Figure 1

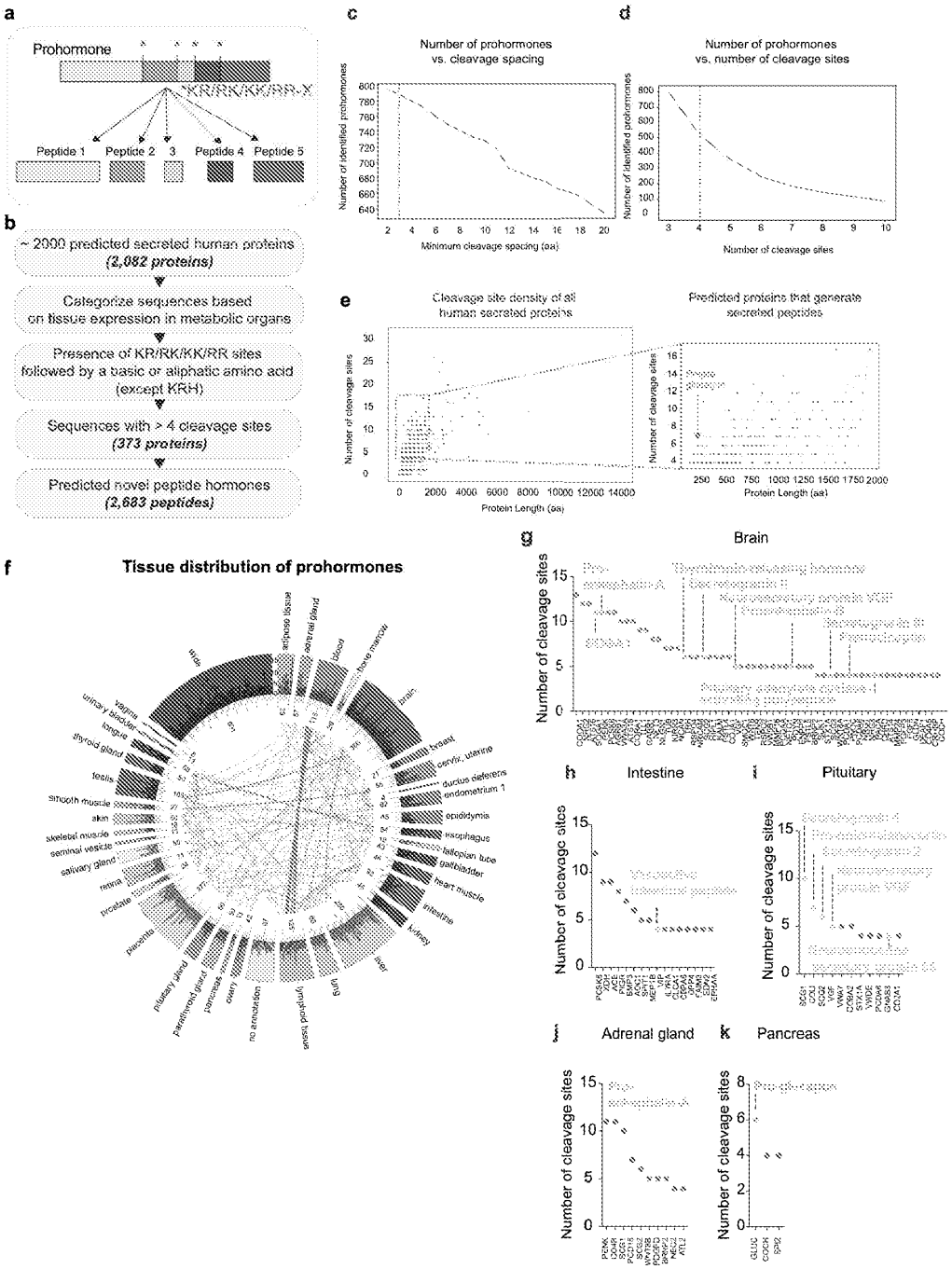

a
Prohormone
KR/RK/KK/RR-X
Peptide 1
Peptide 2
3
Peptide 4
Peptide 5
b
~ 2000 predicted secreted human proteins
(2,082 proteins)
Categorize sequences based on tissue expression in metabolic organs
Presence of KR/RK/KK/RR sites followed by a basic or aliphatic amino acid (except KRH)
Sequences with > 4 cleavage sites
(373 proteins)
Predicted novel peptide hormones
(2,683 peptides)
c
Number of prohormones vs. cleavage spacing
Number of identified prohormones
Minimum cleavage spacing (aa)
d
Number of prohormones vs. number of cleavage sites
Number of identified prohormones
Number of cleavage sites
e
Cleavage site density of all human secreted proteins
Number of cleavage sites
Protein Length (aa)
Predicted proteins that generate secreted peptides
Number of cleavage sites
Protein Length (aa)
f
Tissue distribution of prohormones
adipose tissue
adrenal gland
blood
bone marrow
brain
breast
cervix, uterine
ductus deferens
endometrium 1
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
intestine
kidney
liver
lung
lymphoid tissue
no annotation
ovary
pancreas
parathyroid gland
pituitary gland
placenta
prostate
retina
salivary gland
seminal vesicle
skeletal muscle
skin
smooth muscle
testis
thyroid gland
tongue
urinary bladder
vagina
vein
g
Brain
Number of cleavage sites
h
Intestine
Number of cleavage sites
i
Pituitary
Number of cleavage sites
j
Adrenal gland
Number of cleavage sites
k
Pancreas
Number of cleavage sites

