Supporting Information for:

**Phenylalanine 445 within Oxidosqualene-Lanosterol Cyclase from
Saccharomyces cerevisiae Influences C-Ring Cyclization and Deprotonation
Reactions**

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Materials and methods

Generation of ERG7^{F445X} Site-Saturated Mutants. The plasmid pTKERG7RS314WT was used as a template to construct the ERG7^{F445X} site-saturated mutagenesis plasmids. The oligonucleotide primers used were 5'-AGAAAGGGTGCCTGGGGC~~NNNT~~CAACA-3' and 5'-TGTTGANNNGCCCCAGGCACCCTTTCT-3', which cause the TTC-to-NNN codon change and the GGGGCT-to-GGTGCC changes to generate nearby *BanI* restriction sites. Site-saturated mutagenic DNAs were constructed by use of PCR amplification with the QuickChange Site-Directed Mutagenesis kit (Stratagene Inc., La Jolla, CA), as described previously.^{1,2} The exact amino acid substitution at Phe445 position was determined by sequence of the DNA by use of an ABI PRISM 3100 auto-sequencer, according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). The recombinant pERG7^{F445X} mutated plasmids were transformed into TKW14 by electroporation using a GenPulser with Pulse Controller (BioRad, Hercules, CA). The pRS314 and pTKERG7RSWT plasmids were also transformed as negative and positive controls, respectively. The resulting transformants were selected for growth on SD+Ade+Lys+His+Met+Ura+hemin+G418+Erg plates, and then reselected on

SD+Ade+Lys+His+Met+Ura+hemin+G418+5-FOA plates, to determine the complementation effect on *ERG7* deficiency, as described previously.^{3,4} Transformants were grown in SD+Ade+Lys+His+Met+Ura+hemin+G418 for nonsaponifiable lipid (NSL) extraction and column chromatography.

Analysis of Extracts from $ERG7^{F445X}$ Site-Saturated Mutants. The NSL extracts of TKW14[p $ERG7^{F445X}$] site-saturated mutants were fractionated by silica gel column chromatography using a 19:1 hexane/ethyl acetate mixture. The fractions were assayed by gas-chromatography-mass spectrometry (GC-MS) and examined for the triterpenoid products with a molecular mass of $m/z = 426$. GC analyses were performed with an Agilent 6890N chromatograph equipped with a DB-5HT column (30 m x 0.25 mm I.D., 0.25 μ m film; oven gradient at 50°C for 2 min, and then 20°C per min until 300°C, held at 300°C for 20 min, 300°C injector; 250°C interface; 1/40 split ratio using helium carrier gas at 13 psi column head pressure). GC/MS was performed on an Agilent 6890N chromatograph coupled to an Agilent 5973network mass selective detector (EI⁺, 70 eV, 180°C ion source temperature, 30-650 amu scan range).

Chemical shifts of (13 α H)-isomalabarica-14(26),17E,21-trien-3 β -ol: Chemical shifts were referenced to Si(CH₃)₄ and are generally accurate to + 0.01 ppm. ¹H NMR (600 MHz, CDCl₃): δ 5.11-5.08 (m, 2H, H-17 and H-21), 4.84 and 4.60 (s and s, 1H and 1H, CH₂-14), 3.23 (dd, $J = 11.7, 5.0$ Hz, H-3 α), 2.17-2.11 (m, 2H, H-16), 2.07 (d, $J = 8.9$ Hz, 1H, H-13 α), 2.06 (m, 2H, CH₂-20), 2.04 (m, 1H, H-16), 1.98-1.94 (m, 3H, H-12 β , H-15, and CH₂-19), 1.87-1.83 (m, 1H, H-15), 1.75-1.71 (m, 1H, H-2 α), 1.66 (s, 3H, Me-24), 1.63-1.62 (m, 1H, H-2 β), 1.61 (m, 1H, H-7 β), 1.582 (s, 3H, Me-23), 1.579 (s, 3H, Me-25), 1.55 (s, 1H, H-12 α), 1.54-1.51 (5H, H-5 α , H-9 β , H-11 β , H-6 α),

1.48-1.37 (m, 3H, CH₂-1, H-11 α), 1.28-1.19 (m, 2H, H-6 β , H-7 α), 1.09 (s, 3H, Me-27), 0.96 (s, 3H, Me-29), 0.93 (s, 3H, Me-28), 0.75 (s, 3H, Me-30); ¹³C NMR (149.90 MHz, 30 mM solution in CDCl₃, 25 °C) δ 15.81 (C-30), 16.01 (C-25), 17.68 (C-23), 18.55 (C-6), 20.74 (C-11), 23.11 (C-28), 25.69 (C-24), 26.53 (C-16), 26.72 (C-20), 28.36 (C-12), 29.08 (C-29), 29.17 (C-2), 29.64 (C-27), 31.93 (C-7), 34.27 (C-1), 35.27 (C-10), 39.06 (C-4), 39.22 (C-15), 39.69 (C-19), 44.79 (C-8), 46.70 (C-5), 52.31 (C-9), 56.47 (C-13), 79.53 (C-3), 109.14 (C-26), 124.15 (C-17), 124.37 (C-21), 131.29 (C-22), 135.14 (C-18), 155.08 (C-14).

