Supporting Information

Supplementary Methods

8-oxo-deoxy Guanine assay.

To measure the levels of 8-oxo-deoxy guanine, the oxyDNA assay kit-fluorometric (EMD Chemicals, Inc., Gibbstown, NJ) was used following the manufacturer's instructions. Briefly, following protein knock-down, cells were incubated for additional 24 h with or without exposure to MMS in a 384-well plate, fixed using 4% paraformaldehyde, dehydrated using graded methanol and permeabilized using 99% methanol. Following permeabilization, cells were rehydrated and blocked using blocking solution supplied with the kit. Cells were then incubated with FITC conjugated antibody overnight at 4 ºC, followed by three washes with wash buffer supplied. Images of cells were acquired using a Carl Zeiss Axiovert 200M inverted microscope using 40X objective and 488 nm filter set. The percent of cells positive for fluorescence was determined by counting total cells and labelled cells in each field. Assays were performed in quadruplicate.

Notch reporter assay.

To analyze the function of the Notch pathway, we measured the transcriptional activity of its downstream component RBP-Jk, using a Cignal reporter assay kit (SABiosciences, Fredrick, MD). The assay kit consists of a DNA construct to monitor luciferase activity of the RBP-Jk reporter and renilla luciferase to monitor transfection efficiency. HEK 293 cells were transfected with reporter or control plasmids provided in the kit in 96 well plate, using SureFECT transfection reagent (SABiosciences, Fredrick, MD), following the manufacturer's protocol. Transfected cells were incubated for 48 hours, exposed to damaging agent, and incubated for additional 24 hours. Luciferase activity was quantified and normalized with Renilla luciferase for transfection efficiency, using Dual-Glo luciferase assay kit (Promega). Assays were performed in quadruplicate.

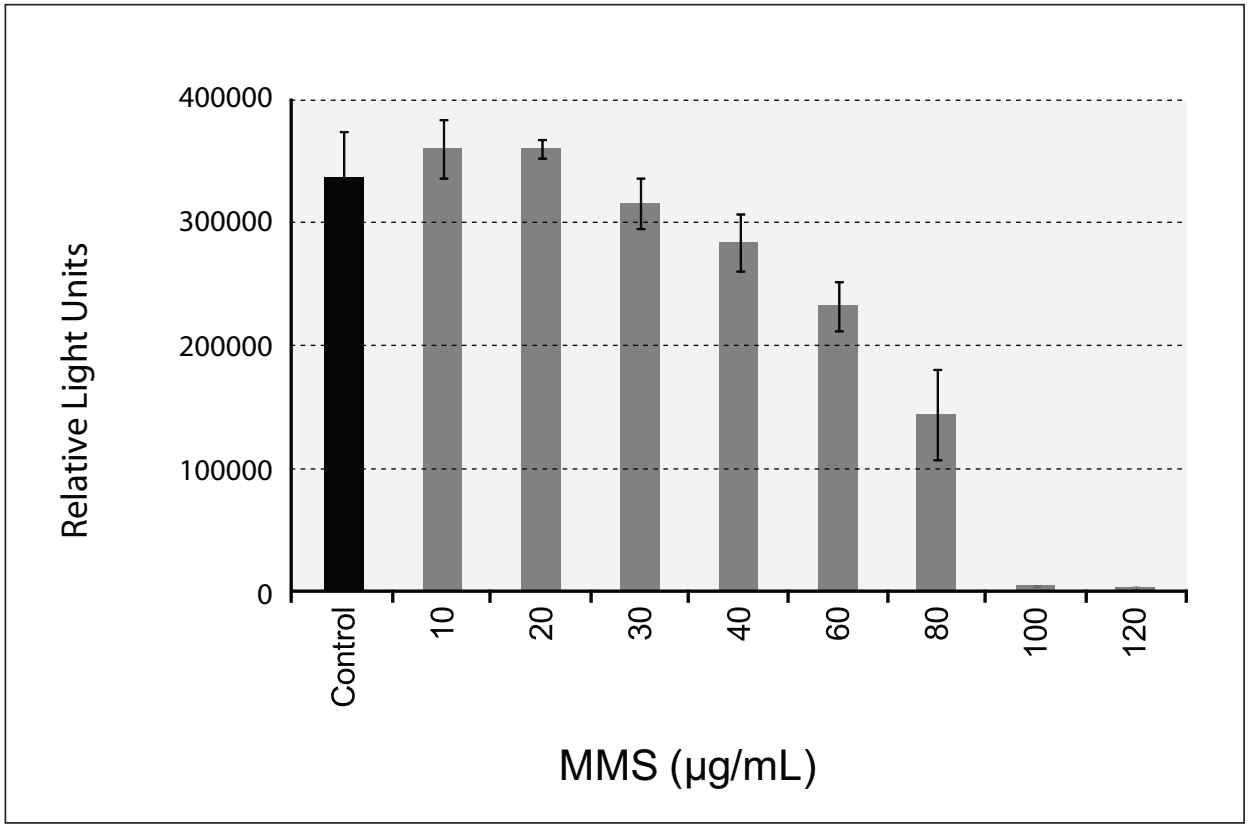

Figure S1.
The MMS dose response as measured by cell survival. Viability of *Drosophila* Kc_{167} cells following exposure to increasing dose of MMS determined using CellTiter-Glo (relative light units (ordinate) for increasing dose of MMS (abscissa)).
doi:10.1371/journal.pgen.1000527.s001
(0.54 MB EPS)

