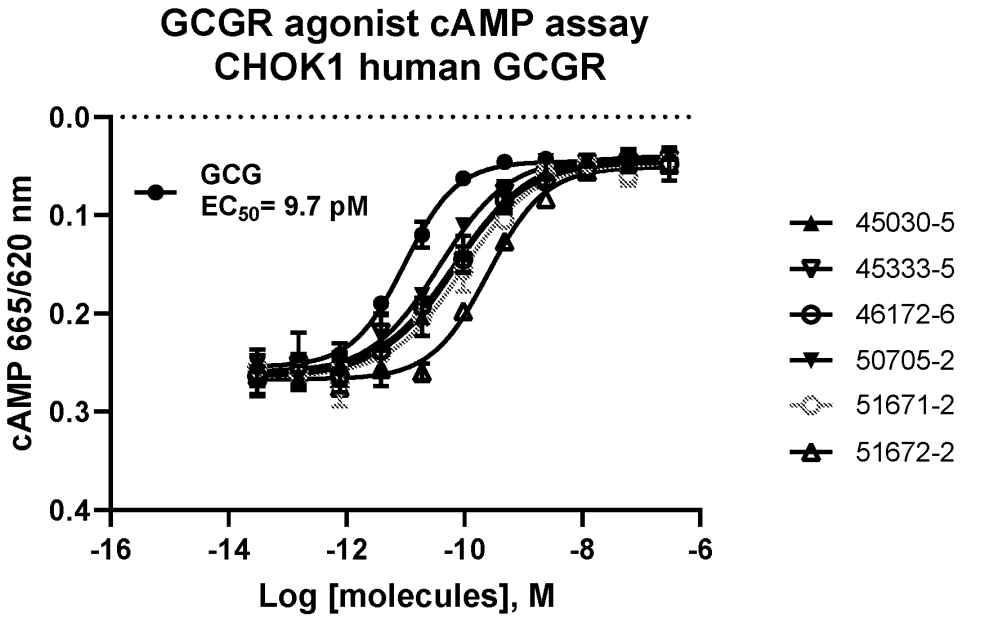

GCGR agonist cAMP assay
CHOK1 human GCGR
cAMP 665/620 nm
0.0
0.1
0.2
0.3
0.4
GCG
EC50= 9.7 pM
-16
-14
-12
-10
-8
-6
Log [molecules], M
45030-5
45333-5
46172-6
50705-2
51671-2
51672-2

FIG. 1A

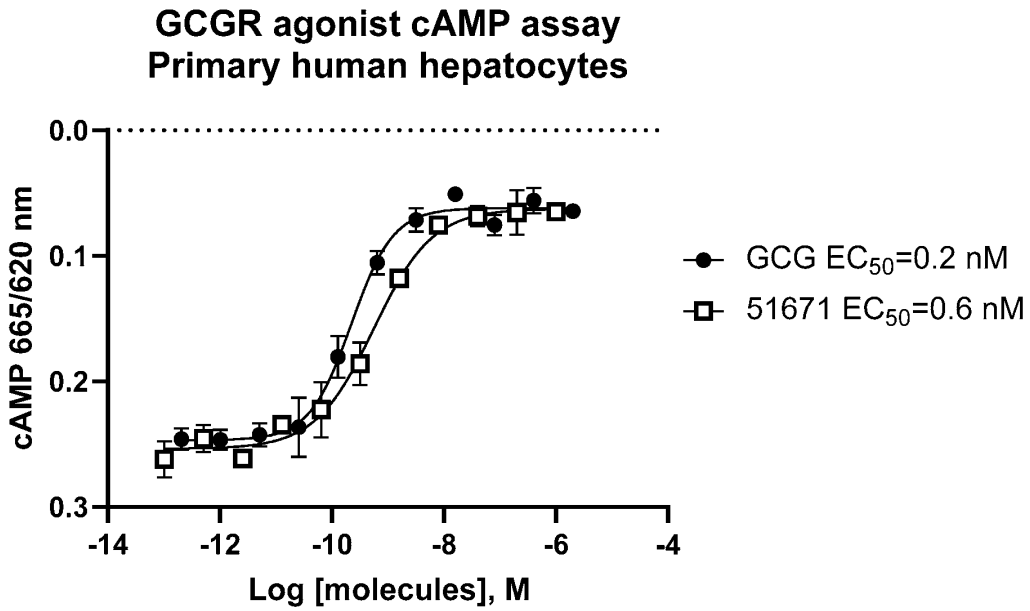

GCGR agonist cAMP assay
Primary human hepatocytes
cAMP 665/620 nm
0.0
0.1
0.2
0.3
-14
-12
-10
-8
-6
-4
Log [molecules], M
GCG EC50=0.2 nM
51671 EC50=0.6 nM

FIG. 1B

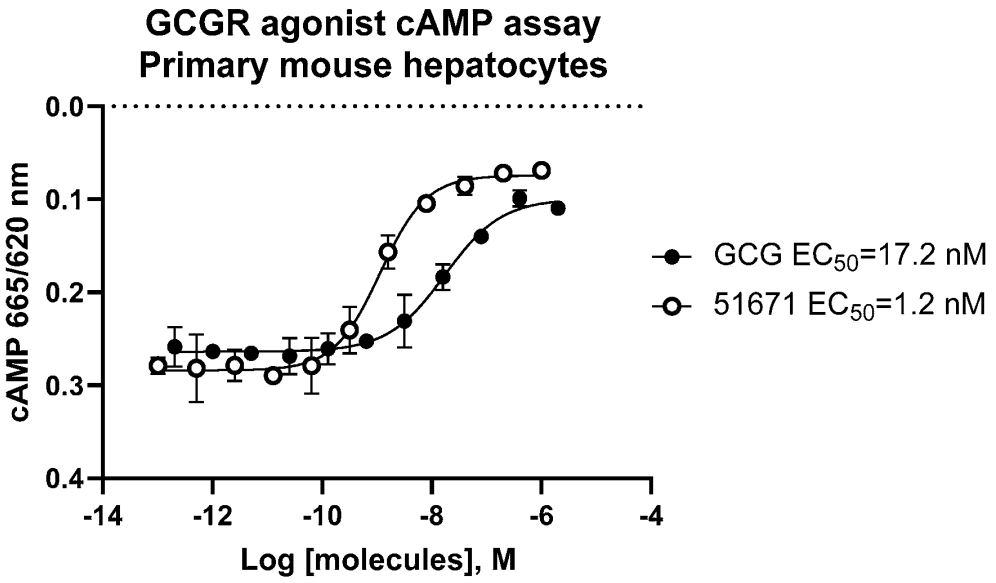

GCGR agonist cAMP assay
Primary mouse hepatocytes
cAMP 665/620 nm
0.0
0.1
0.2
0.3
0.4
-14
-12
-10
-8
-6
-4
Log [molecules], M
GCG EC50=17.2 nM
51671 EC50=1.2 nM

FIG. 1C

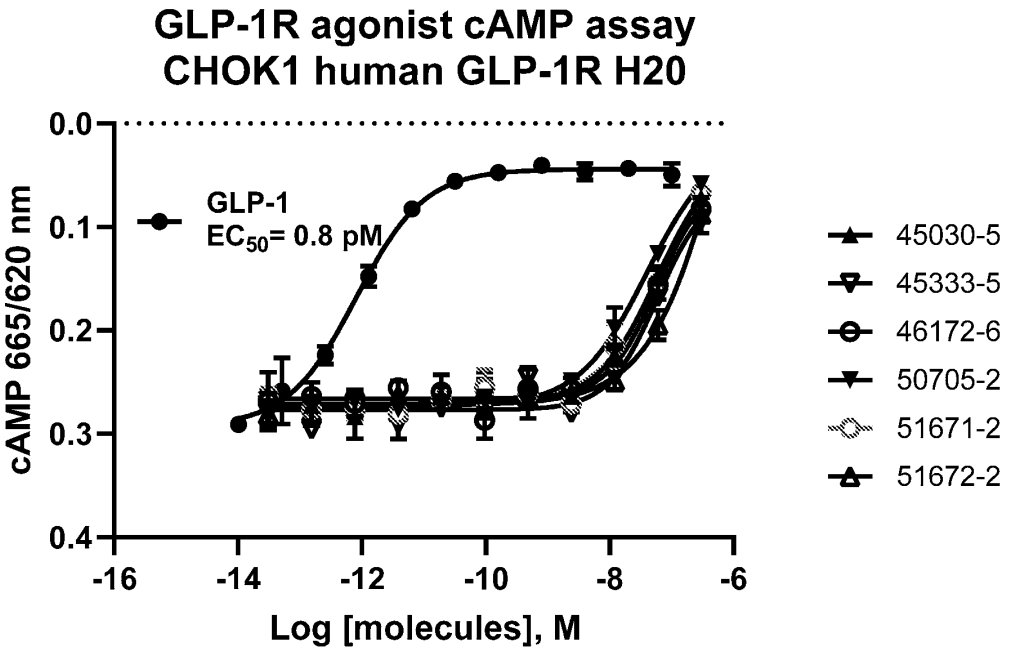

GLP-1R agonist cAMP assay
CHOK1 human GLP-1R H20
cAMP 665/620 nm
0.0
0.1
0.2
0.3
0.4
GLP-1
EC50= 0.8 pM
-16
-14
-12
-10
-8
-6
Log [molecules], M
45030-5
45333-5
46172-6
50705-2
51671-2
51672-2

FIG. 2

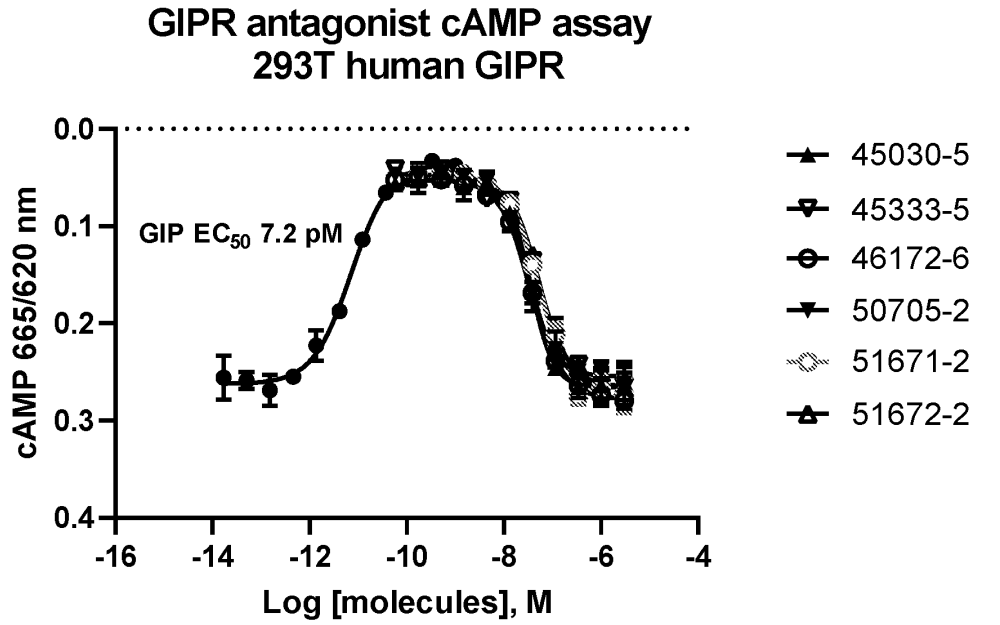

GIPR antagonist cAMP assay
293T human GIPR
cAMP 665/620 nm
0.0
0.1
0.2
0.3
0.4
GIP EC50 7.2 pM
-16
-14
-12
-10
-8
-6
-4
Log [molecules], M
45030-5
45333-5
46172-6
50705-2
51671-2
51672-2

FIG. 3A

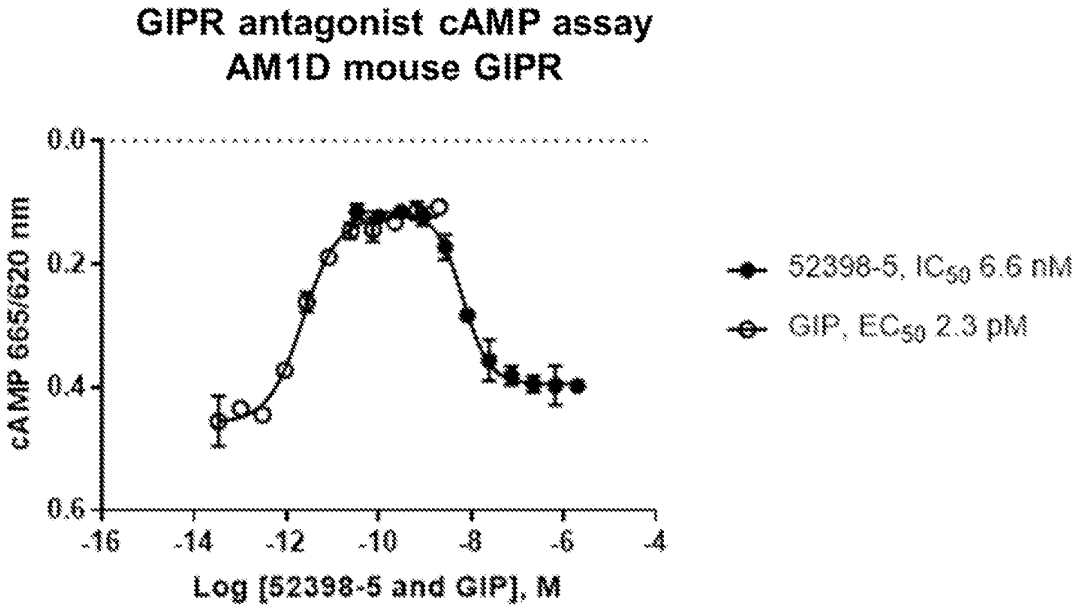

GIPR antagonist cAMP assay
AM1D mouse GIPR
cAMP 665/620 nm
0.0
0.2
0.4
0.6
-16
-14
-12
-10
-8
-6
-4
Log [52398-5 and GIP], M
52398-5, IC50 6.6 nM
GIP, EC50 2.3 pM

