Supporting Information

ZIF-C for Targeted RNA Interference and CRISPR/Cas9 Based Gene Editing in Prostate Cancer

Arpita Poddar, [a,b] *Suneela Pyreddy,* [a] *Francesco Carraro,* [c] *Sudip Dhakal,* [a] *Andrea Rassell,* [a] *Matthew R. Field,* [d] *T. Srinivasa Reddy,* [a] *Paolo Falcaro,* [c] *Cara M. Doherty,* [b] *Ravi Shukla* [a]*

[a] *Ian Potter NanoBiosensing Facility, NanoBiotechnology Research Laboratory (NBRL), School of Science, RMIT University, Melbourne, Victoria 3001, Australia*

[b] *CSIRO Manufacturing, Clayton, Victoria 3168, Australia*

[c] *Institute of Physical and Theoretical Chemistry, Graz University of Technology, 8010 Graz, Austria*

[d] *RMIT Microscopy & Microanalysis Facility, RMIT University, Melbourne, Victoria 3001, Australia*

Materials

Zinc acetate dihydrate, 2-Methylimidazole (2mIM) and ethylenediaminetetraacetic acid disodium salt dihydrate were obtained from Sigma-Aldrich. Lipofectamine™ 3000 Transfection Reagent, GeneArt® CRISPR Nuclease (OFP Reporter) Vector Kit with Competent Cells, PureLink™ HiPure Plasmid DNA Purification Kits and GeneArt® Genomic Cleavage Detection Kits were purchased from Life technologies. siGENOME Human RPSA (3921) siRNA – SMARTpool was purchased from Dharmacon. RPMI media, Opti–MEM, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) and Alexa Fluor®594 tagged goat anti mouse secondary antibody were from Life technologies. Mouse monoclonal [MLuC5] to 67kDa Laminin Receptor was purchased from Abcam. All other reagents were obtained from Sigma-Aldrich and used without further modification.

Methods

RNAi for targeting human RPSA gene (NCBI Gene ID: 3921)

SMARTpool siRNA, M-013303-01-0005 siGENOME, that contained a mixture of 4 sequences targeting RPSA RNA was purchased from Dharmacon. The sequences are as follows:

1. D-013303-01: GGUCAUGCCUGAUCUGUAC
2. D-013303-02: GCACCAAUCUUGACUUCCA
3. D-013303-03: GCGCAUGCGUGGCACCAUU
4. D-013303-04: CAACAAGGGAGCUCACUCA

The SMARTpool siRNA was resuspended following manufactures' protocol (Dharmacon™ siRNA Resuspension) into 20μM stock solution which was subsequently termed as siRPSA.

CRISPR/Cas9 plasmid for targeting human RPSA gene (NCBI Gene ID: 3921)

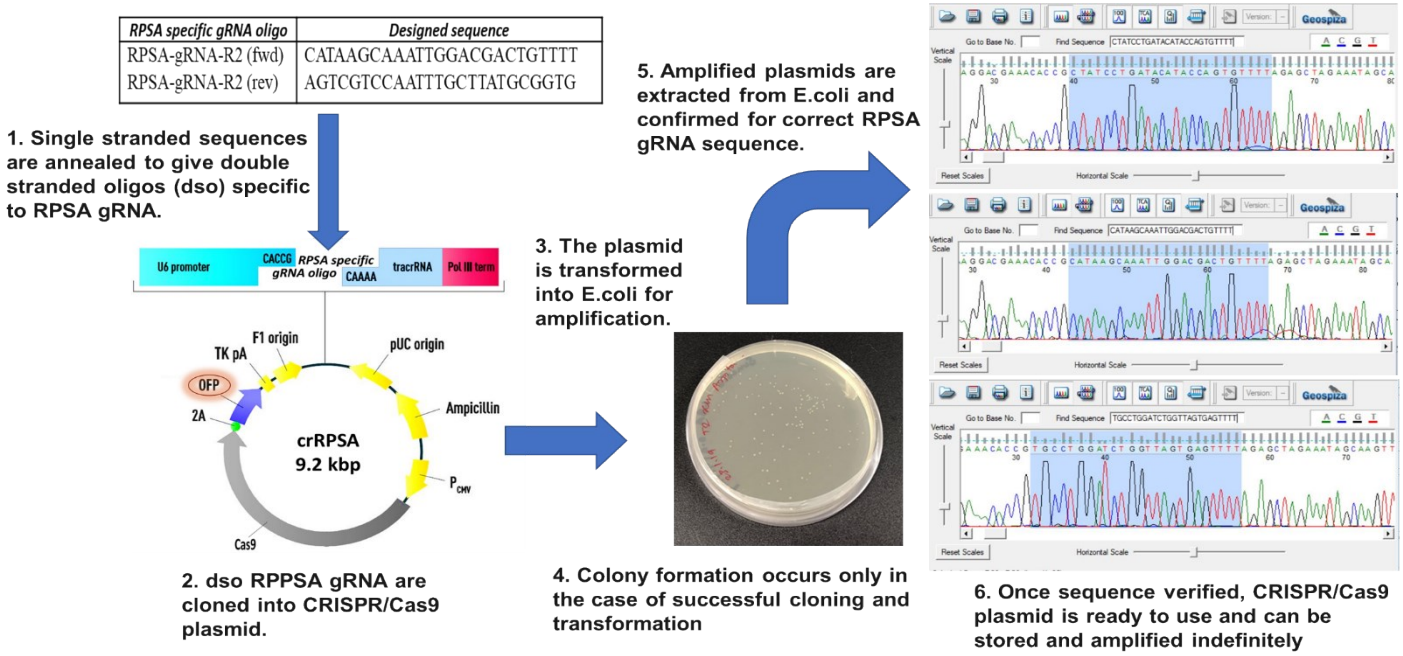
The gRNA sequences to target RPSA gene were designed using the CRISPR guide-RNA (gRNA) in silico tool 'GeneArt CRISPR Search and Design Tool' from Invitrogen TrueDesign Genome Editor software. The single stranded oligo DNA sequences for yielding the gRNAs are as follows:

