Epigenetic Modification of miR-663 Regulates Mitochondria-to-Nucleus Retrograde Signaling and Tumor Progression.

Trevor Carden[1], Bhupendra Singh[1], Ved Mooga[1], Prachi Bajpai and Keshav K Singh [1,2, 3 4]

Departments of Genetics[1], Pathology[2], Environmental Health[2], Center for Free Radical Biology[2], Center for Aging[2] and UAB Comprehensive Cancer Center[2], University of Alabama at Birmingham, Birmingham, Alabama and Birmingham Veterans Affairs Medical Center[3], Birmingham, Alabama, 35294, USA

Supplemental Contents

Table S1
List of PCR primers

GENE	FORWARD	REVERSE
NDUFB8	TGTATGCAGCTCTTCGGTTTC	CTGGTTCTTTGGAGGGATCAC
SDHA	CCCTCCAATTAAACCAAACGC	CAGGTGCTTTAGGTCTCCATAG
SDHB	TTGACTCTACTTTGACCTTCCG	TCCAGTTTCTCACGCTCTTC
SDHC	ATGGAGCGGTTCTGGAATAAG	ATGAGAGGGAAGACAAGTGC
SDHD	TGGGAATTGTCGCCTAAGTG	AAACACTGACAACCCTCTCG
UQCRC1	AATGGGGCAGGCTACTTTTT	GGTCAAGTCTGCACGAGACA
UQCRC2	CAAAGTTGCCCCCAAACTTA	AGCCATGTTTTCCCTTGTTG
UQCRQ	AGTGCAGTGGTGTGATCTCG	CTGTGCCCATTTCCTCATCT
UQCRFS1	GGCAACGGCAGTAATAACCA	CCCACACAGACATCAAGGTG
UCRC	CTTCAAAGCCCTCTGCAAAC	GCCCAAGACAATTCTTCCAA
UQCRB	TTCTCTGTTCGCGATGTGAC	GCTGCATCCACAGACTTCAA
UQCRH	ACTGGAGGACGAGCAAAAGA	TGATGCCCAGATGATGAAGA
CYC1	CCAAAACCATACCCCAACAG	TATGCCAGCTTCCGACTCTT
CYTB	TGAAACTTCGGCTCACTCCT	AATGTATGGGATGGCGGATA
COX5A	AACAAGCCAGATATAGATGCC	TGCTTTGTCCTTAACAACCT
COX4L1	CTTAATGCGATACAACTCGAC	CTCATGAAAGTGTTGTGAAGAG
COX6A1	ACCATACTCTATTCCATAACCC	TTTATTTAAGCCATCTCCTGCC
COX7C	CATTTCATCTGTCCTCATTCTC	AAATGGCAAATTCTTCCCAG
NDUFAF1	GCAGGAGGTCAAGATTCCTTT	CTTGGGTTAAGCTCTGGAGAAT
SDHAF1	AGACTAGCTTGACGAATTGGG	GAGCCAGAAGTGGGTATGTT
SDHAF2	GTGTCTACAGTGTTCTCGACTTC	CTCGTTAATCAGGCGGTCATAG
UQCC1	GCTGGTAGAGTATGTGAGGAAAC	TCGTTGTAAGTCGGAGAATGG
UQCC2	GAGAATACCCAGGTTGCAGAG	TCAGCTTGTACTCTTCCAACG
TTC19	GCCAACTTAGCATTTATACGGG	GGCCAGCTTTAGGGAAATTTC
SCO1	GGAATGAAGCACGTCAAGAAAG	TCCTTGTCAGTTTTACGCTCC
PTCD2	GACATGTGTTCTCGGAGGAAG	GCACAGCATCCAAAGAATCAG
JunB	ACGACTACAAACTCCTGAAACC	GAGCCCTGACCAGAAAAGTAG
TGFβ1	GTCTACACAGTCTTTGCTCCC	CCTCCGCTAACCAGGATTTC
β-Actin	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACAATG

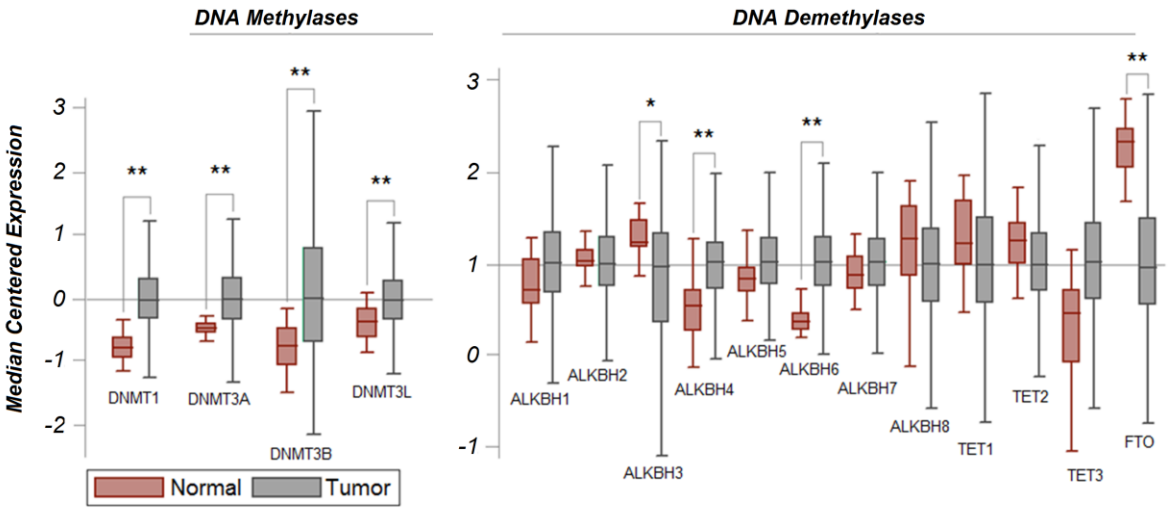

Figure S1
Expression of DNA Methylases and Demethylases in Breast Tumors
Median-centered expression data from TCGA breast samples (n=22 normal and 522 tumor) for four DNA methylases (left panel) and 12 DNA demethylases (right panel). *p<0.05, **p<0.01,***p<0.001

