Overcoming Tamoxifen Resistance of Human Breast Cancer by Targeted Gene Silencing Using Multifunctional pRNA Nanoparticles

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Supplementary information

Table S1. Sequences used for construction of pRNA nanoparticles.

		5' - 3'
pRNA-HER2apt-siScram	p1	GGAucAcGcuucAuAuAcAAAGGAucAAucAuGGccAAucuuAuuucGcccAuGAccuu*
	p2	GGucAuGGGcGAAAuAAGAAAGGccAuGuGuAuGuGGGGGGAGGAcGAuGcGGucuGcuGuGcuuGAuAuGccccAGAcGAcucGccccccAcAuAcuuuGuuGAuccAAuGuAuAuGAAGcGuGAuccuu
pRNA-HER2apt-siMED1	p1	GGAcAGuGAAAGuGAGucAAAGGAucAAucAuGGccAAAGAuGuuAcuuuGAGAGccuu
	p2	GGcucucAAAGuAAcAucuAAGGccAuGuGuAuGuGGGGGGAGGAcGAuGcGGuCuGcuGuGcuuGAuAuGccccAGAcGAcucGccccccAcAuAcuuuGuuGAuccAAuGAcucAcuuucAcuGuccuu
pRNA-HER2apt^{mut}-siMED1	p1	GGAcAGuGAAAGuGAGucAAAGGAucAAucAuGGccAAAGAuGuuAcuuuGAGAGccuu
	p2	GGcucucAAAGuAAcAucuAAGGccAuGuGuAuGuGGG**Au**GAGGA**Gu**AuG**AA**G**AuAc**cuGu**A**cuuGAuAuGccccAGAcGAcAuuccccccAcAuAcuuuGuuGAuccAAuGAcucAcuuucAcuGuccuu

* The lower letters indicated 2'-Fluoro modified nucleotides.

