# Figure S1

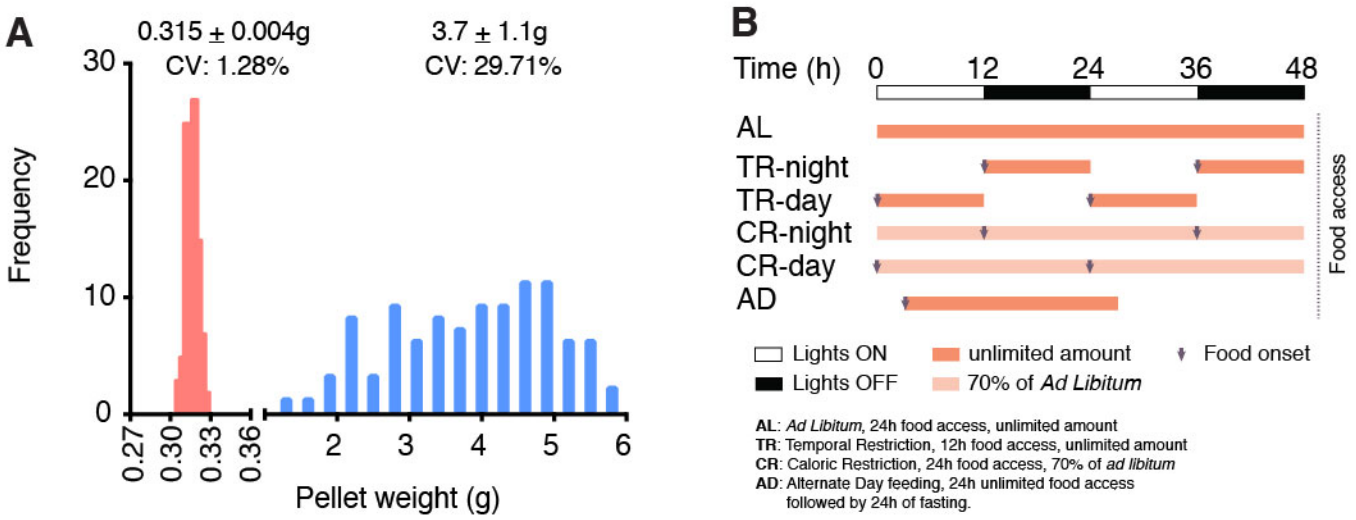



**Figure S1: Pellet Characterization and Feeding Protocol. Related to Figure 1, 2, 3 and 4.**

(A) Comparison of precision pellets and standard laboratory chow (2918) pellets.

(B) Scheme of the feeding conditions imposed after one week of baseline recording (n=6 mice /condition). Specific characteristics of each feeding condition are outlined in the table below the schematic diagram.

Figure S2

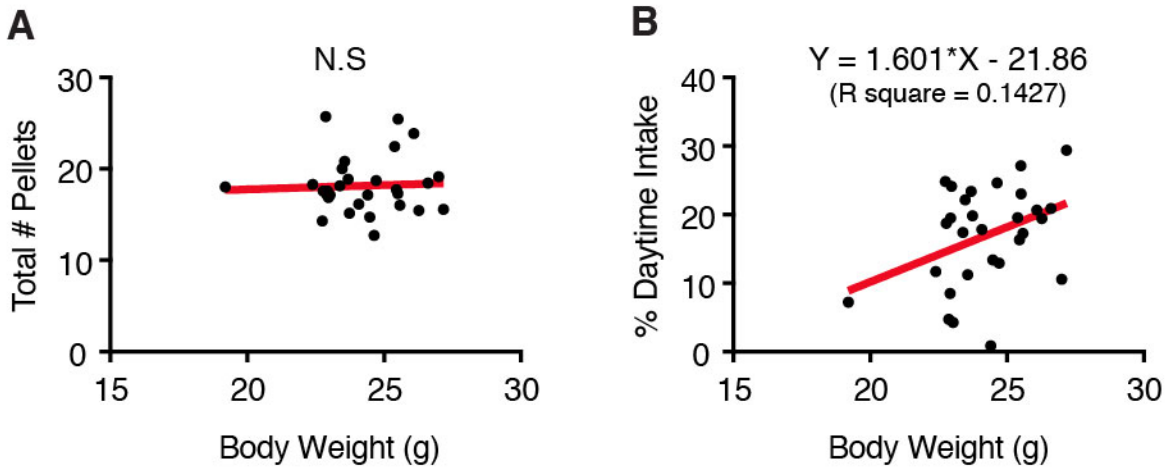
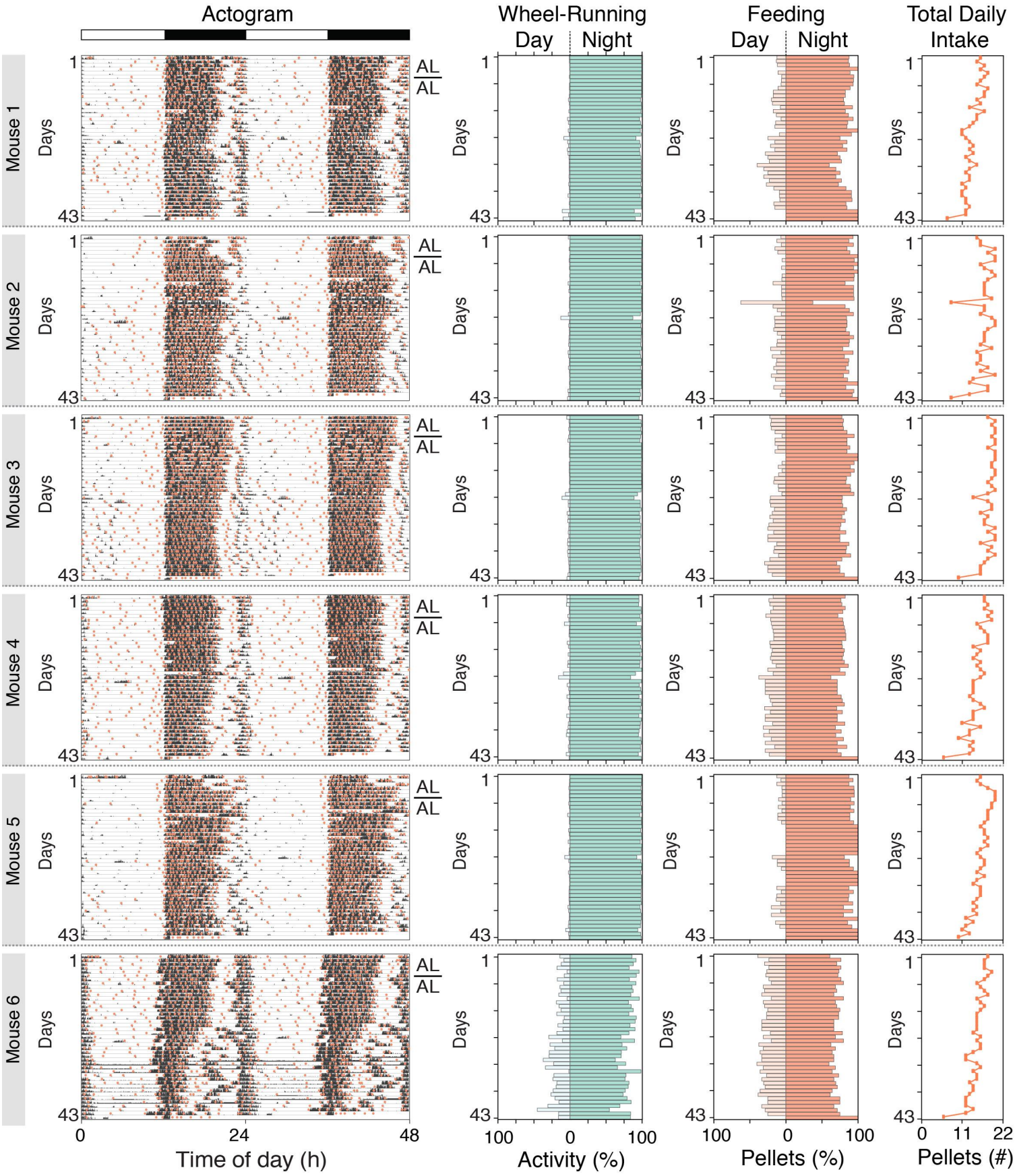


