

1 Oral intake of rice overexpressing ubiquitin ligase inhibitory pentapeptide prevents atrophy in
2 denervated skeletal muscle

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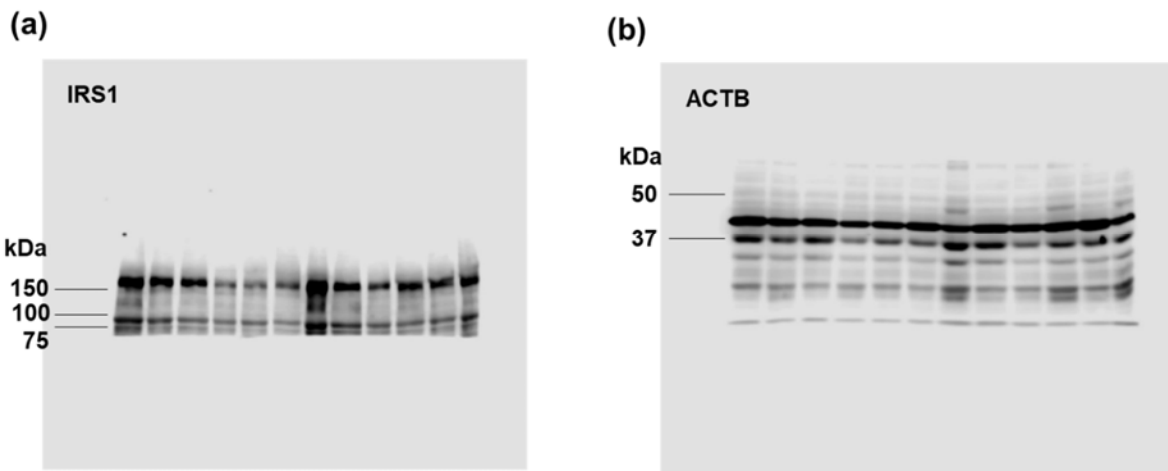
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16 **Supplementary information**



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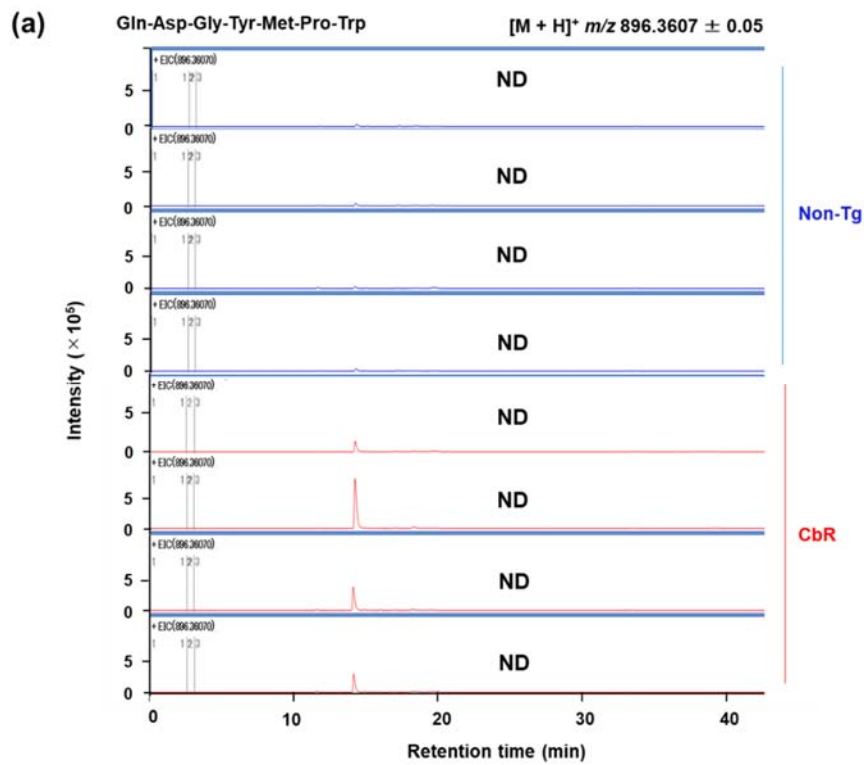
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19 Supplementary Figure 1. Full-size blots of polyacrylamide gels.

