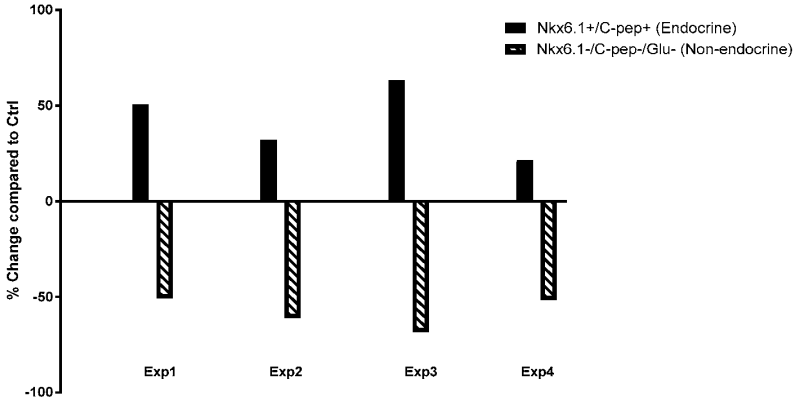
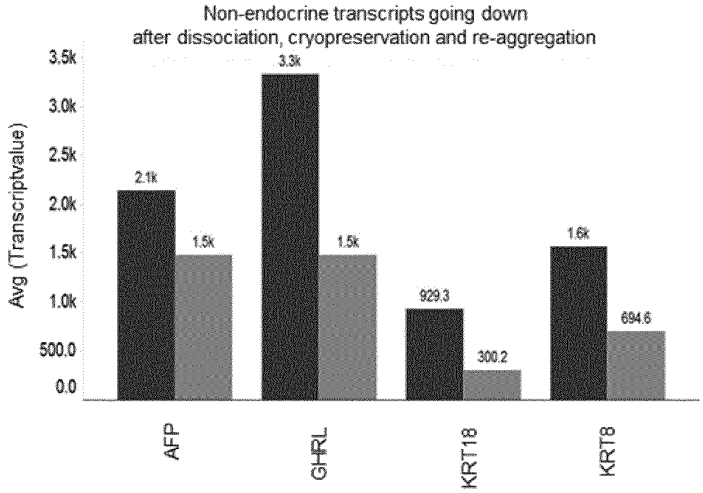

Non-endocrine cells
hESC
Endocrine progenitors (NKX2.2+/NKX6.1+) or Endocrine Cells (NKX6.1+/C-pep+)
Dissociation
Cryopreservation
Thaw, re-aggreation & culture
NKX6.1+/C-pep enriched aggregates

Figure 1

A.


Nkx6.1+/C-pep+ (Endocrine)
Nkx6.1-/C-pep-/Glu- (Non-endocrine)
% Change compared to Ctrl
100
50
0
-50
-100
Exp1
Exp2
Exp3
Exp4


Non-endocrine transcripts going down
after dissociation, cryopreservation and re-aggregation
Avg (Transcriptvalue)
3.5k
3.0k
2.5k
2.0k
1.5k
1.0k
500.0
0.0
2.1k
1.5k
3.3k
1.5k
929.3
300.2
1.6k
694.6
AFP
GHRL
KRT18
KRT8

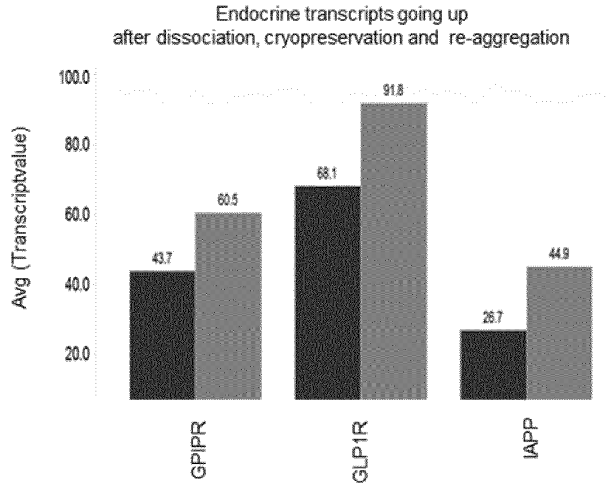
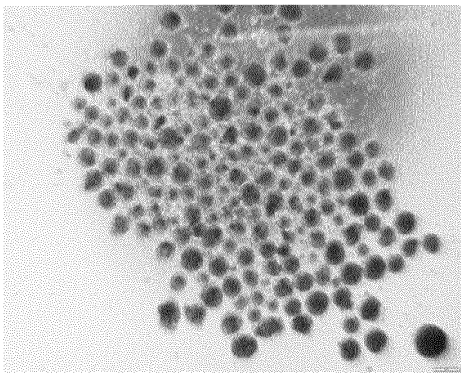
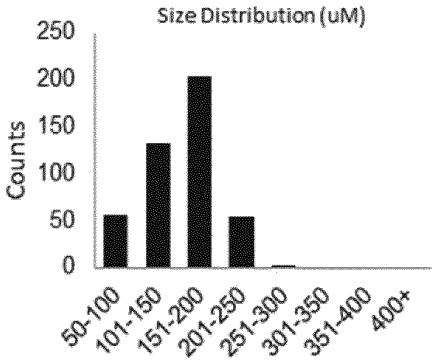

