

1	Supplemental	Figure	1.	Cell	cycle	genes	expression	correlates	with	glioma
2	progression									

A. mRNA expression of cell cycle genes PCNA, CCNB1, CDK1 and CCNA2 in

- 4 oligodendroglioma, oligoastrocytoma, astrocytoma and glioblastoma patients.
- 5 B. Correlation between HELLS and cycling gene MKi67, PCNA, MCM2, CCNB1,
- 6 CDK1 in glioblastoma patients.
- 7 C. H3K27Ac signals at HELLS promoters in GSC models 3691, 3752, 387 and
- 8 corresponding DGC models.
- 9 **D, E.** Relative mRNA expression of HELLS (**D**) and CDCA7 (**E**) in different GSCs and
- 10 corresponding DGCs. Data are presented as Mean ± SD. \*\**P* < 0.01, \*\*\**P*< 0.001 and
- 11 N.S. means no significance, by t-test. Three independent experiments were performed.
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# 13 Supplemental Figure 2. HELLS knockdown in DGCs and NMs

14	A, B. mRNA and protein levels of HELLS in NM 177 (A) and 263 (B) after HELLS
15	knockdown. Data are presented as Mean ± SD. *P <0.05, **P <0.01 and ***P <0.001,
16	by one-way ANOVA with Tukey's multiple comparisons test. Three independent
17	experiments were performed.
18	C, D. CellTiter-Glo assays of NM 177 (C) and NM 263 (D) transduced with shHELLS
19	or shCONT. Data are presented as Mean ± SD. **P <0.01 and ***P <0.001, by two-
20	way ANOVA with Tukey's multiple comparisons test. Three independent experiments
21	were performed. E, F. mRNA and protein levels of HELLS in DGC 387 (E) and 3565
22	(F) after HELLS knockdown. Data are presented as Mean ± SD. ***P<0.01, by one-
23	way ANOVA with Tukey's multiple comparisons test. Three independent experiments
24	were performed.
25	G, H. CellTiter-Glo assays of DGC 387 (G) and 3565 (H) transduced with shHELLS or
26	shCONT. Data are presented as Mean ± SD. ***P< 0.001, by two-way ANOVA with
27	Tukey's multiple comparisons test. Three independent experiments were performed.

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## 30 Supplemental Figure 3. HELLS binds to cycling gene promoters and correlates

#### 31 with their expression

- A. Correlation between SWI/SNF family members and cycling gene expression in
   glioma patients.
- **B**, **C**. ChIP-qPCR analysis of HELLS binding at cycling gene promoters in GSC models

35 387 (B) and 3565 (C). Data are presented as Mean ± SD. Three independent
 acceleration experiments were performed.

- 37 D, E. Cycling gene expression in GSC models 387 (D) and 3565 (E) after HELLS
- knockdown. Data are presented as Mean  $\pm$  SD. \*P <0.05, \*\*P <0.01 and \*\*\*P <0.001,

by two-way ANOVA with Tukey's multiple comparisons test. Three independent
experiments were performed. .

- 41 **F**. Flow cytometry for GSC models 387 and 3565 stained with  $\gamma$ H2AX antibody after
- 42 HELLS knockdown. Y-axis was gated by SSC and x-axis was gated by  $\gamma$ H2AX signals.
- 43 Three independent experiments were performed.
- 44

# Guoxin Zhang

# Supplemental Figure 4

Ranked in Ordered Dataset



2000 3000 4000 5000 6000 7000 Ranked in Ordered Dataset

- 45 Supplemental Figure 4. HELLS is correlated with expression of E2F3 targets
- 46 and MYC targets in glioblastoma patients
- 47 **A**. Heatmap about gene expression profiles from glioblastoma patients based on
- 48 HELLS expression.
- 49 **B**. GSEA (GO) of the differentially expressed genes between HELLS high patients and
- 50 HELLS low patients.
- 51 **C-E**. Plots of the enriched gene sets in GO analysis (**B**).
- 52 F. GSEA (HALLMARK) of the differentially expressed genes between HELLS high
- 53 patients and HELLS low patients.
- 54 **G-I**. Plots of the enriched gene sets in HALLMARK analysis (**E**).



56	Supplemental Figure 5. Analysis of the glioblastoma patient data from TCGA
57	A. Venn diagram of up-regulated genes (fold change>0.5) in HELLS <sup>high</sup> glioblastoma
58	patients, E2F3 targets and MYC targets. Gene set of KONG_E2F3_TARGETS was
59	used as E2F3 targets and HALLMARK_MYC_TARGETS_V1 was used as MYC
60	targets.
61	B. Correlations between HELLS and E2F3 targets or MYC targets in glioblastoma
62	patients.

