Supplementary Text

Supplementary Methods

Data normalization. Raw luminescence values collected from the high throughput screen were normalized to internal reference control samples (cells with no siRNA in wells A1-A8) on each plate to allow for plate-to-plate comparisons. Plates were mean centered to account for additional plate-to-plate variability. The individual datapoints were collected and utilized to calculate ratios (treated/untreated) for each siRNA. The mean and standard deviation of each ratio was calculated, and the distribution of the mean ratio values for each siRNA was utilized to calculate a Z-score for each siRNA. siRNAs with Z-scores <-2 were subjected to further validation.

Secondary validation. Secondary validation studies utilized a similar protocol as described for the high throughput transfection with a couple of modifications. 50 nM pooled or individual siRNAs were used in each transfection. Either 0.15 mg/ml dacarbazine or 10 μM cisplatin was used for the validation studies. For SK-MEL-28 cells, $6x10^3$ cells per well were used for each transfection.

Generation of shRNA expressing or overexpressing melanoma/melanocyte cell lines. Lentiviral shRNAs targeting RhoJ were purchased from Sigma (pLKO.1 vector). Melanoma cells or melanocytes were infected at an MOI<1. Quantitative RT-PCR was used to identify shRNAs that inhibited RhoJ expression. GAPDH, empty vector, and

RhoJ overexpressing lentiviral vectors were purchased from Genecopoiea. A Trans-lentiviral packaging system (Open Biosystems) was used to generate lentivirus. Melanoma cells or melanocytes were infected at an estimated MOI<1. RhoJ overexpression was verified by western blotting.

DNA Microarray Analysis. Microarray experiments using Affymetrix 1.0 ST chips were performed similar to as previously described (14). RNA was isolated and analyzed from 3 replicates of control or RhoJ shRNA expressing C8161 cells. The Cyber-T program (15) was used to determine statistically significant differentially expressed genes. Overrepresented gene ontology biological process categories for differentially expressed genes were determined using the DAVID (Database for Annotation, Visualization and Integrated Discovery) 2007 program (16). Primary microarray data used in this analysis is presented in Table S5.

P53 sequencing. cDNA was prepared from MNT-1 cells using High-Capacity RNA-to-cDNA kit (Applied Biosystem). P53 cDNA was amplified using the following primers (5'-ATGGAGGAGCCGCAGTCA-3' and 5'-TCAGTCTGAGTCAGGCCCTTC-3'). Gel-purified DNA was subjected to DNA sequencing. Alignment to the reference sequence revealed no mutations in the p53 core domain.

Quantitative RT-PCR. Melanoma cells were transfected in 96 well plates with 50 nM candidate siRNA using 0.2 µl Dharmafect 2 reagent. 48 hours after transfection, cDNA was prepared from transfected cells utilizing the Cells-to-Ct kit (Ambion) per the

manufacterer's protocol. In the case of cisplatin treatment, 48hrs post-siRNA transfection, cells were treated with 30μM cisplatin for 24hrs. RNA was harvested from these cells and converted to cDNA using a Cells-to-Ct kit (Applied Biosystems). Taqman assays targeting each candidate gene were purchased from Applied Biosystems. A 7900HT Fast Real-Time PCR System (Applied Biosystems) was utilized to determine Ct values. Values were normalized using actin and analyzed using the relative quantification mathematical model (Pfaffl). For the experiments utilizing quantitative RT-PCR in shRNA expressing cells, RNA was purified from each shRNA expressing cell and RNA levels were normalized. RNA was converted to cDNA by Multiscribe reverse transcriptase (Applied Biosystem) and subjected to quantitative RT-PCR.

Immunofluorescence. For the P-Histone2A.X staining, SK-MEL-28 cells were transfected with RhoJ or scrambled siRNA. Transfected cells were treated with 30μM cisplatin for 16hr followed by fixation and staining with anti-P-H2A.X (Cell Signaling Technologies) using a 1:400 dilution. An AlexaFlour 488-conjugated secondary antibody (Invitrogen) and Rhodamine conjugated phalloidin were utilized to identify H2A.X nuclear staining and actin cytoskelton staining, respectively.

Genotoxicity measurement (Comet assay). The comet assay was performed according to OxiSelect Comet Assay Kit manual (Cell Biolabs, Inc.). Briefly, 100.000 cells were mixed with low-melt agarose per well and seeded into 3-well slides. Cells were lysed in the solidified agarose gel, subjected to alkaline electrophoresis, and stained using a Vista Green DNA dye. Slides were imaged by fluorescence microscopy, and comet tails from

50 cells per siRNA were used to quantify the Olive Tail moments using Comet Scoring Freeware (Tritek). The averages of the Olive Tail Moments were normalized to control siRNA and were plotted ± standard error.