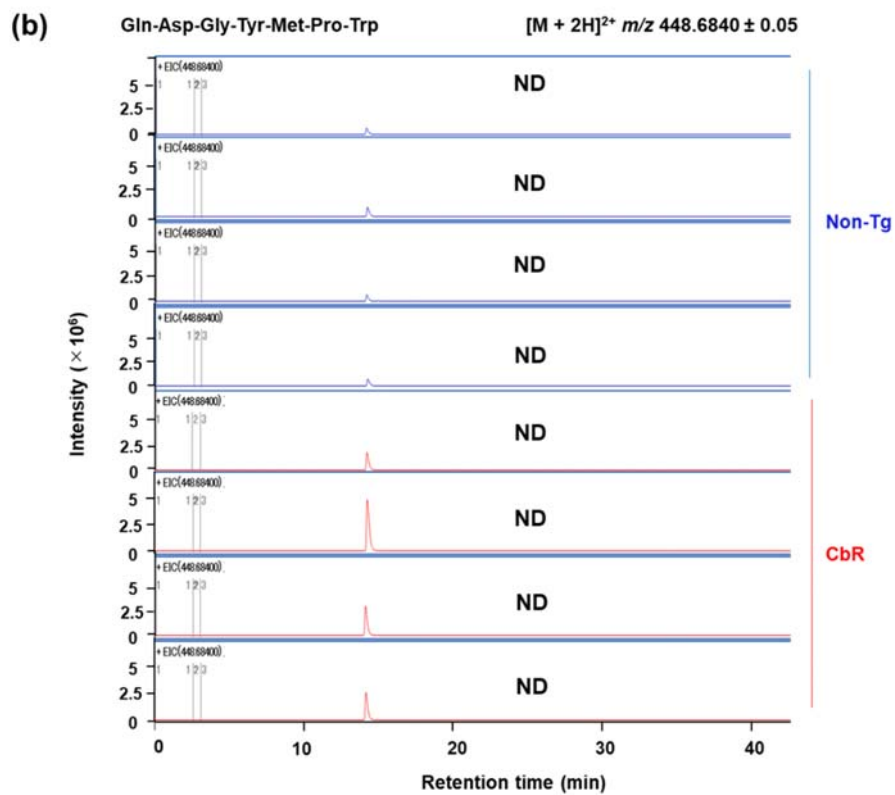
20 Images of actual IRS1 and ACTB blots shown in Figure 5. Image size varies because membrane

21 was cut to blot several proteins.

