Supporting Information

Versatile Redox-Responsive Polyplexes for the Delivery of Plasmid DNA, Messenger RNA, and CRISPR-Cas9 Genome-Editing Machinery

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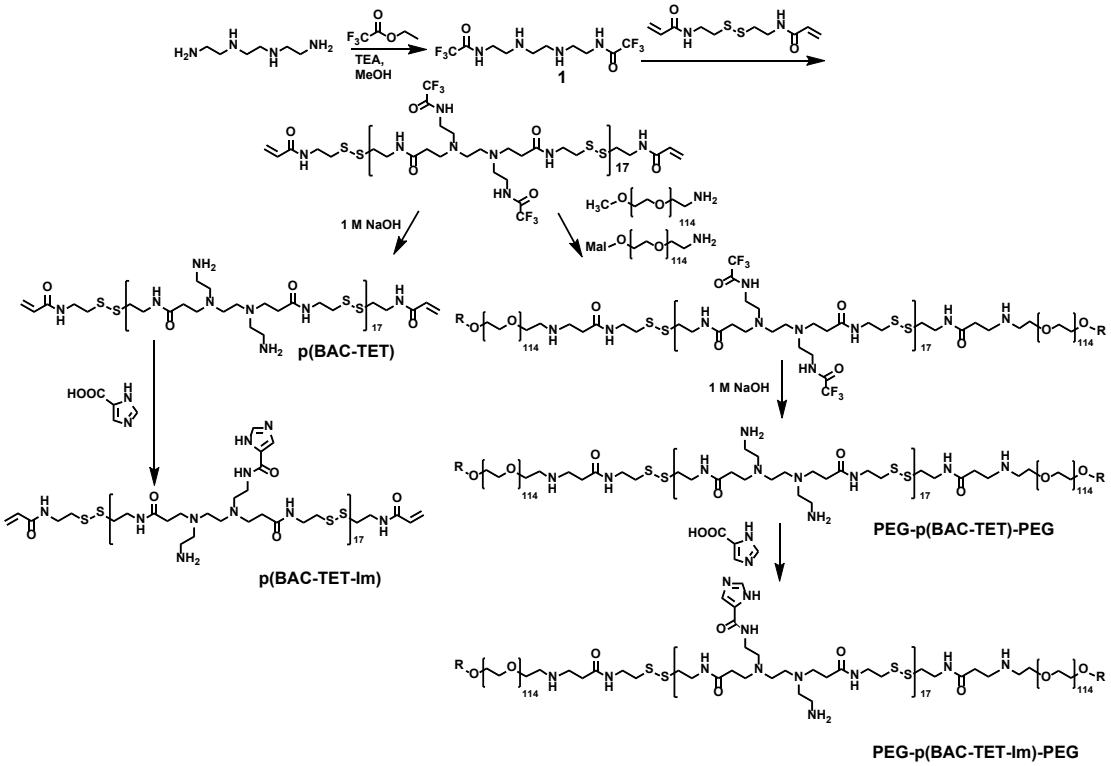

Figure S1. Synthesis scheme for the PBAP polymers, p(BAC-TET-Im) and PEG-p(BAC-TET-Im)-PEG, used to form non-crosslinked PBAP polyplexes.

