Endocytosis-mediated mitochondrial transplantation: Transferring normal human astrocytic mitochondria into glioma cells rescues aerobic respiration and enhances radiosensitivity

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Supplementary figures and figure legends

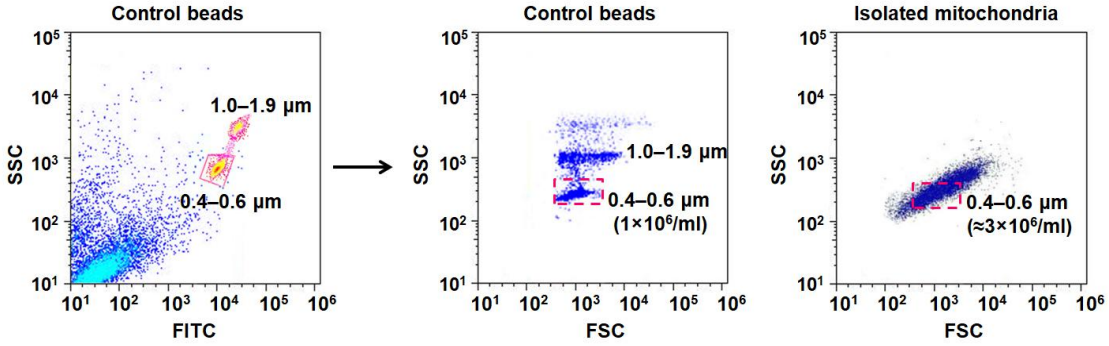
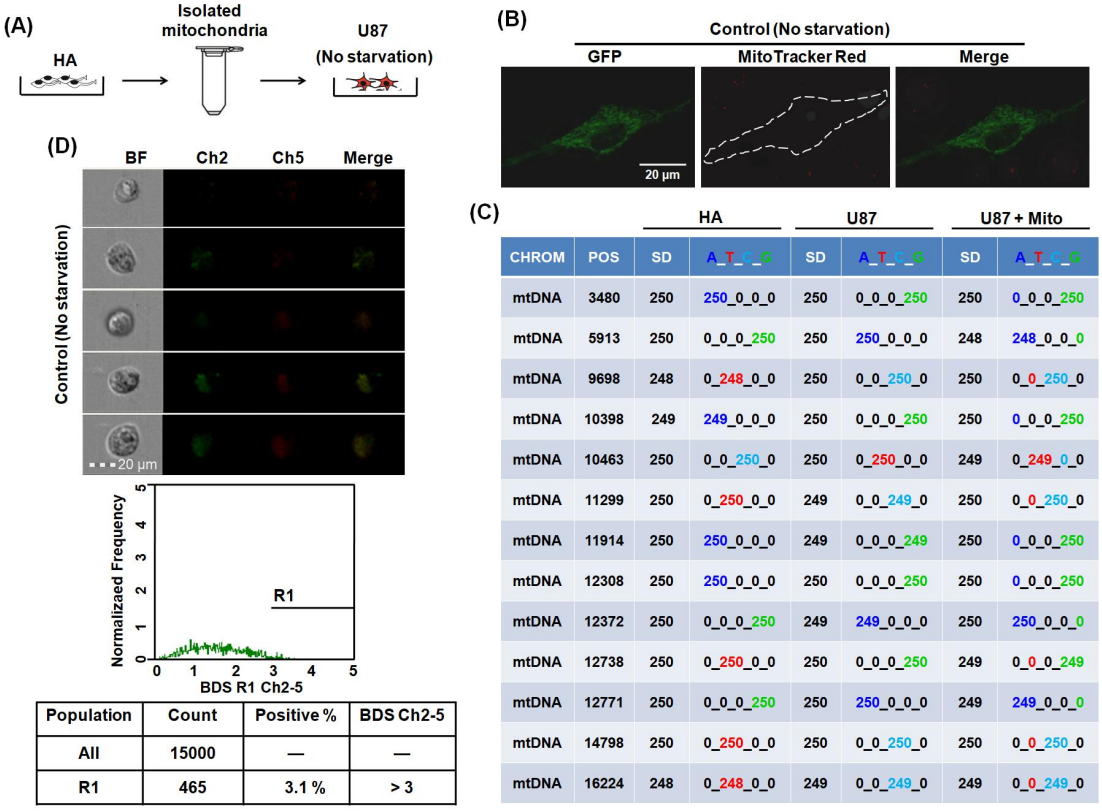


Figure S1. Flow cytometric analysis of isolated mitochondria from human astrocytes. Refer to Mattiasson's work with some modified [1], we try to use flow cytometry and size standard beads (SPHEROTM Nano Polystyrene & Nano Fluorescent Size Standards, Spherotech) for quantitative analysis of isolated mitochondria. The average size of mitochondria is around 0.5 μm. In flow cytometric analysis, control beads were used to gate populations ranging in size from 0.4 to 0.6 μm. The analytical concentration of mitochondria was estimated by comparison of the SSC/FSC signal of the sample with that of standards (control beads: 1×10^6/ml). SSC: side scatter; FSC: forward scatter.



