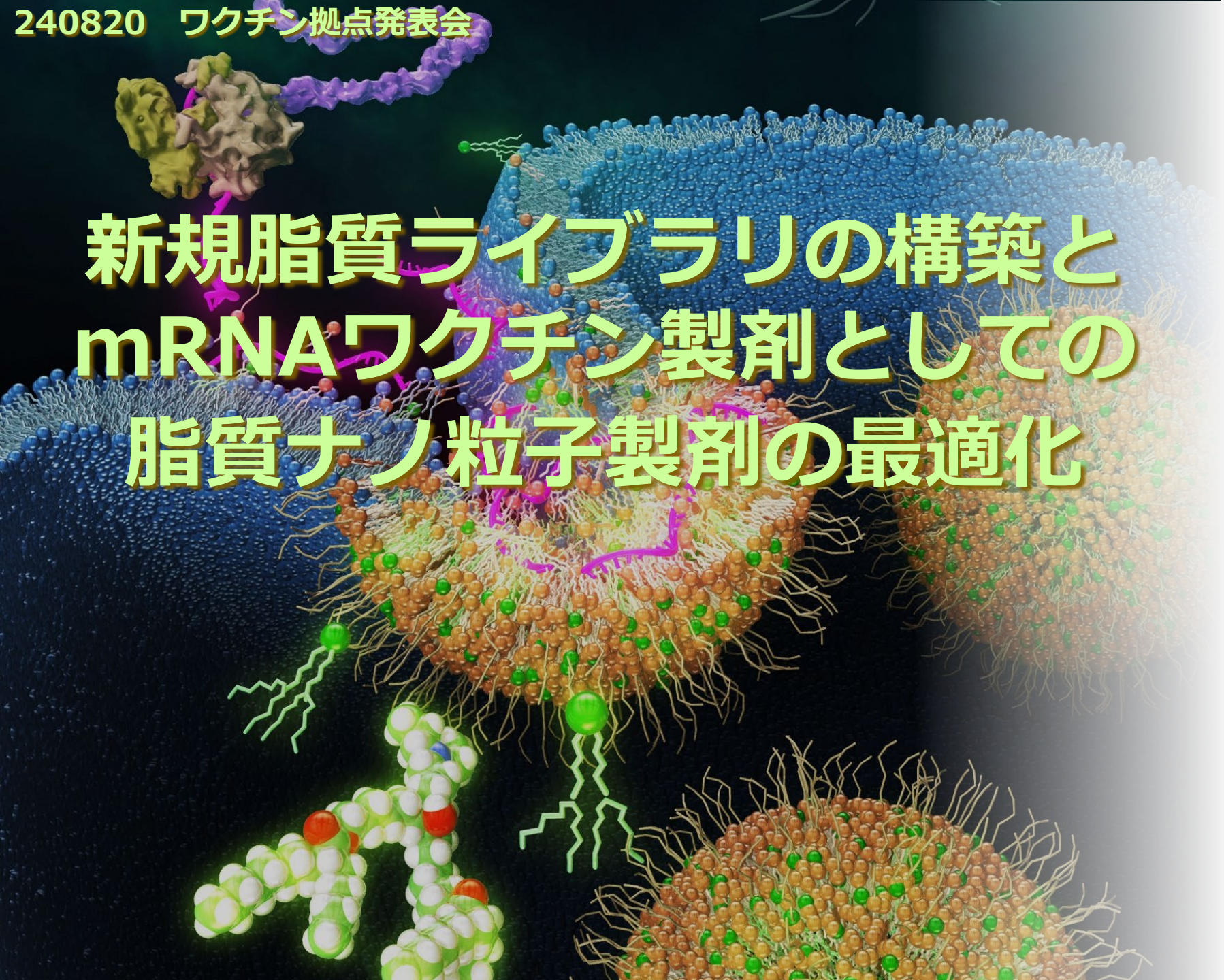
240820　ワクチン拠点発表会



新規脂質ライブラリの構築とmRNAワクチン製剤としての脂質ナノ粒子製剤の最適化

北海道大学
HOKKAIDO UNIVERSITY

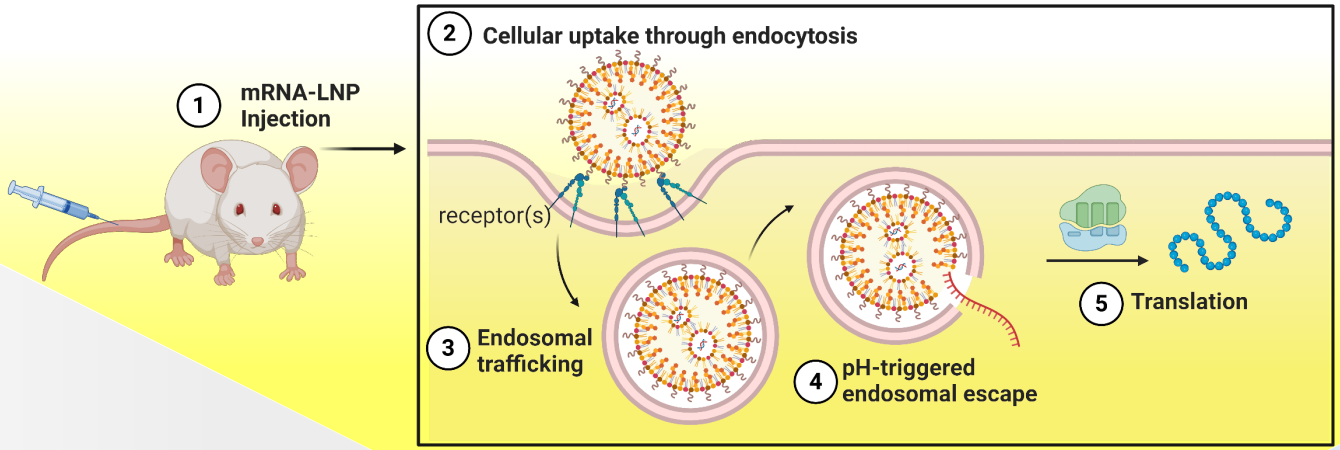
北海道大学　薬学部・大学院薬学研究院
Faculty of Pharmaceutical Sciences, Hokkaido University

IVReD
北海道大学ワクチン研究開発拠点

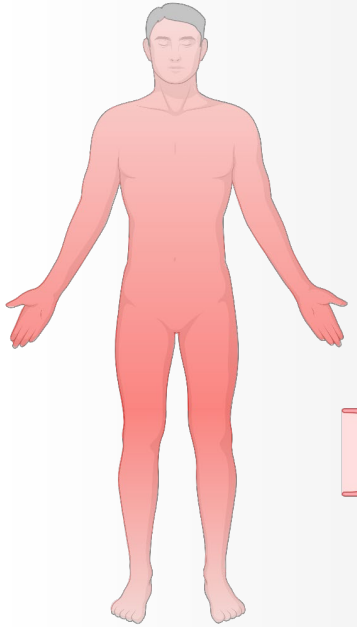
助教　佐藤悠介

大学院薬学研究院

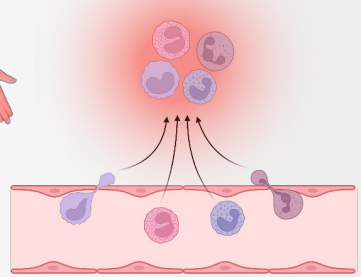
Current limitations of mRNA-LNPs



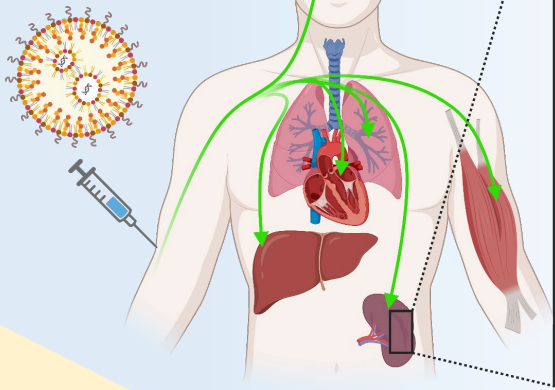
Delivery efficiency

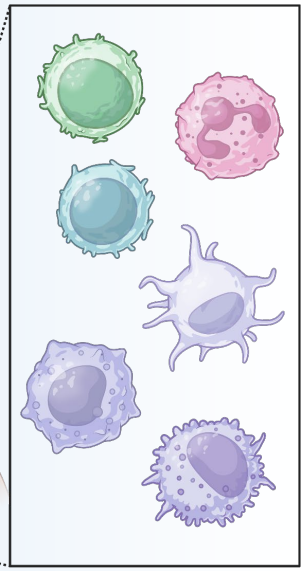


Immunogenicity Toxicity

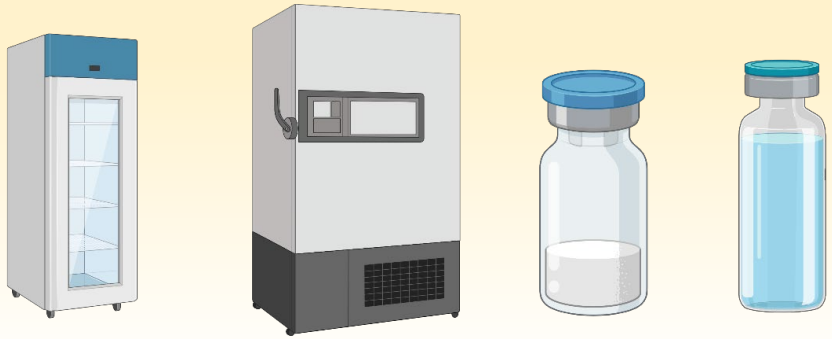


Organ/cell targeting





Storage stability



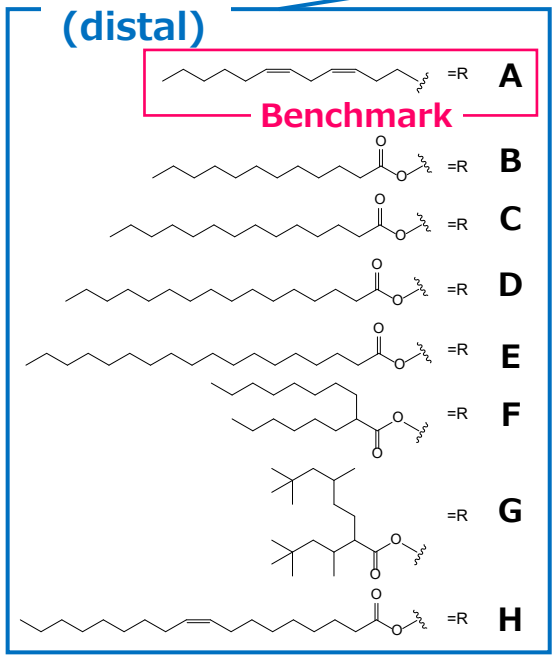
Created with BioRender.com

Development of systematic library of ionizable lipids

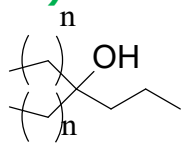
YSK12-C4

US10182987
JP6570188

Scaffold (distal)

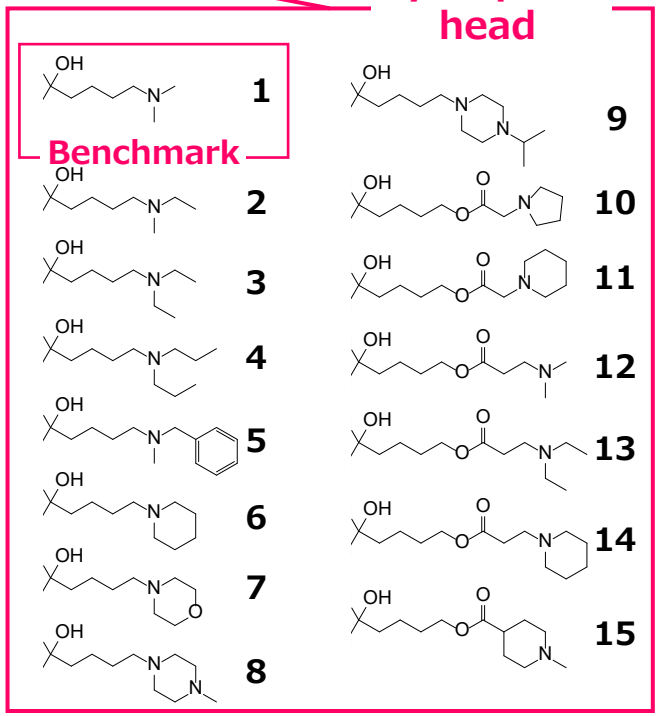


Scaffold (proximal)



n = 6, 8, 10
Benchmark

Hydrophilic head



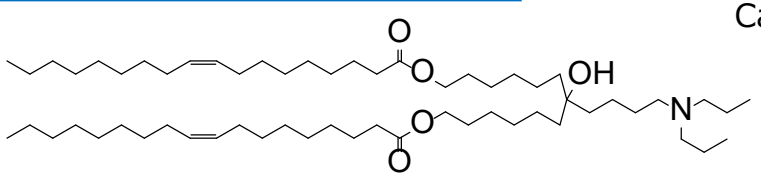
CN110740985, US11517528, JP7202009

Diversity: head x scaffold (distal) x scaffold (proximal)

