Figure 1

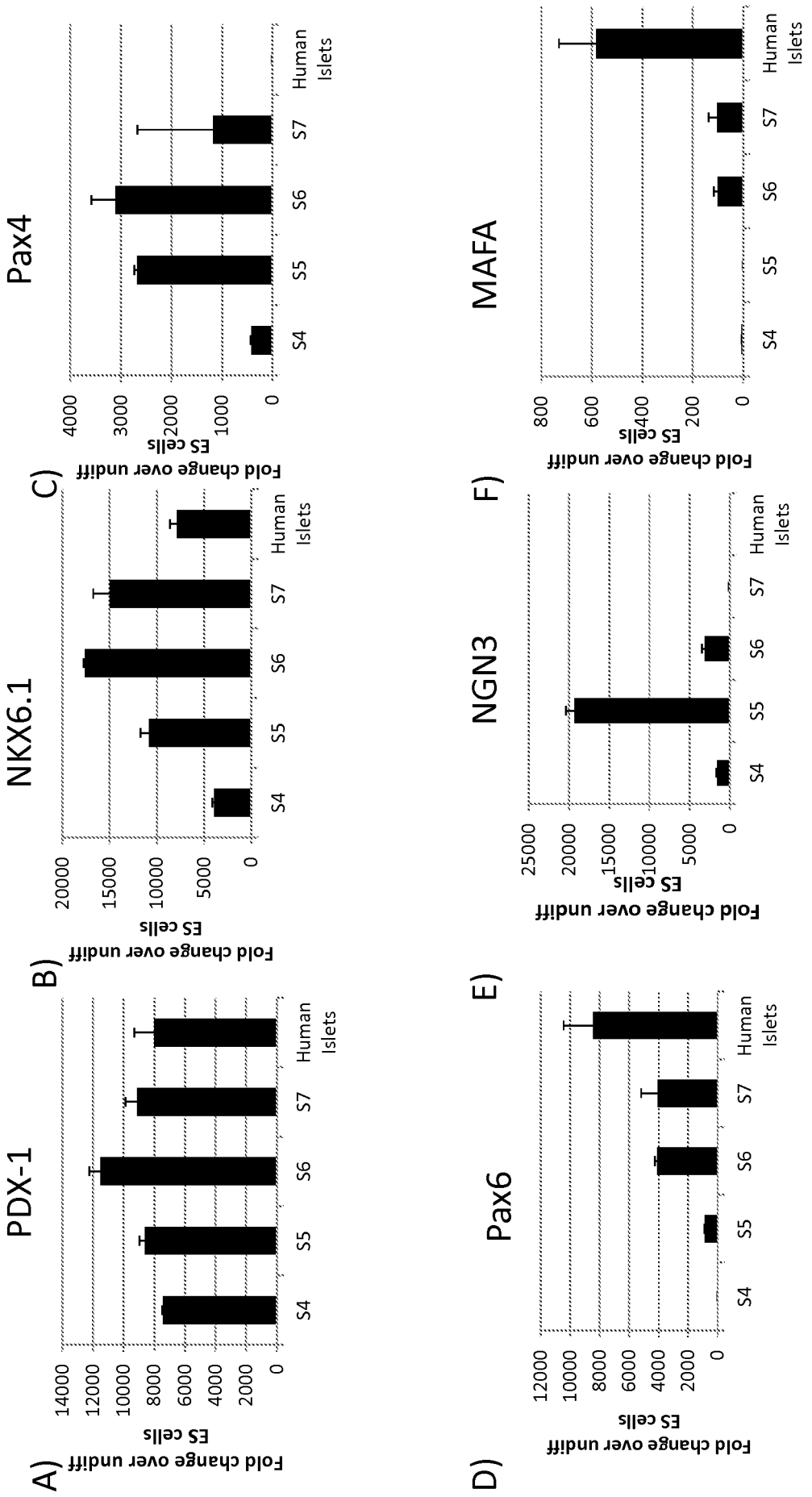

A)
PDX-1
Fold change over undiff
ES cells
14000
12000
10000
8000
6000
4000
2000
0
S4
S5
S6
S7
Human Islets
B)
NKX6.1
Fold change over undiff
ES cells
20000
15000
10000
5000
0
S4
S5
S6
S7
Human Islets
C)
Pax4
Fold change over undiff
ES cells
4000
3000
2000
1000
0
S4
S5
S6
S7
Human Islets
D)
Pax6
Fold change over undiff
ES cells
12000
10000
8000
6000
4000
2000
0
S4
S5
S6
S7
Human Islets
E)
NGN3
Fold change over undiff
ES cells
25000
20000
15000
10000
5000
0
S4
S5
S6
S7
Human Islets
F)
MAFA
Fold change over undiff
ES cells
800
600
400
200
0
S4
S5
S6
S7
Human Islets

Figure 1

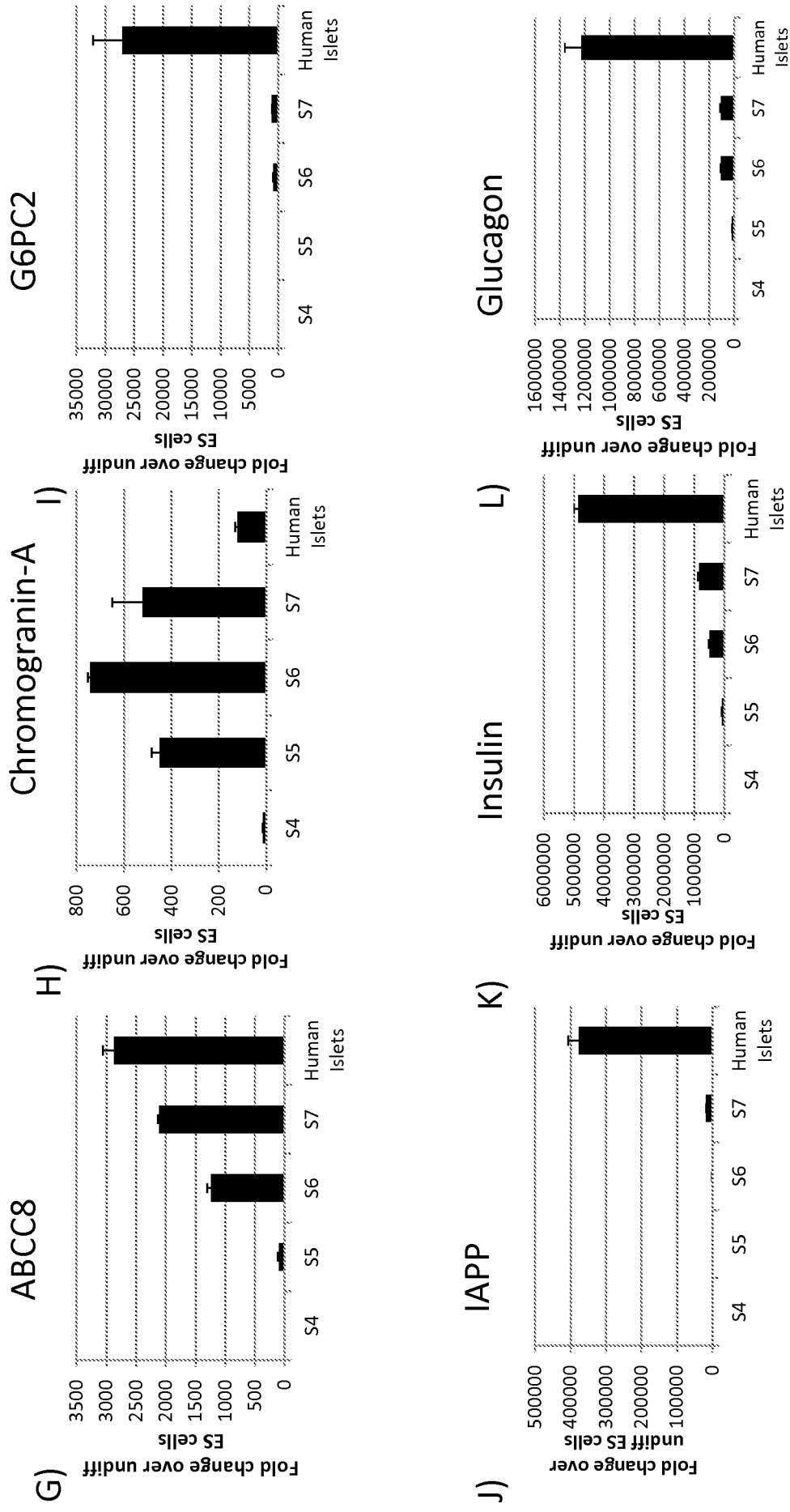

G)
ABCC8
Fold change over undiff ES cells
3500
3000
2500
2000
1500
1000
500
0
S4
S5
S6
S7
Human Islets
H)
Chromogranin-A
Fold change over undiff ES cells
800
600
400
200
0
S4
S5
S6
S7
Human Islets
I)
G6PC2
Fold change over undiff ES cells
35000
30000
25000
20000
15000
10000
5000
0
S4
S5
S6
S7
Human Islets
J)
IAPP
Fold change over undiff ES cells
500000
400000
300000
200000
100000
0
S4
S5
S6
S7
Human Islets
K)
Insulin
Fold change over undiff ES cells
6000000
5000000
4000000
3000000
2000000
1000000
0
S4
S5
S6
S7
Human Islets
L)
Glucagon
Fold change over undiff ES cells
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
S4
S5
S6
S7
Human Islets

