Endogenous targeting: Elucidating the impact of the protein corona on lipid nanoparticle organ-targeting outcomes

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Conflict of interest disclosures

mRNA can be thought of as cellular software to encode a cornucopia of proteins to prevent or treat disease

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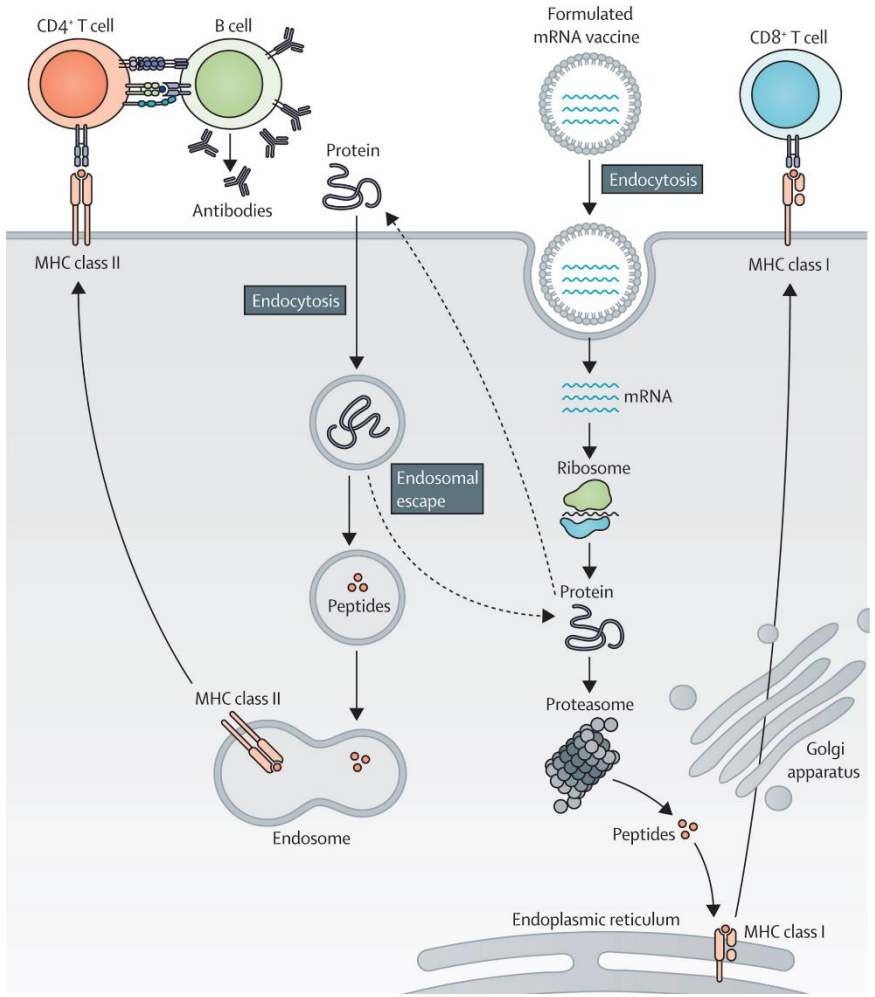
Personalized Cancer Vaccines



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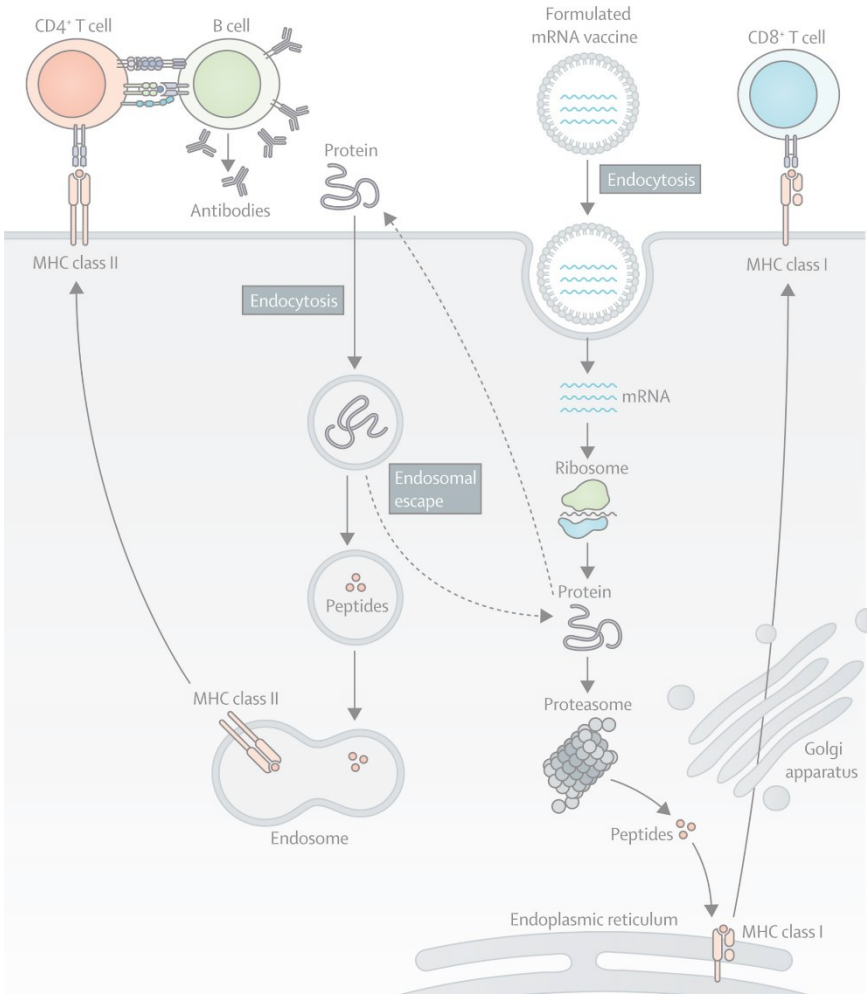
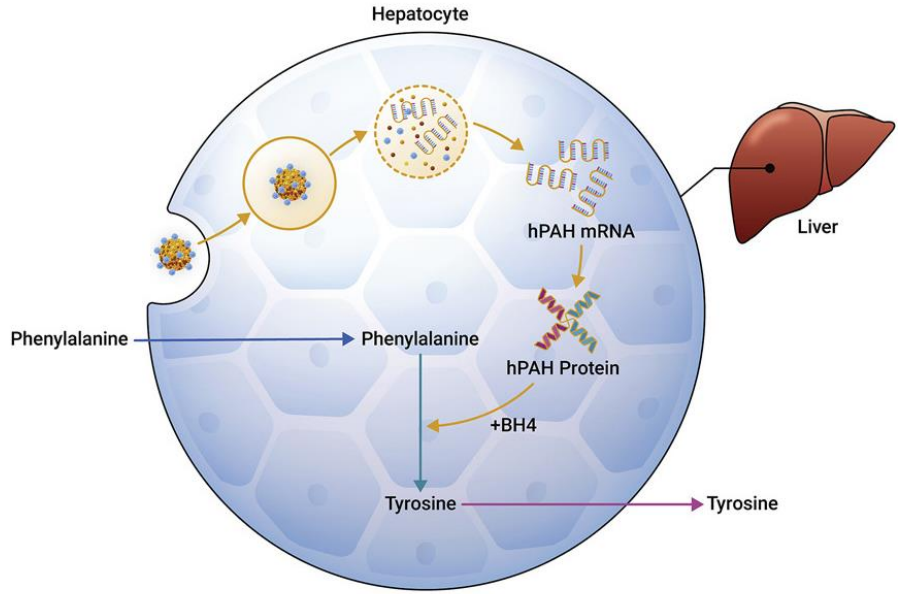


Protein Replacement Therapy



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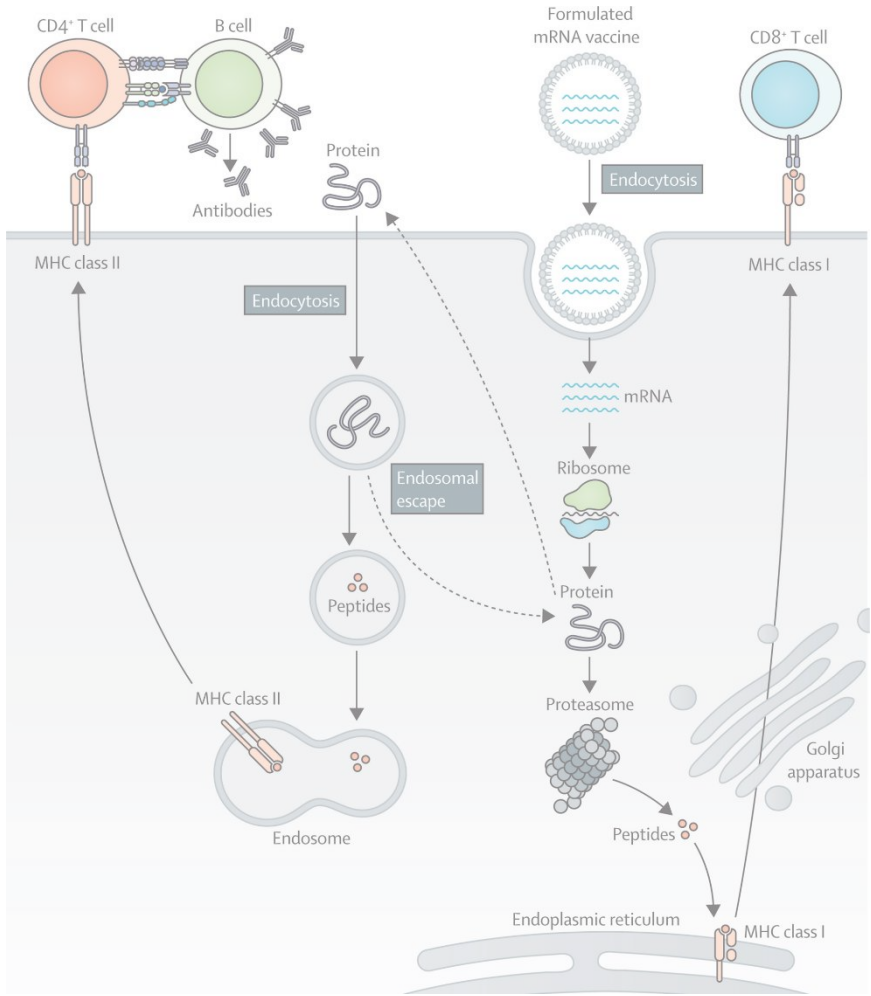

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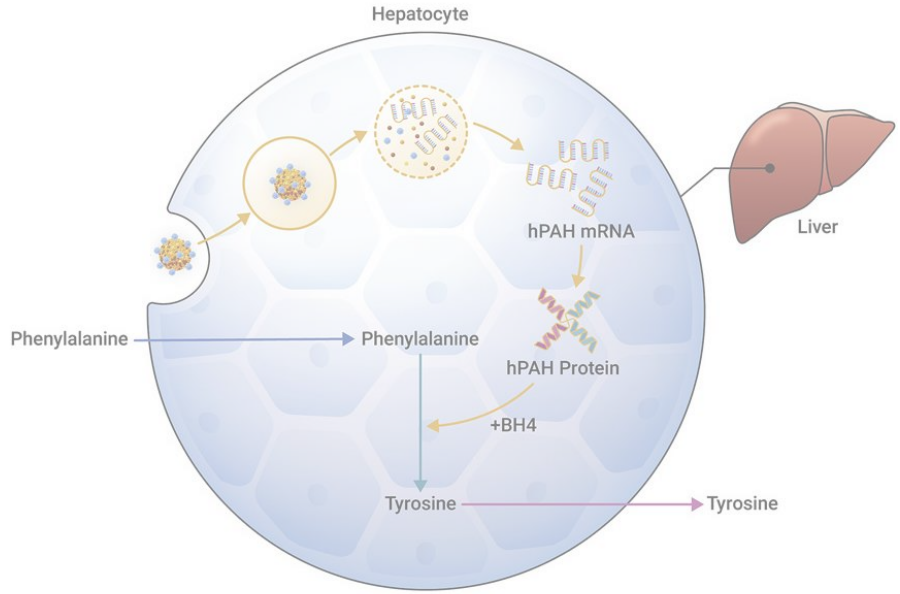
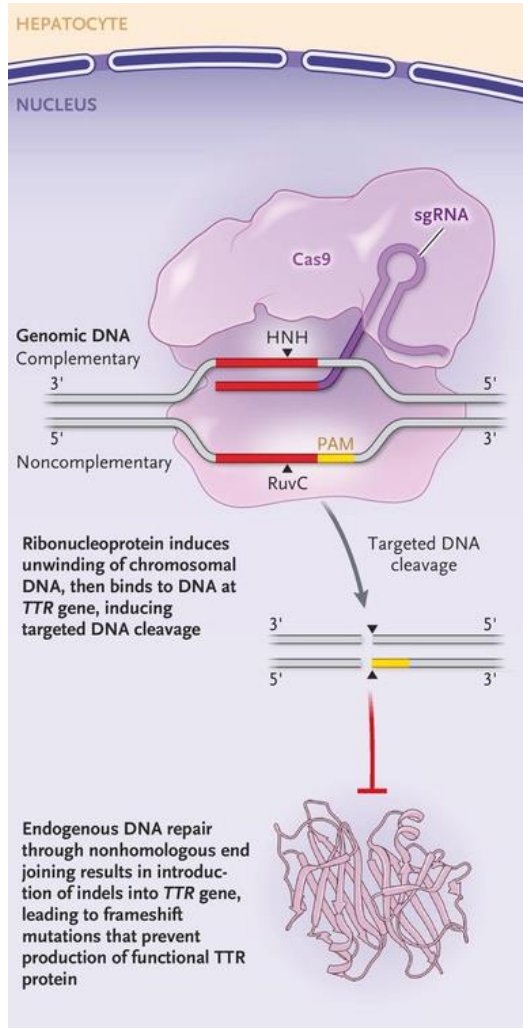


Genome Editing



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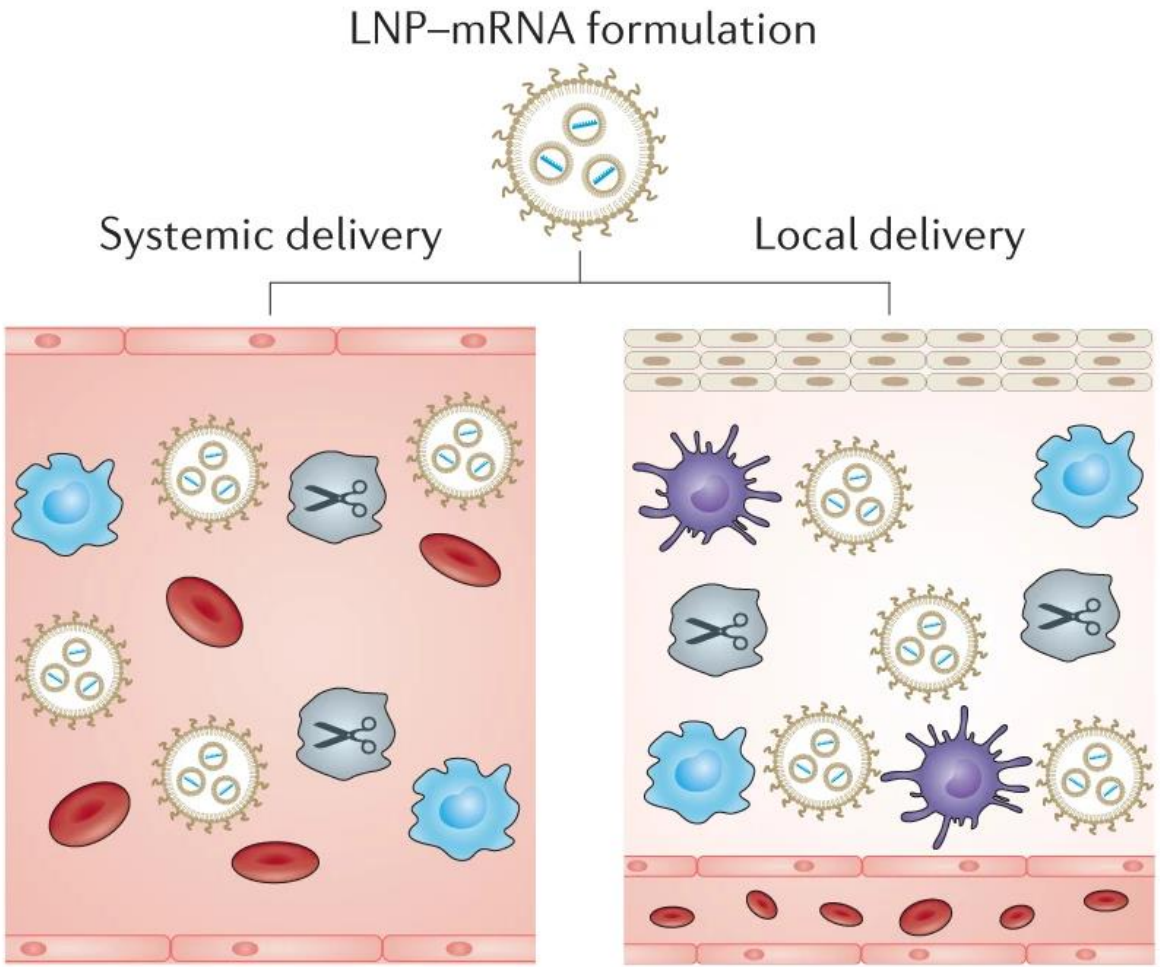
Gillmore et al. **New. Engl. J. Med.** *385,* 493 (2021).

Lipid nanoparticles (LNPs) are used to overcome barriers that hinder the medical use of mRNA and genome editors



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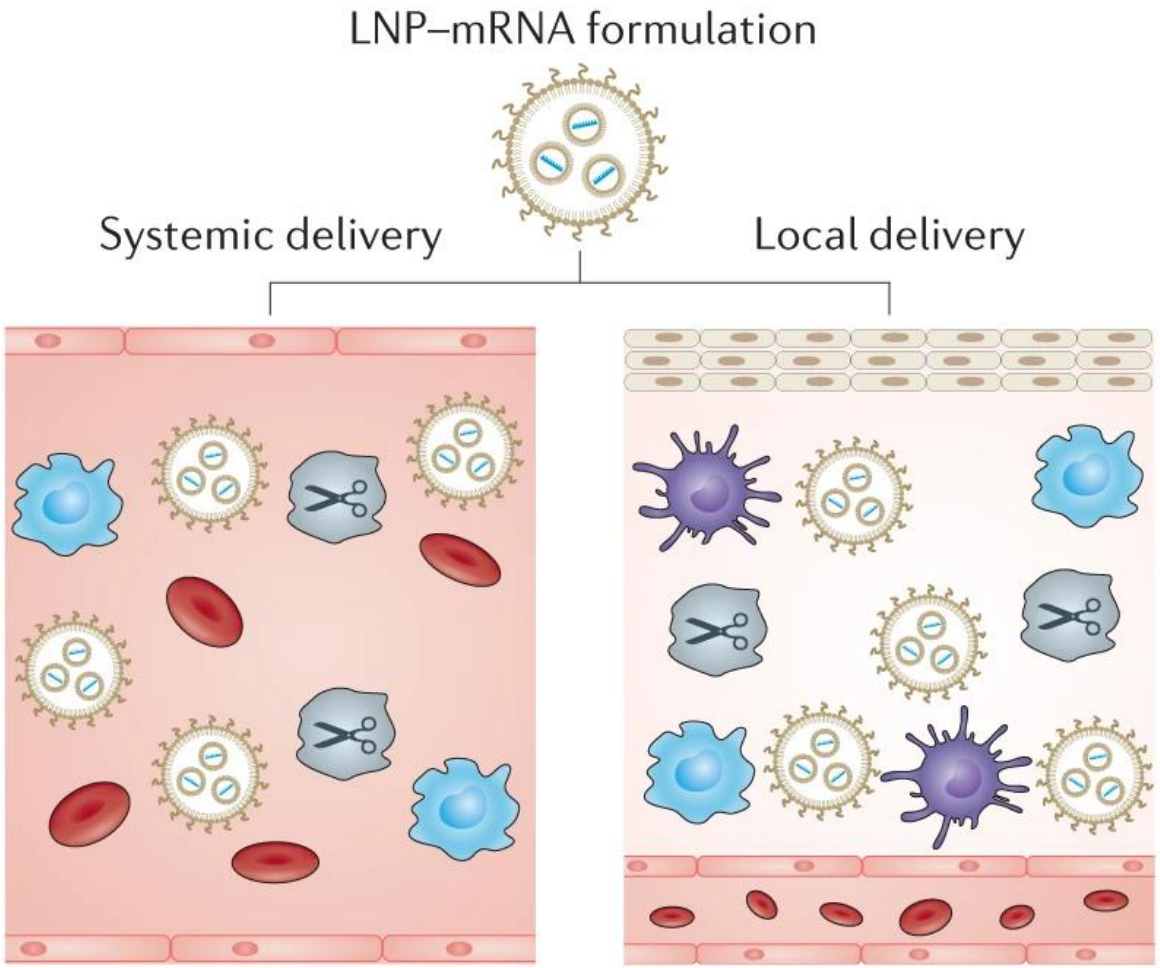