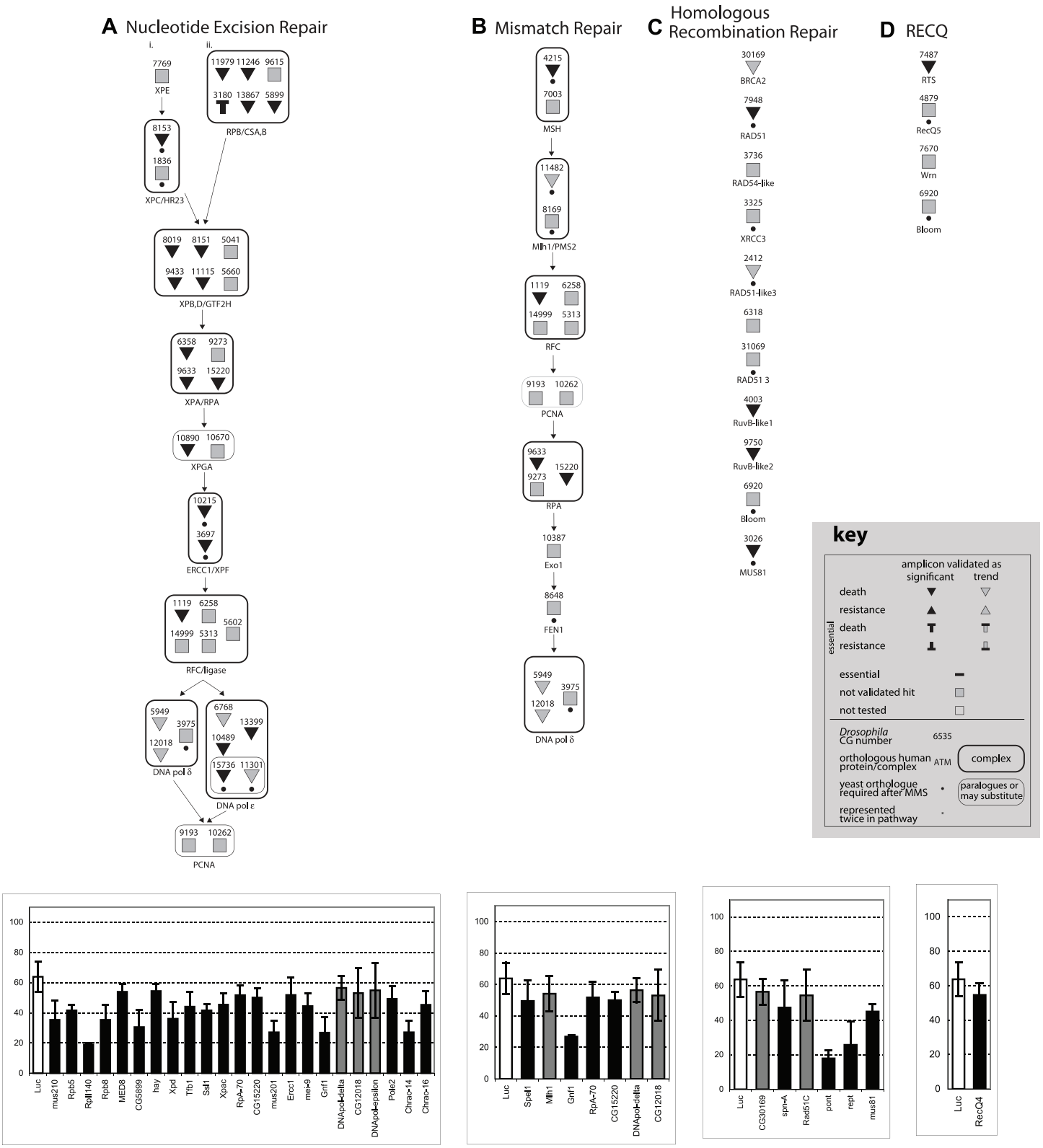

Figure S2.

Four of the eight additional pathways utilized for MMS survival. CG numbers are given for each *Drosophila* pathway component, as well as the protein names or complex names for their human orthologues. Pathway entry points are noted with Roman numerals at the top, and end points are at the bottom. A key for the following symbols is provided. Symbols encircled with thick lines represent proteins that act together or in a complex, while symbols encircled with thin lines represent paralogues or proteins that may substitute for one another. Proteins found to affect MMS survival are

noted as down (death) or up triangles (resistance). Statistically significant proteins are indicated with black triangles, while trend hits are indicated with grey triangles. Essential genes are noted with a thick bar and any with downwards or upwards pointing boxes were also validated as conferring death or resistance, respectively, to MMS upon knock-down. Shaded squares are proteins not found to be hits after validation, and open squares were not tested in our validation. Yeast orthologues previously found to be required for MMS survival [3] are noted with a dot under the symbol. An example of average percent of untreated control survival of validated hits is shown next to each pathway, though this may not represent the actual control for each data point within the graph. Error bars are the standard deviation of quadruplicates. Survival of control cells with dsRNA targeting luciferase is shown in an open bar, protein knock-down that resulted in a significant difference in MMS survival from this control are shown in black (death) or stripped (resistance), and those with a trend effect are shown in grey. A complete list of these proteins and their human and yeast orthologues is given in Table S6. (A) Nucleotide Excision Repair. (B) Mismatch Repair. (C) Homologous Recombination Repair. (D) RecQ Helicases.
doi:10.1371/journal.pgen.1000527.s002
(1.40 MB EPS)

