

## Supplementary information

### Inactivation of the tumor suppressor p53 by long non-coding RNA RMRP

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Running title: RMRP triggers tumor resistance to PARP inhibition

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## Materials and Methods

### Cell culture and transient transfection

Human cancer cell lines HCT116<sup>p53+/+</sup>, HCT116<sup>p53-/-</sup>, RKO (wtp53), LOVO (wtp53), HT-29 (mtp53-R273H), H460 (wtp53), H1299 (p53-null) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. All cells were maintained at 37 °C in a 5% CO<sub>2</sub> humidified atmosphere. Cells were seeded on the plate the day before transfection, and then transfected with plasmids or siRNAs as indicated in figure legends using Hieff Trans<sup>TM</sup> liposomal transfection reagent following the manufacturer's protocol (Yeasen, Shanghai, China). Cells were harvested at 30–48 h post transfection for future experiments. Cycloheximide, MG132, chloroquine, Nutlin-3, the PARP inhibitors Niraparib, Talazoparib and Olaparib, and the chemotherapy drugs Cisplatin and 5-Fu were purchased from MedChemExpress (Shanghai, China).

### Reverse transcription and quantitative RT-PCR analysis

Total RNA was isolated from cells using RNAiso Plus (Takara, Dalian, China) following the manufacturer's protocol. Total RNAs of 0.5 to 1 mg were used as templates for reverse transcription using PrimeScript<sup>TM</sup> RT reagent Kit with gDNA Eraser (Takara, Dalian, China). Quantitative RT-PCR (RT-qPCR) was conducted using TB Green<sup>TM</sup> Premix according to the manufacturer's protocol (Takara, Dalian, China). The RT-qPCR primers used in this study are listed in Supplementary Table 4.

### Cell viability assay

To assess the long-term cell survival, the Cell Counting Kit-8 (CCK-8) (Dojindo, Shanghai, China) was used according to the manufacturer's instructions. Cell suspensions were seeded at 2,000 cells per well in 96-well culture plates at 12 h post-transfection. Cell viability was determined by adding WST-8 at a final concentration of 10% to each well, and the absorbance of the samples was measured at 450 nm using a Microplate Reader every 24 h for 4 days. The cell proliferation curves were plotted using the absorbance at each time point. For assessment of IC<sub>50</sub>, cell suspensions were seeded at 5,000 cells per well in 96-well flat-bottomed plates to determine the concentration that causes 50% inhibition of cell viability. Cells were treated with Olaparib at serial dilutions, or combination of fixed Olaparib with different concentrations of genotoxic agents (Cisplatin or 5-FU). After culturing for 48 h, the cell viability was measured by the CCK-8 assay as described above.

### Colony formation assay

Cells were resuspended in single-cell suspensions 24 h post-transfection and ~500 cells were plated into 6-well plates. The medium was changed every 3 days until the colonies were visible in around two weeks. Puromycin was added in the medium when the stable cell lines

were used in the experiment. Cells were then fixed by methanol and stained with 0.1% crystal violet solution at RT for 30 min. The visible colonies were manually counted.

### **Flow cytometry analysis**

Cells were fixed with ethanol overnight and stained in 500  $\mu$ l of propidium iodide (PI) (Sigma-Aldrich) stain buffer (200  $\mu$ g/ml RNase A, 50  $\mu$ g/ml PI, 0.1% Triton X-100 in phosphate-buffered saline) for 30 min at 37 °C. Then, cells were analyzed for DNA content using a FC500 MPL flow cytometer (Beckham coulter, Indianapolis, IN, USA).

### **In vivo ubiquitination assay**

HCT116 <sup>p53<sup>-/-</sup></sup> cells were transfected with plasmids encoding p53, HA-MDM2, His-Ub, RMRP or Flag-SNRPA1 as indicated in the figure legends. At 48 h after transfection, cells were harvested and split into two aliquots, one for IB and the other for the ubiquitination assay. Briefly, cell pellets were lysed in buffer I (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> (pH 8.0), 10 mM Tris-HCl (pH 8.0), 10 mM  $\beta$ -mercaptoethanol, 5 mM Imidazole and incubated with Ni-NTA beads (Takara, Dalian, China) that capture His-tagged proteins/complex at room temperature for 4-6 h. Beads were washed twice with buffer I, then buffer II (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> (pH 6.3), 10 mM Tris-HCl (pH 6.3), 10 mM  $\beta$ -mercaptoethanol). The captured proteins were eluted and analyzed by IB with the indicated antibodies.

### **RNA interference and generation of lentiviral particles**

The siRNAs used in this study (Supplementary Table 3) were synthesized and purified by GenePharma (Shanghai, China). SiRNAs (50-100 nM) were introduced into cells using Hieff Trans™ liposomal transfection reagent following the manufacturer's protocol (Yeasen, Shanghai, China). Cells were harvested 48-72 h after transfection for IB or RT-qPCR analysis. The pWPXL-RMRP plasmid was generated by inserting the full-length sequence of RMRP into the lentivirus-based pWPXL vector at the BamHI and MluI sites. The primers used are listed in Supplementary Table 6. The HEK293T cells were transfected with pWPXL-vector or pWPXL-RMRP, along with the packaging plasmid psPAX2 and the envelope plasmid pMD2.G. The virus particles were collected 48 h after transfection and then used for cell infection.

### **Luciferase reporter assay**

Constructs of the RMRP promoter regions, -1000 to +268, -500 to +268, and -200 to +268 were generated from the genomic DNA of HCT116 <sup>p53<sup>+/+</sup></sup> cells, and cloned into the pGL3.0 vector. The C/EBP response elements (REs) in the promoter region of RMRP were identified by the online tool PROMO . The primers used for construction were shown in Supplementary Table 6. Cells were seeded into 24-well plates and then co-transfected with C/EBP plasmids (100ng), RMRP promoter constructions (100ng) and the Renilla plasmid (20ng). At 24 h

post-transfection, cells were lysed with the luciferase lysis buffer. Firefly and Renilla luciferase activities were measured by the dual-luciferase assay kit (Promega, Madison, WI, USA). The results were showed by the ratio of firefly luciferase activity to Renilla luciferase activity.

### **RNA-seq**

Total RNAs of ctrl-cas9 and RMRP-sg-2 HCT116 <sup>p53+/+</sup> stable cells were purified by RNAiso Plus. The RNA-seq service was provided by Oebiotech, Shanghai, China. Data were extracted and analyzed according to the manufacturer's standard protocol. Read counts for each gene were normalized to FPKM (Fragments Per Kilobase transcript per Million mapped) values.

### **Cancer patients**

The cDNA array. The Lnc cDNA-HCoLA095Su01 array includes 80 cases of human colorectal cancer tissue and 15 cases of adjacent tissue. Among these, a pair of outliers (one cancer tissue and the matched adjacent tissue) was removed due to the quality control standards. The expression levels of RMRP in 93 cDNA samples (79 cases of cancer tissue and 14 cases of adjacent tissue) were measured through RT-qPCR analysis as described above. The significant differences of survival of the patients were analyzed by Kaplan–Meier statistics.

RNA in situ hybridization. RMRP expression was detected by RNA in situ hybridization on a 4- $\mu$ m-thick paraffin-embedded CRC tissue microarray, including 93 cases of CRC and 87 cases of adjacent tissue. The RMRP-LNA-Detection probe (LNA-modified and 5'- and 3'-DIG labeled oligonucleotide) was designed and synthesized by Exiqon (Qiagen, Hilden, Germany). The sequence is as follows: 5'-TAACTAGAGGGAGCTGACGGAT-3'. Briefly, the TMA were digested with proteinase K, and hybridized with double digoxin-labeled RMRP-LNA-Detection probe overnight at 55°C, then incubated overnight at 4 °C with an anti-Digoxigenin-AP, Fab fragments. The cells nuclei were stained with NBT/BCIP in the dark. Specific RMRP expression level was scored with ImageScope software independently by two blinded pathologists. The staining intensity was scored on a scale of 0–3, as follows: negative (no staining, 0), weak (1), medium (2) or strong (3).

Databases. The survival plot of RMRP in TCGA-READ was generated from The Atlas of ncRNA in Cancer (TANRIC, [https://ibl.mdanderson.org/tanric/\\_design/basic/main.html](https://ibl.mdanderson.org/tanric/_design/basic/main.html)). The differential gene expression in three cohorts of COAD (Colon Adenocarcinoma) in the Cancer Genome Atlas (TCGA, Provisional, TCGA, Nature and TCGA, PanCancer Atlas) was obtained from Cancer Genomics Database (<http://www.cbioportal.org/>). The correlation between the expression of RMRP and that of BTG2, Mdm2, and C/EBP- $\beta$  was evaluated using Pearson correlation coefficient. The significant differences of survival of the patients

were analyzed by the Kaplan-Meier method. The gene expression profiles of colorectal cancer cell lines in Cancer Cell Line Encyclopedia (CCLE, <https://portals.broadinstitute.org/ccle>) were downloaded and the correlation between the expression of RMRP and C/EBP- $\alpha$  was analyzed using Pearson correlation coefficient.

## Supplementary Figure Legends

**Supplementary Figure S1.** (A) Heat-map representation of the RNA-seq results by comparing RMRP-knockout cells with control cells. (B) KEGG enrichment of the cancer-related pathways dysregulated in RMRP-knockout cells. (C, D) Overexpression of RMRP has no effect on p53 mRNA expression in HCT116 <sup>p53+/+</sup> and H460 cells. (E, F) Knockdown of RMRP has no effect on p53 mRNA expression in HCT116 <sup>p53+/+</sup> and H460 cells. (G) Depletion of RMRP by CRISPR/Cas9 does not affect p53 RNA expression in HCT116 <sup>p53+/+</sup> cells. (H, I) The inverse correlation between the expression of RMRP and BTG2 (H) or MDM2 (I) from TCGA database. (J-L) The expression of RMRP is not correlated to the *TP53* status from TCGA database. (M) Knockdown of RMRP does not affect mutant p53 expression. The colon cancer HT-29 cells were transfected with RMRP siRNA or control followed by RT-qPCR and IB analyses. \**p*<0.05, \*\**p*<0.01 by two tailed t-test.

