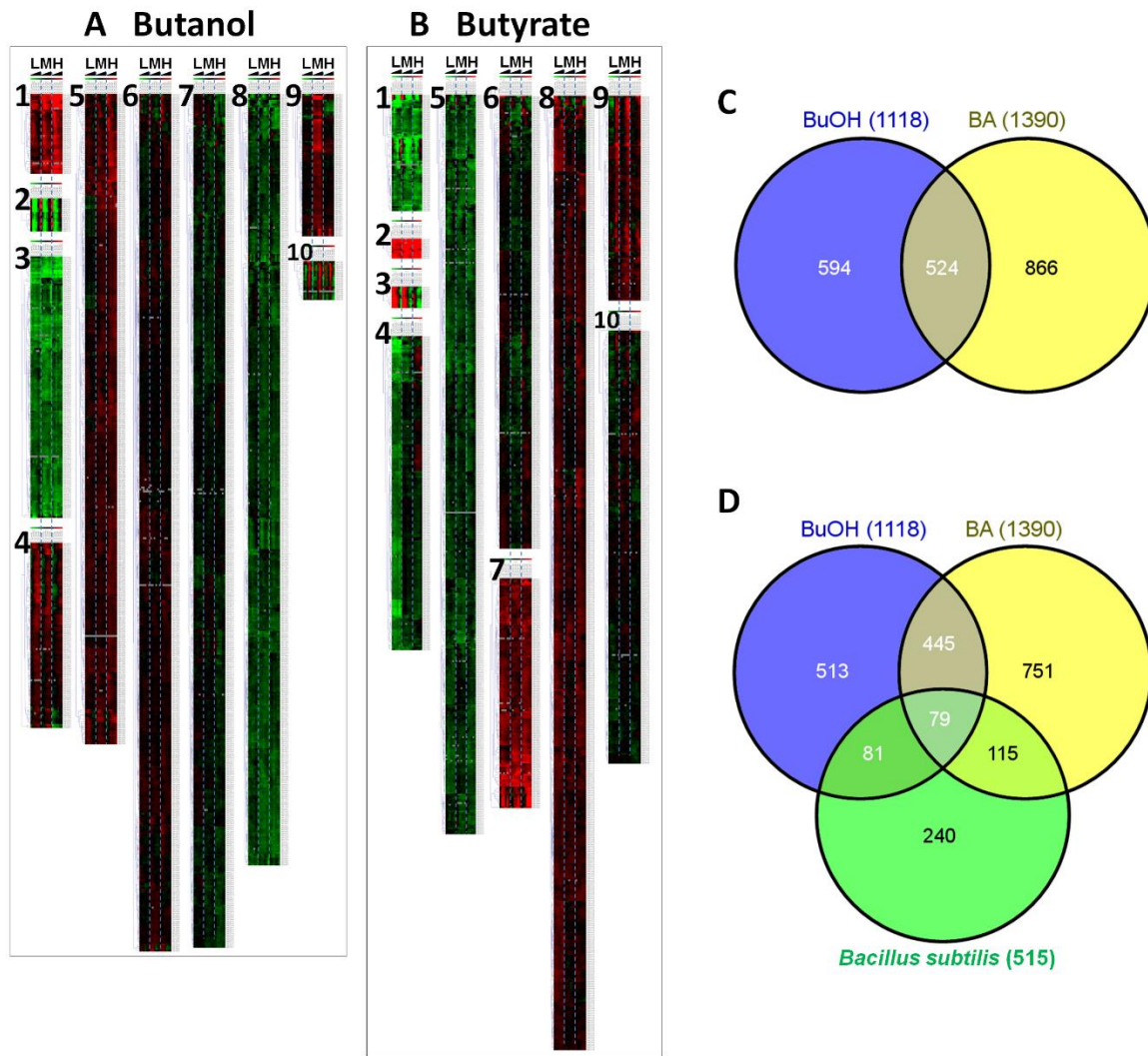
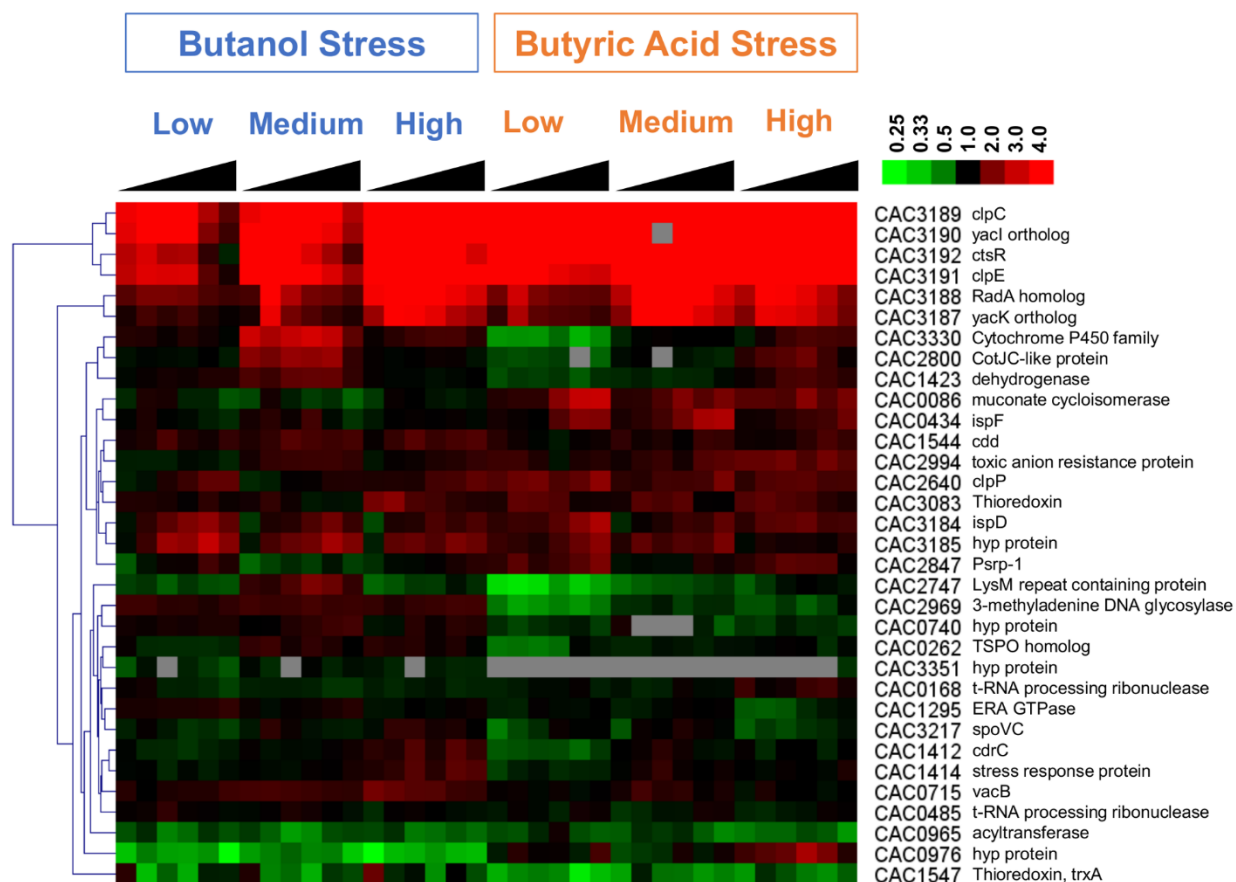


