Supplementary information

Tumor-suppressor *miR-22* determines p53-dependent cellular fate through post-transcriptional regulation of p21

Supplementary information includes Extended Materials and Methods, eight figures and four tables.

Materials and Methods

Cell culture

HCT 116 (HCT 116 $p53^{+/+}$), HCT 116 $p53^{-/-}$ and SW480 colon cancer cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) in humidified air with 5% CO_2. For treatment with genotoxic agents, HCT 116 cells were seeded at 1.0 x 10^5 cells/ml, and incubated with either 100, 200 and 500 ng/ml of ADR or 0.38 mM of 5-FU for specific periods.

Clinical samples

Paired surgical specimens of primary human colon cancers and surrounding non-cancerous counterparts of the colon were obtained from patients treated at the Teikyo University Hospital, Mizonokuchi, Kanagawa, Japan with documented informed-consent in each case. Institutional review board approvals for analysis of

clinical samples were obtained at each institute. Samples were frozen in liquid nitrogen and stored at -80 °C until use. Total RNAs and genomic DNA were prepared by using Trizol (Invitrogen) and DNA extraction kit (Qiagen), respectively, according to the manufacturers' instructions.

Functional miRNA dropout screening

Functional dropout screening to identify tumor-suppressor miRNAs was carried out according to our recent publication (1). In brief, HCT 116 cells were transduced with a pooled lentivirus miRNA expression library (SBI) at a multiplicity of infections (MOI) of 3. Cells were incubated in complete medium for 3 days (P1) and subjected to sequential passages every 3 days. After 9 passages, genomic DNA was prepared from P1, P5 and P9 cells using a DNA extraction kit (Qiagen) and used as a template for PCR amplification of pre-miRNA regions of virus vectors using a primer set corresponding to vector arms as follows; CDH5.1 5'-GCC TGG AGA CGC CAT CCA CGC TG- 3', CDH3.1 5'-GAT GTG CGC TCT GCC CAC TGA C-3'. Amplified DNA from P1, P5 and P9 cells was purified using a gel extraction kit (Qiagen) and subjected to fluorescence labeling. Amplified DNAs from P1 and P5 (or P9) cells were labeled with Cy3 and Cy5-dUTP, respectively, using a Genomic DNA enzymatic labeling kit (Agilent) and purified with a Microcon YM-30 filter (Millipore). An equal amount of Cy3- and Cy5-labeled DNA was combined and loaded onto a custom-made microarray, and hybridized at 65 °C for 24 hr. After washing, microarray was scanned and analyzed according to the CGH protocol ver. 5 (Agilent). The log ratio of each miRNA copy number was calculated by averaging log10 ratios and by excluding the highest and lowest values in 16 replicates.

Real-time PCR

For quantitative expression analysis of miRNAs, total RNAs from colon cancer patients were reverse-transcribed by Multiscribe RT and miRNA-specific primer (ABI), and a PCR reaction was carried out by using a TaqMan microRNA assay kit (ABI). The comparative cycle threshold (Ct) method was applied to evaluate the expression level of miRNA. The *U48* small nuclear RNA was used as an internal standard.

For mRNA expression analysis, total RNAs (~ 1 μg) were reverse-transcribed by SuperScript III reverse transcriptase using a random hexamer primer. Synthesized cDNAs were quantified by TaqMan Gene expression analysis. Relative expression was calculated as described above. *GAPDH* was used as an internal standard.

miRNA microarray analysis

Total RNAs from non-cancerous parts of 4 colon cancer specimens were labeled with pCp-Cy3 using the Agilent miRNA labeling reagent, and then hybridized to an oligonucleotide microarray for human miRNA (8 x 15K, Version 2.0, Agilent) according to the manufacturers' instructions. After hybridization, the array was washed with washing buffer and scanned by an Agilent DNA microarray scanner. The data were numerically converted with Feature Extraction Software (Version 9.5 Agilent), and analyzed by GeneSpring GX10 software.

Array comparative genomic hybridization (aCGH) analysis

For aCGH analysis, 500 ng of genomic DNA from the cancerous and non-cancerous parts of the colon cancer specimens were labeled with Cy5 and Cy3, respectively, and then digested with restriction endonucleases *Alu* I and *Rsa* I. Equal

amounts of labeled DNA were combined and loaded onto a Human Genome CGH microarray 244K (Agilent), and hybridized for 40 hr at 65 °C. After hybridization, the array was washed and scanned as described above, and analyzed by DNA Analytics Software (Ver. 5.0, Agilent).

Expression microarray analysis

HCT 116 cells were transfected with either *miR-22* or miR-NC (final concentration of 1 nM) for 3 days and total RNA was prepared by an RNeasy column (Qiagen). Total RNAs were labeled with Cy3 using an mRNA labeling kit and hybridized to a whole human genome oligo microarray (4 x 44K, Agilent) according to the manufacturers' instructions. Data analysis was performed by GeneSpring GX10.

Copy number assay

The copy number differences of *TP53* or *miR-22* genomic loci in 36 colon cancer patients from NCI were analyzed by quantitative real-time PCR using a TaqMan Copy Number Assay kit according to the manufacturers' instructions. The comparative cycle threshold (Ct) method was applied to evaluate the copy number of these loci using *Line-1* as an internal standard, which is a repetitive element whose copy numbers per haploid genome are similar among all human cells (2, 3). The copy number of *TP53* and *miR-22* in colon cancers were compared to that of their matched normal counterparts.

Cell proliferation assay

HCT 116 cells, seeded at 2.5 x 10^4 cells/ml, were transfected with either *miR-22* or miR-NC using HiperFect reagent (Qiagen) for 3 days. Cells were washed with PBS (-)

and incubated in MST reagent (Promega) for 60 min at 37 °C, and the absorbance at 490 nm was measured using an Arvo plate reader (Stratagene).

Isolation of *C17orf91* cDNA

Total RNA from HCT 116 cells was reverse-transcribed with an oligo(dT) primer, and used as a template for the amplification of *C17orf91* cDNAusing the primer sets described in Fig. S4. Amplified PCR products were ligated into the pPCR2 vector and sequenced, and the *C17orf91* cDNA was subcloned into the pcDNA3.1 (Invitrogen) expression vector.

Chromatin immunoprecipitation (ChIP)

ChIP analysis was carried out as described (4) with modifications. HCT 116 cells, seeded at 2 x 10^5 cells/ml in a 150 mm dish, were treated with 0.38 mM of 5-FU for 16 hrs or 5, 25 nM of Act D for 24 hrs, and fixed by 1% formaldehyde for 10 min at room temperature. After sharing DNA, an extract was incubated with DO-1 p53 antibody or normal mouse IgG for overnight at 4 °C, and precipitated DNA fragments were quantified by real-time PCR analysis using primer sets (Table S1). Data was normalized to input DNA.

ChIP-sequence

HCT 116 cells were treated with 5-FU (0.38 mM) for 9 h, and chromatin immunoprecipitation (ChIP) was performed by using anti-p53, anti-mono or anti histone H3 tri-methylated K4, or anti histone H3 tri-methylated K36 antibodies. ChIP isolated DNA (5-10 ng) was purified by polyacrylamide gel electrophoresis to obtain

100-300 bp DNA fragments. Collected DNA fragments were labeled with a single adenosine nucleotide by using Klenow exo- DNA polymerase (3' and 5' exo minus; Illumina). Illumina adaptors were then added to labeled DNAs, and subjected to PCR amplification for 20 cycles according to the manufacturers' instructions. After purification of PCR products, cluster generation and 36 cycles of sequencing reactions were carried out by an Illumina cluster station and 1G analyzer. Nucleotide sequences derived from 26,737,039 reads were mapped to the reference human genome (NCBI build #36).

AGO2-IP on Chip analysis

AGO2 immunoprecipitation on Chip assay was carried out according to a previous report with minor modifications (5). In brief, HCT 116 cells stably expressing HA-AGO2 cells were transfected with either *miR-22* or miR-NC at a final concentration of 20 nM for 24 hr, and disrupted in lysis buffer composed of 50 mM Tris-Hcl (pH 7.5), 1% NP-40, 1 mM EDTA, 5 mM DTT, 1 x proteinase inhibitor cocktail (Roche), 300 mM NaCl, and RNasin Plus ribonuclease inhibitor (Promega), and immunoprecipitated using anti-HA agarose beads pre-blocked with 1% BSA and 10 μg/ml of yeast tRNA, for 4 hr at 4 ^{o}C. Beads were washed with lysis buffer 5 times, AGO2-bound RNA was eluted in boiling water, and the Trizol-LS reagent was added to extract total RNAs. AGO2-bound total RNAs were further cleaned-up using an RNeasy column and subjected to microarray analysis. After the washing and scanning of the array, data were analyzed. Briefly, normalized signal intensities of AGO2-bound mRNAs in the presence of *miR-22* were divided by those of miR-NC introduced cells. In parallel, an mRNA expression profile of HCT 116 cells in the presence or absence of *miR-22* was

generated, and expression fold-changes were obtained. An enrichment score (E-score) was then calculated by division of fold changes of AGO2-bound mRNA by expression changes. Thus, a high E-score indicates AGO2-bound and down-regulated mRNAs in a *miR-22* dependent manner. Furthermore, cell cycle and apoptosis regulating genes were selected among high E-scored mRNAs.

