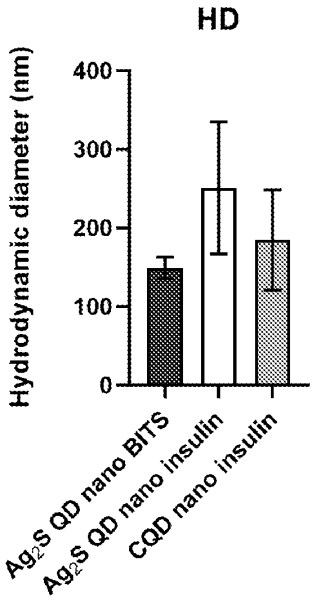
FIGURE 1

A


HD
Hydrodynamic diameter (nm)
400
300
200
100
0
Ag2S QD nano BITS
Ag2S QD nano insulin
CQD nano insulin

B

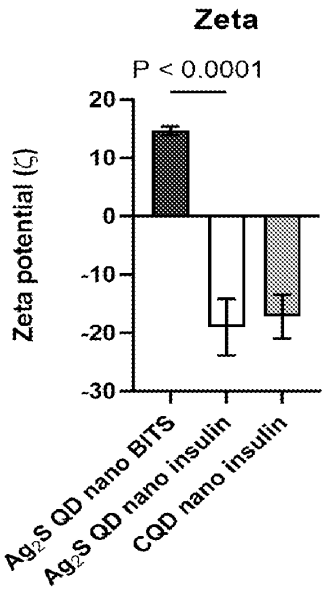
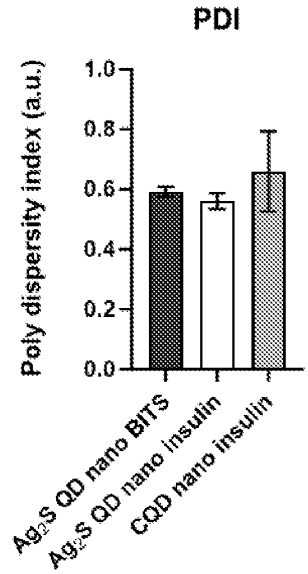

Zeta
P < 0.0001
Zeta potential (ζ)
20
10
0
-10
-20
-30
Ag2S QD nano BITS
Ag2S QD nano insulin
CQD nano insulin

FIGURE 1 - continued

C



D

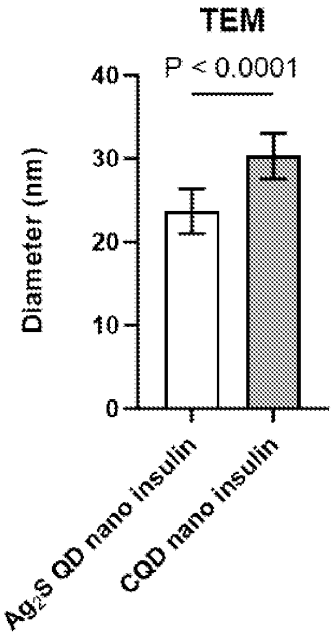


FIGURE 2

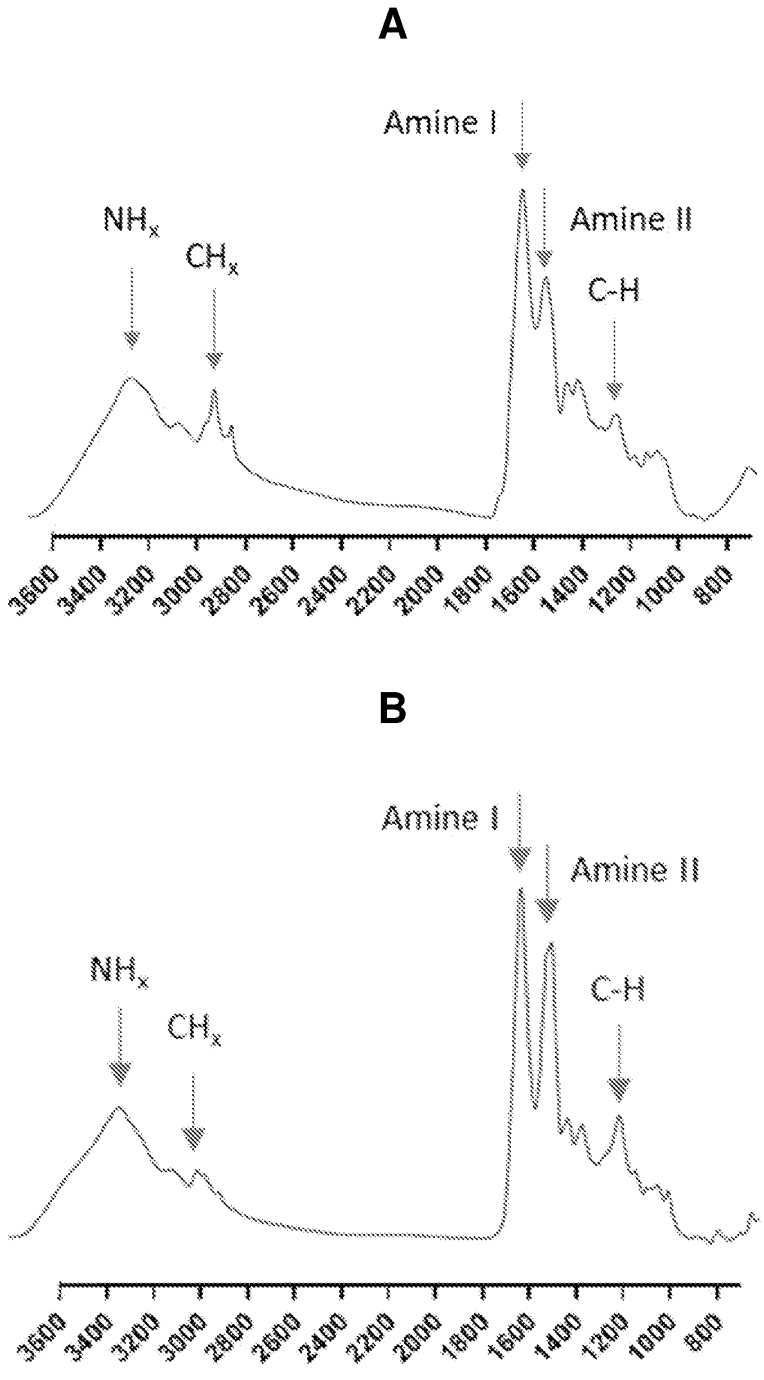

A
Amine I
NHx
CHx
Amine II
C-H
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
B
Amine I
Amine II
NHx
CHx
C-H
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800

FIGURE 3

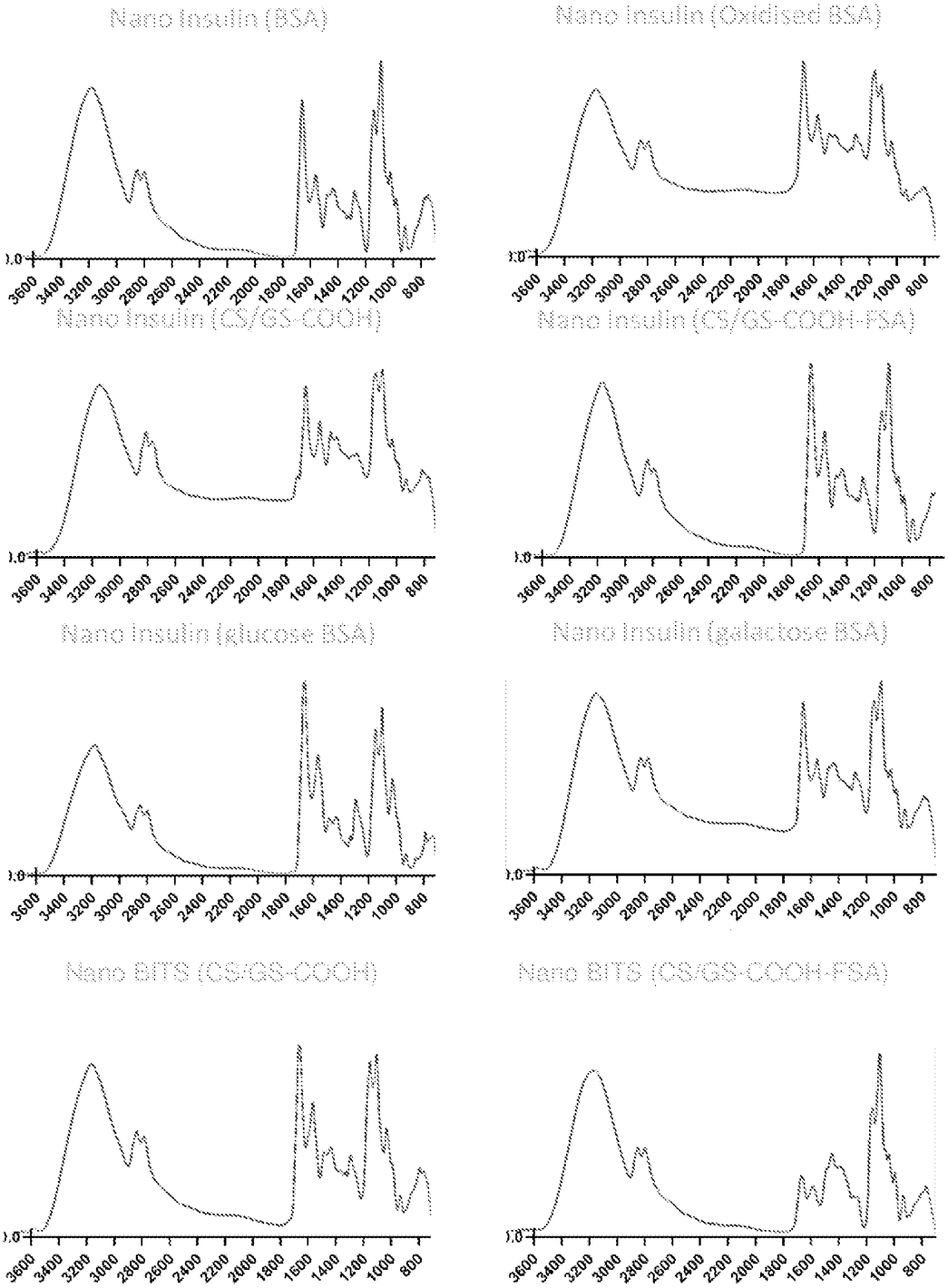

Nano Insulin (BSA)
Nano Insulin (Oxidised BSA)
Nano Insulin (CS/GS-COOH)
Nano Insulin (CS/GS-COOH-FSA)
Nano Insulin (glucose BSA)
Nano Insulin (galactose BSA)
Nano BITS (CS/GS-COOH)
Nano BITS (CS/GS-COOH-FSA)
1.0
3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000 800

