Supporting Information

Delivery of Cas13a/crRNA by self-degradable black phosphorus nanosheets to specifically inhibit Mcl-1 for breast cancer therapy

Huahua Yue [1, 2], Ru Huang[1, 2], Yuanyue Shan[1, 2], and Da Xing[* 1, 2]

Experimental

Materials and Reagents. All DNA oligonucleotides, Poly-L-lysine (PLL), NTP Mixture (10 mM), MTT Cell Proliferation and Cytotoxicity Assay Kit and Annexin V-FITC/PI Apoptosis Detection Kit were purchased from Sangon (Shanghai, China). T7 RNA polymerase was purchased from New England BioLabs. Reverse Transcriptase M-MLV (RNase H-), dNTP Mixture (10 mM), RNase Inhibitor, TB Green™ Premix Ex Taq™ (Tli RNase H Plus), RNAiso Plus reagent and double-labeled (FAM and BHQ1) RNA probe (FQ5U) were purchased from Takara. Black Phosphorus was purchased from XFNANO (Nanjing, China).

The AGS.EGFP cell line was from Professor Zhi Shi at Jinan University as a generous gift. The cell lines of MDA-MB-231, MCF-7, Hela, HepG2 and LO2 were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All of the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and penicillin (100 U/mL)/streptomycin (0.1 mg/mL). Cells were maintained in a humidified cell culture incubator at 37 °C under 5% CO_2.

Expression and purification of Cas13a protein. LwCas13a bacterial expression vector C013-Twinstrep-SUMO-huLwCas13a (Addgene #90097) was transformed into Rosetta (DE3) Competent Cells (Tiangen). A 16 mL starter culture was grown overnight in Terrific Broth (TB) growth media (12 g/L tryptone, 24 g/L yeast extract, 9.4 g/L K_2HPO_4, 2.2 g/L KH_2PO_4), which was used to inoculate 4 L of TB for growth at 37 °C and 300 RPM until an OD_{600} of 0.6. At

this time, protein expression was induced by supplementation with IPTG to a final concentration of 500 μM, and cells were cooled to 16 °C for 12 h for protein expression. After centrifuged at 5000 g for 15 min at 4 °C, cell pellet was harvested and stored at -80 °C. Following cell lysis, protein was bound using a Strep Tactin Sepharose resin (GE) and eluted by SUMO protease digestion. Protein was further purified in a heparin column (GE Healthcare), and eluted with buffer containing 20 mM Tris-HCl pH 7.5, 1 M NaCl, and 10% glycerol. Final fractions containing LwCas13a protein were pooled and concentrated into storage buffer (600 mM NaCl, 50mM Tris-HCl pH 7.5, 50% glycerol and 2 mM DTT) and aliquots were frozen at -80 °C for long-term storage.

Transcription and preparation of target RNA and crRNA. Transcription templates were synthesized by annealing two complementary ssDNAs containing T7 promoter. The target RNA or crRNA sequences were listed in **Table S1** of Supplemental Information. Target RNA or crRNA was synthesized by in vitro transcription using T7 RNA polymerase. Transcription reaction was performed with double-stranded DNA template for 6 h at 37 °C. The transcribed RNAs were purified by RNA Clean Kit (Tiangen), and then analyzed by polyacrylamide gel electrophoresis (PAGE). After quantifying with Nanodrop 2000c (Thermo Scientific), the RNA products were finally stored at -80 °C.

Activity verification of Cas13a/crRNA. The activity of Cas13a/crRNA was verified by gel electrophoresis and fluorescence assays, which were performed with 50 nM target RNA, 100 nM purified LwCas13a, and 200 nM FQ5U. For fluorescence assays, the concentration of crRNA was optimized with different concentrations of 50 nM, 100 nM, 200 nM and 400 nM. All assays were performed in nuclease assay buffer (10 mM Tris-HCl, 10 mM NaCl, 1.5 mM $MgCl_2$). Reactions were performed at 37 °C for 1 h and then terminated by denaturing at 95 °C for 5 min. Finally, fluorescence spectrophotometer (PerkinElmer LS-55) was used to measure

the fluorescence intensity with excitation at 488 nm and an emission range from 500 to 650 nm to verify the trans-cleavage and gel electrophoresis was used to analyze the cis-cleavage.

Preparation and characterization of black phosphorus nanosheets. The BP nanosheets were prepared using a simple liquid phase exfoliation method as a reported work.[1] Briefly, 25 mg of the BP was added into 50 mL N-methyl-2-pyrrolidone (NMP, Aladdin). Then the mixture solution was sonicated in ice bath for 6 h, followed by removing the residual unexfoliated particles and collecting the supernatant. Before use, the BP nanosheets were washed 5 times with deoxygenated distilled water and re-suspended in the aqueous solution. The morphology and thickness of BP nanosheets were characterized by transmission electron microscopy and atomic force microscope (AFM, Agilent Technologies). Hydrodynamic size of BP and PBP were obtained by a Zetasizer Nano ZS (Malvern). The Raman spectra of BP nanosheets were conducted with a Renishaw inVia confocal Raman system (controlled by WiRE 3.4 software) with laser excitation wavelength of 633 nm and coupled to a Leica DM-2500M microscope. Fourier transform infrared spectroscopy (Thermo Scientific) and X-ray photoelectron spectroscopy were employed to characterize BP and PBP.

Degradation assays of BP and PBP under physiological solution. Degradation of bare BP and PBP in PBS were assessed and compared by the changes of absorbance spectra using a UV-Vis spectrophotometer (Lambda-35 UV-Vis spectrophotometer, PerkinElmer).

Preparation and characterization of the PBP/Cas13a/crRNA. For preparing PBP hybrid, 1 mL BP dispersion was mixed with 2 mL 2 mg/mL PLL (prepared with deoxygenated distilled water) and the resultant mixture solution was then incubated at 4 °C in dark for 12 h. After that, the mixture was purified by ultra-filtration tube (MWCO 130 kDa) at 6000 rpm for 20 min, and washed three times with deoxygenated distilled water to remove the excess PLL molecules. The obtained PBP were resuspended in deoxygenated distilled water for further use and stored at 4

°C. Cas13a and crRNA were mixed at a ratio (1:1) in reaction buffer and incubated at room temperature for 10 min, followed by added PBP (final PBP concentration: 30 μg/mL) and incubated at room temperature for 15 min. Zetasizer Nano ZS (Malvern) was used to measure zeta potential of BP, PLL, PBP, Cas13a/crRNA and PBP/Cas13a/crRNA, respectively.

Activity verification of PBP/Cas13a/crRNA. To verify whether PBP/Cas13a/crRNA has cleavage activity to target RNA, Cas13a (200 nM) was incubated with 100 nM crRNA in reaction buffer (10 mM Tris-HCl, 10 mM NaCl, 1.5 mM $MgCl_2$) for 10 min, followed by added PBP (30 μg/mL). After centrifugation, the supernatant was discard, and the pellet was resuspended in reaction buffer, followed by added 10 nM target ssRNA. Gel electrophoresis was used to analyze the cis-cleavage of target ssRNA by PBP/Cas13a/crRNA and Cas13a/crRNA, and fluorescence spectrophotometer was used to measure the trans-cleavage.

Loading and release of Cas13a/crRNA complexes. Loading capacity of Cas13a/crRNA complexes onto PBP was determined by detecting the active Cas13a/crRNA complexes in the supernatant before and after incubates with PBP. Briefly, after the construction of PBP/Cas13a/crRNA, the mixture was centrifuged, and the supernatant was collected and incubated with target ssRNA and FQ5U at 37°C for 30 min, followed by recording maximum fluorescence signal. Cas13a/crRNA complexes without PBP were used as a control. The loading capacity in mass Cas13a/crRNA ($M_{Cas13a\text{-}loaded}/M_{PBP}$) was calculated by using formula 1.[2]

$$\text{Loading capacity (\%)} = \frac{\frac{F_{PBP-} - F_{PBP+}}{F_{PBP-}} \times C_{Cas13a} \times Mw_{Cas13a} \times V}{C_{PBP} \times V} \times 100 \quad (1)$$

where F_{BP-} and F_{BP+} are the maximum fluorescence signals from the control and PBP loaded sample respectively, C_{Cas13a} is the concentration of Cas13a/crRNA complexes, Mw_{Cas13a} is the

molecular weight of Cas13a/crRNA complexes, V is the volume of assembly mixture (100 μL), and the C_{PBP} is the concentration of PBP in the mixture (30 μg/mL).

