

Supplementary Information

Conserved residues in the extracellular loop 2 regulate *Stachel*-mediated activation of ADGRG2

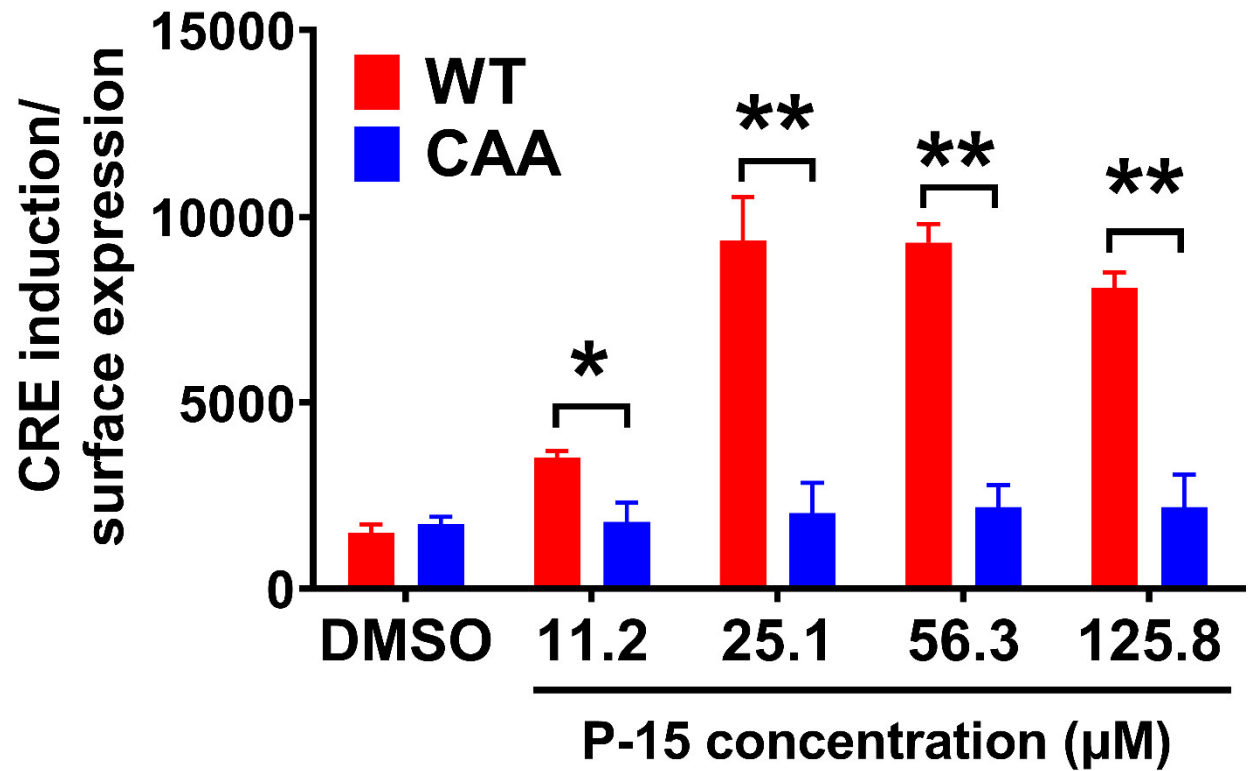
Abanoub A. Gad^{1,2}, Pedram Azimzadeh¹, Nariman Balenga^{1,3, 4 *}

From the ¹Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, ²Graduate Program in Life Sciences, University of Maryland, Baltimore, MD, ³Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD, ⁴Molecular and Structural Biology program at University of Maryland Marlene and Stewart Greenebaum NCI Comprehensive Cancer Center, Baltimore, MD

