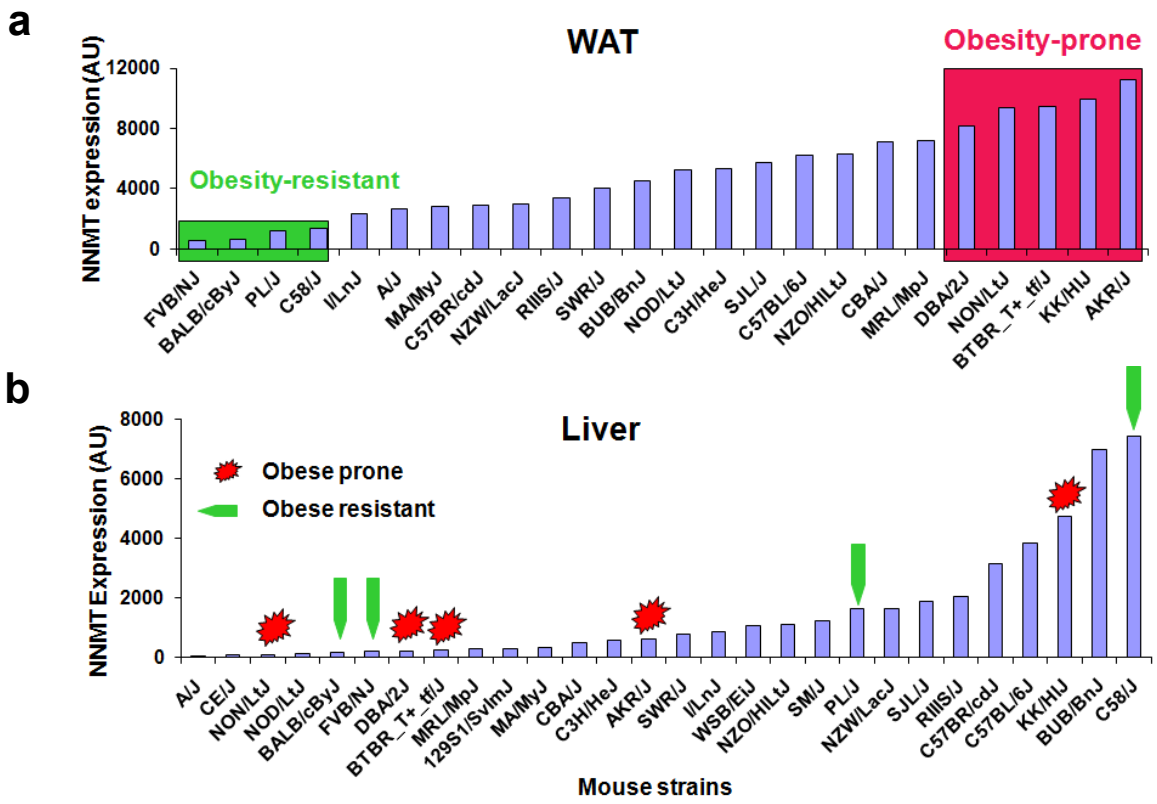
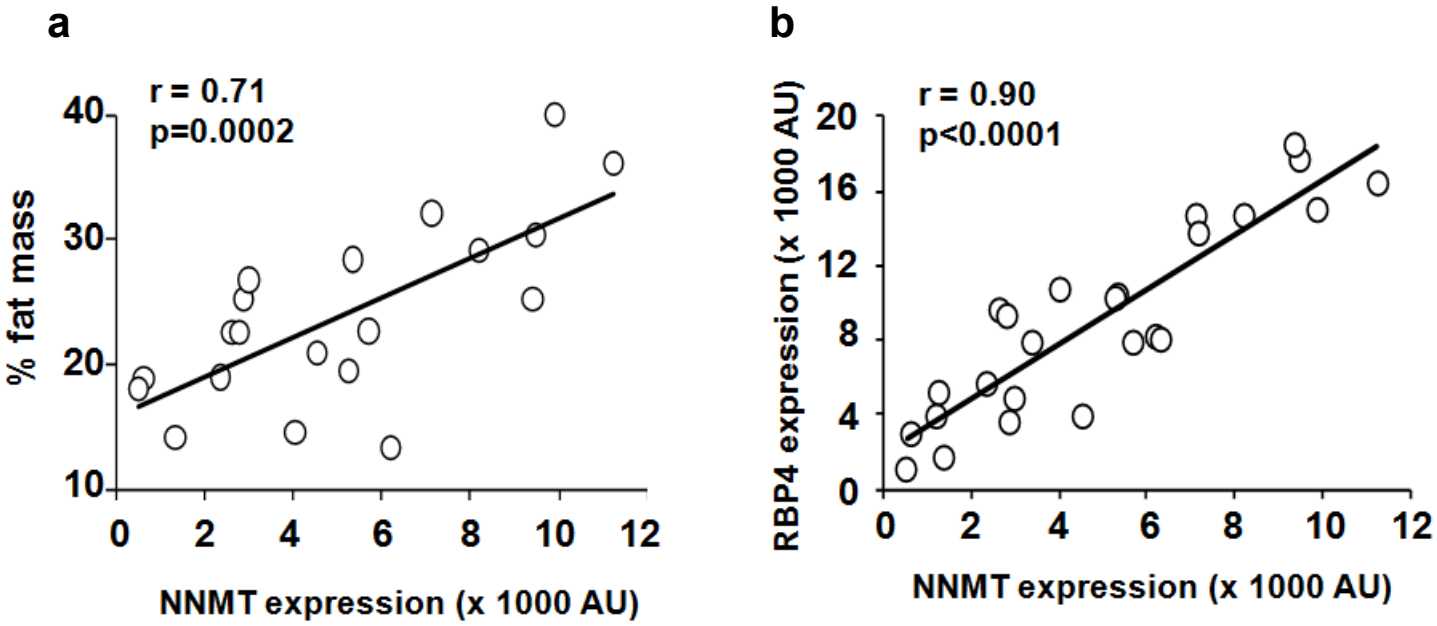
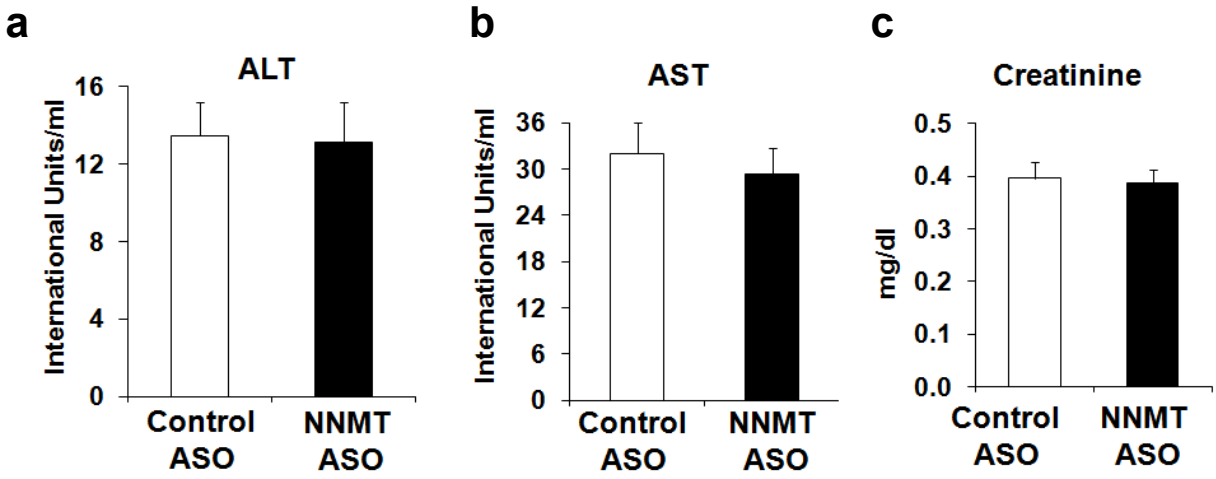
doi:10.1038/nature13198



