SUPPLEMENTARY DATA

Title: The SOS1 Inhibitor MRTX0902 Blocks KRAS Activation and Demonstrates Antitumor Activity in Cancers Dependent on KRAS Nucleotide Loading

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Supplementary Materials and Methods

A. Experimental Preparation of MRTX0902 and Avutometinib

MRTX0902 was prepared as a 50 mg/kg dose with a dosing volume of 10 mL/kg. The required amount of test article (freebase or fumarate) was weighed and transferred to a sterile conical tube, to which 80% of the vehicle (0.5% Methylcellulose (Cat. #M0512, Sigma Aldrich), 0.2% Tween 80 (Cat. #P8074, Sigma Aldrich) in Endotoxin-Free Water (Cat. #786-671, G-Biosciences) was added. The resulting suspension was vortexed for 2 minutes and placed into a water bath sonicator for 5 minutes. The remaining vehicle was added and pH was adjusted to 6-7 with appropriate concentration of hydrochloric acid or sodium hydroxide. Formulations were prepared weekly, dosing solutions were stored protected from light at 4 °C, and suspensions were vortexed prior to dosing animals.

Avutometinib was prepared as a 0.3 mg/kg dose with a dosing volume of 5 mL/kg. A 10X stock solution of 3 mg/kg was prepared in vehicle (5%DMSO: 95%HPCD in water) for the final dosing solution. The required amount of test article was weighed and transferred to a glass sterile vial, to which 5% DMSO (Cat. #472302, Sigma Aldrich) was added. The resulting solution was mixed and the remaining vehicle (10% 2-Hydoxypropyl-beta-Cyclodextrin (HPCD) (Cat. #C0926, Sigma Aldrich) in Endotoxin Free Water (Cat. #786-671, G-Biosciences) was added. Formulations were prepared weekly, dosing solutions were stored protected from light at 4 °C, and solutions were vortexed prior to dosing animals.

B. SOS1 Biochemical Binding Assay

A white 384-well Plate (Greiner Cat. #784075) was dosed by an acoustic dispenser ECHO 650. The assay buffer (25 mM HEPES pH 7.5, 1 mM DTT, 25 mM NaCl, 0.01% Brij35, 0.5 nM of Anti-His-Tb (Cisbio), 100 nM Cy5 labeled Tracer compound prepared in-house) was prepared with 2 nM of Biotin-AviTag-SOS1 protein. Ten microliters of the protein-buffer solution were dispensed using an automatic liquid dispenser (Multidrop Combi Cat. #5840400) into each well of the 384-well plate. The plate was incubated at room temperature (RT) for 1 hour. The homogenous time resolved fluorescence (HTRF) signal was measured using a CLARIOstar® reader (BMG LABTECH Inc.) excitation filter (Ex Tr), dichroic filter (LP TP) and emission filters (F 665-10 and F 620-10) per manufacturer's instructions. The HTRF ratio was calculated using the formula: [emission 665/emission 620] * 10000. K_i's were fit using XLfit software (IDBS) using a Tight-Binding Morrison Competitive equation with the K_d fixed to 6.075 nM, S fixed to 100 nM, and Et fixed to 2 nM:

$$\frac{v_i}{v_0} = 1 - \frac{\left([E]_T + [I]_T + K_i^{app}\right) - \sqrt{\left([E]_T + [I]_T + K_i^{app}\right)^2 - 4[E]_T[I]_T}}{2[E]_T}$$

, where $K_i^{app} = K_i(1+S/K_d)$.

C. SOS1 and SOS2 Functional Assays

A white 384-well Plate (Greiner Cat. #784075) was dosed by an acoustic dispenser ECHO 650. The assay buffer (50 mM HEPES pH 7.5, 5 mM MgCl2, 0.2 mg/mL BSA, 1 mM TCEP) was prepared and used to create the 2x SOS1 binding assay conditions (200 nM Cy5 labeled GTP (Jena Biosciences Cat# NU-820-CY5), assay buffer, and 4 nM 6His-TEV-AviTag SOS1). Five microliters of the SOS1 binding assay reaction mixture were dispensed using an automatic liquid dispenser (Multidrop Combi Cat. #5840400) into each well of the 384-well plate. The plate was incubated at RT for fifteen minutes. Following the incubation, five microliters of a 2X KRAS reaction mixture (60 nM WT-Avi-Biotin KRAS, 1 nM Tb-SA, and assay buffer) was added to each well of the 384-well plate using the same automatic liquid dispenser. The plate was incubated at RT for thirty minutes. The HTRF signal was measured using a CLARIOstar® reader (BMG LABTECH Inc.) excitation filter (Ex Tr), dichroic filter (LP TP) and emission filters (F 665-10 and F 620-10) per manufacturer's instructions. The HTRF ratio was calculated using the formula: [emission 665/emission 620] *10000. IC_{50}s were calculated using a 4-parameter equation:((Top-Bottom)/(1+((x/IC_{50})^Hill)))+Bottom. IC_{50}s were fit using XLFit software (IDBS).

