SUPPORTING INFORMATION

Sex- and Strain-Specific Effects of Mitochondrial Uncoupling on Age-Related Metabolic Diseases in High-Fat Diet-Fed Mice

***Running title*:** Effect of CRMP on Metabolic Disease in Aging Mice

Leigh Goedeke[1], Kelsey N. Murt[2], Andrea Di Francesco[2], João Paulo Camporez[1,3], Ali R. Nasiri[1], Yongliang Wang[1], Xian-Man Zhang[1], Gary W. Cline[1], Rafael de Cabo[2], Gerald I. Shulman[1,4*]

Departments of [1]Internal Medicine and [4]Cellular & Molecular Physiology
Yale School of Medicine, New Haven CT 06520, USA
[2]Translational Gerontology Branch, Intramural Research Program,
National Institute on Aging, NIH, Baltimore, MD 21224, USA
[3]Department of Physiology, Ribeirao Preto School of Medicine,
University of Sao Paulo, Brazil

*To whom correspondence should be addressed: gerald.shulman@yale.edu

This file contains **Supplementary Figures 1–6** and **Supplementary Table 1**.

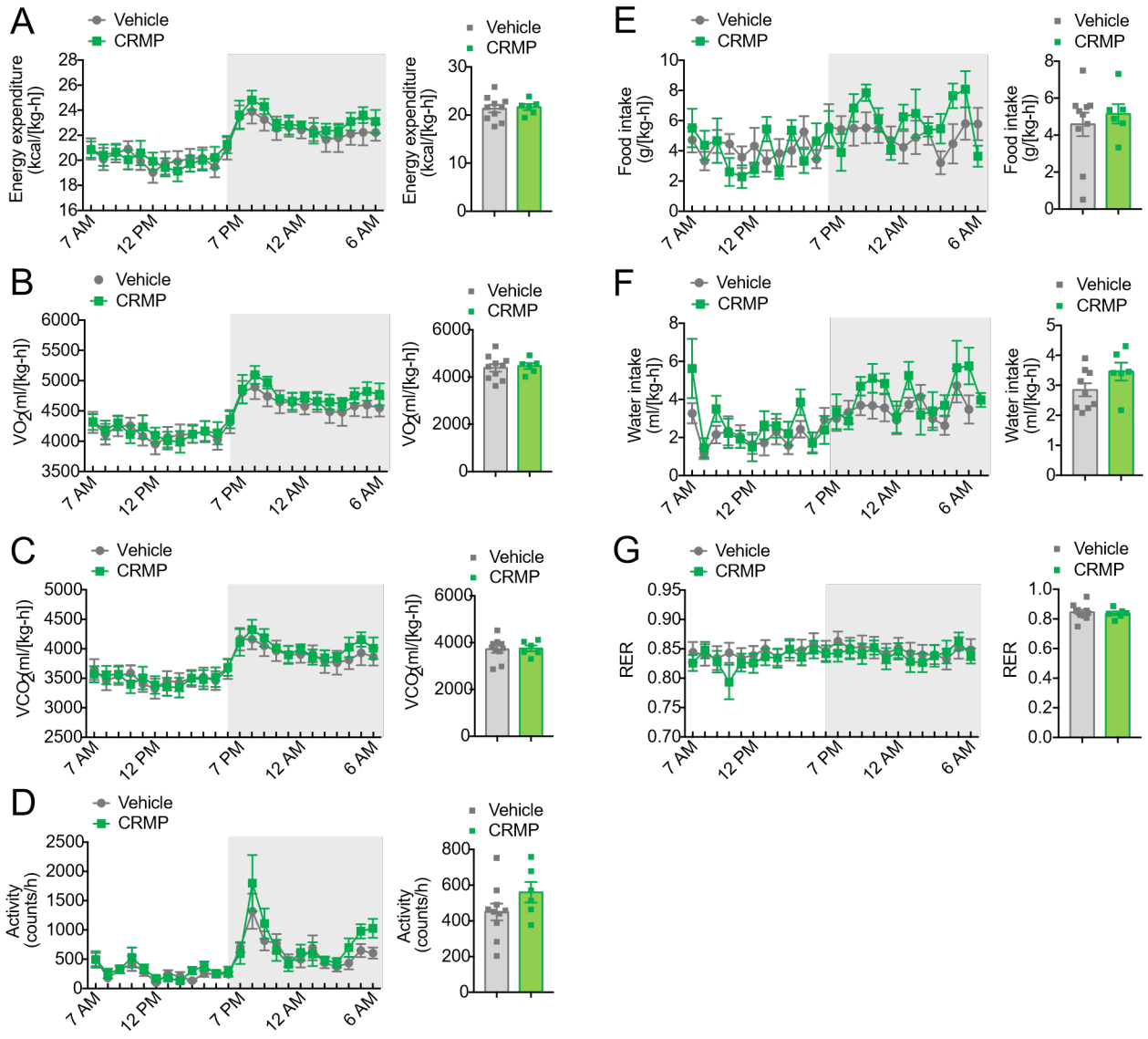

Figure S1: Metabolic parameters of aged HFD-fed male C57BL/6J mice treated with CRMP. (**A**–**D**) Whole-body energy expenditure (**A**), whole-body O_2 consumption (**B**), whole-body VO_2 production (**C**), locomotor activity (**D**), food intake (**E**), water intake (**F**), and RER (**G**) in 74-week-old male C57BL/6J mice fed a high-fat diet (HFD, 45% fat) for 8 weeks and then treated with CRMP (20 mg/[kg-day]) or vehicle control for an additional 4 weeks. In all panels data are presented as mean +/- SEM. *n* = 6–10 per treatment group. Abbreviations: CRMP, controlled-release mitochondrial protonophore; HFD, high-fat diet; RER, respiratory exchange ratio.

