# Supplementary File

**Table S1.** Metabolic model.

| Flux number | Reaction | Carbon transitions |
|---|---|---|
| r1 | Glucose --> G6P | ABCDEF --> ABCDEF |
| r2 | G6P --> F6P | ABCDEF --> ABCDEF |
| r3 | F6P --> G6P | ABCDEF --> ABCDEF |
| r4 | F6P --> FBP | ABCDEF --> ABCDEF |
| r5 | FBP --> DHAP + GAP | ABCDEF --> CBA + DEF |
| r6 | DHAP + GAP --> FBP | CBA + DEF --> ABCDEF |
| r7 | DHAP --> GAP | ABC --> ABC |
| r8 | GAP --> DHAP | ABC --> ABC |
| r9 | GAP --> PGA | ABC --> ABC |
| r10 | PGA --> GAP | ABC --> ABC |
| r11 | PGA --> PEP | ABC --> ABC |
| r12 | PEP --> PGA | ABC --> ABC |
| r13 | PEP --> Pyr | ABC --> ABC |
| r14 | Pyr --> AcCoA + $CO_2$_in | ABC --> BC + A |
| r15 | AcCoA + Oxa --> IsoCit | AB + CDEF --> FEDBAC |
| r16 | IsoCit -->αKG + $CO_2$_in | ABCDEF --> ABCDE + F |
| r17 | αKG --> Suc + $CO_2$_in | ABCDE --> BCDE + A |
| r18 | Suc --> Fum | ABCD --> ABCD |
| r19 | Fum --> Suc | ABCD --> ABCD |
| r20 | Fum --> Mal | ABCD --> ABCD |
| r21 | Mal --> Fum | ABCD --> ABCD |
| r22 | Mal --> Oxa | ABCD --> ABCD |
| r23 | Oxa --> Mal | ABCD --> ABCD |
| r24 | IsoCit + AcCoA --> Mal + Suc | ABCDEF + GH --> ABHG + FCDE |
| r25 | PEP + CO2_in --> Oxa | ABC + D --> ABCD |
| r26 | Oxa --> PEP + $CO_2$_in | ABCD --> ABC + D |
| r27 | Mal --> Pyr + $CO_2$_in | ABCD --> ABC + D |
| r28 | G6P --> 6PG | ABCDEF --> ABCDEF |
| r29 | 6PG --> Ru5P + $CO_2$_in | ABCDEF --> BCDEF + A |
| r30 | Ru5P --> R5P | ABCDE --> ABCDE |
| r31 | R5P --> Ru5P | ABCDE --> ABCDE |
| r32 | Ru5P --> Xu5P | ABCDE --> ABCDE |
| r33 | Xu5P --> Ru5P | ABCDE --> ABCDE |
| r34 | R5P + Xu5P --> S7P + GAP | ABCDE + FGHIJ --> FGABCDE + HIJ |
| r35 | GAP + S7P --> Xu5P + R5P | HIJ + FGABCDE --> FGHIJ + ABCDE |
| r36 | GAP + S7P --> F6P + E4P | ABC + DEFGHIJ --> DEFABC + GHIJ |
| r37 | E4P + F6P --> S7P + GAP | GHIJ + DEFABC --> DEFGHIJ + ABC |
| r38 | E4P + Xu5P --> F6P + GAP | ABCD + EFGHI --> EFABCD + GHI |
| r39 | GAP + F6P --> Xu5P + E4P | GHI + EFABCD --> EFGHI + ABCD |
| r40 | 6PG --> Pyr + GAP | ABCDEF --> ABC + DEF |
| r41 | PGA --> Ser | ABC --> ABC |
| r42 | Ser --> Gly + THF_in | ABC --> AB + C |
| r43 | Gly + THF_in --> Ser | AB + C --> ABC |

**Table S1.** *Cont.*

| Flux number | Reaction | Carbon transitions |
|---|---|---|
| r44 | G6P --> [Biomass] | |
| r45 | F6P --> [Biomass] | |
| r46 | DHAP --> [Biomass] | |
| r47 | Ser --> [Biomass] | |
| r48 | Gly --> [Biomass] | |
| r49 | PEP --> [Biomass] | |
| r50 | Pyr --> [Biomass] | |
| r51 | AcCoA --> [Biomass] | |
| r52 | αKG --> [Biomass] | |
| r53 | Oxa --> [Biomass] | |
| r54 | R5P --> [Biomass] | |
| r55 | E4P --> [Biomass] | |
| r56 | Pyr --> [Lactate_ex] | |
| r57 | Pyr --> AcCoA + [Formate_ex] | ABC --> BC + A |
| r58 | AcCoA --> [Acetate_ex] | |
| R59 | AcCoA --> [EtOH_ex] | |
| r60 | CO2_in --> [CO2_ex] | A --> A |
| r61 | CO2 --> CO2_in | A --> A |
| r62 | THF_in --> [THF_ex] | A --> A |
| r63 | THF --> THF_in | A --> A |

**Figure S1.** Fermentation profile. (**a**) The time course of optical density at 600 nm (Closed diamond) and the concentration of remained glucose (Closed square) and produced ethanol (Closed triangle) are shown. The concentration of glucose was zero constantly, indicating that glucose-limited chemostat culture was achieved. (**b**) The time course of organic acids concentration are shown. Closed circle, square, triangle and diamond indicates pyruvate, lactate, formate and acetate, respectively. The concentration was constant after 30 h from the start of culture, indicating that quasi metabolic steady state was assumable.