FIG. 3B

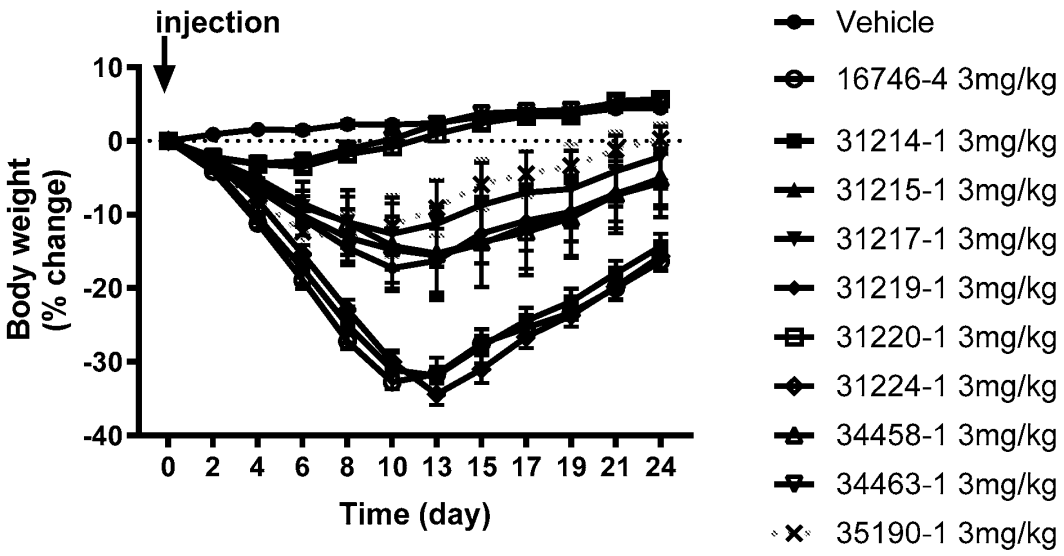

injection
Body weight (% change)
10
0
-10
-20
-30
-40
0 2 4 6 8 10 13 15 17 19 21 24
Time (day)
Vehicle
16746-4 3mg/kg
31214-1 3mg/kg
31215-1 3mg/kg
31217-1 3mg/kg
31219-1 3mg/kg
31220-1 3mg/kg
31224-1 3mg/kg
34458-1 3mg/kg
34463-1 3mg/kg
35190-1 3mg/kg

FIG. 4A

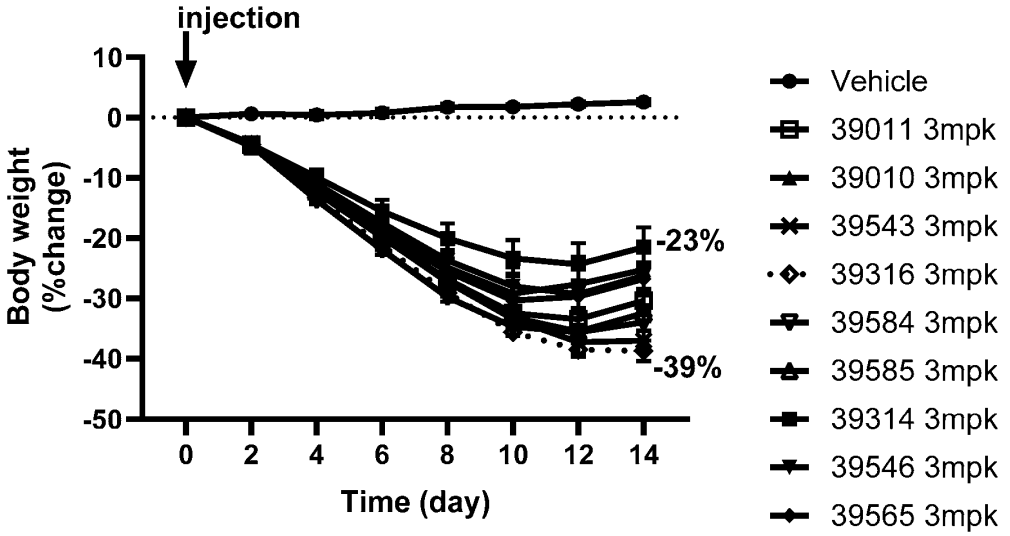

injection
Body weight (%change)
10
0
-10
-20
-30
-40
-50
0 2 4 6 8 10 12 14
Time (day)
-23%
-39%
Vehicle
39011 3mpk
39010 3mpk
39543 3mpk
39316 3mpk
39584 3mpk
39585 3mpk
39314 3mpk
39546 3mpk
39565 3mpk

FIG. 4B

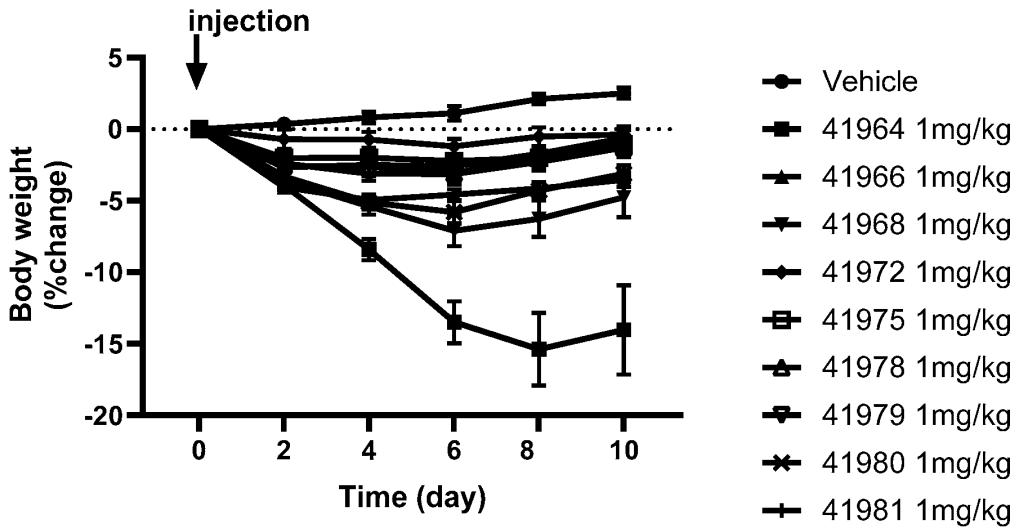

injection
Body weight (%change)
5
0
-5
-10
-15
-20
0
2
4
6
8
10
Time (day)
Vehicle
41964 1mg/kg
41966 1mg/kg
41968 1mg/kg
41972 1mg/kg
41975 1mg/kg
41978 1mg/kg
41979 1mg/kg
41980 1mg/kg
41981 1mg/kg

FIG. 4C

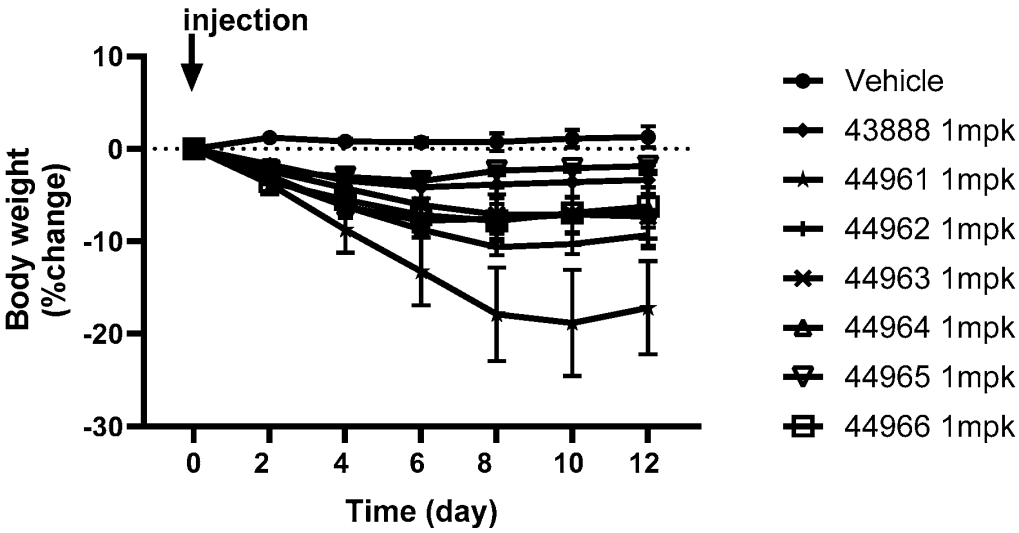

injection
Body weight (%change)
10
0
-10
-20
-30
0
2
4
6
8
10
12
Time (day)
Vehicle
43888 1mpk
44961 1mpk
44962 1mpk
44963 1mpk
44964 1mpk
44965 1mpk
44966 1mpk

FIG. 4D

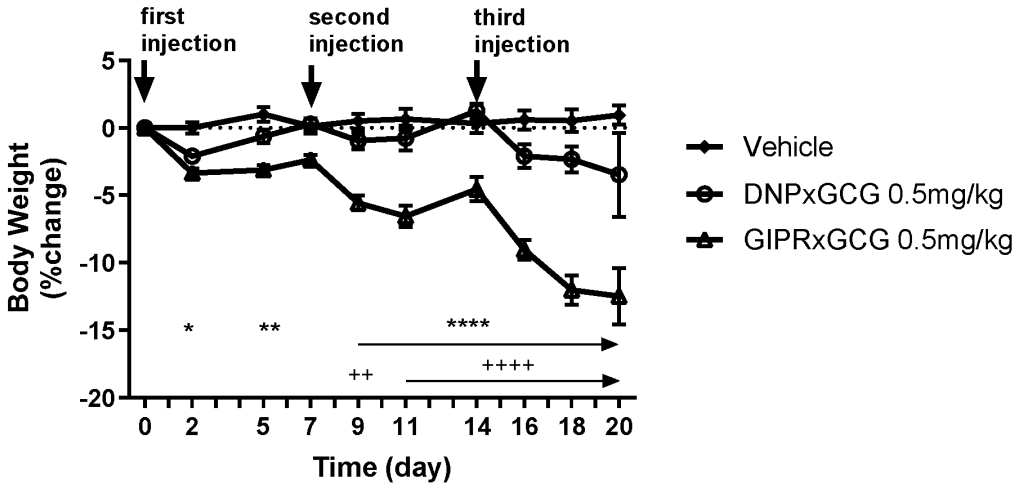

first injection
second injection
third injection
Body Weight (%change)
5
0
-5
-10
-15
-20
*
**
****
++
++++
0
2
5
7
9
11
14
16
18
20
Time (day)
Vehicle
DNPxGCG 0.5mg/kg
GIPRxGCG 0.5mg/kg

FIG. 5A

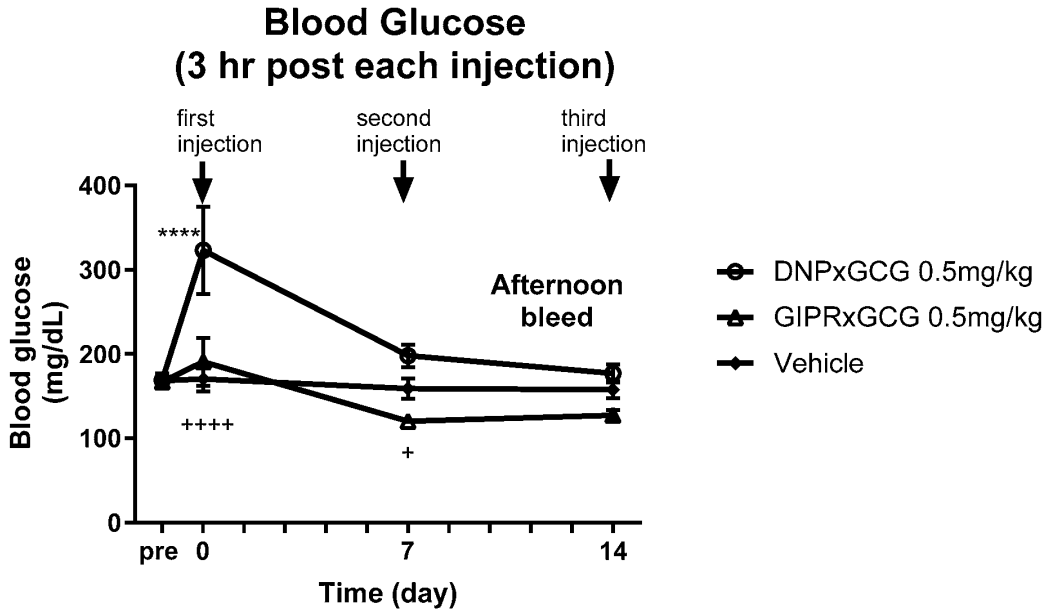

Blood Glucose
(3 hr post each injection)
first injection
second injection
third injection
Afternoon bleed
Blood glucose (mg/dL)
400
300
200
100
0
****
++++
+
pre
0
7
14
Time (day)
DNPxGCG 0.5mg/kg
GIPRxGCG 0.5mg/kg
Vehicle

