Supplemental methods

Cell culture and treatment. Human colorectal cancer cell lines RKO and DLD1 were obtained from American Type Cell Collection (ATCC, Manassas, VA) and cultured in McCoy's 5A modified media (Invitrogen, Carlsbad, CA) supplemented with 10% defined FBS (HyClone, Logan, UT), 100 units/ml penicillin and 1% streptomycin (Invitrogen). Cells were maintained at 37°C with 5% CO2.

In some experiments, cells were grown in medium containing 0.5% serum. For serum-stimulated G1-S transition, cells in 6-well plates were cultured in serum-free media for 48 h prior to the addition of 10% FBS and harvested at various time points for flow cytometry or BrdU incorporation (1). Experiments were carried out in triplicates with similar results.

For radiation-induced transient G2/M checkpoint, cells in 12-well plates (for counting) or in T25 flasks (for Western blotting, RNA isolation and flow cytometry) were irradiated at 12 Gy at a rate of 82 cGy/min using a ^{137}Cs irradiator (Mark I., J.L. Shepherd and Associates). Cells were harvest at 1 h or later after irradiation for various analyses. For UV irradiation, cells in 60 cm^2 plates were exposed to 60 J/m^2 (Spectroline M-X-15 G, Spectronics Corp. Westbury, NY.) , and harvested at 1 h and 4 h post treatment for RNA isolation.

Clonogenic survival was evaluated by colony formation assays. In brief, cells were plated in 6-well plates at several densities and subjected to γ-irradiation at various doses (0, 1, 4 and 8 Gy) in triplicate. The cells were allowed to grow for 10 to 14 days before staining with crystal violet (Sigma) to visualize the colonies. Fractions of colonies following radiation compared with untreated cells were plotted. Only the colonies containing 30 or more were counted**.**

Isolation of microRNAs, Real-Time Polymerase Chain Reaction (PCR) assays, and Northern blotting for mature miRNAs. Total cellular RNA including microRNA was obtained from cells using the mirVana™ miRNA isolation kit (Ambion, Austin, TX). The expression of mature miRNAs was confirmed using real-time polymerase chain reaction (PCR) analysis with sequence specific primers

according to the instructions of the *mir*Vana™ qRT-PCR miRNA Detection Kit (Ambion). The expression of protein coding mRNAs was quantitated by real-time PCR with primers described in Table S5. Total RNA was isolated from cells and tissues using the RNAgents Total RNA Isolation System (Promega, Madison, WI) and CsCl centrifugation method (2), respectively. First-strand cDNA was synthesized from 2-10 μg of total RNA using Superscript II reverse transcriptase (Invitrogen) as described (3). For Northern analysis, 15 μg of total RNA were separated on a 15% polyacrylamide/8 M urea gel and transferred to a Nylon membrane (Pierce) using a Transblot semidry apparatus (Bio-Rad, Hercules, CA). Membranes were crosslinked according to the manufacturer's manual. The *miR-21* antisense oligonucleotide (5'–TCAACATCAGTCTGATAAGCTA-3') as probe was ^{32}P labeled with T4 polynucleotide kinase (New England Biolabs, Ipswich, MA). Prehybridizations and hybridizations were carried out using UltraHyb Hybridization Buffer (Ambion) at 37°C. After washing, membranes were wrapped in plastic wrap, and exposed to film at -80 °C for 24 hours.

Cell growth assays. Cells were plated at 3000 cells/well in 96-well plates. Cell growth was measured by (MTS) assay using a novel tetrazolium compound [3-(4,5-dimethyl-thiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium in CellTiter 96 AQueous One Solution (Promega, Madison, WI) following the manufacturer's instructions. Each experiment was done in triplicate and repeated at least twice (4).

Luciferase reporter constructs. miRNA reporter constructs were created by cloning a PCR generated fragment containing the putative binding site of *miR-21* in the 3'UTR of *Cdc25A* gene into the *Hin*d III and *SpeI* sites of the pMIR-REPORT™ miRNA expression reporter vector (Ambion). The *miR-21* binding site mutated reporter was constructed by site-directed mutagenesis with the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene) following the instructions of the manufacturer. The primers used are listed in Table S2. Cells were plated at 300,000 cells per well in a

12-well plate 24 hours before transfection. Cdc25A 3'UTR reporter or control plasmid (200 ng) plus 100 ng beta-gal miRNA control vector (Ambion) were transfected using Lipofectamine 2000 (Invitrogen). Luciferase and β-galactosidase activities were determined 24 h after transfection using kits from Promega (Madison, WI) and ICN Biomedicals (Costa Mesa, CA), respectively (5). All the experiments were performed in triplicate and repeated at least three times on different days.

Drug treatment. cells were plated in 12-well plates at 20–30% density 24 h before treatment. DMSO stock solutions of anticancer agents were diluted to appropriate concentrations with cell culture medium. The anticancer agents adriamycin, etoposide, camptothecin, and vinblastine were from Sigma (St. Louis, MO); (+)-brefeldin A and staurosporine were from EMD Biosciences (San Diego, CA) and gemcitabine was from Bell Medical Services (Radford, VA).