Release efficiency of Cas13a from PBP/Cas13a/crRNA was determined by detecting active Cas13a/crRNA complexes in the supernatant. Briefly, constructed PBP/Cas13a/crRNA was centrifuged to remove unloaded Cas13a/crRNA complexes in the supernatant and re-suspended in DMEM supplemented with 10% FBS. At different time intervals, 10 μL of the mixture was centrifuged. Active Cas13a/crRNA complexes were determined with target ssRNA and FQ5U. Cas13a release was calculated by using formula 2.[2]

$$\text{Cas13a/crRNA release (\%)} = {}^{F_R}\!/_{F_L} \times 100 \quad (2)$$

where F_R is the mean maximum fluorescence signal of released active Cas13a/crRNA complexes at different time intervals, F_L is the mean maximum fluorescence signal of loaded Cas13a/crRNA complexes based on previous calculation (the maximum fluorescence signal of 200 nM Cas13a/crRNA complexes minus that of PBP unloaded sample).

Cell uptake. To observe the intracellular distribution of Cas13a/crRNA and PBP/Cas13a/crRNA, crRNA was labeled with a Cy5 probe (**Table S2**). MDA-MB-231 cells were seeded into glass bottom cell culture dish at a density of 2.5×10^4 cells per well and cultured for 24 h. Cells were transfected following the same method as above, washed 3 times with cold PBS. The endosomes/lysosomes were counterstained with Lysotracker Green according to the standard protocol provided by the suppliers. The samples were observed by confocal microscopy.

For the cellular uptake of Cy5-labelled crRNA (*crRNA-Cy5), the Cas13a/*crRNA-Cy5 and PBP/Cas13a/*crRNA-Cy5 were formed following the method mentioned above. MDA-MB-231 cells were seeded into 6-well plates at a density of 1×10^5 cells per well and cultured

for 12 h. The Cas13a/*crRNA-Cy5 and PBP/Cas13a/*crRNA-Cy5 were respectively added into the cell-cultured well with FBS free DMEM and sustained for 4 h at 37 °C. Finally, the cells were washed with PBS three times and trypsinized into a single-cell suspension, and then subjected to flowcytometric analysis using a flow cytometer (BD Biosciences).

Determination of endocytosis pathways. Endocytosis inhibitors [3, 4] (methyl-β -cyclodextrin (MCD, 10 mM) for lipid raft, chlorpromazine (CPZ, 10 μM) for clathrin-mediated endocytosis, amiloride (AMI, 2 mM) for macropinocytosis and nystatin (NYS, 25 μg/mL) for caveolin-mediated endocytosis) have been employed to elucidate the underlying cellular uptake mechanism of PBP/Cas13a/crRNA. *crRNA-Cy5 was used to track its uptake. MDA-MB-231 cells were seeded in 6-well plates (~1 × 10^5 cells/well) and cultured for 48 h. Then the cells were pre-incubated with the endocytosis inhibitors above for 1 h at 37 °C, respectively. Afterwards, the cells were incubated with PBP/Cas13a/*crRNA-Cy5 for another 2 h in the presence of the inhibitors. Cells were then washed, trypsinized and resuspended in full serum medium, intracellular Cy5 fluorescence intensities were analyzed by flow cytometry.

In vitro EGFP gene disruption assay. The AGS.EGFP cell line, which has a single copy of destabilized EGFP gene integrated into the genome, was used as the model cell line to explore the feasibility of the proposed method. AGS. EGFP cells were seeded into glass bottom cell culture dish (~ 2.5 × 10^4 cells per well) one day before the PBP/Cas13a/crRNA transfection. When the cells reached 70% confluence, the medium was replaced with fresh DMEM medium without FBS containing the PBP/Cas13a/crRNA complex (PBP concentration at 30 μg/mL and Cas13a/crRNA concentration at 20 μM). After incubation for 4 h, the Cas13a containing medium was replaced with fresh full serum medium incubated for another two days for analyzed by confocal microscope (ZEISS LSM 510). And for flow cytometer analysis, AGS.EGFP cells were seeded into 6-well plates (1 × 10^5 cells per well) and the processing conditions are the same as before. After two days, the cells were washed with PBS three times

and trypsinized with 2.5% trypsin at 37 °C for 1-2 min. Subsequently, the cells were washed and resuspended in PBS and analyzed by a flow cytometer (BD Biosciences).

RNA isolation and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Cells were seeded into 6-well plates at a density of 1×10^5 cells per well. After designated treatments, total RNA of cells was isolated using the RNAiso Plus reagent according to the manufacturer's instructions and quantified with Nanodrop 2000c. Afterwards, RNA was used for the reverse transcription reaction using the Reverse Transcriptase M-MLV (RNase H-) to synthesize complementary DNA. Finally, qRT-PCR was performed using primer pairs with the TB Green™ Premix Ex Taq™ (Tli RNaseH Plus). Actin was used as a loading control. The PCR primers were listed in Supplemental Information **Table S2**.

Immunoblotting assay. Cells were seeded into 6-well plates at a density of 1×10^5 cells per well. After designated treatments, total protein of cells was isolated using the Tissue or Cell Total Protein Extraction Kit (Sangon Biotech) according to the manufacturer's instructions and quantified by Modified BCA Protein Assay Kit (Sangon Biotech). Equivalent proteins were loaded on SDS-PAGE and transferred to PVDF membranes, then incubated with primary antibodies at 4 °C overnight after blocking with 5% non-fat milk, followed by incubation with HRP antibody reaction solution for 1 h at room temperature. The images were taken by a mobile phone.

Cell viability assay. For the cell proliferation and cytotoxicity assays, cells were inoculated into a 96-well plate(5×10^3 per well, 100 μL) and incubated 12 h. Afterwards, for AGS.EGFP cells, the medium in each well replaced with fresh cell culture medium which contain BP and PBP with a serial concentration of 10, 20, 40, 80, and 120 μg/mL. For MDA-MB-231 and MCF-7 cells, cells of 5×10^3 per well were cultured in 96-well plate for 12 h. The cells randomly divided into several groups by designated treatments. Cell viability was assessed with MTT

according to the manufacturer's instructions. Finally, the absorbance intensity at 570 nm was recorded and the cytotoxicity was expressed as a percentage of the control.

Apoptosis analysis. MCF-7 and MDA-MB-231 cells were seeded into 6-well plates at a density of 1×10^5 cells per well and followed by indicated treatments. After 48 h, cells were collected and stained with the Annexin V-FITC Apoptosis Detection Kit according to the manufacturer's instructions. Subsequently, the cells were analyzed by FACSCanto II flow cytometer (BD Bioscience).

***In vivo* antitumor efficacy.** The male BALB/c nude mice (6-week-old) were obtained from Southern Medical University Experimental Animal Center, and all animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. All mice studies were approved by the Institutional Animal Care and Use Committee of South China Normal University (Guangzhou, China). To set up the tumor model, 1×10^7 MDA-MB-231 cells were subcutaneously inoculated into mice. The tumors were allowed to grow to about 100-200 mm^3 before treatment. The tumor volume (V) was estimated by the equation $V = a \times b^2/2$, where a, b are the major and minor axes of the tumor, respectively. For MDA-MB-231 tumor growth inhibition, mice were intratumorally injected with 50 μL of PBP loaded with 50 μM Cas13a/crRNA once every two days, and for a total of ten injections. Mice that were administered with PBS, free Cas13a/crMcl-1 and PBP/Cas13a/crEGFP served as controls. Each group contained six mice. Then, the body weight changes and tumor volume was monitored and recorded every other day for 20 days. Tumor growth was monitored by electronic calipers every 2 days for 3 weeks. The mice were euthanized in the end of the study, and the tumors and major organs were collected for further analysis.

Statistical Analysis. Statistical analysis was performed with Origin 2018 and GraphPad Prism 5. Each experiment was repeated at three times for each sample; and the differences between

two groups were analyzed by one-way ANOVA. The analyses were performed with GraphPad Prism 5. A p-value of < 0.05 was considered statistically significant.

Table S1. Template sequences for in vitro transcription of RNA.

