SUPPLEMENTAL MATERIAL.

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SUPPLEMENTAL METHODS

Chromatin immunoprecipitation, ChIP-seq library preparation and Illumina sequencing.

Chromatin immunoprecipitation was performed as described before (Galbraith et al. 2013). Briefly, subconfluent (50 – 70%) HCT116, MCF7, and SJSA cells were exposed to 10 μM Nutlin or 0.2% DMSO for 12 hours. After the treatment period, culture media was removed and cells were cross-linked using 1% formaldehyde for 15 minutes. Formaldehyde was quenched by glycine (0.125 M final concentration) and after 5 minutes of incubation plates were washed twice with cold PBS. Cross-linked cells were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris pH 8.0, 5 mM EDTA, 1% IGEPAL 630 (NP-40 substituent), 0.5% sodium deoxycholate, 0.1% SDS and protease/phosphatase inhibitors). Whole cell lysates were sonicated to generate fragments of ~200-250 bp. Insoluble material was removed by 20 minute centrifugation at 20,000g. Protein concentration in the supernatant was analyzed using BCA protein assay kit (Pierce, Thermo Fisher Scientific) and diluted to 1 mg/ml in RIPA buffer.

Prior to the immunoprecipitation reaction, Dynabeads M-280 (sheep anti-mouse IgG, Thermo Fisher Scientific) were washed twice with RIPA buffer and used for lysate pre-clearing. Samples were immunoprecipitated overnight with anti-p53 antibody (DO-1, EMD Millipore, 0.5 mg/sample) and Dynabeads at 4°C. Next day beads were washed (5 minutes each washing step) twice with RIPA, four times with IP wash buffer (500 mM LiCl, 100 mM Tris pH 8.5, 1% IGEPAL, 1% sodium deoxycholate), again twice with RIPA and twice briefly with TE (10 mM Tris pH 8, 1 mM EDTA). Next, beads were resuspended in 100 μl of TE and 200 μl of elution buffer (70 mM Tris pH 8, 1 mM EDTA and 1.5% SDS) and incubated at 65°C for 10 minutes. After adding NaCl to final concentration of 200 mM, eluted immunocomplexes were subjected to reverse cross-linking reaction at 65°C for 5 hours. Remaining protein was digested by proteinase K (20 μg/sample) at 45°C for 30 minutes. DNA was recovered by one phenol/chloroform and one chloroform extraction followed by ethanol precipitation and resuspension in 50 μl of TE. Input DNA was extracted from reverse cross-linked lysates using the same extraction protocol as for sample DNA.

Isolated DNA was size-selected using 2% agarose gel electrophoresis (BluePippin, Sage Science) for fragments 80-400 bp-long. 5 ng of the recovered DNA was used to prepare the sequencing library using TruSeq ChIP Library Preparation Kit from Illumina according to manufacturer's recommendations. 10 cycles of the PCR reaction were used to enrich DNA fragments and the reaction products were size-selected for 200-520 bp-long amplicons. The quality and quantity of the library was analyzed by Agilent Bioanalyzer 2100 showing fragment size (including adapters) ranging from 200 to 400 bp. Barcoded libraries were pooled and sequenced to 50 bp (single end) in total of three lanes on an Illumina HiSeq2000.

ChIP-seq data analysis and visualization.