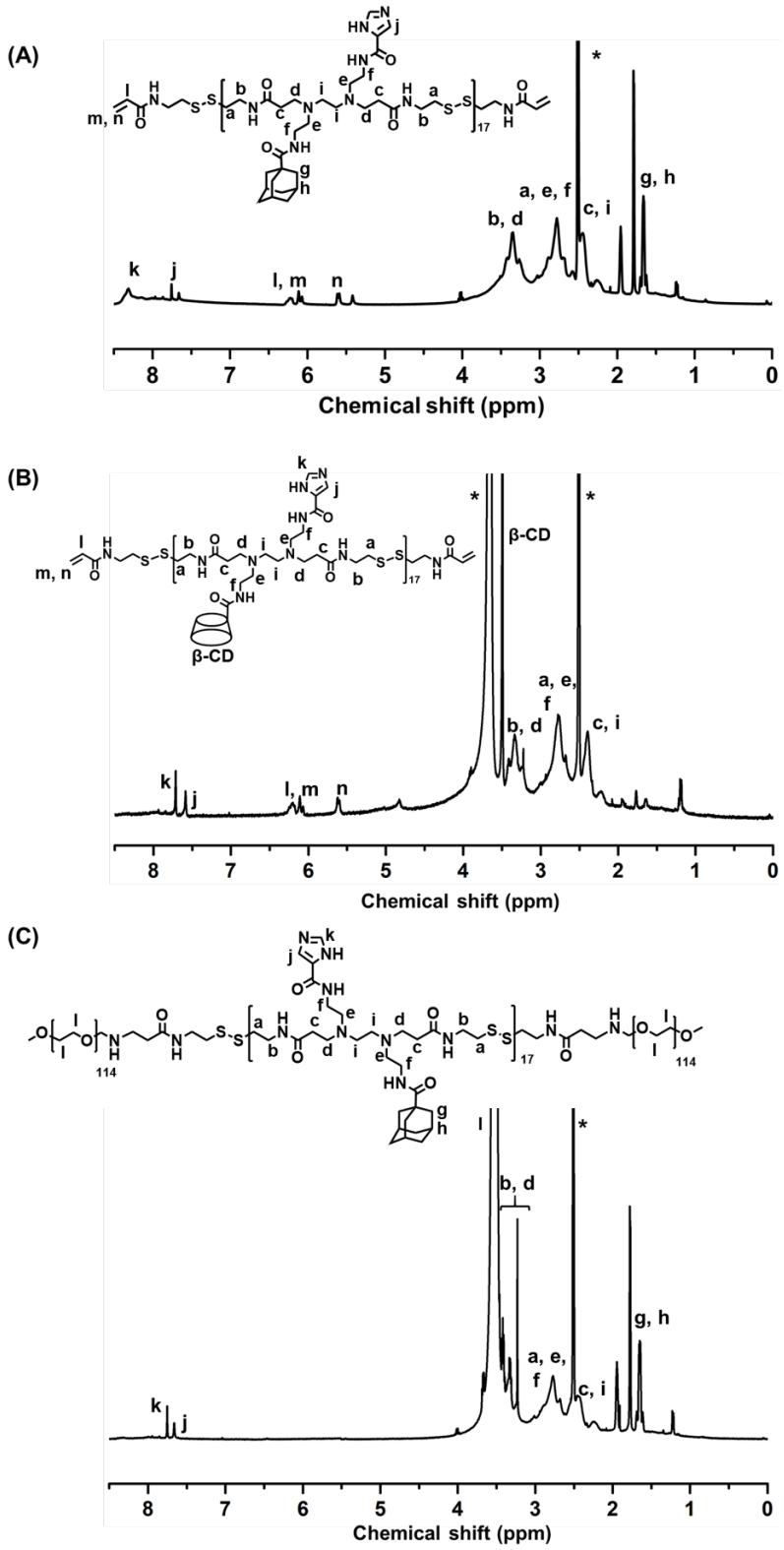

Figure S2. ^{1}H-NMR spectrums of p(BAC-TET-AD/Im) (A), p(BAC-TET-Im/β-CD) (B), and PEG-p(BAC-TET-AD/Im)-PEG (C) used to form crosslinked polyplexes. *: solvent residual peak.

