Supporting Information

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SI Materials and Methods

Yeast Strains and Growth Conditions. All of the experiments were performed using yeast of DBY746 background. Knockout strains were prepared with standard PCR-mediated gene-disruption protocol. The strains used in this study are shown in Table S2.

Yeast cells were grown in minimal medium (SC) containing 2% glucose and supplemented with amino acids as described in ref. 1. For short-term starvation (STS) treatment, day 1 cultures (24 h after initial OD = 0.1 inoculation) were washed three times with sterile distilled water, resuspended in water, and incubated at 30°C with shaking for 48 h (day 3).

Yeast Oxidative Stress Assay. For oxidative stress resistance assays, day 3 cells were diluted to an OD_{600} of 1 in K-phosphate buffer (pH 7.4) and treated with 0.25–1 mM menadione for 1 h. Alternatively, cells were diluted to an OD_{600} of 1 in K-phosphate buffer (pH 6) and treated with 200–400 mM hydrogen peroxide for 30 min. Serial dilutions of untreated and treated cells were spotted onto YPD plates and incubated at 30°C for 2–3 days.

Yeast Viability Assay. Overnight SDC cultures were diluted to OD_{600} 0.1 into fresh SDC medium. After 24 h (day 1), *sch9*Δ*RAS2*^{val19} and *sch9*Δ were mixed at an initial ratio of 1:25 (10 million:250 million cells) and incubated for 2 h at 30°C with shaking. The mixed cultures were then treated with either cyclophosphamide (CP) (0.1 M) or methyl methanesulfonate (MMS) (0.01%; Sigma). MMS was prepared in ddH_20 from stock solution and was diluted directly into the mixed culture to a final concentration of 0.01% (vol/vol). However, because of the high concentration of CP (0.1M) required, CP crystals were dissolved directly into the medium. To do so, mixed cultures were centrifuged for 5 min at 1,875 × *g* and the spent media were collected, in which CP crystals were dissolved to a concentration of 0.1 M. The mixed culture was then resuspended in the CP-containing spent medium. Viability was measured as colony-forming units (CFUs) every 24 h by plating onto appropriate selective media. The viability of individual strains was measured using the same method as for the mixed cultures. Relative survival shown was determined by the percentage of the ratio between the treated and untreated (control) cells.

Cell Cultures. Primary mixed glial cells were obtained from the cerebral cortices of 1- to 3-day-old Sprague–Dawley rat pups (Charles River) as described in ref. 2. Cells cultured for 10–14 days in DMEM/F12 medium (Invitrogen) with 10% FBS were used. C6, A10–85, 9L, and RG2 rat glioma cell lines (kindly provided by T. Chen, University of Southern California), LN229 human glioma cell line (also provided by T. Chen), and SH-SY5Y human neuroblastoma cell line were maintained in DMEM/F12 medium with 10% FBS at 37°C under 5% CO_2.

STS Treatments of Mammalian Cells. Primary glia, glioma, or neuroblastoma cells were seeded into 96-well microtiter plates at 20,000–30,000 cells per well and incubated for 2 days. Cells were washed with PBS before treatments as indicated in the text. All treatments were performed at 37°C under 5% CO_2. Glucose restriction was done by incubating cells in glucose-free DMEM (Invitrogen) supplemented with either low glucose (0.5 g/liter) or normal glucose (1.0 g/liter) for 24 h in 1% serum. Serum restriction was done by incubating cells in DMEM/F12 with either 10% or 1% FBS for 24 h.

***In Vitro* Drug Treatments.** H_2O_2 and menadione were used for *in vitro* mammalian cells oxidative stress experiments. H_2O_2 was dissolved directly into DMEM/F12 to a final concentration of 0–1,000 μM. Menadione (Sigma) was prepared in ethanol at 16 mg/ml and diluted into DMEM/F12 to a final concentration of 0–120 μM. After STS treatments, cells were treated with H_2O_2 or menadione for 24 h. CP (Sigma) was used for *in vitro* chemotherapy studies. CP was prepared in DMEM/F12 with 1% FBS at 40 mg/ml and was filter-sterilized. The stock solution was stored at 4°C for no longer than 2 weeks. After STS treatments, cells were incubated with varying concentrations of CP (6–15 mg/ml) for 10 h in DMEM/F12 with 1% FBS. Glial cells have been reported to express cytochrome P450 and thus are capable of metabolizing the prodrug CP (3, 4). Survival was determined by the MTT/LDH assay and presented as percent ratio of treated to control.

