Supporting Information

Dual Hypoxia-Targeting RNAi Nanomedicine for Precision Cancer Therapy

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MATERIALS AND METHODS

Materials

6-(*t*-Boc-amino)hexyl bromide (BAHB), hydrogen chloride–methanol solution (~1.25 M HCl), methoxy poly(ethylene glycol) (mPEG, M_n = 5000.0 g mol^{-1}), γ-benzyl-L-glutamate (BLG), dichloroacetic acid, hydrogen bromide solution (33 wt.% in acetic acid; HBr/HAc), *N*-hydroxysuccinimide (NHS), *N*,*N*-dimethylformamide (DMF), ethyl acetate, methanol, diethyl ether, dichloromethane, dimethyl sulfoxide (DMSO), rat liver microsomes, sodium iodide (NaI), potassium carbonate (K_2CO_3), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPES; 1 M, pH 7.0 − 7.6), and lithium bromide (LiBr) were supplied by Sigma-Aldrich. 2-Nitroimidazole (NI), triphosgene (TPG), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride (EDC·HCl) were obtained from Tokyo Chemical Industry Co., Ltd. The amino-terminated mPEG (mPEG-NH_2) and γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA) were synthesized according to the previous protocols reported by our team.[1,2] HyClone™ HyPure™ molecular biology grade water (HyPure water) and phosphate-buffered saline (PBS; 1× and 10×) were provided by GE Healthcare Life Sciences.

Dulbecco's modified Eagle's medium (DMEM) was obtained from ATCC. HyClone™ RPMI 1640 and fetal bovine serum (FBS, HyClone™) were supplied by GE Healthcare Life Sciences. Steady-Glo® Luciferase Assay System was provided by Promega Corporation. Annexin V-FITC Apoptosis Detection Kit, Muse® Cell Cycle Assay Kit, and alanine aminotransferase (ALT) Activity Assay Kit were obtained from

Sigma-Aldrich. Penicillin-streptomycin (10,000 U mL^{-1}; Gibco™), Corning™ 0.25% Trypsin (0.1% EDTA in HBSS W/O Calcium, Magnesium and Sodium Bicarbonate), Hoechst 33342 solution (20.0 mM), Alexa Fluor™ 488 Phalloidin (Alexa 488), LysoTracker™ Green DND-26 (LysoTracker DND-26), blood urea nitrogen (BUN) Colorimetric Detection Kit, Pierce™ BCA Protein Assay Kit, and Novex™ WedgeWell™ 10% Tris-Glycine Gels were obtained from Thermo Fisher Scientific. Phenylmethanesulfonyl Fluoride (PMSF), Cell Lysis Buffer (10×), and Protease Inhibitor Cocktail (100×) were supplied by Cell Signaling Technology.

Anti-CDC20 antibody (ab185814) was obtained from Abcam. Anti-cyclin B antibody (A3351) and anti-MCL-1 antibody (Alexa Fluor® 488) (ab197529) were purchased from ABclonal. Goat Anti-Rabbit IgG(H+L) (FITC conjugated) antibody was obtained from Elabscience®. Anti-rabbit IgG, HRP-linked antibody (7074) was purchased from Cell Signaling Technology, Inc. Terminal deoxynucleotidyl transferase FITC-dUTP nick ending labeling (TUNEL) kit was supplied by Roche.

Anti-Luc siRNA (siLuc), DY547-siLuc, Cy5-siLuc, DY677-siLuc, and anti-CDC20 siRNA (siCDC20) were provided by Dharmacon™. The sequences of the siRNA are as follows: siLuc, 5′-CUU ACG CUG AGU ACU UCG AdTdT-3′ (sense) and 5′-UCG AAG UAC UCA GCG UAA GdTdT-3′ (antisense); siCDC20, 5′-CGG AAG ACC UGC CGU UAC AUU-3′ (sense) and 5′-UGU AAC GGC AGG UCU UCC GUU-3′ (antisense). In this study DY547, Cy5, or DY677 were applied in labeling the 5'-end of the sense strand of siLuc.

MCF-7, a human breast cancer cell line, was purchased from ATCC. Luc-HeLa, a stable luciferase-expressing human cervical cancer cell line, was obtained from Alnylam Pharmaceuticals. Axygen® microtubes, cell culture flasks, and Costar® cell culture plates were provided by Corning. 35-mm glass-bottom culture dishes with 14-mm microwell (No. 1.5 coverglass, 0.16−0.19 mm) were purchased from MatTek Corporation.

Data Collection and Bioinformatics Analysis

As shown in Scheme S1, RSEM-normalized RNA-Seq data of 179 donated normal breast tissues retrieved in The Genotype-Tissue Expression (GTEx) database and 1092 breast cancer tissues along with 113 paratumor tissues retrieved in The Cancer Genome Atlas (TCGA) were obtained from UCSC Xena Browser (https://xenabrowser.net/datapages/). RNA-Seq datasets of breast cancer cell lines were obtained from the Cancer Cell Line Encyclopedia (CCLE, https://portals.broadinstitute.org/ccle).[3,4] The prognostic value of CDC20 (Affymetrix ID: 202870_s_at) in breast cancer patients was evaluated by using an online tool, Kaplan-Meier Plotter (http://kmplot.com/analysis/).[5] Version 3.0 of Gene Set Enrichment Analysis (GSEA) was employed to explore CDC20-related hallmarks in breast cancer by using "hallmark.all.v6.1.symbols.gmt" gene set from the Molecular Signatures Database (MSigDB).[6,7]

Bioinformatics Analysis of CDC20 in BRCA samples

RSEM-normalized reads counts of CDC20 in 179 GTEx donated normal tissues, 113 TCGA paratumor tissues and 1092 TCGA breast cancer tissues were extracted, and then one-way analysis of variance (ANOVA) was applied in evaluating the expression difference (Scheme S1).

Bioinformatic Analysis of CDC20 in BRCA Cell Lines

BRCA cell lines were divided into two groups according to the CDC20 expression level, and then the transcriptome data of the two groups was used to perform Gene Set Enrichment Analysis (GSEA) to explore the biological role of CDC20 in breast cancer (Scheme S1).

Characterizations

The proton nuclear magnetic resonance (^{1}H NMR) spectra of synthesized small molecules and polymers were recorded on a 400 MHz Varian NMR spectrometer (Unity Inova 400, Varian, Inc., Palo Alto, USA) using deuterated DMSO (DMSO-d_6) as a solvent and trimethylsilane (TMS) as an internal standard. Number- and weight-average molecular weight (M_n and M_w), and polydispersity index (PDI) = M_w/M_n of polymer were detected by gel permeation chromatography (GPC).

The average hydrodynamic diameter and zeta potential of nanoparticles were measured by dynamic light scattering (DLS; ZetaPALS, Brookhaven Instruments, Holtsville, NY). The morphologies of the nanoparticles were revealed using transmission electron microscopy (TEM) observations on a Tecnai G2 Spirit BioTWIN

(FEI, Hillsboro, OR). A drop of nanoparticle solution at a concentration of 4.0 mg mL^{-1} was dispensed onto a carbon-coated 230 mesh copper grid, followed by a negatively staining with 1% (*W*/*V*) uranyl acetate, and laid to dry in air at room temperature (RT) before measurements.