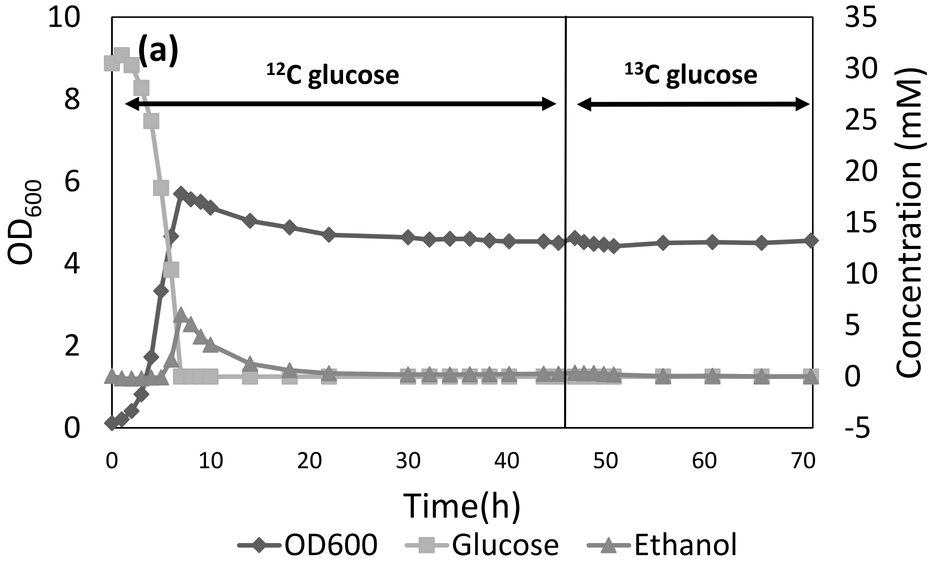


**Figure S1.** *Cont.*

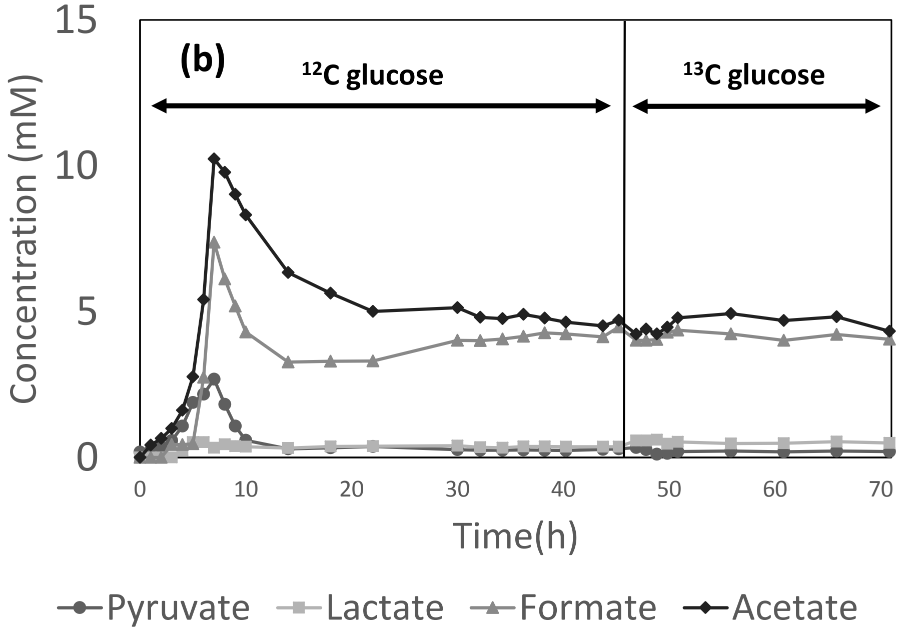


**Figure S2.** Time course of $^{13}$C enrichment of PAAs and FAAs. Fractional labeling of PAAs (Closed circle) and FAAs (Closed square) after the start of tracer feeding is shown in the figure.

