
Dual SORT LNPs for multi-organ base editing

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Additional Supplementary Methods

In vivo mouse *Ttr* editing with Cas9

Cas9 mRNA was synthesized by in vitro transcription (IVT), following previously reported methods^{1, 2}. IVT was performed using the MEGAscript SP6 transcription kit (Invitrogen, AM1330), with uridine triphosphate replaced with N1-methylpseudouridine-5'-triphosphate. After 2 hours of incubation at 37 °C, 1 µL of Turbo DNase was added per reaction. A Cap1 structure was then added to the 5' end of the mRNA using the Cap1 Capping System (Hongene, ON-028 & ON-014). After 1 hour of incubation at 37 °C, the mRNA was subsequently purified by LiCl precipitation. Heavily modified sgTTR (G211) was a kind gift from Hongene, with a modification according to a previously reported study³. The sgTTR sequence is described in **Supplementary Table 4**. *Cas9* mRNA and sgTTR (wt/wt=2:1) were encapsulated in 4A3-SC7 Liver SORT LNPs or 5A2-SC8 DOTAP SORT LNPs (LNP-Cas9) by controlled T-mixing. For 4A3-SC7 Liver SORT LNPs, the molar ratio was 4A3-SC7/DOPE/cholesterol/DMG-PEG2000/Liver SORT lipid=15.04/23.04/38.72/3.2/20 (Total lipid:RNA wt/wt=20:1). For 5A2-SC8 Lung SORT LNP formulation, the molar ratio was 5A2-SC8/DOPE/cholesterol/DMG-PEG2000/DOTAP=21.6/12/25.2/1.2/40 (Total lipid:RNA wt/wt=20:1). C57BL/6J mice (8-to-9-week-old) were IV injected with 1.5 mg kg⁻¹ of 4A3-SC7 Liver SORT LNPs or 2 mg kg⁻¹ of 5A2-SC8 Lung SORT LNPs containing *Cas9* mRNA and sgTTR. Liver and lung tissues were collected 7 days post-injection.

Isolation of bronchial epithelial cells of LNP-Cas9 treated mice by magnetic cell separation

Bronchial epithelial cells from LNP-Cas9 treated mice were isolated using the EasySep™ Biotin Positive Selection Kit II (Stem Cell Technologies, 17665). RBC-depleted lung cells were incubated in 100 µL of cell staining buffer with 1 µL of FcR blocker and 1 µL each of biotin-conjugated anti-mouse CD326 (Ep-CAM) (BioLegend, 118203) for 15 minutes at room temperature. Then, 1 mL of staining buffer was added, and the cells were centrifuged to collect the pellet. The pellet was reconstituted in 100 µL of buffer and then incubated with 10 µL of biotin selection cocktail for 15 minutes. Next, 8 µL of streptavidin-bound magnetic nanoparticles were added and incubated for 10 minutes. An additional 500 µL of staining buffer was added, and the tube was placed on a magnet for 10 minutes. The wash process was performed by adding 500 µL of staining buffer, placing the tube back on the magnet for 10 minutes, and repeating this wash step. Finally, the labeled bronchial epithelial cells were collected.

Next-generation sequencing (NGS) for mouse *Ttr* gene

DNA was extracted from tissues using NucleoSpin Tissue XS kits (MACHEREY-NAGEL, 740901.50), following the manufacturer's protocol. Amplicons were prepared by PCR with Phusion U Green Multiple PCR Master Mix (Thermo Fisher, F564S) with an amplification protocol (98 °C for 30 s; 98°C for 10 s; 65°C for 30 s; 72°C for 20 s) for 30 cycles; 72°C for 2 min). Primers for PCR are described in **Supplementary Table 5**. The amplicons were purified using the Qiagen PCR purification kit (Qiagen, 28106), and the concentration of purified amplicons was confirmed by using a Qubit assay following the manufacturer's protocol. Libraries were sequenced on an Illumina MiSeq Instrument, and the data analysis was performed using CRISPResso2⁴.

DNA template sequences of Cas9 and ABEs

NLS-Cas9-SV40 NLS-Nucleoplasmin NLS:

ATGCGCGCCGCTCCGGCAGCTAAGAAAAAGAACTGGATGGCAGCGTCGACATGGATAAGA
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Methods-only references

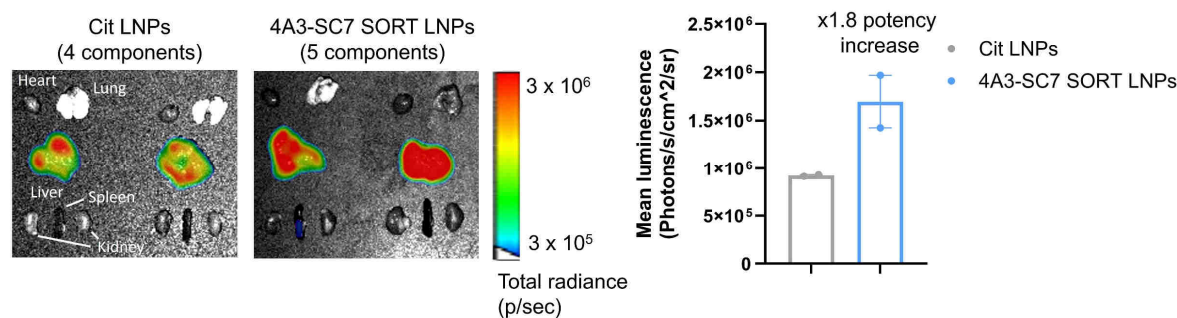
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3. Finn, J.D. et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Rep.* **22**, 2227-2235 (2018).
4. Clement, K. et al. CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat. Biotechnol.* **37**, 224-226 (2019).

Supplementary Figures

a

Name	Molar %					Size (nm)	PDI
	4A3-SC7	DOPE	Cholesterol	PEG-DMG	4A3-Cit		
Cit LNPs (4 components)		19.04	38.72	3.2	39.04	59.6 ± 0.8	0.18 ± 0.004
4A3-SC7 SORT LNPs (5 components)	19.04	19.04	38.72	3.2	20	71 ± 0.25	0.16 ± 0.007