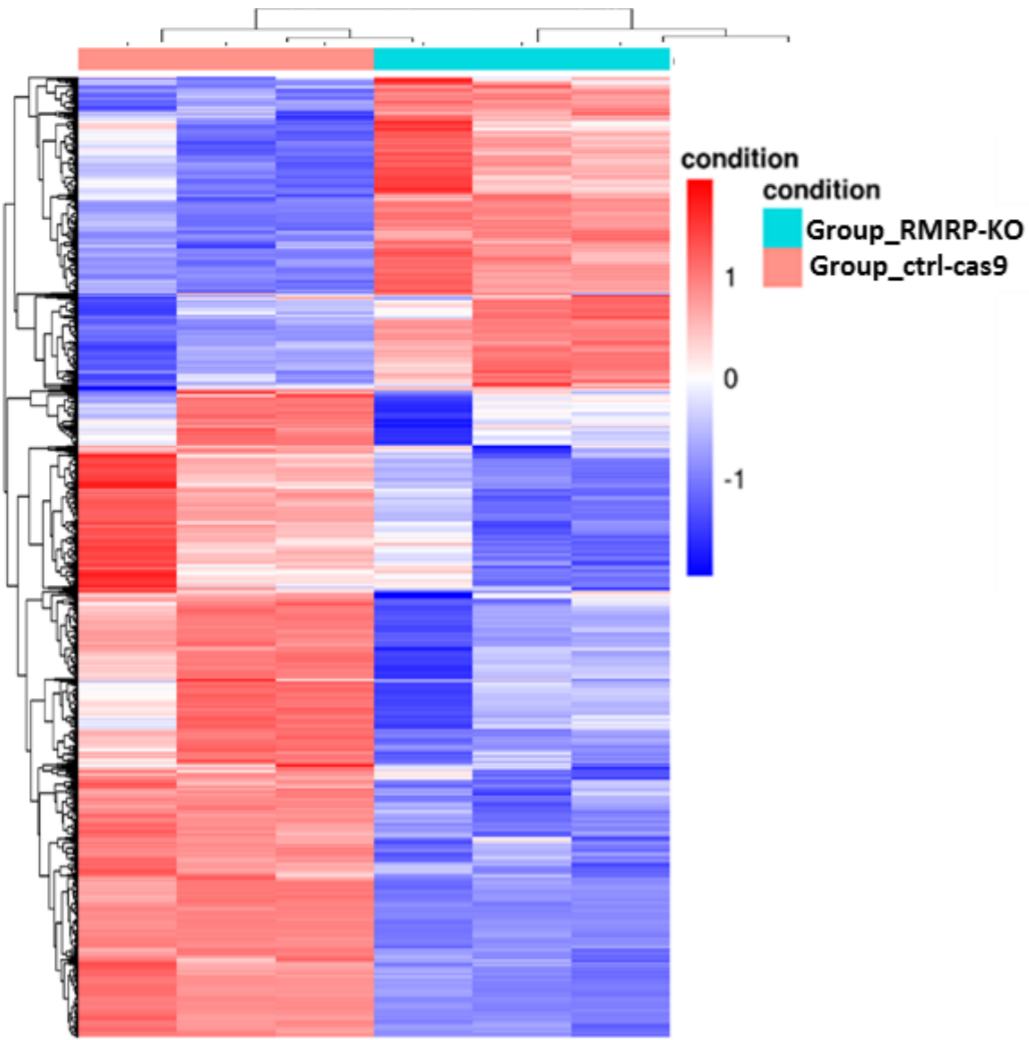
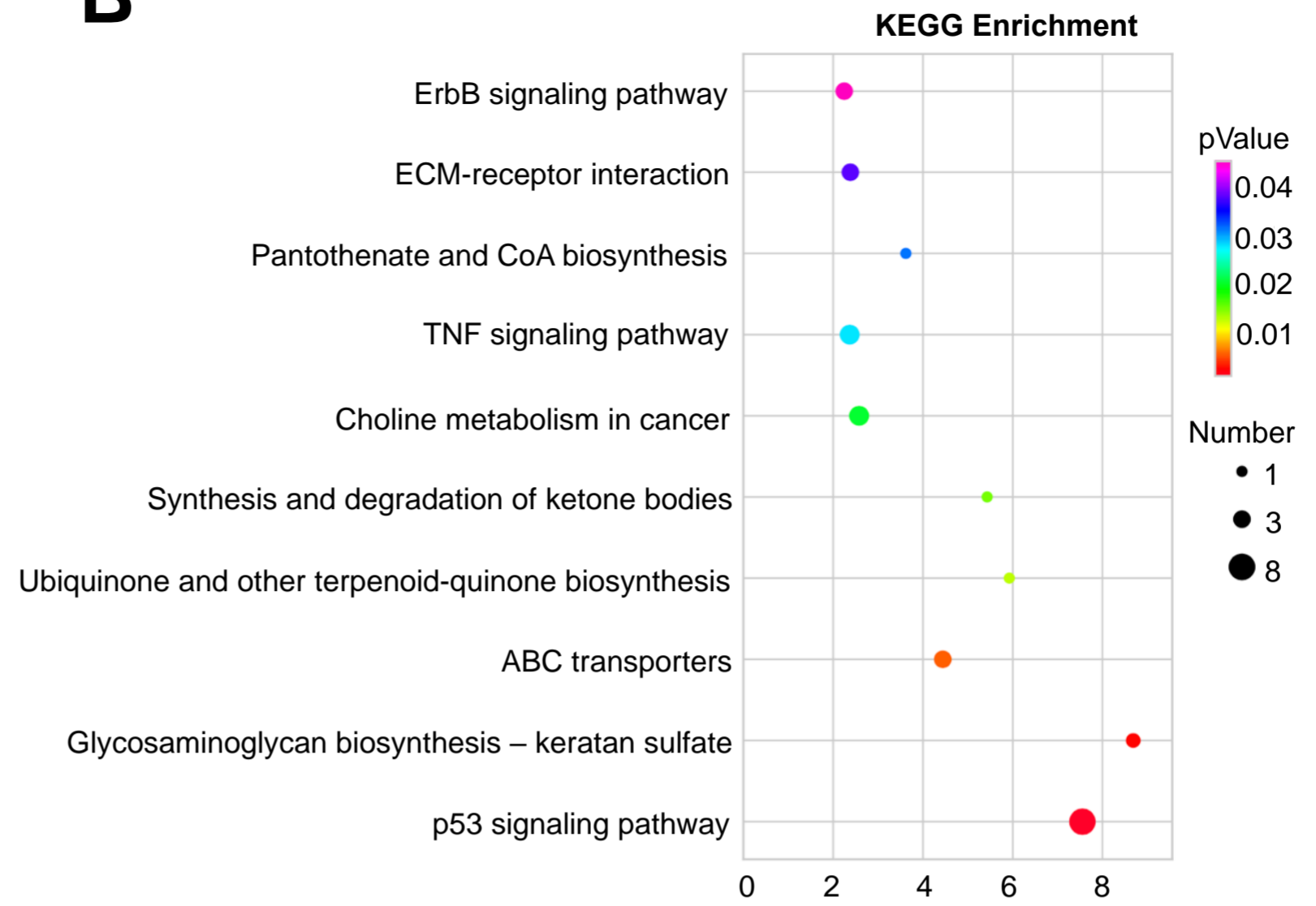
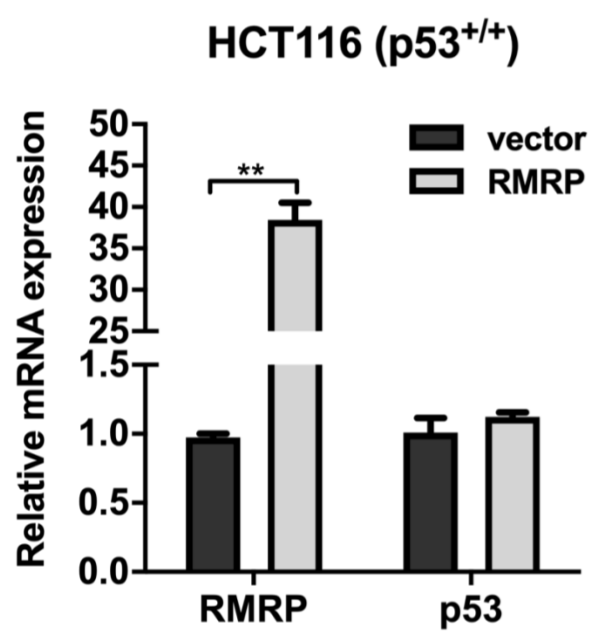
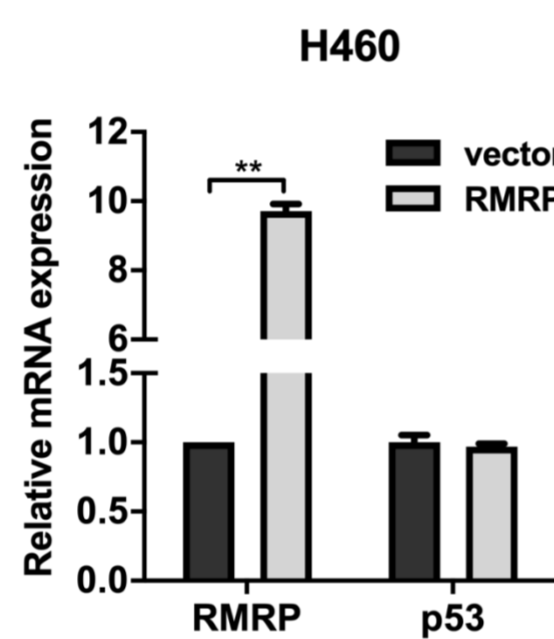
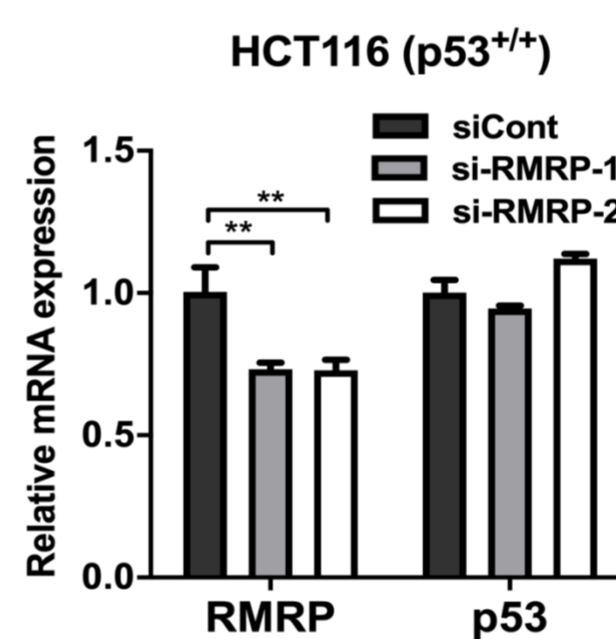
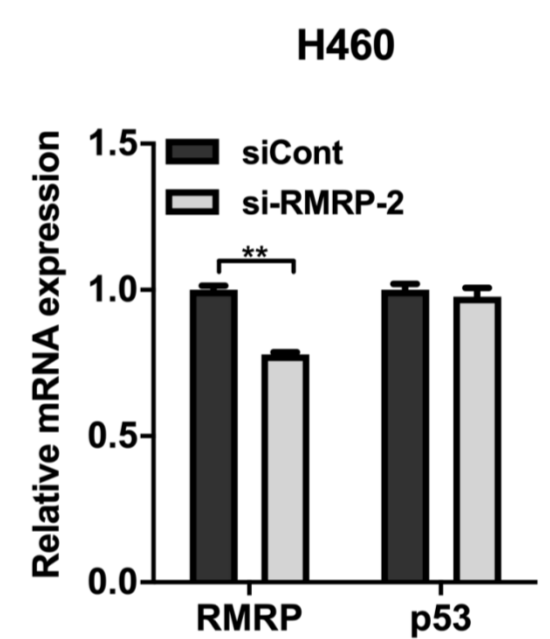
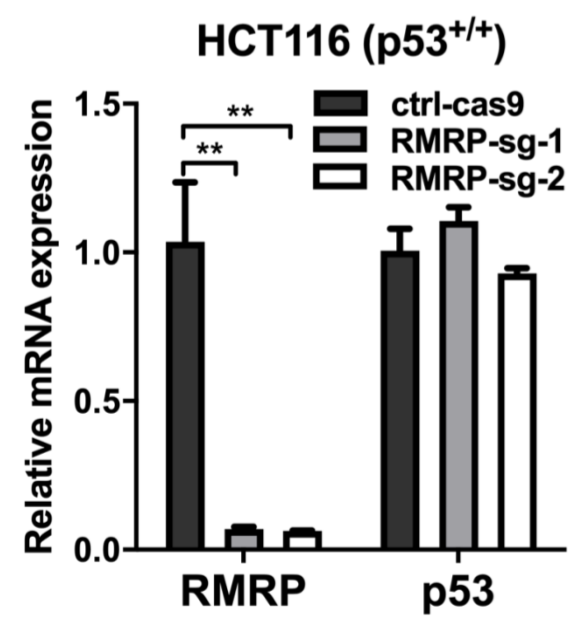
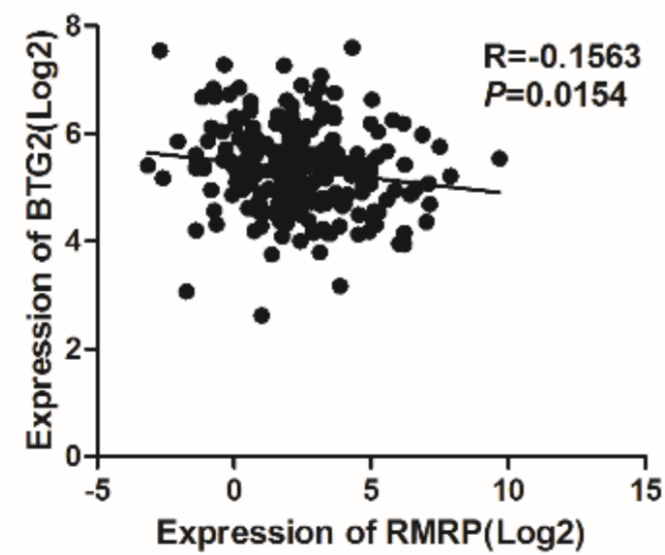
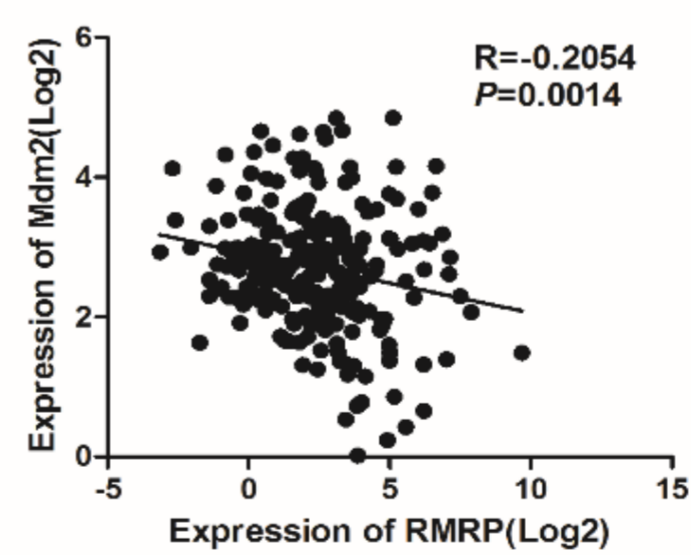
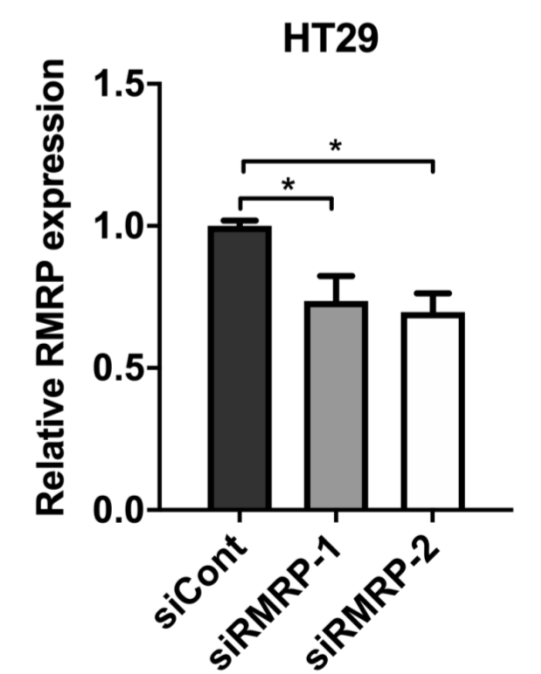
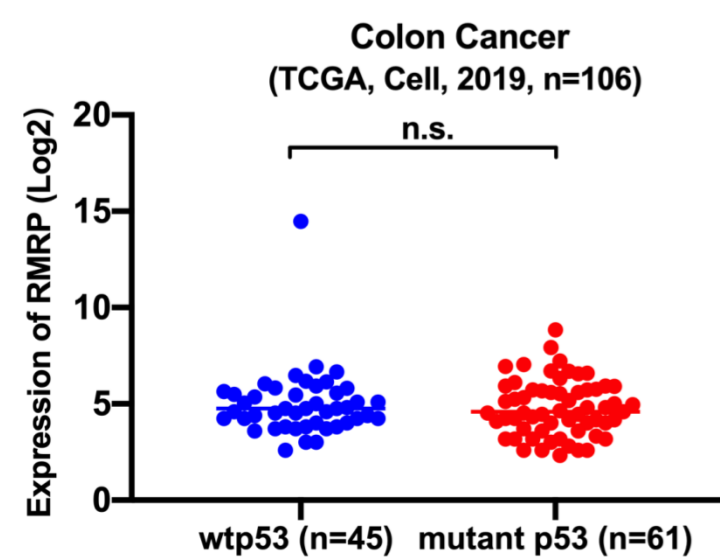
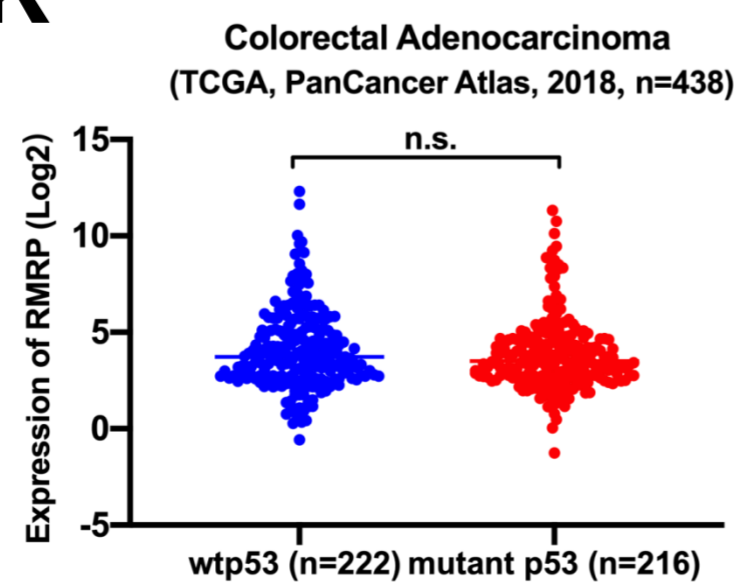
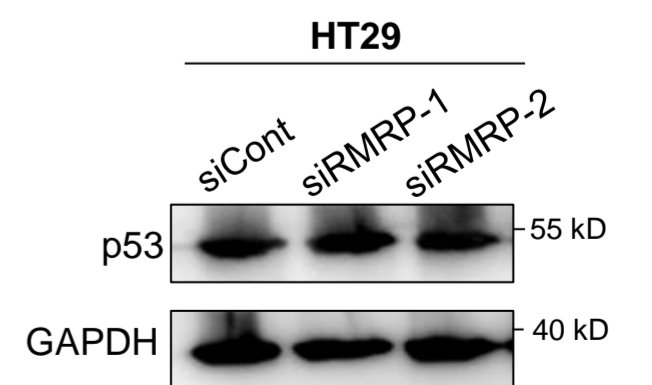
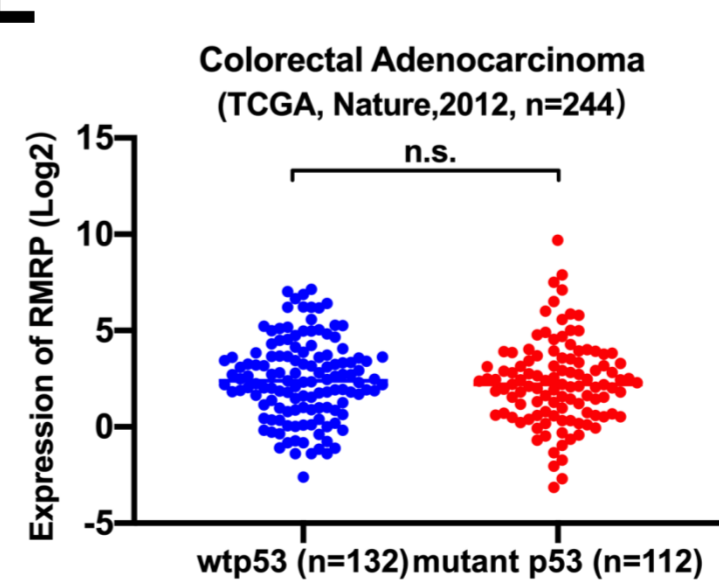
**Supplementary Figure S2.** (A) Mapping the p53 binding domain of SNRPA1. HCT116 <sup>p53-/-</sup> cells were transfected with the plasmid encoding the Myc-SNRPA1 fragment, aa 1–175, aa 176–225, or aa 1–255, along with the Flag-p53 plasmid, followed by a set of co-IP assays. (B) A schematic diagram of the p53 binding region on SNRPA1. (C) Mapping the SNRPA1 binding domain of p53. HCT116 <sup>p53-/-</sup> cells were transfected with the plasmids encoding the Flag-p53 fragment along with the Myc-SNRPA1 plasmid, followed by a set of co-IP assays. (D) A schematic diagram of the SNRPA1 binding region on p53. TAD, transactivation domain (1–42); DBD, DNA-binding domain (101–306); OD, Oligomerization domain (307–355); CTD: C-terminal regulatory domain.