1. RPSA-gRNA-R1 (fwd): CTATCCTGATACATACCAGTGTTTT
   RPSA-gRNA-R1 (rev): ACTGGTATGTATCAGGATAGCGGTG
2. RPSA-gRNA-R2 (fwd): CATAAGCAAATTGGACGACTGTTTT
   RPSA-gRNA-R2 (rev): AGTCGTCCAATTTGCTTATGCGGTG
3. RPSA-gRNA-R3 (fwd): TGCCTGGATCTGGTTAGTGAGTTTT
   RPSA-gRNA-R3 (rev): TCACTAACCAGATCCAGGCACGGTG

Double stranded oligos were generated by annealing the single stranded sequences following manufacturer's protocol (GeneArt® CRISPR Nuclease Vector Kit) and ligated to the CRISPR nuclease vector plasmid (Fig. S1). The successfully ligated plasmid contained the following features to form a complete RPSA targeting CRISPR/Cas9 gene sequence in a single format:

1. Cas9 nuclease coding sequence.
2. Annealed oligonucleotides coding for the target gRNA sequences.
3. tracrRNA: Auxiliary trans-activating crRNA allows loading of Cas9 nuclease onto the gRNA
4. F1 origin of replication: Origin of replication.
5. TK pA: Polyadenylation signal.
6. OFP: Reporter gene for orange fluorescent protein.

7. 2A peptide linker: A self-cleaving peptide linker connecting CD4 or OFP reporter genes to the C-terminal end of Cas9 nuclease. Following translation, the two proteins flanking the 2A peptide are separated from each other.
8. CMV promoter: Allows expression of Cas9 nuclease and OFP reporter genes.
9. Human U6 promoter: Allows RNA Polymerase III-dependent expression of the guide RNA (gRNA) (Kunkel et al., 1986; Kunkel and Pederson, 1988).
10. U6 forward priming site: Allows sequencing of the insert.
11. 3′ overhangs: Allows ligase-mediated directional cloning of the double-stranded oligonucleotide of interest.
12. Pol III terminator: Allows efficient termination of RNA Polymerase III-dependent transcription.
13. Ampicillin resistance gene: Allows selection of the plasmid in E. coli.



14. pUC origin of replication (ori): Permits high-copy replication and maintenance in E. coli.

Once the double stranded circular plasmid was constructed, the plasmid was amplified by transforming into One Shot® TOP10 chemically competent E. coli cells. The transformed cells were plated on LB

plates containing 100 µg/mL ampicillin overnight at 37°C incubator. Growth of colonies indicated successful plasmid construction and ligation (Fig. S1). 3-5 ampicillin resistant colonies were selected and inoculated in LB broth containing 100 µg/mL ampicillin and grown at 37°C with 200rpm overnight. The amplified plasmids were isolated using PureLink™ HiPure Plasmid Midiprep Kit. Sequencing reactions were carried out on the plasmid for final confirmation that the correct RPSA targeting gRNA sequence was constructed. Sequencing was performed at Micromon Genomics facility at Monash University, Australia and matched to the designed gRNA oligoDNA sequence (Fig. S2A-C). The verified plasmid construct was termed as crRPSA and stored at stock concentrations of 0.5-3 µg/µL. This single format CRISPR/Cas9 system (crRPSA) could now be stored, amplified and used indefinitely.

Biomimetic mineralization-based synthesis of siRPSA@ZIF-C and crRPSA@ZIF-C.

Aqueous solution of 160 mM of 2mIM (13 mg/mL) and 40 mM of zinc acetate dihydrate (8.9 mg/mL) were made separately. In a 1.5 mL tube, 100nm siRPSA or 0.49 pmols crRPSA was mixed first with 2mIM (100 µL) and then zinc acetate dihydrate (100 µL). The mixture slowly turned cloudy and was left at room temperature for 10 mins. The products were centrifuged at 10,000 rcf for 10 mins and washed in water three times (Fig. S2).

Synthesis of EsiRPSA@ZIF-C and EcrRPSA@ZIF-C

Synthesized siRPSA@ZIF-C and crRPSA@ZIF-C were dispersed in 250 µM of EGCG solution for 2 hours at 700 rpm. The product is then centrifuged at 10,000 rcf for 10 mins and the pellet is washed in water three times followed by disperseal in 100 µL of water for further use (Fig. S3).