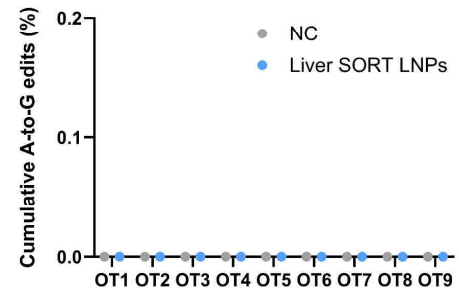
b



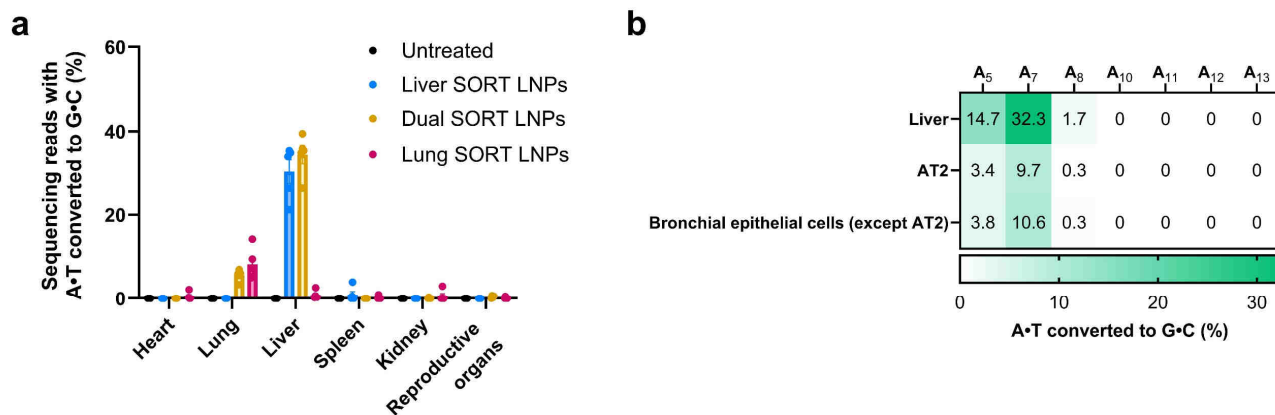
Supplementary Fig. 1. 5-component 4A3-SC7 SORT LNPs (containing 4A3-SC7 and 4A3-Cit) mediated higher mRNA delivery efficiency than 4-component Cit LNPs (containing 4A3-Cit). **a**, Formulation details and physiochemical properties of LNPs. LNPs were formulated by T-mixing. **b**, C57BL/6J mice were intravenously administrated at 0.015 mg kg⁻¹ *Luc* mRNA ($n = 2$ biological replicates). Mice were sacrificed 6 hours after the injection and dissected to collect major organs (heart, lung, liver, spleen, and kidney). Bioluminescence was measured by *ex vivo* imaging using AMI imaging systems. Data are presented as mean ± s.e.m.

a

	Target	Sequence	PAM	Mismatches	Chromosome
On Target	sgSERPINA1	TCGACGAGAAAGGGACTGA	AGC	0	chr14
Off Target (OT)	OT1	TCGggGAGAAAGGGACTGt	TGC	3	chr15
	OT2	TCtggGAGAAAGGGACTGA	GGC	3	chr5
	OT3	TgGAtGAGAAAGGGACTcA	TGC	3	chr20
	OT4	gaGACcAGAAAGGGACTGA	GGC	3	chr12
	OT5	TCcACGAGgAAGGGAgTGA	GGC	3	chr21
	OT6	TCcACGAGAAAGaaACTGA	GGC	3	chr16
	OT7	TCtAtGAGgAAGGGACTGA	GGC	3	chr14
	OT8	gCGAaGAGAAAGaGACTGA	GGC	3	chr10
	OT9	TgGgCGAGAAcGGGACTGA	CGC	3	chr19

b

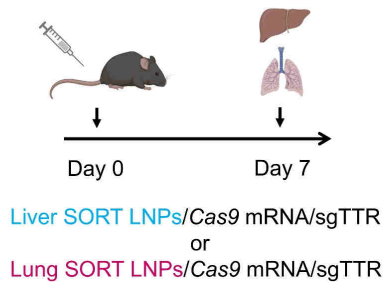
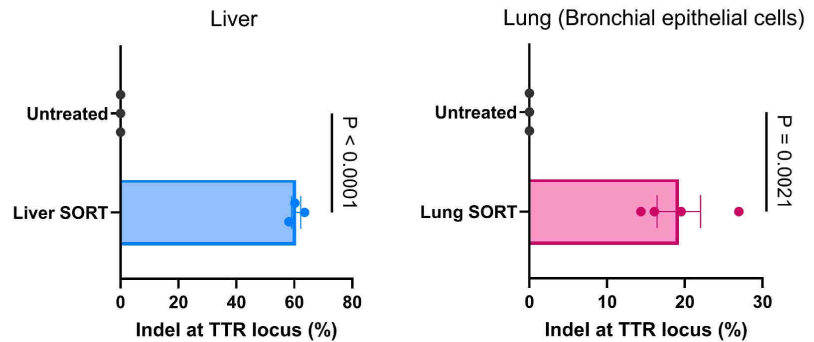
Supplementary Fig. 2. No off-target editing was detected after Liver SORT LNP treatment. Patient-derived liver fibroblasts (GM12445) were treated with optimized all-in-one Liver SORT LNPs. *ABE* mRNA and heavily modified sgSERPINA1 were encapsulated at a weight ratio 1:1. **a**, Sequence information of eight predicted off-target sites of sgSERPINA1 was listed in the table. **b**, The potential off-target sites were amplified by PCR and analyzed using NGS deep sequencing. The non-treated group was used as negative control (NC).



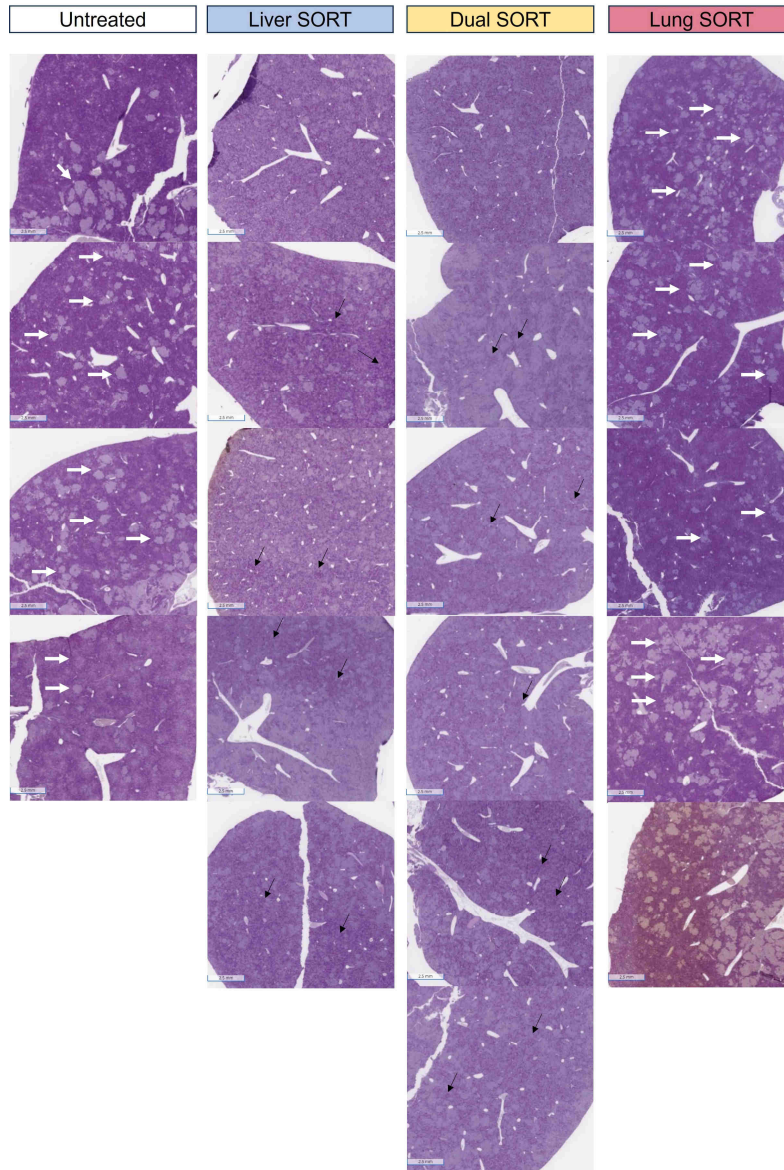
Supplementary Fig. 3. Correction editing profiles across various organs and the editing profile within the protospacer. DNA was extracted from various organs at the 4-week time point. **a**, Editing efficacy profile in various organs of PiZ mice treated with SORT LNPs (Untreated, $n = 3$; Liver SORT and Lung SORT, $n = 5$; Dual SORT, $n = 4,5$ biological replicates). Editing in the *SERPINA1* gene was quantified as the sum of alleles (7G and both 5G+7G edited). Minimal or no editing was detected in organs other than the target organs (lungs and livers). Data are presented as mean $\pm$ s.e.m. **b**, Detailed A-to-G conversion frequency of Dual SORT LNPs within the protospacer in the target tissues.

