Supplementary Information

Selective targeting of the hedgehog signaling pathway by PBM nanoparticles in docetaxel-resistant prostate cancer

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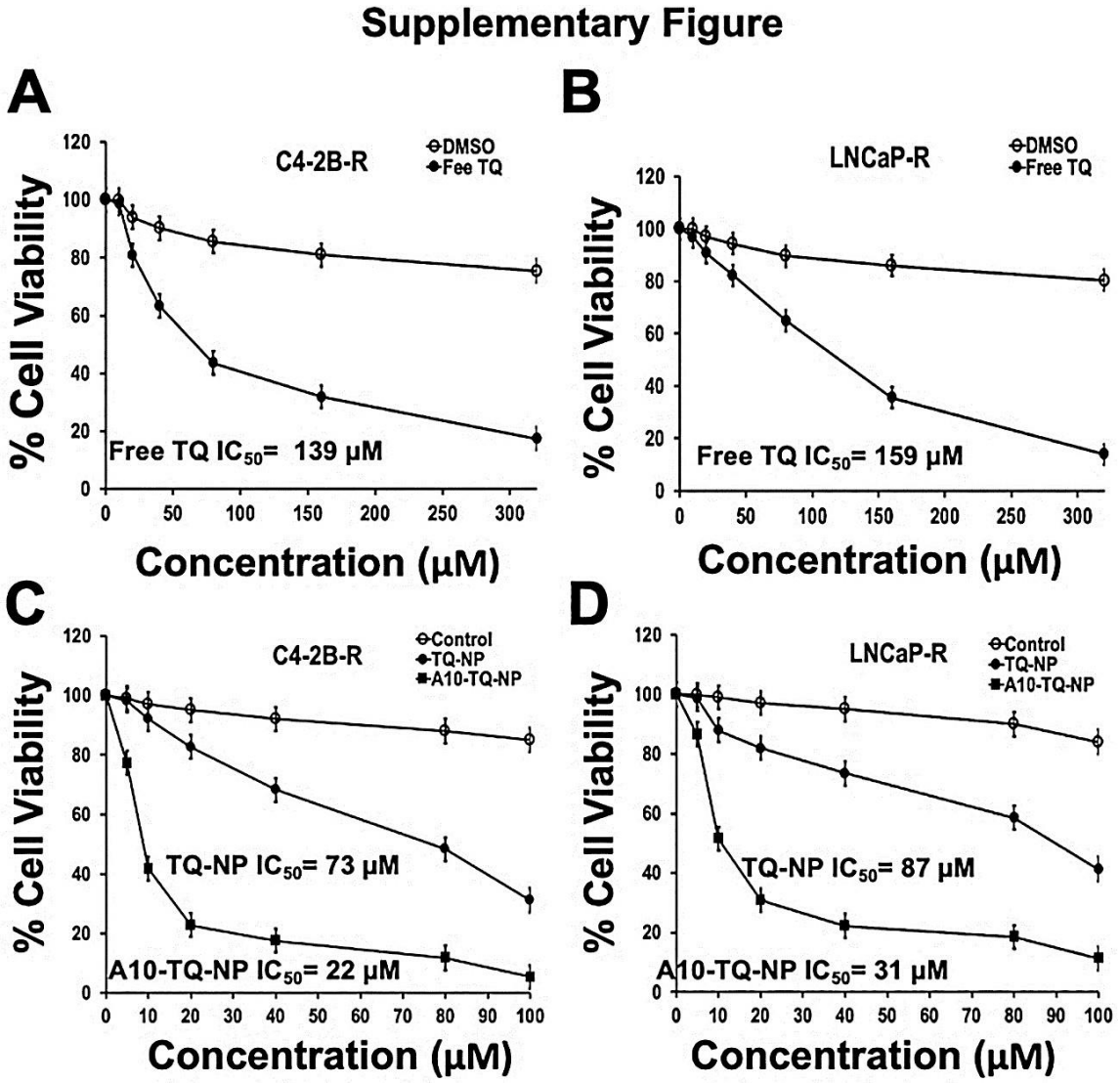


Figure S1: Thymoquinone induces an anti-proliferative effect on docetaxel resistance to prostate cancer cells. Docetaxel resistance cells, C4-2B-R and LNCaP-R were grown on 96-well plates for overnight and then treated with sequential dosages of free TQ (10, 20, 40, 80, 160, 320 µM), and PBM-NPs (TQ-NP, or A10-TQ-NPs; 5, 10, 20, 40, 80, 100 µM) for 48 h at 37°C in a 5% CO_2 incubator. Next, the cell viability was measured by MTT assay. The optimal IC_{50} values of free TQ, and PBM- NPs (TQ-NP, or A10-TQ-NP) are shown in graphs A, B, C, and D, respectively. Data are represented as a mean ± standard error of the mean of at least three independent experiments.