## Supplemental materials

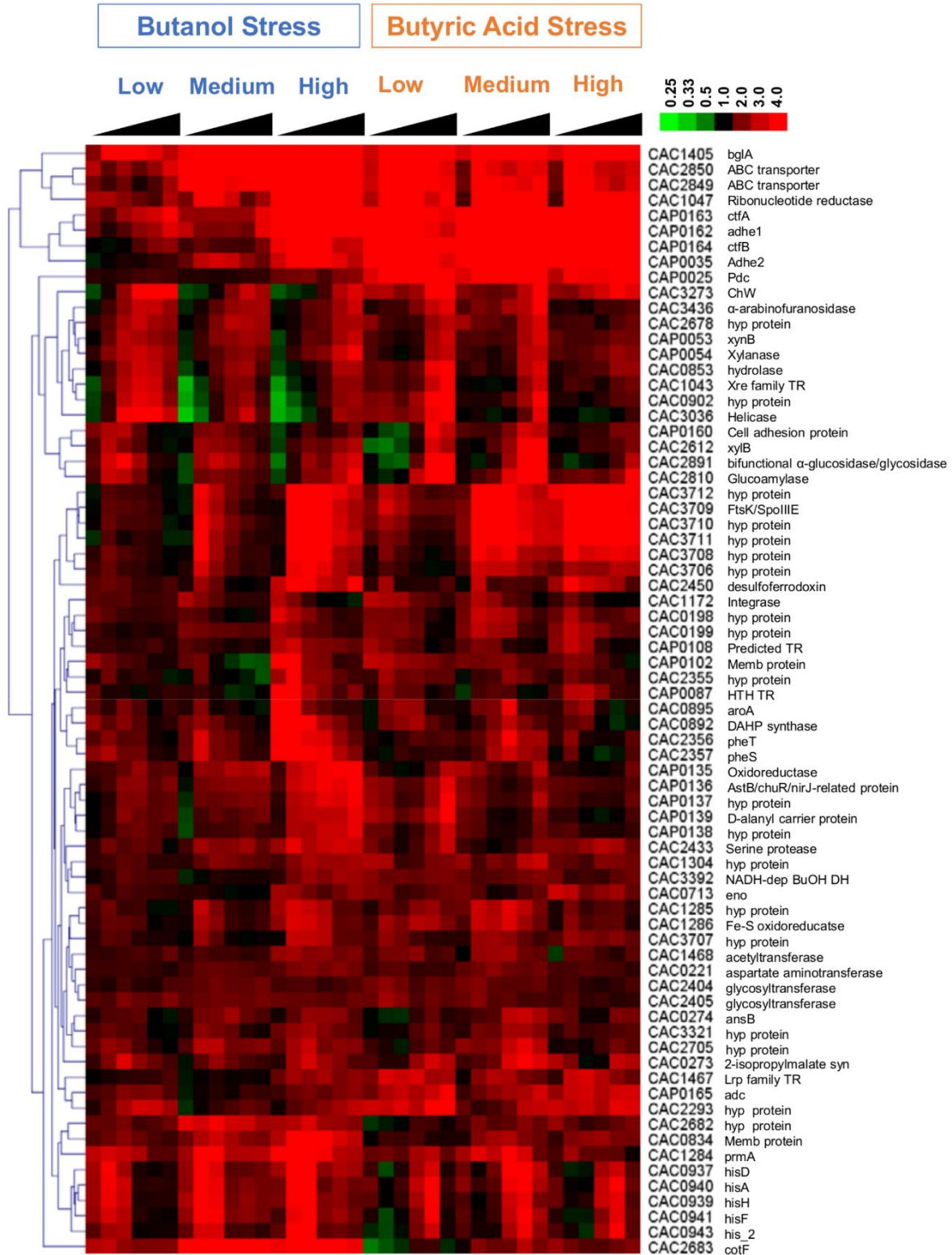


**Fig. S1. Analyses of the microarray expression data.** K-means clustering of 1984 differentially expressed genes under (A) butanol stress and (B) butyrate stress.. For the butanol stress data in (A), cluster 1 is enriched with genes relevant to protein folding and histidine biosynthesis; cluster 2 is enriched with arginine biosynthesis related genes; cluster 5 includes 7 genes involved in aromatic amino acid family biosynthesis, and 6 genes in branched-chain amino acid biosynthesis. For the butyrate stress (B), cluster 1 is enriched with genes related to sulfur