Endocrine transcripts going up
after dissociation, cryopreservation and re-aggregation
Avg (Transcriptvalue)
100.0
80.0
60.0
40.0
20.0
43.7
60.5
68.1
91.8
26.7
44.9
GPIPR
GLP1R
IAPP

Figure 2

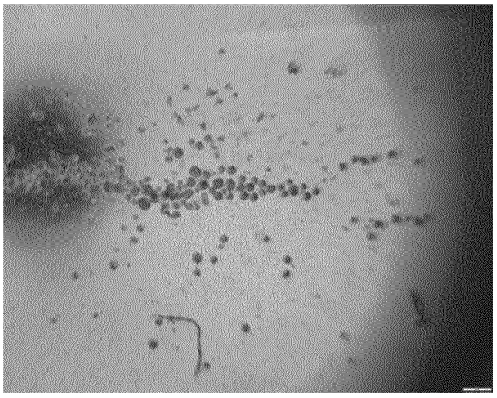
B.

Ctrl





Cryopreserved and re-aggregated



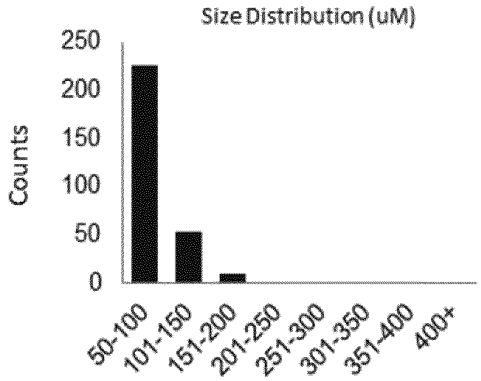
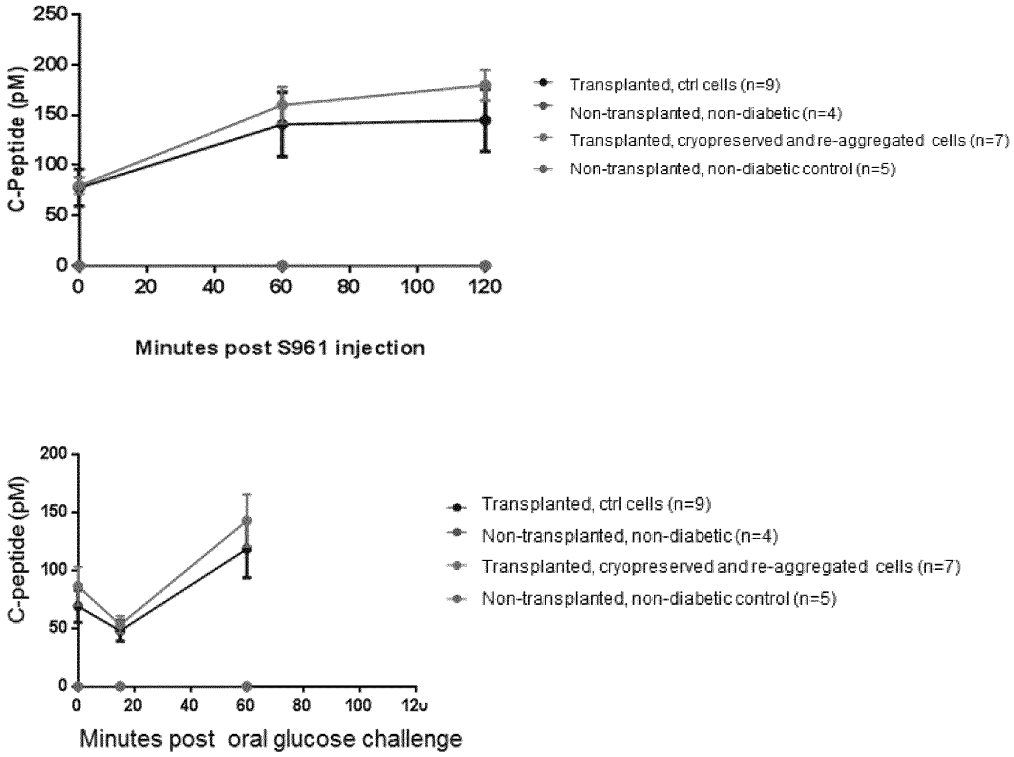


