Figure S1. Screening of candidate miRNAs. (A) A total of 1,749 miRNAs were determined to be targeted to DCLK1 from the TargetScan Human database. Among the 1,749 miRNAs, 30 miRNAs whose target genes were associated with Notch, Wnt/β-catenin or Wnt/Ca^{2+} signaling pathways were extracted for cell viability experiments. (B) Ornithine decarboxylase-degron transduced pancreatic cancer Panc-1 cells were generated as previously described (32-34), and used as a cancer stem cell model to test the effects of these miRNAs on stem [degron (+)] cells and non-stem [degron (-)] cells. Cell viability was evaluated by Cell Counting Kit-8 assay at 72 h after transfection. The cell viability of each group of cells was normalized to that of control cells without transfection. As a putative Anti-OncomiR (5), miR-34a was used as a positive control in this experiment. At 72 h after transfection, among the 30 miRNAs, miR-1291 (the 16th miRNA) significantly inhibited the viability of stem [degron (+)] and non-stem [degron (-)] cell groups compared with either miR-NC group or positive control miR-34a. DCLK1, doublecortin-like kinase 1; OE, overexpressing; miR, microRNA.

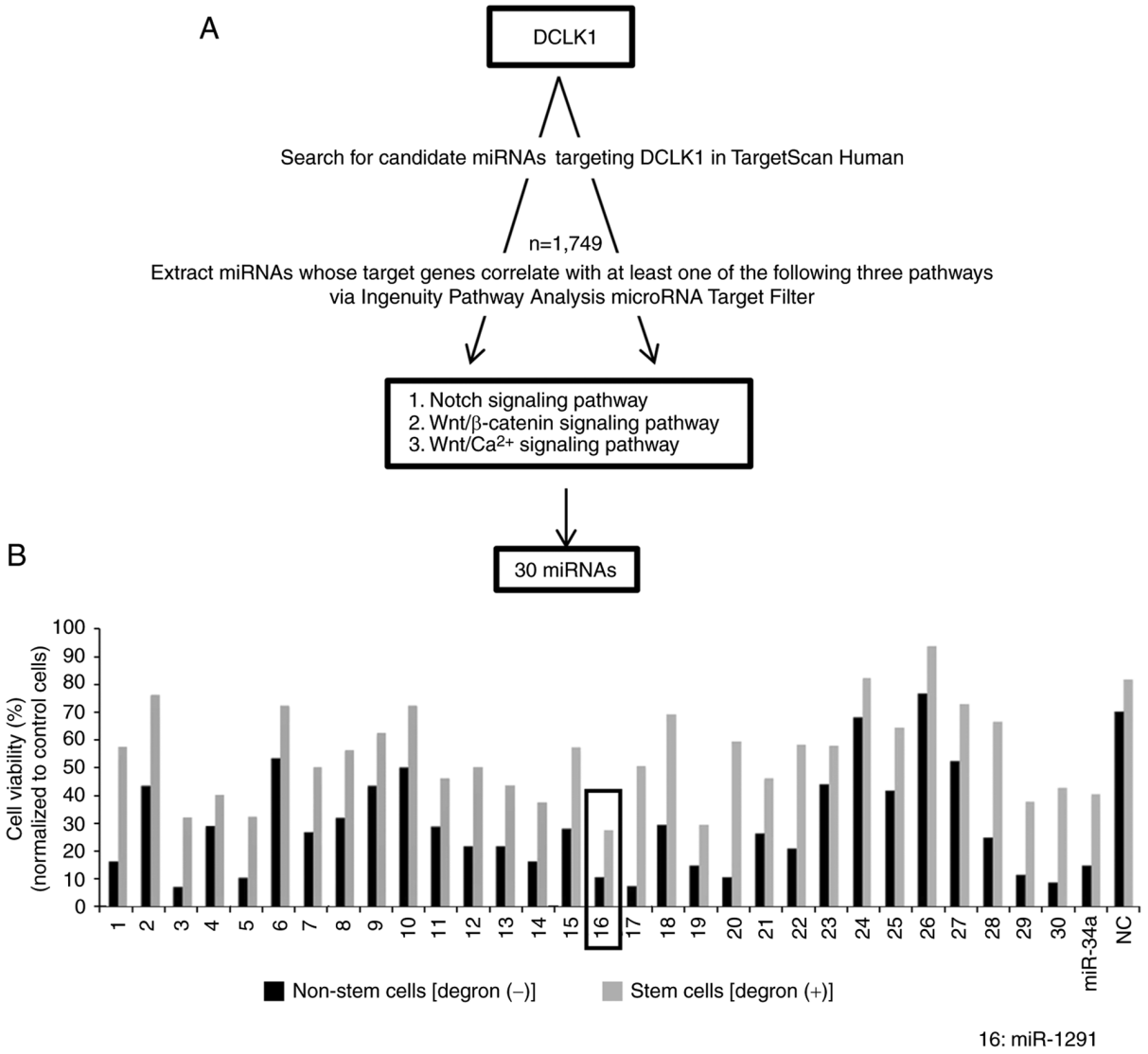

Figure S2. Database analyses for miR-1291 expression levels in human normal tissues. The TissueAtlas (https://ccb-web.cs.uni-saarland.de/tissueatlas/) database was used for analyzing the expression of miRNAs in human normal tissues. (A) miR-1291 expression in human normal colon tissues was ~1/50 of anti-oncomiR (B) miR-34a-5p and 1/1,500 of oncomiR (C) miR-21-5p. miR, microRNA.