**Supplementary Figure S3.** (A) The cellular distribution of lncRNAs, RMRP, U6, and MALAT1, and  $\beta$ -actin mRNA in HCT116 <sup>p53+/+</sup>, HCT116 <sup>p53-/-</sup>, H460, and H1299 cells. (B) CRISPR-Cas9-mediated ablation of RMRP reduces the SNRPA1 level in the nucleus, while increases the cytosolic accumulation of SNRPA1, in HCT116 <sup>p53-/-</sup> cells.

**Supplementary Figure S4.** (A) Overexpression of C/EBP $\alpha$  induces the level of RMRP in HCT116 <sup>p53+/+</sup> cells. (B) Overexpression of C/EBP $\alpha$  triggers RMRP promoter (~500 bp) activity as determined by the luciferase reporter assay. (C) The positive correlation between the expression of C/EBP $\alpha$  and RMRP in the CCLE database. (D) The positive correlation between the expression of C/EBP $\beta$  and RMRP in the TCGA database. (E, F) The Kaplan-Meier survival analysis indicate that higher expression of C/EBP $\beta$  predicts unfavorable patient survival in the two TCGA cohorts of colorectal adenocarcinoma. (G, H) The expression of RMRP was induced by PARP inhibitors, including Olaparib, Niraparib, and Talazoparib, in RKO (G) and LOVO cells (H). (I) The chemotherapeutic drugs, including 5-FU, Cisplatin, and Nutlin-3 do not regulate RMRP expression. (J, K) Neither glucose

deprivation (J) nor serum starvation (K) affects the level of RMRP. (L) Low dose of Olaparib significantly inhibits proliferation of RMRP-knockout, but not RMRP-proficient, RKO cells. (M) Knockout of RMRP sensitizes LOVO cells to Olaparib as determined by the IC50. (N) Knockout of RMRP has a trivial impact on HCT116  $p53^{-/-}$  cell sensitivity to Olaparib. \* $p < 0.05$ , \*\* $p < 0.01$  by two tailed t-test.

