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Appendix Table S1

Primers used for q-RT PCR		
TRINGS	FW:GGACAAGTATGCACTGCCCT	
	RV:AAGAACGCATGAGCTGGTGA	
Actin	FW:CTGTCCCTGTATGCCTCTG	
	RV:ATGTCACGCACGATTTCC	
U1	FW:GGGAGATACCATGATCACGAAGGT	
	RV:CCACAAATTATGCAGTCGAGTTTCCC	
STRAP	FW:GGCATCACGCCTTATGGGTA	
	RV:CAGTGTTGCACCCCAAACAG	
lncRNA-1	FW:CTCCCAGCCAGGAAGAAAGT	
	RV:TCTTGGCCTCATTCCAAGTC	
IncRNA-2	FW:CAGCACGTGGAGACAGTCAA	
	RV:GCATGCGCTGATTGGTCTTG	
lncRNA-3	FW:CGGAGCAGTCTGTGTCAAAA	
	RV:TCTCCTCCCGTGACAATAC	
lncRNA-4	FW:AGAGATTTTCCAGGCCAAGC	
	RV:TTGGATGGTTCTGTGGGTCT	
lncRNA-5	FW:AGCCACTCTACTTGGCCTGA	
	RV:GTAGGTCCCAGCGTGTCTGT	
lncRNA-6	FW:ATTCTACCATGTCGGCATCCA	
	RV:TCGGGATTCTCCTGTACCTCT	
IncRNA-7	FW:CGTGGCCTACCTTGTCAGTT	
	RV:CAGACCCAATGCCCTCAGTT	
lncRNA-8	FW:GAAAGTGGTGAGCCAACGAT	
	RV:GGCCTGAAAAACTGTTGCAT	

lncRNA-9	FW:GGACAAGTATGCACTGCCCT	
	RV:AAGAACGCATGAGCTGGTGA	
lncRNA-10	FW:GGGTAGGTATTCACCTGCACT	
	RV:TGTGCTGTTCCTGCACTTCA	
Primers used for CHIP		
TRINGS	FW:TTATGGTTAAGGGAACAAAT	
	RV:TAAAGCAAAATTAGGAATGCCTTT	
Actin	FW:TCGATATCCACGTGACATCCA	
	RV:GCAGCATTTTTTTACCCCCTC	
p21	FW:CTGTCCTCCCCGAGGTCA	
	RV:ACATCTCAGGCTGCTCAGAGTCT	
Oligonucleotide sequence of shRNAs		
shTRINGS-1	GCACTGCCCTACTTACCTACT	
shTRINGS-2	AACCAGGTAGTCGTACAGTGT	
shSTRAP	GTCTGTTAGTAGTATGGAATA	
shp53	GACTCCAGTGGTAATCTAC	
shGSK3β	CCCAAATGTCAAACTACCAAA	
Oligonucleotide sequence of DNA Probes for Biotin-pulldown		
sense	CCGAGAGACTCACCAAAGACATGAAGAGAA	
Anti-sense	TTCTCTTCATGTCTTTGGTGAGTCTCTCGG	
Oligonucleotide sequence of single-guide RNA		
sgRNA-1	TACTAAACAGACCCAGACTT	
sgRNA-2	ATCTGGACACTCCCAAGTCT	

(A) H1299 cells carrying a p53 tet-on system were treated with DMSO (Dox-) or doxycycline (Dox+, 1ug/ml) for indicated times. Total RNA were analyzed for evaluating indicated lncRNAs expression by real-time RT-PCR using primers against indicated lncRNAs.

(B) U2OS cells were incubated with 2.5 mM glucose starvation medium for 0 h, 12 h or 24 h, and total RNA was then isolated for measuring indicated lncRNAs by real-time RT-PCR.

(C) Schematic illustration of the intron-exon structure of *trings* on chromosome 4.Its corresponding nucleotide sequences are shown in the same color.

(D) Genomic location of TRINGS was shown in the ideogram of chromosome 4, and the genomic tracks with its neighborhood were also represented. Evolutionary conservation of TRINGS was illustrated using multiple alignments with phastCons and phyloP tracks of genome browser. Annotations were taken from UCSC genome browser.

Appendix Figure S2

(A) p53 dependent expression of TRINGS in different types of tumors as compared with mutant p53 in TCGA datasets, the information was extracted and plotted through TANRIC database.

(B-D) U2OS cells were incubated with DMEM medium deprived of FBS (A), Serine (B) or Glutamine (C) for indicated times. Total RNAs were isolated from cells exposed to indicate nutritional stresses and levels of TRINGS were determined by real-time RT-PCR, and cell lysates were analyzed by Western blotting with anti-p53 or anti-Actin antibodies.

(E)H1299 cells (without p53) were cultured under glucose starvation (2.5mM glucose) for the indicated time points. The cells were harvested for total RNA extraction subsequently analyzed by real time RT-PCR.

(A-E) Control or p53 knockdown U87cells (A), Mel-CV cells (B), SMCC7721 cells (C), IMR90 cells (D) or MCF10A cells (E) were incubated with 2.5 mM glucose starvation medium for indicated time periods. Total RNA were analyzed by real-time RT-PCR to examine RNA level of TRINGS. Cell lysates were used to access p53 expression by Western blotting using anti-p53 antibody.

(F) Control shRNA (shctrl) or TRINGS knockdown (shTRINGS-1) U2OS cells were harvested for total RNA extraction, subsequently indicated lncRNAs expression level was measured by real time RT-PCR. Data shown are mean \pm SD (n=3).

