Supplemental Information

α1-Antitrypsin A treatment attenuates neutrophil elastase accumulation and enhances insulin sensitivity in adipose tissue of mice fed a high-fat diet.

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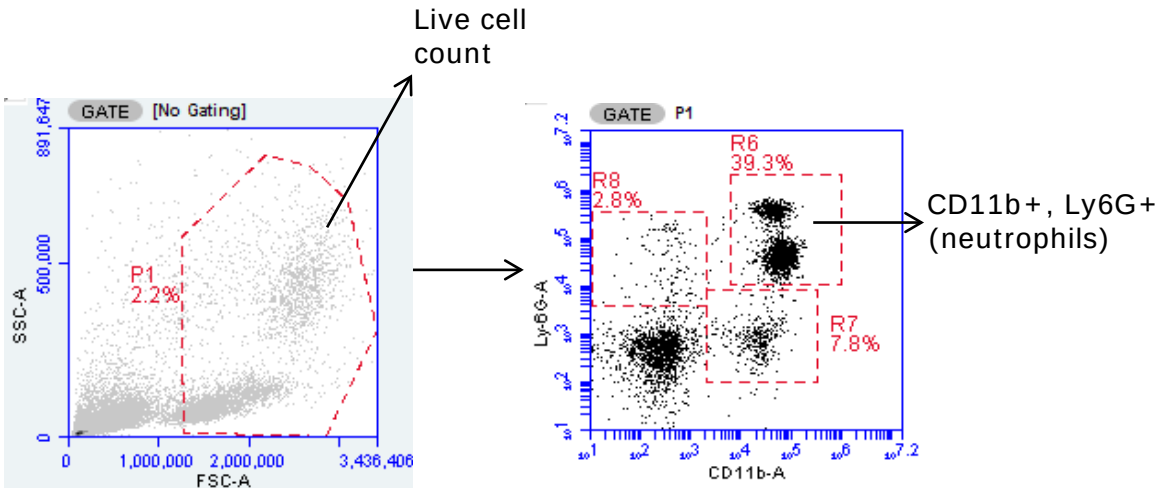
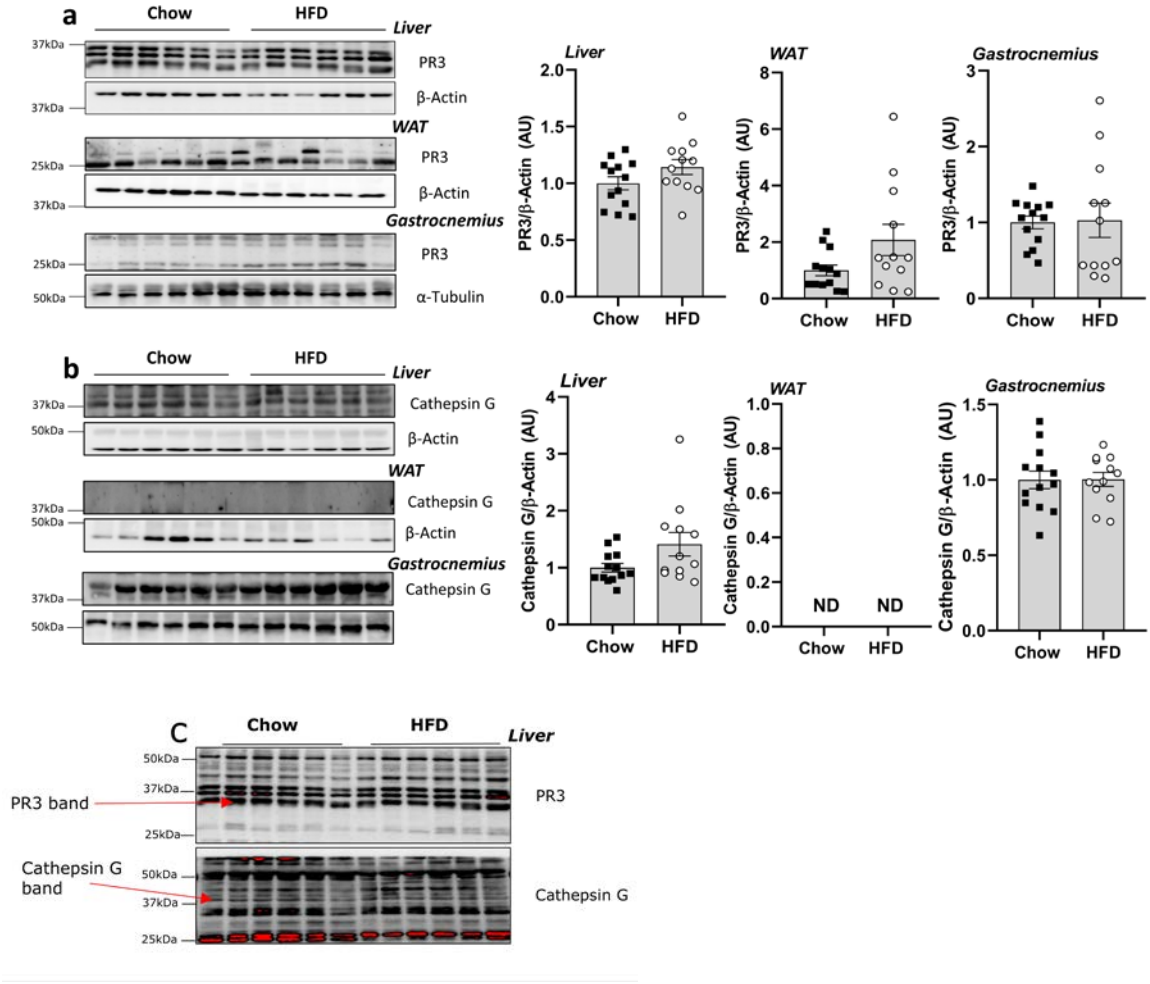
Supplementary figure 1