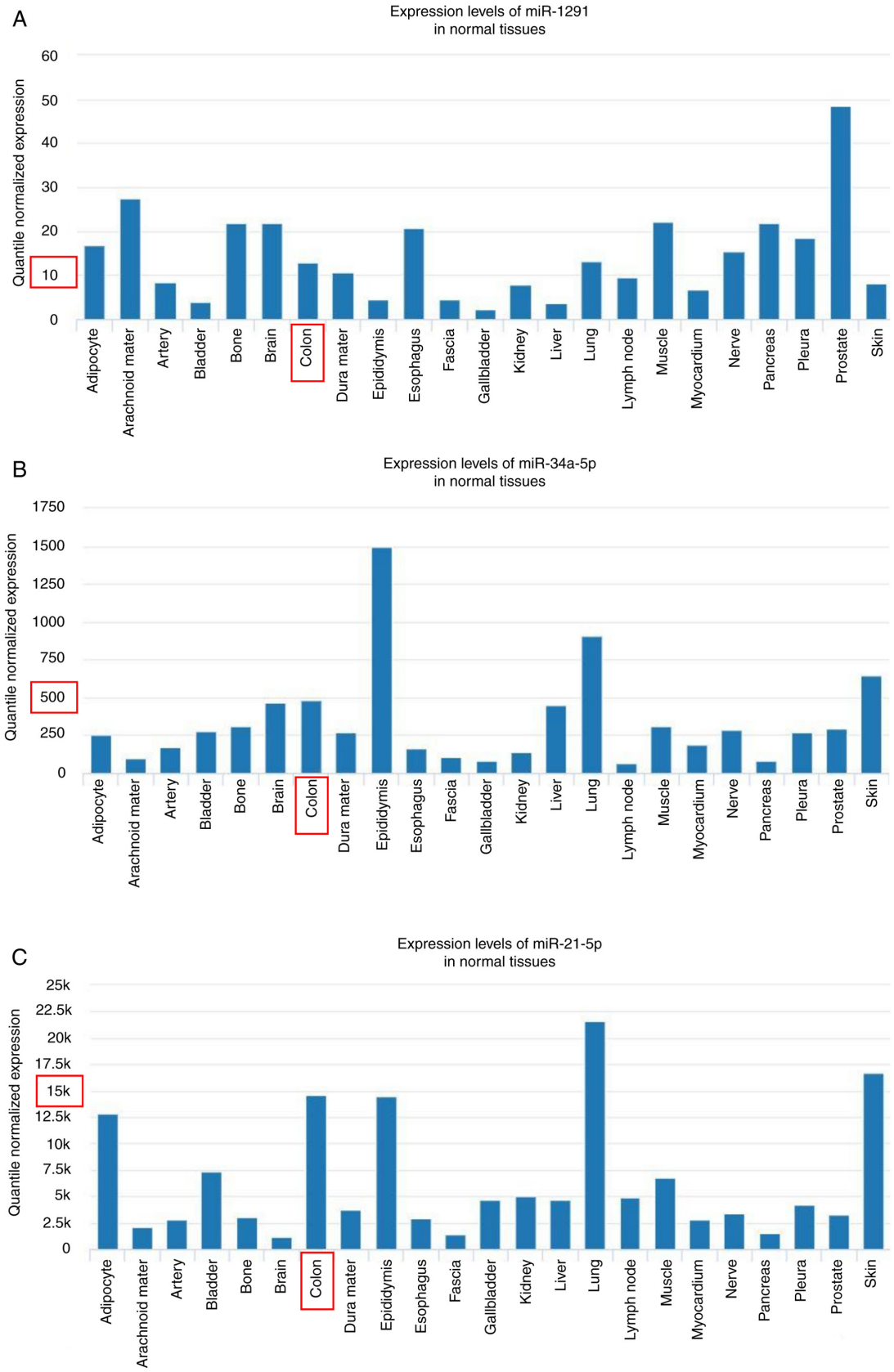

Figure S3. Assessment of CRC cell malignant properties following miR-1291 and antagomir-1291 transfection. (A) Cell growth was assessed in DLD-1, HT29 and HCT116 cells following transfection with miR-1291 and miR-NC at 48 and 72 h. (B) Cell invasion was assessed following miR-1291 transfection in DLD-1 (at 48 h), HT29 (at 96 h) and HCT116 (at 72 h) cell lines. Corning BioCoat Matrigel Invasion Chambers were used for the invasion assays. (C) A gap closure assay following miR-NC, miR-1291 and antagomir-1291 transfection was performed in DLD-1, HT29 and HCT116 cells. All experiments were performed in triplicate. All data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 vs. the NC group. CRC, colorectal cancer; miR, microRNA; NC, negative control; N.S., not significant.

