Expanded View Figures

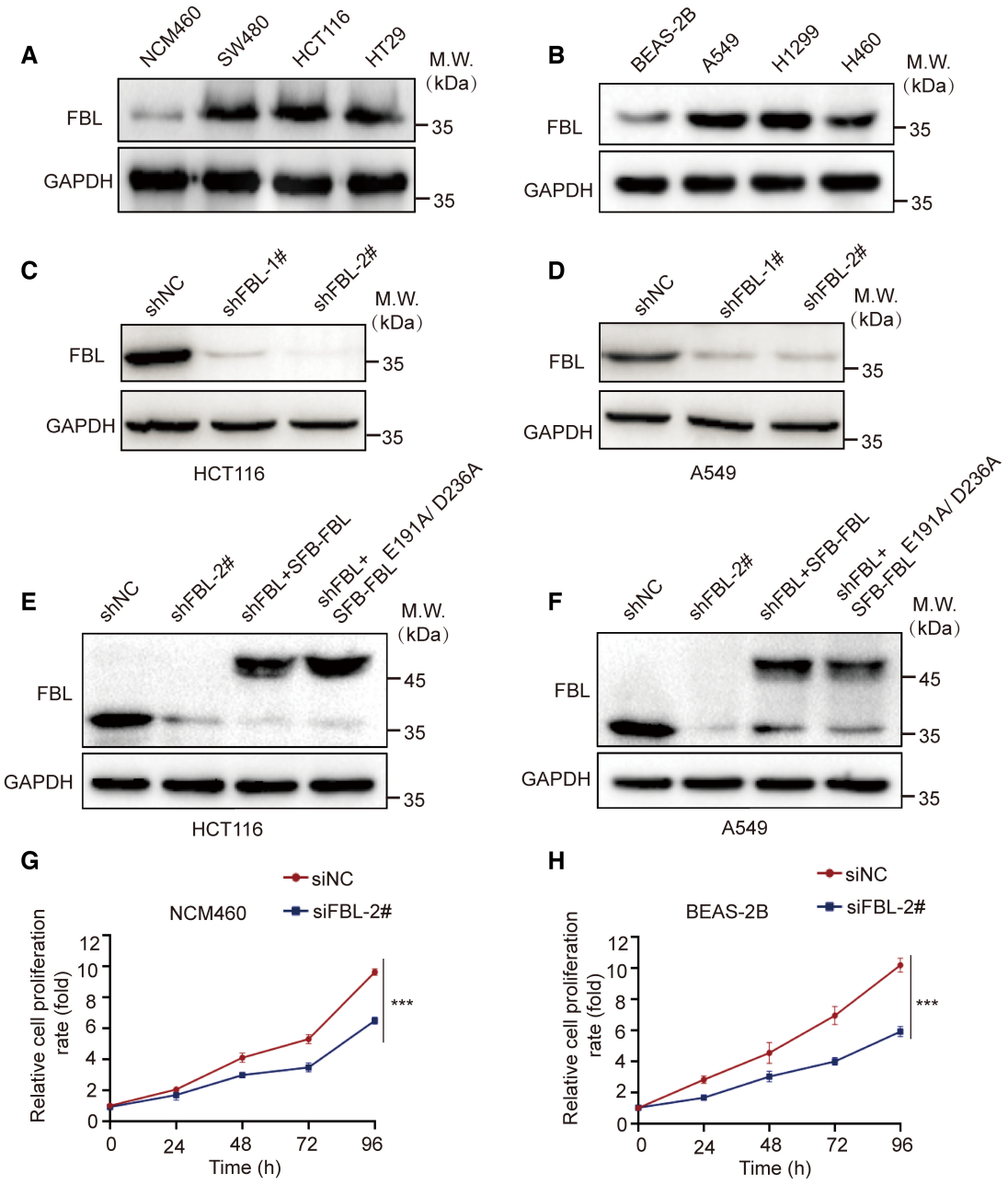

Figure EV1. FBL is overexpressed in cancer cell lines and promotes cell proliferation.

A, B Western blotting analyses of FBL expression in colon/lung cancer and normal cell lines.

