Newman et al., Figure S1

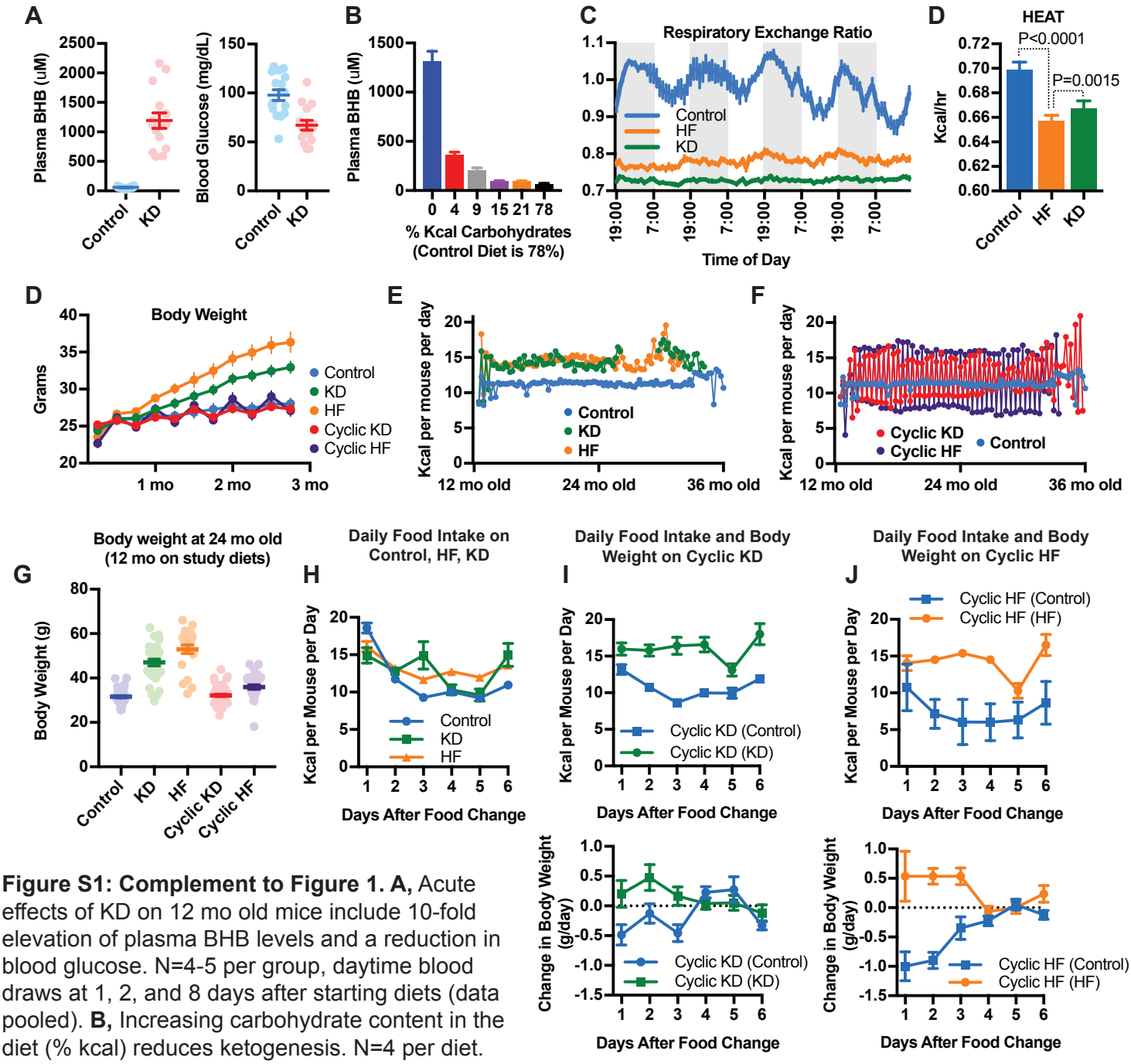

Figure S1: Complement to Figure 1. A, Acute effects of KD on 12 mo old mice include 10-fold elevation of plasma BHB levels and a reduction in blood glucose. N=4-5 per group, daytime blood draws at 1, 2, and 8 days after starting diets (data pooled). **B,** Increasing carbohydrate content in the diet (% kcal) reduces ketogenesis. N=4 per diet. **C-D,** Metabolic cage study of KD and HF. Respiratory exhange ratio (C) shows that mice on both KD and HF rely primarily on fat for energy with minimal circadian variation; KD more so. Both KD and HF reduce energy expenditure (D), HF more so. Mice were observed on Control for one week, then half switched to KD and the other half to HF for an additional week of observation. Control data pooled. 12 mo old C57BL/6, N=6 per group (12 total). **D,** Body weight trajectories for mice in Figure 1C. **E-J, Additional data for lifespan study (Figure 1 D-L). E-F,** Mice in all groups show stable caloric intake throughout life. The gap in intake for KD ~800 days was a time when the consistency of the KD changed suddenly (see Experimental Procedures), making measurement of leftover food unreliable for several weeks, in cages with staggered ages. **G,** Distribution of body weights for mice at 24 mo old. Note the wider distribution for KD and HF. **H,** Daily food intake for Control, HF, and KD groups in the lifespan study, which have no diet cycling. **I-J,** Mice on Cyclic KD (I) or Cyclic HF (J) show stably higher caloric intake on KD/HF weeks, and gradually lower caloric intake on Control weeks. This corresponds to initial gradual weight loss or gain each week, which stabilizes by Day 4. There are no period of fasting or binging. Daily food intake data was collected for all mice in the lifespan study; daily weights were measured on a subset (N=3-8 per group).

