

1    **Supplementary Materials**

2    **Supplementary Figures**

3    **Supplementary Figure 1. Relative expression levels of *cobB* and *yfiQ* genes in the wild-type**

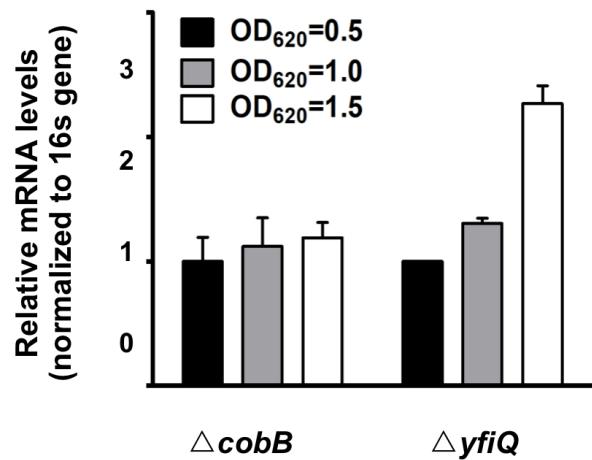
4    ***Y. pestis* strain 201 grown in LB medium.** *Y. pestis* 201 was grown in LB broth and the  
5    bacterial cells were harvested during the different growth phases as indicated. Relative *cobB* and  
6    *yfiQ* transcript levels were determined by qRT-PCR and the expression levels were normalized  
7    to 16s RNA expression.

8    **Supplementary Figure 2. Mutation of *yfiQ* or *cobB* does not influence bacterial growth.**

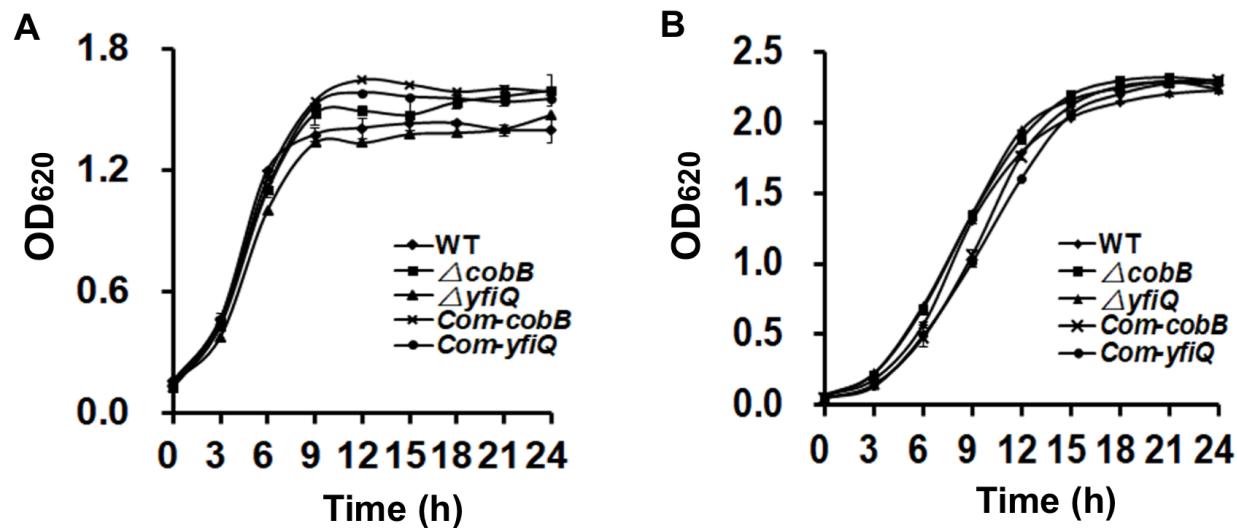
9    The wild-type strain, along with the *cobB* and *yfiQ* mutants and the complemented strains were  
10   inoculated individually into fresh LB broth (A) or TMH medium (B) at an OD<sub>620</sub> of 0.1, and the  
11   growth curves were recorded continuously for 24 h. Each time point is the average of three  
12   measurements and the error bars represent the standard deviations.