(C)

		HA		U87		U87 + Mito	
CHROM	POS	SD	A_T_C_G	SD	A_T_C_G	SD	A_T_C_G
mtDNA	3480	250	250_0_0_0	250	0_0_0_250	250	0_0_0_250
mtDNA	5913	250	0_0_0_250	250	250_0_0_0	248	248_0_0_0
mtDNA	9698	248	0_248_0_0	250	0_0_250_0	250	0_0_250_0
mtDNA	10398	249	249_0_0_0	250	0_0_0_250	250	0_0_0_250
mtDNA	10463	250	0_0_250_0	250	0_250_0_0	249	0_249_0_0
mtDNA	11299	250	0_250_0_0	249	0_0_249_0	250	0_0_250_0
mtDNA	11914	250	250_0_0_0	249	0_0_0_249	250	0_0_0_250
mtDNA	12308	250	250_0_0_0	250	0_0_0_250	250	0_0_0_250
mtDNA	12372	250	0_0_0_250	249	249_0_0_0	250	250_0_0_0
mtDNA	12738	250	0_250_0_0	250	0_0_0_250	249	0_0_0_249
mtDNA	12771	250	0_0_0_250	250	250_0_0_0	249	249_0_0_0
mtDNA	14798	250	0_250_0_0	250	0_0_250_0	250	0_0_250_0
mtDNA	16224	248	0_248_0_0	249	0_0_249_0	249	0_0_249_0

Population	Count	Positive %	BDS Ch2-5
All	15000	—	—
R1	465	3.1 %	> 3

Figure S2. No transplantation of isolated mitochondria into U87 cells without starvation. (A) Experimental schematic for co-incubation studies. (B) Live images of GFP-expressing U87 cells containing MitoTracker Red CMXRos-labelled mitochondria. (C) Mitochondrial genotypes of HA, U87 and U87+Mito (U87 cells without starvation treatment co-incubated with isolated mitochondria) cells were examined by direct sequencing. CHROM: chromosome; POS: position; SD: sequencing depth. (D) The multispectral imaging flow cytometry was used to measure the intracellular colocalization of isolated mitochondria (MitoTracker Red labeling) with the endosomes (stained with pHrodo). Ch2: pHrodo; Ch5: MitoTracker Red; BF: brightfield; BDS Ch2-5: bright detail similarity Ch2-Ch5.

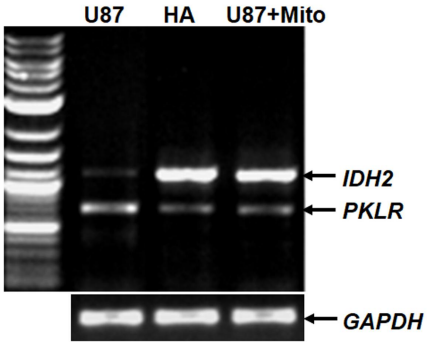


Figure S3. Validation of genes with large errors on the heat map. After completing screening by Human Signal Transduction Pathway Finder PCR Array, 2 genes (*IDH2* and *PKLR*) with large errors were validated by PCR using *IDH2* primers (forward, CCCGTATTATCTGGCAGTTCATC; reverse, ATCAGTCTGGTCACGGTTTGG) and *PKLR* primers (forward, GGACACGGCATCAAGATCATC; reverse, GCAGCGCCCAATCATCATC). Isocitrate dehydrogenase 2 (IDH2) plays an essential role in the oxidative function of the tricarboxylic acid cycle. Pyruvate kinase isozymes R/L (PKLR) is responsible for catalyzing the final step in glycolysis. After mitochondrial transplantation, the changes in transcription levels of *IDH2* and *PKLR* were consistent with the overall trend of energy metabolic phenotypes (shifting from glycolysis to oxidative phosphorylation).