Synthesis of 6-(2-Nitroimidazole) Hexylamine (NIHA)

NIHA was synthesized through the coupling reaction between NI and BAHB, and the subsequent deprotection of *t*-Boc, according to the previously reported protocol (Scheme S2).[8,9] Typically, NI (565.4 mg, 5.0 mmol) was mixed and dissolved in 5.0 mL of DMF, followed by addition of NaI (89.9 mg, 0.6 mmol) and K_2CO_3 (1.2 g, 9.0 mmol), and then BAHB (1.5 g, 5.5 mmol) dissolved in 5.0 mL of DMF was added in a dropwise manner. The solution was then stirred at 60 °C for 48 h. After filtration, ethyl acetate was used to dissolve the residue that was then washed with 5.0% (*W*/*V*) aqueous NaCl solution thrice. After drying over anhydrous Na_2SO_4 and concentrating *t*-Boc-protected NIHA by a rotary evaporator, the concentrated product was further dissolved in 10.0 mL of methanol and cooled to 0 °C. Afterward, there was an addition of 10.0 mL of 1.25 M HCl in methanol to the solution, and it was further stirred at RT for 24 h. After removing the solvent, the crude solid was recrystallized from ethanol to obtain NIHA.

Confirmation of the chemical structure of NIHA was performed by the well-assigned proton nuclear magnetic resonance (^{1}H NMR) spectra, as shown in Figure S3.

Synthesis of Methoxy Poly(ethylene glycol)-*block*-Poly(L-Glutamic Acid) (mPEG-*b*-PLG)

As shown in Scheme S3, mPEG-*b*-PLG was synthesized through the ring-opening polymerization (ROP) of BLG NCA with mPEG-NH_2 as a macroinitiator, followed by deprotection of the benzyl group, according to the protocol described in our previous work.[10] In brief, traces of water in mPEG-NH_2 (5.0 g, 1.0 mmol) was removed by azeotropic distillation with anhydrous toluene at 120 °C, and the mPEG-NH_2 was dissolved in 50.0 mL of anhydrous DMF. Subsequently, BLG NCA (6.6 g, 25.0 mmol) dissolved in 60.0 mL of anhydrous DMF was added, and the mixture was stirred at 25 °C for 72 h for polymerization. Precipitation of the solution was then performed in over-excessive ethyl ether, and the precipitation was collected, redissolved in dichloromethane, and reprecipitated in ethyl ether. The solid product mPEG-*b*-PBLG was obtained after filtration and drying to constant weight *in vacuo* at RT.

For the deprotection of benzyl group, mPEG-*b*-PBLG was dissolved in dichloroacetic acid at a concentration of 0.1 g mL^{-1} at 25 °C, followed by addition of 15.0 mL of HBr/HAc (33 wt.%) while stirring at 30 °C for 1 h, and then precipitation into over-excessive diethyl ether. After drying *in vacuo*, the precipitation was dissolved in DMF, dialyzed against deionized water for three days, and lyophilized to obtain mPEG-*b*-PLG.

The chemical structures of mPEG-*b*-PBLG and mPEG-*b*-PLG were demonstrated by the well assigned ^1H NMR spectra, as shown in Figure S4.

Synthesis of Methoxy Poly(ethylene glycol)-*block*-Poly(L-glutamide-graft-2-nitroimidazole) (mPEG-*b*-(PLG-*g*-NI))

mPEG-*b*-(PLG-*g*-NI) was synthesized by conjugating NIHA onto the side carboxyl group in mPEG-*b*-PLG with EDC·HCl and NHS as the condensing agent and catalyst, respectively (Scheme S3). Typically, 1.0 g (7.5×10^{-5} mol) of mPEG-*b*-PLG containing 1.94 mmol carboxyl groups, EDC·HCl (1.86 g, 9.7 mmol), and NHS (446.6 mg, 3.88 mmol) were dissolved and stirred for 2 h in DMF. This was followed by the addition of 823.7 mg (3.9 mmol) of NIHA dissolved in DMF, and stirring of the mixture for another two days at 10 °C. The end product was transferred into a dialysis bag (molecular weight cut-off (MWCO) = 7,000 Da), which was dialyzed for one day against DMF followed by additional dialysis against deionized water for another two days before being lyophilized.

The chemical structure of mPEG-*b*-(PLG-*g*-NI) was demonstrated by ^{1}H NMR spectrum, as shown in Figure S5.

Preparation of siRNA-Encapsulated Hypoxia-Responsive Nanoparticle

All of the siRNA-encapsulated hypoxia-responsive nanoparticles (HRNP/siRNA) used in this study were prepared by nanoprecipitation according to the following protocol. The amphiphilic copolymer mPEG-*b*-P(LG-*g*-NI) and the cationic lipid G0-C14 were each dissolved in DMF at concentrations of 10.0 and 5.0 mg mL^{-1}, respectively. 200.0 μL of mPEG-*b*-P(LG-*g*-NI) solution was combined with 50.0 μL of G0-C14 solution and 1.0 nmol of siRNA. This solution was added to 5.0 mL of HyPure

water in a dropwise fashion while under stirring at 1,000 rounds per minute (rpm). Next, to remove the organic solvent and any free compounds, the formed siRNA-loaded nanoparticles were transferred to an Amicon® Ultra-15 centrifugal filter units (MWCO = 100,000 kDa) and centrifuged. After washing with 5.0 mL of HyPure water thrice, the HRNP/siRNA were resuspended in HEPES solution to bring the volume to 500.0 μL.

To determine the siRNA encapsulation efficiency (EE), HRNP/DY547-siRNA was prepared, and 5.0 μL of the HRNP/DY547-siRNA solution was removed and mixed with 100.0 μL of DMSO. The standard sample was generated by combining 5.0 μL of DY547-siRNA solution (1.0 nmol/10.0 μL) with 100.0 μL of DMSO. DY547-siRNA's fluorescence intensity was measured using an Infinite M200 PRO microplate reader (Tecan Group Ltd., Männedorf, Switzerland). EE was calculated by Equation 1.

$$\text{EE}\ (\%) = \frac{\text{Fluoresence Intensity of HRNP/DY547 - siRNA}}{\text{Fluoresence Intensity of Standard Sample}} \times 100\% \qquad 1$$

In Vitro Hypoxia-Response of HRNP

HRNP/siLuc was prepared and 500.0 μL of HRNP/siLuc solution was added to a Float-A-Lyzer® G2 Dialysis Device (MWCO = 100 kDa; Spectrum Laboratories, Inc.) and dialyzed against the 20.0 μM HEPES buffer, which contained 100.0 μM NADPH, and 2.0 mg mL^{-1} rat liver microsomes, and gently shaken at 100 rpm, 37 °C. The hypoxic conditions were created and maintained by continually bubbling the nitrogen gas into the medium during the entire period of the experiment. For the normoxic control, only 100.0 μM NADPH was contained in the HEPES buffer without degassing.