FIGURE 4

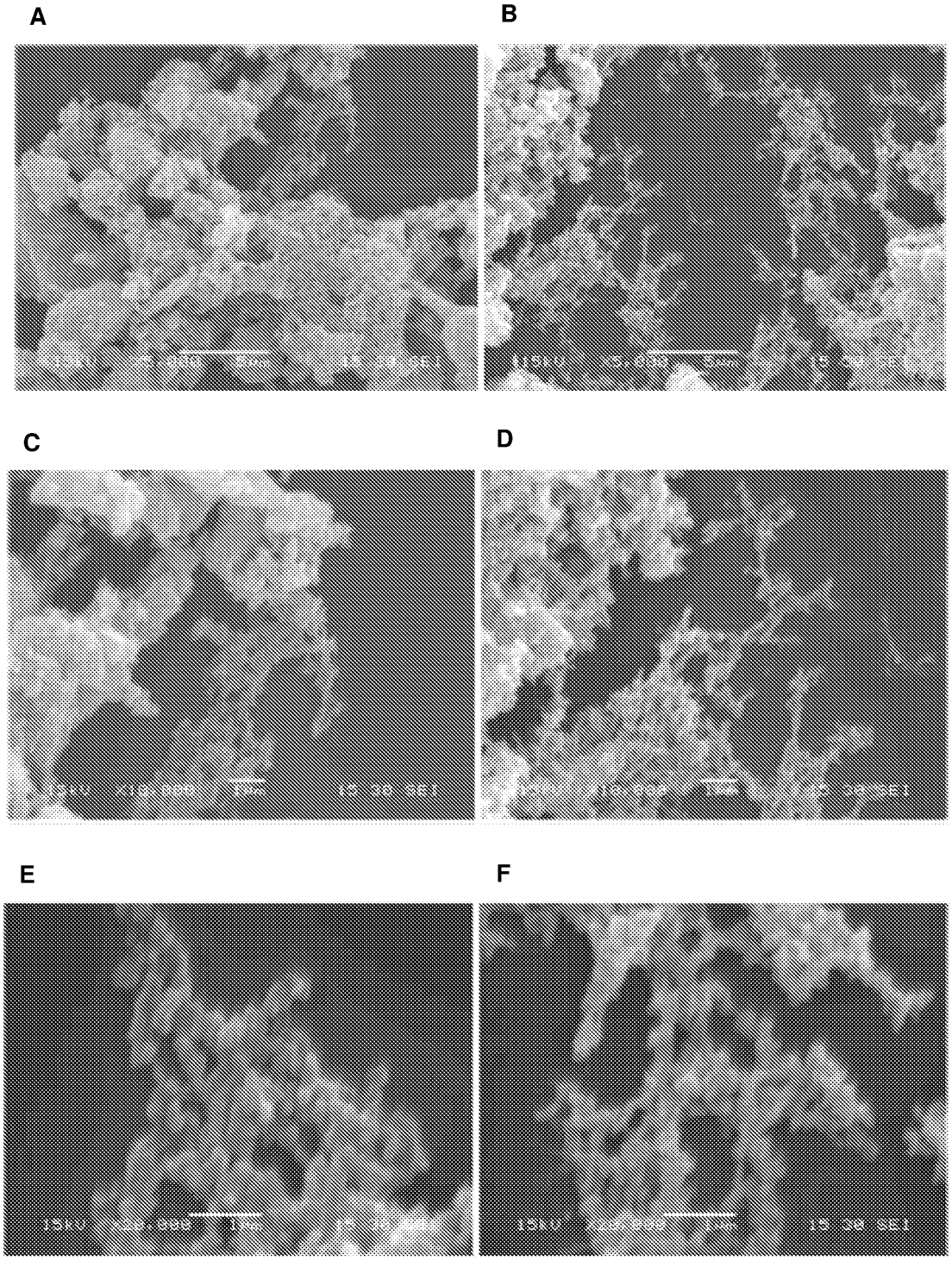


FIGURE 5

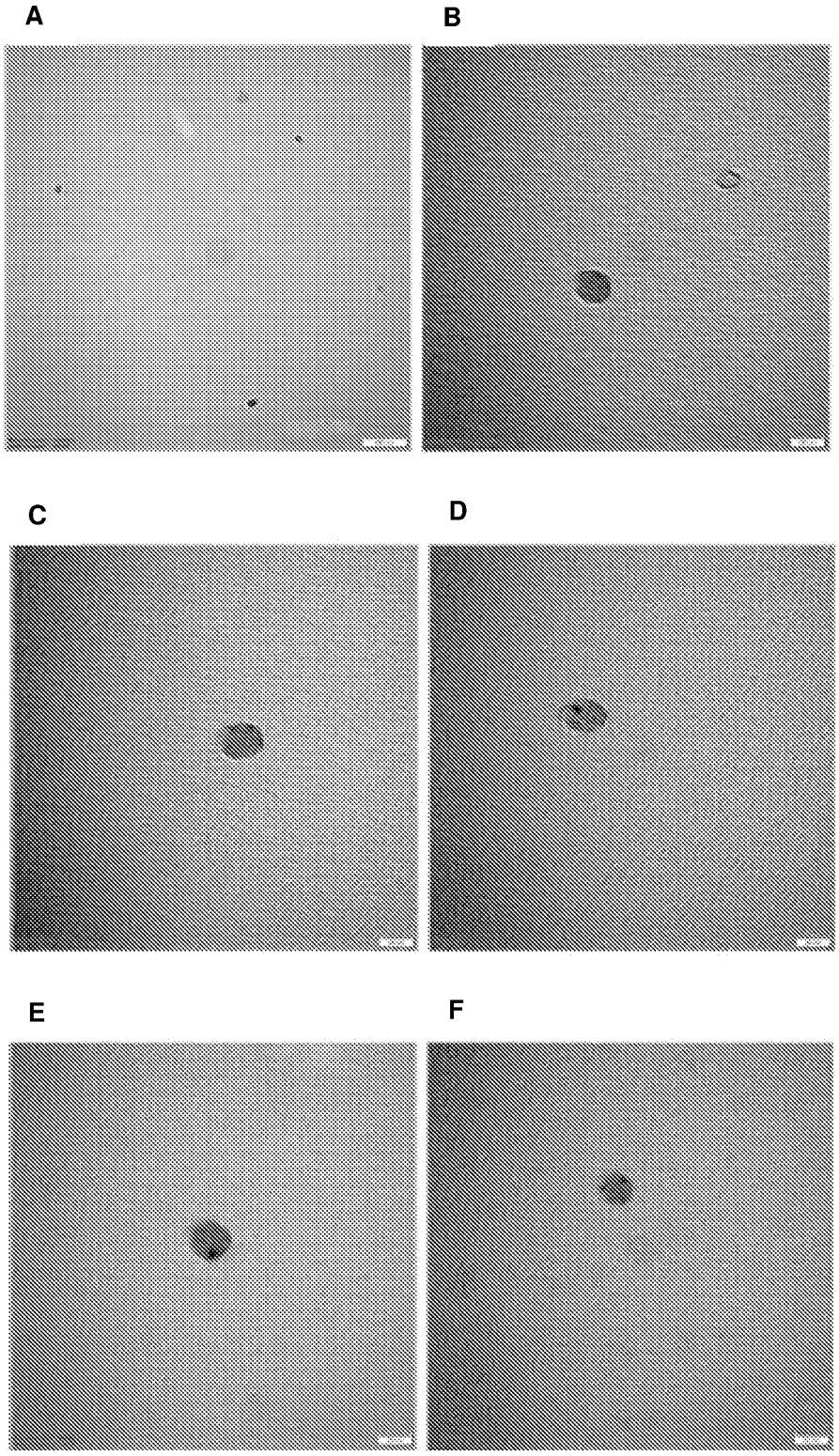


FIGURE 6

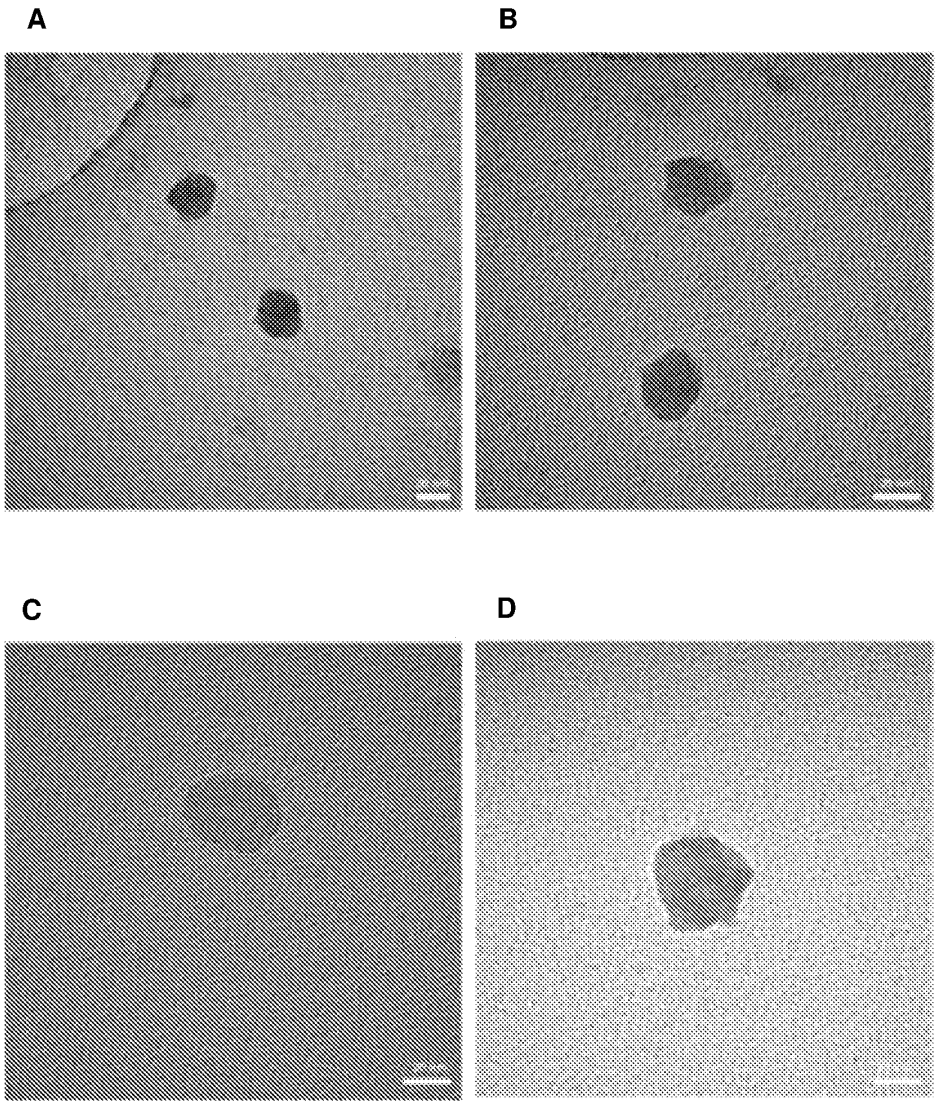


FIGURE 7

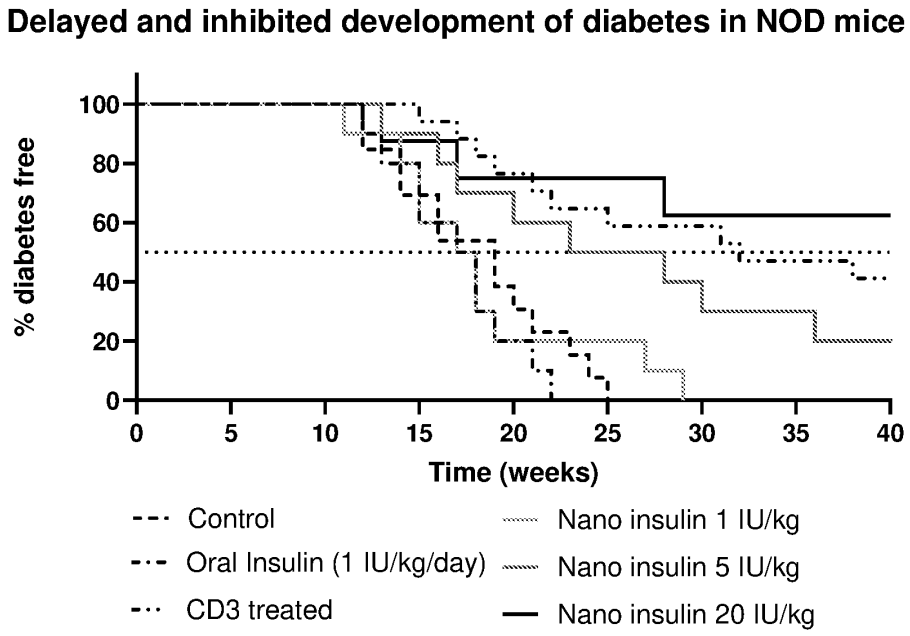

Delayed and inhibited development of diabetes in NOD mice
% diabetes free
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Time (weeks)
Control
Oral Insulin (1 IU/kg/day)
CD3 treated
Nano insulin 1 IU/kg
Nano insulin 5 IU/kg
Nano insulin 20 IU/kg

FIGURE 8

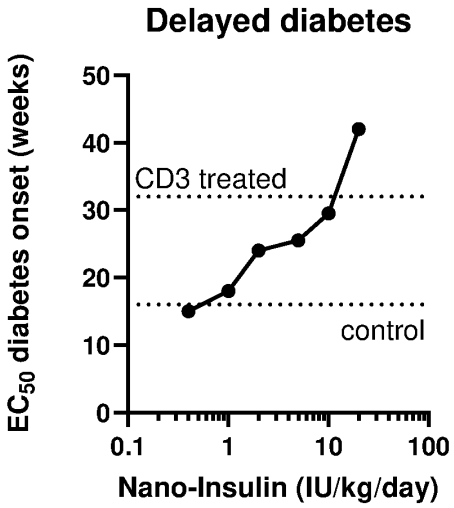

Delayed diabetes
EC50 diabetes onset (weeks)
50
40
30
20
10
0
CD3 treated
control
0.1
1
10
100
Nano-Insulin (IU/kg/day)

FIGURE 9

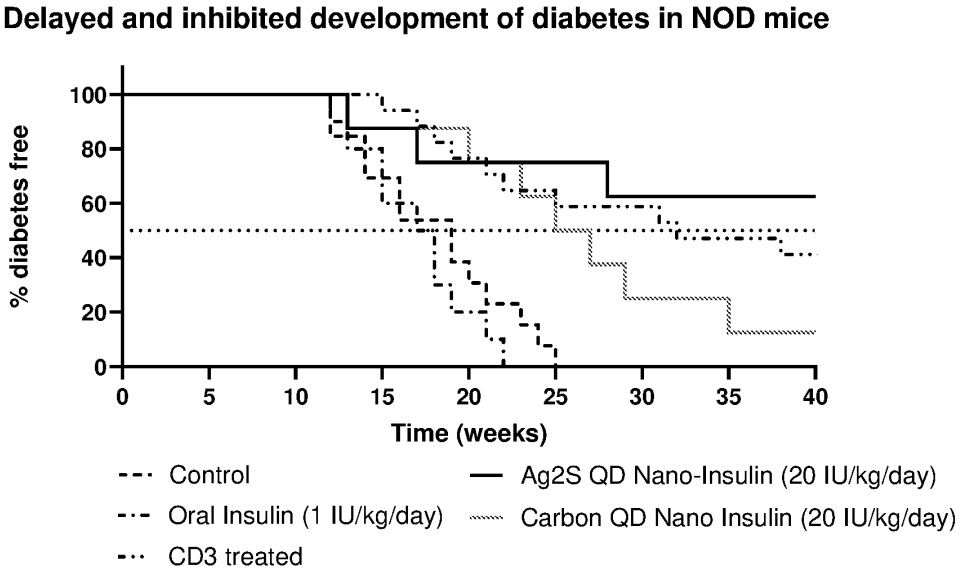


FIGURE 10

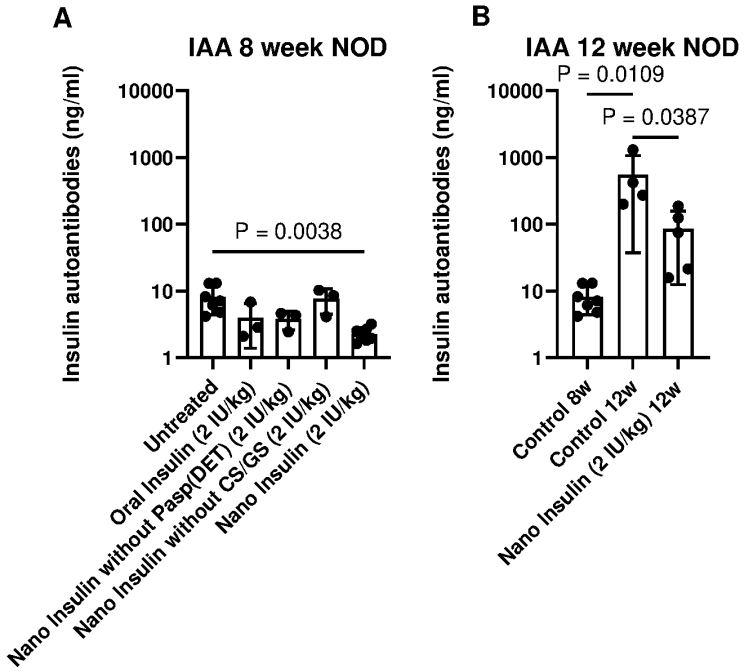


FIGURE 11

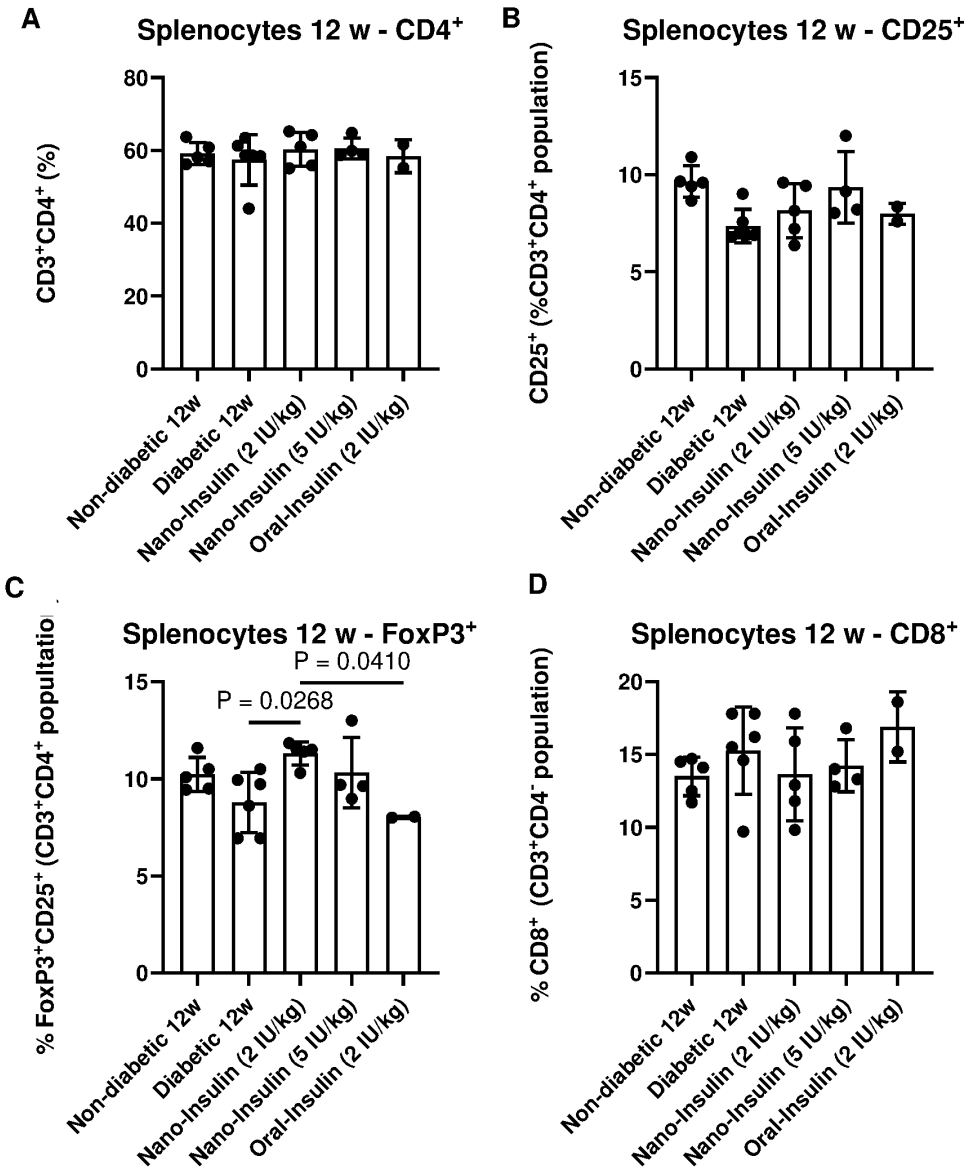

A
Splenocytes 12 w - CD4+
CD3+CD4+ (%)
80
60
40
20
0
Non-diabetic 12w
Diabetic 12w
Nano-Insulin (2 IU/kg)
Nano-Insulin (5 IU/kg)
Oral-Insulin (2 IU/kg)
B
Splenocytes 12 w - CD25+
CD25+ (%CD3+CD4+ population)
15
10
5
0
C
Splenocytes 12 w - FoxP3+
P = 0.0410
P = 0.0268
% FoxP3+CD25+ (CD3+CD4+ populatio
D
Splenocytes 12 w - CD8+
% CD8+ (CD3+CD4- population)
20

FIGURE 12

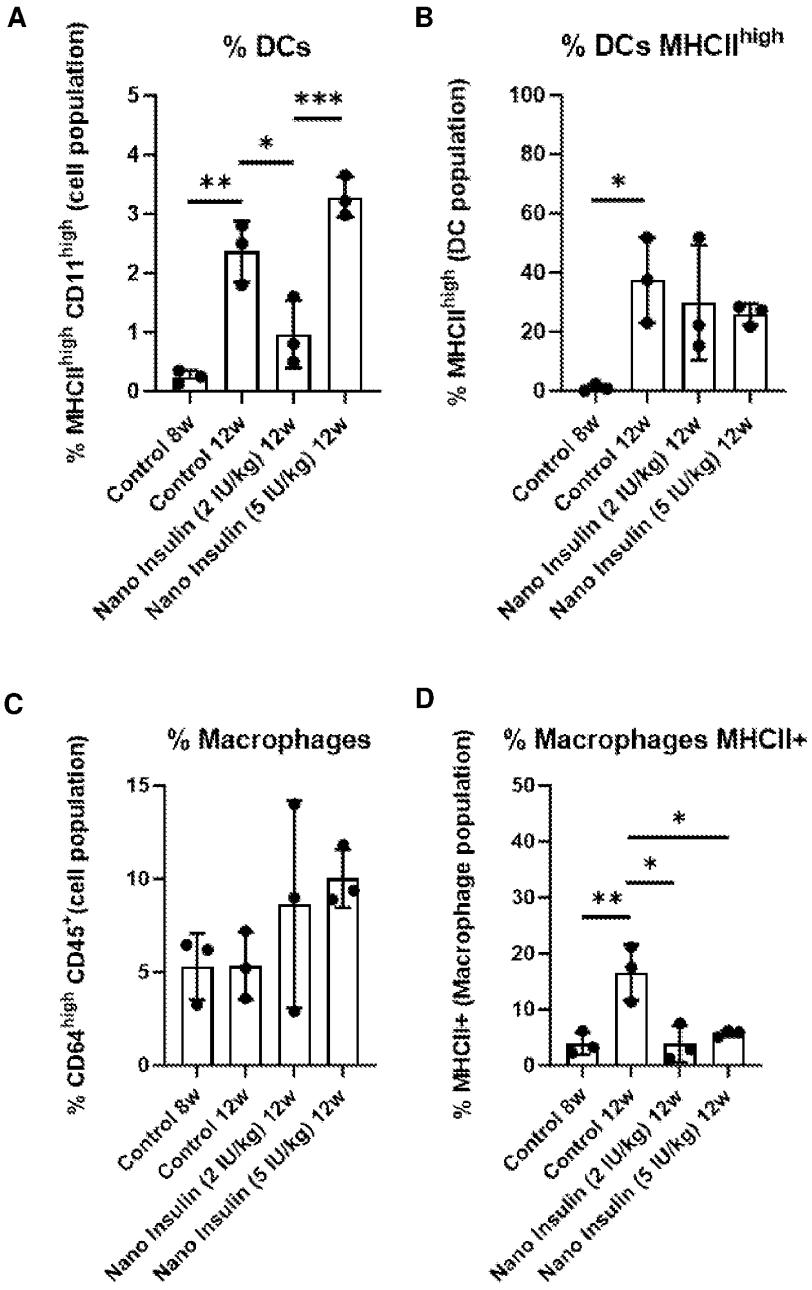

A
% DCs
% MHCII^high CD11^high (cell population)
**
*
***
Control 8w
Control 12w
Nano Insulin (2 IU/kg) 12w
Nano Insulin (5 IU/kg) 12w
B
% DCs MHCII^high
% MHCII^high (DC population)
*
C
% Macrophages
% CD64^high CD45^+ (cell population)
D
% Macrophages MHCII+
% MHCII+ (Macrophage population)

