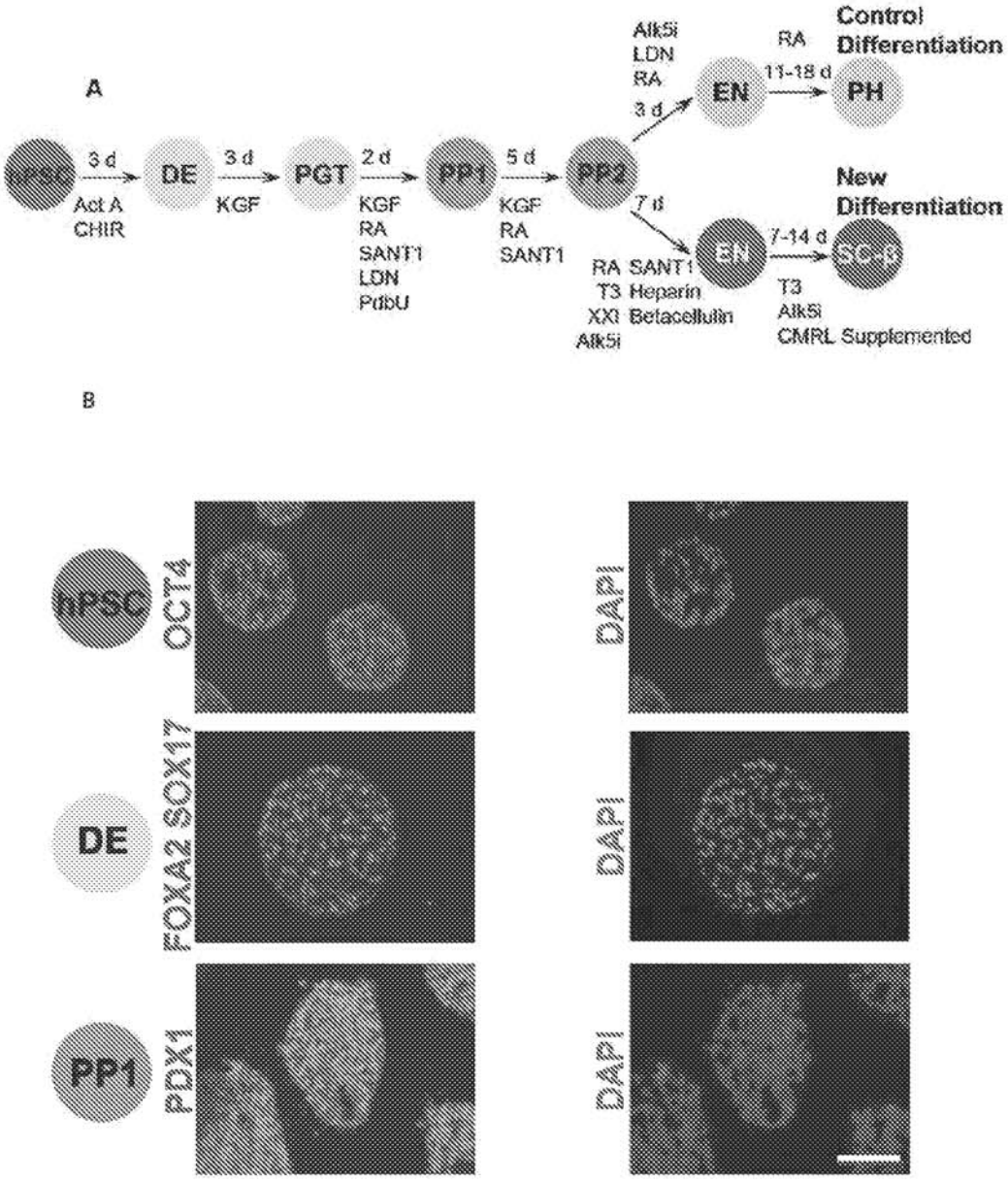
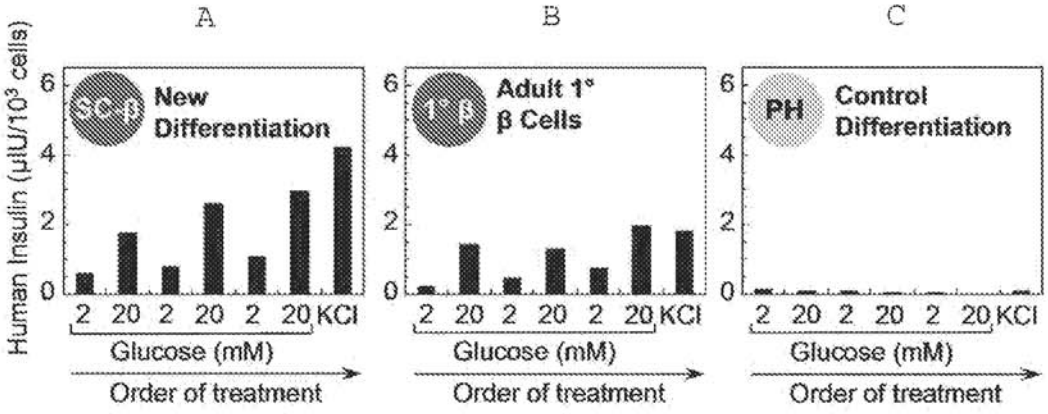
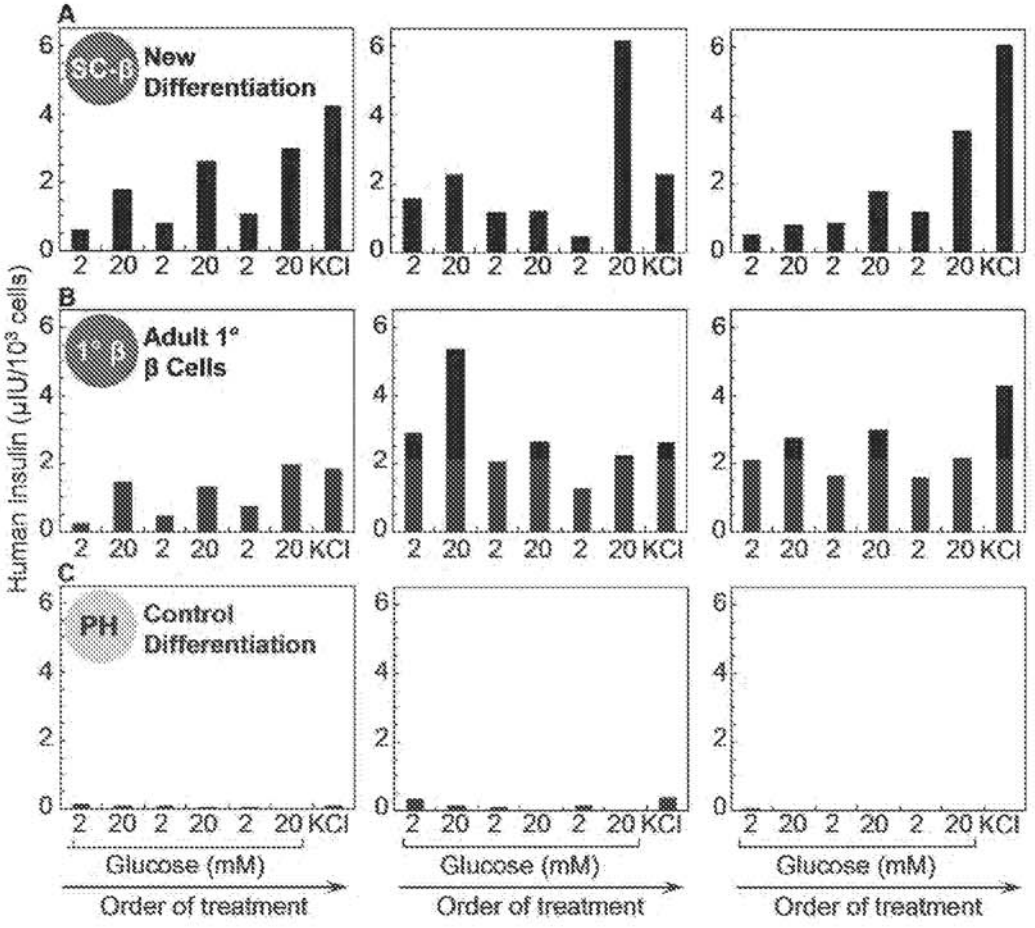

