# Supporting Information

**Harnessing yeast peroxisomes and cytosol acetyl-CoA for sesquiterpene α-humulene production**

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**Table S1 Primers used in this study**

| Name | Primer sequence (5-3) |
|---|---|
| | ***ERG9-PEST* gene expression cassettes construction** |
| ERG9C-F | TGGTCGATGGTAGATCCTTCTGGC |
| PEST-ERG9-R | GTTCAAGTTGGATGCCGCTCTGTGTAAAGTGTATAT |
| ERG9-PEST-F | CTTTACACAGAGCGGCATCCAACTTGAACATTTCG |
| CYC1-PEST-R | CTAATTACATGACTATATTACTTGGGTATTGCCCATAC |
| PEST-CYC1-F | ACCCAAGTAATATAGTCATGTAATTAGTTATGTCACGCT |
| HIS3-CYC1-R | TAGAGTCGACCAAATTAAAGCCTTCGAGC |
| CYC1-HIS3-F | AAGGCTTTAATTTGGTCGACTCTAGAGGATC |
| Terg9-HIS3-R | ATTTGGCGCAGACTATAGCACGTGATGAATTCGAG |
| HIS3-Terg9-F | TCATCACGTGCTATAGTCTGCGCCAAATAACATAAAC |
| Terg9-R | TATACATGTCAACGTTAGCATCTTGAAT |
| | **ZSS1-GFP fusion protein construction** |
| ZSS1-GFP-F | TTGATCGGTGGTGGTATGTCTAAAGGTGAAGAATTAT |
| GFP-ZSS1-R | AGACATACCACCACCGATCAAGAAAGATTCAACGAAG |
| GFP-epts-F | GATGAATTGTACAAAGGTTCATTGGGAAGAGGTAG |
| epts-GFP-R | CTCTTCCCAATGAACCTTTGTACAATTCATCCATAC |
| GFP-CYC1-F | GAATTGTACAAATAATTAGTTATGTCACGCTTACATTC |
| Cyc1-GFP-R | TGACATAACTAATTATTTGTACAATTCATCCATACCAT |
| | **MVA pathway enzyme-GFP fusion proteins construction** |
| LeuAN-F | GATAATAATATTTATAGAATTGTGTAGAATTGCAG |
| Gal1-LeuAN-R | GAAAATTCAATATAATAGAATGGTATATCCTTG |
| LeuAN-Gal1-F | GATATACCATTCTATTATATTGAATTTTCAAAAATTCTTAC |
| Erg10-Gal1-R | ACGTTCTGAGACATTATAGTTTTTTCTCCTTGAC |
| Gal1-Erg10-F | GAGAAAAAACTATAATGTCTCAGAACGTTTACATTG |
| GFP-Erg10-R | TAGACATACCACCACCTATCTTTTCAATGACAAT |
| Erg10-GFP-F | ATTGAAAAGATAGGTGGTGGTATGTCTAAAGGTG |
| TRP1-TCYC1-R | TAGAGTCGACCAAATTAAAGCCTTCGAGC |
| TCYC1-TRP1-F | AAGGCTTTAATTTGGTCGACTCTAGAGGATC |
| LEUBN-TRP1-R | TCTTTTTAAGCAAGTAGCACGTGATGAATTCGAG |
| TRP1-LEUBN-F | TTCATCACGTGCTACTTGCTTAAAAAGATTCTC |
| LEUBN-R | CATCAGATTTGGTATTGGTAGATTTAGT |
| Erg13-Gal1-R | TAGTTGAGAGTTTCATTATAGTTTTTTCTCCTTGAC |
| Gal1-Erg13-F | GAGAAAAAACTATAATGAAACTCTCAACTAAAC |
| GFP-Erg13-R | TAGACATACCACCACCTTTTTTAACATCGTAAGATC |
| Erg13-GFP-F | TACGATGTTAAAAAAGGTGGTGGTATGTCTAAAGGTG |
| Erg8-Gal1-R | TCTCAACTCTGACATTATAGTTTTTTCTCCTTGAC |
| Gal1-Erg8-F | GAGAAAAAACTATAATGTCAGAGTTGAGAGCCTTC |
| GFP-Erg8-R | TAGACATACCACCACCTTTATCAAGATAAGTTTCCGGATC |
| Erg8-GFP-F | AACTTATCTTGATAAAGGTGGTGGTATGTCTAAAGGTG |
| Erg12-Gal1-R | AACGGTAATGACATTATAGTTTTTTCTCCTTGAC |
| Gal1-Erg12-F | GAGAAAAAACTATAATGTCATTACCGTTCTTAAC |
| GFP-Erg12-R | TAGACATACCACCACCTGAAGTCCATGGTAAATTC |