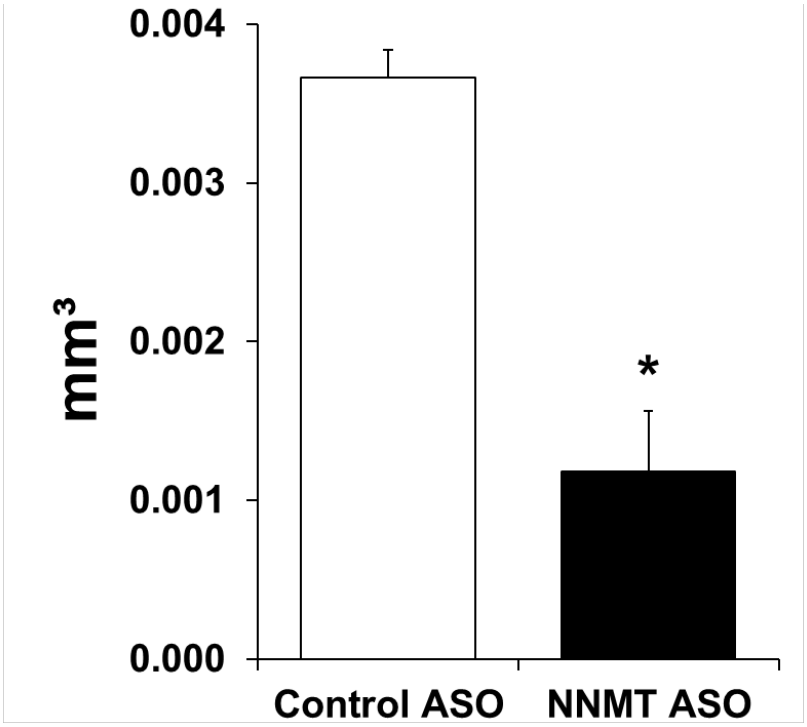
Supplementary Figure 1: *Nnmt* expression in white adipose tissue and liver of 25 strains of mice.
The *Nnmt* expression levels in **a,** white adipose tissue; **b,** liver were obtained from www.BioGPS.org - Adipose (MOE430 V2) (*14*). Obesity-resistant versus obesity-prone is defined based on body composition data from reference (*15*) and "Naggert1" in the Mouse Phenome Database (MPD) (www.jax.org/phnome), in which body composition was measured in 43 different mouse stains that were fed a high fat diet for 8 weeks (*15, 16*). Mouse line PL/J is not studied in Naggert1, but its percentage fat weight at 20 months of age is approximately one standard deviation below the average of 32 mouse strains in the Ackert1 data set (www.jax.org/phnome).



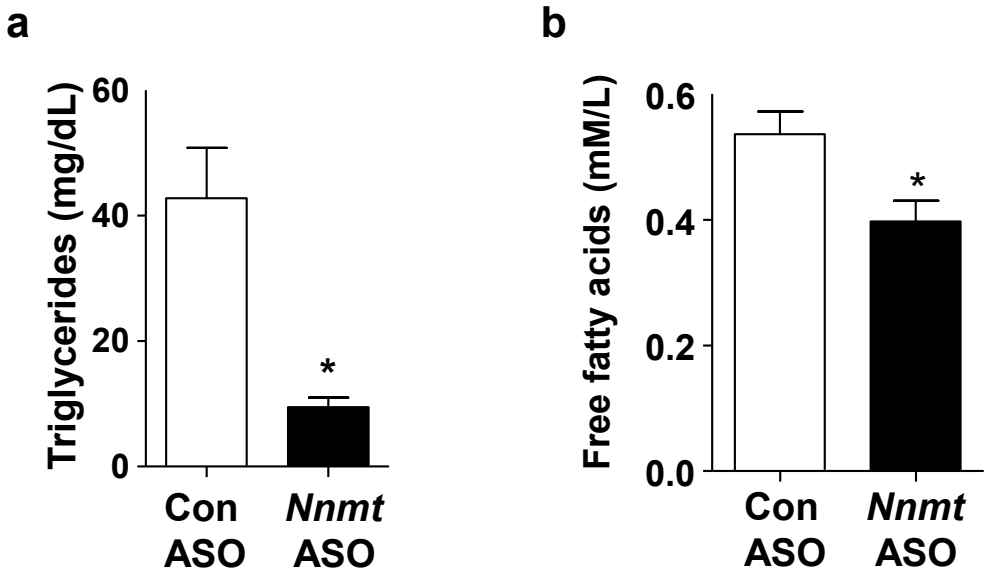
Supplementary Figure 2: Correlation of adipose *Nnmt* expression with % fat mass and RBP4 expression.
Positive correlation between *Nnmt* expression in WAT and (**a**) % fat mass and (**b**) *Rbp4* expression in WAT. The % fat mass was obtained from "Naggert1" dataset in which 43 strains of mice were fed a high fat diet for 8 weeks (www.jax.org/phnome). Among 43 strains, 20 strains have *Nnmt* expression in the BioGPS database. *Nnmt* and *Rbp4* mRNA expression levels were obtained from www.BioGPS.org – Adipose (MOE430 V2) (*14*).



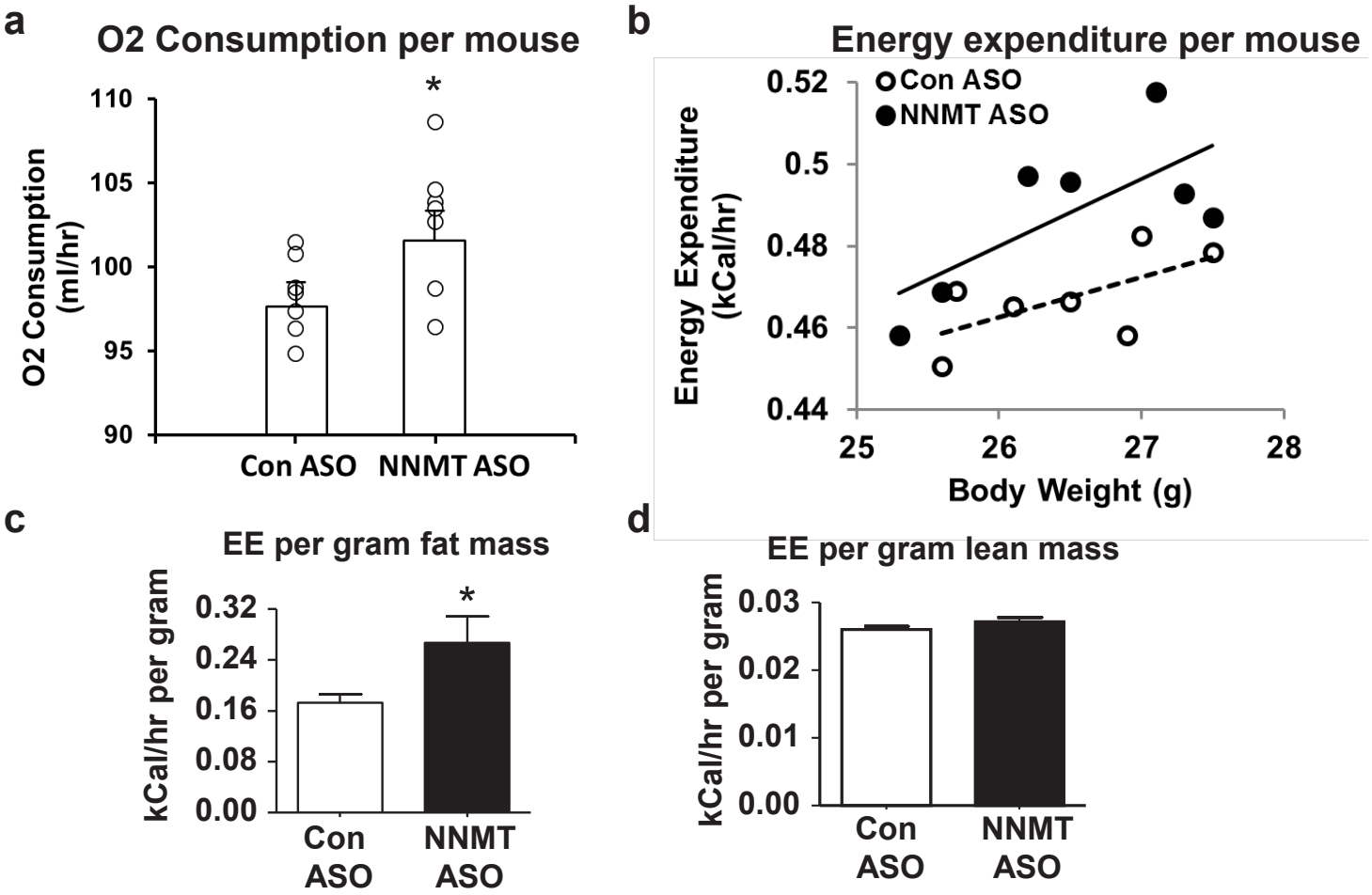
Supplementary Figure 3: Normal liver function and kidney function in *Nnmt*- and control-ASO treated mice.
Serum levels of **a,** alanine aminotransferase (ALT); **b**, aspartate aminotransferase (AST); **c**, creatinine in high fat diet fed mice treated with ASOs for 8 weeks (n=10/group).



