Supplemental Information

Fasting drives the metabolic, molecular, and geroprotective effects of a calorie restricted diet in mice

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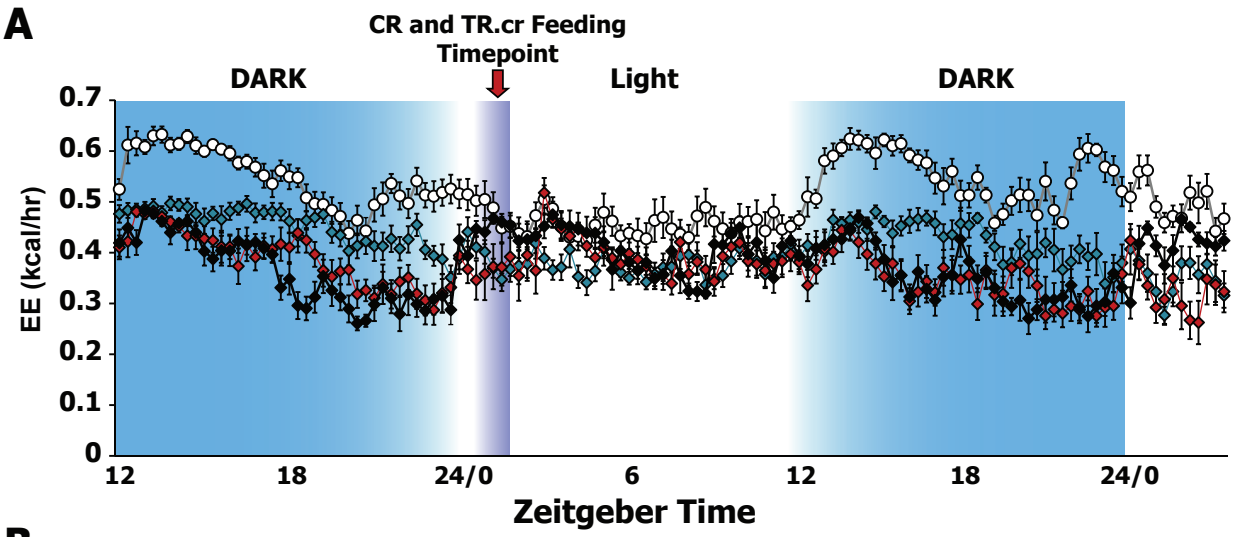
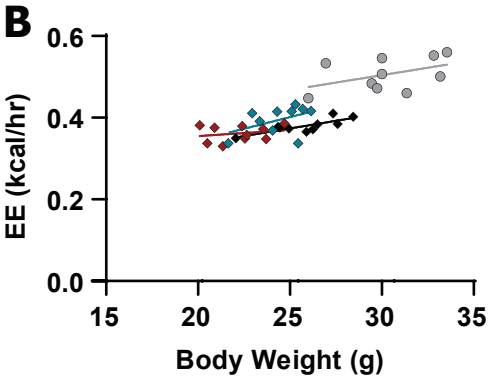
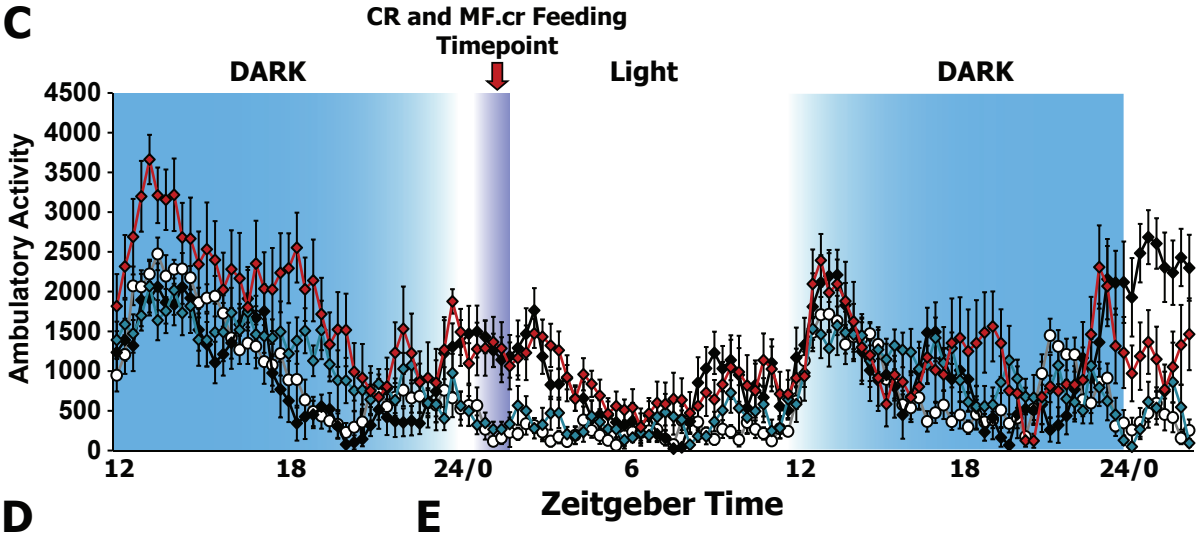
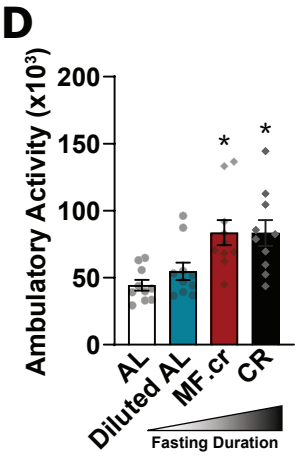
Supplementary Figure 1