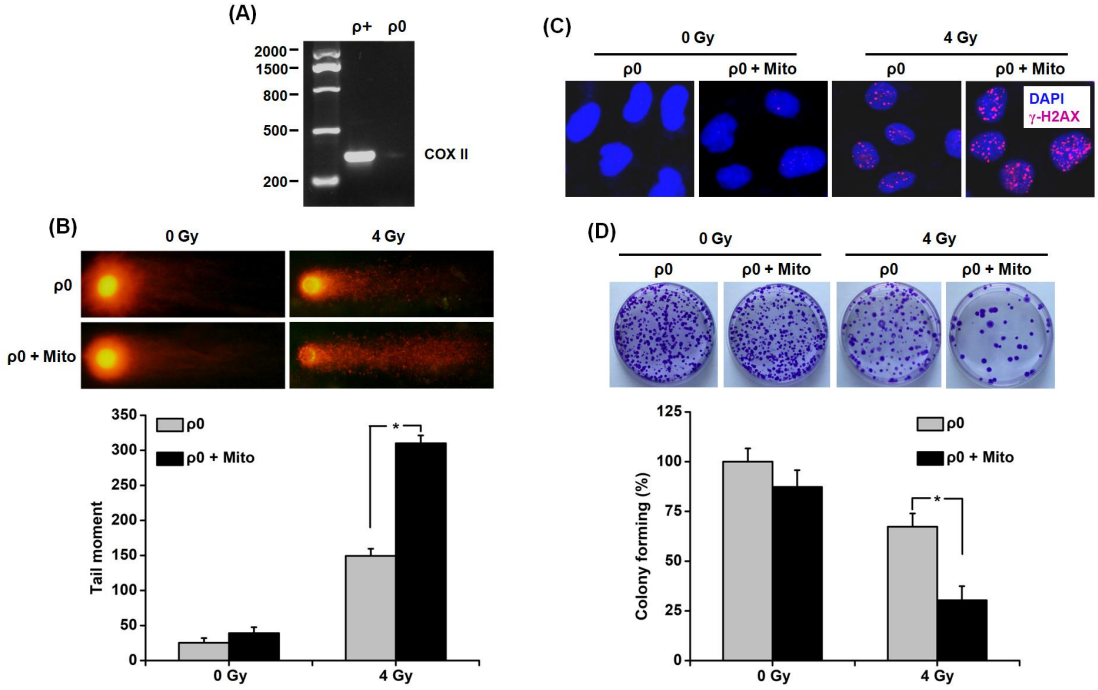


Figure S4. Effects of mitochondrial transplantation on radiosensitivity of ρ^0 cells. Mitochondrial DNA (mtDNA)-depleted U87 cells (ρ^0 cells) were constructed as described previously [2]. U87 cells were cultured in the presence of 200 ng/ml ethidium bromide, 100 μg/ml pyruvate and 50 μg/ml uridine for 40 days. (A) Identification of a mtDNA-deleted cell line by PCR amplification of cytochrome C oxidase subunit II (COX II). (B) After 4 Gy X-ray irradiation, DNA damages of ρ^0 and ρ^0 + Mito (ρ^0 cells co-incubated with isolated mitochondria) cells were measured using comet assay by the method of Jena et al., as described before [3]. (C) After 4 Gy X-ray irradiation, confocal microscopy was used to visualize the intracellular γ-H2AX (a marker of DNA double-strand breakage, red) foci in ρ^0 and ρ^0 + Mito cells. Nuclear staining used DAPI (blue). (D) Clonogenic potential of ρ^0 and ρ^0 + Mito cells.

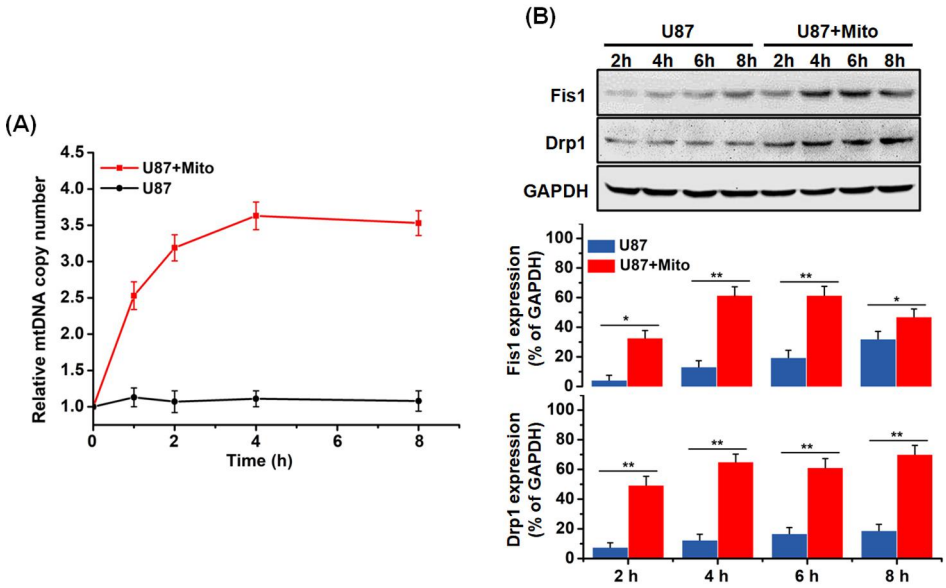


Figure S5. Effects of mitochondrial transplantation on mtDNA replication and mitochondrial fission. (A) After the treatment of mitochondrial transplantation, the mtDNA copy number in U87 cells was quantified by real-time PCR using *ND1* primers (forward, CACCCAAGAACAGGGTTTGT; reverse, TGGCCATGGGTATGTTGTTAA) [4]. The *GAPDH* primers (forward, GGAGCGAGATCCCTCCAAAAT; reverse, GGCTGTTGTCATACTTCTCATGG) were used as a nuclear genome control for normalizing the *ND1* level. (B) After mitochondrial transplantation, expressions of Drp 1 and Fis 1 (two critical proteins involved in the mitochondrial-fission) in U87 cells were measured by western blot. AlphaView SA software was used for quantification of western blot. $^{*}p<0.05$, $^{**}p<0.01$: U87+Mito *vs*. U87.

Reference

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