Supporting Information

Red Blood Cell (RBC) Membrane Coated Poly(lactic-co-glycolic acid) Nanoparticles for Enhanced Chemo and Hypoxia Activated Therapy

Anil Parsram Bidkar,[a] *Pallab Sanpui,* [b] *and Siddhartha Sankar Ghosh* [a,c,*]

[a] Department of Biosciences & Bioengineering, Indian Institute of Technology Guwahati, Guwahati 39, Assam, India
[b]Department of Biotechnology, BITS Pilani, Dubai Campus, Dubai International Academic City, P.O. Box No. – 345055, Dubai, UAE,
[c]Centre for Nanotechnology, Indian Institute of Technology Guwahati, Guwahati-39, Assam, India

*Corresponding author phone: +0361-258-2206; Fax: +0361-258-2249

Email: SSG: sghosh@iitg.ernet.in

Table S1: Nucleotide sequences of the primers used to study gene expression.

Gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
GAPDH	GAAATCCCATCACCATCTTCCAGG	GAGCCCCAGCCTTCTCCATG
Vimentin	AGTCCACTGAGTACCGGAGAC	CATTTCACGCATCTGGCGTTC
Fibronectin	GGTGACACTTATGAGCGTCCTAAA	AACATGTAACCACCAGTCTCATGTG
IL1β	CAGAAGTACCTGAGCTCGCC	AGATTCGTAGCTGGATGCCG
IL6	GGCACTGGCAGAAAACAACC	GCAAGTCTCCTCATTGAATCC
IL8	CAAGAGCCAGGAAGAAACCA	GTCCACTCTCAATCACTCTCAG

Table S2. Loading and encapsulation efficeincy of the curcumin and tirapazamine seperately or in combination with TPZ in PLGA NPs.

	(Drug:PLGA) ratio	*Encapsulation Efficiency (%)*	*Loading Efficiency (%)*
Cur	0.25	80±2.1	20±0.5
	0.5	68±1.8	34±0.9
TPZ	0.25	72±2.3	18±1.1
	0.5	58±3.2	29±1.2
TPZ in Cur+TPZ@PL	0.125	64±2.8	8±0.6
	0.25	25.2±1.6	6.3±0.3
Cur in Cur+TPZ@PL	0.125	56.8±3.2	11±1.2
	0.25	61.2±3.6	15.3±0.9

