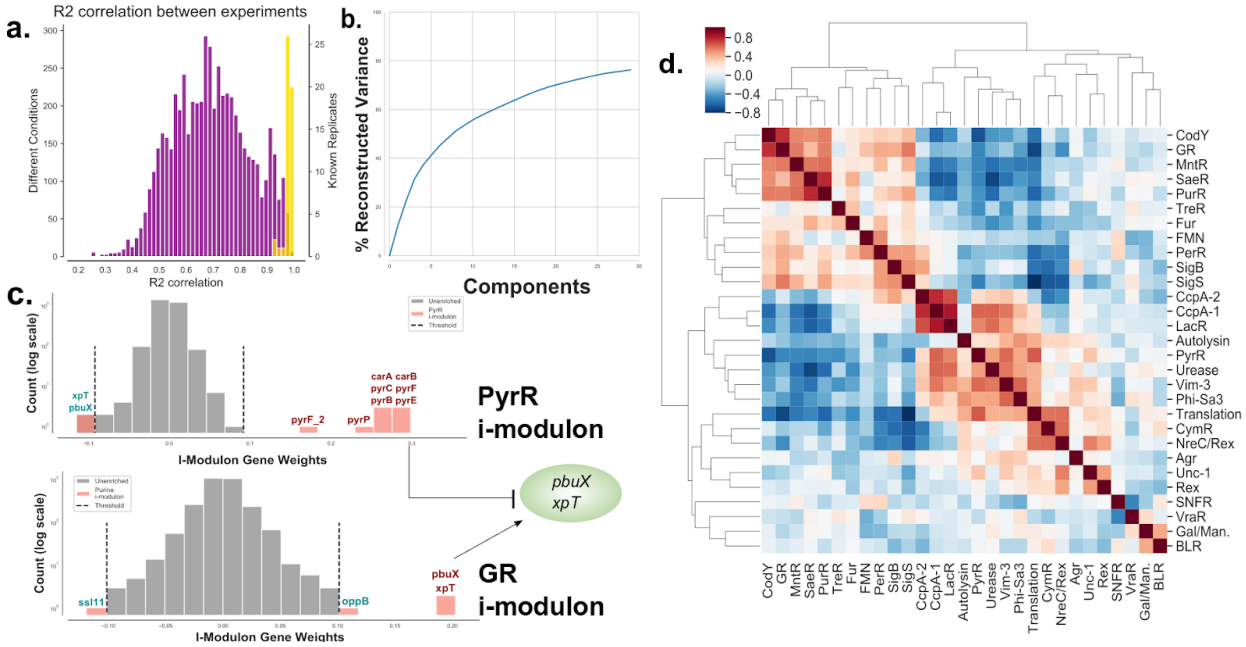
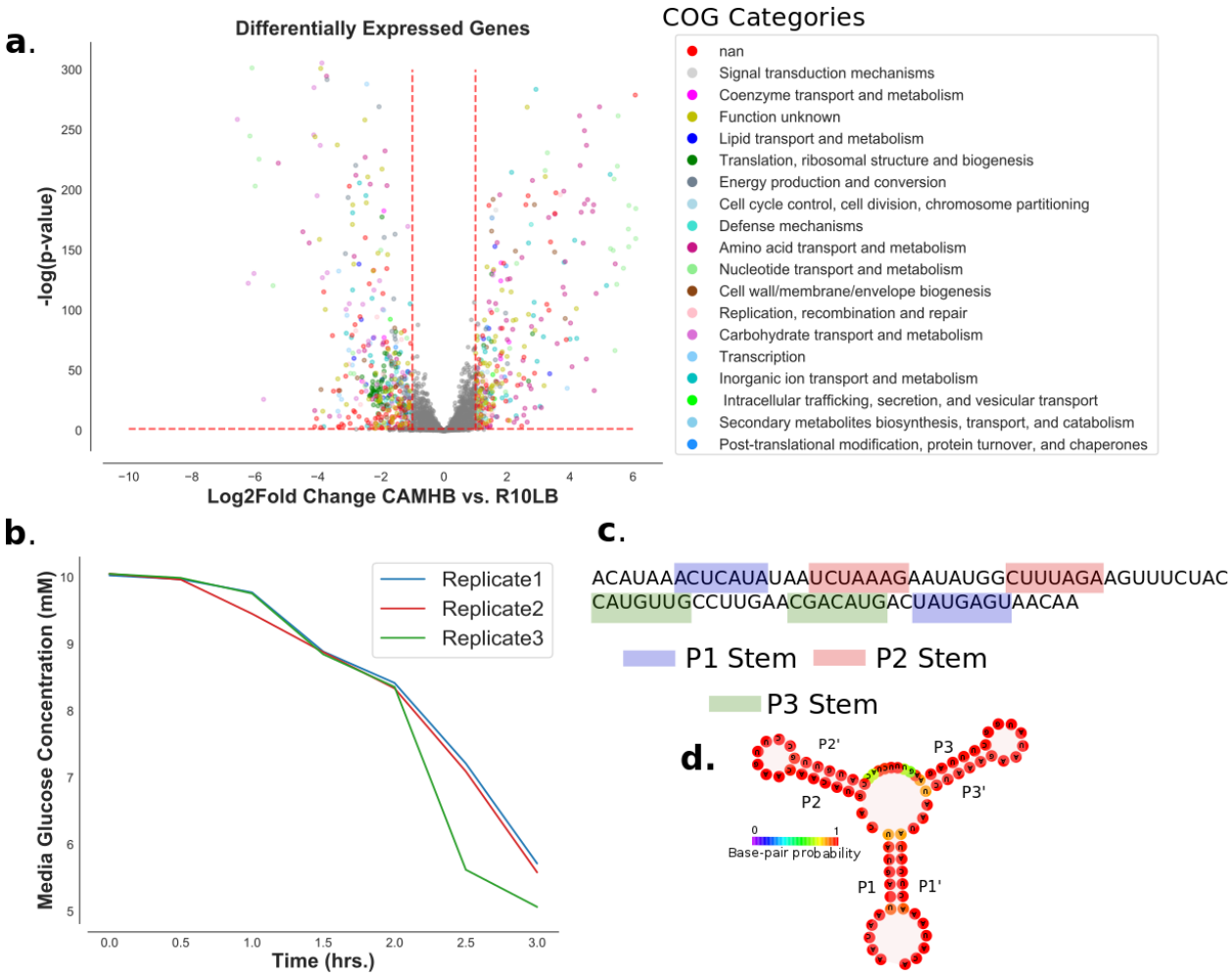
Supplementary Figures