Figure 2

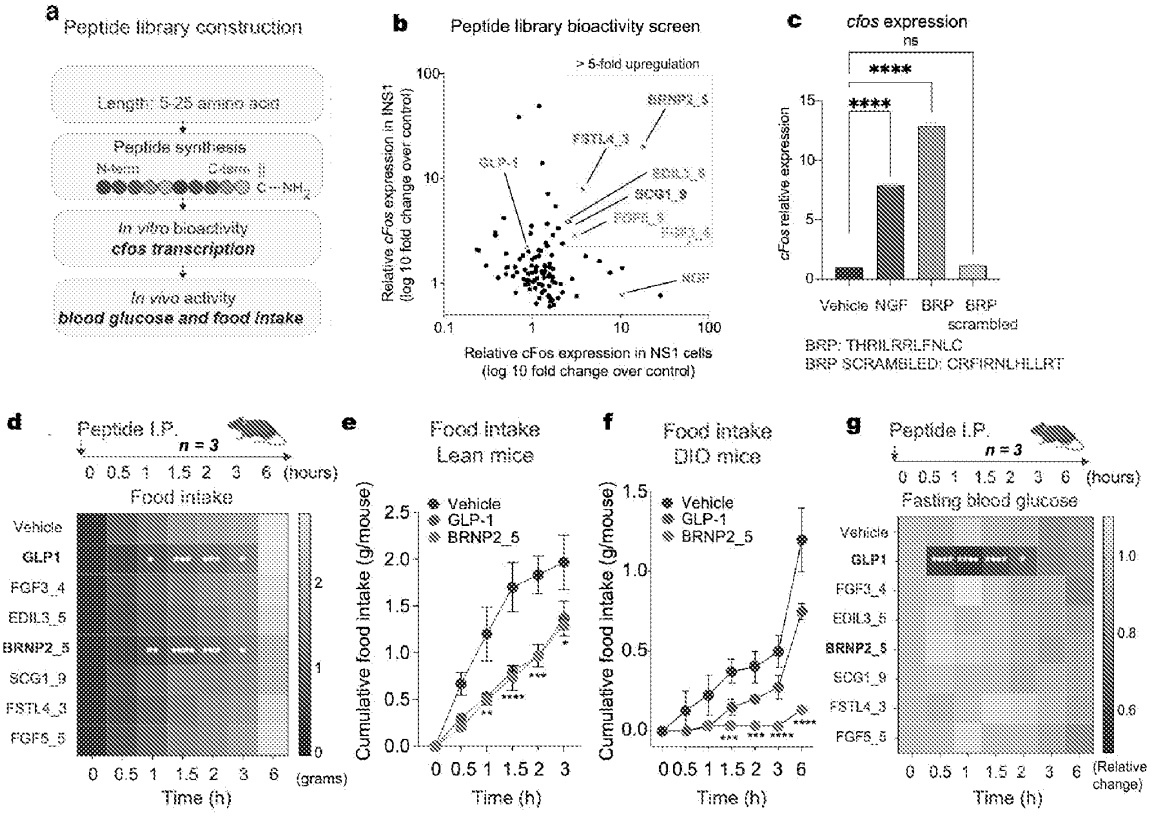

a
Peptide library construction
Length: 5-25 amino acid
Peptide synthesis
N-term
C-term
In vitro bioactivity
cfos transcription
In vivo activity
blood glucose and food intake
b
Peptide library bioactivity screen
> 5-fold upregulation
Relative cFos expression in INS1 (log 10 fold change over control)
Relative cFos expression in NS1 cells (log 10 fold change over control)
BRNP2_5
FSTL4_3
GLP-1
EDIL3_5
SCG1_9
FGF5_5
FGF3_4
NGF
c
cfos expression
ns
****
****
cFos relative expression
Vehicle
NGF
BRP
BRP scrambled
BRP: THRILRRLFNLC
BRP SCRAMBLED: CRFIRNLHLLRT
d
Peptide I.P.
n = 3
0 0.5 1 1.5 2 3 6 (hours)
Food intake
Vehicle
GLP1
FGF3_4
EDIL3_5
BRNP2_5
SCG1_9
FSTL4_3
FGF5_5
2
1
0
(grams)
0 0.5 1 1.5 2 3 6
Time (h)
e
Food intake
Lean mice
Vehicle
GLP-1
BRNP2_5
Cumulative food intake (g/mouse)
2.5
2.0
1.5
1.0
0.5
0.0
0 0.5 1 1.5 2 3
Time (h)
f
Food intake
DIO mice
Vehicle
GLP-1
BRNP2_5
Cumulative food intake (g/mouse)
1.5
1.0
0.5
0.0
0 0.5 1 1.5 2 3 6
Time (h)
g
Peptide I.P.
n = 3
0 0.5 1 1.5 2 3 6 (hours)
Fasting blood glucose
Vehicle
GLP1
FGF3_4
EDIL3_5
BRNP2_5
SCG1_9
FSTL4_3
FGF5_5
1.0
0.8
0.6
(Relative change)
0 0.5 1 1.5 2 3 6
Time (h)

Figure 3

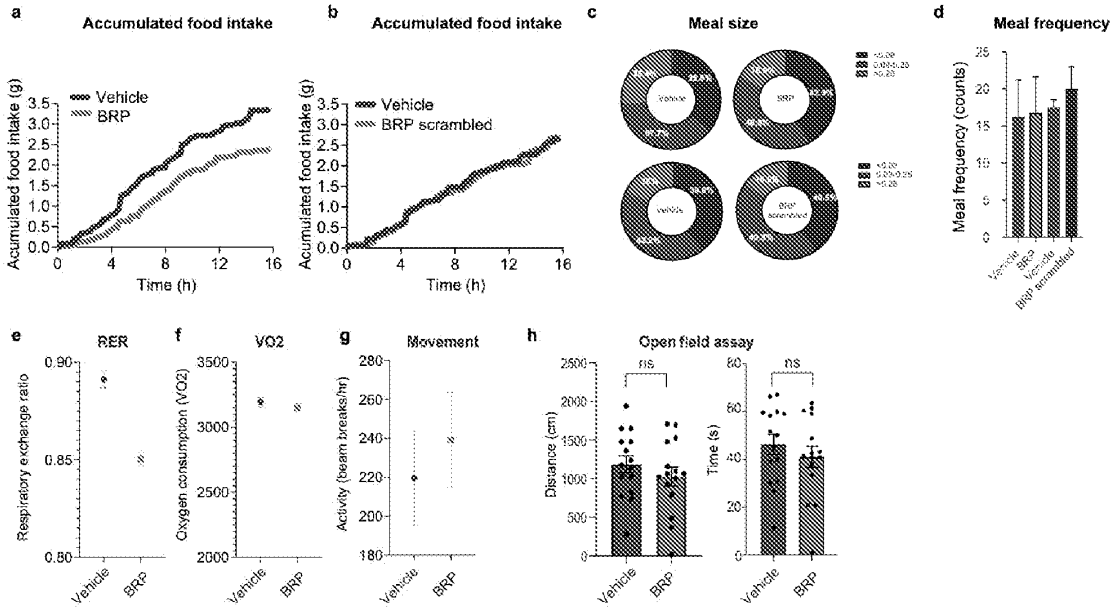

a
Accumulated food intake
Accumulated food intake (g)
Vehicle
BRP
Time (h)
b
Accumulated food intake
Accumulated food intake (g)
Vehicle
BRP scrambled
Time (h)
c
Meal size
d
Meal frequency
Meal frequency (counts)
e
RER
Respiratory exchange ratio
f
VO2
Oxygen consumption (VO2)
g
Movement
Activity (beam breaks/hr)
h
Open field assay
Distance (cm)
Time (s)
ns
Vehicle
BRP

