nature portfolio

https://doi.org/10.1038/s41596-021-00560-y

Supplementary information

Generation of insulin-producing pancreatic β cells from multiple human stem cell lines

In the format provided by the authors and unedited

Supplementary Information

Generation of insulin-producing pancreatic β cells from multiple human stem cell lines

Nathaniel J. Hogrebe[1], Kristina G. Maxwell[1,2], Punn Augsornworawat[1,2], and Jeffrey R. Millman[1,2*]

[1]Division of Endocrinology, Metabolism, and Lipid Research

Washington University School of Medicine

Campus Box 8127

660 South Euclid Avenue

St. Louis, MO 63110

USA

[2]Department of Biomedical Engineering

Washington University in St. Louis

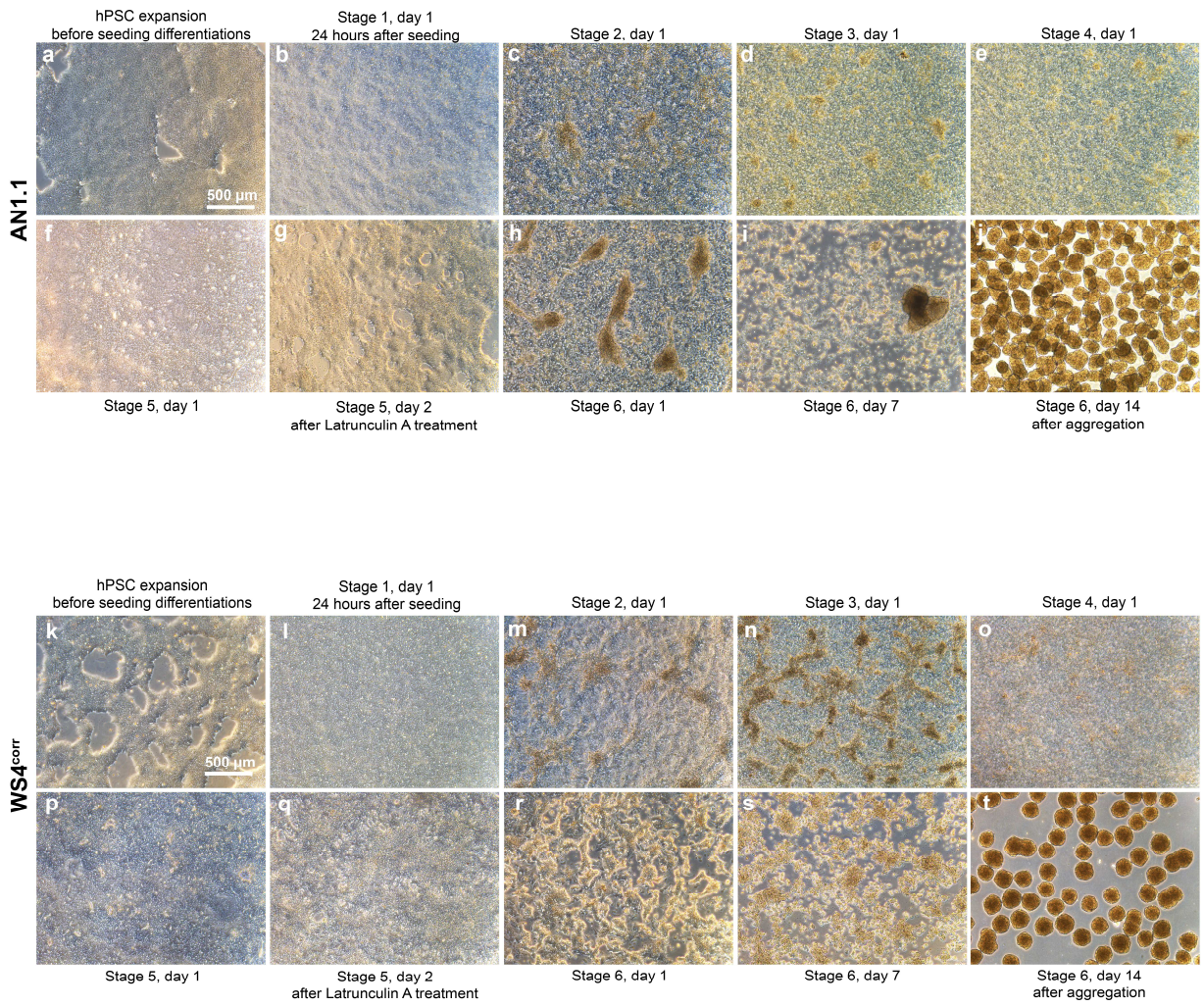
1 Brookings Drive

St. Louis, MO 63130

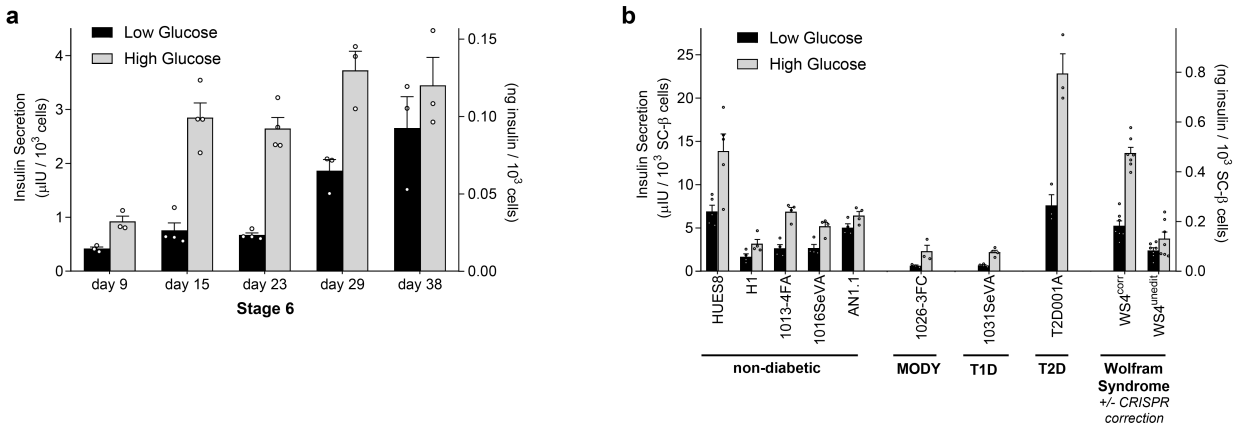
USA

*To whom correspondence should be addressed:

