MICRO-TAG® CELL TARGET ENGAGEMENT REDEFINES GPCR DRUG DISCOVERY WITH FUNCTIONAL RELEVANCE

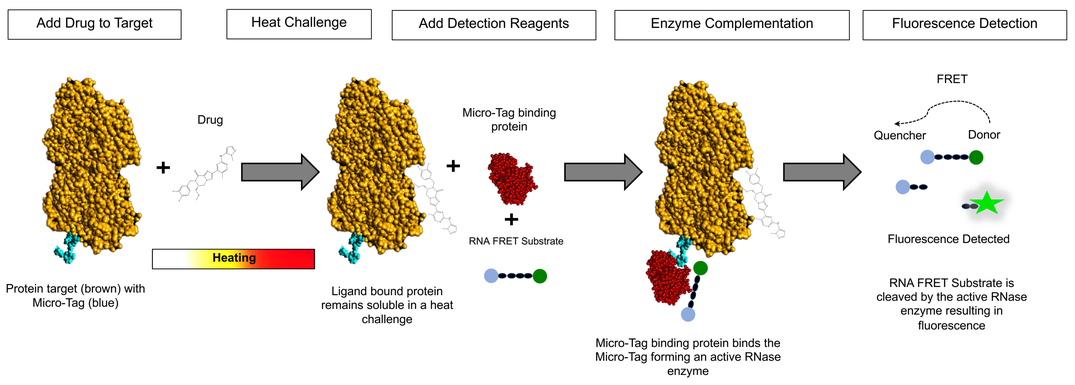
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MICRO-TAG® SYSTEM FOR GPCR TARGETED DRUG SCREENING

ABSTRACT:

GPCRs remain among the most valuable yet technically challenging therapeutic targets. Discovery of drugs targeting these receptors is a challenge in early drug discovery. MICRO-TAG® cell target engagement platform is a cellular and translatable assay system capable of identifying and functionally validating compounds that directly bind GPCRs in their native membrane environment. Here we present drug screening data for GPR75 — a receptor implicated in obesity, cancer, and metabolic syndrome. By integrating direct target engagement with functional pathway interrogation in a cellular environment, MICRO-TAG® enables scalable and physiologically relevant drug discovery for some of the most challenging drug target classes in biology.

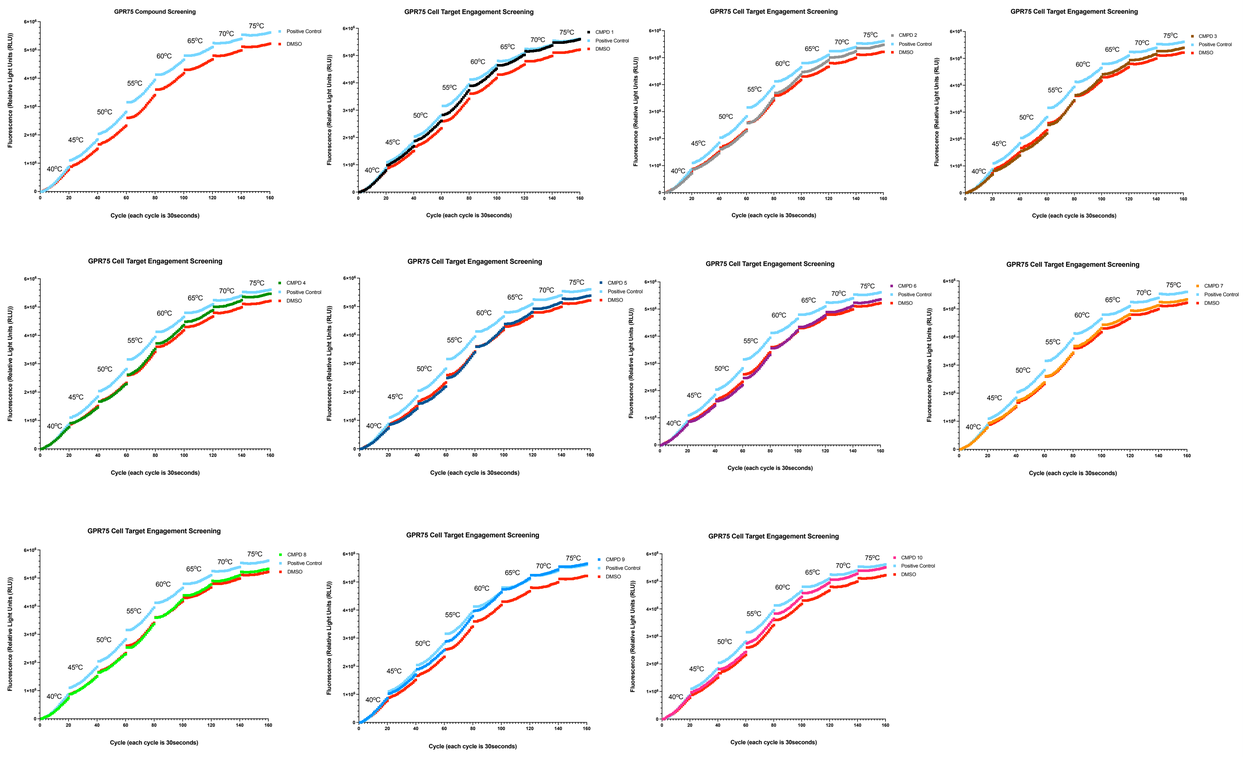
MICRO-TAG® System Used For Target Engagement Focused Drug Discovery



