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Supplemental information

Inter-individual variations in circadian misalignment-induced NAFLD pathophysiology in mice

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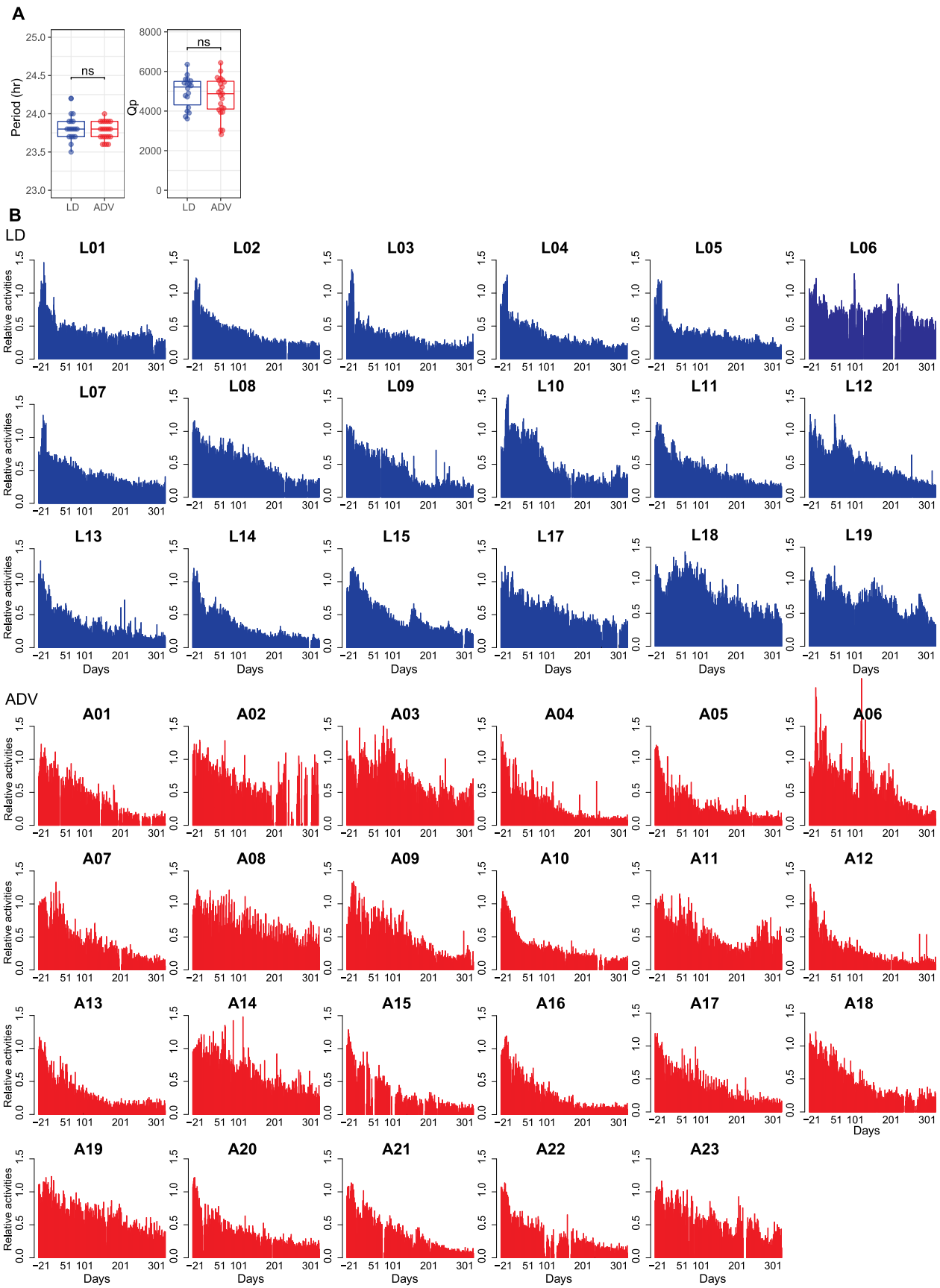

Figure S1. Wheel-running activities in individual mice, Related to Figure 1. (A) Beeswarm box plots of circadian period and power in DD. Chi-square periodogram of wheel-running activities was calculated using 2nd week in DD. No significance was observed in the period and power between ADV and LD (two-sided Welch's t test). **(B)** The daily wheel-running activities in each mouse. The relative to mean activities in 3 weeks before CJL is indicated. The x-axis indicates the days on CJL.

