

The methionine 549 and leucine 552 residues of friedelin synthase from *Maytenus ilicifolia* are important for substrate binding specificity

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Supplementary Material

Figure S1: Multiple global alignment in the wild MiFRS sequence. It is highlighted the mutated residues.

ScLAS	440	KGAWCFSTKTQ	524	PAEVFGNIMV
1W6K	439	KGGFSTLDC	517	PSEVFGDIMI
SAD1	468	KGSWTLSSVDN	547	PSESERNIVV
PEN5	471	KGGWTFQDREQ	550	PVEHVENTIV
PEN6	476	KGGWTFSDQDQ	555	PVERMEDTIV
PEN1	475	KGGWTFSDKQDQ	554	PVEFVQDTVI
PEN4	475	KGGWTFSDQDQ	554	PVEFLEDTIV
MiCAS1	472	KGAWTFSTADH	551	PAETFGDIVI
LcCAS1	475	KGAWTFSTADH	554	PAETFGDIVI
BPX1	477	KGAWTFSTADH	556	PAETFGDIVI
KdCAS	468	KGAWTFSTADH	547	PAETFGDIVI
CAS1	467	KGAWTFSTADH	546	PAETFGDIVI
PNX	467	KGAWTFSTADH	546	PAETFGDIVI
PSX	467	KGAWTFSTADH	546	PAETFGDIVI
OSC5	467	KGAWTFSTADH	546	PAETFGDIVI
GgCAS1	467	KGAWTFSTADH	546	PAETFGDIVI
BPW	466	KGAWTFSMQDH	545	PTEFFEDTLI
OSC3	466	KGAWTFSMHDH	545	PTEFFREETLI
GgLUS1	466	KGAWTFSMHDH	545	PTEFFEDTMI
OEW	467	KGAWTFSMQDH	546	PTEFFEDVLI
TRW	469	KGAWTFSLQDH	548	PTEFFEDVLI
PdFRS	466	KGSWTFSDQDH	545	PMEEFLENIVI
TwOSC1	472	KGSWTFSDQDH	551	PMEEFLENIVI
MiFRS	468	KGSWTFSDQDH	548	PMEEFLENIVI
TwOSC3	468	KGSWTFSDQDH	548	PMEEFLENIVI
OSC1	468	KGAWTFSDRDH	547	PVEFLEDIVI
RsM2	471	KGAWTFSSQDY	550	PLEFFENIVV
LcIMS1	466	KGSWTFSDQDH	545	PVEFLEDLII
LUP1	467	KGAWTFSDRDH	546	PTEFFMANTMV
LUP2	470	KGAWTFSDRDH	549	PTDFFTCVMA
RcLUS	468	KGAWTFSDKQDQ	547	PVERMEDIVV
BgLUS	468	KGSWTFSDKQDH	547	PVEFLADIVI
KcMS	468	KGSWTFSDKQDH	547	PVEFLEDIVI
SHS1	468	KGSWTFSDQDH	547	PVEFLEDIVI
KdFRS	469	KGAWTFSDQDH	548	PTEFFENIVI
KdLUS	469	KGAWTFSDQDH	548	PTEFFENIVI
KdGLS	469	KGSWTFSDQDH	548	PTEFFENIVI
PNY1	470	KGSWTFSDQDH	549	PTEFFADIVI
PNY2	468	KGSWTFSDQDH	547	PTEFFEDIVI
EtAS	469	KGSWTFSDQDH	548	PTEFFADIVI
RsM1	469	KGSWTFSDQDH	548	PTEFFADIVI
KdTAS	488	KGSWTFSDQDH	567	PTEFFADIVI
BPY	469	KGSWTFSDQDH	548	STEFFADIVI
TwOSC2	471	KGSWTFSDQDH	550	PTEFFADIVV
PSM	469	KGSWTFSDKQDH	548	PVEFLEEIVV
AMY2	469	KGAWTFSDQDH	548	PTEFFEDIVI
GgbAS1	469	KGSWTFSDQDH	548	PTEFFADIVV
PSY	469	KGSWTFSDQDH	548	PTEFFADIVV
AMY1	469	KGSWTFSDQDH	548	PTEFFADIVV

Table S1: Sequences of the oxidosqualene cyclase enzymes used in multiple alignment with their accession number on GenBank, species and function.

