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2 **Figure S1.** Expression analysis of TTC36 in RNA-seq and scRNA-seq data. **A.** Cross-
 3 dataset intersection of DEGs from GSE121248 and GSE135631. **B.** pan-cancer analysis

1 of TTC36 expression based on TCGA database (malignant categories with significant
2 TTC36 down-regulation are marked in red fonts). **C.** Analysis of scRNA-seq data for
3 GSE282701: Cell dimensionality reduction clustering and cell annotation results. **D.**
4 Analysis of TTC36 Expression Levels Across Cell Clusters. **E.** Violin Plot of TTC36
5 Expression Level Across Cell Clusters. **F.** Comparative analysis of mean TTC36
6 expression in tumor and matched non-tumor tissues across major cell clusters. **G.**
7 Analysis of immune cell infiltration in HCC stratified by TTC36 expression levels. **H.**
8 Expression profiles of key immune checkpoint molecules in HCC stratified by TTC36
9 expression levels.

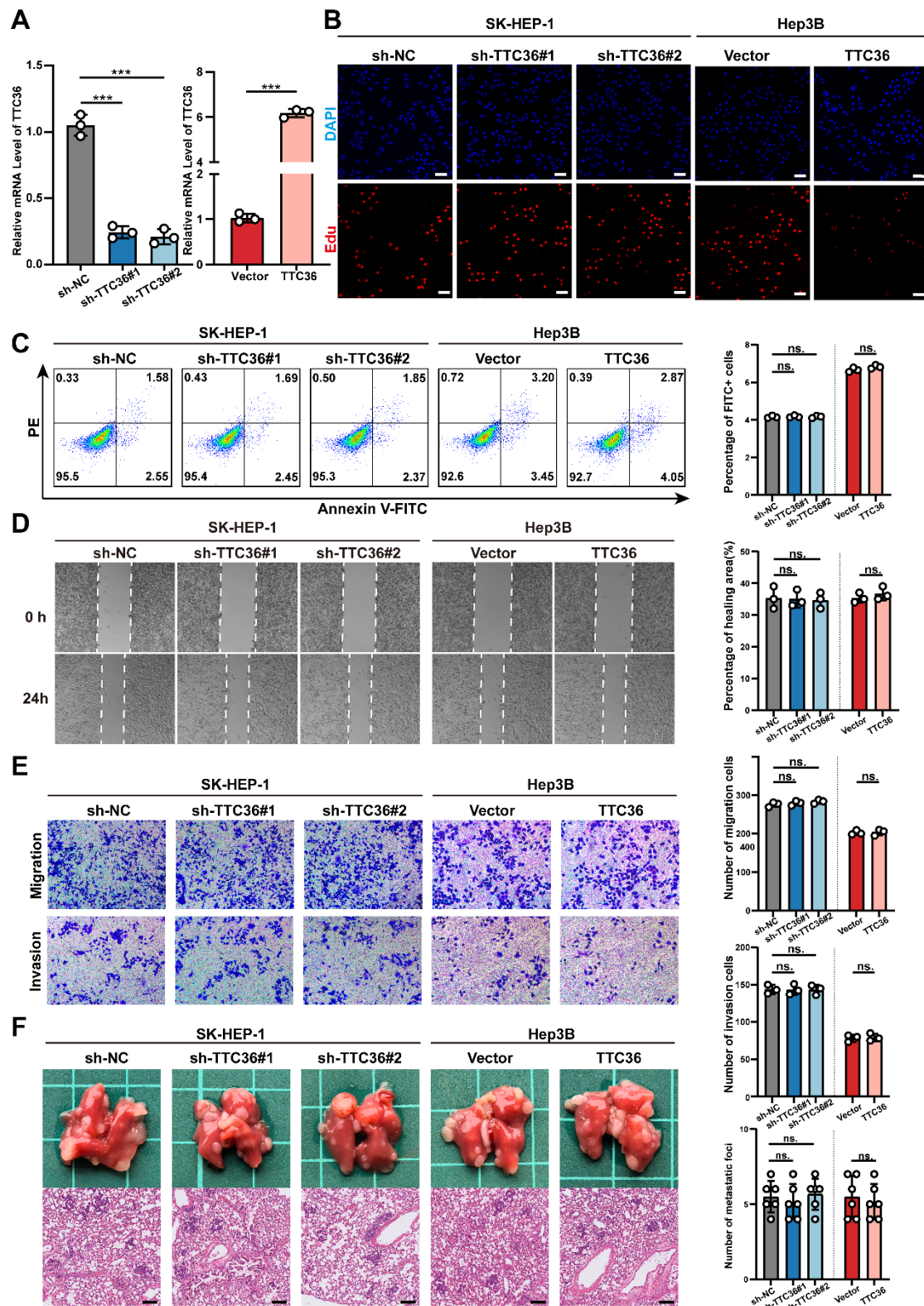
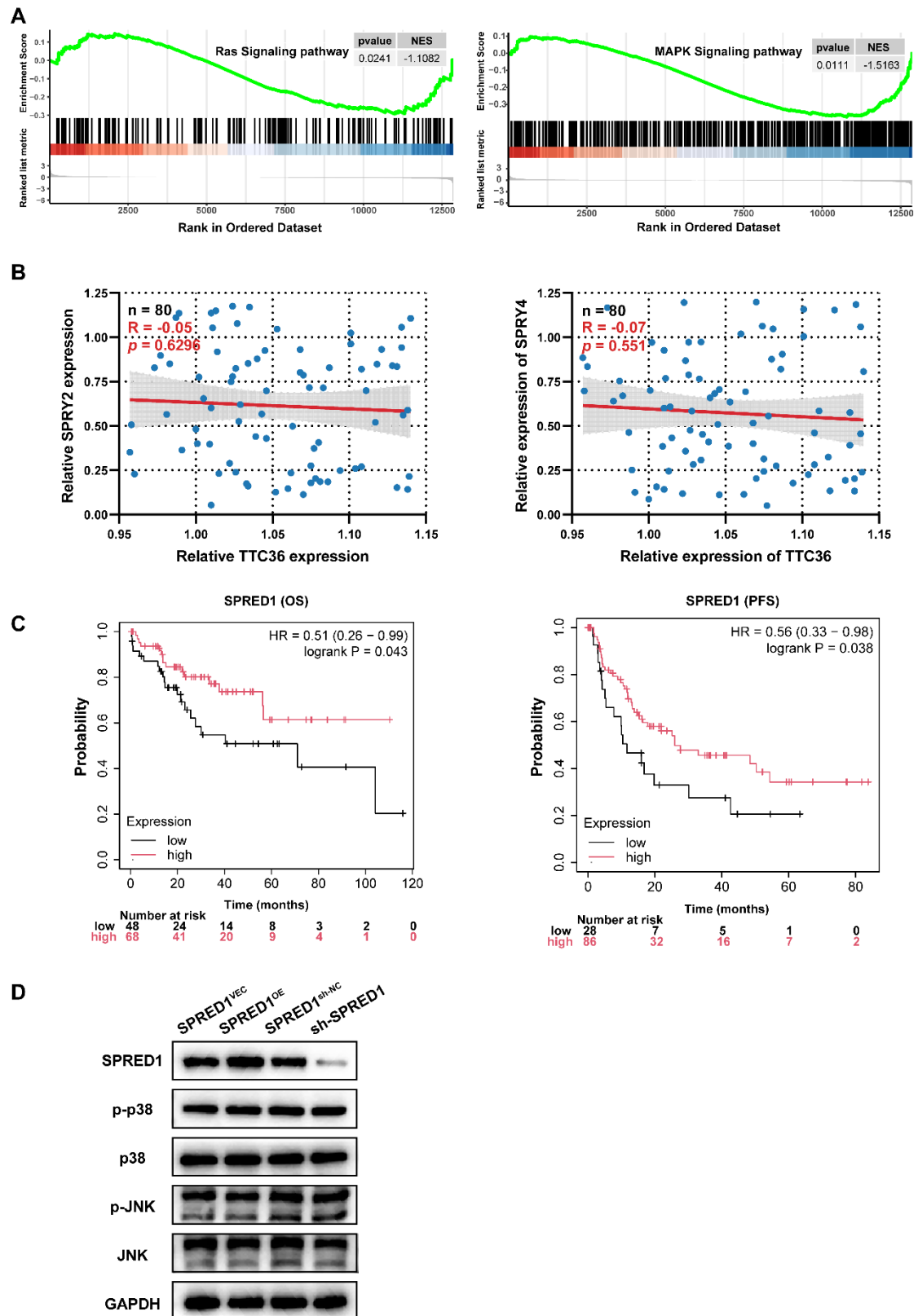


