

Expanded View Figures

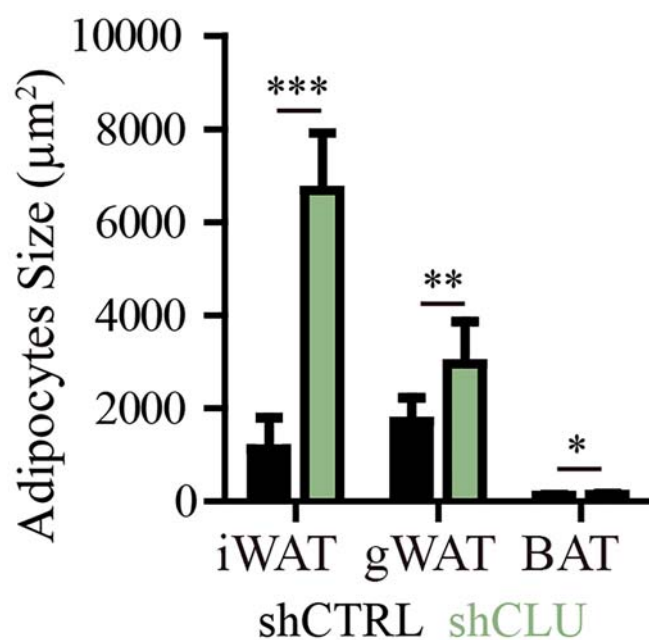


Figure EV1. Related to Fig. 1. Statistical analysis of H&E staining of iWAT, gWAT, BAT in Fig. 1G (shCTRL vs shCLU: iWAT, $P < 0.0001$; gWAT, $P = 0.0014$; BAT, $P = 0.0229$; $n = 10$ per group). The data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical comparisons between groups were performed using unpaired two-tailed Student's *t* tests. Source data are available online for this figure.

