## Materials and methods

### Materials

Minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin and L-glutamine, lysotracker green, and Lipofectamine 3000 (Lipo3000) transfection reagent were purchased from Thermo Fisher Scientific (Waltham, MA, USA). $Zn(NO_3)_2 \cdot 6H_2O$, 2-methylimidazole, methanol, and dimethyl sulfoxide (DMSO) were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Diethylpyrocarbonate (DEPC)-treated and sterile filtered water, Tris acetate-ethylenediaminetetraacetic acid (EDTA) buffer (TAE), Human *ACTB* endogenous reference genes primers, and TRNzol-$A^+$ were purchased from Sangon Biotech (Shanghai, China). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel quick preparation kit, paraformaldehyde, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, agarose, phosphate-buffered saline (PBS), and Hoechst 33258 were purchased from Beyotime Biotechnology (Shanghai, China). KOD DNA polymerase was purchased from TOYOBO Life Science (Shanghai, China). Methylthiazolyldiphenyl-tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). MiniBEST DNA Fragment Purification Kit, in vitro Transcription T7 Kit (for small interfering RNA (siRNA) synthesis), PrimeScript™ RT reagent Kit (Perfect Real Time), and TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) were purchased from TaKaRa Bio (Dalian, China). RNA lording buffer was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China).

### Secondary structure prediction

DNAzyme (DZ)-RNA secondary structure interactions were predicted using RNAstructure software (version 6.4, Mathews Lab) to calculate the hybridization free energy and optimal secondary structure. The secondary structure of single-stranded or partially double-stranded DNA molecules was determined using MXfold2 (http://ws.sato-lab.org/mxfold2).

### Gel electrophoresis analysis

The RNA substrate was transcribed in vitro using a T7 transcription kit and then purified with TRIzol. Next, the RNA substrate (1 μmol/L) was incubated with different DNAzymes (1 μmol/L) or pH-responsive DNAzyme@ZIF-8 in 1× Tri-HCl buffer for 2 h at 37 °C. The samples were then mixed with loading buffer and loaded onto a 2% (0.02 g/mL) agarose gel containing Gel Red. Electrophoresis was performed at 120 V in 1× TAE buffer (0.04 mol/L Tris-acetate, 1 mmol/L EDTA, pH 8.0) for 30 min, and the gel was imaged by a camera.

**Cell culture**

U87MG, HEK293T, and MCF-7 cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). U87MG cells were maintained in MEM, while HEK293T and MCF-7 cells were cultured in DMEM, both supplemented with 10% (volume fraction) FBS and 1% (volume fraction) penicillin/streptomycin.

**RT-qPCR analysis**

Cells were seeded on a 6-well plate at $2\times10^5$ cells per well for 24 h. DZ@ZIF-8 (30 μg/mL) or Lipo3000 transfected DZ (2500 ng) was respectively incubated with cells. After incubation for 24 h or 72 h, cells were washed by PBS and the intracellular total RNA was extracted by TRIzol. mRNA was reverse transcribed by PrimeScript™ RT Reagent Kit. Quantitative PCR analysis (Table 1) was performed using TB Green® Premix Ex Taq™ II (TaKaRa). Amplification was monitored on Applied Biosystems™ QuantStudio™ 3&5.

**Table 1 RT-PCR primer sequences**

| Name | Sequence (5'→3') |
|---|---|
| *CDR1as*-F | ACGTCTCCAGTGTGCTGA |
| *CDR1as*-R | CTTGACACAGGTGCCATC |
| beta-*Actin*-F | CCAACCGCGAGAAGATGA |
| beta-*Actin*-R | GCAGATTTGGCAGAGAGTATAATG |
| *RAF1*-F | TCCATAGAGACATGAAATCCAACA |
| *RAF1*-R | GGCCATCCAGAGGACAGA |
| *PIK3CD*-F | AAGGAGGAGAATCAGAGCGTT |
| *PIK3CD*-R | GAAGAGCGGCTCATACTGGG |
| miR-7-F | CGGCGGTGGAAGACTAGTGATTT |
| miR-7-R | GTGCAGGGTCCGAGGT |

**Synthesis of ZIF-8 nanoparticles**

$Zn(NO_3)_2\cdot6H_2O$ (150 mg) and 2-methylimidazole (330 mg) were separately dissolved in 7 mL of methanol. The zinc nitrate solution was then added to the 2-methylimidazole solution under stirring and incubated for 10 min at room temperature. The resulting mixture was centrifuged at 10000 r/min for 10 min and the precipitate was washed three times with 10 mL of methanol. The ZIF-8 nanoparticles were obtained by vacuum drying at room temperature.