Reporter plasmid construction and luciferase assay

The 3' UTR of *p21* mRNA was amplified by PCR from HCT 116 genomic DNA using a primer set; 5'-TCC GCC CAC AGG AAG CCT GC-3', 5'-GAG CAC CTG CTG TAT ATT CAG C-3'. The DNA fragment was cloned into a pCR2 vector and sequenced, and then fused to the 3' end of a firefly luciferase reporter gene in the pmirGLO dual luciferase vector (Promega). Site directed mutagenesis of a *miR-22* target site of *p21* mRNA was carried out by using a PrimeSTAR Max high fidelity DNA polymerase using the pmirGLO-p21 3'UTR plasmid as a template. Mutagenesis primers are listed below.

p21 5' seed Fw-EcoRV, 5'-TCA GGg ata tcA AGC AGC GAC CGC CCC CT-3'

p21 5' seed Rev-EcoRV 5'-GCT Tga tat cCC TGA GGT AGA ACT AGG G-3'

p21 3' Rev-EcoRI 5'-TGA GGg aat tcT AGG GTG CCC TTC TTC TT-3'

p21 Double Fw-RV-RI 5' CCT Aga att cCC TCA GGg ata tca AAG CA-3'

Nucleotide sequences of the 5' seed and 3' regions of the *miR-22* target site in the *p21* mRNA were replaced with the recognition sequences for *Eco*RV and *Eco*RI, respectively (underlined sequences).

For the luciferase assay, HCT 116 cells, seeded at 5 x 10^4 cells/ml, were co-transfected with 200 ng of reporter plasmid and 10 nM of either *miR-22* or miR-NC using Lipofectamine 2000 according to the manufacturer's protocol for siRNA

introduction. After incubation for 24 hr, cell extracts were prepared by adding passive lysis buffer (Promega) and luciferase activities were determined by using a dual luciferase assay kit (Promega). Luciferase activity was normalized by *Renilla* luciferase activity as an internal standard.

Immunoblot analysis

Cells were lysed in lysis buffer consisting of 25 mM Tris-HCl (pH7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS and 1 x proteinase inhibitor cocktail, and protein concentration was determined by the Bradford method using the Bio-Rad protein assay reagent. For SDS-PAGE and immunoblot analyses, protein samples were mixed with Laemmli sample buffer, and loaded on a 10-20% polyacrylamide gradient gel (ATTO). After electrophoresis, proteins were transferred to a PVDF membrane (Millipore), and analyzed by the standard method.

Indirect immunocytochemistry

HCT 116 cells, transfected with either *miR-22* or miR-NC for 48hr, were treated with 50 ng/ml of ADR for 5 or 10 hr, and fixed with cold methanol for 20 min. Cells were washed twice with PBS and incubated in blocking reagent (2.5% BSA/TBS-T) for 60 min, and further incubated for 60 min with a primary antibody against p21 diluted with blocking buffer. After reaction with a secondary antibody, (Alexa 594 labeled anti-mouse IgG) for 60 min, cells were washed with PBS, and subjected to microscopic observation.

Flow cytometric analysis

HCT 116 cells were transfected with either *miR-22* or miR-NC, incubated for 48

hr, and treated with ADR overnight. Cells were detached from the bottom of the dish by adding trypsin, and stained with FITC-Annexine V and PI (BD Pharmingen). Cells were passed through a cell-strainer filter and subjected to flow cytometry using JSAN. Data were processed by FlowJo software.

Method References

1. Izumiya M, Okamoto K, Tsuchiya N, Nakagama H. Functional screening using a microRNA virus library and microarrays: a new high-throughput assay to identify tumor-suppressive microRNA. Carcinogenesis 2010; 31: 1354-9.
2. Wang TL, Diaz LA, Jr., Romans K, Kinzler KW, Vogelstein B, Lengauer C, et al. Digital karyotyping identifies thymidylate synthase amplification as a mechanism of resistance to 5-fluorouracil in metastatic colorectal cancer patients. Proc Natl Acad Sci U S A 2004; 101: 3089-94.
3. Ugurel S, Houben R, Schrama D, Voigt H, Zapatka M, Schadendorf D, et al. Microphthalmia-associated transcription factor gene amplification in metastatic melanoma is a prognostic marker for patient survival, but not a predictive marker for chemosensitivity and chemotherapy response. Clin Cancer Res 2007; 13: 6344-50.
4. Wei CL, Wu Q, Vega VB, Chiu KP, Ng P, Zhang T, et al. A global map of p53 transcription-factor binding sites in the human genome. Cell 2006; 124: 207-19.
5. Karginov FV, Conaco C, Xuan Z, Schmidt BH, Parker JS, Mandel G, et al. A biochemical approach to identifying microRNA targets. Proc Natl Acad Sci U S A 2007; 104: 19291-6.

Legends to Supplementary Figures

Fig. S1. **A.** Tumor-suppressor miRNA categories identified by the present screening. Experimental strategies used to screen tumor-suppressor miRNAs are also indicated. **B**. Schematic illustration of the functional dropout screen using a lentivirus miRNA expression library. **C**. The number of dropout miRNA clones in 3 settings of microarray analyses. **D.** Expression profile of dropout miRNAs in 4 human normal colon tissues. **E**. Copy number aberration of dropout miRNA genes in 24 human colon cancers.

Fig. S2. Copy number aberrations of *TP53* and *miR-22* loci in 36 human colon cancer patients. Copy number of *TP53* or *miR-22* loci in NCI samples (ID 1 – 36) were calculated by the TaqMan copy number assay system using *Line-1* as an internal standard. Graph indicates copy numbers of *TP53* and *miR-22* loci relative to those in matched normal counterparts. Marked (red circle) samples showed hemizygous loss of *miR-22* locus with intact *TP53*.

Fig. S3. **A**. Cell proliferation assay. HCT 116, HCT 116-p53$^{-/-}$ and SW480 cells were transfected with either 5 nM of *miR-22* or miR-NC, and incubated for 3 days. Cells were subjected to MST assay as described in Materials and Methods. Cell number was indicated by relative values to negative control cells. **B**. Cell cycle analysis. HCT 116 p53-/- and SW480 cells were transfected with either 5 nM of *miR-22* and miR-NC, and incubated for 3 days. Cell were fixed with ethanol and stained with PI, and then cell cycle distribution was analyzed by FACS. Representative cell cycle patterns in both cell lines are indicated. **C**. Quantification of cell number in each phase of cell cycle. Data indicates means from three independent experiments. Error bars show the standard

deviations. Statistical analysis was carried out by *t*-test. **D.** Network analysis. HCT 116 cells were transfected with either miR-NC or *miR-22*, and mRNA expression profiles were generated by means of microarray. Network analysis was carried out by MetaCore software (GeneGo Inc.)

Fig. S4. The nucleotide sequence of *C17orf91* is shown. Underlined sequences indicate the positions of the primers used to isolate the*C17orf91* cDNA from HCT 116 cells. A bold line shows the*pre-miR-22* sequence, and exon junctions are indicated as vertical lines.

Fig. S5. **A**. Expression of *miR-22* after exposure to 5-FU. Cells, seeded at 1.0 x 10^5 cells/ml, were treated with 0.38 mM of 5-FU for 12 h, and *miR-22* expression was quantified by TaqMan real-time RT-PCR. The data indicate the relative expression levels of *miR-22* compared with un-treated cells. **B**. Structure of a luciferase reporter gene construct containing a *miR-22* gene promoter region. The genomic region containing p53 binding sites in a 5' upstream and intron 2 was amplified by PCR from genomic DNA of HCT 116 cells as a template. The DNA fragment was sequenced and subcloned into a pGL3 basic vector. **C**. Reporter gene assay. Cells, seeded at 1.0 x 10^5 cells /ml, were transfected with 200 ng of luciferase reporter gene and 10 ng of control pRL vectors, and incubated for 24 h. Luciferase activity was measured by using a dual luciferase assay kit (Promega), and the data indicates means with standard deviations (n=6). **D. E.** p53-ChIP analysis. Cells, treated with 0.38 mM 5-FU for 16 hrs, were fixed with 1% formaldehyde, and subjected to ChIP analysis. Quantification of precipitated DNA was carried out by real-time PCR analysis in triplicate by using primers spanning

p53 binding site of p21 (positive control), *miR-22* -1104 #1, *miR-22* -1104 #2 (for 5' promoter region), Int2 (for p53BS in intron 2) and IGX1A (ChIP-qPCR human negative control, SABiosciences). Graph indicates fold enrichment of site occupancy for p53 binding relative to non-stressed cells (D), and percent of input (E). Data indicates means with standard deviation of triplicate qPCR. Statistical analysis was carried out by *t*-test. Additional two independent experiments gave similar results.

Fig. S6. **A**. An outline of AGO2-IP on Chip is schematically drawn. HCT 116 cells stably expressing HA-AGO2 were established and transfected with either *miR-22* or miR-NC. After incubation for 24 hr, cells were lysed in lysis buffer and AGO2 complexes were purified by anti-HA antibody. Purified RNAs were used for microarray analysis. **B**. The enrichment score (E-Score) was calculated with the indicated formula, and the criteria used for the selection of *miR-22* target mRNAs are listed. **C**. Distribution of enrichment score (E-Score) of mRNAs selected by AGO2-IP on Chip. Green and red dots indicate the E-score and fold change of mRNA expression in *miR-22* introduced cells, respectively. mRNA species with high E-Score tend to show relatively low expression in the presence of *miR-22*. **D.** Top10 mRNA list enriched in AGO2 complex after *miR-22* introduction. *CDKN1A* (*p21*) is shaded by a yellow line. **E.**The *miR-22* target sequences in the 3' UTR of *p21* mRNA in various mammalian species are aligned. The *miR-22* target sites in the 3' UTR of *p21* mRNA were searched for using the TargetScan database. The conserved target sites in the 3' UTR of *p21* mRNA are aligned against the 5' seed (red) and 3' complementary sequences (blue) from various mammalian species. The nucleotide sequence of *miR-22* is shown at the top.