Nucleic Acid ID	Sequence(5'-3')
EGFP-mRNA-F	GAAATTAATACGACTCACTATAGGGCCTCGCCGACGTAAACGGCC ACAAGTTCAGCGTGTCCGCC
EGFP-mRNA-R	GGCGGACACGCTGAACTTGTGGCCGTTTACGTCGGCGAGGCCCTAT AGTGAGTCGTATTAATTTC
crEGFP–F	GAAATTAATACGACTCACTATAGGGGGGGATTTAGACTACCCCAA AAACGAAGGGGACTAAAACGACACGCTGAACTTGTGGCCGTTTAC GT
crEGFP–R	ACGTAAACGGCCACAAGTTCAGCGTGTCGTTTTAGTCCCCTTCGTT TTTGGGGTAGTCTAAATCCCCCCCTATAGTGAGTCGTATTAATTTC
crCXCR-4-F	GAAATTAATACGACTCACTATAGGGGGGGATTTAGACTACCCCAA AAACGAAGGGGACTAAAACATGATAATGCAATAGCAGGACAGGA TGA
crCXCR-4-R	TCATCCTGTCCTGCTATTGCATTATCATGTTTTAGTCCCCTTCGTTTT TGGGGTAGTCTAAATCCCCCCCTATAGTGAGTCGTATTAATTTC
*crRNA-F	GAAATTAATACGACTCACTATAGGGGACACGCTGAACTTGTGGCC GGGGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACGTGATA CTTTCTGCTAATGGTTCGATGC
*crRNA-R	GCATCGAACCATTAGCAGAAAGTATCACGTTTTAGTCCCCTTCGTT TTTGGGGTAGTCTAAATCCCCGGCCACAAGTTCAGCGTGTCCCCTA TAGTGAGTCGTATTAATTTC
crMcl-1-F	GAAATTAATACGACTCACTATAGGGGGGGATTTAGACTACCCCAA AAACGAAGGGGACTAAAACGTGATACTTTCTGCTAATGGTTCGAT GC

crMcl-1-R	GCATCGAACCATTAGCAGAAAGTATCACGTTTTAGTCCCCTTCGTT TTTGGGGTAGTCTAAATCCCCCCCTATAGTGAGTCGTATTAATTTC

Table S2. Sequences of PCR primers and fluorescence probes.

Nucleic Acid ID	Sequence(5'-3')
CXCR-4-F	GGTGGTCTATGTTGGCGTCT
CXCR-4-R	TGGAGTGTGACAGCTTGGAG
EGFP-F	AGCAAAGACCCCAACGAGAA
EGFP-R	GGCGGCGGTCACGAA
Mcl-1-F	CGGTAATCGGACTCAACCTC
Mcl-1-R	CCTCCTTCTCCGTAGCCAA
Actin-F	TCACCCACACTGTGCCCATCTACGA
Actin-R	CAGCGGAACCGCTCATTGCCAATGG
Cy5 probe	GGCCACAAGTTCAGCGTGTC-CY5
FQ5U probe	BHQ1-UUUUU-FAM

Table S3. Comparison of PBP/Cas13a/crRNA with other NPs-based CRISPR ribonucleoprotein delivery platforms for in vitro applications.

Delivery platform	CRISPR system	Concentration of delivery platform	Concentration of CRISPR	Efficiency	Reference
PBP	Cas13a/crRNA	30 μg/mL	20 μM	58.64%	This study
CRISPR-max	Cas13a/crRNA	According to the manufacturer's instructions	20 μM	94%	[5]
Cas9N3-BPs	Cas9/sgRNA	2 μg/mL	16 nM	32.1%	[2]
Lipid nanoparticles	Cas9/sgRNA	2 μg/mL	25 nM	70 %	[6]
10 nm Gold NPs	Cas9/sgRNA	682.5 μg/mL	62 nM	30%	[7]
15 nm Gold NPs	Cas9/sgRNA	7.59 μg/mL	50 nM	11.3%	[8]
DNA nanoclews	Cas9/sgRNA	Not specified	100 nM	28%	[4]
	Cas12a/crRNA	Not specified	200 nM	48%	[9]
Gold nanowires	Cas9/sgRNA	Not specified	60 nM	80%	[10]
Gold nanoclusters	Cas9/sgRNA	Not specified	150 nM	60%	[11]
ZIF	Cas9/sgRNA	100 μg/mL	240 nM	30%	[12]
Graphene oxide	Cas9/sgRNA	30 μg/mL	100 nM	39%	[13]

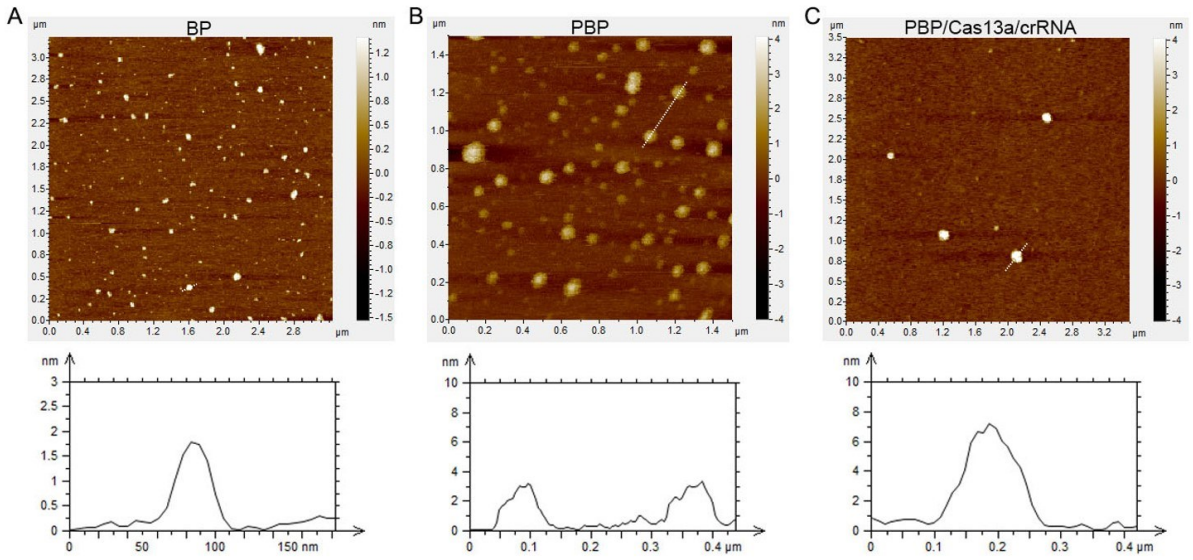

Fig. S1. AFM image of BP nanosheets (A), PBP (B), and PBP/Cas13a/crRNA (C).

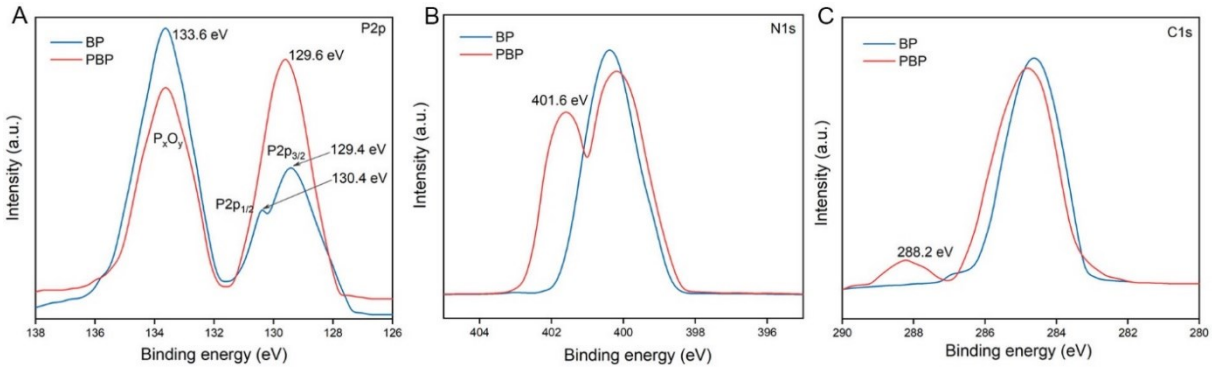

Fig. S2. XPS spectra of BP and PBP. (A) P2p core region; (B) N1s core region; (C) C1score region.

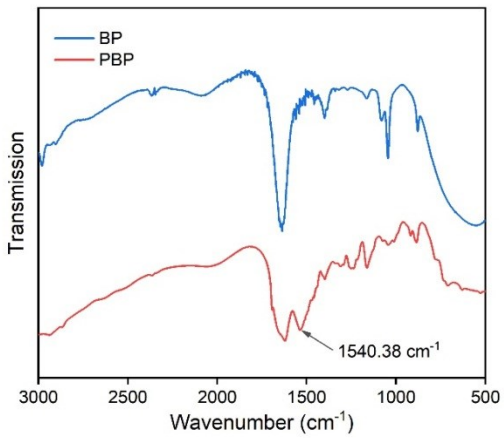

Fig. S3. Fourier transform infrared spectroscopy of BP and PBP.