Cell cycle analysis. Cell cycle profiles were determined by flow cytometry. Briefly, cells were trypsinized and washed with PBS and fixed in 70% ethanol. The nuclei of the cells were stained with propidium iodide (PI, Sigma) (10 μg/ml) and RNase A (Invitrogen) (10 μg/ml) for 30 min. The cell cycle profiles were obtained with a FACStar flow cytometer and analyzed using Cytomation Summit v3.1 (Cytomation, Inc., USA) (6).

Colon cancers and matched normal tissues. Samples of colorectal cancer tissue and matched normal colonic epithelium were obtained from patients undergoing surgery and were frozen immediately (<10 min) after surgical resection. Acquisition of tissue specimens was performed in accordance with Health Insurance Portability and Accountability Act of 1996 (HIPAA) regulations. RNA was isolated from portions of tumors judged by histopathology to contain 60 to 90% tumor cells and adjacent normal tissues containing 90% or above epithelial cells. Total RNA was isolated from bulk tissues using CsCl centrifugation method as preciously described (2, 7).

BrdU incorporation assays. For microscopic examination, cells in 12-well plates were subjected to various treatments and incubated with 10 μM BrdU for 15 min prior to fixation with methanol: acetone (1:1) at -20ºC for 15 min. The cells were treated with 2N HCl for 30 min at 37 ºC, and washed with PBS, and then incubated with monoclonal Alexa Fluor 594 anti-BrdU conjugated antibody (Invitrogen) for 45 min. The cells were finally counter stained with DAPI (20 μg/ml) and analyzed by fluorescence microscopy (3). For flow cytometry, cells in 6-well plates were harvested by trypsinization and fixed with 70% cold ethanol for at least 2 h at 4 ºC. The cells were washed once with PBS and resuspended in 2 N HCl/0.5% Triton X-100 and incubated at 37 ºC for 30 min. Following two washes in PBS, the cells were blocked in PBS/0.5% Tween-20/50% goat serum for 1 h at room temperature and then incubated with PBS/10% goat serum/0.5% anti-BrdU, Alexa Fluor 488 conjugate antibody (Invitrogen) for 1 h at room temperature. After one wash in PBS/0.5% Tween 20/1% BSA, cells were counterstained with PI (5 μg/ml), and analyzed by a FACStar flow cytometer, and the data was analyzed using Cytomation Summit v3.1 (Cytomation, Inc., USA).

Mitotic index measured by phosphorylated histone 3 (H3) staining. For the flow cytometry, cells in T25 flasks were washed with PBS, and fixed with 2% formaldehyde for 15 min at room temperature. After fixation, the cells were washed twice with PBS and permeablized with methanol for 30 min at 4 ºC. Following one wash with PBS, the cells were blocked with 50% goat serum in PBS for 1 h at room temperature and incubated with phosphorylated histone H3 antibody (1: 400 diluted with 10% goat serum in PBS) (Upstate Biotechnology, Lake Placid, NY) 1 h at room temperature. The signals were developed with anti-rabbit antibodies Alexa-488 (Pierce, Rockford, IL) (1:300 dilution) in PBS containing 10% goat serum for 30 min at room temperature. The nuclei were counterstained with PI (2 μg/ml) for 15 min at room temperature. The phosporylated H3-positive cells were analyzed using Cytomation Summit v3.1 (Cytomation, Inc., USA).

Supplemental References

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3. Yue W, Sun Q, Dacic S, et al. Downregulation of Dkk3 activates beta-catenin/TCF-4 signaling in lung cancer. Carcinogenesis 2008;29:84-92.

4. Sun Q, Sakaida T, Yue W, Gollin SM, Yu J. Chemosensitization of head and neck cancer cells by PUMA. Mol Cancer Ther 2007;6:3180-8.

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7. El-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75:817-25.

Supplementary Tables

Table S1 Primers used for *miR-21* KO constructs and screening

Locus	Primer	Sequence
miR-21	Left Arm-Forward	5'-atacatacgcggccgcccaccctcgatgcaatccaccttgtg-3'
	Left Arm-Reverse	5'-atacatacccgcggtggtacagccatggagatgtcacg-3'
	Right Arm-Forward	5'-atacatacggtacccagctgtggcatgctcagaggttcc-3'
	Right Arm-Reverse	5'-atacatacgcggccgcttgtgtaggagcactcaatactgc-3'
	Left Arm-Sequence	5'-gtcttacaagtgagctgacac-3'
	Right Arm-Sequence	5'-cccttaaaacagacaatgtcgg-3'
	Screening-Forward P1	5'-cttaaattgggaggactccaagccg-3'
	Screening-Reverse P2	5'-tagctagtgcatgatccatactc-3'
Neo	Reverse	5'-aagcgcatgctccagactgc-3'

Table S1. The sequences of the primers used for *miR-21* KO targeting vector construction, KO screening, and sequencing the junctions in targeted clones for

verification.

