Development and Application of an Integrated GPCR-Centric Screening Platform for Anti-Obesity Drug Discovery

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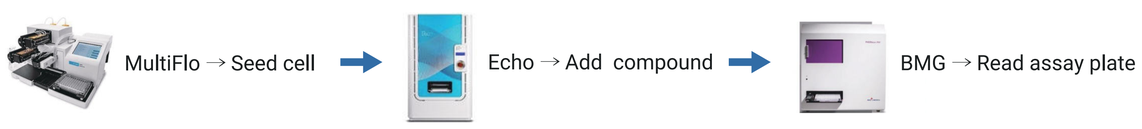
Introduction

Obesity is a significant chronic disease that currently impacts approximately 40% of the global population, with projections from the World Obesity Atlas (WOA)1 indicating this figure could exceed 50% by 2035. Like other chronic conditions, obesity necessitates ongoing, lifelong management. Historically, long-term treatment outcomes for obesity have been disappointing and the challenges for obesity drug discovery include inefficient in vitro systems to evaluate multi-pathway interventions. Our comprehensive platform accelerates GPCR drug discovery by integrating High-throughput screening (HTS) in cell-based assay (reporter assay, HTRF cAMP , β-recruitment) , supporting hit finding, balanced or biased compound (small molecular, peptides, antibody) test.

GPR75 Library Screening in SRE Reporter Assay

HTS by Automation in the function assay



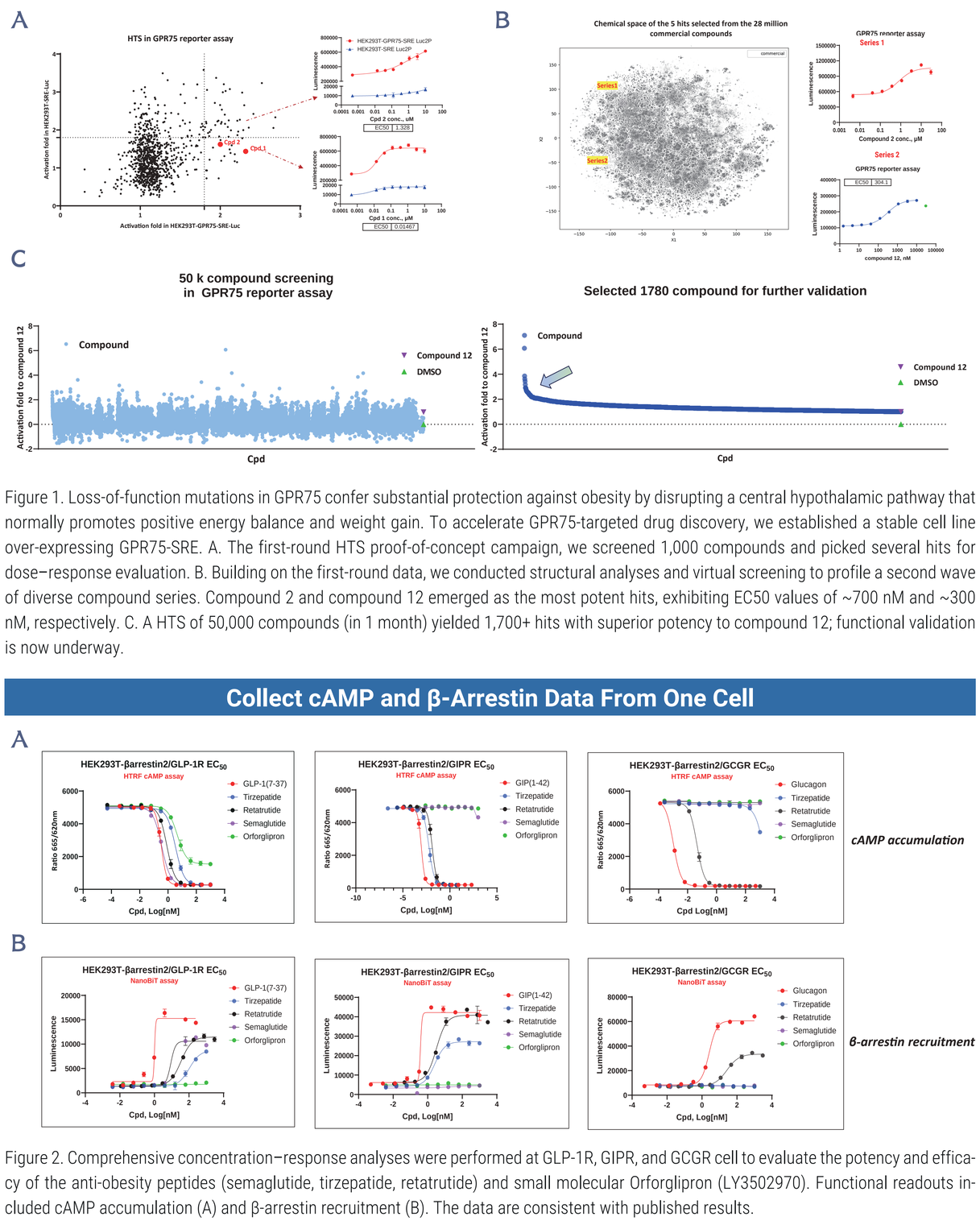
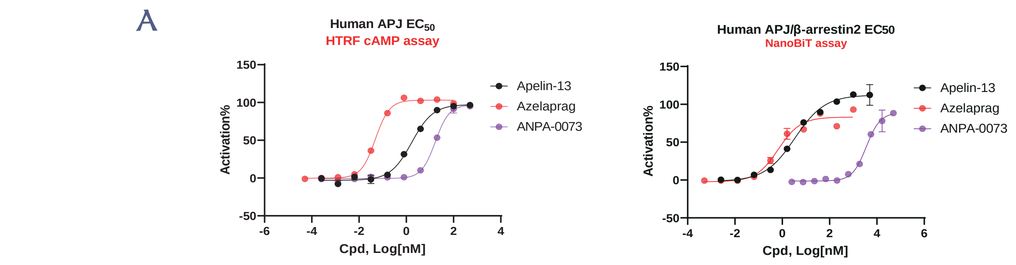


