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SUPPLEMENTAL INFORMATION TITLES AND LEGENDS

Figure S1. Cachectic phenotype of C26 and KPC murine models. (A) Graphical summary of the experimental protocol. (B-C) Weight trajectories of C26 tumor-bearing mice and littermate controls (C), and KPC tumor-bearing mice and PC controls (C) on KD or NF diets since they were enrolled into the study until they reached cachectic endpoint. (D-E) Organ weights of cachectic C26 tumor-bearing mice and littermates (n=10-15) (D), and cachectic KPC tumor-bearing mice and PC controls (n=9-10) (E) fed either KD or NF diets. (F-G) mRNA expression of the PPARα target genes Acadm (F) and Hmgsc2 (G) in C26 tumor-bearing mice and littermate controls fed KD or NF (n=5-7). (H-I) Cumulative food intake of KD- or NF-fed C26-tumor bearing mice and littermates (n=5 LM, n=12 C26) (H), and KD- or NF-fed KPC tumor-bearing mice and PC controls (n=10) (I) during the last 4 days before endpoint.

Data are expressed as the mean ± SEM. One-way ANOVA with Tukey's correction for post hoc testing was used in (D-G). Two-way ANOVA statistical tests with Tukey's correction for post hoc comparisons were performed in (H-I). * p-value < 0.05, ** p-value < 0.01, **** p-value < 0.0001.

Supplemental Table 1. Macronutrient composition and caloric profile of standard and ketogenic diets.

Figure S2. Intratumoral accumulation of lipids and saturation of the GSH system causes ferroptotic cell death. (A) Schematic representation of the GSH pathway for detoxification of LPPs. (B) PCA of tumors from C26 tumor-bearing mice fed NF or KD (n=7). (B) PCA of untargeted metabolomics in the tumors of C26 mice fed KD or NF. (C-F) Quantification of ophthalmate (C), carnosine (D), hypotaurine (E) and taurine (F) metabolites by UPLC-MS/MS in the tumor of C26 mice fed KD or NF (n=7). (G) Quantification by UPLC-MS/MS of GSH/GSSG ratio in the tumor of C26 mice fed KD or NF, untreated or treated with NAC (n=5-7). (H) Oil-Red-O staining of tumors from C26 mice on KD or NF. (I) Western blot of tumor lysates from C26 mice fed KD or NF

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stained for Caspase-3 and BAX apoptotic markers (n=5). (J) Immunohistochemistry staining of tumors from C26 mice fed KD or NF with the proliferation marker Ki67. (K-N) Quantification by flow cytometry of neutrophils (K), T-cells (L), macrophages (M) and monocytes (N), in the tumor of C26 mice fed KD or NF (n=3-4).

Data are expressed as the mean ± SEM. Statistical analysis in (B) is described in the Methods section/Chapter 4. Statistical differences in (C-F, K-N) were examined using an unpaired two-tailed Student's t-test with Welch's correction. One-way ANOVA with Tukey's correction for post hoc testing was used in (G). * p-value < 0.05, ** p-value < 0.01, **** p-value < 0.0001.

Figure S3. Biochemical deficiency in the corticosterone synthesis pathway in the adrenal cortex of tumor-bearing mice fed ketogenic diet. (A) Murine synthetic pathway of corticosterone in the cortex of the adrenal glands. (B) Corticosterone levels at baseline (prior to diet change), 4 days after the start of the experiment, and at endpoint (cachexia) in C26 tumor-bearing mice and littermate controls fed KD or NF (n=5 LM, n=10-14 C26). (C) GSEA pathway analysis of cholesterol homeostasis and steroid biosynthesis in tumor-bearing KD-fed KPC mice compared to NF-fed KPC (n-5). (D) Plasma concentration of the pro-inflammatory cytokine IL-6 in C26 tumor-bearing mice and control littermates on NF or KD diets at endpoint (n=5 LM, n=9-10 C26).

Data are expressed as the mean ± SEM. One-way ANOVA with Tukey's correction for post hoc testing was used in (B, D). Statistical analysis in (C) is described in Methods. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001.