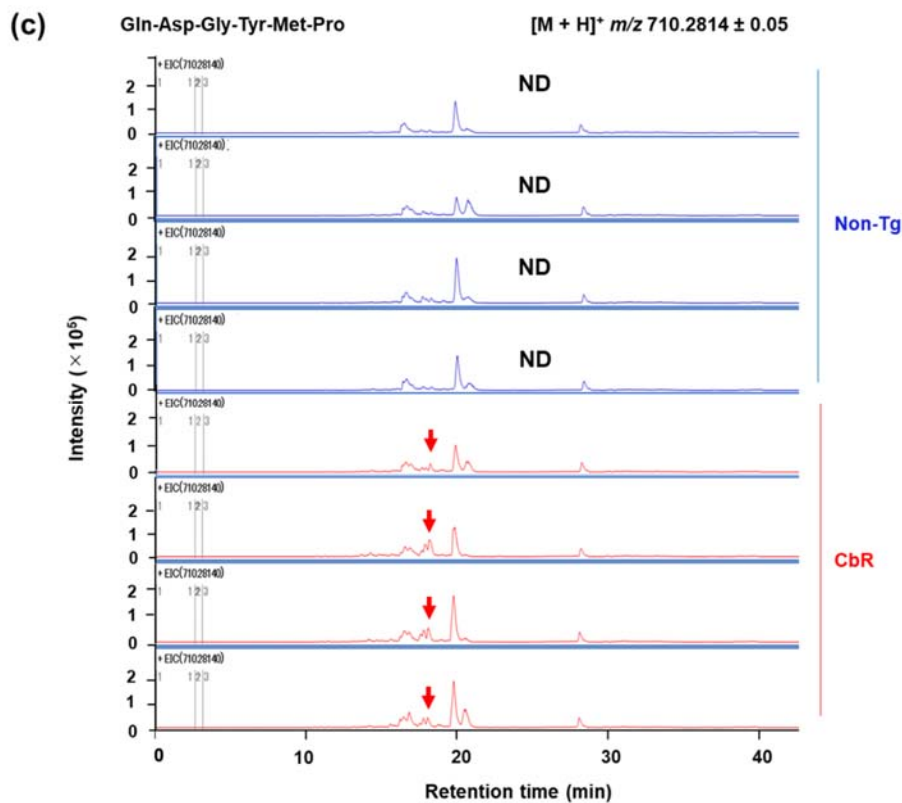
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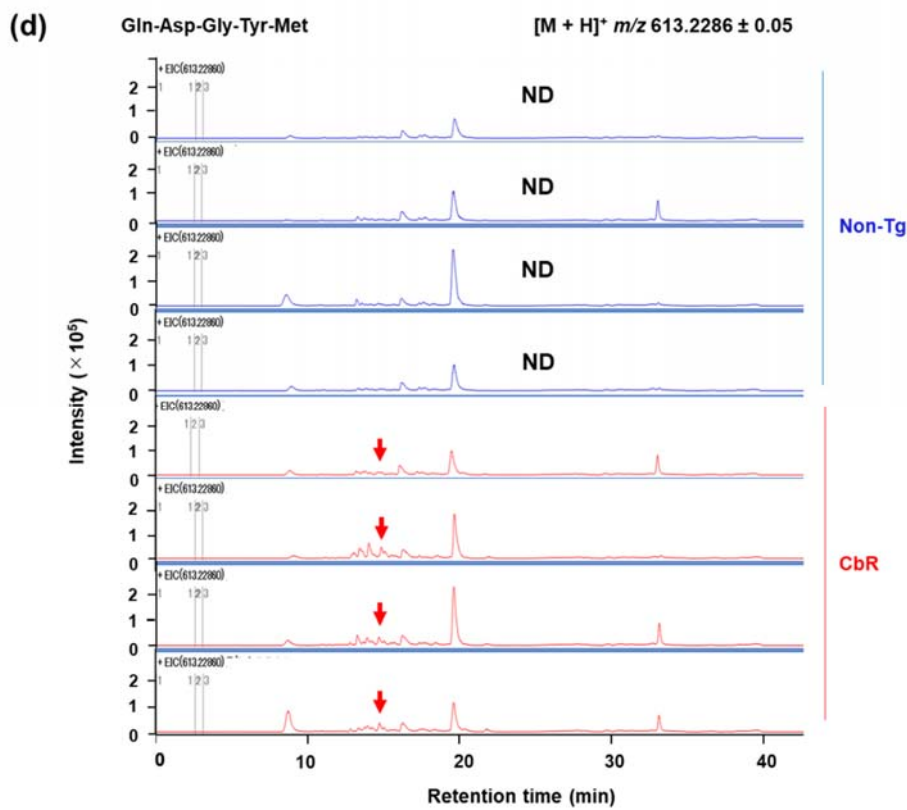
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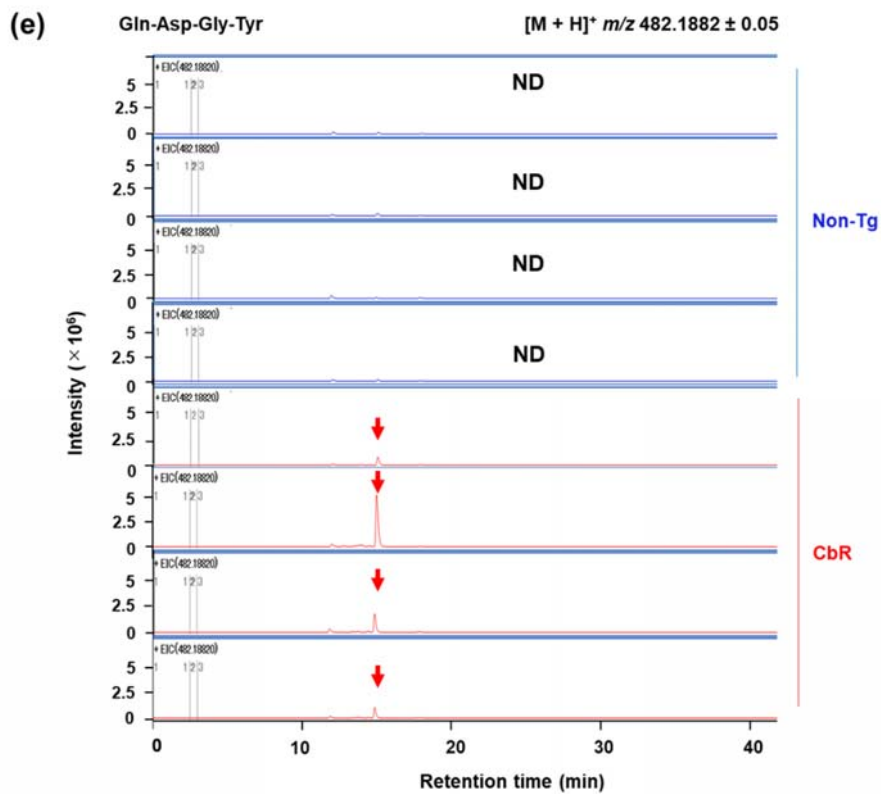
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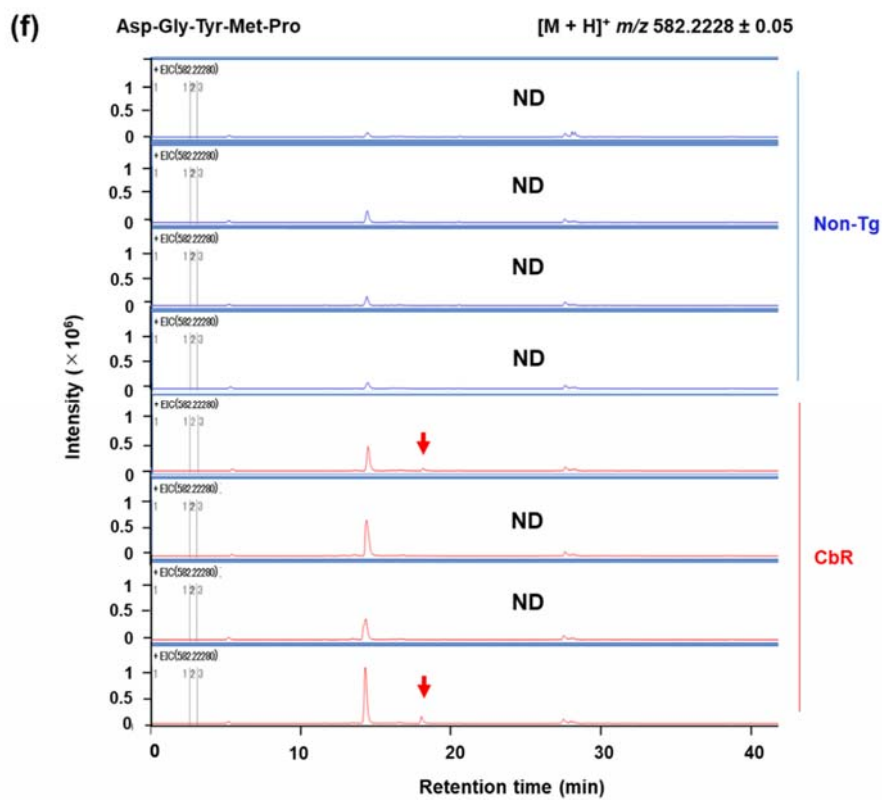