Figure S2. Functional validation of TTC36 *in vivo* and *in vitro* models. **A.** qRT-PCR analysis of TTC36 knockdown and overexpression efficiency in SK-HEP-1 cells and Hep3B cells. **B.** Images of EdU incorporation assays in TTC36-modulated cells (blue: DAPI; red: Edu. Merge images are displayed in Figure 2). **C.** Apoptosis analysis of

1 TTC36-modified SK-HEP-1 and Hep3B cells. **D.** Wound healing assays showing the
2 migratory capacity of TTC36-modulated cells. Wound closure rates were quantified at
3 24h post-scratch. **E.** Transwell migration and invasion assays demonstrating the impact
4 of TTC36 knockdown and overexpression on cellular migration or invasiveness. **F.** *In*
5 *vivo* lung metastasis model constructed via tail injection using TTC36-modified SK-
6 HEP-1 and Hep3B cells. Scale bar: 100µm.



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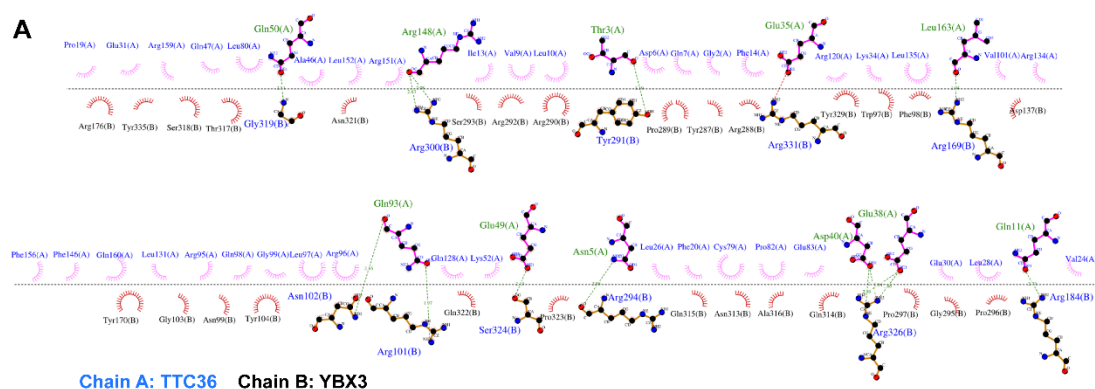
2 **Figure S3.** TTC36 inhibits Ras/MAPK signaling through regulating SPRED1.

3 **A.** GSEA plots showing suppression of Ras and MAPK signaling pathways in TTC36

4 overexpressed cells. **B.** Correlation analysis of TTC36 with SPRY2 and SPRY4 in

1 HCC clinical samples (no significant correlation). **C.** Survival analysis of SPRED1 in
2 HCC patients using Kaplan-Meier plotter (high vs. low, OS, $P = 0.043$; PFS, $P = 0.038$).
3 **D.** Detection of phosphorylation levels in Ras/MAPK downstream branches p38 and
4 JNK via western blot.

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10 **Figure S4.** Structural analysis of the TTC36-YBX3 interaction. **A.** Detailed hydrogen
11 bond network at the TTC36-YBX3 interface.