Figure S4. Extended metabolic profiling of cachectic C26 and KPC mice. (A-B) Plasma urea levels in cachectic C26 tumor-bearing mice and littermate controls (n=5 LM, n=10-11 C26) (A), and cachectic KPC tumor-bearing mice and PC controls (n=5-8) (B) fed either KD of NF diets. (C) PCA of hepatic metabolomics in C26 tumor-bearing mice and control littermates fed with KD or NF (n=5-6).

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Data are expressed as the mean ± SEM. One-way ANOVA with Tukey's correction for post hoc testing was used in (A-B). Statistical analysis in (C) is described in Methods. * p-value < 0.05, ** p-value < 0.01, **** p-value < 0.0001.

Figure S5. Extended data on the systemic effects of Dexamethasone treatment. (A) Weight trajectories of C26 tumor-bearing mice treated with Dexamethasone and fed with either KD or NF. (B) Survival of littermate controls, and C26 tumor-bearing mice treated or untreated with Dexamethasone, fed with KD or NF (n=7 LM, n=17-18 C26, n=7 C26 + Dex). (C) Percentage of mice in each group that were sacrificed because of cachexia (OS) or tumor size (PFS) endpoints. (D) Quantification by UPLC-MS/MS of metabolites involved in fatty acid metabolism in the liver of C26 tumor-bearing mice on either KD or NF diets, untreated or treated with Dexamethasone (n=5). (E) Organ weights of C26 tumor-bearing mice untreated or treated with Dexamethasone after 4 days of treatment (n=10-12 C26, n=3 C26 + Dex). (F-G) Cumulative water intake (F) and total movement (G) during the last 4 days before endpoint in littermate controls and C26 tumor-bearing mice, untreated or treated with Dexamethasone, fed KD or NF (n=7).

Data are expressed as the mean ± SEM. Survival: OS + PFS. Kaplan–Meier curves in (B-C) were statistically analyzed by using the log-rank (Mantel–Cox) test. One-way ANOVA with Tukey's correction for post hoc testing was used in (E). Analysis in (D, F-G) is described in Methods. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001.

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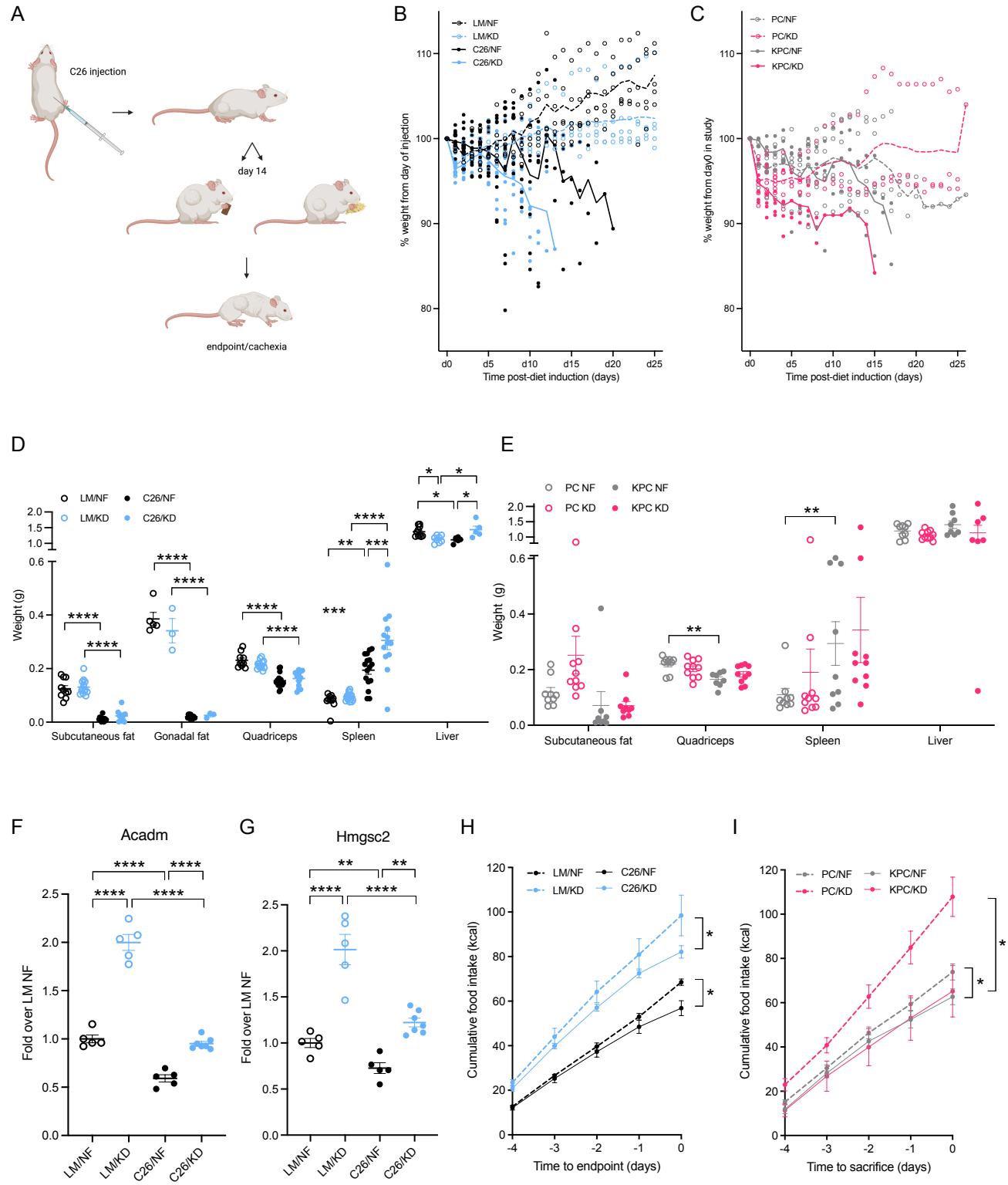