Table S2 Primers for Luciferase reporter construct	
Primer	**Sequence**
Cdc25A-MUT-Forward	5'-tgacaatctagcaatctcccagacccaccactgg-3'
Cdc25A-MUT-Reverse	5'-tgggagattgctagattgtcaggcagccaagcctgg-3'
Cdc25A-Spe I-Forward	5'-attaactagtaggtcctggtgagagcgttggacc-3'
Cdc25A-Hind III-Reverse	5'-atacaagcttcacctcccaccaaatagatatcgg-3'

Table S2. The sequences of the primers used for making *Cdc25A* 3'UTR luciferase reporter constructs.

Table S3 Genes upregulated in *miR-21* KO RKO cells

Gene Name	Accession No.	Fold (KO/WT)	P value
ANLN	NM_018685.1	2.8	0.008
ANXA1	NM_000700.1	2.5	0.000
ATP5D	BE798517	2.7	0.004
BIRC5	NM_001168.1	4.7	0.001
BTG2	NM_006763.1	2.2	0.019
BUB1	AF043294.2	2.6	0.004
BUB1B	NM_001211.2	2.7	0.006
C1ORF2	NM_006589.1	2.7	0.038
C20ORF1	AF098158.1	3.1	0.003
C7orf48	BC004308.1	2.3	0.004
CAP-C	NM_005496.1	2.7	0.000
CBLB	U26710.1	2.7	0.000
CDC25A	AI343459	3.4	0.011
CDKN3	AF213033.1	4.3	0.008
CENPF	NM_005196.1	3.7	0.002
CTGF	M92934.1	6.0	0.001
D6S52E	BG028844	2.0	0.001
DDB2	NM_000107.1	2.4	0.003
DGAT	NM_012079.2	2.1	0.011
DJ616B8.3	BC001068.1	2.0	0.014
DJ742C19.2	NM_004900.1	2.6	0.000
DKFZp434A0530	AL136842.1	4.1	0.006
DKFZp434N0250.1	BF108964	6.1	0.001
DKFZP586O0223	AL096741.1	3.8	0.006
DNMT1	NM_001379.1	2.1	0.003
DTR	NM_001945.1	3.2	0.014
ECM1	U65932.1	2.4	0.014
EGF-like growth factor	M60278	2.6	0.000
ENO2	NM_001975.1	2.4	0.002
EST	BG165011	3.3	0.000
EST	AI813331	3.2	0.000
EST	BG251556	2.8	0.000
EST	AI655524	2.7	0.000
EST	BE207755	2.0	0.000
EST	AA878383	2.8	0.001
EST	AA417878	2.1	0.001

EST	AI208616	5.0	0.002
EST	AW129783	2.9	0.002
EST	AI374739	2.0	0.002
EST	N62196	3.6	0.003
EST	AA702788	3.6	0.004
EST	AW138134	2.2	0.004
EST	AW264125	2.0	0.004
EST	AW151538	2.5	0.006
EST	AV650867	2.6	0.008
EST	A527515	2.2	0.009
EST	BF221525	6.4	0.011
EST	AA704162	2.6	0.011
EST	BE968786	6.1	0.014
EST	AI075194	2.2	0.014
EST	AW451197	6.8	0.019
EST	BF508014	4.0	0.019
EWS	U35622.2	2.1	0.013
FKSG14	BC005400.1	6.1	0.002
FLJ10101	BC002945.1	2.0	0.002
FLJ10350	NM_018067.1	2.7	0.000
FLJ10520	BC002574.1	2.2	0.006
FLJ10624	BG391282	7.3	0.011
FLJ13576	R60866	2.1	0.000
FLJ20085	NM_017660.1	3.4	0.003
FLJ20311	NM_017760.1	2.5	0.011
FLJ20354	AK000490.1	2.4	0.019
FLJ20374	NM_017793.1	2.4	0.008
FLJ20538	AK001039.1	2.4	0.014
FLJ23468	NM_024629.1	3.0	0.000
GCN5L2	AL571424	2.7	0.002
GNAS1	AF064092.1	2.1	0.004
H2AFX	NM_002105.1	2.1	0.004
HAS2	NM_005328.1	2.7	0.011
HCAP-G	NM_022346.1	2.8	0.002
HCAP-G	NM_022346.1	2.3	0.000
HLA-E	X56841.1	2.2	0.000
HMG2	BC000903.1	2.1	0.000
HPCAL1	NM_002149.1	5.4	0.014
HSPC145	AL135396	4.5	0.002
HUMGT198A	NM_013290.1	3.2	0.003
ICBP90	AK025578.1	2.3	0.000
ID-GAP	AU153848	2.8	0.000