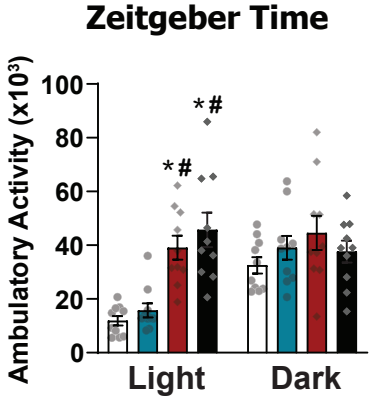




Diet Comparison		Slope		Elevation	
Diet 1	Diet 2	p-Value	Significance	p-Value	Significance
AL	Diluted AL	0.6703	No	0.0394	Yes
AL	MF.cr	0.5922	No	0.0061	Yes
AL	CR	0.9724	No	<0.0001	Yes
Diluted AL	CR	0.5913	No	0.0315	Yes
Diluted AL	MF.cr	0.3540	No	0.2516	No
CR	MF.cr	0.3458	No	0.8312	No

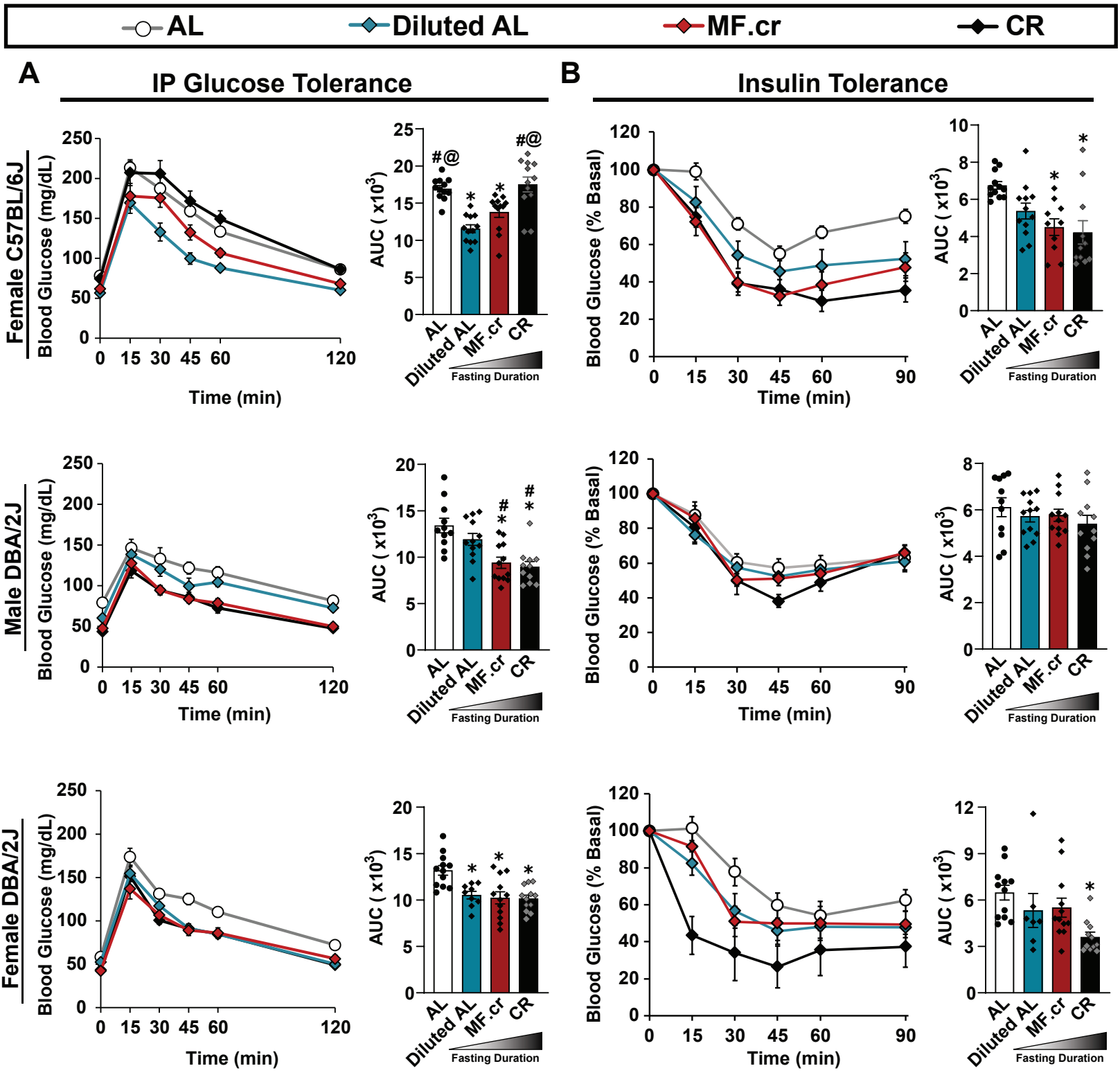






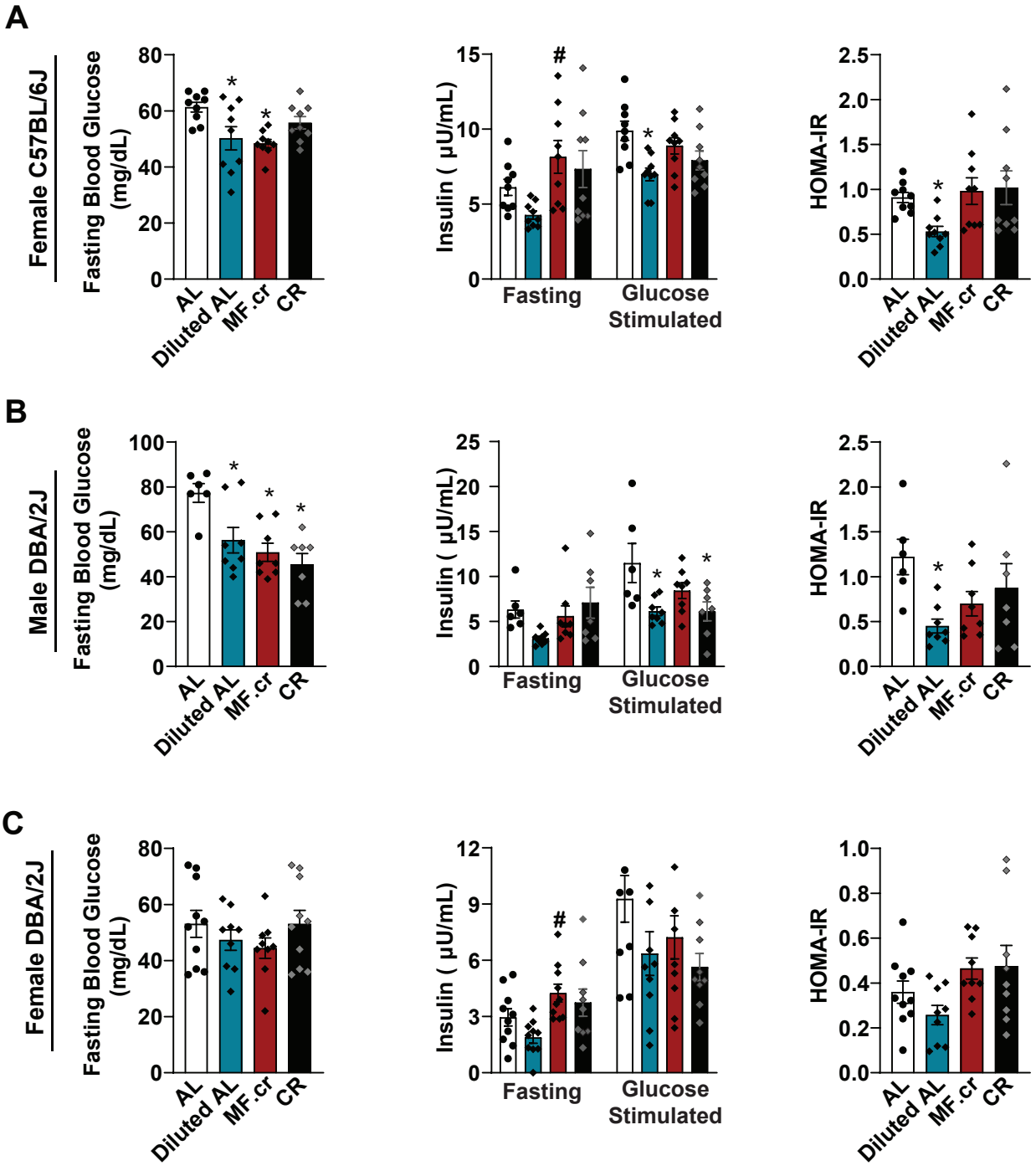
Supplementary Figure 1. Additional metabolic chamber data for C57BL/6J male mice. (A-E) Metabolic chamber analysis of mice fed the indicated diets. (A) Energy expenditure vs. time. (B) Energy expenditure as a function of total body mass was calculated for the 24-hour period following the indicated (arrow) refeeding time; slopes and intercepts were calculated using ANCOVA). (C) Ambulatory activity vs. time (D) Sum of ambulatory activity was calculated for the 24-hour period following the indicated (arrow) refeeding time. * symbol represents a significant difference versus AL (MF.cr, $p = 0.0046$; CR, $p = 0.0060$); based on Kruskal-Wallis test post one-way ANOVA. (E) Sum of ambulatory activity during the light and dark cycle calculated for the 24-hour period following the indicated (arrow) refeeding time. * symbol represents a significant difference versus AL (MF.cr, $p = 0.0003$; CR, $p < 0.0001$); # symbol represents a significant difference versus Diluted AL (MF.cr, $p = 0021$; CR, $p < 0.0001$) based on Tukey's test post one-way ANOVA. (A-E). All data are represented as mean ± SEM. n = 10 biologically independent mice per diet.