Accession number	Species	Function
AB257562.1	<i>Arabidopsis thaliana</i>	Arabidiol synthase (PEN1)
AY327541.1	<i>Arabidopsis thaliana</i>	Talianol synthase (PEN4)
BT020456.1	<i>Arabidopsis thaliana</i>	Marneral synthase (PEN5)
AB274959.1	<i>Arabidopsis thaliana</i>	Triterpene synthase multifuncional (PEN6)
NM_179572.1	<i>Arabidopsis thaliana</i>	Lupeol synthase 1 (LUP1)
NM_106545.3	<i>Arabidopsis thaliana</i>	Lupeol synthase 2 (LUP2)
NM_126681.2	<i>Arabidopsis thaliana</i>	Cycloartenol synthase (CAS1)
AB263204.1	<i>Rhizophora stylosa</i>	Triterpene synthase multifuncional (RsM2)
AB289586.1	<i>Bruguiera gymnorhiza</i>	Lupeol synthase (BgLUS)
AB257507.1	<i>Kandelia candel</i>	Triterpene synthase multifuncional (KcMS)
DQ268869.1	<i>Ricinus communis</i>	Lupeol synthase (RcLUS)
AB058643.1	<i>Luffa cylindrica</i>	Isomultiflorenol synthase (LcIMS1)
<u>AB037203.1</u>	<i>Glycyrrhiza glabra</i>	β -amyrin synthase (GgbAS1)
AB181244.1	<i>Lotus japonicus</i>	β - amyrin synthase (OSC1)
AB034802.1	<i>Pisum sativum</i>	β - amyrin synthase (PSY)
AF478453.1	<i>Medicago truncatula</i>	β -amyrin synthase (AMY1)
AF478455.1	<i>Lotus japonicus</i>	β -amyrin synthase multifuncional (AMY2)
AB034803.2	<i>Pisum sativum</i>	amyrin synthase mista (PSM)
AB009030.1	<i>Panax ginseng</i>	β -amyrin synthase (PNY1)
AB014057.1	<i>Panax ginseng</i>	β -amyrin synthase (PNY2)
AB055512.1	<i>Betula platyphylla</i>	β -amyrin synthase (BPY)
HM623868.1	<i>Kalanchoe daigremontiana</i>	Taraxerol synthase (KdTAS)
AB263203.1	<i>Rhizophora stylosa</i>	Triterpene synthase multifuncional (RsM1)
AB206469.1	<i>Medicago tirucalli</i>	β -amyrin synthase (EtAS)
HM623870.1	<i>Kalanchoe daigremontiana</i>	Friedelin synthase (KdFRS)
HM623869.1	<i>Kalanchoe daigremontiana</i>	Glutanol synthase (KdGLS)
HM623871.1	<i>Kalanchoe daigremontiana</i>	Lupeol synthase (KdLUS)
AB025343.1	<i>Olea europaea</i>	Lupeol synthase (OEW)
AB025345.1	<i>Taraxacum officinale</i>	Lupeol synthase (TRW)
AB181245.1	<i>Lotus japonicus</i>	Lupeol synthase (OSC3)
AB116228.1	<i>Glycyrrhiza glabra</i>	Lupeol synthase (GgLUS1)
AB055511.1	<i>Betula platyphylla</i>	Lupeol synthase (BPW)
AB181246.1	<i>Lotus japonicus</i>	Cycloartenol synthase (OSC5)
AB025968.1	<i>Glycyrrhiza glabra</i>	Cycloartenol synthase (GgCAS1)
AB009029.1	<i>Panax ginseng</i>	Cycloartenol synthase (PNX)
HM623872.1	<i>Kalanchoe daigremontiana</i>	Cycloartenol synthase (KdCAS)
AB055509.1	<i>Betula platyphylla</i>	Cycloartenol synthase (BPX1)
APG38073.1	<i>Maytenus ilicifolia</i>	Friedelin synthase (MiFRS)
AAA16975.1	<i>Saccharomyces cerevisiae</i>	Lanosterol synthase (ScLAS)
P48449.1	<i>Homo sapiens</i>	Lanosterol synthase (1W6K)
BAK52535.1	<i>Aster tataricus</i>	Shionone synthase (SHS1)
AJ311789.1	<i>Avena strigosa</i>	β -amyrin sintase (SAD1)

ART66198.1	<i>Populus davidiana</i>	Friedelin synthase (PdFRS)
AWK97810.1	<i>Tripterygium wilfordii</i>	Friedelin synthase (TwOSC1)
AWK97811.1	<i>Tripterygium wilfordii</i>	β -amyrin synthase (TwOSC2)
AWK97812.1	<i>Tripterygium wilfordii</i>	Friedelin synthase (TwOSC3)

Table S2 - List of forward and reverse primers for each site-directed mutation reaction.

