Supplementary Figure S1. mRNA and microRNA species induced by p53

(A) SJSA cells were incubated with 8 μM Nutlin-3 for 24 hours. Immunoblot analysis was performed to detect the known p53-responsive gene product Mdm2. Actin staining served as a loading control.

(B) SJSA cells were incubated with 8 μM Nutlin-3 for 24 hours. qRT-PCR analysis was performed to detect the known p53 responsive gene products PIG3 and p21. Expression levels are shown as ratios between mRNA levels upon treatment with Nutlin-3 vs. DMSO alone ("fold induction").

Supplementary Figure S2. Genes encoding the microRNA molecules 194-1, 215, 194-2, and 192

(A) Locus of MicroRNA Molecules 194-1 and 215 on Chromosome 1. **A** indicates the stemloop sequence of MicroRNA Molecule 194-1 with highlighted mature sequence, **B** indicates the stemloop sequence of MicroRNA Molecule 215 with highlighted mature sequence.

(B) Locus of MicroRNA Molecules 194-2 and 192 on Chromosome 11. **A** indicates the stemloop sequence of MicroRNA Molecule 194 with highlighted mature sequence, **B** indicates the stemloop sequence of MicroRNA Molecule 192 with highlighted mature sequence.

(C) Sequence comparison reveals structural analogy of MicroRNA Molecules 215 and 192.

Supplementary Figure S3. microRNA expression levels in patient material

microRNA expression levels from patient material were determined by array hybridization. The log2 of hybridization intensities were determined for each sample, grouped according to its origin: normal colon mucosa, colon cancer with microsatellite instability (MSI), and colon cancer with stable microsatellites (MSS). The data from this study are summarized in a box plot format in Fig. 3B, including the level of significance that distinguishes the MSS tumors from normal tissue for each microRNA. Corresponding raw data of fluorescence intensities are provided in Table S2, clinical data in Table S3, and analysis of significance in Table S4.

Supplementary Figure S4. Alterations in p53-responsive gene product levels in response to miR-192

HCT116 cells with or without a targeted disruption of TP53 were transfected with 10nM of either control microRNAs or miR-192, or mock-transfected, and incubated for 2 days; cell lysates were then subjected to immunoblot analysis to detect the indicated gene products.

Supplementary Figure S5. DNA content in response to an LNA antagonizing miR-192

A549 cells were transfected with either scrambled LNA or anti-miR-192-LNA (100nM) and then (24 hrs post-transfection) treated with 500µM 5-FU or the DMSO solvent alone. Flow cytometry was performed 48 hours of incubation with 500µM 5-FU or DMSO alone. The cells were trypsinized, fixed with ethanol and stained with propidium iodide. Three experiments of this kind are summarized in Fig. 4C.

Supplementary Figure S6. DNA content in response to p53-responsive microRNAs

After transfection with the indicated PreMir molecules and 48 hours of incubation, the DNA content of HCT116 cells with or without p53 was determined by flow cytometry. The cells were trypsinized, fixed with ethanol and stained with propidium iodide. The percentages of cells with a DNA content below 2N (Sub-G1, gated to the total number of cells), and the percentages of cells with a 4N DNA content (G2/M Phase, gated to total number of cells without SubG1) are indicated in each case. Three experiments of this kind are summarized in Fig. 5, A and B.

Supplementary Figure S7. Requirement for p21 in miR-192-induced cell cycle arrest.

(A) HCT116 cells with a targeted disruption of the CDKN1A/p21 gene (p21 -/-) were transfected with 10nM of either control microRNAs or miR-192 and incubated for 2

days and harvested for flow cytometry analysis. The percentage of cells in the sub-G1-fraction are shown.

B) HCT116 p21 -/- cells transfected with 10nM of the indicated microRNAs were incubated for 2 days and then treated with Nocodazole (100ng/ml) for another 18hrs. Cell cycle profiles were monitored as described in the Materials and Methods. The percentage of cells in the sub-G1-fraction are shown.

Supplementary Figure S8. G1-arrest induced by p53-responsive microRNAs (nocodacole trap)

A549 cells transfected with 10nM microRNAs (control, miR-34a, miR-192, miR-194, and miR-215) were incubated for 2 days; subsequently, the cells were treated with Nocodazole (100ng/ml) for another 18 hrs. Cell cycle profiles were monitored as described in the Materials and Methods. The percentage of cells in the G1-fraction are shown.

Supplementary Table S1. Changes in micro-RNA expression levels in response to p53

6.6 micrograms of RNA enriched for small RNA molecules, extracted from SJSA cells treated with 8 µM Nutlin-3 or DMSO, was conjugated with Cy3 or Cy5 dyes and hybridized overnight to six microarrays (3 glass slides with 2 microarrays each). A dye-swap was performed in each case. The table shows the data derived from all spots with a detected fluorescence signal above 1000 relative units. The last column on the right shows the relative induction of all microRNA molecules.

Supplementary Table S2. microRNA expression levels in patient material.

Expression levels were determined by array hybridization, and the log2 intensities are presented for each microRNA in each sample. Samples were taken either from normal colon mucosa, or cancer with microsatellite instability (MSI), or cancer with stable microsatellites (MSS).

Supplementary Table S3: Clinical and histopathological data for 59 samples used for microRNA expression profiling

Clinical data corresponding to the samples shown in Table S2 are presented.

Supplementary table S4: Differentially expression of has-miR-192, has-miR-194, and has-miR-215 in normal mucosa and MSS and MSI colon cancer

The significance levels of downregulation are shown for normal mucosa vs. cancer as indicated.