13   **Supplementary Figure 3. Functional classification of genes that were differently expressed**  
14   **in the *cobB* and *yfiQ* mutants compared with the parental *Y. pestis* strain.** DEGs in  $\Delta$ *cobB*  
15   (A) and  $\Delta$ *yfiQ* (B) were classified according to their functional categories.

16   **Supplementary Figure 4. Correlations between the fold changes in gene expression**  
17   **measured by qRT-PCR and RNA-seq.** Correlations between the qRT-PCR and the RNA-seq  
18   data were analyzed using a linear regression method. The correlation coefficient ( $R^2$ ) is shown  
19   for  $\Delta$ *cobB*/WT (A) and  $\Delta$ *yfiQ*/WT (B).

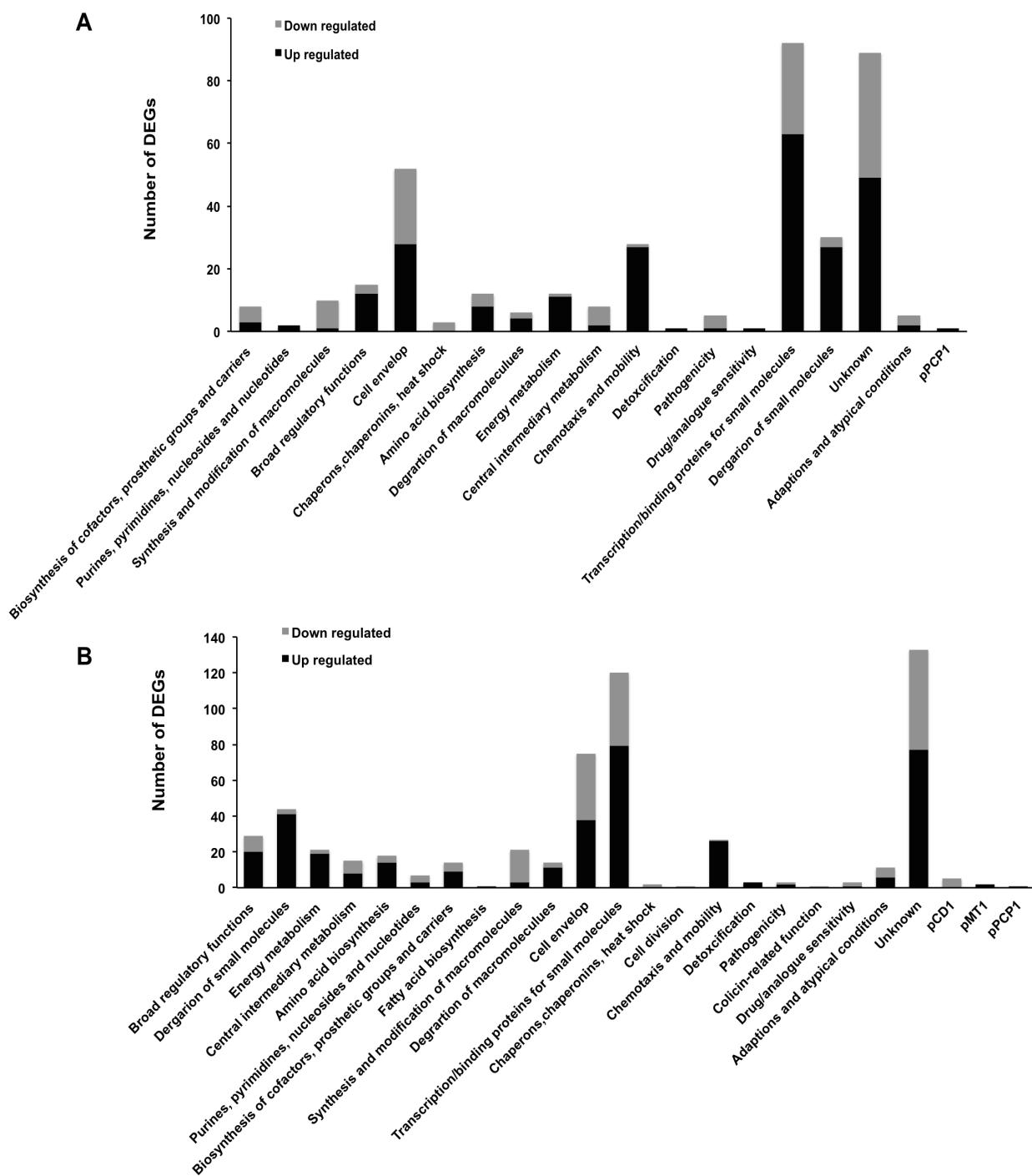


Supplementary Figure 1



Supplementary Figure 2

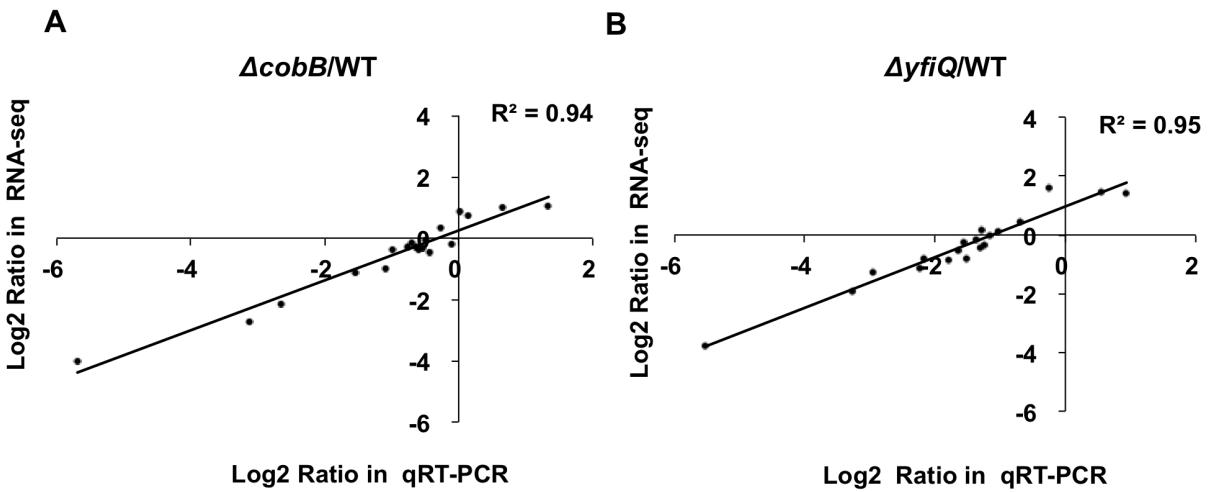
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**Supplementary Figure 3**

