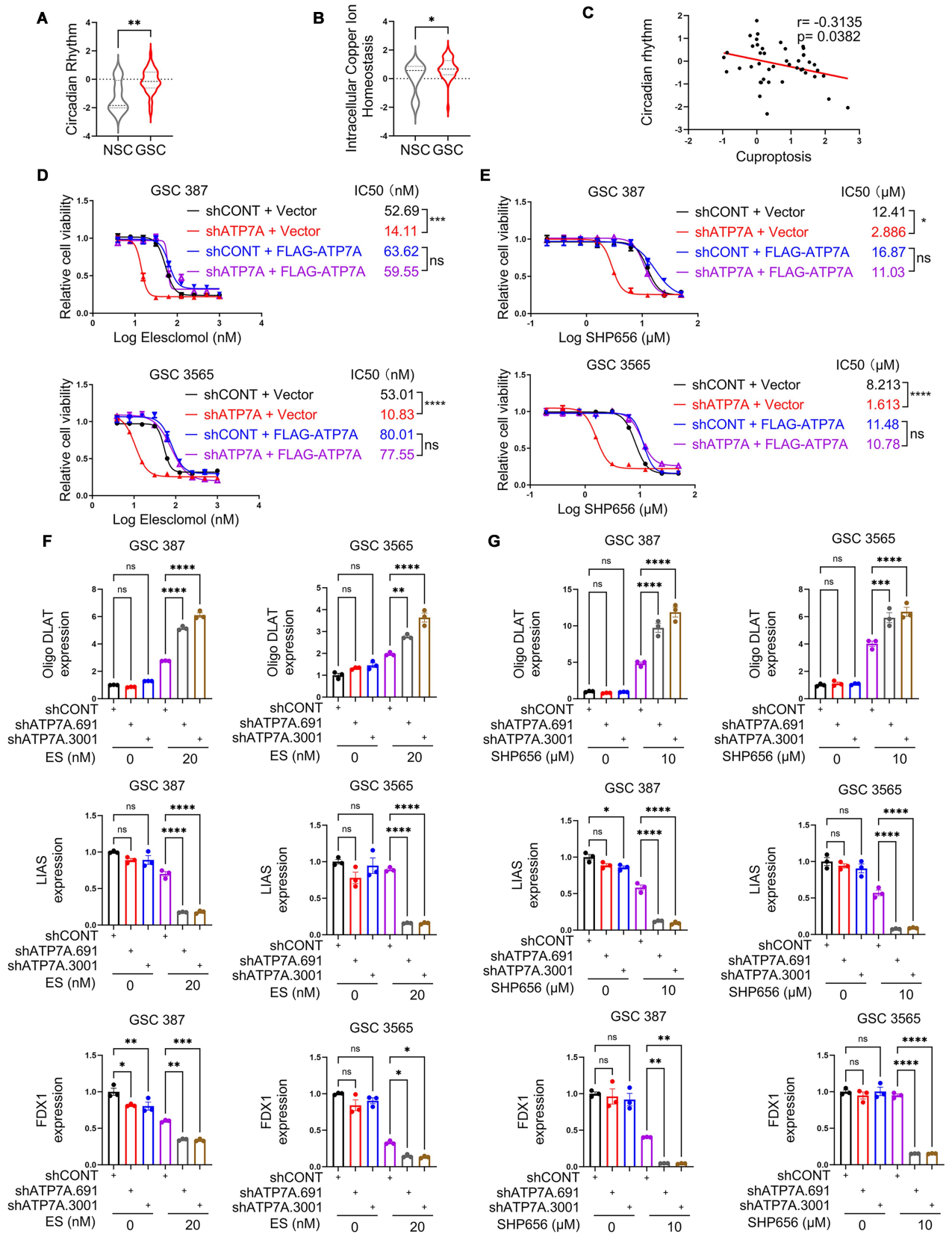


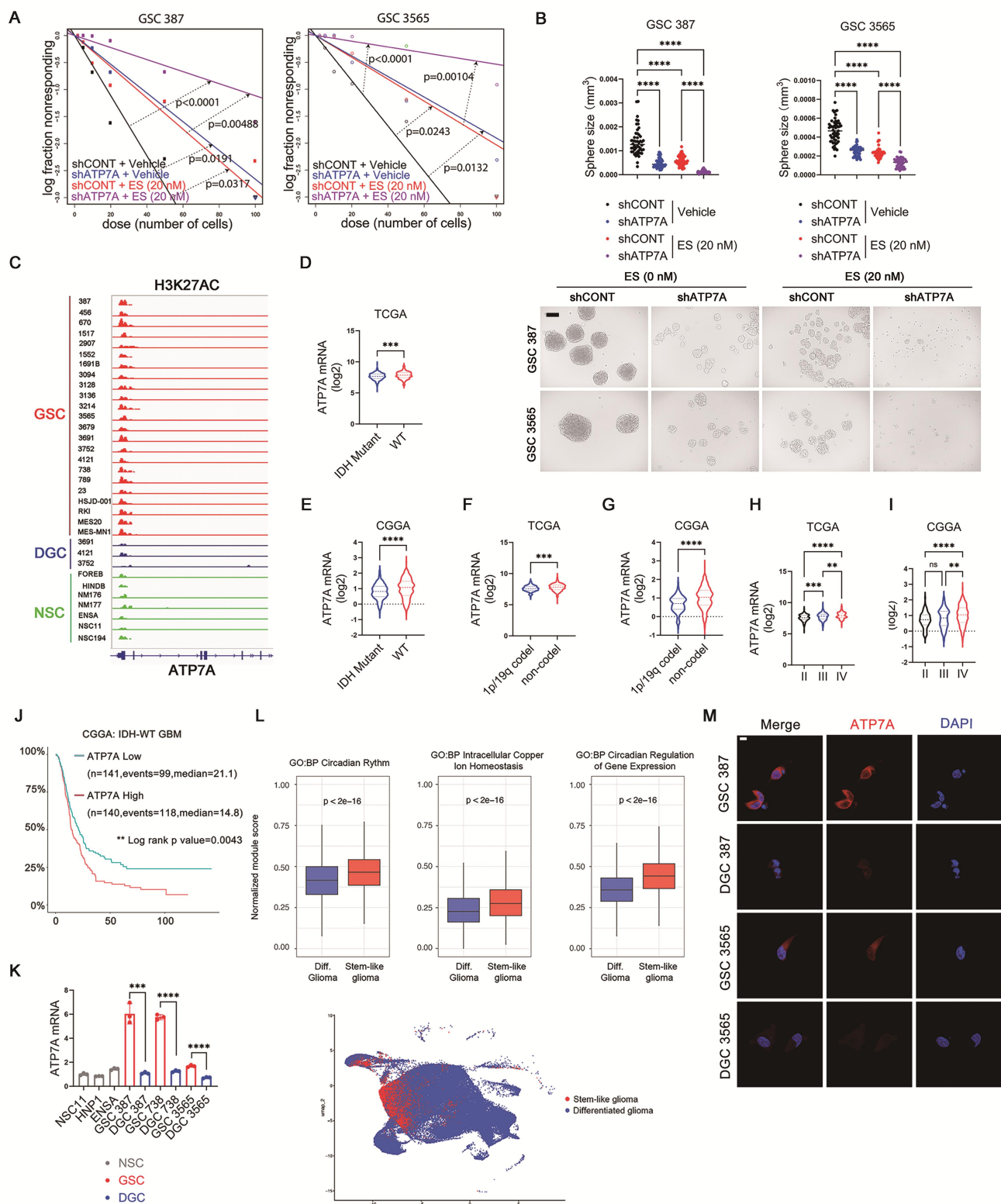
Supplemental Figure 1.

(A) Cell viability after elesclomol treatment with or without 1 μ M of the indicated metals. (B) Western blots of DLAT, LIAS, and FDX1 in GSCs treated with elesclomol $\pm$ 1 μ M CuCl₂; band intensities quantified by ImageJ and normalized to β -actin. One-way ANOVA with