

Supplemental information

**Calorie restriction and calorie dilution have
different impacts on body fat, metabolism,
behavior, and hypothalamic gene expression**

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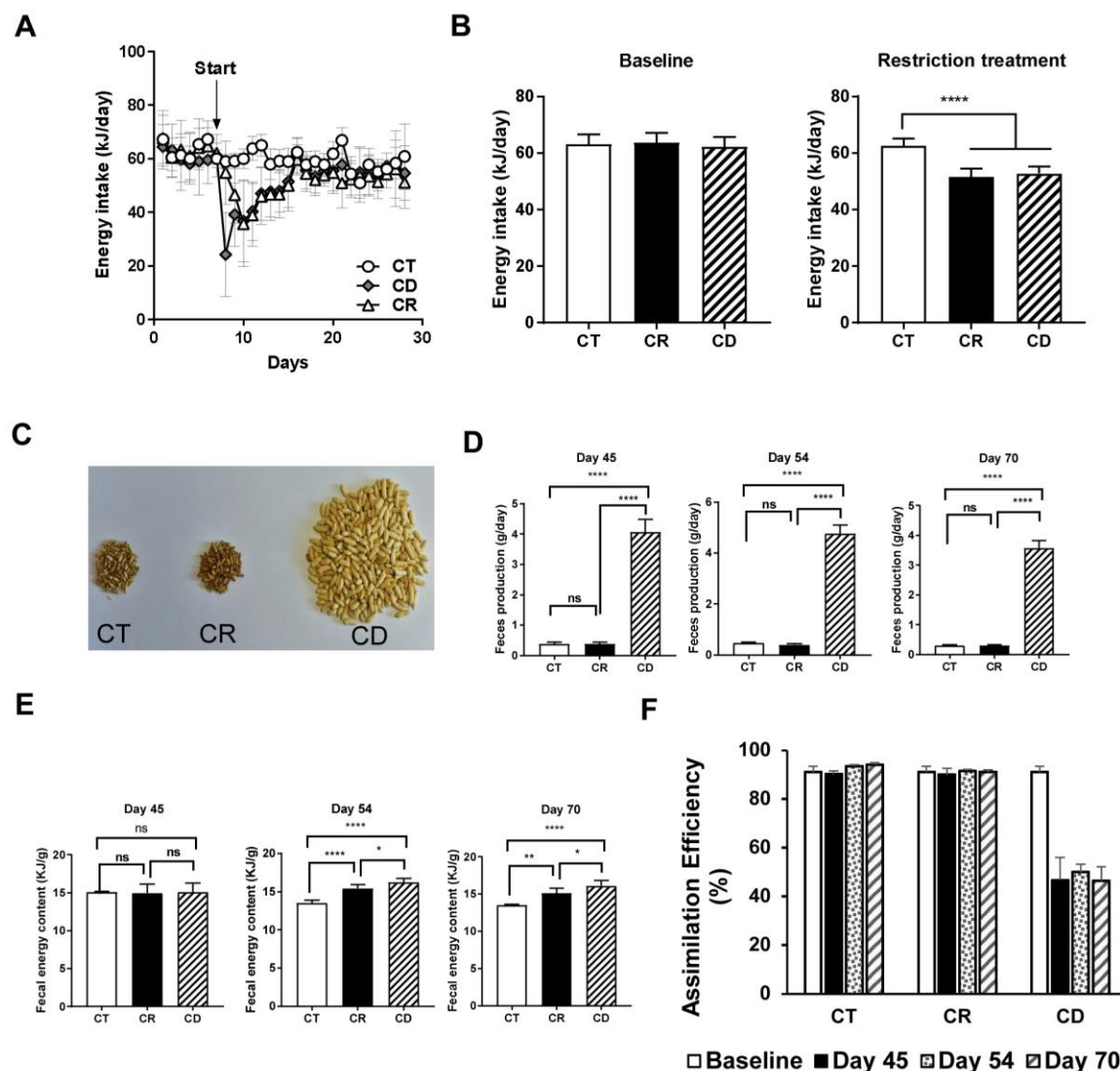
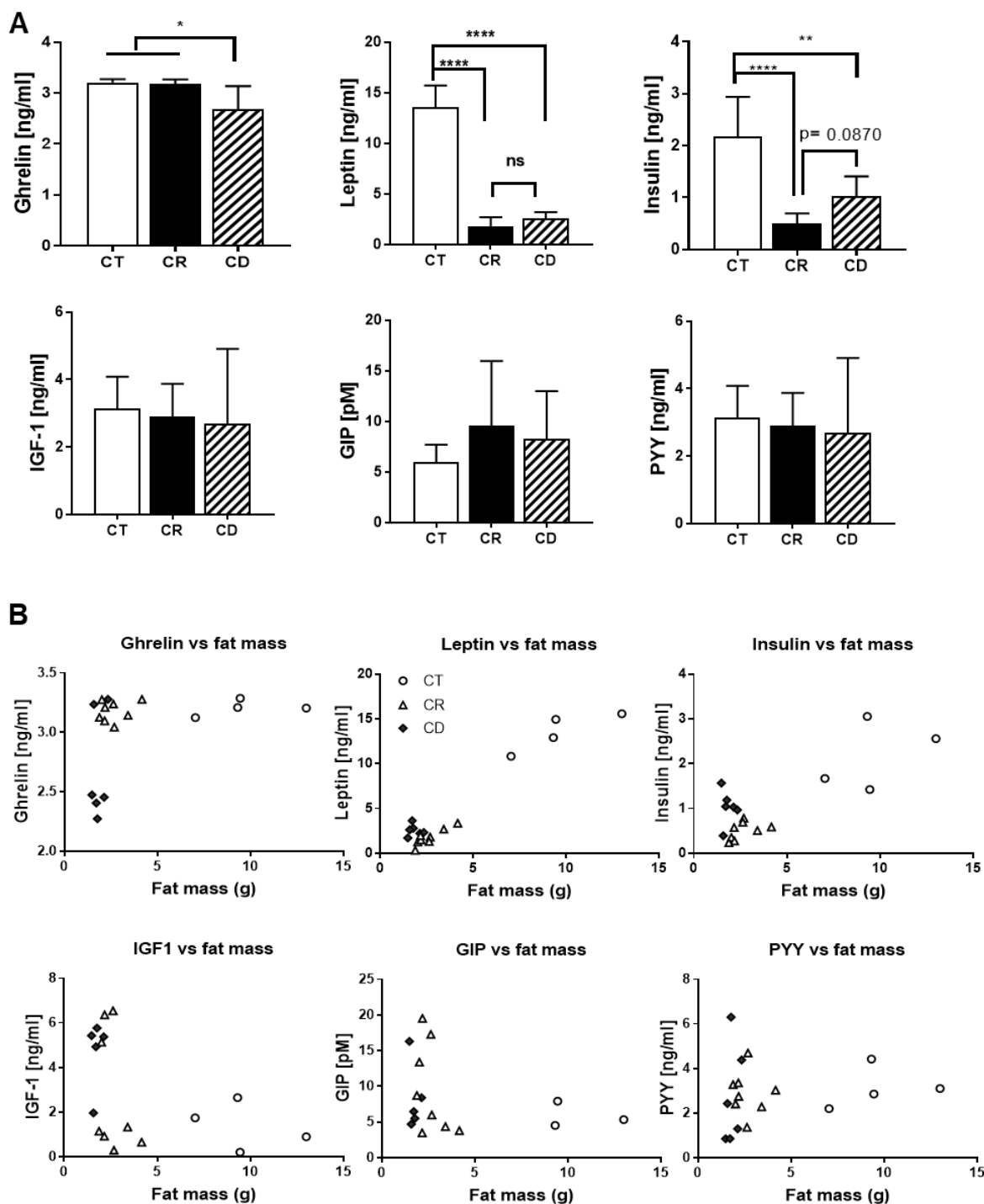


