Rational design of mRNA folding architecture to enhance protein production

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CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

RNA therapy: bridging niche to mainstream medicine

First siRNA drug approved by FDA (2018)

Polyneuropathy of hereditary transthyretin-mediated (hATTR) amyloidosis

First mRNA drug approved by FDA (2021)

COVID-19

	Therapeutic	Developer	Type	Indication	Target organ	Administration route	Approval year
	Fomivirsen (Vitravene)	Ionis	ASO	CMV	Eye	Intravitreal	FDA 1998 EMA 1999
	Eteplirsen (Exondys 51)	Sarepta	ASO	DMD	Muscle	Intravenous	FDA 2016
	Mipomersen (Kynamro)	Ionis	ASO	HoFH	Liver	Subcutaneous	FDA 2013 EMA 2012
	Nusinersen (Spinraza)	Ionis	ASO	SMA	CNS	Intrathecal	FDA 2016 EMA 2017
	Inotersen (Tegsedi)	Ionis	ASO	hATTR-PN	Liver	Subcutaneous	FDA 2018 EMA 2018
	Volanesoren (Waylivra)	Ionis/Akcea	ASO	FCS	Liver	Subcutaneous	EMA 2019
	Golodirsen (Vyondys 53)	Sarepta	ASO	DMD	Muscle	Intravenous	FDA 2019
	Casimersen (Amondys 45)	Sarepta	ASO	DMD	Muscle	Subcutaneous	FDA 2021
	Viltolarsen (Viltepso)	NS Pharma	ASO	DMD	Muscle	Intravenous	FDA 2020
	Eplonterson (Wainua)	Ionis	ASO	hATTR-PN	Liver	Subcutaneous	FDA 2023
	Tofersen (Qalsody)	Ionis	ASO	ALS	CNS	Intrathecal	FDA 2023 EMA 2024
	Givosiran (Givlaari)	Alnylam	siRNA	AHP	Liver	Subcutaneous	FDA 2019 EMA 2020
	Inclisiran (Leqvio)	Novartis/Alnylam	siRNA	ASCVD, HeFH	Liver	Subcutaneous	EMA 2020 FDA 2021
	Lumasiran (Oxlumo)	Alnylam	siRNA	PH1	Liver	Subcutaneous	FDA 2020 EMA 2020
*	Patisiran (Onpattro)	Alnylam	siRNA	hATTR-PN	Liver	Intravenous	FDA 2018 EMA 2018
	Lumasiran (Oxlumo)	Alnylam	siRNA	PH1	Liver	Subcutaneous	FDA 2020 EMA 2020
	Vutrisiran (Amvuttra)	Alnylam	siRNA	hATTR-PN	Liver	Subcutaneous	FDA 2022 EMA 2022
	Alipogene tiparvovec (Glybera)	uniQure	DNA	LPLD	Muscle	Intramuscular	EMA 2012
	Voretigene neparvovec (Luxturna)	Spark	DNA	IRD/RPE65 gene	Eye	Subretinal	FDA 2017 EMA 2018
	Onasemnogene abeparvove (Zolgensma)	AveXis/Novartis	DNA	SMA	CNS	Intravenous	FDA 2019 EMA 2020
	Oxford–AstraZeneca (Vaxzevria)	AstraZeneca	DNA	COVID-19	Lymphoid organ	Intramuscular	FDA 2021 EMA 2021
	Ad26.COV2.S (Jcovden)	Johnson & Johnson	DNA	COVID-19	Lymphoid organ	Intramuscular	FDA 2021 EMA 2021
	Etranacogene dezaparvovec (Hemgenix)	uniQure	DNA	Hemophilia B	Liver	Intravenous	FDA 2022 EMA 2023
	Nadofaragene firadenovec (Adstiladrin)	Merk	DNA	Bladder cancer	Bladder	Intravesical	FDA 2022
	Valoctocogene roxaparvovec (Roctavian)	BioMarin	DNA	Hemophilia A	Liver	Intravenous	FDA 2023 EMA 2022
	Eladocagene exuparvovec (Upstaza)	PTC Therapeutics	DNA	ADCC deficiency	Brain	Brain infusion	FDA 2024 EMA 2022
	Beremagene geperpavec (Vyjuvek)	Krystal Biotech	DNA	DEB	Wounds	Topical	FDA 2023
*	Tozinameran (Comirnaty)	BioNTech/Pfizer	mRNA	COVID-19	Lymphoid organ	Intramuscular	FDA 2021 EMA 2020
*	mRNA-1273	Moderna/NIAID/BARDA	mRNA	COVID-19	Lymphoid organ	Intramuscular	FDA 2020 EMA 2020

The major hurdle for RNA therapeutics:

Delivery, Delivery, Delivery

Ni Q, Peng L, ***Nature Review Bioengineering***, In Press

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What is the future of nanotechnology in nucleic acids therapy?

mRNA

Hard to reach organs, tissues, and cells

We develop platform technologies for mRNA delivery and modification

Major scientific challenges

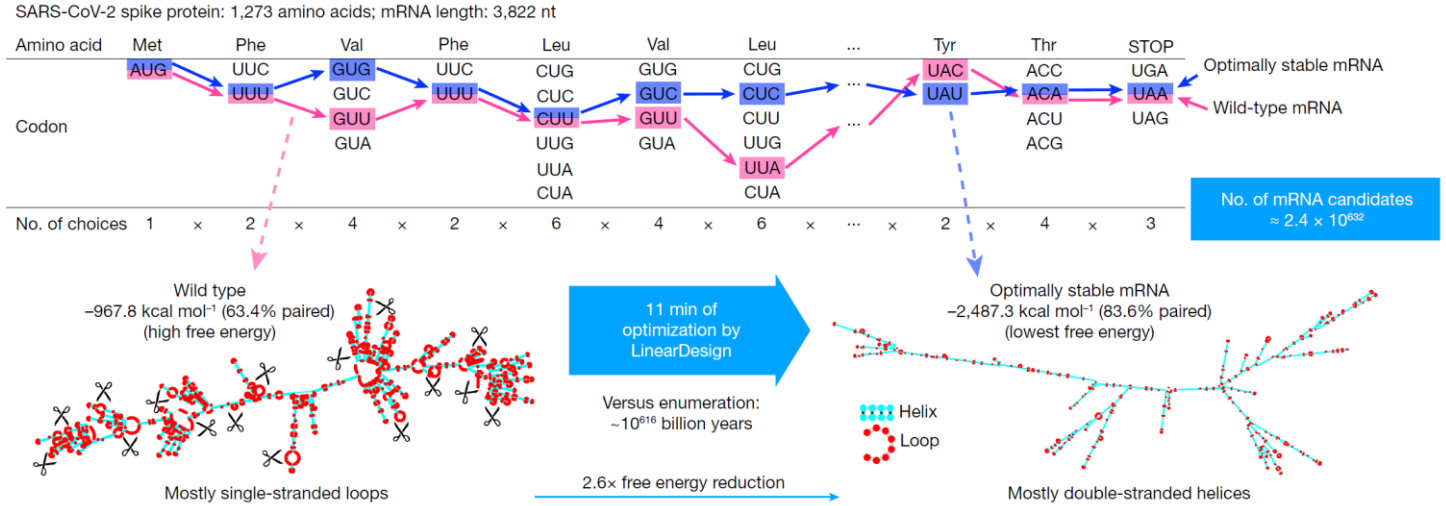
- **Improve protein production of mRNA therapeutics**
- **Target RNA therapeutics to specific cells and organs**

How can we improve protein production of mRNA therapeutics?

