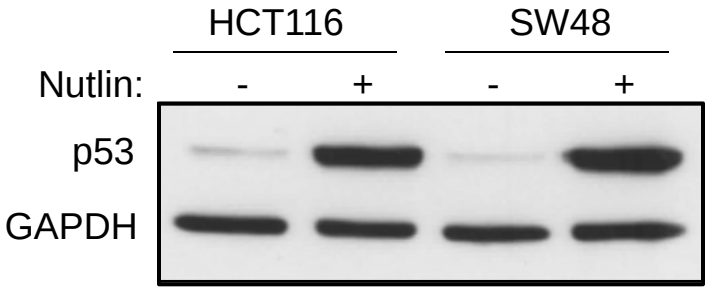
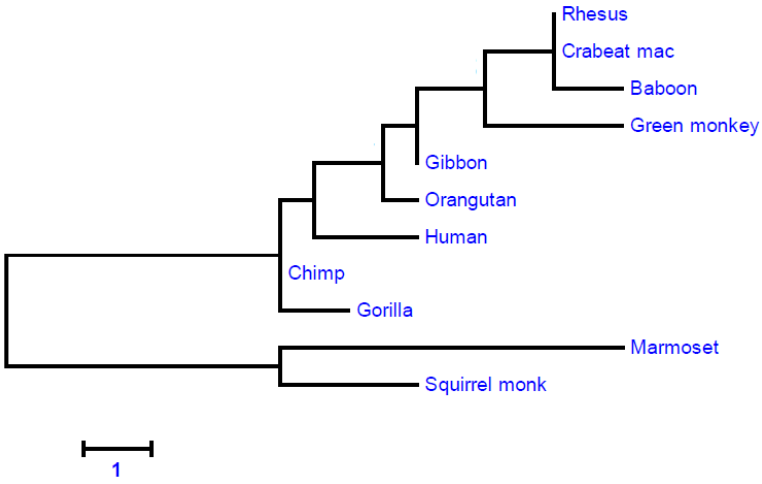


Figure S1. Nutlin-3 treatment induces p53 protein.
HCT116 and SW48 cells were left untreated or treated for 8 hr with Nutlin-3 (10 μM) to up-regulate p53. Whole cell lysates were analyzed by immunoblotting. p53 protein was strongly induced following Nutlin treatment. GAPDH was used as loading control.

```
Human          GCCTCAGTTGCCCCATCTGTGC------CCTGGGTAGGAATATCCTGGATCCCCTTG------GGTCT----------GATGGGGTAGCCGATGC
Chimp          GCCTCAGTTGCCCCATCTGTGC------CCTGGGTAGGAACATCCTGGACCCCCTTG------GGTCT----------GATGGGGTAGCCGATGC
Orangutan      GCCTCAGTTGCCCCATCTGTGC-15nt-CCTGGGTAGGAACATCCTGGATCCCCTTG------GGTCT----------GATGGGGTAGCTGATGC
Gibbon         GCCTCAGTTGCCCCATCTGTGC------CCTGGGTAGGAACATCCTGGACCCCCTTG------GGTCT----------GATGGGGTAGCTGATGC
Gorilla        GCCTCAGTTGCTCCATCTGTGC------CCTGGGTAGGAACATCCTGGACCCCCTTG------GGTCT----------GATGGGGTAGCCGATGC
Green          GCCTCAGTTGCCCTGTCTGTGC------CCTGGGTAGGAACGTCCTGGACCCCCTTG------GGTCT----------AATGGGGTAGCTGATGC
Rhesus         GCCTCAGTTGCCCCGTCTGTGC------CCTGGGTAGGAACGTCCTGGACCCCCTTG------GGTCT----------GATGGGGTAGCTGATGC
Crabeat_monk   GCCTCAGTTGCCCCGTCTGTGC------CCTGGGTAGGAACGTCCTGGACCCCCTTG------GGTCT----------GATGGGGTAGCTGATGC
Baboon         GCCTCAGTTGCCCCGTCTGTGC------CCTGGGTAGGAACGTCCTGGACCCCCTTG------GGTCT----------GATAGGGTAGCTGATGC
Marmoset       ACCTCAGTTGCTCCGCCTGTGC------CCTGGGTAGGAACATTCTGGACCCCTTTA------GGTCT--------------GAAACCCGATGC
Squirrel_monk  ACCTCAGTTGCCCC-CCTGTGC------CCTGGGTAGAAACATCCTGGACCCCCTTA------GGTCT----------GATGGGGTGCCCGATGC
Mouse          GACTCAGTTTCCCCTGCTGTGA------CCT---CTGGAGCTGCCAGCAGC-----------GGGCTCAGCCAG---AGTAGGACGGATGA---
Rat            GCCTCAGTTTCCCCTGCTGTGA------CCT---CTGGAGTTGCCAGCAGTCCCCCGTGCAGTGGGCTCAGCCAG---AGTAGGAAGGACGA---
Dog            GCCTCAGTCTCCCCACCTGTGA------ATCGGACAAGAACATCT-----CCCCTTG------GGGCTGACGGTGGGGGGGAGGGCGGCGGGCGG
Cat            GCCTCAGTTTCCTTATCTGTGA------ACCGGACAAGAACATCC-----CCCCTAG------GGGCTTCGGGGGGTGGGGGGGGGAGGTGGTG-
                 ******  *     *****                 *                      ** **              *      *
```