Lipid name: **CL 1 A 6** → **YSK12-C4**

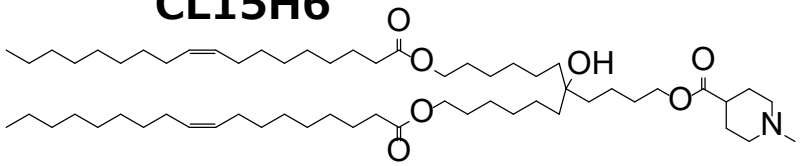
Cationic Lipid

CL4H6



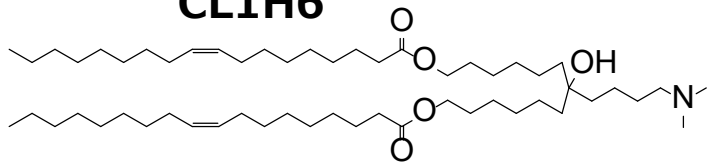
siRNA/mRNA hepatocytes (Sato Y *et al.*, ***J Control Release***, 2019; Hashiba A *et al.*, ***J Control Release***, 2020), **mRNA platelets** (Leung J *et al.*, ***Sci Adv***, 2023), **siRNA/mRNA splenic DCs** (Okuda K *et al.*, ***J Control Release***, 2022; Sasaki K *et al.*, ***Pharmaceutics***, 2022), **Cas9 RNP vitro/hepatocytes** (Suzuki Y *et al.*, ***J Control Release***, 2021; Onuma H *et al.*, ***J Control Release***, 2023)

CL15H6



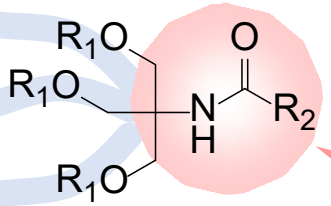
siRNA/mRNA activated hepatic stellate cells (Younis MA *et al.*, ***J Control Release***, 353: 685-698, 2023; Younis MA *et al.*, ***J Control Release***, 361: 592-603, 2023)

CL1H6



siRNA/mRNA NK cells (Nakamura T *et al.*, ***Int J Pharm***, 2021; Nakamura T *et al.*, ***Int J Pharm***, 2023)

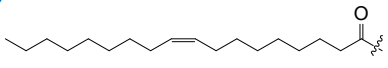
Construction of novel iTxT library for mRNA delivery



iTxT

PCT/JP2023/020385
PCT/JP2024/020084

R_1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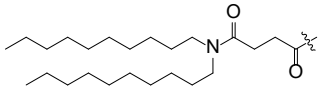
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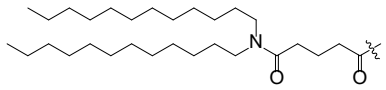
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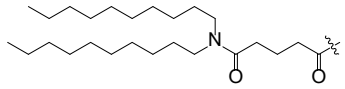
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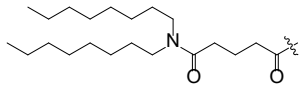
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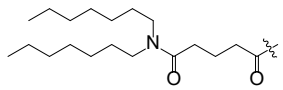
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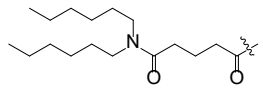
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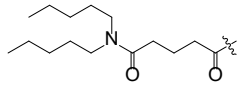
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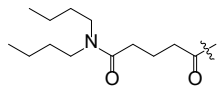
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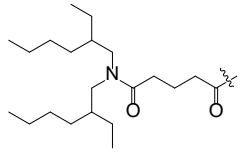
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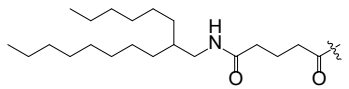
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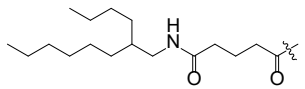
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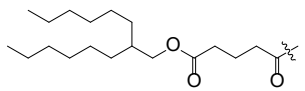
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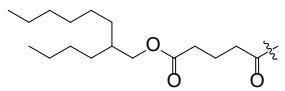
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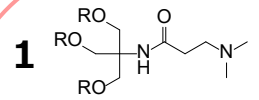
R_2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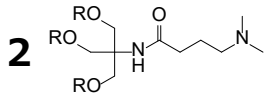
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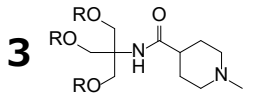
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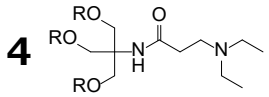
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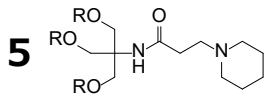
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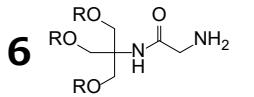
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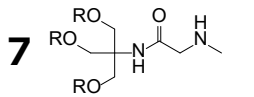
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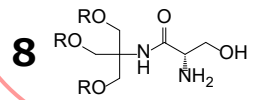
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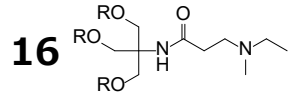
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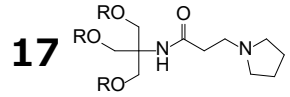
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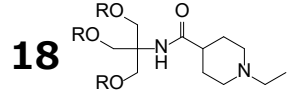
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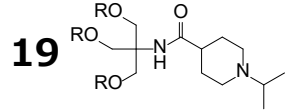
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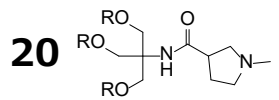
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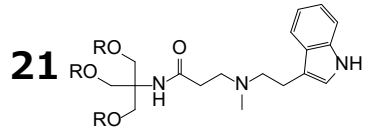
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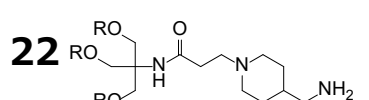
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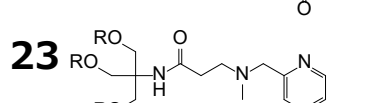
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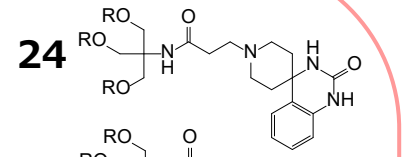
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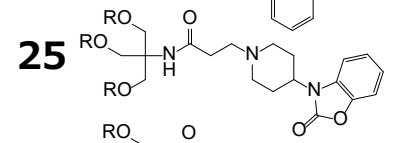
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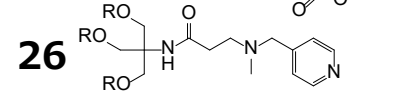
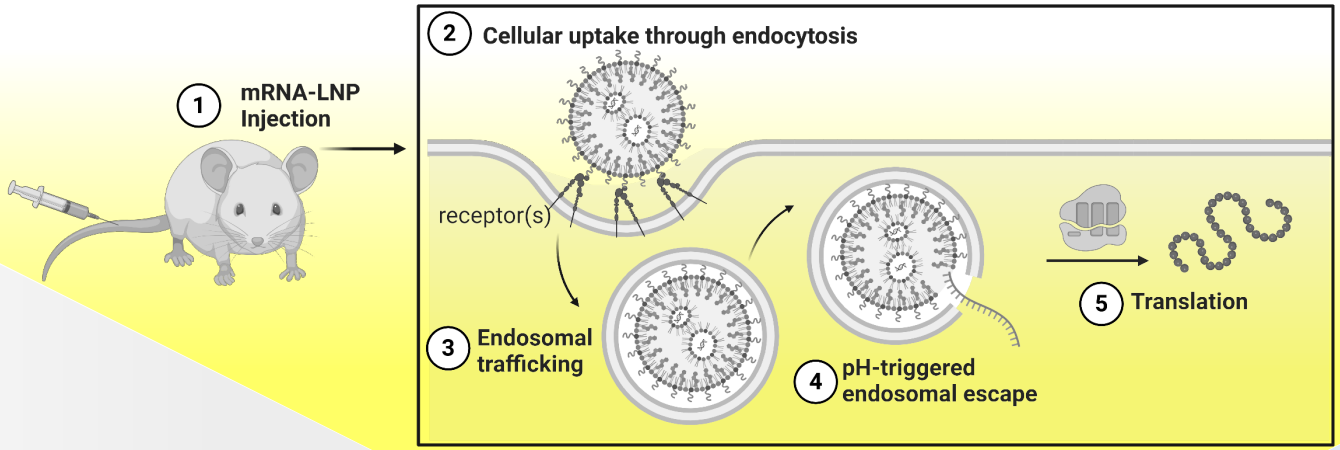

26

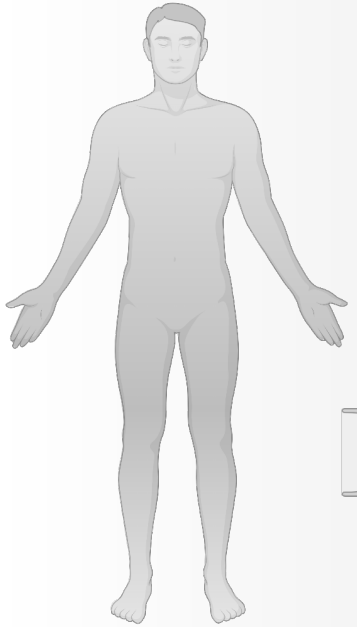
- **Ionizable lipid with various physchem properties can be synthesized based on combination of R_1 and R_2.**

