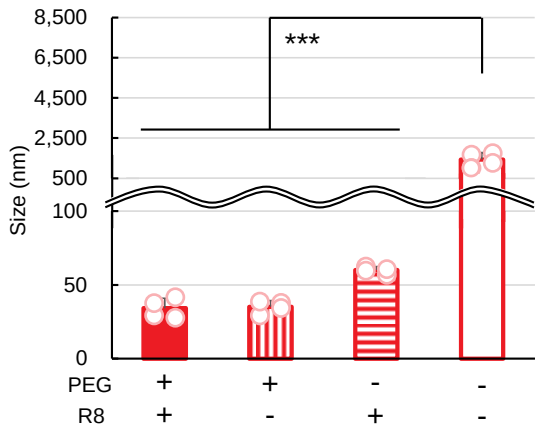


SUPPLEMENTARY INFORMATION

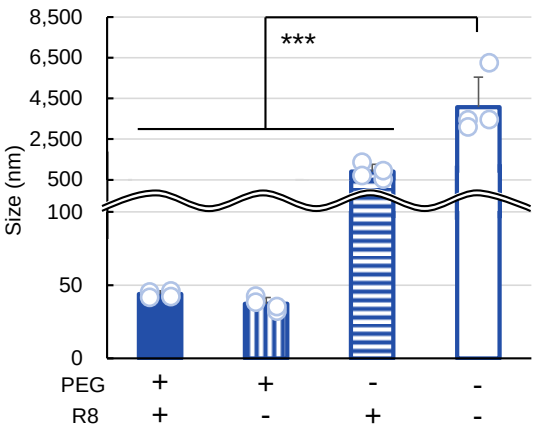
Supplementary Figure

**A** Particle size

**(a) Empty-MITO-Porter**

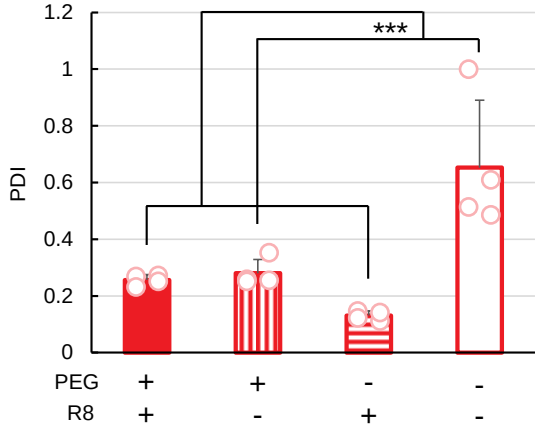


**(b) CoQ<sub>10</sub>-MITO-Porter**

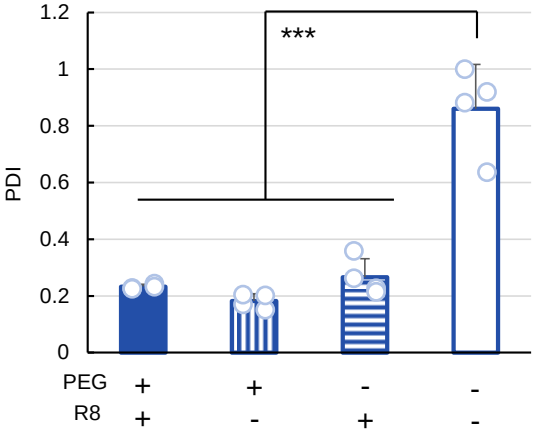


**B** Dispersibility

**(a) Empty-MITO-Porter**

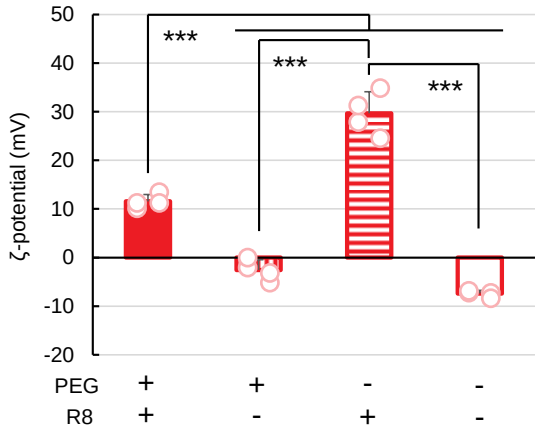


**(b) CoQ<sub>10</sub>-MITO-Porter**

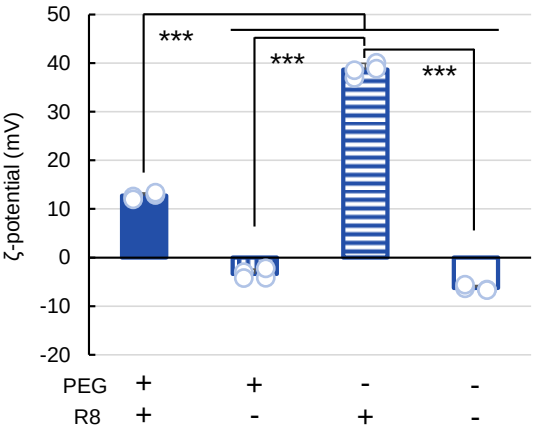


**C** ζ-potential

**(a) Empty-MITO-Porter**



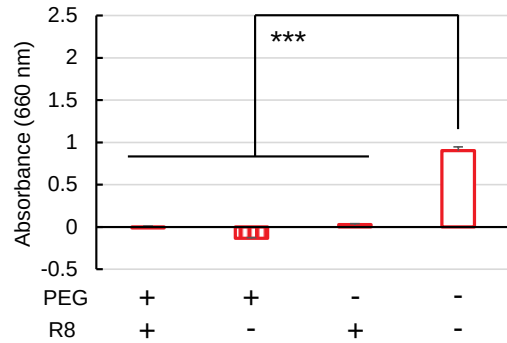
**(b) CoQ<sub>10</sub>-MITO-Porter**



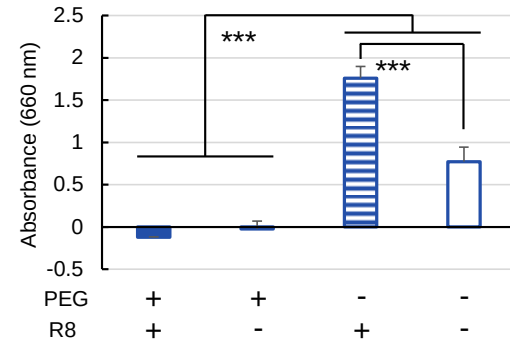
**Figure S1 Physical properties of the MITO-Porter before dialysis.** The physical properties of the MITO-Porter with or without PEG, R8 and CoQ<sub>10</sub> after being prepared by the microfluidic device