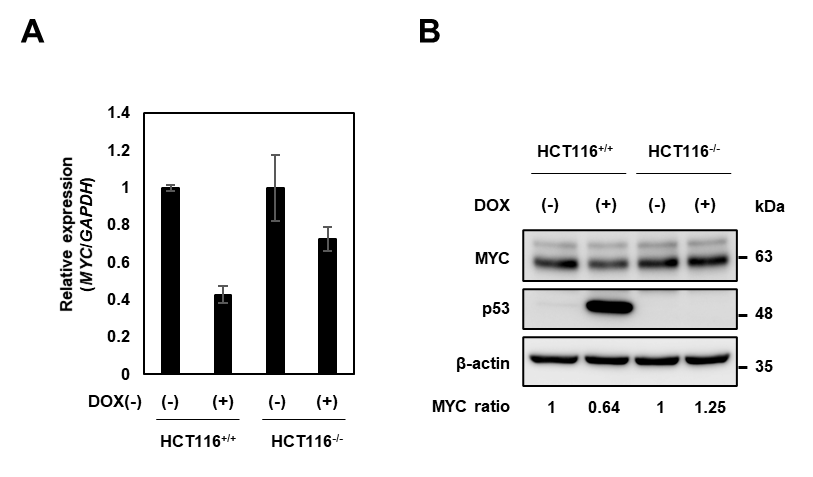
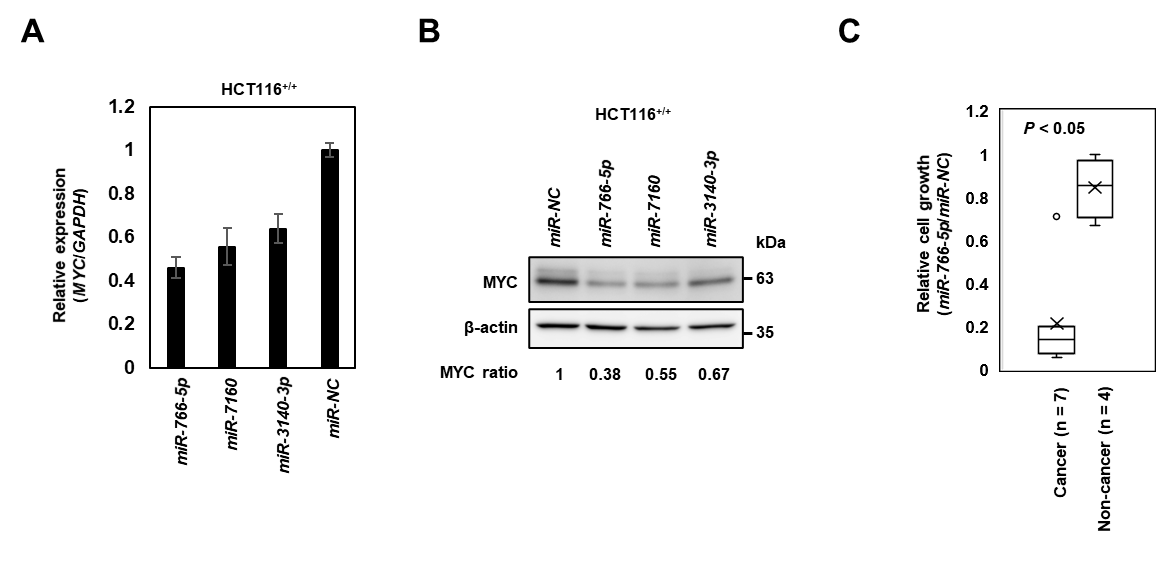
**Figure S1**



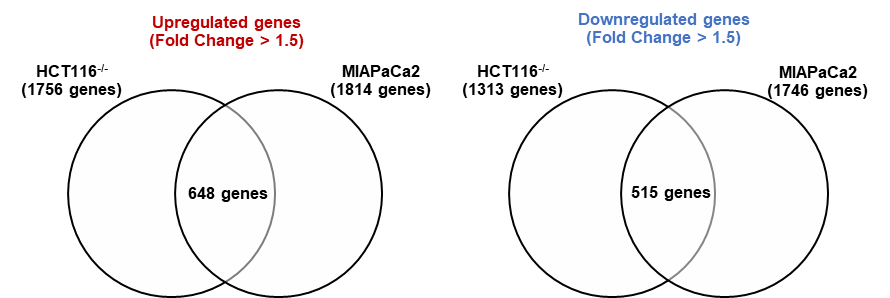
**(A)** qRT-PCR of *MYC* mRNA in HCT116+/+ and HCT116-/- cells after treatment with doxorubicin (0.5 µg/mL) for 24 hours. *GAPDH* was an internal control. Bar, SD for triplicate experiments. **(B)** Western blot analysis of MYC and p53 in HCT116+/+ and HCT116-/- cells after treatment with doxorubicin (0.5 µg/mL) for 24 hours.

**Figure S2**



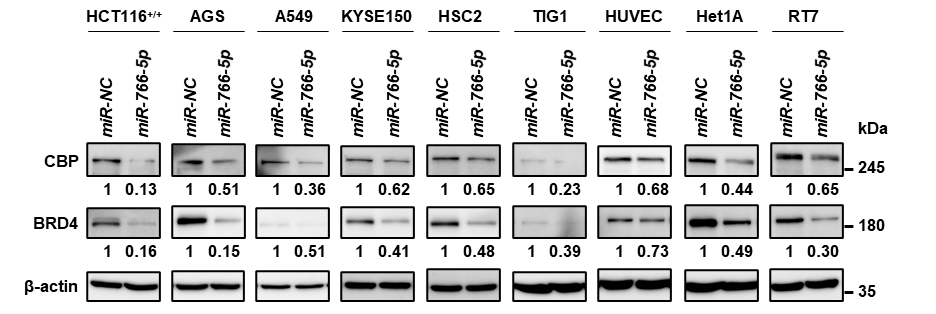
**(A)** qRT-PCR of *MYC* mRNA in HCT116+/+ cells 48 hours after transfection with indicated miRs. *GAPDH w*as an internal control. Bar, SD for triplicate experiments. **(B)** Western blot analysis of MYC in HCT116+/+ cells 48 hours after transfection with indicated miRs. **(C)** Relative number of cancer cells or non-cancer cells 96 hours (at day 4) after transfection with 10 nmol/L of *miR-766-5p* in Figure 1F. The cell growth rate was evaluated using a relative ratio compared with that of *si-NC*-transfected cells.