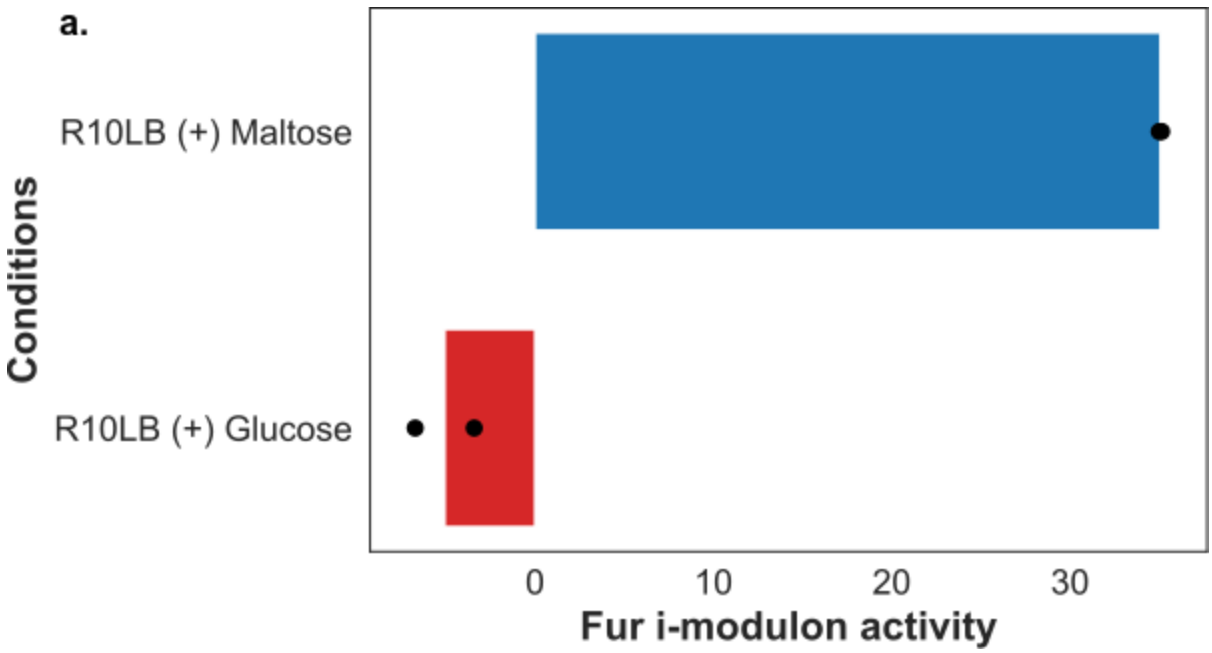
Supplementary Figure 1: Mathematical representation of *S. aureus* TRN. (a) RNA sequencing data were collected in duplicates and their reproducibility was verified using Spearman correlation of TPM values . Correlation between replicates (yellow bars) for all samples had $R^2 > 0.9$, with most samples having $R^2 > 0.95$. Correlation between different samples (purple bars) had a wide range of correlation, indicating the presence of diverse expression states. (b) The ICA decomposition captured most of the information in the input RNA sequencing compendium (**Dataset S7**). 76% of the total variance could be reconstructed from the product of **S** (**Dataset S8)** and **A** (**Dataset S9**). (c) Histogram of gene coefficient in two example components (containing i-modulon for pyrmindine above and GR below). While most genes in a component have weights close to 0, few statistically significant outliers (outside of the vertical dashed lines) with high weightings (red bars) form an independently modulated set of genes (called an i-modulon). Genes can have both positive and negative coefficients and can be present in multiple i-modulons. The genes *xpt* and *pbuX* have negative coefficient in the PyrR i-modulon (top histogram)

