# Supplementary Information

# Super mitochondria-enriched extracellular vesicles enable enhanced mitochondria transfer

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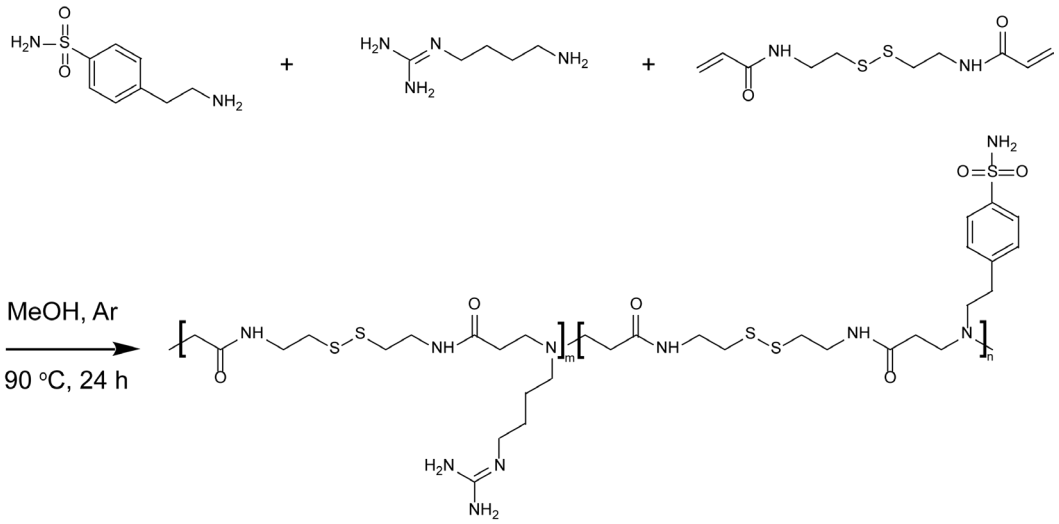
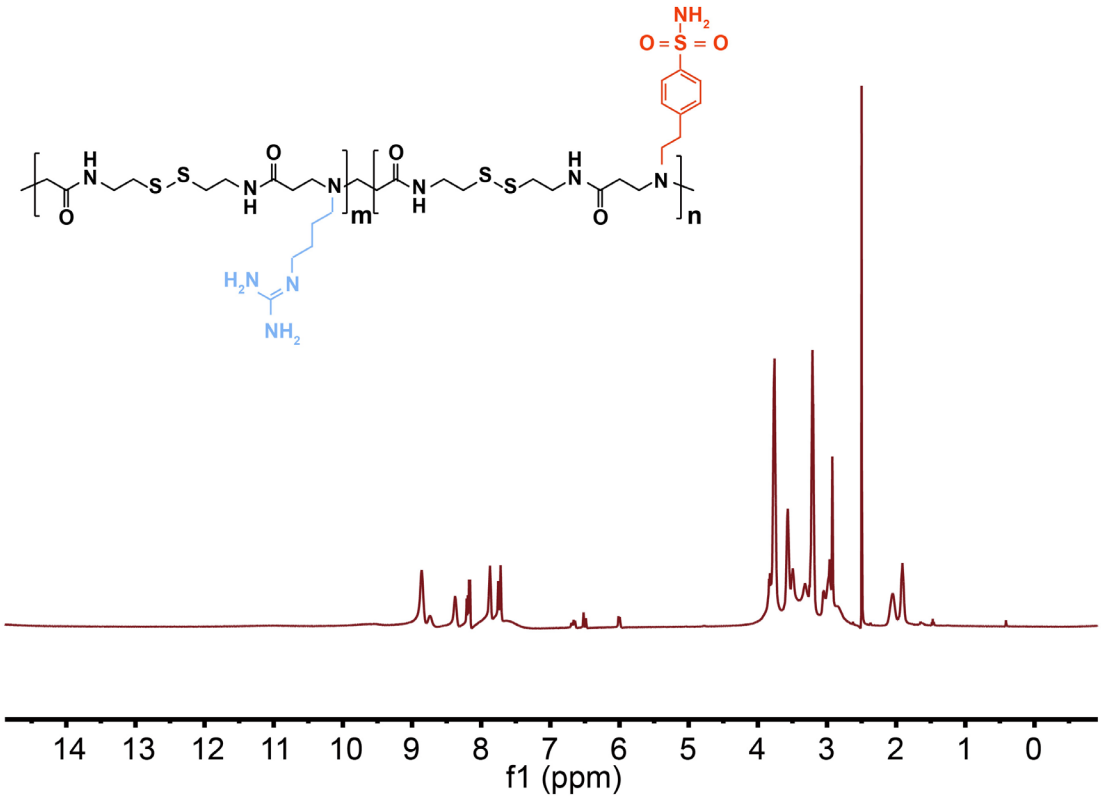
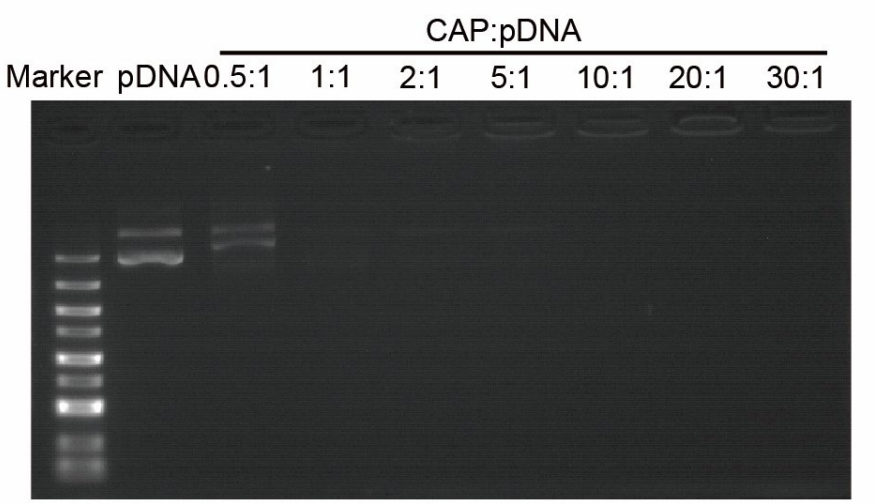