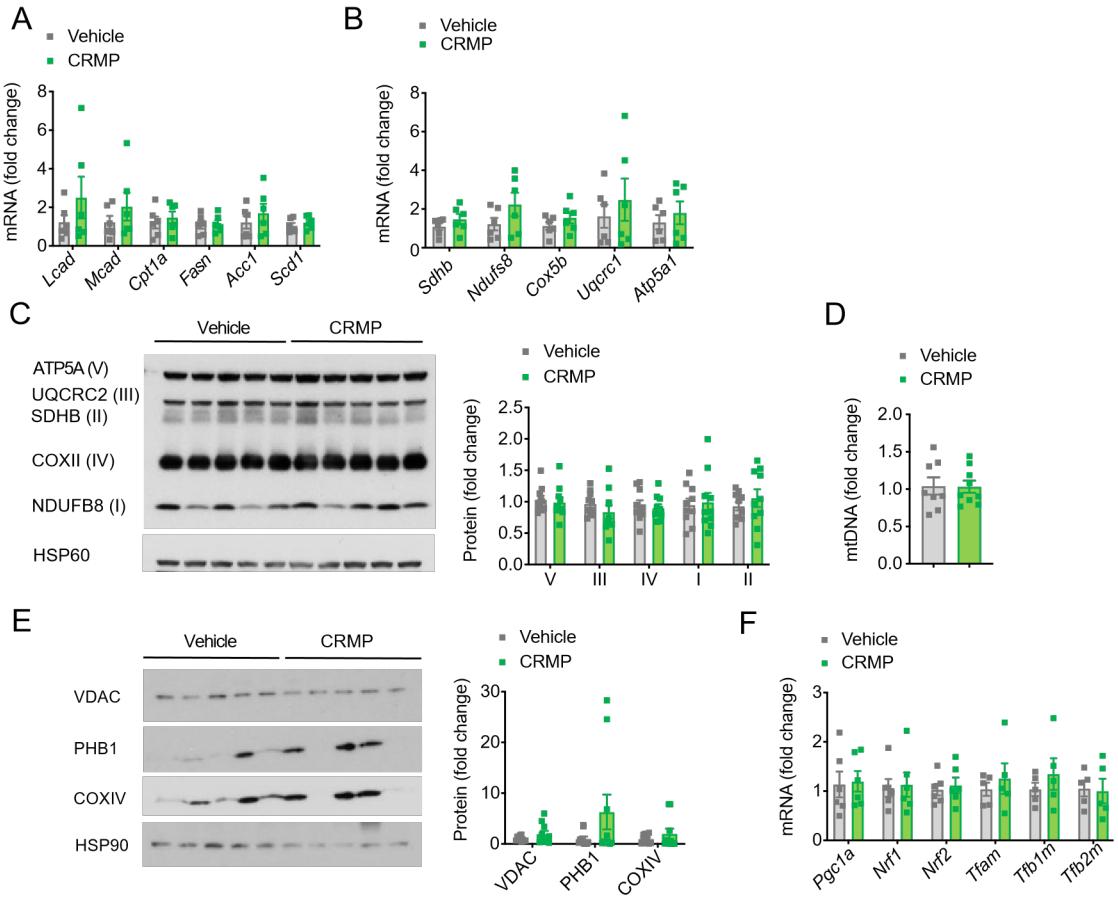

Figure S2: CRMP treatment does not alter hepatic fatty acid metabolism gene expression or markers of mitochondrial mass in aged HFD-fed male C57BL/6J mice. (**A**) qRT-PCR analysis of fatty acid metabolism genes in the livers of 74-week-old male C57BL/6J mice fed a high-fat diet (HFD, 45% fat) for 8 weeks and then treated with CRMP (20 mg/[kg-day]) or vehicle control for an additional 4 weeks. (**B–C**) qRT-PCR and representative Western blot analysis of mitochondrial ETC components in the livers of mice treated as in (**A**). HSP60 was used as a loading control. Quantification of blot shown to the right. (**D**) Mitochondrial DNA content in the livers of mice treated as in (**A**). **(E**) Representative Western blot analysis of mitochondrial markers in the livers of mice treated as in (**A**). HSP90 was used as a loading control. Quantification of blot shown to the right. (**F**) qRT-PCR analysis of *Pgc1a* targets in the livers of mice treated as in (**A**). In all panels data are presented as mean +/- SEM. *n* = 6 (**A–B**; **F**), 10 (**C, E**) or 8-9 (**D**) per treatment group. Abbreviations: CRMP, controlled-release mitochondrial protonophore; ETC, electron transport chain; HFD, high-fat diet.

