

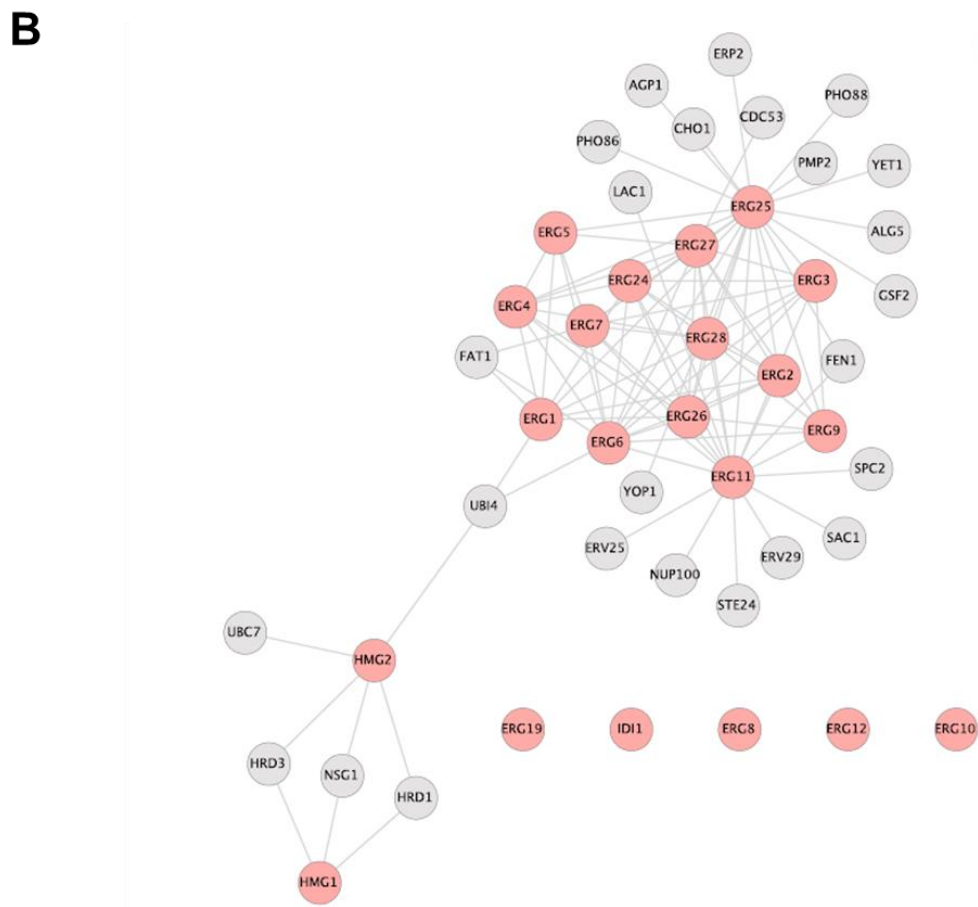
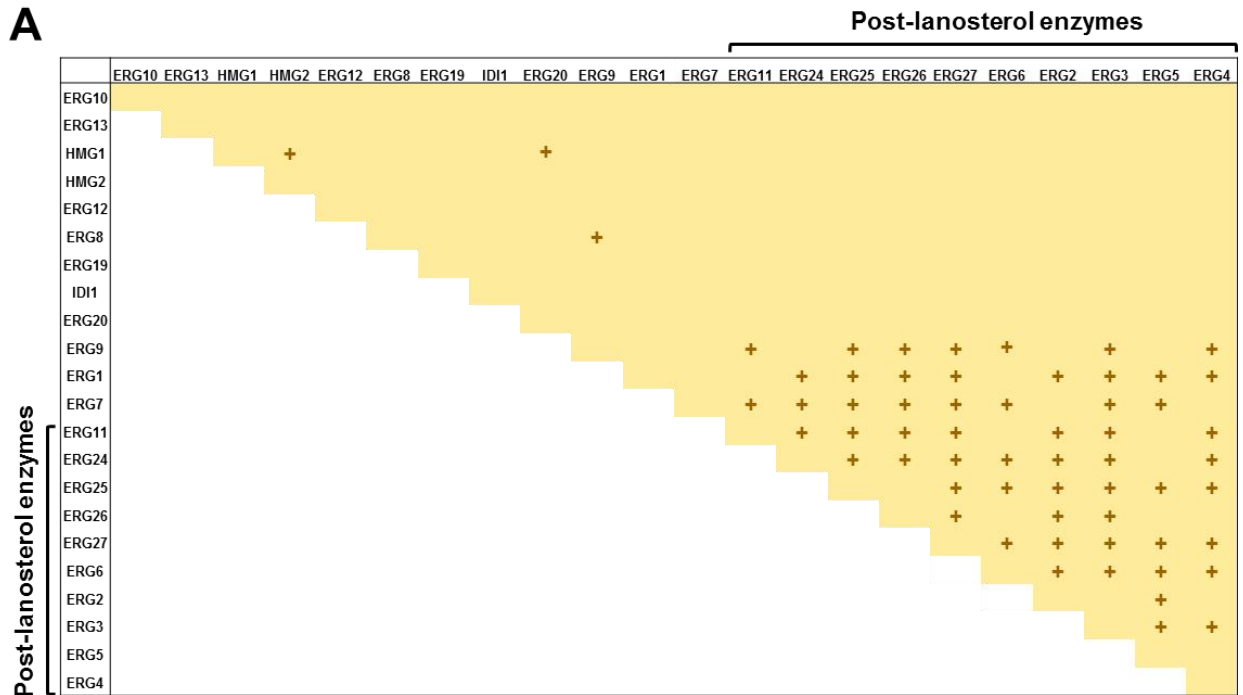
Supplementary Figure Legends

