SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. β-cell replication-inducing activities of Jak 3 inhibitor VI and tivozanib are not shared by similar-acting molecules. (a) Rat β-cell replication in response to treatment with JAK3 inhibitors (Jak3 inhibitor VI, tofacitinib and ruxolitinib) and VEGF inhibitors (tivozanib and VEGF R1/FLT-1-Fc). Data are presented as the mean ± standard deviation (n=4 wells per treatment condition;*, $p<0.01$). (b) Fold induction of human β-cell replication ($Ki67^{+}Insulin^{+}/Insulin^{+}$) following 48 hours treatment with the indicated compounds with and without treatment with Exenatide [10 nM]. Replication indices of individual wells (n=5, >1,500 β-cells per well) and means ± standard deviation are shown (*, $p<0.05$ vs DMSO or as indicated).

Supplemental Figure 2. *In vivo* stability, replication-inducing potency and lineage-specific replication-promoting activities of CC-401. (a) Plasma levels of CC-401 at multiple time points following i.p. injection (25 mg/kg). Data are presented as the mean ± standard deviation (n=3 mice per time point). (b) *In vitro* rat β-cell replication indices following CC-401 treatment (48 hours). Data are presented as the mean ± standard deviation (n=4 wells per treatment condition;*, $p<0.05$). (c) *In vitro* fold-increase in rat β- (PDX1), α- (glucagon) and δ-cell (somatostatin) replication indices following treatment with dipyridamole [15 μM] or CC-401 [10 μM] compared to the DMSO treatment condition. Data are presented as the mean ± standard deviation (n=8 wells per treatment condition;*, $p<0.05$). (d) Percent mouse primary fibroblast replication ($ki67^{+}fibronectin^{+}/fibronectin^{+}$) following treatment with DMSO or CC-401(48h). Data are presented as the mean ± standard deviation (n=8 wells per treatment condition).

Supplemental Figure 3. FACS-based purification of rat β-cells for RNA-Seq. Representative fluorescence image of rat islet cultures following (48 hours) infection with a HIP-GFP lentiviral reporter. Cultures were stained for DAPI, insulin and GFP (upper panels). HIP-GFP infected rat islet cultures were FACS purified based upon GFP expression, seeded into tissue culture wells and analyzed by DAPI and insulin staining 48h after plating.

Supplemental Figure 4. Replication promoting activity of DYRK1inhibitors and islet CSNK1 expression (a) (b) Rat β-cell replication response to 48 hours treatment with the DYRK1A/B inhibitor Leucettine L41 at the indicated compound concentrations (n=4 wells per treatment condition;*, $p<0.05$ shown vs. DMSO-treated condition). (c) Rat β-cell replication response to DYRK1A/B inhibitor treatment (n=4 wells per treatment condition;*, $p<0.05$ shown vs. DMSO-treated condition).

SUPPLEMENTAL TABLE LEGENDS

Supplemental Table 1. Primary Screening results for β-cell replication index

Supplemental Table 2. Rat β-cell replication response to a subset of compounds selected for confirmatory dose-response curves. **CC-401-treated RNA-Seq results**

Supplemental Table 3. Kinome screening results of highly active β-cell replication-inducing DYRK1A/B inhibitors (CC-401 and Harmine) and less active β-cell replication-inducing DYRK1A/B inhibitors (AZ191 and KH CB19). Values reflect residual kinase activity following incubation with the indicated compound at [10 μM].

Supplemental Table 4. RNA-Sequencing results obtained from sorted rat β-cells following treatment with CC-401 (48 hours). Fold-change is obtained by comparison to sorted rat β-cells following treatment with DMSO (48 hours).

Supplemental Experimental Procedures:

