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Supplementary Materials for

SCAN1-TDP1 trapping on mitochondrial DNA promotes mitochondrial dysfunction and mitophagy

Arijit Ghosh, Sangheeta Bhattacharjee, Srijita Paul Chowdhuri, Abhik Mallick, Ishita Rehman, Sudipta Basu, Benu Brata Das*

*Corresponding author. Email: pcbbd@iacs.res.in

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Supplementary Figures

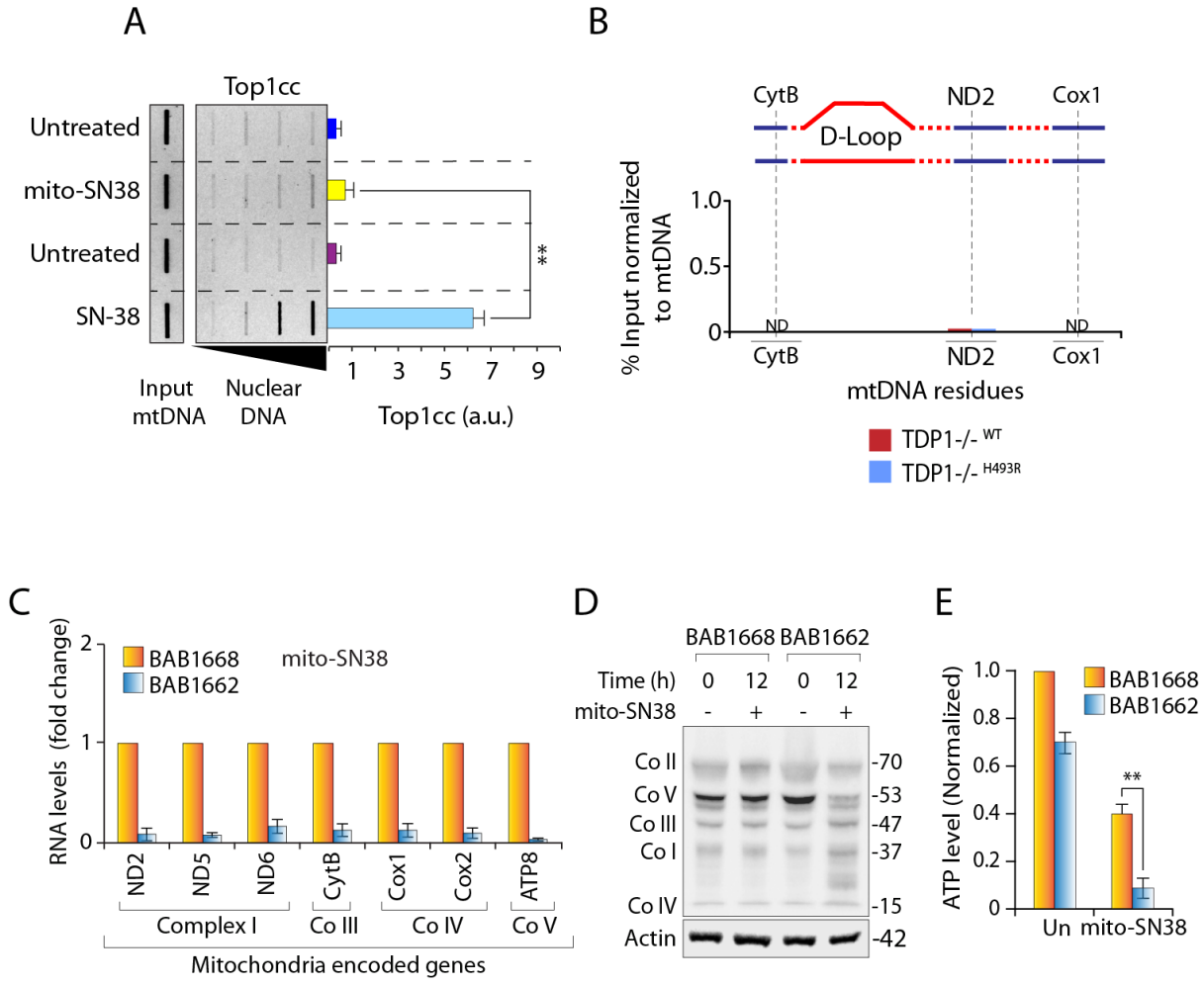

Fig. S1. Mito-SN38 does not trap nuclear-Top1cc but impairs mitochondrial metabolism through SCAN1-TDP1 trapping in the mitochondria. (A) Nuclear Top1cc was detected by ICE bioassay with nuclear Top1 specific antibody after treated with mito-SN38 (5 μM, 3 h) or SN-38 (1 μM, 3 h). Total DNA at increasing concentrations (0.5, 1, 2 and 4 μg) was immunoblotted with an anti-nuclear Top1 specific antibody. The DNA input was probed with anti-dsDNA antibody. Densitometry analysis of trapped Top1 band intensity were quantified and expressed as fold increase relative to DNA input. (Error bar: mean values ± SEM). Asterisks denote statistically significant difference (**P<0.01; t-test) **(B)** Detection of $TDP1^{H493R}$ -trapping