# Supplemental Figure 6

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#### 64 Supplemental Figure 6. Protein level of E2F3 and binding of E2F3 to its targets

#### 65 in GSCs after HELLS knockdown

A, B. E2F3 protein expression in GSC models 387 and 3565 36 hours (A) or 48 hours
 (B) after HELLS knockdown. ACTIN was used as input. Three independent
 experiments were performed.

69 **C**, **D**. ChIP-qPCR analysis of FLAG-E2F3a (**C**) or FLAG-E2F3b (**D**) binding at the E2F3

target gene promoters after HELLS knockdown. Data are presented as Mean ± SD.

71 Three independent experiments were performed.

72 E. qPCR analysis of E2F3 target genes expression after HELLS knockdown in GSC

73 387- and 3565. Data are presented as Mean ± SD. \*\**P* <0.01 and \*\*\**P* <0.001, by two-

vay ANOVA with Tukey's multiple comparisons test. Three independent experiments

vere performed.

**F**. mRNA expression and protein expression of E2F3 in GSC 387 (left) and 3565 (right) after E2F3 knockdown. Data are presented as Mean  $\pm$  SD. \*\*\**P* <0.001, by one-way ANOVA with Tukey's multiple comparisons test. Three independent experiments were performed.

6. CellTiter-Glo assay with GSC 387 (left) and GSC 3565 (right) after E2F3 knockdown. Data are presented as Mean  $\pm$  SD. \*\*\**P* <0.001, by two-way ANOVA with Tukey's multiple comparisons test. –Three independent experiments were performed.

H. Rescue assays of HELLS knockdown in GSC models 387 (left) and 3565 (right)
with E2F3 overexpression. Data are presented as Mean ± SD. \*\**P* < 0.01 and \*\*\**P* <</li>

- 85 0.001, by two-way ANOVA with Tukey's multiple comparisons test. Three independent
- 86 experiments were performed.



## 88 Supplemental Figure 7. E2F3 is required for GSC maintenance

- A. Immunoblot of MYC in GSC 387 and 3565 after HELLS knockdown. ACTIN was
   used as input. Three independent experiments were performed.
- **B**. qPCR analysis of MYC target genes expression after HELLS knockdown in GSC
- models 387 (upper) and 3565 (bottom). Data are presented as Mean  $\pm$  SD. \**P* <0.05
- and \*\*\**P* <0.001, by two-way ANOVA with Tukey's multiple comparisons test. Three
- 94 independent experiments were performed.
- 95 C. Immunoblot of MYC in GSC 387 and 3565 overexpressing GFP or MYC. Three
- <sup>96</sup> independent experiments were performed. ACTIN was used as input.
- 97 **D.** Rescue assays of HELLS knockdown in GSC models 387 (left) and 3565 (right) 98 with MYC overexpression. Data are presented as Mean  $\pm$  SD. \*\*\**P* <0.001, by two-
- 99 way ANOVA with Tukey's multiple comparisons test. Three independent experiments
- 100 were performed.
- 101



### **Supplemental Figure 8. HELLS and Cell cycle signature inform prognosis**

- A. The index of cell cycle signature in tumor and non-tumor tissues of glioma patients
   in TCGA datasets. Data are presented as Mean ± SD. Significance was tested by t test.
- **B**. The index of cell cycle signature in different grades of gliomas in TCGA datasets.
- 107 Data are presented as Mean ± SD. Statistical significance was examined by one-way
- 108 ANOVA with Tukey's comparison test.

C. Kaplan-Meier plot of glioma patient survival based on the index of cell cycle
 signature in TCGA datasets. Statistical significance was determined by Mantel-Cox
 log-rank test.

D. Kaplan-Meier plot of glioblastoma patient survival based on the index of cell cycle
 signature in TCGA datasets. Statistical significance was determined by Mantel-Cox
 log-rank test.

E. mRNA expression of HELLS in glioblastoma tissues and non-tumor brain tissues in
 TCGA datasets. Data are presented as Mean ± SD. Statistical significance was
 examined by t-test.

**F**. Kaplan-Meier plot of glioma patient survival based on the HELLS expression in
 datasets of REMBRANDT. Statistical significance was determined by Mantel-Cox log rank test.

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### 122 Supplemental Figure 9. HELLS correlates with E2F3 or MYC expression and

### 123 inform prognosis in other cancers

- 124 A-F. Correlation between HELLS and E2F3 (A-C) or HELLS and MYC (D-F) in TCGA
- dataset. COADREAD, colon adenocarcinoma (COAD) and rectum adenocarcinoma
- 126 (READ); KIPAN, pan-kidney cohort (kidney chromophobe + KIRC + kidney renal
- 127 papillary cell carcinoma); LUAD, lung adenocarcinoma.
- 128 G-L. Kaplan-Meier plots of patients with higher or lower HELLS expression in breast
- 129 cancer (G), lung cancer (H), gastric cancer (I), liver cancer (J), sarcoma (K) and kidney
- 130 renal papillary cell carcinoma (L).