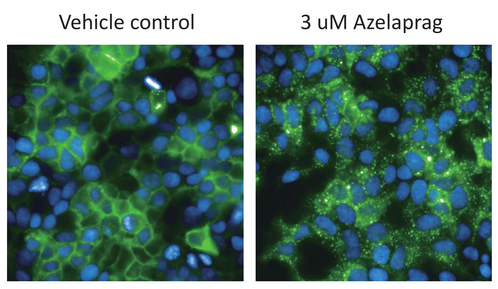
Figure 1. Loss-of-function mutations in GPR75 confer substantial protection against obesity by disrupting a central hypothalamic pathway that normally promotes positive energy balance and weight gain. To accelerate GPR75-targeted drug discovery, we established a stable cell line over-expressing GPR75-SRE. A. The first-round HTS proof-of-concept campaign, we screened 1,000 compounds and picked several hits for dose–response evaluation. B. Building on the first-round data, we conducted structural analyses and virtual screening to profile a second wave of diverse compound series. Compound 2 and compound 12 emerged as the most potent hits, exhibiting EC50 values of ~700 nM and ~300 nM, respectively. C. A HTS of 50,000 compounds (in 1 month) yielded 1,700+ hits with superior potency to compound 12; functional validation is now underway.

Collect cAMP and β-Arrestin Data From One Cell

Figure 2. Comprehensive concentration–response analyses were performed at GLP-1R, GIPR, and GCGR cell to evaluate the potency and efficacy of the anti-obesity peptides (semaglutide, tirzepatide, retatrutide) and small molecular Orforglipron (LY3502970). Functional readouts included cAMP accumulation (A) and β-arrestin recruitment (B). The data are consistent with published results.