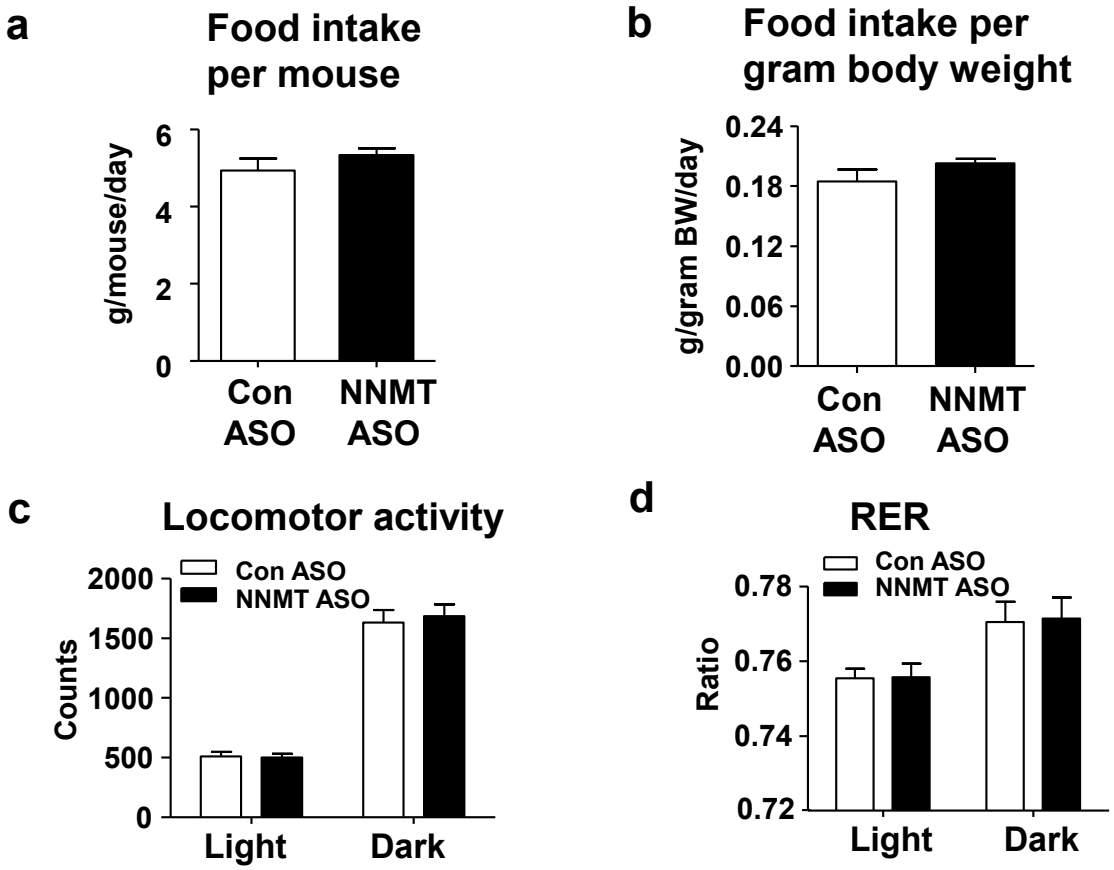
Supplementary Figure 4: *Nnmt* knockdown in adipose tissue and liver in mice decreases adipocyte volume.
Adipocyte volume in mice treated with *Nnmt*- or control-ASO for 8 weeks. Adipocyte volume was calculated from the radius obtained from adipocyte cross-sections (see example in Fig. 2l) using the formula $v = 4/3\pi r^3$. n=8 mice per group. Four sections were examined per mouse with 700-1100 cells per section. *p<0.05.



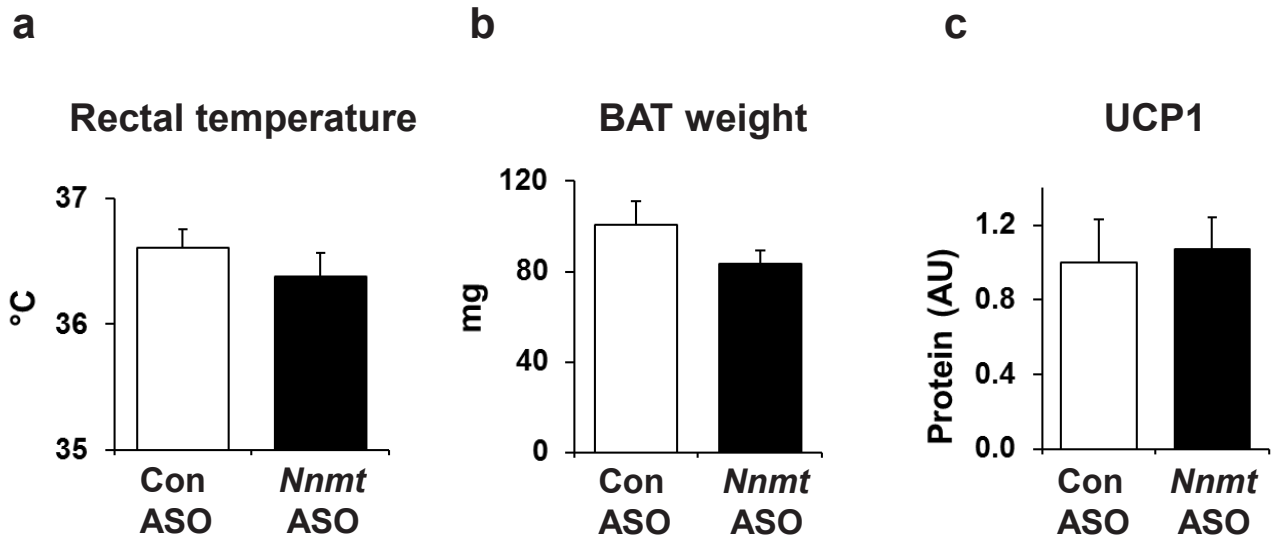
Supplementary Figure 5: *Nnmt* knockdown in adipose tissue and liver in mice fed a high fat diet decreases serum triglyceride and free fatty acid levels.
a, serum triglycerides (n=10 Control ASO; n=12 *Nnmt* ASO treated mice) and **b**, free fatty acids (n=8 Control ASO; n=10 *Nnmt* ASO) in control ASO and *Nnmt* ASO-treated mice. Mice were fed a high fat diet and were treated with ASOs for 6 weeks. *p<0.05.