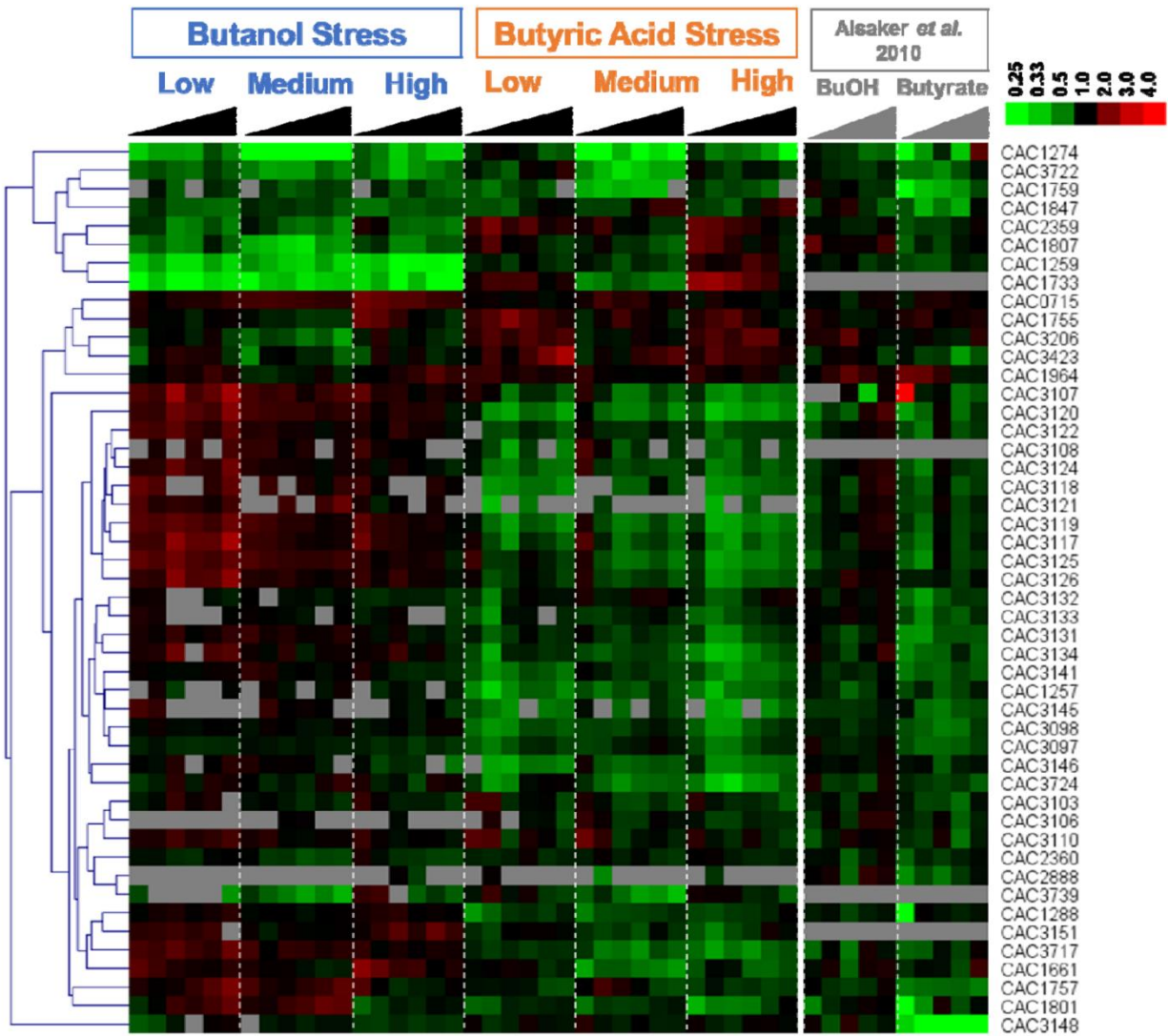
compound metabolic processes, and inorganic ion transport and metabolism; cluster 2 includes many Class III and IV HSPs; cluster 3 has genes involved in arginine biosynthesis; cluster 5 is enriched with genes for structural constituents of ribosomes, and cobalamin biosynthesis; cluster 7 has 8 genes involved in purine ribonucleoside monophosphate biosynthesis, and several Class I HSPs; cluster 9 is enriched with genes involved in histidine biosynthesis, and carbohydrate transport and metabolism. (C) Venn diagram of the distribution of the differentially expressed genes between the two conditions (BuOH – Butanol and BA- Butyrate). (D) Comparison of the differentially expressed genes with the 515 genes with certain homology to *B. subtilis* stress-related proteins (see manuscript Methods section for details).



**Fig. S2.** Orthologs of *B. subtilis* Class II HSPs in *C. acetobutylicum* based on orthology analysis (OMA [1, 2]). Genes CAC3187-CAC3192 belong to Class III HSPs regulated by CtsR and the other genes show no significant differential expression under stress, which confirms the absence of a functional  $\sigma^B$  in *C. acetobutylicum*.