A
hPSC
3 d
Act A
CHIR
DE
3 d
KGF
PGT
2 d
KGF
RA
SANT1
LDN
PdbU
PP1
5 d
KGF
RA
SANT1
PP2
Alk5i
LDN
RA
3 d
EN
RA
11-18 d
PH
Control
Differentiation
7 d
RA SANT1
T3 Heparin
XXI Betacellulin
Alk5i
EN
7-14 d
T3
Alk5i
CMRL Supplemented
SC-β
New
Differentiation
B
hPSC
OCT4
DAPI
DE
FOXA2 SOX17
DAPI
PP1
PDX1
DAPI

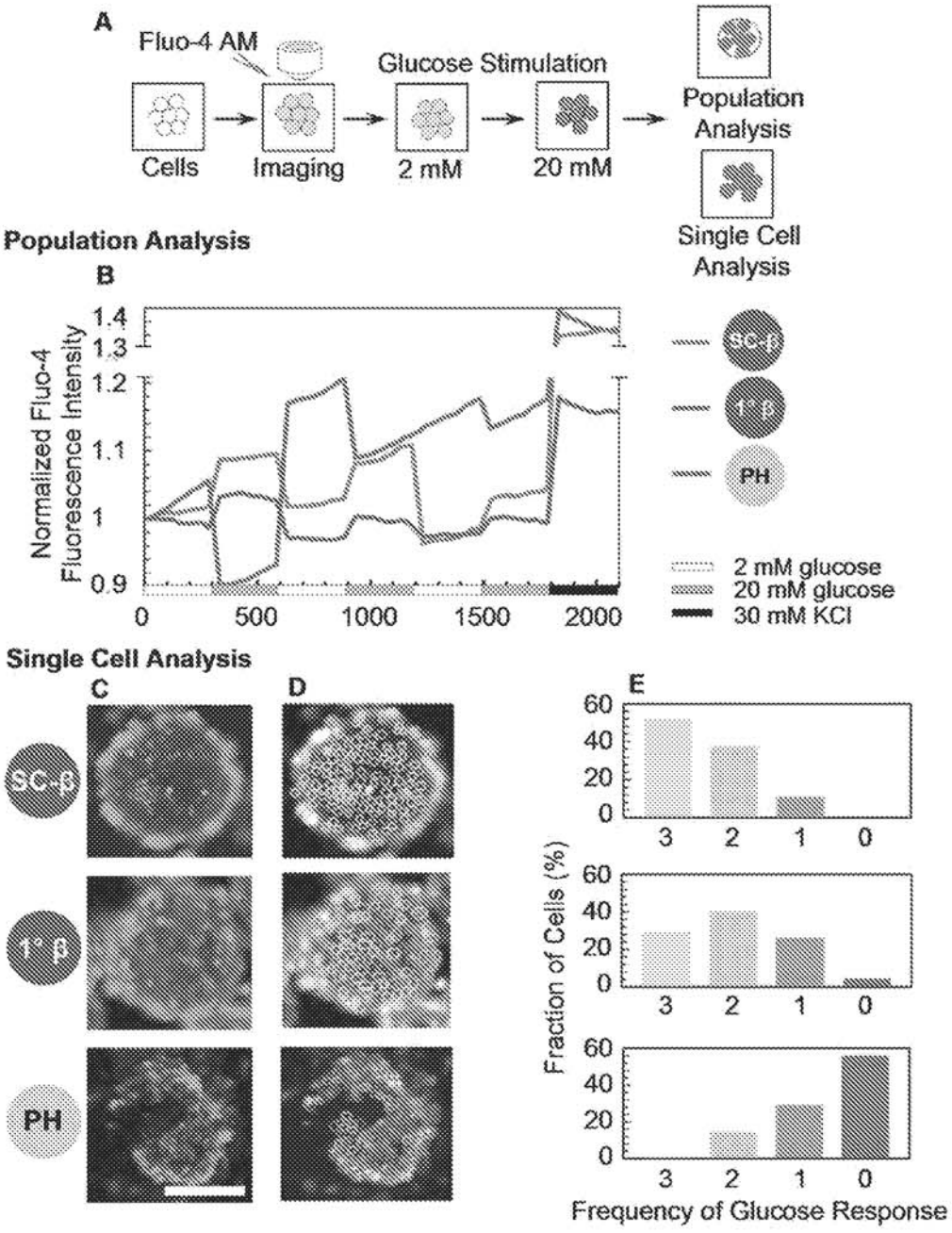
FIGS. 1A-1B


A
B
C
Human Insulin (μIU/10³ cells)
6
4
2
0
SC-β
New
Differentiation
1° β
Adult 1°
β Cells
PH
Control
Differentiation
2 20 2 20 2 20 KCl
Glucose (mM)
Order of treatment

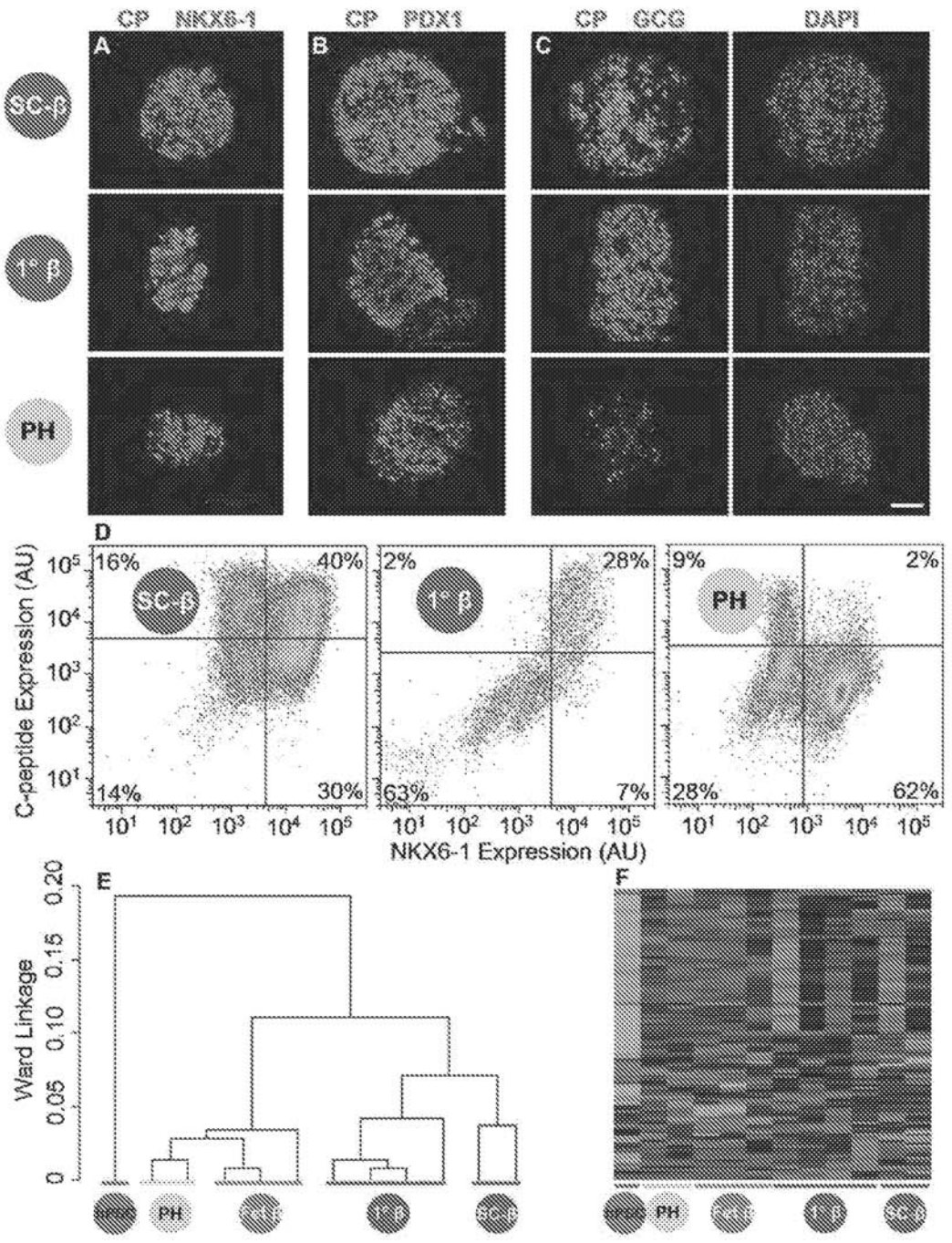
FIGS. 2A-2C


A
SC-β
New
Differentiation
B
Adult 1°
β Cells
C
PH
Control
Differentiation
Human insulin (μIU/10³ cells)
0
2
4
6
2 20 2 20 2 20 KCl
Glucose (mM)
Order of treatment