Supplementary Figure 1

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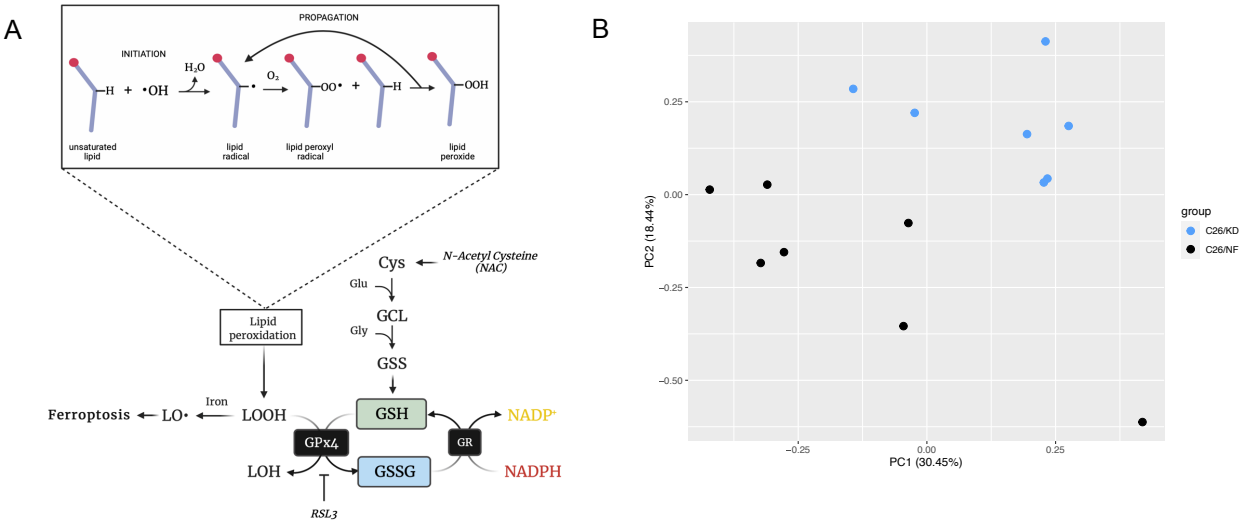