***In Vitro* Cytotoxicity Assays.** Cytotoxicity was measured by either lactate dehydrogenase released using the CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega) or the ability to reduce methylthiazolyldiphenyl-tetrazolium bromide (MTT). LDH released into the medium by lysed cells were measured with a 10-min enzymatic assay that converts a tetrazolium salt (INT) into a red formazan product. A 96-well based colorimetric assay measured the amount of the red formazan formed, which is proportional to the number of dead cells. Percent LDH release was determined with reference to the maximum and background LDH release of control cells. MTT is reduced in the mitochondria (metabolically active cells) by mitochondrial reductase enzymes to form insoluble purple formazan crystals, which are solubilized by the addition of a detergent (5). Briefly, MTT was prepared at 5 mg/ml in PBS and was diluted in DMEM/F12 1% FBS media to a final concentration of 0.5 mg/ml for assays. After experimental treatments, media were replaced with 100 μl of MTT and incubated for 3~4 h at 37°C. Formazan crystals were dissolved overnight (16 h) at 37°C with 100 μl of lysis buffer [15% (wt/vol) SDS, 50% (vol/vol) dimethylformamide (pH 4.7)]. MTT assay results were presented as percentage of MTT reduction level of treated cells to control cells. Absorbance was read at 490 and 570 nm for LDH and MTT assays, respectively, using a microplate reader (SpectraMax 250; Molecular Devices) and SoftMax Pro 3.0 software (Molecular Devices). *p*-values were calculated with Student's *t* test in GraphPad Prism 4 software.

1. Fabrizio P, Longo VD (2003) *Aging Cell* 2:73–81.
2. McCarthy KD, de Vellis J (1980) *J Cell Biol* 85:890–902.
3. Geng J, Strobel HW (1998) *Brain Res* 784:276–283.
4. Kempermann G, Knoth R, Gebicke-Haerter PJ, Stolz BJ, Volk B (1994) *J Neurosci Res* 39:576–588.
5. Hansen MB, Nielsen SE, Berg K (1989) *J Immunol Methods* 119:203–210.

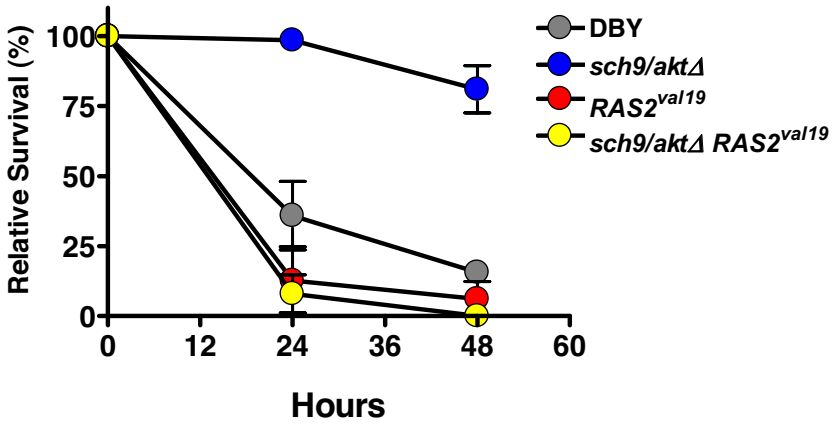

Fig. S1. DSR against chronic CP treatment. Wild-type (DBY746), *RAS2*^{val19}, *sch9/aktΔ*, and *sch9/aktΔ RAS2*^{val19} strains were inoculated at OD = 0.1, grown separately in glucose media, and treated with CP (0.1 M) 24 h after initial inoculation. Viability was measured as colony forming units (CFU) at 24 and 48 h.