**Figure S3**



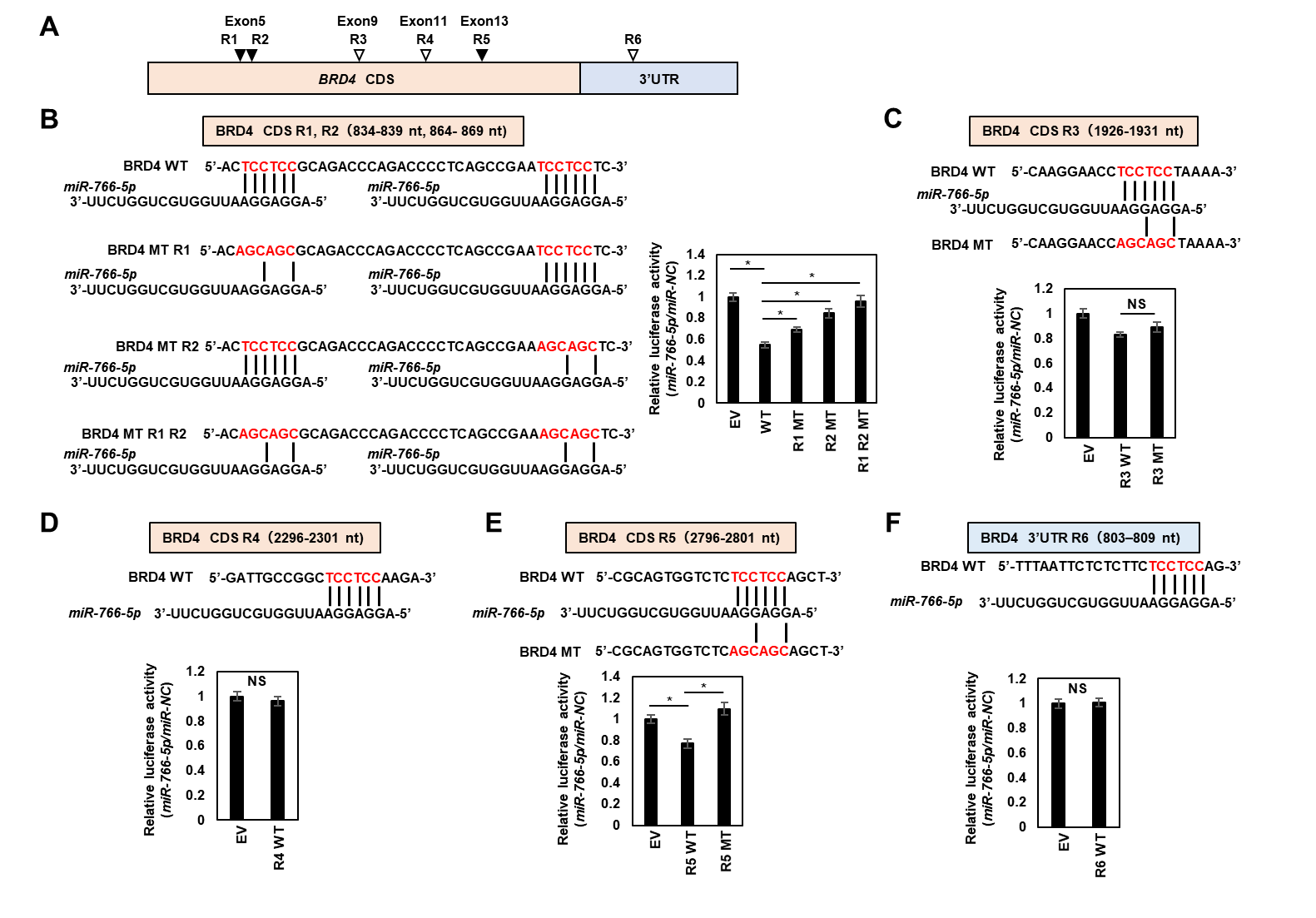
The Venn diagram shows that 648 genes were commonly upregulated (fold change >1.5) (left) and 515 genes were commonly downregulated (fold change >1.5) (right) by transfection of *miR-766-5p* in HCT116-/- and MIAPaCa2 cells.

**Figure S4**



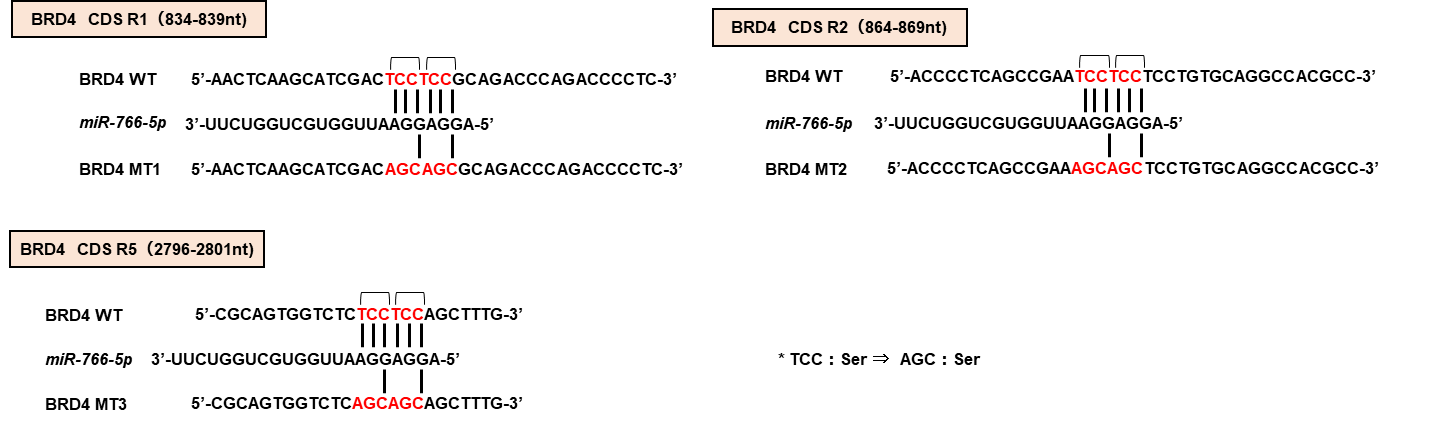
Western blot analysis of CBP and BRD4 in indicated cells 48 hours after transfection with 10 nmol/L of *miR-NC* or *miR-766-5p*. The numbers under the blots correspond to densitometric analysis of each protein normalized to β-actin. The results are expressed as fold change relative to the *miR-NC* control.

