

## **Ginsenoside Rg3-containing ionizable lipid nanoparticles for siRNA delivery**

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**Table S1.** Formulation of LNPs with diverse molar ratio of MC3, DSPC, PEG and Rg3  
**L16(4<sup>4</sup>) orthogonal table**

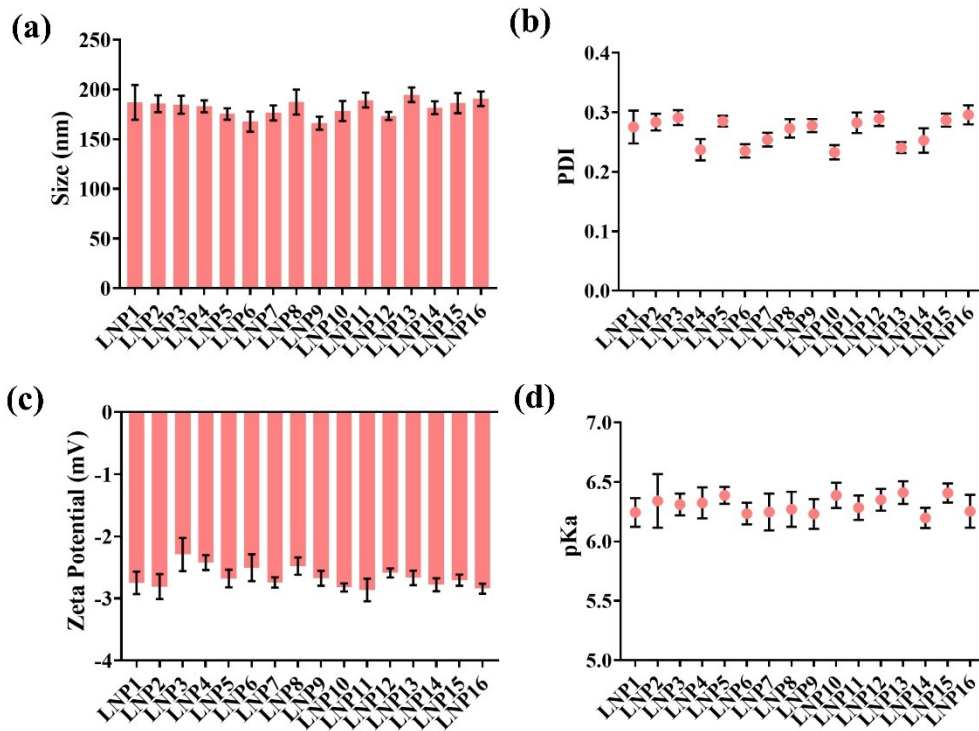
|              | <b>MC3</b> | <b>DSPC</b> | <b>PEG</b> | <b>Rg3</b> |
|--------------|------------|-------------|------------|------------|
| <b>LNP1</b>  | 15         | 10          | 0.5        | 10         |
| <b>LNP2</b>  | 15         | 20          | 1          | 20         |
| <b>LNP3</b>  | 15         | 30          | 2.5        | 30         |
| <b>LNP4</b>  | 15         | 40          | 5          | 40         |
| <b>LNP5</b>  | 25         | 10          | 1          | 30         |
| <b>LNP6</b>  | 25         | 20          | 0.5        | 40         |
| <b>LNP7</b>  | 25         | 30          | 5          | 10         |
| <b>LNP8</b>  | 25         | 40          | 2.5        | 20         |
| <b>LNP9</b>  | 35         | 10          | 2.5        | 40         |
| <b>LNP10</b> | 35         | 20          | 5          | 30         |
| <b>LNP11</b> | 35         | 30          | 0.5        | 20         |
| <b>LNP12</b> | 35         | 40          | 1          | 10         |
| <b>LNP13</b> | 45         | 10          | 5          | 20         |
| <b>LNP14</b> | 45         | 20          | 2.5        | 10         |
| <b>LNP15</b> | 45         | 30          | 1          | 40         |
| <b>LNP16</b> | 45         | 40          | 0.5        | 30         |