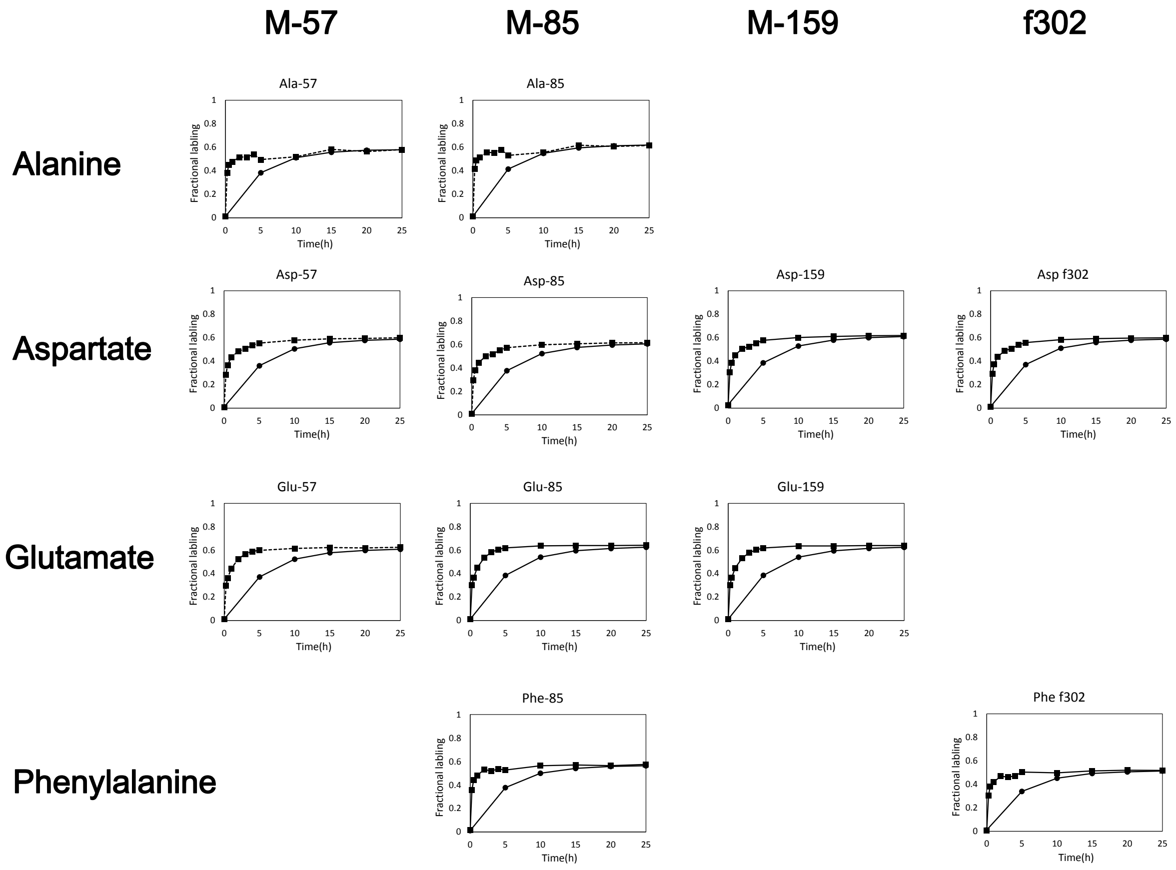
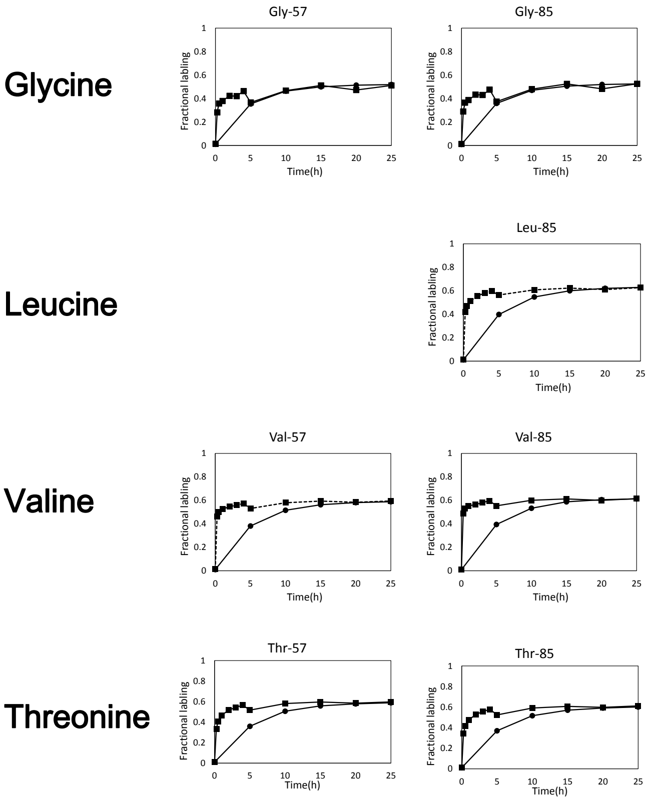


**Figure S2.** *Cont.*



## Supplementary Data 1

## Results of metabolic flux analysis based on the PAAs_fullset

**Table S1-1.** Estimated flux distribution and its 95% confidence interval.

| Reaction | Lower | Best fit | Upper |
|---|---|---|---|
| G6P <-> F6P | 74 | 75 | 77 |
| F6P -> FBP | 83 | 85 | 86 |
| FBP <-> DHAP + GAP | 83 | 85 | 86 |
| DHAP <-> GAP | 82 | 84 | 85 |
| GAP <-> PGA | 172 | 175 | 176 |
| PGA <-> PEP | 164 | 166 | 167 |
| PEP -> Pyr | 122 | 129 | 136 |
| Pyr -> AcCoA | 122 | 128 | 136 |
| AcCoA + Oxa -> Cit | 75 | 77 | 78 |
| Cit ->αKG | 44 | 51 | 58 |
| αKG -> Suc | 38 | 45 | 52 |
| Suc <-> Fum | 69 | 70 | 71 |
| Fum <-> Mal | 69 | 70 | 71 |
| Mal <-> Oxa | 48 | 53 | 57 |
| Glyoxylate shunt | 19 | 25 | 32 |
| PEP + $CO_2$ <-> Oxa | 29 | 34 | 40 |