Figure S2. miR-3189 is conserved among apes and old-world monkeys.
Multiple alignment of miR-3189 candidate sequence between mammalian species. Mammalian orthologous regions for the human miR-3189 precursor showed a mosaic pattern of similarity wherein conserved sites are located at the 5'end of hairpin. The rest of the precursor sequence is poorly conserved in mammals, although synteny of genomic alignments in the vicinity of this region is unambiguous. Mature human miR-3189-3p and -5p sequences are shown in red. Positions of 100% sequence conservation are marked by stars.

A



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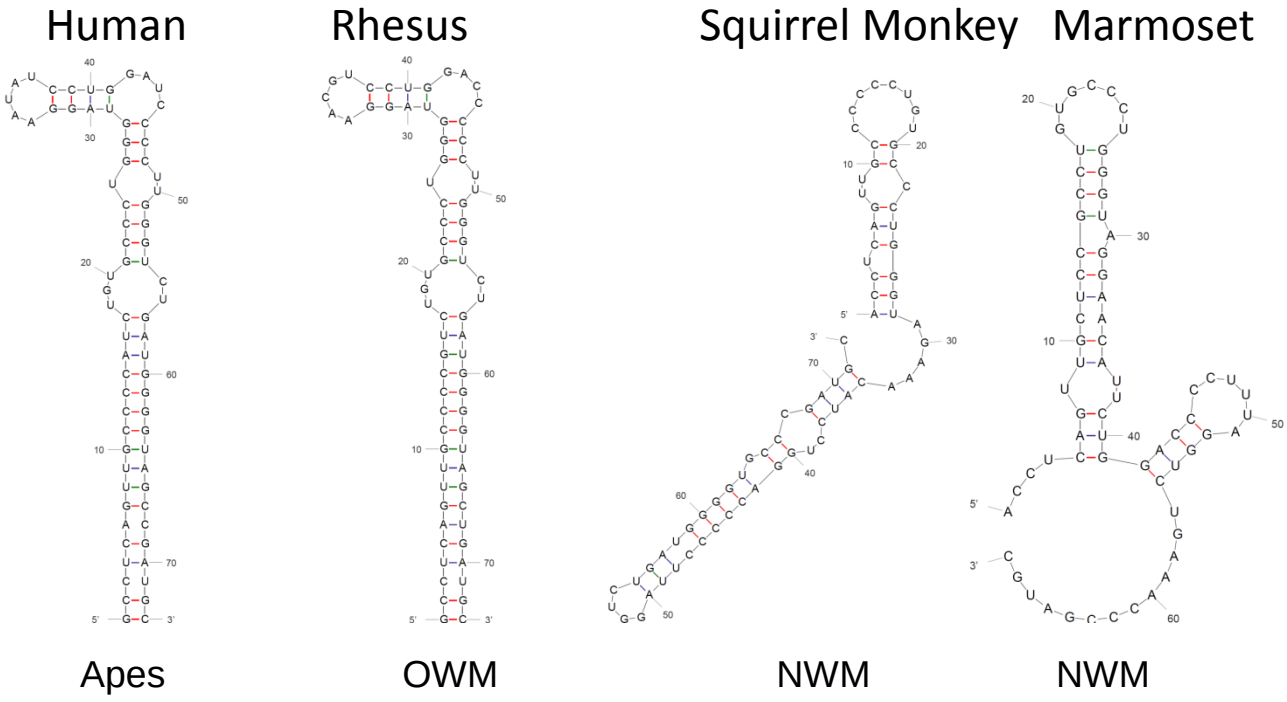


Figure S3. Pre-miR-3189 structure is conserved among apes and old-world monkeys.
(A) Phylogenetic tree of miR-3189 hairpin regions based on the multiple alignment of 11 primate candidate sequences (apes, old-world monkey, and new-world monkey. (B) Secondary structure prediction for miR-3189 stem-loop sequence. Only apes and old-world monkey (OWM), but not new-world monkey (NWM) sequences adopt stable miRNA-like structures.