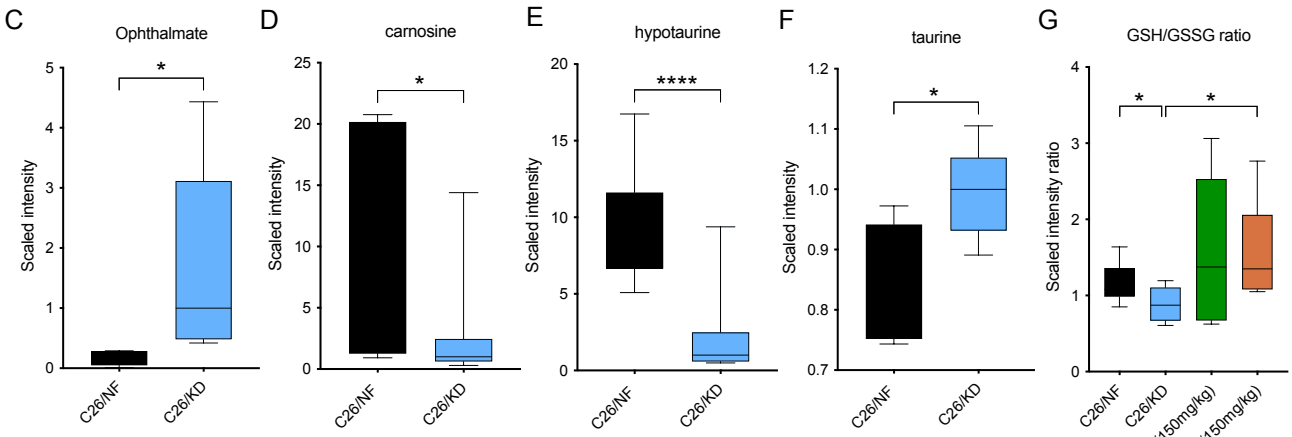

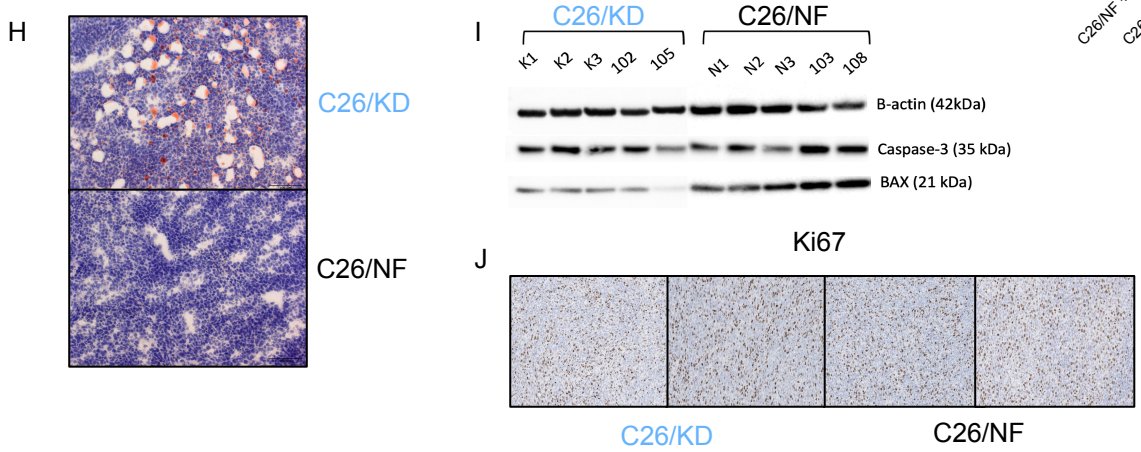

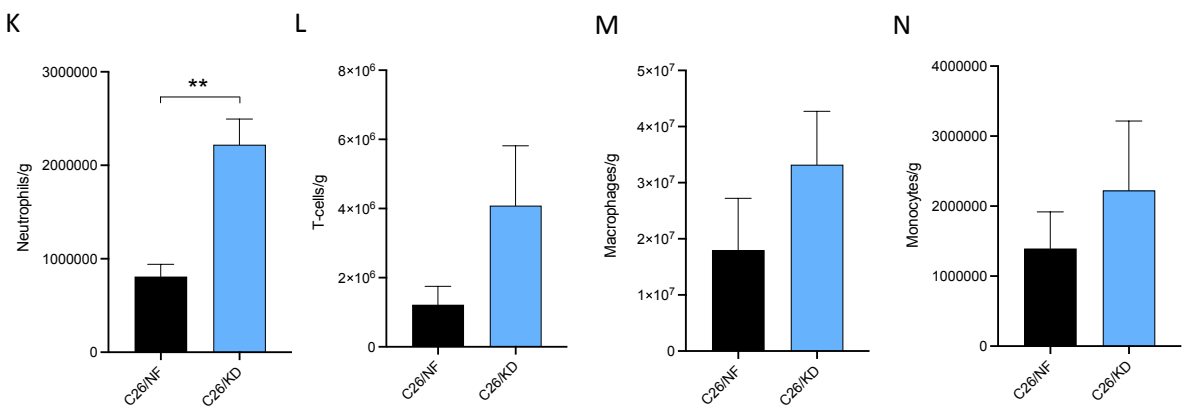