Figure S2. Densitometry readings/intensity ratios in C4-2B-R and LNCaP-R immunoblots

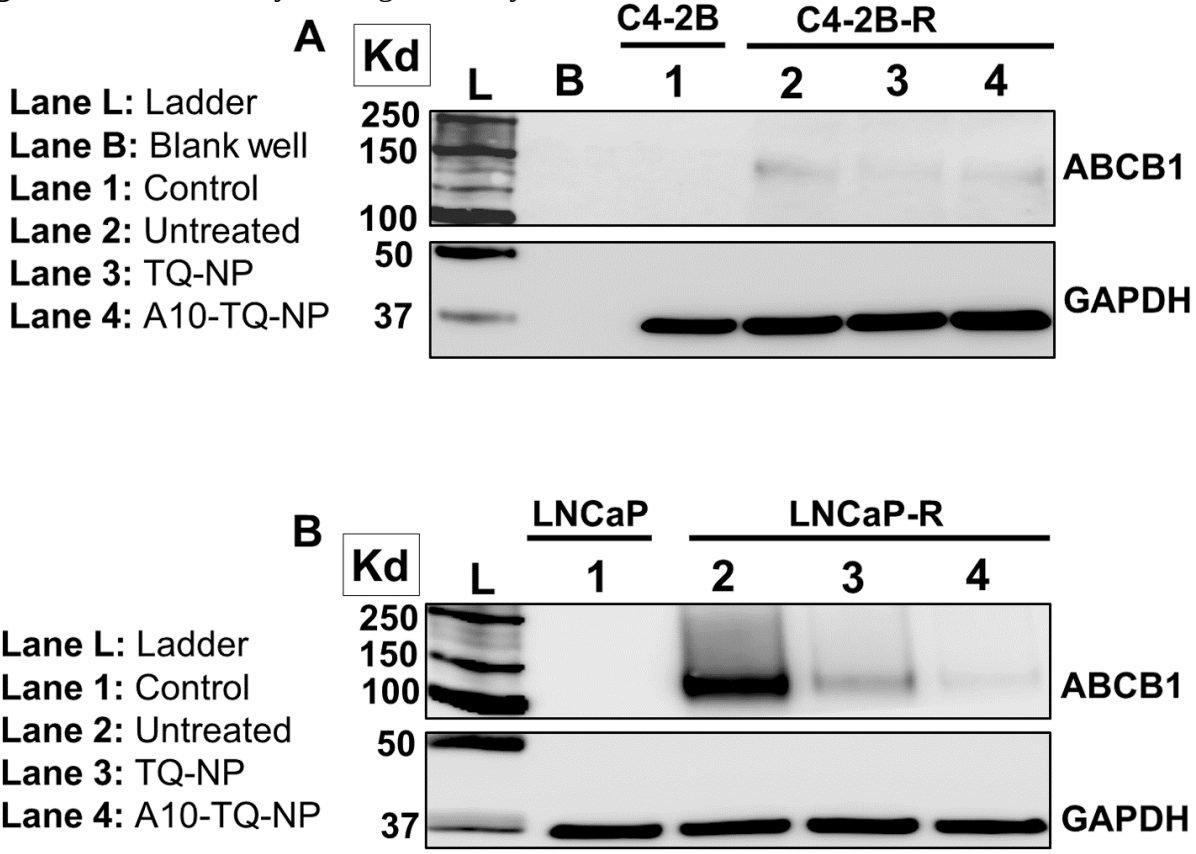


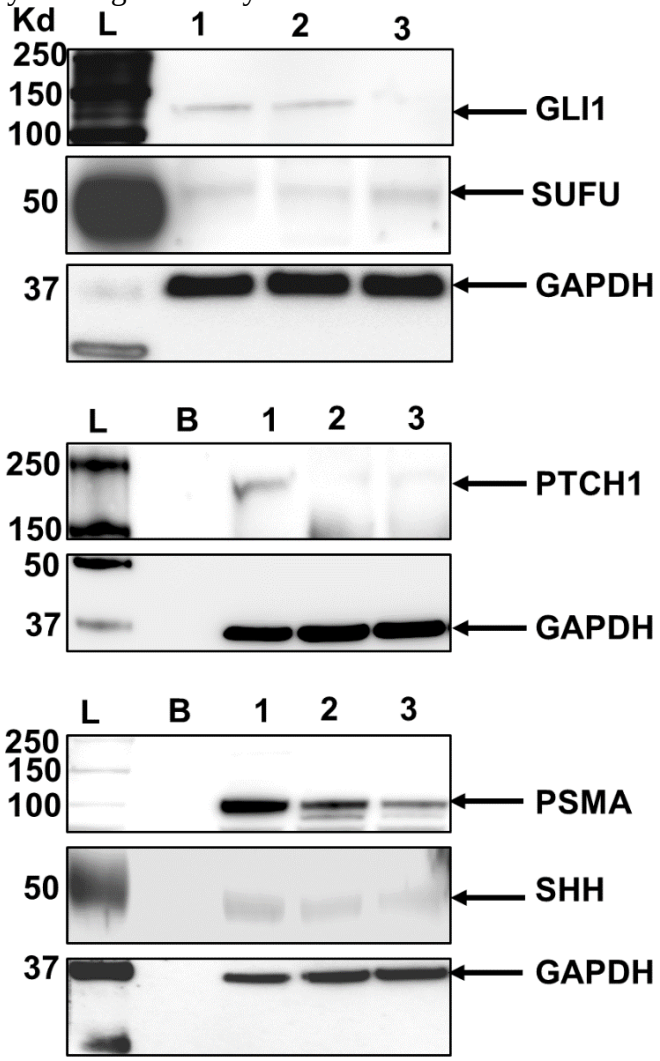
Table. Densitometry readings/intensity ratios

	ABCB1/GAPDH
C4-2B-Control	0
C4-2B-R (UT)	3.317917
TQ-NP	0.59683
A10-TQ-NP	0.560444

	ABCB1/GAPDH
LNCaP-Control	0
LNCaP-R (UT)	3.913951
TQ-NP	0.695781
A10-TQ-NP	0.137985

Figure S2. Densitometry readings/intensity ratio of ABCB1 marker in C4-2B-R and LNCaP-R immunoblots. PCa cells (parental control and DTX- resistant cell) were treated with or without A10-conjugated TQ-NPs for 48 h, and ABCB1 (~140KD) protein expression was assessed by Western blots. Tables shows the density intensity ratio of C4-2B-R and LNCaP-R cell lines. GAPDH (37KD) was used as an internal standard for equalizing the samples. The ratios for immunoblot densitometry readings/intensity shown are representative of three independent experiments.