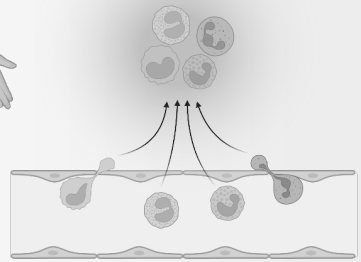
Current limitations of mRNA-LNPs



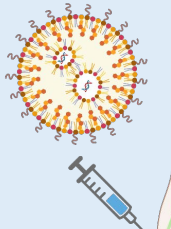
Delivery efficiency

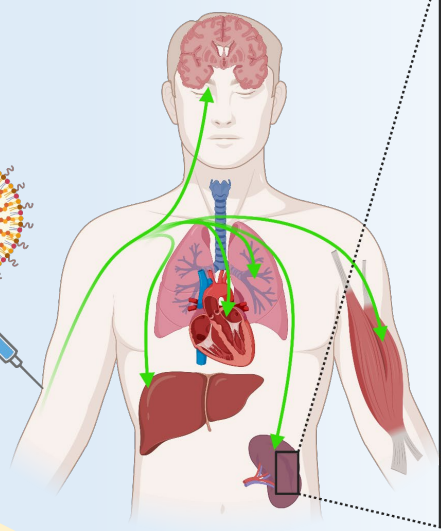
Immunogenicity Toxicity

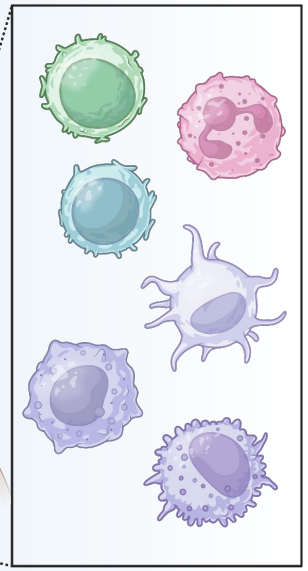




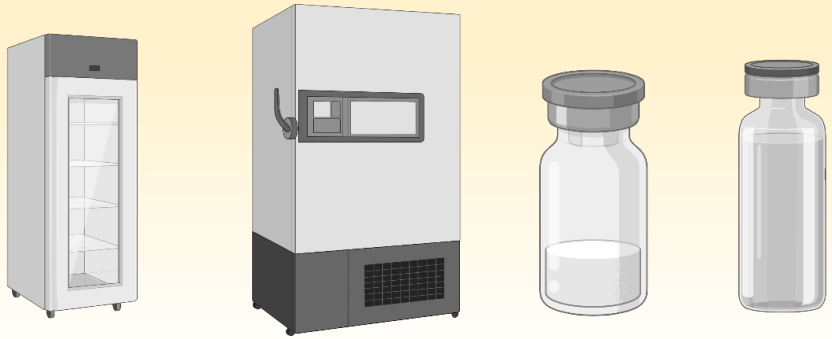
Organ/cell targeting



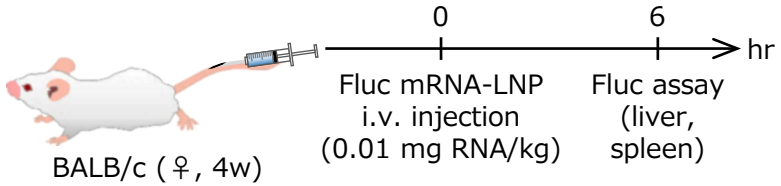




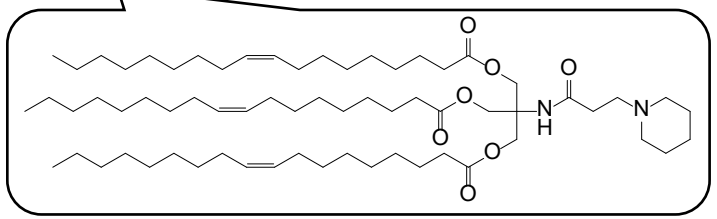
Storage stability

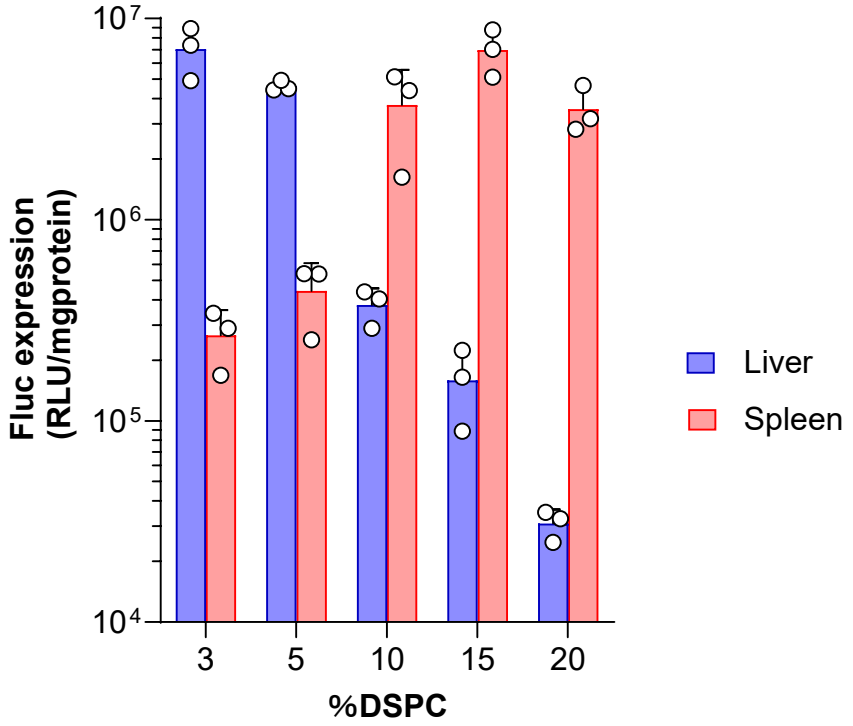


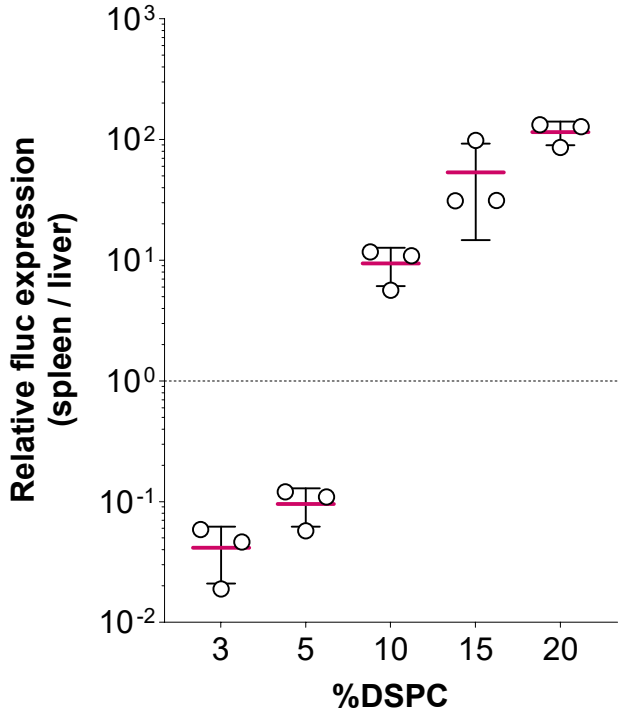
Impact of DSPC content in TOT-5-LNPs on organ-selectivity



[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/3-15/20-32/1.5 (mol%)



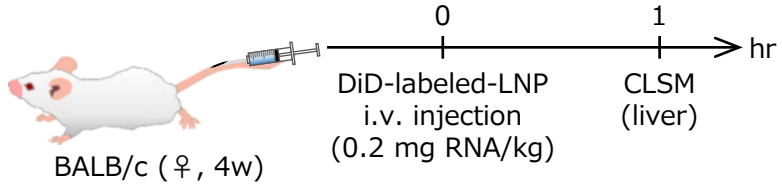


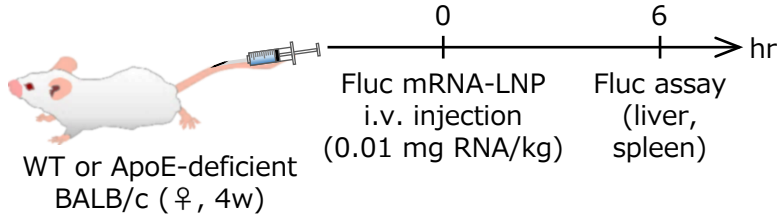


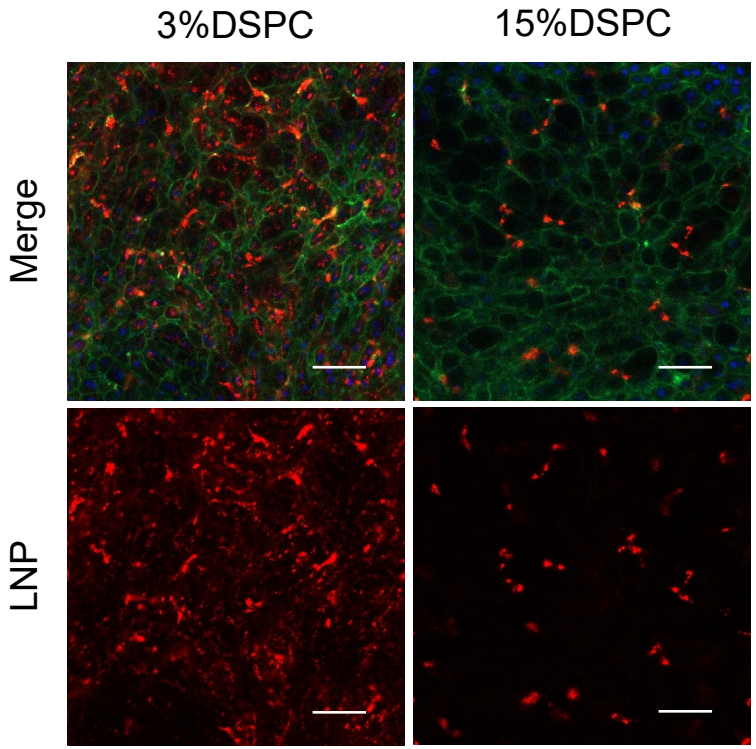
- TOT-5-LNPs functionally delivered mRNA into spleen depending on DSPC content.

Impact of DSPC on biodistribution of TOT-5-LNPs

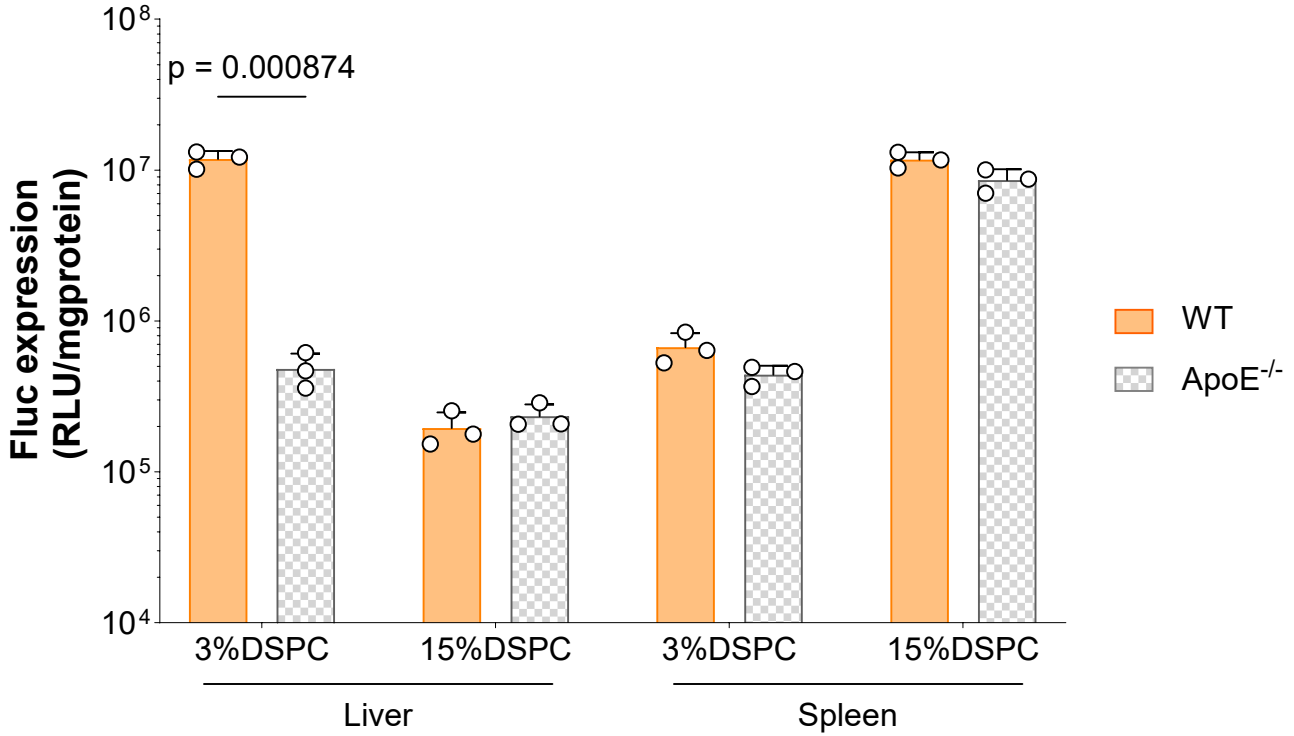
[lipid composition] TOT-5/DSPC/Chol/PEG/DiD = 65/X/35-X/1.5/0.5 (mol%)







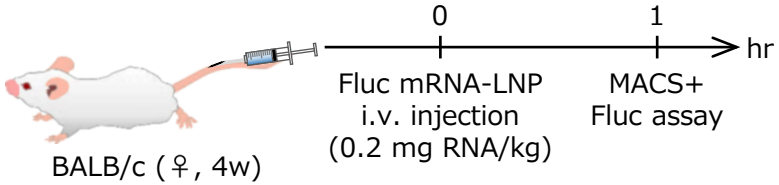
Blood vessels (Tomato-lectin FITC) LNPs (DiD) Bars: 100 μm

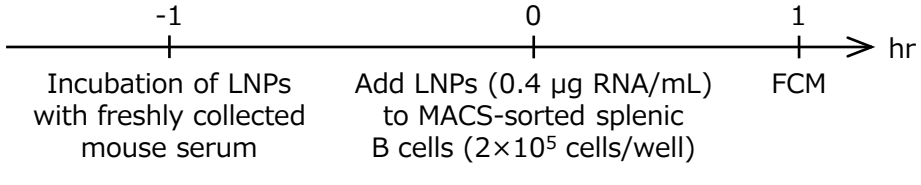


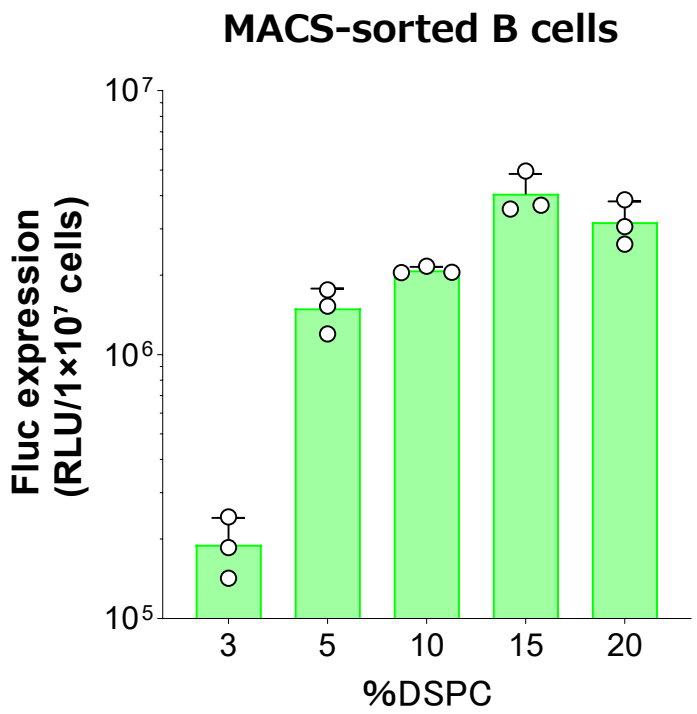
- 15%DSPC failed to accumulate in hepatocytes.
- 15%DSPC functionally delivered mRNA to the spleen through some uptake pathway, not because of passive transfer to the spleen due to lack of hepatic clearance by ApoE-LDLR pathway.

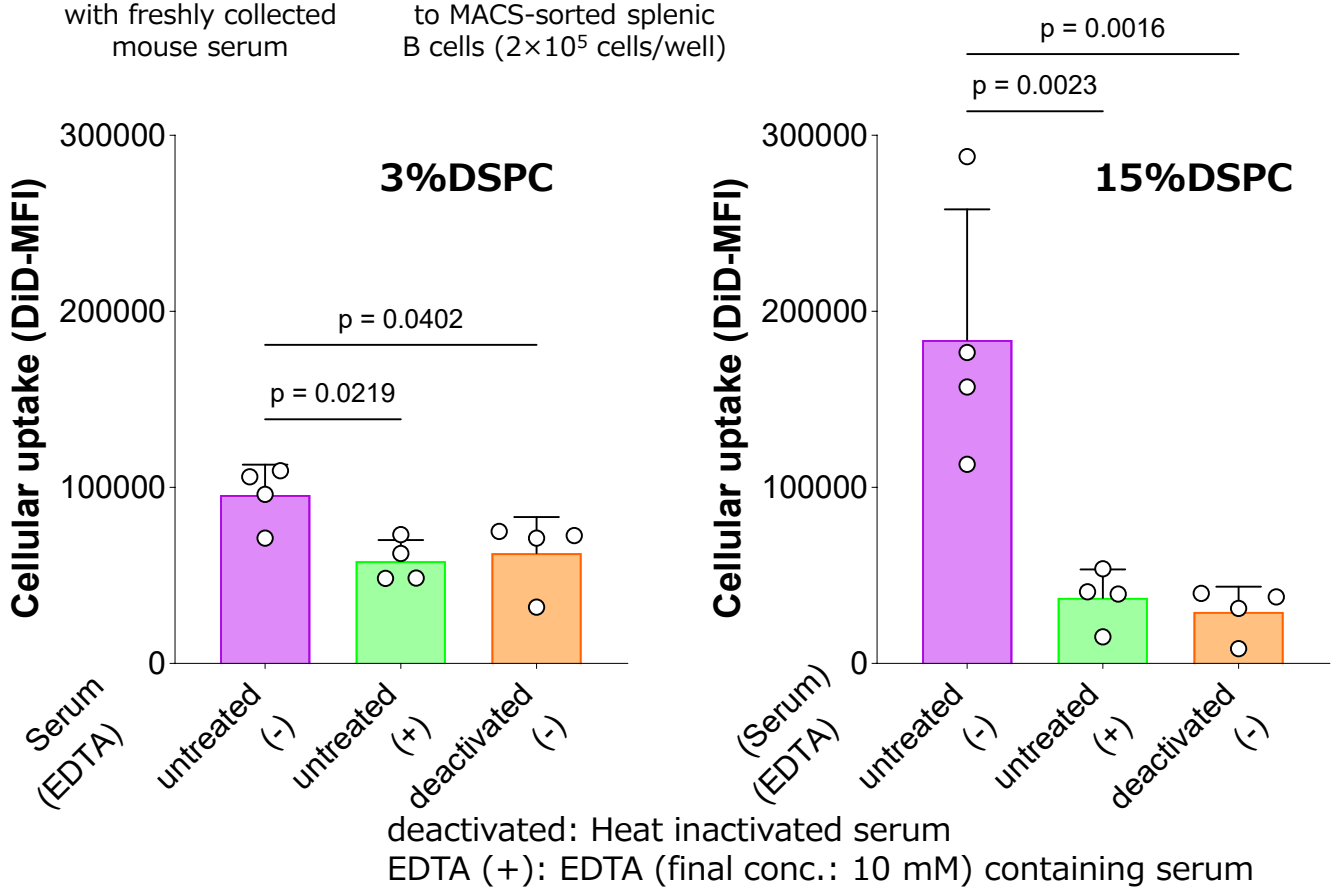
Impact of DSPC on cellular uptake of TOT-5-LNPs in splenic B cells

