Supplementary Data

Incorporation of tyrosine and glutamine residues into the soluble guanylate cyclase heme distal pocket alters NO and O_2 binding

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Fig. S1. Effect of GTP and YC-1 on the temperature-dependent equilibrium between 5- and 6-coordinate Fe^{II}-NO complexes in sGC α1β1 I145Y and α1β1 F74Y. Spectra (500 - 650 nm) are shown for 5 (black solid line), 30 (black dashed line) and 45 ºC (gray solid line) for α1β1 I145Y and for 5 (black solid line), 20 (black dashed line) and 30 ºC (gray solid line) for α1β1 F74Y.

Fig. S2. Activity of sGC β1 distal pocket mutants in the absence and presence of NO (100 μM DEA/NO) and YC-1 (150 μM) at 37 ºC. The bars, from left to right, refer to wild-type α1β1, α1β1 V5Y, α1β1 F74Y, α1β1 I145Y and α1β1 I149Y. NO-stimulated activity and YC-1 synergism is reduced in all of the mutants.

Fig. S3. Characterization of sGC α1β1 L9W/I145Y. (**A**) Electronic absorption spectra of Fe^{II}-unligated (black solid line), Fe^{II}-CO (black dashed line) and Fe^{II}-NO (gray solid line) complexes at 20 ºC. (**B**) Activity of wild-type α1β1 (black bars) and α1β1 L9W/I145Y (white bars) in the presence and absence of NO, CO, and YC-1 at 37 ºC.

Fig. S4. Activity of wild-type α1β1 (black bars) and α1β1 I145Y/I149Q (white bars). Reduced protein was assayed in the unligated state and after exposure to NO, CO and O_2 at 25 ºC. α1β1 I145Y/I149Q was monitored by electronic absorption spectroscopy to ensure the Soret absorbance maximum shifted after addition of each ligand. The Fe^{II} protein was oxidized with potassium ferricyanide (1 mM) and then the enzyme was assayed in the presence and absence of sodium cyanide (10 mM) at 25 ºC.

Table S1. Activity of various α1β1 sGC mutants at 37 ºC[a]

sGC	Ligand	Specific Activity (nmol cGMP min^{-1} mg^{-1}) - YC-1	Specific Activity (nmol cGMP min^{-1} mg^{-1}) + YC-1
wt	-	185 ± 0	985 ± 125
	NO	13282 ± 477	26655 ± 5344
	CO	199 ± 19	6976 ± 899
β1 I5Y	-	33 ± 12	161 ± 65
	NO	1588 ± 588	5162 ± 2845
	CO	43 ± 0.3	423 ± 90
β1 F74Y	-	433 ± 3	1070 ± 157
	NO	5004 ± 137	5079 ± 1142
	CO	143 ± 52	1253 ± 398
β1 I145Y	-	94 ± 11	181 ± 67
	NO	1197 ± 68	3216 ± 448
	CO	105 ± 5	142 ± 24
β1 I149Y	-	139 ± 0	245 ± 42
	NO	416 ± 23	1829 ± 351
	CO	250 ± 82	185 ± 61

[a]Values for sGC determined in duplicate. The concentration of YC-1 was 150 μM.

Figure S1

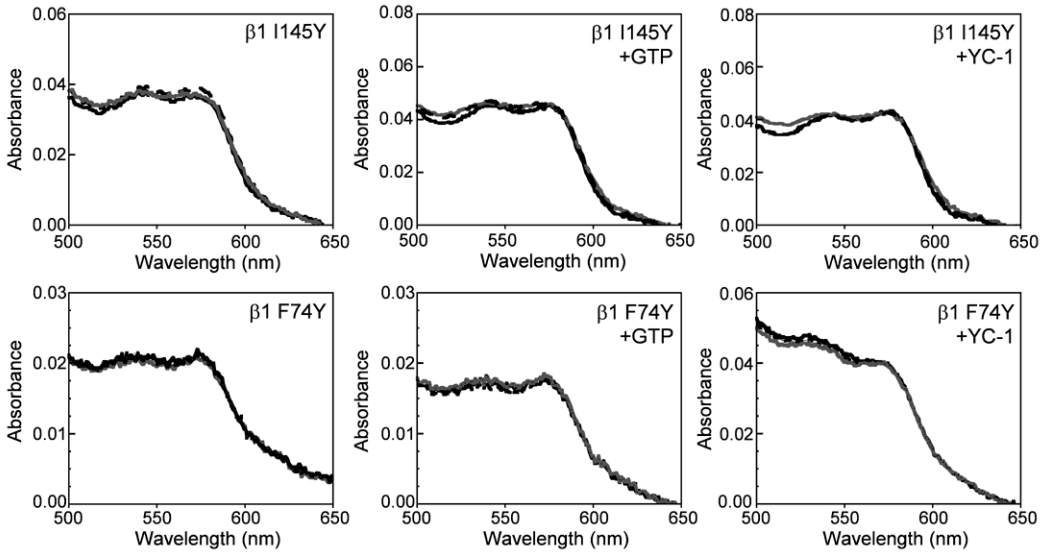

β1 I145Y
β1 I145Y +GTP
β1 I145Y +YC-1
β1 F74Y
β1 F74Y +GTP
β1 F74Y +YC-1
Absorbance
Wavelength (nm)

