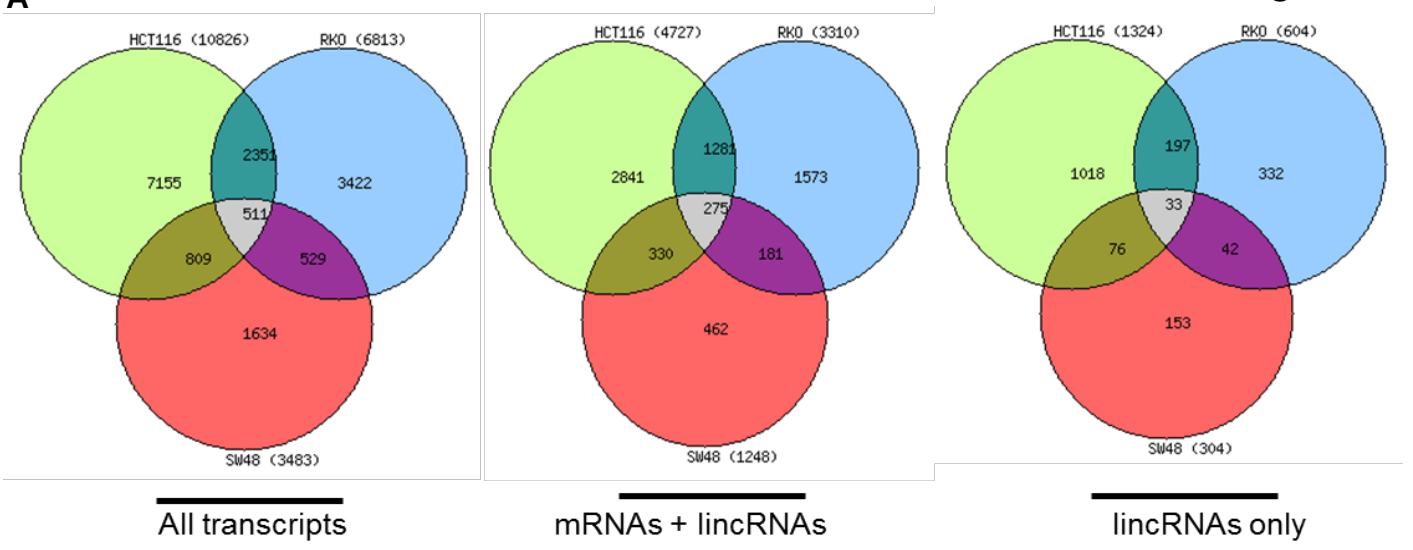
A



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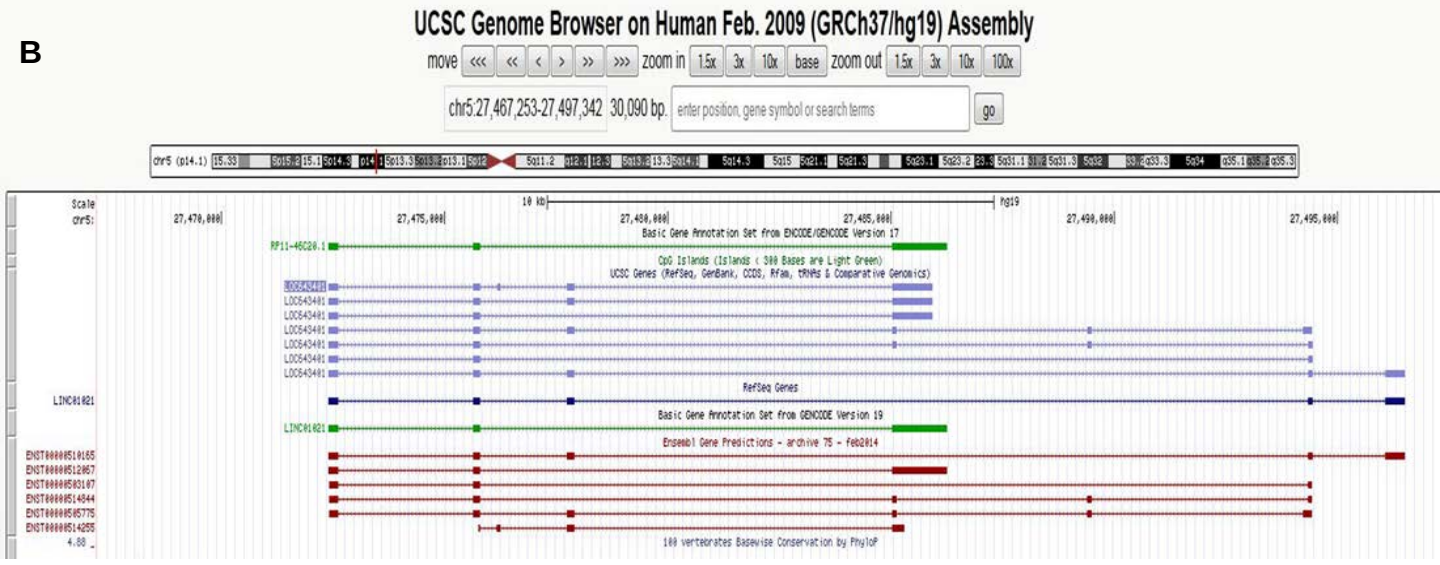

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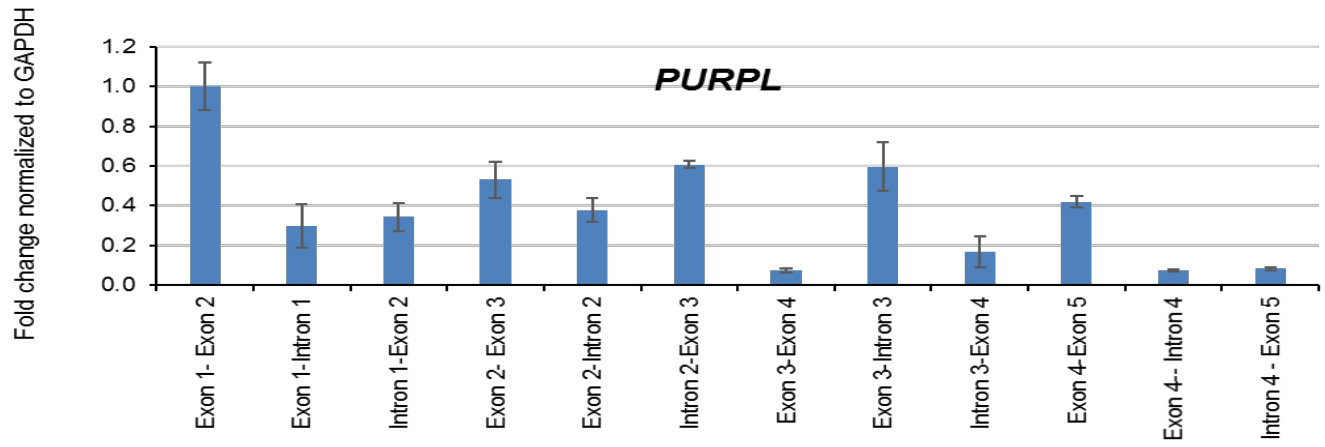


Figure S1. Overlap between the list of RNAs induced by DNA damage in multiple CRC lines, Related to Figure 1.
(A) Venn diagram shows the number of all transcripts, mRNAs and lincRNAs, and only lincRNAs upregulated in a p53-dependent manner after DOXO treatment in HCT116, RKO and SW48 cells and the overlap between the 3 lines. (B) UCSC tracks shows multiple isoforms expressed from the *PURPL* locus. (C) Parental HCT116 cells were treated with DOXO for 16 hr and RT-qPCR for *LINC01021* was performed using exon-exon and exon-intron primers annotated by RefSeq. As shown, the relative abundance of intron 2 as measured by RT-qPCR with exon 2-intron 2 or intron 2-exon 3 is similar to exon 2 – exon 3. Thus, intron 2 appears to be retained. This result is consistent with the RNA-seq snapshot shown in Figure 1B. Error bars represent SD from 2 independent experiments.