Supplementary Figure 2



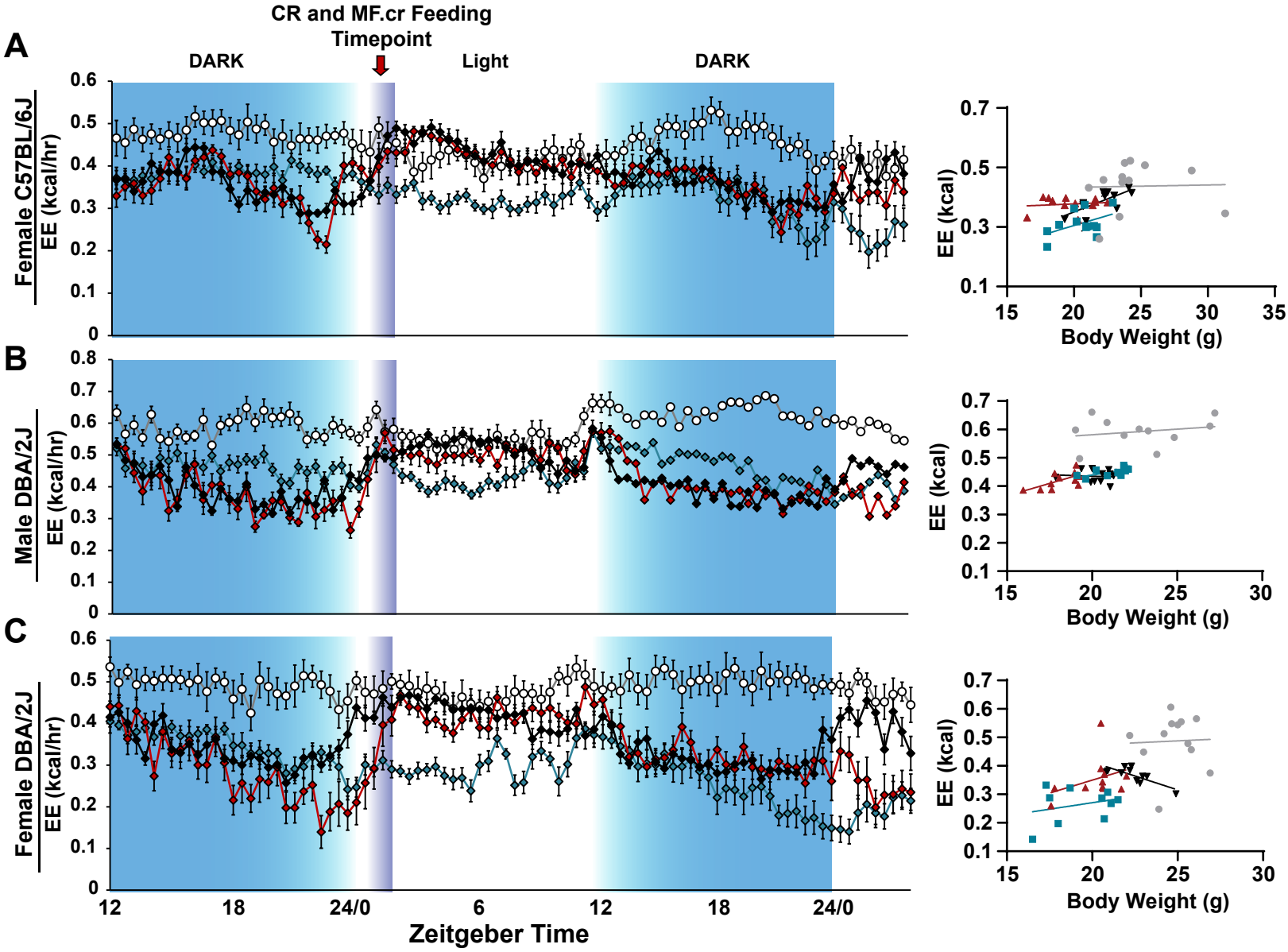
Supplementary Figure 2. Additional measures of glucose homeostasis in female C57BL/6J mice and DBA/2J male and female mice. (A) Glucose tolerance test performed on female C57BL/6J (AL, n = 12; Diluted AL, n = 12; MF.cr, n = 12; CR, n = 12 biologically independent mice), male DBA/2J (AL, n = 11; Diluted AL, n = 12; MF.cr, n = 12; CR, n = 11 biologically independent mice), and female DBA/2J (AL, n = 12; Diluted AL, n = 9; MF.cr, n = 12; CR, n = 12 biologically independent mice) mice of both sexes after 13 weeks on the indicated diets. (B) Insulin tolerance test performed on female C57BL/6J (AL, n = 12; Diluted AL, n = 12; MF.cr, n = 10; CR, n = 11 biologically independent mice), male DBA/2J (AL, n = 11; Diluted AL, n = 12; MF.cr, n = 12; CR, n = 11 biologically independent mice), and female DBA/2J (AL, n = 12; Diluted AL, n = 7; MF.cr, n = 12; CR, n = 11 biologically independent mice) mice of both sexes after 14 weeks on the indicated diets. Female C57BL/6J mice were given 0.5U insulin per kg of body weight; DBA/2J male and female mice were given 0.75U insulin per kg of body weight. * symbol represents a significant difference