Total number of reads per sample ranged between $4.9*10^7$ and $6.7*10^7$. Quality of the sequenced reads was analyzed using FastQC (version 0.10.1, http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low quality reads were removed (phred < 10, ea-utils_1.1.2, http://code.google.com/p/ea-utils). Read duplication level ranged from 1.8 to 9.2%. Next, reads were mapped to the reference human genome (GRCh37/hg19) using Bowtie2 (bowtie2-2.2.3, --sensitive –-end-to-end) and reads with low mapping quality scores (MAPQ < 10) as well as reads mapped to multiple loci were removed. The Homer suite (version 4.3) (Heinz et al. 2010) was used for identification of peak regions and motif discovery. Peak calling criteria for each cell line were normalized based on the fraction of background sequences. Peaks common in different cell lines were identified by Homer mergePeak tool using 100 bp as offset maximum. IP efficiency factors calculated by Homer findPeaks as a ratio of reads associated with peaks versus background reads varies between 0.03% (MCF7) and 0.83% (SJSA) in samples treated with DMSO and 1.96% (HCT116) and 6.19% (MCF7) in samples treated with Nutlin. Importantly, virtually all TP53 peaks found in DMSO-treated samples showed increased read density following Nutlin treatment indicating very low presence of non-specific or "phantom" peaks (Jain et al. 2015). Normalized read densities were calculated by Homer (annotatePeaks.pl –size given, default normalization, getDifferentialPeaks). Identification of sequence motifs associated with TP53 binding was performed by findMotifsGenome.pl. The canonical TP53 motif was found in more than 80% of peaks in all three cell lines (see Supplemental Table S2). Distance of peaks from TSS of adjacent genes was measured by bedtools2 (http://bedtools.readthedocs.io/en/latest/#, version 2.22.0). The same data processing pipeline was

used for analysis of ChIP-seq raw data files obtained from the Encyclopedia of DNA Elements (ENCODE) (Consortium 2012) listed in Supplemental Table S3. For peak calling of the H3K4me3 and H3K36me3 ChIP-seq, as well as the DNaseI-seq we used the setting "–histone style" better reflecting the nature of the signal distribution.

To compare TP53 read densities between two different cell lines at individual loci we used MAnorm (Shao et al. 2012) with default settings. For visualization of ChIP-seq data, we generated bigwig tracks for genome browsers using deepTools (Ramirez et al. 2016) (version 2.2.2, settings --binSize=1 –extendReads FRAGMENT_LENGTH --minMappingQuality 10 –normalizeUsingRPKM). Read density was displayed at 1 bp resolution as reads per million of mapped reads per 1 kb (RPM/kb).

GRO-seq library preparation and Illumina sequencing.

Both nuclear run-on and sequencing library preparation were carried out using modified protocol described by Wang et al. (Wang et al. 2011). After the treating cell cultures with either DMSO (0.2%) or Nutlin (10 μM) for 1 hour, plates were washed three timed with ice-cold PBS and placed on ice. Cells were scraped into 10 ml of lysis buffer (10 mM Tris pH 7.4, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.5% IGEPAL, 10% glycerol, 1 mM DTT, protease inhibitor cocktail (cOmplete Mini, Roche), 4U/ml SUPERase-In RNase inhibitor (Thermo Fisher Scientific)) and centrifugated at 1000g for 10 minutes at 4°C. Pelleted nuclei were washed in 10 ml of lysis buffer, transferred to Eppendorf tubes and resuspended in freezing buffer (50 mM Tris pH 8.3, 5 mM $MgCl_2$, 40% glycerol, 0.1 mM EDTA and 4U/ml SUPERase-In), dispensed in 100 μl aliquots ($5*10^6$ nuclei each), snap frozen and stored at -80°C. Nuclear run-on was performed with 8 aliquots per sample. The reaction was started by mixing each aliquot with 100 μl of the pre-warmed reaction buffer (10 mM Tris pH 8.0, 5 mM $MgCl_2$, 1 mM DTT, 300 mM KCl, rATP, rCTP, rGTP and Br-UTP (0.5 mM each), 20 U SUPERase-In, 1% sarcosyl). After 5 minutes of incubation at 30°C, the run-on reaction was terminated by adding 1 ml of TRI Reagent (Sigma-Aldrich, St. Louis, MO) to the reaction mix. RNA was extracted by using the total volume of 2 ml of TRI Reagent and 440 μl of chloroform per sample. The aqueous phase was subjected to one phenol/chloroform and one chloroform extraction and ethanol precipitation of the RNA. GlycoBlue coprecipitant (Ambion, Thermo Fisher Scientific) was used in every precipitation step throughout the protocol. RNA pellets were washed with 70% ethanol, resuspended in DEPC-treated water, combined and split in three 20 μl aliquots, which were hydrolyzed with 2.5 μl of 1 M NaOH for 22 minutes. Base hydrolysis was stopped by neutralizing the reaction mix with 40 μl of 1M Tris pH 6.8. Next, samples were purified using Micro Bio-Spin P-30 columns (RNase-free, BioRad) and treated with amplification grade RNase-free DNAaseI (Invitogen, Thermo Fisher Scientific) for 10 minutes at 37°C. After second P-30 purification, samples were treated with Antarctic Phosphatase (M0289, New England Biolabs) in the presence of 20 U of SUPERase-In and purified with P30 columns for the third time. Prior to immunoprecipitation, RNA samples were incubated at 65°C for 5 minutes and placed on ice.