IL8	NM_000584.1	2.6	0.001
ISG20	NM_002201.2	3.8	0.006
KDELR1	NM_006801.1	2.4	0.003
KIAA0101	NM_014736.1	2.0	0.000
KIAA0186	NM_021067.1	2.4	0.000
KIAA0233	NM_014745.1	2.1	0.000
KIAA0286	AB006624.1	2.1	0.002
KIAA0542	AB011114	2.4	0.015
KIAA0729	AW117717	2.4	0.008
KIAA0964	BF346592	2.6	0.000
KIAA1020	AI912206	2.1	0.011
KIAA1595	AB046815.1	2.3	0.000
KIF4A	NM_012310.2	3.2	0.001
KNSL1	NM_004523.2	4.1	0.019
KNSL4	NM_007317.1	2.7	0.019
KNSL6	U63743.1	2.5	0.000
KRTAP4.9	AJ406941.1	2.2	0.000
L2DTL	NM_016448.1	3.0	0.001
LBP-32	BE566136	2.1	0.002
LOC51203	NM_016359.1	3.0	0.000
LOC51210	NM_016372.1	2.8	0.008
LRP16	NM_014067.2	2.8	0.000
MAD1L1	NM_003550.1	2.5	0.000
MAD2L1	NM_002358.2	2.4	0.000
MCM2	NM_004526.1	2.2	0.000
MCM5	AA807529	2.1	0.001
MDS030	NM_018465.1	2.0	0.003
MEA	NM_014623.1	2.4	0.001
MGAT4B	NM_014275.1	2.7	0.002
MGC12904	NM_031219.1	6.4	0.011
MGC2479	AL121829	2.1	0.019
MGC3121	NM_024031.1	2.6	0.030
MGC4342	NM_024329.1	2.2	0.002
MLF1IP	NM_024629.1	3.0	0.000
MLL3	AA121529	2.3	0.001
NDUFS8	NM_002496.1	2.6	0.002
NESP55	NM_016592.1	2.0	0.000
NET1	AW263232	2.0	0.001
NFKB2	BC002844.1	2.7	0.014
NPIP	AC002045	2.1	0.002
NTKL	AF297709.1	2.3	0.011
NUSAP1	NM_016359.1	3.0	0.000

OGFR	NM_007346.1	2.3	0.019
OIP5	BE045993	3.3	0.001
P1cdc47	D55716.1	2.1	0.019
PEF	NM_012392.1	2.2	0.000
PIR51	BE966146	2.2	0.000
pknbeta	NM_013355.1	2.3	0.001
PLAB	NM_007317.1	2.7	0.002
PLXNB2	BC004542.1	2.3	0.014
POLRMT	NM_005035.1	2.0	0.011
PPM1G	NM_002707.1	2.0	0.003
PRC1	NM_003981.1	4.9	0.003
PRIM1	NM_000946.1	2.2	0.004
PRO1843	NM_018507.1	3.2	0.000
proto-LBC	AF127481.1	2.8	0.000
PTD017	AA152202	2.4	0.011
PTPRF	NM_002840.1	2.5	0.006
RELA	NM_021975.1	2.1	0.003
RRM2	BE966236	3.7	0.000
RRM2	BC001886.1	3.6	0.000
RTN4	AF333336.1	2.0	0.000
semaZ	AB022433.1	2.2	0.000
SIAT1	AI743792	2.3	0.000
Similar to PRO1992	BC004144.1	2.2	0.000
SLC25A1	U25147.1	2.6	0.000
SLC27A4	AK000722.1	3.0	0.003
SLC4A2	NM_003040.1	2.0	0.001
SLC7A6	NM_003983.1	2.1	0.014
SPINT1	NM_003710.1	2.1	0.001
STK6	NM_003158.1	3.4	0.002
TCF3	M31523.1	2.6	0.000
TCFL5	NM_006602.1	2.0	0.001
TFDP1	NM_007111.1	3.3	0.008
TGFB1	BC000125.1	2.5	0.001
TOP2A	AU159942	5.2	0.006
TOPK	NM_018492.1	2.8	0.001
TR	AB019695.1	2.8	0.006
TRXR2A	AF201385.1	6.5	0.002
TTK	NM_003318.1	2.9	0.014
TUFM	NM_003321.1	2.2	0.000
TYMS	NM_001071.1	3.1	0.001
UBCH10	NM_007019.1	3.3	0.001
UBE2M	NM_003969.1	2.6	0.008

WDR1	NM_017491.1	2.3	0.000
XAP4	NM_006462.1	2.5	0.001
YWHAH	NM_003405.1	2.1	0.001
ZNF144	AI744229	2.1	0.000

Table S3. The transcripts that were upregulated by at least two-fold in *miR-21* KO cells transfected with control siRNA compared with parental RKO cells transfected with pre-*miR-21*. The cut-off P value was set at 0.02.

Table S4 Expression of several *miR-21* targets

Gene Name	Accession No.	Fold (KO/WT)	P value
Pdcd4	NM_014456.1	1.1	0.0024
PTEN	NM_000314.1	1.2	0.018
Spry 1	BF508662	1.8	0.001
Spry 2	NM_005842.1	1.44	0.046
TMP1	NM_000366.1	1.2	0.001

Table S4. The ratio (fold) was obtained using expression of a transcript in *miR-21* KO cells transfected with control siRNA divided by that in parental RKO cells transfected with pre-*miR-21*.