[lipid composition] TOT-5/DSPC/Chol/PEG/DiD = 65/X/35-X/1.5/0.5 (mol%)







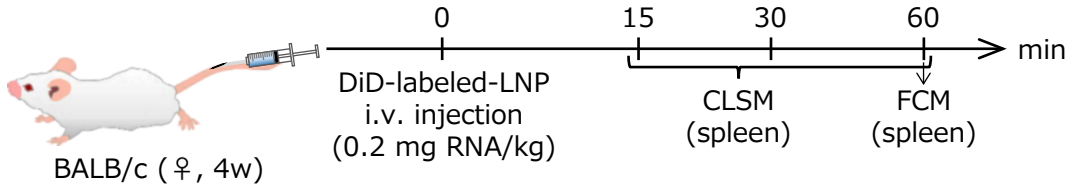


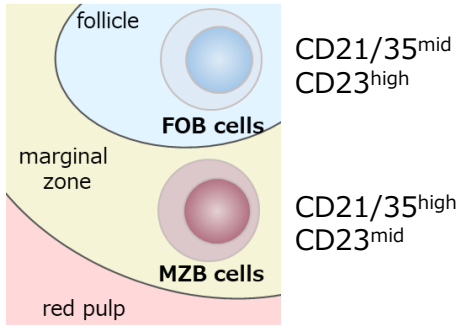
deactivated: Heat inactivated serum
EDTA (+): EDTA (final conc.: 10 mM) containing serum

- TOT-5-LNPs with 15% DSPC showed the highest transgene expression level in splenic B cells.
- Complement pathway would be involved in B cell uptake of 15%DSPC.

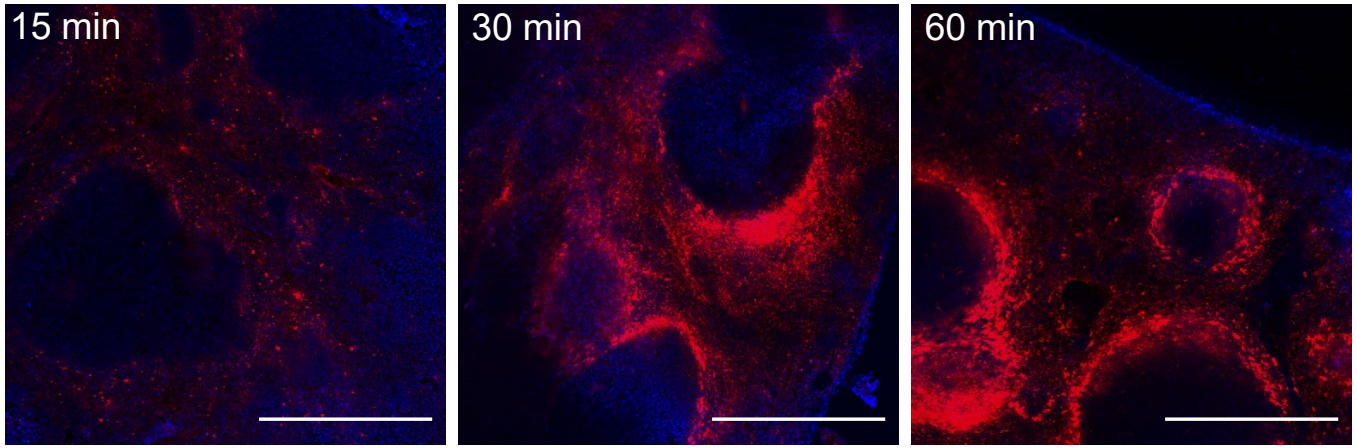
Uptake of 15%DSPC in marginal zone B (MZB) cells

[lipid composition] TOT-5/DSPC/Chol/PEG/DiD = 65/15/20/1.5/0.5 (mol%)

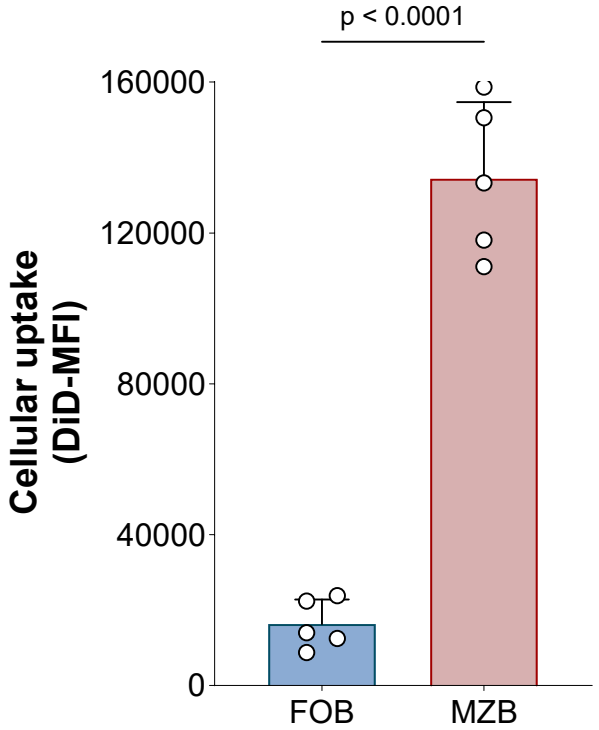




MZB cells are located in MZ where particles can access and express high level of CD21/35.

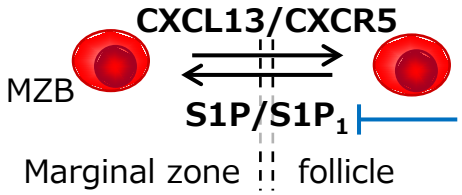


Nuclei (Hoechst33342) LNPs (DiD) Bars: 500 μm



- 15%DSPC highly accumulated in marginal zones in spleen within 30 min.
- Flowcytometric analysis revealed high accumulation of 15%DSPC in MZB cells.

Importance of location of MZB cells on cellular uptake of 15%DSPC

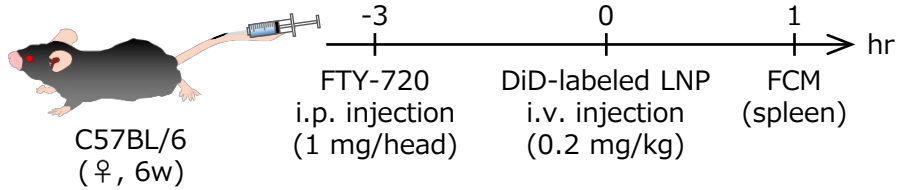


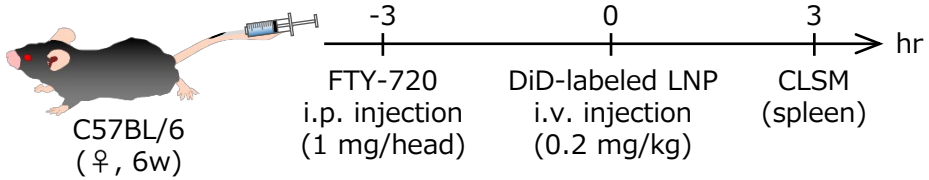
FTY-720 (Fingolimod)

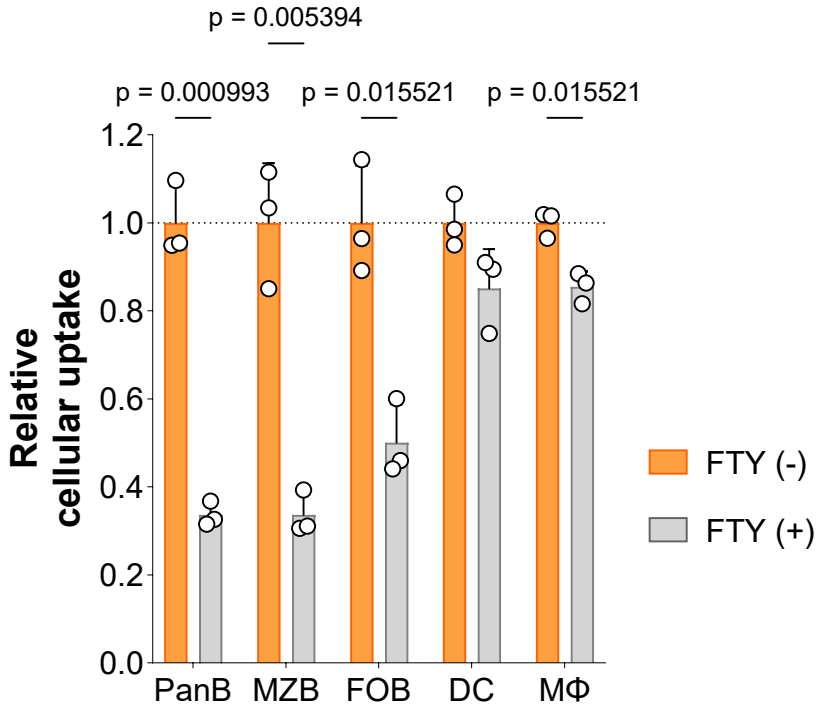
Administration of the $S1P_1$ antagonist FTY causes MZB cells to localize to the follicle

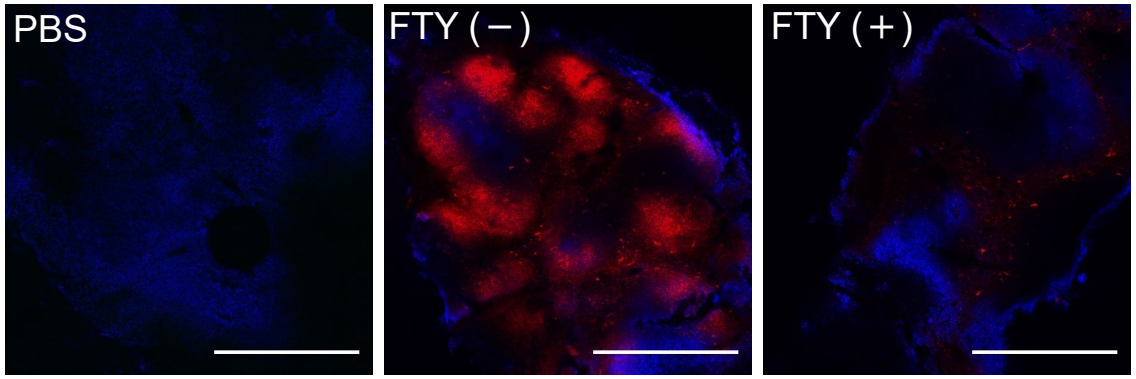
Cinamon G et al., Nat Immunol, 5: 713-720 (2014)

[lipid composition] TOT-5/DSPC/Chol/PEG/DiD = 65/15/20/1.5/0.5 (mol%)





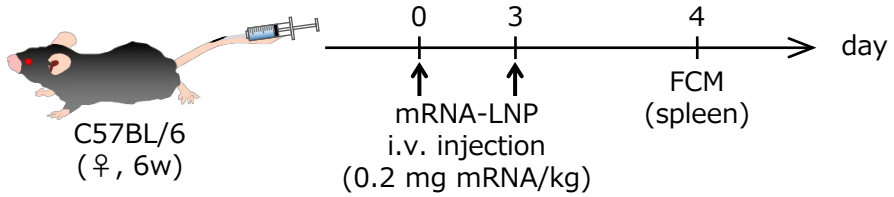




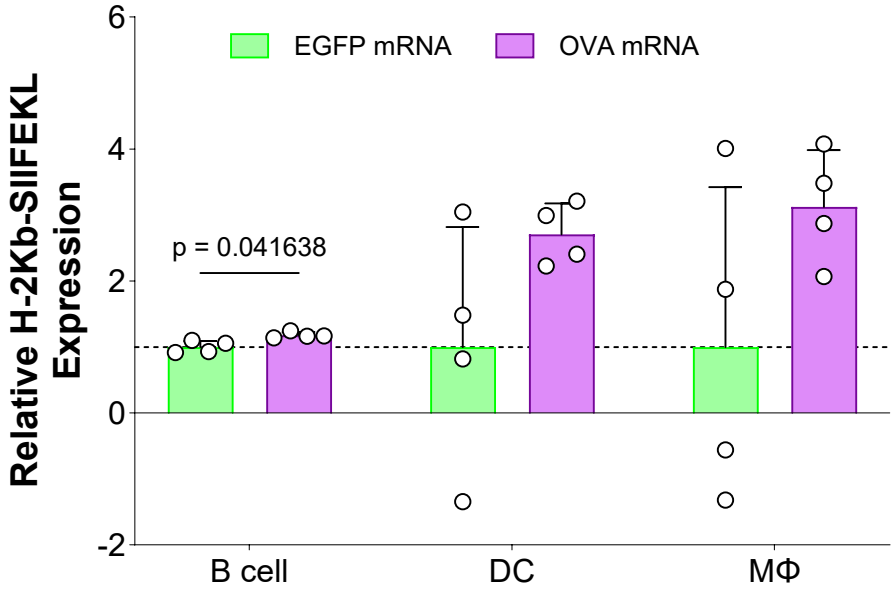
Nuclei (Hiechst33342) LNPs (DiD) Bars: 500 μm