a

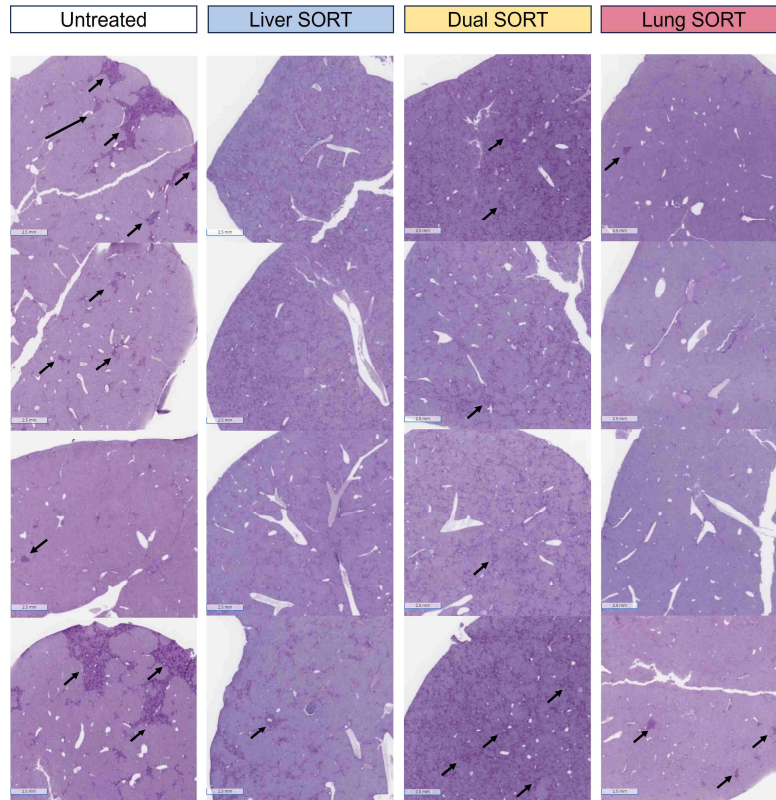
Name	Molar %					Size (nm)	PDI
	4A3-SC7/ 5A2-SC8	DOPE	Cholesterol	PEG-DMG	4A3-Cit/ DOTAP		
Liver SORT LNPs	15.04 (4A3-SC7)	23.04	38.72	3.2	20 (4A3-Cit)	64.6 ± 0.34	0.13 ± 0.006
Lung SORT LNPs	21.6 (5A2-SC8)	12	25.2	1.2	40 (DOTAP)	75.4 ± 0.5	0.15 ± 0.002

b**c**

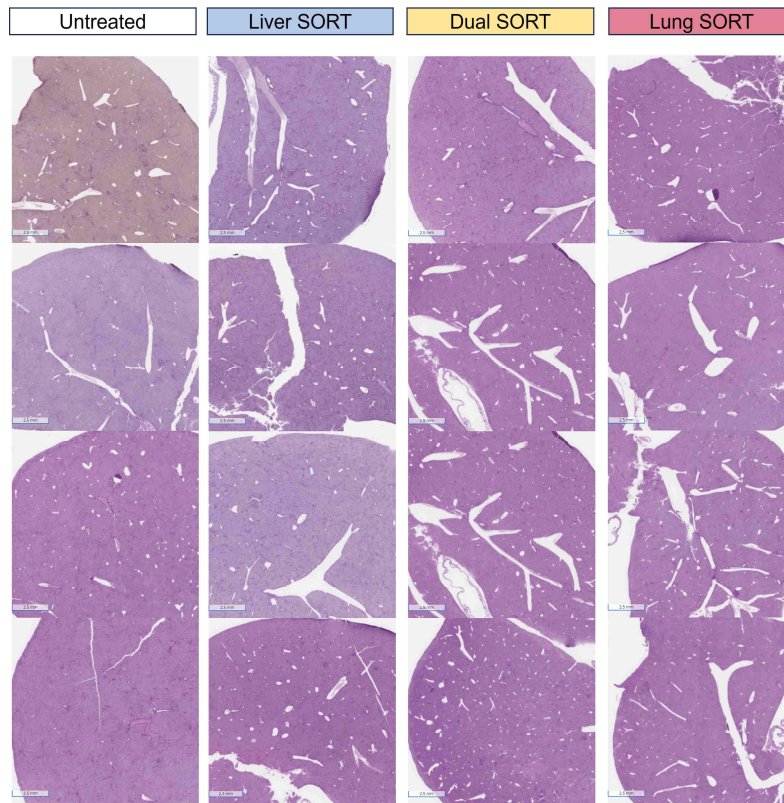
Supplementary Fig. 4. Substantial *in vivo* gene editing in the liver and lungs of mice using Cas9. **a**, Formulation details and physiochemical properties of LNPs prepared by controlled T-mixing. **b**, A schematic representation of the experimental design used to evaluate gene editing efficiency in liver and lung cells of C57BL/6J mice. The mouse *Ttr* gene was targeted by co-delivery of *Cas9* mRNA and sgTTR. 4A3-SC7 Liver SORT LNPs or 5A2-SC8 Lung SORT LNPs were used to co-deliver *Cas9* mRNA and heavily modified sgTTR. Mice were IV injected with each Liver SORT (1.5 mg kg⁻¹) and Lung SORT LNPs (2 mg kg⁻¹), with a *Cas9* mRNA:sgTTR ratio of 2:1 (wt/wt). Mice were sacrificed 7 days post-injection. **c**, Insertion and deletion (Indel) rate in the liver ($n = 3$ biological replicates) and bronchial epithelial cells ($n = 4$ biological replicates). Data are presented as mean ± s.e.m. Two-tailed *t*-test.