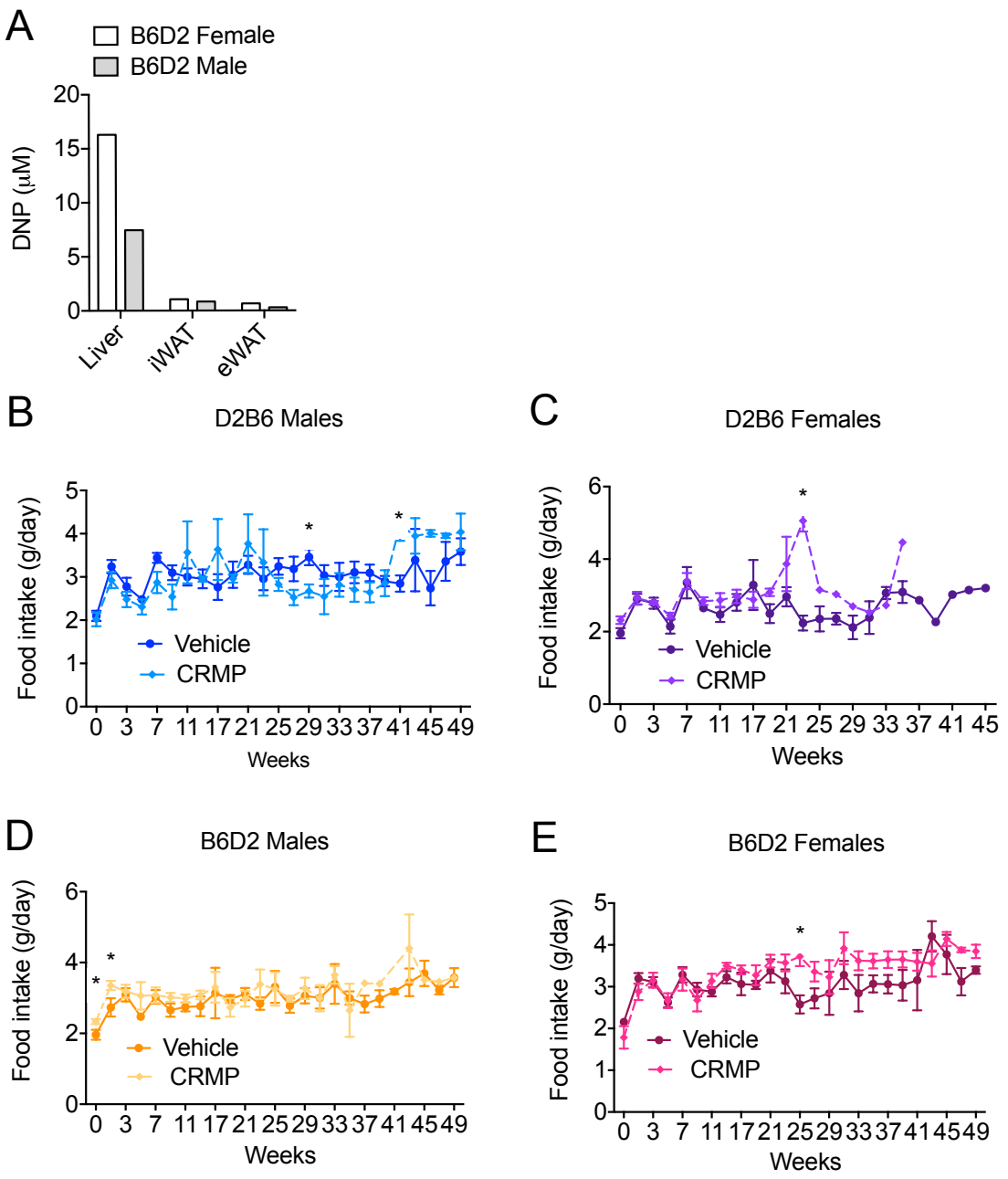

Figure S3: Food intake in aged HFD-fed male and female mice. (**A**) Tissue DNP concentrations in 6 h fasted 94-104 week-old HFD-fed D2B6 male and female mice treated with CRMP (10 mg/[kg-day]) for 3 months. *n* =1 per group. (**B**–**E**) Food intake trajectories of 94-104 week-old male and female F1 offspring of DBA/2J (D2) and C57BL/6J (B6) mice fed a HFD (45% fat) or HFD containing CRMP (10 mg/[kg-day]) for the remainder of their lives. In panels (**B–E**) data are presented as mean +/- SEM. *n* = 1129 per treatment group. **P*< 0.05 by two-sided unpaired Student's *t*-test compared to vehicle control. Abbreviations: C57BL/6J (B6)**;** CRMP, controlled-release mitochondrial protonophore; DBA/2J (D2); DNP, 2-4 dinitrophenol; HFD, high-fat diet.

