# Kidney-targeted rhein-loaded liponanoparticles for diabetic nephropathy therapy via size control and enhancement of renal cellular uptake

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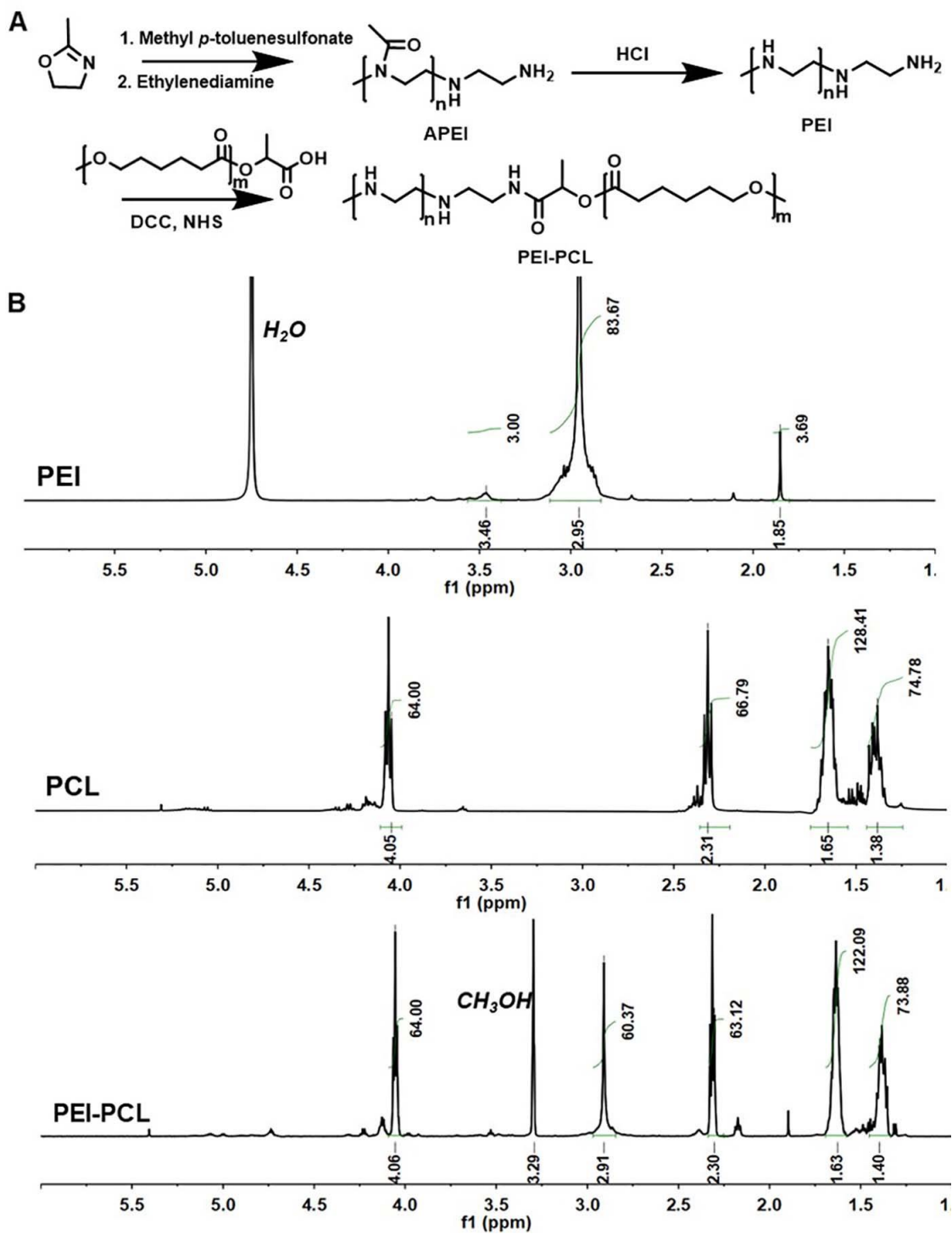
**Supplementary Material**