**Mutations of HER2 aptamers are indicated as Bold.

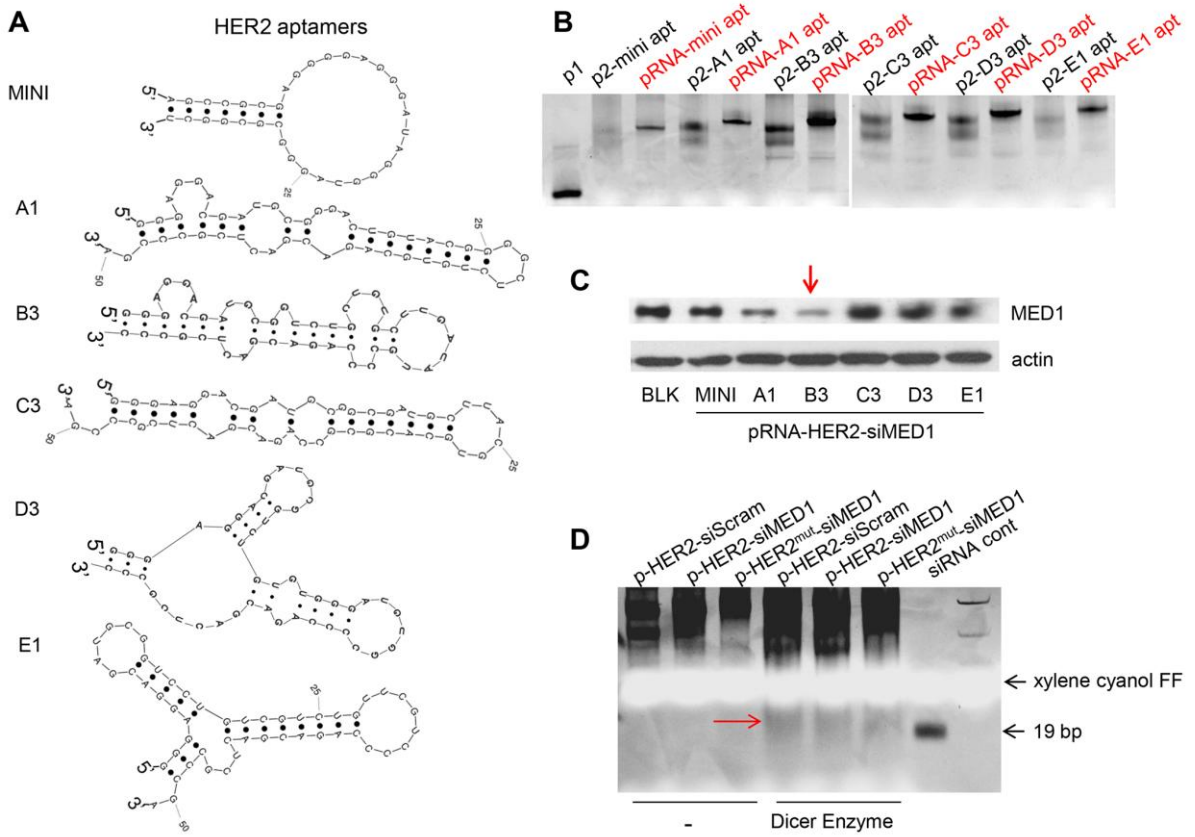

Figure S1. Testing of HER2-targeting RNA aptamers for MED1 siRNA delivery and siRNA release from pRNA nanoparticles. (A) Six HER2-targeting RNA aptamers were selected from previous literatures, and their secondary structures were predicted using Srna-fold software . (B) Six p2 strands containing the six different HER2 RNA aptamers were synthesized and annealed with the same p1 strand to generate six different pRNA-HER2apt-MED1 siRNA nanoparticles, respectively, followed by examination using 8% native PAGE gel electrophoresis. (C) BT474 cells were treated with above pRNA nanoparticles for 48 h, and then MED1 protein level was determined by western blotting. Red arrow indicates the pRNA nanoparticles with B3 aptamer had the best efficiency in MED1 silencing. (D) 1 μg pRNA nanoparticles were incubated with recombinant Dicer enzyme (Genlantis) at 37 ^{o}C for 12 h, the product was separated by 8% native PAGE gel.