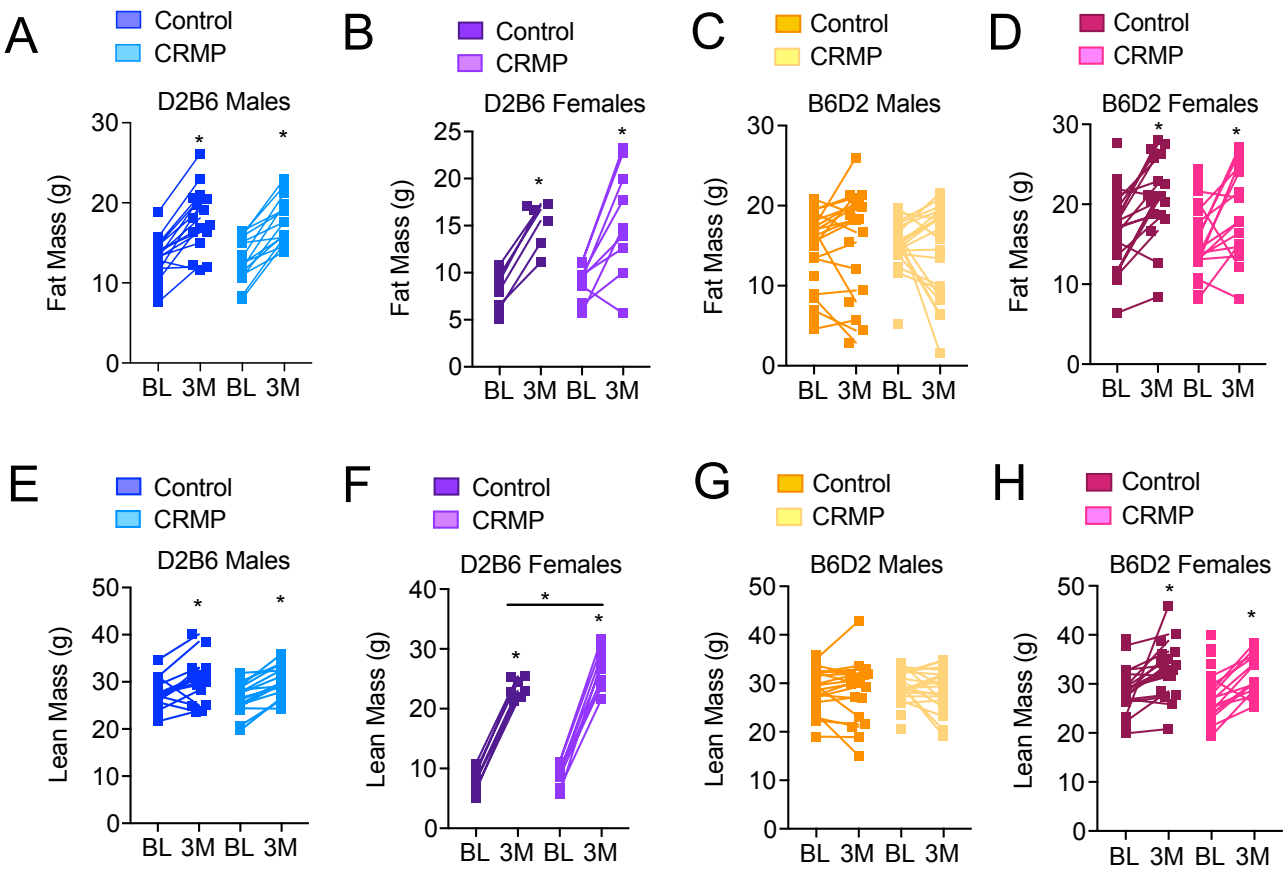

Figure S4: Sex and strain-specific effects of CRMP on lean and fat mass. Fat mass (**A–D**) and lean mass (**E**–**H**) in male and female F1 offspring of DBA/2J (D2) and C57BL/6J (B6) mice fed a HFD (45% fat; baseline [BL]) or HFD containing CRMP (10 mg/[kg-day]) for 3 months (3M). In all panels, *n* = 7-28 per group. Data are presented as mean +/- SEM. **P*<0.05 by two-sided unpaired or paired Student's *t-test* (**A–B**, **D–H**) or Wilcoxon matched-pairs signed rank test (**C**). Abbreviations: C57BL/6J (B6)**;** CRMP, controlled-release mitochondrial protonophore; DBA/2J (D2); HFD, high-fat diet; F, fat mass; L, lean mass.

