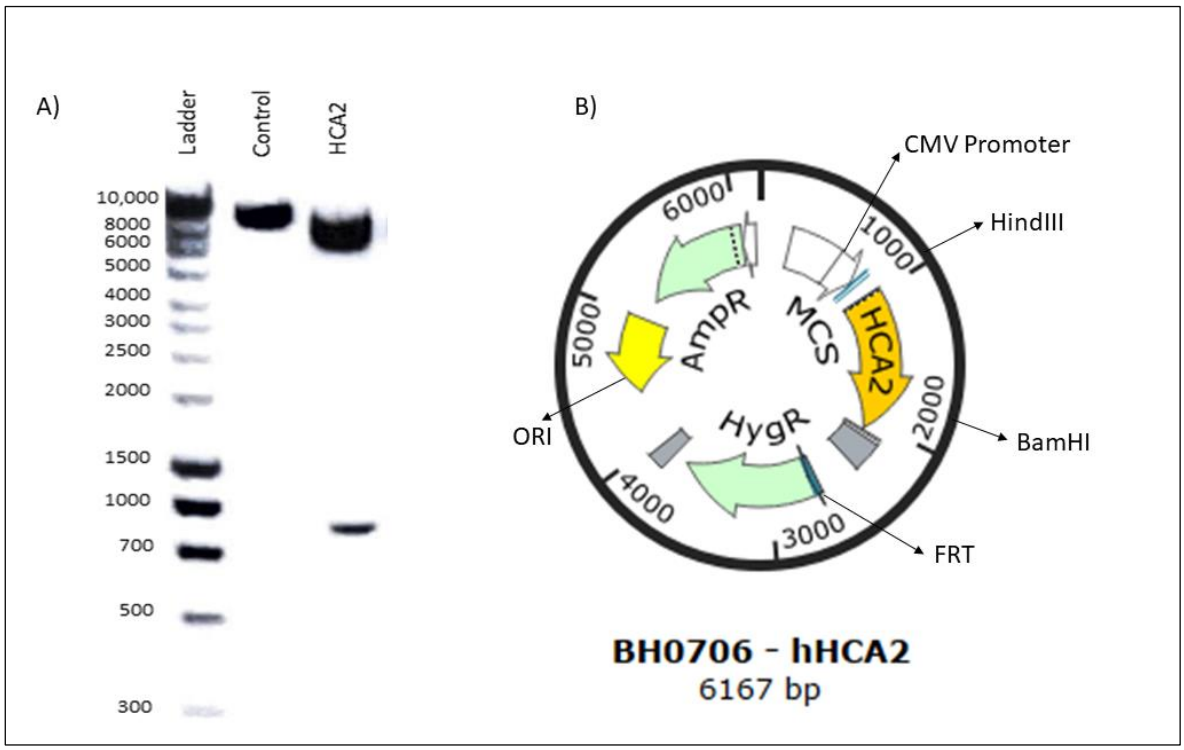
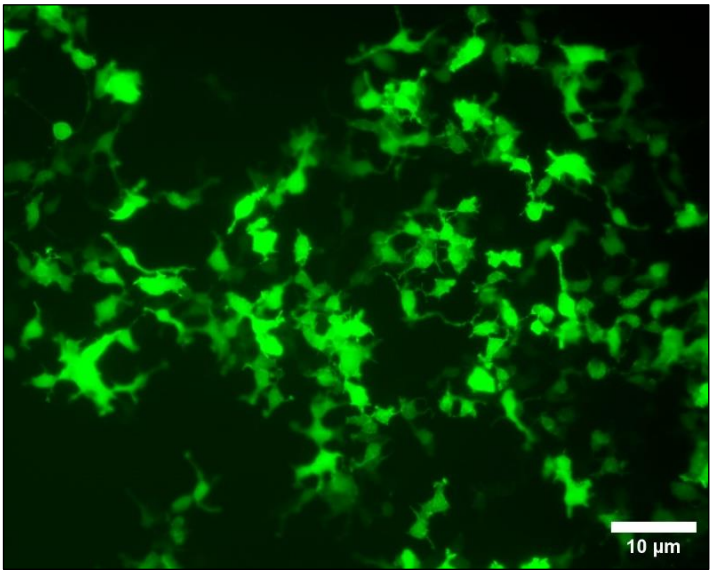
Significant Results of the Project

1.