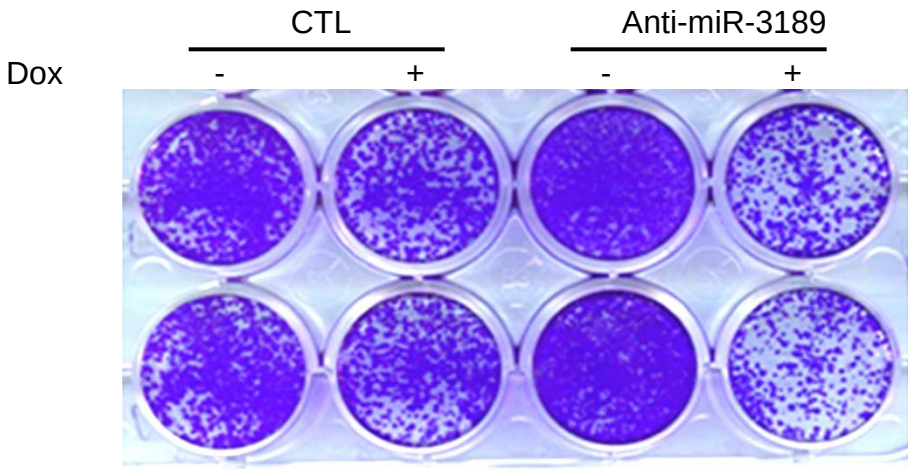


Figure S4. miR-3189-3p knockdown reduces colony formation after Doxorubicin treatment.
As shown in Figure 2, HCT116 cells were reverse transfected with Anti-miR-3189-3p for 48 hr, then treated with 300nM Dox for 8 hr and seeded for colony formation on plastic for 10 days.