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**Supplementary Figure 4**

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26 **Supplementary Tables**

27 **Supplementary Table 1. Primers used in this study.**

| Name       | Primer (5'-3')   |
|------------|--|
| CobB-K-F   | ATGCGCATTGCCATAGGCTGTGCGGTTCGTAAGAGTAGTGTAGGCTGGAGCTGCTTC    |
| CobB-K-R   | TCAATCCCCCTGACGATTCTCACCGCATGTGGTCAGAAACCATAATGAATATCCTCCTTA |
| CobB-I-F   | AGCGTTCTGCATTGGCAG   |
| CobB-I-R   | GACGACCTGCAACACTAATC   |
| CobB-F     | CGTTGTGCTTACTGGTGCTG   |
| CobB-R     | ATGGCCTGAAGTGCCAATTG   |
| CobB-COM-F | CGCGCATGCGCCATCGCGGTGTATAGAG                                 |
| CobB-COM-R | ACCGTCGACAGTTATCTGAATGAGTTGAG                                |
| CobB-EXP-F | CGCGGATCCATGCGATTGCCATAGGCTGTG                               |
| CobB-EXP-R | ACCGTCGACTCAATCCCCCTGACGATTCTCAC                             |
| YfiQ-K-F   | ATGAGCCAACGTGGATTAGAACGACTGTTACGTCCGAAATGTGTAGGCTGGAGCTGCTTC |
| YfiQ-K-R   | TCAAAGTGTAGCTCCAGATTACGATCCCCTTCTATTCAATGAATATCCTCCTTA       |
| YfiQ-I-F   | AGTCGCCACCGCGCTGCTCC   |
| YfiQ-I-R   | ACAGTGCCTTCTCTTCTT   |
| YfiQ-F     | GCGGTGCTTGGCTTACGCC  |
| YfiQ-R     | GATCATCTTCTCAAGTAATG   |
| YfiQ-COM-F | CGCGGATCCCTGCGTCAACCTTATGTG                                  |
| YfiQ-COM-R | ACCGTCGACATCACGCTGATTGGTGTAAAC                               |
| YfiQ-EXP-F | CGCGGATCCATGAGCCAACGTGGATTAGAACGAC                           |
| YfiQ-EXP-R | ACCGTCGACTCAAAGTGTCAAGCTCCAGATTACG                           |
| Kana-I-R   | GCCGATTGTCTGTTGTGCC  |
| Kana-I-F   | GGCTGGGTGTGGCGGACCGC   |
| CobB-RT-F  | CATCAACGTTTCGCTCCC   |
| CobB-RT-R  | ACGGAAAGTACGGATAACCC   |
| YfiQ-RT-F  | TGCTGCGAGTACAGGATAACCC                                       |
| YfiQ-RT-R  | CTGACAATCAACAGCCGTTCA  |
| hmsS-RT-F  | ACGGAACAGCGTCTGCTAC  |
| hmsS-RT-R  | GATATAATGCAATCGTCGTC   |
| wzxE-RT-F  | CACTGTCTGGCTGAAGG  |
| wzxE-RT-R  | TTAATACATGCCACC  |
| katA-RT-F  | GTGCTAAATGTCCGTTCC   |
| katA-RT-R  | AAATCAGGCTGTTCTTGG   |
| yscL-RT-F  | AGAGGTGACTGCCGATGC   |
| yscL-RT-R  | TATTGCCAGAGAAAGTGC   |
| yscM-RT-F  | AAACTAACCCACTCACAGAG   |

|            |                        |
|------------|------------------------|
| yscM-RT-R  | TGGTAATAAGCAGGCAAT     |
| Pla-RT-F   | GGAGTGCAGGTAAATAGGT    |
| Pla-RT-R   | GTGAGCCGGATGTCTTCT     |
| adiA-RT-F  | GTCACAAGAACGCAACG      |
| adiA-RT-R  | ACAACGAAGAGTCGGAGA     |
| hmsT-RT-F  | CAGTATGCTATCATCGTCGC   |
| hmsT-RT-R  | GTAGACCGATGAGGATTG     |
| cspC3-RT-F | AGGCTTCGGTTTCATCAC     |
| cspC3-RT-R | GCAGACGGACCTTTAGCA     |
| caflR-RT-F | ATTCGCAACAGACATTCA     |
| caflR-RT-R | CCTTCTACCCAACAAACC     |
| ilvC-RT-F  | CAGCACTGGGTTACTCGC     |
| ilvC-RT-R  | CTTCGCCTTAGGGTCGT      |
| uspB-RT-F  | GTGACCCGTTGGTATC       |
| uspB-RT-R  | CTAAACACCATAACGCACT    |
| uspA2-RT-F | GCCAAACCGTACAATGCC     |
| uspA2-RT-R | ATCACCGCTGCCGCTTAA     |
| lytT-RT-F  | GGCGAAGACACTGACACG     |
| lytT-RT-R  | GCTGGGTATAACCTGATTGC   |
| htpG-RT-F  | CTCCTCCATTGATGATTG     |
| htpG-RT-R  | CAGGGTTAAAGTACGTTCT    |
| grpE-RT-F  | AGAGCAGCAAGTGGAAAGC    |
| grpE-RT-R  | TCAGTACGACGACGGATA     |
| ibpB-RT-F  | CCACTATCGCATCTCACTG    |
| ibpB-RT-R  | CAAACGGGCATTATCACAC    |
| phoR-RT-F  | GCCTGCATTATTACTGGG     |
| rcsB-RT-F  | GGGATAAGTATGGTGATGG    |
| rcsB-RT-R  | AGGTAAGTCAGCGGGTGCG    |
| phoR-RT-R  | CGGTGTCATACTGCGATC     |
| 16S-RT-F   | GCCACACTGGAACTGAGACACG |
| 16S-RT-R   | CGCTGAAAGTGCCTTACAACCC |