Newman et al., Figure S2

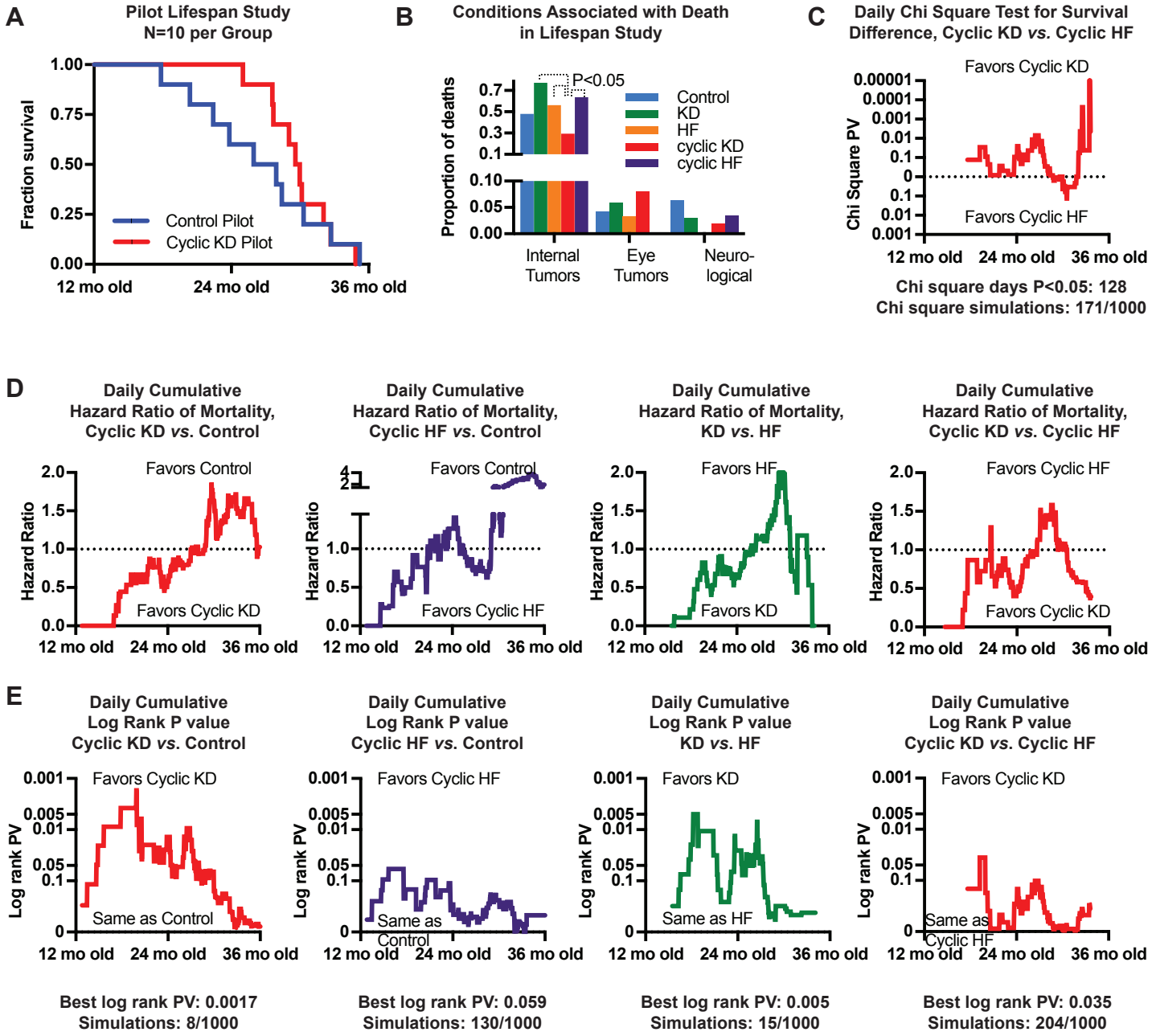

Figure S2: Complement to Figure 1. A, Survival curve from pilot lifespan study, enrolling C57BL/6 males at 17-19 mo old (N=10 per group). Conditions were otherwise identical to the main lifespan study in Figure 1. **B,** Proportion of deaths in the main lifespan study associated with neurological conditions or visible tumors on necropsy. **C,** Daily chi square tests comparing survival in Cyclic KD vs. Cyclic HF, with random-data simulation results. **D,** Daily calculations of the cumulative hazard ratio for four pairwise group comparisons. Plot for Cyclic KD vs. Control (left) appears to show that Cyclic KD mortality is delayed and then lower than Control but increasing later in the study. A similar pattern is seen in the KD vs. HF comparison. **E**, Daily calculation of the log rank test for four pairwise group comparisons. This tests the difference between survival curves from the beginning up through each day (e.g. day 365-440, day 365-441, etc.). The difference between Cyclic KD and Control (left) reaches significance for most of the duration from the study start through 24-30 mo. The difference between Cyclic HF and Control follows a similar pattern but only approaches modest significance. The difference between KD and HF is also most significant from the study start through 24-30 mo. The difference between Cyclic KD and Cyclic HF only briefly reached modest significance. We performed monte carlo simulations with 1000 randomized data sets to find how often random data generated a “best” log rank P value better than what was seen in the actual data (below plots).

