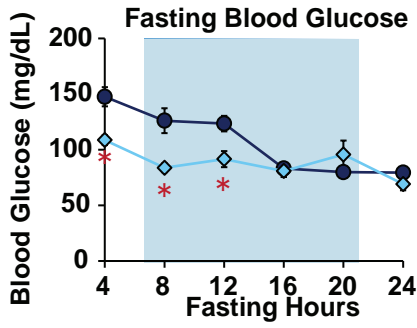


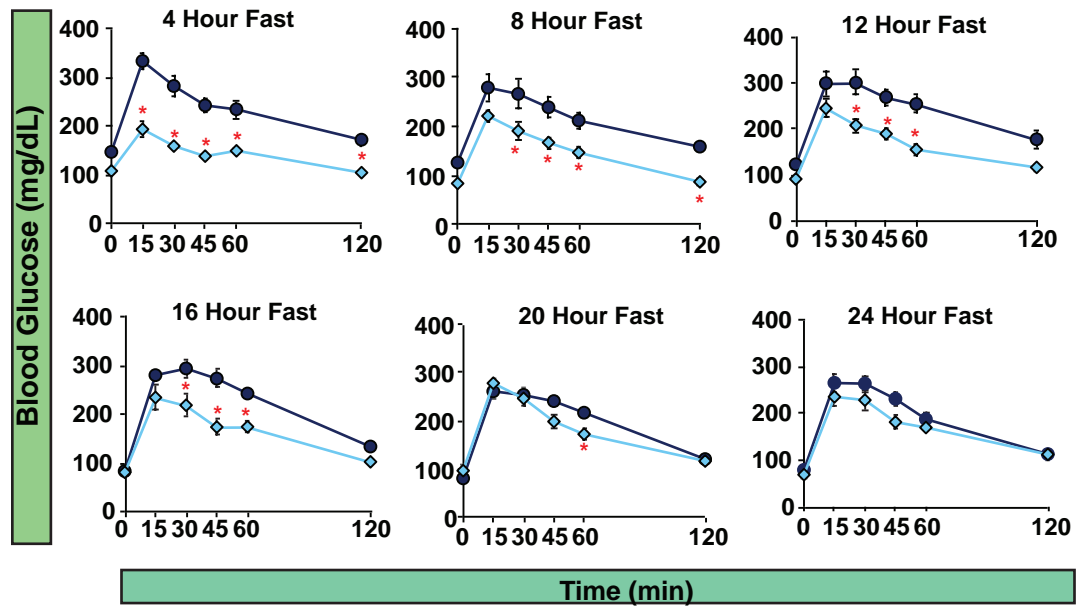
# Supplementary Figure 1

**A**

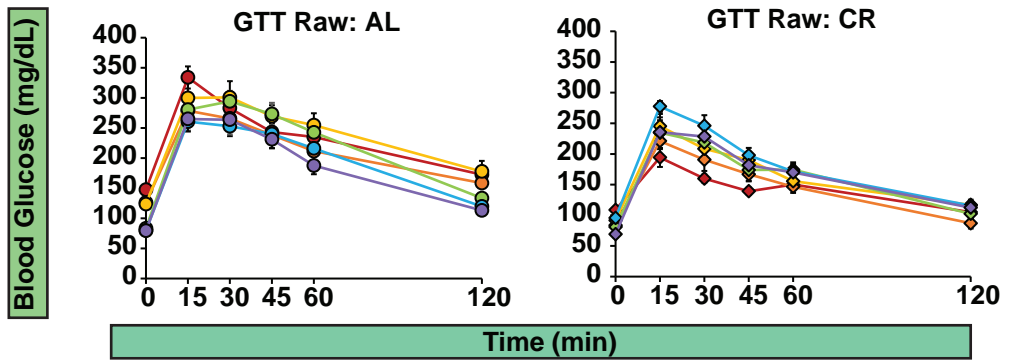


Fasting Duration	
AL	CR
● 4hr	◆ 4hr
● 8hr	◆ 8hr
● 12hr	◆ 12hr
● 16hr	◆ 16hr
● 20hr	◆ 20hr
● 24hr	◆ 24hr

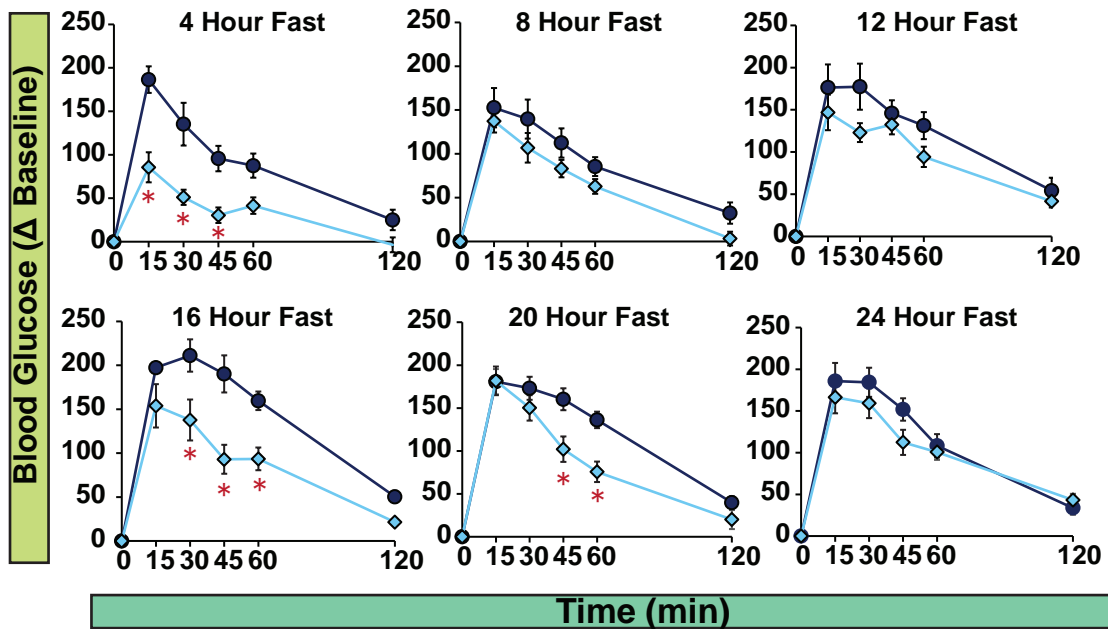
**B GTT Raw**



**C**



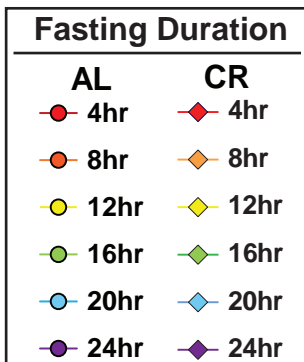
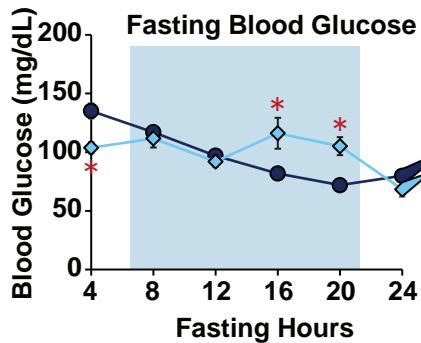
**D ΔGTT**



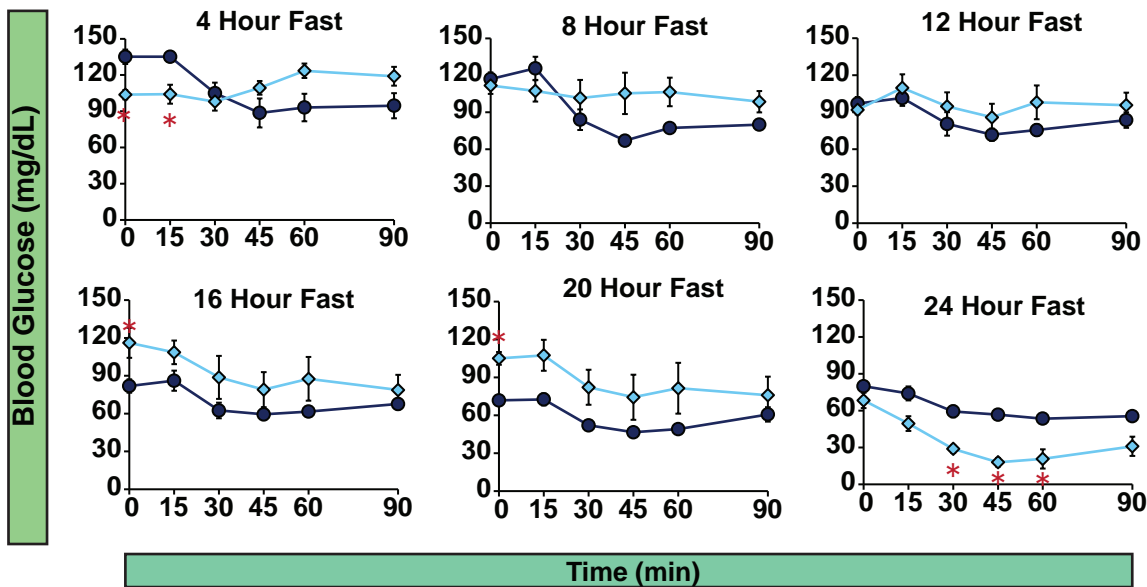
**Supplementary Figure 1. Raw and delta values for glucose tolerance tests in Morning-Fed males, related to Figure 1. (A)** Fasting blood glucose prior to GTT. **(B)** Raw GTT values. **(C)** Combined raw GTT for AL and CR. **(D)** Delta values for GTT. **(A-D)** AL, n = 7-8 per time point; CR, n = 7-8 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