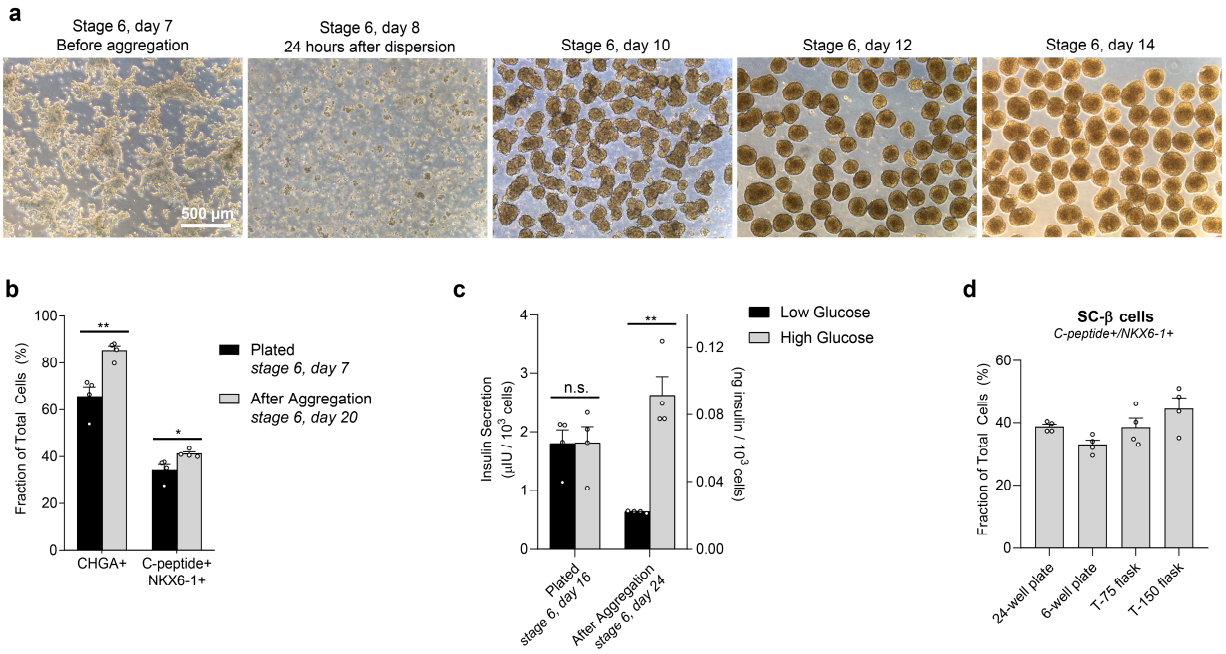
Jeffrey R. Millman, jmillman@wustl.edu



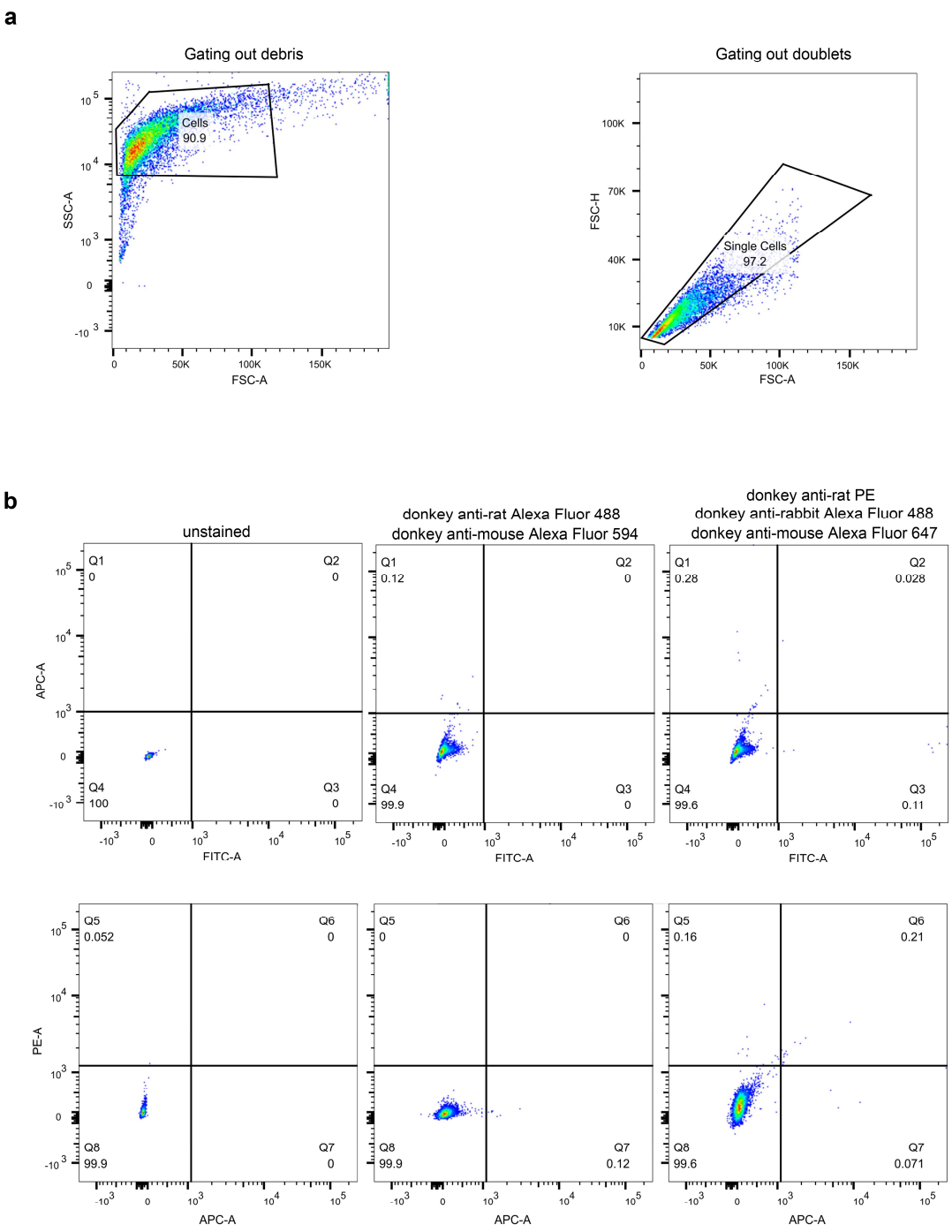
Supplementary Figure 1. Cell morphology shown at each stage for the AN1.1 (**a-j**) and WS4^{corr} cell lines (**k-t**). Scale bars = 500 µm.