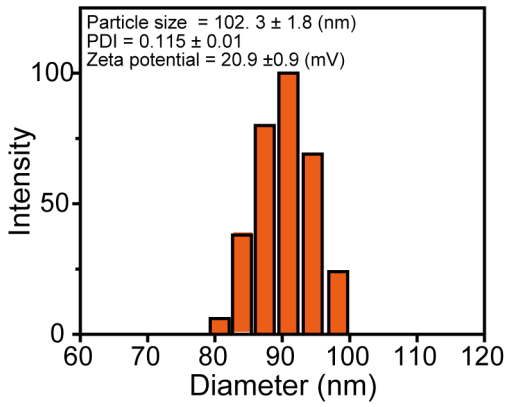

**Supplementary Fig. 1.** Schematic representation of the synthetic route of CAP through Michael's addition.



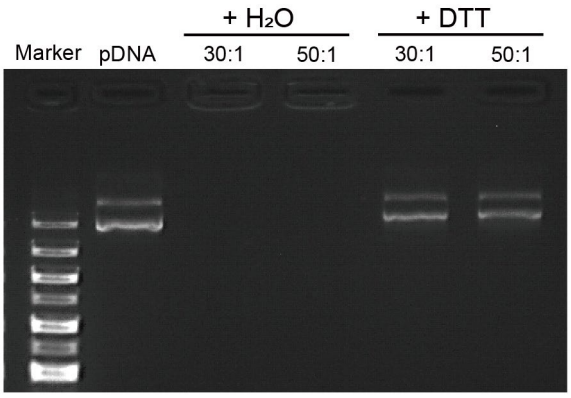

**Supplementary Fig. 2.** $^{1}$H NMR spectrum (500 MHz, d6-Dimethyl Sulfoxide) of CAP.



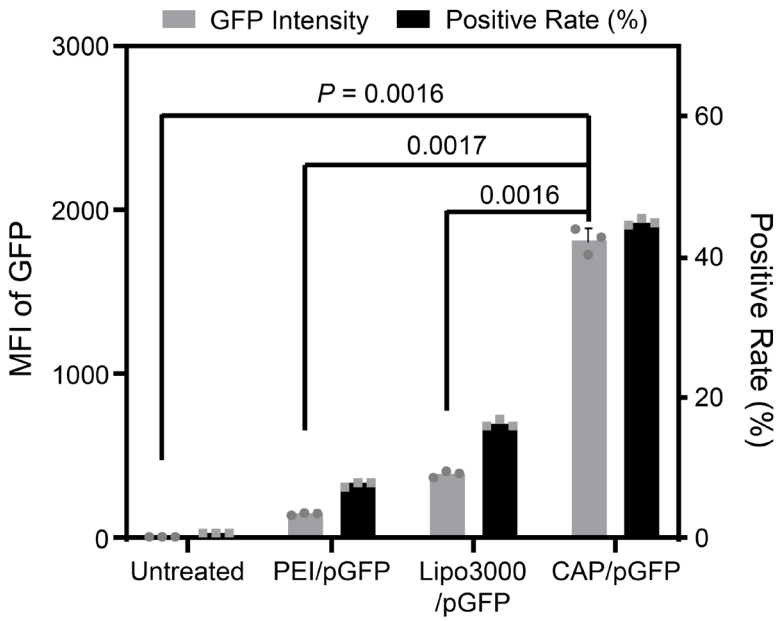

**Supplementary Fig. 3.** Agarose gel electrophoresis assay of CAP/pDNA complexes. Source data are provided as a Source Data file.



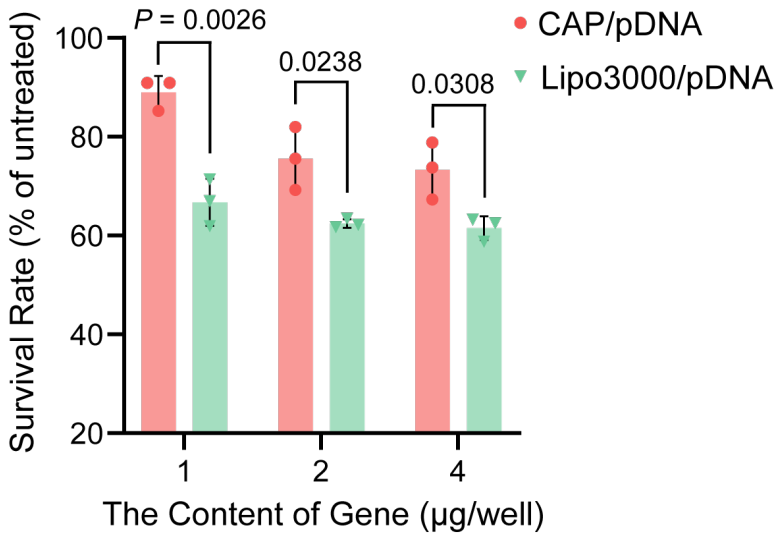