**Preparation of Dz@ZIF-8 and Alexa647-DZ@ZIF-8 nanoparticles**

DNAzymes (4 μmol/L) or Alexa647-DNAzymes (4 μmol/L) were incubated with ZIF-8 (600 μg/mL) in HEPES buffer (10 mmol/L, pH 7.4) for 1 h. The mixture was then centrifuged at 15000 r/min for 10 min and the pellet was washed with HEPES buffer.

**Preparation of DZ/MB@ZIF-8 nanoparticles**

DNAzymes (4 μmol/L) and molecular beacon (MB) (2 μmol/L) were incubated with ZIF-8 (600 μg/mL) in HEPES buffer (10 mmol/L, pH 7.4) for 1 h. The solution was then centrifuged at 15000 r/min for 10 min and the precipitate was washed with HEPES buffer.

**Characterization of Dz@ZIF-8**

The morphology, size, and potential of DZ@ZIF-8 were characterized by the following methods. (1) Scanning electron microscopy (SEM): ZIF-8 and DZ@ZIF-8 were drop-casted on conductive tape and dried. The SEM images of the samples were obtained using a Hitachi S-4800 SEM instrument (S-4800, Hitachi, Japan). (2) Dynamic light scattering (DLS) and zeta potential: ZIF-8 and DZ@ZIF-8 were dispersed in water and measured for their hydrodynamic diameter and surface charge using a Malvern Nano ZS90 particle sizer (Nano ZS90, Malvern, UK).

**Calculation of loading capacity**

To determine the loading capacity of ZIF-8, a standard curve was first established using known concentrations of cyanine 3 (Cy3)-labeled DNAzyme (Cy3-DZ) by measuring the fluorescence intensity at 570 nm. Subsequently, varying concentrations of Cy3-DZ (1–6 μmol/L) were incubated with a fixed concentration of ZIF-8 (600 μg/mL). After centrifugation, the fluorescence of the unbound DNA in the supernatant was measured. The amount of loaded DNA was calculated by subtracting the unbound DNA amount from the initial input, utilizing the standard curve for quantification. The loading capacity ($C$, μg/mg) was calculated using the following formula:

$$C = \frac{m_{\text{total}} - m_{\text{supernatant}}}{m_{\text{ZIF-8}}}$$

where $m_{\text{total}}$ is the initial mass of the input DNA, and $m_{\text{supernatant}}$ is the mass of the unbound DNA remaining in the supernatant after centrifugation, determined via the Cy3 fluorescence standard curve, and $m_{\text{ZIF-8}}$ is the mass of ZIF-8.

**MTT assay**

MCF-7 cells were seeded on a 96-well plate at $1\times10^4$ cells per well for 24 h. The cells were then treated with different concentrations of DZ@ZIF-8 for 24 h. After washing with PBS, the cells were incubated with 100 μL MTT (0.5 mg/mL in PBS) at 37 °C for 4 h. The MTT solution was then carefully removed, and100 μL DMSO was added to each well for incubation at 37 °C for 20 min.

Finally, the absorbance was measured at 490 nm.

**Confocal fluorescence microscopy**

Cells were imaged using a Leica TCS SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany). Alexa647 was excited with a 633 nm HeNe laser, Cy3 were excited with a 561 nm helium-neon laser, the lysotracker green was excited with a 488 nm Ar-Kr laser, and Hoechst 33258-labeled nuclei was excited with a 405 nm diode laser. The imaging channels were set at 650–680, 570–620, 500–550, and 450–500 nm, respectively.

**Cellular uptake and lysosomal escape**

To study the cellular uptake and lysosomal escape of Alexa647-DZ@ZIF-8, MCF-7 cells were seeded on glass-bottom culture dishes (35 mm in diameter) at a density of $4\times10^4$ cells per well and incubated for 24 h. Then, the cells were exposed to 30 μg/mL of Alexa647-DZ@ZIF-8 or Alexa647-DZ for 24 h. After the incubation, the cells were washed with PBS and fixed with 4% (0.04 g/mL) paraformaldehyde for 15 min. The nuclei were stained with Hoechst solution (5 μg/mL) for 10 min and washed with PBS. The fluorescence images of the cells were acquired by a Leica TCS SP8 confocal microscope.

To monitor the lysosomal escape of Alexa647-DZ@ZIF-8, the cells were treated with 30 μg/mL of Alexa647-DZ@ZIF-8 for 16 h and then washed with PBS. The cells were incubated with serum-free medium for another 2 h. The lysosomes were labeled with LysoTracker Green (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The confocal images of the cells were captured at different time points. Alexa647 was excited with a 633 nm HeNe laser, Cy3 were excited with a 561 nm helium-neon laser, the lysotracker green was excited with a 488 nm Ar-Kr laser, and Hoechst 33258-labeled nuclei was excited with a 405 nm diode laser.

