SUPPLEMENTAL MATERIAL

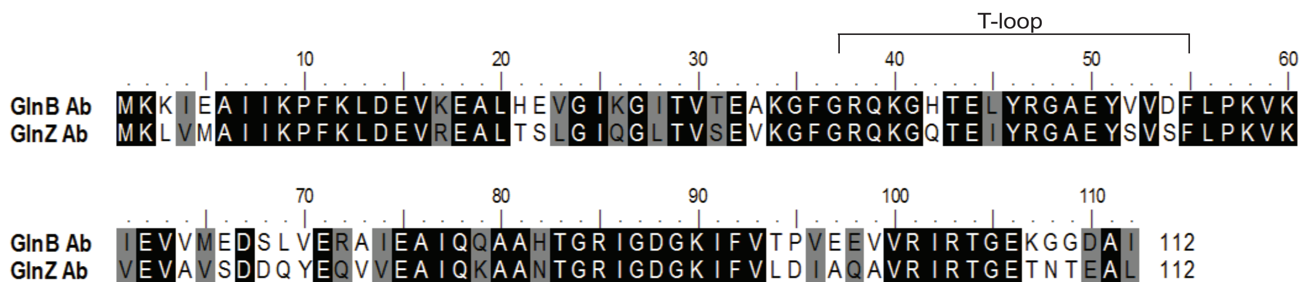

FIGURE S1. Alignment of P_{II} amino acids sequence for *A. brasilense* GlnB and GlnZ. The position of the T-loop is indicated above the alignment, the identical and similar residues are identified by black and grey shading, respectively.

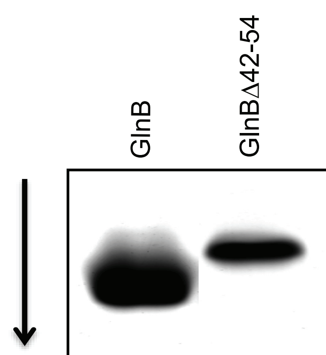

FIGURE S2. Migration profile of GlnB and GlnBΔ42-54 variant on native-PAGE (7.5%). The gel was stained with Coomassie blue. The arrow indicate the migration direction from the negative to the positive pole. The slightly reduced migration of the GlnBΔ42-54 in comparison to the wild-type GlnB is a result of increased net positive charge as expected based on the GlnB primary sequence analysis.

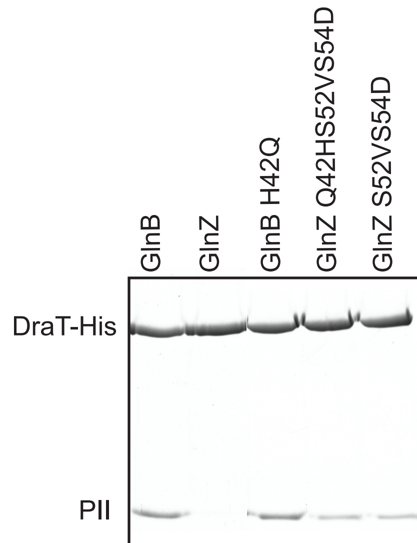

FIGURE S3. *In vitro* interaction between His-tagged DraT and various P_{II} mutant proteins. Complex formation was assessed by co-precipitation using Ni^{2+} beads. Reactions were performed in the presence of 1 mM ADP. Binding reactions were conducted in 500 μl of buffer adding His-tagged DraT 5 μg and the indicated purified P_{II} protein (10 μg). The eluted fractions was subjected to SDS-PAGE and the gels were Coomassie blue stained.

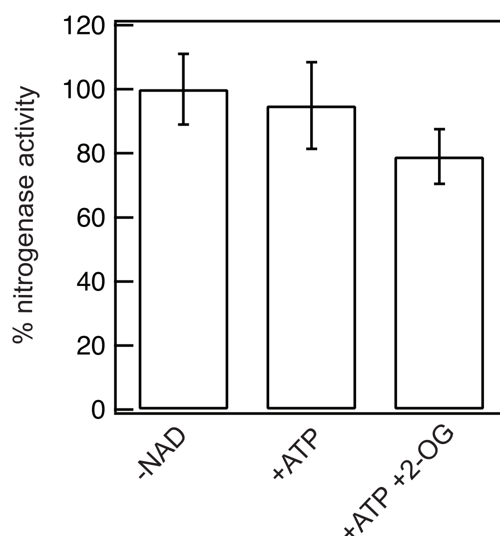

FIGURE S4. Effect of ATP and 2-oxoglutarate without ADP on the activity of the DraT-GlnB complex. DraT activity was assessed indirectly by Fe protein activity measuring the hydrogen evolution. Reactions for DraT assay contained 4 mM $MgCl_2$, 2 mM ATP, 2 mM 2-OG, co-purified DraT-GlnB complex, 100 μg *A. vinelandii* Fe protein and the effectors as indicated. For the control, the reaction was performed in the presence of co-purified DraT-GlnB complex, 2 mM ATP or ADP in the absence of NAD^+. The DraT activity was stopped by reducing the NAD^+ using dithionite solution. The Fe protein activity was determined adding 500 μg of MoFe protein and the hydrogen production was measured.