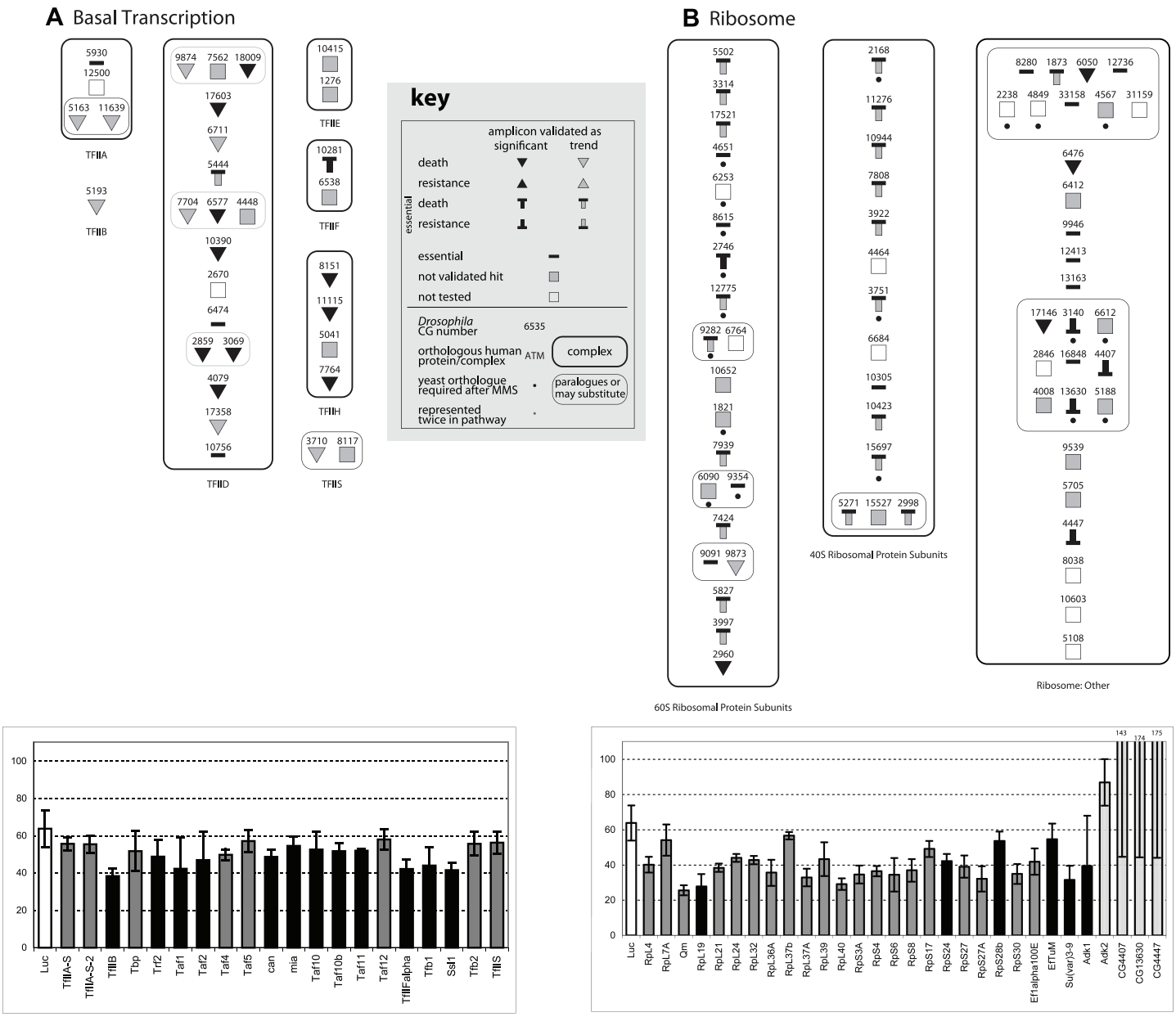

Figure S3.

Two of the eight additional pathways utilized for MMS survival. CG numbers are given for each *Drosophila* pathway component, as well as the protein names or complex names for their human orthologues. Pathway entry points are noted with Roman numerals at the top, and end points are at the bottom. A key for the following symbols is provided. Symbols encircled with thick lines represent proteins that act together or in a complex, while symbols encircled with thin lines represent paralogues or proteins that may substitute for one another. Proteins found to affect MMS survival are noted as down (death) or up triangles (resistance). Statistically significant proteins are indicated with black triangles, while trend hits are indicated with grey triangles. Essential genes are noted with a thick bar and any with downwards or upwards pointing boxes were also validated as conferring death or resistance, respectively, to MMS upon knock-down. Shaded squares are proteins not found to be hits after validation, and open squares were not tested in our validation. Yeast orthologues previously found to be required for MMS survival [3] are noted with a dot under the symbol. An example of average percent of untreated control survival of validated hits is shown next to each pathway, though this may not represent the actual control for each data point within the graph. Error bars are the standard deviation of quadruplicates. Survival of control cells with dsRNA targeting luciferase is shown in an open bar, protein knock-down that resulted in a significant difference in MMS survival

from this control are shown in black (death) or stripped (resistance), and those with a trend effect are shown in grey. A complete list of these proteins and their human and yeast orthologues is given in Table S6. (A) Basal Transcription. (B) Ribosome.
doi:10.1371/journal.pgen.1000527.s003
(3.26 MB EPS)