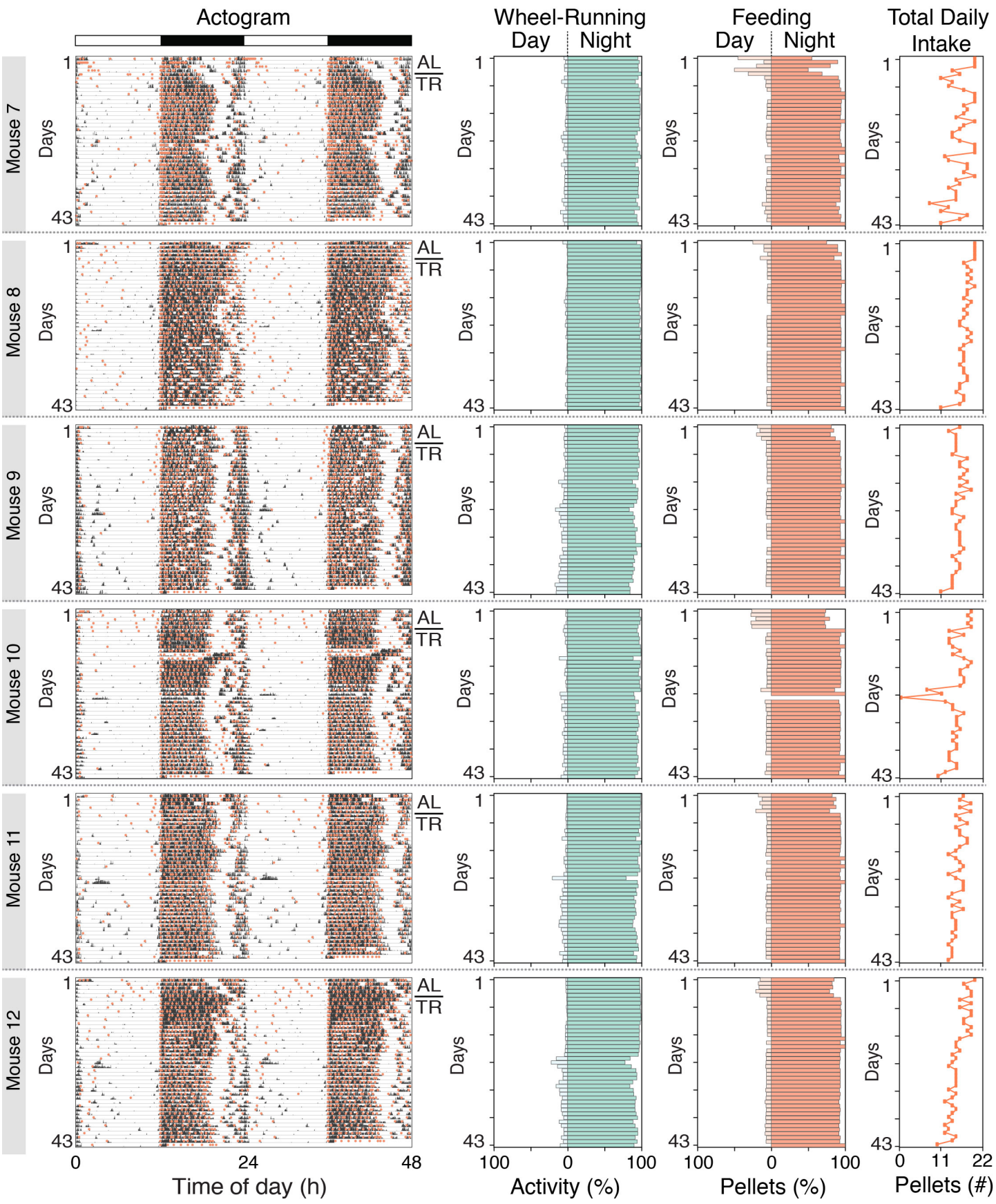

**Figure S2: Correlation of Body Weight, Total Daily Intake and % Daytime Feeding for Ad Libitum Fed Mice. Related to Figure 1.**

Body weight correlation with A) total daily intake and B) % daytime feeding for mice in AL condition. Mice were tested in 3 batches at 61d (n=11); 81d (n=12) and 88d (n=6) of age. Body weight was normalized to 61 days of age (n=29 mice). Linear regression indicates the slope in B) is different from zero; p=0.0433; F=4.494.