FIG. 5B

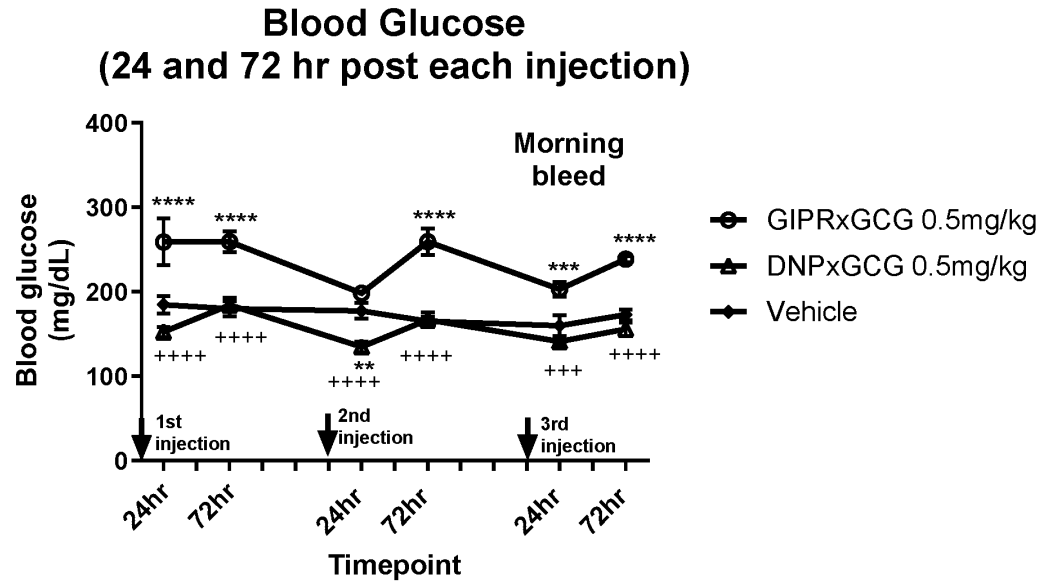

Blood Glucose
(24 and 72 hr post each injection)
Morning bleed
Blood glucose (mg/dL)
400
300
200
100
0
****
++++
**
+++
1st injection
2nd injection
3rd injection
24hr
72hr
Timepoint
GIPRxGCG 0.5mg/kg
DNPxGCG 0.5mg/kg
Vehicle

FIG. 5C

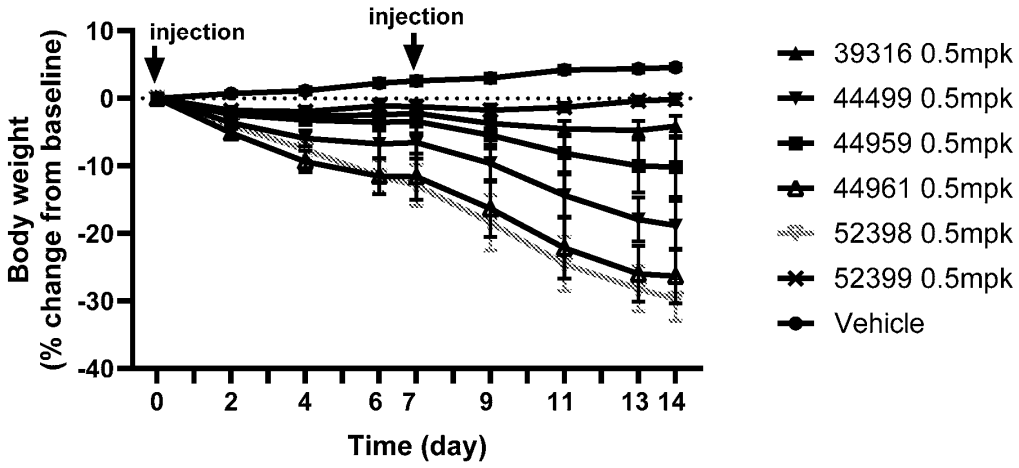

injection
injection
Body weight
(% change from baseline)
10
0
-10
-20
-30
-40
0
2
4
6
7
9
11
13
14
Time (day)
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk
Vehicle

FIG. 6A

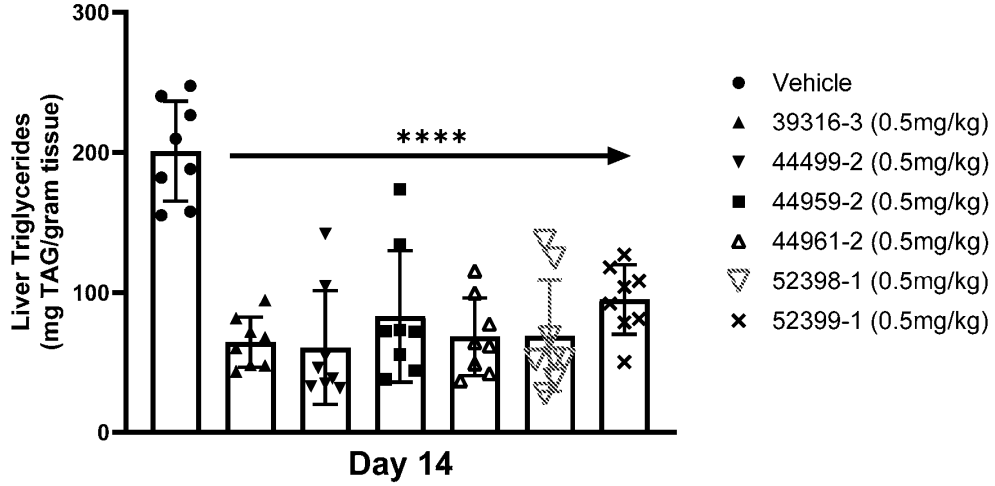

Liver Triglycerides
(mg TAG/gram tissue)
300
200
100
0
****
Day 14
Vehicle
39316-3 (0.5mg/kg)
44499-2 (0.5mg/kg)
44959-2 (0.5mg/kg)
44961-2 (0.5mg/kg)
52398-1 (0.5mg/kg)
52399-1 (0.5mg/kg)

FIG. 6B

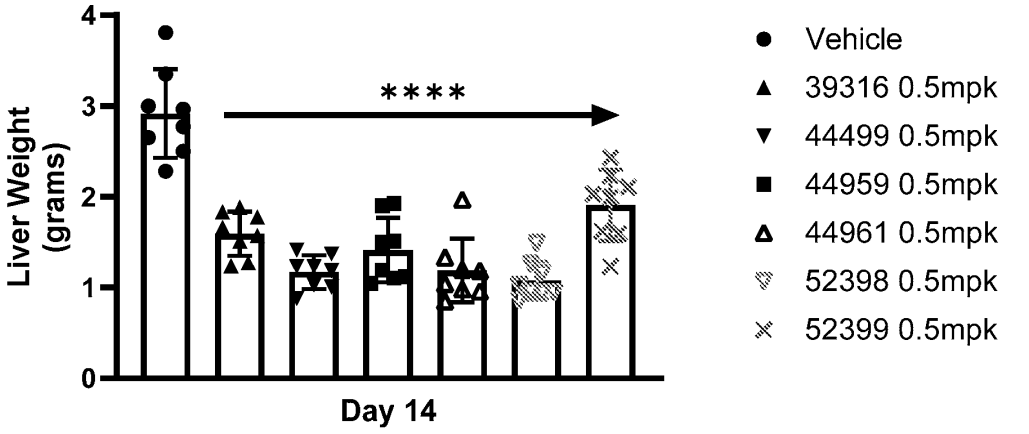

Liver Weight (grams)
4
3
2
1
0
****
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6C

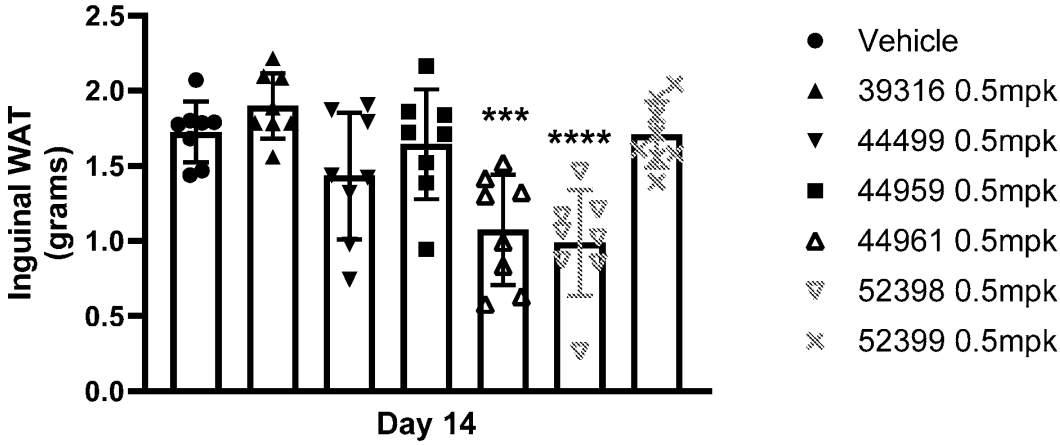

Inguinal WAT (grams)
2.5
2.0
1.5
1.0
0.5
0.0
***
****
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6D

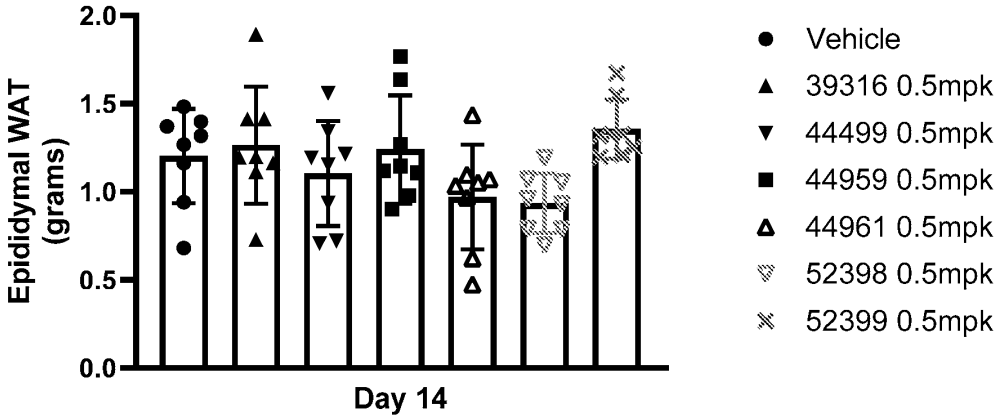

Epididymal WAT (grams)
2.0
1.5
1.0
0.5
0.0
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6E

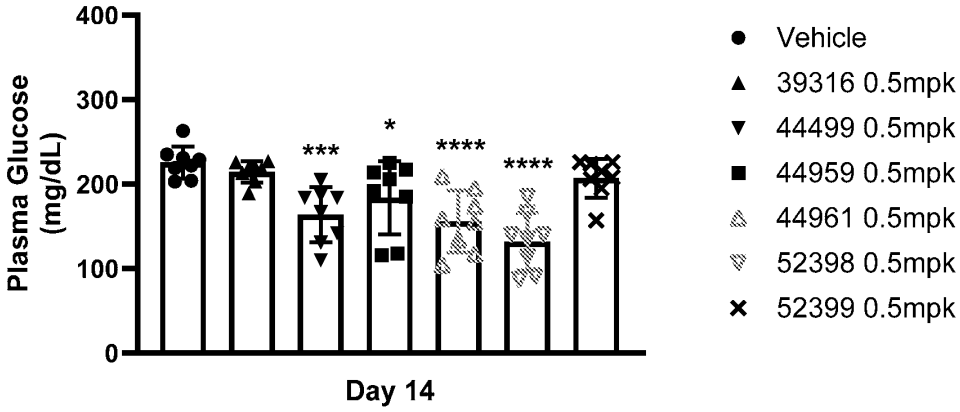

Plasma Glucose (mg/dL)
400
300
200
100
0
***
*
****
****
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6F