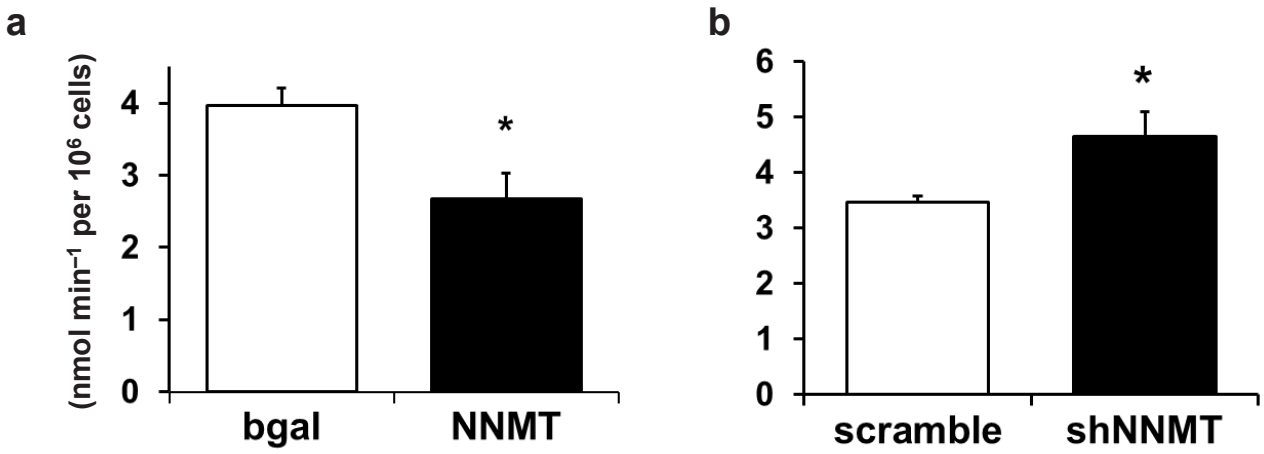
Supplementary Figure 6: ***Nnmt*** **knockdown in adipose tissue and liver increases energy expenditure in CLAMS studies.** Energy expenditure determined by CLAMS studies performed in mice treated with ASOs before body weights diverged. **a**, O2 consumption per mouse**; b**, scatter plot of the relationship between body weight and energy expenditure in the CLAMS studies. **c**, energy expenditure (EE) adjusted for fat mass; **d**, energy expenditure (EE) adjusted for lean mass. n=7 per group, *p<0.05.



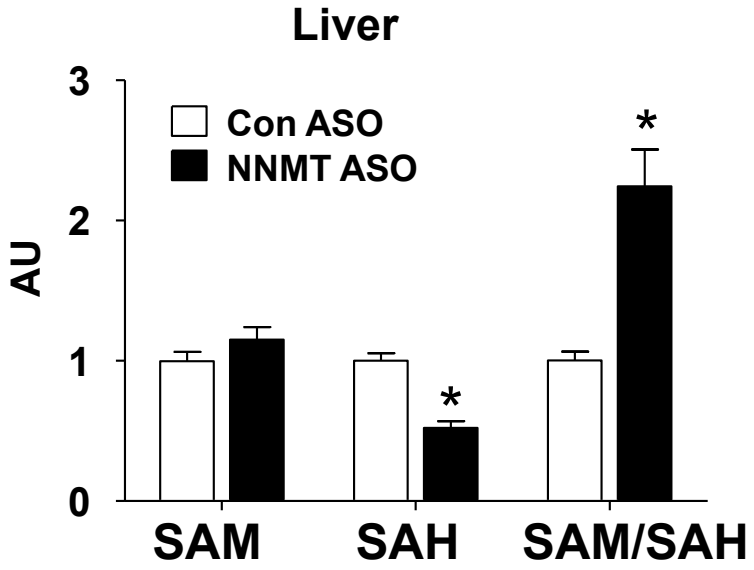
Supplementary Figure 7: NNMT knockdown in adipose tissue and liver does not alter food intake, locomotor activity or RER. **a**, food intake per mouse per day; **b**, food intake per gram body weight per day; **c,** locomotor activity and **d,** respiratory exchange ratio (RER) measured by CLAMS were not different in NNMT-ASO compared to control-ASO treated mice. The mice were fed a HFD for 5 weeks and treated with NNMT or control ASOs in the last three weeks. n=7 per group.



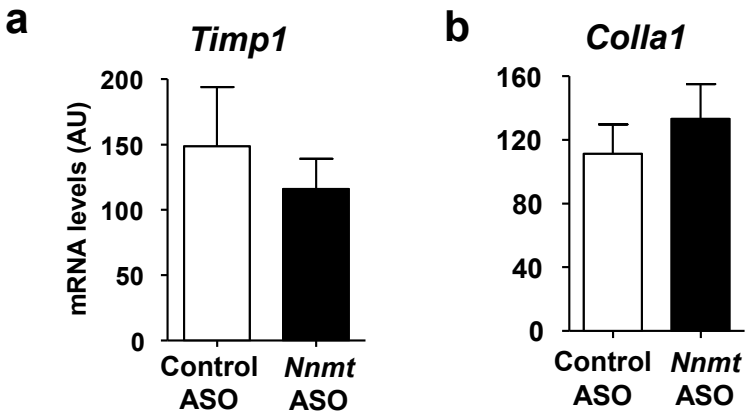
Supplementary Figure 8: *Nnmt* knockdown does not alter body temperature or brown adipose tissue activity.
a, rectal temperature; **b**, brown adipose tissue (BAT) weight; and **c**, UCP1 expression in BAT were not different in *Nnmt-ASO* compared to control ASO treated mice. The HFD fed mice were treated with *Nnmt* or control ASOs for 8 weeks. n=8 per group.