Immunoprecipitation of BrU-containing RNA was performed using anti-BrdU agarose beads (60 μl per sample, sc-32323AC, Santa Cruz Biotechnology). Beads were equilibrated by two washes with binding buffer (0.25x SSPE, 1 mM EDTA, 0.05% Tween-20, 37.5 mM NaCl and 4 U/ml of SUPERase-In) and blocked for 1 hour at RT in the blocking buffer (binding buffer with 0.1% PVP and 1 μg/ml of Ambion ultrapure BSA). After two washes with binding buffer, beads were combined with RNA samples and the total volume was adjusted to 500 μl with binding buffer. Following 1 hour incubation at room temperature, beads were washed (5 minutes each washing step with the exception of high-salt buffer wash) once with binding buffer, once with low salt buffer (0.2x SSPE, 1 mM EDTA, 0.05% Tween-20 and 4 U/ml of SUPERase-In), once briefly with high-salt buffer (0.25x SSPE, 1 mM EDTA, 0.05% Tween-20, 137.5 mM NaCl and 4 U/ml of SUPERase-In), and twice with TET buffer (10 mM Tris pH 8, 1 mM EDTA, 0.05% Tween-20 and 4 U/ml of SUPERase-In). Finally, RNA was eluted with 125 μl of elution buffer (50 mM Tris pH 7.5, 20 mM DTT, 1 mM EDTA, 150 mM NaCl, 0.1% SDS and 4 U/ml of SUPERase-In) in four 10-minute incubations at 42°C, extracted once with phenol/chloroform, once with chloroform and precipitated with cold ethanol, 300 mM of NaCl (final concentration) and GlycoBlue. Pelleted RNA was washed with 70% ethanol, resuspended in 25 μl of DEPC-treated water, 5.2 μl of T4 PNK buffer, 1 μl of SUPERase-In and phosphorylated with T4 PNK (1 μl/sample, M0201, New England Biolabs) for 1 hour at 37°C. After phenol/chloroform and chloroform extractions, ethanol precipitation (300 mM NaCl, GlycoBlue) and 70% ethanol wash RNA pellets were reconstituted in 5 μl of DEPC-treated water, 0.8 μl of poly-A polymerase buffer, 0.8 μl 10 mM ATP, 0.5 μl SUPERase-In and 0.75 μl Poly-A Polymerase (M0276,

