**Supplemental Information:**

# Autotaxin-Lysophosphatidic Acid Signaling Contributes to Obesity-Induced Insulin Resistance in Muscle and Impairs Mitochondrial Metabolism

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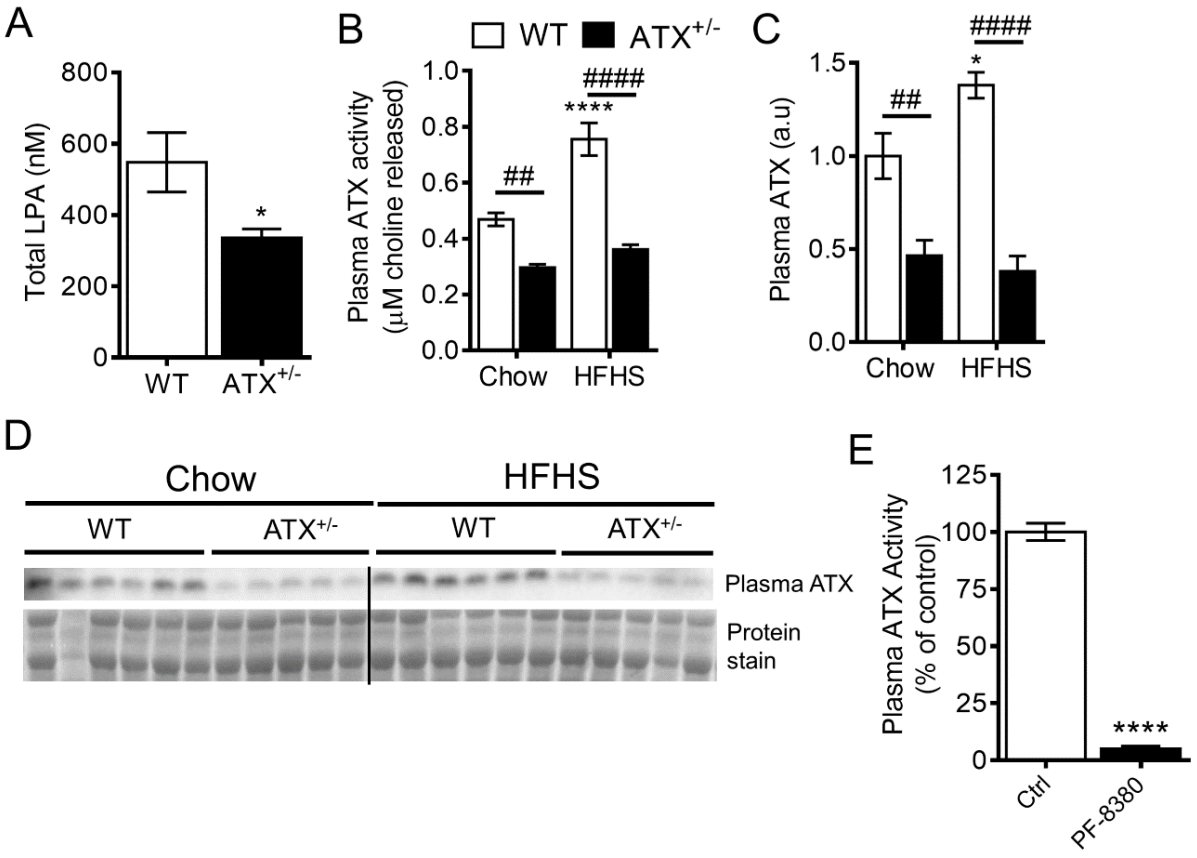
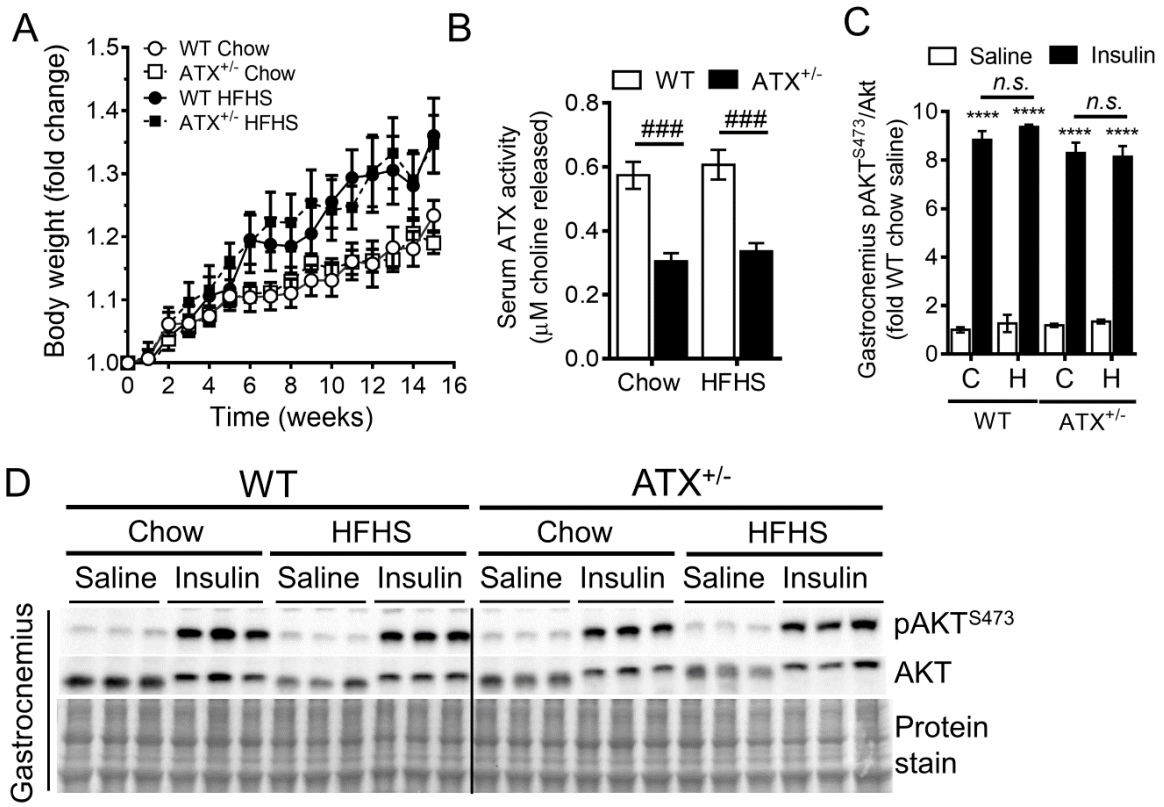
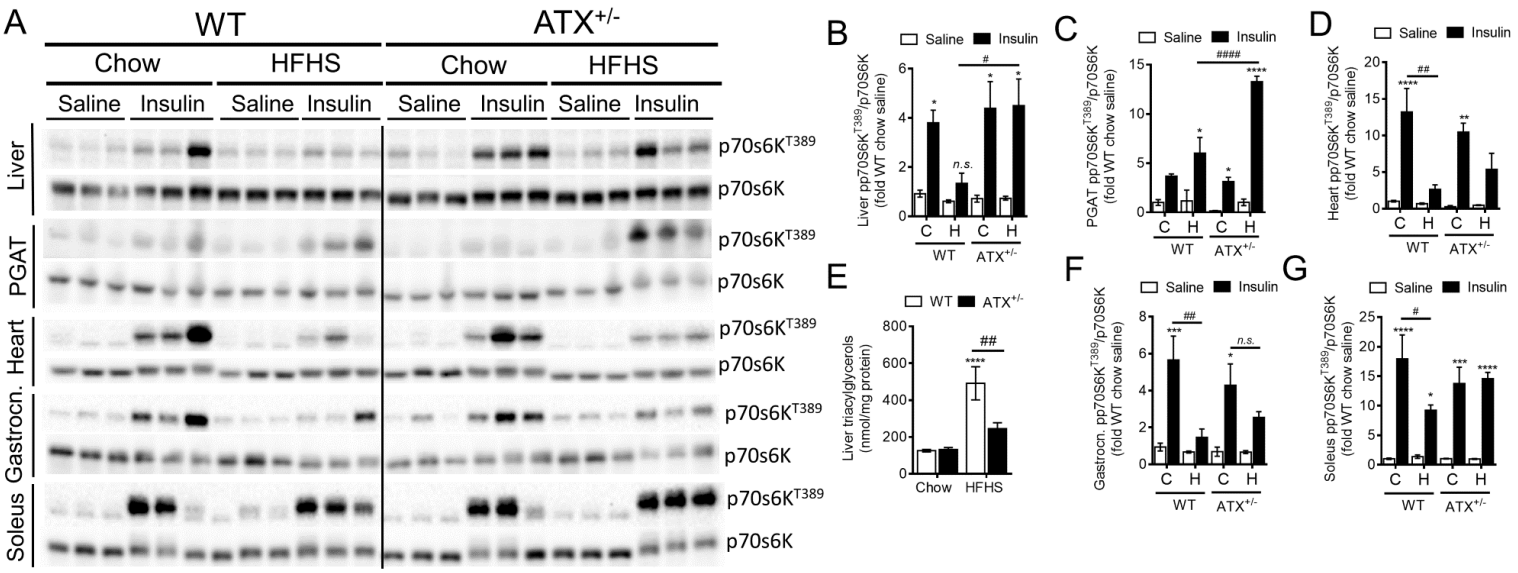