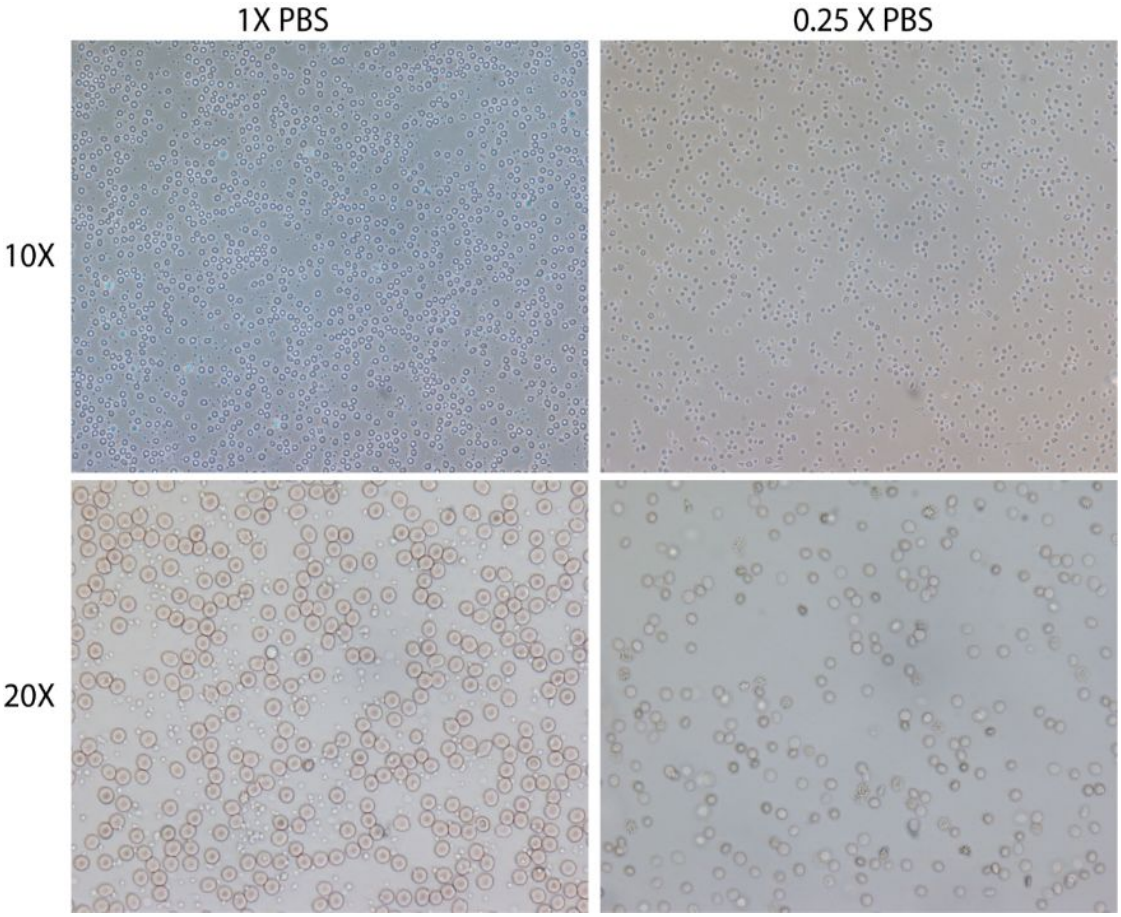

Figure S1. Microscopic images of RBCs in 1X PBS and 0.25X PBS, RBCs in 0.25X showed loss in the membrane integrity due to hypotonic treatment.

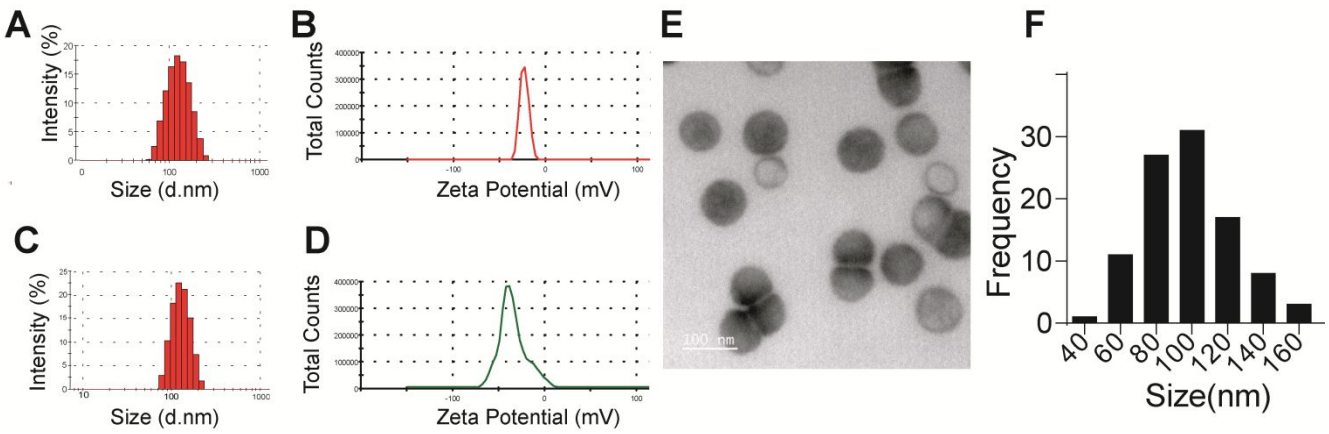

Figure S2. Characterization of PLGA NPs and RBC-NPs. (**A-D**) Hydrodynamic diameter of the PLGA NPs (**A**) and RBC-NPs (**C**) along with zeta potential of PLGA NPs (**B**) and RBC-NPs (**D**). (**E**) TEM micrograph for PLGA NPs. (**F**) Size distribution of the RBC-NPs calculated from TEM images.

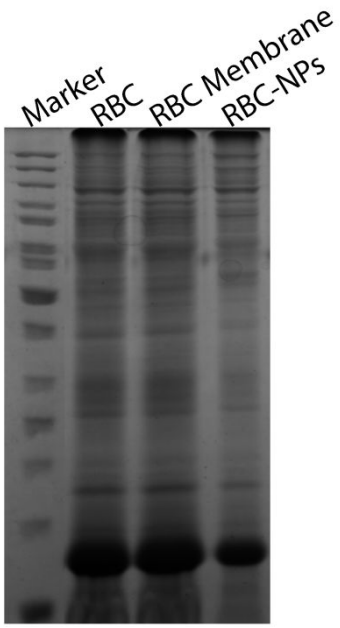

Figure S3. SDS-PAGE of the RBC membrane proteins. RBC, isolated RBC membrane and RBC-NPs showed similar protein content confirming the coating of PLGA NPs without loss of the membrane proteins.

