Supplementary Information

Self-Targeted, Bacillus-Shaped, and Controlled-Release MTX Prodrug Polymeric Nanoparticles for Intratumoral Administration with Improved Therapeutic Efficacy in Tumor-Bearing Mice

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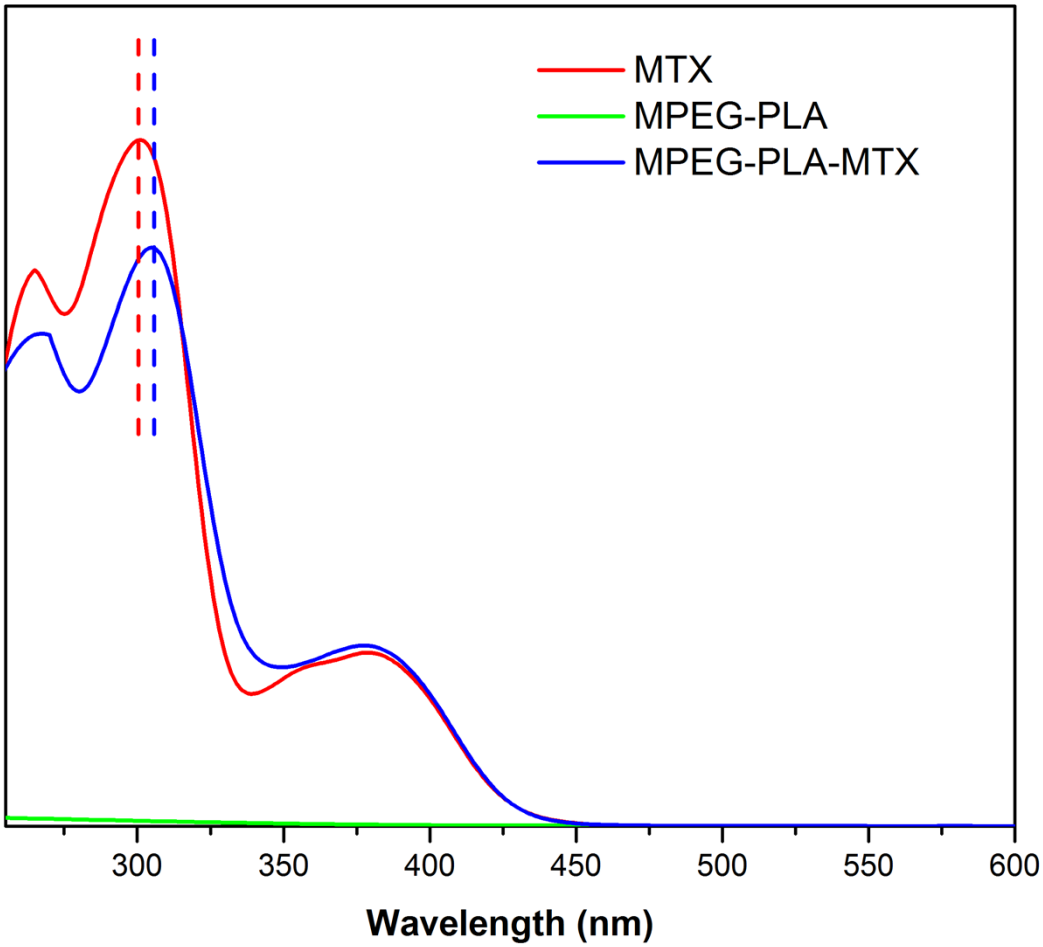

Fig. S1 UV-vis absorption spectra of MTX, MPEG-PLA, and MPEG-PLA-MTX.

The UV-vis spectrum of MPEG-PLA-MTX (Fig. S1) clearly exhibited an absorption peak at 306 nm originated from the structure of MTX. The result indicated that MTX was linked to MPEG-PLA.