**Supplementary Fig. 4.** Particle size distribution of CAP/pDNA complexes (w/w =30/1). Source data are provided as a Source Data file.



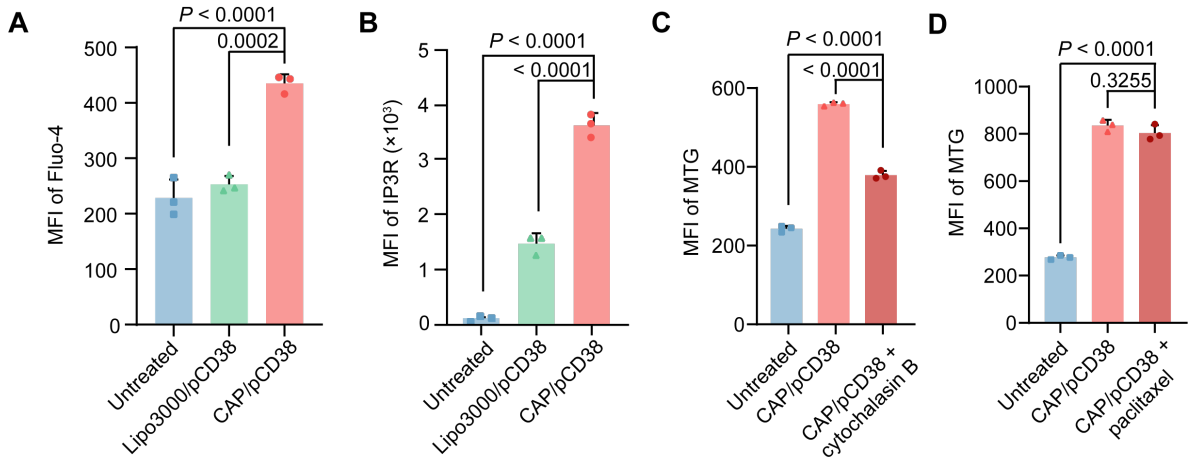

**Supplementary Fig. 5.** Agarose gel electrophoresis of CAP/pDNA complexes after incubation with 10 mM DTT at different weight ratios (30/1 or 50/1) for 2 h. Source data are provided as a Source Data file.