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30 **Supplementary Table 2. The CobB-dependent or YfiQ-dependent genes involved in stress**  
 31 **response and virulence as determined by RNA-seq and quantitative RT-PCR analyses.**

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| geneID    | Name         | Product   | log2 Ratio ( $\Delta cobB/WT$ ) |         | log2 Ratio ( $\Delta yfiQ/WT$ ) |         |
|-----------|--------------|---|---------------------------------|---------|---------------------------------|---------|
|           |              |   | qRT-PCR                         | RNA-seq | qRT-PCR                         | RNA-seq |
| YP_pCD21  | <i>yscM</i>  | putative type III secretion regulatory protein YscM   | -0.61                           | -0.36   | -1.16                           | -0.02   |
| YP_pCD22  | <i>yscL</i>  | type III secretion system protein                     | -0.43                           | -0.47   | -1.51                           | -0.79   |
| YP_3187   | <i>wzxE</i>  | lipopolysaccharide biosynthesis protein               | -0.53                           | -0.32   | -2.17                           | -0.80   |
| YP_3332   | <i>uspB</i>  | universal stress protein UspB                         | -0.75                           | -0.29   | -1.29                           | 0.15    |
| YP_3333   | <i>uspA2</i> | universal stress protein A                            | 0.02                            | 0.89    | -0.25                           | 1.58    |
| YP_0919   | <i>rcsB</i>  | transcriptional regulator RcsB                        | -0.69                           | -0.16   | -1.31                           | -0.42   |
| YP_pPCP08 | <i>pla</i>   | outer membrane protease                               | 1.34                            | 1.04    | 0.94                            | 1.43    |
| YP_0728   | <i>phoR</i>  | phosphate regulon sensor protein                      | -0.10                           | -0.20   | -1.65                           | -0.52   |
| YP_0397   | <i>lytT</i>  | two-component response-regulatory protein YehT        | 0.65                            | 1.00    | 0.54                            | 1.45    |
| YP_0931   | <i>katA</i>  | catalase  | -0.99                           | -0.38   | -1.03                           | 0.11    |
| YP_3158   | <i>ilvC</i>  | ketol-acid reductoisomerase                           | -3.11                           | -2.71   | -2.24                           | -1.13   |
| YP_3993   | <i>ibpB</i>  | heat shock chaperone IbpB                             | -1.10                           | -0.98   | -1.24                           | -0.34   |
| YP_0811   | <i>htpG</i>  | heat shock protein 90                                 | -1.53                           | -1.15   | -1.79                           | -0.84   |
| YP_1698   | <i>hmss</i>  | hemin storage system protein                          | -2.64                           | -2.14   | -3.28                           | -1.94   |
| YP_1049   | <i>grpE</i>  | heat shock protein GrpE                               | -0.64                           | -0.27   | -1.56                           | -0.23   |
| YP_0936   | <i>adiA</i>  | amino acid decarboxylase                              | -5.69                           | -4.00   | -5.53                           | -3.77   |
| YP_3756   | <i>hmst</i>  | HmsT protein  | 0.15                            | 0.76    | -0.70                           | 0.45    |
| YP_1786   | <i>cspC3</i> | cold shock protein                                    | -0.50                           | -0.05   | -2.94                           | -1.28   |
| YP_pMT085 | <i>cafIR</i> | putative F1 operon positive regulatory protein, cafIR | -0.27                           | 0.34    | -1.38                           | -0.17   |

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