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Supplemental Information

Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice

Sarah J. Mitchell, Michel Bernier, Miguel A. Aon, Sonia Cortassa, Eun Young Kim, Evandro F. Fang, Hector H. Palacios, Ahmed Ali, Ignacio Navas-Enamorado, Andrea Di Francesco, Tamzin A. Kaiser, Tyler B. Waltz, Ning Zhang, James L. Ellis, Peter J. Elliott, David W. Frederick, Vilhelm A. Bohr, Mark S. Schmidt, Charles Brenner, David A. Sinclair, Anthony A. Sauve, Joseph A. Baur, and Rafael de Cabo

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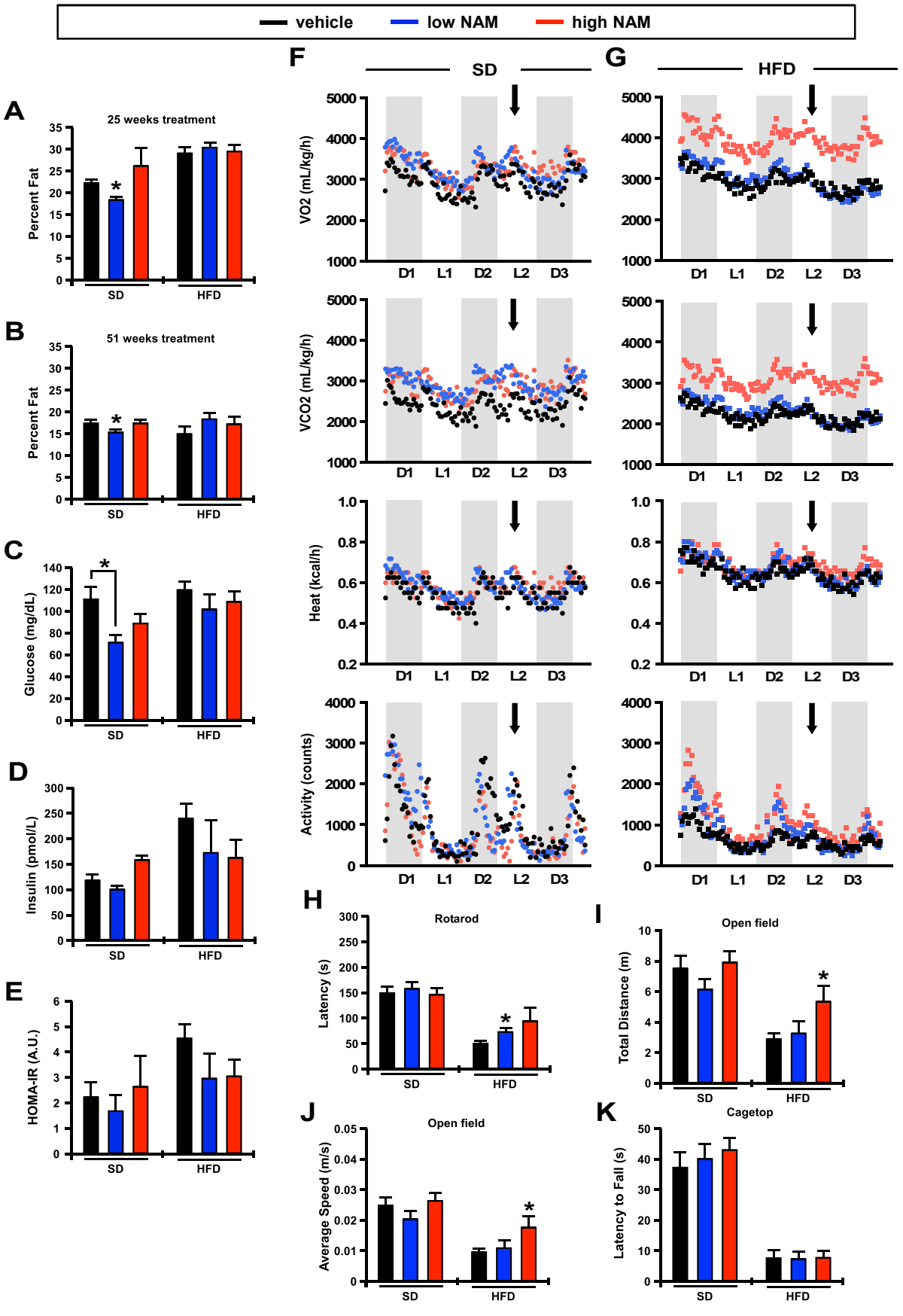