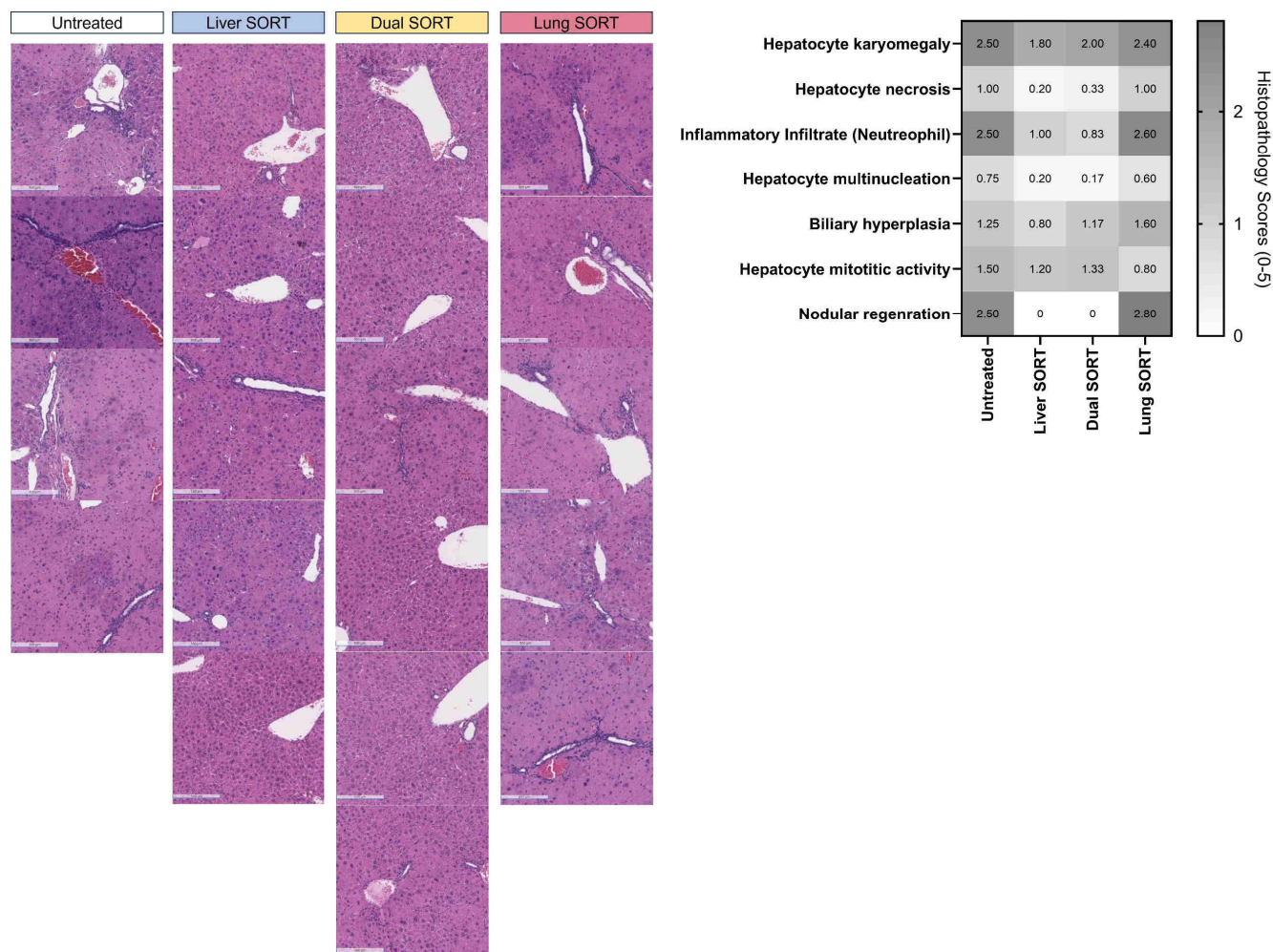
Supplementary Fig. 5. Images of Periodic Acid-Schiff with diastase (PAS-D)-stained liver slides from SORT LNP-treated PiZ mice at the 4-week time point. White arrows indicate non-Z-A1AT aggregates detected area. Black arrows indicate Z-A1AT aggregates (globules). Dual SORT LNP treatment successfully lowered the Z-A1AT. Scale bar, 2.5 mm.



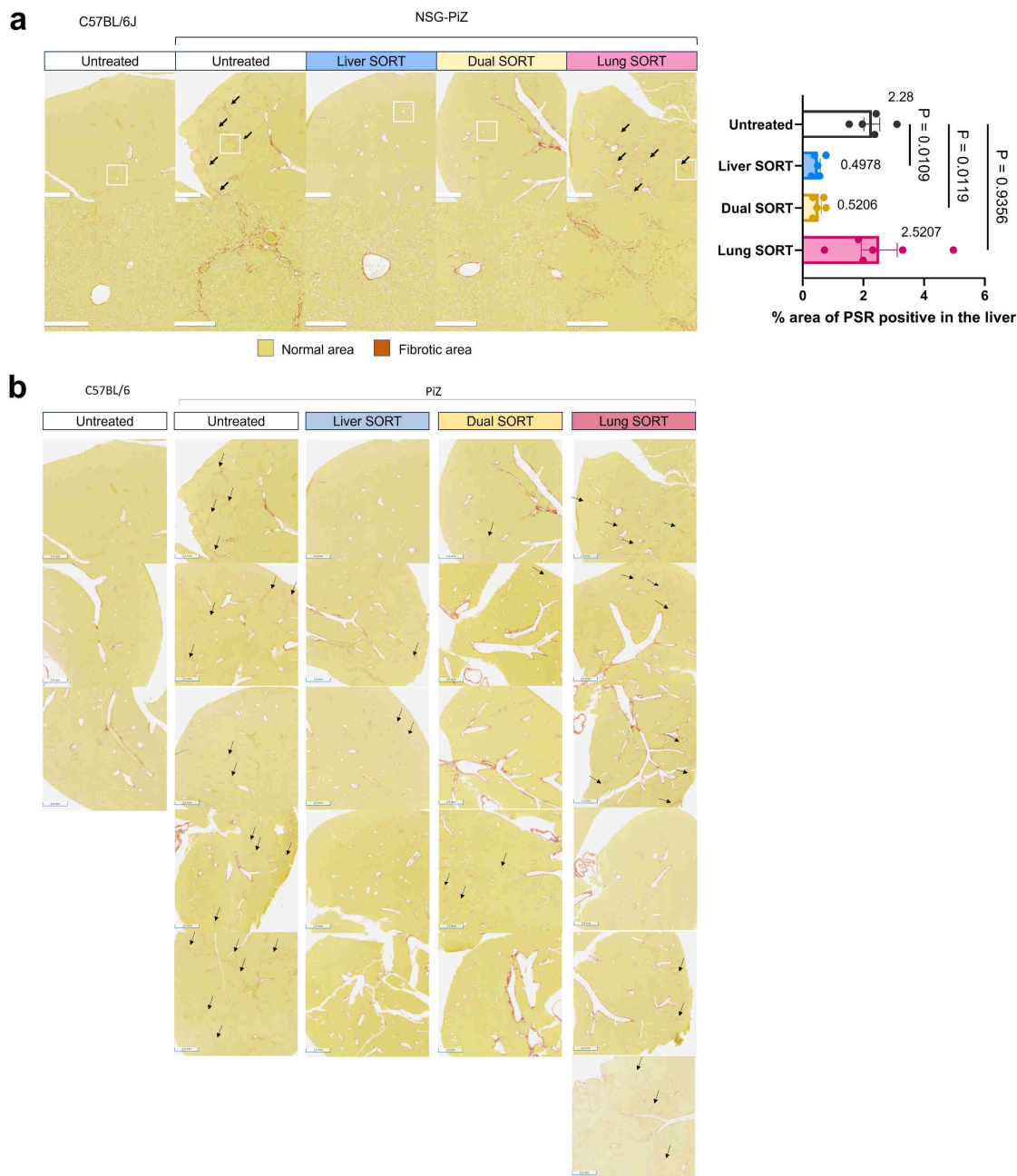
Supplementary Fig. 6. Images of PAS-D-stained liver slides from SORT LNP-treated PiZ mice at the 12-week time point. Black arrows indicate Z-A1AT aggregates (globules). Minimal Z-A1AT aggregates were detected in the untreated PiZ mice. Scale bar, 2.5 mm.



