

**Supplementary Materials for:**

**Revealing the Mechanisms of Enhanced  $\beta$ -Farnesene Production in  
*Yarrowia lipolytica* through Metabolomics Analysis**

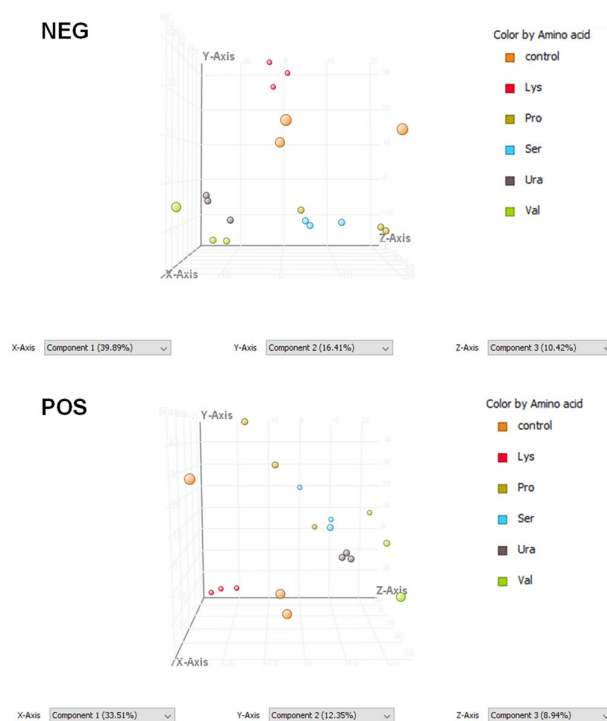
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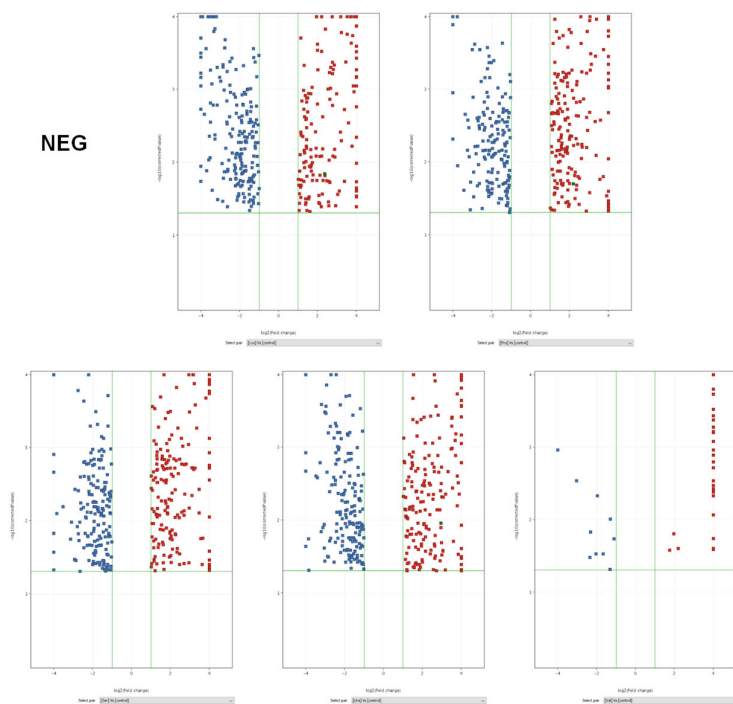
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## Supplementary Figures