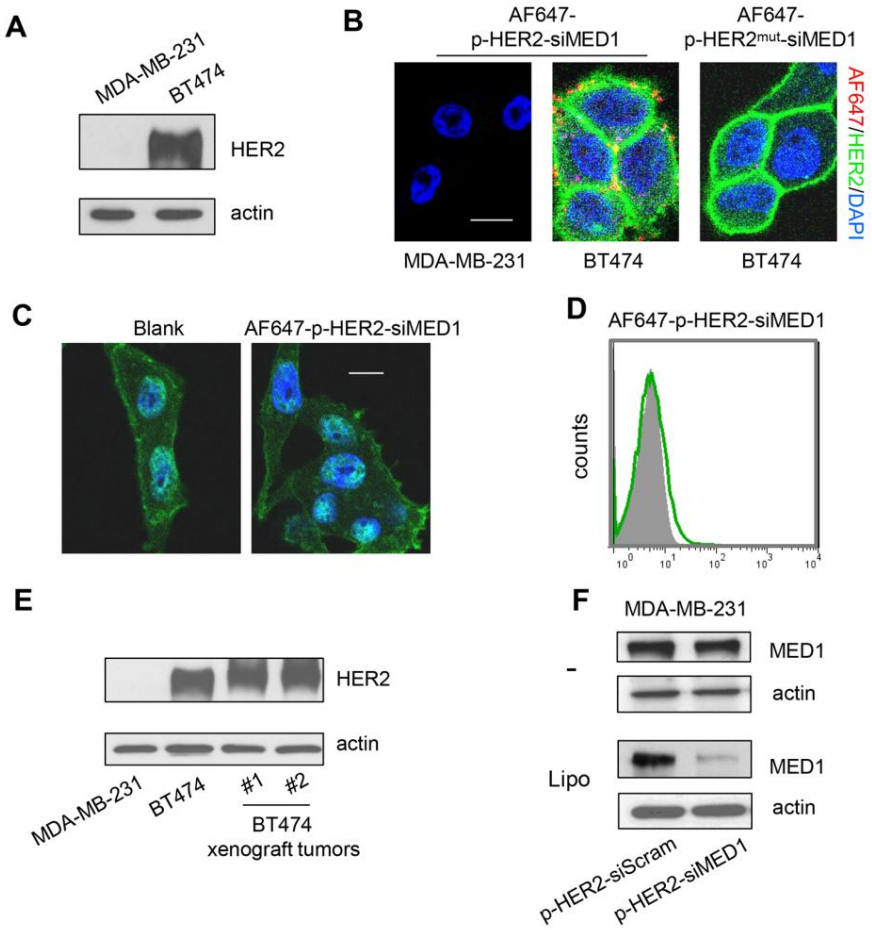

Figure S2. pRNA-HER2apt-siMED1 nanoparticles targeted HER2-overexpressing BT474 cells but not HER2-negative MDA-MB-231 cells. (A) HER2 expression in BT474 and MDA-MB-231 cells determined by western blot analyses. (B) The binding of AF-647-labeled pRNA-HER2apt-siMED1 and its mutant nanoparticles to MDA-MB-231 and BT474 cells examined by confocal microscopy. Green represents the immunofluorescent staining of HER2, and blue represents nuclear staining with DAPI. (C) The internalization of AF647-labeled pRNA nanoparticles by MDA-MB-231 cells examined by confocal microscopy. Green represents the immunofluorescent staining of β-actin. Scale bar: 10 μm. (D) The cellular uptake of AF647-labeled pRNA nanoparticles by MDA-MB-231 cells quantified by flow cytometry. (E) HER2 expression in BT474 xenograft tumors was examined by western blot analyses. (F) MDA-MB-231 cells were incubated directly with (as indicated by -) or transfected with pRNAs using lipofectamine 2000. At 48 h post treatment, MED1 protein levels were determined by western blots.

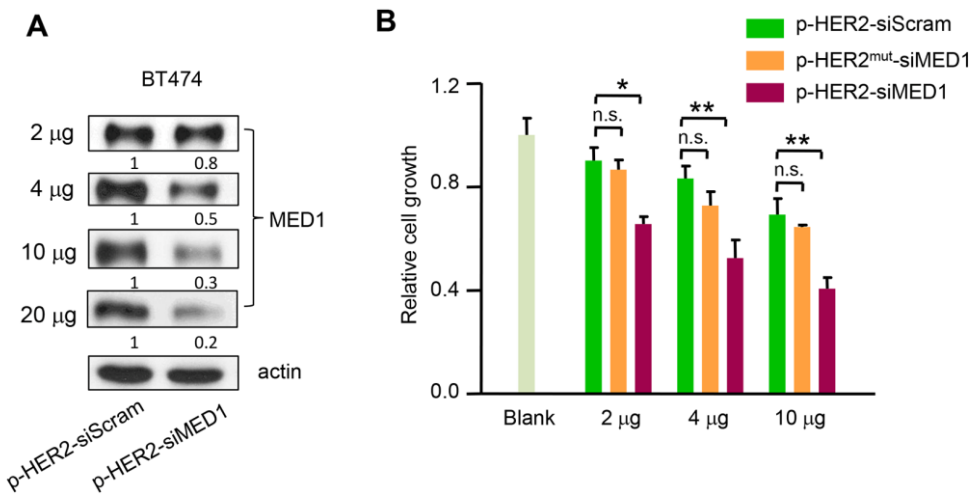

Figure S3. pRNA-HER2apt-siMED1 nanoparticles dose-dependently knockdown MED1 expression and inhibited the growth of BT474 cells. BT474 cells were treated with different concentrations of pRNA nanoparticles as indicated for 48 h. After that, MED1 protein level was determined by western blots (A), and the cell viability was determined by MTT assay (B).