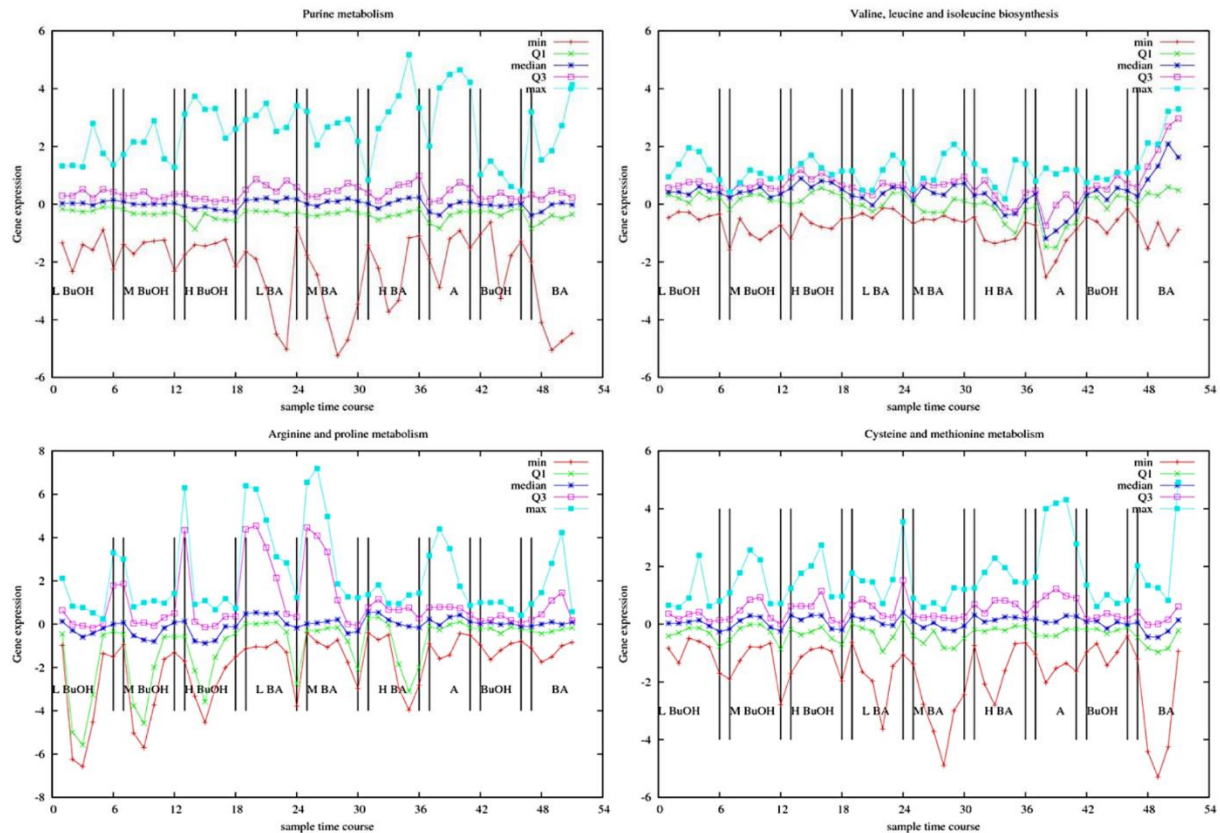


**Fig. S3.** Class IV stress genes in *C. acetobutylicum* based on their upregulation under both butanol and butyrate stress, and by excluding genes regulated by either HrcA or CtsR.



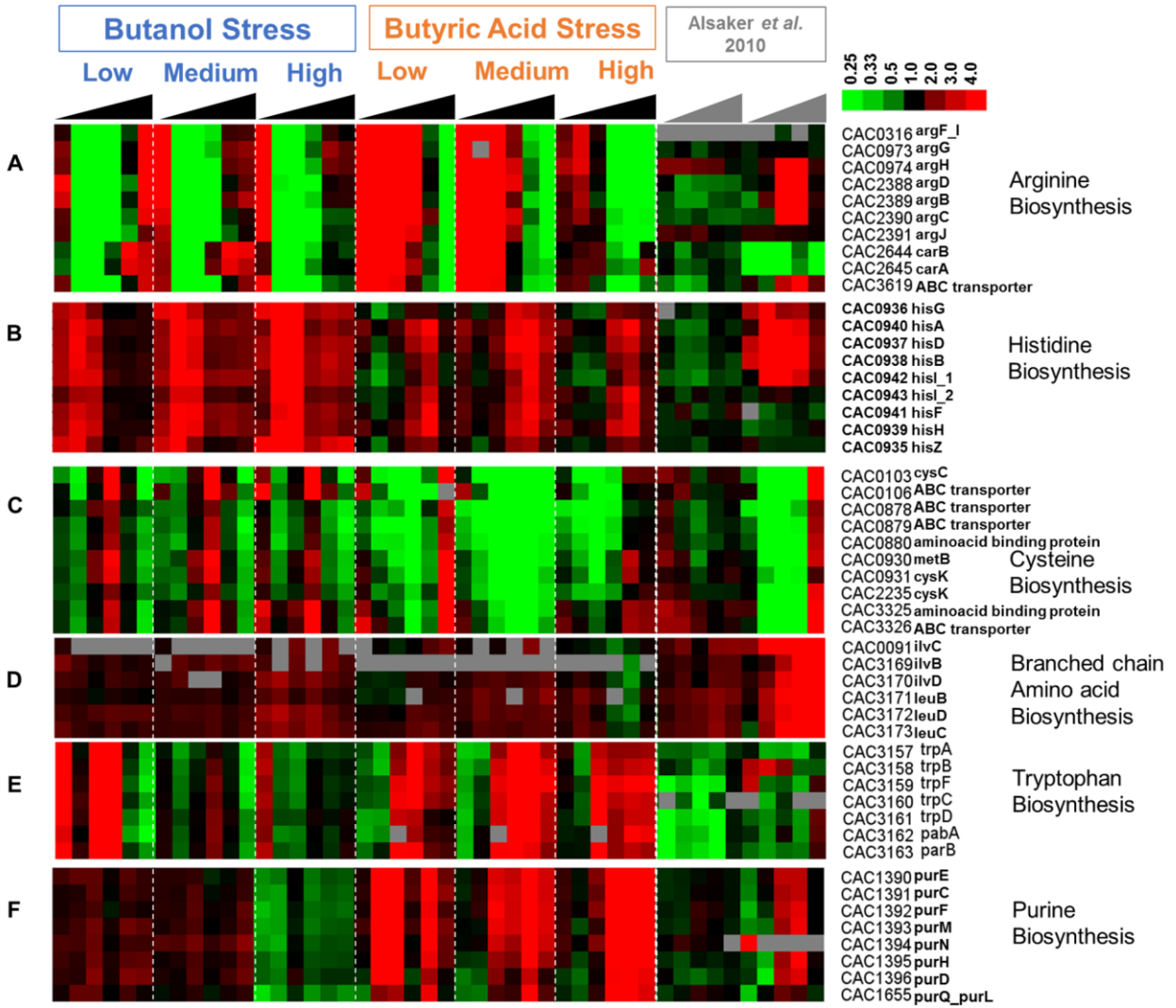
**Fig. S4.** Hierarchical clustering of 48 ribosomal proteins in *C. acetobutylicum* under butanol and butyrate stress and comparison with the data from Alsaker *et al.* 2010 study [3].