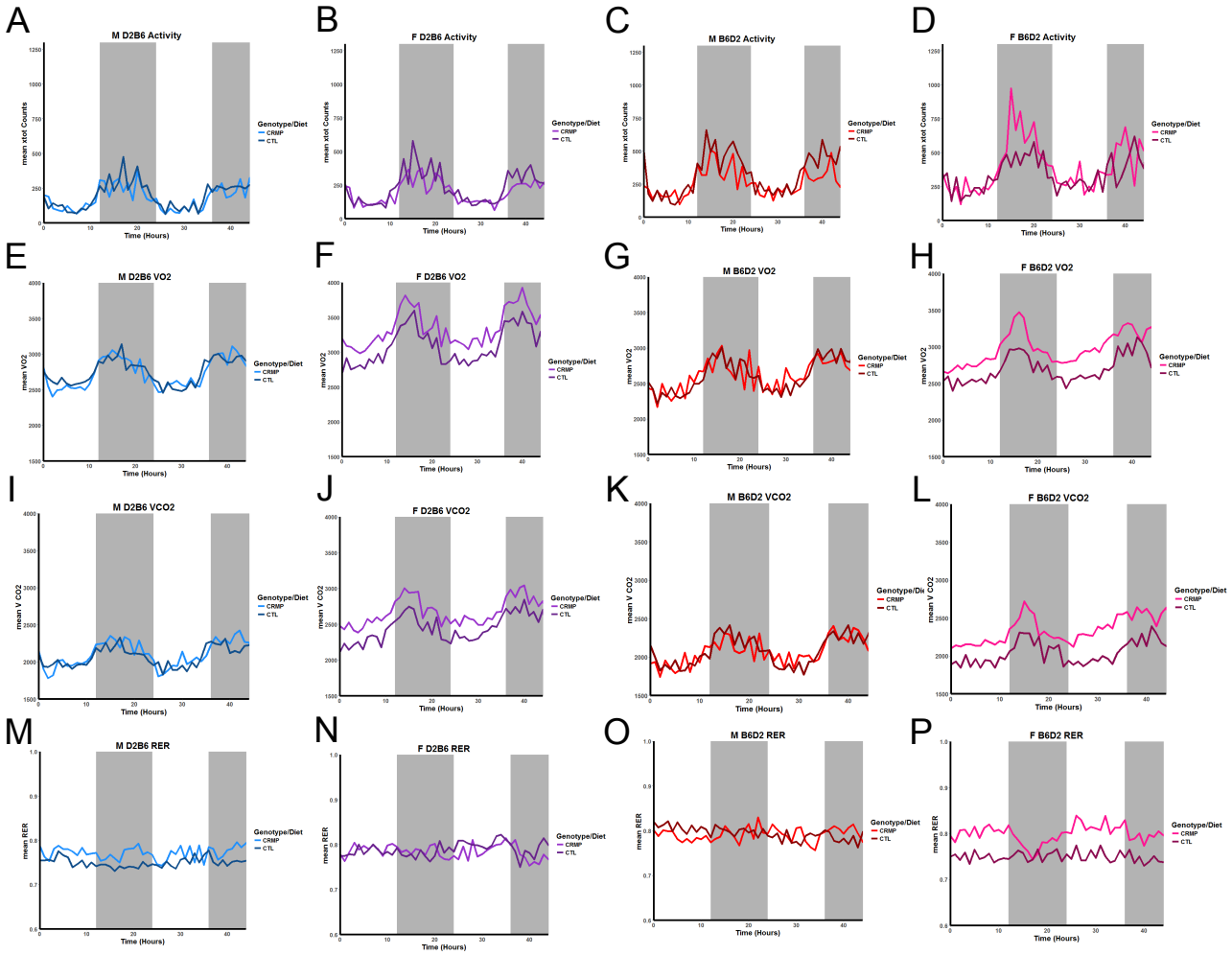

Figure S5: Sex and strain-specific effects of CRMP on whole-body metabolic parameters. Locomotor activity (**A–D**), whole-body O_2 consumption (**E**–**H**), whole-body VO_2 production (**I–L**), and RER (**M–P**) in 94-104 week-old male and female F1 offspring of DBA/2J (D2) and C57BL/6J (B6) mice fed a HFD (45% fat) or HFD containing CRMP (10 mg/[kg-day]) for 3 months. Data are presented as mean +/- SEM. *n* = 4 per treatment group. Abbreviations: C57BL/6J (B6)**;** CRMP, controlled-release mitochondrial protonophore; DBA/2J (D2); HFD, high-fat diet; RER, respiratory exchange ratio.