**Figure S5**



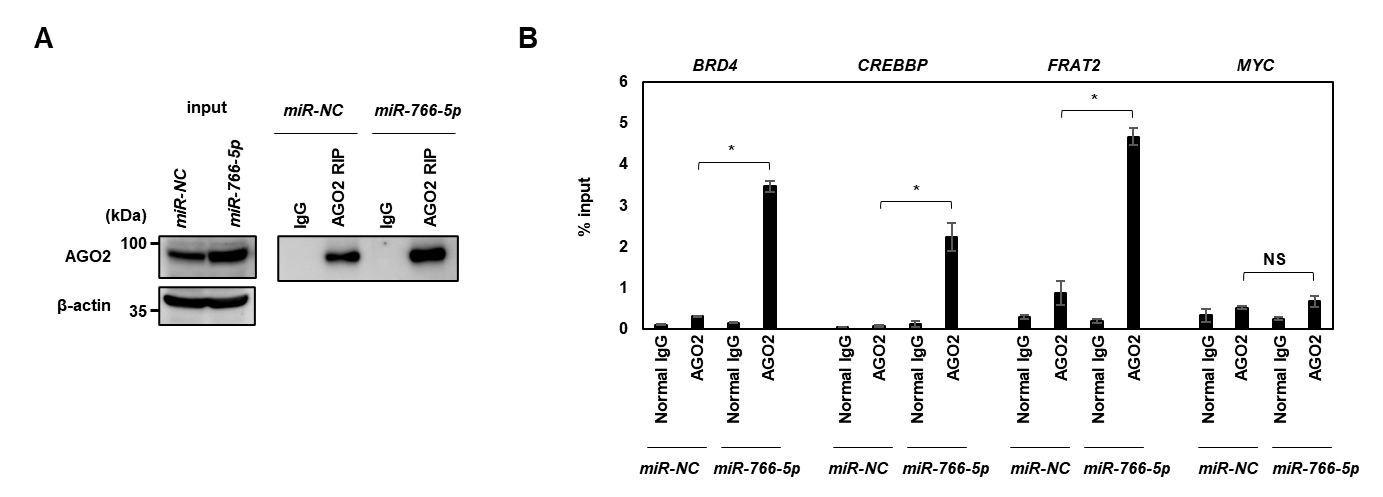
**(A)** R1, R2, R3, R4, R5, and R6 indicate putative binding sites of each *miR-766-5p* within the coding sequence (CDS) and 3’ UTR of *BRD4*. **(B-F)** Luciferase reporter assay. HCT116-/- cells were transfected with the pmirGLO Dual Luciferase vectors containing wild-type (WT) or MT BRD4, or empty vector (EV), and after 6 hours, either *miR-NC* or *miR-766-5p* was additionally transfected. Putative binding sequence of *miR-766-5p* within the CDS of BRD4 and mutant sequences are indicated. Graphs show the results of luciferase assay; NS, not significant. \**P* < 0.05.

**Figure S6**



Putative binding sites of *miR-766-5p* within the coding sequence of *BRD4* and synonymous mutation sequences. Ser, Serine.

**Figure S7**

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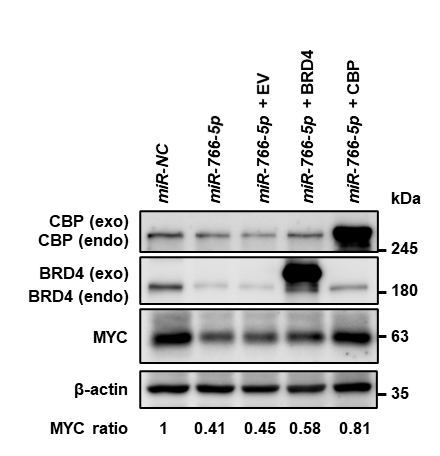
**(A)** RNA immunoprecipitation (RIP) analysis using AGO2 antibody. Western Blot analysis of proteins immunoprecipitated with anti-AGO2 antibody in HCT116-/- cells transfected with *miR-NC* or *miR-766-5p*. IgG was used as a negative control. **(B)** RIP-PCR analysis of the *BRD4*, *CREBBP*, *FRAT2* and *MYC* gene. Relative RIP enrichment values in the indicated genes are expressed as percentages relative to input DNA. Bar, SD for triplicate experiments. \* *P* < 0.05 (One-way ANOVA with Bonferroni adjustment).

**Figure S8**



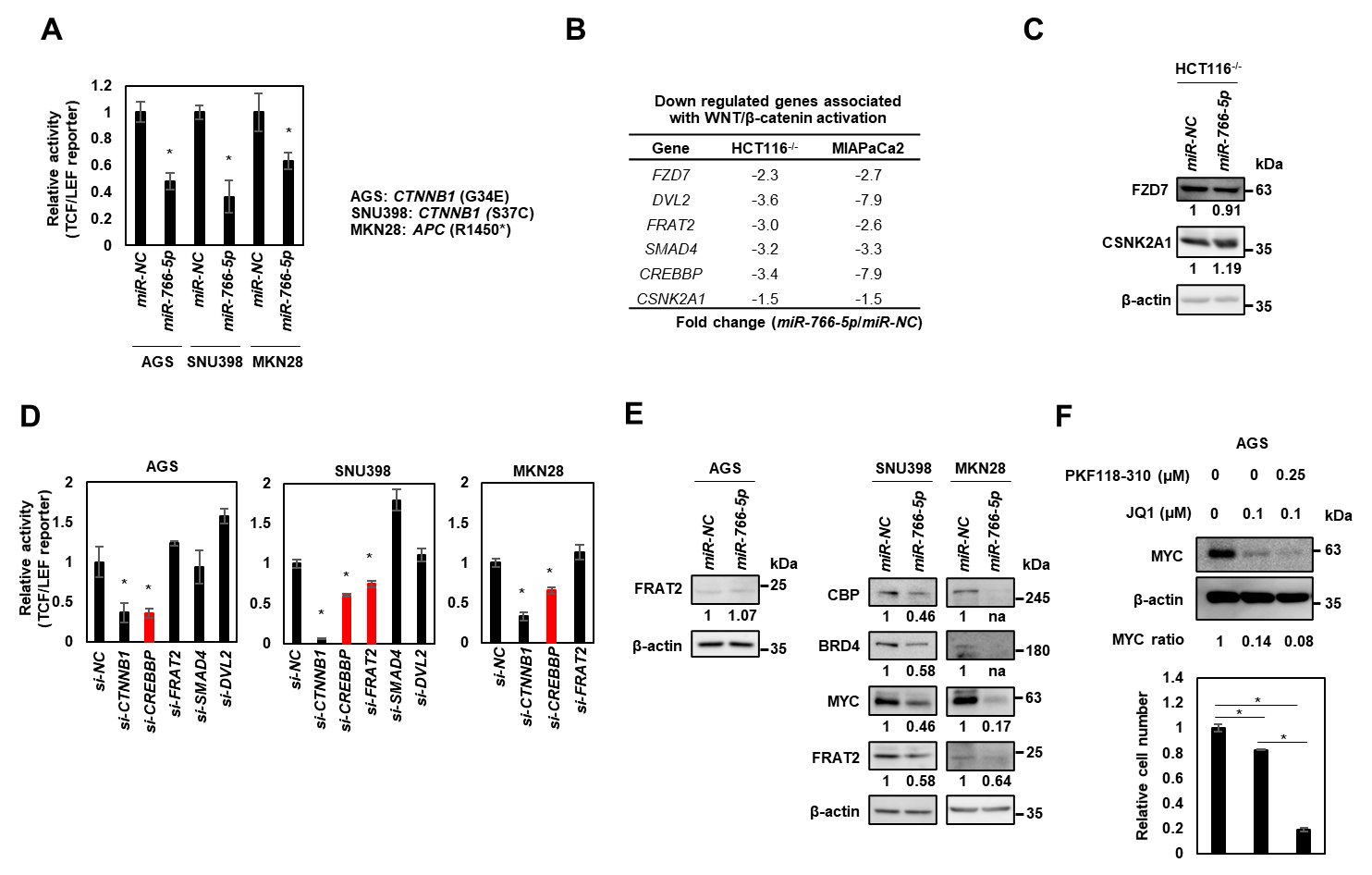
**(A)** Western blot analysis of CBP, BRD4, and MYC in the indicated cell lines 48 hours after transfection with siRNA (*si-NC* (40 nmol/L); *si-BRD4*, *si-CREBBP*, *si-BRD4*, and *si-CREBBP* (each 20 nmol/L)).The intensity of MYC bands was quantified by densitometry using Image J software and shown as the fold change after normalization with β-actin. **(B)** Cell growth assay in the indicated cell lines after transfection with siRNA (*si-NC* (40 nmol/L); *si-BRD4*, *si-CREBBP*, *si-BRD4*, and *si-CREBBP* (each 20 nmol/L)). Seventy-two hours after transfection with the siRNAs, the cell growth rate was assessed by the CV staining assay using a relative ratio compared with that of *si-NC*-transfected cells. Error bar, SD for triplicate experiments. NS, not significant. \**P* < 0.05.