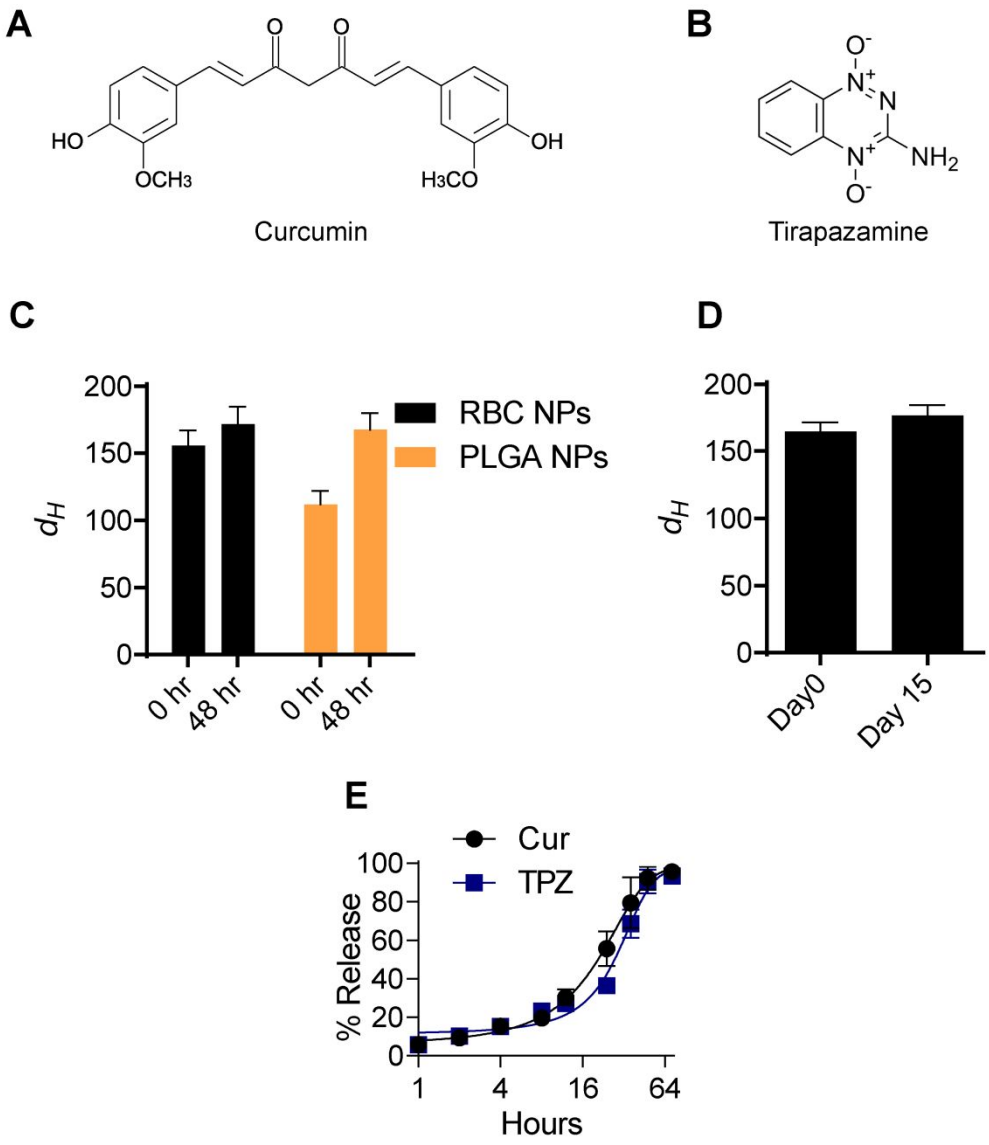

Figure S4 (**A, B**) Chemical structure of the curcumin and tirapazamine. (**C**)Size of the PLGA NPs and RBC NPs when stored at 37 °C in FBS for 48 hr. (**D**) Hydrodynamic diameter measured at day 0 and day 15 after storage at -80 °C. (**E**) Release profile of Cur and TPZ from Cur+TPZ@RB NPs.