Fig S1. Mice of CD and CR groups were well paired feeding and assimilation efficiency keep consistent during caloric restriction A. Mice daily energy intake (kJ/day) since 1 week before treatment and 2 weeks after treatment. B. The average daily energy intake during baseline and treatment. For A-B, CT n=8; CR and CD n=19. C. Dried feces produced by one mouse from CT, CR and CD group during 3 days respectively. D. Feces production during dietary treatment E. Fecal energy content during dietary treatment. F. Assimilation efficiency of three groups at baseline, day 45, day 54 and day 70. For (D-F) CT n=4, CR and CD n=6. All data is presented as mean \pm SD. Statistics analysis by One-way ANOVA followed by Tukey's multiple comparisons test, compare each group with every other groups. Related to Fig 1.



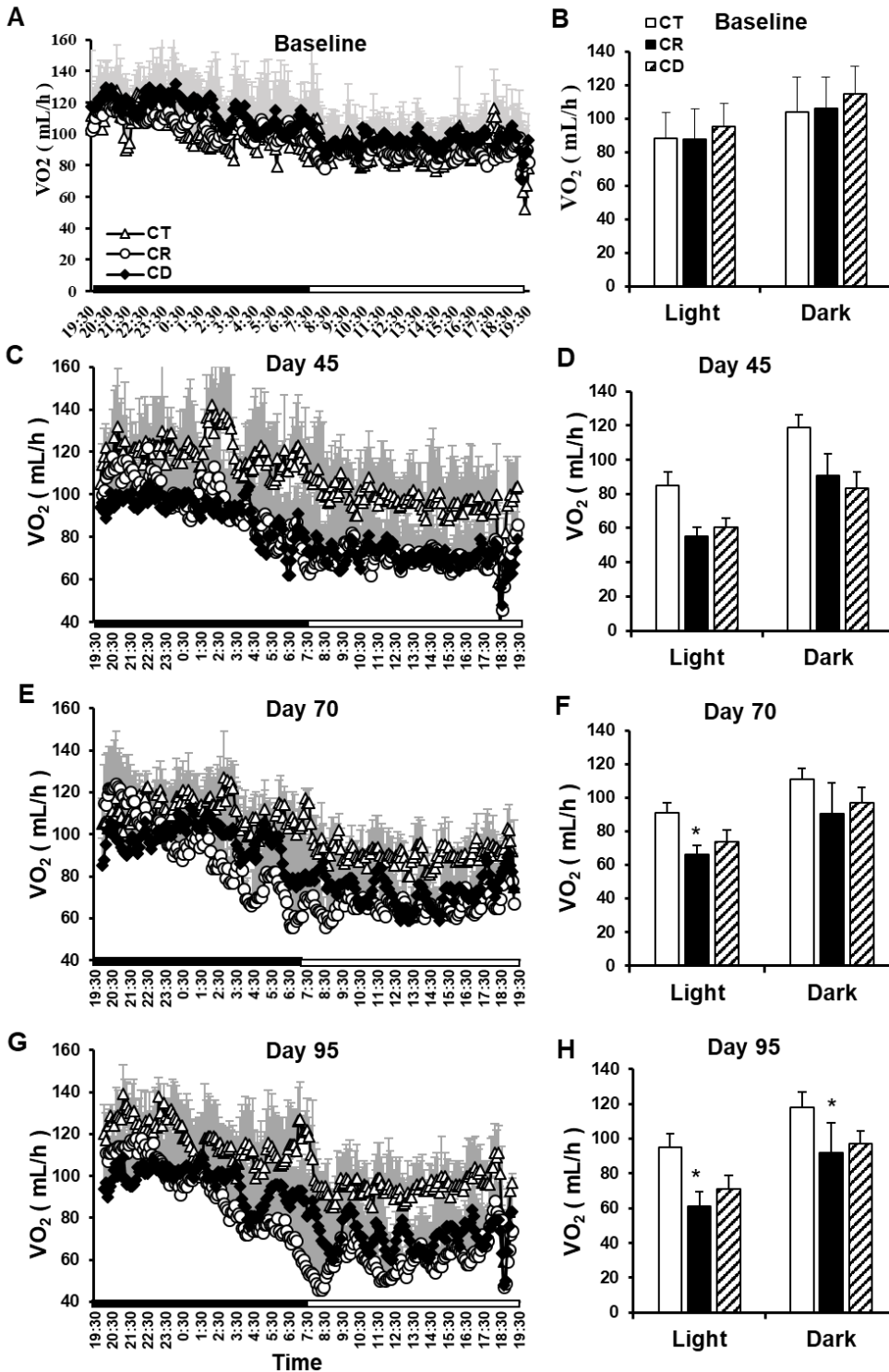


Fig S3. Energy expenditure in CT, CR and CD three groups. (A), (C), (E), (G) Total oxygen consumption (ml O₂/6-min time interval) during 24 hours in CT, CR and CD groups at baseline, day 45, day 70 and day 95 respectively. (B), (D), (F), (H) shows the average of total oxygen consumption (ml O₂/6-min time interval) during a light circle and a dark circle. CT n=4, CR and CD n=6. Analyzed by ANCOVA. Related to Fig. 3.