Supplementary Figure 1. **Evidence of protein-protein interaction of yeast ergosterol synthetic enzymes.** (A) Table compiled using data from the STRING database (<http://string-db.org/>) based on experimental evidence, where '+' indicates high confidence for interaction, with scores of at least 0.7. (B) Yeast ergosterol synthesis protein network compiled using the high-confidence yeast protein-protein interaction dataset updated from [1] as described in [2]. The pink nodes are the ergosterol synthesis enzymes, and the grey nodes are their interaction partners.

Supplementary Figure 2. **Ion m/z values monitored during selective ion monitoring (SIM) analysis of the N,O -bis(trimethylsilyl)trifluoroacetamide derivatives.** (A) A simplified schematic illustrating our metabolic labelling strategy. (B) Chromatograms and mass spectra of the [$^2\text{D}_6$]-desmosterol and [$^2\text{D}_7$]-7DHC standards, and their relative retention times with respect to the 5α -cholestane internal standard (IS). (C) The SIM ions for 5α -cholestane were monitored from 27 to 32 min (width 0.1 Da, dwell 120 ms); for [$^2\text{D}_6$]-cholesterol and [$^2\text{D}_7$]-cholesterol from 32 to 35.5 min (width 0.1 Da, dwell 120 ms); for [$^2\text{D}_6$]-desmosterol and [$^2\text{D}_7$]-7DHC from 35.5 to 38 min (width 0.1 Da, Dwell 200 ms).