Figure S1, related to Figure 1. Impact of NAM supplementation on body composition and neurobehavioral outcomes in mice. (A, B) Percent fat mass at 25 and 51 weeks of treatment was determined in mice by nuclear magnetic resonance (n = 42-84 per group for both time points). (C-E) Analyses were carried out in mice after a16-h fasting period: (C) Blood glucose levels; (D) Serum insulin levels; and (E) Homeostatic measure of insulin resistance (HOMA-IR), n = 6-8 per group. Data are shown as mean ± SEM. *$P \leq 0.05$ in comparison to diet without NAM. (F and G) After 49 weeks of treatment mice were placed into metabolic cages for the measure of VO_2 consumption, VCO_2 generation, heat production, and ambulatory activity as detailed in Experimental Procedures, n= 6 per group. We observed the occurrence of an event (e.g., power outage or the like) that has caused a burst in metabolic/activity pattern during the 'L2' period (black arrow). Nevertheless, all mice behaved equally. (H-K) After 59 weeks of treatment, the following behavioral tests were performed: (H) Time to fall from an accelerating rotarod; total distance (I) and average speed (J) of mice in the open field test; and (K) time to fall from an elevated wire cage top. Data are shown as mean ± SEM, n= 8-16 per group.

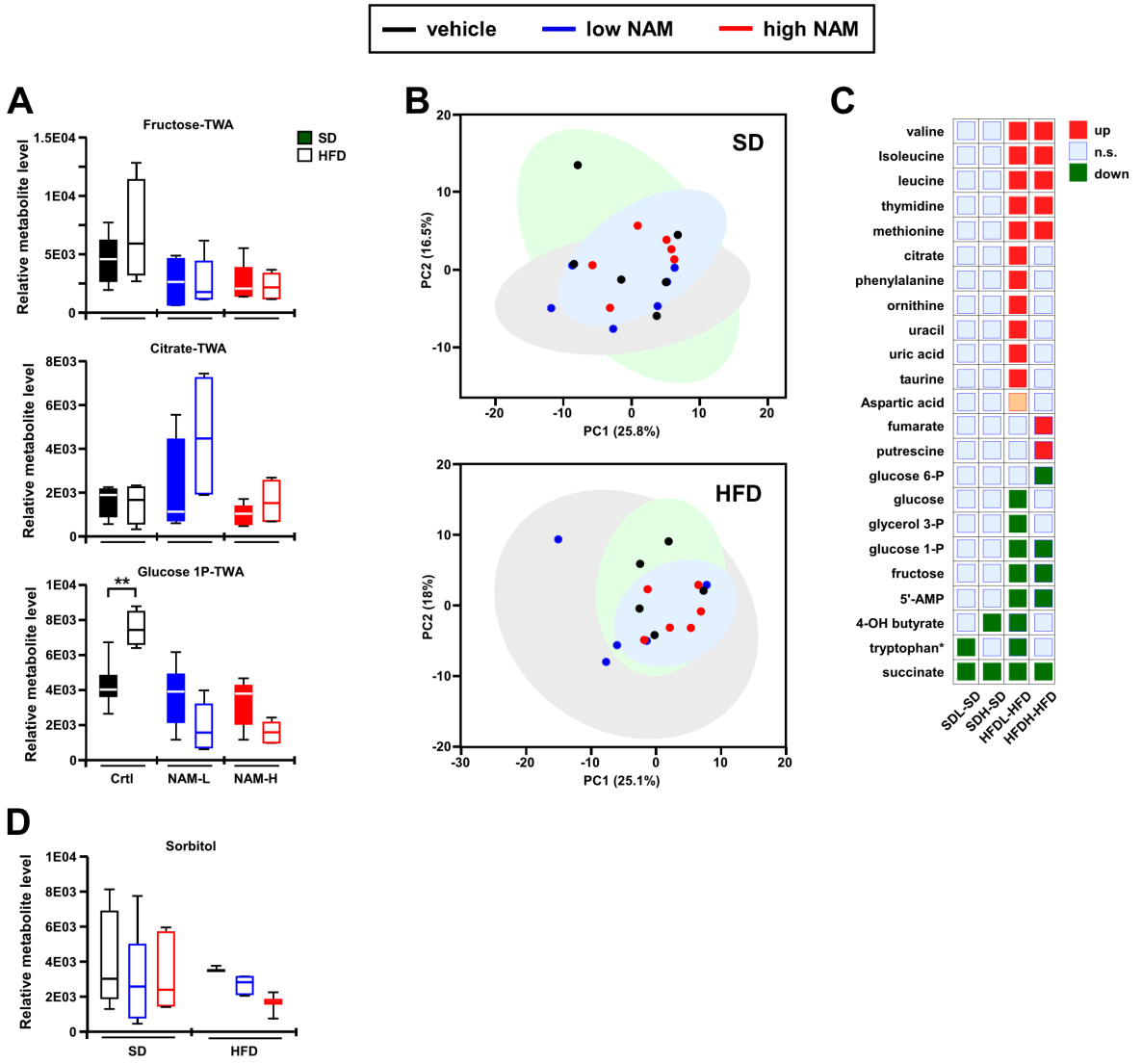

Figure S2, related to Figure 2. Impact of NAM supplementation in the liver metabolomics profile of mice fed SD versus HFD. (A) Two-way ANOVA was used to interrogate the differences between SD and HFD diets at each level of treatment (control, low and high NAM) of a select group of metabolites. (B) PCA scatterplot analysis revealed the impact of NAM supplementation (control, low and high NAM) in male mice fed SD or HFD. The first and second components (i.e., 2 separate weighted sums of the original biochemicals) explained the variability of the metabolites to a comparable degree in SD vs. HFD [~43.1% vs. ~42.3%]. (C) Binary representation of a select group of biochemicals significantly impacted among the four pairwise comparisons. Upregulated, red squares; downregulated, green squares, not significant, beige squares. (D) Shown are the relative levels of sorbitol. For (A, D), the data are the mean ± SEM, n= 6 mice per group.