Fig S7. A. Introduction of *miR-22* significantly reduced the p21 protein levels after exposure to ADR. Cells transfected with either *miR-22* or miR-NC were treated with indicated concentrations of ADR for 24 h, and p21 levels were determined by IB analysis. The data were prepared from three independent experiments, and indicated relative p21 protein levels compared with miR-NC transfected cells (lane 2). **B**. Repression of PARP-1 cleavage by introduction of p21 ORF. The data are related to Fig. 5C in the text. Two independent experiments of IB images are indicated. **C**. siRNA knockdown promotes PARP-1 cleavage. Cells were transfected with 5 nM of p21 siRNA (Qiagen #7) and negative control (Qiagen) for 48 h, and cells treated with indicated concentrations of ADR for 24 h, then cleaved PARP-1 was determined by IB. **D**. Inhibition of *miR-22* caused reduction of the S-phase of cell cycle. HCT 116 cells were infected either pZip-22 or control lentivirus, and selected by puromycin. Cells were reseeded at 1.0 x 10^5 cells /ml and treated with ADR for 24 h. FACS was carried out by PI staining, and the number of cells in each phase of the cell cycle was analyzed by FlowJo software.

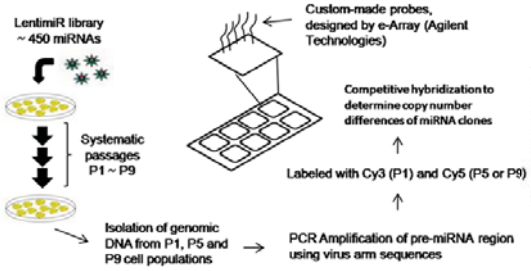
Fig. S8. A. B. p53-ChIP analysis. HCT 116 cells, seeded at 2 x 10^5 cells/ml in 150 mm dish, were treated with 5 or 25 nM of Act D for 24 hrs, and fixed with 1% formaldehyde fo 10 min, then subjected to ChIP analysis as indicated in Supplementary Materials and Methods. Quantification of precipitated DNA was carried out by quantitative real-time PCR analysis in triplicate with primers spanning p53 binding site of *p21*, *miR-22* -1104#1, *miR-22* -1104#2, Int2 and *IGX1A* (negative control, SABiosciences). Graph indicates fold enrichment of site occupancy for p53 binding relative to non-stressed cells (A), and percent of input (B). Data indicates means with standard deviation of triplicate

qPCR. Statistical analysis was carried out by *t*-test. Additional two independent experiments gave similar results.

A

Criteria	Experimental Strategy
1. Repressor of cell proliferation	Functional dropout screening using colon cancer cell line
2. Expressed in normal colon	Comprehensive expression profile in non-cancerous colon tissues
3. High frequency of chromosomal loss	Comparative genomic hybridization (aCGH) in colon cancer patients
4. Down-regulated in cancer patients	Quantitative RT-PCR using colon cancer samples

B



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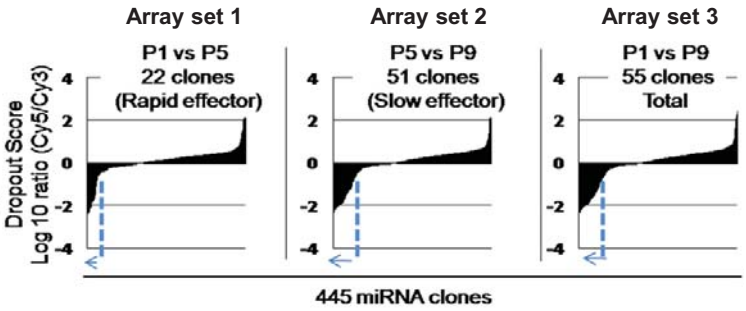

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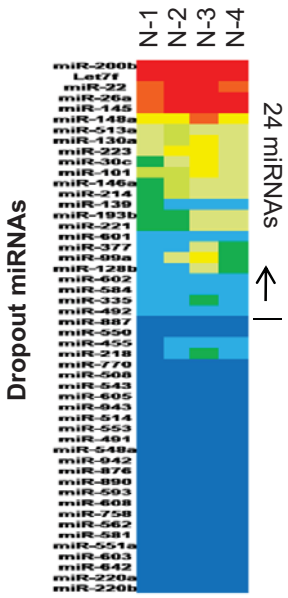

E

miRNA	Chromosomal localization		Copy number aberration Loss (n=24)
mir_22	17: 1563947-1564031 [-]		19
mir_200b	1: 1092347-1092441 [+]		11
mir_101	1: 65,524,117-65,524,191 [-]	9: 4,850,291-4,850,381[+]*	9
mir_30c	1: 41,222,956-41,223,044 [+]	6: 72,086,663-72,086,734[-]*	8
mir_377	14: 100598140-100598208 [+]		8
mir_130a	11: 57,408,671-57,408,759 [+]		8
mir_145	5: 148790402-148790489 [+]		5
mir_146a	5: 159,912,359-159,912,457[+]		5
mir_128	2: 136139437-136139518 [+]	3: 35760972-35761055 [+]*	4
mir_139	11: 72,326,107-72,326,174[-]		4
mir_584	5: 148422069-148422165 [-]		4
mir_602	9: 139852692-139852789 [+]		4
mir_26a	3: 37985899-37985975 [+]	12: 56504659-56504742 [-]*	3
mir_335	7: 129923188-129923281 [+]		3
mir_492	12: 93752305-93752420 [+]		3
mir_99a	21: 17,911,409-17,911,489[+]		3
mir_193b	16: 14,397,824-14,397,906[+]		2
mir_601	9: 126,164,804-126,164,882 [-]		2
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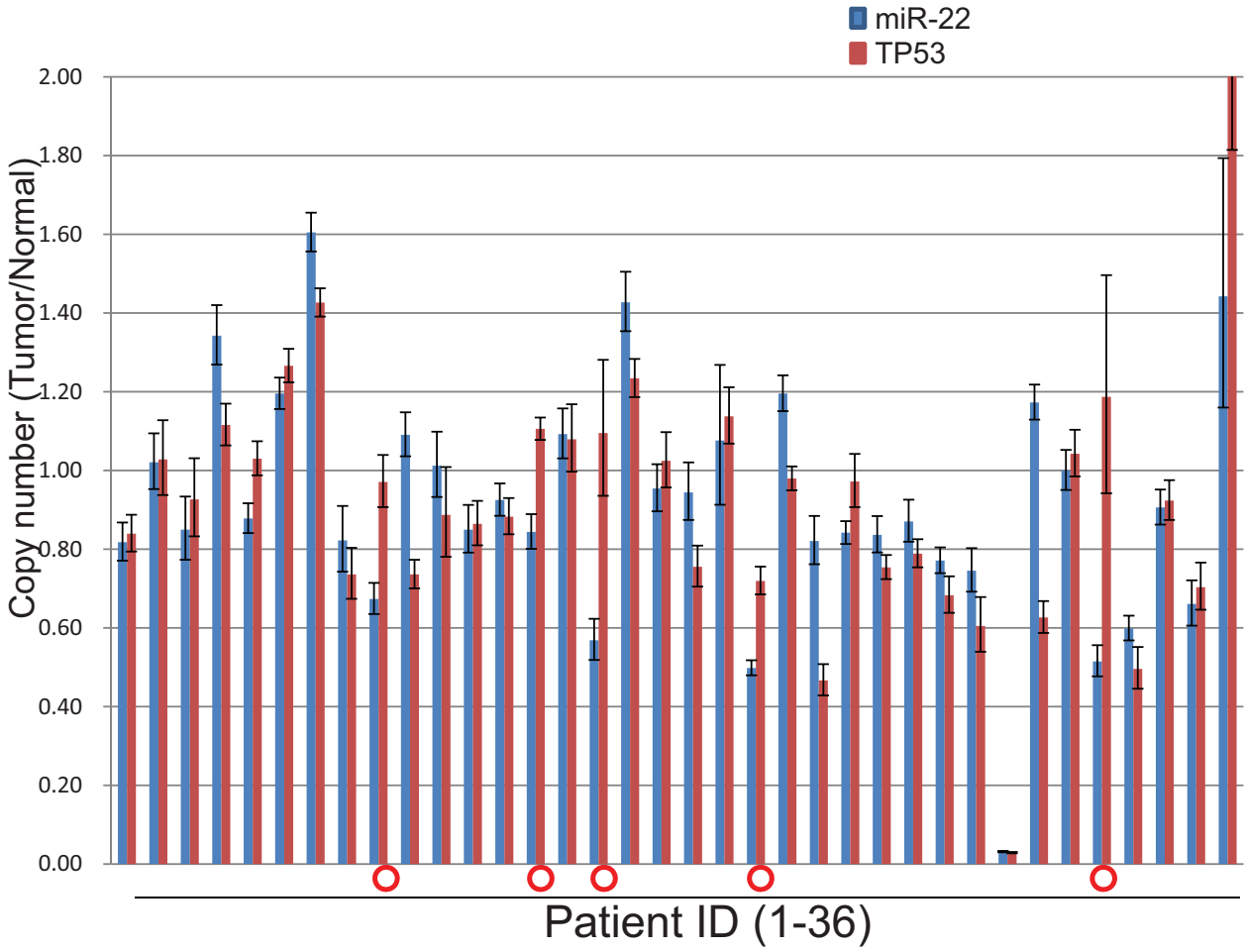
Fig. S1