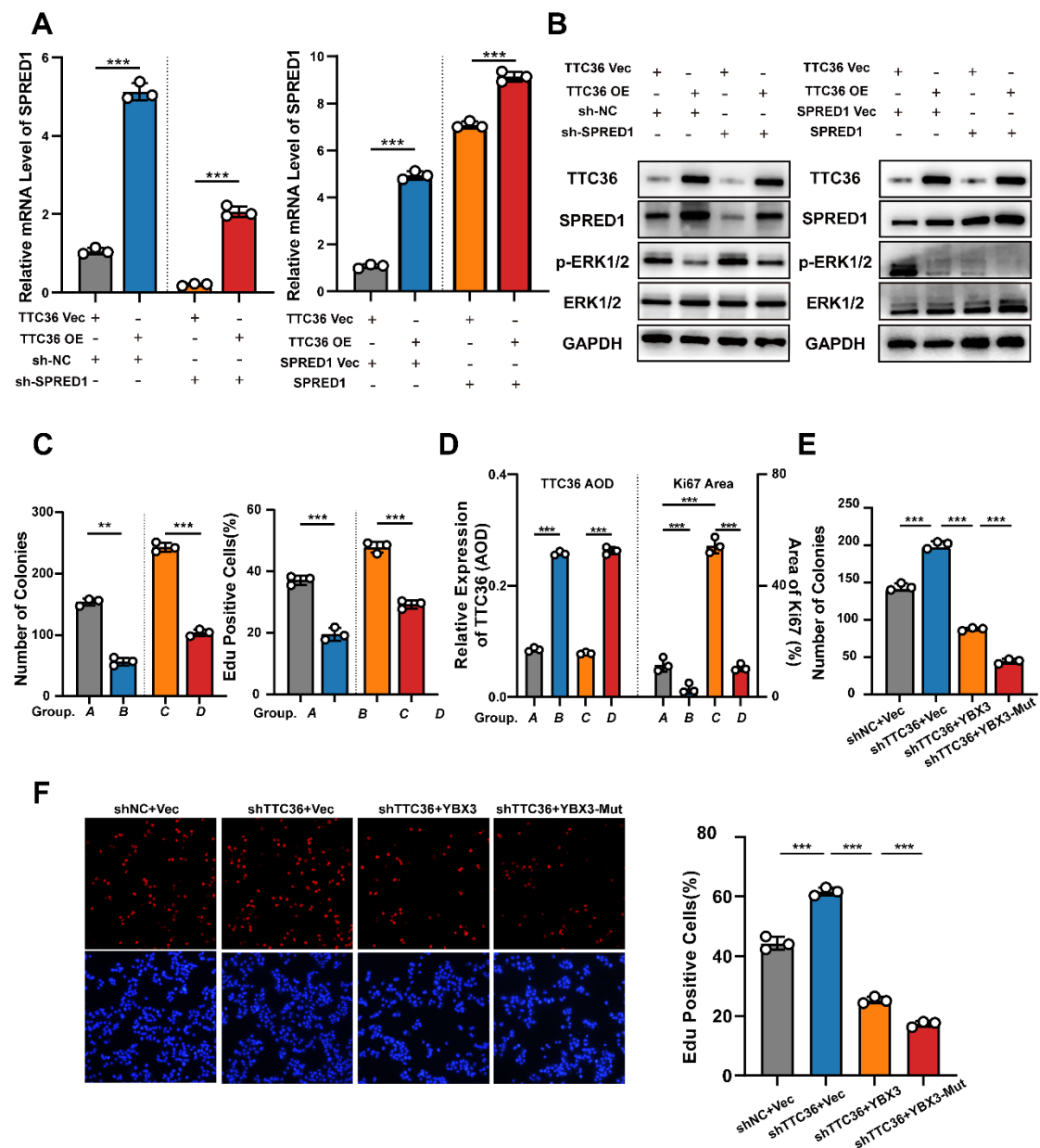
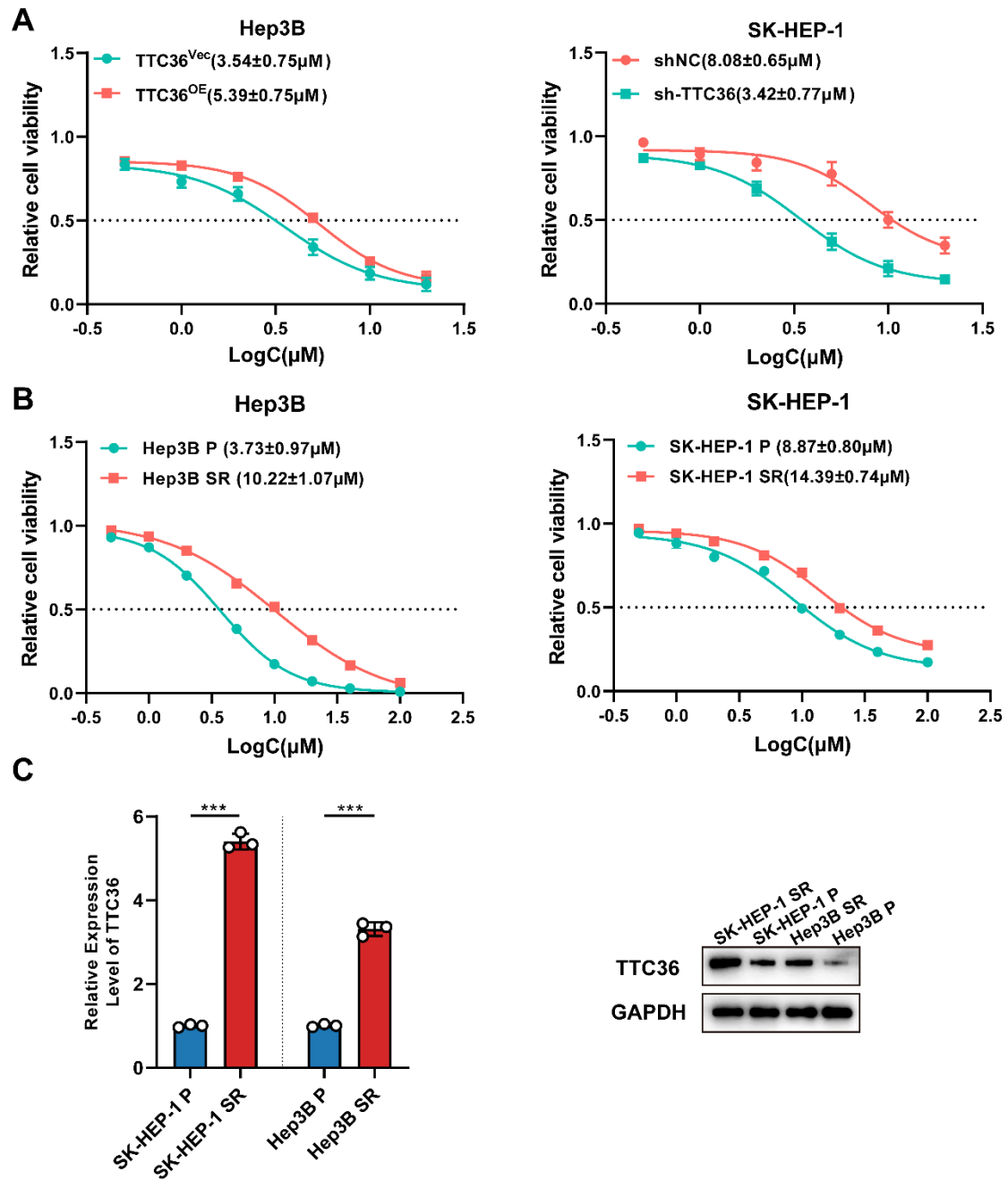


