Supplementary information for

Suppression of Mic60 compromises mitochondrial transcription and oxidative phosphorylation

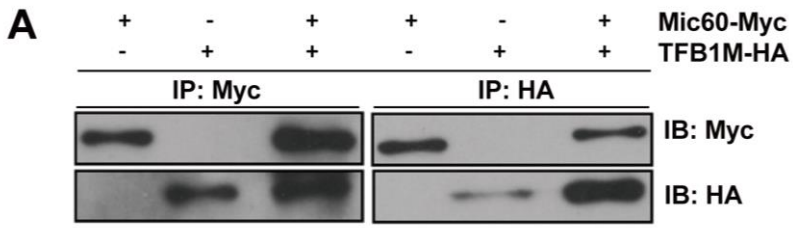
Rui-Feng Yang[1#], Li-Hong Sun[1#], Ran Zhang[1#], Yuan Zhang[1], Yu-Xuan Luo[1], Wei Zheng[1], Zhu-Qin Zhang[1], Hou-Zao Chen[1] & De-Pei Liu[1]*

1 State Key Laboratory of Medical Molecular Biology, Department of Biochemistry and Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, P.R. China

* Correspondence and requests for materials should be addressed to D.P.L. (liudp@pumc.edu.cn)

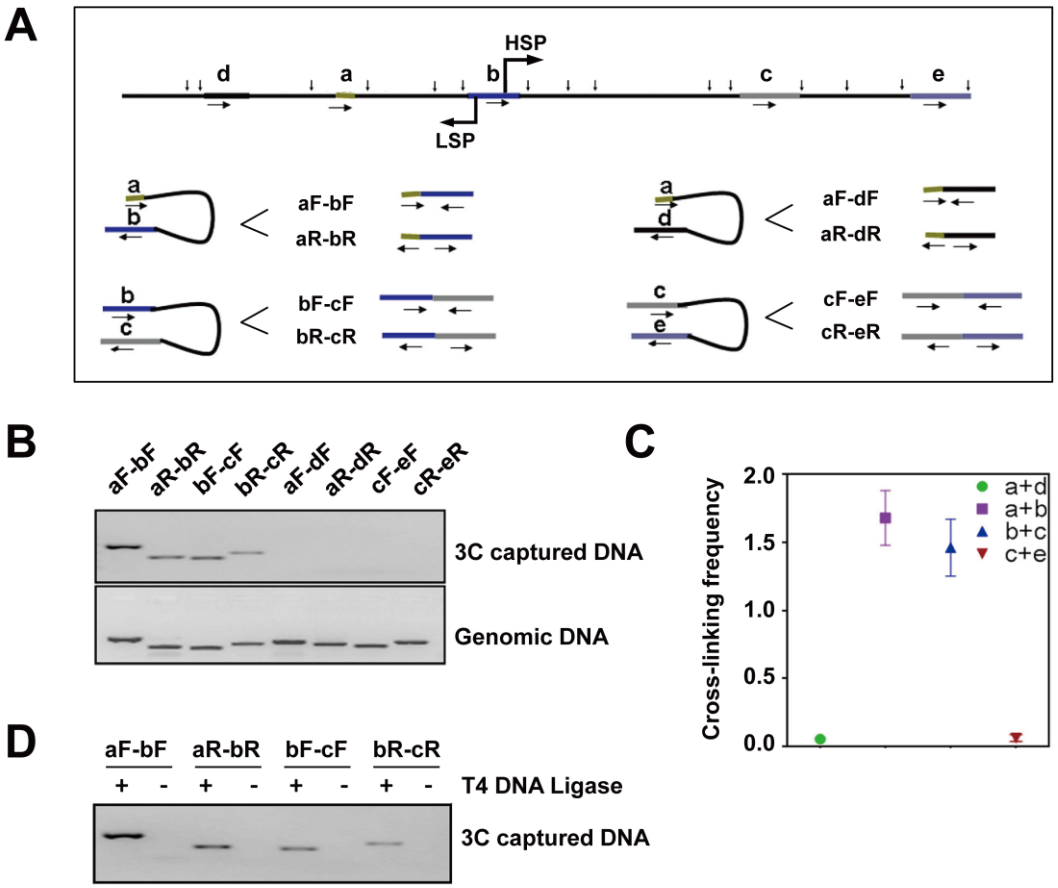
[#] These authors contributed equally to this work

Supplementary Figure S1.



Supplementary Figure S1. Mic60 interacts with TFB1M. (A) Co-immunoprecipitation (IP) of Mic60 and TFB1M in cell lysates using antibodies specific for the c-Myc tag or the HA tag. Cell lysates were obtained from HEK293T cells transfected with Mic60-Myc and TFB1M-HA. Input, 10%. Immunoblotting assays were performed after IP.

Supplementary Figure S2.