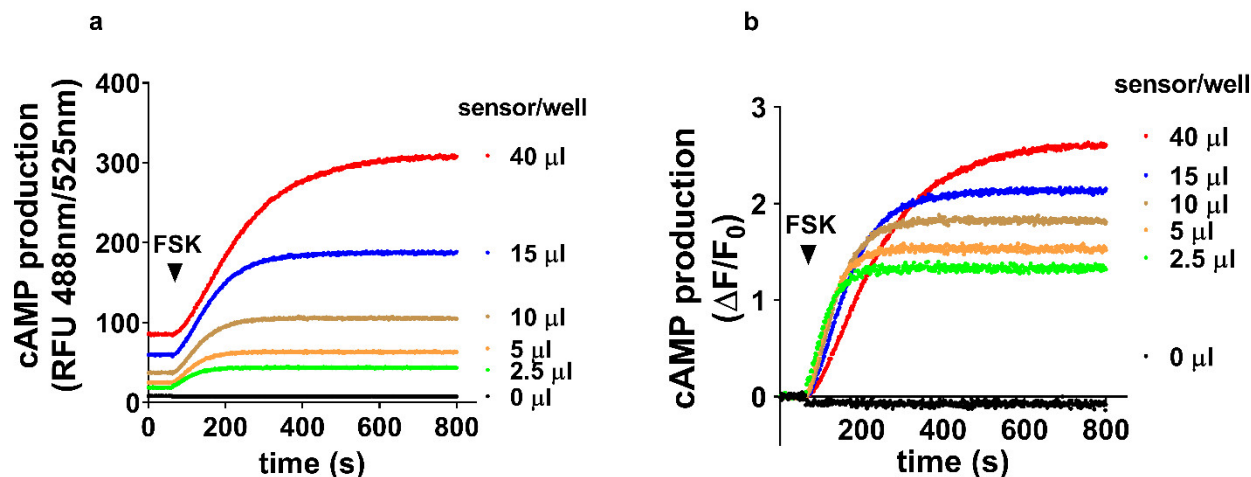
*To whom correspondence should be addressed: Nariman Balenga; Department of Surgery and Pharmacology, University of Maryland School of Medicine, 655 W. Baltimore Street, Room 10-027, Baltimore, MD 21201; Tel.:410-706-3261; Fax.:410-706-3260. E-mail: nbalenga@som.umaryland.edu

Consensus	-----XXXXYXXXXXCWLXXXXXXXX-----
ADGRA1	-----RNYGTE-----DEDTAYCWM AWEPS-----
ADGRA2	-----HNY-----RDHSPYCWL VWRPS-----
ADGRA3	-----KNYGS-----RPNAPYCWM AWEPSLGA----
ADGRB1	-----AKGYSTMNYCWL SLEGGLLY----
ADGRB2	-----RTKGYGTSSYCWL SLEGGLLY----
ADGRB3	-----RTKGYGTDHYCWL SLEGGLLY----
ADGRC1	-----PQGYGNPDFCWL SLQDTL-----
ADGRC2	-----PEGYGNPDFCWL SIYDTL-----
ADGRC3	-----PEGYGNPDFCWL ISVHEP-----
ADGRD1	-----DSYGTSNNCWL SLASG-----
ADGRD2	-----PHDYVAPGHCWL NVHTN-----
ADGRE1	-----QPQGYGMHNR CWNTE-----
ADGRE2	-----RPHLYGTPSRCWL QPEKG-----
ADGRE3	-----WPHLYGTADRCWL HLDQGF MWSF--
ADGRE4P	-----PQNYGTF-TCWL KLDKG-----
ADGRE5	-----SKGYGRPRYCWL DFEQG-----
ADGRF1	-----TQPSNTYKRKDV CWNWSNGSKPL---
ADGRF2	-----VAATEPGKGYLRPEI CWNWDMTKALLA--
ADGRF3	-----GLYLPQGQYLREGECWL DGKGGALYT---
ADGRF4	-----TEPEKGYMRPEACWL NWDNTKALLA--
ADGRF5	-----TQPREVYTRKNVCWL NWEDTKALL---
ADGRG1	-----DNYGP I I--LAVHRTPEGVIYPSM CWIRDSLVSYITNLG
ADGRG2	-----DNYGLGS-----YGKFPNGSPDDFCW INNNAV FYIT---
ADGRG3	-----GTGSANSYGLY-----TIRDRENRTSLEL CWFREGTTMYALYIT
ADGRG4	-----SVKKDLYGTL-----SPTTPFCW IKDDS-----
ADGRG5	-----SVKSSVYG PCTIPVFD SWENGTGFQNM SICWVRSPV VHS----
ADGRG6	-----SRNNNEVYGKES-----YGK---EKGDEF CWIQDPVIFYVT---
ADGRG7	-----GVIYSQNGNNPQWELD YRQEKI CWLAIPEPNGVIKSP
ADGRL1	-----YRSYGTEKACWL RVDNY-----
ADGRL2	-----KSYGTEKACWL HVDNYF-----
ADGRL3	-----DYRSYGTDKV CWLRLD TYF-----
ADGRL4	-----RYYGTTKV CWLSTENNF IWS---
ADGRV1	LKGIYHQSMSQIYGLI-----HGDLCFIPNVYA-----

Supplementary Figure 1. Multiple alignments of the ECL2 of all 33 members of the aGPCR family. Predicted amino acid sequences of the ECL2 for each aGPCR were derived from Uniprot. The alignment was conducted in SnapGene software using the Clustal Omega algorithm.