versus AL (Diluted AL, $p \leq 0.0064$; MF.cr, $p \leq 0.0145$; CR, $p \leq 0.0012$); **#** symbol represents a significant difference versus Diluted AL (MF.cr, $p = 0.0378$, CR, $p \leq 0.0136$); **@** symbol represents a significant difference versus MF.cr (AL, $p = 0.0058$; CR, $p \leq 0.0029$) based on Tukey's test post one-way ANOVA. All data are represented as mean ± SEM.

Supplementary Figure 3



Supplementary Figure 3. Additional measures of glucose and insulin in female C57BL/6J mice and DBA/2J male and female mice. Mice were fasted overnight and blood glucose, fasting insulin, glucose stimulated insulin secretion (after 15 minutes) were determined, and the HOMA-IR was calculated after 11 weeks on the specified diets for (A) female C57BL/6J, (B) male DBA/2J, and (C) female DBA/2J mice (AL, n = 10; Diluted AL, n = 9; MF.cr, n = 9; CR, n = 9 biologically independent mice). * symbol represents a significant difference versus AL (Diluted AL, $p \leq 0.0314$; MFcr, $p \leq 0.0053$; CR, $p \leq 0.0240$); **#** symbol represents a significant difference versus Diluted AL (MF.cr, $p \leq 0.0187$; CR, $p = 0.0478$) based on Tukey's test post one-way ANOVA. All data are represented as mean ± SEM.