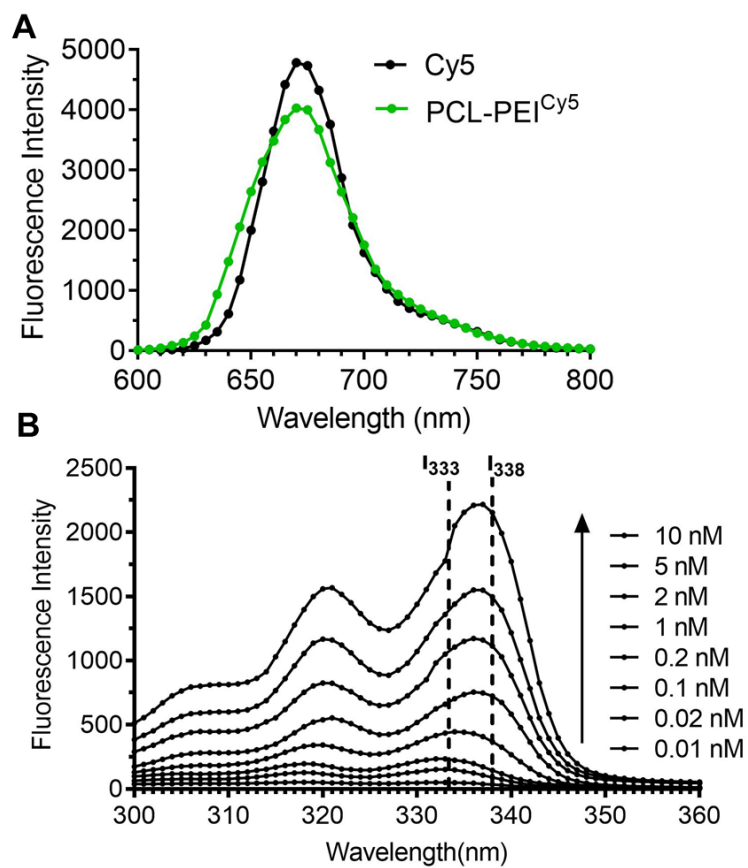**Figure S1.** Synthetic route (A) and $^1$H NMR spectra of the polymers (B).