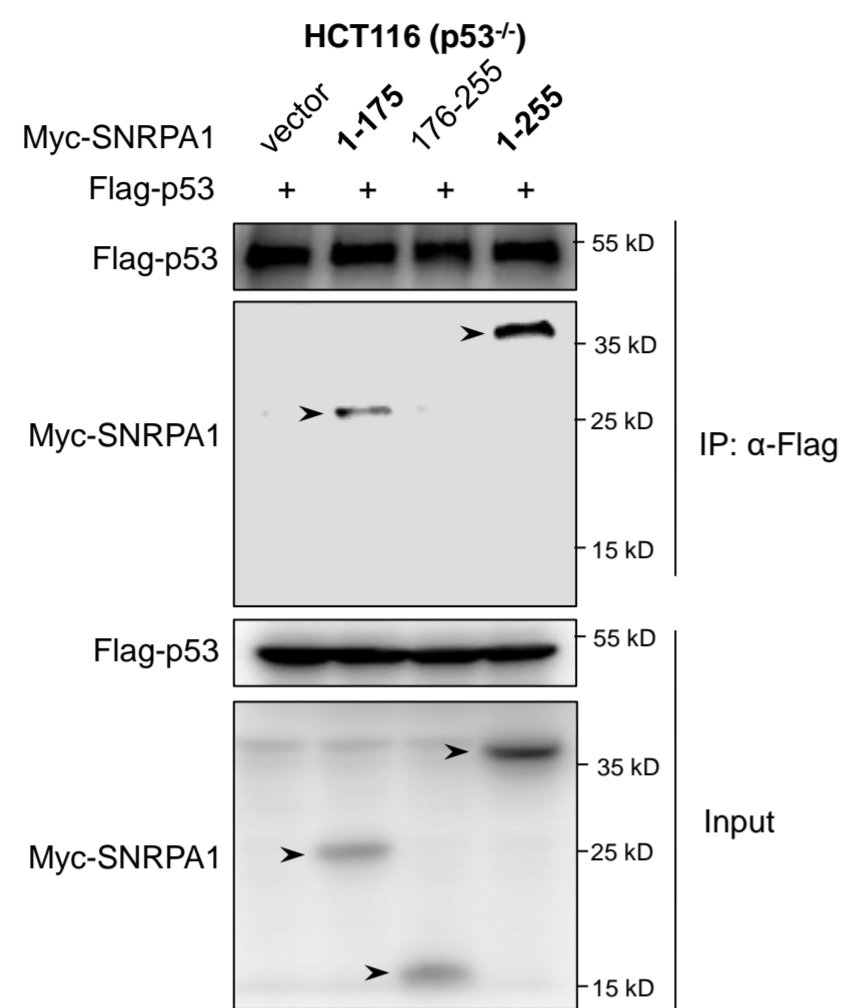
# Figure S1

**A****B****C****D****E****F****G****H****I****M****J****K****L**

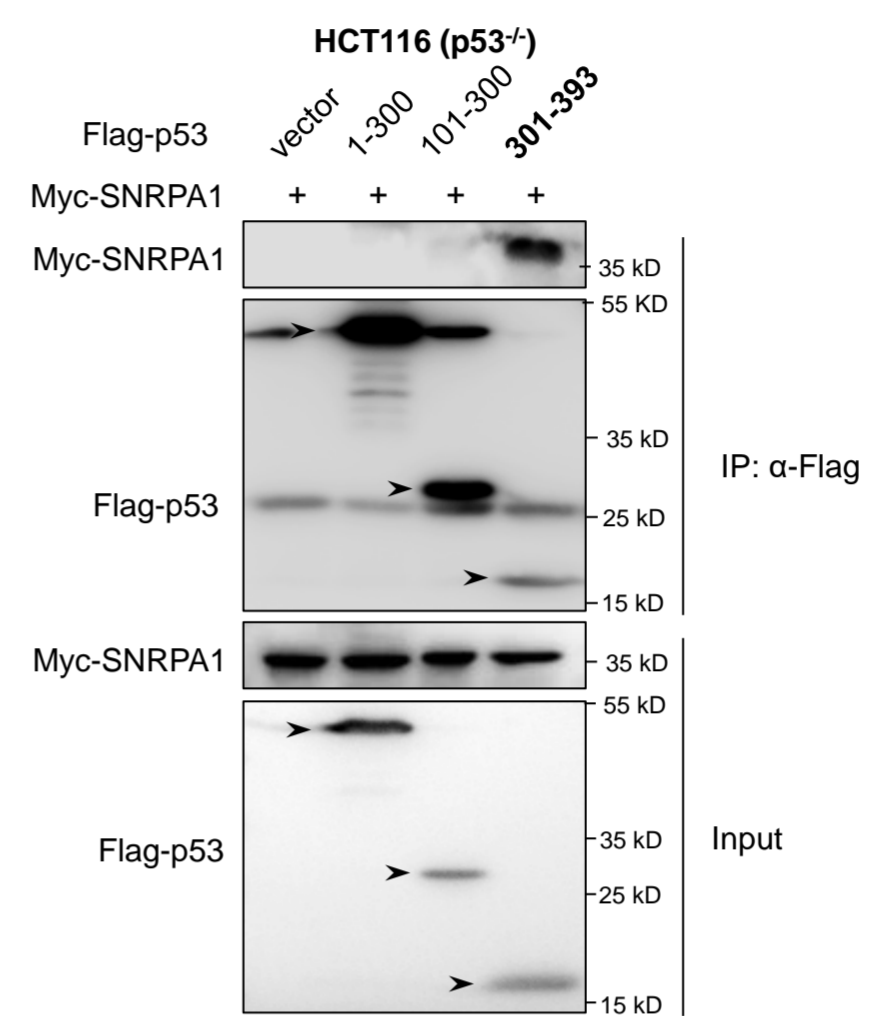


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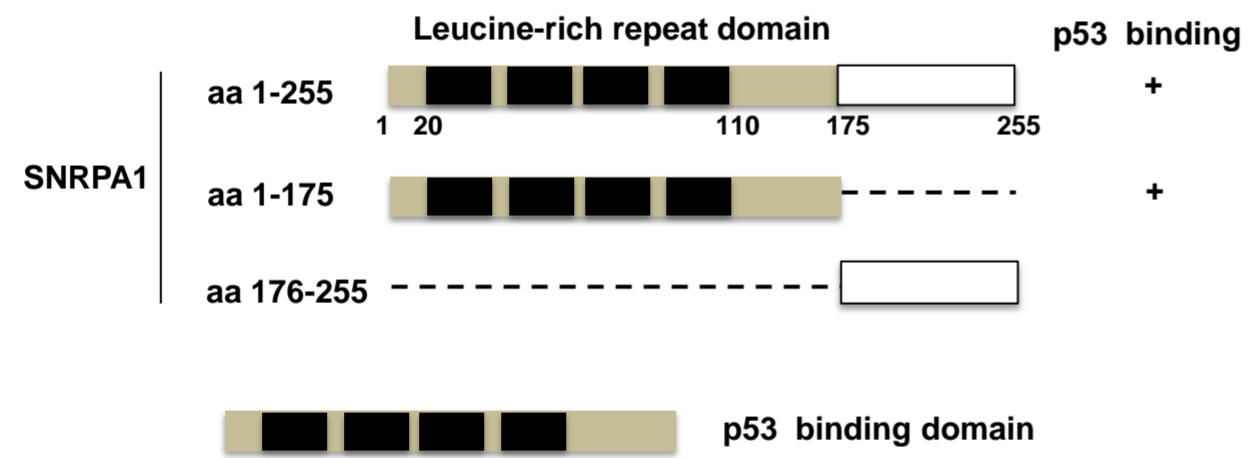
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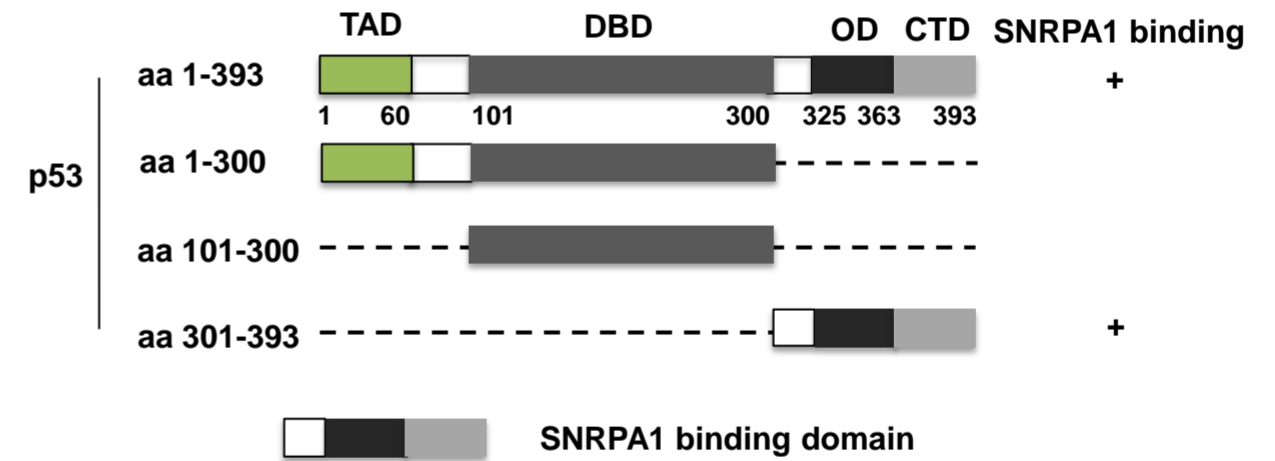
## C



## B

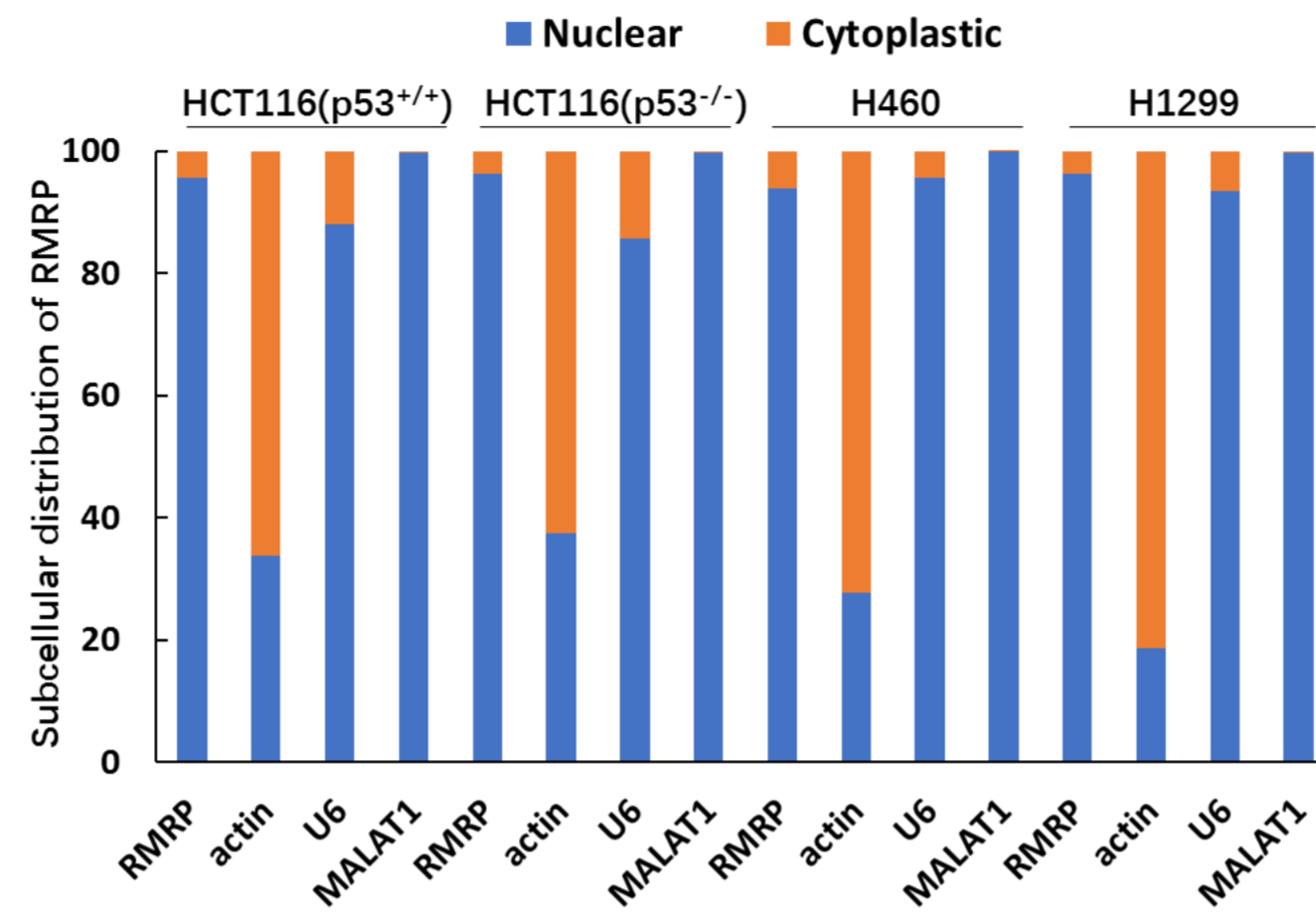


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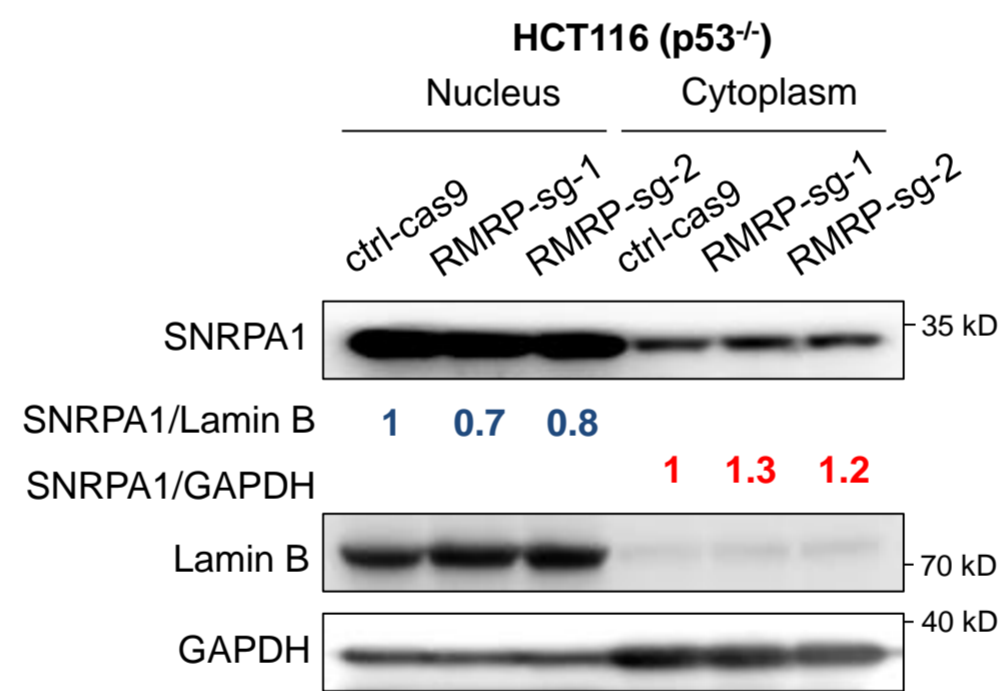


# Figure S3

## A

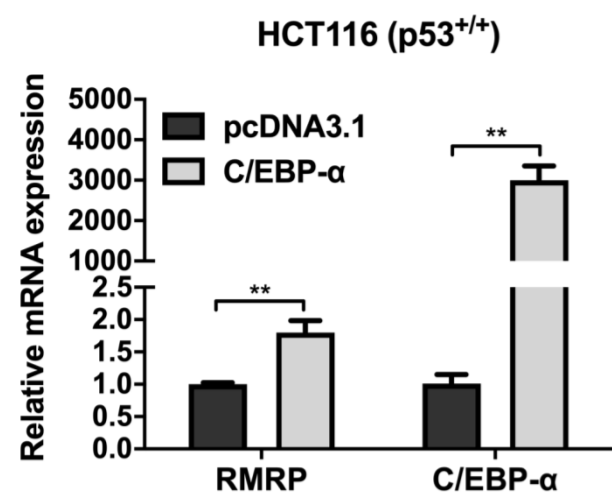


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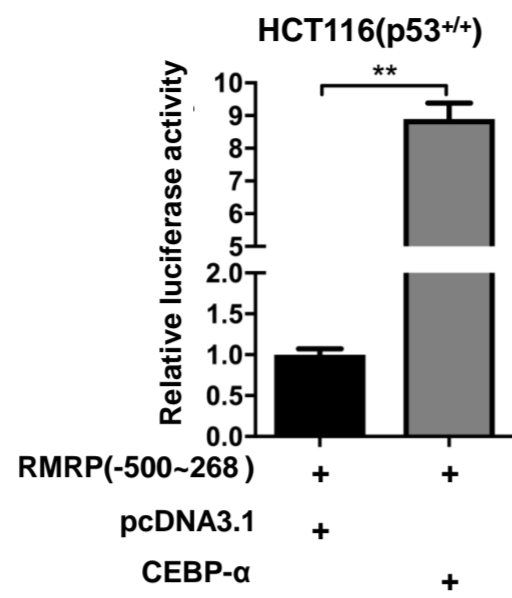