Cellular Assay Establishment for APJ Bias Compound Screening





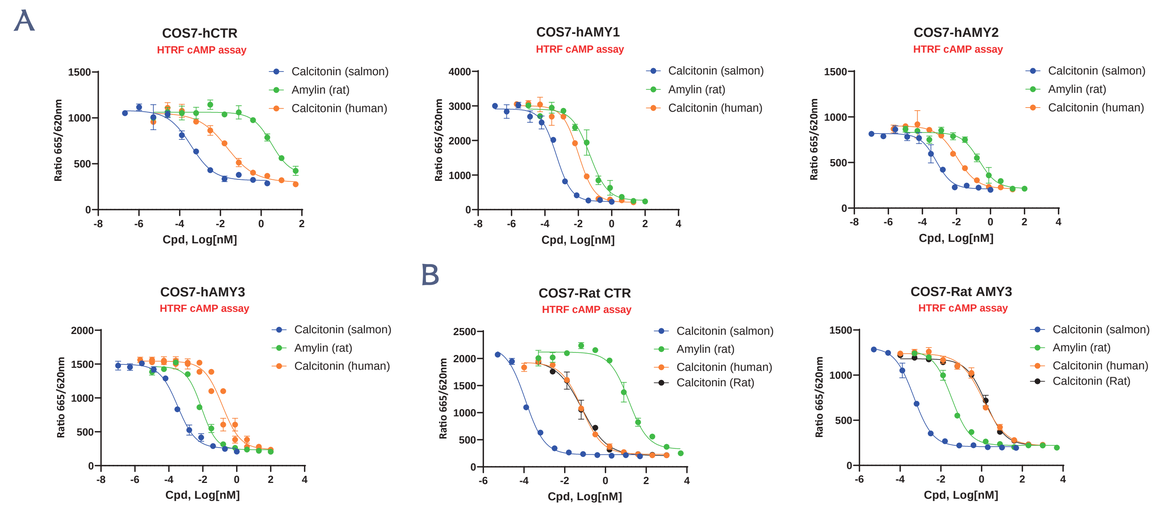
C

Item	Description	Assay	Apelin-13 (EC50 nM)
Cell based assay	hAPJ function assay	HTRF cAMP	1.7
		β-arrestin2 NanoBiT assay	3.4
		Internalization assay	/
	Selectivity	CHO-Mouse APJ	3.35
		CHO-Rat APJ	2.69
		CHO-Monkey APJ	1.77
		CHO-Dog APJ	16.85
	Off-target screening	PDE selectivity panel (10 and 26 targets)	Ready
		GPCR panel (13 targets)	Ready
		Safety panel (44 and 90 targets)	Ready

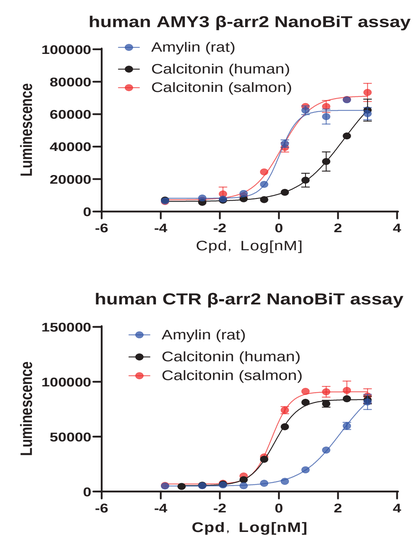
Figure 3. A. HTRF cAMP assay was constructed for compound screening, which is formatted as an immunoassay for quantifying cAMP levels. Azelaprag and ANPA-0073 were used as references, yielding an EC50 of 0.049 nM and 17 nM, respectively. The β-arrestin2 NanoBiT assay was constructed to characterize the APJ and β-arrestin2 recruitment stimulated by Azelaprag and ANPA-0073 in APJ/β-arrestin2 co-expression stable cells. B. HEK293T-APJ-GFP stable cell was constructed to observe the APJ internalization in live cells. C. Multiple cell-based assays were developed for compound validation, meeting target MOA study, species selectivity, and safety panel screening.

Cellular Assay Establishment for AMY3 And CTR

Human and Rat AMY3 And CTR HTRF cAMP assay in COS7 cell



Ligand	cAMP accumulation in hCTR and AMY3-COS7 stable cell, EC50 (pM)						
	hCTR	AMY1R	AMY2R	AMY3R	CTR/AMY1R	CTR/AMY2R	CTR/AMY3R
Calcitonin (salmon)	0.4	0.487	0.661	0.33	0.82	0.61	1.21
Calcitonin (human)	21.17	10.23	10.71	62.24	2.07	1.98	0.34
Rat amylin	3199	48.89	215.8	8.54	65.43	14.82	374.59



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Obesity-Relevant Target Profiling

Target	Cellular Assay Format
5-HT2C*	Agonist
APJ *	Agonist
CALCR *	Agonist
CALCR-RAMP3*	Agonist
CCKAR*	Agonist
CB1R*	Antagonist
GCGR *	Agonist
GIPR*	Agonist/Antagonist
GLP1R*	Agonist
MC4R*	Agonist
NPY1R*	Agonist
NPY2R*	Agonist
NPY4R*	Agonist
GPR40*	Agonist
GPR75*	Antagonist
OX1R*	Antagonist
OX2R*	Antagonist