**Figure S2.** Fluorescent scanning spectra of Cy5 labeled PCL-PEI (A). Fluorescence intensity of pyrene against various concentrations of polymers (B).
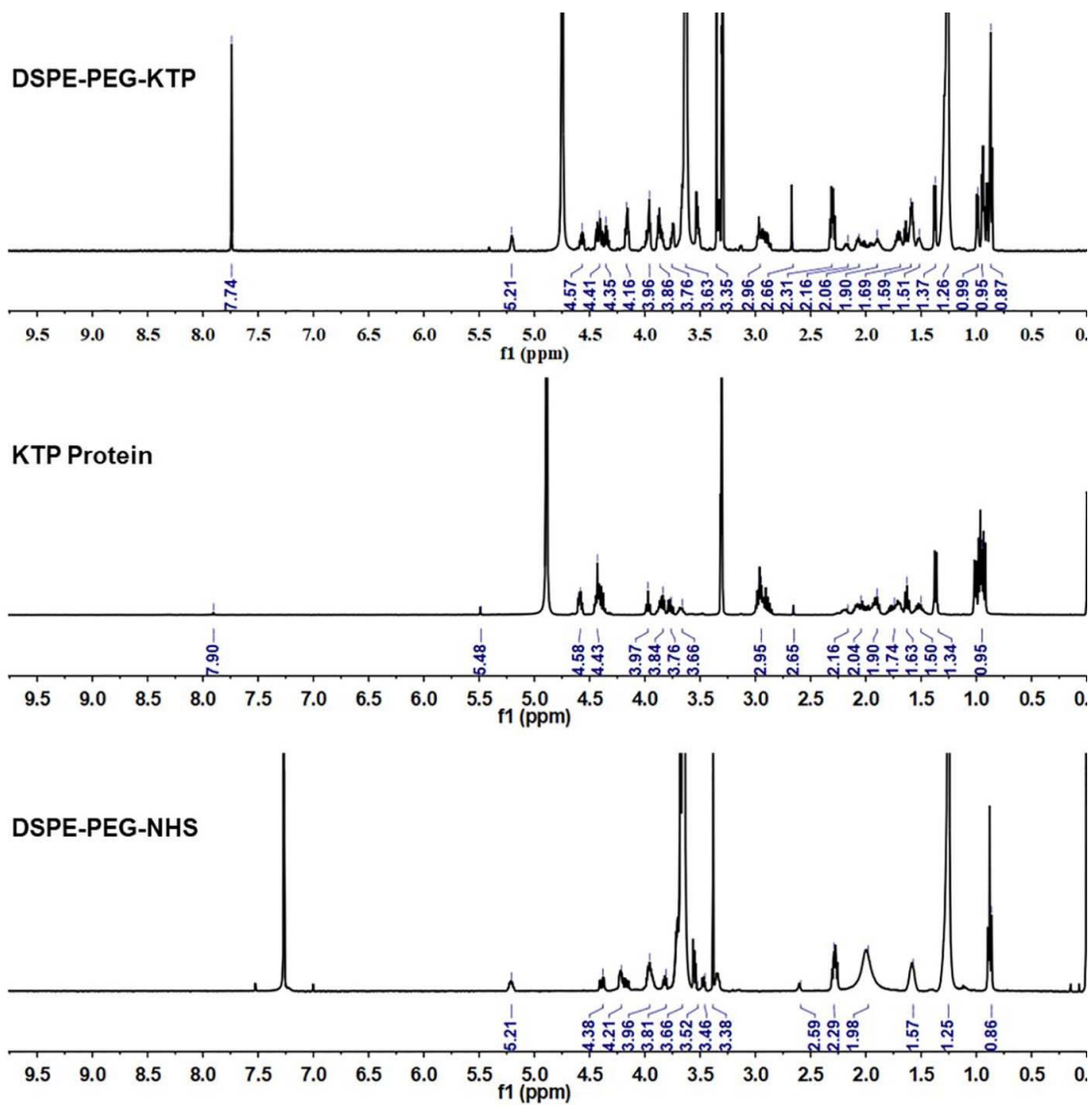
