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Supplementary Materials for

Controlled-release mitochondrial protonophore (CRMP) reverses dyslipidemia and hepatic steatosis in dysmetabolic nonhuman primates

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This PDF file includes:

SUPPLEMENTARY FIGURES

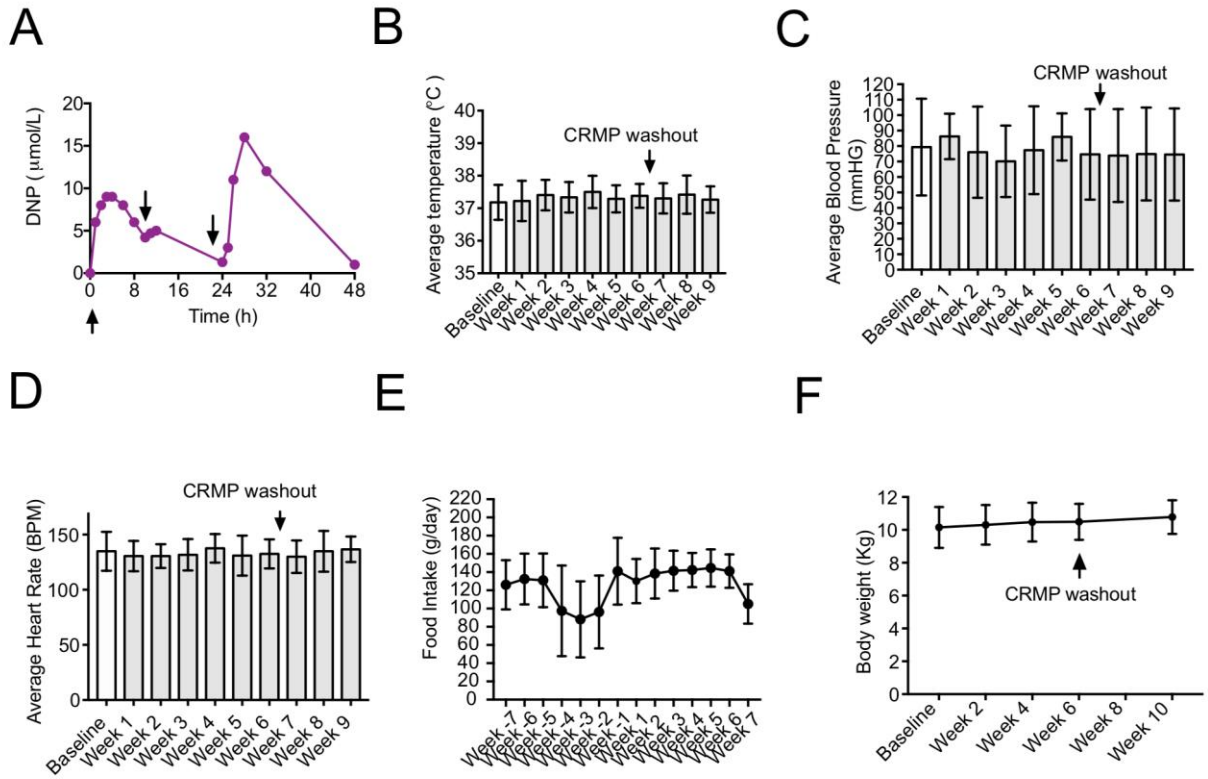

Fig. S1. Safety and efficacy profile of CRMP-treated, high-fat, fructose-fed cynomolgus macaques. (**A**) Representative plasma DNP concentrations after CRMP dosing (0.8 mg/Kg BID). Arrowheads denote dosing. (**B-F**) Weekly body temperature (**B**), blood pressure (**C**), heart rate (**D**), food intake (**E**) and body weight (**F**) at baseline, during 6 weeks of CRMP treatment (0.8 mg/Kg BID), and washout. Data are mean ± SD. *n* =6.