**Figure S9**

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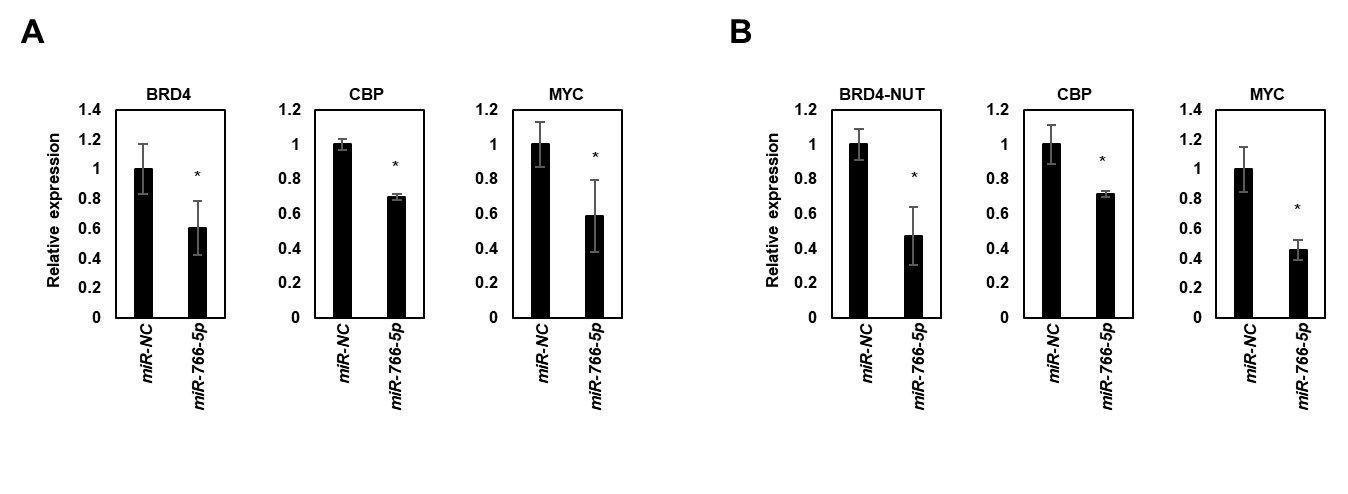
Western blot analysis of CBP, BRD4, MYC, and β-actin. *miR-NC* or *miR-766-5p* was transfected into HCT116-/- cells and followed by transfection with the CBP or BRD4 expression vector or the empty vector. CBP expression vector does not contain its 3’UTR and BRD4 expression vector harbor three synonymous mutations as indicated in **Fig. S6** so that *miR-766-5p*-targeted regions were not contained in these vectors.

**Figure S10**



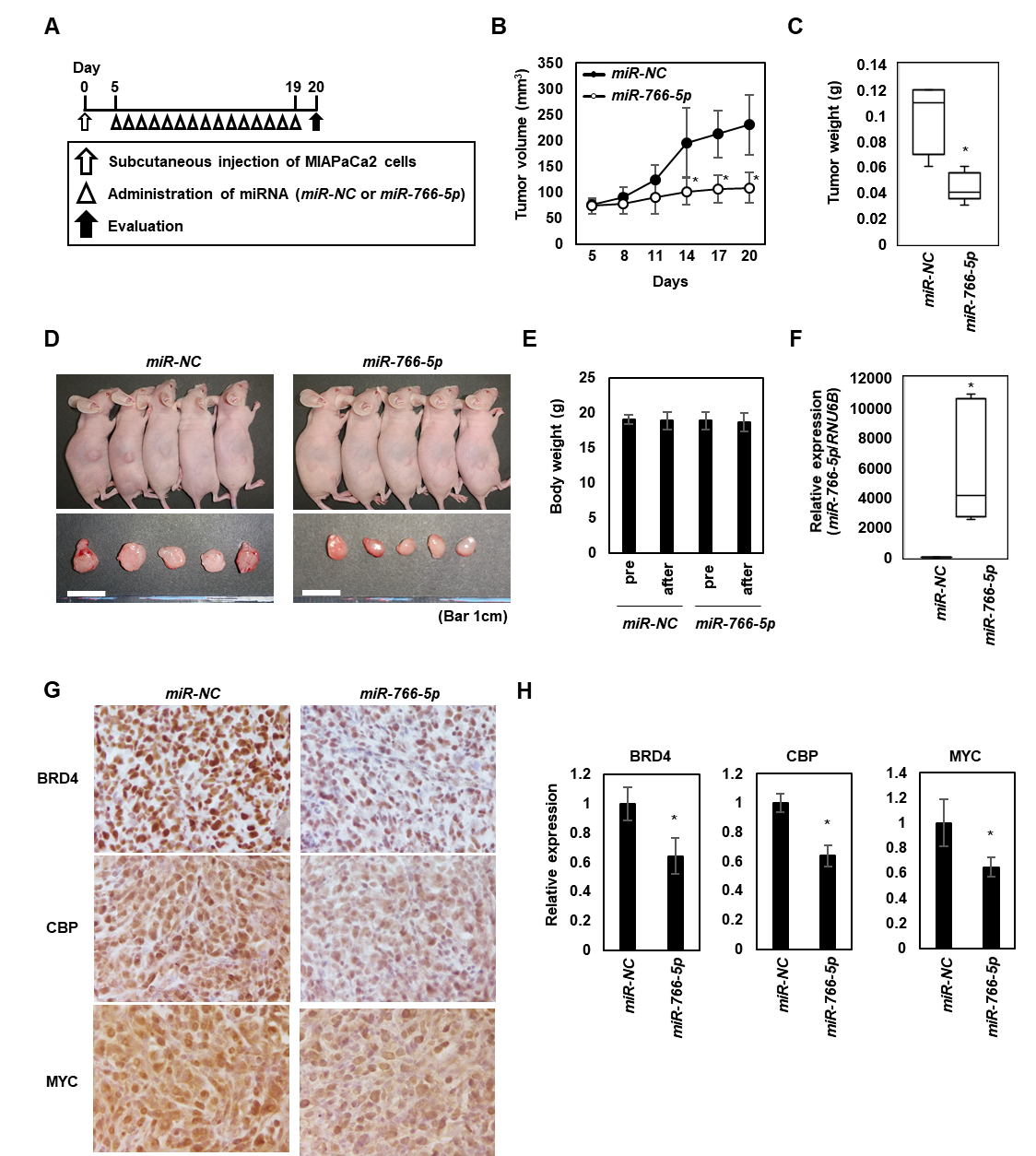
**(A)** TCF/LEF reporter assay in AGS, SNU398, and MKN28 cells transfected with 10 nmol/L of *miR-NC* or *miR-766-5p*. The mutation status of *CTNNB1* and *APC* was based on Cancer Cell Line Encyclopedia (CCLE, https://portals.broadinstitute.org/ccle). Bar, SD for triplicate experiments. \* *P* < 0.05. **(B)** The table shows the genes associated with WNT/β-catenin signaling among the 324 candidate target genes of *miR-766-5p* (see, **Table S5**). **(C)** Western blot analysis of FZD7 and CSNK2A1 in HCT116-/- cells transfected with 10 nmol/L of *miR-NC* or *miR-766-5p*. The numbers under the blots correspond to densitometric analysis of each protein normalized to β-actin. The results are expressed as fold change relative to the *miR-NC* control. **(D)** TCF/LEF reporter assay in AGS, SNU398, and MKN28 cells transfected with 20 nmol/L of *si-NC* or indicated siRNAs. Bar, SD for triplicate experiments. \* *P* < 0.05. **(E)** Western blot analysis of indicated proteins in AGS, SNU398, and MKN28 cells transfected with 10 nmol/L of *miR-NC* or *miR-766-5p*. The numbers under the blots correspond to densitometric analysis of each protein normalized to β-actin. The results are expressed as fold change relative to the *miR-NC* control. **(F)** Western blot analysis (upper) and cell growth assay (lower) of AGS cells after treatment with DMSO, JQ1, or the combination of JQ1 and PKF118-310 (β-catenin inhibitor). The cell growth rate was assessed by crystal violet staining using a relative ratio compared with that of DMSO-treated cells. Bar, SD for triplicate experiments. \* *P* < 0.05.