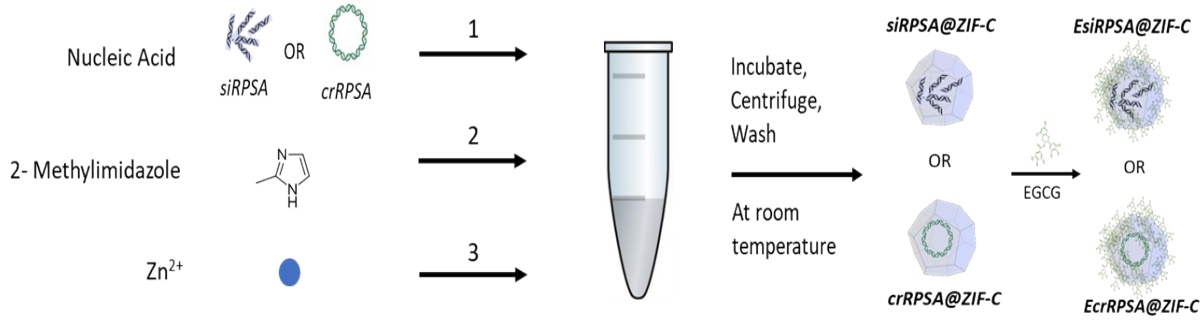


Fig. S2. Synthesis and characterisation of NA@ZIF-C. (A) Schematic of synthesis of crRPSA@ZIF-C, siRPSA@ZIF-C, EcrRPSA@ZIF-C, or EsiRPSA@ZIF-C.

Scanning electron microscopy (SEM)

SEM was used to investigate the particle morphology using a Carl Zeiss Gemini Field Emission Scanning Electron Microscope (FESEM). Approximately 2 µL of the synthesized (siRPSA@ZIF-C, crRPSA@ZIF-C, EsiRPSA@ZIF-C and EcrRPSA@ZIF-C) sample mixture was drop-cast on a silicon wafer, allowed to air dry and followed by sputter coating with 5-6 nm of iridium. Imaging was done under high resolution visualization at EHT 5.0 kV with High Efficiency (HE-SE2) detector for collecting secondary electrons.

X Ray Diffraction

Diffraction patterns were collected using a Bruker D8 Discover GADDS Diffractometer. The x ray diffraction beam monochromator was a Cu target x ray tube with Kα radius 1.544 Å, set to 40kV generator intesity and 40mA generator current. The step size was 0.01°. All data were collected at room temperature. The simulated sod-ZIF-8 and ZIF-C patterns were obtained from Cambridge Structural Database (CSD) OFERUN and [10.26434/chemrxiv.10059935, 10.1021/ic5027174] respectively[1, 2]. Diffraction patterns were run on the web application *ZIF phase analysis* (https://rapps.tugraz.at/apps/porousbiotech/ZIFphaseanalysis/) and the results are shown in Table S1.

Only in the case of crRPSA@ZIF-C we observed the presence of traces of sodalite. The wt% ratio ZIF-C/sod=95%/5% (Table S1) was quantified by the "ZIF phase analysis" [10.26434/chemrxiv.10059935] using the (220) peak at 12.75°.

Sample	***sod***	***ZIF-C***
crRPSA@ZIF-C	5%	95%
EcrRPSA@ZIF-C	-	100%
EsiRPSA@ZIF-C	-	100%
siRPSA@ZIF-C	-	100%

Table S1. ZIF phase analysis of biocomposites

Fluorescence Spectroscopy

To determine loading efficiency of short chain nucleic acids (NAs), a DNA oligomer labelled with TAMRA fluorophore (excitation maxima at 561 nm and emission maxima at 583 nm) was used. Different concentrations of the oligomer (110 ng/µL, 230 ng/µL, 450 ng/µL, 560 ng/µL and 850 ng/µL) were prepared according to the biomimetic mineralisation protocol. Following synthesis, the particles were

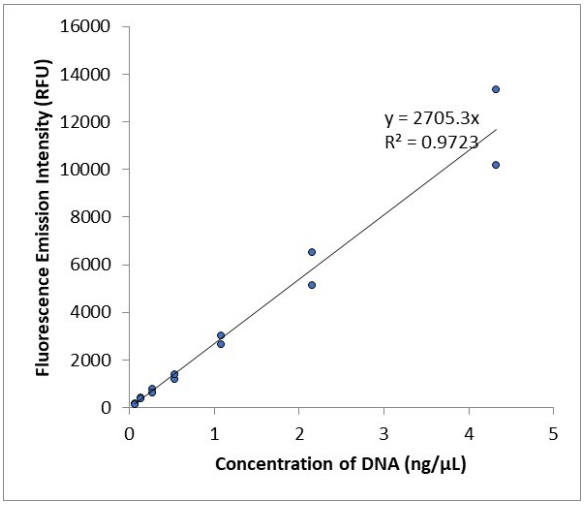


Fig. S3. Standard curve for determination of oligoDNA concentration.

dissolved using EDTA (20mM) and the fluorescence intensity from the samples were recorded using a SpectraMax Paradigm Multi-Mode Microplate Reader. The concentration of associated DNA was calculated from the fluorescence intensity using the standard curve method determined using known concentrations of DNA dilutions. The standard curve (Fig. S3) was obtained from a scatter diagram of concentration versus fluorescent emission intensity of the known DNA concentration diluted two-fold.

