Supplementary Text 1. Supplementary information for long-acting injectable *in situ* treprostinil depot for pulmonary hypertension.

1 Methods

1.1 Preparation of TRE-Cy5.5

Cy5.5-COOH, 1,3-dicyclohexyldiamine, 4-dimethylpyridine, and TRE were accurately weighed at a mass ratio of 2:3:1:2. Cy5.5-COOH, 1,3-dicyclohexyldiamine, and 4-dimethylpyridine were dissolved in 5 mL of acetone and stirred at 25℃ for 1 h to ensure complete activation. Subsequently, the acetone solution of TRE was gradually added to the reaction mixture, followed by a 24-h reaction at 25℃ under light-protected conditions with continuous stirring at 600 rpm. Acetone and unreacted free TRE were removed from the reaction system by dialysis. The final product, TRE-Cy5.5 was obtained after freeze-drying.

1.2 Characterization of TRE-MPs

The particle size and polydispersity index (PDI) of TRE-MPs were measured by dynamic light scattering (DLS) using a ZetaPlus nanoparticle size analyzer (Brookhaven Instruments, USA) at 25℃. The morphology of TRE-MPs was visualized by scanning electron microscopy (SEM, SIGMA HD SEM, Zeiss, Germany) with an acceleration voltage ranging from 0.02 to 30 kV.

To characterize the crystalline phase composition of the microcrystals, freeze-dried samples of TRE, P188, physical mixtures (PM), and TRE-MPs were analyzed using differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). DSC measurements were performed under a N_2 atmosphere with a flow rate of 60 mL/min and 80 mL/min, over a temperature range of 25-300℃, and a heating rate of 10℃/min. PXRD analysis employed a Cu target, with a scanning range of 3° to 40°, a step size of 0.02° per step, and a scanning speed of 10°/min.

Furthermore, to examine the microcrystalline structure and intermolecular interactions, freeze-dried samples of TRE, P188, PM, and TRE-MPs were analyzed by Fourier-transform infrared spectroscopy (FTIR) using the KBr pellet method (Bruker Tensor 27). Spectra were recorded in the range of 4000-400 cm^{-1} with a resolution of 1.0 cm^{-1} and a scan rate of 20 scans per second.

1.3 *In vitro* stability of TRE-MPs

Freeze-dried TRE-MPs were stored in sealed containers for stability tests: (1) storage at 4℃ and 25℃; (2) high-temperature at 60℃; (3) high-humidity at 25℃ with a relative humidity of 90 ± 5%; (4) strong light exposure under an illumination intensity of 4500 ± 500 Lx (stress factor test); and (5) accelerated test at 40℃ and 75 ± 5% relative humidity. For both the storage stability and stress factor tests (high temperature, high humidity, strong light), samples were taken at 0, 5, and 10 days. For accelerated stability test, samples were collected at 0 and 10 days. All collected samples were analyzed by DLS for particle size and PDI, and by HPLC to quantify drug content.

1.4 Extraction of hydrogel extract

The hydrogel extract was prepared following a previously reported method. TRE-MPs-Gel solutions at various concentrations (10, 50, 100, 200, 300, and 400 μg/mL) were exposed to UV light for 30 min, then dispensed into 24-well plates and transferred to a cell culture incubator to allow gel formation. The gels were incubated with 1.8 mL of complete culture medium for 24 h. Afterwards, the supernatant was collected to obtain the hydrogel extract.

1.5 Pharmacokinetic studies

The plasma concentration of TRE was quantified using triple quadrupole liquid chromatography-tandem mass spectrometry (LC-MS/MS). MS spectrometry conditions were as follows: Electrospray ionization (ESI) in negative ion mode, curtain gas pressure at 35 psi, ionization voltage of -4500 V, source temperature of 550°C, nebulizer gas pressure of 55 psi, and auxiliary gas pressure of 55 psi. For TRE, the precursor ion was m/z 389.2, fragment ion m/z 331.1, declustering potential of -122.19 V, and collision energy of 35.08 eV. The internal standard had a precursor ion of m/z 200.9, fragment ion m/z 143.1, declustering potential of -73.87 V, and collision energy of 30.48 eV.