**Table S2.** Physical properties of Rg3-LNPs-siRNA and Chol-LNPs-siRNA

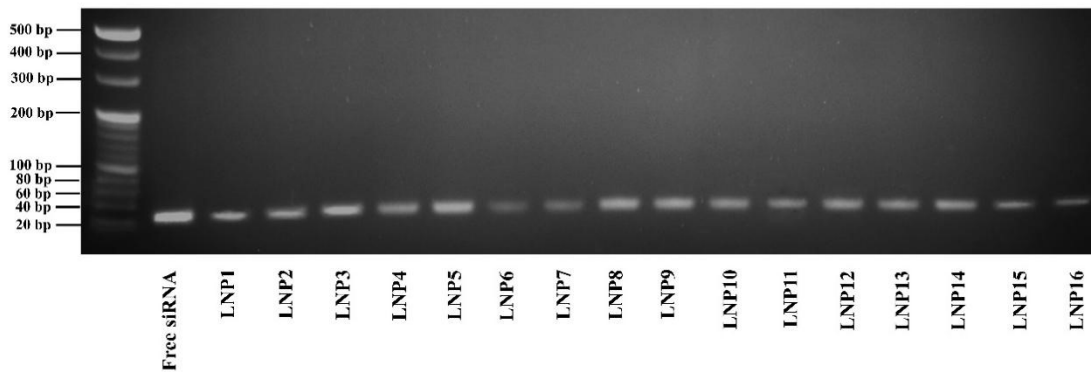
|                        | <b>Size (nm)</b> | <b>PDI</b>   | <b>Zeta Potential (mV)</b> |
|------------------------|------------------|--------------|----------------------------|
| <b>Rg3-LNPs-siRNA</b>  | 172 ± 6          | 0.273 ± 0.13 | -0.83 ± 0.10               |
| <b>Chol-LNPs-siRNA</b> | 165 ± 10         | 0.289 ± 0.07 | -0.77 ± 0.22               |

**Table S3.** Physical properties of Rg3-LNPs-siSTAT3 and Chol-LNPs-siSTAT3

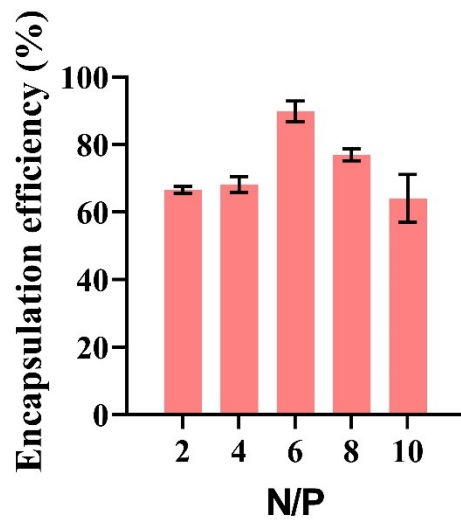
|                          | <b>Size (nm)</b> | <b>PDI</b>   | <b>Zeta Potential (mV)</b> |
|--------------------------|------------------|--------------|----------------------------|
| <b>Rg3-LNPs-siSTAT3</b>  | 166 ± 17         | 0.235 ± 0.09 | -0.50 ± 0.17               |
| <b>Chol-LNPs-siSTAT3</b> | 169 ± 8          | 0.242 ± 0.07 | -0.66 ± 0.13               |



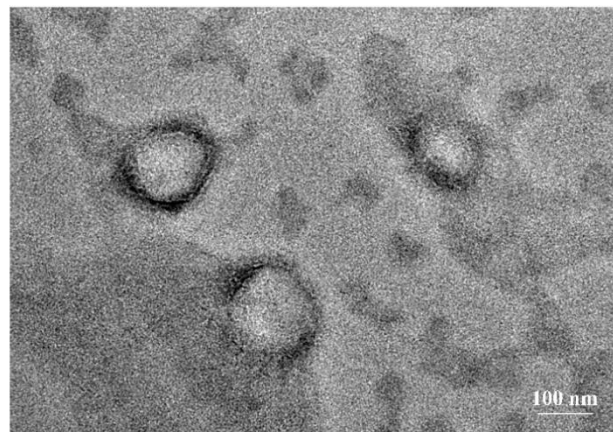
**Fig. S1.** Particle size of sixteen groups of Rg3-LNPs (a), PDI (b), Zeta potential (c) and pKa values (d).