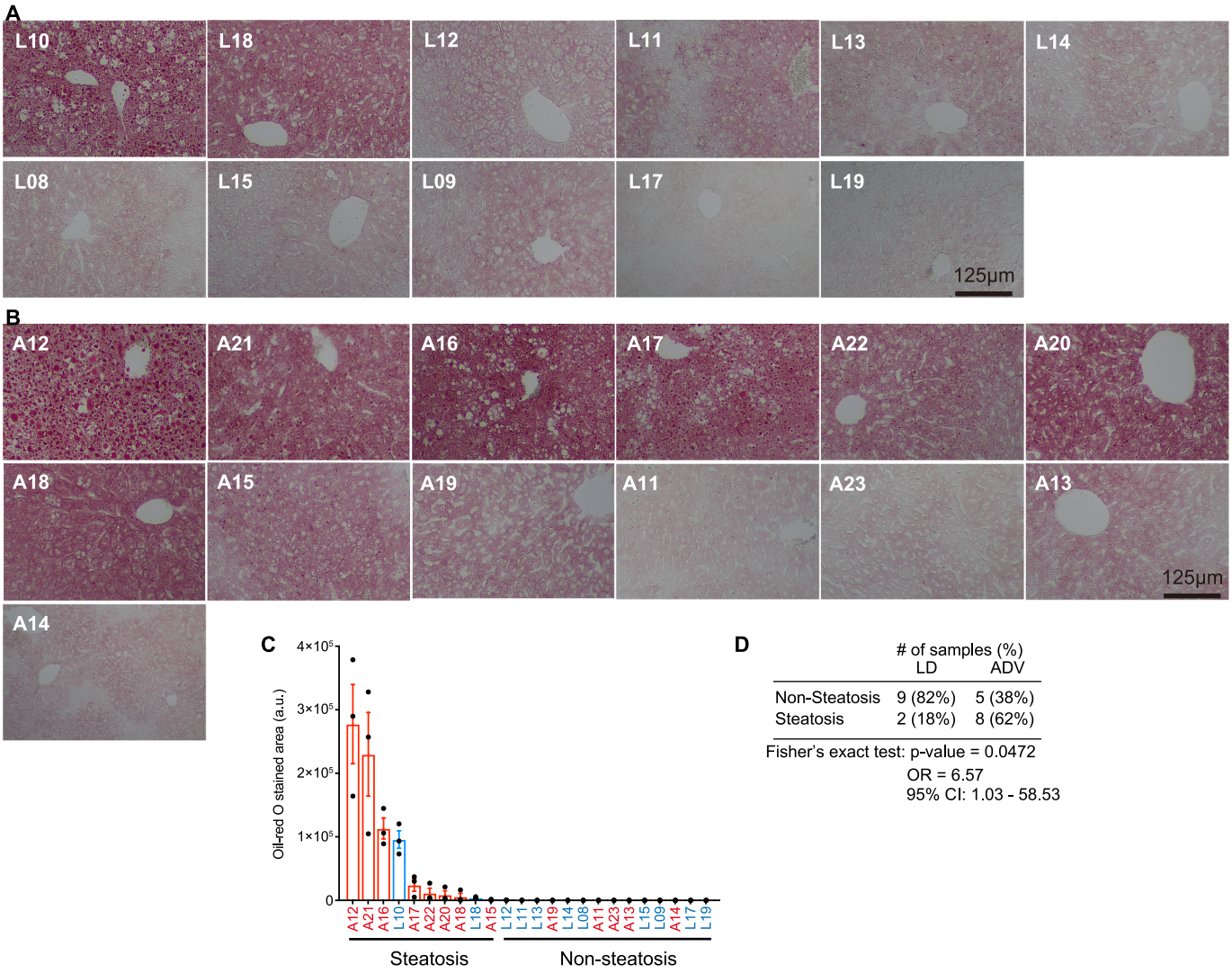

Figure S2. Elevated likelihood of steatosis in the ADV mice, Related to Figure 2. (A and B) Oil Red O stained liver from LD (A) and ADV (B) mice in the 2nd experiment. **(C)** Quantification of Oil Red O staining for analysis of hepatic lipid accumulation in ADV (*n* = 13) and LD (*n* = 11). Values are means ± SD from triplicate. **(D)** The number of animals showed hepatic steatosis in LD and ADV. P value, OR and 95% CI is indicated from Fisher's exact test.