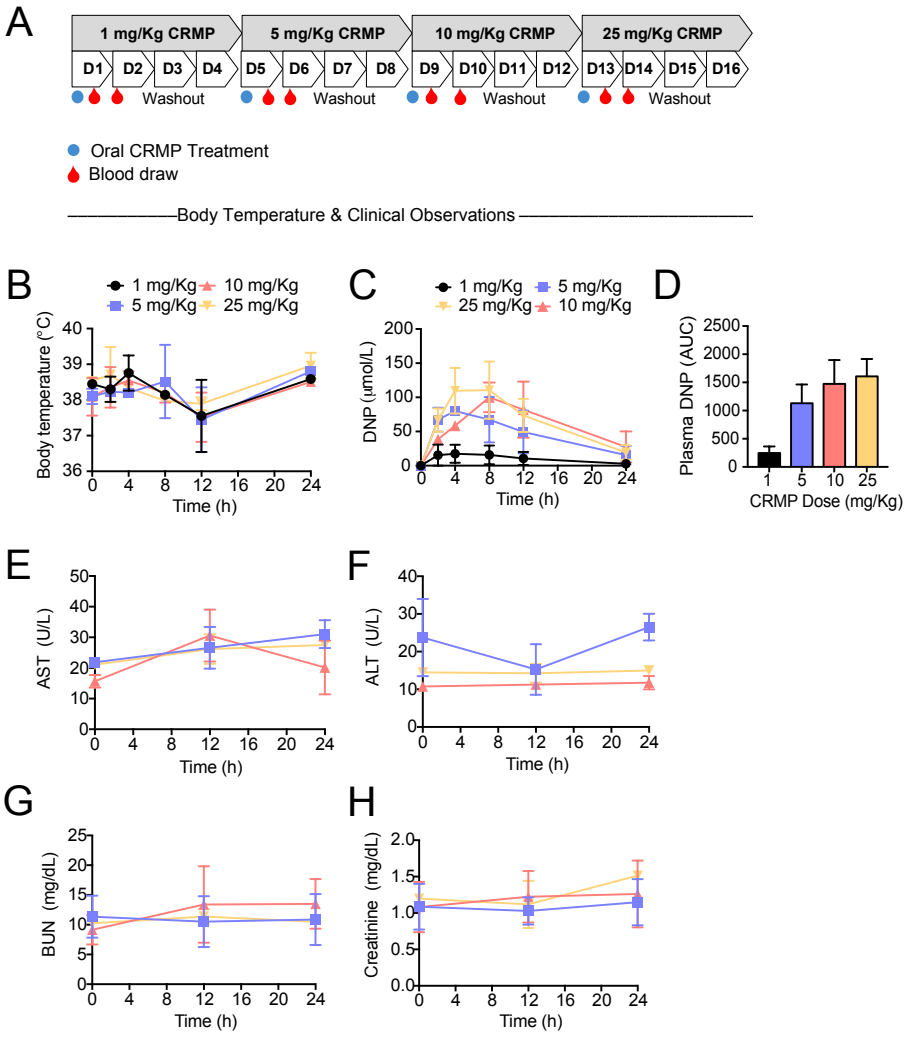

Figure S2: Oral CRMP dose escalation study in dysmetabolic rhesus macaques. (**A**) Experimental outline of dose escalation study. Dysmetabolic Rhesus monkeys were given escalating doses of CRMP (1, 5, 10, and 25 mg/Kg body weight-day) over a period of 16 days. Blood was drawn before and 2, 4, 8, 12, and 24 h after each dose for subsequent analyses. (**B**) Body temperature before (t = 0 h) and 2, 4, 8, 12 and 24 h after escalating doses of CRMP. (**C-D**) Plasma DNP levels (**C**) and AUC (**D**) in Rhesus monkeys treated as in (**A**). (**E-H**) Plasma AST (**E**), ALT (**F**), BUN (**G**), and creatinine (**H**) before (t = 0 h) and 2-24 h after escalating doses of CRMP (1-25 mg/Kg-day). In panels (**B-H**), data are mean ± SD. n = 2 per treatment group.

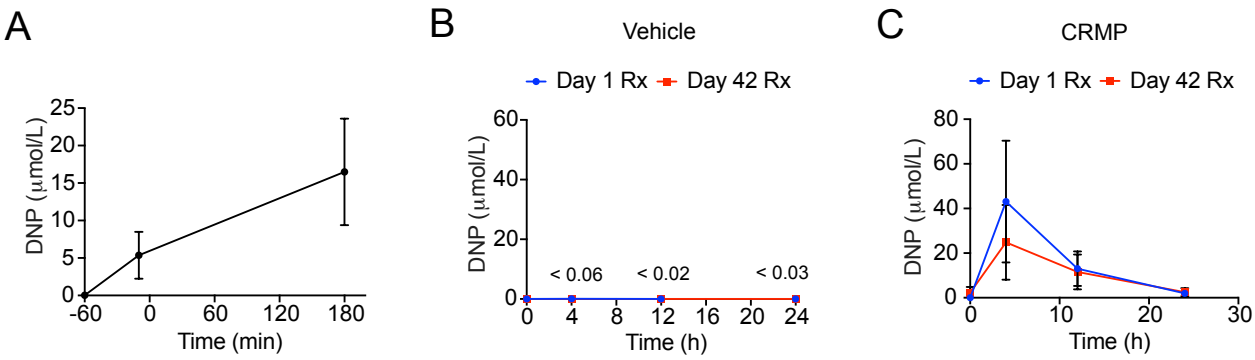

Figure S3: Plasma DNP concentrations in dysmetabolic rhesus macaques. (**A**) Average plasma DNP concentrations in dysmetabolic Rhesus monkeys (n = 10) treated with an acute dose of 5 mg/Kg CRMP and infused with 10 µmol/(Kg-min) [3-^{13}C] lactate, 0.1 mg/(Kg-min) [d_7] glucose, and 0.02 mg/(Kg-min) [$^{13}C_4$] β-OHB for 180 minutes. (**B-C**) Time course of average plasma DNP levels in dysmetabolic Rhesus monkeys on day 1 and day 42 of dosing with vehicle (**B**) or 5 mg/Kg CRMP (**C**). n = 6 vehicle, 12 CRMP. In panels all panels, data are mean ± SD.