miR-22
TP53
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1.80
1.60
1.40
1.20
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0.80
0.60
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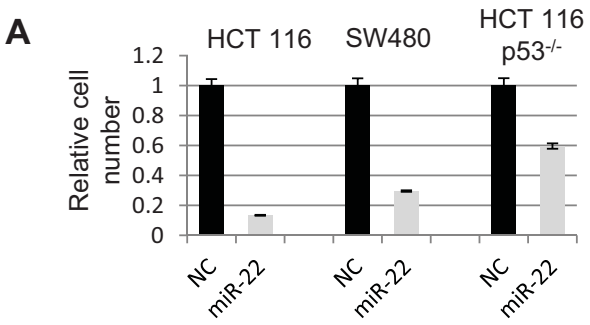
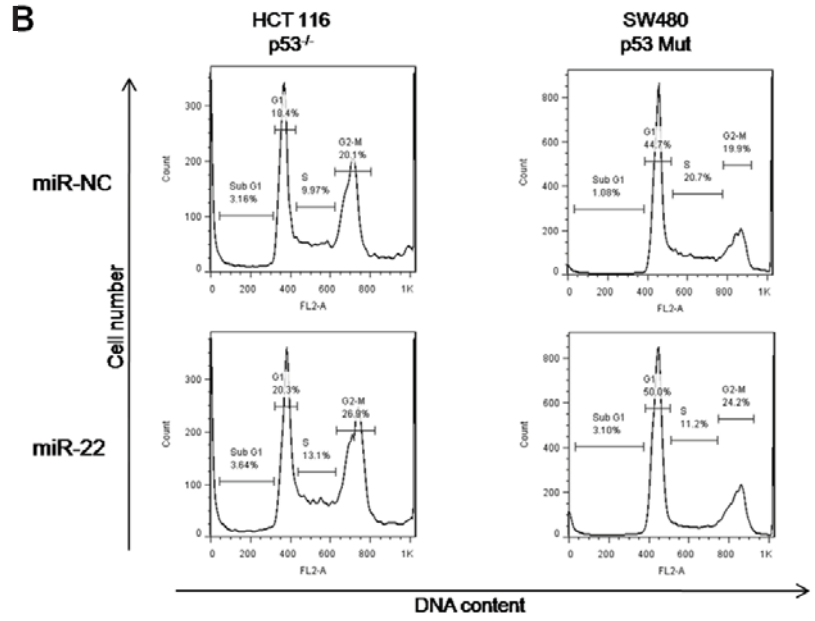
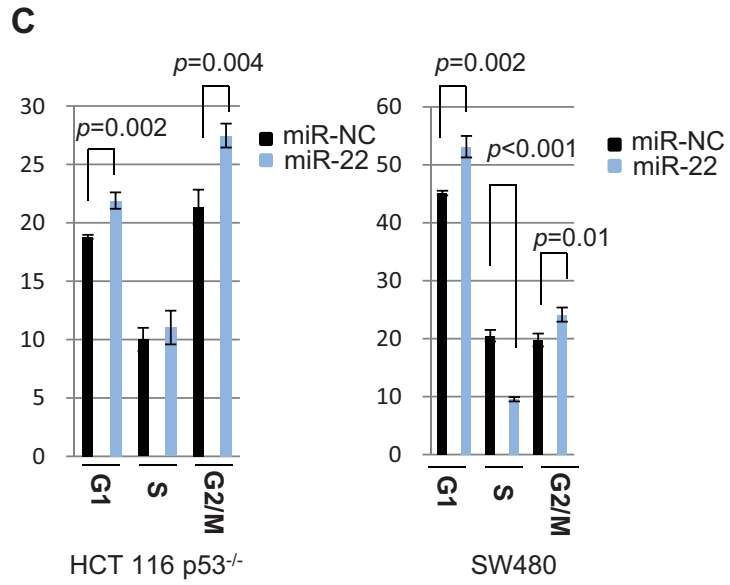
0.00
Copy number (Tumor/Normal)
Patient ID (1-36)

Fig. S2







D

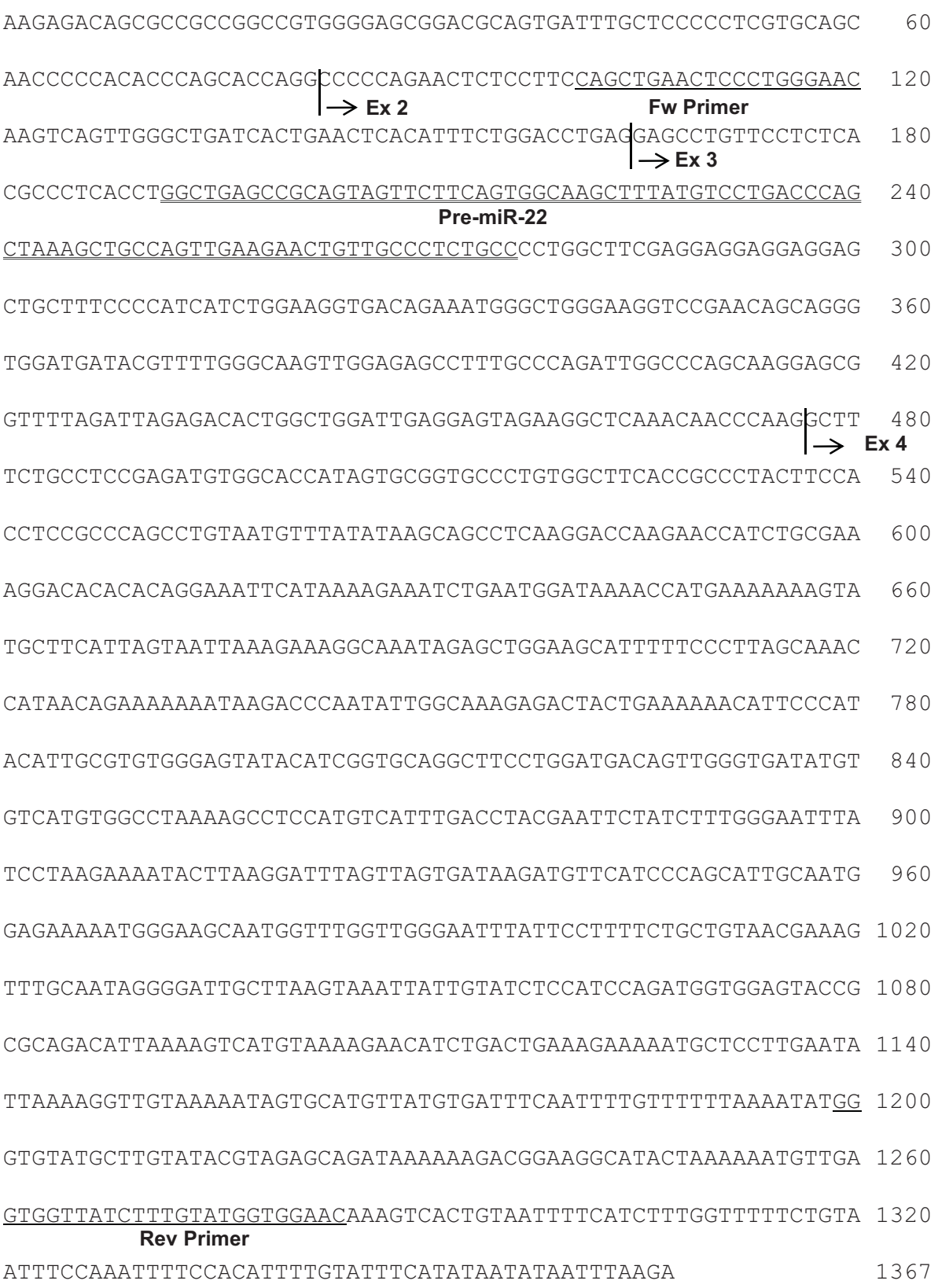
Up-regulated

Rank	Network	p-Value
1	p53	3.76E-25
2	c-Jun	3.76E-25
3	c-Myc	5.48E-22
4	Androgen receptor	7.65E-19
5	HIF1A	1.01E-15

Down-regulated

Rank	Network	p-Value
1	SP1	6.38E-32
2	CREB1	4.63E-19
3	c-Jun	4.63E-19
4	Androgen receptor	9.19E-13
5	p53	1.17E-09

Fig. S3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Ex 3
CGCCCTCACCTGGCTGAGCCGCAGTAGTTCTTCAGTGGCAAGCTTTATGTCCTGACCCAG 240
Pre-miR-22
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Ex 4
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Rev Primer
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Fig. S4

