

# Engineered cross-feeding creates inter- and intra-species synthetic yeasts communities with enhanced bioproduction

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**Supplementary Table 1.** Growth rate and maximal OD from syntrophic cocultures of *Y. lipolytica* auxotroph strains (inoculation ratio = 1:1). Values represent average and standard deviation (n=3).

| Auxotrophic pair   | maximal growth rate (h <sup>-1</sup> ) |    | maximal OD    |    |
|--------------------|----------------------------------------|----|---------------|----|
| <i>Δura3-Δleu2</i> | 0.035 ± 0.011                          | +  | 0.244 ± 0.010 |    |
| <i>Δura3-Δlys5</i> | 0.066 ± 0.019                          | ++ | 0.329 ± 0.009 | +  |
| <i>Δura3-Δtrp2</i> | 0.032 ± 0.005                          | +  | 0.360 ± 0.019 | +  |
| <i>Δura3-Δtrp4</i> | 0.072 ± 0.023                          | ++ | 0.700 ± 0.025 | ++ |
| <i>Δura3-Δmet5</i> | 0.047 ± 0.003                          | +  | 0.646 ± 0.055 | ++ |
| <i>Δleu2-Δlys5</i> | 0.016 ± 0.006                          |    | 0.221 ± 0.013 |    |
| <i>Δleu2-Δtrp2</i> | 0.035 ± 0.007                          | +  | 0.332 ± 0.005 | +  |
| <i>Δleu2-Δtrp4</i> | 0.048 ± 0.010                          | +  | 0.609 ± 0.038 | ++ |
| <i>Δleu2-Δmet5</i> | 0.011 ± 0.007                          |    | 0.262 ± 0.023 |    |
| <i>Δlys5-Δtrp2</i> | 0.031 ± 0.005                          | +  | 0.359 ± 0.041 | +  |
| <i>Δlys5-Δtrp4</i> | 0.040 ± 0.008                          | +  | 0.566 ± 0.043 | ++ |
| <i>Δlys5-Δmet5</i> | 0.013 ± 0.002                          |    | 0.393 ± 0.047 | +  |
| <i>Δtrp2-Δtrp4</i> | 0.084 ± 0.003                          | ++ | 0.513 ± 0.041 | +  |
| <i>Δtrp2-Δmet5</i> | 0.019 ± 0.006                          |    | 0.304 ± 0.041 |    |
| <i>Δtrp4-Δmet5</i> | 0.029 ± 0.006                          | +  | 0.627 ± 0.063 | ++ |

| growth rate                   |    | OD                    |    |
|-------------------------------|----|-----------------------|----|
| $\mu_{\max} \geq 0.06$        | ++ | $OD \geq 0.55$        | ++ |
| $0.02 \leq \mu_{\max} < 0.06$ | +  | $0.32 \leq OD < 0.55$ | +  |
| $\mu_{\max} \leq 0.02$        |    | $OD \leq 0.32$        |    |

**Supplementary Table 2.** Primer sequences used in this study.

| Primer             | Sequence (5' - 3')                                     |
|--------------------|--------------------------------------------------------|
| pTEF-internal-Fw   | CCATGCCGGACGCAAAATAGACTAC                              |
| Tlip2-internal-Rev | CGATTTGTCTTAGAGGAACGCATATACAGTAATC                     |
| hrGFP-internal-Rev | GCTGTAGAACTTGCCGCTGTTTCAG                              |
| mRFP1-internal-Rev | CGGTAACAACACCACCGTCTTCG                                |
| URA3-500-Rev       | CTGGCCAGCTTCTCGTTGGG                                   |
| URA3-ATG-Fw        | ATGCCCTCTACGAAGCTCGAG                                  |
| LEU2-800-Rev       | GGCCTCGTCGGAGATGATATCGC                                |
| LEU2-ATG-Fw        | ATGGAACCCGAAACTAAGAAGACCAAGAC                          |
| P-LYS5-Fw          | CCCATCACACGGATTACTTAGGTGG                              |
| P-LYS5-Rev         | CGTGCAATTACCCTGTTATCCCTAGCCTGGTTGAAGTCGTTCTGGG         |
| T-LYS5-Fw          | GGCTAGGGATAACAGGGTAATGCACGTCTAGAATTTGAACCTATCG         |
| T-LYS5-Rev         | GGCCAGAAATTTCTGGTACGACATG                              |
| Ex-LYS5-Fw         | CGACATTGAGATGATTTCTCAGGTGCC                            |
| Ex-LYS5-Rev        | CCCGTTTGAACAGTACACTTGGC                                |
| P MET5-Fw          | ATAAGAATGCGGCCGCGGTACAAGTACCAAGCCAAGG                  |
| P MET5-Rev         | CGATTACCCTGTTATCCCTACCGTTGCAGTCTCCATTGAGG              |
| T MET5-Fw          | GGTAGGGATAACAGGGTAATCGGTCATCAAGGAGACCACTGC             |
| T MET5-Rev         | ATAGTTTAGCGGCCGCCACGATCATGTGACCAGACC                   |
| Ex-MET5-Fw         | CGGTGCTGTACCTGTACAGTAC                                 |
| Ex-MET5-Rev        | GCTTCTAGCAGGTTCACTTGG                                  |
| P TRP2-Fw          | ATAAGAATGCGGCCGCGAGCTGATGTGGCAAGGTC                    |
| P TRP2-Rev         | CGATTACCCTGTTATCCCTACCCGAAATGTATCCGATGGCTCC            |
| T TRP2-Fw          | GGTAGGGATAACAGGGTAATCGGAGGCGATGAAGATGCACTTC            |
| T TRP2-Rev         | ATAGTTTAGCGGCCGCCAAGTATGGCTGTCTCGGAAG                  |
| P TRP4-Fw          | ATAAGAATGCGGCCGCGCACTTGGTCAGGTATAGCG                   |
| P TRP4-Rev         | CGATTACCCTGTTATCCCTACCGTGTGAGAGGCAGAGACATG             |
| T TRP4-Fw          | GGTAGGGATAACAGGGTAATCGGAAGGAGGCATAAACGCTCG             |
| T TRP4-Rev         | ATAGTTTAGCGGCCGCGAGTCGGAGTTGTGTTCAACC                  |
| GG-BAPAT-Fw        | GCATCGTCTCATCGGGGTCTCAAATGGAATTAATGATCGTTCAAGTAACTGAGC |
| BAPAT-600-Rev      | GGATTTCTGTAGGTATCAGGTGGG                               |
| GG-BAPAT-Rev       | CTGACGTCTCAGGTCGGTCTCATAGATCAGGATCCTAGTTGAGCAAGACATTCC |
| GG-YDFG-Fw         | GCATCGTCTCATCGGGGTCTCAAATGATAGTCTTGGTCACAGGTGCAAC      |
| YDFG-700-Rev       | CGGGCATCATTTCCAAGG                                     |
| GG-YDFG-Rev        | CTGACGTCTCAGGTCGGTCTCATAGATCAGGATCCTTGCCTATGCACG       |
| GG-PAND-Fw         | GCATCGTCTCATCGGGGTCTCAAATGCCTGCAACAGGAGAGGAC           |
| PAND-500-Rev       | GGGTCAAAGCGTCAGTTGCC                                   |
| GG-PAND-Rev        | CTGACGTCTCAGGTCGGTCTCATAGATCAGGATCCTAAATCAGAACCTAGACG  |