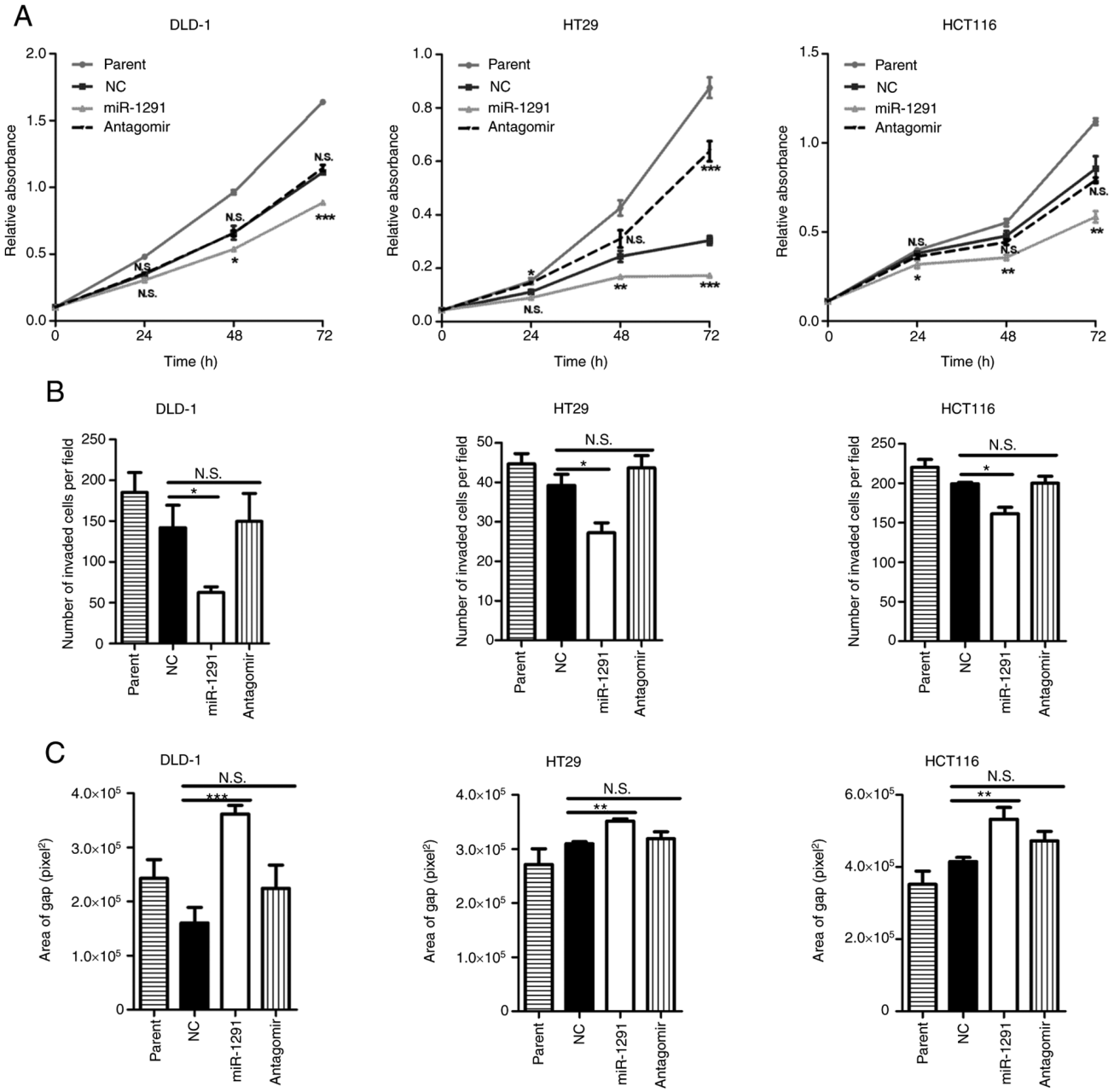

Figure S4. miR-1291 scarcely decreases the expression of stem cell surface marker CD166. Flow cytometric analysis using PE Mouse Anti-Human CD166 antibody was performed using HCT116. NC, negative control.