Supplementary Figure 2

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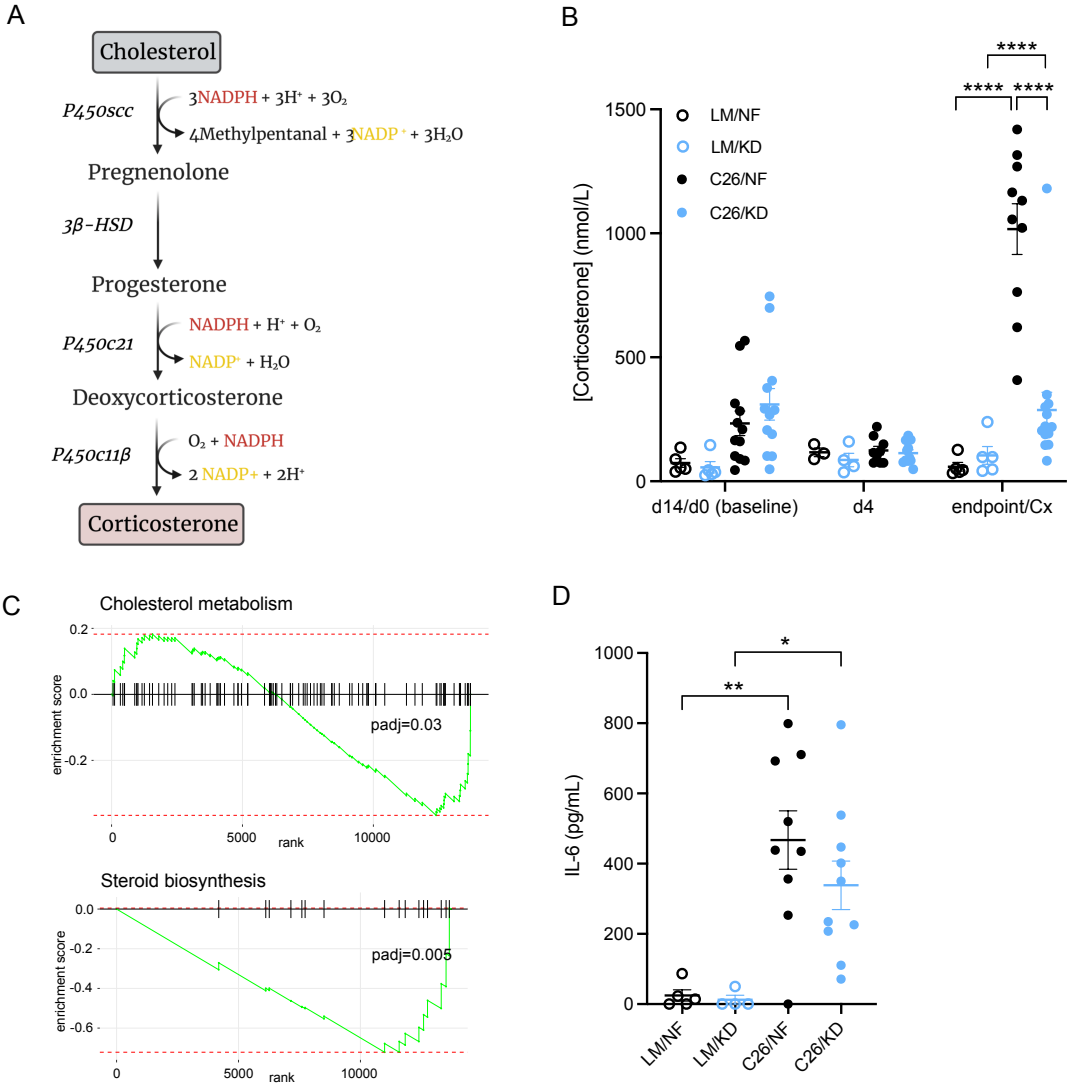