multiple comparisons. (C) Representative images of GSC sphere formation under indicated elesclomol concentrations (1 μ M CuCl₂). Scale bar: 100 μ m. (D) Quantification of Figure 1D; relative intensities normalized to β -actin, analyzed by one-way ANOVA. ****p<0.0001; ***p<0.001; **p<0.01; *p<0.05; ns, not significant.



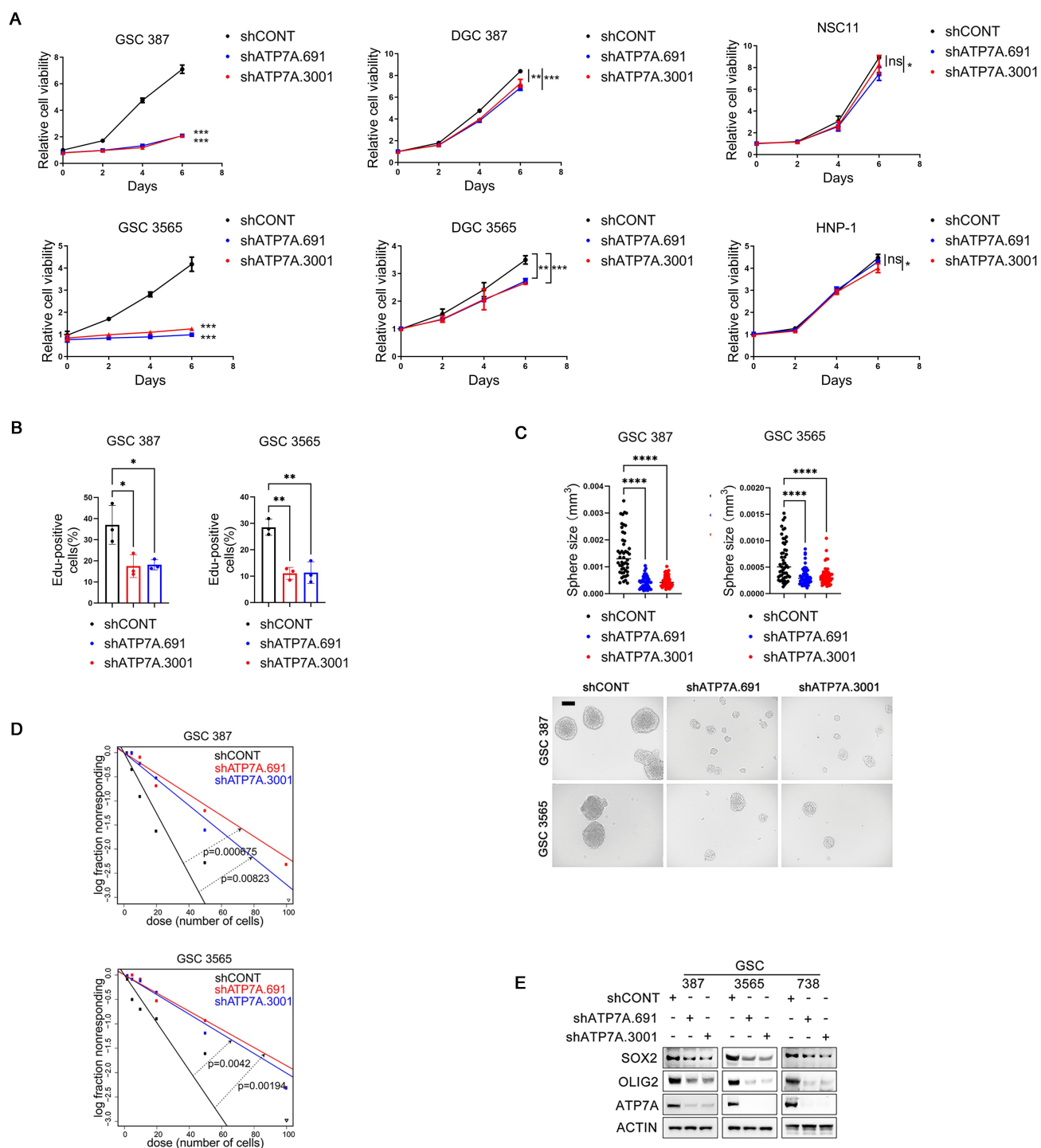
Supplemental Figure 2.