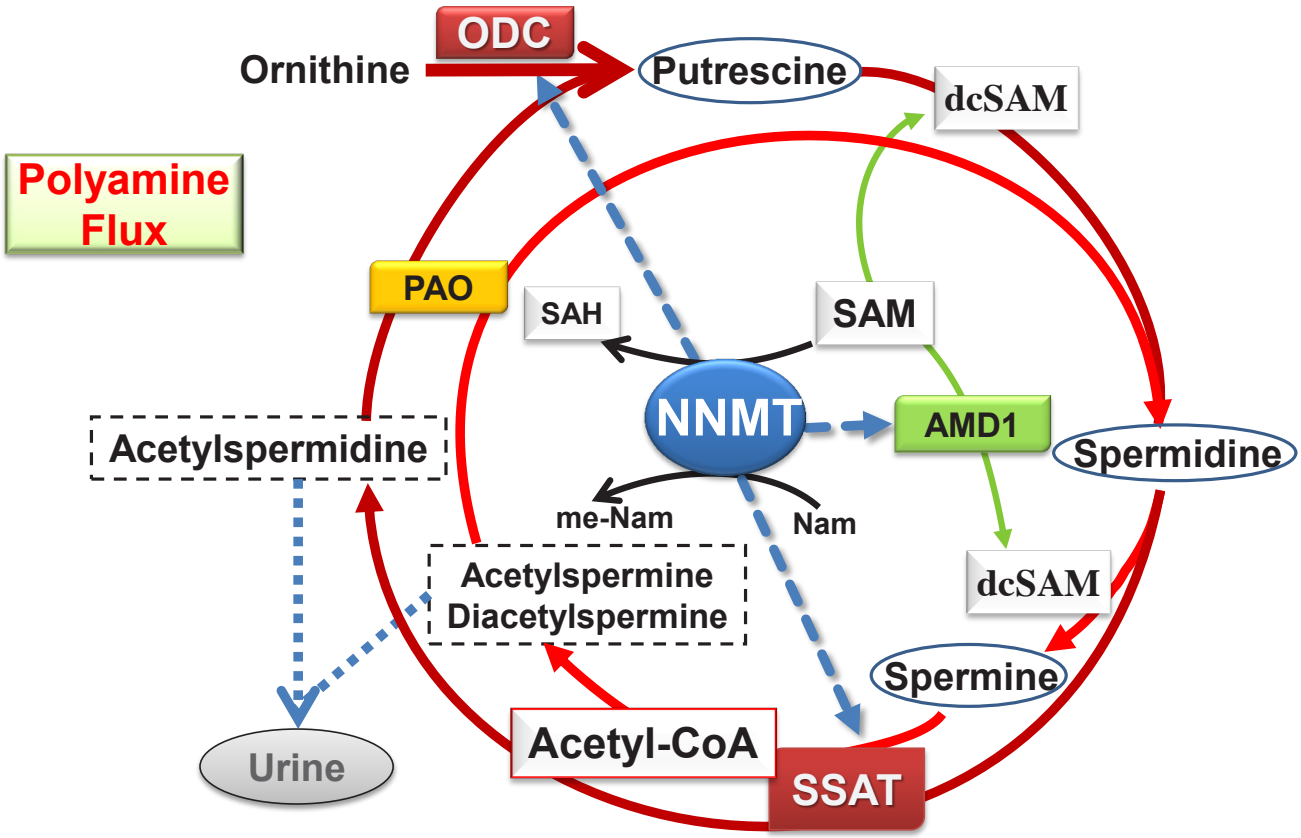
Supplementary Figure 9: ***Nnmt*** **regulates oxygen consumption in H2.35 hepatocytes**
H2.35 hepatocytes were transduced with adenovirus expressing (**a**) β-galactosidase (b-gal) or *Nnmt* (n=6 wells/condition); and (**b**) 'scrambled' shRNA or *Nnmt* shRNA (n=4 wells/condition). Oxygen consumption was measured by Clark-type oxygen electrode. *p< 0.05.



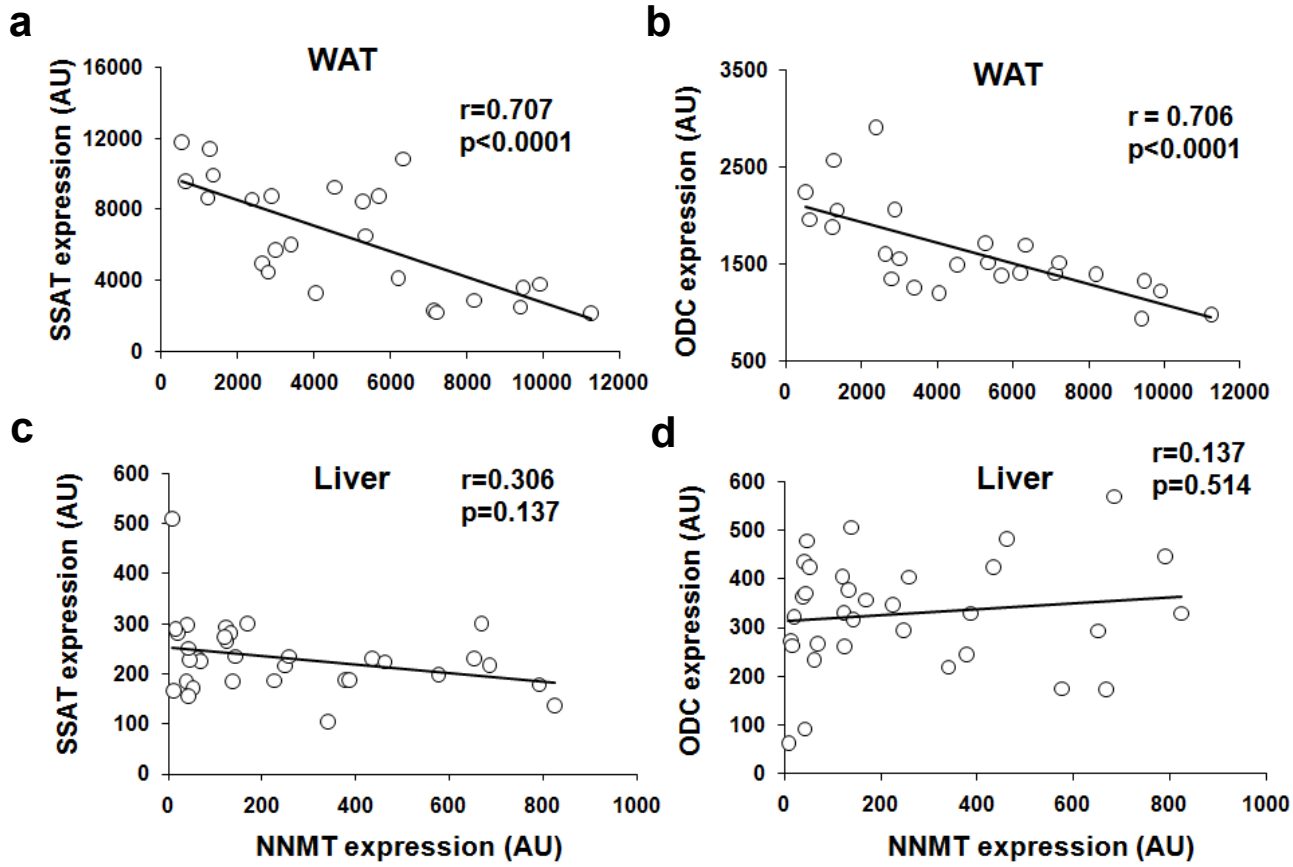
Supplementary Figure 10: S-adenosylmethionine (SAM), s-adenosylhomocysteine (SAH) and SAM/SAH in liver with *Nnmt* knockdown *in vivo*.
The metabolites were measured with targeted tandem mass spectrometry (LC-MS/MS). The mice were fed a HFD and were treated with control or *Nnmt* ASO for 8 weeks. n=4/group, *p<0.05.



Supplementary Figure 11: *Nnmt* knockdown does not alter mRNA levels of markers of hepatic fibrosis - tissue inhibitor of metalloproteinase 1 (*Timp1*) and collagen a1 type 1 (*Colla1*).
a, *Timp1* and **b,** *Colla1* mRNA expression in liver of high fat diet fed mice treated with ASOs for 8 weeks. *Timp1* and *Colla1* are markers of hepatic fibrosis. mRNA levels were measured by Taqman qPCR using probes from ABI. n=8/group.