FIGS. 3A-3C


A
Fluo-4 AM
Glucose Stimulation
Cells
Imaging
2 mM
20 mM
Population Analysis
Single Cell Analysis
Population Analysis
B
Normalized Fluo-4 Fluorescence Intensity
1.4
1.3
1.2
1.1
1
0.9
0
500
1000
1500
2000
sc-β
1° β
PH
2 mM glucose
20 mM glucose
30 mM KCl
Single Cell Analysis
C
D
E
SC-β
1° β
PH
60
40
20
0
3
2
1
0
Fraction of Cells (%)
Frequency of Glucose Response

FIGS. 4A-4E


CP NKX6-1
CP PDX1
CP GCG
DAPI
A
B
C
SC-β
1° β
PH
D
C-peptide Expression (AU)
NKX6-1 Expression (AU)
16%
40%
14%
30%
2%
28%
63%
7%
9%
2%
28%
62%
E
Ward Linkage
0
0.05
0.10
0.15
0.20
PH
1° β
SC-β
F

FIGS. 5A-5F

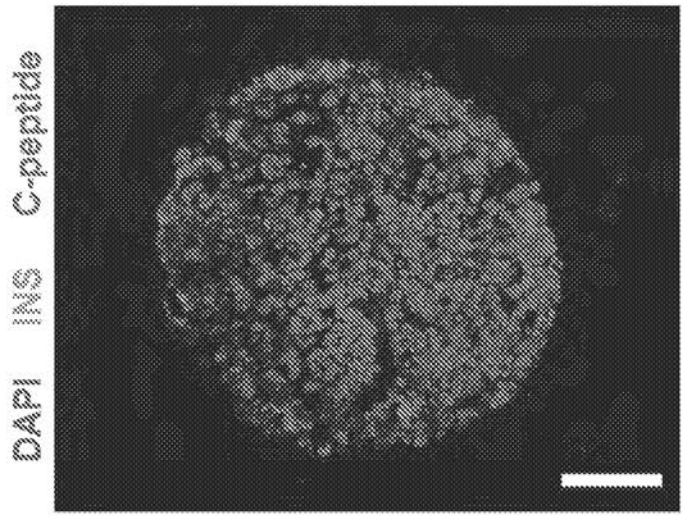
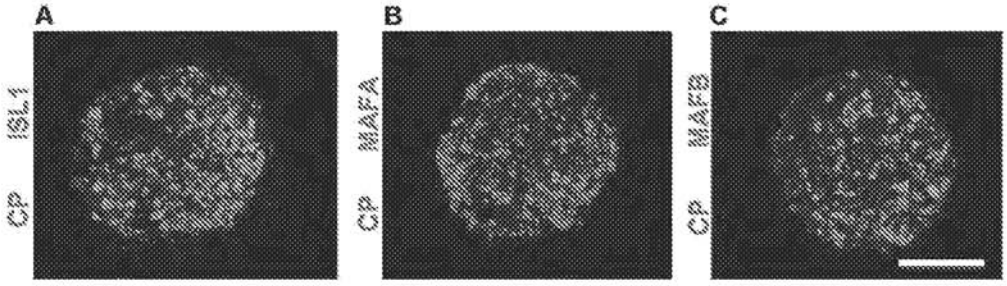
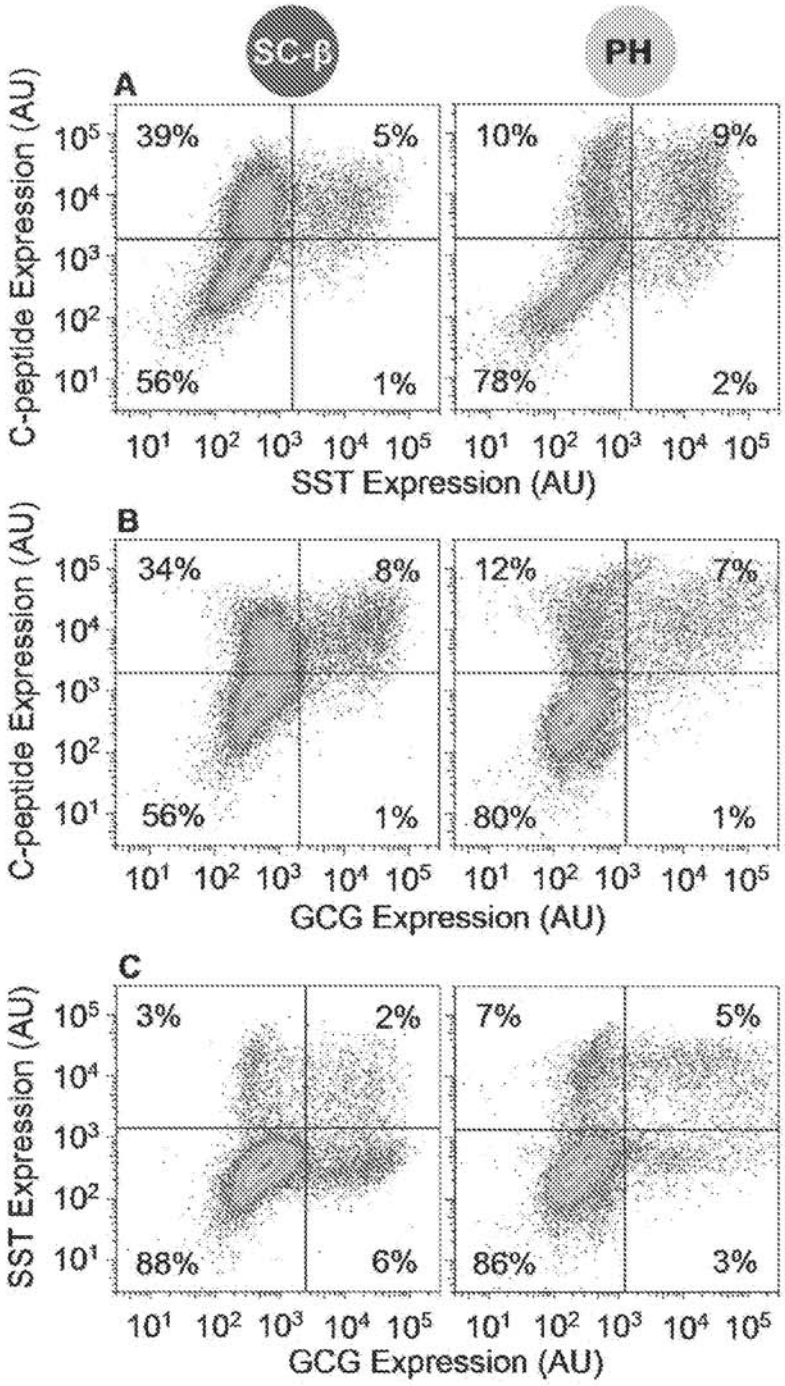