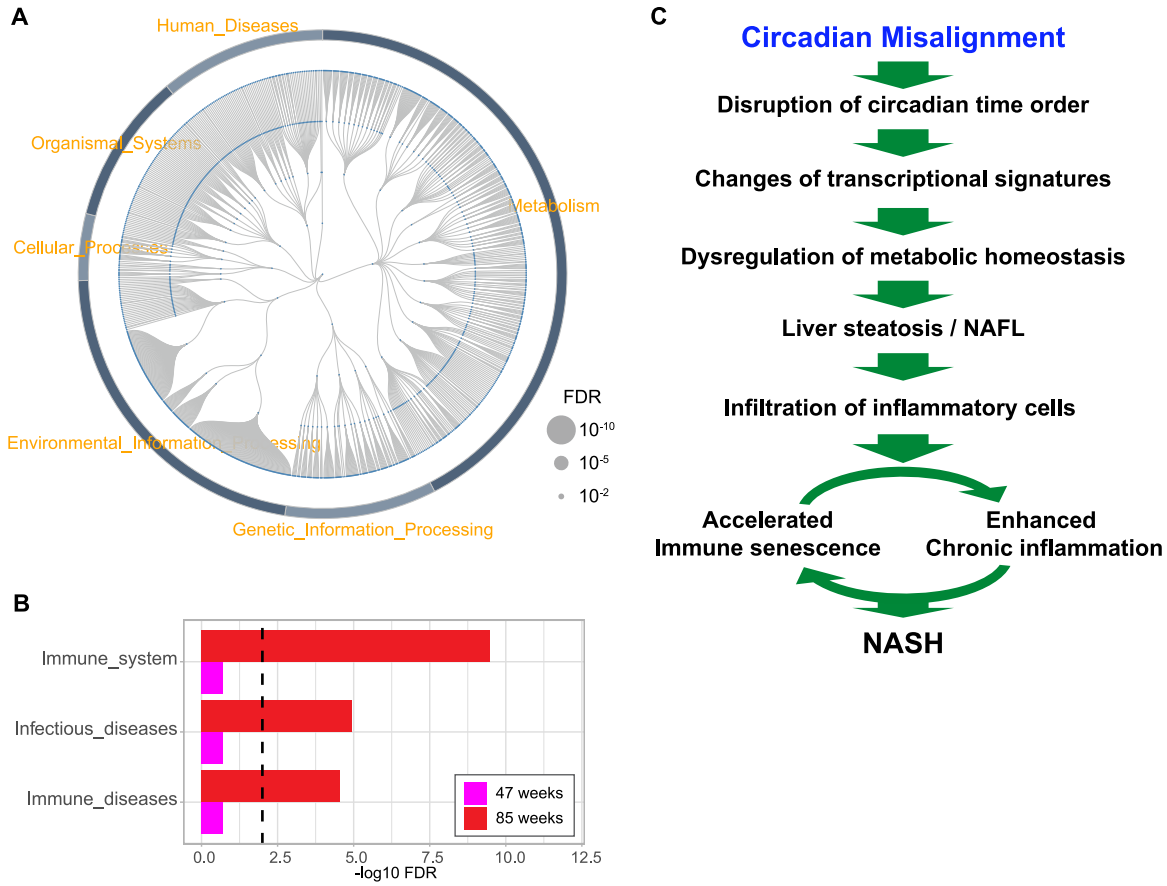

Figure S3. The immune pathways were not significantly activated in the liver from 47-week ADV mice compared with LD mice, Related to Figure 3. (A) Functree visualization showing no terms from the enrichment analysis based on KEGG functional hierarchy for gene expressions in the liver. Significance was determined by FDR < 0.01. **(B)** Activation of immune-related biological processes in the liver in a more prolonged jet-lag. Previously published RNA-seq data (GSE142248) of liver from mice exposed 85 weeks CJL were reanalyzed as described in the methods. Immune system, infectious diseases and immune diseases are significantly (FDR < 0.01) up-regulated in ADV condition for 85 weeks compared with LD, whereas they are remained in subtle in ADV for 47 weeks. The black broken lines indicate FDR of 0.01. **(C)** Schematic representation of pathological process of circadian-related diseases in the liver, NAFLD / NASH.