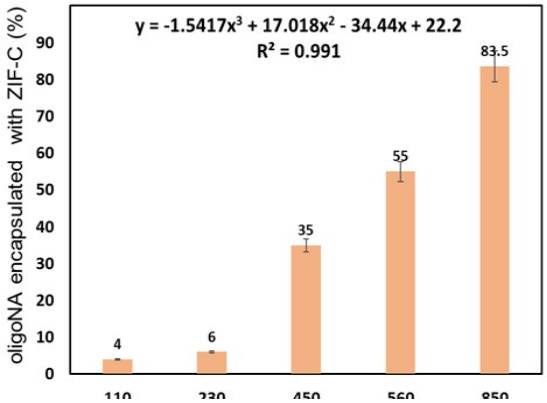


Fig. S4. (G) Determination of loading efficiency of oligoDNA.

A fluorescent TAMRA-labelled (excitation/emission 565/580nm) 19-mer oligonucleotide was used to evaluate loading efficiency of short-chain NAs (Fig. S4).

RNAi stability of ZIF-C encapsulated siRPSA

RNase treatment was used to evaluate nuclease stability of the siRNA biocomposites. Treatment was done using Ribonuclease A from bovine pancreas (Sigma-Aldrich) following manufacturer's protocol. RNase (final concentration of 10 µg/mL) was added to siRPSA@lip, siRPSA@ZIF-C and EsiRPSA@ZIF-C. The reaction was allowed to occur for 15 minutes at 37°C. For siRPSA@lip, standard DharmaFECT lipofectamine was used as per manufacturer's protocol. The nuclease treatment was done post formation of the siRPSA@lip complex. Cell transfection and RNAi efficiency studies were subsequently carried out

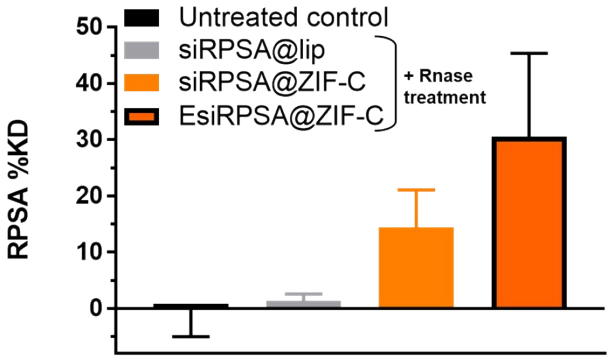


Fig. S5. %KD of RPSA mRNA expression on transfection with RNase treated siRPSA@lip, siRPSA@ZIF-C and EsiRPSA@ZIF-C.

as outlined below at 96 hours post treatment (Fig. S5).

The results show that RNase treated transfection of siRPSA using standard lipofectamine reagent (siRPSA@lip) did not give any RPSA KD. However, despite the nuclease treatment, c.a. 13% KD of RPSA mRNA expression is obtained using siRPSA@ZIF-C and c.a. 30% KD using EsiRPSA@-ZIF-C; which is similar to results obtained using RNase untreated siRPSA@ZIF-C and EsiRPSA@ZIF-C (Fig 3A). Thus, complexing of siRPSA with ZIF-C provides stability and protection to the siRNAs from nuclease treatment.

Cell Transfection

PC-3 prostate cancer cells were kindly provided by Prof John Mariadoson's lab in the Olivia Newton-John Cancer Research Centre. PC-3 cells were seeded in 6 well plate with the seeding density of 0.35×10^6 cells/well and incubated in RPMI medium supplemented with 4.5 g/L D-glucose, 25 mM Hepes, 0.11 g/L sodium pyruvate, 1.5 g/L sodium bicarbonate, 2 mML-glutamine 10% FBS and 1% antibiotic at 37°C with 5% CO_2 overnight. Before transfection, siRPSA@ZIF-C, crRPSA@ZIF-C, EsiRPSA@ZIF-C or EcrRPSA@ZIF-C were dispersed in 200 µL Opti-MEM. Cells were given a medium change with 2 mL of antibiotic free RPMI media with 10% FBS followed by addition of Opti-MEM containing siRPSA@ZIF-C, crRPSA@ZIF-C, EsiRPSA@ZIF-C or EcrRPSA@ZIF-C dropwise in wells and incubated at 5% CO_2 37°C incubators. After 3.5 hours, the treatment media is replaced with 2 mL of fresh RPMI medium containing 10% FBS and 1% antibiotic and continued for incubation for 24, 48, 72 or 96 hours. Cells were then observed under Bio-Rad ZOE™ fluorescent cell imager (Fig. S6) or fixed with 4% paraformaldehyde, stained with Hoechst nuclear stain and imaged in a confocal laser scanning microscope

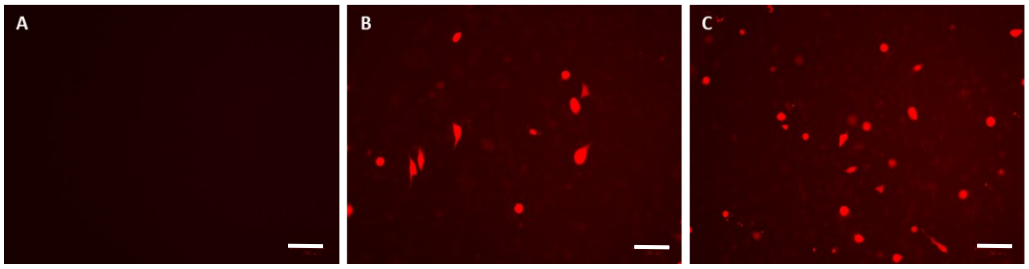


Fig. S6. Cellular delivery of CRISPR/Cas9 using ZIF-C. Cell fluorescence imaging of PC3 cells transfected with (A) control untreated cells, (B) crRPSA@ZIF-C, and (C) EcrRPSA@ZIF-C. All transfections were carried out with 0.49 pmols of crRPSA and imaged at 96 hours. Red – fluorescence due to orange fluorescence protein (OFP) production. Scale bar 100 µm.