# Figure S4

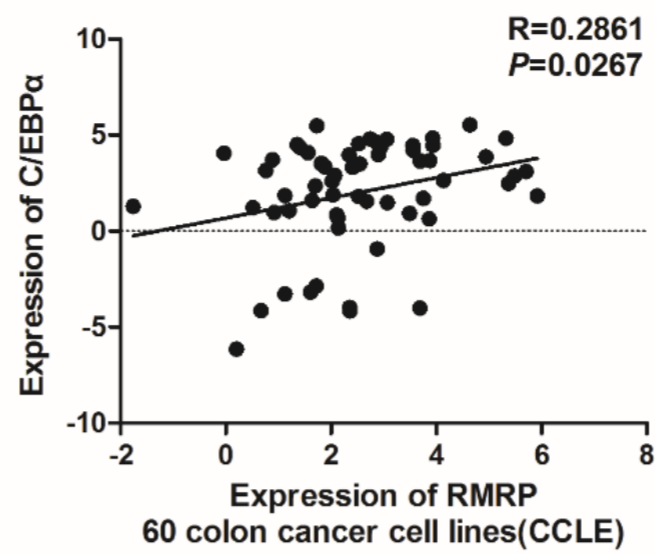
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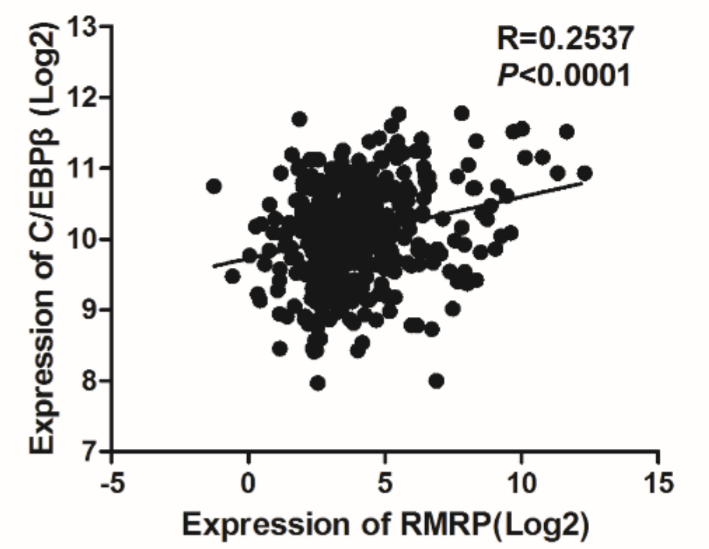
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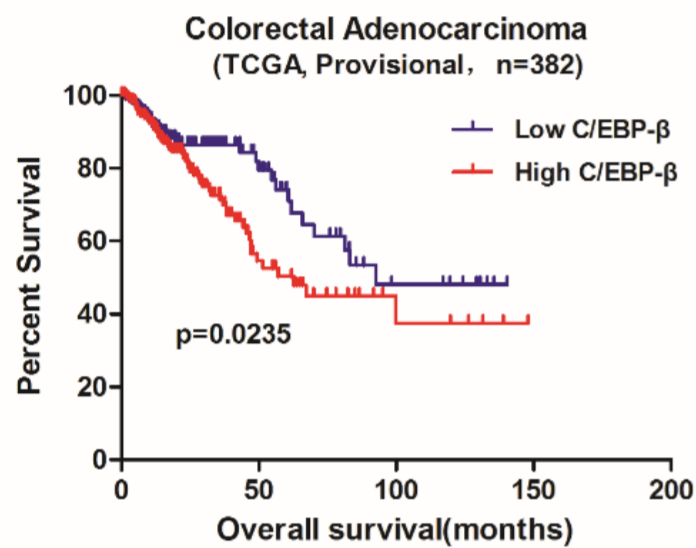
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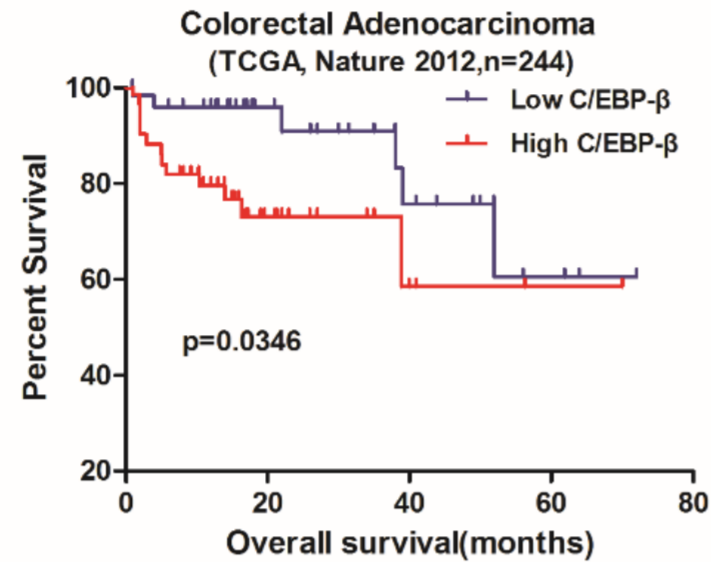
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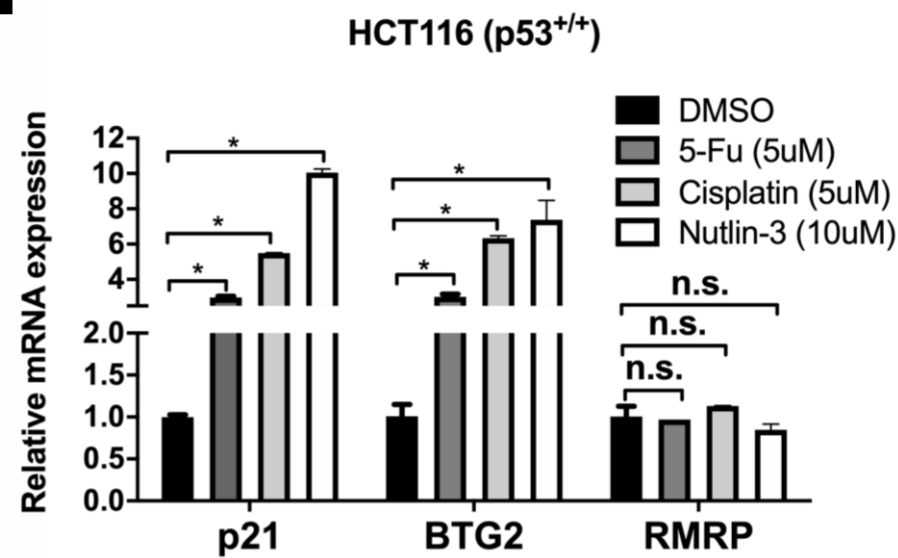
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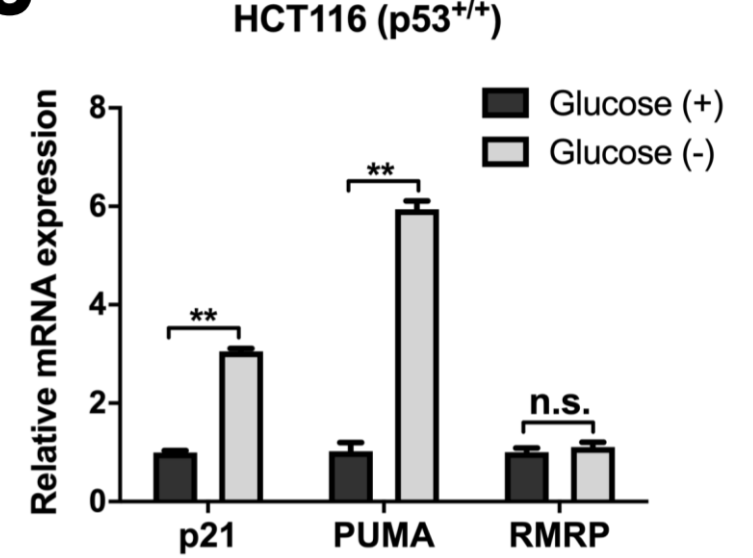
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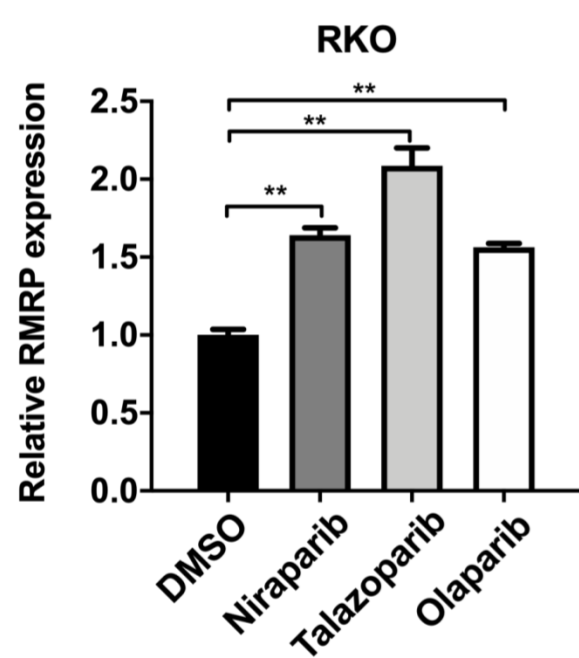