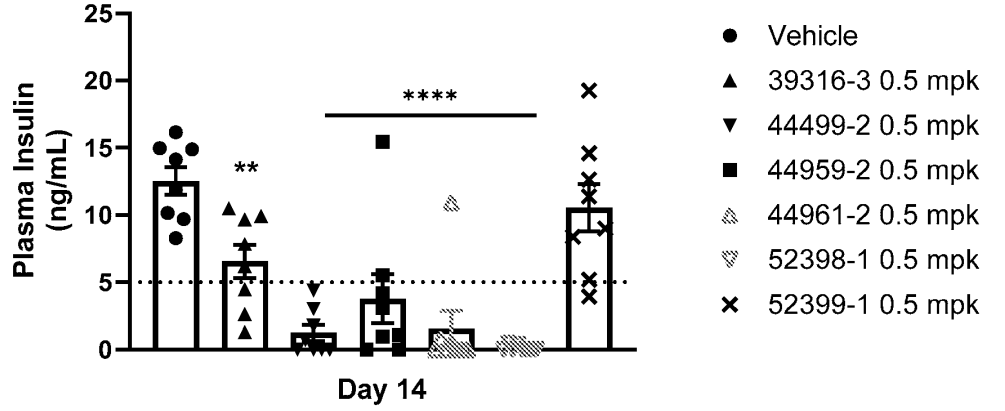

Plasma Insulin (ng/mL)
25
20
15
10
5
0
**
****
Day 14
Vehicle
39316-3 0.5 mpk
44499-2 0.5 mpk
44959-2 0.5 mpk
44961-2 0.5 mpk
52398-1 0.5 mpk
52399-1 0.5 mpk

FIG. 6G

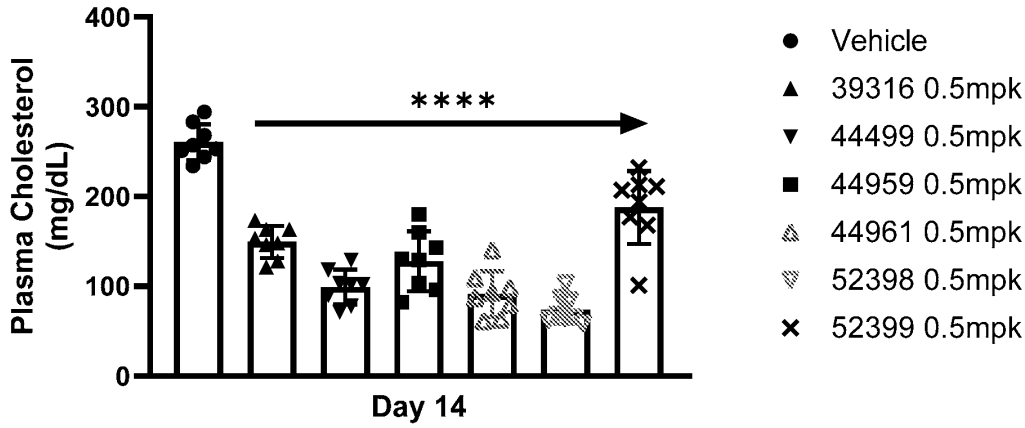

Plasma Cholesterol (mg/dL)
400
300
200
100
0
****
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6H

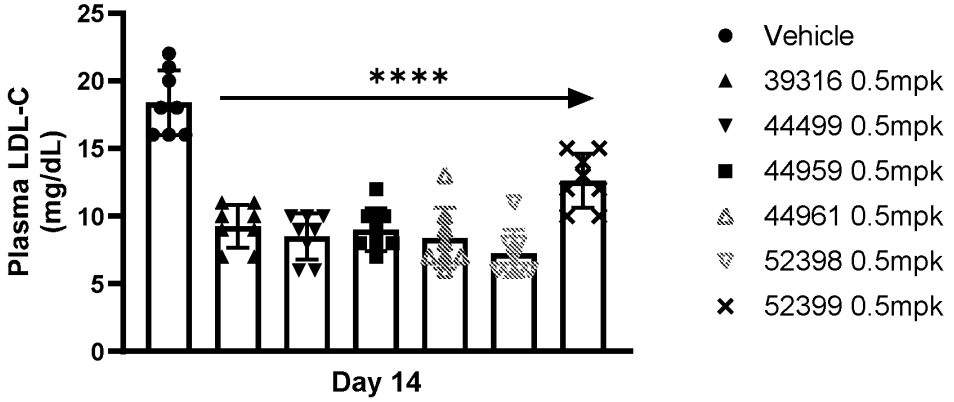

Plasma LDL-C (mg/dL)
25
20
15
10
5
0
****
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6I

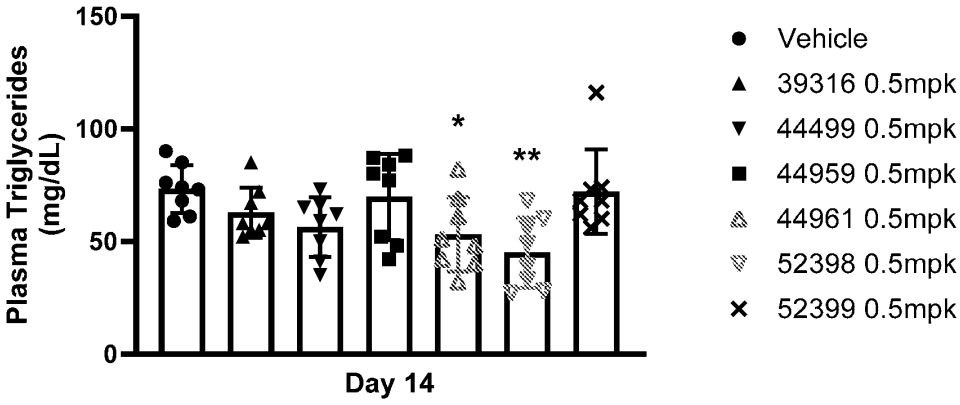

Plasma Triglycerides (mg/dL)
150
100
50
0
*
**
Day 14
Vehicle
39316 0.5mpk
44499 0.5mpk
44959 0.5mpk
44961 0.5mpk
52398 0.5mpk
52399 0.5mpk

FIG. 6J

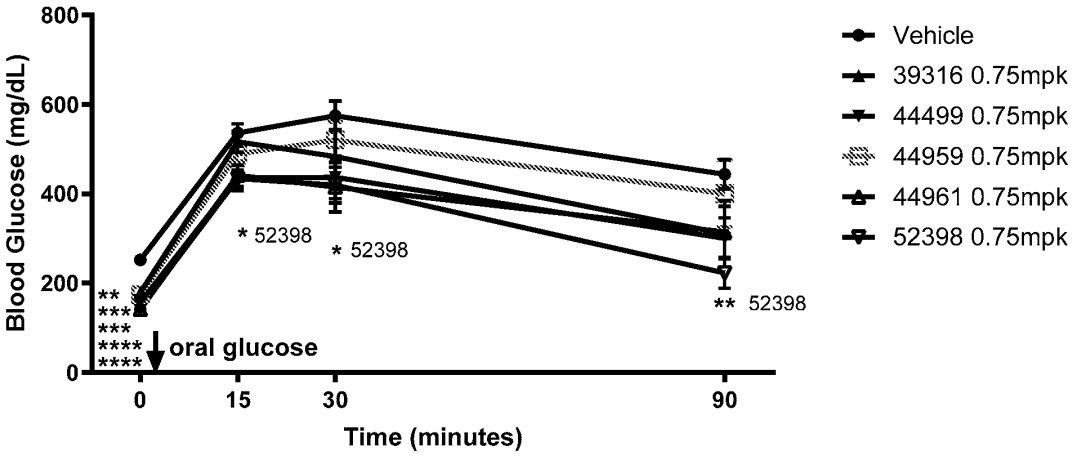

800
600
400
200
0
Blood Glucose (mg/dL)
** 52398
* 52398
* 52398
** 52398
**
***
***
****
****
oral glucose
0
15
30
90
Time (minutes)
Vehicle
39316 0.75mpk
44499 0.75mpk
44959 0.75mpk
44961 0.75mpk
52398 0.75mpk

FIG. 7A

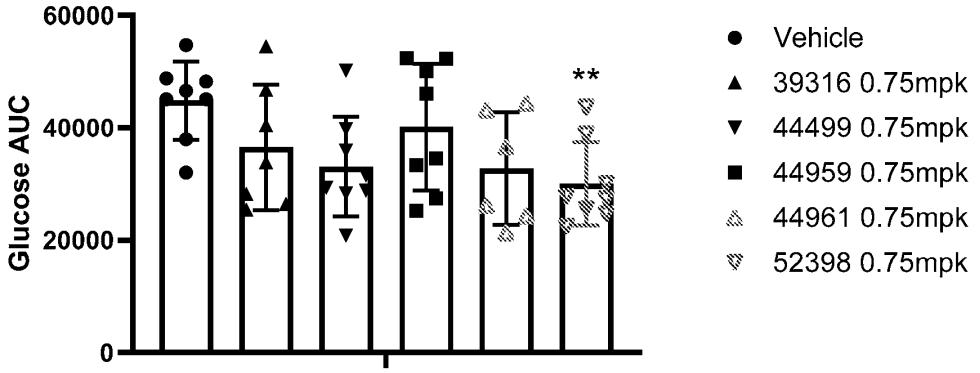

60000
40000
20000
0
Glucose AUC
**
Vehicle
39316 0.75mpk
44499 0.75mpk
44959 0.75mpk
44961 0.75mpk
52398 0.75mpk

FIG. 7B

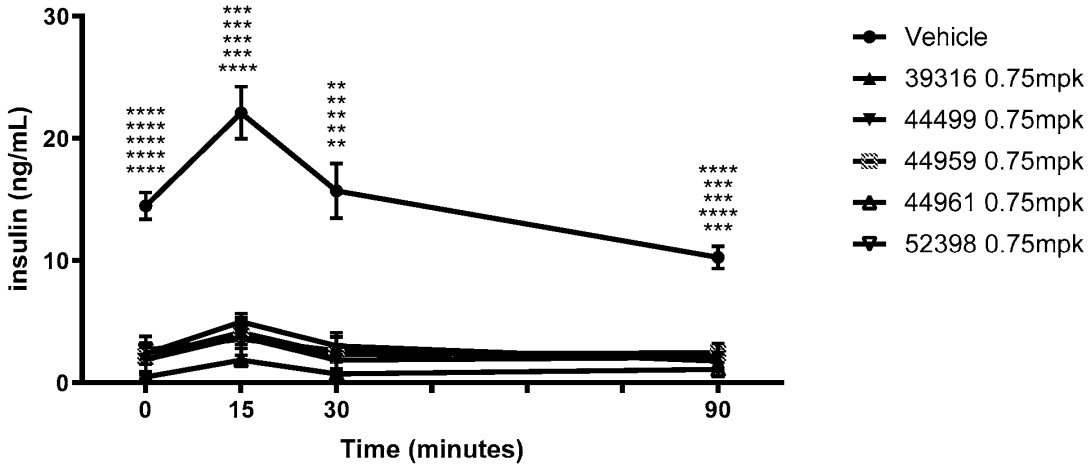

insulin (ng/mL)
30
20
10
0
0
15
30
90
Time (minutes)
Vehicle
39316 0.75mpk
44499 0.75mpk
44959 0.75mpk
44961 0.75mpk
52398 0.75mpk

FIG. 7C

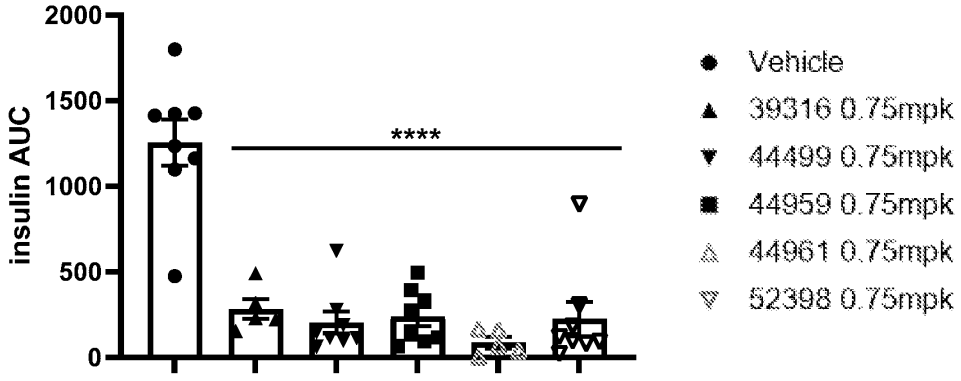

insulin AUC
2000
1500
1000
500
0
****
Vehicle
39316 0.75mpk
44499 0.75mpk
44959 0.75mpk
44961 0.75mpk
52398 0.75mpk