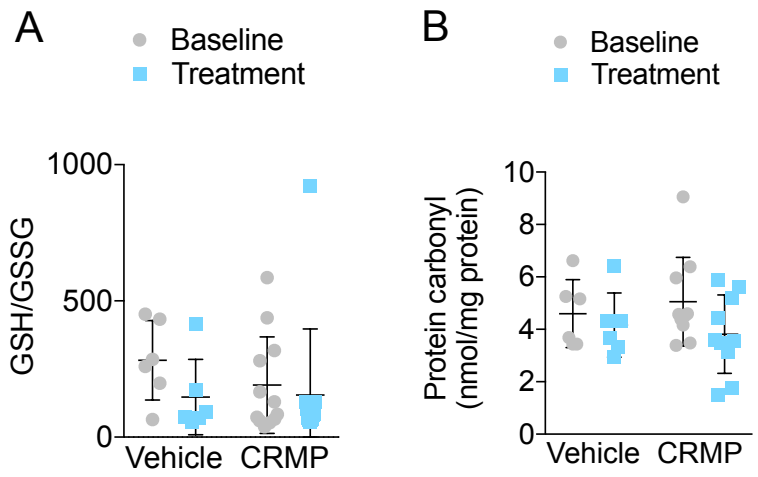

Figure S4: CRMP treatment does not alter oxidative stress in dysmetabolic rhesus macaques. Hepatic GSH/GSSG (**A**) and protein carbonyl content (**B**) in the livers of dysmetabolic Rhesus monkeys before (baseline) and after treatment with 5 mg/(Kg-day) CRMP (n = 10) or vehicle control (n = 6) for 6 weeks (treatment). In all panels, data are mean ± SD.

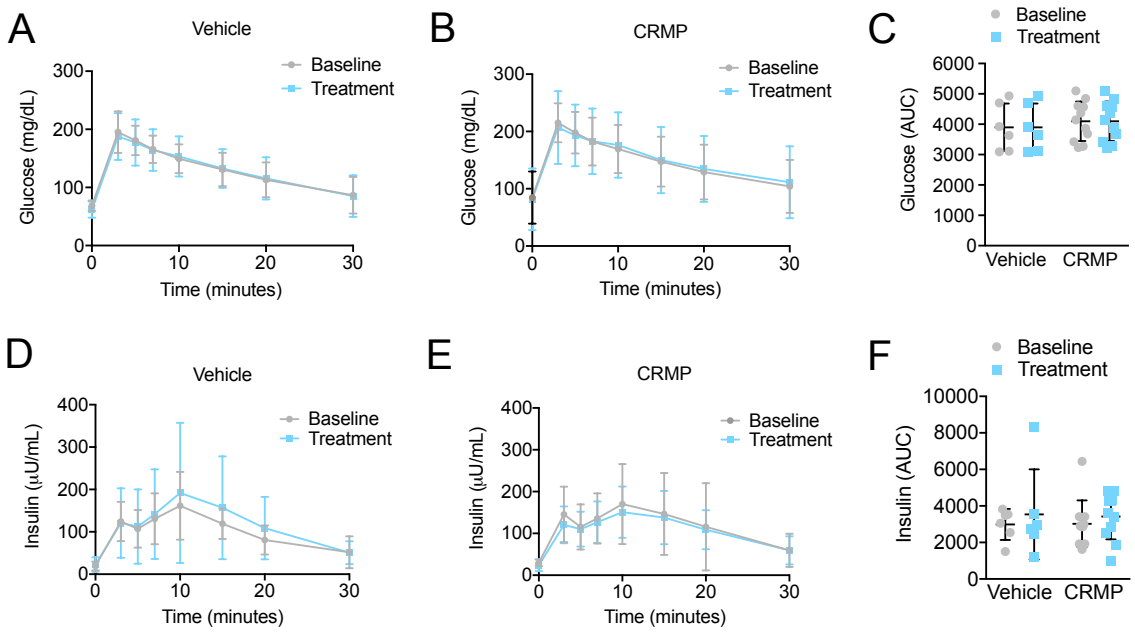

Figure S5: CRMP treatment does not alter whole-body glucose tolerance in dysmetabolic rhesus macaques. Plasma glucose concentrations (**A-B**), glucose AUC, plasma insulin concentrations (**D-E**) and insulin AUC (**F**) during an intravenous glucose tolerance test in dysmetabolic Rhesus monkeys before (baseline) and after 6 weeks of treatment with 5 mg/Kg CRMP (n = 12) or vehicle control (n = 6). In all panels, data are mean ± SD.

