Supporting Information

Biodegradable Polyester Nanoparticle Vaccines Deliver Self-Amplifying mRNA in Mice at Low Doses

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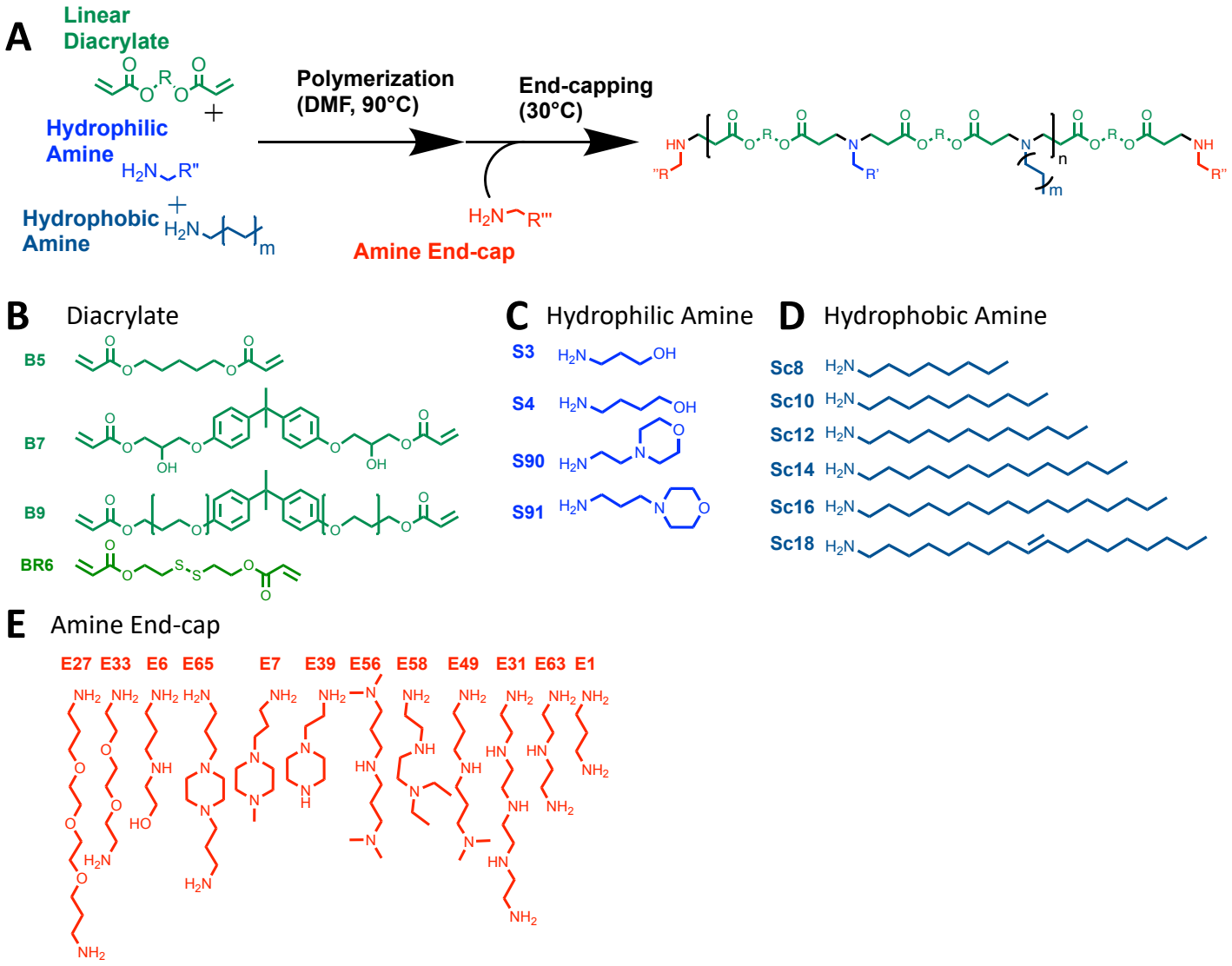


Figure S1. PBAE Synthesis. (A) Generalized synthesis of PBAEs in DMF followed by polymer end-capping with small molecule amine endcaps to yield linear, amphiphilic PBAEs. **(B)** Selected diacrylate monomers. **(C)** Selected hydrophilic amine monomers. **(D)** Selected hydrophobic amine monomers. **(E)** Selected amine end-cap monomers used in synthesis of the presented PBAEs.