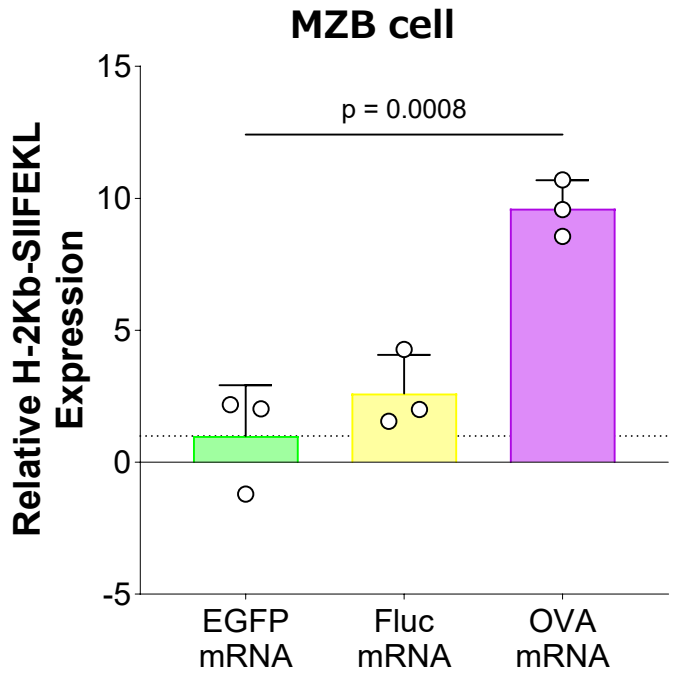
- Pre-treatment of FTY significantly suppressed cellular uptake of 15%DSPC in splenic B cells and localization in follicles.

Detection of MHC class I antigen presentation



[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/15/20/1.5 (mol%)

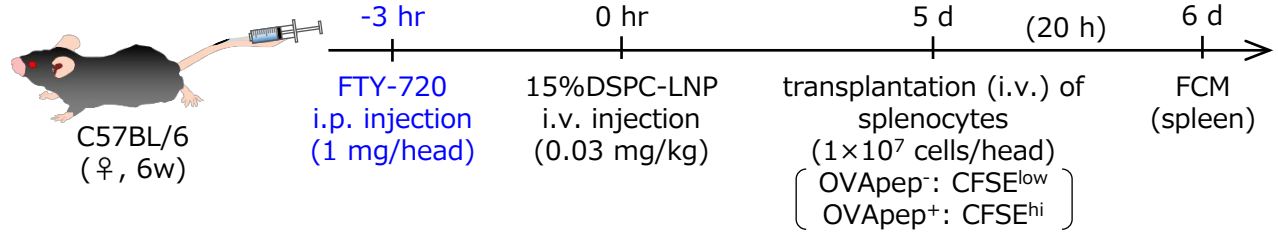




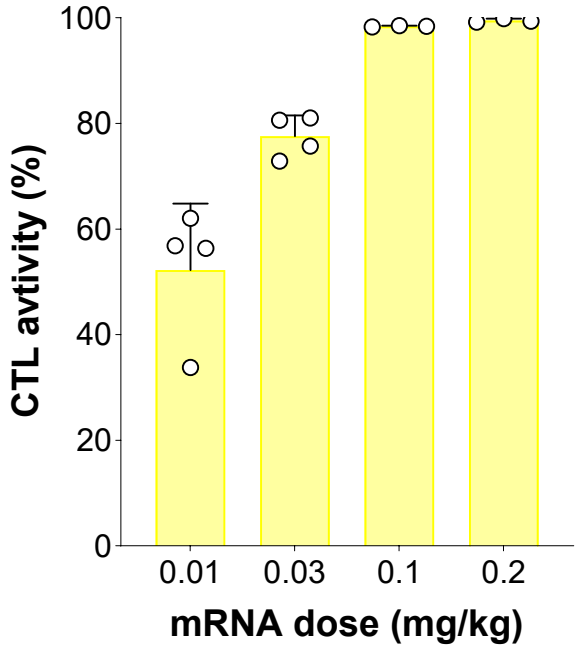
- MHC class I antigen presentation by MZB cells was confirmed.

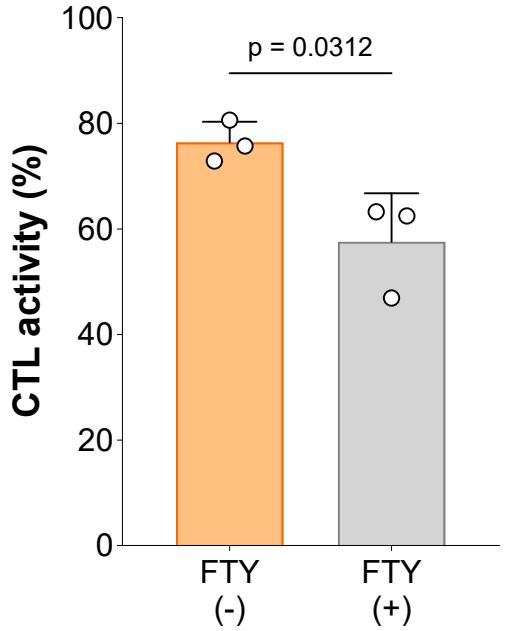
Induction of OVA-specific CTL activity in vivo

[lipid composition] TOT-5/DSPC/Chol/PEG/DiD = 65/15/20/1.5/0.5 (mol%)



※ OVApep: a class I (Kb)-restricted peptide epitope (257-264, SIINFEKL) of the OVA protein

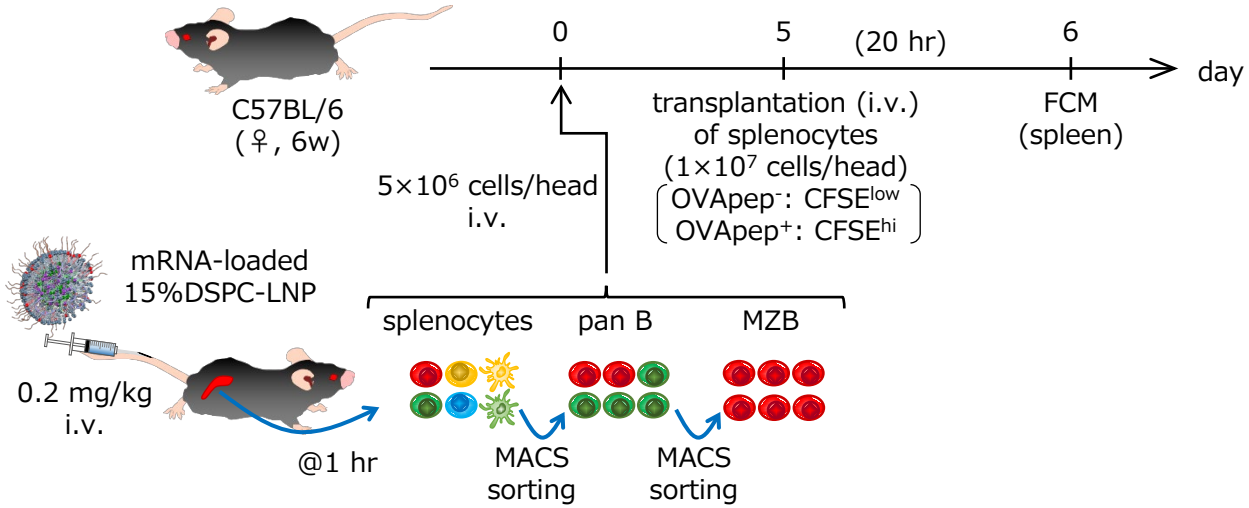


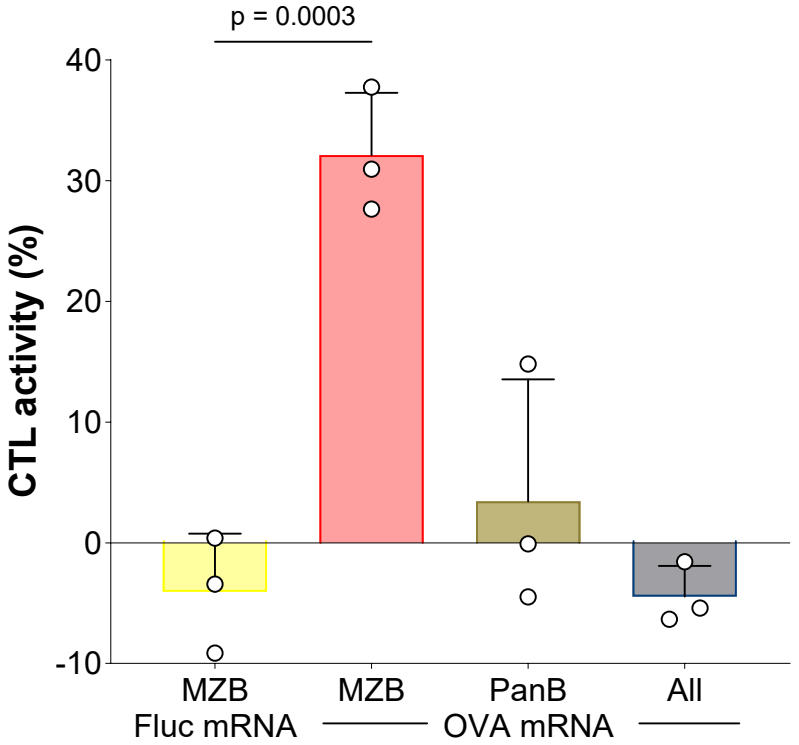


- Nearly 100% CTL activity was achieved at a dose of 0.1 mg mRNA/kg.
- Significant decrease in CTL activity was observed by pre-treatment of FTY, indicating substantial contribution of MZB cells on CTL activity.

Confirming contribution of splenic MZB cells on inducing CTL activity

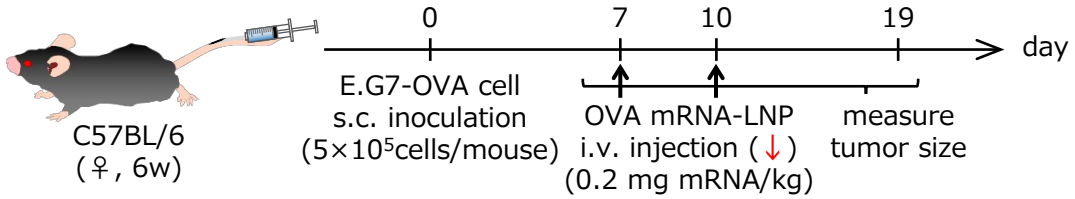
[lipid composition] TOT-5/DSPC/Chol/PEG/DiD = 65/15/20/1.5/0.5 (mol%)





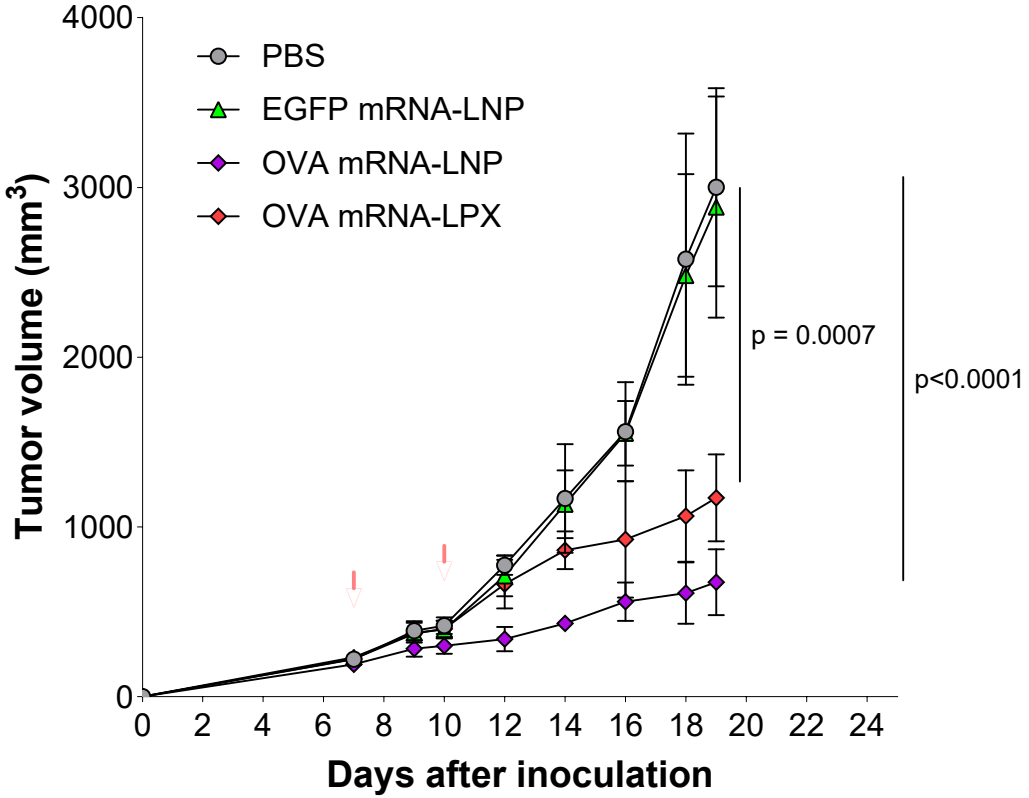
- Significant induction of CTL activity was detected by transplantation of MZB cells collected from OVA mRNA-treated mice, proving contribution of splenic MZB cells on inducing CTL activity.