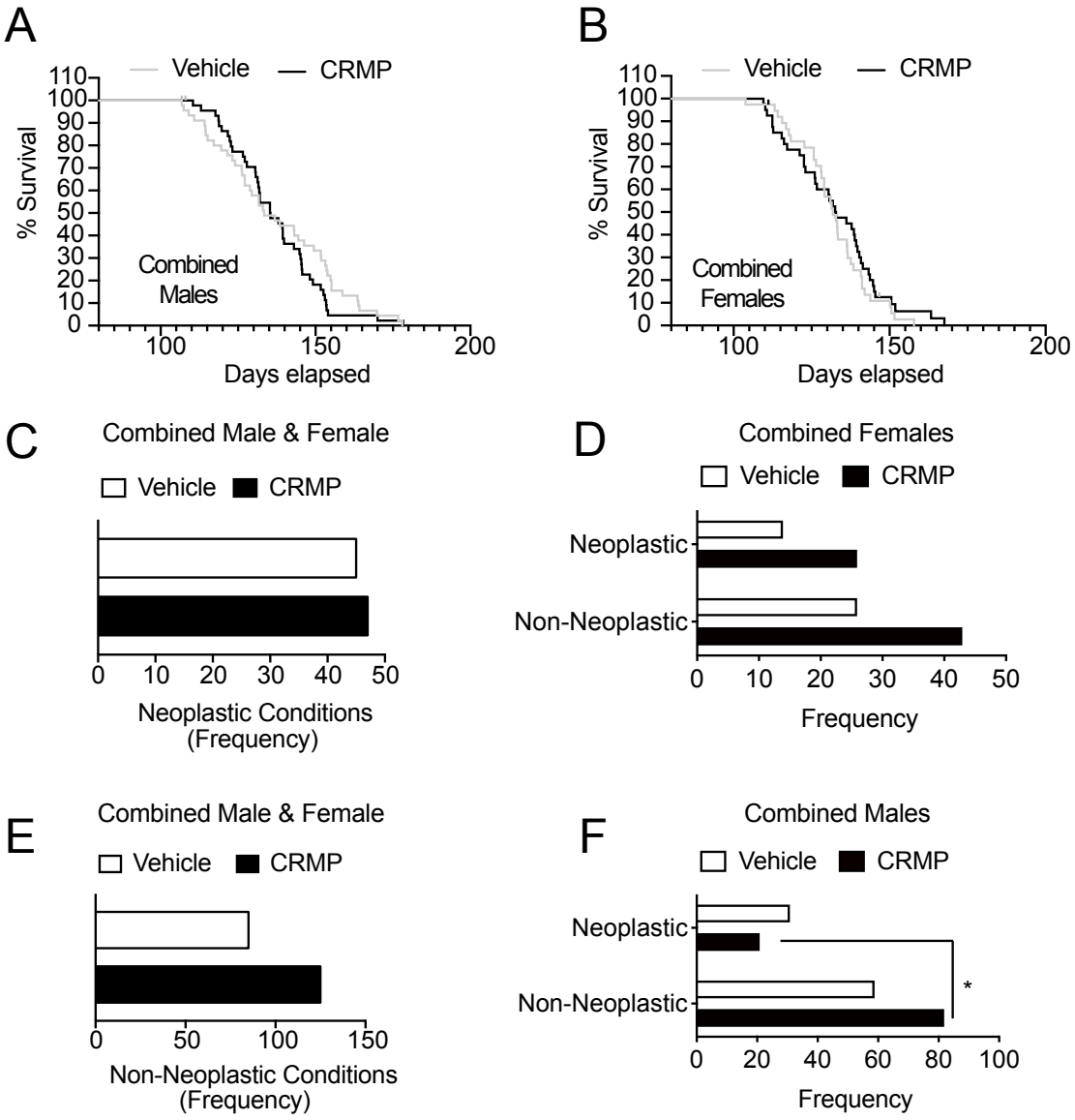

Figure S6: Sex-specific effects of CRMP on neoplastic and non-neoplastic disease incidence in HFD-fed mice. (**A–B**) Kaplan-Meier survival curves for D2B6/B6D2 males (**A**) and D2B6/B6D2 females (**B**) fed a HFD (45%) or HFD containing CRMP (10 mg/[kgday]). (**C**) Frequency of all neoplastic disease incidence in male and female mice treated as in (**A**). (**D**) Frequency of neo- and non-neoplastic disease incidence in female mice treated as in (**A**). (**E**) Frequency of all non-neoplastic disease incidence in male and female mice treated as in (**A**). (**F**) Frequency of neo- and non-neoplastic disease incidence in male and female mice treated as in (**A**). *n* = 30-31 per treatment group. **P*<0.05 by Fisher's exact test (neoplastic vs non-neoplastic disease). Abbreviations: C57BL/6J (B6)**;** CRMP, controlled-release mitochondrial protonophore; DBA/2J (D2); HFD, high-fat diet.

Table S1: Survival statistics for mice stratified by gender and strain.

	Males						Females					
	B6D2				Combined		B6D2				Combined	D2B6