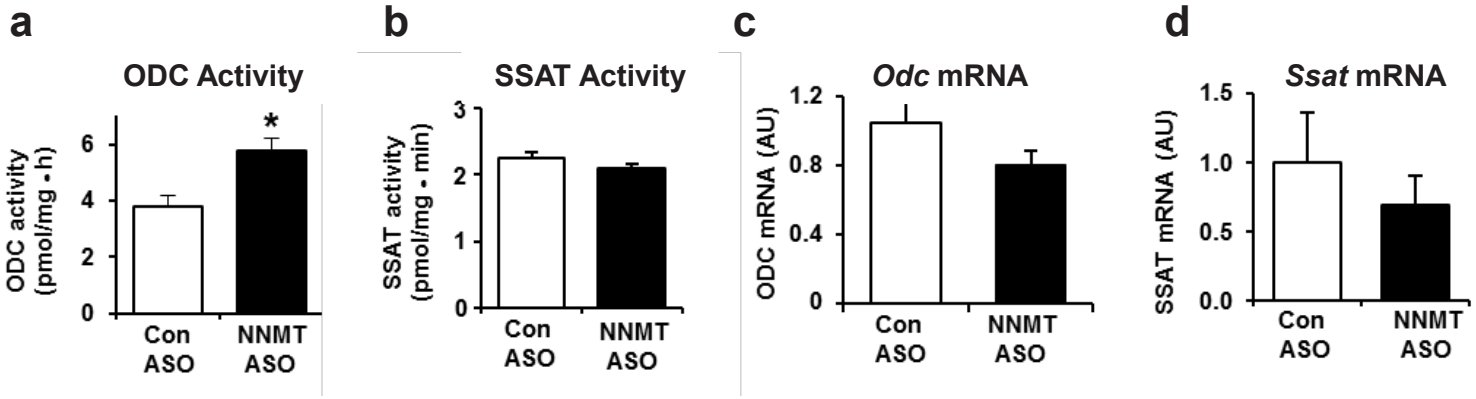


Supplementary Figure 12: NNMT regulates polyamine flux.
Polyamines (putrescine, spermidine and spermine) are polycations that play essential roles in basic cell function including DNA stability and cell growth. Polyamine metabolism is tightly controlled by synthesis, catabolism and excretion. The synthesis is controlled by ornithine decarboxylase (ODC) producing putrescine and by adenosylmethionine decarboxylase (AMD1) providing decarboxylated adenosylmethionine (dcSAM) that is used for the synthesis of spermidine and spermine. The catabolism is controlled by spermidine-spermine N^1-acetyltransferase (SSAT), which acetylates spermidine and spermine using acetyl-CoA as a substrate. The acetylated products, acetylspermine, diacetylspermine, and acetylspermidine are oxidized by polyamine oxidase (PAO) or excreted in the urine. Both routes cause a net loss of acetyl-CoA from cells. NNMT regulates polyamine flux by providing SAM as a substrate for AMD1 and also by altering *Odc* and *Ssat* expression through modulating histone methylation.

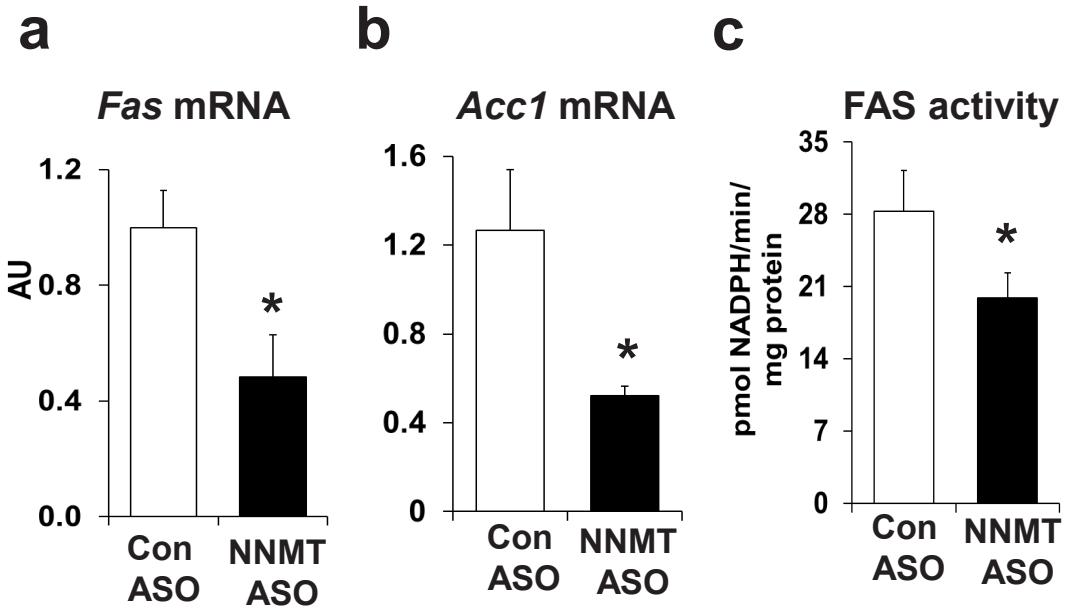


Supplementary Figure 13: Inverse correlation between *Nnmt* expression and *Ssat* or *Odc* expression in adipose tissue but not in liver of 25 mouse stains.

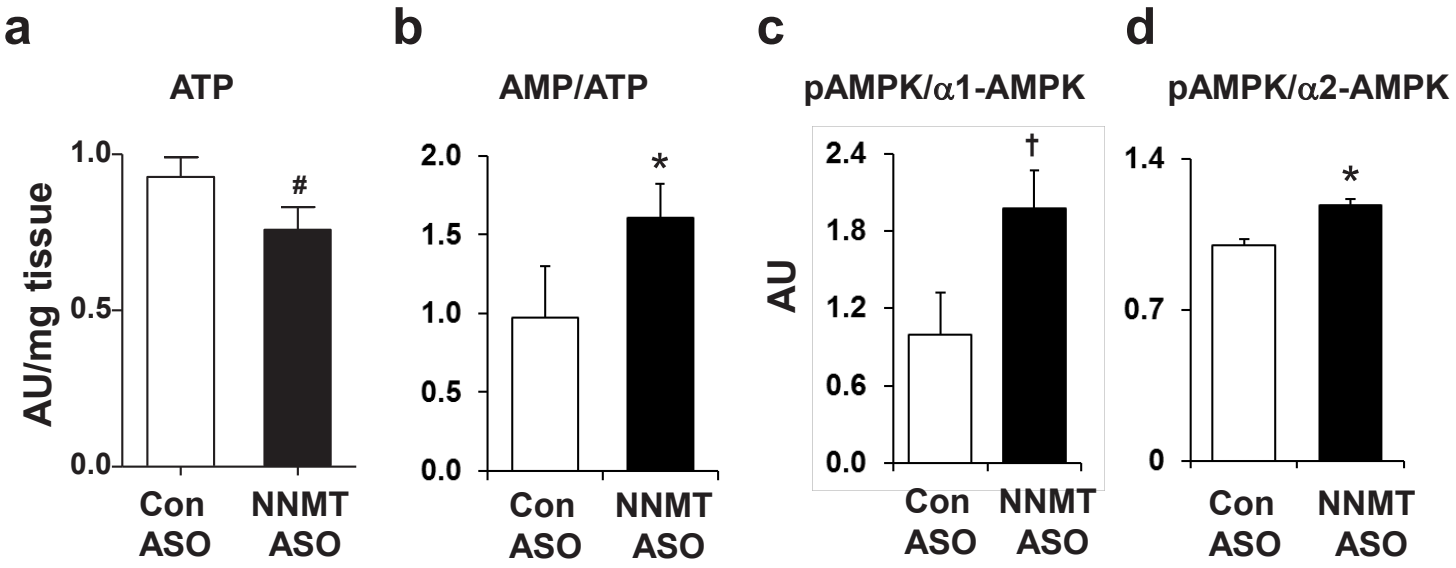
a-b, *Nnmt* expression correlates negatively with *Ssat* and *Odc* expression in WAT. **c-d**, *Nnmt* expression does not correlate with *Ssat* or *Odc* expression in liver. All expression levels were from www.BioGPS.org - Adipose (MOE430 V2) and Liver (GNF1M) (*14*).