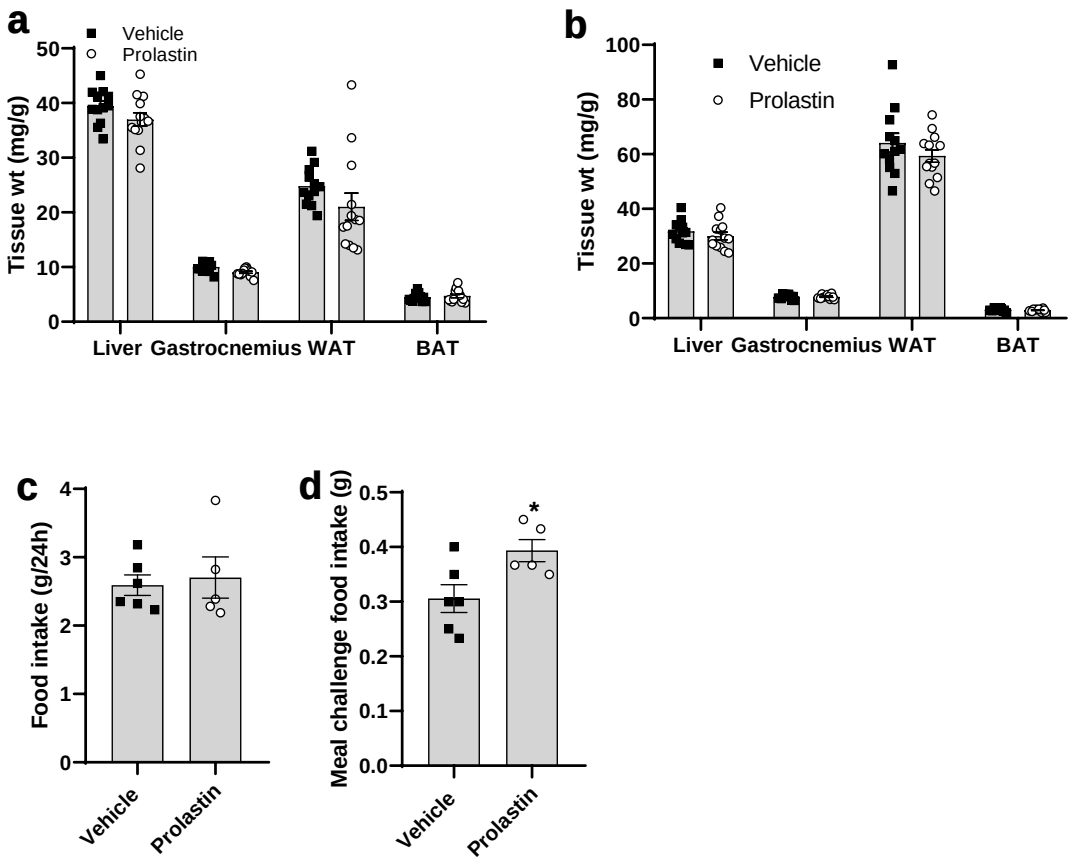
Supplementary Figure 1. FACS gating strategy for identification of neutrophil content in blood cells. Gate P1 contains all cells that were deemed acceptable immune cell events as per forward and side scatter criteria. Within P1, the R7 gate contains all cells that were $Cd11b^{+}$, $Ly6G^{-}$whilst the R8 gate reflects all events that were $Cd11b^{-}$, $Ly6G^{+}$. The R6 gate contained all cell events that were both $Cd11b^{+}$, $Ly6G^{+}$ which were identified as neutrophils for the present analyses.