**Supplementary Table 3.** Gene sequences for 3-HP production used in this study.

| Gene  | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PAND  | ATGCCTGCAACAGGAGAGGACCAAGACCTAGTTCAAGACTTAATAGAGGAACCAGCAACATTTAGTGATG<br>CCGTATTATCCAGCGACGAGGAGCTATTTTCATCAGAAGTGCCCTAAGCCTGCTCCTATTTATAGCCCGGTGT<br>CTAAACCCGTGTCTTTGAATCATTGCCTAACCGTAGGTTGCACGAGGAGTTTCTAAGGTCTTCTGTGGACG<br>TTTTATTACAGGAAGCTGTTTTCGAAGGTACGAACAGGAAGAACAGAGTCCTTCAGTGAGAGAGCCAGA<br>GGAGCTAAGAAGGTTGATGGACTTCGGAGTCAGGAGTGCCCTTCTACCCACGAGGAGTTACTAGAGGTT<br>TTAAAGAAAGTAGTAACCTATAGTGTTAAACAGGACACCCATATTTCTGTTAACCACTATTTAGCGCGGT<br>GACCTTATGGATTAGTTGCACAATGGGCAACTGACGCTTGAACCTAGTGATATACTTACGAGGTGAG<br>TCCCGTATTTGTATTGATGGAAGAGTCTGCTTAGGGAGATGAGGGCAATCGTCGGATTTGAGGGCGGT<br>AAAGGGGACGGTATCTTCTGTCTGGCGGGTCAATAGCCAATGGCTACGCTATCTCCTGTGCAAGATATCG<br>TTTTATGCCTGACATCAAGAAGAAGGGGTTGCATTCTTGCCAAGATTAGTATTGTTACATCTGAGGATGC<br>GCACTATTCCATAAAGAACTTGCATCTTCCAGGGCATTGGGACCGACAACGTATATTTGATACGTACCG<br>ACGCAAGAGGCAGGATGGACGTTTCACACTTAGTTGAAGAGATAGAGCGTAGCTTGAGGGAAGGGGCAG<br>CACCTTTCATGGTTTCGGCTACCGCAGGGACCACTGTTATCGGAGCTTTCGATCCTATAGAAAAGATCGCC<br>GATGCTGCCAAAAGTACAAGCTTTGGCTACATGTAGACGCTGCGTGGGGTGGCGGGCATTGGTCAAGCG<br>CGAAGCACCGTCATCTACTGAAAGGAATTGAGAGGGCTGATTCAGTTACATGGAACCTCATAAGCTATTA<br>ACCGCCCCACAGCAGTGCTCACTTTATTATTGAGACATGAGGGTGTCTAGCTGAAGCACATTCCTACTAAT<br>GCCGCTTACCTGTTCCAGAAGGACAAGTTTTATGATACTAAGTACGACACAGGAGACAAACACATTCAGTG<br>TGGAAGGAGAGCAGACGTTCTAAAATTCTGGTTCATGTGGAAGCAAAGGGCACAAGCGGGCTTGAGAA<br>ACACGTGGACAAAGTTTTCGAGAAGCCAGGTTCTTTACAGATTGATTAAGAACCCTGAGGGCTTTGAGA<br>TGGAATTGCAGAGCCAGAATACACAAACATATGTTTCTGGTATGTCCCAAAGTCTCTACGTGGGCGTAA<br>GACGAGGCCGACTATAAAGACAACTACACAAAGTCGCTCCAAGAATCAAGGAGCGTATGATGAAAGAG<br>GGCTCTATGATGGTAACCTATCAGGCACAGAAGGGTCACCTAATCTTTCTGATAGTCTTTCAAACCTCT<br>GGACTAGACAAAGCCGACATGGTACATTTGGTCTGAAGAGATTGAGCGTCTAGGTTCTGATTAGGATCCT<br>GA |
| BAPAT | ATGGAATTAATGATCGTTCAAGTAAGTACGCAACACAGAGCTTAAGAAGACCGACGAGAAATATTTAT<br>GGCATGCCATGAGGGGCGCAGCGCCATCTCCGACTAATTTGATCATTACAAAGCAGAGGGTGCCTGGGT<br>CACCGATATTGATGGTAATCGTTATCTAGATGGCATGTCTGGCTTGTTGGTGTGTTAACGTCGGCTATGGGA<br>GAAAAGAAGTAGCAAGGGCTGCATTGCAACAACCTGAAGAAATGCCTTACTTCCCTTTAACTCAGTCTCAT<br>GTACCTGCAATAAAGCTAGCAGAGAAATGAATGAGTGGCTGGATGACGAATACGTATTTCTTCTCAA<br>CTCTGGGTCGGAAGCTAATGAAACGGCATTCAAATAGCAAGACAATATCACCACAGAAAGGTGACCAC<br>GGTAGATATAAGTTTATTTCAAGATATCGTGCATCATGGAATTCATGGGTGCCCTGGCTGCAACTGG<br>TCAAGCCCAAAGAAAATACAAGTACGAGCCACTAGGGCAAGGATTCCTACAGTAGCCCCACTGATACCT<br>ACAGAAATCCAGAAGATGTCCATACCTGGCATCAGCTGAGGAAATCGATCGTGTATGACGTGGGAATT<br>GTCTCAAACCGTGGCTGGAGTGATCATGGAACCCATCATTACGGGTGGTGGTATTCTGATGCCACCTGATG<br>GATATATGGAAGGTAAAGGAAATTTGCGAAAAGCAGGAGCACTTTTAACTGCGATGAGGTTATCTG<br>CGGATTTGGCCGTACTGGGAACCGTTTGGGTTTCATGAATATGGTGCAAGCCGGACATTATTAATGAG<br>CGAAAGGTATCACCAGCGCATACCTACCTTTCTGCCACAGCTGTGCGTGAAGTGACGAGGCTTTT<br>GTAGGTTCCGACGATTACGACAGATTTAGACATGTAAACACCTTTGGTGGAATCCAGCAGCCTGTGCATT<br>GGCTTTAAAGAACCTGGAATAATGAAAACGAAAAGCTGATTGAAAGATCAAAGGAAGTGGGTGAAAG<br>ATTGTTGTACGAATTAGAGGATGTGAAGGAACATCCCAATGTGGGTGATGTAAGGGGCAAAGGTTTGTG<br>CTGGGAATCGAACTAGTTGAGGATAAACAACTAAGGAACCTGCATCTATAGAAAAGATGAATAAGGTTA<br>TTAATGCATGCAAGGAGAAAGGACTGATCATCGGGAAGAATGGGGATACAGTAGCTGGGTATAACAATAT<br>CCTACAAGTGGCGCCCCGTGTCAATACCGAAGAGGACTTCACTTTTATAGTTAAACGATGAAGGAAT<br>GTCTTGCTCAACTAGGATCCTGA                                                                                                                                                                                                                                                                                                       |
| YDFG  | ATGATAGTCTTGGTCACAGGTGCAACGGCGGGTTTCGGTGAATGTATTACTCGTAGGTTCATACAACAGGG<br>GCATAAGGTTATAGCGACGGGAAGGAGACAGGAAAGATTGCAAGAATTAAGACGAACTTGGCGATAA<br>TTTGTATATTGCTCAATTGGATGTACGTAACAGGGCAGCTATAGAAGAAATGTTAGCCTCATTGCCAGCAG<br>AGTGGTGTAAACATAGACATTCTAGTAAACAATGCCGGTCTTGCCTTAGGAATGGAACAGCGCACAAAGGC<br>CTCTGTTGAAGATTGGGAGACTATGATCGATACGAACAATAAGGGTTTGGTCTATATGACGCGTGGCGTCT<br>TGCCGGGTATGGTGGAAGAAACCATGGCCACATAATTAATTTGGGTCCACAGCGGGTTCATGGCCATA<br>CGCTGGCGGGAACGTGTACGGTGCTACTAAAGCTTTTGAAGGCAATTTAGCCTGAATTTGAGGACCGATT<br>TGATGGCACTGCCGTTAGAGTAACAGACATTGAGCCCGTCTTGTGGCGGAACCGAATTTAGTAACGTG<br>AGGTTTAAAGGGTACGATGGTAAGGCAGAGAAAACCTATCAAAATACGGTAGCCTTGACACCAGAAGATG<br>TGTCAGAGGCTGTATGGTGGGTATCACTTTACCGGCGCATGTAATATAAATACCTTGGAATGATGCC<br>GTCACACAATCCTACGAGGCCTTAACGTGCATAGGCAAGGATCCTGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

**Supplementary Table 4.** Production of metabolites in the 3-HP biosynthetic pathway from the intra- and interspecies synthetic communities. Values represent average and standard deviation (n=2).

| Strain(s)                             | Inoculation ratio | $\beta$ -Alanine (mM) | MSA (mM)           | 3-HP (mM)          |
|---------------------------------------|-------------------|-----------------------|--------------------|--------------------|
| SC mono (P+B)                         |                   | 0.194 $\pm$ 0.0259    | 0.051 $\pm$ 0.0085 | 0.112 $\pm$ 0.0175 |
| YL mono (P+B)                         |                   | 0.071 $\pm$ 0.0007    | 0.021 $\pm$ 0.0017 | 0.242 $\pm$ 0.0177 |
| SC WT-P/SC WT-B                       | 1:1               | 0.190 $\pm$ 0.0021    | 0.061 $\pm$ 0.0029 | 0.065 $\pm$ 0.0047 |
| YL WT-P/YL WT-B                       | 1:1               | 0.092 $\pm$ 0.0007    | 0.019 $\pm$ 0.0005 | 0.113 $\pm$ 0.0264 |
| SC WT-B/YL WT-P                       | 1:1               | 0.051 $\pm$ 0.0027    | 0.038 $\pm$ 0.0042 | nd                 |
| SC $\Delta$ trp2-B/SC $\Delta$ trp4-P | 10:1              | nd                    | 1.599 $\pm$ 0.0267 | 0.104 $\pm$ 0.0316 |
|                                       | 1:1               | nd                    | 0.058 $\pm$ 0.0142 | 0.14 $\pm$ 0.0317  |
|                                       | 1:10              | nd                    | 0.066 $\pm$ 0.0066 | nd                 |
| YL $\Delta$ trp2-B/YL $\Delta$ trp4-P | 10:1              | 0.02 $\pm$ 0.0196     | 1.089 $\pm$ 0.0167 | 4.671 $\pm$ 0.0635 |
|                                       | 1:1               | 0.261 $\pm$ 0.0347    | 1.761 $\pm$ 0.0910 | 2.666 $\pm$ 0.0473 |
|                                       | 1:10              | 0.103 $\pm$ 0.0026    | 0.039 $\pm$ 0.0009 | 0.265 $\pm$ 0.0355 |
| SC $\Delta$ trp2-B/YL $\Delta$ trp4-P | 10:1              | nd                    | 1.466 $\pm$ 0.0637 | 4.500 $\pm$ 0.0119 |
|                                       | 1:1               | nd                    | 0.159 $\pm$ 0.0210 | 0.204 $\pm$ 0.0008 |
|                                       | 1:10              | 0.088 $\pm$ 0.0014    | 1.453 $\pm$ 0.0029 | 0.076 $\pm$ 0.0150 |
| YL $\Delta$ trp2-B/SC $\Delta$ trp4-P | 10:1              | 0.163 $\pm$ 0.0030    | 1.473 $\pm$ 0.0236 | 3.962 $\pm$ 0.0009 |
|                                       | 1:1               | 0.182 $\pm$ 0.0032    | 0.163 $\pm$ 0.0613 | 0.706 $\pm$ 0.1052 |
|                                       | 1:10              | 0.201 $\pm$ 0.0213    | 0.950 $\pm$ 0.1025 | 1.119 $\pm$ 0.0477 |