Supplementary Figure S2. A higher-order mtDNA structure detected by 3C assay using *CviA* II digested fragments. (A) Schematic representation of 3C design and proposed ligation fragment. *CviA* II restriction endonuclease sites are depicted by arrows above the sequence. The primer sets used in the *CviA* II-digested samples are shown, with F representing the forward direction of DNA and R representing the reverse direction. DNA was digested and intermolecularly ligated for use as a positive control. (B) 3C analysis of *CviA* II-digested samples. As described in Methods, mtDNA-protein complexes were digested by *CviA* II and ligated, and the ligated DNA were eluted for PCR analysis. PCR products were generated with primer pairs aF-bF, aR-bR, bF-cF and bR-cR. (C) Cross-linking frequency determination of *CviA* II-digested samples by 3C assays. Cross-linking frequencies were determined in triplicate, and means and SEM are plotted for every primer pair. (D) 3C analysis of *CviA* II-digested samples. PCR products were obtained between a-b and b-c ligated fragments in the presence of DNA ligase. Unligated samples were used as negative controls.

Supplementary Table S1. RT-PCR primers for real-time PCR.

Gene	Species	Forward primer sequence	Reverse primer sequence
COXI	Mouse	TTGGAGGCTTTGGAAACTGAC	GAGAAGGAGAAATGATGGTGGTAG
COXII	Mouse	TGCTCTGAAATTTGTGGATCTAACC	TTTTTTTTTTTTTTTTTTTTTTAAATTAAA
COXIII	Mouse	GAAACCACATAAATCAAGCCCTAC	TGAAGAATGTAGAACCATAGATACC
Cyto b	Mouse	CCAAATCTCCACGGTCTGTTC	CTCTCCCCAGGTGATGCCT
ND1	Mouse	AGTTCCCCTACCAATACCACACC	GGAGTTTGAGGCTCATCCTGATC
ND2	Mouse	CATAGGGGCATGAGGAGGACT	TGAGTAGAGTGAGGGATGGGTTG
ND4	Mouse	CCTCACATCATCACTCCTATTCTG	GGCTATAAGTGGGAAGACCATTTG
ND5	Mouse	GCCTGATAATAGTGACGCTAGGA	CTATGAATGATTGAGCCAGAGCAT
COXIV	Human	CATGTGGCAGAAGCACTATGTGT	GGTTCACCTTCATGTCCAGCA
Cyto c	Human	GTGCCAGCGACTAAAAAGAGAAT	AGTCTTGTGCTTGCCTCCCTT
SDH	Human	CTGGAGATCCGAGAAGGAAGAG	AGCGAAGATCATGGCTGTCTC
12S rRNA	Human	CACTACGAGCCACAGCTTAA	TCAGGGTTTGCTGAAGATGG
16S rRNA	Human	GGCATGCTCATAAGGAAAGG	GGCCGTTAAACATGTGTCAC
COXI	Human	GATTTTTCGGTCACCCTGAAG	CTCAGACCATACCTATGTATC
COXII	Human	CTATCCTGCCCGCCATCATC	GATTAGTCCGCCGTAGTCGG
COXIII	Human	CACATCCGTATTACTCGCATC	GAAGTACTCTGAGGCTTGTAG
Cyto b	Human	CAAACTAGGAGGCGTCCTTG	CTGGTTGTCCTCCGATTCAG
ND1	Human	CCTAGGCCTCCTATTTATTC	GAATGATGGCTAGGGTGAC
ND2	Human	CTACGCCTAATCTACTCCAC	CTTTGAAGGCTCTTGGTCTG
ND4	Human	GGACTCCACTTATGACTCCC	GGTTGAGAATGAGTGTGAGGC
ND5	Human	CTATCACCACTCTGTTCGCAG	GTGGTTGGTTGATGCCGATTG
ND6	Human	CTAAAACACTCACCAAGACC	GGAATGATGGTTGTCTTTGG
Mitofilin	Human	GGGTACAAGAACAGGAATTGAAGT	AGAAACGGGCTCTAAGGGTCTC
GAPDH	Human	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT

Supplementary Table S2. PCR primers for 3C assays.

Primer	Primer sequences
aF	TGTGGGGGGTGTCTTTGG
aR	CCACAGCACTTAAACACATCTCTG
bF	GGGAGGGGGTTTTGATGTG
bR	GTACCATAAATACTTGACCACCTGTAG
cF	GCGTGAAGGTAGCGGATGA
cR	ACTTTATTGACTCCTAGCCGCAG
dF	AACTCACTGGAACGGGGATG
dR	GACCTGGCGGTGCTTCATATC
eF	GCGAGAATAATGATGTATGCTTTG
eR	AACAGAAACAAAGCATACATCATTATTC
a1F	AAAAAAGTAAAAGGAACTCGGCAAAC
a1R	CGGAGATGTTGGATGGGGTG
a2F	GGATAATGCCGATGTTTCAGGTT
a2R	ACACAGCAAGACGAGAAGACCCT
a3F	GCCAAGGAGTGAGCCGAAGTT
a3R	AGCCTGCGTCAGATCAAAACACT
a4F	CCTACAAACAACTAACCTGCCACT
a4R	TCTTCTATGATAGGGGAAGTAGCGT