**Intracellular gene regulation of DZ/MB@ZIF-8**

U87MG cells ($6\times10^4$ cells/well), MCF-7 cells ($4\times10^4$ cells/well), and HEK293T cells ($8\times10^4$ cells/well) were seed on a glass-bottom culture dish with a diameter of 35 mm for 24 h. Then, the cells were incubated with MB@ZIF-8 or DZ/MB@ZIF-8. After incubating for 24 h, cells were washed by PBS and then stained with Hoechst solution (5 μg/mL) for 30 min, followed by confocal imaging.

**Flow cytometry**

U87MG cells ($2\times10^5$ cells per well), HEK293T cells ($3\times10^5$ cells per well), and MCF-7 cells ($2\times10^5$ cells per well) were seeded in 6-well plates and incubated overnight. Then, the cells were treated with 30 μg/mL of MB@ZIF-8, DZ/MB@ZIF-8, or 10-23 DNAzyme (10DZ)/MB@ZIF-8 for 24 h. After the treatment, the cells were washed with PBS and detached with trypsin. The cells were collected by centrifugation at 1500 r/min for 5 min at room temperature and resuspended in PBS. The intracellular fluorescence of the cells was measured by flow cytometry at 571 nm.

**Statistical analysis**

The statistical analysis was performed using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. All quantitative data are presented as mean±standard deviation (SD). Student's *t*-test was used to compare the two groups, while one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple comparisons. A *P*-value of less than 0.05 was considered statistically significant. The significance levels are represented as $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

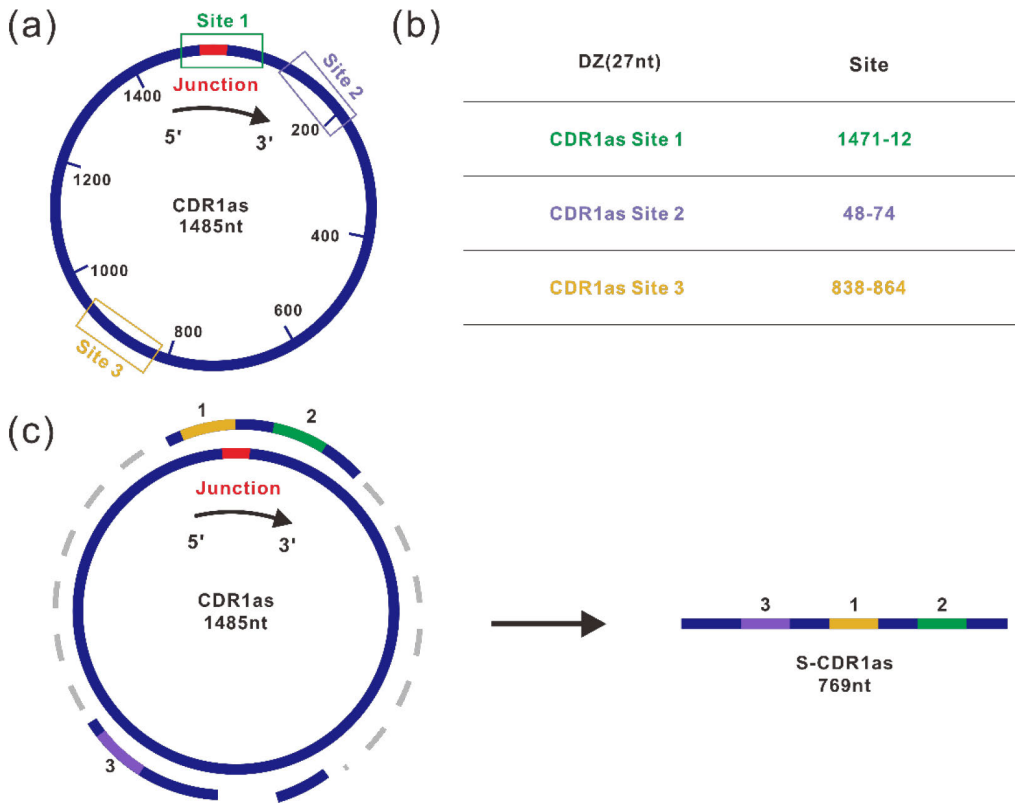


**Fig. S1** **Design and target site characterization of 8-17 DZs and the RNA substrate mimic. (a, b) Schematic representation of *CDR1as* and the three cleavage sites (Site 1, Site 2, and Site 3) selected by 8-17 DNAzyme (DZ). Site 1 is located in the back-splice junction (BSJ) region (1471–12), while Site 2 is proximal to the BSJ (48–74) and Site 3 is distal from the BJS (838–864). (c) Design of an RNA sequence, S-*CDR1as*, that contains three cleavage sites for DNAzymes. Abbreviations: *CDR1as*, cerebellar degeneration-related protein 1 antisense.**