Figure 1

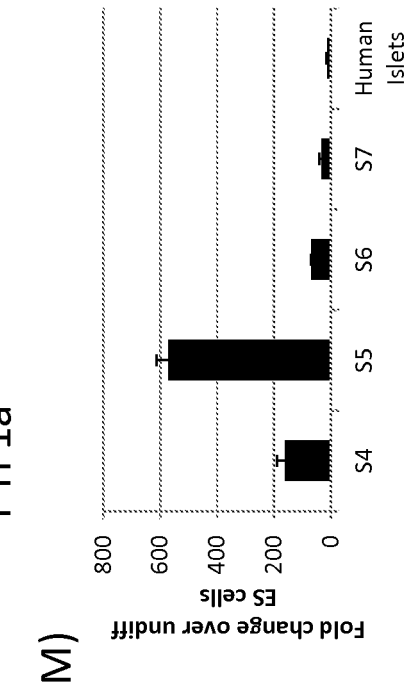

M)
PTF1a
Fold change over undiff
ES cells
800
600
400
200
0
S4
S5
S6
S7
Human Islets

Figure 2

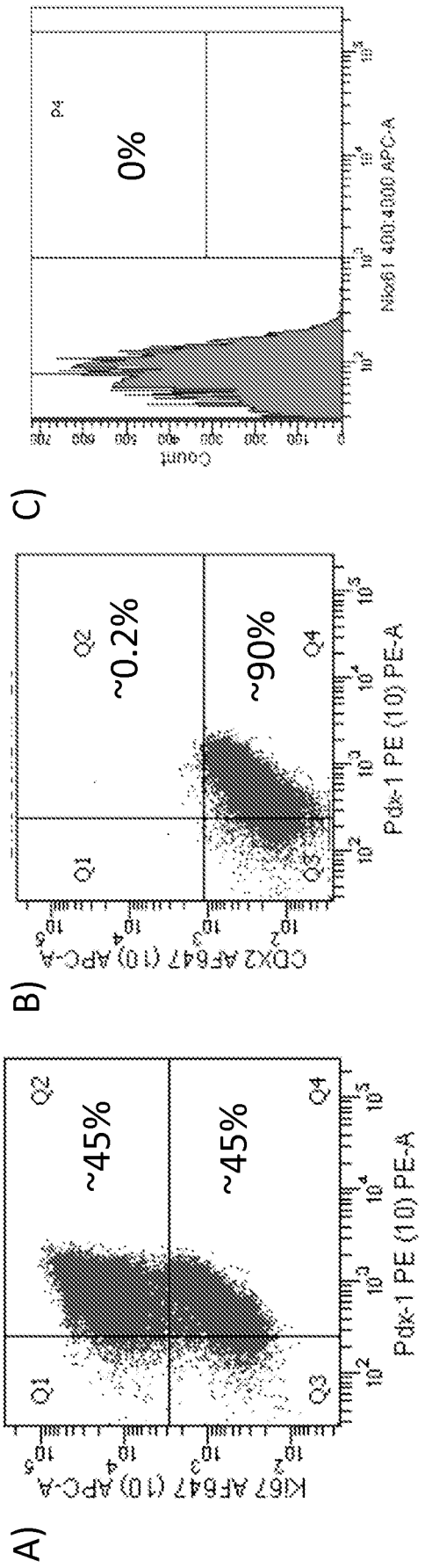


Figure 3

Stage 4

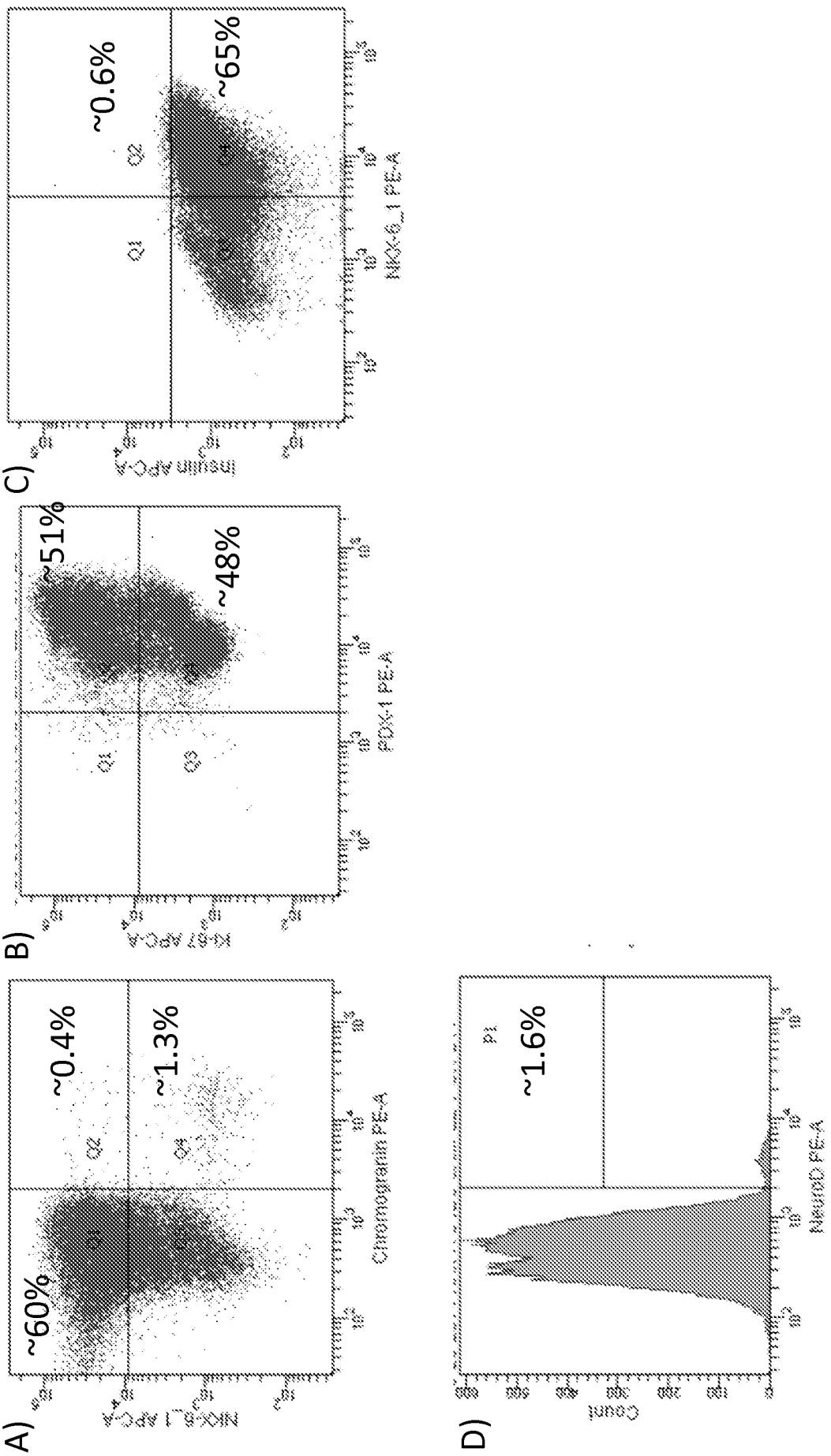


Figure 4

Stage 5

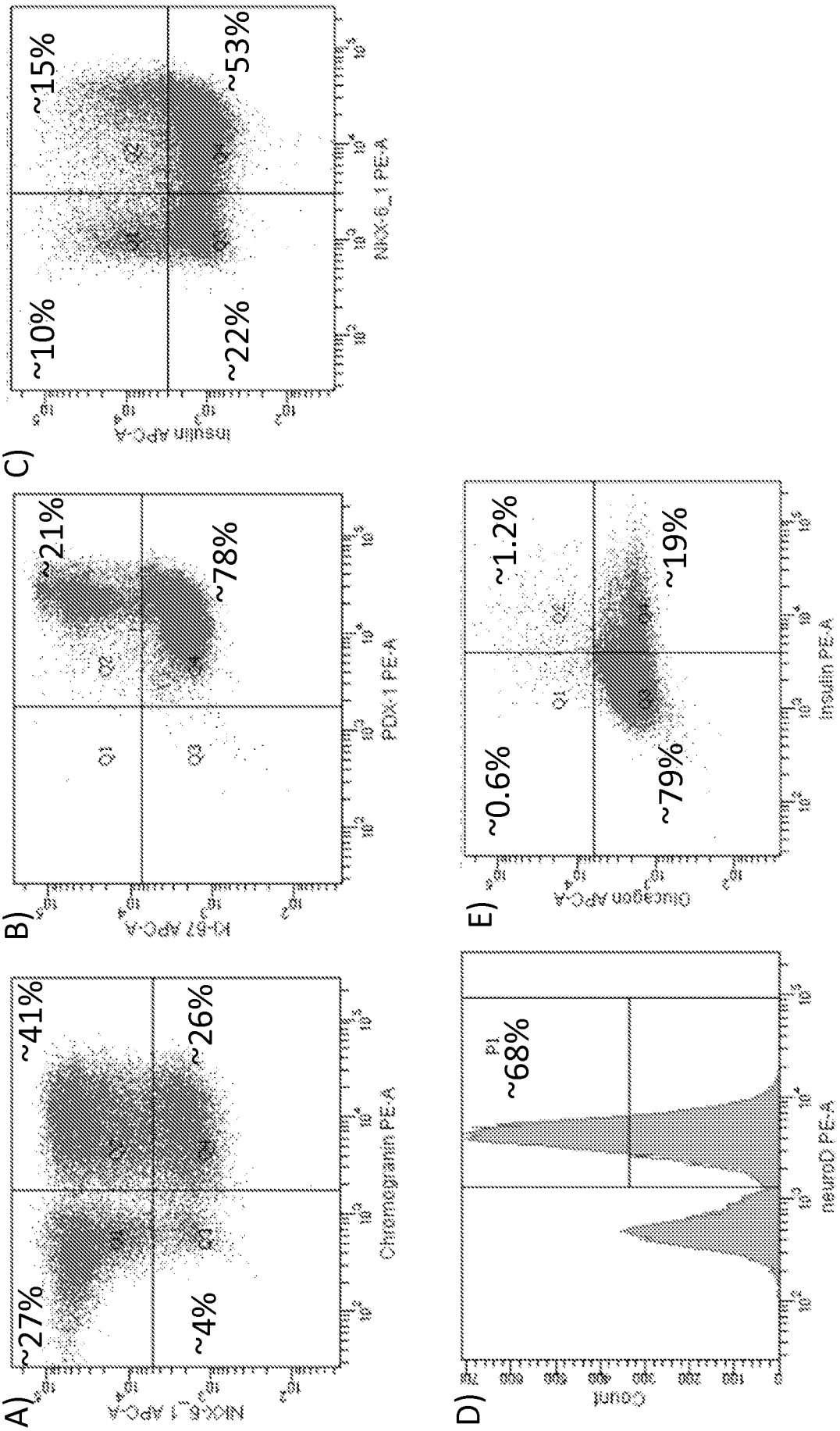


Figure 5

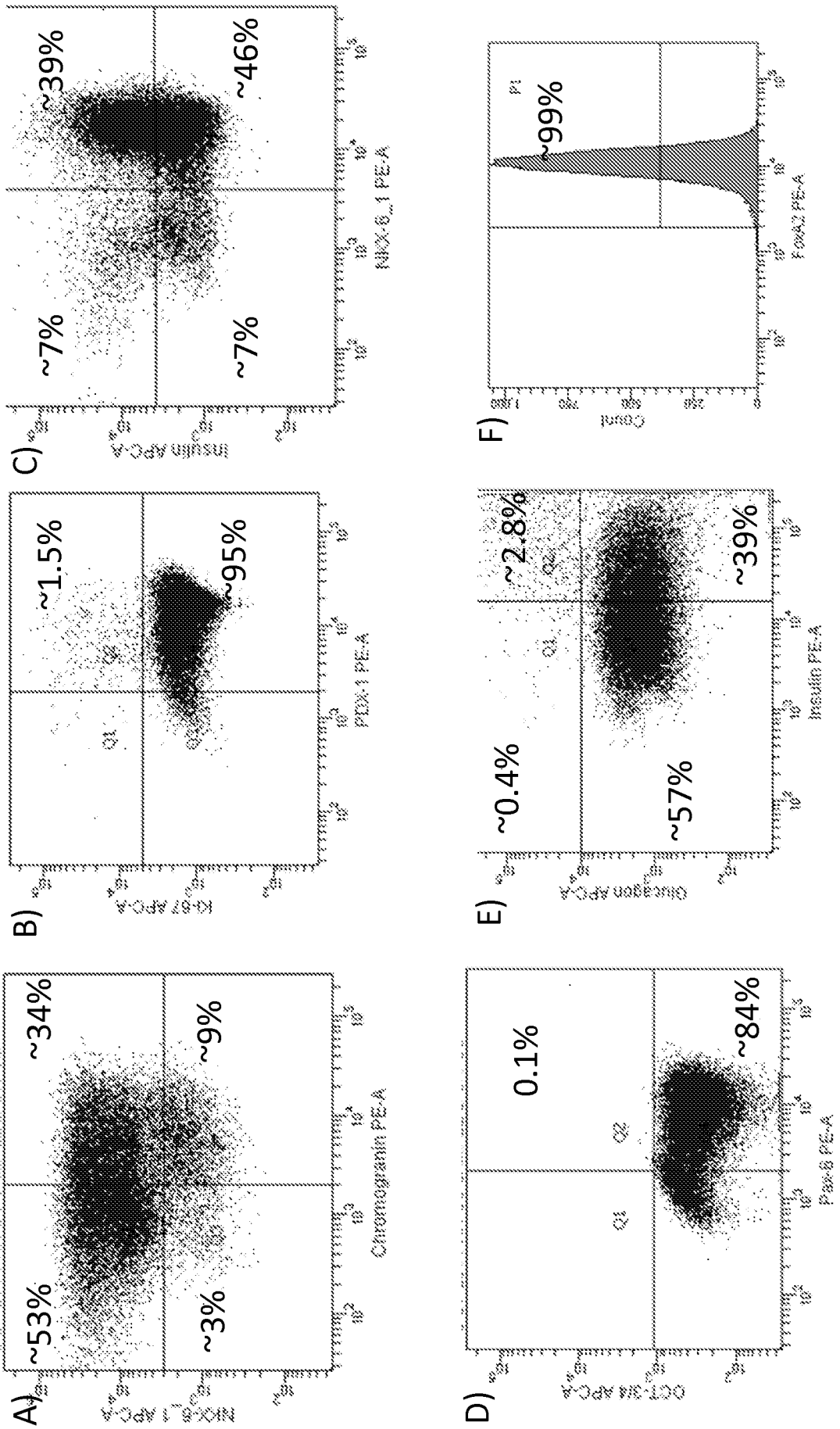

Stage 6
A)
~53%
~34%
~3%
~9%
NKX-6.1 APC-A
Chromogranin PE-A
B)
~1.5%
~95%
Ki-67 APC-A
PDX-1 PE-A
C)
~7%
~39%
~7%