**Table S1-1.** *Cont.*

| **Reaction** | **Lower** | **Best fit** | **Upper** |
|---|---|---|---|
| Mal -> Pyr | 36 | 43 | 50 |
| G6P -> 6PG | 22 | 24 | 25 |
| 6PG -> Ru5P | 18 | 21 | 24 |
| Ru5P <-> R5P | 10 | 11 | 12 |
| Ru5P <-> Xu5P | 8 | 10 | 12 |
| R5P + Xu5P <-> S7P + GAP | 5 | 6 | 7 |
| GAP + S7P <-> E4P +F6P | 5 | 6 | 7 |
| E4P + Xu5P <-> GAP + F6P | 3 | 4 | 5 |
| ED pathway | 0 | 3 | 6 |

**Table S1-2.** Measured and estimated MID of PAAs_fullset.

| **Name** | **Mass isotopomer** | **Measured MID** | **Estimated MID** |
|---|---|---|---|
| PAA_Ala-57 | M | 0.213 | 0.215 |
| | M+1 | 0.228 | 0.219 |
| | M+2 | 0.160 | 0.160 |
| | M+3 | 0.398 | 0.406 |
| PAA_Ala-85 | M | 0.248 | 0.249 |
| | M+1 | 0.269 | 0.270 |
| | M+2 | 0.483 | 0.481 |
| PAA_Asp-57 | M | 0.083 | 0.083 |
| | M+1 | 0.181 | 0.178 |
| | M+2 | 0.245 | 0.245 |
| | M+3 | 0.276 | 0.277 |
| | M+4 | 0.215 | 0.216 |
| PAA_Asp-85 | M | 0.120 | 0.121 |
| | M+1 | 0.251 | 0.248 |
| | M+2 | 0.318 | 0.321 |
| | M+3 | 0.310 | 0.310 |
| PAA_Asp-159 | M | 0.119 | 0.121 |
| | M+1 | 0.247 | 0.248 |
| | M+2 | 0.320 | 0.321 |
| | M+3 | 0.313 | 0.310 |
| PAA_Asp302 | M | 0.267 | 0.270 |
| | M+1 | 0.290 | 0.287 |
| | M+2 | 0.443 | 0.443 |
| PAA_Glu-57 | M | 0.033 | 0.030 |
| | M+1 | 0.095 | 0.094 |
| | M+2 | 0.205 | 0.205 |
| | M+3 | 0.283 | 0.283 |
| | M+4 | 0.237 | 0.238 |
| | M+5 | 0.148 | 0.149 |

**Table S1-2.** *Cont.*

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| PAA_Glu-85 | M | 0.048 | 0.045 |
| | M+1 | 0.138 | 0.139 |
| | M+2 | 0.294 | 0.299 |
| | M+3 | 0.300 | 0.298 |
| | M+4 | 0.220 | 0.219 |
| PAA_Glu-159 | M | 0.049 | 0.045 |
| | M+1 | 0.139 | 0.139 |
| | M+2 | 0.295 | 0.299 |
| | M+3 | 0.298 | 0.298 |
| | M+4 | 0.219 | 0.219 |
| PAA_Phe-57 | M | 0.023 | 0.020 |
| | M+1 | 0.045 | 0.043 |
| | M+2 | 0.082 | 0.080 |
| | M+3 | 0.120 | 0.118 |
| | M+4 | 0.139 | 0.136 |
| | M+5 | 0.171 | 0.172 |
| | M+6 | 0.143 | 0.147 |
| | M+7 | 0.123 | 0.125 |
| | M+8 | 0.086 | 0.087 |
| | M+9 | 0.067 | 0.074 |
| PAA_Phe-85 | M | 0.026 | 0.021 |
| | M+1 | 0.049 | 0.048 |
| | M+2 | 0.117 | 0.117 |
| | M+3 | 0.139 | 0.134 |
| | M+4 | 0.169 | 0.171 |
| | M+5 | 0.142 | 0.143 |
| | M+6 | 0.162 | 0.165 |
| | M+7 | 0.118 | 0.117 |
| | M+8 | 0.078 | 0.084 |
| PAA_Phe302 | M | 0.424 | 0.420 |
| | M+1 | 0.129 | 0.128 |
| | M+2 | 0.447 | 0.451 |
| PAA_Gly-57 | M | 0.419 | 0.425 |
| | M+1 | 0.130 | 0.123 |
| | M+2 | 0.451 | 0.452 |
| PAA_Gly-85 | M | 0.480 | 0.481 |
| | M+1 | 0.520 | 0.519 |
| PAA_Ile-85 | M | 0.034 | 0.030 |
| | M+1 | 0.092 | 0.094 |
| | M+2 | 0.199 | 0.205 |
| | M+3 | 0.283 | 0.283 |
| | M+4 | 0.239 | 0.238 |
| | M+5 | 0.153 | 0.149 |

