STAT3 potentiates RNA polymerase I-directed transcription and tumor growth by activating RPA34 expression

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Fig. S1

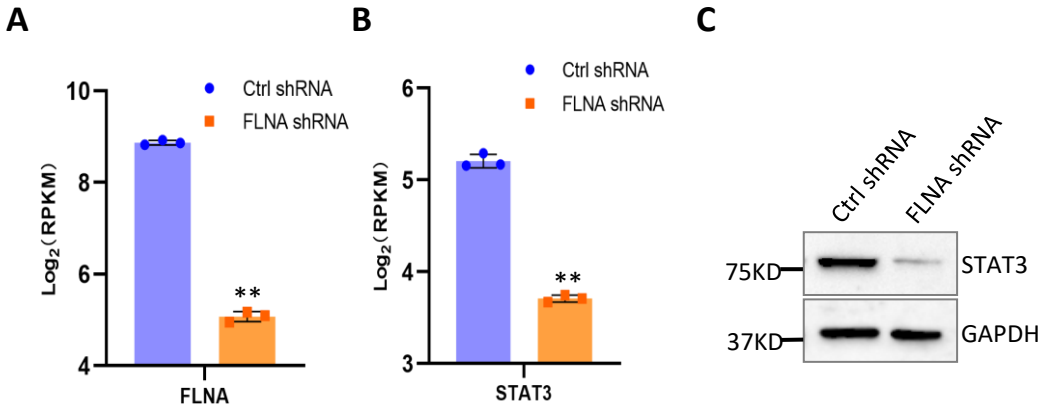
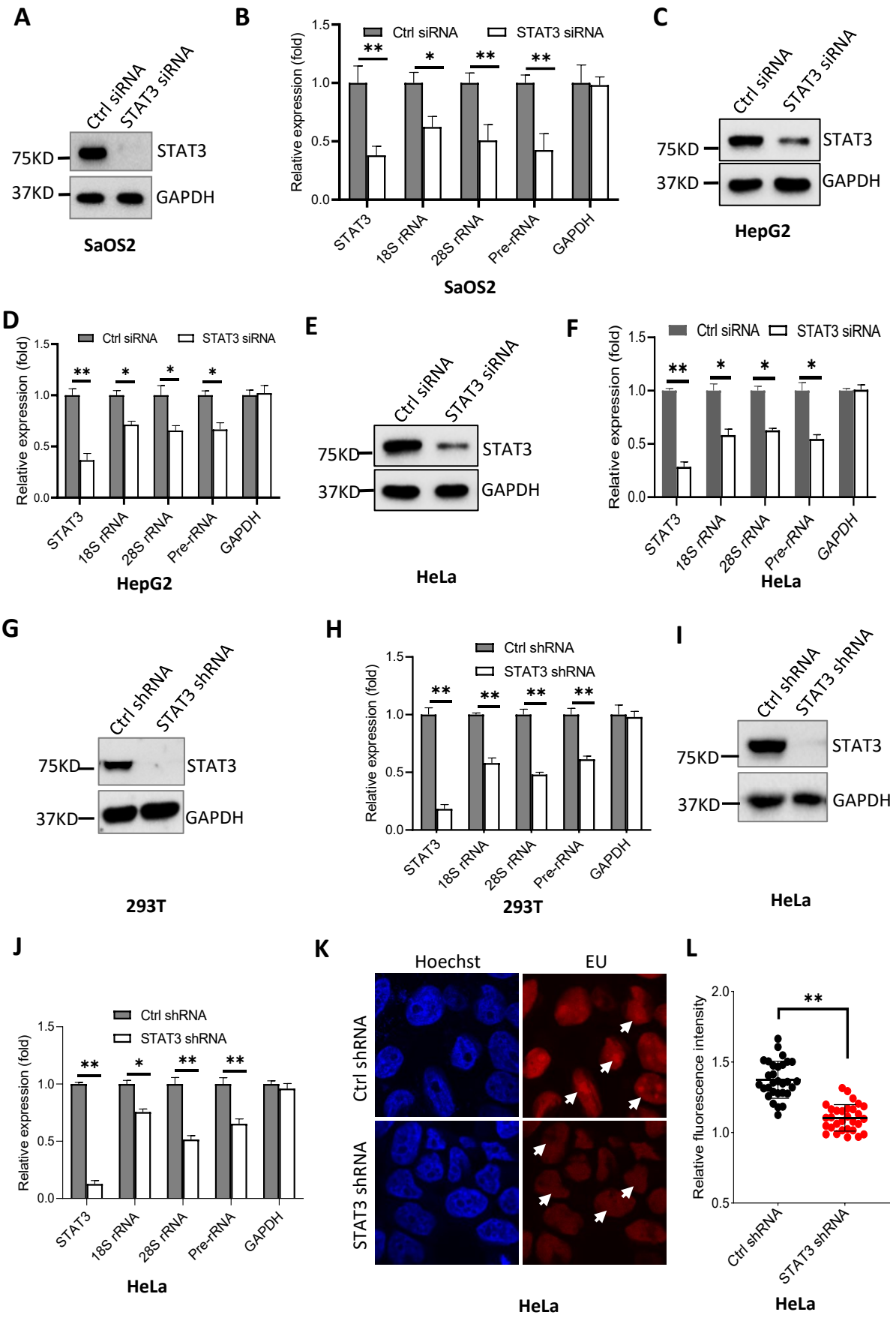


Fig. S1 Filamin A silencing reduced STAT3 expression. **A**, Analysis of FLNA mRNA reads using the RNA-seq data of SaOS2 cells stably expressing FLNA shRNA or control shRNA. **B**, Analysis of STAT3 mRNA reads using the RNA-seq data of SaOS2 cells stably expressing FLNA shRNA or control shRNA. **C**, STAT3 protein was detected by Western blot using SaOS2 cells stably expressing FLNA shRNA or control shRNA. Each column in the histograms represents the mean ± SD of three biological replicates , **, $P<0.01$. *P* values were obtained by Student's *t* test performed with control and treatment groups

Fig. S2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STAT3 siRNA
75KD
STAT3
37KD
GAPDH
SaOS2
B
Ctrl siRNA
STAT3 siRNA
Relative expression (fold)
STAT3
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
SaOS2
C
Ctrl siRNA
STAT3 siRNA
75KD
STAT3
37KD
GAPDH
HepG2
D
Ctrl siRNA
STAT3 siRNA
Relative expression (fold)
STAT3
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
HepG2
E
Ctrl siRNA
STAT3 siRNA
75KD
STAT3
37KD
GAPDH
HeLa
F
Ctrl siRNA
STAT3 siRNA
Relative expression (fold)
STAT3
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
HeLa
G
Ctrl shRNA
STAT3 shRNA
75KD
STAT3
37KD
GAPDH
293T
H
Ctrl shRNA
STAT3 shRNA
Relative expression (fold)
STAT3
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
293T
I
Ctrl shRNA
STAT3 shRNA
75KD
STAT3
37KD
GAPDH
HeLa
J
Ctrl shRNA
STAT3 shRNA
Relative expression (fold)
STAT3
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
HeLa
K
Hoechst
EU
Ctrl shRNA
STAT3 shRNA
HeLa
L
Relative fluorescence intensity
Ctrl shRNA
STAT3 shRNA
HeLa

Fig. S2 STAT3 knockdown reduced the synthesis of Pol I products. **A** and **B,** STAT3 siRNA transfection reduced the expression of Pol I products. SaOS2 cells were transfected with STAT3 siRNA; after 48h, cells were harvested and STAT3 expression was analyzed by Western blot (A), where Pol I products was detected by RT-qPCR (B). **C** and **D,** STAT3 siRNA transfection decreased the expression of Pol I products in HepG2 cells. STAT3 siRNA transfection was performed with HepG2; STAT3 (C) and Pol I products (D) were analyzed by Western blot and RT-qPCR, respectively. **E** and **F**, STAT3 siRNA transfection decreased the expression of Pol I products in HeLa cells. STAT3 siRNA transfection was performed with HepG2; STAT3 (E) and Pol I products (F) were analyzed by Western blot and RT-qPCR, respectively. **G** and **H**, STAT3 shRNA stable expression reduced the synthesis of Pol I products in 293T cells. 293T cell lines stably expressing STAT3 shRNA or control shRNA were generated using lentiviral particles. STAT3 expression was detected by Western blot (G), where Pol I products were analyzed by RT-qPCR (H). **I** and **J**, STAT3 shRNA stable expression decreased the synthesis of Pol I products in HeLa cells. HeLa cell lines expressing STAT3 shRNA or control shRNA were generated using lentiviral particles. STAT3 expression were analyzed by Western blot (I), where Pol I products were monitored by RT-qPCR (J). **K** and **L,** Effect of STAT3 silencing on rRNA synthesis was determined by EU assays. HeLa cell lines expressing STAT3 shRNA or control shRNA were labeled by EU, and the EU-labeled cells were monitored by an EU detection kit. Images were captured under a fluorescence microscope (K). The relative fluorescence intensity for nucleoli was obtained by ImageJ software (L). The scale bars in images represent 5 μm. Each column in histograms represents the mean $\pm$ SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by Student's *t* test performed with control and treatment groups