(A and B) ssGSEA of circadian rhythm (GO:0007623) and copper homeostasis (GO:0006878) gene sets in 44 GSCs and 10 NSCs (GSE119834). Unpaired t-test used for comparison. (C) Correlation between circadian rhythm and cuproptosis signatures (10 genes: FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, CDKN2A); Pearson correlation with linear regression. (D and E) IC_{50} and dose-response curves of elesclomol (ES) and SHP656 in GSC387 and GSC3565 expressing shATP7A (UTR-targeting) $\pm$ shRNA-resistant FLAG-ATP7A or vector control (1 μ M $CuCl_2$, 48 h). (F and G) Quantification of Figures 3F and G; band intensities normalized to β -actin via ImageJ. One-way ANOVA followed by multiple comparisons for D-G. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.



Supplemental Figure 3.

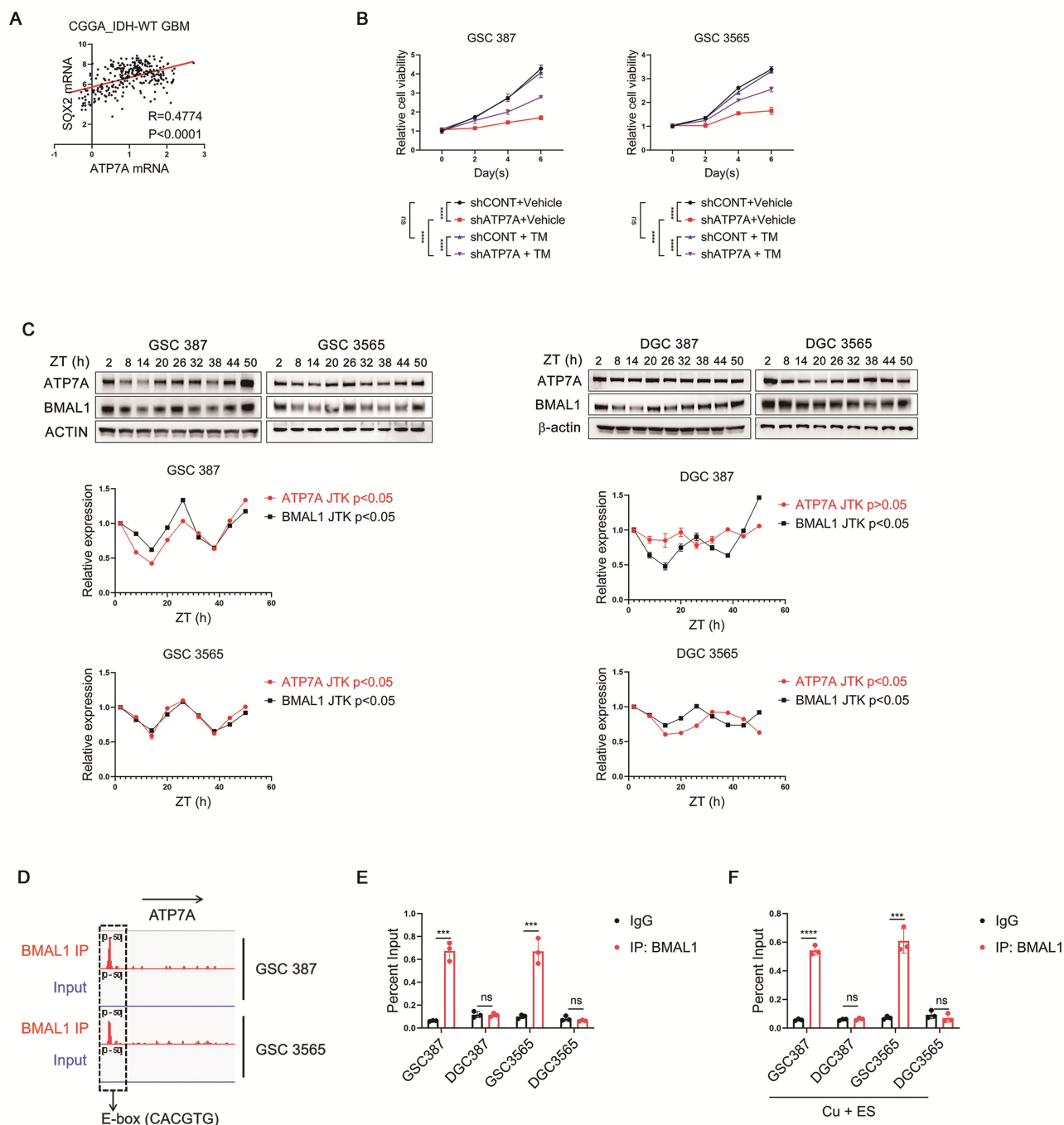
(A) Extreme limiting dilution and (B) sphere formation assays of control and ATP7A-knockdown GSCs treated with elesclomol (ES, 0–20 nM, 1 μ M CuCl₂). Representative images shown; scale bar: 100 μ m. (C) H3K27ac signal at the ATP7A locus across 23 GSCs, 3 DGCs, and 7 normal brain (NB) cells. (D–I) ATP7A mRNA expression in glioma subgroups from TCGA and CGGA: IDH status, 1p/19q codeletion, and WHO grades II–IV. (J) Kaplan–Meier survival of IDH-WT GBM patients (CGGA) stratified by median ATP7A expression. (K) qPCR of ATP7A in GSCs, DGCs, and NSCs (normalized to NSC11 = 1; mean $\pm$ SEM, n = 3). (L) Single-cell dataset (GSE174554) showing normalized module scores for circadian (GO:0007623, GO:0032922) and copper homeostasis (GO:0006878) programs in stem-like vs. differentiated glioma. (M) Immunofluorescence showing ATP7A localization in GSCs and DGCs. Scale bar: 10 μ m. Two-tailed likelihood-ratio test for A. Two-tailed unpaired t test for D–G. One-way ANOVA followed by multiple comparison for B, H, I and K. Log-rank analysis for J. ****p<0.0001; ***p<0.001; **p<0.01; *p<0.05; ns, not significant.