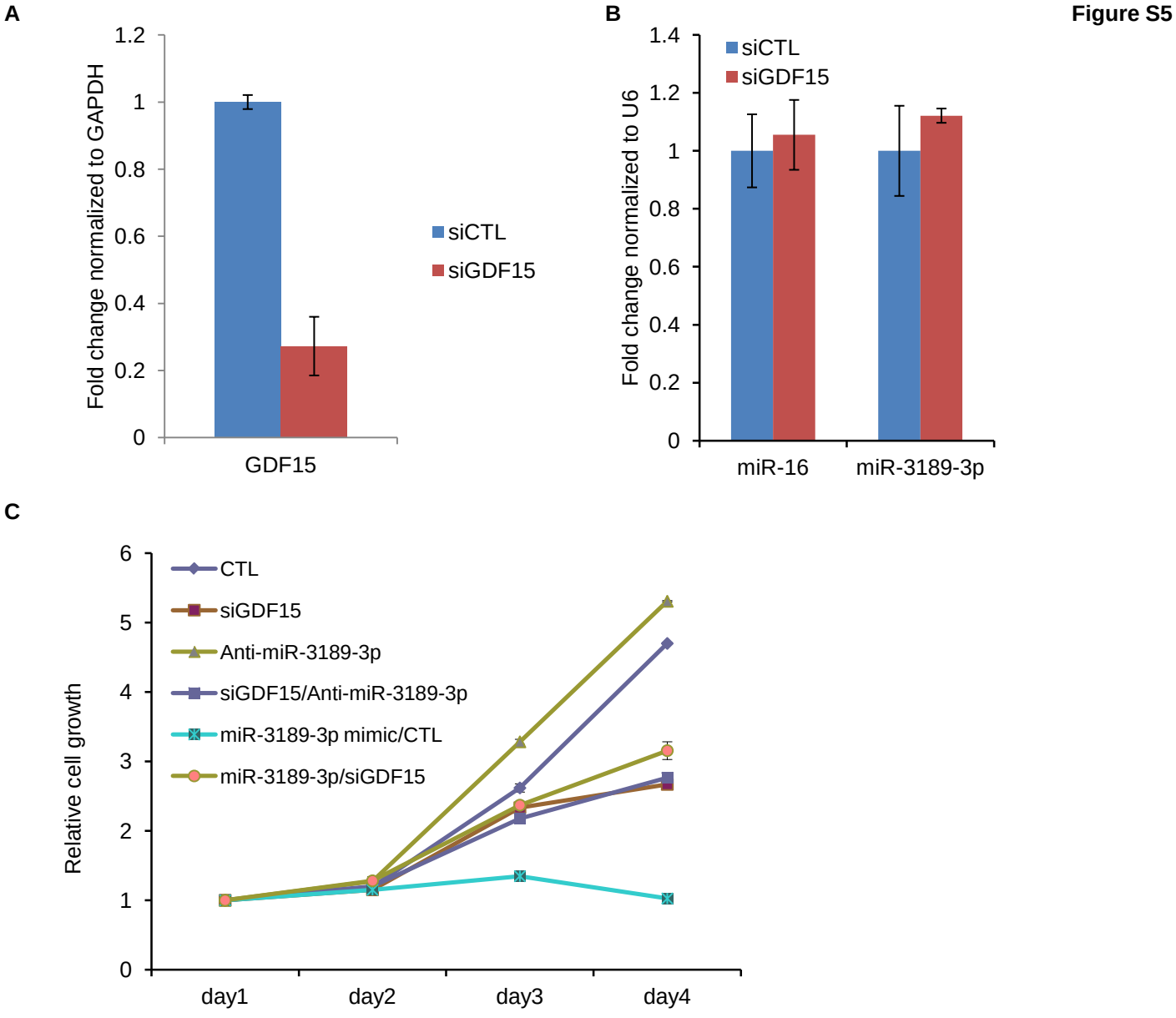
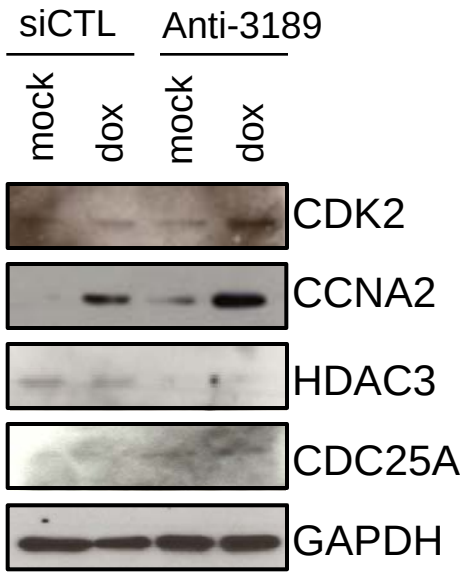


Figure S5. Comparison of miR-3189 and GDF15 knockdowns

HCT116 cells were reverse transfected with siRNA targeting GDF15. (A) 48 hours after transfection, GDF15 mRNA levels were measured by SYBR-Green qRT-PCR. GDF15 was down-regulated by over %70. (B) miR-3189-3p levels were quantitated following GDF15 knockdown by TaqMan qRT-PCR. The unrelated miR-16 is shown as a control. (C) HCT116 cells were reverse transfected with the indicated molecules and seeded in 96-well plates. Relative proliferation was measured over a 4 day period using WST-8 colorimetric assays.

A



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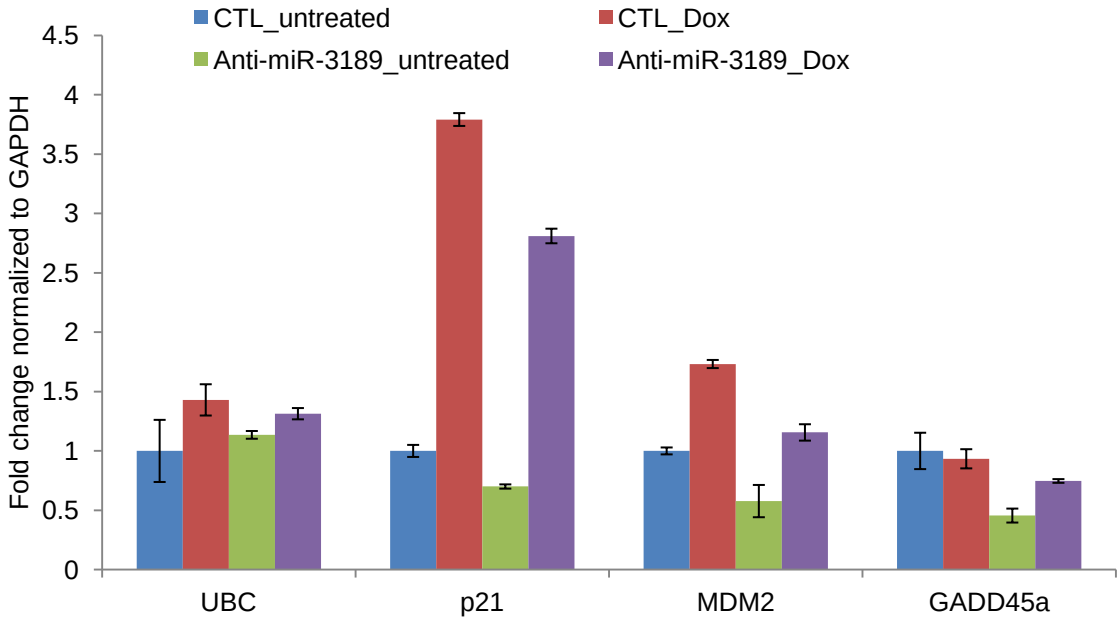


