Supporting Information for

Nanoengineered mitochondria enable ocular mitochondrial disease therapy *via* the replacement of dysfunctional mitochondria

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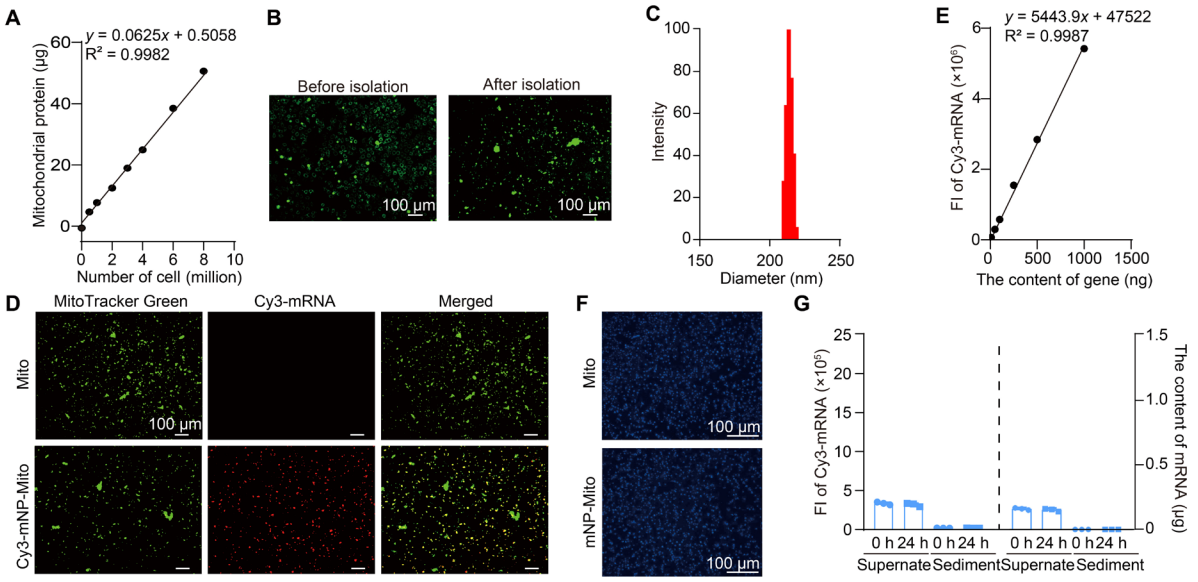

Figure S1 The characteristics of mNP-Mito. (A) The standard curve of mitochondrial proteins and the number of donor cells; (B) Before isolation from donor cells and after isolation in a culture dish, the mitochondrial mass was detected by MitoTracker Green probe; (C) The nanoparticle size of mNP; (D) The binding of Cy3-mNP to extracted mitochondria checked by the colocalization of green-labeled mitochondria and red-labeled mRNA; (E) The relationship between fluorescence intensity (FI) of Cy3-mRNA and the contents of mRNA; (F) The mitochondrial activity before and after binding with mNP detected by Janus green-B dye; (G) FI of Cy3-mRNA was used to investigate the stability of mNP-Mito at different times.