**Figure S11**



Evaluation of immunohistochemistry in the xenograft model of HCT116-/-. The relative expression of each protein by immunohistochemistry was calculated using the immunohistochemistry image analysis toolbox developed in ImageJ, and the results were normalized to the values of tumors treated with *miR-NC*. \**P* < 0.05.

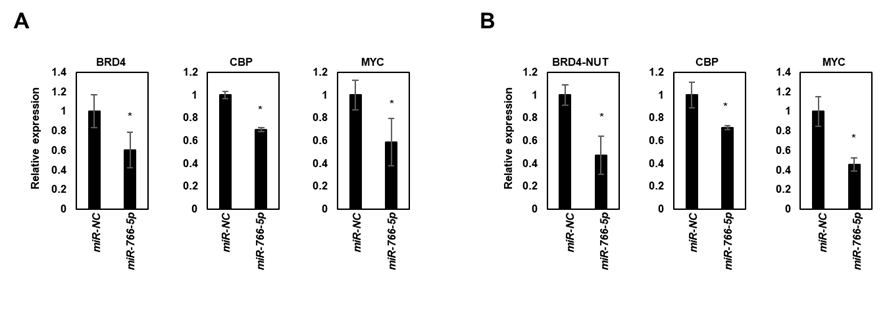
**Figure S12**



**(A)** The experimental schedule for *miR-766-5p* treatment using the ionic liquid transdermal system (ILTS) in nude mice. On day 5 after inoculation of MIAPaCa2 cells, the local administration of *miR-NC* or *miR-766-5p* to subcutaneous tumors was initiated. **(B)** Tumor growth curves of xenograft mouse models treated with *miR-NC* or *miR-766-5p* (n = 5, each). The tumor volume was calculated using the following formula: (shortest

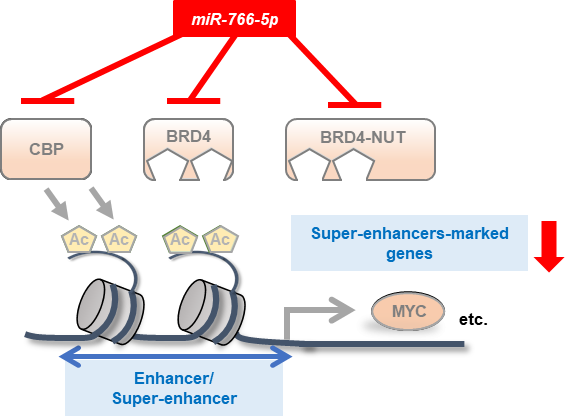
diameter) 2 × (longest diameter) × 0.5. Bar, SD for 5 mice; \**P* < 0.05. **(C)** Weights of the resected tumors. Tumor weights are shown as a box plot. Bar, SD for 5 mice; \**P* < 0.05. **(D)** Representative images of tumor-bearing nude mice and resected tumors at 20 days after the injection of MIAPaCa2 cells. Scale bar, 10 mm. **(E)** Body weights on day 5 (before the treatment) and on day 20 (after the treatment) were measured. **(F)** qRT-PCR of *miR-766-5p* in the resected tumors. Each experiment was performed in triplicate. The relative ratio was normalized to the expression of *RNU6B*. Bar, SD for 5 mice; \**P* < 0.05. **(G)** Representative images of immunohistochemical staining for BRD4, CBP, and MYC in resected tumors. Original magnification, ×200. **(H)** The relative expression of each protein by immunohistochemistry in **(G)** was calculated using the immunohistochemistry image analysis toolbox developed in ImageJ. The positive-staining area was calculated in each of three images of sections from three tumors treated with *miR-NC* or *miR-766-5p*. The results were normalized to the mean positive-staining area of tumors treated with *miR-NC*. \**P* < 0.05.

**Figure S13**

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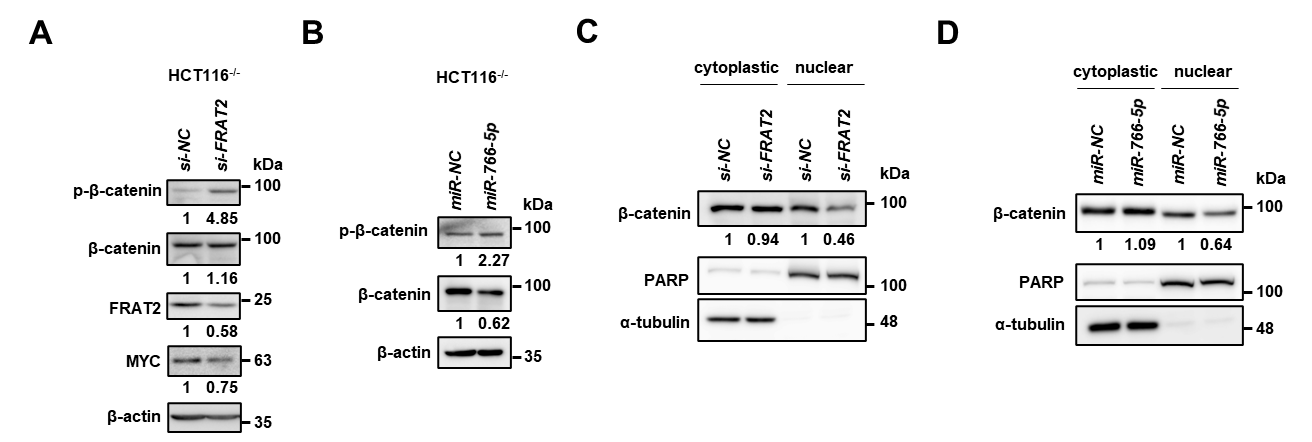
Evaluation of immunohistochemistry in the xenograft model of Ty82-JQ1R. The relative expression of each protein by immunohistochemistry was calculated using the immunohistochemistry image analysis toolbox developed in ImageJ, and the results were normalized to the values of tumors treated with *miR-NC*. \**P* < 0.05.