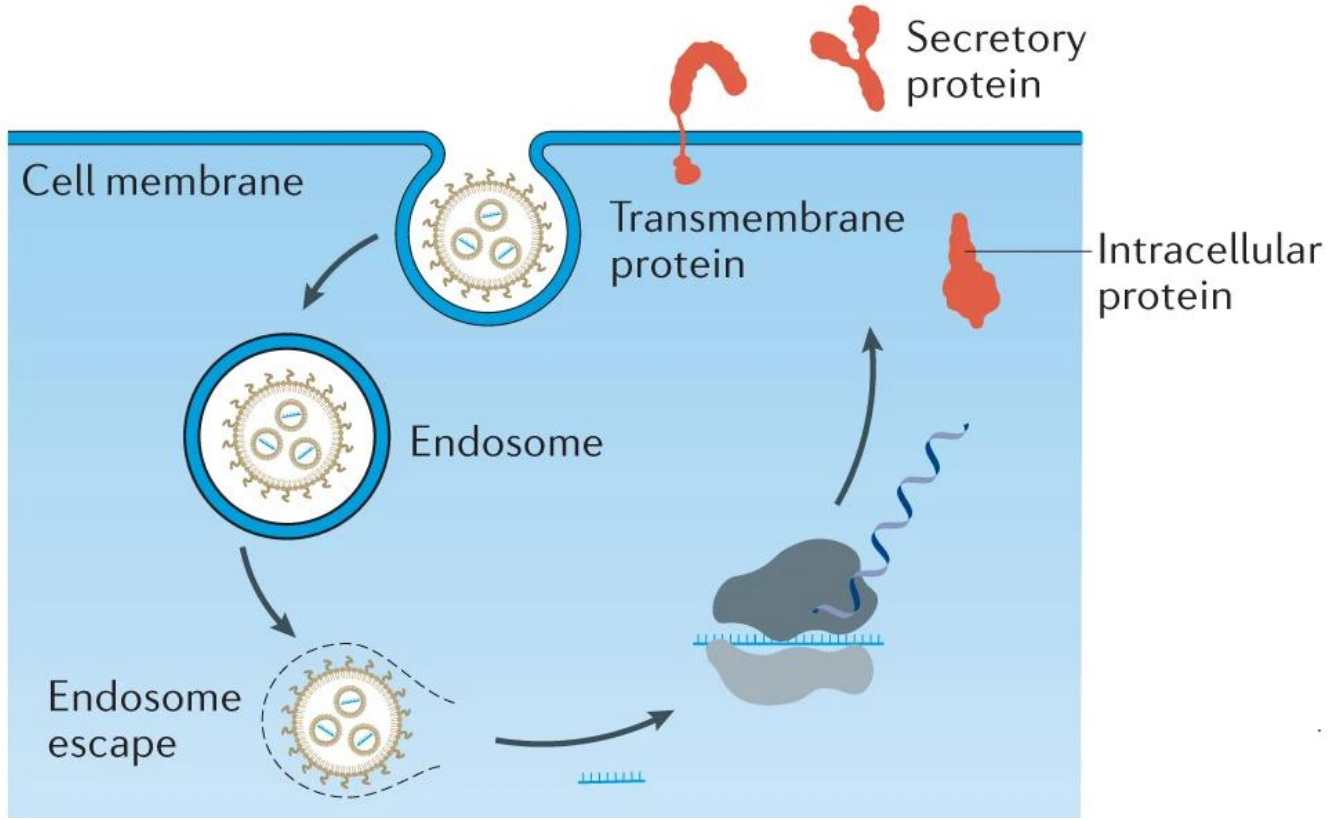

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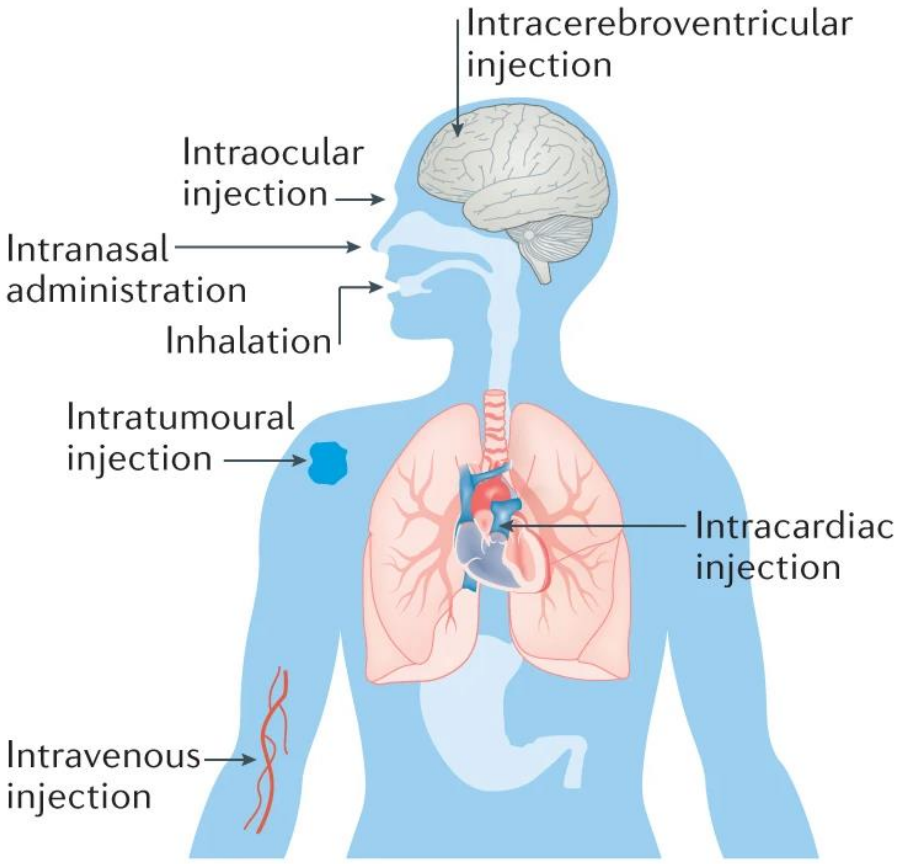
Local delivery barriers are also present and unique to specific sites



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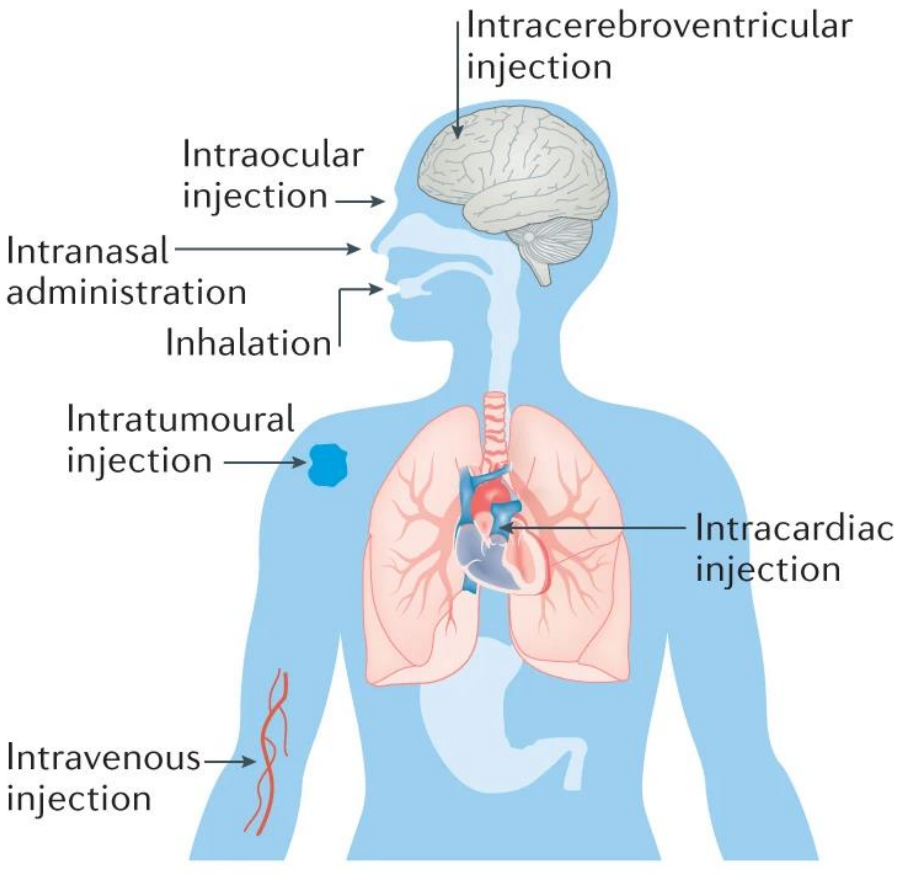

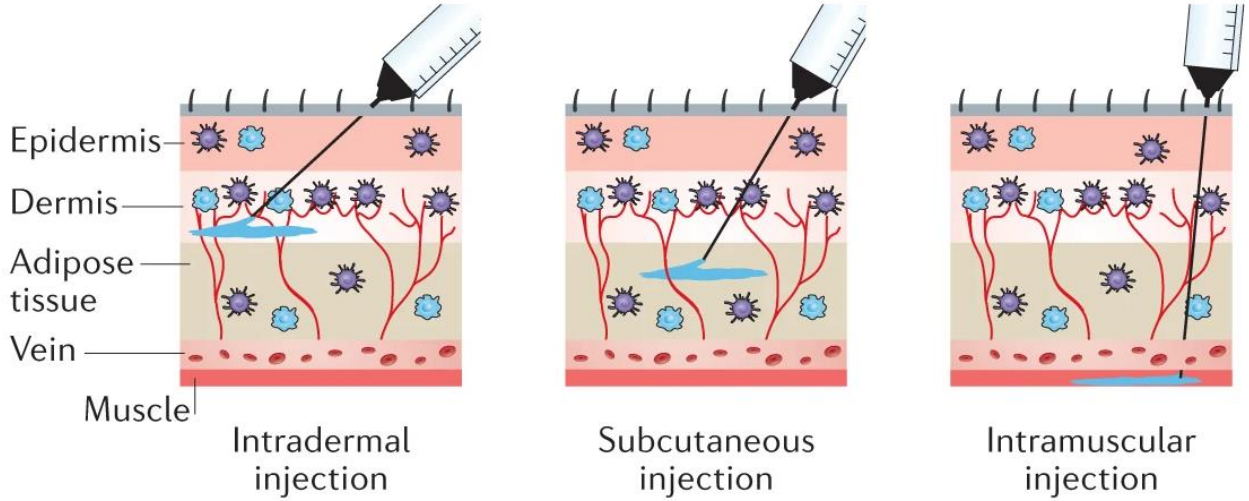

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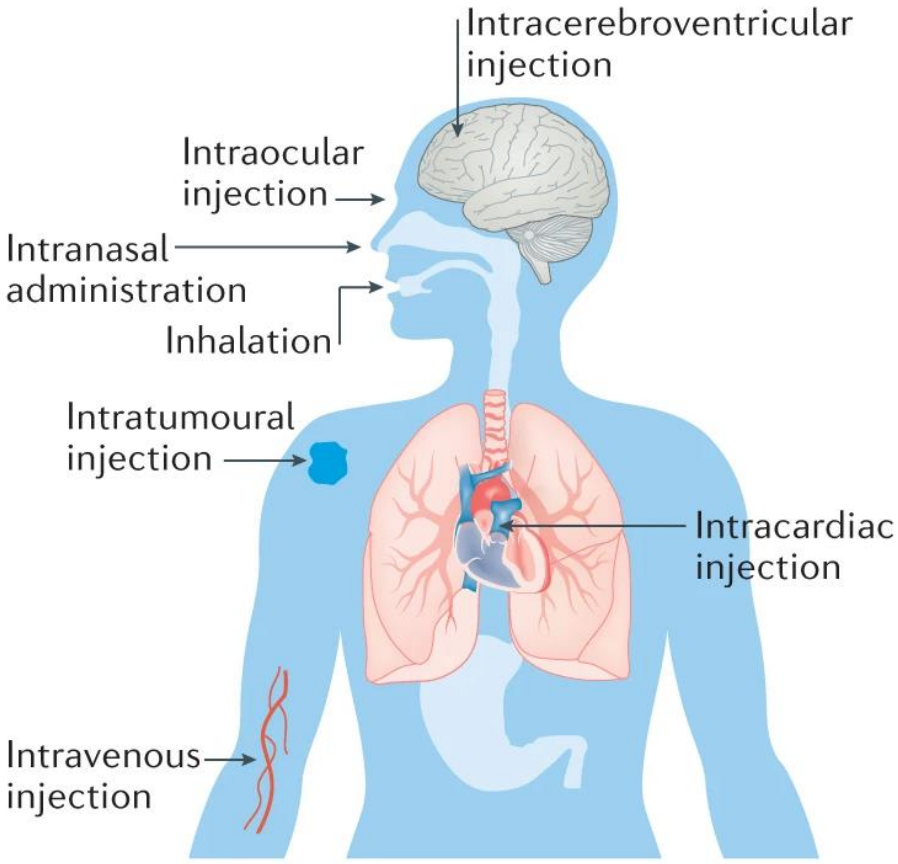

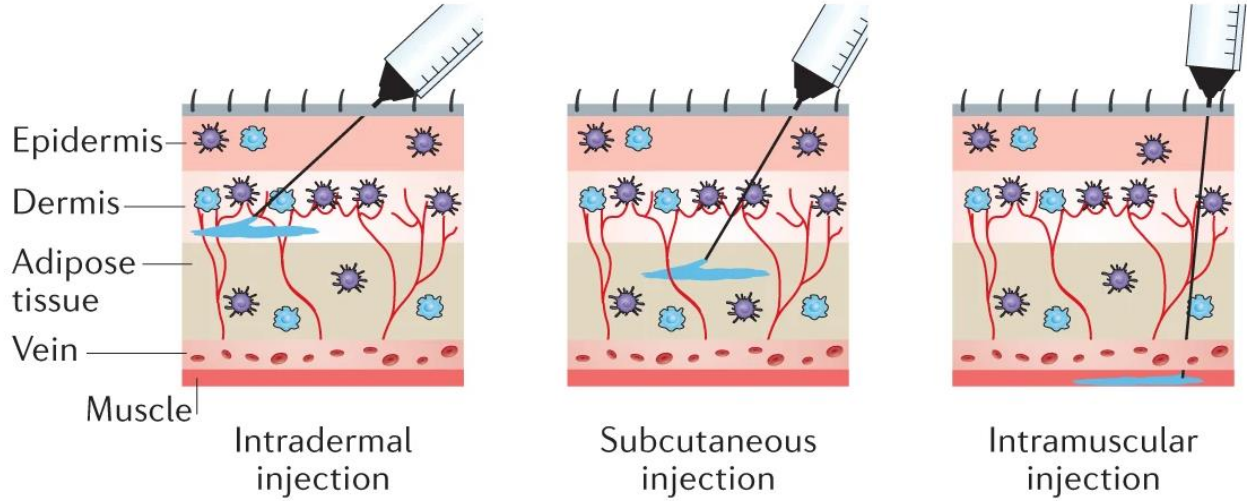

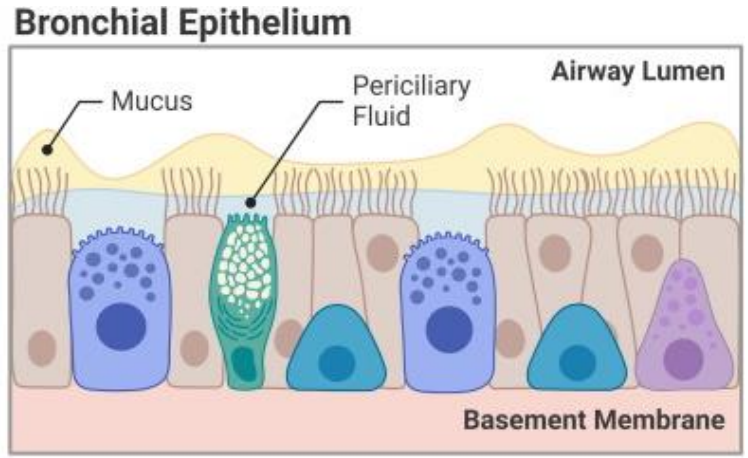

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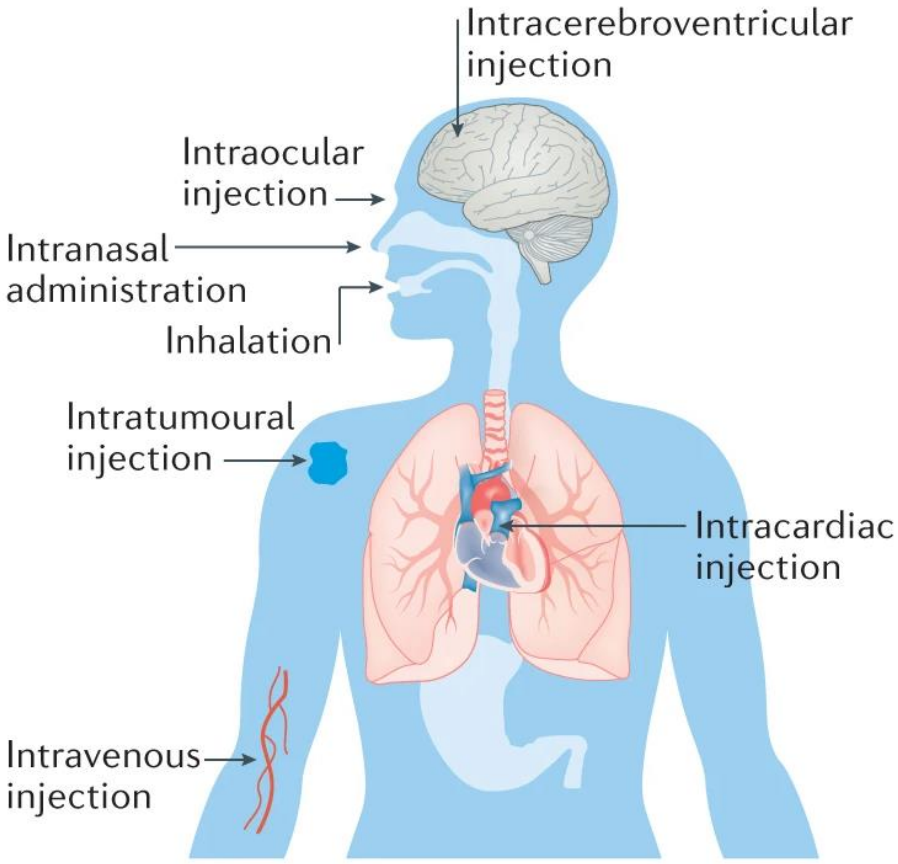

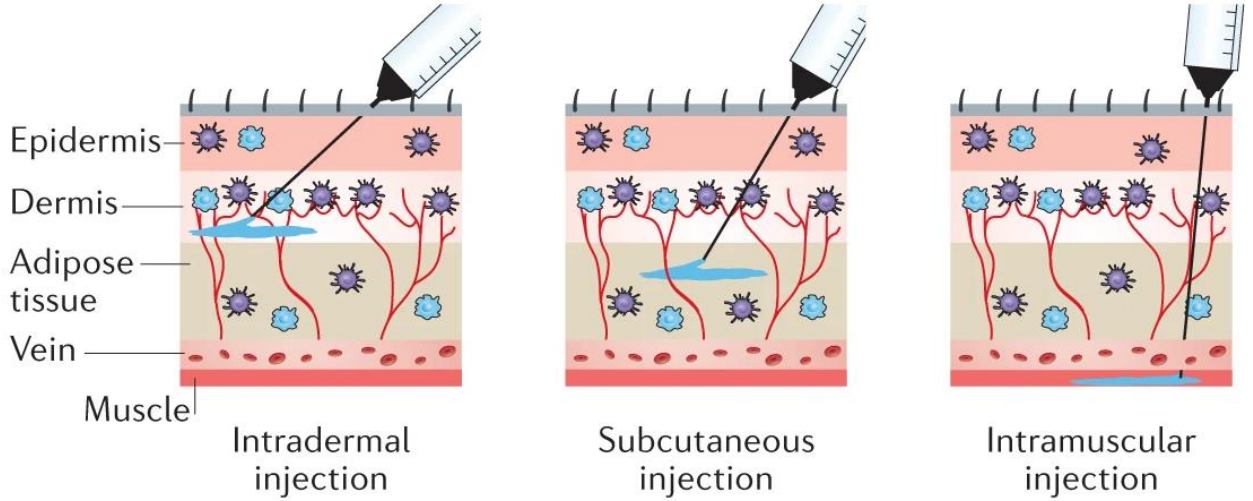

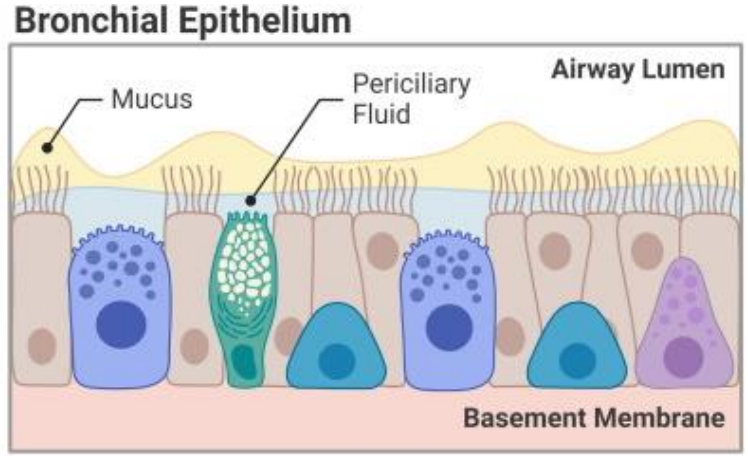

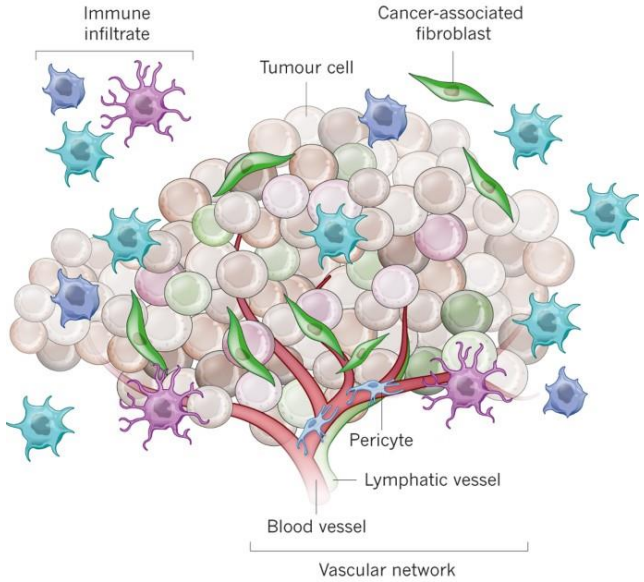

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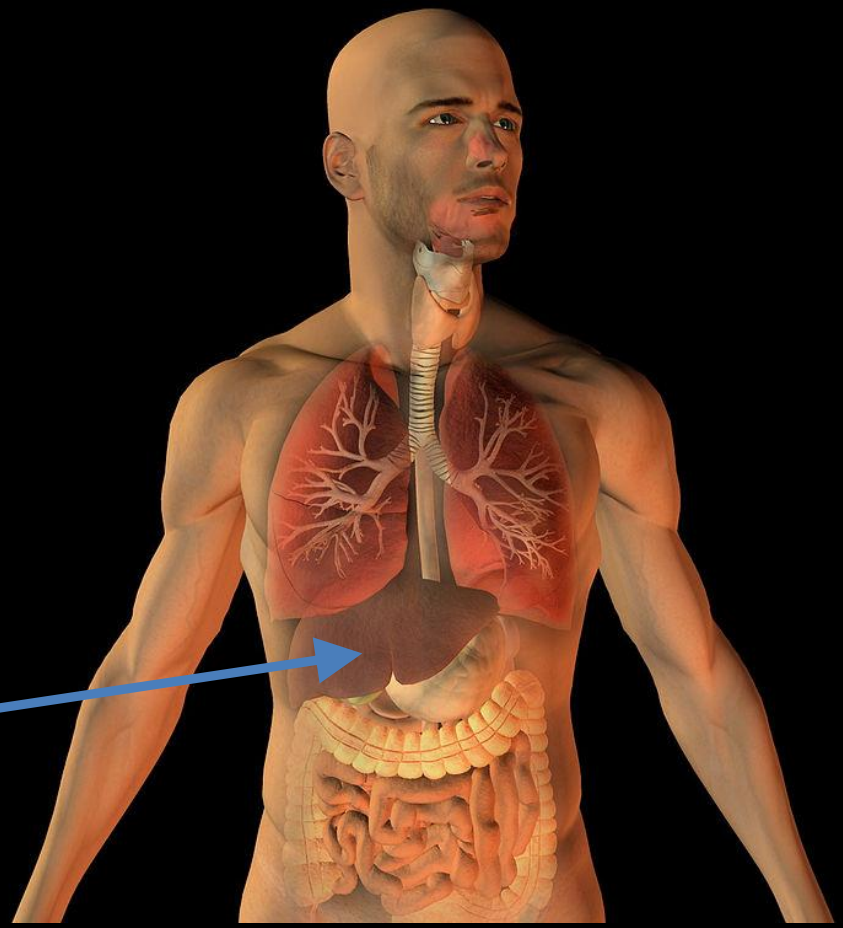
Extrahepatic (non-liver) delivery represents a major hurdle for LNPs

Liver hepatocytes

Plus Kupffer, stellate, sinusoidal endothelial…

onpattro® (patisiran) lipid complex injection

Many more in development and trials across cargoes



http://www.alnylam.com/wp-content/uploads/2018/08/ONPATTRO-Prescribing-Information.pdf

Extrahepatic (non-liver) delivery represents a major hurdle for LNPs

Brain / CNS?
Neurons, glia, tumors…

Muscle?
Cardiac, smooth, skeletal…

Stomach / intestines?