| | |
|---|---|
| Erg12-GFP-F | TACCATGGACTTCAGGTGGTGGTATGTCTAAAGGTG |
| IDI1-Gal1-R | TTGTCGGCAGTCATTATAGTTTTTTCTCCTTGACGT |
| Gal1-IDI1-F | GAGAAAAAACTATAATGACTGCCGACAACAATAGTATG |
| GFP-IDI1-R | TAGACATACCACCACCTAGCATTCTATGAATTTGCCTG |
| IDI1-GFP-F | TTCATAGAATGCTAGGTGGTGGTATGTCTAAAGGTG |
| MVD1-Gal1-R | TGTGTAAACGGTCATTATAGTTTTTTCTCCTTGACGT |
| Gal1-MVD1-F | GAGAAAAAACTATAATGACCGTTTACACAGCATC |
| GFP-MVD1-R | TAGACATACCACCACCTTCCTTTGGTAGACCAGTCTTTG |
| MVD1-GFP-F | GTCTACCAAAGGAAGGTGGTGGTATGTCTAAAGGTG |
| Erg20-Gal1-R | TTCTGAAGCCATTATAGTTTTTTCTCCTTGACG |
| Gal1-Erg20-F | GAGAAAAAACTATAATGGCTTCAGAAAAAGAAATTAGGAG |
| GFP-Erg20-R | TAGACATACCACCACCTTTGCTTCTCTTGTAAACTTTG |
| Erg20-GFP-F | ACAAGAGAAGCAAAGGTGGTGGTATGTCTAAAGGTG |
| tHMG1-Gal1-R | TTGGTTAAAACCATTATAGTTTTTTCTCCTTGAC |
| Gal1-tHMG1-F | GAGAAAAAACTATAATGGTTTTAACCAATAAAACAG |
| GFP-tHMG1-R | TAGACATACCACCACCGGATTTAATGCAGGTGACGGAC |
| tHMG1-GFP-F | CCTGCATTAAATCCGGTGGTGGTATGTCTAAAGGTG |

**$P_{ADH2}$-*ZSS1-ePTS-*$T_{CYC1}$ gene expression cassette construction**

| | |
|---|---|
| TRP1L-F | GGAAGAGGAGTAGGGAATATTACTGGC |
| LEU2-TRP1L-R | TCCTCTAGAGTCGACCTGCAAGCCGCAAACTTTCAC |
| TRP1L-LEU2-F | TGCGGCTTGCAGGTCGACTCTAGAGGATCC |
| PADH2-LEU2-R | CTCTTCAAACAAACATAGCACGTGATGAATTCGAG |
| LEU2-PADH2-F | TTCATCACGTGCTATGTTTGTTTGAAGAGACTAATC |
| ePTS1-ZSS1-R | GATCTTCTACCTCTTCCCAATGAACCGATCAAGAAAGATTCAAC G |
| ePTS1-TCYC1-F | GGTTCATTGGGAAGAGGTAGAAGATCCAAATTGTAATTAGTTA TGTCACGCTTAC |
| TRP1NR-TCYC1-R | TATTTCTTAGCACAAATTAAAGCCTTCGAGCGTC |
| TCYC1-TRP1NR-F | AGGCTTTAATTTGTGCTAAGAAATAGGTTATTACTGAGTAGT |
| TRP1NR-R | CATTGATGAGGCAACGCTAATTATCAAC |