Figure 4

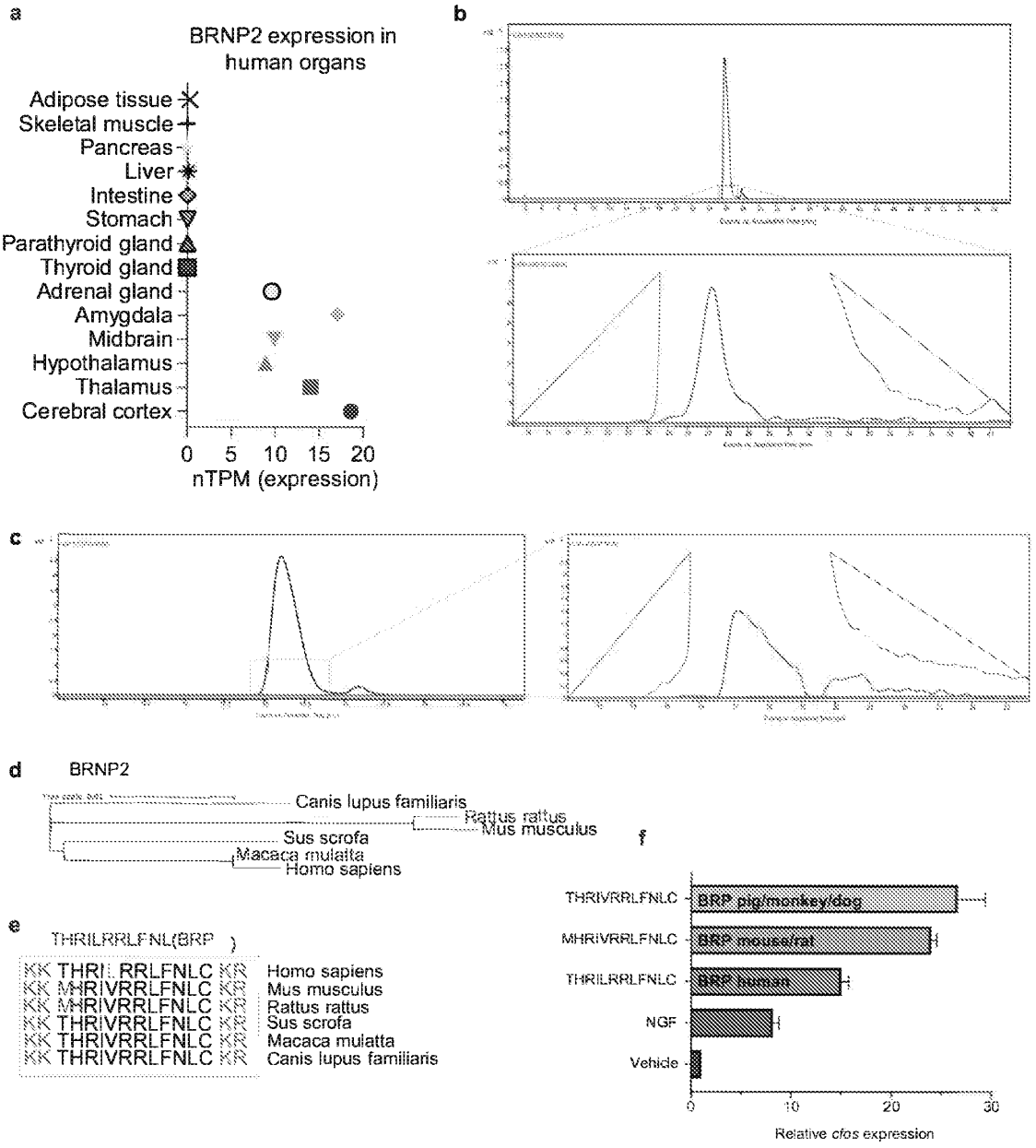

a
BRNP2 expression in human organs
Adipose tissue
Skeletal muscle
Pancreas
Liver
Intestine
Stomach
Parathyroid gland
Thyroid gland
Adrenal gland
Amygdala
Midbrain
Hypothalamus
Thalamus
Cerebral cortex
0 5 10 15 20
nTPM (expression)
b
c
d
BRNP2
Canis lupus familiaris
Rattus rattus
Mus musculus
Sus scrofa
Macaca mulatta
Homo sapiens
e
THRILRRLFNL(BRP)
KK THRI RRLFNLC KR Homo sapiens
KK MHRIVRRLFNLC KR Mus musculus
KK MHRIVRRLFNLC KR Rattus rattus
KK THRIVRRLFNLC KR Sus scrofa
KK THRIVRRLFNLC KR Macaca mulatta
KK THRIVRRLFNLC KR Canis lupus familiaris
f
THRIVRRLFNLC
MHRIVRRLFNLC
THRILRRLFNLC
NGF
Vehicle
BRP pig/monkey/dog
BRP mouse/rat
BRP human
0 10 20 30
Relative cfos expression