D. KRAS-SOS1 Protein-Protein Interaction (PPI) HTRF Assay

MRTX0902 was evaluated in the RAS:SOS1 Protein:Protein Interaction (PPI) assay as described by Reaction Biology (RBC). Briefly, 10 µL of 1.5x SOS1 protein (His-tagged SOS1 (aa 564-1049), prepared internally by RBC) was delivered to assay wells, and MRTX0902 was added via an automatic liquid dispenser (Echo®, Labcyte) followed by a 15-minute incubation at room temperature. MRTX0902 was tested in duplicate starting at 10 µM (10-point, 3-fold dilution curve). Next, a 3-fold solution of WT or mutant KRAS protein (aa 2-169, prepared internally by RBC)/detection mix (Mab Anti-6His Tb cryptate Gold with Streptavidin-XL 665) was prepared in a reaction buffer consisting of 10 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM DTT, 10 mM EDTA, 0.05% BSA, 0.005% Triton X100. Afterwards, 5 µL of the KRAS-detection mix-buffer solution was added to all assay wells and the HTRF signal was measured following 45-60 minutes using a BMG LABTECH Inc. instrument with excitation filter/emission filters (337/665; 620) per manufacturer's instructions. IC_{50}s were determined using Sigmoidal dose response (variable slope) equation.

E. In-Cell Western Assay

To prepare assay plates for the pERK In-Cell Western (ICW) assay, cells were trypsinized, resuspended in fresh media, and viable cells were counted utilizing a Cellometer Mini (Nexcelom Bioscience, Lawrence, MA) and trypan blue exclusion. All cell lines were diluted in complete growth media and seeded at 30,000 cells/well in 96-well black walled, clear bottom assay plates (Corning Cat. #3904). PBS was added to the outer wells of each 96-well plate. Cells were incubated for 24 hours at 37 °C, 5% CO_2, 100% humidity to adhere prior to treatment. MRTX0902 was serially diluted (1:3) in DMSO. A 10X dosing plate was prepared from the DMSO serial dilutions using an

intermediate dilution (1:100) into complete growth media without serum. Cells were dosed with 10 µl of the 10X intermediate drug dilutions added to the 96-well cell plates. Six vehicle (DMSO) wells were included on each assay plate. After a 30-minute drug treatment, cells were fixed by adding freshly prepared 4% formaldehyde to each well and incubated for 20 minutes at room temperature. Formaldehyde was removed and cells were permeabilized with ice-cold methanol for 30 minutes at -20 °C. Following permeabilization, methanol was removed from each well and blocking buffer (Odyssey Blocking Buffer Cat. #927-50000; LI-COR, Lincoln, NE) with 0.05% Tween-20, was added to each well and incubated for 1 hour at room temperature. Blocking buffer was removed and the pERK (Cell signaling Technology #9101L; Rabbit, 1:500 in blocking buffer) and GapDH (Millipore #MAB374; Mouse; 1:5000 in blocking buffer) primary antibodies were added and incubated overnight at 4 °C on a rocking platform. Plates were then washed three times with PBS+0.1%Tween 20 (PBST) and incubated with IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody (LI-COR Biosciences Cat. #926-32211) and IRDye® 680RD Goat anti-Mouse IgG Secondary Antibody (LI-COR Biosciences Cat. #926-68070) (1:800 in blocking buffer) for 1.5 hours at room temperature. Plates were washed three times with PBS-T and then imaged using LI-COR Odyssey CLx Imaging system (LI-COR, Lincoln, NE) set to Plate Acquisition format for both the 700 and 800 wavelength channels to measure the signal intensities from each well for the IRDye® 680RD Goat anti-Mouse IgG Secondary Antibody and IRDye® 800CW Goat anti-Rabbit IgG secondary antibodies, respectively. The signal output for both channels was exported to excel for further quantitative analysis.
For the ICW assay, the fluorescence intensities for both pERK and GapDH proteins were quantified in each selected vehicle or drug-treated well. A normalized value for pERK (versus GapDH) was calculated, divided by the average fluorescence in vehicle-treated wells, and multiplied by 100 to yield the "percent (%) of vehicle control" value. "Percent (%) of vehicle control" values were plotted as log(inhibitor) vs. response – Variable slope (four parameters) for curve fitting and IC_{50} value determination in GraphPad Prism.

