Supporting Information

Phenylboronic acid-functionalized polyamidoamine-mediated miR-34a delivery for the treatment of gastric cancer

Zheyu Song,[a,b] Xiao Liang,[b] Yudi Wang,[b] Haobo Han,[b] Jiebing Yang,[b]

Xuedong Fang,[a,*] Quanshun Li[b,*]

[a]*Department of Gastrointestinal Colorectal and Anal Surgery, China-Japan Union Hospital, Jilin University, Changchun 130033, China*

[b]*Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, Changchun 130012, China*

*Corresponding authors.

Tel.: +86-431-85155201; Fax: +86-431-85155200.

E-mail: quanshun@jlu.edu.cn (Q. Li); fangxd1961@163.com (X. Fang).

Materials and methods

1. Cell cytotoxicity analysis of PAMAM and PPP

The human gastric cancer cell BGC-823 and gastric epithelial cell GES-1 were seeded at a density of 7,000 cells per well in 96-well plates and cultured in 10% FBS-containing medium at 37 °C for 24 h. Subsequently, the cells were treated with different concentrations of PAMAM and PPP for another 48 h. Then, 20 μL of MTT solution (5 mg/mL in PBS) was added into each well and the plates were incubated at 37 °C for 4 h. After discarding the MTT solution from each well, the formazan crystals were thoroughly dissolved in 150 μL dimethyl sulfoxide (DMSO). The absorbance at 492 nm was measured on GF-M3000 microplate reader (Shandong, China). The cell viability (%) was calculated as the ratio of A_{sample} and $A_{control}$, which represented the absorbance value of treated and untreated cells, respectively.

2. Cell colony formation assay

The BGC-823 cells were cultured in 6-well plates at a density of 2.5×10^5 cells per well for 24 h. The cells were then incubated with PAMAM/miR-34a (N/P of 50), PPP/miR-34a (N/P ratio of 100) and PPP/NC (N/P ratio of 100) nanoparticles harboring 100 nM miR-34a in serum-free medium for 6 h. After the incubation in 10% FBS-containing medium for 48 h, the cells were collected and planted into 6-well plates at a density of 1.0×10^4 cells/well for a week. Prior to the straining with 0.2% crystal violet solution, the cells were fixed with 70% cold ethanol at 4 °C for 20 min. Finally, the cells were rinsed with PBS and captured with IX71 fluorescence microscopy (Olympus, Tokyo, Japan).

3. Live/Dead cell staining assay

The BGC-823 cells were cultured and treated as described in section 2 “Cell colony formation assay”. The cells were then washed with PBS and stained with live/dead solution at room temperature for 30 min in the dark according to the manufacturer’s protocol. Afterwards, the cells were rinsed with PBS and observed with IX71 fluorescence microscopy (Olympus, Tokyo, Japan).