Liver hepatocytes
Plus Kupffer, stellate, sinusoidal endothelial…

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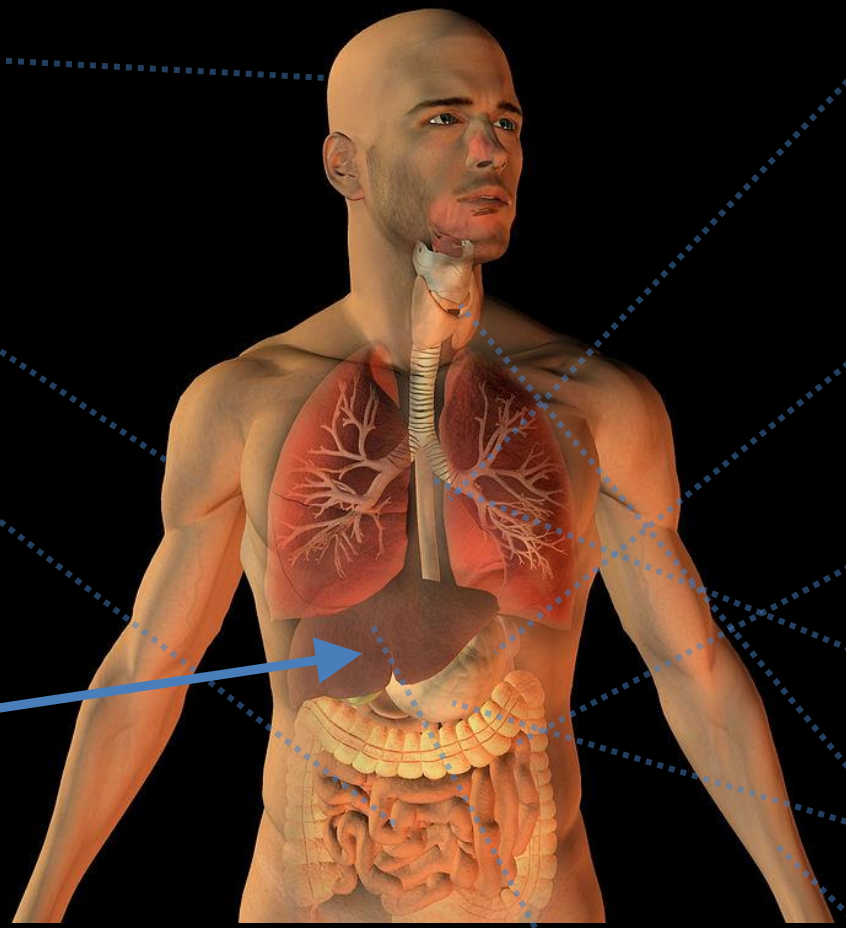

Tumors?

Lungs?
Endothelium, epithelium, basal cells…

Spleen?
Immune cells, T cells, B cells…

Kidneys?
Epithelium, glomerular endothelium and basement membrane, podocytes, mesangial cells…

Heart?
Cardiomyocytes, smooth muscle, endothelial, fibroblasts, mast…

Pancreas?

Lymph nodes?

Bone marrow?

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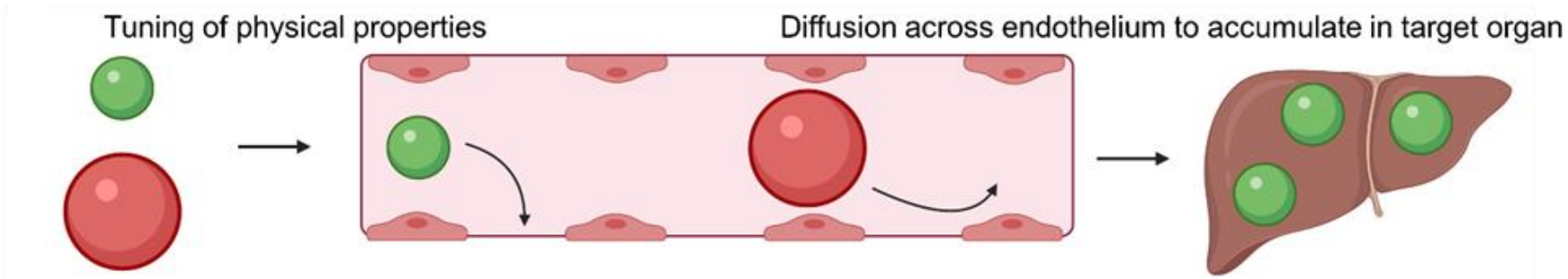
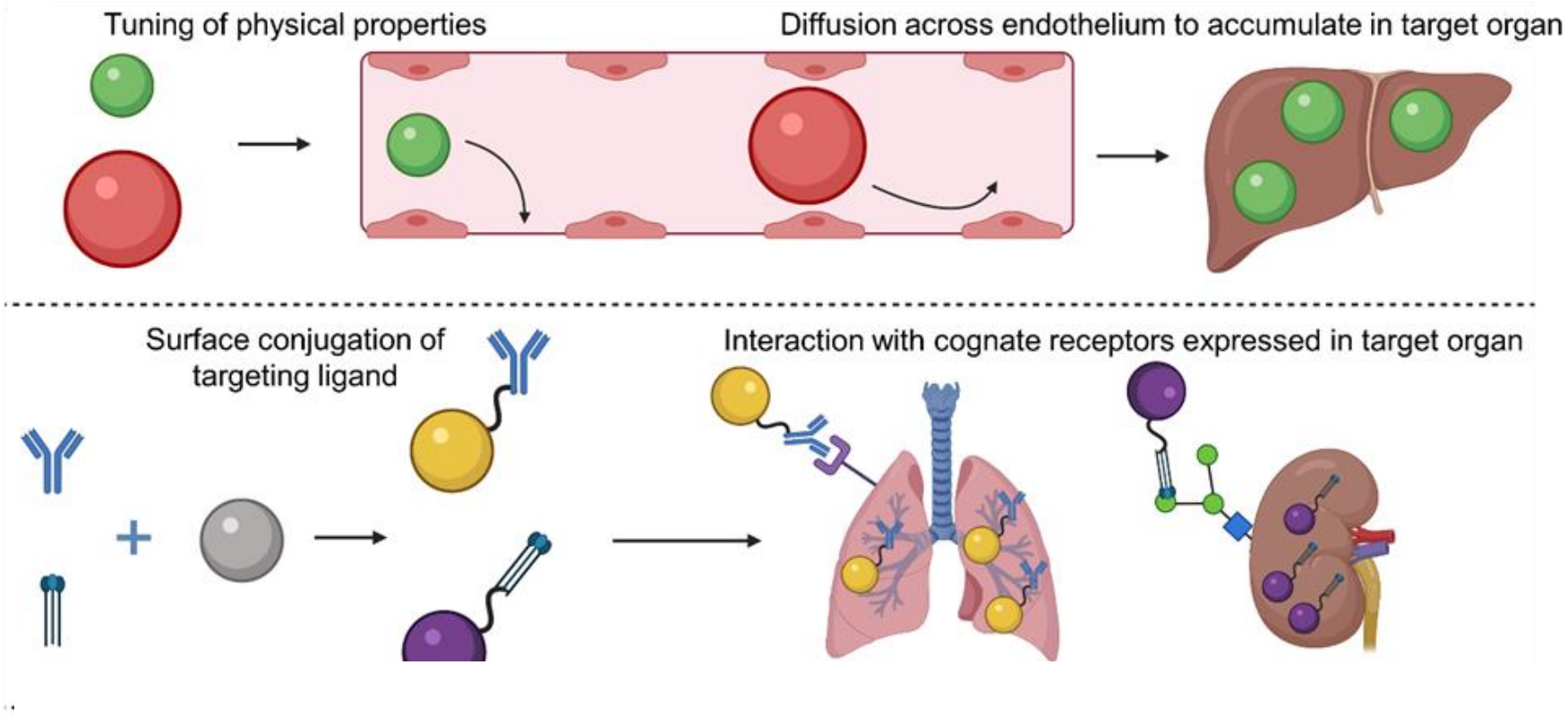
Passive, active, and endogenous mechanisms can enable nanoparticle targeting



Dilliard and Siegwart. **Nat. Rev. Mater.** *8,* 282 (2023).

Passive, active, and endogenous mechanisms can enable nanoparticle targeting



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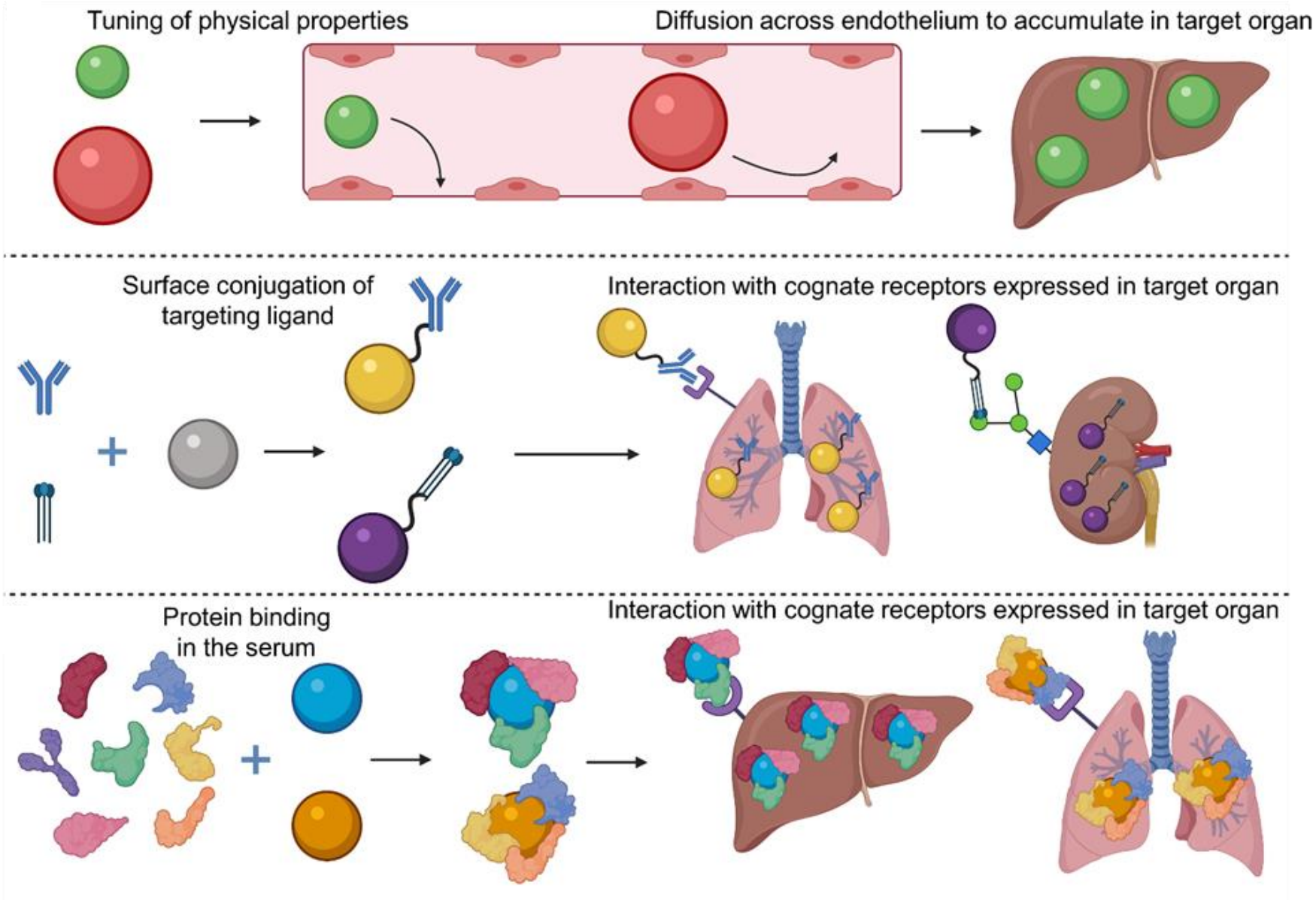

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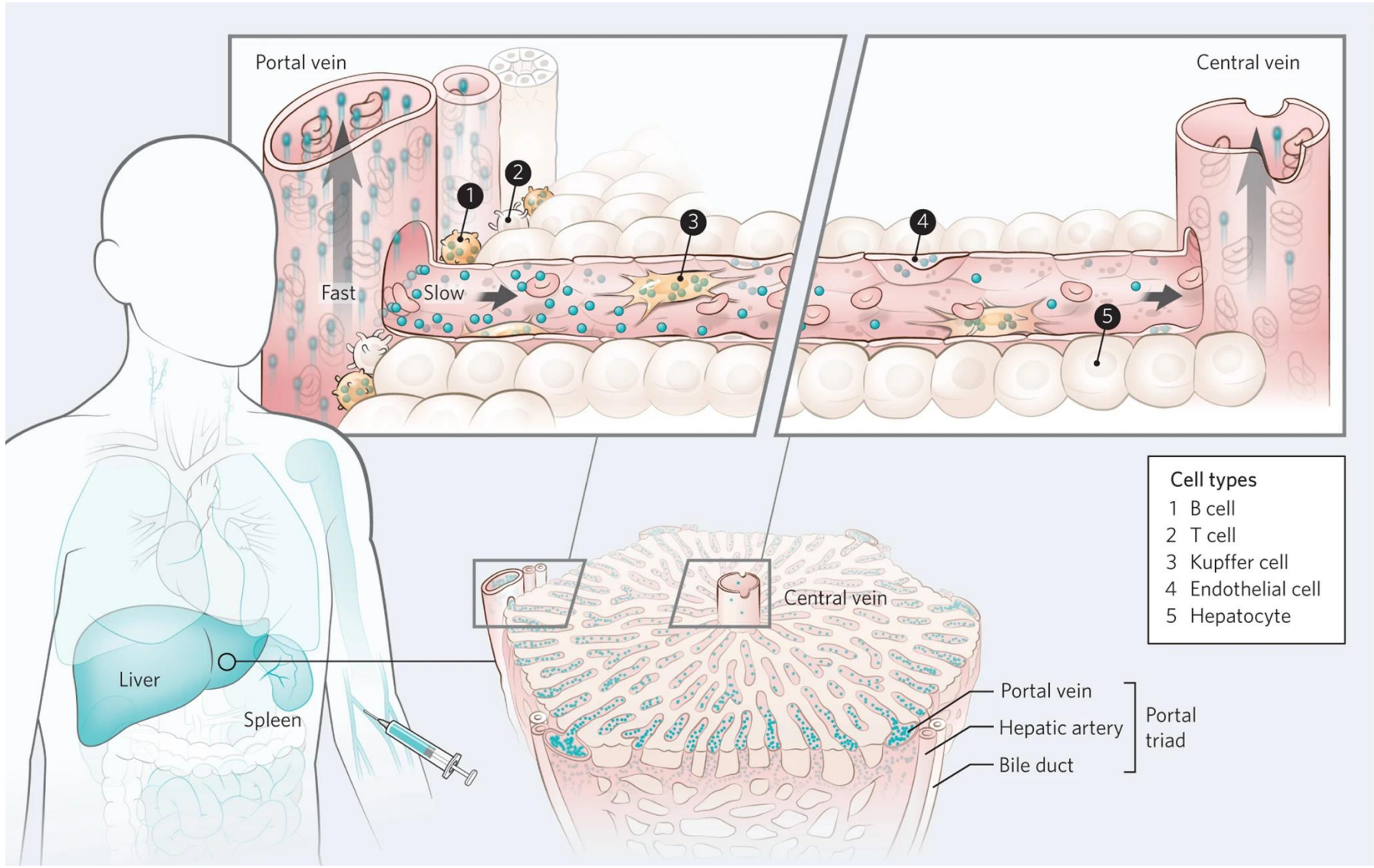
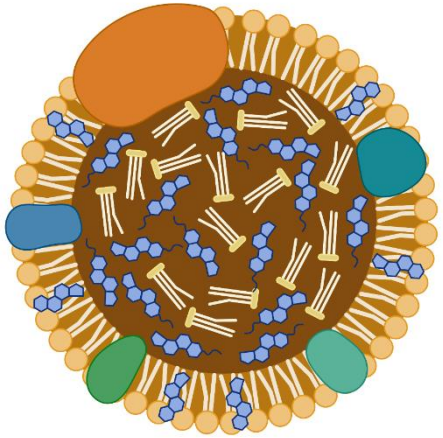
Liver anatomy permits passive accumulation of nanoparticles

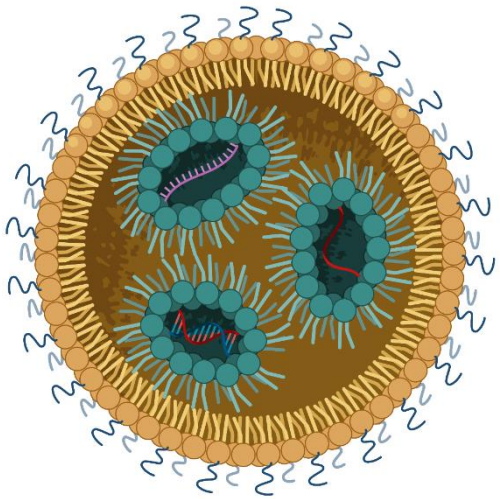


Tsoi et al. **Nat. Mater.** *15,* 1212 (2016).