Supplementary figure 2



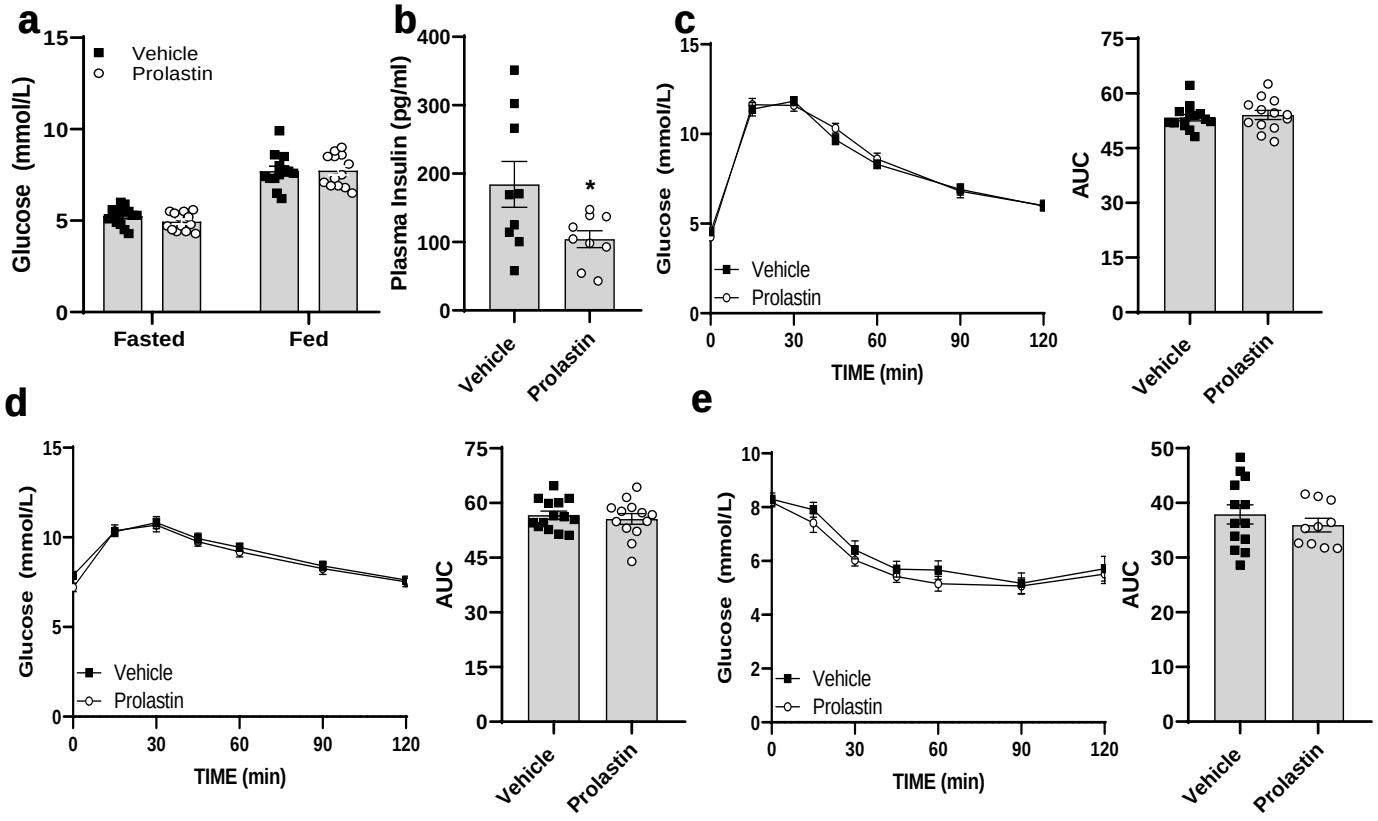
Supplementary Figure 2: The effect of high fat diet (HFD) on PR3 and Cathepsin G expression in insulin sensitive tissues. (a-b) Liver, visceral white adipose tissue and gastrocnemius muscle tissue expression of PR3 and Cathepsin G (chow n=12, HFD n=12). **(c)** Liver whole blot image for both PR3 and Cathepsin G showing multiple banding pattern. Results are presented as means ± SE. Significance was determined using two tailed student's t-test, *p < 0.05. ND, not detected.