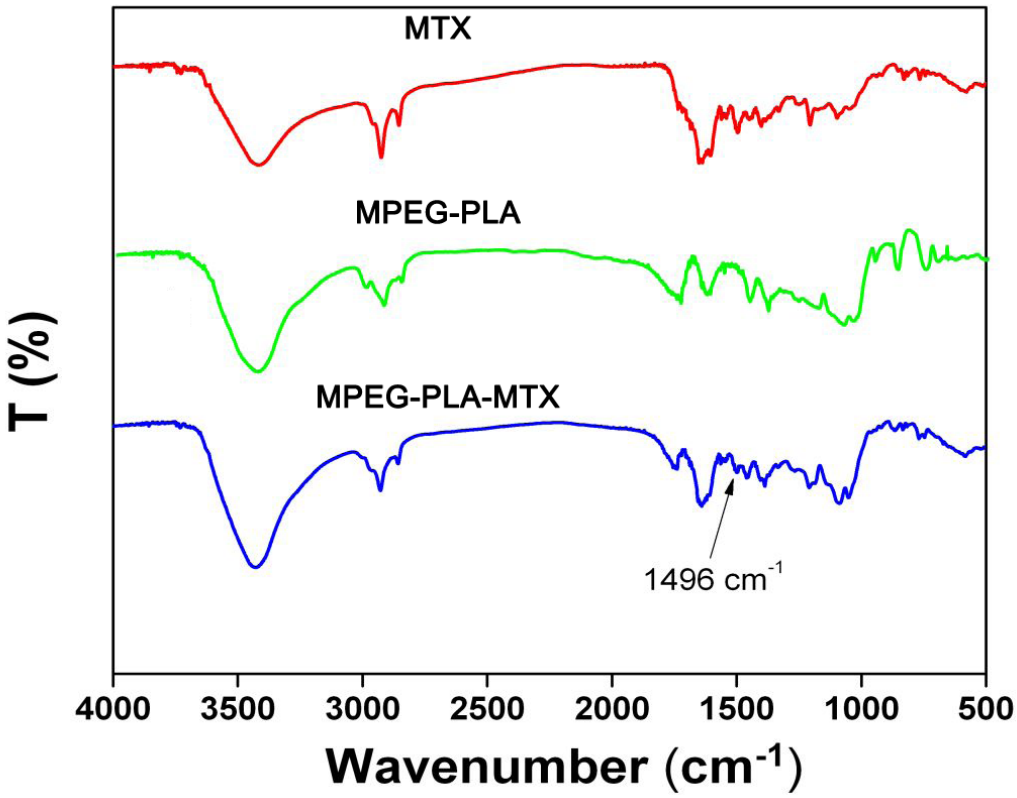

Fig. S2 FTIR spectra of MTX, MPEG-PLA, and MPEG-PLA-MTX.

As seen in FTIR spectra of MPEG-PLA-MTX compared with MTX and MPEG-PLA (Fig. S2), the peak at 1496 cm^{-1} in the MPEG-PLA-MTX indicated the benzene ring carbon skeleton stretching vibration native to the structure of MTX.