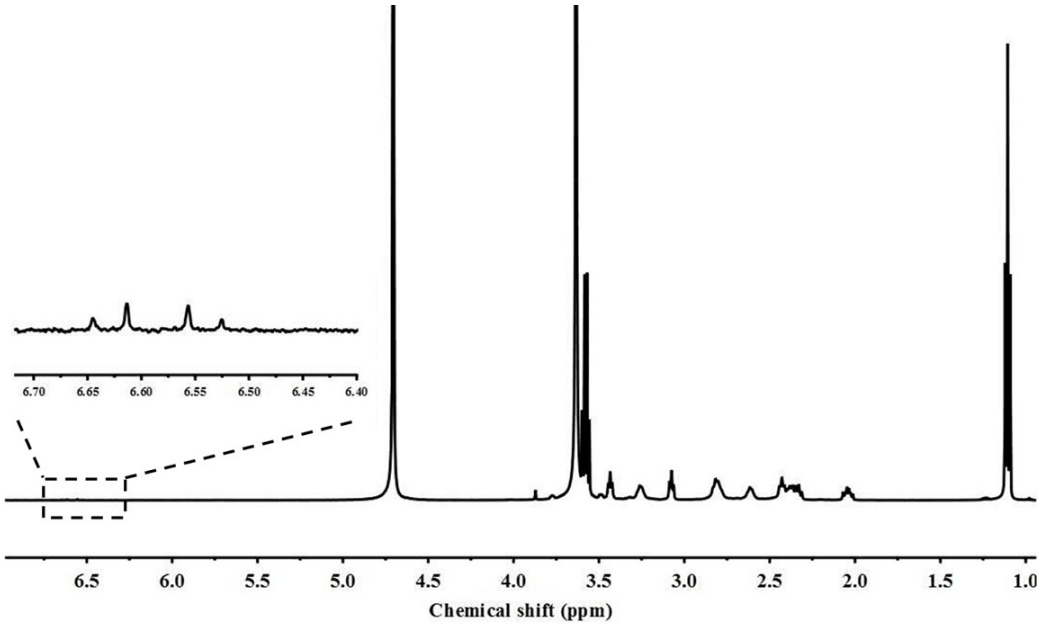

Figure S1. ^{1}H NMR spectrum of PPP in D_2O.

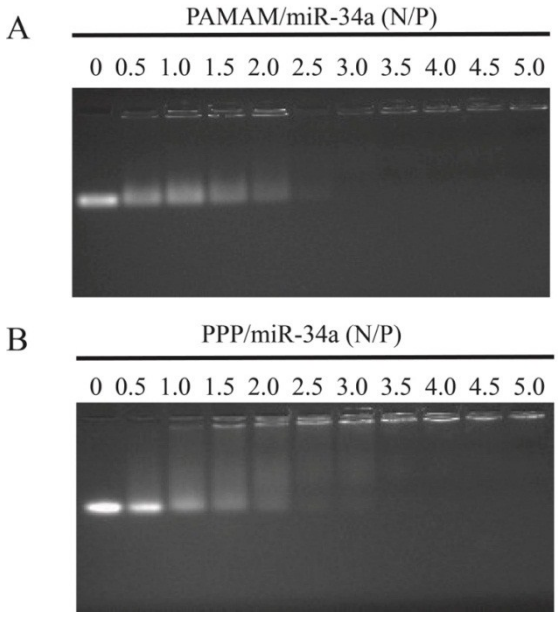

Figure S2. Gel retardation assay of PAMAM (A) and PPP (B) with miR-34a at different N/P ratios.

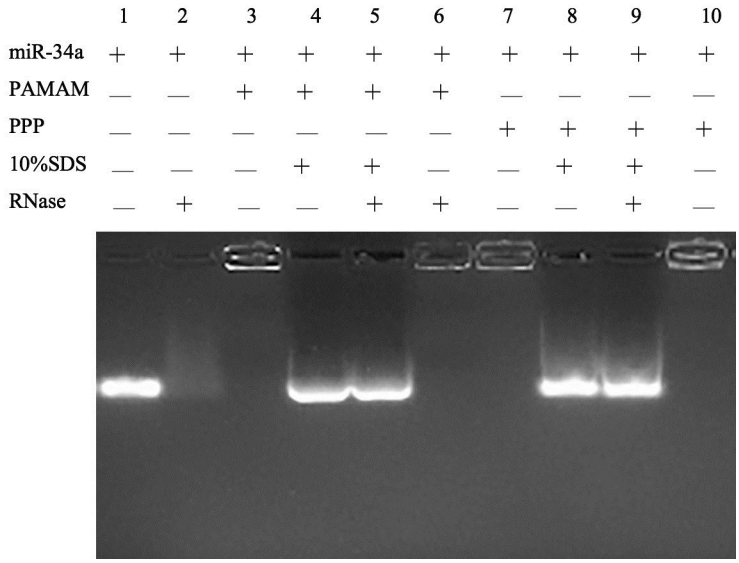

Figure S3. Gel retardation assay of carrier/miR-34a nanoparticles at a N/P ratio of 10 after RNase treatment (10 μg/mL for 15 min).