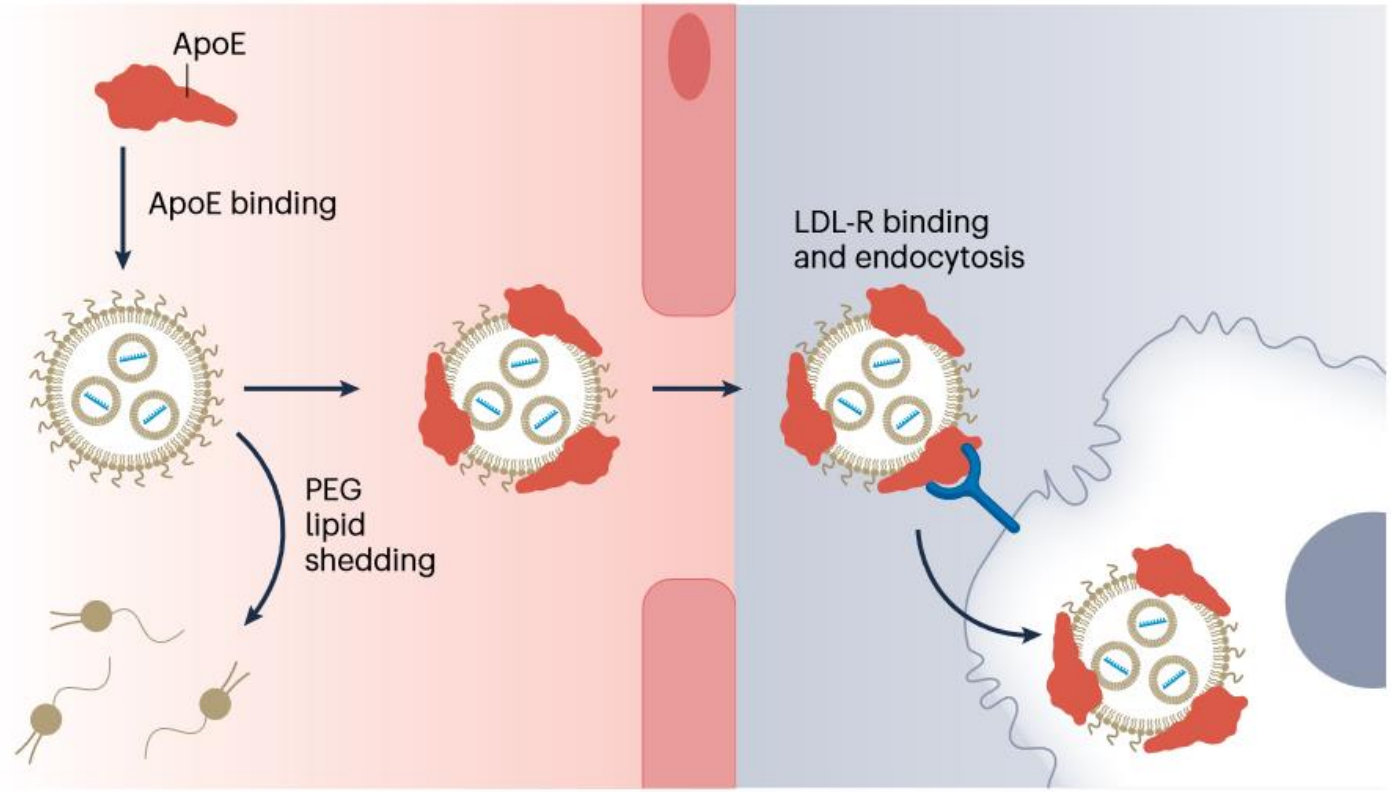
Proteins involved in transport of endogenous lipid particles through the blood direct LNPs to liver hepatocytes



Low-density lipoprotein



Lipid nanoparticle



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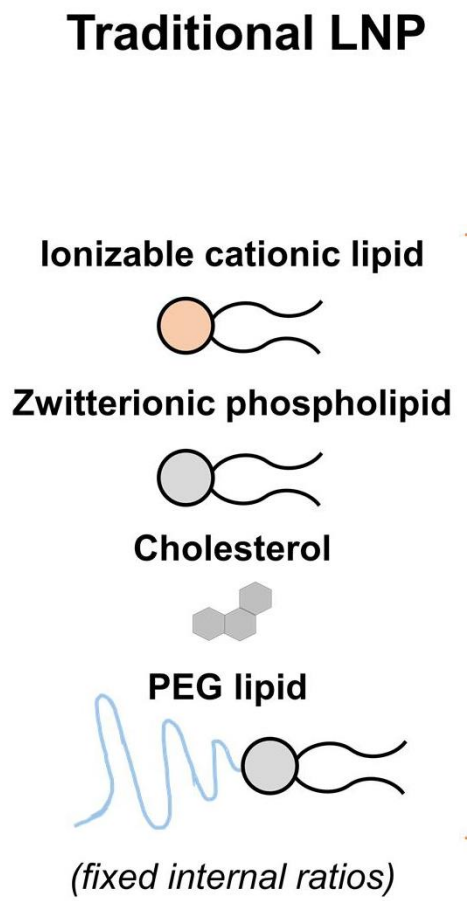
Alteration of lipid molar ratios is necessary for efficient delivery of different nucleic acid cargoes but still results in hepatic delivery



siRNA formulations: Zhou et al. **PNAS** *113*, 520 (2016).

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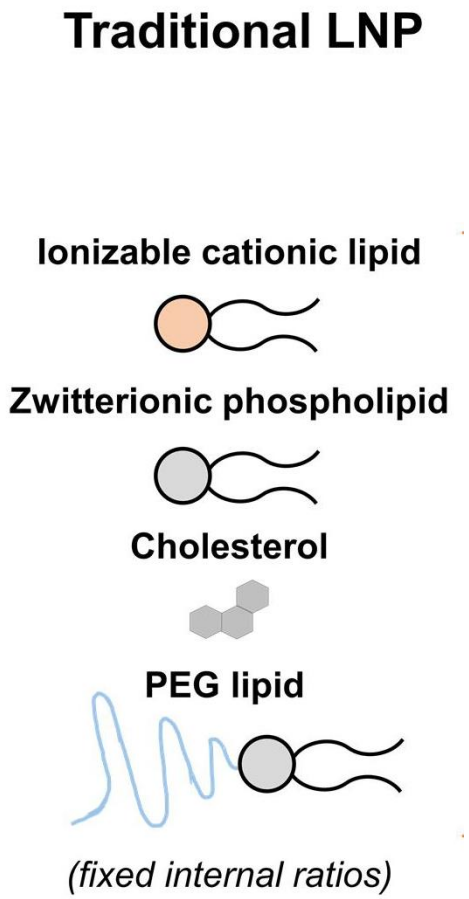

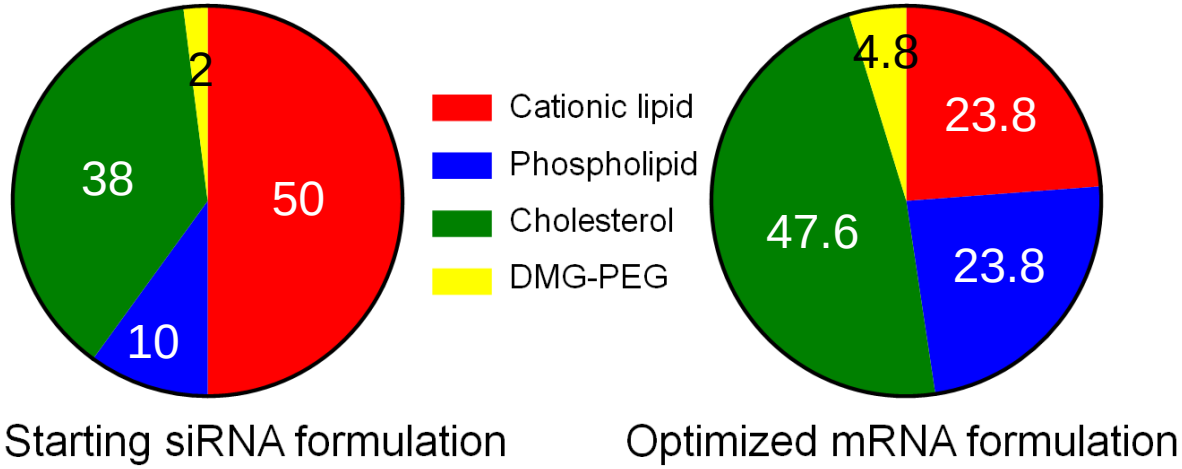

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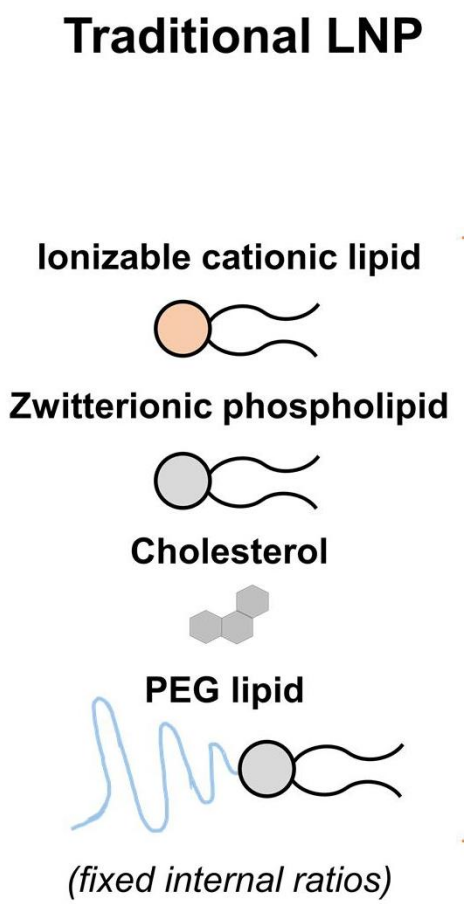
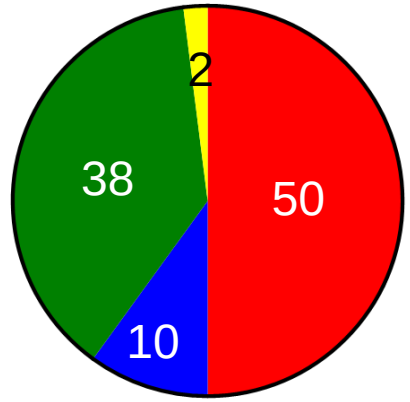
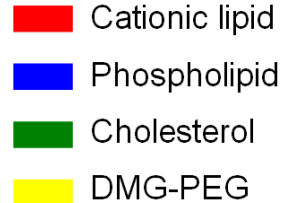
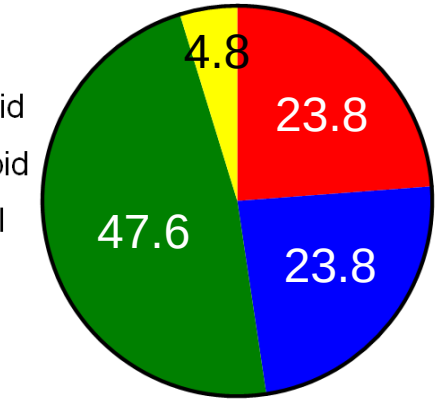


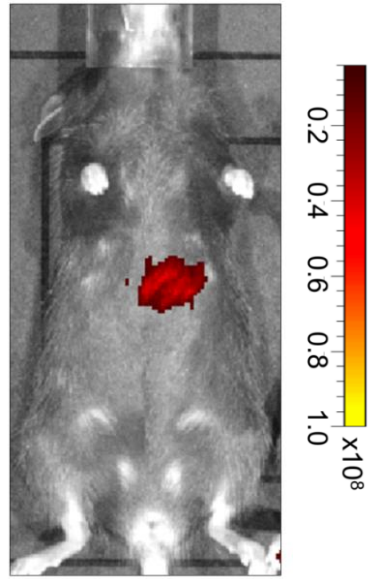


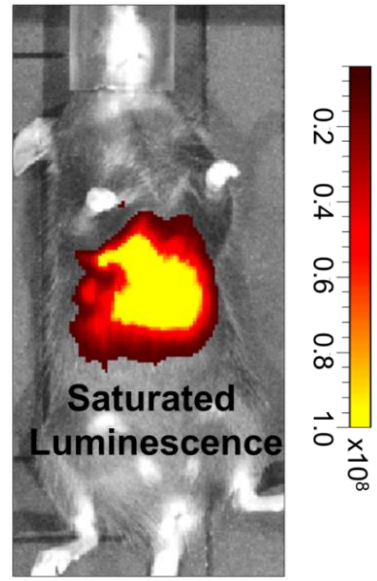




Starting siRNA formulation

Optimized mRNA formulation





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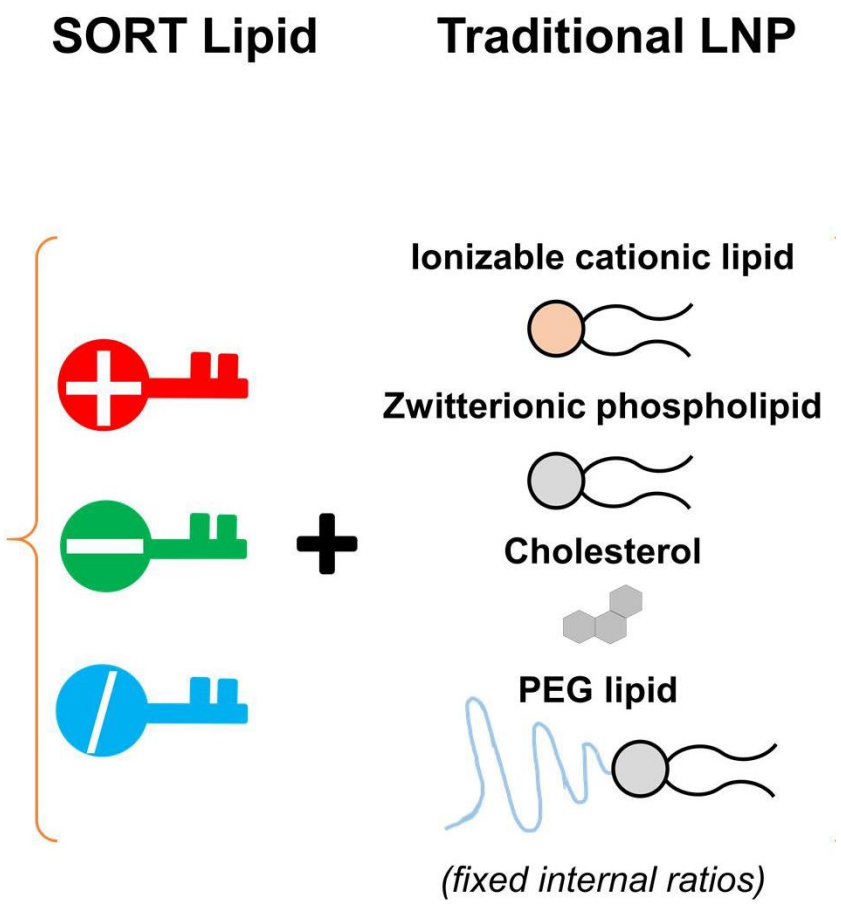
Selective ORgan Targeting (SORT) allows lipid nanoparticles (LNPs) to be systematically and predictably engineered to accurately edit cells in specific organs



Yan et al. **PNAS** *113*, E5702 (2016).
Miller et al. **Angew. Chem. Int. Ed.** *56*, 1059 (2017).

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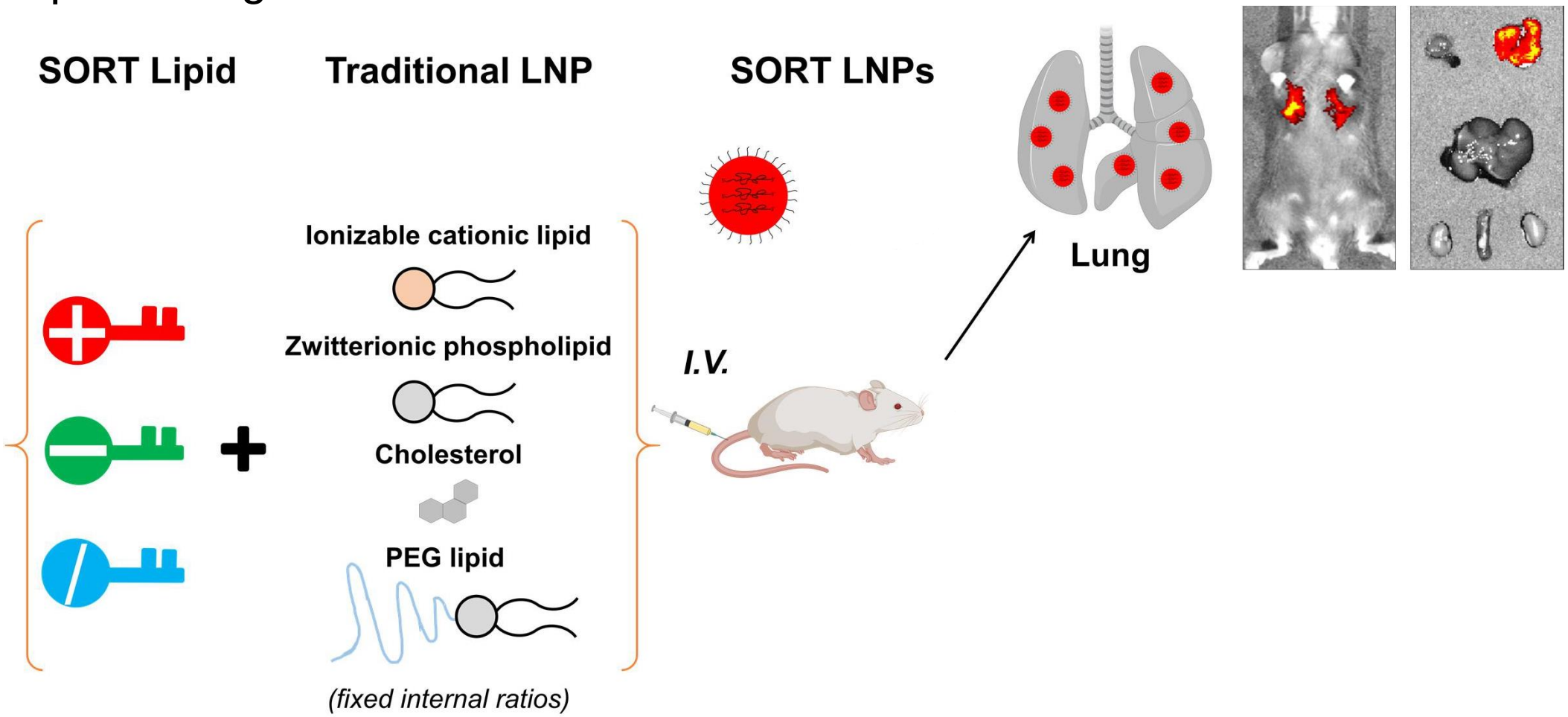

Cheng et al **Nat. Nanotechnol.** *15*, 313 (2020).
Wei et al. **Nat. Commun.** *11*, 3232 (2020).

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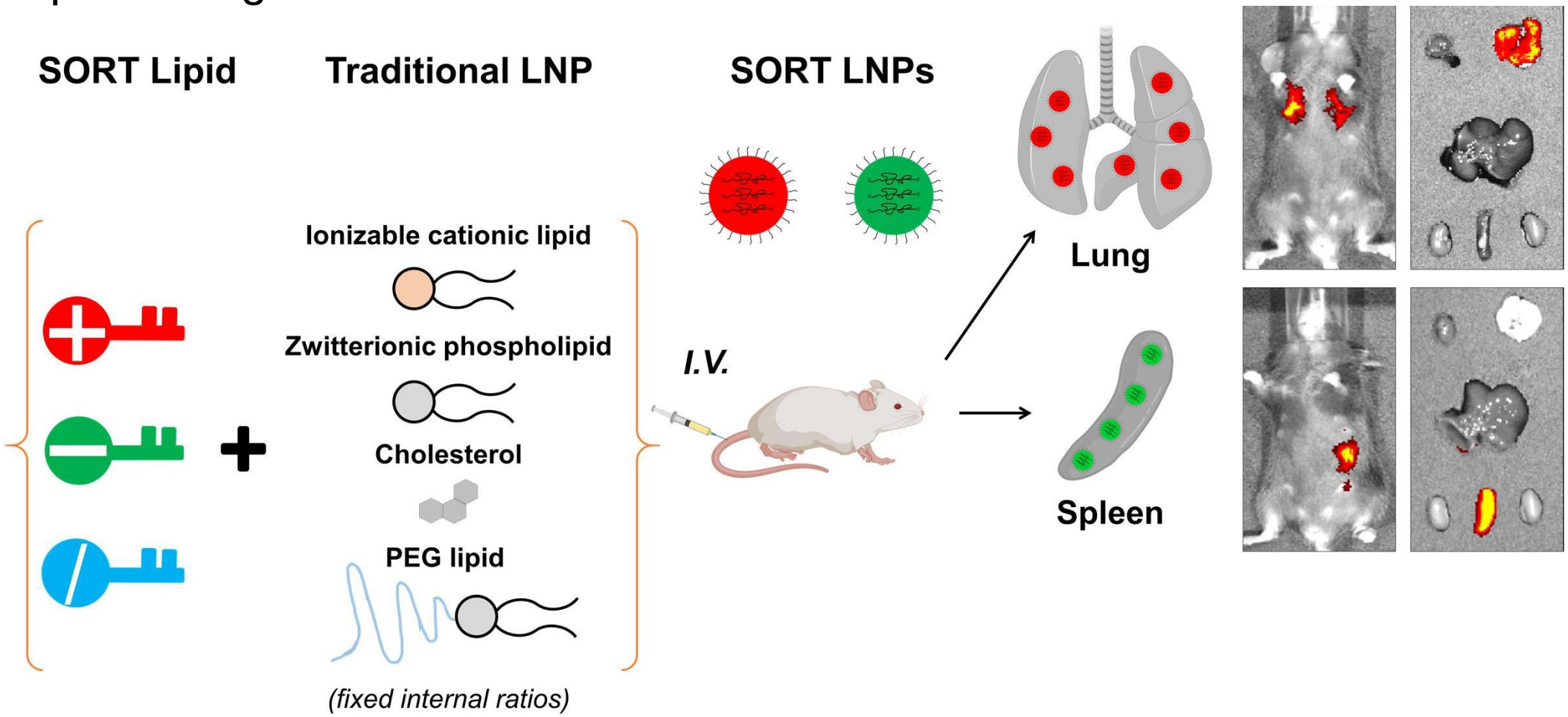