mRNA payload design

mRNA delivery carrier

mRNA structural optimization

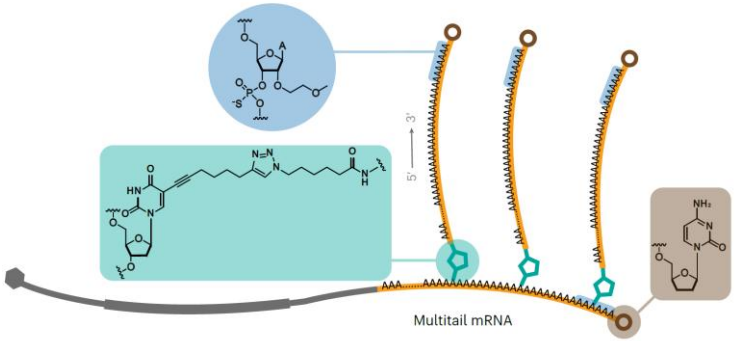


Folded secondary structure, such as helices and loops that arise from nucleotide pairing, influences RNA stability

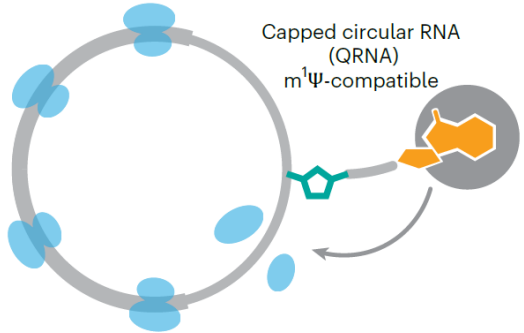
Zhang H, Huang L, et al. ***Nature*** (2023)

mRNA structural modification

Branched chemically modified poly(A) tails



Multicapped mRNA



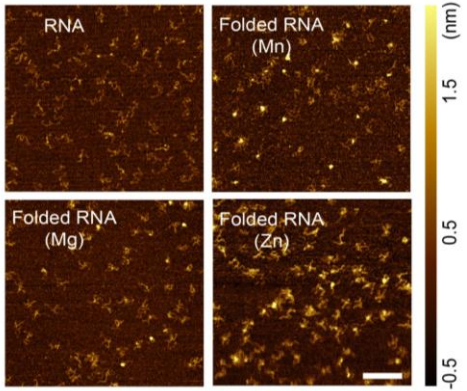
Enhance mRNA translation

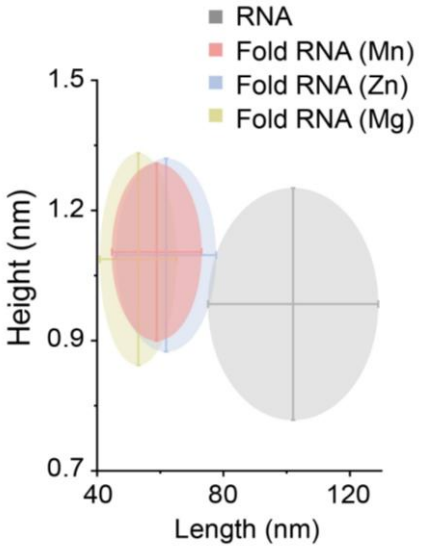
Enhance mRNA stability

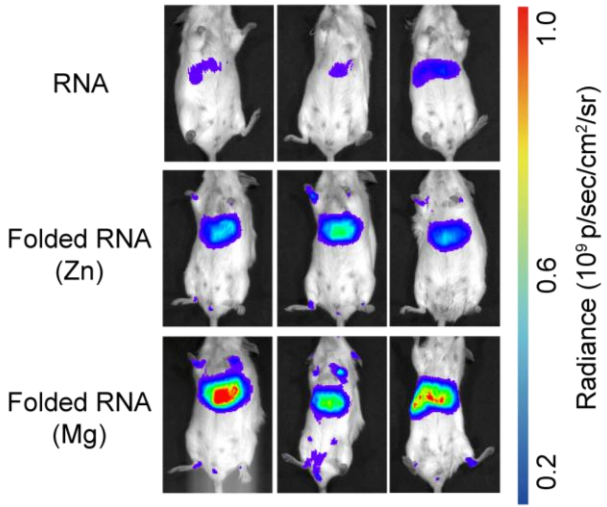
Wang X, et al., Nat. Biotechnol. (2024)

Whether RNA tertiary structures engineering could influence mRNA molecules for expressing proteins?

Rational design of mRNA folding architecture to enhance intracellular processing and protein production

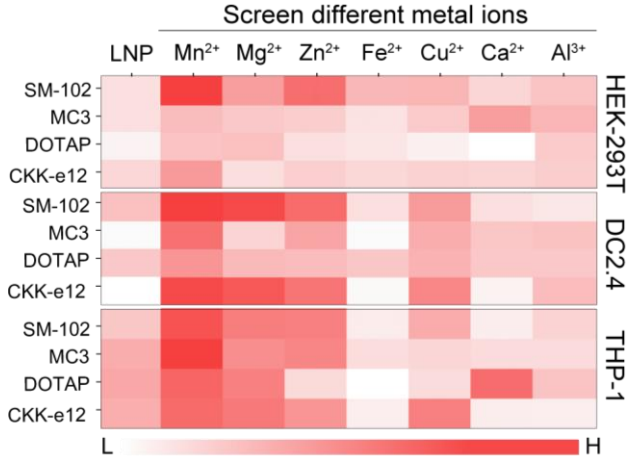






Yang B, et al, Ni Q*. *Nature Nanotechnology,* Under Revision

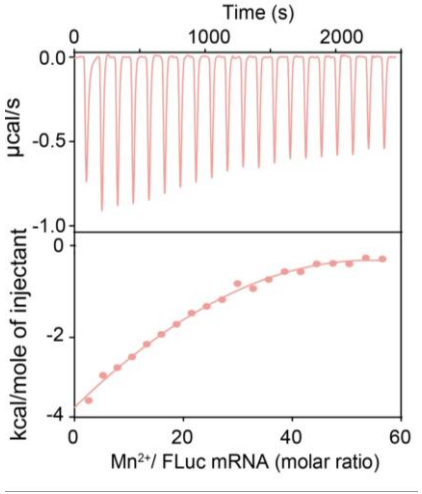
MARF strategy enhances protein production



Thermodynamics of Different Metal Ions Binding to FLuc mRNA

Metal ions	Binding number *n*	Dissociation constant *Kd* (M) × 10^{-4}	△*H* (kJ mol^{-1})	△*S* (J $mol^{-1}K^{-1}$)	△*G* (KJ mol^{-1})
Mn^{2+}	10	0.59	-12.79	38.04	-24.13
Mg^{2+}	10	0.78	-62.08	-129.6	-23.45
Zn^{2+}	2.1	2.4	-99.99	-266.0	-20.72
Cu^{2+}	8.6	3.4	-77.69	-194.1	-19.88
Ca^{2+}	5.8	4.4	-100.0	-271.1	-19.24
Fe^{2+}	9.2	9.2	-100.0	-277.2	-17.45