***In Vitro* β-Cell Replication Assay (detailed)**

Freshly isolated islets were incubated (37°C, 5%CO_2) overnight in low glucose (1g/liter) DMEM (Hyclone) supplemented with 10% FBS (Hyclone), penicillin/streptomycin (Gibco) and glutamax (Gibco). The following morning, islets were trypsinized (0.25%, Gibco) into cellular clusters of 1-3 cells, re-suspended in the same media and plated into the wells of a 384-well plate (~25,000 islet-cells/well) that had been coated with the conditioned media of 804G rat bladder carcinoma cells. The islet-cells were allowed 48-hours to adhere at which time the media was changed to Islet Media (Mediatech 99-786-CV) supplemented with 2% serum, 5mM glucose, penicillin/streptomycin and glutamax and cells were compound treated. For screening, compounds were tested at 10 μM concentrations in singlicate or duplicate. After 48-hours of compound treatment, cells were fixed with fresh 4%PFA. Based upon prior observation that glucose- and phosphodiesterase inhibitor (PDE-Is)-dependent induction of β-cell replication required >24h of treatment, a 48h treatment duration was used (Annes et al., 2012; Zhao et al., 2014). Antigen retrieval was performed by heating the cells to 70 ºC in 95% formamide and 50mM citrate. Cells were then washed and permeablized with PBS/0.3% Triton X-100. Staining was performed by overnight incubation with primary antibody in PBS at 4 ºC. For the primary screen, PDX1 antibody (R&D AF2419; 1:300) was used to reveal beta-cells and Ki67 antibody (BD Bioscience 556003, 1:300) to visualize proliferating cells. Additional replication assays used antibodies raised against insulin (Dako A0564, 1:300), glucagon (Dako A0565, 1:500) somatostatin (A0566, 1:300) and vimentin (Abcam ab24525, 1:200). Replication was assessed via automated image acquisition and analysis using a Cellomics ArrayScanVTI. The acquisition thresholds/parameters were established such that the computer-based calls of replication events were consistent with human-based calls.

Measurement of *in vivo* pharmacokinetics

To measure CC-401 stability in mice, 17 eight-week old female C57BL/6J (The Jackson Laboratory, 0664) mice were injected with vehicle (0.7%NaCl, 50mM citrate. pH2.5 adjusted to pH5.0) or CC-401 (25 mg/kg; 10 μL/g). Plasma was serially collected (n=3 per time point, n=2 time 0) by terminal bleed and analyzed by LC/MS/MS using prepared standards for intact CC-401 peak identification and quantitation (Integrated Analytical Solutions, Berkley CA).

Caco-2 P_{app} Determination

Caco-2 cells were maintained in DMEM in an atmosphere of 5% CO_2. Cells (5×10^5 cells/well) were seeded and maintained (21 ± 4 days) on polycarbonate filter inserts prior to experimentation. Apparent permeability coefficients were determined for A → B and B → A directions in the presence and absence of elacridar [5μM], a transporter inhibitor. Reference compounds [10μM] were dissolved in HBSS at pH 7.4. Assays were performed in HBSS containing 25 mM HEPES (pH 7.4) at 37 °C with test compounds added to the donor side. Aliquots of the receiver side were taken over the 2 h incubation period; aliquots of the donor side were taken at 0 h and 2 h. Aliquots were diluted with an equal volume of methanol/water with 0.1% formic acid containing the internal standard and analyzed by LC−MS/MS. The apparent permeability coefficients (P_{app}) were calculated using the formula: $P_{app} = (dC_{rec}/dt)/(A \times C_{0,donor})] \times 106$ with dC_{rev}/dt being the change in concentration in the receiver compartment with time,

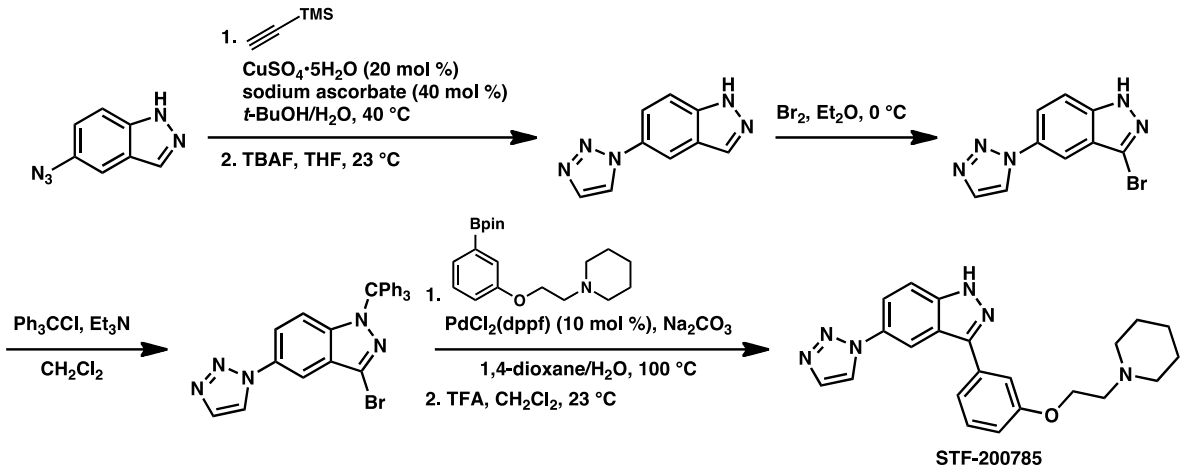
$C_{0,donor}$ the concentration in the donor compartment at time 0, and A the area of the compartment with the cells.