Newman et al., Figure S3

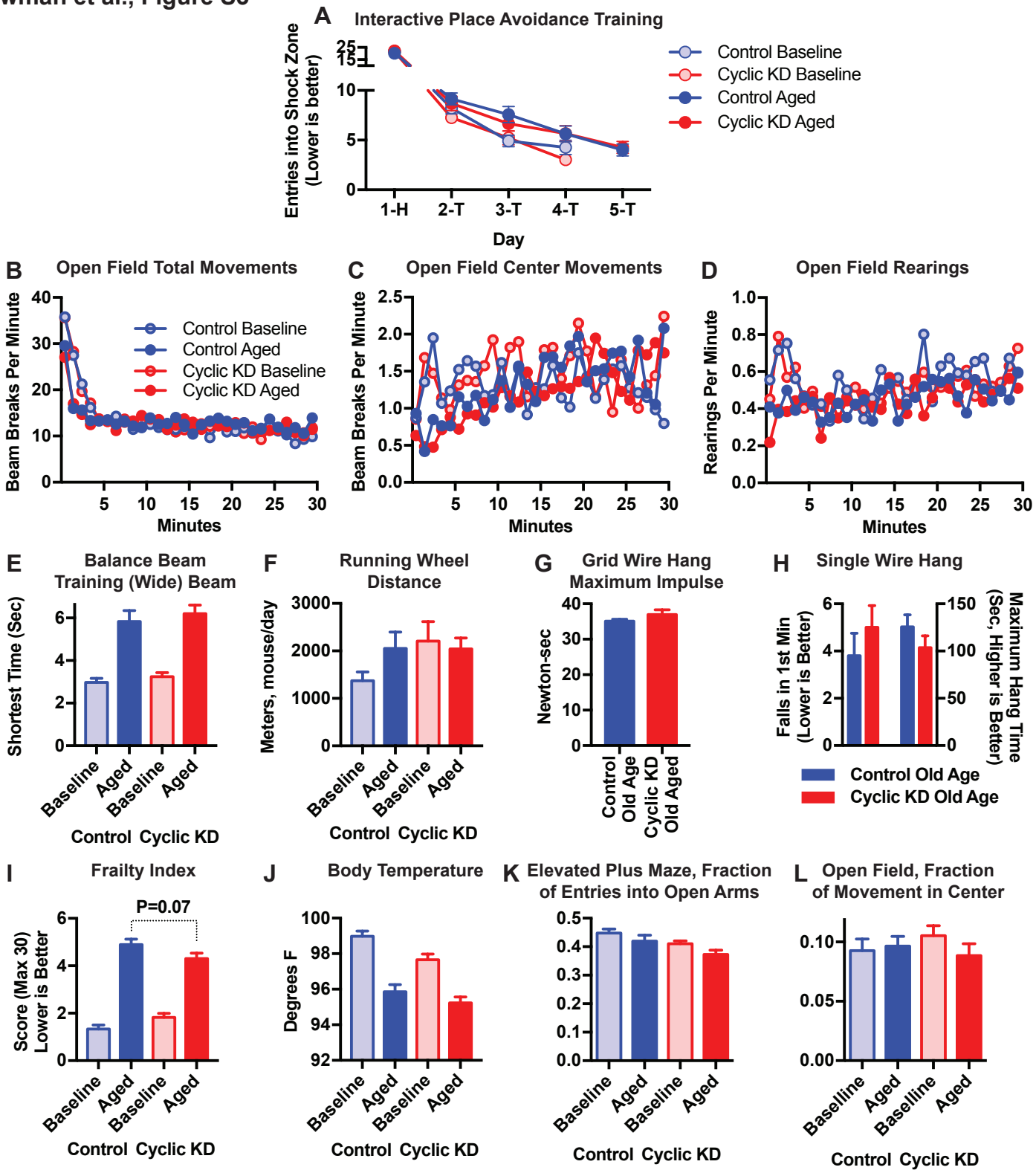

Figure S3: Complement to Figure 2. A, Learning/training phase of the Place avoidance cognitive test. Day 1 is habituation with no shocks. Days 2-4/5 mice are training to avoid the shock zone. Note overall slower training with age, but similar final training with an extra day. **B-D,** Detailed Open Field data. The only change between Baseline and Aged was a reduction in the vey early exploratory period (1-5 min) in Aged mice, with no difference between Control and Cyclic KD. **E,** The "training" (1/2") balance beam discrimated age performance better than the "test" (1/4") beam in Figure 2H. **F,** In-cage group running wheels showed no difference between groups. **G-H,** "Old Age" testing at 28-30 months included Grid Wire Hang (J) and Single Wire Hang (K). Both showed no difference between groups. **I,** Mouse frailty index increased with age, and trended lower in Cyclic KD. **J,** Surface body temperature declined with age. **K-L,** Anxiety-associated parameters in both the Elevated Plus Maze (K) and the Open Field (L) showed no difference between Control and Cyclic KD.