T = 298 K



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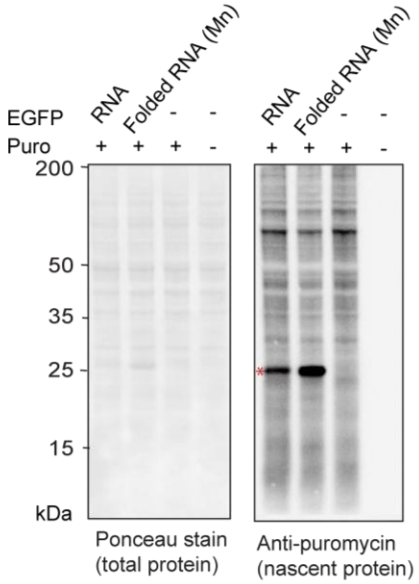
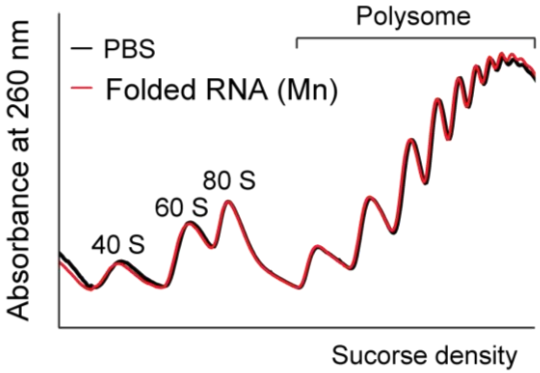
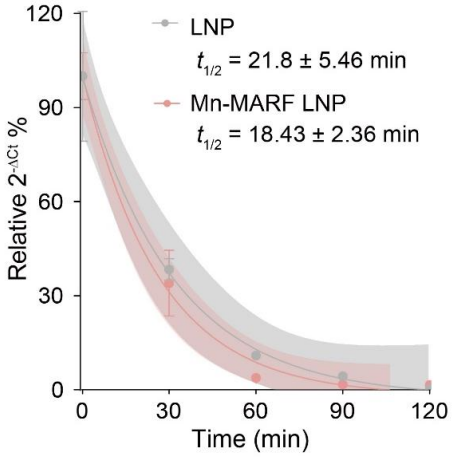
Three key factors involved in enhancing mRNA protein production:

Intracellular delivery, translation efficiency and mRNA stability

MARF strategy has negligible effect on translation activity and cellular stability







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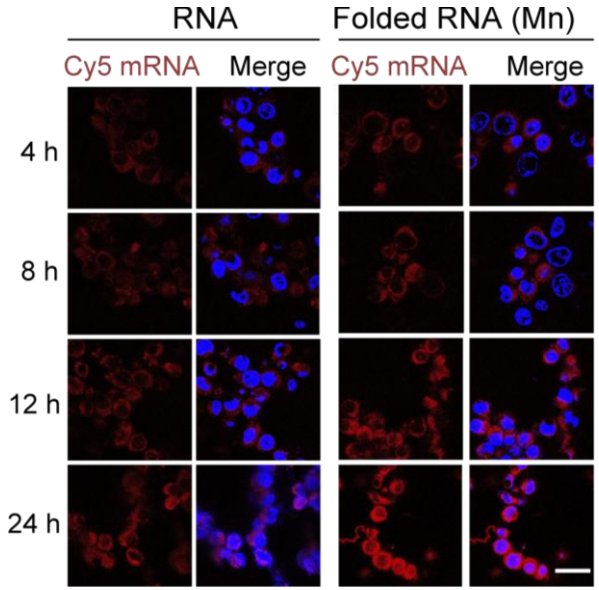
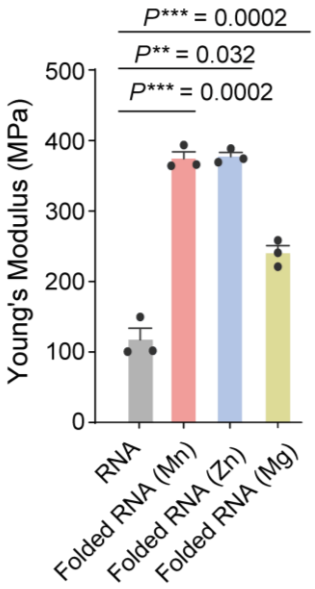
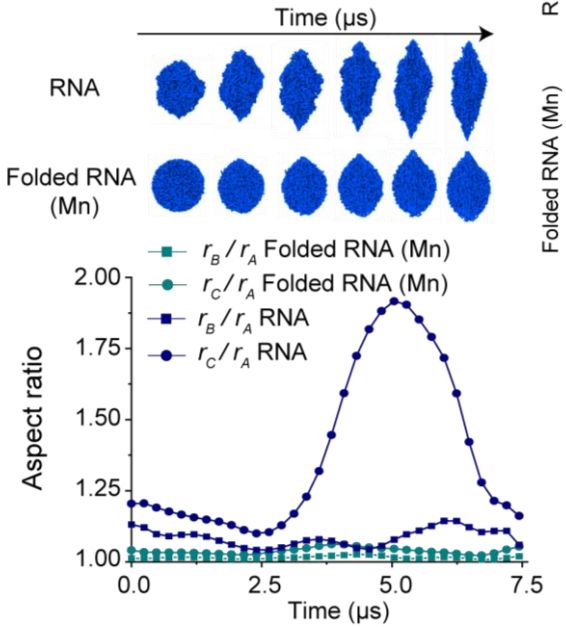
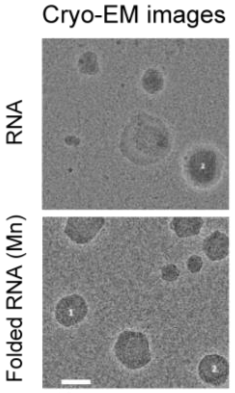
MARF strategy improves cellular uptake of mRNA









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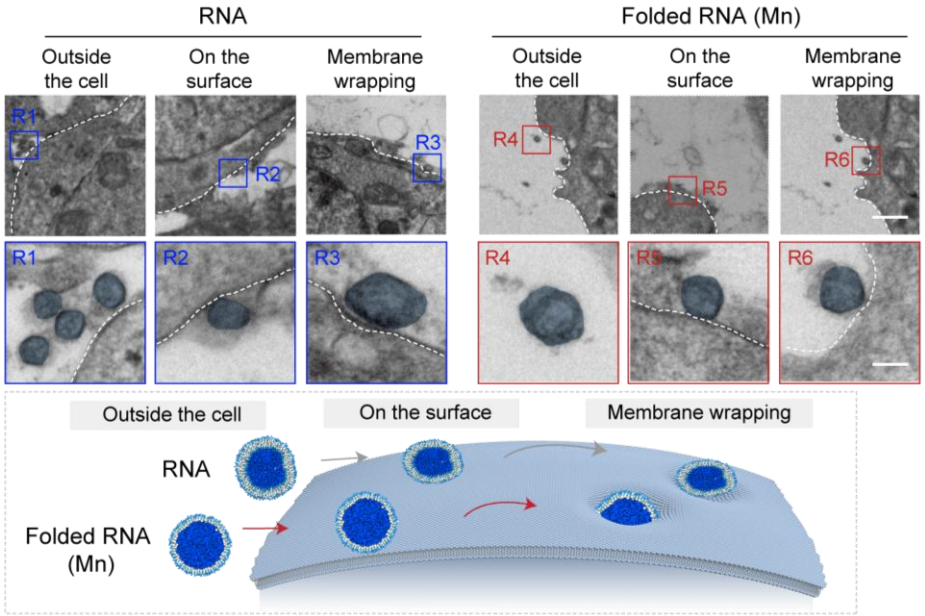
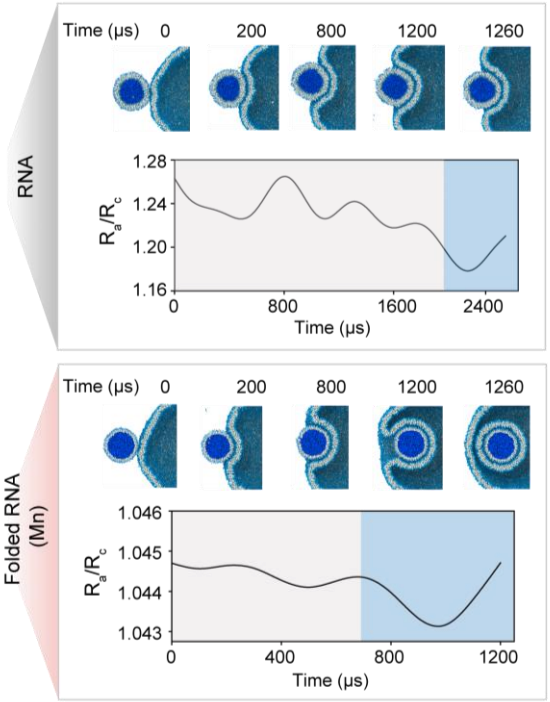
MARF strategy enhances RNA delivery through rigidity-dependent nano-bio interactions





