

Supplemental Information

Poly(ethylene glycol)-poly(beta-amino ester)-based nanoparticles for suicide gene therapy enhance brain penetration and extend survival in a preclinical human glioblastoma orthotopic xenograft model

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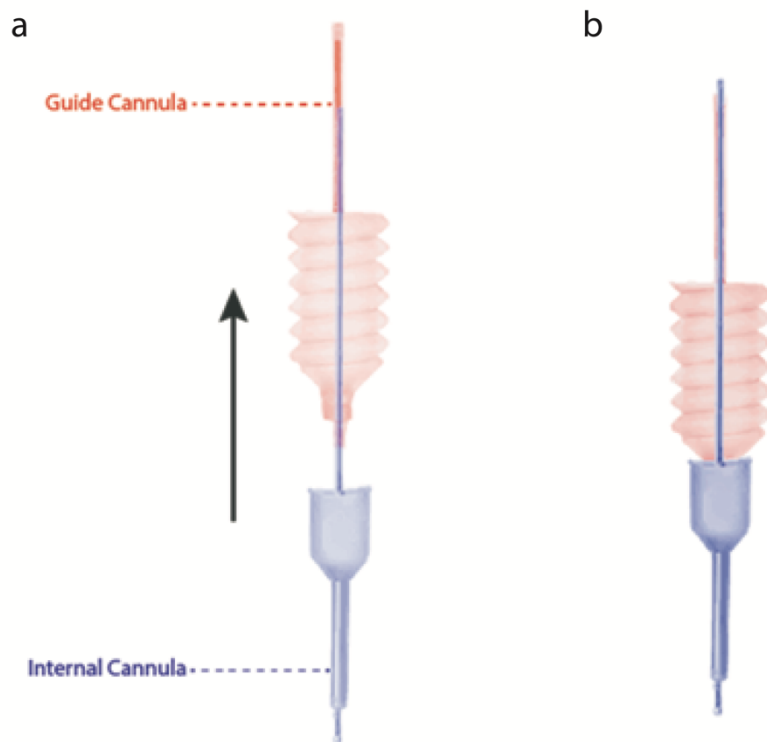


Figure S1. (a) Schematic depicting the components and assembly of the cannula and (b) the final assembled cannula.

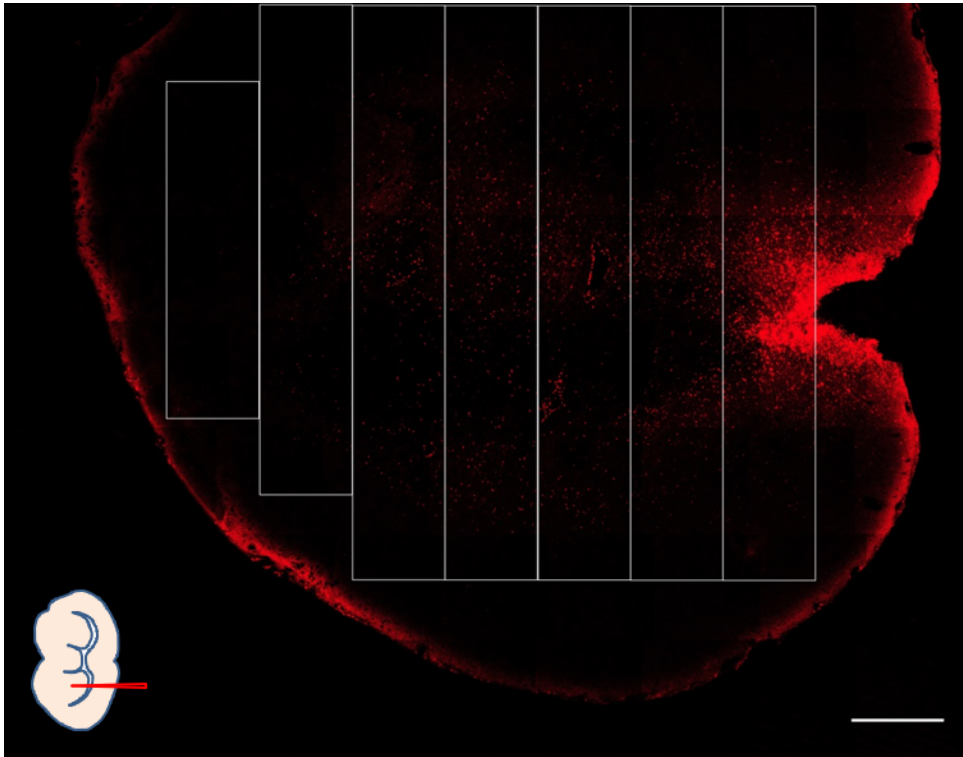


Figure S2. Representative image of nanoparticle penetration measurement. Width of each rectangle is 500 μm (scale bar = 500 μm).