Therapeutic antitumor activity of 15%DSPC in E.G7-OVA tumor model



[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/15/20/1.5 (mol%)

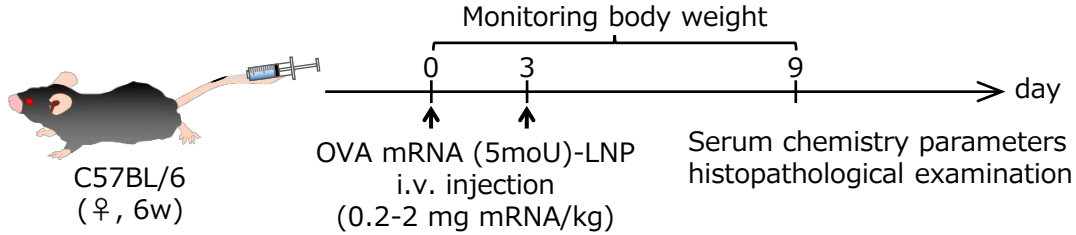
※ LPX: a formulation for cancer mRNA vaccines developed by BioNTech.



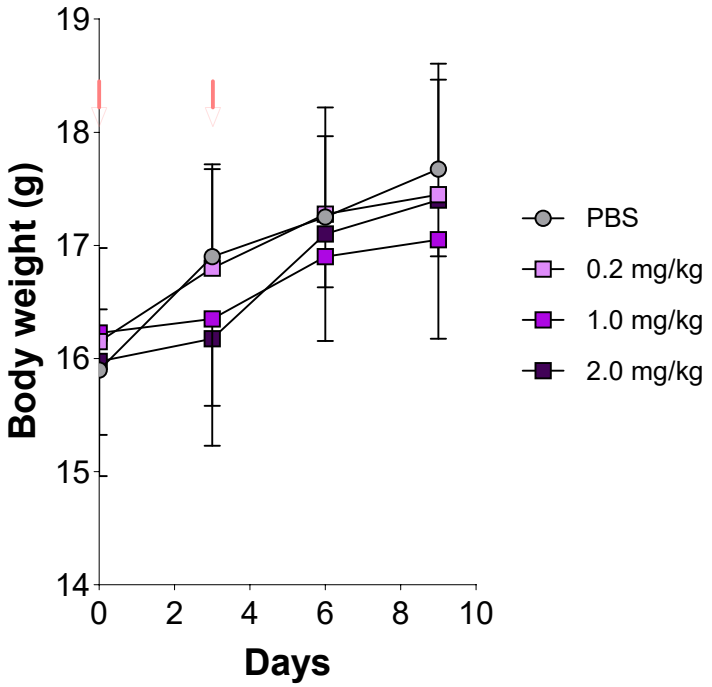
n=3-4, $^{**}p<0.01$, Turkey's test

- OVA mRNA-loaded TOT-5-LNPs showed clear therapeutic antitumor effect in E.G-OVA tumor-bearing mice, suggesting their ability to induce antigen-specific cell-mediated immunity.

Safety of 15%DSPC



[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/15/20/1.5 (mol%)



	PBS	0.2 mg/kg	1.0 mg/kg	2.0 mg/kg		PBS	0.2 mg/kg	1.0 mg/kg	2.0 mg/kg
TP (g/dL)	5.1±0.1	5.2±0.1	5.2±0.1	5.1±0.1*	ALT (IU/L)	18.0±0.7	18.3±1.6	17.0±2.1	18.5±1.1
ALB (g/dL)	3.7±0.04	3.7±0.07	3.6±0.07	3.5±0.07	LDH (IU/L)	228.8±43.0	227.0±30.1	289.5±50.1	304.3±15.8
BUN (mg/dL)	22.2±1.0	21.3±1.0	19.9±1.4	19.7±1.7	AMY (IU/L)	1911±78	1763±20	2053±66	1901±116
CRE (mg/dL)	0.1±0.01	0.±0.01	0.1±0.01	0.1±0.00	γ-GT (IU/L)	<3	<3	<3	<3
Na (mEq/L)	150.0±1.0	150.3±0.4	152.5±0.5**	149.5±0.5	T-CHO (mg/dL)	71.5±2.1	67.5±2.1	71.0±2.7	71.5±8.0
K (mEq/L)	7.9±0.6	7.2±0.4	6.1±0.3**	7.0±0.2	TG (mg/dL)	47.5±6.3	42.3±2.5	60.5±18.0	44.8±11.1
Cl (mEq/L)	107.0±0.7	108.3±0.8	109.0±1.6	107.5±0.9	HDL-C (mg/dL)	44.3±1.8	41.5±1.1	44.3±2.2	42.8±5.7
Ca (mg/dL)	11.0±0.1	10.9±0.2	10.7±0.4	10.6±0.2	T-BIL (mg/dL)	0.1±0.01	0.1±0.01	0.1±0.00	0.1±0.04
IP (mg/dL)	10.8±0.4	10.0±0.1	10.0±0.7	10.0±0.5	GLU (mg/dL)	381.0±24.8	339.3±28.6	364.8±41.2	357.5±46.5
AST (IU/L)	43.0±2.7	45.8±2.2	46.8±2.6	51.3±8.0	*p<0.05, **p<0.01, nrANOVA followed by Dunnett's test vs. PBS				

	PBS				0.2 mg/kg				1.0 mg/kg				2.0 mg/kg			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Liver																
Abnormal findings	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spleen																
Enlargement of the white pulp	-	-	-	-	±	±	±	±	±	±	±	±	±	+	±	+

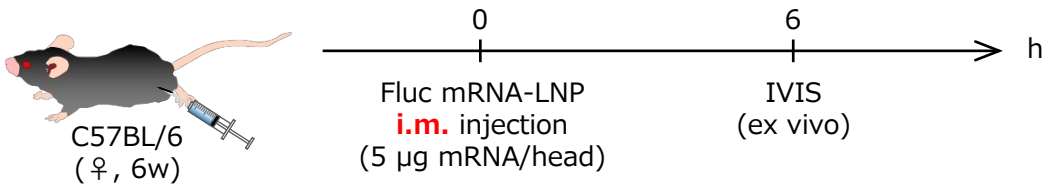
− : no abnormal ± : slightly weak + : weak ++ : medium +++ : high

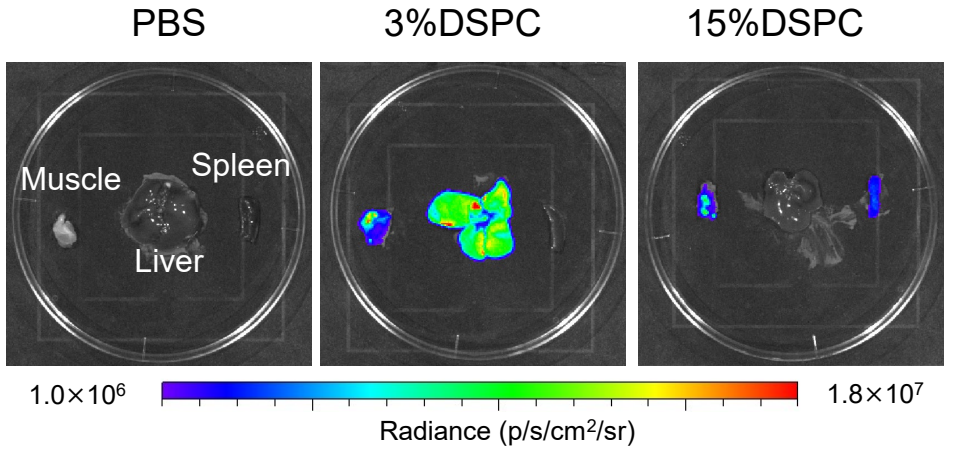
n = 4, mean±SD

- No obvious signs of toxicity were detected.

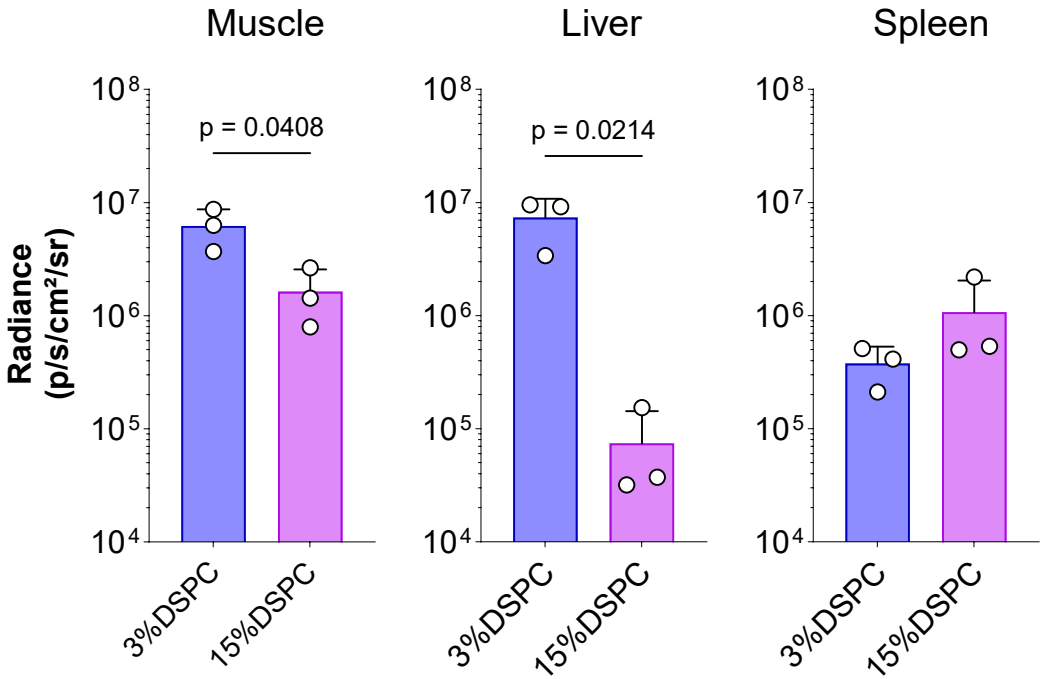
Application of 15%DSPC-TOT-5-LNPs for intramuscular vaccines

[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/3 or 15/20 or 32/1.5 (mol%)

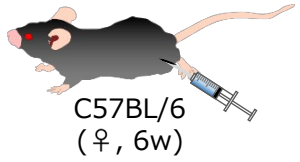


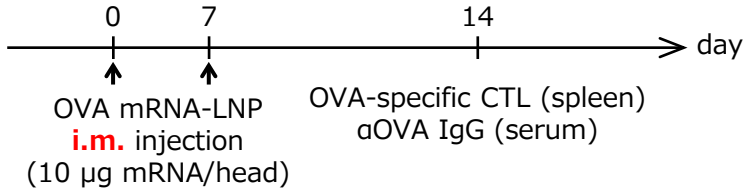


- Fluc expression at injection site was low for 15%DSPC.
- Fluc expression in the liver was significantly suppressed in 15%DSPC, reflecting organ selectivity after intravenous administration.



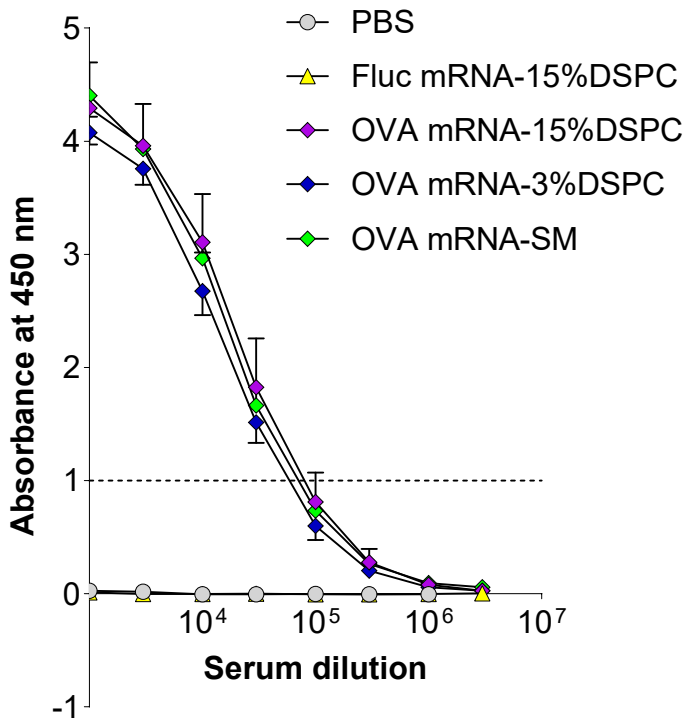
Application of 15%DSPC-TOT-5-LNPs for intramuscular vaccines