\*nd: not detected

**Supplementary Table 5.** Raw OD<sub>600</sub> data and normalized OD<sub>600</sub> data of Figure 4.

a) OD<sub>600</sub> data from the spectrophotometer

| Strain                                | Inoculation ratio |       | Time (hr) |      |      |       |       |      |
|---------------------------------------|-------------------|-------|-----------|------|------|-------|-------|------|
|                                       |                   |       | 0         | 24   | 48   | 72    | 96    | 120  |
| SC mono (P+B)                         |                   | rep 1 | 0.1       | 14.1 | 22.7 | 27.7  | 30.7  | 37.5 |
|                                       |                   | rep 2 | 0.1       | 13.7 | 23.2 | 27.2  | 28.2  | 37.6 |
| YL mono (P+B)                         |                   | rep 1 | 0.1       | 42.4 | 53.8 | 52.2  | 59.3  | 58.2 |
|                                       |                   | rep 2 | 0.1       | 41.4 | 54.9 | 56.2  | 57.9  | 61.8 |
| SC WT-P/SC WT-B                       | 1:1               | rep 1 | 0.1       | 21.7 | 28.9 | 35.2  | 34.6  | 42   |
|                                       |                   | rep 2 | 0.1       | 23.6 | 30   | 36.4  | 38.9  | 43.7 |
| YL WT-P/YL WT-B                       | 1:1               | rep 1 | 0.1       | 39.1 | 49.9 | 52.3  | 53.7  | 57.9 |
|                                       |                   | rep 2 | 0.1       | 36   | 43.6 | 49.1  | 46.5  | 49.4 |
| SC WT-B/YL WT-P                       | 1:1               | rep 1 | 0.1       | 28.7 | 42.3 | 37.4  | 30.5  | 43.5 |
|                                       |                   | rep 2 | 0.1       | 33   | 45.3 | 40.5  | 42    | 44   |
| SC $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:10              | rep 1 | 0.1       | 0.96 | 16.5 | 20.3  | 25.4  | 31.3 |
|                                       |                   | rep 2 | 0.1       | 1.28 | 16.6 | 22.7  | 28.8  | 33.9 |
|                                       | 1:1               | rep 1 | 0.1       | 8.94 | 43.1 | 32.6  | 36    | 39.4 |
|                                       |                   | rep 2 | 0.1       | 9.04 | 43.7 | 33.4  | 35.8  | 41.3 |
|                                       | 10:1              | rep 1 | 0.1       | 2.58 | 4.3  | 11.74 | 15.12 | 19.3 |
|                                       |                   | rep 2 | 0.1       | 2.68 | 4.14 | 11.54 | 15.4  | 20.4 |
| YL $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:10              | rep 1 | 0.1       | 2.54 | 46   | 49.9  | 51.8  | 52.2 |
|                                       |                   | rep 2 | 0.1       | 1.66 | 51.8 | 51.3  | 64.2  | 58   |
|                                       | 1:1               | rep 1 | 0.1       | 4.34 | 26.5 | 21.6  | 21.4  | 29.1 |
|                                       |                   | rep 2 | 0.1       | 4.74 | 31.3 | 24.7  | 22.4  | 28.7 |
|                                       | 10:1              | rep 1 | 0.1       | 0.82 | 2.62 | 5.78  | 7.74  | 8.96 |
|                                       |                   | rep 2 | 0.1       | 0.92 | 3.16 | 6.02  | 7.94  | 9.5  |
| SC $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:10              | rep 1 | 0.1       | 0.66 | 10.6 | 22.3  | 24.1  | 25.7 |
|                                       |                   | rep 2 | 0.1       | 0.98 | 9.9  | 26.2  | 25.4  | 24   |
|                                       | 1:1               | rep 1 | 0.1       | 7.98 | 39.7 | 34.3  | 41.1  | 46.3 |
|                                       |                   | rep 2 | 0.1       | 8.02 | 39.1 | 32.4  | 38.5  | 42.4 |
|                                       | 10:1              | rep 1 | 0.1       | 2.48 | 2.38 | 3.06  | 3.9   | 4.6  |
|                                       |                   | rep 2 | 0.1       | 2.76 | 2.24 | 3.64  | 4.38  | 5.2  |
| YL $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:10              | rep 1 | 0.1       | 0.34 | 16.4 | 39.3  | 49.3  | 44.6 |
|                                       |                   | rep 2 | 0.1       | 0.64 | 16.3 | 42.2  | 52    | 53.1 |
|                                       | 1:1               | rep 1 | 0.1       | 3.3  | 52.8 | 51.2  | 47.8  | 56.9 |
|                                       |                   | rep 2 | 0.1       | 3.44 | 53.1 | 54.8  | 48.9  | 58.9 |
|                                       | 10:1              | rep 1 | 0.1       | 0.84 | 4.22 | 13.08 | 18.58 | 27.3 |
|                                       |                   | rep 2 | 0.1       | 0.1  | 4.2  | 12.46 | 19.5  | 28.2 |

(b) Normalized OD<sub>600</sub> data

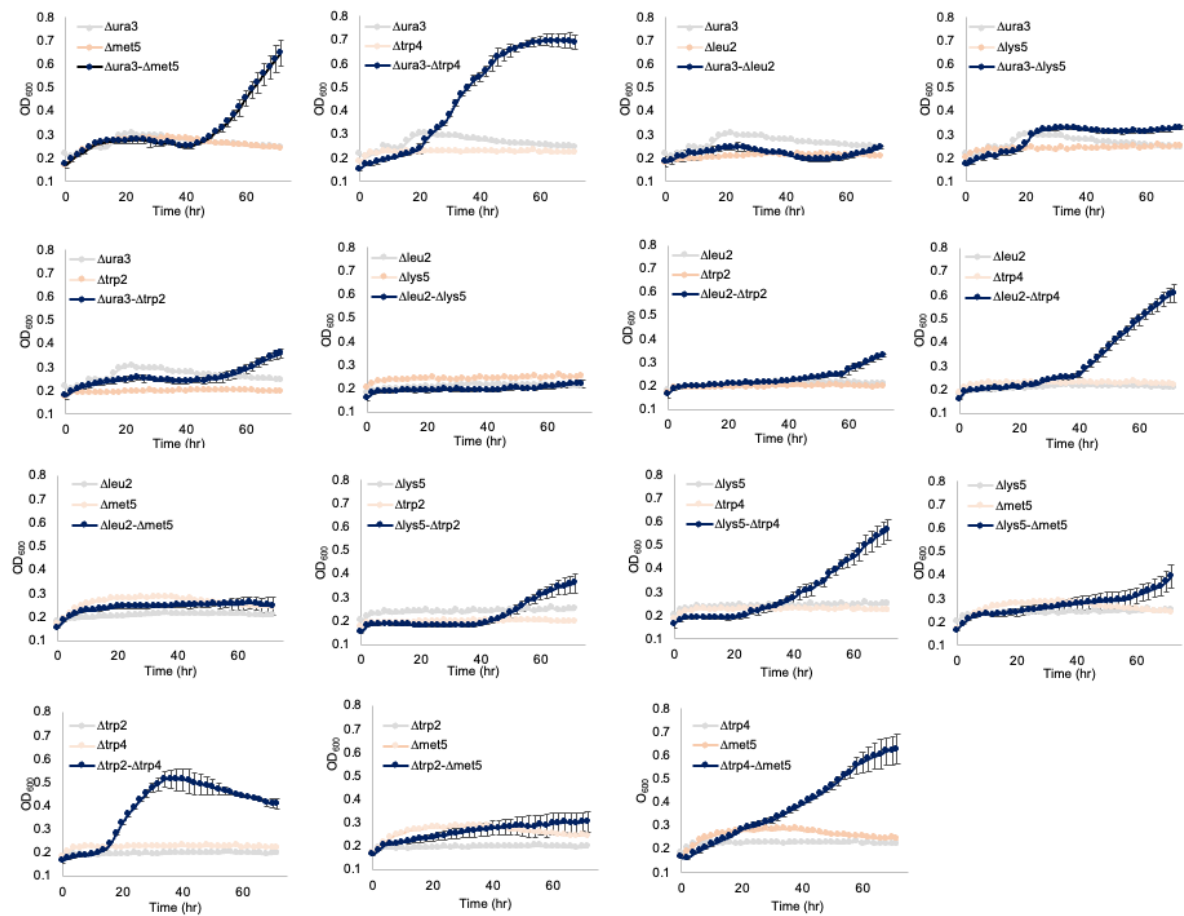
| Strain                                | Inoculation ratio |       | Time (hr) |      |      |      |      |      |
|---------------------------------------|-------------------|-------|-----------|------|------|------|------|------|
|                                       |                   |       | 0         | 24   | 48   | 72   | 96   | 120  |
| SC mono (P+B)                         |                   | rep 1 | 0.06      | 0.87 | 1.37 | 1.66 | 1.83 | 2.22 |
|                                       |                   | rep 2 | 0.06      | 0.85 | 1.40 | 1.63 | 1.69 | 2.23 |
| YL mono (P+B)                         |                   | rep 1 | 0.06      | 2.51 | 3.17 | 3.07 | 3.49 | 3.42 |
|                                       |                   | rep 2 | 0.06      | 2.45 | 3.23 | 3.31 | 3.40 | 3.63 |
| SC WT-P/SC WT-B                       | 1:1               | rep 1 | 0.06      | 1.31 | 1.73 | 2.09 | 2.06 | 2.48 |
|                                       |                   | rep 2 | 0.06      | 1.42 | 1.79 | 2.16 | 2.31 | 2.58 |
| YL WT-P/YL WT-B                       | 1:1               | rep 1 | 0.06      | 2.32 | 2.94 | 3.08 | 3.16 | 3.40 |
|                                       |                   | rep 2 | 0.06      | 2.14 | 2.58 | 2.90 | 2.74 | 2.91 |
| SC WT-B/YL WT-P                       | 1:1               | rep 1 | 0.06      | 1.72 | 2.50 | 2.22 | 1.82 | 2.57 |
|                                       |                   | rep 2 | 0.06      | 1.96 | 2.68 | 2.40 | 2.48 | 2.60 |
| SC $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:10              | rep 1 | 0.06      | 0.11 | 1.01 | 1.23 | 1.52 | 1.87 |
|                                       |                   | rep 2 | 0.06      | 0.13 | 1.02 | 1.37 | 1.72 | 2.02 |
|                                       | 1:1               | rep 1 | 0.06      | 0.57 | 2.55 | 1.94 | 2.14 | 2.33 |
|                                       |                   | rep 2 | 0.06      | 0.58 | 2.58 | 1.99 | 2.13 | 2.44 |
|                                       | 10:1              | rep 1 | 0.06      | 0.20 | 0.30 | 0.73 | 0.93 | 1.17 |
|                                       |                   | rep 2 | 0.06      | 0.21 | 0.29 | 0.72 | 0.95 | 1.24 |
| YL $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:10              | rep 1 | 0.06      | 0.20 | 2.72 | 2.94 | 3.05 | 3.07 |
|                                       |                   | rep 2 | 0.06      | 0.15 | 3.05 | 3.02 | 3.77 | 3.41 |
|                                       | 1:1               | rep 1 | 0.06      | 0.31 | 1.59 | 1.30 | 1.29 | 1.74 |
|                                       |                   | rep 2 | 0.06      | 0.33 | 1.87 | 1.48 | 1.35 | 1.72 |
|                                       | 10:1              | rep 1 | 0.06      | 0.10 | 0.21 | 0.39 | 0.50 | 0.57 |
|                                       |                   | rep 2 | 0.06      | 0.11 | 0.24 | 0.40 | 0.51 | 0.60 |
| SC $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:10              | rep 1 | 0.06      | 0.09 | 0.67 | 1.35 | 1.45 | 1.54 |
|                                       |                   | rep 2 | 0.06      | 0.11 | 0.63 | 1.57 | 1.52 | 1.44 |
|                                       | 1:1               | rep 1 | 0.06      | 0.52 | 2.35 | 2.04 | 2.43 | 2.73 |
|                                       |                   | rep 2 | 0.06      | 0.52 | 2.32 | 1.93 | 2.28 | 2.51 |
|                                       | 10:1              | rep 1 | 0.06      | 0.20 | 0.19 | 0.23 | 0.28 | 0.32 |
|                                       |                   | rep 2 | 0.06      | 0.21 | 0.18 | 0.27 | 0.31 | 0.36 |
| YL $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:10              | rep 1 | 0.06      | 0.07 | 1.00 | 2.33 | 2.91 | 2.63 |
|                                       |                   | rep 2 | 0.06      | 0.09 | 1.00 | 2.50 | 3.06 | 3.13 |
|                                       | 1:1               | rep 1 | 0.06      | 0.25 | 3.11 | 3.02 | 2.82 | 3.35 |
|                                       |                   | rep 2 | 0.06      | 0.25 | 3.13 | 3.22 | 2.88 | 3.46 |
|                                       | 10:1              | rep 1 | 0.06      | 0.10 | 0.30 | 0.81 | 1.13 | 1.63 |
|                                       |                   | rep 2 | 0.06      | 0.06 | 0.30 | 0.78 | 1.18 | 1.69 |