Supplementary Figure Legends

Figure S1. Negative regulators of melanoma chemoresistance include known melanoma oncogenes. A. Z-score distribution for genome-wide dacarbazine sensitizer screen. Z-score for the ratio distribution was calculated. siRNAs with Z scores less than -2 (putative targets) are shown in pink. Note the large number of genes with Z-score greater than 4. **B. Negative regulators of melanoma chemoresistance are enriched in single gene lethals.** SiRNAs that promoted chemoresistance that had Z-scores greater than 4 were examined for their impacts on melanoma survival. A majority of the genes with Z-score greater than 4 impacted MNT-1 cell survival in the absence of dacarbazine, including BRAF (red bar). **C. Negative regulators of melanoma chemoresistance are enriched in oncogenes.** The GO annotation of siRNAs that negatively impact melanoma chemoresistance (Z-score>4) was examined. A significant number of these genes were kinases, including several kinases (BRAF, ABL, MET) that are known to impact melanoma survival. **D. BRAF-depleted cells are not sensitized to DNA damage agents.** MNT-1 melanoma cells were transfected with BRAF or control siRNAs. 72 hours after transfection, transfected cells were treated with the indicated doses of dacarbazine or cisplatin. Relative survival of treated and untreated cells was measured using a cell titer glo assay.

Figure S2. **Secondary Validation of siRNA Screen Hits**. **A. Primary Validation of Hits from siRNA Screen.** MNT-1 cells were transfected with the indicated siRNA pools (50 nM final concentration) targeting 44/140 siRNAs identified in our screen. 48 hours after transfection, transfected cells were incubated in the presence and absence of 0.15 mg/ml light activated dacarbazine. A Cell-Titer-Glo assay was used to quantify the impact of each individual siRNA and DTIC treatment on cell survival. The ratio of celltiter glo values for treated and untreated samples were calculated and data was normalized to the ratio seen in nontargeting siRNA transected cells. The graph is representative of three experiments performed in triplicate. **B. Pool Deconvolution analysis reveals that screen hits were not a result of siRNA off-target effects.** Four independent siRNAs targeting the genes that potently sensitize MNT-1 cells to dacarbazine (Figure S2B) were separately tested for the capacity to sensitize cells to DTIC treatment. The ratio of Cell-Titer-Glo values for treated and untreated samples were calculated and data was normalized to the ratio seen in nontargeting siRNA transected cells. The graph is representative of three experiments performed in triplicate. **C**. **Identification of gene targets that enhances dacarbazine sensitivity in SK-MEL-28 cells.** SK-MEL-28 melanoma cells were transfected with siRNAs that sensitized MNT-1 cells to dacarbazine. 48 hours after transfection, cells were incubated in the presence and absence of 0.15 mg/ml light activated dacarbazine. Data is normalized to the values obtained from control transfected samples.

Figure S3. RhoJ Supresses cisplatin-induced Apoptosis in p53 mutant cells by suppresing NBS1 phosphorylation. A. RhoJ depletion sensitizes p53 mutant cells to

cisplatin-induced apoptosis. P53 mutant SK-MEL-28 cells were transfected with the indicated siRNA for 48hrs, then were treated with 30μM cisplatin for 24hrs. Cells were stained with Annexin-V and analyzed by Flow Cytometry. **B. RhoJ Depletion sensitizes p53 mutant cells to early stage apoptosis.** SK-MEL-28 cells were transfected with indicated siRNA for 48hrs, then were treated with 30μM cisplatin for 24hrs, Cells were stained with Annexin-V and Propidium iodide and analyzed by Flow Cytometry. Note the accumulation of Annexin-V positive, PI negative cells. **C. NBS1 depletion mitigates the effect of RhoJ Depletion on Cisplatin Induced Apoptosis in p53 mutant cells.** SK-MEL-28 cells were transfected with indicated siRNA for 48hrs and treated with 30μM cisplatin for 40hrs. Early stage apoptosis was measured by quantifying the accumulation of Annexin V positive, PI negative cells. ##, $p<0.05$ vs. siCTL; *, $p<0.05$; **, $p<0.01$ vs. siRhoJ determined by Student's t-test. The efficacy of indicated siRNAs was measured by immunoblotting. **D. RhoJ depletion induces ATR-dependent NBS1 phosphorylation upon Cisplatin treatment.** SK-MEL-28 cells were treated with indicated siRNA for 48 hrs, then were treated with 30μM cisplatin for 24hrs as indicated. Accumulation of NBS1 and NBS1 phosphorylated at the ATR dependent site (phospho-Ser343 NBS1) was measured by immunoblotting.