Supplementary Figure 14: Liver ODC and SSAT activity and mRNA levels with *Nnmt* knockdown.
a, Ornithine decarboxylase (ODC) activity (n=4 Con-ASO, n=7 NNMT-ASO); **b**, spermidine-spermine acetyltransferase (SSAT activity) (n=10/group); **c**, *Odc* mRNA (n=8/group) and **d**, *Ssat* mRNA in liver of control and *Nnmt*-ASO-treated mice (n=8/group). The mice were fed a HFD and were treated with ASOs for 8 weeks. *p<0.05.

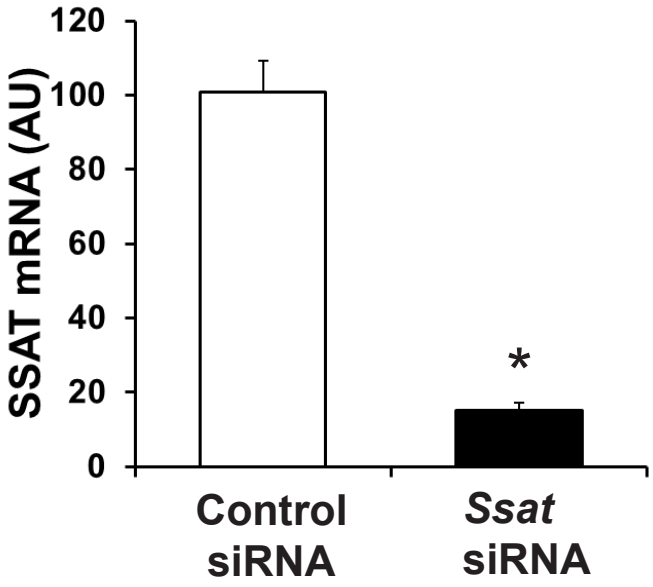


Supplementary Figure 15: *Nnmt* knockdown in adipose tissue reduces fatty acid synthesis.
a, Fatty acid synthase (*Fas*) (n=6 Con ASO; n=8 *Nnmt* ASO); **b**, acetyl-CoA carboxylase 1 (*Acc1*) (n=8/group) mRNA expression; and **c**, FAS activity (n=6/group) in adipose tissue of *Nnmt* and control ASO treated mice. The mice were fed a high fat diet and treated with *Nnmt* or control ASOs for 6 weeks. *Fas* and *Acc1* mRNA levels were measured using quantitative PCR. FAS activity was measured using NADPH absorbance assay. *p<0.05.

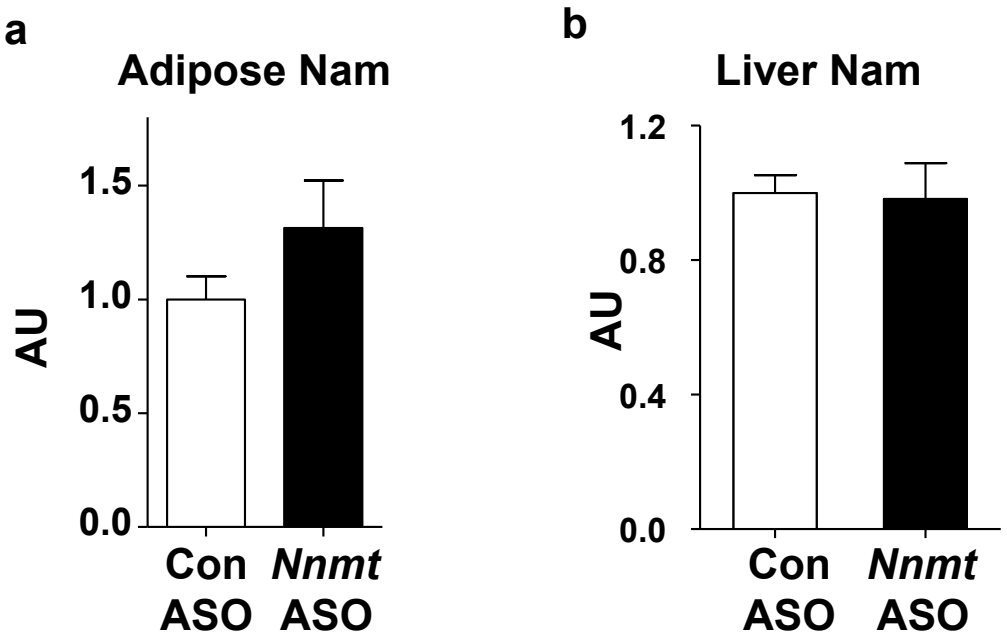


Supplementary Figure 16: *Nnmt* knockdown in adipose tissue alters ATP levels and AMP/ATP ratio and enhances AMPK phosphorylation.

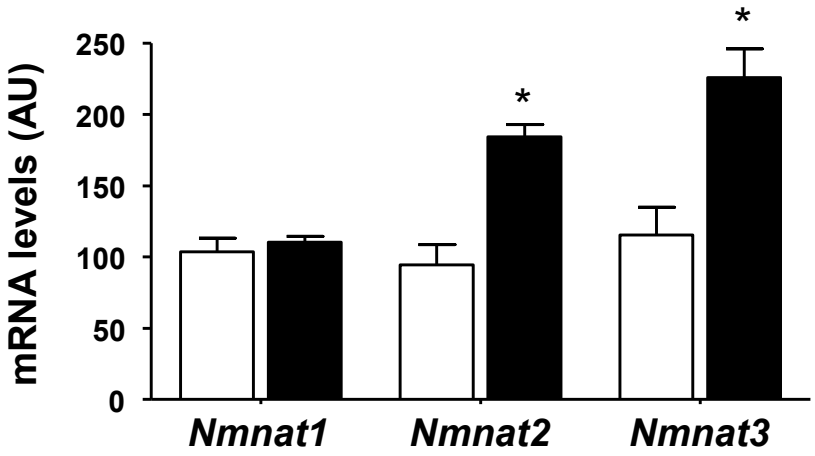
a. ATP levels; **b**. AMP/ATP ratio (n=7 Con ASO; n=10 *Nnmt* ASO); **c-d**. AMPK phosphorylation (Threonine 172) corrected with (c) a1-AMPK (n=8 Con ASO; n=18 *Nnmt* ASO) and (d) a2-AMPK (n=10 Con ASO; n=11 *Nnmt* ASO) in adipose tissue of *Nnmt* and control ASO treated mice. The mice were fed a high fat diet and treated with *Nnmt* or control ASOs for 6 weeks. ATP, AMP levels were measured by Mass Spectrometry. Phosphorylated and total AMPK were measured by Western blotting. *p<0.05, †p=0.066.