Figure S6 Effects of miR-3189 knockdown on gene expression following DNA damage.
A) HCT116-p53WT or –p53KO cells were transfected with 50nM Anti-miR-3189 for 48 hours, then treated with 300 nM doxorubicin for 8 hours. Whole cell lysates were probed with the indicated antibodies, GAPDH was used as a loading control. B) HCT116 cells were transfected and treated with doxorubicin as in (A), RNA was isolated and the expression of p53 pathway genes was measured using qRT-PCR.

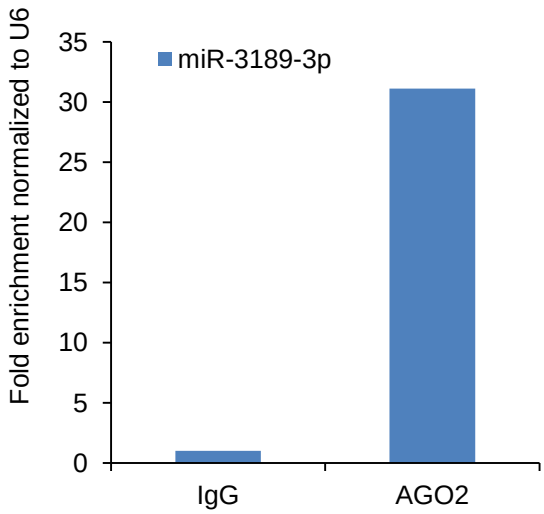


Figure S7. miR-3189-3p is enriched in RISC after mimic transfection

To ensure that the levels of miR-3189-3p incorporated into RISC after miR-3189-3p over-expression achieved by transient mimic transfection were physiologically reasonable, we immunoprecipitated Ago2 48 hr after transfecting HCT116-p53WT cells with miR-3189-3p mimics. RNA was isolated from the IP material and TaqMan RT-qPCR quantitation of miR-3189-3p in the RISC IP compared to input showed approximately 30-fold enrichment.