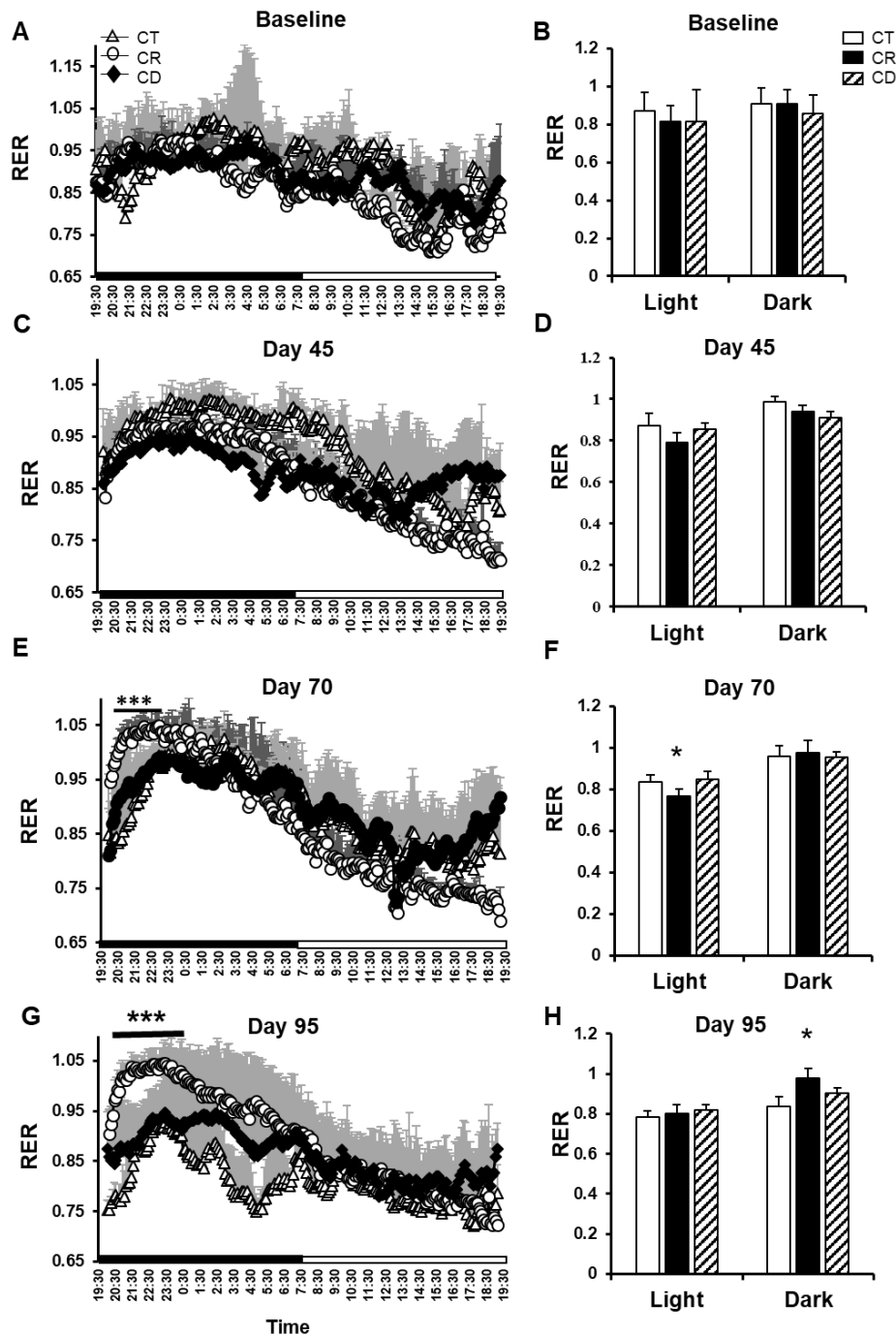


Fig S4. Calorie restricted (CR) mice had different 24h profiles of respiratory exchange ratio (RER) than calorie diluted (CD) or control (CT) mice. (A), (C), (E), (G) Average RER (6-min time interval) during 24 hours in CT, CR and CD groups at baseline, day 45, day 70 and day 95 respectively. (B), (D), (F), (H) shows the average RER (6-min time interval) during a light and dark cycles. All data is presented as mean \pm SD. CT n=4, CR and CD n=6. Analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Related to Fig 3.

