M	ACG>CTG, GAA>ATG
T133L-E165A	ACG>CTG, GAA>GCT
T133L-E165T	ACG>CTG, GAA>ACT
T133G-E165F	ACG>GGG, GAA>TTT
T133Q-E165F	ACG>CAG, GAA>TTT
T133M-E165F	ACG>ATG, GAA>TTT
T133L-E165I	ACG>CTG, GAA>ATA
T133M-E165I	ACG>ATG, GAA>ATA
T133L-E165F-L139Q	ACG>CTG, GAA>TTT, CTT> CAG
T133L-E165I-F175I	ACG>CTG, GAA>ATA, TTC>ATT
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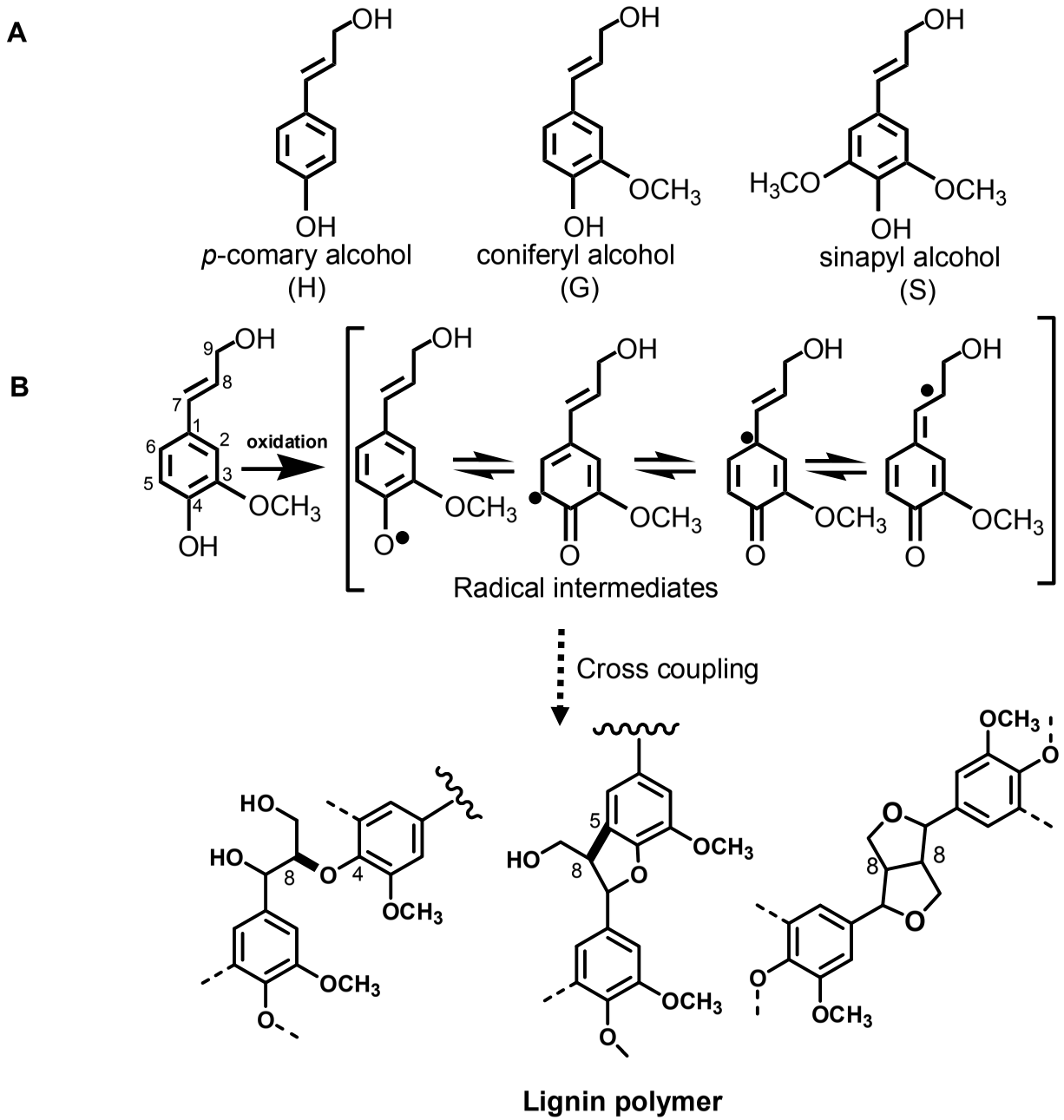
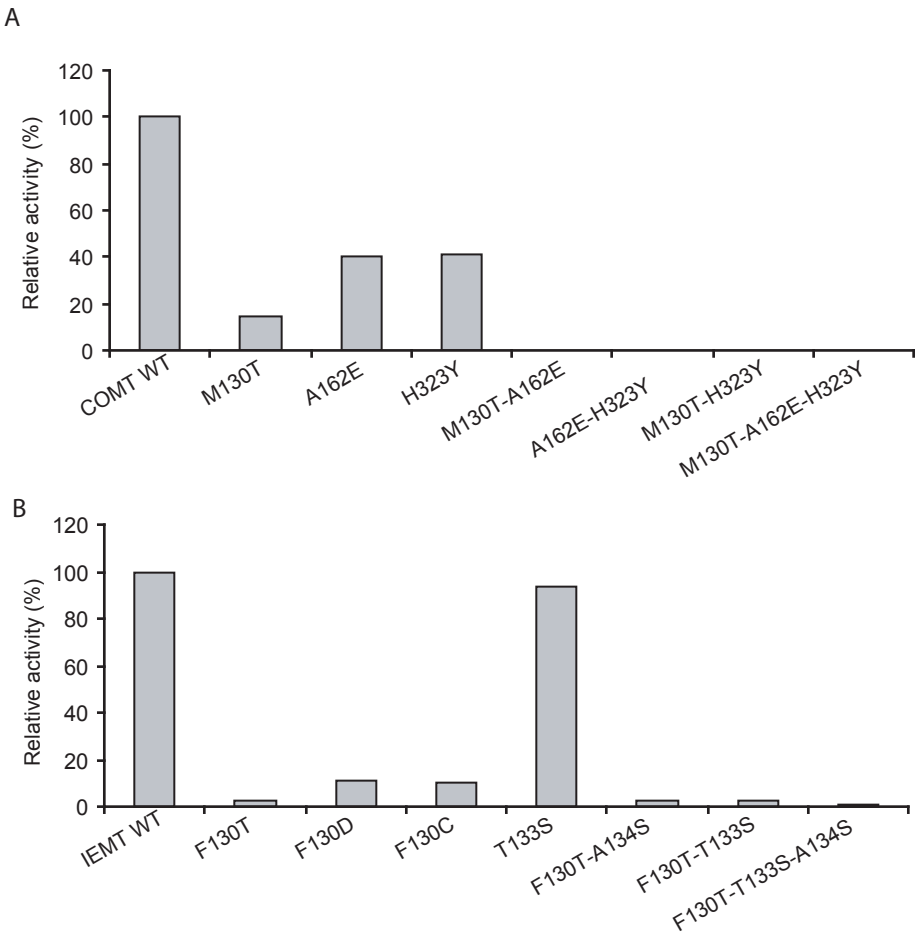
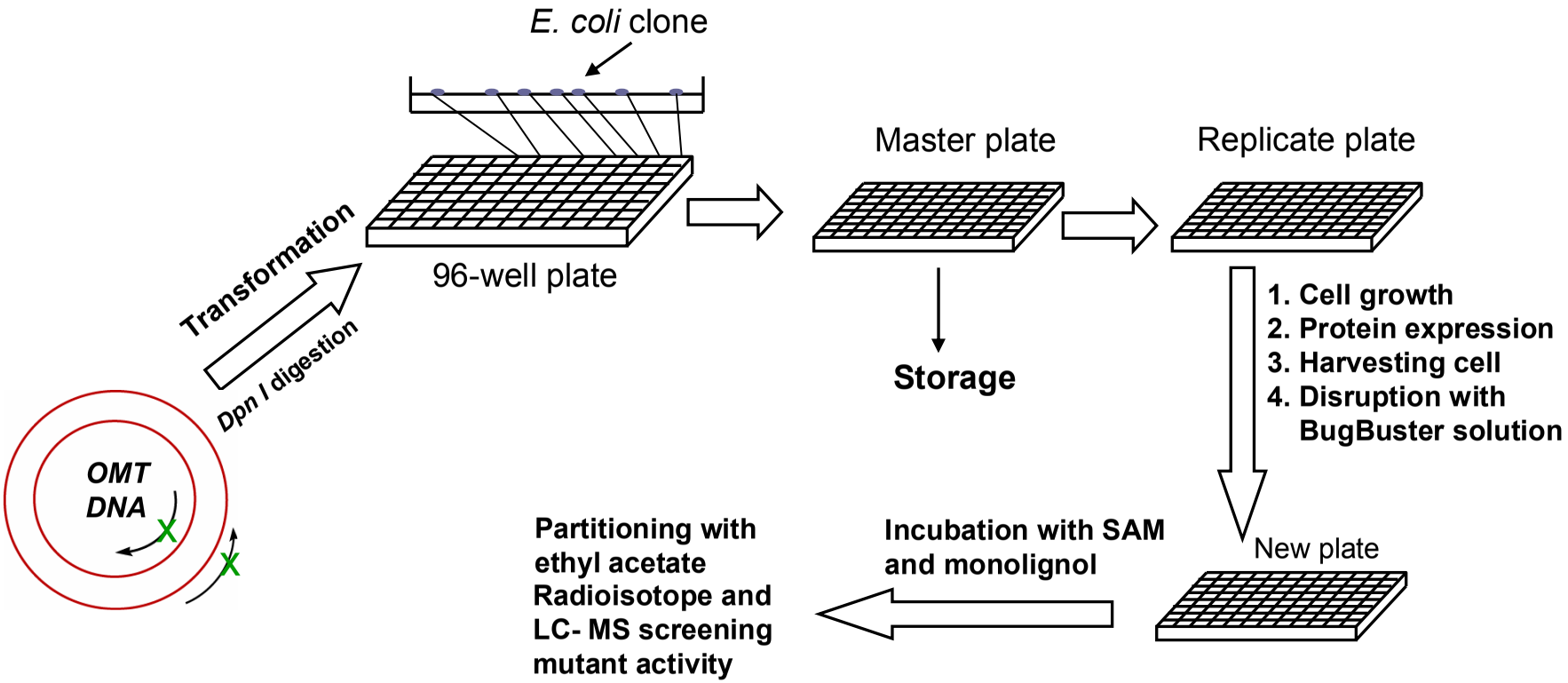