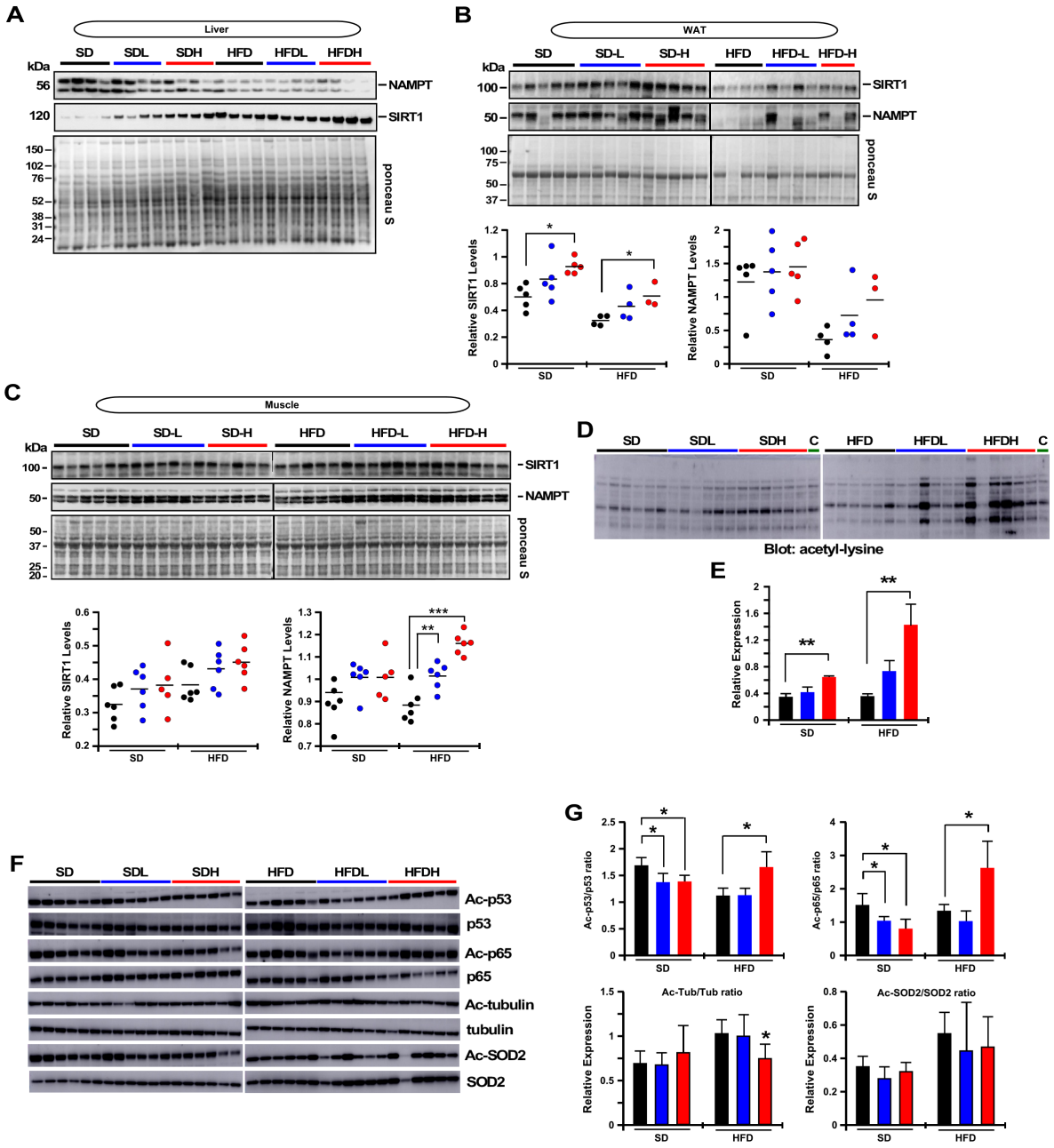

Figure S3, related to Figure 4. Effect of NAM treatment on the expression of NAMPT, SIRT1, and acetylation level of downstream targets. (A-C) Western blots depicting expression level of SIRT1 and NAMPT in (A) liver extracts, (B) white adipose tissue (WAT) and (C) skeletal muscle within the six experimental groups. Protein bands were normalized to Ponceau S staining of the membranes, and scatter plots are depicted (B and C only). (D) Whole lysates were prepared from mouse liver tissues at 118-week of age (62 weeks on NAM) and immunoblotted using anti-acetyl-lysine antibody. (E) Protein bands were normalized to Coomassie Brilliant Blue R-250-stained gels. (F) Western blots depicting expression level of acetylated and total forms of p53, p65Rel, tubulin, and SOD2 in the liver. (G) Determination of acetylated/total protein ratios for p53, p65Rel, tubulin, and SOD2 out from Western blot densitometric data. Bars represent the average ± SEM, n=5-6 per group. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ versus control.