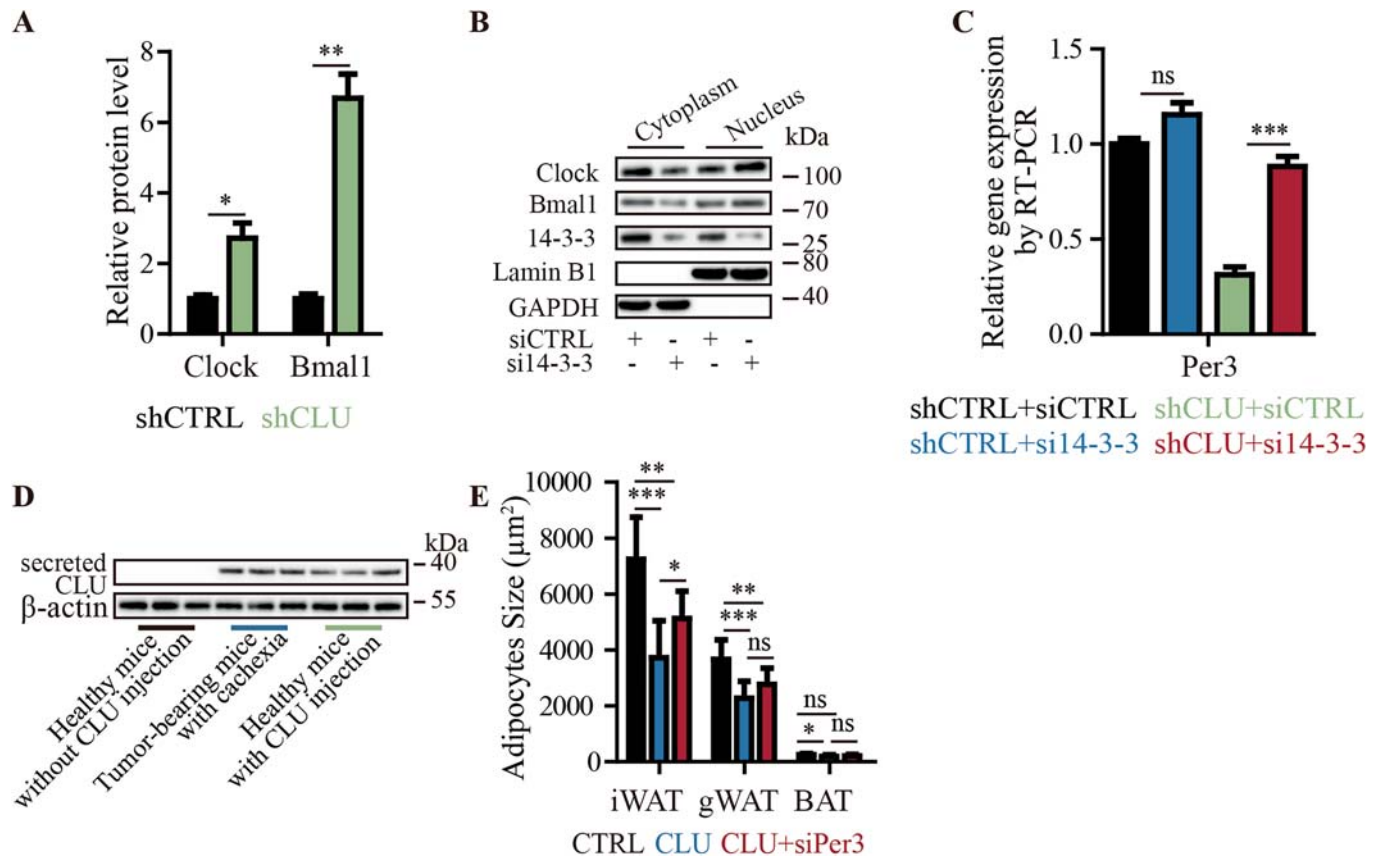


Figure EV2. Related to Fig. 2. (A) Quantification of western blot by Co-IP assays in Fig. 2F (shCTRL vs shCLU: Clock, $P = 0.0181$; Bmal1, $P = 0.0012$; $n = 3$ per group). (B) Representative Western blot images of a nucleocytoplasmic separation assay showing the levels of cytoplasmic and nuclear Clock, Bmal1, and 14-3-3 in iWAT cells with and without 14-3-3 knockdown in mice bearing CLU knockdown tumors. (C) Per3 expression in iWAT cells with and without 14-3-3 knockdown by RT-qPCR. The shCLU and shCTRL stand mice bearing tumor with and without CLU knockdown, respectively (shCTRL+siCTRL vs shCTRL+si14-3-3: Per3, $P = 0.0691$; shCLU+siCTRL vs shCLU+si14-3-3: Per3, $P = 0.0001$; $n = 4$ per group). (D) circulating CLU levels in iWAT of mice by western blot. (E) Statistical analysis of H&E staining of iWAT, gWAT, BAT in Fig. 2L (iWAT: CTRL vs CLU, $P < 0.0001$; CTRL vs CLU+siPer3, $P = 0.0016$; CLU vs CLU+siPer3, $P = 0.0151$; gWAT: CTRL vs CLU, $P = 0.0001$; CTRL vs CLU+siPer3, $P = 0.0057$; CLU vs CLU+siPer3, $P = 0.0819$; BAT: CTRL vs CLU, $P = 0.0137$; CTRL vs CLU+siPer3, $P = 0.0511$; CLU vs CLU+siPer3, $P = 0.5122$; $n = 10$ per group). The data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns for not significant. Statistical comparisons between groups were performed using unpaired two-tailed Student's t tests. Source data are available online for this figure.