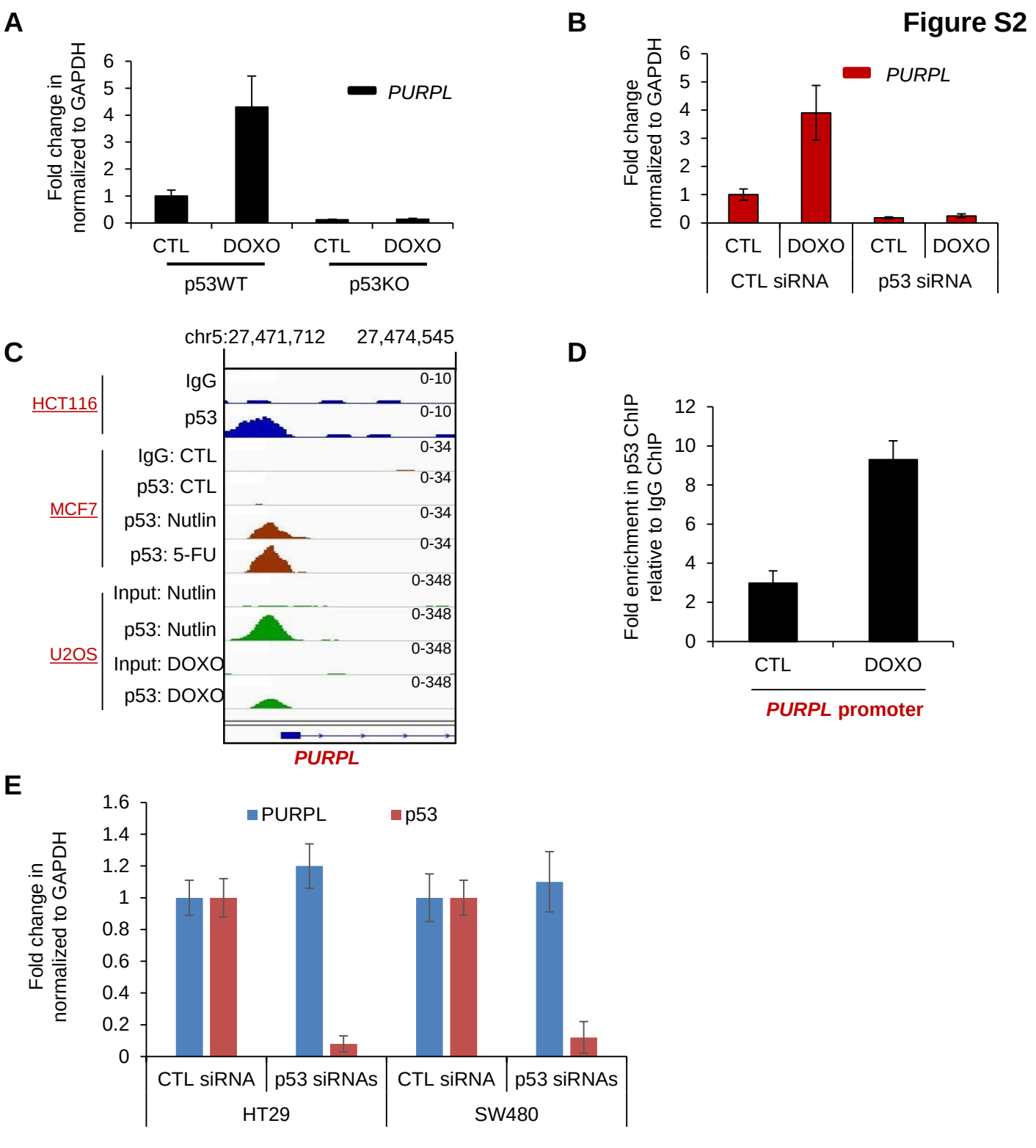
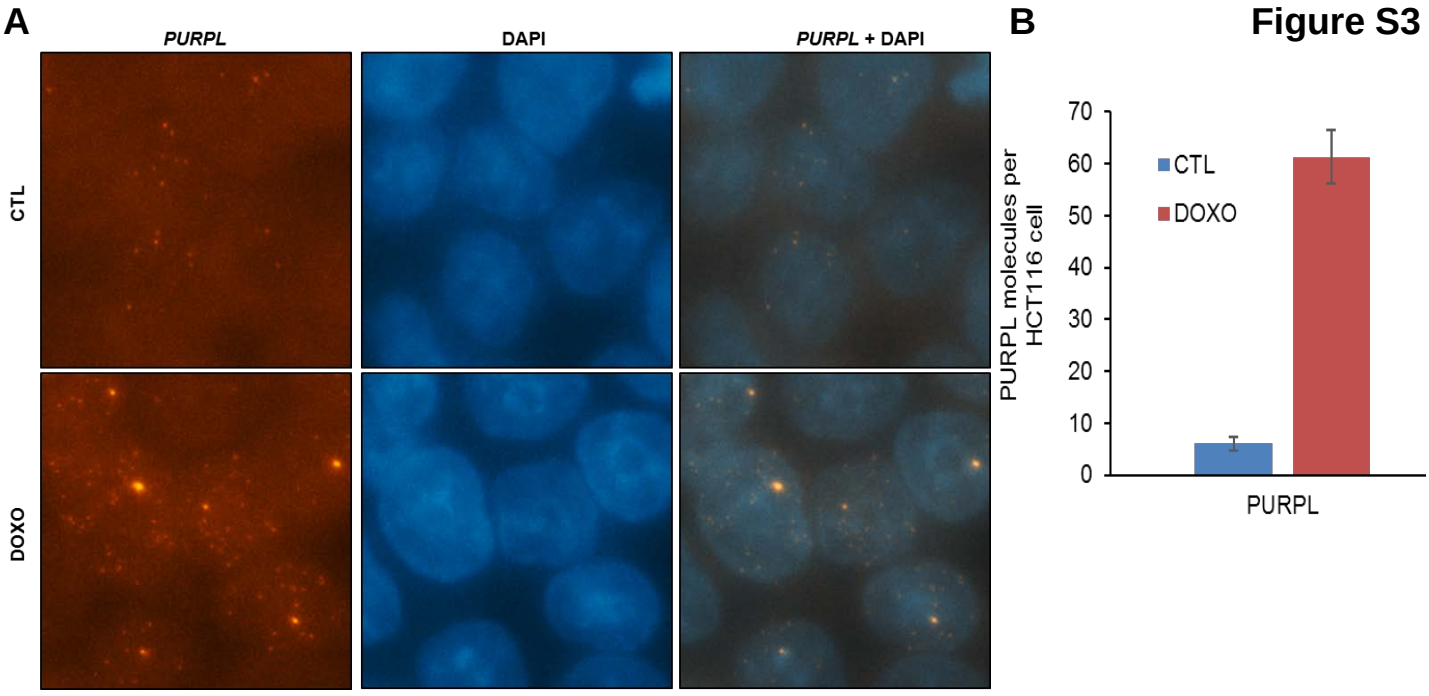


Figure S2. *PURPL* is a direct target of p53, Related to Figure 1.

(A) RT-qPCR analysis of *PURPL* expression in isogenic p53WT and p53KO-HCT116 cells with or without treatment with DOXO for 16 hr. (B) HCT116 cells were transfected with CTL siRNA or p53 siRNAs for 48 hr and RT-qPCR was performed after DOXO treatment for 16 hr. (C) Snapshot of p53 ChIP-seq signal at *PURPL* promoter from HCT116, MCF7 and U2OS cells untreated or treated with Nutlin or 5-FU or DOXO. (D) HCT116 cells were untreated or treated with DOXO for 16 hr and qPCR using primers spanning the p53-ChIP-seq peak was performed from IgG-ChIP and p53-ChIP. (E) RT-qPCR analysis of *PURPL* expression in the mutant p53-expressing HT29 and SW480 cells 72 hr after knockdown of mutant p53 with siRNAs. Error bars represent SD from 3 experiments.

Figure S3



C

>gi|336391099|ref|NR_038848.1| Homo sapiens long intergenic non-protein coding RNA 1021 (LINC01021), long non-coding RNA

CTGGGAATCAATGTGTGAGGTGGTGCTATGTAGCAGGAACCCCTCTTGCTTTGCAAATAGTTTTTTGTTTGTTTTCCTTTTTGCCCAATAGAG
CCCTGCTCTACTGACCCTTCAATGTGCCCGTGTGCCTAAATATTCCTGGTCGTGTGAAAAGAACCCAGGTATTAGCTGAACTAAGGAGCACA
ATTCTGCAACATTTTGGCACCCAAACACGGGGCTTGAGAAATGAATGCAATTGGAGAAACTGGTTGTTTTACCAGGCGTTGATTGGAAATGT
GTGCTTCCCTTTAAGCAGTCAAGCTCAACTTGCAGAACTGATGGGAACCCCTTGGGAAAACTGGCCTCAAATGTTTGTCTACACAGTCCACA
TACAGGGTTCTTAACCTGCGATGAGGGAAACTTCCCAGGGCTTGTCTGGGTATGCCCACAGTGGACTGGAGCCCAAAATGCACACTGGAG
GAAGTGGATGGAGCCACGTGGATGTCATGCCTTATGCAGGGGAGGAGCCTGCTCTCTTCAGCTCCTGTGGTAATGTGGGAATCGATCTGT
GAGCATATTTAACTATCCGGCTATTTTGGAGATTGAAAAAAGAAGACCTGATTTATCATGTGCAATATCTCACACATCTGTCATTTCTATTCTA
CCGCAATTCGATGGAGTCTTGTTCTGTGGTCCAGGCTAGAGGGCAGTGGCATGATCTCGGCTCACTACAAGCTCTGCCTCCCGGGTTCAC
GCCATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGGCGCCCGCCACCACGCCTGGCTAATTTTTTGTATATTTACTAGAGATGG
CTAGTGTTAGCCAAGATGGTCTCGATCTCCTGACCTCGTGATCTGCCCACCTCAGCCTGCCAAAGTGCTGGGATTACTGGCGTGAGCCACC
GTGCCTGGCCAGCATCCTCTAATTATTATTCATAGTTTGTGAATTTAGGCCTACGTGAATAATAATATAATATTTACTATGATGTTACTATCTAC
CAACCTTATTTTTCTTTTCTATAAAATAGGAGCCAAAATAATATTTATGTCATACCTTATGAAGTTAAAATAAAATATAACGAATGAGGGGCTTA
AAAAAAAAAAAAAAA