<i>Primer</i>	<i>Sequence 5'-3'</i>	<i>Tm (° C)</i>
F473Wf (VZO2355)	CAAGGGTAGTTGGACAT <u>G</u> GTCCGATCAAGACCACGG	67,1
F473Wr (VZO2356)	CCGTGGTCTTGATCGGAC <u>C</u> ATGTCCAACCTACCTTG	67,1
M549Sf (VZO2361)	GATGGAAATGTTGAACCCAT <u>C</u> TGAATTCTTGAAAACATCG	62,8
M549Sr (VZO2362)	CGATGTTTTCCAAGAATTCAGATGGGTTCAACATTCCATC	62,8
L552Fr (VZO2364)	GATGACGATGTTTTCA <u>A</u> AGAATTCATTGGG	58,5

The underlined region indicates the bases of the respective amino acid residue mutated.
The letters *f* and *r* in the primers correspond to forward and reverse, respectively.

Table S3 - List of forward primers used in sequencing for each site-directed mutation reaction.

<i>Primer</i>	<i>Sequence 5'-3'</i>	<i>Verified mutations</i>
369/484seq (VZO2372)	GGGACTCTTTGTATGTTGCATC	F473W
534/612seq (VZO2373)	GTTGCTTGTTAGCTGCAACC	M549S, L552

Figure S2: Interaction between Phe473 residue and Trp612 residue.

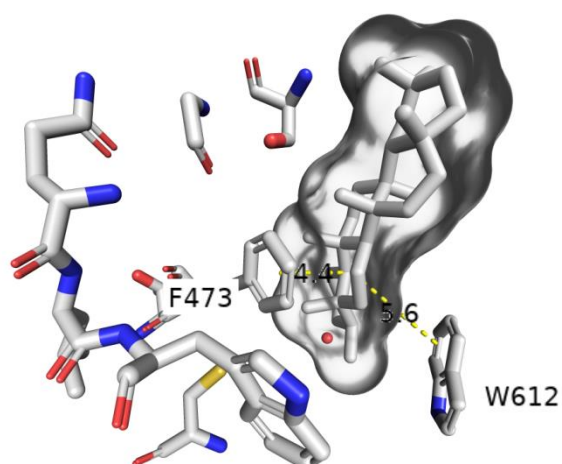
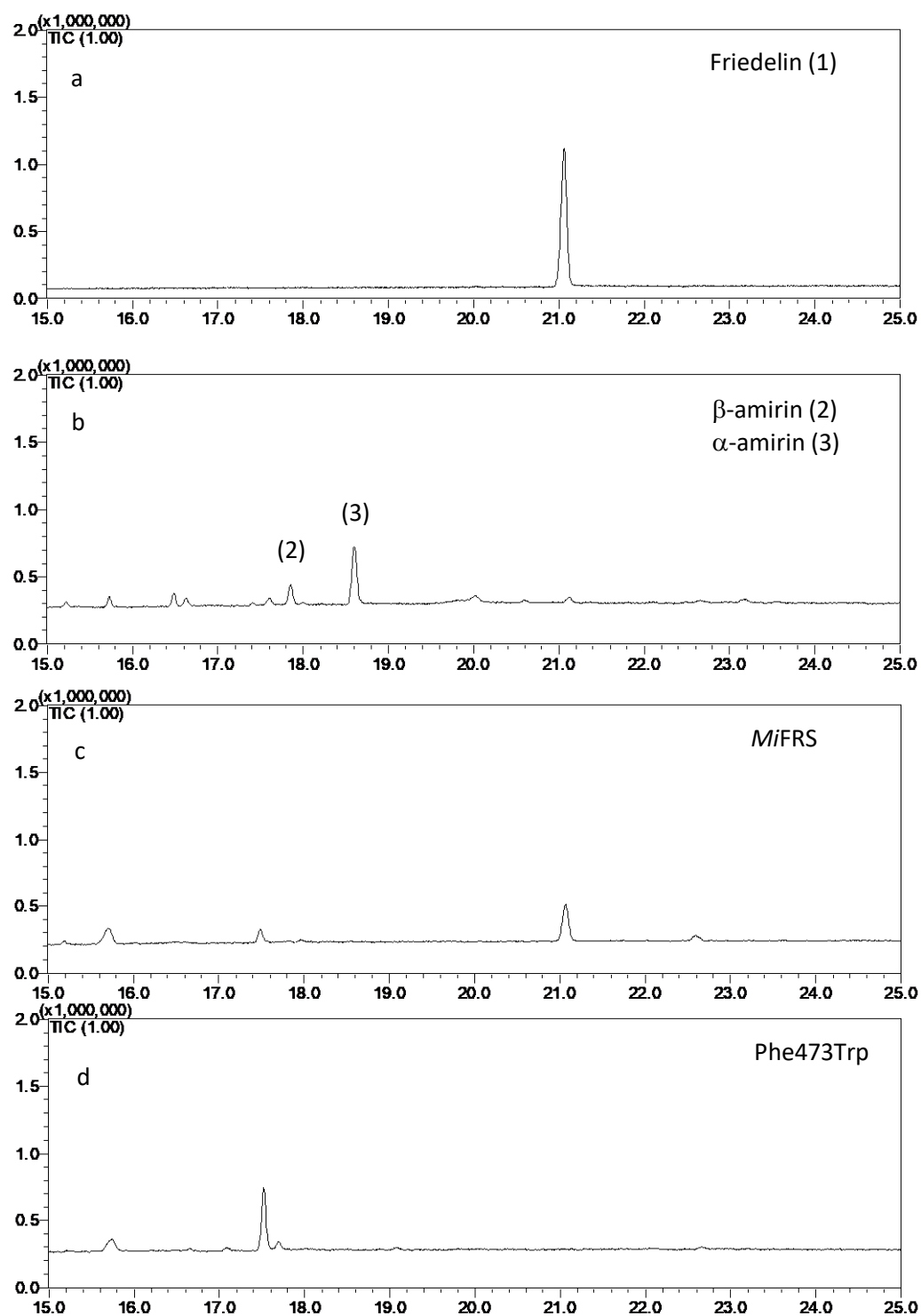


