Table S1 Differentially intracellular metabolites in BW25113-ΔastE when compared to that those in BW25113 after exposing to 5 g/L butanol

| Name                                      | Log2f old | qvalue   | Up/down | Mzmed | Rtmed  |
|-------------------------------------------|-----------|----------|---------|-------|--------|
| L-leucine                                 | 14.5      | 0.017584 | UP      | 117.1 | 35.34208 |
| 11-deoxycorticosterone                    | -13.5     | 0.014418 | DOWN    | 314.2 | 35.35  |
| 2-oxoglutarate                            | -12       | 0.010417 | DOWN    | 130   | 38.552 |
| γ-butyrobetaine                           | 11.6      | 0.019996 | UP      | 130.1 | 38.541 |
| 9-mercaptodethiobiotin                    | -9.7      | 0.029138 | DOWN    | 245.1 | 32.4445 |
| 2,3-dihydroxybenzoate                     | -8        | 0.046539 | DOWN    | 117   | 19.082 |
| 1-myristoyl-2-palmitoleoyl phosphatidate  | 7.9       | 0.057484 | UP      | 308.2 | 33.844 |
| Pyrazine-2-carboxylate                    | -7.7      | 0.045552 | DOWN    | 87    | 19.08  |
| 6-deoxy-6-sulfo-D-fructose-1-phosphate    | -7.4      | 0.031078 | DOWN    | 163   | 8.713  |
| 2-oxo-4-methylthiobutanoate               | -7.1      | 0.014468 | DOWN    | 132   | 38.8645 |
| S-sulfanylglutathione                     | -6.1      | 0.017348 | DOWN    | 358.1 | 30.294 |
| α-ribazole 5’-phosphate                   | -6.1      | 0.016339 | DOWN    | 359.1 | 30.294 |
| Glyoxylate                                | -5.9      | 0.041394 | DOWN    | 119   | 18.31433 |
| Malate                                    | -5.9      | 0.053315 | DOWN    | 118   | 19.0755 |
| 5-amino-1-(5-phospho-β-D-ribosyl)imidazole| -5.3      | 0.042877 | DOWN    | 314.1 | 18.3   |
| Sulfate                                   | -5.3      | 0.009955 | DOWN    | 116   | 38.552 |
| 5-amino-6-((D-ribitylamino)uracil)        | -5        | 0.021238 | DOWN    | 259.1 | 24.4715 |
| 6,7-dimethyl-8-(1-D-ribityl)lumazine      | -4.9      | 0.019453 | DOWN    | 307.1 | 19.824 |
| N2-succinyl-L-ornithine                   | -4.6      | 0.00941  | DOWN    | 214.1 | 29.373 |
| Ethylene glycol                           | -4.5      | 0.04192  | DOWN    | 101   | 19.162 |
| 3-keto-L-gulonate 6-phosphate             | -4.4      | 0.016669 | DOWN    | 149   | 43.749 |
| (1R,6R)-6-hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate | -4.3 | 0.008353 | DOWN    | 258.1 | 24.46 |
| Compound                                           | Change | p-value  | Fold Change | p-value  |
|----------------------------------------------------|--------|----------|-------------|----------|
| Oxaloacetate                                      | -3.4   | 0.050957 | DOWN        | 131      | 29.539   |
| Betaine aldehyde hydrate                          | -3.2   | 0.012421 | DOWN        | 165.1    | 34.9585  |
| 6-carboxy-5,6,7,8-tetrahydropterin                 | 3      | 0.008353 | UP          | 229.1    | 22.217   |
| L-phenylalanine                                   | 3      | 0.04148  | UP          | 225.1    | 30.29642 |
