Supporting Information for:

Nitric oxide-responsive inter-domain regulation targets the σ^{54} - interaction surface in the enhancer binding protein NorR

By **Matthew Bush, Tamaswati Ghosh, Nicholas Tucker, Xiaodong Zhang and Ray Dixon**

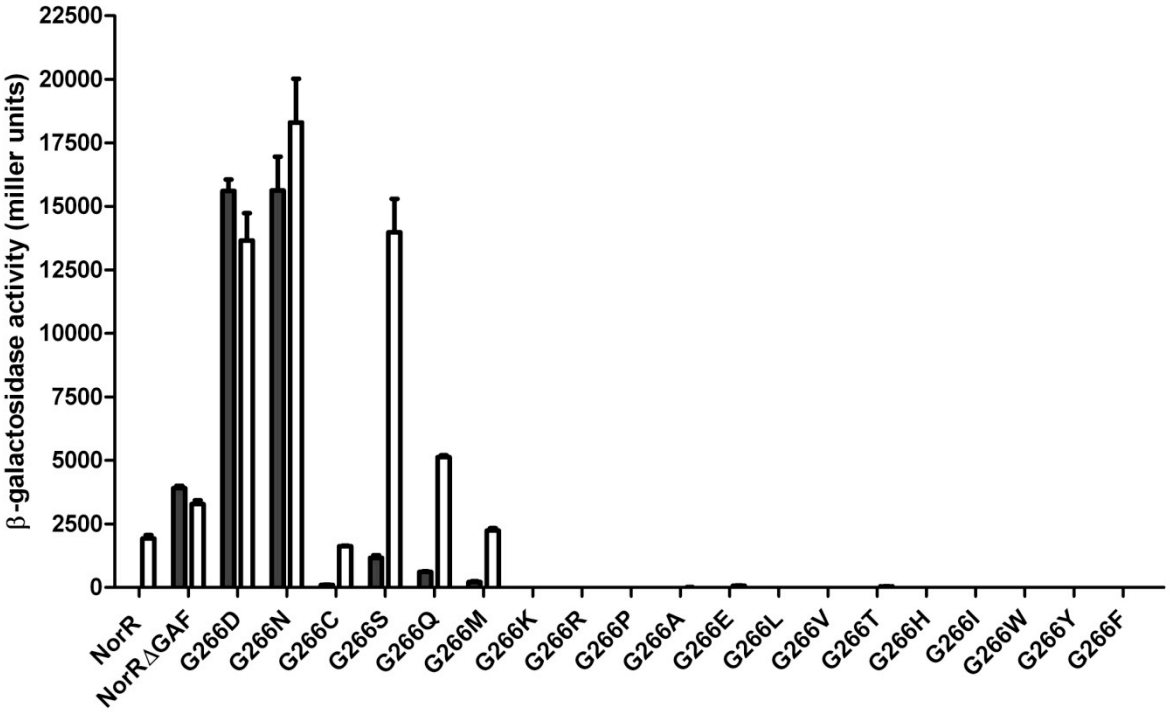

Figure S1. Activities of G266 variants *in vivo* as determined by the *norV-lacZ* reporter assay "NorR" represents the wild-type protein and "NorRΔGAF", the N-terminally truncated (Δ1-170) protein. Cultures were grown either in the absence (black bars) or presence (white bars) of 4 mM potassium nitrite, which induces endogenous NO production. Error-bars show the standard error of the three replicates carried out for each condition.

