Supplementary Information

A human islet cell-culture system for high-throughput screening

Deepika Walpita[1], Thomas Hasaka[†2], James Spoonamore[†2], Amedeo Vetere[1], Karen K. Takane[3], Dina Fomina-Yadlin[1,4], Nathalie Fiaschi-Taesch[3], Alykhan Shamji[1], Paul A. Clemons[1], Andrew F. Stewart[3], Stuart L. Schreiber[1,4,5,6], and Bridget K. Wagner[*1]*

Affiliations:

[1] Chemical Biology Program, [2] Chemical Biology Platform, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142

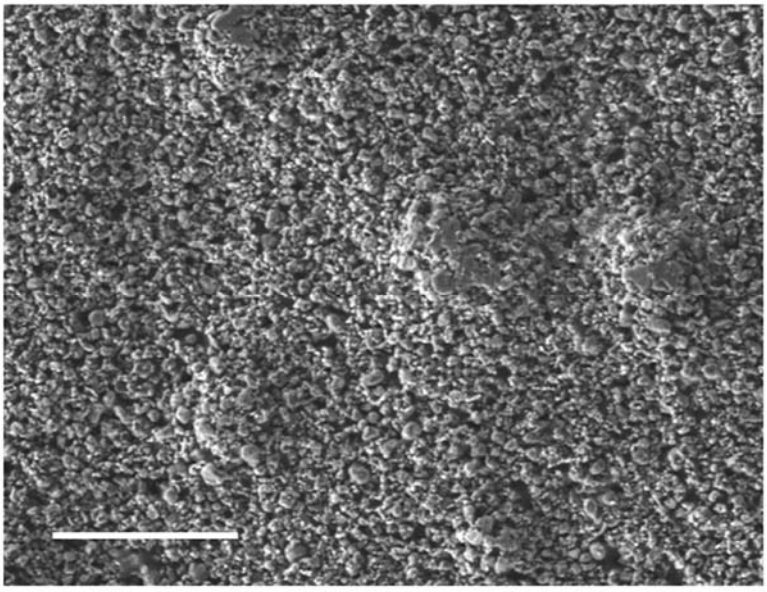
[3] Department of Endocrinology, University of Pittsburgh Medical School, Pittsburgh, PA 15213

[4] Department of Molecular and Cellular Biology, [5] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

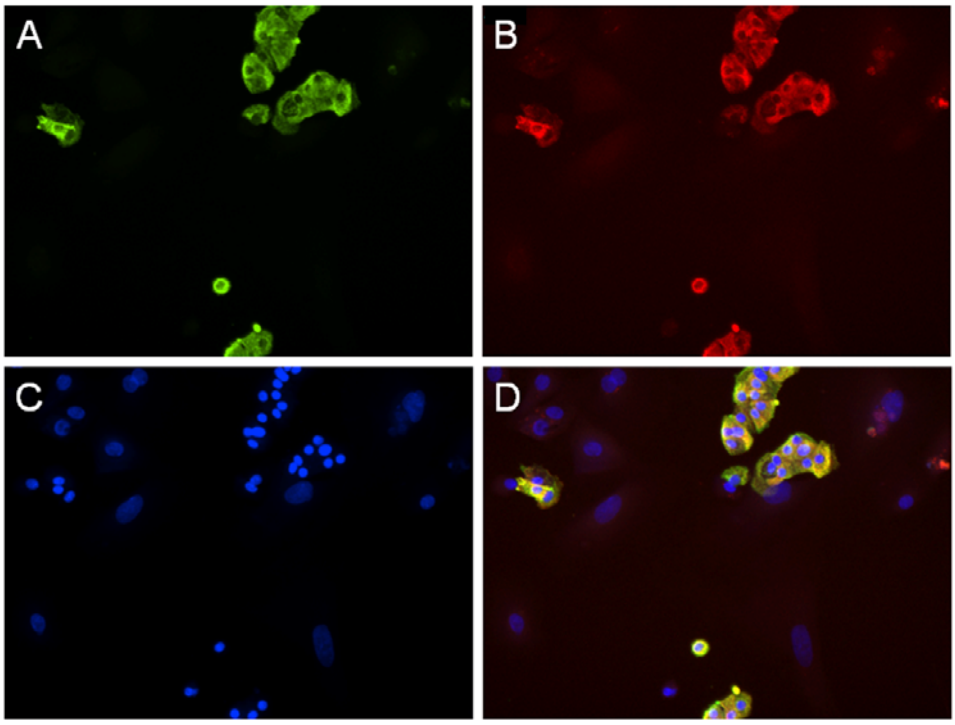
[6] Howard Hughes Medical Institute, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142

* Corresponding author email: bwagner@broadinstitute.org.

