

Supplementary information

The transition of photoreceptor guanylate cyclase type 1 to the active state

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Table S1. Oligonucleotide sequences used in the present study.

Mutants	Oligonucleotide Sequence 5'-3'
1 Alanine (1Ala)	FP- 5'- AGG CAC CGG CTA CTT CAC ATG CAA ATG -3' RP- 5'- AGC CAC ATA ATG GGC CAG GAA GGC -3'
2 Alanine (2Ala)	FP- 5'- AGG CAC CGG CTA CTT CAC ATG CAA ATG -3' RP 5'- TGC AGC CAC ATA ATG GGC CAG GAA GGC -3'
3 Alanine (3Ala)	FP 5'- GCC AGG CAC CGG CTA CTT CAC ATG -3' RP 5'- TGC AGC CAC ATA ATG GGC CAG GAA GGC -3'
4 Alanine (4Ala) Set a	FP 5'- GCC GCT AGG CAC CGG CTA CTT CAC ATG -3' RP 5'- TGC AGC CAC ATA ATG GGC CAG GAA GGC -3'
4 Alanine (4Ala) Set b	FP 5'- GCC GCT GCT AGG CAC CGG CTA CTT CAC ATG -3' RP- 5'- AGC CAC ATA ATG GGC CAG GAA GGC -3'
5 Alanine (5Ala)	FP 5'- GCC GCT GCT AGG CAC CGG CTA CTT CAC ATG -3' RP 5'- TGC AGC CAC ATA ATG GGC CAG GAA GGC -3'

Figure S1

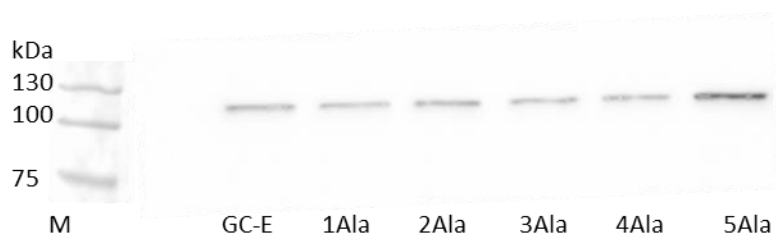


Figure S1. Western blot for verification and quantification of poly alanine mutants. Expression level of mutants was compared to that of GC-E wildtype in HEK293 cell membranes. Five μg of total protein amount for each sample was analysed by SDS-PAGE. The samples were detected by the specific antibody anti-GC1#3. The slight difference between each mutant band intensity was considered for the activity measurement of amount of cGMP synthesized with each sample.

Figure S2

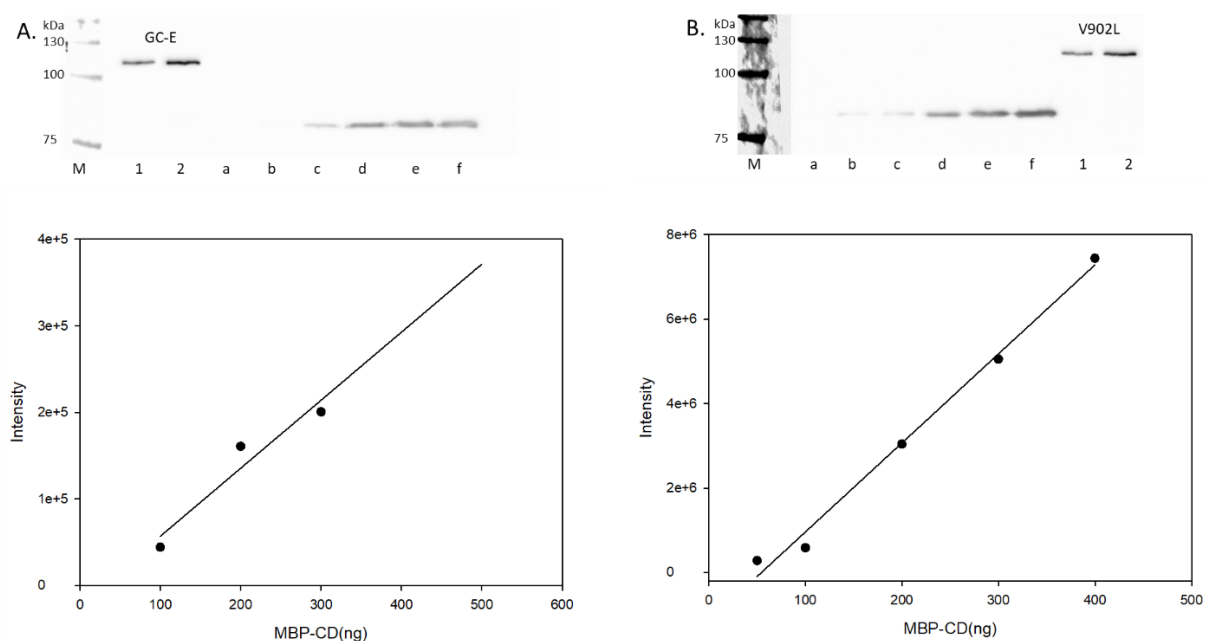


Figure S2. Quantification of GC-E and V902L in HEK293 cell membranes. Purified MBP-CD protein samples were electrophoresed and blotted in increasing amount (a- 20ng, b-50ng, c- 100ng, d-200ng, e-300ng, f-400ng) were used to create a calibration curve along with a sample of wildtype GC-E (**A**) or V902L (**B**) present in HEK293 membrane fractions (1- 2.5 μg , 2- 5 μg). The samples were detected by GC1 # 3 antibody and ECL imaging. Band intensity was linear within the tested range for **B**, for **A** the plot was extended manually based on linearity. Quantification yielded for 5 μg membrane protein 292 ng of GC-E wildtype, and 212 ng of V902L, taking a purity of 73% into account.