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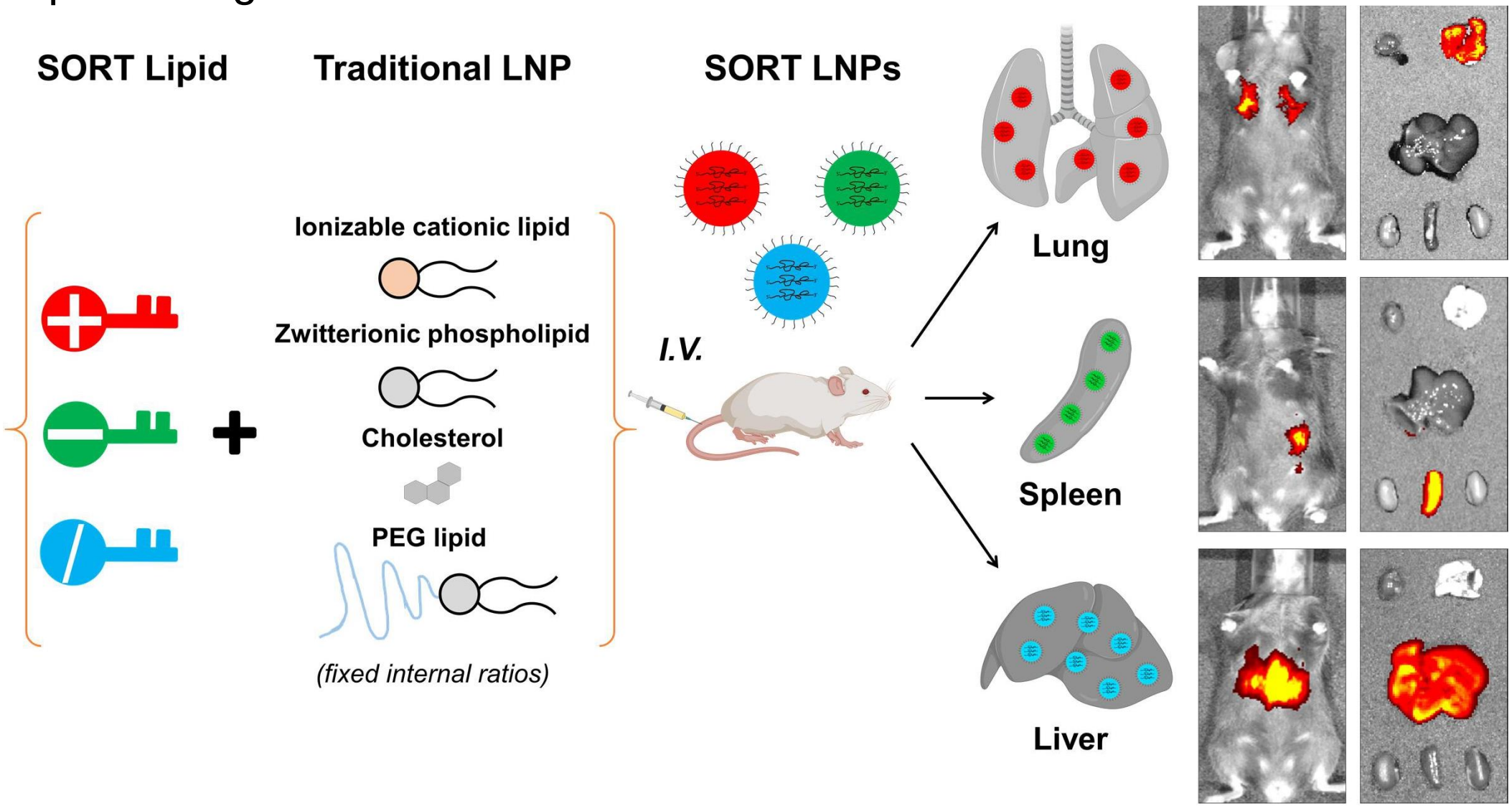
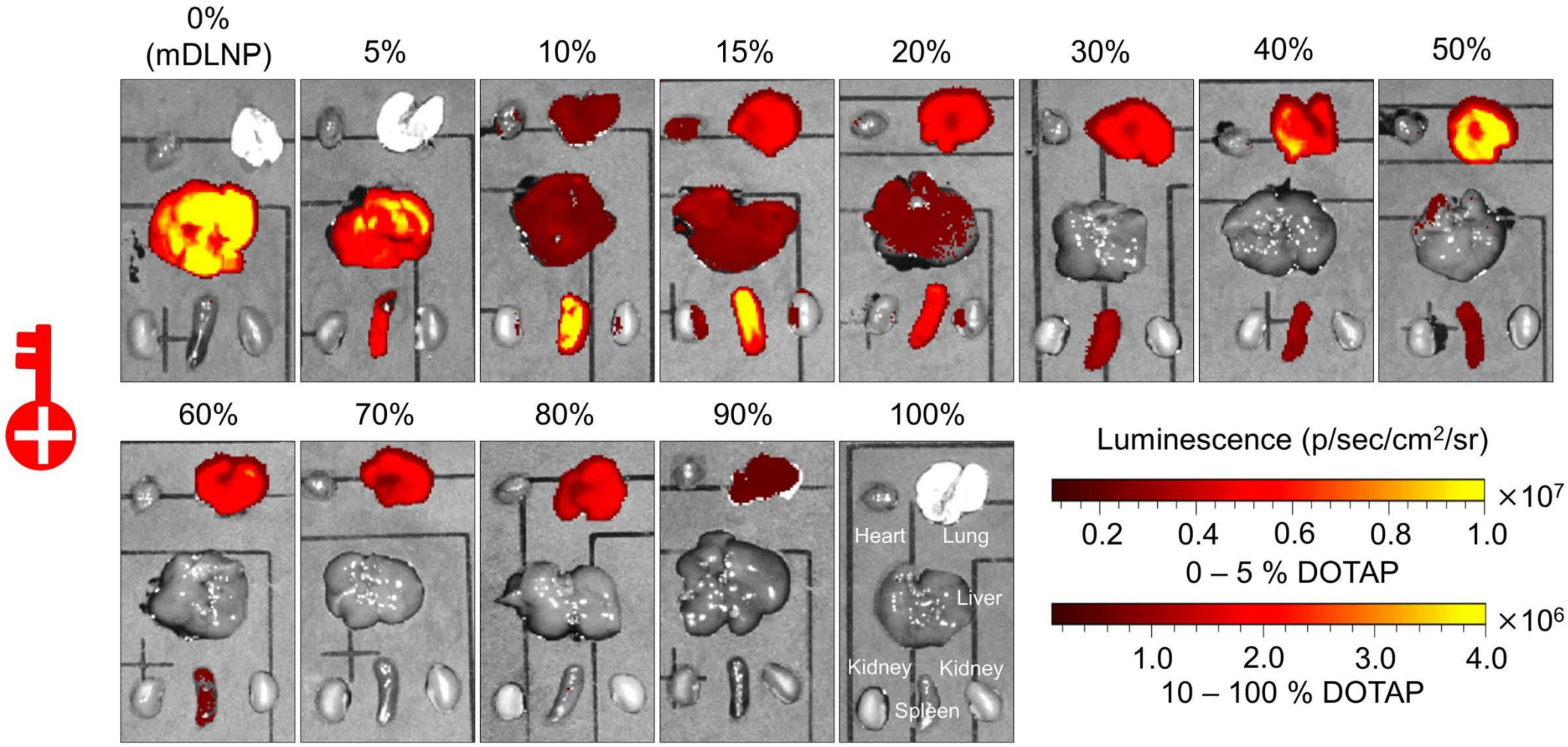


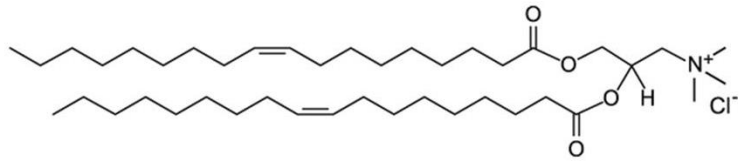
Cheng et al **Nat. Nanotechnol.** *15*, 313 (2020).
Wei et al. **Nat. Commun.** *11*, 3232 (2020).

Lee et al. **Angew. Chem. Int. Ed.** *60*, 5848 (2021).
Liu et al. **Nat. Mater.** *20*, 701-710 (2021).

Inclusion of a permanently cationic lipid into 5A2-SC8 LNPs systematically shifted luciferase protein as a function of SORT lipid percentage



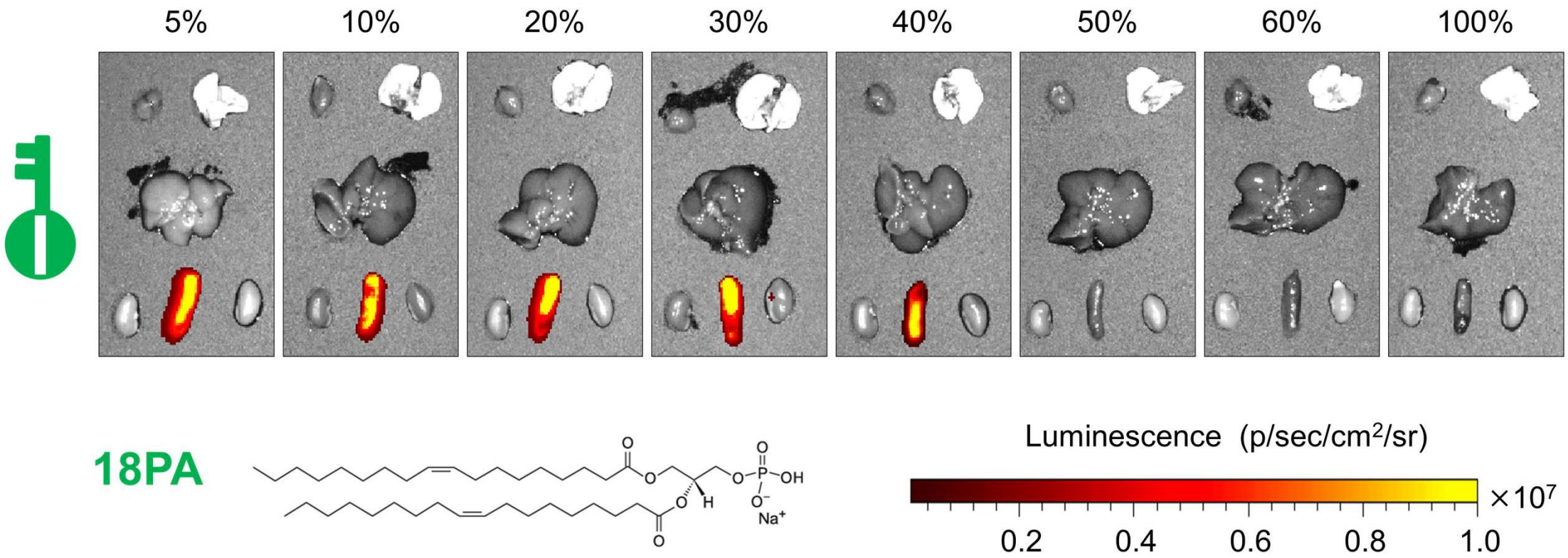
DOTAP



5A2-SC8/DOPE/Chol/DMG-PEG/DOTAP =15/15/30/3/X
where X = 0 – 1200 (mol/mol)

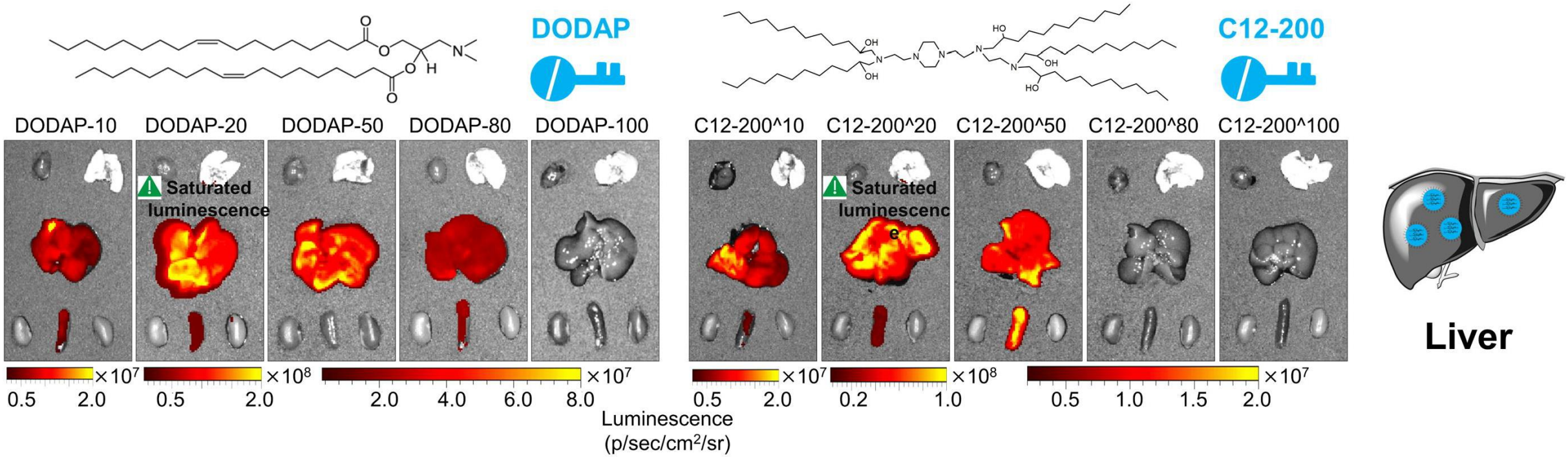
0.1 mg/kg Luc mRNA, IV, 6h

Inclusion of an anionic lipid completely switched luciferase protein expression from liver to spleen



5A2-SC8 SORT LNPs
0.1 mg/kg Luc mRNA, IV, 6h

Interestingly, inclusion of ionizable cationic SORT lipids enhanced liver delivery



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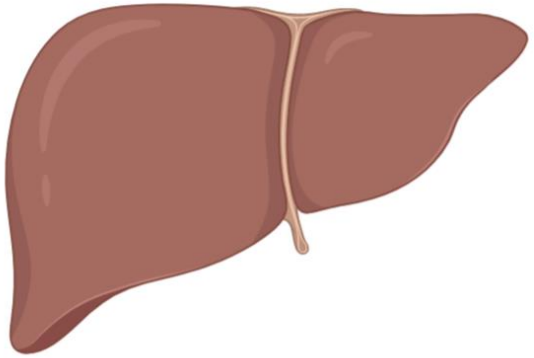
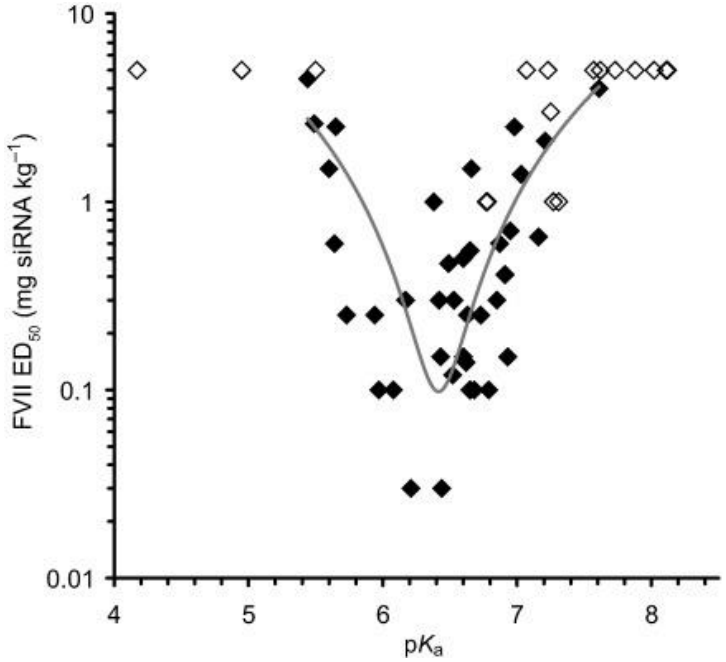
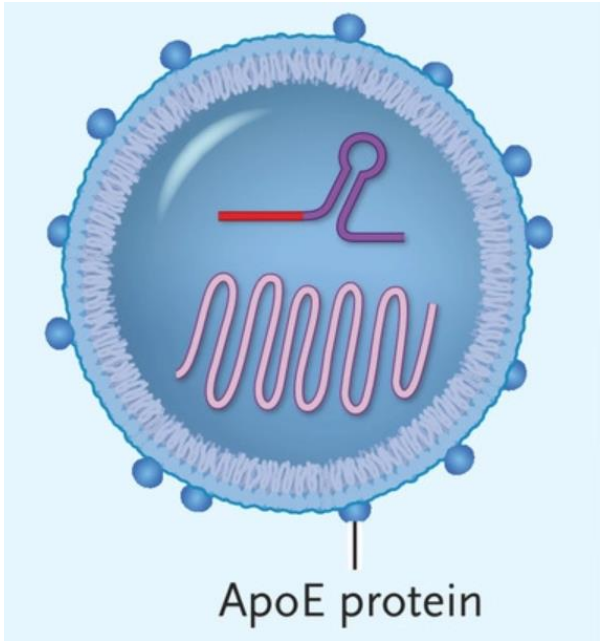
Three essential dogmas have been established for liver delivery of LNPs







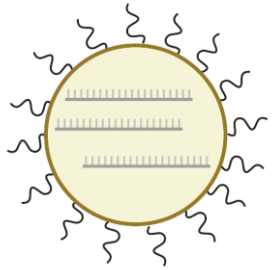
1. Liver biodistribution

2. pK_a = 6.4

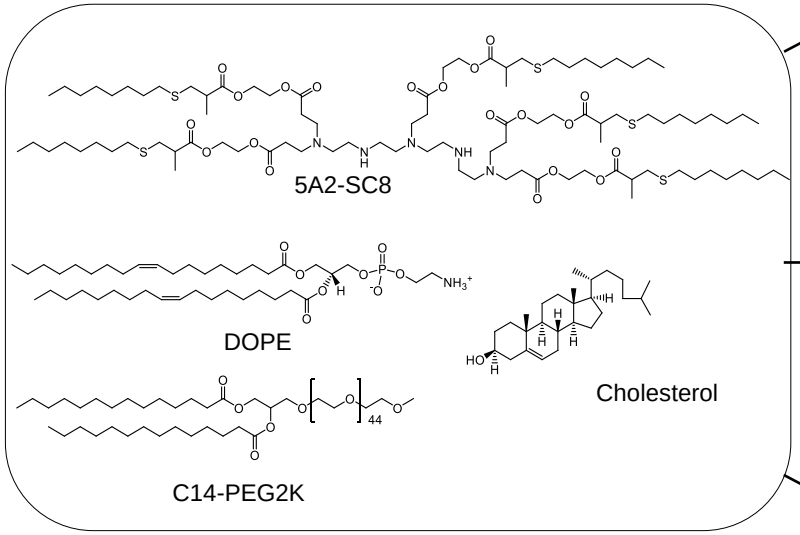
3. ApoE adsorption

Gillmore et al. **New Engl. J. Med.** *85*, 493 (2021).
Jayaraman et al. **Angew. Chem. Int. Ed.** *51*, 8529 (2012).

Liver, Spleen, and Lung SORT LNPs were formulated and characterized

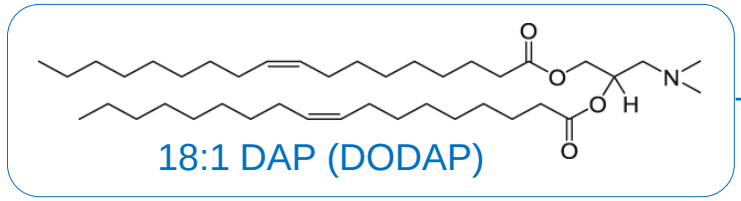


Conventional 4-Component LNP

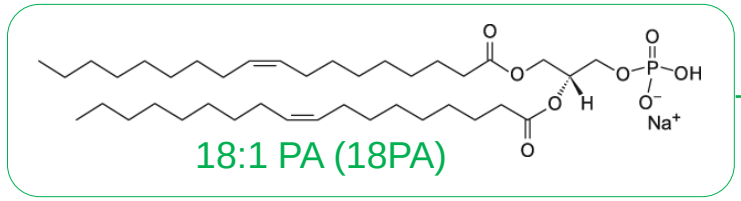


5A2-SC8/DOPE/Chol/C14PEG =
23.8/23.8/47.6/4.8 (mol. %)

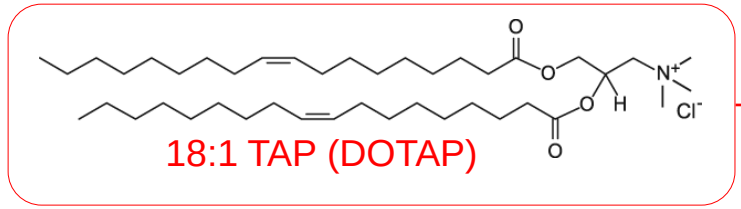
Addition of 5th SORT molecule



5A2-SC8/DOPE/Chol/C14PEG/DODAP =
19/19/38/4/20 (mol. %)

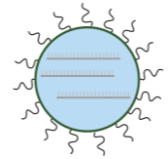


5A2-SC8/DOPE/Chol/C14PEG/18PA =
16.7/16.7/33.3/3.3/30 (mol. %)

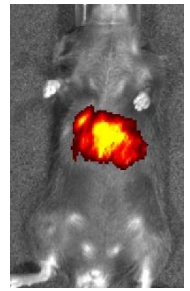


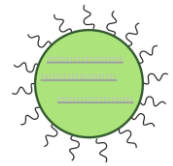
5A2-SC8/DOPE/Chol/C14PEG/DOTAP =
11.9/11.9/23.8/2.4/50 (mol. %)

LNP functional readout:
Luc mRNA translation to protein

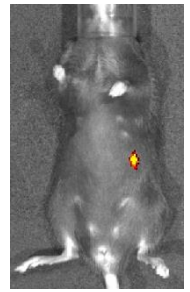


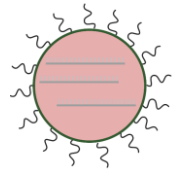
Liver
SORT



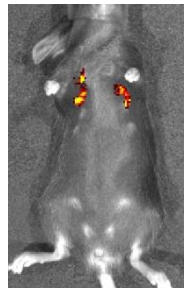


Spleen
SORT



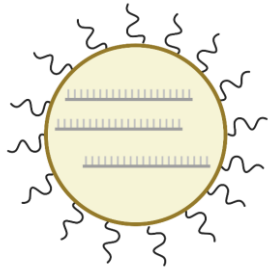


Lung
SORT



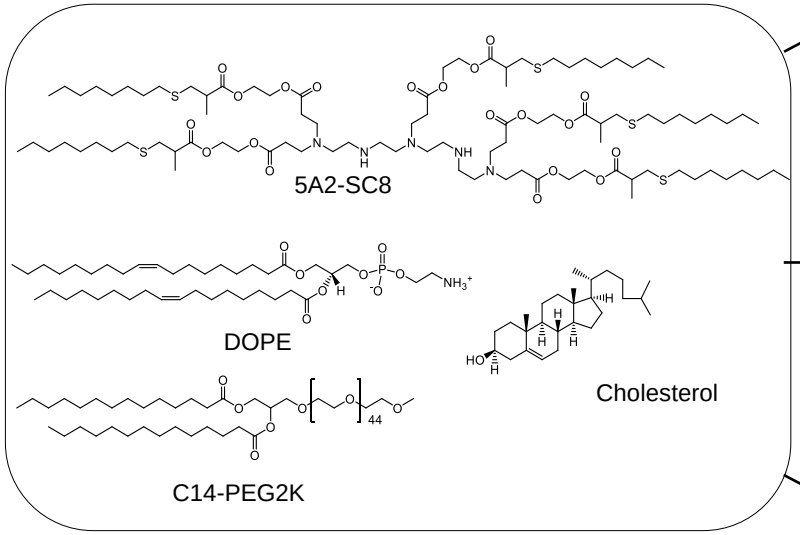
Dilliard et al. **PNAS** *118*, e2109256118 (2021).
Wang et al. **Nat. Protocols** *18*, 265 (2023).

