Supplemental Information

Fig. S1. Comparison of C-terminal amidated and carboxy CAP-TAC1 with targeted LCMSMS method. The 463.2 m/z b4 extracted ion chromatograms for mouse plasma, the carboxy synthetic standard, and amidated synthetic standard (m/z = 724.3 parent ion with 1.3 m/z isolation window).

Fig. S2. Determination of capped peptide sequence repeats in whole mouse proteome. Distribution of the number of precursor proteins (1, 2, or 3) the detected mouse capped peptide sequences (detected sequence + glycine-dibasic motif, N=64) are found in by a BlastP search.

Fig. S3. Additional measurements from metabolic chambers of mice treated with CAP-GDF15. (A,B) VO2 (A) and VCO2 (B) of 12-16-week diet-induced obese male mice following a single treatment of CAP-GDF15 (50 mg/kg, intraperitoneal) or vehicle control. (C,D) VO2 (C) and VCO2 (D) of 12-16-week diet-induced obese male mice following a single treatment of scrambled CAP-GDF15 (50 mg/kg, intraperitoneal, scrambled sequence: pQGLEALRARLRV-NH2) or vehicle control. Data are shown as means ± SEM. For (A-B), N=12/group; for (C-D), N=8/group. For (A-D), injection occurred at time T=0 (5:00pm) and data was collected for the subsequent 16 hrs.

Fig. S4. Prediction, detection, and composition analysis of human capped peptides. (A) Schematic of numbers of predicted human capped peptides. (B) Quantification of detectable human capped peptide concentrations in human plasma (N=3). (C) Comparison of frequency of each amino acid between human capped peptides and known peptide hormones. For (B), data are shown as means ± SEM, N=3.

Fig. S5. Tissue distribution of mRNAs for home genes corresponding to human capped peptide. H-clustered heat map of mRNA expression for capped peptide preproprecursor home genes across human tissues and cell types, using GTEx as the reference database.

Table S1. List of classically secreted mouse proteins from Uniprot.

Table S2. Predicted and detected mouse capped peptides.

Table S3. List of known peptide hormones and neuropeptides from Uniprot.

Table S4. List of classically secreted human proteins from Uniprot.

Table S5. Predicted and detected human capped peptides.

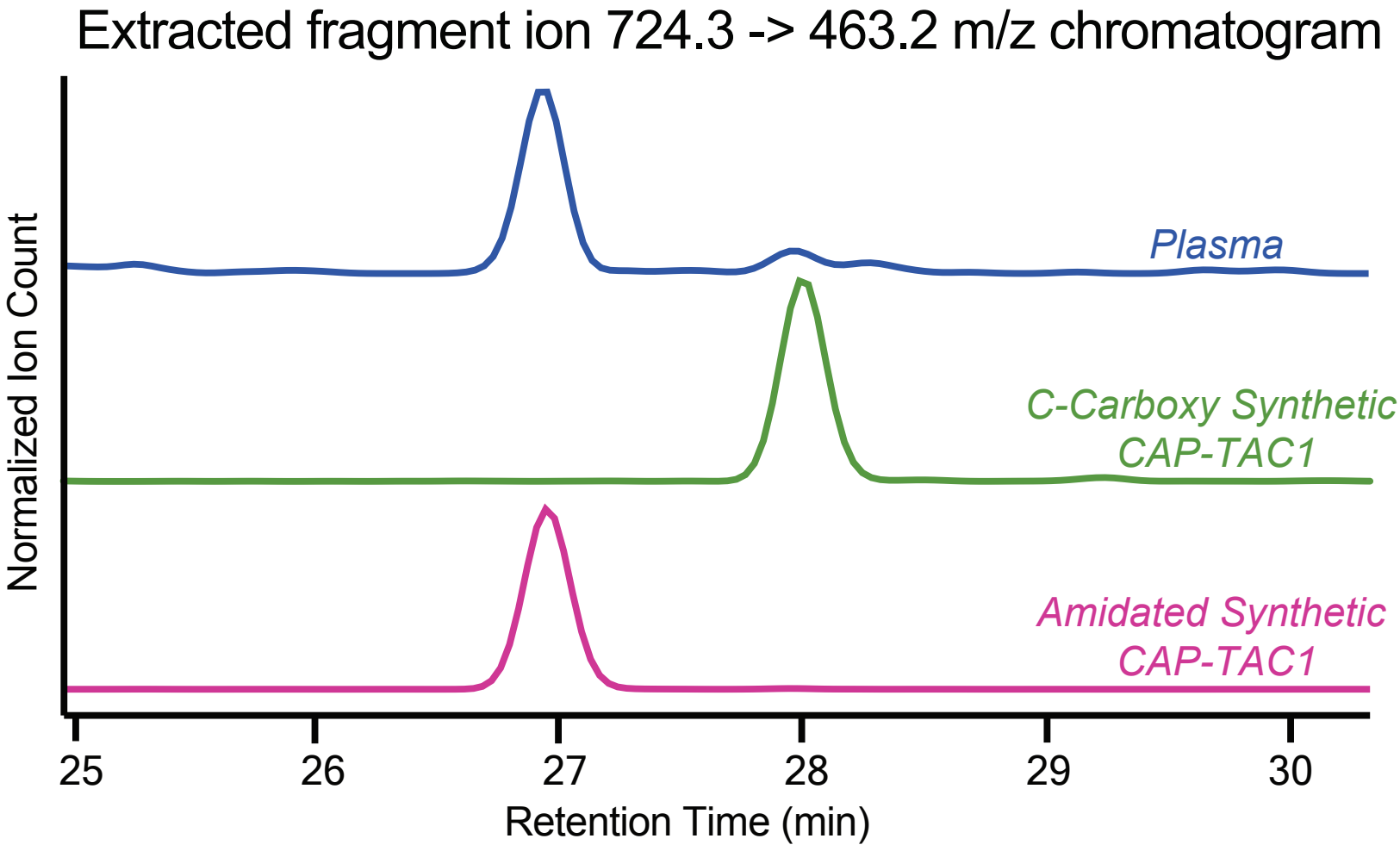
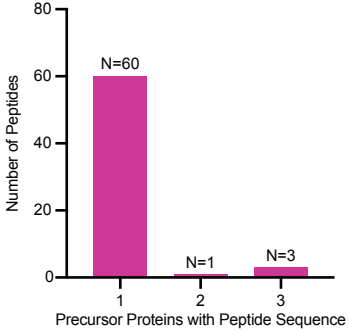