Figure S5. Functional validation of TTC36/YBX3/SPRED1 axis in HCC suppression.

A. qRT-PCR analysis of SPRED1 mRNA in TTC36-modulated Hep3B cells co-transfected with sh-SPRED1 or SPRED1 expression plasmid. **B.** Western blot analysis of SPRED1 expression and ERK1/2 activation level in Hep3B cells co-transfected with TTC36 plasmid and sh-SPRED1/SPRED1 plasmid. **C.** Quantitative statistical analysis of colony formation assay and Edu staining assay displayed in Figure 7B. **D.** Quantitative statistical analysis of TTC36 expression and percentage of Ki67 area in the IHC staining displayed in Figure 7D. **E.** Quantitative statistical analysis of colony formation assay displayed in Figure 7F. **F.** Images and quantitative statistical analysis of EdU staining assay in sh-TTC36-modulated SK-HEP-1 cells co-transfected with

1 wild type YBX3 or K311/K350 mutant YBX3 (blue: DAPI; red: EdU. Merge images
2 are displayed in Figure 7G)



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4 **Figure S6.** Examination associated with sorafenib resistance in Hep3B and SK-HEP-1
5 cells.
6 **A.** IC₅₀ curves of TTC36-modulated Hep3B and SK-HEP-1 cells treated with sorafenib
7 at the concentration 0.5 μ M, 1 μ M, 2 μ M, 5 μ M, 10 μ M, and 20 μ M for 48 hours. **B.**
8 IC₅₀ curves of sorafenib resistant and parental Hep3B and SK-HEP-1 cells. (Labeled
9 as Hep3B SR, SK-HEP-1 SR, Hep3B P and SK-HEP-1 P, respectively.) **C.** qRT-PCR

- 1 and western blot analysis of TTC36 expression in sorafenib resistant or parental SK-
- 2 HEP-1 and Hep3B cells.