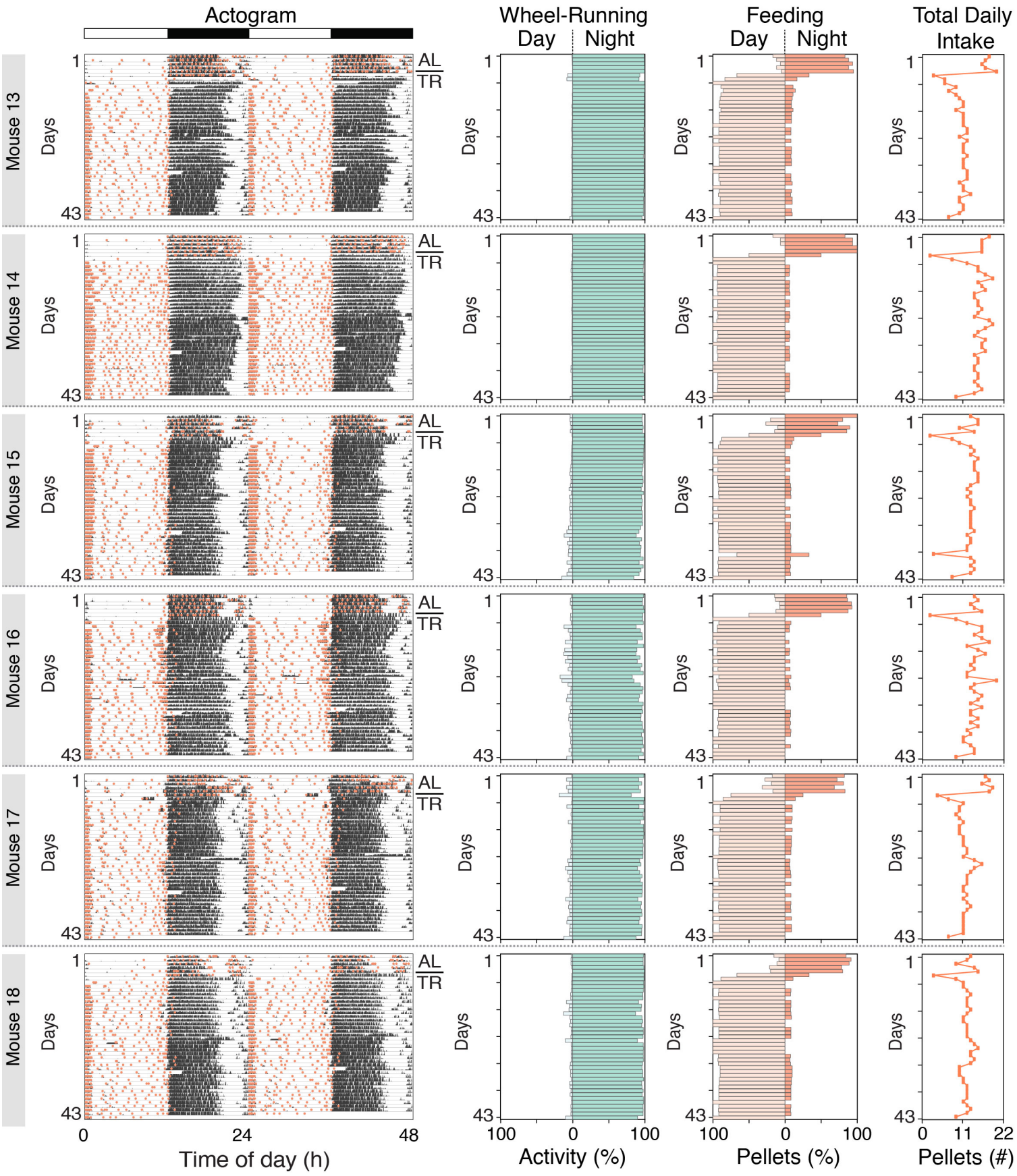
# Figure S3.A: AL



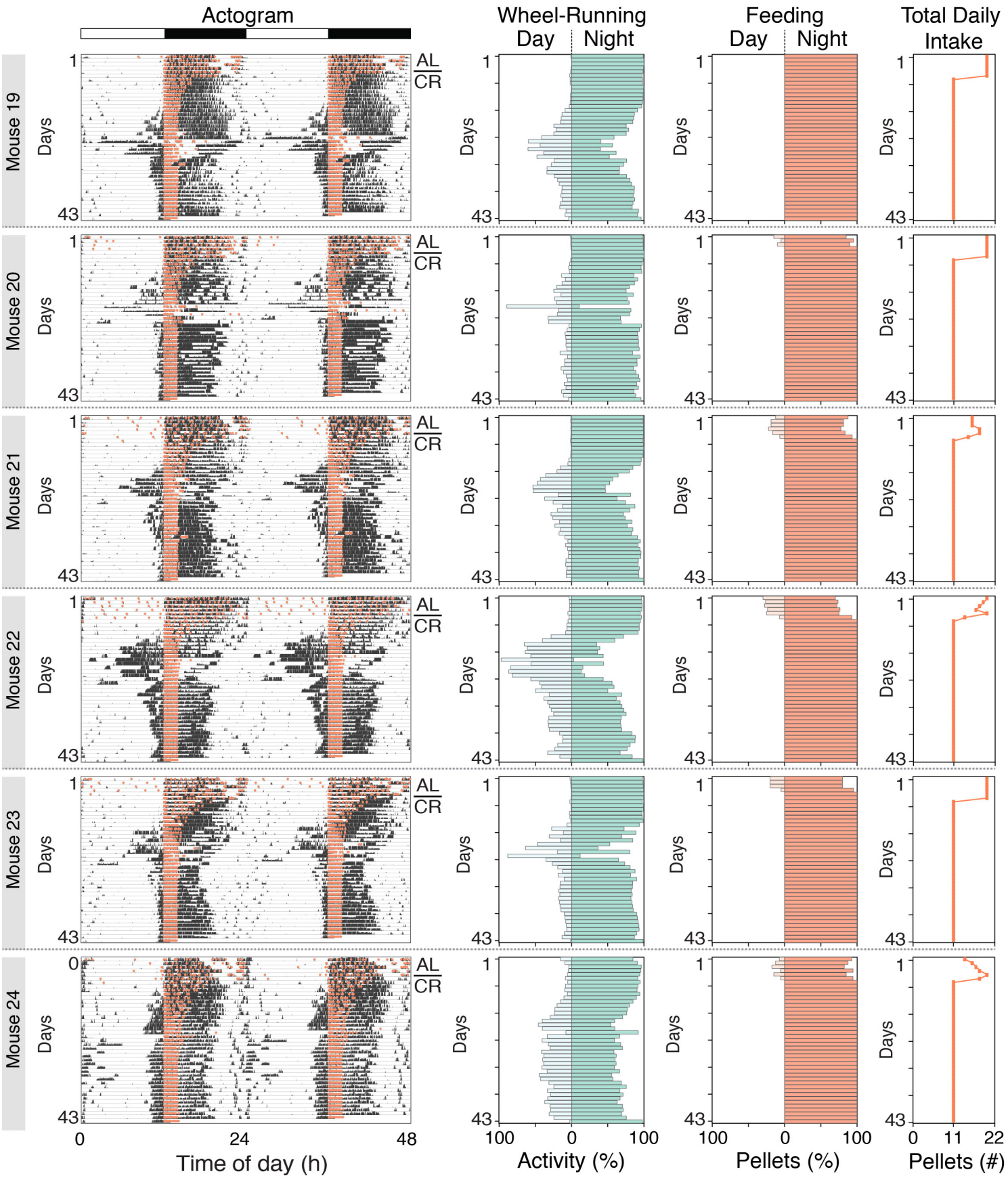
# Figure S3.B: TR-night



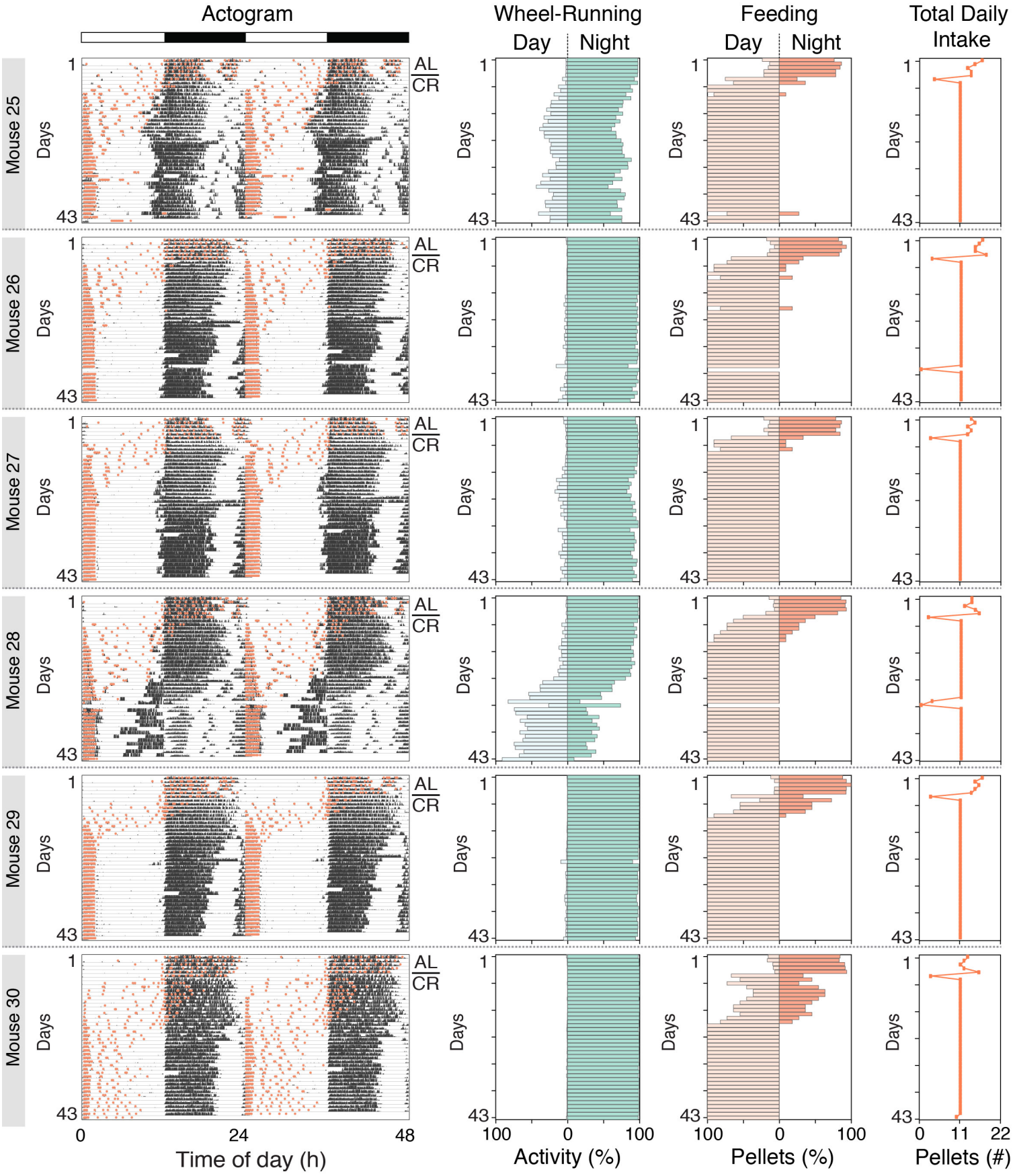
# Figure S3.C: TR-day



# Figure S3.D: CR-night



# Figure S3.E: CR-day



# Figure S3.F: AD

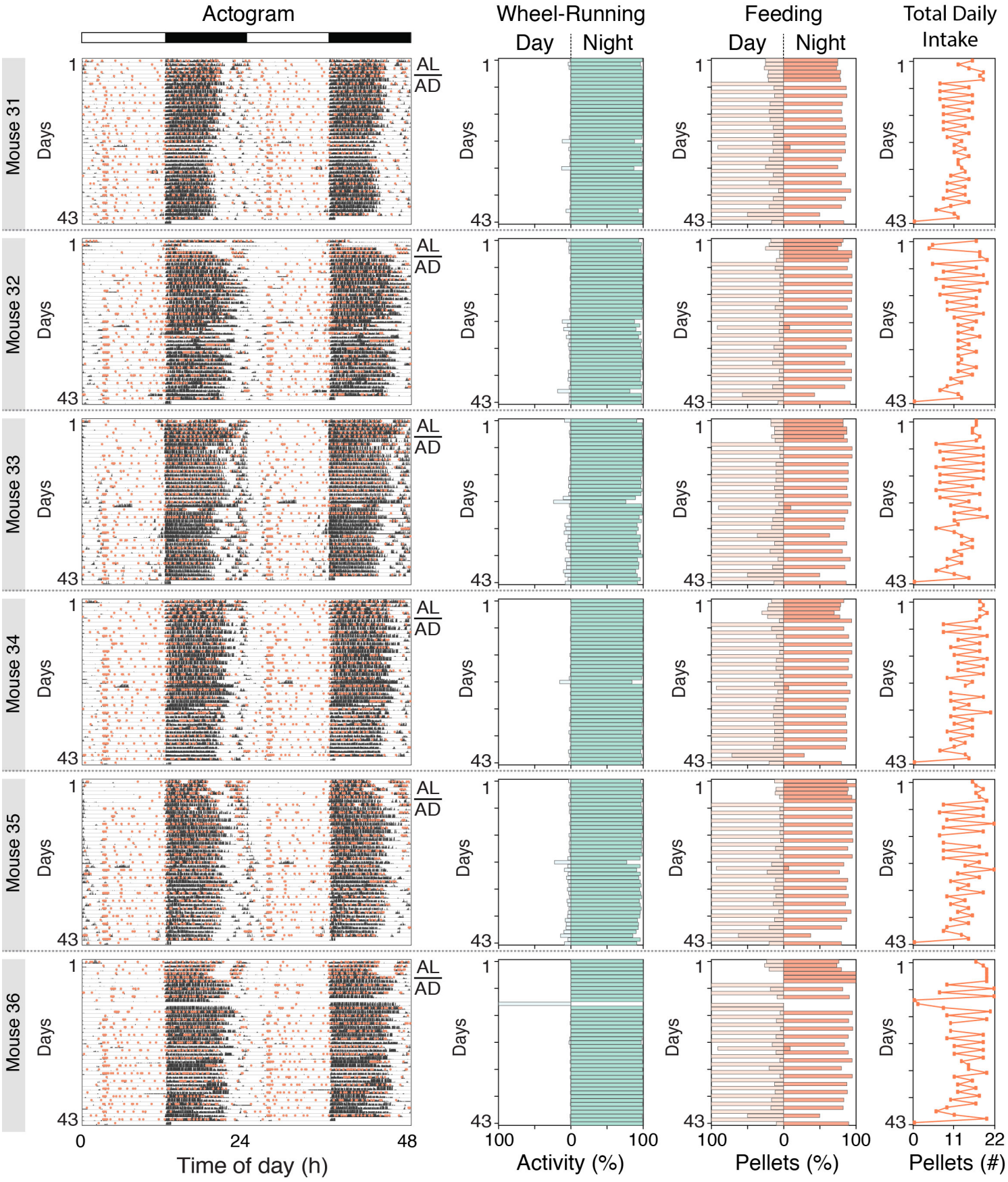


**Figure S3: Overlay Actograms, LD Distribution of Activity and LD Intake Patterns of Individual Mice. Related to Figures 2, 3 and 4.**