C, D Cell lysates of monoclonal cell lines were immunoblotted with FBL antibodies to confirm the knockdown efficiency in HCT116/A549 cells.

E, F Cells were reconstituted with full-length FBL and $FBL^{E191A/D236A}$ in HCT116/A549 cells. Protein expressions were examined by western blot with indicated antibodies.

G, H Cell viability assays were performed on control (siNC) and FBL knockdown (siFBL) NCM460/BEAS-2B cells using CCK-8 assays. The data are presented as mean ± SD from three independent experiments. The statistical significance was determined using Student's *t*-test. ***$P < 0.001$.

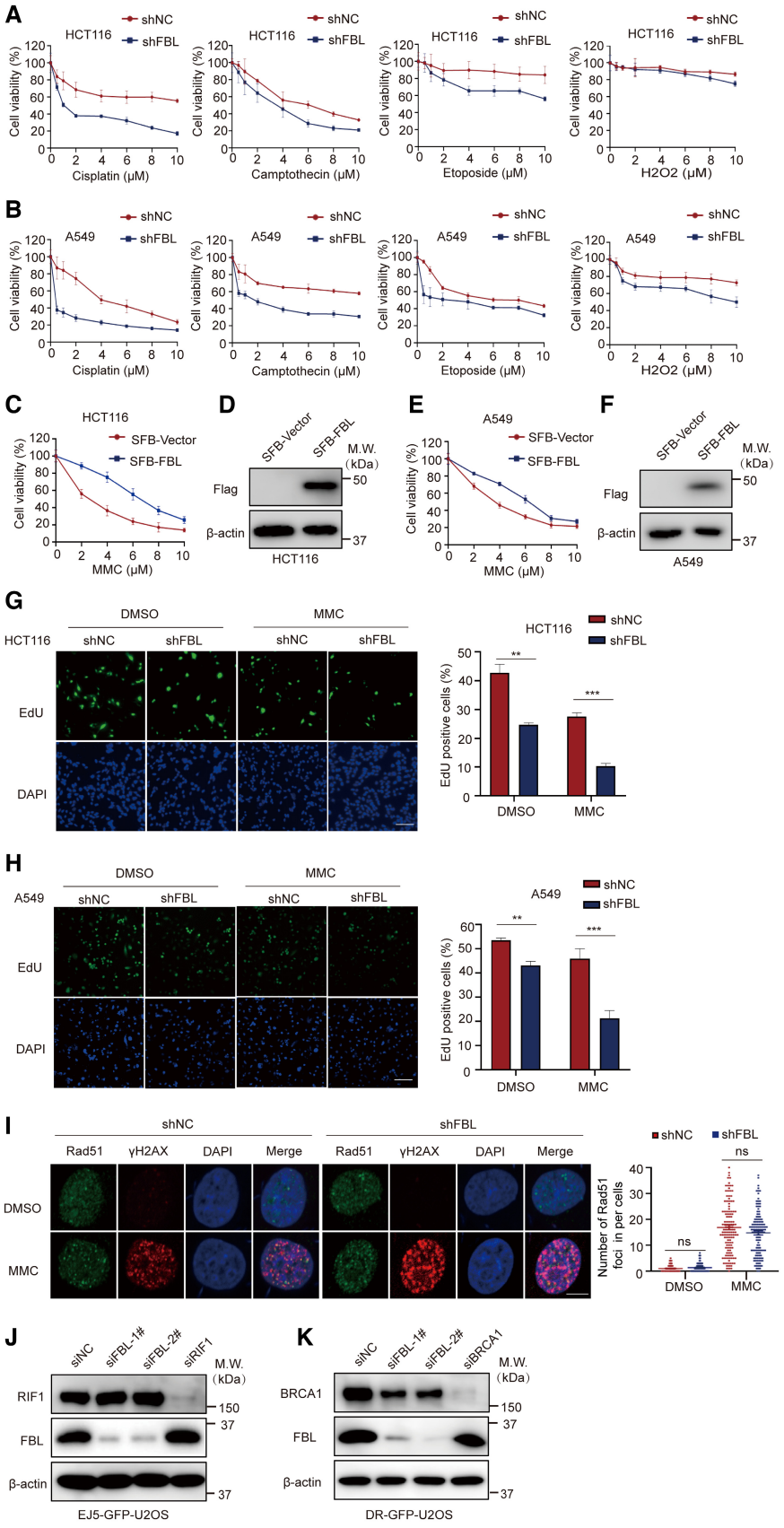

Figure EV2. FBL plays a crucial role in regulating cell sensitivity to DNA damage and HR-mediated DNA repair.

