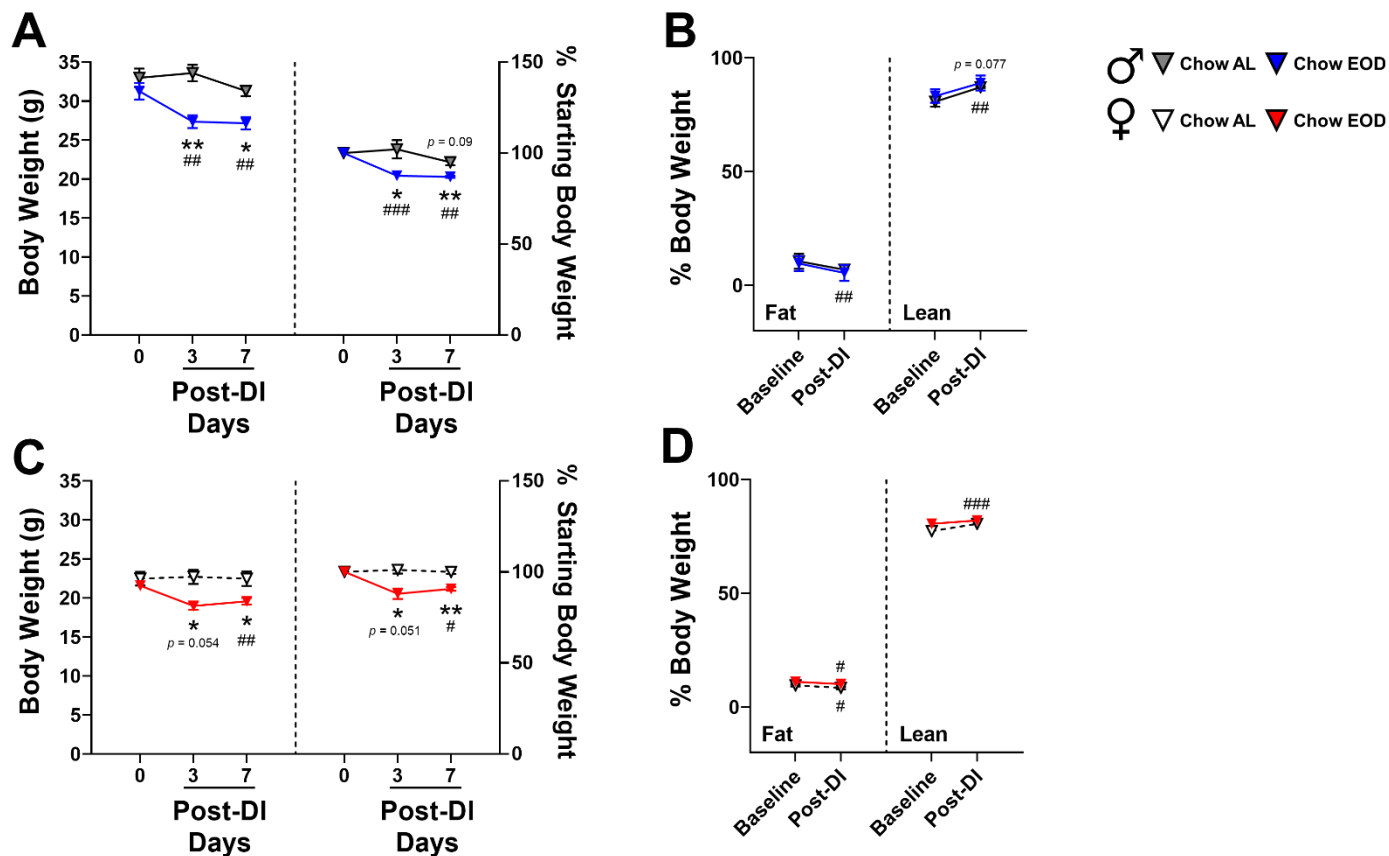