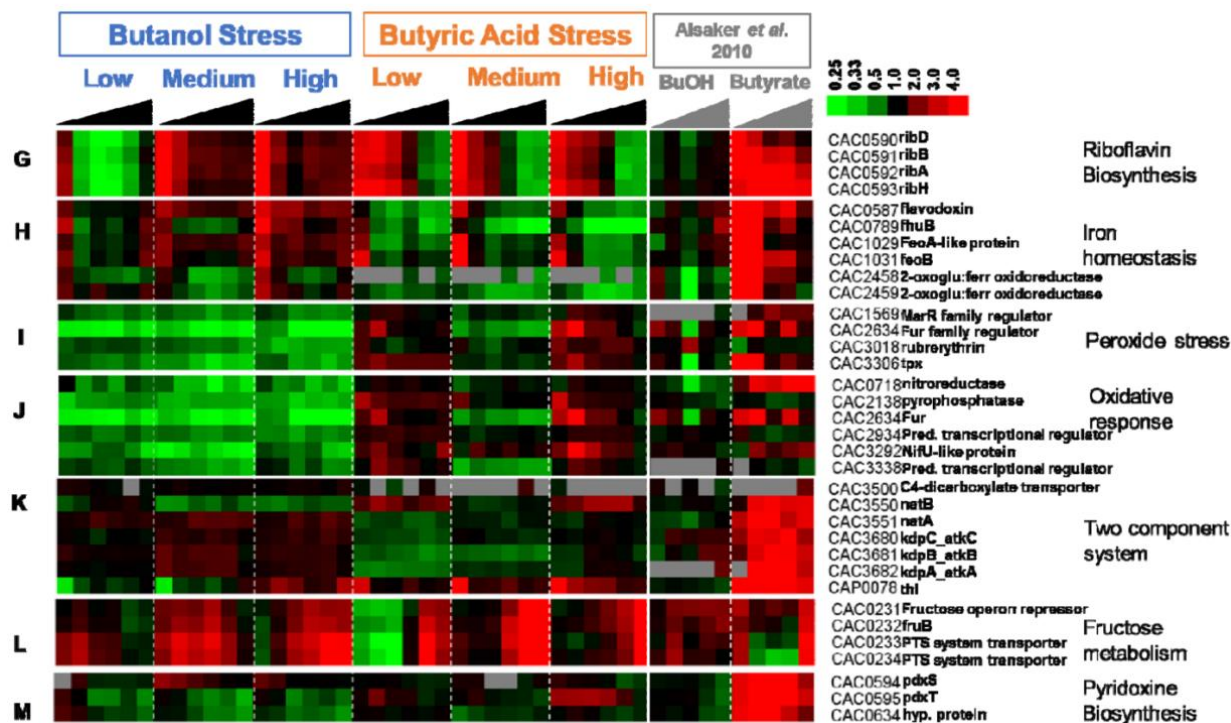


**Fig. S5.** The so-called 5-number summary (i.e., the median, the upper and lower quartiles, and the minimum and maximum) of a dataset for the logarithmic (to the basis of 2) expression ratios under this study (i.e., BuOH L(ow) M(edium) H(igh), BA L M H, at the tested time points) and in the 2010 Alsaker study (i.e. “A”, “BuOH”, “BA”) for the genes of four KEGG pathways in *Clostridium acetobutylicum*. The x-axis is indexing a sample (e.g., the sample L BuOH at time 0 min is shown at 1 for x-axis, whereas L BA at time 0 min is shown at 19). The y-axis is the logarithm of gene expression ratios. The four panels include purine metabolism (top left, 58 genes), branched chain amino acid (ILV) biosynthesis (Top right, 13 genes); arginine and proline metabolism (bottom left, 23 genes); and cysteine and methionine metabolism (bottom right, 32 genes). An interesting finding is that, frequently, not all genes in a given pathway show consistent expression patterns. For example, it is not uncommon that only few genes in a

pathway are strongly differentially expressed, whereas the majority is not. For example, among the 58 *C. acetobutylicum* genes involved in purine metabolism (upper left panel), most do not experience a significant change in expression under stress (i.e., their logarithmic expression ratios are close to 0). This holds true also for the valine/leucine/isoleucine biosynthesis and cysteine/methionine metabolism pathways as displayed in these graphs. These data suggest that a pathway could be regulated by changing the expression of a subset of its genes, rather than the whole gene set, and this is consistent with the well-established notion of the rate- or process controlling genes, enzymes or reactions. On the other hand, arginine/proline metabolism pathway is an example for pathways with a significant subset of genes consistently differentially expressed (lower left panel).