FIGURE 12 - continued

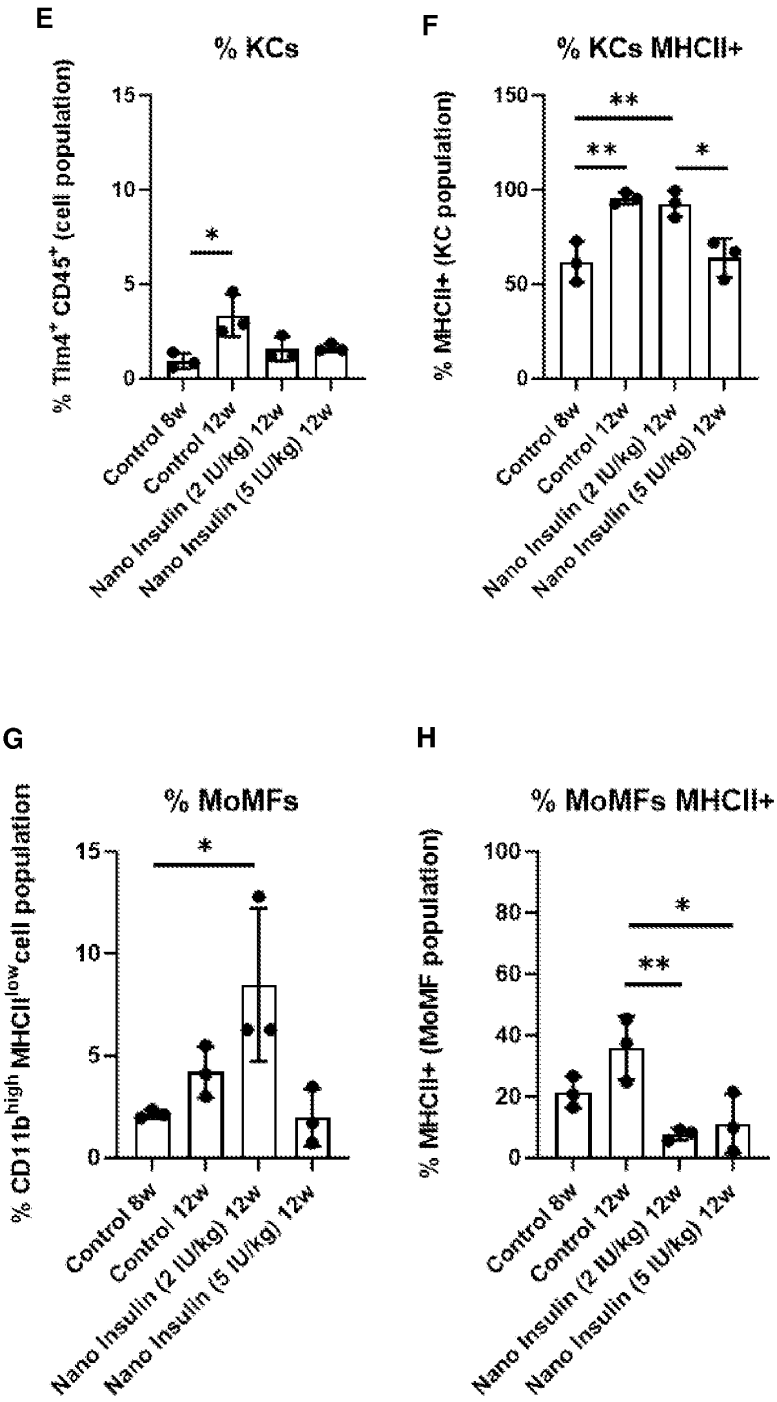


FIGURE 12 - continued

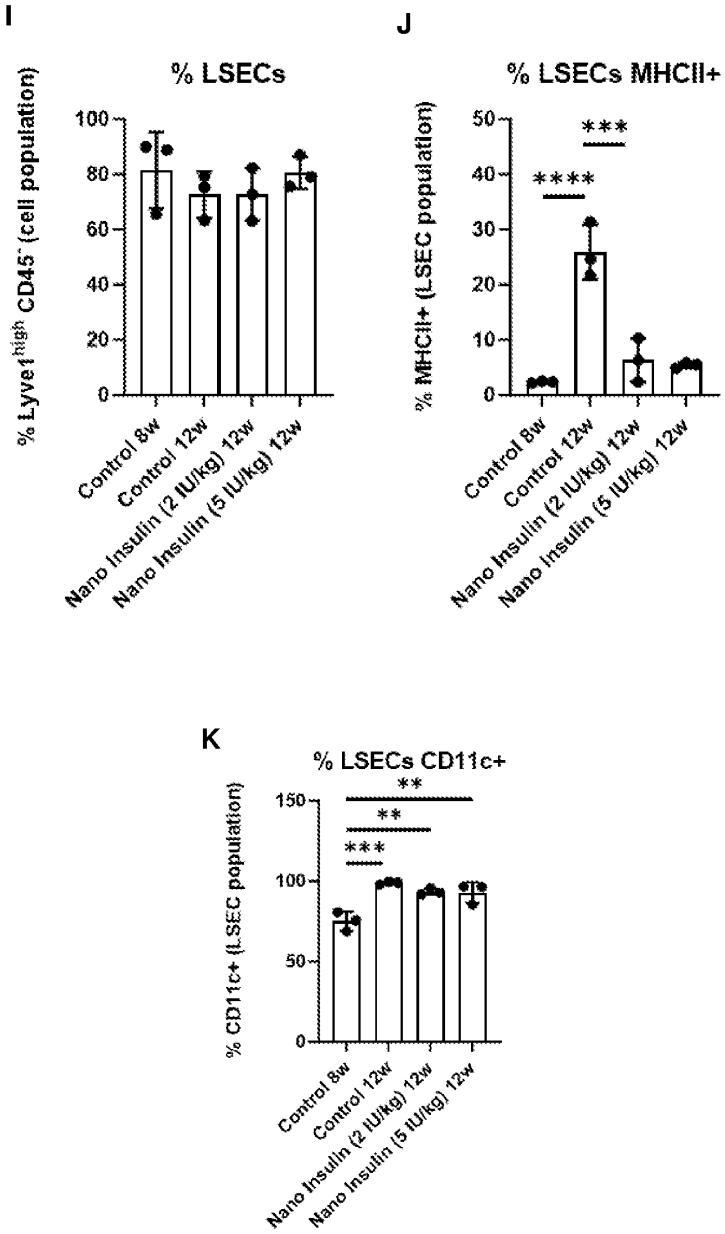
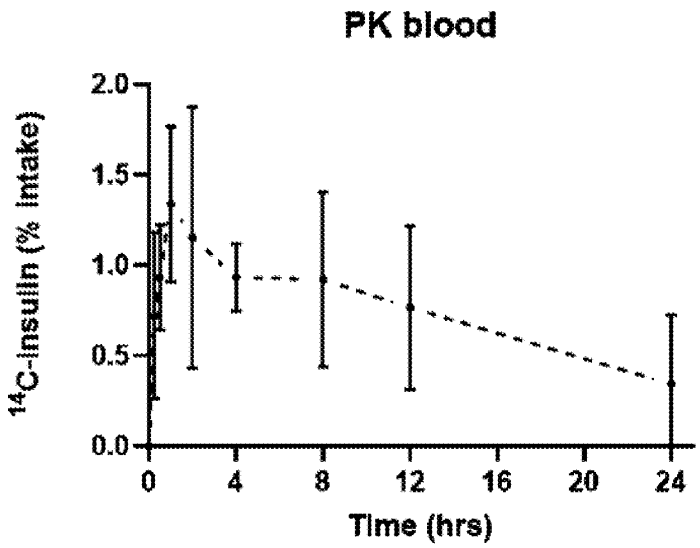


FIGURE 13

A


PK blood
^{14}C-insulin (% intake)
2.0
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
Time (hrs)

B

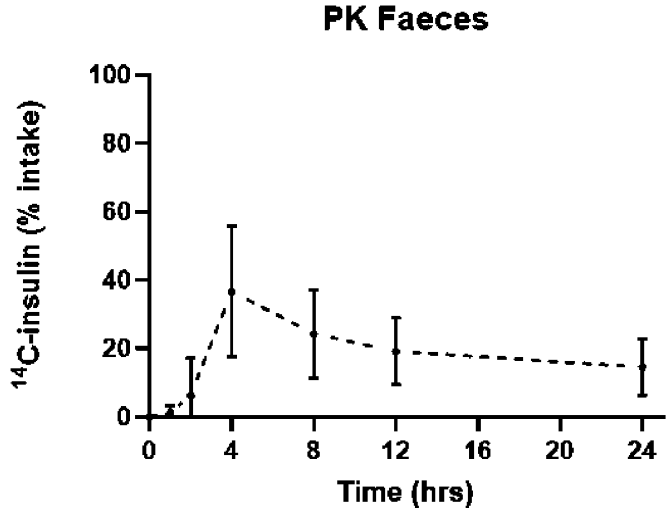
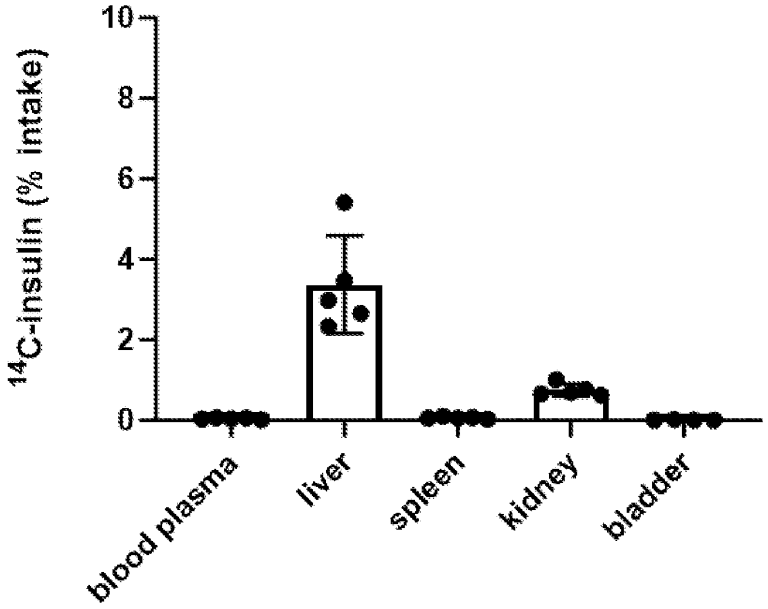

PK Faeces
^{14}C-insulin (% intake)
100
80
60
40
20
0
0
4
8
12
16
20
24
Time (hrs)

FIGURE 13 - continued

C



D

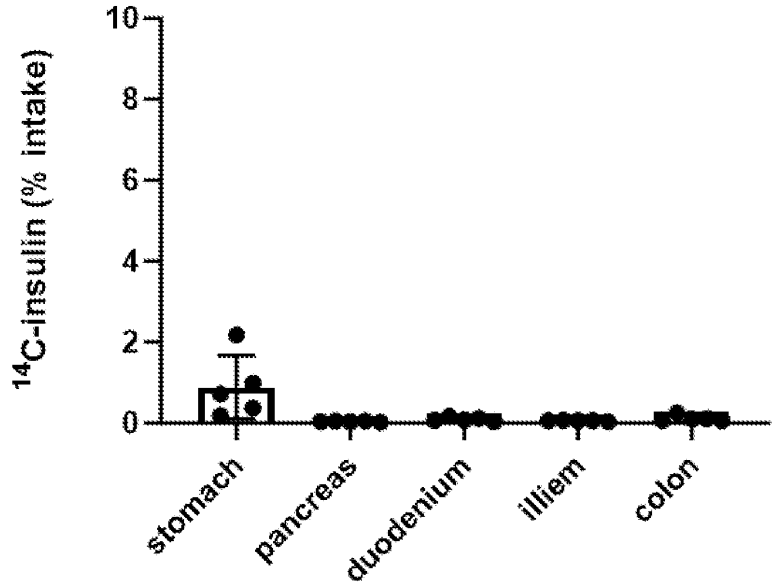
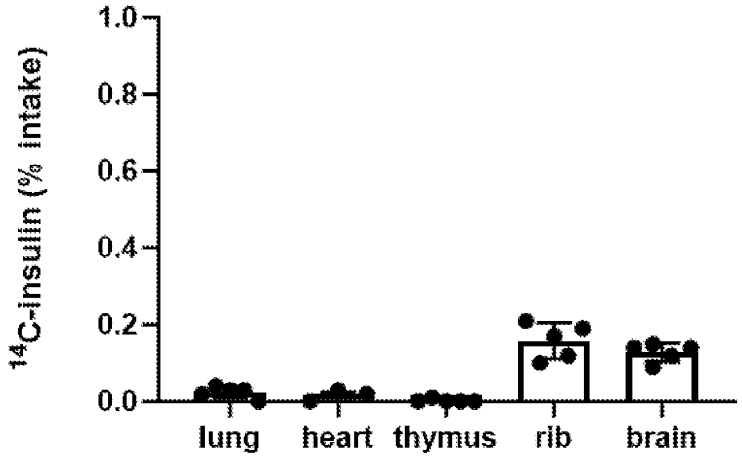


FIGURE 13 - continued

E



F

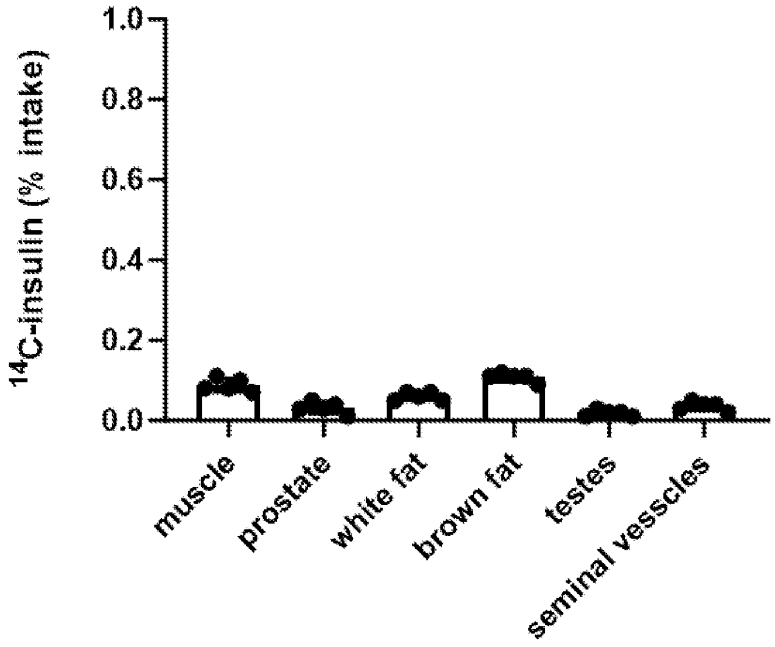
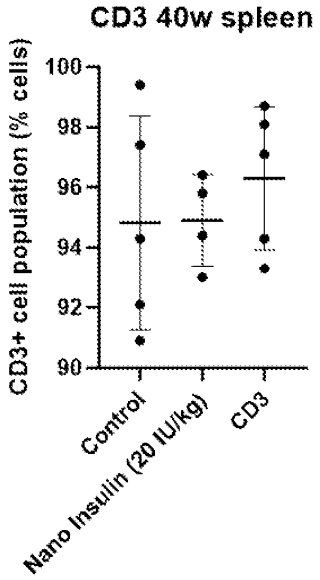


FIGURE 14

A


CD3 40w spleen
CD3+ cell population (% cells)
100
98
96
94
92
90
Control
Nano Insulin (20 IU/kg)
CD3

B

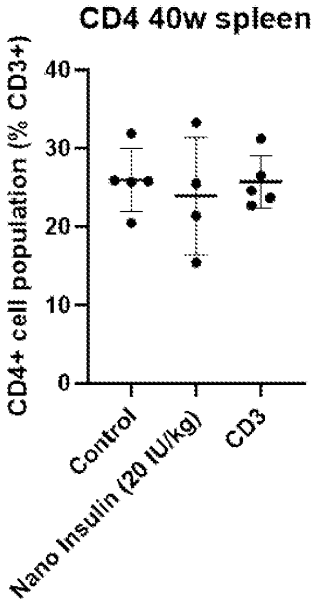
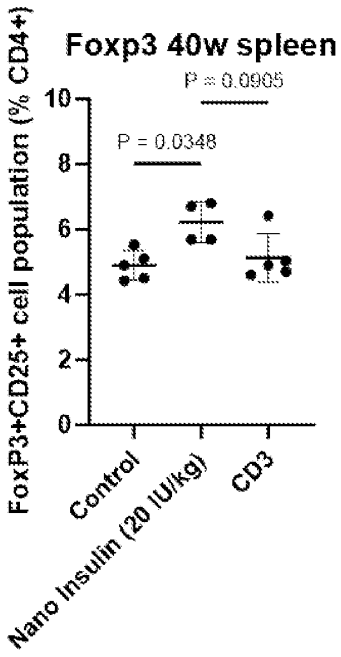

CD4 40w spleen
CD4+ cell population (% CD3+)
40
30
20
10
0
Control
Nano Insulin (20 IU/kg)
CD3

FIGURE 14 - continued

C



D

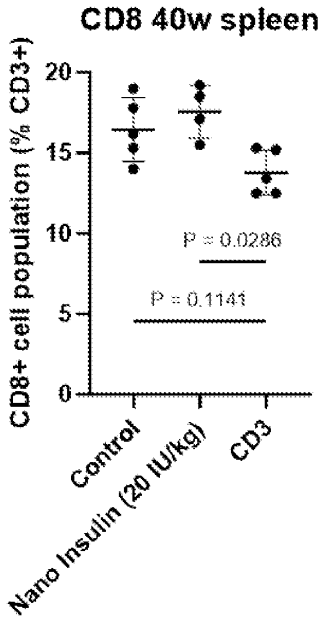
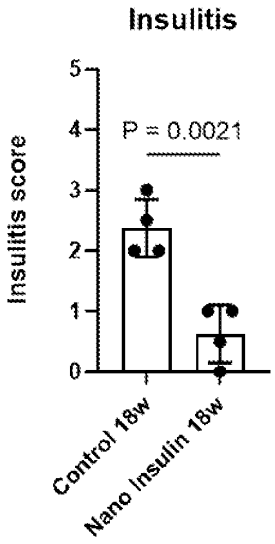


FIGURE 15

A


Insulitis
Insulitis score
P = 0.0021
0
1
2
3
4
5
Control 18w
Nano Insulin 18w

B

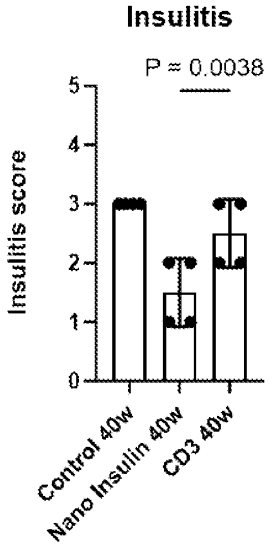

Insulitis
Insulitis score
P = 0.0038
0
1
2
3
4
5
Control 40w
Nano Insulin 40w
CD3 40w