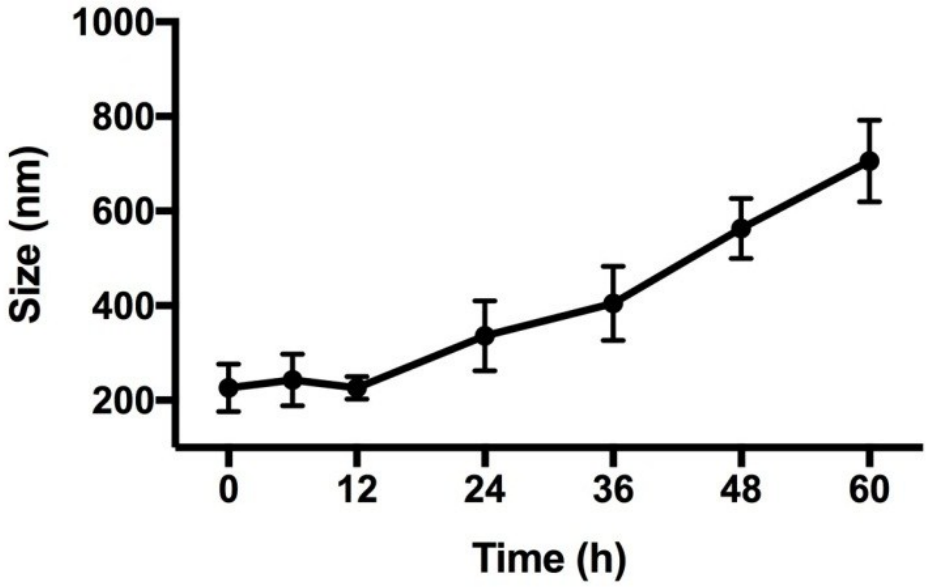

Figure S4. The hydrodynamic diameter of PPP/miR-34a nanoparticles (N/P ratio of 100) in serum-free medium for 60 h.

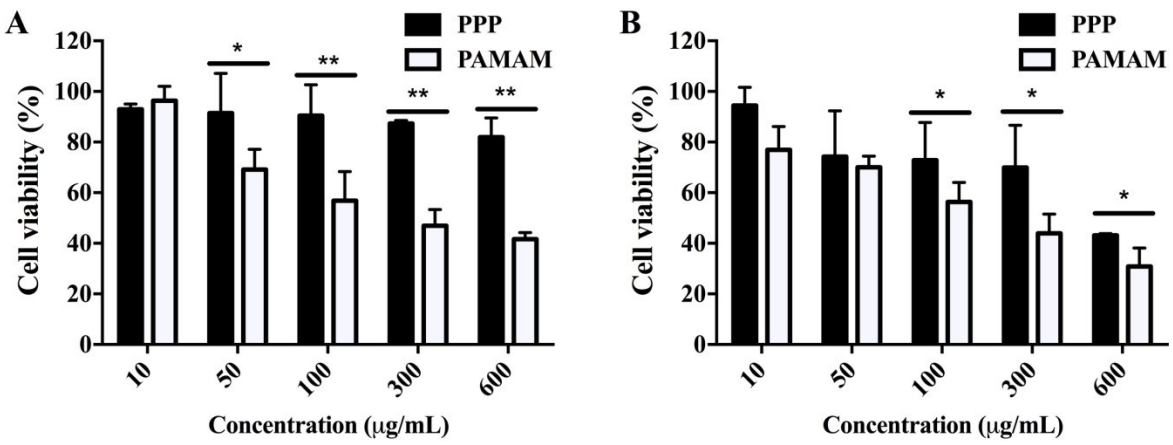

Figure S5. Cell viability of PAMAM and PPP at different concentrations using BGC-823 (A) and GES-1 cells (B) as models.