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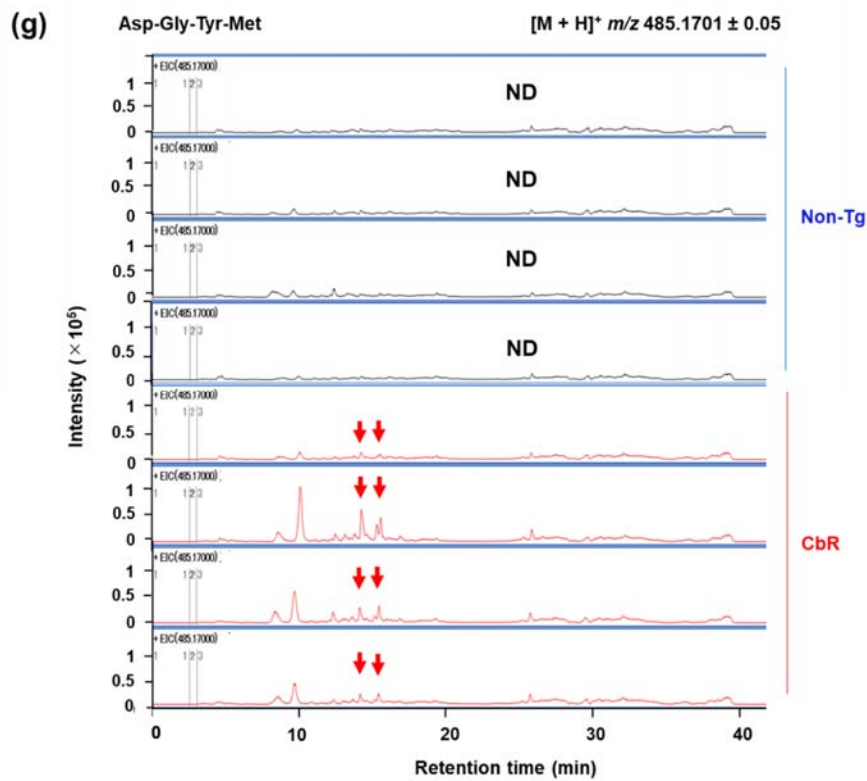
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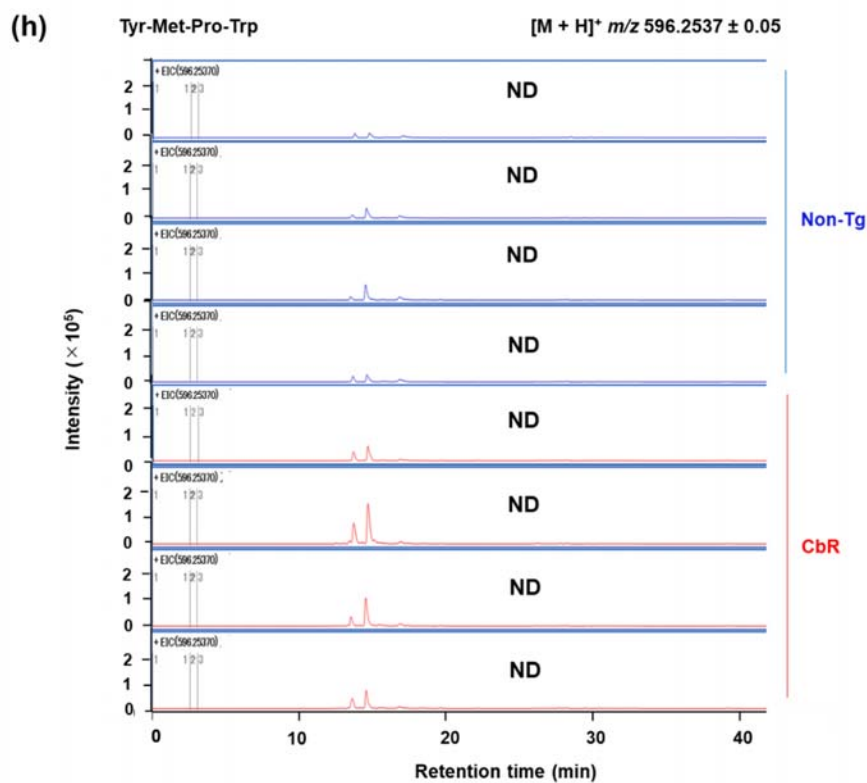
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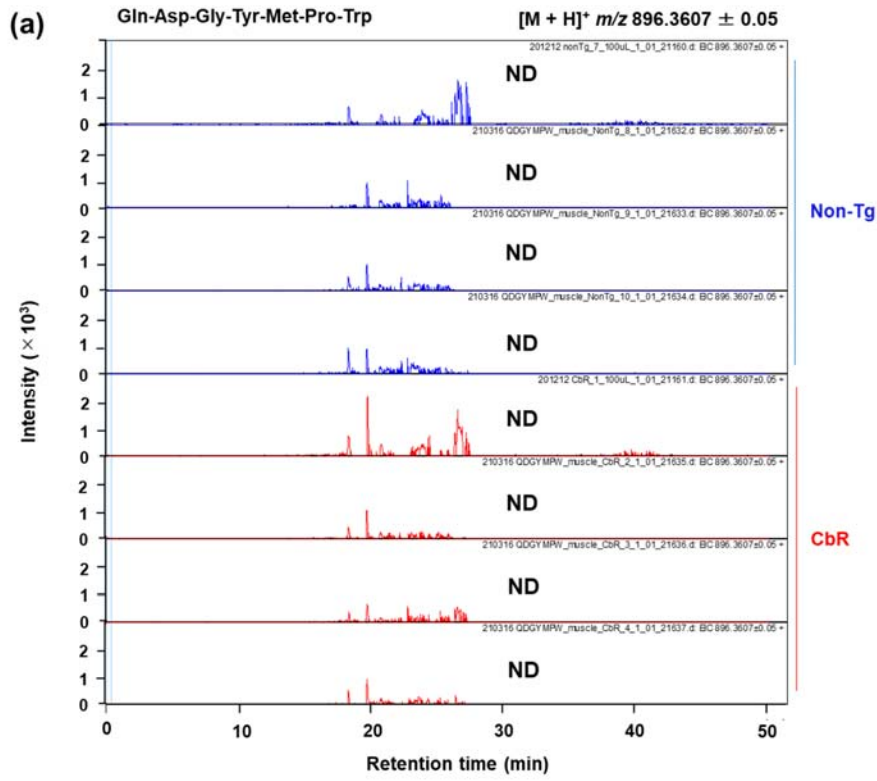