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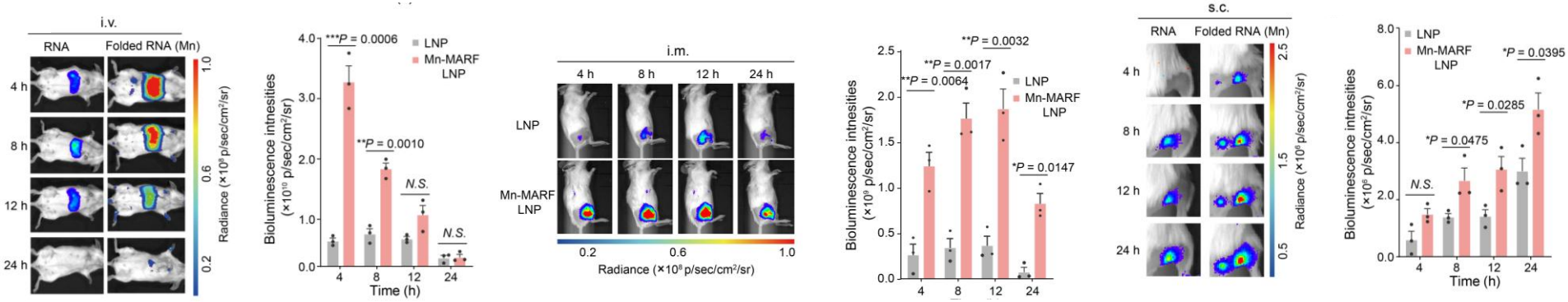
How can we use this MARF strategy in therapeutic applications?

In vivo performance of MARF strategy



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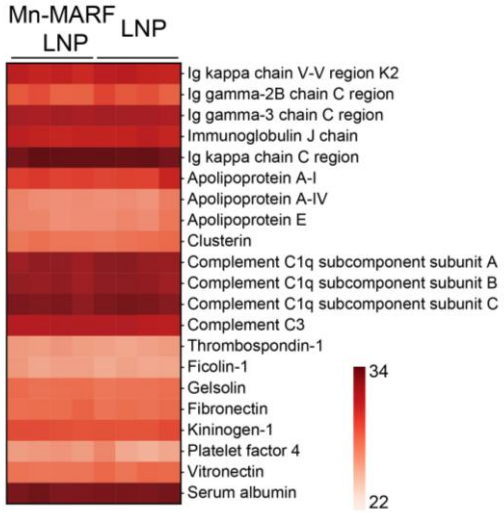
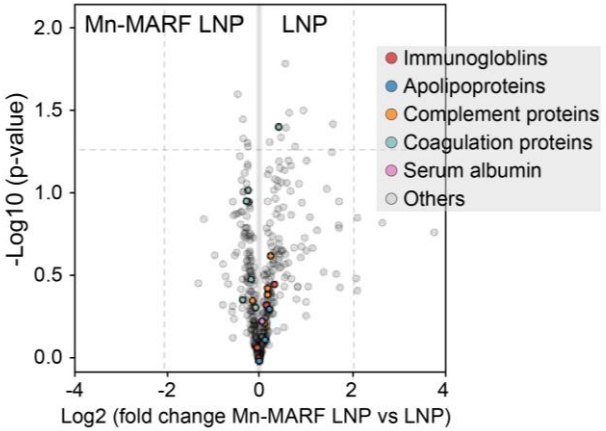
Why liver? Surface protein corona?





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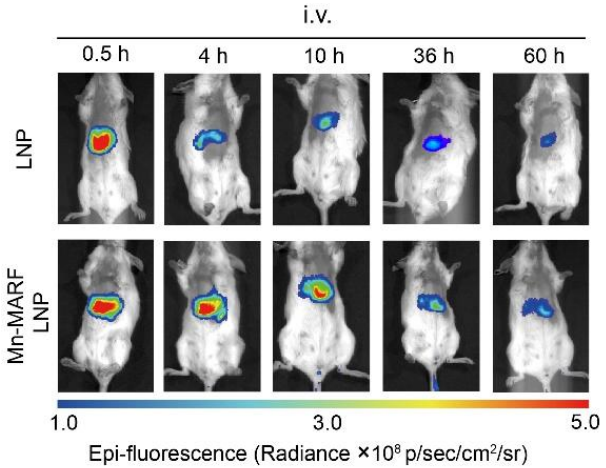
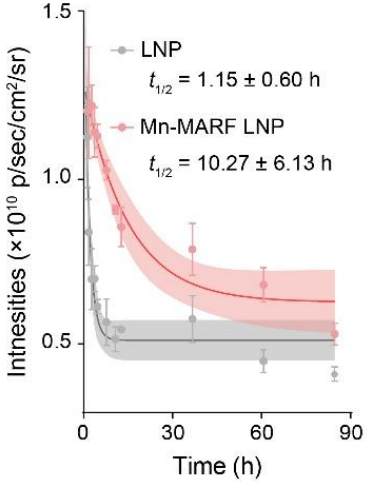
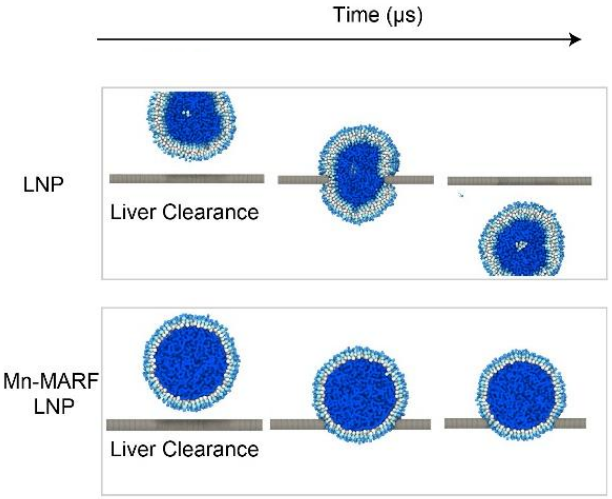
MARF strategy enhances NP retention in liver tissues