FIG. 7D

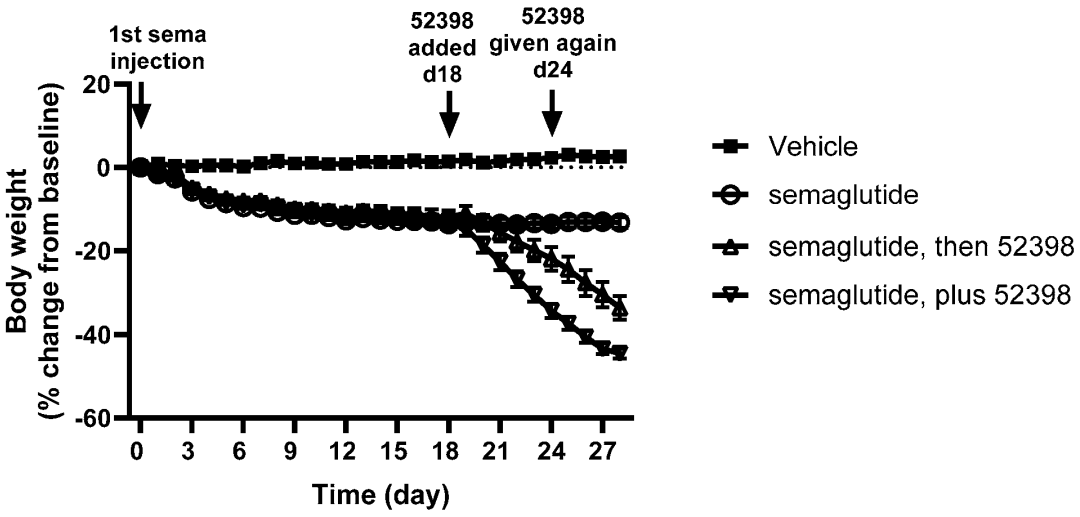
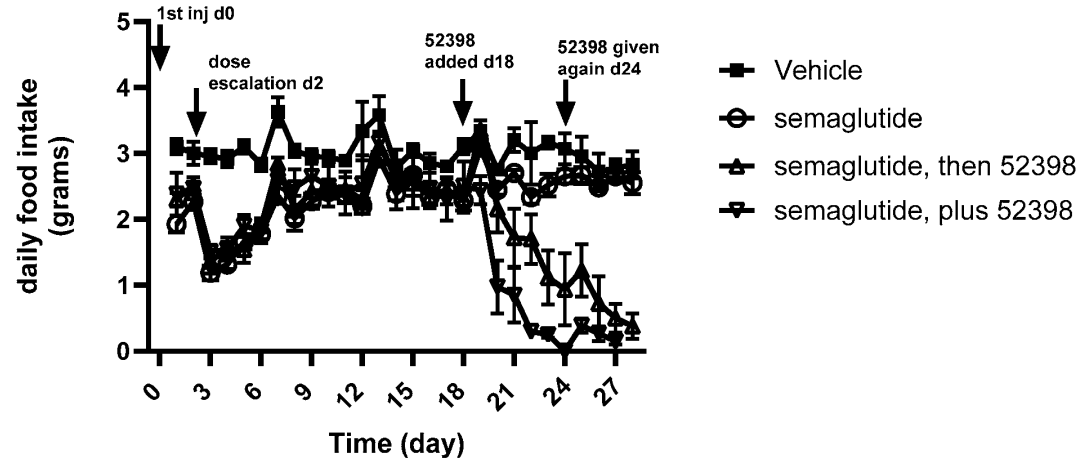



FIG. 8A




NO food intake values for sema plus 52398 for d28; all shredders

FIG. 8B

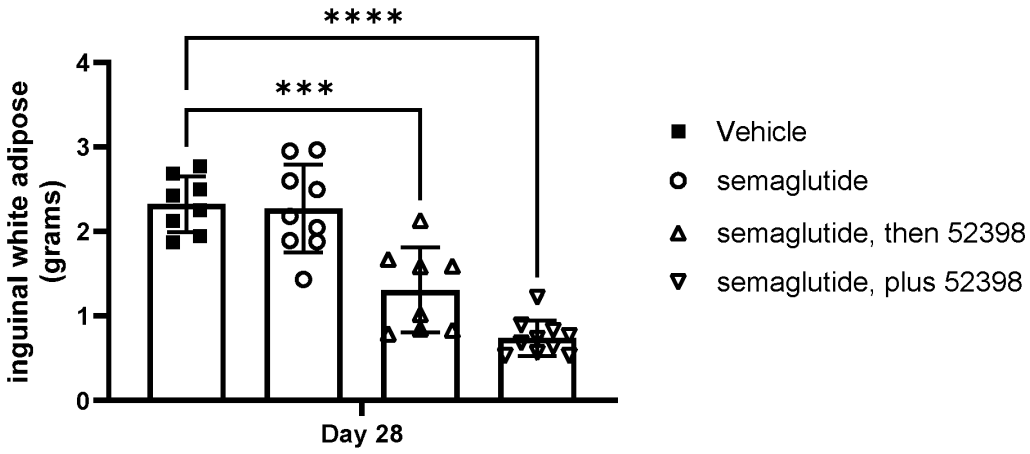

****
***
inguinal white adipose (grams)
4
3
2
1
0
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8C

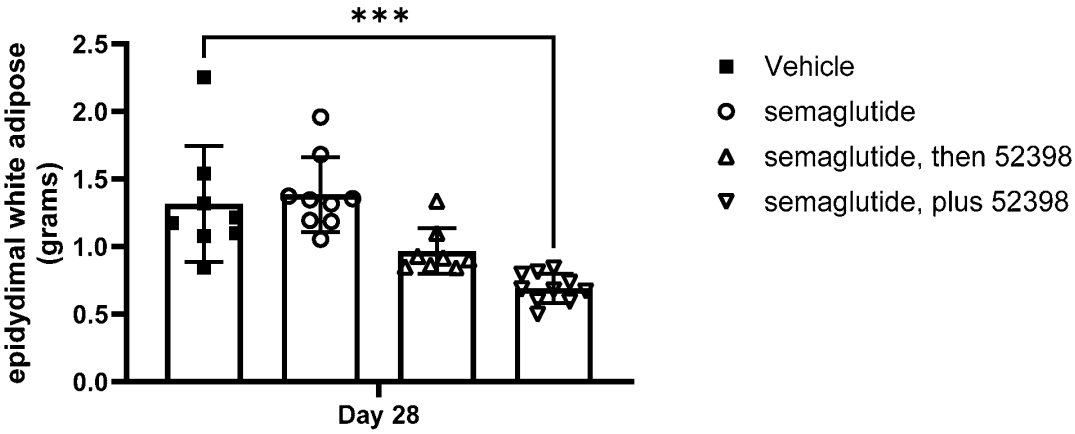

***
epidydimal white adipose (grams)
2.5
2.0
1.5
1.0
0.5
0.0
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8D

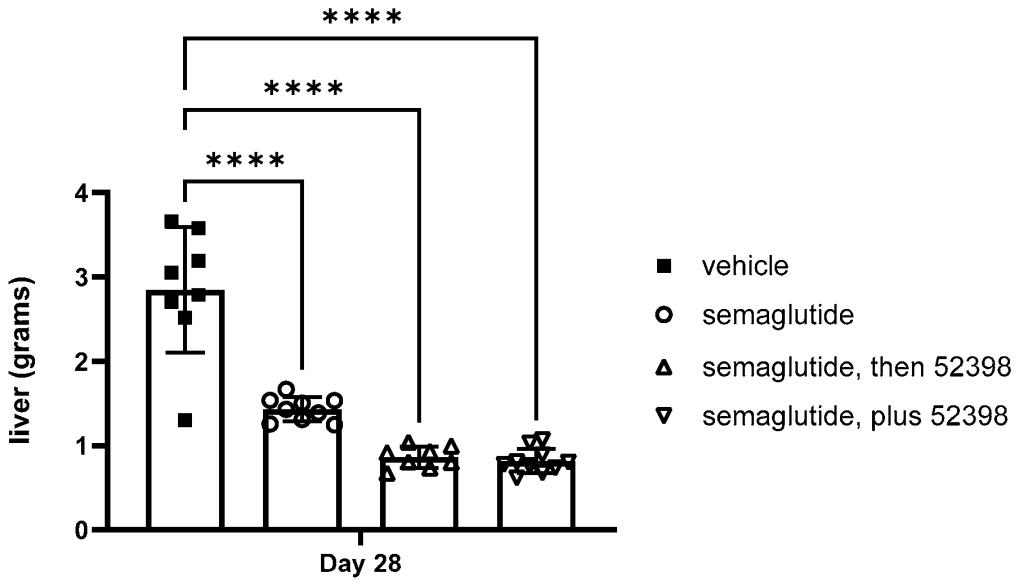

****
****
****
liver (grams)
4
3
2
1
0
Day 28
vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8E

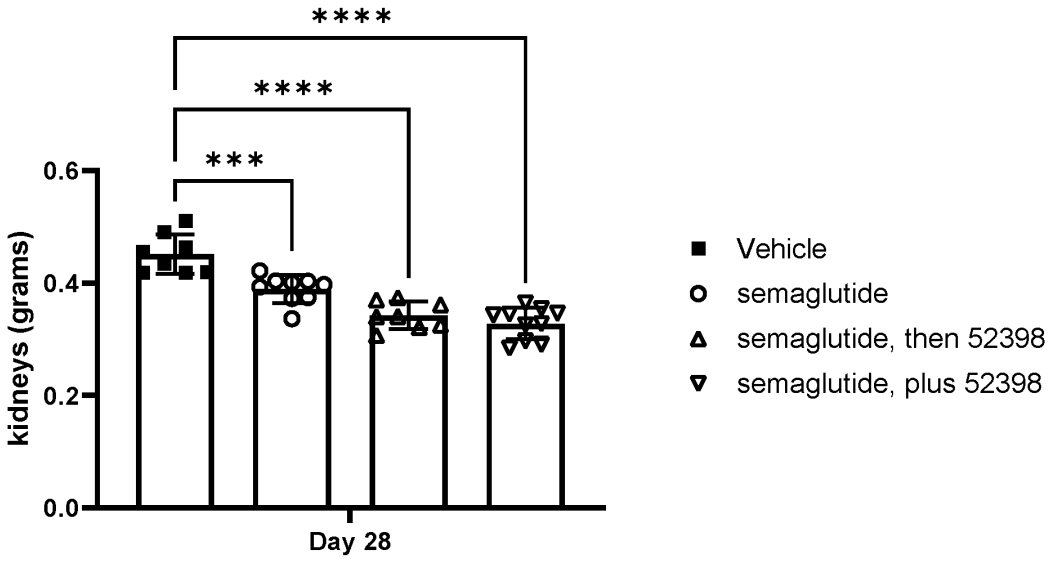

****
****
***
kidneys (grams)
0.6
0.4
0.2
0.0
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8F

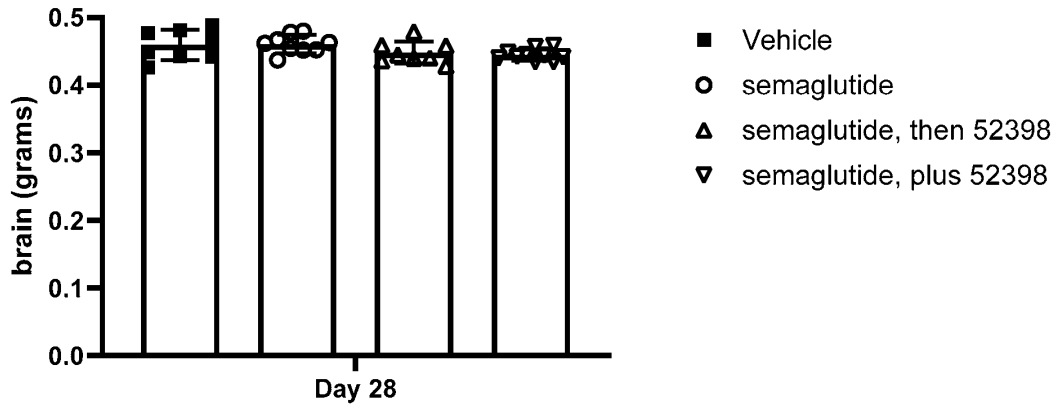

brain (grams)
0.5
0.4
0.3
0.2
0.1
0.0
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8G