At predetermined time points (*i.e.*, 0, 1, 2, 4, 8, 12, and 24 h), the HRNP/siLuc solution was taken out from the dialysis device, and the size of the HRNP/siLuc was determined using DLS.

Cell Culture

Luc-HeLa cells were cultured in RPMI 1640 supplemented with 10% (*V*/*V*) FBS. MCF-7 cells were cultured in DMEM supplemented with 10% (*V*/*V*) FBS. For normoxic conditions, the cells were grown at 37 °C in a humidified atmosphere supplemented with 21% oxygen (O_2) and 5% carbon dioxide (CO_2). In contrast, the hypoxic conditions were generated by culturing the cells in a hypoxic chamber supplemented with 1% O_2 in a 5% CO_2 humidified atmosphere at 37 °C.

***In Vitro* Luciferase Silencing**

At a density of 5,000 cells per well, Luc-HeLa cells were seeded in 96-well plates and incubated for 24 h at a temperature of 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. Subsequently, the cells were treated with HRNP/siLuc with the siLuc concentration at 0, 1, 5, 10, 20, or 40 nM for 24 h under normoxic or hypoxic conditions, followed by a change of media and further incubation for 48 h. After that, the cytotoxicity of HRNP/siLuc was measured through an Alamar Blue assay in accordance with the manufacturer's protocol. The level of luciferase expression in HeLa cells was measured using the Steady-Glo® Luciferase Assay

System and a microplate reader in accordance with the manufacturer's protocol. The average value of six independent experiments was calculated.

Cell Uptake Assays

The cell internalization of HRNP/siRNA was determined by both flow cytometry (FCM) and confocal laser scanning microscope (CLSM).

For the FCM test, MCF-7 cells were seeded at a density of 100,000 cells per well in 6-well plates and incubated for 24 h at 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. After changing the media, cells were then treated with HRNP/Cy5-siRNA and incubated for 4 h under normoxic or hypoxic conditions. After incubation, the media was removed, and PBS was used to wash the cells followed by digestion with trypsin. Cells were washed an additional time with PBS, filtered, and collected for flow cytometry quantitative analysis.

For CLSM detection, MCF-7 cells were seeded in glass-bottom culture dishes at a density of 40,000 cells per dish and incubated for 24 h at a temperature of 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. After changing the media, cells were treated with HRNP/Cy5-siRNA and incubated for 4 h under normoxic or hypoxic conditions. The media was then discarded, and cells were washed thrice with PBS followed by staining of the nuclei and endosomes with Hoechst 33342 and LysoTracker DND-26, respectively. Cells were detected on an Olympus FV1000 CLSM (Olympus America, Center Valley, PA).

Western Blot Tests

MCF-7 cells were seeded in 6-well plates (100,000 cells per well) and incubated for 24 h at a temperature of 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. After changing the media, the cells were treated with HRNP/siCDC20 and incubated for 24 h under normoxic or hypoxic conditions. Afterward, the media was replaced with fresh media for an additional 48 h incubation. Then, cells were collected, and proteins were then extracted using lysis buffer supplemented with protease inhibitor cocktail and 1.0 mM phenylmethanesulfonyl fluoride (PMSF). Before running the SDS-PAGE gel, the protein was confirmed with a Pierce™ BCA Protein Assay Kit in accordance with the manufacturer's protocol. 20.0 μg of protein for each sample was added to 10% Tris-Glycine Gels and separated by gel electrophoresis followed by transfer of the protein to a polyvinylidene difluoride membrane. Subsequently, the blots were then blocked with 3% (*W*/*V*) bovine serum albumin (BSA) in Tris-buffered saline containing 0.2%−0.4% Tween-20 (TBST) and incubated in the dark overnight at 4 °C with a mixture of anti-CDC20 and anti-β-actin antibodies. After washing thrice for 15 min each with TBST, the secondary antibody (anti-rabbit IgG HRP-linked antibody) was applied, followed by incubation at RT for 1 h in the dark. After incubation with the secondary antibody, enhanced chemiluminescence (ECL) detection system was added followed by the detection of CDC-20 expression through the Syngene PXi imaging system.

***In Vitro* Cell Proliferation**

MCF-7 cells were seeded at a density of 20,000 cells per well in 6-well plates and incubated for 24 h at a temperature of 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. After that, the initial cell viability was determined through an Alamar Blue assay on day 0 in accordance with the manufacturer's protocol. After removing the Alamar Blue and adding fresh media, cells were treated with either siCDC20, HRNP/siCtrl, or HRNP/siCDC20 for 24 h under normoxic or hypoxic conditions. Then, the media was changed for further incubation, and additional Alamar Blue assays were performed in accordance with the manufacturer's protocols at predetermined intervals on day 0, 2, 4, 6, and 8.

Cell Cycle Analysis

MCF-7 cells were seeded in 6-well plates (100,000 cells per well) and incubated for 24 h at 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. After changing the serum-free media for cell cycle synchronization, the cells were treated with HRNP/siCDC20 and incubated for 24 h under normoxic or hypoxic conditions. Afterward, the cells were harvested and fixed in 70% cold ethanol overnight at 4 °C. This was followed by two washes with cold PBS and staining using the Muse® Cell Cycle Assay Kit (Sigma) according to the manufacturer's protocol. A DXP11 Flow Cytometry Analyzer conducted the cell cycle analysis.

Apoptosis Analysis

MCF-7 cells were seeded at a density of 100,000 cells per well in 6-well plates and incubated for 24 h at a temperature of 37 °C in a 5% CO_2 humidified atmosphere to allow attachment of the cells. After changing the media, the cells were treated with HRNP/siCDC20 and incubated for 24 h under normoxic or hypoxic conditions. Afterward, the media was substituted with fresh media for another 72 h of incubation. The cells were then collected, and apoptosis analysis was conducted using Annexin V-FITC Apoptosis Detection Kit on a DXP11 Flow Cytometry Analyzer in accordance with the manufacturer's protocol.

Animal Procedures

BALB/c nude mice (female, 6−8 weeks) were acquired from Charles River Laboratory and Beijing HFK Bioscience Co Ltd. All animal studies were performed under the animal protocol approved by the Institutional Animal Care and Use Committees of Harvard Medical School and Jilin University.

Pharmacokinetics Study

Healthy female BALB/c mice were separated into two groups (n = 3) at random and intravenously injected either DY677-siRNA or HRNP/DY677-siRNA at a siRNA dose of 1.0 nmol per mouse through the tail vein. At set time intervals, 20.0 μL of blood was extracted using a tube containing heparin from the orbital veil, and the blood's fluorescence intensity of DY677-siRNA was measured by a microplate reader (Tecan).

Biodistribution Study

BALB/c nude female mice bearing MCF-7 tumor were separated into three groups at random and received an intravenous injection of either PBS, DY677-siRNA, or HRNP/DY677-siRNA at a siRNA dose of 1.0 nmol per mouse through the tail vein. After 24 h, the mice were sacrificed, and the major organs were harvested and imaged under a Syngene PXi imaging System (Synoptics Ltd).