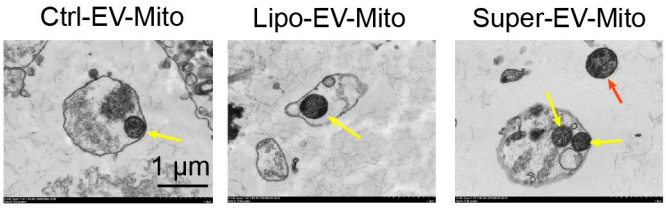

**Supplementary Fig. 6.** Transfection efficiency of CAP/pGFP complexes (w/w = 30/1) in MSCs. Data are presented as mean ± SD. $n$ = 3 biologically independent samples. Statistical significances were performed via one-way ANOVA with two-sided Games-Howell test. Source data are provided as a Source Data file.



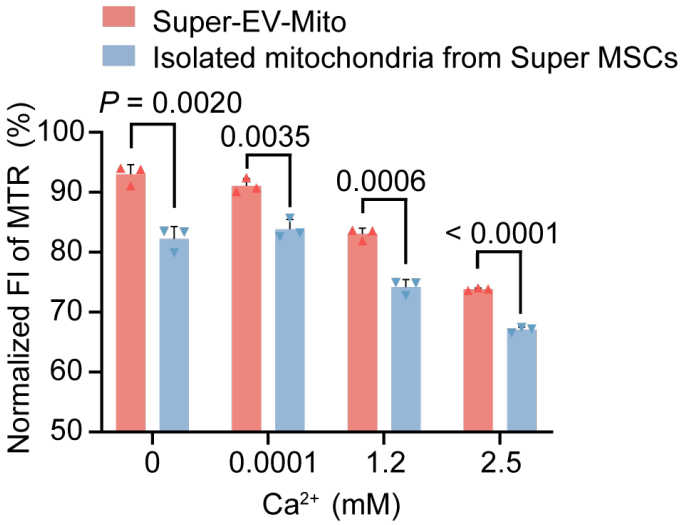

**Supplementary Fig. 7.** The relative cell survival rate after treatment with CAP/pDNA or Lipo3000/pDNA measured by MTT assay. Data are presented as mean ± SD. $n$ = 3 biologically independent samples. Statistical significances were performed via two-sided t-test. Source data are provided as a Source Data file.



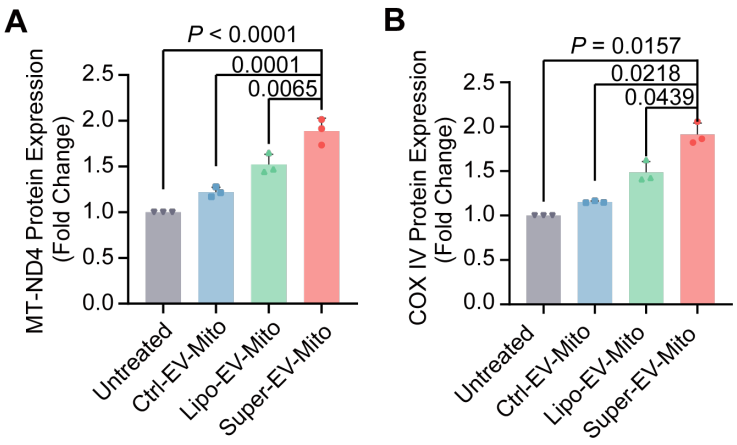

**Supplementary Fig. 8.** IP3R protein expression in MSCs following pCD38 transfection and the release of EV-Mito under different inhibitors. (A) Cytoplasmic calcium levels in MSCs after different treatments. (B) IP3R protein expression quantified by immunofluorescence. (C) The release of EV-Mito with or without actin inhibitors (cytochalasin B). (D) The release of EV-Mito with or without a tubulin inhibitor (paclitaxel). Data are presented as mean ± SD. $n$ = 3 biologically independent samples. Statistical significances were performed via one-way ANOVA with two-sided Tukey's HSD post hoc test (A-D). Source data are provided as a Source Data file.