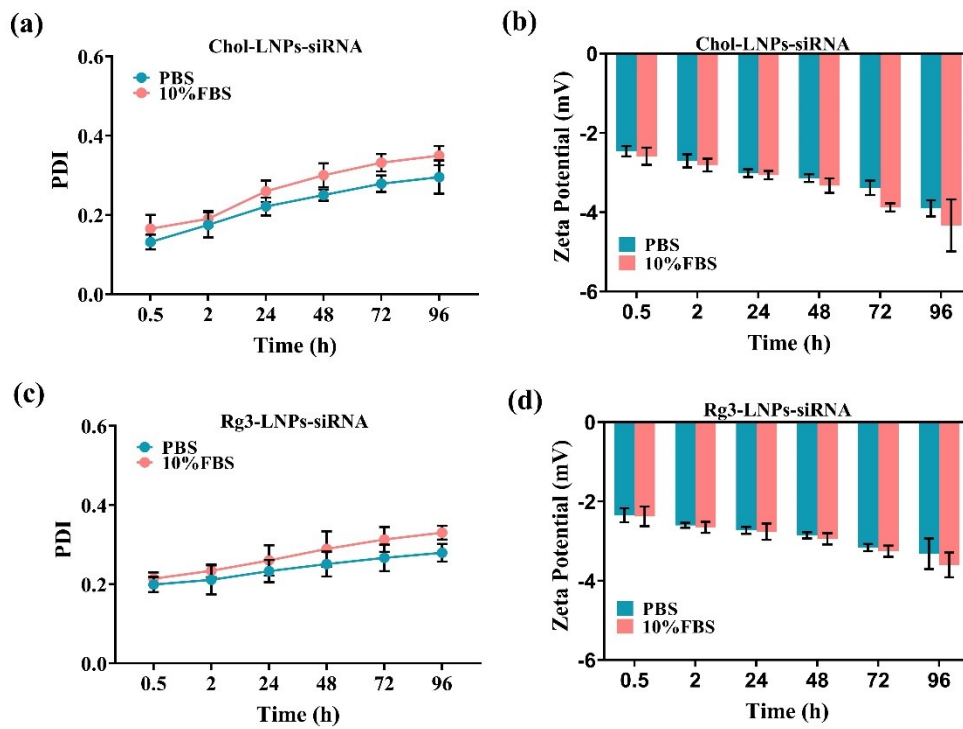
**Fig. S2.** Gel electrophoresis analysis of siRNA loading efficiency for 16 groups of LNPs.



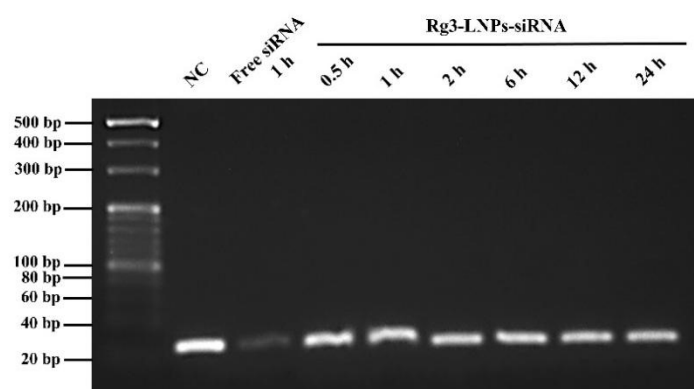
**Fig. S3.** Encapsulation efficiency of LNP6 under different N/P.



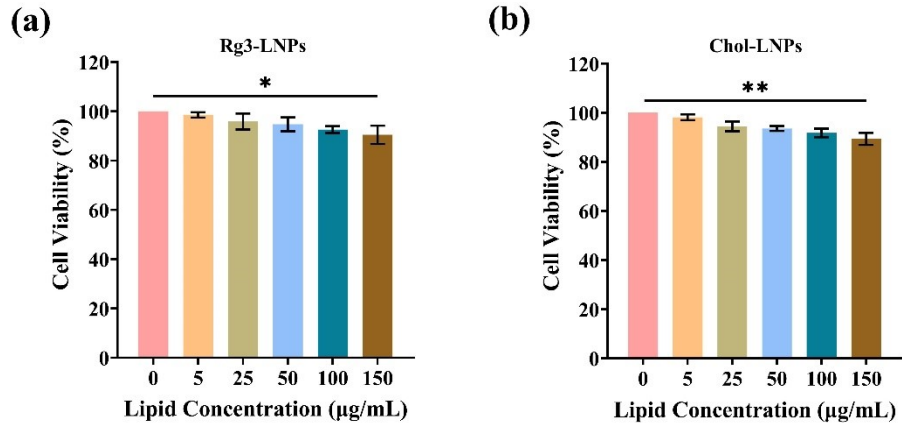
**Fig. S4.** TEM image of Rg3-LNPs-siRNA.