New England Biolabs) and incubated for 10 minutes at 37°C. The reaction was terminated by adding 12 µl of 500 µM EDTA, 12 µl of 5M NaCl and 268 µl of DEPC-treated water. Next, the samples were extracted and precipitated as described above. RNA pellets were resuspended in 50 µl of DEPC-treated water and subjected to second round of immunoprecipitation with anti-BrdU beads as before. Isolated RNA was resuspended in 8 µl of DEPC-treated water then used in reverse transcriptase reactions. First, RNA was incubated for 3 minutes at 75°C with 1 µl of dNTPs (10 mM) and 2.5 µl of NTI233HIseq primer (12.5 µM, pGATCGTCGGACTGTAGAACTCT/idSp/CCTTGGCACCCGAGAATTCCATTTTTTTTTTTTTTTTTTVN - p indicates 5' phosphorylation, idSp indicates dSpacer Furan and VN stands for degenerate nucleotides) and briefly chilled on ice. Second, each reaction mix received 0.5 µl of SUPERase, 3.75 µl of 0.1 M DTT, 2.5 µM $MgCl_2$, 5 µl reverse transcription buffer, 2 µl of SuperScript III reverse transcriptase (Invitrogen) and the reaction cocktail was incubated at 48°C for 30 minutes. Finally, remaining primer was eliminated by 1 hour incubation at 37°C with 4 µl of Exonuclease I and 3.2 µl of Exonuclease I buffer (EN0581, Fermentas, Thermo Fisher Scientific). RNA in samples was hydrolyzed by NaOH (50 mM final concentration) during 20 minutes incubation at 98°C. Next, the reaction mix was neutralized with 1.6 µl of 1M HCl. cDNA was extracted once with phenol/chloroform, once with chloroform and precipitated with cold ethanol, 300 mM of NaCl (final concentration) and GlycoBlue. The pellet was washed with 70% ethanol, reconstituted in 30 µl of water and subjected to 200-600bp size selection in 2% agarose gel (BluePippin). cDNA from collected fraction was precipitated as described above, resolved in 8 µl of water and circularized by ssDNA circular ligase in a reaction mix containing 1 µl of CircLigase buffer, 0.5 µl 1 mM ATP, 0.5 µl 50 mM $MnCl_2$ and 0.5 µl CircLigase (CL4111K, Epicentre, Illumina). After 1 hour incubation at 60°C, circular ligase was heat-inactivated for 20 minutes at 80°C and ssDNA was re-linearized using 1.5 µl of APEI with 1.3 µl of NEB Buffer 4 (M0282, New England Biolabs) for 1 hour incubation at 37°C. Linear ssDNA was extracted with phenol/chloroform, precipitated, dissolved and all three aliquots of each sample were combined in total volume of 65 µl of water. Next, each library was amplified in 8 parallel PCR reactions using 4 µl of Phusion HF buffer, 0.4 µl of HiSeq forward primer, 0.4 µl of HiSeq Indexed reverse primer (for primer sequences see Supplemental Table S4), 0.4 µl of $MgCl_2$, 0.4 µl of dNTPs, 0.6 µl of DMSO, 0.2 µl Phusion DNA polymerase (F530, Thermo Fisher Scientific), 4 µl of template cDNA and 9.6 µl of water. Amplification was performed in following steps: 30 seconds initial denaturation followed by 10 to 12 cycles of 98°C for 10 seconds, 62°C for 30 seconds and 72°C for 20 seconds, followed by final extension at 72°C for 10 minutes. PCR product was extracted with phenol/chloroform, precipitated, dissolved in 30 µl of water and size-selected for DNA fragments from 200 to 600 bp (BluePippin). Recovered DNA was extracted with phenol/chloroform, precipitated, dissolved in 10 µl of water and quantified with an Agilent Bioanalyzer 2100 instrument. Two biological replicates of barcoded GRO-seq libraries were combined and sequenced in two lanes on Illumina HiSeq2000.

GRO-seq data analysis and visualization.

The quality of the sequenced libraries was analyzed by FastQC. fastqc-mcf from ea-utils (v1.1.2) was used to detect and remove sequencing adapters, detect limited skewing at the ends of reads and clip, detect poor quality at the ends of reads and clip, detect Ns and remove from ends, remove reads with CASAVA "Y" flag (purity filtering) and discard sequences that were too short after all of the above (<30 bp). Given the high quality of the sequencing reactions, less than 5% of the reads were discarded. Reads were then mapped to the reference human genome (GRCh37/hg19) using Bowtie2 (bowtie2-2.2.3, --sensitive –-end-to-end) and reads with low mapping quality scores (MAPQ<10) were removed using samtools_0.1.19, which also removed multi-mapping reads. Reads were then coordinate sorted using PICARD (v1.129). After read processing and mapping, regions of active transcription were identified with FStitch (https://github.com/azofeifa/FStitch) (Azofeifa et al. 2014). Selection of active transcripts was determined by active transcription at the transcription start site (TSS) and in at least of 70% of the gene body. Custom scripts were written in python for this purpose, utilizing the pybedtools_0.7.1 library. To avoid discarding transcripts with inaccurately annotated TSS, we accepted transcripts with any active transcription within 250 bp from the annotated TSS. Gene body was defined as the region from 1 kb downstream of the TSS down to the annotated transcription termination site (TTS). Transcripts shorter than 2000 bp were excluded from further analysis. In case of genes with multiple active transcripts we chose the transcript with the highest RPKM value. R_3.1.0 and DESeq2 (v1.6.3) were used to identify genes with significantly different transcriptional activity between DMSO- and Nutlin-treated cells (padj < 0.05, 0.5 cpm cut-off). lncRNAs were annotated using Gencode hg19 (GRCH37.p13,) file. lncRNA