Poly(A) signal

D

Sequence Name	Coding Probability	Coding Label
LINC01021 (*PURPL*)	0.001479502	no

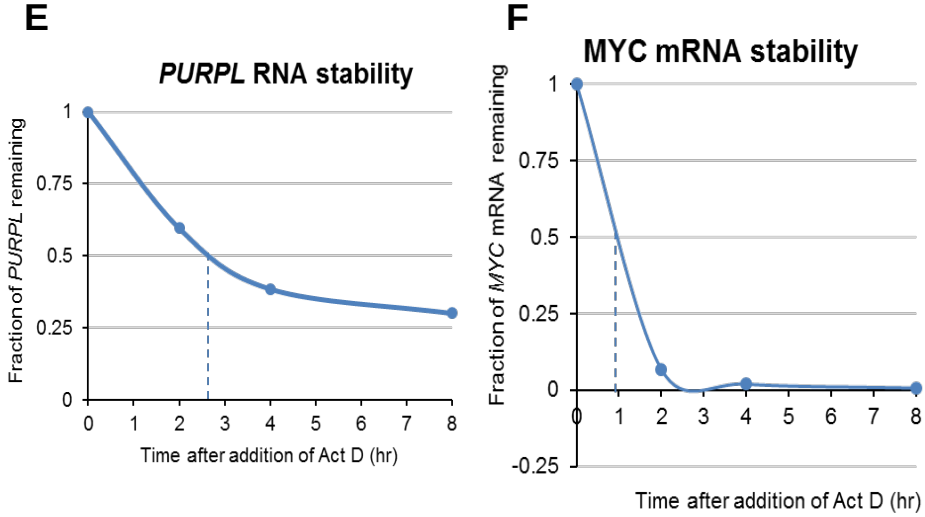


Figure S3. *PURPL* is a unstable, nuclear noncoding RNA, Related to Figure 1.

(A) RNA-FISH of *PURPL* in HCT116 untreated or treated with DOXO for 16 hr. DNA was counterstained with DAPI. (B) Number of *PURPL* molecules per HCT116 was measured using *in vitro* transcribed *PURPL* RNA and HCT116 total RNA followed by RT-qPCR. (C) Polyadenylation signal (AATAAA) at the 3'end of *PURPL* RNA is shown. (D) Coding potential analysis of *PURPL* was determined using CPAT (http://lilab.research.bcm.edu/cpat/index.php). (E, F) RT-qPCR for *PURPL* (E) and *MYC* mRNA (F) from HCT116 treated with Act D for the indicated times. *GAPDH* mRNA was used as loading control.

Figure S4

A

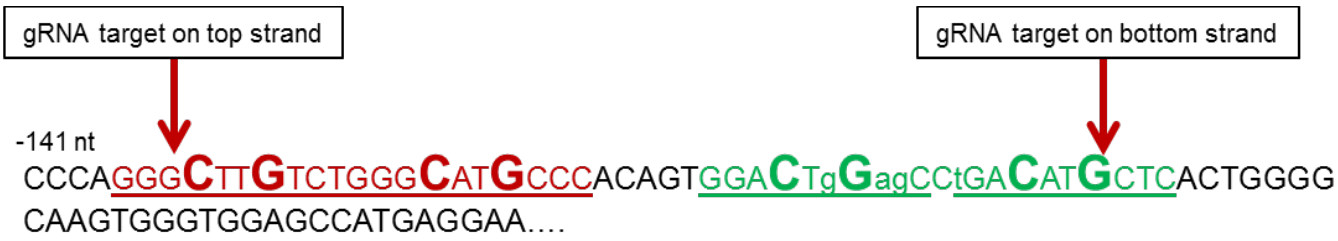
Canonical p53RE: RRR**C**WW**G**YYY (**N**)$_{0-13}$ RRR**C**WW**G**YYY

R=A or G; W =A or T; Y = C or T; N= 0 to 13 nt

B

5'gRNA target sequence	GGGCATGCCCAGACAAGCCC
3'gRNA target sequence	CTGGAGCCTGACATGCTCAC

C



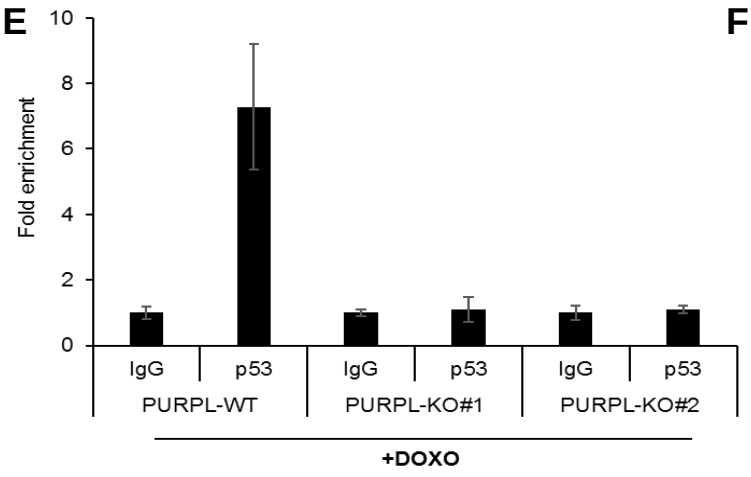
D

HCT116_*PURPL*-KO#1

TGTTTACATTAGATGCTTTTGTGCAGATGAGGGAACCTGCCCAGGGCTTGTCTGGGCATGCCCACAGTGGACTgAGTG
GACTGGAGCCTGACATGCTATGCCCACAGTGGACTGAGTTGTCTGAGCATGCCCACAGTGGACTGAGTGGACTGGAG
CCTGACATGCTATGCCCACAGTGGACTGAGTGGACTGGAGCCTGACATGCTCACTGGGGCAAGTGGGTGGAGCCATG
AGGAATTCATGCCTTGCAAAGGGGAGGAGCCTGCCCTCTTGAGCTCTTGTGGTGACCTGGGAATCAATGTGTGAGGT
GGTGCTA

HCT116_*PURPL*-KO#2

AGATAAAAAGGGGTCCCTAGAGAATTTCTGGCCTTCTTCACAAGTGTTTACATTAGATGCTTTTGTGCAGATGAGGGA
ACCTGCCCAGGGCTTGTCTGGGCATGCCCACAGTGGACTgGagC(T/C)(T/C)(A/G)(C/T)(T/C)(G/T)GGCTCACTGGG
GCAAGTGGGTGGAGCCATGAGGAATTCATGCCTTGCAAAGGGGAGGAGCCTGCCCTCTTGAGCTCTTGTGGTGACCT
GGGAATCAATGTGTGAGGTGGTGCTATGTAGCAGGA



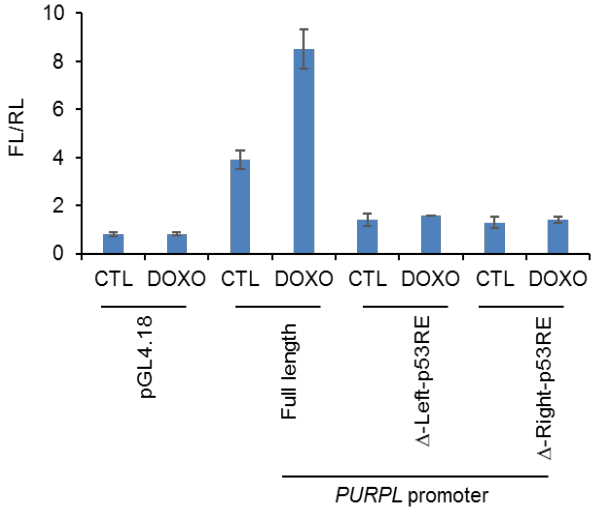


Figure S4. Targeted disruption of p53RE in the *PURPL* promoter in HCT116, Related to Figure 2. (A) Shown is the canonical sequence of a p53 response element (p53RE) derived from http://p53.iarc.fr/TargetGenes.aspx. (B) The targeting sequence of each gRNA is shown. (C) The 2 p53REs in the *PURPL* promoter are shown in "red" and "green". Arrows indicate the target site of each gRNA. Bases that do not correspond to the p53RE consensus sequence are in lowercase. (D) Genomic sequence of the p53REs in the *PURPL* promoter in *PURPL*-KO clones is shown. The left p53RE sequences are shown in "red", the right p53RE sequences are shown in "green" and the insertions or mutations are shown in "blue". As shown in blue, there is a ~120 nucleotide insertion in the right p53RE in *PURPL*-KO#1. In *PURPL*-KO#2 clone, there are mutations in the right p53RE shown in blue. (E) *PURPL*-WT and *PURPL*-KO clones were treated with DOXO for 16 hr and p53 binding to the *PURPL* promoter was measured by ChIP-qPCR. (F) Luciferase assays from HCT116 transfected in triplicate with pGL4.18 or pGL4.18 in which a 1 kb region of the *PURPL* promoter (Full length) was inserted. Δ-Left-p53RE and Δ-Right-p53RE refer to pGL4.18 in which the left or right p53RE of the 1 kb *PURPL* promoter was deleted. Co-transfections with pRL-TK was used as internal control. FL refers to Firefly luciferase; RL refers to Renilla luciferase. Error bars are SD from 2 independent experiments.