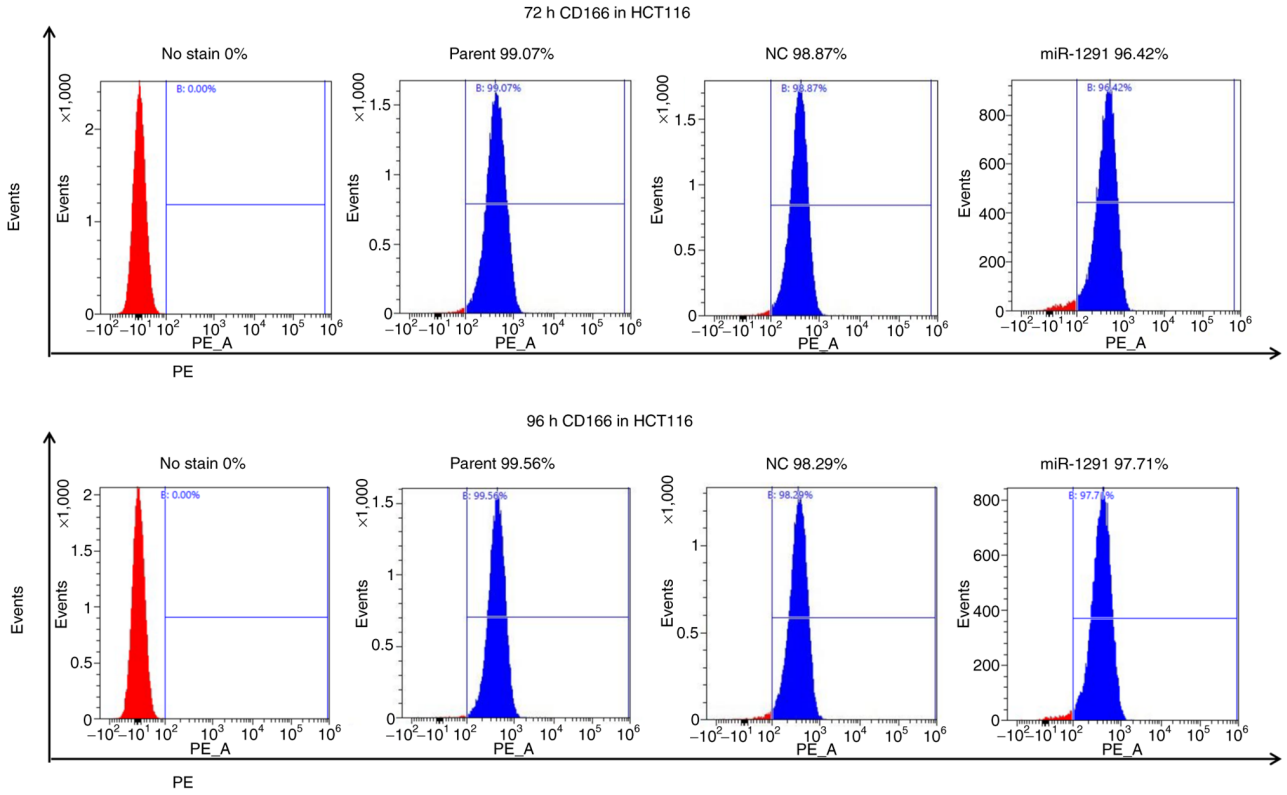

Figure S5. Continued.