Tumor Growth Inhibition

MCF-7 tumor-bearing BALB/c nude mouse models were constructed by subcutaneous injections of MCF-7 cells at a cell density of 3.0×10^6, and when the tumor volume reached about 50 or 120 mm^3. The mice were assigned into four groups (n = 5 per group) at random and were administered either PBS, siCDC20, HRNP/siCtrl, or HRNP/siCDC20 at the siRNA dose of 1.0 nmol per mouse on days 1, 4, 7, 10, or 13. The volume of the tumor and body weight were monitored every other day until day 19.

Hematological Examination

On day 19, the blood was obtained from the mice through retro-orbital blood collection, and the serum was separated for hematologic examination. The alanine aminotransferase (ALT) and blood urea nitrogen (BUN) levels in serum were determined by ALT Activity Assay Kit (Sigma-Aldrich) and BUN Colorimetric

Detection Kit (Thermo Fisher Scientific), respectively, and according to the manufacturer's protocols.

Histological Analysis

The mice were sacrificed on day 19, and major organs were harvested, fixed with 4% (*W*/*V*) paraformaldehyde, then embedded in paraffin. Afterward, staining of the tissue sections was done with hematoxylin and eosin (H&E).

For immunofluorescence assessments of tumors, the tissue sections were incubated with the FITC-linked primary antibodies of CDC20, cyclin B, and Mcl-1. DAPI was also used to stain the nuclei. For TUNEL assays, the tumor tissue sections were implemented with a commercial kit in accordance with the manufacturer's instructions. The fluorescence images of the tumor tissue sections were taken on a CLSM.

Statistical Analysis

Statistical significance among experimental groups was calculated through a one-way analysis of variance with the statistical software, SPSS 17.0 (SPSS Inc., Chicago, USA). When $^{*}P < 0.05$, it was considered statistically significant, and when $^{**}P < 0.01$ or $^{***}P < 0.001$, it was considered highly significant.

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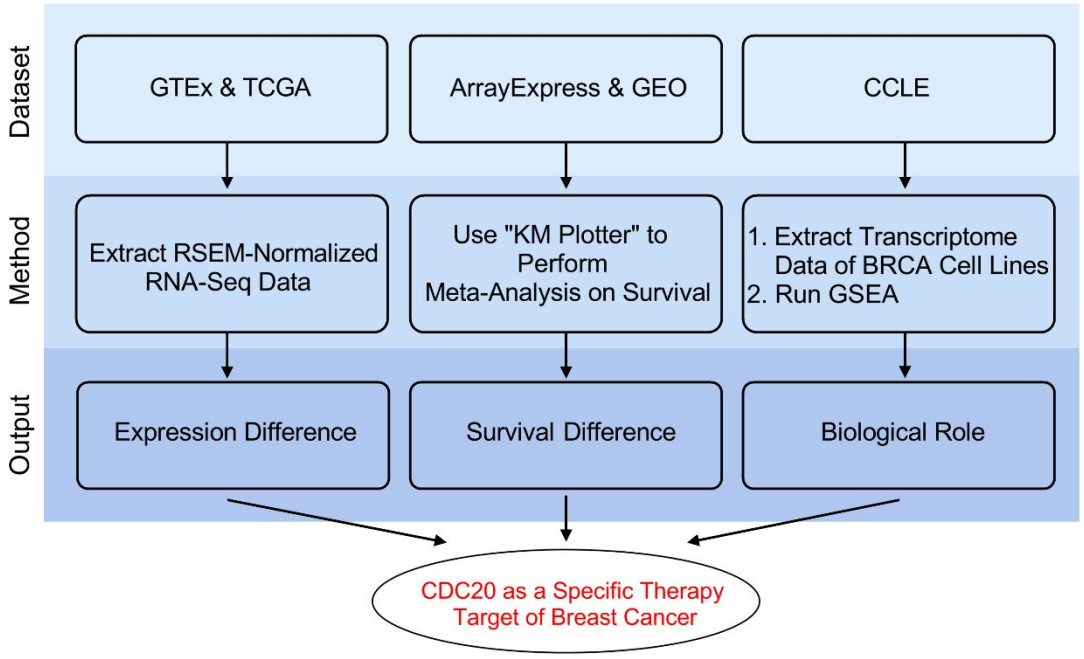
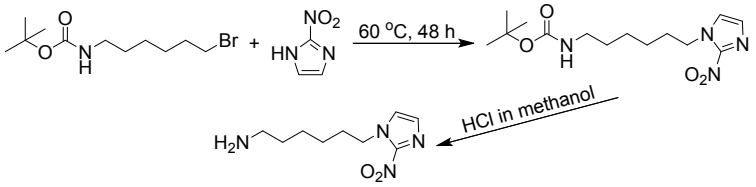
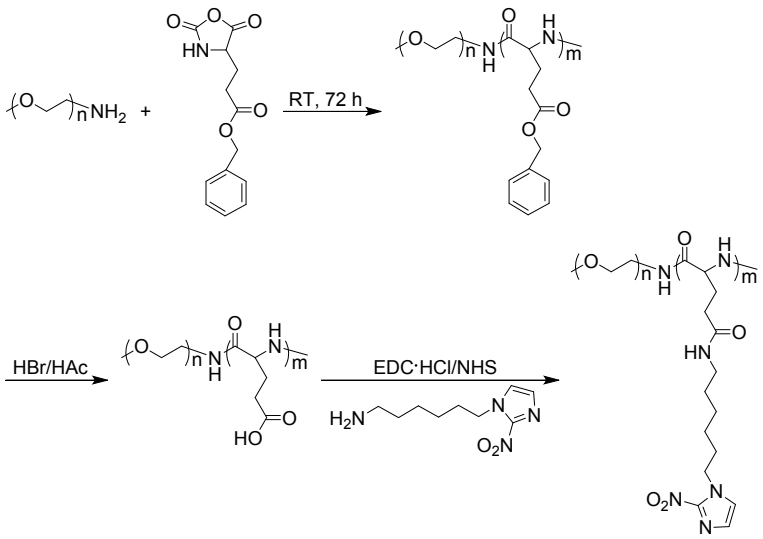


Scheme S1. Flow diagram to identify CDC20 as a promising therapeutic target in breast cancer. Evidence from clinical significance and biological function.



Scheme S2. Synthesis of 6-(2-nitroimidazole)hexylamine (NIHA).



Scheme S3. Synthesis proposal of mPEG-*b*-P(LG-*g*-NIHA).