indicating that these genes are contra-regulated to genes with positive coefficient in the same i-modulon (.e.g *carAB*). *Xpt* and *pbuX* are also present in the GR i-modulon (bottom histogram), indicating that these two genes are regulated by multiple regulators. The first row of the matrix also contains the threshold used to call i-modulons. (d) Though i-modulons represent independently regulated set of genes, their activities are coordinated with one another. The coordination is visualized as a heatmap depicting Pearson correlation of i-modulon activities across all 108 samples.

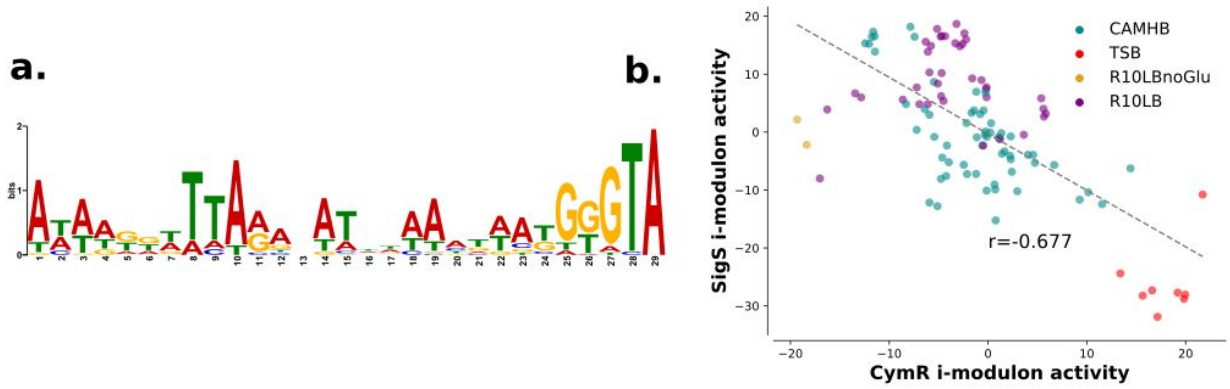


Supplementary Figure 2: Differential activation analysis and verification. (a) Volcano plot of differential expression levels of genes between CAMHB and R10LB. 848 genes spanning at least 17 COG categories (as determined by EggNog v4.5) were significantly differentially expressed[1]. Genes with greater than 2-fold change in expression and with p-value < 0.05 were considered significantly differentially expressed. (b) Glucose uptake was measured in R10LB and CAMHB. *S. aureus* actively took up glucose in R10LB while no glucose was detected in CAMHB. Each line represents a biological replicate in R10LB. (c) Riboswitch in conserved sequence upstream of *xpt* gene was verified using