**Figure S14**

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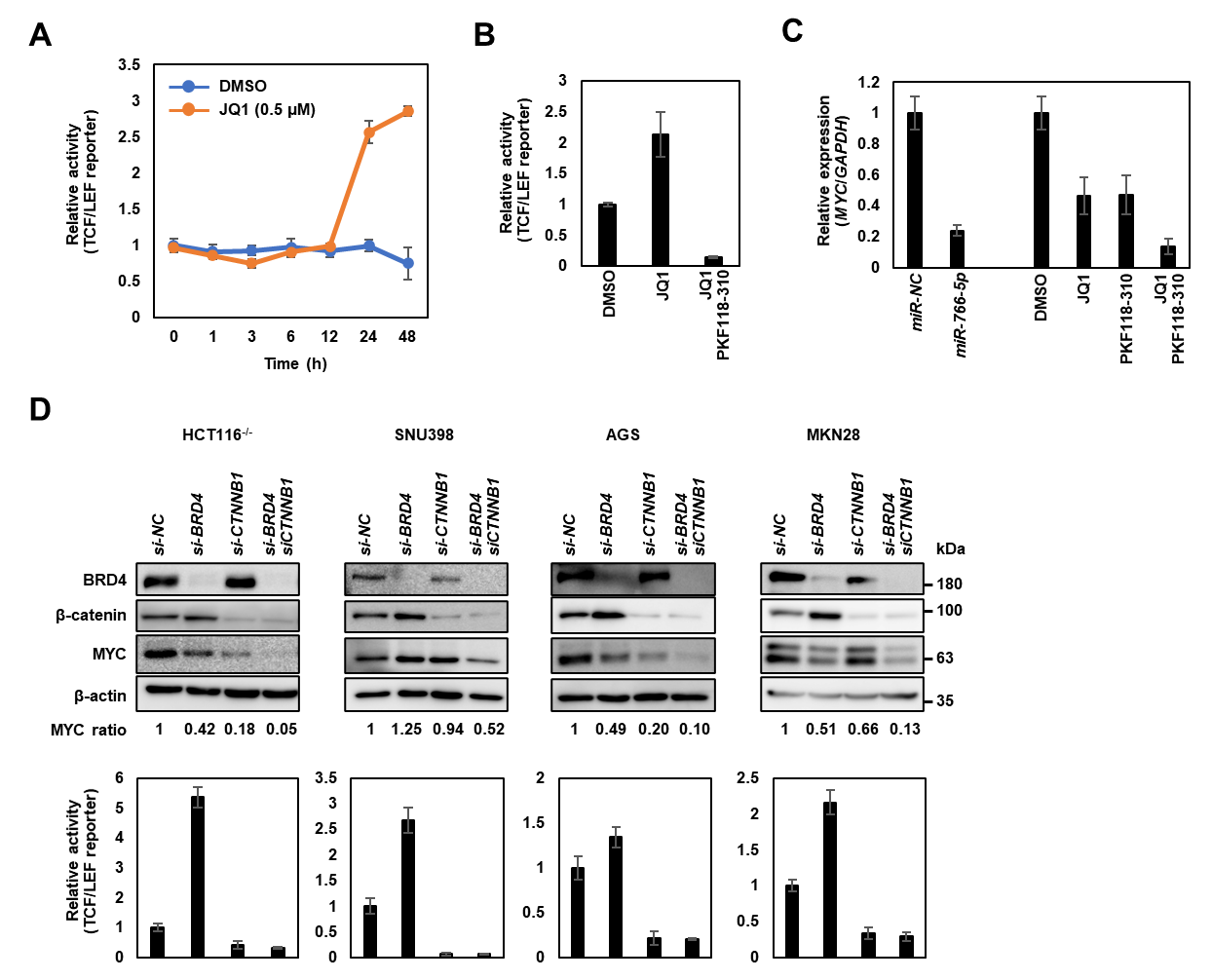
Schematic models of the mechanism by which *miR-766-5p* suppresses MYC expression.

**Figure S15**

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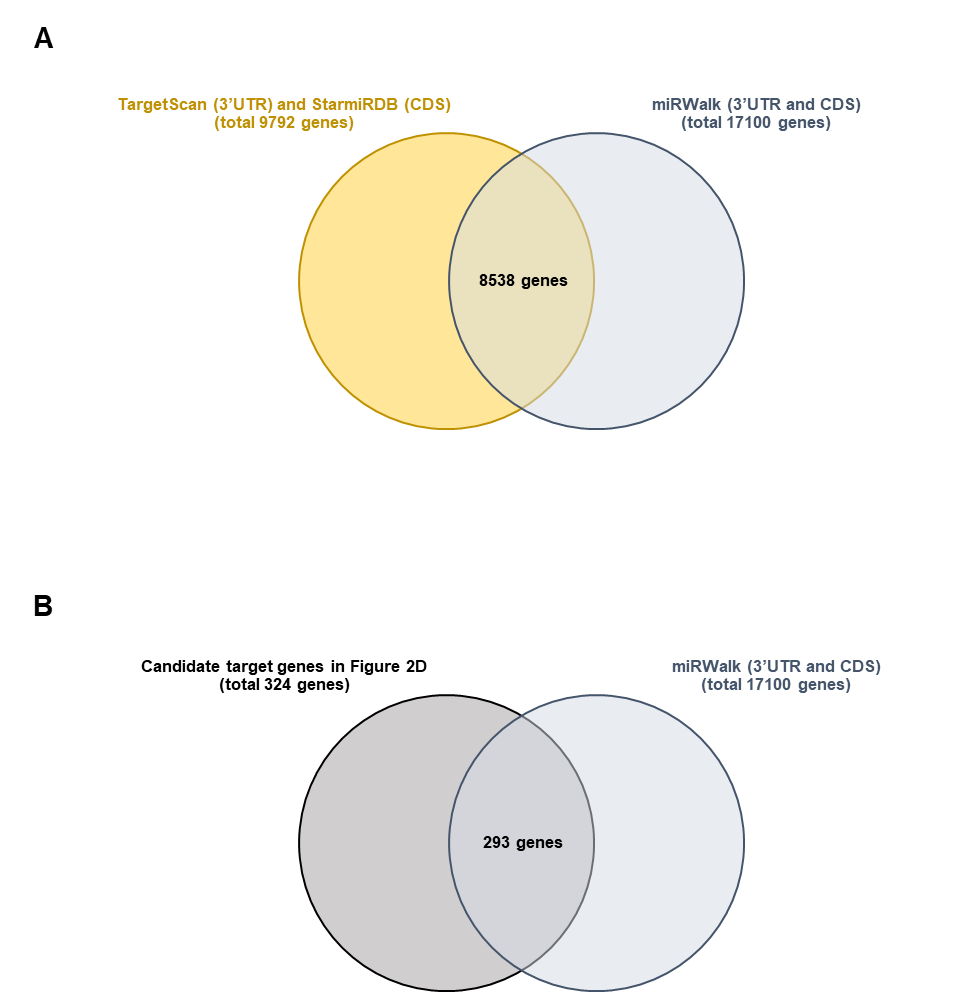
**(A, B)** Western blot analysis of indicated proteins in HCT116-/- cells transfected with 20 nmol/L of *si-NC* or *si-FRAT2* (A), or 10 nmol/L of *miR-NC* or *miR-766-5p* (B). The numbers under the blots correspond to densitometric analysis of each protein normalized to β-actin. The results are expressed as fold change relative to the *si-NC* or *miR-NC* control. **(C, D)** Cytoplasmic and nuclear extracts of HCT116-/- cells transfected with 20 nmol/L of *si-NC* or *si-FRAT2* (C), or 10 nmol/L of *miR-NC* or *miR-766-5p* (D) were analyzed for β-catenin by Western blotting. PARP and α-tubulin were used as a nuclear and cytoplasmic marker, respectively. The numbers under the blots correspond to densitometric analysis of each protein normalized to α-tubulin or PARP. The results are expressed as fold change relative to the *si-NC* or *miR-NC* control.