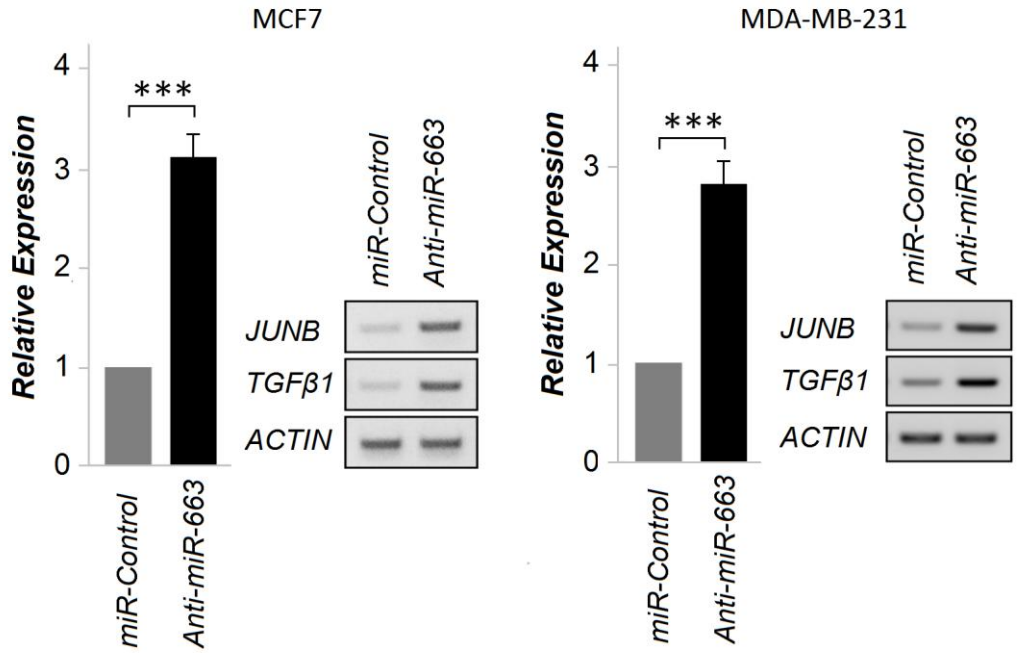

Figure S2
Stable Over-expression and Knockdown of miR-663 in Breast Cancer Cell Lines
A) Expression of the miR-663 primary transcript in MCF7 breast cancer cells stably expressing a pre-miR-663 expression plasmid (left side). Increased RNA Expression of TGFβ1 and JunB, two known target genes of miR-663, demonstrates inhibition of miR-663 by the anti-miR-663 construct (Right side). B) Expression of the miR-663 primary transcript in MDA-MB-231 breast cancer cells stably expressing pre-miR-663 (left side). Increased RNA Expression of TGFβ1 and JunB demonstrates inhibition of miR-663 (Right side). ***p<0.001. Real-Time PCR data represent three biological replicates and error bars denote standard deviation. Semi-quantitative PCRs are representative of two biological replicates.

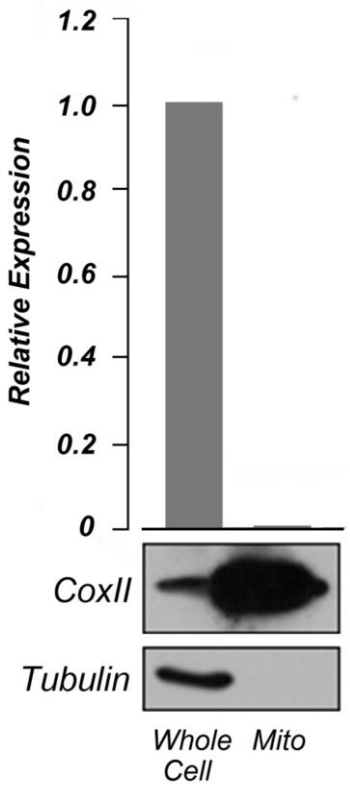

Figure S3
miR-663 Does Not Localize to Mitochondria
Real Time PCR of mature miR-663 in MCF7 mitochondria. Crude mitochondria were isolated from MCF7 cells and purified on a sucrose gradient. cDNA was prepared from RNA of the purified mitochondria.

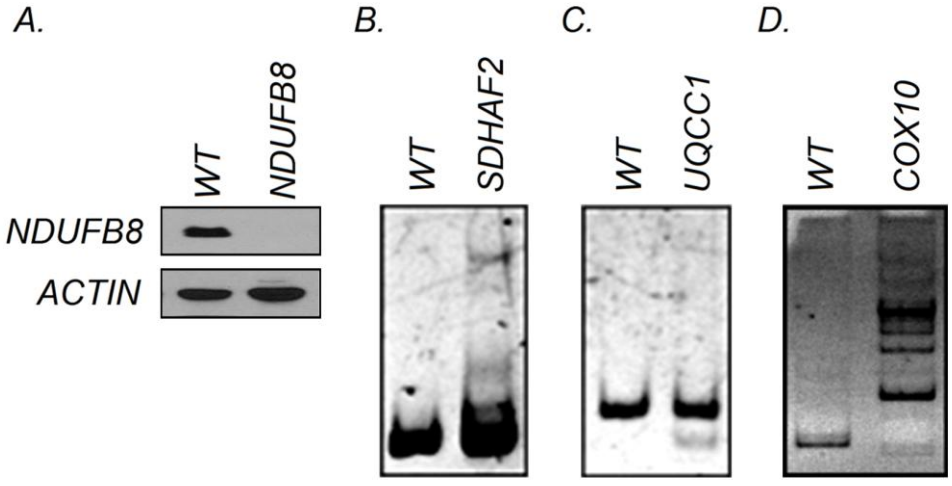

Figure S4
Confirmation of Gene Dysruption by CRISPRs
A) Western blot was used to assess disruption of NDUFB8 in MCF7 WT cells transfected with a CRISPR targeting NDUFB8. Heteroduplex Mobiliy Assays were used to assess gene disruption of MCF7 cells transfected with CRISPRs against B) SDHAF2 C) UQCC1 and D) COX10.