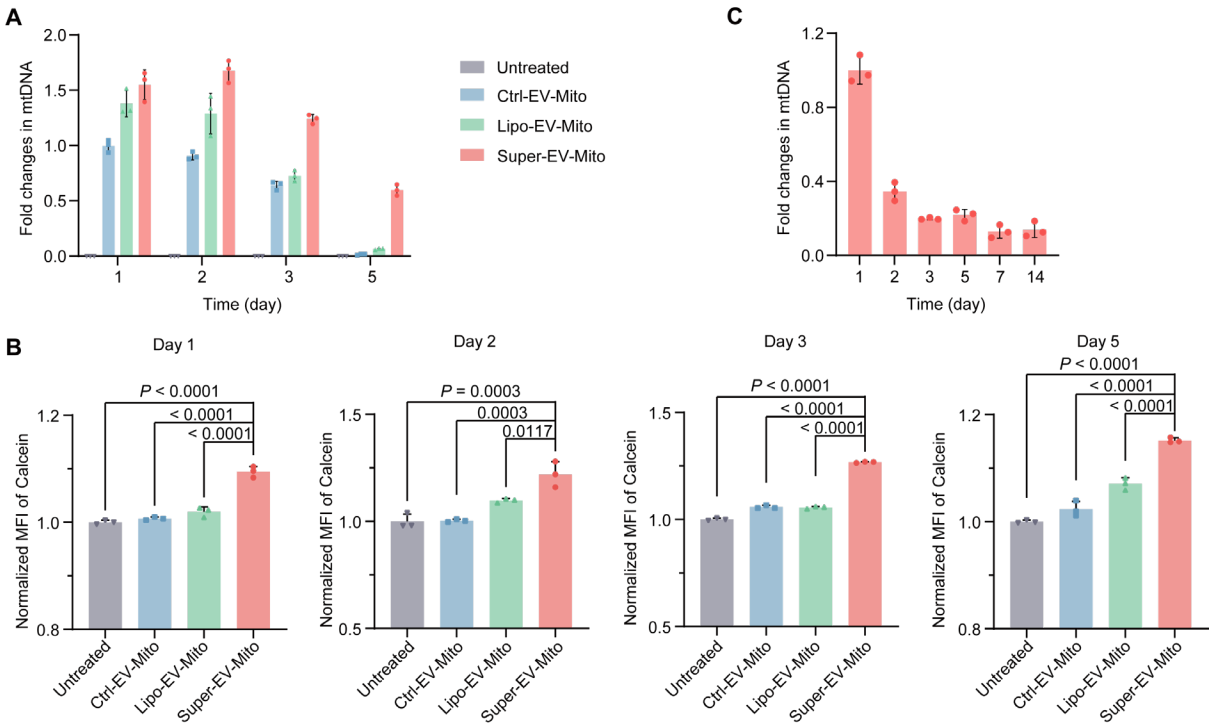

**Supplementary Fig. 9.** TEM images of different EV-Mito groups. Yellow arrows: released mitochondria enclosed in EVs; red arrows: free mitochondria not enclosed in EVs. Representative images from $n$ = 3 independent experiments.