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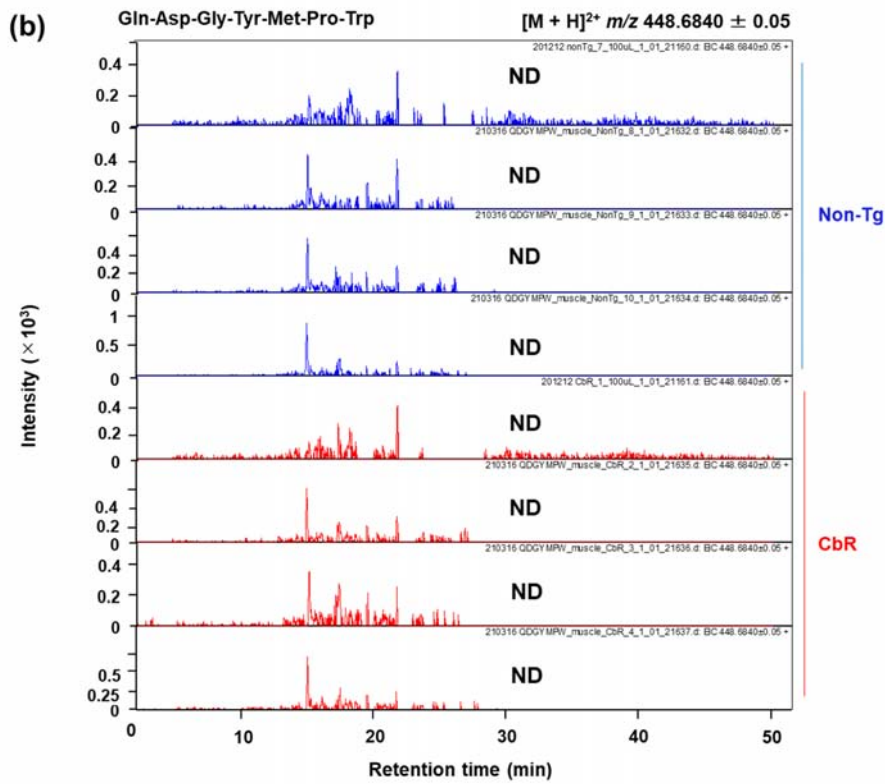
31 Supplementary Figure 2. Analysis of serum collected from mice fed with Non-Tg or CbR using
32 LC-TOF/MS.