D2B6	Vehicle	CRMP	Vehicle	CRMP	Vehicle	CRMP	Vehicle	CRMP	Vehicle	CRMP	Vehicle	CRMP
Log-rank (Mantel-Cox) test												
Chi square	0.07974		1.386		0.6776		1.725		1.977		0.763	
df	1		1		1		1		1		1	
P value	0.7776		0.2391		0.4104		0.1890		0.1597		0.3824	
Median survival	143	145.4	132.4	131.9	133.4	135.4	131.4	132.9	135.6	130.4	131.9	132.7
Ratio (and reciprocal)	0.9833	1.017	1.004	0.9962	0.9858	1.014	0.9892	1.011	1.04	0.9616	0.9935	1.007
95% CI of ratio	0.5111-1.892	0.5286-1.957	0.5858-1.72	0.5814-1.707	0.6506-1.494	0.6695-1.537	0.5831-1.678	0.5958-1.715	0.4417- 2.449	0.4084-2.264	0.6338-1.558	0.6419-1.578
Hazard Ratio (logrank)	A/B	B/A	A/B	B/A	A/B	B/A	A/B	B/A	A/B	B/A	A/B	B/A
Ratio (and reciprocal)	1.096	0.9126	0.709	1.41	0.8342	1.199	1.405	0.712	0.5747	1.74	1.216	0.8225
95% CI of ratio	0.5701-2.106	0.4748-1.754	0.4-1.257	0.7956-2.5	0.5418-1.284	0.7786-1.846	0.8202-2.405	0.4157-1.219	0.2389- 1.383	0.7232-4.187	0.7728-1.913	0.5228-1.294
Number of rows	38	38	53	53	92	92	57	57	21	21	79	79
# of blank lines	19	19	27	26	47	46	29	28	11	10	41	39
# censored subjects	0	2	0	0	0	2	1	1	0	0	1	1
# deaths/events	19	17	26	27	45	44	27	28	10	11	131.9	132.7