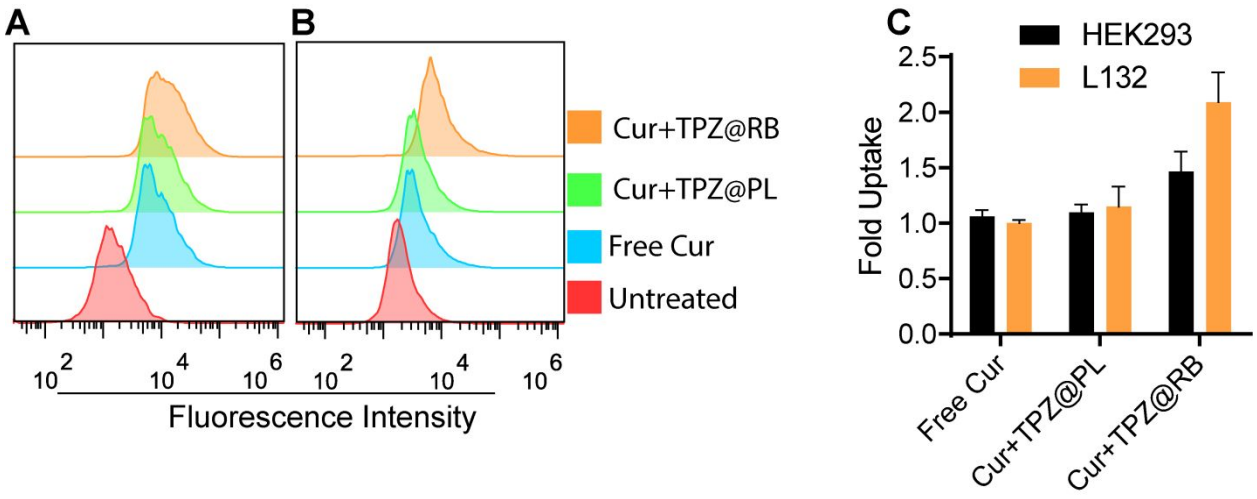

Figure S5. Uptake of the Cur, Cur+TPZ@PL and Cur+TPZ@RB in HEK293 (**A**) and L132 (**B**) cells. (**C**) Comparison of the uptake of Cur, Cur+TPZ@PL and Cur+TPZ@RB.

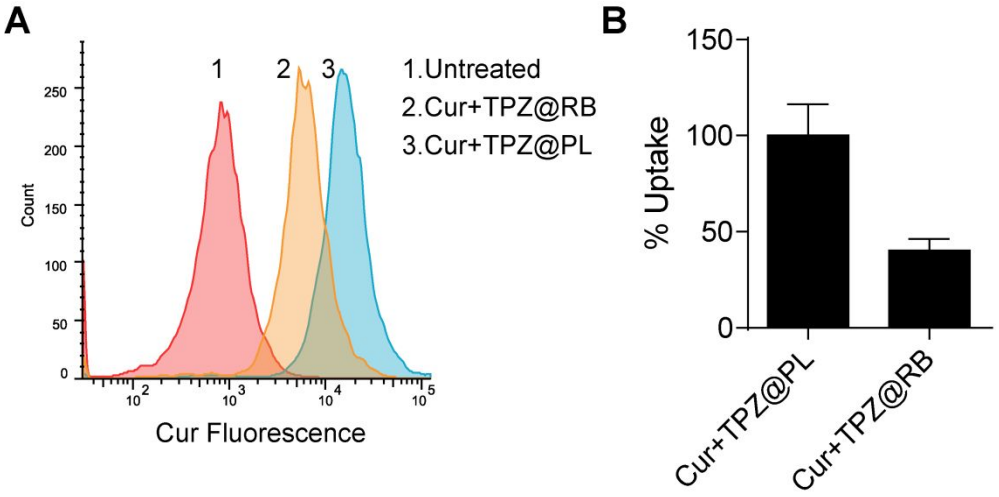

Figure S6. Uptake study showing histograms (**A**) of the Cur+TPZ@PL and Cur+TPZ@RB uptake in macrophage cells. (**B**) % Uptake of the Cur+TPZ@RB NPs as compared to Cur+TPZ@PL.

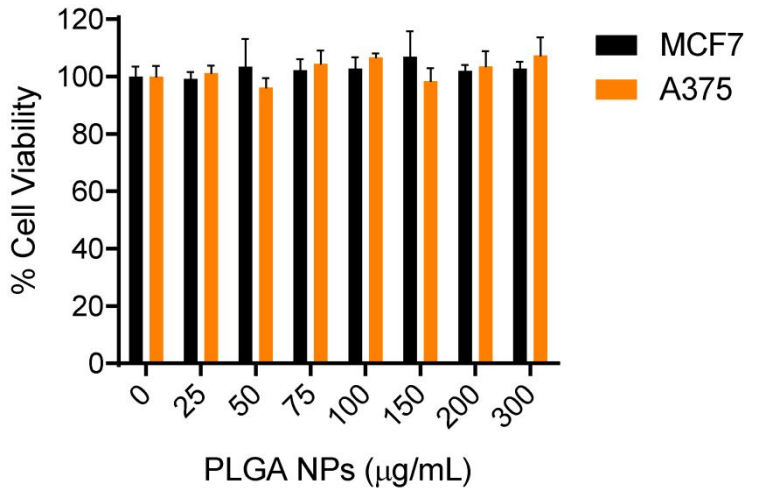

Figure S7. Toxicity of the PLGA nanoparticles was studied in MCF7 and A375 cells for 48 h treatment.

