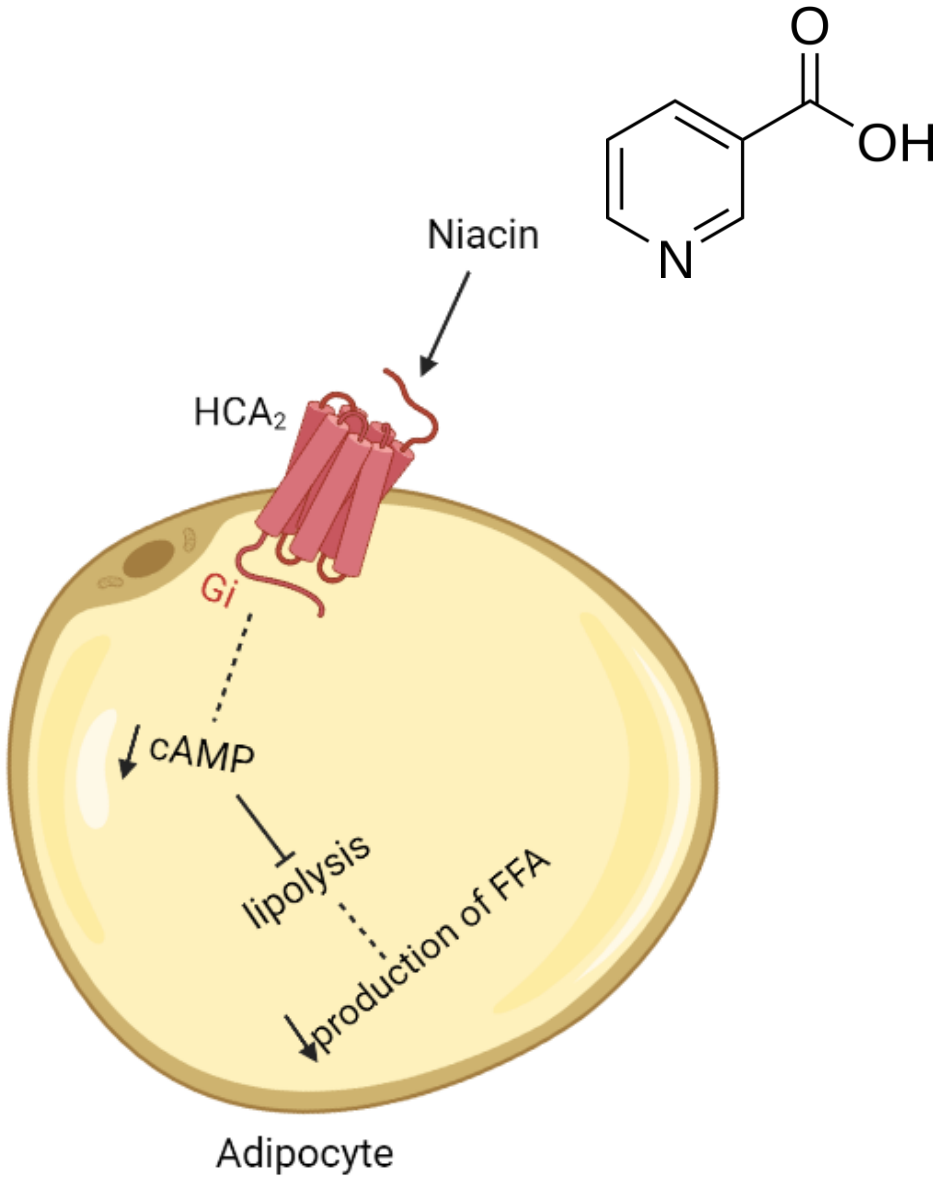
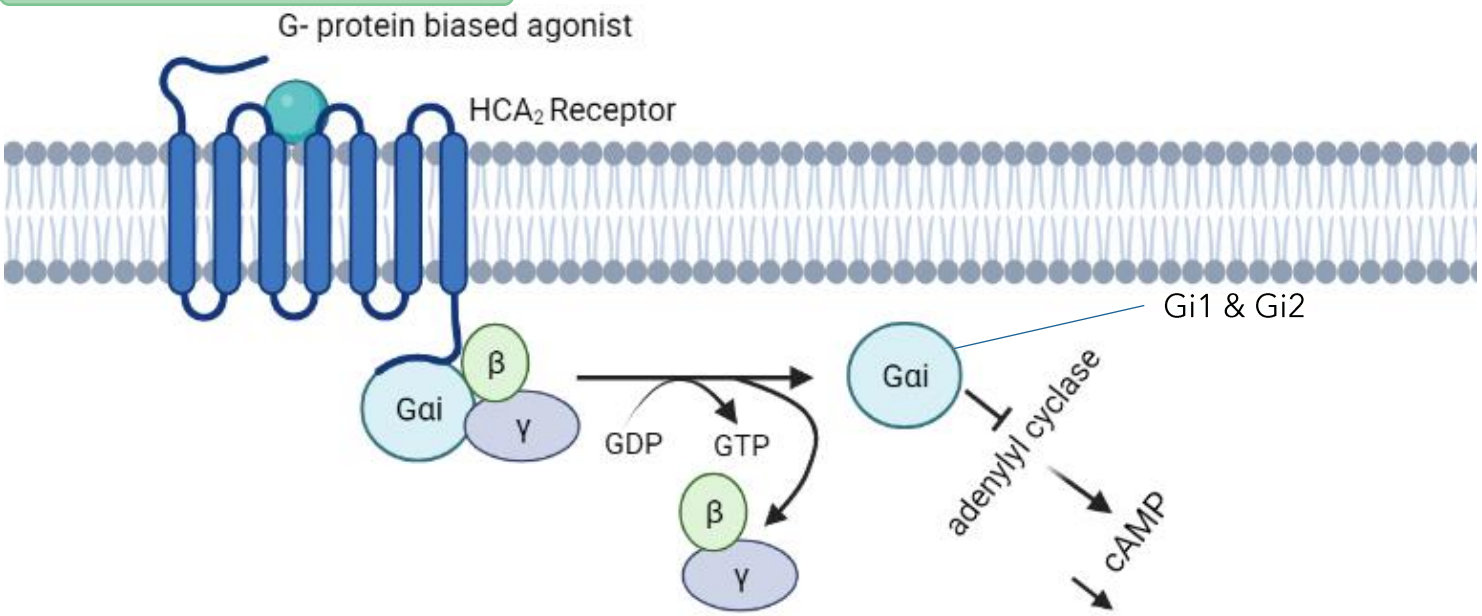
Background- HCA_2 receptor