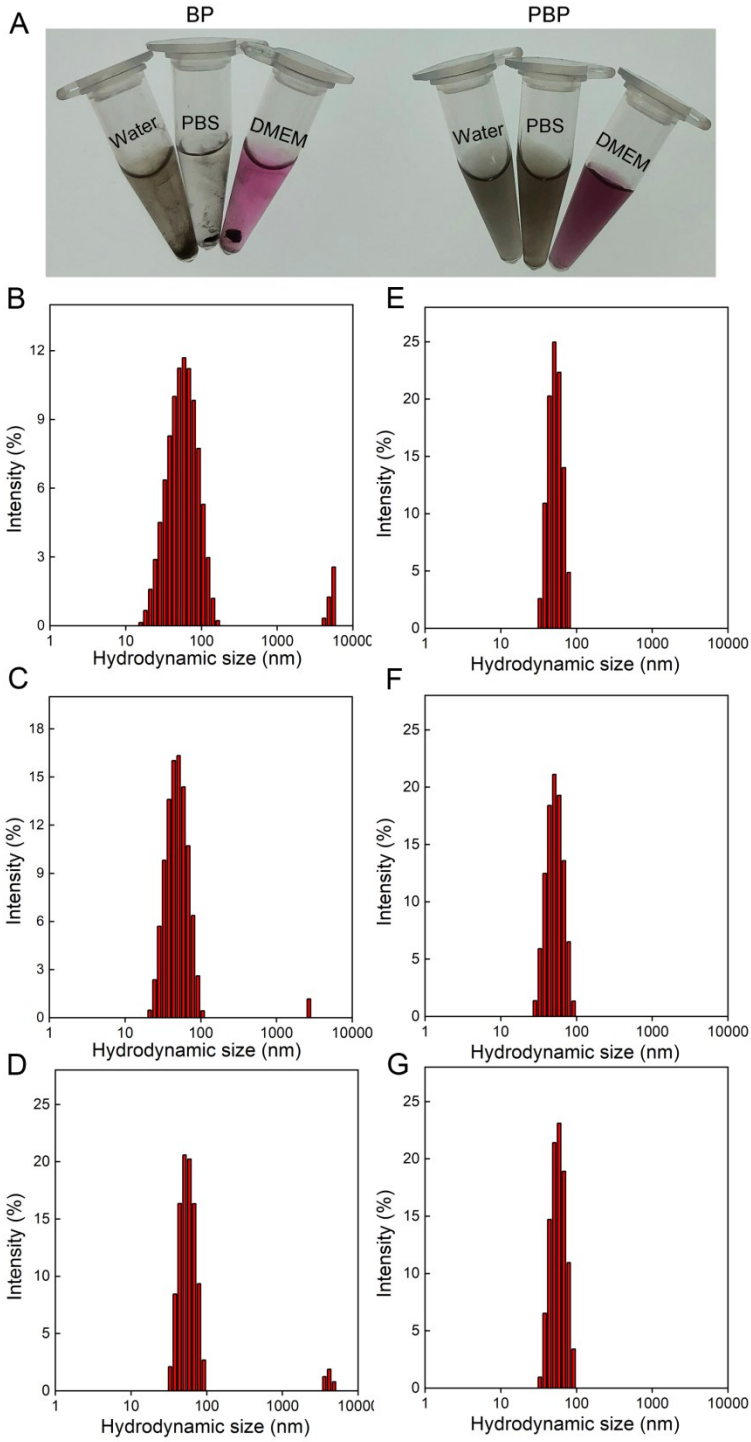

Fig. S4. Stability analysis of BP and PBP. (A) Photos of BP and PBP in water, PBS and cell culture medium after 1 week-standing. DLS hydrodynamic sizes distribution of BP in water (B), PBS (C) and DMEM (D). DLS hydrodynamic sizes distribution of BP in water (E), PBS (F) and DMEM (G).

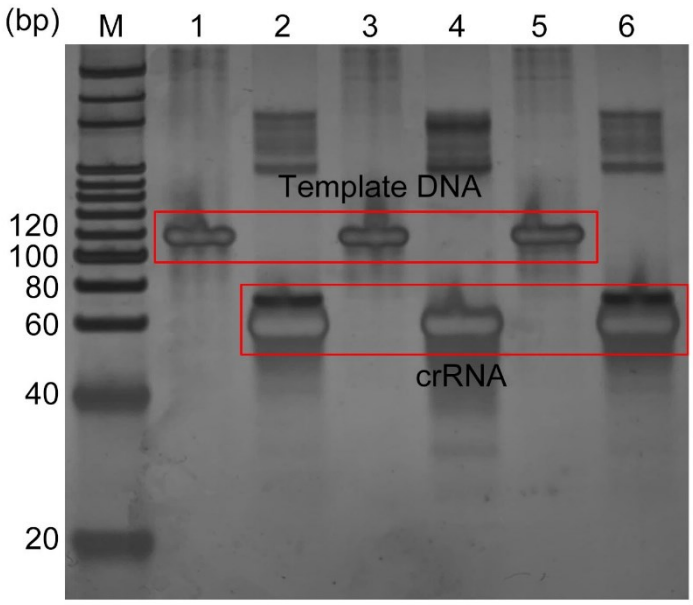

Fig. S5. Gel electrophoresis analysis of the crRNA. Lane M: Marker; lane1, 3, 5: Template DNA for T7 RNA polymerase-mediated *in vitro* transcription; lane 2, 4, 6: crRNA.

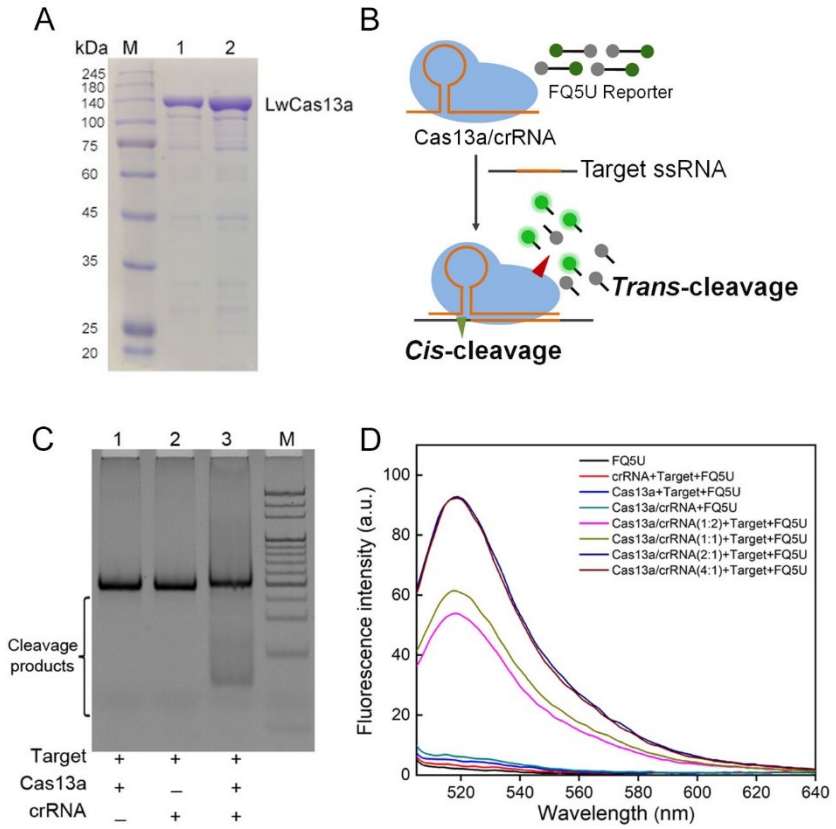

Fig. S6. Analysis of the RNA-guided RNA cleavage activity of LwCas13a. (A) SDS-PAGE (10%) and Coomassie blue staining of purified LwCas13a. Lane M: Marker; lane 1 and 2: purified LwCas13a. The molecular weights of LwCas13a protein is 138.6 kDa. (B) Schematic diagram of tans- and cis-cleavage of Cas13a/crRNA. (C) Gel electrophoresis analysis of the activity of Cas13a/crRNA. Lane M: Marker; lane 1: Cas13a+Target ssRNA; lane 2: crRNA+Target ssRNA; lane 3: Cas13a/crRNA+Target ssRNA. (D) Fluorescence spectra analysis of the activity of LwCas13a/crRNA.