**Table S1-2.** *Cont.*

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| PAA_Ile-159 | M | 0.034 | 0.030 |
| | M+1 | 0.093 | 0.094 |
| | M+2 | 0.202 | 0.205 |
| | M+3 | 0.285 | 0.283 |
| | M+4 | 0.239 | 0.238 |
| | M+5 | 0.147 | 0.149 |
| PAA_Leu-85 | M | 0.024 | 0.020 |
| | M+1 | 0.084 | 0.085 |
| | M+2 | 0.186 | 0.191 |
| | M+3 | 0.295 | 0.296 |
| | M+4 | 0.248 | 0.251 |
| | M+5 | 0.162 | 0.158 |
| PAA_Ser-57 | M | 0.189 | 0.189 |
| | M+1 | 0.278 | 0.270 |
| | M+2 | 0.155 | 0.166 |
| | M+3 | 0.378 | 0.375 |
| PAA_Ser-85 | M | 0.206 | 0.207 |
| | M+1 | 0.366 | 0.367 |
| | M+2 | 0.429 | 0.426 |
| PAA_Ser-159 | M | 0.208 | 0.207 |
| | M+1 | 0.369 | 0.367 |
| | M+2 | 0.423 | 0.426 |
| PAA_Val-57 | M | 0.059 | 0.054 |
| | M+1 | 0.117 | 0.113 |
| | M+2 | 0.204 | 0.202 |
| | M+3 | 0.250 | 0.250 |
| | M+4 | 0.182 | 0.187 |
| | M+5 | 0.188 | 0.195 |
| PAA_Val-85 | M | 0.067 | 0.062 |
| | M+1 | 0.134 | 0.135 |
| | M+2 | 0.310 | 0.313 |
| | M+3 | 0.258 | 0.260 |
| | M+4 | 0.231 | 0.231 |
| PAA_Tyr302 | M | 0.424 | 0.420 |
| | M+1 | 0.129 | 0.128 |
| | M+2 | 0.448 | 0.451 |
| PAA_Thr-57 | M | 0.082 | 0.083 |
| | M+1 | 0.180 | 0.178 |
| | M+2 | 0.244 | 0.245 |
| | M+3 | 0.278 | 0.277 |
| | M+4 | 0.217 | 0.216 |
| PAA_Thr-85 | M | 0.121 | 0.121 |
| | M+1 | 0.251 | 0.248 |
| | M+2 | 0.323 | 0.321 |
| | M+3 | 0.305 | 0.310 |

## Supplementary Data 2

## Results of metabolic flux analysis based on the FAAs_fullset

**Table S2-1.** Estimated flux distribution and its 95% confidence interval.

| **Reaction** | **Lower** | **Best fit** | **Upper** |
|---|---|---|---|
| G6P <-> F6P | 69 | 74 | 80 |
| F6P -> FBP | 76 | 81 | 86 |
| FBP <-> DHAP + GAP | 76 | 81 | 86 |
| DHAP <-> GAP | 76 | 81 | 85 |
| GAP <-> PGA | 167 | 172 | 176 |
| PGA <-> PEP | 158 | 163 | 167 |
| PEP -> Pyr | 122 | 128 | 142 |
| Pyr -> AcCoA | 116 | 133 | 145 |
| AcCoA + Oxa -> Cit | 75 | 78 | 81 |
| Cit ->αKG | 37 | 49 | 60 |
| αKG -> Suc | 30 | 43 | 53 |
| Suc <-> Fum | 68 | 71 | 75 |
| Fum <-> Mal | 68 | 71 | 75 |
| Mal <-> Oxa | 46 | 56 | 68 |
| Glyoxylate shunt | 18 | 29 | 40 |
| PEP + $CO_2$ <-> Oxa | 20 | 32 | 43 |
| Mal -> Pyr | 30 | 44 | 56 |
| G6P -> 6PG | 19 | 25 | 29 |
| 6PG -> Ru5P | 9 | 18 | 27 |
| Ru5P <-> R5P | 7 | 10 | 14 |
| Ru5P <-> Xu5P | 2 | 8 | 14 |
| R5P + Xu5P <-> S7P + GAP | 2 | 5 | 8 |
| GAP+ S7P <-> E4P + F6P | 2 | 5 | 8 |
| E4P + Xu5P <-> GAP + F6P | 0 | 3 | 6 |
| ED pathway | 0 | 7 | 14 |

**Table S2-2.** Measured and estimated MID of FAAs_fullset.

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| FAA_Ala-57 | M | 0.217 | 0.215 |
| | M+1 | 0.225 | 0.211 |
| | M+2 | 0.173 | 0.173 |
| | M+3 | 0.385 | 0.401 |
| FAA_Ala-85 | M | 0.252 | 0.254 |
| | M+1 | 0.270 | 0.267 |
| | M+2 | 0.478 | 0.479 |
| FAA_Asp-57 | M | 0.072 | 0.079 |
| | M+1 | 0.182 | 0.170 |
| | M+2 | 0.252 | 0.257 |
| | M+3 | 0.284 | 0.279 |
| | M+4 | 0.210 | 0.215 |
| FAA_Asp-85 | M | 0.108 | 0.114 |
| | M+1 | 0.256 | 0.247 |
| | M+2 | 0.330 | 0.330 |
| | M+3 | 0.306 | 0.309 |
| FAA_Asp-159 | M | 0.107 | 0.114 |
| | M+1 | 0.251 | 0.247 |
| | M+2 | 0.330 | 0.330 |
| | M+3 | 0.311 | 0.309 |
| FAA_Asp302 | M | 0.254 | 0.261 |
| | M+1 | 0.299 | 0.295 |
| | M+2 | 0.447 | 0.444 |
| FAA_Glu-57 | M | 0.014 | 0.029 |
| | M+1 | 0.094 | 0.093 |
| | M+2 | 0.207 | 0.204 |
| | M+3 | 0.288 | 0.285 |
| | M+4 | 0.244 | 0.240 |
| | M+5 | 0.152 | 0.148 |
| FAA_Glu-85 | M | 0.028 | 0.043 |
| | M+1 | 0.140 | 0.141 |
| | M+2 | 0.298 | 0.297 |
| | M+3 | 0.309 | 0.303 |
| | M+4 | 0.225 | 0.217 |
| FAA_Glu-159 | M | 0.029 | 0.043 |
| | M+1 | 0.141 | 0.141 |
| | M+2 | 0.299 | 0.297 |
| | M+3 | 0.307 | 0.303 |
| | M+4 | 0.223 | 0.217 |
| FAA_Phe-85 | M | 0.021 | 0.021 |
| | M+1 | 0.047 | 0.047 |
| | M+2 | 0.113 | 0.115 |
| | M+3 | 0.139 | 0.134 |