were evaluated. The prepared LNPs are characterized by three indices: (A) Particle size, (B) Dispersibility, (C)  $\zeta$ -potential. The physical properties of (a) Empty-MITO-Porter and (b) CoQ<sub>10</sub>-MITO-Porter are compared to consider the effect of CoQ<sub>10</sub>, a poorly water-soluble molecule. Circles represent the values of 4 individual samples and bars are the mean (n=4). Data represented the mean  $\pm$  S.D. (n=4). The significant differences were calculated by two-way ANOVA, followed by Tukey test (\*\*\*)  $p < 0.001$ ).

**A** Turbidity of LNP solution after preparing by microfluidic devise

**(a) After preparing by microfluidic devise**

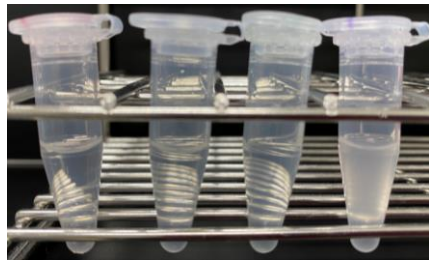


**(b) After dialysis**



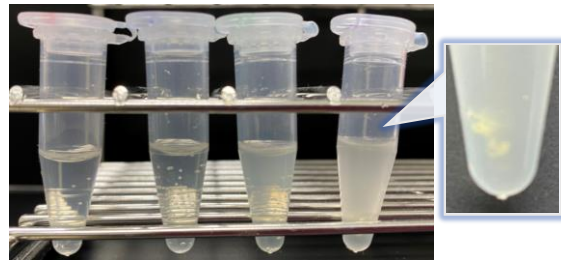
**B** Appearance of LNP solution after preparing by microfluidic devise