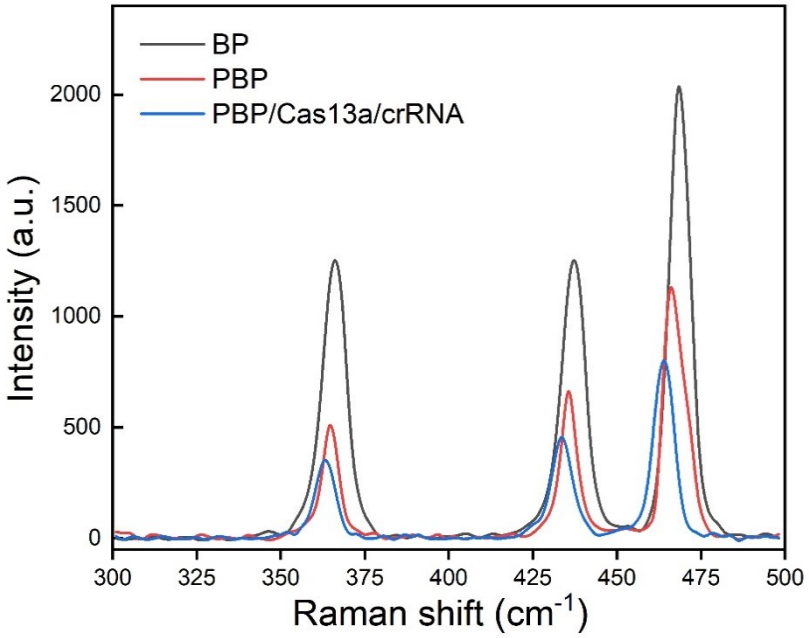

Fig. S7. Raman spectrum of bulk BP, BP nanosheets and PBP/Cas13a/crRNA.

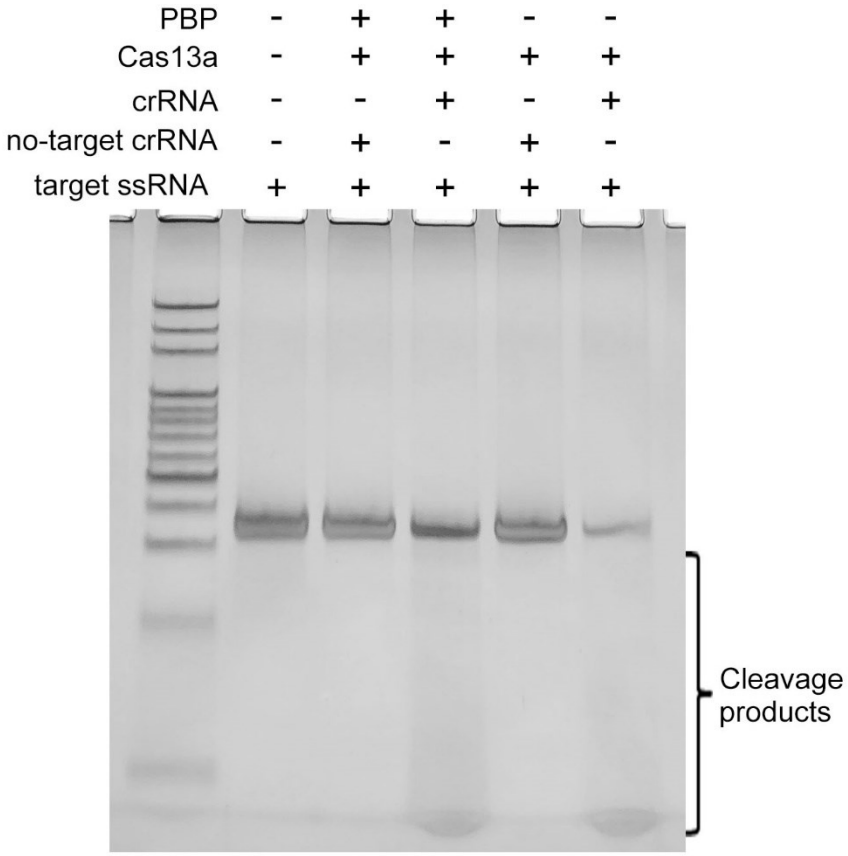

Fig. S8. Gel electrophoresis analysis of the activity of PBP/Cas13a/crRNA and Cas13a/crRNA.

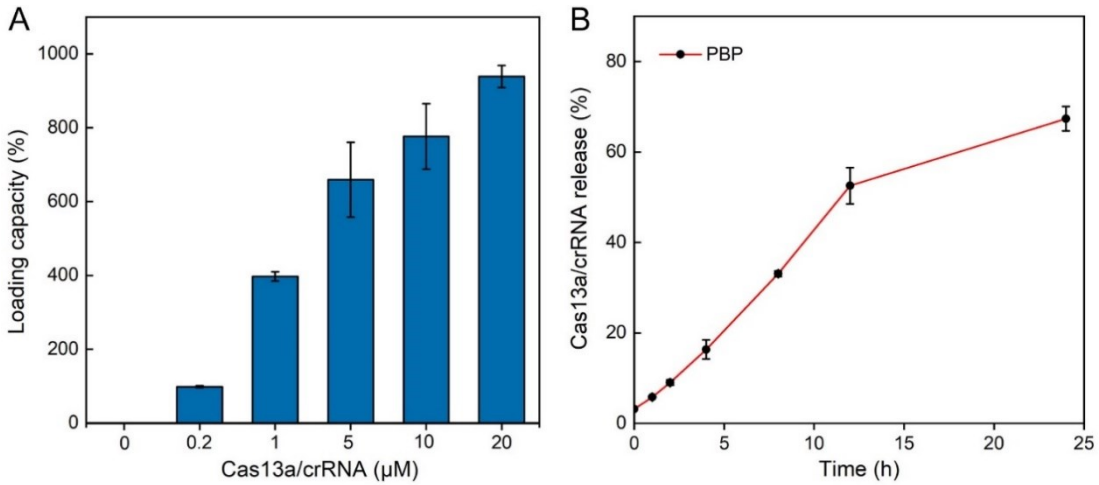

Fig. S9. Loading capacity and release profile of Cas13a/crRNA from PBP/Cas13a/crRNA. (A) Loading capacity of PBP for Cas13a/crRNA at various concentrations. (B) Percentages of active Cas13a/crRNA complexes released from PBP/Cas13a/crRNA at different time points.

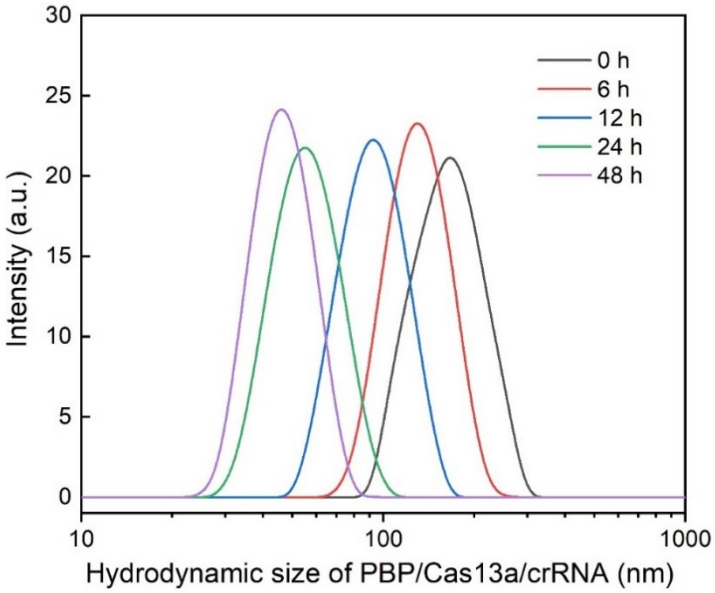

Fig. S10. The time dependent DLS size distribution of as-made PBP/Cas13a/crRNA in DMEM with 10% serum.

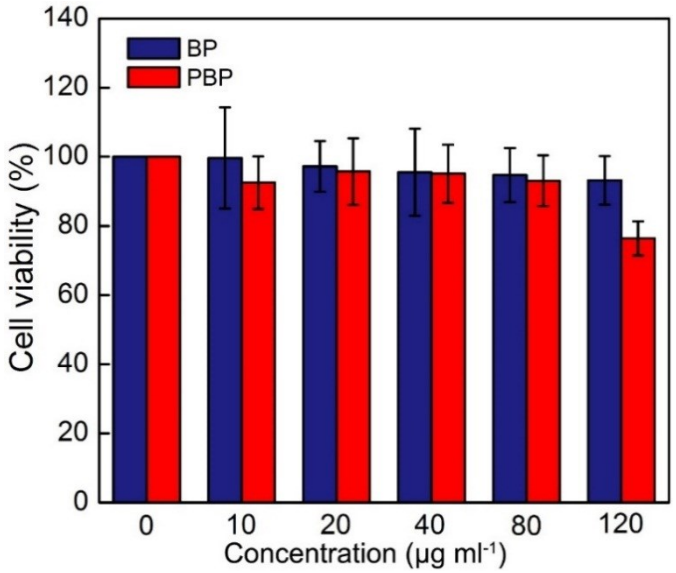

Fig. S11. Relative viability of AGS.EGFP cells after being treated with BP and PBP (0-120 μg/mL) at 48 h. The percentage of cell viability was measured by MTT assay.