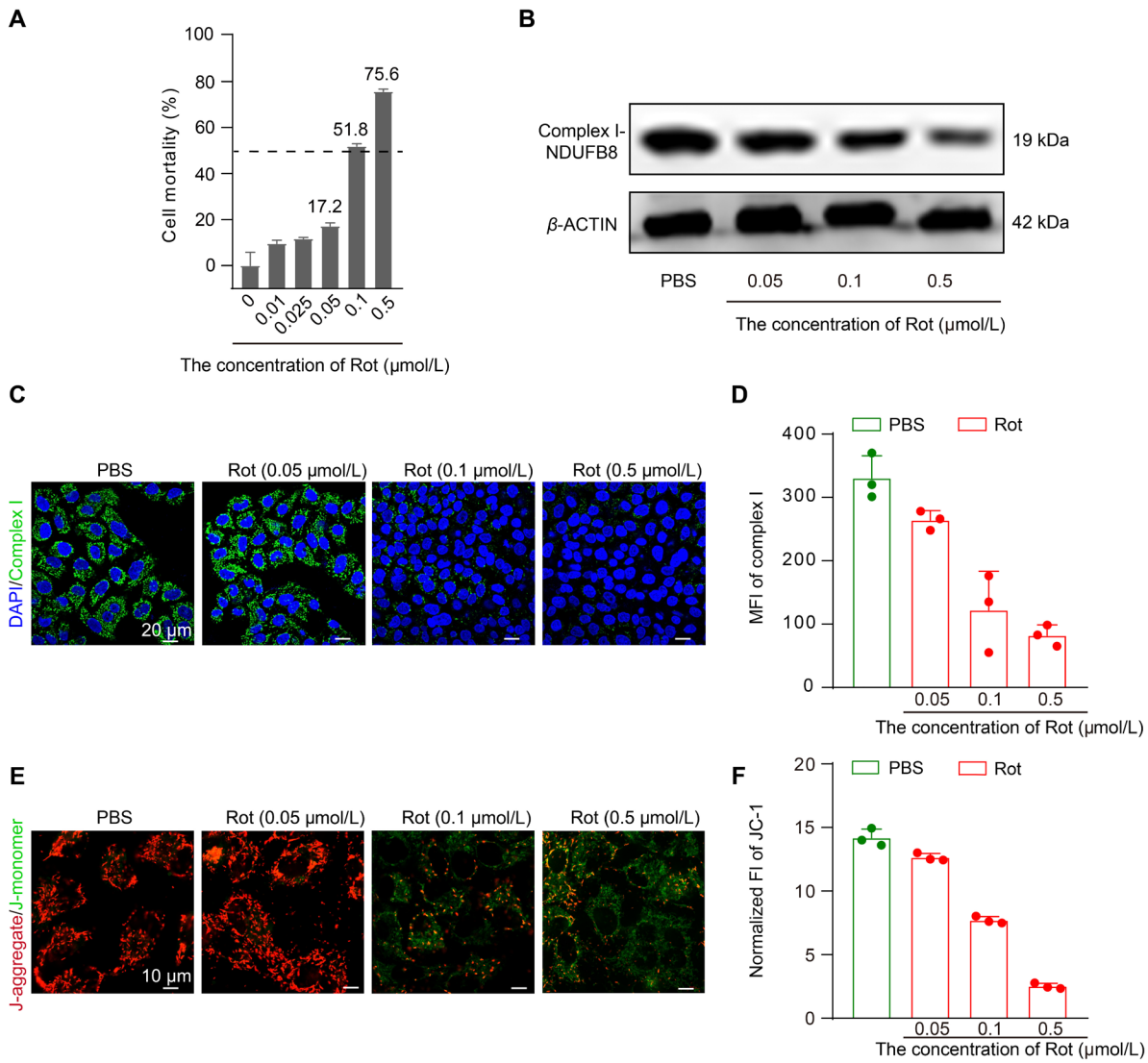

Figure S2 Impairment of mitochondrial complex I tightly correlates with Rot concentrations in the Rot-induced cell model. (A) The relationship between Rot concentration and cell mortality; (B) The expression of mitochondrial protein NDUFB8 was evaluated under different concentrations of Rot *in vitro*; (C, D) Decreasing complex I contents detected by CLSM (C) and flow cytometry of extracted mitochondria (D); (E, F) The changes in MMP detected by CLSM (E) and multifunctional microplate reader (F), normalized FI = FI/cellular whole-protein contents. In (A), (D), and (F), data are mean ± SD (n = 3).