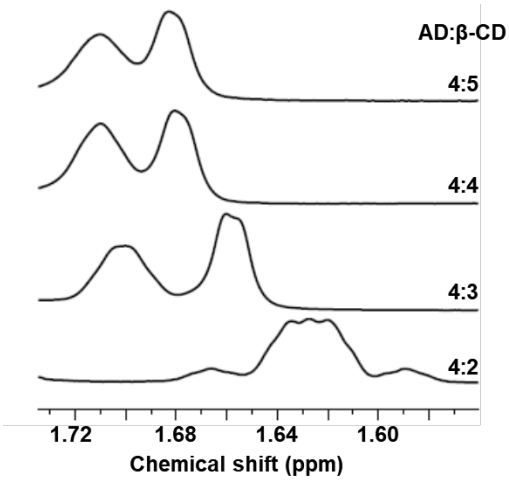

Figure S3. Confirmation of host–guest interactions between β-CD and AD present in the CLPBAP polymers by ^{1}H-NMR.

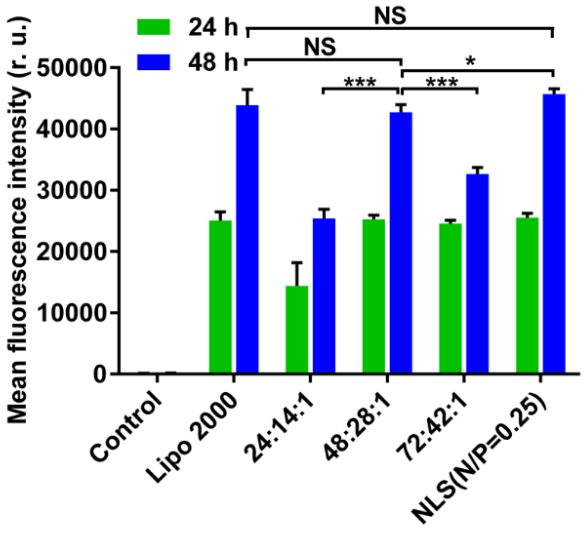

Figure S4. Effects of DNA polyplex formulation on the mean fluorescence intensity (MFI) of non-crosslinked DNA polyplexes. Cells were treated with Lipo 2000, non-crosslinked polyplexes with three different PBAP:PEG-PBAP-PEG:DNA weight ratios, and a polyplex with a PBAP:PEG-PBAP-PEG:DNA weight ratio of 48:28:1 with NLS (N/P ratio of 0.25). NS: not significant; *: $p < 0.05$; ***: $p < 0.001$.

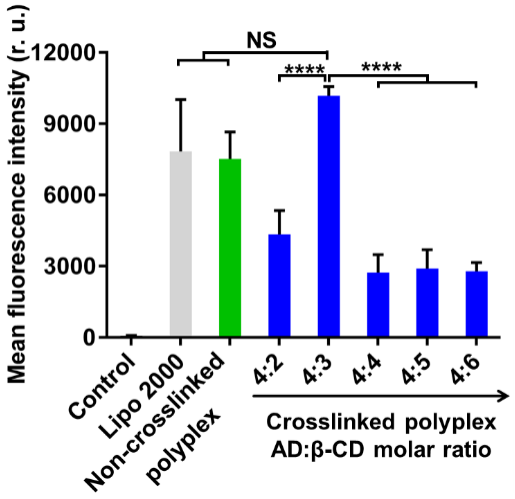

Figure S5. Effects of AD:β-CD molar ratios on the MFI of cells treated with crosslinked DNA polyplexes with different AD:β-CD molar ratios. HEK 293 cells were treated with Lipo2000, non-crosslinked PBAP polyplexes, and crosslinked CLPBAP polyplexes with different crosslinker molar ratios. The MFI was measured 48 h post treatment. NS: not significant; ****: $p < 0.0001$.