Figure 5

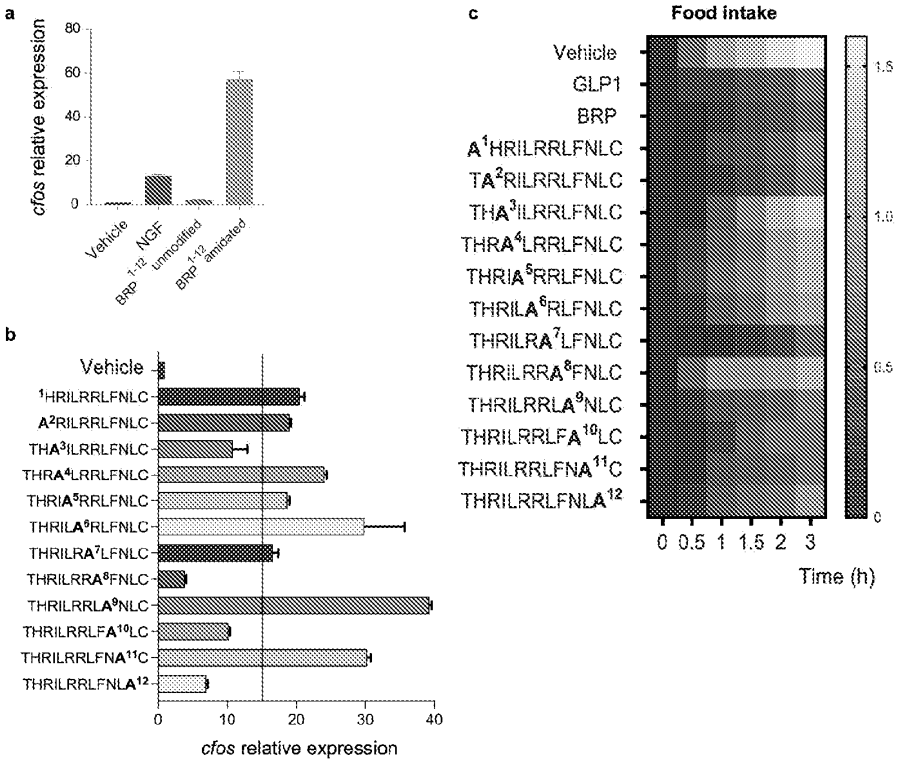

a
cfos relative expression
80
60
40
20
0
Vehicle
NGF
BRP 1-12 unmodified
BRP 1-12 amicated
b
Vehicle
1HRILRRLFNLC
A2RILRRLFNLC
THA3ILRRLFNLC
THRA4LRRLFNLC
THRIA5RRLFNLC
THRILA6RLFNLC
THRILRA7LFNLC
THRILRRA8FNLC
THRILRRLA9NLC
THRILRRLFA10LC
THRILRRLFNA11C
THRILRRLFNLA12
0
10
20
30
40
cfos relative expression
c
Food intake
Vehicle
GLP1
BRP
A1HRILRRLFNLC
TA2RILRRLFNLC
THA3ILRRLFNLC
THRA4LRRLFNLC
THRIA5RRLFNLC
THRILA6RLFNLC
THRILRA7LFNLC
THRILRRA8FNLC
THRILRRLA9NLC
THRILRRLFA10LC
THRILRRLFNA11C
THRILRRLFNLA12
1.5
1.0
0.5
0
0 0.5 1 1.5 2 3
Time (h)