**Supplementary Table 6.** Raw data of metabolite analysis by LC/MS.

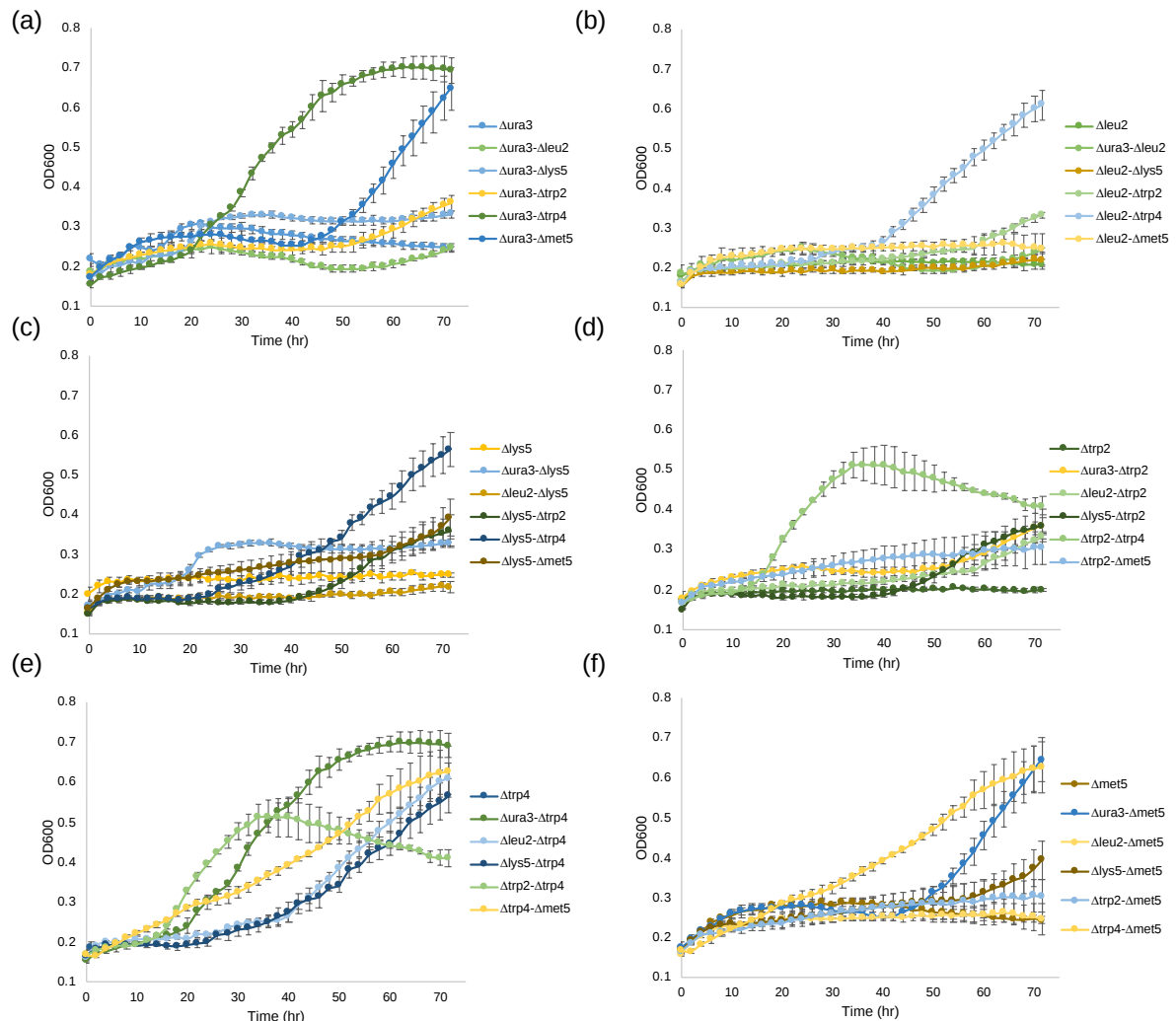
| Strain                                | Inoculation ratio |       | $\beta$ -Alanine     |           | MSA                  |           | 3-HP                 |           |
|---------------------------------------|-------------------|-------|----------------------|-----------|----------------------|-----------|----------------------|-----------|
|                                       |                   |       | Retention time (min) | Peak area | Retention time (min) | Peak area | Retention time (min) | Peak area |
| SC mono (P+B)                         | Mono culture      | rep 1 | 2.915                | 9036      | 2.598                | 6856      | 2.982                | 4859      |
|                                       |                   | rep 2 | 2.909                | 11823     | 2.609                | 4896      | 2.992                | 6669      |
| YL mono (P+B)                         | Mono culture      | rep 1 | 2.842                | 3875      | 2.558                | 2271      | 2.908                | 11548     |
|                                       |                   | rep 2 | 2.853                | 3803      | 2.553                | 2671      | 2.919                | 13380     |
| SC WT-P/SC WT-B                       | 1:1               | rep 1 | 2.904                | 10328     | 2.587                | 6710      | 2.987                | 3586      |
|                                       |                   | rep 2 | 2.904                | 10107     | 2.587                | 7386      | 2.937                | 3097      |
| YL WT-P/YL WT-B                       | 1:1               | rep 1 | 2.839                | 4908      | 2.606                | 2234      | 2.906                | 4474      |
|                                       |                   | rep 2 | 2.838                | 4986      | 2.588                | 2108      | 2.905                | 7200      |
| SC WT-B/YL WT-P                       | 1:1               | rep 1 | 2.835                | 2908      | 2.585                | 3850      | nd                   | nd        |
|                                       |                   | rep 2 | 2.837                | 2619      | 2.57                 | 4831      | nd                   | nd        |
| SC $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:10              | rep 1 | nd                   | nd        | 2.603                | 6904      | nd                   | nd        |
|                                       |                   | rep 2 | nd                   | nd        | 2.578                | 8426      | nd                   | nd        |
| SC $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:1               | rep 1 | nd                   | nd        | 2.571                | 5103      | 2.938                | 8875      |
|                                       |                   | rep 2 | nd                   | nd        | 2.587                | 8391      | 2.92                 | 5600      |
| SC $\Delta$ trp2-B/SC $\Delta$ trp4-P | 10:1              | rep 1 | nd                   | nd        | 2.586                | 181695    | 2.902                | 3714      |
|                                       |                   | rep 2 | nd                   | nd        | 2.583                | 187868    | 2.916                | 6974      |
| YL $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:10              | rep 1 | 2.843                | 5687      | 2.577                | 4372      | 2.91                 | 11835     |
|                                       |                   | rep 2 | 2.841                | 5412      | 2.574                | 4584      | 2.891                | 15503     |
| YL $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:1               | rep 1 | 2.833                | 15945     | 2.583                | 213951    | 2.9                  | 135148    |
|                                       |                   | rep 2 | 2.83                 | 12201     | 2.597                | 192932    | 2.914                | 140034    |
| YL $\Delta$ trp2-B/YL $\Delta$ trp4-P | 10:1              | rep 1 | nd                   | nd        | 2.589                | 123887    | 2.906                | 244342    |
|                                       |                   | rep 2 | 2.837                | 2115      | 2.571                | 127735    | 2.904                | 237788    |
| SC $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:10              | rep 1 | 2.84                 | 4801      | 2.606                | 168162    | 2.89                 | 3147      |
|                                       |                   | rep 2 | 2.838                | 4653      | 2.605                | 167499    | 2.939                | 4699      |
| SC $\Delta$ trp2-B/YL $\Delta$ trp4-P | 1:1               | rep 1 | nd                   | nd        | 2.573                | 15980     | 2.906                | 10553     |
|                                       |                   | rep 2 | nd                   | nd        | 2.584                | 20822     | 2.901                | 10468     |
| SC $\Delta$ trp2-B/YL $\Delta$ trp4-P | 10:1              | rep 1 | nd                   | nd        | 2.568                | 161980    | 2.901                | 232860    |
|                                       |                   | rep 2 | nd                   | nd        | 2.583                | 176699    | 2.9                  | 231634    |
| YL $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:10              | rep 1 | 2.839                | 9685      | 2.589                | 121604    | 2.905                | 60209     |
|                                       |                   | rep 2 | 2.839                | 11981     | 2.589                | 97910     | 2.906                | 55284     |
| YL $\Delta$ trp2-B/SC $\Delta$ trp4-P | 1:1               | rep 1 | 2.836                | 9653      | 2.569                | 25960     | 2.902                | 41879     |
|                                       |                   | rep 2 | 2.833                | 9997      | 2.566                | 11793     | 2.899                | 31016     |
| YL $\Delta$ trp2-B/SC $\Delta$ trp4-P | 10:1              | rep 1 | 2.836                | 8613      | 2.586                | 167435    | 2.903                | 204517    |
|                                       |                   | rep 2 | 2.833                | 8941      | 2.583                | 172882    | 2.9                  | 204426    |