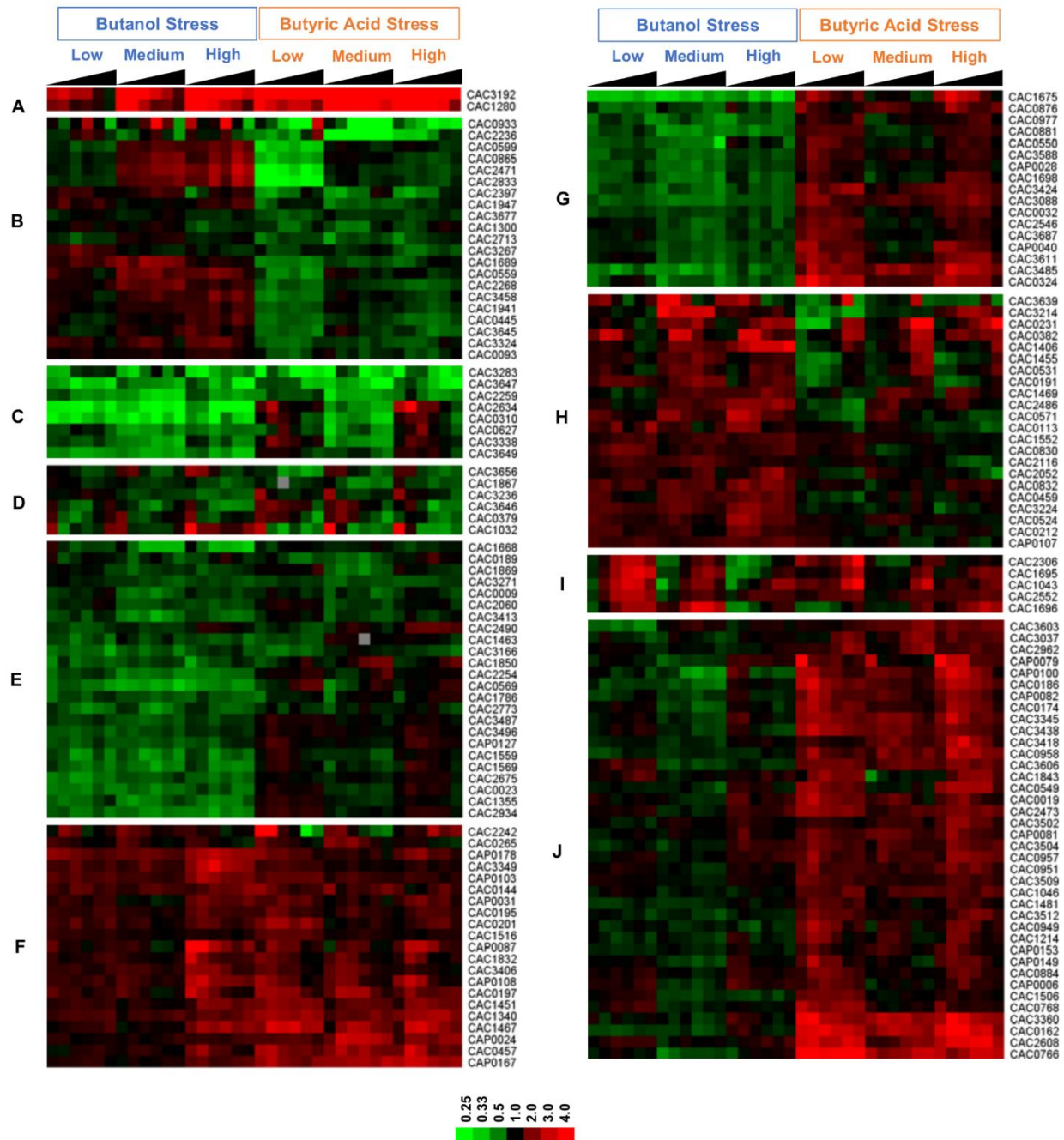






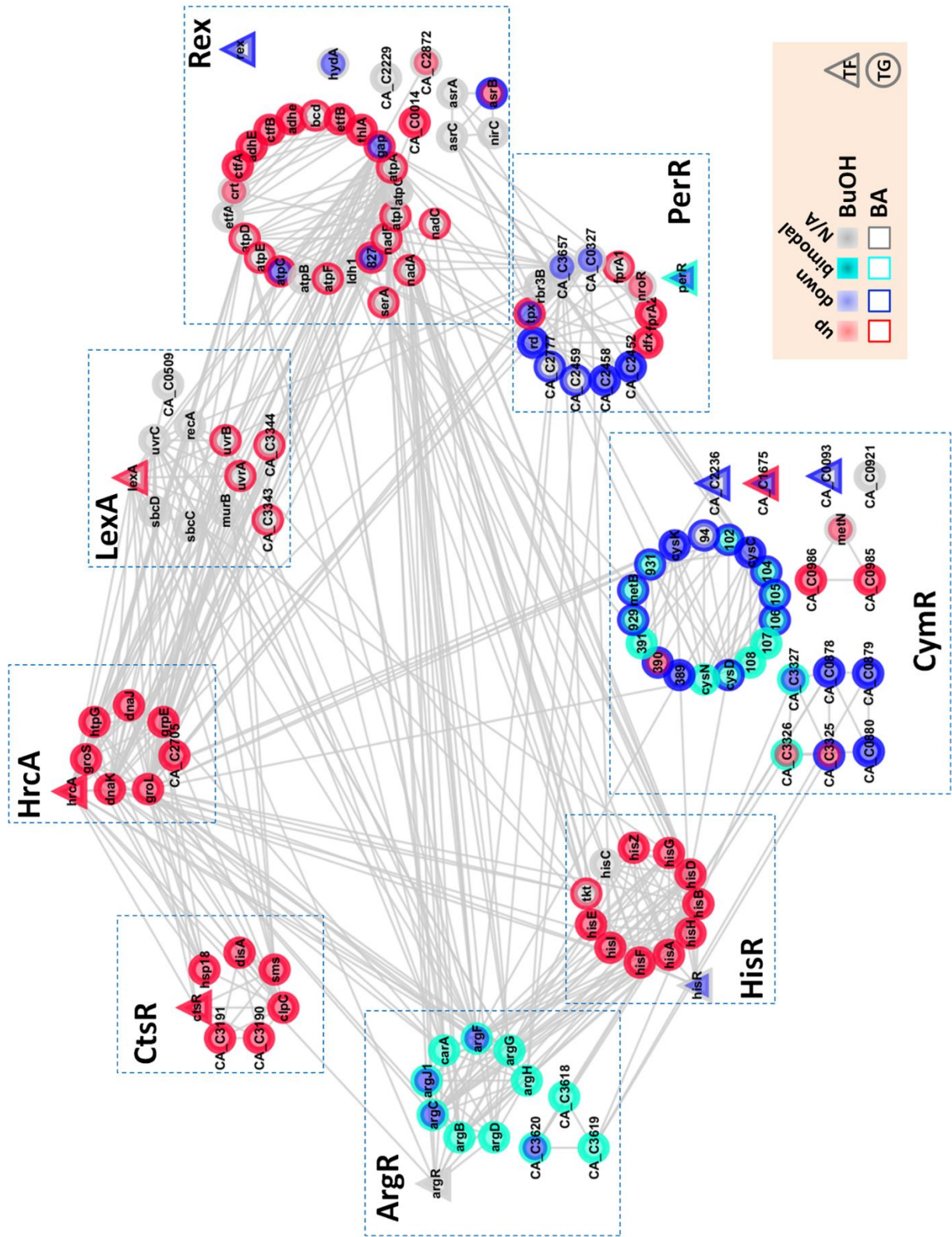
**Fig. S6.** Comparisons on selected genes between this study and the previous study by Alsaker et al. in 2010 [3].

- (A) 10 genes in Arg biosynthesis.
- (B) 9 genes in His metabolism.
- (C) 10 genes in Cys metabolism.
- (D) 6 genes in Ile, Leu, Val biosynthesis.
- (E) 7 genes in Trp biosynthesis
- (F) 8 genes in Purine metabolism.
- (G) 4 genes in Riboflavin Biosynthesis
- (H) 6 genes involved in iron homeostasis show strong difference in response to 30mM butyrate stress.
- (I) 4 genes in Peroxide stress
- (J) 6 genes in Oxidative response
- (K) 7 genes related to two-component system
- (L) 4 genes in Fructose metabolism
- (M) 3 genes in Pyridoxine metabolism