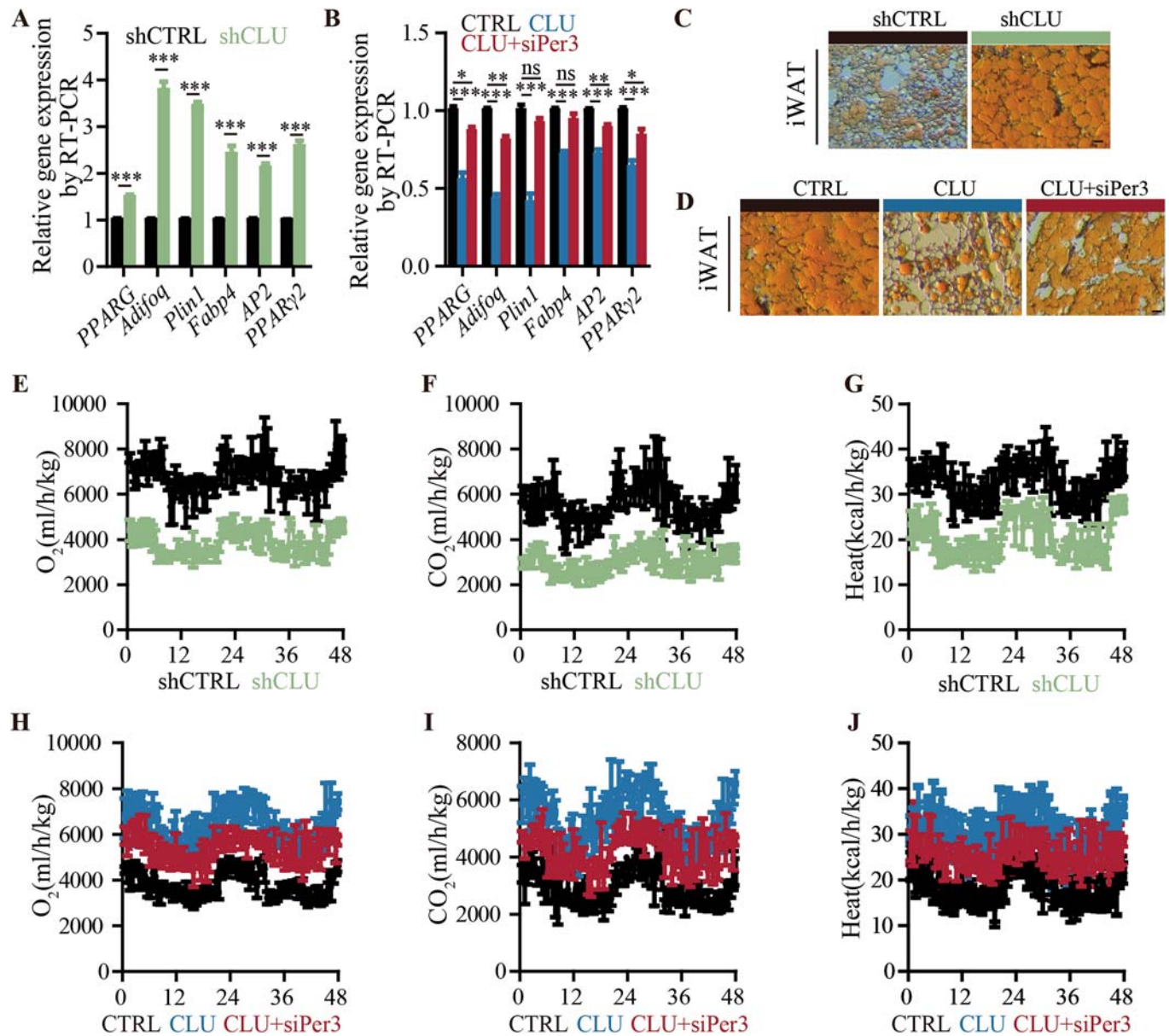


Figure EV3. The CLU-Per3 axis contribute to adipogenesis and energy expenditure.