\*nd: not detected

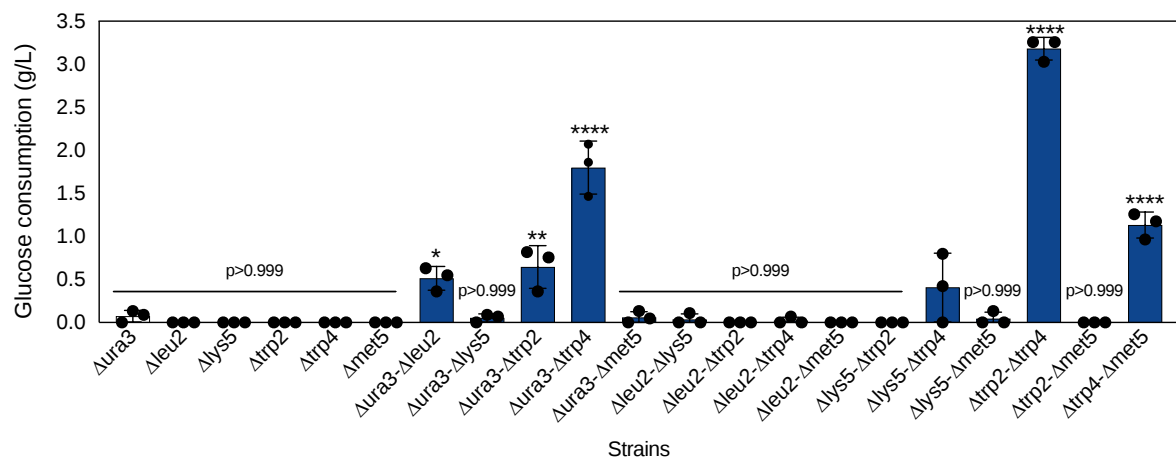
**Supplementary Figure 1.** Growth profile of syntrophic coculture of *Y. lipolytica* auxotroph strains (inoculation ratio = 1:1). Values represent averages and error bars denote standard deviation (n=3).



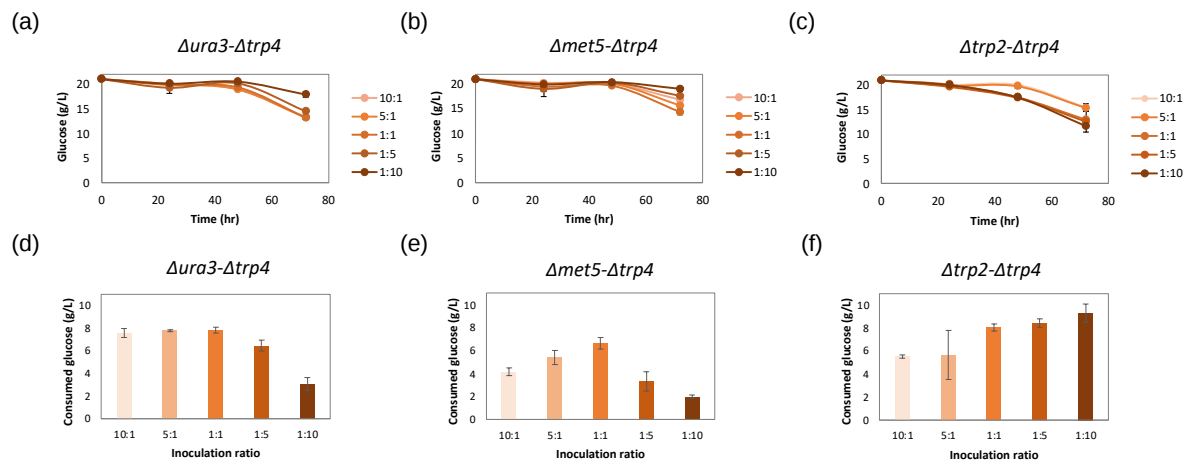
**Supplementary Figure 2.** Growth profile of syntrophic coculture of *Y. lipolytica* auxotroph strains with a specific auxotroph (inoculation ratio = 1:1). (a) coculture pairs with  $\Delta ura3$ , (b) coculture pairs with  $\Delta leu2$ , (c) coculture pairs with  $\Delta lys5$ , (d) coculture pairs with  $\Delta trp2$ , (e) coculture pairs with  $\Delta trp4$ , (f) coculture pairs with  $\Delta met5$ . Values represent averages and error bars denote standard deviation(n=3).



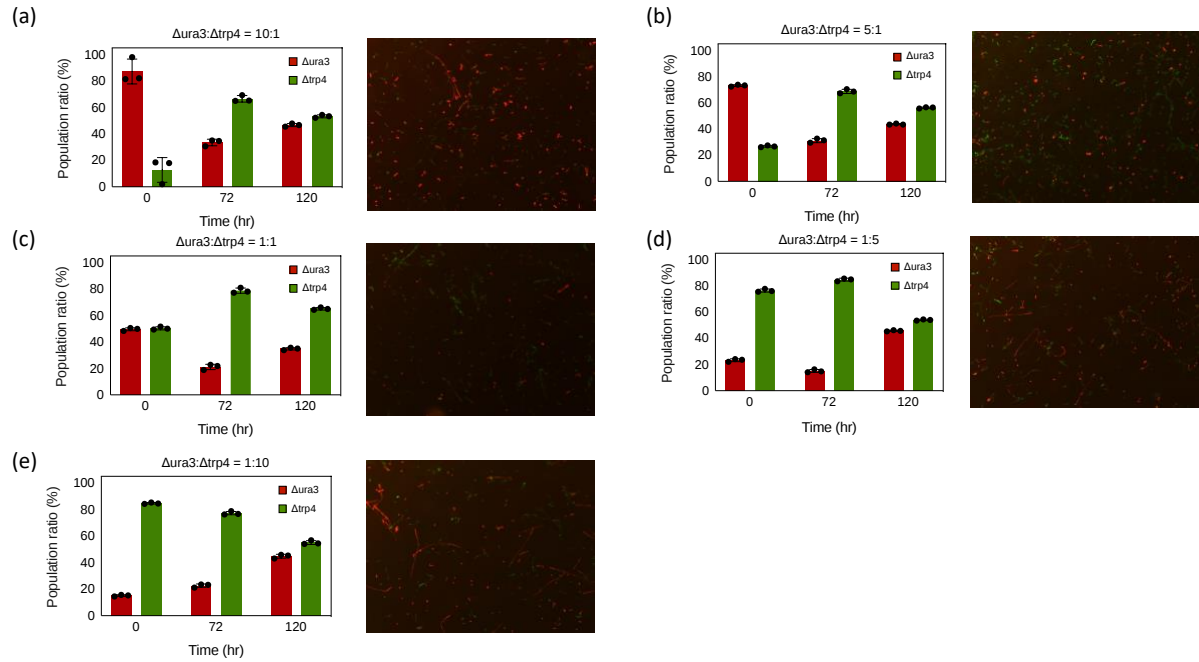
**Supplementary Figure 3.** Glucose consumption of monocultures (a single auxotroph) and cocultures (a pair of two auxotroph with 1:1 inoculation ratio) at 72 hours of cultivation. Values represent averages and error bars denote standard deviation (n=3). One-way ANOVA, followed by Bonferroni's multiple comparisons test with 95% confidence intervals were performed using GraphPad Prism 9.5.0 software and *p* values are indicated as asterisks in the graph (\*:*p* < 0.05, \*\*: *p* < 0.005, \*\*\*: *p* < 0.0005, \*\*\*\*: *p* < 0.0001).



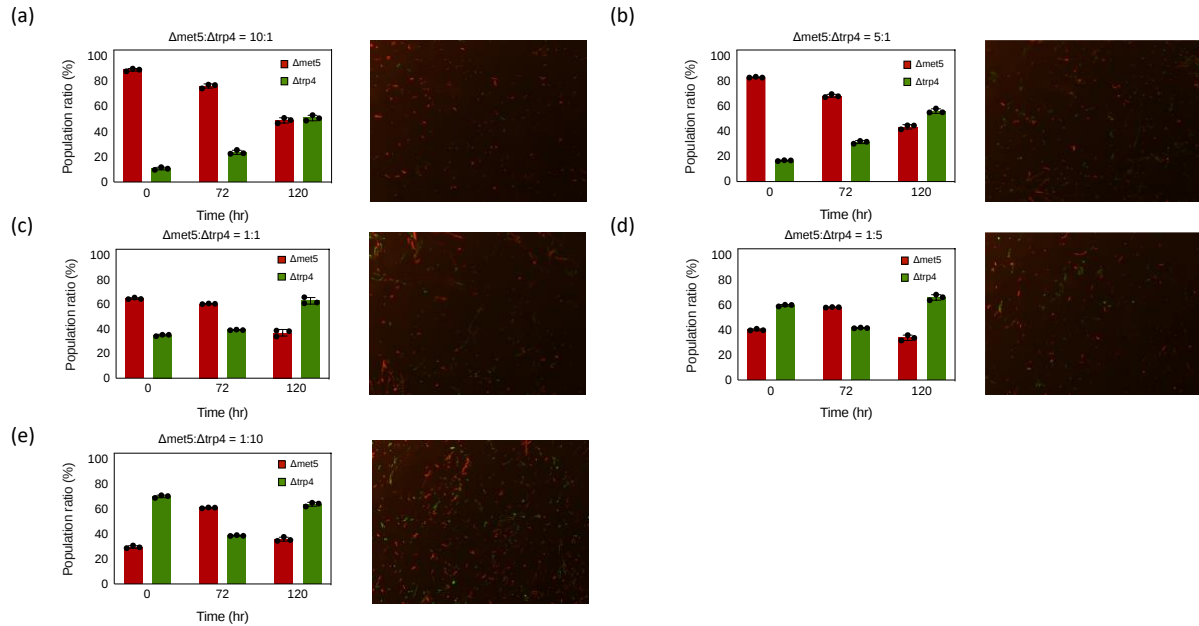
**Supplementary Figure 4.** Glucose consumption of selected syntrophic coculture of *Y. lipolytica* auxotroph strains at different inoculation ratio from 10:1 to 1:10. Values represent averages and error bars denote standard deviation (n=3).