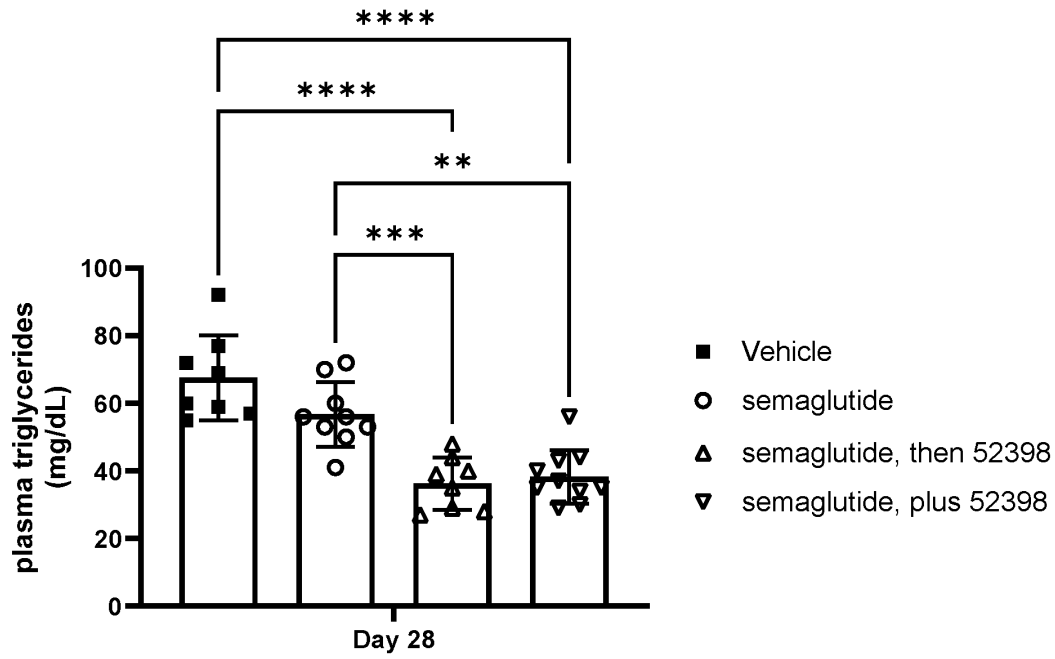

****
****
**
***
plasma triglycerides (mg/dL)
100
80
60
40
20
0
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8H

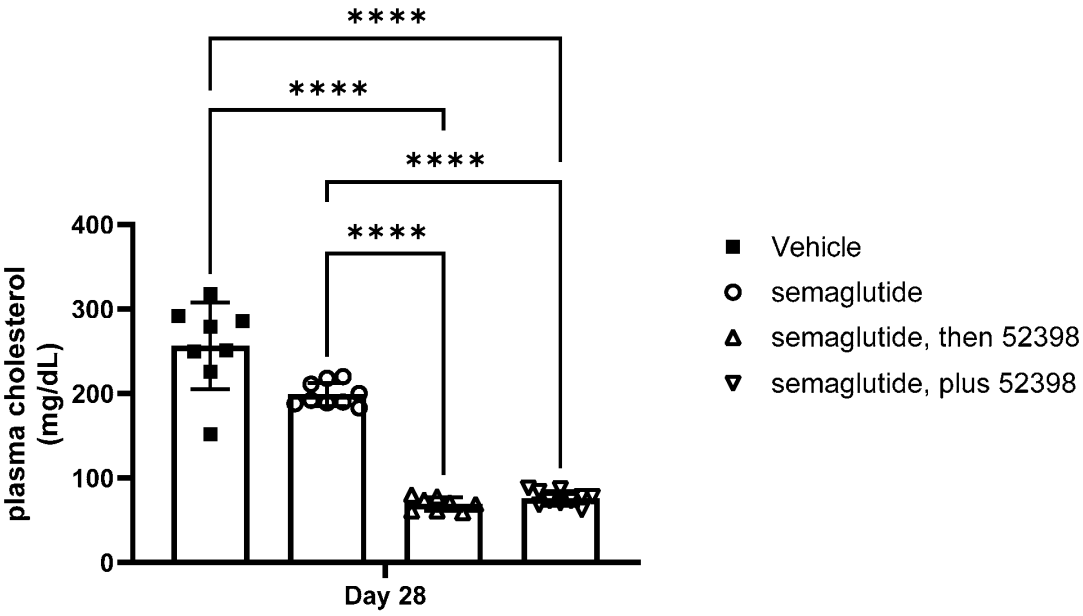

****
****
****
****
400
300
200
100
0
plasma cholesterol (mg/dL)
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8I

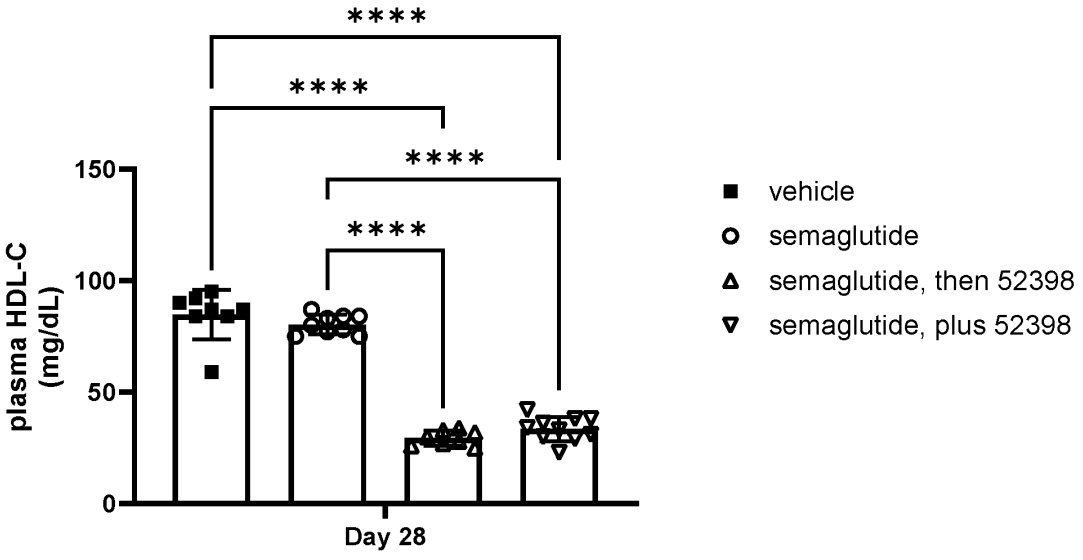

****
****
****
****
150
100
50
0
plasma HDL-C (mg/dL)
Day 28
vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8J

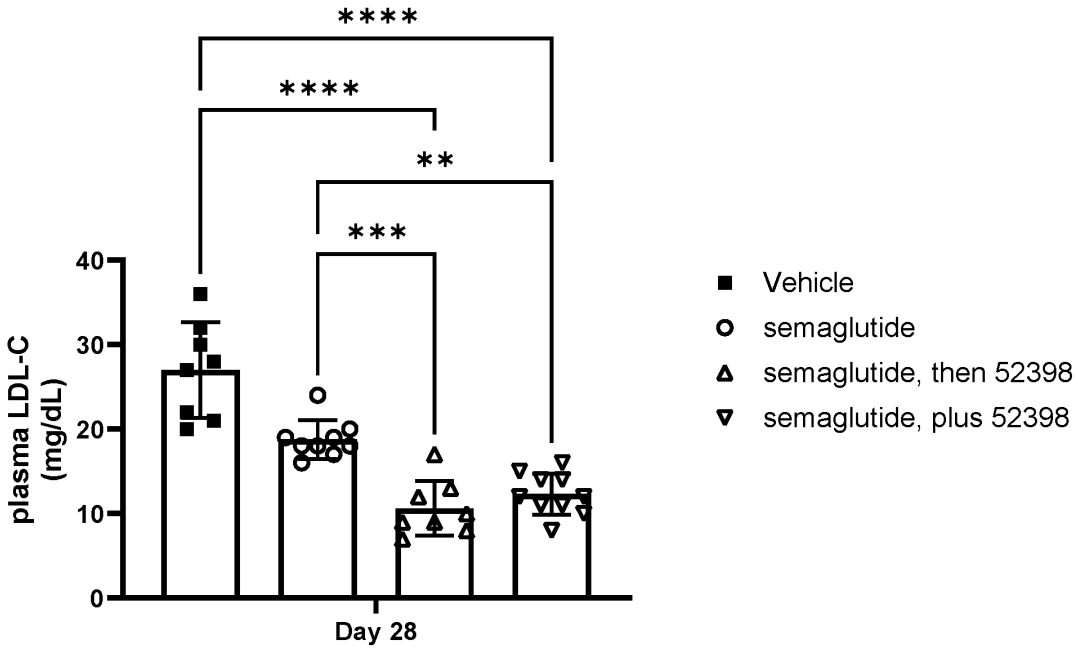

****
****
**
***
40
30
20
10
0
plasma LDL-C (mg/dL)
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8K

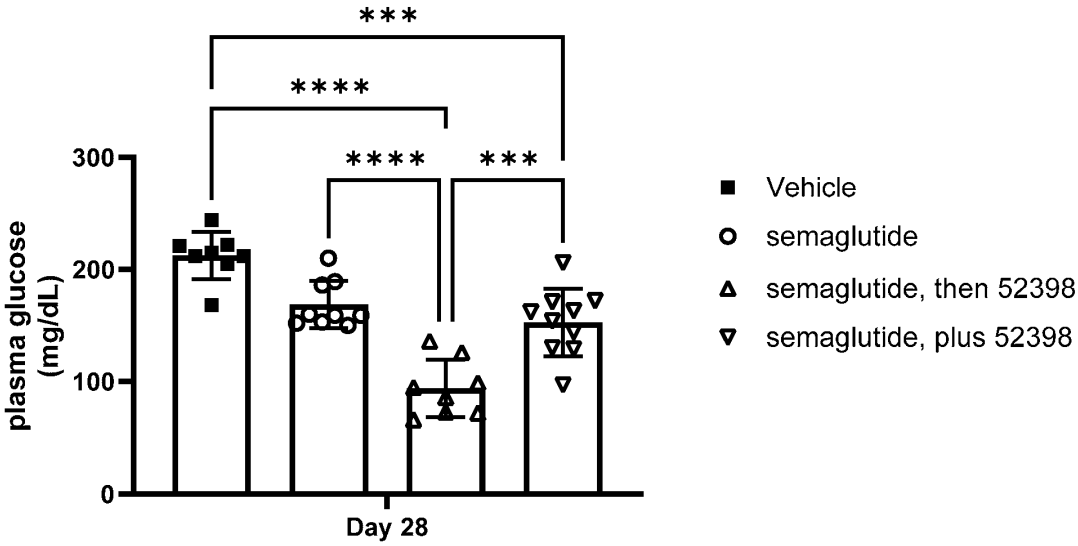

***
****
****
***
300
200
100
0
plasma glucose (mg/dL)
Day 28
Vehicle
semaglutide
semaglutide, then 52398
semaglutide, plus 52398

FIG. 8L

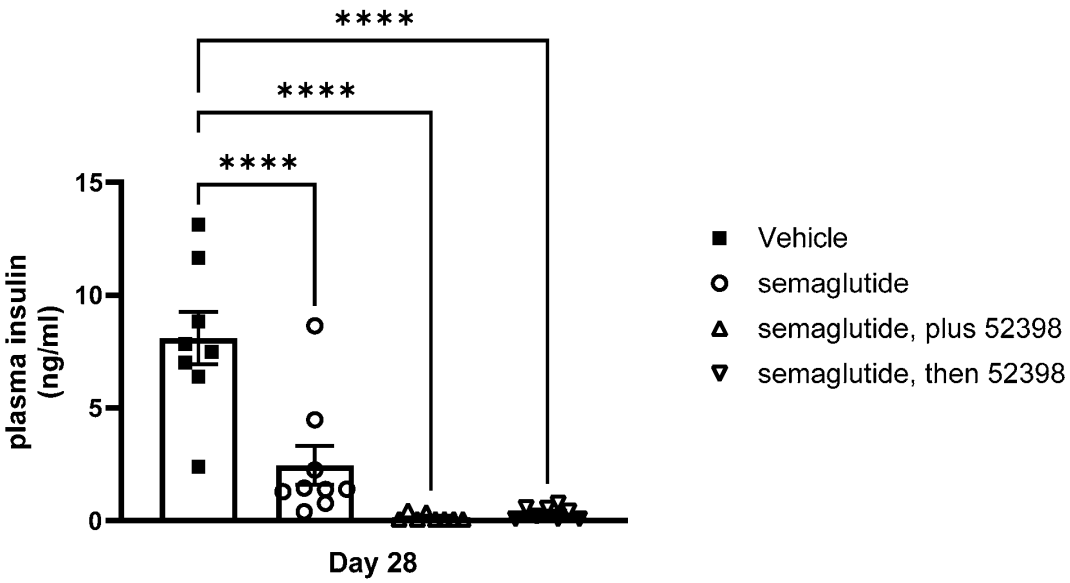

****
****
****
15
10
5
0
plasma insulin (ng/ml)
Day 28
Vehicle
semaglutide
semaglutide, plus 52398
semaglutide, then 52398