**Supplemental Figure S1. Plasma ATX activity and protein levels are reduced in male chow and HFHS-fed ATX$^{+/-}$ mice. (A)** Plasma LPA levels ($n$ = 7-8) in chow-fed WT and ATX$^{+/-}$ mice. **(B)** Plasma ATX activity ($n$ = 9-11) and **(C, D)** ATX protein content ($n$ = 5-6) in chow and HFHS-fed WT and ATX$^{+/-}$ mice. **(E)** Inhibition of ATX activity using PF-8380 blunts choline release, demonstrating that choline release is highly ATX dependant. **(A, E)** Statistical analysis was performed using a Student's t-test or **(B, C)** two-way ANOVA followed by a Tukey's multiple comparison test; (**A-C, E**) *$p < 0.05$, ****$p < 0.0001$ vs. chow/WT/ctrl; $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$ as indicated.




**Supplemental Figure S2. Female mice are protected from HFHS diet-induced insulin resistance and upregulation of ATX. (A)** Body weight gain and **(B)** serum ATX activity in chow and HFHS-fed WT and $ATX^{+/-}$ mice. **(C-D)** Immunoblot and densitometric analysis of AKT phosphorylation at $S^{473}$ in gastrocnemius muscle from chow and HFHS-fed WT and $ATX^{+/-}$ mice subjected to a 3-h food withdrawal, followed by the intraperitoneal injection of saline or 10 U/kg insulin ($n$ = 3-5). **(A-C)** Statistical analysis was performed using a two-way ANOVA followed by a Tukey's multiple comparison test; **(B)** $^{###}p < 0.001$ as indicated; **(C)** $^{****}p < 0.0001$ vs. saline.




**Supplemental Figure S3. Male HFHS-fed ATX$^{+/-}$ mice show improved insulin signaling in liver, PGAT, heart, gastrocnemius and soleus muscle.** Immunoblot and densitometric analysis of p70S6K phosphorylation at T$^{389}$ in **(A, B)** liver, **(A, C)** PGAT, **(A, D)** heart, **(A, F)** gastrocnemius and, **(A, G)** soleus muscle from chow and HFHS-fed WT and ATX$^{+/-}$ mice subjected to a 3-h food withdrawal, followed by the intraperitoneal injection of saline or 10 U/kg insulin ($n$ = 4-6). **(E)** Liver TGs from chow and HFHS-fed WT and ATX$^{+/-}$ mice subjected to a 3-h food withdrawal ($n$ = 9). **(B-G)** Statistical analysis was performed using a two-way ANOVA followed by a Tukey's multiple comparison test; *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001, ****p < 0.0001 vs. saline; $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01, $^{\#\#\#\#}p$ < 0.0001 as indicated; C, chow; H, HFHS.