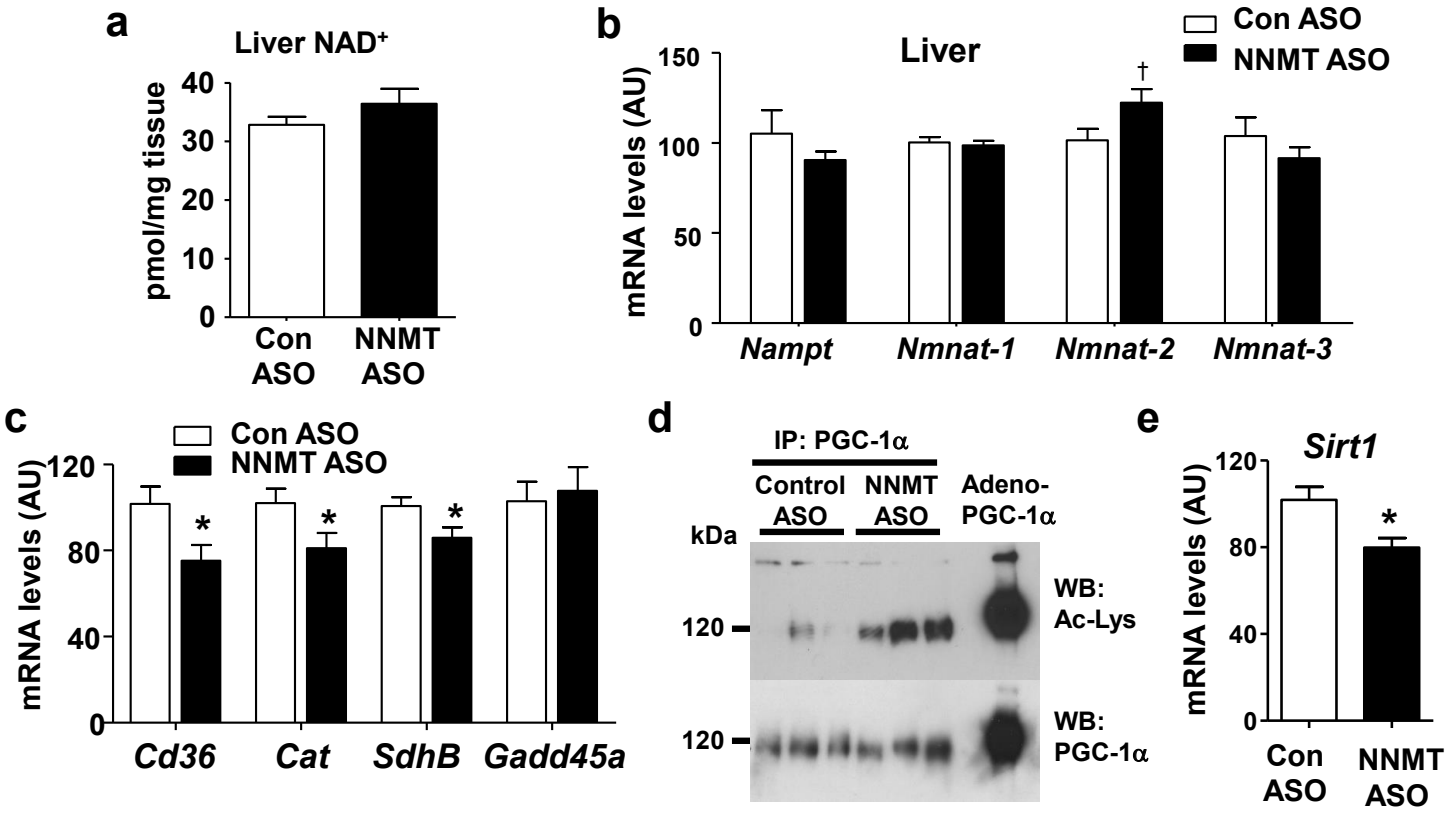
Supplementary Figure 17: *Ssat* mRNA levels with *Ssat* siRNA treatment in 3T3-L1 adipocytes.
Ssat mRNA expression in 3T3-L1 adipocytes treated with *Ssat* siRNA. 100 nM *Ssat* siRNA was transfected into differentiated adipocytes by electroporation. Total RNA was extracted 24 hours later and *Ssat* expression was measured using Taqman quantitative PCR. n=6 per condition, *p<0.01.



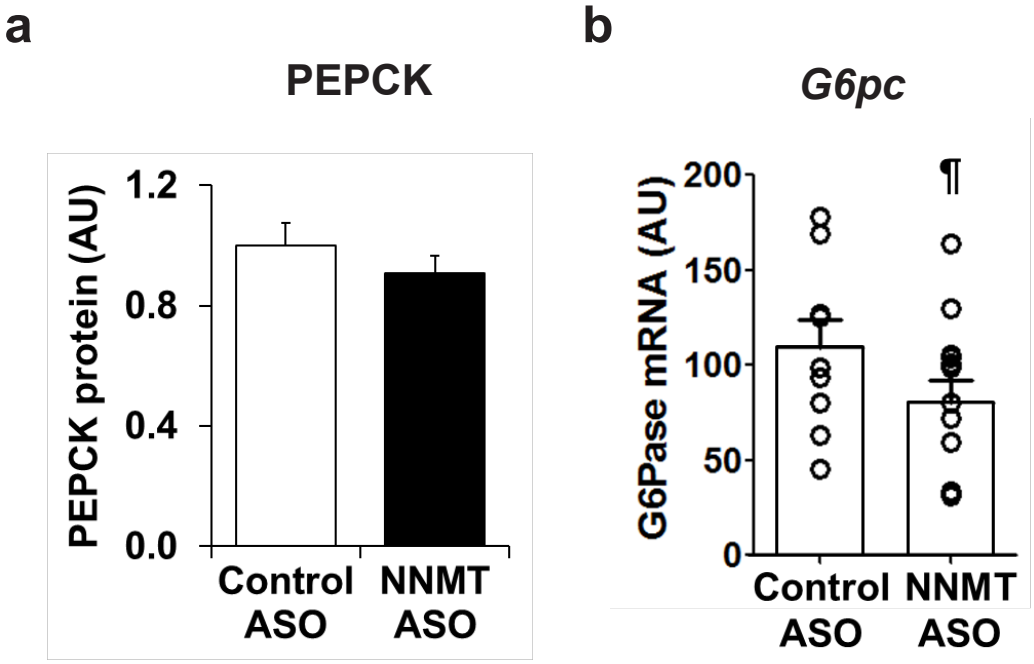
Supplementary Figure 18: *Nnmt* knockdown *in vivo* does not alter nicotinamide levels in adipose tissue and liver. Nicotinamide (Nam) levels in **(a)** adipose tissue (n=8 Con ASO; n=12 *Nnmt* ASO); **(b)** liver (n=4/group) of control and *Nnmt*-ASO-treated mice. Nicotinamide was measured with targeted tandem mass spectrometry (LC-MS/MS). The mice were fed a HFD and were treated with control or *Nnmt* ASO for 8 weeks.



Supplementary Figure 19: *Nnmt* knockdown in adipose tissue regulates enzymes involved in NAD⁺ synthesis.
The final step of NAD^+ synthesis in a salvage pathway is controlled by nicotinamide mononucleotide adenylyltransferase (NMNAT). Three distinct isoforms of NMNATs, NMNAT 1-3, are localized in nucleus, cytoplasm and mitochondria, respectively. *Nnmt* knockdown increases the expression of cytosolic *Nmnat2* and mitochondrial *Nmnat3*, but not nuclear *Nmnat1*. mRNA levels were measured by qPCR. n=9 Con ASO; n=11 *Nnmt* ASO. *$p<0.05$.



Supplementary Figure 20: NAD+, Sirt1 and Sirt1 target genes in liver with NNMT knockdown.
a, NAD+ levels (n=4/group); **b**, The expression of rate-limiting enzymes involving in NAD+ salvage synthesis pathway; Nampt: Nicotinamide phosphoribosyltransferase, NMNAT: nicotinamide mononucleotide adenylyltransferase (n=8 Con-ASO; n=13 NNMT-ASO); **c,** mRNA levels of Sirt1 target genes: *Cd36*, catalase (*Cat*), succinate dehydrogenase B (*SdhB*) and growth arrest and DNA-damage-inducible protein (*Gadd45a*) (n=8 Con-ASO; n=13 NNMT-ASO); **d**, Acetylation of PGC-1α; and **e,** Sirt1 mRNA levels in liver of control and NNMT-ASO-treated mice. *p<0.05, †p=0.06.



Supplementary Figure 21: *Nnmt* knockdown does not alter *PEPCK* or *G6pc* expression.
a, PEPCK protein levels (n=8); and **b**, *G6pc* mRNA levels (n=9 Con ASO; n=13 *Nnmt* ASO) in liver of *Nnmt* ASO and control ASO treated mice. The mice were treated with ASOs for 3-5 weeks, ¶p=0.14.