Compound Synthesis

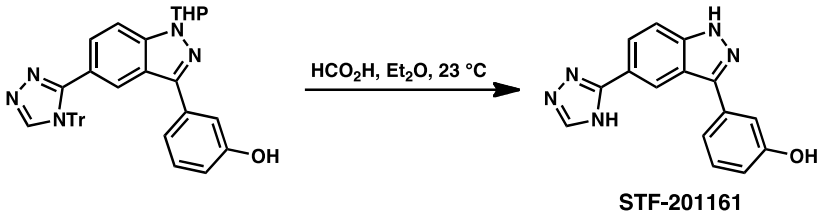
Reactions were performed under ambient atmosphere unless otherwise noted. Qualitative TLC analysis was performed on 250 mm thick, 60 Å, glass backed, F254 silica (Silicycle, Quebec City, Canada). Visualization was accomplished with UV light and exposure to *p*-anisaldehyde or $KMnO_4$ stain solutions followed by heating. Flash chromatography was performed on a Teledyne Isco purification system using silica gel flash cartridges (SiliCycle®, SiliaSep^{TM} 40-63 □m, 60Å). HPLC was performed on an Agilent 1260 Infinity preparative scale purification system using an Agilent PrepHT Zorbax Eclipse XDB-C18 reverse-phase column (21.2 X 250 mm). Structure determination was performed using ^{1}H and ^{13}C NMR spectra that were recorded on a Bruker AV-500 spectrometer, and low-resolution mass spectra (ESI-MS) that was collected on a Shimadzu 20-20 ESI LCMS instrument.

Materials for Chemical Synthesis:
Dry THF, CH_2Cl_2, DMF, DME, MeCN were purchased from Acros Organics or EMD Millipore Corporation and stored under a nitrogen atmosphere. Deuterated solvents were purchased from Cambridge Isotope laboratories, Inc. **CC-401** was purchased from MedChem Express, Inc. **STF-200866** and **STF-200894** are known compounds and were prepared following literature procedures (Bhagwat, 1998: 542693). The synthesis of **STF-200785** from known 5-azido-1H-indazole is described in Scheme 1 (Sanphanya et al., 2013). The synthesis of **STF-201161** was performed commercially except for the last step (Scheme 2). The synthesis of **STF-201162** from known *N*-Boc indazole is described in Scheme 3 (Furuya and Ritter, 2009).

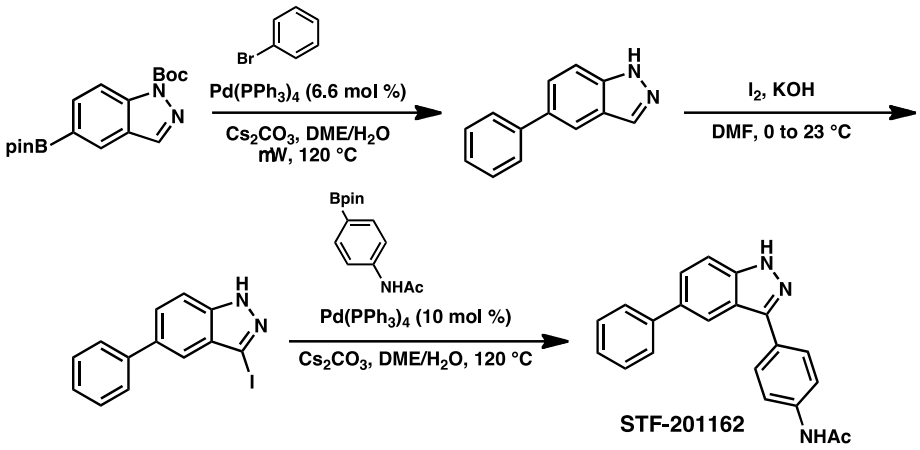
Scheme 1: Synthesis: STF-200785



Scheme 2: Synthesis of STF-201161



Scheme 3: Synthesis of STF-201162



Quantitative PCR Primers

Human *MYBL2*

FWD: 5'- GGCATCGAACTCATCATCGAG-3'
REV: 5'-CAGTTGTCGGCAAGGATAGAG-3'