Fig. S3

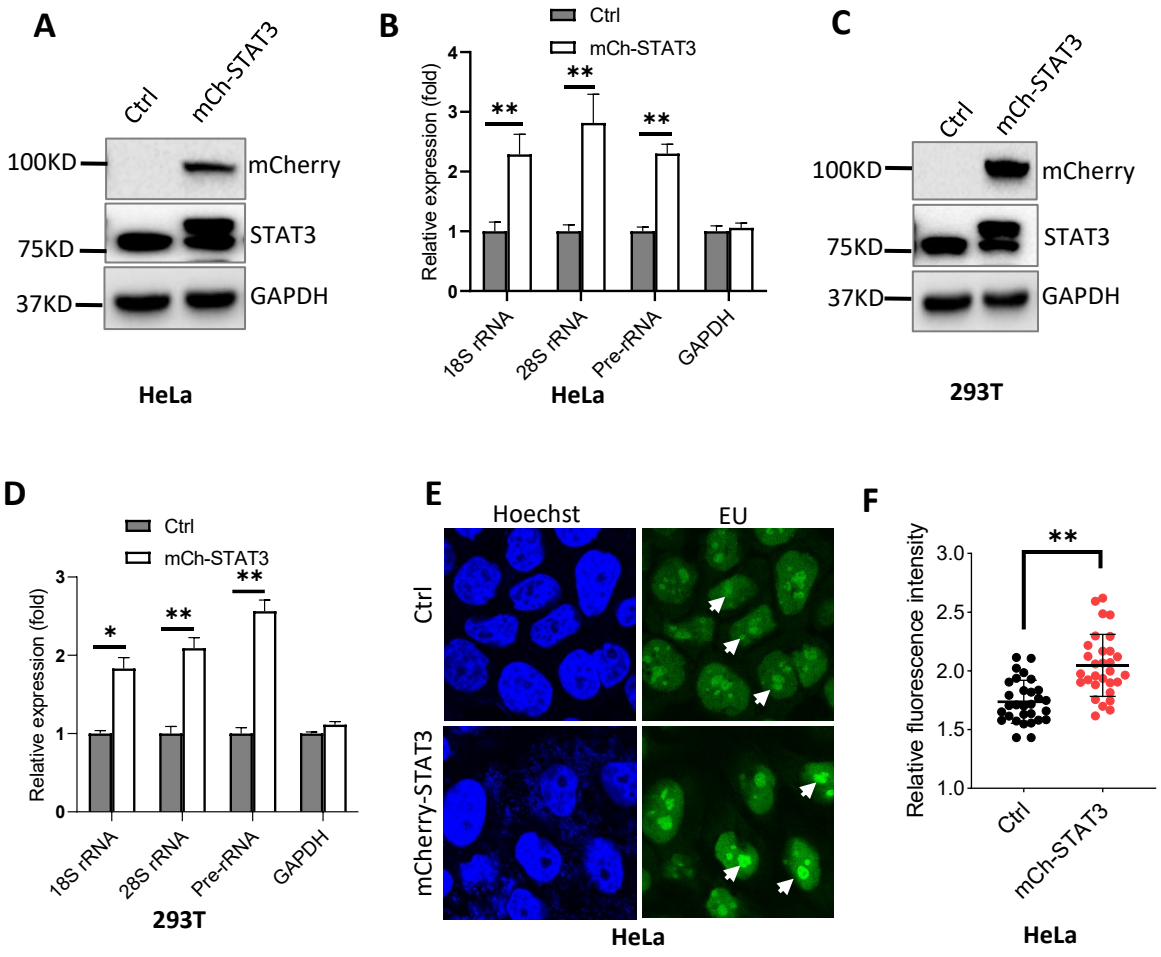


Fig. S3 STAT3 overexpression enhanced the synthesis of Pol I products. A and **B**, STAT3 overexpression increased the synthesis of Pol I product in HeLa cells. A HeLa cell line stably expressing mCherry -STAT3 and its control cell line were generated by a lentiviral infection system. STAT3 protein expression and Pol I products were detected by Western blot (A) and RT-qPCR (B), respectively. **C** and **D**, STAT3 overexpression enhanced the synthesis of Pol I products in 293T cells. A 293T cell line expressing mCherry-STAT3 and its control cell line were generated as for HeLa cells. STAT3 protein expression and Pol I products were analyzed by RT-qPCR (C) and Western blot (D), respectively. **E** and **F**, EU assays showing the effect of STAT3 overexpression on rRNA synthesis was examined. A HeLa cell line expressing mCherry-STAT3 and its control cell line were labeled by EU, and the EU-labeled cells were monitored by the EU detection kit. Images were captured under a fluorescence microscope (E), and the relative fluorescence intensity for nucleoli was obtained by the ImageJ software (F). The scale bars in the images represent 5 μm. Each column in the bar graphs represents the mean ± SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by Student's *t* test performed with control and treatment groups

Fig. S4

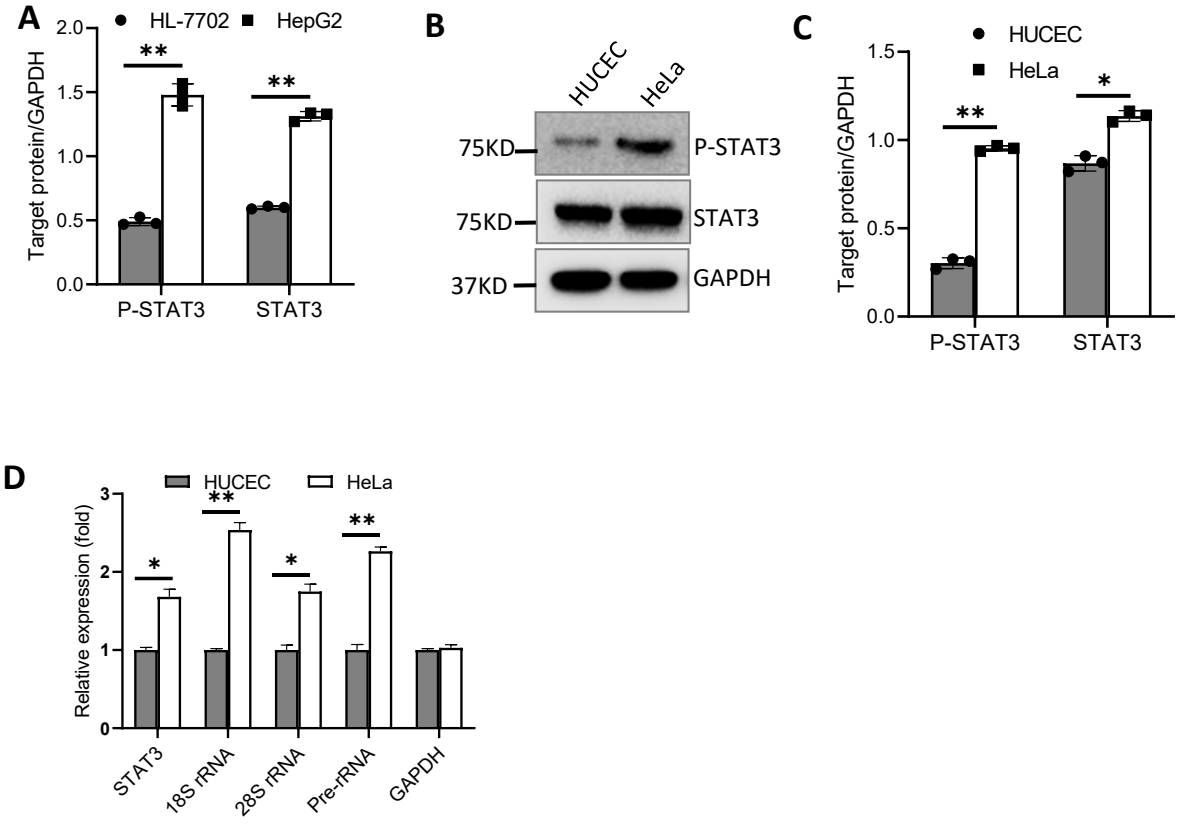
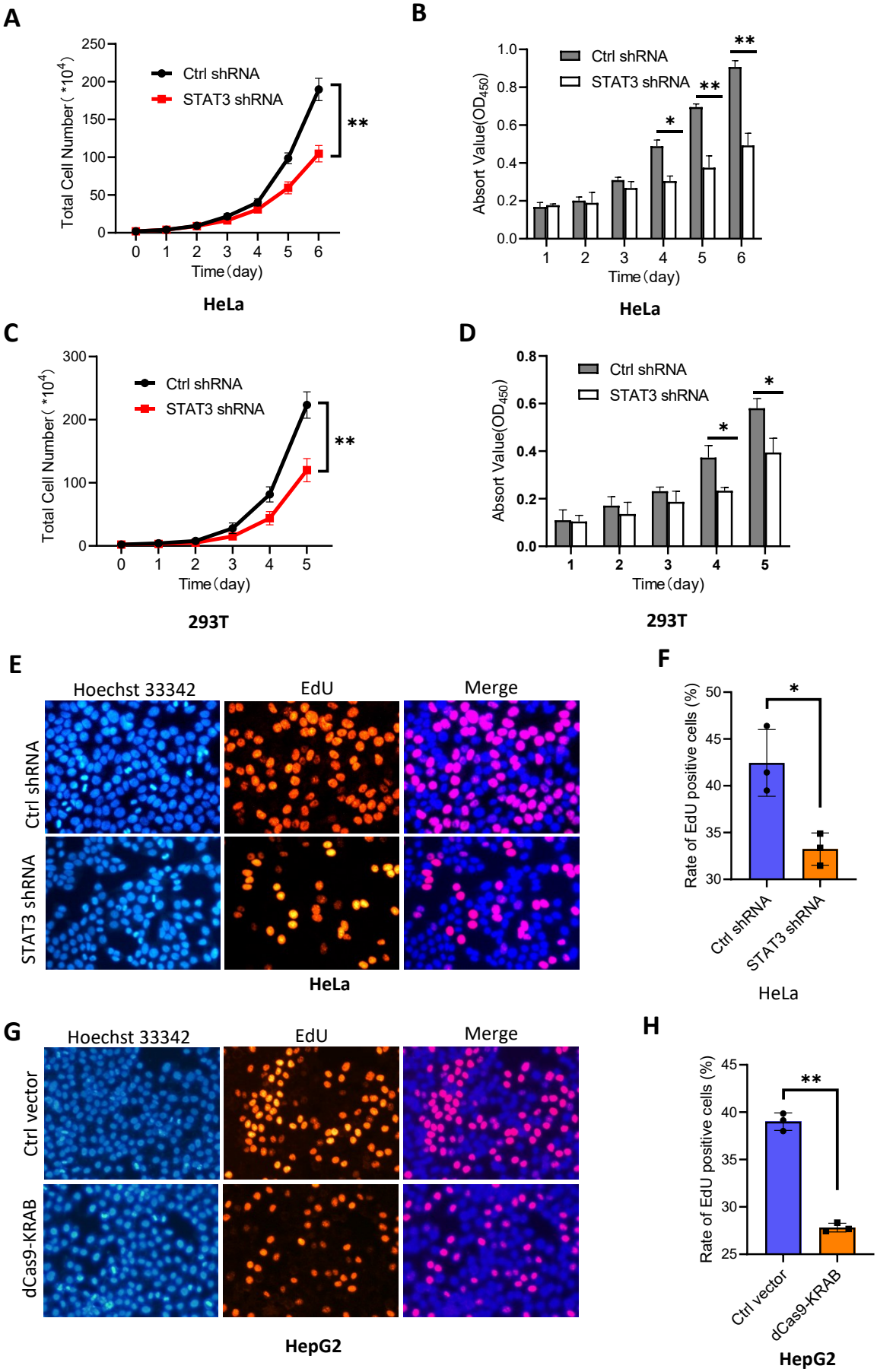