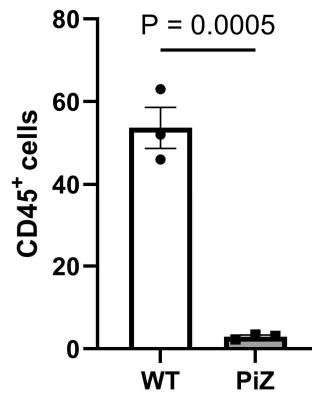
Supplementary Fig. 7. Images of PAS-D-stained liver tissues from SORT LNP-treated PiZ mice at the 32-week time point. Black arrows indicate Z-A1AT aggregates (globules). Minimal Z-A1AT aggregates were detected in the untreated PiZ mice. Scale bar, 2.5 mm.



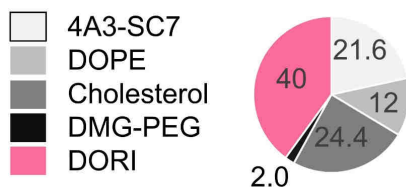
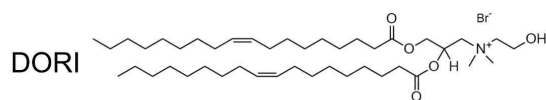
Supplementary Fig. 8. The histopathology assessment of liver slides from SORT LNP-treated PiZ mice at the 4-week time point. Images of H&E-stained liver tissue slides from PiZ mice. Scale bar, 500 μ m. On the right side of the images are the mean individual histopathology scores (0-5) of liver tissue slides from PiZ mice (Untreated, $n = 7$; Liver SORT and Lung SORT, $n = 5$; Dual SORT, $n = 6$ biological replicates).



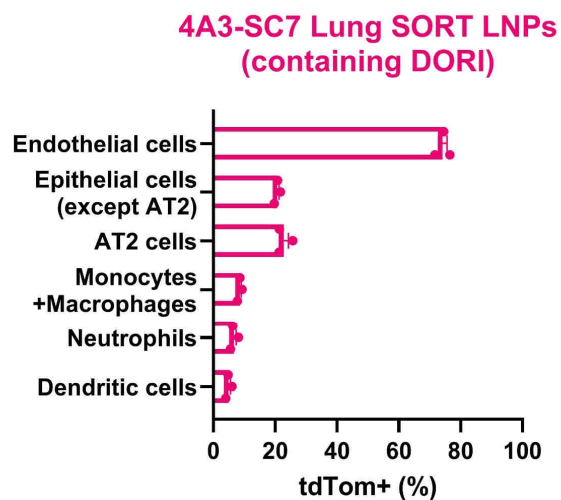
Supplementary Fig. 9. Images of Picro-Sirius red (PSR)-stained liver slides from SORT LNP-treated PiZ mice at the 32-week time point. Thirty-eight- to forty-week-old C57BL/6J mice were used as a control. Black arrows indicate collagens. **a**, Representative images of the PSR-stained liver slides at the 32-week time point (Untreated, Liver SORT, and Dual SORT, $n = 5$; Lung SORT, $n = 6$ biological replicates). Scale bar, top panel, 2.5 mm; scale bar, bottom panel, 500 μm . On the right side of the images is the quantification of the PSR-positive area in the liver (Data are presented as mean $\pm$ s.e.m). One-way analysis of variance (ANOVA). **b**, Images of the PSR-stained liver slides at the 32-week time point. Scale bar, 2.5 mm.



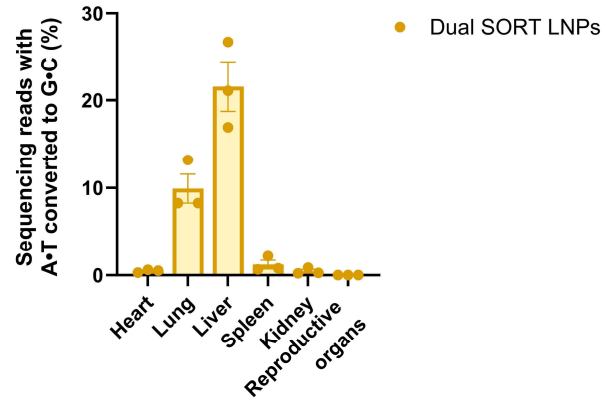
Supplementary Fig. 10. The percentage of CD45⁺ lung immune cells varies depending on the mouse strain. Lungs were extracted from 8-week-old C57BL/6J (WT) and NSG-PiZ mice ($n = 3$ biological replicates). CD45⁺ immune cell population was confirmed by flow cytometry. PiZ mice (NSG background) have significantly lower CD45⁺ immune cell population levels compared to C57BL/6J mice (WT). Data are presented as mean $\pm$ s.e.m. Two-tailed t -test.

a

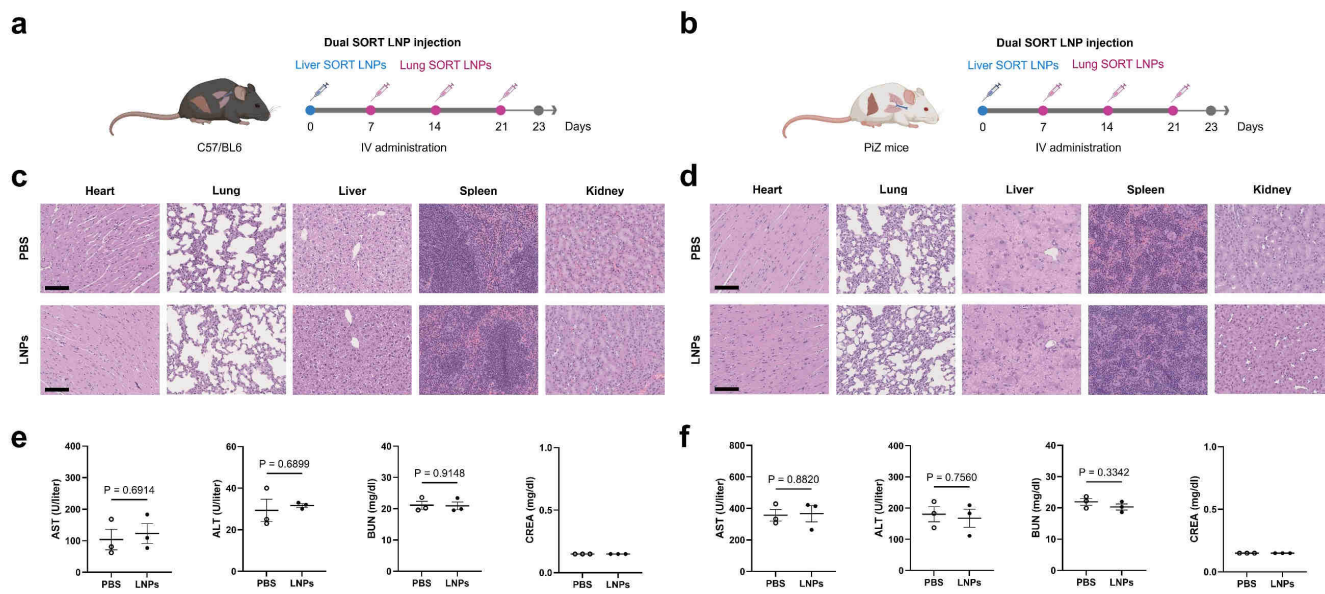
Size (nm)	PDI	Zeta potential
69 ± 0.7	0.12 ± 0.009	8.4 ± 0.76

