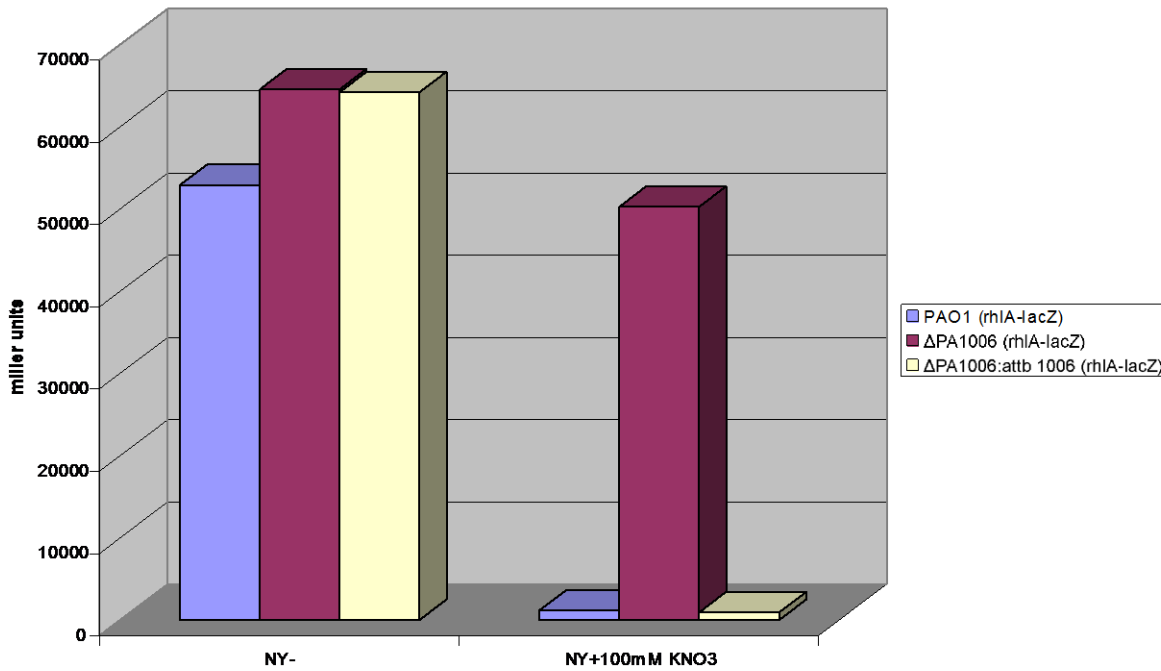
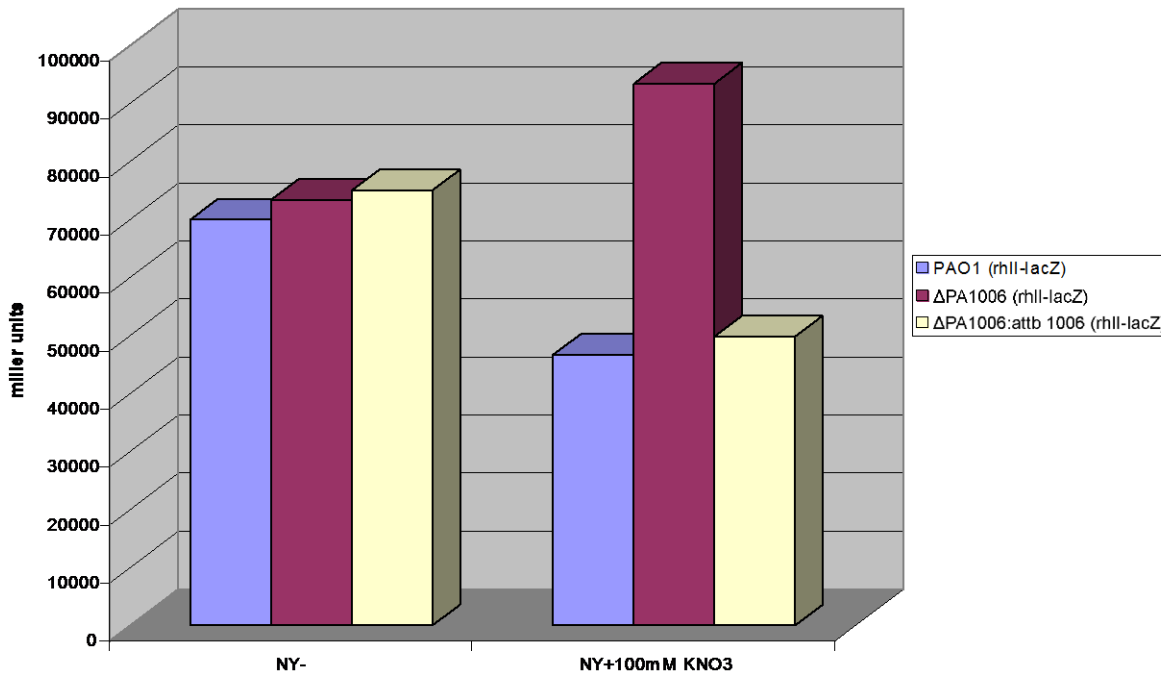