Figure 2 Continue

A.


C-Peptide (pM)
250
200
150
100
50
0
0 20 40 60 80 100 120
Minutes post S961 injection
Transplanted, ctrl cells (n=9)
Non-transplanted, non-diabetic (n=4)
Transplanted, cryopreserved and re-aggregated cells (n=7)
Non-transplanted, non-diabetic control (n=5)
C-peptide (pM)
200
150
100
50
0
0 20 40 60 80 100 12υ
Minutes post oral glucose challenge
Transplanted, ctrl cells (n=9)
Non-transplanted, non-diabetic (n=4)
Transplanted, cryopreserved and re-aggregated cells (n=7)
Non-transplanted, non-diabetic control (n=5)

B.

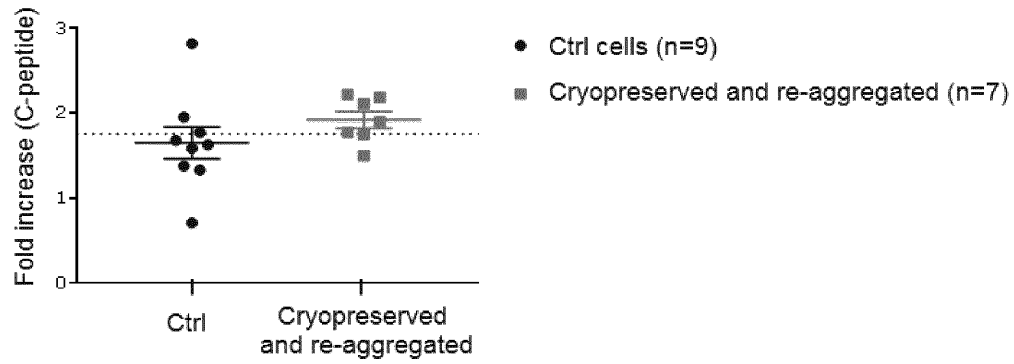

Fold increase (C-peptide)
3
2
1
0
Ctrl
Cryopreserved and re-aggregated
Ctrl cells (n=9)
Cryopreserved and re-aggregated (n=7)

Figure 3

C.