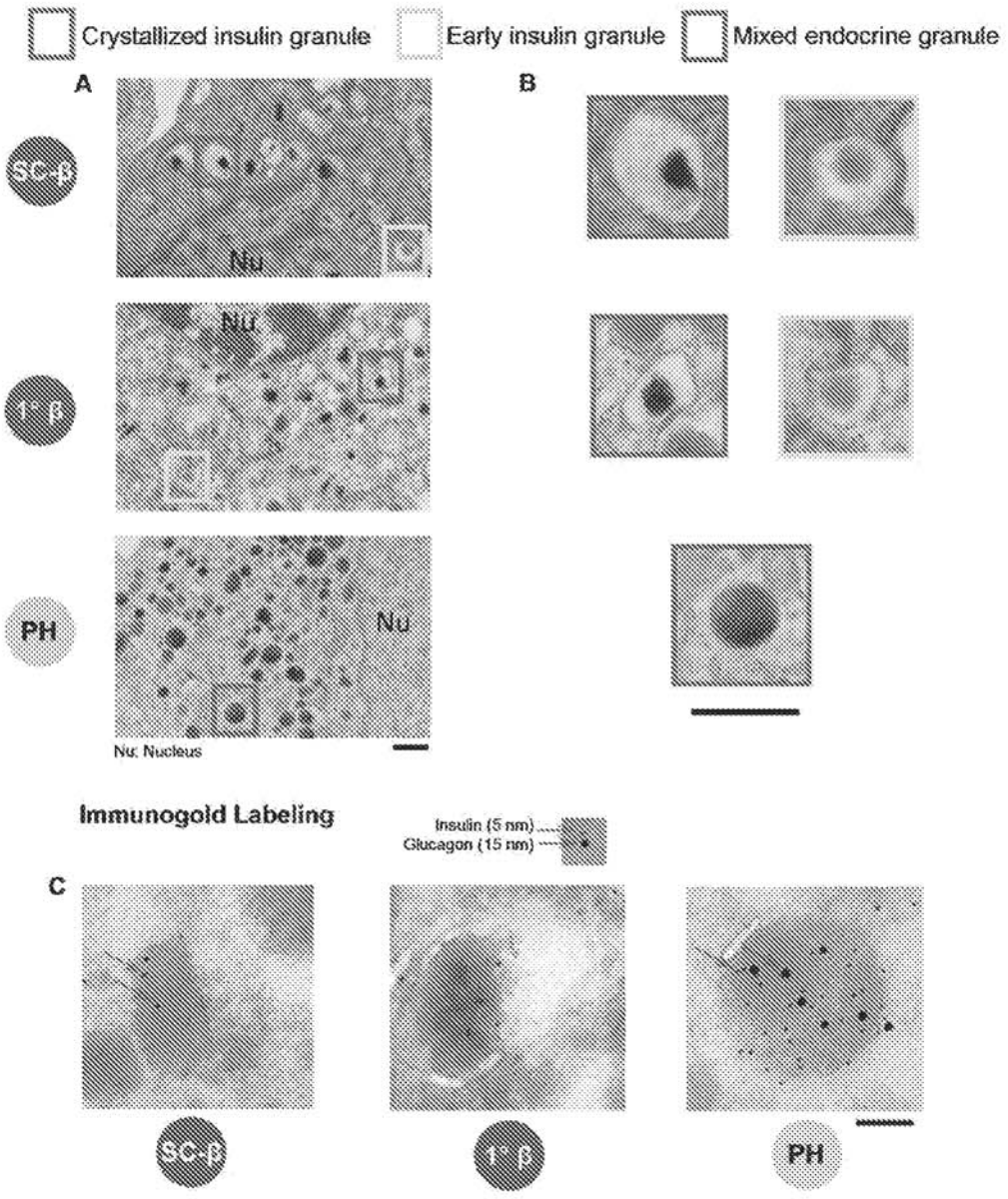
FIG. 6



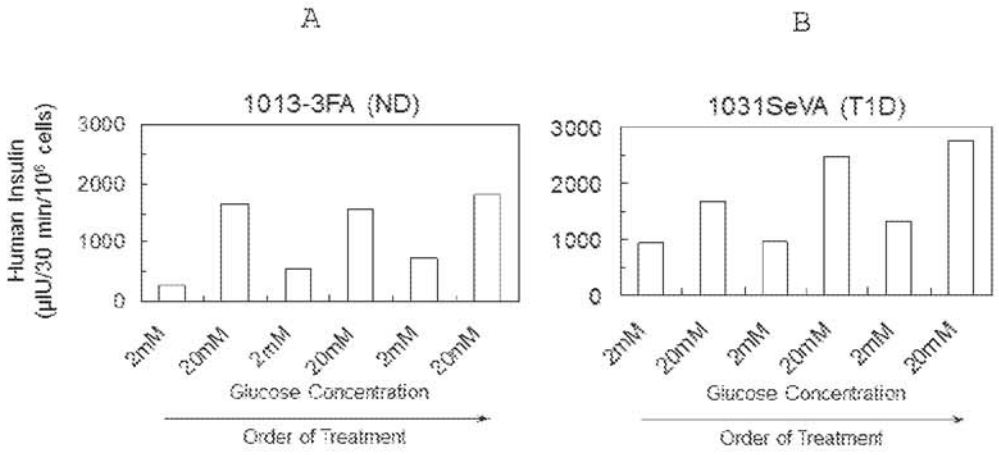
FIGS. 7A-7C


SC-β
PH
A
C-peptide Expression (AU)
39%
5%
56%
1%
10%
9%
78%
2%
SST Expression (AU)
B
C-peptide Expression (AU)
34%
8%
56%
1%
12%
7%
80%
1%
GCG Expression (AU)
C
SST Expression (AU)
3%
2%
88%
6%
7%
5%
86%
3%
GCG Expression (AU)

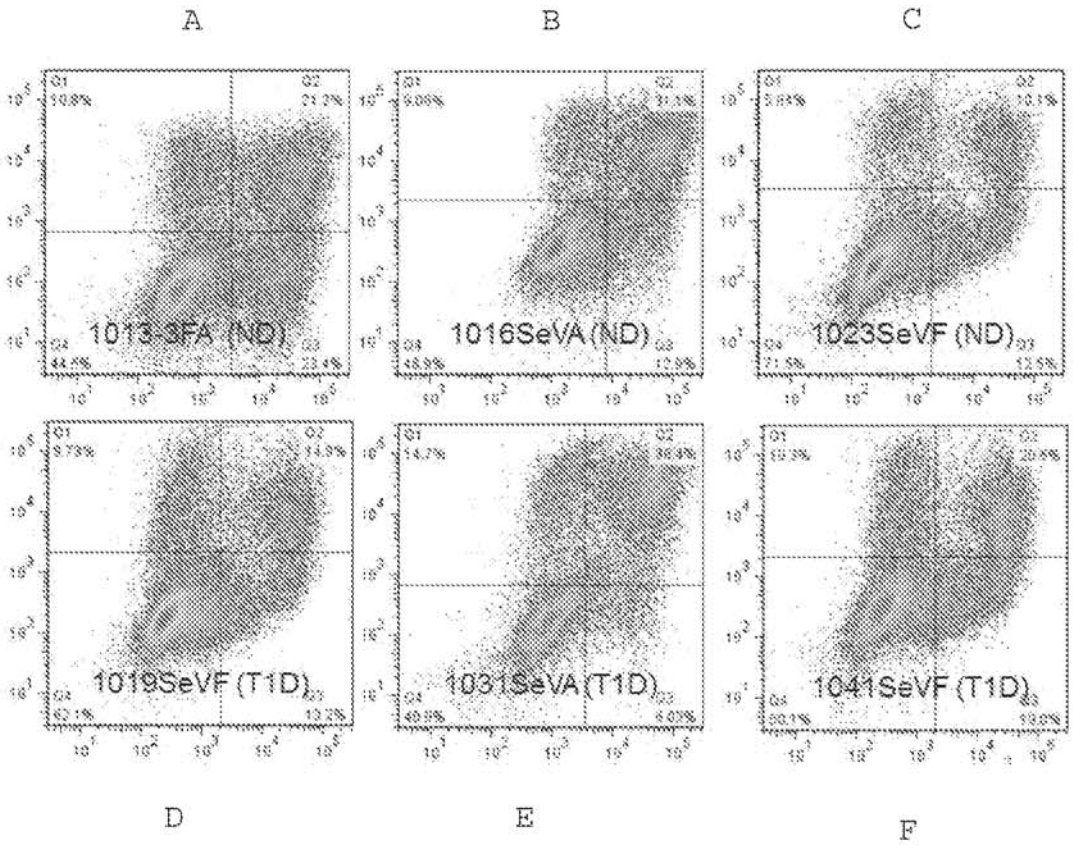
FIGS. 8A-8C


Crystallized insulin granule
Early insulin granule
Mixed endocrine granule
A
B
SC-β
1° β
PH
Nu
Nu: Nucleus
Immunogold Labeling
Insulin (5 nm)
Glucagon (15 nm)
C
SC-β
1° β
PH