Extracted fragment ion 724.3 -> 463.2 m/z chromatogram
Normalized Ion Count
Plasma
C-Carboxy Synthetic
CAP-TAC1
Amidated Synthetic
CAP-TAC1
25
26
27
28
29
30
Retention Time (min)

Fig. S1

A



B Alignment of capped peptides that appear >1 time in the proteome

CAP-DEFA-RS7

```
...QDAALGWGRR... DEFA-RS7
...QDAALGWGRR... DEFA-RS2
```

CAP-EPHB6

```
...QPRGRR... EPHB6
...QPRGRR... CILP2
...QPRGRR... KIFC2
```

CAP-FGF18

```
...QVLGRR... FGF18
...QVLGRR... TP73
...QVLGRR... TP63
```

CAP-WNT3A

```
...QFRGRR... WNT3A
...QFRGRR... WNT3
...QFRGRR... MGAT1
```

Fig. S2

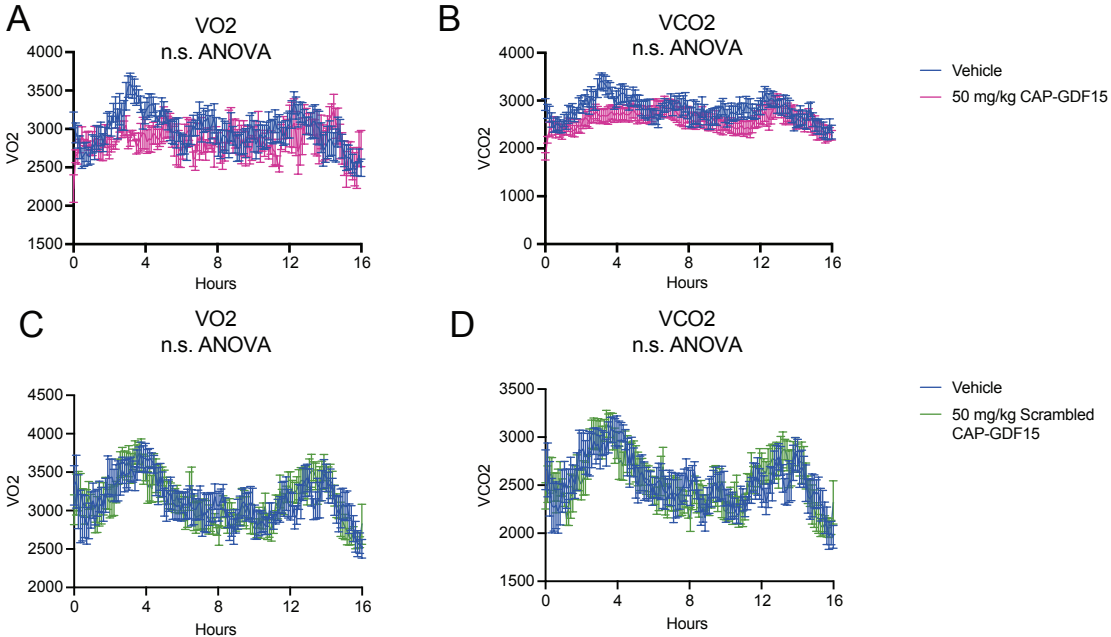

