Supplementary Methods

Exome Sequencing. Exome library construction was completed using the Roche NimbleGen V3 (44.1 Mbp) Exome Enrichment Kit from 1µg of total cell line DNA. Paired-end sequencing (2 × 150 bp) for NimbleGen V3 libraries on an Illumina HiSEQ 4000 at the University of Michigan DNA sequencing core according to standard core protocol.

Variant Calling. We first controlled for total read quality using FastQC analysis (1). We aligned reads to hg19 reference genome using BWA v0.7.8 (2), and then mapping was completed and duplicates marked using PicardTools v1.79 (Broad Institute). We then performed INDEL realignment and base quality score recalibration using GATK v3.2-2 (3). Variant calling was then performed using the HaplotypeCaller and Genotype GVCFs following the GATK best practices workflow guideline (4) in order to jointly filter variants across all three samples. Varseq v1.4.0 (Golden Helix, Inc., Bozeman, MT) was used to annotate and filter the variants. Filters were set to remove false positive variant calls due to any sequencing artifacts. The variants were also required to have 5 or more reads supporting the alternate allele to be considered positive and be found in <1% in a normal population according to the 1000 genomes project (5). Intronic and intergenic variants were also filtered out from analysis with the exception of the variants in splice donor or accepter regions, which were still analyzed.

References (for Supplementary Methods)

1. Andrews S. FastQC A Quality Control tool for High Throughput Sequence Data. http://wwwbioinformaticsbabrahamacuk/projects/fastqc/. doi: citeulike-article-id:11583827.

2. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754-60.

3. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce

framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297-303.

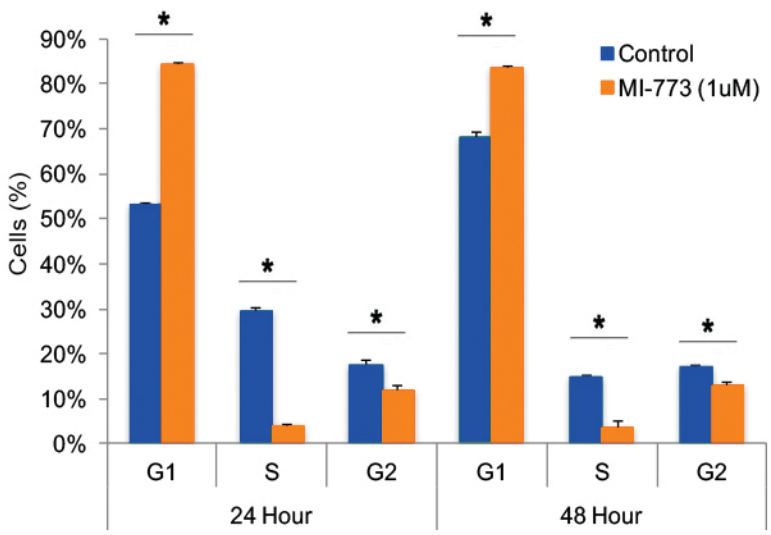
4. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011;43:491-8.