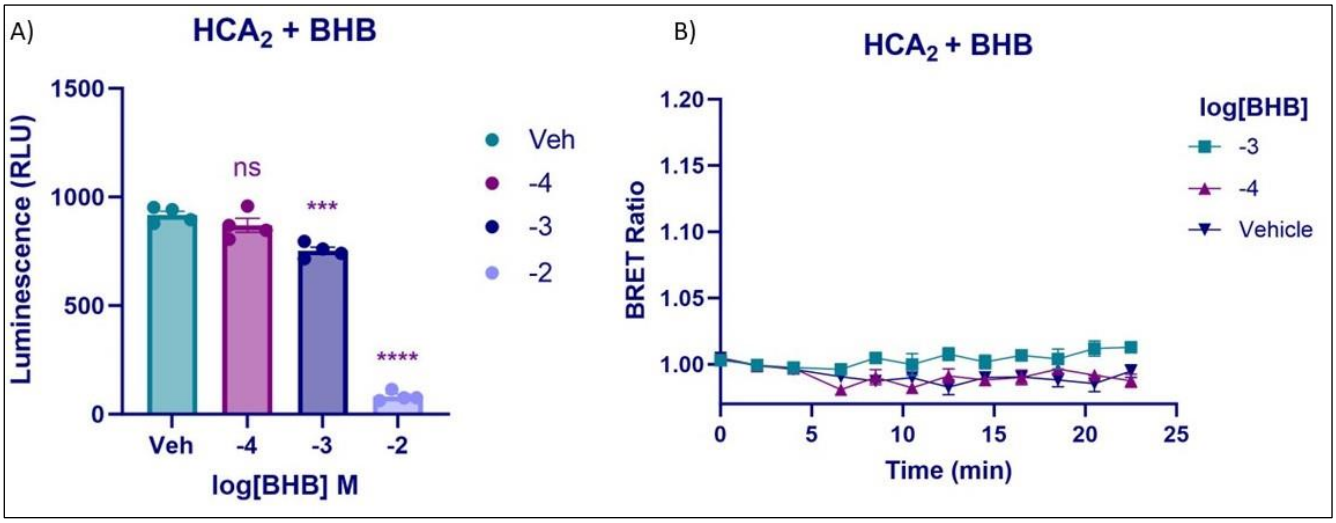
The Human HCA_2 sequence was successfully cloned into a pcDNA5 FRT/TO plasmid. A) Image from the GelDoc of a 1% agarose gel electrophoresis. The first well has the standard DNA ladders with known molecular weights. The second and third well have the control (parental plasmid lacking BamHI site) and HCA_2 construct respectively, which were cut using BamHI and HindIII restriction enzymes. B) The plasmid map of the HCA_2 receptor depicts the key components of the circular plasmid of length 6167 basepairs, including the multiple cloning sites and Ampicillin and Hygromycin resistance along with the HCA_2 gene fragment.

2.