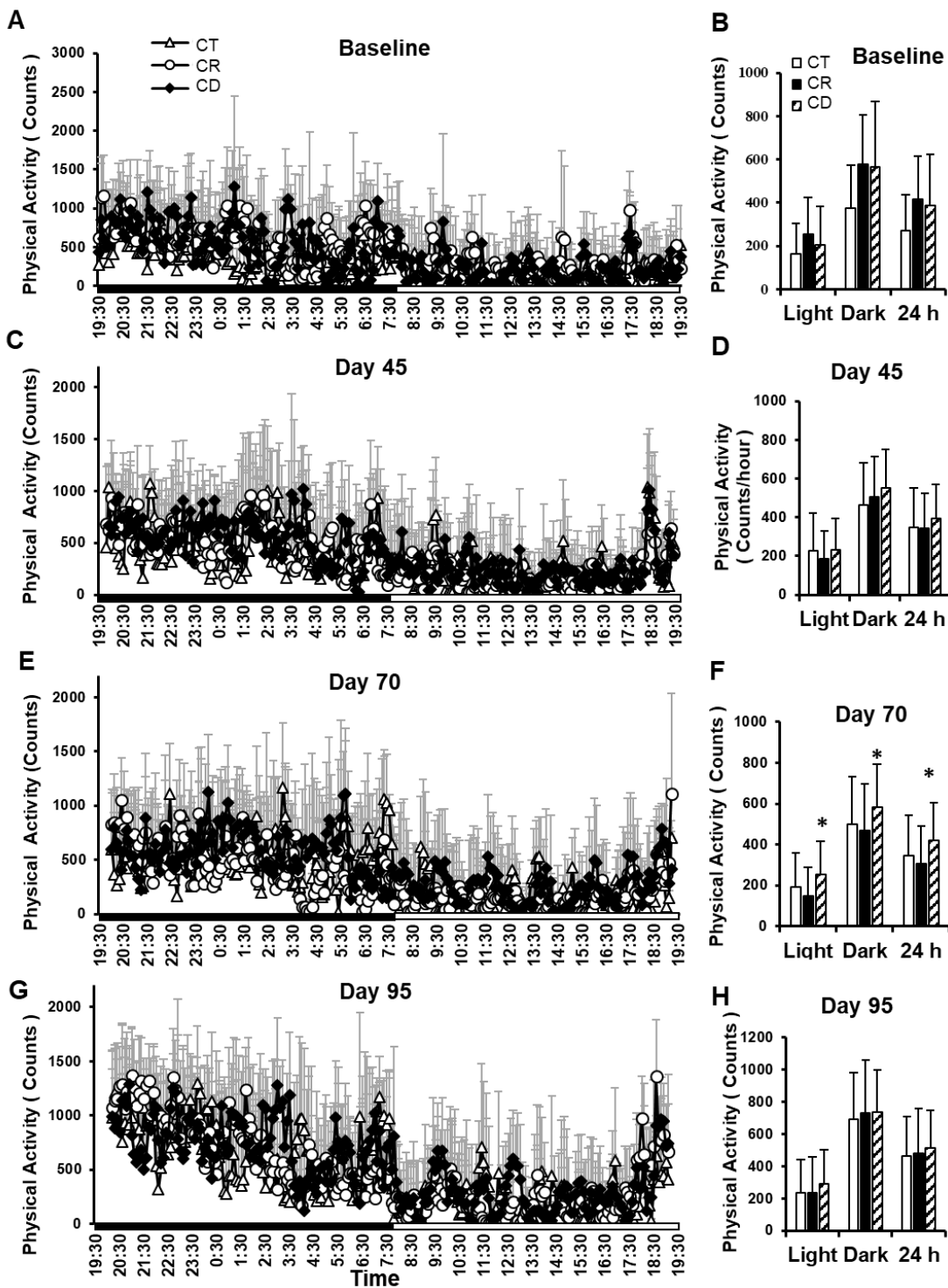
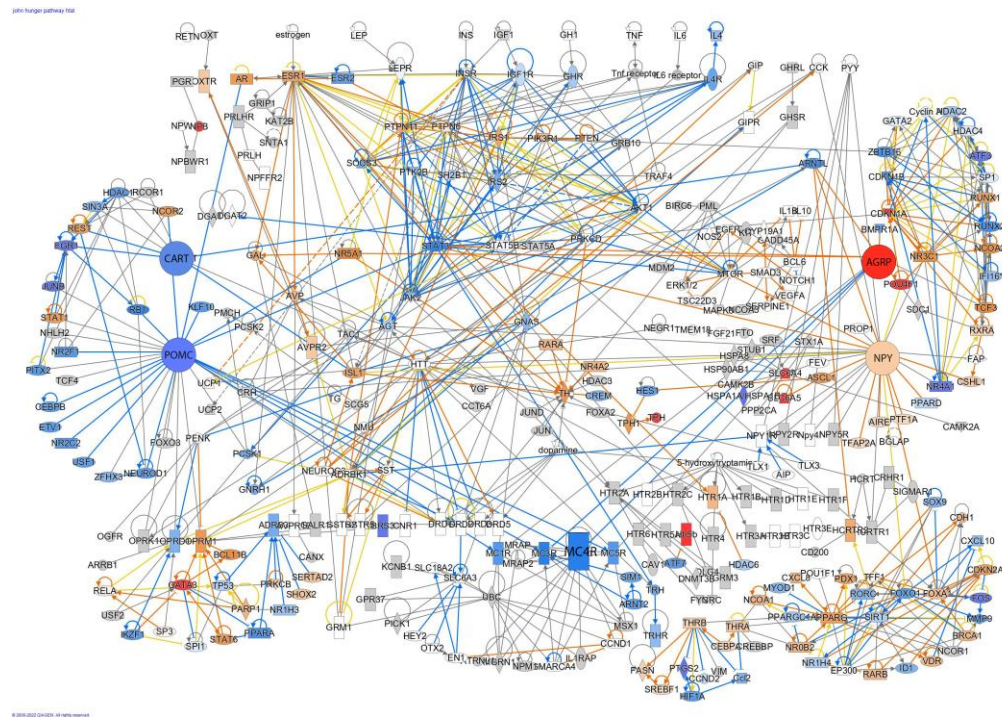