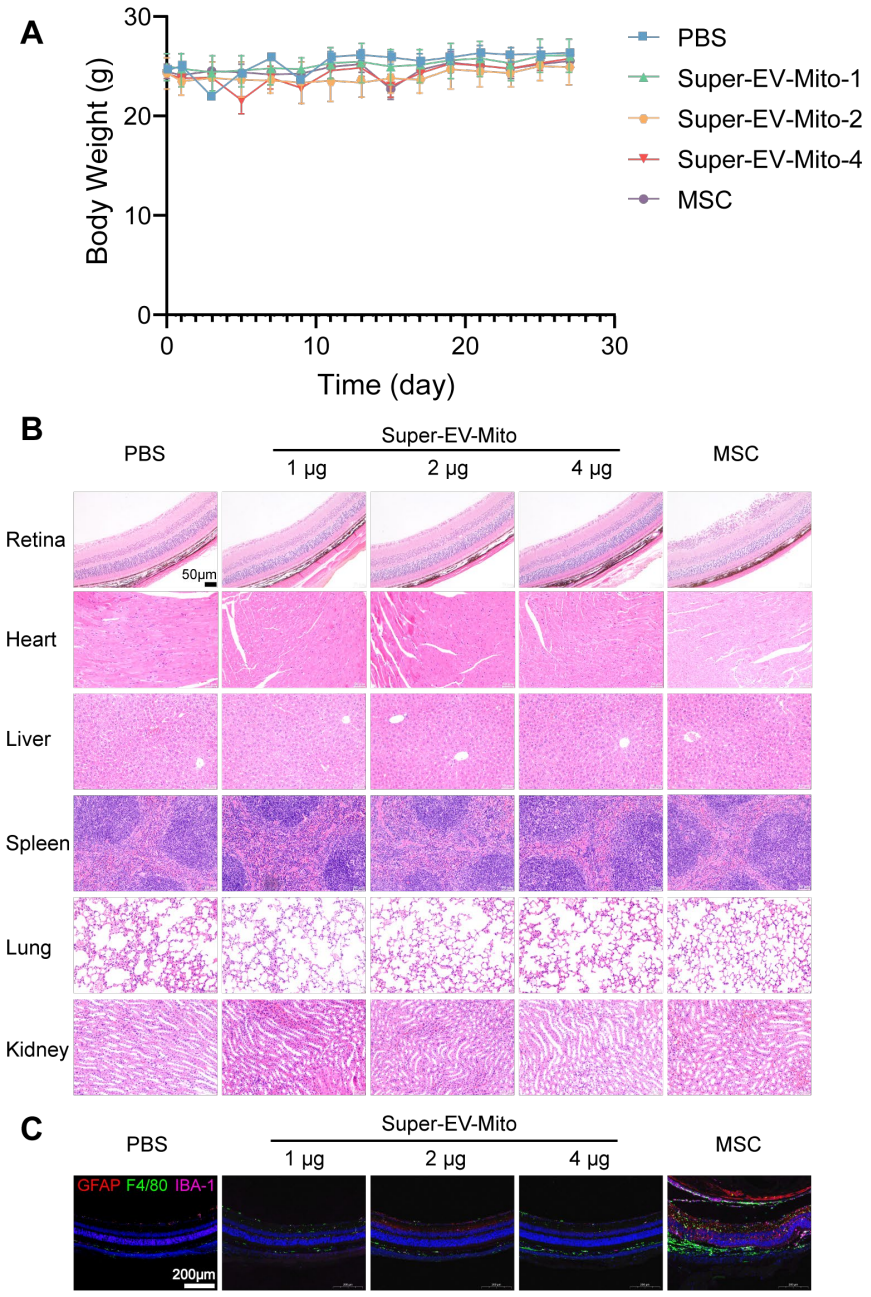

**Supplementary Fig. 10.** The stability of mitochondria in Super EV-Mito under $Ca^{2+}$ conditions. MTR intensity of Super-EV-Mito and isolated mitochondria incubated in different concentrations of $Ca^{2+}$. Data are presented as mean ± SD. $n = 3$ biologically independent samples. Statistical significances were performed via two-sided t-test. Source data are provided as a Source Data file.



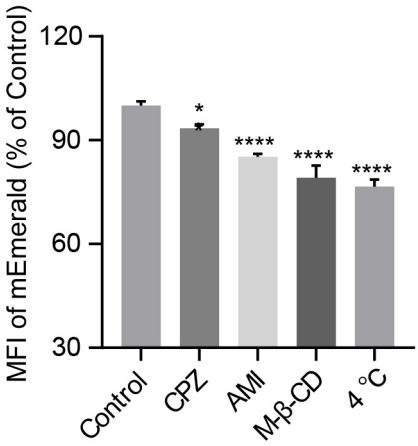

**Supplementary Fig. 11.** Mitochondrial protein expression after different treatments in GM10742 cells. (A) MT-ND4 protein. (B) COX IV protein. Data are presented as mean ± SD. $n$ = 3 biologically independent samples. Statistical significances were performed via one-way ANOVA with two-sided Tukey's HSD post hoc test (A) or two-sided Games-Howell test (B). Source data are provided as a Source Data file.