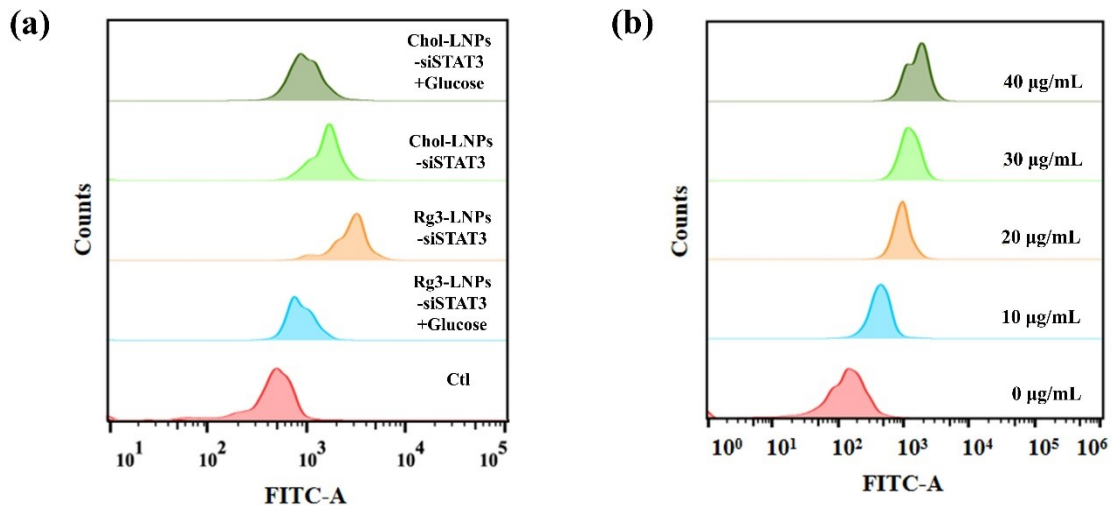
**Fig. S5.** The PDI and Zeta potential of Chol-LNPs-siRNA (a, b) and Rg3-LNPs-siRNA (c, d) in different medium conditions with different incubation time periods characterized by DLS.



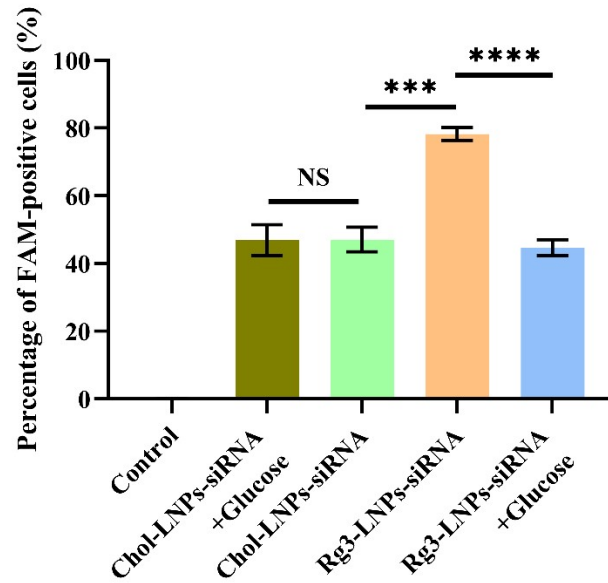
**Fig. S6.** Gel electrophoresis analysis of the RNase stability of siRNA in Rg3-LNPs.