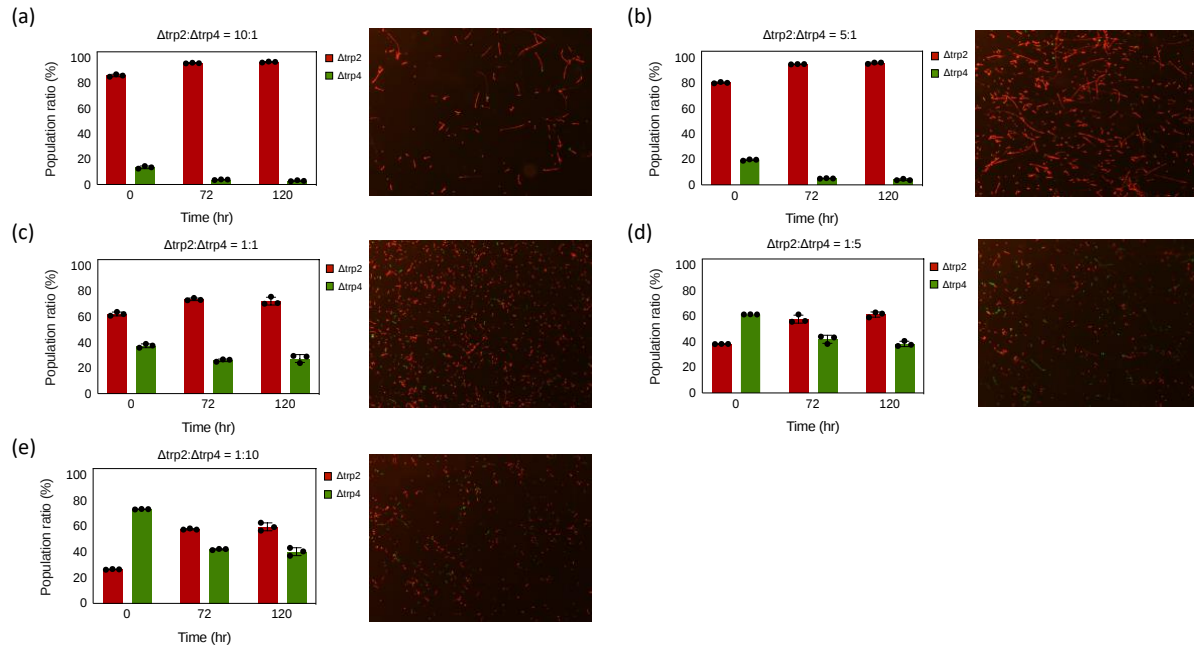
**Supplementary Figure 5.** Population of co-culture between YL $\Delta$ *ura3* and YL $\Delta$ *trp4* at different inoculation ratios. The populations were measured by Flow Cytometry and corresponding fluorescence microscope images were taken at 120 hours of cultivation. Values represent averages and error bars denote standard deviation (n=3).



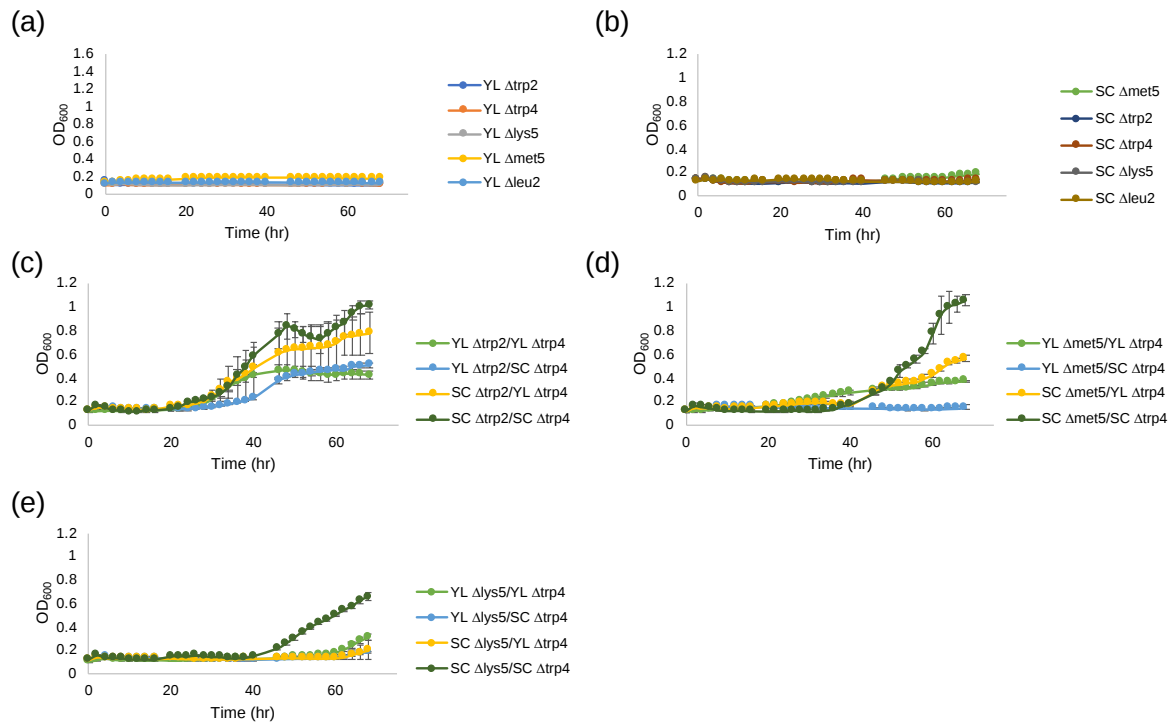
**Supplementary Figure 6.** Population of co-culture between YL $\Delta met5$  and YL $\Delta trp4$  at different inoculation ratios. The populations were measured by Flow Cytometry and corresponding fluorescence microscope images were taken at 120 hours of cultivation. Values represent averages and error bars denote standard deviation (n=3).



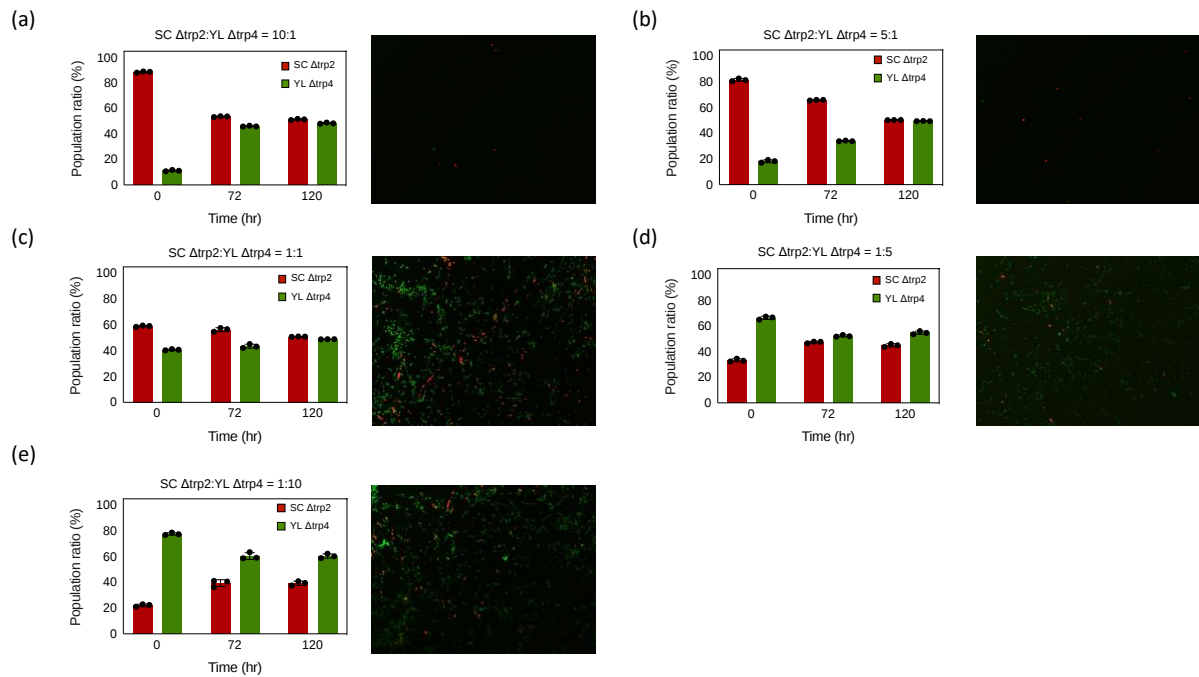
**Supplementary Figure 7.** Population of co-culture between  $Y\Delta trp2$  and  $Y\Delta trp4$  at different inoculation ratios. The populations were measured by Flow cytometry and corresponding fluorescence microscope images were taken at 120 hours of cultivation. Values represent averages and error bars denote standard deviation (n=3).