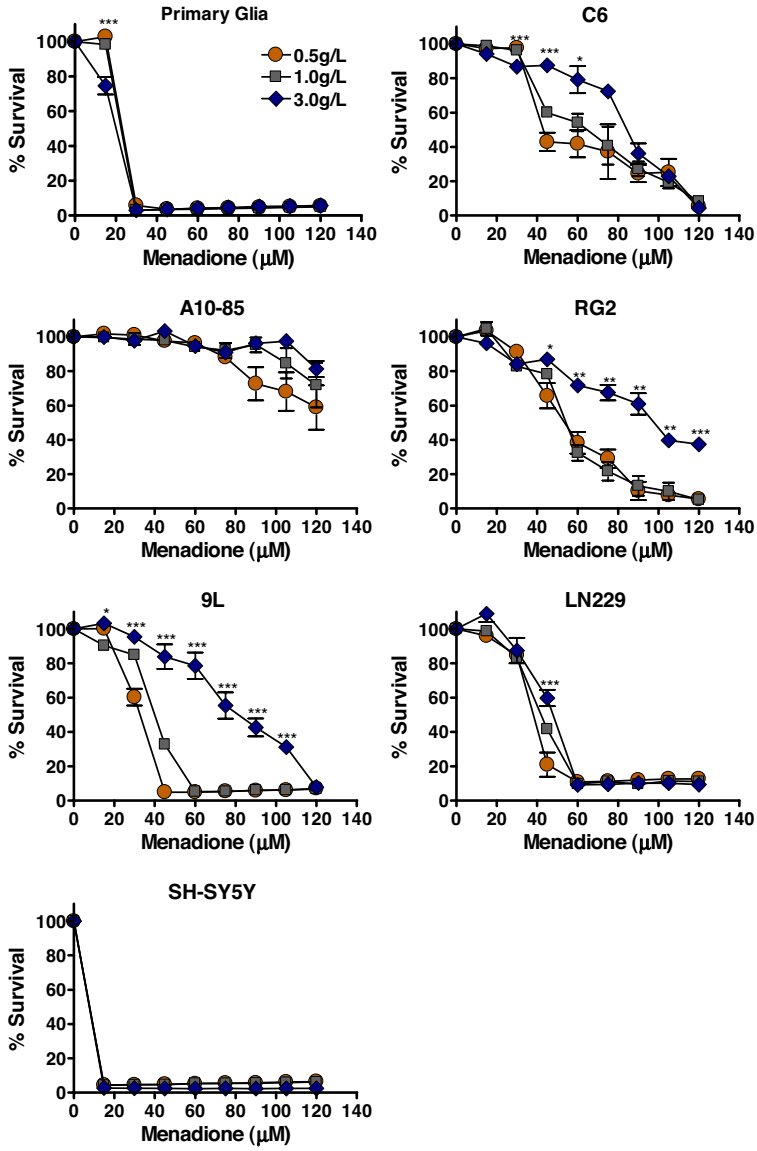

Fig. S2. *In vitro* differential stress response (DSR) to menadione treatments. Primary rat glial cells, rat glioma cell lines (C6, A10–85, RG2, and 9L), a human glioma cell line (LN229), and a human neuroblastoma cell line (SH-SY5Y) were tested for glucose restriction-induced differential stress resistance. Cells were incubated in either low glucose (0.5 g/liter) (STS), normal glucose concentration (1.0 g/liter), or default media glucose (3.0g/liter), supplemented with 1% serum for 24 h. Viability (MTT assay) was determined after a 24-h treatment with 15–120 μM menadione. All data are presented as mean ± SD. *p*-values were calculated by one-way ANOVA (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

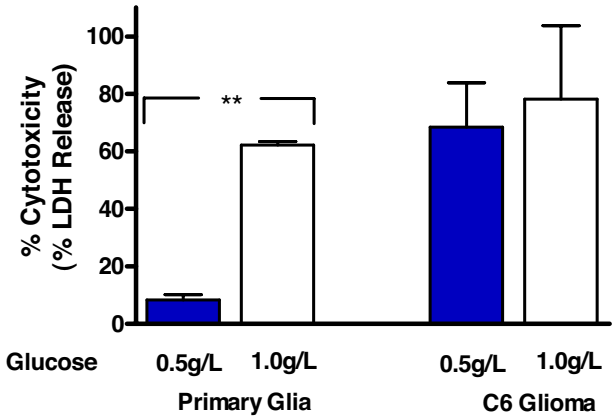

Fig. S3. *In vitro* STS effect on DSR to CP treatments. Primary rat glial cells and the C6 rat glioma cells were grown to 70% confluency and then incubated in either low glucose (0.5g/L) (STS) or normal glucose (1.0g/L), supplemented with 1% serum for 24 h followed by CP (12 mg/ml) treatment. Cytotoxicity was measured by LDH release. Data are represented as mean ± SD. *p*-values were calculated with Student's *t* test (**, $P < 0.005$).