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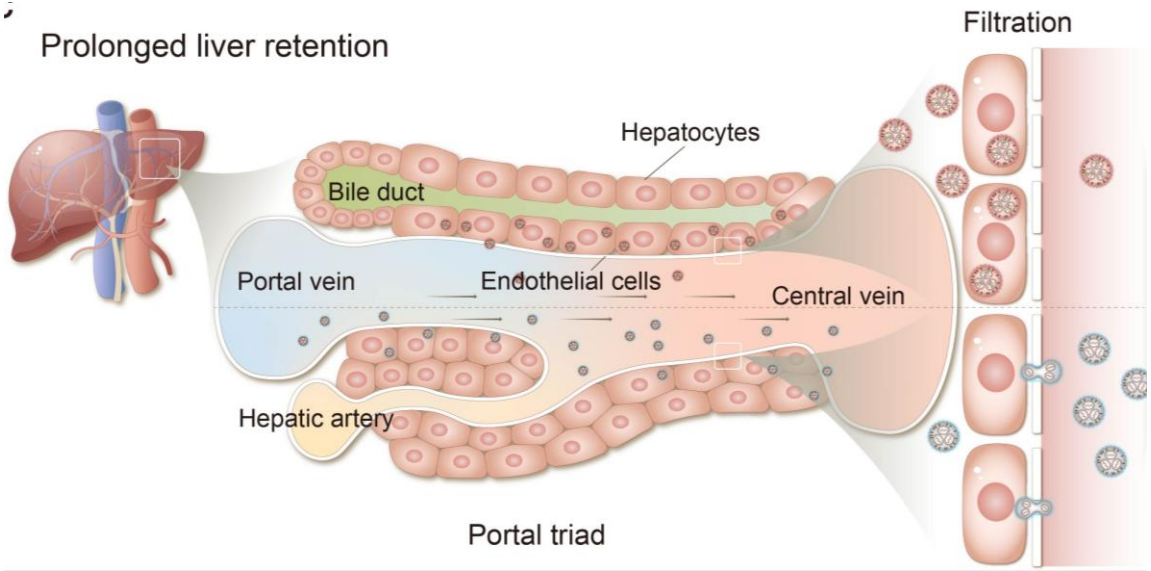

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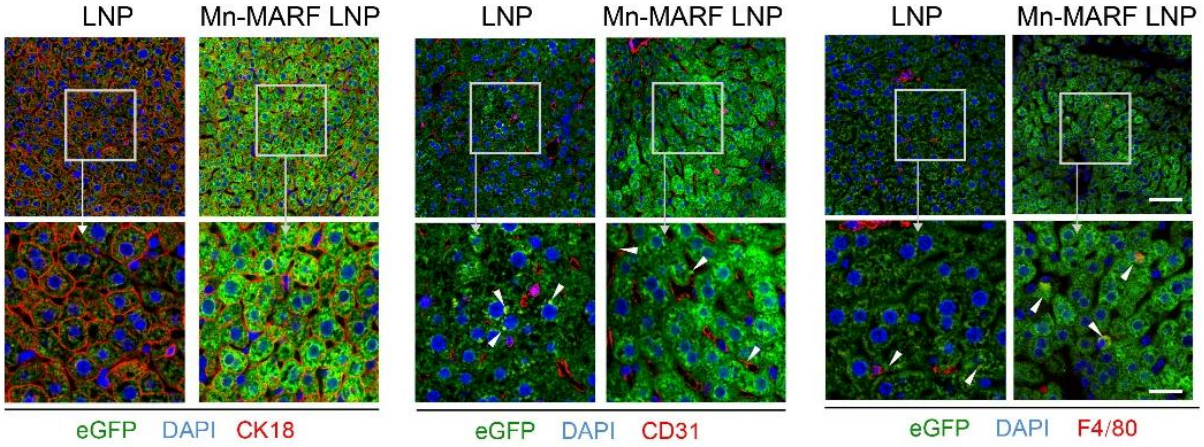

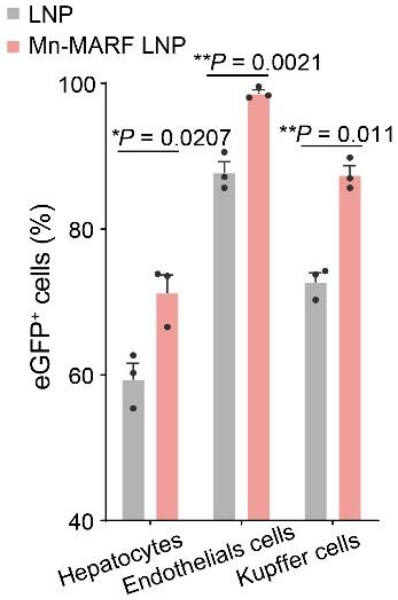

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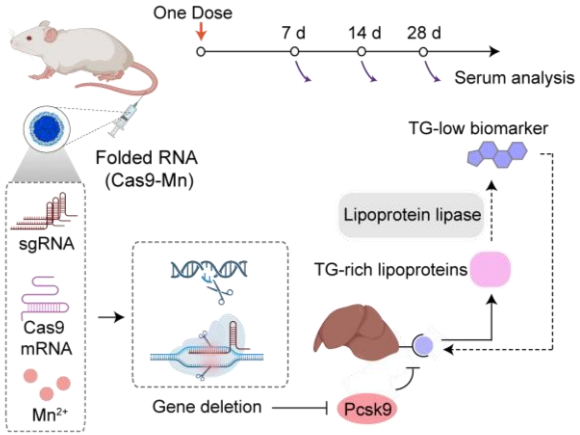
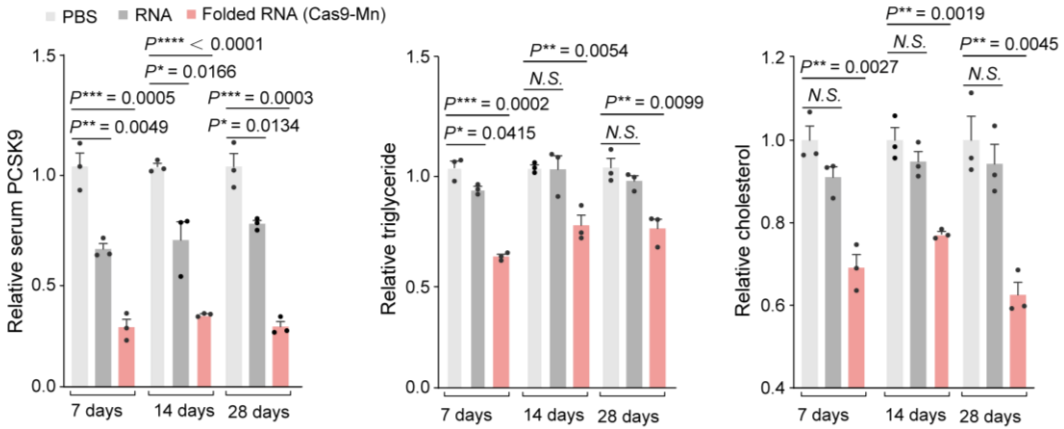
MARF strategy improves PCSK9 targeted genome editing





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Summary

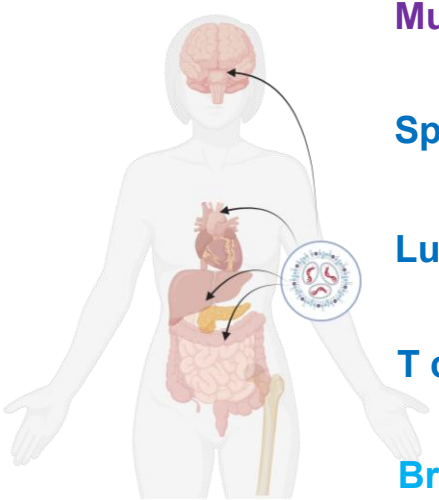
- Tertiary structures of mRNA may influence the performance and adaptation of LNP towards surrounding physiological environment.

- We answered the questions from industry – “whether and how metal ions can be used in RNA therapy and RNA vaccine”

- We opened up a new avenue for investigating basic nano-bio interactions to fully harness their capabilities in drug delivery.

What else can we do beyond liver?