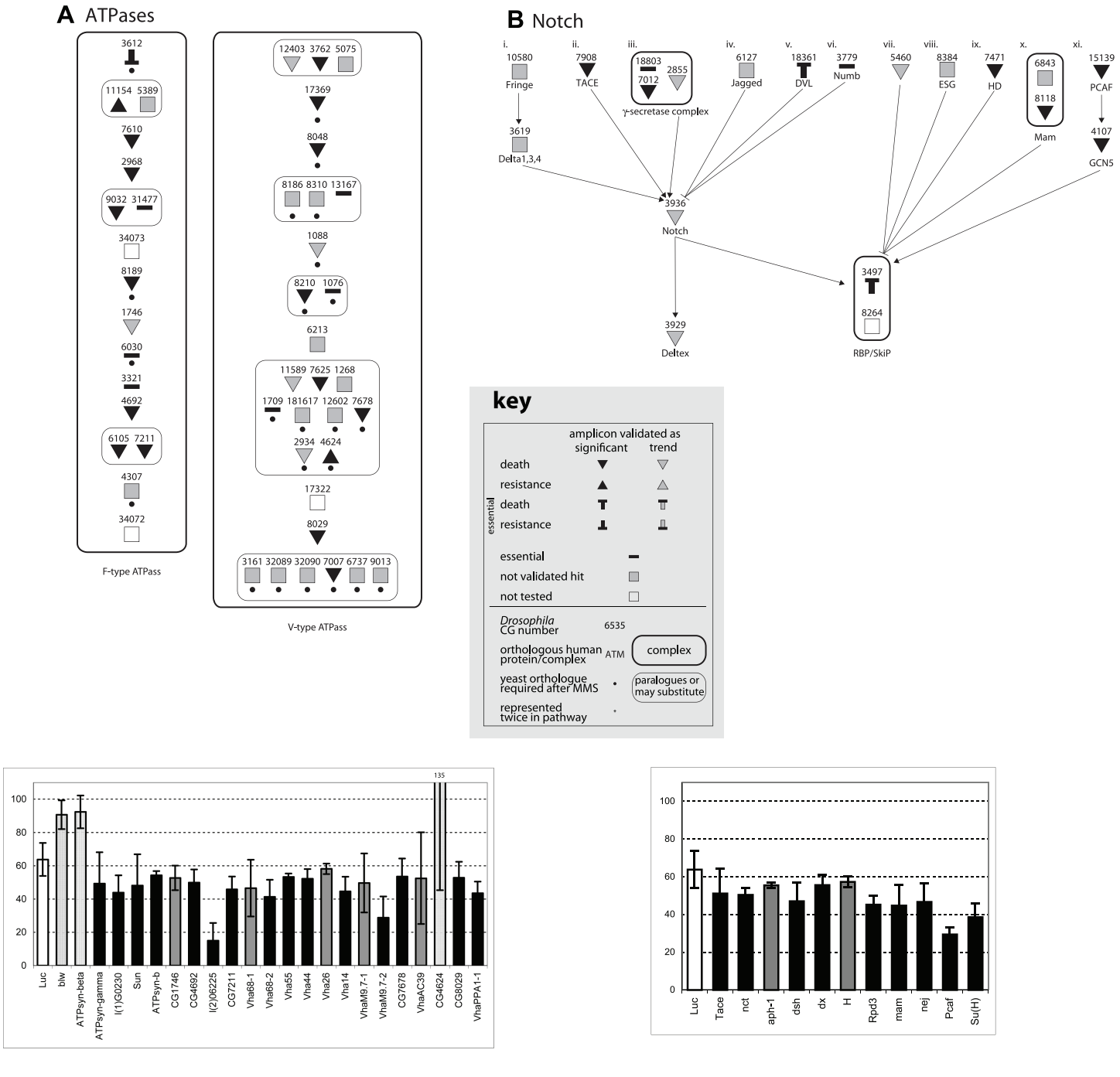

Figure S4.

Two of the eight additional pathways utilized for MMS survival. CG numbers are given for each *Drosophila* pathway component, as well as the protein names or complex names for their human orthologues. Pathway entry points are noted with Roman numerals at the top, and end points are at the bottom. A key for the following symbols is provided. Symbols encircled with thick lines represent proteins that act together or in a complex, while symbols encircled with thin lines represent paralogues or proteins that may substitute for one another. Proteins found to affect MMS survival are noted as down (death) or up triangles (resistance). Statistically significant proteins are indicated with black triangles, while trend hits are indicated with grey triangles. Essential genes are noted with a thick bar and any with downwards or upwards pointing boxes were also validated as conferring death or resistance, respectively, to MMS upon knock-down. Shaded squares are proteins not found to be hits after validation, and open squares were not tested in our validation. Yeast orthologues previously

found to be required for MMS survival [3] are noted with a dot under the symbol. An example of average percent of untreated control survival of validated hits is shown next to each pathway, though this may not represent the actual control for each data point within the graph. Error bars are the standard deviation of quadruplicates. Survival of control cells with dsRNA targeting luciferase is shown in an open bar, protein knock-down that resulted in a significant difference in MMS survival from this control are shown in black (death) or stripped (resistance), and those with a trend effect are shown in grey. A complete list of these proteins and their human and yeast orthologues is given in Table S6. (A) ATPases. (B) Notch.
doi:10.1371/journal.pgen.1000527.s004
(2.47 MB EPS)

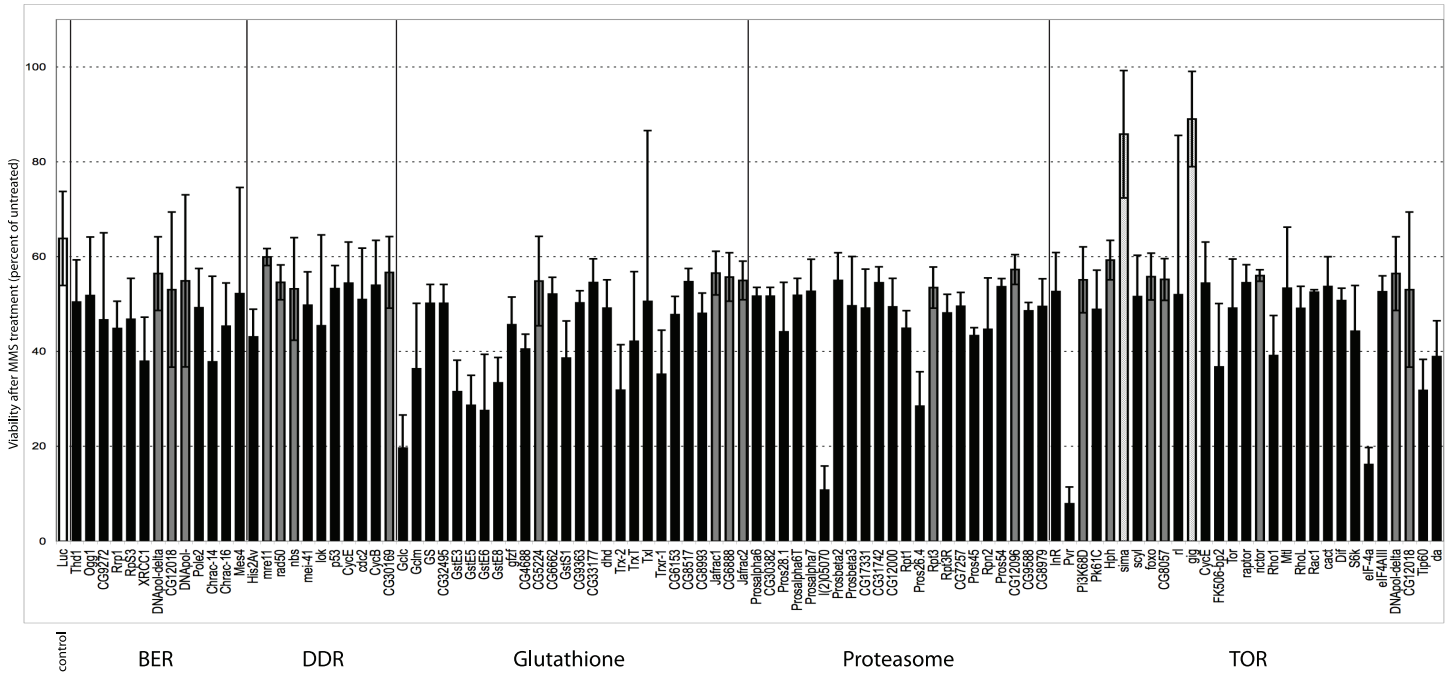

Figure S5.
Average percent of untreated control survival of validated hits in each pathway represented in Figures 2 and 3. Error bars are the standard deviation of quadruplicates. Survival of control cells with dsRNA targeting luciferase is shown in an open bar as an average across all plates as a general reference. It should be noted that each plate had its own luciferase controls against which all plate values were compared. Protein knock-down that resulted in a significant difference in MMS survival from their internal plate control are shown in black (death) or dashed (resistance), and those with a trend effect are shown in grey. A complete list of these proteins and their human and yeast orthologues is given in Table S6.