Supplemental Figure 4

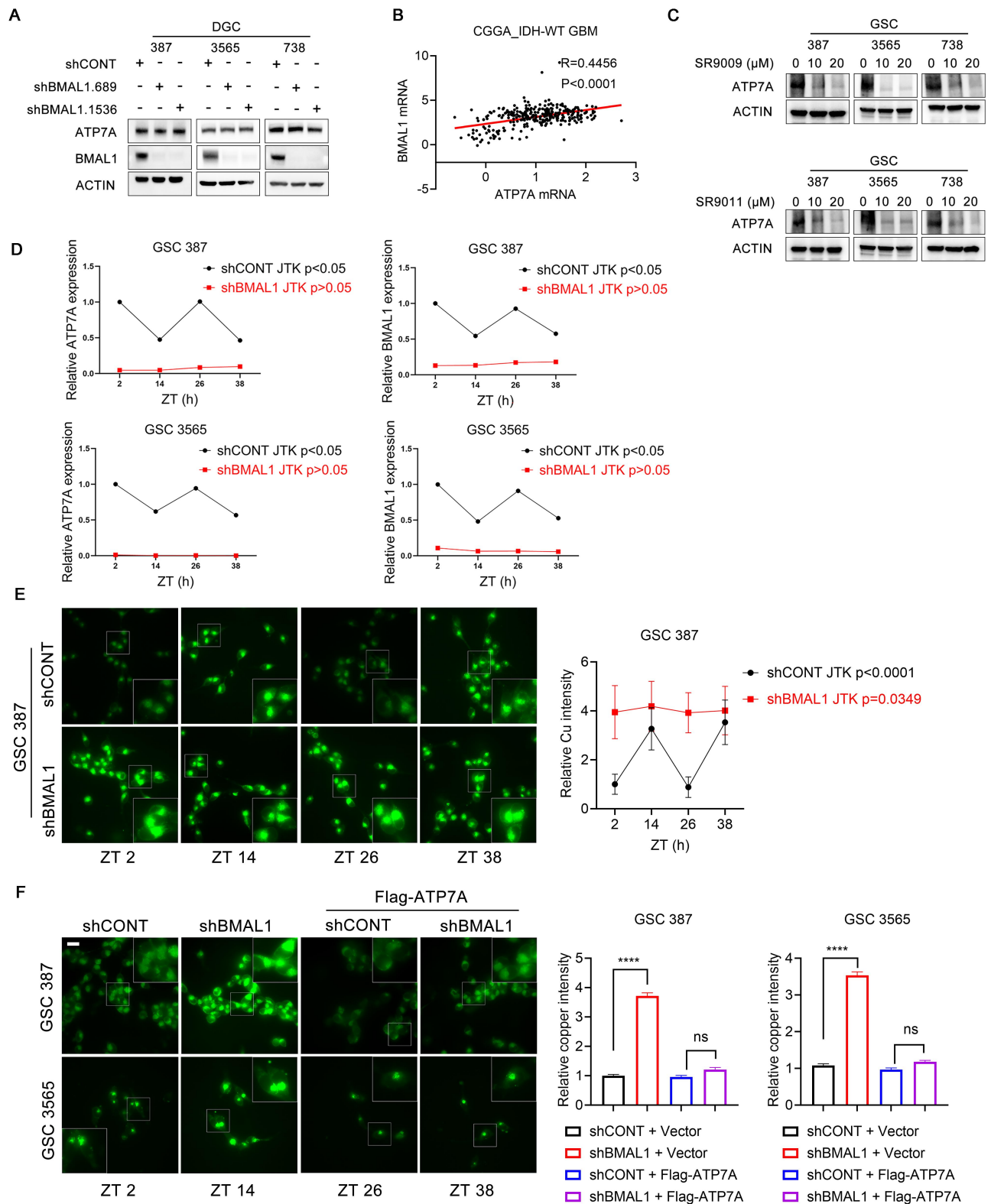
(A) CCK-8 assay measuring viability of GSCs, DGCs, and NSCs over 6 days after ATP7A knockdown (shATP7A.691, shATP7A.3001; n=3). (B) EdU incorporation in control and ATP7A-knockdown GSCs (n=3). (C) Sphere formation and (D) extreme limiting dilution assays of GSCs with control or ATP7A knockdown. Scale bar: 100 μ m. (E) Western blots showing SOX2 and OLIG2 expression after ATP7A knockdown.