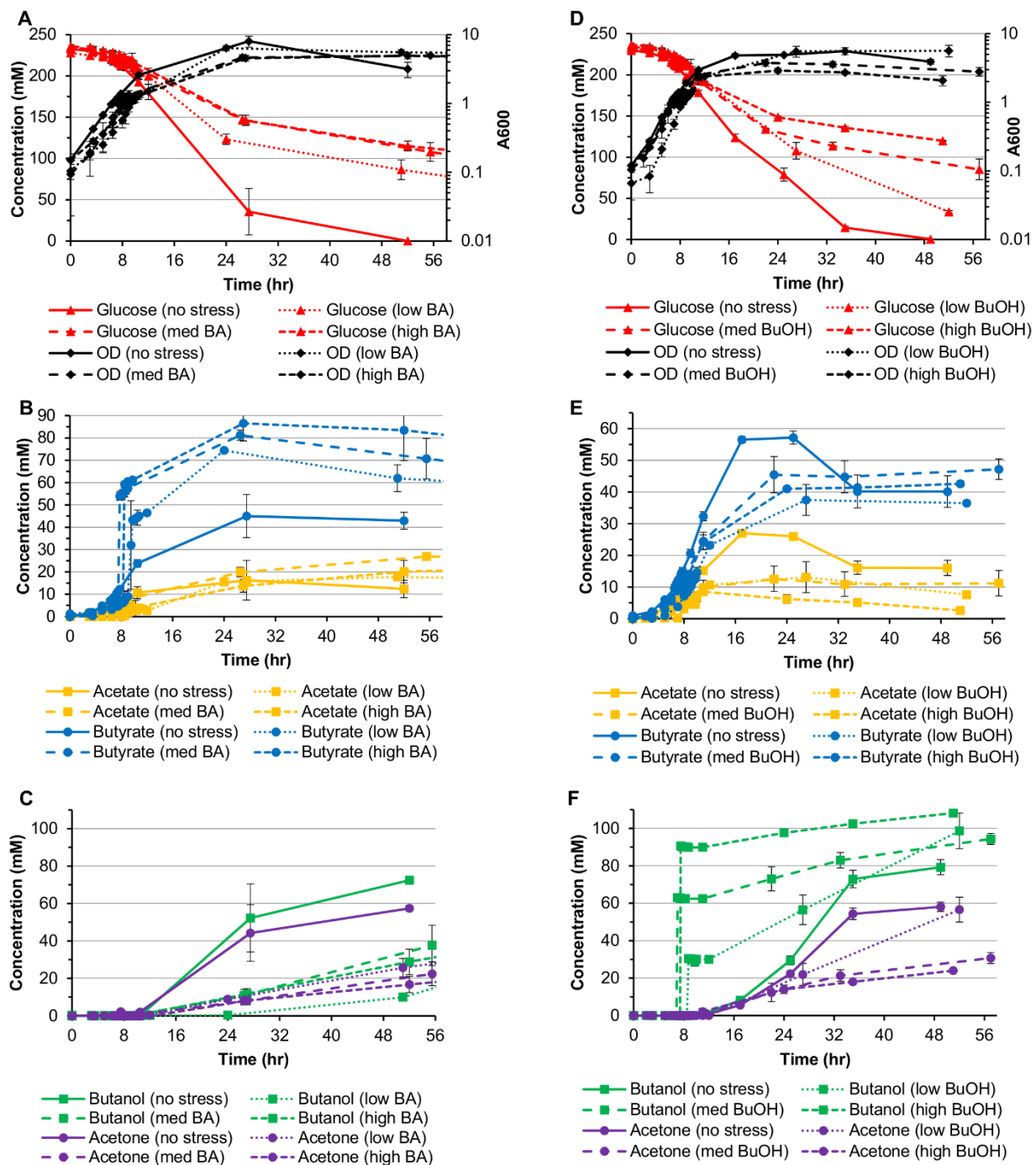


**Fig. S7.** K-means clustering into 10 user-defined clusters of 164 differentially expressed transcriptional regulators in *C. acetobutylicum* under butanol and butyrate stress. Clusters A, F & I represent upregulated TRs, while C, D & E contain downregulated TRs (general stress response). On the other hand, clusters B, G, H & J consist of TRs with distinct expression patterns for each stress and are involved in orchestrating the stressor-specific response.

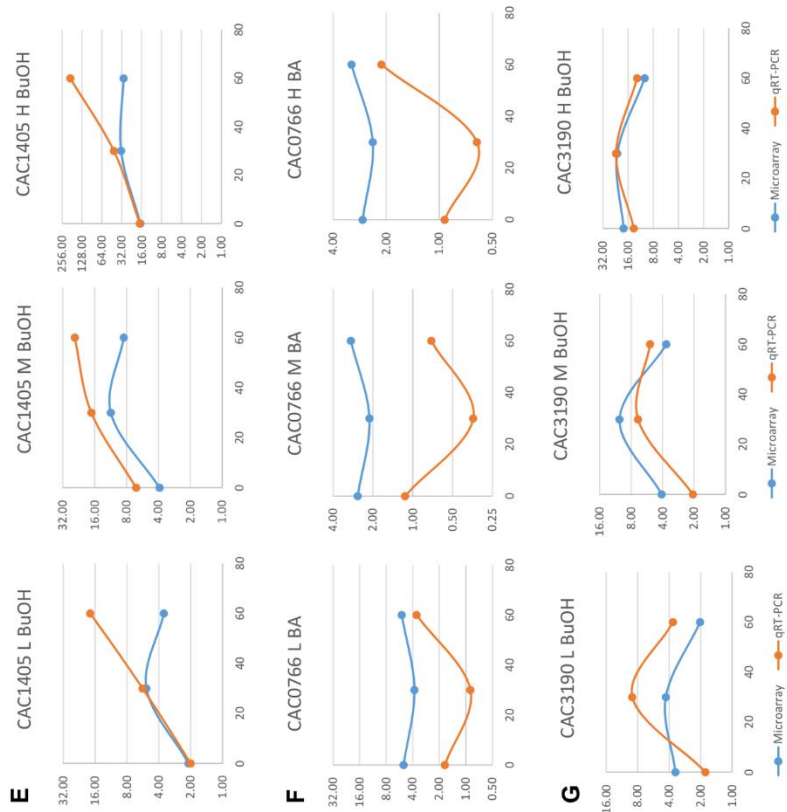
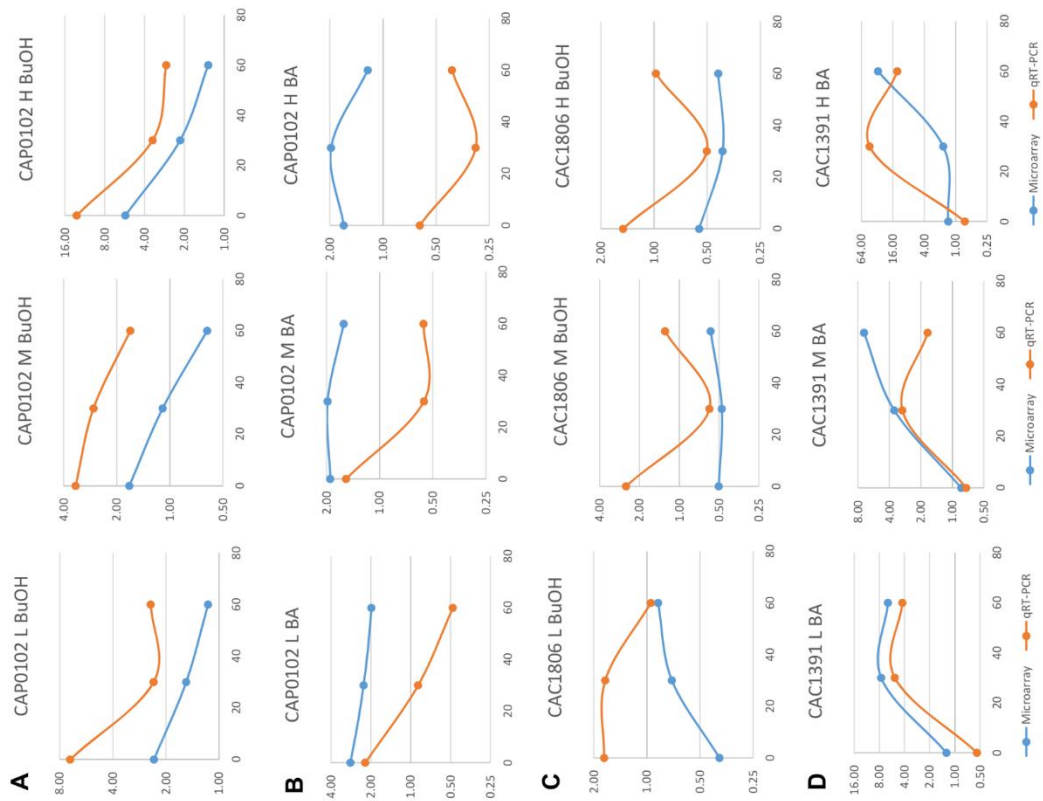