Liver, Spleen, and Lung SORT LNPs were formulated and characterized



Size = 144 nm
PDI = 0.13
ζ-potential = -3.5 mV

Conventional 4-Component LNP



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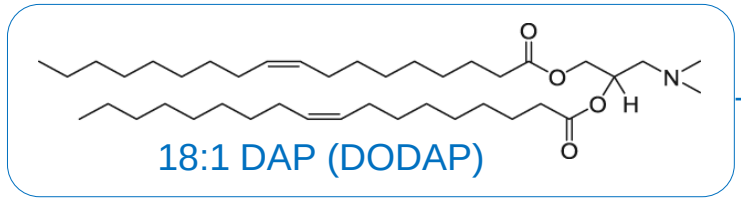
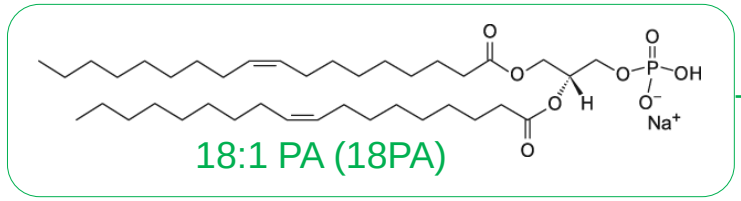
23.8/23.8/47.6/4.8 (mol. %)

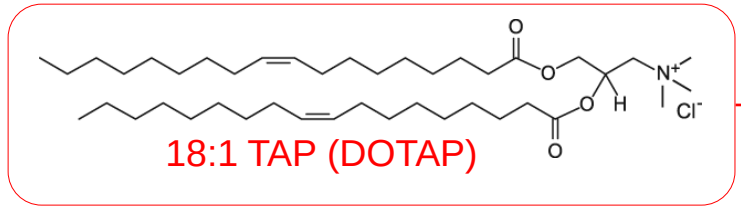
Addition of 5th SORT molecule



5A2-SC8/DOPE/Chol/C14PEG/DODAP =
19/19/38/4/20 (mol. %)



5A2-SC8/DOPE/Chol/C14PEG/18PA =
16.7/16.7/33.3/3.3/30 (mol. %)



5A2-SC8/DOPE/Chol/C14PEG/DOTAP =
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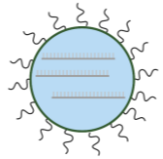
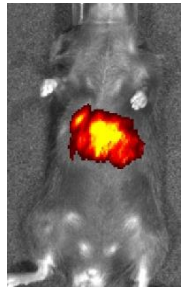
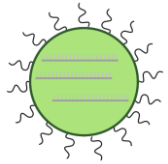
LNP functional readout:
Luc mRNA translation to protein



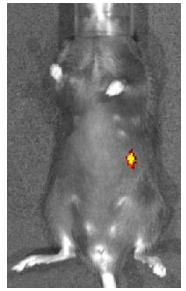
Liver SORT



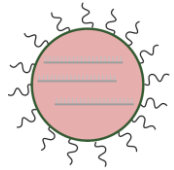
Size = 154 nm
PDI = 0.11
ζ-potential = -3.8 mV



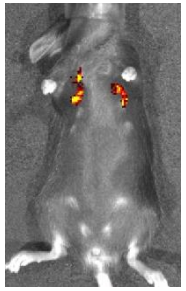
Spleen SORT



Size = 167 nm
PDI = 0.14
ζ-potential = -1.8 mV



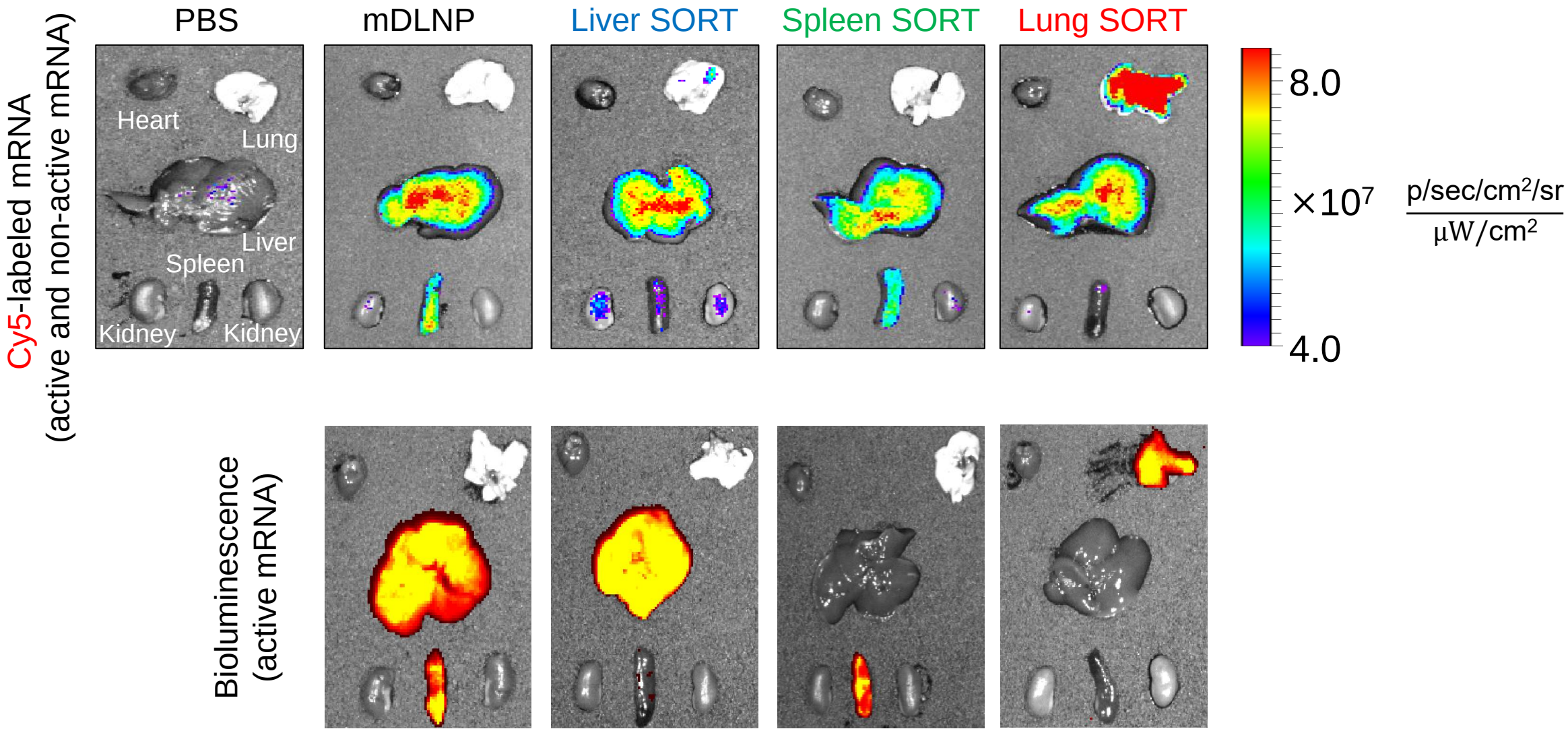
Lung SORT



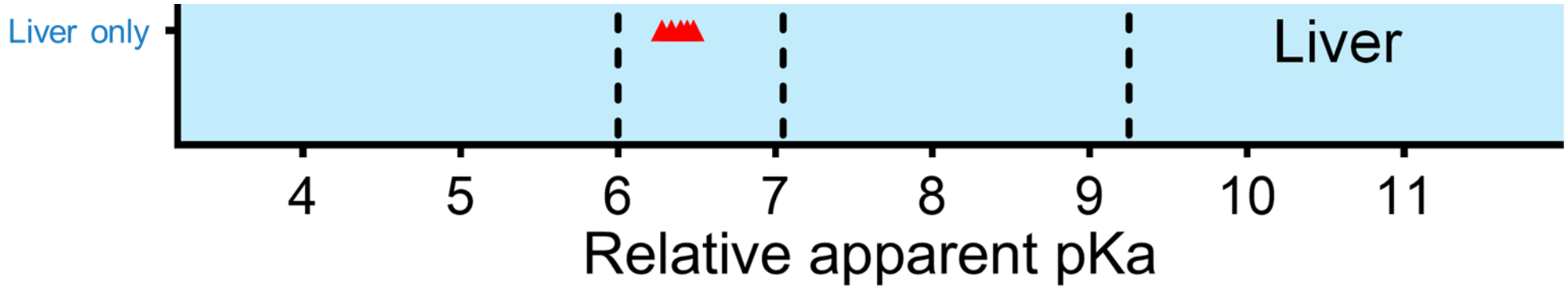
Size = 114 nm
PDI = 0.15
ζ-potential = -0.8 mV

Dilliard et al. **PNAS** *118*, e2109256118 (2021).
Wang et al. **Nat. Protocols** *18*, 265 (2023).

SORT molecules promote biodistribution to the target organ but biodistribution is not sufficient for tissue-specific mRNA activity

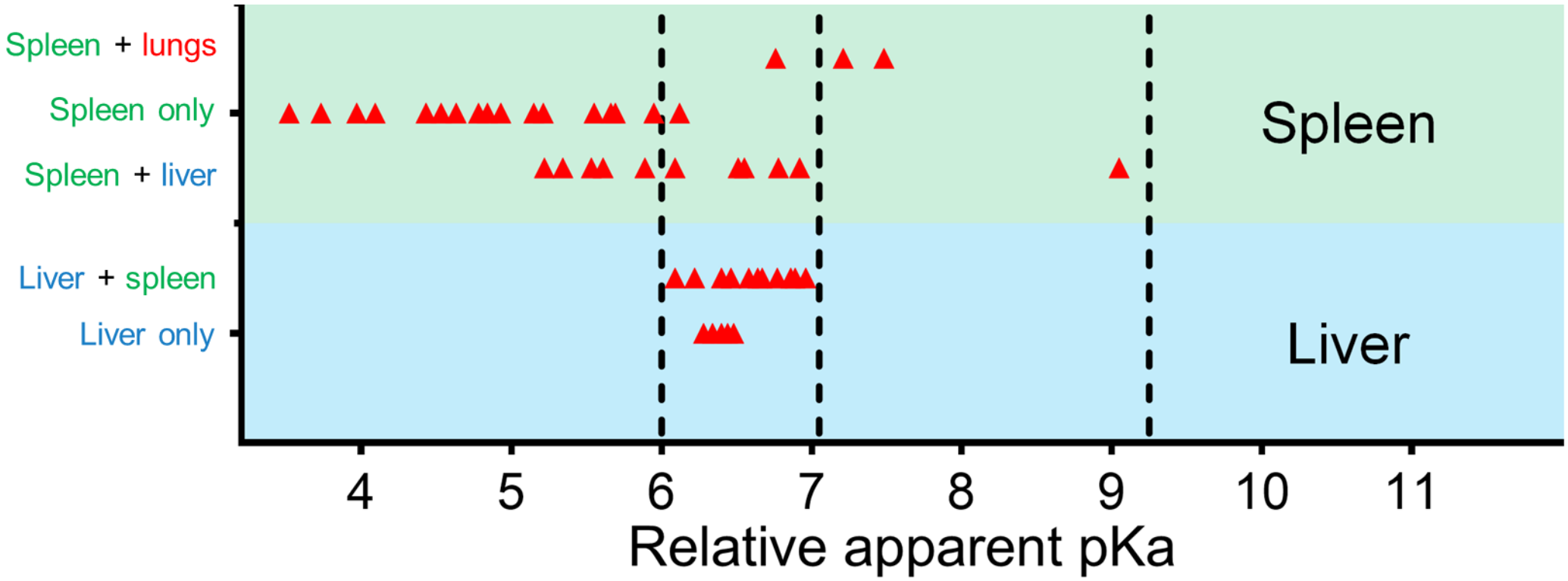


SORT LNP pK_a correlates with organ-specific efficacy



67 distinct SORT LNPs, all with high *in vivo* delivery efficacy
Global/apparent LNP pKa measured by the 6-(*p*-toluidino)-2-naphthalenesulfonic acid (TNS) assay

SORT LNP pK_a correlates with organ-specific efficacy



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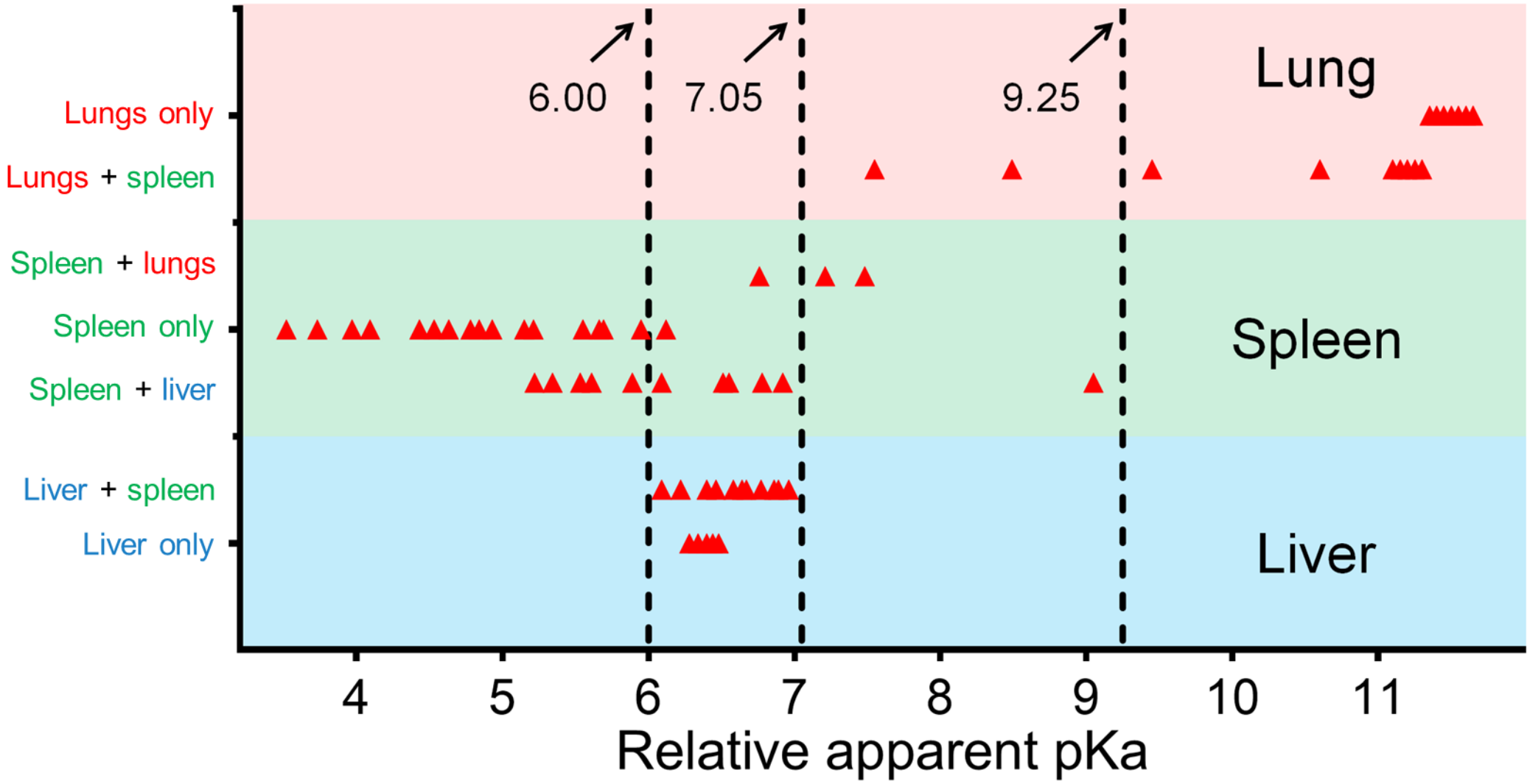
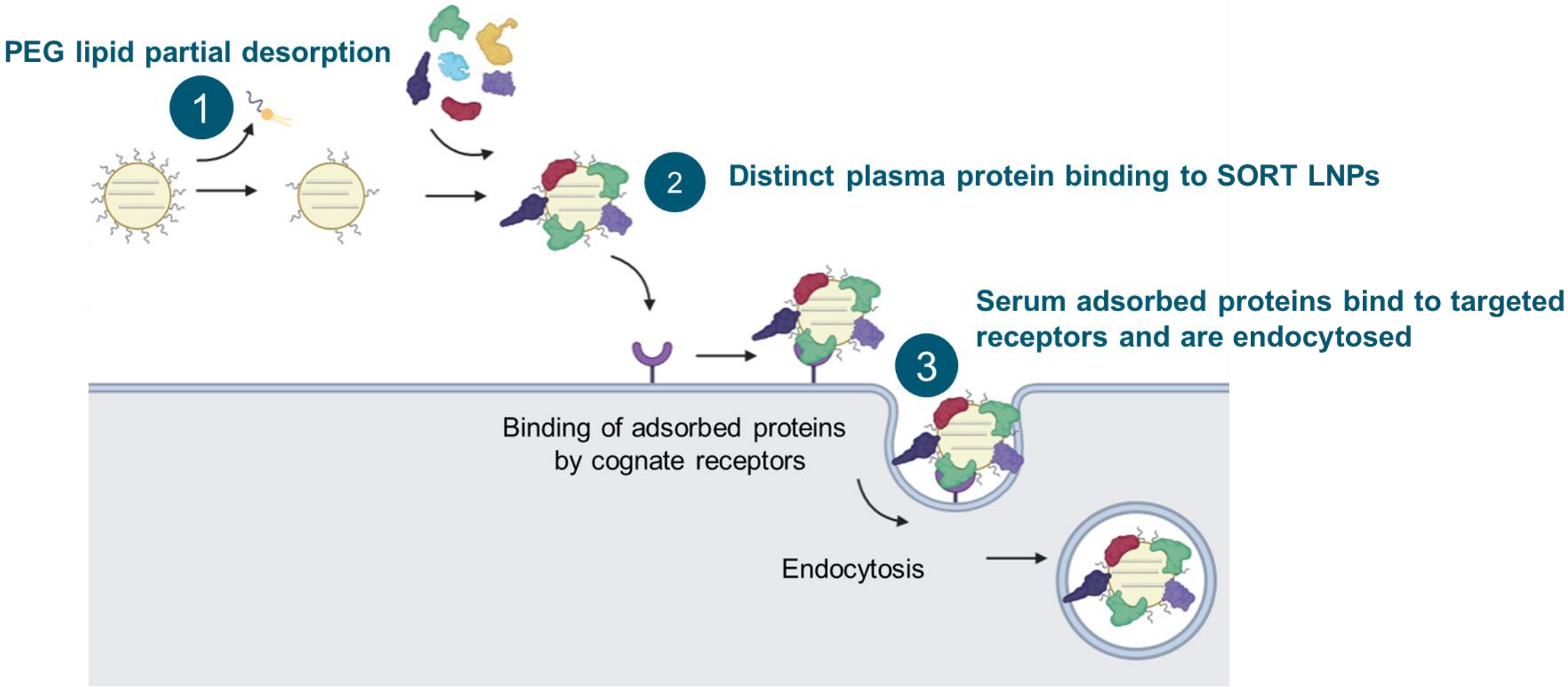
SORT LNP pK_a correlates with organ-specific efficacy

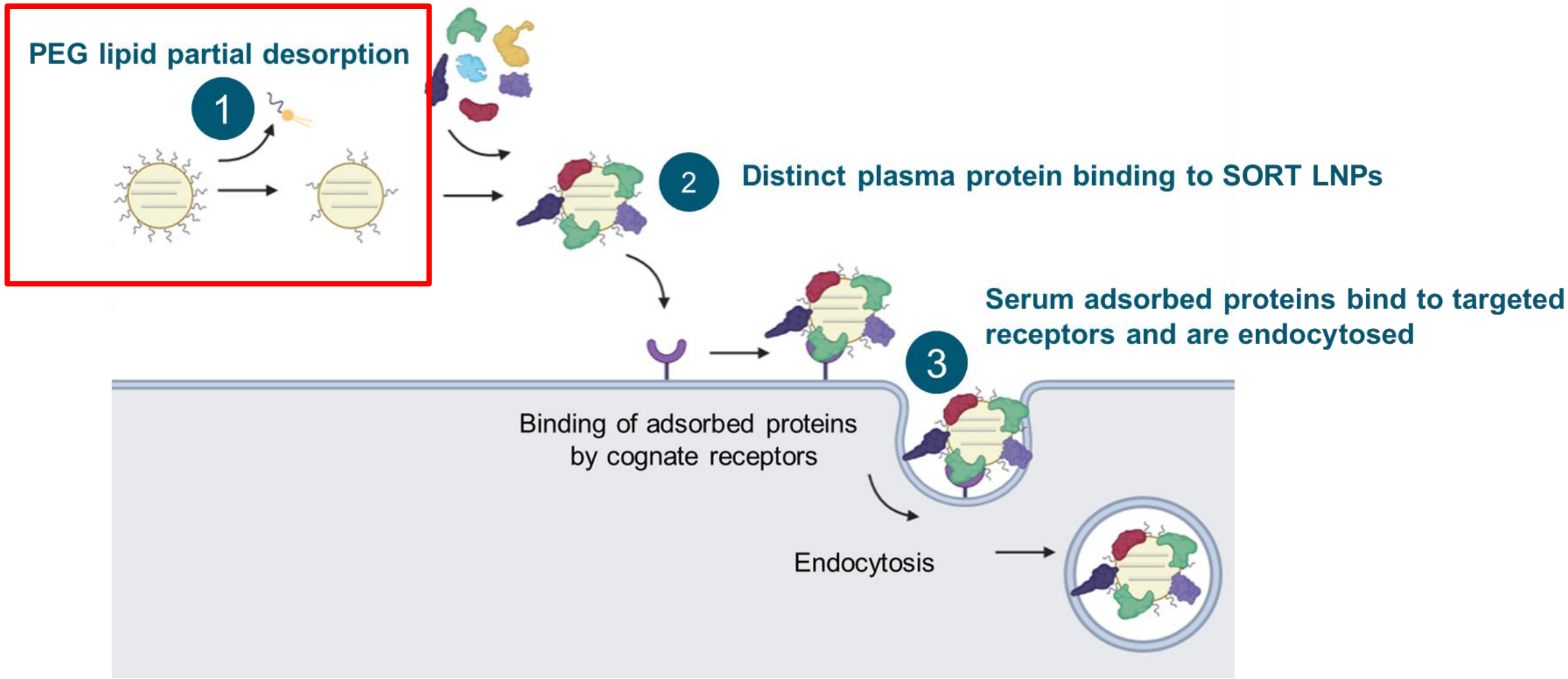


67 distinct SORT LNPs, all with high *in vivo* delivery efficacy
Global/apparent LNP pKa measured by the 6-(*p*-toluidino)-2-naphthalenesulfonic acid (TNS) assay