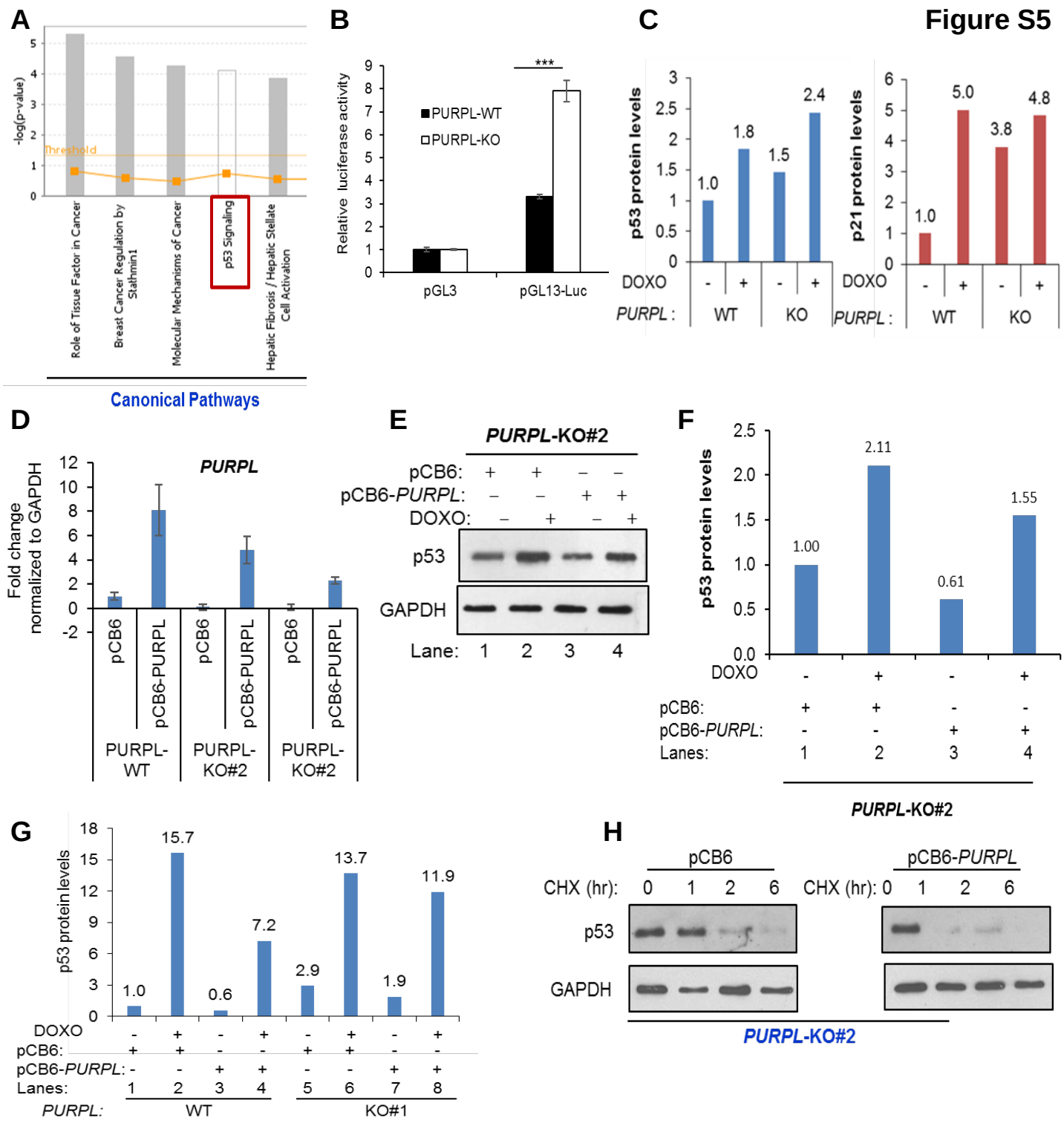


Figure S5. *PURPL* regulation of basal p53 levels, Related to Figure 3.

(A) Pathway analysis for the genes upregulated upon loss of *PURPL* in the microarrays from untreated *PURPL*-WT and *PURPL*-KO cells. (B) Luciferase assays were performed from untreated *PURPL*-WT and *PURPL*-KO cells transfected for 24 hr with pGL3 or the p53-responsive pGL13-Luc. (C) Densitometric quantitation of changes in p53 and p21 protein levels (normalized to GAPDH) for the immunoblot in Figure 3B are shown. (D) RT-qPCR was performed from *PURPL*-WT and *PURPL*-KO cells 48 hr after transfection with pCB6 or pCB6-PURPL. GAPDH was used as loading control. (E) Immunoblotting was performed from *PURPL*-KO#2 cells transfected for 48 hr with pCB6 or pCB6-*PURPL* followed by DOXO for 16 hr. GAPDH was used as loading control. (F, G) Densitometric quantitation of changes in p53 protein levels (normalized to GAPDH) for the immunoblot in Figure S5E (F) and 3C (G) are shown. (H) *PURPL*-KO#2 cells were transfected for 48 hr with pCB6 or pCB6-*PURPL* and then treated with Cycloheximide for the indicated times; immunoblotting for p53 and the loading control GAPDH was performed. Error bars represent SD from 3 independent experiments. ***p<0.001.

A **Figure S6**

Deletion: CTTGTCTGGGCATGCCC

SW48_*PURPL*-KO

TTTCTGGCCTTCTTCACAAGTGTTTACATTAGATGCTTTTGTGCAGATGAGGGAACCTGCCCAGGGACAGTGGACTgG
agCCtGACATGCTCACTGGGGCAAGTGGGTGGAGCCATGAGGAATTCATGCCTTGCAAAGGGGAGGAGCCTGCCCT
CTTGAGCTCTTGTGGTGACCTGGGAATCAATGTGTGAGTGGGTG

DLD1_*PURPL*-KO

GCAGATAAAAAGGGGTCCCTAGAGAATTTCTGGCCTTCTTCACAAGTGTTTACATTAGATGCTTTTGTGCAGATGAGGG
AACCTGCCCA(C/T)TG(T/G)CTGGGCATGCCCACAGTGGACTgGagCCtGACATGCTCACTGGGGCAAGTggGTGGAGC
CATGAGGAATTCATGCCTTGCAAAGGGGAGGAGCCTGCCCTCTTGAGCTCTTGTGGT

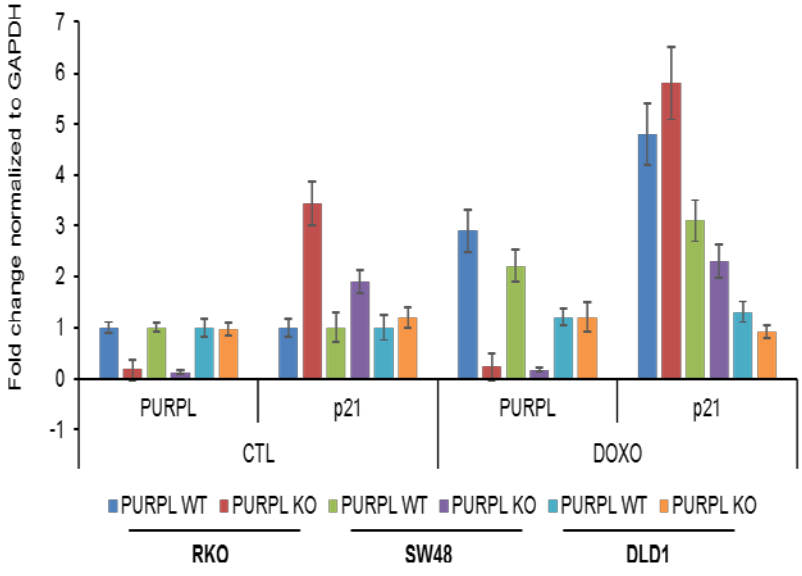
Deletion: GGG

RKO_*PURPL*-KO

CCTAGAGAATTTCTGGCCTTCTTCACAAGTGTTTACATTAGATGCTTTTGTGCAGATGAGGGAACCTGCCCAG(G/C)
(G/T)(C/T)(G/A)(C/T)(C/T)(G/T)GGGCA(A/T)G(C/T)(G/C)(G/C)(A/G)(C/T)(A/G)G(T/A)G(G/C)A(C/A)Tg(G/A)
(a/g)g(C/A)(C/A)t(G/T)(A/C)(C/A)(A/T)(T/G)(G/C)CT(C/T)(A/G)CTGGGGCAAGTGGGTGGAGCCATGAGGAAT
TCATGCCTTGC