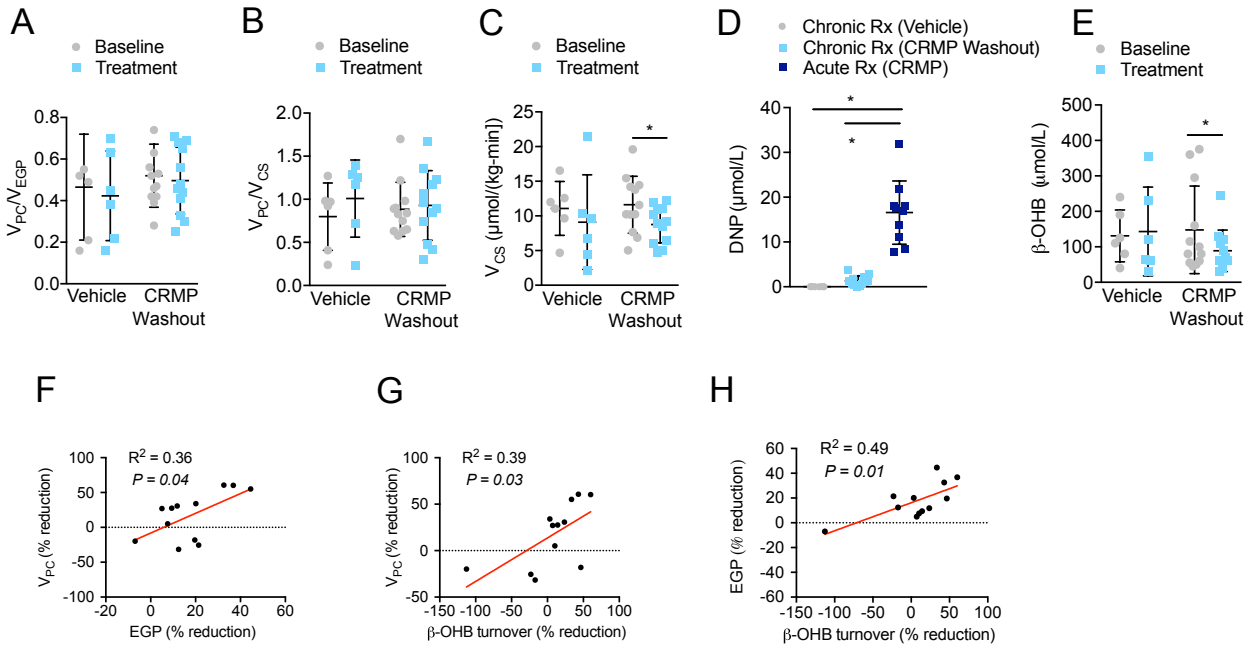

Figure S6: Hepatic mitochondrial fluxes in dysmetabolic rhesus macaques treated with CRMP. (**A-C**) V_{PC}/V_{EGP} (**A**), V_{PC}/V_{CS} (**B**) and V_{CS} (**C**) in dysmetabolic Rhesus monkeys before (baseline) and after treatment with 5 mg/Kg-day CRMP (n = 12) or vehicle control (n = 6) for 6 weeks (treatment). (**D**) Average plasma DNP concentrations in the plasma of dysmetabolic Rhesus monkeys 180 minutes after an infusion with 10 µmol/(Kg-min) [3-^{13}C] lactate, 0.1 mg/(Kg-min) [d_7] glucose, and 0.02 mg/(Kg-min) [$^{13}C_4$] β-OHB and treated with 5 mg/Kg-day CRMP (n = 12; CRMP washout) or vehicle control (n = 6; vehicle) for 6 weeks or after an acute dose of 5 mg/Kg CRMP (CRMP). *$P \leq 0.05$ by Dunn's multiple comparison test. (**E**) Plasma β-OHB levels in dysmetabolic Rhesus monkeys treated as in (**A-C**). *$P \leq$ 0.05 by Wilcoxon matched-pairs signed rank test. (**F-H**) Linear regression analysis of % reductions in V_{PC}, EGP and β-OHB turnover in dysmetabolic Rhesus monkeys after treatment with 5 mg/Kg-day CRMP (n = 12) for 6 weeks. In panels (**A-E**), data are mean ± SD.