**$P_{GAL7}$-*ERG13-ePTS1-*$T_{ADH2}$ and $P_{GAL2}$-*ERG10-ePTS1-*$T_{ADH3}$ gene expression cassettes construction**

| | |
|---|---|
| MET17L-F | GGCACCTTGTCCAATTGAACACG |
| Ttdh2-MET17A-R | TGGCTTTTCGCCCTAAATCTGACGCCTTAAAC |
| MET17A-Ttdh2-R | TCAGATTTAGGGCGAAAAGCCAATTAGTGTGAT |
| ePTS-Ttdh2-F | GGTTCATTGGGAAGAGGTAGAAGATCCAAATTGTAAATTTAACTCC TTAAGTTACT |
| GAL7-ERG13-F | TATTCCCTCAAAAATGAAACTCTCAACTAAACTTTGT |
| ePTS-ERG13-R | CTTCTACCTCTTCCCAATGAACCTTTTTTAACATCGTAAGATC |
| GAL7-ERG13-F | TATTCCCTCAAAAATGAAACTCTCAACTAAACTTTGT |
| ERG13-GAL7-R | TGAGAGTTTCATTTTTGAGGGAATATTCAACTG |
| GAL2-GAL7-F | ATTCCGGAAGCTGTACCACCATGGAGACATC |
| GAL7-GAL2-R | CATGGTGGTACAGCTTCCGGAATGGCTTAAGTAG |
| ERG10-GAL2-R | GTTCTGAGACATTATGAAAGAATTATTTTTTTTATTATGTTAATC |
| GAL2-ERG10-F | TAATTCTTTCATAATGTCTCAGAACGTTTACATTGTATC |

| | |
|---|---|
| ePTS1-ERG10-R | ATCTTCTACCTCTTCCCAATGAACCTATCTTTTCAATGACAATAGAG |
| ePTS1-TADH3-F | GGTTCATTGGGAAGAGGTAGAAGATCCAAATTGTAATAGCGTGTT ACGCACCCAAAC |
| TRP1-TADH3-R | CTCTAGAGTCGACGATGTTTTAGTTCGAATGTCTCCTATTG |
| TADH3-TRP1-F | GAACTAAAACATCGTCGACTCTAGAGGATC |
| MET17R-TRP1-R | TACGCACACTCATAGCACGTGATGAATTCGAG |
| TRP1-MET17R-F | CATCACGTGCTATGAGTGTGCGTAATGAGTTGT |

***$P_{GAL10}$-ERG12-ePTS1-$T_{CYC1}$* and *$P_{GAL1}$-ERG8-ePTS1-$T_{ADH1}$* gene expression cassettes construction**

| | |
|---|---|
| HOL-F | TCCCAGGCGTAGAACAGTTTATCAG |
| Tcyc1-HOL-R | GAAGGCTTTAATTTGGATCCAAGCTATCTACTGAG |
| HOL-Tcyc1-F | TAGATAGCTTGGATCCAAATTAAAGCCTTCGAGCGT |
| eTCYC1-ERG12-R | TCTTCCCAATGAACCTGAAGTCCATGGTAAATTCGTG |
| GAL10-ERG12-F | TGAAAATTCAATATAAATGTCATTACCGTTCTTAACTTC |
| ERG12-GAL10-R | ACCCAAGTAATATAGTCATGTAATTAGTTATGTCACGCT |
| ERG8-GAL1-R | CTCAACTCTGACATTATAGTTTTTTCTCCTTGACGTTAAAG |
| GAL1-ERG8-F | AGGAGAAAAAACTATAATGTCAGAGTTGAGAGCCTTC |
| ePTS1-ERG8-R | ATCTTCTACCTCTTCCCAATGAACCTTTATCAAGATAAGTTTCCGGA T |
| ePTS1-TADH1-F | GGTTCATTGGGAAGAGGTAGAAGATCCAAATTGTAAGCGAATTTCT TATGATTTATG |
| MET17-TADH1-R | AACCCTTGCATCCGAGATCATGATACATAAAAGCGATATAAC |
| TADH1-MET17-F | ATGTATCATGATCTCGGATGCAAGGGTTCGAATC |
| HOR-MET17-R | CTGACATACCAAGAACTGTTCTTTGATGTTAG |
| MET17-H0R-F | AACATCAAAGAACAGTTCTTGGTATGTCAGCTACT |
| HOR-R | CACTTCACGTGCTTCTGGTACATACTTG |