F. 3D Ultra-Low Attachment (ULA) Viability Assay

To prepare assay plates for viability assays, cells were trypsinized, resuspended in fresh media, and viable cells were counted utilizing a Cellometer Mini (Nexcelom Bioscience (Lawrence, MA)) and trypan blue exclusion. All cell lines were diluted in complete growth media and seeded at 5000 cells/well in round bottom ultra-low attachment (ULA) microplate (S BIO, Cat. #MS-9096 UZ). PBS was added to the outer wells of each 96-well plate. After seeding, cell plates were incubated for 24 hours at 37 °C, 5% CO_2, 100% humidity to adhere prior to treatment. The following day, MRTX0902 was serially diluted (1:3) in 100% DMSO. A 10X dosing plate was prepared from the DMSO serial dilutions using an intermediate dilution (1:100) into complete growth media without serum. Cells were dosed with 10 µl of the 10X intermediate drug dilutions added to the 96-well cell plates, incubated for 7-14 days, and replenished with fresh media containing 1X compound on days 4, 7, and 10. Six vehicle (0.2% DMSO) wells were included on each assay plate. After drug treatment, 45 µl of CellTiter-Glo® (CTG) reagent (3D Cell Viability Assay (Cat. #G79681, Madison, WI) was added to each well,

covered in aluminum foil to protect from light, incubated at room temperature for 30 minutes on a microtiter plate shaker and luminescence readings were collected using a CLARIOstar® microplate reader (BMG LABTECH, Inc., Germany).
For the 3D viability assay, percent inhibition values were calculated by dividing relative luminescence unit (RLU) values from each treated well by the average RLU values in the vehicle-treated wells and multiplying by 100. Percent of vehicle control values were plotted as log(inhibitor) vs. response – Variable slope (four parameters) for curve fitting and IC_{50} value determination in GraphPad Prism.

G. Immunoblotting and Densitometry Analysis

Tumor fragments were frozen in 2 mL screw top microtubes with ceramic beads. An equal volume of ice cold 1X Lysis/Binding/Wash Buffer from Active Motif's GTPase Ras ELISA kit (Active Motif, Carlsbad, CA) and tumors were homogenized using the MP FastPrep-24 homogenizer (MP Biomedicals, Santa Ana, CA) with high-speed shaking 3 – 5 times for 20 seconds while keeping the tumor lysate on ice between cycles. After homogenization, tubes were spun at 15,000 rpm for 10 minutes at 4 °C and supernatant was collected. Protein concentrations of each lysate sample were determined using a Pierce BCA protein assay kit (Cat. #PI23227; Thermo Fisher Scientific, Waltham, MA) per the manufacturer's instructions.

Approximately 30 µg of total protein was added to 1X Lysis/Binding/Wash Buffer (Cat. #52097; Active Motif, Carlsbad, CA), 10X reducing agent (Cat. #NP0009; Invitrogen, Carlsbad, CA), and 4X XT Sample Buffer (Cat. #1610791; Bio-Rad, Hercules, CA). Samples were boiled for 7-10 minutes. Processed samples were then loaded onto 12% Criterion™ XT Precast Gel (Cat. #345-0118; Bio-Rad, Hercules, CA) using MOPS 1X Running Buffer (Cat. #161-0788; Bio-Rad, Hercules, CA). Proteins were transferred from the gels to a nitrocellulose membrane using the iBlot 2 Dry Blotting System (Cat. #IB23001; Thermo Fisher Scientific, Waltham, MA) and run at 20 volts for 1 minute, 23 volts for 4 minutes and 25 volts for 7 minutes.