Table S5 Primers for RT-PCR of candiate genes

Primer	Sequence
Cdc25A-Forward	5'-tcaccaacctgaccgtcactatgg-3'
Cdc25A-Reverse	5'-tgtgtgaagagatctttaccctcc-3'
NUSAP1-Forward	5'-tgcagaacttagccaagagtctgg-3'
NUSAP1-Reverse	5'-agctctgagatcctggctttcctg-3'
MLF1IP-Forward	5'-agcttccagtcgctcgagagcggag-3'
MLF1IP- Reverse	5'-atgggcctgagctttcttcctggc-3'
DDB2-Forward	5'-caagaagctctgtgcgaagggctc-3'
DDB2- Reverse	5'-tgtctcctgtgaccaccattcggc-3'
BTG2-Forward	5'-gagcgagcagaggcttaagg-3'
BTG2-Reverse	5'-gtacaagacgcagatggagc-3'
CTGF-Forward	5'-aaccgcaagatcggcgtgtgcacc-3'
CTGF-Reverse	5'-ggctctaatcatagttgggtctgg-3'
CENPF-Forward	5'-agttggaactacgcctgcaaggac-3'
CENPF-Reverse	5'-ccagttcagcctgcaggacgttgtg-3'
Pdcd4-Forward	5'-aggaggtggatgtgaaagatcc-3'
Pdcd4-Reverse	5'-cagaagcacggtagccttatcc-3'
GAPDH Forward	5'-ctcagacaccatggggaaggtga-3'
GAPDH Reverse	5'-atgatcttgaggctgttgtcata-3'

Table S5. The sequences of the primers used for RT-PCR.

Supplementary Figure Legends

Figure S1. Generation of *miR-21* KO DLD1 cells

A. Identifying *miR-21* KO DLD1 clones following removal of *Neo* by PCR. WT and the recombinant (*miR-21* KO) alleles were indicated. **B.** Cdc25A levels analyzed by RT-PCR in RKO WT and *miR-21* KO cells with or without Pre-*miR-21* transfection. *GAPDH* was used as a loading control.

Figure S2. RT-PCR validation of microarray expression results

We selected 12 cell cycle and DNA damage response genes from the list of genes whose expression was upregulated in *mirR-21* KO cells based on the microarray analysis. The expression of 7 candidates was validated using semi-quantitative RT-PCR in RKO WT or *miR-21 KO* cells.

Figure S3. ***Cdc25A* is regulated by *miR-21* in DLD 1 cells**

The expression of *Cdc25A* and the effect of pre-*miR-21* on its expression were analyzed in DLD1 cells as in RKO cells (Fig. 2A-D). **A.** Relative *Cdc25A* mRNA expression was measured by real-time RT-PCR in DLD1 wild-type (WT) and *miR-21* knockout (KO) cells. The expression of *Cdc25A* mRNA was normalized to that of *GAPDH*. Values are means ± SD, n=3. *P<0.02. **B.** Expression of Cdc25A protein levels was determined by Western blotting. **C**. The effect of precursor *miR-21* on *Cdc25A* levels. Cells were transfected with precursor *miR-21* or control siRNA for 48 h and analyzed for *Cdc25A* expression by RT-PCR. Values are means ± SD, n=3. *P<0.05. Expression levels were normalized to those in control siRNA transfected cells. **D.** Cdc25A levels in cells treated as in **C** were analyzed by Western blotting.

Figure S4. The effects of *miR-21* on the expression of Cdc25B, Cdc25C and known miR-21 targets, drug-induced apoptosis or proliferation

The medium contained 10% serum in all experiments. **A.** The expression of Cdc25A, B and C, p-Cdc2, Chk1, and β-Trcp was analyzed in the indicated cells by Western blotting. **B.** The expression of several miR-21 regulated genes or proteins. PTEN and Bcl-2 was measured by Western blotting. α-tubulin was used as a loading control. The expression of *Pcdc4* mRNA was measured by real-time RT-PCR. Values are means ± SD, n=3. *P>0.3. **C**. The growth rate of indicated cell lines was followed for 7 days by MTS assay. Values are means ± SD, n=3. **D.** The parental or *miR-21 KO* RKO cells were treated with indicated anticancer agents for 48 h. Apoptosis was quantified by nuclear fragmentation assay. As least 300 cells were scored in each determination and repeated at least twice.

Figure S5. *miR-21* regulates serum-induced G1-S transition through Cdc25A

A. An example of BrdU incorporation assay in *miR-21* KO RKO cells. The cells were stimulated with serum for 15 h and incubated with BrdU for 15 min. BrdU

incorporation was determined using an anti-BrdU antibody (red), and the nuclei were counterstained using DAPI (blue). **B**. The indicated RKO cells were cultured in serum free medium for 48 h and then stimulated with 10% fetal calf serum. Levels of Cdc25A in indicated cells following serum stimulation were analyzed in the indicated time by Western blotting**.** **C**. The indicated RKO and DLD1 cell lines in log phase in the presence of 10% serum were analyzed for cell cycle by flow cytometry. No significant differences in cell cycle distribution were found between the parental and *miR-21* KO cells.