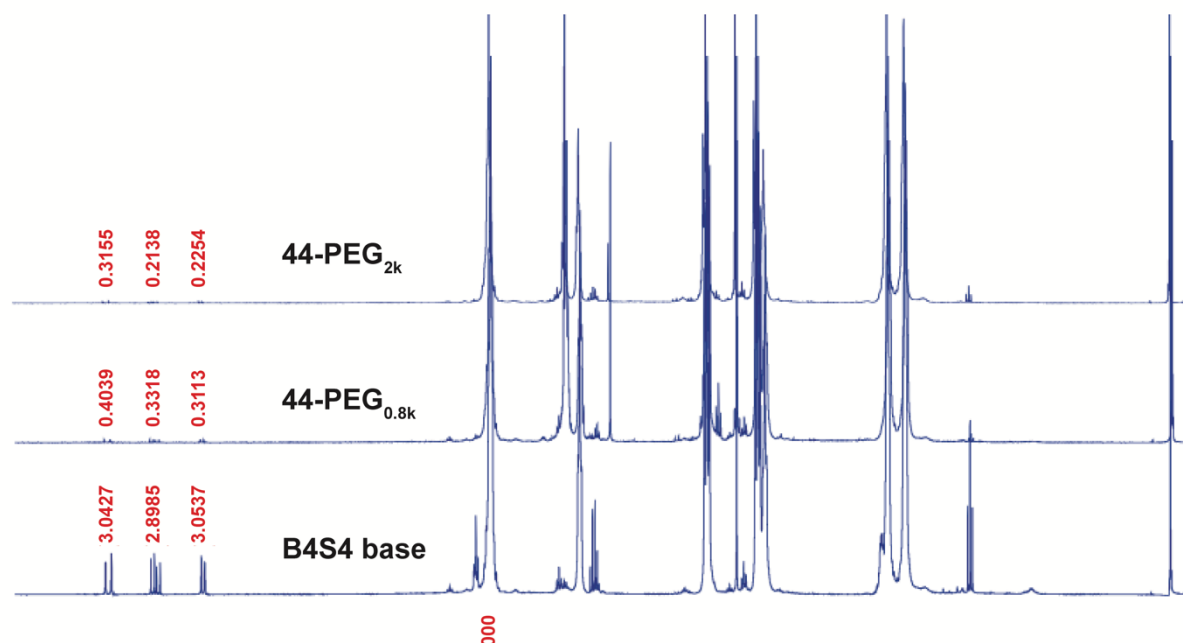


Figure S3. ¹H-NMR. ¹H-NMR spectra of B4S4 base PBAE polymer and 44-PEG_{0.8k} and 44-PEG_{2k} PEG-PBAE polymers. The absence of peaks for hydrogens from acrylate groups in 44-PEG_{0.8k} and 44-PEG_{2k} PEG-PBAE polymers shows complete reaction of PEG-thiol conjugation.

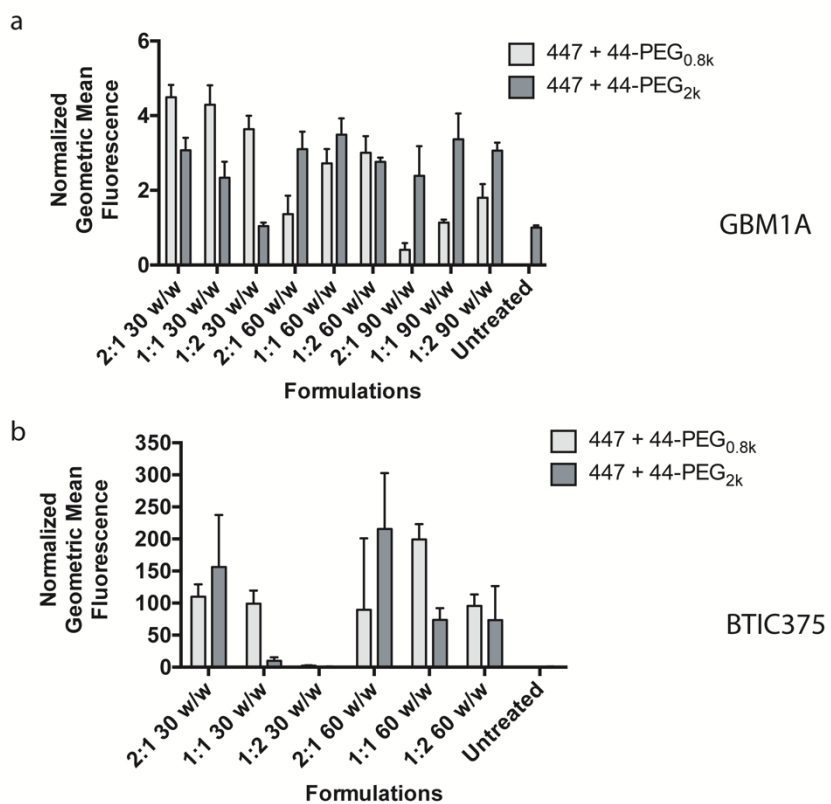


Figure S4. Transfection efficacy of PEG-PBAE-based NPs in GBM1A and BTIC375 cells. Geometric mean fluorescence intensity of pEGFP expression after treatment with 18 or 12 PEG-PBAE NP formulations normalized to untreated control in (a) GBM1A and (b) BTIC375 cells (n=4, mean ± s.d.).