Deletion: TT

B



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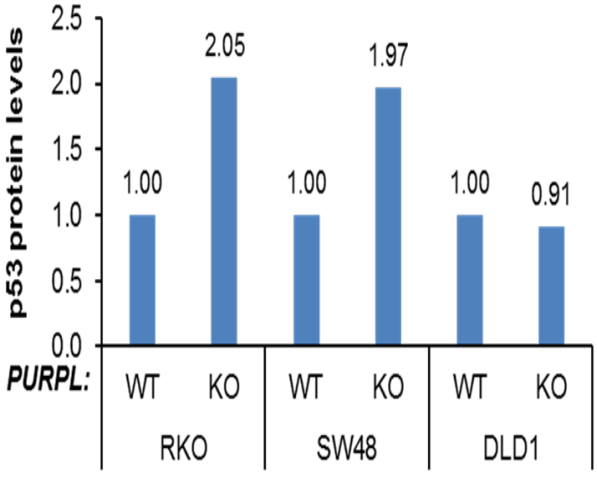
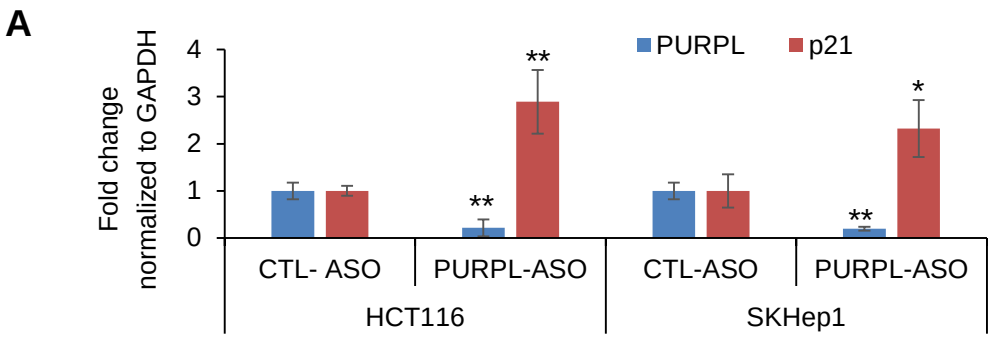
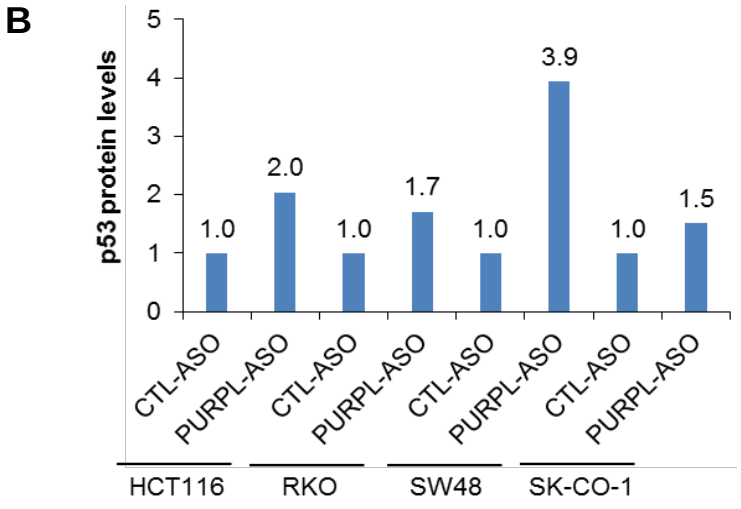
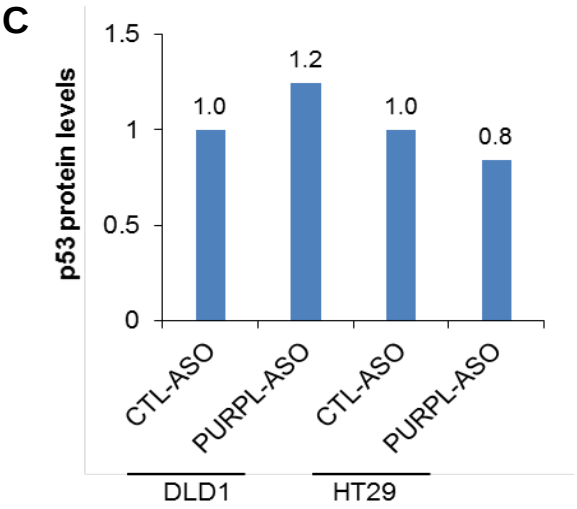


Figure S6. Targeted disruption of the p53RE in the *PURPL* promoter in SW48, DLD1 and RKO cells, Related to Figure 3. (A) Genomic sequence of the p53REs in the *PURPL* promoter in *PURPL*-KO SW48, DLD1 and RKO cells is shown. The left p53RE sequences are shown in "red", the right p53RE sequences are shown in "green" and the insertions, deletions or mutations are shown in "blue". Bases that do not correspond to the p53RE consensus sequence are in lowercase. As shown: (1) in SW48_*PURPL*-KO, there was a deletion in the left p5RE but the right p53RE was unaltered, (2) in DLD1_*PURPL*-KO, there were mutations and deletion in the left p5RE but the right p53RE was unaltered, and (3) in RKO_*PURPL*-KO, there were mutations and deletions in both the p53REs. (B) RT-qPCR analysis from *PURPL*-WT and *PURPL*-KO RKO, SW48 and DLD1 cells untreated or treated with DOXO for 16 hr. (C) Densitometric quantitation of changes in p53 protein levels (normalized to GAPDH) for the immunoblot in Figure 3F is shown. Error bars represent SD from 2 independent experiments.

Figure S7







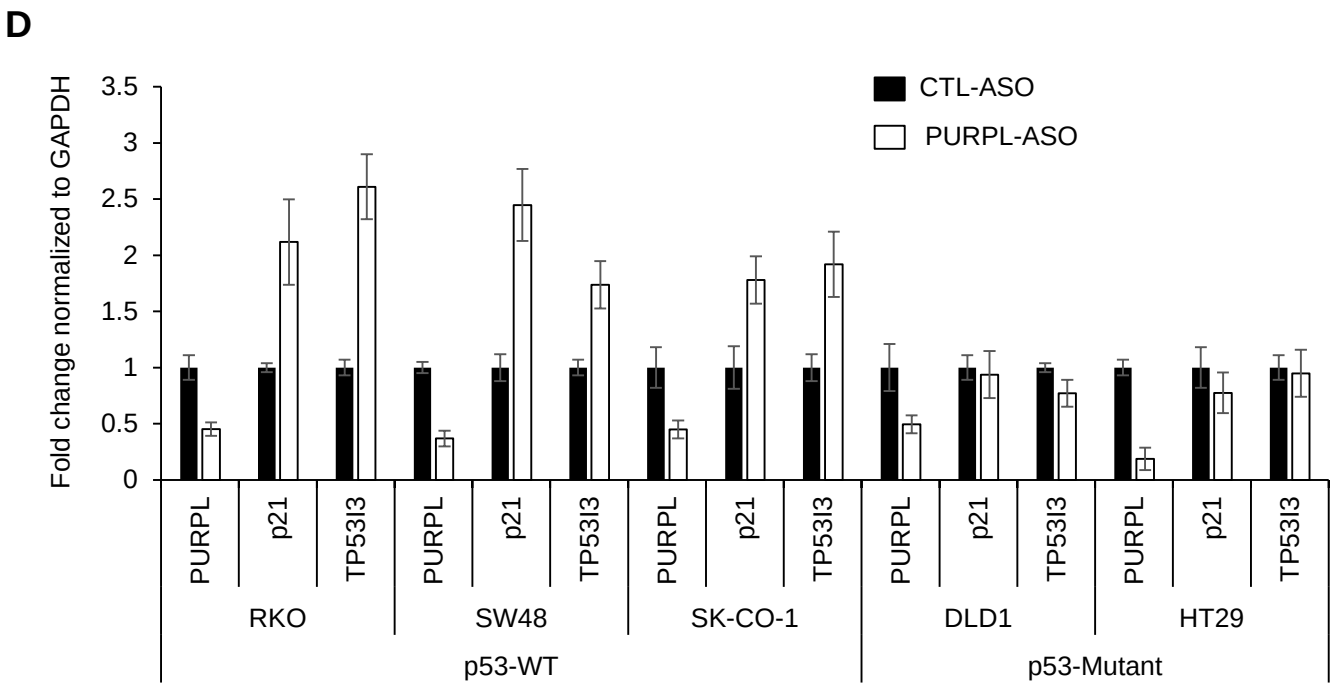


Figure S7. Transient knockdown of *PURPL* upregulates p53 and the p53-regulated p21 mRNA, Related to Figure 3.
(A) Parental HCT116 or SKHep1 cells were transfected with a CTL antisense oligo (ASO) or *PURPL*-ASO for 48 hr and the levels of *PURPL* and p21 mRNA were assessed by RT-qPCR. Densitometric quantitation of changes in p53 protein levels (normalized to GAPDH) for the immunoblot in Figure 3G is shown in "B" and 3H is shown in "C". (D) Parental RKO, SW48, SK-CO-1, DLD1 or HT29 cells were transfected with a CTL antisense oligo (ASO) or *PURPL*-ASO for 48 hr and the levels of *PURPL*, p21 and TP53I3 mRNAs were assessed by RT-qPCR. Error bars in "A" represent SD from 3 experiments and in "D" represent SD from 2 independent experiments. *p<0.05, **p<0.01 and ****p<0.00001.