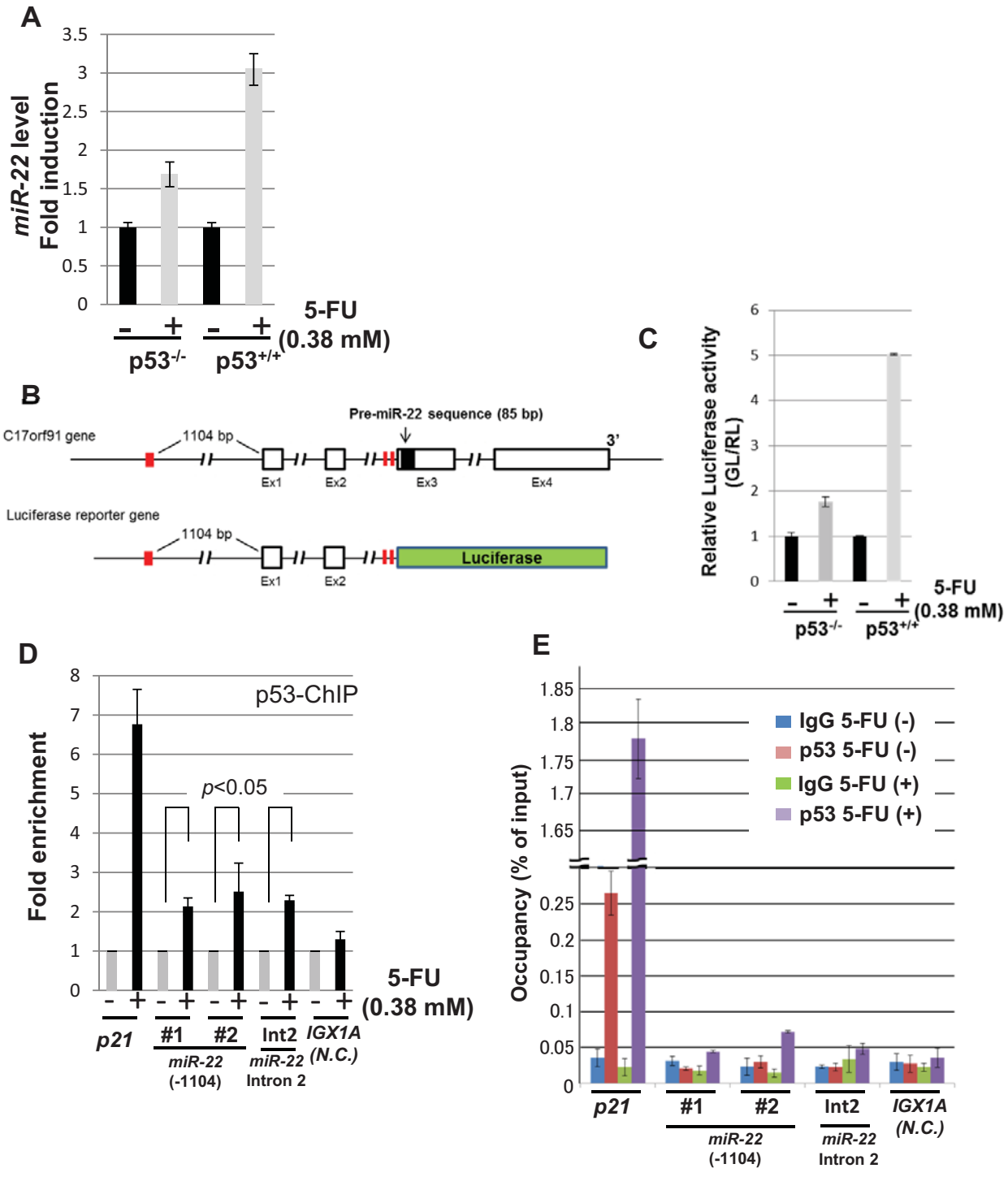
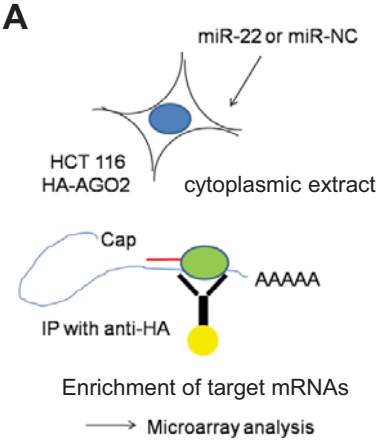

A
miR-22 level
Fold induction
3.5
3
2.5
2
1.5
1
0.5
0
- + - +
5-FU
(0.38 mM)
p53-/-
p53+/+
B
Pre-miR-22 sequence (85 bp)
C17orf91 gene
1104 bp
3'
Ex1
Ex2
Ex3
Ex4
Luciferase reporter gene
1104 bp
Luciferase
Ex1
Ex2
C
Relative Luciferase activity
(GL/RL)
6
5
4
3
2
1
0
- + - +
5-FU
(0.38 mM)
p53-/-
p53+/+
D
Fold enrichment
p53-ChIP
p<0.05
8
7
6
5
4
3
2
1
0
- + - + - + - + - +
5-FU
(0.38 mM)
p21
#1
#2
Int2
IGX1A
(N.C.)
miR-22
(-1104)
miR-22
Intron 2
E
Occupancy (% of input)
1.85
1.8
1.75
1.7
1.65
0.25
0.2
0.15
0.1
0.05
0
IgG 5-FU (-)
p53 5-FU (-)
IgG 5-FU (+)
p53 5-FU (+)
p21
#1
#2
Int2
IGX1A
(N.C.)
miR-22
(-1104)
miR-22
Intron 2

Fig. S5

A

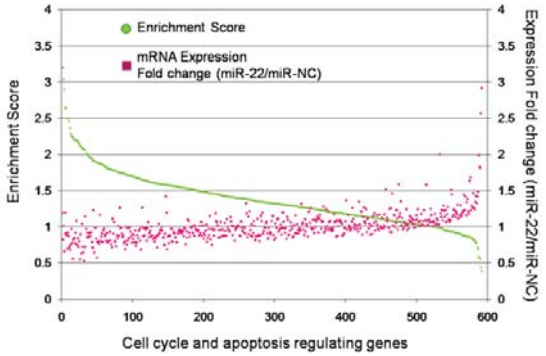


B

$$\text{Enrichment Score} = \frac{\text{Signal intensity of IP with miR-22/with miR-NC}}{\text{Expression fold change (miR-22/miR-NC)}}$$

Category for target	
Enrichment score (E-Score)	high
Expression in HCT 116 cells	+
Gene Ontology	cell cycle apoptosis

C



D

Gene	Enrichment Score	Fold change (22/nc)	
SON	15.614	1.189	anti-apoptosis
TNFRSF10C	3.199	0.821	apoptosis, signal transduction
NUPR1	3.035	1.011	induction of apoptosis
C9orf127	2.899	0.656	regulation of progression through mitotic cell cycle
ASPM	2.649	1.198	cell cycle, mitosis, cell division
CLN3	2.635	0.906	negative regulation of apoptosis
KATNB1	2.503	0.888	negative regulation of microtubule depolymerization, cell cycle, mitosis
CDKN1A*	2.500	0.697	cell cycle arrest, negative regulation of apoptosis
TXNL4B	2.496	0.919	mRNA processing, cell cycle, mitosis
CHMP1A	2.488	0.876	mitotic chromosome condensation

E

3′- UGUCAAGAAGUUGACCGUCGAA-5′ *has-miR-22*

```
Human       AAGAAGGGCACCCUAGUUCUACCUCAGGCAGCUCAAGCAGCGACCGC---
Chimpanzee  AAGAAGGGCACCCUAGUUCUACCUCAGGCAGCUCAAGCAGCGACCGC-
Rhesus      AAGAAGGGCACCCUAGUUCUACCUCAGGCAGCUCAAGCAGCGACUCCG--
Mouse       AAGUGGGAUUCCCUGGUCUUACCUUAGGCAGCUCCAGUGGCAACCC----
Rat         AAGUGGGGUUUCCUGGUCCUACCUCAGGCAGCUCCAGUGGCAACUG-
Dog         AAGUGGGGGUCCCCAGUUCUACCUCAGGCAGCUCAAGCAGCGACCACC-
Cat         AAGUAGGGGUCCCCGGUUCUACCUCAGGCAGCUCAAGCAGUGG--------
Horse       AAGCAGGGCUCUCCAGUUCUCCCUCAGGCAGCUCAAGCAGCGACCGCC-
Armadillo   AAGCCGGGCCGCCCCAUUCUACCUCAGGCAGCUCAGGCAGCGACUGCCUC
Elephant    AAGUUGGGAUUCCAAGUUUUACCUCAGGCAGCUCAAGCAGUGACCACCUC
Tenrec      AAGUAGGGAUCCCCAGUUCUACCUCAGGCAGCUCAAGCAGCGACCACCUC
```

Fig. S6

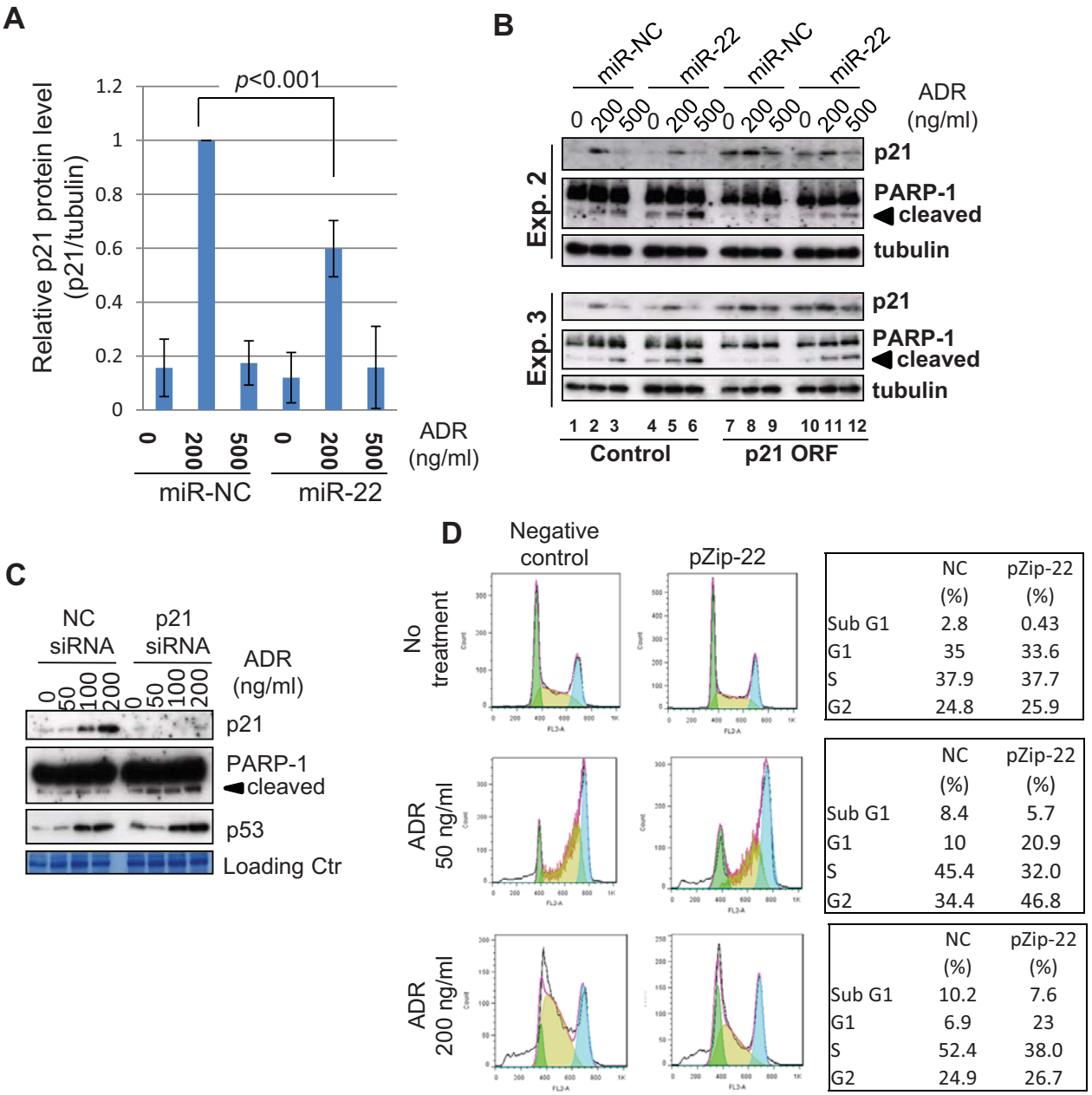
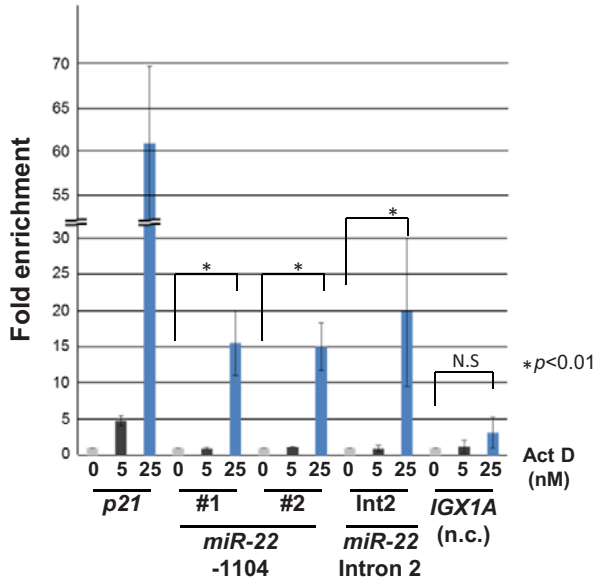

