

A New Tool to Identify and Pharmacologically Characterize GLP-1 Stimuli

Valentina Casa¹, Sara Montelatici², Laura Stucchi¹, Camilla Trovesi¹, Paola Bonetti¹, Stefania De Cesare¹, Alice Segnali¹, Loredana Redaelli¹, Viviana Agus¹, Alberto Di Silvio¹, Marcel Winnig¹

¹Axxam S.p.A. OpenZone, Via Meucci 3, 20091 Bresso (Milan), Italy

²Kaiserslautern University of Applied Sciences, Amerikastr.1, 66482, Zweibrücken, Germany

Introduction

In the intestinal epithelium, enteroendocrine L-cells serve as specialized chemosensory cells that detect luminal nutrients and secrete Glucagon-like Peptide-1 (GLP-1), a peptide hormone critical for the regulation of glucose homeostasis and appetite suppression.

To detect GLP-1 secretion from enteroendocrine cell supernatants, we optimized and validated a GLP-1 secretion assay. We employed NCI-H716 and STC-1 cell lines, well-characterized models for human and murine intestinal L-cells, respectively, along with a reporter cell line that expresses the recombinant GLP-1 Receptor in combination with the CNG reporter system.

Our high-throughput screening (HTS)-grade GLP-1 secretion assay was functionally assessed using carbohydrate stimuli, including fructose. Additionally, by testing specific receptor agonists, we confirmed a role for Bitter Taste Receptor 38 (TAS2R38) and Free Fatty Acid Receptor 4 (FFAR4, also known as GPR120) in stimulating GLP-1 release from NCI-H716 and STC-1 cells, respectively.

Overall, we developed a powerful tool for identifying and pharmacologically profiling novel GLP-1 stimuli, opening new avenues for obesity and diabetes therapies.

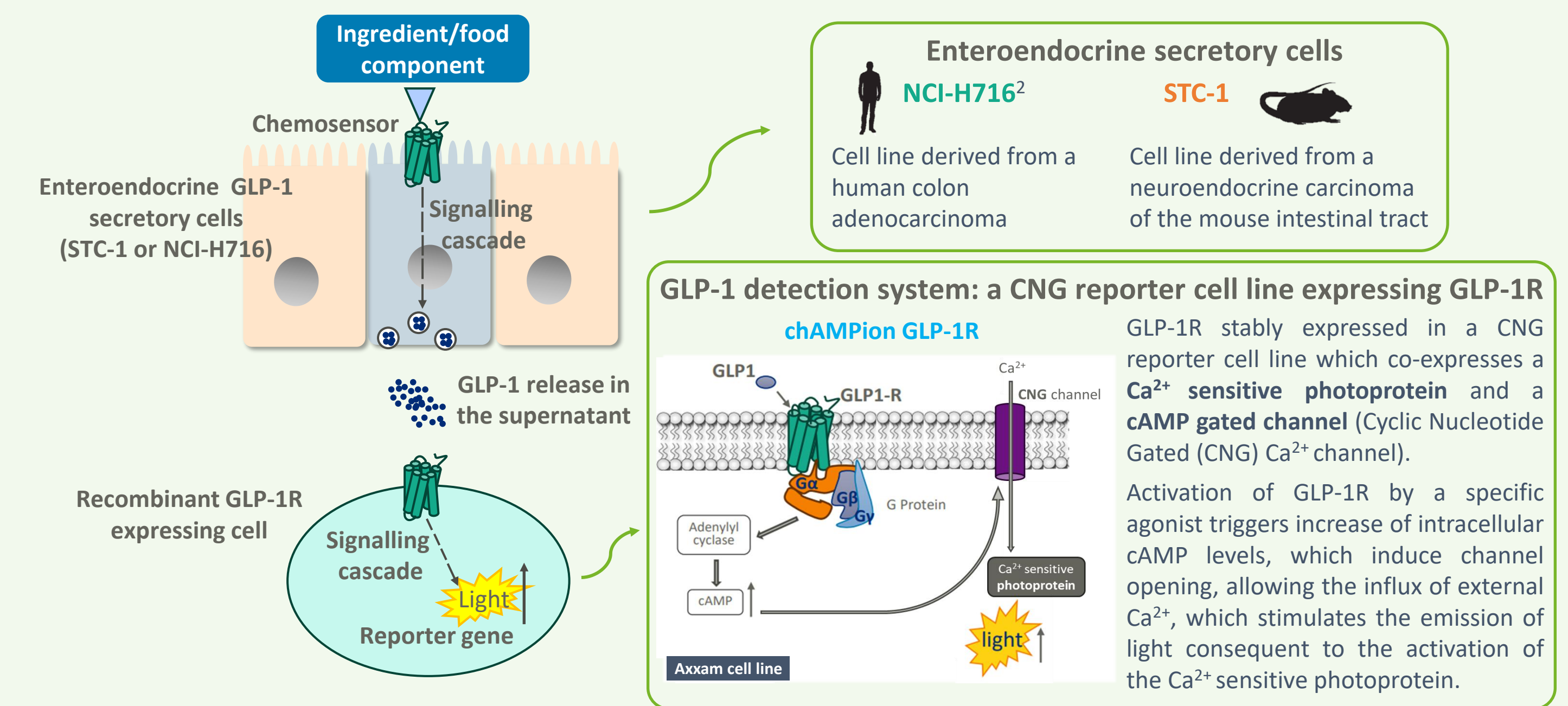
Figure 1.