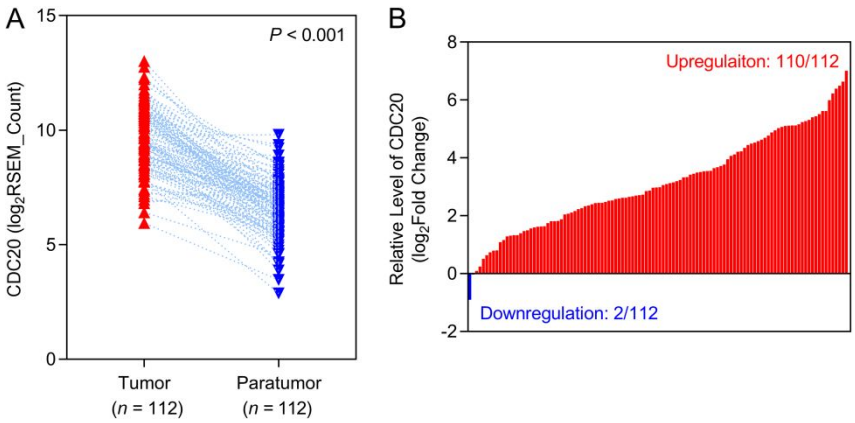


Figure S1. CDC20 level in BRCA tissues. (A) Significantly upregulated CDC20 in tumor tissues compared to those of paratumor tissues. ***P < 0.001. (B) Widely upregulated CDC20 in 110/112 tumor tissues of patients.

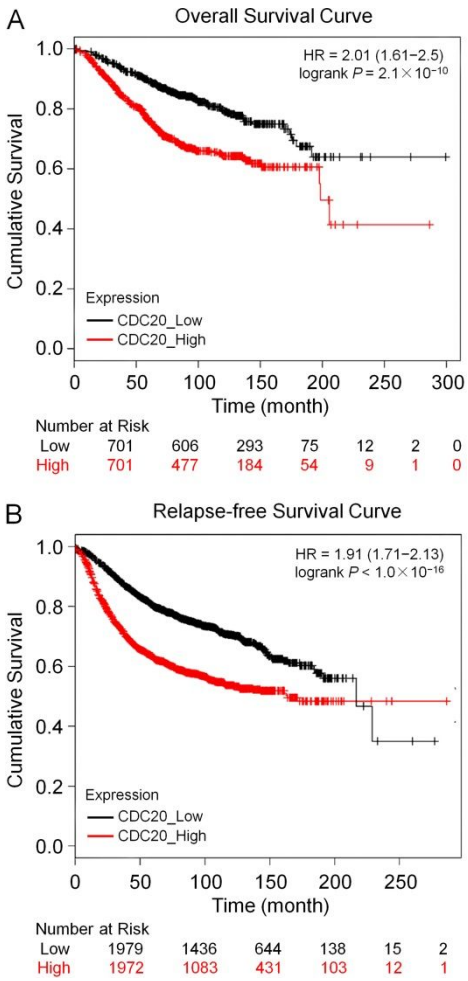


Figure S2. Prognostic values of CDC20 for OS and RFS in total patients by Kaplan-Meier Plotter.

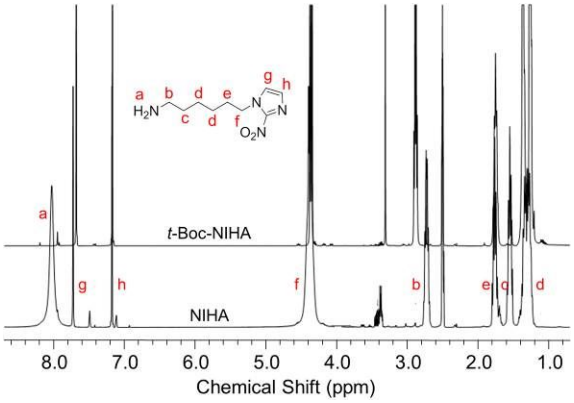


Figure S3. ^{1}H NMR spectra of *t*-Boc-NIHA and NIHA in DMSO-d_6.

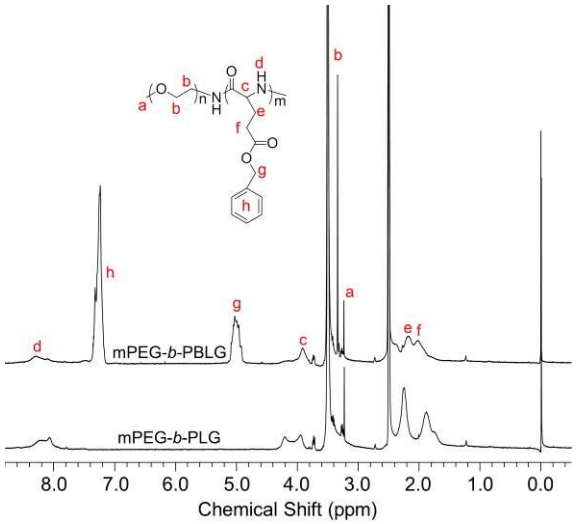


Figure S4. ^{1}H NMR spectra of mPEG-*b*-PBLG and mPEG-*b*-PLG in DMSO-d_6.

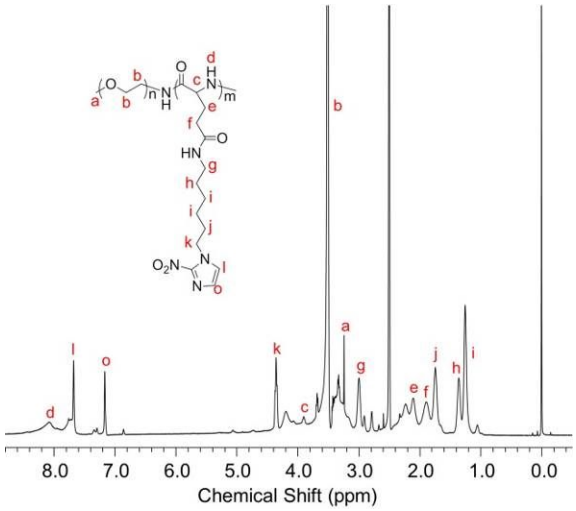


Figure S5. ^{1}H NMR spectrum of mPEG-*b*-P(LG-*g*-NI) in DMSO-d_6.

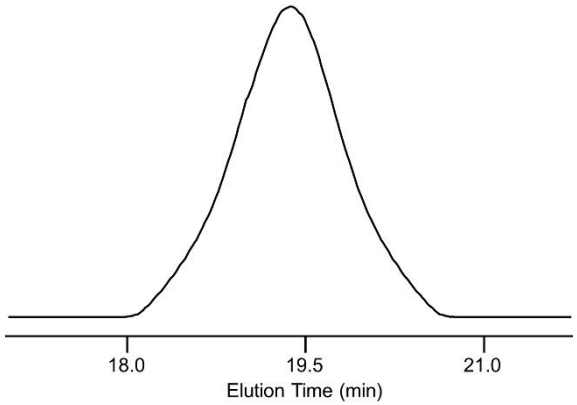


Figure S6. GPC of mPEG-*b*-P(LG-*g*-NI).