A
Relative p21 protein level (p21/tubulin)
p<0.001
1.2
1
0.8
0.6
0.4
0.2
0
0 200 500 0 200 500
ADR (ng/ml)
miR-NC
miR-22
B
miR-NC
miR-22
miR-NC
miR-22
ADR (ng/ml)
0 200 500 0 200 500 0 200 500 0 200 500
Exp. 2
p21
PARP-1
cleaved
tubulin
Exp. 3
p21
PARP-1
cleaved
tubulin
1 2 3 4 5 6 7 8 9 10 11 12
Control
p21 ORF
C
NC siRNA
p21 siRNA
ADR (ng/ml)
0 50 100 200 0 50 100 200
p21
PARP-1
cleaved
p53
Loading Ctr
D
Negative control
pZip-22
No treatment
ADR 50 ng/ml
ADR 200 ng/ml
NC (%) pZip-22 (%)
Sub G1 2.8 0.43
G1 35 33.6
S 37.9 37.7
G2 24.8 25.9
NC (%) pZip-22 (%)
Sub G1 8.4 5.7
G1 10 20.9
S 45.4 32.0
G2 34.4 46.8
NC (%) pZip-22 (%)
Sub G1 10.2 7.6
G1 6.9 23
S 52.4 38.0
G2 24.9 26.7

Fig. S7

A


Fold enrichment
70
65
60
55
30
25
20
15
10
5
0
*
*
*
N.S
*p<0.01
0 5 25 0 5 25 0 5 25 0 5 25 0 5 25
Act D (nM)
p21
#1
#2
Int2
IGX1A (n.c.)
miR-22 -1104
miR-22 Intron 2

B

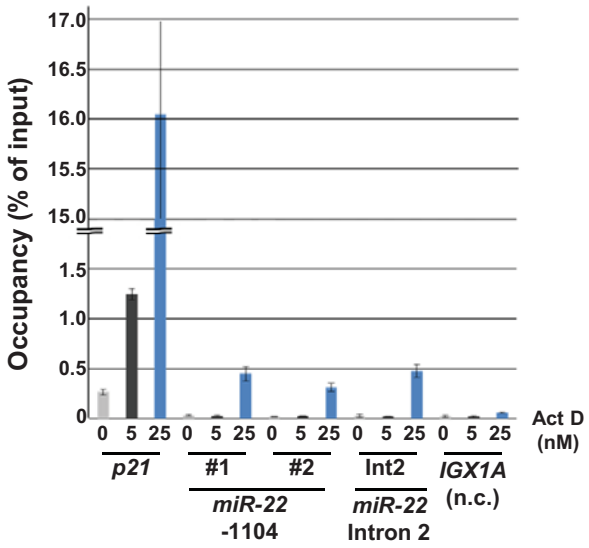

Occupancy (% of input)
17.0
16.5
16.0
15.5
15.0
1.5
1.0
0.5
0
0 5 25 0 5 25 0 5 25 0 5 25 0 5 25
Act D (nM)
p21
#1
#2
Int2
IGX1A (n.c.)
miR-22 -1104
miR-22 Intron 2

Fig. S8

Table S1 Primer sequences used for this study

Primer name	Nucleotide sequence
Vector primer CDH5.1	5'–GCC TGG AGA CGC CAT CCA CGC TG–3'
Vector primer CDH3.1	5'–GAT GTG CGC TCT GCC CAC TGA C–3'
p21 3' UTR Fw	5'–TCC GCC CAC AGG AAG CCT GC–3'
p21 3' UTR Rev	5'–GAG CAC CTG CTG TAT ATT CAG C–3'
Mutagenesis p21 5' seed Fw	5'–TCA GGg ata tcA AGC AGC GAC CGC CCC CT–3'
Mutagenesis p21 5' seed Rev	5' GCT Tga tat cCC TGA GGT AGA ACT AGG G–3'
Mutagenesis p21 3' Rev	5'–TGA GGg aat tcT AGG GTG CCC TTC TTC TT–3'
Mutagenesis Double Fw	5'–CCT Aga att cCC TCA GGg ata tcA AGC A–3'
p21 –2238 ChIP Fw	5'–AGC AGG CTG TGG CTC TGA TT–3'
p21 –2238 ChIP Rev	5'–CAA AAT AGC CAC CAG CCT CTT CT–3'
ChIP miR–22 –1104 #1 Fw	5'–GCA TGG ATT TCA GTC CAC AT–3'
ChIP miR–22 –1104 #1 Rev	5'–ATG AAC CCA GTG ACT TGG CA–3'
ChIP miR–22 –1104 #2 Fw	5'–CAG CTG ATT CGT CCC CAG AGG TCA–3'
ChIP miR–22 –1104 #2 Rev	5'–GGA GCT CCA AAA TTC TGA GCC TGC G–3'
ChIP miR–22 Int2 Fw	5'–GAC CTT CTT TCA AAC GCT AG–3'
ChIP miR–22 Int2 Rev	5'–ACG CAG GTA CTA GAA GTG TG–3'