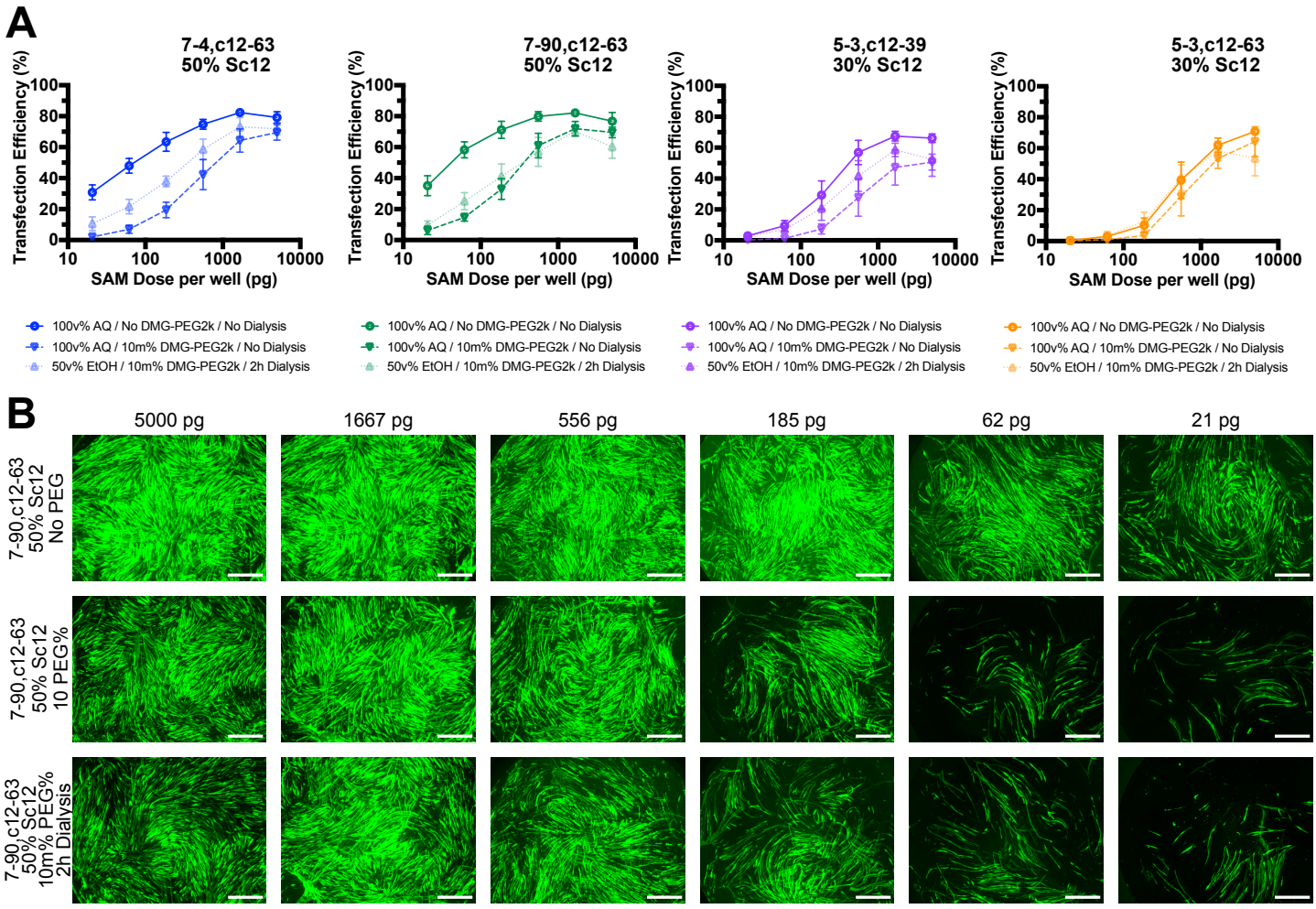


Figure S2. Inclusion of PEG-lipid does not improve transfection *in vitro*. (A) Transfection efficiency of SAM nanoparticles encoding eGFP added to confluent monolayers of C2C12 cells with SAM nanoparticles encoding eGFP formed under three different conditions. Solid lines show results for NPs prepared in 100v% aqueous $MgAc_2$ buffer with no DMG-PEG2k. Dashed lines show NPs prepared in 100v% aqueous $MgAc_2$ buffer with 10m% DMG-PEG2k. Dotted lines show NPs prepared in 50v% aqueous $MgAc_2$ buffer and 50v% EtOH with 10m% DMG-PEG2k followed by dialysis for 75 minutes into 150 mM PBS, pH 7.4. Inclusion of DMG-PEG2k reduced transfection efficiency *in vitro*, which was partially rescued in some formulations by dialysis. Points show ± SD of four wells of a 96 well plate. **(B)** Representative microscope images of transfected wells of C2C12 myoblasts showing reduction in efficiency of transfection of PEGylated formulations for polymer 7-4,c12-63, 50% Sc12 *in vitro* at doses below 185 pg per well. Scale bars indicate 1 μm.