Figure S2

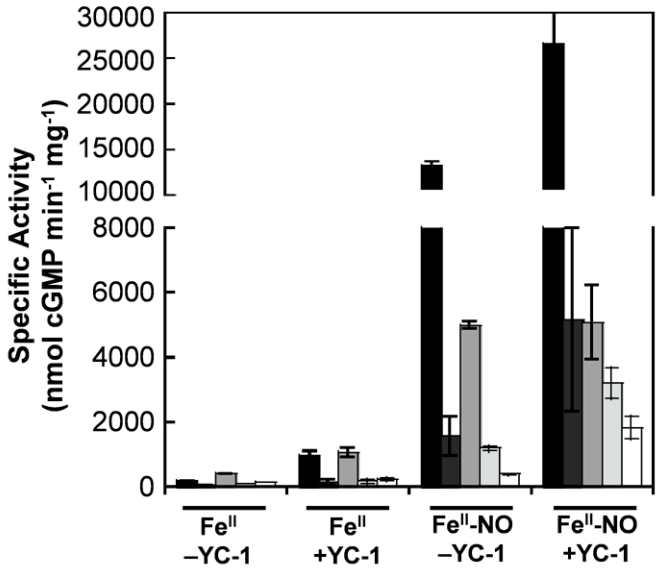

Specific Activity
(nmol cGMP min-1 mg-1)
30000
25000
20000
15000
10000
8000
6000
4000
2000
0
FeII
–YC-1
FeII
+YC-1
FeII-NO
–YC-1
FeII-NO
+YC-1

Figure S3

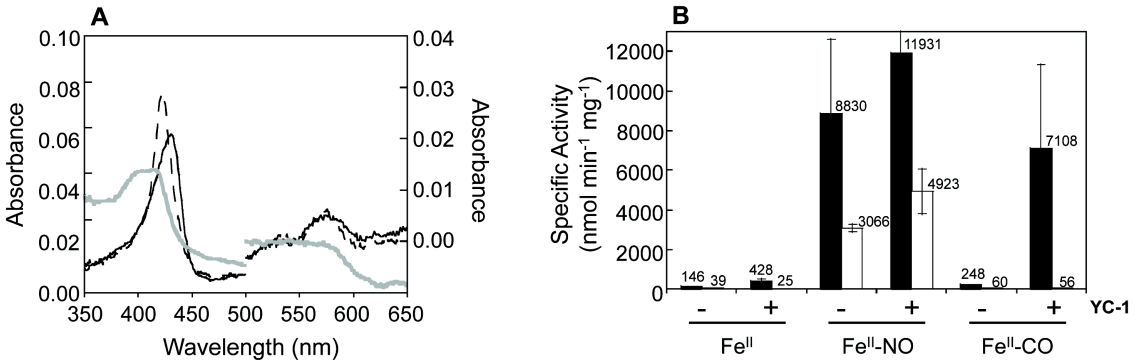
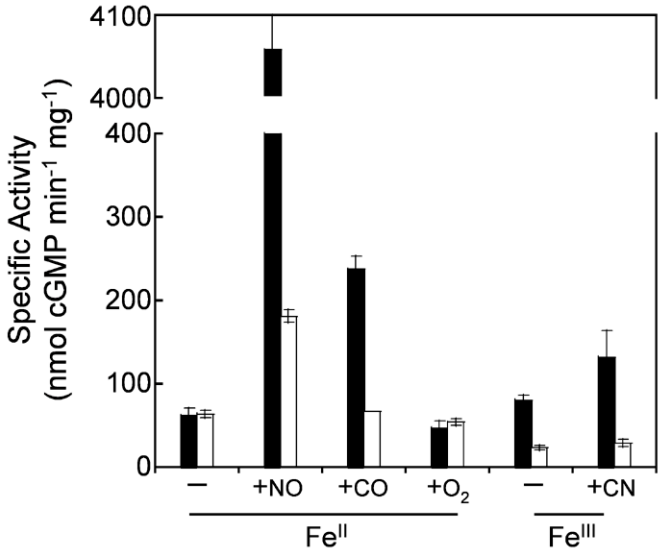

A
Absorbance
Absorbance
Wavelength (nm)
0.10
0.08
0.06
0.04
0.02
0.00
0.04
0.03
0.02
0.01
0.00
350 400 450 500 550 600 650
B
Specific Activity
(nmol min-1 mg-1)
12000
10000
8000
6000
4000
2000
0
146 39
428 25
8830
3066
11931
4923
248 60
7108
56
- + - + - +
YC-1
FeII
FeII-NO
FeII-CO

Figure S4


4100
4000
400
300
200
100
0
Specific Activity
(nmol cGMP min-1 mg-1)
—
+NO
+CO
+O2
—
+CN
FeII
FeIII