A, B Cell viability analysis was performed on control (shNC) and shFBL-HCT116/shFBL-A549 cells treated with indicated concentrations of cisplatin, etoposide, camptothecin, or H_2O_2 using CCK-8 assays. Each data point represents the mean ± SD from three replicates.

C–F (C, E) FBL overexpression cells are sensitive to MMC. FBL WT or empty vector HCT116/A549 cells were treated with MMC. Cell survival was measured by CCK-8 assay. Each data point represents the mean ± SD from three replicates. (D, F) Cells were reconstituted with full-length FBL in HCT116/A549 cells. Protein expressions were examined by western blot with indicated antibodies.

G, H Immunofluorescence analysis of 5-ethynyl,2′-deoxyuridine (EdU) incorporation and quantitative analysis of EdU-positive cells with 5 μM MMC or DMSO treatment. Scale bar, 50 μm. The bar graphs present data as mean ± SD from three independent experiments. The statistical significance was determined using one-way ANOVA. $^{**}P < 0.01$ and $^{***}P < 0.001$.

I Immunofluorescence staining showed the formation of γH2AX and Rad51 foci in shFBL and shNC HCT116 cells treated with 5 μM MMC or mock treatment. The scale bar represents 10 μm. The right panel displays quantification of the left panel: indicating the number of Rad51 foci per cell ($n \geq 100$ cells). The bar graphs present data as mean ± SD from three independent experiments. The statistical significance was determined using one-way ANOVA. ns. no significance.

J, K Western blotting analysis of FBL levels in FBL knockdown EJ5-GFP-U2OS/DR-GFP-U2OS cells.

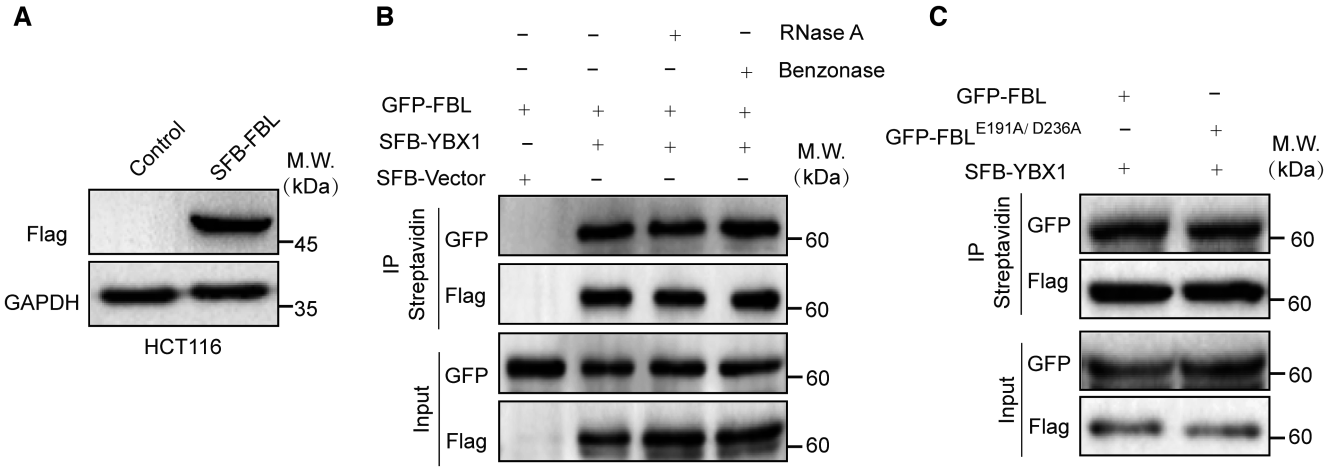

Figure EV3. FBL interacts with YBX1 independent of DNA or RNA and its methyltransferase activity.