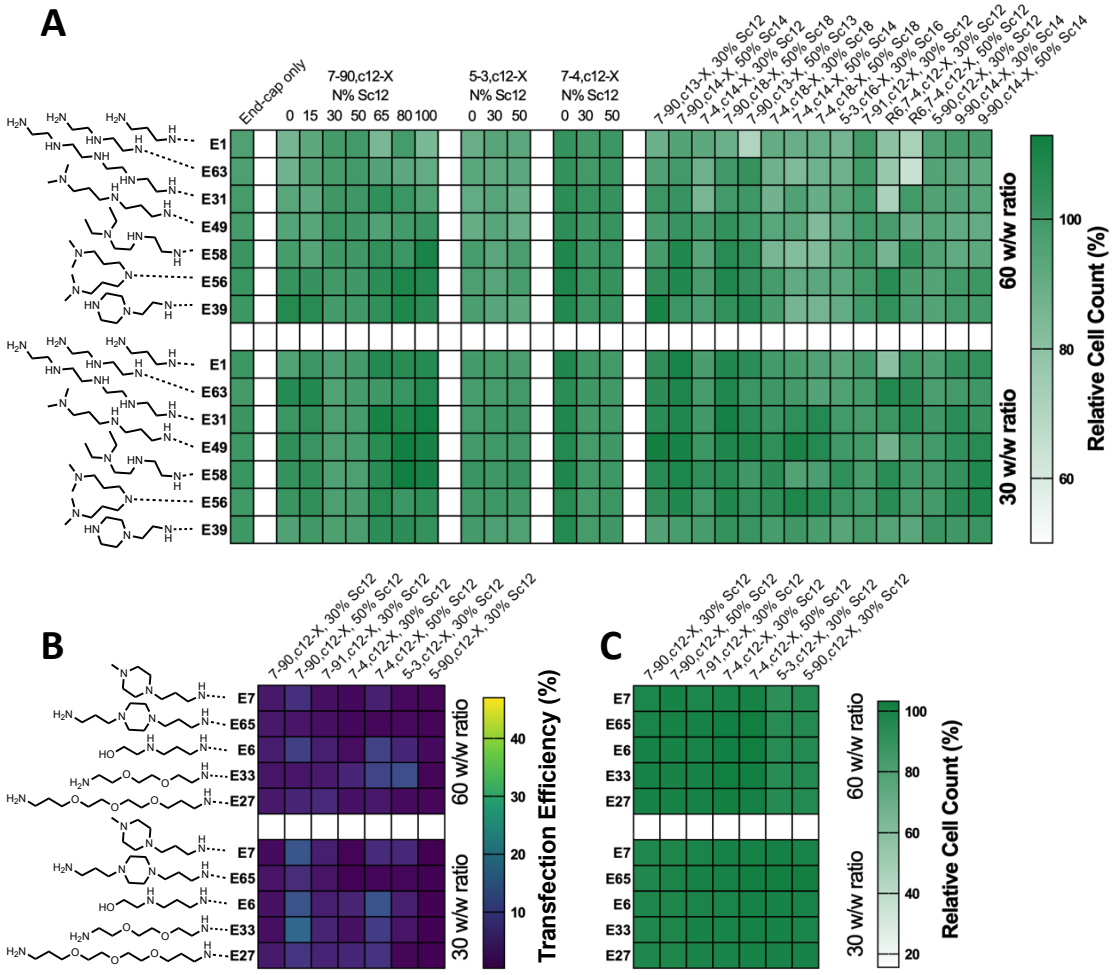
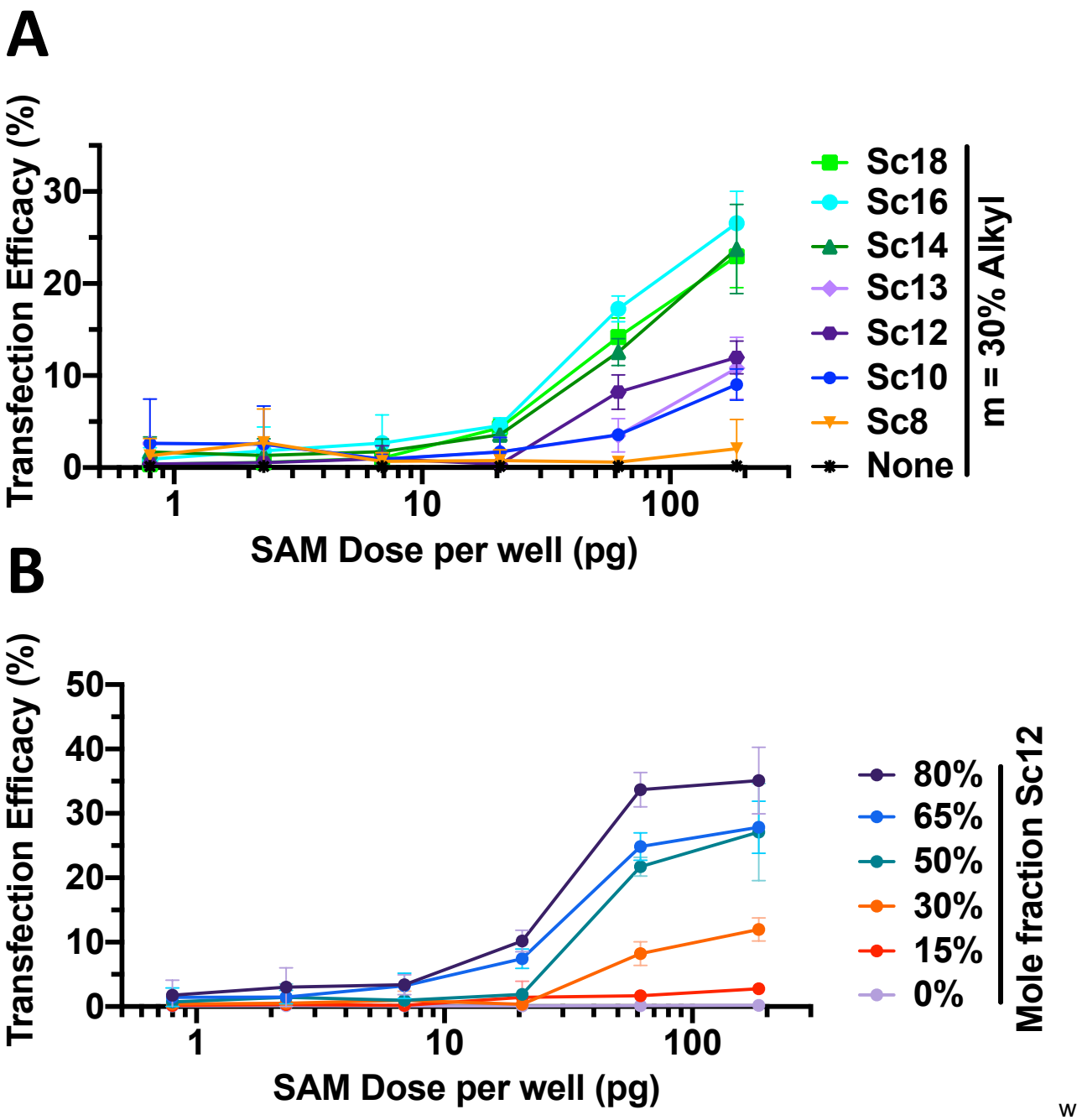
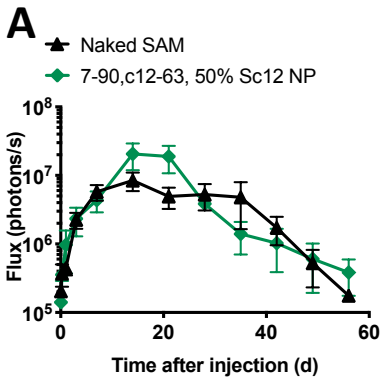