Fig S5. Effects of two protocols on physical activity of CT, CR and CD. (A), (C), (E), (G) Average total physical activity (counts/6-min time interval) during 24 hours in CT, CR and CD groups at baseline, day 45, day 70 and day 95 respectively. (B), (D), (F), (H) shows the average total physical activity (counts/6-min time interval) during a light circle and a dark circle. CT n=4, CR and CD n=6. Analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. Related to Fig. 3.

A



B

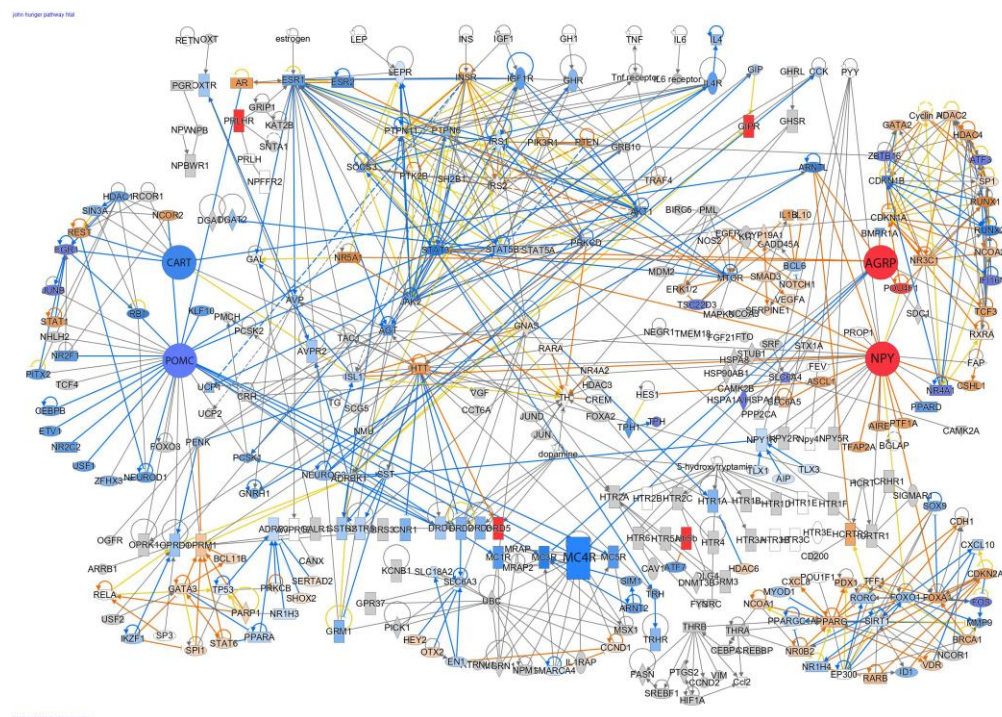


Fig.S6 Pathway diagram for canonical hunger signaling pathway A: CR vs CT and B: CD vs CT. Blue indicates the down regulated, red indicates up regulated in CR or CD group relative to the CT group, gray indicates no significance. Generated by Ingenuity Pathway Analysis. CT n=6, CR n=5 and CD n=6. Related to Fig. 4.

Table S1. Wet weight (g) of organs from CT, CR and CD at the end of diet treatment.

