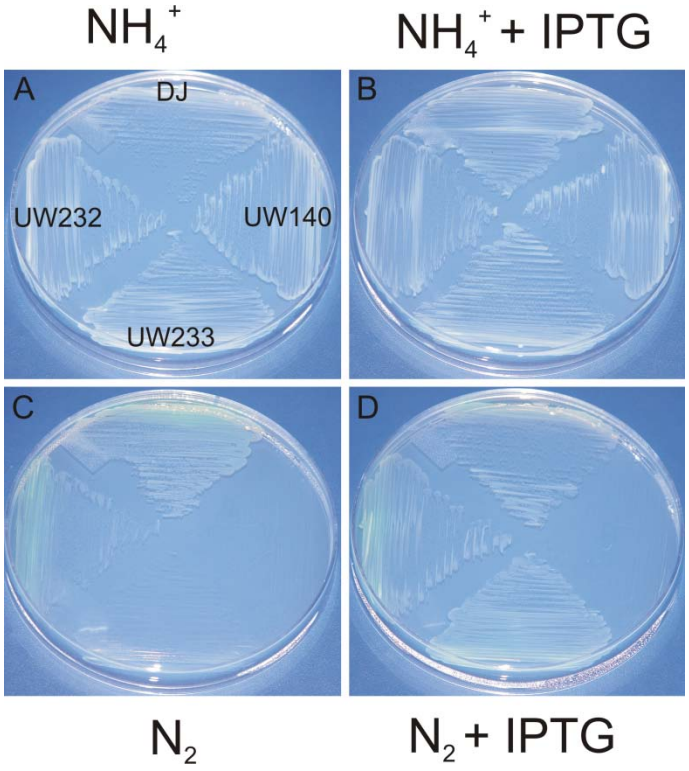


**Fig. S1.** Artificially-controlled expression of *A. vinelandii* NifB. *A. vinelandii* strains DJ (*wt*), UW140 (Δ*nifB*), UW232 (Δ*nifB, nifH*$_P$*::his6::nifB),* UW233 (Δ*nifB,* IPTG-controlled *tac*$_P$*::gst::nifB*) were cultured in Burk's modified medium containing ammonium (A and B) or lacking ammonium (C and D). To induce GST-NifB protein expression, the medium was supplemented with 1 mM IPTG (B and D). UW140 and UW232 strains have been described in (Curatti *et al*., 2006. *Proc Natl Acad Sci USA* 103: 5297-301).

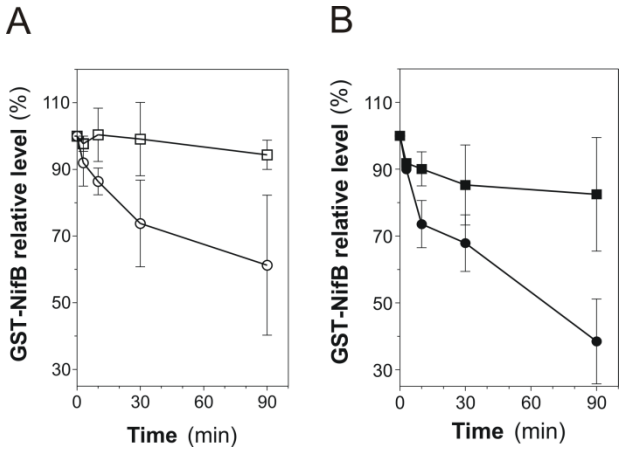


**Fig. S2.** *In vitro* degradation of *A. vinelandii* GST-NifB. UW233 cells were loaded with GST-NifB *in vivo* by culturing in the presence of ammonium and 10 mM IPTG for 2 h. UW233 cell-free extracts were desalted through PD10 columns (GE Healthcare) and mixed with desalted cell-free extracts obtained from strain UW140 (Δ*nifB*) cultured in the presence (squares) or in the absence (circles) of ammonium. The reaction mixtures were incubated at 30°C and samples were withdrawn at different time points for immunoblot analysis developed with antibodies to NifB. Plots were generated by densitometric quantitation of GST-NifB levels using the ImageJ software. A, no supplements were added to the reaction mix. B, ATP and an ATP regenerating system (1.25 mM ATP, 18 mM phosphocreatine and 40 μg.ml$^{-1}$ creatine phosphokinase) were added. Results shown are the mean and standard deviation from two independent experiments.

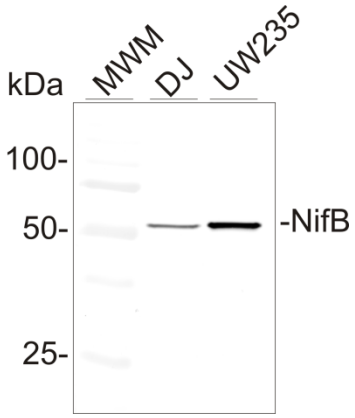


**Fig. S3.** NifB accumulation in an *A. vinelandii* mutant strain lacking *nifENX*. Immunoblot analysis of NifB accumulation in *A. vinelandii* strains DJ (*wt*) and UW235 (Δ*nifENX::kn*) 4 h after ammonium removal from the medium. Equal amounts of cells ($OD_{600}$) from both strains were collected, boiled in SDS sample buffer, and loaded in each lane of the SDS-PAGE.

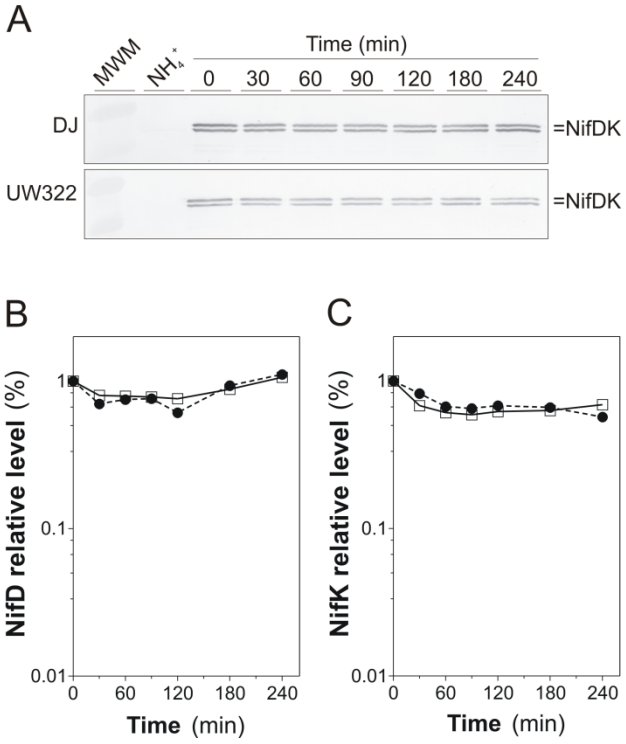


**Fig. S4.** Time-course of NifD and NifK polypeptides degradation in the wild-type and the Δ*clpX2* mutant strains. A, Time-course analysis of NifDK protein accumulation in cell-free extracts from wild-type (DJ) and Δ*clpX2* (UW322) strains derepressed for nitrogenase and treated with spectinomycin (10 μg.ml$^{-1}$) to stop protein synthesis. Cell-free extracts obtained from cultures grown with ammonium were used as controls. Immunoblots were developed with antibodies to NifDK. Representative immunoblots are shown. Relative levels of NifD (B) and NifK (C) polypeptides in the wild-type (closed circles) and the Δ*clpX2* (open squares) strains estimated by densitometric quantification of immunoblots as shown in A.