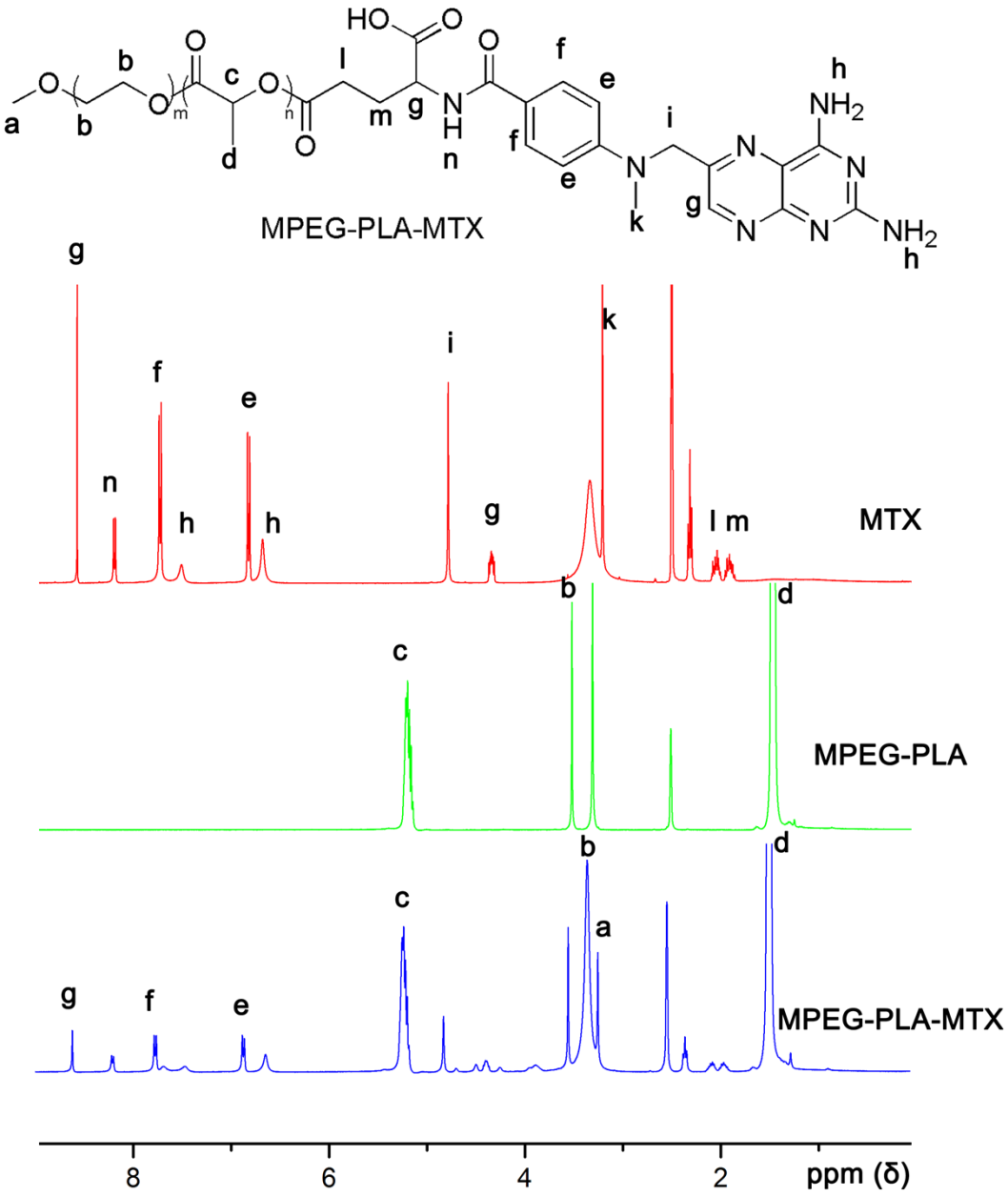

Fig. S3 ^{1}H NMR spectra of MTX, MPEG-PLA, and MPEG-PLA-MTX.

MPEG-PLA-MTX was synthesis from MPEG-PLA and MTX by adding DCC, NHS, and DMAP to trigger the formation of ester bonds. In NMR spectrum (Fig. S3), the solvent peak of DMSO was found at 2.5 ppm. The NMR spectrum of MPEG-PLA-MTX showed that PEG and PLA units appeared at

peaks of 3.2, 3.3 (CH_3-O- and $-CH_2-CH_2O-$ in MPEG), 1.5, and 5.2 ppm (CH_3- and $-CH-$ in PLA, respectively). And, the characteristic peaks related to MTX (6.8, 7.7 and 8.6 ppm) were visible, which were assigned to the protons of the pteridine ring and p-phenyl ring. The result indicated the successful preparation of MPEG-PLA-MTX. Thus, both results of UV-vis, FTIR, ^1H NMR data testified to the successful preparation of MPEG-PLA-MTX.