Fig. S4 Cancer cell lines exhibited the increased STAT3 phosphorylation and Pol I –directed transcription compared to their normal cells. **A**, Qantification of Western blot data obtained from Fig 2K and other repeated assays. **B** and **C**, STAT3 expression and phosphorylation were analyzed by Western blot using HUCEC and HeLa cells. Western blot data was quantified and presented graphically in C. **D**, HeLa cells showed the increased Pol I-directed transcription compared to HUCEC. Each column in the bar graphs represents the mean±SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by Student's *t* test performed with control and treatment groups.

Fig. S5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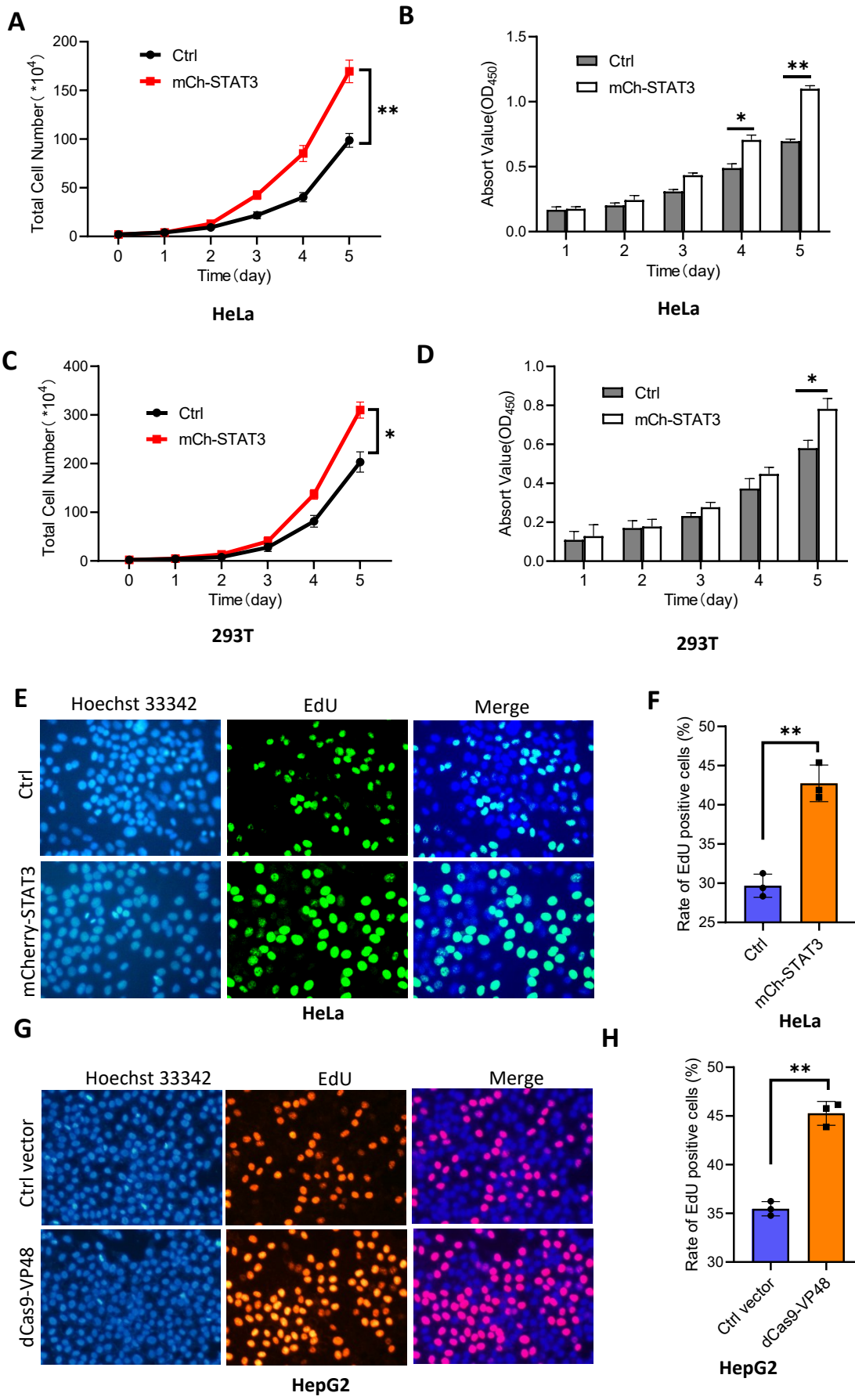
Ctrl shRNA
STAT3 shRNA
**
Time (day)
HeLa
B
Absort Value(OD450)
Ctrl shRNA
STAT3 shRNA
*
**
**
Time (day)
HeLa
C
Total Cell Number (*10^4)
Ctrl shRNA
STAT3 shRNA
**
Time (day)
293T
D
Absort Value(OD450)
Ctrl shRNA
STAT3 shRNA
*
*
Time (day)
293T
E
Hoechst 33342
EdU
Merge
Ctrl shRNA
STAT3 shRNA
HeLa
F
Rate of EdU positive cells (%)
*
Ctrl shRNA
STAT3 shRNA
HeLa
G
Hoechst 33342
EdU
Merge
Ctrl vector
dCas9-KRAB
HepG2
H
Rate of EdU positive cells (%)
**
Ctrl vector
dCas9-KRAB
HepG2

Fig. S5 STAT3 downregulation reduced cell proliferative activity . A and **B**, STAT3 knockdown reduced HeLa cell proliferation. HeLa cell lines expressing STAT3 shRNA or control shRNA were cultured for proliferation analysis using cell counting (A) and CCK8 (B) methods. **C** and **D**, STAT3 knockdown decreased 293T cell proliferation. The 293T cell lines stably expressing STAT3 shRNA or control shRNA were cultured for proliferation analysis with cell counting (C) an CCK8 (B)methods. **E** and **F**, The effect of STAT3 knockdown on HeLa cell proliferation was analyzed by EdU assays. EdU assays were performed using HeLa cell lines expressing STAT3 shRNA or control shRNA. Images were captured under a fluorescence microscope (E), and EdU-labelled cells from the images were subjected to statistical analysis (F). The scale bars in the images represent 100 μm. **G** and **H**, Endogenous STAT3 downregulation caused by dcas9-KRAB expression inhibited HepG2 cell proliferation. EdU assays were performed using HepG2 cells transfected with the vector expressing STAT3 promoter gRNA and dcas9-KRAB. The quantification result from EdU images (G) is presented in H. The scale bars in the images represent 100 μm. Each point/column in histograms represents the mean±SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by two-way ANOVA (A-D) or Student's *t* test (F and H).