A To establish cell lines stably expressing epitope-tagged proteins, HCT116 cells were transfected with plasmids encoding SFB-FBL. Protein expressions were examined by western blotting with indicated antibodies.

B The interaction between FBL and YBX1 is not mediated by DNA or RNA. The association between FBL and YBX1 was examined by coimmunoprecipitation assays in HCT116 cells with RNase A or benzonase treatment.

C The catalytically inactive mutant of FBL also binds YBX1 *in vivo*. Co-IP analyses of GFP-FBLE191A/D236A and SFB-YBX1 in HCT116 cells.

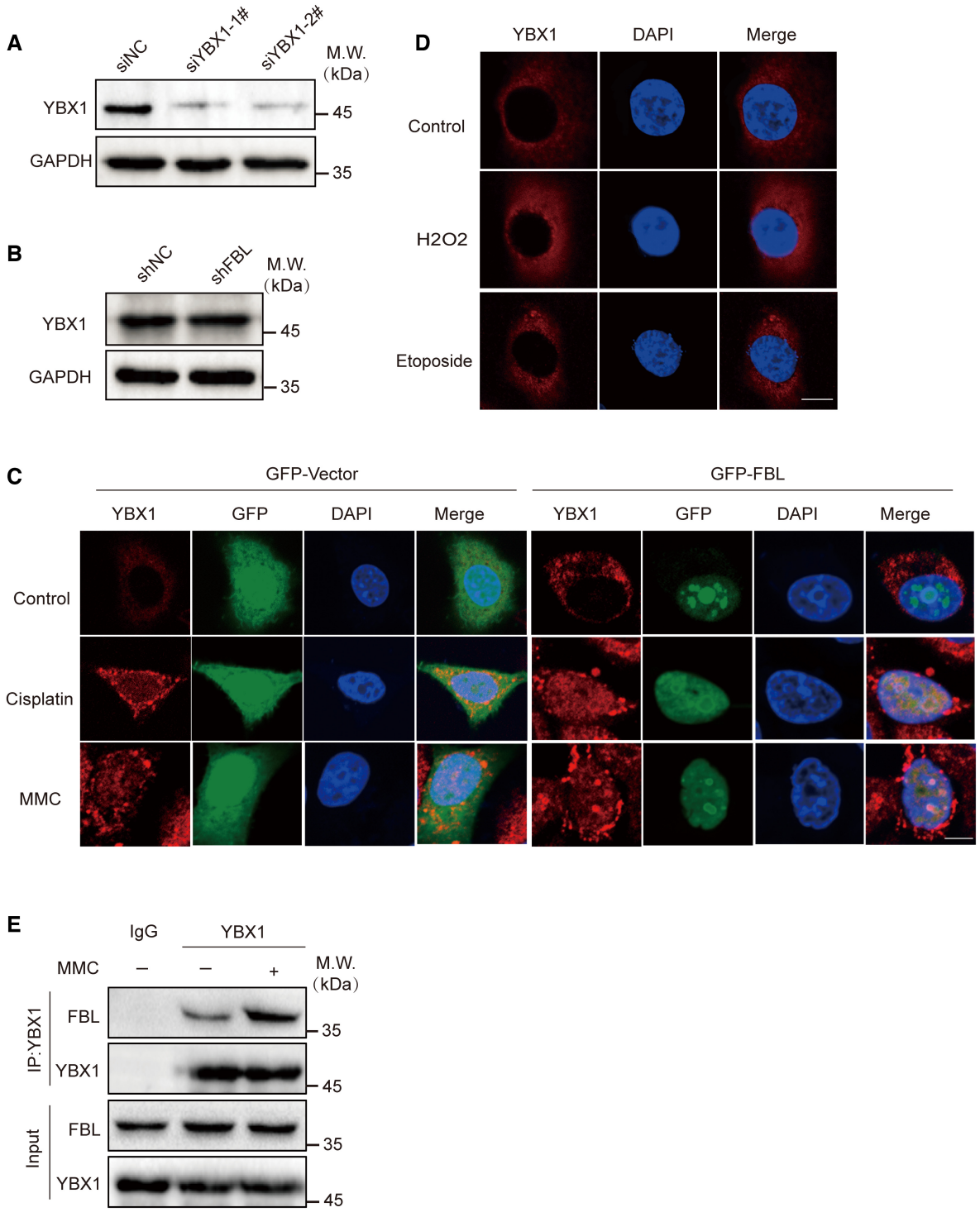

Figure EV4. The effects of FBL on the expression/subcellular localization of YBX1 and the increased interaction between YBX1 and FBL induced by MMC.