- Hydroxycarboxylic Acid Receptor 2 (GPR109A) is a G protein-coupled receptor
- Activated by short-chain hydroxycarboxylic acids, notably niacin and beta hydroxybutyrate
- Expressed in **adipocytes** and **immune cells** like macrophages
- Suggesting a possible role it could play in the immune responses and inflammation

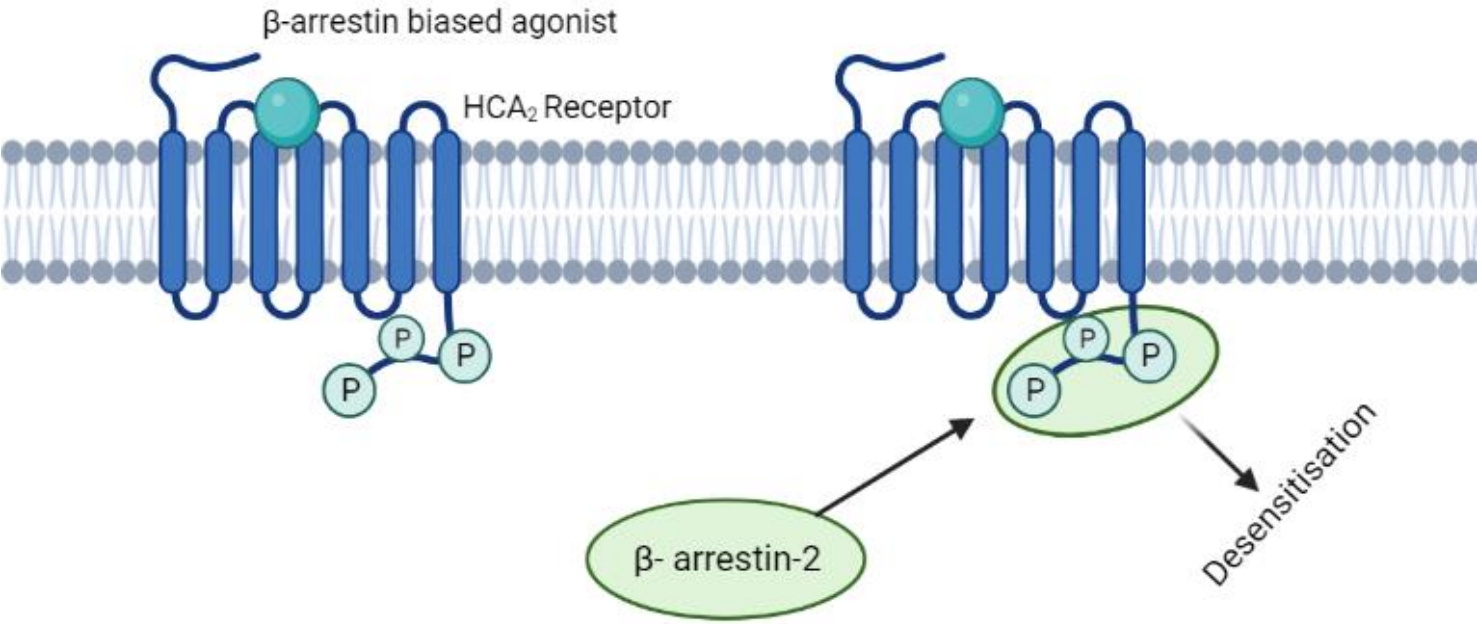


HCA_2 Signalling Pathways