Supplementary Figure 4



D Female C57BL/6J

Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	Diluted AL	0.4097	No	0.0060	Yes
AL	MF.cr	0.9333	No	0.1428	No
AL	CR	0.2478	No	0.1889	No
Diluted AL	CR	0.6721	No	0.0033	Yes
Diluted AL	MF.cr	0.2000	No	<0.0001	Yes
CR	MF.cr	0.0267	Yes	Cannot be compared	

E Male DBA/2J

Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	Diluted AL	0.6826	No	<0.0001	Yes
AL	MF.cr	0.3090	No	<0.0001	Yes
AL	CR	0.6716	No	<0.0001	Yes
Diluted AL	CR	0.171	No	0.1783	No
Diluted AL	MF.cr	0.2663	No	0.4334	No
CR	MF.cr	0.0665	No	0.7964	No

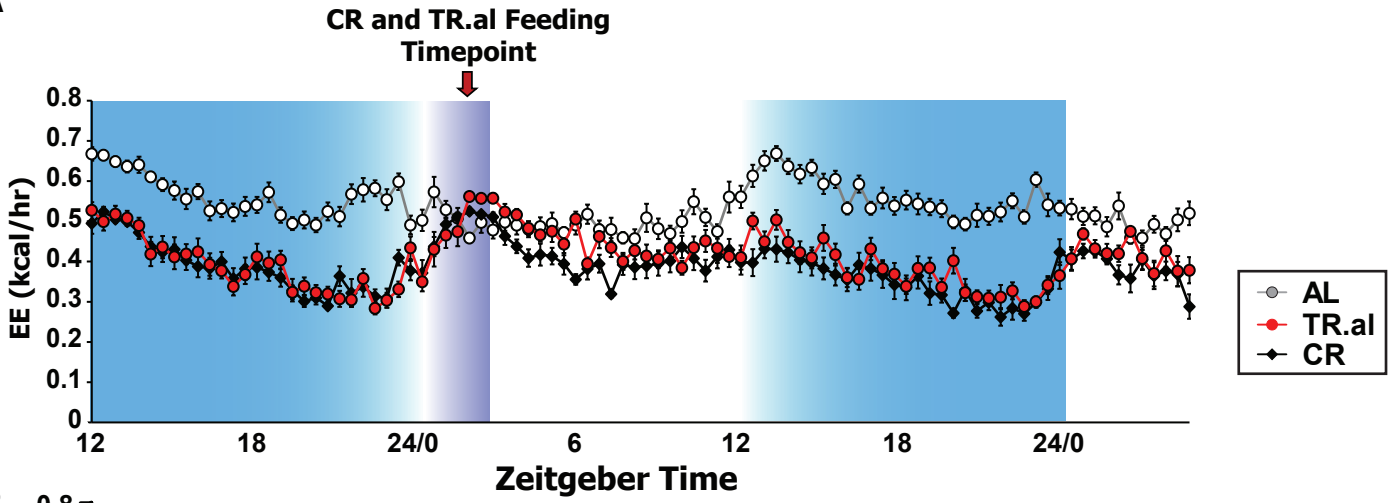
F Female DBA/2J

Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	Diluted AL	0.8032	No	0.0219	Yes
AL	MF.cr	0.4223	No	0.0062	Yes
AL	CR	0.5978	No	0.2633	No
Diluted AL	CR	0.0687	No	0.0042	Yes
Diluted AL	MF.cr	0.6474	No	0.0190	Yes
CR	MF.cr	0.0634	No	0.8511	No

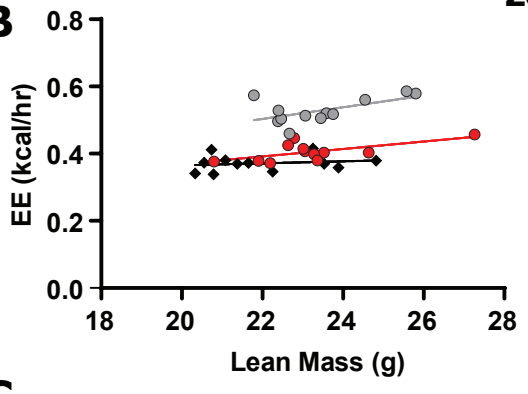
Supplementary Figure 4. Additional energy expenditure data for C57BL/6J female and DBA/2J male and female mice. A-C) Energy expenditure vs. time and energy expenditure as a function of total body mass was calculated for the 24-hour period following the indicated (arrow) refeeding time for A) C57BL/6J female mice (n = 12 biologically independent mice per diet) (B) DBA/2J male (AL, n = 12; Diluted AL, n = 11; MF.cr, n = 11; CR, n = 11 biologically independent mice) C) DBA/2J female (AL, n = 11; Diluted AL, n = 11; MF.cr, n = 11; CR, n = 10 biologically independent mice). Data for each individual mouse is plotted; slopes and intercepts were calculated using ANCOVA. Data are represented as mean ± SEM.