~46%
Insulin APC-A
NKX-6.1 PE-A
D)
0.1%
~84%
OCT-3/4 APC-A
Pax-6 PE-A
E)
~0.4%
~ 2.8%
~57%
~39%
Glucagon APC-A
Insulin PE-A
F)
~99%
Count
FOXA2 PE-A

Figure 6

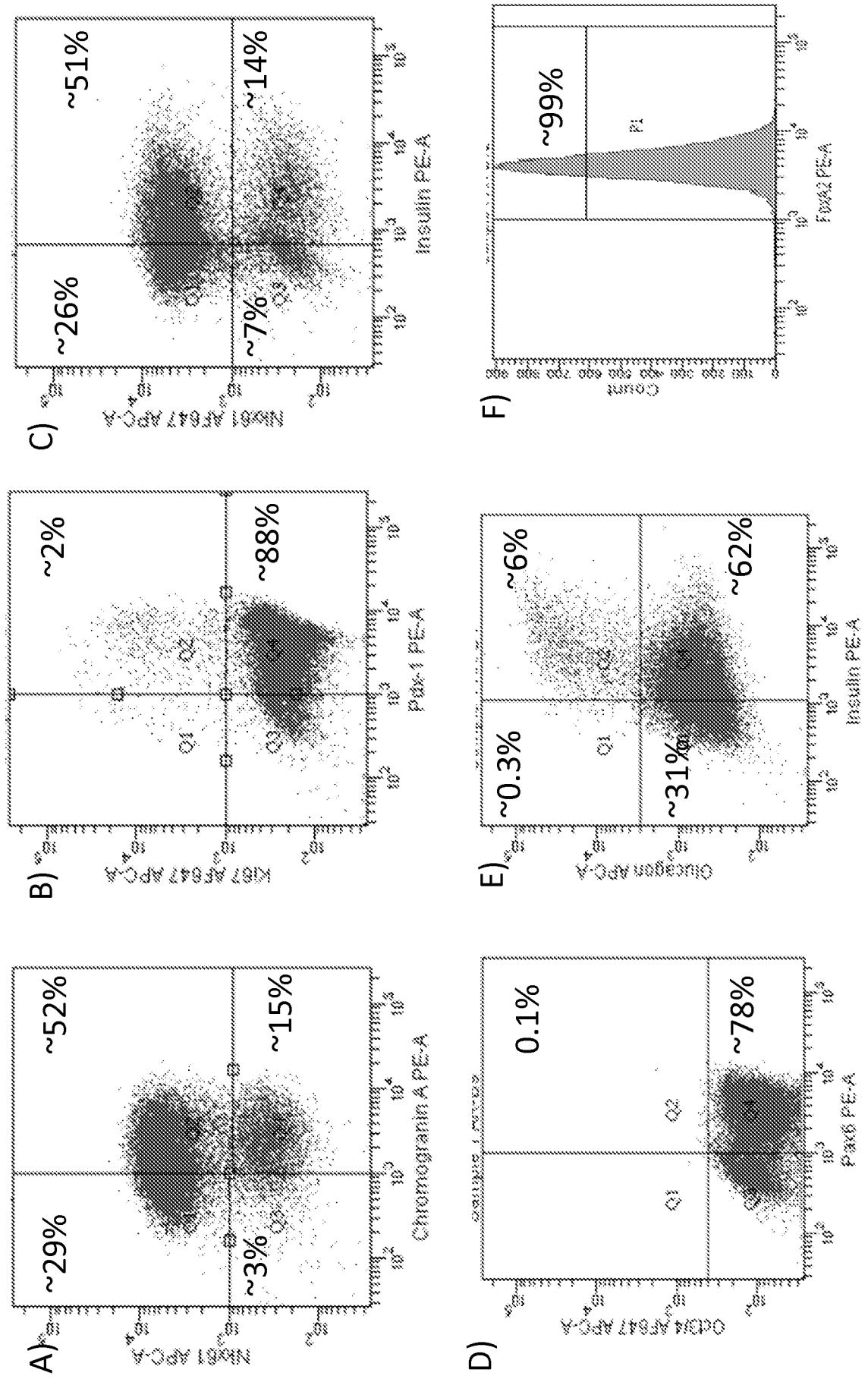

Stage 7
A)
~52%
~15%
~29%
~3%
NKX6.1 APC-A
Chromogranin A PE-A
B)
~2%
~88%
KI67 AF647 APC-A
PDX-1 PE-A
C)
~51%
~14%
~26%
~7%
NKX6.1 AF647 APC-A
Insulin PE-A
D)
0.1%
~78%
OCT3/4 AF647 APC-A
PAX6 PE-A
E)
~6%
~62%
~0.3%
~31%
Glucagon APC-A
Insulin PE-A
F)
~99%
FOXA2 PE-A
Count

