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Supporting Information

Highly Efficient *In vivo* Editing of Macrophages through Systemic Delivery of CRISPR-Cas9-Ribonucleoprotein-Nanoparticle Nanoassemblies

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SUPPORTING NOTES

Cas9E20 sequence: (E20 at the N-terminus represents E-tag; NLS is underlined at the C-

terminus)

MEEEEEEEEEEEEEEEEEEEMDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDD SFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAIL SARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDT **YDDDLDNLLAOIGDOYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEH** HQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTF RIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLP NEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEOKKAIVDLLFKTNRKVT VKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDI VLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIK KGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLK DDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKL VSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDV RKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELOKGNELALPSKYVNFLYLASHYEKLKGSPE DNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDS RADPKKKRKVAAALEHHHHHH

SUPPORTING FIGURES



Figure S1. DLS characterization of Cas9En-RNP/AuNPs composites. Intensity histogram of composite hydrodynamic size (n=5). Zeta potential consistently remained between 0 and -5.



Figure S2. Serum stability test of nanocomposites in the presence of serum over a 24-hour period.



Figure S3. Biodistribution of gold in mouse different organs, quantitatively analyzed by ICP-MS. Delivery vehicle (+++) n=3, controls n=1.



Figure S4. LA-ICP-MS and optical images of liver and spleen mouse organs of mouse injected with nanocomposites. Optical images are shown with ¹⁹⁷Au, ⁵⁷Fe, and ⁶⁶Zn metal distribution images, Au/Fe distribution is also shown. White arrows signal a vein; yellow arrows signal the red pulp while red arrows show the white pulp. The high sensitivity and spatial resolution of this technique allows us to determine how nanocomposites distribute *in vivo*.



Figure S5. Single line scans of the spleen. The similarity of gold and iron indicates that the gold nanocomposites co-localized with the red pulp.



Figure S6. Efficient gene editing selectively present in organ homogenates resulting from Cas9En-RNP delivery. Experimental conditions and control conditions were shown as En and Cn. The red arrows indicate the two cutting bands after activities of the T7E1 assay.



Figure S7. Quantitative tracking of weight change in mice throughout 6-day experiment.

Tuble 51. Thinle bequeices.				
ID	Sequence (5'->3')			
surv-PtenF	AAACCTCCCGTCCGCCGCCGC			
surv-PtenR	ACCAGGCAAGAGTTCCGTCTAGCCAAACAC			
PTENoff1-F	TCAGACTTTCCGTGCAGTCT			

Table S1. Primer sequences.

PTENoff1-R	ACAAGCGGAAACATCCACAC
PTENoff2-F	TGGACTCTGCTTTGAAGACA
PTENoff2-R	ATAAGCCCTGGGTAAGAGCC
PTENoff3-F	TCCCAGTTCTTCCCAACCTC
PTENoff3-R	ACACACGTTCAGTTTCTGCC
PTENoff4-F	CCAACTCACCCTTCTCCCTT
PTENoff4-R	ACCTTGTGTGAGACTTCCCA



Figure S8. % purity of extracted immune cells from (a) liver and (b) spleen.



Figure S9. Insertion/deletion (indel) frequencies were observed in splenic and hepatic macrophages specifically, as analyzed by T7E1 assay.