Table S4: Concentration of friedelin present in fractions extracted from *S. cerevisiae* yeast, expressing *MiFRS* mutants.

Sample	Friedelin peak area average $t_R \sim 21,100$ min	Cholesterol peak area average $t_R \sim 14,706$ min	Friedelin area/ Cholesterol area	[friedelin] (mg/L)*
<i>MiFRS</i> – Hex	897656,7	3948327	0,227351	0,206±0,002
M549S - Hex	5548441	2112643	2,626303	0,403±0,002
L552F - Hex	268982,7	4824556	0,055753	0,192±0,001

* Concentration of friedelin in mg/L of culture medium.

Figure S3: Chromatogram of substances used as standard: (a) friedelin, (b) β -amyrin and α - amyrin. Chromatogram of the hexane fractions of *MiFRS* (c), F473W (d), L552F (e) and M549S (f).



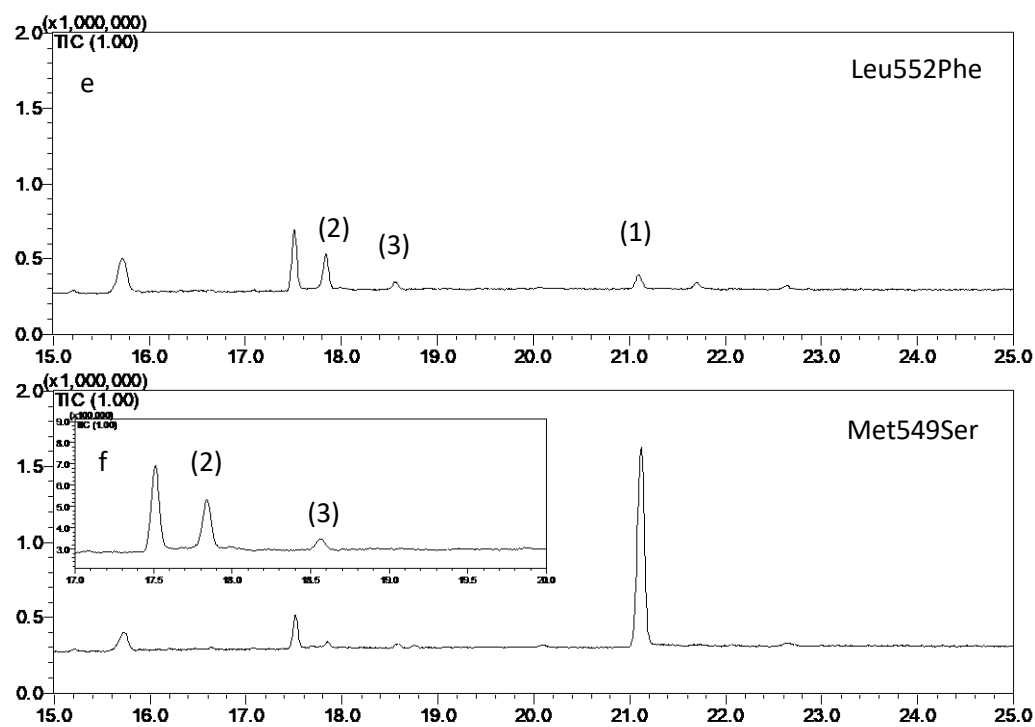


Figure S4: Chromatogram of the hexane fractions of wild type of MiFRS: (a) squalene, (b) oxidosqualene, (c) cholesterol and (d) friedelin.