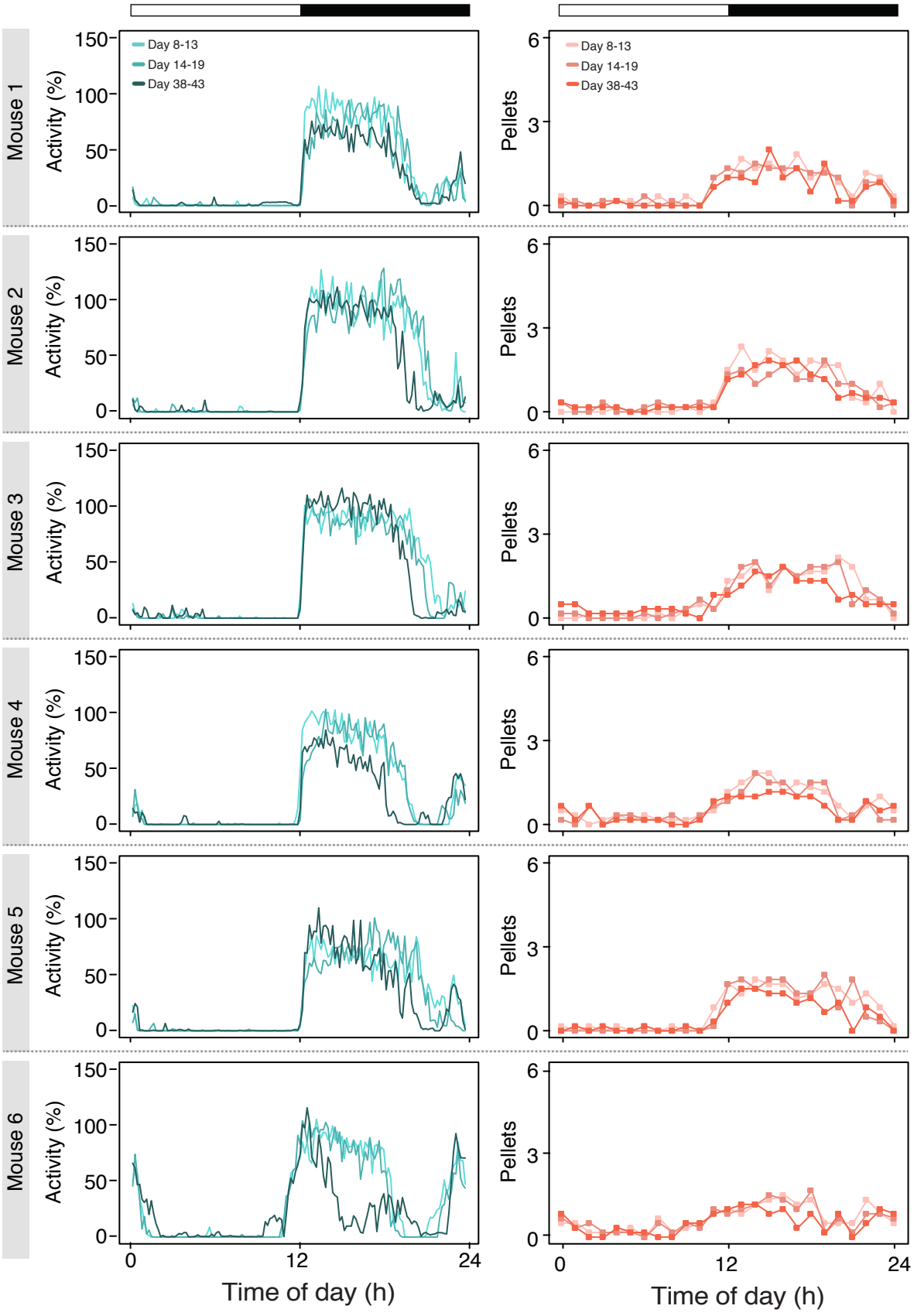
Left: Representative double-plotted actograms showing both wheel running (black bars) and pellets taken (pink dots) are plotted for each of the 6 mice in A (AL), B (TR-night), C (TR-day), D (CR-night), E (CR-day), and F (AD). The LD cycle is shown in the bar across the top. Pink shading represents time of food availability in each condition. All mice had free access to food for the first seven days of recording and were then subjected to the feeding conditions as denoted on the right side of the actogram.

Middle-left: A stacked histogram plot showing the wheel-running activity distributed during the day or the night along the 43 experimental days for each individual mouse in A (AL), B (TR-night), C (TR-day), D (CR-night), E (CR-day), and F (AD). Daytime activity is plotted as light-green bars extended from the center to the left. On the contrary, night activity recordings are plotted as green bars extended to the right.

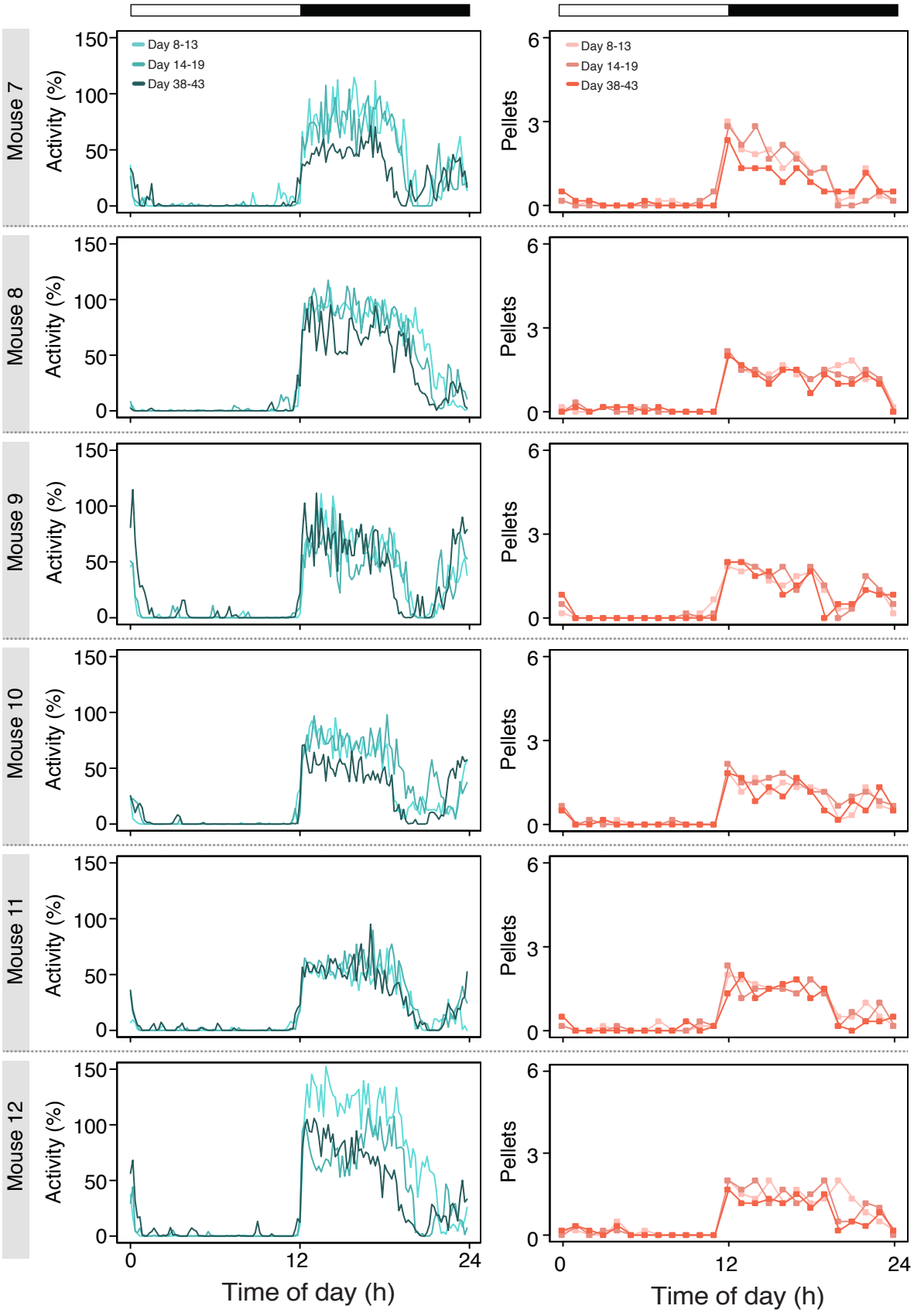
Middle-right: A stacked histogram plot displaying the food intake during the day or the night along the 43 experimental days for each individual mouse in A (AL), B (TR-night), C (TR-day), D (CR-night), E (CR-day), and F (AD). Light-pink bars extended from the center to the left represents the daytime activity, whereas, night activity recordings are plotted as pink bars extended to the right.

Right: Total number of pellets consumed for each day of the experiment for each individual mouse in A (AL), B (TR-night), C (TR-day), D (CR-night), E (CR-day), and F (AD).