Mouse *Mybl2*

FWD: 5'- CTTCCCAGTTTCTGAACTTCTG -3'
REV: 5'- ATGATGGATACTTCTGGTGCAG -3'

Human FOXM1

FWD: 5' GTGTTTAAGCAGCAGAAACGAC - -3'
REV: 5'- TGGATAGGTACCAGGTATGAGC -3'

Mouse *Foxm1*

FWD: 5'- TGGAATCACAGCAGAAACGAC -3'
REV: 5'- GACTGGTTCACTGGGAACTG -3'

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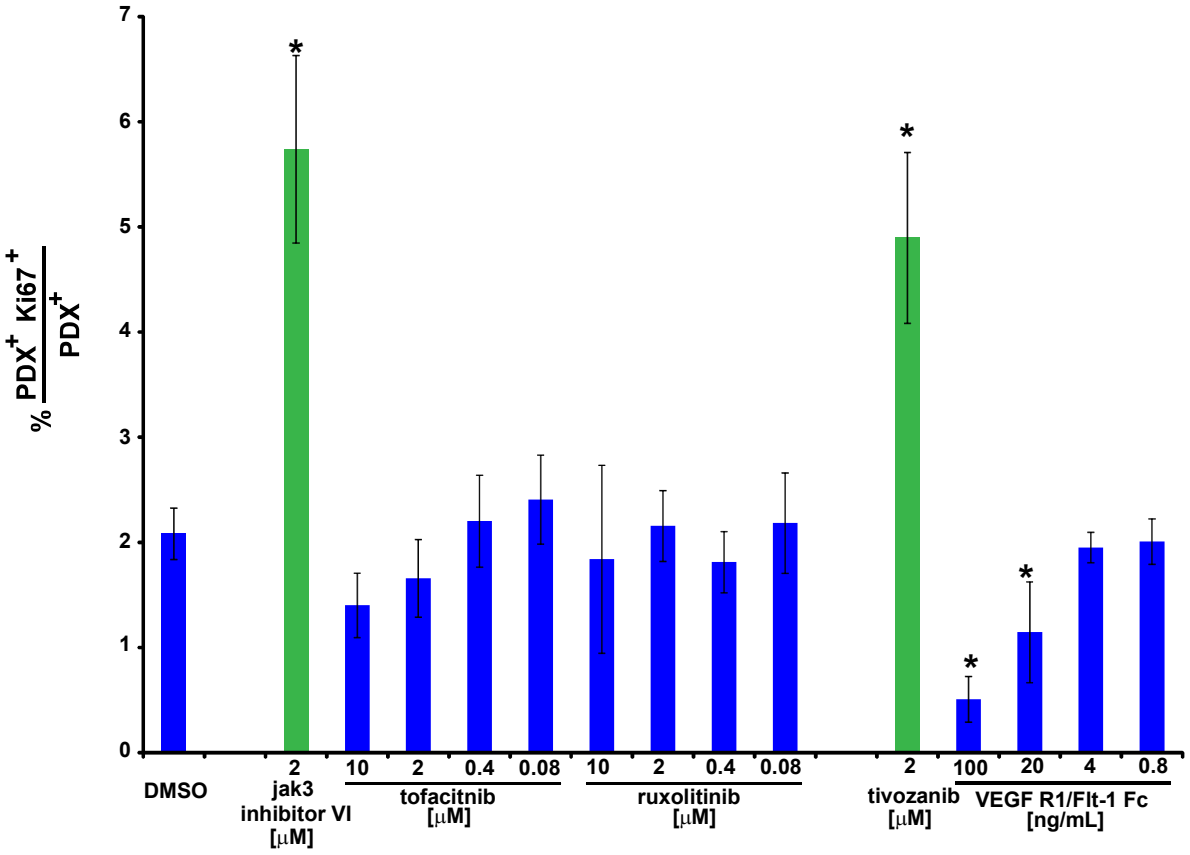
Supplemental Figure 1.