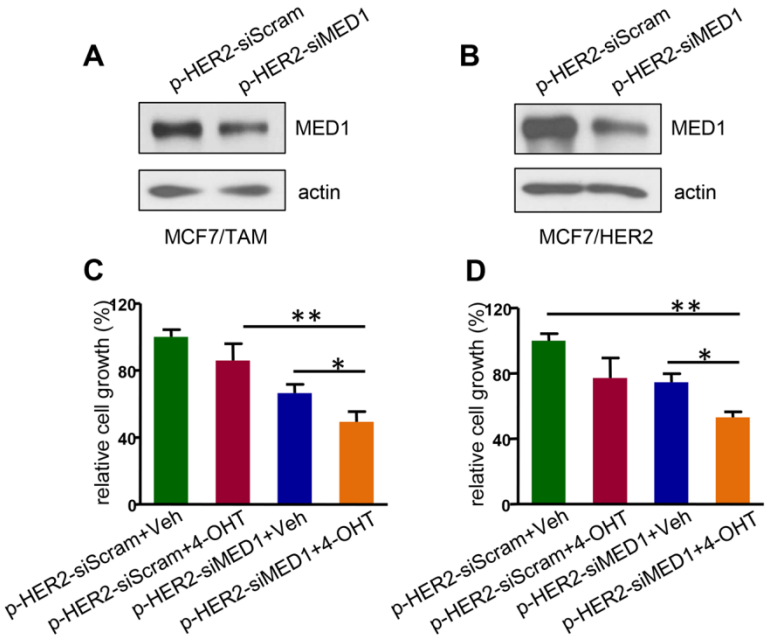

Figure S4. pRNA-HER2apt-siMED1 nanoparticles silenced MED1 expression and sensitized two other HER2-overexpressing breast cancer cells to tamoxifen treatment. (A and B) Two HER2-overexpressing breast cancer cell lines MCF7/TAM (A) and MCF7/HER2 (B), were treated with 10 μg/mL pRNA-HER2apt-siMED1 nanoparticles or scramble control for 48 h, and then MED1 protein level was determined using western blots. (C and D) MCF7/TAM (C) and MCF7/HER2 (D) cells were treated with 10 μg/mL pRNA nanoparticles and/or 1μM 4-hydroxytamoxifen (4-OHT) as indicated for 48 h, and then cell growth was determined using an MTT assay.

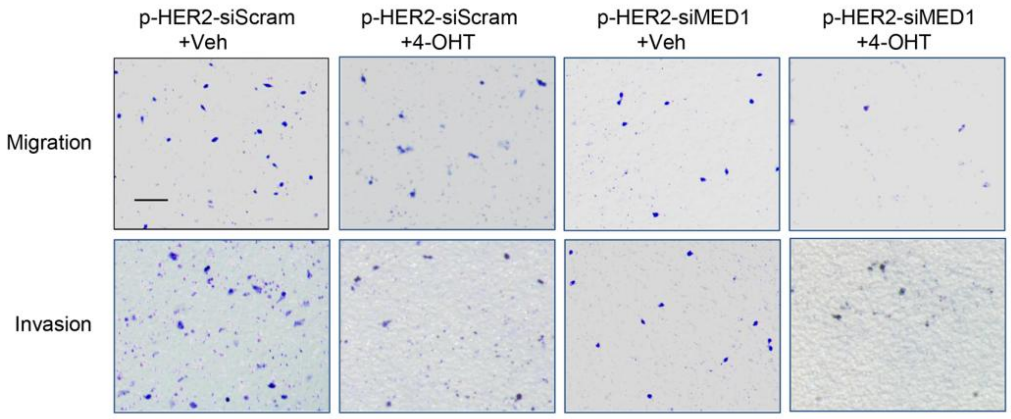

Figure S5. pRNA-HER2apt-siMED1 nanoparticles strongly inhibited the migration and metastasis of BT474 cells in combination with tamoxifen treatment. BT474 cells were treated with pRNA nanoparticles alone or together with 1 μM 4-OHT for 48 h. Cell migration and invasion were then examined using transwell assay. Photos were recorded using an Olympus SZX12 microscope. Scale bar: 100 μm.