Supplementary figure 3



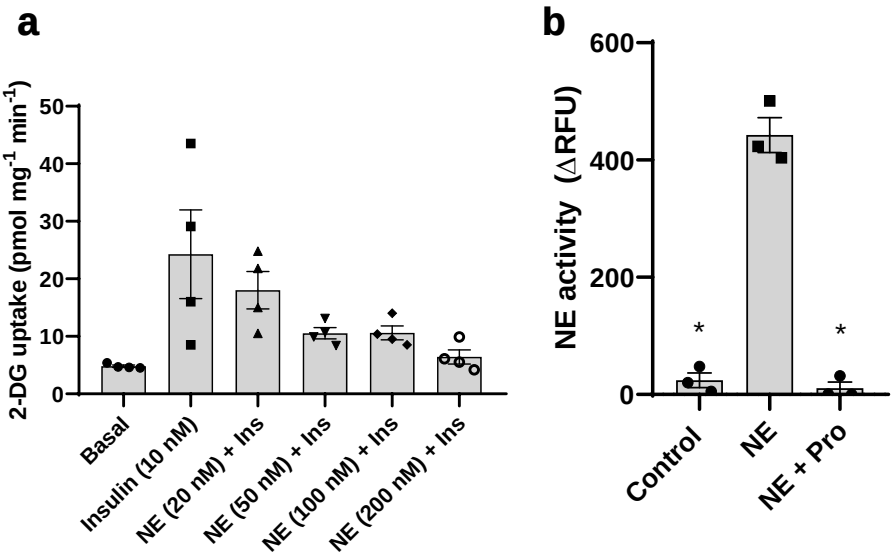
Supplementary Figure 3: Effect of Prolastin on body composition, diet consumption responses to a single meal. Body composition for chow **(a)** and high fat diet fed mice respectively **(b)**. **(c)** High fat diet fed mice feed consumption per day, and in an acute 3 hour feed **(d)** following an overnight fast. Data shown as means ± SE. **(a-d)** n=12 per group except HFD + Prolastin group n=13. Significance for **(a-d)** was determined using two tailed student's t-test. *$p < 0.05$.

Supplementary figure 4



Supplementary Figure 4. The effect of Prolastin on blood glucose homeostasis, insulin sensitivity, and liver function in standard chow fed mice. (a) Overnight fasting and fed blood glucose, **(b)** 3h fasted plasma insulin, **(c-e)** temporal glucose in response to GTT (overnight fast followed by 1mg/g oral gavage), PTT (4h fast followed by 1mg/g sodium pyruvate ip) and ITT (4h fast followed by 0.5mU/g insulin ip) respectively. Data shown as means ± SE, n=12 per group. Significance for **(a, b)** was determined using two tailed student's t-test. *p < 0.05. Significance for **(c-e)** was determined using two-way repeated measures ANOVA with Prolastin as a between-group factor and time as repeated factor. *p <0.05 between groups at respective timepoints.