Gi-Protein Signalling Pathway

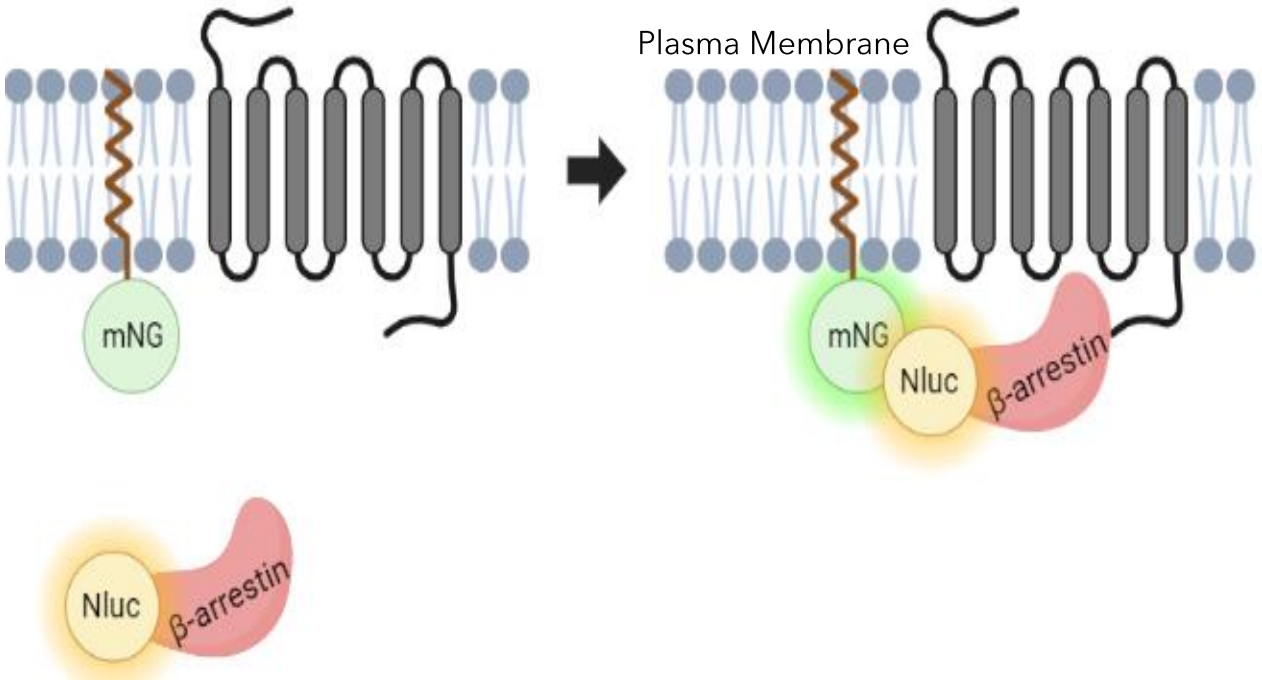


β-Arrestin signalling pathway



How do we study the real-time function of the HCA_2 receptor in living cells?

Bystander biosensors to investigate β-arrestin signalling



TRUPATH biosensors to investigate G protein signalling

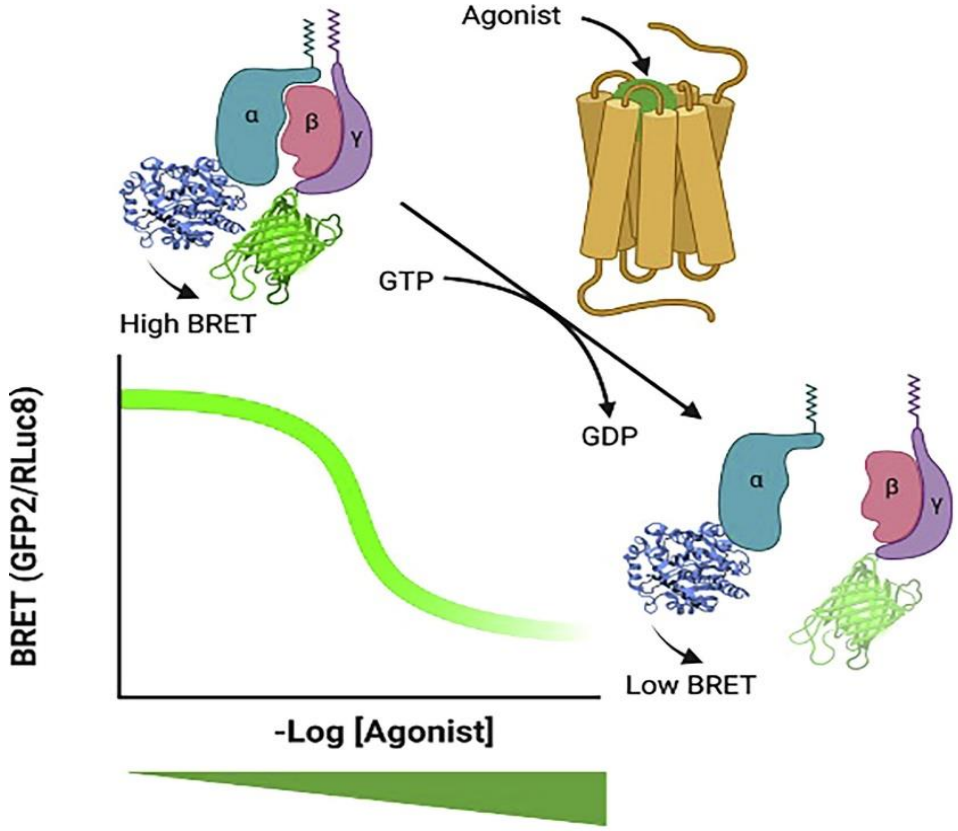
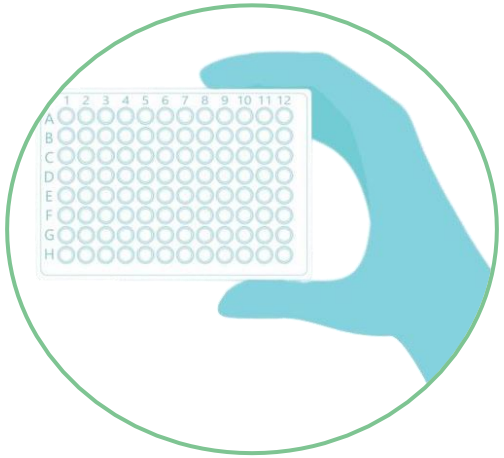


