Supporting Information

Cationic polymer nanoparticles mediated delivery of miR-124 impairs tumorigenicity of prostate cancer cells

Raffaele Conte,^{a,‡} Anna Valentino,^{b,‡} Ilenia De Luca,^b Gianfranco Peluso,^a Pierfrancesco Cerruti,^{c,*} Anna Di Salle,^a Anna Calarco^{a,*}

^aResearch Institute on Terrestrial Ecosystems (IRET-CNR), Via P. Castellino 111 80131 Napoli, Italy

^bElleva Pharma Srl, Via P. Castellino 111 80131 Napoli, Italy

^cInstitute for Polymers, Composites and Biomaterials (IPCB-CNR), Via Campi Flegrei 34 80078 Pozzuoli (Na), Italy

Correspondence to: Pierfrancesco Cerruti. email: <u>cerruti@ipcb.cnr.it</u>; Anna Calarco. email: <u>anna.calarco@cnr.it</u>

Contents

Table	S1.	Size	of	PHB	NPs	(nm)	as	а	function	of	PHB	and	Pluronic®	F-127
concent	tration	l	•••••		•••••					•••••		•••••		
Table S	5 2. Co	ncentr	atior	ı (mmo	1 -NH2	/g) of -	NH ₂	gro	ups on the	PHB	-PEI N	Ps sur	face	S4
Figure	S1. E	valuati	ion c	of cytot	oxicity	v in Ca	co-2,	, M	CF-7 and l	MCF	10A ce	ells aft	er 6, 24, 48	and 72
hours o	f incu	bation	with	PHB-	PEI NI	Ps		••••		••••			•••••••••••••••••••••••••••••••••••••••	S5
Figure	S2. D	Degrada	ation	assay	of fre	e miRN	NA o	or m	iR-124 N	Ps at	N/P ra	atio of	1:1, 5:1, a	nd 10:1
treated	with 1	RNase	A f	or 60 1	ninute	s. Rele	ased	nu	cleotides v	vas r	neasure	ed by	spectrophot	ometric
continu	ous de	etection	n (λ	= 260 r	۱m)				• • • • • • • • • • • • •					S6

PHB concentration	Pluronic [®] F-127 concentration (wt%)								
(mg/ml)	0.1	0.5	1	2					
8.33	298.9 ± 48.8	153.3 ± 44.1	191.7 ± 49.7	138.3 ± 44.9					
16,7	455.4 ± 95.7	254.5 ± 43.2	231.4 ± 55.0	167.7 ± 44.1					
33,3	644.6 ±115.8	425.2 ± 87.9	381.0± 65.0	305.4 ± 59.1					
41.7	705.5 ± 235.8	554.4 ± 97.7	502.5± 85.4	450.3 ± 73.5					

Table S1. Size of PHB NPs (nm) as a function of PHB and Pluronic[®] F-127 concentration.

PEI concentration	Reaction conditions											
(% w/v)												
	5 min at	15 min at	30 min at	60 min at	5 min at	15 min at	30 min	60 min	5 min at	15 min at	30 min at	60 min at
	25 °C	25 °C	25 °C	25 °C	50 °C	50 °C	at 50 °C	at 50 °C	100 °C	100 °C	100 °C	100 °C
12	12.3	15.4	14.4	13.4	33.1	32.2	34.3	36.4	29.3	30.3	30.4	31.2
24	13.3	16.4	14.4	15.2	31.3	38.2	36.4	35.3	34.2	34.1	32.4	33.3
48	11.2	14.1	11.4	10.2	35.1	33.2	34.3	33.3	33.4	31.2	29.1	26.3

Table S2. Concentration (mmol -NH $_2$ /g) of -NH $_2$ groups on the PHB-PEI NPs surface.





Figure S1. Cytotoxicity was determined in Caco-2, MCF-7 and MCF10A cells after 6, 24, 48 and 72 hours of incubation with varying concentrations of PHB-PEI NPs using the CCK-8 assay: (a, b and c; Caco-2, MCF-7 and MCF10A respectively), and the LDH assay (d, e and f; Caco-2, MCF-7 and MCF10A respectively). Untreated cells were used as control. Data represent the mean±SD for three independent measurements.





Figure S2. Degradation assay of free miRNA or miR-124 NPs at N/P ratio of 1:1, 5:1, and 10:1 treated with RNase A for 60 minutes. Released nucleotides were measured by UV spectrophotometric continuous detection ($\lambda = 260$ nm).