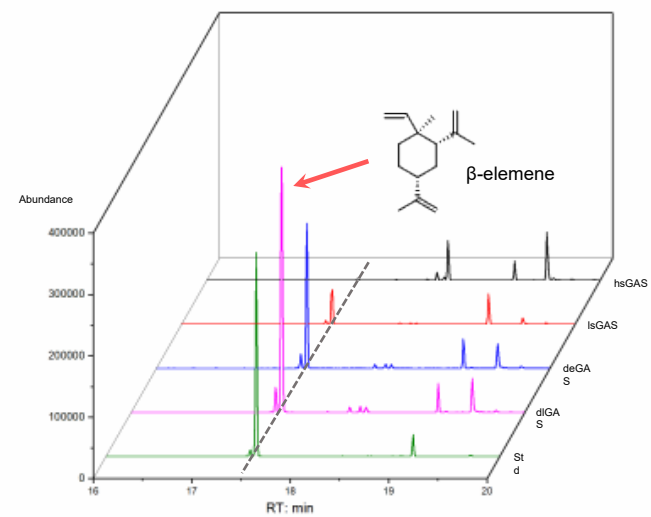


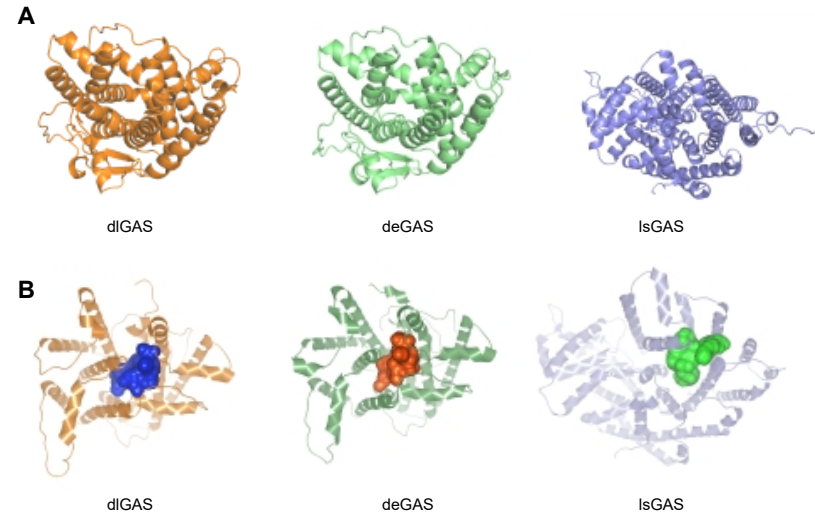
**Fig. 1** The intermediate germacrene A can be converted into various products and their bioactivity. The sesquiterpenes and sesquiterpene lactone derived from germacrene A are :1,  $\beta$ -elemene; 2,  $\delta$ -guaiene; 3, bulnesol; 4, pogostol; 5, patchoulol; 6, germacranolide; 7, parthenolide; 8, costunolide. Fig. S1 was created by Figdraw.



**Fig. 2 Construction of a phylogenetic tree, exhibiting the evolution of germacrene A synthases.** The enzyme sequences were download from NCBI or Uniport. The germacrene A synthases originating from bacteria, red algae, fungi and plant are shown in grayish, pink, light blue and reseda, respectively.