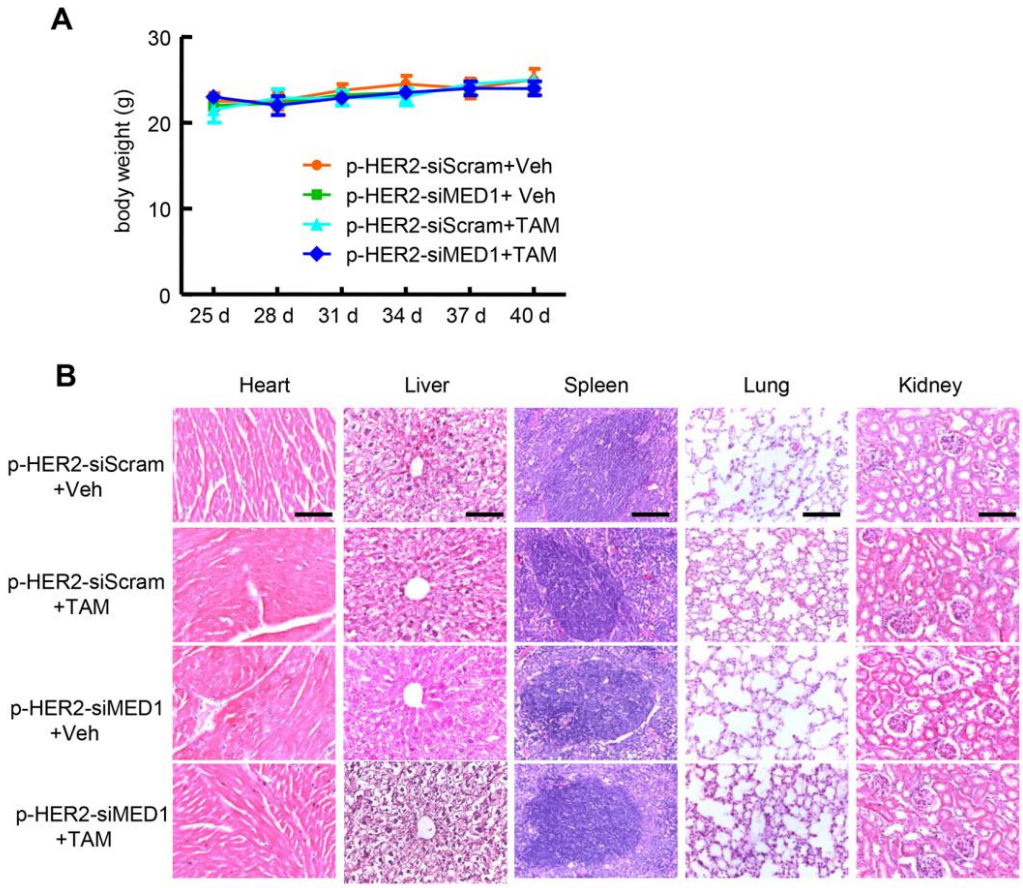

Figure S6. pRNA-HER2apt-siMED1 nanoparticles exhibited excellent biosafety. (A) During therapeutic treatment to orthotopic xenograft mouse models, the body weights of mice were recorded every three days. (B) After therapeutic treatment, major organs were excised, fixed in 10% neutral formalin buffer, embedded in paraffin, sectioned and examined for histopathology by H&E staining. Scale bar: 100 μm.