| Oxalosuccinate                                    | -2.9   | 0.045327 | DOWN        | 213      | 18.265   |
| 7,8-dihydromonapterin                             | 2.9    | 0.039561 | UP          | 256.1    | 30.295   |
| N,N-dimethyl-p-phenylenediamine                   | -2.9   | 0.010324 | DOWN        | 181.1    | 34.961   |
| 2'-deoxycytidine                                  | 2.8    | 0.008353 | UP          | 228.1    | 22.222   |
| 4-(γ-glutamylamino)butanal                        | -2.6   | 0.013737 | DOWN        | 201.1    | 31.50392 |
| Propanoate                                         | -2.5   | 0.029839 | DOWN        | 113      | 19.8575  |
| N-acetylmuramate                                   | -2.2   | 0.051255 | DOWN        | 316.1    | 18.26433 |
| Thymidine                                          | 2.2    | 0.010074 | UP          | 243.1    | 22.217   |
| Succinate semialdehyde                            | -2.1   | 0.05022  | DOWN        | 137      | 18.2685  |
| 4-(γ-L-glutamylamino)butanoate                    | -1.8   | 0.010305 | DOWN        | 256.1    | 19.8605  |
| L-Valine                                           | 1.8    | 0.03812  | UP          | 132.1    | 38.547   |
| Amino acids | BW25113 | BW25113-ΔastE |
|-------------|---------|---------------|
|             | Supernate (mg/100ml) | Intracellular (mg/100ml) | Supernate (mg/100ml) | Intracellular (mg/100ml) |
| Ala         | 0.25 ± 0.03 | 0.37 ± 0.04 | 0.73 ± 0.10 | 0.21 ± 0.15 |
| Gly         | 0.13 ± 0.01 | 0.54 ± 0.14 | 0.21 ± 0.01 | 0.20 ± 0.09 |
| Val         | 0.33 ± 0.07 | 0.32 ± 0.09 | 0.01 ± 0.02 | 0.33 ± 0.07 |
| Met         | 0.15 ± 0.02 | 0.17 ± 0.06 | 0.48 ± 0.16 | 0.21 ± 0.05 |
| Ile         | 0.05 ± 0.06 | 0.19 ± 0.05 | 0.69 ± 0.10 | 0.12 ± 0.08 |
| Phe         | 0.50 ± 0.14 | 0.17 ± 0.06 | 0.28 ± 0.07 | 0.19 ± 0.13 |
| NH3         | 17.2 ± 0.08 | 0.40 ± 0.02 | 23.0 ± 0.16 | 0.36 ± 0.07 |
| His         | 0.00 ± 0.00 | 0.10 ± 0.07 | 0.00 ± 0.00 | 0.12 ± 0.12 |
| Asp         | 0.17 ± 0.03 | 0.10 ± 0.005 | 0.19 ± 0.03 | 0.03 ± 0.01 |
| Thr         | 0.23 ± 0.01 | 0.25 ± 0.05 | 0.00 ± 0.00 | 0.28 ± 0.16 |
| Ser         | 0.05 ± 0.01 | 0.22 ± 0.03 | 0.07 ± 0.02 | 0.13 ± 0.09 |
| Pro         | 0.00 ± 0.00 | 0.12 ± 0.07 | 0.00 ± 0.00 | 0.05 ± 0.03 |
| Glu         | 0.36 ± 0.03 | 0.79 ± 0.22 | 2.84 ± 0.34 | 0.30 ± 0.21 |
| Cys         | 0.04 ± 0.04 | 0.00 ± 0.00 | 0.27 ± 0.01 | 0.69 ± 0.10 |
| Leu         | 0.32 ± 0.11 | 0.18 ± 0.13 | 0.59 ± 0.02 | 0.34 ± 0.09 |
| Tyr         | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.36 ± 0.02 |
| Lys         | 0.06 ± 0.01 | 0.00 ± 0.00 | 0.22 ± 0.03 | 0.04 ± 0.01 |
| Arg         | 0.05 ± 0.00 | 0.00 ± 0.00 | 0.41 ± 0.03 | 0.00 ± 0.00 |
| Strains/plasmids | Relevant characteristics | Reference          |
|------------------|--------------------------|---------------------|
| **Plasmids**     |                          |                     |
| pKD46            | Red recombinant plasmid, Temperature-sensitive, L-arabinose induction, Amp\(^r\) | Lab. collected     |