Figure 7

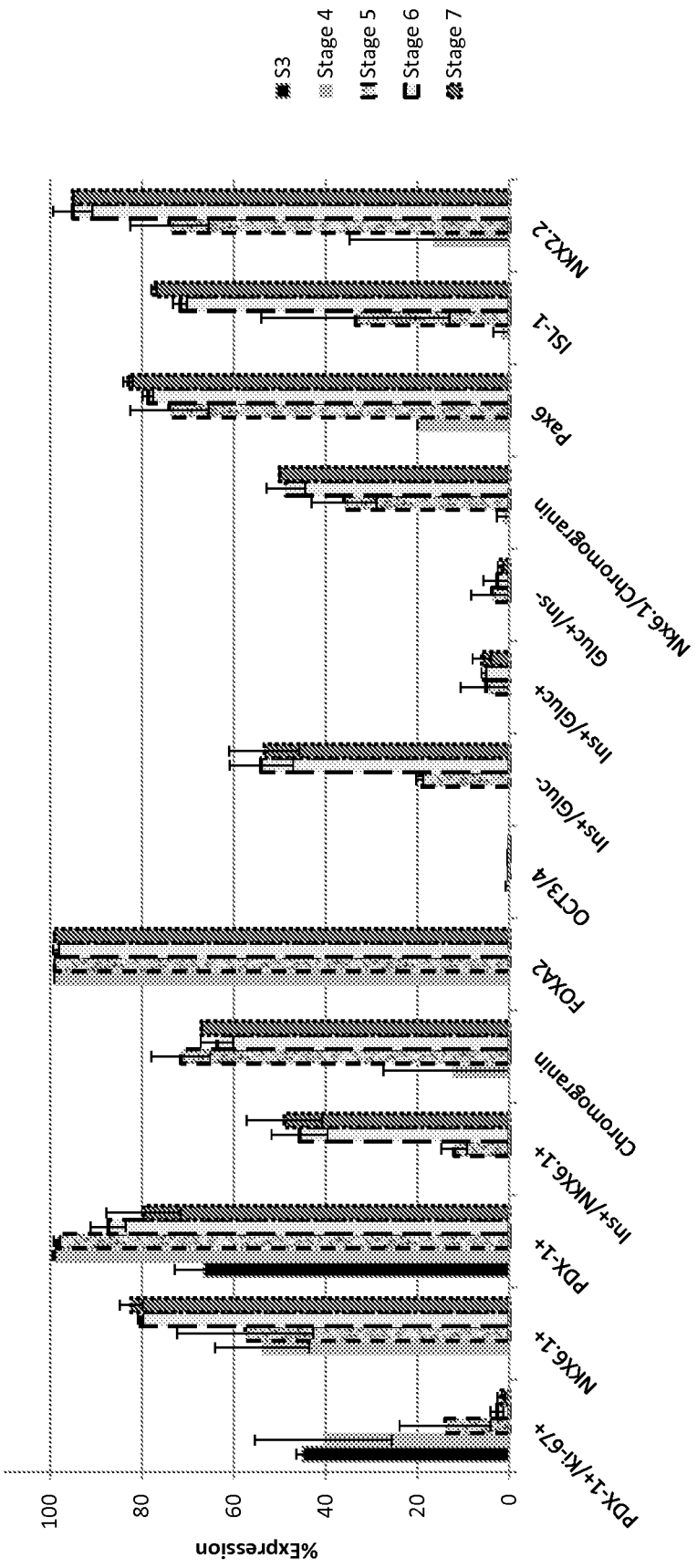

S3
Stage 4
Stage 5
Stage 6
Stage 7
%Expression
0
20
40
60
80
100
PDX-1+/Ki-67+
NKX6.1+
PDX-1+
ins+/NKX6.1+
Chromogranin
FOXA2
OCT3/4
ins+/Gluc-
ins+/Gluc+
Gluc+/ins-
NKX6.1/Chromogranin
Pax6
ISL-1
NKX2.2

Figure 8

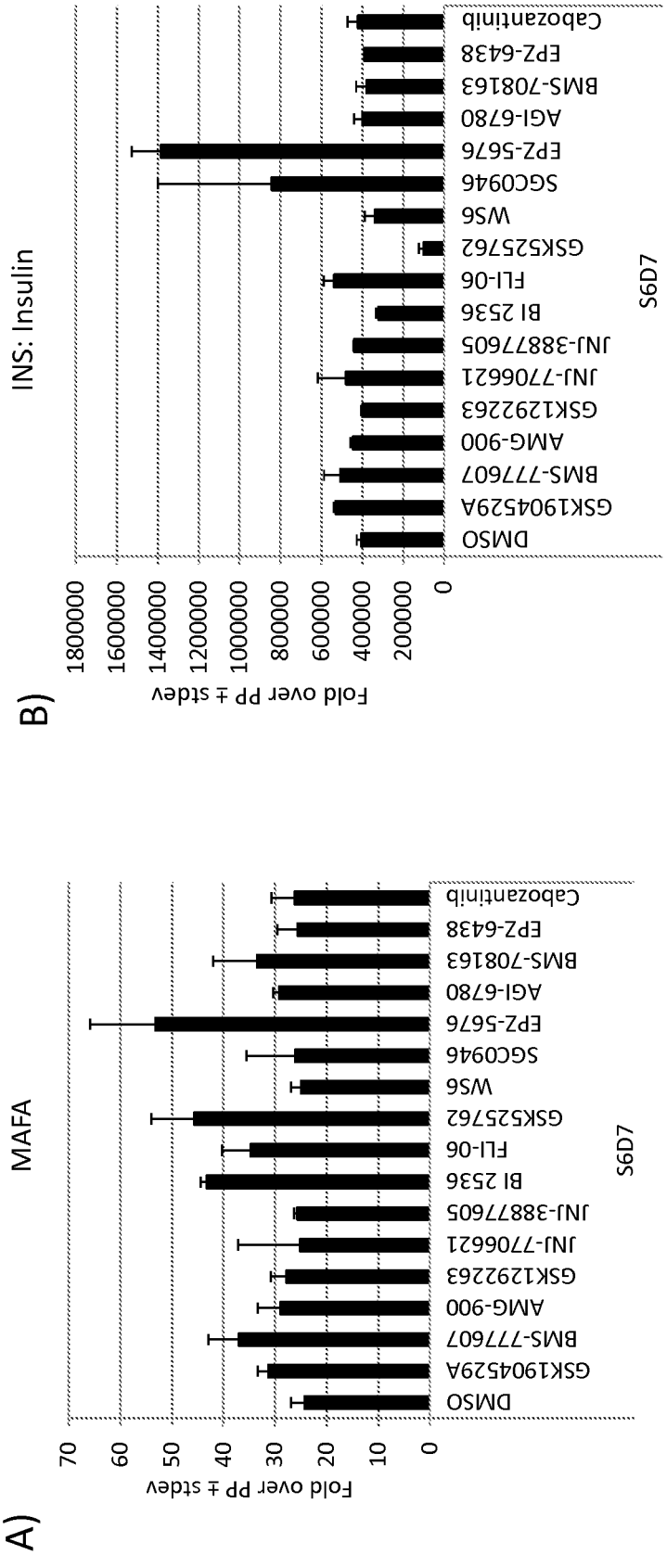

A)
MAFA
Fold over PP ± stdev
0
10
20
30
40
50
60
70
DMSO
GSK1904529A
BMS-777607
AMG-900
GSK1292263
JNJ-770662 1
JNJ-38877605
BI 2536
FLI-06
GSK525762
WS6
SGC0946
EPZ-5676
AGI-6780
BMS-708163
EPZ-6438
Cabozantinib
S6D7
B)
INS: Insulin
Fold over PP ± stdev
0
200000
400000
600000
800000
1000000
1200000
1400000
1600000
1800000
DMSO
GSK1904529A
BMS-777607
AMG-900
GSK1292263
JNJ-770662 1
JNJ-38877605
BI 2536
FLI-06
GSK525762
WS6
SGC0946
EPZ-5676
AGI-6780
BMS-708163
EPZ-6438
Cabozantinib
S6D7