# Supplementary Figure 2

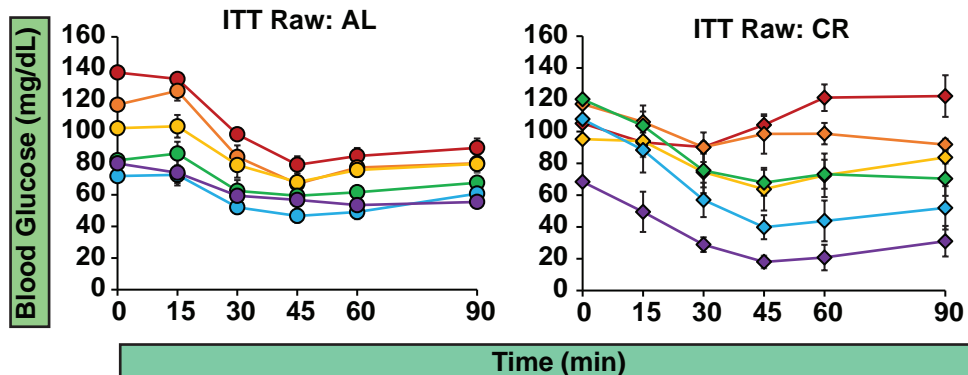
**A**



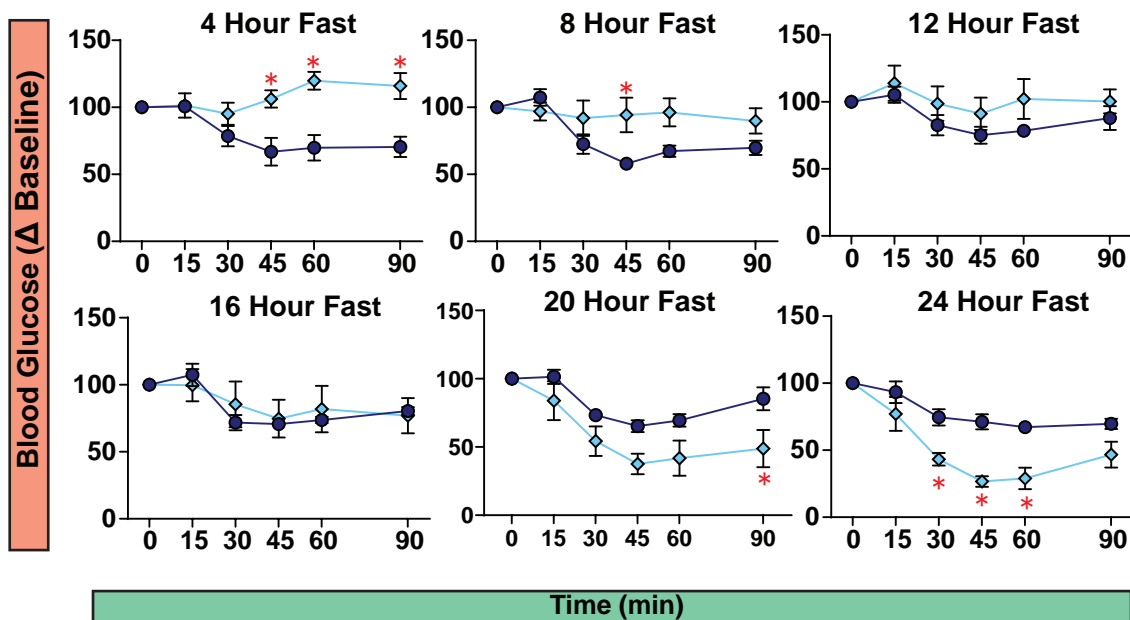
**B ITT Raw**



**C**



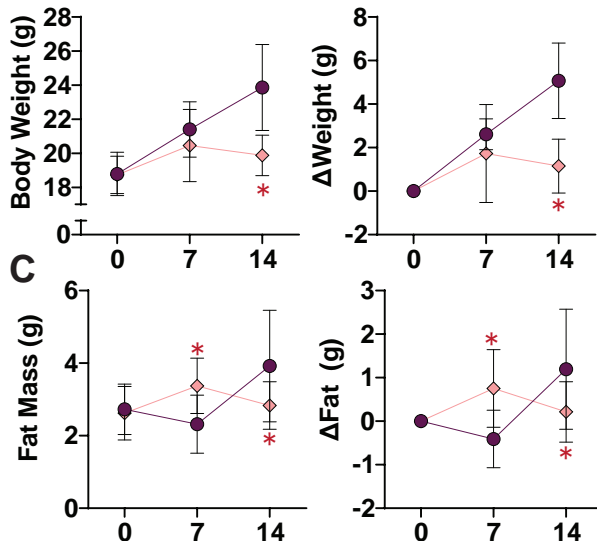
**D ΔITT**



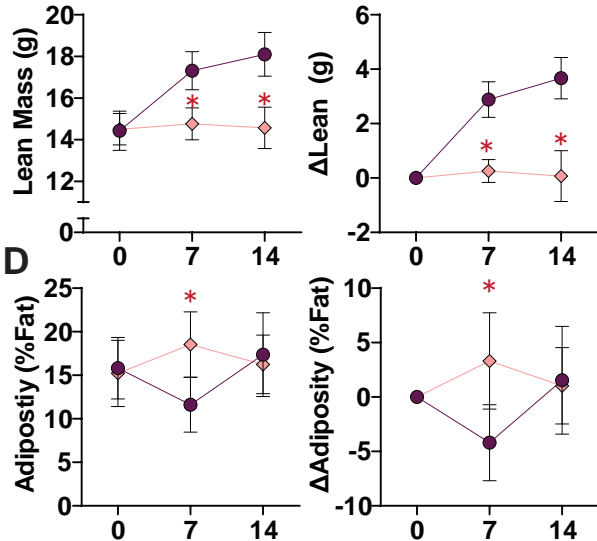
**Supplementary Figure 2. Raw and delta values for insulin tolerance tests in Morning-Fed males, related to Figure 1. (A)** Fasting blood glucose prior to ITT. **(B)** Raw values for ITT **(C)** Combined raw ITT for AL and CR. **(D)** Delta values for ITT. **(A-D)** AL, n = 7-8 per time point; CR, n = 7-8 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

# Supplementary Figure 3

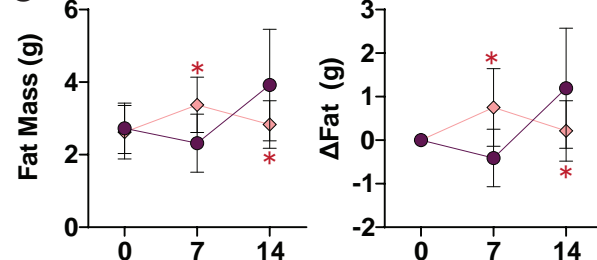
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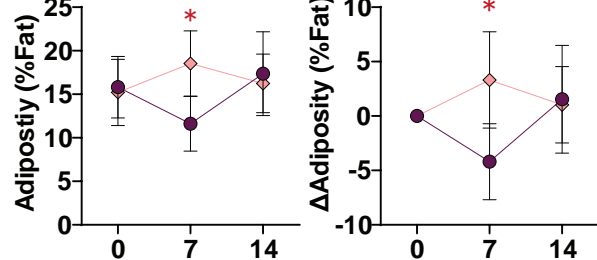
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**C**



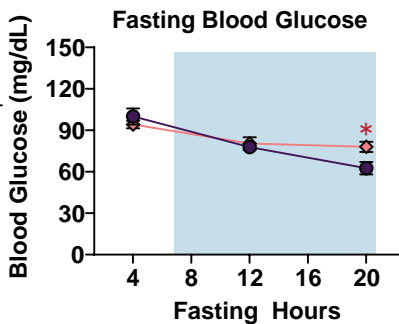
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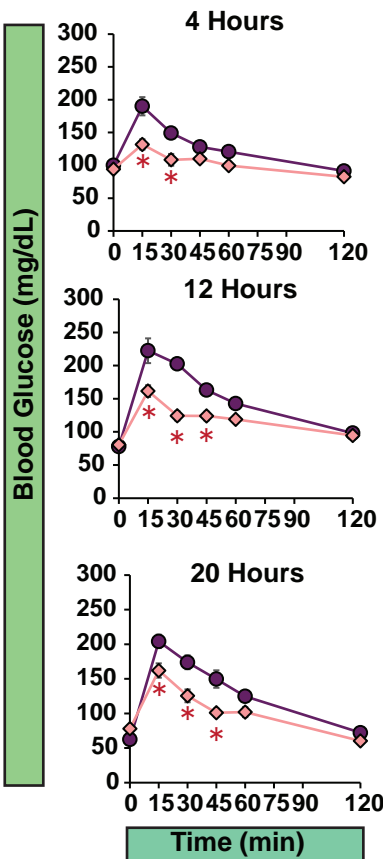
Weeks on Diet

♀ AL CR

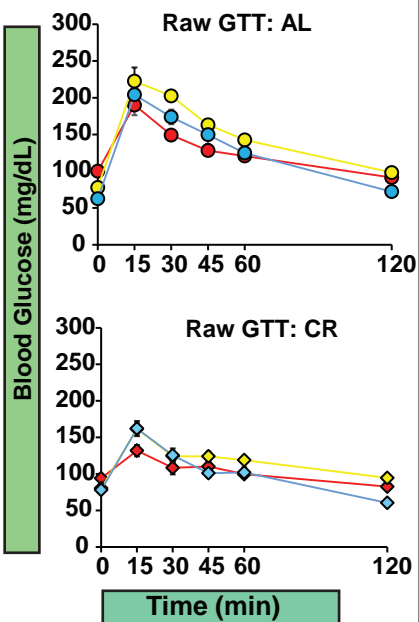
**E**



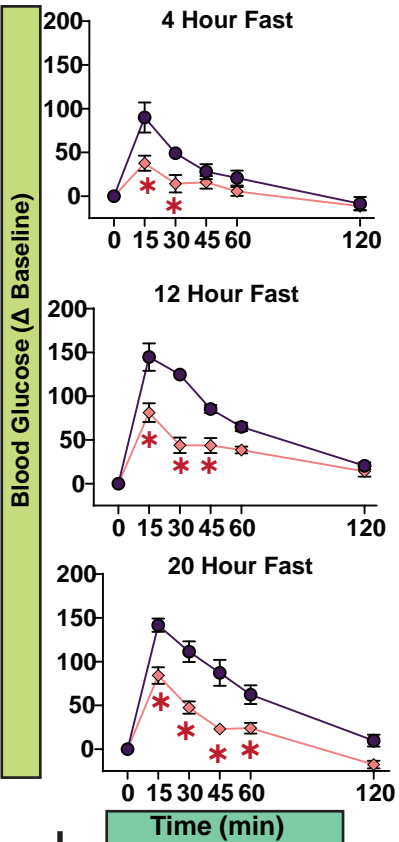
**F** GTT Raw



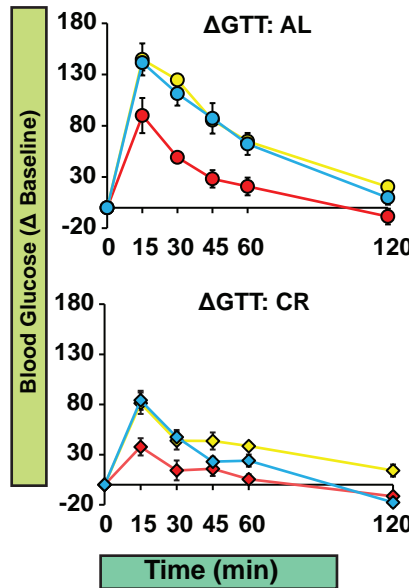
**H**



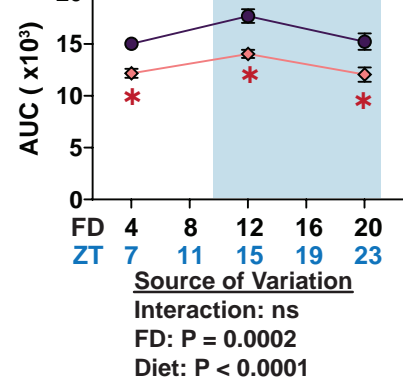
**I**  $\Delta$ GTT



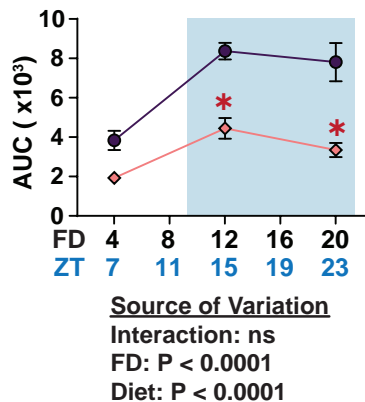
**K**



**G**



**J**



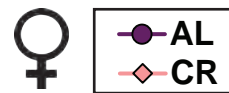
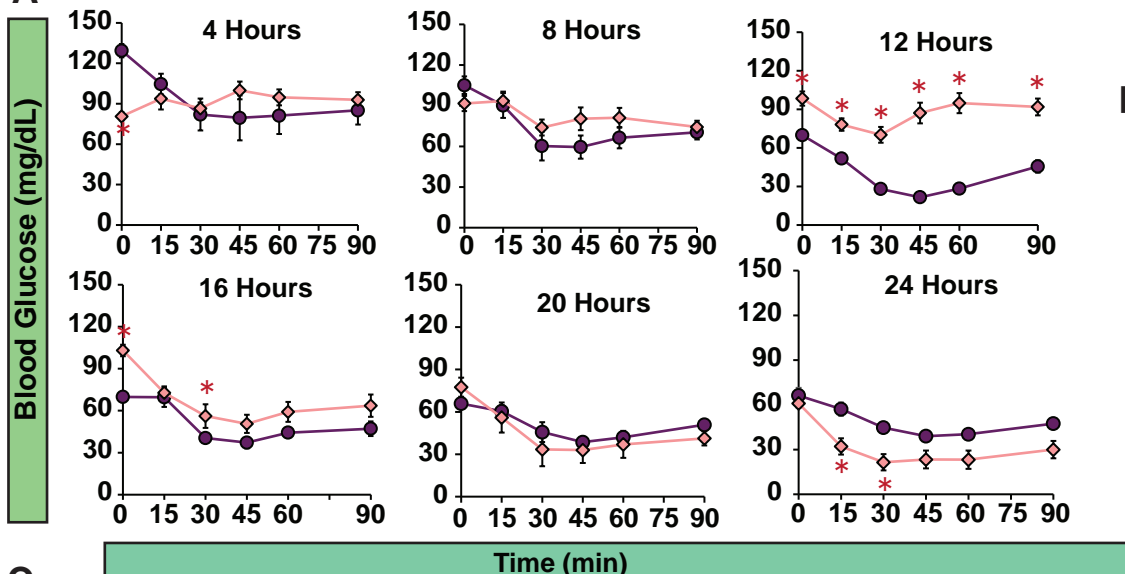
Fasting Duration