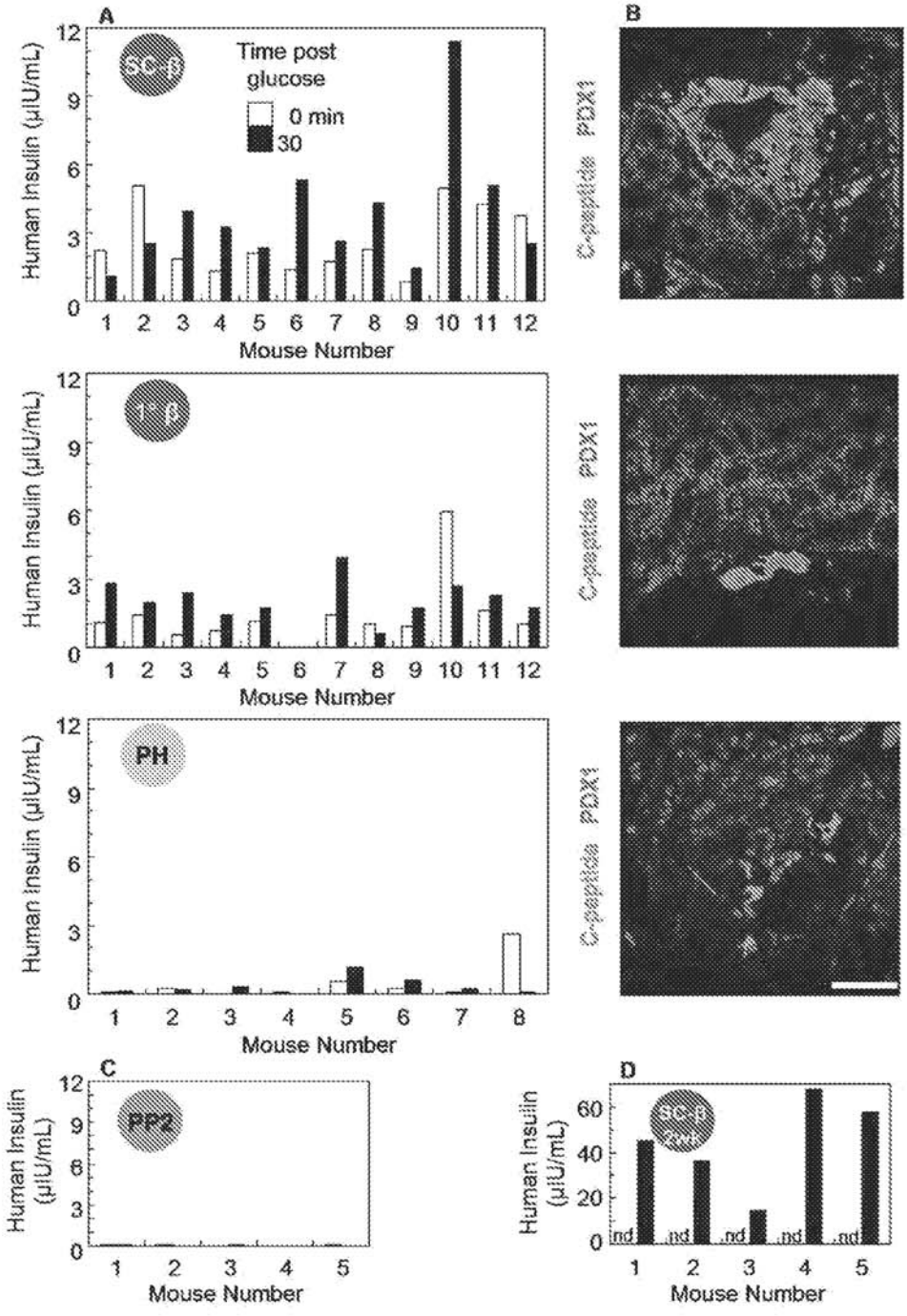
FIGS. 9A-9C


A
B
1013-3FA (ND)
1031SeVA (T1D)
Human Insulin
(μIU/30 min/10^6 cells)
3000
2000
1000
0
2mM
20mM
2mM
20mM
2mM
20mM
Glucose Concentration
Order of Treatment

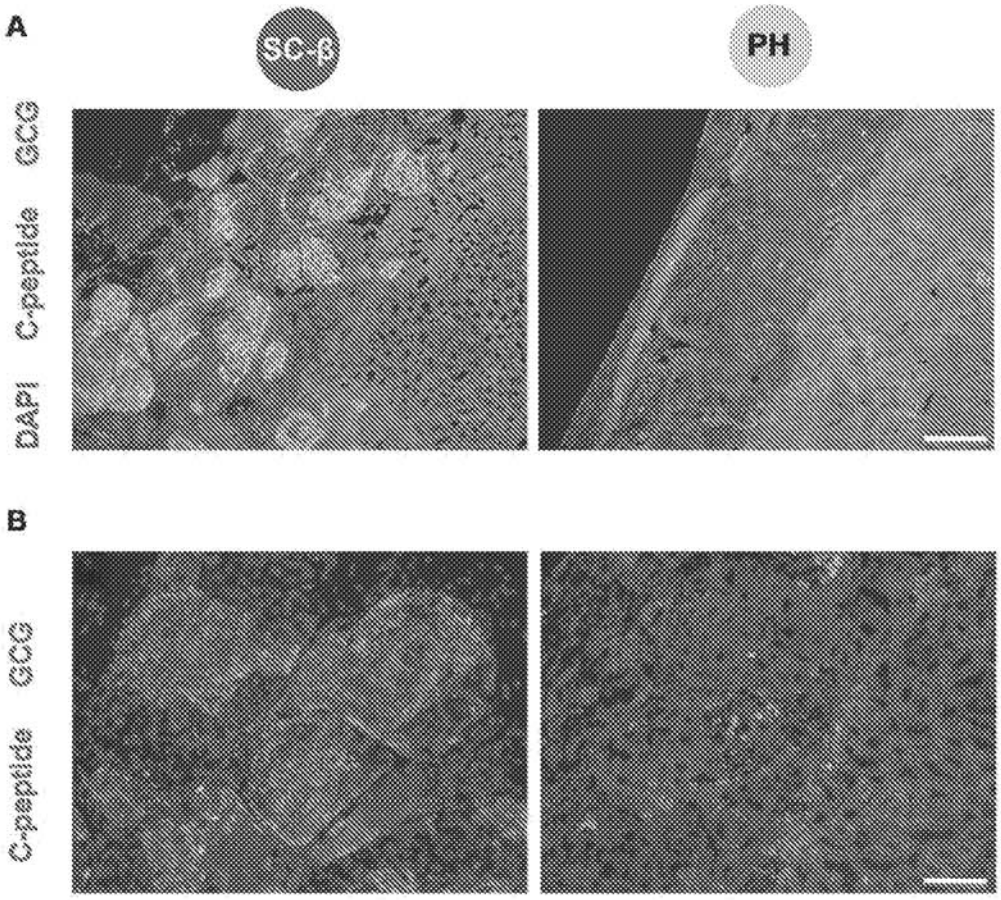
FIGS. 10A-10B


A
B
C
1013-3FA (ND)
1016SeVA (ND)
1023SeVF (ND)
1019SeVF (T1D)
1031SeVA (T1D)
1041SeVF (T1D)
D
E
F

FIGS. 11A-11F


A
B
SC-β
Time post glucose
0 min
30
Human Insulin (μIU/mL)
Mouse Number
C-peptide PDX1
T-β
PH
C
PP2
D
SC-β
Cyt.
nd