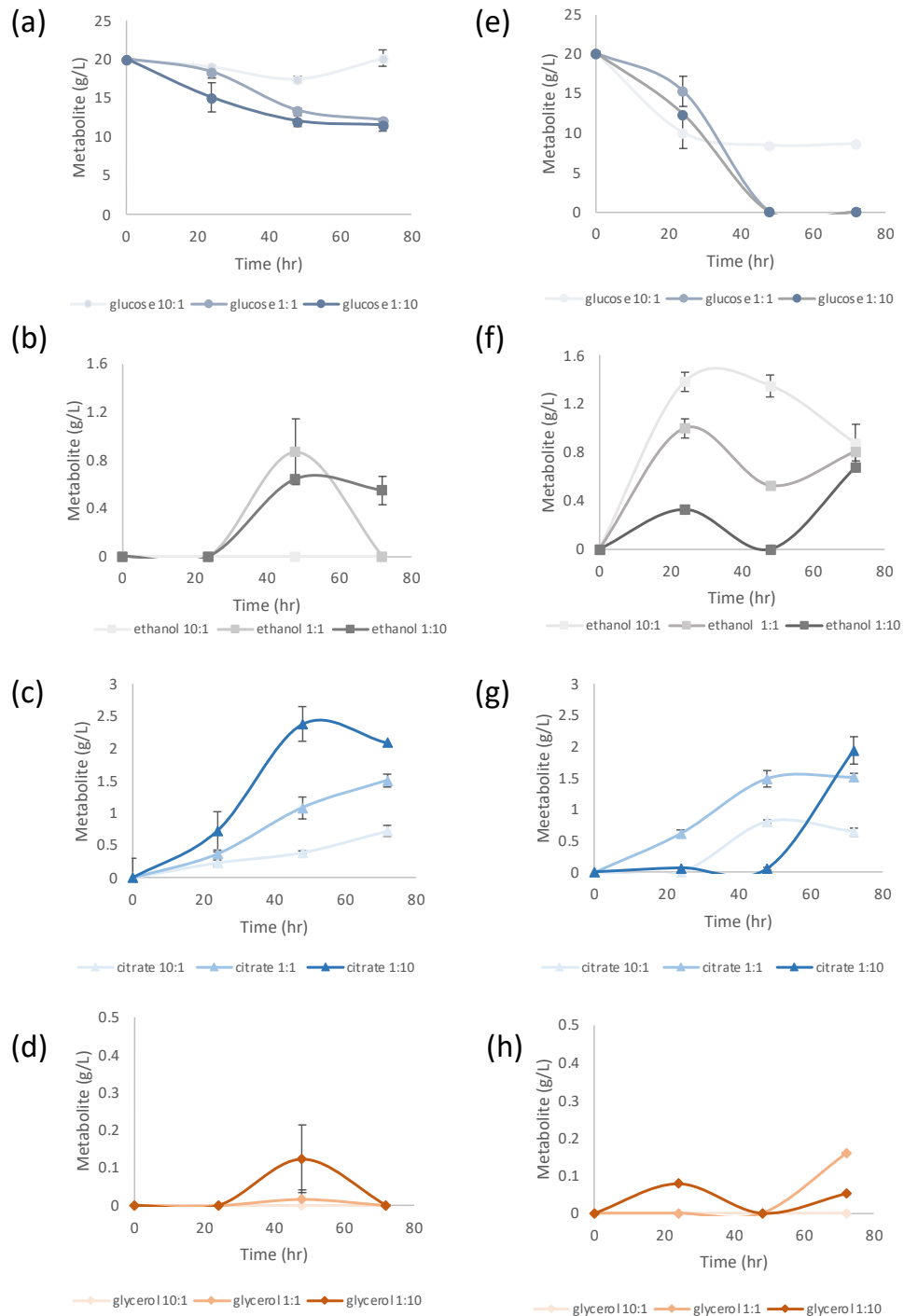
**Supplementary Figure 8.** Growth profile of interspecies syntrophic coculture between *Y. lipolytica* and *S. cerevisiae* (inoculation ratio = 1:1). (a) monoculture of *Y. lipolytica* auxotroph strains, (b) monoculture of *S. cerevisiae* auxotroph strain, (c) coculture pairs of  $\Delta trp2$  and  $\Delta trp4$ , (d) coculture pairs of  $\Delta met5$  and  $\Delta trp4$ , and (e) coculture pairs of  $\Delta lys5$  and  $\Delta trp4$ . Values represent averages and error bars denote standard deviation (n=3).



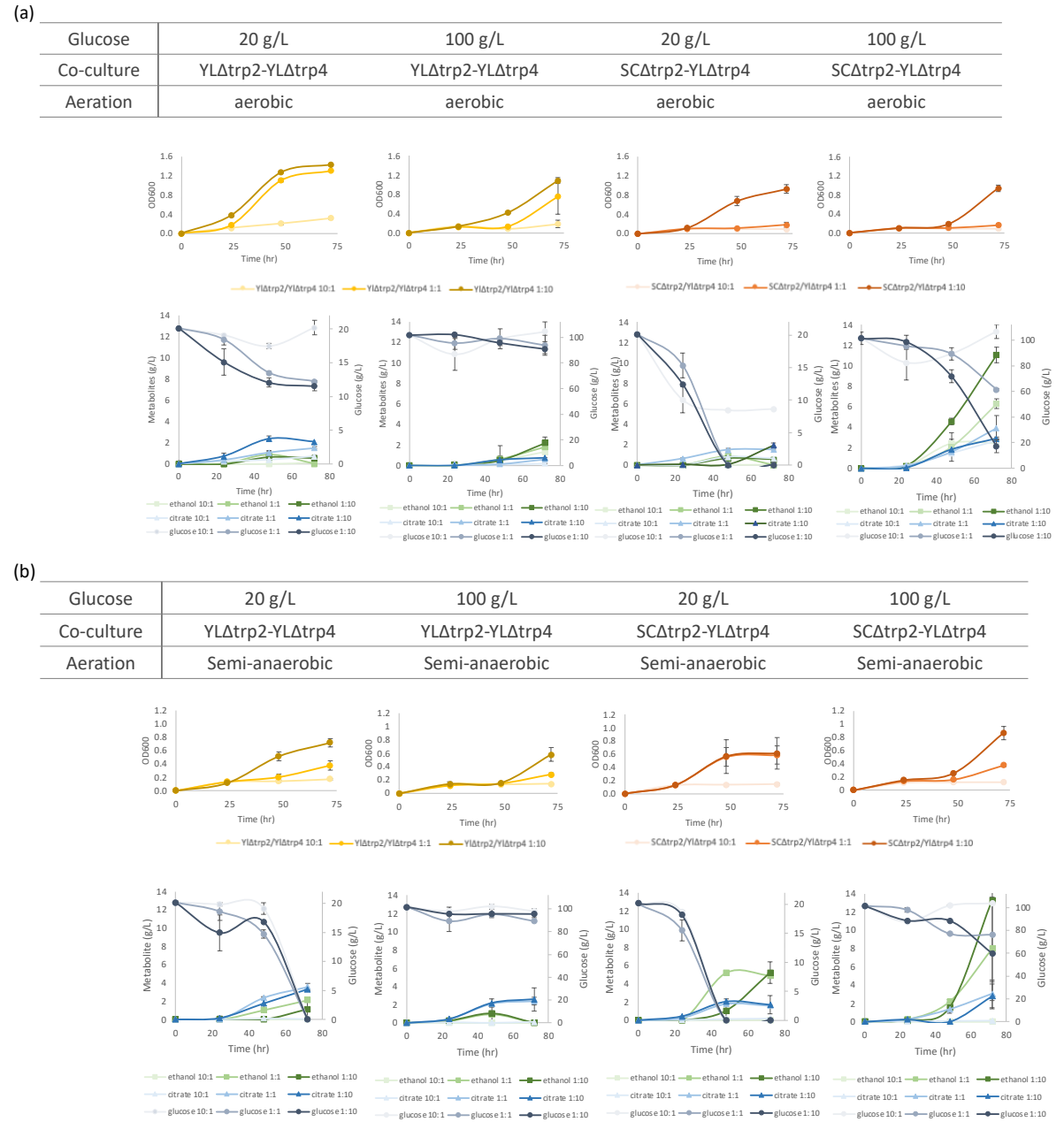
**Supplementary Figure 9.** Population of co-culture among  $YL\Delta trp2$ ,  $YL\Delta trp4$ ,  $SC\Delta trp2$ , and  $SC\Delta trp4$  at different inoculation ratios. The populations were measured by Flow Cytometry and corresponding fluorescence microscope images were taken at 120 hours of cultivation. Values represent averages and error bars denote standard deviation (n=3).



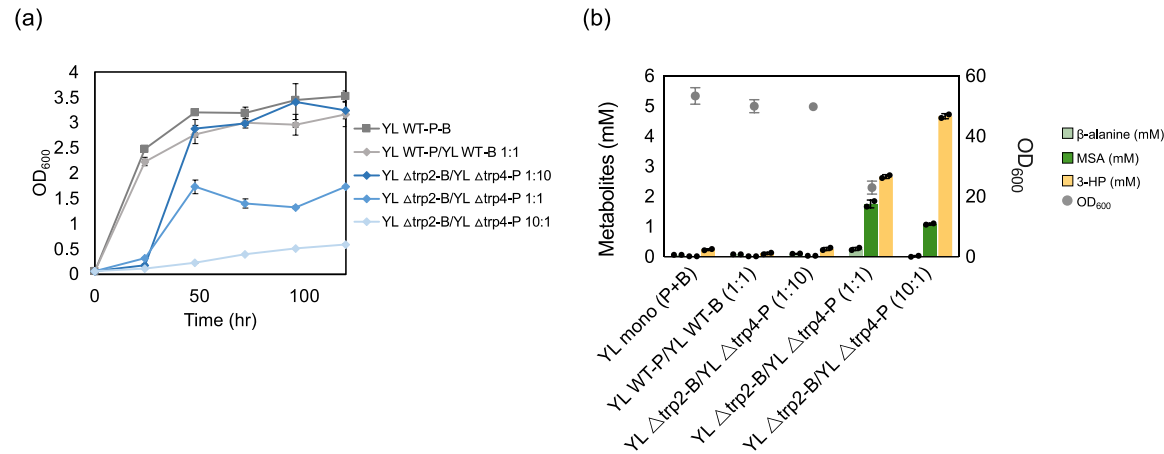
**Supplementary Figure 10.** Profiles of metabolites in co-culture among  $Y\Delta trp2$ ,  $Y\Delta trp4$ ,  $SC\Delta trp2$ , and  $SC\Delta trp4$  at different inoculation ratios in aerobic condition. Metabolites (glucose, ethanol, citrate, and glycerol) from the co-culture of (a-d)  $Y\Delta trp2$  -  $Y\Delta trp4$  and (e-h)  $SC\Delta trp2$  -  $Y\Delta trp4$ . Values represent averages and error bars denote standard deviation (n=3).