A
OH
OH
p-comary alcohol
(H)
OH
OCH3
OH
coniferyl alcohol
(G)
OH
H3CO
OCH3
OH
sinapyl alcohol
(S)
B
oxidation
Radical intermediates
Cross coupling
Lignin polymer

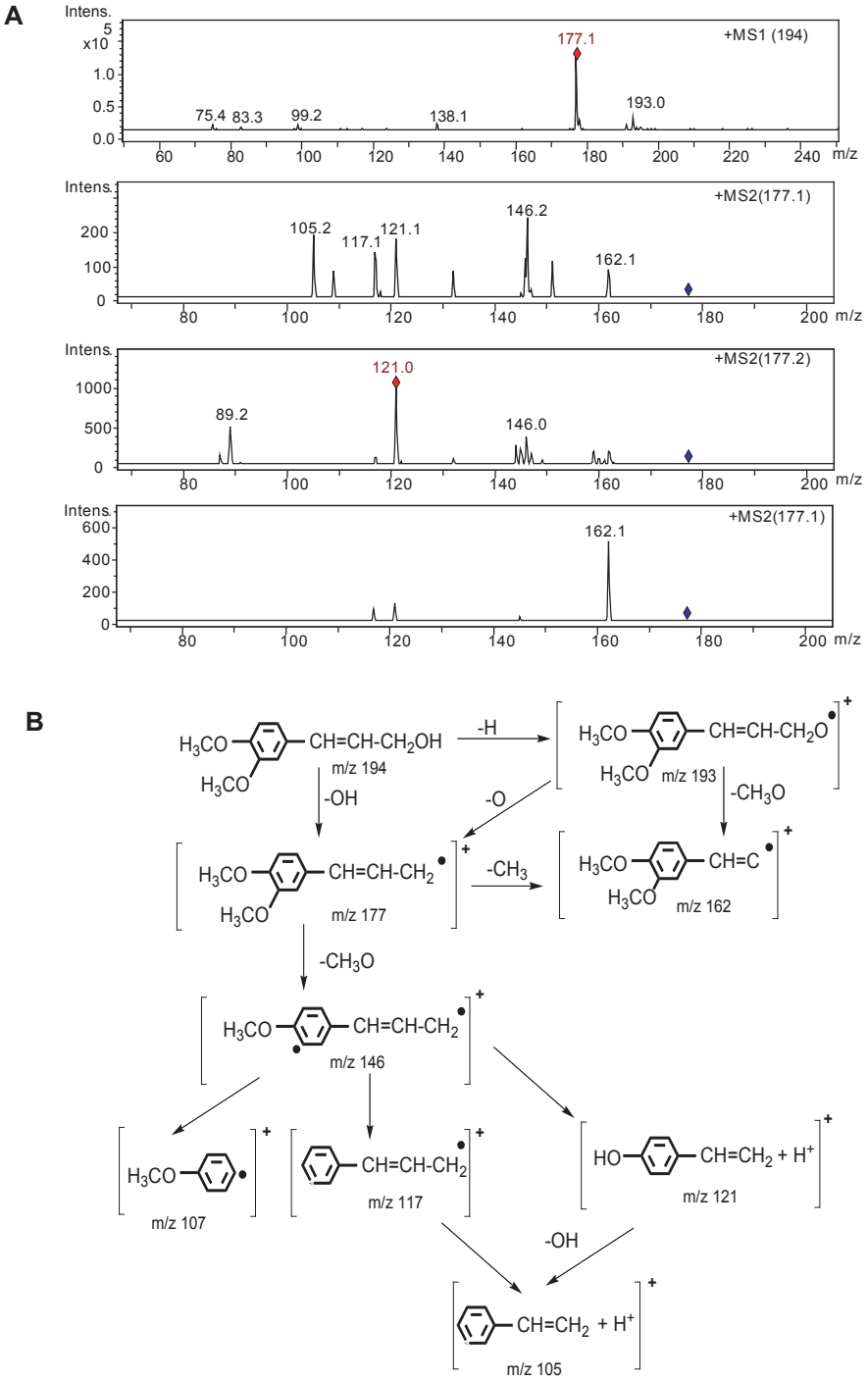
SI Fig. 1


A
Relative activity (%)
120
100
80
60
40
20
0
COMT WT
M130T
A162E
H323Y
M130T-A162E
A162E-H323Y
M130T-H323Y
M130T-A162E-H323Y
B
Relative activity (%)
120
100
80
60
40
20
0
IEMT WT
F130T
F130D
F130C
T133S
F130T-A134S
F130T-T133S
F130T-T133S-A134S