T7E1 analysis			
		Spleen Macrophag	ge Liver Macrophage
		=	
		-	
		_	
		-	
		EI EZ E3 E4 E5 CI AuNPs + + + + -	$C_2 C_3 = C_1 C_2 C_3 = C_1 C_2 C_2 C_3 + - AuNPs + + + + - + - + - + + + + + + + + + +$
ICE analysis		Cas 9 + + + + + -	+ + Cas 9 + + + + + - +
Spleen Macropha	iae	sgRNA + + + + + -	— + sgRNA + + + + + — —
INDEL	CONTRIBUTION -	- SEQUENCE	
E1 + 🛛	72.7%	C A T C A A A G A G A T C G T T A G C A G A A A C	A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+2	4.6%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C
-30	2%	C A T C A A A G A G	
-20	1.6%	G A T C A A A G A G A T C G T T A G C	
-23	0.7%	CATCAAAGAGATCGTTA	
+4	0.6%	с а т с а а а б а б а т с б т т а б с а б а а а с	N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A
-11	0.4%	C A T C A A A G A G A T C G T T A G C A G A A A C	
+12	0.1%	с а т с а а а а а а а а т с в т т а в с а в а а а с	
-23	0.1%	C A T C A A A G A G A T C G T	
INDEL	CONTRIBUTION -	SEQUENCE	
E2 + 0	81.3%	C A T C A A A G A G A T C G T T A G C A G A A A C	A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+2	• 3%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N A A A A G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C
-30	1.4%	CATCAAAGAG	
-20	1.2%	C A T C A A A G A G A T	
-21	0.8%	C A T C A A A G A G A T	A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-19	0.6%	C A T C A A A G A G	G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+5	0.4%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T
-1/ +9	0.3%	C A T C A A A G A G A T C G T T A G C A G A A A C	G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G A A C T T G A C
-10	0%	C A T C A A A G A G A T C G T	A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+4	0%	C A T C A A A G A G A T C G T T A G C A G A A A C	: N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A
INDEL	CONTRIBUTION -	SEQUENCE	
E3 + 🛛 0	77.8%	C A T C A A A G A G A T C G T T A G C A G A A A C	A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+2 +6	· 4.4%	C A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A G C A G A A A C	N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G A C C T G
-20	1.796	C A T C A A A G A G A T C G T T A G C A	G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-30	1.6%	C A T C A A A G A G	
-23	0.9%	C A T C A A A G A G A T C G T T A G C	
+4	0.6%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A
+12	0.1%	C A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A	N N N N N N N N N N N A A A A G G A G A
+1	0.196	C A T C A A A G A G A T C G T T A G C A G A A A C	N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C
-11	0%	C A T C A A A G A G A T C G T T A G C A G A A A C	
-19	0%	CATCAAAGAGATCGTT	; ATCAAGAGGATGGATTCGACTTAGACTTGACCTGTATCCA
INDEL	CONTRIBUTION -	· SEQUENCE	
E4 + 0	77.5%	C A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A G C A G A A A C	; A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-30	1.8%	C A T C A A A G A G	
+6	1.8%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G
-20	196	CATCAAAGAGATCGTTAGCA	N N N N A A A A G G A G A T A T C A A G A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-23	0.5%	C A T C A A A G A G A T C G T	
-23	0.5%	C A T C A A A G A G	A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-11	0.4%	C A T C A A A G A G A T C G T T A G C A G A A A C	
-21	0.2%	C A T C A A A G A G	A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+12	0.1%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N N N N N N A A A A G G A G A
-19	0%	CATCAAAGAGA	G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
INDEL F5 + 0	CONTRIBUTION - 78%		
+2	• 4.196	C A T C A A A G A G A T C G T T A G C A G A A A C	N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C
+6	1.9%	CATCAAAGAGATCGTTAGCAGAAAC	N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G
-30	. 1.7%	C A T C A A A G A G A T C G T T A G C A	
-21	1.2%	C A T C A A A G A G	A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-19	0.8%	C A T C A A A G A G A	;
-20	0.6%	C A T C A A A G A G A	A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
-8	0.6%	C A T C A A A G A G A T C G T T A G C A G A	G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+4 -8	0,1%	G A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A G C A G A A A C	A T A T C A A G A G A T A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A
+15	0.196	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N N N N N N N N N A A A A G G A G A
-11	0.1%	C A T C A A A G A G A T C G T T A G C A G A A A C	