FIGS. 12A-12D



FIGS. 13A-13B

A

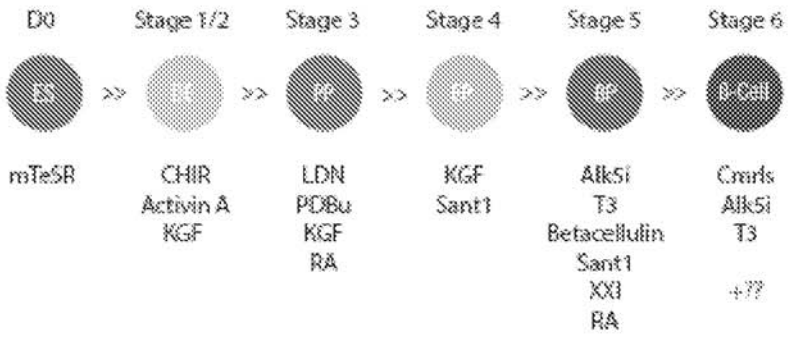
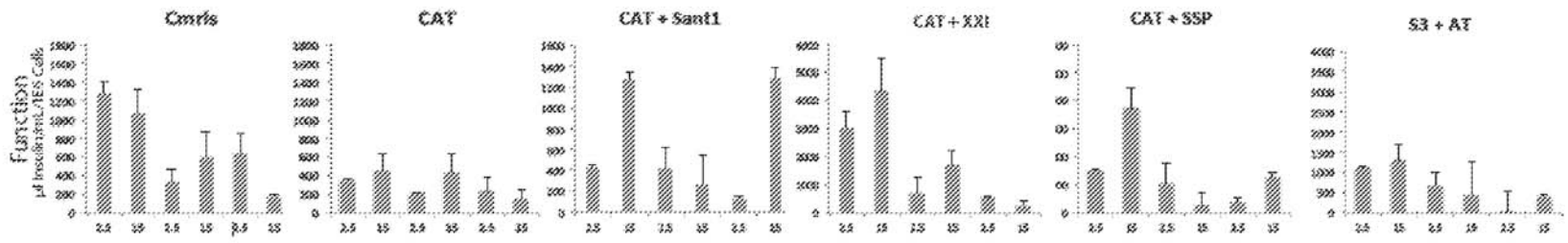
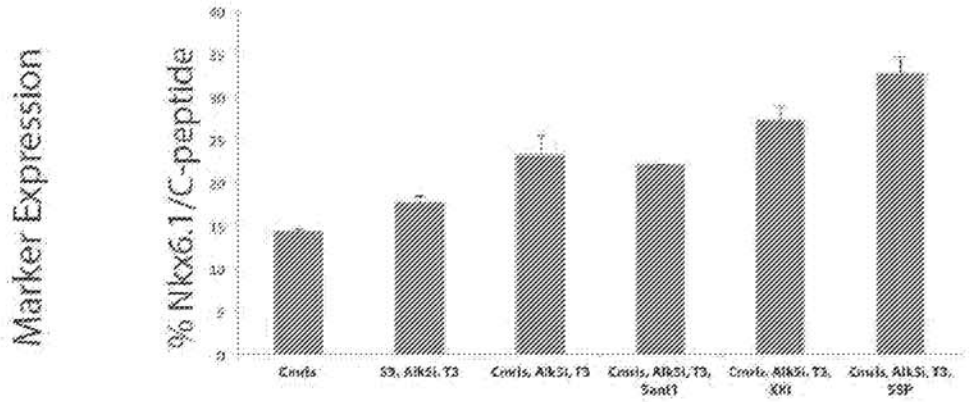

D0
Stage 1/2
Stage 3
Stage 4
Stage 5
Stage 6
ES
>>
>>
>>
>>
>>
β-Cell
mTeSR
CHIR
Activin A
KGF
LDN
PDBu
KGF
RA
KGF
Sant1
Alk5i
T3
Betacellulin
Sant1
XXI
RA
Cmrls
Alk5i
T3
+??

