

Supplementary Information

Table S1: qPCR primers

	Forward	Reverse
CD68	TCACCTTGACCTGCTCTCTC	AGGCCAATGATGAGAGGCAG
IL-6	GTCGGAGGCTTAATTACACATGT	CAAGTGCATCATCGTTGTTCA
Npy	ACCGGTGGTCTCTTCTCTCA	CTTGTTACCTAGCATCGTGGC
Pepck	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCGTACTCC
Pgc1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Pomc	GCGAGGCAAACAAGATTGG	GAGGCACTGAACATCTTTGTC
Prdm16	CCGCTGTGATGAGTGTGATG	GGACGATCATGTGTTGCTCC
Tbp	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
TNFα	CCCACGTCGTAGCAAACCA	GTCTTTGAGATCCATGCCGTTG
Ucp1	CTGCCAGGACAGTACCCAAG	TCAGCTGTTCAAAGCACACA

Supplementary Figure 1. L-serine supplementation does not affect white adipose (WAT) tissue inflammation or cell size in ad libitum fed mice.

(a) Body weight of ad libitum CD and HFD fed mice with or without L-serine supplementation starting at 8 weeks of age (CD: $n=5$; HFD: $n=4$; CD+L-ser, HFD+L-ser: $n=6$). **(b)** Expression of inflammatory marker genes in SCF and PGF of CD and HFD fed mice with or without L-serine supplementation after 8 weeks starting at 4 weeks of age ($n=8$). **(c)** Adipocyte size distribution of subcutaneous fat (SCF) and perigonadal fat (PGF) of random fed mice in CD and HFD with or without L-serine supplementation for 8 weeks starting at 4 weeks age ($n=3$ animals >10000 cells per tissue and mouse). Adipocytes were grouped in different size categories. **(d)** Triglyceride content of liver from CD and HFD fed mice with or without L-serine supplementation for 8 weeks starting at 4 weeks of age **(e)** Serine content in liver of CD or HFD fed mice with or without L-serine supplementation for 8 weeks starting at 4 weeks of age ($n=8$). **(f)** Liver weight of CD or

HFD fed mice with or without L-serine supplementation for 8 weeks starting at 4 weeks of age ($n=8$). **(g)** Glycogen levels in livers of CD or HFD fed mice with or without L-serine supplementation for 8 weeks starting at 4 weeks of age ($n=8$). **(h)** Pepck gene expression in liver of CD or HFD fed mice with or without L-serine supplementation for 8 weeks starting at 4 weeks of age ($n=8$). **(i)** Pyruvate Tolerance test (PTT) of CD and HFD fed mice with or without L-serine supplementation after 1 week starting at 8 week of age (2mg/kg pyruvate; $n=6$). Data are shown as mean \pm SEM, one or two way ANOVA with Tukey's post-hoc test. $**p<0.01$.

Supplementary Figure 2. L-serine supplementation reduces white adipocyte size and white adipose tissue (WAT) inflammation upon fasting.

(a) Liver weight of CD and HFD fed mice with or without L-serine supplementation after 8 weeks and repeated overnight fasting starting at 8 weeks of age ($n=6$). **(b)** Triglyceride content 8 weeks ($n=6$). **(c)** Representative H&E stainings of liver after 8 weeks. **(d)** Adipocyte sizes of SCF and PGF after 8 weeks and repeated overnight fasting starting at 8 weeks of age ($n=4$ mice; >7000 adipocytes per group for PGF and >10000 adipocytes per group for SCF). Statistical analysis using a one-way ANOVA with a Tukey's post-hoc test revealed statistically significant differences between all groups in SCF and PGF. **(e)** Expression of inflammatory marker genes in PGF and SCF of fasted CD and HFD fed mice with or without L-serine supplementation after 8 weeks and repeated overnight fasting starting at 8 weeks of age (CD, HFD, HFD+L-ser : $n=6$; CD+L-ser: $n=4-5$). Data are shown as mean \pm SEM, one way ANOVA with Tukey's post-hoc test. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure 3. L-serine supplementation increases cumulative high fat diet intake in fasted mice.

(a) Experimental setup for 16-week L-serine supplementation and indirect calorimetry measurement (TSE) in CD and HFD fed mice with and without L-serine supplementation. The figure was generated using Biorender.com. **(b)** Cumulative food intake of random fed and fasted CD and HFD fed mice with or without L-serine supplementation, starting at 8 weeks of age, at week 7(CD) or 8 (HFD) (CD random fed: $n=5$; CD fasted, CD+L-ser random fed, CD+L-ser fasted: $n=6$; HFD random fed: $n=4$; HFD fasted, HFD+L-ser random fed, HFD+L-ser fasted: $n=6$). **(c)** Average water intake (g) day total, light and dark phases of random fed and fasted CD and HFD fed mice with or without L-serine supplementation, starting at 8 weeks of age, at week 7(CD) or 8 (HFD) (CD random fed: $n=5$; CD fasted, CD+L-ser random fed, CD+L-ser fasted: $n=6$; HFD random fed: $n=3$; HFD fasted: $n=5$; HFD+L-ser random fed, HFD+L-ser fasted: $n=6$). **(d)** mRNA expression of NPY and POMC from the hypothalamus of random fed and fasted CD and HFD fed mice with or without L-serine supplementation starting at 8 weeks of age, at week 16 (CD random fed: $n=4$; CD fasted, CD+L-ser random fed, CD+L-ser fasted: $n=6$; HFD random fed: $n=4$; HFD fasted, HFD+L-ser random fed, HFD+L-ser fasted: $n=6$). **(e)** Respiratory exchange ratio (RER) of random fed and fasted mice at week 7(CD) or 8 (HFD) (CD random fed: $n=5$; CD fasted, CD+L-ser random fed, CD+L-ser fasted: $n=6$; HFD random fed: $n=4$; HFD fasted, HFD+L-ser random fed, HFD+L-ser fasted: $n=6$). **(f)** Western blots of phosphorylated-HSL 563, Ser660, Ser565 and total HSL in PGF and SCF of random fed and fasted CD or HFD fed mice with or without L-serine supplementation for 8 weeks starting at 8 weeks of age ($n=6$). Data are shown as mean \pm SEM, two way ANOVA with

Tukey's post-test. Data are shown as mean \pm SEM, two way ANOVA with Tukey's post-hoc test. * $p < 0.05$, ** $p < 0.01$, $p^{***} < 0.001$, $p^{****} < 0.0001$.

Supplementary Figure 4. L-serine supplementation does not alter thermogenic gene expression in brown adipose tissue (BAT).

(a) Average locomotor activity (counts) of random fed and fasted CD and HFD fed mice with or without L-serine supplementation at week 7(CD) or 8 (HFD) (CD random fed: $n=5$; CD fasted, CD+L-ser random fed, CD+L-ser fasted: $n=6$; HFD random fed: $n=4$; HFD fasted, HFD+L-ser random fed, HFD+L-ser fasted: $n=6$). **(b)** BAT weight of fasted CD and HFD fed mice with or without L-serine supplementation for 8 weeks starting at 8 weeks of age ($n=6$). **(c)** mRNA expression of thermogenesis-related genes in BAT after 8 weeks starting at 8 weeks of age ($n=6$). **(d)** Western blots for UCP1 of random fed and fasted CD or HFD fed mice with or without L-serine supplementation for 8 weeks starting at 8 weeks of age. Data are shown as mean \pm SEM, one or two way ANOVA with Tukey's post-hoc test. * $p < 0.05$.