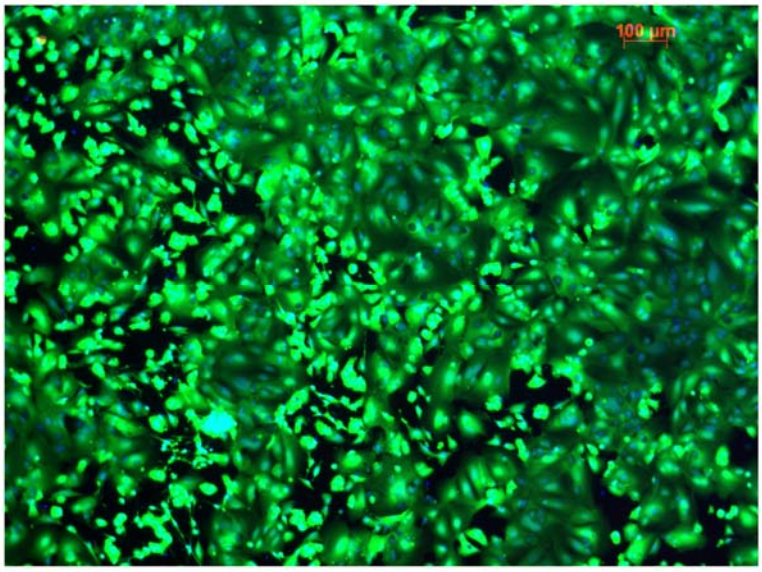
[†] These authors contributed equally to this work.



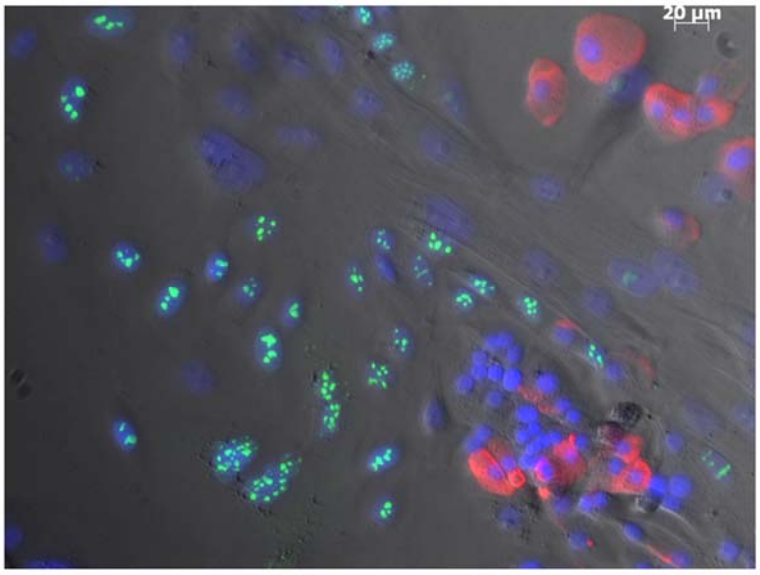
Supplementary Figure S1. Electron micrograph of the surface of a 96-well plate containing extracellular matrix from HTB-9 cells grown and denuded. Scale bar = 50μm.