***$P_{GAL7}$-MVD1-ePTS1-$T_{CYC1}$* and *$P_{GAL2}$-IDI1-ePTS1-$T_{ADH1}$* gene expression cassettes construction**

| | |
|---|---|
| LPPA-F | CCAATCATGGTTTCATGGTCACTGGTAAG |
| CYC1t-LPPA-R | GAAGGCTTTAATTTGGAATATGACGAGTTTCCTTAGC |
| LPPA-CYC1t-F | CGTCATATTCCAAATTAAAGCCTTCGAGCG |
| MVD1-eTCYC1-F | GTCTACCAAAGGAAGGTTCATTGGGAAGAGGTAGAAG |
| eTCYC1-MVD1-R | TCTTCCCAATGAACCTTCCTTTGGTAGACCAGTC |
| GAL7-MVD1-F | ATATTCCCTCAAAAATGACCGTTTACACAGCATC |
| MVD1-GAL7-R | GTGTAAACGGTCATTTTTGAGGGAATATTCAACTG |
| ID1- Gal2-R | TTGTCGGCAGTCATTATGAAAGAATTATTTTTTTTATTATGTTAATC |
| Gal2-ID1-F | TAATTCTTTCATAATGACTGCCGACAACAATAG |
| eADH1-IDI1-R | CTTCCCAATGAACCTAGCATTCTATGAATTTGC |
| IDI1-eADH1-F | TCATAGAATGCTAGGTTCATTGGGAAGAGGTAGAAG |
| HIS3-ADH1-R | CATGAACAAGGAAGTACAGGACAGATCATGATACATAAAAGCGAT ATAAC |
| ADH1-HIS3-F | GTTATATCGCTTTTATGTATCATGATCTGTCCTGTACTTCCTTGTTC ATGTG |
| LPPB-HIS3-R | GTACTGAAAGTAGTATAGCTTATAGCACGTGATGAATTCG |
| His3-LPPB-F | CGAATTCATCACGTGCTATAAGCTATACTACTTTCAGTAC |
| LPPB-R | CTGTAAGTGTTACTAAACACTAACCGGTGAAG |