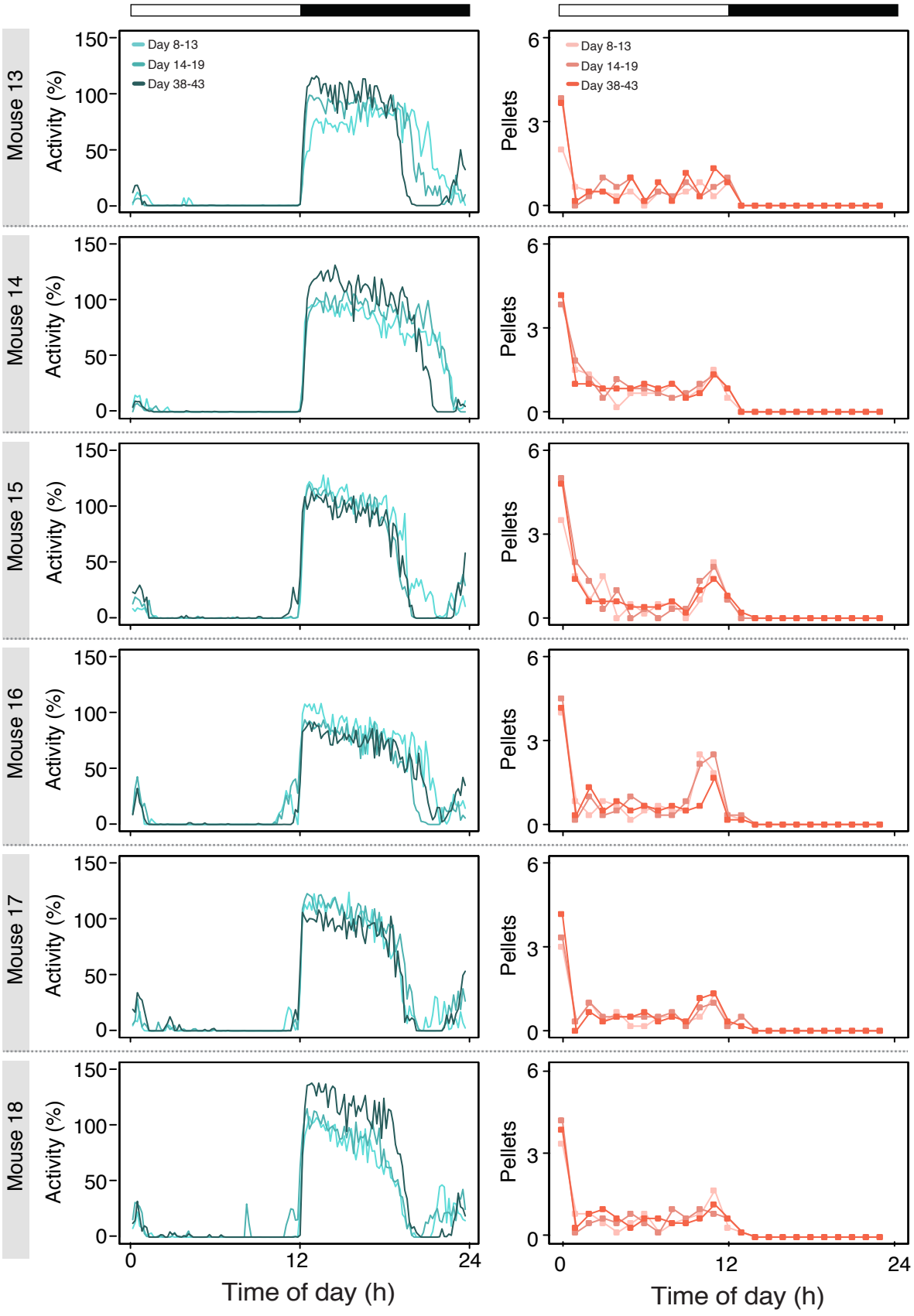
# Figure S4.A: AL



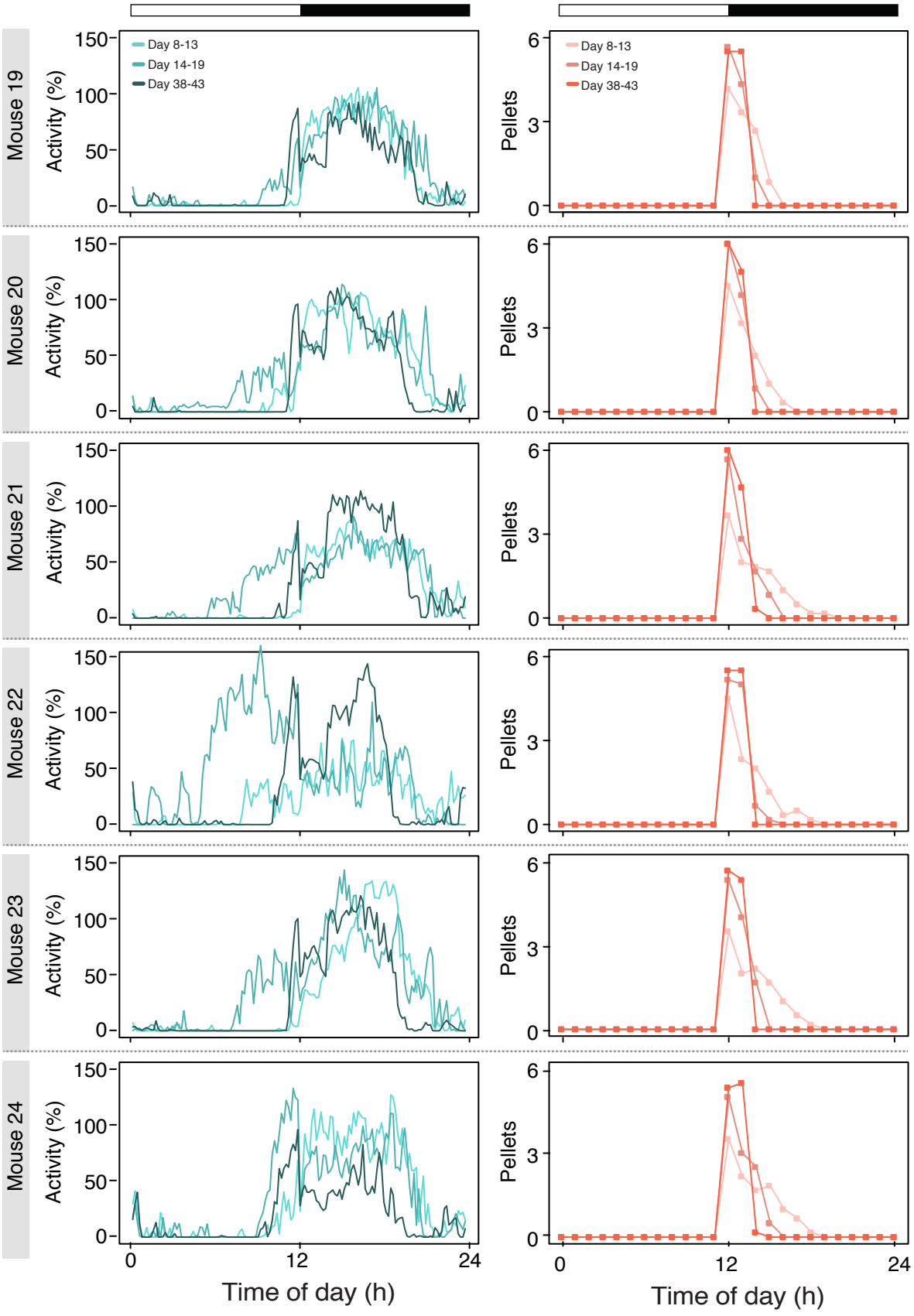
# Figure S4.B: TR-night



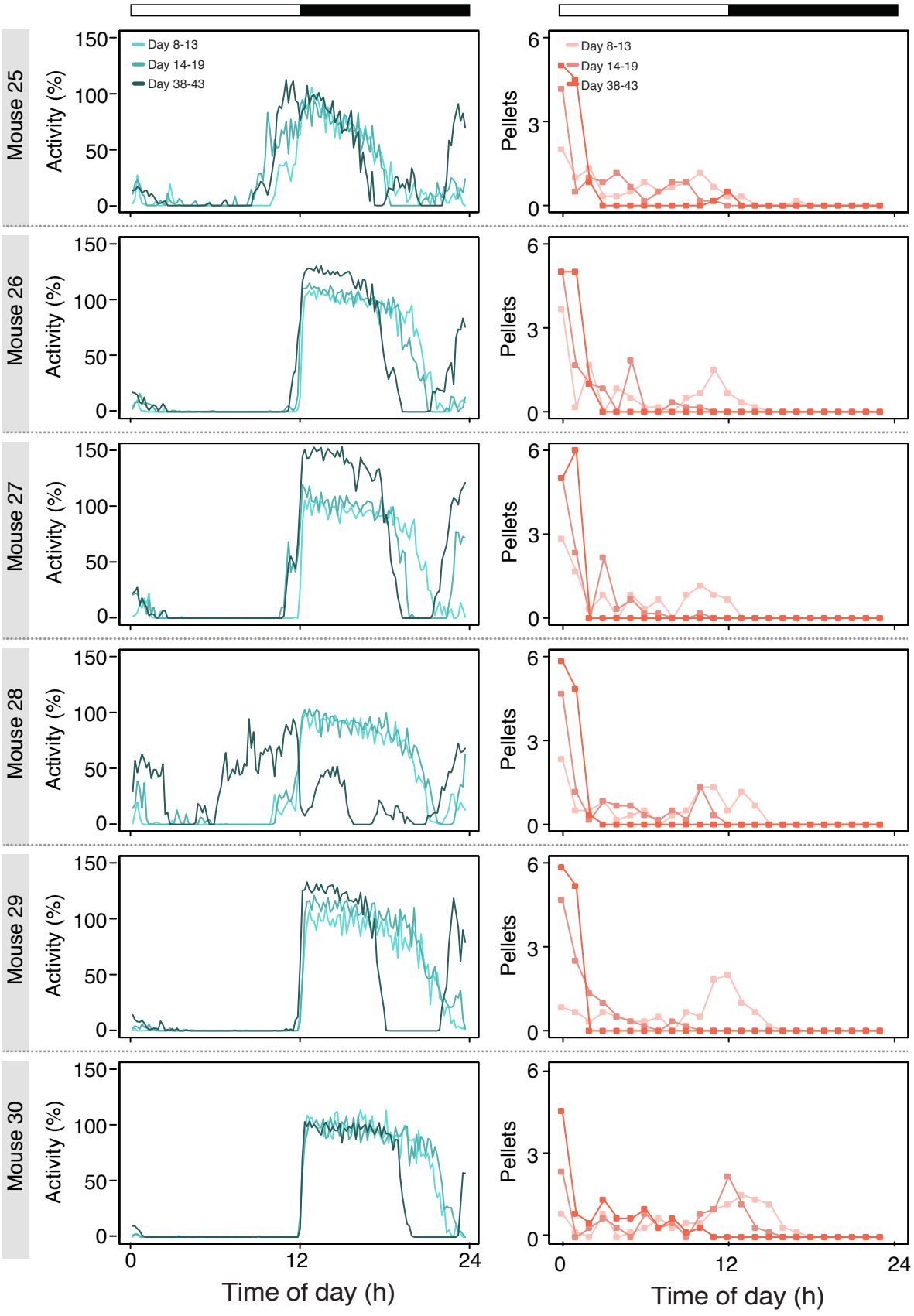
# Figure S4.C: TR-day



# Figure S4.D: CR-night



# Figure S4.E: CR-day



# Figure S4.F: AD

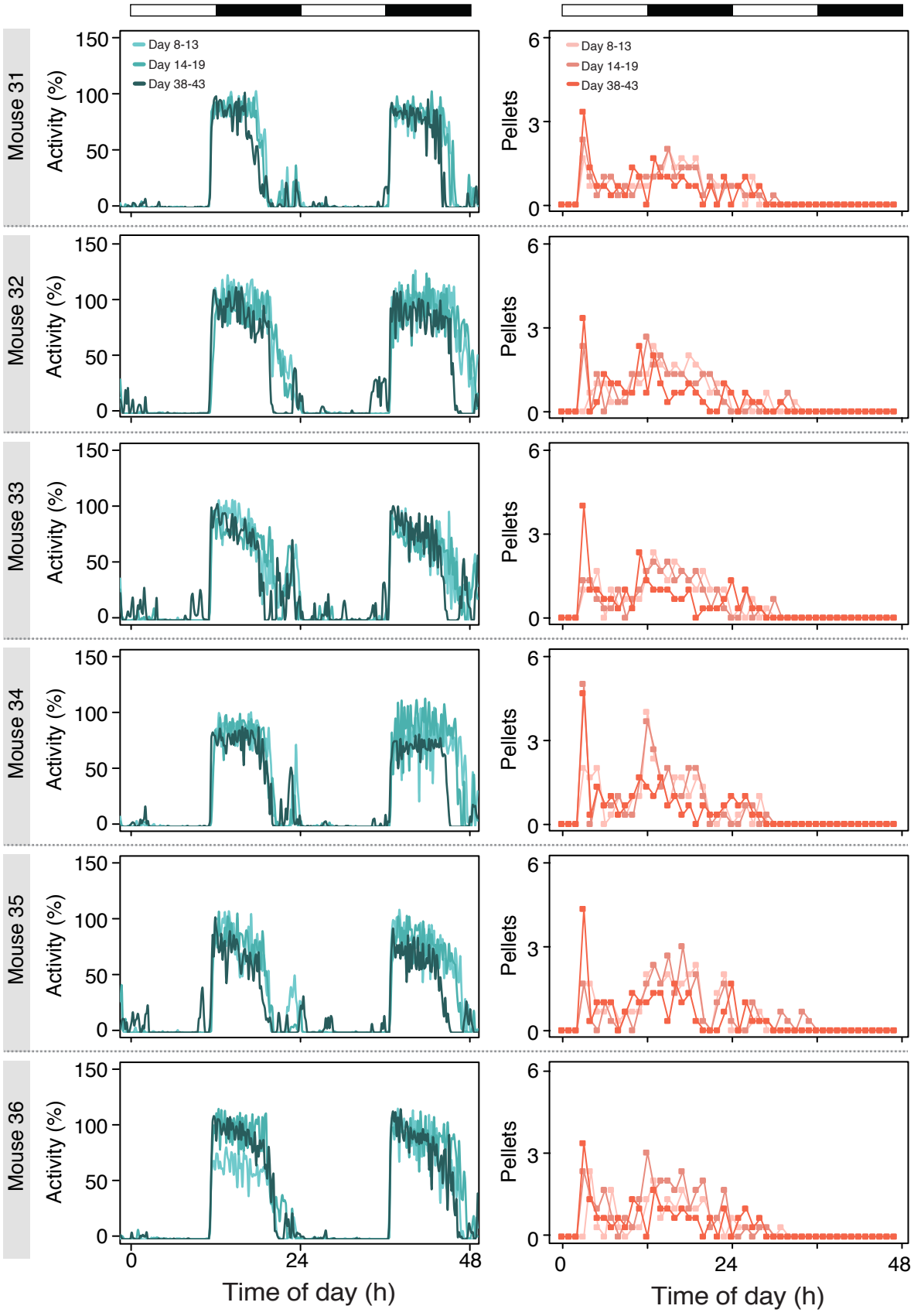


**Figure S4: Activity Patterns, Intake Patterns and Total Pellet Consumption for Individual Mice. Related to Figures 2, 3 and 4.**

Left: Average numbers of wheel revolutions recorded (normalized to maximum wheel counts recorded during baseline) as a function of time of day for individual mice in each of the feeding conditions for 6 day time blocks during the first, second and last week of recording under the imposed feeding paradigms are shown in A (AL), B (TR-night), C (TR-day), D (CR-night), E (CR-day), and F (AD). The LD cycle is shown in the bar across the top.