* Proteins available for antibody screening and structure analysis

Figure 4. Drug discovery targeting the AMY3–CTR receptor heterocomplex is yielding small-molecule and peptide modulators that fine-tune satiety signaling for the treatment of metabolic diseases. A. Cellular assay construction for AMY1, 2, 3, and CTR compound validation. HTRF cAMP assay was constructed for compound screening, which is formatted as an immunoassay for quantifying cAMP levels. Calcitonin (salmon), Calcitonin (human) and Rat amylin were used as references, yielding EC50 in human CTR, AMY1/2/3 stable cell, respectively. B. Rat CTR and AMY3 stable cells were constructed to confirm species selectivity Calcitonin (salmon), Calcitonin (human) , Rat amylin and Calcitonin (Rat) were used as reference compounds. The result was consistent with published data. C. The β-arrestin2 NanoBiT assay was constructed to characterize the hCTR and hAMY3 and β-arrestin2 recruitment stimulated by Calcitonin (salmon), Calcitonin (human) and Rat amylin in HEK-293T-hCTR/arrestin2 and HEK293T-hAMY3/arrestin2 co-expression stable cells. D. Obesity-Relevant Target Profiling. GPCR receptors related to obesity and metabolic disease were chosen to form the ICE_Obesity Panel. Supporting compound validation, meeting target MOA study, species selectivity, and panel screening with multiple cell-based assays.

In VIVO DIO Mode (Semaglutide Test)

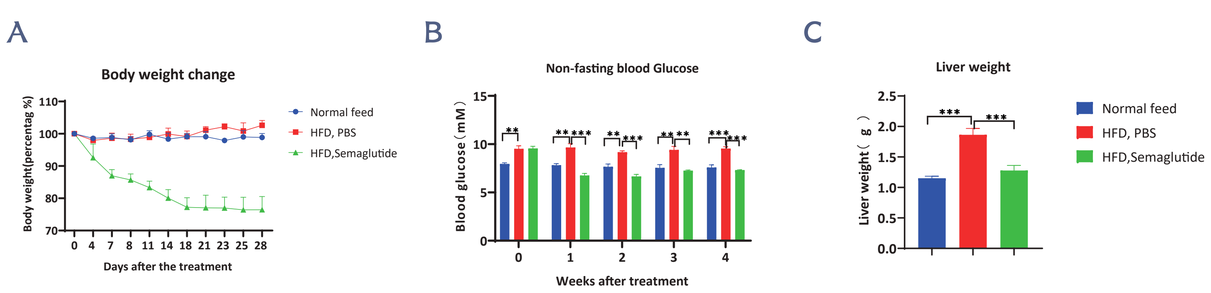

Figure 5. Semaglutide test on DIO mouse model. A. Bodyweight change. B. Non-fasting blood glucose change during experiment. C. Liver weight of endpoint.**p＜0.01, ***p＜0.001. One−way ANOVA.

Summary

This integrated platform enables rapid identification of multi-target anti-obesity agents by combining traditional pharmacology with systems biology approaches. The creation of customed GPCR panel, coupled with the diverse functional assays, offers robust tools for discovery and screening of new drugs.

References

[1] World Obesity Federation, World Obesity Atlas 2023. https://data.worldobesity.org/publications/?cat= 1
[2] A. Manuel Liaci, Vasudha Gathey, Aniket Magarkar, Tobias Kiechle, Camilla Mayer, Giuseppe Bruschetta. The CryoEM Structure of Human GPR75: Insights into ECL2-Mediated Activation. bioRxiv preprint doi: https://-doi.org/10.1101/2025.07.30.667585.
[3] Chen J, Li Z, Zhao Q, Chen L. Roles of apelin/APJ system in cancer: Biomarker, predictor, and emerging therapeutic target. J Cell Physiol. 2022 Oct;237(10):3734-3751. doi: 10.1002/jcp.30845. Epub 2022 Aug 7. PMID: 35933701.
[4] Fletcher MM, Keov P, Truong TT, Mennen G, Hick CA, Zhao P, Furness SGB, Kruse T, Clausen TR, Wootten D, Sexton PM. AM833 Is a Novel Agonist of Calcitonin Family G Protein-Coupled Receptors: Pharmacological Comparison with Six Selective and Nonselective Agonists. J Pharmacol Exp Ther. 2021 Jun;377(3):417-440. doi: 10.1124/jpet.121.000567. Epub 2021 Mar 16. PMID: 33727283.
[5] Kruse T, Hansen JL, Dahl K, Schäffer L, Sensfuss U, Poulsen C, Schlein M, Hansen AMK, Jeppesen CB, Dornonville de la Cour C, Clausen TR, Johansson E, Fulle S, Skyggebjerg RB, Raun K. Development of Cagrilintide, a Long-Acting Amylin Analogue. J Med Chem. 2021 Aug 12;64(15):11183-11194. doi: 10.1021/acs.jmedchem.1c00565. Epub 2021 Jul 21. PMID: 34288673.