**(a) Empty-MITO-Porter**



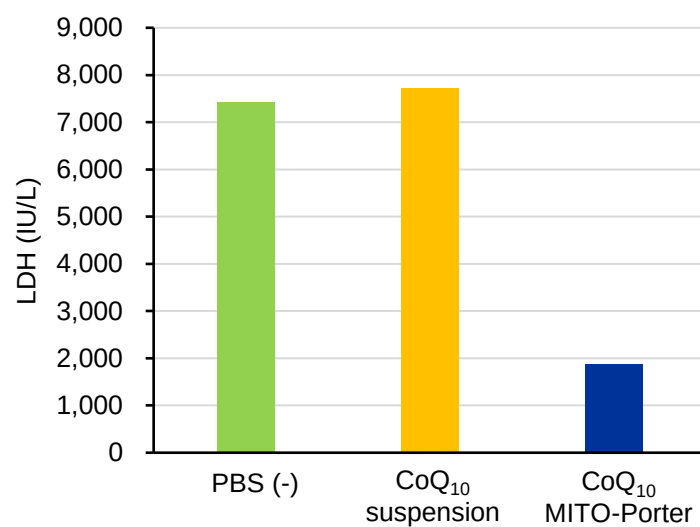
|     |   |   |   |   |
|-----|---|---|---|---|
| PEG | + | + | - | - |
| R8  | + | - | + | - |

**(b) CoQ<sub>10</sub>-MITO-Porter**

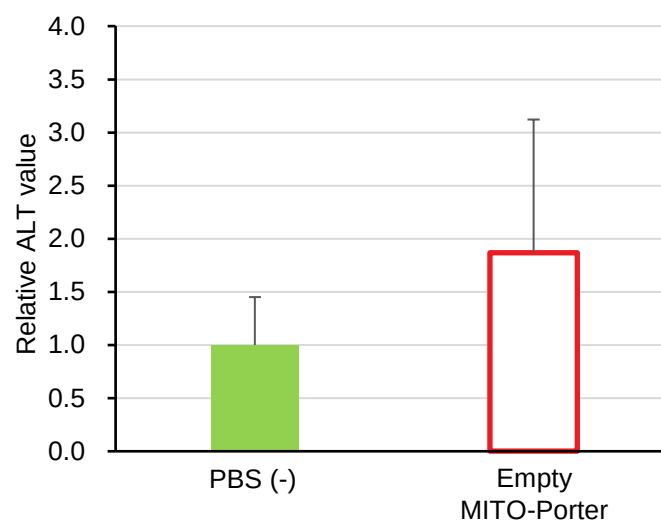


|     |   |   |   |   |
|-----|---|---|---|---|
| PEG | + | + | - | - |
| R8  | + | - | + | - |

**Figure S2 Appearance of a MITO-Porter solution before dialysis.** The appearance of a MITO-Porter solution with or without PEG, R8 and CoQ<sub>10</sub> after being prepared by a microfluidic device were evaluated. (A) Turbidity of LNP solution. Data represented the mean  $\pm$  S.D. (n=4). The significant differences were calculated by two-way ANOVA, followed by Tukey test (\*\*p<0.001). (B) Appearance of LNP solution. (a) Empty-MITO-Porter and (b) CoQ<sub>10</sub>-MITO-Porter are compared.



**Figure. S3 Measurement of serum LDH levels.** Serum lactate dehydrogenase (LDH) was evaluated, as biochemical evaluation other than serum ALT, a marker of liver function, in the APAP-induced liver injury model mice. Data represented the mean (n=2-4).



**Figure. S4 The values for relative ALT.** The empty-MITO-Porter was performed as a control experiment to confirm that the delivery of CoQ<sub>10</sub>-MITO-Porter system protects against APAP liver injury. Data represented the mean  $\pm$  S.D. (n=4). In [Fig. S4](#), significant differences between PBS (-) group and empty-MITO-Porter group were calculated by the Unpaired t-test, and no significant difference was found. The physical properties of the administered empty-MITO-Porter are reported [Table S8](#) in supplementary information.