33 Mice were handled as described in Methods. Briefly, 6-week-old male Jcl:ICR mice (Japan SLC
34 Inc., Shizuoka, Japan) were housed with access to a standard diet and water *ad libitum*. The mice
35 were then fed with a diet containing either CbR or Non-Tg for 7 days, then their sciatic nerves
36 were unilaterally transected under anesthesia. Seven days later, serum was separated from blood
37 by centrifugation for 10 min at $5,800 \times g$, then stored at $-80\text{ }^{\circ}\text{C}$. Serum was deproteinized by
38 precipitation with ice cold ACN containing 0.1% FA then centrifuged at $14,000 \times g$ for 15 min.
39 The supernatant was collected and dried using a centrifugal evaporator, and pellets were
40 resuspended in 50 μL of 0.1% FA and analyzed by LC-TOF/MS under conditions described in
41 Methods. Extracted ions correspond to those of possible metabolites. Arrows indicate MS signals
42 specific for CbR group. ND, not detected.

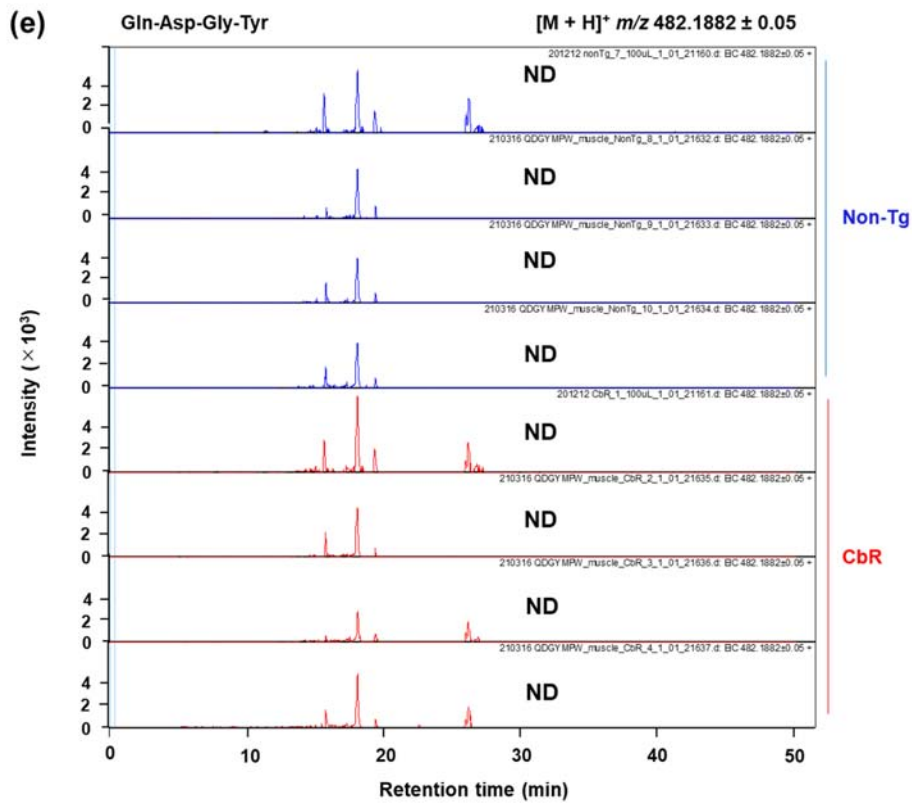
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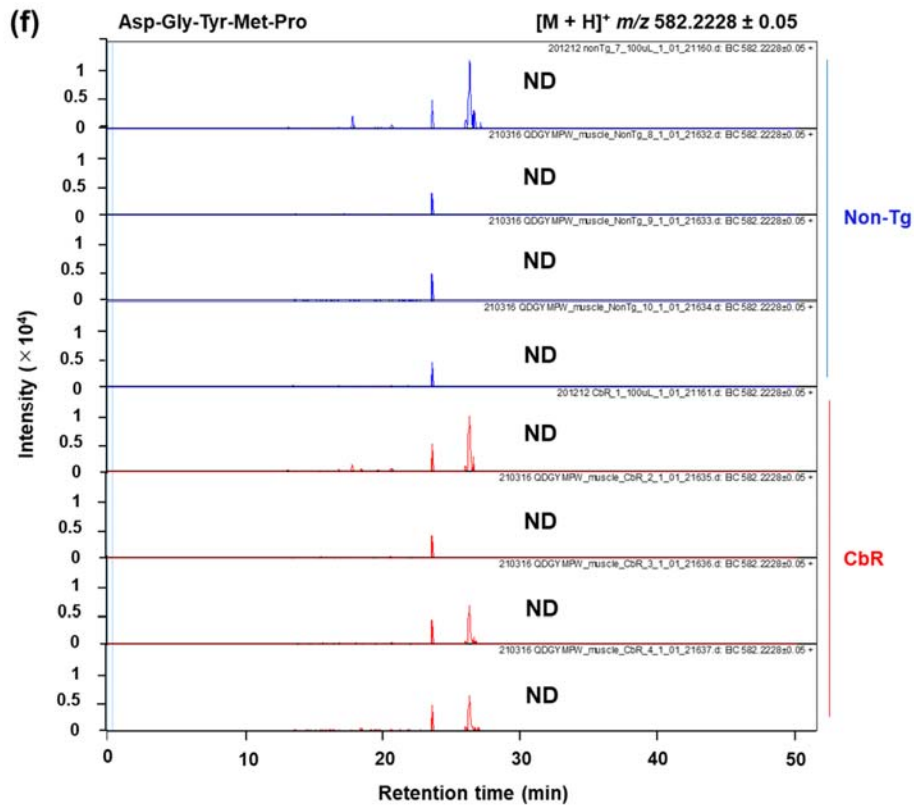