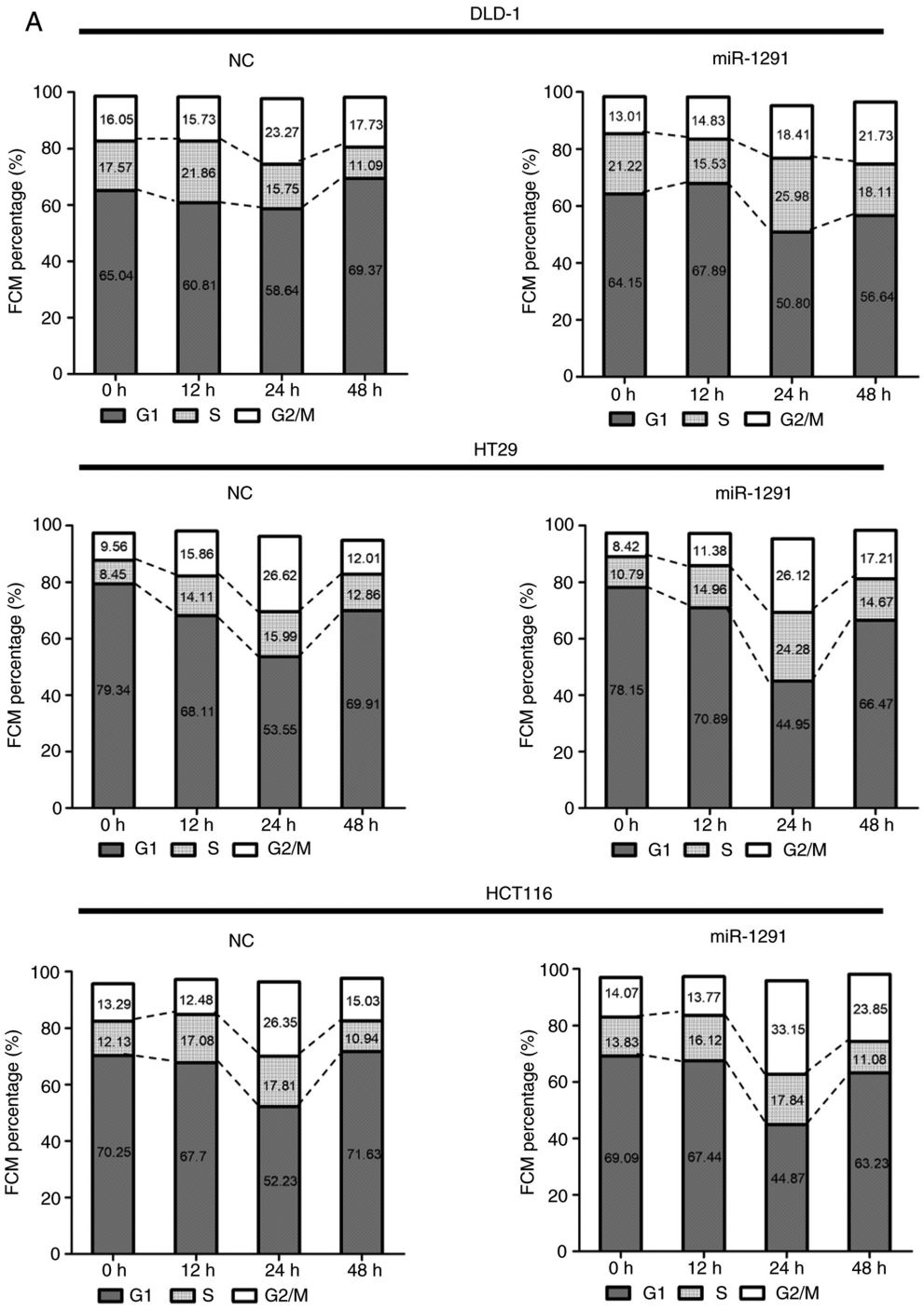

Figure S5. Analyses of the effects of miR-1291 on the cell cycle and related protein expressions in DLD-1, HT29 and HCT116 cell lines. (A) Experimental process of cell cycle assay. Cells were seeded and cultured overnight before starvation. Cells were then starved in FBS-free medium (RPMI-1640 or DMEM) for 48 h. A total of 24 h before the end of starvation, cells were transfected with miR-NC or miR-1291. After starvation, cells were cultured with 10% FBS-supplemented medium (RPMI-1640 or DMEM) and collected for cell cycle analyses at the indicated times (0, 12, 24 and 48 h). The ratio of cell population was detected at G1, S and G2/M phases of cell cycle using flow cytometric analyses. (B) Expression of cell cycle-related proteins other than $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ in DLD-1, HT29 and HCT116 cells. ACTB was used as a loading control. All data are presented as the mean ± SEM. For the reference protein, ACTB was constructed by stripping the membrane or by running the same lysates on a separate gel in case that the dense bands overlap on the actin band around 45 kDa. Cyclin D1 (36 kDa), cdc2 (34 kDa), P-cdc2 (34 kDa), Cyclin E1 (42, 50 kDa), CDK4 (34 kDa), and CDK6 (36 kDa) tended to overlap with the actin band. Each rectangle indicates simultaneously performed western blot data and each contains the corresponding actin bands. $^{*}P<0.05$ $^{**}P<0.01$ and $^{***}P<0.001$. miR, microRNA; NC, negative control; CDC25A, cell division cycle 25A; CDC25B, cell division cycle 25B; CDC25C, cell division cycle 25C; CDK4, cyclin dependent kinase 4; CDK6, cyclin dependent kinase 6; P-cdc2, phospho-cdc2; Rb, retinoblastoma; ACTB, actin beta; N.S., not significant.