AL CR  
 ● 4hr ◆ 4hr  
 ● 12hr ◆ 12hr  
 ● 20hr ◆ 20hr

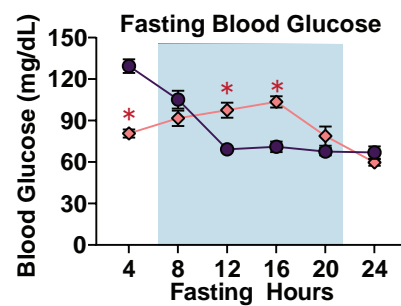
**Supplementary Figure 3. Body composition and glucose tolerance tests of female C57BL/6J mice in Morning-Fed study.** (A-D) Body composition measurement of female C57BL/6J mice under Morning-Fed conditions (AL, n = 7-8 and CR, n = 7-8 biologically independent mice) \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM. (A) Total body weight with change in body weight from baseline. (B) Fat mass with change in fat mass from baseline. (C) Lean mass with change in fat mass from baseline. (D) Adiposity with change in adiposity from baseline. (E) Fasting blood glucose prior to GTT. (F) Raw GTT values (G) with AUC. (H) Combined raw GTT for AL and CR. (I) Delta values for GTT (J) with AUC. (K) Combined delta GTT for AL and CR. (A-K) AL, n = 7-8 per time point; CR, n = 7-8 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

# Supplementary Figure 4

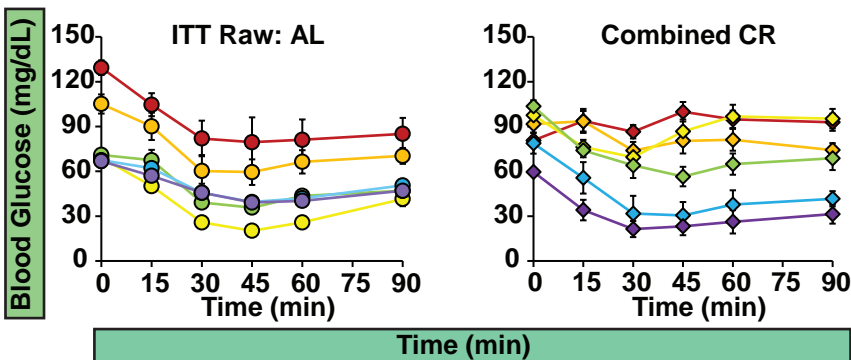
## A ITT Raw



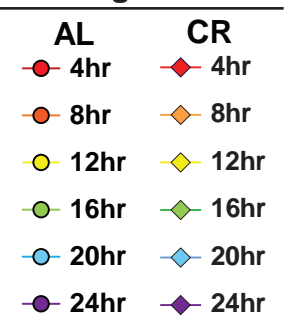
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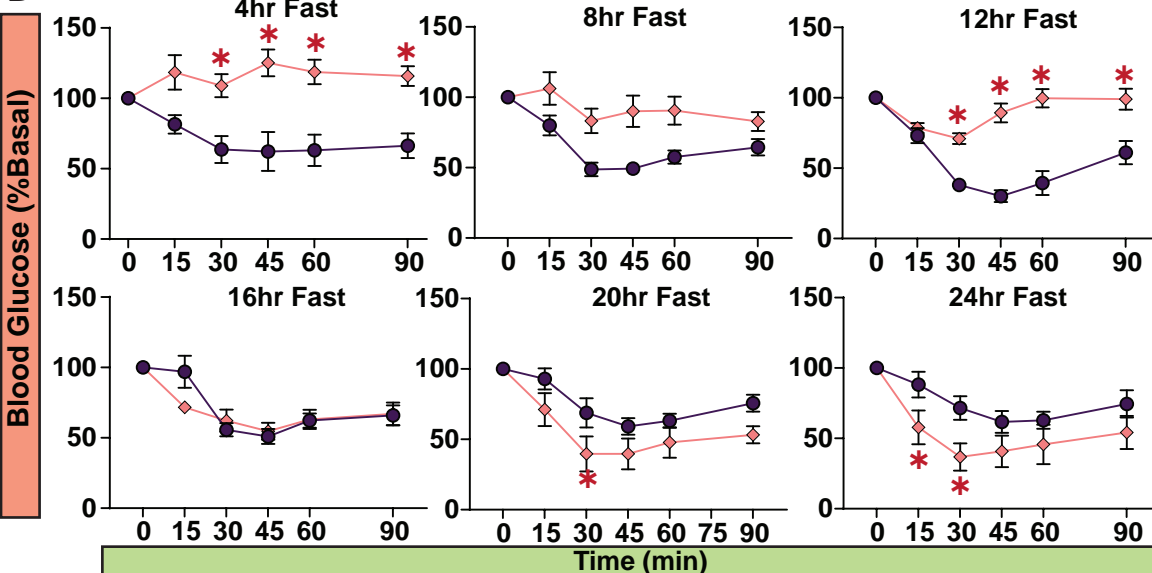
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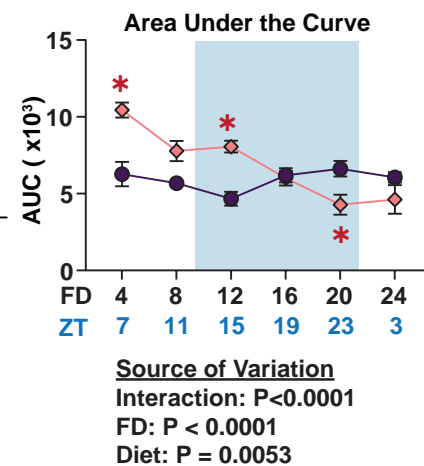
## Fasting Duration



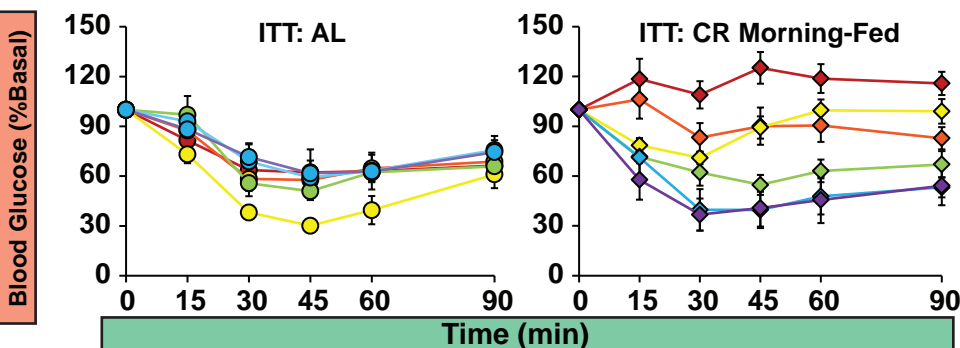
## D ΔITT



## E



## F

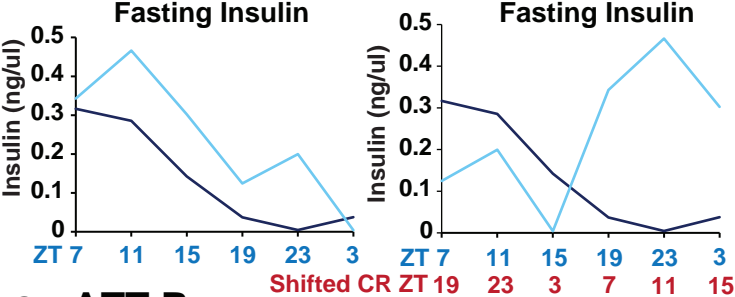


**Supplementary Figure 4. Insulin tolerance tests of female C57BL/6J mice in Morning-Fed study.** (A) Raw ITT values (B) with fasting blood glucose prior to ITT. (C) Combined raw ITT for AL and CR. (D) Delta values for ITT (E) with AUC. (F) Combined delta ITT for AL and CR (A-F) AL, n = 7-8 per time point; CR, n = 7-8 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