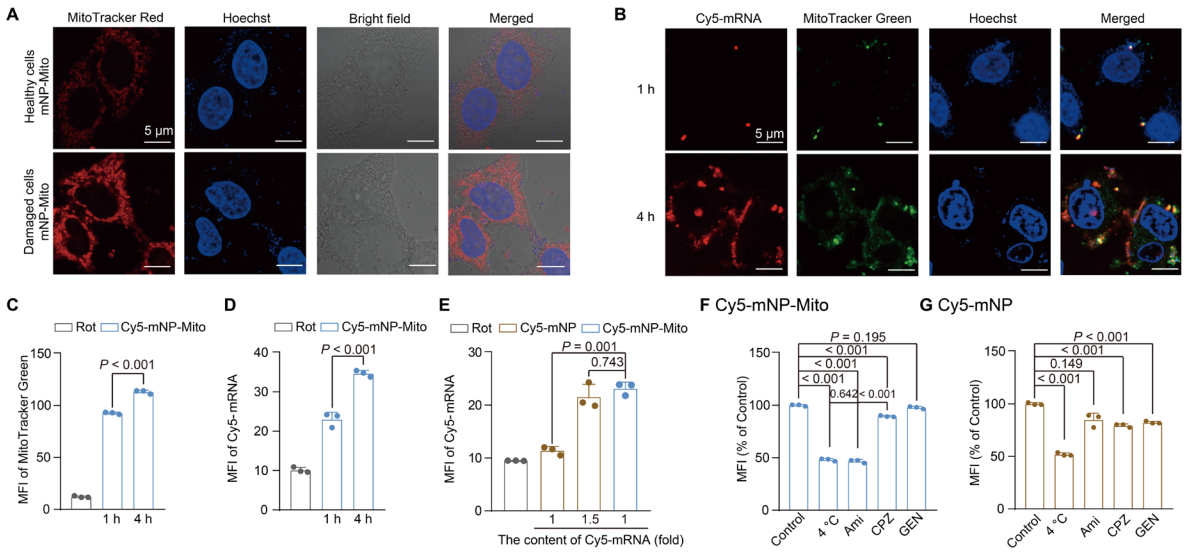

Figure S3 The cellular uptake of mNP-Mito. (A) The cellular uptake of exogenous mitochondria in normal HeLa cells and Rot-damaged cells (24 h); (B–D) The cellular uptake of MitoTracker Green-labeled Cy5-mNP-Mito after different treatment times detected by CLSM (B) and flow cytometer (C, D); (E) The cellular uptake of Cy5-mNP-Mito after different treatments at 4 h detected by flow cytometer; (F, G) The cellular endocytosis pathway studies of Cy5-mNP-Mito (F) and Cy5-mNP (G). Data are shown as mean ± SD (*n* = 3 in C–G). Statistical significance was analyzed by one-way ANOVA with the Tukey's honest significant difference (HSD) post hoc test (C, D, F) and Games-Howell test (E, G).