One-way ANOVA followed by multiple comparisons for B and C. Two-way ANOVA followed by multiple comparisons for A. Two-tailed likelihood-ratio test for D



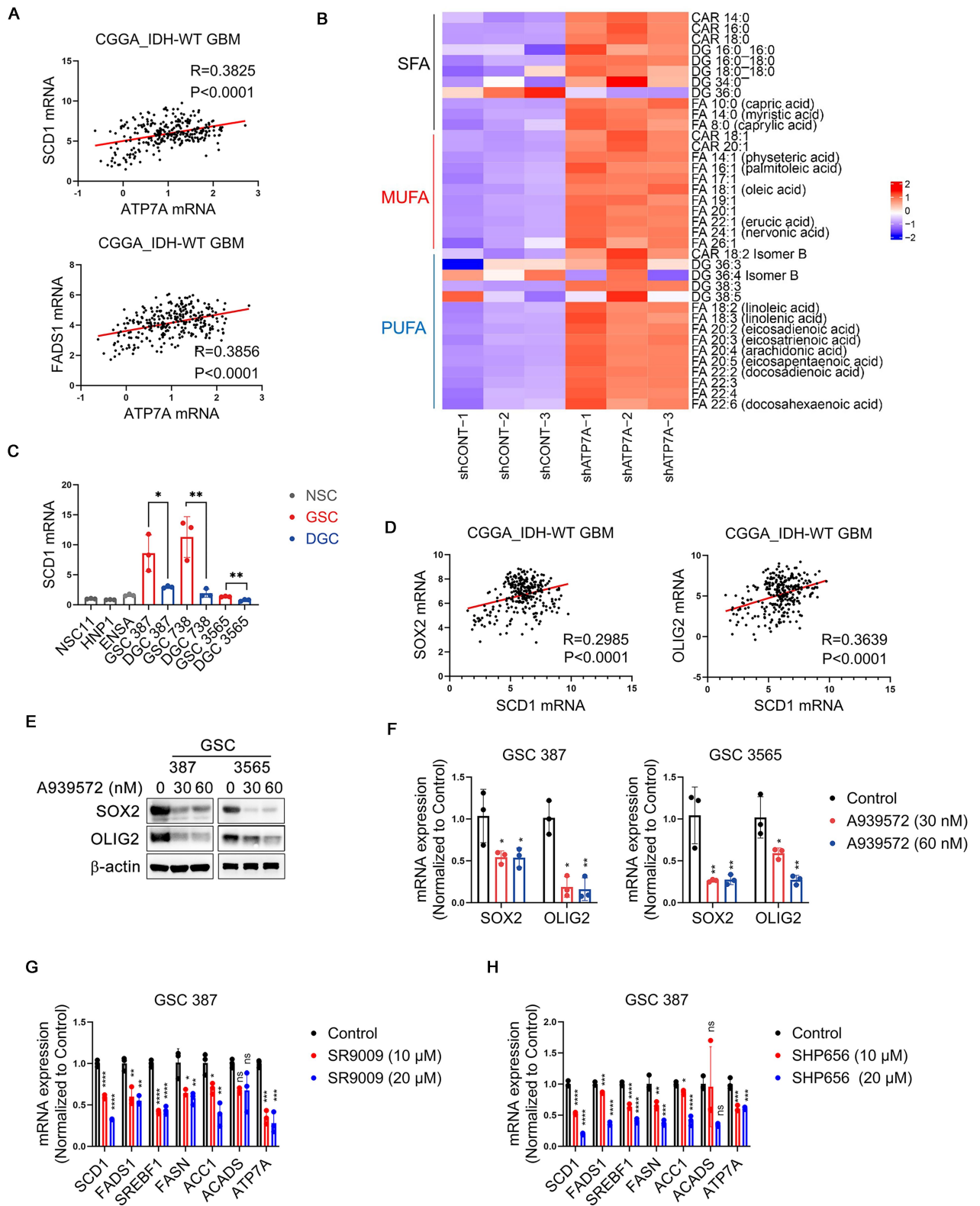
Supplemental Figure 5

(A) Pearson correlation between ATP7A and SOX2 expression in IDH-WT GBM (CGGA, n=287); red line indicates linear regression. (B) CCK-8 assay of GSC viability over 6 days after ATP7A knockdown $\pm$ TM treatment (n=3). (C) Time-course Western blots of ATP7A and BMAL1 at 6-h intervals in synchronized GSCs and DGCs (n=3). (D) UCSC genome tracks showing BMAL1 ChIP-seq enrichment at the ATP7A locus in GSCs. (E and F) BMAL1 ChIP-qPCR confirming ATP7A promoter binding in GSCs and DGCs (n=3). Rhythmicity analysis using the RTK algorithm in C. $P < 0.05$ indicates significant rhythmicity. Two-way ANOVA followed by multiple comparisons for B. T-test for E and F. Pearson correlation analysis for A. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.