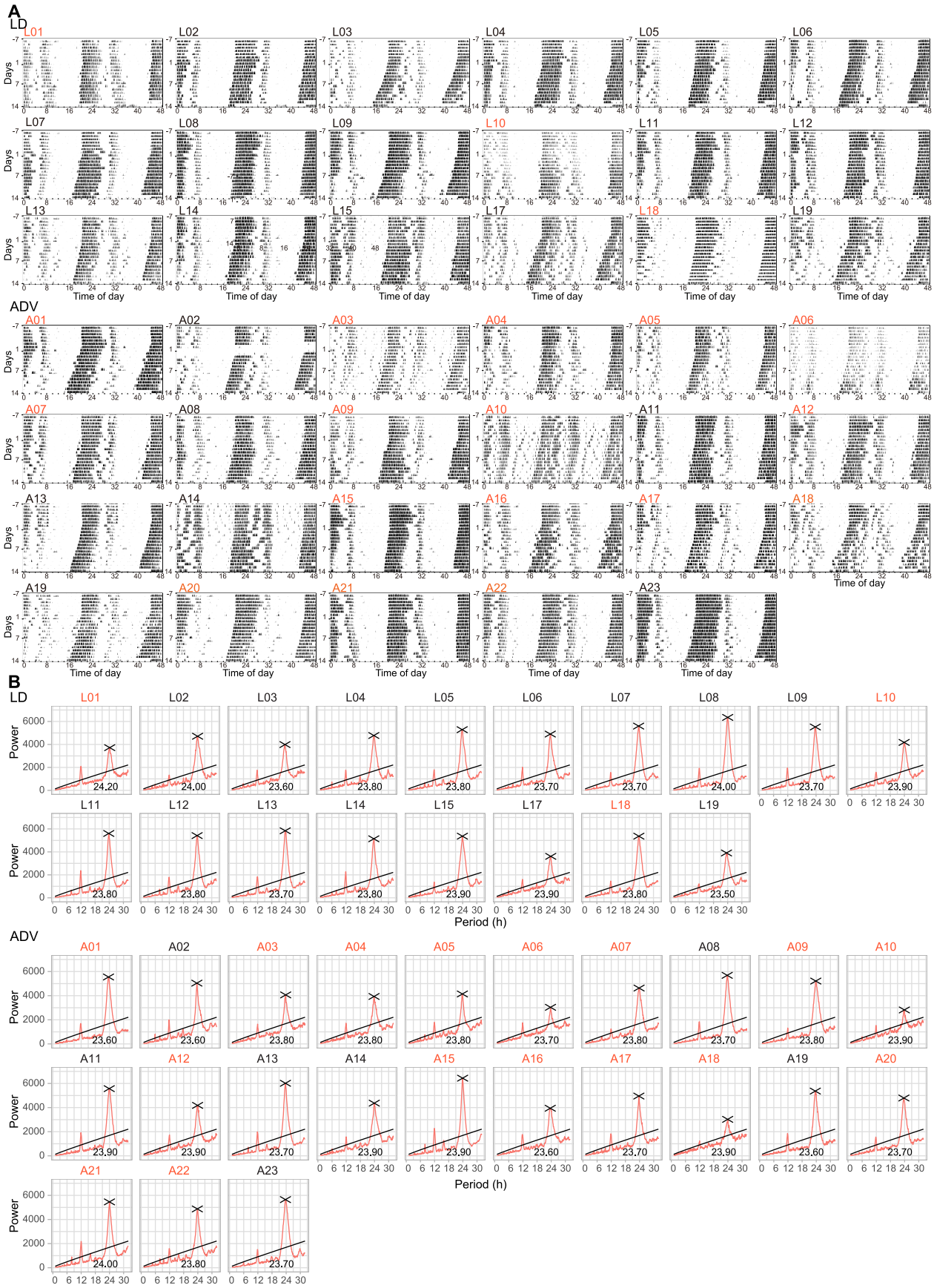

Figure S4. The free-running circadian period in the ADV mice, Related to Figure 5. (A) Double-plotted actogram of the ADV mice during the 7 days in LD and 14 days in DD before initiating CJL. The mice showed hepatic steatosis are indicated by orange in the text labels. **(B)** Chi-square periodogram of wheel-running activities in DD. The periodogram was calculated using 2nd week in DD. The oblique line in the periodogram indicates the significance level of α = 0.001. The highest peak value above the line is indicated. The mice with hepatic steatosis are indicated in orange.