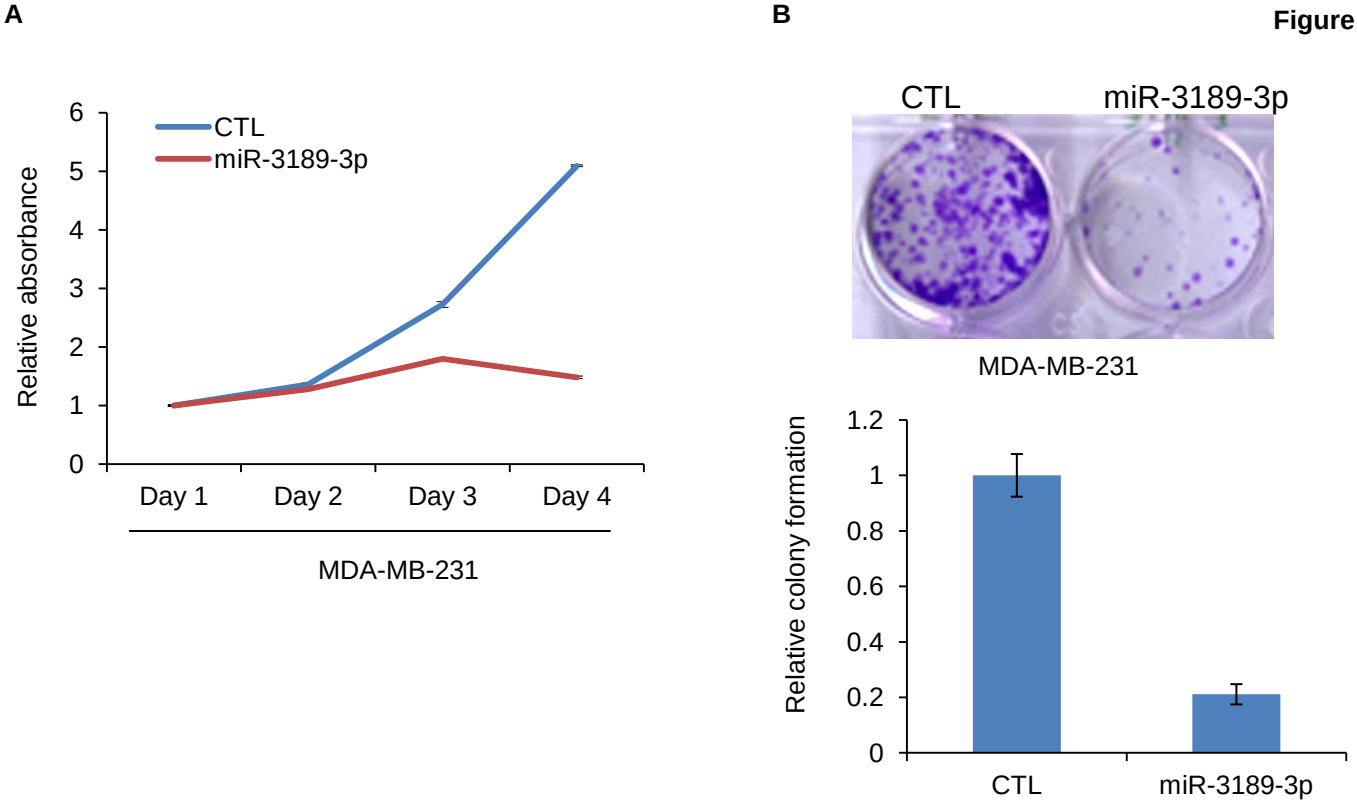
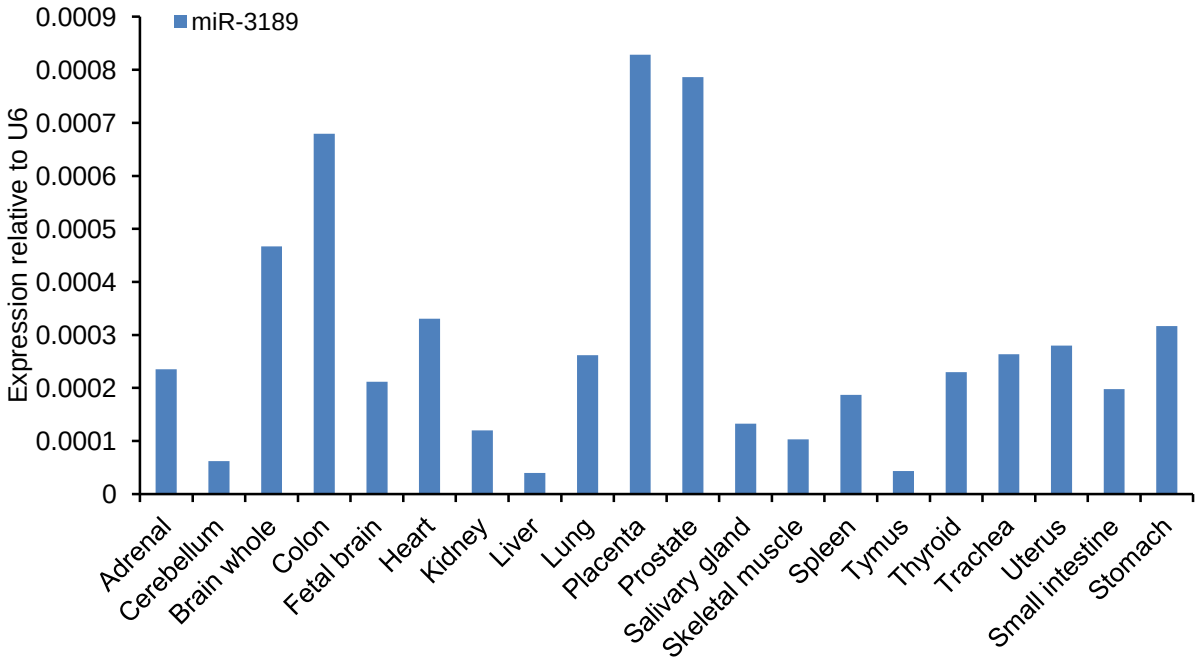


Figure S8. MiR-3189-3p over-expression reduces the viability of MDA-MB-231 cells.
(A) The mutant p53 cell line MDA-MB-231, was transiently transfected with miR-3189-3p mimic or control and seeded in a 96-well plate. MTT assays were performed over 4 days to measure proliferation. (B) MDA-MB-231 cells were transfected as above, and re-seeded at 500 cells/well in a 24-well plate (n=3). After 10 days, colonies were fixed with ice-cold methanol, stained with crystal violet, and counted (lower panel).