**Figure S3.** $^1$H NMR spectra of DSPE-PEG-KTP, KTP protein and DSPE-PEG-NHS.
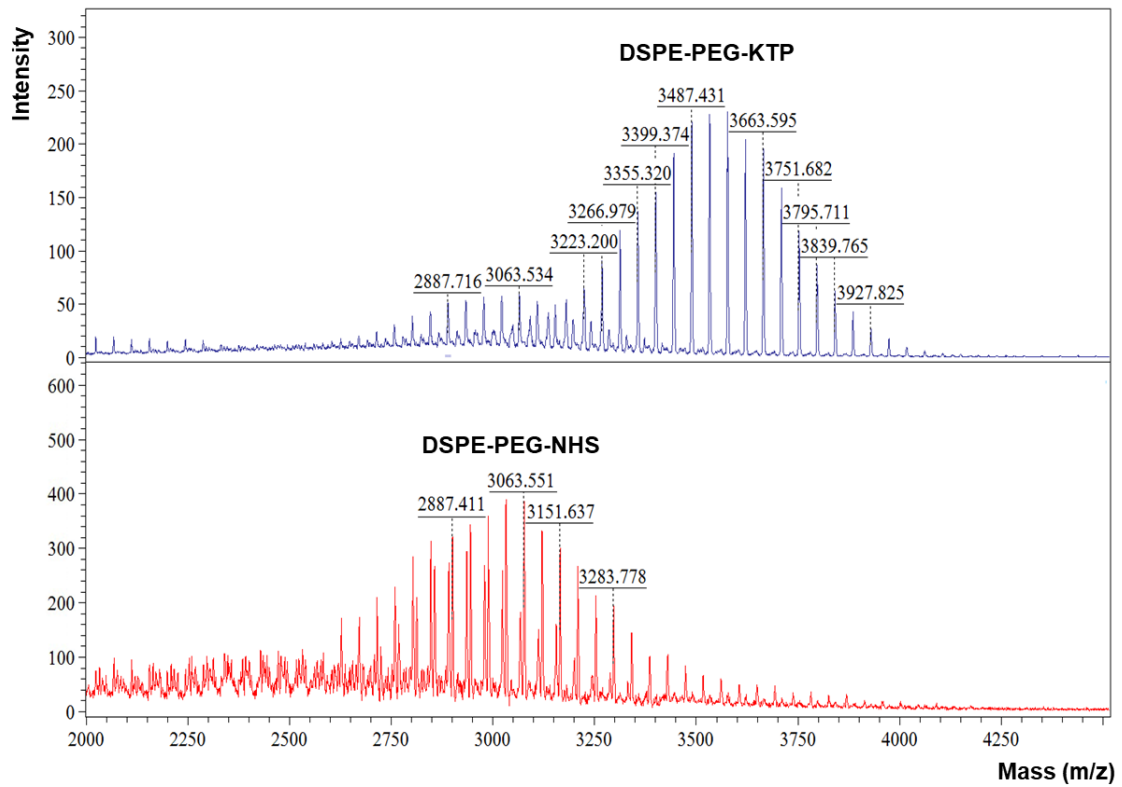
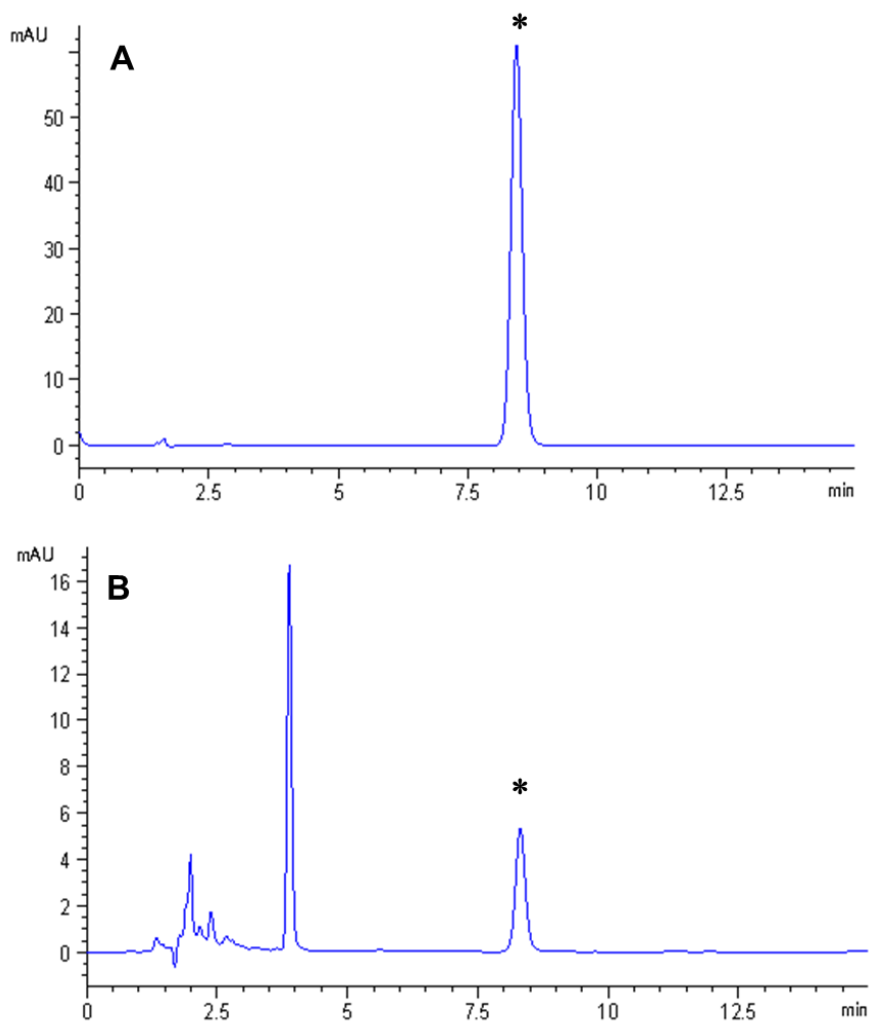**Figure S4.** The MALDI-TOF-MS spectrum of DSPE-PEG-KTP and DSPE-PEG-NHS.