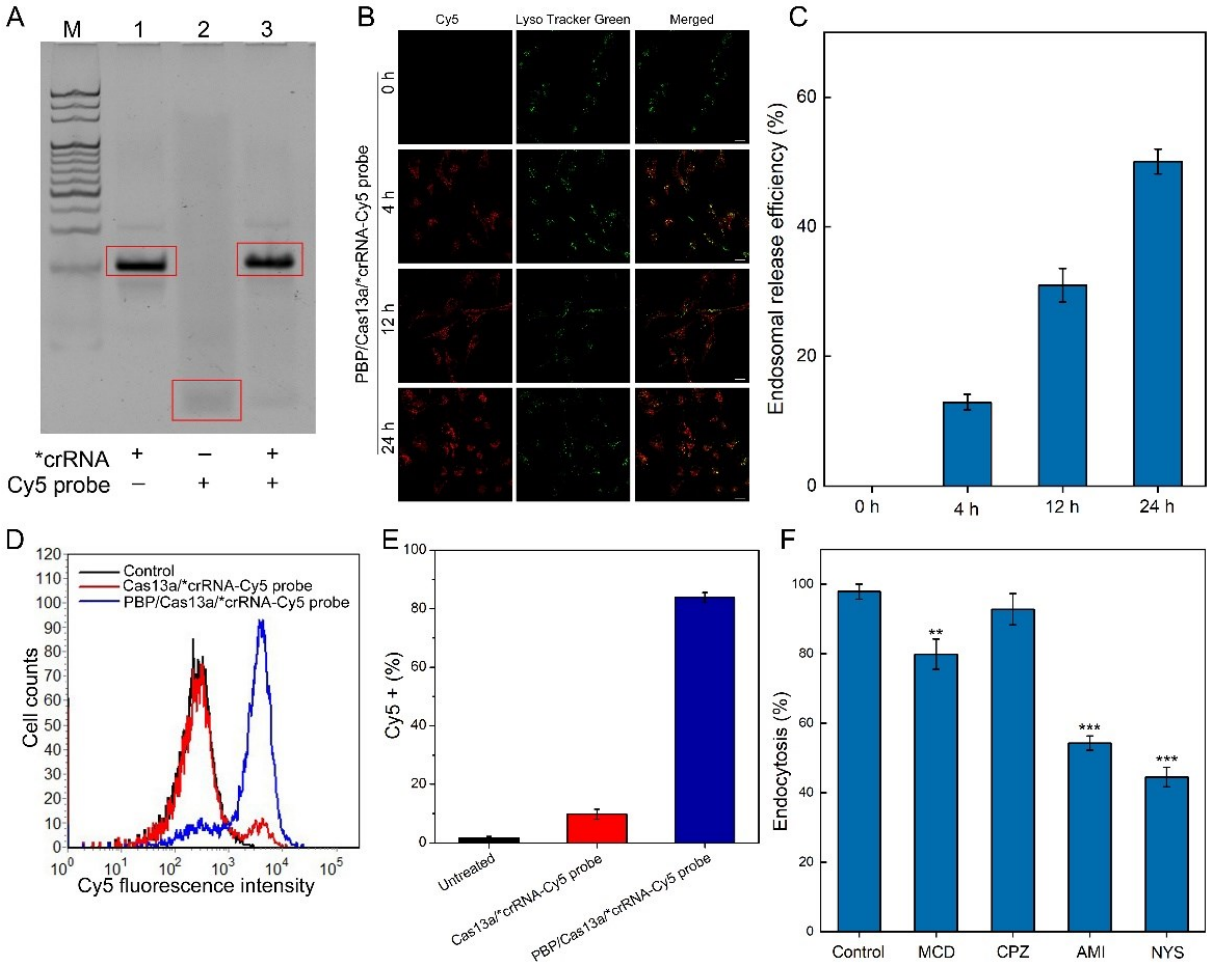

Fig. S12. Cellular uptake. (A) Analysis of hybridization of *crRNA and Cy5 probe by gel electrophoresis. Lane M: Marker; lane 1: *crRNA; lane 2: Cy5 probe; lane 3: *crRNA+Cy5 probe. (B) Confocal laser scanning fluorescence imaging of MDA-MB-231 cells treated with PBP/Cas13a/*crRNA-Cy5 probe for different time intervals. Red and green fluorescence images show Cy5 and Lysotracker Green, respectively. (Scale bar, 20 μm). (C) Quantification of fluorescence intensity from (B). (D, E) Flow cytometry analysis of cell uptake level of nanoparticles loaded with Cy5 probe-labelled crRNAs. (F) Relative PBP/Cas13a/crRNA uptake by MDA-MB-231 cells in the presence of different endocytosis inhibitors.

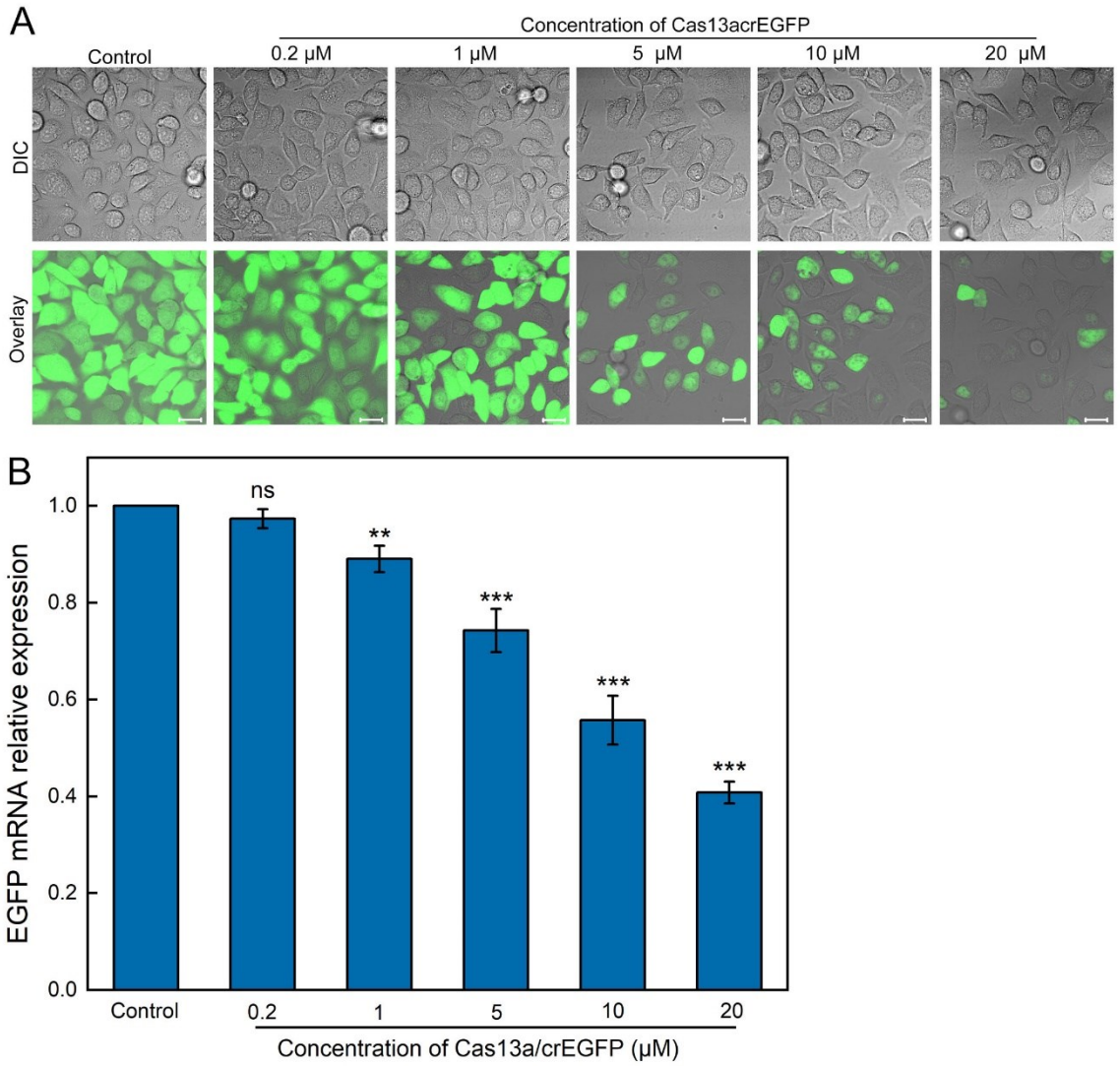

Fig. S13. Time-dependent knockdown of targeted gene by using PBP/Cas13a/crRNA. (A) Confocal microscope images of AGS.EGFP reporter cells treated with different concentrations of PBP/Cas13a/crEGFP. (B) Quantitative RT-PCR analysis of relative levels of EGFP mRNA in AGS.EGFP reporter cells after treating with different concentrations of PBP/Cas13a/crEGFP.