A



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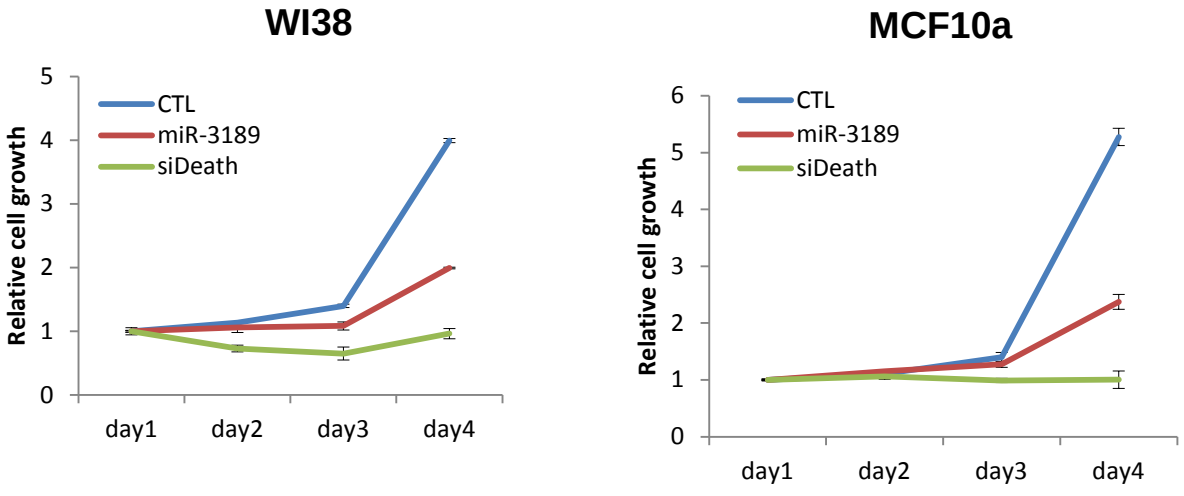

Figure S9. mir-3189-3p is less potent in normal fibroblasts

A) RNA isolated from the indicated normal tissues was purchased from Clontech. MiR-3189 expression was measured in the tissue panel by TaqMan qRT-PCR and expression values are given as a fraction of U6 expression. (B) The normal fibroblast cell lines WI38 and MCF10A were transfected with miR-3189-3p or control mimic, and their proliferation over 4 days was measured using MTT colorimetry. The effect of miR-3189-3p was much less than that of siDeath.

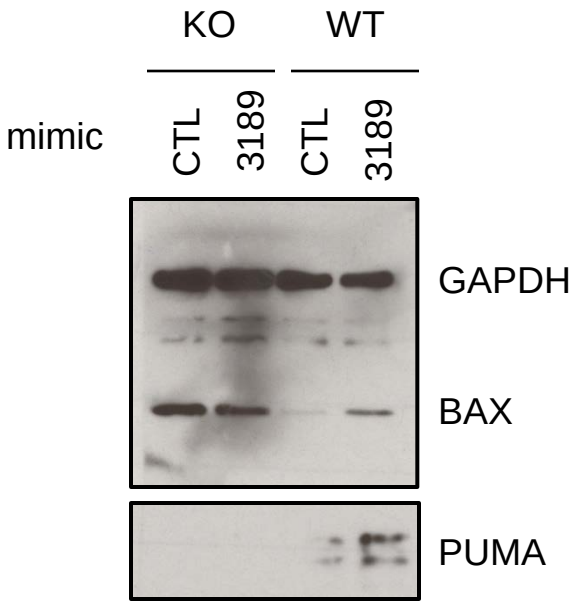

Figure S10. miR-3189 mimic transfection induces p53-associated mediators of apoptosis
HCT116-p53WT or –p53KO cells were transfected with 20nM miR-3189 mimic for 48 hours**.** Whole cell lysates were probed with the indicated antibodies, GAPDH was used as a loading control.