(a)



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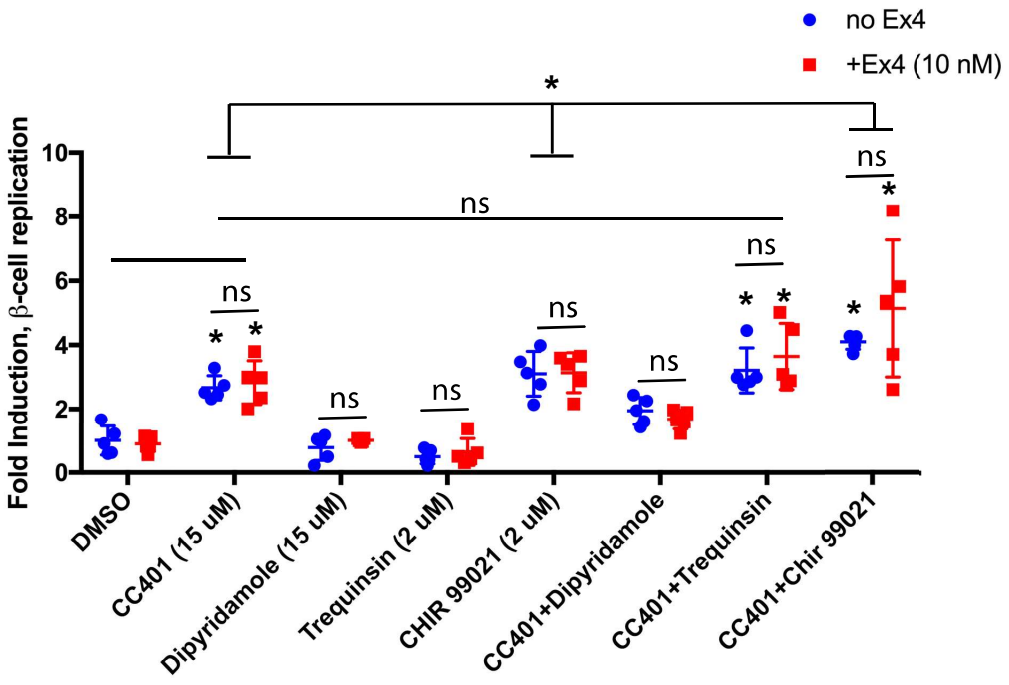