## Supplementary Table

**Table S1** Characteristics of the MITO-Porters.

| Sample                                                   | Diameter (nm)         | Polydispersity index | $\zeta$ -potential (mV) |
|----------------------------------------------------------|-----------------------|----------------------|-------------------------|
| After preparing by microfluidic devise (before dialysis) |                       |                      |                         |
| Empty-MITO-Porter                                        | $34.2 \pm 6.7$        | $0.257 \pm 0.0187$   | $11.6 \pm 1.4$          |
| [PEG (+), R8 (+)]                                        |                       |                      |                         |
| Empty-MITO-Porter                                        | $35.2 \pm 4.2$        | $0.280 \pm 0.0488$   | $-2.6 \pm 2.2$          |
| [PEG (+), R8 (-)]                                        |                       |                      |                         |
| Empty-MITO-Porter                                        | $60.1 \pm 2.4$        | $0.131 \pm 0.0164$   | $29.7 \pm 4.4$          |
| [PEG (-), R8 (+)]                                        |                       |                      |                         |
| Empty-MITO-Porter                                        | $1,433.3 \pm 352.0$   | $0.657 \pm 0.238$    | $-7.4 \pm 0.6$          |
| [PEG (-), R8 (-)]                                        |                       |                      |                         |
| After dialysis                                           |                       |                      |                         |
| Empty-MITO-Porter                                        | $59.3 \pm 2.9$        | $0.528 \pm 0.0284$   | $10.1 \pm 0.6$          |
| [PEG (+), R8 (+)]                                        |                       |                      |                         |
| Empty-MITO-Porter                                        | $54.9 \pm 3.8$        | $0.342 \pm 0.104$    | $-4.7 \pm 0.9$          |
| [PEG (+), R8 (-)]                                        |                       |                      |                         |
| Empty-MITO-Porter                                        | $75.4 \pm 1.8$        | $0.119 \pm 0.0152$   | $26.7 \pm 1.3$          |
| [PEG (-), R8 (+)]                                        |                       |                      |                         |
| Empty-MITO-Porter                                        | $5,077.8 \pm 558.5$   | $0.593 \pm 0.260$    | $-8.3 \pm 0.5$          |
| [PEG (-), R8 (-)]                                        |                       |                      |                         |
| After preparing by microfluidic devise (before dialysis) |                       |                      |                         |
| CoQ <sub>10</sub> -MITO-Porter                           | $43.9 \pm 2.1$        | $0.233 \pm 0.00787$  | $12.7 \pm 0.6$          |
| [PEG (+), R8 (+)]                                        |                       |                      |                         |
| CoQ <sub>10</sub> -MITO-Porter                           | $37.2 \pm 4.4$        | $0.183 \pm 0.0255$   | $-3.4 \pm 0.9$          |
| [PEG (+), R8 (-)]                                        |                       |                      |                         |
| CoQ <sub>10</sub> -MITO-Porter                           | $899.9 \pm 363.3$     | $0.266 \pm 0.0651$   | $38.7 \pm 1.3$          |
| [PEG (-), R8 (+)]                                        |                       |                      |                         |
| CoQ <sub>10</sub> -MITO-Porter                           | $4,075.3 \pm 1,467.8$ | $0.860 \pm 0.156$    | $-6.3 \pm 0.5$          |
| [PEG (-), R8 (-)]                                        |                       |                      |                         |
| After dialysis                                           |                       |                      |                         |
| CoQ <sub>10</sub> -MITO-Porter                           | $54.4 \pm 4.8$        | $0.289 \pm 0.0188$   | $12.6 \pm 1.4$          |
| [PEG (+), R8 (+)]                                        |                       |                      |                         |
| CoQ <sub>10</sub> -MITO-Porter                           | $56.4 \pm 3.6$        | $0.206 \pm 0.246$    | $-4.8 \pm 0.2$          |

|                                |                   |               |            |
|--------------------------------|-------------------|---------------|------------|
| [PEG (+), R8 (-)]              |                   |               |            |
| CoQ <sub>10</sub> -MITO-Porter | 387.9 ± 54.2      | 0.804 ± 0.119 | 37.6 ± 1.5 |
| [PEG (-), R8 (+)]              |                   |               |            |
| CoQ <sub>10</sub> -MITO-Porter | 7,447.5 ± 1,302.1 | 0.813 ± 0.232 | -8.6 ± 0.8 |
| [PEG (-), R8 (-)]              |                   |               |            |

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Particle size, polydispersity index and  $\zeta$ -potential were summarized for the empty-MITO-Porter and the CoQ<sub>10</sub>-MITO-Porter after being prepared by a microfluidic device and after dialysis. Data represents the mean  $\pm$  S.D. (n=4).

