Supporting Information

Bioluminescent Assay for the Quantification of Cellular Glycogen Levels

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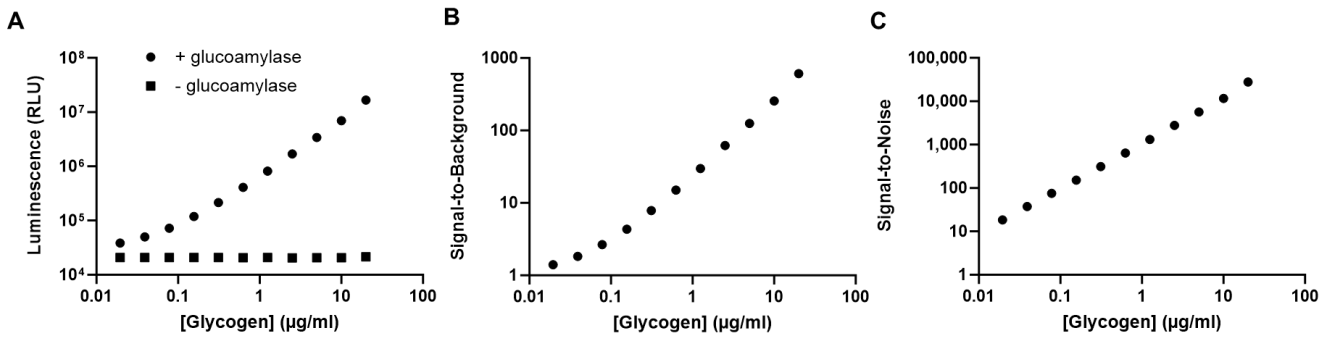


Figure S1. Parameters of the glycogen detection assay. (A) A purified stock of glycogen was serially diluted two-fold in PBS, starting from 20 µg/ml, and assayed in the presence and absence of glucoamylase. A negative control (assay background) containing PBS without glycogen was included. Each concentration was tested in quadruplicate in the wells of a 96-well plate. The average RLU are plotted. Error bars are +/- 1 SD. Percent CVs were <5%. (B) The average RLU was used to calculate signal-to-background ratios. (C) The average net RLU and standard deviation of the background control were used to calculate the signal-to-noise ratio.

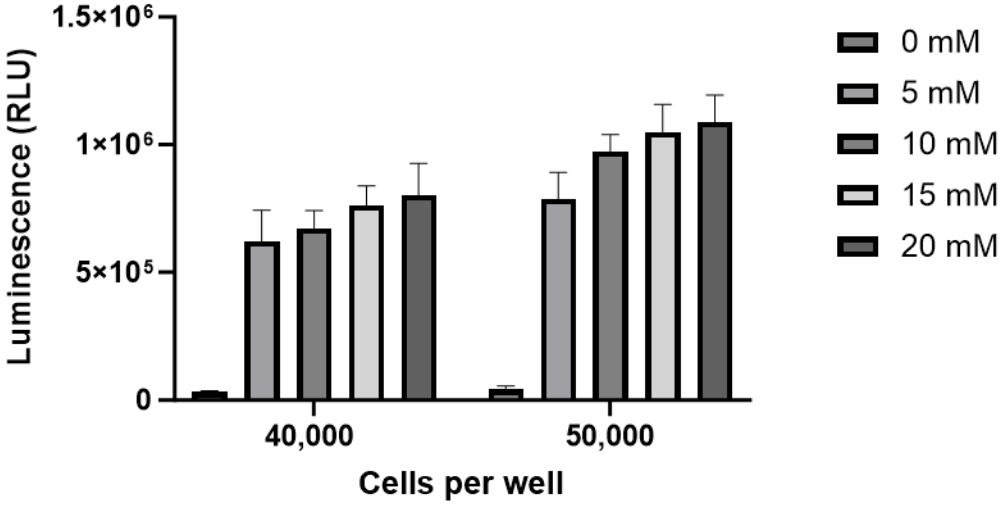


Figure S2: Accumulation of glycogen. After an overnight starvation in flasks, HeLa cells were collected and washed and plated in the wells of a 96-well plate at the indicated cell number per well. They were then incubated overnight in the presence of medium containing 0 to 20 mM glucose before measuring glycogen. Each condition was assayed in quadruplicate and the average RLU are plotted. Error bars are +/- 1 SD.