Table S2 Dropout Clones during culture of HCT 116 cells

P1 vs P5			P5 vs P9			P1 vs P9		
miRNA clones	log 10 ratio	mature form	miRNA clones	log 10 ratio	mature form	miRNA clones	log 10 ratio	mature form
mir_455	-2.3558	miR-455	mir_492	-2.3219	miR-492	mir_455	-2.2504	miR-455
mir_34a	-2.2627	miR-34a	mir_593	-2.1573	miR-593	mir_26a_1	-2.2009	miR-26a
mir_486	-2.1658	miR-486	mir_220a	-2.1532	miR-200a	mir_145	-2.1592	miR-145
mir_608	-2.1589	miR-608	mir_145	-2.1503	miR-145	mir_492	-2.1258	miR-492
mir_942	-2.1364	miR-942	mir_26a_1	-2.1494	miR-26a	mir_593	-2.1207	miR-593
mir_379	-2.1319	miR-379	mir_758	-2.1210	miR-758	mir_513a_1	-2.0899	miR-513a
mir_562	-2.0680	miR-562	mir_345	-2.0961	miR-345	mir_758	-2.0882	miR-758
mir_605	-2.0587	miR-605	mir_543_	-2.0699	miR-543	mir_513a_2	-2.0478	miR-513a
mir_30d	-1.9143	miR-30d	mir_346	-2.0607	miR-346	mir_220a	-2.0454	miR-220a
mir_891a	-1.8138	miR-891a	mir_601	-2.0571	miR-601	mir_942	-2.0220	miR-492
mir_22	-1.8014	miR-22	mir_602	-2.0347	miR-602	mir_608	-2.0194	miR-608
mir_513a_2	-1.7761	miR-513a	mir_770	-2.0275	miR-770	mir_601	-2.0144	miR-601
mir_891b	-1.7693	miR-891	mir_584	-2.0273	miR-584	mir_602	-1.9996	miR-602
mir_218_1	-1.7527	miR-218	mir_548a_2	-2.0261	miR-548a	mir_543_	-1.9873	miT-543
mir_513a_1	-1.7165	miR-513a	mir_539	-2.0213	miR-539	mir_223	-1.9530	miR-223
mir_934	-1.6903	miR-934	mir_551a	-1.9773	miR-551a	mir_770	-1.9518	miR-770
mir_514_1	-1.6660	miR-514	mir_551b	-1.9654	miR-551b	mir_562	-1.9499	miR-562
mir_890	-1.5240	miR-890	mir_30c_2	-1.9639	miR-30c	mir_551a	-1.9498	miR-551a
mir_508	-1.4822	miR-508	mir_148a	-1.9621	miR-148	mir_605	-1.9373	miR-605
mir_200b	-1.2109	miR-200b	mir_642	-1.9431	miR-642	mir_148a	-1.9164	miR-148a
mir_514_2	-1.1794	miR-514	mir_221	-1.9229	miR-221	mir_548a_2	-1.8718	miR-548a
mir_491	-1.0582	miR-491	mir_223	-1.9089	miR-223	mir_642	-1.8560	miR-642
			mir_603	-1.8850	miR-603	mir_30c_2	-1.8510	miR-30c
			mir_889	-1.8614	miR-889	mir_200b	-1.8254	miR-200b
			mir_214	-1.8330	miR-214	mir_603	-1.8165	miR-603
			mir_220b	-1.8076	miR-220b	mir_221	-1.7971	miR-221
			mir_876	-1.7357	miR-876	mir_214	-1.7799	miR-214
			mir_99a	-1.7116	miR-99a	mir_584	-1.7428	miR-584
			mir_550_1	-1.7049	miR-550	mir_22	-1.6873	miR-22
			mir_30c_1	-1.7027	miR-30c	mir_491	-1.6667	miR-491
			mir_550_2	-1.6782	miR-550	mir_220b	-1.6575	miR-220b
			mir_491	-1.5603	miR-491	mir_218_1	-1.6445	miR-218
			mir_335	-1.5452	miR-335	mir_99a	-1.6321	miR-99a
			mir_146a	-1.5304	miR-146a	mir_876	-1.6228	miR-876
			mir_801	-1.5265	miR-801	mir_550_1	-1.6215	miR-550
			mir_553	-1.4319	miR-553	mir_934	-1.5791	miR-934
			mir_133a_2_	-1.4203	miR-133a	mir_30c_1	-1.5773	miR-30c
			mir_887	-1.4108	miR-887	mir_514_1	-1.5629	miR-514
			let_7f_2	-1.3337	let7f	mir_335	-1.5575	miR-335
			mir_129_2	-1.3250	miR-129	mir_550_2	-1.5543	miR-550
			mir_128b	-1.3042	miR-128	mir_146a	-1.4840	miR-146a
			mir_581	-1.2894	miR-581	mir_890	-1.4535	miR-890
			mir_193b	-1.2738	miR-193b	mir_801	-1.3823	miR-801
			mir_101_1_	-1.2460	miR-101	mir_508	-1.3787	miR-508
			mir_139	-1.2234	miR-139	mir_553	-1.3490	miR-553
			mir_513a_1	-1.2180	miR-513a	mir_887	-1.3411	miR-887
			mir_609	-1.1896	miR-609	let_7f_2	-1.2749	let7f
			mir_873	-1.1794	miR-873	mir_581	-1.2729	miR-581
			mir_513a_2	-1.1105	miR-513a	mir_101_1_	-1.2454	miR-101
			mir_377_	-1.0887	miR-377	mir_193b	-1.2075	miR-193b
			mir_130a	-1.0315	miR-130a	mir_128b	-1.1754	miR-128b
						mir_139	-1.1155	miR-139
						mir_514_2	-1.1105	miR-514
						mir_377_	-1.0870	miR-377
						mir_130a	-1.0173	miR-130a

The list of dropout clones identified by functional screening. Three assay conditions (P1 vs P5, P5 vs P9 and P1 vs P9) are indicated in 3 different columns. The left of each column indicates the name of lentivirus miRNA clones, the center is dropout score (Cy5/Cy3, log10 ratio), and the right is the name of mature miRNA derived from the lentivirus clone.

Table S3. mRNAs up-regulated by miR-22

fold change (> 2-fold)				
1st	2nd	3rd		
miR22/miRnc2	miR22/miRnc2	miR22/miRnc2	Common name	GeneSymbol
2.346	2.369	3.023	NM_016270	KLF2
1.497	2.296	2.748	NM_016201	AMOTL2
1.252	2.039	2.317	NM_001964	EGR1
1.506	2.949	3.679	NM_001554	CYR61
2.664	2.601	3.147	NM_130469	JDP2
1.828	2.024	2.244	NM_032865	TNS4
1.387	2.723	3.350	NM_001554	CYR61
2.545	3.043	3.485	NM_021913	AXL
1.866	2.010	2.316	NM_001001555	GRB10
1.548	2.732	3.515	NM_001554	CYR61
2.278	2.348	2.791	NM_032463	LAT2
2.145	2.626	3.126	NM_016352	CPA4
2.967	3.910	4.185	NM_198951	TGM2
2.128	1.582	2.175	NM_019083	CCDC76
1.988	2.798	3.641	NM_014572	LATS2
4.464	8.188	9.668	NM_182507	KRT80
2.216	1.377	2.127	NM_030753	WNT3
2.406	2.763	3.280	NM_016352	CPA4
7.268	4.898	6.251	DA438590	DA438590
2.254	1.526	2.250	THC2732721	THC2732721
2.333	2.469	3.619	NM_014572	LATS2
2.656	3.391	4.250	NM_004734	DCLK1
2.273	3.901	4.815	NM_001901	CTGF
69.503	0.842	19.518	AF116677	AF116677
2.117	2.539	2.835	NM_144677	MGAT5B
2.906	1.524	2.404	THC2579650	THC2579650
2.094	1.923	2.786	DQ080207	MIG7
2.989	2.372	3.041	NM_005358	LMO7
2.277	2.810	3.674	ENST00000367013	C1orf133
1.524	2.128	2.166	AB028976	SAMD4A
3.256	4.654	5.850	NM_001955	EDN1
2.171	2.114	2.510	AK057956	AK057956
1.953	2.509	2.867	NM_001008540	CXCR4
1.602	2.091	2.293	NM_020349	ANKRD2
2.181	2.967	3.176	NM_004613	TGM2
2.473	5.143	0.850	NM_030639	BHLHB9
3.394	6.101	8.744	BC073764	IGKC
1.496	3.602	2.643	BC019234	ZNF273
1.616	2.183	2.289	NM_013962	NRG1
2.139	2.756	3.304	NM_003246	THBS1
2.099	1.932	2.911	BX647685	BX647685
7.790	1.407	2.450	THC2612011	THC2612011
2.170	1.648	2.331	AK002200	SMC4
7.721	4.703	0.601	BC031641	C10orf128
1.936	2.290	2.999	NM_020632	ATP6V0A4
20.544	2.068	0.099	AF020645	AF020645
3.891	1.609	4.452	NM_003015	SFRP5
2.696	0.721	4.139	NM_005527	HSPA1L
1.908	2.132	2.489	NM_001031741	NEK10
2.392	1.254	2.142	THC2609820	THC2609820
2.110	3.702	3.806	NM_015881	DKK3
2.063	2.200	1.486	NR_001589	HBBP1
23.044	20.421	1.073	AK026149	TUSC3
3.290	2.722	1.043	NM_006352	ZNF238
1.154	2.280	3.304	NM_182898	CREB5

2.490	0.251	4.784	NM_001012391	RUNDC2B
2.043	1.830	2.383	NM_001008394	EID3
2.009	1.494	2.072	NM_032464	LAT2
2.251	1.703	2.257	THC2604590	THC2604590
2.568	1.427	2.480	NM_173077	CPO
15.544	22.956	10.852	NM_182898	CREB5
19.193	9.800	1.054	AA827683	AA827683
3.001	2.960	4.395	NM_000584	IL8
2.225	1.445	2.183	NM_182527	CABP7
3.165	2.471	2.645	NM_020182	TMEPAI
2.558	2.692	4.165	NM_000509	FGG
12.737	10.072	0.996	BC041467	C17orf67
3.562	2.065	4.434	ENST00000299694	ENST00000299694
2.957	2.294	0.732	AU157551	AU157551
1.718	2.242	2.645	ENST00000370548	ENST00000370548
3.308	5.512	2.110	AK025303	KIF13A
1.876	2.433	2.585	NM_018993	RIN2
3.367	3.865	1.791	AF035035	IGKV1D-8
1.601	6.019	2.253	BX112970	BX112970
1.156	3.513	2.511	NM_018996	TNRC6C
2.383	1.793	2.196	AL137653	CTBP1
3.784	2.773	3.754	CV570707	CV570707
10.305	7.556	0.049	NM_058172	ANTXR2
1.368	2.903	2.329	H28997	H28997
2.155	2.013	1.901	NM_005771	DHRS9
2.290	1.124	2.119	NM_002203	ITGA2
1.411	2.915	3.440	NM_144777	SCEL
1.665	2.643	2.159	NM_001011703	FAM125B
2.981	0.231	4.217	NM_172311	SALF
2.054	0.659	3.375	AK097149	UNQ1887
8.982	1.026	4.279	BE858685	BE858685
2.017	1.379	9.313	NM_001080542	FBF1
3.048	2.001	5.801	ENST00000367012	SERTAD4
1.108	2.244	3.719	AK057304	FLJ32742
4.627	2.489	4.186	NM_182507	KRT80
2.727	1.858	10.636	NM_025047	ARL14
2.056	2.841	4.001	NM_000728	CALCB
1.621	2.002	2.704	THC2516687	THC2516687
1.735	2.452	2.839	NM_001299	CNN1
1.810	2.689	3.256	NM_000800	FGF1
1.818	3.674	3.038	NM_032782	HAVCR2
2.266	2.416	3.302	NM_003038	SLC1A4
3.685	0.697	25.119	AY326436	RIC3
3.047	2.518	2.328	AK025758	AK025758
2.160	1.365	6.630	NM_152756	RICTOR
2.163	2.225	0.764	NM_002145	HOXB2
0.731	3.752	3.709	BC039082	TMPRSS6
2.391	1.282	2.410	AY055760	CD55
1.254	2.081	2.469	XM_001132569	LOC730130
2.542	1.296	3.353	NM_024600	C16orf30
1.244	2.131	3.035	AK096488	C1orf83
3.262	5.277	0.612	NM_004440	EPHA7
3.695	4.391	1.571	NM_153046	TDRD9
4.978	1.846	3.227	NM_004543	NEB
2.488	1.262	2.432	NM_206855	QKI
1.400	4.490	2.399	NM_174927	SPATA19
2.139	1.172	2.795	NM_001116	ADCY9
4.661	4.646	4.781	NM_019605	SERTAD4
2.570	1.183	3.589	ENST00000378204	EXT1
1.747	2.490	2.642	NM_018661	DEFB103A