FIG. 14A

B

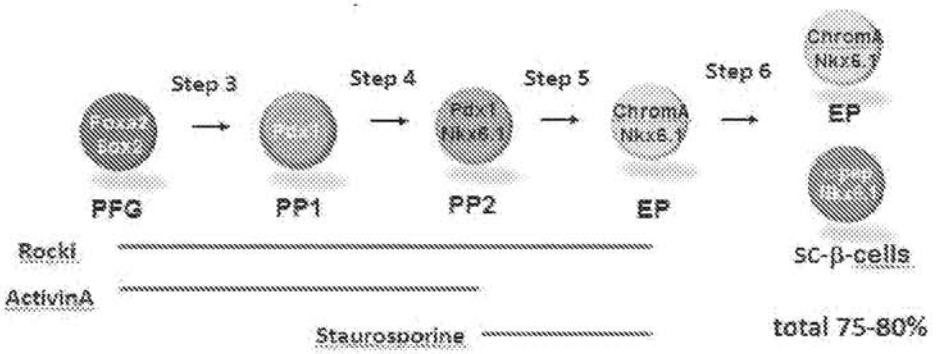


C

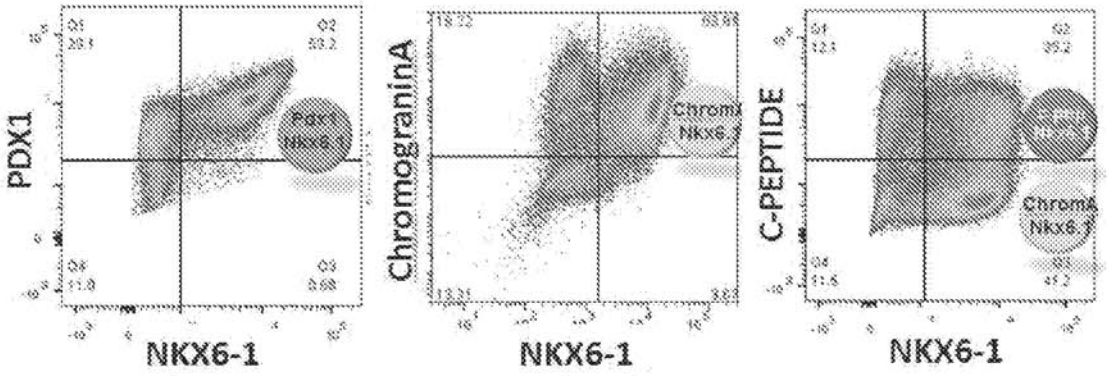


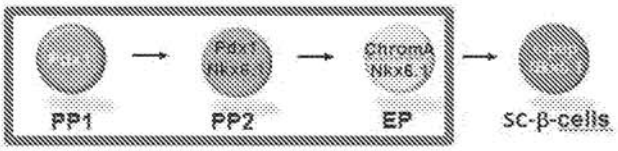
FIGS. 14B-14C

A

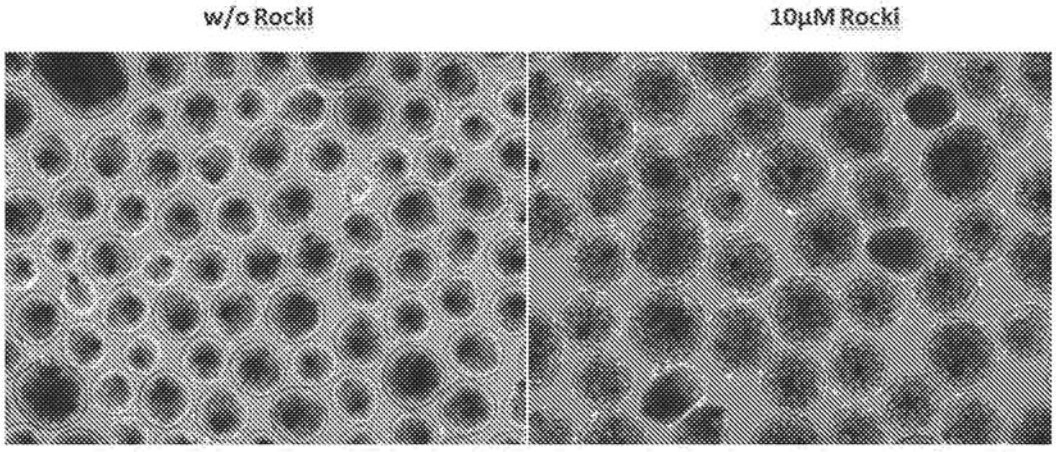


B



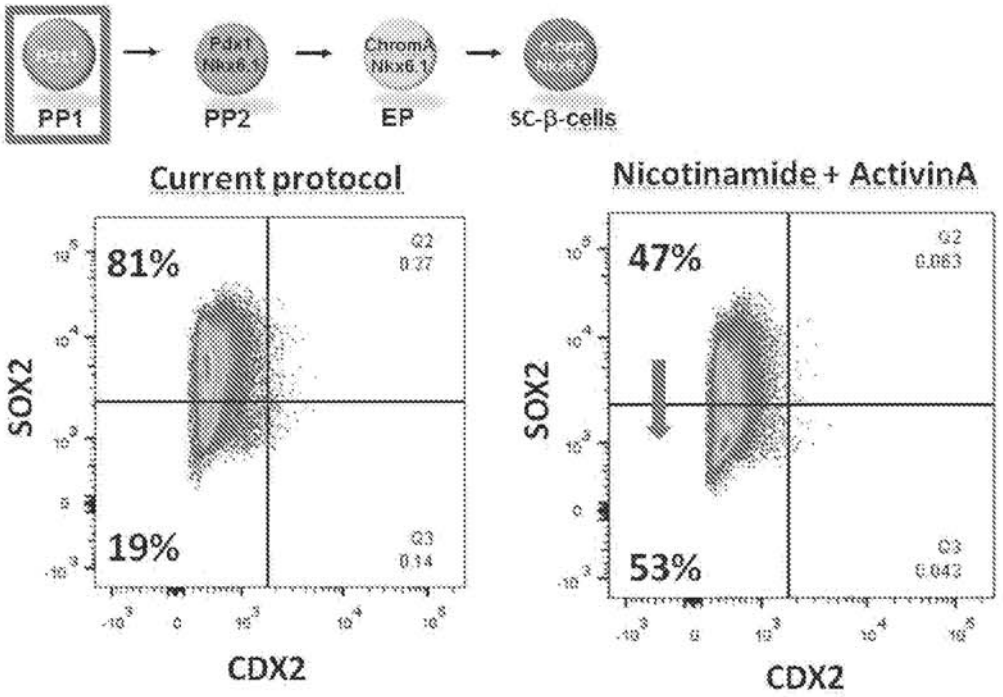


C

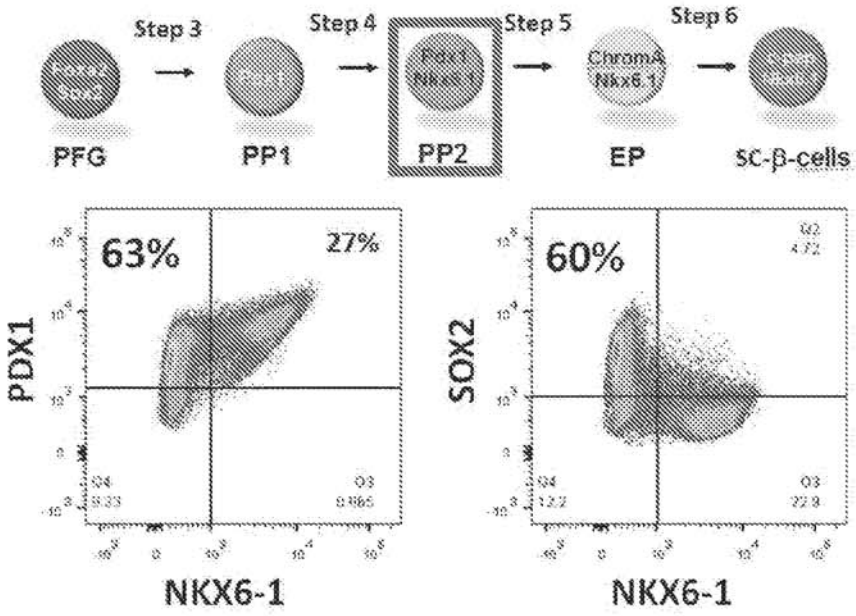


FIGS. 15A-15C

D

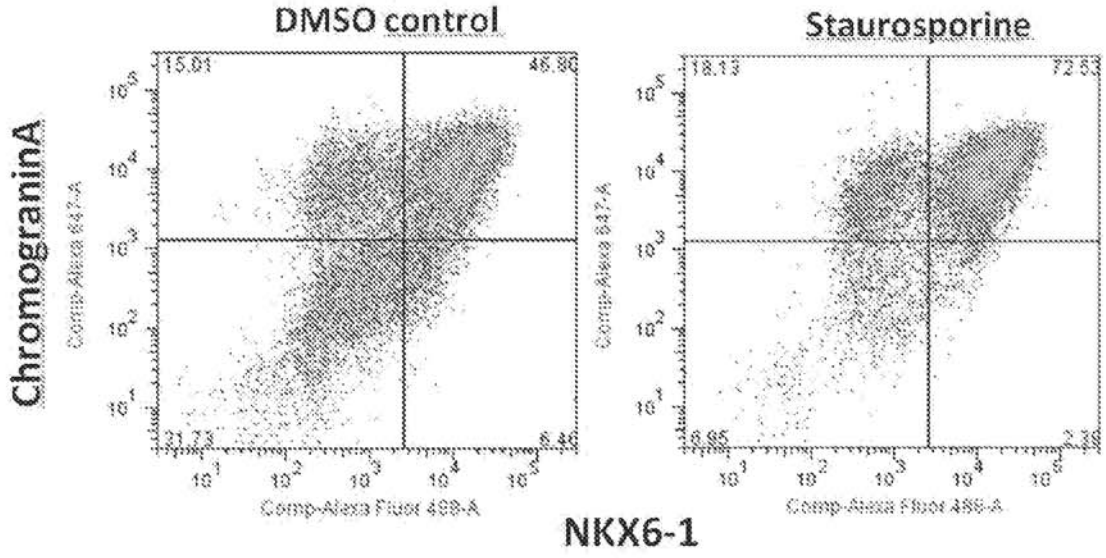


E

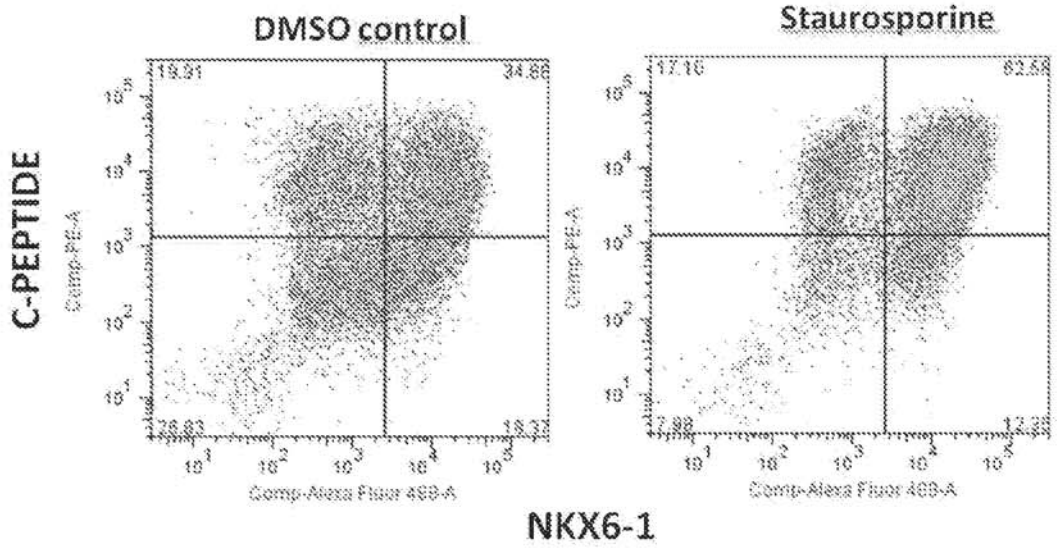


FIGS.15D-15E

F

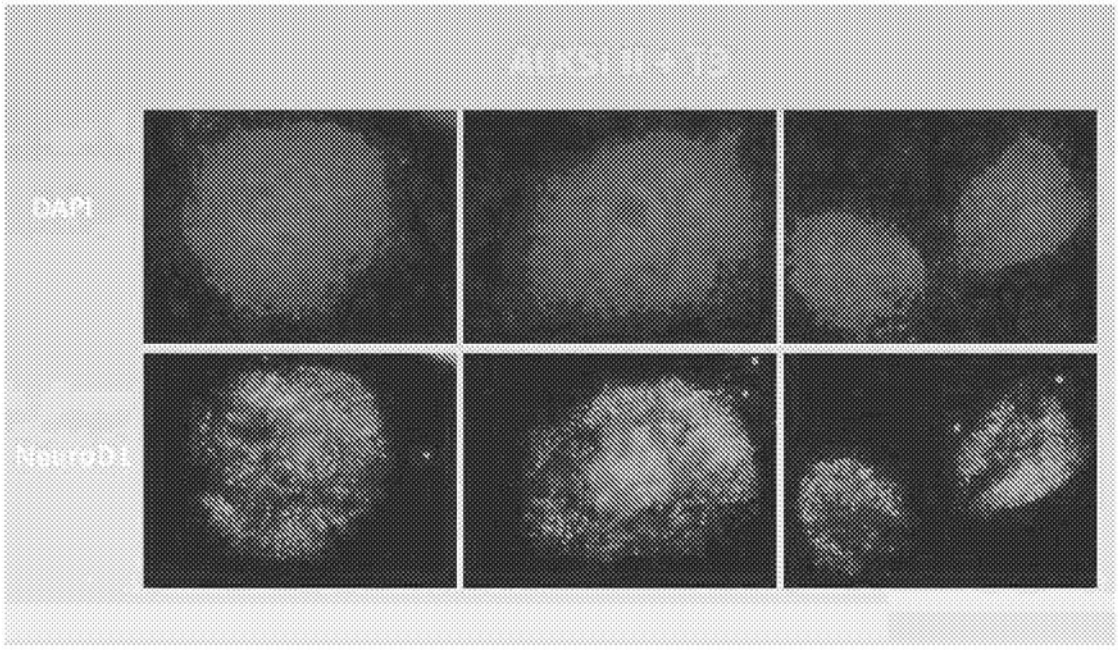


G

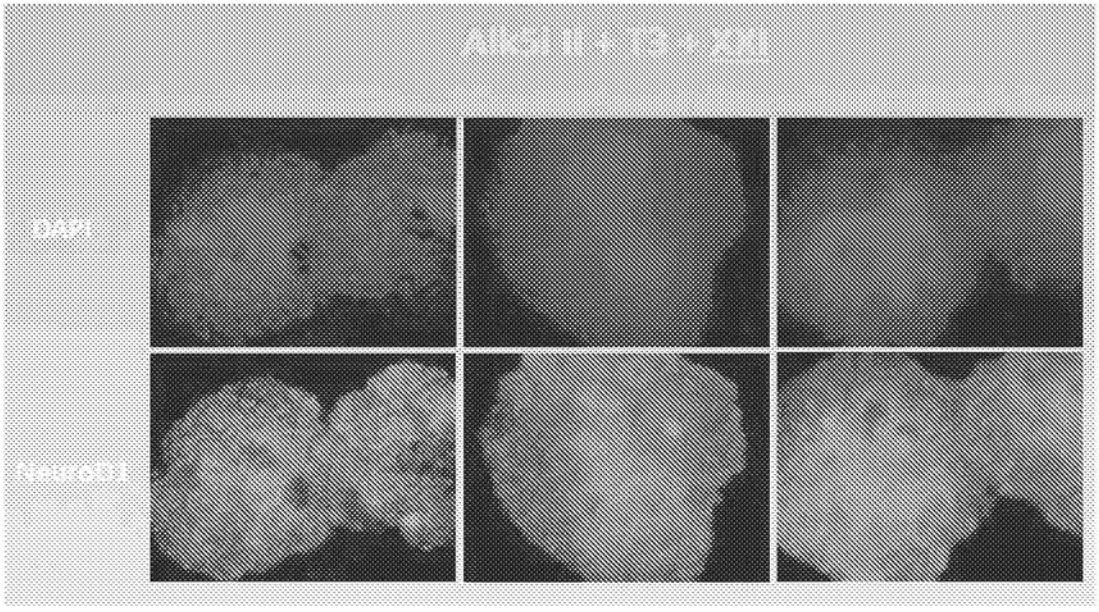


FIGS. 15F-15G

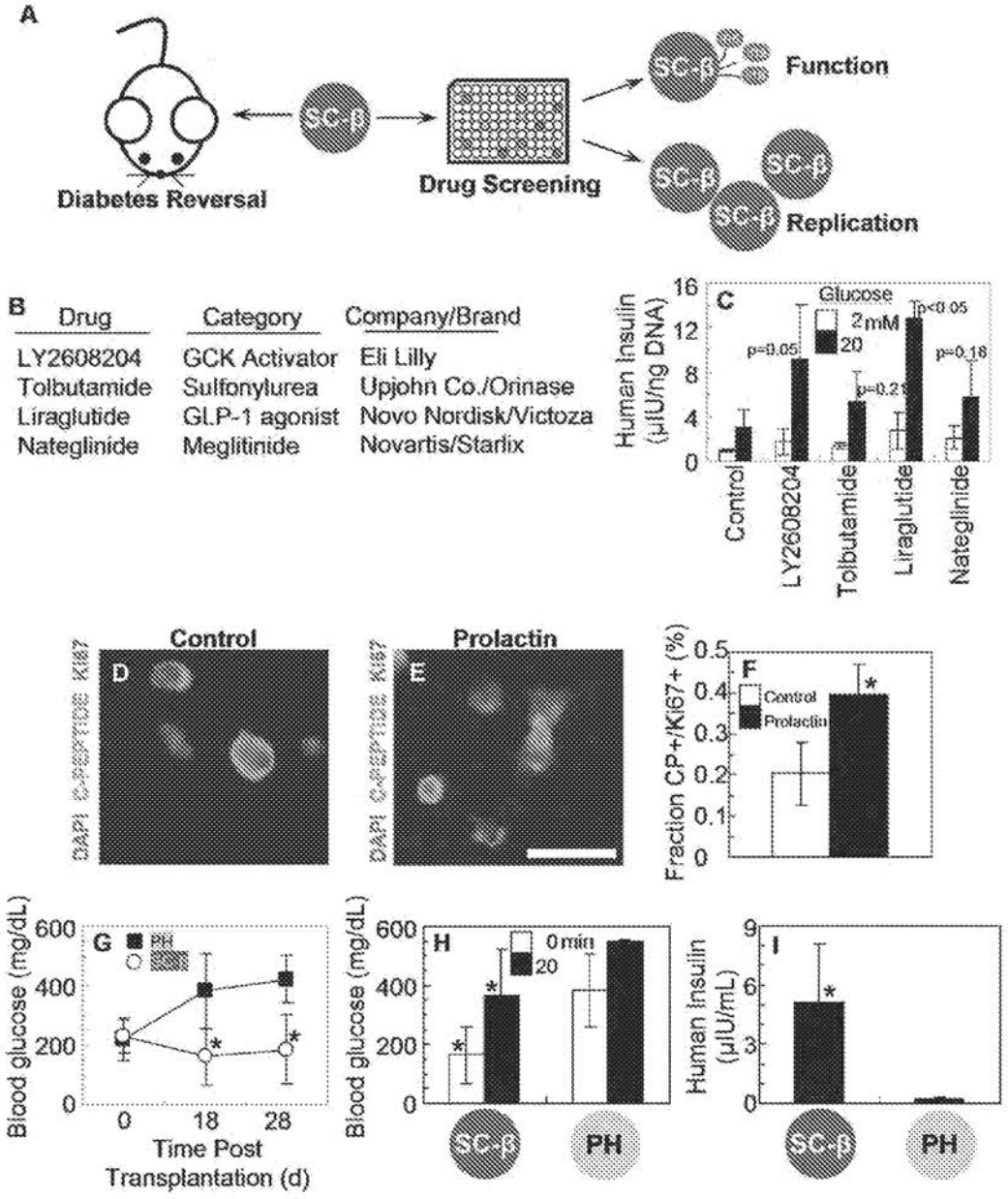
H



I



FIGS. 15H-15I


A
SC-β
Diabetes Reversal
Drug Screening
Function
Replication
B