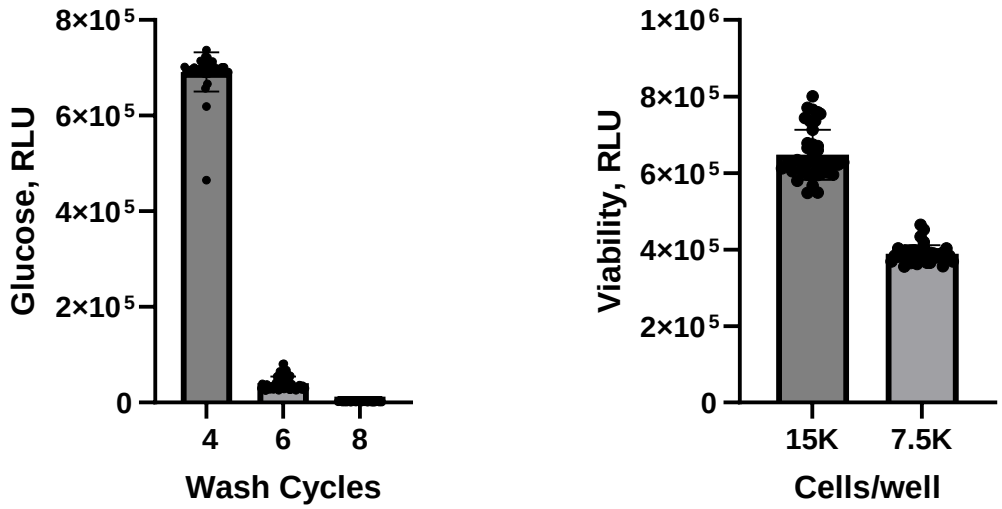


Figure S3. Validation of the automated protocol. (A) Measurement of residual glucose after the indicated number of wash cycles. (B) Viability measurements after the 8th wash cycle using the CellTiter-Glo Assay. In both panels, each data set is for 40 wells.

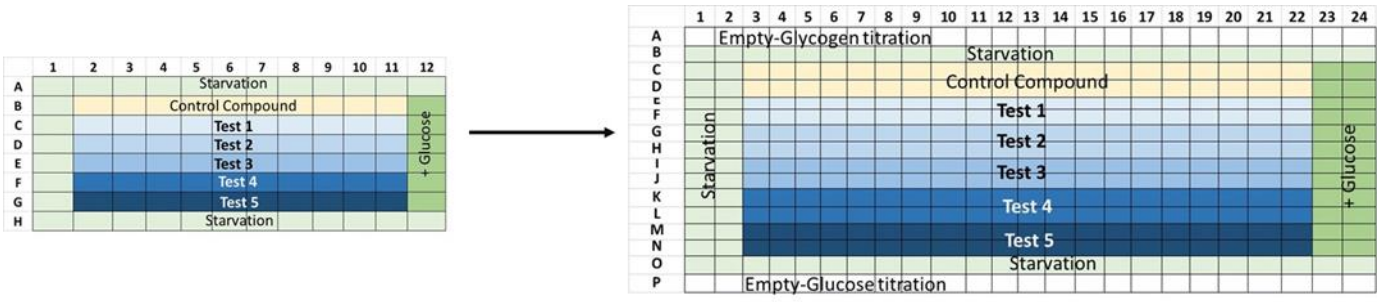
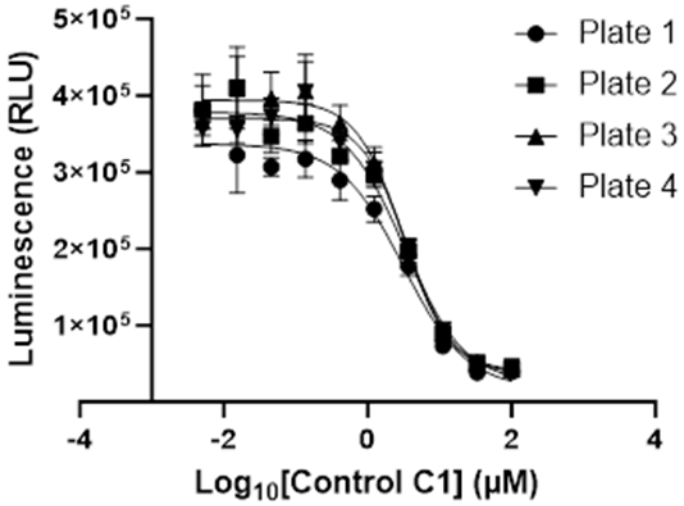


Figure S4. Layout of inhibitor dilution (96-well) and cell-based screening (384-well) plates. Compounds were diluted in each 96-well plate and then transferred to a corresponding 384-well plate. Rows A and P were reserved for glycogen and glucose titrations in the final assay plates.