Supplementary Figure 5

A

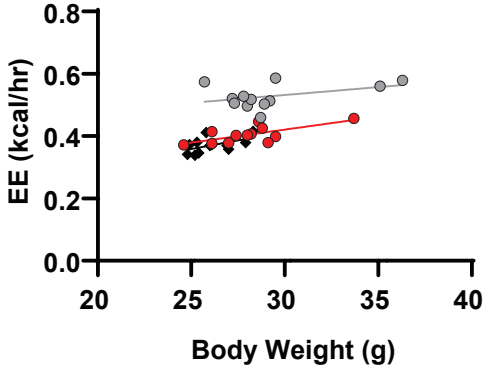


B



Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	CR	0.1270	No	<0.0001	Yes
AL	TR.cr	0.4677	No	<0.0001	Yes
CR	TR.cr	0.2255	No	0.0256	Yes

C

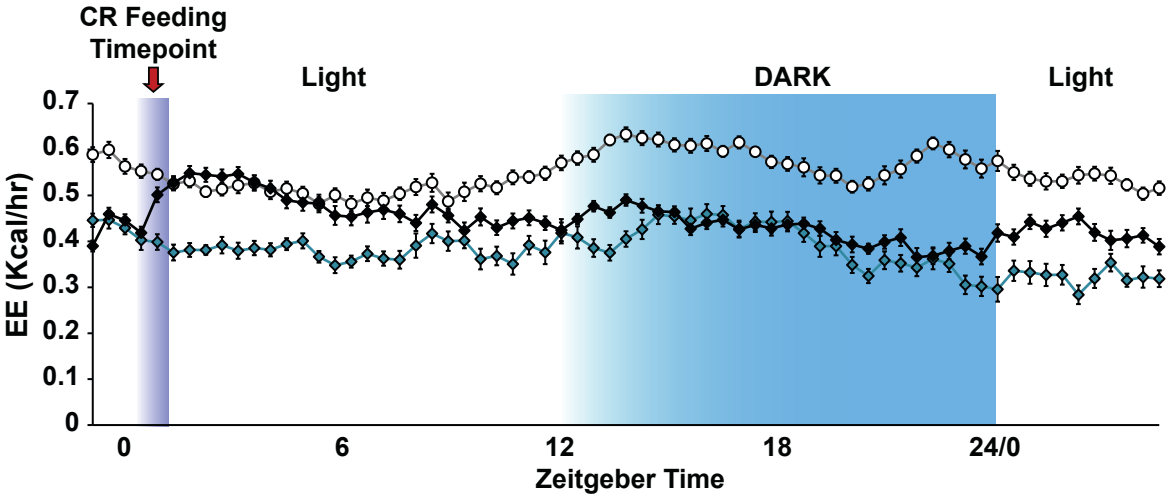


Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	CR	0.5051	No	<0.0001	Yes
AL	TR.cr	0.4760	No	<0.0001	Yes
CR	TR.cr	0.6577	No	0.1079	No

Supplementary Figure 5. Additional energy expenditure data for C57BL/6J male mice fed CR or TR.al diets. A) Energy expenditure vs. time B) Energy expenditure as a function of lean mass was calculated for the 24-hour period following the indicated refeeding time C) Energy expenditure as a function of total body mass was calculated for the 24-hour period following the indicated (arrow) refeeding time (n = 12 biologically independent mice per diet, data for each individual mouse is plotted; slopes and intercepts were calculated using ANCOVA). Data are represented as mean ± SEM.