| Organ (g) | Treatment | | | F values | P values |
|------------------------|-----------|------|------|----------|----------|
| | CT | CR | CD | | |
| Stomach | 0.16 | 0.19 | 0.21 | | |
| StDev | 0.02 | 0.02 | 0.03 | | |
| Stats | B | AB | A | 7.45 | 0.004 |
| Colon | 0.17 | 0.33 | 0.45 | | |
| StDev | 0.03 | 0.22 | 0.07 | | |
| Stats | B | AB | A | 5.91 | 0.011 |
| Caecum | 0.11 | 0.14 | 0.30 | | |
| StDev | 0.02 | 0.01 | 0.04 | | |
| Stats | B | B | A | 94.66 | <0.0001 |
| Small intestine | 1.33 | 1.74 | 2.35 | | |
| StDev | 0.14 | 0.20 | 0.18 | | |
| Stats | C | B | A | 25.42 | <0.0001 |
| Pancreas | 0.29 | 0.16 | 0.22 | | |
| StDev | 0.04 | 0.02 | 0.06 | | |
| Stats | A | C | B | 15.97 | <0.0001 |
| Spleen | 0.08 | 0.05 | 0.07 | | |
| StDev | 0.01 | 0.01 | 0.01 | | |
| Stats | A | C | B | 25.95 | <0.0001 |
| Sub. WAT | 1.24 | 0.28 | 0.27 | | |
| StDev | 0.31 | 0.07 | 0.07 | | |
| Stats | A | B | B | 64.96 | <0.0001 |
| BAT | 0.19 | 0.07 | 0.08 | | |
| SD | 0.05 | 0.02 | 0.01 | | |
| Stats | A | B | B | 29.75 | <0.0001 |
| Retro. WAT | 0.56 | 0.09 | 0.07 | | |
| StDev | 0.16 | 0.04 | 0.04 | | |
| Stats | A | B | B | 58.88 | <0.0001 |
| Ep. WAT | 1.27 | 0.35 | 0.23 | | |
| StDev | 0.42 | 0.15 | 0.05 | | |
| Stats | A | B | B | 35.3 | <0.0001 |
| Mesent WAT | 0.56 | 0.16 | 0.16 | | |
| StDev | 0.23 | 0.06 | 0.05 | | |
| Stats | A | B | B | 20.62 | <0.0001 |

| | | | | | |
|----------------|-------|-------|-------|-------|---------|
| Skin | 5.66 | 4.36 | 3.80 | | |
| StDev | 0.41 | 0.48 | 0.47 | | |
| Stats | A | B | B | 27.73 | <0.0001 |
| Carcass | 14.91 | 11.27 | 10.89 | | |
| StDev | 1.10 | 0.75 | 0.55 | | |
| Stats | A | B | B | 48.25 | <0.0001 |
| Tail | 0.82 | 0.73 | 0.70 | | |
| StDev | 0.04 | 0.07 | 0.05 | | |
| Stats | A | B | B | 7.38 | 0.005 |
| Kidneys | 0.48 | 0.40 | 0.43 | | |
| StDev | 0.07 | 0.03 | 0.02 | | |
| Stats | A | B | AB | 7.16 | 0.005 |
| Liver | 1.80 | 1.58 | 1.68 | | |
| StDev | 0.25 | 0.19 | 0.08 | | |
| Stats | A | A | A | 3.17 | 0.07 |
| Testes | 0.17 | 0.18 | 0.19 | | |
| StDev | 0.04 | 0.02 | 0.02 | | |
| Stats | A | A | A | 2.06 | 0.16 |
| Lungs | 0.21 | 0.23 | 0.21 | | |
| StDev | 0.05 | 0.03 | 0.03 | | |
| Stats | A | A | A | 0.81 | 0.46 |
| Heart | 0.17 | 0.16 | 0.16 | | |
| StDev | 0.02 | 0.01 | 0.01 | | |
| Stats | A | A | A | 0.84 | 0.45 |
| Brain | 0.47 | 0.49 | 0.45 | | |
| StDev | 0.12 | 0.12 | 0.06 | | |
| Stats | A | A | A | 0.17 | 0.84 |

Sub. WAT, Mesent WAT, Retro. WAT, Ep. WAT and BAT refer to subcutaneous, mesenteric, retroperitoneal, epididymal white adipose tissue and brown adipose tissue respectively. CT n=4; CR=8 and CD n=7. Statistics analysis by One-way ANOVA followed by Tukey's multiple comparisons test, compare each group with every other groups. Related to Fig. 2

Table S2. Gradients of regression equations between logged final body weight and the final organ weight in CT, CR and CD groups.