Lipid engineering for mRNA delivery



Mucosal mRNA delivery

Spleen targeted delivery

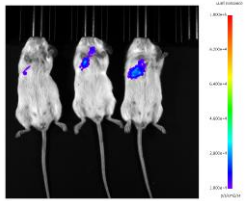
Lung targeted delivery

T cell targeted delivery

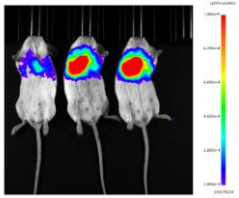
Brain targeted delivery

>250 ionizable lipid

Moderna-SM102



E10



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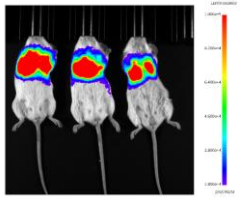

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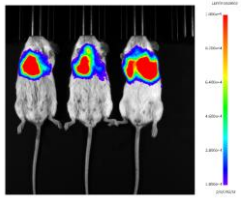

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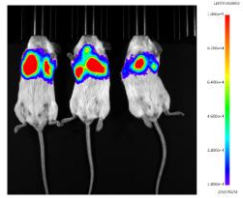
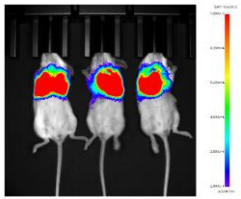


F11



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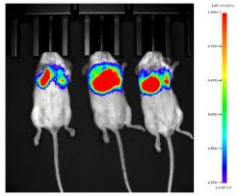
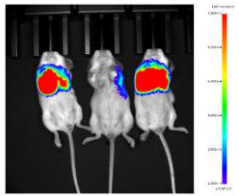
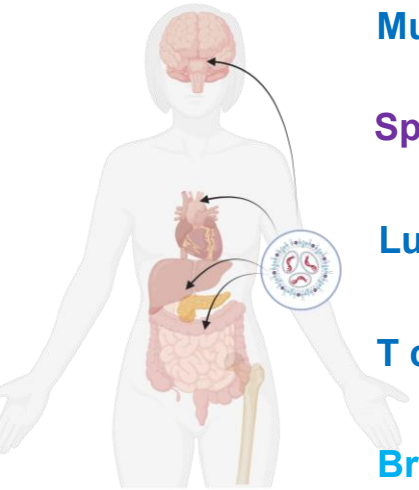


F13



Unpublished data

Lipid engineering for mRNA delivery



Mucosal mRNA delivery

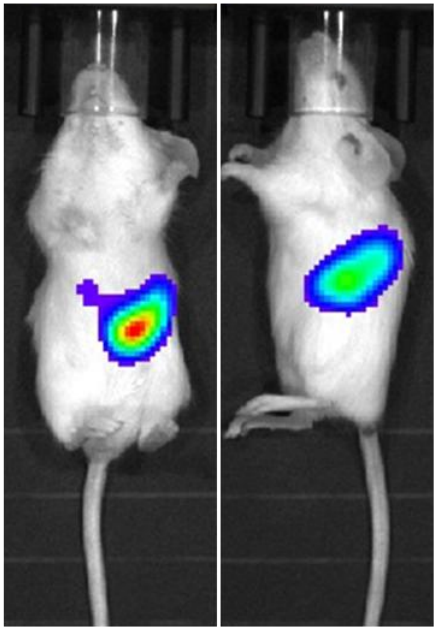
Spleen targeted delivery

Lung targeted delivery

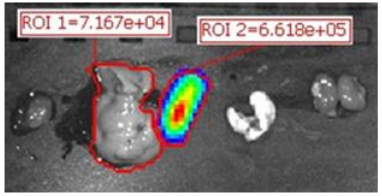
T cell targeted delivery

Brain targeted delivery

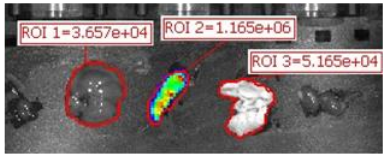
Spleen targeting (intravenous injection)



6h post injection



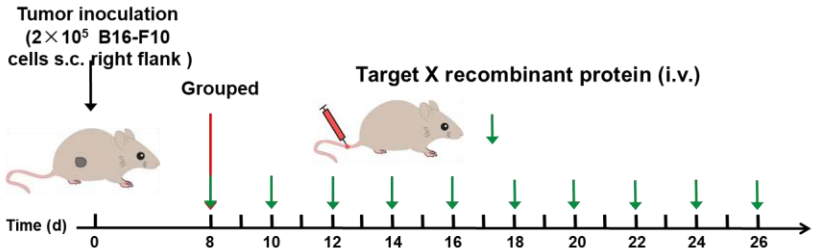
12h post injection

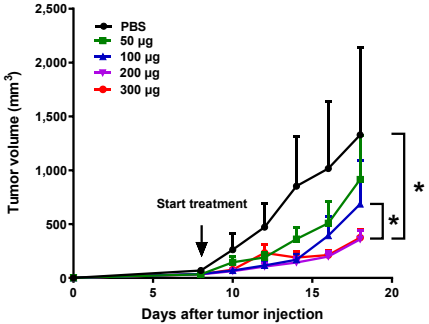


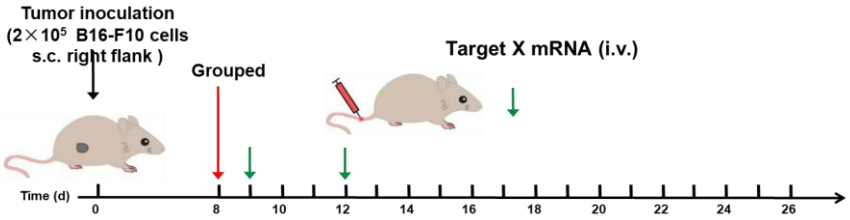
Spleen: liver
30:1

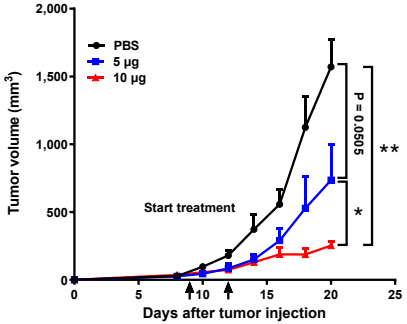
Unpublished data

Spleen targeted mRNA delivery









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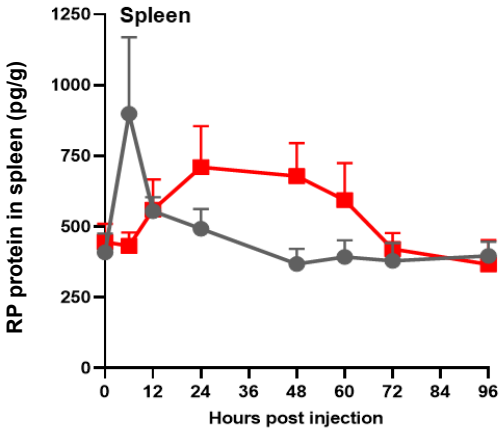
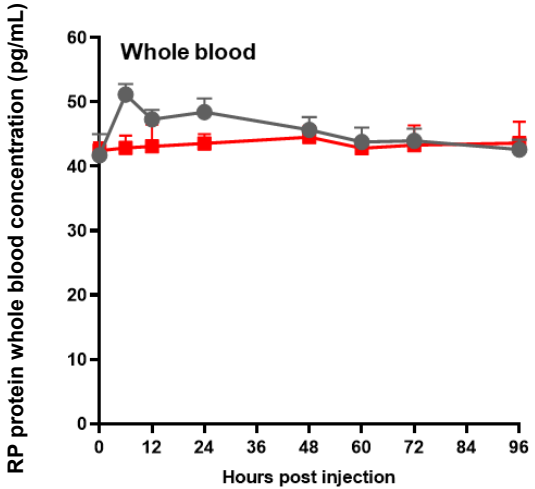
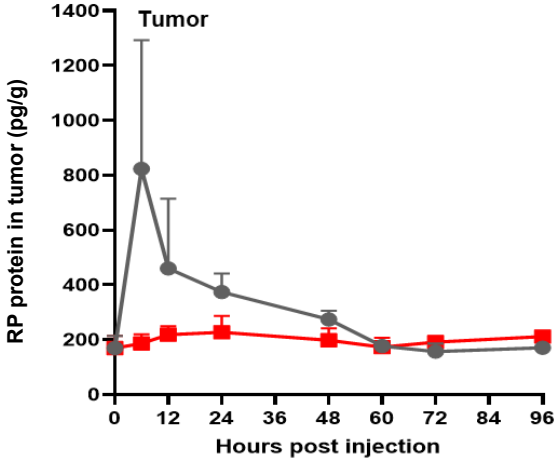
Spleen targeted mRNA delivery