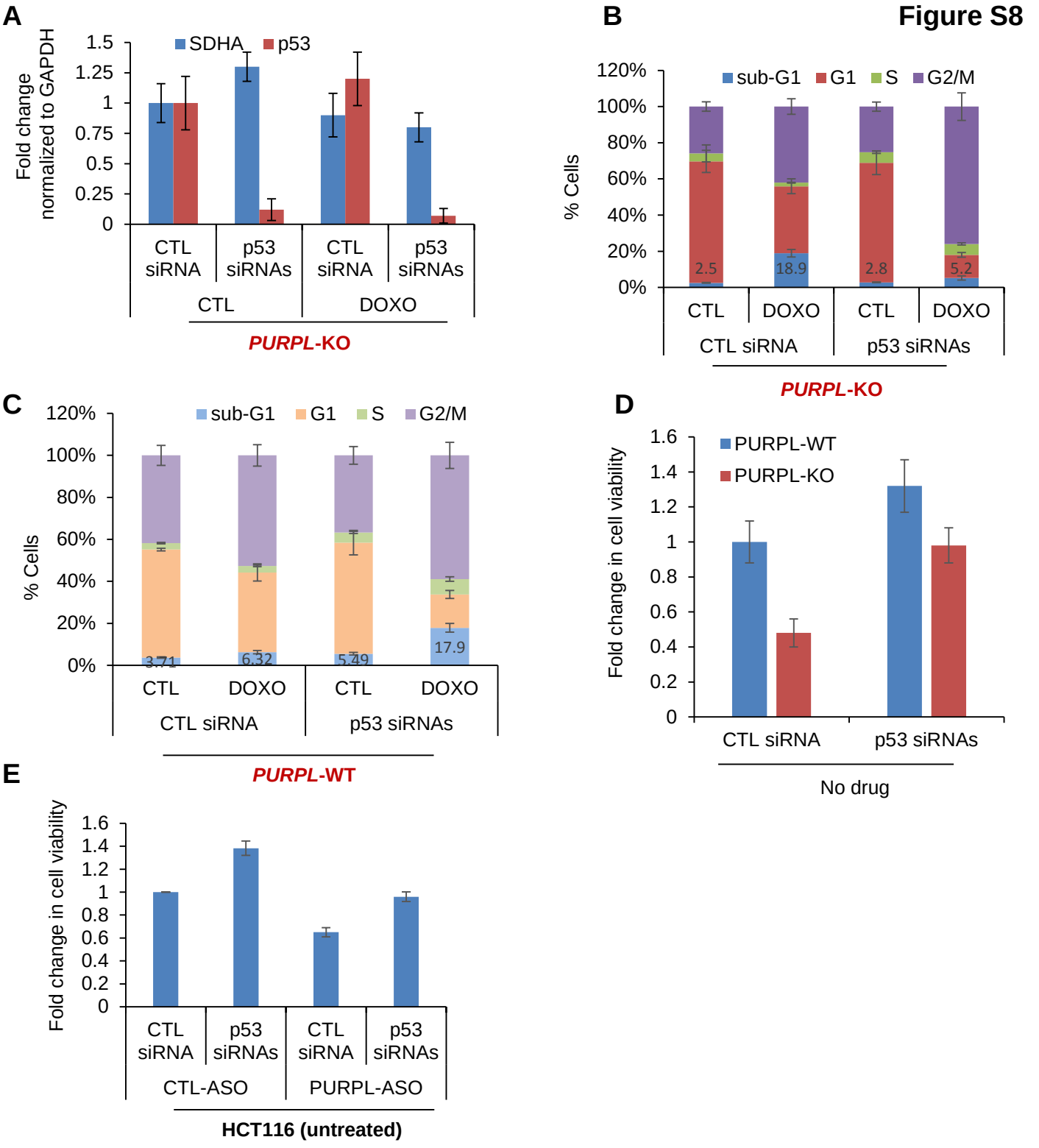
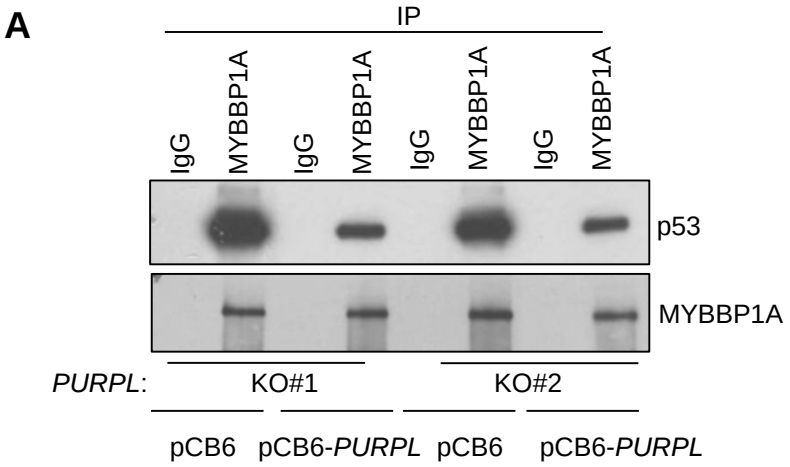


Figure S8. Silencing p53 rescues the hypersensitivity of *PURPL*-depleted cells to DNA damage, Related to Figures 3 and 4. (A) *PURPL*-KO cells were transfected for 48 hr with a CTL siRNA or p53 siRNAs and RT-qPCR was performed from untreated or DOXO-treated (48 hr) cells. *SDHA* was used as negative control. (B, C) *PURPL*-KO cells (B) and PURPL-WT cells (C) were transfected with a CTL siRNA or p53 siRNAs for 48 hr and then left untreated or treated with DOXO for 48 hr; cell death (%sub-G1 cells) was accessed by Propidium iodide staining and FACS analysis. (D) The effect on cell viability after knockdown of p53 in untreated (no drug) *PURPL*-WT and *PURPL*-KO cells was determined by cell count assay 72 hr after transfection with CTL siRNA or p53 siRNAs. (E) Parental HCT116 cells were co-transfected with CTL-ASO with CTL or p53 siRNAs and *PURPL*-ASO with CTL or p53 siRNAs for 48 hr. The effect on cell viability was determined by trypan blue cell exclusion assay. Error bars represent SD from 2 independent experiments.



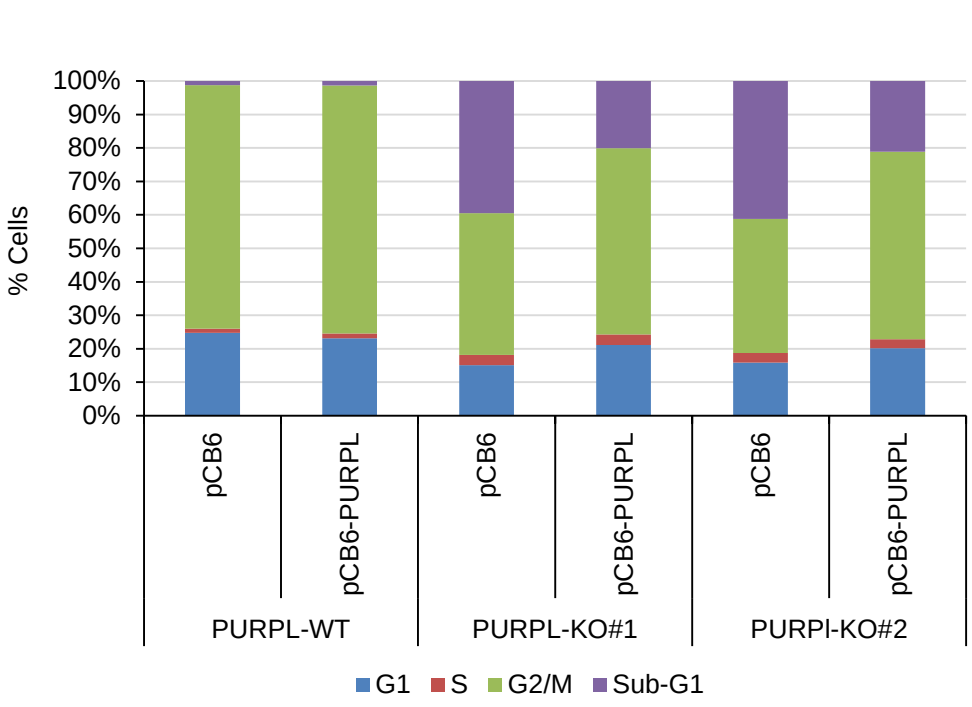


Figure S9. Reintroduction of *PURPL* in *PURPL*-KO cells reduces MYBBP1A-p53 interaction and partially rescues hypersensitivity to DNA damage, Related to Figure 5.