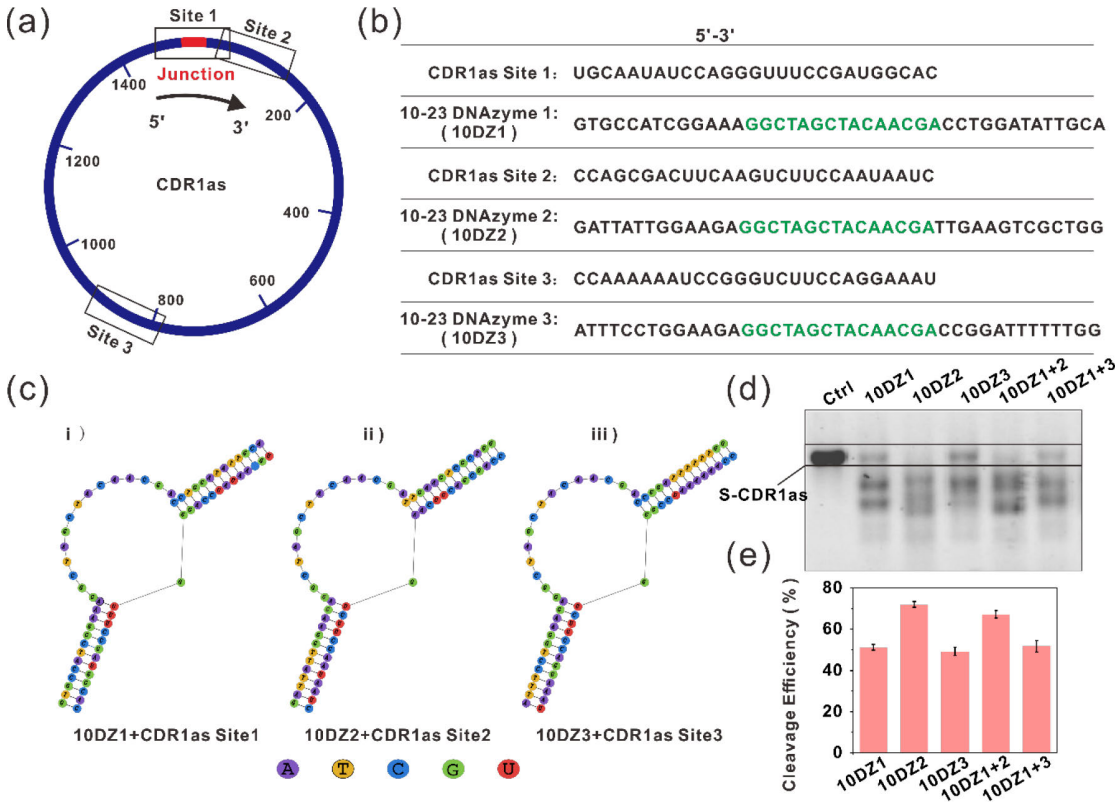


**Fig. S2 Design, structure prediction, and in vitro cleavage efficiency of 10-23 DZs targeting CDR1as. (a) Schematic representation of *CDR1as* and the three cleavage sites (Site 1, Site 2, and Site 3) selected by 10-23 DNAzymes (10DZ). (b) Sequences of three cleavage sites. The catalytic core of the 10-23 DNAzyme is shown in green. (c) Predicted secondary structures of the 10DZ-target complexes using RNAstructure software. (d) Gel electrophoresis analysis of the cleavage of 10DZ and S-*CDR1as*. (e) Cleavage efficiency of individual or combined 10DZ quantified using ImageJ software. The data are expressed as mean±standard deviation (SD) (*n*=3). Abbreviations: DZ, DNAzyme; *CDR1as*, cerebellar degeneration-related protein 1 antisense; S-*CDR1as*, substrate RNA derived from *CDR1as* containing three DZ-argeted sequences.**