**Table S2-2.** *Cont.*

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| | M+4 | 0.170 | 0.173 |
| | M+5 | 0.148 | 0.149 |
| | M+6 | 0.164 | 0.166 |
| | M+7 | 0.123 | 0.116 |
| | M+8 | 0.076 | 0.079 |
| FAA_Phe302 | M | 0.414 | 0.418 |
| | M+1 | 0.138 | 0.143 |
| | M+2 | 0.449 | 0.439 |
| FAA_Gly-57 | M | 0.425 | 0.421 |
| | M+1 | 0.154 | 0.141 |
| | M+2 | 0.421 | 0.439 |
| FAA_Gly-85 | M | 0.489 | 0.488 |
| | M+1 | 0.511 | 0.512 |
| FAA_Leu-85 | M | 0.027 | 0.021 |
| | M+1 | 0.095 | 0.087 |
| | M+2 | 0.189 | 0.193 |
| | M+3 | 0.286 | 0.296 |
| | M+4 | 0.243 | 0.247 |
| | M+5 | 0.159 | 0.157 |
| FAA_Val-57 | M | 0.054 | 0.055 |
| | M+1 | 0.118 | 0.111 |
| | M+2 | 0.206 | 0.203 |
| | M+3 | 0.250 | 0.249 |
| | M+4 | 0.184 | 0.190 |
| | M+5 | 0.187 | 0.192 |
| FAA_Val-85 | M | 0.067 | 0.064 |
| | M+1 | 0.140 | 0.135 |
| | M+2 | 0.319 | 0.315 |
| | M+3 | 0.238 | 0.256 |
| | M+4 | 0.236 | 0.230 |
| FAA_Tyr302 | M | 0.422 | 0.418 |
| | M+1 | 0.136 | 0.143 |
| | M+2 | 0.442 | 0.439 |
| FAA_Thr-57 | M | 0.079 | 0.079 |
| | M+1 | 0.179 | 0.170 |
| | M+2 | 0.247 | 0.257 |
| | M+3 | 0.278 | 0.279 |
| | M+4 | 0.216 | 0.215 |
| FAA_Thr-85 | M | 0.118 | 0.114 |
| | M+1 | 0.253 | 0.247 |
| | M+2 | 0.324 | 0.330 |
| | M+3 | 0.305 | 0.309 |

## Supplementary Data 3

## Results of metabolic flux analysis using FAAs_Glu+Asp

**Table S3-1.** Estimated flux distribution and its 95% confidence interval.

| **Reaction** | **Lower** | **Best fit** | **Upper** |
|---|---|---|---|
| G6P <-> F6P | 55 | 89 | 99 |
| F6P -> FBP | 67 | 91 | 94 |
| FBP <-> DHAP + GAP | 67 | 91 | 94 |
| DHAP <-> GAP | 67 | 90 | 93 |
| GAP <-> PGA | 157 | 181 | 184 |
| PGA <-> PEP | 149 | 172 | 176 |
| PEP -> Pyr | 52 | 183 | 233 |
| Pyr -> AcCoA | 99 | 137 | 187 |
| AcCoA + Oxa -> Cit | 6 | 80 | 84 |
| Cit ->αKG | 6 | 49 | 84 |
| αKG -> Suc | 0 | 43 | 77 |
| Suc <-> Fum | 63 | 74 | 77 |
| Fum <-> Mal | 63 | 74 | 77 |
| Mal <-> Oxa | −23 | 105 | 154 |
| Glyoxylate shunt | 0 | 31 | 77 |
| PEP + $CO_2$ <-> Oxa | −60 | −14 | 115 |
| Mal -> Pyr | 0 | 0 | 196 |
| G6P -> 6PG | 0 | 10 | 43 |
| 6PG -> Ru5P | 0 | 10 | 43 |
| Ru5P <-> R5P | 4 | 8 | 20 |
| Ru5P <-> Xu5P | −4 | 2 | 25 |
| R5P + Xu5P <-> S7P + GAP | −1 | 2 | 30 |
| GAP + S7P <-> E4P + F6P | −1 | 2 | 30 |
| E4P + Xu5P <-> GAP + F6P | −3 | 0 | 28 |
| ED pathway | 0 | 0 | 26 |

