Supporting Information

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SI Materials and Methods

Cells and Cell Transfection. Human lung H460 and H1299, liver HepG2, and brain HTB-15 cells were obtained from American Type Culture Collection. Human colon HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were a generous gift from B. Vogelstein at Johns Hopkins University. V138/H1299 cells were a generous gift from J. Chen at H. Lee Moffitt Cancer Center. LN-2024 cells were a generous gift from E. Van Meir at Emory University. The siRNA oligo against GLS2 and p53 (Ambion) was transfected into cells using Oligofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Plasmids were transfected into cells using Lipofectamine 2000 (Invitrogen).

Chromatin Immunoprecipitation Assay. Chromatin immunoprecipitation (ChIP) assays were performed using the Upstate ChIP Assay Kit according to the manufacturer's instructions. Primer sets were designed to encompass the potential p53-binding elements in the human *GLS2* gene. The sequences are as follows: for promoter, 5′-GGCCTCCCAAGT CACCAGTTCA-3′ and 5′-TGTTTTTGCTTGTTTTCGCCTTCT-3′; for potential binding element A in intron 1, 5′-CCCCAGAAGCAGGAAAACT-3′ and 5′-TGGGCAACAGAGCGAGACT-3′; and for potential binding element B in intron 1, 5′-CTTTGTGTCAGGCAGT-TTTTCA-3′ and 5′-AATCCACCCTCTCCTCCTTTTC-3′. The p53-binding element in the human MDM2 promoter region was used as a positive control for ChIP assays. The primers are as follows: 5′-GGTTGACTCAGCTTTT CCTCTTG-3′ and 5′-GGAAAATGCATGGTTTAAATAGCC-3′.

Construction of Plasmids. For construction of GLS2 expression vector to express GLS2 protein with C-terminal Flag tag, human full-length GLS2 cDNA was generated by RT-PCR and inserted into p3xFlag-CMV-14 plasmid (Sigma) at HindIII and EcoRI sites. The primers used for PCR are as follows: 5′-AAGCTTGGCAT-GCGCTCCATGAAG-3′ and 5′-TGAATTCGCTACCATGCTT-TCTAAGTTCTC-3′. For construction of the luciferase reporter gene, the TOPO II vector (Invitrogen) was used to clone PCR fragments containing the putative p53-binding elements in the human GLS2 gene by using PCR primers for ChIP assays. The sequence-confirmed clones were subcloned into pGL2 luciferase reporter plasmid (Promega) at XhoI and HindIII sites.

Luciferase Activity Assay. The pGL2 reporter plasmids containing one copy of each putative p53-binding element in the human GLS2 gene were transfected into p53 null H1299 and HCT116 $p53^{-/-}$ cells by using Lipofectamine 2000 (Invitrogen) along with 1 μg of pRC-wtp53 (wild-type human p53 expression plasmid) or pRC-273H (mutant human p53 expression plasmid containing a substitution at R273H) and 0.5 ng of pRL-SV40 plasmid expressing renilla luciferase as an internal control to normalize transfection efficiency. The luciferase activity was measured 24 h after transfection. The reporter activity was calculated as luciferase activity of reporter plasmids in cells with wild-type p53 compared with that in cells with mutant p53.

Quantitative Real-Time PCR. Total RNA was prepared with the RNeasy kit (Qiagen) and treated with DNase I to remove residual genomic DNA. The cDNA was prepared with random primers using the TaqMan reverse transcription kit (Applied Biosystems). Real-time PCR was done in triplicate with TaqMan PCR mixture (Applied Biosystems). The expression of genes was normalized to the Actin gene.

Western-Blot Analysis. Rabbit polyclonal antibody to GLS2 was raised against a 15-aa peptide corresponding to the GLS2 human protein (PFAKDRWGNIPLD DC) (Genescript). Antibodies against p53 and DO-1 were purchased from Santa Cruz Biotechonology. Antibodies against Actin (A5441) and Flag (M2) (Sigma) were purchased from Sigma. For detection of endogenous GLS2 protein, mitochondrial protein was enriched. Mitochondria were isolated from cells by using mitochondrial isolation kits (Qiagen) according to manufacturer's instructions. The isolated mitochondria were lysated in RIPA buffer for the detection of GLS2 protein by Western-blot assays. For the detection of other proteins, whole cell lysates were prepared using RIPA buffer. Protein samples were separated by 4–20% SDS/PAGE and transferred to PVDF membranes. The protein levels were quantified by digitalization of the x-ray film and analyzed with Scion Image software (Scion Corporation).

Immunofluorescence Staining. Cells grown on slides were fixed with 3% paraformaldehyde for 45 min and treated with 0.5% Triton X-100 for 5 min, blocked with 1% bovine serum albumin for 1 h, and stained with anti-Flag (M2; Sigma) to detect Flag-tagged GLS2. Slides were washed and then incubated with fluorescein isothiocyanate-conjugated secondary antibody against mouse immunoglobulin G (Molecular Probes). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma). For MitoTracker (Molecular Probes) staining, cells were incubated in 50 nm MitoTracker for 30 min and then fixed with paraformaldehyde.

Measurement of Intracellular Levels of Glutamate, α-Ketoglutarate, and ATP. Intracellular glutamate levels were measured by using the Amplex Red Glutamine Acid/Glutamate oxidase assay kit (Invitrogen) according to manufacturer's instructions. Intracellular α-ketoglutarate levels were measured by using the α-ketoglutarate assay kit (Biovision), and intracellular cellular ATP levels were measured by using the ATP Bioluminescence assay kit (Roche).

Measurement of Levels of ROS. Cells were treated with or without H_2O_2 (100–600 μM) for 4 h. After treatment cells were trypsinized and resuspended in PBS. Cells (1×10^6) were then incubated with 3 mM dihydrorhodamine 123 (Sigma) in PBS for 30 min at 37 °C. Cells were washed with PBS and then applied to a flow cytometer for measuring ROS levels.

Measurement of Levels of Glutathione and NADH. The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured by using a glutathione detection kit (Biovision) according to manufacturer's instructions. In brief, cell lysates were prepared using Assay Buffer (Biovision). After centrifugation, supernatants were assayed for reduced glutathione by adding o-phthalaldehyde to react only with reduced glutathione and generate fluorescence. For GSSG, a reducing agent was added to convert GSSG to GSH. Fluorescence intensities were monitored using an excitation wavelength of 340 nm and emission wavelength of 420 nm. NADH levels were measured by using an NAD+/NADH quantification kit (Biovision) according to manufacturer's instructions.

Measurement of Oxygen Consumption. Oxygen consumption in cells was measured by using the BD Oxygen Biosensor System according to manufacturer's instructions. This system is an oxygen-sensitive fluorescent compound embedded in a gas-permeable and hydrophobic matrix attached to the bottom of a multiwell plate. The

amount of fluorescence correlates directly to the rate of oxygen consumption in the well. In brief, cells were seeded in a 96-well plate covered with microcarrier beads (Cytodex-3; Sigma), for cells to attach better. Oxygen consumption was measured by a fluorescence plate reader using *Ex*/*Em* = 485/630 nm at different hours after cell seeding.