Figure 8

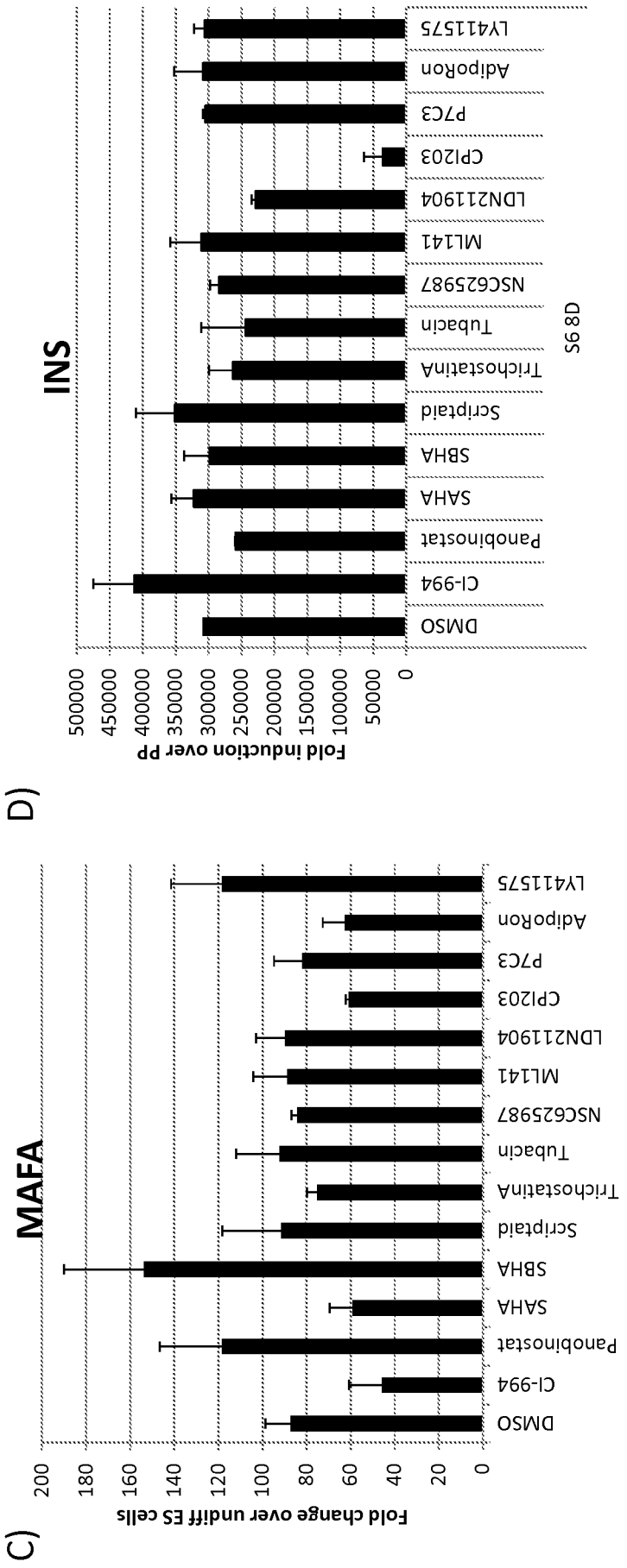

C)
MAFA
Fold change over undiff ES cells
0
20
40
60
80
100
120
140
160
180
200
DMSO
Cl-994
Panobinostat
SAHA
SBHA
Scriptaid
TrichostatinA
Tubacin
NSC625987
ML141
LDN211904
CPI203
P7C3
AdipoRon
LY411575
D)
INS
Fold induction over PP
0
50000
100000
150000
200000
250000
300000
350000
400000
450000
500000
DMSO
Cl-994
Panobinostat
SAHA
SBHA
Scriptaid
TrichostatinA
Tubacin
NSC625987
ML141
LDN211904
CPI203
P7C3
AdipoRon
LY411575
S6 8D

Figure 8

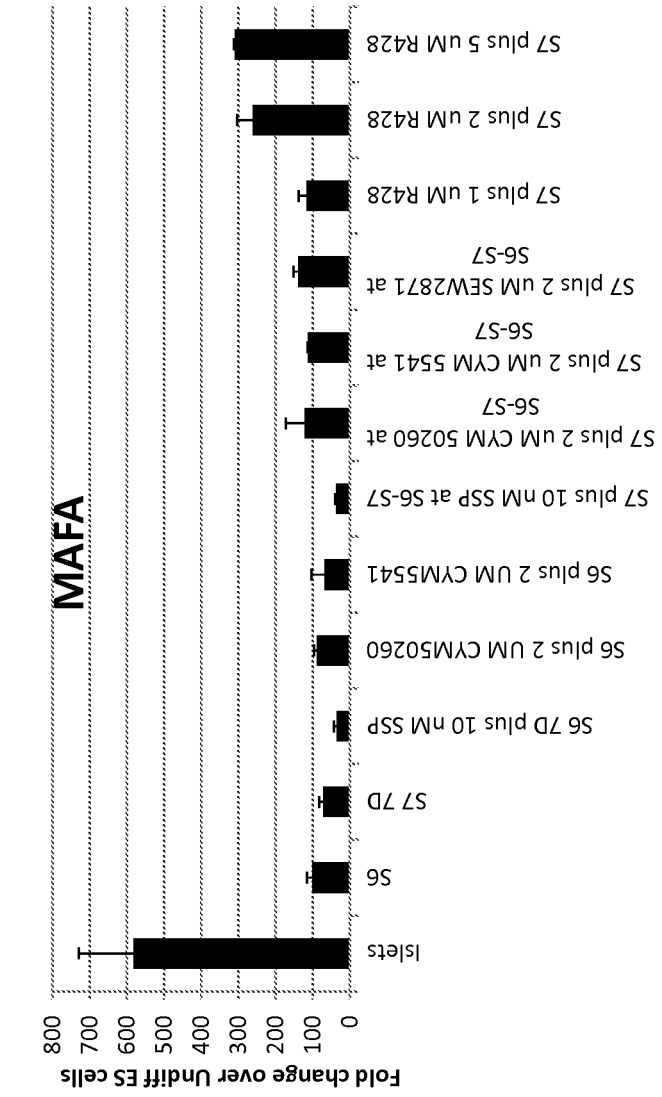

E)
MAFA
Fold change over Undiff ES cells
800
700
600
500
400
300
200
100
0
Islets
S6
S7 7D
S6 7D plus 10 nM SSP
S6 plus 2 UM CYM5026O
S6 plus 2 UM CYM5541
S7 plus 10 nM SSP at S6-S7
S7 plus 2 uM CYM 5026O at S6-S7
S7 plus 2 uM CYM 5541 at S6-S7
S7 plus 2 uM SEW2871 at S6-S7
S7 plus 1 uM R428
S7 plus 2 uM R428
S7 plus 5 uM R428

Figure 9

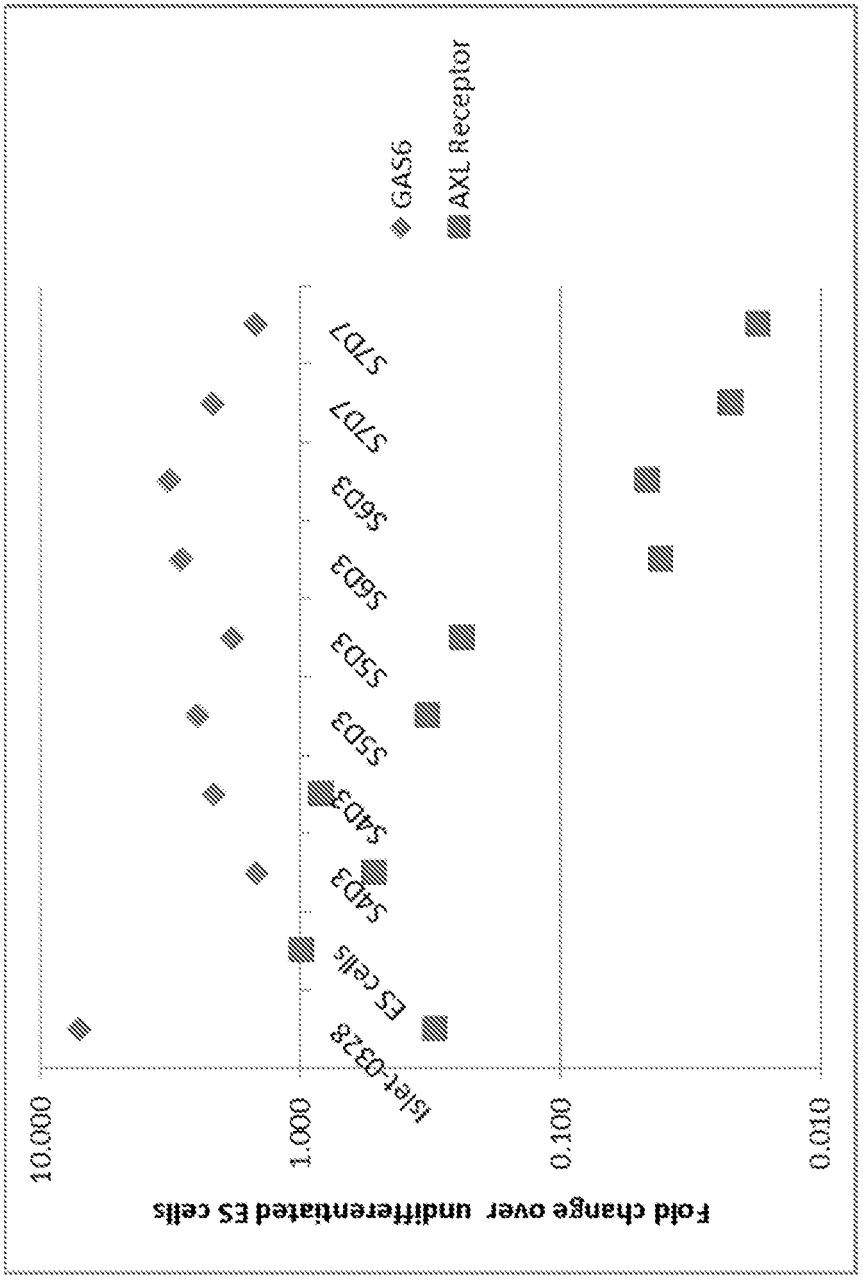

Fold change over undifferentiated ES cells
10.000
1.000
0.100
0.010
bler-0328
ES cells
5AD3
5A03
5SD3
5503
5603
5603
57D7
57D7
57D1
GAS6
AXL Receptor