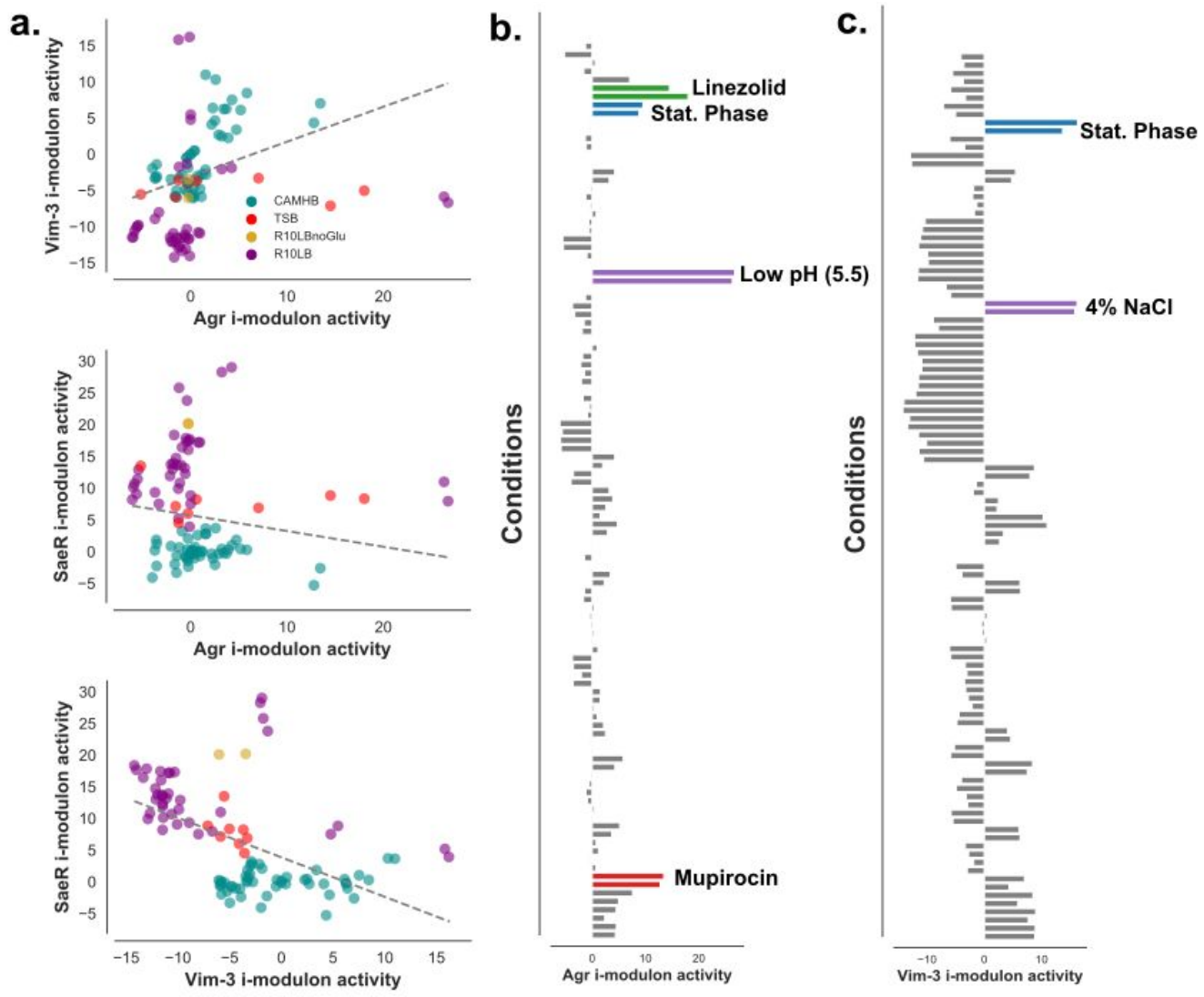
RiboSwitch[2]. (d) The structure of the riboswitch was verified with RNAfold within the ViennaRNA Package 2.0[3].



Supplementary Figure 3: Fur activity in response to changes in carbon source. (a) The activity of Fur i-modulons increased when the carbon source in R10LB was changed from glucose to maltose.



Supplementary Figure 4: Sigma Factor I-modulons. (a) Regulatory region of SigB i-modulon contained a conserved motif that closely matched SigB motif of B. *subtilis*. (b) The activity level of SigS i-modulon was negatively correlated with the activity of CymR i-modulon (PearsonR=0.677, p-val=8.29e-16).