Fig. S6


A
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Ctrl
mCh-STAT3
**
Time (day)
HeLa
B
Absort Value(OD450)
Ctrl
mCh-STAT3
*
**
Time (day)
HeLa
C
Total Cell Number (*10^4)
Ctrl
mCh-STAT3
*
Time (day)
293T
D
Absort Value(OD450)
Ctrl
mCh-STAT3
*
Time (day)
293T
E
Hoechst 33342
EdU
Merge
Ctrl
mCherry-STAT3
HeLa
F
Rate of EdU positive cells (%)
**
Ctrl
mCh-STAT3
HeLa
G
Hoechst 33342
EdU
Merge
Ctrl vector
dCas9-VP48
HepG2
H
Rate of EdU positive cells (%)
**
Ctrl vector
dCas9-VP48
HepG2

Fig. S6 STAT3 upregulation promoted cell proliferation . A-D, STAT3 overexpression enhanced HeLa cell proliferation. A HeLa cell line expressing mCherry-STAT3 and its control cell line were cultured for proliferation analysis using cell counting (A) and CCK8 (B). **C** and **D,** STAT3 overexpression enhanced 293T cell proliferation . A 293T cell line expressing mCherry-STAT3 and its control cell line were cultured for proliferation analysis using cell counting (C) and CCK8 (D) methods. **E** and **F**, The effect of STAT3 overexpression on HeLa cell proliferation was analyzed by EdU assays. EdU assays were performed using a HeLa cell line expressing mCherry-STAT3 and its control cell line. Images were captured under a fluorescence microscope (E), and EdU-labelled cells from the images were subjected to statistical analysis (F). Scale bars in the images represent 100 μm**. G** and **H**, The effect of endogenous STAT3 upregulation on cell proliferative activity. EdU assays were performed using HepG2 cells transfected with the vectors expressing the STAT3 promoter gRNA and dcas9-VP48. Images were captured under a fluorescence microscope (G), and EdU-labelled cells from the images were subjected to statistical analysis (H). Scale bars in the images represent 100 μm**.** Each point/column in graphs represents the mean±SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by two-way ANOVA (A-D) or Student's *t* test (F and H).

Fig. S7

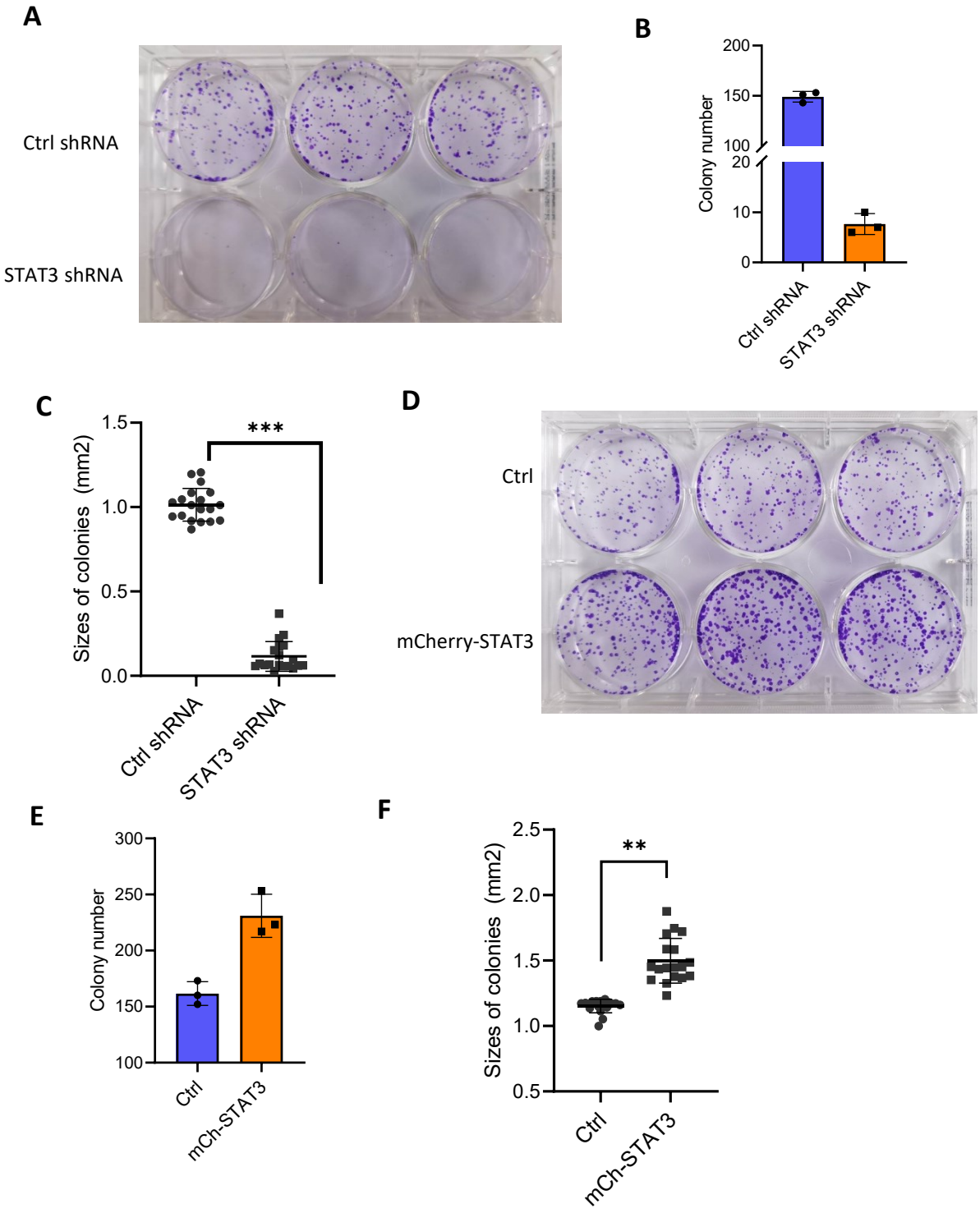

A
Ctrl shRNA
STAT3 shRNA
B
Colony number
200
150
100
20
10
0
Ctrl shRNA
STAT3 shRNA
C
Sizes of colonies (mm2)
1.5
1.0
0.5
0.0
***
Ctrl shRNA
STAT3 shRNA
D
Ctrl
mCherry-STAT3
E
Colony number
300
250
200
150
100
Ctrl
mCh-STAT3
F
Sizes of colonies (mm2)
2.5
2.0
1.5
1.0
0.5
**
Ctrl
mCh-STAT3

Fig. S7 STAT3 positively regulates cell survival and growth. **A-C**, STAT3 knockdown in HepG2 cells reduced the number of colony formation and the sizes of colonies. HepG2 cell lines expressing STAT3 shRNA or control shRNA were seeded in 6-well plates. After growing 10 days, cells were stained with 0.2% crystal violet and photographed with a camera (A). The number of colonies in each well was counted (B), and the sizes of individual colonies were obtained by calculating the area of each colony (C). The data obtained were subjected to statistical analysis. **D-F**, STAT3 overexpression in HepG2 cells increased the number of colony formation and the colony size. A HepG2 cell lines stably expressing mCherry-STAT3 and its control cell line were grown in 6-well plates. After growing 10 days, cells were stained and photographed (D). The number of colonies in each well was counted (E), and the sizes (area) of individual colonies randomly selected was calculated (F). The data obtained were subjected to statistical analysis. Each column in histograms represents the mean $\pm$ SD of three biological replicates. *, $P<0.05$; **, $P<0.01$. *P* values were obtained Student's *t* test