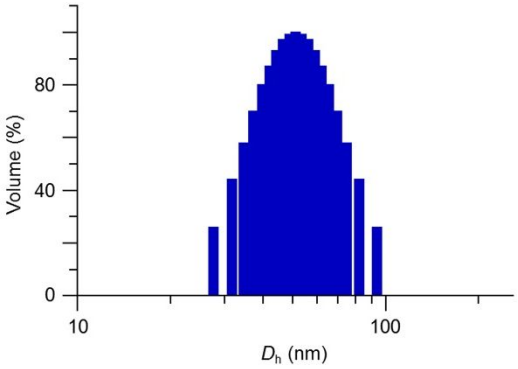


Figure S7. D_h of HRNP/siRNA.

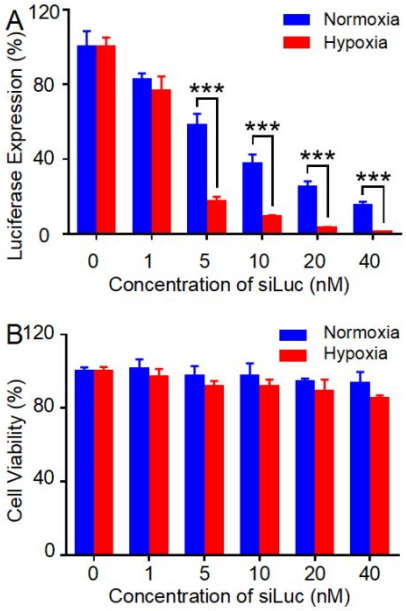


Figure S8. Luciferase silence and cytotoxicity of HRNP/siLuc in Luc-Hela cells after 24 h incubation at different concentrations under normoxic or hypoxic conditions. The statistical data are presented as mean ± SD (standard deviation; $n = 3$; ***$P < 0.001$).

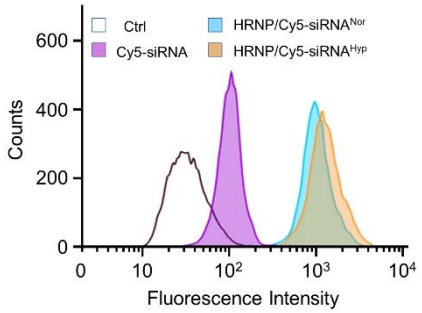


Figure S9. FCM profiles of MCF-7 cells incubated with Cy5-siRNA or HRNP/Cy5-siRNA at 37 °C for 4 h under normoxic *vs.* hypoxic conditions.

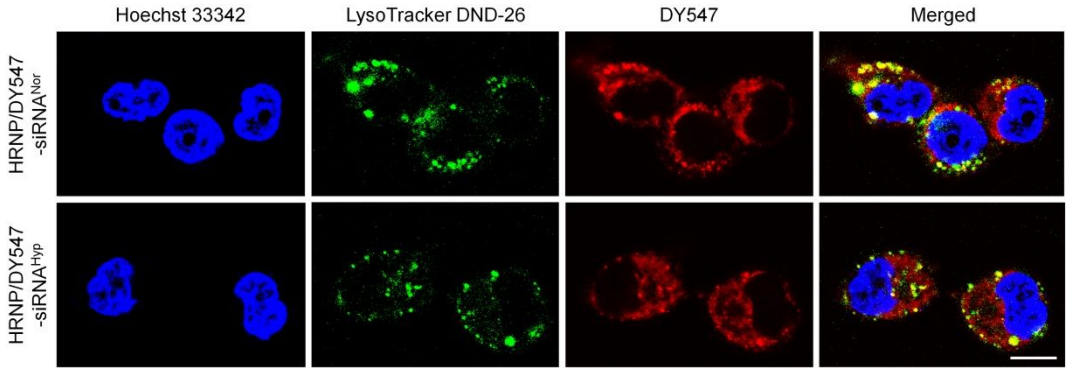


Figure S10. CLSM images of endosomal escape in MCF-7 cells incubated with HRNP/DY547-siRNA at 37 °C for 4 h under normoxic *vs.* hypoxic conditions. Hoechst 33342 and LysoTracker DND-26 are used to stain nuclei (blue) and endosomes (green), respectively. Scale bar is 10 μm.

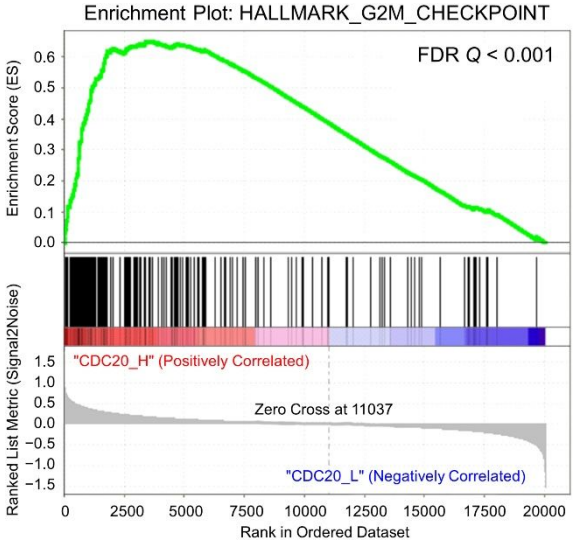


Figure S11. GSEA output about CDC20 in breast cancer cell lines.

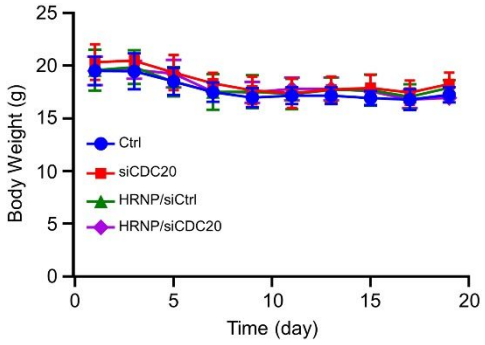


Figure S12. Body weight changes of mice bearing MCF-7 tumors with initial volume at 55 mm^3 treated with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20.

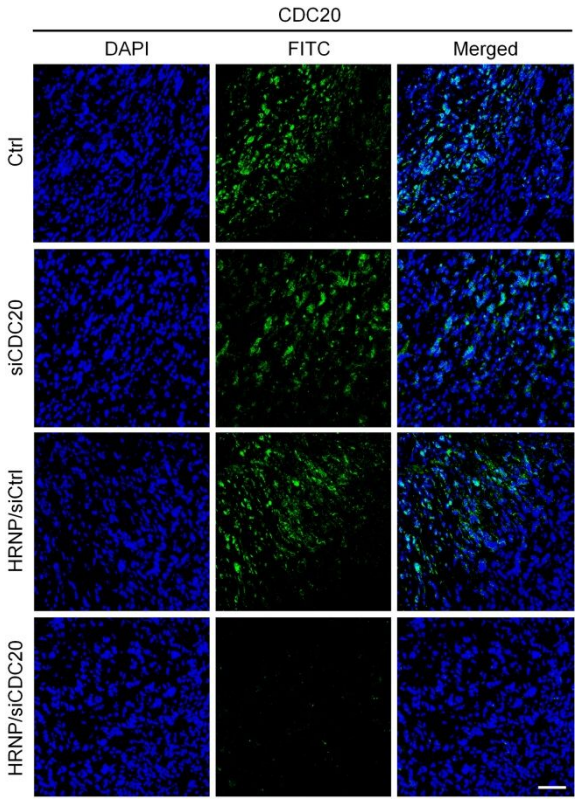


Figure S13. Immunofluorescence staining of CDC20 expression in MCF-7 tumor tissues of xenograft mice after treatment with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20. Scale bar is 50 μm.