doi:10.1371/journal.pgen.1000527.s005
(4.48 MB EPS)

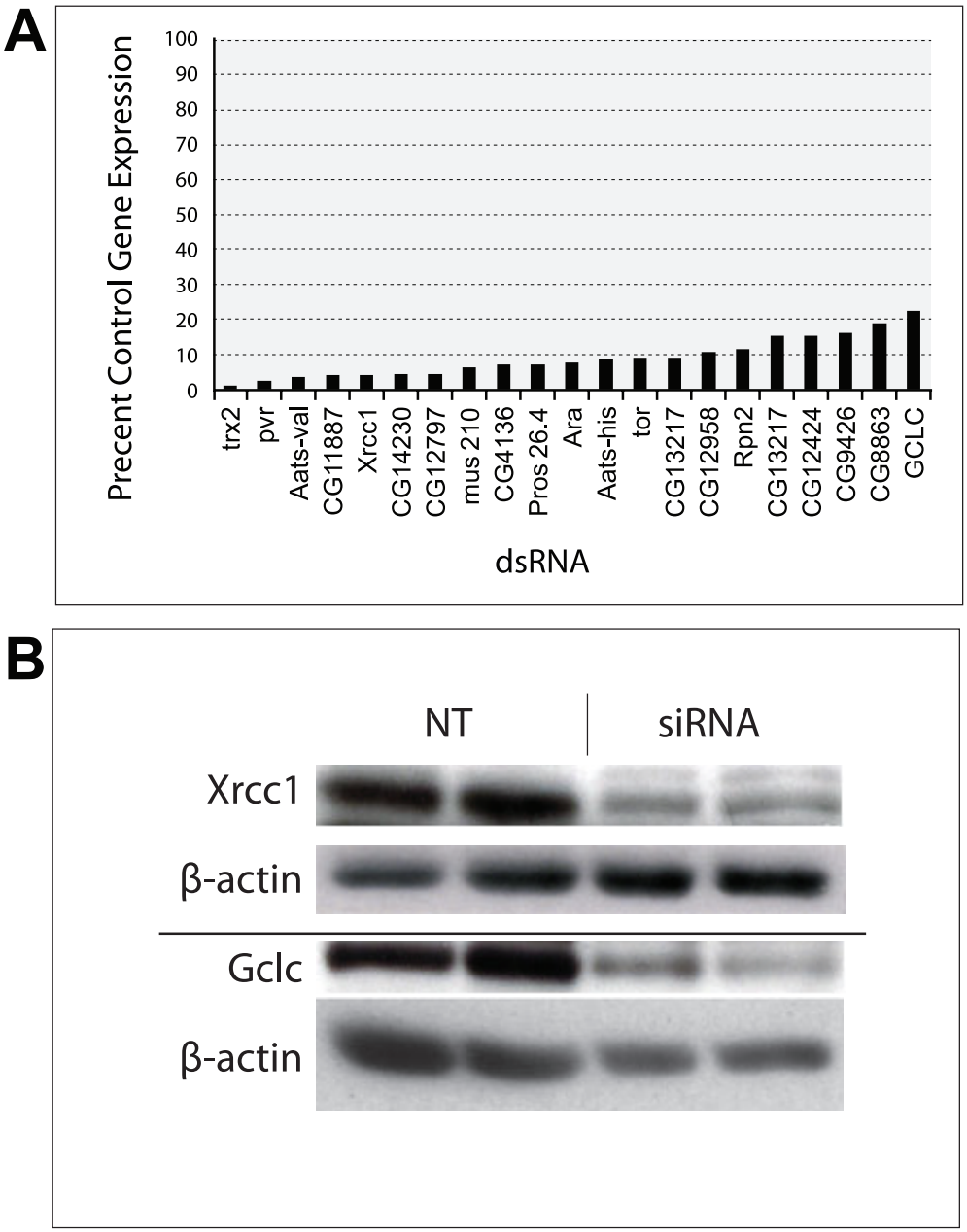

Figure S6.
Efficiency of RNAi mediated silencing of gene expression. (A) RNAi transfection resulted in decreased expression of target mRNA in *Drosophila* Kc_{167} cells, measured by quantitative real time PCR as indicated percent control gene expression (ordinate) for the genes (abscissa) tested, with expression normalized to endogenous control (CG6905). (B) Western blot analysis for efficiency of RNAi in siRNA transfected primary mouse embryonic fibroblast cells.
doi:10.1371/journal.pgen.1000527.s006
(0.74 MB EPS)