Figure 6

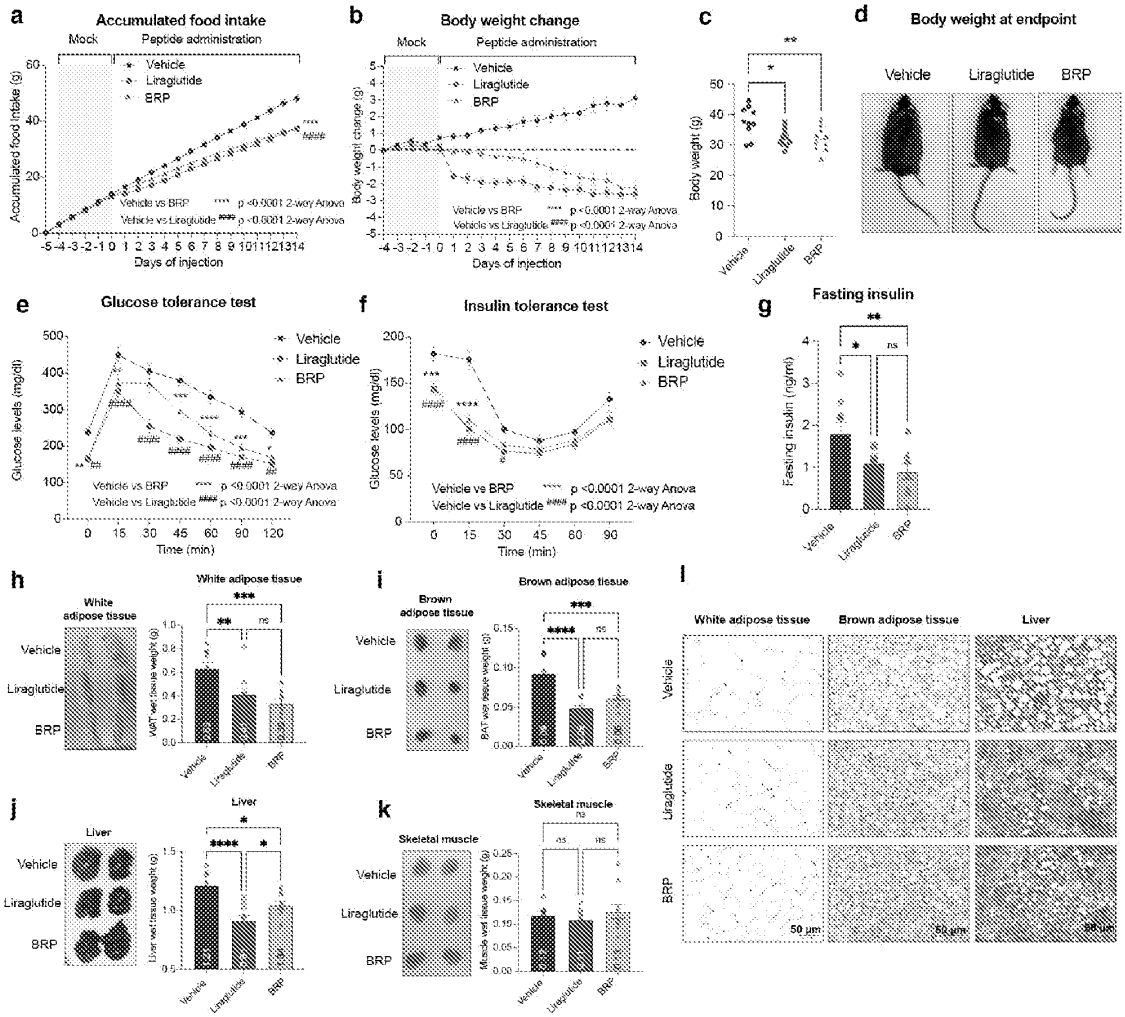

a
Accumulated food intake
Mock
Peptide administration
Vehicle
Liraglutide
BRP
Accumulated food intake (g)
Days of injection
Vehicle vs BRP **** p <0.0001 2-way Anova
Vehicle vs Liraglutide **** p <0.0001 2-way Anova
b
Body weight change
Body weight change (g)
c
Body weight (g)
d
Body weight at endpoint
e
Glucose tolerance test
Glucose levels (mg/dl)
Time (min)
f
Insulin tolerance test
g
Fasting insulin
Fasting insulin (ng/ml)
h
White adipose tissue
i
Brown adipose tissue
j
Liver
k
Skeletal muscle
l
White adipose tissue
Brown adipose tissue
Liver
50 μm