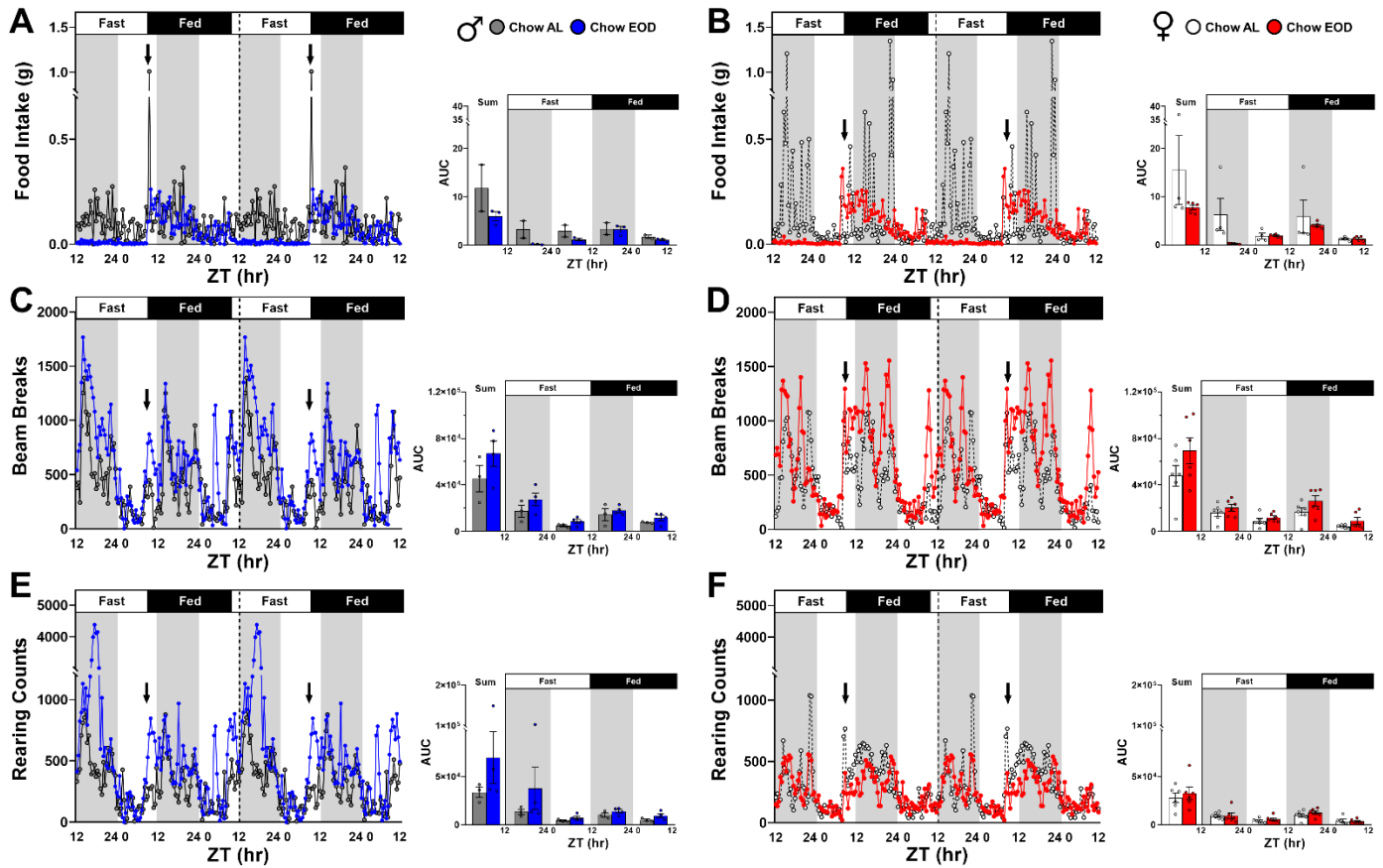