Supplementary Figure 5: The virulence i-modulons of *S. aureus*. (a) Activity of Agr was poorly correlated with the activities of the other two virulence associated i-modulons, SaeR and Agr. However, SaeR and Vim-3 activities were negatively correlated. (b) Agr activity in most samples were close to 0. Its activity could be induced by translation inhibitors, growth to OD600 of 1 (Stat. Phase), and low pH (5.5). (c) The Vim-3 i-modulon had the highest activity in stationary phase and when *S. aureus* was challenged with 4% NaCl.

Supplementary Note 1
Analysis of complex *in vitro* and *ex vivo* expression profiling data with ICA and StaphNet

ICA analysis provides a low dimensional and biologically relevant decomposition of expression profiling data. This decomposition recasts the expression data into activity of independently modulated sets of genes (i-modulons), making the data far more interpretable. To demonstrate this, we reanalyzed the *ex vivo* serum data from Figure 6 using a graph based model of *S. aureus* TRN named StaphNet[4], and traditional differential expression analysis. Our analysis demonstrates that output of ICA analysis is more tractable than those provided by differential expression analysis or by StaphNet.

StaphNet is a probabilistic functional gene network of USA300 strain FPR3757 built by combining genomic data from multiple sources. Users can use this model to explore their differential expression data using a method called 'Context-centric Search.' Given a set of differentially expressed genes (DEGs) this algorithm finds hub genes (genes with >= 20 connections) which have neighbors that are significantly overlapped with input DEGs. This allows the users to understand which genetic hubs the DEGs are centered around. Unlike ICA analysis, StaphNet does not provide any form of activity or expression levels as output and therefore cannot be used to generate time-series data as presented in Figure 6a. On the other hand, while differential expression analysis gave gene expression levels for each of the time-point, each time point had over 100 DEGs which could not be conveyed clearly in a time-series plot. Therefore, we chose to compare the 2 hour time point.

Traditional comparison of gene expression after two hour growth in serum revealed that at this time-point there were 848 genes spanning dozen COG categories that were differentially expressed which made it difficult to fully characterize the response of *S. aureus*[5]. Analyzing the top 500 DEGs with StaphNet (the maximum number allowed by the algorithm) yielded at least 100 gene hubs that were enriched in proximity to the DEGs (**Dataset S10**). These hubs are ranked by StaphNet and the products of the top 5 hub genes were DNA-directed RNA polymerase subunit delta (SAUSA300_RS14555), polysaccharide deacetylase (SAUSA300_RS14530), DUF3816 family protein (SAUSA300_RS14550), L-threonine dehydratase biosynthetic IlvA (SAUSA300_RS11075), dihydroxy-acid dehydratase (SAUSA300_RS11035) (Table1).

