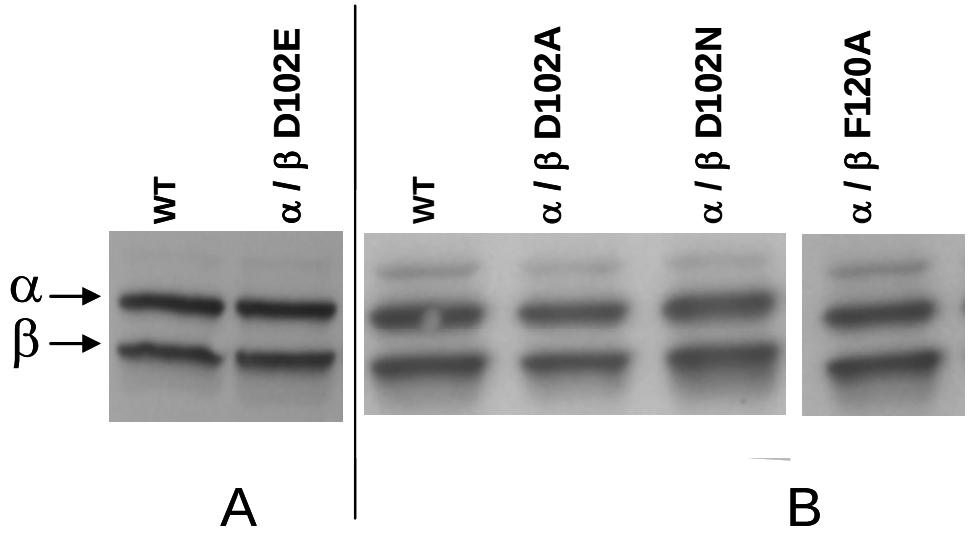
Fig.1 Supplemental data:



Western blot analysis with anti-sGC antibodies of WT and HNOX mutants transiently expressed in COS-7 cells.
10μg of cytosolic fraction were electrophorated on 7.5% SDS-gel. A and B are 2 separate transfections and are representative to 3 transfections.

Fig. 2 Supplemental data: Elution profiles of the purification of WT and mutants. Absorbance at 280nm (red), 431nm (green) and 393 nm (blue indicated for WT and D012N, same scale than OD431) was monitored by the UV900 detector of the GE AKTA FPLC.. Black line indicates the salt gradient.