(A) Adipogenesis-related gene expression by RT-qPCR in iWAT of MDA-MB-468 tumor-bearing mice with indicated treatment (shCTRL vs shCLU: PPARG, $P = 0.0003$; Adipoq, $P < 0.0001$; Plin1, $P < 0.0001$; Fabp4, $P = 0.0002$; AP2, $P < 0.0001$; PPARG2, $P < 0.0001$; $n = 4$ per group). (B) Adipogenesis-related gene expression by RT-qPCR in iWAT of healthy nude mice with indicated treatment (CTRL vs CLU: PPARG, $P = 0.0002$; Adipoq, $P < 0.0001$; Plin1, $P = 0.0002$; Fabp4, $P = 0.0001$; AP2, $P = 0.0002$; PPARG2, $P = 0.0003$; CTRL vs CLU+siPer3: PPARG, $P = 0.0142$; Adipoq, $P = 0.0018$; Plin1, $P = 0.1590$; Fabp4, $P = 0.2300$; AP2, $P = 0.0088$; PPARG2, $P = 0.0163$; $n = 10$ per group). (C, D) Adipogenesis by using Oil Red O staining in iWAT of mice. (E, H) Oxygen consumption, (F, I) carbon dioxide generation and (G, J) heat production by metabolic cages in mice ($n = 3$ per group). The data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns for not significant. Statistical comparisons between groups were performed using unpaired two-tailed Student's t tests. Source data are available online for this figure.