b

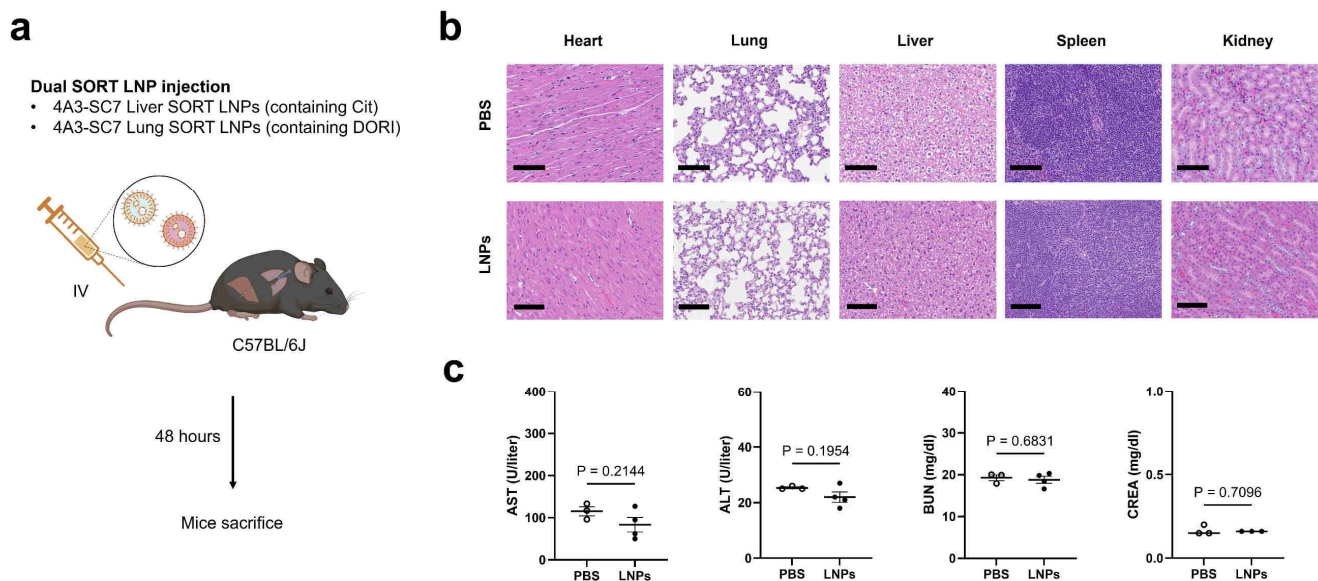
Supplementary Fig. 11. 4A3-SC7 Lung SORT LNPs (containing DORI) allowed editing of therapeutically relevant types of lung cells. Ai14 mice were dosed with *Cre* mRNA at 0.3 mg kg^{-1} . The lungs were collected 2 days after the injection. **a**, Formulation details and the physiochemical properties of LNPs formulated by T-mixing. **b**, Percentage of tdTomato-positive lung cells in mice ($n = 3$ biological replicates). Data are presented as mean $\pm$ s.e.m.



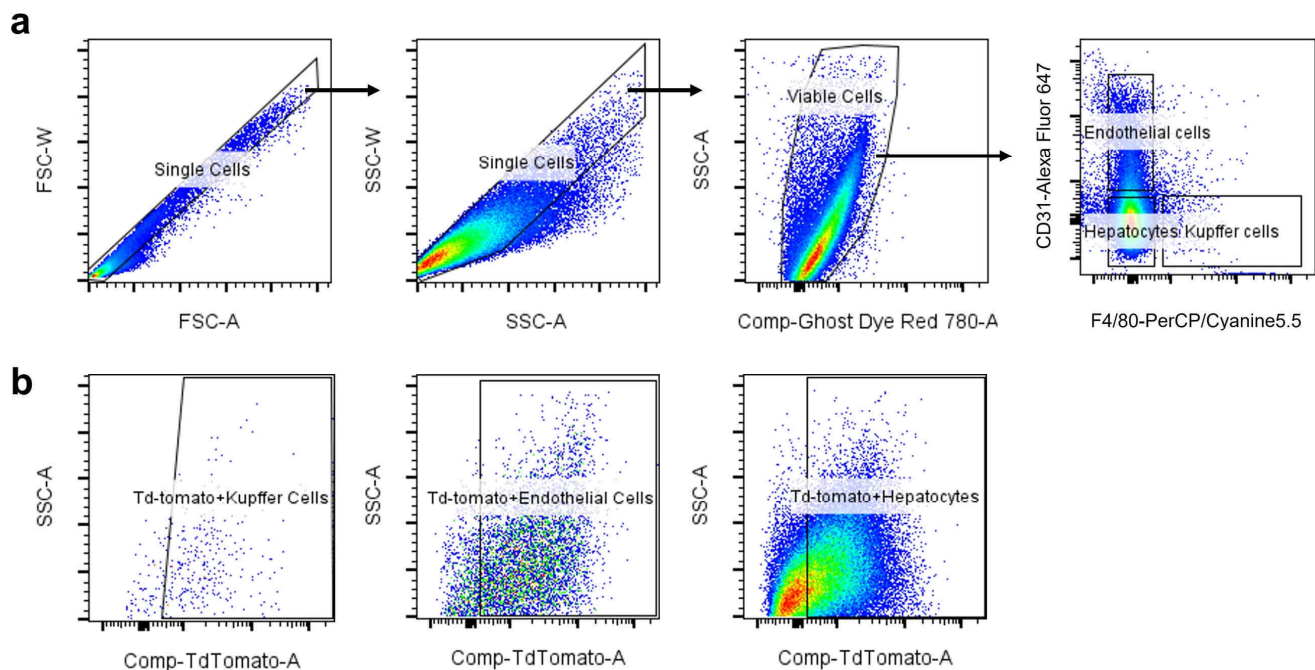
Supplementary Fig. 12. Correction editing efficacy profiles across various organs of PiZ mice treated with updated Dual SORT LNPs. Eight- to ten-week-old PiZ mice were administered IV with all-in-one Dual SORT LNPs. 4A3-SC7 Liver SORT LNPs (containing Cit, 1.5 mg kg^{-1}) and 4A3-SC7 Lung SORT LNPs (containing DORI, 1.5 mg kg^{-1}) were injected simultaneously. Mice were sacrificed 6 days post-injection. Correction editing in the organs was confirmed by NGS and CRISPResso2 analysis ($n = 3$ biological replicates). Data are presented as mean $\pm$ s.e.m.