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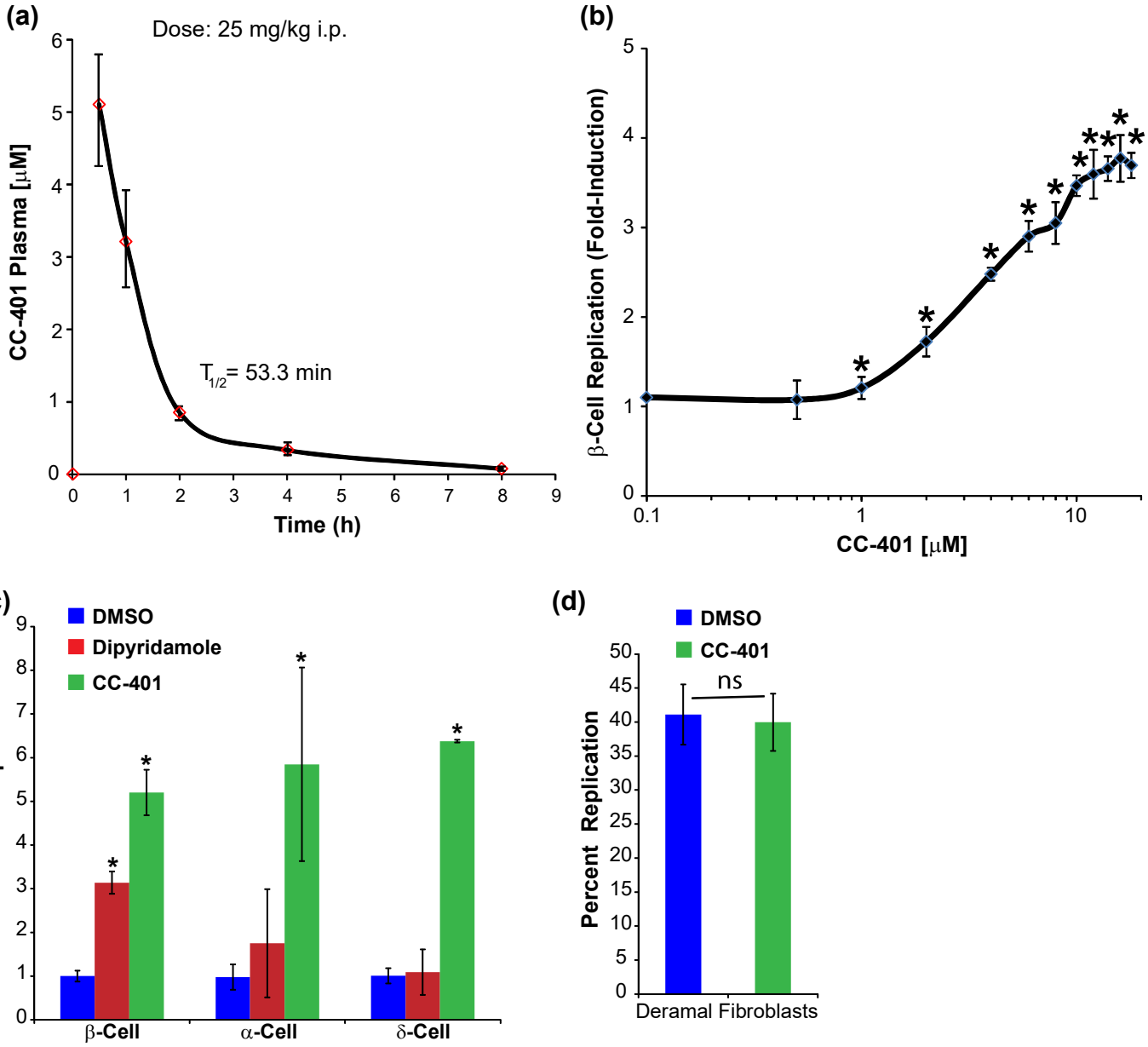
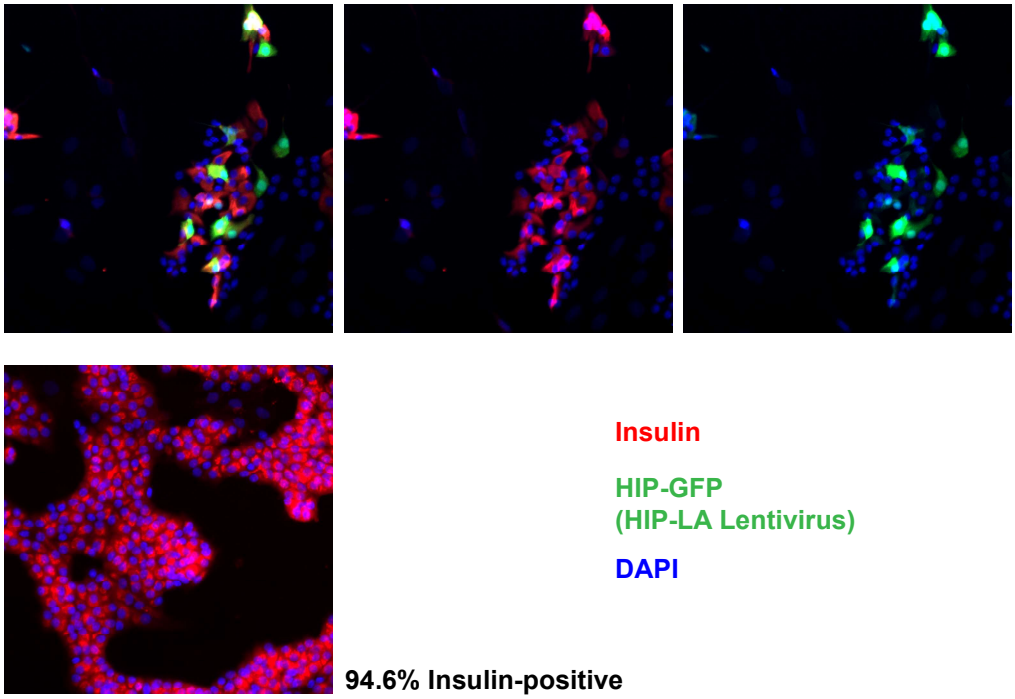


Supplemental Figure 3.



Supplemental Figure 4.