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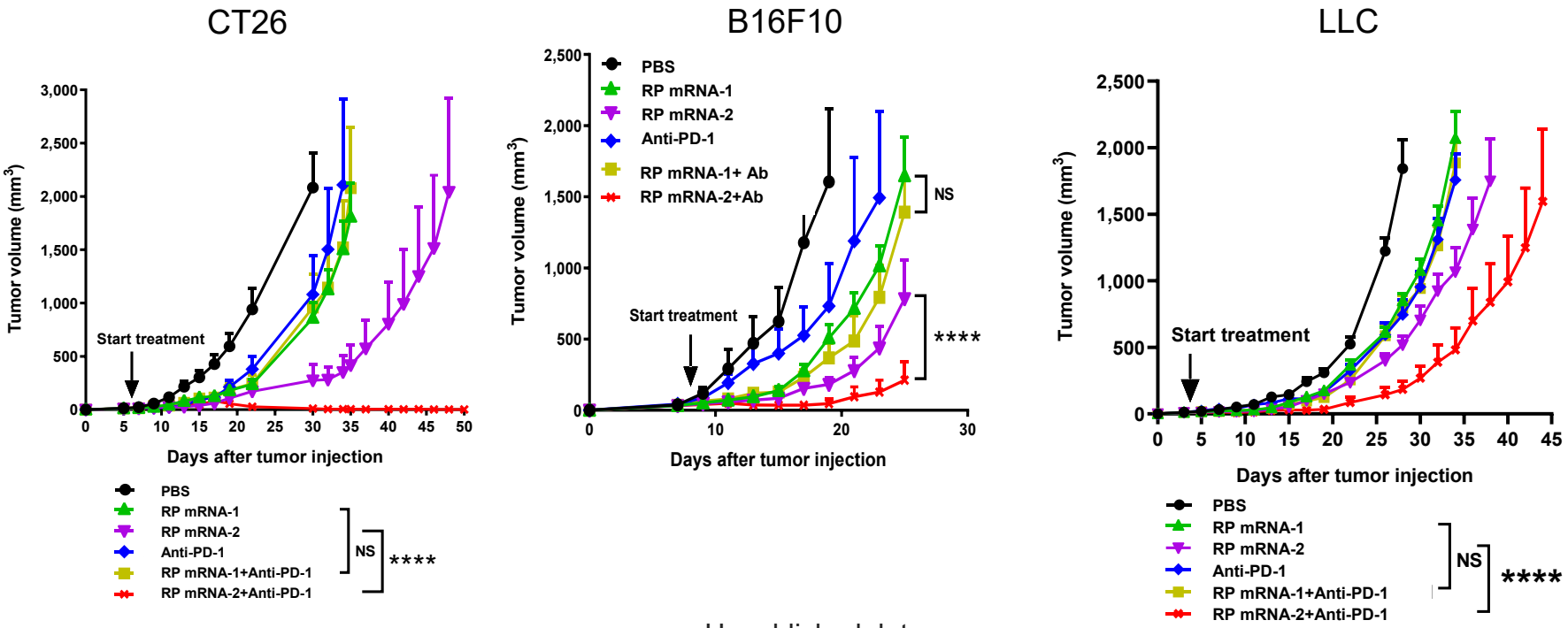
Spleen targeted mRNA synergizes with PD-1 blockade



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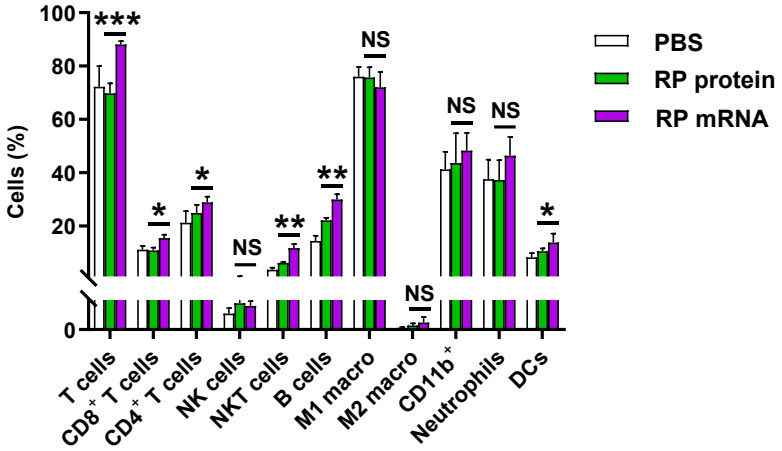
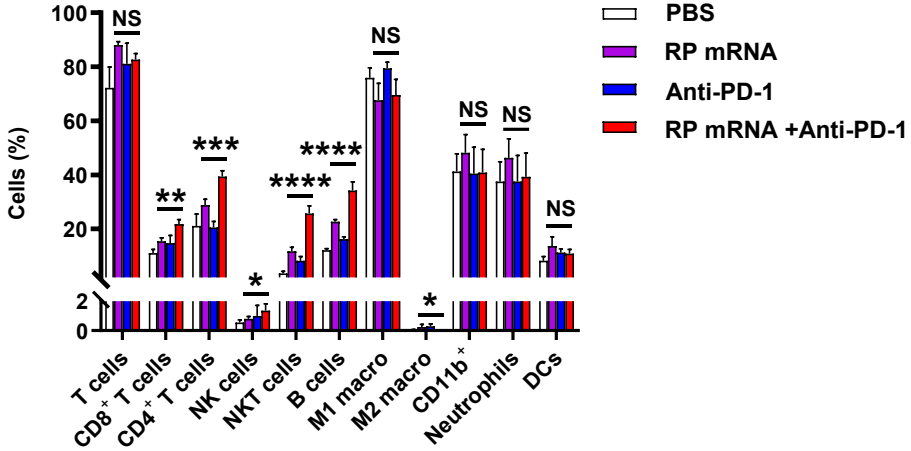
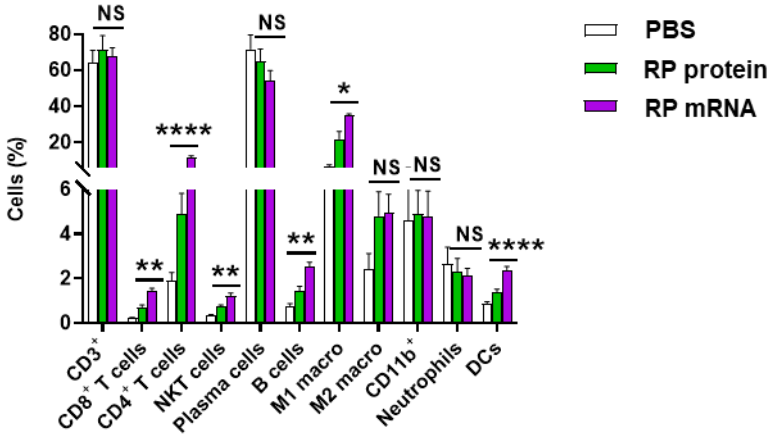
Spleen targeted mRNA facilitates immune cell proliferation in spleen