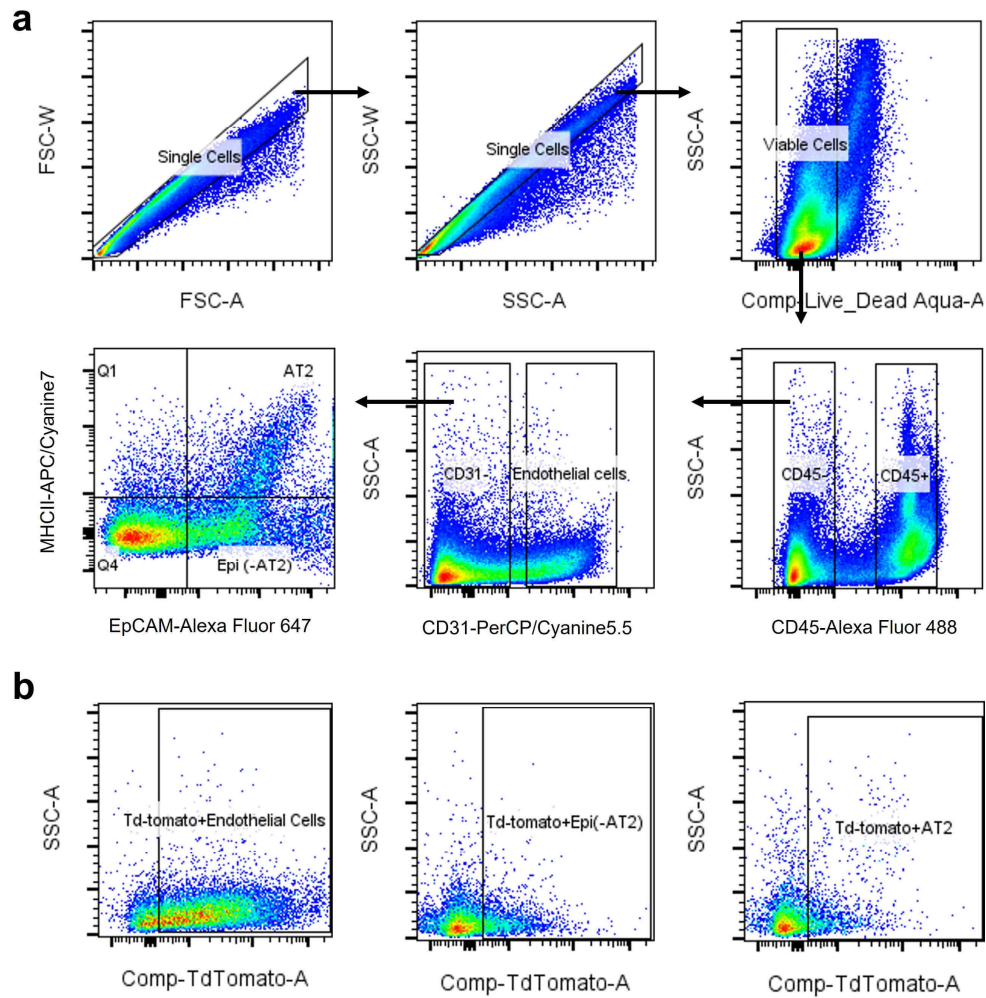
Supplementary Fig. 13. *In vivo* safety evaluation of Dual SORT LNPs through histological assessment and blood biochemical analysis. Male C57BL/6J (**a**, **c**, and **e**) and PiZ mice (**b**, **d**, and **f**) were injected with either PBS or *Cre* mRNA encapsulated 4A3-SC7 Liver SORT (containing Cit) and 5A2-SC8 Lung SORT LNPs (containing DOTAP) with the same treatment dosing regimen. Two days after the last injection, the mice were euthanized and indicated organs were prepared for the slide section and stained with H&E. Scale bar, 100 μ m. Blood was analyzed for the indicated biochemicals. Data are presented as mean $\pm$ s.e.m of $n = 3$ biological replicates. Two-tailed t -test.



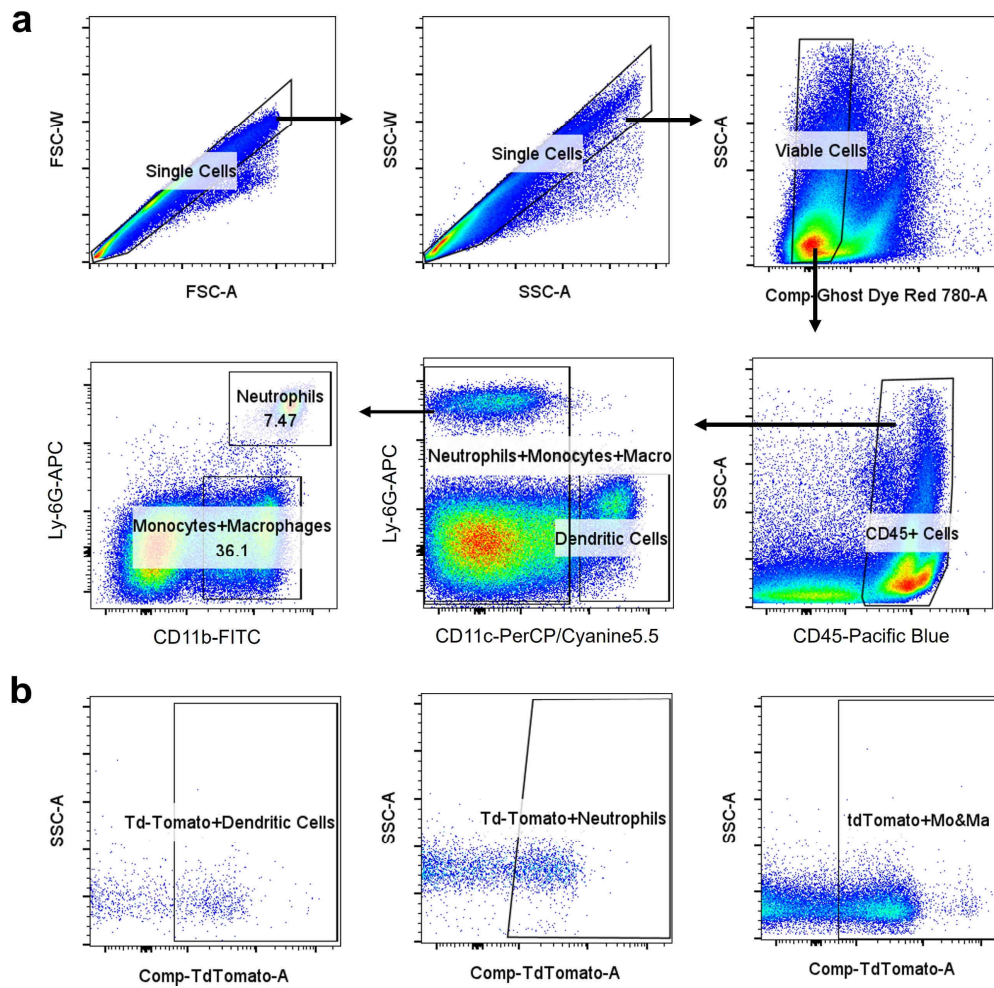
Supplementary Fig. 14. *In vivo* safety evaluation of updated Dual SORT LNPs through histological assessment and blood biochemical analysis. **a**, Eight- to ten-week-old C57BL/6J mice were IV injected with Dual SORT LNPs. *Cre* mRNA was encapsulated in 4A3-SC7 Liver SORT LNPs (containing Cit) and 4A3-SC7 Lung SORT LNPs (containing DORI). Mice were administered 1.5 mg/kg of *Cre* mRNA with each LNP simultaneously and were sacrificed 48 hours after the injection. **b**, Representative H&E-stained slides of heart, lung, liver, spleen, and kidney. Scale bar, 100 μ m. **c**, Blood chemical analyses, including AST, ALT, BUN, and CREA. No signs of inflammation were detected in the Dual SORT LNP-treated group. Data are presented as mean $\pm$ s.e.m of $n = 3, 4$ biological replicates. Two-tailed *t*-test.