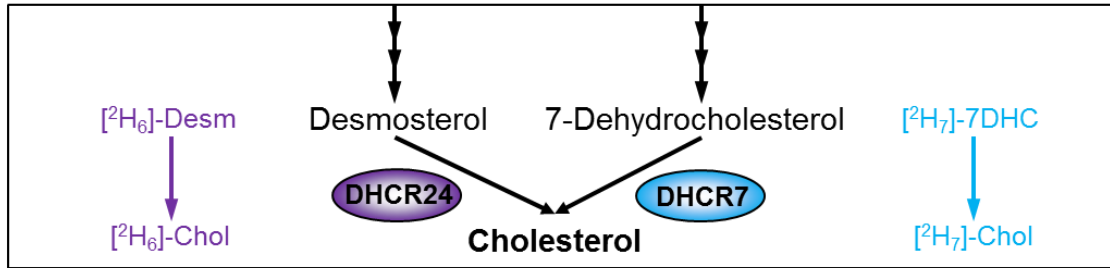
Supplementary Figures

Supplementary Figure 1

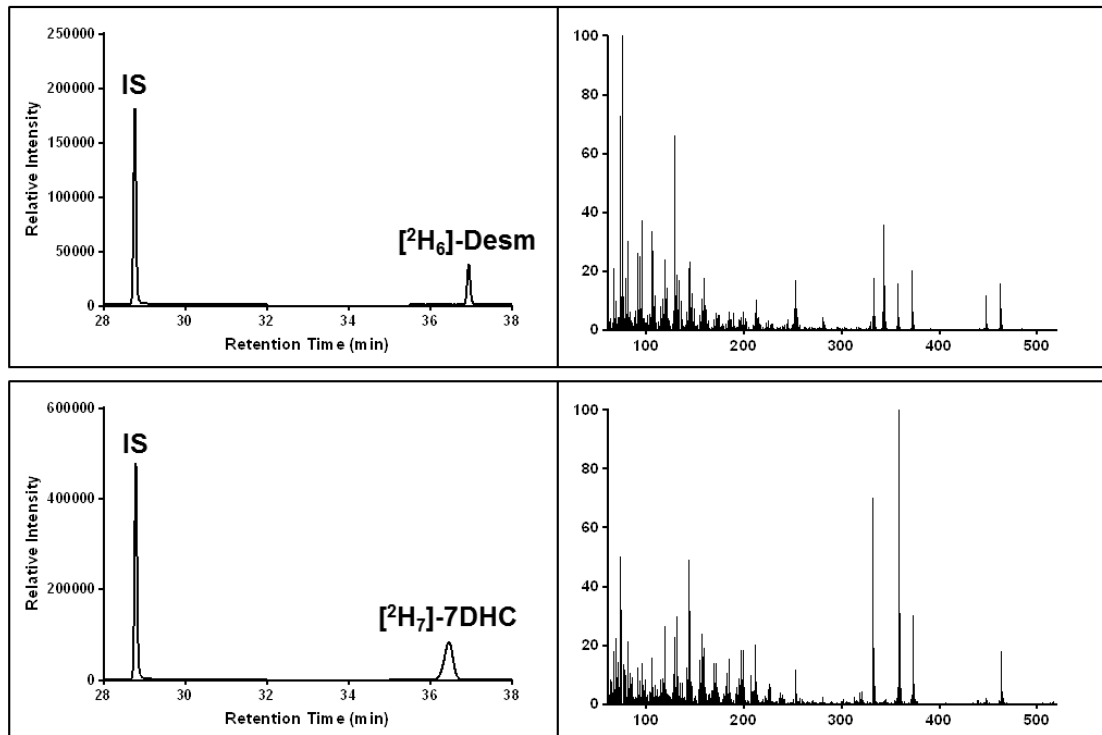


Supplementary Figure 2

A



B



C

Compound	Retention time (min)	Quantification/confirmation ion m/z	Molecular ion m/z
5 α -Cholestane (IS)	28.92	217	372
$[^2\text{D}_6]$ -Cholesterol	34.57	374	464
$[^2\text{D}_6]$ -Desmosterol	37.08	333	462
$[^2\text{D}_7]$ -Cholesterol	34.53	336	465
$[^2\text{D}_7]$ -7-Dehydrocholesterol	36.58	358	463
Cholesterol	34.04	368	458
Desmosterol	37.37	327	456

References

1. Bertin N, Simonis N, Dupuy D, Cusick ME, Han JD, Fraser HB, Roth FP, Vidal M (2007) Confirmation of organized modularity in the yeast interactome. *PLoS biology* 5 (6):e153. doi:10.1371/journal.pbio.0050153
2. Erce MA, Pang CN, Hart-Smith G, Wilkins MR (2012) The methylproteome and the intracellular methylation network. *Proteomics* 12 (4-5):564-586. doi:10.1002/pmic.201100397