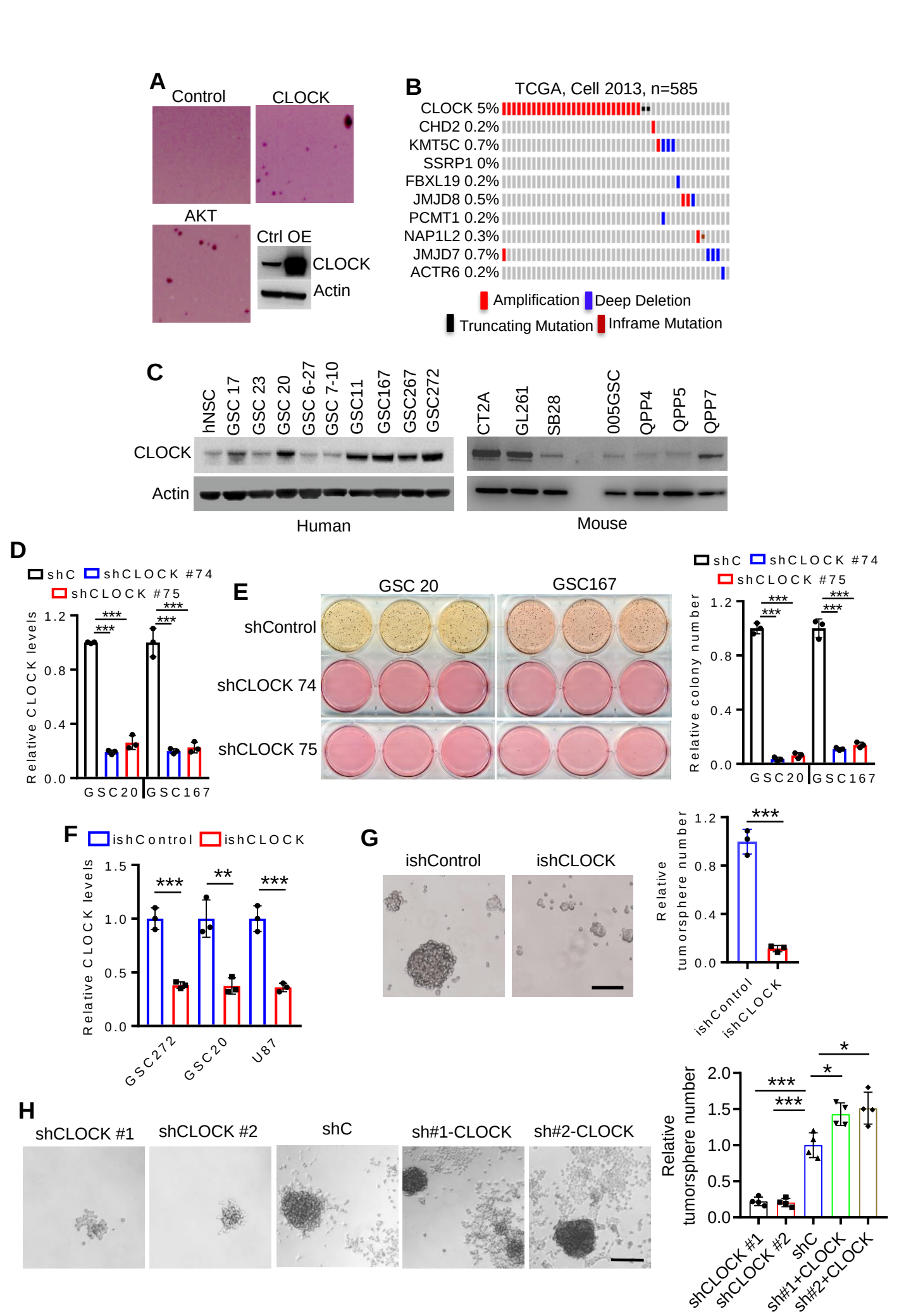
A
Control
CLOCK
AKT
Ctrl OE
CLOCK
Actin
B
TCGA, Cell 2013, n=585
CLOCK 5%
CHD2 0.2%
KMT5C 0.7%
SSRP1 0%
FBXL19 0.2%
JMJD8 0.5%
PCMT1 0.2%
NAP1L2 0.3%
JMJD7 0.7%
ACTR6 0.2%
Amplification
Deep Deletion
Truncating Mutation
Inframe Mutation
C
hNSC
GSC 17
GSC 23
GSC 20
GSC 6-27
GSC 7-10
GSC11
GSC167
GSC267
GSC272
CT2A
GL261
SB28
005GSC
QPP4
QPP5
QPP7
CLOCK
Actin
Human
Mouse
D
shC
shCLOCK #74
shCLOCK #75
Relative CLOCK levels
GSC20
GSC167
E
GSC 20
GSC167
shControl
shCLOCK 74
shCLOCK 75
shC
shCLOCK #74
shCLOCK #75
Relative colony number
GSC20
GSC167
F
ishControl
ishCLOCK
Relative CLOCK levels
GSC272
GSC20
U87
G
ishControl
ishCLOCK
Relative tumorsphere number
ishControl
ishCLOCK
H
shCLOCK #1
shCLOCK #2
shC
sh#1-CLOCK
sh#2-CLOCK
Relative tumorsphere number
shCLOCK #1
shCLOCK #2
shC
sh#1+CLOCK
sh#2+CLOCK

Figure S1. CLOCK/BMAL1 is essential for GSC self-renewal, but not required for glioma cell proliferation.

(A) Representative images of soft agar colony formation of p53DN-hNSCs overexpressing CLOCK, AKT (positive control). Immunoblotting shows CLOCK overexpression (OE) efficiency in hNSCs. Ctrl, Control.

(B) Genomic alterations of *CLOCK*, *CHD2*, *KMT5C*, *SSRP1*, *FBXL19*, *JMJD8*, *PCMT1*, *NAP1L2*, *JMJD7* and *ACTR6* in TCGA GBM database (*Cell*, 2013 dataset; n = 585).

(C) Immunoblots for CLOCK in cell lysates of human GSCs (p53DN-hNSCs, GSC17, GSC23, GSC20, GSC6-27, GSC7-10, GSC11, GSC167, GSC267 and GSC272), mouse GSCs (005 GSC, QPP4, QPP5 and QPP7) and mouse glioma cell lines (CT2A, GL261 and SB28). GSC20, GSC167, GSC272, CT2A and QPP7 cell lines were identified as having high expression of CLOCK.

(D) qRT-PCR shows CLOCK shRNA knockdown efficiency in GSC20 and GSC167. n = 3 biological replicates; ***P <0.001.

(E) Representative images (*left panel*) and quantification (*right panel*) of soft agar colony formation assays in GSC20 and GSC167 expressing *CLOCK* shRNAs or control. n = 4 biological replicates; ***P <0.001.

(F) qRT-PCR shows CLOCK inducible shRNA knockdown efficiency in GSC272, GSC20 and U87. n = 3 biological replicates; ***P <0.001.

(G) Conditional depletion of CLOCK suppresses GSC20 tumorsphere formation. Representative images (*left panel*) and quantification (*right panel*). n = 3 biological replicates; ***P <0.001.

(H) Depletion of CLOCK suppresses QPP7 tumorsphere formation, which is rescued by re-expression of CLOCK. Representative images (*left panel*) and quantification (*right panel*). n = 4 biological replicates; *P <0.05 and ***P <0.001.