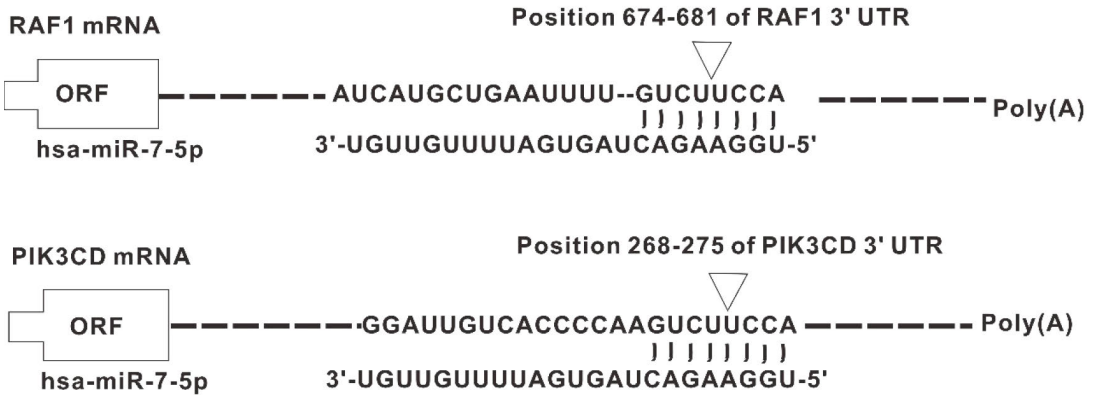


**Fig. S3 Target site alignment of miR-7 on downstream target mRNAs. Identification of two downstream mRNAs (*RAF1* mRNA and *PIK3CD* mRNA) regulated by miR-7 using TargetScan software. The diagram shows the sequence pairing and predicted binding sites of miR-7 within the coding region (or 3' UTR) of RAF1 and PIK3CD mRNAs. Abbreviations: mRNA, messenger RNA; *RAF1*, Raf-1 proto-oncogene, serine/threonine kinase; *PIK3CD*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta.**

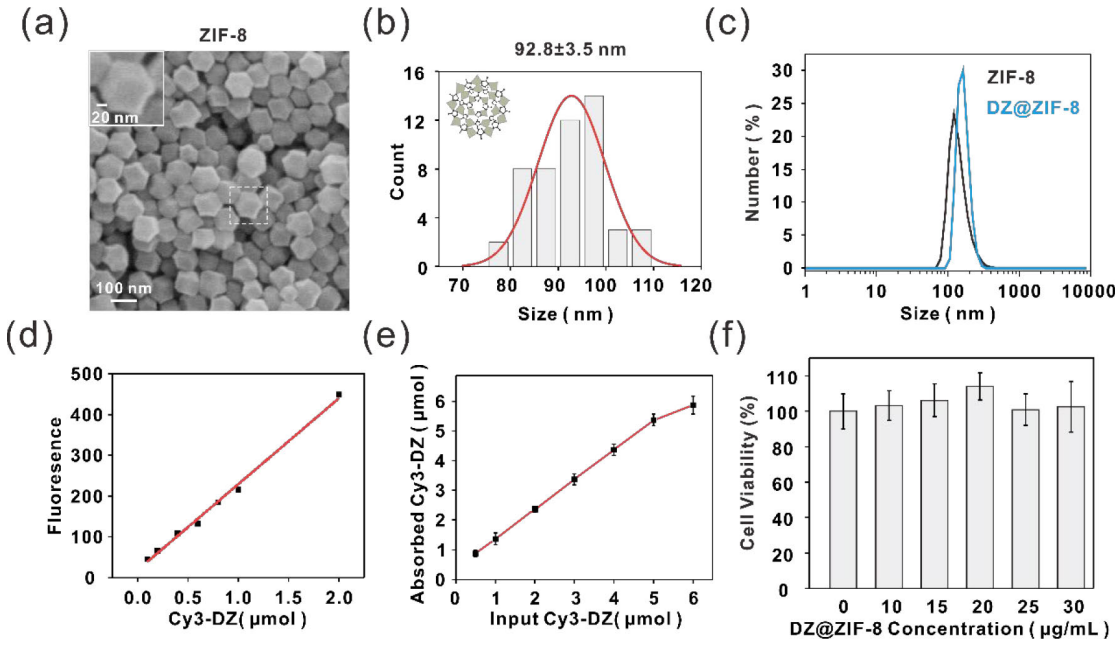


**Fig. S4 Characterization, nucleic acid loading capacity, and biocompatibility of ZIF-8 nanoparticles (a, b) SEM images and corresponding particle size distribution analysis of ZIF-8. (c) Dynamic light scattering (DLS) measurements showing the hydrodynamic size distributions of ZIF-8 and 8DZ1@ZIF-8 nanoparticles. The average hydrodynamic diameters of ZIF-8 and DZ@ZIF-8 were 122.4 nm and 164.2 nm, respectively. (d) Standard fluorescence intensity calibration curve of Cy3-DZ at 570 nm. (e) Quantification of adsorbed Cy3-DZ on ZIF-8 to calculate DNA loading capacity (55.54 μg/mg). (f) Biocompatibility and cell viability of MCF-7 cells treated with different concentrations of DZ@ZIF-8 nanoparticles determined by MTT assay after 24 h. The data are expressed as mean ± standard deviation (SD) (n = 3). Abbreviations: SEM, scanning electron microscope; ZIF-8, zeolitic imidazolate framework-8; DZ, DNAzyme.**