FIGURE 15 - continued

C

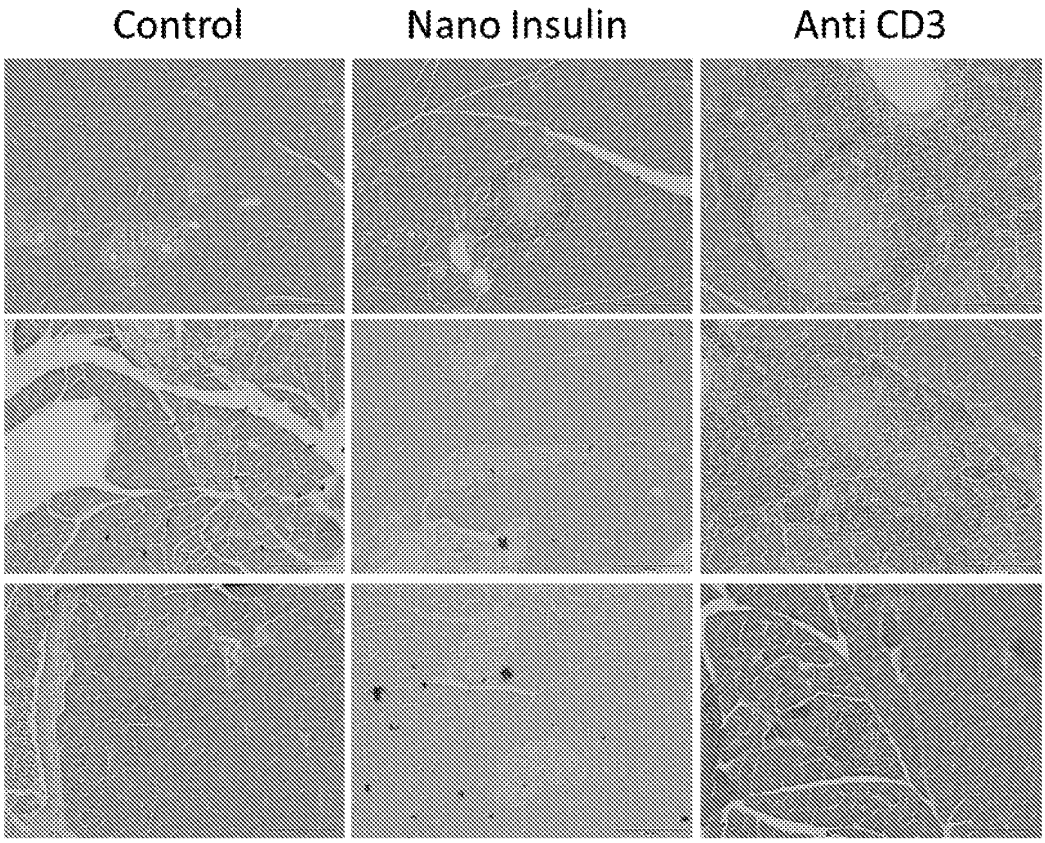


FIGURE 16

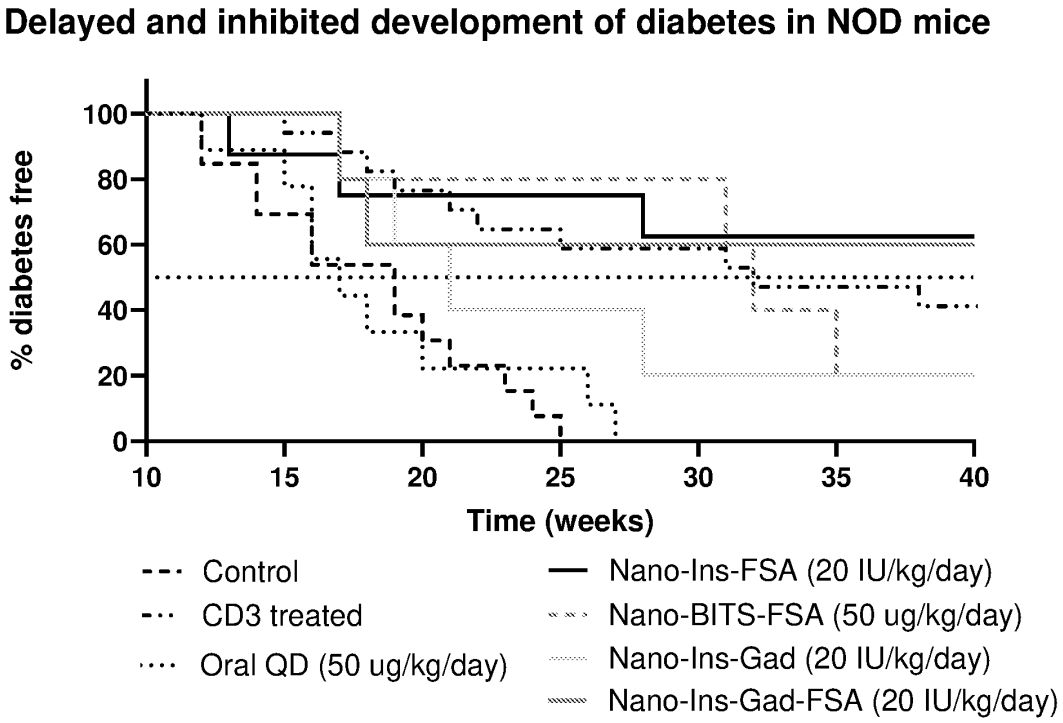
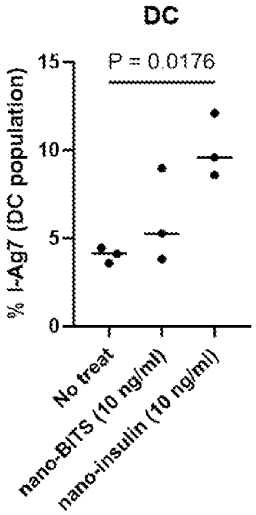

Delayed and inhibited development of diabetes in NOD mice
% diabetes free
100
80
60
40
20
0
10
15
20
25
30
35
40
Time (weeks)
Control
CD3 treated
Oral QD (50 ug/kg/day)
Nano-Ins-FSA (20 IU/kg/day)
Nano-BITS-FSA (50 ug/kg/day)
Nano-Ins-Gad (20 IU/kg/day)
Nano-Ins-Gad-FSA (20 IU/kg/day)

FIGURE 17

A


DC
P = 0.0176
% I-Ag7 (DC population)
15
10
5
0
No treat
nano-BITS (10 ng/ml)
nano-insulin (10 ng/ml)

B

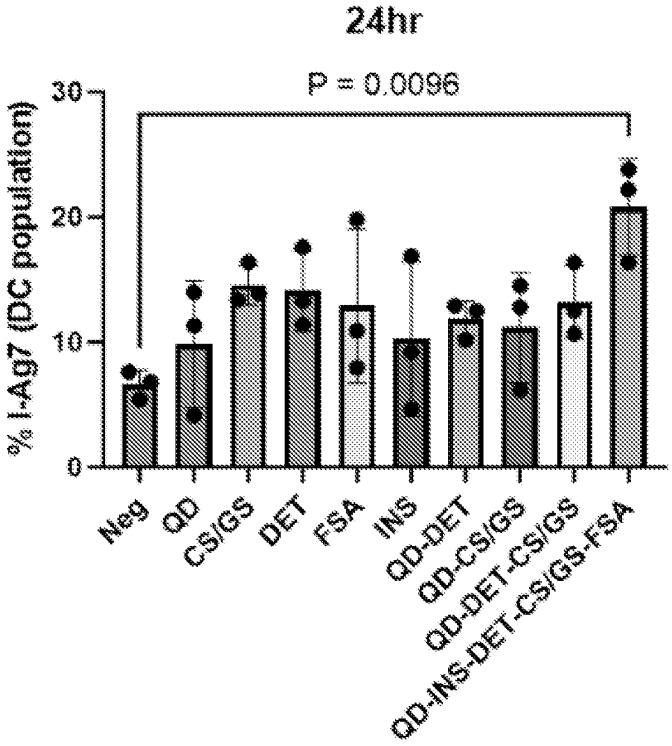
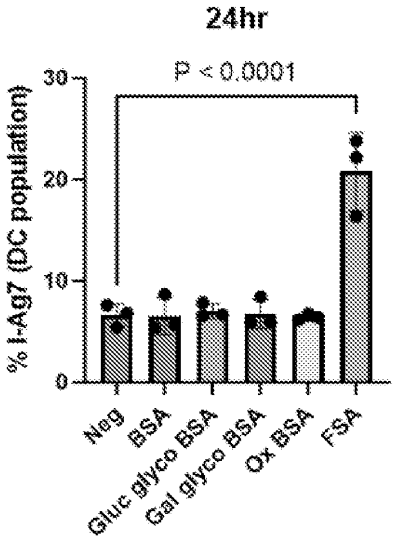

24hr
P = 0.0096
% I-Ag7 (DC population)
30
20
10
0
Neg
QD
CS/GS
DET
FSA
INS
QD-DET
QD-CS/GS
QD-DET-CS/GS
QD-INS-DET-CS/GS-FSA

FIGURE 17 - continued

C



D

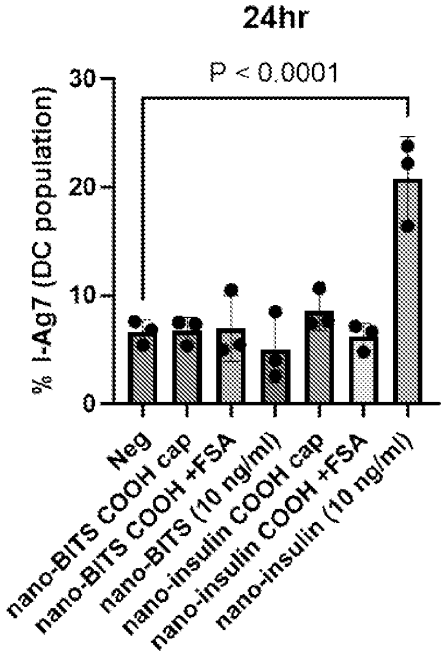
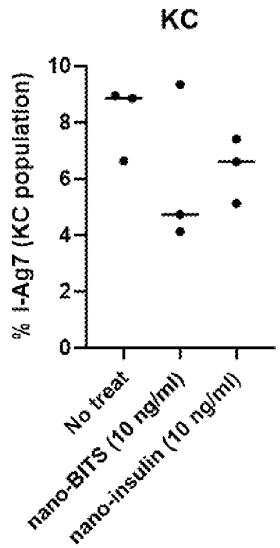


FIGURE 18

A


KC
% I-Ag7 (KC population)
10
8
6
4
2
0
No treat
nano-BITS (10 ng/ml)
nano-insulin (10 ng/ml)

B

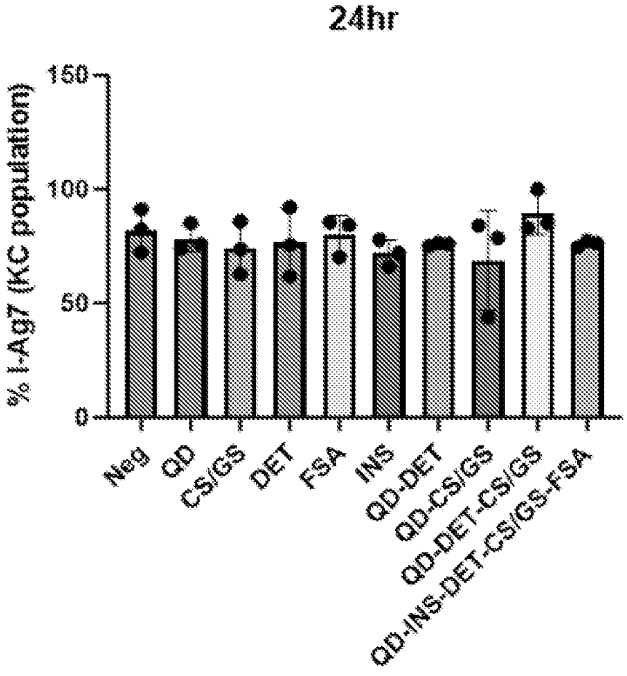
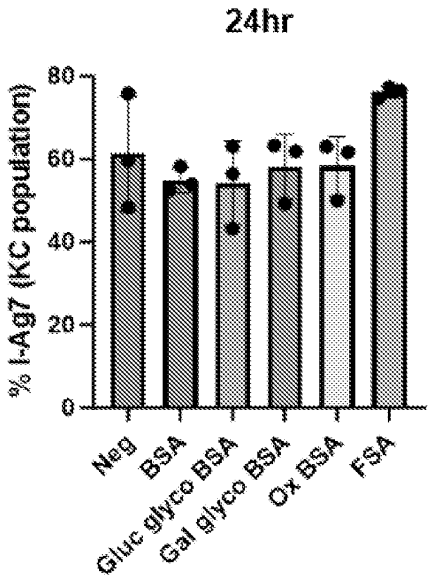

24hr
% I-Ag7 (KC population)
150
100
50
0
Neg
QD
CS/GS
DET
FSA
INS
QD-DET
QD-CS/GS
QD-DET-CS/GS
QD-INS-DET-CS/GS-FSA

FIGURE 18 - continued

C



D

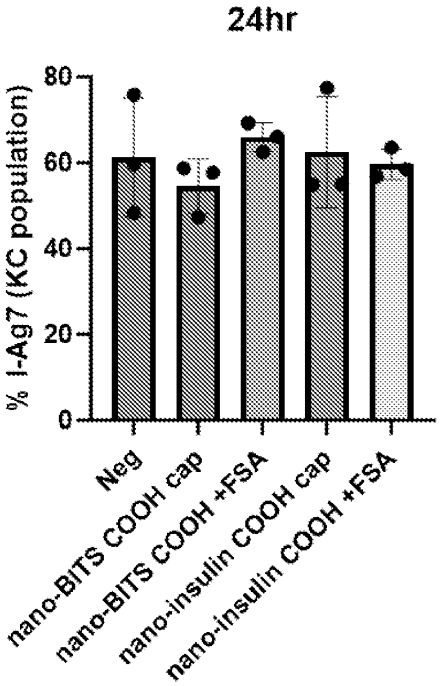
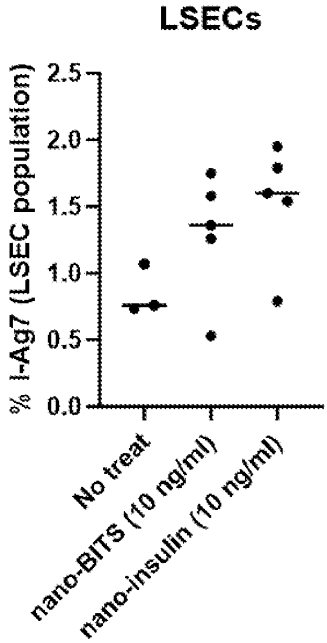


FIGURE 19

A


LSECs
% I-Ag7 (LSEC population)
2.5
2.0
1.5
1.0
0.5
0.0
No treat
nano-BITS (10 ng/ml)
nano-insulin (10 ng/ml)

B

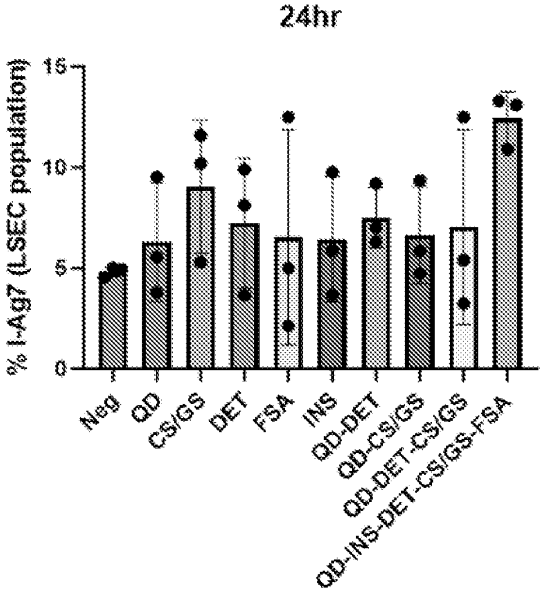
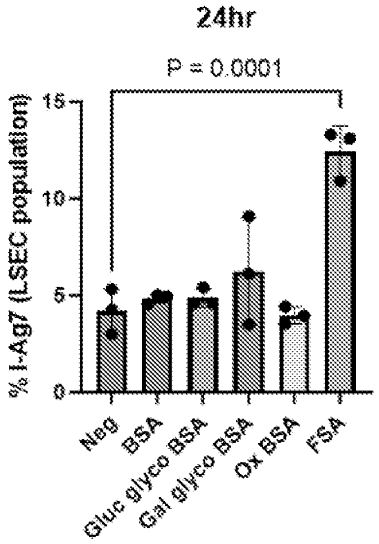

24hr
% I-Ag7 (LSEC population)
15
10
5
0
Neg
QD
CS/GS
DET
FSA
INS
QD-DET
QD-CS/GS
QD-DET-CS/GS
QD-INS-DET-CS/GS-FSA

FIGURE 19 - continued

C



D

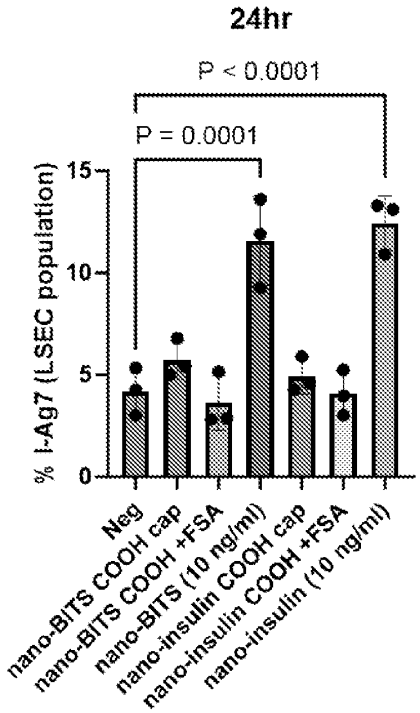
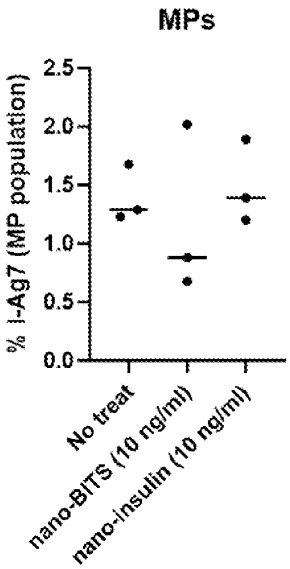


FIGURE 20

A


MPs
% I-Ag7 (MP population)
2.5
2.0
1.5
1.0
0.5
0.0
No treat
nano-BITS (10 ng/ml)
nano-insulin (10 ng/ml)