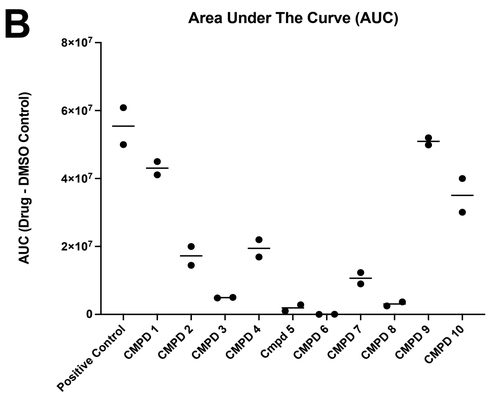
CellarisBio Step Gradient Cell Target Engagement



A GPR75 MICRO-TAG Cell Target Engagement Screening Assay



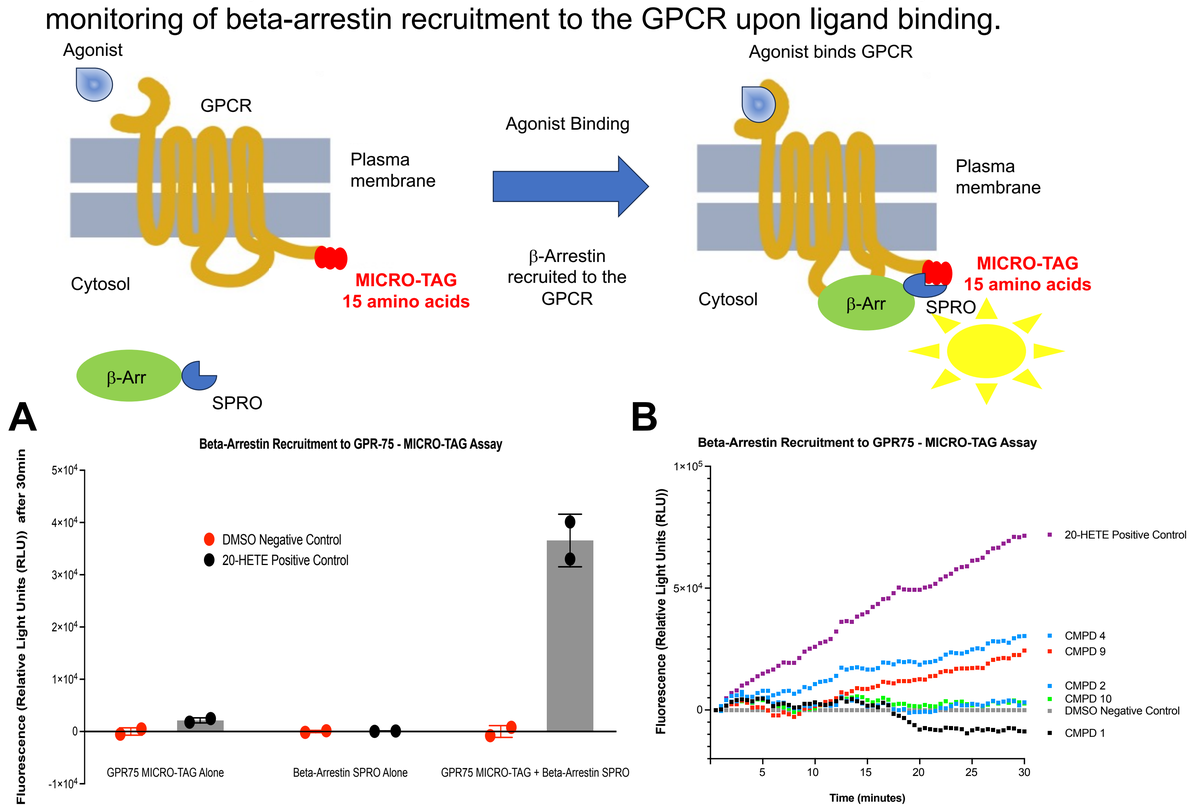
B



Real-time STEP gradient results for testing ligand binding to GPR75 Micro-Tag target.
(A) Real-time data testing 10 different compounds along with the positive control compound AAA for direct binding to GPR75 in real-time.
(B) Area under the curve (AUC) determinations from the cellular target engagement assay identifies compounds that bind and stabilize GPR75 in cells.

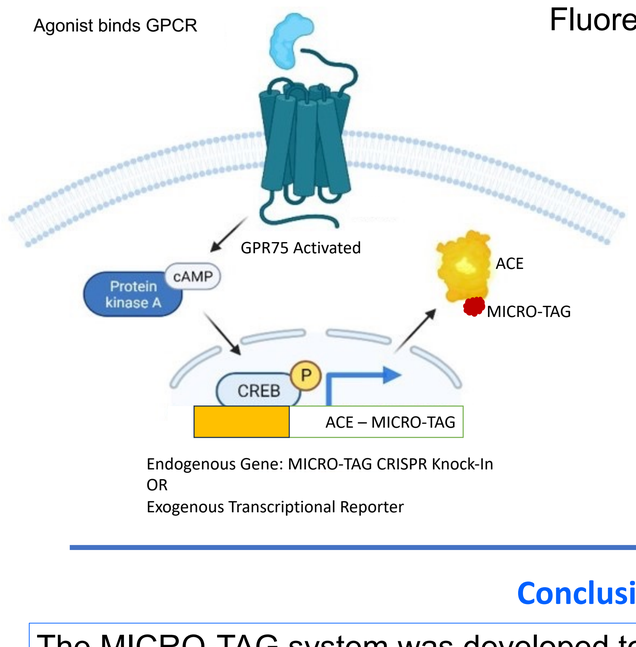
GPCR Agonist Activation Assay With MICRO-TAG®

Cellular target engagement assay is a useful primary screen for identifying potential drug candidates. The binding assay must be complimented with a functional read-out to identify molecules that not only bind to the receptor of interest but also promote a measurable cellular response. CellarisBio has developed a real-time GPCR agonist activation assay with the MICRO-TAG system. This offers highly sensitive real-time monitoring of beta-arrestin recruitment to the GPCR upon ligand binding.



MICRO-TAG assay system measures β-Arrestin recruitment to GPR75 in real-time.
(A) GPR75 MICRO-TAG with β-Arrestin SPRO can be used to monitor agonist (20-HETE) induced β-Arrestin recruitment to the GPCR.
(B) Compounds identified from the GPR75 cell target engagement MICRO-TAG screening assay were tested for agonist activity by monitoring β-Arrestin recruitment. Compounds 4 and 9 demonstrated agonist activity in this assay.

Custom Functional MICRO-TAG® Bioassay - monitoring cellular response downstream of GPCR activity



Endogenous Gene: MICRO-TAG CRISPR Knock-In
OR
Exogenous Transcriptional Reporter

Fluorescence based assay compatible with:

1. **Plate readers**
2. **Real-time instruments**
3. **Flow cytometry**

MICRO-TAG Bioassay:

Custom built fluorescence cell-based reporter bioassays for monitoring downstream transcription.

Conclusions:

The MICRO-TAG system was developed to provide a differentiated highly sensitive method for real-time monitoring of drug-target interactions within cells. The system has now been expanded to offer assay tools for GPCR drug discovery. The MICRO-TAG assay system can be used to:

(1) detect direct ligand–receptor binding in cells
(2) monitor downstream GPCR signaling
(3) provide sensitive cell-based reporter readouts of agonist or antagonist activity