Chemical Shifts of 9 β -lanosta-7,24-dien-3 β -ol 3-acetate: ¹³C NMR (150.74 MHz, 20 mM solution in CDCl₃, 25 °C) δ 17.47 (C-28), 17.62 (C-27), 18.31 (C-21), 21.32 (CH₃CO-), 22.91 (C-11), 22.91 (C-6), 23.60 (C-18), 24.31 (C-2), 24.47 (C-19), 25.04 (C-23), 25.71 (C-26), 28.61 (C-16), 28.74 (C-29), 30.53 (C-30), 33.37 (C-15), 35.17 (C-1), 35.22 (C-12), 35.72 (C-10), 35.99 (C-20), 36.10 (C-22), 37.78 (C-4), 43.48 (C-13), 48.39 (C-9), 48.55 (C-5), 52.75 (C-14), 53.48 (C-17), 81.11 (C-3), 121.11 (C-7), 125.18 (C-24), 130.92 (C-25), 149.08 (C-8), 171.00 (CH₃CO-). Chemical shifts ($\pm$ 0.02 ppm) were referenced to the CDCl₃ signal at δ 77.0 ppm. ¹³C NMR analyses yield spectra consistent with the data published in the literature.⁵

Lanosteryl acetate. ¹³C NMR (150.68 MHz, 30 mM solution in CDCl₃, 25 °C) δ 15.73 (C-18), 16.51 (C-29), 17.62 (C-27), 18.10 (C-6), 18.61 (C-21), 19.16 (C-19), 20.97 (C-11), 21.32 (CH₃CO-), 24.15 (C-2), 24.22 (C-30), 24.90 (C-23), 25.71 (C-26), 26.35 (C-7), 27.89 (C-28), 28.18 (C-16), 30.80 (C-15), 30.94 (C-12), 35.24 (C-1), 36.24 (C-20), 36.32 (C-22), 36.87 (C-10), 37.78 (C-4), 44.45 (C-13), 49.78 (C-14), 50.35 (C-17), 50.47 (C-5), 80.91 (C-3), 125.22 (C-24), 130.89 (C-25), 134.21 (C-9), 134.46 (C-8), 171.00 (CH₃CO-). Chemical shifts ($\pm$ 0.02 ppm) were referenced to

the CDCl₃ signal at δ 77.0 ppm. ¹³C NMR analyses yield spectra consistent with the data published in the literature.²

References:

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¹H-NMR spectrum of lanosteryl acetate and 9β-lanosta-7,24-dien-3β-ol 3-acetate mixture

Handwritten labels in the spectrum:

- lanosteryl acetate
- 9β-lanosta-7,24-dien-3β-ol 3-acetate

Integration values (from left to right):

- 1.901
- 1.901
- 1.901
- 1.405
- 24.795
- 24.802
- 24.811
- 1.901
- 1.901
- 1.901
- 1.901

Chemical shifts (δ) (from right to left):

- 9.96363
- 9.94724
- 9.95864
- 9.97416
- 9.98276
- 9.99303
- 9.91780
- 9.94032
- 9.99252
- 9.99113
- 8.99216
- 1.58012
- 1.67828
- 1.64164
- 1.64635
- 1.64635
- 1.64635
- 1.65915
- 2.01078
- 2.01548
- 2.01868
- 2.02294
- 2.02675
- 2.02847
- 4.42960
- 4.41875
- 4.44221
- 4.44620
- 4.45557
- 4.46149
- 4.47094
- 4.48124
- 4.49078
- 5.06459
- 5.07623
- 5.08690
- 5.08804
- 5.11498
- 5.12062
- 5.12558

^{13}C -NMR spectrum of lanosteryl acetate and 9 β -lanosta-7,24-dien-3 β -ol 3-acetate mixture

DEPT spectrum of lanosteryl acetate and 9 β -lanosta-7,24-dien-3 β -ol 3-acetate mixture

Expanded DEPT spectrum of lanosteryl acetate and 9 β -lanosta-7,24-dien-3 β -ol 3-acetate mixture (1)

Expanded DEPT spectrum of lanosteryl acetate and 9 β -lanosta-7,24-dien-3 β -ol 3-acetate mixture (2)

Expanded DEPT spectrum of lanosteryl acetate and 9 β -lanosta-7,24-dien-3 β -ol 3-acetate mixture (3)

Expanded DEPT spectrum of lanosteryl acetate and 9 β -lanosta-7,24-dien-3 β -ol 3-acetate mixture (4)