Axxam's GLP-1 secretion assay is based on the use of human NCI-H716 or murine STC-1 enteroendocrine secretory cells, widely described as model for intestinal L-cells¹.

After stimulation of the secretory cells, the supernatant (possibly containing secreted GLP-1) is collected.

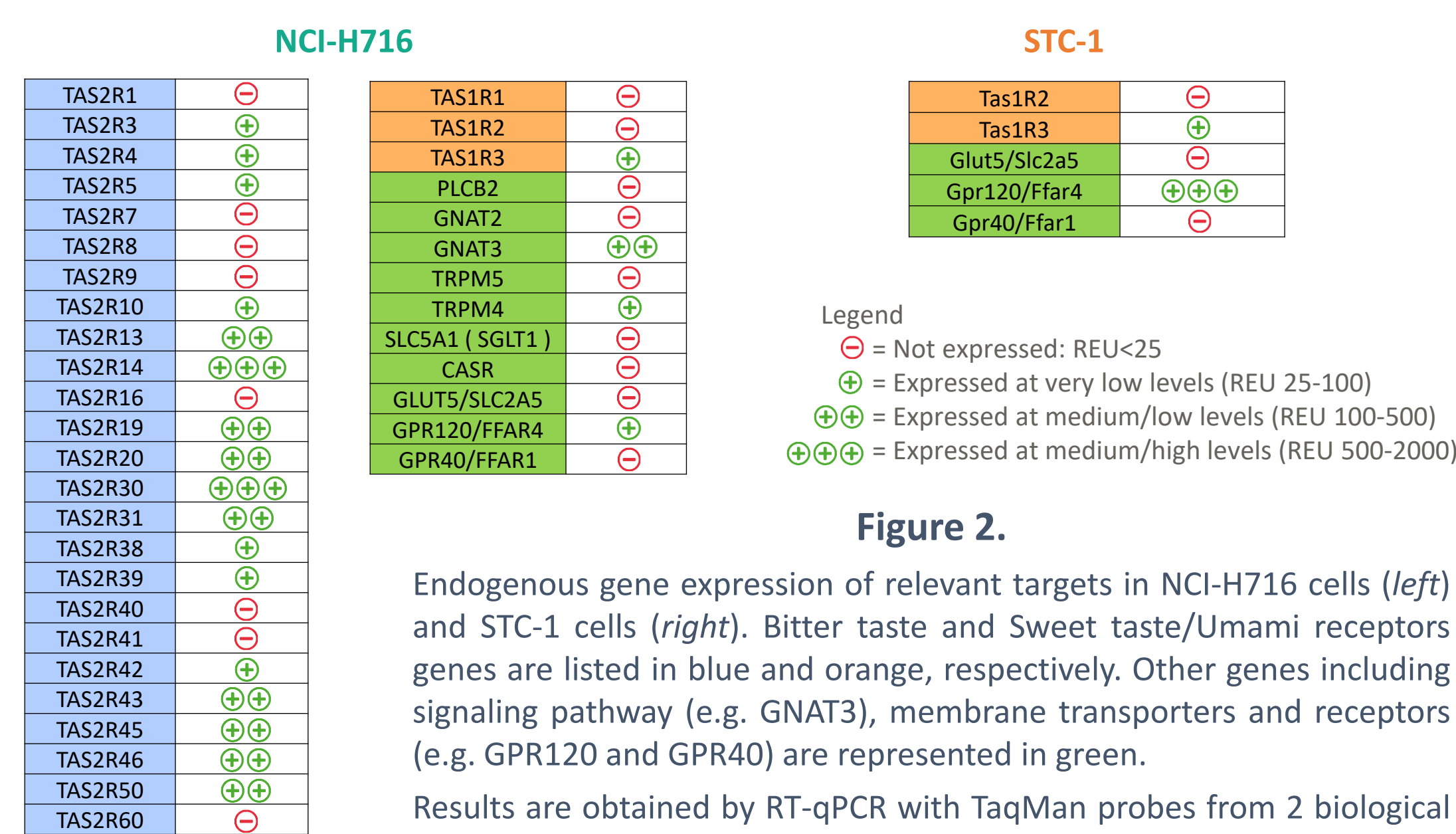
The supernatant is then injected into a GLP-1 receptor (GLP1-R) recombinant cell line that co-expresses an appropriate functional readout. This setup enables the measurement of GLP1-R activation in relation to the concentration of GLP-1 present in the supernatant.