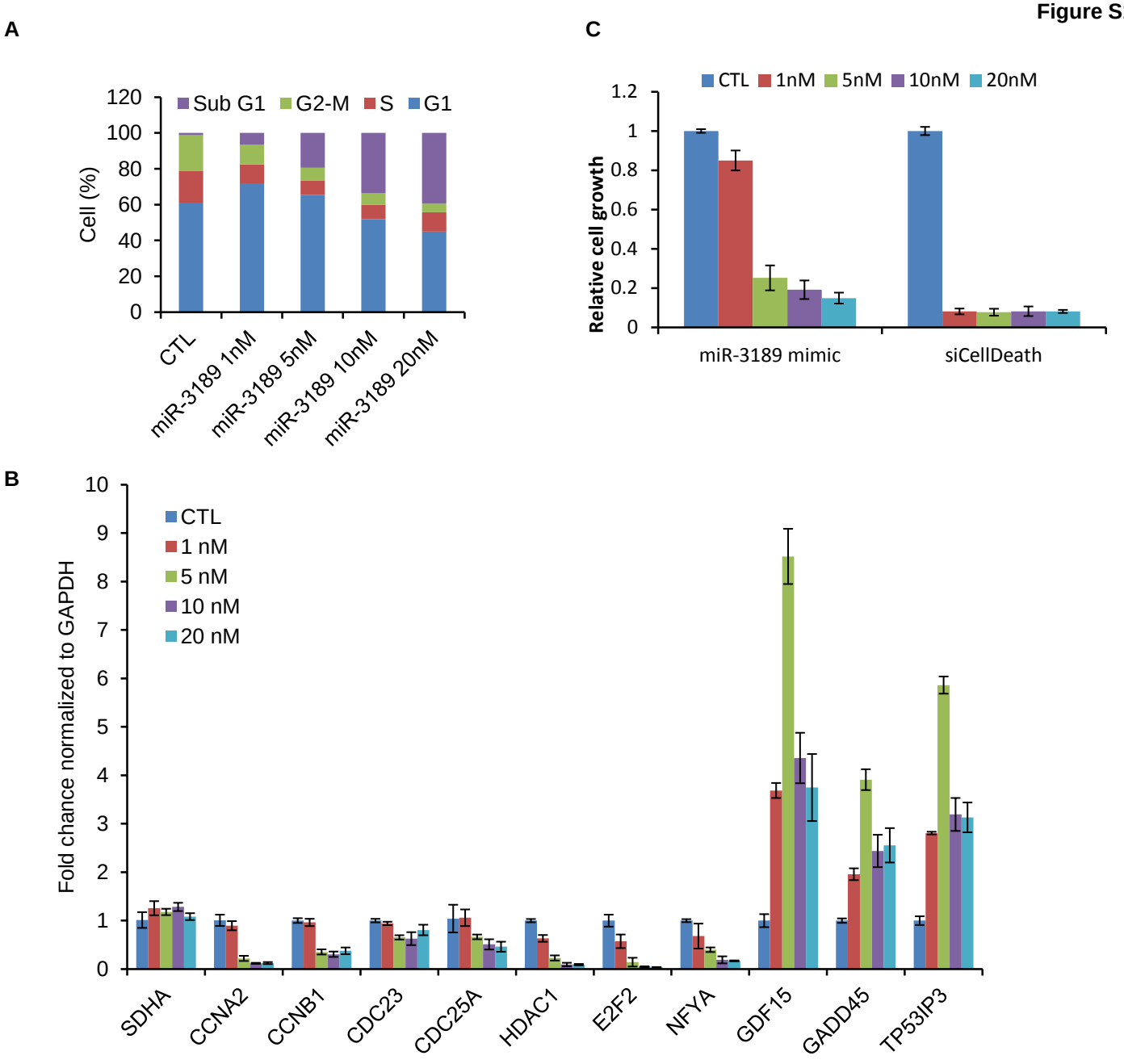

Figure S11. Dose-dependence of miR-3189 mimic transfection

In order to test the effects of different concentrations of miR-3189 mimic, we transfected HCT116-p53WT cells with 1, 5, 10, or 20 nM miR-3189-3p mimic or 20 nM CTL mimic. (A) After 48 hours, cells were fixed and stained with PI, and cell cycle was analyzed by flow cytometry. (B) RNA was harvested 48 hours after transfection, and the expression of miR-3189 targets was measured by SYBR-Green qRT-PCR. SDHA is shown as a negative control. (C) Cells were reverse transfected and seeded in 96 well plates, and relative growth after a further 72 hours was measured by WST-8 assay.