**Figure S5.** HPLC chromatograms of RH standard solution (A) and plasma added with RH standard solution (B). The retention time of RH was 8.44 min. ∗ indicated the chromatograph peak of RH.

**Table S1.** Release kinetics of KLPPR in PBS (pH 7.4).

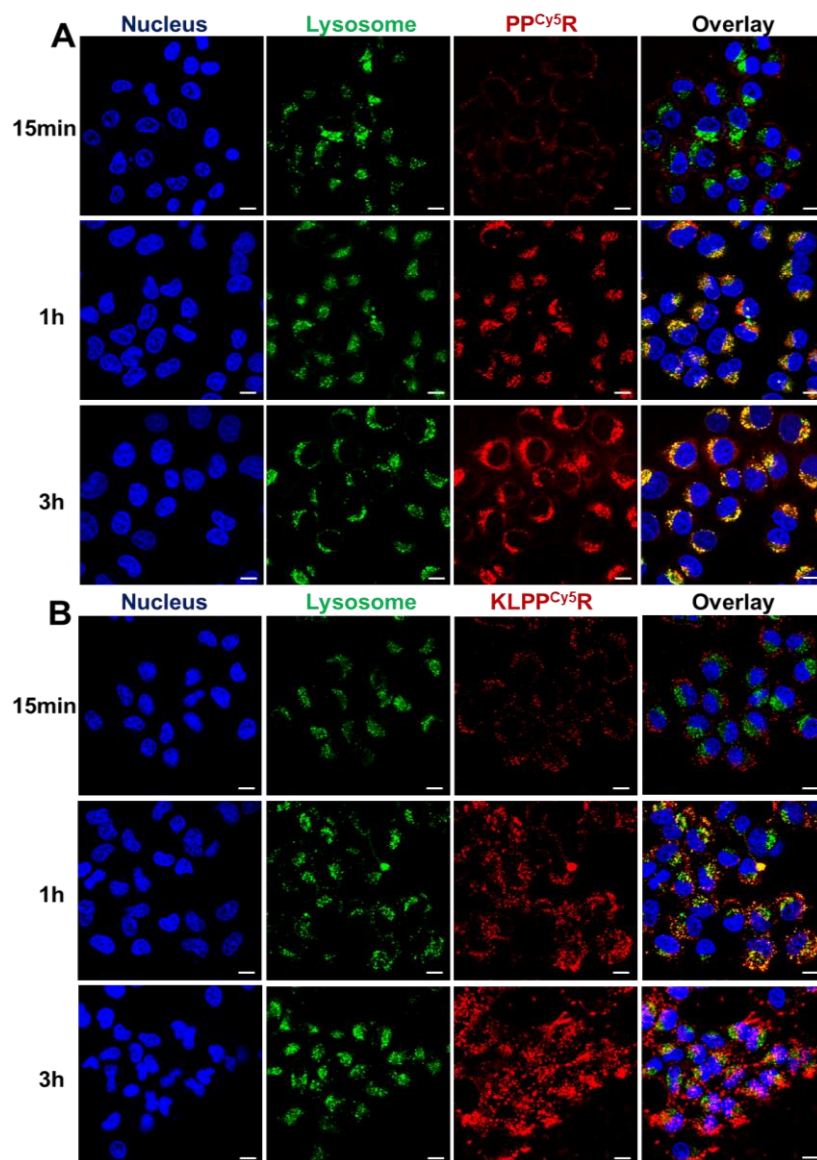| Model | Regression equation | $R^2$ |
|---|---|---|
| Zero-order | $Q = 0.0117t + 0.1867$ | 0.6105 |
| First-order | $\ln(1-Q) = -0.0191t - 0.2168$ | 0.7218 |
| Higuchi | $Q = 0.979t^{1/2} + 0.0648$ | 0.8655 |