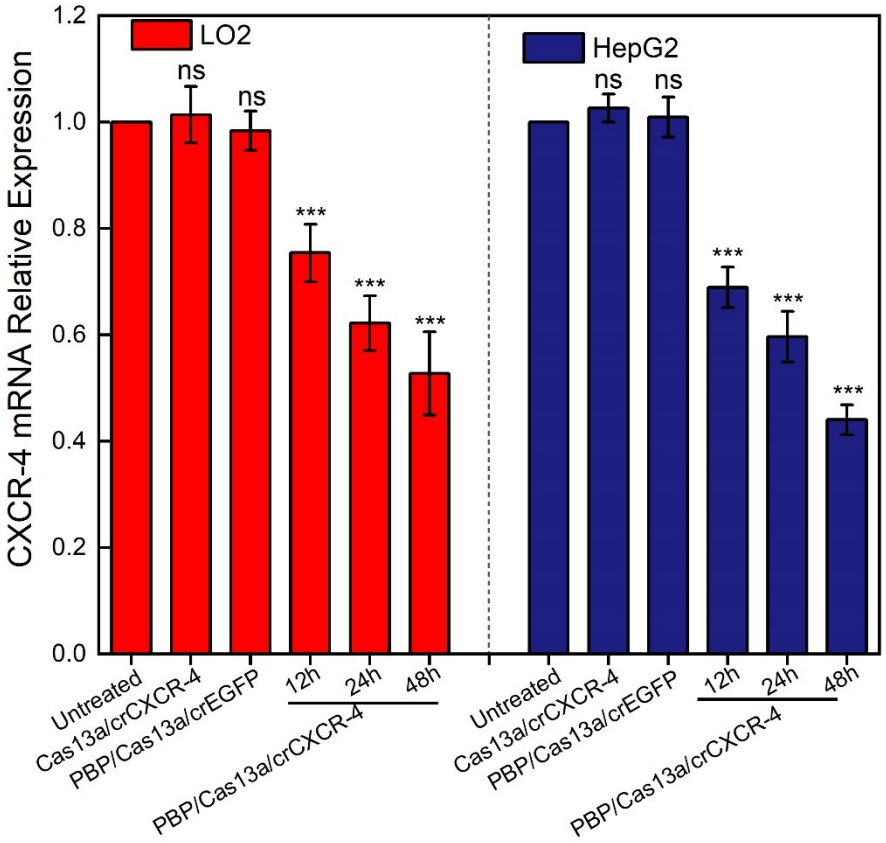

Fig. S14. Relative levels of CXCR-4 mRNA in LO2 and HepG2 cells evaluated by qPCR after being treated by Cas13a/crCXCR-4, PBP/Cas13a/crEGFP, and PBP/Cas13a/crCXCR-4 for 12h, 24h and 48 h, respectively.

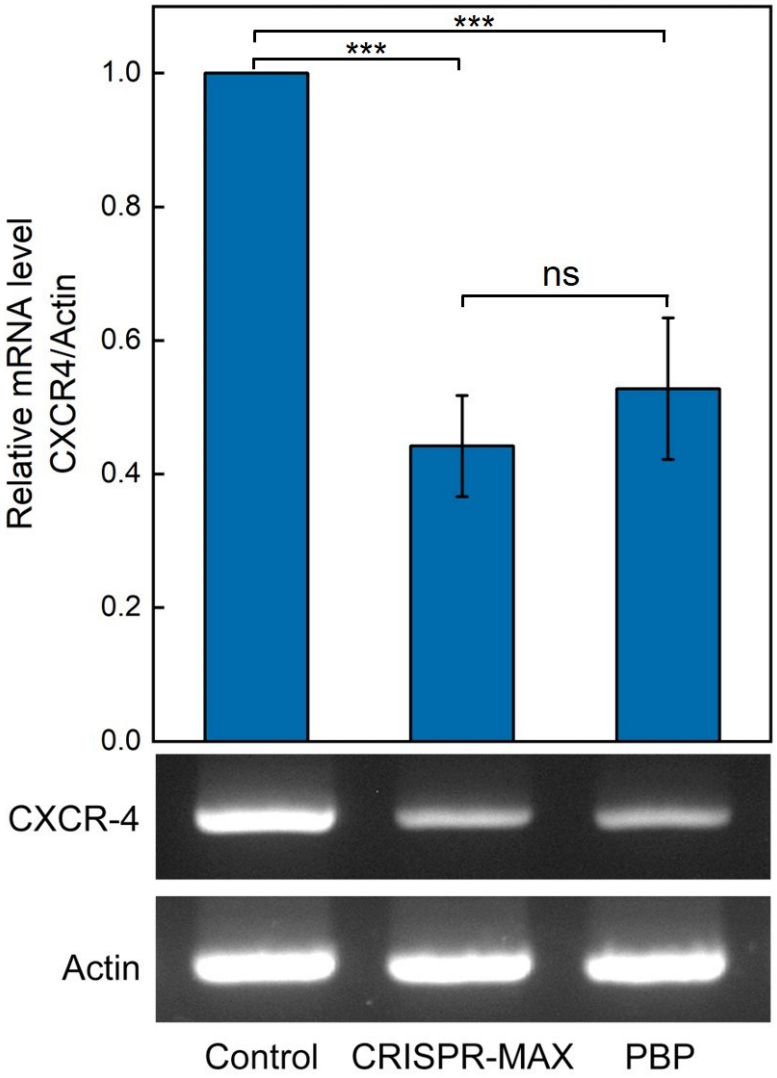

Fig. S15. RT-PCR analysis of Hela cells treated with Cas13a/crRNA via PBP delivery and CRISPR-MAX transfection.

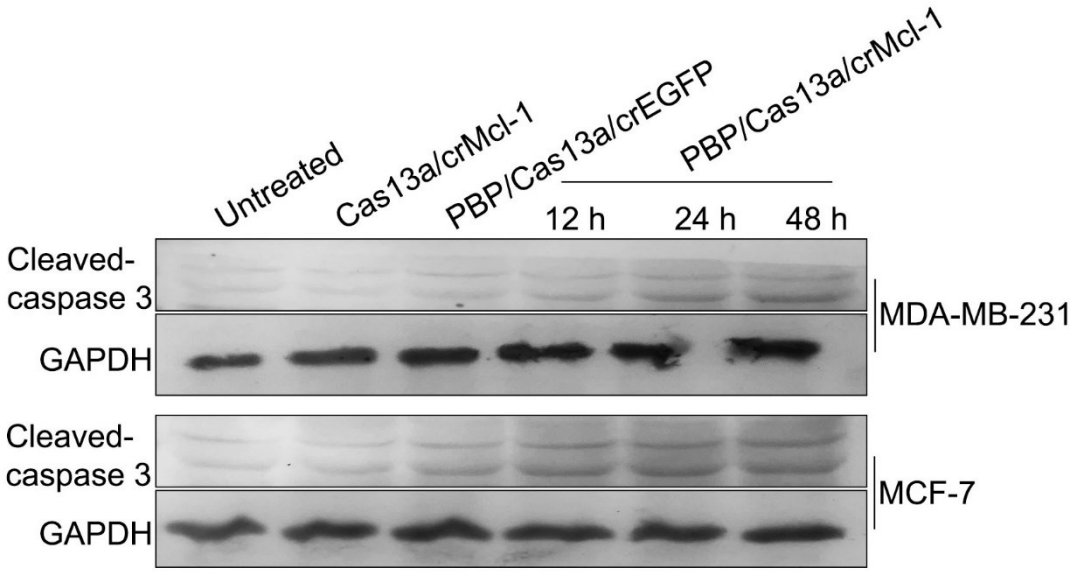

Fig. S16. Immunoblotting analysis of expression levels of Cleaved-caspase 3 in breast cancer cell lines after being treated with Cas13a/crMcl-1, PBP/Cas13a/crEGFP, and PBP/Cas13a/crMcl-1 for 12h, 24h and 48h, respectively. GAPDH served as a loading control.