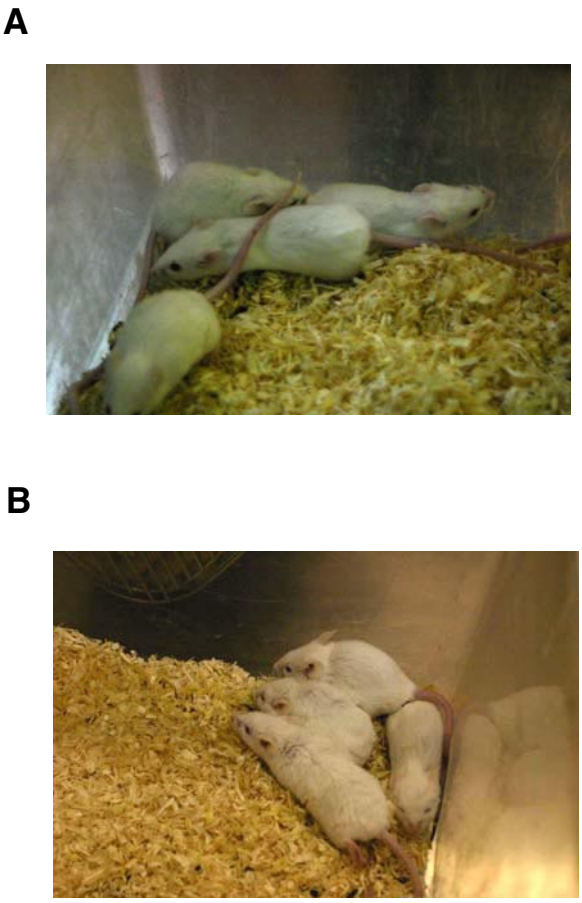

Fig. S4. NXS2/STS/Eto group (*A*) and NXS2/Eto group (*B*) shown after etoposide treatment.

Table S1. Frequency of metastases and survival in NXS2-injected mice (STS, etoposide treatments)

Groups	No. mice	No. toxicity deaths	Liver	Kidneys	Ovaries	Adrenal gland	Hemorrhagic ascites	Median survival, days	Survival range, days
NXS2	16	0/16	16/16	14/16	13/16	3/16	16/16	32	32–38
NXS2/STS	8	0/8	8/8	5/8	7/8	1/8	8/8	30	26–35
NXS2/STS/Eto	16	1/16	15/15	9/15	9/15	0/15	14/15	44	35–60
NXS2/Eto	6	3/6	3/3	3/3	0/3	0/3	3/3	130	87–140

Groups represent the mice shown in Fig. 5 *A* and *B*. A/J mice were i.v. inoculated with 200,000 NXS2 cells per mouse and treated as described in *Materials and Methods*. All mice were followed until death and necropsies were performed. Only three mice from the NXS2/Eto group survived the initial etoposide treatment. The deaths caused by etoposide toxicity in the early days that occurred were not considered in the calculation of median survival.

Table S2. Strains used in this study

Strain	Genotype
DBY746	*MAT*α, *leu2–3*, *112*, *his3*Δ*1*, *trp1–289*, *ura3–52*, *GAL*$^{+}$
sch9/aktΔ	DBY746 *sch9*::*URA3*
ras2Δ	DBY746 *ras2*::*LEU2*
tor1Δ	DBY746 *tor1*::*HIS3*
RAS2^{val19}	DBY746 *RAS2*^{val19} (*CEN*, *URA3*)*
tor1Δ*RAS2*^{val19}	DBY746 *tor1*::*HIS3 RAS2*^{val19} (*CEN*, *URA3*)*
sch9/aktΔ*RAS2*^{val19}	DBY746 *sch9*::*TRP RAS2*^{val19} (*CEN*, *URA3*)*
SCH9/AKT	DBY746 *SCH9* (*CEN*, *URA3*)*

*pRS416-*RAS2*^{val19} was constructed by inserting a 1.9-kb ClaI–HindIII fragment from pMF100 (provided by Dr. J. Broach, Princeton University) into pRS416. Plasmid overexpressing *SCH9* was provided by Dr. K. A. Morano (University of Texas Medical School, Houston).