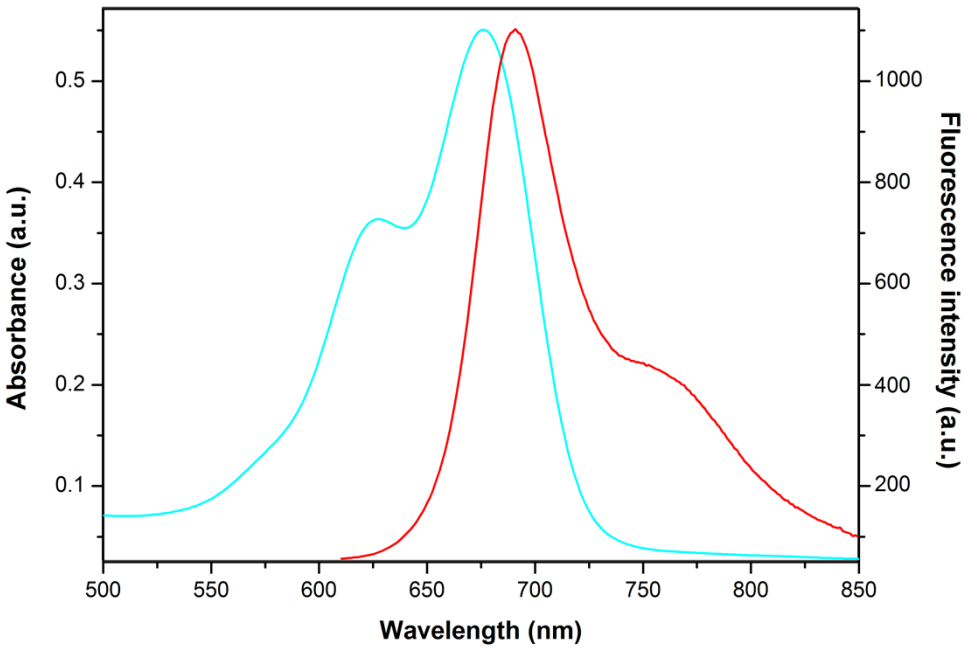

Fig. S4 UV-Vis absorption and fluorescence spectrum of the synthesized MPEG-PLA-Cy5.5.