B

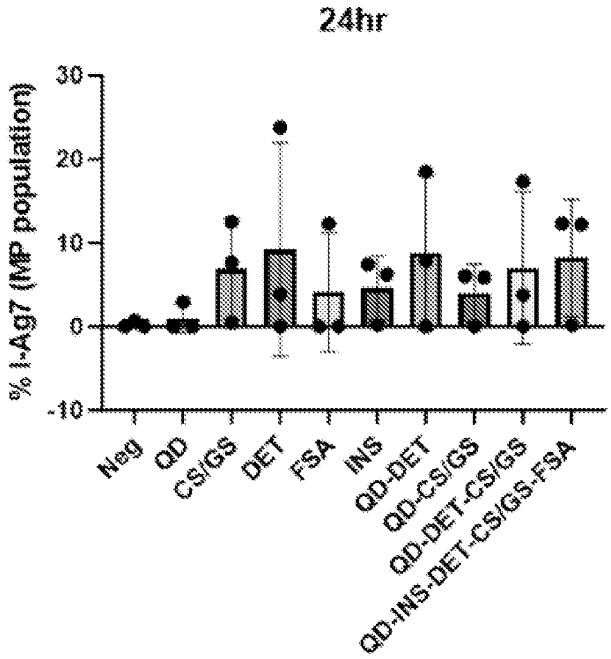
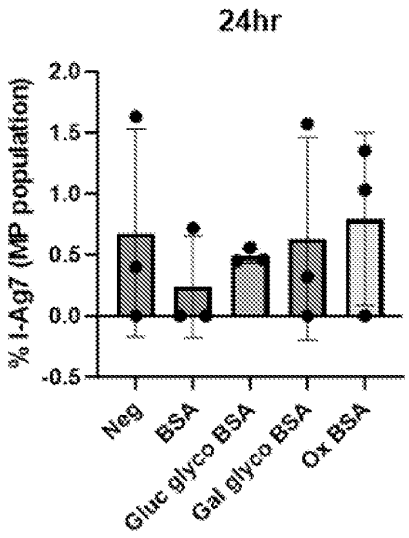

24hr
% I-Ag7 (MP population)
30
20
10
0
-10
Neg
QD
CS/GS
DET
FSA
INS
QD-DET
QD-CS/GS
QD-DET-CS/GS
QD-INS-DET-CS/GS-FSA

FIGURE 20 - continued

C



D

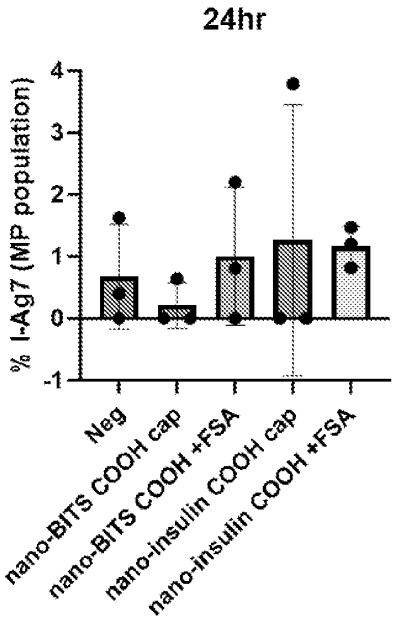
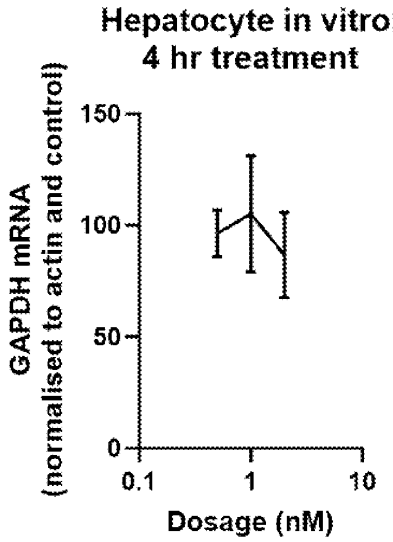


FIGURE 21

A


Hepatocyte in vitro:
4 hr treatment
GAPDH mRNA
(normalised to actin and control)
150
100
50
0
0.1
1
10
Dosage (nM)

B

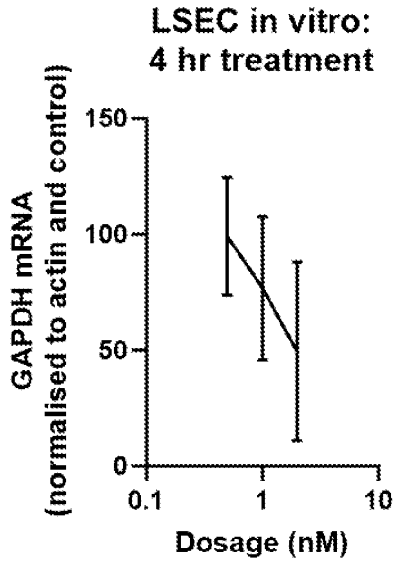

LSEC in vitro:
4 hr treatment
GAPDH mRNA
(normalised to actin and control)
150
100
50
0
0.1
1
10
Dosage (nM)

FIGURE 21 - continued

C

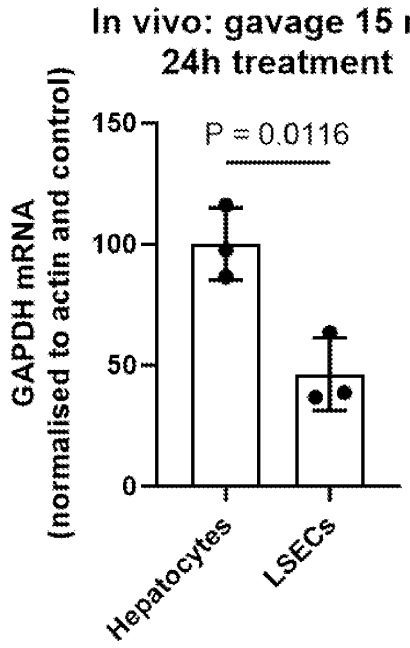


FIGURE 22

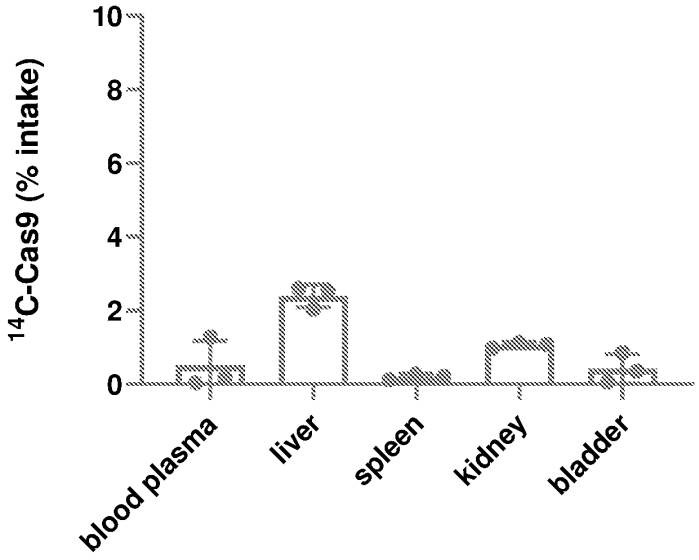


FIGURE 23

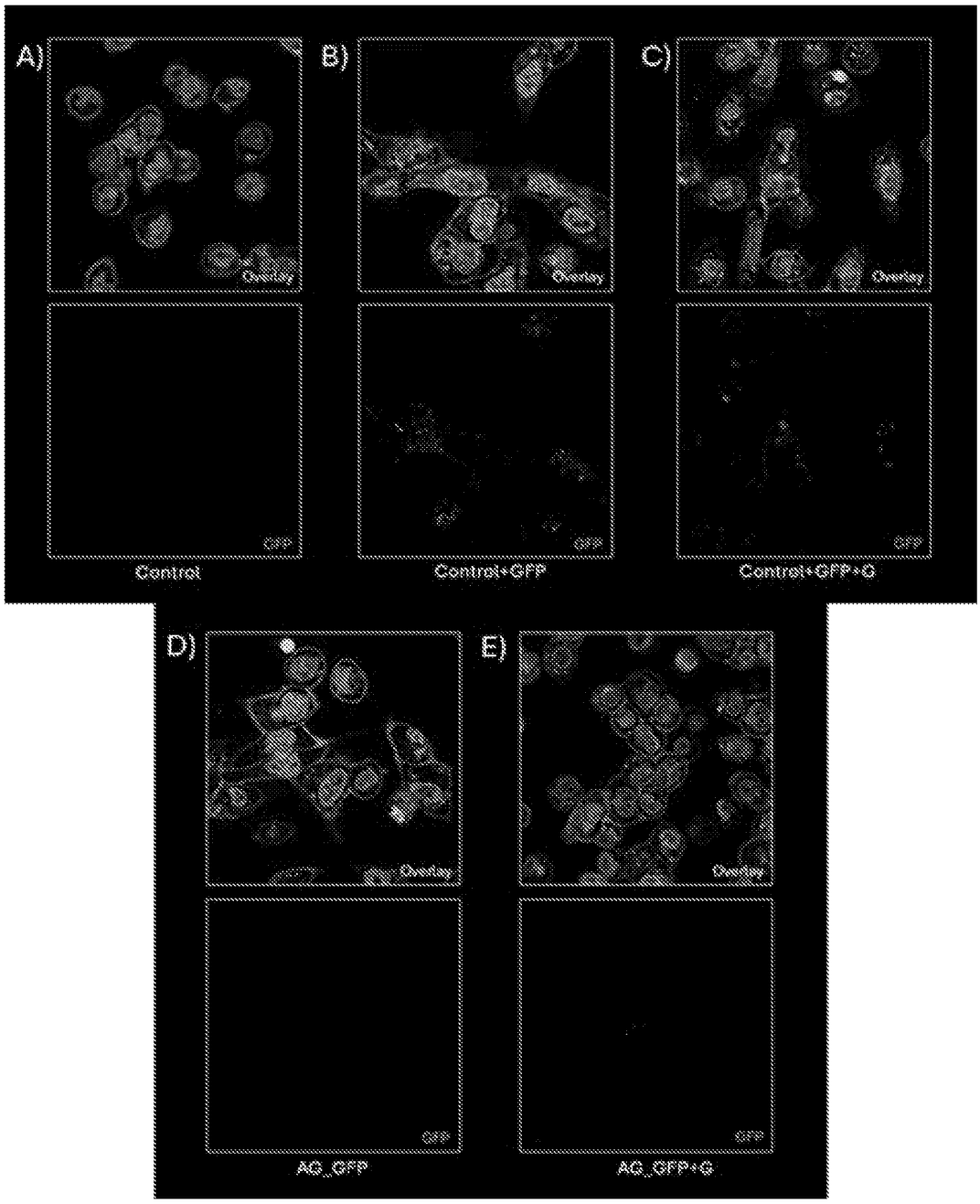

A)
B)
C)
Overlay
GFP
Control
Control+GFP
Control+GFP+G
D)
E)
AG_GFP
AG_GFP+G

FIGURE 24

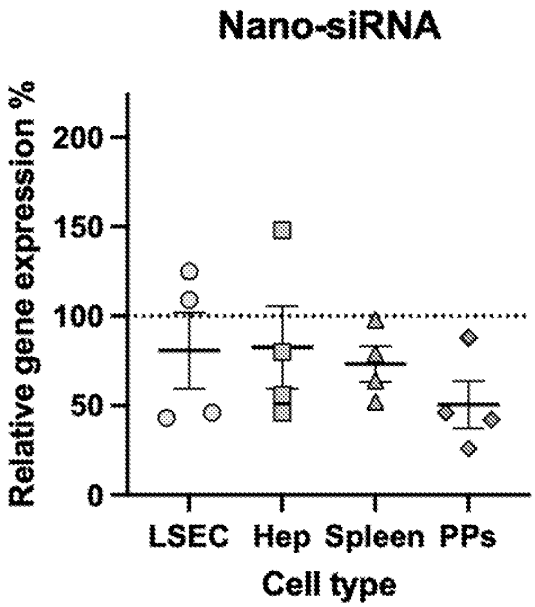

Nano-siRNA
Relative gene expression %
200
150
100
50
0
LSEC
Hep
Spleen
PPs
Cell type

FIGURE 25

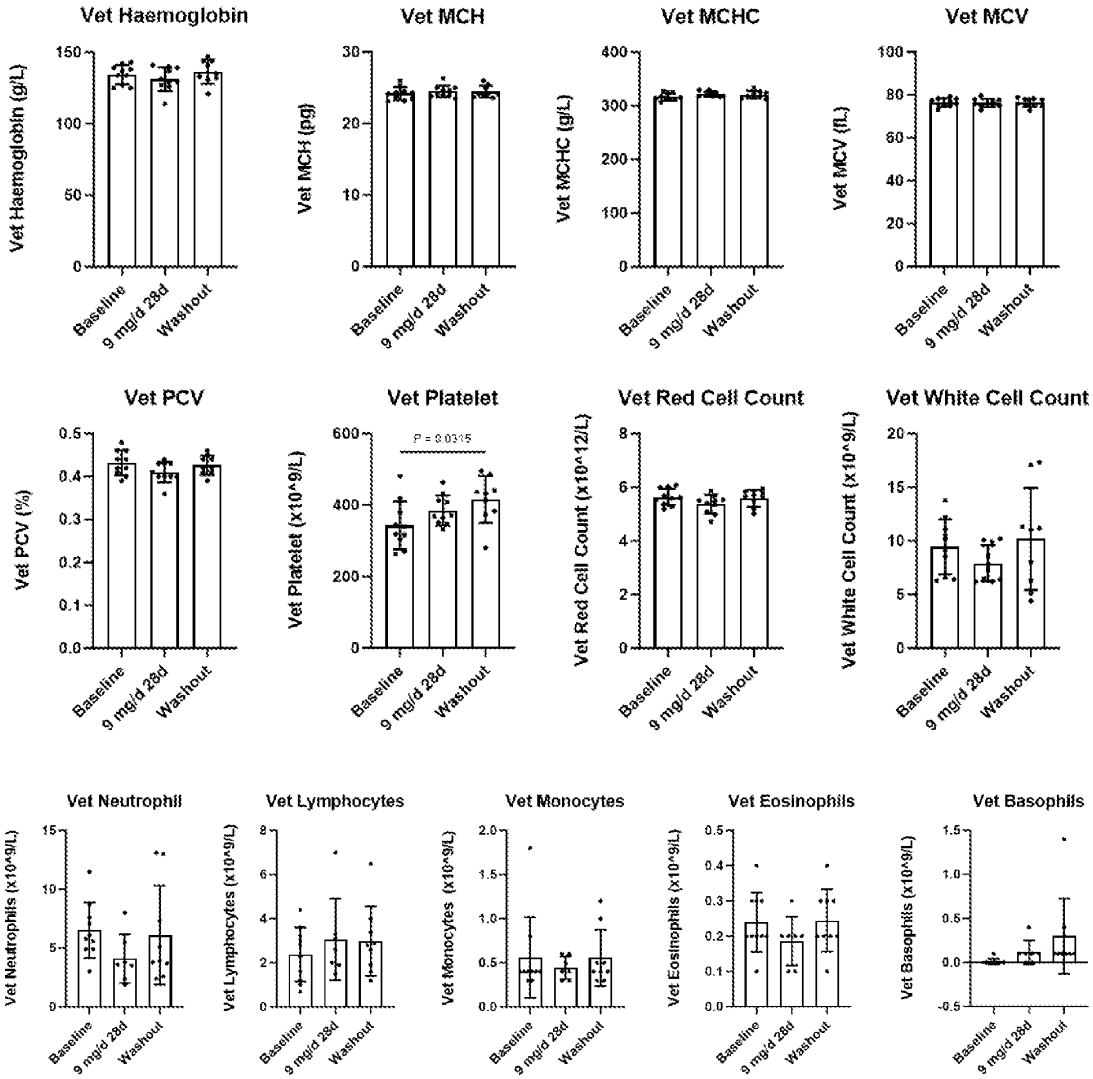

Vet Haemoglobin
Vet Haemoglobin (g/L)
Vet MCH
Vet MCH (pg)
Vet MCHC
Vet MCHC (g/L)
Vet MCV
Vet MCV (fL)
Vet PCV
Vet PCV (%)
Vet Platelet
Vet Platelet (x10^9/L)
P = 0.0315
Vet Red Cell Count
Vet Red Cell Count (x10^12/L)
Vet White Cell Count
Vet White Cell Count (x10^9/L)
Vet Neutrophil
Vet Neutrophils (x10^9/L)
Vet Lymphocytes
Vet Lymphocytes (x10^9/L)
Vet Monocytes
Vet Monocytes (x10^9/L)
Vet Eosinophils
Vet Eosinophils (x10^9/L)
Vet Basophils
Vet Basophils (x10^9/L)
Baseline
9 mg/d 28d
Washout

FIGURE 26

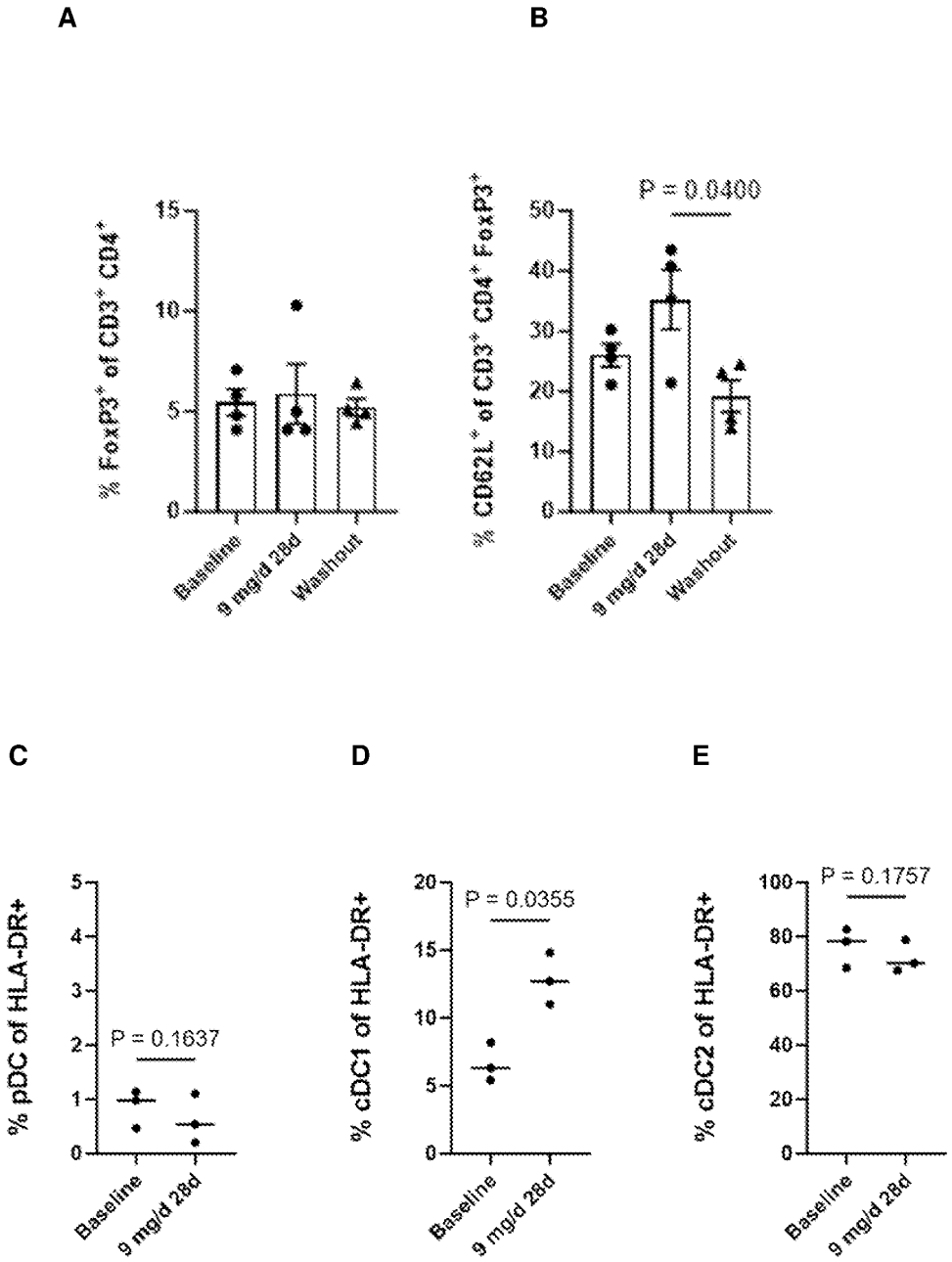

A
B
% FoxP3+ of CD3+ CD4+
% CD62L+ of CD3+ CD4+ FoxP3+
P = 0.0400
Baseline
9 mg/d 28d
Washout
C
D
E
% pDC of HLA-DR+
% cDC1 of HLA-DR+
% cDC2 of HLA-DR+
P = 0.1637
P = 0.0355
P = 0.1757
Baseline
9 mg/d 28d