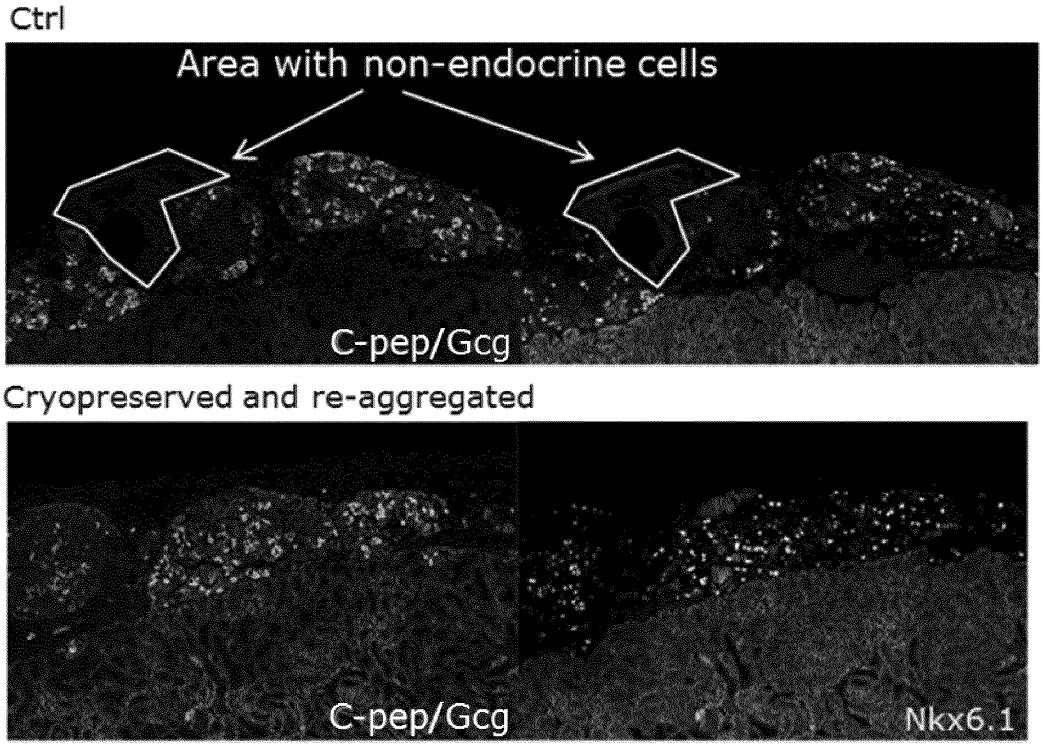
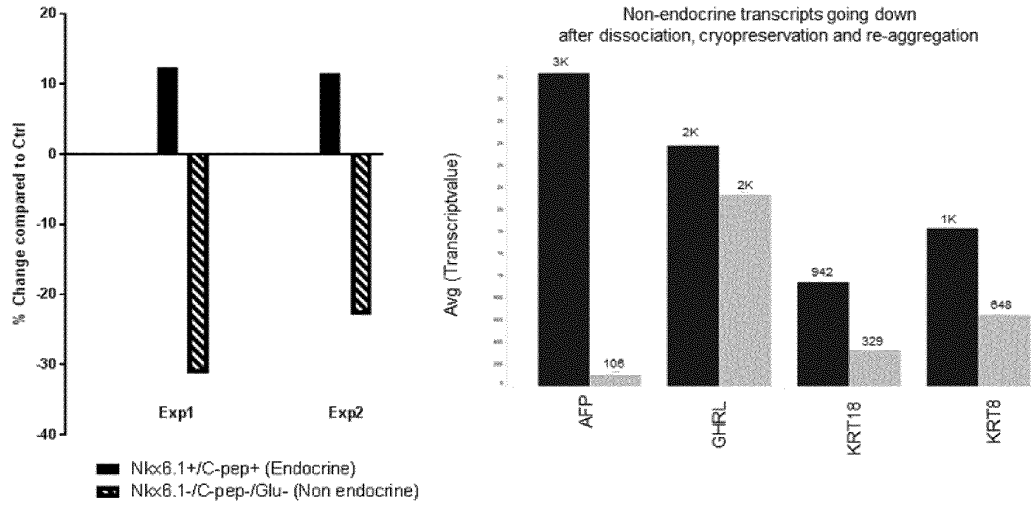


Figure 3. Continue

A.


% Change compared to Ctrl
20
10
0
-10
-20
-30
-40
Exp1
Exp2
Nkx6.1+/C-pep+ (Endocrine)
Nkx6.1-/C-pep-/Glu- (Non endocrine)
Non-endocrine transcripts going down
after dissociation, cryopreservation and re-aggregation
Avg (Transcriptvalue)
3K
106
2K
2K
942
329
1K
648
AFP
GHRL
KRT18
KRT8

B.

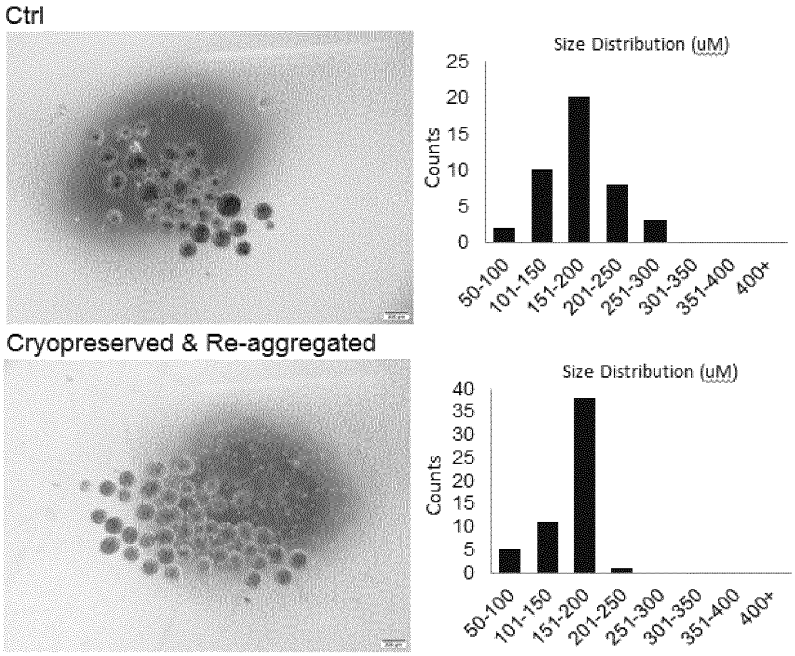
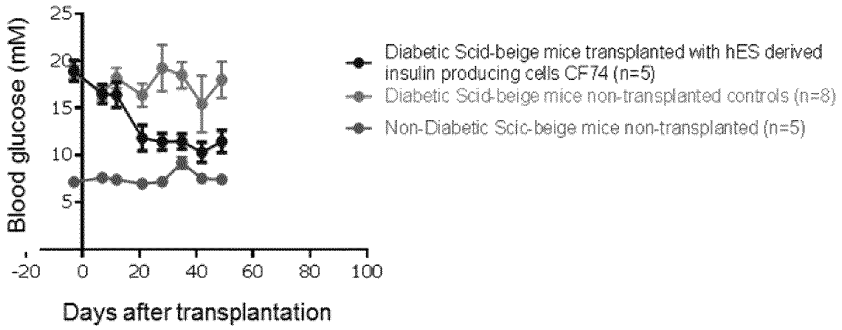
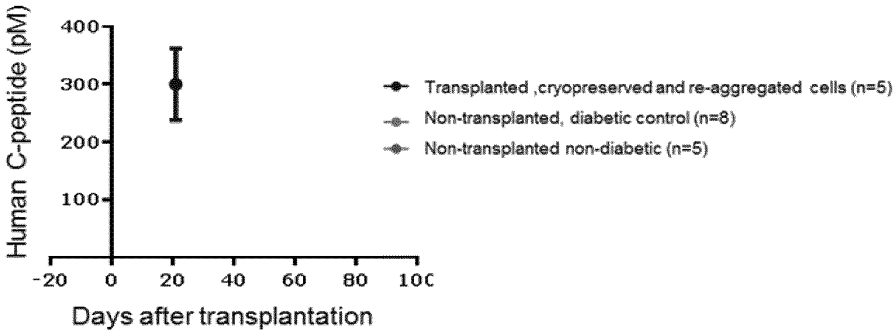

Ctrl
Size Distribution (uM)
Counts
25
20
15
10
5
0
50-100
101-150
151-200
201-250
251-300
301-350
351-400
400+
Cryopreserved & Re-aggregated
Size Distribution (uM)
Counts
40
35
30
25
20
15
10
5
0
50-100
101-150
151-200
201-250
251-300
301-350
351-400
400+

Figure 4

A.


Blood glucose (mM)
25
20
15
10
5
-20
0
20
40
60
80
100
Days after transplantation
Diabetic Scid-beige mice transplanted with hES derived insulin producing cells CF74 (n=5)
Diabetic Scid-beige mice non-transplanted controls (n=8)
Non-Diabetic Scic-beige mice non-transplanted (n=5)

B.


Human C-peptide (pM)
400
300
200
100
-20
0
20
40
60
80
100
Days after transplantation
Transplanted ,cryopreserved and re-aggregated cells (n=5)
Non-transplanted, diabetic control (n=8)
Non-transplanted non-diabetic (n=5)

C.