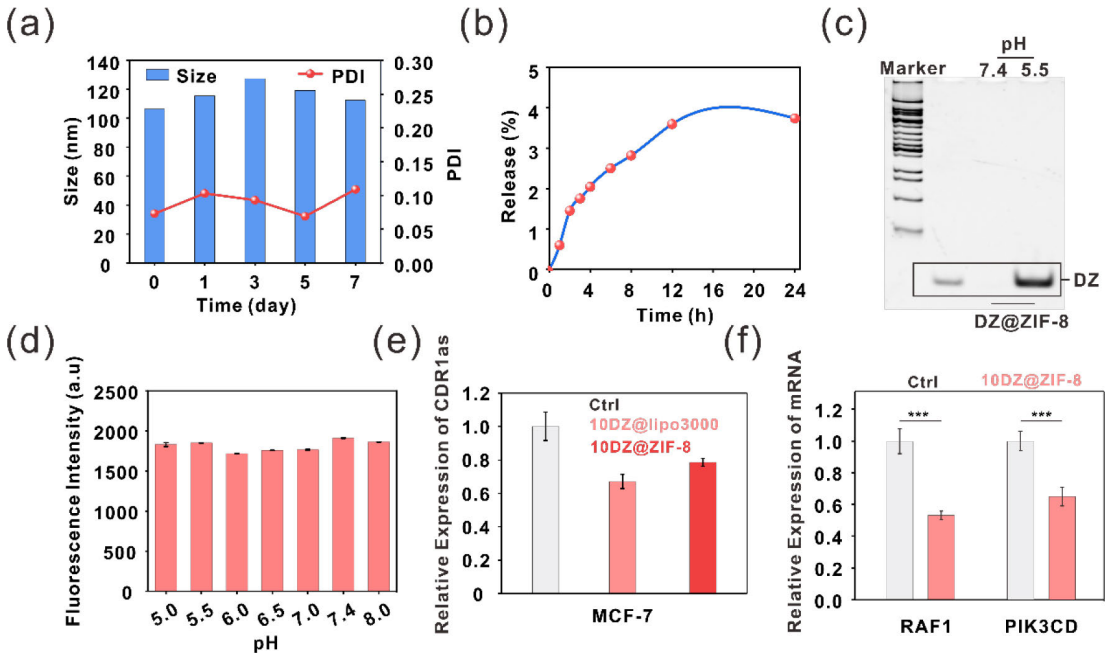


**Fig. S5 Stability, pH-responsive release, and regulation of CDR1as and downstream pathways by 10-23 DZs (a) Hydrodynamic diameter and polydispersity index (PDI) monitoring of DZ@ZIF-8 in deionized water (25 °C) over 7 d. (b) Cumulative release profile of Cy3-DZ from ZIF-8 in physiological media over 24 h. (c) PAGE electrophoresis analysis of pH stability of DZ@ZIF-8 nanoparticles. DZ1@ZIF-8 nanoparticles were dispersed in solutions with different pH values and incubated at room temperature for 2 h. Then, the solutions were centrifuged and the supernatants were analyzed by 12% polyacrylamide gel electrophoresis. (d) Detection of Cy3 fluorescence intensity in buffers of different pH values. (e) RNA levels of *CDR1as* upon treated with 10DZ@Lipo3000 or 10DZ@ZIF-8 analyzed by RT-qPCR. (f) RT-qPCR analysis of mRNA levels of *RAF1* and *PIK3CD* treated with 10DZ@ZIF-8. The data are expressed as mean±standard deviation (SD) (*n*=3). $^{***}P$<0.001. Abbreviations: ZIF-8, zeolitic imidazolate framework-8; DZ, DNAzyme; PAGE, polyacrylamide gel electrophoresis; *CDR1as*, cerebellar degeneration-related protein 1 antisense; Lipo3000, Lipofectamine 3000.**