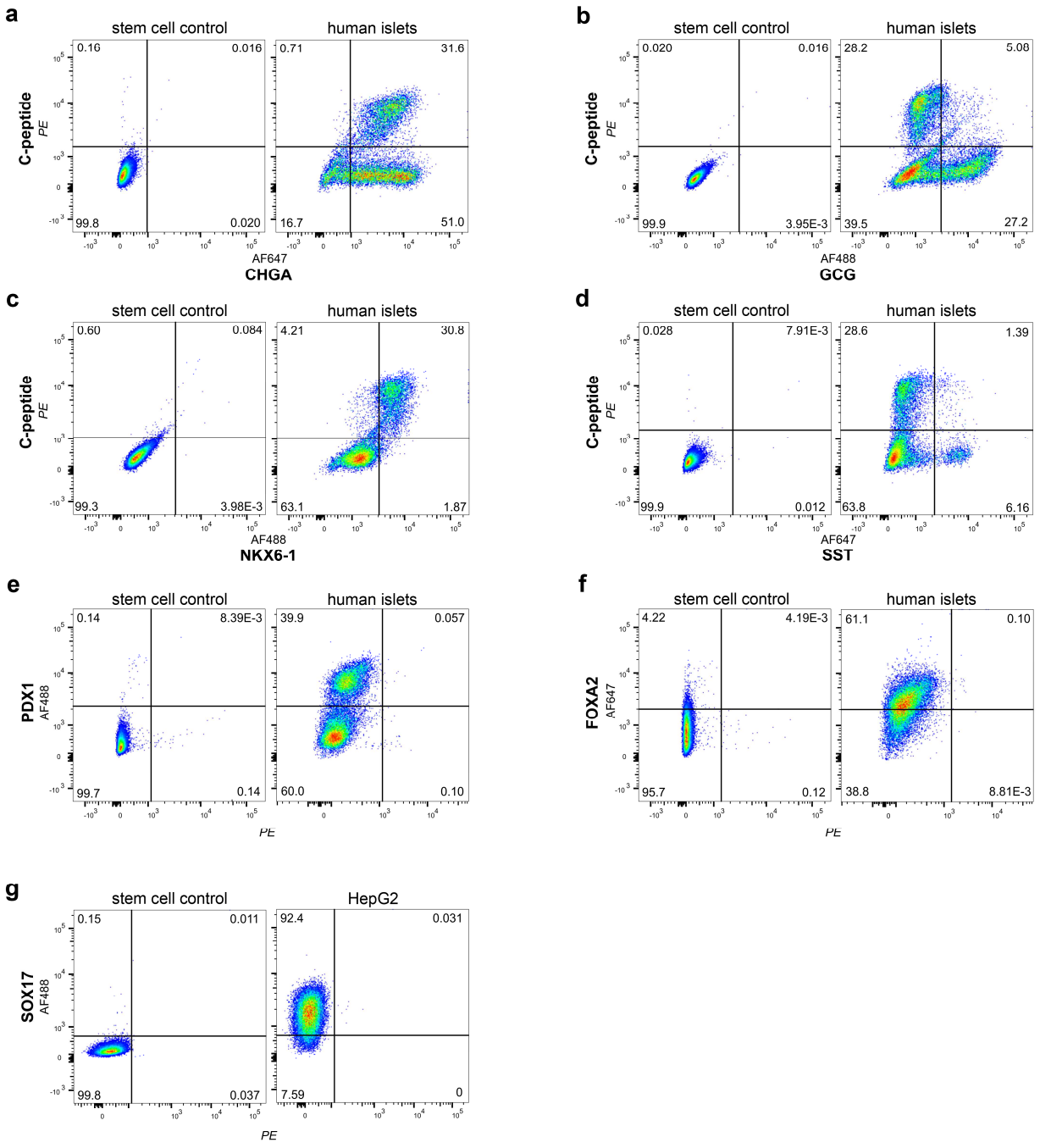
Supplementary Figure 2. (**a**) Static GSIS data of aggregated SC-β cell clusters on the indicated day of stage 6 demonstrating that the cells remain functional for weeks *in vitro*. (**b**) Static GSIS data from Figure 5b that has been normalized to the number of SC-β cells rather than total number of cells, which was calculated from the percentage of C-peptide+/NKX6-1+ cells shown in Figure 5a. All data are represented as the mean, and all error bars represent SEM. Individual data points are shown for all bar graphs, where n = number of separate wells from one or more independent differentiations.