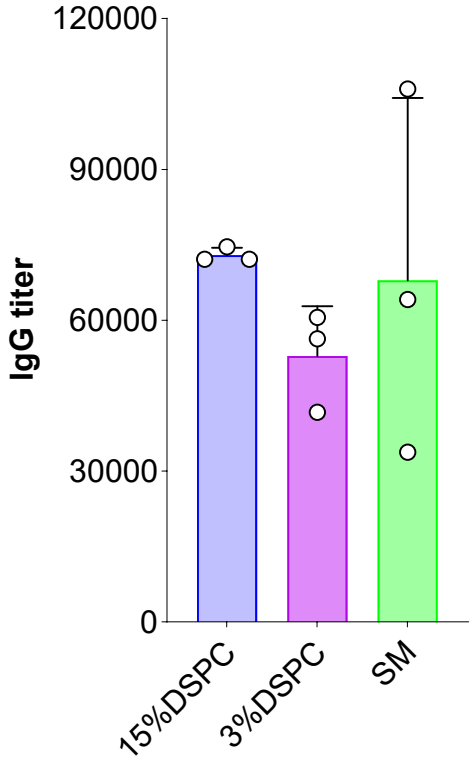




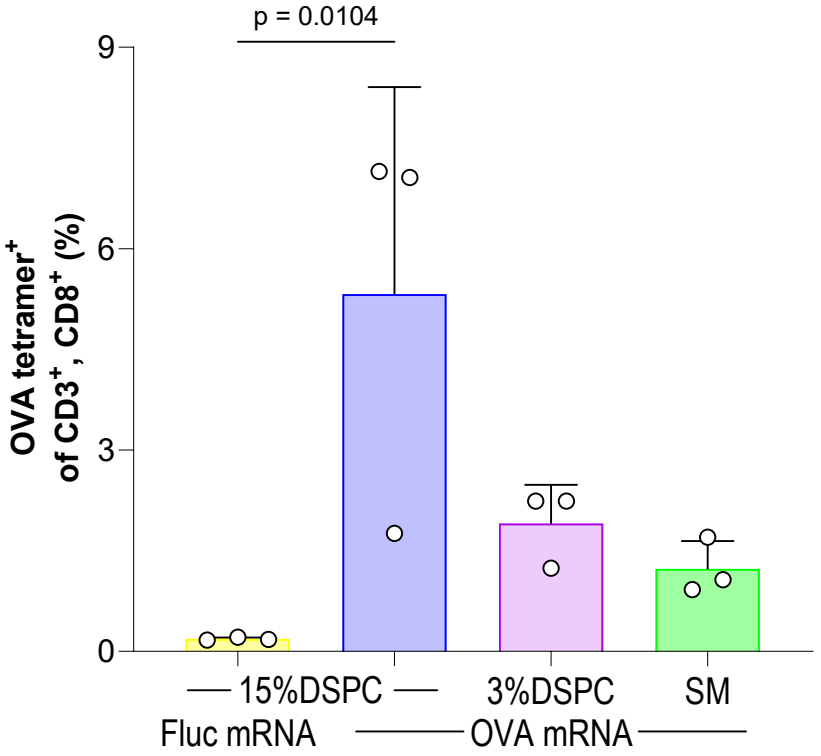
[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/3 or 15/20 or 32/1.5 (mol%)
SM-102/DSPC/Chol/PEG = 50/10/40/1.5 (mol%)

αOVA IgG (serum)



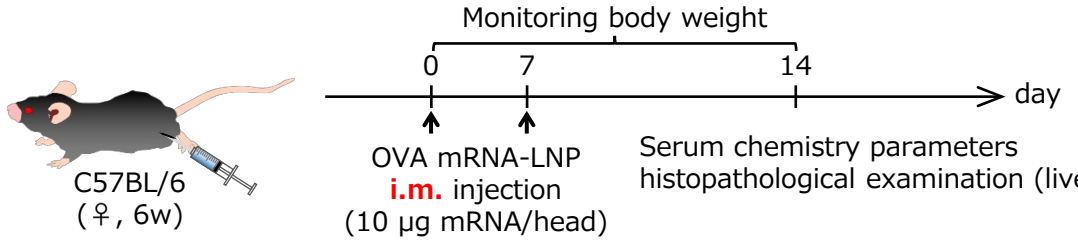


OVA-specific CTL (spleen)

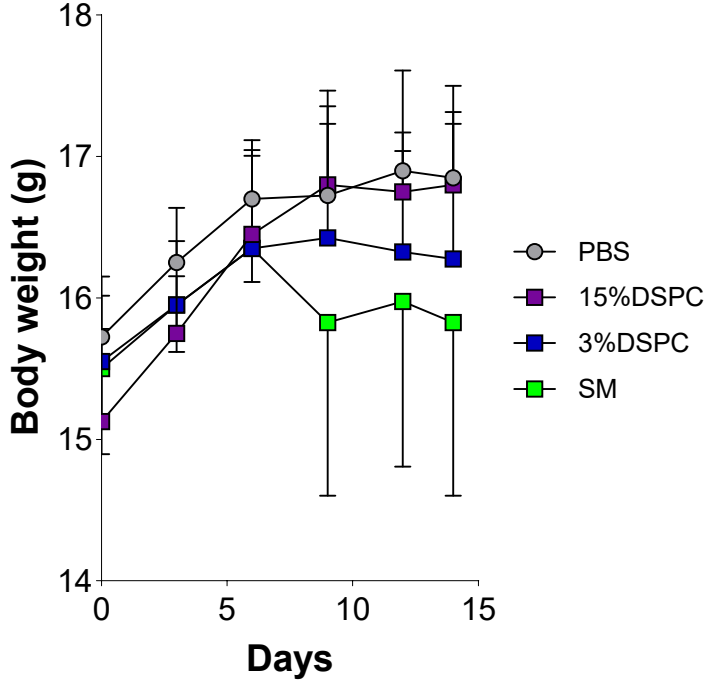


- 15%DSPC-LNP showed comparable and better induction of anti-OVA IgG level and OVA-specific CTL, respectively, compared with SM-LNPs (Moderna's vaccine-mimic).

Application of 15%DSPC-TOT-5-LNPs for intramuscular vaccines



[lipid composition]
TOT-5/DSPC/Chol/PEG = 65/3 or 15/20 or 32/1.5 (mol%)
SM-102/DSPC/Chol/PEG = 50/10/40/1.5 (mol%)



	PBS	15%DSPC	3%DSPC	SM		PBS	15%DSPC	3%DSPC	SM
TP (g/dL)	5.3±0.2	5.4±0.2	5.3±0.2	5.3±0.3	ALT (IU/L)	17.8±1.6	20.5±2.3	68.3±29.2*	72.0±29.8*
ALB (g/dL)	3.8±0.1	3.7±0.1	3.6±0.1	3.6±0.2	LDH (IU/L)	347.5±76.9	344.0±225.5	615.0±48.6	362.0±104.8
BUN (mg/dL)	16.8±0.5	20.8±2.2	19.3±3.0	24.6±3.0	AMY (IU/L)	1720.0±157.6	1802.5±45.1	1829.0±112.8	1851.3±120.5
CRE (mg/dL)	0.1±0.01	0.2±0.02	0.1±0.01	0.1±0.01	γ-GT (IU/L)	<3	<3	<3	<3
Na (mEq/L)	156.0±1.7	153.8±0.8	152.8±1.5*	155.8±1.1	T-CHO (mg/dL)	87.3±3.9	82.8±4.3	77.8±5.9	76.3±11.8
K (mEq/L)	6.2±0.4	6.7±1.5	6.5±0.2	6.6±0.7	TG (mg/dL)	52.3±8.8	41.3±9.6	44.8±7.7	33.3±12.9
Cl (mEq/L)	109.0±1.4	110.8±1.8	109.3±2.0	112.3±0.8	HDL-C (mg/dL)	51.3±1.8	48.3±4.1	45.8±1.5	41.8±8.1
Ca (mg/dL)	10.7±0.3	10.3±0.2	10.5±0.2	10.6±0.3	T-BIL (mg/dL)	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.02*
IP (mg/dL)	12.9±0.6	11.3±3.0	12.7±1.0	12.7±0.6	GLU (mg/dL)	225.5±30.2	214.5±38.4	224.5±5.4	166.5±17.9*
AST (IU/L)	42.8±2.2	45.3±6.5	85.3±25.3	159.3±110.4	*p<0.05, nrANOVA followed by Dunnett's test vs. PBS				

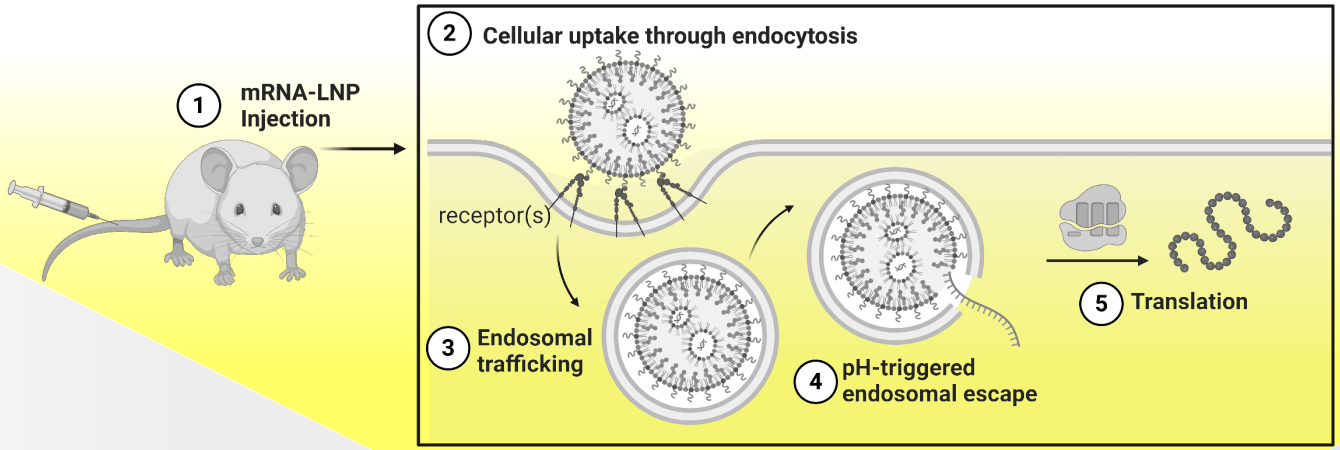
	PBS				15%DSPC				3%DSPC				SM			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Liver																
Hepatocellular necrosis	-	-	-	-	-	-	-	-	-	-	-	±	±	±	-	-
Enlargement of the portal region hepatic cell	-	-	-	-	-	-	-	-	-	-	-	-	+	±	±	±
Enlargement of the Kupffer cell	-	-	-	-	-	-	-	-	±	±	±	±	+	±	±	±
Increased mitotic count	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±
Spleen																
Extramedullary hematopoiesis	-	-	-	-	++	±	++	±	+	+	±	-	++	+	++	++
Enlargement of the white pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	±	±	-

− : no abnormal ± : slightly weak + : weak ++ : medium +++ : high

- 15%DSPC-LNP showed better safety profile compared with SM-LNPs
- Spleen selective 15%DSPC-LNPs would be effective and safe IM mRNA vaccines.

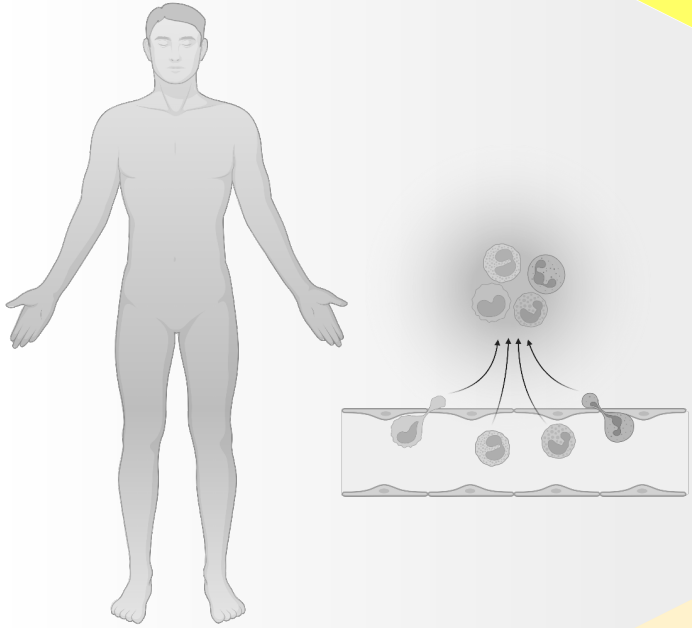
n = 4, mean±SD

Current limitations of mRNA-LNPs

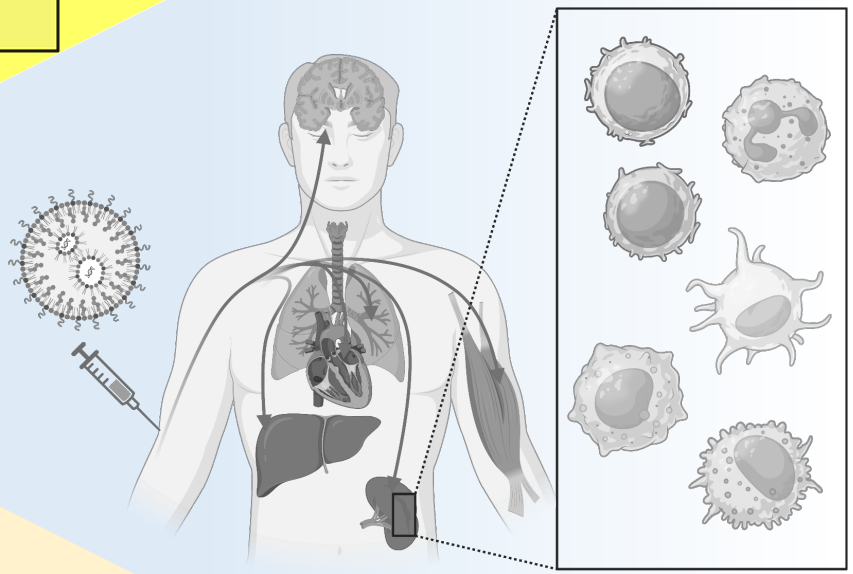


Delivery efficiency

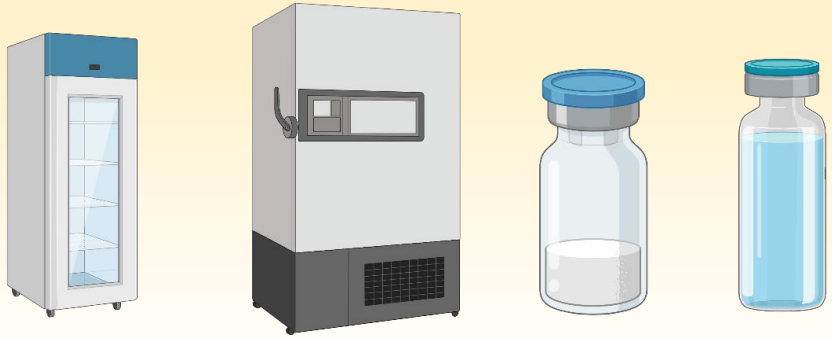
Immunogenicity Toxicity



Organ/cell targeting



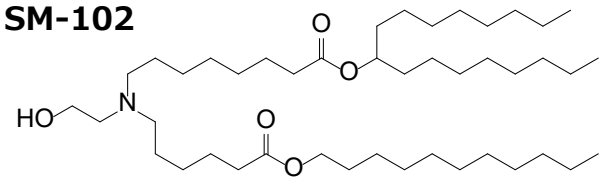
Storage stability



Created with BioRender.com

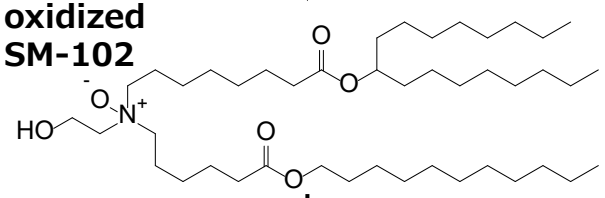
Inactivation of mRNA through adduct formation reported by Moderna