Figure 10

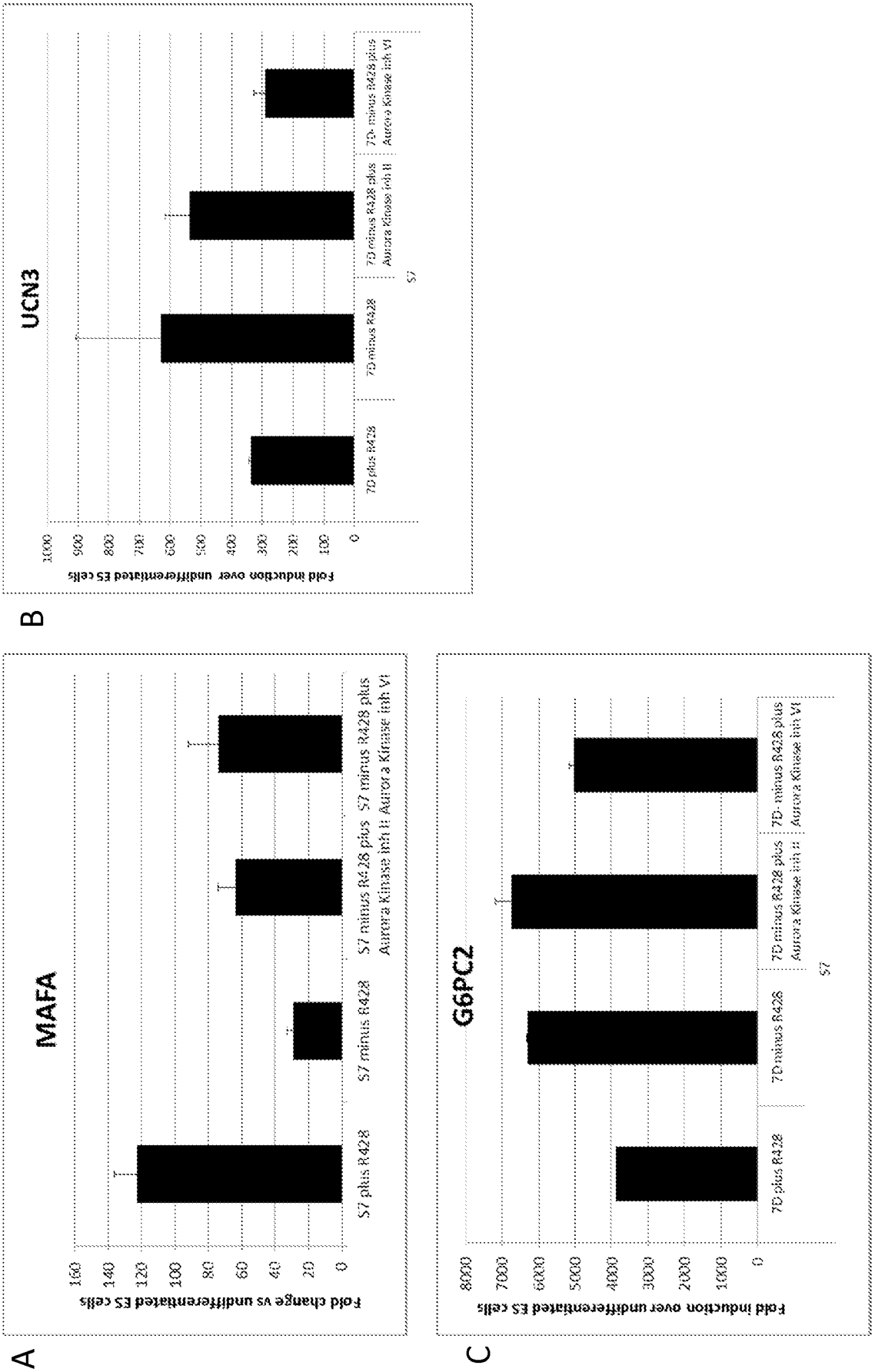

A
MAFA
Fold change vs undifferentiated ES cells
160
140
120
100
80
60
40
20
0
S7 plus R428
S7 minus R428
S7 minus R428 plus Aurora a Kinase Inh II
S7 minus R428 plus Aurora Kinase Inh VI
B
UCN3
Fold induction over undifferentiated ES cells
1000
900
800
700
600
500
400
300
200
100
0
7D plus R428
7D minus R428
7D minus R428 plus Aurora Kinase Inh II
7D- minus R428 plus Aurora Kinase Inh VI
S7
C
G6PC2
Fold induction over undifferentiated ES cells
8000
7000
6000
5000
4000
3000
2000
1000
0
7D plus R428
7D minus R428
7D minus R428 plus Aurora Kinase Inh II
7D- minus R428 plus Aurora Kinase Inh VI
S7

Figure 10D

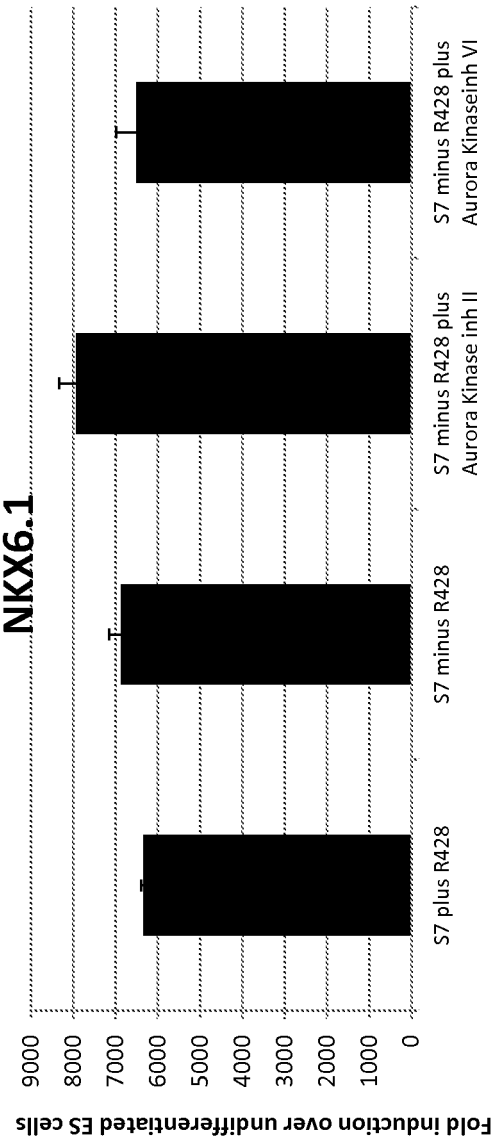

NKX6.1
Fold induction over undifferentiated ES cells
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
S7 plus R428
S7 minus R428
S7 minus R428 plus Aurora Kinase inh II
S7 minus R428 plus Aurora Kinaseinh VI

Figure 10E

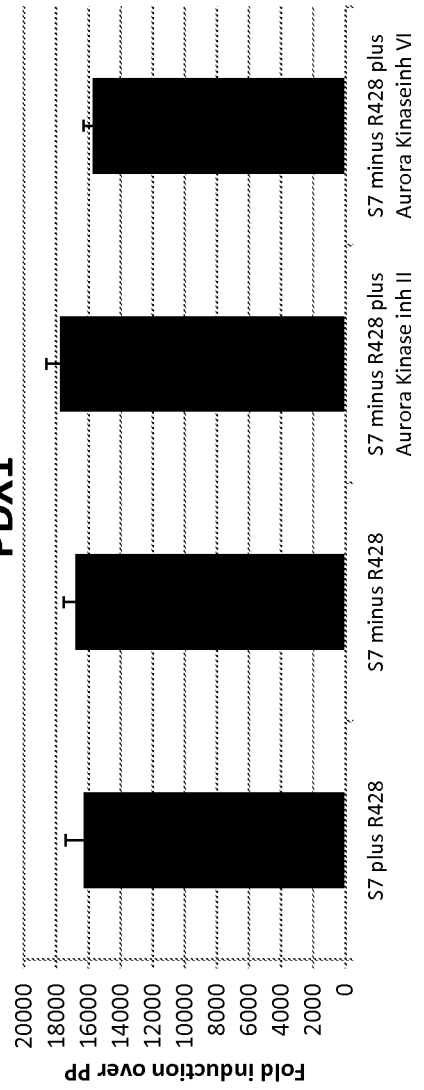

PDX1
Fold induction over PP
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
S7 plus R428
S7 minus R428
S7 minus R428 plus Aurora Kinase inh II
S7 minus R428 plus Aurora Kinaseinh VI