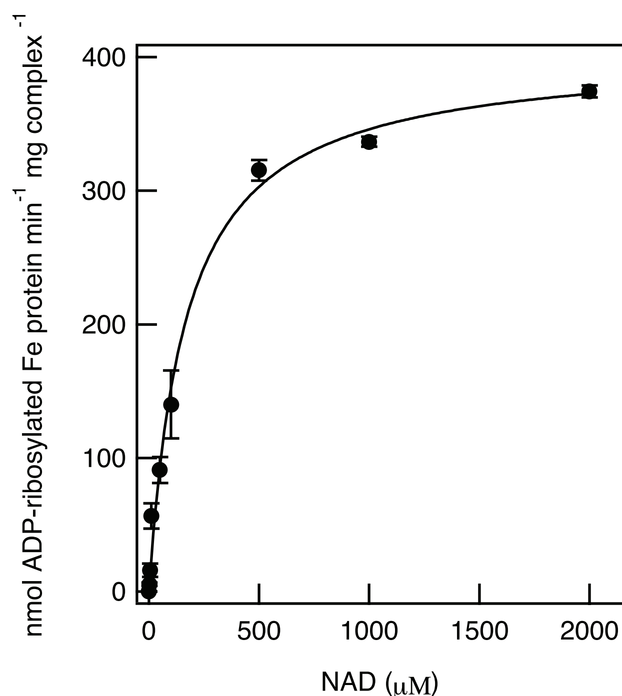

FIGURE S5. Determination of the K_m for NAD^+ using the DraT-GlnB complex. ADP-ribosylation of *Azotobacter vinelandii* Fe protein. Complex reactions contained different NAD^+ concentrations, 1 mM $MgCl_2$, DraT-GlnB complex, 1 mM ADP and 100 μg Fe protein were incubated 5 min, 130 rpm at 30°C. The reactions were performed using Fe protein:DraT-GlnB complex molar ratio of 125:1. For the control, the reaction was performed in the presence of either co-purified complex or DraT in the absence of NAD^+. The DraT activity was stopped reducing the NAD^+ using dithionite solution. The Fe protein activity was determined adding 500 μg of MoFe protein and hydrogen production was measured. The K_m (166 ± 24 μM) and V_{max} (403 ± 15 nmol ADP-ribosylated Fe protein min^{-1} mg $complex^{-1}$) values were determined using Igor Pro program).

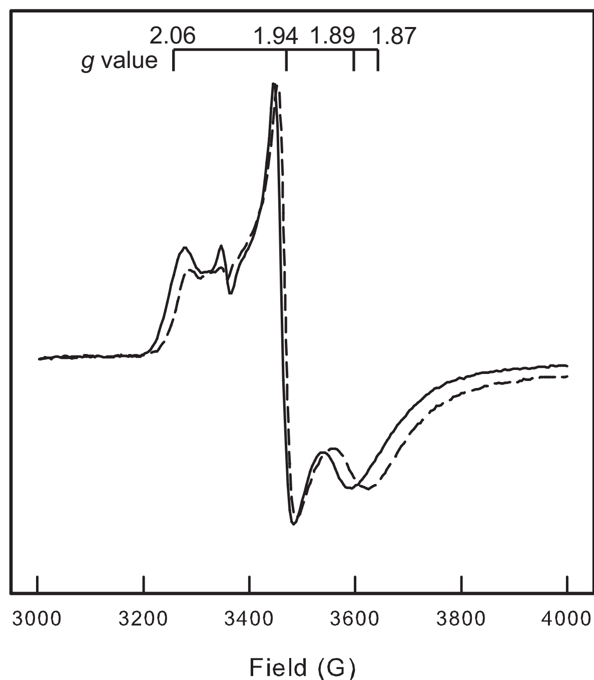

FIGURE S6. EPR Spectra of *Azotobacter vinelandii* wild type Fe protein and ADP-ribosylated Fe protein. Unmodified and ADP-ribosylated Fe protein (dashed line) samples were maintained in the reduced state by the presence of 2 mM dithionite. Spectra were recorded at 12 K, 9.50 GHz, and 1 mW microwave power. The *g* value for the each peak is shown.