Supplemental Figure 6.

(A) Western blot showing ATP7A expression in DGCs after BMAL1 knockdown (shBMAL1.689, shBMAL1.1536). (B) Correlation between ATP7A and BMAL1 mRNA expression in IDH-WT GBM (CGGA, n=287; Gliovis). (C) Western blot of ATP7A in GSCs treated with circadian inhibitors SR9009 or SR9011. (D) Quantification of Figure 4F. (E) Live-cell copper imaging of synchronized GSC387-shCONT and shBMAL1 cells at 12 h intervals (100 nM dexamethasone). Scale bar: 20 μ m. (F) Copper imaging in GSCs with control or BMAL1 knockdown $\pm$ ATP7A overexpression (Flag-ATP7A). Scale bar: 20 μ m. Pearson correlation analysis for B. Rhythmicity analysis using the RTK algorithm in D and E. $P < 0.05$ indicates significant rhythmicity. One-way ANOVA followed by multiple comparison for F.

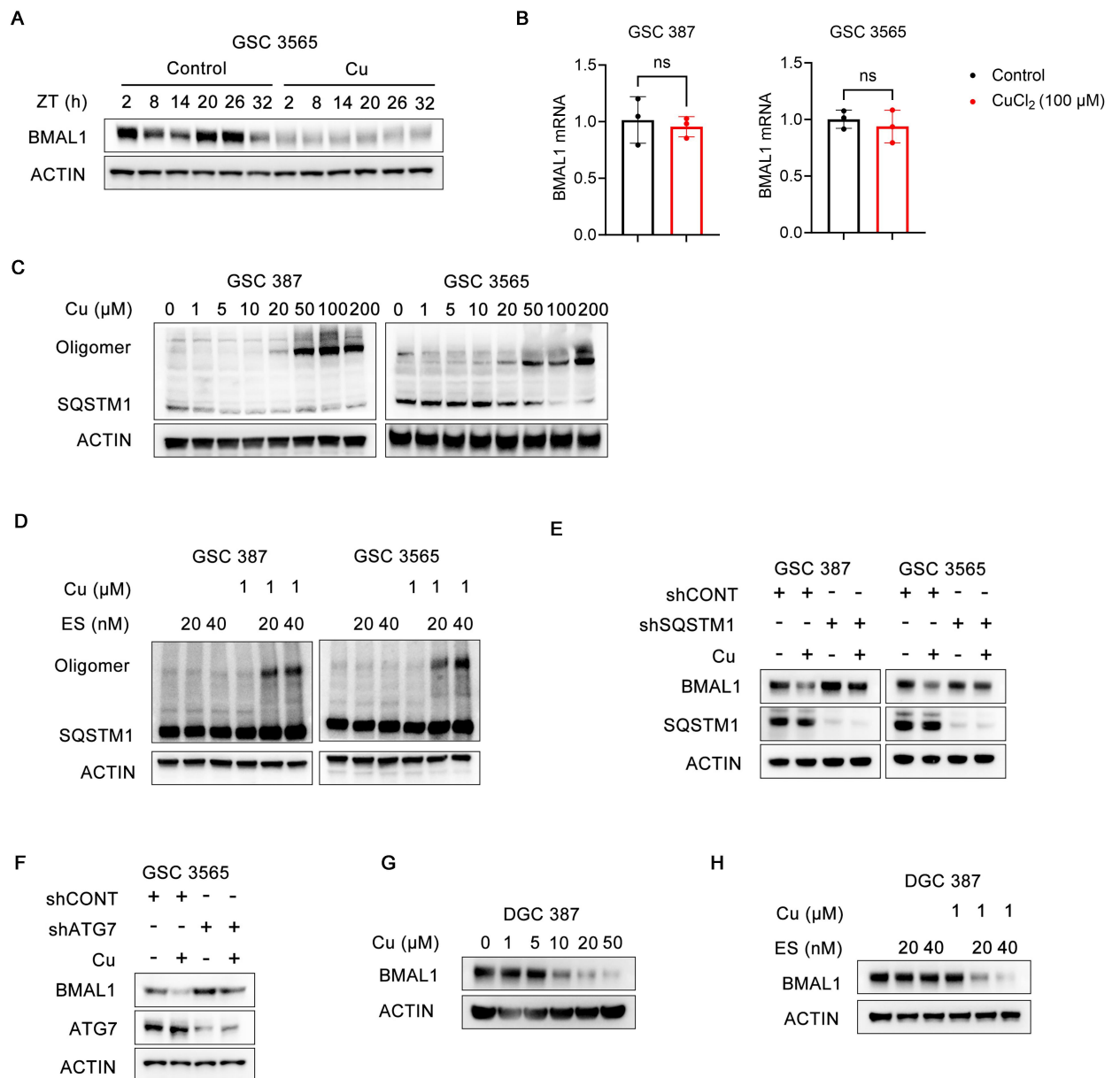


Supplemental Figure 7.