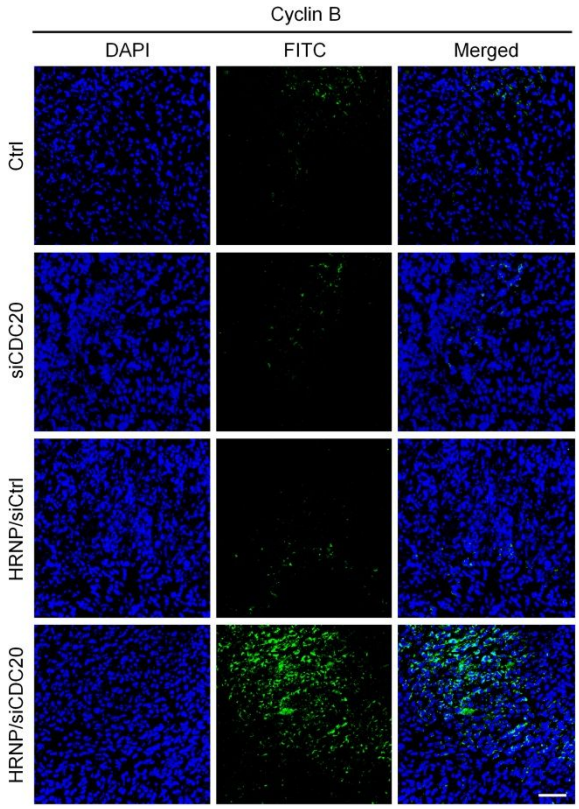


Figure S14. Immunofluorescence staining of cyclin B expression in MCF-7 tumor tissues of xenograft mice after treatment with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20. Scale bar is 50 μm.

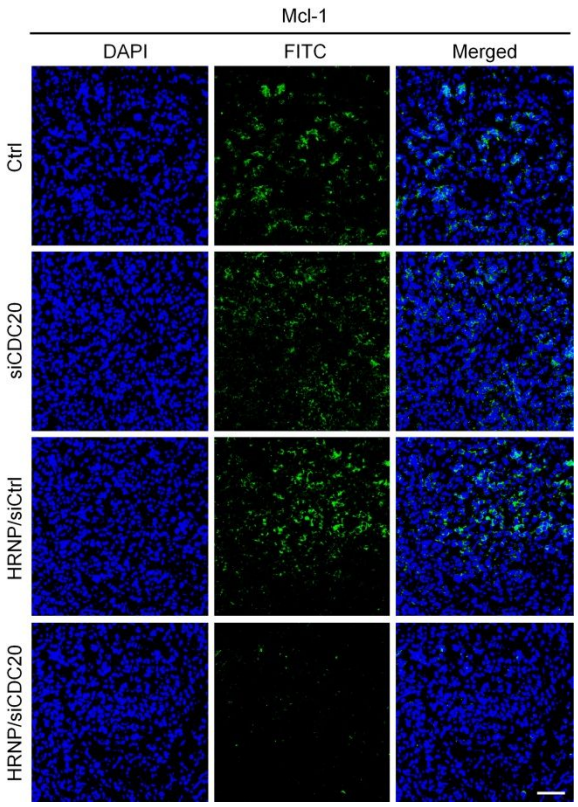


Figure S15. Immunofluorescence staining of Mcl-1 expression in MCF-7 tumor tissues of xenograft mice after treatment with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20. Scale bar is 50 μm.

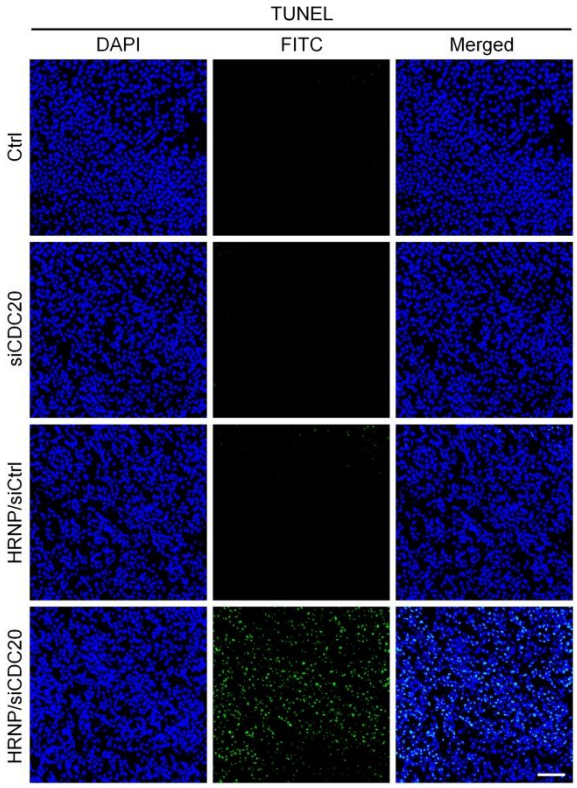


Figure S16. TUNEL staining of MCF-7 tumor tissue of xenograft mice after treatment with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20. Scale bar is 50 μm.