A LDH-Glo Cytotoxicity assay (from Promega) is run in parallel to rule out any unspecific release of GLP-1 due to a putative loss of cell membrane integrity.

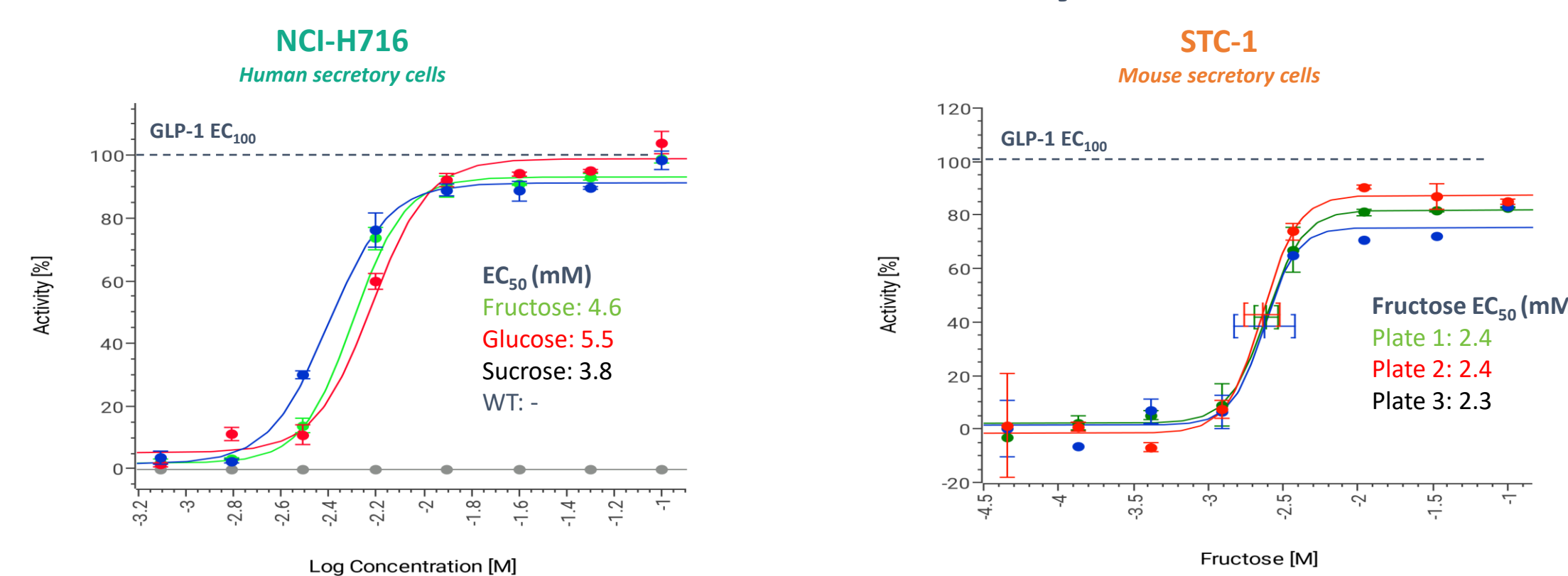


Results

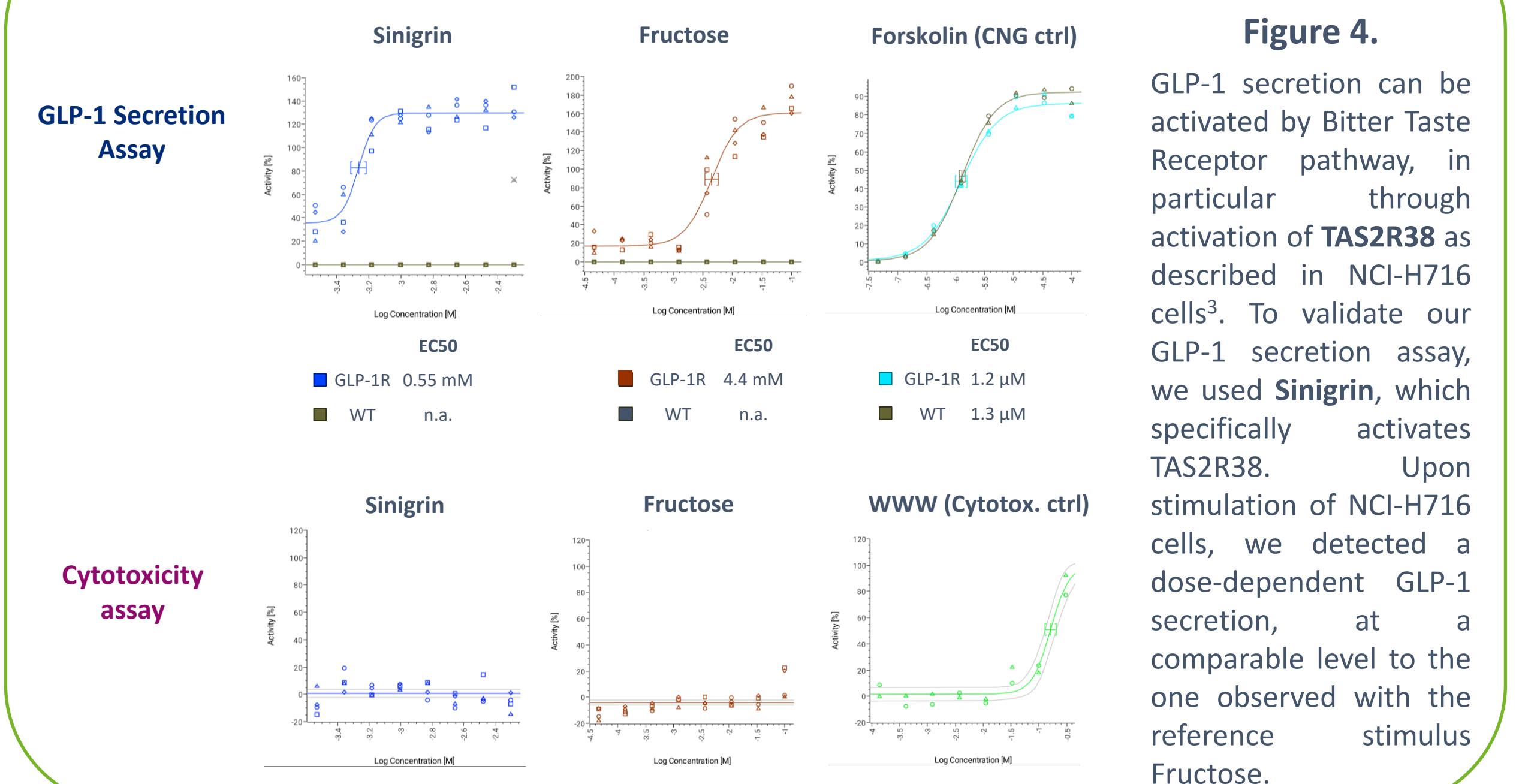
Endogenous gene expression levels of relevant targets



GLP-1 Secretion Assay

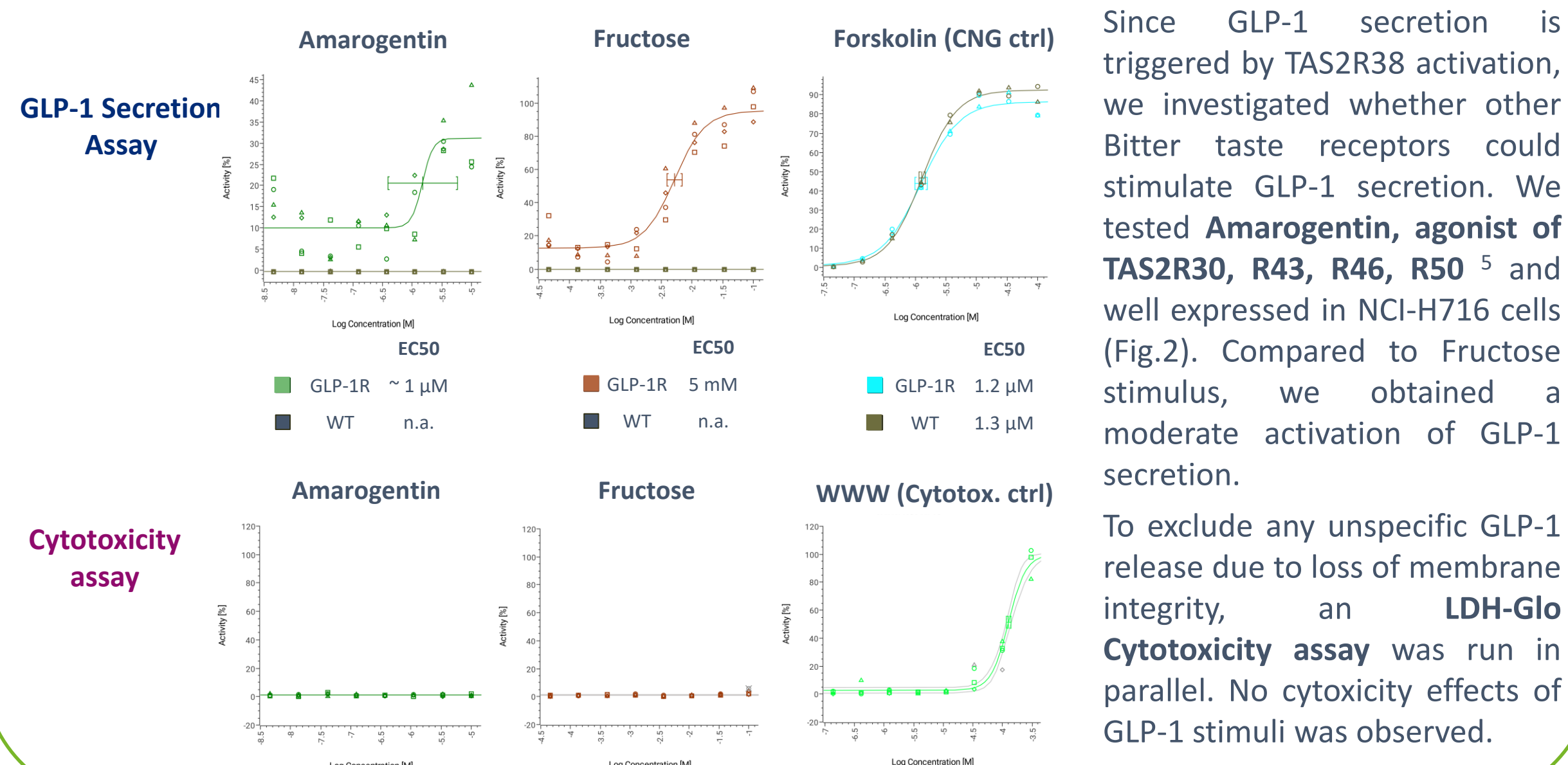


NCI-H716 GLP-1 Secretion Assay: Sinigrin (TAS2R38 agonist)



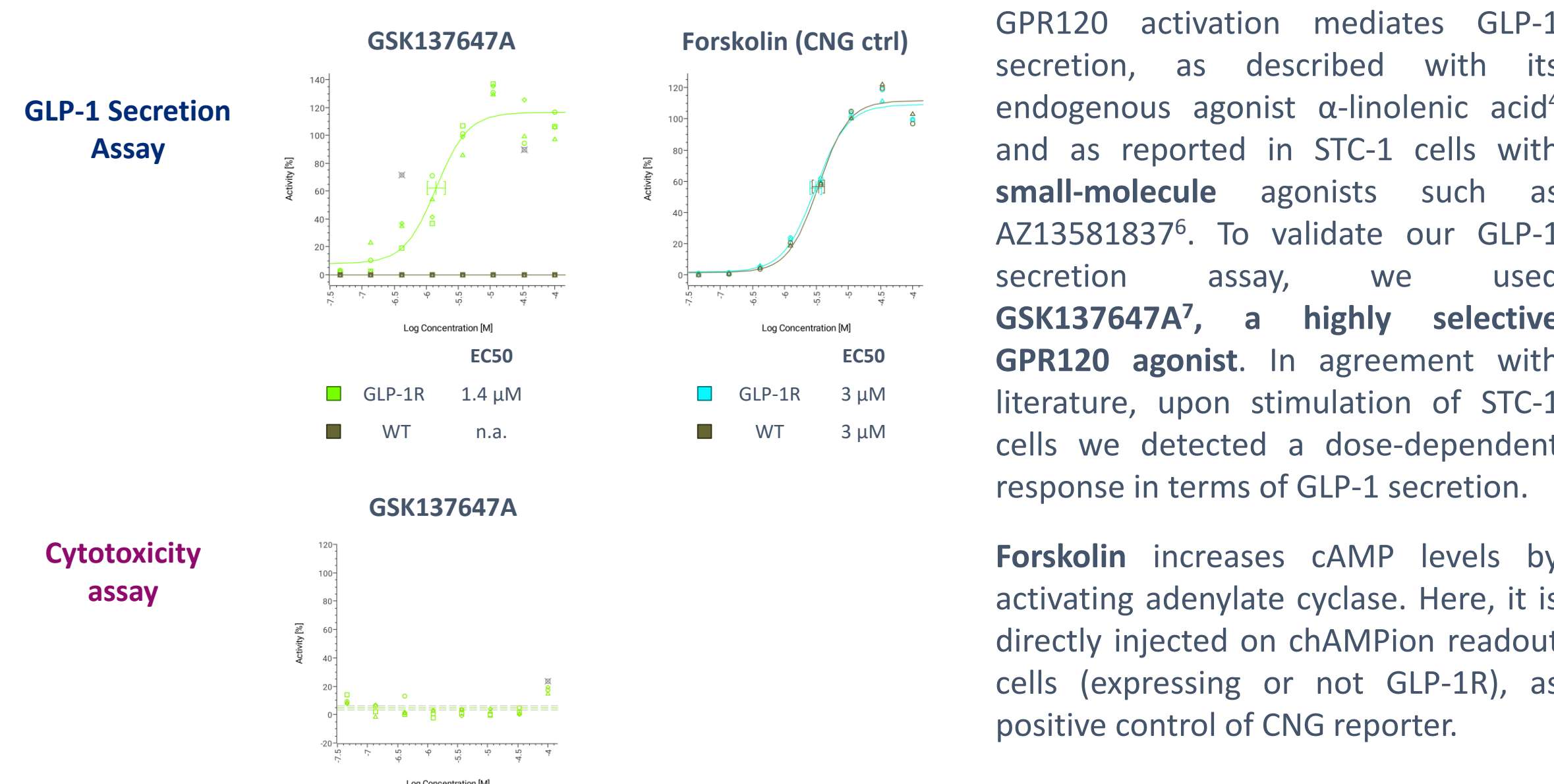
NCI-H716 GLP-1 Secretion Assay: Amarogentin (TAS2R30, R43, R46, R50 agonist)

Figure 5.

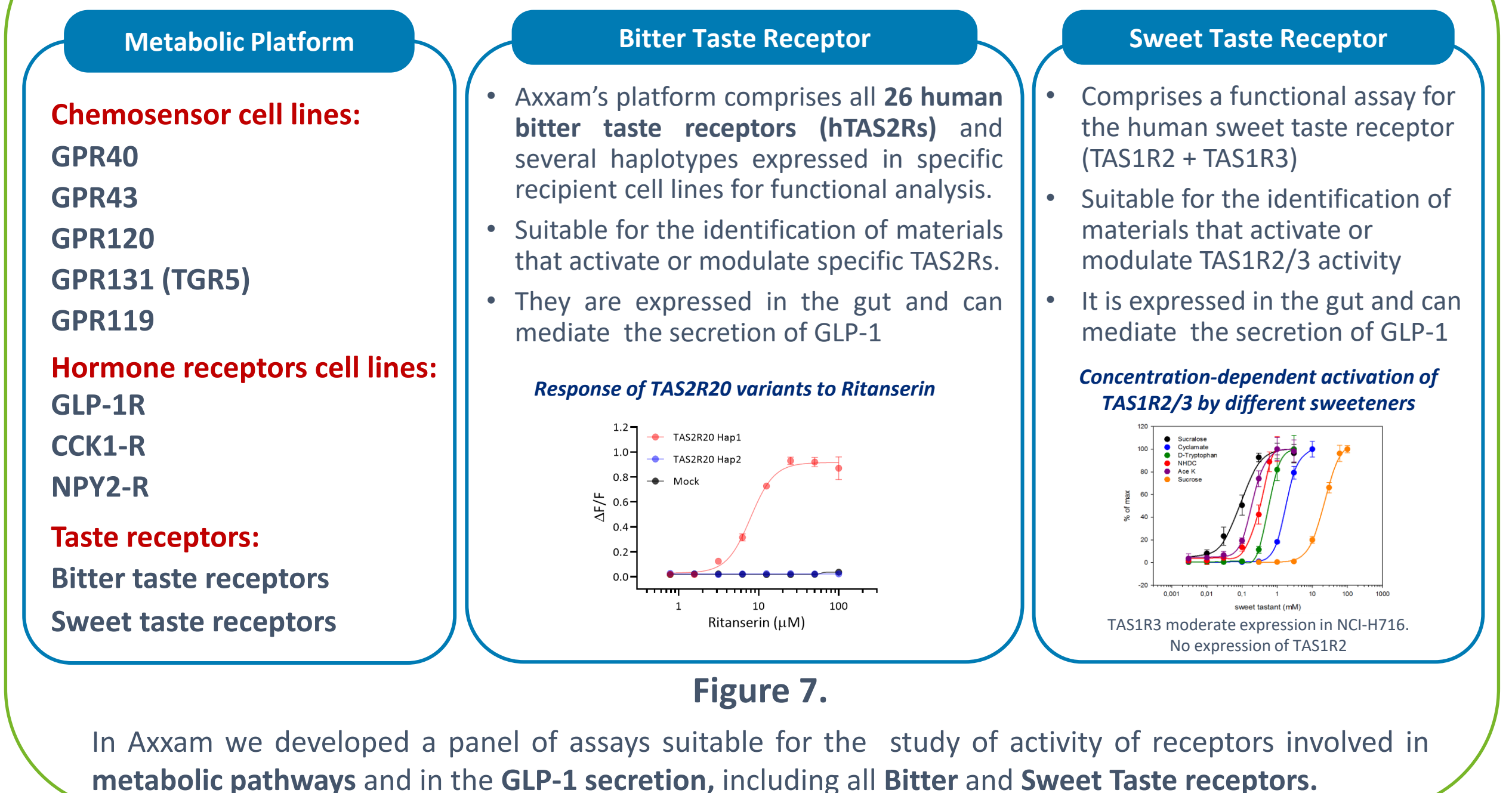


STC-1 GLP-1 Secretion Assay: GSK137647A (GPR120 agonist)

Figure 6.



Axxam's metabolic platform



Conclusions

AXXAM developed a screening platform for the identification of novel stimuli for GLP-1 secretion, based on the use of human NCI-H716 or murine STC-1 enteroendocrine secretory cells, widely described as a model for intestinal L-cells. Our tool has been validated with targets described in literature, including Bitter Taste Receptor 38 (TAS2R38) and Free Fatty Acid Receptor 4 (FFAR4/GPR120) respectively in NCI-H716 and STC-1 cells, demonstrating its applicability for the identification of new therapeutic strategies in the treatment of diabetes and obesity.

¹ Kuhre et al., 2016; doi: 10.1530/JME-15-0293

² Axxam hereby acknowledges that the NCI-H716 cell line (ATCC Ref. No. CCL-251), as described in R. Phelps et al. Journal of Cellular Biochemistry Supplement (1996) 24:32-91 (HHS Ref. No. E-112-2007-0), has been developed in the laboratories of Drs. Gazdar and Minna at the National Cancer Institute.

³ Yu et al., 2015. doi: 10.1016/j.bcp.2015.07.012.

⁴ Hirasawa et al., 2005; DOI: 10.1038/nm1168

⁵ BitterDB: Ziaikin et al., 2024; doi: 10.1093/nar/gkae1044

⁶ Sundstrom et al., 2017; https://doi.org/10.1371/journal.pone.0189060

⁷ Martin et al., 2012; DOI 10.1194/jlr.M025874

