Table S5. Role of metal acquisition systems in plague

| METAL ACQUISITION SYSTEMS |                  | MUTANT LACKING               |                                | DIFFERENTIAL                       | VIRULENCE DATA OBTAINED USING              |           |                                |                     | ,                  |
|---------------------------|------------------|------------------------------|--------------------------------|------------------------------------|--|-----------|--------------------------------|---------------------|--------------------|
|                           |                  |                              |                                |                                    | PER POOL MUTANTS <sup>†</sup>              |           | INDIVIDUAL MUTANT <sup>‡</sup> |                     | _                  |
|                           |                  | ORF(s)                       | GENE(s)                        | EXPRESSION<br>IN VIVO <sup>3</sup> | ARCI ± SE IN                               |           | SURVIVAL<br>(mutant vs WT)     |                     | ROLE IN VIRULENCE* |
|                           |                  |                              |                                |                                    | LYMPH NODE                                 | SPLEEN    | PERCENT                        | MEDIAN<br>(in days) |                    |
|                           | Siderophores     | YPO0989-YPO0994 <sup>¢</sup> | iucABCD iutA                   | +                                  | 20.2 ± 13                                  | 10.1 ± 10 | 0 vs 0                         | 4 vs 4              | No                 |
| Iron                      |                  | YPO1753                      | fcuA                           | +                                  | 31.2 ± 13                                  | 26.9 ± 13 | Not determined                 |                     | No                 |
|                           |                  | YPO1528-YPO1538              | ysuFJIHGDCBARED                | +                                  | 50.4 ± 15                                  | 47.4 ± 14 | Not determined                 |                     | No                 |
|                           |                  | YPO0955-YPO0956              | -                              | +                                  | 63.2 ± 14                                  | 53.7 ± 16 | Not det                        | ermined             | No                 |
|                           |                  | YPO1008-YPO1012 <sup>a</sup> | -ynp1 ynp2                     | +                                  | Not dete                                   | ermined   | 12.5 vs 12.5 5 vs 4            |                     | No                 |
|                           |                  | YPO3340 <sup>a</sup>         | -                              | +                                  | Not dete                                   | ermined   | 0 vs 0                         | 4 vs 4              | No                 |
|                           |                  | YPO1906-YPO1916              | fyuA ybtETU irp1 irp2 ybtAPQXS | +                                  | Not dete                                   | ermined   | Previously studied [10]        |                     | Yes                |
|                           |                  | YPO0770-YPO0777              | ynp3 to ynp11                  | $\leftrightarrow$                  | Not determined Not determin                |           | ermined                        | To be tested        |                    |
|                           | ABC transporters | YPO2958-YPO2960              | yfuABC                         | +                                  | 23.0 ± 13                                  | 17.9 ± 10 | Previously studied [11]        |                     | No                 |
|                           |                  | YPO4022-YPO4025              | fitABCD                        | +                                  | 34.4 ± 14                                  | 39.2 ± 14 | Not determined                 |                     | No                 |
|                           |                  | YPO1343-YPO1345              | fiuABC                         | +                                  | 35.6 $\pm$ 15 27.3 $\pm$ 14 Not determined |           | ermined                        | No                  |                    |
|                           |                  | YPO1310-YPO1313              | yiuABCR                        | +                                  | Not determined                             |           | 0 vs 12.5                      | 4 vs 4              | No                 |
|                           |                  | YPO2439-YPO2442              | yfeABCD                        | +                                  | Not determined                             |           | Previously studied [12]        |                     | Yes                |
|                           |                  | YPO3390-YPO3392              | fhuCDB                         | $\leftrightarrow$                  | Not determined                             |           | Not determined                 |                     | To be tested       |
|                           | Permeases        | YPO1854-YPO1856              | efeUOB                         | +                                  | Not determined                             |           | 12.5 vs 12.5                   | 4 vs 4.5            | No                 |
|                           |                  | YPO1941-YPO1948              | -                              | +                                  | Not determined                             |           | 0 vs 0                         | 4 vs 4              | No                 |
|                           | Heme uptake      | YPO0286-YPO0279              | -hmuRSTUV                      | +                                  | Not dete                                   | ermined   | 0 vs 0                         | 4.5 vs 5.5          | No                 |
|                           |                  | YPO1951-YPO1954              | hmsHFRS                        | +                                  | 57.4 ± 15                                  | 37.8 ± 14 | Previously s                   | studied [13]        | No                 |
|                           |                  | YPO3918-YPO3923              | hasRADEB                       | _                                  | Not dete                                   | ermined   | Previously s                   | studied [14]        | No                 |
|                           | Ferrous          | YPO0131-YPO133               | feoABC                         | $\leftrightarrow$                  | Not determined                             |           | Previously studied [15]        |                     | No                 |
| Manganese                 | ABC transporter  | YPO2439-YPO2442              | yfeABCD                        | +                                  | Not determined                             |           | Previously studied [12]        |                     | Yes                |
|                           | Permease         | YPO2982                      | mntH                           | +                                  | Not determined                             |           | 0 vs 0                         | 4 vs 4              | No <sup>*</sup>    |
| Zinc                      | ABC transporter  | YPO2439-YPO2442              | yfeABCD                        | +                                  | Not determined                             |           | Previously studied [12]        |                     | Yes                |
|                           |                  | YPO2059-YPO2061              | znuBC znuA                     | +                                  | Not dete                                   | ermined   | 25 vs 0                        | 6.5 vs 6.5          | No                 |

<sup>\*,</sup> according to our previous data [16]

<sup>&</sup>lt;sup>†</sup>, ARCIs were determined in groups of 10 rats inoculated with a pool of 5 mutants (with 20 CFU of each mutant)

<sup>&</sup>lt;sup>‡</sup>, virulence was determined after intradermal inoculation of ~10 CFU. Groups of 8 animals were used

Y, confirms recently published work

<sup>&</sup>lt;sup>Ф</sup> pseudogene

<sup>\*,</sup> a gene was considered to be necessary ("Yes") or not necessary ("No") for virulence if the survival curve for animals infected with the mutant and the wild-type strain were significantly different (p<0.05) or not significantly different (p>0.05) in a Gehan-Breslow-Wilcoxon test, or because the mutant was outcompeted (ARCI<20) or not outcompeted (ARC≥20) in the per-pool screening, or because it has been previously studied elsewhere. In the latter case, a reference (Text S1) is provided.