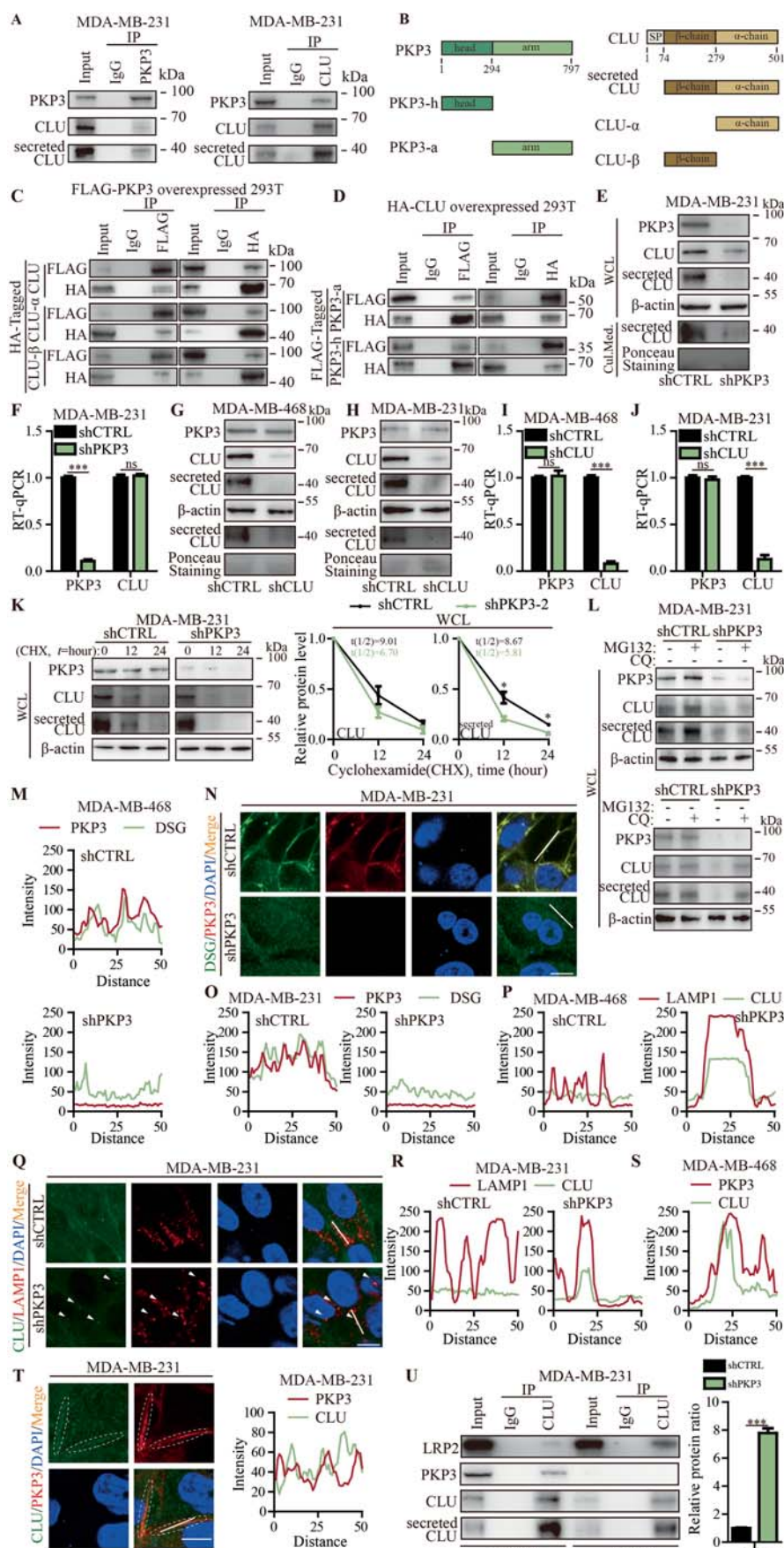


Figure EV4. CLU stabilization by PKP3 in TNBC cancer cells.

(A) Representative Western blot images showing PKP3, CLU and secreted CLU levels in Co-IP assays using TNBC MDA-MB-231 cells. (B) Schematic representation of full-length PKP3, CLU, secreted CLU and their domain truncates. (C) Representative Western blot images of Co-IP assays using 293 T cells with ectopic overexpression of FLAG-PKP3 and HA-CLU or its domain truncates (HA-CLU- α , HA-CLU- β). (D) Representative Western blot images of Co-IP assays using 293 T cells with ectopic overexpression of HA-CLU and PKP3 truncates (FLAG-PKP3-a, FLAG-PKP3-h). (E) Representative Western blot images showing PKP3, CLU and secreted CLU protein levels in MDA-MB-231 cells and secreted CLU protein levels in cell culture medium (Cul. Med.) upon PKP3 knockdown compared to controls. (F) PKP3 and CLU mRNA levels in MDA-MB-231 cells by RT-qPCR. (G–J) PKP3 and CLU mRNA and PKP3, CLU and secreted CLU protein levels in TNBC MDA-MB-468 and MDA-MB-231 cancer cells in cell culture medium (Cul. Med.) upon CLU knockdown compared to controls. (G, H) Representative Western blot images. (I, J) RT-qPCR ($n = 4$ per group). (K) Representative Western blot images and their quantification of PKP3, CLU and secreted CLU protein levels in MDA-MB-231 cells upon PKP3 knockdown compared to control after CHX treatment (shCTRL vs shPKP3-2: secreted CLU, 12-hour, $P = 0.0261$; 24-hour, $P = 0.0137$). (L) Representative Western blot images showing PKP3, CLU and secreted CLU protein levels in cancer cells