**Table S2 Absorbance measurements of MITO-Porter suspension.**

| Sample                                                   | 660 nm Absorbance |   |        |
|----------------------------------------------------------|-------------------|---|--------|
| After preparing by microfluidic devise (before dialysis) |                   |   |        |
| Empty-MITO-Porter [PEG (+), R8 (+)]                      | -0.0107           | ± | 0.0222 |
| Empty-MITO-Porter [PEG (+), R8 (-)]                      | -0.134            | ± | 0.0092 |
| Empty-MITO-Porter [PEG (-), R8 (+)]                      | 0.0270            | ± | 0.0106 |
| Empty-MITO-Porter [PEG (-), R8 (-)]                      | 0.902             | ± | 0.0442 |
| CoQ <sub>10</sub> -MITO-Porter [PEG (+), R8 (+)]         | -0.122            | ± | 0.0055 |
| CoQ <sub>10</sub> -MITO-Porter [PEG (+), R8 (-)]         | -0.0237           | ± | 0.0932 |
| CoQ <sub>10</sub> -MITO-Porter [PEG (-), R8 (+)]         | 1.76              | ± | 0.139  |
| CoQ <sub>10</sub> -MITO-Porter [PEG (-), R8 (-)]         | 0.772             | ± | 0.171  |
| After dialysis                                           |                   |   |        |
| Empty-MITO-Porter [PEG (+), R8 (+)]                      | 0.225             | ± | 0.0071 |
| Empty-MITO-Porter [PEG (+), R8 (-)]                      | 0.204             | ± | 0.0384 |
| Empty-MITO-Porter [PEG (-), R8 (+)]                      | 0.205             | ± | 0.0175 |
| Empty-MITO-Porter [PEG (-), R8 (-)]                      | 0.783             | ± | 0.0322 |
| CoQ <sub>10</sub> -MITO-Porter [PEG (+), R8 (+)]         | 0.0117            | ± | 0.0032 |
| CoQ <sub>10</sub> -MITO-Porter [PEG (+), R8 (-)]         | 0.0347            | ± | 0.0402 |
| CoQ <sub>10</sub> -MITO-Porter [PEG (-), R8 (+)]         | 1.10              | ± | 0.123  |
| CoQ <sub>10</sub> -MITO-Porter [PEG (-), R8 (-)]         | 0.657             | ± | 0.0407 |

Absorbance intensity was summarized regarding empty-MITO-Porter and CoQ<sub>10</sub>-MITO-Porter after microfluidic device preparation and after dialysis. Data represented the mean ± S.D. (n=4).

**Table S3** Two-way ANOVA analysis results of the physicochemical properties of MITO-Porters prepared using a microfluidic device (before dialysis).

| Sample                         | Parameter          | Factors                         | P-value by 2-way ANOVA | Significant difference |
|--------------------------------|--------------------|---------------------------------|------------------------|------------------------|
| Empty-MITO-Porter              | Particle size      | PEG                             | $3.4 \times 10^{-6}$   | $< 0.001$              |
|                                |                    | R8                              | $4.8 \times 10^{-6}$   | $< 0.001$              |
|                                |                    | Interaction between two factors | $4.9 \times 10^{-6}$   | $< 0.001$              |
|                                | Dispersibility     | PEG                             | $6.6 \times 10^{-2}$   | NS                     |
|                                |                    | R8                              | $7.7 \times 10^{-4}$   | $< 0.001$              |
|                                |                    | Interaction between two factors | $1.5 \times 10^{-3}$   | $< 0.01$               |
|                                | $\zeta$ -potential | PEG                             | $2.4 \times 10^{-4}$   | $< 0.001$              |
|                                |                    | R8                              | $1.5 \times 10^{-10}$  | $< 0.001$              |
|                                |                    | Interaction between two factors | $1.6 \times 10^{-6}$   | $< 0.001$              |
| CoQ <sub>10</sub> -MITO-Porter | Particle size      | PEG                             | $3.1 \times 10^{-5}$   | $< 0.001$              |
|                                |                    | R8                              | $1.3 \times 10^{-3}$   | $< 0.01$               |
|                                |                    | Interaction between two factors | $1.2 \times 10^{-3}$   | $< 0.01$               |
|                                | Dispersibility     | PEG                             | $2.6 \times 10^{-6}$   | $< 0.001$              |
|                                |                    | R8                              | $3.8 \times 10^{-5}$   | $< 0.001$              |
|                                |                    | Interaction between two factors | $7.1 \times 10^{-6}$   | $< 0.001$              |
|                                | $\zeta$ -potential | PEG                             | $6.3 \times 10^{-12}$  | $< 0.001$              |
|                                |                    | R8                              | $5.7 \times 10^{-17}$  | $< 0.001$              |
|                                |                    | Interaction between two factors | $4.4 \times 10^{-13}$  | $< 0.001$              |