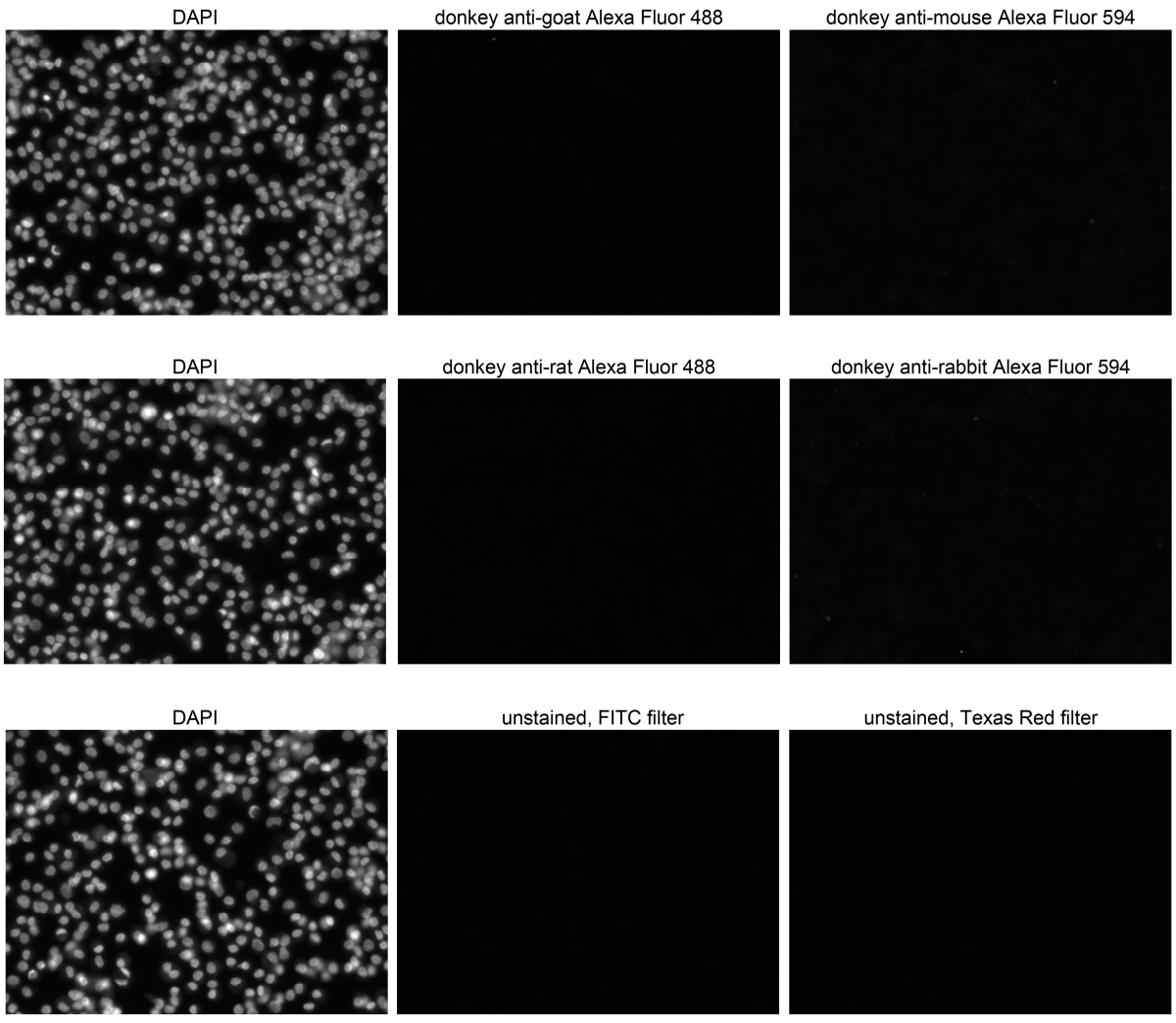
Supplementary Figure 3. (**a**) Images of cells before aggregation (stage 6, day 7) and on days after aggregation (stage 6, days 8-14), demonstrating formation of the clusters. (**b**) Flow cytometry of cells before and after aggregation, showing enrichment of endocrine (CHGA+) and SC-β cells (C-peptide+/NKX6-1+). (**c**) If SC-β cells are not glucose-responsive while remaining plated, the aggregation step can improve their function, measured here in a static GSIS assay. (**d**) Flow cytometry data demonstrating that the percentage of SC-β cells is similar in aggregated clusters that were generated from different culture plate sizes. All data are represented as the mean, and all error bars represent SEM. Individual data points are shown for all bar graphs, where n = number of separate wells from one or more independent differentiations.