A



B

	Plate 1	**Plate 2**	**Plate 3**	**Plate 4**
IC_{50}, µM	3.1	3.2	3.1	3.5
Signal Window	9.6	8.8	8.6	7.8

C

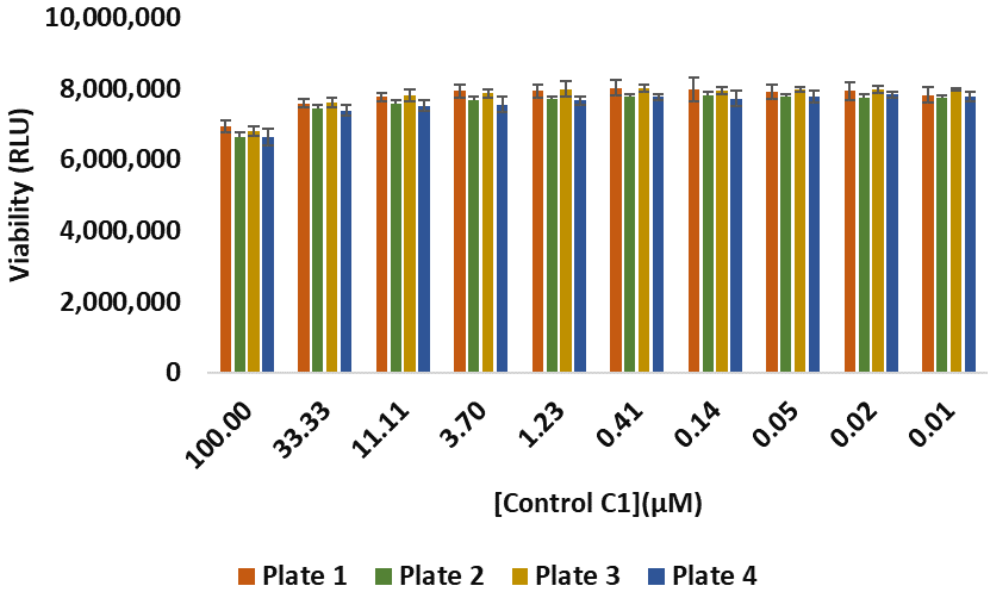


Figure S5. Control compound C1 performance across four plates. (A) Control compound C1 was assayed in a 10-point dose response curve on each of the four screening plates in Figure 6. Each concentration was tested in quadruplicate. The average RLU was calculated. Error bars are +/- 1 SD. (B) The IC_{50} and maximum signal window for each of the plates. The signal window was calculated as the ratio of signal at low and high compound concentrations. (C) Cell viability was assessed using the RealTime-Glo Assay prior to cell lysis as described in the Methods section.

Table S1: Automated Screening Protocol

	Step	Volume, time	Details
1	Cells	24 h	Culture in T75 flasks overnight in starvation medium.
2	Cells	25 µl	Collect cells, wash, and resuspend to 600,000 cells/ml in starvation medium. Transfer 25 µl (15,000 cells) to the wells of a 384-well plate.
3	Library Compounds		10mM compounds in DMSO. Prepare 10-point dilution series starting with 200 µM (or 60 µM) compound in recovery medium with 20 mM glucose and 2% DMSO.
4	Add Compounds to Cells	25 µl	Transfer 25 µl to cells in 384-well plate. (Cells are now in 10 mM glucose.) Assay each compound dilution in quadruplicate wells.
5	Incubate	24 h	37°C, 5% CO_2
6	Cell Viability measurement	30-60 min	RealTime-Glo Assay Record luminescence.
7	Wash	8 cycles	Remove media. Wash with PBS, 70 µl per wash cycle. After final wash, remove PBS, leaving 15 µl.
8	Lyse	10 min	Add 7.5 µl 0.3N HCl to cells. Followed by addition of 7.5 µl 450 mM Tris pH 8.0.
9	Freeze plates	overnight	Seal plates and freeze overnight at -20°C
10	Glycogen digestion	30 µl	Thaw cell lysate plates. Prepare glucoamylase solution and add 30 µl to well containing cell lysate.
11	Incubation	1 h	37°C or Room temperature
12	Glucose measurement	8 µl	Transfer 8 µl to wells of 384-well plate for the glucose detection assay.
13	Glycogen Assay readout	90 min	Add 8 µl glucose detection reagent. Incubate 90 min at RT. Record luminescence.

Table S2: Summary of data for experiment in Figure 8

	Avg RLU	SD	%CV
Cells, 10 mM glucose	350,201	21,184	6.0%
Cells, 0 mM glucose	35,519	6,407	18.0%
No cells (assay background)	11,111	1,788	16.1%

Signal Window = ratio cells 10mM/ cells 0 mM = 9.9

Signal to Background = ratio cells 10 mM / no cells = 32

Signal to Background = ratio cells 0 mM/ no cells = 3.2

Signal to Noise = (cells 10 mM – cells 0 mM)/ SD 0 mM = 49

Signal to Noise = (cells 10 mM – no cells)/ SD no cells = 190

Signal to Noise = (cells 0 mM – no cells)/ SD no cells = 14