A
VO2
n.s. ANOVA
VO2
Hours
B
VCO2
n.s. ANOVA
VCO2
Hours
Vehicle
50 mg/kg CAP-GDF15
C
VO2
n.s. ANOVA
VO2
Hours
D
VCO2
n.s. ANOVA
VCO2
Hours
Vehicle
50 mg/kg Scrambled CAP-GDF15

Fig. S3

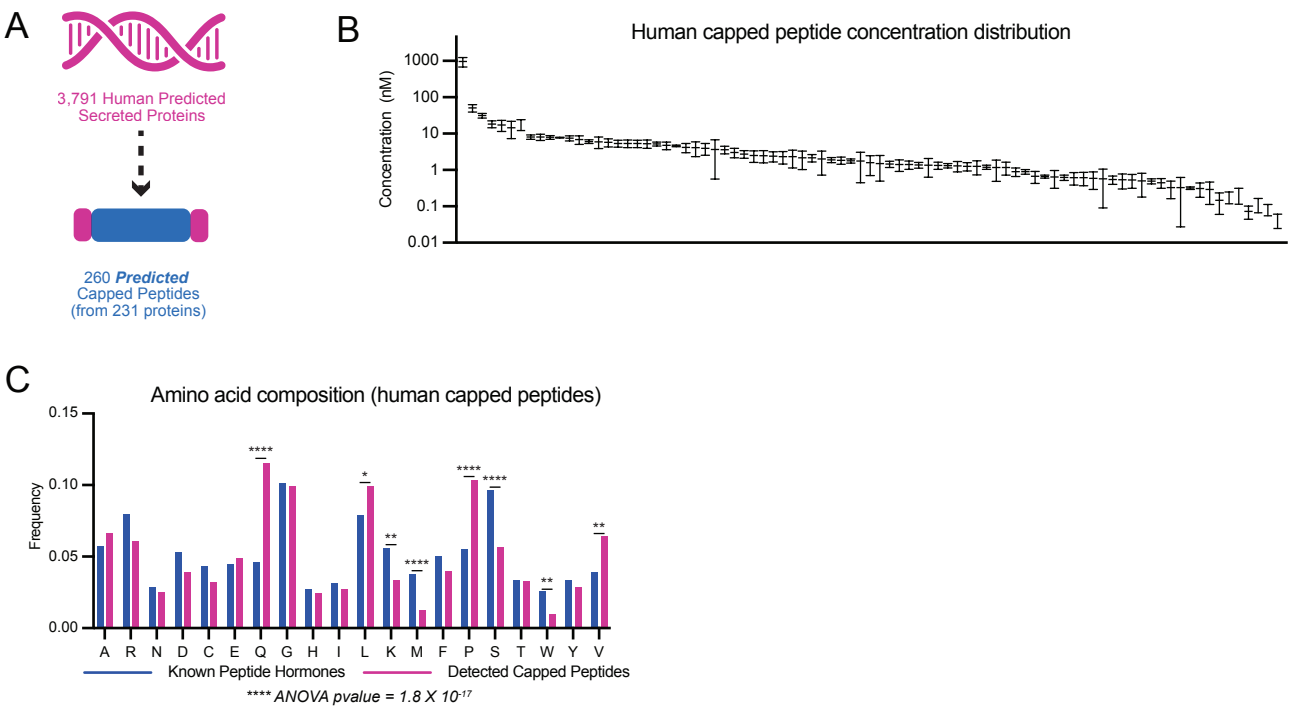

A
3,791 Human Predicted Secreted Proteins
260 Predicted Capped Peptides (from 231 proteins)
B
Human capped peptide concentration distribution
Concentration (nM)
1000
100
10
1
0.1
0.01
C
Amino acid composition (human capped peptides)
Frequency
0.15
0.10
0.05
0.00
A R N D C E Q G H I L K M F P S T W Y V
Known Peptide Hormones
Detected Capped Peptides
**** ANOVA pvalue = 1.8 X 10-17