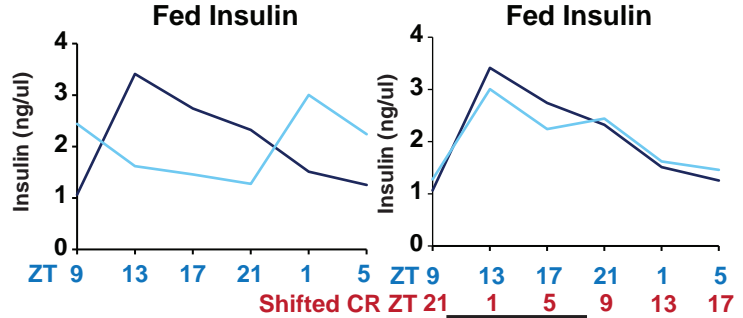


# Supplementary Figure 5

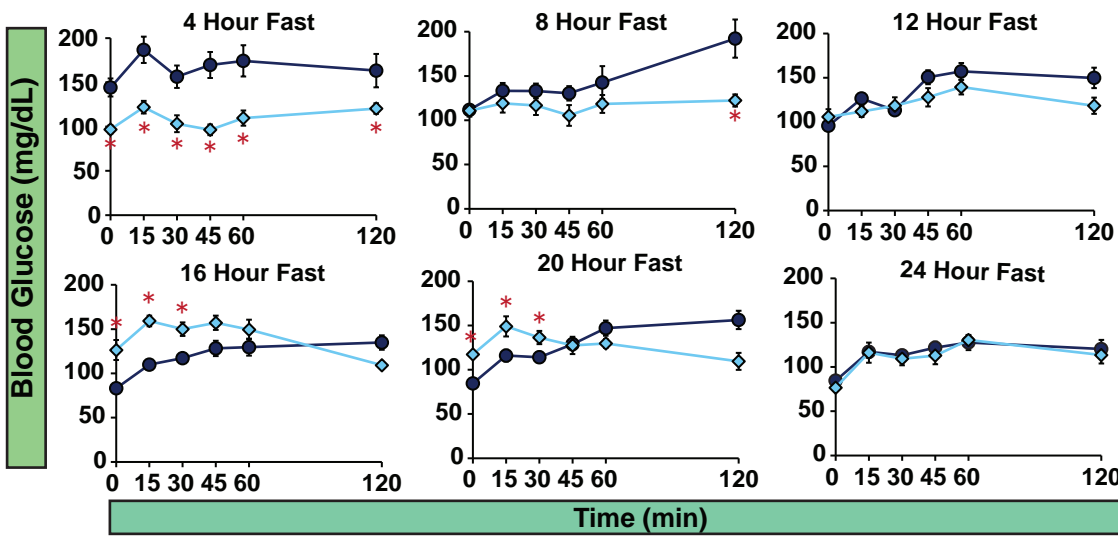
**A**



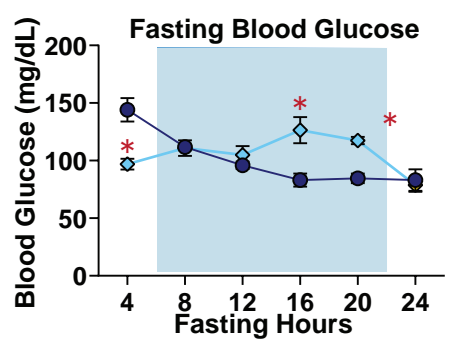
**B**



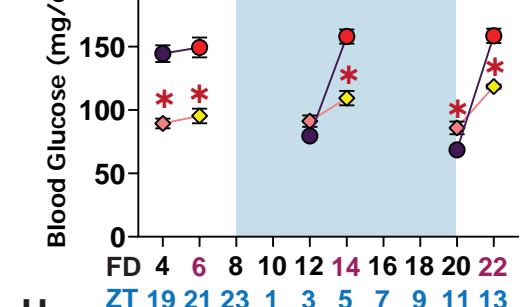
**C ATT Raw**



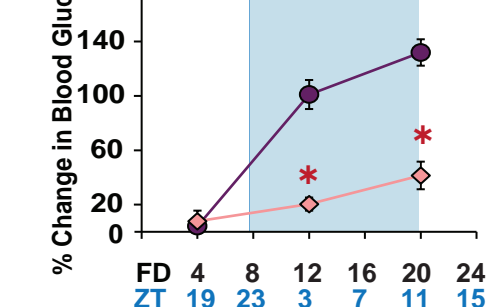
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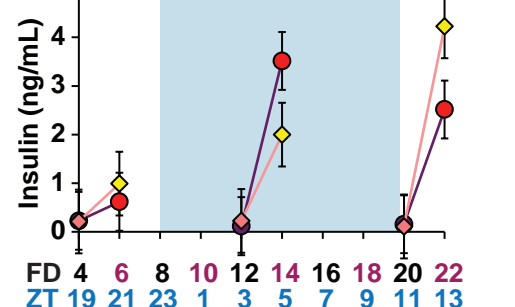
**E**



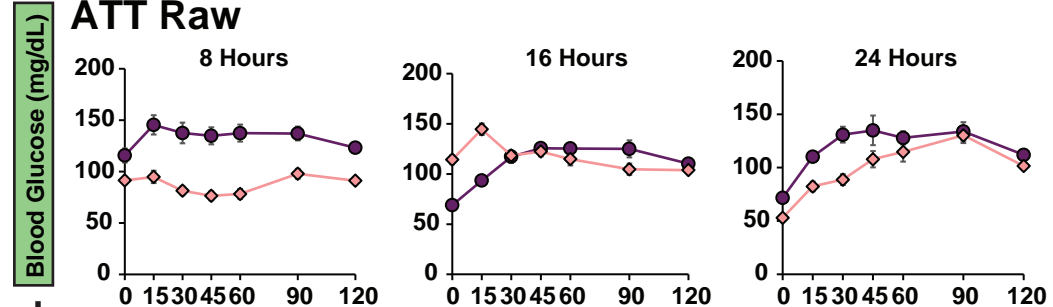
**F**



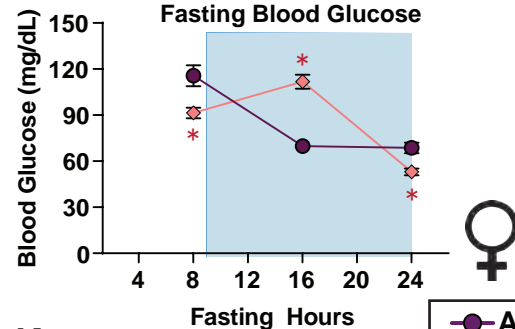
**G**



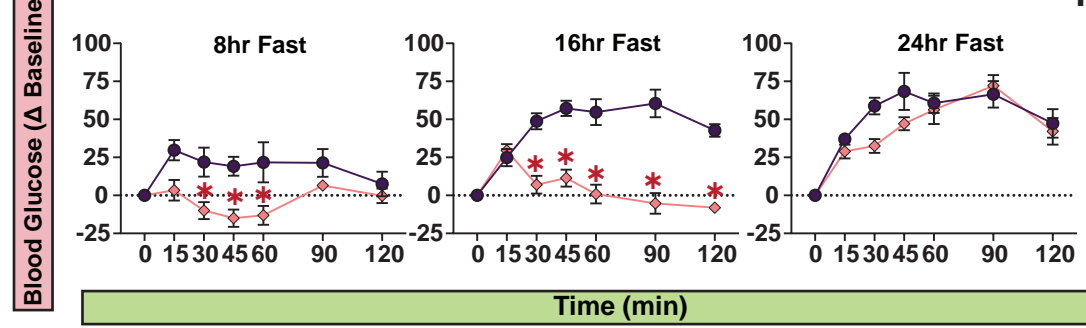
**H**



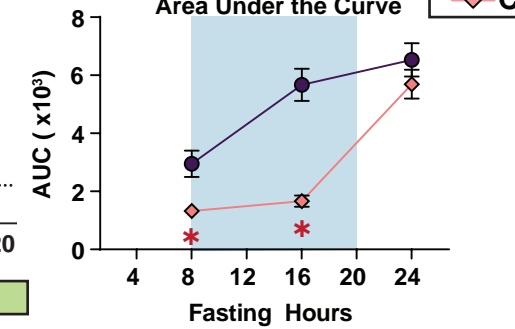
**I**



**J ΔATT**



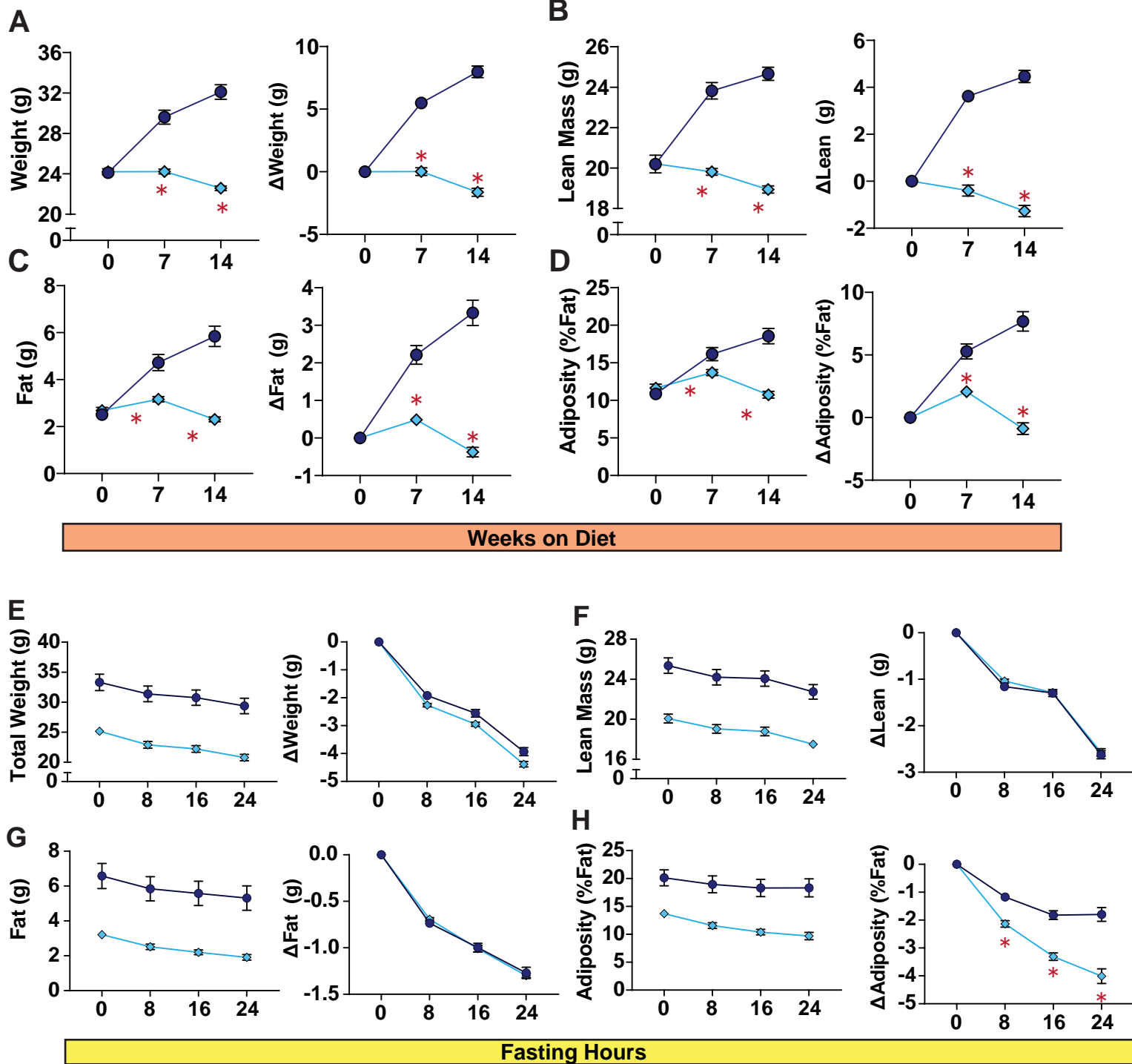
**K**



**Supplementary Figure 5. Meal-stimulated insulin level and raw values for alanine tolerance test in Morning-Fed males and females, related to Figure 2.** (A) Simplified representation of fasted insulin level related to Figure 2D-E. (B) Simplified representation of fed insulin level related to Figure 2D-E. (C) Raw values for ATT with (D) fasting blood glucose. (E-F) Blood glucose level of fasted and fed state during respective fasting timepoints (E), percent change in blood glucose level from fasted to fed state (F). (G) Insulin level of fasted and fed state mice during respective fasting/refed timepoints. (H) Raw values for ATT with (I) fasting blood glucose. (J) Delta values for ATT and (K) AUC (A-K) AL, n = 7-16 per timepoint; CR, n = 7-16 per timepoint, biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean ± SEM.

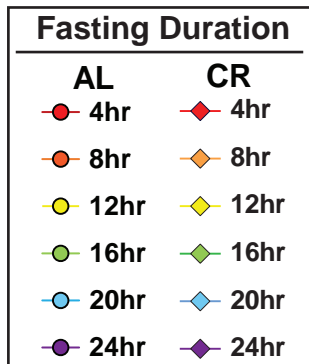
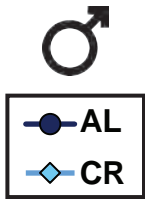
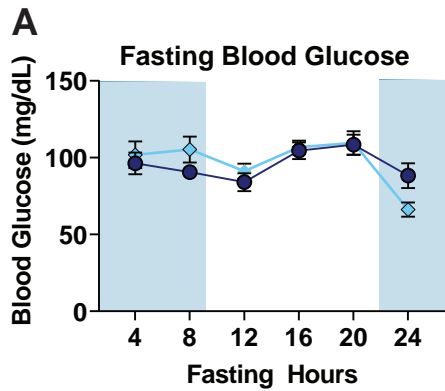
# Supplementary Figure 6