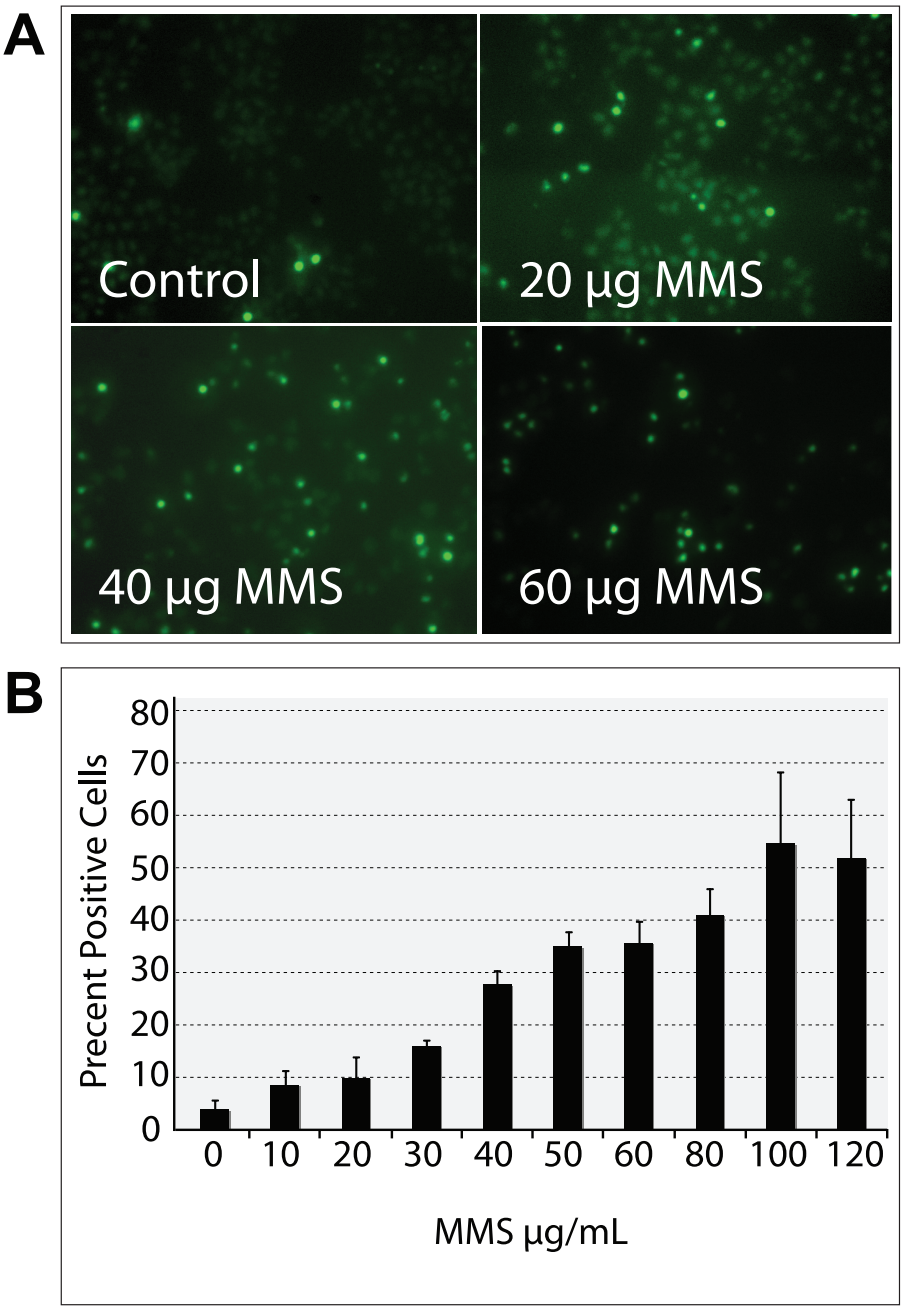

Figure S7.
MMS exposure results in a dose-dependent increase in 8-oxoguanine DNA modifications. The MMS-dependent increase in 8-oxoguanine is (A) observed qualitatively by microscopic examination with 8-oxoguanine containing cells observed by fluorescence in the FITC channel and this (B) can be quantified as a percentage of fluorescent within a field (ordinate) for increasing dose of MMS (abscissa).
doi:10.1371/journal.pgen.1000527.s007
(7.71 MB EPS)

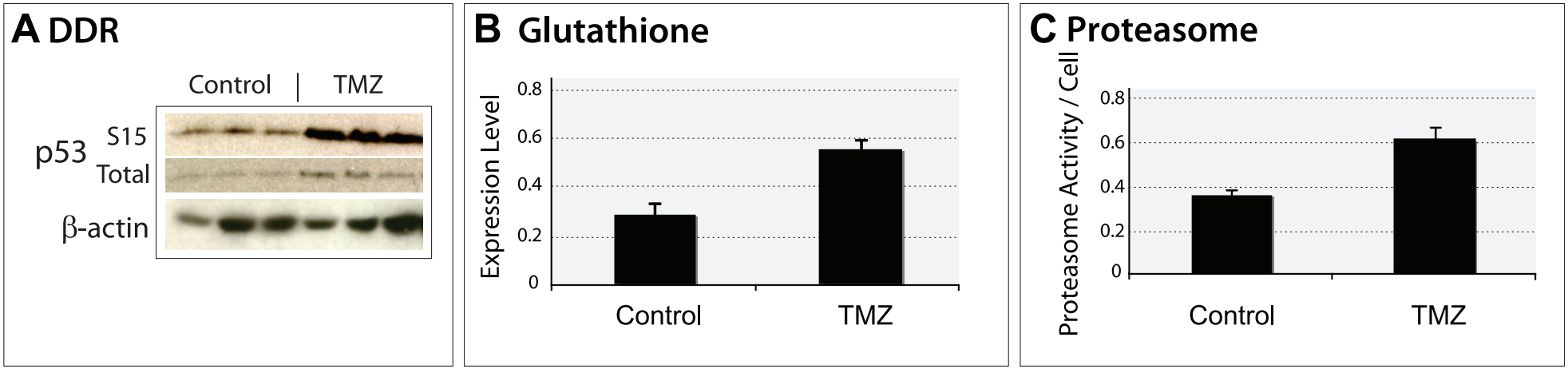

Figure S8.
Temozolomide exposure results in functional response by MMS survival pathways in mouse embryonic fibroblasts. (A) Temozolomide exposure results in phosphorylation of p53 and in an accumulation of total p53 levels. (B) Temozolomide exposure results in an increased amount of total glutathione. The intracellular glutathione concentration is expressed as units of activity/cell. (C) Proteasome activity is increased following temozolomide exposure, normalizing the activity to the number of cells using a parallel viability assessment. Proteasome activity is expressed as units of activity/cell, normalizing the activity to the number of cells using a parallel viability assessment.
doi:10.1371/journal.pgen.1000527.s008
(0.71 MB EPS)

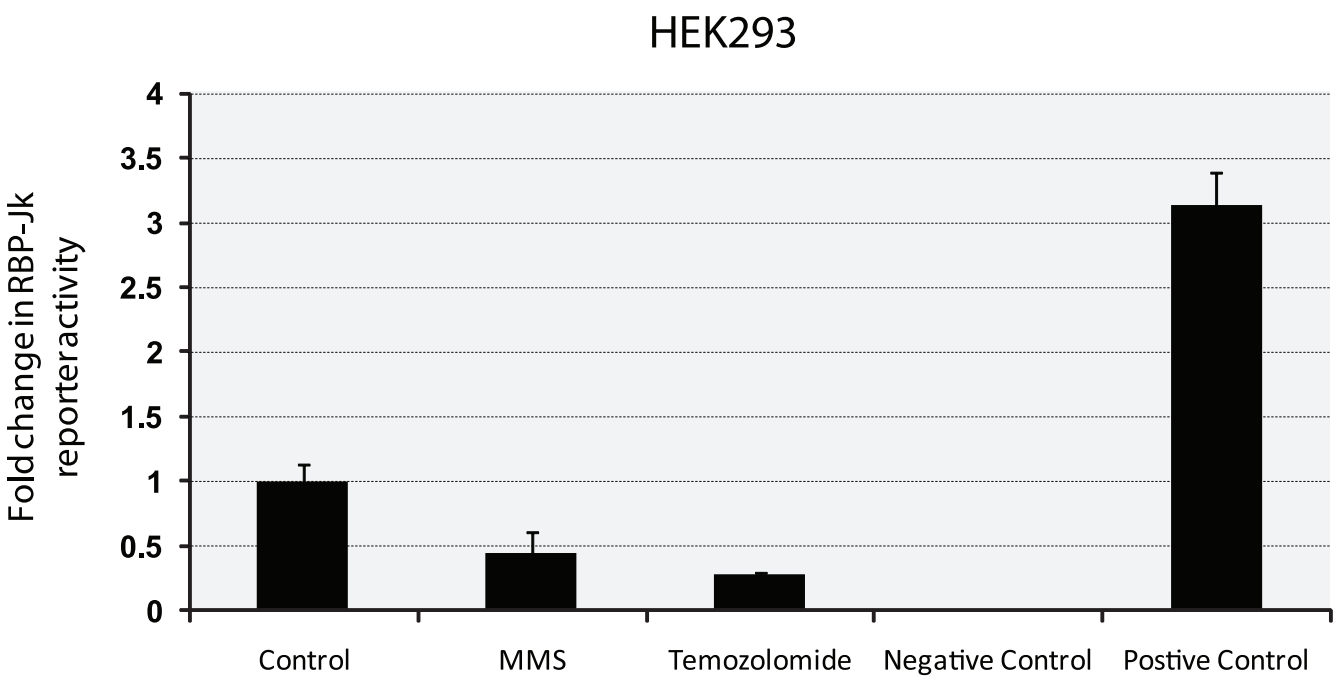

Figure S9.
MMS and Temozolomide exposure results in functional response by notch signalling pathway in HEK 293 cells. MMS and temozolomide exposure results in decreased luciferase activity of the notch reporter RBP-Jk, normalized for transfection efficiency in HEK 293 cells using renilla luciferase.
doi:10.1371/journal.pgen.1000527.s009
(0.41 MB EPS)

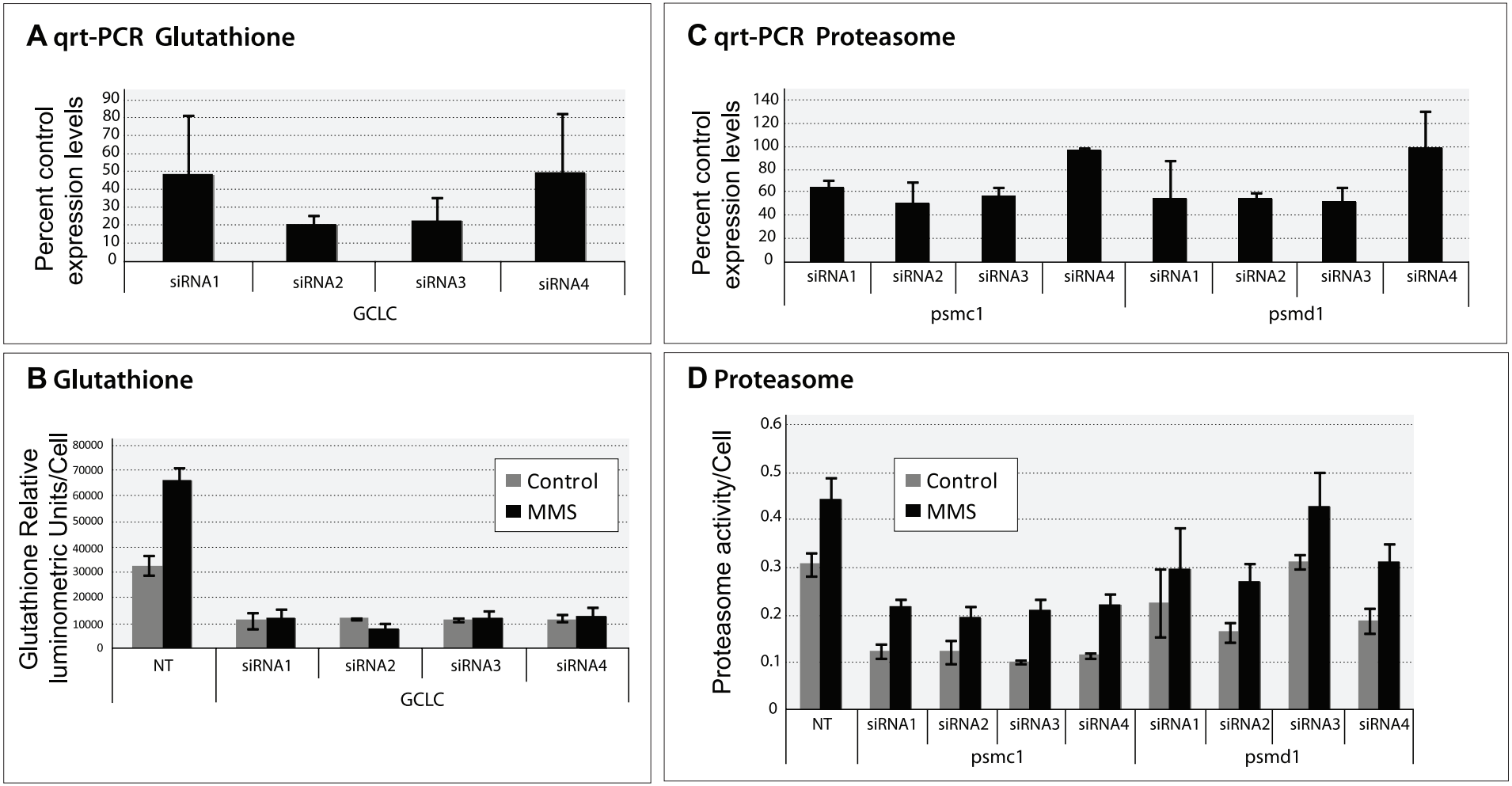

Figure S10.