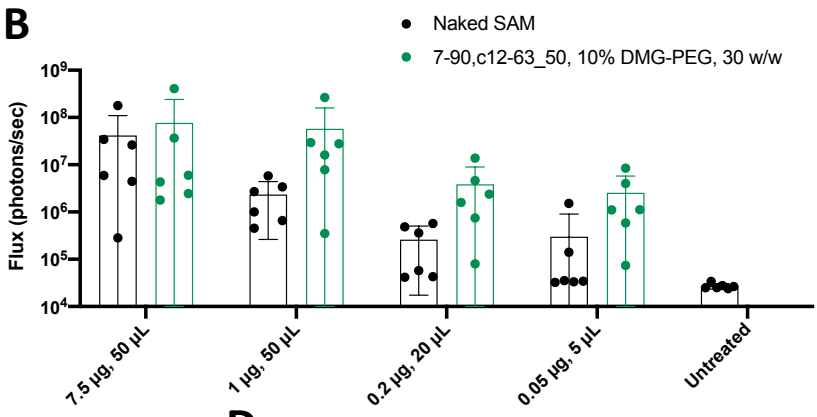
Figure S3. Supporting data for *in vitro* screening of PBAE polymers. (A) *In vitro* transfection relative cell viability assessed by nuclei counting normalized to untreated wells. Each well represents the mean of two wells of a 384-well plate. **(B)** Additional minimally effective polymers screened for SAM delivery in 384-well plates for transfection efficiency using eGFP SAM at a dose of 1 ng/well relative to untreated cells and **(C)** Cellular viability of these polymers as assessed by nuclei counting relative to untreated cells.



w

Figure S4. Alkyl-side chain hydrophobicity influence on *in vitro* transfection of SAM to differentiated C2C12 myoblasts. Experiments were performed using polymer 7-90,Sc_n-63 with alkyl side-chains of length n and m% mole fraction alkyl side-chain monomer. All nanoparticles were prepared with GFP SAM at 30 w/w ratio without DMG-PEG2k**. (A)** Increasing the alkyl-amine side-chain length while maintaining mole fraction of alkyl side-chain at 30% demonstrated that more hydrophobic alkyl-side chains increased efficacy of transfection *in vitro.* **(B)** Increasing the alkyl-amine side-chain mole fraction while keeping the alkyl length constant at 12 carbons similarly increased transfection *in vitro*.





C

Vaccine	Dose (µg)	Injection volume (µL)	mRNA concentration injected (ng/µL)
BioNTech	30	300	100
Moderna	100	500	200

D

Dose (µg)	Injection volume (µL)	SAM concentration injected (ng/µL)	Fold NP luminescence over naked SAM
7.5	50	150	1.84
1	50	20	24.64
0.2	20	10	14.86
0.05	5	10	8.51

E

	Mouse	Human	Scaling Factor:
Body Surface Area (m^2)	0.007	1.6	(*228)

F

Injection volume scaling by body surface area	
Mouse (µL)	Human (mL) (*228)
50	11.4
20	4.6
5	1.1

G

Injection dose scaling by body surface area		Nucleic Acid Concentration Required for 0.5 mL injection volume (ng/µL)
Mouse mRNA dose (µg)	Human mRNA Surface Area Scaled Dose (µg) (*228)	
7.5	1710	3420
1	228	456
0.2	45.6	91
0.05	11.4	23