Appendix Figure S4

(A-C) Fluorescence microscopy images of control or TRINGS knockdown U87 cells (A), Mel-CV cells (B) or SMCC7721 (C) cells incubated with 2.5 mM glucose starvation medium for indicated times and followed by double staining with PI and Hochest 33342.

(D) U2OS cells were treated with 1mM H_2O_2 indicated time. The total RNA was analyzed for TRINGS expression by real time RT PCR. Data shown are mean \pm SD (n=3). Cell lysates were also analyzed by Western blotting with anti-p53 and anti-Actin antibodies.

(E) U2OS cells containing control shRNA (shctrl), shTRINGS-1 or shTRINGS-2 were cultured in DMEM medium with 1mM H_2O_2 for 12 h. Cells were then stained with PI and Hochest33342 and visualized by fluorescent microscope.

(F) U2OS cells infected with control shRNA (shctrl), shTRINGS-1 or shTRINGS-1 plus shSTRAP were incubated in DMEM medium with 1mM H_2O_2 for 12 h. Cells were then stained with PI and Hochest33342, and visualized by fluorescent microscope. PI positive cells were counted from 300 Hochest33342 positive cells. Bars represent means \pm SD of three independent experiments. Cell lysates were analyzed by Western blotting using anti-STRAP or anti-Actin antibody. (***, p < 0.001; error bars are \pm S.E.).

(G) Control shRNA (shctrl) or shTRINGS U2OS cells were incubated with 2.5 mM glucose starvation medium with or without 5μ M CHIR98014 (inhibitor of GSK3 β activity) for 32 h. Cells were then stained with PI and Hochest33342, and visualized

by fluorescent microscope. PI positive cells were counted in 300 Hochest 33342 positive cells and plotted. Bars represent means \pm SD of three independent experiments. (***, p < 0.001).

Appendix Figure S5

(A) Control shRNA (shctrl), shTRINGS-1 or shTRINGS-2 U2OS cells were incubated under normal condition, growth curves of cells were measured for the indicated periods of time. Data shown are mean \pm SD (n=3).

(B) 10^3 shctrl, shTRINGS-1 or shTRINGS-2 U2OS cells were seeded into soft agar for each well of 6-well plate. Two weeks later, cells were subjected to crystal violet staining and photographs were taken.

(C) $2x10^6$ shctrl or shTRINGS-1 U2OS cells were then injected subcutaneously into nude mice (n = 8 for each group). Representative photographs of xenografts were taken 2 weeks after injection.

(D) Lysates from HCT116 cells expressing Flag or Flag-STRAP and Flag-STRAP mutants as indicated were used for coimmunoprecipitation (co-IP) using anti-Flag resin. Eluted products were used for examining TRINGS and STRAP by real-time RT-PCR and Western blotting analysis respectively.

Appendix Figure S6

(A) The distribution of strap was detected by western blot. H2A and GAPDH were used as markers for nucleus and cytosol, respectively.

(B)Control, p53 knockdown (shp53) or p53 overexpressed (Flag-p53) U2OS cells were harvested, and cell lysates were analyzed by Western blotting using indicated antibodies.

(C) Control or p53 knockdown U2OS cells were treated with 50μ g/ml of cycloheximide (CHX) for indicated times. Subsequently cell lysates were analyzed by western blotting with anti-p53, anti-STRAP and anti-Actin antibodies. Total RNA was analyzed by real-time RT-PCR analysis for TRINGS expression. Data shown are mean ±SD (n=3).

(D) Control or p53 overexpression U2OS cells were treated with 50μ g/ml of cycloheximide (CHX) for indicated times. Subsequently cell lysates were analyzed by western blotting with anti-p53, anti-STRAP and anti-Actin antibodies. Total RNA was analyzed by real-time RT-PCR analysis for TRINGS expression. Data shown are mean ±SD (n=3).

(E) Control shRNA (shctrl) or TRINGS knockdown U2OS cells were transfected with control vector or Flag-STRAP separately. 24 h after transfection, cell lysates were used for co-immunoprecipitation assay with anti-Flag coupled resin. The input and co-immunoprecipitates were analyzed by Western blotting using anti-Flag or anti-GSK3 β antibodies. The intensity of STRAP-binding GSK3 β bands was quantified by densitometry analysis.

(F) U2OS cells under normal condition or glucose starvation were harvested, and the cell lysates were used for co-immunoprecipitation assay with anti-GSK3 β antibody. The input and co-immunoprecipitates were analyzed by Western blotting using anti-STRAP or anti-GSK3 β antibodies. The intensity of GSK3 β -binding STRAP bands were quantified by densitometry analysis.

(G) Lysates from U2OS cells under normal condition or glucose starvation were used for co-immunoprecipitation (co-IP) using lgG or anti-STRAP antibody. Eluted products were used for assessing TRINGS and STRAP by real-time RT-PCR and Western blotting analysis respectively.

Appendix Figure S7

(A) U2OS cells expressing control shRNA (shctrl) or shTRINGS-1,-2 were treated with Doxorubicin (1 μ g/ml), Nutlin-3 (Mdm2 inhibitor) (20 μ M) or glucose starvation (2.5mM, 32h) individually, and the cell lysates were assayed by Western blotting using antibodies against PARP, p53 and Actin.

(B) U2OS cells were treated with DMSO (control), Doxorubicin (1 μ g/ml), Nutlin-3 (20 μ M) or glucose starvation (2.5mM, 32h), and the levels of TRINGS were measured by real time RT-PCR. Cell lysates were used for monitoring p53 activation by Western blot analysis.



D





(2.5mM)











Anti-Flag