(Fig. 2A-C).

RNAi efficiency by using ZIF-C delivered siRPSA

Total RNA was extracted from control untreated and experimental treated cells using guanidinium phenol reagent (TRIzol reagent; Invitrogen) following manufacturer's protocol. The RNA pellet obtained was dissolved in 30µl DEPC water and purity and concentration were quantified using OPTIZEN NanoQ. Reverse transcription was immediately carried out on 2µg RNA from each sample using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher) following manufacturer's protocol. Reaction was

conducted in a T100™ Thermal Cycler (BioRad) with the following step details: -

Lid 105°C, Reaction vol. 20 µL

Temperature	Time
1. 25°C	10:00
2. 37°C	2:00:00
3. 85°C	5:00
4. 4°C	∞

For real time quantitative evaluation of RPSA knockdown using siRNA, qPCR was carried out on the cDNA with RPSA-specific qPCR primers. The TaqMan™ Fast Universal PCR Master Mix (2X), no AmpErase™ UNG (ThermoFisher) was used with TaqMan™ Gene Expression Assay (FAM) ID Hs03046712_g1 (for target RPSA) and Hs01060665_g1 (for housekeeping normalized control ACTB). Manufacturer's protocol was followed, and reaction was conducted in a 7500 Fast Real-Time PCR System (Applied Biosystems) with the following cycle specifications:

Reaction vol per well. 10 µL

Temperature	Time	
1. 95°C	00:20	
2. 95°C	00:03	x40 cycles
3. 60°C	00:30	

The CT values obtained for RPSA were normalized to untreated cells. The "fold-over untreated" method was used to calculate the expression level and %KD of RPSA in treated samples as compared to untreated samples[3].

CRISPR/Cas9 efficiency by using ZIF-C delivered crRPSA

Untreated, crRPSA@ZIF-C and EcrRPSA@ZIF-C treated PC-3 cells were harvested at 96 hours and lysed using protein degrader in a T100™ Thermal Cycler (BioRad) at 68°C 15 min, 95°C 10 min and 4°C hold. Cell lysate was PCR amplified at the reaction rate of 95°C 10 min 1 X, [95°C 30 sec 55°C (Tm) 30 sec 40 X Extend 72°C 30 sec] 40 X, 72°C 7 min 1 X hold and 4°C hold 1 X using Froward and Reverse primers for the designed oligos. The PCR primers specific to the gRNA sequence were designed using the CRISPR guide-RNA (gRNA) in silico tool 'GeneArt CRISPR Search and Design Tool' from Invitrogen TrueDesign Genome Editor software. The synthesized primer sequences are as follows:

RPSA - gRNA	gRNA Oligo Sequence	Primer Sequence	Amplicon Size (parental band) for PCR verification	Cleavage bands for CRISPR cleavage verification
R1 (fwd)	CTATCCTGATACA TACCAGTGTTTT	CTCAGTGGGTT TGATGTGGTGG	634 bp	418 + 216 bp
R1 (rev)	ACTGGTATGTATC AGGATAGCGGTG	CTGAGCGCTCC AGTCTTCTGTT		
R2 (fwd)	CATAAGCAAATTG GACGACTGTTTT	GTGCATAAGAA TTGCCCAG	606 bp	416 +189 bp
R2 (rev)	AGTCGTCCAATTT GCTTATGCGGTG	AGTCTGCAACC TCAGGCT		
R3 (fwd)	TGCCTGGATCTGG TTAGTGAGTTTT	GCTGTAGAATG AACTGAGTG	614 bp	370 + 244 bp
R3 (rev)	TCACTAACCAGAT CCAGGCACGGTG	CTCTAACAATG TGCACAGGA		

Table S2. RPSA targeting CRISPR/Cas9 plasmid details

The resulting PCR product was run on a 2% agarose gel to verify the presence of a single band corresponding to the amplicon size for the respective gRNA used (Fig. S7). A single band is expected to be detected at the same position for treated and untreated cells to confirm target amplification.

The PCR product was subsequently used for detecting genomic cleavage efficiency, performed by GeneArt™ Genomic Cleavage Detection Kit (Life Technologies). Cleavage assay was performed by

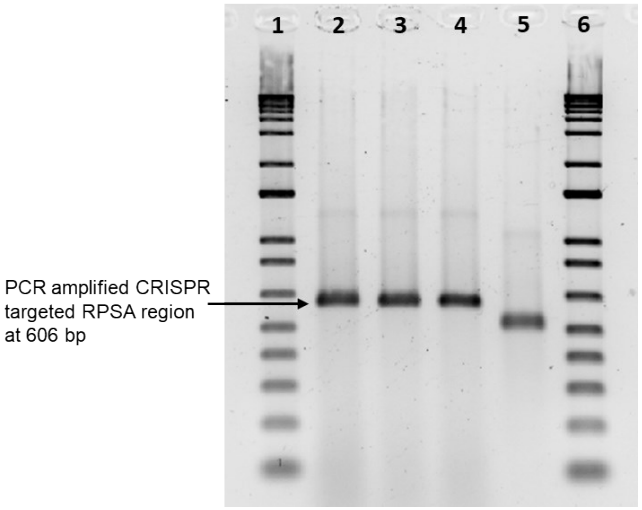


Fig. S7. Agarose gel electrophoresis for the verification of PCR amplification of target RPSA genomic region on using RPSA-gRNA-R2. 1, 6 – 1 kbp DNA ladder, 2 – crRPSA@ZIF-C, 3 - EcrRPSA@ZIF-C, 4 – untreated and 5 – nonspecific kit control.