upon MG132 or CQ treatment in PKP3 knockdown MDA-MB-231 cells compared to control. (M) Colocalization analysis of Fig. 3F. (N) Representative immunofluorescence images and (O) colocalization analysis showing PKP3 and DSG localization in MDA-MB-231 cells after PKP3 knockdown compared to the control. Green for DSG, red for PKP3, blue for DAPI and orange for DSG-PKP3 colocalization. Scale bar: 10 μ M. (P) Colocalization analysis of Fig. 3G. (Q) Representative immunofluorescence images and (R) colocalization analysis showing PKP3 and CLU localization in MDA-MB-231 cells. White dashed lines highlight the intercellular plaque regions, green for CLU, red for PKP3, blue for DAPI, orange for CLU-PKP3 colocalization. Scale bar: 10 μ M. (S) Colocalization analysis of Fig. 3H. (T) Representative immunofluorescence images and colocalization analysis showing CLU and LAMP1 localization in MDA-MB-231 cells upon PKP3 knockdown compared to the control. Green for CLU, red for LAMP1, blue for DAPI and orange for CLU-LAMP1 colocalization. Scale bar: 10 μ M. (U) Representative Western blot images and quantification of LRP2, PKP3, CLU and secreted CLU levels in Co-IP assays using MDA-MB-231 cells upon PKP3 knockdown compared to the control (shCTRL vs shPKP3: LRP2, $P < 0.0001$; $n = 3$ per group). The data are presented as mean $\pm$ SEM ($n = [X]$ biologically independent samples). * $p < 0.05$, *** $p < 0.001$, ns for not significant. Statistical comparisons between groups were performed using unpaired two-tailed Student's t tests. Source data are available online for this figure.