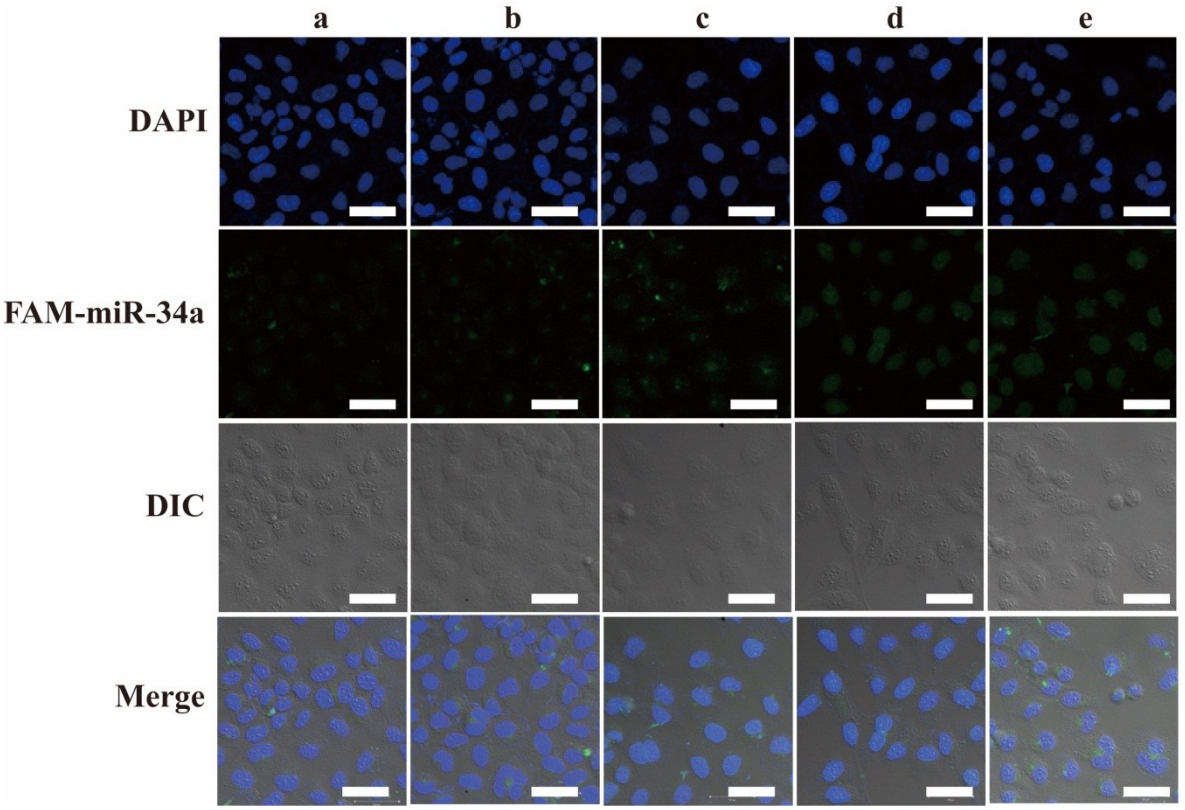

Figure S6. CLSM images of GES-1 cells exposed to miR-34a (a), PAMAM/miR-34a (b), PAMAM/miR-34a with pre-treatment using 1 mM PBA for 1 h (c), PPP/miR-34a (d) and PPP/miR-34a with pre-treatment using 1 mM PBA for 1 h (e). Blue (DAPI, nucleic), green (FAM-labeled miR-34a). The scale bar is 50 μm.

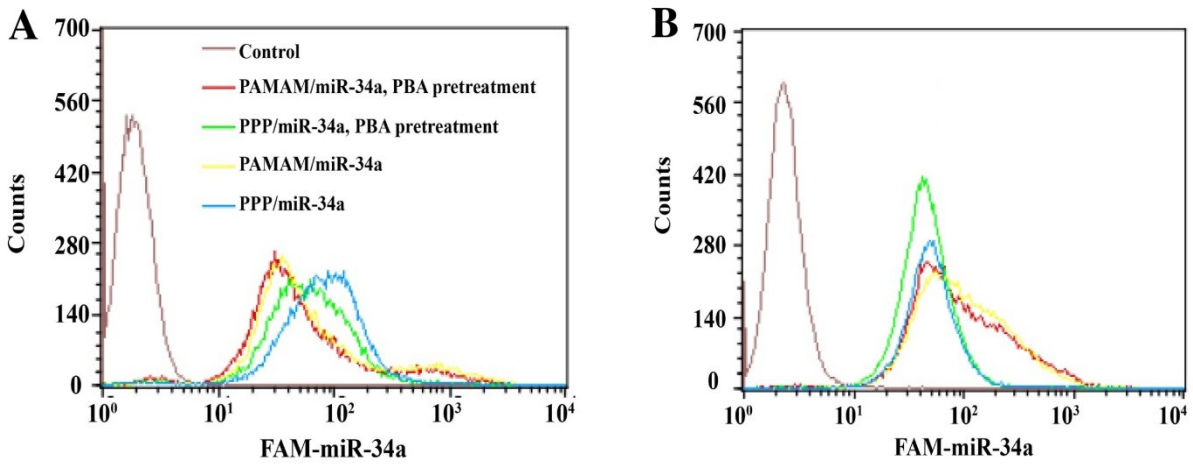

Figure S7. Flow cytometer analysis of the different nanoparticles in BGC-823 (A) and GES-1 cells (B).