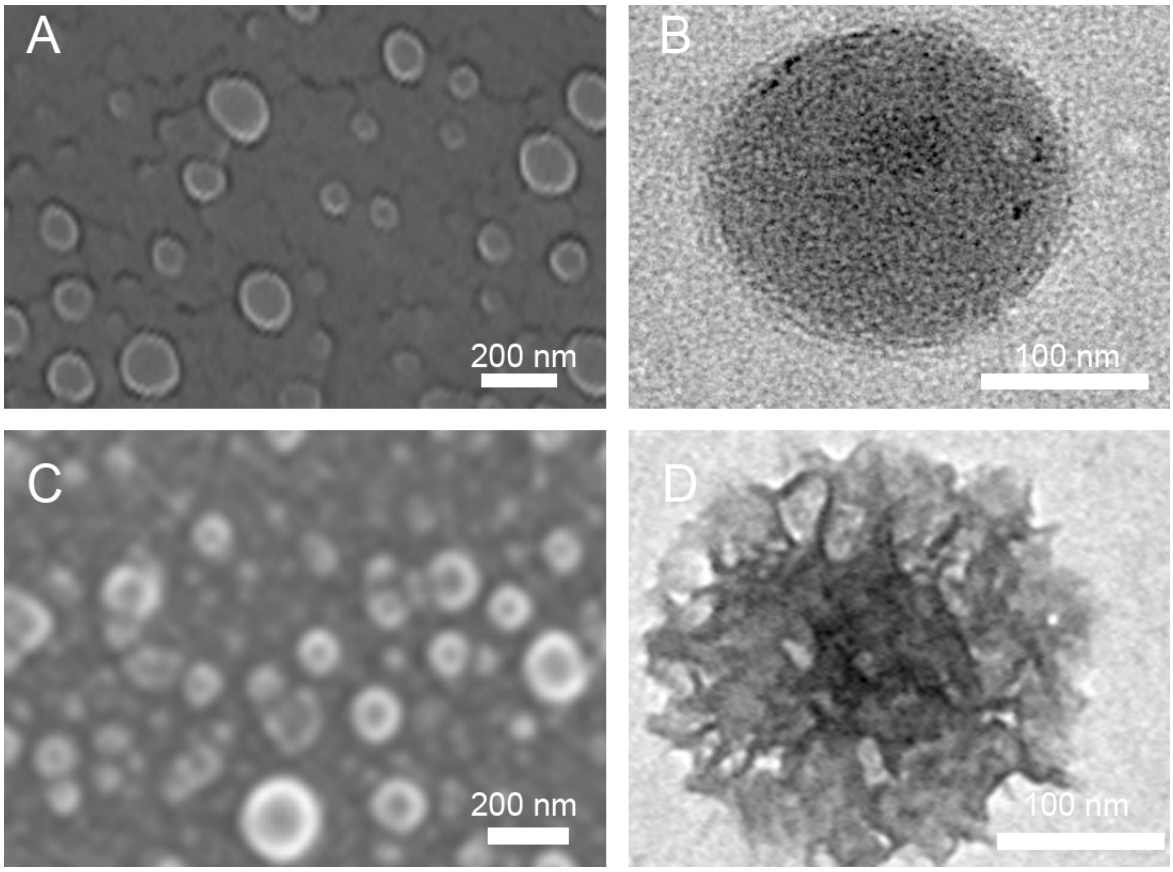

Fig. S5 (A) SEM image of the MPEG-PLA-Cy5.5 NSs. (B) TEM image of the MPEG-PLA-Cy5.5 NSs. (C) SEM image of the MPEG-PLA-MTX-Cy5.5 NSs. (D) TEM image of the MPEG-PLA-MTX-Cy5.5 NSs.

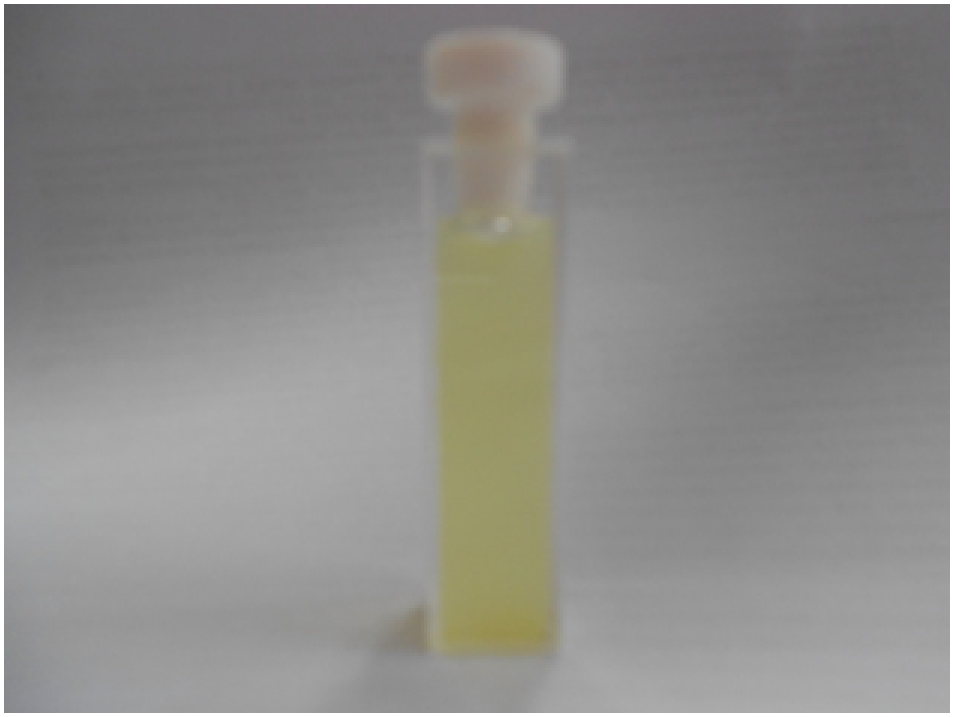

Fig. S6 Optical photograph of the MPEG-PLA-MTX NBs suspension.