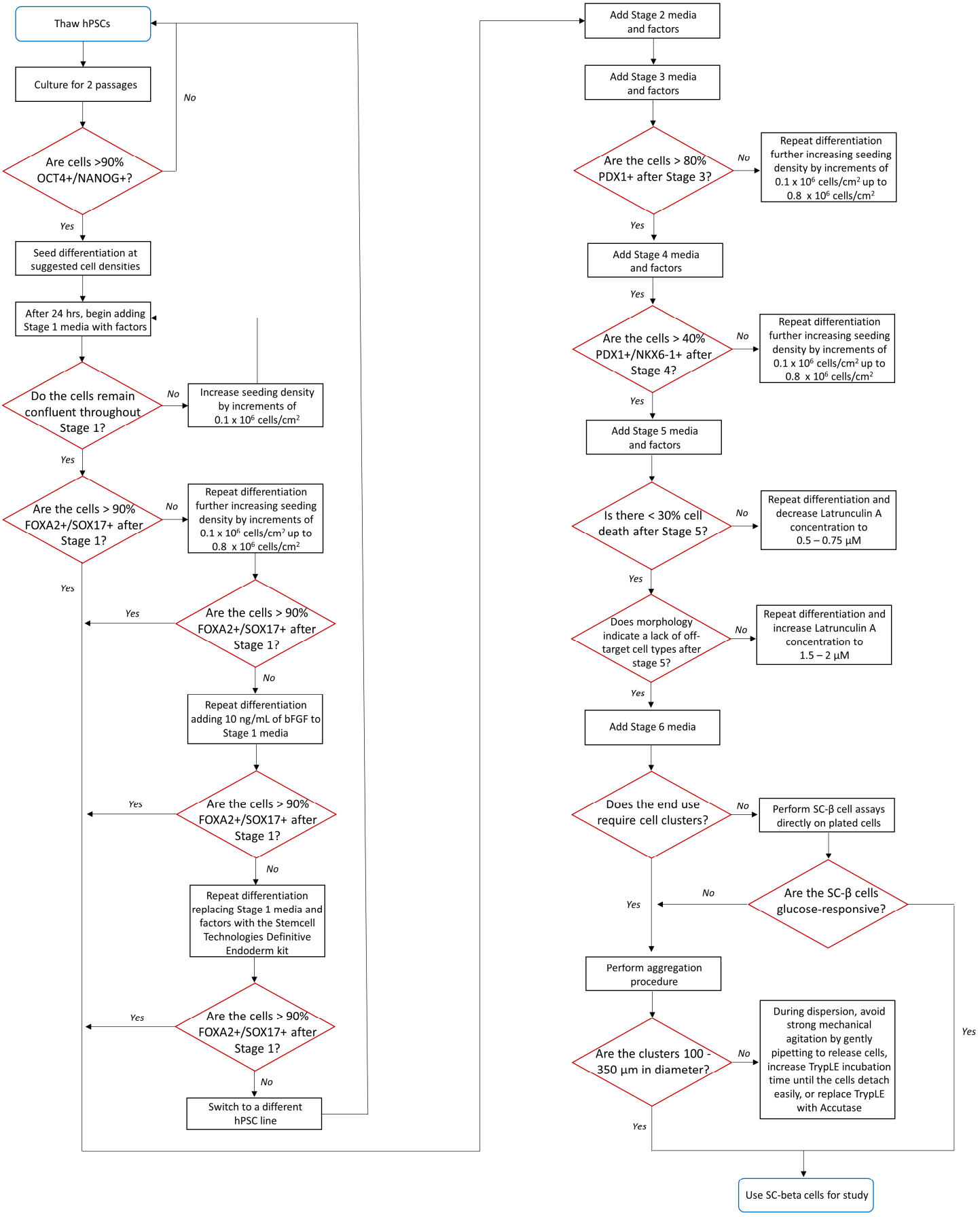
Supplementary Figure 4. (**a**) An example gating scheme that removes debris and doublets when analyzing flow cytometry data. (**b**) Flow cytometry plots demonstrating negative staining of stage 6 cells when omitting primary antibodies and only using the indicated secondary antibodies.