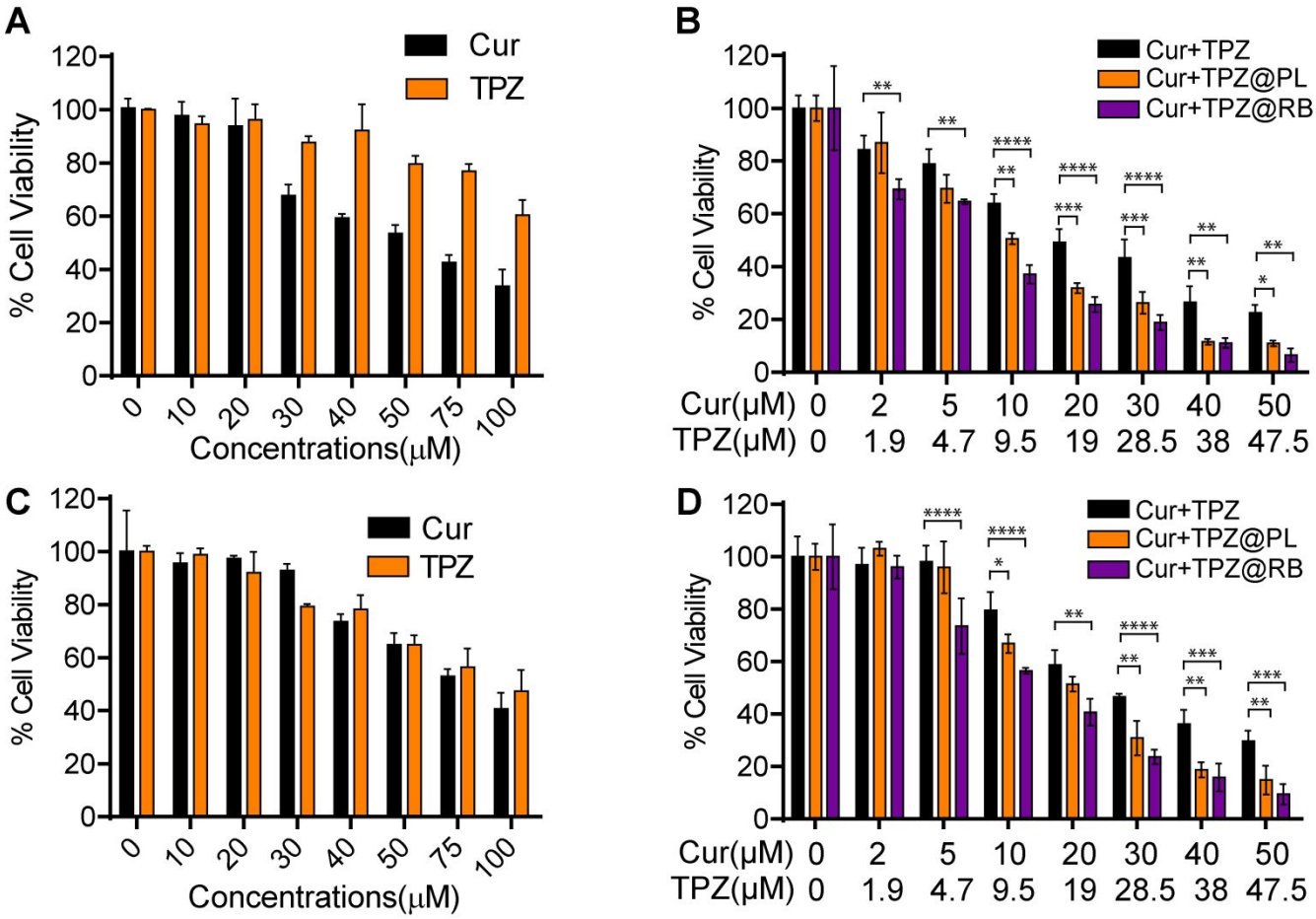

Figure S8. Antiproliferative assays of the Cur, TPZ and their formulations. (**A)** MTT assay results of Cur and TPZ in MCF7 cells. (**B**) MTT assay results on MCF7 cells for Cur+TPZ, Cur+TPZ@PL and Cur+TPZ@RB. (**C**) MTT assay results of Cur and TPZ treatment in A375 cells. (**D**) MTT assay results on A375 cells for Cur+TPZ, Cur+TPZ in PLGA NPs and RBC NPs. (Data are shown as mean ± SD, n = 3; $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.00001$ are denoted as *, **, ***, and **** respectively).