FIG. 8M

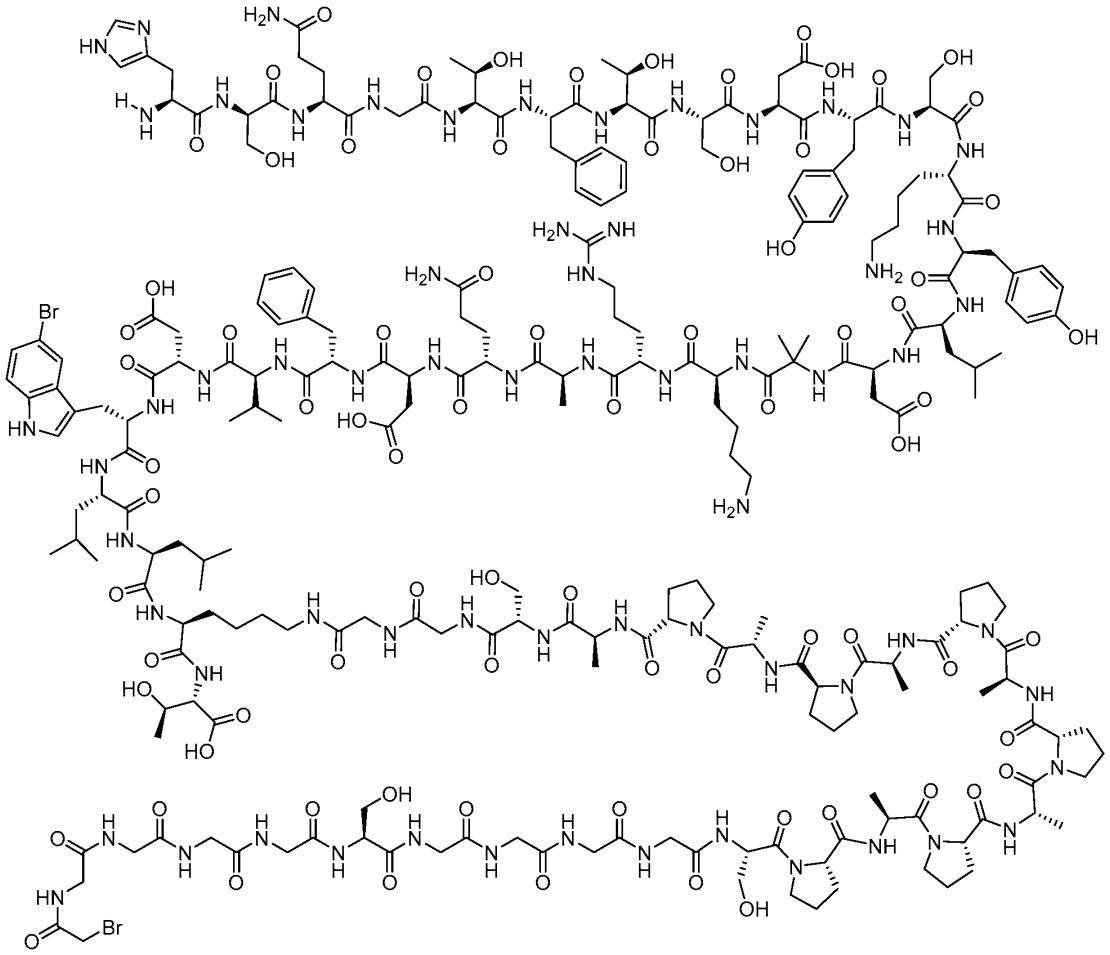


FIG. 9

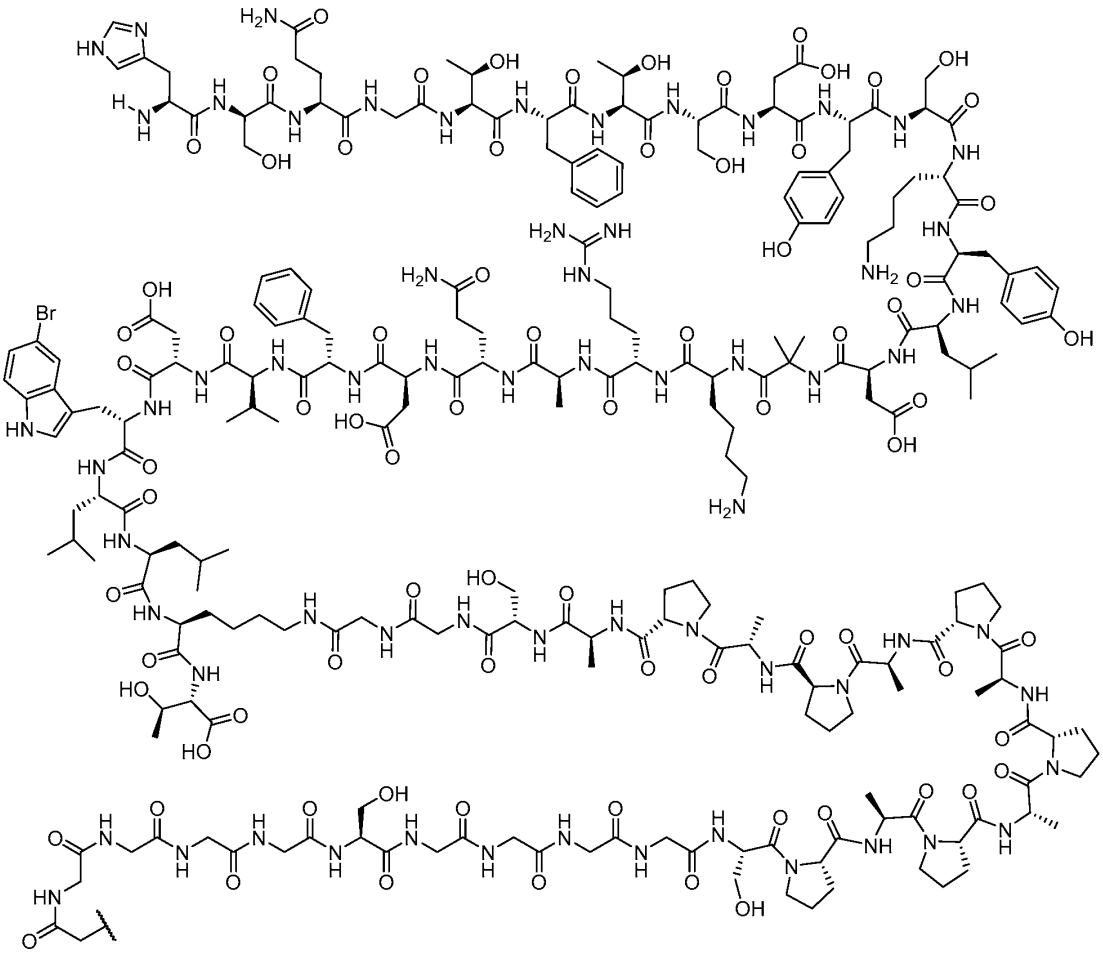


FIG. 10

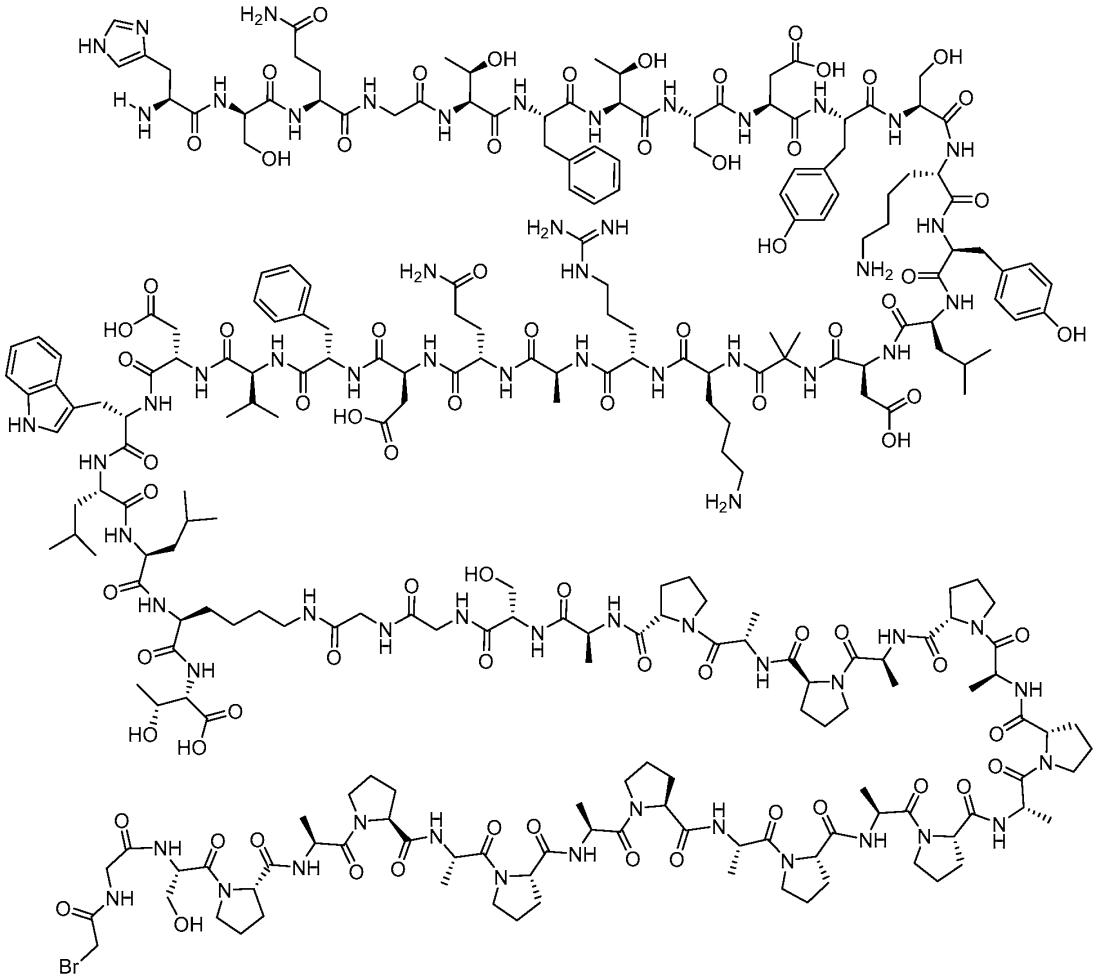


FIG. 11

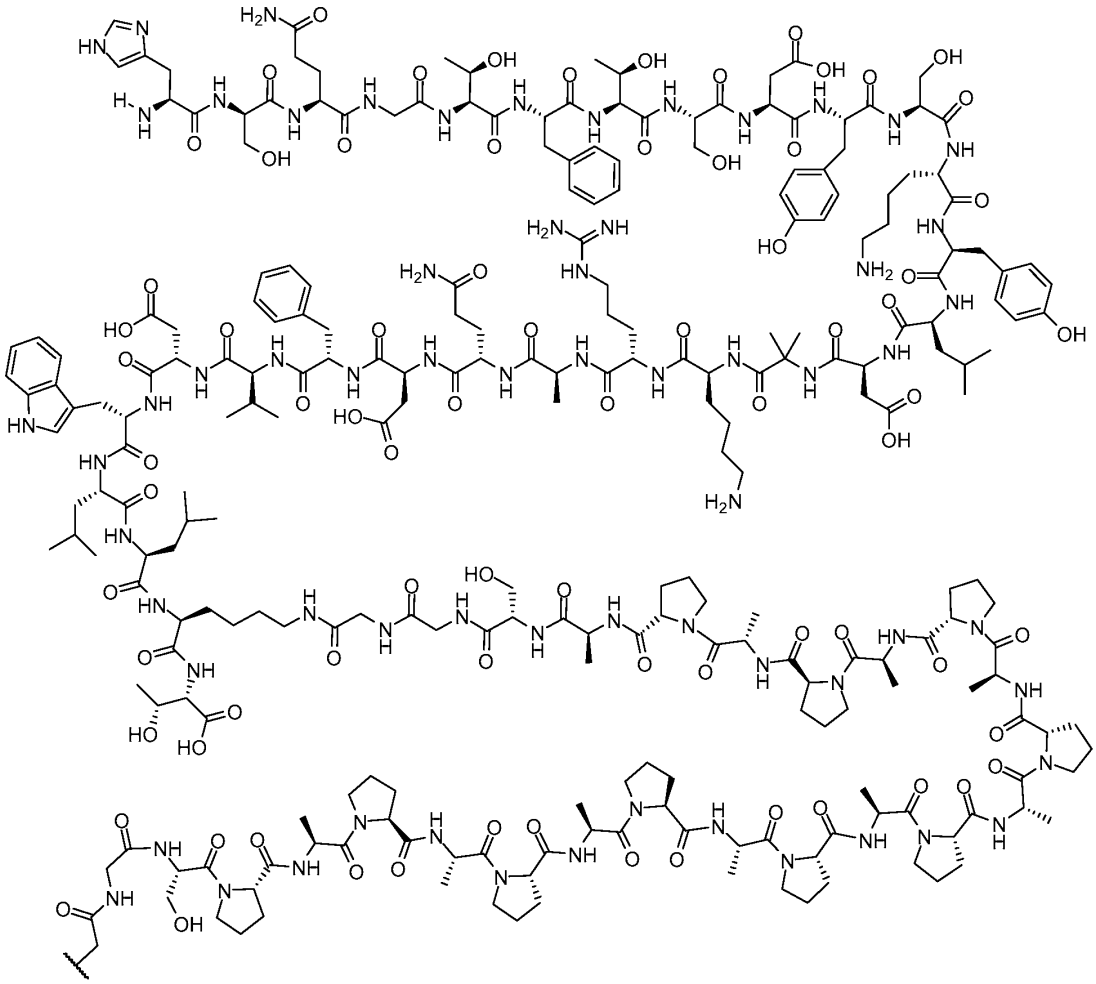