**Fig. S8.** The Stress Response Network (SRN) displaying the inter-relationships among the eight core, stress-related, regulons.

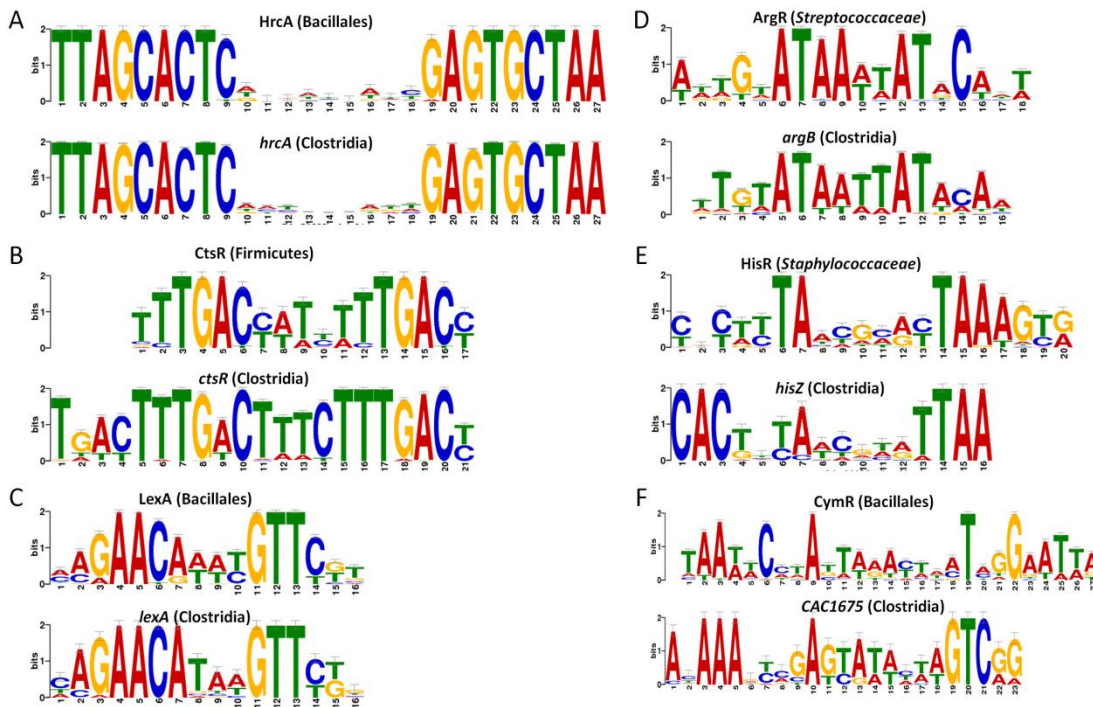


**Fig. S9.** Growth curve for butyrate stress (A) and butanol stress (D). Product formation for butyrate stress (B & C) and butanol stress (E & F).



**Fig. S10.** Comparison of microarray (blue points and lines) against Q-RT-PCR (orange points and lines). A. CAP0102 – butanol (BuOH) stress; B. CAP0102 – butyrate (BA) stress; C. CAC1806; D. CAC1391; E. CAC1405; F. CAC0766; G. CAC3190.





**Fig. S11.** Comparison of the DNA motifs for the binding of (A) HrcA, (B) CtsR, (C) LexA, (D) ArgR, (E) HisR and (F) CymR from RegPrecise [4] (the upper sequence logos in each panel) with those for the identified *cis*-elements in the class of Clostridia for the given genes, i.e., *hrcA*, *ctsR*, *lexA*, *argB*, *hisZ* and CAC1675 (the lower sequence logos in each panel).

## Reference

1. Schneider, A., C. Dessimoz, and G.H. Gonnet, *OMA Browser - Exploring orthologous relations across 352 complete genomes*. Bioinformatics, 2007. **23**(16): p. 2180-2182.
2. Altenhoff, A.M., et al., *OMA 2011: orthology inference among 1000 complete genomes*. Nucleic Acids Research, 2011. **39**: p. D289-D294.
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4. Novichkov, P.S., et al., *RegPrecise: a database of curated genomic inferences of transcriptional regulatory interactions in prokaryotes*. Nucleic Acids Research, 2009. **38**(Database): p. D111-D118.