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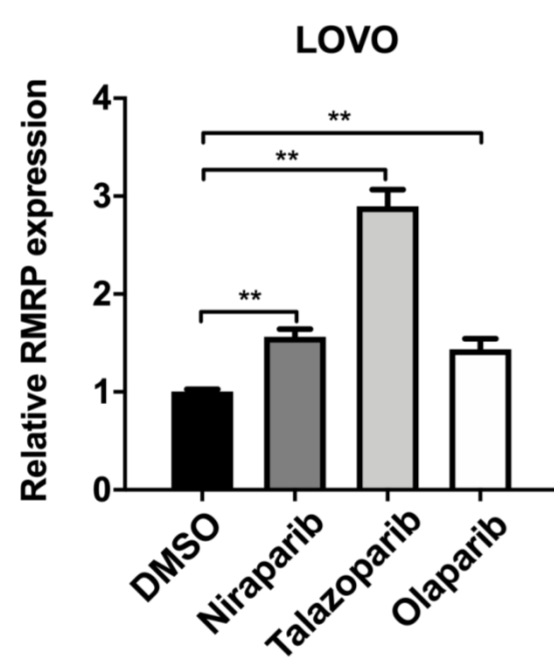
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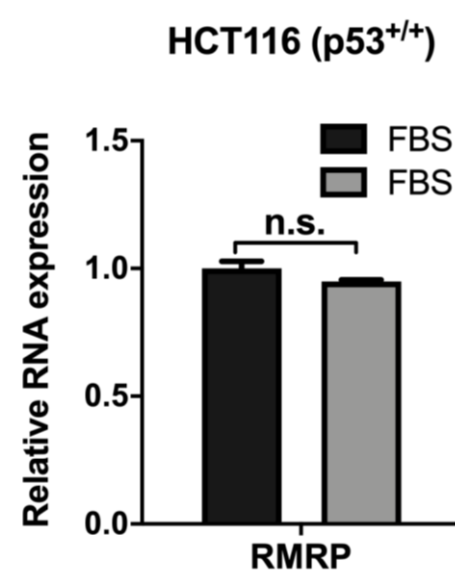
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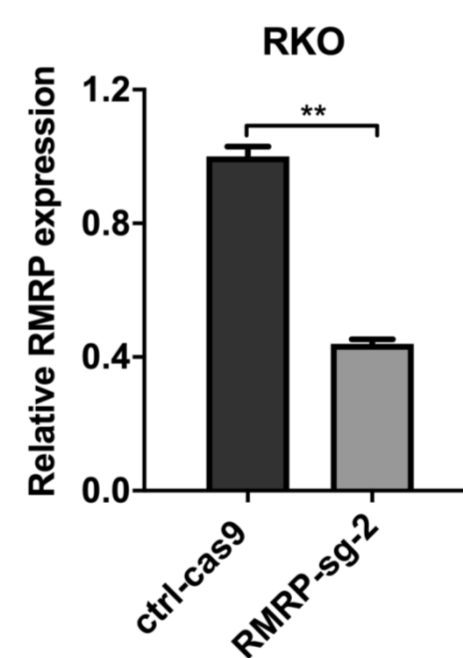
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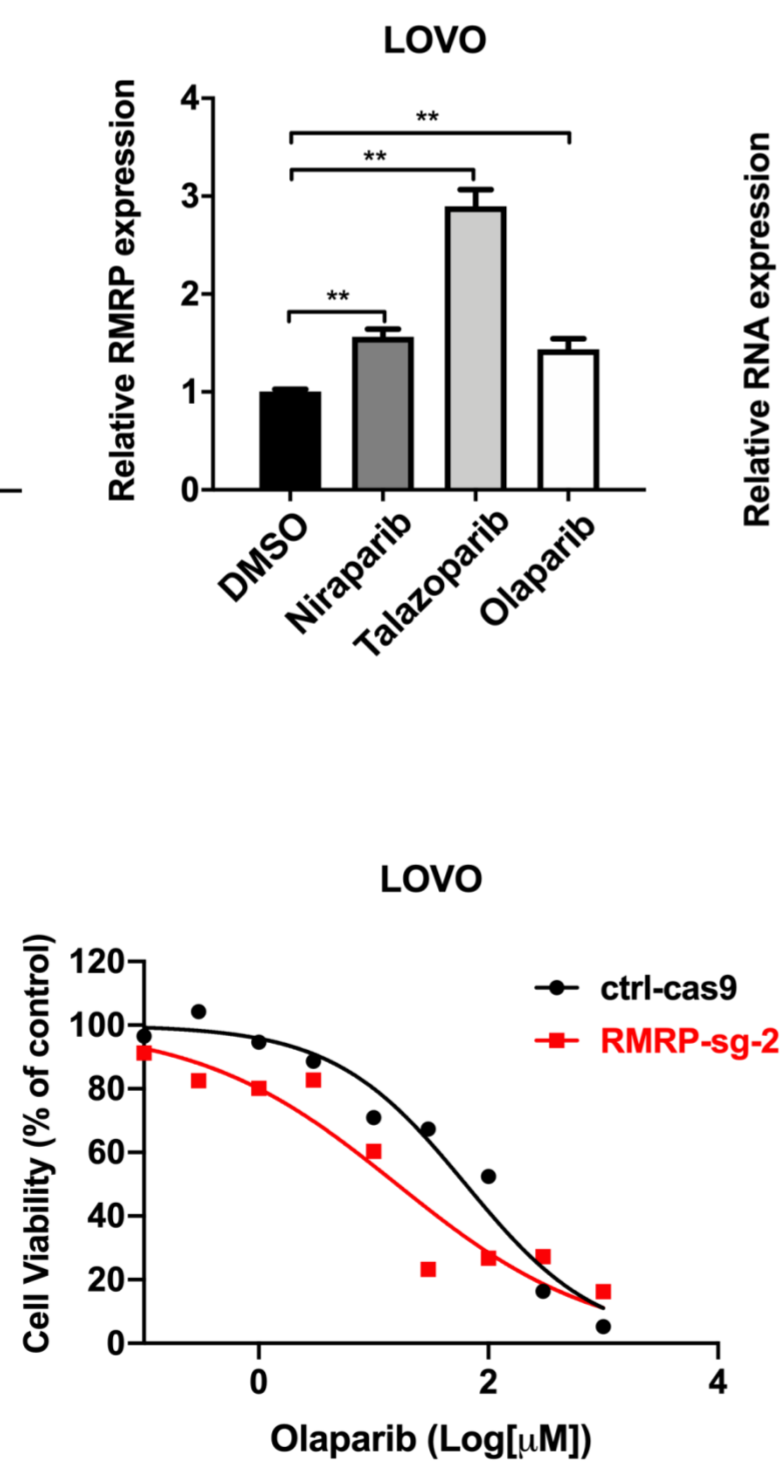
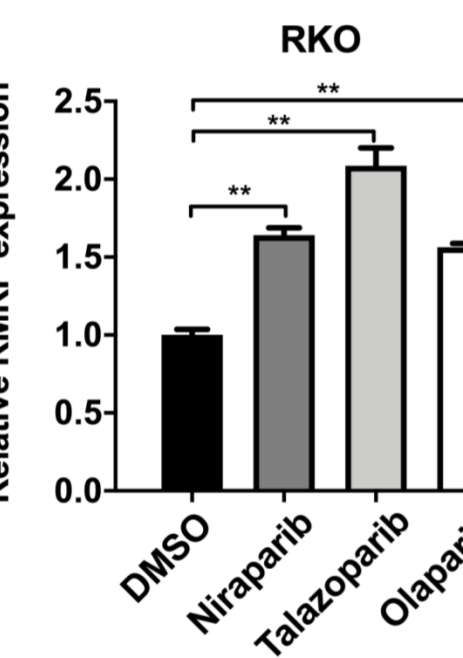
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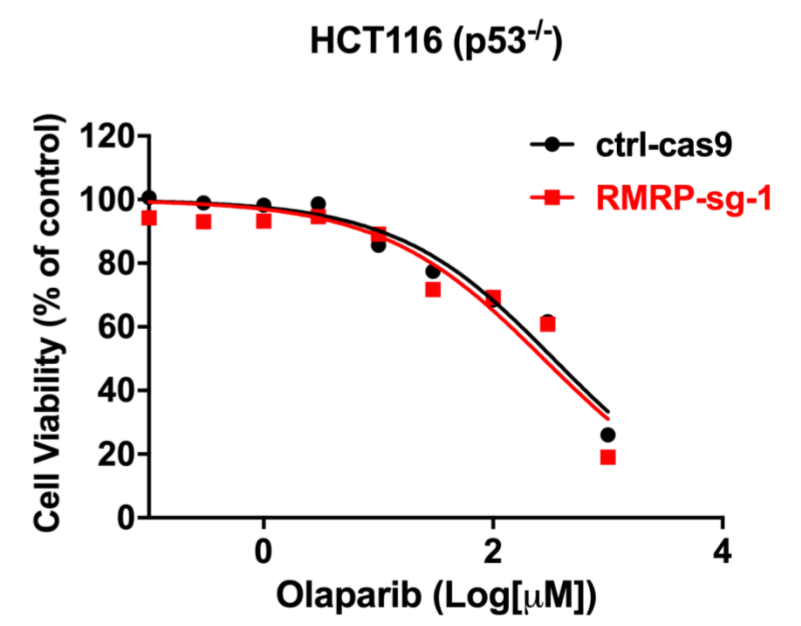
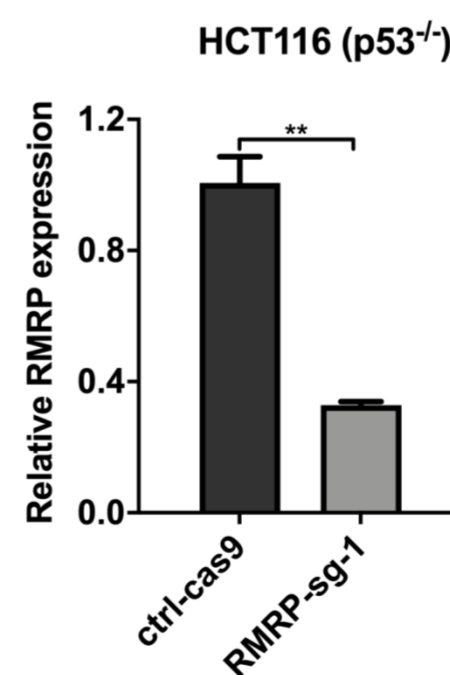