Fig. S4

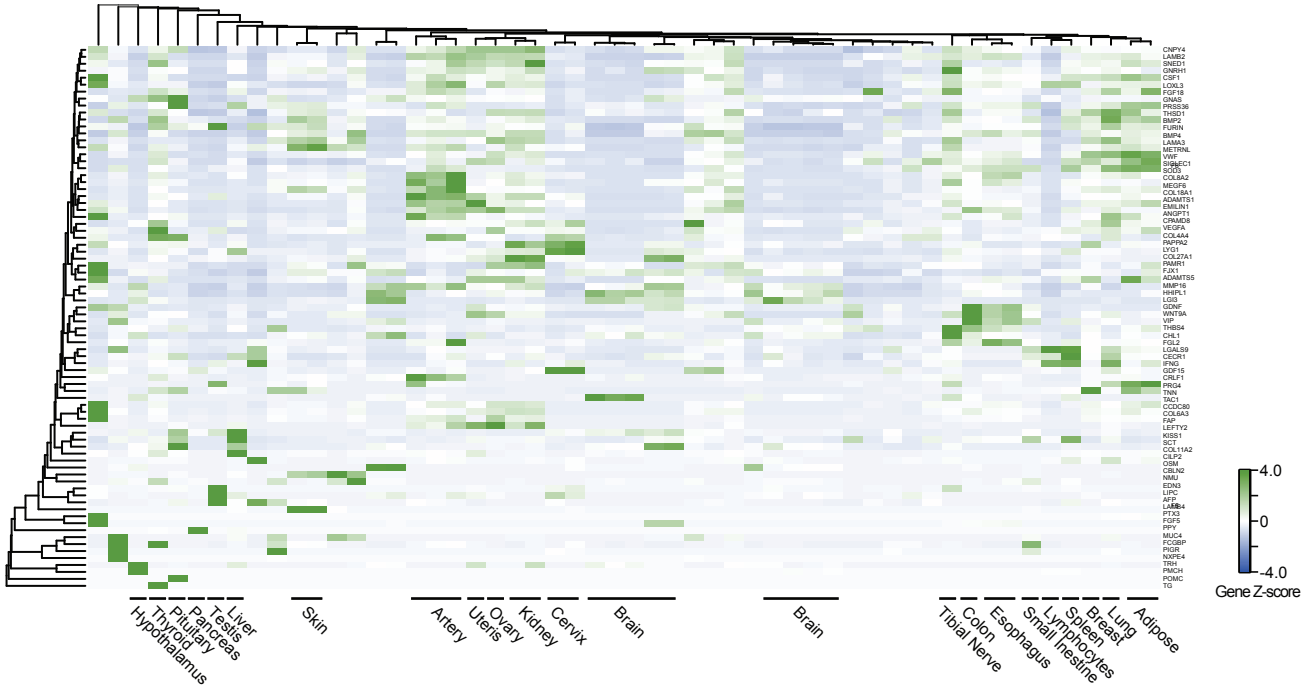

CNPY4
LAMB2
SNED1
GNRH1
CSF1
LOXL3
FGF18
GNAS
PRSS36
THSD1
BMP2
FURIN
EMR4
LAMA3
METRNL
VWF
SH3BGRL2
SOD3
COL8A2
MEGF6
COL18A1
ADAMTS1
EMILIN1
ANGPT1
CPAMD8
VEGFA
COL4A4
PAPPA2
LYG1
COL27A1
PAMR1
FJX1
ADAMTS5
MMP16
HHIPL1
LGI3
GDNF
WNT9A
VIP
THBS4
CHL1
FGL2
LGALS9
CECR1
IFNG
GDF15
CRLF1
PRG4
TNN
TAC1
CCDC80
COL6A3
FAP
LEFTY2
KISS1
SCT
COL11A2
CILP2
OSM
CBLN2
NMU
EDN3
LIPC
APOB
LAMB4
PTX3
FGF5
PPY
MUC4
FCGBP
PIGR
NXPE4
TRH
PMCH
POMC
TG
Hypothalamus
Thyroid
Pituitary
Pancreas
Testis
Liver
Skin
Artery
Uterus
Ovary
Kidney
Cervix
Brain
Brain
Tibial Nerve
Colon
Esophagus
Small Intestine
Lymphocytes
Spleen
Breast
Lung
Adipose
4.0
0
-4.0
Gene Z-score

Fig. S5