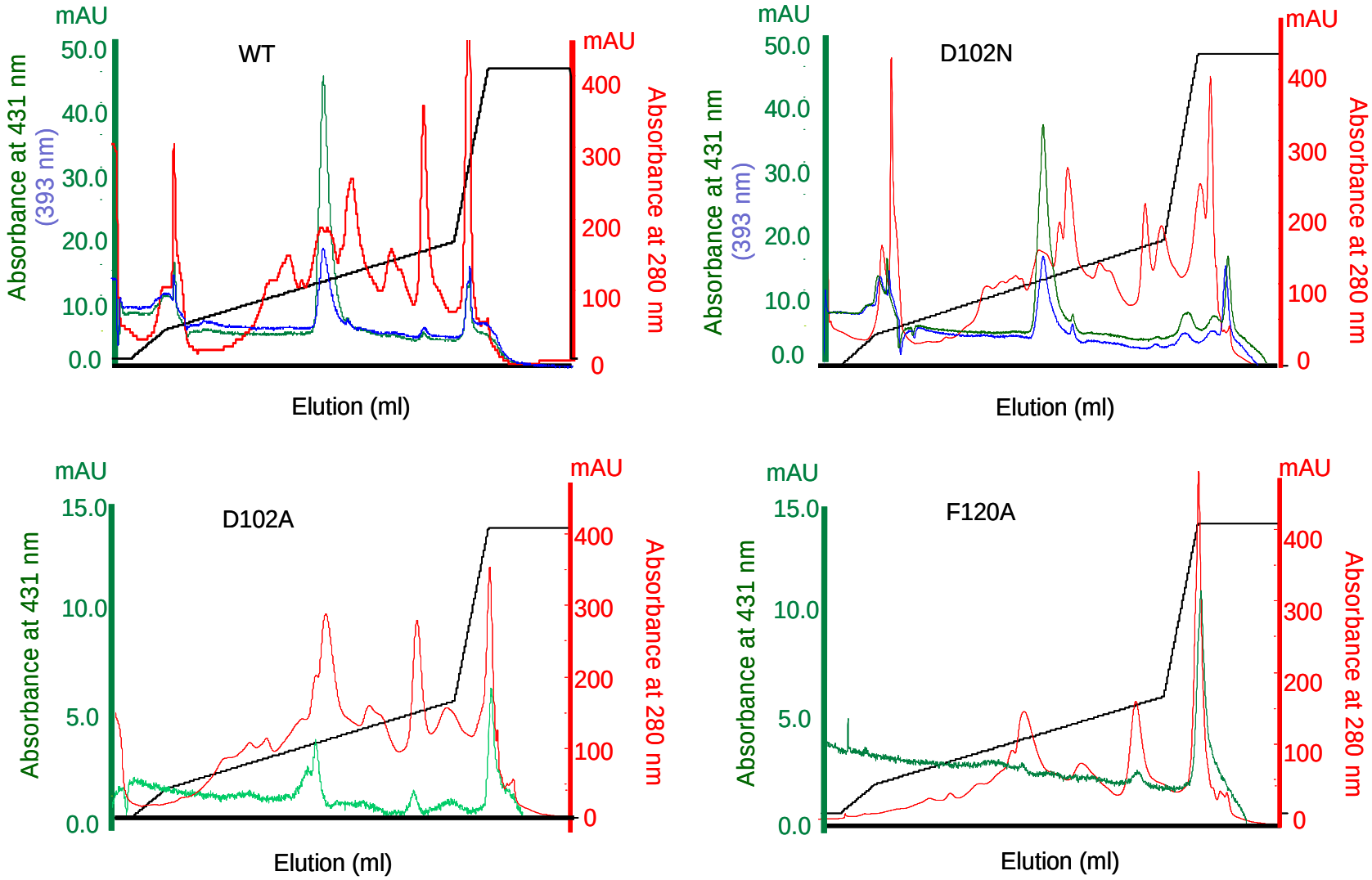


Table 2 : Characterization of sGC and mutants purification. The protocol of purification is described in *Experimental Methods*. The purification level was calculated as a function of the protein total concentration (280nm). All the mutants and WT were purified at least twice. The ratio 280/431 estimates the heme content. WT and mutants eluted at a salt concentration comprised between 250 and 280mM salt. Abs at 393nm is an indicator of oxidized heme.

sGC / mAU	**WT**	**D102N**	**D102A**	**F120A**
431nm	57.9	39.6	4.7	2.6
280nm	174.6	139.0	248.0	152.0
393nm	22.3	16.3	13.9	3.4
Ratio 280/431	3.0	3.5	52.7	58.5
elution (mM NaCl)	260	250	277	280

Figure 3: Coomassie stained gel of sGC and D102A, D102N mutants indicated a similar degree of purification (F120A was not detectable by Coomassie)

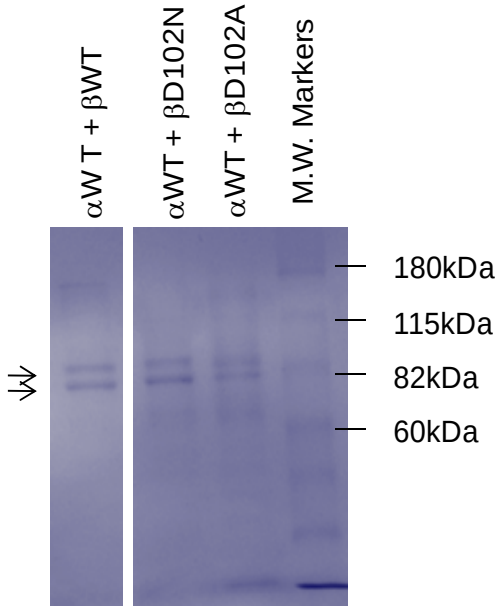


Table 3: Comparison of purified WT and mutants basal and stimulated activities. The values correspond to Fig.3 and include the fold stimulation of each activators compared to basal activity for WT and mutants and percentage of basal activity of the mutants compared to WT. Specific activity is expressed in nmol.min^{-1}.mg^{-1}± SEM.

	BASAL	% WT	PPIX, 10μM	fold stimulation	YC-1, 100μM	fold-stimulation	DEA-NO, 1μM	fold-stimulation	DEA-NO, 1μM + YC-1, 10μM	fold stimulation
WT	73.1 ± 2.0	100	886.1 ±99.6	12.1	561.5 ±75.2	7.9	4310 ±298.8	59	6566.9 ±208.2	89.8
D102A	47.1 ± 6.7	64	64.9 ±19.0	1.4	84.2 ±12.1	1.8	166.1 ±43.0	3.5	224.6 ±46.0	4.8
D102N	88.5 ±3.6	120	138.5 ± 29.8	1.6	169.3 ±27.1	2.2	282.0 ±26.6	3.2	1189.3 ±186.7	13.4
F120A*	142.9 ±12.8	----	153.8 ± 37.8	1.1	168.3 ±31.8	1.1	458.0 ±53.5	3.2	351.0 ±51.6	2.2

*: The higher basal activity of F120A is explained by a higher amount of sGC in the fraction used, i.e. the fraction eluted with high salt (~500mM) with some absorbance at 431nm as well. To better appreciate the synergistic effect, YC-1 and DEA-NO were kept at 10μM and 1μM, respectively. On the other hand, the COS-7 cells cytosolic extracts were treated with SNAP and YC-1 at 100μM to detect the highest production of cGMP (Table 1 of main text).