Supplementary Figure 5. Primary human islets can be used to show positive staining for (**a-d**) C-peptide, (**a**) CHGA, (**b**) GCG, (**c**) NKX6-1, (**d**) SST, (**e**) PDX1 and (**f**) FOXA2. (**g**) The HepG2 cell line can be used to show positive staining for SOX17. Stem cells can be used as a negative control for these stains.



Supplementary Figure 6. Fluorescence images demonstrating negative staining of stage 6 cells when omitting primary antibodies and only using the indicated secondary antibodies.


Thaw hPSCs
Culture for 2 passages
Are cells >90% OCT4+/NANOG+?
No
Yes
Seed differentiation at suggested cell densities
After 24 hrs, begin adding Stage 1 media with factors
Do the cells remain confluent throughout Stage 1?
No
Increase seeding density by increments of 0.1 x 10^6 cells/cm^2
Yes
Are the cells > 90% FOXA2+/SOX17+ after Stage 1?
No
Repeat differentiation further increasing seeding density by increments of 0.1 x 10^6 cells/cm^2 up to 0.8 x 10^6 cells/cm^2
Yes
Are the cells > 90% FOXA2+/SOX17+ after Stage 1?
Yes
No
Repeat differentiation adding 10 ng/mL of bFGF to Stage 1 media
Are the cells > 90% FOXA2+/SOX17+ after Stage 1?
Yes
No
Repeat differentiation replacing Stage 1 media and factors with the Stemcell Technologies Definitive Endoderm kit
Are the cells > 90% FOXA2+/SOX17+ after Stage 1?
Yes
No
Switch to a different hPSC line
Add Stage 2 media and factors
Add Stage 3 media and factors
Are the cells > 80% PDX1+ after Stage 3?
No
Repeat differentiation further increasing seeding density by increments of 0.1 x 10^6 cells/cm^2 up to 0.8 x 10^6 cells/cm^2
Yes
Add Stage 4 media and factors
Yes
Are the cells > 40% PDX1+/NKX6-1+ after Stage 4?
No
Repeat differentiation further increasing seeding density by increments of 0.1 x 10^6 cells/cm^2 up to 0.8 x 10^6 cells/cm^2
Yes
Add Stage 5 media and factors
Is there < 30% cell death after Stage 5?
No
Repeat differentiation and decrease Latrunculin A concentration to 0.5 – 0.75 µM
Yes
Does morphology indicate a lack of off-target cell types after stage 5?
No
Repeat differentiation and increase Latrunculin A concentration to 1.5 – 2 µM
Yes
Add Stage 6 media
Does the end use require cell clusters?
No
Perform SC-β cell assays directly on plated cells
Are the SC-β cells glucose-responsive?
No
Yes
Yes
Perform aggregation procedure
Are the clusters 100 - 350 µm in diameter?
No
During dispersion, avoid strong mechanical agitation by gently pipetting to release cells, increase TrypLE incubation time until the cells detach easily, or replace TrypLE with Accutase
Yes
Yes
Use SC-beta cells for study

Supplementary Figure 7. A troubleshooting flowchart depicts the suggested steps to take when the SC-β cell differentiation fails to meet the indicated quality control thresholds at various stages of the protocol. Optimizing the seeding density for new cell lines is a particularly important step in assuring quality differentiations.