**Table S3-2.** Measured and estimated MID of FAAs_Glu+Asp+Ala.

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| FAA_Asp-57 | M | 0.072 | 0.074 |
| | M+1 | 0.182 | 0.174 |
| | M+2 | 0.252 | 0.253 |
| | M+3 | 0.284 | 0.285 |
| | M+4 | 0.210 | 0.213 |
| FAA_Asp-85 | M | 0.108 | 0.108 |
| | M+1 | 0.256 | 0.251 |
| | M+2 | 0.330 | 0.333 |
| | M+3 | 0.306 | 0.308 |
| FAA_Asp-159 | M | 0.107 | 0.108 |
| | M+1 | 0.251 | 0.251 |
| | M+2 | 0.330 | 0.333 |
| | M+3 | 0.311 | 0.308 |
| FAA_Asp302 | M | 0.254 | 0.259 |
| | M+1 | 0.299 | 0.294 |
| | M+2 | 0.447 | 0.447 |
| FAA_Glu-57 | M | 0.014 | 0.026 |
| | M+1 | 0.094 | 0.091 |
| | M+2 | 0.207 | 0.202 |
| | M+3 | 0.288 | 0.288 |
| | M+4 | 0.244 | 0.246 |
| | M+5 | 0.152 | 0.148 |
| FAA_Glu-85 | M | 0.028 | 0.039 |
| | M+1 | 0.140 | 0.136 |
| | M+2 | 0.298 | 0.295 |
| | M+3 | 0.309 | 0.310 |
| | M+4 | 0.225 | 0.220 |
| FAA_Glu-159 | M | 0.029 | 0.039 |
| | M+1 | 0.141 | 0.136 |
| | M+2 | 0.299 | 0.295 |
| | M+3 | 0.307 | 0.310 |
| | M+4 | 0.223 | 0.220 |

## Supplementary Data 4

## Results of metabolic flux analysis using FAAs_Glu+Asp+Ala

**Table S4-1.** Estimated flux distribution and its 95% confidence interval.

| **Reaction** | **Lower** | **Best fit** | **Upper** |
|---|---|---|---|
| G6P <-> F6P | 59 | 73 | 95 |
| F6P -> FBP | 72 | 85 | 93 |
| FBP <-> DHAP + GAP | 72 | 85 | 93 |
| DHAP <-> GAP | 71 | 84 | 92 |
| GAP <-> PGA | 162 | 175 | 183 |
| PGA <-> PEP | 153 | 167 | 174 |
| PEP -> Pyr | 108 | 188 | 222 |
| Pyr -> AcCoA | 113 | 143 | 179 |
| AcCoA + Oxa -> Cit | 70 | 75 | 83 |
| Cit ->αKG | 7 | 34 | 68 |
| αKG -> Suc | 0 | 28 | 62 |
| Suc <-> Fum | 64 | 69 | 77 |
| Fum <-> Mal | 64 | 69 | 77 |
| Mal <-> Oxa | 34 | 110 | 146 |
| Glyoxylate shunt | 10 | 41 | 72 |
| PEP + CO2 <-> Oxa | −54 | −24 | 52 |
| Mal -> Pyr | 0 | 0 | 77 |
| G6P -> 6PG | 4 | 26 | 40 |
| 6PG -> Ru5P | 0 | 25 | 40 |
| Ru5P <-> R5P | 4 | 13 | 18 |
| Ru5P <-> Xu5P | −4 | 12 | 23 |
| R5P + Xu5P <-> S7P + GAP | −1 | 7 | 12 |
| GAP + S7P <-> E4P + F6P | −1 | 7 | 12 |
| E4P + Xu5P <-> GAP + F6P | −3 | 5 | 10 |
| ED pathway | 0 | 1 | 20 |

**Table S4-2.** Measured and estimated MID of FAAs_Glu+Asp+Ala.

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| FAA_Asp-57 | M | 0.072 | 0.074 |
| | M+1 | 0.182 | 0.174 |
| | M+2 | 0.252 | 0.253 |
| | M+3 | 0.284 | 0.285 |
| | M+4 | 0.210 | 0.213 |
| FAA_Asp-85 | M | 0.108 | 0.108 |
| | M+1 | 0.256 | 0.251 |
| | M+2 | 0.330 | 0.333 |
| | M+3 | 0.306 | 0.308 |
| FAA_Asp-159 | M | 0.107 | 0.108 |
| | M+1 | 0.251 | 0.251 |
| | M+2 | 0.330 | 0.333 |
| | M+3 | 0.311 | 0.308 |
| FAA_Asp302 | M | 0.254 | 0.259 |
| | M+1 | 0.299 | 0.294 |
| | M+2 | 0.447 | 0.447 |
| FAA_Glu-57 | M | 0.014 | 0.026 |
| | M+1 | 0.094 | 0.091 |
| | M+2 | 0.207 | 0.202 |
| | M+3 | 0.288 | 0.288 |
| | M+4 | 0.244 | 0.246 |
| | M+5 | 0.152 | 0.148 |
| FAA_Glu-85 | M | 0.028 | 0.039 |
| | M+1 | 0.140 | 0.136 |
| | M+2 | 0.298 | 0.295 |
| | M+3 | 0.309 | 0.310 |
| | M+4 | 0.225 | 0.220 |
| FAA_Glu-159 | M | 0.029 | 0.039 |
| | M+1 | 0.141 | 0.136 |
| | M+2 | 0.299 | 0.295 |
| | M+3 | 0.307 | 0.310 |
| | M+4 | 0.223 | 0.220 |