## ♂ NIGHT STUDY

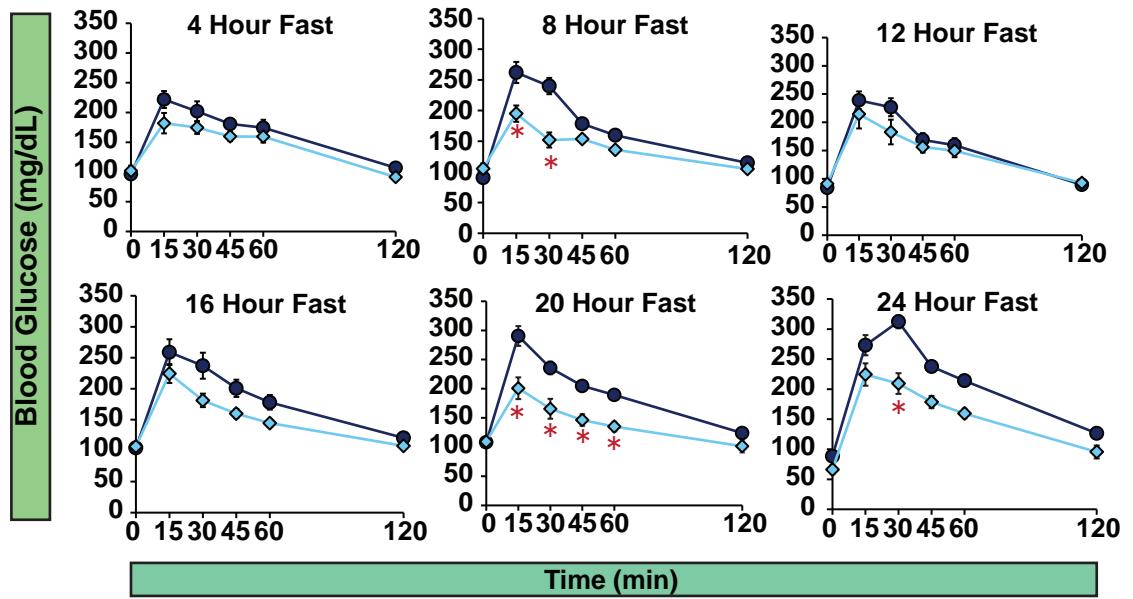


**Supplementary Figure 6. Body composition of Night Study male C57BL/6J mice and change in body composition during a 24hr fast, related to Figure 3. (A)** Total body weight with % change in body weight from baseline. **(B)** Lean mass with % change in lean mass from baseline. **(C)** Fat mass with % change in fat mass from baseline. **(D)** adiposity with % change in adiposity from baseline. **(A-D)** AL, n = 27 and CR, n = 27 biologically independent mice. \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM. **(E-H)** Mice were fed at ZT 12, and body composition was measured starting at ZT 15 (0hr fasted). All mice were fasted after the initial baseline measurements and body composition was measured again at ZT 20 (8hr fasted), ZT 4 (16hr fasted), and ZT 12 (24hr fasted). **(E)** Total body weight with % change in body weight from baseline, **(F)** lean mass with % change in lean mass from baseline **(G)**, fat mass with % change in fat mass from baseline, **(H)** adiposity with % change in adiposity from baseline. **(F-H)** AL, n = 12 and CR, n = 12 biologically independent mice. \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

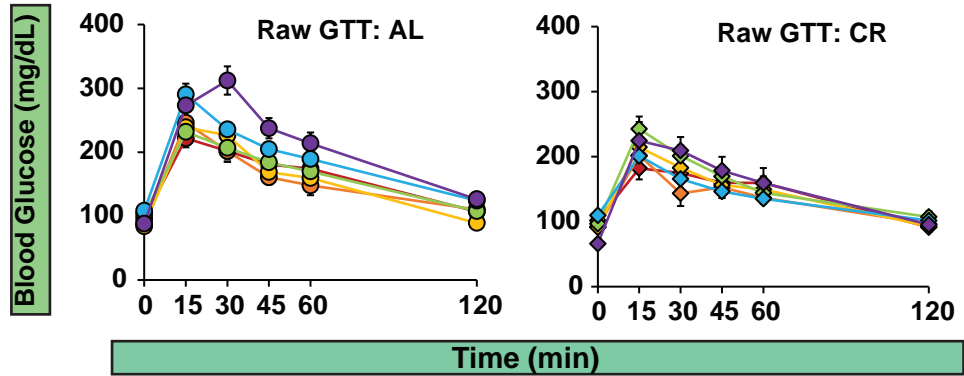
# Supplementary Figure 7



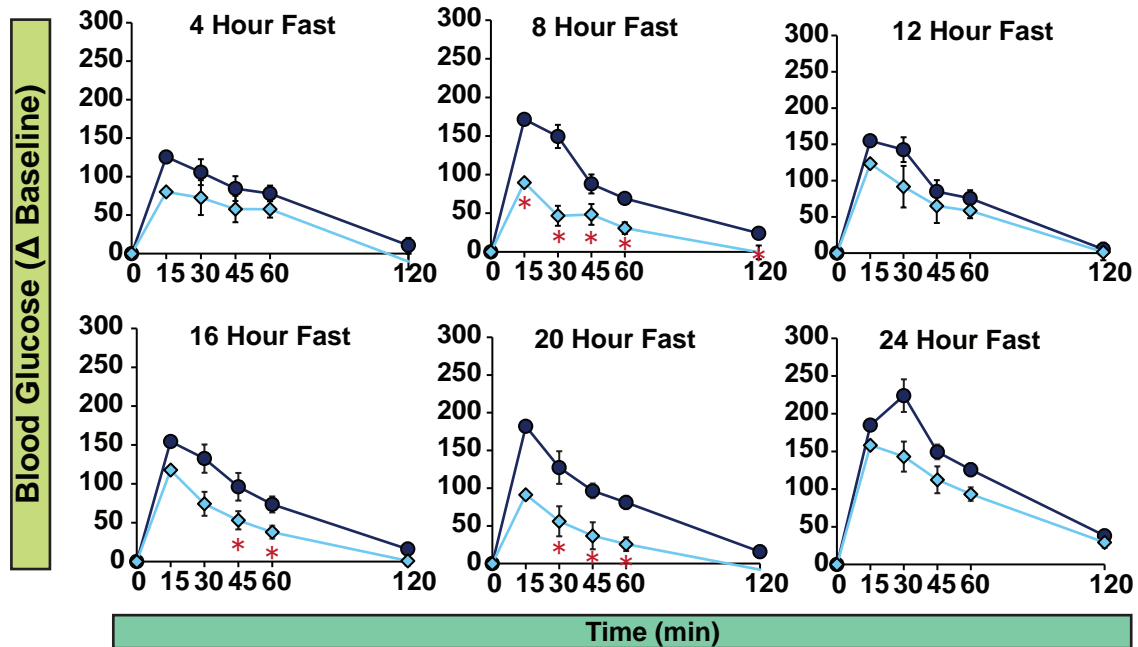
**B** **GTT Raw**



**C**



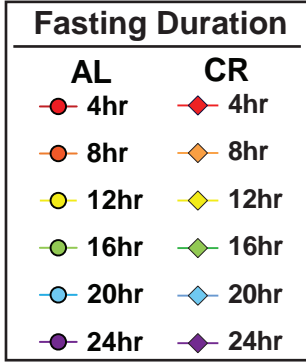
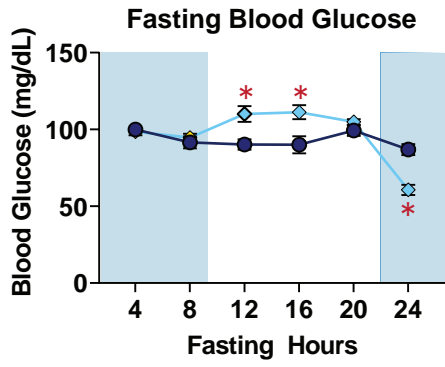
**D**  **$\Delta$ GTT**



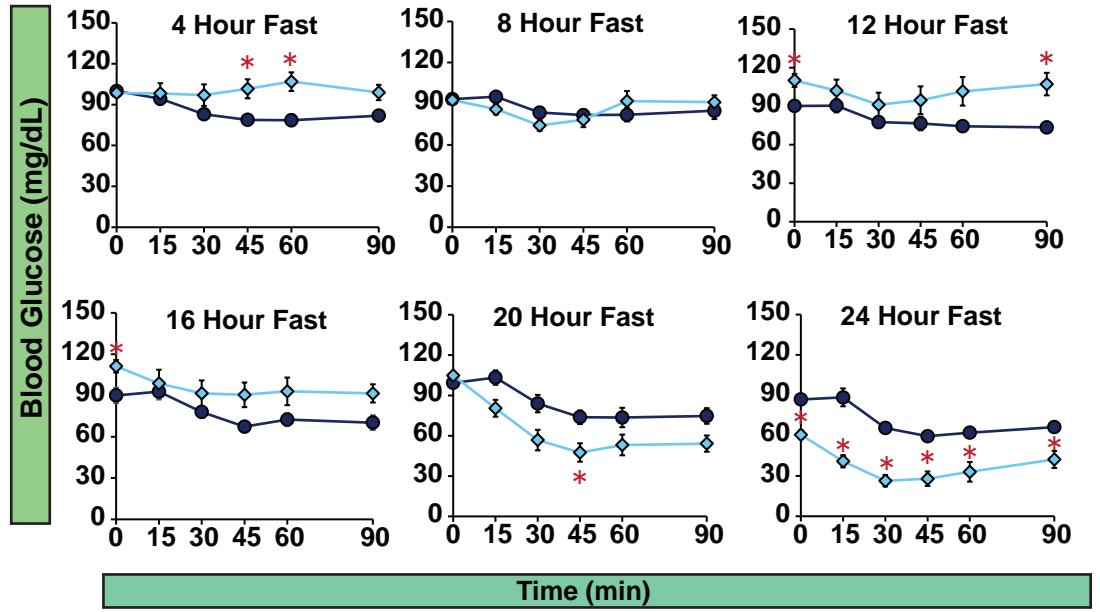
**Supplementary Figure 7. Raw and delta values for glucose tolerance tests of C57BL/6J mice in Night-Fed study, related to Figure 3.** (A) Fasting blood glucose prior to GTT. (B) Raw GTT values. (C) Combined raw GTT for AL and CR (D) Delta values for GTT. (AL, n = 7-16 per time point; CR, n = 7-16 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM. (A-D) AL, n = 7-16 per time point; CR, n = 7-16 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