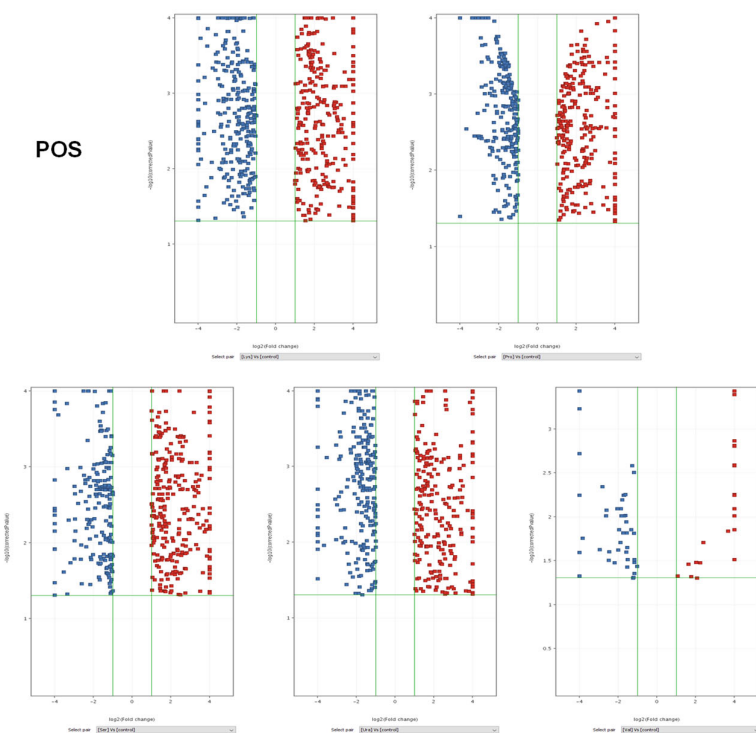


**Figure S1.** 3D PCA plot of different experimental groups in positive and negative polarity.

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4 **Figure S2.** Volcano plot of metabolites under different amino acid/nucleobase

5 additions in positive and negative polarity.

## 6 Supplementary Tables

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8 **Table S1.** Strains used in this study.

Strains	Genotype or characteristic	Sources
<i>E. coli</i>	Trans10	TransGen
AYL119	AYL101, pMO- <i>P<sub>TEFin</sub>-mmACL-T<sub>xpr2</sub>-P<sub>TEFin</sub>-ylAMPD-T<sub>xpr2</sub>-T<sub>xpr2</sub>-P<sub>TEFin</sub>-ylYHM2-T<sub>xpr2</sub></i>	[14]
AYL119-1	AYL119, pMO- <i>P<sub>TEFin</sub>-ylPanK-T<sub>lip2</sub></i>	This study
AYL119-2	AYL119, pMO- <i>P<sub>TEFin</sub>-ylPanL-T<sub>lip2</sub></i>	This study
AYL119-3	AYL119, pMO- <i>P<sub>TEFin</sub>-ylETNK-T<sub>lip2</sub></i>	This study
AYL119-4	AYL119, pMO- <i>P<sub>TEFin</sub>-ylPAP-T<sub>lip2</sub></i>	This study
AYL119-5	AYL119, pMO- <i>P<sub>TEFin</sub>-ylERG3-T<sub>lip2</sub></i>	This study
AYL119-6	AYL119, $\Delta$ <i>ylPAP::3HA</i>	This study
AYL119-7	AYL119, $\Delta$ <i>ylERG3::3HA</i>	This study

9 **Table S2.** Plasmids used in this study.

Plasmids	Description	Sources
pMO	Amp, mtOri, <i>URA3</i> marker, TEFin promoter and XPR2 terminator	Laboratory storage
pRSF	Kan, 3HA- <i>URA3</i> marker-3HA, TEFin promoter and XPR2 terminator	Laboratory storage
pMO-ylPanK	<i>P<sub>TEFin</sub>-ylPanK-T<sub>lip2</sub></i>	This study
pMO-ylPanL	<i>P<sub>TEFin</sub>-ylPanL-T<sub>lip2</sub></i>	This study
pMO-ylETNK	<i>P<sub>TEFin</sub>-ylETNK-T<sub>lip2</sub></i>	This study
pMO-ylPAP	<i>P<sub>TEFin</sub>-ylPAP-T<sub>lip2</sub></i>	This study
pMO-ylERG3	<i>P<sub>TEFin</sub>-ylERG3-T<sub>lip2</sub></i>	This study
pRSF- ylPAP	<i>PAP<sub>up</sub>-3HA-URA3-3HA-PAP<sub>down</sub></i>	This study
pRSF- ylERG3	<i>ERG3<sub>up</sub>-3HA-URA3-3HA-ERG3<sub>down</sub></i>	This study

11 **Table S3.** List of primers used in this study.