Drug | Category | Company/Brand
LY2608204 | GCK Activator | Eli Lilly
Tolbutamide | Sulfonylurea | Upjohn Co./Orinase
Liraglutide | GLP-1 agonist | Novo Nordisk/Victoza
Nateglinide | Meglitinide | Novartis/Starlix
C
Glucose
2mM
20
Human Insulin (μIU/mg DNA)
p=0.05
p<0.05
p=0.18
Control
LY2608204
Tolbutamide
Liraglutide
Nateglinide
Control
Prolactin
DAPI C-PEPTIDE KI67
D
E
F
Control
Prolactin
Fraction CP+/Ki67+ (%)
G
PH
Blood glucose (mg/dL)
Time Post Transplantation (d)
H
0 min
20
Blood glucose (mg/dL)
I
Human Insulin (μIU/mL)
SC-β
PH

FIGS. 16A-16I

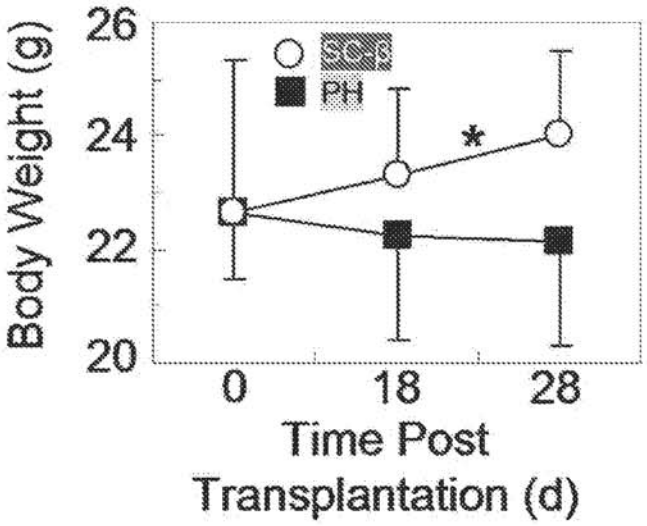

Body Weight (g)
26
24
22
20
SC-β
PH
*
0
18
28
Time Post
Transplantation (d)

FIG. 17