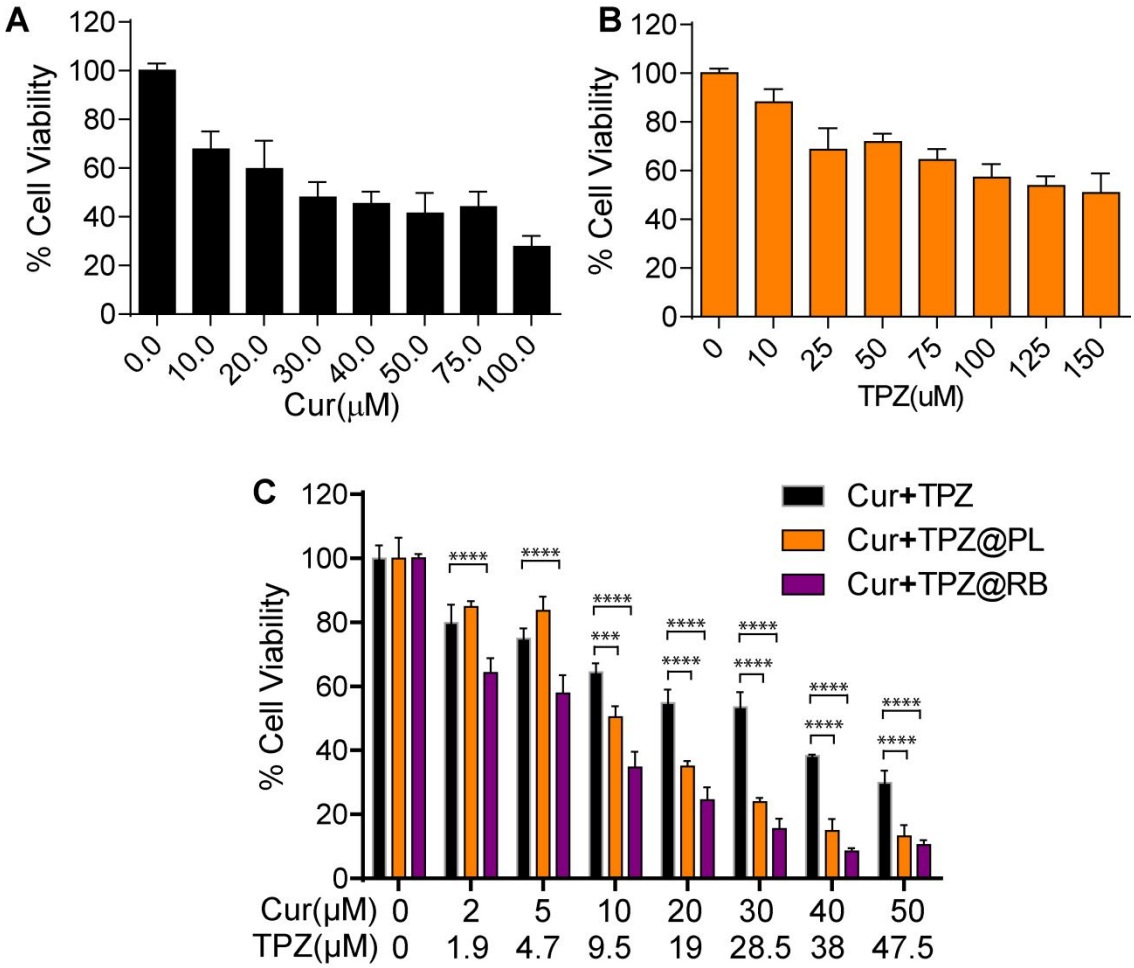

Figure S9 (**A, B**) MTT assay results of Cur and TPZ in HEK293 cells. **C**: MTT assay results on HEK293 cells for Cur+TPZ, Cur+TPZ@PL and Cur+TPZ@RB. (Data are shown as mean ± SD, n = 3; $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.00001$ are denoted as *, **, ***, and **** respectively).