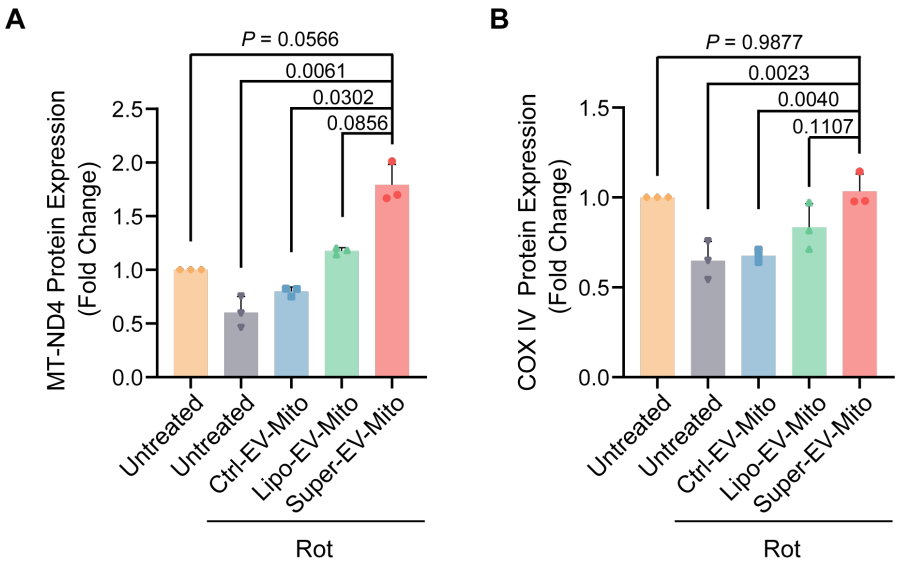

**Supplementary Fig. 12.** Single-dose expression duration of Super-EV-Mito. (A) Time-course analysis of rat-derived mtDNA persistence in GM10742 cells following treatment with different EV-Mito, $n$ = 3 biologically independent samples. (B) Evaluation of the long-term mitochondrial permeability transition pore (mPTP) repair potential in GM10742 cells after a single administration of Super-EV-Mito, $n$ = 3 biologically independent samples. (C) Persistence of rat-derived mtDNA in mouse eyes following a single administration of Super-EV-Mito, $n$ = 3 biologically independent samples. Data are presented as mean ± SD. Statistical significances were performed via one-way ANOVA with two-sided Tukey's HSD post hoc test (B). Source data are provided as a Source Data file.




**Supplementary Fig. 13.** Safety evaluation of Super-EV-Mito in normal C57BL/6J male mice. (A) Body weight, data are presented as mean ± SD, $n$ = 5 biologically independent samples. (B) H&E staining. (C) Inflammation-related proteins in retina (red: GFAP, green: F4/80, pink: IBA-1, blue: DAPI). Source data are provided as a Source Data file.




**Supplementary Fig. 14.** The cellular uptake mechanism study of Super-EV-Mito. CPZ: chlorpromazine, AMI: amiloride; M-β-CD: methyl-β-cyclodextrin. Data are presented as mean ± SD. $n$ = 3 biologically independent samples. Statistical significances were performed via one-way ANOVA with two-sided Tukey's HSD post hoc test. Source data are provided as a Source Data file.




**Supplementary Fig. 15.** Mitochondrial protein expression after different treatments in Rot-induced model cells. (A) MT-ND4 protein. (B) COX IV protein. Data are presented as mean ± SD. $n$ = 3 biologically independent samples. Statistical significances were performed via one-way ANOVA with two-sided Tukey's HSD post hoc test (B) or two-sided Games-Howell test (A). Source data are provided as a Source Data file.

## Supplementary Table

**Supplementary Table 1.** NTA concentration and zeta potential of EV-Mito. Data are presented as mean ± SD. *n* = 3 biologically independent samples in zeta potential.

| | Ctrl-EV-Mito | Lipo-EV-Mito | Super-EV-Mito |
|---|---|---|---|
| Concentration ($10^{11}$ particles/mg) | 3.60 | 5.68 | 5.19 |
| Zeta potential (mV) | -1.37 ± 0.13 | -1.35 ± 0.06 | -1.76 ± 0.27 |

**Supplementary Table 2.** The hematological parameters of the mice in various groups. The reference in the last column indicates the normal range of each parameter. WBC: white blood cells, Lymph: lymphocyte cells, Mon: monocytes, Gran: granulocytes, RBC: red blood cells, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelet, MPV: mean platelet volume, PDW: platelet distribution width. Error bars: mean ± SD ($n$ = 3 biologically independent samples). Source data are provided as a Source Data file.