(A) *PURPL*-KO HCT116 cells were transfected for 48 hr with pCB6 or pCB6-PURPL and the interaction between p53 and MYBBP1A was determined by immunoblotting after MYBBP1A IP. IgG IP was used as negative control. (B) PURPL-WT or *PURPL*-KO HCT116 cells were transfected for 48 hr with pCB6 or pCB6-PURPL and then treated with DOXO for 48 hr. PI staining and FACS analysis was performed to determine the effect on cell cycle and cell death.

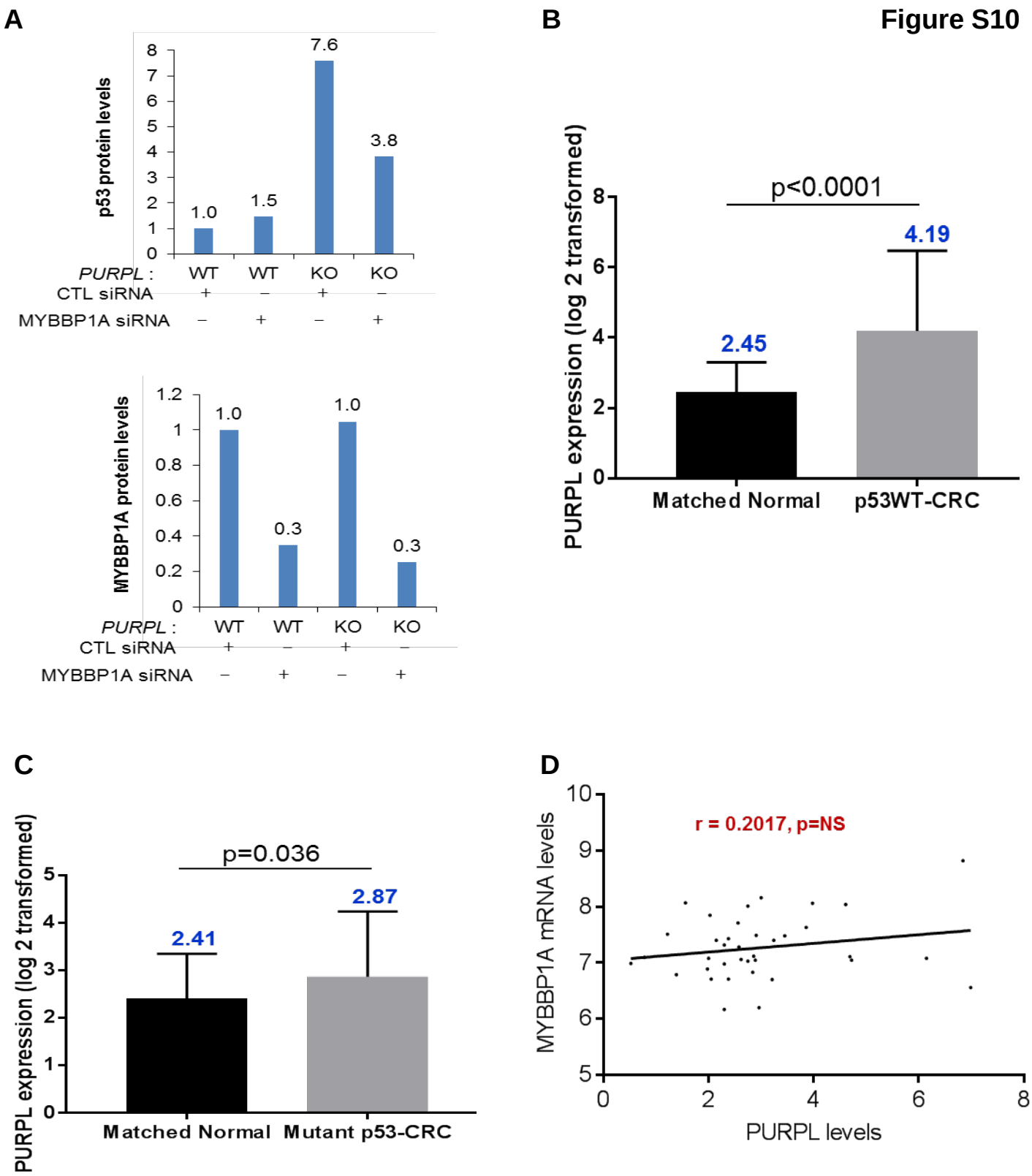


Figure S10. Lack of significant correlation between *PURPL* and MYBBP1A mRNA levels in p53 mutant tumors, Related to Figure 7.

(A) Densitometric quantitation of changes in p53 (top) and MYBBP1A (bottom) protein levels (normalized to GAPDH) for the immunoblot in Figure 7A are shown. (C,D) *PURPL* levels (log2 transformed) were compared between p53WT CRC tumors vs matched normal (B) or Mutant p53 CRC tumors vs matched normal (C). (D) Correlation between *PURPL* RNA and *MYBBP1A* mRNA levels was assessed in the p53 mutant CRC tumors (N=37) in the UMMC cohort.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture, siRNA transfections, cell viability, colony formation assays and luciferase assays

HCT116, RKO, DLD1, HT29, SK-CO-1, SW48, SW480 and SKHep1 cells were obtained from ATCC. The isogenic p53WT and p53KO HCT116, RKO and SW48 were previously generated (1,2) by Bert Vogelstein's lab (Johns Hopkins University). The final concentration of Doxorubicin (DOXO) used in this study was 300 nM. All cell lines were maintained in Dulbecco's modified Eagle's (DMEM) (Thermo Fisher Scientific) medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin-streptomycin and grown at 37 °C, 5% CO_2. All cell lines were routinely checked for mycoplasma using the Venor Gem Mycoplasma detection kit (Sigma-Aldrich). SMARTpool siRNAs for HuR, p53 and MYBBP1A were purchased from Thermo Scientific. The Allstars Negative control (CTL) was purchased from Qiagen. SiRNAs were reverse transfected at a final concentration of 20 nM, using Lipofectamine RNAiMAX (Thermo Fisher Scientific) as directed by the manufacturer. To determine the effect of silencing *PURPL* on the proliferation of HCT116 and SkHep1, the cells were reverse transfected with CTL-ASO or PURPL-ASO (50 nM) and cell proliferation was determined by Cell counting Kit-8 (Dojindo). For colony formation on plastic, 0.5 x 10^5 *PURPL*-WT and *PURPL*-KO cells were seeded in 6-well plates. After 24 hr, the cells were seeded in a 12-well plate at a density of 500 cells per well. After 2 weeks, colonies were fixed with ice-cold 100% methanol for 5 min, stained with crystal violet and colonies were counted. Luciferase assays were performed as previously described (3).

Fluorescence RNA *in situ* hybridization (RNA-FISH)

Forty-eight smFISH probes, spanning and antisense to *PURPL* were designed using Stellaris Probe Designer and ordered from Biosearch Technologies (http://www.biosearchtech.com). Each probe was 20 nt long and its 3'end was modified with mdC (TEG-Amino). Probes were dissolved individually in water to a final concentration of 100 µM. Equal volumes of each probe were combined for labeling. Approximately 1/9th of the reaction volume of 1 M sodium bicarbonate (pH = 8.5) was added such that the final concentration of sodium bicarbonate was 0.1 M. Then, 0.05 mg Cy®3 Mono NHS Ester (GE Healthcare, Cat#: PA13101, dissolved in 1 µl DMSO) was mixed with 25 µl of the above probe solution. The labeling reaction was incubated in the dark at 37 °C with gentle vortexing overnight. The labeling was stopped with the addition of 1/9th reaction volume of 3 M sodium acetate (pH = 5), followed by ethanol precipitation. Labeled probes were further purified from unconjugated free dye via P-6 Micro Bio-Spin Column (Bio-Rad). Probes concentration and labeling efficiency were determined by Nanodrop. Parental HCT116 cells were grown on coverslips, with or without 8 hr of 300 nM Doxorubicin treatment. Then, the cells were then rinsed once with PBS, and fixed with 75% methanol + 25% glacial acetic acid for 10 min at RT. The fixed cells were washed once with washing buffer (10% formamide in 2X SSC). The probe was added to Hybridization buffer (10% dextran sulfate, 10% formamide in 2X SSC) at a final concentration of 125 nM. Then the hybridization was set up in a humidified chamber, in dark at 37°C for 2 hr. After the hybridization, the coverslips were transferred into fresh plates and washed with washing buffer by incubation in dark at 37°C for 30 minutes. Then DAPI nuclear stain (Washing buffer with 20 ng/ml DAPI) was performed with 30 min incubation in dark at 37 °C. Finally, the coverslips were rinsed with 2X SSC, and mounted with ProLong Gold antifade reagent (ThermoFisher, Cat#: P36930). Pictures were taken using a Zeiss Axiovert 200M microscope, with Cascade 512b high sensitivity camera.

RNA isolation, RT-qPCR and RNA stability assays

Total RNA from cell lines was isolated using RNeasy kit (Qiagen). RNA stability assays from HCT116 cells were performed by treating the cells with Act D (2 µg/ml) followed by isolating RNA using Trizol reagent (Invitrogen). For RT-qPCR analysis, 500 ng total RNA was reverse transcribed using iScript Reverse Transcription kit (Bio-Rad) and qPCR was performed using Fast SYBR Green Master Mix (Roche) and StepOnePlus Real-time PCR system (ThermoFisher Scientific). Primer sequences are listed in Table S4.