Figure 10F

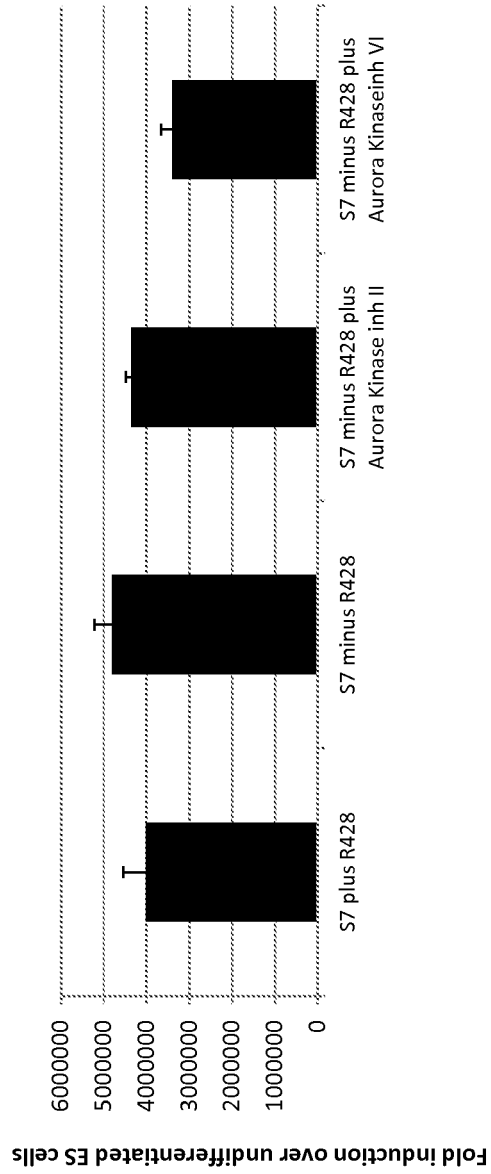

INS
Fold induction over undifferentiated ES cells
6000000
5000000
4000000
3000000
2000000
1000000
0
S7 plus R428
S7 minus R428
S7 minus R428 plus Aurora Kinase inh II
S7 minus R428 plus Aurora Kinaseinh VI

Figure 11A

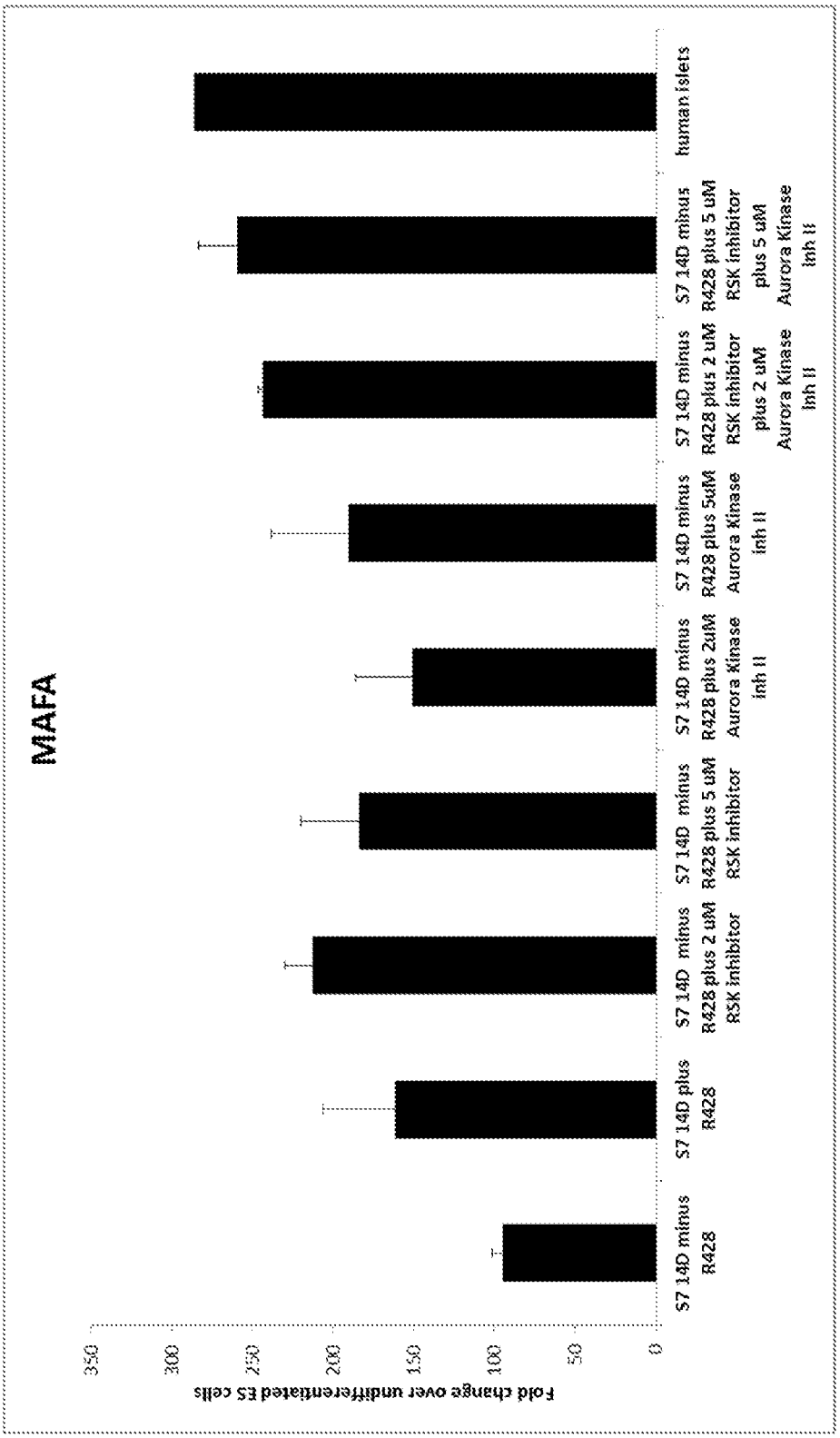

MAFA
Fold change over undifferentiated ES cells
0
50
100
150
200
250
300
350
S7 14D minus R428
S7 14D plus R428
S7 14D minus R428 plus 2 uM RSK inhibitor
S7 14D minus R428 plus 5 uM RSK inhibitor
S7 14D minus R428 plus 2uM Aurora Kinase inh II
S7 14D minus R428 plus 5uM Aurora Kinase inh II
S7 14D minus R428 plus 2 uM RSK inhibitor plus 2 uM Aurora Kinase inh II
S7 14D minus R428 plus 5 uM RSK inhibitor plus 5 uM Aurora Kinase inh II
human islets