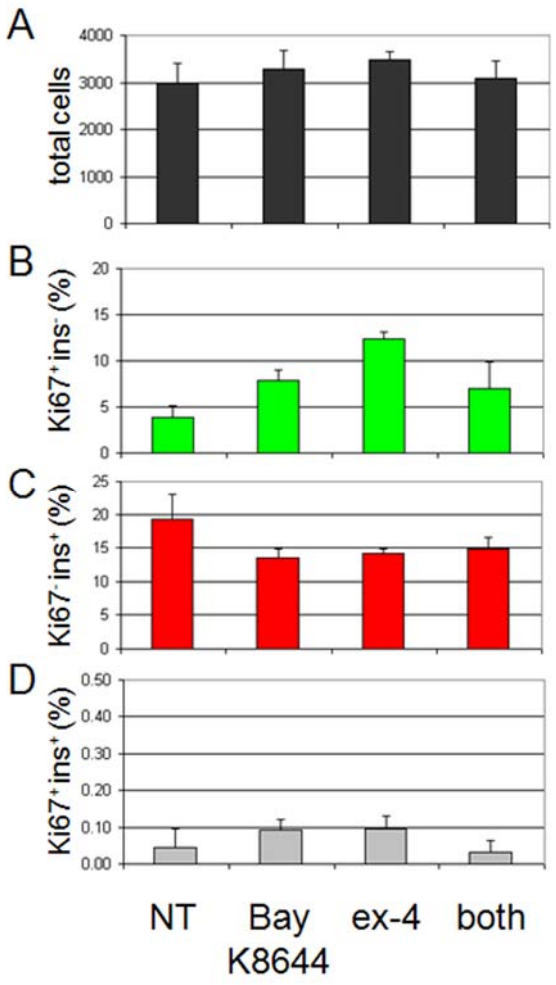
Supplementary Figure S2. Immunofluorescence of dissociated islet cells cultured on ECM. Evaluation of overlap between C-peptide (a cleavage product of proinsulin) and insulin stains. Cells were stained for **(A)** C-peptide (green), **(B)** insulin (red), and **(C)** nuclei were stained with DAPI (blue). **(D)** Image overlay.



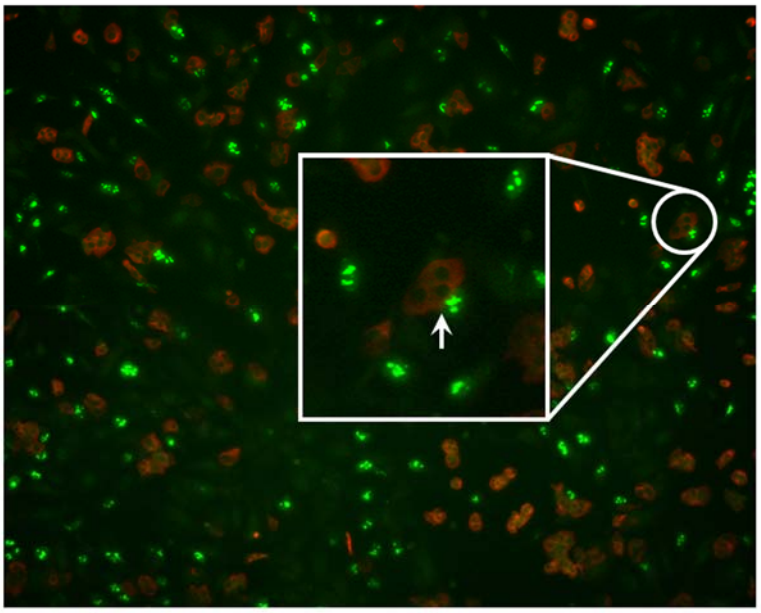
Supplementary Figure S3. Dissociated islet cells were plated in a 96-well plate, and viability assessed by the fluorescent vital stain calcein-AM.



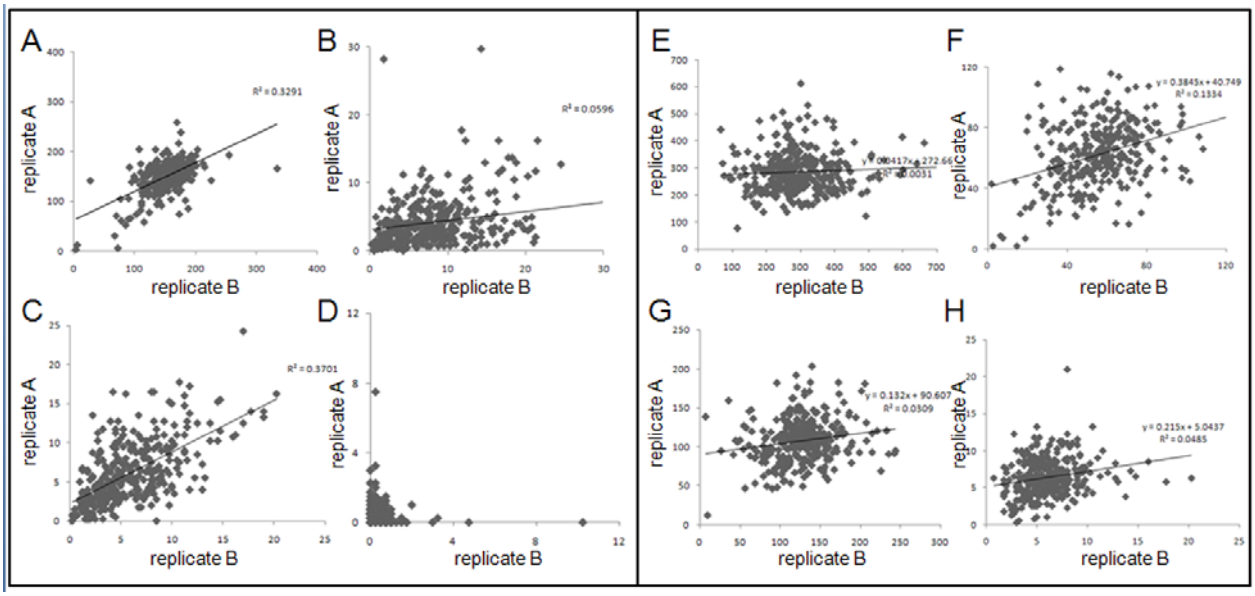
Supplementary Figure S4. Proliferating cells within dissociated islets appear fibroblastic in nature. Overlay of bright-field, nuclei (blue), Ki67 for proliferation (green), and C-peptide for beta cells (red).