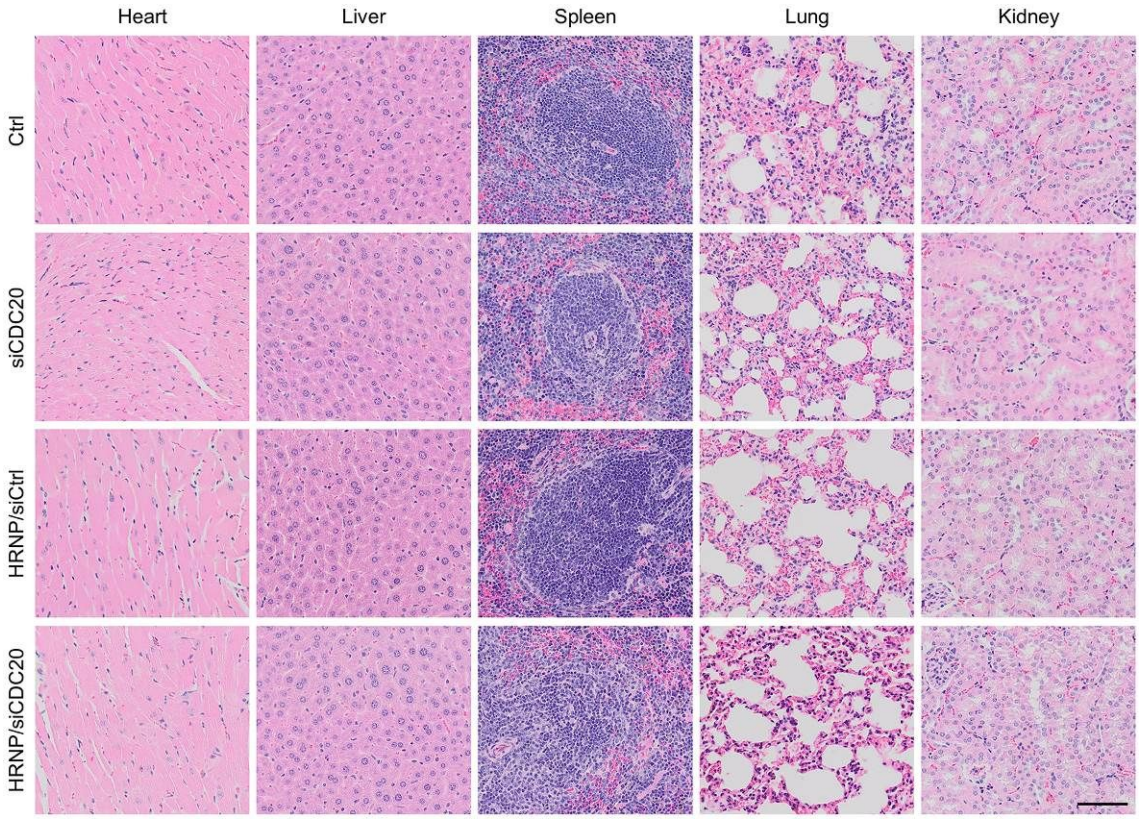


Figure S17. H&E staining of major organs after treatment with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20. The scale bar is 100 μm.

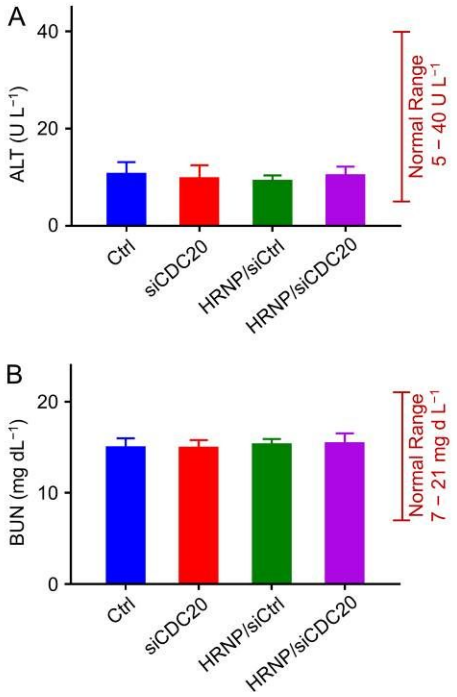


Figure S18. ALT and BUN levels in serum from xenograft mice after treatment with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20.

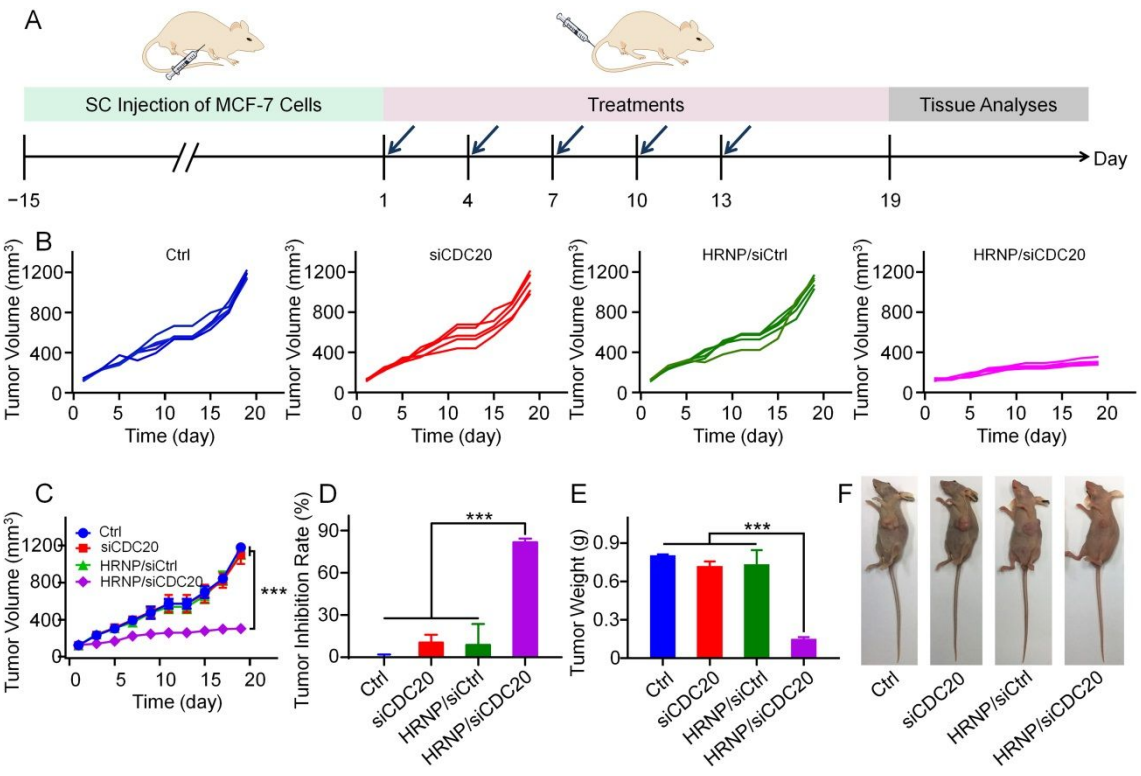


Figure S19. *In vivo* antitumor efficacy and gene silencing of HRNP toward MCF-7 tumor-bearing BALB/c nude mice. (A) Timeline of construction of MCF-7 tumor-bearing BALB/c nude mouse model and treatment strategy. (B) Tumor growth curves for individual mice, (C) average tumor volume growth curves, (D) tumor inhibition rate in each treatment group, and (E) weight of tumor in each treatment group. (F) Representative image of MCF-7 tumor-bearing nude mouse in each group after a 19-day evaluation. The statistical data are presented as mean ± SD (n = 5; ***P < 0.001).

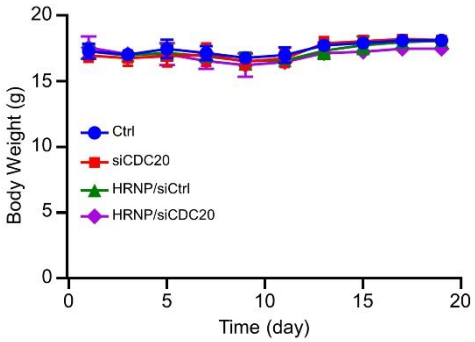


Figure S20. Body weight changes of mice bearing MCF-7 tumors with initial tumor volume at about 120 mm^3 treated with Ctrl, siCDC20, HRNP/siCtrl, or HRNP/siCDC20.