Figure 7

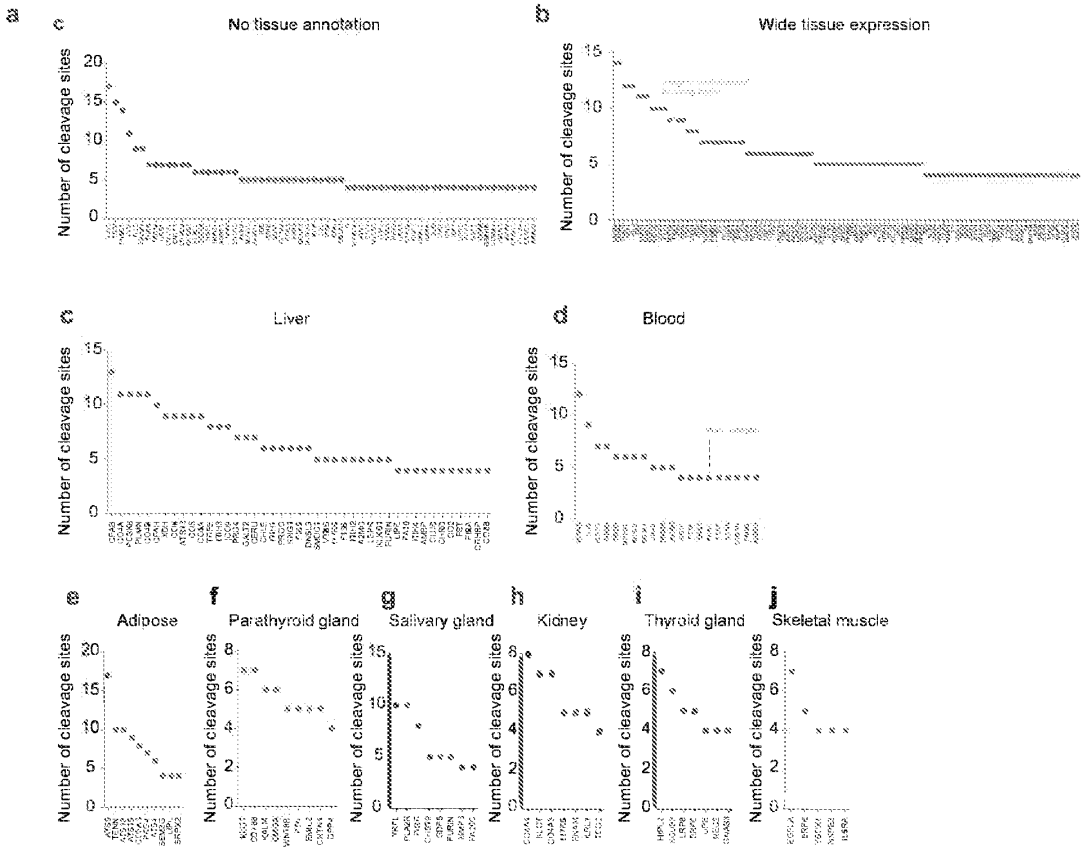

a
c
No tissue annotation
b
Wide tissue expression
Number of cleavage sites
c
Liver
d
Blood
e
Adipose
f
Parathyroid gland
g
Salivary gland
h
Kidney
i
Thyroid gland
j
Skeletal muscle