Figure S6. *miR-21* modulates DNA damage-induced G2/M checkpoint in colon cancer cells.

A. The indicated RKO cells were harvested at 1 h after irradiation (IR, 12Gy) followed by staining with phospho-histone H3 (Ser10) antibody. The nuclei were counter stained by propidium iodide (PI). The fractions of p-H3 positive cells were analyzed by flow cytometry with representative profiles shown. **B.** The indicated DLD1 cells were subjected to irradiation (IR, 12Gy) and harvested at 1 h after radiation followed by staining with phospho-histone H3 (Ser10) antibody as in A (right panel). The phospho-histone H3 positive cells were quantified (the middle panel). **C.** The levels of Cdc25A were analyzed by Western blotting in the indicated DLD1 cells 1h after irradiation. **D.** The levels of *miR-21* in RKO cells normalized to those of *U6* at indicated time points after UV irradiation (60 J/m^2) were determined by realtime RT-PCR. Values are means ± SD, n=3.

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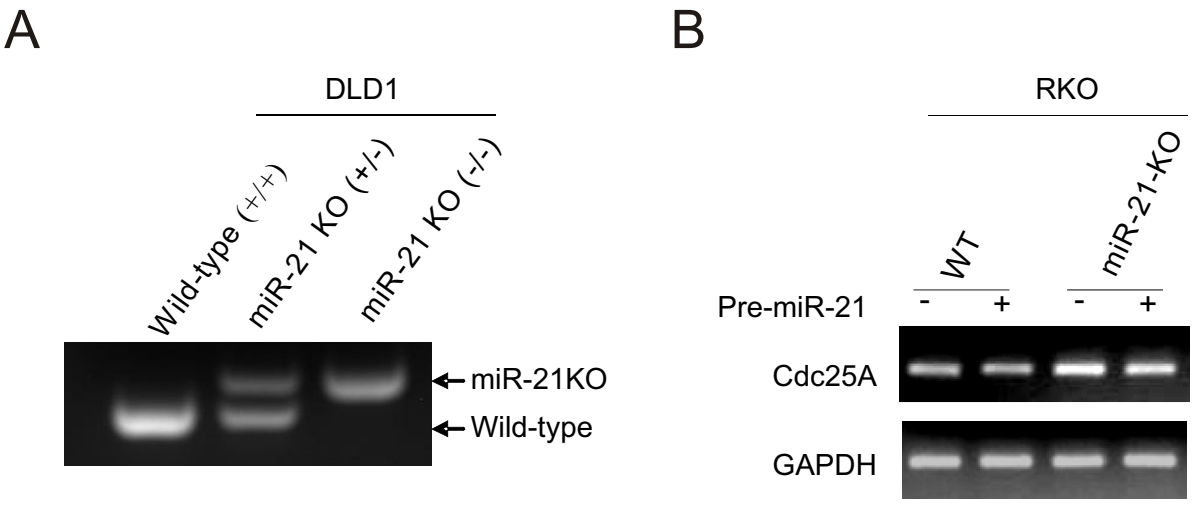
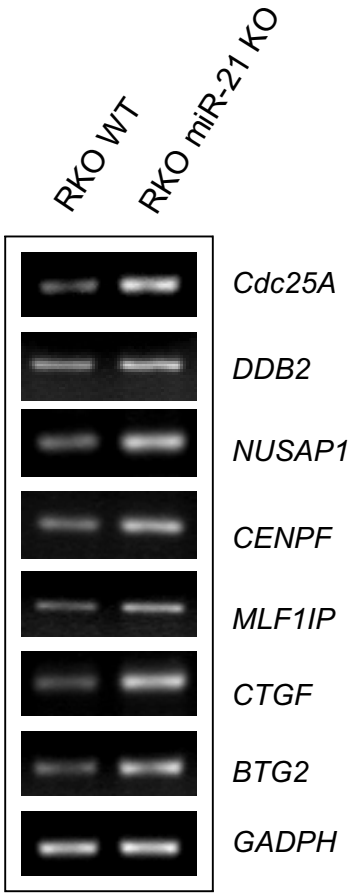
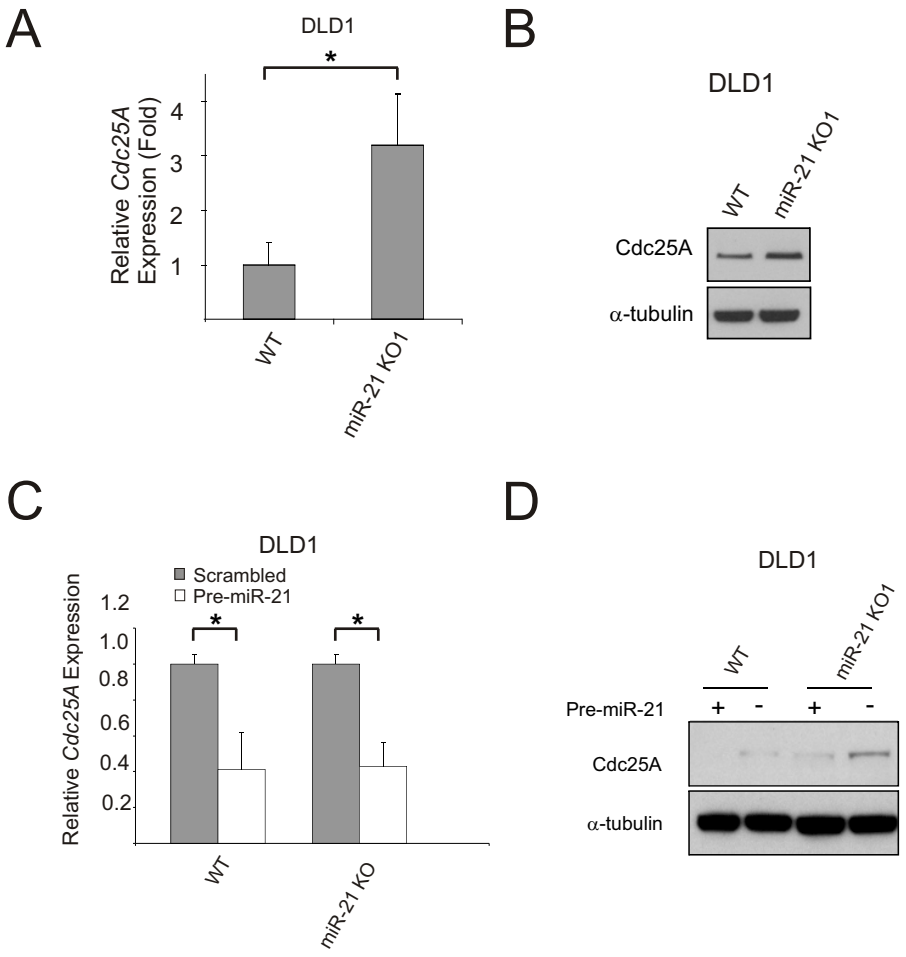