Supplementary Figure S5. Dissociated islets were treated for three days with DMSO (NT), 10μM Bay K8644, 100nM exendin-4 (ex-4), or both. Cells were fixed and stained as described in the text, and populations quantified: (A) total cells, (B) percentage of proliferating non-beta cells, (C) percentage of quiescent beta cells, and (D) percentage of proliferating beta cells.



Supplementary Figure S6. Untreated dissociated islet cells were stained for Ki67 (green) and C-peptide (red). Inset shows single cells falsely called positive during automated image analysis.



Supplementary Figure S7. Analysis of screening performance reproducibility with donors 2 and 3 (see Supplementary Table S1). The two replicates are plotted against each other examining **(A,E)** total cells, **(B,F)** proliferating C-peptide-negative cells, **(C,G)** quiescent C-peptide-positive beta cells, and **(D,H)** proliferating beta cells. The correlations of each dataset to a linear fit are shown. Note that the scales for each donor are very different, due to donor variability and variability in islet isolation procedures.

Supplementary Table S1. Donor information for islets used in assay development and screening. [a] Samples used for pilot screening. [b] Samples used for replicate analysis.

Donor	Age	Race	Gender	BMI	Purity	Note
1[a]	12	--	--	34.0	95%	
2[a]	47	W	F	27.6	85%	
3[a]	36	--	F	37.4	95%	
4[b]	58	B	M	66.6	75%	T2D
5	49	W	M	30.5	75%	T1D
6	52	W	F	38.8	85%	
7[b]	42	--	--	22.0	70%	
8[b]	38	W	F	31.6	90%	
9	47	W	M	23.0	85%	
10	30	W	M	30.4	45%	
11	55	W	F	23.3	85%	
12	52	W	F	29.1	75%	
13	40	W	F	24.4	85%	
14	39	W	M	26.9	90%	
15	32	W	M	27.2	85%	
16	31	W	M	30.7	85%	
17	33	W	F	26.0	80%	
18	53	W	M	31.0	30%	

Supplementary Table S2. Performance analysis of 256 DMSO-containing wells during pilot screening. Nine parameters were calculated by image analysis (MetaXpress, Molecular Devices).

	Average ± SD			CV		
Parameter	**Donor1**	**Donor2**	**Donor3**	**Donor1**	**Donor2**	**Donor3**
Total cells	412 ± 124	353 ± 60	264 ± 87	30%	17%	33%
Positive Ki67	39 ± 27	27 ± 10	78 ± 39	70%	39%	51%
% Positive Ki67	9 ± 4	8 ± 3	29 ± 7	45%	35%	25%
Positive C-peptide	168 ± 36	121 ± 27	70 ± 21	21%	22%	30%
% Positive C-peptide	42 ± 8	34 ± 3	27 ± 6	18%	9%	23%
Positive scoring profiles:						
Hoechst only	208 ± 91	208 ± 32	123 ± 43	44%	16%	35%
Hoechst & Ki67	36 ± 26	24 ± 10	71 ± 37	71%	40%	52%
Hoechst & C-peptide	165 ± 35	118 ± 26	62 ± 19	21%	22%	31%
All three stains	3 ± 2	2 ± 1	7 ± 4	67%	51%	53%