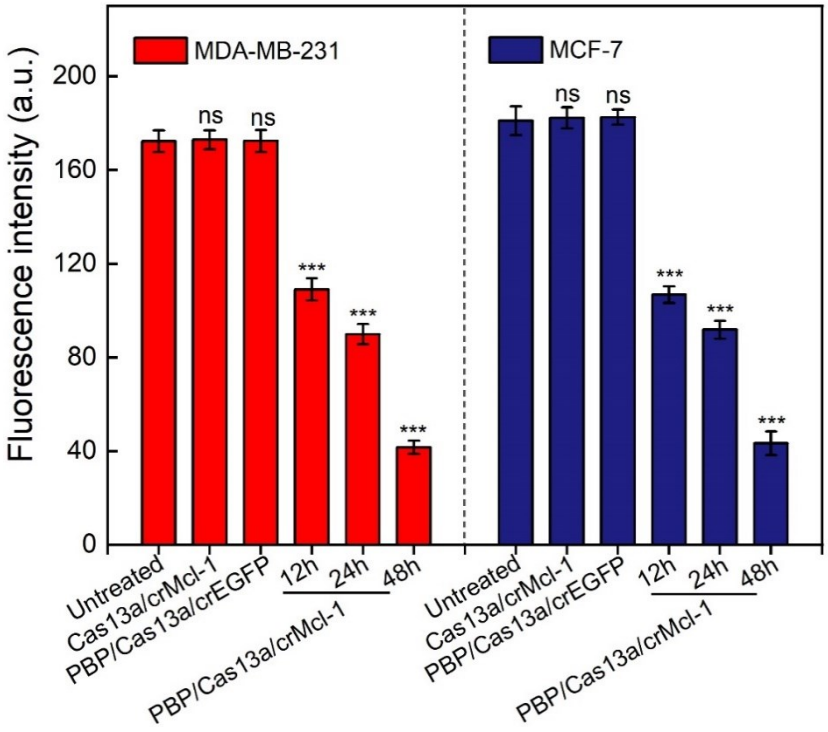

Fig. S17. Statistical analysis of the mean fluorescence intensity in Fig. 4D and 4E.

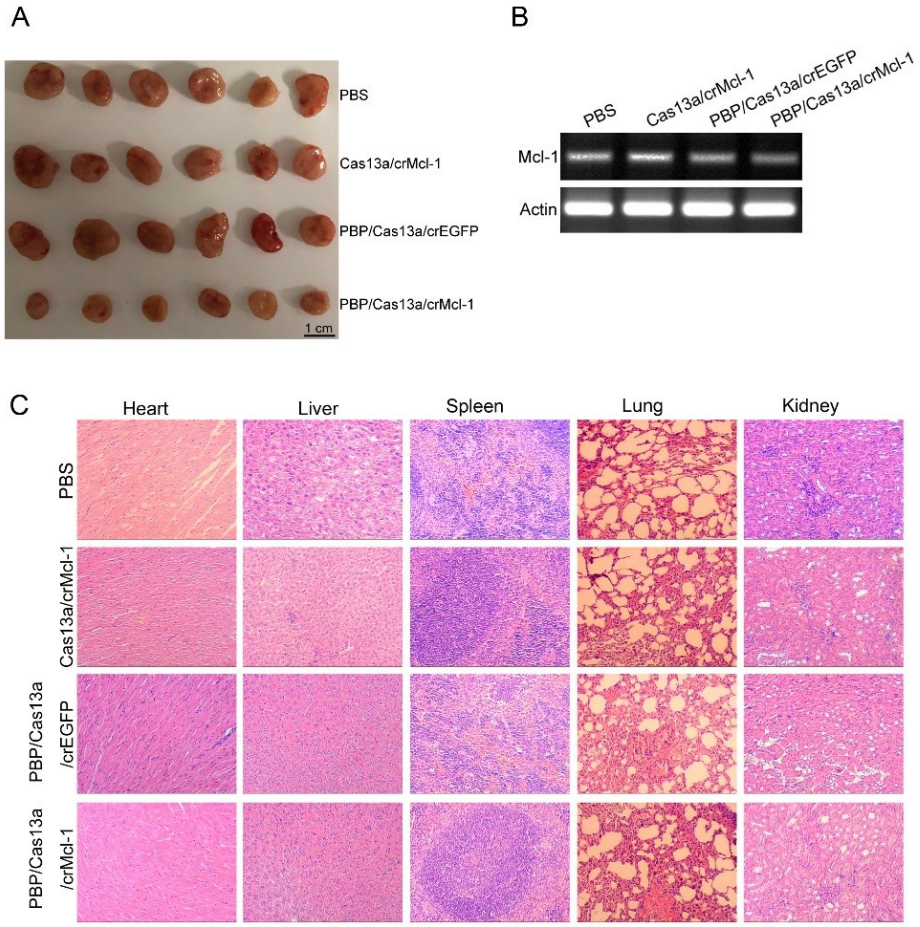

Fig. S18. Delivery of Cas13a/crRNA by PBP for potently in vivo tumor growth suppression (A) Representative images of tumors collected from tumor bearing nude mice following treatments as indicated for 3 weeks. (B) Relative levels of Mcl-1 mRNA in collected tumors from different groups. (C) H&E-stained images of the major organs of the mice treated with PBS, Cas13a/crMcl-1, PBP/Cas13a/crEGFP, and PBP/Cas13a/crMcl-1.

Reference

1. Y. Zhao, Y.-H. Zhang, Z. Zhuge, Y.-H. Tang, J.-W. Tao and Y. Chen, *Analytical Chemistry*, 2018, **90**, 3149-3155.
2. W. Zhou, H. Cui, L. Ying and X.-F. Yu, Angewandte Chemie, 2018, **130**, 10425-10429.
3. K. Saha, S. T. Kim, B. Yan, O. R. Miranda, F. S. Alfonso, D. Shlosman and V. M. Rotello, *Small*, 2013, **9**, 300-305.
4. W. Sun, W. Ji, J. M. Hall, Q. Hu, C. Wang, C. L. Beisel and Z. Gu, *Angewandte Chemie*, 2015, **127**, 12197-12201.
5. X. Zhao, L. Liu, J. Lang, K. Cheng, Y. Wang, X. Li, J. Shi, Y. Wang and G. Nie, *Cancer Letters*, 2018, **431**, 171-181.
6. M. Wang, J. A. Zuris, F. Meng, H. Rees, S. Sun, P. Deng, Y. Han, X. Gao, D. Pouli, Q. Wu, I. Georgakoudi, D. R. Liu and Q. Xu, *Proceedings of the National Academy of Sciences*, 2016, **113**, 2868-2873.
7. R. Mout, M. Ray, G. Yesilbag Tonga, Y.-W. Lee, T. Tay, K. Sasaki and V. M. Rotello, *ACS Nano*, 2017, **11**, 2452-2458.
8. K. Lee, M. Conboy, H. M. Park, F. Jiang, H. J. Kim, M. A. Dewitt, V. A. Mackley, K. Chang, A. Rao, C. Skinner, T. Shobha, M. Mehdipour, H. Liu, W.-c. Huang, F. Lan, N. L. Bray, S. Li, J. E. Corn, K. Kataoka, J. A. Doudna, I. Conboy and N. Murthy, *Nature Biomedical Engineering*, 2017, **1**, 889-901.
9. W. Sun, J. Wang, Q. Hu, X. Zhou, A. Khademhosseini and Z. Gu, *Science Advances*, 2020, **6**, eaba2983.
10. M. Hansen-Bruhn, B. E.-F. de Ávila, M. Beltrán-Gastélum, J. Zhao, D. E. Ramírez-Herrera, P. Angsantikul, K. Vesterager Gothelf, L. Zhang and J. Wang, *Angewandte Chemie International Edition*, 2018, **57**, 2657-2661.
11. L. Zhang, L. Wang, Y. Xie, P. Wang, S. Deng, A. Qin, J. Zhang, X. Yu, W. Zheng and X. Jiang, *Angewandte Chemie International Edition*, 2019, **58**, 12404-12408.
12. S. K. Alsaiari, S. Patil, M. Alyami, K. O. Alamoudi, F. A. Aleisa, J. S. Merzaban, M. Li and N. M. Khashab, Journal of the American Chemical Society, 2018, **140**, 143-146.
13. H. Yue, X. Zhou, M. Cheng and D. Xing, *Nanoscale*, 2018, **10**, 1063-1071.