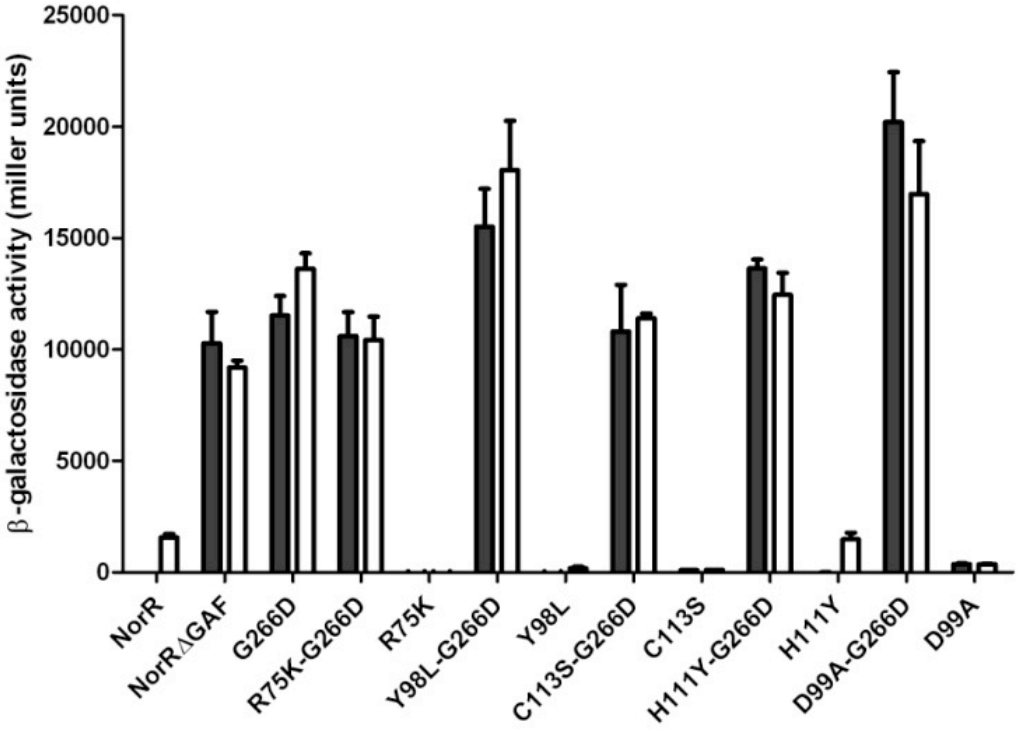

Figure S2. Activities of G266 variants *in vivo* when additional amino acid changes are made in the GAF domain to substitute residues that are predicted to coordinate the iron. "NorR" represents the wild-type protein and "NorRΔGAF", the N-terminally truncated (Δ1-170) protein. Cultures were grown either in the absence (black bars) or presence (white bars) of 4 mM potassium nitrite, which induces endogenous NO production. Error-bars show the standard error of the three replicates carried out for each condition.

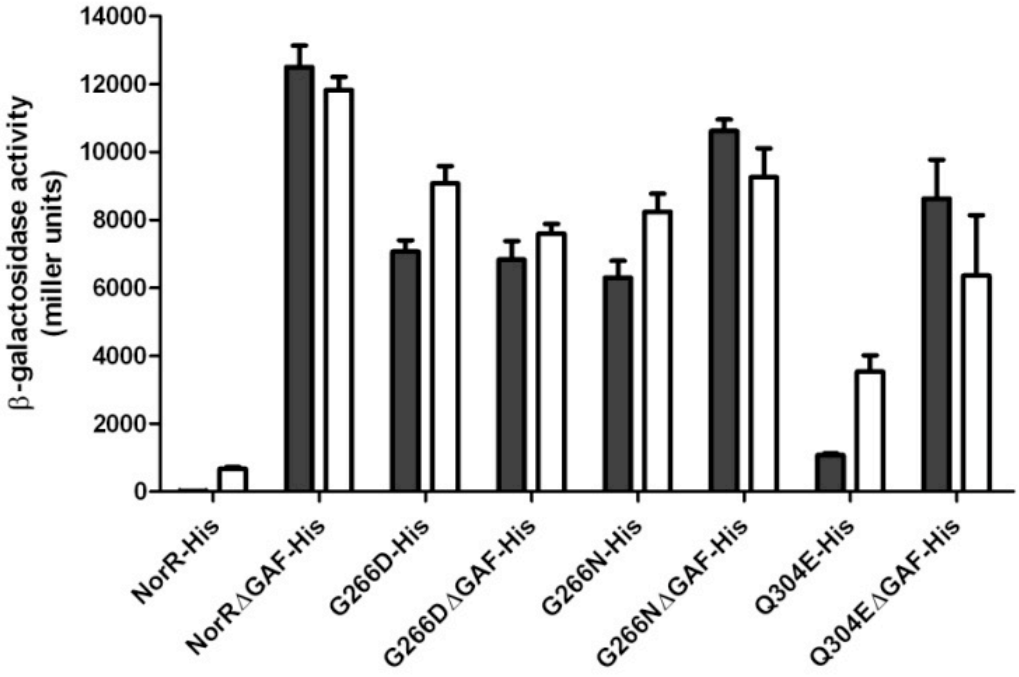

Figure S3. *In vivo* activities of the NorR-His, G266D-His, G266N-His and Q304E-His proteins in their full length and N-terminal GAF-truncated forms (Δ1-170) as determined by the *norV-lacZ* reporter assay. Cultures were grown either in the absence (black bars) or presence (white bars) of 4 mM potassium nitrite, which induces endogenous NO production. Error-bars show the standard error of the three replicates carried out for each condition.

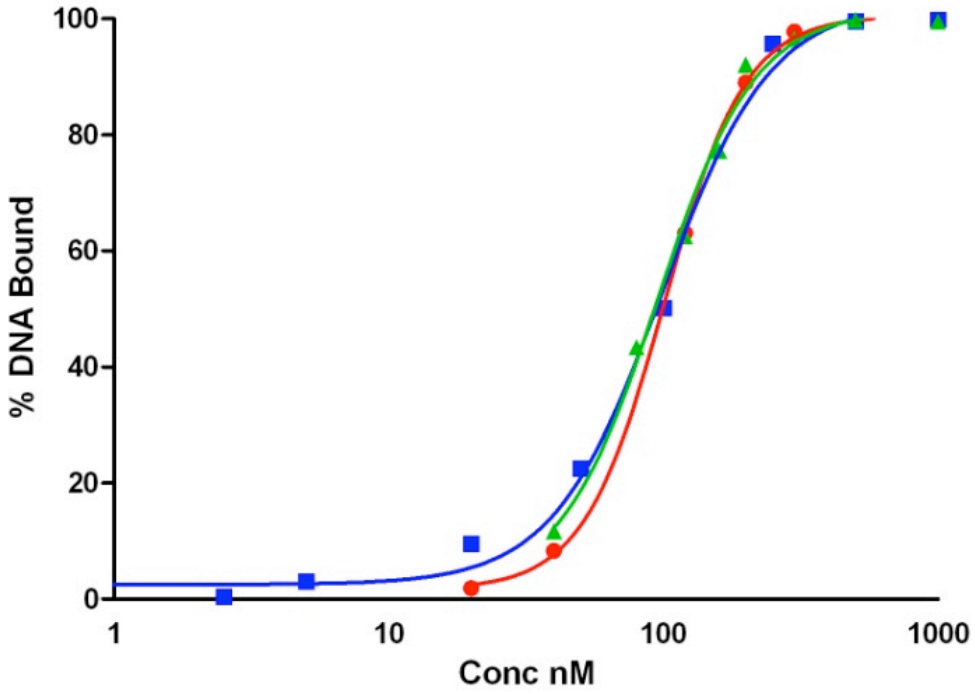

Figure S4. Enhancer binding activity of the G266DΔGAF-His (blue squares) and G266NΔGAF-His (green triangles) variants compared to NorRΔGAF-His (red circles) as determined by gel retardation assays. The percentage of fully shifted DNA was quantified using a Fujix BAS 1000 phosphoimager**.** The G266 substitutions do not significantly affect the affinity of NorR for the 361bp fragment of the *norR-norVW* intergenic region that contains the 3 NorR binding sites.

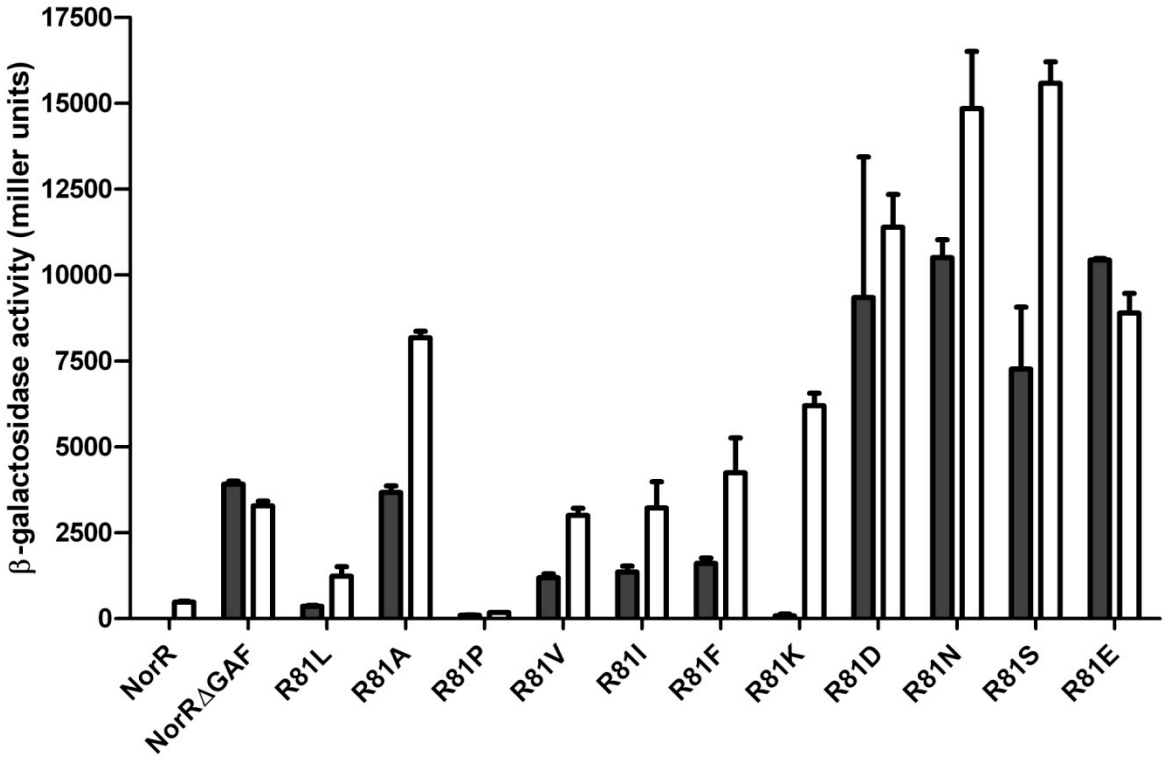

Figure S5. Activities of R81 variants *in vivo* as determined by the *norV-lacZ* reporter assay "NorR" represents the wild-type protein and "NorRΔGAF", the N-terminally truncated (Δ1-170) protein. Cultures were grown either in the absence (black bars) or presence (white bars) of 4 mM potassium nitrite, which induces endogenous NO production. Error-bars show the standard error of the three replicates carried out for each condition.

