

**Table S1** Impact of various *nif*-gene inactivations on nitrogenase activity in different engineered strains. Relative activities with respect to that for the respective engineered strain with wild-type *nif* cluster.

| Genotype            | <i>E. coli</i> <sup>a</sup> | <i>E. coli</i> <sup>b</sup> | <i>Synechocystis</i><br>6803 <sup>c</sup> |
|---------------------|-----------------------------|-----------------------------|---|
| wild-type cluster   | 100%                        | 100%                        | 100%                                      |
| $\Delta nifT$       | -                           | -                           | 4%  |
| $\Delta nifV$       | 1%                          | 25%                         | -   |
| $\Delta nifB$       | 0%                          | 0%                          | -   |
| $\Delta nifS$       | 5%                          | -                           | -   |
| $\Delta nifU$       | 10%                         | -                           | -   |
| $\Delta nifX$       | -                           | 50%                         | <1%                                       |
| $\Delta nifW$       | -                           | -                           | 7%  |
| $\Delta hesA$       | -                           | 50%                         | -   |
| $\Delta hesB$       | -                           | -                           | 9%  |
| $\Delta nifQ$       | 90%                         | -                           | -   |
| $\Delta nifF$       | 50%                         | -                           | -   |
| $\Delta nifJ$       | 30%                         | -                           | -   |
| $\Delta nifTZ$      | -                           | -                           | <1%                                       |
| $\Delta hesAB$      | -                           | -                           | 6%  |
| $\Delta nifXhesA$   | -                           | 35%                         | -   |
| $\Delta nifVhesA$   | -                           | 25%                         | -   |
| $\Delta nifVXhesA$  | -                           | 7%                          | -   |
| $\Delta nifVXNhesA$ | -                           | 2%                          | -   |
| $\Delta nifENX$     | 110%                        | -                           | -   |
| $\Delta nifUSV$     | 0%                          | -                           | -   |
| $\Delta nifWZM$     | 100%                        | -                           | -   |

<sup>a</sup>Nitrogen fixation genes from *A. vinelandii* and *K. oxytoca*. Data from (1).

<sup>b</sup>Nitrogen fixation genes from *Paenibacillus*. Data from (2).

<sup>c</sup>Activities shown here are based on the strain TSyNif-2 with 100% activity (this study)