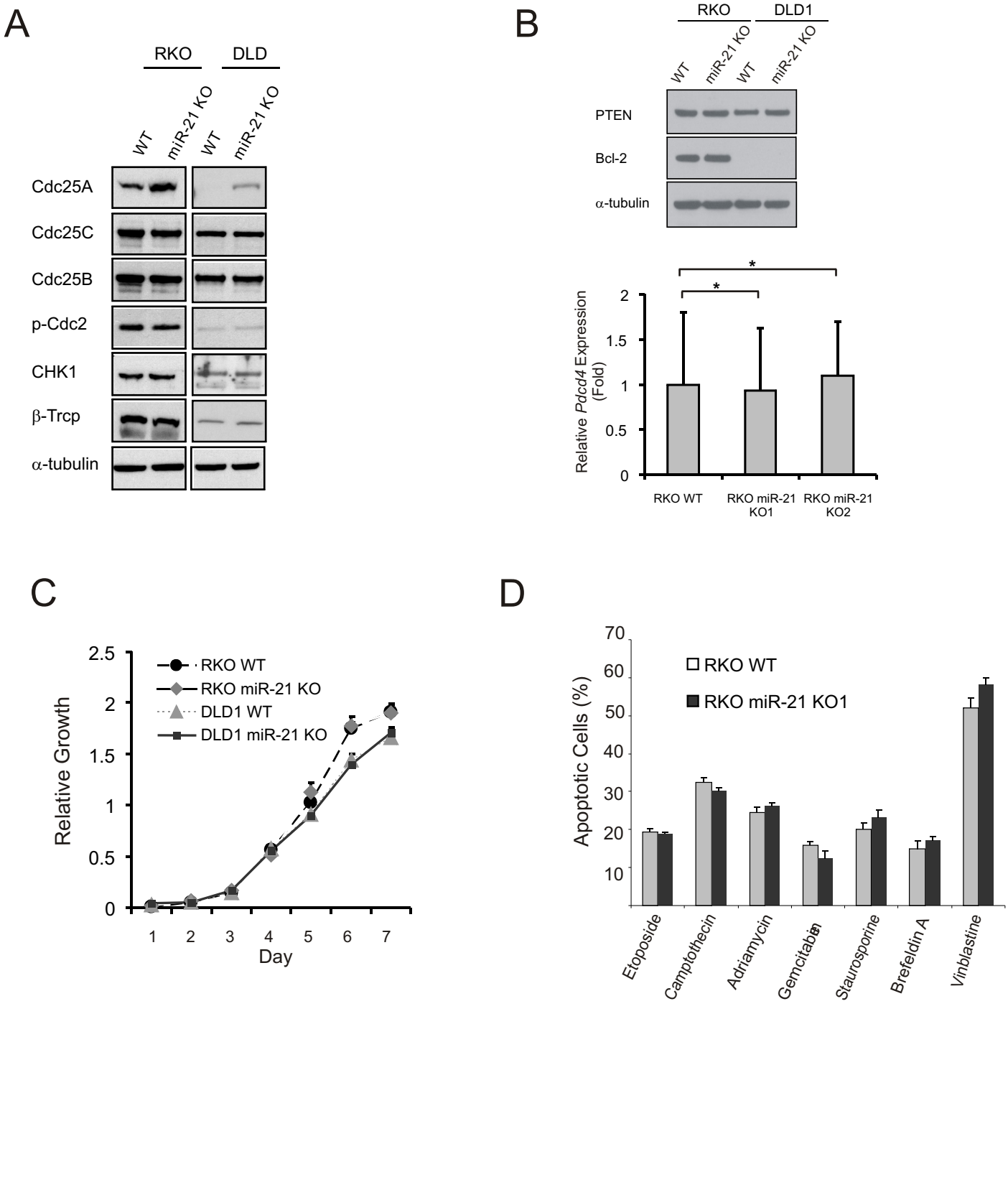
Wang *et. al* Figure S2



Wang *et al*. Figure S3

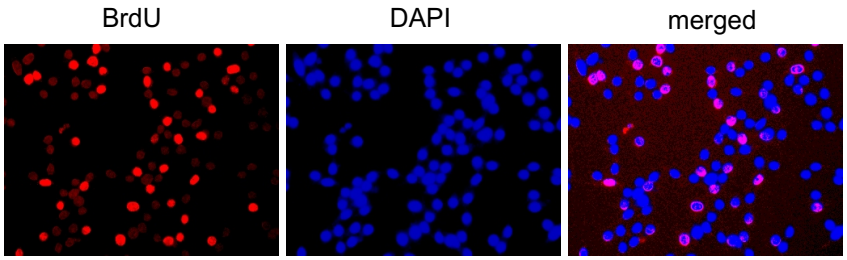


Wang *et al*. Figure S4