Right: Average number of pellets consumed as a function of time of day by individual mice in each of the feeding conditions for 6 day time blocks during the first, second and last week of recording under the imposed feeding paradigms is shown in A (AL), B (TR-night), C (TR-day), D (CR-night), E (CR-day), and F (AD). The LD cycle is shown in the bar across the top.

# Figure S5

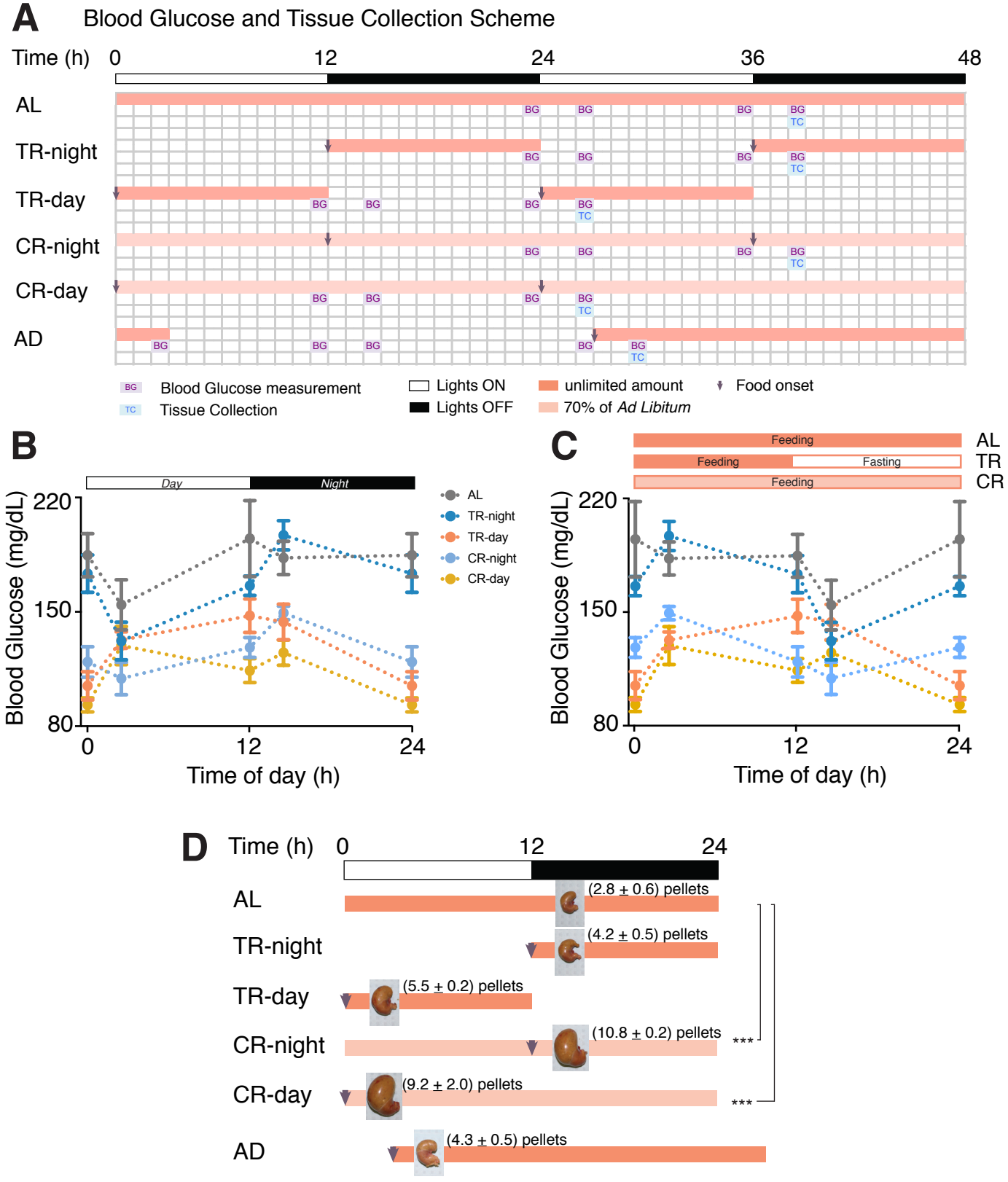



**Figure S5: Blood Glucose and Tissue Collection. Related to Figure 5.**
(A) Schematic of the schedule of the blood glucose samples taken from mice in each of the feeding conditions in relation to the LD cycle and food availability.
(B) Schematic of the schedule of final tissue collection in relation to the LD cycle and food availability.
(C) Blood glucose levels (± SD) plotted in relation to feeding time. Values measured just before food becomes available for ease of visualization. The LD cycle is shown in the bars across the top.
(D) Stomach size and pellet consumption (± SD) 2.5h after food onset. Note this is a combination of Fig 5C and 5E.