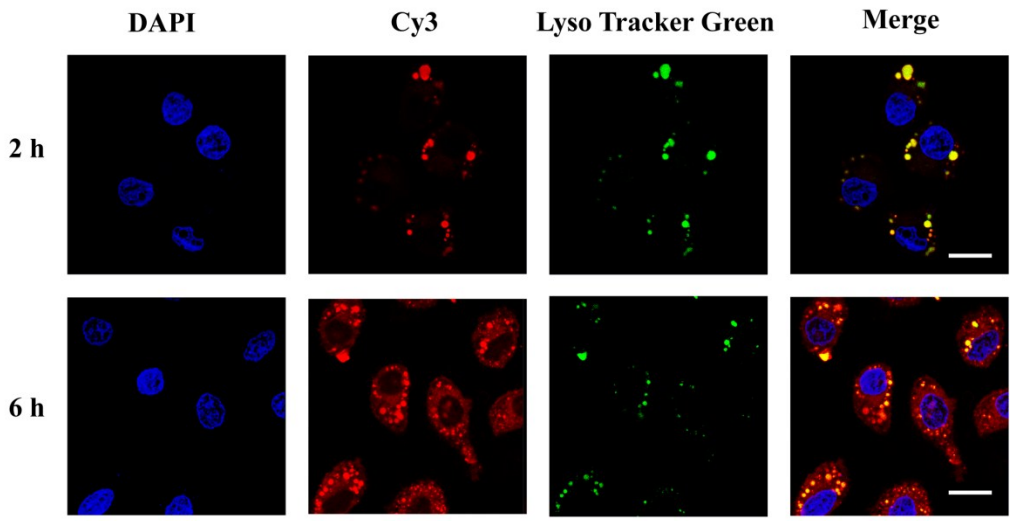

Figure S8. CLSM images of BGC-823 cells exposed to PPP/miR-34a nanoparticles for 2 and 6 h. Blue (DAPI, nucleus), red (Cy3-labeled miR-34a) and green (LysoTracker Green). The scale bar is 50 μm.

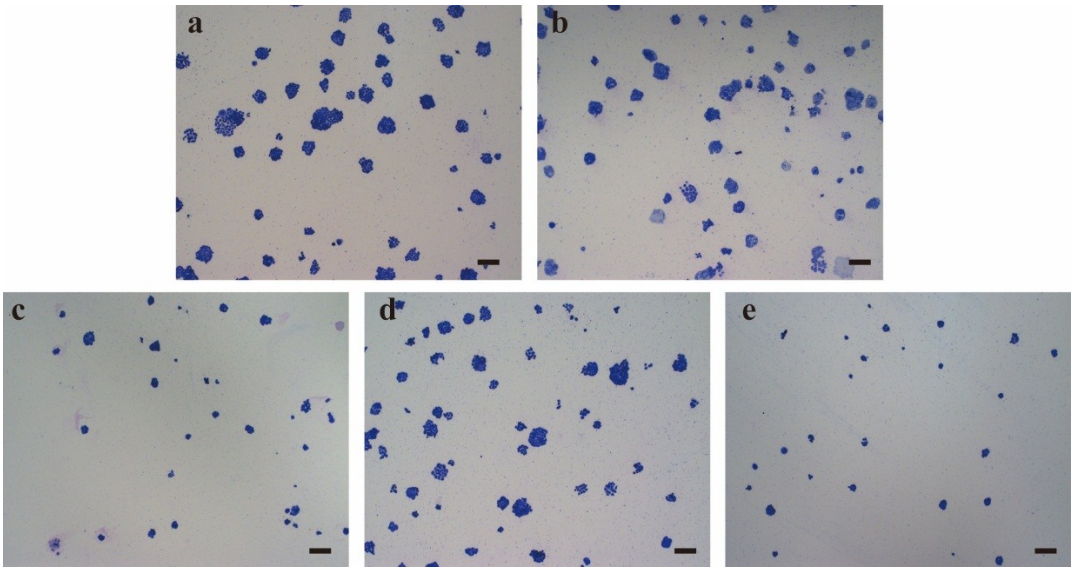

Figure S9. Colony formation assay after miR-34a transfection mediated by different nanoparticles: (a) control, (b) PPP, (c) PPP/miR-34a nanoparticles, (d) PPP/NC nanoparticles and (e) PAMAM/miR-34a nanoparticles. The scale bar is 200 μm.