A Western blotting analysis of YBX1 levels in YBX1-knockdown HCT116 cells. GAPDH was used as the protein-loading control.

B Western blotting analysis of total YBX1 proteins in FBL KD (shFBL) HCT116 cells. GAPDH was used as the protein-loading control.

C Subcellular localization of YBX1/FBL was confirmed by immunofluorescence assay after MMC (5 μM, overnight) or cisplatin (25 μM, 2 h) treatment in FBL WT or empty vector cells. Scale bar, 10 μm.

D Subcellular localization of YBX1 was confirmed by immunofluorescence assay after H_2O_2 (2 mM, 5 min)/etoposide (10 μM, 6 h) treatment. Scale bar, 10 μm.

E The endogenous association between FBL and YBX1 in HCT116 cells was examined by coimmunoprecipitation assays with 5 μM MMC treatment. Cells were lyzed with NETN300 buffer, and nuclear lysates were examined by IP and western blot with indicated antibodies.

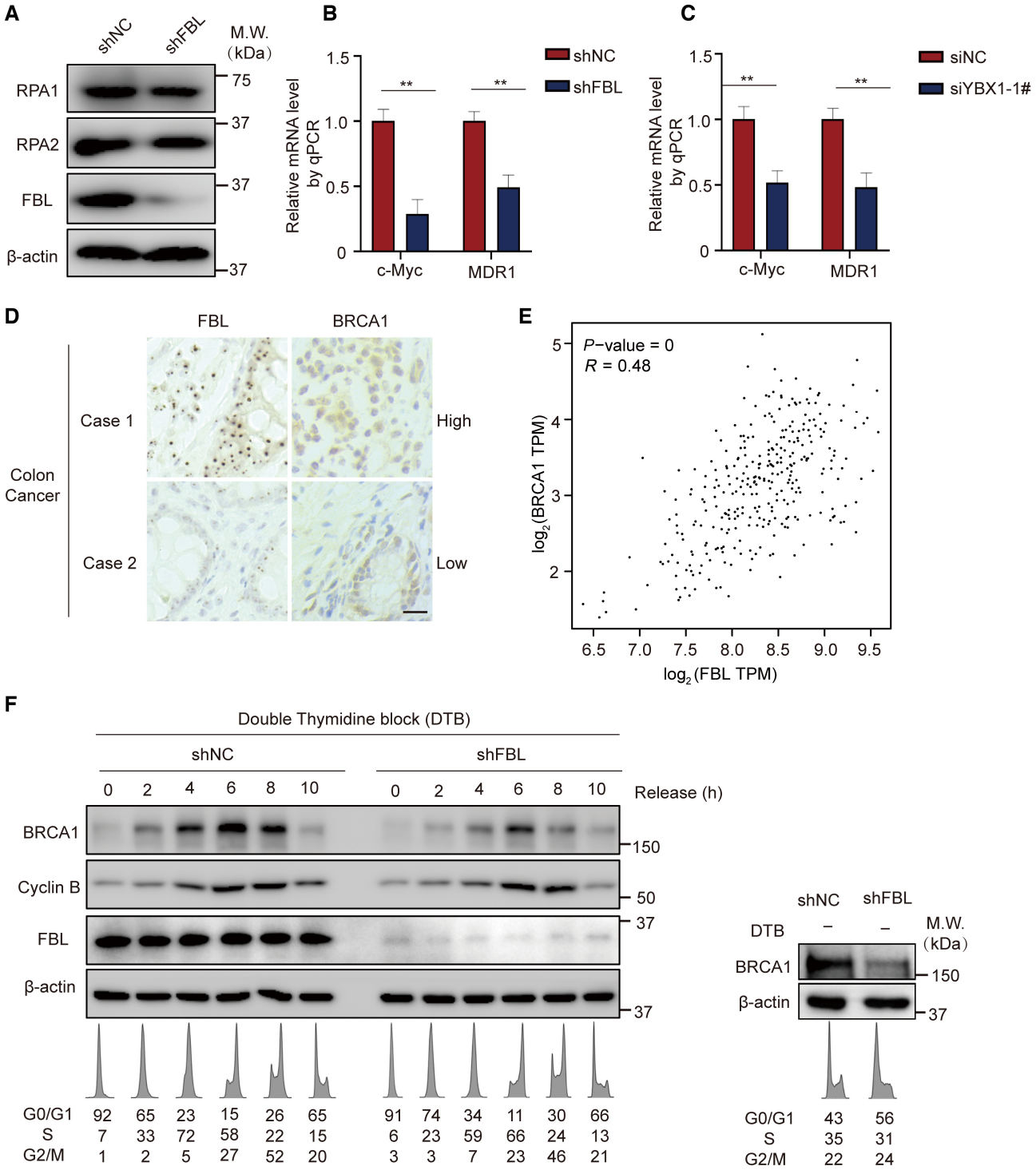
A
shNC
shFBL
M.W. (kDa)
RPA1
RPA2
FBL
β-actin
75
37
37
37
B
shNC
shFBL
Relative mRNA level by qPCR
1.5
1.0
0.5
0
**
**
c-Myc
MDR1
C
siNC
siYBX1-1#
Relative mRNA level by qPCR
1.5
1.0
0.5
0
**
**
c-Myc
MDR1
D
FBL
BRCA1
Colon Cancer
Case 1
Case 2
High
Low
E
P−value = 0
R = 0.48
log2(BRCA1 TPM)
5
4
3
2
6.5
7.0
7.5
8.0
8.5
9.0
9.5
log2(FBL TPM)
F
Double Thymidine block (DTB)
shNC
shFBL
0 2 4 6 8 10
0 2 4 6 8 10
Release (h)
BRCA1
Cyclin B
FBL
β-actin
150
50
37
37
G0/G1 92 65 23 15 26 65 91 74 34 11 30 66
S 7 33 72 58 22 15 6 23 59 66 24 13
G2/M 1 2 5 27 52 20 3 3 7 23 46 21
shNC
shFBL
DTB – –
M.W. (kDa)
BRCA1
β-actin
150
37
G0/G1 43 56
S 35 31
G2/M 22 24