# Supplementary Figure 8

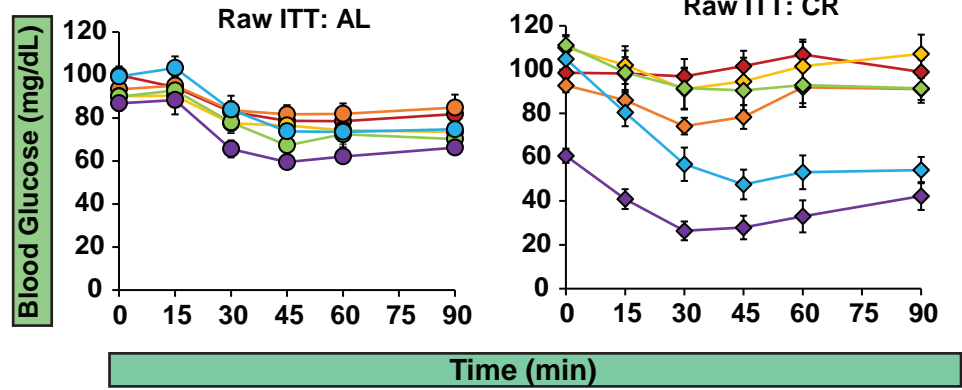
**A**



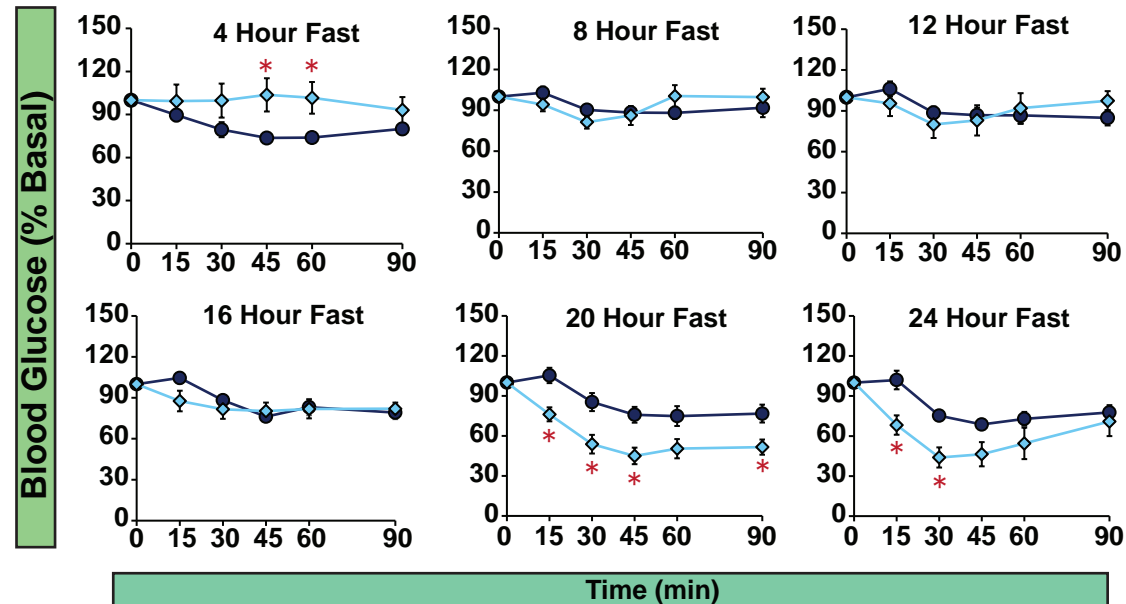
**B Raw ITT**



**C**



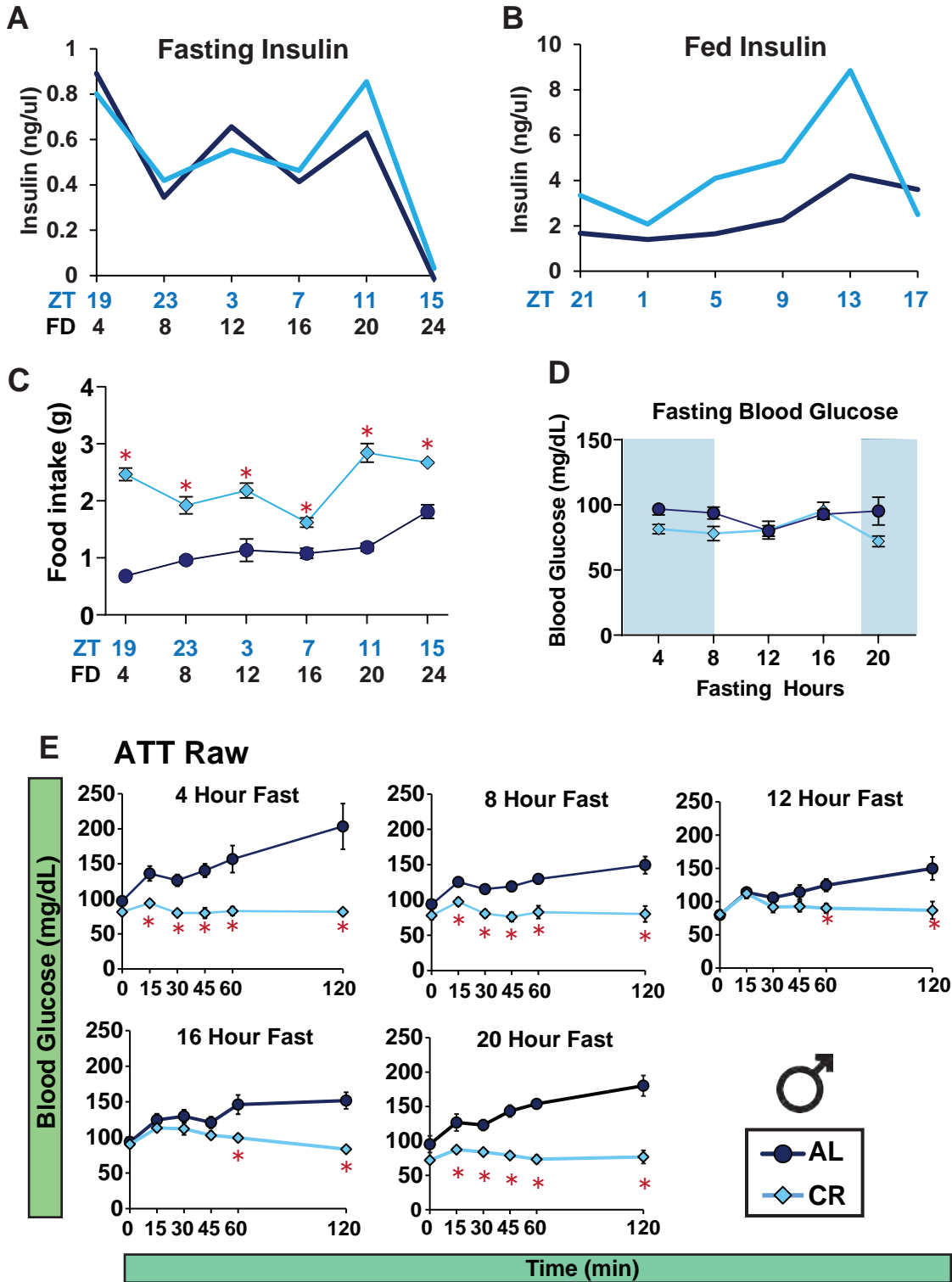
**D  $\Delta$ ITT**



**Supplementary Figure 8. Raw and delta values for insulin tolerance tests of C57BL/6J mice in Night-Fed study, related to Figure 3.** (A) Fasting blood glucose prior to ITT. (B) Raw ITT values. (C) Combined raw ITT for AL and CR (D) Delta values for ITT. (A-D) AL, n = 7-16 per time point; CR, n = 7-16 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

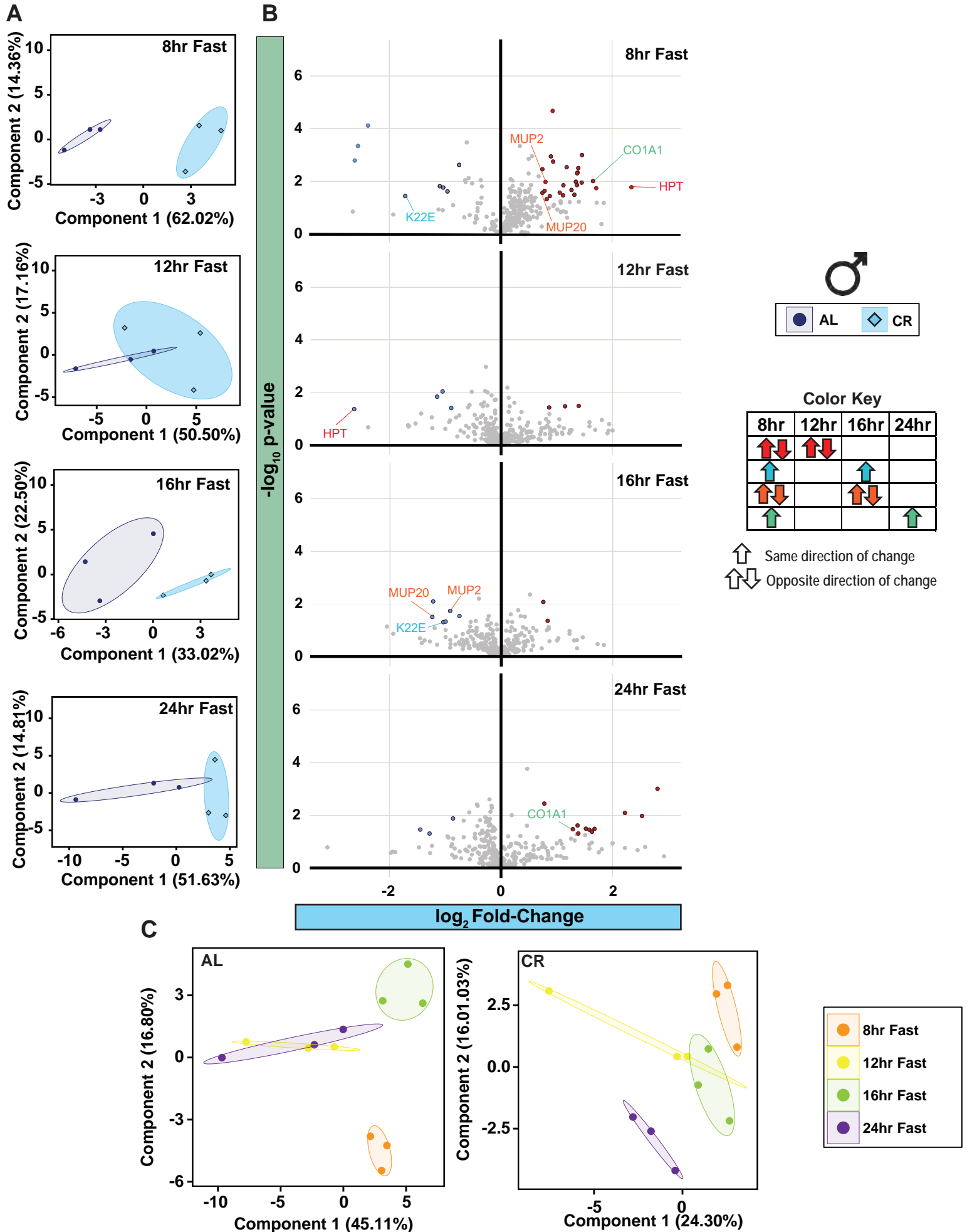


# Supplementary Figure 9



**Supplementary Figure 9. Meal-stimulated insulin level and raw values for alanine tolerance test in Night-Fed males, related to Figure 3.** (A) Simplified representation of fasted insulin level related to Figure 3H. (B) Simplified representation of fed insulin level related to Figure 3H. (C) Food intake during MSIS test. (D) Fasting blood glucose prior to ATT. (E) Raw values for ATT. (A-E) AL, n = 7-16 per time point; CR, n = 7-8 per time point biologically independent mice; \*p<0.05 AL-fed vs. CR-fed mice at each time point, Sidak's test post two-way repeated measures ANOVA. Data represented as mean  $\pm$  SEM.