Figure S5. **Selection of murine intramuscular dose and timepoint for luminescence measurements. (A)** Identification of peak expression of SAM following intramuscular injection between 10-21 days with a dose of 2 µg of SAM packaged in 7-90,c12-63, 50% Sc12 nanoparticles formulated with 10 mass% DMG-PEG2k. **(B)** Intramuscular injection volume and dose titration in mice of naked SAM and PBAE nanoparticles. **(C)** Injection doses and volumes used for SARS-CoV-2 mRNA vaccines used clinically. **(D)** Injection dose, volume, SAM concentration and fold luminescence over naked SAM achieved using PBAE nanoparticles. **(E)** Body surface area scaling between mice and adult humans results in a scaling factor of 228 using a lower end body surface area of 1.6 m^2 for an adult human female. Using a surface area of 1.9 m^2 for an adult human male would result in a higher scaling factor. **(F)** Scaling intramuscular injection volumes by body surface area results in injection volumes greater than acceptable for human intramuscular injection volumes (red), where 0.5 mL is considered a maximum acceptable injection volume. **(G)** Calculations for scaling intramuscular mRNA doses from those evaluated in mice (0.05 µg to 7.5 µg) to an equivalent surface area scaled dose for administration in humans. Given the dosage constraints, a 0.2 µg dose (green) was selected for evaluation of intramuscular administration in mice.

Table S1. Monomers used for PBAE Synthesis

	Chemical Name	Supplier	Cas Number	Product Number
Linear Diacrylate Monomers				
B5	1,5-Pentanediol diacrylate	Bajae Labs	36840-85-4	9281
B7	Bisphenol A glycerolate (1 glycerol/phenol) diacrylate	Sigma Aldrich	4687-94-9	411167
B9	Bisphenol A ethoxylate (1.5 EO/phenol) diacrylate	Sigma Aldrich	64401-02-1	413550
BR6	2,2-disulfanediylbis(ethane-2,1-diyl) diacrylate	Synthesized according to Kozielski et al. 2013	N/A	N/A
Hydrophilic Amino Monomers				
S3	3-amino-1-propanol	Alfa Aesar	156-87-6	B23041
S4	4-amino-1-butanol	Fisher Scientific	13325-10-05	AC176350050
S90	4-(2-Aminoethyl)morpholine	Sigma Aldrich	2038-031	A55004
S91	3-Morpholinopropylamine	Sigma Aldrich	123-00-2	A9028
Hydrophobic Amino Monomers				
Sc8	Octylamine	Sigma Aldrich	111-86-6	O5802
Sc10	1-decylamine	Sigma Aldrich	2016-57-1	30692
Sc12	1-dodecylamine	Alfa Aesar	124-22-1	A15515
Sc13	Tridecylamine	TCI America	2869-34-3	A0762
Sc14	Tetradecylamine	Acros Organics	2016-42-4	AC138100500
Sc16	Hexadecylamine	Acros Organics	143-27-1	AC120510050
Sc18	Oleylamine	Sigma Aldrich	112-90-3	O7805
End-cap Amino Monomers				
E1	1,3-diaminopropane	Sigma Aldrich	109-76-2	D23602
E6	2-(3-Aminopropylamino)ethanol	Sigma Aldrich	4461-39-6	9293
E7	1-(3-Aminopropyl)-4-methylpiperazine	Alfa Aesar	4572-031	L04876
E27	4,7,10-Trioxa-1,13-tridecanediamine	Sigma Aldrich	4246-51-9	369519
E31	Tetraethylenepentamine	Sigma Aldrich	1112-57-2	T11509
E33	2,2′-(Ethylenedioxy)bis(ethylamine)	Sigma Aldrich	929-59-9	385506
E39	1-(2-Aminoethyl)piperazine	Sigma Aldrich	140-31-8	A55209

E49	N,N-Dimethyldipropylenetriamine	Sigma Aldrich	10563298	550019
E56	3,3′-Iminobis(N,N-dimethylpropylamine)	Santa Cruz	6711484	SC-231967
E58	N,N-Diethyldiethylenetriamine	Sigma Aldrich	24426-16-2	518832
E63	Diethylentriamine	Sigma Aldrich	111-40-0	803274
E65	1,4-Bis(3-aminopropyl)piperazine	TCI America	7209-38-3	B1041

Table S2. PBAE synthesis conditions. All were synthesized at 90°C for 48 hours in DMF. Arranged to match Figure 2A.