Figure EV5.

Figure EV5. Effects of FBL or YBX1 on other genes expression and the impact of FBL on the cell cycle process.

A Western blotting analysis of RPA1 and RPA2 proteins in FBL KD (shFBL) HCT116 cells. β-actin was used as the protein-loading control.

B, C The mRNA levels of c-Myc or MDR1 were validated by RT–qPCR after FBL or YBX1 silencing in HCT116 cells. Ct values were normalized to GAPDH. A mean value ± SD of three independent experiments is shown. Student's *t*-test. $^{**}P < 0.01$.

D The correlation of FBL and BRCA1 gene expressions. Representative images of immunohistochemical analysis of FBL and BRCA1 expressions in 40 human colon tumors. Scale bar, 25 μm.

E The correlation analysis of FBL and BRCA1 gene expressions obtained from GEPIA database (http://gepia.cancer-pku.cn/). TPM stands for transcripts per million; R stands for correlation coefficient. Each spot indicates FBL and BRCA1 expression in a specific sample.

F The levels of BRCA1 protein fluctuate during cell cycle progression, with its expression being regulated by FBL. HCT116 cells and shFBL-HCT116 cells were grown asynchronously (right panel) or were synchronized at the G1/S transition by a double thymidine block (DTB) and then released by addition of fresh medium (left panel). At various time points post-release, cell extracts were prepared and analyzed by western blotting using indicated antibodies. Cyclin B serves as a marker of S, G2, and M phases. Similarly, cells induced at specific time points were subjected to flow cytometry analysis. The resulting flow cytometry profiles and the cell cycle distributions are listed below the immunoblots. β-actin was used as the protein-loading control.