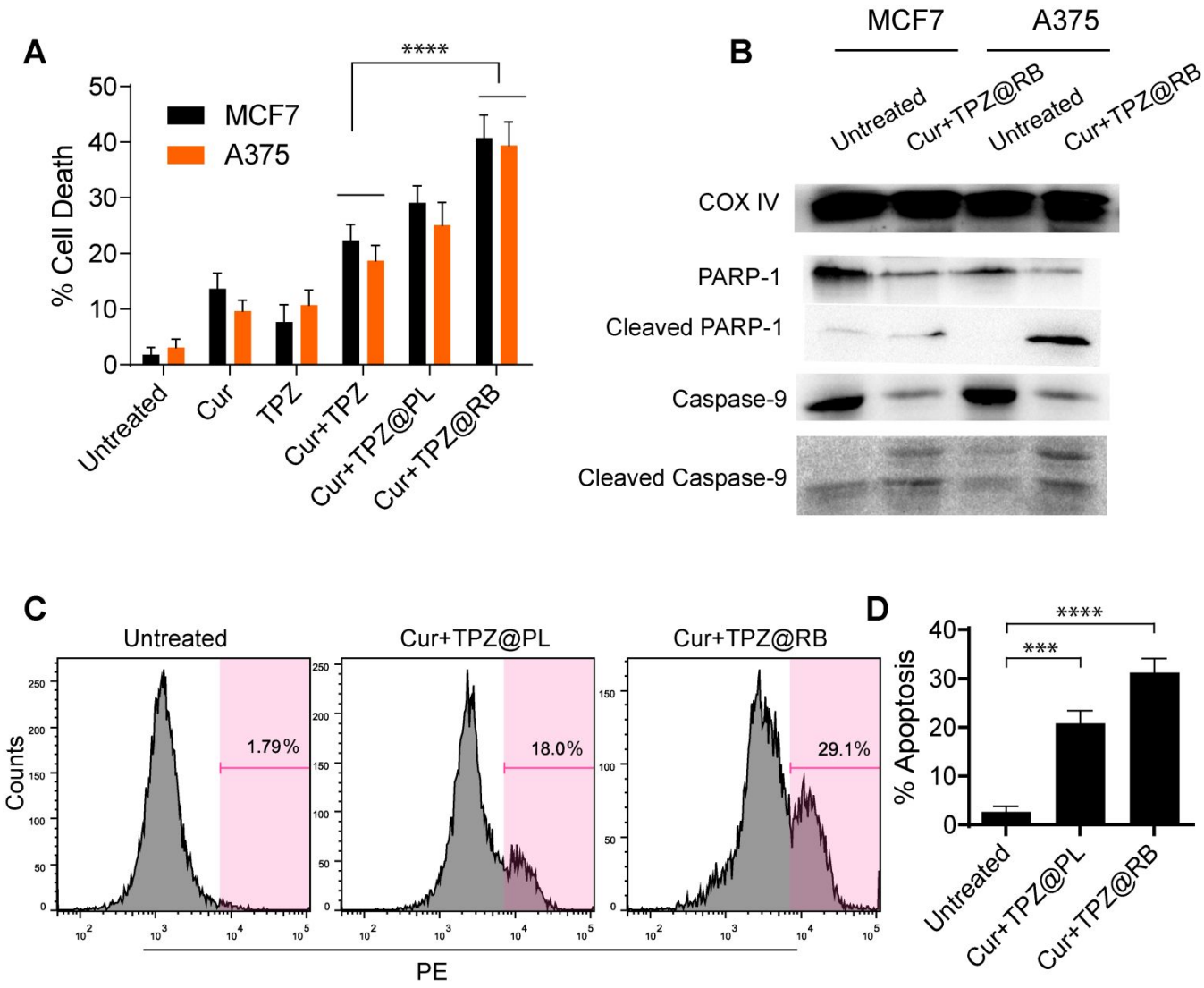

Figure S10. Apoptosis and cell death measurements. (**A**) Measurements of % cell death in treated MCF7 and A375 cells by flow cytometry. (**B**) Western blots showing cleaved PARP-1 and cleaved caspase-9 after treatment in MCF7 and A375 cells. (**C**, **D**) Caspase-3 assay results showing number of apoptotic cells positive for active caspase-3. (Data are shown as mean ± SD, n = 3; $p < 0.001$ and $p < 0.00001$ are denoted as *** and ****, respectively).

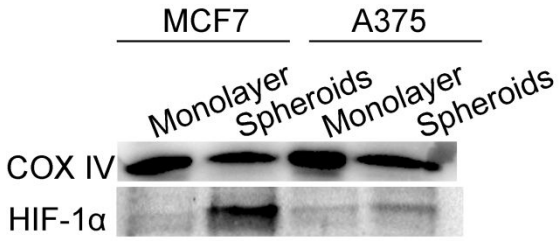

Figure S11. Western blot for detection of the HIF-1α protein in MCF7 and A375 spheroids.