Base polymer Name	Overall Vinyl : Amine Monomer Ratio	Alkyl Side-chain Monomers	Bioreducible Monomers	Synthesis Conc (mg/mL)
7-90-	2.173	0	0	400
7-90,c12-	2.3	15% Sc12	0	600
7-90,c12-	2.3	30% Sc12	0	600
7-90,c12-	2.3	50% Sc12	0	600
7-90,c12-	2.3	65% Sc12	0	600
7-90,c12-	2.3	80% Sc12	0	600
7-90,c12-	2.4	100% Sc12	0	300
5-3-	2.187	0	0	Neat
5-3,c12-	2.1	30% Sc12	0	600
5-3,c12-	2.1	50% Sc12	0	600
5-3,c12-	2.1	50% Sc12	0	600
7-4-	2.182	0	0	400
7-4,c12-	2.205	30% Sc12	0	400
7-4,c12-	2.235	50% Sc12	0	400
7-90,c13-	2.300	30% Sc13	0	
7-90,c13-	2.3	50% Sc13	0	600
7-90,c14-	2.4	50% Sc14	0	600
7-90,c18-	2.3	50% Sc18	0	600
5-3,c16-	2.1	30% Sc16	0	600
7-4,c14-	2.3	30% Sc14	0	600
7-4,c14-	2.3	50% Sc14	0	600
7-4,c18-	2.3	30% Sc18	0	600
7-4,c18-	2.3	50% Sc18	0	600
R6,7-4,c12-	2.040	30% Sc12	60% BR6	500
R6,7-4,c12-	2.040	50% Sc12	60% BR6	500
7-91,c12-	2.400	30% Sc12	0	400
5-90,c12-	2.100	30% Sc12	0	600
9-90,c14-	2.4	30% Sc14	0	600
9-90,c14-	2.4	50% Sc14	0	600
7-90,c8-	2.3	30% Sc8	0	600
7-90,c10-	2.3	30% Sc10	0	600
7-90,c12-	2.3	30% Sc12	0	600
7-90,c14-	2.3	30% Sc14	0	600
7-90,c16-	2.3	30% Sc16	0	600
7-90,c18-	2.3	30% Sc18	0	600

Table S3. PBAE characterization properties assessed by gel permeation chromatography. Arranged to match Figure 2A.

Base polymer Name	GPC MN (Da)	GPC MW (Da)	GPC MP (Da)	GPC PDI
7-90-	7164	20896	8158	2.917
7-90,c12-	7473	12142	8637	1.620
7-90,c12-	7299	15946	7554	2.180
7-90,c12-	5880	17707	6394	3.012
7-90,c12-	5820	14530	7070	2.500
7-90,c12-	7217	17190	10092	2.380
7-90,c12-	5083	9148	6083	1.800
5-3-	5316	15919	6394	2.994
5-3,c12-	5446	7425	6602	1.363
5-3,c12-	5168	6535	6404	1.264
5-3,c12-	7492	8253	7241	1.100
7-4-	6851	21161	7520	3.089
7-4,c12-	4762	10408	5844	2.186
7-4,c12-	4291	9892	4920	2.305
7-90,c13-	5746	13596	6259	2.366
7-90,c13-	9382	45293	227896	4.830
7-90,c14-	7493	33512	16646	4.472
7-90,c18-	5816	20701	6146	3.559
5-3,c16-	6741	7737	7006	1.150
7-4,c14-	5513	12770	6450	2.316
7-4,c14-	6589	34004	187514	5.161
7-4,c18-	5694	14412	6611	2.531
7-4,c18-	7516	35262	193540	4.691
R6,7-4,c12-	4514	11360	5270	2.517
R6,7-4,c12-	3348	4784	3809	1.429
7-91,c12-	6080	17560	7299	2.876
5-90,c12-	6009	6485	5983	1.079
9-90,c14-	4290	5263	5667	1.227
9-90,c14-	4414	5406	5818	1.225
7-90,c8-	4358	4993	4980	1.15
7-90,c10-	9330	28457	16255	3.05
7-90,c12-	7299	15946	7554	2.18
7-90,c14-	7578	15557	7508	2.05
7-90,c16-	8774	26958	14872	3.07
7-90,c18-	8759	26516	15197	3.03