SI Figure 2

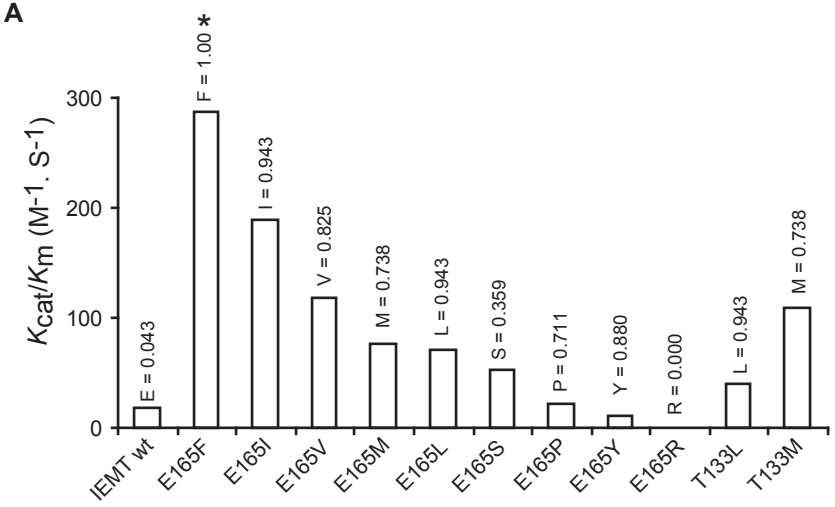

E. coli clone
96-well plate
Transformation
Dpn I digestion
OMT
DNA
Master plate
Storage
Replicate plate
1. Cell growth
2. Protein expression
3. Harvesting cell
4. Disruption with
BugBuster solution
New plate
Incubation with SAM
and monolignol
Partitioning with
ethyl acetate
Radioisotope and
LC- MS screening
mutant activity

SI Fig. 3

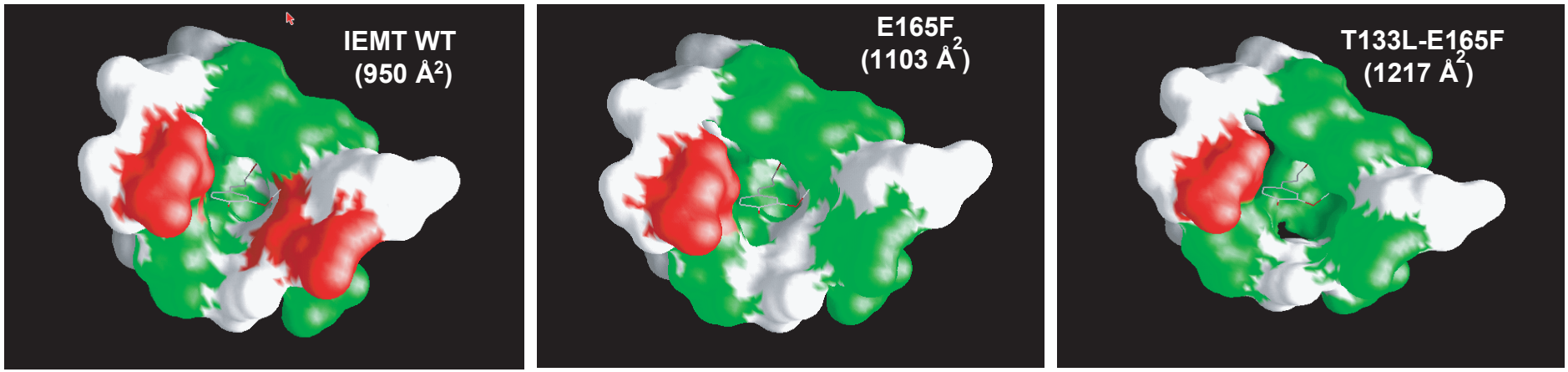

A
Intens.
x10 5
1.0
0.5
0.0
177.1
+MS1 (194)
75.4
83.3
99.2
138.1
193.0
60
80
100
120
140
160
180
200
220
240
m/z
Intens.
200
100
0
+MS2(177.1)
105.2
117.1
121.1
146.2
162.1
80
100
120
140
160
180
200
m/z
Intens.
1000
500
0
+MS2(177.2)
121.0
89.2
146.0
80
100
120
140
160
180
200
m/z
Intens.
600
400
200
0
+MS2(177.1)
162.1
80
100
120
140
160
180
200
m/z
B
H3CO
H3CO
CH=CH-CH2OH
m/z 194
-H
-OH
-O
H3CO
H3CO
CH=CH-CH2O
m/z 193
-CH3O
H3CO
H3CO
CH=CH-CH2
m/z 177
-CH3
H3CO
H3CO
CH=C
m/z 162
-CH3O
H3CO
CH=CH-CH2
m/z 146
H3CO
m/z 107
CH=CH-CH2
m/z 117
HO
CH=CH2 + H+
m/z 121
-OH
CH=CH2 + H+
m/z 105