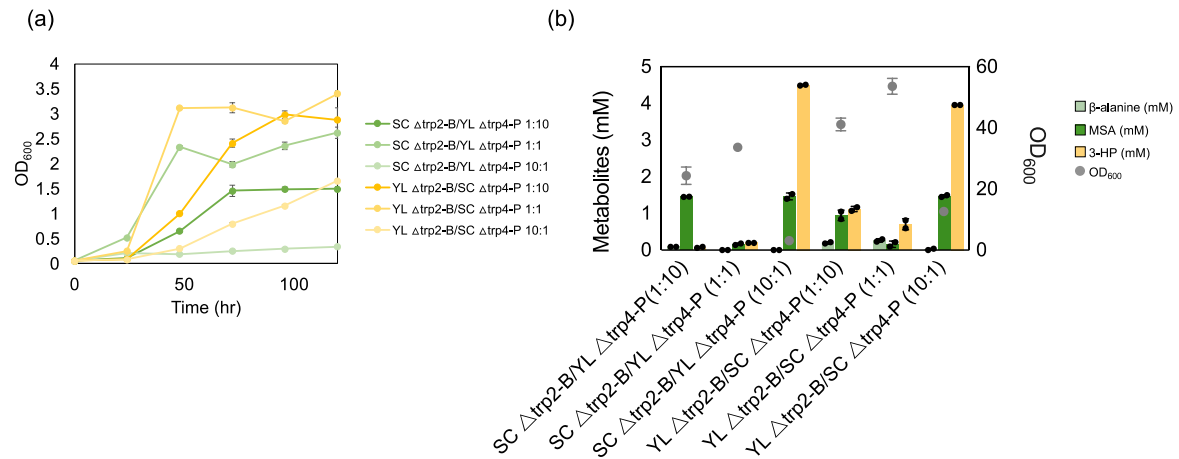
**Supplementary Figure 11.** Profiles of growth and metabolites in co-culture among *YLΔtrp2*, *YLΔtrp4*, *SCΔtrp2*, and *SCΔtrp4* at different inoculation ratios in different culture condition. (a) Aerobic condition (continuous shaking), and (b) semi-anaerobic condition (static and closed condition). Values represent averages and error bars denote standard deviation (n=3).



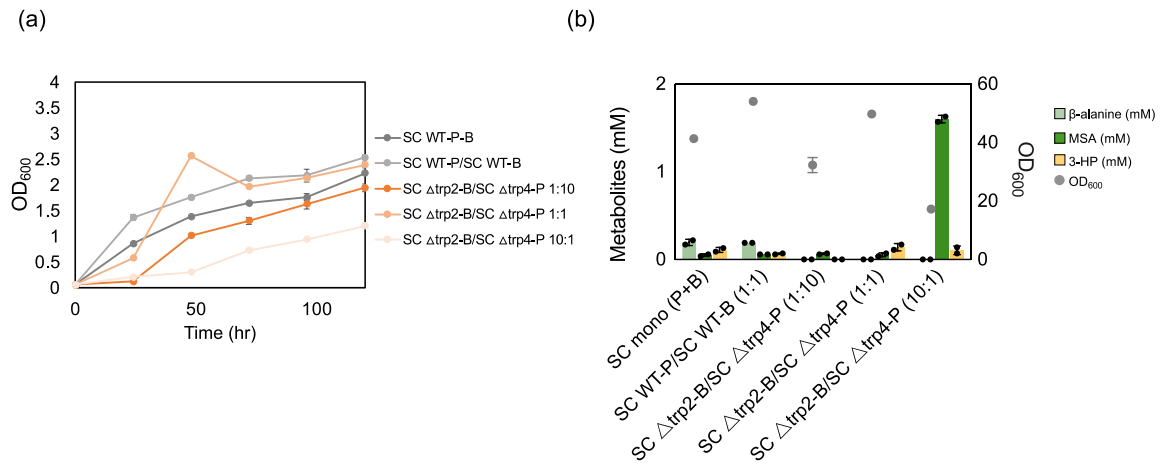
**Supplementary Figure 12.** Division of labor in syntrophic community of *Y. lipolytica* for bioproduction of 3-hydroxypropionic acid. (a) Growth of monoculture and cocultures of pairs with  $\Delta trp2$  and  $\Delta trp4$  at different inoculation ratios, (b) production of metabolites in the 3-HP synthetic pathway from monoculture and cocultures. The strains were incubated in flask at 30 °C with 250 rpm for 120 hours. Values represent averages and error bars denote standard deviation (n=2).



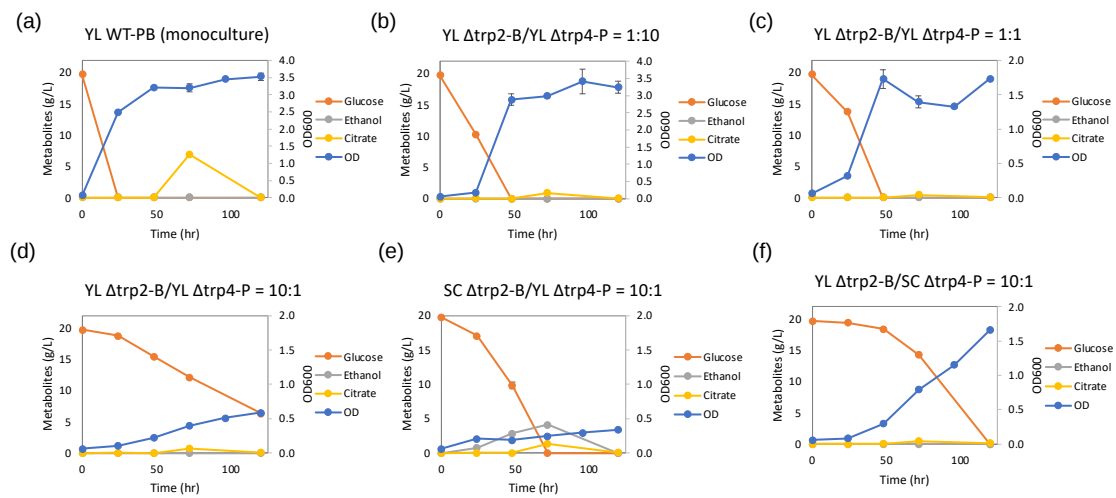
**Supplementary Figure 13.** Division of labor in interspecies syntrophic community of *Y. lipolytica* and *S. cerevisiae* for bioproduction of 3-hydroxypropionic acid. (a) Growth of interspecies cocultures of pairs with  $\Delta trp2$  and  $\Delta trp4$  at different inoculation ratios, (b) production of metabolites in the 3-HP synthetic pathway from monoculture and cocultures. The strains were incubated in flask at 30 °C with 250 rpm for 120 hours. Values represent averages and error bars denote standard deviation (n=2).



**Supplementary Figure 14.** Division of labor in syntrophic community of *S. cerevisiae* for bioproduction of 3-hydroxypropionic acid. (a) Growth of monoculture and cocultures of pairs with  $\Delta trp2$  and  $\Delta trp4$  at different inoculation ratios, (b) production of metabolites in the 3-HP synthetic pathway from monoculture and cocultures. The strains were incubated in flask at 30 °C with 250 rpm for 120 hours. Values represent averages and error bars denote standard deviation (n=2).

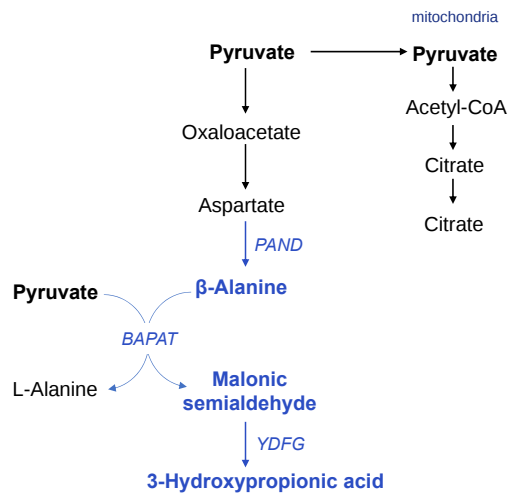


**Supplementary Figure 15.** Profiles of glucose consumption and byproduct formation in the co-cultures for 3-HP production. The strains were incubated in flask at 30 °C with 250 rpm for 120 hours. Values represent averages and error bars denote standard deviation (n=2).

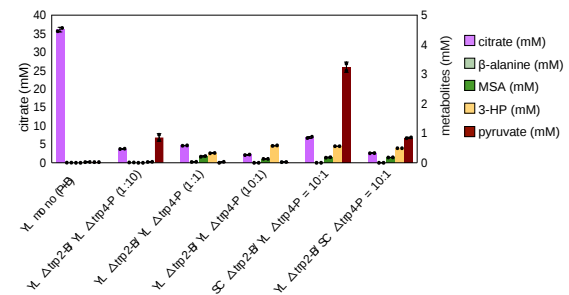


**Supplementary Figure 16.** (a) Metabolic pathway including synthetic 3-HP pathway (b) Production of metabolites (citrate, pyruvate,  $\beta$ -alanine, malonic semialdehyde, and 3-hydroxypropionic acid) from mono- and co-cultures. The strains were incubated in flask at 30 °C with 250 rpm for 120 hours. Values represent averages and error bars denote standard deviation (n=2). One-way ANOVA, followed by Bonferroni's multiple comparisons test with 95% confidence intervals were performed using GraphPad Prism 9.5.0 software and  $p$  values are indicated as asterisks in the graph (\*:  $p < 0.05$ , \*\*:  $p < 0.005$ , \*\*\*:  $p < 0.0005$ , \*\*\*\*:  $p < 0.0001$ ).

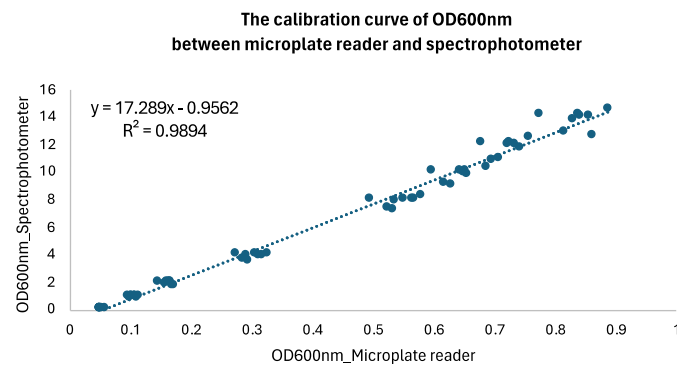
(a)



(b)



**Supplementary Figure 17.** Calibration curve of OD<sub>600</sub> between microplate reader and spectrophotometer. The OD<sub>600</sub> from spectrophotometer was calibrated to the value of microplate reader in figures in the main manuscript and Supplementary Figures.



**Supplementary Figure 18.** Comparison of division of labor in the *S. cerevisiae* synthetic community between previous study and this study. Supplementary Data 8 of previous study (Aulakh et al. 2013) was used.