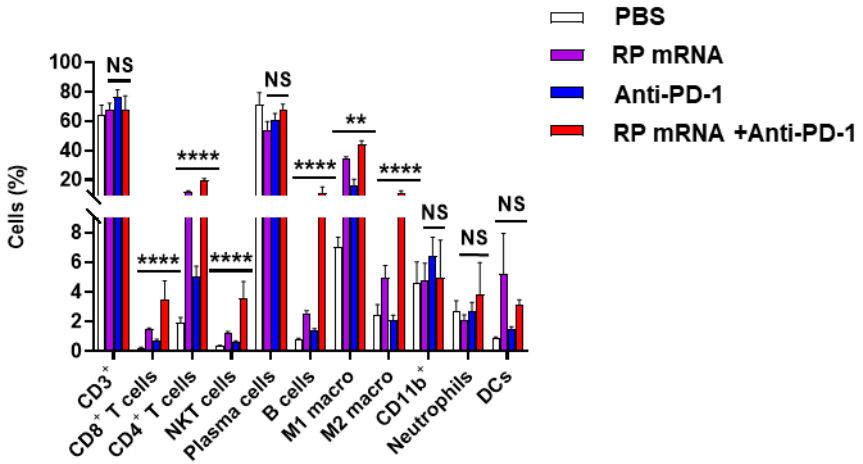




Unpublished data

Spleen targeted mRNA facilitates tumor infiltration of effector cells





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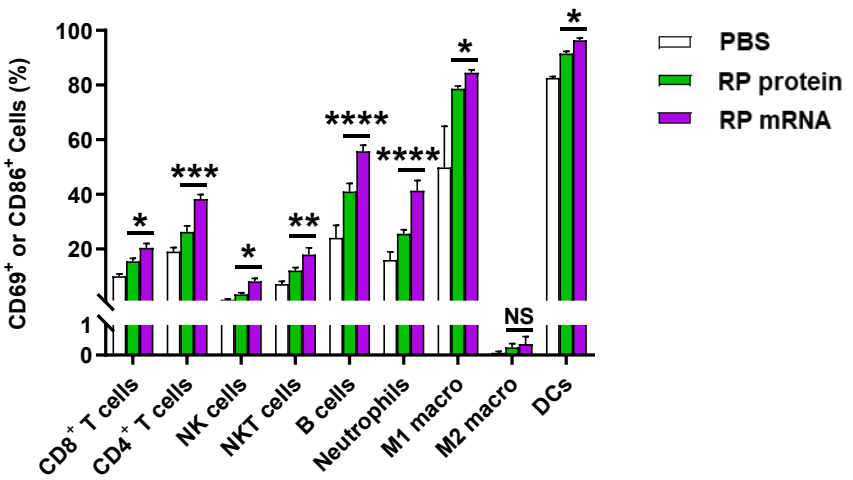
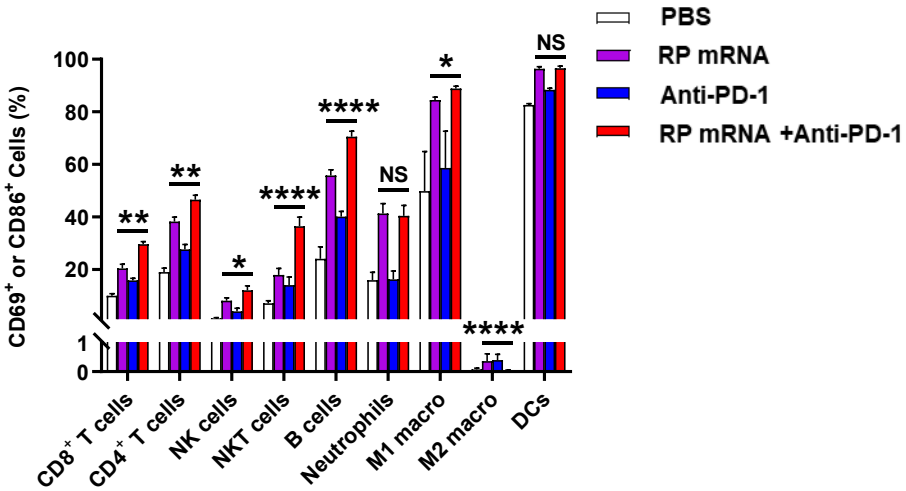
Spleen targeted mRNA induces potent potent activation of APCs and effector cells





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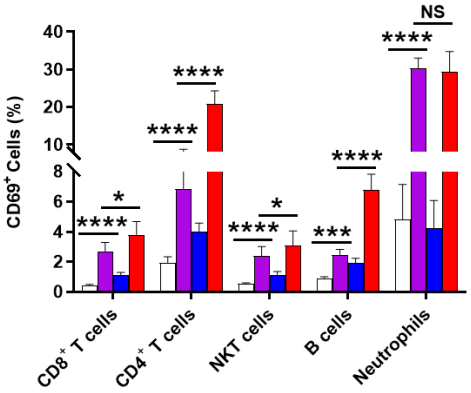
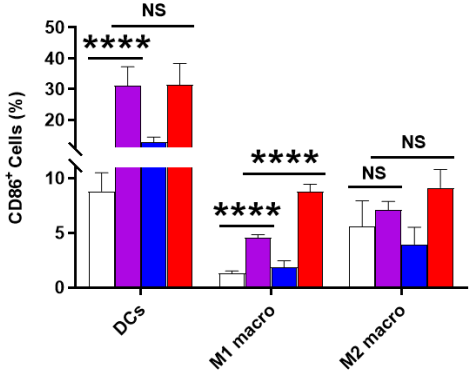
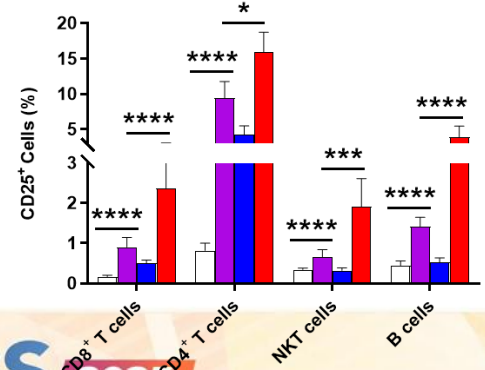
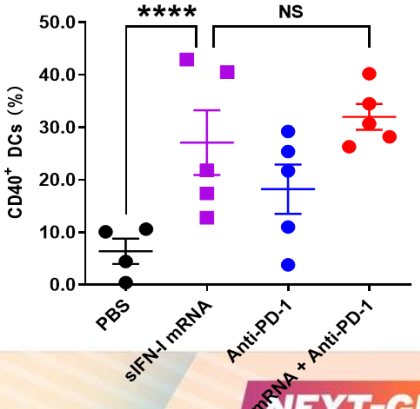
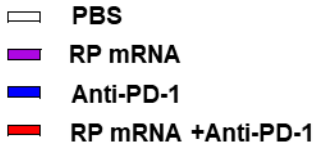
Spleen targeted mRNA induces potent potent activation of APCs and effector cells











Unpublished data

Validation in non-human primates

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NMRC National Medical Research Council Singapore

NATi

NUS National University of Singapore

NRF SINGAPORE

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Thank you for your attention!