(A) Pearson correlation of ATP7A, SCD1, and FADS1 expression in IDH-WT GBM (CGGA, n=287); red lines indicate linear regression (R-values shown). (B) Lipidomic heatmap of SFA, MUFA, and PUFA in GSC3565 with control or ATP7A knockdown. (C) qPCR of SCD1 in GSCs, DGCs, and NSCs (normalized to NSC11 = 1; n=3). (D) Correlation of SCD1 with SOX2 and OLIG2 expression in IDH-WT GBM (CGGA, n=287; Gliovis). (E and F) Western blot and qPCR showing SOX2 and OLIG2 expression in GSCs treated with A939572. (G and H) qPCR of fatty acid-related genes (SCD1, FADS1, SREBF1, FASN, ACC1, ACADS) and ATP7A in GSCs treated with SR9009 (G) or SHP656 (H) (n=3).

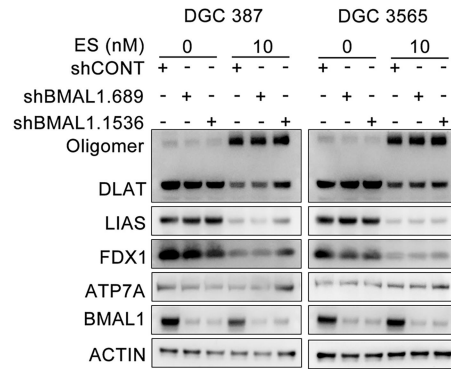
Data are mean $\pm$ SEM. One-way ANOVA for C, F–H; Pearson correlation for A, D.

****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns not significant.

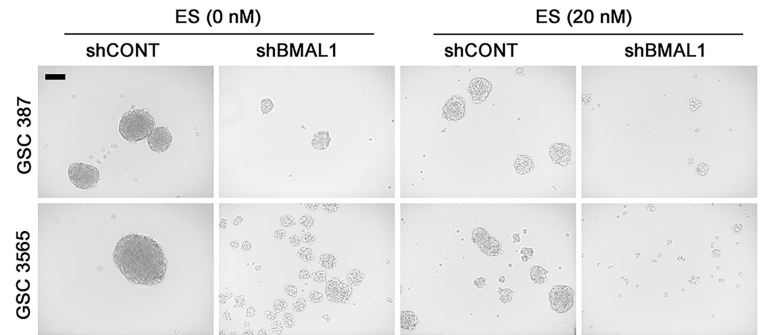


Supplemental Figure 8. (A) Western blot of BMAL1 at 6-h intervals in synchronized GSCs $\pm$ 100 μ M CuCl₂. (B) qPCR of GSCs treated with 100 μ M CuCl₂ for 48 h. (C–D) Western blots showing SQSTM1 and its oligomers in GSCs treated with CuCl₂ or elesclomol $\pm$ 1 μ M CuCl₂. (E and F) Western blots of GSCs with SQSTM1 (shP62) or ATG7 knockdown $\pm$ 100 μ M CuCl₂. (G and H) BMAL1 expression in DGCs treated with CuCl₂ or elesclomol $\pm$ 1 μ M CuCl₂. Data are mean $\pm$ SEM. T test was conducted for B. ns not significant.