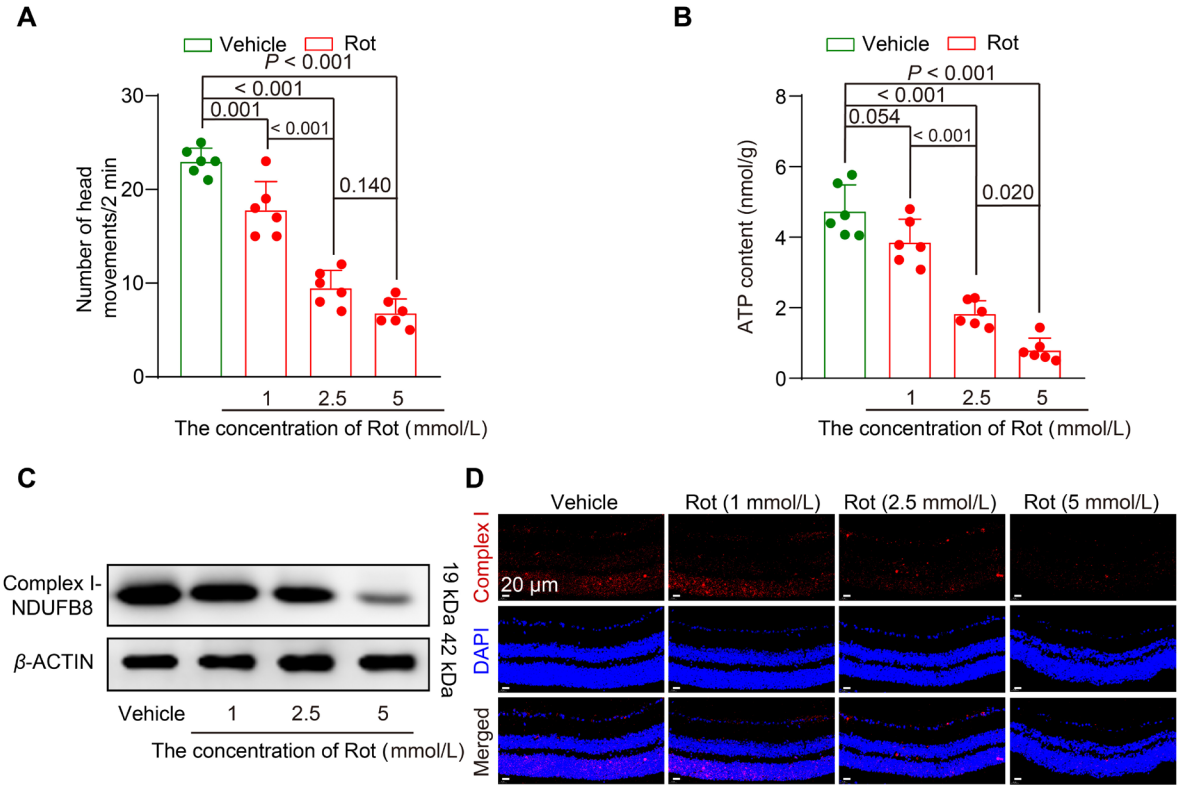

Figure S4 Impairment of mitochondrial complex I tightly correlates with disorder severity in the Rot-induced experimental LHON-like mouse model. (A) The optomotor test and counting the number of head movements following different Rot concentrations; (B) ATP contents in retina after treatment with different concentrations of Rot; (C) The expression of mitochondrial protein NDUFB8 located in mitochondrial complex I after Rot treatment; (D) The contents of complex I detected by immunofluorescence staining after Rot treatment. Data are shown as mean ± SD (n = 6 in A, B). Statistical significance was analyzed by one-way ANOVA with the Tukey's honest significant difference (HSD) post hoc test (A, B).

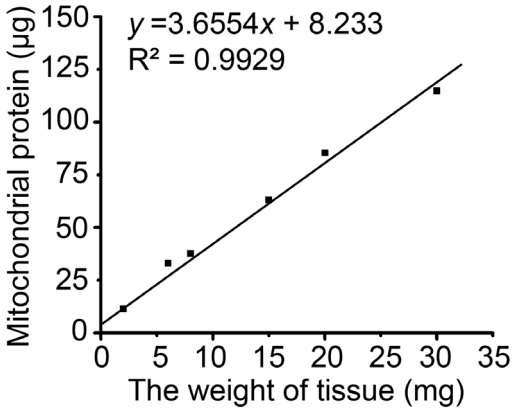

Figure S5 The standard curve between the weight of Balb/c mouse heart tissues and the contents of mitochondrial proteins.

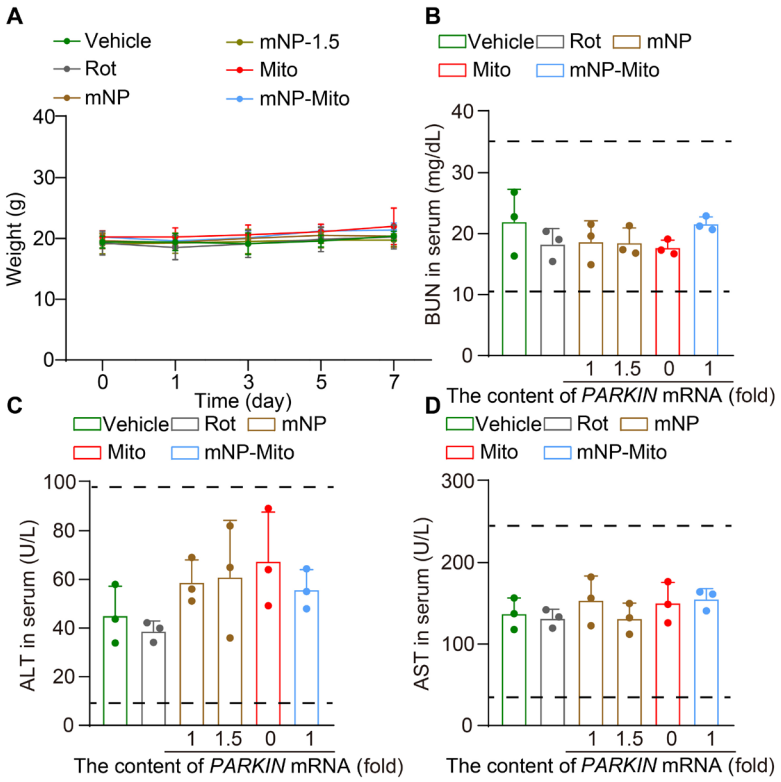

Figure S6 The safety of mNP-Mito in Rot-induced mice model. (A) The weight change in different formulation treatments; (B–D) The detection of blood urea nitrogen (BUN, B), alanine transaminase (ALT, C), and aspartate aminotransferase (AST, D) in serum after Day 7 of treatments, error bars: mean ± SD (*n* = 7 per group in A, *n* = 3 mice per group in B–D). The dashed area represents the normal range of the indicator.