denaturing and reannealing the PCR amplified product to form heterogeneous DNA duplexes using T100™ Thermal Cycler (BioRad) at reaction conditions of 95°C 5 min, 95°C–85°C [ramp rate -2°C/sec], 85°C–25°C [ramp rate –0.1°C/sec] and 4°C -hold. The amplified strands are denatured and reannealed because cleavage by CRISPR/Cas9 results in a mismatch in the target DNA strand, and this mismatch can be detected by a mismatch detection enzyme in the reannealed strands. Immediately, the re-annealed product is proceeded for enzyme digestion by incubating the samples with (or without) the detection enzyme for 1 hour at 37°C to detect the heteroduplex DNA containing the insertion, deletion, or mismatched DNA (indel).

Genomic cleavage was visualized by immediately loading the entire enzyme treated sample on a 2% agarose gel with 10 µL water using 1 kb DNA ladder in parallel as a reference and sample without enzyme treated as negative control and allowed to run at 100V for 1 hours. Bands are imaged through Bio-Rad

Gel Doc™ XR+ Imager. With CRISPR/Cas9 cleavage of the target, two cleavage bands are formed on treatment with the detection enzyme. Densitometry analysis on the agarose gel obtained bands were quantitated using FIJI [4]. In FIJI software, the LUTs were inverted and the rectangle tool was used to isolate the regions of interest. Band intensity peaks were graphed for calculating area under the curve for each peak. The peak percentage of each experimental band (crRPSA@ZIF-C or EcrRPSA@ZIF-C) was divided by the peak percentage of the control band (nonspecific control) to estimate the relative percent of DNA quantity associated with the crRPSA cleaved bands for determining %GCD.

Cellular Viability Assays

Cytotoxicity of siRPSA@ZIF-C, crRPSA@ZIF-C, EsiRPSA@ZIF-C and EcrRPSA@ZIF-C on PC-3 cells were assessed by MTT assay. PC-3 cells were seeded in 96 well plates with density of 10000, 7500, 5000 and 2500 cells/well for 24, 48, 72, 96 hours respectively and incubated at 37°C with 5%CO_2 overnight. Next day cells were treated with 100 µL RPMI medium (10% FBS) containing test ZIF-C biocomposites (siRPSA@ZIF-C, crRPSA@ZIF-C, EsiRPSA@ZIF-C and EcrRPSA@ZIF-C) and incubated for 3.5 hours at 37°C with 5% CO_2. Then, the medium was replaced with 100 µL RPMI medium containing 10% FBS and 1% antibiotic. Later at each time point medium was aspirated in respective plate and 100 µL of serum free medium containing 0.5 mg/mL of MTT was added to each well and incubated for 4 hours in dark at 37°C. After incubation medium containing MTT was replaced with DMSO for dissolving purple formazan crystals and the absorbance was measured in a micro plate reader at 570 nm with the reference wavelength of 630 nm. The percentage cell viability is calculated by the formula [(absorbance of treated cells/absorbance of untreated cells) *100].

The non-specific NA@ZIF-C using non-targeting oligomer NA or green fluorescent protein (GFP) plasmid were used as controls to assess the toxicity of ZIF-C as gene delivery systems. To check for toxicity of the delivery system, the short chain TAMRA labelled oligomer and a green fluorescent plasmid (plGFP gene) were used to synthesise oligoNA@ZIF-C, geneNA@ZIF-C, EoligoNA@ZIF-C and

EgeneNA@ZIF-C (with and without EGCG coating). MTT assays were carried out on PC-3 cells following treatment with these particles in the same protocol as described above (Fig. S8).

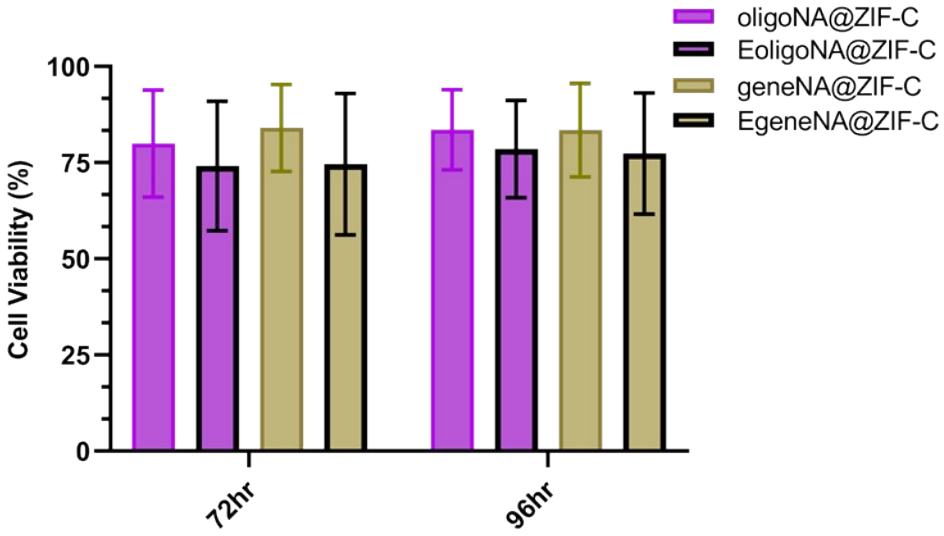


Fig. S8. Cellular viability of PC-3 cells after 3.5-hour treatment with non-targeting NA@ZIF-C biocomposites at 72 and 96 hours.