| | |
|---|---|
| **$P_{GAL10}$-*tHMG1-ePTS1-*$T_{TDH2}$ and $P_{GAL1}$-*ERG20-ePTS1-*$T_{ADH3}$ gene expression cassettes construction** | |
| DPPA-F | GTCCGATAAACACAGTTGTGATCTGTCTTG |
| eTDH2-DPPA-R | TTGGCTTTTCGCTATGATTTAATTCTGATTCTTTG |
| DPPA-eTDH2-F | AGAATTAAATCATAGCGAAAAGCCAATTAGTGTG |
| tHMG1-eTDH2-F | CTGCATTAAATCCGGTTCATTGGGAAGAGGTAG |
| Gal10-tHMG1-F | AAATTCAATATAAATGGTTTTAACCAATAAAACAG |
| eTDH2-tHMG1-R | TCCCAATGAACCGGATTTAATGCAGGTGACGGAC |
| tHMG1-Gal10-R | TGGTTAAAACCATTTATATTGAATTTTCAAAAATTCTTAC |
| ERG20-GAL1-R | TTCTGAAGCCATTATAGTTTTTTCTCCTTGACG |
| Gal1-ERG20-F | GAGAAAAAACTATAATGGCTTCAGAAAAAGAAATTAGGAG |
| eADH3-ERG20-R | TCCCAATGAACCTTTGCTTCTCTTGTAAACTTTG |
| ERG20-eADH3-F | CAAGAGAAGCAAAGGTTCATTGGGAAGAGGTAGAAG |
| LEU2-eADH3-R | CTCTAGAGTCGACGATGTTTTAGTTCGAATGTCTCCTATTG |
| TADH3-LEU2-F | GAACTAAAACATCGTCGACTCTAGAGGATC |
| DPPB-LEU2-R | CGGTTGTTGGTAATACACTGTAGCACGTGATGAATTC |
| LEU2-DPPB-F | GAATTCATCACGTGCTACAGTGTATTACCAACAACCG |
| DPP1B-R | CGGGATCCTCTGATGGGTGACTG |
| ***GAL80* deletion** | |
| GAL80L-F | TGAAGCCATAGAACCACCACCCAATTCGGATAAGTGGTCTAT |
| TRP1-GAL80L-R | TCTAGAGTCGACGATCTCTTGTTGTAGTCCATG |
| GAL80L-TRP1- F | CAACAAGAGATCGTCGACTCTAGAGGATCCCCGGGAT |
| GAL80R-TRP1-R | GCAAGATGCTTTAGCACGTGATGAATTCGAG |
| HIS3-GAL80R-F | ATTCATCACGTGCTAAAGCATCTTGCCCTGTGCTTG |
| GAL80R-R | TTCATCGTGTTGTTTTGGCCTTGGAAT |
| **$P_{HXT1}$-*GAL80*** | |
| HXT1-TRP1-R | TTCATCACGTGCTACAGGTCTCATCTGGAATATAAT |
| TRP1-HXT1-F | CATAAACAAACAAAATGTCAGCTGCAGTTAAATGTTCATTGACT |
| GAL80-HXT1-R | TTGTTGTAGTCCATGATTTTACGTATATCAACTAGTTG |
| HXT1-GAL80-F | GATATACGTAAAATCATGGACTACAACAAGAGATC |
| GAL80R-R | TTCATCGTGTTGTTTTGGCCTTGGAAT |
| HXT1-TRP1-R | TTCATCACGTGCTACAGGTCTCATCTGGAATATAAT |
| ***PEX5* deletion** | |
| PEX5L-F | ACGATAGGATCATATCCGCTCAGAG |
| URA3-PEX5L-R | CTCTAGAGTCGACGTGTTATGATATATTGTTTATTG |
| PEX5L-URA3-F | AATATATCATAACACGTCGACTCTAGAGGATC |
| PEX5R-URA3-R | GGAGAACCATATAGCACGTGATGAATTCGAG |
| PEX5L-F | ACGATAGGATCATATCCGCTCAGAG |
| URA3-PEX5L-R | CTCTAGAGTCGACGTGTTATGATATATTGTTTATTG |
| URA3-PEX5B-F | AATTCATCACGTGCTATATGGTTCTCCGCAATTTTATG |
| PEX5B-R | GGATTTCTGCTTATGGTGGAATCGC |
| **$P_{ADH2}$-*ZSS1-*$T_{CYC1}$ gene expression cassette construction** | |
| δ2-F | GCTTCGGTTACTTCTAAGGAAGTCCACAC |
| ADH2-δ2-R | GTCTCTTCAAACAAACATTGGAAAGTCATTAG |
| δ2-ADH2-F | CTAATGACTTTCCAATGTTTGTTTGAAGAGAC |