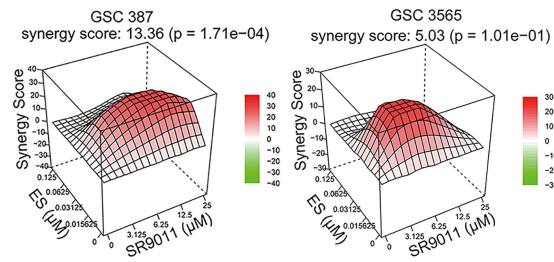
A



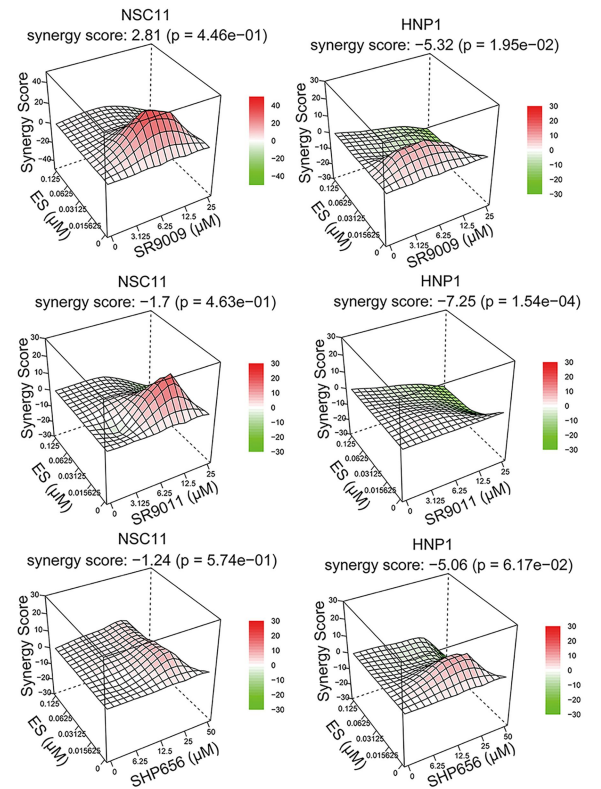
B



C

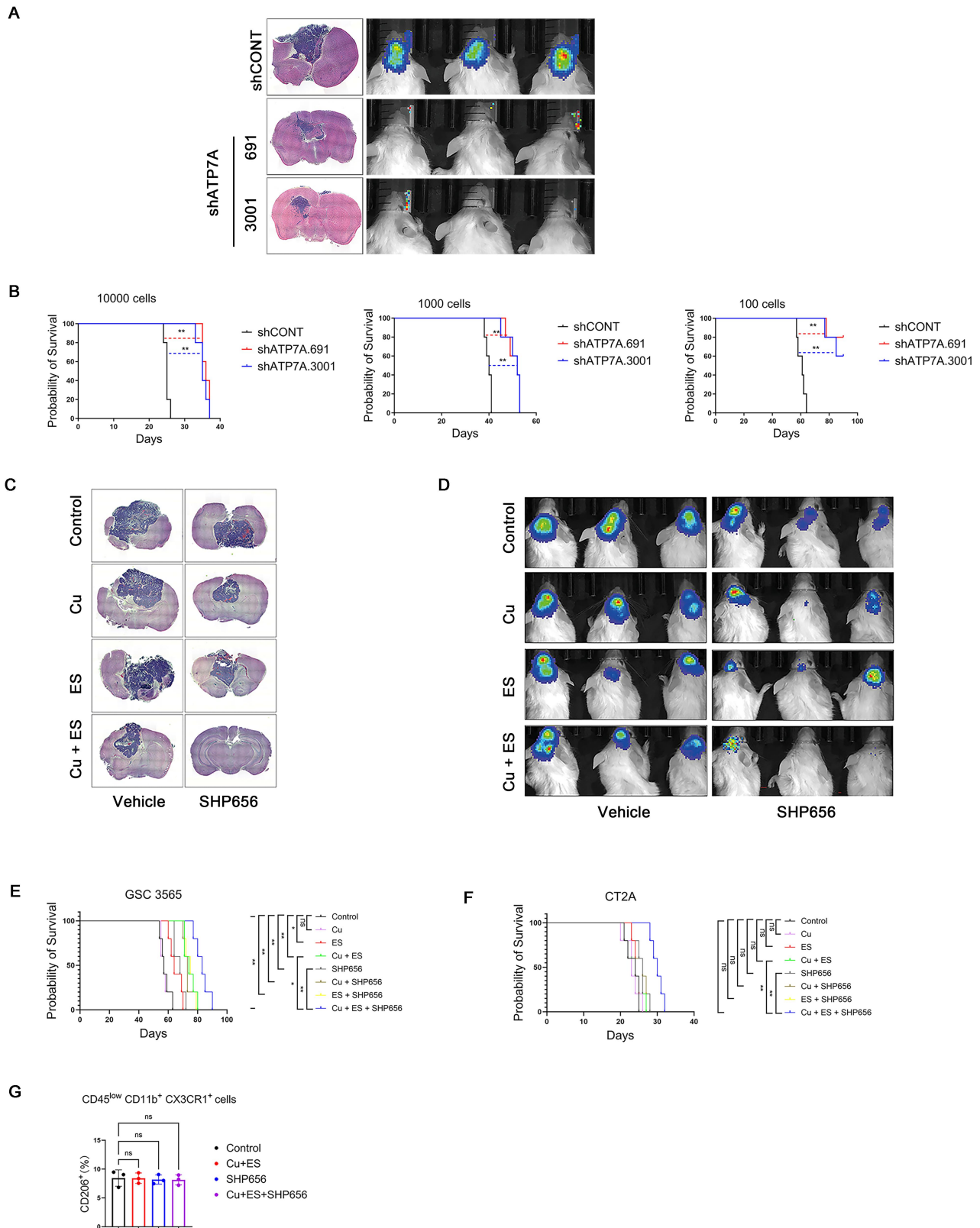


D



Supplemental Figure 9.

(A) Immunoblots showing ES (20 nM, 48 h)–induced cuproptosis in DGCs with control or BMAL1 knockdown (shBMAL1.689, shBMAL1.1536; 1 μ M CuCl₂). (B) Representative sphere formation images from Figure 7D. Scale bar: 100 μ m. (C and D) SynergyFinder plots showing combined effects of ES with SR9011 in GSCs (C) and with SHP656, SR9009, or SR9011 in NSCs (D).



Supplemental Figure 10.

(A) Bioluminescence imaging and total flux quantification (n=5) of mice bearing GSC3565 tumors $\pm$ ATP7A knockdown. Representative H&E-stained brain sections shown; scale bar: 1 mm. (B) Kaplan–Meier survival of mice implanted with 10^4 , 10^3 , or 10^2 GSC387 cells expressing control or ATP7A shRNAs (n=5/group). (C and D) Intracranial GSC387 tumors treated with copper gluconate, elesclomol, SHP656, or combination; H&E staining (C) and bioluminescence imaging (D). (E) Kaplan–Meier survival of GSC3565 tumor–bearing mice receiving indicated treatments (n=5/group). (F) Kaplan–Meier survival of CT2A-bearing immunocompetent mice treated as above (n=5/group). (G) Flow cytometry of CD206⁺ cells among CD45^{low}CD11b⁺CX3CR⁺ populations. Log-rank test was performed for B, E, and F. One-way ANOVA for G. **p<0.01; *p<0.05; ns, not significant.

SUPPLEMENTAL TABLES

Supplemental Table 1. sgRNA in crispr library

Supplemental Table 2. sgRNA counts

Supplemental Table 3. MAGECK test

Supplemental Table 4. Lipdomic data

Supplemental Table 5. Peptides in MS

Supplemental Table 6. MS for BMAL1-binding proteins

Supplemental Table 7. GOBP:SELECTIVE_AUTOPHAGY, GO0061912

Supplemental Table 8. Primers

Supplemental Table 9. Reagents and antibodies