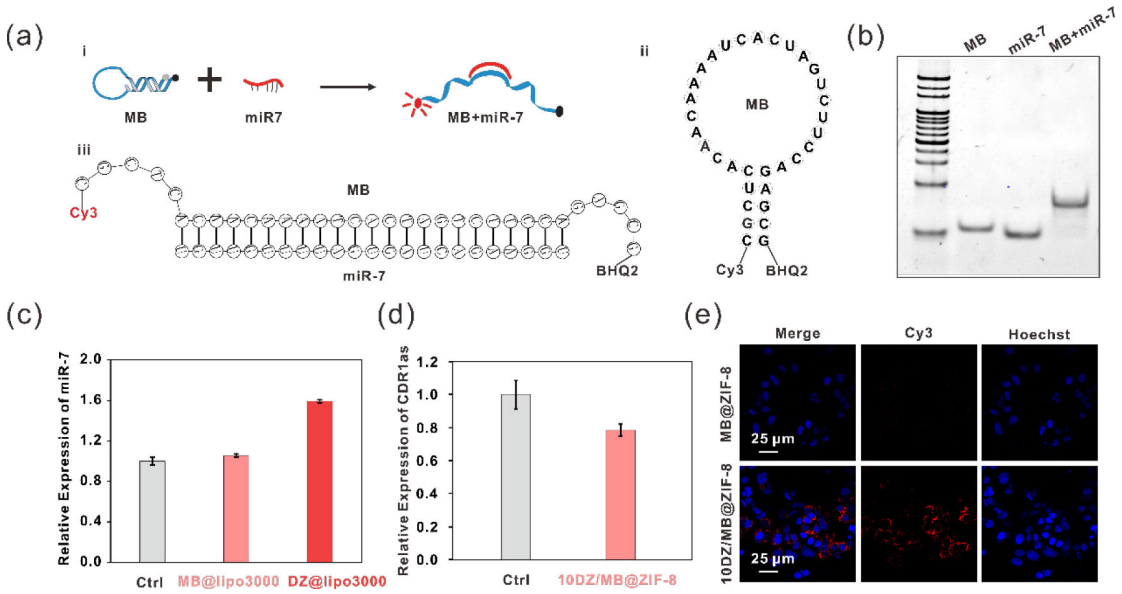


**Fig. S6 Characterization of the molecular beacon (MB) probe and miR-7 sensing performance in live cells (a) Schematic diagram of MB, a hairpin-shaped oligonucleotide with a fluorophore (Cy3) and a quencher (BHQ-2) at the stem ends. MB binding to miR-7, which opens the hairpin and releases the fluorophore from the quencher, resulted in fluorescence emission. (b) PAGE electrophoresis analysis of MB binding affinity to miR-7. (c) Levels of miR-7 treated with MB@Lipo3000 and DZ@Lipo3000 analyzed by RT-qPCR. (d) RNA levels of *CDR1as* treated with 10DZ/MB@ZIF-8 analyzed by RT-qPCR. (e) Confocal images of MCF-7 cells treated with MB@ZIF-8 or 10DZ/MB@ZIF-8 (Cy3: red; Hoechst: blue). Abbreviations: ZIF-8, zeolitic imidazolate framework-8; DZ, DNAzyme; MB, molecular beacon; Cy3, cyanine 3; BHQ-2, black hole quencher 2; miR-7, microRNA-7; *CDR1as,* cerebellar degeneration-related protein 1 antisense; Lipo3000, Lipofectamine 3000.**

