# Identification of FoxO1 inhibitors using large scale transiently transfected assay-ready cells in HTS

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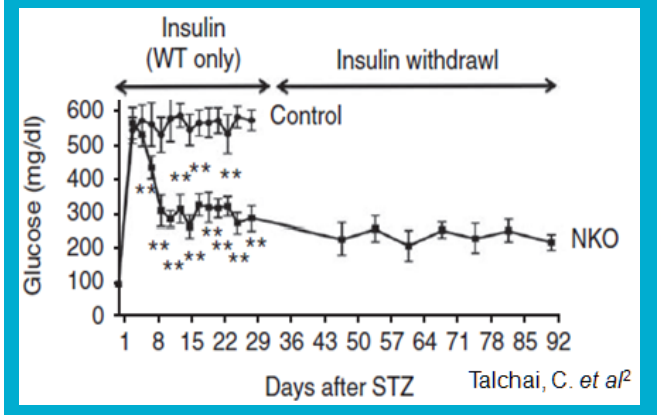
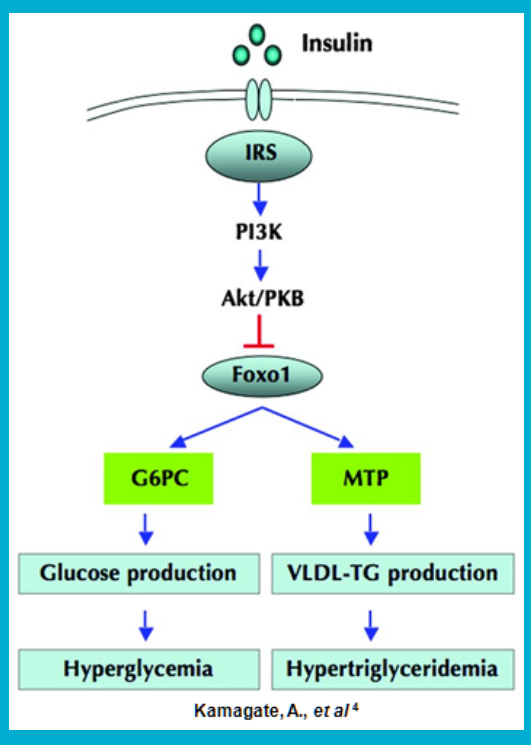
## Introduction

There is a large unmet medical need for improved treatment of Type I diabetes which is increasing at a rate of 3% per year[1] and is a result of autoimmune destruction of insulin-producing β-cells of the pancreas.

Pancreatic and enteric endocrine cells, including β-cells, arise from Neurog3+ progenitors in which FoxO1 is widely expressed. Ablation, or inhibition, of FoxO1 in mouse Neurog3+ enteroendocrine progenitor cells or in human iPS cells-derived gut organoid cultures generates insulin-producing cells that secrete insulin in response to glucose[2,3].




FoxO1 is regulated by a complex signalling cascade and by phosphorylation, acetylation, ubiquitination and sub-cellular localization. For example, insulin inhibits FoxO1 activity via Akt-dependent phosphorylation, resulting in FoxO1 nuclear exclusion. In relation to diabetes, loss of insulin inhibition of FoxO1 activity results in excessive production of both glucose and VLDL-TG, contributing to hyperglycemia and hypertriglyceridemia in diabetes[4].




FoxO1 is an intrinsically disordered transcription factor with no known ligand binding domain resulting in the inability to use biochemical or biophysical assay strategies to identify small molecule inhibitors.

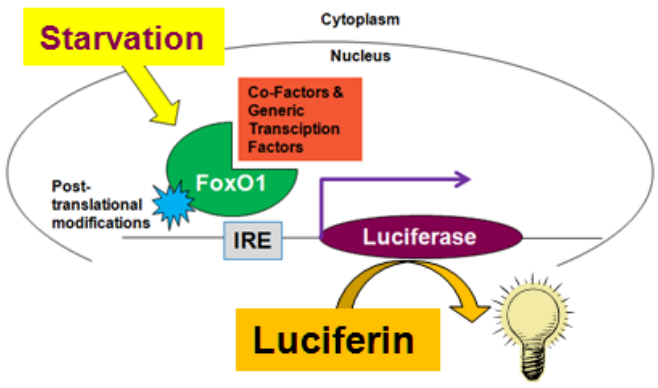
We developed cell-based assays using large scale transiently-transfected assay-ready cells. Optimization of the cell generation process resulted in reduced costs and improved assay performance. These cells were used in HTS and downstream screening cascade to identify FoxO1 inhibitors that have the potential to be therapeutically beneficial.

## Methods

**Assay-ready cells and reporter gene assay:** Reporter gene assays were chosen for hit identification to increase the likelihood of identifying compounds affecting FoxO1-dependent transcriptional activity through numerous mechanisms (e.g., signalling, nuclear export, degradation, protein-protein interactions).

**Cell scale up**: HEK293s cells expanded into 26 Corning 10-layer Cell Stacks over 12 days yielding ~2x10[10] cells.

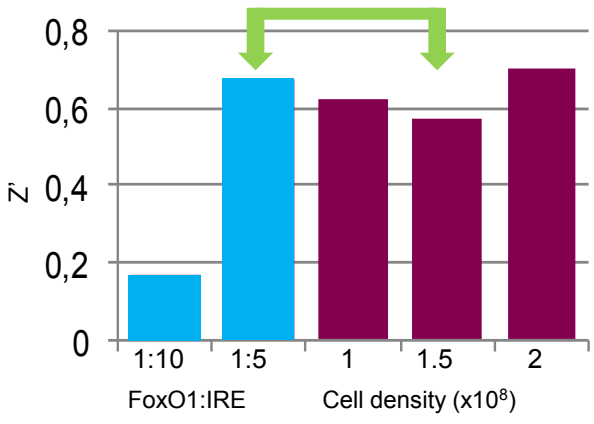
**Transfection**: Cells were MaxCyte flow electroporated with 2 vectors (FoxO1 and luciferase reporter containing 4x insulin response element (IRE)) then cryopreserved.




**Assay**: Thawed cells were added directly to plates containing compounds in starvation media (5mM glucose & 1% FBS) to activate FoxO1, incubated 24 hours then measured for luciferase activity.

## Results

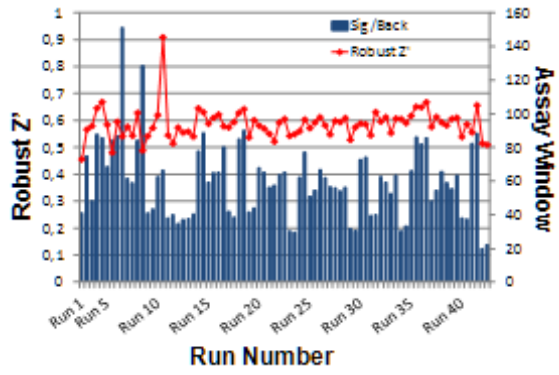
**Optimization of assay-ready cell generation:** Cell quality and assay performance enhanced through optimization of the FoxO1:IRE DNA ratio and increasing transfection cell density resulting in 33% reduction of DNA used while maintaining assay performance.



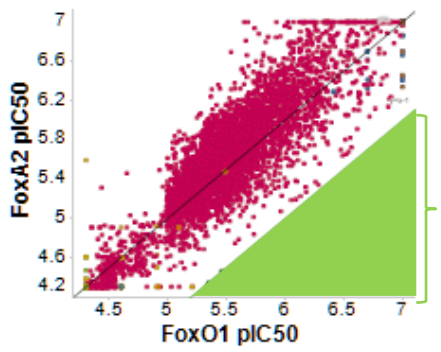

**Reproducible assay-ready cell batches:** 4 transfection batches generated 2.8x10[10] assay-ready cells with consistent yields and assay performance.

| Batch | Cell Yield | Assay z' |
|---|---|---|
| 1 | 7.7x10⁹ | 0.60 |
| 2 | 7.2x10⁹ | 0.67 |
| 3 | 7.0x10⁹ | 0.61 |
| 4 | 6.2x10⁹ | 0.68 |

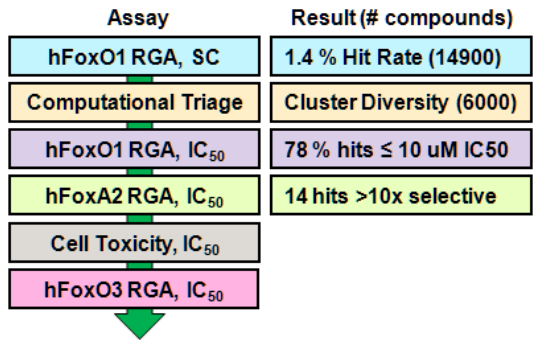
**Primary high-throughput screen:** 1 million compounds screened in 42 runs using pooled cell batches with robust assay performance and consistent data throughout the screen.



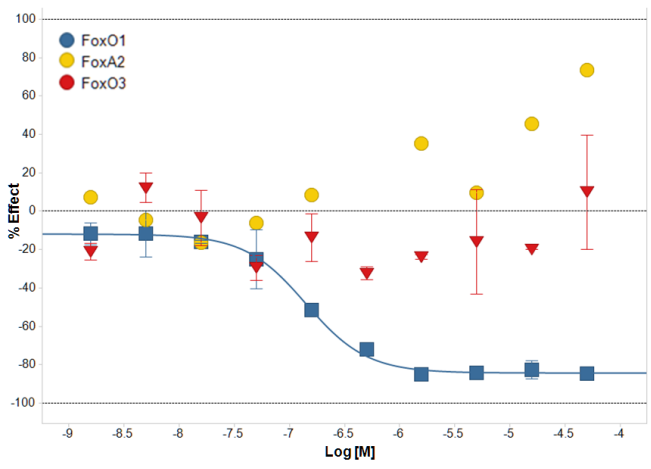

**Hit IC50 and selectivity:** Primary hits IC50s and selectivity against FoxA2 were determined. A low frequency of selective hits was observed.




**Screening cascade:** Transiently transfected assay-ready cells were used to drive the screening cascade.




**Selectivity profile:** Selective, potent FoxO1 inhibitors have been identified.




## Conclusions & next steps

- Optimization provided a 33% reduction in DNA and transfection consumable costs
- Transiently transfected assay-ready cells were generated to support HTS and screening cascade
- Potent, selective FoxO1 inhibitors identified
- Work is ongoing to determine inhibitor MoA and their ability to generate insulin-producing cells from enteroendocrine progenitors

**References**

1.International Diabetes Federation. Diabetes Atlas, 5th ed., 2011.
2.Talchai, C. *et al* (2012) *Nat Genet.*, 44, 406-412.
3.Bouchi, R. *et al* (2014) *Nat Commun.*, 30, 4242.
4.Kamagate, A., *et al* (2008) *J. Clin. Invest.* 118:2347.

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