Figure 11B

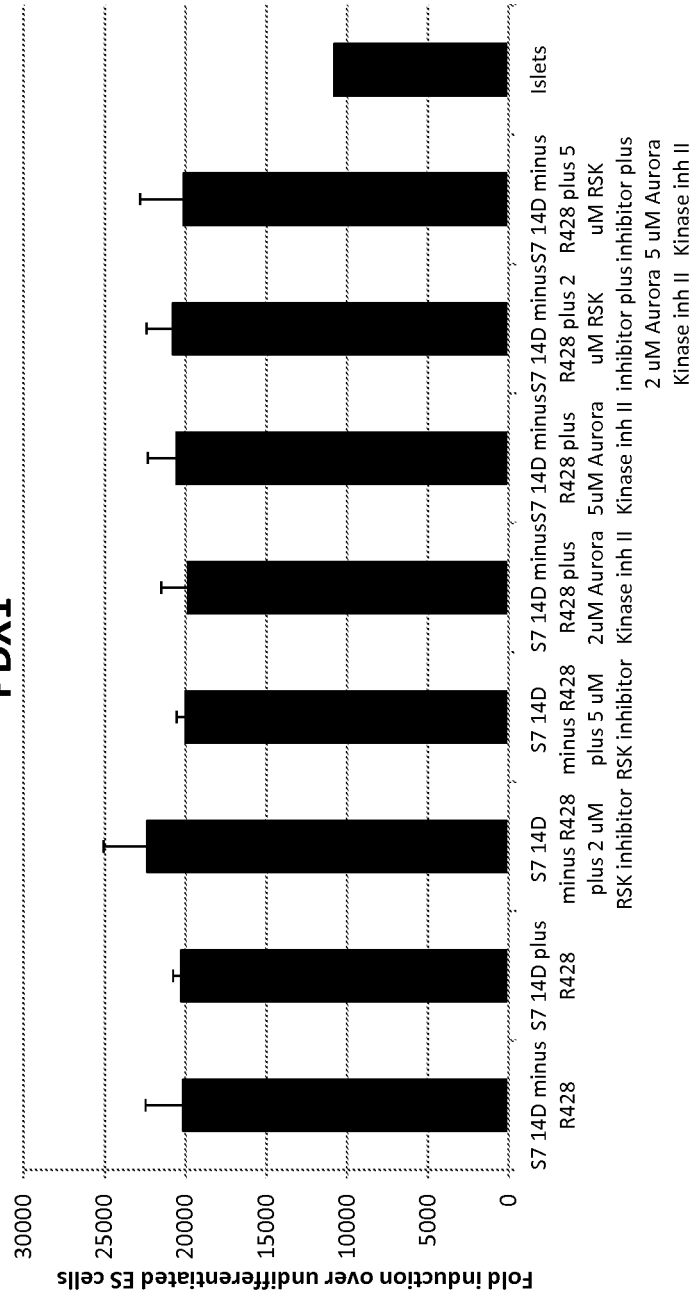

PDX1
Fold induction over undifferentiated ES cells
30000
25000
20000
15000
10000
5000
0
S7 14D minus R428
S7 14D plus R428
S7 14D minus R428 plus 2 uM RSK inhibitor
S7 14D minus R428 plus 5 uM RSK inhibitor
S7 14D minus R428 plus 2uM Aurora Kinase inh II
S7 14D minus R428 plus 5uM Aurora Kinase inh II
S7 14D minus R428 plus 2 uM Aurora inhibitor plus uM RSK Kinase inh II
S7 14D minus R428 plus 5 uM Aurora inhibitor plus 5 uM RSK Kinase inh II
Islets

Figure 11C

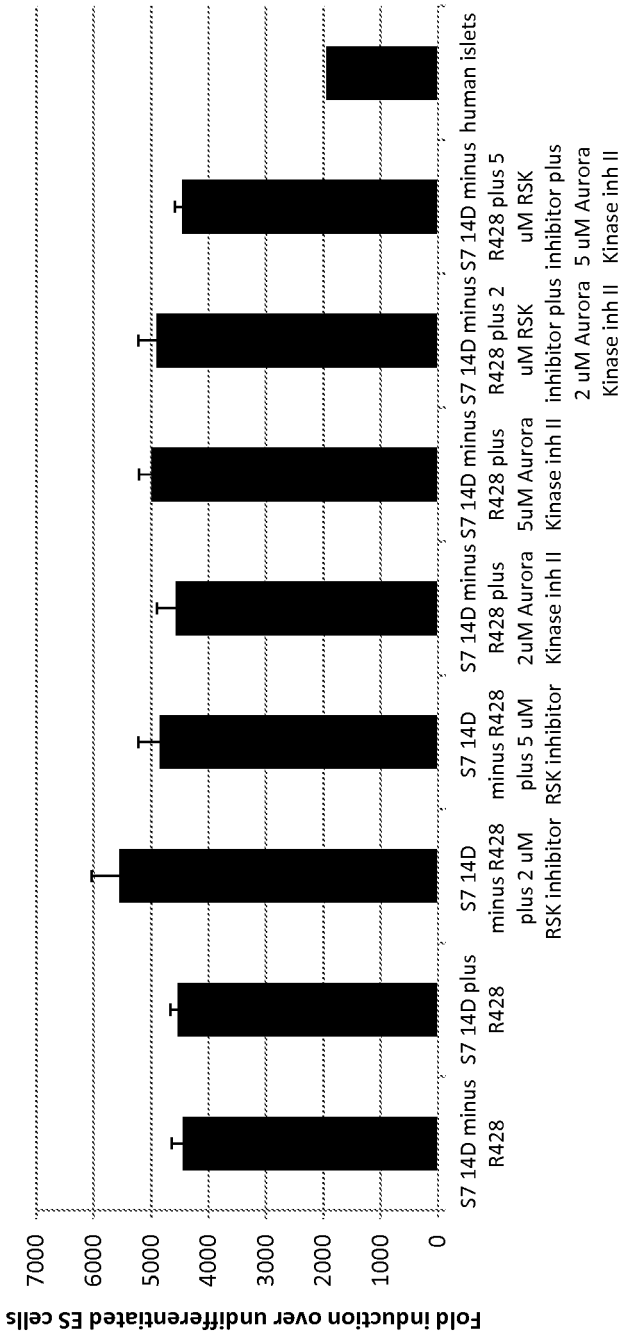
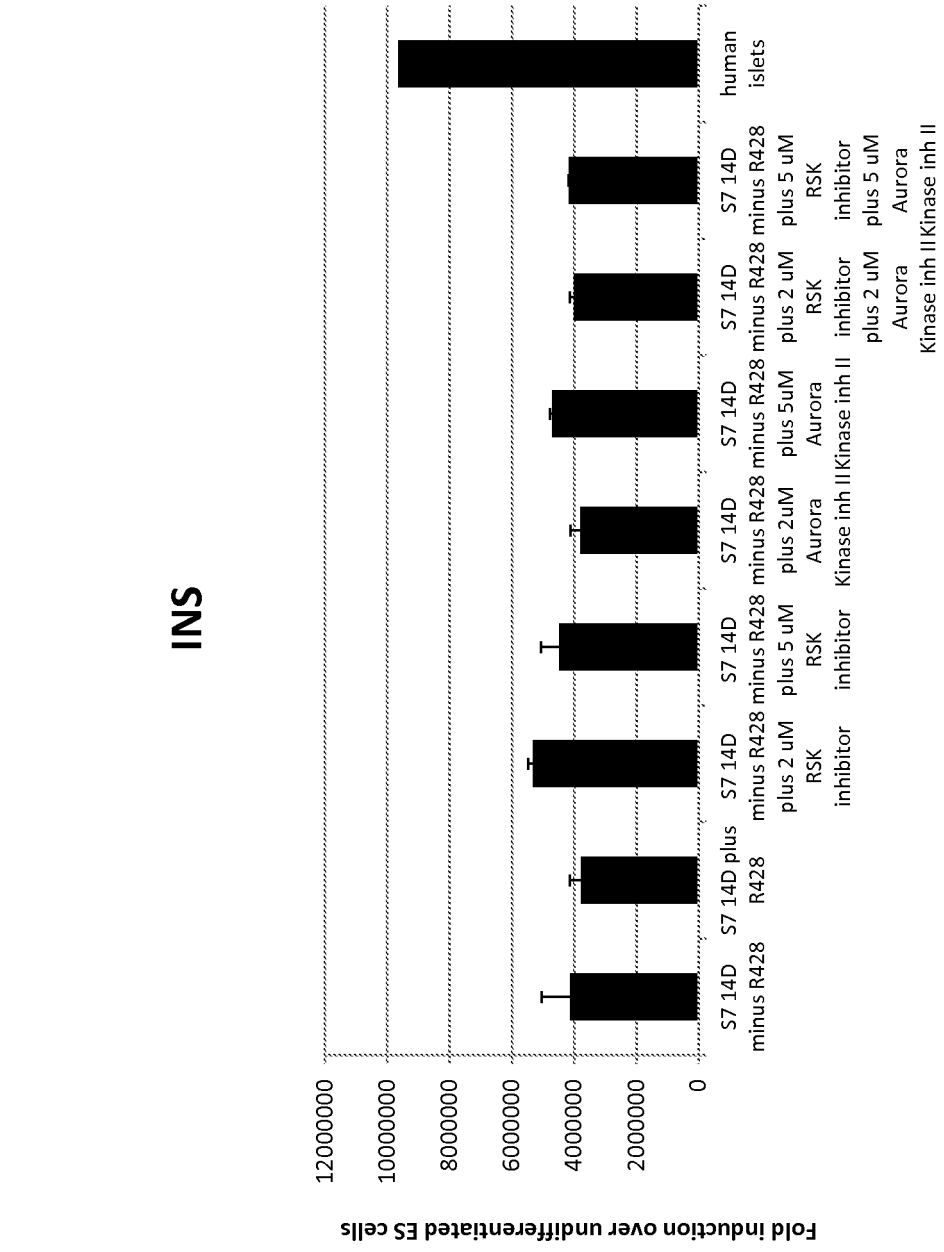

NKX6.1
Fold induction over undifferentiated ES cells
7000
6000
5000
4000
3000
2000
1000
0
S7 14D minus R428
S7 14D plus R428
S7 14D minus R428 plus 2 uM RSK inhibitor
S7 14D minus R428 plus 5 uM RSK inhibitor
S7 14D minus R428 plus 2uM Aurora Kinase inh II
S7 14D minus R428 plus 5uM Aurora Kinase inh II
S7 14D minus R428 plus 2 uM RSK inhibitor plus 2 uM Aurora Kinase inh II
S7 14D minus R428 plus 5 uM RSK inhibitor plus 5 uM Aurora Kinase inh II
human islets

Figure 11D


INS
Fold induction over undifferentiated ES cells
12000000
10000000
8000000
6000000
4000000
2000000
0
S7 14D minus R428
S7 14D plus R428
S7 14D minus R428 plus 2 uM RSK inhibitor
S7 14D minus R428 plus 5 uM RSK inhibitor
S7 14D minus R428 plus 2uM Aurora Kinase inh II
S7 14D minus R428 plus 5uM Aurora Kinase inh II
S7 14D minus R428 plus 2 uM RSK inhibitor
S7 14D minus R428 plus 5 uM RSK inhibitor
S7 14D minus R428 plus 2 uM Aurora Kinase inh II
S7 14D minus R428 plus 5 uM Aurora Kinase inh II
human islets