transcripts with at least 70% of the gene body (from TSS to TTS) identified by FStitch as actively transcribed were selected for further analysis. For visualization of GRO-seq data, we generate bigwig files using deepTools with the same setting like for ChIP-seq tracks (see above). For preparation of metagene plots depicting the average presence of transcriptionally engaged RNA polymerases we employed deepTools bamCoverage (--binSize=1 --minMappingQuality 10 –normalizeUsingRPKM --filterRNAstrand forward/reverse) and computeMatrix (--beforeRegionStartLength=3000 --afterRegionStartLength=10000 --binSize=200 --averageTypeBins=mean --regionBodyLength=46000) modules to count base pair resolution coverage and score regions respectively. Finally, we used python (2.7.9) and its library matplotlib (1.4.3) to parse and plot the gene activation tracks. To mitigate the problem of outliers in the heatmaps washing out the signal intensity, we used a median-absolute-deviation (MAD) based approach to replace them with the maximum non-outlier value. Each value calculated by deepTools was converted to a modified z-score (based on the median absolute deviation). The detection was done column-wise for every point on the heatmap, and a modified z-score greater than 5 was classified as an outlier.

Pausing index calculation.

To calculate pausing indexes, we use similar an approach similar to that developed by Core et al, 2008 (Core et al. 2008). We limited analysis of pausing indexes to transcriptionally active genes and the transcripts with the highest transcriptional activity as defined by FStitch (see GRO-seq Data Analysis section above). First, we identified promoter proximal peak (PPP) by using a 500 bp sliding window shifting by 5 bp steps between 1 kb upstream and 1 kb downstream from the annotated TSS by counting the sense bases. In each window we counted sense strand reads and normalized the result by base mappability factor (Core et al. 2008). Second, we counted the number of sense reads within the gene body and normalized it by body length and base mappability. Third, we calculated pausing index as a ratio of length-normalized PPP and length-normalized reads in the gene body.

RNA-seq library preparation and sequencing.

HCT116 TP53+/+, HCT116 TP53 -/-, MCF7 and SJSA cells were plated and treated as described above. Polysomal fractions were prepared as described previously (Zaccara et al. 2014). Briefly, samples were loaded in 15–50% linear sucrose gradients, ultra-centrifugated and fractionated with an automated fraction collector. All the fractions containing polysomal RNA were identified and pooled together. RNA was purified by extraction with 1 volume of phenol–chloroform and adding a washing step in 70% v/v ethanol in order to remove phenol contaminations. Total RNA was extracted using TRI Reagent, according to the manufacturer's instructions. Total RNA quality was assessed using Bioanalyzer RNA Pico chips (Agilent). PolyA+ RNA was purified from 15 µg of total RNA using the Dynabeads mRNA Direct micro kit (Life Technologies) according to the manufacturer's instructions, and ribosomal RNA contamination assessed using Bioanalyzer RNA Pico chips. Libraries for Ion Torrent sequencing were prepared using the Ion Total RNAseq v2 kit (Life Technologies), according to the manufacturer's instructions. Yield and insert-size distribution were measured using Bioanalyzer High Sensitivity DNA chips (Agilent). The final Ion Torrent RNAseq libraries were subjected to an additional size-selection for 120-200 bp on a Blue Pippin (Sage Science) using a 2% dye-free gel with marker V1. Accounting for ~90 bp of Ion adapter sequences, this ensured a library insert size range of ~30-110 bp. Ion Torrent template preparation was carried out using the Ion PI Template OT2 200 Kit v2 and library sequencing was carried out on an Ion Torrent Proton sequencer using the Ion PI Sequencing 200 Kit v2 (Life Technologies), according to the manufacturer's instructions.