## Supplementary Data 5

## Results of metabolic flux analysis using FAAs_Glu+Asp+Ala+Phe

**Table S5-1.** Estimated flux distribution and its 95% confidence interval.

| **Reaction** | **Lower** | **Best fit** | **Upper** |
|---|---|---|---|
| G6P <-> F6P | 67 | 75 | 88 |
| F6P -> FBP | 76 | 84 | 90 |
| FBP <-> DHAP + GAP | 76 | 84 | 90 |
| DHAP <-> GAP | 75 | 83 | 89 |
| GAP <-> PGA | 165 | 174 | 181 |
| PGA <-> PEP | 157 | 165 | 172 |
| PEP -> Pyr | 123 | 141 | 165 |
| Pyr -> AcCoA | 125 | 140 | 162 |
| AcCoA + Oxa -> Cit | 73 | 77 | 83 |
| Cit ->αKG | 20 | 40 | 58 |
| αKG -> Suc | 14 | 34 | 52 |
| Suc <-> Fum | 67 | 70 | 77 |
| Fum <-> Mal | 67 | 70 | 77 |
| Mal <-> Oxa | 49 | 66 | 88 |
| Glyoxylate shunt | 22 | 36 | 54 |
| PEP + $CO_2$ <-> Oxa | 0 | 21 | 40 |
| Mal -> Pyr | 20 | 41 | 59 |
| G6P -> 6PG | 10 | 24 | 32 |
| 6PG -> Ru5P |  | 20 | 32 |
| Ru5P <-> R5P | 4 | 11 | 15 |
| Ru5P <-> Xu5P | −4 | 9 | 17 |
| R5P + Xu5P <-> S7P + GAP | −1 | 6 | 10 |
| GAP + S7P <-> E4P + F6P | −1 | 6 | 10 |
| E4P + Xu5P <-> GAP + F6P | −3 | 4 | 8 |
| ED pathway | 0 | 3 | 16 |

**Table S5-2.** Measured and estimated MID of FAAs_Glu+Asp+Ala+Phe.

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| FAA_Ala-57 | M | 0.217 | 0.212 |
| | M+1 | 0.225 | 0.215 |
| | M+2 | 0.173 | 0.171 |
| | M+3 | 0.385 | 0.401 |
| FAA_Ala-85 | M | 0.252 | 0.244 |
| | M+1 | 0.270 | 0.277 |
| | M+2 | 0.478 | 0.479 |
| FAA_Asp-57 | M | 0.072 | 0.077 |
| | M+1 | 0.182 | 0.170 |
| | M+2 | 0.252 | 0.259 |
| | M+3 | 0.284 | 0.280 |
| | M+4 | 0.210 | 0.213 |
| FAA_Asp-85 | M | 0.108 | 0.111 |
| | M+1 | 0.256 | 0.249 |
| | M+2 | 0.330 | 0.331 |
| | M+3 | 0.306 | 0.308 |
| FAA_Asp-159 | M | 0.107 | 0.111 |
| | M+1 | 0.251 | 0.249 |
| | M+2 | 0.330 | 0.331 |
| | M+3 | 0.311 | 0.308 |
| FAA_Asp302 | M | 0.254 | 0.261 |
| | M+1 | 0.299 | 0.295 |
| | M+2 | 0.447 | 0.444 |
| FAA_Glu-57 | M | 0.014 | 0.027 |
| | M+1 | 0.094 | 0.092 |
| | M+2 | 0.207 | 0.203 |
| | M+3 | 0.288 | 0.286 |
| | M+4 | 0.244 | 0.244 |
| | M+5 | 0.152 | 0.148 |
| FAA_Glu-85 | M | 0.028 | 0.040 |
| | M+1 | 0.140 | 0.138 |
| | M+2 | 0.298 | 0.295 |
| | M+3 | 0.309 | 0.308 |
| | M+4 | 0.225 | 0.218 |
| FAA_Glu-159 | M | 0.029 | 0.040 |
| | M+1 | 0.141 | 0.138 |
| | M+2 | 0.299 | 0.295 |
| | M+3 | 0.307 | 0.308 |
| | M+4 | 0.223 | 0.218 |

**Table S5-2.** *Cont.*

| Name | Mass isotopomer | Measured MID | Estimated MID |
|---|---|---|---|
| FAA_Phe-85 | M | 0.021 | 0.019 |
| | M+1 | 0.047 | 0.047 |
| | M+2 | 0.113 | 0.114 |
| | M+3 | 0.139 | 0.136 |
| | M+4 | 0.170 | 0.172 |
| | M+5 | 0.148 | 0.149 |
| | M+6 | 0.164 | 0.165 |
| | M+7 | 0.123 | 0.119 |
| | M+8 | 0.076 | 0.079 |
| FAA_Phe302 | M | 0.414 | 0.412 |
| | M+1 | 0.138 | 0.141 |
| | M+2 | 0.449 | 0.447 |