| - | PBS | Super-EV-Mito-1 μg | Super-EV-Mito-2 μg | Super-EV-Mito-4 μg | MSC | Reference |
|---|---|---|---|---|---|---|
| WBC ($10^9$/L) | 5.10 ± 1.42 | 5.83 ± 0.66 | 4.03 ± 1.02 | 3.27 ± 1.53 | 3.87 ± 0.76 | 0.8-10.6 |
| Lymph# ($10^9$/L) | 4.27 ± 0.93 | 4.97 ± 0.55 | 3.50 ± 0.95 | 2.83 ± 1.21 | 3.30 ± 0.78 | 0.6-8.9 |
| Mon# ($10^9$/L) | 0.17 ± 0.21 | 0.07 ± 0.06 | 0.03 ± 0.06 | 0.03 ± 0.06 | 0.03 ± 0.06 | 0.04-1.4 |
| Gran# ($10^9$/L) | 0.67 ± 0.29 | 0.80 ± 0.17 | 0.50 ± 0.10 | 0.40 ± 0.26 | 0.53 ± 0.06 | 0.23-3.6 |
| Lymph% (%) | 84.34 ± 4.97 | 85.33 ± 1.40 | 86.50 ± 3.44 | 87.07 ± 3.99 | 84.67 ± 2.90 | 40-92 |
| Mon% (%) | 3.40 ± 2.97 | 1.40 ± 0.36 | 1.40 ± 0.44 | 1.93 ± 0.91 | 1.97 ± 0.21 | 0.9-18 |
| Gran% (%) | 12.27 ± 2.08 | 13.27 ± 1.05 | 12.10 ± 3.00 | 11.00 ± 3.12 | 13.37 ± 2.70 | 6.5-50 |
| RBC ($10^{12}$/L) | 9.98 ± 0.68 | 9.27 ± 0.53 | 9.39 ± 0.31 | 10.07 ± 1.16 | 8.43 ± 0.39 | 6.5-11.5 |
| HGB (g/L) | 142.67 ± 10.41 | 135.33 ± 5.13 | 136.67 ± 5.86 | 144.00 ± 16.52 | 119.33 ± 6.03 | 110-165 |
| HCT (%) | 45.67 ± 2.64 | 42.13 ± 3.38 | 41.70 ± 1.97 | 43.57 ± 9.39 | 36.97 ± 1.86 | 35-55 |
| MCV (fL) | 45.87 ± 1.10 | 45.67 ± 2.72 | 44.43 ± 0.67 | 43.03 ± 4.47 | 43.97 ± 2.74 | 41-55 |
| MCH (pg) | 14.27 ± 0.21 | 14.57 ± 0.32 | 14.50 ± 0.35 | 14.27 ± 0.25 | 14.10 ± 0.10 | 13-18 |
| MCHC (g/L) | 312.00 ± 12.12 | 321.67 ± 22.94 | 327.33 ± 8.02 | 334.33 ± 31.39 | 322.67 ± 20.82 | 300-360 |
| PLT ($10^9$/L) | 1038.67 ± 238.51 | 1219.67 ± 160.03 | 1029.00 ± 65.20 | 1201.33 ± 38.08 | 889.33 ± 114.15 | 400-1600 |
| MPV (fL) | 5.53 ± 0.51 | 5.77 ± 0.25 | 5.83 ± 0.12 | 5.57 ± 0.40 | 5.67 ± 0.23 | 4.0-6.2 |
| PDW | 16.20 ± 0.30 | 16.23 ± 0.42 | 16.20 ± 0.17 | 15.93 ± 0.15 | 16.33 ± 0.25 | 12.0-17.5 |

**Supplementary Table 3.** The blood biochemistry parameters of the mice in various groups. The reference in the last column indicates the normal range of each parameter. ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN: blood urea nitrogen. Error bars: mean ± SD (*n* = 3 biologically independent samples). Source data are provided as a Source Data file.

| - | PBS | Super-EV-Mito -1 μg | Super-EV-Mito -2 μg | Super-EV-Mito -4 μg | MSC | Reference |
|---|---|---|---|---|---|---|
| ALT (U/L) | 39.29 ± 15.22 | 61.76 ± 11.86 | 52.61 ± 3.62 | 53.76 ± 11.26 | 60.09 ± 16.38 | 10.06-96.47 |
| AST (U/L) | 109.54 ± 11.01 | 192.32 ± 30.86 | 166.71 ± 17.04 | 152.60 ± 41.27 | 141.98 ± 25.60 | 36.31-235.48 |
| BUN (mg/dL) | 15.61 ± 2.65 | 15.12 ± 0.69 | 13.57 ± 0.86 | 14.45 ± 4.42 | 20.59 ± 7.47 | 10.81-34.74 |

## Supplementary gene sequences

**Supplementary Sequence 1.** The gene sequences of siIP3R.
CCAGAGAAAGAGGATATCA

**Supplementary Sequence 2.** The gene sequences of plasmid CD38.
ATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGCCACCATGGCCAACTATGAATTTTC
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