Supplementary Fig. 15. Flow cytometry gating strategy for various liver cell types. a, Single cells prepared from Ai14 mouse lungs were gated. Viable cells were gated out as Ghost Red negative cells. Endothelial cells were gated out as CD31-positive cells. Kupffer cells were gated out as F4/80 positive cells. Hepatocytes were gated out as CD31 and F4/80 double negative cells. **b**, tdTomato-positive F4/80⁺ Kupffer cells, tdTomato-positive CD31⁺ endothelial cells, and tdTomato-positive hepatocytes.



Supplementary Fig. 16. Flow cytometry gating strategy for various lung cell types. a, Single cells prepared from Ai14 mouse lungs were gated. Viable cells were gated out as LIVE/DEAD™ Fixable Aqua negative cells. Endothelial cells (CD31 positive), epithelial cells, except alveolar type II (AT2) cells (EpCAM positive, MHCII negative), and AT2 cells (CD45 negative, CD31 negative, EpCAM positive, MHCII positive) were gated. **b**, Tdtomato-positive CD31⁺ endothelial cells, EpCAM⁺MHCII⁻ epithelial cells, and EpCAM⁺MHCII⁺AT2 cells.



Supplementary Fig. 17. Flow cytometry gating strategy for various lung immune cell types. **a**, Single cells prepared from Ai14 mouse lungs were gated. Viable cells were gated out as Ghost Red negative cells. Total immune cells (CD45 positive), dendritic cells (CD45 positive and CD11c positive), neutrophils (CD45 positive and Ly-6G positive), and monocytes and macrophages (CD45 positive and CD11b positive) were gated out. **b**, tdTomato-positive dendritic cells, tdTomato-positive neutrophils, and tdTomato-positive monocytes and macrophages.

Formulation	Benchmark DOTAP40	High DOPE	High Cholesterol	Low DMG-PEG
Lipid:RNA (wt/wt)	20:1	20:1	20:1	20:1
Ionizable lipid (Molar ratio)	21.6	11.6	11.6	21.6
DOPE (Molar ratio)	12	22	12	12
Cholesterol (Molar ratio)	24	24	34	25.2
DMG-PEG (Molar ratio)	2.4	2.4	2.4	1.2
DOTAP SORT lipid (Molar ratio)	40	40	40	40
Encapsulation Efficiency	93 ± 2 %	90 ± 3 %	95 ± 1 %	91 ± 4 %
Size	87 ± 3 nm	88 ± 2 nm	85 ± 2 nm	90 ± 3 nm
PDI	0.24 ± 0.05	0.24 ± 0.02	0.22 ± 0.01	0.15 ± 0.02

Supplementary Table 1. Detailed formulation molar ratios for Lung SORT LNPs and their corresponding physiochemical properties. Encapsulation efficiency, size, and PDI are presented as mean ± s.d.

Liver SORT LNPs	Size of RNA (nucleotides)	Encapsulation Efficiency	Size	PDI
sgRNA-LNPs	~100	91 ± 3%	71 ± 5 nm	0.11 ± 0.005
Cre mRNA-LNPs	~1500	89 ± 2%	81 ± 4 nm	0.14 ± 0.003
ABE mRNA-LNPs	~5000	48 ± 10%	98 ± 20 nm	0.25 ± 0.05

Supplementary Table 2. Physiochemical properties of Liver SORT LNPs depending on the size of RNA cargo. Encapsulation efficiency, size, and PDI are presented as mean ± s.d.

Formulation # (ABE mRNA LNPs)	1 (original)	2	3	4	5	6	7	8	9	10	11	12 (Lead formulation)
Lipid:RNA (wt/wt)	20:1	30:1	40:1	20:1	20:1	20:1	20:1	20:1	20:1	20:1	20:1	20:1
Ionizable lipid (Molar ratio)	19.04	19.04	19.04	21.42	21.42	12.42	12.42	30.42	19.04	11.04	15.04	15.04
DOPE (Molar ratio)	19.04	19.04	19.04	21.42	12.42	30.42	21.42	12.42	11.04	27.04	19.04	23.04
Cholesterol (Molar ratio)	38.72	38.72	38.72	43.56	52.56	43.56	52.56	43.56	46.72	38.72	42.72	38.72
DMG-PEG (Molar ratio)	3.2	3.2	3.2	3.6	3.6	3.6	3.6	3.6	3.2	3.2	3.2	3.2
Cit (Molar ratio)	20	20	20	10	10	10	10	10	20	20	20	20
Encapsulation Efficiency (%)	58 ± 3	65 ± 2	53 ± 2	49 ± 1	0	61 ± 2	0	0	3 ± 0.5	64 ± 1	84 ± 2	87 ± 5
Size (nm)	130 ± 41	96 ± 5	90 ± 7	62 ± 5	251 ± 10	147 ± 8	203 ± 9	355 ± 11	186 ± 7	114 ± 5	71 ± 5	74 ± 4
PDI	0.28 ± 0.03	0.20 ± 0.02	0.28 ± 0.03	0.25 ± 0.03	0.08 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.27 ± 0.02	0.19 ± 0.02	0.25 ± 0.03	0.21 ± 0.01	0.17 ± 0.02

Supplementary Table 3. Detailed lipid component molar ratios and physiochemical properties of ABE mRNA Liver SORT LNPs. Encapsulation efficiency, size, and PDI are presented as mean ± s.d.

Name	Sequences (5' to 3')	PAM (5' to 3')	Notes
18 nt sgSERPINA1	CGACGAGAAAGGGACUGA	NGC	Chemically modified sgRNAs were used. Detailed modification patterns are indicated in methods.
19 nt sgSERPINA1	UCGACGAGAAAGGGACUGA	NGC	
20 nt sgSERPINA1	AUCGACGAGAAAGGGACUGA	NGC	
sgTTR	UUACAGCCACGUCUACAGCA	NGG	

Supplementary Table 4. All sgRNA sequences used in this research work are listed.

Target	Forward Primers (5' to 3')	Reverse Primers (5' to 3')	Notes
	TTGGACCCTCGTACAGAAGCTAATACG	T ₁₂₀ CTTCCTACTCAGGCTTTATTCAAAGACCA	For IVT template
On Target	GGAGGTGTCCACGTGAGCCTTG	TTGGGTGGGATTCACCACTTTTCCCATG	For NGS amplicon of <i>SERPINA1</i>
OT1	TTGAGGCACTGCTGGAGGAGGG	CCCCAGGAGTGGAAGCCAGGAA	
OT2	ACGAGGTCGAGGCTGTAAGGGG	CAATTGCGGGAGGAGGTGCCAG	
OT3	GCCCCGGCAGCAATCCACGTAA	GCTGTCATTGAGGCCACCAGC	
OT4	TGACTTGGTGGCTGCTCCGTT	GCCTGGTGATTGTACACAACATACATGTAAAC	
OT5	ACCTCGAGGAGAGTGTGGGGGA	ACTCTTGGCAGCTGCAGCAAGG	
OT6	GGCGATGTGGGAACAGGAGGGA	TCAACTCTGTGGGACCGAGGCA	
OT7	TTGAACCCAGGAGGCGGAGGTT	GCATGGTGCCTCCCCTCCAATC	
OT8	ACAGTCAGCAAACTGGAGAACA	TCCAGGTTTACATCTTCCACGA	
OT9	GGAAAGCGACGCTGCGGGTTAT	CCCTCGACTGCCTCCAGAACA	
On Target	TGGGCTTTCTACAAGCTTACC	CGGTTTACTCTGACCCATTTC	For NGS amplicon of <i>Ttr</i>

Supplementary Table 5. Information on primer sequences used in this research work.