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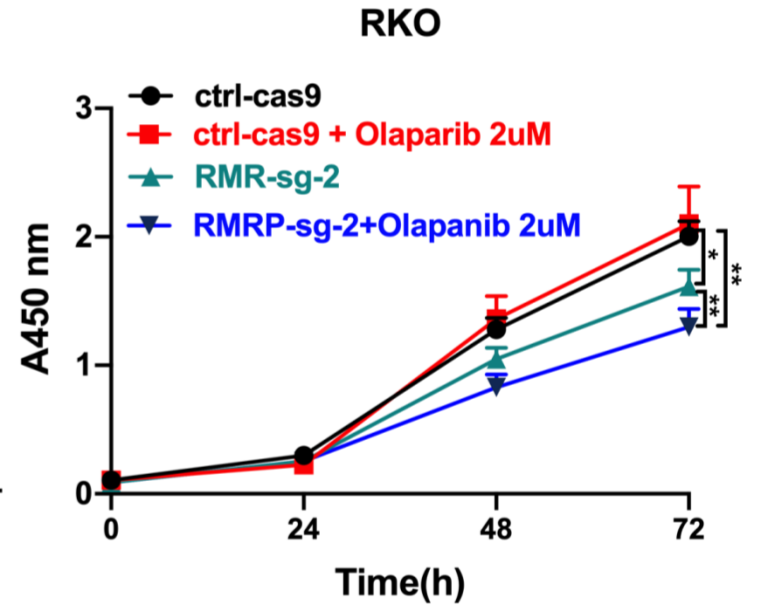
Group	IC50
ctrl-cas9	63.34 $\mu$ M
RMRP-sg-2	15.97 $\mu$ M

**N**



Group	IC50
ctrl-cas9	335.8 $\mu$ M
RMRP-sg-1	274.7 $\mu$ M

**O**



**Supplementary Table 1.** Relationships between LncRNA RMRP expression and Clinicopathologic features of the patients with colon cancer

Feature	No. of patients	RMRP (Low)	RMRP (High)	P-value
Gender				0.9241
Male	46	22	24	
Female	33	17	16	
Age(years)				0.5806
≥ 60	50	23	27	
< 60	29	16	13	
Positive lymph nodes				0.7360
0	41	22	19	
≥ 1	34	16	18	
Differentiation grade				0.4491
I	2	0	2	
I - II	3	2	1	
II	54	26	28	
II -III	12	6	6	
III	7	5	2	
TNM				0.7360
early stage	41	22	19	
late stage	34	16	18	

**Supplementary Table 2.** Univariate and multivariate Cox regression analyses of LncRNA RMRP for overall survival of the patients with colon cancer

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	P-value	HR	95%CI	P-value
Gender	0.9967	0.5434-1.8280	0.9910	1.0630	0.5532-2.0430	0.8544
Age	1.5350	0.7988-2.9490	0.1980	1.6060	0.7723- 3.3400	0.2047
Positive lymph nodes	1.8610	0.9868-3.5100	0.0550 <sup>#</sup>	1.7380	0.9125-3.3100	0.0927
Differentiation grade	1.5440	0.7898-3.0190	0.2040	1.6620	0.8111- 3.4040	0.1652
TNM	1.8610	0.9868-3.5100	0.0550 <sup>#</sup>	1.7380	0.9125-3.3100	0.0927
RMRP	1.4130	1.0280-1.8410	0.0331 <sup>*</sup>	1.3740	0.9677-1.9500	0.0757 <sup>#</sup>

CI, confidence interval; HR, hazard ratios; OS, overall survival;  
Statistical significance (\* P<0.05); Tendency toward statistical significance (<sup>#</sup> P>0.05)

**Supplementary Table 3. Sequences of siRNAs**

<b>siRNA</b>	<b>Sequences (5'-3')</b>
siCont	UUCUCCGAACGUGUCACGUTT
siRMRP-1	CCUAGGCUACACACUGAGGACUTT
siRMRP-2	GUUCGUGCUGAAGGCCUGUAU
siSNRPA1-1	GAA AUGUUCAAGGGCAA
siSNRPA1-2	CGGUAACCAAUAAGAAGCA
siLAMP2A-1	GCAGUGCAGAUGACGACAATT
siLAMP2A-2	GCCUUGGCAGGAGUACUUATT
siC/EBP- $\alpha$	CCAAGAAGUCGGUGGACAAdTdT
siC/EBP- $\beta$	UGCCUUUAAAUCCAUGGAA
siATF3	CTGTGAGATAAGCGGGACTCAG
siCTCF	CAUCAGAAGUCACACAAGA
siE2F1	GCATTAGAGATCTCTTTGA
siEGR1	GGACUAAAAGGCUCUUAU
siRela	GCUGAUGUGCACCGACAAG
siREST	GGCAA AUGUGGCCUUAACU
siRUNX3	GCCAA AU CUUGCGAUTCCU
siTEAD4	GGAACAACUGUGCCUGAATT
siZBTB7A	GCUGGACCUUGUAGAUCAAT

**Supplementary Table 4. Primers for RT-qPCR**

<b>Primer</b>	<b>Sequences (5'-3')</b>
GAPDH-F	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG
ACTB-F	CATGTACGTTGCTATCCAGGC
ACTB-R	CTCCTTAATGTCACGCACGAT
U6-F	GCTTCGGCAGCACATATACTAAAAT
U6-R	CGCTTCACGAATTTGCGTGTGCAT
RMRP-F	TGCTGAAGGCCTGTATCCT
RMRP-R	TGAGAATGAGCCCCGTGT
TP53-F	CCCAAGCAATGGATGATTTGA
TP53-R	GGCATTCTGGGAGCTTCATCT
CDKN1A(p21)-F	CTGGACTGTTTTCTCTCGGCTC
CDKN1A(p21)-R	TGTATATTCAGCATTGTGGGAGGA
MDM2-F	ATGAATCCCCCCTTCCAT
MDM2-R	CAGGAAGCCAATTCTCACGAA
BTG2-F	CCAGGAGGCACTCACAGAGCA
BTG2-R	ACCCACAGGGTCAGCTCGCT
BAX-F	CTGCAGAGGATGATTGCCGCCG
BAX-R	TCCGGCACCTTGGTGCACAG
PUMA-F	ACAGTACGAGCGGCGGAGACAA
PUMA-R	GGCGGGTGCAGGCACCTAATT
MALAT1-F	GATCTAGCACAGACCCTTCAC
MALAT1-R	CGACACCATCGTTACCTTGA
SNRPA1-F	CAGAATATGCCGTATAGGTGA
SNRPA1-R	CTTATTGGTTACCGGATTTCC
C/EBP- $\alpha$ -F	GGCCGCCTTCAACGACGAGTT
C/EBP- $\alpha$ -R	GCCCGGGTAGTCAAAGTCGC
C/EBP- $\beta$ -F	CGCGCTTACCTCGGCTACCA
C/EBP- $\beta$ -R	GCGCCGGATCTTGTACTCGTC

**Supplementary Table 5. Primers for CHIP-qPCR**

<b>Primer</b>	<b>Sequences (5'-3')</b>
GAPDH-CHIP-F	TACTAGCGGTTTTACGGGCG
GAPDH-CHIP-R	TCGAACAGGAGGAGCAGAGAGCGA
RMRP-CHIP-F	GTCATTCTAGCTTTCCTGTA
RMRP-CHIP-R	ACGTCCTCAGCTTCACAGA

**Supplementary Table 6. Primers for construction and others**

<b>Primer</b>	<b>Sequences (5'-3')</b>
pWPXL-RMRP-F (BamH I)	CGGGATCCGGTTCGTGCTGAAGGCC
pWPXL-RMRP-R (Mlu I)	TAGCGCTAGGACGCGTACAGCCGCGCTGAGAAT
RMRP-gRNA-1-F	CACCGTATCCTAGGCTACACACTG
RMRP-gRNA-1-R	AAACCAGTGTGTAGCCTAGGATAC
RMRP-gRNA-2-F	CACCGTTCCTCCCCTTTCCGCCTAG
RMRP-gRNA-2-R	AAACCTAGGCGGAAAGGGGAGGAAC
RMRP-gRNA-3-F	CACCGTATGATTAGGGTGAGAAAGT
RMRP-gRNA-3-R	AAACACTTTCTCACCTAATCATAC
RMRP-sense-F	TAATACGACTCACTATAGGGAGAGGTTTCGTGCTGAA GGCCTGTATC
RMRP-sense-R	ACAGCCGCGCTGAGAATGAGCC
RMRP-antisense-F	GGTTCGTGCTGAAGGCCTGTATC
RMRP-antisense-R	TAATACGACTCACTATAGGGAGAACAGCCGCGCTGA GAATGAGCC
pGL3.0-(-200~268)-F (Mlu I)	TAGCGCTAGGACGCGTGTTCATTCTAGCTTTTCCTGTAT TTG
pGL3.0-(-200~268)-R (Hind III)	CCCAAGCTTACAGCCGCGCTGAGAAT
pGL3.0-(-500~268)-F (Mlu I)	TAGCGCTAGGACGCGTTCCCAGCCCTCCTCCA
pGL3.0-(-500~268)-R (Hind III)	CCCAAGCTTACAGCCGCGCTGAGAAT
pGL3.0-(-1000~268)-F (Mlu I)	TAGCGCTAGGACGCGTTGGGTGACCTCGAGCAAG
pGL3.0-(-1000~268)-R (Hind III)	CCCAAGCTTACAGCCGCGCTGAGAAT
Myc-SNRPA1-F (Xba I)	GCTCTAGAATGGTCAAGCTGACGGCGG
Myc-SNRPA1-R (Not I)	ATAAGAATGCGGCCGCGGACCCGTTTGTGACTGTGT
Myc-SNRPA1-(1-175)-F (Xba I)	GCTCTAGAATGGTCAAGCTGACGGCGG
Myc-SNRPA1-(1-175)-R (Not I)	ATAAGAATGCGGCCGCGGCAATATCCTTTGCAAGCT GTGC
Myc-SNRPA1-(176-225)-F (Xba I)	GCTCTAGAATGAGGAGAAGCAAACCTTTAATCCAG GT
Myc-SNRPA1-(176-225)-R (Not I)	ATAAGAATGCGGCCGCGGACCCGTTTGTGACTGTGT