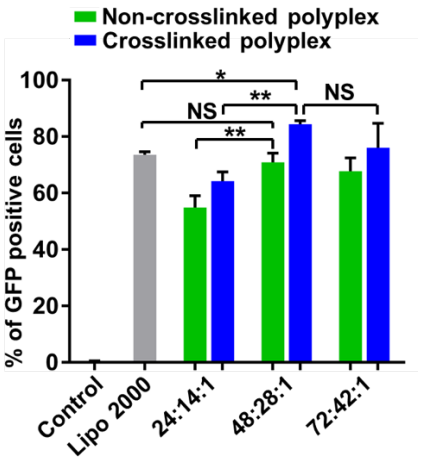

Figure S6. Optimization of PBAP-based polymers and mRNA weight ratios for mRNA transfection. HEK293 cells were treated with Lipo 2000, non-crosslinked polyplexes with three different PBAP:PEG–PBAP–PEG:mRNA weight ratios, and crosslinked polyplexes with three different CLPBAP:PEG–CLPBAP–PEG:mRNA weight ratios. In the crosslinked polyplexes, the AD:β-CD molar ratio was fixed at 4:3. NS: not significant; *: $p < 0.05$; **: $p < 0.01$.

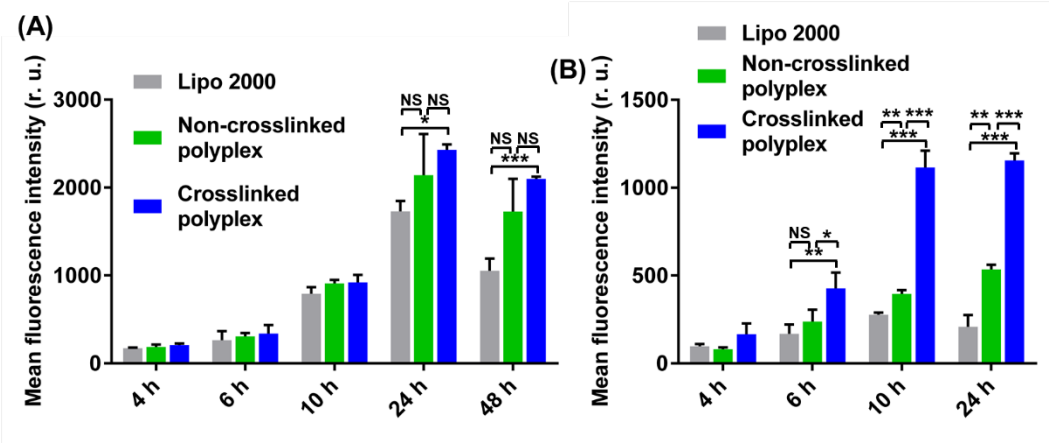

Figure S7. MFI analysis of non-crosslinked and crosslinked mRNA polyplexes in (A) HEK 293 cells and (B) RAW 264.7 cells at various time points. The weight ratio of PBAP:PEG–PBAP–PEG:mRNA in the non-crosslinked polyplexes and the weight ratio of CLPBAP:PEG–CLPBAP–PEG:mRNA in the crosslinked polyplexes were both fixed at 48:24:1. NS: not significant, *: $p < 0.01$; **: $p < 0.01$; ***: $p < 0.001$.