# Supplementary Figure 10

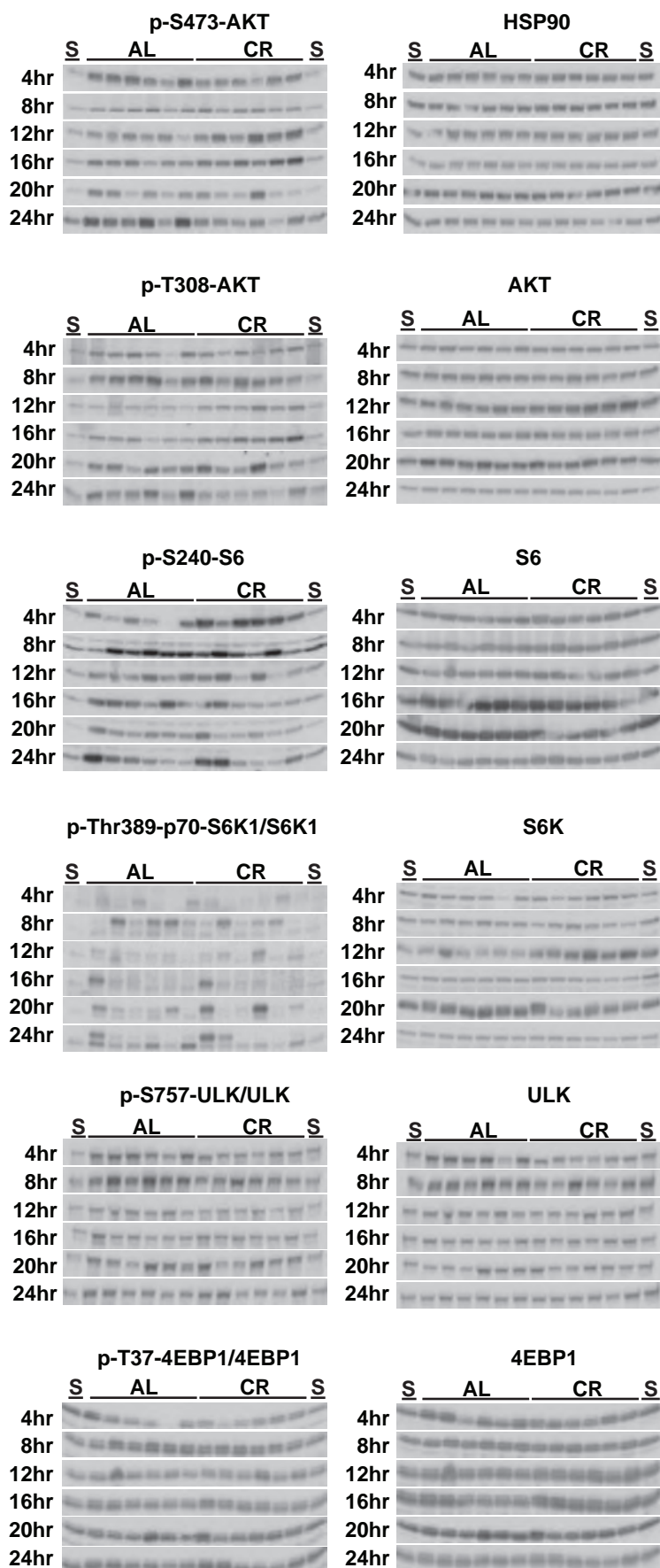


**Supplementary Figure 10. Calorie restriction has minimal effects on the plasma proteome.**

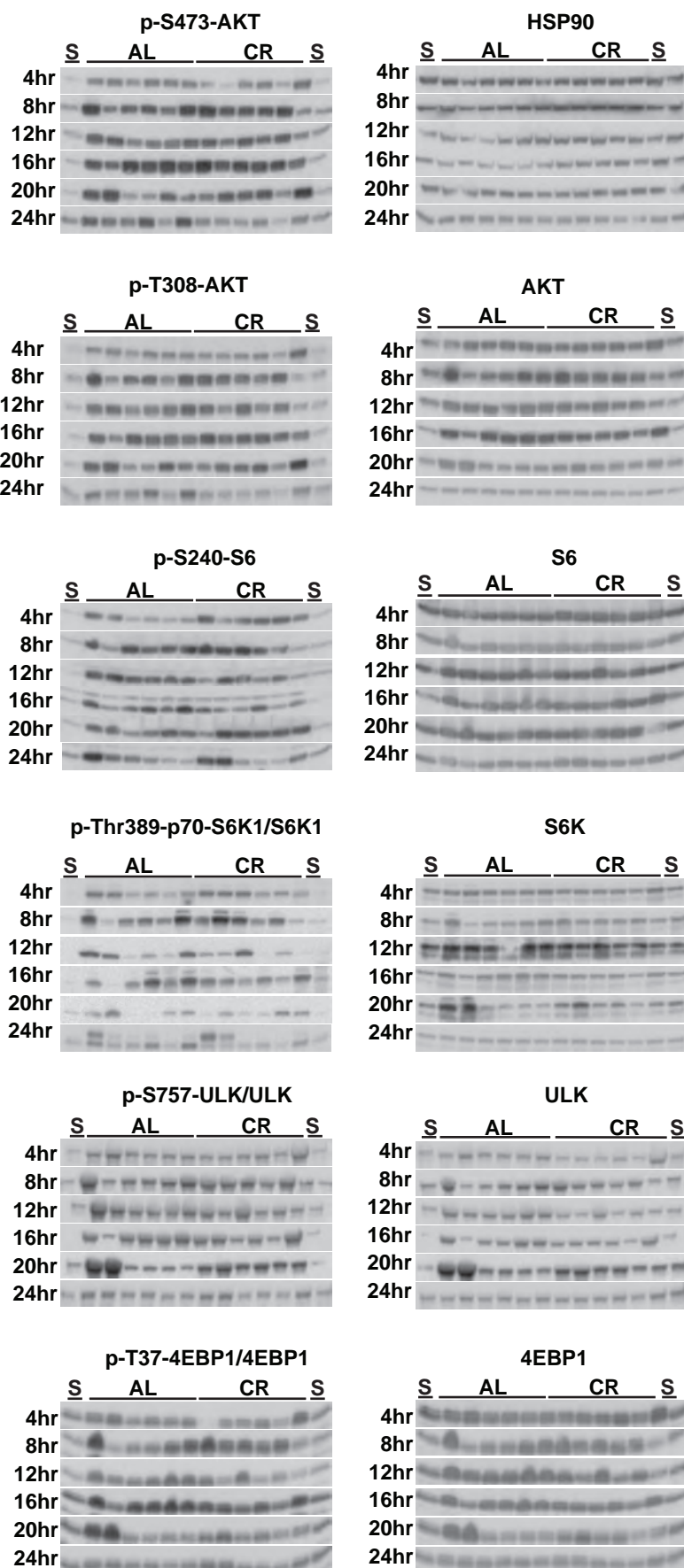
(A) Untargeted proteomics were performed on the plasma of male C57BL/6J mice fed AL and CR diet from the Morning Study PCA and volcano plot of plasma proteins with AL and CR mice collected after an 8hr, 12hr, 16hr and 24hr fast. (B) Volcano plots show the statistical significance (p-value; y-axis) versus magnitude of change (fold-change ( $\log_2$ ; x-axis). Significantly decreased metabolites are colored blue and significantly increased metabolites are colored red. Metabolite names are colored coded based on significant change between AL and CR at more than one time point (color key). (C) PCA of plasma protein collected after an 8hr, 12hr, 16hr and 24hr fast for AL- or CR-fed mice. n = 3 biologically independent mice per diet.

# Supplementary Figure 11

## A Morning Study Liver



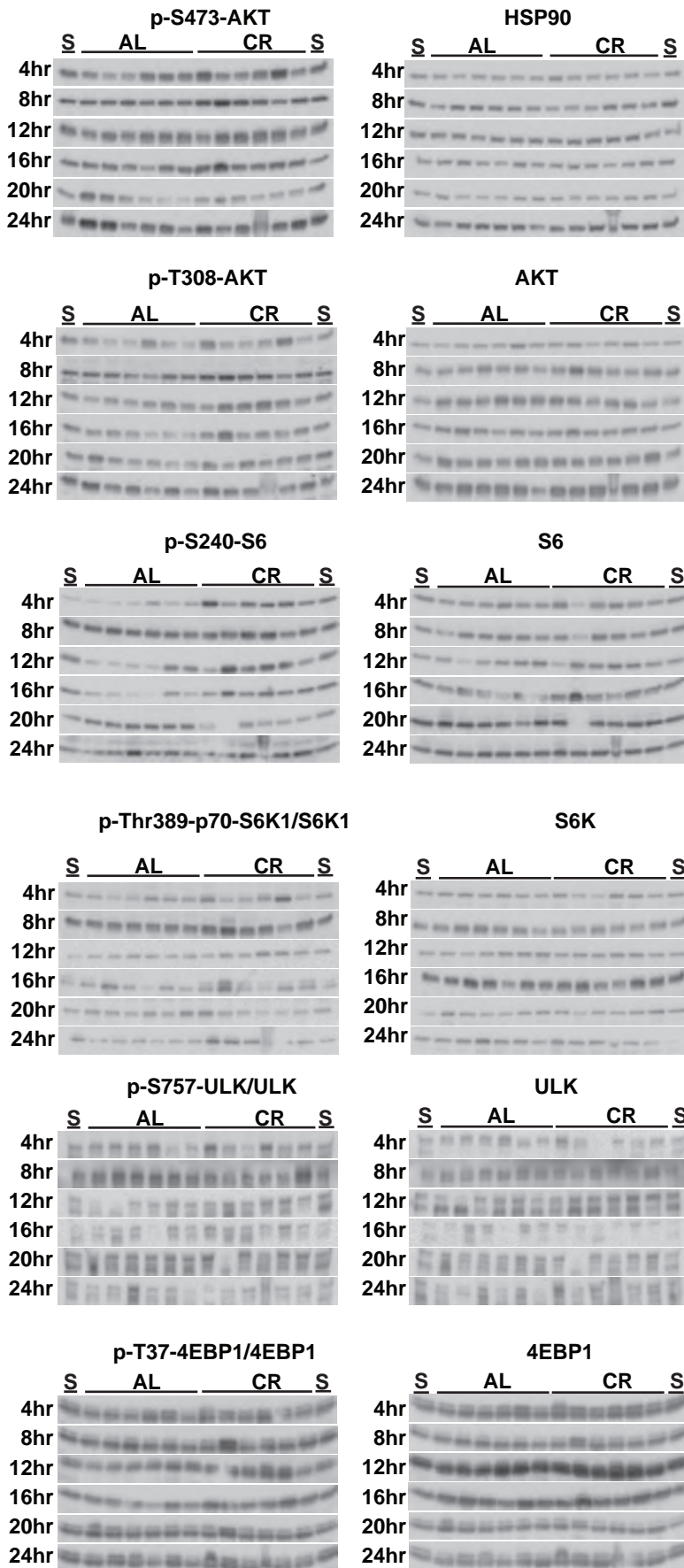
## B Night Study Liver



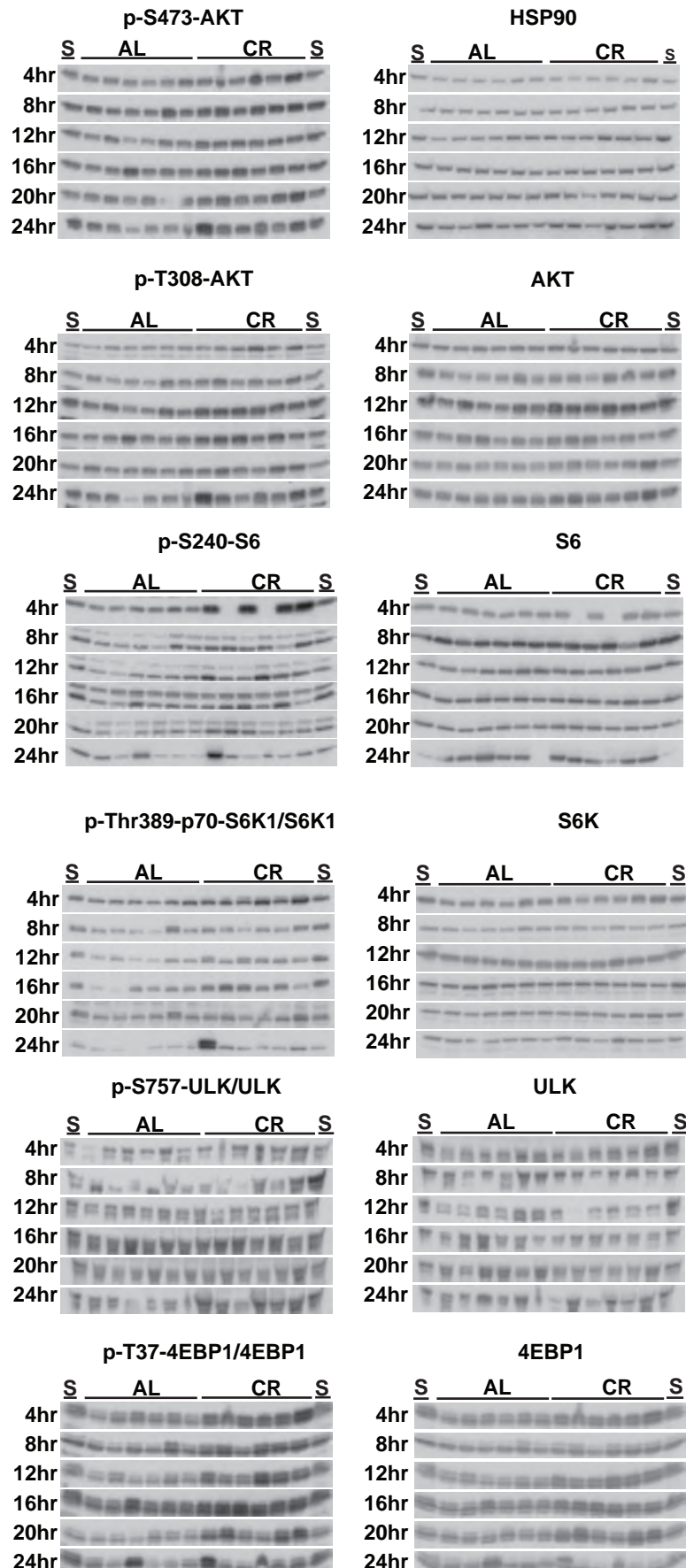
**Supplementary Figure 11. Liver western blots, related to Figure 5. (A) Morning-Study Liver. (B) Night-Study Liver.**