SM-102



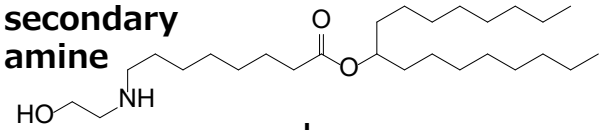
oxidation

oxidized SM-102



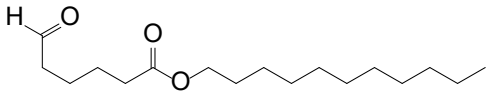
hydrolysis

secondary amine



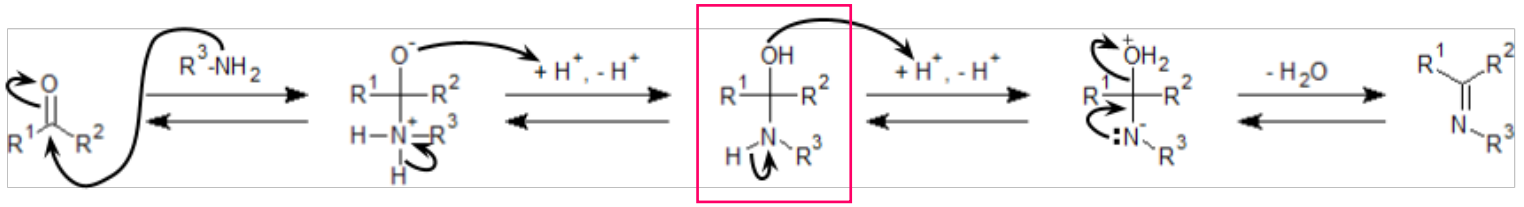
+

fatty aldehyde



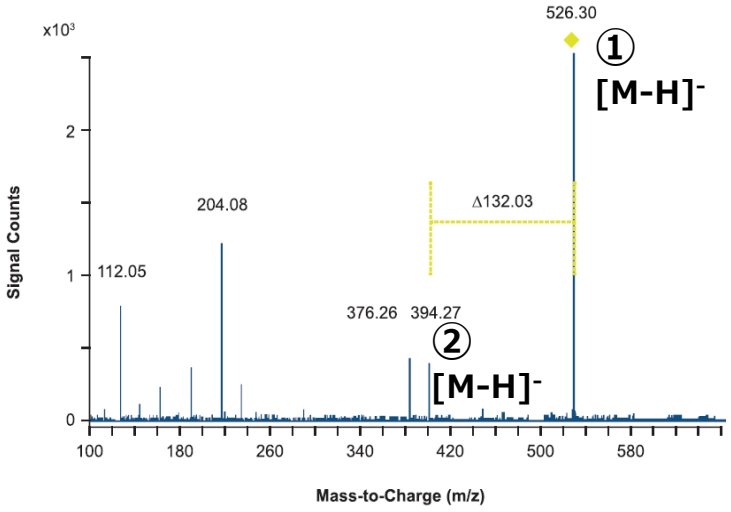
Addition to nucleobases of mRNA
→ inactivation of mRNA

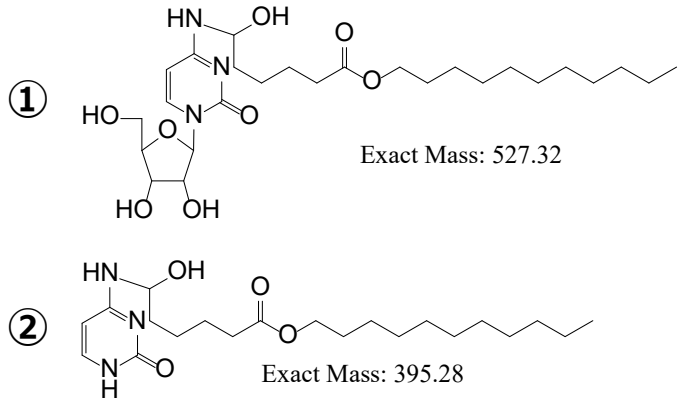
Addition reaction of aldehyde to nucleobase (amine)



hemiaminal

Ex) addition to cytosine base





Adduct formation was observed in >100 types of the tested Moderna's ionizable lipids.

Packer M *et al*., *Nat Commun*, 12: 6777 (2021)

General inactivation pathways for tertiary amine-based ionizable lipids

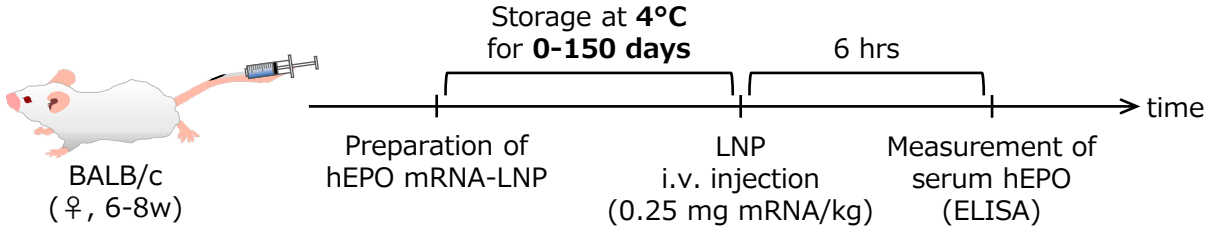
Ex) 4000 nt length of mRNA and N/P ratio of 6

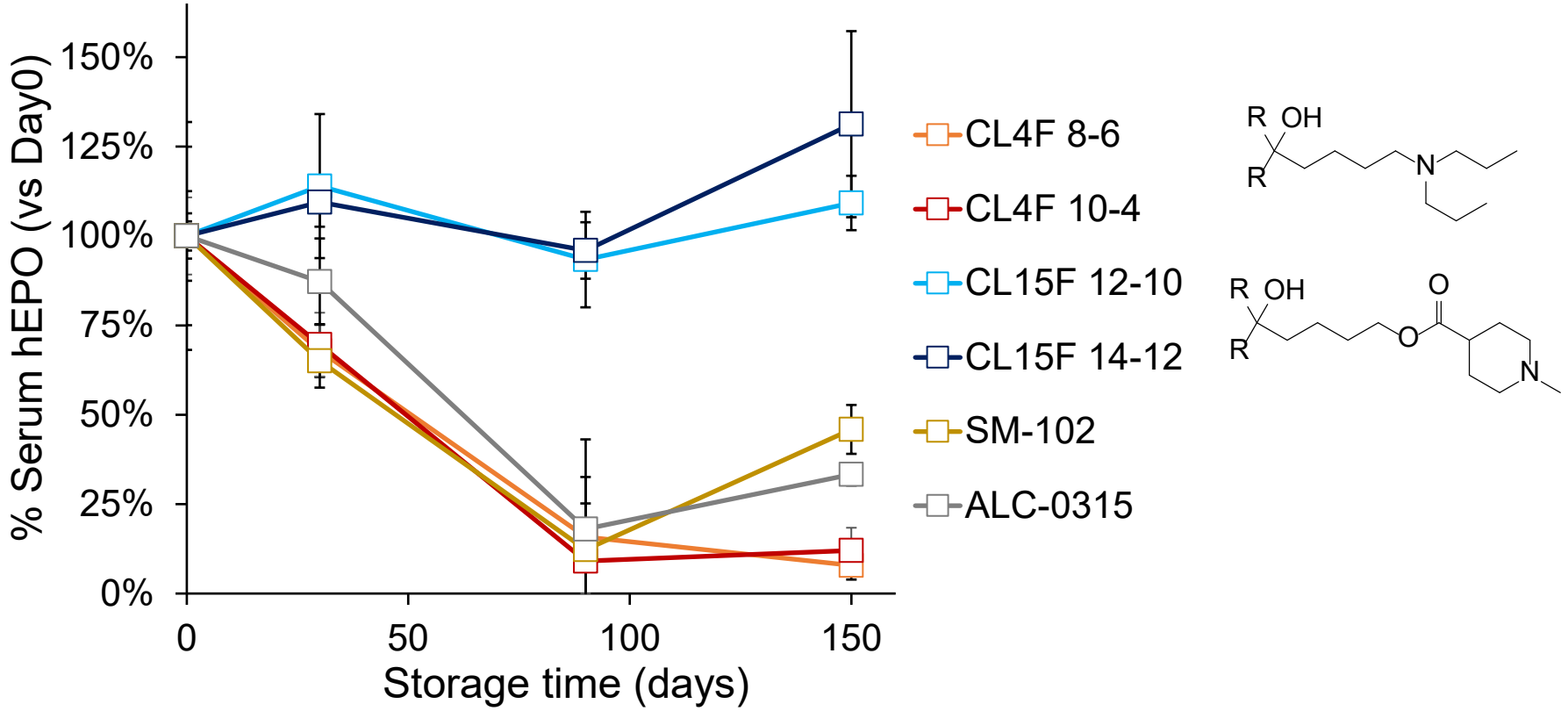
24,000 molar excess ionizable lipids against mRNA exist in LNPs

→ 24 molar excess amounts of aldehydes are produced after only 0.1% of ionizable lipids are oxidized/hydrolyzed

→ **Strategy to minimize generation of fatty aldehydes is desired in addition to manufacturing process control**

Piperidine-based CL15 lipids showed enhanced stability at 4°C in vivo

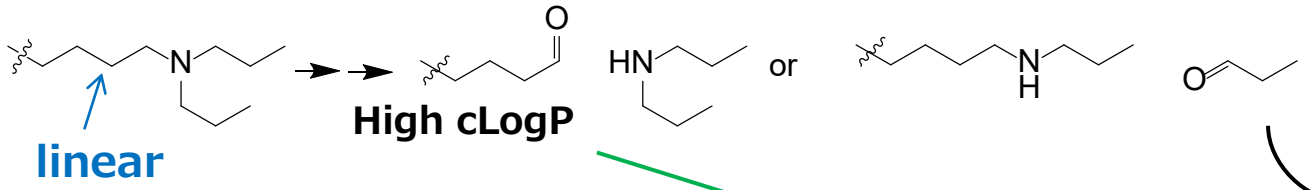




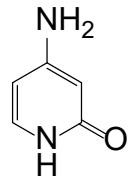
- **Activity of all conventional ionizable lipids dropped down in a time-dependent manner while both CL15 lipids showed no detectable loss of activity**

Hypothesis: relationship between headgroup structure and adduct formation

General ionizable lipid with linear linker to amine

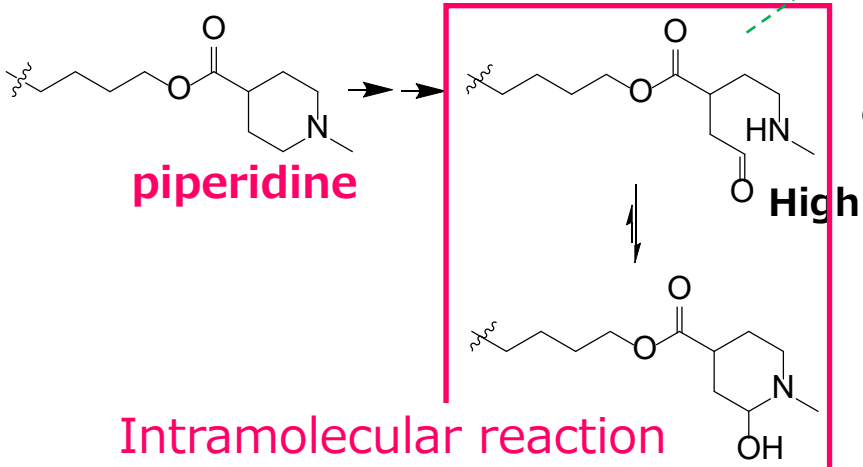


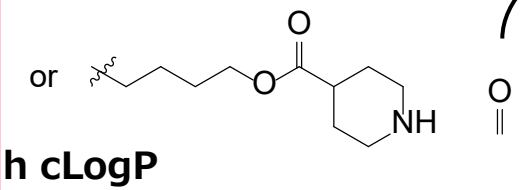
Adduct formation



diffuse out of LNPs (highly diluted)
⇒ limited adduct formation

Ionizable lipid with piperidine linker





⇒ limited adduct formation

(cLogP values were calculated by ChemDraw)

Piperidine structure in headgroup could limits adduct formation and suppress inactivation of mRNA

Summary

- Use of TOT-5 and increasing DSPC content achieved specific delivery of mRNA into MZB cells and strong antigen-specific immune response after i.v. and i.m. administration with improved safety compared with clinically approved vaccine formulation.

- Further optimization of vaccine formulation for improving safety is currently under examination.

- Ionizable lipids with improved thermostability suitable for vaccine formulation are currently being synthesized.

Future plans

- Submission of new patent regarding ionizable lipids with improved safety and thermostability.