| pKD3             | Harboring the gene of chloramphenicol resistance, Amp\(^r\), Cm\(^r\) | Lab. collected     |
| pCP20            | Harboring the gene of FLP recombinase, Temperature-sensitive, Amp\(^r\), Cm\(^r\) | Lab. collected     |
| **Strains**      |                          |                     |
| *E. coli* BW25113| Host for homologous recombination | Lab. collected     |
| BW25443-Δast E   | Knocking-out of *astE* gene in BW25113, Cm\(^r\) | This study         |

Amp\(^r\), Cm\(^r\) are ampicillin, chloramphenicol resistance, respectively
| Primers       | Sequences (5’-3’)                                                                 | Application          |
|--------------|----------------------------------------------------------------------------------|----------------------|
| astE-cms     | TTAGCTTATTTTTTTTCGAGCATTAATCCCCGCGGTAA                                          | Knocking-out astE   |
|              | TGCTTTGAGCGATTGTGTAGG                                                             |                      |
| astE-cmas    | ATGGATAATTTTCTTGCTCTGACCTAACCAGGTAA                                             |                      |
|              | ACTTAACGGCTGACATGGGAA                                                            |                      |
| astE-yans    | CCAATTACTGAATCTCGGT                                        | Identification of   |
| astE-yanas   | AGAAAGCAACTTAATACCCG                                                            | ΔastE               |

*astEcm represents the primers for plate pKD3; astEyan represents the identifying primers. Underline represents the homology sequences to the aim gene.
| Gene name | Sense Primer | Sequence (5’to3’) |  | Anti-sense Primer | Sequence (5’to3’) |
|-----------|--------------|------------------|---|------------------|------------------|
| astE      | astE-qF      | ATATGGCAAGTGACACGCTGAAT | astE-qR | CCAACGCTACCAACGGAT TAGG |
| prpB      | prpB-qF      | TTGGCACCATAACGCCTAATCAT | prpB-qR | GTCGGTCAGCACATCATC AAGG |
| prpD      | prpD-qF      | AGGTGAATCGGTTCGCTTCCA | prpD-qR | GCTGCTTCAACTGGCCTCTG |
| ybfA      | ybfA-qF      | ACAGAGAATATCCTGCAATGGCTTAT | ybfA-qR | TCGGCCTGATCCATCCACAC |
| ymgA      | ymgA-qF      | CAGGCCTGGTCGCCAGATTACCT | mgA-qR | GCTGTCGCTGTTCTTCGATT |
| ymgC      | ymgC-qF      | ATGACGCATGGGTATGTGTTATG | mgC-qR | AGAGAGCACGGATTCCTGTCG |
| iraD      | iraD-qF      | TTAATTCCTCTCATTCGGCATACT | iraD-qR | GAGTGTGGCAGTACGCTTT |
| psuK      | psuK-qF      | TGAAGAGGCACCTGGCATTGATT | psuK-qR | TGGCTTGAGAGTGTGGATTCTGAT |
| gadC      | gadC-qF      | ACTGGGTGTTAGTTTCCGGTGTTG | gadC-qR | TGGTGGCTTCTGACCTGGGAT |
| YbaL      | YbaL-qF      | CTGATATCCTTGCTTGTTGATTG | YbaL-qR | TTCCTGTGGTCGCGCATATG |
| rpoA      | rpoA-qF      | ATTCGTCGTCGGGCAACCA | rpoA-qR | ACAGTCAATTCCAGATCGTCAACAG |
| astE      | astE-qF      | ATATGGCAAGTGACACGCTGAAT | astE-qR | CCAACGCTACCAACGGATTAGG |
| prpC      | prpC-qF      | ATCTCGACTGTTCTCTCTCCT | prpC-qR | CATATTGGCCGAGGACGGATCAGT |