Supplementary Figure 6

A



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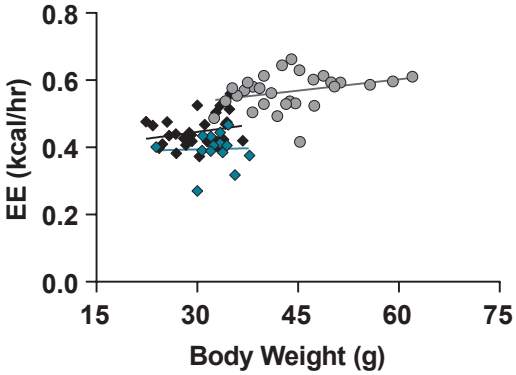
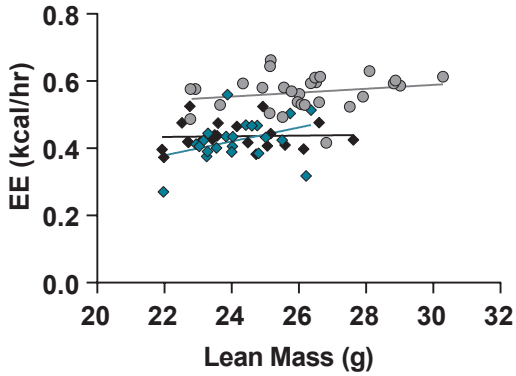


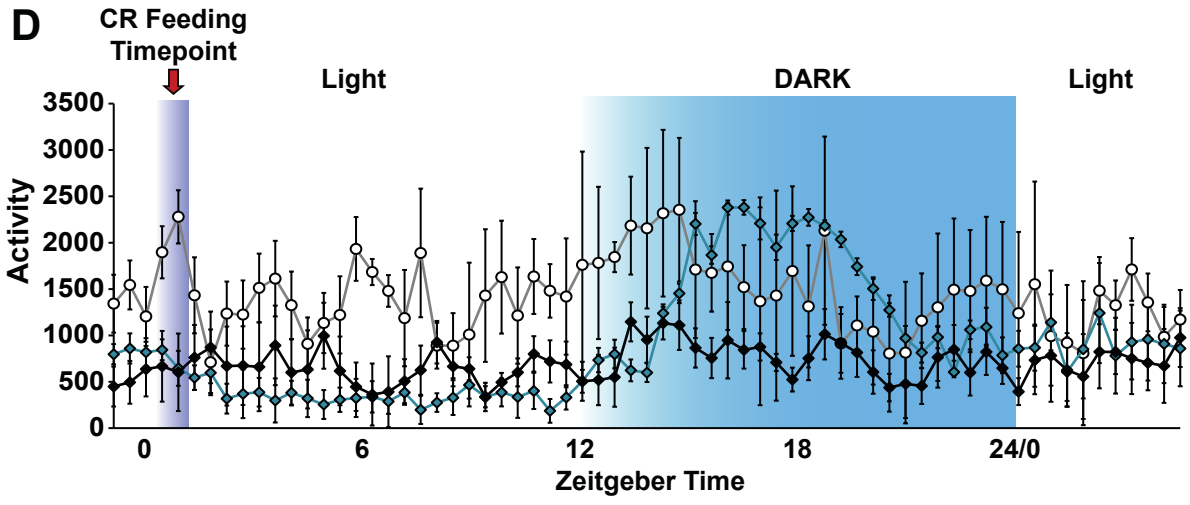
Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	Diluted AL	0.6959	No	<0.0001	Yes
AL	CR	0.8613	No	<0.0001	Yes
CR	Diluted AL	0.6313	No	0.0011	Yes

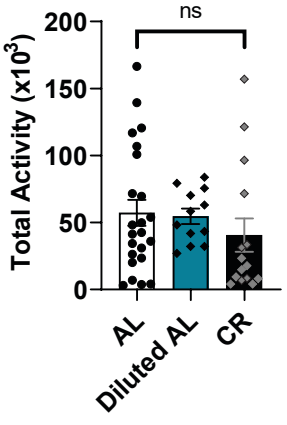
C



Diet Comparison		Slope		Elevation	
		p-Value	Significance	p-Value	Significance
AL	Diluted AL	0.2238	No	<0.0001	Yes
AL	CR	0.5741	No	<0.0001	Yes
CR	Diluted AL	0.1229	No	0.4463	No

D





Supplementary Figure 6. Additional energy expenditure data for 19-month-old C57BL/6J male. A) Energy expenditure vs. time B) Energy expenditure as a function of total body mass was calculated for the 24 hour period following the indicated refeeding time C) Energy expenditure as a function of lean mass was calculated for the 24 hour period following the indicated refeeding time (D) Ambulatory activity vs. time and average sum of ambulatory activity was calculated for the 24 hour period following the indicated refeeding time (AL, n = 29; Diluted AL, n = 14; CR, n = 29 biologically independent mice). Data for each individual mouse is plotted; slopes and intercepts were calculated using ANCOVA). Data are represented as mean ± SEM.