A consistent reduction in viability was seen on treatment with EGCG coated biocomposites as compared to uncoated ones. However, >80% viability with uncoated particles and >70% viability with coated particles up to 96 hours indicated the lack of significant toxicity from the delivery system itself.

The effects of ZIF-C biocomposites (siRPSA@ZIF-C, crRPSA@ZIF-C, EsiRPSA@ZIF-C and EcrRPSA@ZIF-C) were further assessed in less metastatic PC cell line DU145 and normal prostate epithelial cell line PNT1A following the protocol outlined above (Fig. S9). crRPSA showed consistently higher toxicity than siRPSA for all cell lines up to 96 hours. Normal PNT1A cells showed least toxicity with 67% viability. Out of the cancer cells, the metastatically advanced PC-3 showed highest toxicity with 49% viability and the less metastatic DU145 gave viability of 64%. Viability was further reduced on coating with EGCG. The viability pattern seen for the cell lines supports the reason that RPSA gene overexpression is higher in the more advanced forms of PC, thus targeting RPSA is more lethal for advanced PC cell lines than normal prostate cells. For siRPSA, all the cells showed relatively lower toxicity than with crRPSA. However, siRPSA gave higher knockdown of RPSA expression than crRPSA (Fig. 4A). Cytoplasmic KD of RPSA reduced expression further than genomic knockdown, while

genomic knockdown had the more pronounced cytotoxic effect. These results suggest that genomic disruption of RPSA interrupts cellular processes that have greater regulatory impact on viability than disruption at the mRNA level.

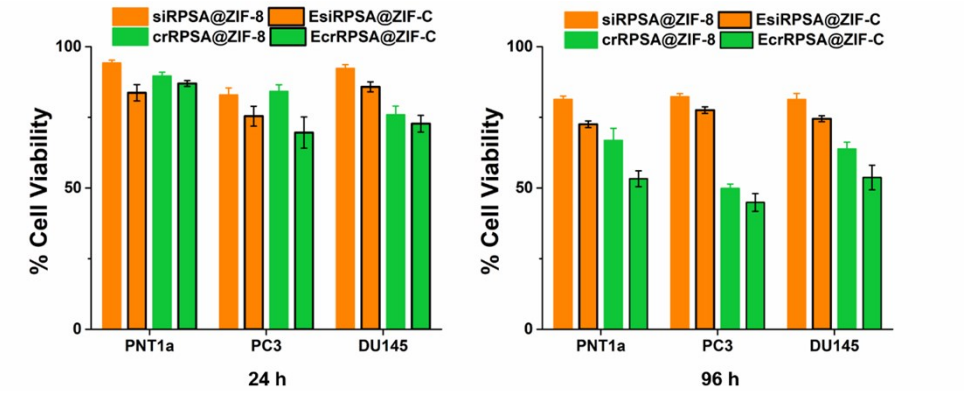


Fig. S9. Cellular viability of PNT1A, PC-3 and DU145 cells after 3.5-hour treatment with siRPSA/crRPSA delivered via uncoated and EGCG coated ZIF-C biocomposites at 24 and 96 hours.

Immunofluorescence Assays

Immunofluorescence staining was carried out in order to assess the impact on LAM67R, protein product of RPSA, following ZIF-C mediated KD of RPSA. In order to see changes in protein level resulting from genomic disruption using CRISPR/Cas9, while at the same time maintaining consistent experimental conditions, seeding with crRPSA@ZIF-C or EcrRPSA@ZIF-C required allowing the cells to grow beyond 96 hours without passaging in order to see changes at the protein level. This led to high cell death rates which is a commonly observed phenomenon in mammalian cells when allowed to grow beyond confluency[5]. Thus, as siRNAs are faster in impacting protein expression due to being downstream to the genomic pathway, siRPSA mediated KD results gave clearer representation of disrupted LAM67R protein expression. PC-3 cells were seeded on coverslips in 6 well plate with the seeding density of 0.35×10^6 cells/well and incubated in RPMI medium supplemented with 4.5 g/L D-glucose, 25 mM Hepes, 0.11 g/L sodium pyruvate, 1.5 g/L sodium bicarbonate, 2 mM L-glutamine 10% FBS and 1% antibiotic at 37°C with 5% CO_2 overnight. Before transfection, siRPSA@ZIF-C or EsiRPSA@ZIF-C were dispersed in 200