| prpE      | prpE-qF      | CTGGTCCTGGGAAAGTCGGTTA | prpE-qR | CGGATCCTGGTCTCCAGCTAGT |
| ycgZ      | ycgZ-qF      | GCGGAGCAATCACAAGTGTAC | ycgZ-qR | GCGTGGTCCAGTCGGCAAGT |

Table S5 Quantitative PCR primers of selected genes
| Gene  | Primer Set | Forward Sequence | Reverse Sequence |
|-------|------------|------------------|------------------|
| ariR  | ariR-qF    | TTAGAAGAAGAATCA  | ariR-qR          | TGTTGTTCAGGAGTG  |
|       |            | GCAGTGTTAGG      |                  | TATCAGA          |
|       | spy-qF     | ATGCAGCAGACACCAC | spy-qR           | CTCAGGTTTCAAGGT  |
|       |            | TACC             |                  | TCT              |
|       | psuG-qF    | TTGTTGTTTCCAAGGAGT | psuG-qR       | CGCCGCAATACATCGT |
|       |            | TATCAGA          |                  | TGAAG            |
|       | csrB-qF    | GGAGTCAGACACGAA  | csrB-qR          | GCGTCTGGTGCTCCTCTT|
|       |            | GTGAACATC        |                  | T                |
|       | adiA-qF    | CGCTGTCAAGGATTCTT | adiA-qR          | TCAACCGCTTTCGTAATC|
|       |            | TATATTCA         |                  | ACTTC            |
|       | gadC-qF    | ACTGGTTGTTAGTTTCC| gadC-qR          | TGGTGCGTTCTGACTGTG|
|       |            | TGGTGTA          |                  | TTT              |
|       | YbaT-qF    | TGGCGGTATTGTGGCG | YbaT-qR          | CCAACGCTCAACAGGTCA|
|       |            | ATGT             |                  | GTAAC            |
|       | yidE-qF    | CCGCTGATTACTGTGG | yidE-qR          | GCACCGCTGTTGGATGA|
|       |            | CATTCT           |                  | AG               |
|       | cysB-qF    | ATATCTTCAGCCACAGT| cysB-qR          | CAGCCGCACTCAACGACAT|
|       |            | ACAACCAA         |                  | CA               |
Fig. S1 The catabolism pathway of L-arginine in *E. coli*. *ast*A, Arginine N-succinyltransferase; *ast*B, N-succinylarginine dihydrolase; *ast*C, Succinylornithine transaminase; *ast*D, Succinylglutamate-semialdehyde dehydrogenase; *ast*E, Succinylglutamate desuccinylase.
Fig. S2 Cell growth against 8 g/L butanol stress: Application of a spotting assay
Fig. S3 Metabolomic cloud plot of strains in the presence and absence of butanol (Green, up/down-regulated metabolites; red, up/down-regulated metabolites; p<0.01; FC≥1).
Fig. S4 Overview of the differentially expressed genes between BW25113 and BW25113-ΔastE against 5 g/L butanol stress (Green, downregulated genes; red, upregulated genes; p<0.05; FC≥2).
Fig. S5 GO enrichment analysis of differentially expressed genes between BW25113 and BW25113-ΔastE after exposure to 5 g/L butanol stress.
Fig. S6 KEGG enrichment analysis of differentially expressed genes between BW25113 and BW25113-ΔastE after exposure to 5 g/L butanol.
Fig. S7 KEGG enrichment analysis of significantly downregulated genes
Fig. S8 Different metabolites: Clustering heat map. analysis related to the different metabolites of BW25113-ΔastE and BW25113 after exposure to 5 g/L butanol and metabolic pathways (red, upregulated; blue and green, downregulated)