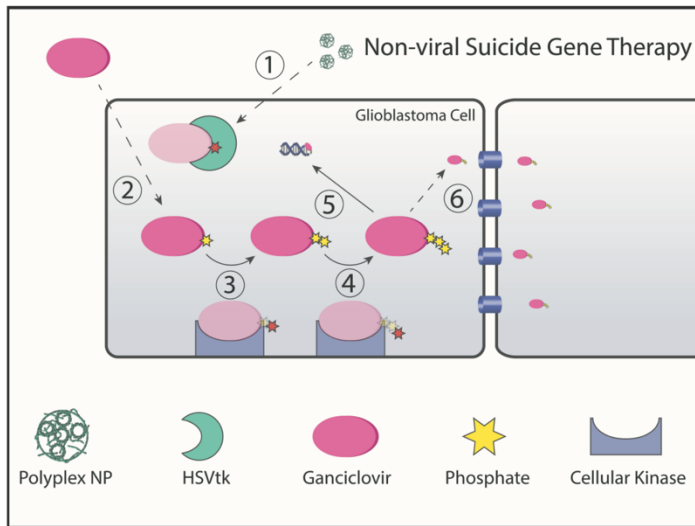


Figure S5. Schematic diagram illustrating GCV activation and bystander effect. (1) Gene delivery, (2) GCV entry and HSVtk-mediated activation, (3-4) GCV cellular activation, (5) inhibition of DNA synthesis, and (6) bystander effect.

ePBAE	PEG-PBAE	ePBAE:PEG-PBAE	Total polymer:DNA	ePBAE:PEG-PBAE:DNA
any	-	-	30	30 : 0 : 1
any	-	-	60	60 : 0 : 1
any	-	-	90	90 : 0 : 1
447	44-PEG _{0.8k}	2:1	30	20:10:1
447	44-PEG _{0.8k}	1:1	30	15:15:1
447	44-PEG _{0.8k}	1:2	30	10:20:1
447	44-PEG _{0.8k}	2:1	60	40:20:1
447	44-PEG _{0.8k}	1:1	60	30:30:1
447	44-PEG _{0.8k}	1:2	60	20:40:1
447	44-PEG _{0.8k}	2:1	90	60:30:1
447	44-PEG _{0.8k}	1:1	90	45:45:1
447	44-PEG _{0.8k}	1:2	90	30:60:1
447	44-PEG _{2k}	2:1	30	20:10:1
447	44-PEG _{2k}	1:1	60	15:15:1
447	44-PEG _{2k}	1:2	90	10:20:1
447	44-PEG _{2k}	2:1	30	40:20:1
447	44-PEG _{2k}	1:1	60	30:30:1
447	44-PEG _{2k}	1:2	90	20:40:1
447	44-PEG _{2k}	2:1	30	60:30:1
447	44-PEG _{2k}	1:1	60	45:45:1
447	44-PEG _{2k}	1:2	90	30:60:1

Table S1. Effective mass ratios of ePBAE and PEG-PBAE polymers in NP formulations.

Day 6		PBAE						
PEG-PBAE	GCV (µg/mL)	0	5	10	20	50	100	200
	0	-	ns	***	****	****	****	****
	5	-	-	ns	****	****	****	****
	10	-	-	-	ns	****	****	****
	20	-	-	-	-	****	ns	ns
	50	-	-	-	-	-	***	****
	100	-	-	-	-	-	-	ns
	200	-	-	-	-	-	-	-

Day 6		PBAE						
PBAE	GCV (µg/mL)	0	5	10	20	50	100	200
	0	-	ns	***	****	****	****	****
	5	-	-	***	****	****	****	****
	10	-	-	-	****	****	****	****
	20	-	-	-	-	****	****	****
	50	-	-	-	-	-	****	****
	100	-	-	-	-	-	-	ns
	200	-	-	-	-	-	-	-

Day 6		PEG-PBAE						
PEG-PBAE	GCV (µg/mL)	0	5	10	20	50	100	200
	0	-	ns	****	****	****	****	****
	5	-	-	****	****	****	****	****
	10	-	-	-	****	****	****	****
	20	-	-	-	-	**	****	****
	50	-	-	-	-	-	*	***
	100	-	-	-	-	-	-	ns
	200	-	-	-	-	-	-	-

Table S2. Statistical significance in day 6 cell viability between conditions treated with PBAE, PEG-PBAE NP, and different GCV concentrations.