FIGURE 27

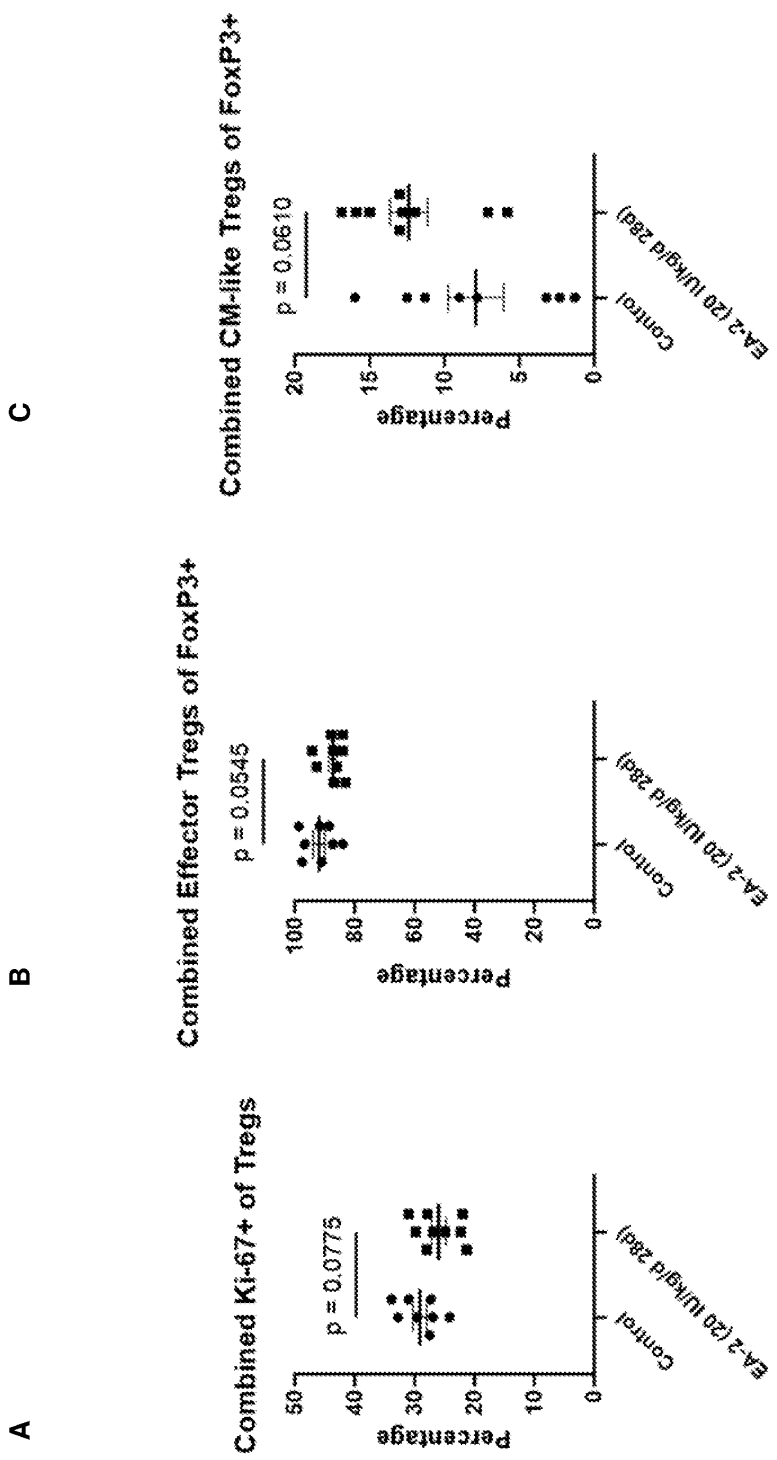

A
Combined Ki-67+ of Tregs
p = 0.0775
Percentage
50
40
30
20
10
0
Control
EA-2 (20 IU/kg/d 28d)
B
Combined Effector Tregs of FoxP3+
p = 0.0545
Percentage
100
80
60
40
20
0
Control
EA-2 (20 IU/kg/d 28d)
C
Combined CM-like Tregs of FoxP3+
p = 0.0610
Percentage
20
15
10
5
0
Control
EA-2 (20 IU/kg/d 28d)

FIGURE 28

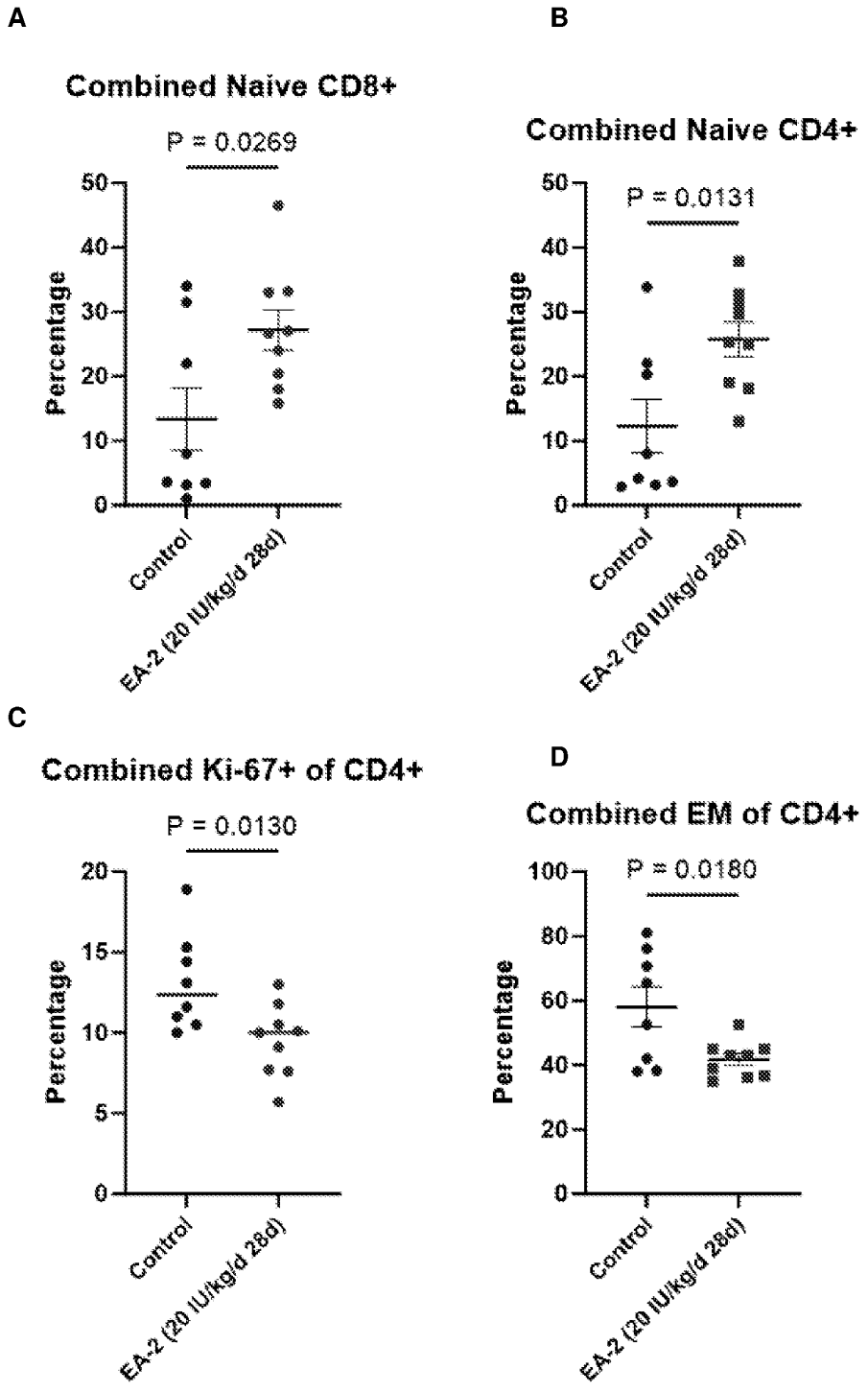

A
Combined Naive CD8+
P = 0.0269
Percentage
50
40
30
20
10
0
Control
EA-2 (20 IU/kg/d 28d)
B
Combined Naive CD4+
P = 0.0131
Percentage
50
40
30
20
10
0
Control
EA-2 (20 IU/kg/d 28d)
C
Combined Ki-67+ of CD4+
P = 0.0130
Percentage
20
15
10
5
0
Control
EA-2 (20 IU/kg/d 28d)
D
Combined EM of CD4+
P = 0.0180
Percentage
100
80
60
40
20
0
Control
EA-2 (20 IU/kg/d 28d)

FIGURE 29

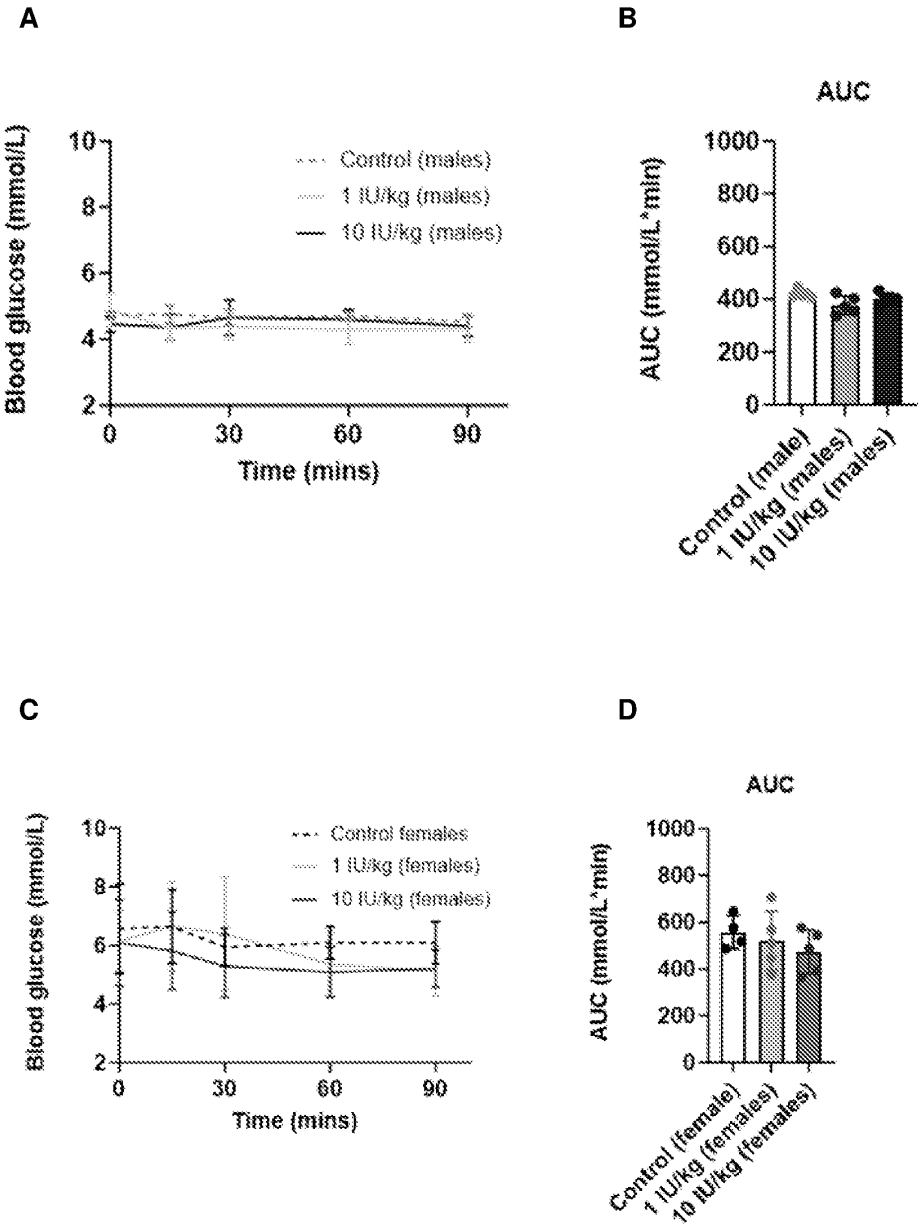

A
Blood glucose (mmol/L)
10
8
6
4
2
0
30
60
90
Time (mins)
Control (males)
1 IU/kg (males)
10 IU/kg (males)
B
AUC
AUC (mmol/L*min)
1000
800
600
400
200
0
Control (male)
1 IU/kg (males)
10 IU/kg (males)
C
Blood glucose (mmol/L)
Control females
1 IU/kg (females)
10 IU/kg (females)
Time (mins)
D
AUC
AUC (mmol/L*min)
Control (female)
1 IU/kg (females)
10 IU/kg (females)

FIGURE 29 - continued

E

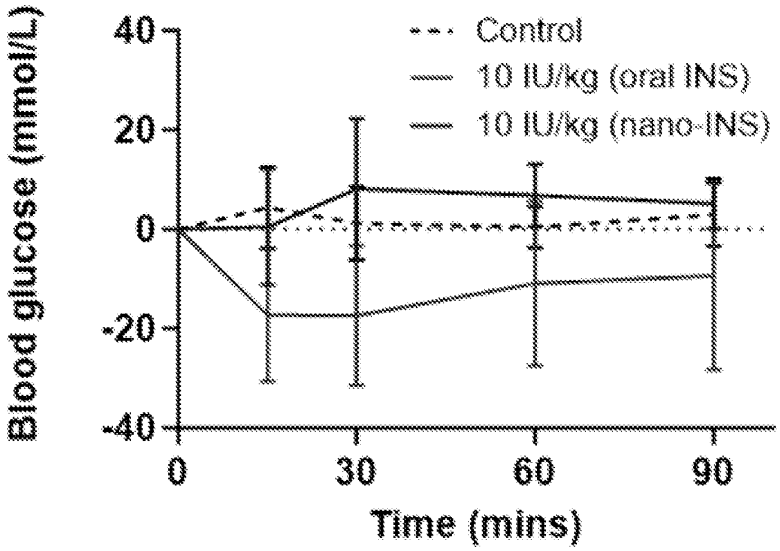


FIGURE 30

A

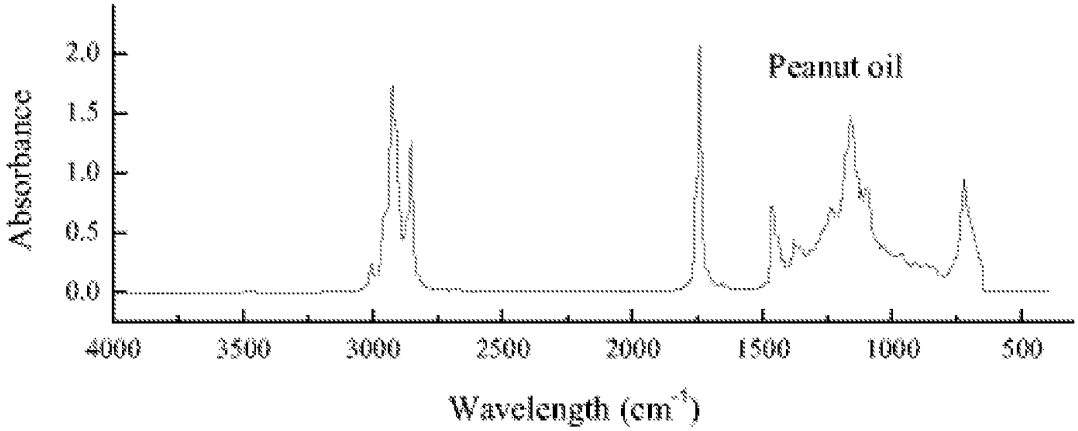
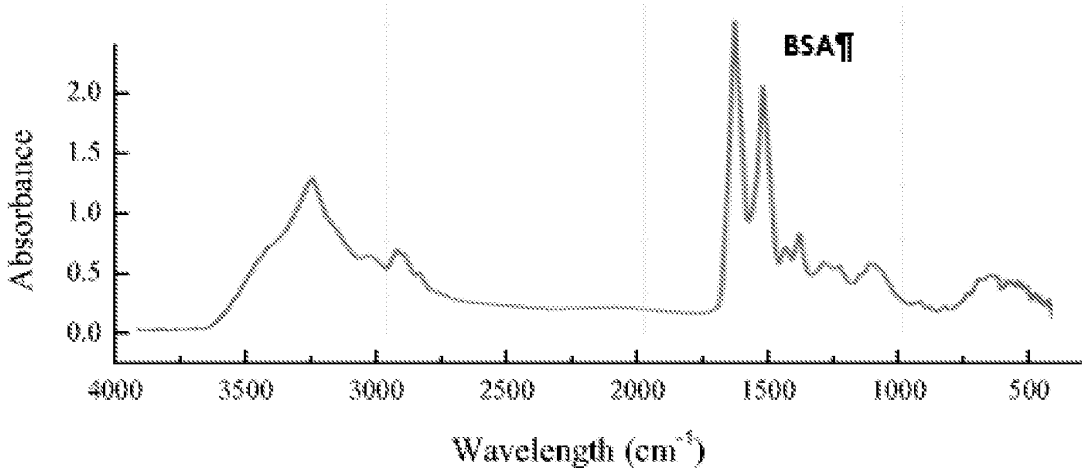