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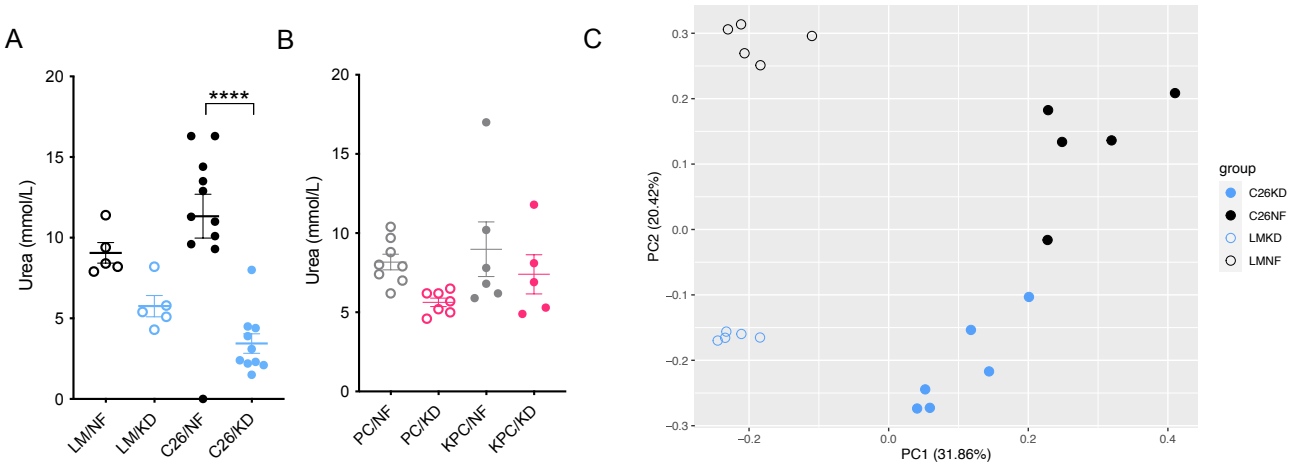

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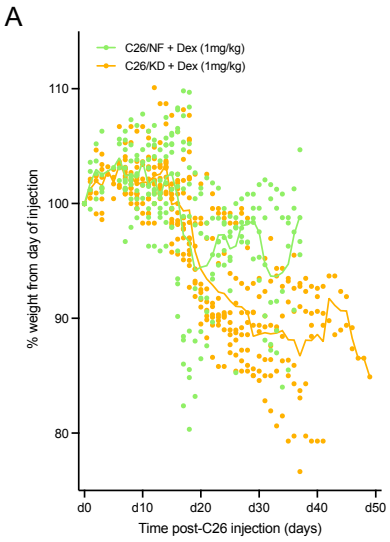
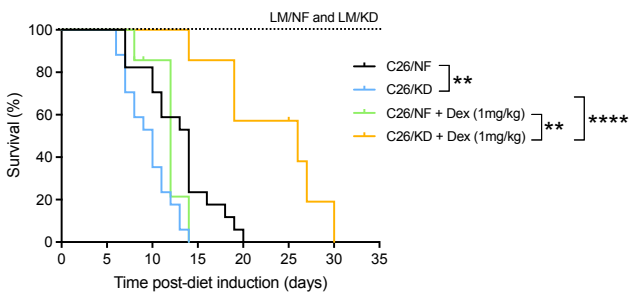
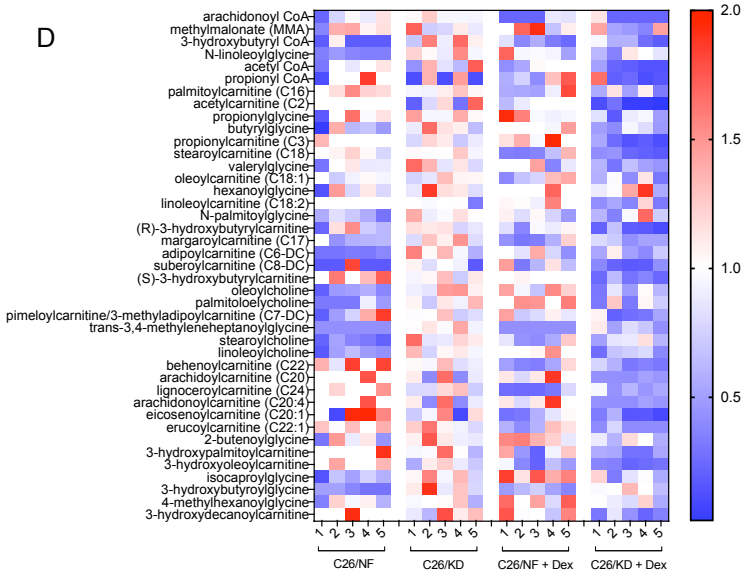
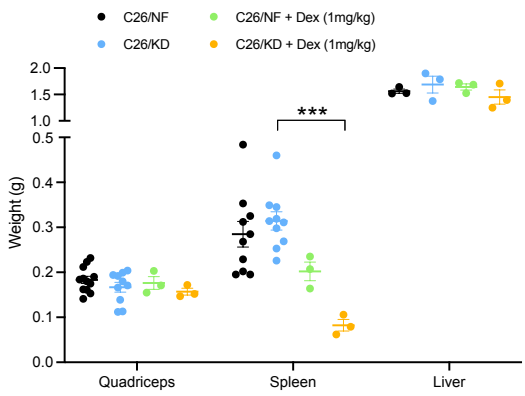