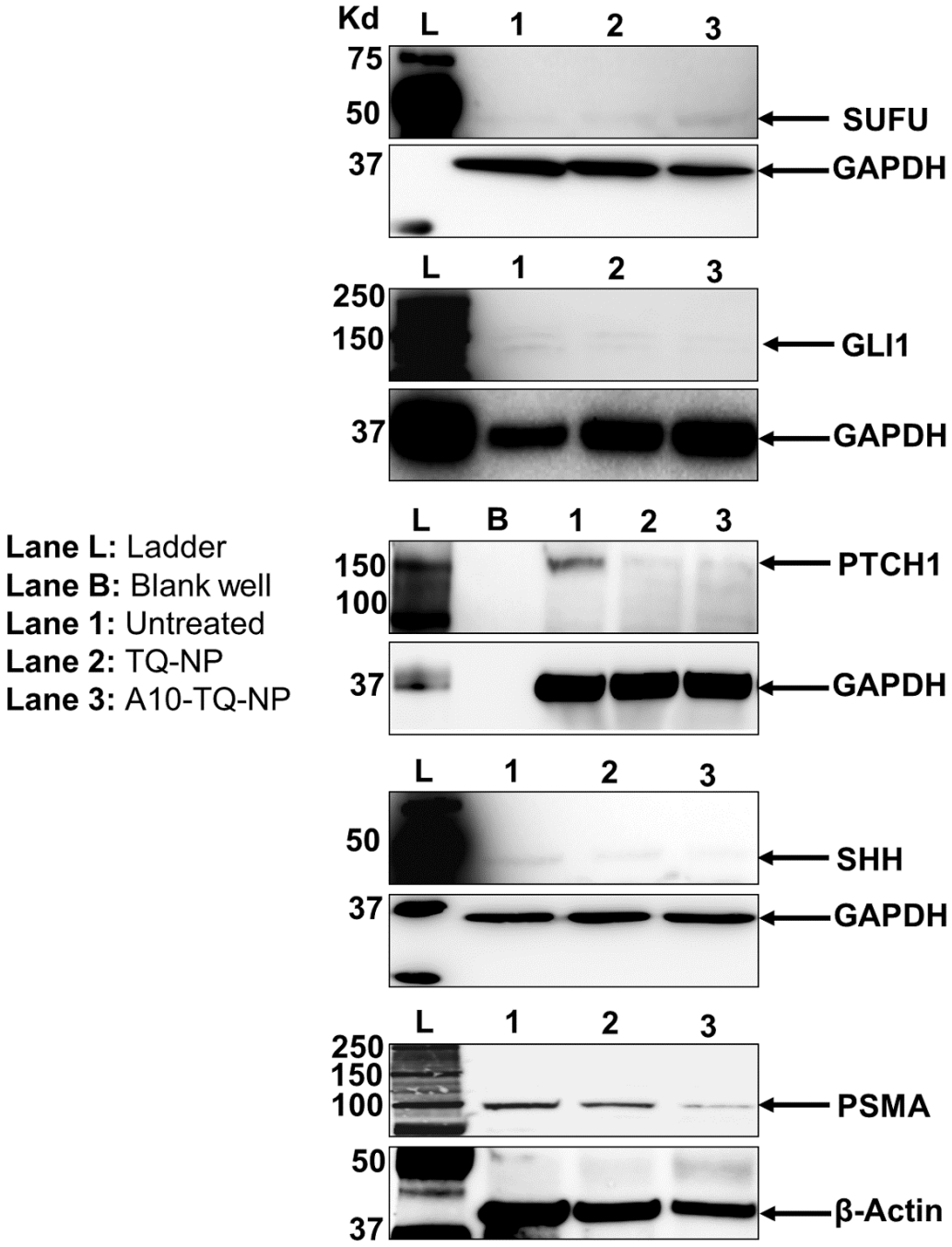
Figure S3. Densitometry readings/intensity ratios in C4-2B-R cell line



Lane L: Ladder
Lane B: Blank well
Lane 1: Untreated
Lane 2: TQ-NP
Lane 3: A10-TQ-NP

	C4-2B-R				
	PSMA/GAPDH	**SHH/GAPDH**	**PTCH1/GAPDH**	**GLI1/GAPDH**	**SUFU/GAPDH**
UT	2.116074	1.550368	2.396878	1.586341	0.941512
TQ-NP	0.723468	0.809105	0.349075	1.368609	0.747718
A10-TQ-NP	0.137971	0.636629	0.398266	0.06786	1.439391

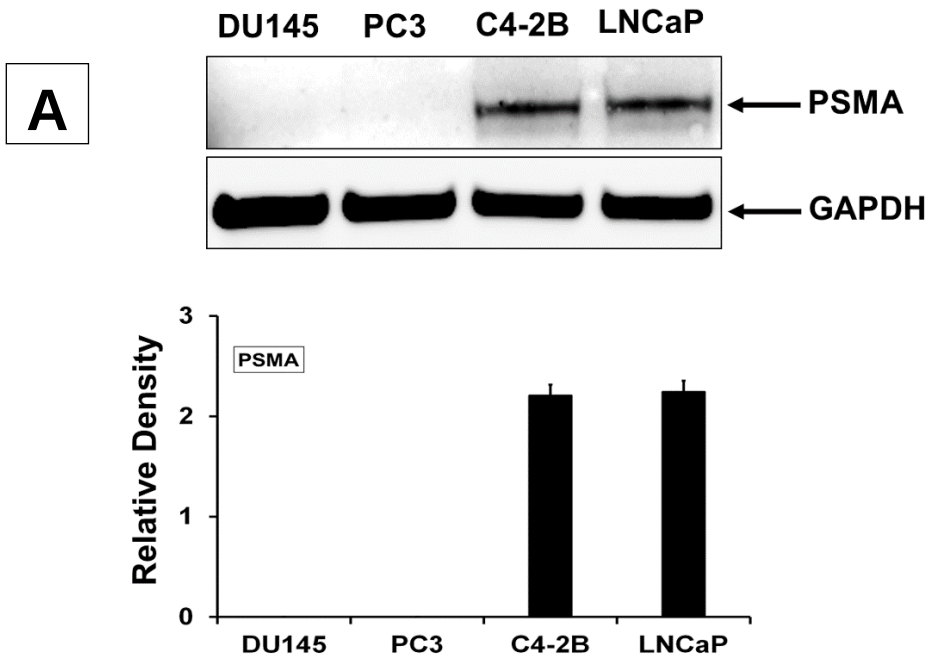
Figure S3. Densitometry readings/intensity ratios in LNCaP-R cell line