Image Sourced from : DiBerto, J.F., Smart, K., Olsen, R.H.J. and Roth, B.L. (2022). Agonist and antagonist TRUPATH assays for G protein-coupled receptors. *STAR Protocols*, 3(2), p.101259.

PROJECT AIMS

Use molecular biology techniques to clone the untagged human HCA_2 receptor from linear DNA.



Utilize genetically encoded biosensors to investigate the signalling pathways of the HCA_2 receptor.

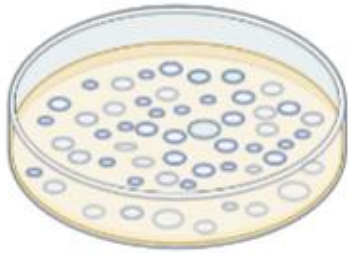
- Bystander BRET for arrestin recruitment
- TRUPATH for Gi1 and Gi2

METHODS

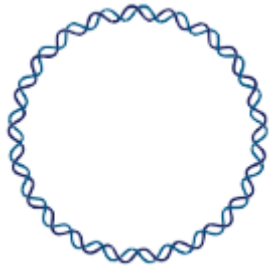
1. Amplifying the HCA_2 sequence using PCR

2. Amplified PCR products extracted using gel electrophoresis

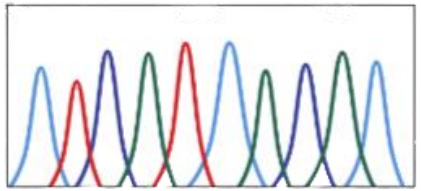
3. AQUA cloning to ligate vector and HCA_2 insert



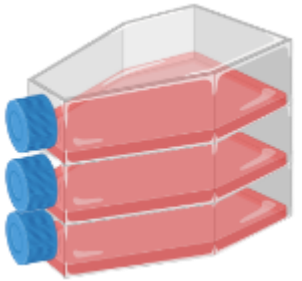
4. Isolated HCA_2 plasmid DNA from transformed cells



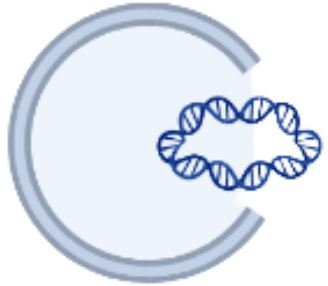
5. Construct verified by DNA sequencing



6. HEK cells cultured and maintained in flasks

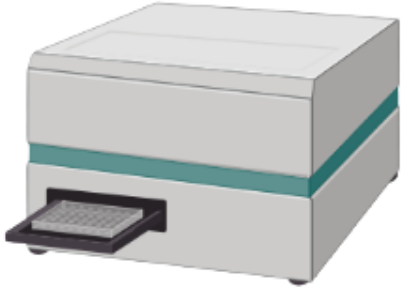


7. HCA_2 receptor transfected with biosensor into the cells



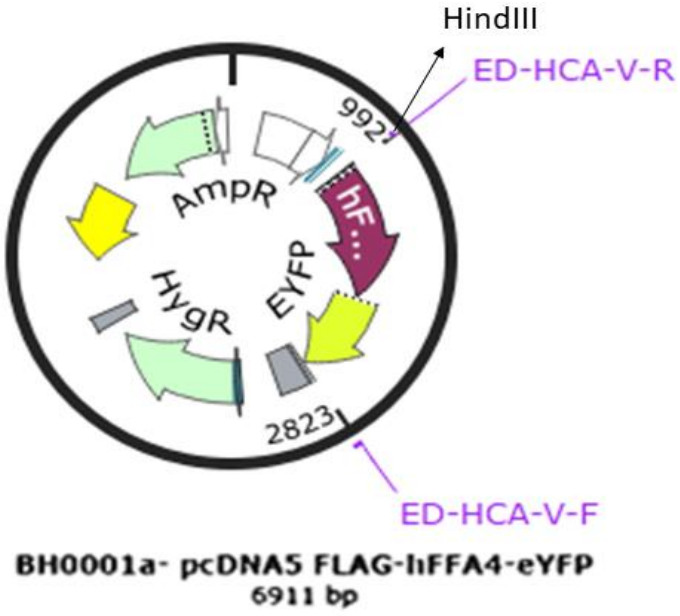
8. Cells treated with substrate and drugs