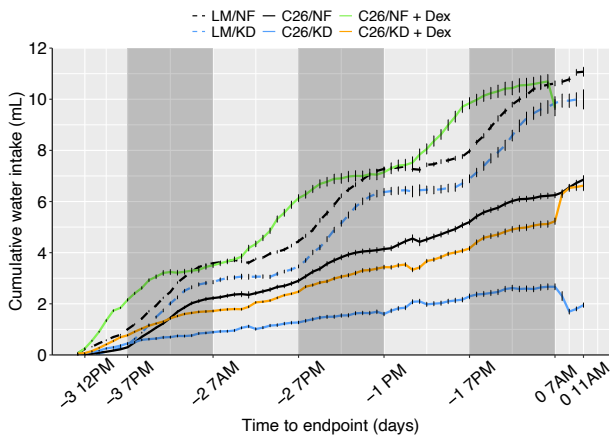


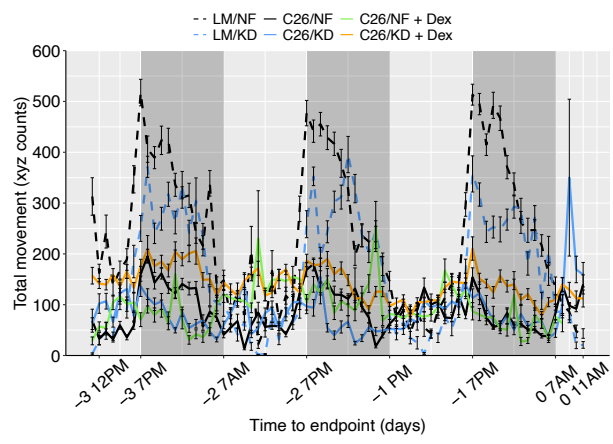
C

	OS	PFS
C26/NF	70.6%	29.4%
C26/KD	100%	0%
C26/NF + Dex	42.9%	57.1%
C26/KD = Dex	71.4%	28.6%









Supplementary Figure 5

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	Standard diet (PicoLab Rodent Diet 20)	Ketogenic diet (AIN-76A Modified)
Fat (%)	10.6	75.1
Protein (%)	20	8.6
Carbohydrates (%)	52.9	3.2
Fiber (%)	4.7	4.8
Ash (%)	6.1	3
Moisture (%)	<10	<10
Caloric profile (kcal/g)	4.07	7.24
Cystine (g/kg)	2.8	0.3
Methionine (g/kg)	7	2.2

Extended Data Table 1