| Organ | Gradient - CT | Gradient - CR | Gradient - CD |
|-----------------|----------------------|----------------------|----------------------|
| Colon | 0,8633 | -8,886 | 0,2183 |
| Pancreas | -0,7667 | -0,4112 | 4,6002 |
| Lungs | 0,5941 | -0,1232 | -1,0856 |
| Heart | 2,013 | 0,4796 | -0,3426 |
| Caecum | 2,2782 | 0,6323 | 2,246 |
| Tail | 0,5312 | 0,664 | 1,887 |
| Brain | 1,3755 | 0,6804 | -0,3735 |
| Kidneys | 2,2439 | 0,8948 | 0,8503 |
| BAT | -2,5605 | 1,2962 | -2,1166 |
| Spleen | 1,3053 | 1,4797 | -1,2842 |
| Carcass | 0,771 | 1,6956 | 0,5733 |
| Small intestine | 0,0717 | 1,7418 | 1,1392 |
| Stomach | 0,8609 | 1,7619 | -2,0078 |
| Skin | 1,1307 | 2,1772 | 0,0363 |
| Sub. WAT | -0,1084 | 2,549 | -2,6197 |
| Other WAT | -0,6453 | 2,6676 | 0,0477 |
| Testes | 1,8279 | 2,7971 | 1,0862 |
| Retro. WAT | 0,7731 | 2,915 | -12,491 |
| Liver | 0,0261 | 3,2471 | 0,242 |
| Ep. WAT | 0,8948 | 4,6312 | -1,9783 |
| Mesent WAT | 0,3683 | 9,9629 | -4,1474 |

Values less than 0 reflect tissues were invested in, and values less than 1 reflect the protection of the tissue and values greater than 1 indicate disproportional utilization during weight loss. CR n=11, CD=13. Each tissue analyzed by one-way ANOVA and difference indicated from post-hoc Tukey tests. Related to Fig 2.

Table S3 Effects of two restriction protocols on food anticipatory activity.

| Time | Physical Activity (Counts/measurement point) | | |
|-----------------|--|-----------|-----------|
| | CT | CR | CD |
| B4h-B2h | 165 ± 43 | 188 ± 97 | 219 ± 65 |
| B2h-dark | 278 ± 88 | 582 ± 275 | 335 ± 216 |

CT n=4; CR and CD n=6. Each measurement point is 6 min. Related to Fig3.

Table S4 Significant upregulated hypothalamic genes in CR and CD groups.

| Upregulated Group | Upregulated genes | fold change CD to CR | t-test CD-CR (p values) | log2 fold change |
|--------------------------|--------------------------|-----------------------------|--------------------------------|-------------------------|
| CR | Hif3a | 0.376905 | 0.000198 | -1.40773 |
| | RP24-187P11.4 | 0.387399 | 0.0062 | -1.36811 |
| | Fmo2 | 0.460795 | 0.001258 | -1.1178 |
| | Fam83d | 0.526235 | 0.001435 | -0.92622 |
| | D430018E03Rik | 0.528956 | 0.003668 | -0.91878 |
| | Gimap8 | 0.56715 | 0.00693 | -0.8182 |
| | RP23-240E15.3 | 0.59028 | 0.001395 | -0.76053 |
| | Gm12096 | 0.596641 | 0.00351 | -0.74506 |
| | 4930562C15Rik | 0.611463 | 0.008025 | -0.70966 |
| | Plekhf1 | 0.632531 | 0.002106 | -0.66079 |
| | Tspear | 0.643929 | 0.008464 | -0.63503 |
| | Pdk4 | 0.647249 | 0.005006 | -0.62761 |
| | Mertk | 0.649698 | 0.002805 | -0.62216 |
| | Gabrr2 | 0.662099 | 0.009429 | -0.59488 |
| | Rab39 | 0.685286 | 0.00643 | -0.54522 |
| | Tsc22d3 | 0.703499 | 0.00055 | -0.50738 |
| CD | Spata33 | 1.421879 | 0.001501 | 0.507799 |
| | Nes | 1.424234 | 0.000336 | 0.510186 |
| | S100a11 | 1.424654 | 0.005521 | 0.510611 |
| | Rab26os | 1.446832 | 0.003352 | 0.532898 |
| | Cnn2 | 1.449661 | 0.002662 | 0.535716 |
| | Gm37182 | 1.453536 | 0.000402 | 0.539567 |
| | Gm14966 | 1.470483 | 0.005872 | 0.55629 |
| | C230035I16Rik | 1.518406 | 0.005021 | 0.602558 |
| | Gm8173 | 1.536511 | 0.00517 | 0.619658 |
| | Fabp7 | 1.590592 | 0.00788 | 0.669564 |
| | Itgae | 1.605602 | 0.004798 | 0.683115 |
| | Sp7 | 1.612214 | 0.007503 | 0.689043 |
| | Alpk3 | 1.694101 | 0.003937 | 0.76052 |
| | Gm37333 | 1.722933 | 0.001706 | 0.784867 |
| | RP24-544J6.5 | 1.792102 | 0.002121 | 0.841653 |
| | Fat2 | 2.986068 | 0.007743 | 1.578247 |

CT n=6, CR n=5, CD n=6. Related to fig4.