Fig. S8

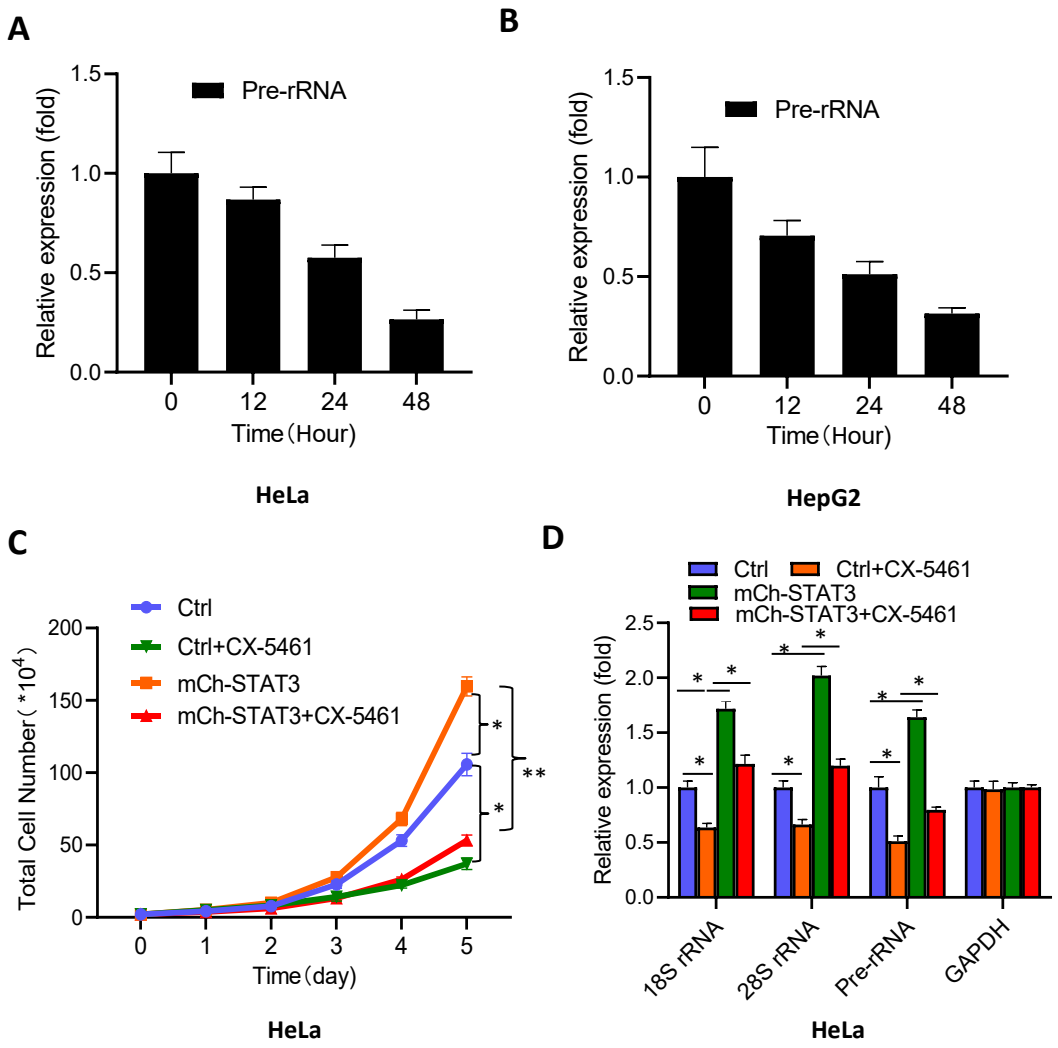


Fig. S8 Pol I-specific inhibitor represses cell proliferation and pre-rRNA synthesis. A, Effect of CX-5461 on the expression of pre-rRNA in HeLa cells. HeLa cells were seeded in 6-well plates and cultured in the medium containing 5 μM CX-5461. Cells were harvested at different time points, and pre-rRNA expression was analyzed by RT-qPCR. **B,** Effect of CX-5461 on the expression of pre-rRNA in HepG2 cells. Cell culture and RT-qPCR were performed as described for A. **C**, CX-5461 inhibited the enhancement of proliferation induced by STAT3 overexpression. **D**, CX-5461 inhibited the activation of Pol I-directed transcription induced by STAT3 overexpression. Each point/column in graphs represents the mean±SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values in C and D were obtained by two-way ANOVA

Fig. S9

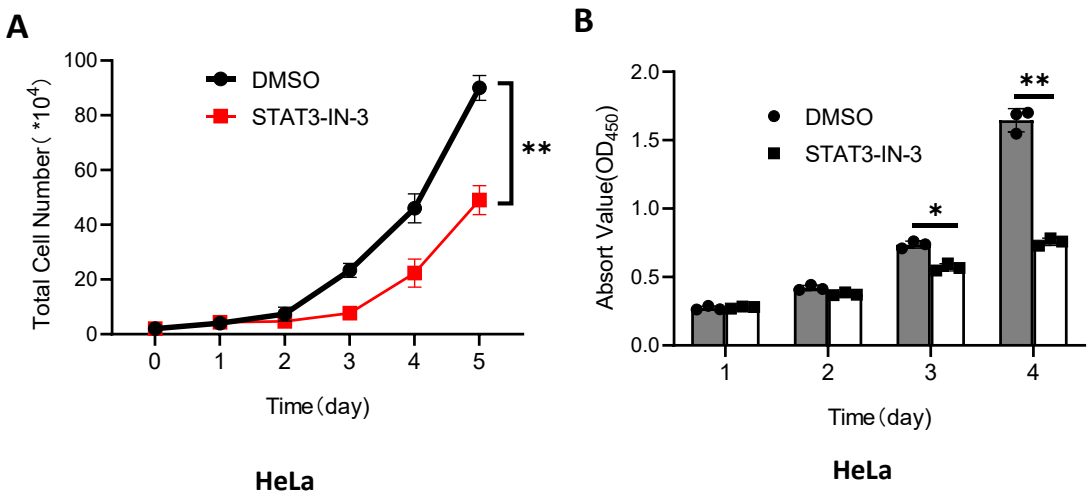
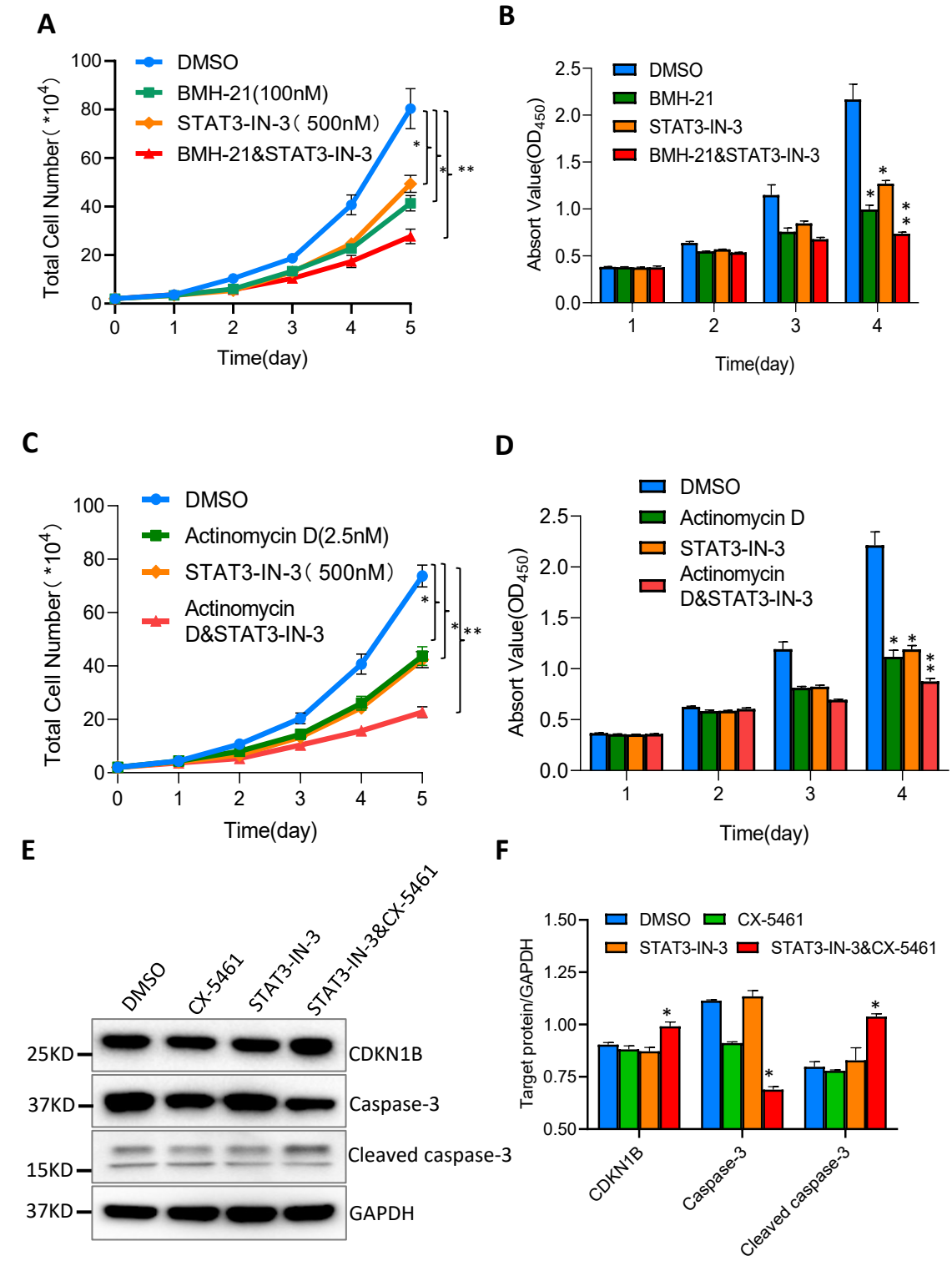


Fig. S9 STAT3-IN-3 inhibited HeLa cell proliferation. HeLa cells were seeded in 12-well and 96-well plates, and cell proliferative activity was assessed by cell counting (**A**) and CCK-8 (**B**) methods. Each point or column in histograms represents the mean ±SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by two-way ANOVA.

Fig. S10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STAT3-IN-3（500nM）
BMH-21&STAT3-IN-3
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Time(day)
B
DMSO
BMH-21
STAT3-IN-3
BMH-21&STAT3-IN-3
Absort Value(OD450)
Time(day)
C
DMSO
Actinomycin D(2.5nM)
STAT3-IN-3（500nM）
Actinomycin D&STAT3-IN-3
Total Cell Number（*10^4）
Time(day)
D
DMSO
Actinomycin D
STAT3-IN-3
Actinomycin D&STAT3-IN-3
Absort Value(OD450)
Time(day)
E
DMSO
CX-5461
STAT3-IN-3
STAT3-IN-3&CX-5461
25KD
CDKN1B
37KD
Caspase-3
15KD
Cleaved caspase-3
37KD
GAPDH
F
DMSO
CX-5461
STAT3-IN-3
STAT3-IN-3&CX-5461
Target protein/GAPDH
CDKN1B
Caspase-3
Cleaved caspase-3

Fig. S10 Combination of STAT3-IN-3 and Pol I transcription inhibitors showed additive effect on HepG2 cell growth compared to the application of single drug. A and **B**, Effect of STAT3-IN-3 (500 nM) and BMH-21 (100 nM) on HepG2 cell growth. Cell growth was monitored by cell counting (A) and CCK8 (B) methods. **C** and **D,** Effect of STAT3-IN-3 (500 nM)and Actinomycin D (2.5 nM) on HepG2 cell growth. Cell growth was monitored by cell counting (C) and CCK8 (D) methods. **E** and **F**, Effect of STAT-IN-3 and CX-5461 on the expression of CDKN1B, caspas-3 and cleaved caspas-3. F represents the quantified result of the Western blot in E. Each point or column in histograms represents the mean ±SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by two-way ANOVA.