| | |
|---|---|
| ZSS1-ADH2-R | CATAGATTGTCTTTCCATTGTGTATTACGATATAGTTAATAG |
| ADH2-ZSS1-F | CTTTTTACAACAAATATAAAACAATGGAAAGACAATCTATGGC |
| HIS3-TCYC1-R | CCTCTAGAGTCGACCAAATTAAAGCCTTCGAGCGTC |
| TCYC1-HIS3-F | AAGGCTTTAATTTGGTCGACTCTAGAGGATC |
| δ1-HIS3-R | GTTGATTTCTATTCCAACACCCGGGATAACTTCGTATAATG |
| HIS3-δ1-F | CATTATACGAAGTTATCCCGGGTGTTGGAATAGAAATCAAC |
| δ1-R | CACAGGCGCTACCATGAGAATTG |

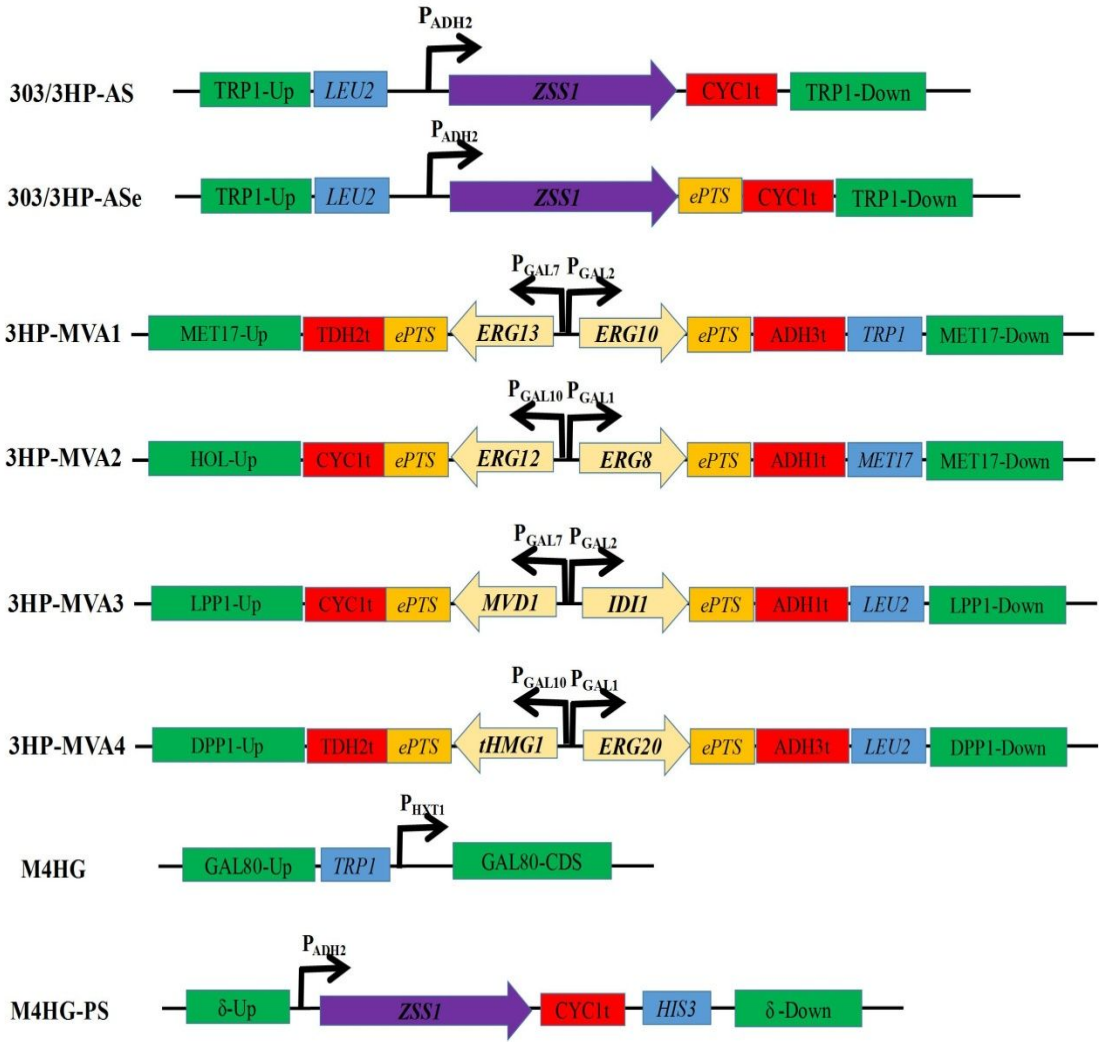