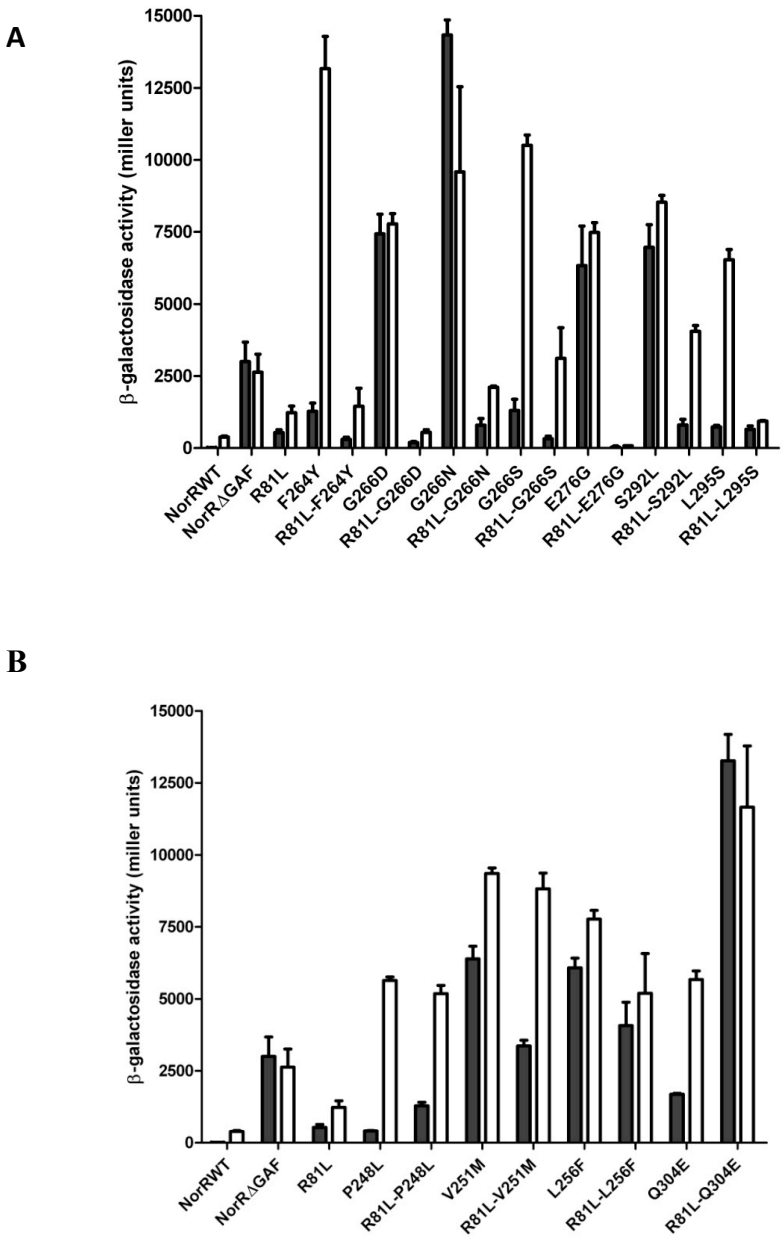

Figure S6. Influence of the R81 substitution on the activity of AAA+ domain variants **(A)** NorR AAA+ variants that are effectively suppressed by the R81L substitution in the GAF domain. **(B)** NorR AAA+ variants that are not significantly suppressed by the R81L substitution in the GAF domain. "NorR" is the wild-type protein. "NorRΔGAF" is the N-terminally truncated (Δ1-170) protein. Activities *in vivo* (as determined by the *norV-lacZ* reporter assay) were measured either in the absence (black bars) or presence (white bars) of 4 mM potassium nitrite, which induces endogenous NO production. Error-bars show the standard error of the three replicates carried out for each condition.