FIG. 12

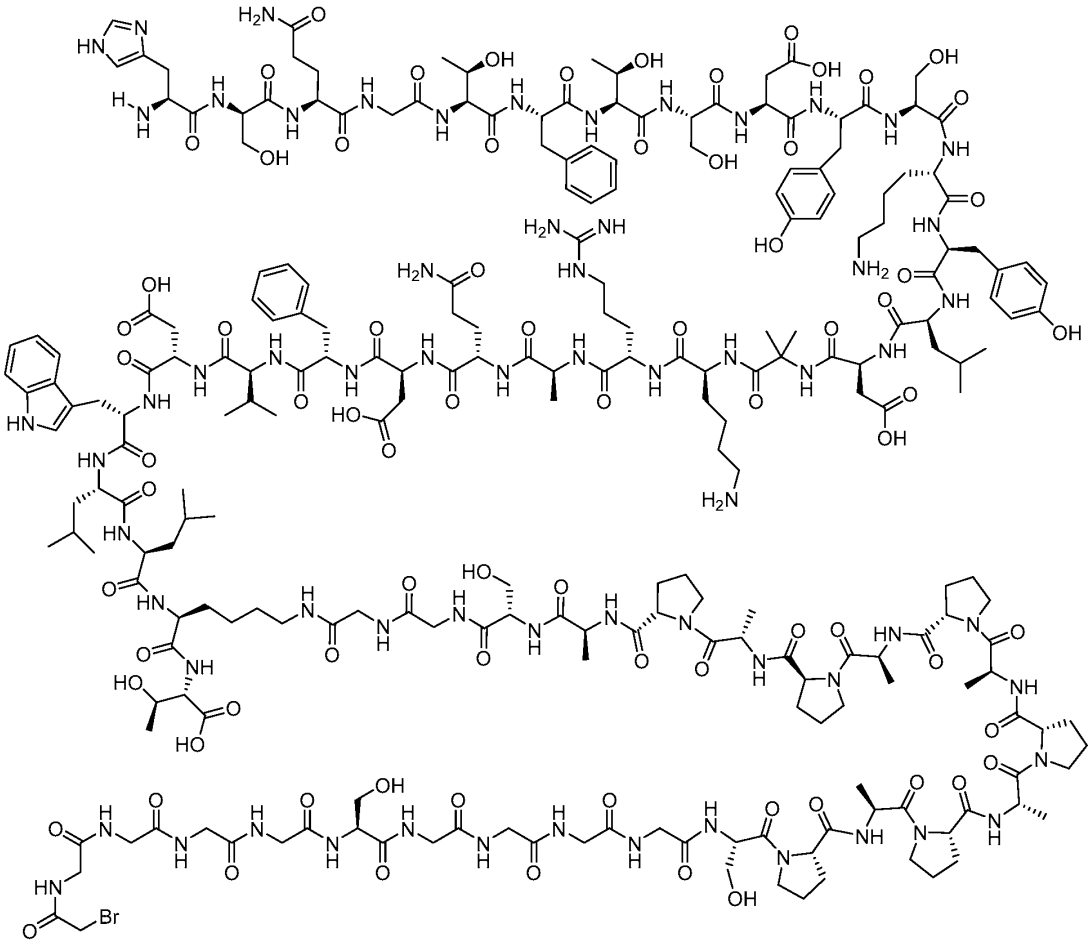


FIG. 13

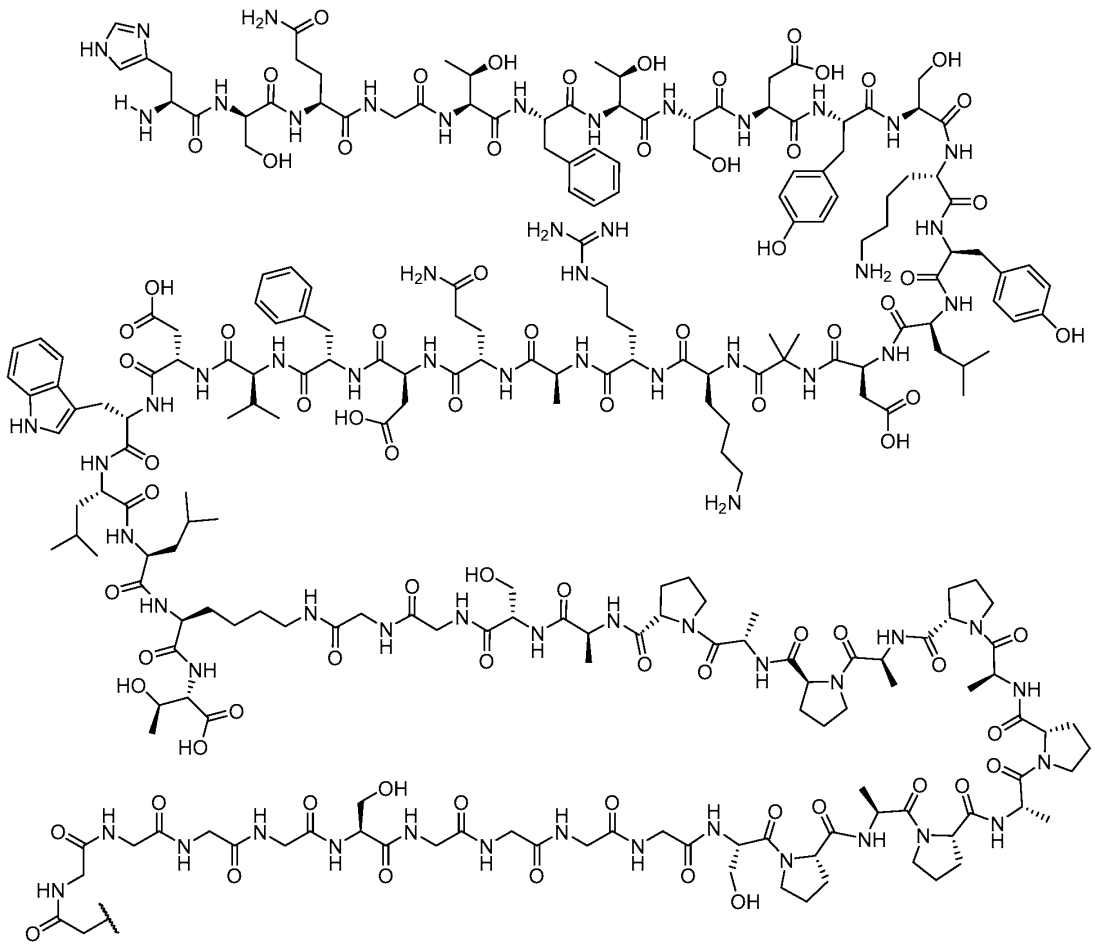


FIG. 14

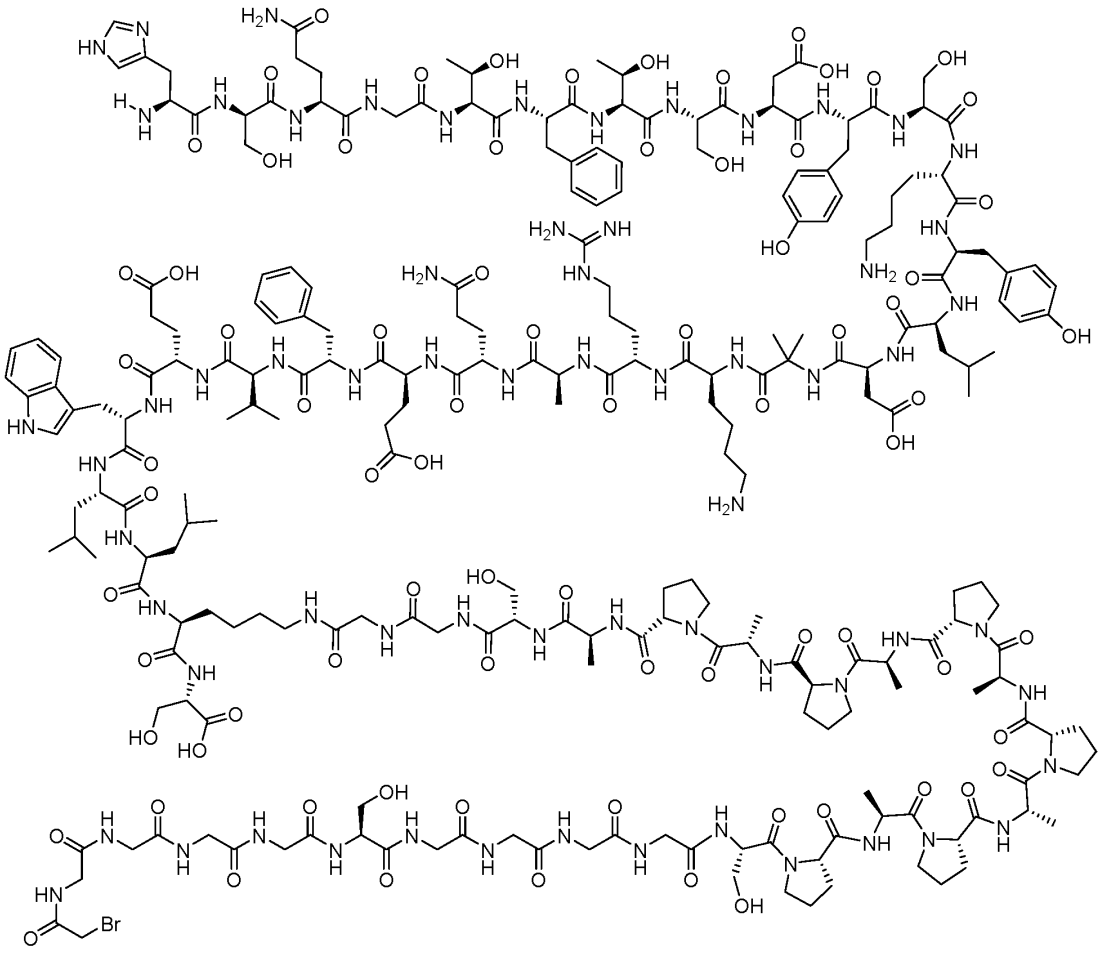


FIG. 15

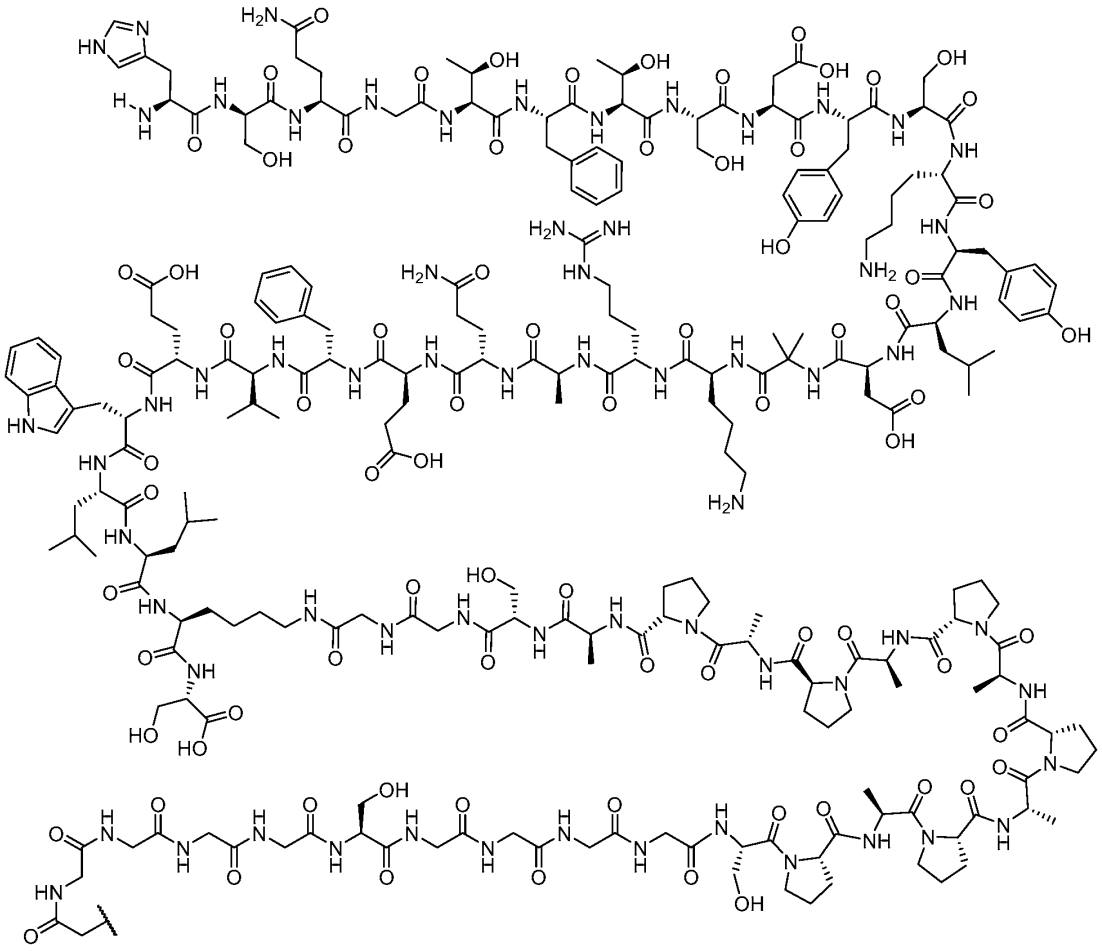


FIG. 16