**Figure S6.** Subcellular distribution of (A) PP$^{Cy5}$R and (B) KLPP$^{Cy5}$R. HK-2 cells were imaged with a CLSM and the nuclei were stained with Hoechst 33342 (blue), the lysosomes were labeled with LysoTracker® Green DND26 (green) and the nanoparticles were labeled with Cy5 (red). The scale bar is 50 μm.
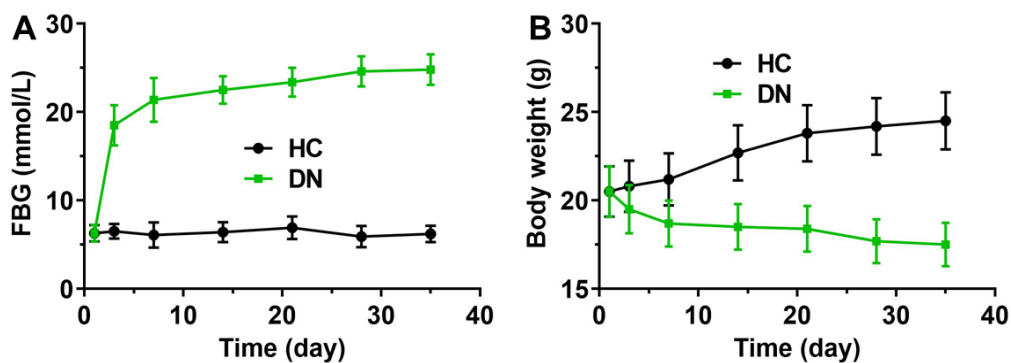
**Figure S7.** FBG (A) and body weight (B) changes of healthy control mice and diabetic nephropathy mice in five weeks after STZ-injected.

**Table S2**. Pharmacokinetic parameters of RH-sol and KLPPR (intravenously, dose equivalent to RH 5 mg/kg, 4 mice in each group). Data were presented as mean ± SD.

| Parameters | Unit | RH-sol | KLPPR |
|:---:|:---:|:---:|:---:|
| $T_{1/2}$ | h | $0.42 \pm 0.17$ | $1.44 \pm 0.28$ |
| CL | $(\mu g)/(\mu g/mL)/h$ | $5.09 \pm 1.48$ | $1.60 \pm 0.33$ |
| $AUC_{0-t}$ | $\mu g /mL \cdot h$ | $19.64 \pm 3.55$ | $62.26 \pm 7.29$ |
| MRT | h | $0.65 \pm 0.13$ | $2.18 \pm 0.45$ |

$T_{1/2}$, CL, $AUC_{0-t}$ and MRT refer to elimination half-time, clearance, area under curve and mean retention time respectively.
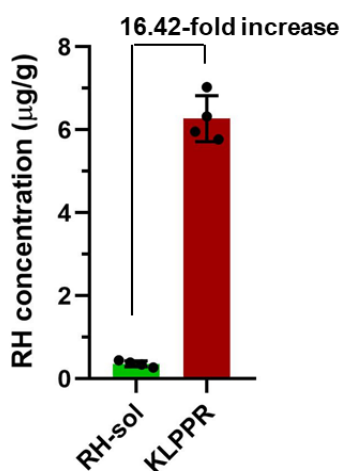


**Figure S8.** The concentration of Rhein in kidney at 12 h post-injection in diabetic

nephropathy mice. The DN mice were intravenously injected with RH-sol and KLPPR (dose equivalent to RH 5 mg/kg, 4 mice in each group).