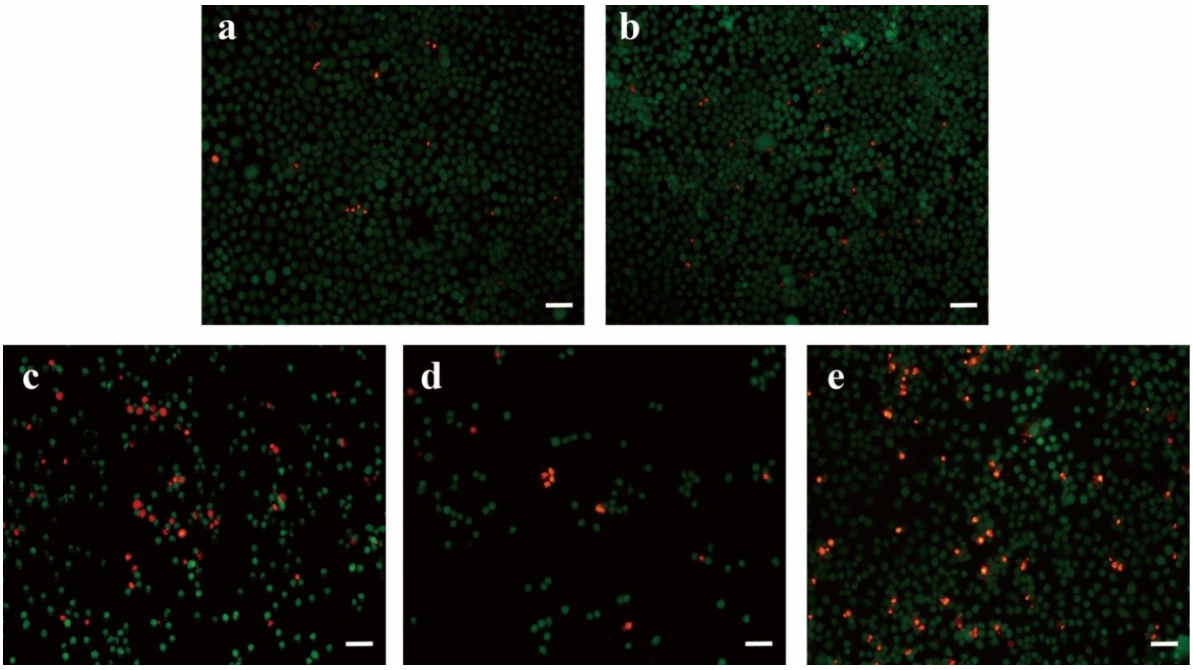

Figure S10. Live/dead staining assay of BGC-823 cells after miR-34a transfection. (a) Control, (b) PPP, (c) PPP/miR-34a nanoparticles, (d) PPP/NC nanoparticles and (e) PAMAM/miR-34a nanoparticles. The scale bar is 50 μm.