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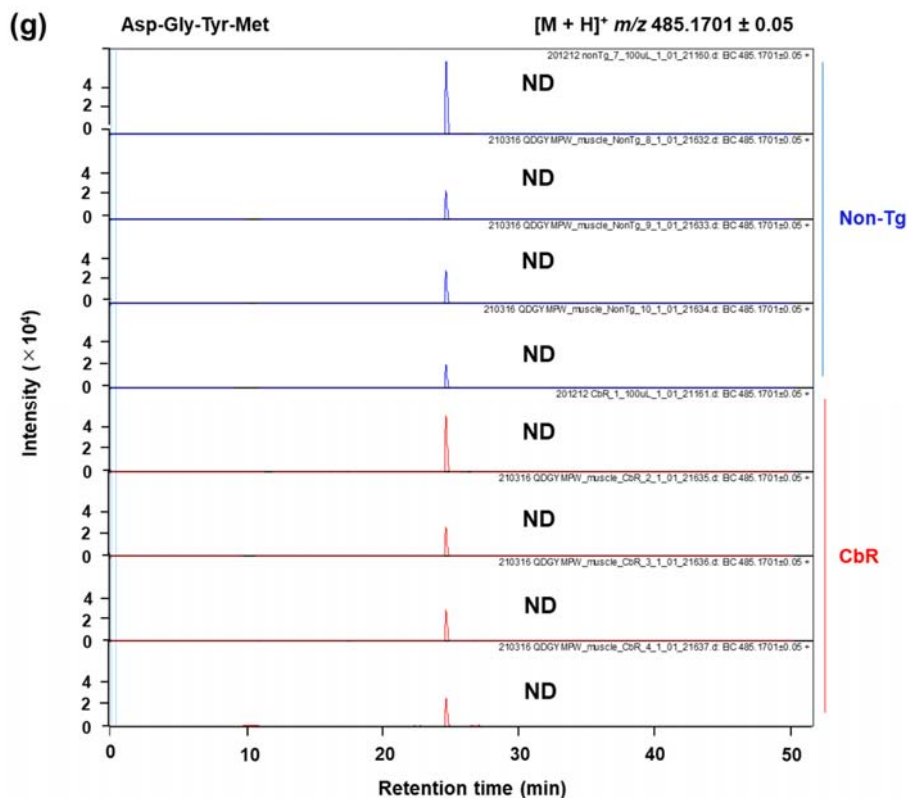
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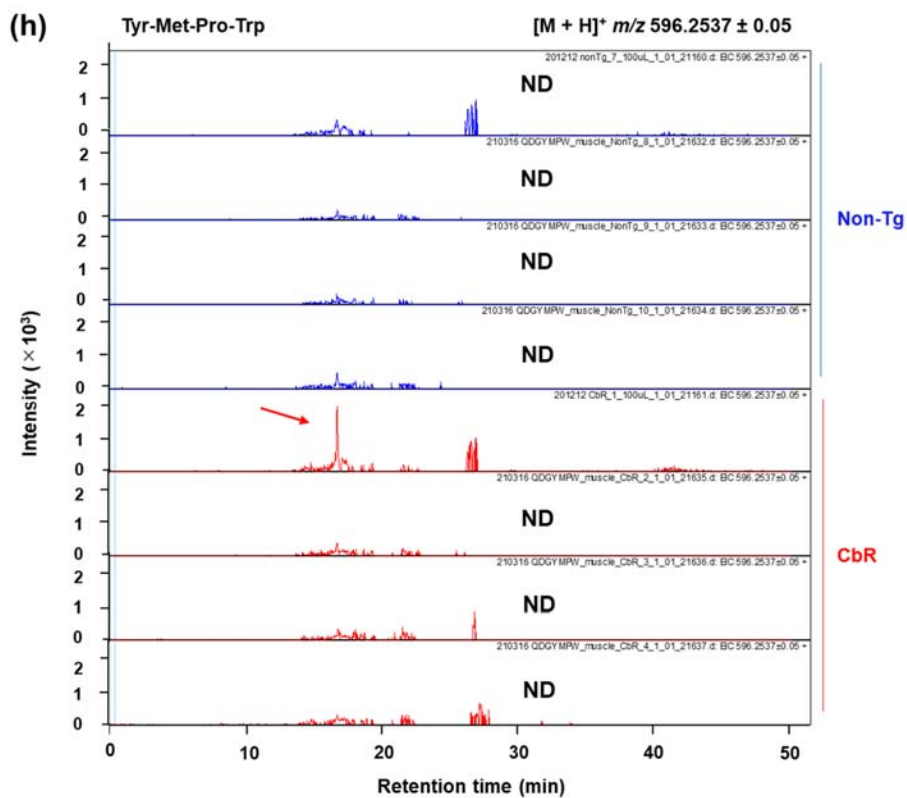
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52 Supplementary Figure 3. LC-TOF/MS analysis of skeletal muscle collected from mice fed with
53 Non-Tg or CbR.
54 Mice were handled as shown in Methods and in Supplementary Fig. 2. Gastrocnemius (Ga)
55 muscles stored at -80°C were homogenized in Tris-HCl buffer, pH 7.5, containing 150 mM
56 NaCl, 5 mM EDTA, 1% TritonX-100, 10 mM NaF, 2 mM Na₃VO₄, 10 μM MG132, and protease
57 inhibitor tablets (Roche Diagnostics). Lysates were precipitated with ice cold ACN containing
58 0.1% FA, then separated by centrifugation at 14,000 × *g* for 15 min. Supernatants were collected
59 and dried using a centrifugal evaporator. Pellets were resuspended in 100 μL of 0.1% FA and
60 analyzed by LC-TOF/MS under conditions described in Methods. Extracted ions correspond to
61 those of possible metabolites. Arrows indicate MS signals specific to CbR. ND, not detected.