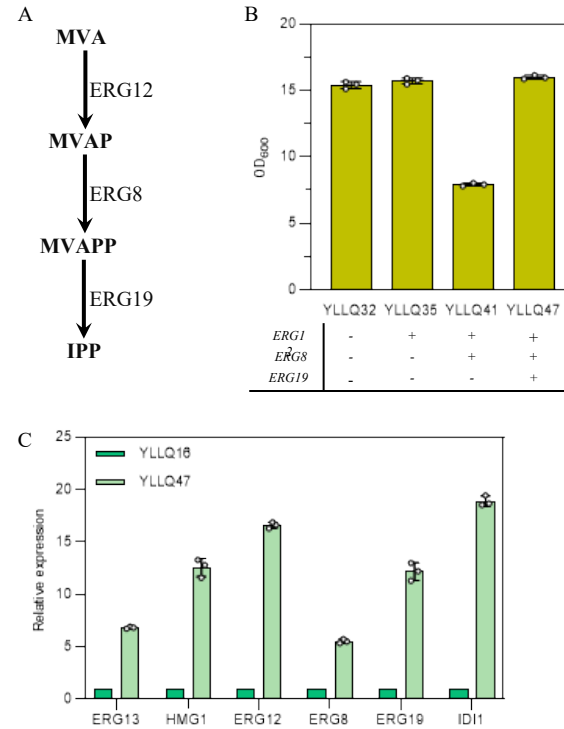


**Fig. 3 GC-MS analysis of shake flask fermentation products in strains expressing dlGAS, deGAS, lsGAS and hsGAS. GC-MS profiles show the same retention time (17.5 minutes) as the  $\beta$ -elemene standard.**

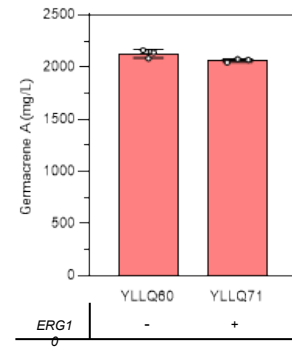


Structure ID	Pocket Volume (Å <sup>3</sup> )
dIGAS	624.9
deGS	757.1
IsGAS	1617.8

**Fig. 4 Computational analysis of the active cavities of dIGAS, deGAS and IsGAS.** (A) Protein models of GASs predicted using AlphaFold<sup>2</sup><sup>1</sup>. (B) The volume of cavities calculated by CAVER Analyst 2.0<sup>2</sup>.



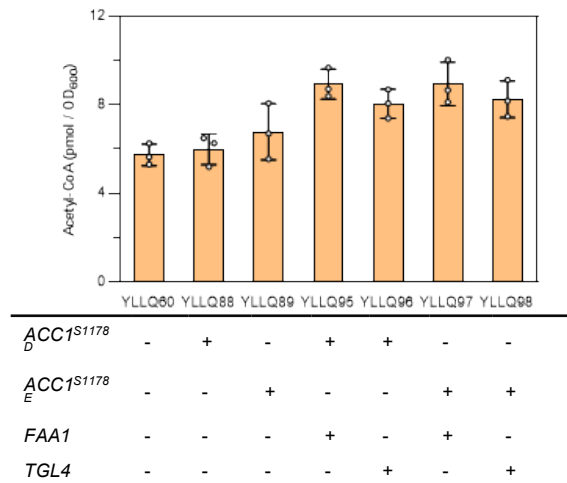
**Fig. 5 Engineering the downstream mevalonate pathway to improve germacrene A production.** (A) Schematic illustration of the enhanced conversion of mevalonate to IPP. (B) Effects of increasing downstream mevalonate pathway on the biomass of engineered strains. MVA, mevalonate; MVAP, 5-phosphomevalonate; MVAPP, 5-diphosphomevalonate; IPP, isopentenyl diphosphate. (C) qRT-PCR analysis of MVA pathway gene transcription levels of engineered strains YLLQ16 and YLLQ47. Error bars correspond to the SD of the mean (n = 3, corresponding to triplicate biological replicates).



**Fig. 6 Germacrene A production for the ERG10-engineered strain (YLLQ71) based on strain YLLQ60.** Error bars correspond to the SD of the mean (n = 3, corresponding to triplicate biological replicates).



**Fig. 7 Alignment of ACC1p amino acid sequences from *Y. lipolytica* and *S. cerevisiae*.** BLAST analysis of the amino acid sequences was performed with ClustalW and the final format was generated with DNAMAN. Homologous residues are highlighted in deep blue, the brown frames represent the mutated sites and the red arrows represent the phosphorylation sites<sup>3,4</sup>.



**Fig. 8 Quantification of acetyl-CoA levels in engineered strains cultured for 24 h.** Error bars correspond to the SD of the mean (n = 3, corresponding to triplicate biological replicates)