Microarrays and Pathway analysis

Total RNA was isolated from *PURPL*-WT and *PURPL*-KO HCT116 in triplicate using the RNeasy plus mini kit (Qiagen) and labeled using the IlluminaTotalPrep RNA amplification kit (Ambion). Microarrays were performed with the HumanHT-12 v4 Expression BeadChip kit (Illumina) and analyzed in the R/Bioconductor package (Bioconductor). Pathway analysis and Gene ontology analysis was performed using Ingenuity pathways (www.ingenuity.com).

Immunoblotting and subcellular fractionation

Whole cell lysates were prepared using radioimmunoprecipitation (RIPA) buffer containing protease inhibitor cocktail (Roche) as previously described (4). To measure the stability of p53 protein, the cells were treated with Cycloheximide at a final concentration of 50µg/ml and immunoblotting was performed from whole cell lysates. Subcellular fractionation followed by RT-qPCR for nuclear and cytoplasmic fractions was performed as previously described (5). Proteins were quantified using the bicinchoninic acid protein quantitation (BCA) kit (Thermo Scientific). For immunoblotting, 10 µg whole cell lysate per lane was loaded onto a 12% SDS-PAGE gel and transferred to nitrocellulose membrane (4). The following antibodies were used: anti-p53 (DO-1), anti-HuR (3A2) and anti-Tubulin from SantaCruz Biotech, and anti-p21 (C19), anti-MYBBP1A (A301-328A) and anti-Nucleolin from Bethyl Labs, anti-Histone H3 and anti-GAPDH from Cell Signaling.

Nucleolar isolation was performed from 30 x 10^6 *PURPL*-WT and *PURPL*-KO HCT116 cells following the protocol of the Lamond lab (6) (http://www.lamondlab.com/f5nucleolarprotocol.htm). Final volume of each extract was 1 ml. For protein analysis, the nucleolar, nucleoplasmic and cytoplasmic fractions were diluted in 2x SDS-PAGE sample buffer and the subjected to SDS-PAGE and immunoblotting. For RNA analysis, 800 µl Trizol LS reagent (Invitrogen) was added to 200 µl of nucleoplasmic or nucleolar extract and RNA was isolated and subjected to RT-qPCR.

Flow cytometry assays

Cells were fixed with ice-cold 75% ethanol for 24 hr and stained with propidium iodide (Sigma) in the presence of RNase A (Qiagen). DNA content was analyzed on a FACSCalibur flow cytometer (BD Biosciences) and the data were analyzed using FlowJo software.

Immunostaining

PURPL-WT and *PURPL*-KO HCT116 cells were seeded at 300,000 cells per well of a 6-well plate. After 24 hr, the cells were untreated or treated with 300 nM Doxorubicin for 72 hr and fixed by 4% paraformaldehyde for 10 minutes at room temperature (RT). Fixed cells were permeabilized by 0.5% Triton X-100 for 10 min at RT and stained with primary antibodies anti-Mab414 (mouse) for Nucleoporin and active Caspase-3 (rabbit) for apoptotic cells at RT for 1 hr. DNA was stained with DAPI (blue) and secondary antibodies, anti-mouse 586 (orange) and anti-rabbit 488 (green) at RT for 1 hr. Images were taken using a Ziess immunofluorescence microscope with x63 lens. Anti-Mab414 was purchased from Covance (Cat # MMS120P), anti-caspase 3 cleaved (Cell signaling 9661S), anti-rabbit 488 (Alexa Fluor 488 donkey anti-rabbit IgG, life Technology, Cat number A21206) and anti-mouse 586 (Alexa Fluor 586 goat anti-mouse IgG, Life Technology, Cat # A11031).

Xenograft assays

Animal protocols were approved by the National Cancer Institute Animal Care and Use Committee following AALAAC guidelines and policies. HCT116 *PURPL*-WT and *PURPL*-KO cells were trypsinized and washed with PBS. Live cells were counted with trypan blue exclusion and equal numbers of live cells were injected for each clone. Cells (1×10^6) were mixed with 30% matrigel in PBS on ice and the mixture was injected into the flanks of 6–8-week-old female athymic nude mice (Animal Production Program, Frederick, MD, USA) (each group N=10). Tumor volume was measured twice a week after 1 week of injection.

Biotin-RNA pulldowns and Mass Spectrometry

PURPL and the control firefly luciferase cDNA were PCR amplified from a plasmid DNA using a forward primer containing the T7-promoter sequence at its 5'end and a gene-specific reverse primer (Table S4). We then performed *in vitro* transcription to generate biotinylated *PURPL* and the control luciferase RNAs using MEGAscript *in vitro* transcription kit (Ambion) and biotin RNA labeling mix (Roche). The *in vitro* transcribed RNA was treated with DNase (Ambion) and purified with RNeasy kit (Qiagen). Twenty-five pmole biotinylated RNA was incubated with 1 mg whole lysate prepared from HCT116 cells for 4 hr at 4°C. The biotinylated RNA-protein complexes were pulled down by incubation with Dynabeads M-280 Streptavidin (Thermo Fisher Scientific) for 4 hr at 4ºC. Proteins bound to the beads were eluted with SDS-PAGE sample buffer and fractionated by SDS-PAGE and each lane was cut into 10 slices. The protein bands were then in-gel digested with trypsin (Sigma) overnight at 37°C, as described (7). Peptides were extracted following cleavage and lyophilized. The dried peptides were solubilized in 2% acetonitrile, 0.5% acetic acid, 97.5% water for mass spectrometry analysis. They were trapped on a trapping column and separated on a 75 µm x 15 cm, 2 µm Acclaim PepMap reverse phase column (Thermo Scientific) using an UltiMate 3000 RSLCnano HPLC (Thermo Scientific). Peptides were separated at a flow rate of 300 nL/min

followed by online analysis by tandem mass spectrometry using a Thermo Orbitrap Fusion mass spectrometer. Peptides were eluted into the mass spectrometer using a linear gradient from 96% mobile phase A (0.1% formic acid in water) to 55% mobile phase B (20% water, 80% acetonitrile, 0.08% formic acid) over 30 minutes. Parent full-scan mass spectra were collected in the Orbitrap mass analyzer set to acquire data at 120,000 FWHM resolution; ions were then isolated in the quadrupole mass filter, fragmented within the HCD cell (HCD normalized energy 32%, stepped ± 3%), and the product ions were analyzed in the ion trap. Proteome Discoverer 2.0 (Thermo Fisher Scientific) was used to search the data against human proteins from the UniProt database using SequestHT. The search was limited to tryptic peptides, with maximally two missed cleavages allowed. Cysteine carbamidomethylation was set as a fixed modification, and methionine oxidation set as a variable modification. The precursor mass tolerance was 10 ppm, and the fragment mass tolerance was 0.6 Da. The Percolator node was used to score and rank peptide matches using a 1% false discovery rate.

RNA IP after UV-crosslinking or formaldehyde-crosslinking

HCT116 cells were seeded onto 10 cm culture dish one day before the experiment. For UV-crosslinking, cells were UV-crosslinked at 254 nm at a dose of 400 mJ/cm2 using UV Stratalinker1800 (Stratagene) as previously described (8). Cells then washed with 5 ml PBS and 1 ml RIPA buffer containing protease inhibitors and RNase inhibitor was added to lyse the cells. After centrifugation at 16,000 x g for 30 min at 4°C, the whole cell lysate was precleared by incubation with 25 µl Protein A/G magnetic beads (Pierce, USA) for 2 hr at 4°C. The precleared lysate was divided into 2 parts and each part was incubated overnight at 4°C with 2 µg anti-HuR antibody or 2 µg mouse IgG antibody. Next, 25µl Protein A/G magnetic beads was added to the lysate-antibody mixture and incubated for 2 hr at 4°C. After washing the beads 5 times with RIPA buffer and digestion of proteins in the IP material with proteinase K, RNA was extracted from the beads using phenol-chloroform and precipitated with Ethanol. The enrichment of *PURPL* RNA in the HuR IPs was determined by RT-qPCR with specific primers (Table S4). For formaldehyde crosslinking, cells were crosslinked used 4% formaldehyde for 10 minutes at room temperate. After removing the fixing solution and washing with PBS, the crosslinking was stopped with 1x glycine for 5 minute at room temperate. Cells were collected and IP performed as described above. For RNA pulldown using Bi-*PURPL* and GST-HuR Bi-*PURPL* and the control Bi-Luc RNAs were prepared as described above. 2 ug of Bi-*PURPL* or Bi-Luc was incubated with 500 ng of recombinant protein GST-HuR or GST alone in 500 ul of binding buffer for 4 hours, at 4°C with rotation. Then 25 ul of Dynabeads M-280 Streptavidin (Thermo Fisher Scientific) was added and pulldown was performed for additional 4 hours. The pulldown material was washed 4 times with binding and washing buffer. Proteins bound to the beads were eluted with 1x SDS-PAGE sample buffer, and subjected to SDS-PAGE and immunoblotting with anti-HuR antibody to detect the binding of *PURPL* to HuR.

Supplemental References

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