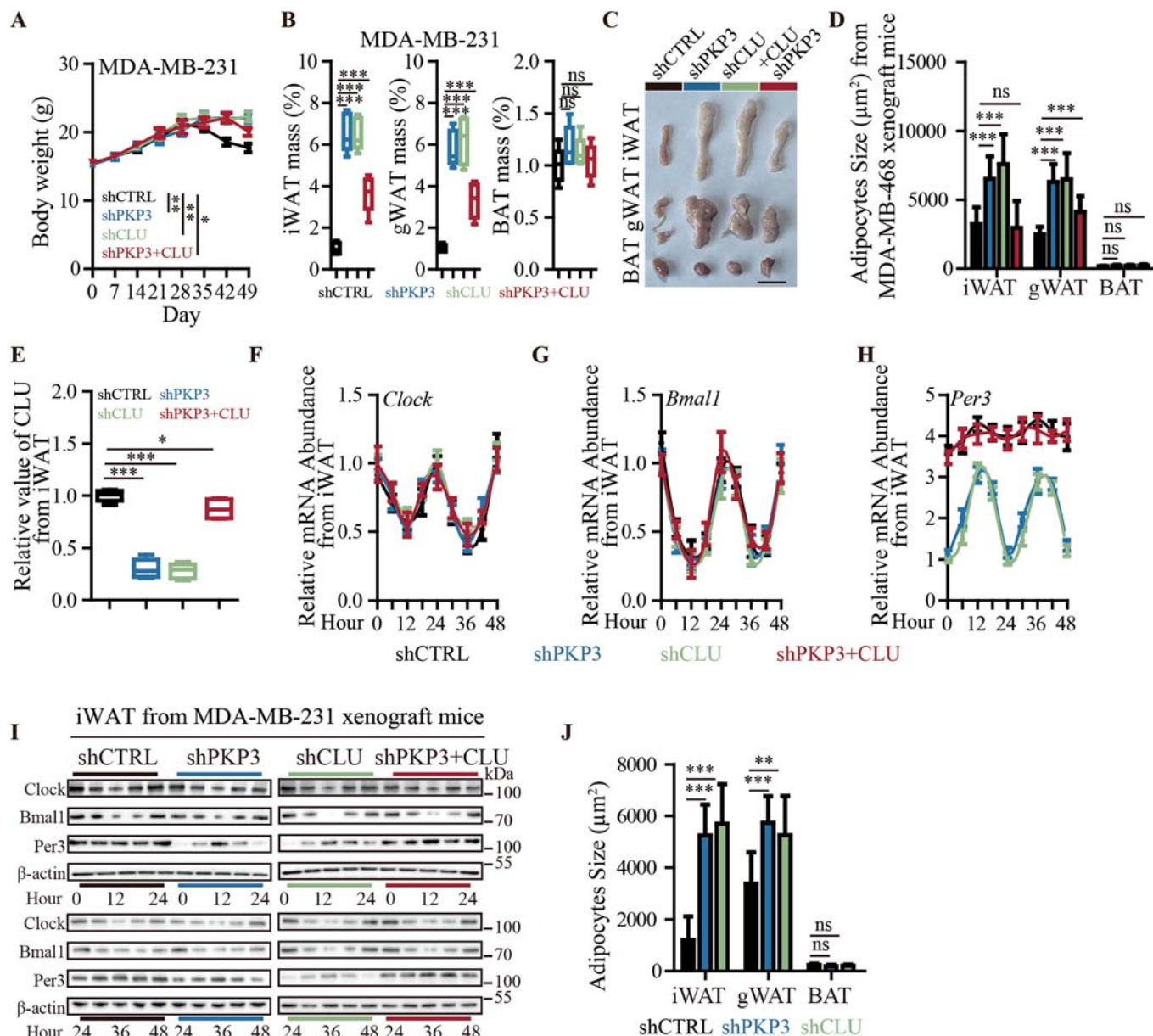


Figure EV5. Disruption of WAT's circadian rhythm by tumor PKP3-CLU axis for cachectic fat mass loss.

(A–I) MDA-MB-231 tumor-bearing mice with PKP3 knockdown, CLU knockdown, or PKP3 knockdown combined with ectopic overexpression of CLU ($n = 5$ per group): (A) Body weight (shCTRL vs shPKP3, $P = 0.0015$; shCTRL vs shCLU, $P = 0.0012$; shCTRL vs shPKP3+CLU, $P = 0.0260$; $n = 5$ per group); (B) Weight change of iWAT, gWAT, BAT (iWAT: shCTRL vs shPKP3, $P < 0.0001$; shCTRL vs shCLU, $P < 0.0001$; shCTRL vs shPKP3+CLU, $P = 0.0003$; gWAT: shCTRL vs shPKP3, $P < 0.0001$; shCTRL vs shCLU, $P < 0.0001$; shCTRL vs shPKP3+CLU, $P < 0.0001$; BAT: shCTRL vs shPKP3, $P = 0.1656$; shCTRL vs shCLU, $P = 0.1322$; shCTRL vs shPKP3+CLU, $P = 0.7618$; $n = 5$ per group), in a box-plot, the center line represents the median of the data, while the lower and upper limits of the box correspond to the first quartile and third quartile, respectively; (C) Representative iWAT, gWAT, BAT photos, Scale bar: 1 cm; (D) Statistical analysis of H&E staining of iWAT, gWAT, BAT in Fig. 4D (iWAT: shCTRL vs shPKP3, $P < 0.0001$; shCTRL vs shCLU, $P < 0.0001$; shCTRL vs shPKP3+CLU, $P = 0.7042$; gWAT: shCTRL vs shPKP3, $P < 0.0001$; shCTRL vs shCLU, $P < 0.0001$; shCTRL vs shPKP3+CLU, $P = 0.0009$; BAT: shCTRL vs shPKP3, $P = 0.2816$; shCTRL vs shCLU, $P = 0.1173$; shCTRL vs shPKP3+CLU, $P = 0.0980$; $n = 10$ per group). (E) Tumor-secreted CLU levels in iWAT by ELISA assay (shCTRL vs shPKP3, $P < 0.0001$; shCTRL vs shCLU, $P < 0.0001$; shCTRL vs shPKP3+CLU, $P = 0.0277$; $n = 5$ per group); (F–H) CLOCK, BMAL1, PER3 expression in WAT in a 48-hour cycle by (F–H) RT-qPCR ($n = 4$ per group) and (I) Western blot assay. (J) Statistical analysis of H&E staining of iWAT, gWAT, BAT in Fig. 6G (iWAT: shCTRL vs shPKP3, $P < 0.0001$; shCTRL vs shCLU, $P < 0.0001$; gWAT: shCTRL vs shPKP3, $P = 0.0002$; shCTRL vs shCLU, $P = 0.0068$; BAT: shCTRL vs shPKP3, $P = 0.0745$; shCTRL vs shCLU, $P = 0.1963$; $n = 3$ per group). The data are presented as mean $\pm$ SEM ($n = [X]$) biologically independent samples). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns for not significant. Statistical comparisons between groups were performed using unpaired two-tailed Student's *t* tests. Source data are available online for this figure.