Liver Macrophage						
		INDEL	CONTRIBUTION -	SEQUENCE		
E	1 +	0	86.2%	C A T C A A A G A G A T C G T T A G C A G A A A C	A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		+2	· 3.1%	C A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A G C A G A A A C	N N A A A A G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C A	
		-21	1.1%	C A T C A A A G A G A T C G T T A G C		
		+6	0.9%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G	
		-30	0.8%	C A T C A A A G A G	G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-23	0.8%	C A T C A A A G A G A T C G T		
		-5	0.4%	C A T C A A A G A G A T C G T T A G C A G A A A C		
		-23	0.2%	C A T C A A A G A G A T C		
		-19	0.2%	C A T C A A A G A G A	G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-19	0.1%	C A T C A A A G A G A T C G T T A G C A G	;	
		INDEL	CONTRIBUTION -	REGUENCE		
F	:2 +	0	93.5%	CATCAAAGAGATCGTTAGCAGAAAC		
	-2	+2	196	C A T C A A A G A G A T C G T T A G C A G A A A C		
		-8	D.8%	C A T C A A A G A G A T C G T T A G C A G		
		-21	0.8%	C A T C A A A G A G	A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-19	0.5%	C A T C A A A G A G A		
		-30	0.5%	C A T C A A A G A G		
		+4	0.5%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A	
		-6	0.2%	C A T C A A A G A G A T C G T T A G C A G	A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		+5	0.2%	C A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A G C A G A A A C	. N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T	
		-28	0%	C A T C A A A G A G		
		+14	0%	C A T C A A A G A G A T C G T T A G C A G A A A C		
,	·	INDEL	CONTRIBUTION -	SEQUENCE		
t	:3 +	+2	90.9%	C A T C A A A G A G A T C G T T A G C A G A A A C C A T C A A A G A G A T C G T T A G C A G A A A C		
		+5	0.4%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T	
		-20	0.4%	C A T C A A A G A G A T C G T T A G C		
		-21	0.4%	C A T C A A A G A G A T C G T T A G C		
		+4	0.2%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A	
		-8	0.1%	CATCAAAGAGATCGTTAGC		
		-10	0%	C A T C A A A G A G A T C G T T A G C A G	A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-19	0%	C A T C A A A G A G A		
		-30	0%	C A T C A A A G A G	G A G G A T G G A T C G A C T T A G A C T T G A C C T G T A T C C A	
		INDEL	CONTRIBUTION -	SEQUENCE		
E	4 +	0	88%	C A T C A A A G A G A T C G T T A G C A G A A A C	A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		+2	• 2.9%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C	
		-20	1.3%	C A T C A A A G A G A T C G T T A G C		
		+4	0.9%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A	
		-30	0.8%	C A T C A A A G A G	G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-23	0.6%	C A T C A A A G A G		
		-19	0.5%	C A T C A A A G A G A	G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-20	0.4%		A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-8	0.2%	C A T C A A A G A G A T C G T T A G C A G A		
		+6	0.2%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G	
		-11	O.1%	C A T C A A A G A G A T C G T T A G C A G A A A C		
		+10	0.1%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A	
		-5	0%	C A T C A A A G A G A T C G T T A G C A G A	A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		INDEL	CONTRIBUTION -	SEQUENCE		
E	5 +	· 0	92.7%	C A T C A A A G A G A T C G T T A G C A G A A A C	: A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-20	196	C A T C A A A G A G A T C G T T A G C A G A T A G	AGAGGATGGATGGATGGATTCGACTTAGACTTGACCTGTATCCA	
		-19	0.6%	C A T C A A A G A G A		
		+6	0.5%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G A	
		-21	0.5%	C A T C A A A G A G	A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-23	0.4%		ATATCAAGAGGATGGATTCGACTTAGACTTGACCTGTATCCA	
		-21	0.3%	C A T C A A A G A G A T C G T T A G C		
		-10	0.2%	C A T C A A A G A G A T C G T T A G C A G A		
		+15	0.2%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N N N N N N N N N N N N N N	
		-8	0.2%	C A T C A A A G A G A T C G T T A G C A G A	G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		-17	0.2%	C A T C A A A G A G A T C G T T A G C A		
		+4	0%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A	
Control Experiment (AuNPs: - Cas0++ soRNA++)						
		INDEL	CONTRIBUTION -			
	+	• 0	99.6%		N N N N N A A A A G G A G A T A T C A A G A G A G A G A G A T G G A T T C G A C T T A G A C T T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C T G A C C C C C C C C C C C C C C C C C C	
Control Experiment (AuNPs Cas9 saRNA)						
contro	^1	INDEL	CONTRIBUTION -	SEQUENCE		
	4		99.2%		A A A A G G A G A T A T C A A G A G G A T G G A T T C G A C T T A G A C T T G A C C T G T A T C C A	
		+5	0.2%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N A A A A G G A G A T A T C A A G A G A T G G A T T C G A C T T A G A C T T G A C C T G T	
		-21	0.196	C A T C A A A G A G A T C G T T A G C		
		+10	0.1%	C A T C A A A G A G A T C G T T A G C A G A A A C	N N N N N N N N N A A A A G A G A T A T C A A G A G A T G G A T T C G A C T T A G A C T T G A	

Figure S10. T7E1 gel and normalized contributions of each sequence obtained from Interference for CRISPR Edits (ICE) analysis. Experimental conditions and control conditions

were shown as En and Cn. Dash line indicates the expected cutting site; orange plus indicates the wild-type sequence.



Figure S11. Off-Target editing results for the *PTEN* gene at 4 loci as measured by T7E1 assay.