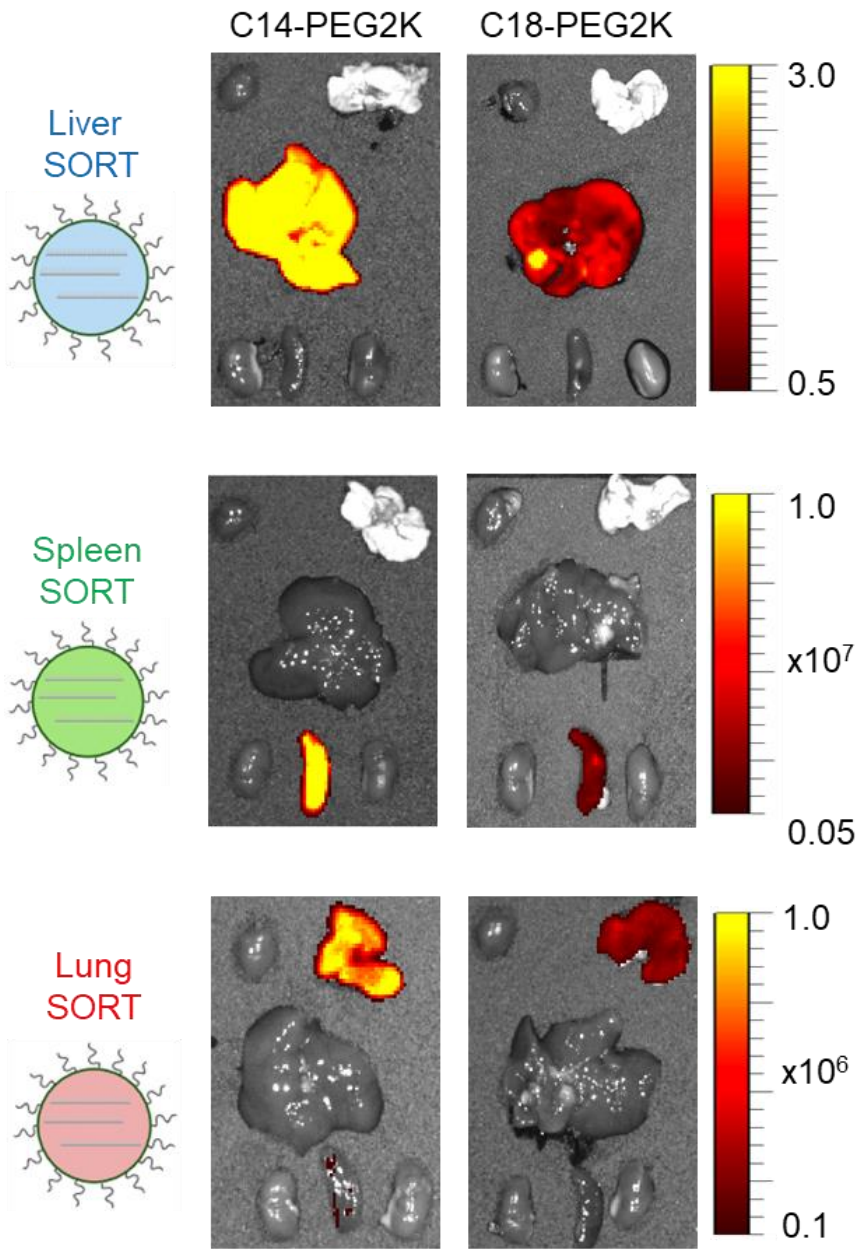
We hypothesize that SORT LNPs also use an **“endogenous targeting”** mechanism via adsorption of different proteins

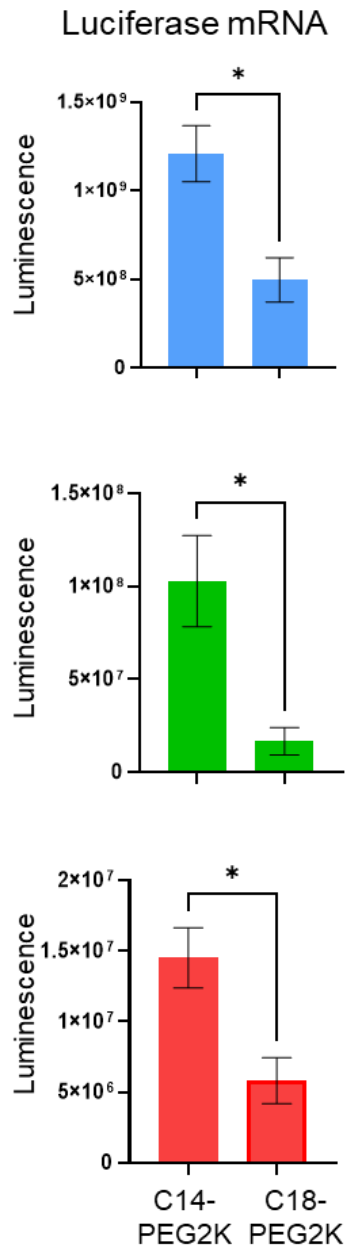


Does PEG-lipid desorption impact SORT LNP activity?



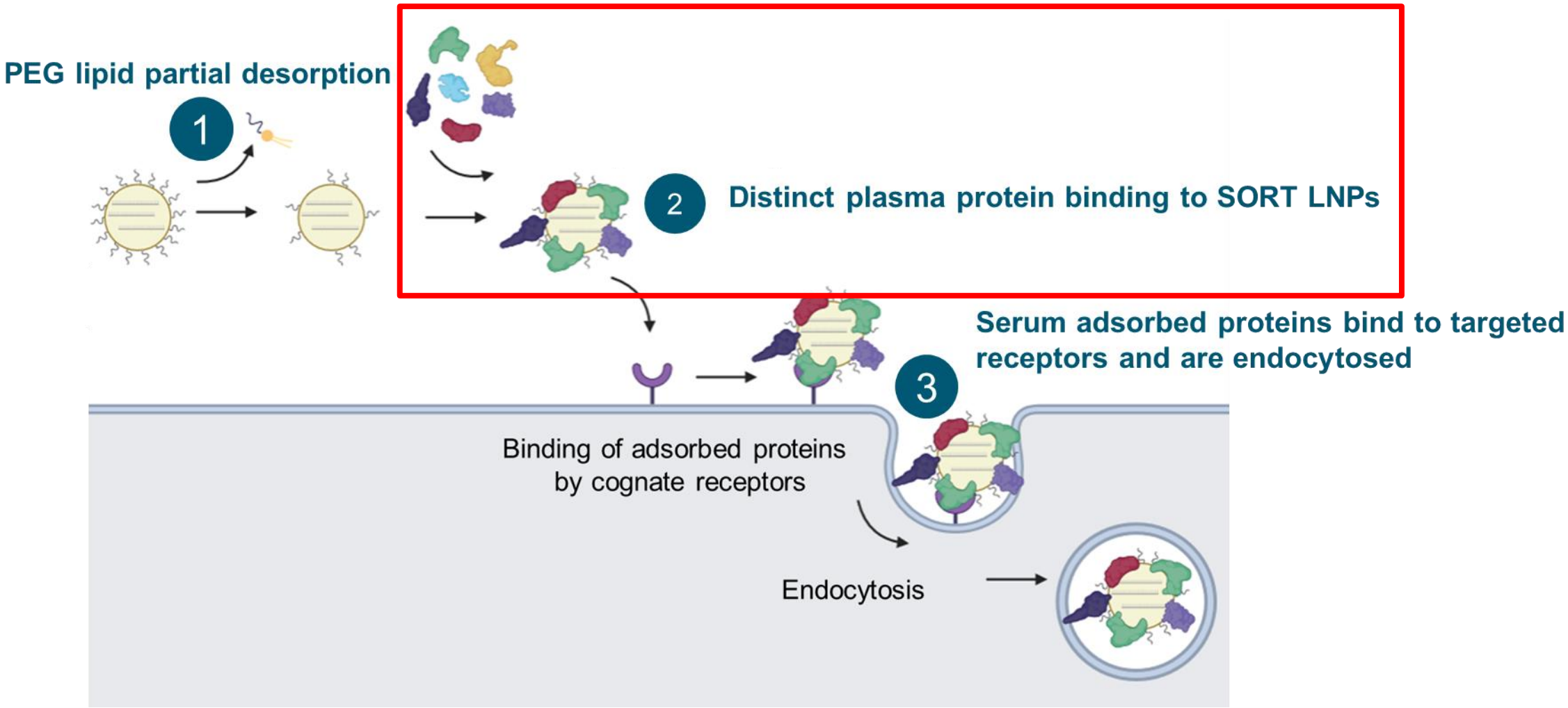
Replacing C14-PEG2K with slower shedding C18-PEG2K impairs mRNA delivery



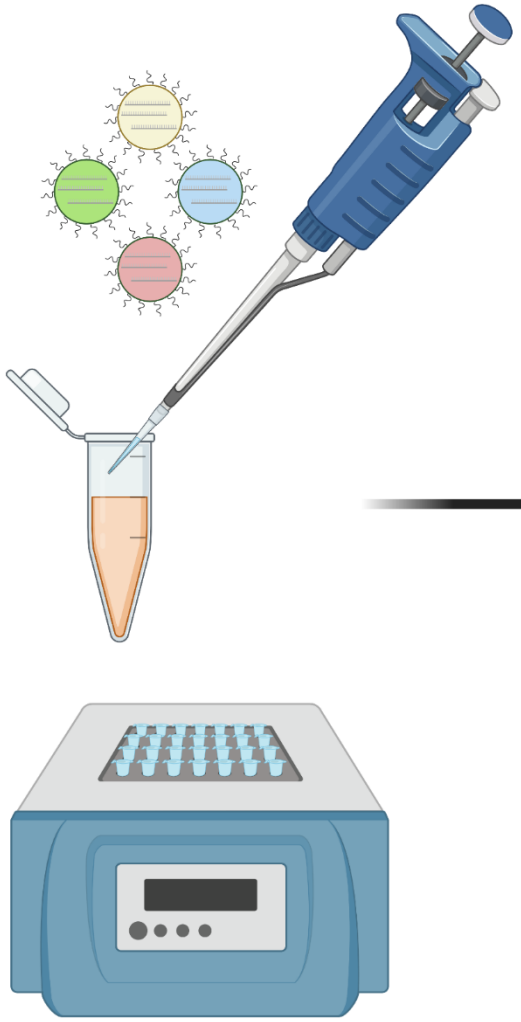


0.1 mg/kg mRNA

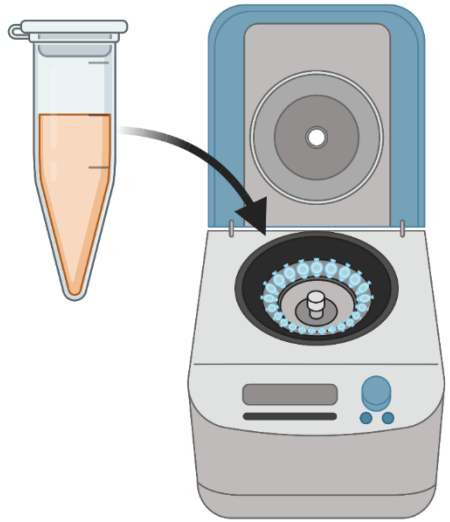
Do SORT LNPs have distinct protein corona compositions?



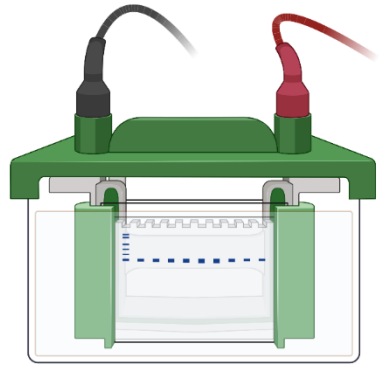
Density gradient centrifugation was used to isolate the protein corona of SORT LNPs incubated in plasma *ex vivo*



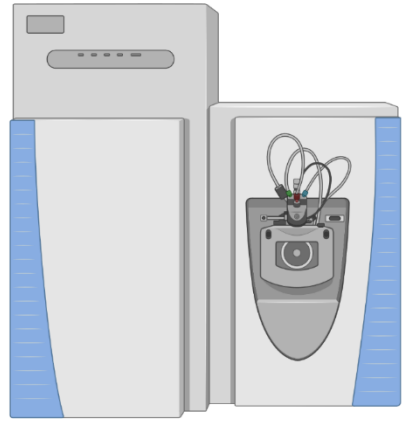
Incubation of SORT LNPs with mouse plasma



Centrifugation to isolate protein corona

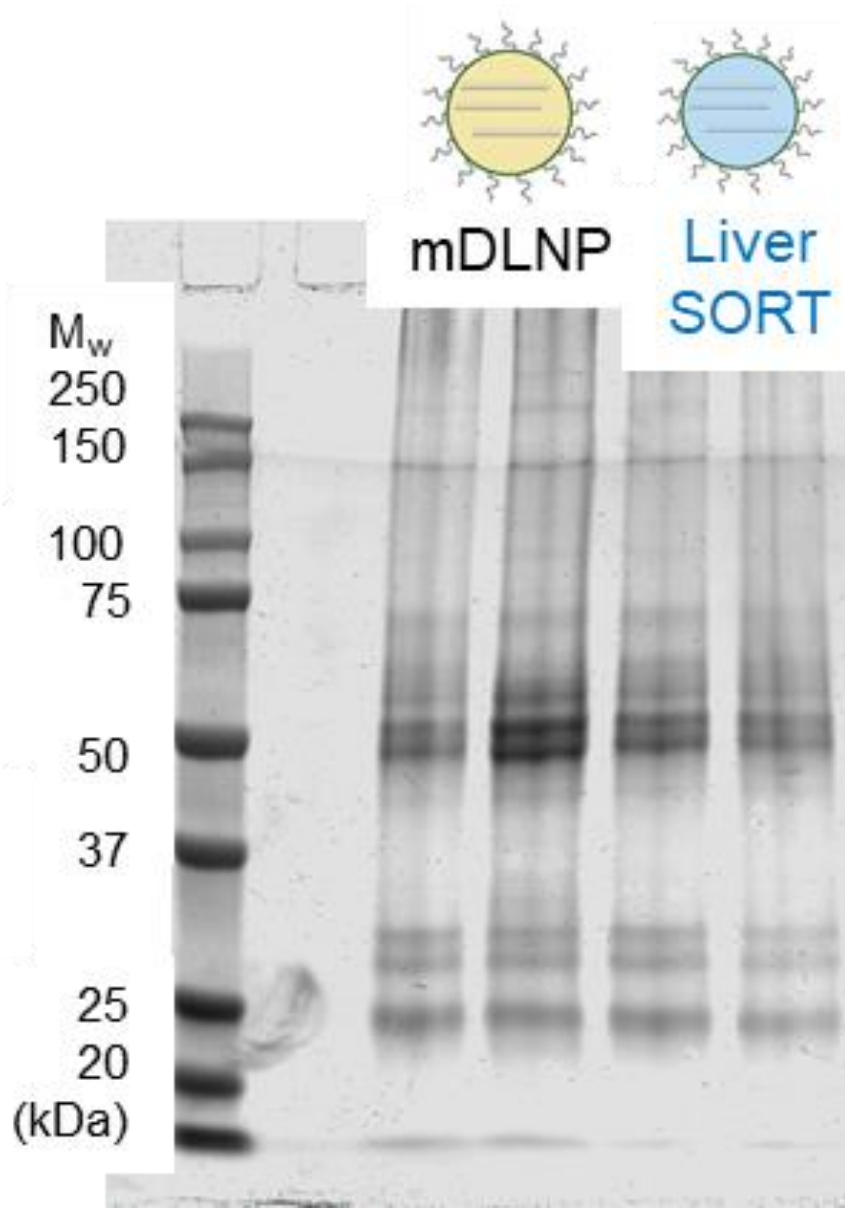


SDS PAGE

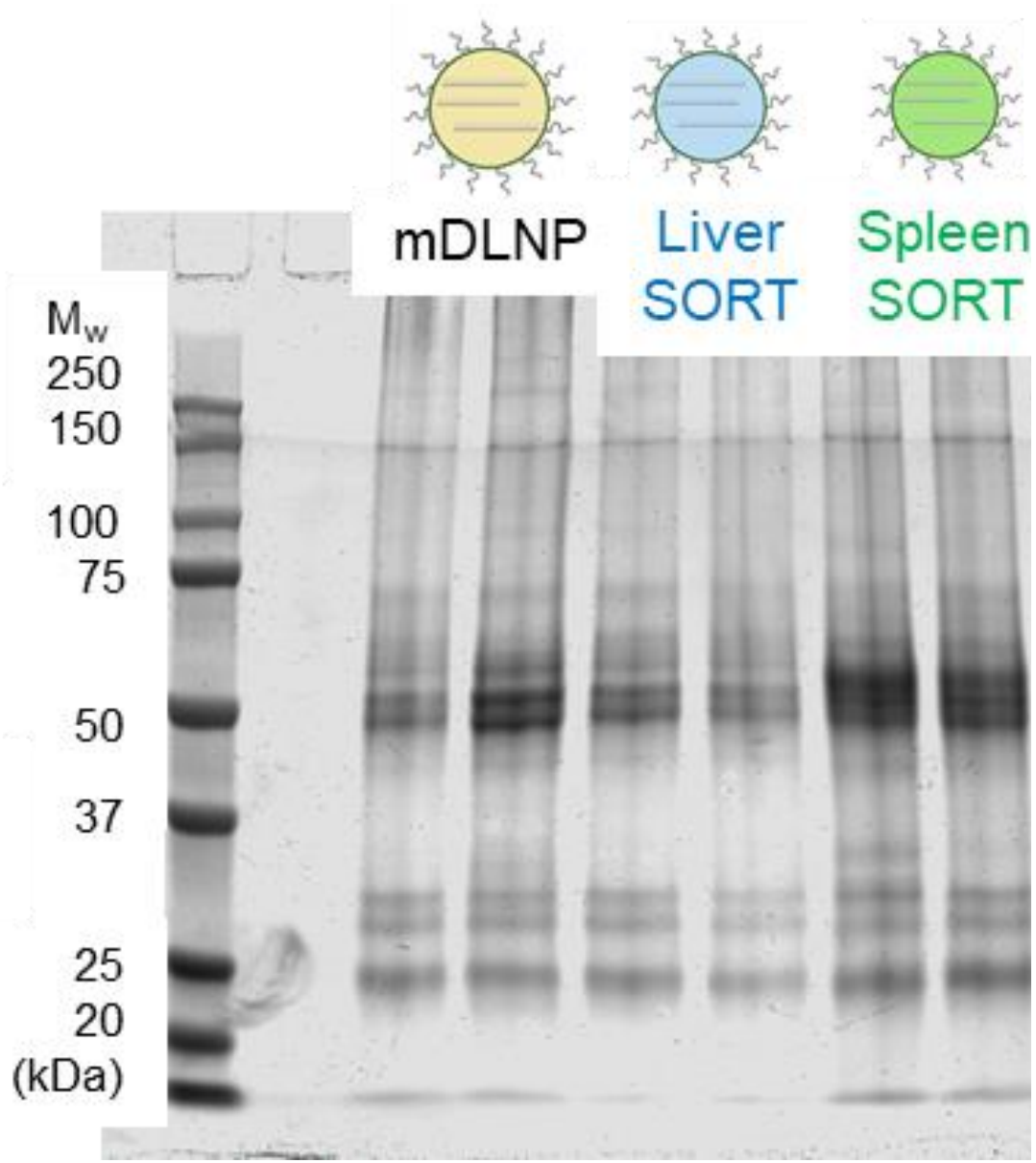


Mass Spectrometry Proteomics

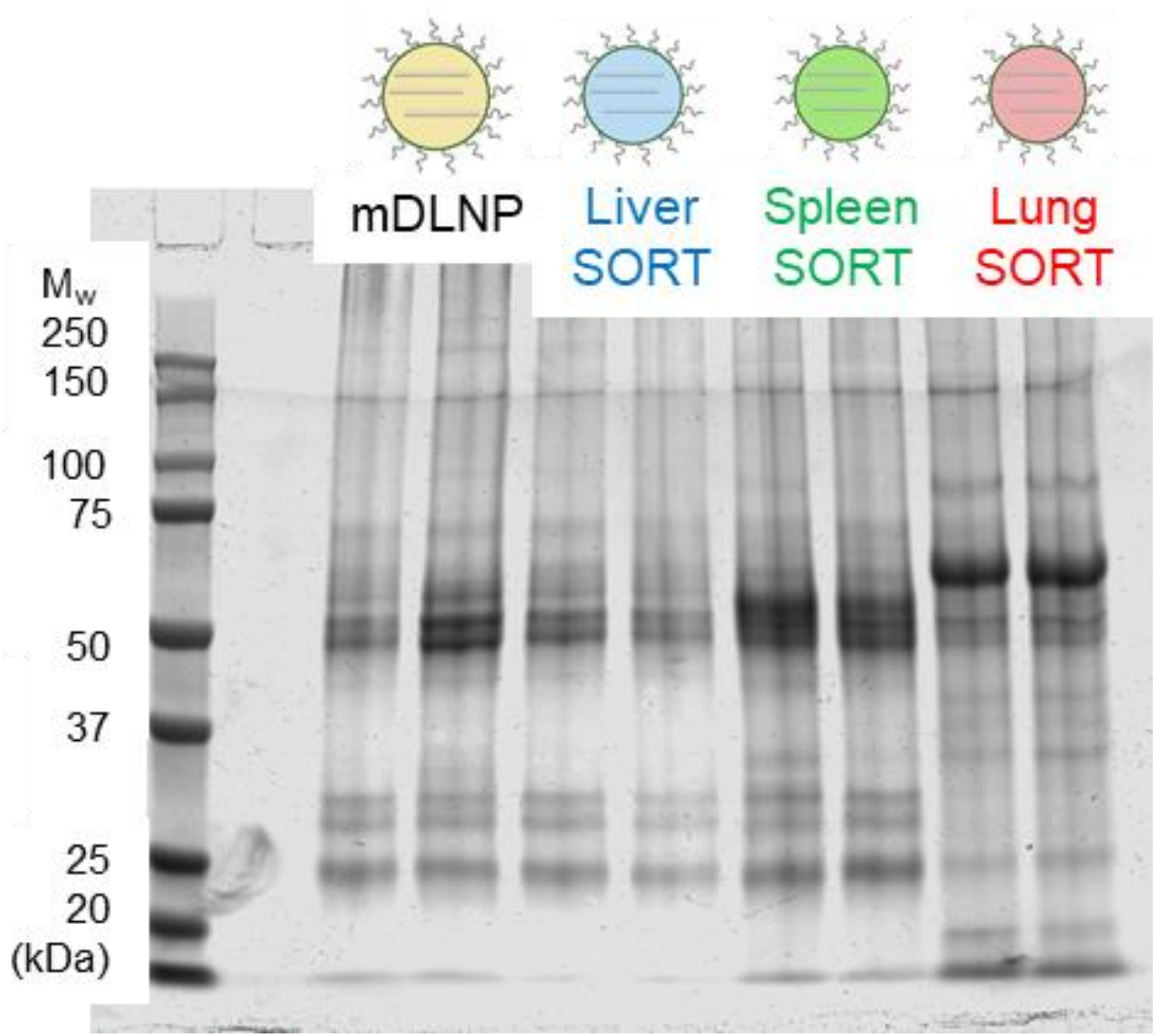
The choice of SORT molecule yields a unique protein corona