μL Opti-MEM. Cells were given a medium change with 2 mL of antibiotic free RPMI media with 10% FBS followed by addition of Opti-MEM containing siRPSA@ZIF-C or EsiRPSA@ZIF-C dropwise in wells and incubated at 5% CO_2 37°C incubators. After 3.5 hours, the treatment media is replaced with 2 mL of fresh RPMI medium containing 10% FBS and 1% antibiotic and continued for incubation for 96 hours. Cells were then fixed in 4% paraformaldehyde for 10 minutes and imaged in a phase contrast microscope. (Fig. S10 A-C). Cells were washed thrice in PBS and stored in PBS at 4°C until antibody staining could be performed. For the staining, PBS was removed, and cells were permeabilised with 0.02% Triton X-100 in PBS for 90 seconds. Cells were washed once with PBS, followed by blocking with 2% bovine serum albumin (BSA) in PBS for 30 minutes. Cells were then incubated with 1:50 dilution of mouse monoclonal LAM67R antibody (MLuC5) in blocking buffer, for 1 hour. After washing thrice with PBS, cells were incubated with 1:500 dilution of goat anti mouse secondary antibody tagged with Alexa Fluor®594 in blocking buffer, for 30 minutes, in the dark. Cells were washed 5 times in PBS followed by staining with Hoechst 33342 (2 μg/mL in PBS) for 10 minutes. After final three PBS washes, cells were mounted on to glass slides using antifade mounting medium and imaged in a confocal laser scanning microscope (Fig. S10 D-F).

Brightfield images taken using a phase contrast microscope further visualised the increased cytotoxicity due to RPSA knockdown. As compared to untreated cells, distinct morphological changes and increased cell death can be seen in both EGCG uncoated and coated ZIF-C treatments to knockdown RPSA. In order to visualise RPSA protein expression that caused these changes, the cells were treated with antibodies against LAM67R (MLuc5), the protein product of RPSA. LAM67R is a cell-surface receptor protein[6], thus when untreated cells are incubated with MLuc5, LAM67R expression (red) can be observed clearly around the cell membrane (Fig. S10 D). On ZIF-C based treatment, a distinct reduction in the red fluorescence (indicating LAM67R protein) can be clearly seen. Moreover, morphological changes and increased cytotoxicity on RPSA targeting is further visualised, as can also be seen from bright field images.

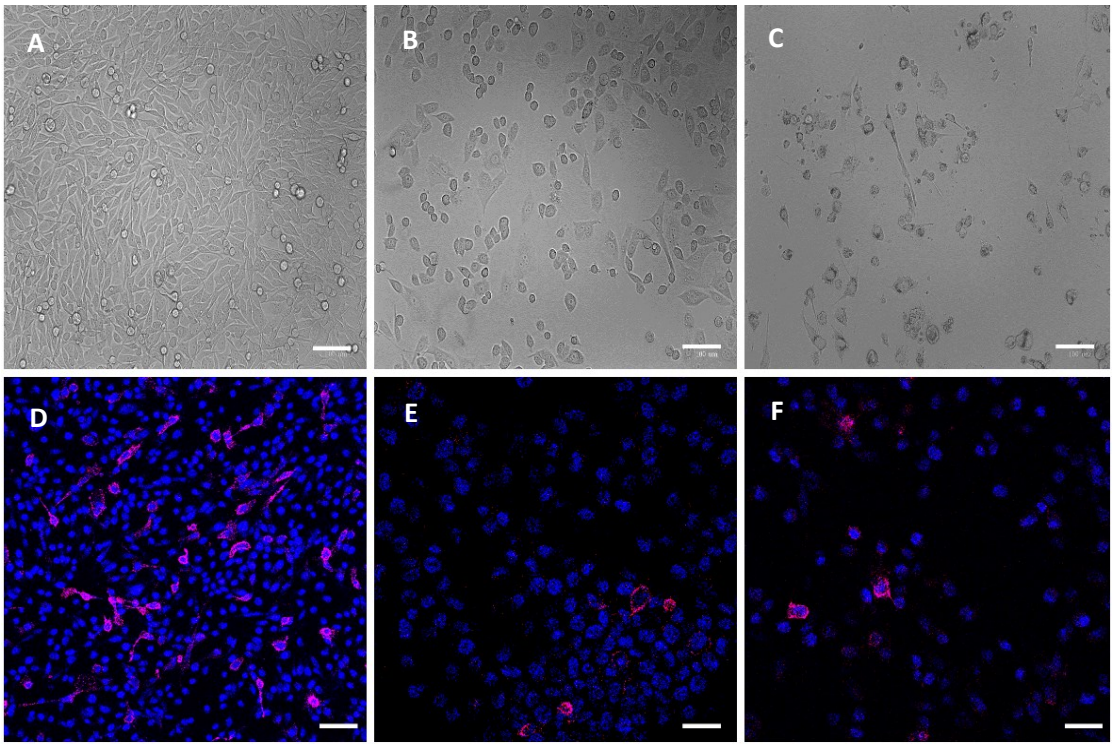


Fig. S10. Brightfield images of PC-3 cells (A) untreated, (B) treated with siRPSA@ZIF-C, and (C) treated with EsiRPSA@ZIF-C. Immunofluorescence images of LAM67R antibody stained PC-3 cells (D) untreated, (E) treated with siRPSA@ZIF-C, and (F) treated with EsiRPSA@ZIF-C. Images taken at 96 hours post treatment. Blue – cell population as seen by Hoechst 33342 nuclear stain. Red – fluorescence due to antibody staining of LAM67R protein, scale bar (A-D) 100 µm, (E-F) 50 µm.

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