Membranes were blocked with LI-COR Odyssey TBS Blocking Buffer (Cat. #927-50000; LI-COR, Lincoln, NE) for 1 hour at room temperature on a rocking platform. Primary antibodies were diluted in LI-COR Blocking Buffer and incubated overnight at 4 °C on a rocking platform. Membranes were then washed three times for 10 minutes with Tris-buffered saline-Tween 20 (TBS-T) and incubated with LI-Cor IR Dye secondary antibodies for 1 hour at room temperature. Membranes were washed three times for 10 minutes with TBS-T. Images were acquired from probed nitrocellulose membranes using the LI-COR Odyssey CLx Imaging system (LI-COR, Lincoln, NE) set to the AutoScan channel for both the 700 and 800 wavelength channels to measure the signal intensity from the IRDye® 680RD goat anti-rabbit and IRDye® 800CW goat anti-mouse secondary antibodies, respectively. Images were imported into LiCor's Image Studio software version 4.0 and then .tif files were exported for annotation.

To quantify the pixel intensity for each selected protein band, the "Add Rectangle" tool in the image viewer was used to identify a consistently sized area of interest for each band of a given target protein as well as a representative background region of the immunoblot. The signal output column from the software subtracts background pixel intensity and was used to determine the target pixel intensity for each

protein band. This corrected signal intensity was determined for each target protein of interest and data were exported to Excel.

Drug treatment is defined as either single agent administration of MRTX0902, adagrasib, avutometinib, osimertinib or dual administration of MRTX0902 and partnering drug. Target protein sample loading normalization of each sample was determined by dividing the signal output of the target protein by the signal output of the loading control protein (β-actin or α-tubulin). Each target protein was also averaged within each vehicle or drug treatment group. The vehicle value was normalized to “1” by dividing all average values by the vehicle value and standard deviation was calculated from the normalized values. To measure the degree of pERK/pAKT/pS6/p4EBP1 phosphorylation, the normalized pERK/pAKT/pS6/p4EBP1 signal for each sample was determined by dividing the pERK/pAKT/pS6/p4EBP1 signal output by the signal output of the total ERK1/2/AKT/S6/α-tubulin protein. Percent inhibition of normalized pERK/pAKT/pS6/p4EBP1 in drug-treated tumors compared to vehicle-treated control tumors was calculated by dividing the average drug-treated tumor normalized pERK/pAKT/pS6/p4EBP1 by the average vehicle-treated normalized pERK/pAKT/pS6/p4EBP1 signal and multiplying by 100. GraphPad Prism 8 was used to graph the data and complete a two-tailed Student’s *t* test statistical analysis between the vehicle- and drug-treated cohorts.

Antibodies for Western blotting were purchased from Cell Signaling Technology (Danvers, MA): Phospho-p44/42 MAPK (Erk1/2) (Cat. #9101L; Rabbit, 1:500 in blocking buffer); p44/42 MAPK (Erk1/2) (Cat. #9102L; Rabbit, 1:500 in blocking buffer); S6 Ribosomal Protein (5G10) (Cat. #2217; Rabbit, 1:500 in blocking buffer); Phospho-S6 Ribosomal Protein (Ser235/236) (Cat. #2211; Rabbit, 1:500 in blocking buffer); Phospho-4E-BP1 (Cat. #2855; Rabbit, 1:500); DUSP4 (Cat. #5149; Rabbit, 1:500); Phospho-AKT (Ser473) (Cat. #4060, Rabbit, 1:500); Akt (Cat. #9272, Rabbit, 1:500); α-Tubulin (Cat. #3873, Mouse, 1:5000). DUSP6 (Cat. #ab76310, Rabbit, 1:500) and ß-actin (Cat. #ab8227, Rabit, 1:5000) antibodies were purchased from Abcam (Waltham, MA). KRAS antibody was from Sigma Aldrich (Cat. #SAB1404011, Mouse, 1:250).