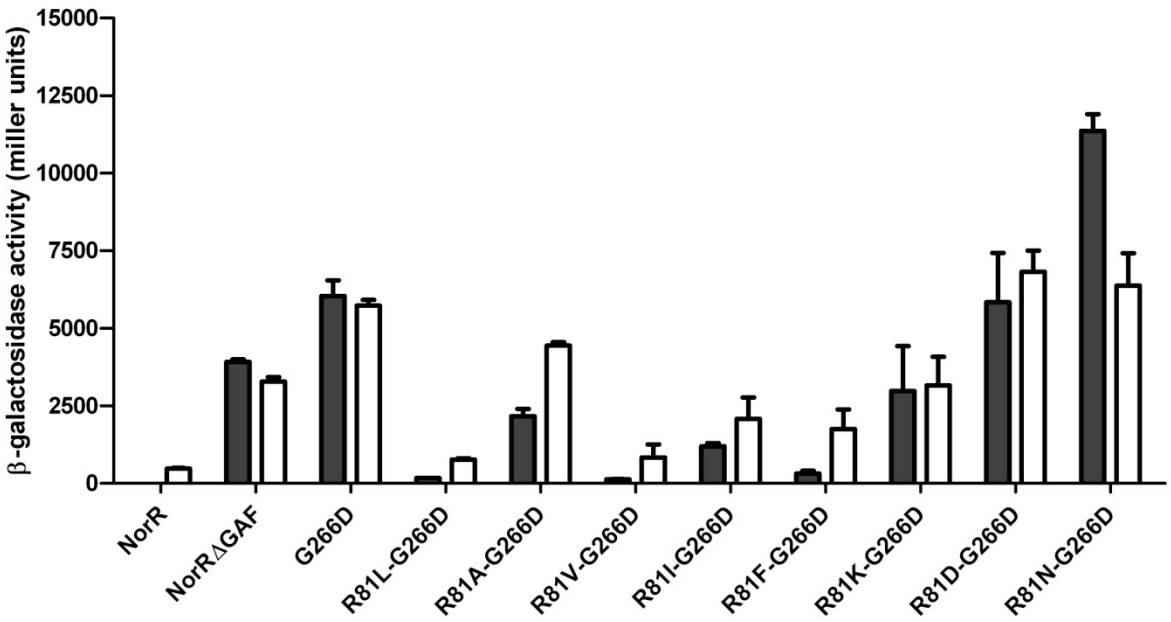

Figure S7. Suppression of the constitutive phenotype of the NorRG266D variant by hydrophobic substitutions made at the R81 residue in the GAF domain. "NorR" is the wild-type protein. "NorRΔGAF" is the N-terminally truncated (Δ1-170) protein. Activities *in vivo* (as determined by the *norV-lacZ* reporter assay) were measured either in the absence (black bars) or presence (white bars) of 4 mM potassium nitrite, which induces endogenous NO production. Error-bars show the standard error of the three replicates carried out for each condition.

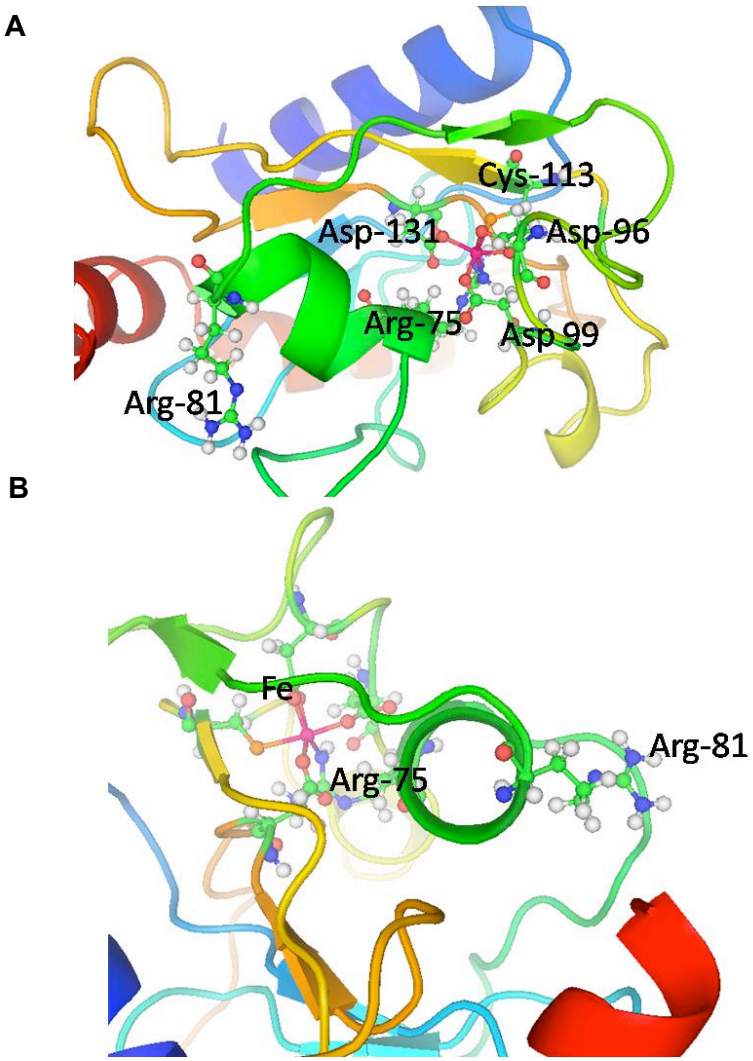

Figure S8. The R81-mediated mechanism of inter-domain repression (**A)** Structural model of the GAF domain of NorR based on the GAF-B domain of 3',5'-cyclic nucleotide phosphodiesterase (Tucker et al., *J Biol Chem* 283:908-918, 2008) showing the iron centre and proposed ligands, relative to the R81 residue. (**B**) The model predicts that the residue is surface exposed and may therefore be well positioned for AAA+ contact.