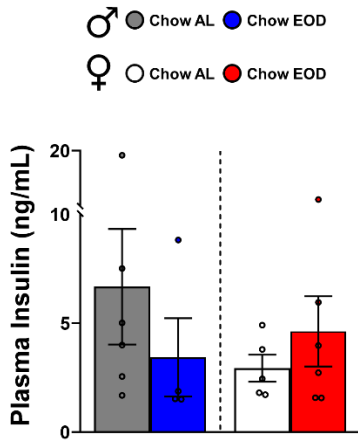
Supplemental Data Figures and their Legends for both review purposes and intended for publication as an online data supplement for the manuscript titled “**Late-life intermittent fasting decreases aging-related frailty and increases renal hydrogen sulfide production in a sexually dimorphic manner**” by Yoko O. Henderson, Nazmin Bithi, Christopher Link, Jie Yang, Rebecca Schugar, Natalia Llarena, J. Mark Brown, and Christopher Hine



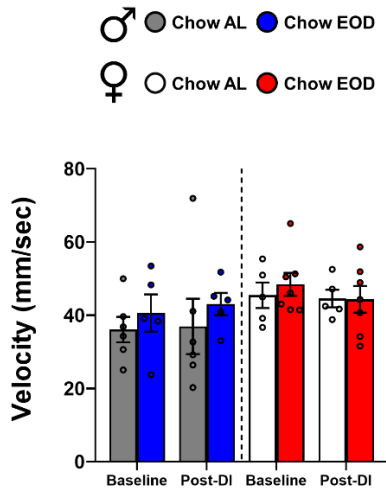
Supplemental Figure 1: EOD fasting decreases body weight and alters body composition in a sexually dimorphic manner in young adult mice. (A-D) Body mass and body composition in 6-month old male and female mice on Chow AL and Chow EOD feeding. Absolute body weight (*left*) and % body weight compared with baseline (*right*) in the male (**A**) and female (**C**) Chow AL and Chow EOD groups from baseline to post-dietary intervention (post-DI). (**B, D**) % fat mass adjusted to body weight (*left*) and % lean mass adjusted to body weight (*right*) in the male (**B**) and female (**D**) Chow AL and Chow EOD groups at baseline and post-DI. The figures depict the mean with error bars (\pm SEM) and $n = 3$ mice/diet group per sex. The asterisks indicate the significant difference between the same-sex Chow AL and Chow EOD groups. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. The pound signs indicate the significant within group difference between the baseline and a post-DI time point. $\#p < 0.05$, $\##p < 0.01$, $\###p < 0.001$, and $\####p < 0.0001$. See also Figure 1.



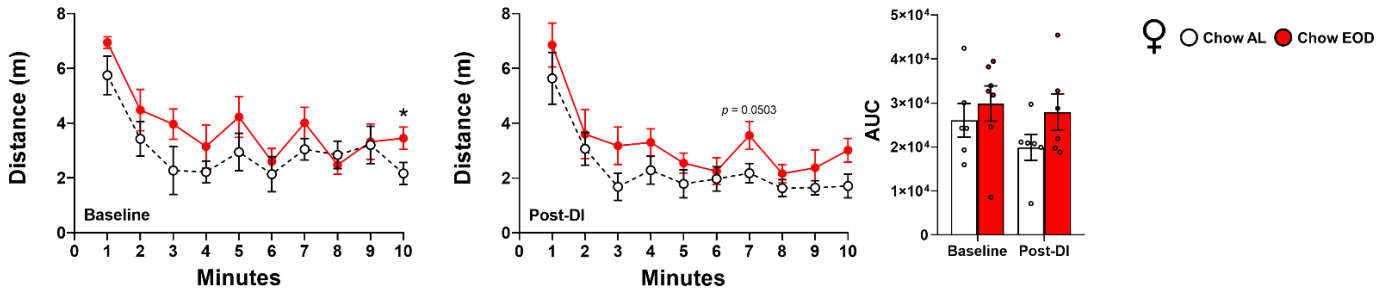
Supplemental Figure 2: Late-life EOD fasting does not affect circadian patterns of food intake, ambulation, or rearing. (A-F) Food intake (g) per mouse, beam breaks, and rearing counts over the 4-day period in the metabolic chamber were measured approximately every 20 min in the male (A, C, E) and female (B, D, F) Chow AL and Chow EOD groups ($n = 3-6$ mice per sex per group). The figures (A-F) depict the mean with no error bars for a representation purpose, while the insets show the AUC for combined/summation of area, fast day 12:12 light:dark cycle, and fed day 12:12 light:dark cycle for each measure. The inset figures depict the mean with error bars (\pm SEM). See also Figure 2.