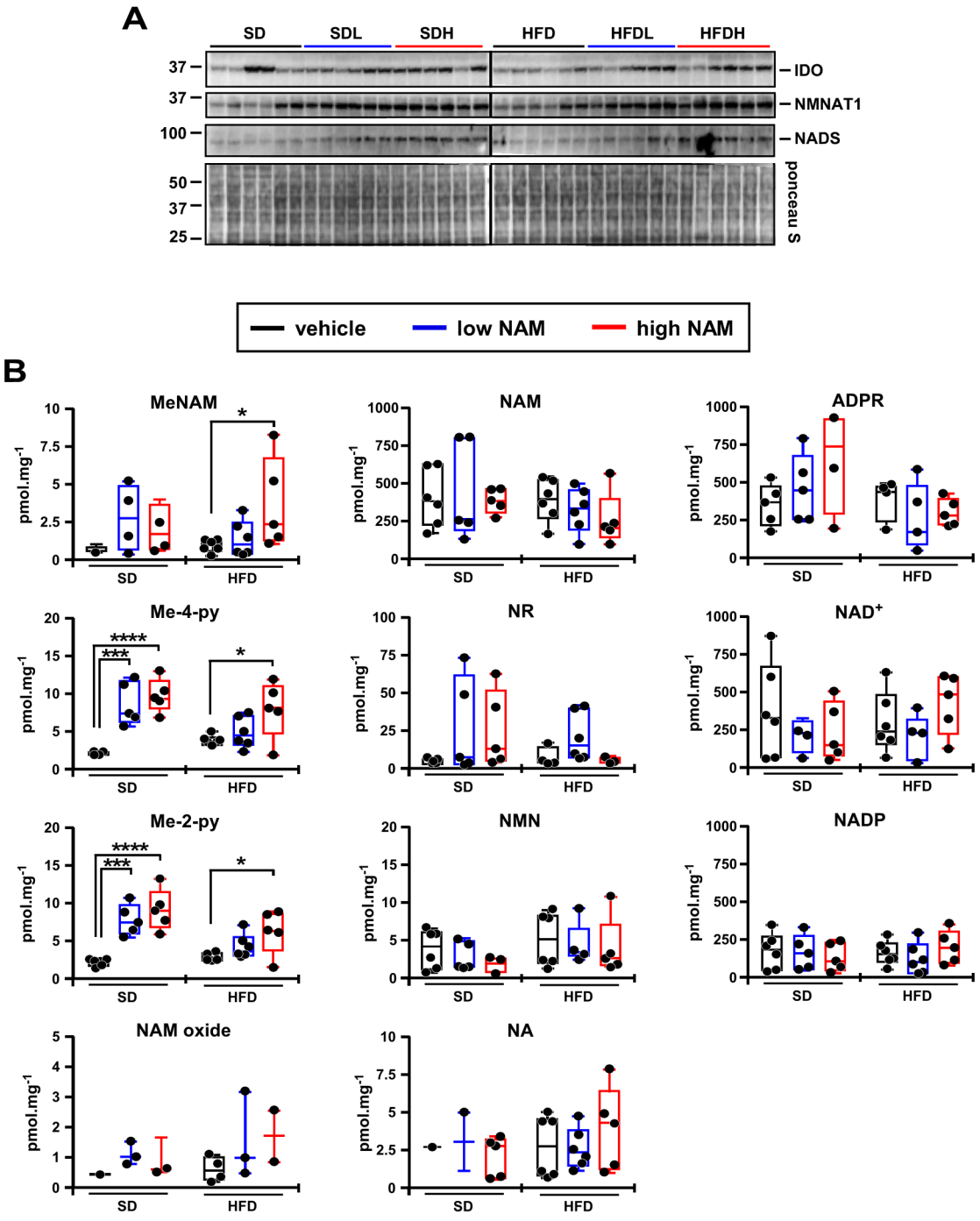

Figure S4, related to Figure 4. (A) Immunoblot analysis of liver lysates using antibodies specific for IDO, NMNAT1, and NADS. Protein bands were normalized to Ponceau S staining of the membranes, and scatter plots are depicted in Figure 4C. (B) Histograms show the concentrations of hepatic NAD^+ metabolites in response to NAM supplementation. Data are represented as scatter plots from 5-6 mice per group and was analyzed by two-way ANOVA with Tukey's post hoc test using Prism. Outliers were excluded from the statistics when values were above or below 1.5 times the interquartile range comprised between the 75% and 25% percentiles, respectively (Aitken et al., 2010). * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$.

Table S1, related to Figures 1A and 1B. Gross histopathology analysis of tissues collected at death or euthanasia in male C57BL/6J mice fed a standard or high-fat diet supplemented with or without nicotinamide.

		Diet treatment (number of mice)					
		SD	SDL	SDH	HFD	HFDL	HFDH
		74	75	75	73	80	78
Age (wks)		120	116	117	100	102	100
Heart	Enlarged	5	9	4	19	16	25
	Tumor	0	0	0	0	0	0
Lungs	Enlarged	7	4	2	2	2	4
	Tumor	5	3	6	4	2	5
Liver	Enlarged	21	24	23	19	25	20
	Tumor	13	17	16	5	14	11
Kidney	Enlarged	3	4	2	9	9	9
	Tumor	0	1	0	1	0	0
Spleen	Enlarged	21	23	26	12	22	25
	Tumor	4	2	4	1	3	0

SD, standard diet supplemented with low (SDL) or high dose (SDH) of nicotinamide (NAM); HFD, high-fat diet supplemented with low (HFDL) or high dose (HFDH) of NAM.