FIGURE 30 - continued

B



C

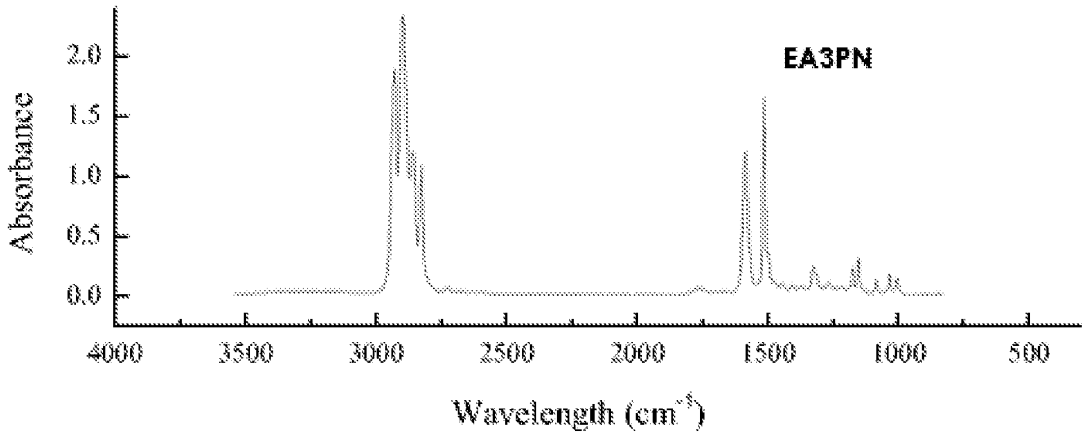
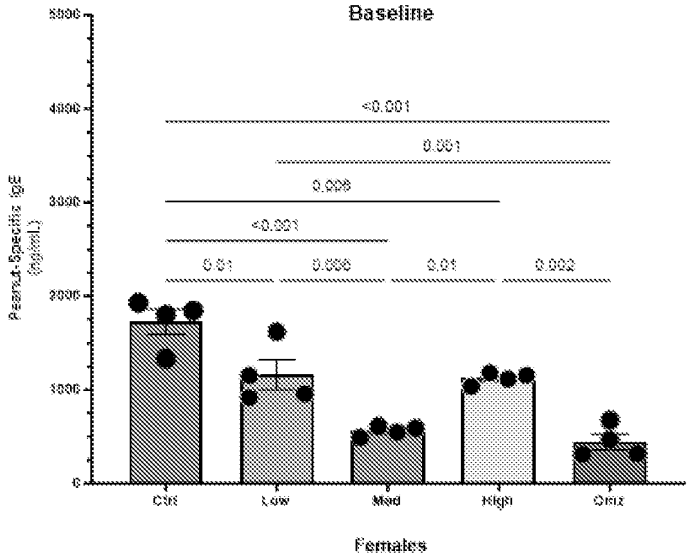


FIGURE 31

A


Baseline
<0.001
0.001
0.006
<0.001
0.01
0.006
0.01
0.002
Peanut-Specific IgE (ng/mL)
Ctrl
Low
Med
High
Omz
Females

B

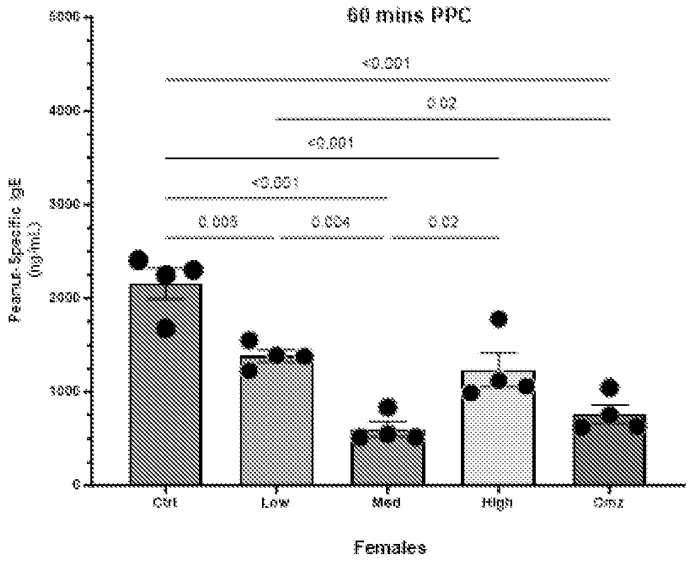
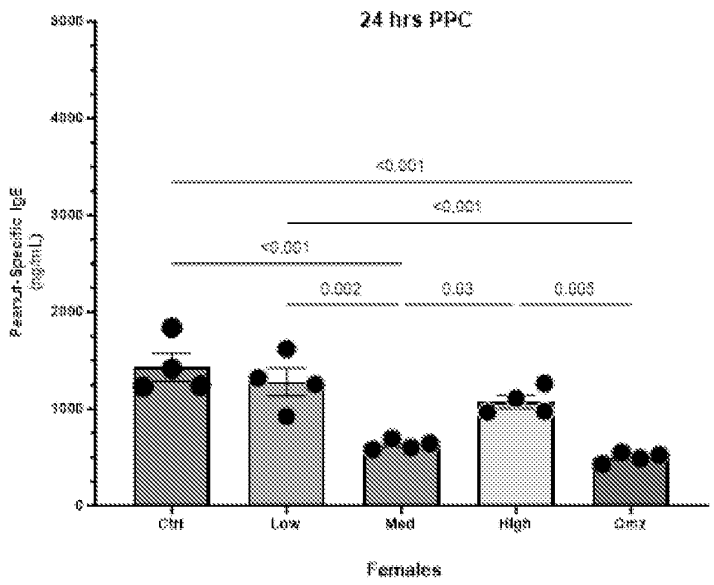

60 mins PPC
<0.001
0.02
<0.001
<0.001
0.008
0.004
0.02
Peanut-Specific IgE (ng/mL)
Ctrl
Low
Med
High
Omz
Females

FIGURE 31 - continued

C



D

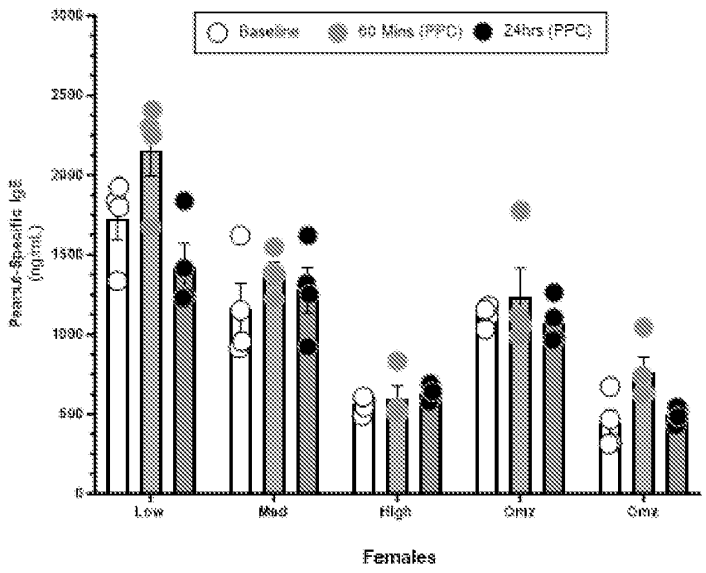
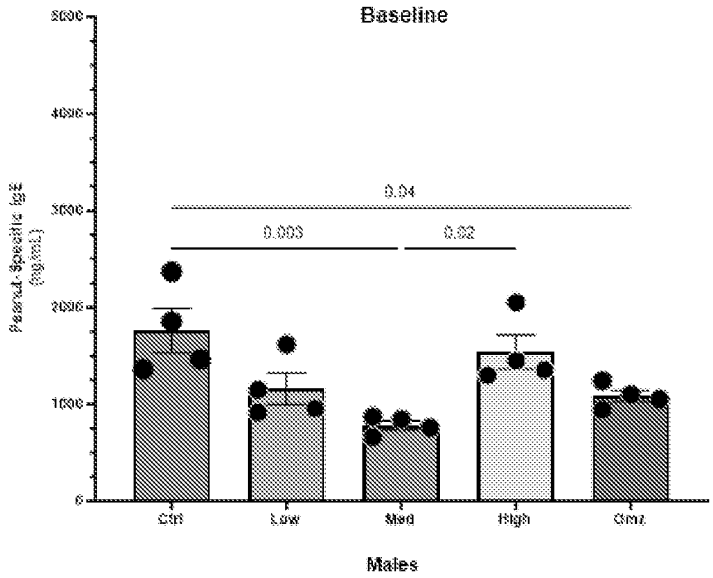


FIGURE 31 - continued

E



F

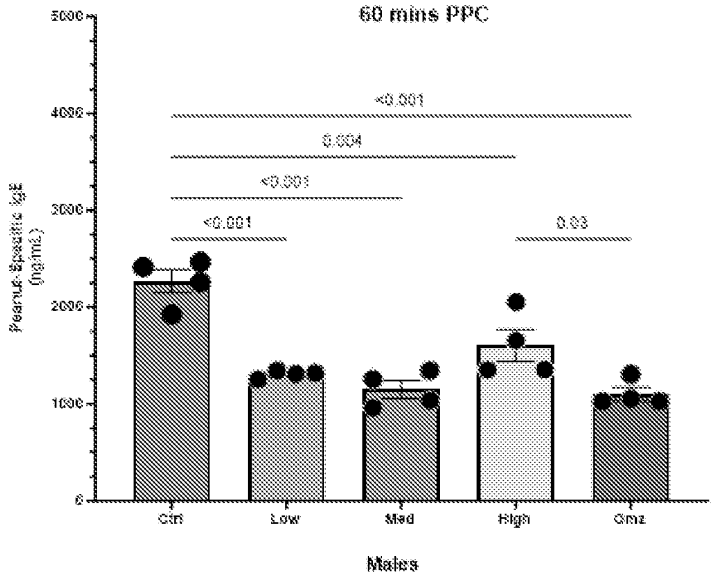
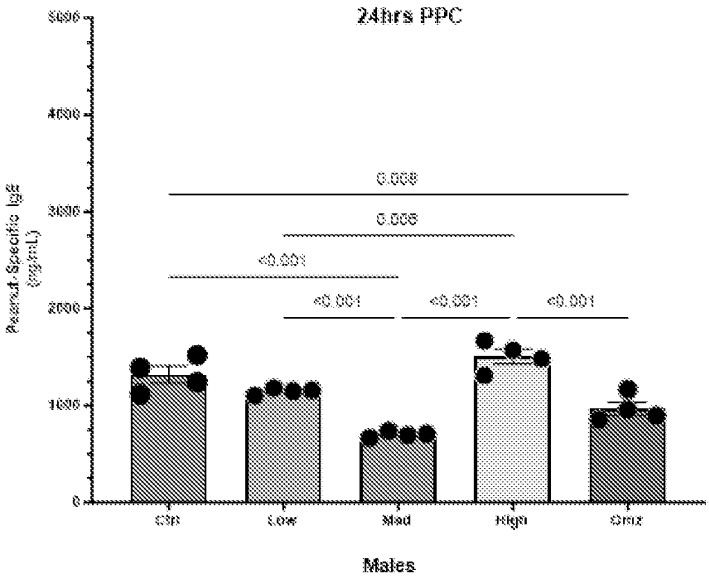


FIGURE 31 - continued

G



H

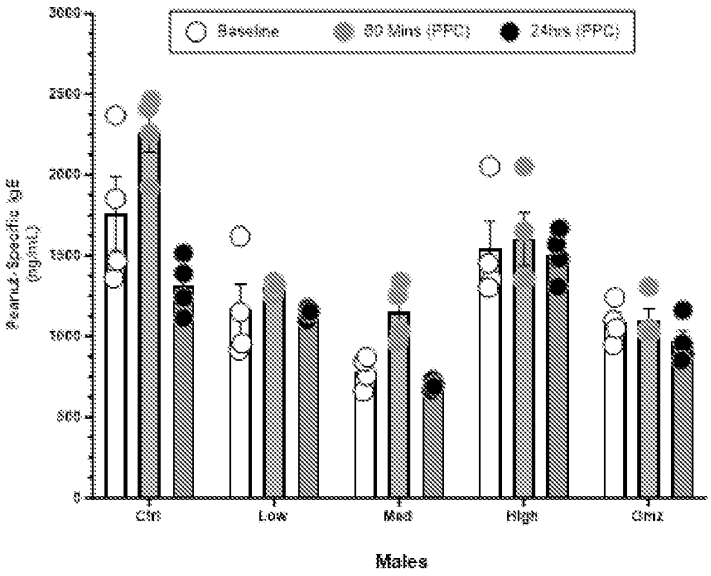
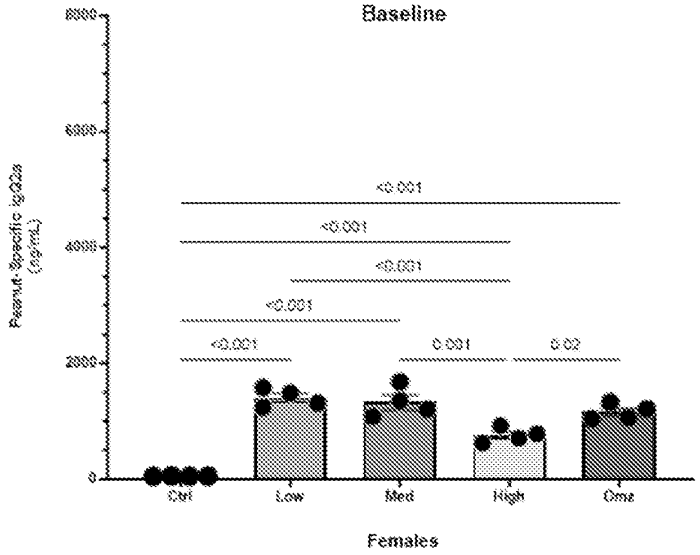


FIGURE 32

A


Baseline
Peanut-Specific IgG2a (ng/mL)
8000
6000
4000
2000
0
<0.001
<0.001
<0.001
<0.001
<0.001
0.001
0.02
Ctrl
Low
Med
High
Oma
Females

B

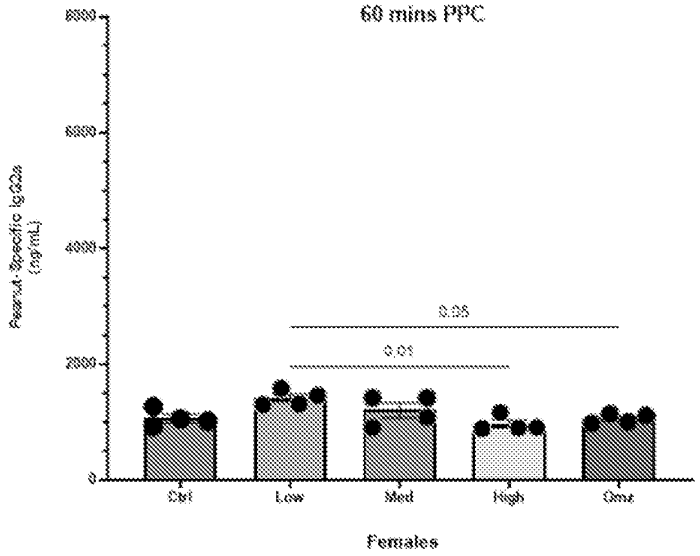
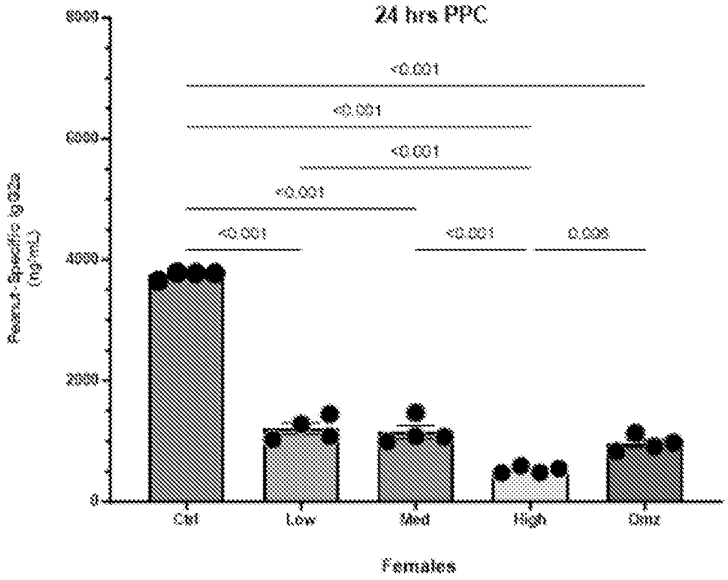

60 mins PPC
Peanut-Specific IgG2a (ng/mL)
8000
6000
4000
2000
0
0.05
0.01
Ctrl
Low
Med
High
Oma
Females

FIGURE 32 - continued

C



D

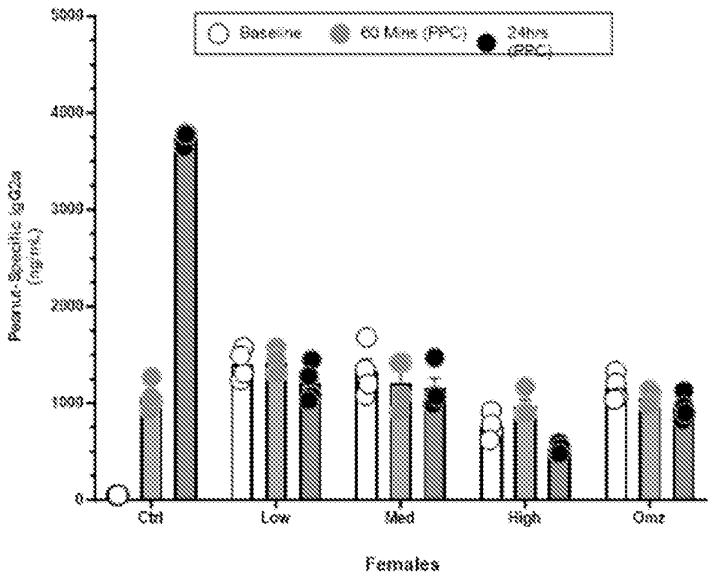
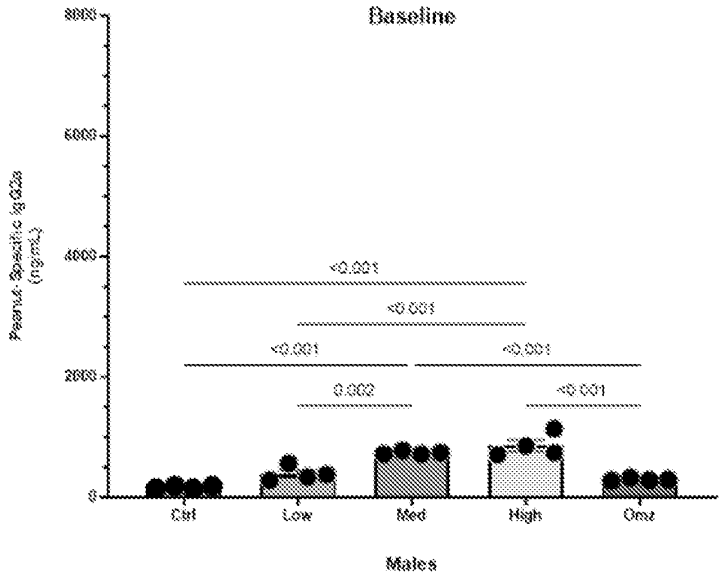