62 Supplementary Table 1. Pharmacokinetics after a single oral administration of Asp-Gly-Tyr-Met-
63 Pro at lower or higher doses in 8-wk SD rats.

Measured parameters	Oral peptide dose (mg/kg BW)	
	50	200
C_{\max} (pmol/mL plasma)	0.79	27.25
t_{\max} (min)	10	30
$AUC_{0-60 \text{ min}}$ (pmol · min/mL plasma)	14.61	648.91
$t_{1/2}$ (min)	8.99	5.58

64 Data are expressed as means (n = 1 per dose). $AUC_{0-60 \text{ min}}$, area under plasma concentration vs.
65 time curve; BW, body weight; C_{\max} , maximum plasma concentration; t_{\max} , elapsed time to peak
66 plasma concentration; $t_{1/2}$, elimination half-life.

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68 Supplementary Table 2. Composition of diets.

	Standard diet for acclimation	Non-Tg	CbR
Non-transgenic rice (g)	-	50.0	-
Cblin peptide-enriched rice (g)	-	-	50.0
Casein (g)	19.8	16.6	16.6
Corn starch (g)	46.2	-	-
Lard (g)	4.4	3.5	3.5
Sucrose (g)	23.1	23.4	23.4
Cellulose (g)	2.0	2.0	2.0
AIN93 vitamin mix (g)	1.0	1.0	1.0
AIN93 mineral mix (g)	3.5	3.5	3.5
Total (g)	100	100	100

69 CbR, rice enriched with transgenic Cblin pentapeptide; Non-Tg, non-transgenic rice.

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72 Supplementary Table 3. Primer sequences.

Gene	Forward (5'→3')	Reverse (5'→3')
<i>Mafbx</i>	GGAAGCTTTCAACAGACTGGA	CTCAGGGATGTGAGCTGTGA
<i>Murfl</i>	ACGAGAAGAAGAGCGAGCTG	CTTGGAAGGAGGAGGAGGAAGG
<i>Actb</i>	CACACCTTCTACAATGAGCTGC	CATGATCTGGGTCACTTTTCA

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75 Supplementary Table 4. Mass spectrometry of CbR-specific signals in mouse serum.

m/z [M + H] ⁺	Predicted peptide sequence	RT (min)
710.2814	Gln-Asp-Gly-Tyr-Met-Pro	17.5
613.2286	Gln-Asp-Gly-Tyr-Met	14.6
482.1882	Gln-Asp-Gly-Tyr	15.5
582.2228	Asp-Gly-Tyr-Met-Pro	18.0
485.1701	Asp-Gly-Tyr-Met	14.2, 15.4

76 Values (m/z) correspond to predicted metabolites with ≥ 4 amino acid residues. CbR, rice
77 enriched with transgenic Cblin pentapeptide; RT, retention time under LC-MS conditions
78 described in legend to Supplementary Figure 2.