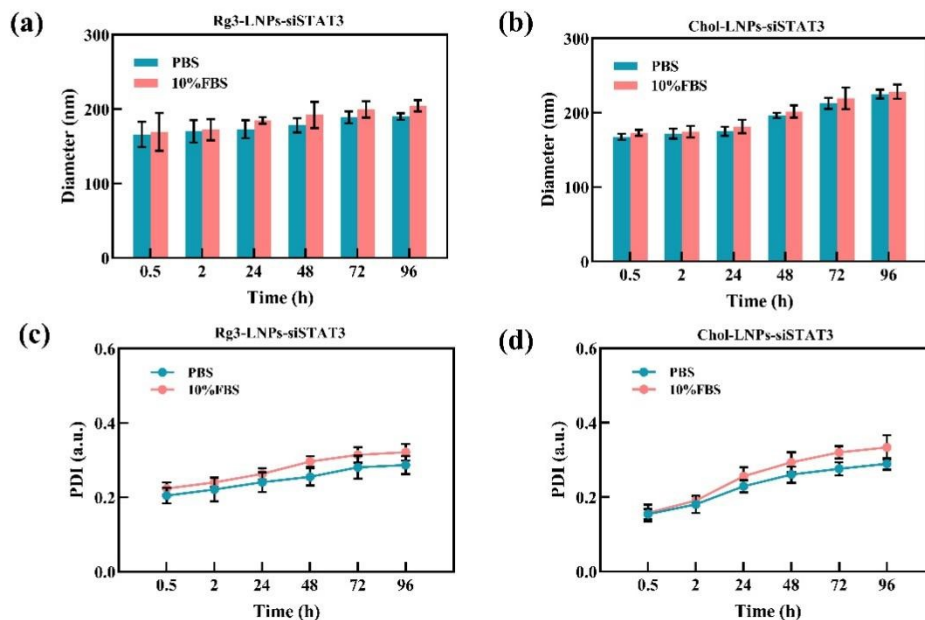
**Fig. S7.** Cell viability of Rg3-LNPs (a) and Chol-LNPs (b) after incubation with different lipid concentration for 24h. Data in a, b are expressed as mean±standard deviation (n=3 biologically independent samples). Statistical significance was analyzed by two-tailed unpaired t test: \*P < 0.05, \*\*P < 0.01.



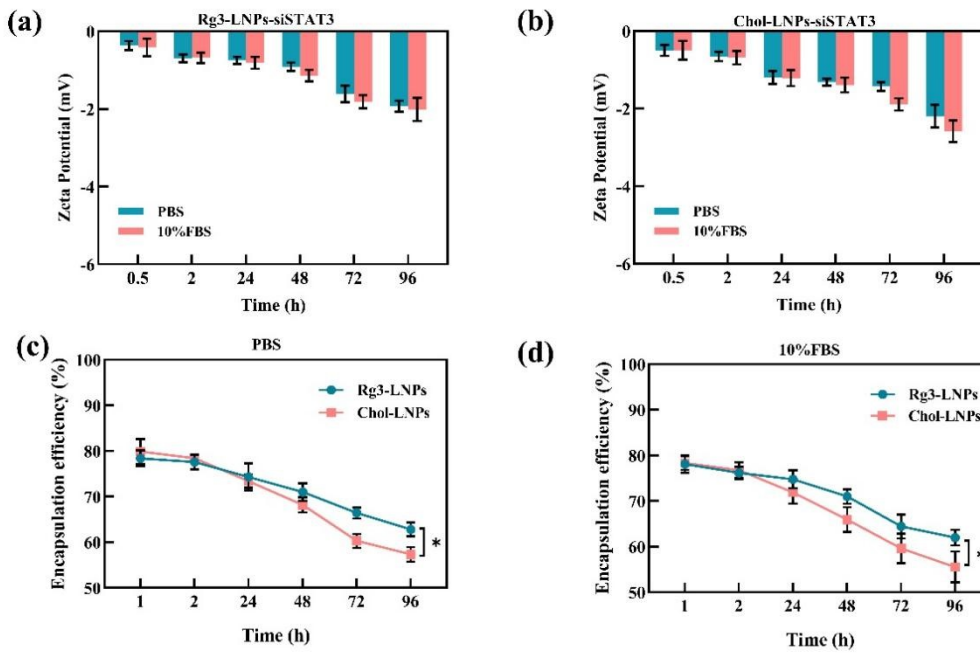
**Fig. S8.** (a) The uptake of Rg3-LNPs-siRNA, Chol-LNPs-siRNA, Rg3-LNPs-siRNA+Glucose, and Chol-LNPs-siRNA+Glucose by 4T1 cells analyzed by flow cytometry. (b) Dose-dependent cellular uptake of FAM-siRNA in 4T1 cells treated with different concentrations of Rg3-LNPs-siRNA detected by flow cytometry.