**Supplemental File 3: Confirmation of altered *rhIA*, *rhII*, and *rhIR* gene expression in the  $\Delta PA1006$  mutant to WT PAO1 when grown in the presence of nitrate.  $\beta$ -galactosidase *rhI*-promoter fusion reporter constructs were used to determine expression levels.**

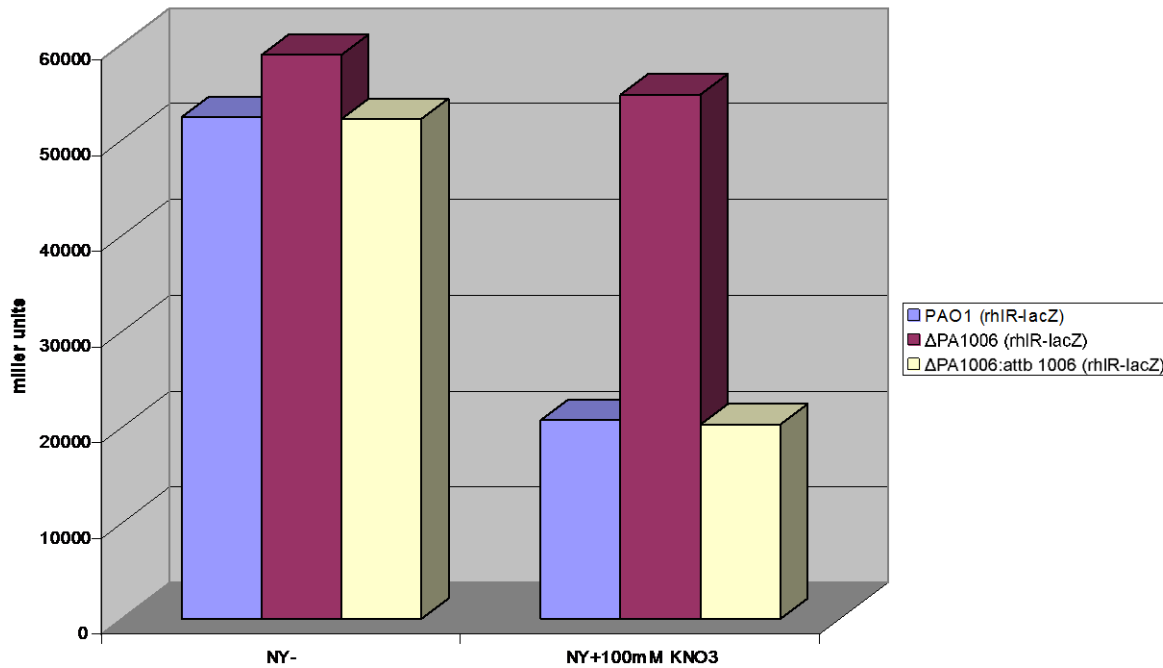
**B-gal activity of *rhIA-lacZ* fusion**



**B-gal activity of *rhII-lacZ* fusion**



### B-gal activity of rhIR-lacZ fusion



**Supplemental method for transcriptional fusion assays.**  $\beta$ -galactosidase activity was determined at 30°C as described previously [90]. Plasmids containing the *rhlA'*-*lacZ* [48], *rhlI'*-*lacZ* (Van Delden C), and *rhlR'*-*lacZ*, [48] were introduced into *Pae*WT strain,  $\Delta$ *nbvF* mutant, and complemented  $\Delta$ *nbvF* mutant by electroporation and transformants were selected by growth on PTSB with 200  $\mu$ g/ml carbenicillin (PTSB<sup>carb</sup>). Overnight cultures (16 h) were grown in PTSB<sup>carb</sup> at 37°C and then diluted to OD<sub>600</sub> of 0.05 in PTSB<sup>carb</sup>. Cells were collected at OD<sub>600</sub> ~1.2 and assayed  $\beta$ -galactosidase activity as described [90]. Data presented are representative of at least two independent experiments.