sites on mtDNA by chromatin immunoprecipitation (ChIP) followed by mtDNA specific qPCR analysis. FLAG-TDP1-DNA adducts was immunoprecipitated with anti-FLAG antibody in indicated cells after treatment with mito-SN38 treatment (5 μM, 3 h), and the putative TDP1-binding site was quantified by qPCR. The mtDNA copy numbers of each cell line were concomitantly measured using primers for the mitochondrial (ND2, Cox1 and CytB) and nuclear (B2M) genes. Enrichment of TDP1-bound mtDNA is expressed as percent input, which is then normalized to the mtDNA copy number of the cell line. Data represents the mean ± standard error of independent experiments. (**C)** Gene expression profile of mitochondria-encoded ND2, ND5, ND6, CytB, Cox1, Cox2 and ATP8 by RT-PCR in SCAN1 patient derived lymphoblastoid cell lines (BAB1662) and their wild type counterpart (BAB1668) after treatment with mito-SN38 (5 μM, 6 h). Data represents the mean ± standard error of three independent experiments. (**D**) Representative western blot of nuclear-encoded OXPHOS subunits in indicated cells before and after treatment with mito-SN38 (5 μM, 12 h). Actin is shown as the loading control. (**E**) ATP level estimation in BAB1668 and BAB1662 cells treated with or without mito-SN38 (5 μM, 6 h). Data represents the mean ± standard error of three independent experiments. Asterisks denote statistically significant difference (**P<0.01; t-test).