Names	Sequences (5' > 3')
pMOvec-F	GCTATTTATCACTCTTTACAACCTTCTACCTCAACTATC
pMOvec-R	CTGCGGTTAGTACTGCAAAAAGTGCTG
ylPanK-F	CTTTTTGCAGTACTAACCGCAGATGCAACAAGCAACACAGGA ACTG
ylPanK-R	GTTGTAAAGAGTGATAAATAGCCTACTGCATGAAACGCTCCAA CTCC
ylPanL-F	CTTTTTGCAGTACTAACCGCAGATGTTGCGACCGGTGATTCTG
ylPanL-R	GTTGTAAAGAGTGATAAATAGCTTAACACAGAACATTGTCAAT GATTCTAGTCT
ylETNK-F	CTTTTTGCAGTACTAACCGCAGATGTCGCAACCAACATACCCA ATG
ylETNK-R	GTTGTAAAGAGTGATAAATAGCTTACTTTGCCTCTCTTCCCTTC TTCC
ylPAP-F	CTTTTTGCAGTACTAACCGCAGATGTTGTCTTCCAGCTCCACCC T
ylPAP-R	GTTGTAAAGAGTGATAAATAGCTTAAACCTGGTTCTCGAGCTG AACATCG
ylERG3-F	CTTTTTGCAGTACTAACCGCAGATGGATATCGCTCTGGAGACCA TCG
ylERG3-R	GTTGTAAAGAGTGATAAATAGCTTAATCCTGCTTGGTGTTCGC TTGACA
ylERG3-UP-F	TTGCGTTGCGCCAATCTATACACTAGCCAGATGGCTTCTTTG
ylERG3-UP-R	CGTTTTACAACGGCGGCAGGTGTGTGTGTC
ylERG3-DM-F	TCTCTGTCTGCCATCTTTTGACCATGAAGCGAAGGACAAG
ylERG3-DM-R	ATGCCTGCCATGATGATACACGAGAGAGAGAGATAGC
pRSF-ERG3vec-F	CGTGTATCATCATGGCAGGCATTTGAGAAGCACACGGTC
pRSF-ERG3vec-R	GTGTATAGATTGGCGCAACGCAATTAATGTAAGTTAGCTCA
ylERG3-3HA-F	CCTGCCGCCGTTGTAAAACGACGGCCAGTCGAAC
ylERG3-3HA-R	TGGTCAAAAGATGGCAGACAGAGAGGTGAAGAAGAGG

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ylPAP-UP-F	GTTGCGCCTGGGGCAAGAAGTTTCCTGGC
ylPAP-UP-R	CGTTTTACAAC TTTGGGTGCGGCAAAGTTGATTTTCAG
ylPAP-DM-F	CTCTGTCTGCCACCAAACCAAACAATATAAAAACGGATCTGTA G
ylPAP-DM-R	CAAATGCCTGAGAGGGTCCTCCTTGAGGCAG
pRSF-PAPvec-F	GGAGGACCCTCTCAGGCATTTGAGAAGCACACGG
pRSF-PAPvec-R	CTTCTTGCCCCAGGCGCAACGCAATTAATGTAAGTTAGCTCAC
ylPAP-3HA-F	GCCGCACCCAAAGTTGTAAAACGACGGCCAGTCGAAC
ylPAP-3HA-R	TTGGTTTGGTGGCAGACAGAGAGGTGAAGAAGAGG

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## 13   **References**

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15       Tan, T. Enhancing precursor supply and modulating metabolism to achieve high-level  
16       production of  $\beta$ -farnesene in *Yarrowia lipolytica*. *Bioresour. Technol.* **2023**, 382, 129171.

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