RNA-seq data analysis and visualization.

Signal processing, base-calling and removal of barcode and adapter sequences was carried out automatically by the Torrent Suite Software (Life Technologies). Data quality was assessed using Fastqc v0.11.2. Fastx toolkit (version 0.0.13.2) (Gordon 2012) was used to trim reads to 150 bp and to remove reads shorter than 30 bp. Alignment to the Human genome (hg19/GRCh37) was carried out using GSNAP (gsnap_2015-06-12) (Wu and Nacu 2010) with a mismatch setting of 3%. Reads were then filtered to remove low quality mapped reads (MAPQ < 10) and non-unique reads were removed by SAMtools (0.1.19). In total, the number of mappable reads ranged from $3.9*10^7$ to $8.0*10^7$ per sample. Next, we used Picard Tools (1.129) for sorting and duplicate

marking. Finally, we generated gene counts with HTSeq_0.6.1 using following options: --stranded=reverse –minaqual=10 –type=exon –idattr=gene --mode= intersection-nonempty. To identify significant differences in mRNA expression we used R_3.1.0 and DESeq2 (v1.6.3). Only genes with cpm value > 0.5 were considered to be detected at levels sufficient for meaningful analysis. Differentially expressed genes were selected based on padj value < 0.05. For data visualization, Bigwig files were prepared using deepTools v2.2.2 (--binSize=1 --minMappingQuality 10 –normalizeUsingRPKM). Read density was displayed with 1 bp resolution as reads per million of mapped reads per 1 kb (RPM/kb).

shRNA screens.

Our genome-wide library of shRNAs was acquired from the Functional Genomics Facility at the University of Colorado (http://functionalgenomicsfacility.org) and consisted of the TRC1, 1.5 and 2 libraries from the RNAi Consortium (Sigma-Aldrich). The genome-wide collection covers more than 20,000 human genes with >165,000 shRNAs in the library. The smaller TP53TARGET library contains ~3100 shRNAs targeting 342 genes characterized as direct TP53 target genes either by the data in this report or based on previously published works (Nikulenkov et al. 2012; Menendez et al. 2013; Allen et al. 2014) more refs. A complete list of the genes targeted by this library is found in Supplemental File S7. 12.5 µg of DNA for each library were co-transfected along with lentiviral packaging plasmids into HEK293FT cells in a 15 cm dish previously plated with $1x10^7$ cells. After three days of virus production, culture media was collected, filtered, and added to SJSA target cells at a multiplicity of infection (MOI) of 0.5. SJSA cells were selected with 2 µg/ml puromycin (Sigma Aldrich-P8833) starting 48 hours post transduction. Cells were grown for at least 1 week in selection media for clearance of essential genes. After one week, cells were plated at equal densities ($5.3*10^4$ cells/cm^2) in separate plates (in triplicate for each treatment), grown for 24 hours and treated with either 0.2% DMSO or 10 µM Nutlin. After 48 hours of treatment, cells were washed three times with PBS, and put back in regular culture conditions to allow their recovery. Upon reaching 80% confluency, cells were harvested and re-plated for second round of treatment with either DMSO or Nutlin. After 48 hours, cells were washed with PBS and allowed to recover and grow back to ~80% confluency in drug-free media. After recovery, $5x10^6$ cells were harvested and gDNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The shRNA cassettes were isolated from genomic DNA with a first PCR reaction using the following primers: pLKO ForHTm primer (GGGCCTATTTCCCATGATTCCTTC), and Rev-pLKO.1 (CCAAAGTGGATCTCTGCTGTCCC). After verification of a single PCR product at 497 bp size, PCR reactions were pooled and cleaned up with QIAquick PCR Purification Kit (Qiagen). Next, 1µg of the resulting DNA was digested with XhoI overnight at 37°C (for ligation of barcode linkers to allow multiplex sequencing), gel excised and the appropriate band was isolated using QIAquick Gel Extraction Kit (Qiagen). Selected barcode linkers were added to ligation reactions with 100 ng of the purified XhoI fragment. Ligations were performed overnight at 16°C and run on 2% TAE agarose gel. The resulting ligation product was excised from the gel and purified as before. A second PCR was performed for addition of Illumina adapters with PCR2 primers (PCR2 Fwd: CAAGCAGAAGACGGCATACGATGGAAAGGACGAAACACCGG, PCR2 rev: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT). These PCR reactions were run again on a 2% TAE agarose gel, and the appropriate final product was excised and purified as above. Before sequencing, we assessed the purity and quantity of our sequencing libraries with the Bioanalyzer High Sensitivity DNA Kit (Agilent), and confirmed the presence of only one DNA fragment. The resulting samples were submitted to the University of Colorado Cancer Center Genomics and Microarray Shared Resource and sequenced using an Illumina HiSeq 2000 instrument.