FIGURE 32 - continued

E



F

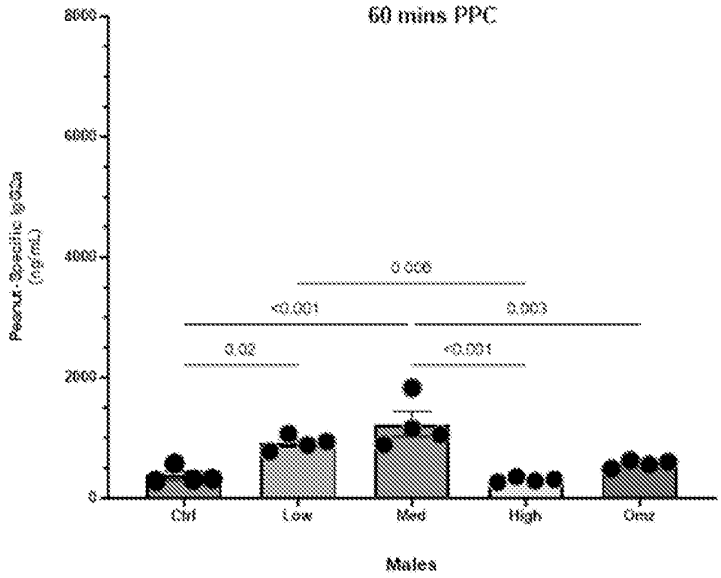
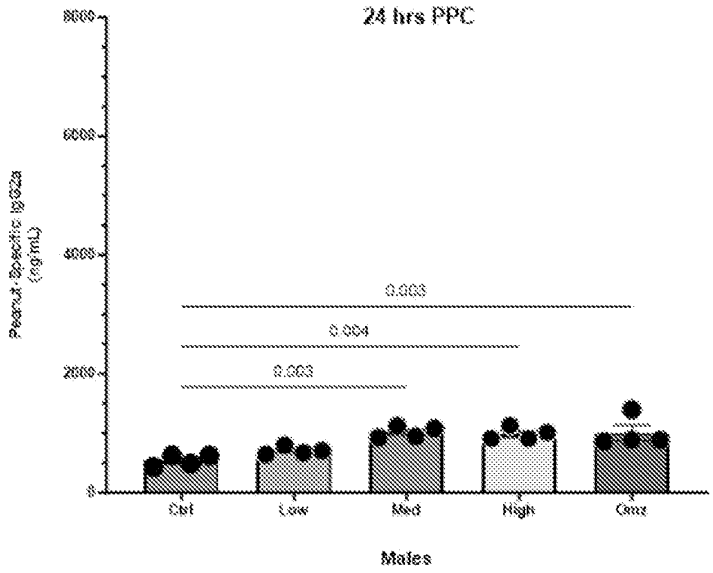


FIGURE 32 - continued

G



H

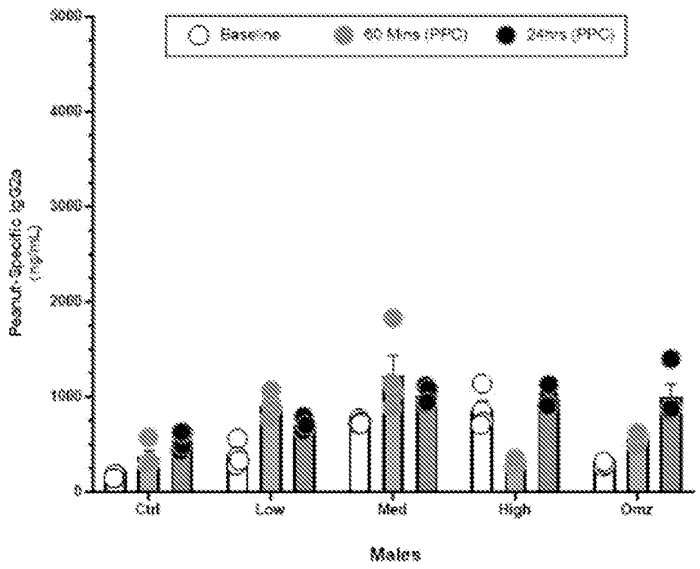
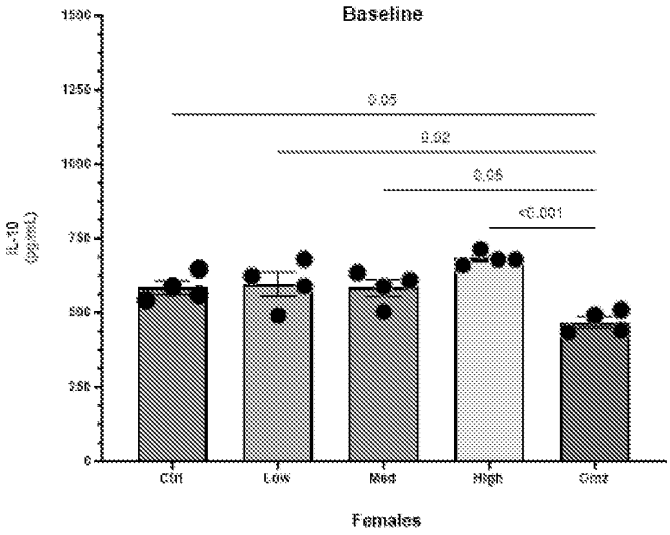


FIGURE 33

A


Baseline
1500
1250
1000
750
500
250
0
IL-10 (pg/mL)
0.05
0.02
0.05
<0.001
Ctrl
Low
Med
High
Cmz
Females

B

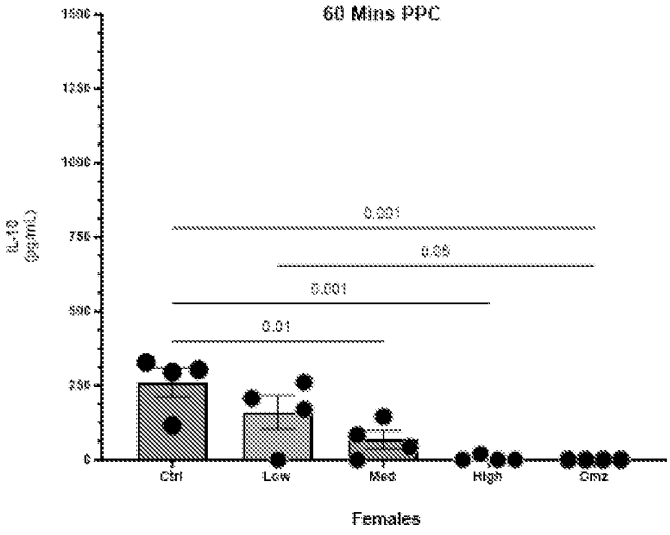
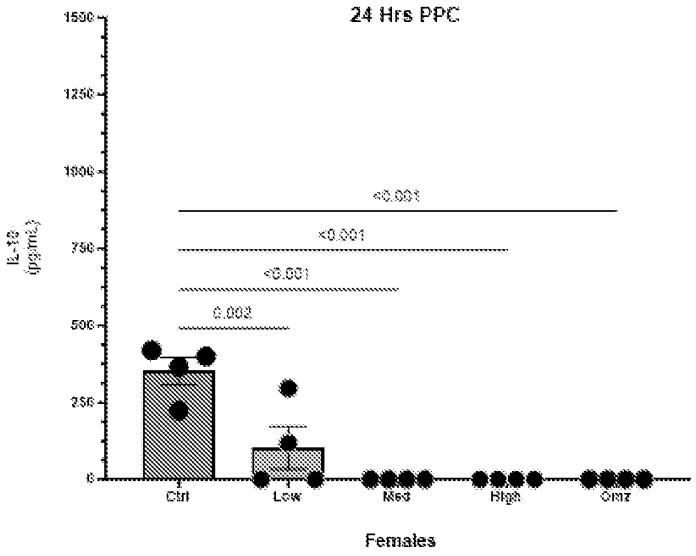

60 Mins PPC
1500
1250
1000
750
500
250
0
IL-10 (pg/mL)
0.001
0.05
0.001
0.01
Ctrl
Low
Med
High
Cmz
Females

FIGURE 33 - continued

C



D

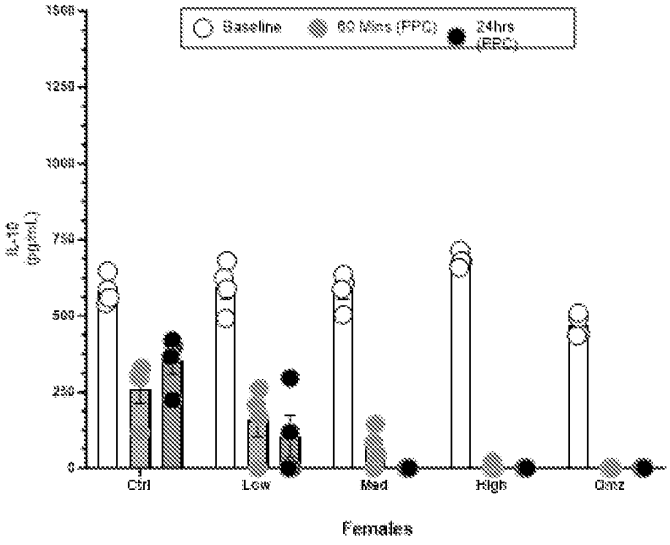
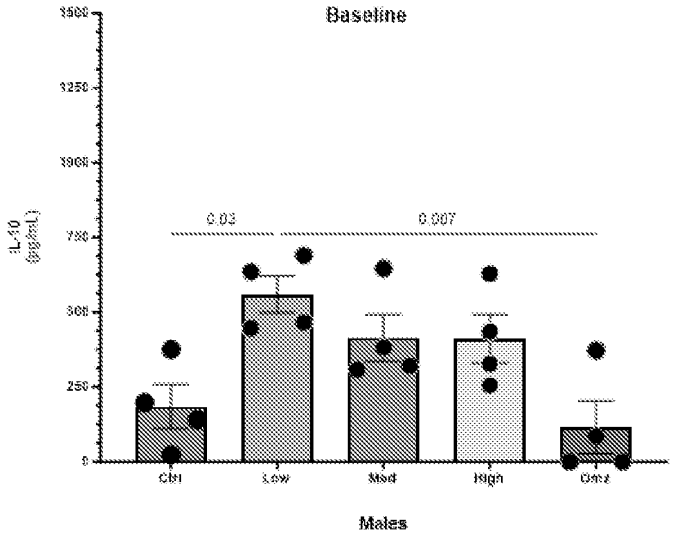


FIGURE 33 - continued

E



F

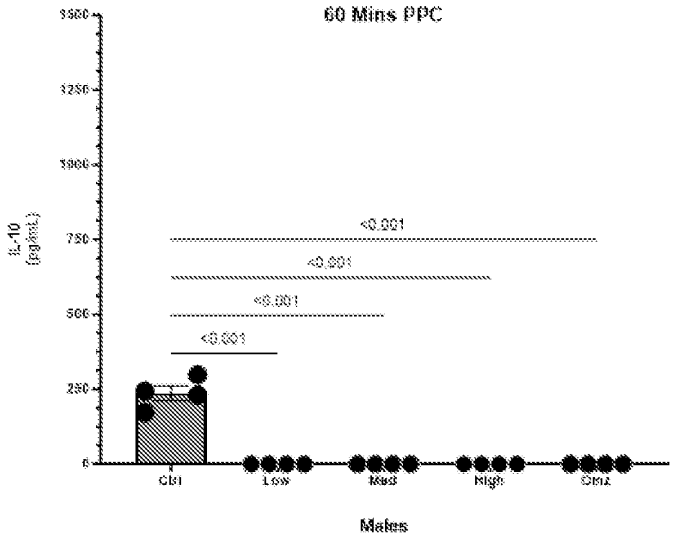
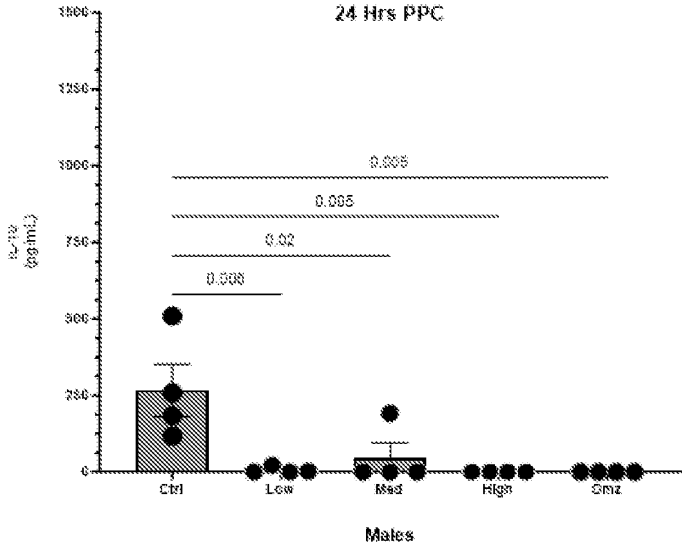


FIGURE 33 - continued

G



H

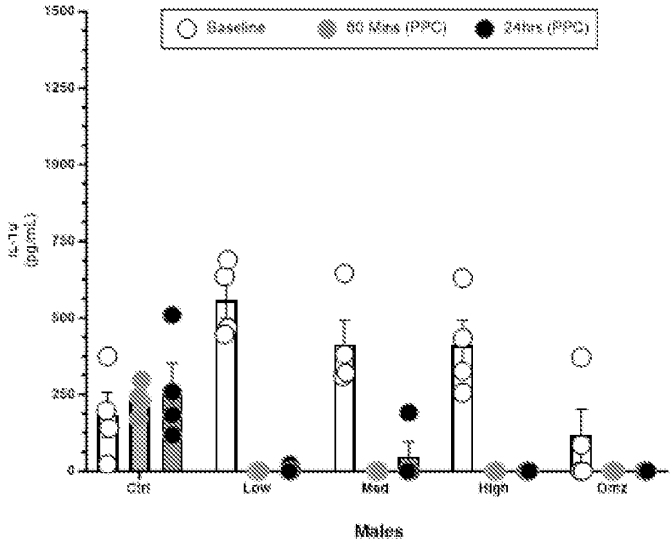
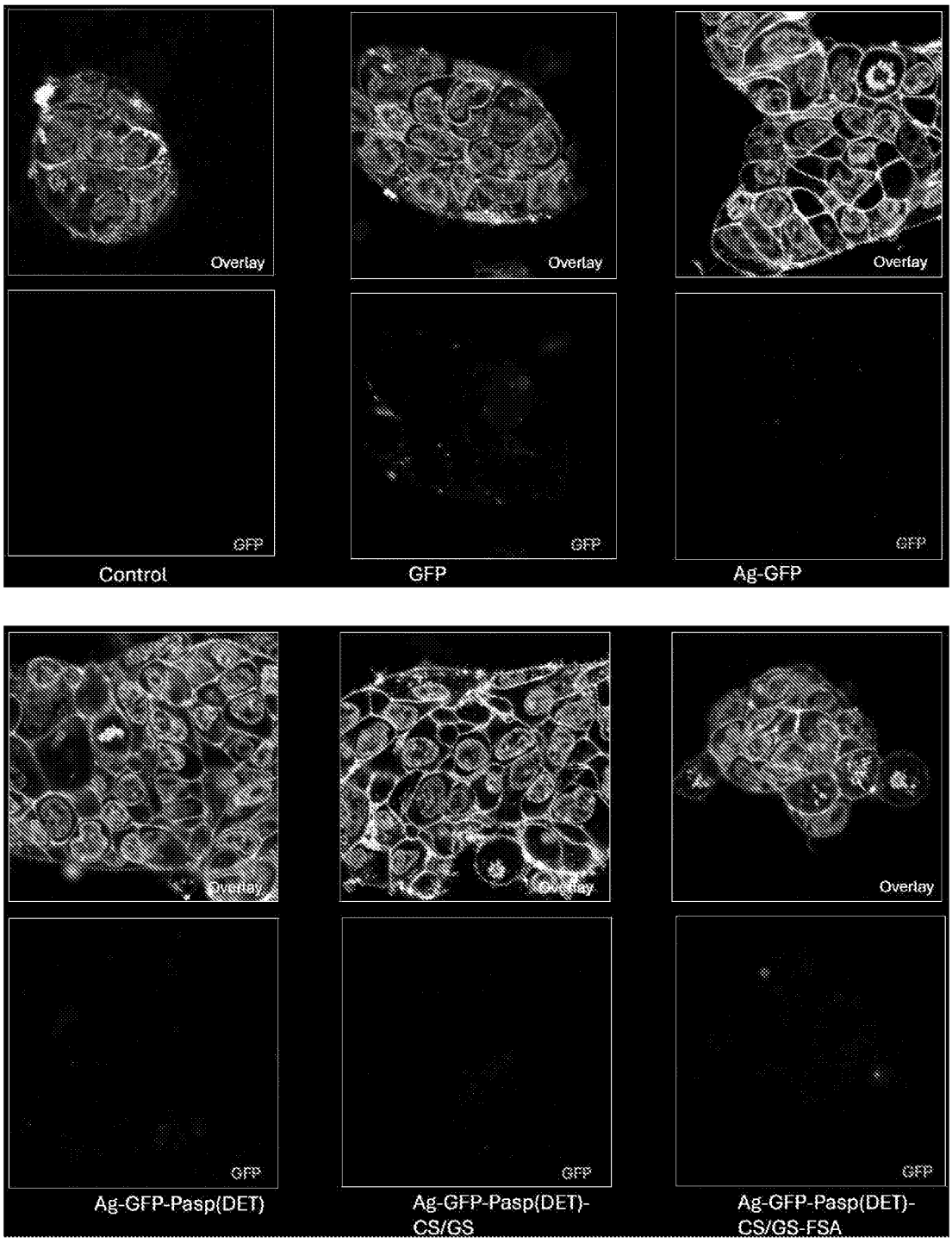


FIGURE 34


Overlay
Overlay
Overlay
GFP
GFP
GFP
Control
GFP
Ag-GFP
Overlay
Overlay
Overlay
GFP
GFP
GFP
Ag-GFP-Pasp(DET)
Ag-GFP-Pasp(DET)-CS/GS
Ag-GFP-Pasp(DET)-CS/GS-FSA