0.998	2.320	2.201	NM_014070	C6orf15	
2.159	1.229	4.982	L10403	PPBPL2	
2.638	2.192	1.524	NM_152592	C14orf49	
2.539	4.233	7.770	AK094154	AK094154	
4.181	2.274	1.830	THC2655194	THC2655194	
2.709	7.568	1.014	BC034285	BC034285	
2.546	4.266	5.838	THC2634493	THC2634493	
1.658	3.374	2.200	NM_000267	NF1	
1.336	4.795	11.241	NM_145004	ADAM32	
1.943	5.438	6.682	NM_000800	FGF1	
3.653	4.409	1.294	AK096956	AK096956	
2.165	2.892	1.716	BC034497	FAM124A	
1.125	2.407	2.977	BX114358	BX114358	
4.689	6.061	1.067	NM_031409	CCR6	
2.121	1.844	9.732	THC2675521	THC2675521	
2.388	0.882	11.897	NM_134431	SLCO1A2	
1.436	2.317	3.156	NM_001003811	TEX11	
3.926	4.878	0.222	U79301	U79301	
1.424	2.168	2.213	AI161251	AI161251	
3.152	1.330	5.063	BC044620	C9orf57	
2.611	22.220	1.005	AB063115	NAV2	
1.055	5.418	2.656	NM_005337	NCKAP1L	
1.349	6.872	11.582	NM_152868	KCNJ4	
1.284	4.013	2.744	NM_024574	C4orf31	
1.512	2.287	2.414	NM_144992	MGC26733	
2.104	2.197	3.296	NM_000887	ITGAX	
2.067	5.382	6.431	NM_020814		39876
1.820	3.122	8.193	NM_000936	PNLIP	
2.739	2.686	28.921	NM_201648	GLYAT	
0.711	7.677	2.941	L17328	FEZ2	
2.033	4.487	5.254	U09850	ZNF143	
1.563	9.012	10.782	NM_000547	TPO	
1.498	4.903	5.556	NM_000353	TAT	
1.443	6.188	12.767	CA946373	CA946373	
0.979	13.619	8.049	NM_000433	NCF2	
1.140	6.766	24.352	BC025666	BC025666	
0.711	15.406	34.697	AK074236	SLC14A2	

mRNAs were selected to show the up-regulation in at least two experiments

Table S4. mRNA down-regulated by miR-22

fold change (< 0.5 fold)				
1st	2nd	3rd		
miR22/miRnc2	miR22/miRnc2	miR22/miRnc2	Common name	GeneSymbol
0.498	0.445	0.577	NM_002129	HMGB2
0.411	0.315	0.409	ENST00000229270	ENST00000229270
0.609	0.392	0.495	NM_002961	S100A4
0.508	0.393	0.478	NM_022126	LHPP
0.495	0.464	0.425	NM_024336	IRX3
0.390	0.311	0.377	NM_002923	RGS2
0.448	0.326	0.430	NM_003721	RFXANK
0.456	0.445	0.549	THC2474831	THC2474831
0.545	0.405	0.484	NM_152832	FAM89B
0.472	0.356	0.423	NM_004192	ASMTL
0.408	0.369	0.477	NM_032284	PRPF38A
0.753	0.412	0.485	NM_001002021	PFKL
0.489	0.394	0.534	NM_032932	RAB11FIP4
0.472	0.338	0.412	NM_004192	ASMTL
0.475	0.381	0.487	NM_018664	SNFT
0.412	0.273	0.364	NM_033285	TP53INP1
0.487	0.422	0.498	NM_000314	PTEN
0.457	0.327	0.465	AB016898	LOC653483
0.625	0.460	0.496	NM_025083	EDC3
0.377	0.263	0.327	NM_207578	PRKACB
0.637	0.407	0.497	NM_001038	SCNN1A
0.377	0.317	0.408	NM_003385	VSNL1
0.473	0.407	0.521	ENST00000244221	PAIP2B
2.053	0.296	0.385	NM_006889	CD86
0.541	0.489	0.481	NM_002055	GFAP
0.099	3.151	0.189	CB854194	CB854194
0.467	0.436	0.490	BX648343	KRTAP4-7
0.552	0.468	0.498	NM_003118	SPARC
0.620	0.396	0.483	NM_002868	RAB5B
0.295	0.483	1.003	NM_001005273	CHD3
0.216	0.711	0.168	BC038840	NAALAD2
0.450	0.369	0.521	NM_001040152	PEG10
0.615	0.449	0.500	NM_003469	SCG2
0.468	0.438	0.396	NM_004041	ARRB1
0.479	0.370	0.487	NM_054014	FKBP1A
0.516	0.411	0.469	NM_005269	GLI1
0.533	0.245	0.426	NM_005540	INPP5B
0.458	0.379	0.386	NM_001017915	INPP5D
0.441	0.429	0.480	NM_030929	KAZALD1
0.568	0.373	0.431	NM_006454	MXD4
1.015	0.277	0.382	NM_004917	KLK4
0.024	0.225	1.137	AK124129	DKFZP434H168
0.636	0.454	0.495	NM_001001438	LSS
0.382	1.067	0.248	NM_005408	CCL13
0.464	0.343	0.449	NM_004664	LIN7A
0.123	0.256	0.607	NM_001037231	LOC285735
0.332	0.458	1.077	ENST00000344523	NR6A1
0.140	0.053	1.170	BC037791	BC037791
1.029	0.455	0.307	NM_018265	C1orf106
0.241	0.222	0.801	AF217973	AF217973
0.364	0.190	1.075	XM_001128267	LOC730704
0.372	0.231	0.272	NM_005540	INPP5B
0.395	0.086	0.524	NM_014668	GREB1
0.637	0.377	0.458	AB039791	ARP11
0.333	0.451	3.961	NM_015025	MYT1L

0.083	0.159	0.388	NM_001080437	SNED1
0.499	0.843	0.465	AJ278120	TANC2
0.130	0.312	1.024	ENST00000305752	FAM98B
0.695	0.308	0.174	THC2554861	THC2554861
0.958	0.058	0.306	ENST00000377525	ENST00000377525
0.356	0.302	0.767	NM_172249	CSF2RA
0.443	0.399	0.403	BG719660	BG719660
0.681	0.193	0.254	NM_003896	ST3GAL5
0.686	0.206	0.326	NM_025212	CXXC4
0.460	0.952	0.349	NM_145208	MBD3L1
0.486	0.393	0.555	AK022997	AK022997
0.528	0.200	0.460	NM_006608	PHTF1
0.596	0.308	0.449	ENST00000305749	CXXC4
0.848	0.436	0.399	AB051473	PLEKHA5
0.481	0.355	0.307	AK054626	FLJ30064
1.047	0.274	0.391	AK057476	AK057476
0.069	0.060	1.003	NM_024867	FLJ23577
0.396	0.462	1.245	NM_015596	KLK13
0.618	0.464	0.327	NM_000834	GRIN2B
0.808	0.320	0.449	NM_001850	COL8A1
1.836	0.326	0.435	NM_025193	HSD3B7
0.764	0.463	0.486	NM_021200	PLEKHB1
0.225	0.294	0.854	NM_000651	CR1
0.356	0.343	2.250	NM_144727	CRYGN
0.810	0.229	0.227	NM_005036	PPARA
0.273	0.224	0.555	BQ775944	BQ775944
0.216	1.968	0.184	AK024328	ABCA1
0.857	0.475	0.095	NM_014660	PHF14
1.220	0.246	0.274	BF959143	BF959143
0.737	0.286	0.227	BC026179	SEC61A2
0.322	0.146	1.263	THC2746042	THC2746042
0.458	0.130	0.221	THC2732175	THC2732175
0.499	0.487	0.950	AK023372	FLJ13310
1.330	0.284	0.280	NM_001836	CMA1
0.242	0.837	0.212	NM_022360	FAM12B
0.885	0.223	0.138	NM_018371	ChGn
0.698	0.193	0.489	NM_173081	ARMC3
1.288	0.261	0.129	NM_023914	P2RY13
0.885	0.348	0.188	NM_153236	GIMAP7
0.408	0.500	0.333	NM_001010978	LDLRAD1
0.429	0.291	1.477	NM_021225	PROL1
0.917	0.173	0.171	NM_012298	CAND2
0.745	0.366	0.321	NM_017820	FLJ20433
2.680	0.313	0.463	AW502318	AW502318
1.105	0.325	0.195	AK126097	LOC285827
0.708	0.406	0.192	NM_017855	ODAM
1.659	0.177	0.391	NM_014333	CADM1
3.479	0.085	0.286	NM_004464	FGF5
0.884	0.203	0.214	NM_133448	TMEM132D
3.085	0.048	0.186	NM_016592	GNAS
0.614	0.068	0.210	NM_001005733	C21orf34
0.704	0.178	0.207	BC030609	BC030609
0.626	0.049	0.132	NM_001001317	TRY1
0.627	0.113	0.049	NM_000689	ALDH1A1
0.710	0.099	0.115	NM_080818	OXGR1
2.618	0.113	0.066	NM_025113	C13orf18
0.638	0.035	0.103	NM_173645	FLJ37357

mRNA down-regulated by miR-22 in at least two experiments were selected