Supplemental Methods on Data Analysis

Project Achilles RNAi and CRISPR-Cas9 Screens

We analyzed a previously published genome-scale RNAi screen in 501 cancer cell lines, which included 10 MRT lines (1). In brief, each cell line was screened with one of two genome-scale shRNA libraries of ~55,000 or ~100,000 hairpins. The libraries were transduced into each cell line at a low multiplicity of infection, and cells passaged for 16 doublings or 40 days, whichever came first. Depletion or enrichment of each shRNA was assessed through high-throughput sequencing of the final cell populations in comparison with the starting shRNA plasmid library. Hairpin-level data was collapsed to gene-level dependency scores using the DEMETER algorithm, which computationally corrects for seed effects and outlier shRNAs. Z-scores were then calculated over the population of all cell lines for their comparative depletion or enrichment for each gene.

We also analyzed an updated version of the Broad Institute's GeCKOv2 library screen of 43 cancer cell lines, which included 8 MRT lines (2, 3). This library contained approximately 123,411 sgRNAs with an average of 6 sgRNAs per gene and 1,000 negative control sgRNAs. Briefly, cell lines were transduced with Cas9 using a lentiviral system and assayed for sufficient Cas9 activity (2). The pooled GeCKOv2 CRISPR library was then transduced into a population of cells and cultured for approximately 21 days. Barcodes for each sgRNA were then amplified and sequenced to determine relative amounts in the starting and end populations. Sequencing was performed in duplicate. Poor replicate reproducibility or sgRNAs not well represented in the initial pool were removed from the analysis. The CERES algorithm (4) was then used to collapse the sgRNA level data into gene scores. Scores for each cell line were then scaled such that

the mean of negative control sgRNAs was 0 and the mean of a subset of positive control genes in required housekeeping pathways was -1.

For both the RNAi and CRISPR-Cas9 datasets, genetic dependencies that are enriched in MRT cell lines were identified using linear-model analysis from the limma R package (5) by performing a two-tailed *t*-test for the difference in distribution of gene dependency scores in MRT cell lines compared to all other cell lines screened. Statistical significance was calculated as a Q value derived from the P value corrected for multiple hypothesis testing using the Benjamini & Hochberg method (6). When comparing individual genes when divided by p53 status, one-way ANOVA with Holm-Sidak's multiple comparisons correction was performed using GraphPad Prism (La Jolla, CA).

p53 Status Annotations

We adapted the functional and genetic *TP53* status annotations from (7). Briefly, we defined the functional and genetic *TP53* status of cell lines using the Cancer Cell Line Encyclopedia (CCLE - https://portals.broadinstitute.org/ccle/) (8), Genomics of Drug Sensitivity in Cancer (GDSC - http://www.cancerrxgene.org) (9), and The Cancer Genome Atlas (TCGA accessed via cBioPortal - http://www.cbioportal.org) (10) databases. To determine the functional p53 status of each cell line, we considered p53 target gene expression (11) computed using data from the CCLE. Cell lines were included in the "p53 functional" class if their functional score calculated as [Target Genes CCLE Z-score] was greater than 0, and included in the "p53 non-functional" class if the functional score was less than 0. "p53 functional" cell lines were ultimately determined to be "p53 wild-type (WT)" if no genetic *TP53* alterations were found by CCLE, GDSC, or TCGA (n = 252), and discarded as discordant if any *TP53* alterations

were found (n = 82). "p53 non-functional" cell lines were determined to be "p53 mutant" if they harbored any genetic *TP53* alteration (n = 516) and discarded as discordant if no *TP53* alterations were found (n = 116). All scores and classifications are detailed in Supplemental Table S2.

RNA-sequencing

Deconvoluted data sets were aligned to human genome hg19 using STAR (12) with ENCODE standard options (--outFilterType BySJout, --outFilterMultimapNmax 20, --alignSJoverhangMin 8, --alignSJDBoverhangMin 1, --outFilterMismatchNmax 999, --outFilterMismatchNoverLmax 0.04, --alignIntronMin 20, --alignIntronMax 1000000, --alignMatesGapMax 1000000). RSEM (13) with the options of --bam --estimate-rspd --calc-ci --no-bam-output --seed 12345 --paired-end was used to quantify gene expression. A hierarchical clustering method was performed on the read counts of all genes across all samples, which discovered the idasanutlin, replicate 3 sample as an outlier. Differential expression analysis between the remaining samples was performed using DESeq2 (14).

Primary Tumor Expression Analysis

TARGET MRT and paired normal kidney samples (dbGaP phs000218.v19.p7) were aligned or re-aligned with STAR and transcript quantification performed with RSEM to generate TPM expression per gene. Expression values were log2 transformed and floored at -3. For the PANCAN dataset, samples annotated as normal, cell line, or xenograft were excluded.

The 13-gene expression signature for predicting sensitivity to MDM2 inhibition was adapted from Jeay et al (11). The genes included in the analysis were *AEN, BAX, CCNG1, CDKN1A, DDB2, FDXR, MDM2, RPS27L, RRM2B, SESN1, TNFRSF10B,*

XPC, and *ZMAT3.* For each gene, z-scores were calculated based on the TPM expression values for all samples used in our analysis (MRT, normal kidney, and other pediatric cancers). Then, the sum of the 13 z-scores was calculated to achieve an "MDM2-sensitivity combined z-score" for each sample. Higher values predict a greater sensitivity to MDM2 inhibition. Finally, we compared MRT samples with their normal kidney pairs using a two-sided paired *t*-test, all MRT samples to all normal kidney samples using a two-sided unpaired *t*-test, and all MRT samples to all other pediatric cancer samples using a two-sided unpaired *t*-test, all in GraphPad Prism.

References

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