H. DUSP6 Quantification from Naïve and Tumor-Bearing Mouse Blood

Naïve nu/nu or MIA PaCa-2 tumor-bearing mice were randomized into five groups and dosed with vehicle, MRTX0902 (BID), adagrasib (QD), adagrasib + MRTX0902, and avutometinib (BID Q2D; positive control) for 6 days. Blood was collected from animals at 1 hour, 4 hours, and 6 hours post last dose and RNA was purified using the Mouse RiboPure™-Blood RNA Isolation Kit (Cat. #AM1951; Thermo Fisher Scientific, Waltham, MA) in accordance with manufacturer’s instructions. cDNA synthesis was performed using the 5X iScript™ Reverse Transcription Supermix kit (Cat. #170-8841; Bio-Rad, Hercules, CA) in accordance with manufacturer’s instructions. Transcripts were quantified using the TaqMan™ Real-Time PCR assay kit with the following primers: CLTC (Mm01303974_m1), GAPDH (Mm99999915_g1), SMARCA4 (Mm01151944_m1), DUSP6 (Mm00518185_m1). Samples were tested in triplicate in optically clear 384-well plates (Applied Biosystems, Cat. #4309849) using the QuantStudio 7 Flex System (Applied Biosystems). The Comparative $\Delta\Delta C_T$ analysis was completed using the Real-Time PCR Software (Applied Biosystems) to compare

the relative expression of DUSP6 gene transcripts before and after MRTX0902 dosing. GraphPad Prism 8 was used to graph the data and complete a two-tailed Student's *t* test statistical analysis between the vehicle- and drug-treated cohorts.

I. Bioanalysis and Pharmacokinetic Analysis

Plasma was collected from tumor-bearing immunocompromised mice on the last day of the study post last dose. The MRTX0902 plasma concentrations were determined by liquid chromatograph-mass spectrometry/mass spectrometry (LC-MS/MS) after protein precipitation. MRTX0902 concentration in plasma samples were quantitated against an eight-point plasma calibration curve ranging in from 1–5000 ng/mL with quality controls (QCs) prepared at 2, 8, 80, 400, and 2000 ng/mL. Plasma samples with MRTX0902 concentrations above 5000 ng/mL were diluted either 10- or 100-fold as appropriate and corresponding dilution QCs were included. The measured concentrations of MRTX0902 in plasma were subjected to non-compartmental pharmacokinetic (PK) analysis in Phoenix 64 software (version 8.3.1.5014, Certara, Princeton, NJ). The calculation of all pharmacokinetic parameters used nominal dose levels and nominal sampling times.

J. Synergy Analysis

A custom R-script was created, integrating open-source Bioconductor packages, to batch process metadata files containing experimental parameters and raw data files. Various numerical and graphical outputs were generated to summarize the data. Single agent parameters were generated using GRmetrics (1) while the synergyfinder package was used to determine whether the two test compounds demonstrate synergy using four independent mathematical reference models (Loewe additivity, Bliss independence, Highest Single Agent and ZIP) (2). The output of the data from each mathematical model is the assignment of a relative synergy score. The data reported in Figures S6 and S7 are the aggregate sum of the Loewe additivity, Bliss independence, Highest Single Agent and ZIP scores ("Composite Synergy Score").

K. CRISPR/Cas9 Screening and Data Analysis Methodology

A custom sgRNA lentiviral library was generated at Cellecta (Mountain View, CA) comprised of 8-10 sgRNAs targeting ~400 genes including ~10 positive and negative controls. Positive controls consisted of sgRNAs targeting essential genes whereas negative controls consisted of sgRNAs targeting introns of various genes. LU99 (*KRAS* G12C-mutant) and MIA PaCa-2 (*KRAS* G12C-mutant) cells were first transduced with a separate Cas9 lentiviral construct and selected with blasticidin. After determining the functional titer of the concentrated lentiviral sgRNA library prep, Cas9-expressing cells were then transduced with the sgRNA lentiviral library and selected with puromycin for 3 days. Cells were transduced such that at least 500 cells per sgRNA construct on average were transduced with each sgRNA construct (4,000), for each replicate (2E10^6 transduced cells/replicate). The multiplicity of infection (MOI) was ~0.25 to ensure the majority of transduced cells were transduced with only one lentivirus (~8E10^6 cells