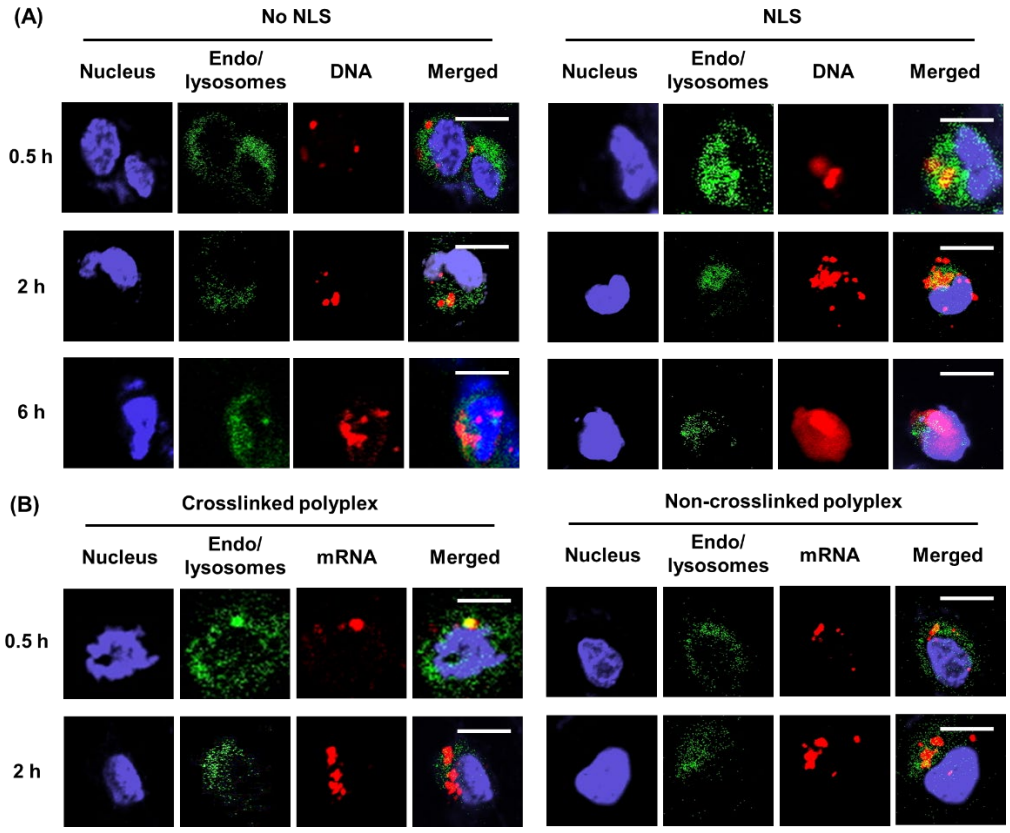

Figure S8. **(A)** Intracellular trafficking of the non-crosslinked DNA polyplexes with and without NLS. **(B)** Intracellular trafficking of the non-crosslinked and crosslinked mRNA polyplexes. Scale bar: 10 μm.

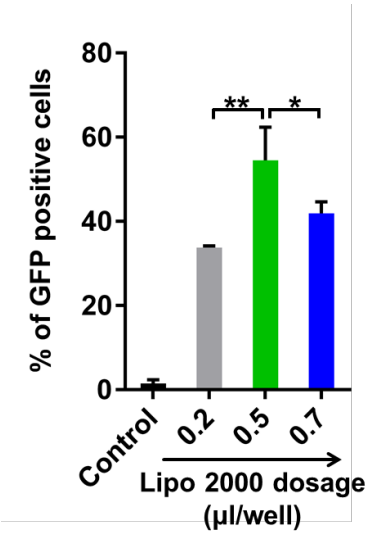

Figure S9. Optimization of the Lipo 2000 dosage using DNA as the payload. *: $p < 0.01$; **: $p < 0.01$; $n = 3$.

Table S1. Molecular weights of the polymers used to form the non-crosslinked and crosslinked polyplexes measured by GPC.

Polymer	Number-Average Molecular Weight (M_n)	Polydispersity Index (PDI)
p(BAC-TET)	8.5 kDa	1.6
PEG- p(BAC-TET)-PEG	19.1 kDa	1.7
p(BAC-TET-Im/AD)	9.2 kDa	1.7
p(BAC-TET-Im/β-CD)	12.3 kDa	1.9
PEG-p(BAC-TET-Im/AD)-PEG	19.5 kDa	2.1

Table S2. Size and zeta-potential of non-crosslinked PBAP and crosslinked CLPBAP polyplexes.

Polyplex	Payload	Size by DLS (nm)	Zeta potential (mV)
Non-crosslinked	DNA	136	8.6
	mRNA	150	2.0
	RNP	143	6.7
	S1mplex	151	3.5
Crosslinked	DNA	168	7.9
	mRNA	191	-1.5
	RNP	170	5.4
	S1mplex	179	4.5