Supplementary Table S1: Site-directed mutagenesis. Oligonucleotides and PCR reactions used to generate the plasmid for expression of GC-A S487E.

Number	Name	Sequence	Tm [°C]
1	S487A/E fwd I	ctg gcc tct ggg ata tcc acc tc	66.0°C
4	S487A/E rev II	ctt agg gca atc tcc tgc agg atg	64.4°C
5	S487E fwd II	gag tta gaa cgc cac tta cgg agc gct ggc agc cgg ctg acc	80.2°C
6	S487E rev I	gtg gcg ttc taa ctc gct ggg ctg caa gtc ctc cca gcg cac	80.2°C

Legend to supplemental Table S1:

The wild type FLAG-tagged GC-A from rat in pCMV5 expression vector was used as a template for PCR mediated mutagenesis of serine 487 to glutamate. Two independent PCRs (PCR I: primer 1 + primer 4; PCR II: primer 2 + primer 3) were set up at an annealing temperature of 55 °C using 5 ng of template DNA, a final primer concentration of 20 pM and 1 unit of Taq DNA Polymerase (Invitrogen, Karlsruhe, Germany). PCR products were separated by gel electrophoresis and extracted using a commercial available kit (Macherey-Nagel, Düren, Germany). The products of PCRI (~ 250bp) and PCRII (~ 650bp) were fused to generate a DNA strain carrying the S487E mutation. Briefly, a 20 µl PCR reaction was set up with 1 µl of each PCR product, 1 unit of Taq DNA polymerase and 2 nM dNTP were annealed at 60°C. After 10 cycles reaction was cooled down to 4°C, 20 pM of primer 1 and primer 2 were added and the reaction was continued for 25 cycles. Q-Solution (Qiagen, Hilden, Germany) was added according to the manufacturer to all PCR reactions to improve the specificity. Fused PCR products (879bp) were purified by Chloroform/Isoamyl alcohol extraction. The pCMV5-FLAG-GC-A plasmid and the purified DNA strain carrying the expected mutation were subjected to double digestion with 5 units of EcoRV and SbfI (New England Biolabs, Frankfurt/Main, Germany) and ligated with the mutated strain using T4 DNA Ligase (New England Biolabs, Frankfurt/Main, Germany). Resulting constructs were verified through sequencing using internal primers.