**Table S1: Comparison of Types of Experimenter Imposed Feeding Paradigms Used in Mouse Longevity Studies**

**Studies Limiting the Amount of Food (CR)**

| Phase | Frequency | Amount (% of AL) | Duration | Light Cycle | Housing Density | Length of Study | Reference |
|---|---|---|---|---|---|---|---|
| Day | Daily | 34% to 80% | Not stated or until consumed or 2.75 to 4h | LD 12:12 | Individually or group | 1300 to 1540 days | (Nelson and Halberg, 1986a) (Nelson and Halberg, 1986b) (Koizumi et al., 1996) (Ikeno et al., 2005) (McCarter et al., 2007) (McDonald et al., 2008) |
| | < once per day | 50% to 90% | Not stated or until consumed (3-4h) | LD12:12 or LD14:10 | Not stated or individually or group | 1200 to 1541 days | (Harrison et al., 1984) (Harrison and Archer, 1987) (Koizumi et al., 1992) (Dhahbi et al., 2004) |
| Night | Daily | 60% to 75% | Not stated or 2.75 to 4h | not stated or LD 12:12 | Not stated or individually or group | 1100 to 1650 days | (Nelson and Halberg, 1986a) (Nelson and Halberg, 1986b) (Forster et al., 2003) (Flurkey et al., 2010) (Liao et al., 2010) (Patel et al., 2016) |
| | > once per day (6 meals) | 75% | 9-12 min | LD12:12 Lon 6:00 | Group | 1526 days | (Nelson and Halberg, 1986a) |
| Not stated or morning or evening | Daily | 50% to 95% | Not stated | not stated or LD12:12 or LD16:8 | Individually or group | 55 to 1580 days | (Ball et al., 1947) (Fernandes et al., 1976) (Fernandes et al., 1978) (Means et al., 1993) (Blackwell et al., 1995) (Sheldon et al., 1995) (Troyer et al., 1995) (Turturro et al., 1999) (Turturro et al., 2002) (Buschemeyer et al., 2010) |
| Not stated or in the morning | < once per day | 36% to 95% | Not stated or until consumed | not stated or LD12:12 | Not stated or individually or group | 1300 to 1650 days | (Cheney et al., 1980) (Weindruch and Walford, 1982) (Weindruch et al., 1986) (Pugh et al., 1999) (Lee et al., 2004) (Rikke et al., 2010) |
| Not stated | Not stated | 55% to 75% | Not stated | not stated or LD12:12 or LD14:10 | Not stated or individually or group | 308 to 1680 days | (Volk et al., 1994) (Yoshida et al., 1997) (Mattison et al., 2000) (Bartke et al., 2001) (Bonkowski et al., 2006) (Harper et al., 2006) (Boily et al., 2008) (Pearson et al., 2008b) (Harper et al., 2010) |

**Studies Limiting the Frequency or Duration of Food Access (TR or AD/EOD or IF)**

| Phase | Frequency | Amount | Duration | Light Cycle | Housing Density | Length of Study | Reference |
|---|---|---|---|---|---|---|---|
| Day or Night | Daily (TR) | *Ad libitum* | 4h or 6h | LD12:12 Lon 9:00 or 21:00 | Individually | 30 days | (Wu et al., 2004) |
| Night | Daily (TR) | *Ad libitum* | 14h | LD10:14 Lon 8:00 | Group | 170 days | (Skillings et al., 2014) |
| Not stated | < once per day (AD/EOD) | *Ad libitum* | 24h | LD12:12 | Not stated or group | 1120 to 1400 days | (Talan and Ingram, 1985) (Goodrick et al., 1990) (Pearson et al., 2008a) (Arum et al., 2009) |
| Not stated | < once per day (IF) | *Ad libitum* | N/A | Not stated | Individually or group | 55 to 90 days | (Buschemeyer et al., 2010) (Thomas et al., 2010) |

For studies in which food intake is manipulated, researchers have controlled the phase, frequency, amount and duration of the food made available to the mice.

**DEFINITIONS**

**Phase:** the time that food is made available to the mouse. This can be during the day (light phase) when the mouse is normally resting, or during the night (dark phase) when the mouse would be active and would normally be consuming most of its food. All of the various feeding paradigms, except for AL, involve this parameter, but only a small number of the papers surveyed included the time or phase of the day that the mice were fed.

**Frequency:** the number of times food is presented to the mouse during a given period of time. This can be more than once per day (as in meal feeding), once per day (CR, TR), or less than once per day (AD/EOD/IF).

**Amount:** the amount of food or food component (i.e. macronutrient) that is made available to the mouse. This can be either in excess (AL) of what a mouse would consume (TR, AD/EOD/IF), or some percentage of normal daily intake (CR).

**Duration:** the length of time that the food is available to the mouse. This can be until it is consumed (CR, in most cases this is not measured), or until it is removed after a specified amount of time (TR, AD/EOD/IF).

**AL – *ad libitum*:** food made available in excess of normal consumption at all times.

**AD – alternate day** (also referred to as EOD – every other day)**:** food (in excess of normal consumption or AL) is provided for 24 h (feeding day) and is then withdrawn for 24 h (fasting day).

**CR – caloric restriction** (also referred to as DR – dietary restriction)**:** a percentage (range 34% to 95%) of normal or baseline intake level when food is freely available, is provided once daily or in portions throughout the week.

**IF – intermittent fasting:** food is withheld for one or more days per week

**TR – temporal restriction:** food is made available in excess of normal consumption (or AL) for a limited amount of time each day (e.g. only during the night or only during the day), and is removed thereafter.

**SUPPLEMENTAL REFERENCES**

Arum, O., Bonkowski, M.S., Rocha, J.S., and Bartke, A. (2009). The growth hormone receptor gene-disrupted mouse fails to respond to an intermittent fasting diet. Aging Cell *8*, 756-760.

Ball, Z.B., Barnes, R.H., and Visscher, M.B. (1947). The effects of dietary caloric restriction on maturity and senescence, with particular reference to fertility and longevity. Am J Physiol *150*, 511-519.

Bartke, A., Wright, J.C., Mattison, J.A., Ingram, D.K., Miller, R.A., and Roth, G.S. (2001). Extending the lifespan of long-lived mice. Nature *414*, 412.

Blackwell, B.N., Bucci, T.J., Hart, R.W., and Turturro, A. (1995). Longevity, body weight, and neoplasia in ad libitum-fed and diet-restricted C57BL6 mice fed NIH-31 open formula diet. Toxicol Pathol *23*, 570-582.

Boily, G., Seifert, E.L., Bevilacqua, L., He, X.H., Sabourin, G., Estey, C., Moffat, C., Crawford, S., Saliba, S., Jardine, K., et al. (2008). SirT1 regulates energy metabolism and response to caloric restriction in mice. PLoS One *3*, e1759.

Bonkowski, M.S., Rocha, J.S., Masternak, M.M., Al Regaiey, K.A., and Bartke, A. (2006). Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. Proc Natl Acad Sci U S A *103*, 7901-7905.

Buschemeyer, W.C., 3rd, Klink, J.C., Mavropoulos, J.C., Poulton, S.H., Demark-Wahnefried, W., Hursting, S.D., Cohen, P., Hwang, D., Johnson, T.L., and Freedland, S.J. (2010). Effect of intermittent fasting with or without caloric restriction on prostate cancer growth and survival in SCID mice. Prostate *70*, 1037-1043.

Cheney, K.E., Liu, R.K., Smith, G.S., Leung, R.E., Mickey, M.R., and Walford, R.L. (1980). Survival and disease patterns in C57BL/6J mice subjected to undernutrition. Exp Gerontol *15*, 237-258.

Dhahbi, J.M., Kim, H.J., Mote, P.L., Beaver, R.J., and Spindler, S.R. (2004). Temporal linkage between the phenotypic and genomic responses to caloric restriction. Proc Natl Acad Sci U S A *101*, 5524-5529.

Fernandes, G., Yunis, E.J., and Good, R.A. (1976). Influence of diet on survival of mice. Proc Natl Acad Sci U S A *73*, 1279-1283.

Fernandes, G., Yunis, E.J., Miranda, M., Smith, J., and Good, R.A. (1978). Nutritional inhibition of genetically determined renal disease and autoimmunity with prolongation of life in kdkd mice. Proc Natl Acad Sci U S A *75*, 2888-2892.

Flurkey, K., Astle, C.M., and Harrison, D.E. (2010). Life Extension by Diet Restriction and N-Acetyl-l-Cysteine in Genetically Heterogeneous Mice. J Gerontol A Biol Sci Med Sci *65*, 1275-1284.

Forster, M.J., Morris, P., and Sohal, R.S. (2003). Genotype and age influence the effect of caloric intake on mortality in mice. FASEB J *17*, 690-692.