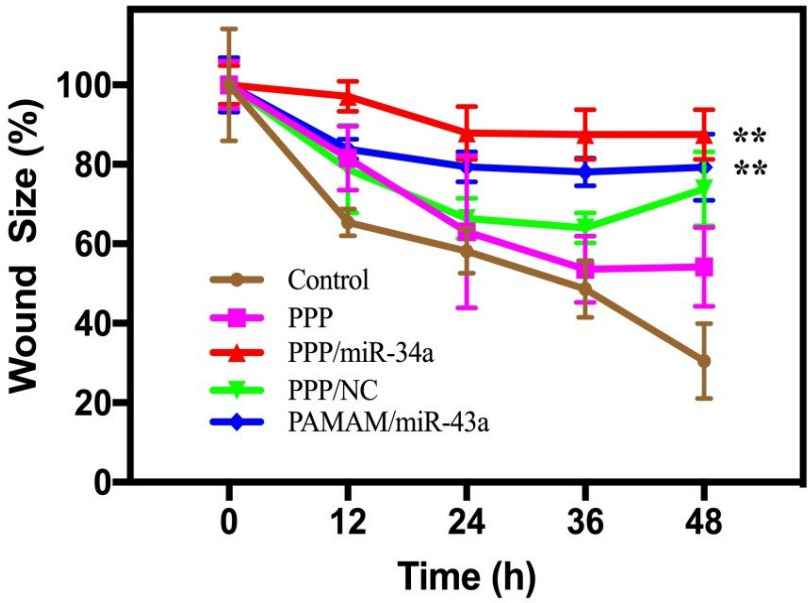

Figure S11. Quantitative analysis of wound healing size after carrier-mediated miR-34a transfection. The data are represented as mean value ± SD of triplicate experiments (*p < 0.05, **p < 0.01).

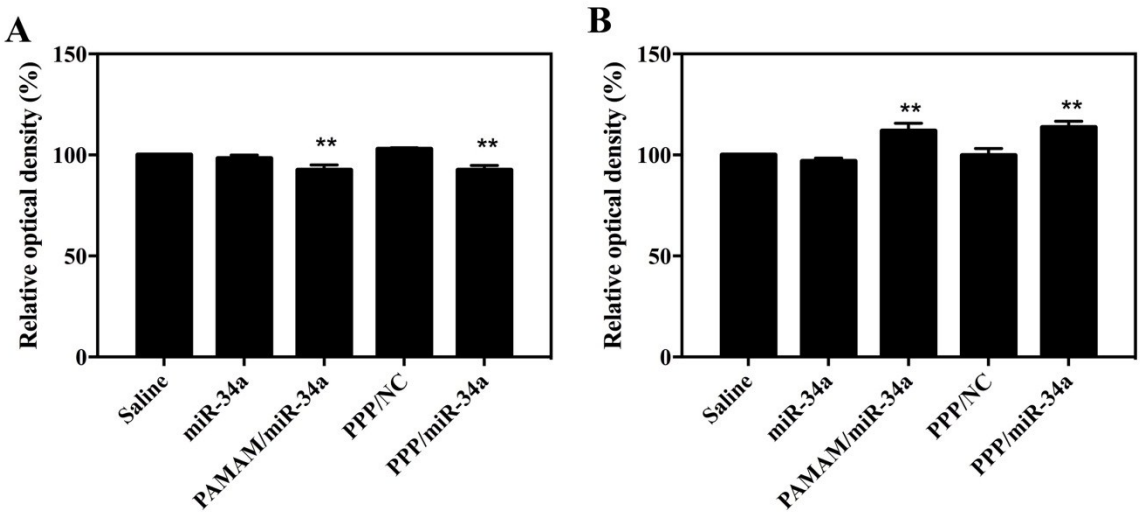

Figure S12. Quantitative analysis of positive staining of TUNEL (A) and Ki67 (B) using Image J software. The data are represented as mean value ± SD of triplicate experiments (*$p < 0.05$, **$p < 0.01$).

Table S1. The hydrodynamic diameter and zeta potential of carrier/miR-34a nanoparticles at different N/P ratios. Data were presented as mean value ± SD of triplicate experiments.

Nanoparticle	N/P ratio	Size (nm)	PDI	Zeta potential (mV)
PAMAM/miR-34a	2:1	830.56 ± 37.14	0.088	+18.33 ± 0.32
	5:1	765.28 ± 127.51	0.126	+28.73 ± 1.31
	20:1	749.31 ± 6.39	0.131	+32.8 ± 2.48
	50:1	697.38 ± 22.16	0.132	+38.4 ± 1.31
	75:1	689.32 ± 14.76	0.118	+39.37 ± 1.59
PPP/miR-34a	2:1	298.20 ± 15.06	0.439	+1.19 ± 0.82
	5:1	287.60 ± 30.12	0.408	+3.67 ± 0.69
	75:1	234.89 ± 25.40	0.209	+5.71 ± 0.37
	100:1	226.19 ± 50.59	0.311	+7.55 ± 1.09
	150:1	135.13 ± 15.61	0.379	+8.39 ± 1.54