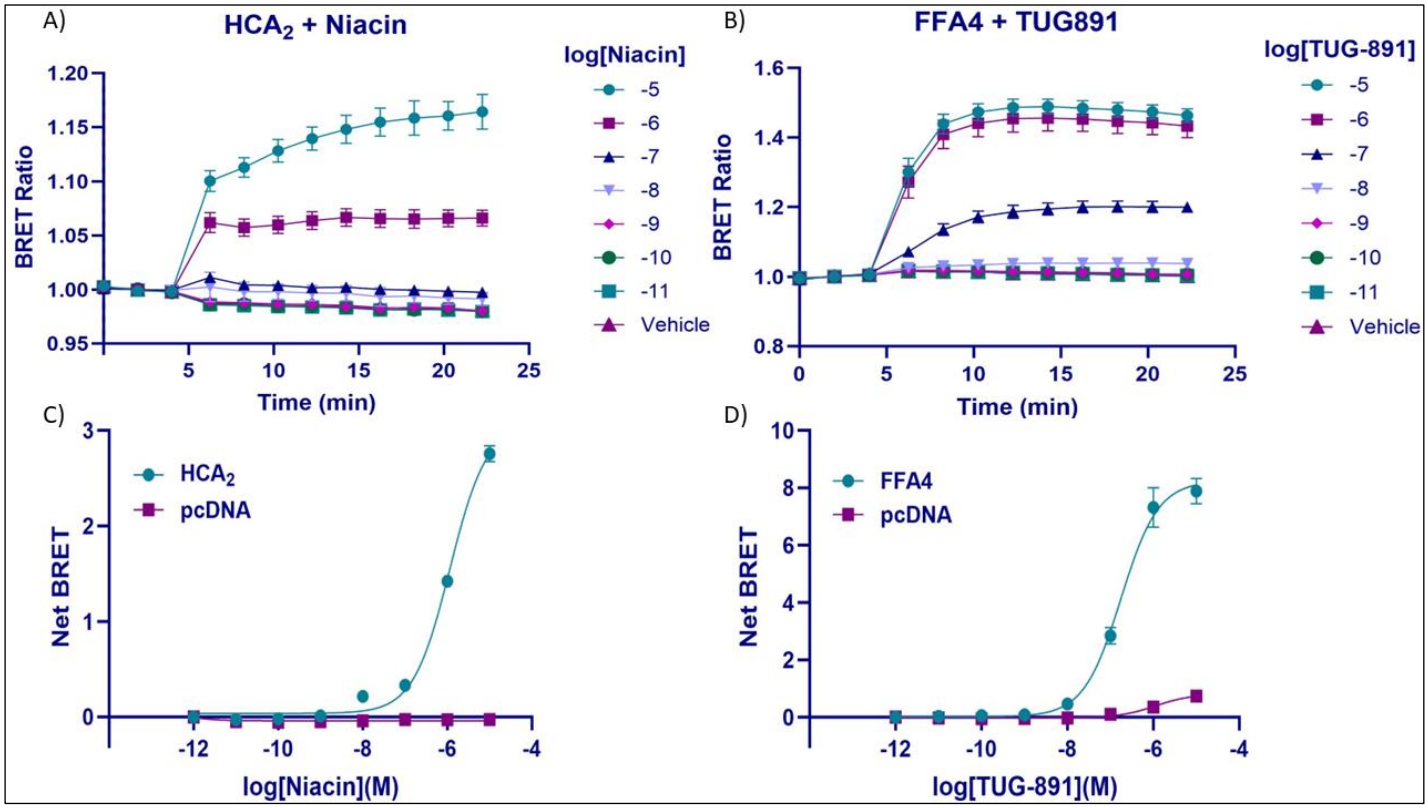
Lyn11-mNeonGreen is successfully transfected into HEK293 cells. The fluorescence microscopy image shows the successful transfection of the target cells with mNeonGreen. Green fluorescence indicates the expression of the transfected gene, confirming the efficient incorporation of the foreign DNA into the host cells. The scale bar this image is set is at 10µm.

3.