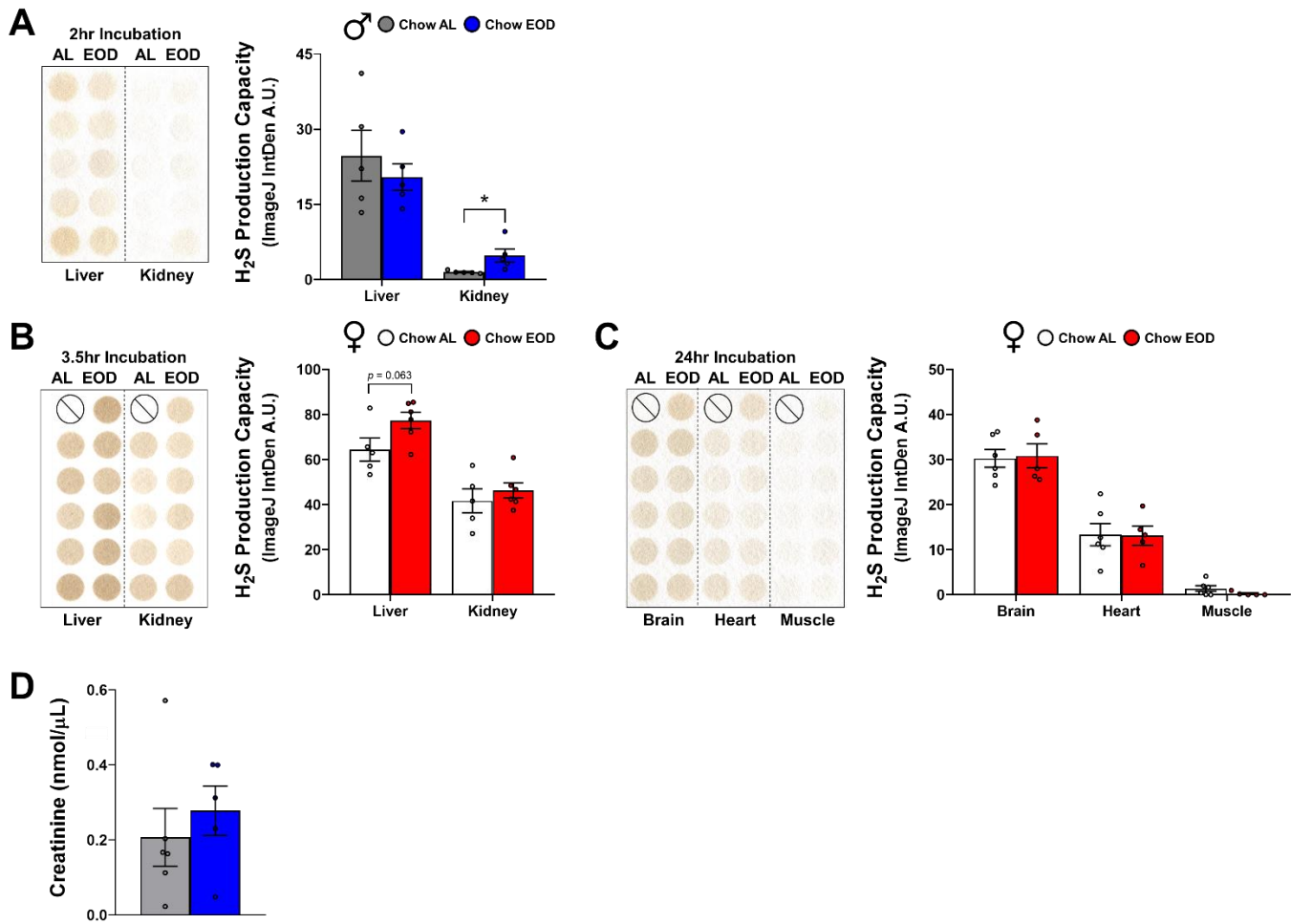
Supplemental Figure 4: Late-life EOD fasting did not affect circulating insulin levels. Plasma insulin levels (ng/mL) in the male (*left*) and female (*right*) Chow AL and Chow EOD groups (n = 4-6 mice per sex per group). The data depict the mean with error bars (\pm SEM). See also Figure 4.



Supplemental Figure 6: Late-life EOD fasting does not affect locomotion during the hippocampal-dependent short-term memory test. Velocity in the Y Maze forced alternation task at baseline and post-DI in the male (*left*) and female (*left*) Chow AL and Chow EOD groups (n = 5-7 mice per sex per group). The figure depicts the mean with error bars (\pm SEM). See also Figure 6.



Supplemental Figure 7: Late-life EOD fasting does not affect motor activity in females during the open field task. Velocity in the open field at baseline and post-DI in the female Chow AL ($n = 6$) and Chow EOD groups ($n = 6$). The figure depicts the mean with error bars (\pm SEM). The asterisks indicate the significant difference between the same-sex Chow AL and Chow EOD groups. $*p < 0.05$. See also Figure 7.



Supplemental Figure 9: Renal H₂S production capacity is enhanced by late-life EOD fasting in a sex-specific manner and without affecting circulating plasma creatinine levels. (A) H₂S production capacity in liver and kidney in the male Chow AL (n = 5) and Chow EOD groups (n = 5). Images with quantitated lead sulfide spots on the filter paper following a 2-hr incubation. (B, C). H₂S production capacity in liver and kidney (B) and in brain, heart, and muscle (C) in the female Chow AL (n = 5) and Chow EOD groups (n = 6). Images with quantitated lead sulfide spots on the filter paper following a 3.5-hr incubation (B) and a 24-hr incubation (C). (D) Plasma creatinine levels in the male Chow AL (n = 6) and Chow EOD groups (n = 5). The figures (A-D) depict the mean with error bars (± SEM). The asterisks indicate the significant difference between the male Chow AL and Chow EOD groups. **p* < 0.05. See also Figure 9.