Fig. S11

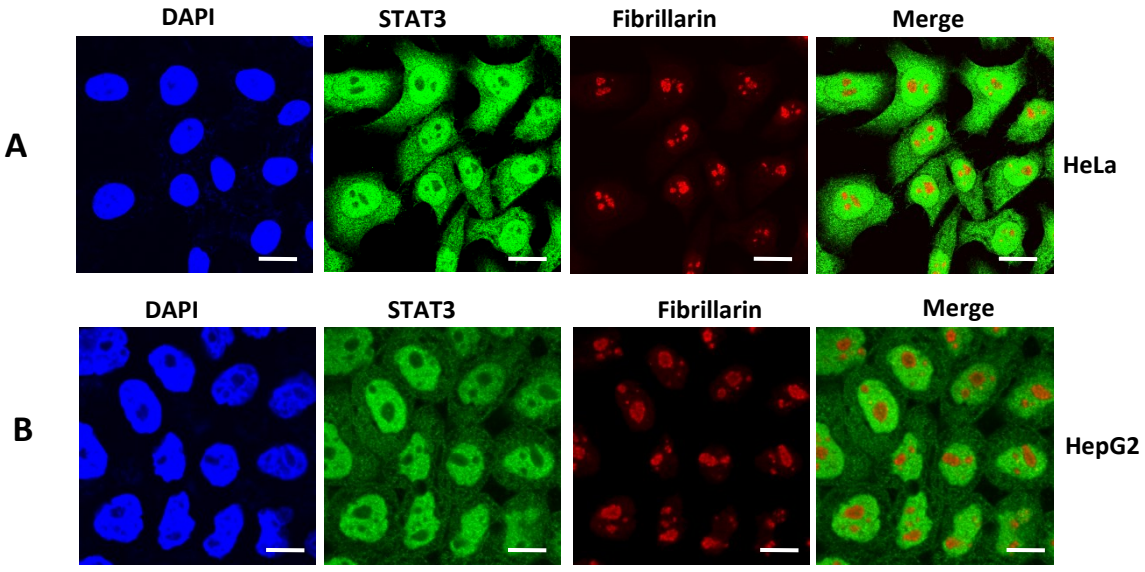
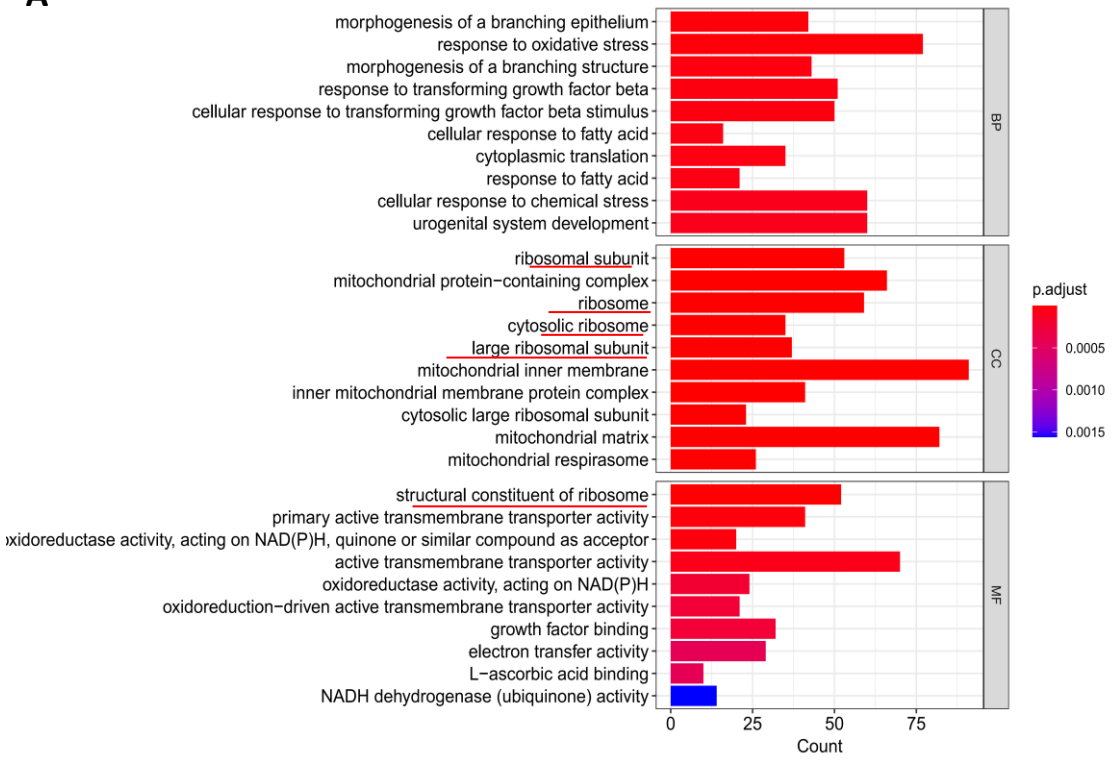


Fig. S11 The co-localization analysis of STAT3 and Fibrillarin in HeLa and HepG2 cells. A, The co-localization analysis between STAT3 and Fibrillarin in HeLa cells using the antibodies against STAT3 or Fibrillarin. Images were captured by a confocal fluorescence microscope. The scale bars in images represent 2.5 μm. **B**, The co-localization analysis between STAT3 and Fibrillarin in HepG2 cells using the antibodies against STAT3 or Fibrillarin. Images were captured by a confocal fluorescence microscope. The scale bars in the images represent 2.5 μm.

Fig. S12

A



B

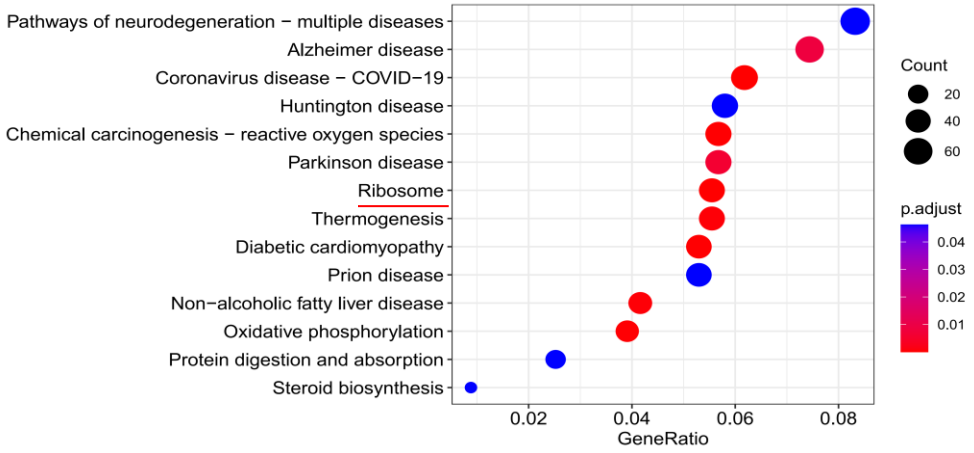


Fig. S12 STAT3 silencing affected expression of the genes that are related to ribosome activity in HepG2 cells. A, Analysis of gene ontology based on the significant differential expression genes obtained from the RNA-seq data of HepG2 cell lines expressing STAT3 shRNA or control shRNA; **B**, Analysis of KEGG (Kyoto enclopedia of genes and genomes) pathways based on the significant differential expression genes obtained from the RNA-seq data of HepG2 cell lines expressing STAT3 shRNA or control shRNA.