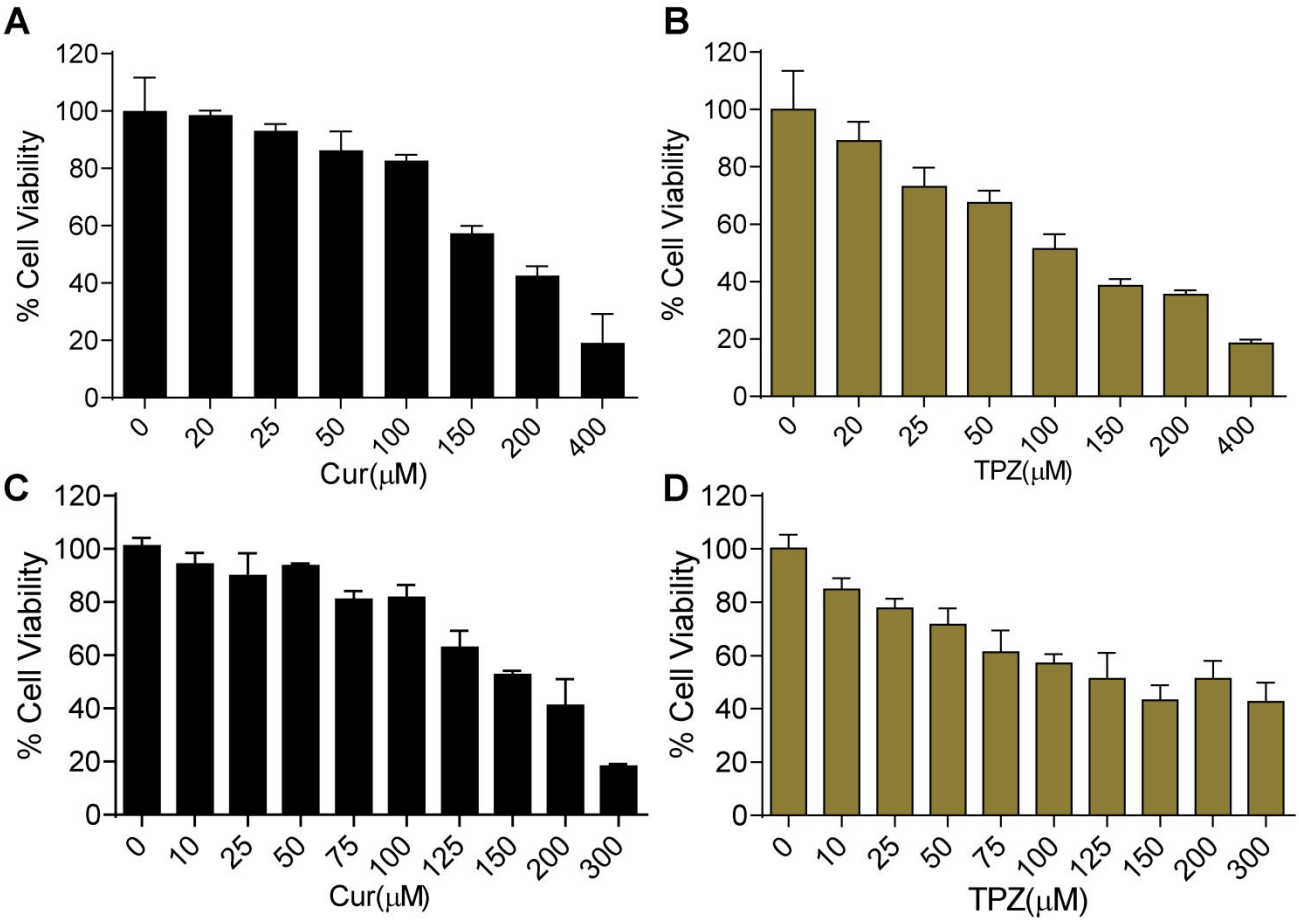

Figure S12. Viability assay results after treatment with increasing concentrations of Cur and TPZ on MCF7 (**A**, **B**) and A375 (**C**, **D**) Spheroids.

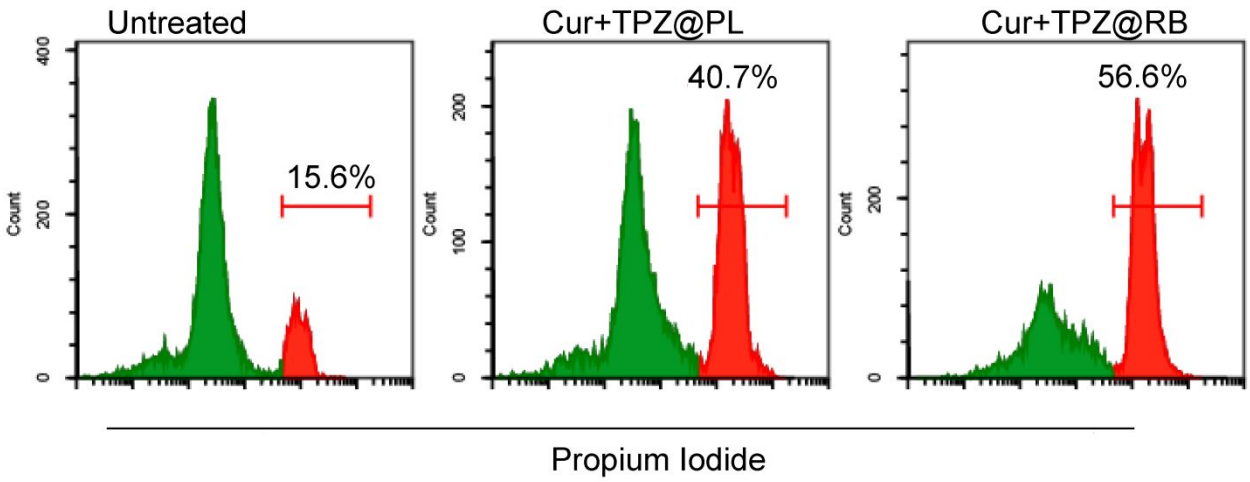

Figure S13. Flow cytometric measurement of the PI positive cells in MCF7 spheroids after Cur+TPZ@PL and Cur+TPZ@RB treatment.