**Figure S16**

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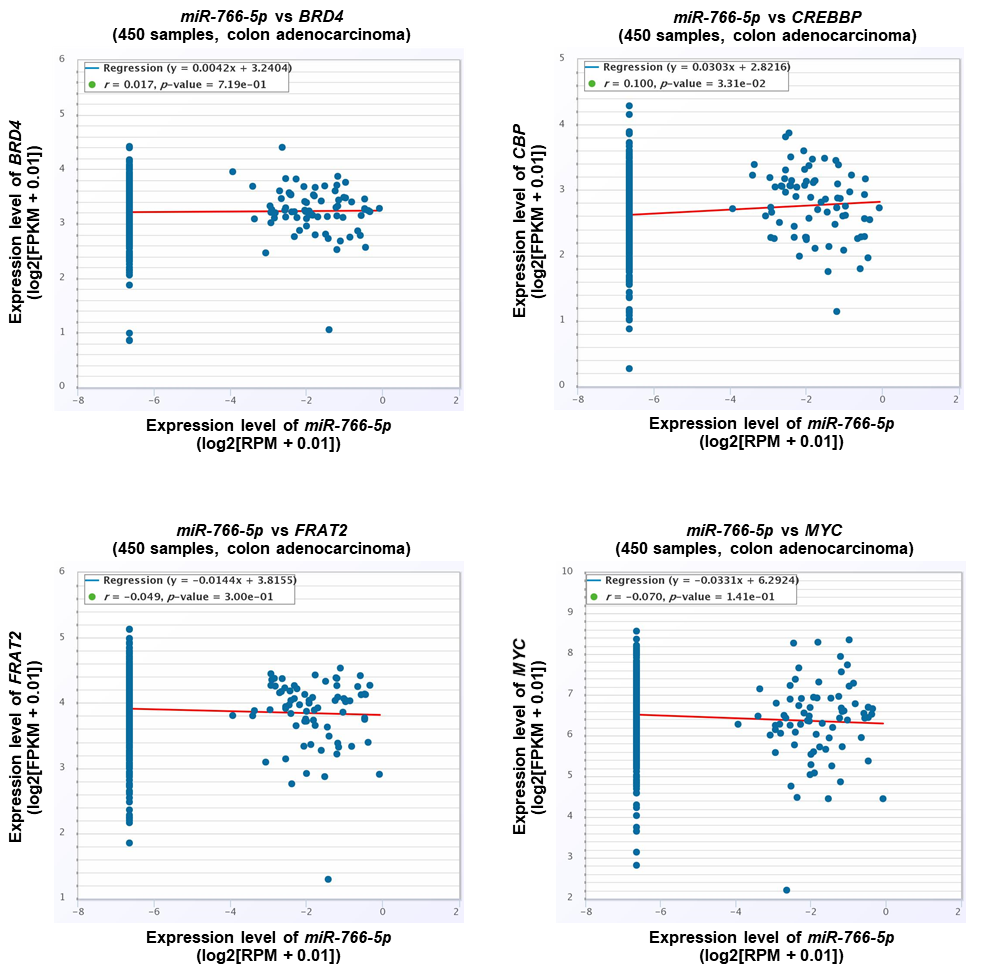
**(A)** TCF/LEF reporter assay in HCT116-/- cells treated with DMSO or 0.5 µmol/L of JQ1. TCF/LEF reporter activity was calculated as a relative ratio compared with time 0. Bar, SD for triplicate experiments. **(B)** TCF/LEF reporter assay in HCT116-/- cells treated with DMSO, JQ1 (0.5 µmol/L), or JQ1 and PKF118-310 (each 0.5 µmol/L) for 24 hours. The results were normalized to the values of cells treated with DMSO. **(C)** qRT-PCR for MYC in HCT116-/- cells 48 hours after treatment with DMSO, JQ1 (0.5 µmol/L), or JQ1 and PKF118-310 (each 0.5 µmol/L). The results were normalized to the values of cells treated with DMSO. Bar, SD for triplicate experiments. **(D)** Western blot analysis (upper) and TCF/LEF reporter assay (lower) of the indicated cells after transfection with siRNA (*si-NC* (40 nmol/L); *si-BRD4*, *si-CTNNB1*, *si-BRD4*, and *si-CTNNB1* (each 20 nmol/L)). The TCF/LEF activity was calculated using a relative ratio compared with that of *si-NC* transfected cells. Bar, SD for triplicate experiments.