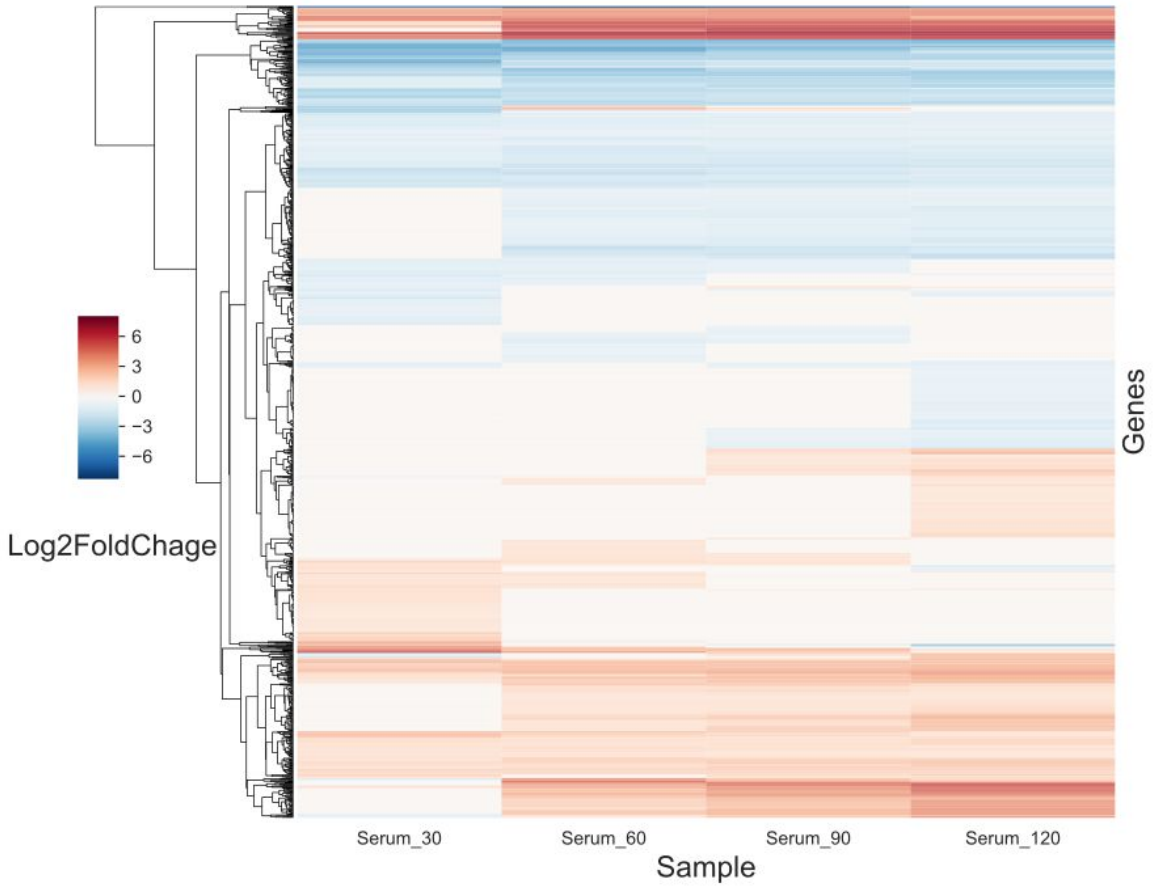
In contrast, ICA analysis provided clear differential activation of different regulators in the serum (e.g. SaeR, AgrA, CodY, Fur).The analysis also outputs the activity of each of these regulator associated i-modulons, which allows us to follow their dynamics through the time-course. For example, while both Fur and CodY activity are very high in serum at 2 hour time-point, Fur activity jumps immediately when introduced to serum while the CodY activity steadily increases over time to match Fur by 2 hours (Figure 6a). Indeed these dynamics not cannot be readily inferred from the expression levels of 1177 genes that were differentially expressed in at least one of the serum time-points (Figure S6).

Methods

For methods used for ICA analysis of serum data, please see the main text. The differentially expressed genes and their expression level in serum was used as reported by the original paper[5]. The top 500 differentially expressed genes in Serum at 2 hour time-point was submitted to the online implementation of the StaphNet Context-centric Search algorithm (https://www.inetbio.org/staphnet/Network_regulon_form.php). The products of the top 5 hub genes were determined using Aureowiki (Table 1)[6].

Rank	ORF_ID	USA300_FPR3757 (old locus tag)	submitted DEG	S. aureus GO terms	p-value
1	SAUSA300_RS14555	SAUSA300_2619	DEG		6.65E-28
2	SAUSA300_RS14530	SAUSA300_2614	DEG	GO:0005975-carbohydrate metabolic process	2.02E-27
3	SAUSA300_RS14550	SAUSA300_2618	DEG		4.20E-27
4	SAUSA300_RS11075	SAUSA300_2014	DEG	GO:0009097-isoleucine biosynthetic processGO:0006566-threonine metabolic process	2.13E-25
5	SAUSA300_RS11035	SAUSA300_2006	DEG	GO:0009097-isoleucine biosynthetic processGO:0009099-valine biosynthetic process	3.31E-23

Table 1: Top 5 hubs (degree >= 20) enriched from the top 500 differentially expressed genes in serum at 2 hour time-point. The hubs were determined using the 'Context-centric Search' method from StaphNet.



Supplementary Figure 6: Differentially expressed genes in Serum. Clustermap of 1177 genes that were differentially expressed in at least one of the serum samples.

Supplementary References

1. Huerta-Cepas, J. *et al.* eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res.* **44**, D286–93 (2016).

2. Bengert, P. & Dandekar, T. Riboswitch finder--a tool for identification of riboswitch RNAs. *Nucleic Acids Res.* **32**, W154–9 (2004).
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4. Kim, C. Y., Lee, M., Lee, K., Yoon, S. S. & Lee, I. Network-based genetic investigation of virulence-associated phenotypes in methicillin-resistant Staphylococcus aureus. *Sci. Rep.* **8**, 10796 (2018).
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