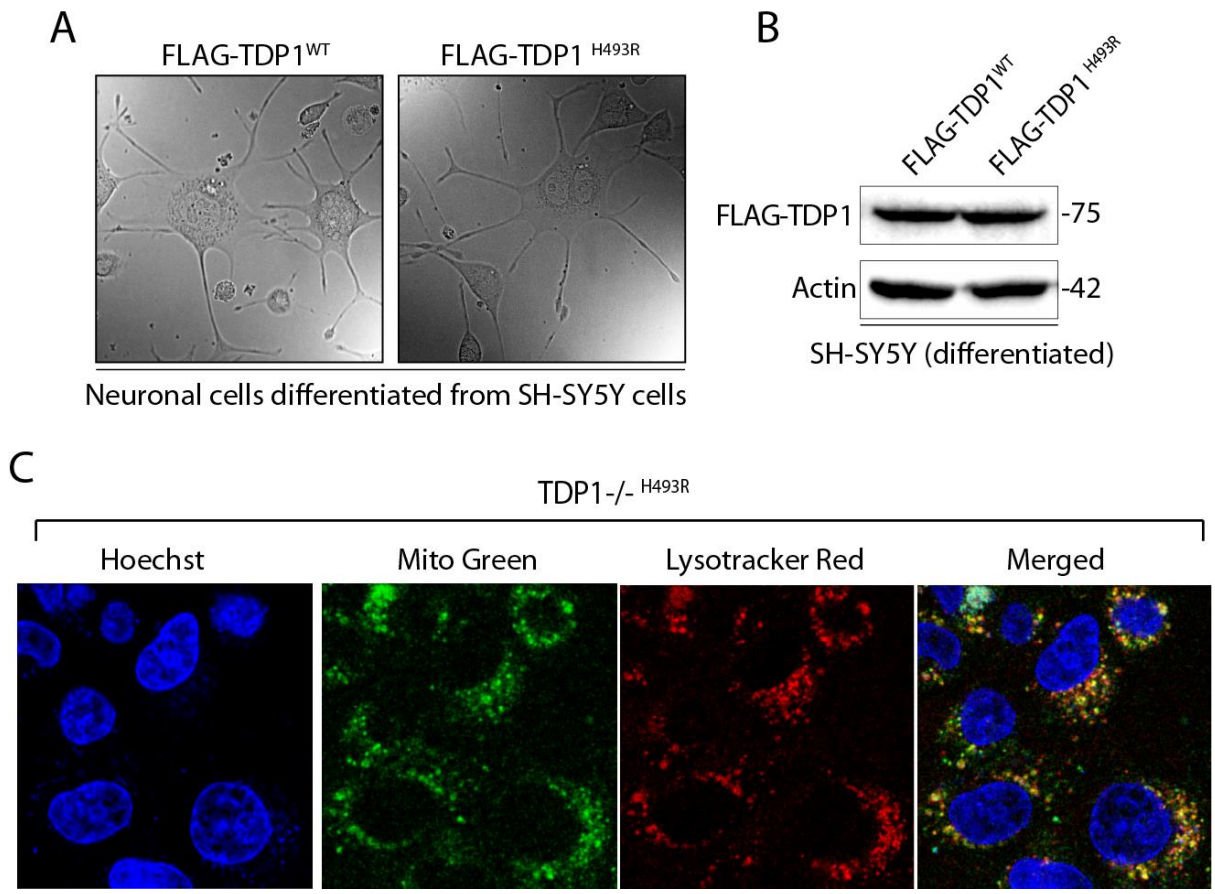

Fig. S2. Differentiation of SH-SY5Y cells showing expression of FLAG-TDP1 and lysosomal localization of SCAN1 mitochondria showing mitophagy. (**A**) Representative images of retinoic acid induced differentiated neuronal cells from human neuroblastoma (SH-SY5Y) cells transfected with lentiviral constructs FLAG-TDP1^{WT} or FLAG-TDP1^{H493R} under live cell confocal microscopy. (**B**) Western blot analysis of FLAG-TDP1 in whole cell lysates obtained from differentiated neuronal cell expressing FLAG-TDP1^{WT} or FLAG-TDP1^{H493R} detected with anti-FLAG antibody. Actin served as the loading control. **(C)** Representative images of TDP1-/-^{H493R} MEFs treated with mito-SN38 (2.5 μM, 24 h) analysed under live cell microscopy. The green fluorescence signal of MitoTracker green denote mitochondrial network;

red fluorescence signal of LysoTracker red denote lysosomal vesicles and the yellow fluorescence signal denote colocalization of mitochondria with lysosomes (merged image) .

Table S1. List of primers used.

List of primers used in Real Time PCR

Gene Name	Species	5'-Forward Primer	5'-Reverse Primer
ACTINrt	Human	GACCCAGATCATGTTTGAGACC	CATCACGATGCCAGTGGTAC
ATP8rt	Human	ACCGTATGGCCCACCATAATTACC	TTTATGGGCTTTGGTGAGGGAGGT
ND2rt	Human	ACTGCGCTAAGCTCGCACTG	ATTATGGATGCGGTTGCTTG
ND5rt	Human	GGTTTCATCCTCGCCTTAGC	ACCTAATTGGGCTGATTTGC
ND6rt	Human	AGGATTGGTGCTGTGGGTGAAAGA	ATAGGATCCTCCCGAATCAACCCT
Cox1rt	Human	ACCCTAGACCAAACCTACGC	TAGGCCGAGAAAGTGTTGTG
Cox2rt	Human	ACAGATGCAATTCCCGGACG	GGCATGAAACTGTGGTTTGC
CytBrt	Human	CTCCCGTGAGGCCAAATATC	GAATCGTGTGAGGGTGGGAC
TFAMrt	Human	GACTTCTGCCAGCATAATAC	GAGTTCTGCCTGCTTTATG
NRF1rt	Human	GGAGTGATGTCCGCACAGAA	CGCTGTTAAGCGCCATAGTG
PGC1rt	Human	GTCACCACCCAAATCCTTAT	ATCTACTGCCTGGAGACCTT

List of primers used in ChIP analyses

Gene Name	Species	5'-Forward Primer	5'-Reverse Primer
B2M	Mouse	GCTACGTAACACAGTTCC	GTGAGCCAGGATATAGAAAG
MTNCR15420	Mouse	CCAAAGCTGGTATTCTA	TATGACCTGAACCATTG
MTNCR15690	Mouse	CTTCCATATGACTATCCC	CCTGAAGTAAGAACCAG
MTNCR16060	Mouse	CTACGGTGAAGAATCATTAG	GTTTGGCATTAAGAGGAG
MT Heavy & Light Strand Promoter (HSP-LSP)	Mouse	CTCCTCTTAATGCCAAAC	GAATTGATCAGGACATAGG
CYTBrt	Mouse	ATTCCTTCATGTCGGACGAG	ACTGAGAAGCCCCCTCAAAT
Cox1rt	Mouse	TTTTCAGGCTTCACCCTAGATGA	CCTACGAATATGATGGCGAAGTG
ND2rt	Mouse	CCATTCCACTTCTGATTACC	GTCATGTAAGAAGAATAAGTCC

List of primers used in Long Range PCR

Gene Name	Species	5'-Forward Primer	5'-Reverse Primer
LRMT	Mouse	GCCAGCCTGACCCATAGCCATAATAT	GAGAGATTTTATGGGTGTAATGCGG
SRMT	Mouse	CCCAGCTACTACCATCATTCAAGT	GATGGTTTGGGAGATTGGTTGATGT
LRMT	Human	TTTCATCATGCGGAGATGTTGGATGG	TCTAAGCCTCCTTATTCGAGCCGA
SRMT	Human	CCCACAAACCCCATTACTAAACCCAC	TTTCATCATGCGGAGATGTTGGATGG

List of primers used in Site Directed Mutagenesis

Gene Name	Species	5'-Forward Primer	5'-Reverse Primer
TDP1 H493R	Human	CGCAGCAATGCCATGCCACGTATTAA GACATATATGAGG	CCTCATATATGTCTTAATACGTGGCA TGGCATTGCTGCG
TDP1 C-Ter	Human	GGAGAAGAATTCATGATCAAGGATA TTTTATCTCC	GGAGAAGGATCCCGGAGGGCACCCA CATGTTG