Validation of pathway functions with additional siRNA knock down in mouse embryonic fibroblasts. (A) Knock-down of GCLc with four different siRNA results in decreased GCLc expression in mouse cells, by quantitative real-time PCR analysis; expression level is provided as fold-change compared to an endogenous control (mouse β-actin). (B) MMS results in an increased amount of total glutathione, and this increase is dependent upon the rate limiting glutathione metabolizing enzyme glutamate-cysteine ligase (GCLc). (C) Knock-down of proteasome components Psmc1 or Psmd1 with four different siRNA results in decreased gene expression in mouse cells, by quantitative real-time PCR analysis; expression level is provided as fold-change compared to an endogenous control (mouse β-actin). (D) Proteasome activity is increased following MMS exposure in a manner that is dependent upon proteasome components Pmsc1 and Psmd1.

doi:10.1371/journal.pgen.1000527.s010

(0.81 MB EPS)

Table S1.
Summary of MMS screen hits and pathway genes selected for validation. The 534 *Drosophila* MMS screen hits selected for validation analysis and 298 pathway genes tested for pathway analysis, provided with FlyBase gene number, corresponding CG number, and the dsRNA used for validation, noted by DRSC identification number, either library dsRNA or validation dsRNA. For those genes that had no validation amplicon designed or those whose library amplicon had no potential off-target effects at 19 nt, data from [18] was used (asterisks). For each dsRNA, significant death, trend death or significant resistance to MMS treatment is noted (MMS survival).
doi:10.1371/journal.pgen.1000527.s011
(0.94 MB XLS)

Table S2.
Raw, normalized, and survival data for validation experiment. For data normalization, raw data of untreated control and MMS experiments were normalized using luciferase (Luc) and high MMS controls and statistical significance was determined as described [18].
doi:10.1371/journal.pgen.1000527.s012
(0.23 MB XLS)

Table S3.
List of yeast MMS hits and their fly orthologues. Yeast MMS hits as determined by Begley et al. [3], and their fly orthologues that were neither a hit in our MMS screen nor in a pathway identified from the screen. Also given is whether each fly gene was essential, a resistance hit, a death hit, or a death tread after validation with an independent dsRNA.
doi:10.1371/journal.pgen.1000527.s013
(0.04 MB XLS)

Table S4.
Drosophila GST family members and their involvement in MMS survival. The five GST families and their component members. Provided is the gene name, corresponding CG number, whether they validated as a significant MMS survival gene (death), a trend (death trend) or not involved in MMS survival (no). For each validation observed to have an effect, the type of amplicon, library or validation is given. It is also noted for any validated MMS survival gene whether the effect was also observed in the screen. If validation was not performed this is also noted (not tested).
doi:10.1371/journal.pgen.1000527.s014
(0.02 MB XLS)

Table S5.
Summary of the number of MMS survival genes identified per MMS survival pathway (BER: base excision repair; DDR: DNA damage response; Proteasome; GSH: glutathione synthesis; TOR: TOR pathway; NER: nucleotide excision repair; MMR: mismatch repair; HRR: homologous recombination repair; RECQ: RecQ helicases; Transcription: basal transcription; Ribosome; ATPase; Notch: Notch signaling pathway), and the percentage non-essential genes of each pathway that this represents. Protein enrichment within each pathway compared to the number of genes that validated (202 of 13826 prior to pathway analysis and 307 of 13826 including pathway analysis) is determined using a Fisher's Exact Test (NS: Not Significant; NA: Not Applicable).
doi:10.1371/journal.pgen.1000527.s015
(0.51 MB PDF)

Table S6.
The orthologous relationship between the MMS survival genes identified in the *Drosophila* and yeast MMS survival screens. Provided are the 13 MMS survival pathway, the gene names of each *Drosophila* component and their corresponding CG number. For each of these *Drosophila* genes, the

yeast ORF is provided for any identifiable yeast orthologue and whether it was observed as being involved in MMS survival (hit) in *S. cerevisiae* by Begley et al. [3], if it was not identified (not hit) or if it is essential (essential). Where orthologues between the species are known and taking into account the yeast essential genes that could not be assayed for their effect on MMS survival, there is a clear enrichment in MMS survival genes within the majority of pathways examined between the two species. A complete list of these proteins and their human orthologues are also given.
doi:10.1371/journal.pgen.1000527.s016
(0.11 MB XLS)

Table S7.
Connectivity analysis of MMS survival genes identified by the RNAi genomic screen and following validation analyses. Provided are the number of proteins identified, the number of those proteins that are within the protein:protein interactome (PPI), the number of direct connections between these proteins, the average distance between every possible pair of proteins within the network compared to the expected values, the global efficiency and the clustering coefficient. For each measurement the expected number of direct interactions is derived from the same number of proteins randomly selected 1000 times from the PPI. Analyses are provided for the proteins identified by the MMS screen prior to validation (Screen hits), those screen hits that actually validated (Validated hits), the validated hits as well as essential proteins that are connected to two or more hits (Validated+essential connectors), the total number of validated MMS survival proteins identified by both screen and pathway validation (Validated+pathway hits), the Validated and pathway hits and the essential proteins that are connected to two or more hits (Validated+pathway hits+essential connectors), the total number of validated MMS survival proteins and all other components of the 13 MMS survival pathways (Validated+all in pathways), and finally the total number of validated MMS survival proteins, all other components of the 13 MMS survival pathways and the essential proteins that are connected to two or more hits or pathway proteins (Validated+all in pathways+essential connectors). Analyses were also provided to compare connectivity of real and randomly rewired PPI.
doi:10.1371/journal.pgen.1000527.s017
(0.19 MB DOC)

Text S1.
Methods for 8-oxo-guanine assay and notch reporter analysis.
doi:10.1371/journal.pgen.1000527.s018
(0.03 MB DOC)