Figure S4. siRNAs inhibit the expression of their cognate targets. A. Quantitative RT-PCR was employed to measure the impact of select pooled siRNAs on target mRNA levels. Actin primers were employed to control for total mRNA concentrations. Results are representative of three experiments performed in triplicate. A student's t-test was utilized to validate that siRNAs significantly inhibited the expression of the

corresponding gene. All of the siRNAs tested in this analysis significantly inhibited the expression of the corresponding gene (p<0.05 determined by Student's t-test) in MNT-1 cells. **B.** MNT-1 and SK-MEL-28 cells were transfected with control or RhoJ siRNAs for 48hrs and then were treated with or without 30μM cisplatin for 24hrs. The relative levels of RhoJ and Actin were determined by immunolblotting. **C. Relative Pak3 expression in the melanoma cells studied was measured.** HEK293T cells were utilized to verify that the Pak3 antibody can recognize its target.

Figure S5. RhoJ Uncouples ATR From Chk1. A. RhoJ does not inhibit the DNA damage foci formation. SK-MEL-28 cells were transfected with control or RhoJ siRNA for 48hrs, then were treated with 30μM cisplatin for 16hrs, fixed, incubated with a P-Ser139-H2A.X antibody and following by a Alexa Flour 488-conjugated secondary antibody. Rhodamine-Phalloidin was used as the counter staining. Relative staining in RhoJ siRNA treated and control siRNA treated nuclei (1000 nuclei in each group) was quantified as described (1). **B. RhoJ does not inhibit cisplatin-inducd DNA damage.** SK-MEL-28 cells were transfected with control or RhoJ siRNA for 48hrs, and were treated with or without 30μM cisplatin for 20 hrs before being subjected to the comet assay. Olive Tail Moments of RhoJ siRNA treated cells were normalized to control siRNA treated cells. Representative images are shown on the right. **C. Other RNAi screen targets regulate p53 accumulation, but not Chk1 activation.** MNT-1 cells were transfected with the indicated siRNAs for 48hrs and incubated in the presence and absence of 30 μM cisplatin for 24hrs. The accumulation of pChk1, Chk1, p53and cleaved

PARP was measured via immunoblotting. **D. RhoJ depletion Suppresses Cellular Proliferation.** Representative images used to generate the graph in Figure 4B was shown.

Figure S6. RhoJ Regulates the Expression of Sox10. A. RhoJ Regulates the Expression of Genes that Control Neural Differentiation. Significantly enriched sets of genes in control and RhoJ depleted cells was determined using DAVID. Enriched gene sets and their p-values are shown. Note; Regulators of Neural Differentiation were either significantly upregulated or downregulated. **B. RhoJ Regulates the Expression of Sox10 and Sox10 Target genes.** SOX10 and SOX10 targets whose expression was significantly downregulated is shown. **C. Sox10 depletion impacts melanoma chemoresistance.** SK-MEL-28 melanoma cells were transfected with the indicated siRNAs and incubated with 10 μM cisplatin for 72hrs. Relative cell number was determined using a sulforhodamine B assay as described to quantify the percent of surviving cells.

Table S1. Genome-wide siRNA dataset. MNT-1 melanoma cells were transfected with 84,920 siRNA duplexes targeting 21,230 genes in a one-well, one-gene reverse transfection format as we have previously described (2). 72 hrs post transfection, cells were incubated in the presence and absence of 0.1 mg/ml dacarbazine. Cell-Titer-Glo values were collected for each well and normalized to internal reference samples on each plate, followed by normalization to the experimental mean for each well calculated from the full data set to control for variations due to plate and position effects. Similarly adjusted luminescence values from untreated wells were used to calculate the ratio of

Cell-Titer-Glo for each siRNA. Data for each individual ratio, the mean, standard deviation, and ratio Z-score is shown for all of the genes examined. Additionally gene ontology information for each gene, accession number, and chromosome position is indicated. Using the calculated Z scores from the genome wide dataset, we identified those siRNAs with a Z-score less than two as putative siRNAs that impact dacarbazine sensitivity (second tab). 140 siRNAs were identified and the data for each individual ratio, mean, standard deviation, and Z-score is shown. The retested siRNAs are shown in bold.

Table S2. RhoJ is upregulated in advanced tumors. The GEO database was queried to identify melanoma datasets in which the expression of RhoJ was assessed. One published dataset was identified which compared RhoJ gene expression in 16 primary and 40 metastatic flash-frozen melanoma tumors (23). Bayesian-based statistical approaches were utilized to calculate the p-value and fold change for each of the seven different probe sets for RhoJ between primary and metastatic melanoma specimens.

Table S3. Microarray Dataset. The microarray data from each replicate for RhoJ shRNA expressing SK-MEL-28 melanoma cells (E_3, E_4, E_5) and control shRNA expressing SK-MEL-28 cells (C_0, C_1, C_2) were analyzed using a Bayesian-based statistical approach (Cyber-T). The output from the Cyber-T analysis is presented, along with a list of genes that were differentially expressed ($p<.005$). Genes that were significantly ($p<.005$) upregulated in RhoJ depleted cells greater than 5 fold are listed in

the first tab. Genes that were significantly ($p<0.005$) downregulated in RhoJ depleted cells greater than 5 fold are listed in the second tab.