Figure 8

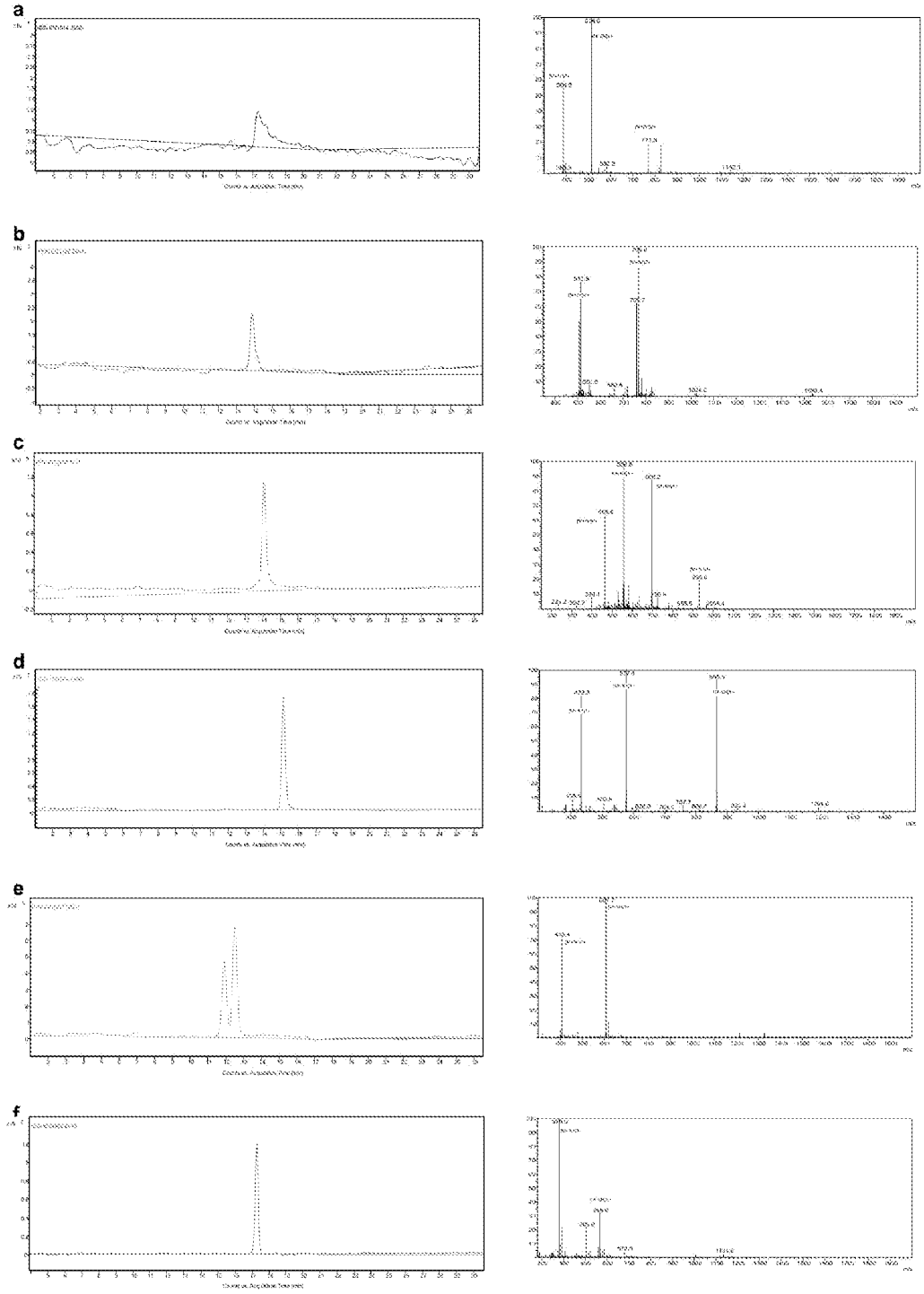
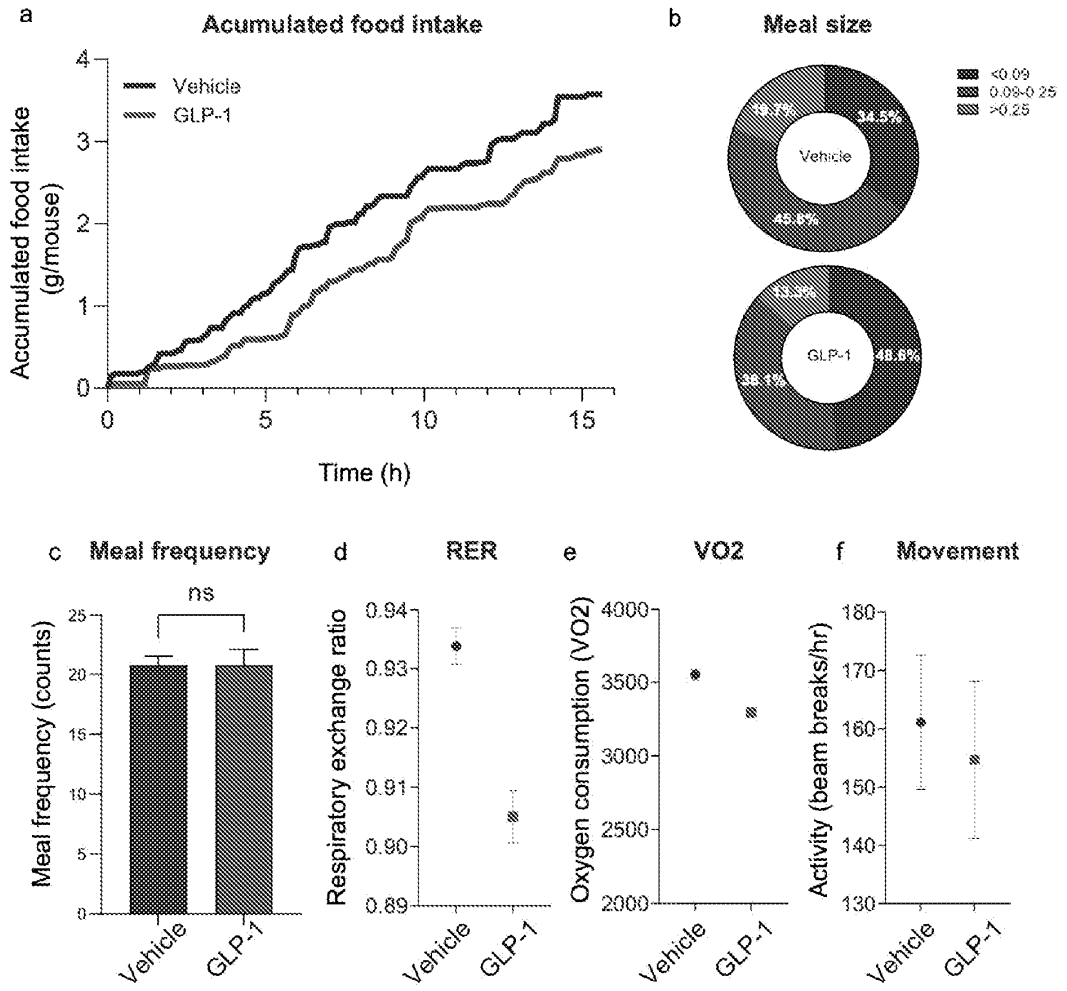


Figure 9


a
Acumulated food intake
Accumulated food intake (g/mouse)
Vehicle
GLP-1
Time (h)
b
Meal size
<0.09
0.09-0.25
>0.25
Vehicle
GLP-1
c
Meal frequency
ns
Meal frequency (counts)
d
RER
Respiratory exchange ratio
e
VO2
Oxygen consumption (VO2)
f
Movement
Activity (beam breaks/hr)