SI Figure 4

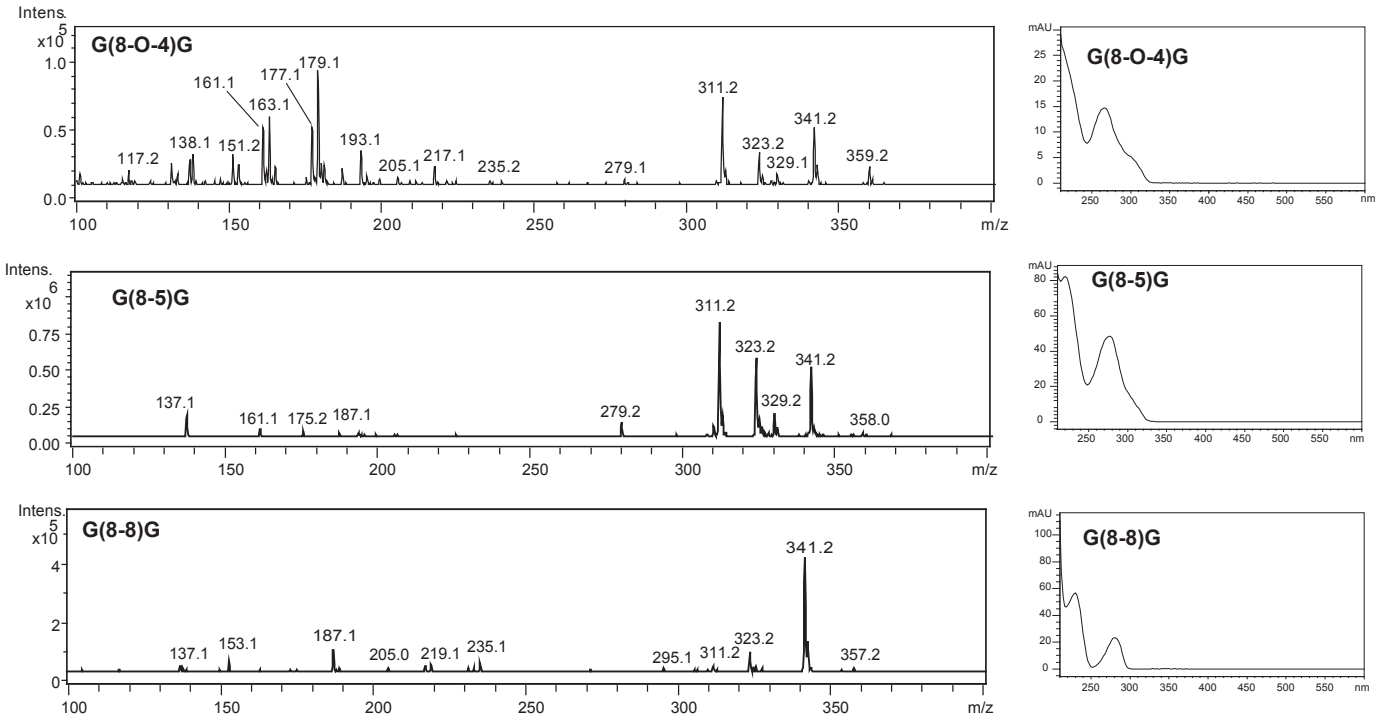
A



B



SI Figure 5


Intens.
x10 5
G(8-O-4)G
1.0
0.5
0.0
117.2
138.1
151.2
161.1
163.1
177.1
179.1
193.1
205.1
217.1
235.2
279.1
311.2
323.2
329.1
341.2
359.2
100
150
200
250
300
350
m/z
mAU
G(8-O-4)G
nm
Intens.
x10 6
G(8-5)G
0.75
0.50
0.25
0.00
137.1
161.1
175.2
187.1
279.2
311.2
323.2
329.2
341.2
358.0
mAU
G(8-5)G
Intens.
x10 5
G(8-8)G
4
2
0
137.1
153.1
187.1
205.0
219.1
235.1
295.1
311.2
323.2
341.2
357.2
mAU
G(8-8)G

SI Figure 6