5. The Genomes Project C. A global reference for human genetic variation. Nature 2015;526(7571):68-74.

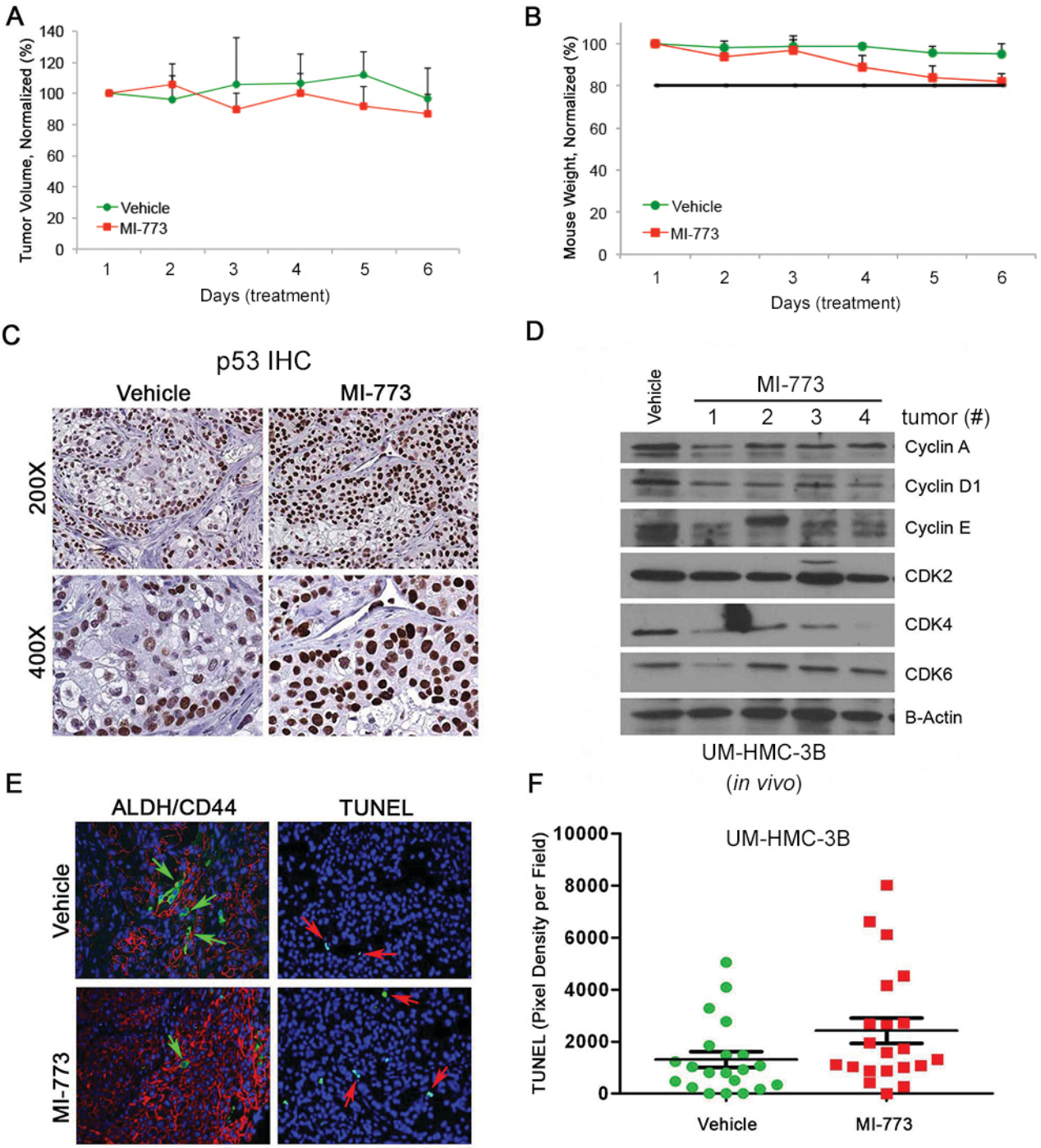
Supplementary Figure Legends

Supplementary Figure 1. Cell cycle analysis of UM-HMC-1 cells treated with MI-773 for 24 or 48 hours. Control cells were treated with DMSO. Asterisks indicate $p<0.05$. Bars indicate the standard deviation.

Supplementary Figure 2. Effect of treatment of UM-HMC-3B xenograft tumors with MI-773. A, Graph depicting tumor volume upon short-term treatment with MI-773. Briefly, 600,000 UM-HMC-3B cells were mixed with 400,000 human endothelial cells (HDMEC), seeded on biodegradable scaffolds, and transplanted into the subcutaneous space of SCID mice (N=10 tumors per experimental condition). Once tumors reached an average volume of 500 mm^3, mice were treated by daily oral gavage with 200 mg/kg MI-773 or a vehicle control for 5 days. B, Graph depicting the average mouse weight during treatment. C, Immunohistochemistry staining for p53 (brown color) in sections from the vehicle and MI-773 treated tumors. D, Western blot analysis of vehicle or MI-773-treated tumor tissues for cell cycle-associated proteins. E, Immunofluorescence staining for ALDH/CD44 (cancer stem cell markers) or TUNEL (apoptosis) in vehicle or MI-773-treated tumors. F, Graph depicting the pixel density per field of sections stained for TUNEL in MI-773 or vehicle treated tumors. Seven fields at 200X were evaluated per tumor (n=3 tumors per experimental condition) and quantified using the ImageJ software. Bars indicated the standard error of the mean.



Supplementary Figure 1 - Andrews et al



Supplementary Figure 2 - Andrews et al