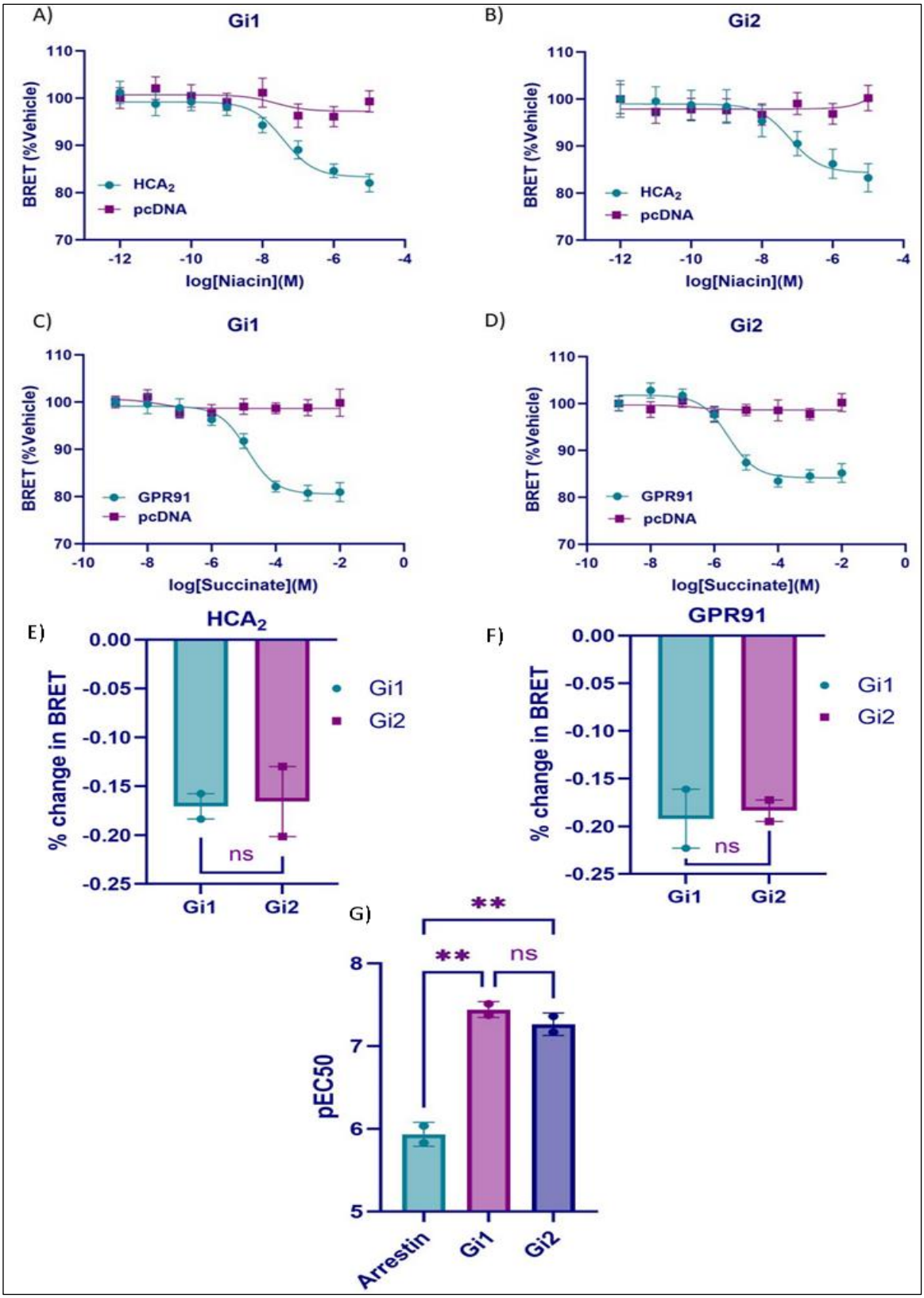
No significant BRET signal observed in cells expressing the HCA_2 receptor when treated with BHB at any tested concentration. A) raw luminescence value at 475nm for cells having HCA_2 receptor treated with BHB and a control as vehicle. Data are presented as mean ± SEM from n=1 experiment done in triplicates. Comparisons between 10^{-2}, 10^{-3}, 10^{-4} concentrations made with the vehicle showing **** = $p < 0.0001$, *** = $p<0.001$ and no significance respectively by performing one-way ANOVA with Tukey's post hoc test. B) depicts the time-course kinetics graph. The concentrations of the drugs are their log molar concentrations and BHB was added after 4 mins

4.



Significant increase in BRET signal in cells expressing the HCA_2 receptor upon Niacin treatment suggesting Arrestin recruitment. A and B) Kinetic BRET binding experiments for both HCA_2 with niacin and FFA4 with TUG-891. The drugs are added after 4 minutes on completion of 3 cycles. C and D) Concentration-dependant curves for both receptors with respective ligands. The concentrations of the drugs are their log molar concentrations of both Niacin and TUG-891. Vehicles are plotted at 10^{-12} M for graphs with both Niacin and TUG-891. Data are presented as mean ± SEM from n=2 independent experiments completed in quadruplicates.

5.



Concentration-dependent decrease in BRET signal observed in cells expressing the HCA_2 receptor, suggesting disassociation of G-protein subunits. Concentration-dependent response curve with the specific log ligand concentrations. BRET % of each of its vehicle baseline corrected values. Signal from cells with A) HCA_2 receptor with Gi1 B) HCA_2 receptor with Gi2, C) GPR91 receptor (control) with Gi1

and D) GPR91 receptor (control) with Gi2, E) % change in BRET showing no significant difference between the Gi1 and Gi2 protein in HCA_2 with Niacin, F) % change in BRET showing no significant difference between the Gi1 and Gi2 protein in GPR91 with Succinate, G) Comparison done by performing one-way ANOVA with Tukey's post hoc test and ** = $p<0.01$. Data are presented as mean ± SEM from n=2 independent experiments completed in quadruplicates. The concentrations of the drugs are their log molar concentrations of both Niacin and Succinate. Vehicles are plotted at 10^{-12} M and 10^{-9} M for graphs with Niacin and Succinate respectively