9. Signal measured on a microplate reader

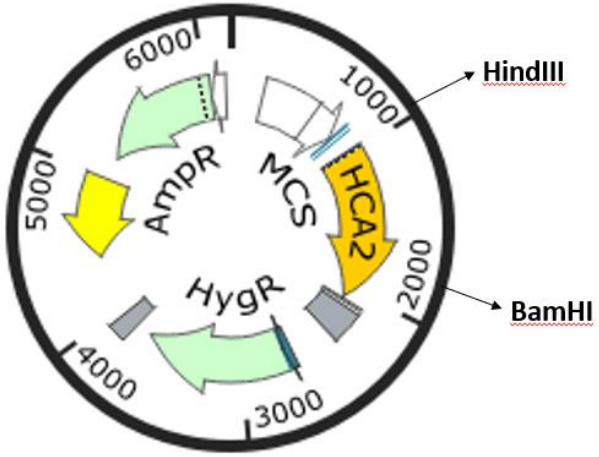


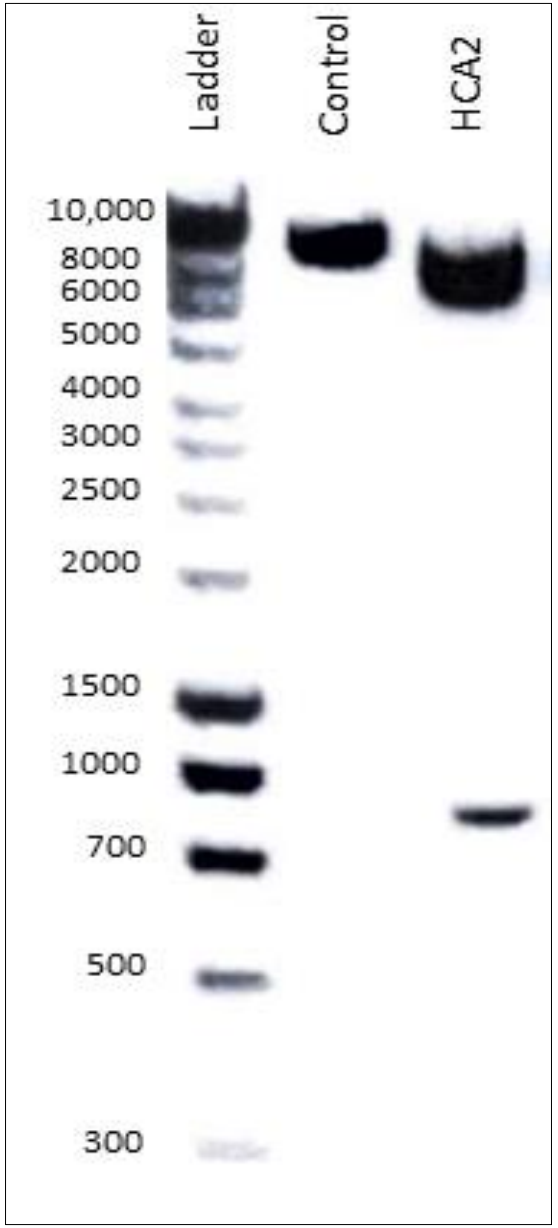
Successful Construction of the untagged HCA_2 Plasmid