Supplementary figure 5



Supplementary Figure 5: NE impairs insulin stimulated glucose uptake in 3T3L1 adipocytes. (a) Dose-response of NE impairing glucose uptake and **(b)** NE maintains activity in cell media. Data shown as means ± SE. **(b)** Significance was determined via one way ANOVA. *$p<0.05$ between NE and other groups.

Supplementary tables

Supplementary Table 1: Antibody list

Antibody	Company	Catalog	Dilution
CD11b	BD Biosciences	564985	1:100
LY6G	Biolegend	127605	1:50
Neutrophil Elastase	Abcam	ab68672	1:1000
PR3	Abcam	ab103632	1:500
Cathepsin G	Abcam	ab197354	1:500
A1AT	ThermoFisher Scientific	PA5-16661	1:2500
Akt^{Ser473}	Cell Signalling	CST9271	1:1000
Akt	Cell Signalling	CST9279	1:1000
Caspase 1	Abcam	ab138483	1:1000
p-IR1β	Cell Signalling	CST3024	1:1000
IR	Cell Signalling	CST3020	1:1000
p-IRS-1	Cell Signalling	CST3203	1:1000
IRS-1	Cell Signalling	CST2382	1:1000
α-Tubulin	Sigma-Aldrich	T9026	1:5000
β-Actin	Sigma-Aldrich	A2228	1:5000
Secondary Mouse	ThermoFisher Scientific	G21240	1:10000
Seocondary Rabbit	ThermoFisher Scientific	G21234	1:10000
Secondary Rat	ThermoFisher Scientific	A10549	1:5000

Supplementary table 2: qPCR primer sequences

Gene name	Forward primer	Reverse primer
36B4	GGCCCTGCACTCTCGCTTTC	TGCCAGGACGCGCTTGT
B2m	TGACCGGCCTGTATGCTATC	GGCGGGTGGAACTGTGTTAC
Gapdh	CTTTGGCATTGTGGAAGGGC	CAGGGATGATGTTCTGGGCA
Itgam	TGGCCTATACAAGCTTGGCTTT	AAAGGCCGTTACTGAGGTGG
Ly6G	GGAGGGGCTGAGAGAAAGTAAA	GCTGCACAGATAAAACTTCCTC
Elane	AGGCGTGGAGGTCATTTCTG	TGACCGGAAATTTAGGCCGT
Prtn3	ACGGTGGTCACCTTCCTATG	GAATGCCATTGCAGATCAAG
Ctsg	AGAAGACTTCGTCCTAACAGCA	CCTTTCTCGCATTTGGATGTTGT
Cd68	ACTGGTGTAGCCTAGCTGGT	CCTTGGGCTATAAGCGGTCC
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
ChREBP	CTGGGGACCTAAACAGGAGC	GAAGCCACCCTATAGCTCCC
G6P	TGCAAGGGAGAACTCAGCAA	GGACCAAGGAAGCCACATG
GK	CCCTGAGTGGCTTACAGTTC	ACGGATGTGAGTGTTGAAGC
PEPCK	GTGTTTGTAGGAGCAGCCATGAGA	GCCAGTGGGCCAGGTATTTG
Tnf-α	CCCACACCGTCAGCCGATTT	GTCTAAGTACTTGGGCAGATTGACC
Il-6	TCCTCTCTGCAAGAGACTTCC	TTGTGAAGTAGGGAAGGCCG
Mcp-1	AGGTCCCTGTCATGCTTCTGG	AGGAGCTGTCATTAGGGACATC
IL-1β	GCCACCTTTTGACAGTGATGAG	GACAGCCCAGGTCAAAGGTT
Mrc	GGCTGATTACGAGCAGTGGA	CATCACTCCAGGTGAACCCC
Cd163	TCTCCTGGTTGTAAAAGGTTTGT	CAGTTGTTTTCACCACCCGC
Casp1	GGGACCCTCAAGTTTTGCC	GACGTGTACGAGTGGTTGTATT