

1 **Supplemental information:**

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3 **METHODS**

4 *Repeat administration of NMU and NMS.* The effect of repeated administration of NMU and
5 NMS (2 nmol per day per mouse) was examined with respect to food intake and metabolic
6 output. During feeding experiments, mice received daily injections of NMU or NMS at ZT
7 12 for 3 consecutive days. Food intake was monitored hourly for the first 4 hr post injection,
8 as well as 12 hr and 24 hr post injection. In addition, daytime (ZT0-12) and night time
9 (ZT12-24) food intake was also measured on the day following the last peptide injection
10 (referred to as day 4).

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12 **Supplemental Figure 1. NMU and NMS alter feeding behaviour and metabolic**
13 **rate.** NMU (*A*) and NMS (*B*) were administered (i.c.v.) to mice at the onset of the
14 dark period (8-15 mice/group). A dose-dependent reduction in food intake was
15 observed following administration of either NMU or NMS, which was most
16 pronounced over the first 4 hr post injection. NMU and NMS (2 nmol) caused a
17 significant reduction in body weight over 24 hr (*C*). Further, VO_2 was increased
18 significantly following administration of either NMU or NMS at ZT2 (*D-E*; 4-6
19 mice/group). In contrast, when the peptides were administered at ZT12, during the
20 normal nocturnal rise in VO_2 , no further elevation was observed (*F-G*). A decrease
21 was observed in respiratory quotient (RQ) over approximately 4 hr post injection,
22 likely reflecting decreased food intake in these mice (*G*). * = $P < 0.05$, ** = $P < 0.01$,
23 one-way ANOVA with Dunnet's *post hoc* test.

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25 **Supplemental Figure 2. Effects of repeated NMU or NMS administration.** Short-term
26 anorexigenic effects of both NMU and NMS diminished upon repeated dosing (*A*). The
27 peptide-induced reduction in feeding over 24 hour post injection was also lost by day 2 and 3
28 of administration, primarily due to increased daytime feeding in these mice relative to
29 vehicle-treated animals (*B*). Interestingly, 24-hour food intake was increased compared with
30 vehicle-treated animals on day 3 and 4, a phenomenon which was especially pronounced in
31 the NMS-treated mice. Body weight showed a similar trend to food intake. While a
32 significant drop in body weight was observed in peptide-treated mice on day 1, by day 4
33 NMU- and NMS-treated mice were significantly heavier than mice treated with vehicle (*C*).
34 $n = 8$ mice/group. ** = $P < 0.01$, * = $P < 0.05$ one way ANOVA (repeated measures) with
35 Bonferonni's *post hoc* test.