Control

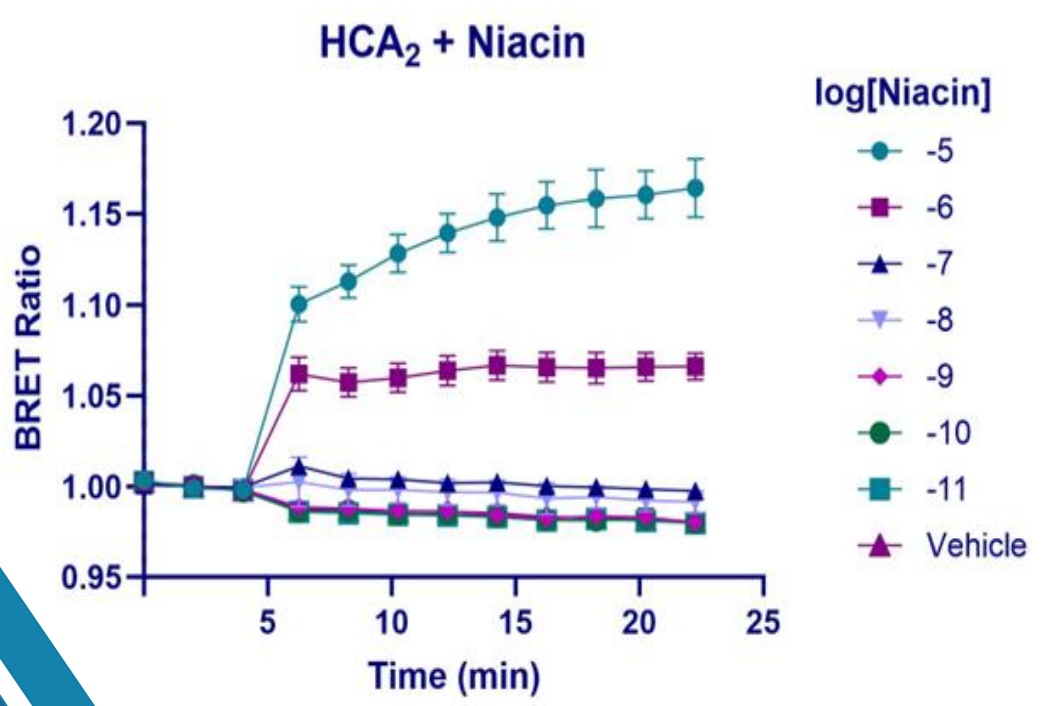


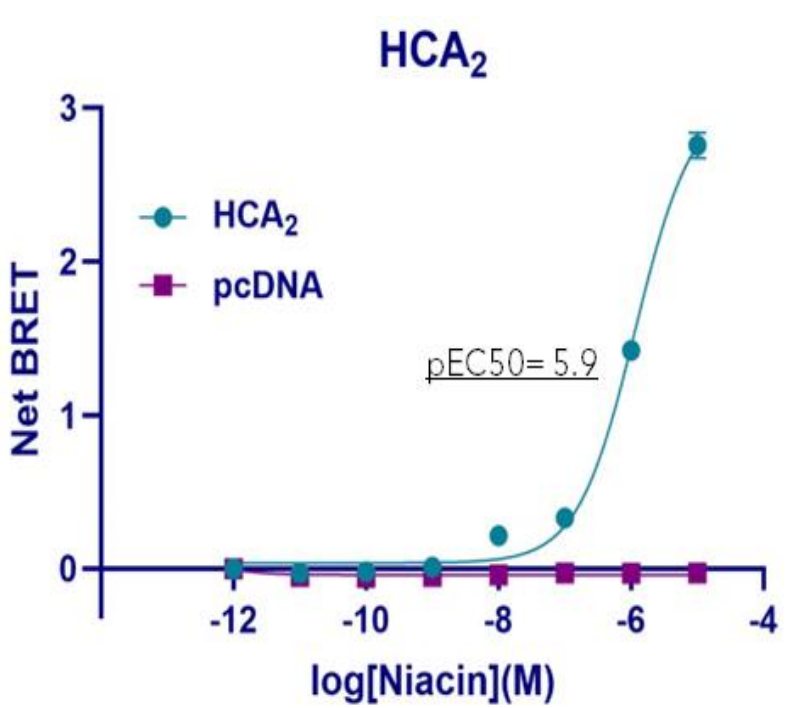
HCA_2 Plasmid

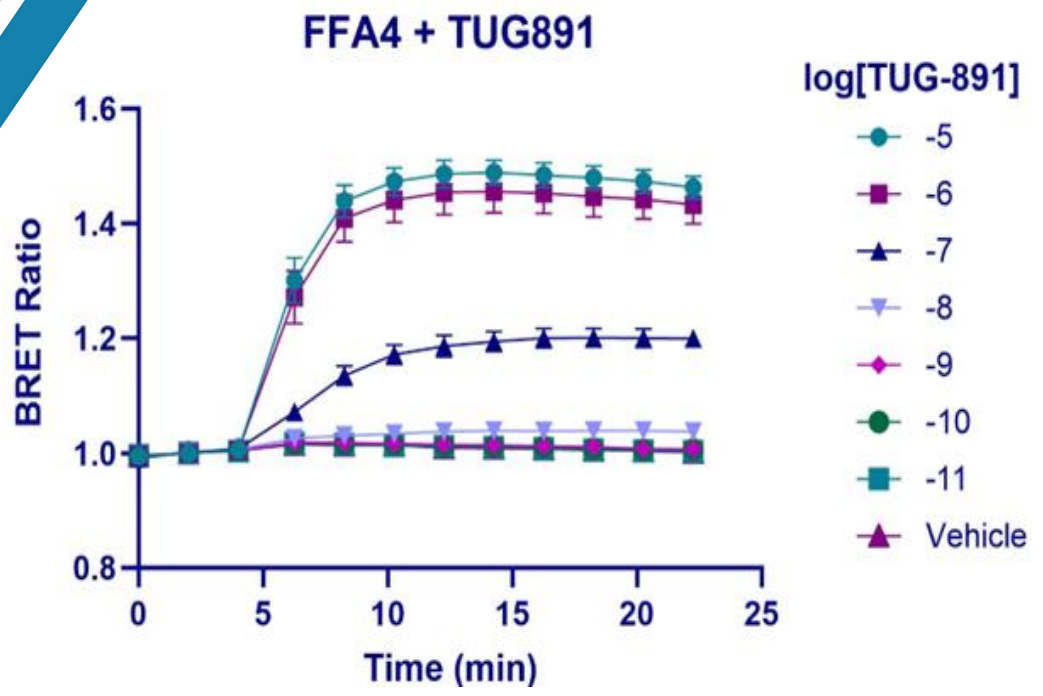


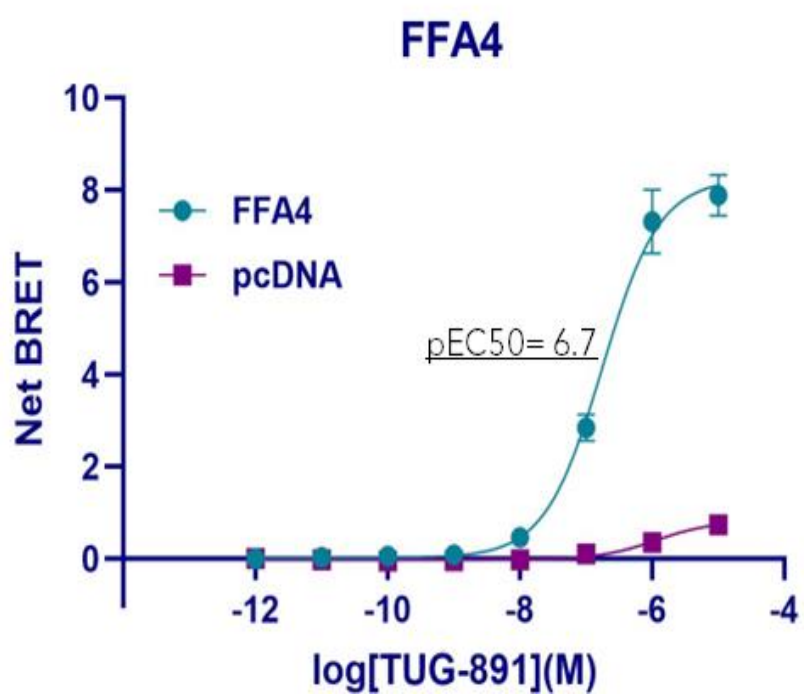


Treatment of HCA_2 receptor with Niacin leads to Arrestin Recruitment

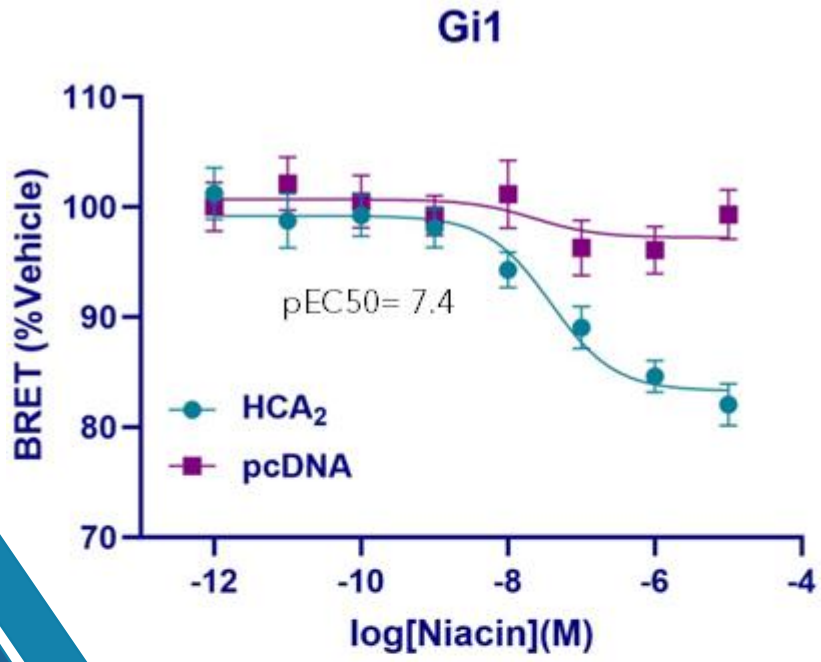