Fig. S13

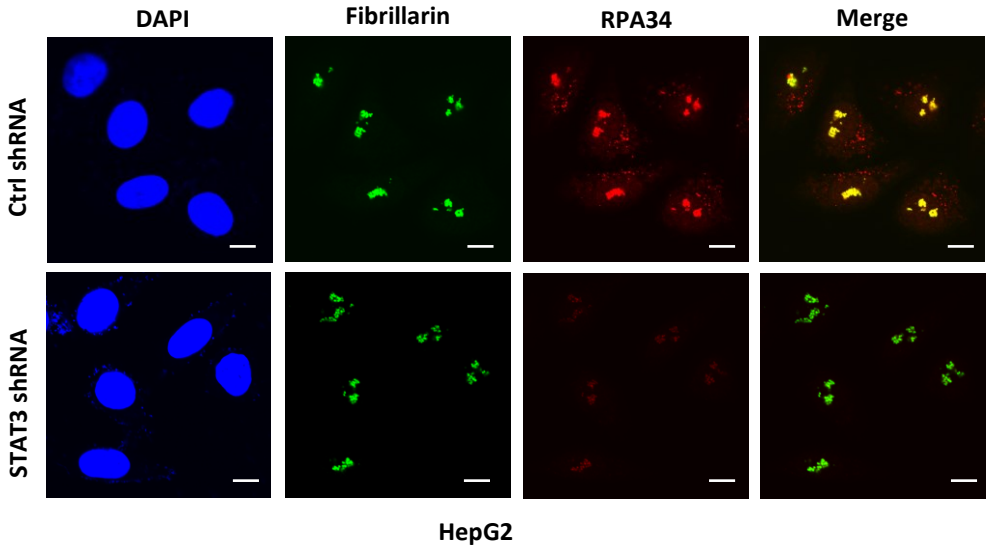


Fig. S13 STAT3 silencing reduced the localization of RPA34 in the nucleoli of HepG2 cells. Immunofluorescence staining assays were performed using HepG2 cells expressing STAT3 shRNA or control shRNA cultured on the round coverslips with 14-mm in diameter and antibodies against RPA34 and fibrillarin. Images were captured under a confocal fluorescence microscope; scale bars in the images represent 2.5 μm.

Fig. S14

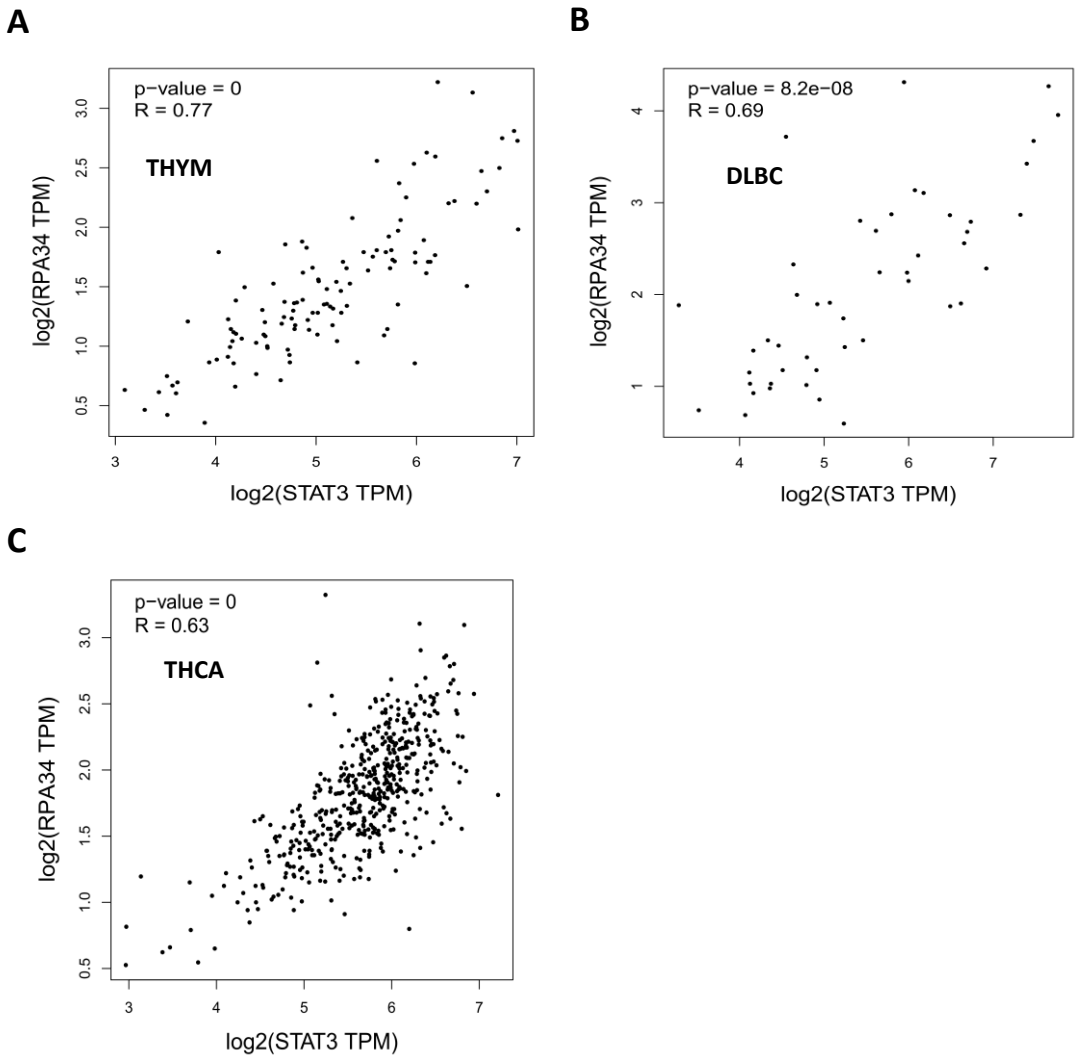


Fig. S14 Pearson correlation analysis between RPA34 and STAT3 in cancer tissues. Pearson based on the TCGA dataset of clinical cancer samples, including thymoma (**A**), diffuse large B-cell lymphoma (**B**) and thyroid carcinoma (**C**), using the GEPIA online tool (http://gepia2.cancer-pku.cn/#index)

Fig. S15

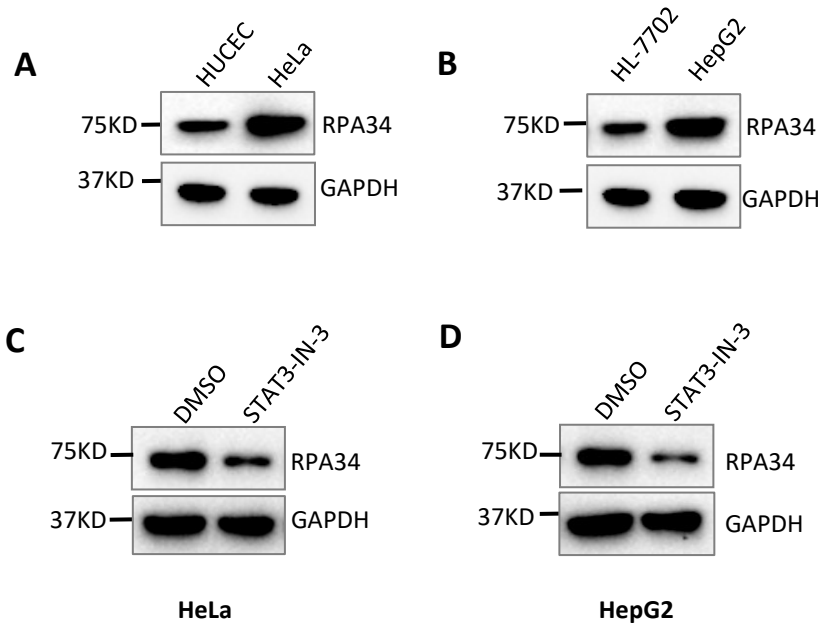


Fig. S15 RPA34 expression was analyzed by Western blot. **A**, RPA34 expression in HeLa cells is higher than that in HUCEC cells. **B**, RPA34 expression in HepG2 cells is higher than that in HL-7702 cells. **C**, STAT3-IN-3 inhibited RPA34 expression in HeLa cells. **D**, STAT3-IN-3 inhibited RPA34 expression in HepG2 cells.