Physical property values of the MITO-Porters were subjected to a 2-way ANOVA analysis to compare the effect of 2 factors, “PEG” and “R8”. A significant interaction between the two factors was found and a simple main effect test was performed. Significant differences (NS: not significant,  $p < 0.01$ ,  $p < 0.001$ ) were calculated by a simple main effect test, followed by Tukey test.

**Table S4** Two-way ANOVA analysis of the turbidity of a MITO-Porter solution prepared using a microfluidic device (before dialysis).

| Sample                         | Factors                         | P-value by 2-way ANOVA | Significant difference |
|--------------------------------|---------------------------------|------------------------|------------------------|
| Empty-MITO-Porter              | PEG                             | $6.4 \times 10^{-10}$  | $< 0.001$              |
|                                | R8                              | $1.8 \times 10^{-9}$   | $< 0.001$              |
|                                | Interaction between two factors | $4.9 \times 10^{-10}$  | $< 0.001$              |
|                                |                                 |                        |                        |
| CoQ <sub>10</sub> -MITO-Porter | PEG                             | $5.2 \times 10^{-10}$  | $< 0.001$              |
|                                | R8                              | $4.1 \times 10^{-5}$   | $< 0.001$              |
|                                | Interaction between two factors | $5.2 \times 10^{-6}$   | $< 0.001$              |
|                                |                                 |                        |                        |

Absorbance intensity of the MITO-Porters were subjected to a 2-way ANOVA analysis to compare the effect of 2 factors that are “PEG” and “R8”. A significant interaction between the two factors was found and then a simple main effect test performed. Significant differences ( $p < 0.001$ ) are calculated by a simple main effect test, followed by Tukey test.

**Table S5 Characteristics of LNPs.**

| Sample                         | Diameter (nm) | Polydispersity index | ζ -potential (mV) |
|--------------------------------|---------------|----------------------|-------------------|
| Empty-MITO-Porter              | 40.8          | 0.371                | 11.2              |
| CoQ <sub>10</sub> -MITO-Porter | 51.5          | 0.327                | 8.5               |

Particle properties of the empty-MITO-Porter and CoQ<sub>10</sub>-MITO-Porter for SAXS analysis (Fig. 4B) are summarized. Number of data is 1.

**Table S6 Characteristics of the CoQ<sub>10</sub>-MITO-Porter labelled with DiD.**

| Sample                             | Diameter (nm) | Polydispersity index | ζ-potential (mV) |
|------------------------------------|---------------|----------------------|------------------|
| DiD-CoQ <sub>10</sub> -MITO-Porter | 52.8 ± 2.7    | 0.269 ± 0.03         | 9.5 ± 1.3        |

Particle properties of the CoQ<sub>10</sub>-MITO-Porter labeled with DiD for pharmacokinetics analysis (Fig. 5) are summarized. Data represent the mean ± S.D. (n=3).

**Table S7 Characteristics of the CoQ<sub>10</sub>-MITO-Porter.**

| Sample                         | Diameter (nm) | Polydispersity index | ζ-potential (mV) |
|--------------------------------|---------------|----------------------|------------------|
| CoQ <sub>10</sub> -MITO-Porter | 52.2 ± 4.0    | 0.307 ± 0.05         | 14.0 ± 1.8       |

Particle properties of the CoQ<sub>10</sub>-MITO-Porter for animal studies against APAP liver injury (Fig. 6) are summarized. Data represent the mean ± S.D. (n=3).

**Table S8 Characteristics of the empty-MITO-Porter.**

| Sample            | Diameter (nm) | Polydispersity index | ζ-potential (mV) |
|-------------------|---------------|----------------------|------------------|
| Empty-MITO-Porter | 53.8 ± 1.7    | 0.473 ± 0.121        | 12.9 ± 1.3       |

Particle properties of the empty-MITO-Porter for animal studies against APAP liver injury (Fig. S4) are summarized. Data represent the mean ± S.D. (n=4).