Goodrick, C.L., Ingram, D.K., Reynolds, M.A., Freeman, J.R., and Cider, N. (1990). Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. Mech Ageing Dev *55*, 69-87.

Harper, J.M., Leathers, C.W., and Austad, S.N. (2006). Does caloric restriction extend life in wild mice? Aging Cell *5*, 441-449.

Harper, J.M., Wilkinson, J.E., and Miller, R.A. (2010). Macrophage migration inhibitory factor-knockout mice are long lived and respond to caloric restriction. FASEB J *24*, 2436-2442.

Harrison, D.E., and Archer, J.R. (1987). Genetic differences in effects of food restriction on aging in mice. J Nutr *117*, 376-382.

Harrison, D.E., Archer, J.R., and Astle, C.M. (1984). Effects of food restriction on aging: separation of food intake and adiposity. Proc Natl Acad Sci U S A *81*, 1835-1838.

Ikeno, Y., Hubbard, G.B., Lee, S., Richardson, A., Strong, R., Diaz, V., and Nelson, J.F. (2005). Housing density does not influence the longevity effect of calorie restriction. J Gerontol A Biol Sci Med Sci *60*, 1510-1517.

Koizumi, A., Tsukada, M., Wada, Y., Masuda, H., and Weindruch, R. (1992). Mitotic activity in mice is suppressed by energy restriction-induced torpor. J Nutr *122*, 1446-1453.

Koizumi, A., Wada, Y., Tuskada, M., Kayo, T., Naruse, M., Horiuchi, K., Mogi, T., Yoshioka, M., Sasaki, M., Miyamaura, Y., et al. (1996). A tumor preventive effect of dietary restriction is antagonized by a high housing temperature through deprivation of torpor. Mech Ageing Dev *92*, 67-82.

Lee, C.K., Pugh, T.D., Klopp, R.G., Edwards, J., Allison, D.B., Weindruch, R., and Prolla, T.A. (2004). The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. Free Radic Biol Med *36*, 1043-1057.

Liao, C.Y., Rikke, B.A., E., J.T., Diaz, V., and Nelson, J.F. (2010). Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. Aging Cell *9*, 92-95.

Mattison, J.A., Wright, C., Bronson, R.T., Roth, G.S., Ingram, D.K., and Bartke, A. (2000). Studies of aging in ames dwarf mice: Effects of caloric restriction. J Am Aging Assoc *23*, 9-16.

McCarter, R., Mejia, W., Ikeno, Y., Monnier, V., Kewitt, K., Gibbs, M., McMahan, A., and Strong, R. (2007). Plasma glucose and the action of calorie restriction on aging. J Gerontol A Biol Sci Med Sci *62*, 1059-1070.

McDonald, R.B., Walker, K.M., Warman, D.B., Griffey, S.M., Warden, C.H., Ramsey, J.J., and Horwitz, B.A. (2008). Characterization of survival and phenotype throughout the life span in UCP2/UCP3 genetically altered mice. Exp Gerontol *43*, 1061-1068.

Means, L.W., Higgins, J.L., and Fernandes, T.J. (1993). Mid-Life Onset of Dietary Restriction Extends Life and Prolongs Cognitive Function. Physiol Behav *54*, 503-508.

Nelson, W., and Halberg, F. (1986a). Meal-timing, circadian rhythms and life span of mice. J Nutr *116*, 2244-2253.

Nelson, W., and Halberg, F. (1986b). Schedule-shifts, circadian rhythms and lifespan of freely-feeding and meal-fed mice. Physiol Behav *38*, 781-788.

Patel, S.A., Chaudhari, A., Gupta, R., Velingkaar, N., and Kondratov, R.V. (2016). Circadian clocks govern calorie restriction-mediated life span extension through BMAL1- and IGF-1-dependent mechanisms. FASEB J *30*, 1634-1642.

Pearson, K.J., Baur, J.A., Lewis, K.N., Peshkin, L., Price, N.L., Labinskyy, N., Swindell, W.R., Kamara, D., Minor, R.K., Perez, E., et al. (2008a). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab *8*, 157-168.

Pearson, K.J., Lewis, K.N., Price, N.L., Chang, J.W., Perez, E., Cascajo, M.V., Tamashiro, K.L., Poosala, S., Csiszar, A., Ungvari, Z., et al. (2008b). Nrf2 mediates cancer protection but not prolongevity induced by caloric restriction. Proc Natl Acad Sci U S A *105*, 2325-2330.

Pugh, T.D., Oberley, T.D., and Weindruch, R. (1999). Dietary intervention at middle age: caloric restriction but not dehydroepiandrosterone sulfate increases lifespan and lifetime cancer incidence in mice. Cancer Res *59*, 1642-1648.

Rikke, B.A., Liao, C.Y., McQueen, M.B., Nelson, J.F., and Johnson, T.E. (2010). Genetic dissection of dietary restriction in mice supports the metabolic efficiency model of life extension. Exp Gerontol *45*, 691-701.

Sheldon, W.G., Bucci, T.J., Hart, R.W., and Turturro, A. (1995). Age-related neoplasia in a lifetime study of ad libitum-fed and food-restricted B6C3F1 mice. Toxicol Pathol *23*, 458-476.

Skillings, E.A., Wood, N.I., and Morton, A.J. (2014). Beneficial effects of environmental enrichment and food entrainment in the R6/2 mouse model of Huntington's disease. Brain Behav *4*, 675-686.

Talan, M.I., and Ingram, D.K. (1985). Effect of intermittent feeding on thermoregulatory abilities of young and aged C57BL/6J mice. Arch Gerontol Geriatr *4*, 251-259.

Thomas, J.A., 2nd, Antonelli, J.A., Lloyd, J.C., Masko, E.M., Poulton, S.H., Phillips, T.E., Pollak, M., and Freedland, S.J. (2010). Effect of intermittent fasting on prostate cancer tumor growth in a mouse model. Prostate Cancer Prostatic Dis *13*, 350-355.

Troyer, D.A., Chandrasekar, B., Thinnes, T., Stone, A., Loskutoff, D.J., and Fernandes, G. (1995). Effects of Energy Intake on Type 1 Plasminogen Activator Inhibitor Levels in Glomeruli of Lupus-Prone B/W mice. Am J Pathol *146*, 111-120.

Turturro, A., Duffy, P., Hass, B., Kodell, R., and Hart, R. (2002). Survival characteristics and age-adjusted disease incidences in C57BL/6 mice fed a commonly used cereal-based diet modulated by dietary restriction. J Gerontol A Biol Sci Med Sci *57*, B379-389.

Turturro, A., Witt, W.W., Lewis, S., Hass, B.S., Lipman, R.D., and Hart, R.W. (1999). Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. J Gerontol A Biol Sci Med Sci *54*, B492-501.

Volk, M.J., Pugh, T.D., Kim, M., Frith, C.H., Daynes, R.A., Ershler, W.B., and Weindruch, R. (1994). Dietary restriction from middle age attenuates age-associated lymphoma development and interleukin 6 dysregulation in C57BL/6 mice. Cancer Res *54*, 3054-3061.

Weindruch, R., and Walford, R.L. (1982). Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. Science *215*, 1415-1418.

Weindruch, R., Walford, R.L., Fligiel, S., and Guthrie, D. (1986). The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. J Nutr *116*, 641-654.

Wu, M.W., Li, X.M., Xian, L.J., and Levi, F. (2004). Effects of meal timing on tumor progression in mice. Life Sci *75*, 1181-1193.

Yoshida, K., Inoue, T., Nojima, K., Hirabayashi, Y., and Sado, T. (1997). Calorie restriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in C3H/He mice. Proc Natl Acad Sci U S A *94*, 2615-2619.

**Table S2:** Comparison of available systems for measuring food intake in rodents

| Feeder | Food Intake Quantification Method | Control timing of food access | Control amount of food access | Standard Wheel Cages[i] | Pellet type | Maximum housing duration | Fits in light tight cabinet |
|---|---|---|---|---|---|---|---|
| **Actimetrics** [a] | Counts by sensor | yes | yes | yes | Precision | unlimited | yes |
| **FED** [b] | Counts by sensor | no | no | no | Precision | 7 days | yes |
| **Coulbourn Instruments** [c] | Counts by sensor | no | no | no | Standard or precision | not specified | no |
| **CLAMS** [d] | Weight with scale | yes | yes | no | Standard (powdered) | 3 months | no |
| **BioDAQ** [e] | Counts by sensor | yes | no | no | Standard or precision | unlimited | no |
| **Bioseb** [f] | Weight with scale | no | no | no | Standard or precision | not specified | no |
| **Promethion** [g] | Counts by sensor | not specified | not specified | no | Standard or precision | not specified | no |
| **PhenoMaster** [h] | Weight with scale | yes | yes | no | Standard or precision | not specified | no |

[a] http://actimetrics.com/products/clocklab/clocklab-feeder-control/

[b] Nguyen et al., 2016

[c] http://www.coulbourn.com/product_p/h14-23m.htm

[d] http://www.colinst.com/products/clams-comprehensive-lab-animal-monitoring-system

[e] http://www.researchdiets.com/biodaq

[f] http://www.bioseb.com/bioseb/anglais/default/item_id=23_cat_id=6_Food%20and%20drink%20monitoring%20system.php

[g] http://www.sablesys.com/products/promethion-line/promethion-high-definition-behavioral-phenotyping-system-for-mice/

[h] http://www.tse-systems.com/products/behavior/home-cage/phenomaster/index.htm

[i] Standard polycarbonate mouse cages containing a 4.75 in diameter stainless steel running wheel (Siepka and Takahashi, 2005).