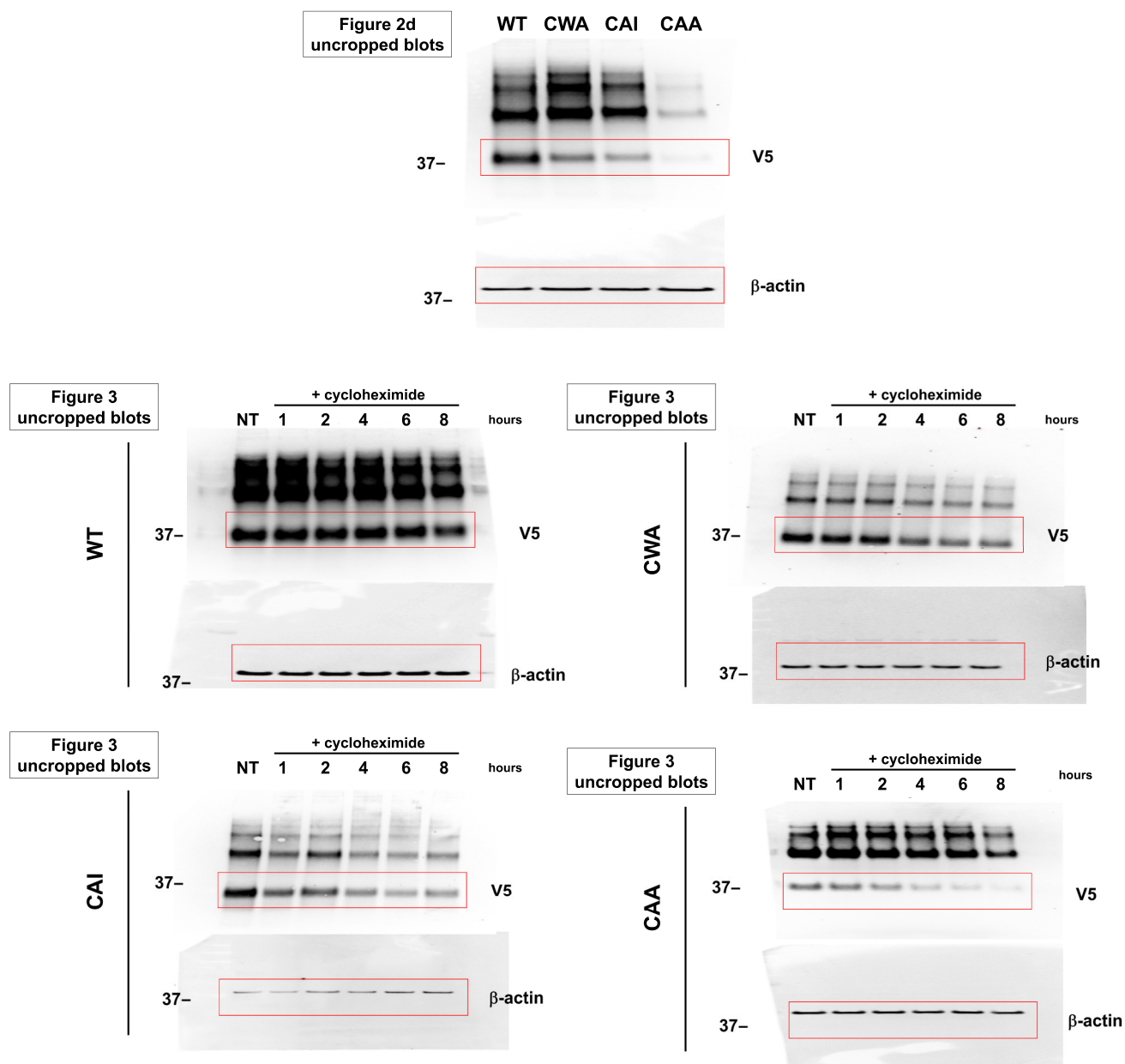


Supplementary Figure 2. HEK cells were transfected with the same dose of WT and CAA plasmids along with CRE-Luc plasmid. Luciferase activity in response to increasing concentrations of P-15 was measured in a luminescence-based assay. Relative light units (RLU) were normalized to the surface expression data derived from an ELISA assay and are presented as mean $\pm$ S.E.M from 3 independent experiments performed in duplicate. * $P < 0.05$, ** $P < 0.01$. Data were compared with WT at each concentration of P-15 with Holm-Sidak multiple comparison test.



Supplementary Figure 3. Characterization of a fluorescent genetically encoded cAMP sensor.

HEK cells were transduced with various amounts of Upward Green cADDis cAMP sensor in 96-well plates overnight. Cells were washed with assay buffer and then the fluorescence (Excitation at 488 nm/Emission at 525 nm) was recorded for 1 min before and 12 min after addition of 10 μ M FSK. (a) Raw data showing the relative fluorescence units (RFU) for each amount of sensor. (b) Data were analyzed in GraphPad Prism and are presented as change in RFU divided by the initial RFU ($\Delta F/F_0$).



Supplementary Figure 4. Uncropped blots related to Figure 2d and Figure 3 are provided. Red boxes show the cropped images used for Figure 2 and 3. The upper bands in the V5 blots (not shown in Figures 2 and 3) are ubiquitinated forms of the receptors. Auto-contrast in the iBright imaging system was used for unbiased handling of western blot images.