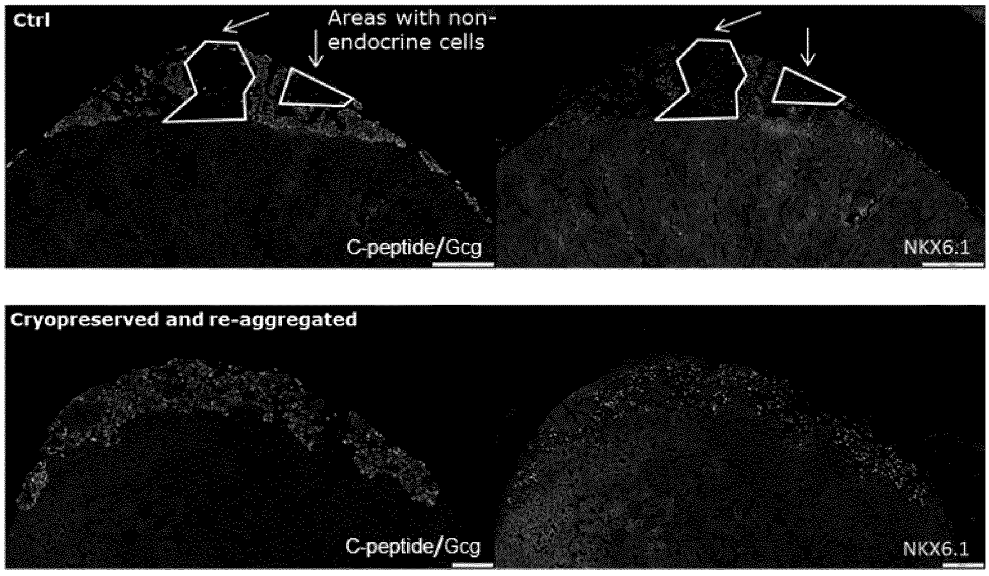
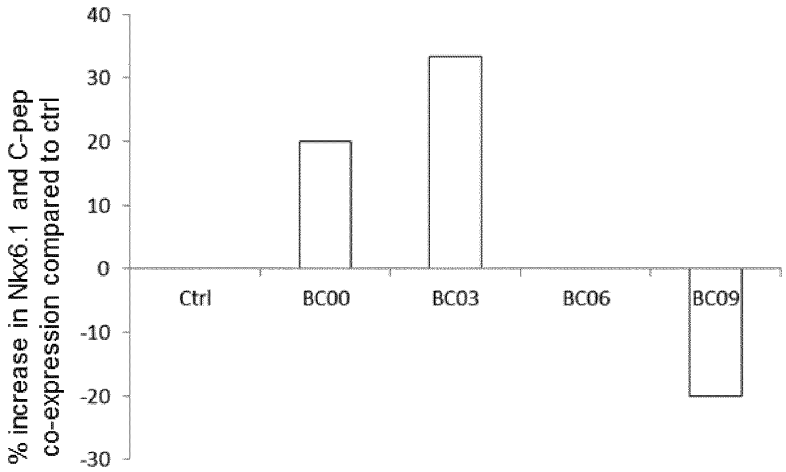

Ctrl
Areas with non-endocrine cells
C-peptide/Gcg
NKX6.1
Cryopreserved and re-aggregated
C-peptide/Gcg
NKX6.1

Figure 5

A.

Day of differentiation	Time of cryopreservation	Expression	Glucose response (final BC day14)
16	PE	NKX6.1/PDX1	Failed
20	BC00	NKX6.1/NKX2.2	Stimulation index of 3.2
23	BC03	NKX6.1/C-pep	Stimulation index of 2.6
26	BC06	NKX6.1/C-pep	Stimulation index of 1.7
29	BC09	NKX6.1/C-pep	Stimulation index of 1.5

B.



C.

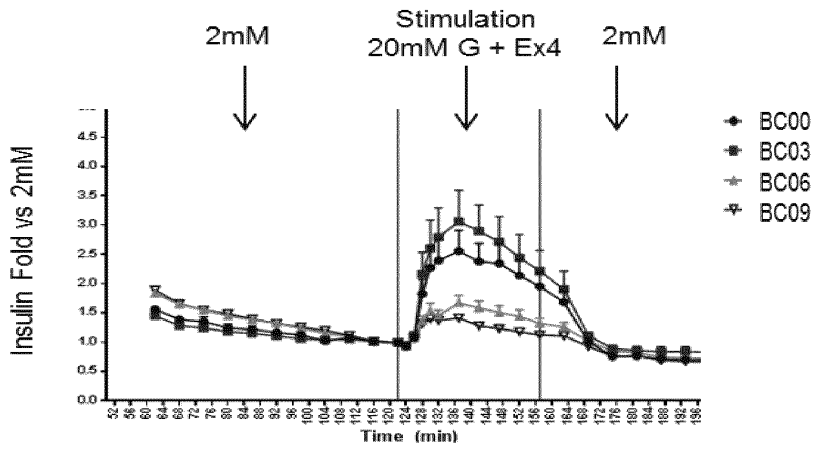


Figure 6