LNCaP-R					
	PSMA/β-Actin	**SHH/GAPDH**	**PTCH1/GAPDH**	**GLI1/GAPDH**	**SUFU/GAPDH**
UT	1.198227	0.921141	1.234737	0.993969	0.126035
TQ-NP	1.071506	0.54844	0.290596	0.728331	0.44728
A10-TQ-NP	0.357167	0.055435	0.258125	0.344001	2.498563

Figure S3. Densitometry readings/intensity ratio of hedgehog pathways in C4-2B-R and LNCaP-R immunoblots. DTX-resistant (C4-2B-R and LNCaP-R) PCa cells were treated with or without A10-conjugated TQ-NP for 48 h, and the expression of PSMA(110KD), SHH (45Kd precursor), PTCH1 (180KD), GLI1 (160KD), and SUFU (54KD) proteins were analyzed by Western blots. Table shows the corresponding densitometry reading intensity ratio in C4-2B-R and LNCaP-R cells. GAPDH (37KD) and β-actin (42KD) was used as a loading control. UT: untreated (DTX resistant). The ratios for immunoblot densitometry readings/intensity shown are representative of three independent experiments.

Figure S4. Densitometry readings/intensity ratios of PSMA in PCa cell lines



	PSMA/GAPDH
DU145	0
PC3	0
C4-2B	2.204945
LNCaP	2.24207

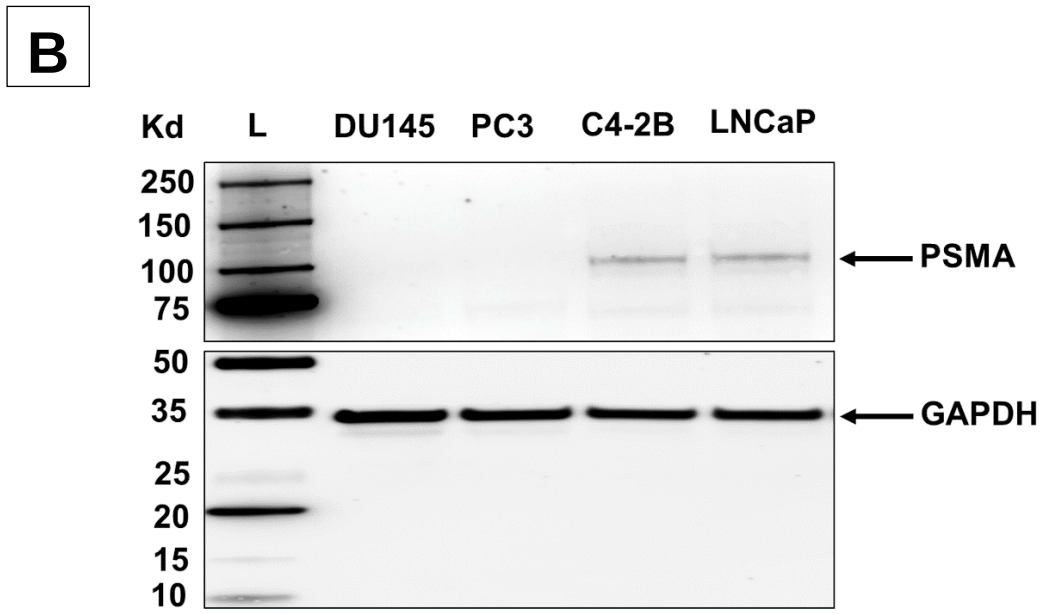


Figure S4. **Densitometry readings/intensity ratio and immunoblots of PSMA expressing PCa cell lines.** (A) Immunoblots shows the expression of PSMA (110KD) protein in untreated PCa cells (DU145, PC3, C4-2B, and LNCaP). Table shows the corresponding densitometry reading intensity ratio. GAPDH (37KD) was used as a loading control. The ratios for immunoblot densitometry readings/intensity shown are representative of three independentexperiments. PCa cell lines, PC-3 and DU145, were purchased from American Tissue Culture Collection (ATCC, Manassas, VA, USA) and maintained in RPMI-1640 media supplemented with 10% FBS, nonessential amino acids, HEPES, 2 mM L-glutamine, and penicillin/streptomycin antibiotic solution (Fisher Scientific, USA). (B) Shows the original blot of PSMA and GAPDH proteins expression.