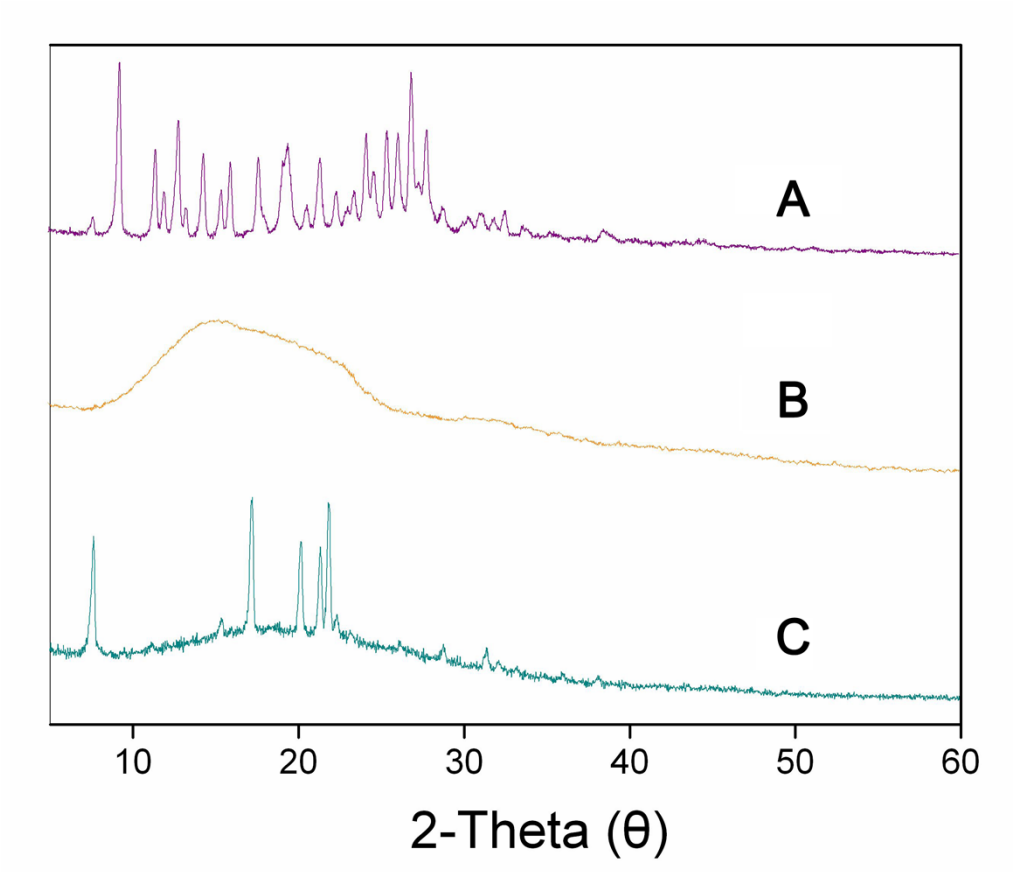

Fig. S7 XRD analysis of (A) MTX, (B) MPEG-PLA NSs, (C) MPEG-PLA-MTX NBs. Some characteristic crystalline drug signals were detected in the MPEG-PLA-MTX NBs, indicating the presence of MTX in the MPEG-PLA-MTX NBs.

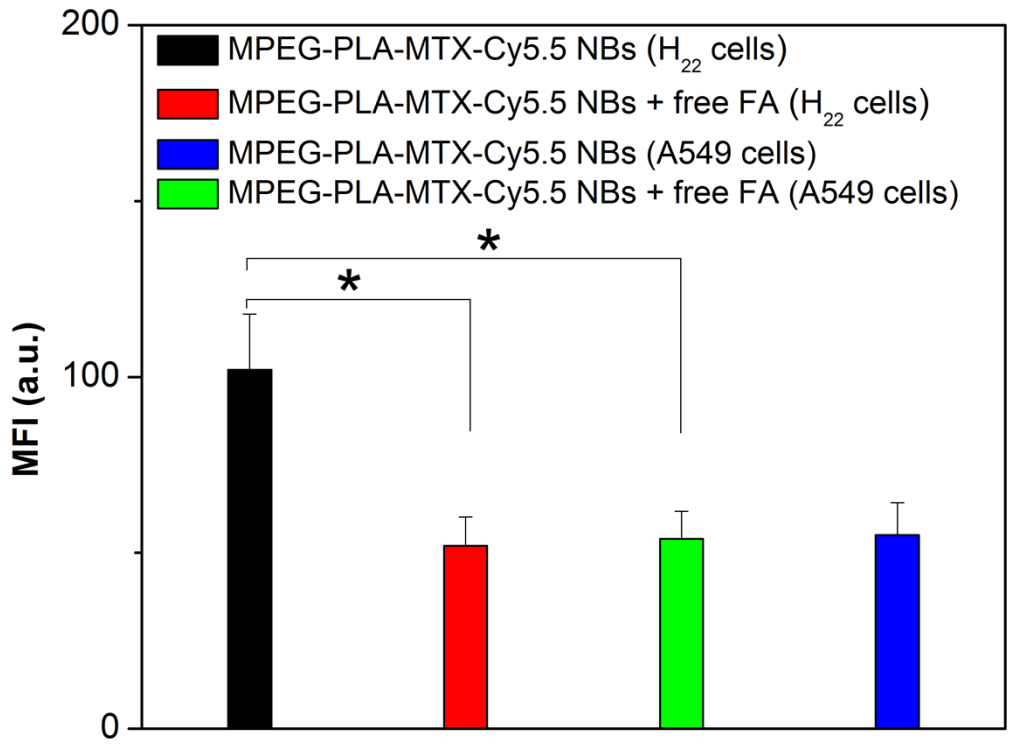

Fig. S8 Mean fluorescence intensity (MFI) of H_{22} cells (FA receptors high-expressing tumor cell line) or A549 cells (FA receptors low-expressing tumor cell line) incubated with the MPEG-PLA-MTX-Cy5.5 NBs (equivalent Cy5.5 concentration) in the absence or presence of an excess of free FA for the same incubation time (6 h) measured by flow cytometer tests. Data were presented as mean ± SD (n = 3). * $P < 0.05$ (two-tailed Student's *t-test*).

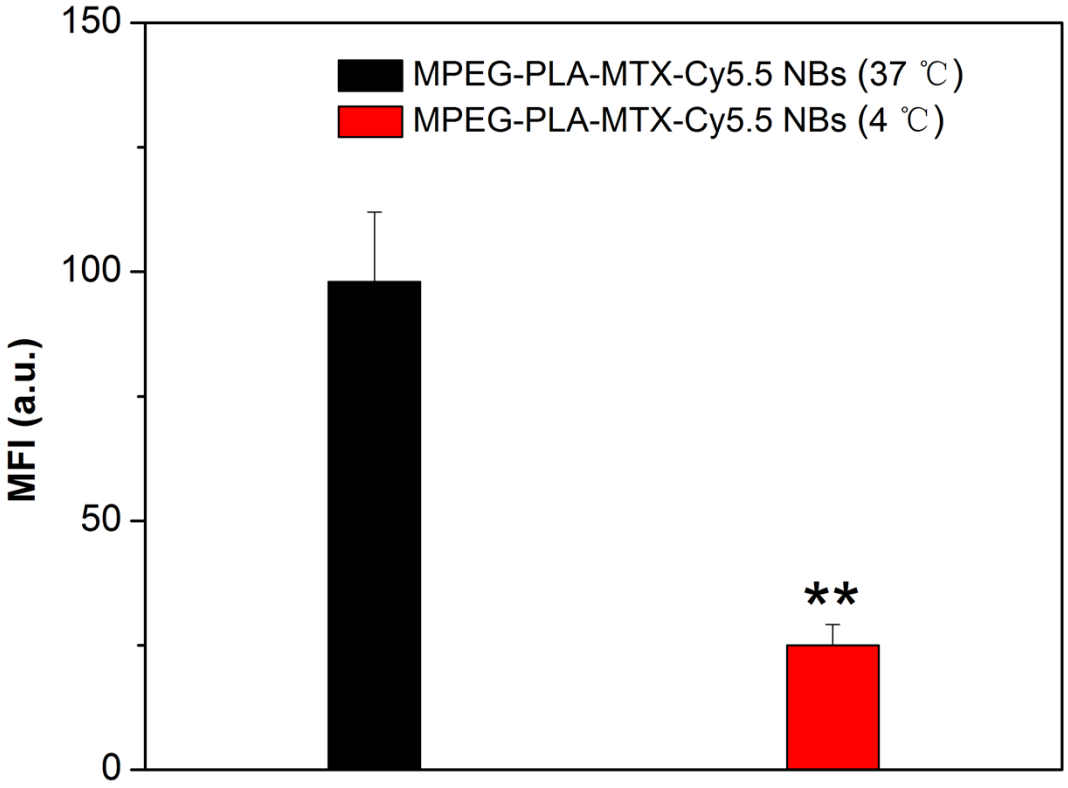

Fig. S9 Mean fluorescence intensity (MFI) of H_{22} cells incubated with the MPEG-PLA-MTX-Cy5.5 NBs (equivalent Cy5.5 concentration) at physiological temperature (37°C) or low temperature (4°C) for the same incubation time (6 h) measured by flow cytometer tests. Data were presented as mean ± SD (n = 3). * $P < 0.05$ (two-tailed Student's *t-test*).

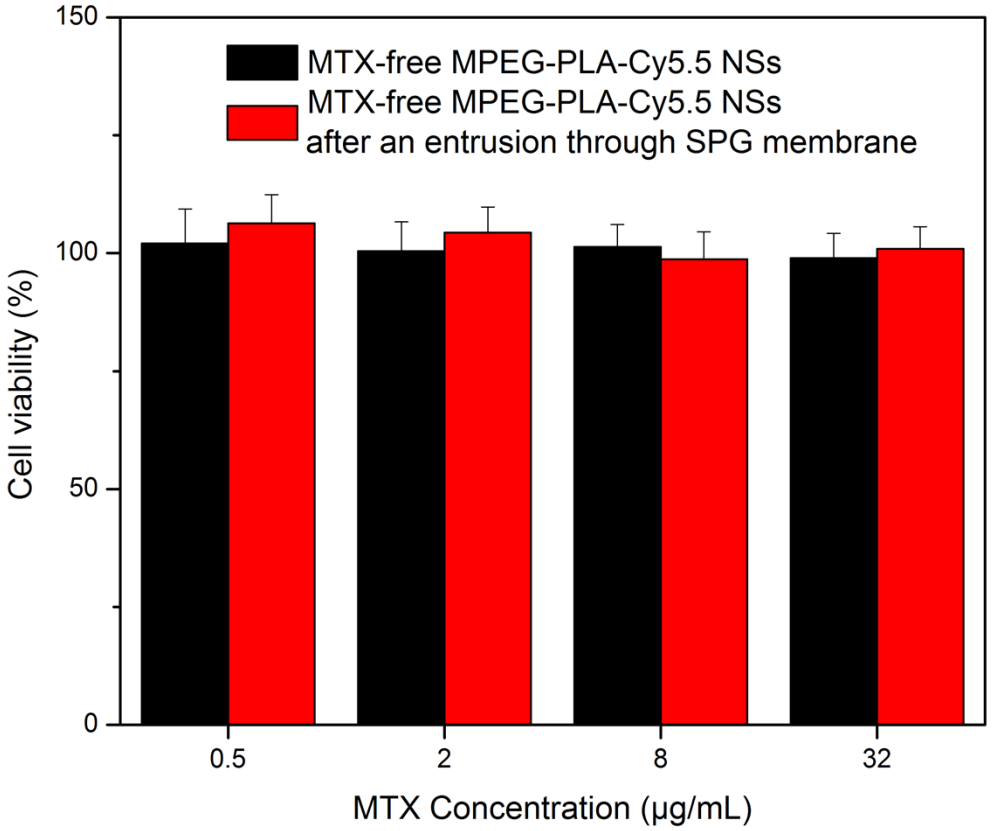

Fig. S10 *In vitro* cell viability of the MTX drug-free MPEG-PLA-Cy5.5 NSs after incubation for 48 h. Data were presented as mean ± SD (n = 6). * $P < 0.05$ (two-tailed Student's *t-test*).