shRNA screen data analysis.

Number of sequencing reads ranged from $7.5*10^6$ to $2.3*10^7$ for the TP53TARGET library and from $1.4*10^7$ to $2.7*10^7$ for the genome-wide library. Reads were trimmed to the 21 bp sequences containing the shRNA targeting sequences using fastx_trimmer (Fastx Toolkit, fastx_0.0.13.2) (Gordon 2012) and filtered by fastq_quality_filter (options: -q 10 -p 94). Next, reads were mapped with bowtie (bowtie2-2.2.3, options: --end-to-end --very-sensitive) to the Broad Institute public TRC library (http://portals.broadinstitute.org/gpp/public/dir?dirpath=shrna_annot/legacy). Mapped reads were then filtered using samtools (0.1.19) keeping reads with MAPQ > 10. On average, 3.2% of reads were lost during these

steps. Next, all mapped reads were converted to counts per million (CPM) by a custom script and 1 count was added to all values to allow ratio calculations in cases where CPMs were zero. Median values were calculated from biological replicates for every shRNA. Then, median values were calculated for all shRNAs targeting the same gene, and used to determine fold change between DMSO- and Nutlin-treated samples (see Supplemental File S7 for screen results).

TP53 mutation analysis.

To analyze *TP53* mutations in SJSA cells exposed to Nutlin, we used the Illumina TruSight Tumor 26 kit. According to the manufacturer, the detection limit of the kit is below 5% variant allele frequency. Samples were prepared in the same way as in the shRNA screen experiment. Briefly, SJSA cells plated at $5.3*10^4$ cells/cm^2 were cultivated for 24 hours and exposed to either Nutlin or DMSO for another 48 hours. Next, the media with drug (or vehicle) was washed away with PBS and replaced with fresh cultivation media to allow recovery of Nutlin-treated cells. After cell populations reached ~80% density, the plating, treatment and recovery process was repeated three times. After each round, a genomic DNA sample was collected. Library preparation was performed using the Illumina TruSight Tumor 26 kit according to the manufacturer's instructions. This kit amplifies selected regions of 26 cancer-related genes including *TP53*. Libraries were sequenced on the Illumina MiSeq platform for a targeted depth of no less than 500x coverage for any individual amplicon. A custom-built bioinformatics pipeline utilizing GSNAP for sequence alignment and FreeBayes (https://github.com/ekg/freebayes) (Garrison and Marth 2012) for variant calling was employed for data analysis. All genomic regions were verified to be covered by at least 500 sequencing reads and identified variants were manually inspected using Integrative Genomics Viewer (Broad Institute).

Mutation and Copy Number Variation analysis in tumor samples.

All sample data were obtained from the Synapse website (http://www.synapse.org), accession no. syn1729383. Sample collection, genome sequencing, sequence data processing and mutation calling were described before (Lawrence et al. 2014). For mutation analysis we used MutSigCV 1.4 with default parameters (Lawrence et al. 2014).

CNV analysis was performed by using GISTIC 2.0.22 with default setting (Mermel et al. 2011). Segmentation data were downloaded from The Cancer Genome Atlas (2016_01_28) using firehose_get v 0.4.5 (https://confluence.broadinstitute.org/display/GDAC/Download). Only data from samples published by Lawrence et al. (Lawrence et al. 2014) were used for CNV analysis.

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