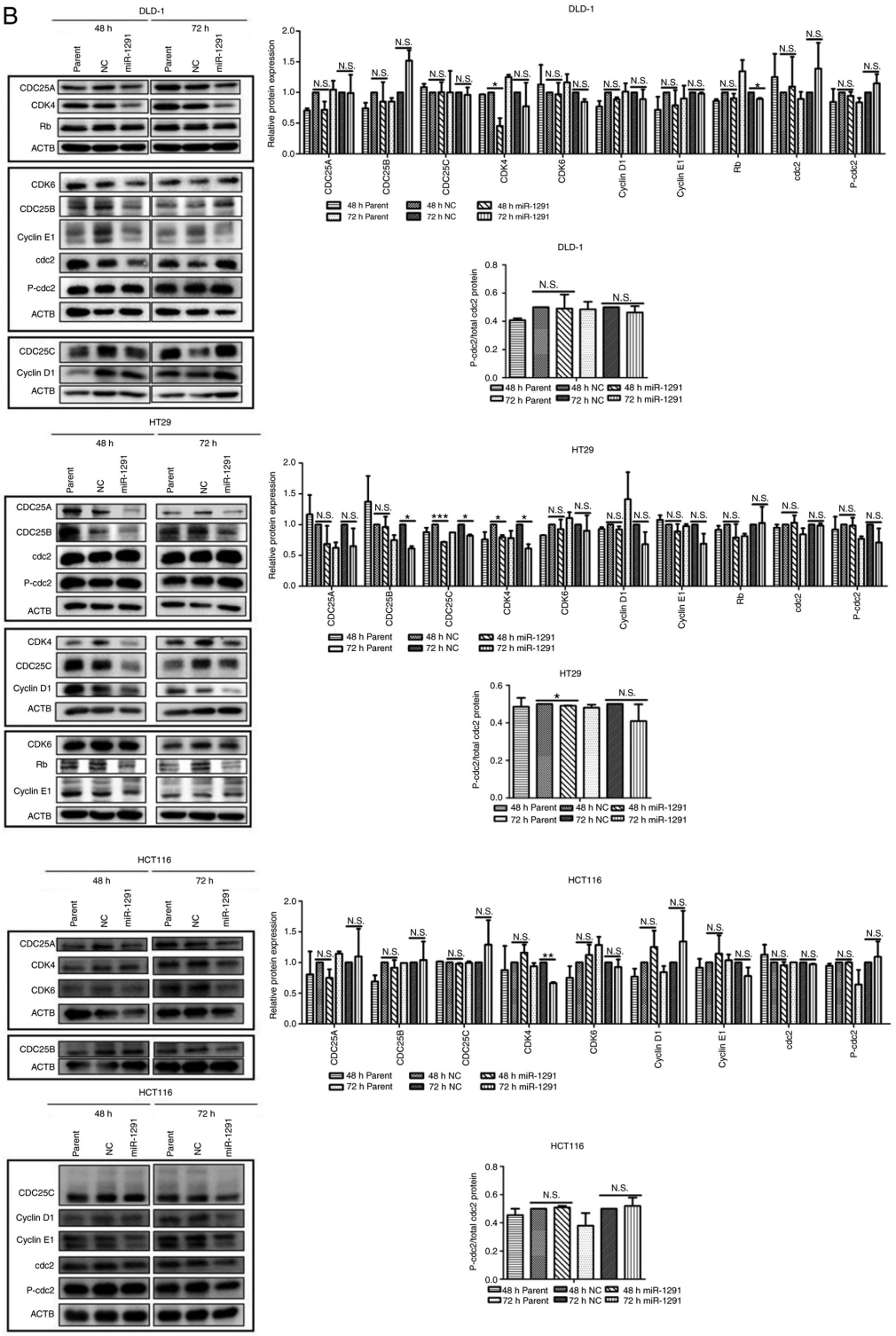

Table SI. List of primers used in reverse transcription-quantitative PCR.

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	CAACTACATGGTTTACATGTTC	GCCAGTGGACTCCACGAC
DCLK1	AGTCTTCCGATTCCGAGTTGAG	CAGCAACCAGGAATGTATTGGA
BMI1	TGTAAAACGTGTATTGTTCGTTAC	CAATATCTTGGAGAGTTTTATCTGACC
CD133	TGACAAGCCCATCACAACAT	CGCCTGAGTCACTACGTTGC
DCLK1 3' UTR 2-nucleotide mutated type plasmid primer	CCATGCTGAATGGGCCGAGCATT CTTCTGAGCTG	CAGCTCAGAAGAATGCTCGGCCCATTCAGCAT GG
DCLK1 3' UTR 3-nucleotide deleted type plasmid primer	CCATGCTGAATGGGTGCATTCTTC TGAGCTGCC	GGCAGCTCAGAAGAATGCACCCATTCAGCAT GG

DCLK1, doublecortin-like kinase 1; BMI1, B cell-specific Moloney murine leukemia virus integration site 1; UTR, untranslated region.

Table SII. Relationship between various clinicopathological parameters and miR-1291 levels.

Variables	miR-1291 expression level		P-value
	High expression (n=10)	Low expression (n=10)	
Sex (male/female)	5/5	5/5	1.000
Age, mean (SD)	58.7 (5.94)	67.6 (5.94)	0.303
Tumor size (mm), mean (SD)	49.1 (4.87)	57.8 (4.87)	0.223
Tumor location, (colon/rectum)	6/4	6/4	1.000
Differentiation, (tub1 and tub2/muc and por)	9/1	9/1	1.000
Tumor depth (T1 and T2/T3 and T4)	2/8	0/10	0.474
Lymph node metastasis (negative/positive)	6/4	2/8	0.170
Lymphatic invasion (negative/positive)	5/5	4/6	>0.999
Venous invasion (negative/positive)	3/7	4/6	>0.999

A Student's t-test (two-tailed, unpaired) was used for the analysis of age and tumor size, while a Fisher's exact test was used for the remaining variables. tub1, well differentiated adenocarcinoma; tub2, moderately differentiated adenocarcinoma; muc, mucinous carcinoma; por, poorly differentiated adenocarcinoma; T1, involvement of submucosa; T2, involvement of muscularis propria; T3, involvement of subserosa; T4, involvement of serosal surface or direct invasion to other organs; miR, microRNA.