Fig. S16

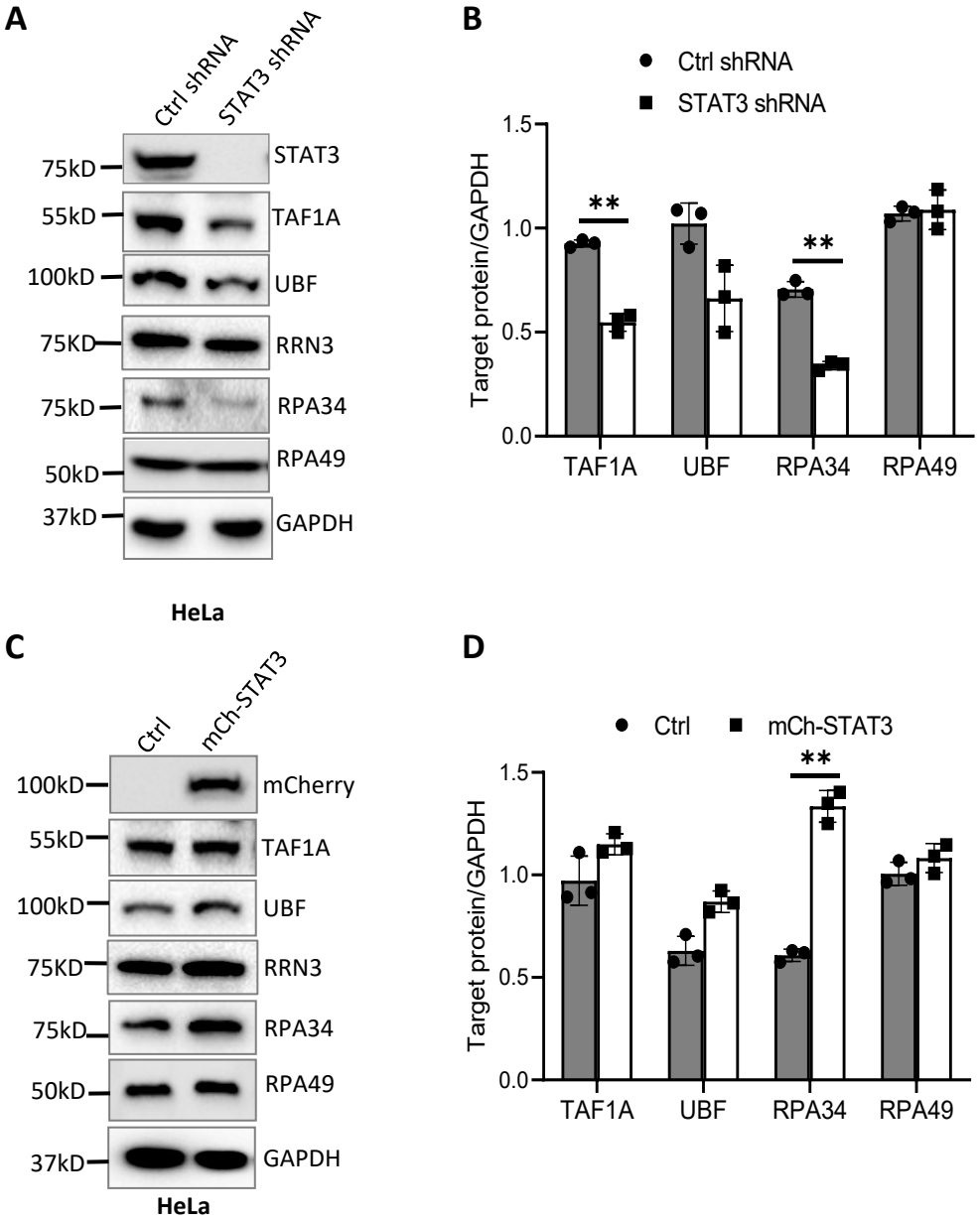
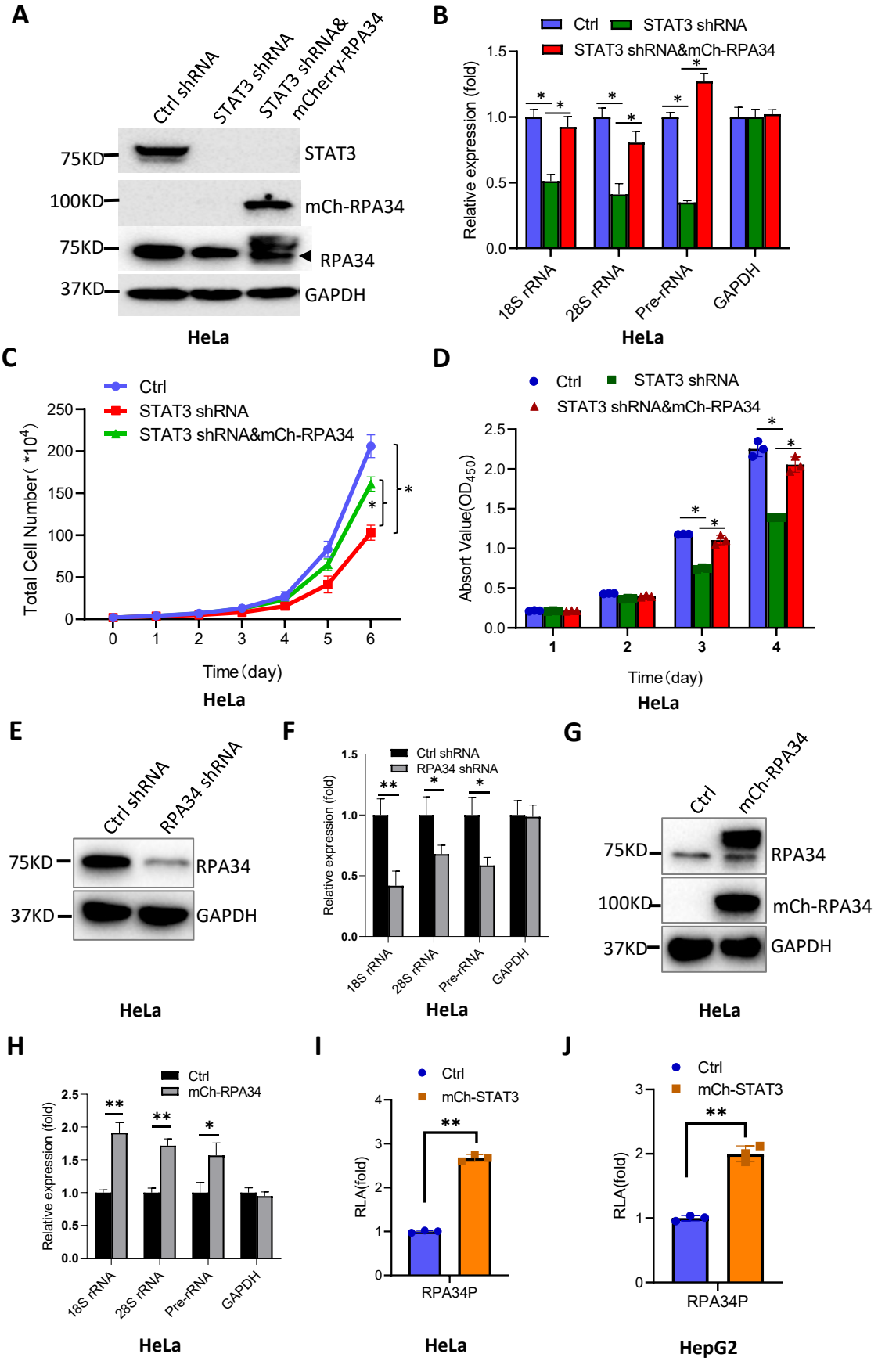


Fig. S16 STAT3 positively regulates RPA34 expression. A and **B,** STAT3 knockdown reduced RPA34 and RPA49 expression in HeLa cells. B is the quantified result of Western blot in A. **C** and **D**, STAT3 overexpression increased RPA34 and UBF expression in HeLa cells. D is the quantified result of Western blot in C. Each column in histograms represents the mean ±SD of tree independent intensity caculations. *, $P<0.05$; **, $P<0.01$. P values were obtained by Student's t test.

Fig. S17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STAT3 shRNA& mCherry-RPA34
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STAT3
100KD
mCh-RPA34
75KD
RPA34
37KD
GAPDH
HeLa
B
Ctrl
STAT3 shRNA
STAT3 shRNA&mCh-RPA34
Relative expression (fold)
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
HeLa
C
Ctrl
STAT3 shRNA
STAT3 shRNA&mCh-RPA34
Total Cell Number(*10^4)
Time (day)
HeLa
D
Ctrl
STAT3 shRNA
STAT3 shRNA&mCh-RPA34
Absort Value(OD450)
Time (day)
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75KD
RPA34
37KD
GAPDH
HeLa
F
Ctrl shRNA
RPA34 shRNA
Relative expression (fold)
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
HeLa
G
Ctrl
mCh-RPA34
75KD
RPA34
100KD
mCh-RPA34
37KD
GAPDH
HeLa
H
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mCh-RPA34
Relative expression (fold)
18S rRNA
28S rRNA
Pre-rRNA
GAPDH
HeLa
I
Ctrl
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RLA(fold)
RPA34P
HeLa
J
Ctrl
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RLA(fold)
RPA34P
HepG2

Fig. S17 RPA34 is required for the regulation of Pol I-directed mediated by STAT3. **A**, Western blot showing the generation of a HeLa cell line expressing both STAT3 shRNA and mCherry-RPA34. A HeLa cell line expressing STAT3 shRNA was incubated with lentiviral particles expressing mCherry-RPA34. Cells were selected by puromycin, and expression of STAT3 and mCherry-RPA34 was examined by Western blot with antibodies as indicated. **B**, mCherry-RPA34 expression reversed the inhibition of Pol I-directed transcription caused by STAT3 silencing. Pol I products were detected by RT-qPCR. **C** and **D**, mCherry-RPA34 expression alleviated the inhibition of cell proliferation caused by STAT3 silencing. Cell proliferation assays was performed using cell counting and CCK-8 methods. **E** and **F,** RPA34 knockdown reduced the synthesis of Pol I products. RPA34 and Pol I products were analyzed by Western blot (E) and RT-qPCR (F), respectively. **G** and **H**, RPA34 overexpression increased the synthesis of Pol I products. RPA34, mcherry-RPA34 and Pol I products were analyzed by Western blot (G) and RT-qPCR (H), respectively. **I** and **J**, STAT3 overexpression enhanced the RPA34 promoter activity. Luciferase assays were performed using HeLa (I) or HepG2 (J) cell lines expressing mCherry-STAT3 and their corresponding control cells, where cells were transfected with the RPA34 promoter-driven reporter vector. Luciferase activity was monitored using a luciferase detection kit (Promega). Relative luciferase activity (RLA) was obtained by comparing luciferase activity in treatments to that of controls, where the luciferase activity in control was arbitrarily set as 1. Each column or point in histograms represents the mean $\pm$SD of three independent experiments. *, $P<0.05$; **, $P<0.01$. *P* values were obtained by two-way ANOVA (C-D) or Student's *t* test (B, E, F, H and J).