Table S1 The organ indexes of the normal Balb/c mice in PBS, Low-dose (1-fold mNP-Mito), Mid-dose (2-fold mNP-Mito) and High-dose (4-fold mNP-Mito) groups after Day 14 treatments (intravitreal administration, organ index = organ/body weight × 100), *n* = 6 in the PBS, Low-dose and Mid-does groups, *n* = 5 in the High-dose group. Statistical significance was measured using one-way ANOVA with the Scheffe or Games-Howell test. Each group was compared with the PBS group, with a $P > 0.05$. Except for PBS (liver) *versus* High-dose (liver) group, *P* = 0.022; and PBS (liver) *versus* Mid-dose (liver) group, *P* = 0.045. Error bars: mean ± SD.

Organ	PBS	Low-dose group	Mid-dose group	High-dose group
Heart	0.49 ± 0.06	0.48 ± 0.02	0.48 ± 0.07	0.49 ± 0.04
Liver	4.33 ± 4.25	4.84 ± 0.21	4.88 ± 0.23	4.97 ± 0.44
Spleen	0.51 ± 0.08	0.59 ± 0.06	0.56 ± 0.04	0.57 ± 0.12
Lung	0.70 ± 0.07	0.79 ± 0.04	1.11 ± 0.90	0.74 ± 0.08
Kidney	1.44 ± 0.13	1.58 ± 0.06	1.58 ± 0.06	1.39 ± 0.14

Table S2 The hematological parameters of the mice in various groups after different treatments in the LHON mice model. Error bars: mean ± SD (*n* = 3 mice per group).

–	Vehicle	Rot	mNP	mNP-1.5	Mito	mNP-Mito	Reference
WBC	5.50 ± 1.61	6.87 ± 5.05	4.57 ± 1.63	2.23 ± 1.60	3.33 ± 0.72	5.30 ± 1.57	0.80–6.80
Lymph	3.57 ± 0.83	3.50 ± 2.17	3.30 ± 1.39	1.63 ± 1.14	2.50 ± 0.66	3.90 ± 1.30	0.70–5.70
Gran	1.30 ± 0.17	1.30 ± 0.44	1.03 ± 0.31	0.53 ± 0.42	0.73 ± 0.23	1.23 ± 0.59	0.10–1.80
Lymph%	65.33 ± 3.11	62.17 ± 9.51	70.40 ± 7.57	73.07 ± 1.07	75.40 ± 6.56	72.93 ± 9.78	55.80–90.60
Mon%	5.13 ± 0.40	6.47 ± 3.32	6.10 ± 3.77	3.67 ± 0.32	3.47 ± 1.01	3.93 ± 1.72	1.80–6.00
Gran%	29.20 ± 3.33	31.37 ± 10.37	23.50 ± 3.80	23.27 ± 1.01	21.13 ± 5.61	23.13 ± 8.17	8.60–38.90
HGB	141.67 ±10.01	146.33 ± 24.17	148.67 ± 4.04	137.33 ± 6.35	140.67 ± 16.04	134.00 ± 3.00	110.00–143.00
HCT	39.90 ± 2.69	34.97 ± 2.06	39.93 ± 4.53	37.23 ± 4.46	35.30 ± 3.05	35.73 ± 2.93	34.60–44.60
MCHC	319.33 ± 17.16	347.00 ± 22.61	333.33 ± 30.50	337.33 ± 21.08	332.67 ± 19.66	313.00 ± 12.49	302.00–353.00

WBC: white blood cells; Lymph: lymphocytes; Gran: granulocytes; Lymph%: lymphocyte percentage; Mon%: monocyte percentage; Gran%: granulocyte percentage; HGB: hemoglobin; HCT: hematocrit; MCHC: mean corpuscular hemoglobin concentration.