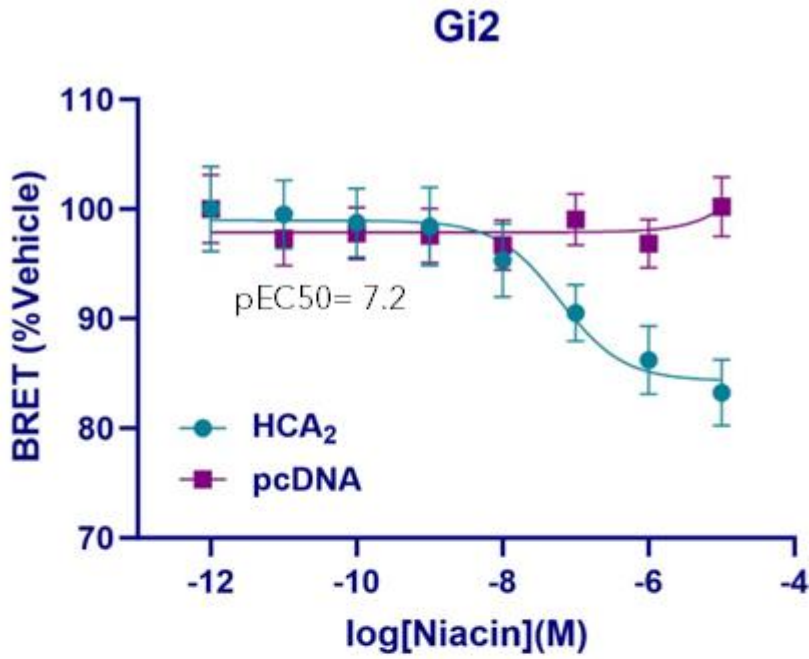


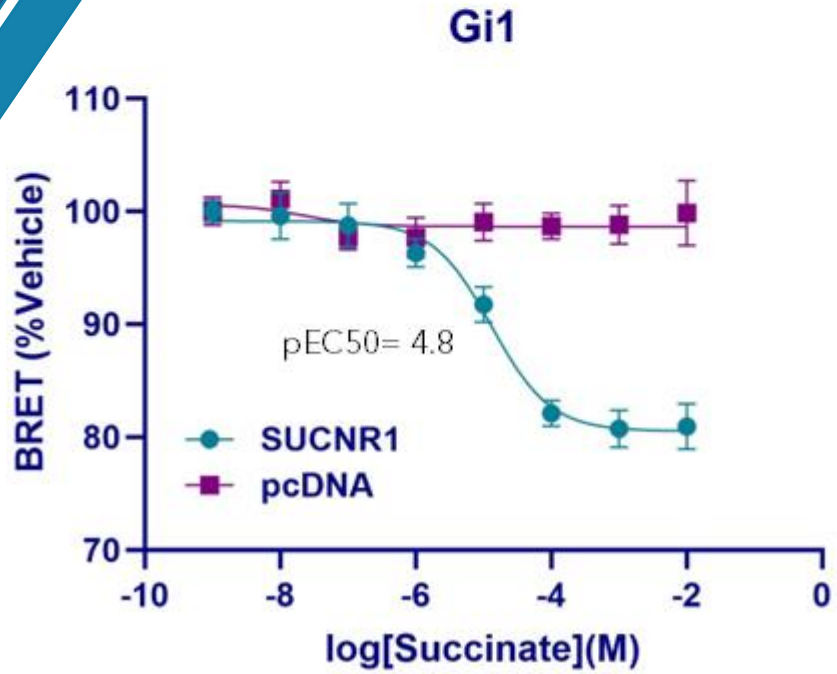


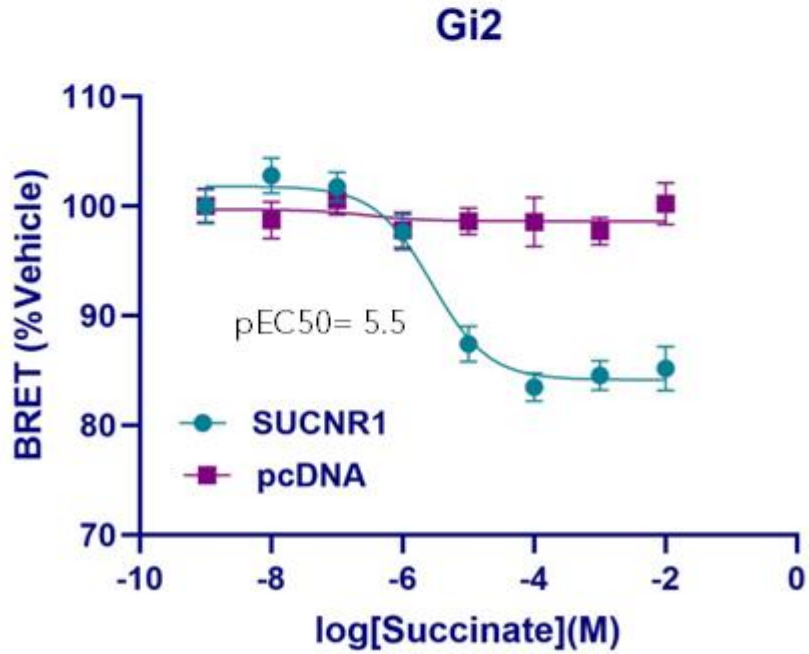


Treatment of HCA_2 receptor with Niacin activates G protein signalling









Conclusion

- Successful generation of the plasmid containing HCA_2 receptor
- It was used to show arrestin recruitment using a Bystander Assay
- G protein activation of Gi1 and Gi2 was seen using a TRUPATH assay
- HCA_2 receptor displays stronger potency of Niacin in G-protein assay than in arrestin assay.
- BRET biosensors proved effective tools for studying HCA_2 receptor function