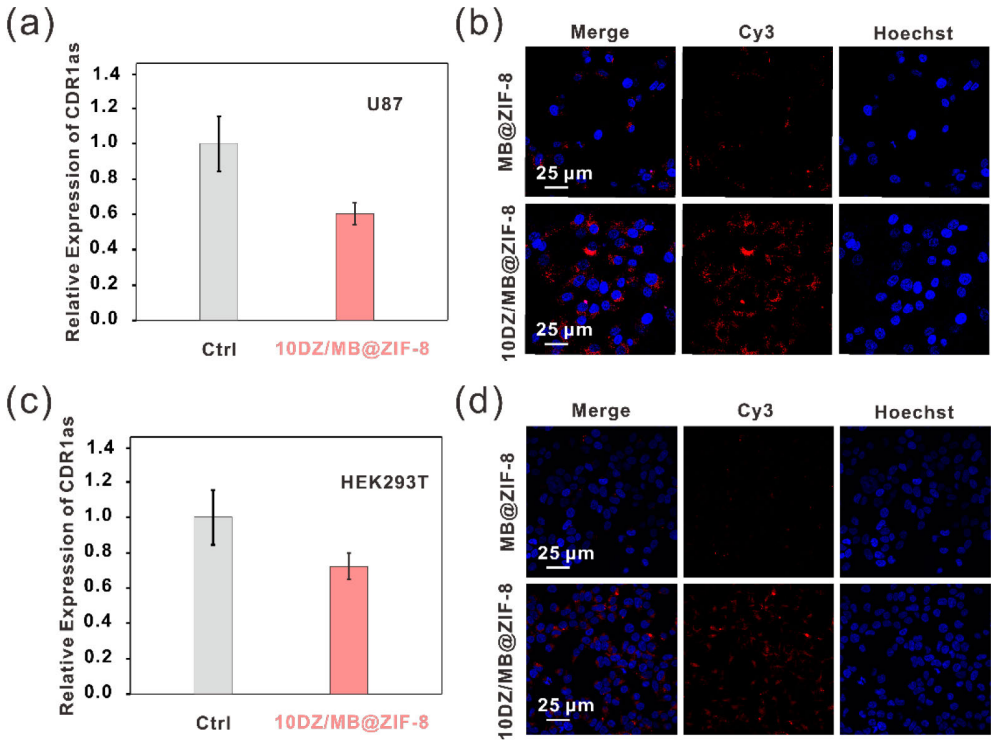


**Fig. S7 Universality evaluation of 10DZ/MB@ZIF-8 for CDR1as degradation and miR-7 sensing in U87MG and HEK293T cells (a) Relative RNA levels of *CDR1as* in U87MG cells treated with 10DZ/MB@ZIF-8 analyzed by RT-qPCR. (b) Confocal images of U87MG cells treated with MB@ZIF-8 or 10DZ/MB@ZIF-8 (Cy3: red; Hoechst: blue). (c) Relative RNA levels of *CDR1as* in HEK293T cells treated with 10DZ/MB@ZIF-8 analyzed by RT-qPCR. (d) Confocal images of HEK293T cells treated with MB@ZIF-8 or 10DZ/MB@ZIF-8 (Cy3: red; Hoechst: blue). Abbreviations: ZIF-8, zeolitic imidazolate framework-8; DZ, DNAzyme; MB, molecular beacon; miR-7, microRNA-7; *CDR1as,* cerebellar degeneration-related protein 1 antisense.**

**Tables: DNA and RNA sequences used in this study**

All DNA oligonucleotides were synthesized and purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified by high performance liquid chromatography (HPLC). The sequences were shown in Table S1. S-*CDR1as* RNA was prepared from a synthesized DNA template using the T7 in vitro Transcription Kit.

**Table S1 DNAzyme sequence**

| Name | Sequence (5'→3') |
|---|---|
| 10-23DNAzyme 1 | GTGCCATCGGAAAGGCTAGCTACAACGACCTGGATATTGCA G |
| 10-23DNAzyme 2 | GATTATTGGAAGAGGCTAGCTACAACGATTGAAGTCGCTGG |
| 10-23DNAzyme 3 | ATTTCCTGGAAGAGGCTAGCTACAACGACCGGATTTTTTGG |
| 8-17DNAzyme 1 | CCATCGGAAACCTCCGAGCCGGTCGAAGGATATTGCAGAC |
| 8-17DNAzyme 2 | ATTATTGGAAGATCCGAGCCGGTCGAATGAAGTCGCTGGA |
| 8-17DNAzyme 3 | ATTTTTTGGAAGTCCGAGCCGGTCGAAATAGATTTAAGGG |

The catalytic core of the DNAzyme is shown in green.

**Table S2 S-*CDR1as* sequences**

| Name | Sequence (5'→3') |
|---|---|
| S-*CDR1as* | GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUA<br>CAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAA<br>GACGAGGGUAAAGAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCG<br>AAAGCUGCAAGAGAAUGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGU<br>UCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGAAAAACAAAA<br>AACAAAAAAACCAACGACCUUAUCUGAACAUAAUGCUACCGUUUAAUAU<br>UGCGUCACCUCCAGACUAUCCAUGUCUUCCAGAAAAUCCUUGUCUUCCC<br>UUAAAUCUAUAGCUUCCAAAAAAUCCGGGUCUUCCAGGAAAUCCGUGUC<br>UUCCAGCAAGUGCUGAUCUUCUGACAUUCAGGUCUUCCAGUGUCUGCAA<br>UAUCCAGGGUUUCCGAUGGCACCUGUGUCAAGGUCUUCCAACAACUCCG<br>GGUCUUCCAGCGACUUCAAGUCUUCCAAUAAUCUCAAGGUCUUCCAGAU<br>AAUCCUGAGUGGGUCUUCCUGAAAAUCUACGUCUUCCAAAAAAACAAAA<br>AACAAAACGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUA<br>AUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGG<br>GAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGU<br>AGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG |