Supporting Information for

Improved Therapeutic Efficacy of Quercetin-Loaded Polymeric Nanoparticles on Triple-Negative Breast Cancer by Inhibiting uPA

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Table S1. The parameters of particle size and zeta potential about Qu-NPs.

Qu-NPs	**First day**	**After one week**
Particle size (nm)	198.4 ± 7.8	216.3 ± 16.5
Zeta potential (mV)	-22.5 ± 2.5	-17.4 ± 7.1
PDI	0.120	0.184

Table S2. The sequences of the primers involved in RNAi knockdown and qPCR.

Primer	Sequence
RNAi-uPA-F	CCGGGGGCGAACGACAATAGCTTTACTCGAGTAAAG CTATTGTCGTTCGCCCTTTTTG
RNAi-uPA-R	AATTCAAAAAGGGCGAACGACAATAGCTTTACTCGA GTAAAGCTATTGTCGTTCGCCC
qPCR-uPA-F1	GGGAATGGTCACTTTTACCGAG
qPCR-uPA-R1	GGGCATGGTACGTTTGCTG
qPCR-uPA-F2	GCTTGTCCAAGAGTGCATGGT
qPCR-uPA-R2	CAGGGCTGGTTCTCGATGG

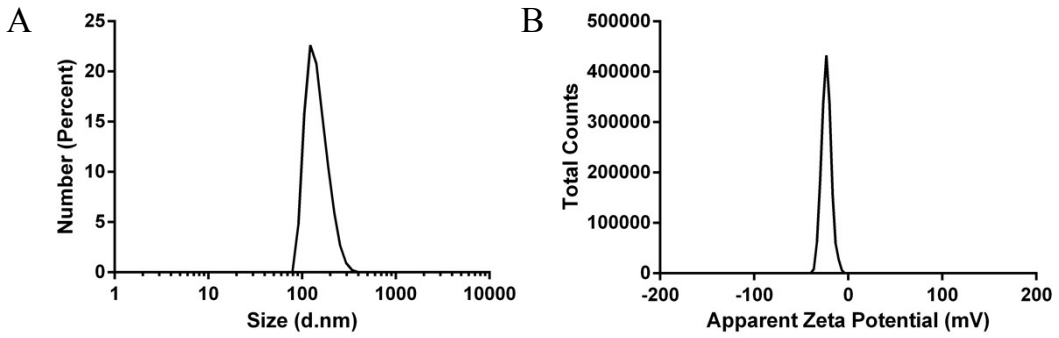

Figure S1. Particle size and zeta potential distribution of pure PLGA/TPGS nanoparticles without quercetin. (A) Particle size intensity distribution map of pure PLGA/TPGS nanoparticles without quercetin. The average particle size (Z-Average) of the obtained quercetin nanoparticles is 173.6 d.nm, the dispersion coefficient (PDI) is 0.073. (B) Zeta potential profile of pure PLGA/TPGS nanoparticles without quercetin. The measured zeta potential result is -22.5 mV.

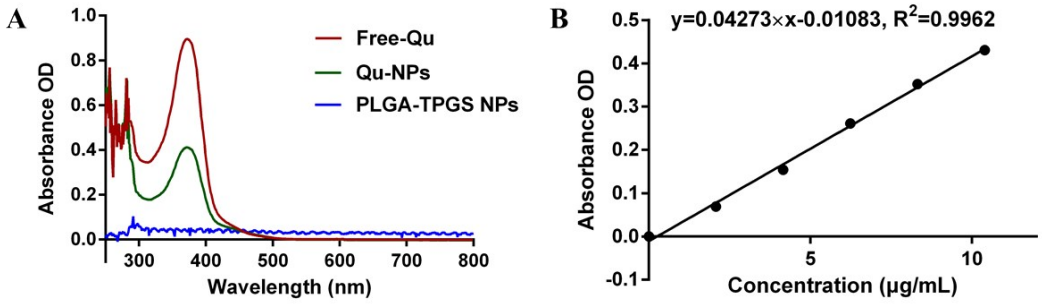

Figure S2. Determination of quercetin content in Qu-NPs by UV spectrophotometry. (A) Determine the maximum absorption wavelength of quercetin (Qu). The free-Qu has a maximum absorption peak at 370 nm, and the Qu-NPs also has a maximum absorption peak at 370 nm. But PLGA-TPGS NPs have no obvious absorption peak at 370 nm. UV spectrophotometry is suitable for the determination of quercetin content in Qu-NPs, and the optimum measurement wavelength is 370 nm. (B) Drawing of the standard curve of quercetin. Take 0, 0.1, 0.2, 0.3, 0.4 mL and 0.5 mL quercetin standard solution and 10 mL volumetric flask in turn, make up to 10 mL with methanol solution, and use methanol solution as blank zero solution, at the determined maximum absorption wavelength. The absorbance was measured at 370 nm and measured in parallel for 3 times. The linear regression equation was obtained as y = 0. 04273x-0.01083, R^2=0. 9962.

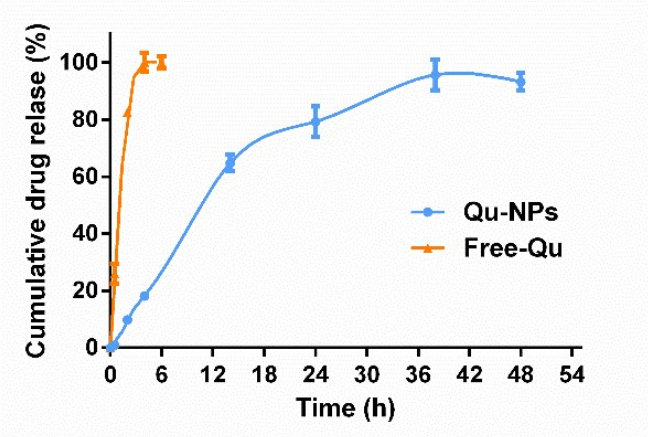

Figure S3. In vitro release of Qu-NPs and free quercetin. Diffusion 2mL of Qu-NPs (360ug/mL)

and free quercetin (360ug/mL) through the dialysis bag into 20mL of dialysate (phosphate buffer pH 7.4, 0.1% SDS (v / v), 2.5 % Tween 80 (v / v)), dialysis at 37 ± 1°C, 100 rpm with stirring, 100 μL samples are taken out at certain time intervals (Qu-NPs: 0h, 0.5h, 2h, 4h, 14h, 24h, 38h, 48h; free quercetin: 0h, 0.5h, 2h, 4h, 6h), and replaced with an equal amount of fresh dialysate buffer. After analyzing the sample at 370 nm using an ultraviolet-visible spectrophotometer. Each data point is represented as mean ± SD (n = 3).

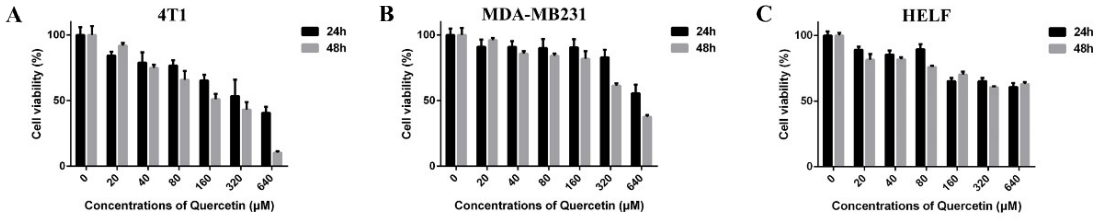

Figure S4. A comparison of the toxic effects of quercetin alone on a range of cells. Treatment of (A) 4T1, (B) MDA-MB231 and (C) HELF cells with a series of concentrations of quercetin (0, 20, 40, 80, 160, 320, 640 μM) for 24 hours and 48 hours. Cell viability was measured by MTT assay. The cytotoxic effect of quercetin on breast cancer cells is weak, and it is also slightly cytotoxic to human embryonic lung fibroblasts HELF cells.

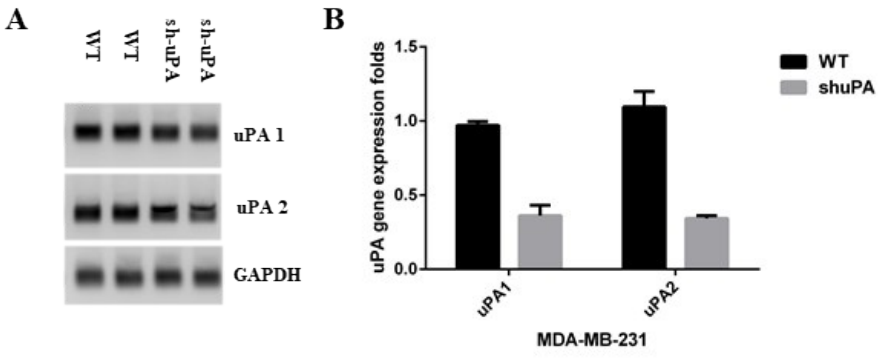

Figure S5. RNAi knockdown of uPA expression in the triple-negative breast cancer cell line MDA-MB231. (A) The quantitative real-time polymerase chain reaction (qPCR) of RNA extracted

from shRNA-transfected MDA-MB231 cells as described under “Experimental Procedures”. The GAPDH mRNA was co-amplified as a control. (B) The quantitative processing of the data obtained by qPCR showed that the expression of uPA gene in MDA-MB231 cells transfected with shuPA was much lower than that of wild-type MDA-MB231 cells. uPA 1 and uPA 2 represent the two designed qPCR primers, respectively.