plated for transduction/replicate). After puromycin selection, ~2E10^6 cells were harvested for the baseline sample, and at least 1-2E10^6 cells were plated in triplicate and treated with DMSO or an IC_{75}-IC_{90} concentration of adagrasib with MRTX0902 for 2 weeks and harvested. Throughout the 2 weeks, at least 2E10^6 cells were kept in the experiment at all times to help ensure the library complexity was maintained and not artifactually affected by low abundance, for example, after splitting back. Cells were also grown up for less than a week and 10E10^6 cells/mouse were implanted (with Matrigel) into 15 immunocompromised mice. Tumors were grown until they reached a volume of ~250-300 mm^3 and 5 xenografts were harvested for *in vivo* Day 0. Five tumor-bearing animals were then dosed with either vehicle or 100 mg/kg adagrasib with 50 mg/kg BID MRTX0902 for 2 weeks and whole tumors were harvested. *In vitro* and *in vivo* samples were sent to Cellecta for DNA isolation, sgRNA sequence amplification by PCR and next generation sequencing. Replicates exhibited good agreement as did the different sgRNAs targeting the same gene and controls behaved as expected.

sgRNA counts were normalized by sample. Log2 fold change in sgRNA abundance relative to baseline or Day 0 *in vivo* were calculated and average log 2-fold change for all sgRNAs targeting each gene were calculated and plotted. sgRNA raw counts were log2 transformed and normalized by the sum of log-transformed target gDNAs per sample. All samples clustered by treatment and cell line in the Principal Component Analysis (PCA) and no batch effects were identified. Differential dropout analysis was performed using Limma (v 3.40.6) in R (v.3.6.1). Average log2 fold change of sgRNAs targeting each gene were calculated and plotted using customized python (v3.7) and R scripts (v4.0.2).

L. RNAseq Pre-Processing

Mouse read contamination were removed for each fastq read pair using Xenome (3) with the “pairs” and “preserve-read-order” options by comparing against the human GRCh38 and mouse mm10 genomes. Reads with unique alignments to the mouse genome were removed and the remaining reads were merged together and used for further analysis. The reads were trimmed, and low-quality reads were removed using Trimgalore (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with the “paired” parameter and length of 151 bps. Trimmed fastq sequences where aligned to the human reference genome (GRCh38) (4) using STAR aligner v020201 (5) with the produced bam files sorted by coordinate by using option “--outSAMtype BAM SortedByCoordinate”. Raw read gene counts were obtained by using STAR aligner with options “--quantMode GeneCounts” and “--sjdbGTFfile” with gene models in GTF format obtained from EnsEMBL release 83. Alignment QC and read mapping statistic were obtained from Picard tools using function “CollectMultipleMetrics” (http://broadinstitute.github.io/picard/).

M. RNAseq Data Analysis

Raw gene counts were used for quality control and differential expression analysis. The “prcomp” function in the stats R library was applied for the Principal Component Analysis (PCA). All samples cluster according to treatment in the Principal Component Analysis (PCA) and 300 – 500 mg vehicle tumors were selected as controls because they are virtually indistinguishable. No batch effects were found relative to RNA-extraction or RNA-seq run (Association to first 10 principal components; Kruskal-Wallis p-value >0.05). Differential expression analysis was performed with limma-voom (version 3.36.2; (6)) in R (version 3.5.1). Very lowly expressed genes were eliminated for differential gene expression analysis using “filterByExpr” in edgeR version 3.22.3 (7); genes that passed this filter had a minimum of 10 reads in at least one sample and a minimum of 20 reads across all samples. Gene Set Enrichment Analysis tool (GSEA; gsea2-2.2.4) and Molecular Signature Database (MSigDB; msigdb_v6.0) were used for gene enrichment analysis (8). RNA-seq data will be deposited to databases upon acceptance.

N. Whole Genome Sequencing

Whole genome sequencing (short-read, non-human somatic variant) was performed by Azenta Life Sciences (Next Generation Sequencing) on ten tumor samples as paired-end 150 bp reads with an average of approximately 864 million reads. Sequence reads were aligned to the GRCm38 version of the mouse genome using BWA(v 0.7.17). Reads were trimmed and low-quality reads were removed using Trim Galore (v0.6.3_dev). Picard tools (v2.20.3) was used for alignment QC. CNV calling was performed using CNVkit (v0.9.9) (9). Vehicle treated samples were used as reference samples for CNVkit. AnnotSV (v2.3) (10) was used to annotate the bed-format regions from the CNVkit output. The linear copy number values from CNVkit were divided by two to provide the copy number ratios to avoid the assumption that the control genomes were diploid.

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