Newman et al., Figure S4

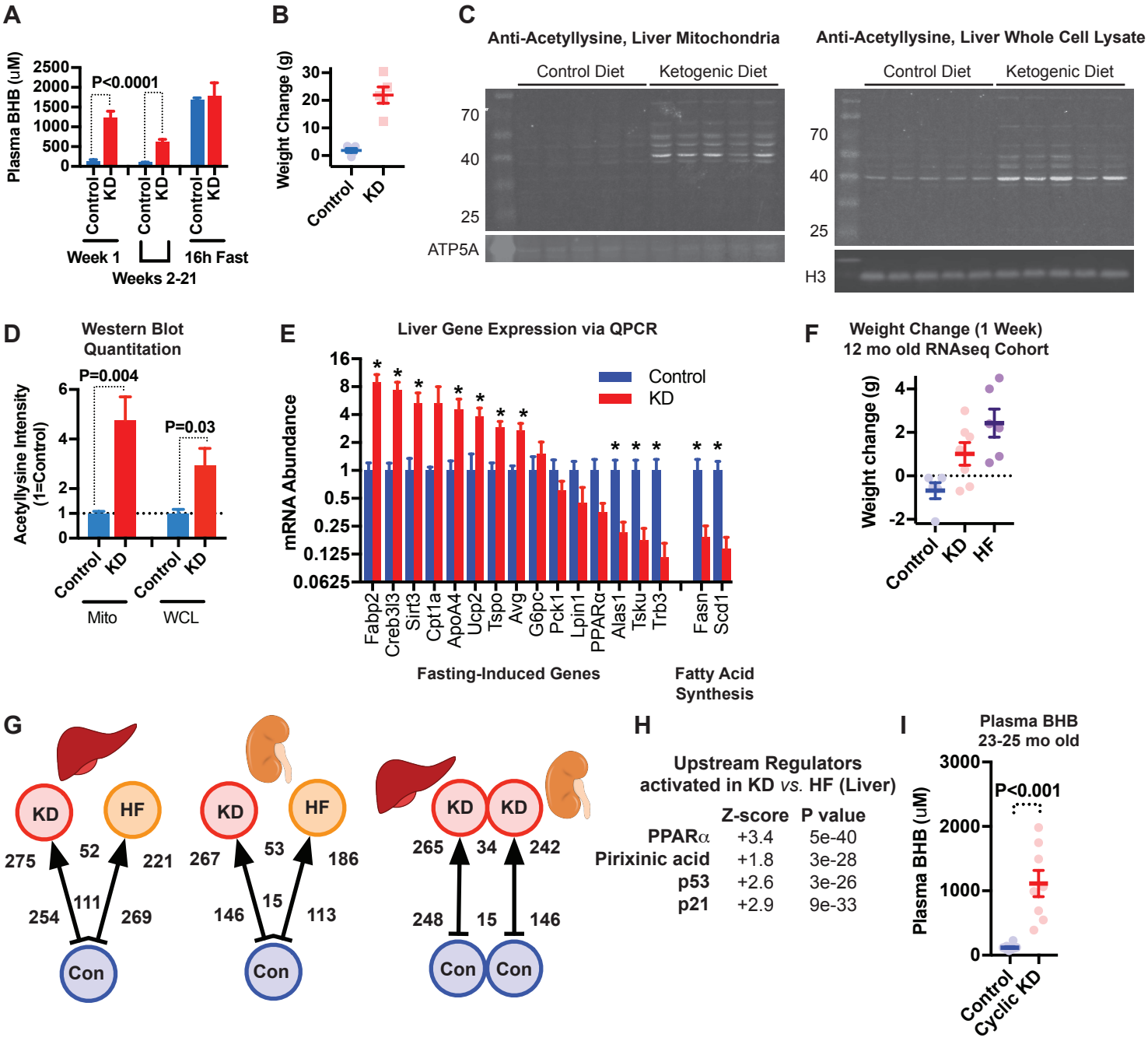

Figure S4: Complement to Figure 3. A-E, Preliminary study of 4 mo old C57BL/6 males fed Control or KD for 5 months (N=5 per diet). **A,** Daytime plasma BHB levels were highest during the first week. Mice also underwent a 16-hour fast during week 2, to provide context for BHB levels on KD. **B,** Mice gained weight when fed KD. **C,** KD increases total protein acetylation in both mitochondria (left) and whole cell lysates (right) from liver. **D,** LiCor Odyssey quantitation of acetyllysine band intensity, over the full lanes as pictured, normalized to the pictured loading control antibody, and expressed as fold-change of KD compared to Control. **E,** QPCR shows that KD increases expression of fasting-induced genes and suppresses genes involved in fatty acid synthesis in liver *, P<0.05. **F-H,** Additional data from RNAseq study shown in Figure 3. Mice are 12 mo old C57BL/6 males on diets for one week, with tissue and blood collected during night-time. **F,** Weight change over one week on the diets. **G,** Counts of genes defined as significantly regulated (P<=0.02 and FC>1.3) by KD and HF vs. Control in the liver or kidney, with overlaps. Arrow points to up-regulation; bar faces down-regulation. Counts only include genes detected in both data sets being compared, and so vary slightly between comparisons. **H,** Ingenuity upstream regulators identified via direct comparison of KD to HF, showing p21, p53, PPARα, and pirixinic acid among those most activated by KD *vs.* HF. **I,** Plasma BHB levels of representative 23-25 mo old mice in lifespan study, collected at night during KD-fed week for Cyclic KD group (N=8-10 per group).