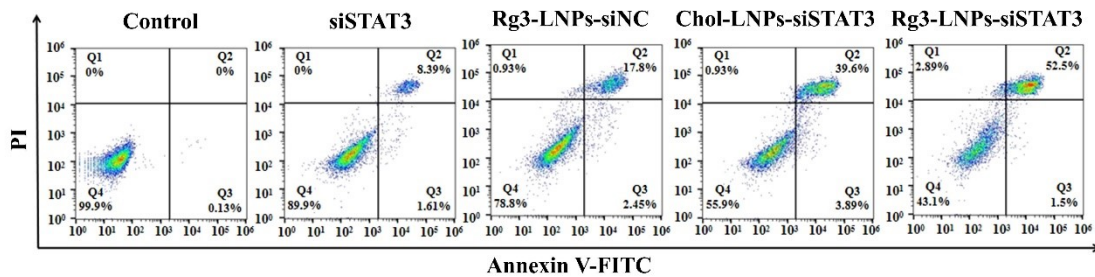
**Fig. S9.** Flow cytometry was used to determine the percentage of FAM-positive cells in total 4T1 cells treated with Rg3-LNPs-siRNA, chol-LNPs-siRNA, Rg3-LNPs-siRNA+Glucose, and chol-LNPs-siRNA+Glucose. Data are expressed as mean±standard deviation (n=3 biologically independent samples). Statistical significance was analyzed by two-tailed unpaired t test: ns, not significant, \*\*\*P < 0.001, \*\*\*\*p < 0.0001.



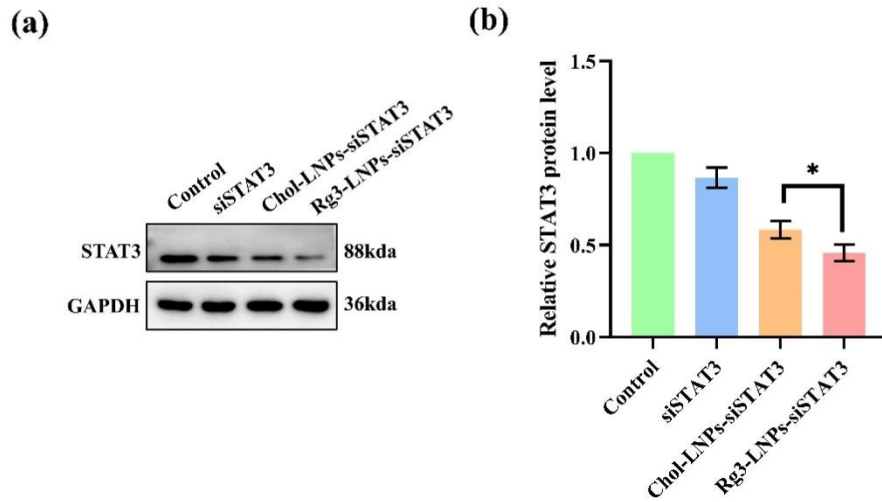
**Fig. S10.** The Diameter (a, b) and PDI (c, d) of Chol-LNPs-siSTAT3 and Rg3-LNPs-siSTAT3 in different medium conditions with different incubation time periods characterized by DLS.



**Fig. S11.** The Zeta potential (a, b) and EE (c, d) of Chol-LNPs-si STAT3 and Rg3-LNPs-siSTAT3 in different medium conditions with different incubation time periods characterized by DLS. Data in c, d are expressed as mean  $\pm$  standard deviation ( $n=3$  biologically independent samples). Statistical significance was analyzed by two-tailed unpaired t test: \* $P < 0.05$ .



**Fig. S12.** Flow cytometric analysis of apoptosis in 4T1 cells after 48 h of incubation with PBS, siSTAT3, Rg3-LNPs-siNC, Chol-LNPs-siSTAT3 and Rg3-LNPs-siSTAT3.



**Fig. S13.** (a) Western blot analysis of STAT3 proteins. (b) Quantitative analysis of the Western blot. Data are expressed as mean  $\pm$  standard deviation (n=3 biologically independent samples). Statistical significance was analyzed by two-tailed unpaired t test: \*P < 0.05.