**Figure S17**

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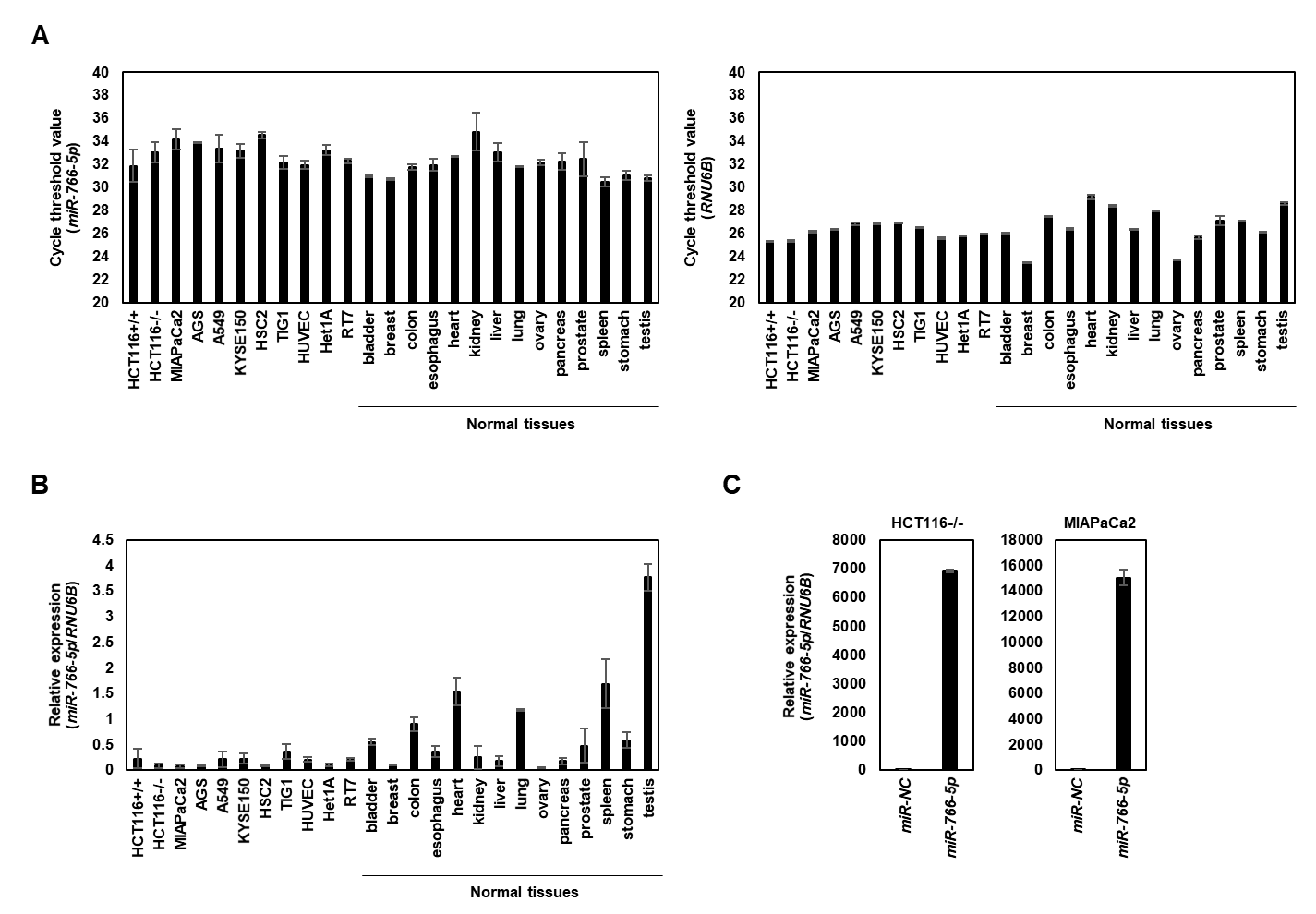
**(A)** Prediction of candidate targets regulated by *miR-766-5p* via their 3’UTR or coding sequences (CDS). The Venn diagram shows that 9792 genes or 17100 genes were predicted as candidate target genes of *miR-766-5p* by the TargetScan and STarMirDB database, or miRWalk database (http://mirwalk.umm.uni-heidelberg.de/), respectively. A total of 8538 genes were overlapped in both analyses. (**B)** Among 324 candidate target genes in **Fig. 2D** and **Table S5**, 293 genes were included in the predicted target genes of *miR-766-5p* by miRWalk database.

**Figure S18**

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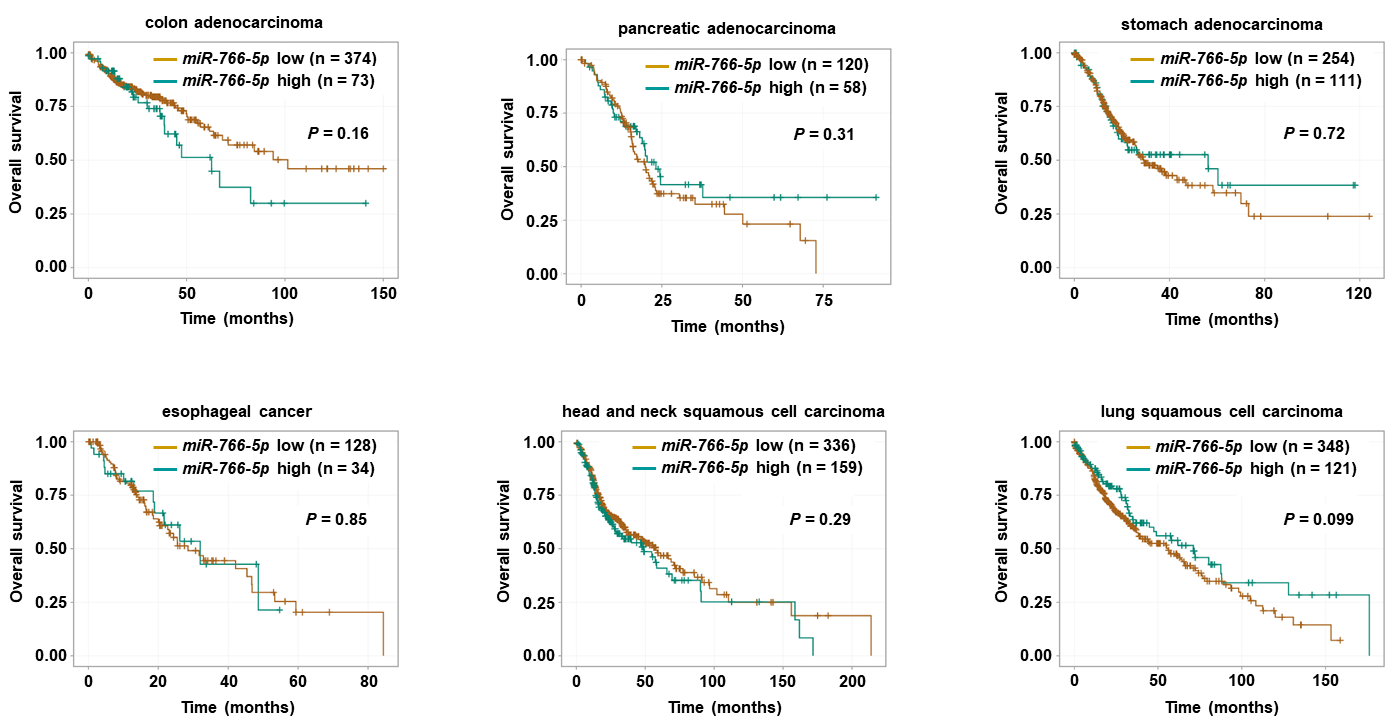
The expression data of *BRD4*, *CREBBP*, *FRAT2*, and *MYC* or *miR-766-5p* in colon adenocarcinoma of TCGA project were obtained from ENCORI database (http://starbase.sysu.edu.cn/index.php). The expression values of each mRNA or *miR-766-5p* from RNA-seq or miRNA-seq were scaled with log2(FPKM + 0.01) or log2(RPM + 0.01), respectively.

**Figure S19**

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**(A, B)** Expression analysis of *miR-766-5p* and *RNU6B* (internal control) in indicated cells and normal tissues using Taqman qRT-PCR (A; cycle threshold values of *miR-766-5p* or *RNU6B*, B; the relative ratio normalized based on the expression of *RNU6B*). Each experiment was performed in triplicate. Bar, SD. **(C)** The expression level of *miR-766-5p* was measured by Taqman qRT-PCR using the relative ratio normalized based on the expression of *RNU6B* in HCT116-/- and MIAPaCa2 cells transfected with 10 nmol/L *miR-NC* or *miR-766-5p*.

**Figure S20**

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Kaplan-Meier curves for overall survival rates of patients with indicated types of cancer in TCGA project. Data were obtained from ENCORI database (http://starbase.sysu.edu.cn).