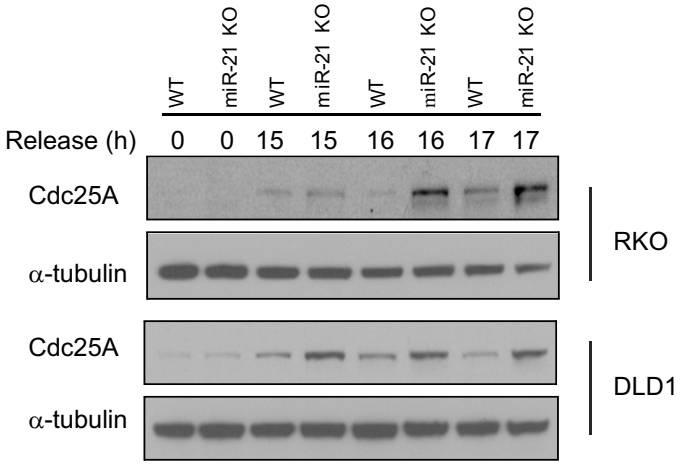


Wang *et. al* Figure S5

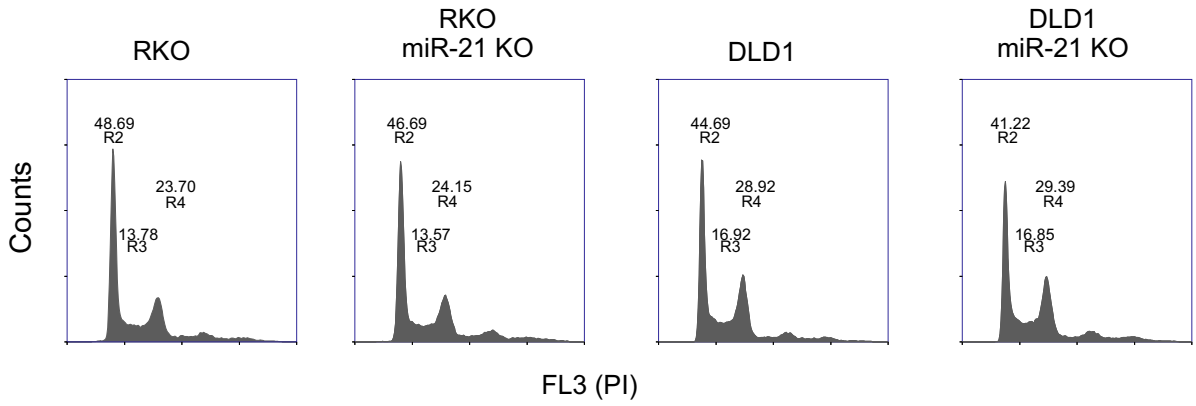
A



B



C



Wang *et al*. Figure S6