**Figure S1 Construction and insertion of expression cassettes.**

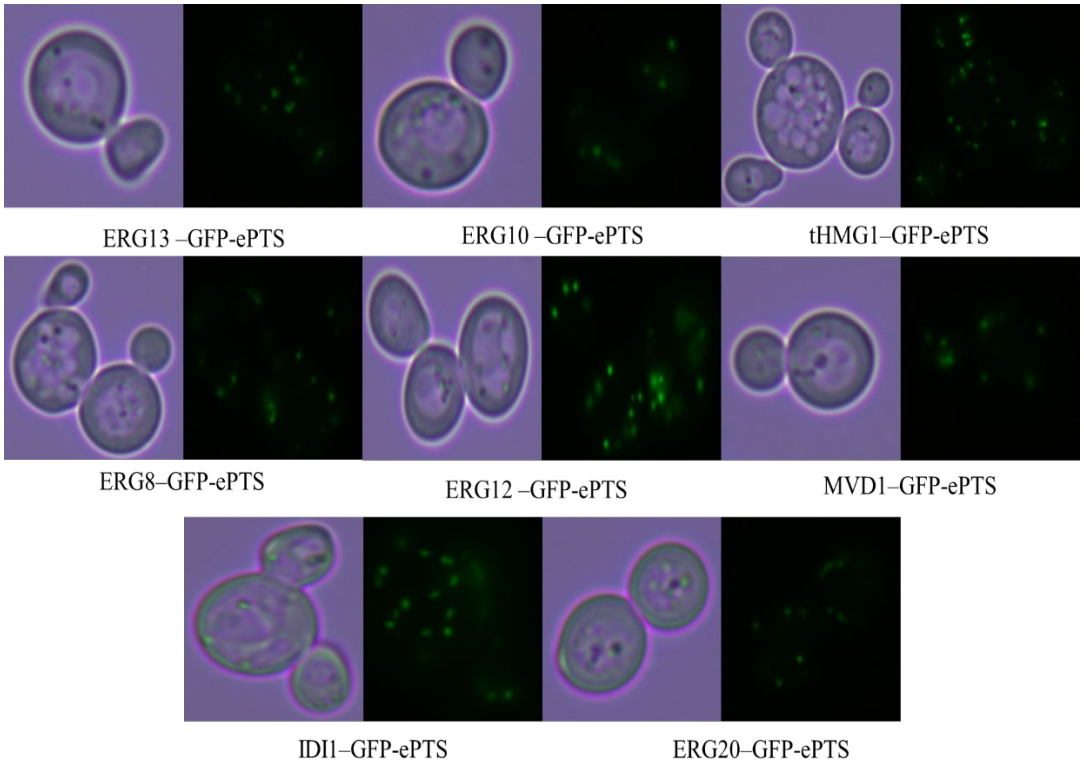


**Figure S2 Fluorescence microscopy (10×100) of yeast strains containing MVA pathway enzyme and GFP fusion proteins.** MVA pathway enzymes were fused with GFP-ePTS respectively, and the constructs were inserted into the genome of W303-1a at *leu2* site and expressed under the control of $P_{GAL1}$. Yeast strains were observed at 8 h after adding 20 g/L of galactose.