Alkylation sensitivity screens reveal a conserved cross-species functionome

Supplemental Information

Supplemental Figures

Figure S1. Screen methodology, vehicle effects to verify screening conditions, and hit selection criteria. **A)** A wet-reverse transfection was used to knockdown genes with siRNA. Transfection consisted of 3 siRNAs targeting the same gene in every well. Transfection reagents were mixed and split into two different plates before the addition of T98G cells to the plates. After 48hrs one plate was treated with 1mM TMZ in DMSO (1% DMSO, final concentration) while the other was treated with 1% DMSO. **B)** The vehicle (1%DMSO) is nontoxic after 48hrs of incubation before determining growth inhibition by an MTS assay. **C)** Methodology used to create high confidence hit list from two distinct methods

Figure S2. Different methods of analysis yield similar gene enrichments. The diagram demonstrates the large overlap of gene enrichment groups determined by NIH DAVID and Princeton GO term finder.

Figure S3. Lentivirus and siRNA mediated knockdown of UNG, UBE3B, and ICMT. The mRNA was quantified by qRT-PCR. **A)** Quantification of protein modification processes mRNA knockdown in lentiviral infected cells as determined by qRT-PCR. TaqMan probes were used to quantify mRNA levels on an Applied Biosystems StepOnePlus machine. The qRT-PCR data was analyzed using the $_{\Delta\Delta}C_t$ method and was normalized to GFP infected plate controls. Gene expression of each gene was normalized to the expression of human ß-actin. The mean of three independent experiments is plotted ± SEM. **B)** Quantification of UBE3B and UNG mRNA knockdown in siRNA transfected cells as determined by qRT-PCR. Dark gray bars denote UBE3B mRNA levels while light gray bars denote UNG mRNA levels. TaqMan probes were used to quantify mRNA levels as described above. The qRT-PCR data was normalized to scrambled siRNA controls.

Supplemental Tables

Table S1. Significantly conserved biological processes as determined by cross-species Functionome Analysis.

Supplemental Datasets

Dataset 1 - Genes of interest from the siRNA screen that satisfied both selection criteria: a p-value less than 0.05 and a viability ratio in the lowest 5%

Dataset 2 - Second group of genes from the siRNA screen analyzed for gene enrichment using selection criteria of a p-value less than 0.05 and a viability ratio in the lowest 2.5%

Dataset 3 - Third group of genes from the siRNA screen analyzed for gene enrichment using selection criteria of a p-value less than 0.05 and a viability ratio in the lowest 7.5%

Dataset 4 - Fourth group of genes from the siRNA screen analyzed for gene enrichment using selection criteria of a p-value less than 0.05 and a viability ratio in the lowest 10%

Dataset 5 - Enriched genes identified by both NIH DAVID and Princeton GO Term Finder

Dataset 6 - Enriched biological processes and associated genes as determined by Princeton GO Term Finder