# Supplementary Figure 12

## A Morning Study Muscle



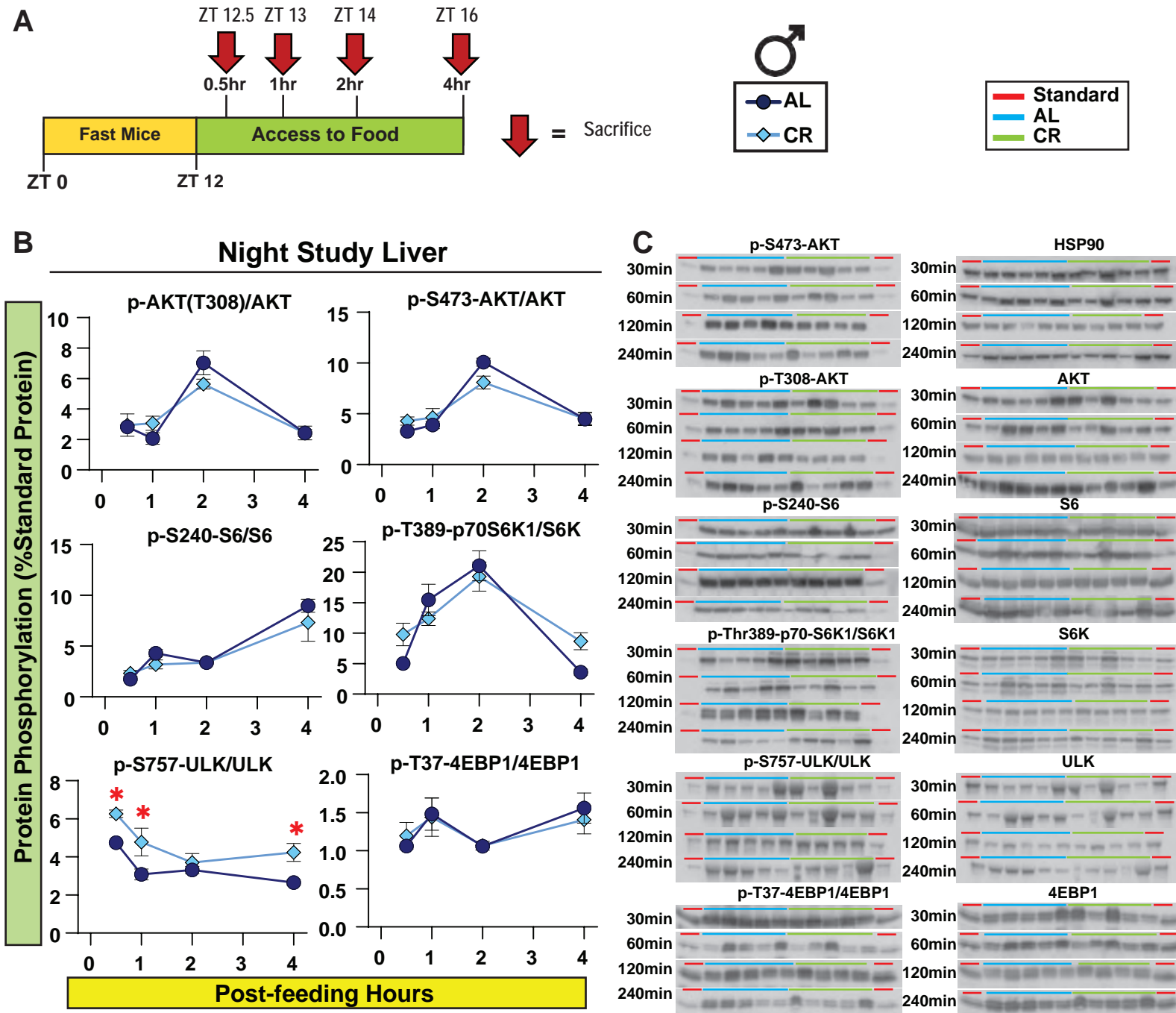
## B Night Study Muscle



**Supplementary Figure 12. Muscle Western blots, related to Figure 5. (A) Morning-Study Muscle. (B) Night-Study Muscle.**



# Supplementary Figure 13

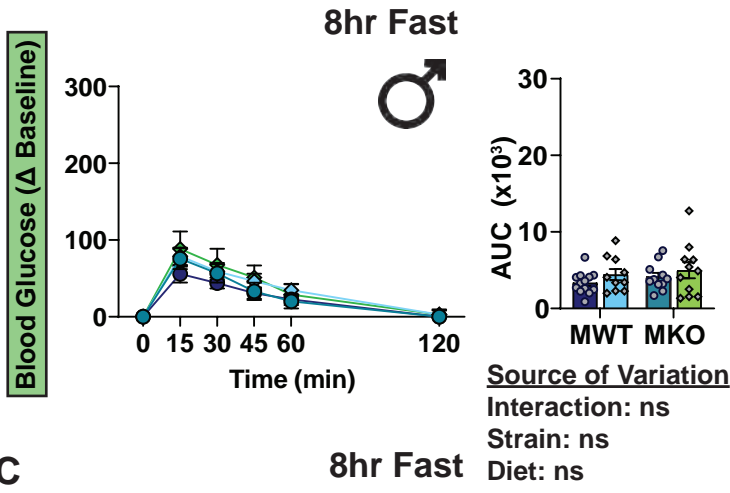


**Supplementary Figure 13. Liver mTORC1 activity is dependent on feeding status, related to Figure 5. (A) Experimental design. (B) Western Blot analysis of refeed Night-Study Liver and (C) Western Blots. (n = 5 AL-fed and CR-fed biologically independent mice per time point; \*p<0.05, Sidak's test post 2-way ANOVA). Data represented as mean ± SEM.**

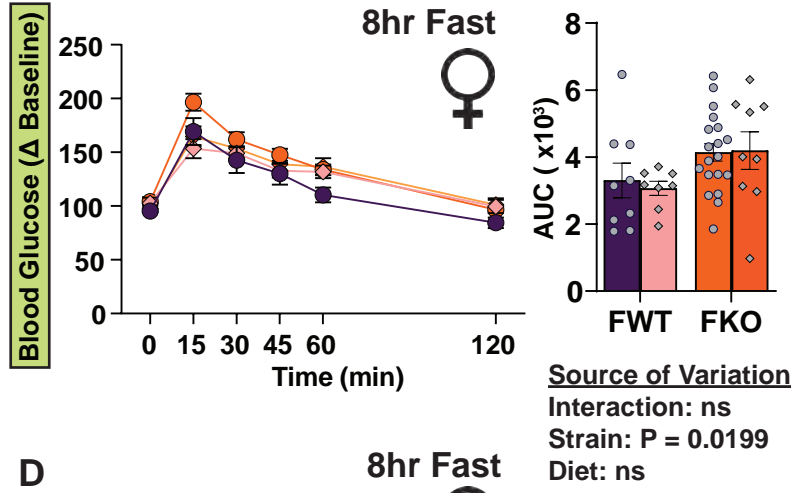
# Supplementary Figure 14



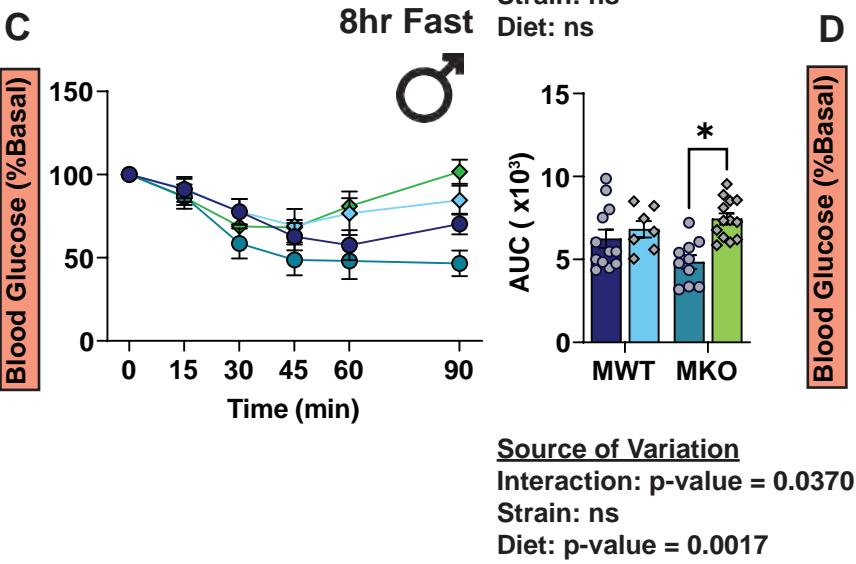
**A**



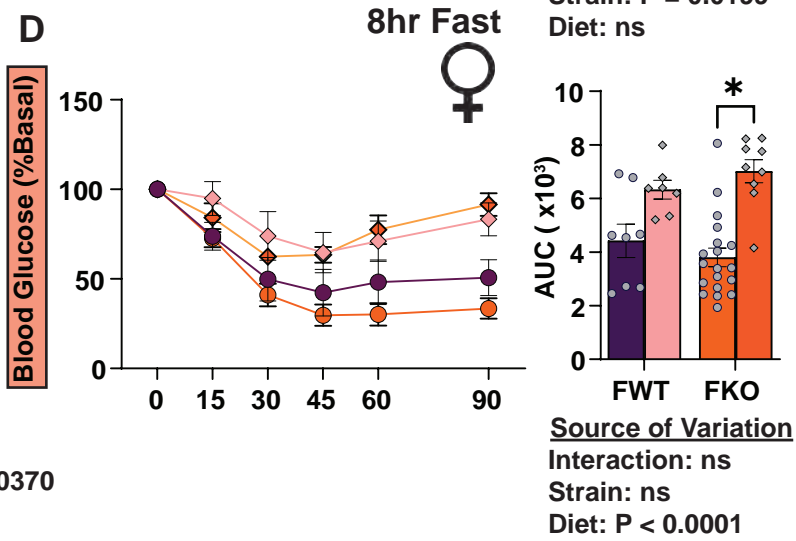
**B**



**C**



**D**



**Supplementary Figure 14. Glucose and insulin tolerance test in *Tsc1* (KO) after an 8hr fast, related to Figure 6.** (A-B) Glucose tolerance tests in male (A) and female (B) mice lacking hepatic *Tsc1* (KO) and their wild-type (WT) littermates. (C-D) Insulin tolerance tests in male (C) and female (D) mice lacking hepatic *Tsc1* (KO) and their wild-type (WT) littermates. (A,C) MWT AL, n = 13; MWT CR, n = 10; MKO AL, n = 11; MKO CR, n = 10 biologically independent mice. (B,D) FWT AL, n = 9; FWT CR, n = 8; FKO AL, n = 20; FKO CR, n = 9 biologically independent mice. (A-D) statistics for the overall effects of genotype, diet, and the interaction represent the p value from a two-way ANOVA; \*p<0.05, from a Sidak's post-test examining the effect of parameters identified as significant in the 2-way ANOVA. Data represented as mean  $\pm$  SEM.