The choice of SORT molecule yields a unique protein corona

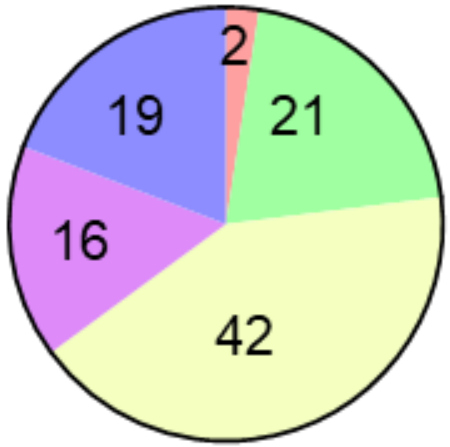


The choice of SORT molecule yields a unique protein corona

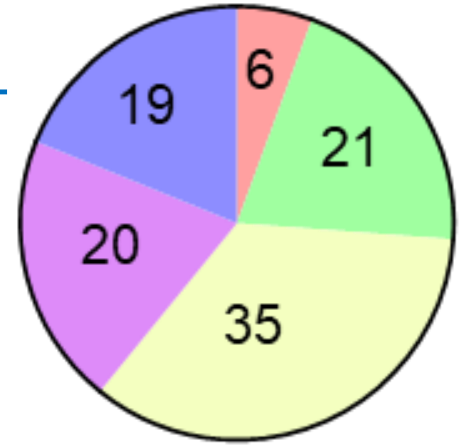


The functional composition of the protein corona depends on the choice of SORT molecule

mDLNP



Liver SORT

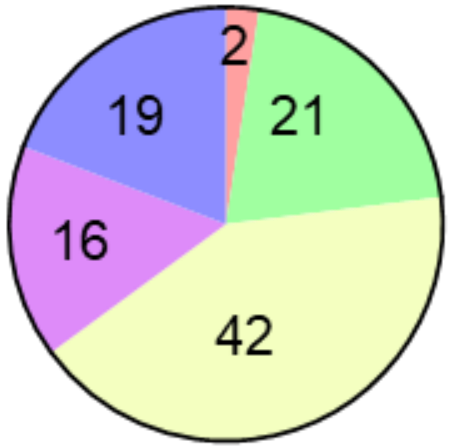


Apolipoproteins
Coagulation Proteins
Complement Proteins
Immune Proteins
Other

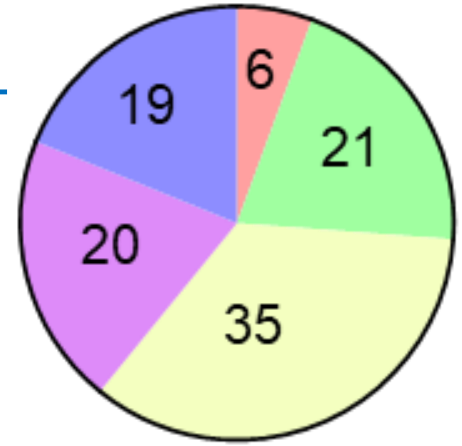
n = 3, top 80% of all adsorbed plasma proteins

The functional composition of the protein corona depends on the choice of SORT molecule

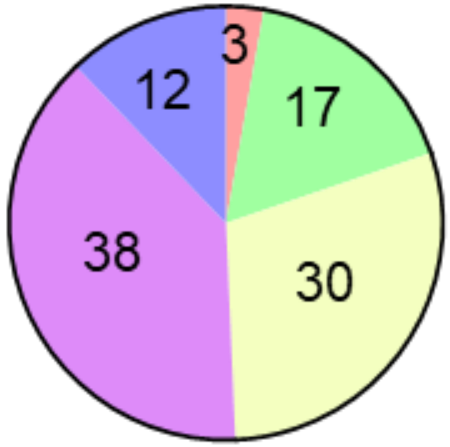
mDLNP



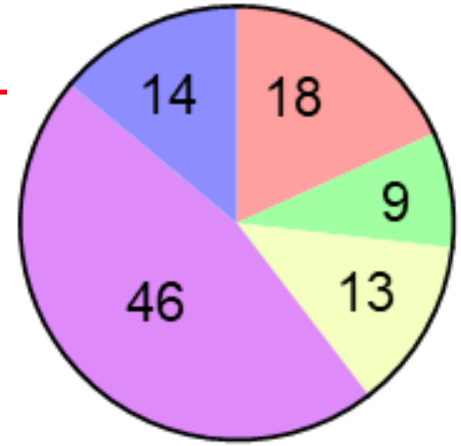
Liver SORT



Spleen SORT



Lung SORT



Apolipoproteins
Coagulation Proteins
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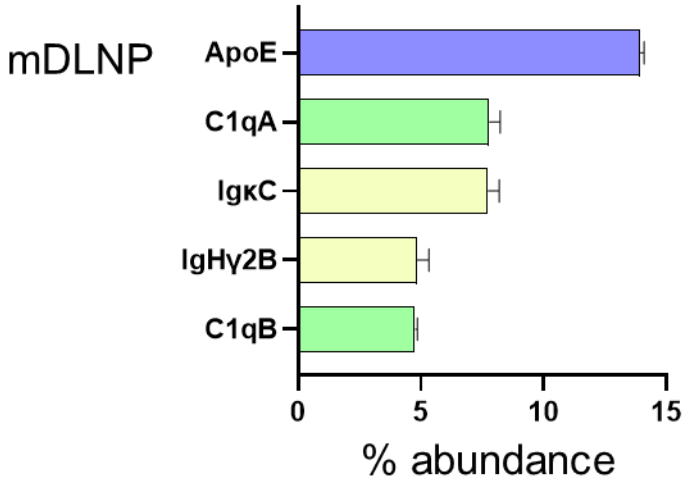
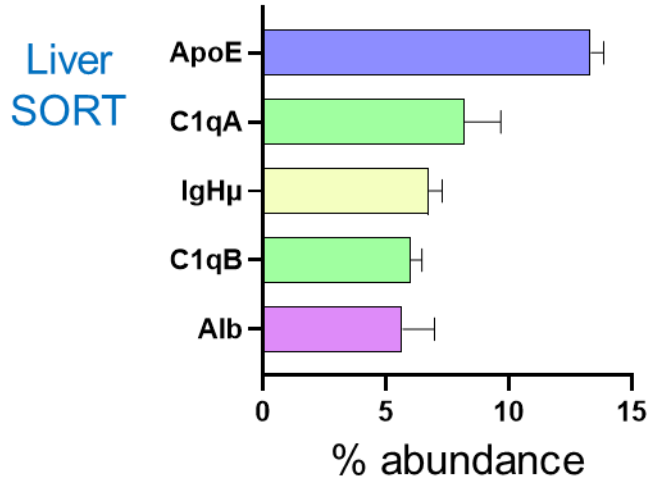
n = 3, top 80% of all adsorbed plasma proteins

The serum proteins which most avidly bind an LNP depends on the chemical structure of a given SORT molecule





n = 3, top 80% of all adsorbed plasma proteins

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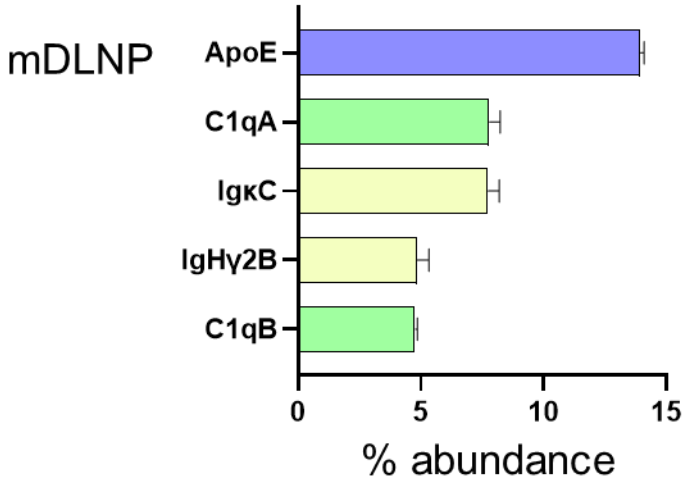
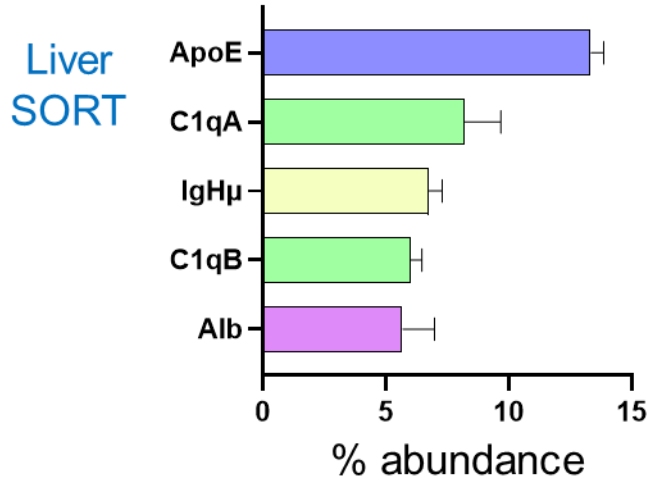
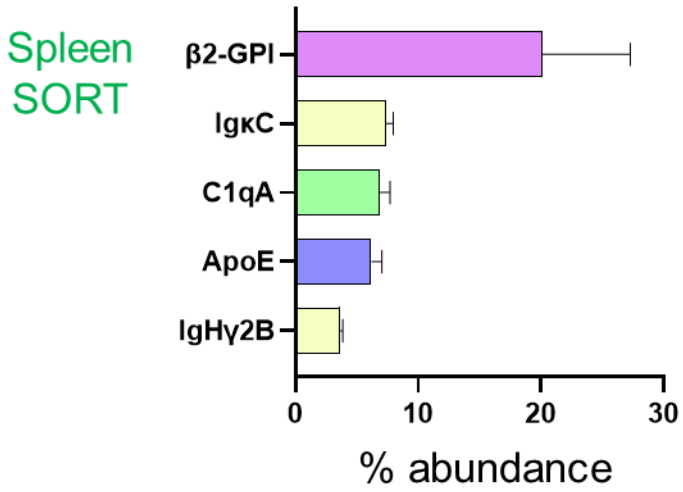
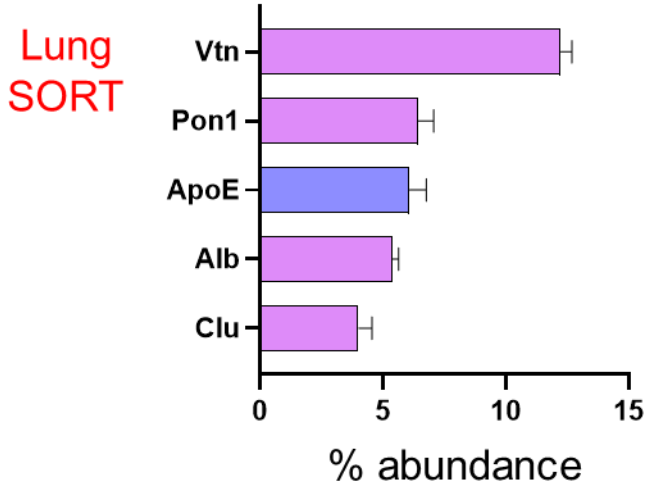


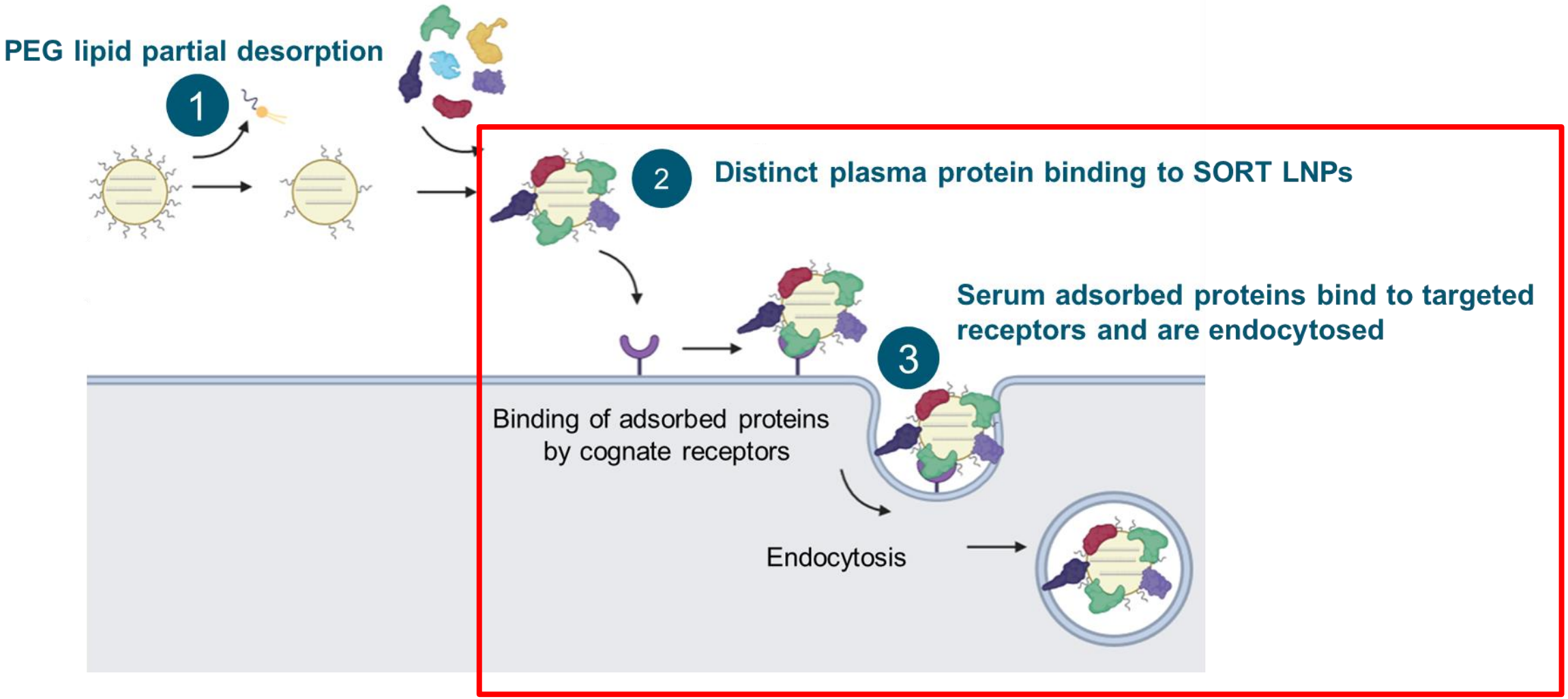




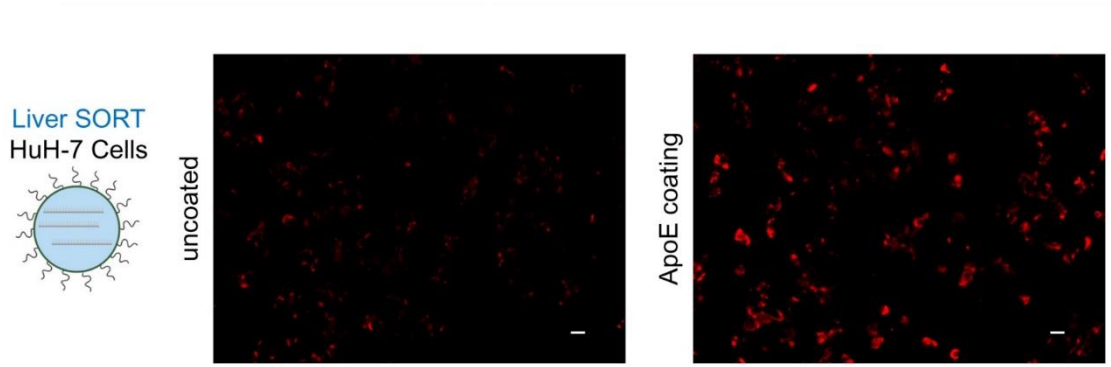


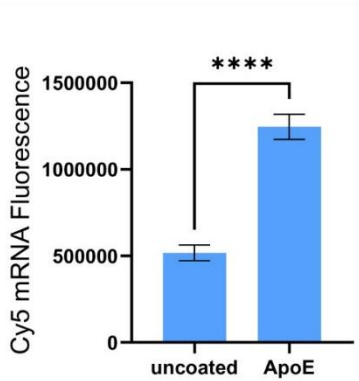
n = 3, top 80% of all adsorbed plasma proteins

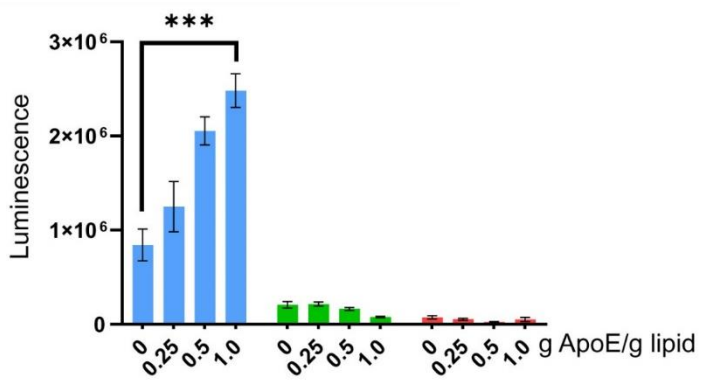
Do bound serum proteins impact receptor-mediated uptake and delivery?



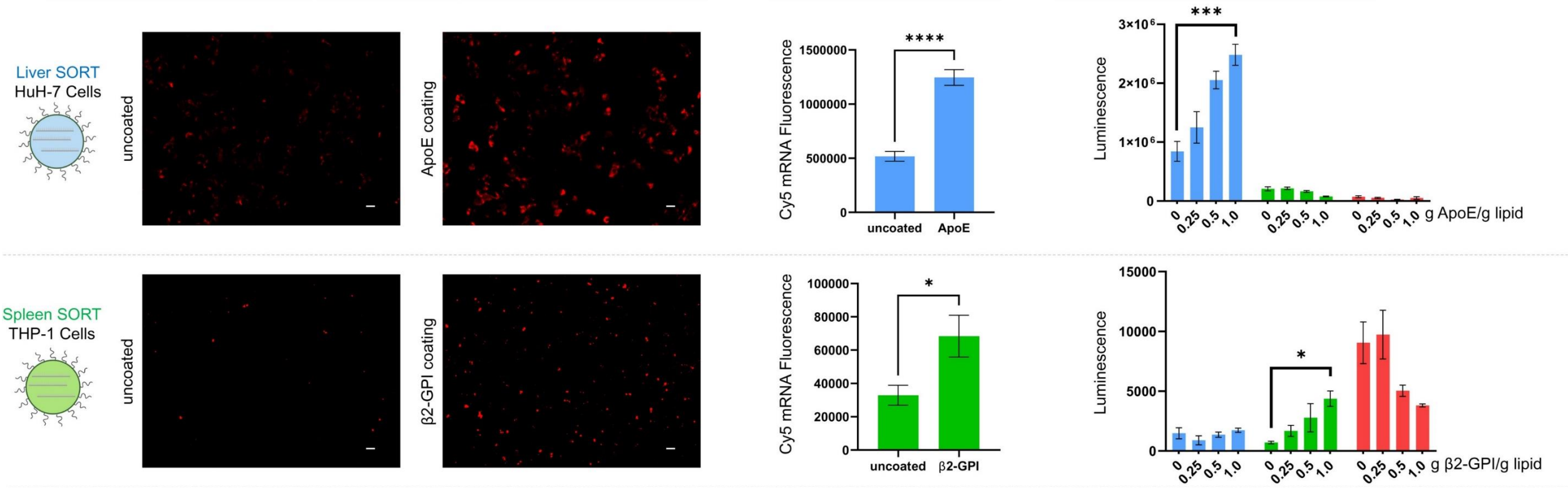
Key proteins selectively enhance SORT LNP uptake and delivery to cells highly expressing their cognate receptor







Key proteins selectively enhance SORT LNP uptake and delivery to cells highly expressing their cognate receptor



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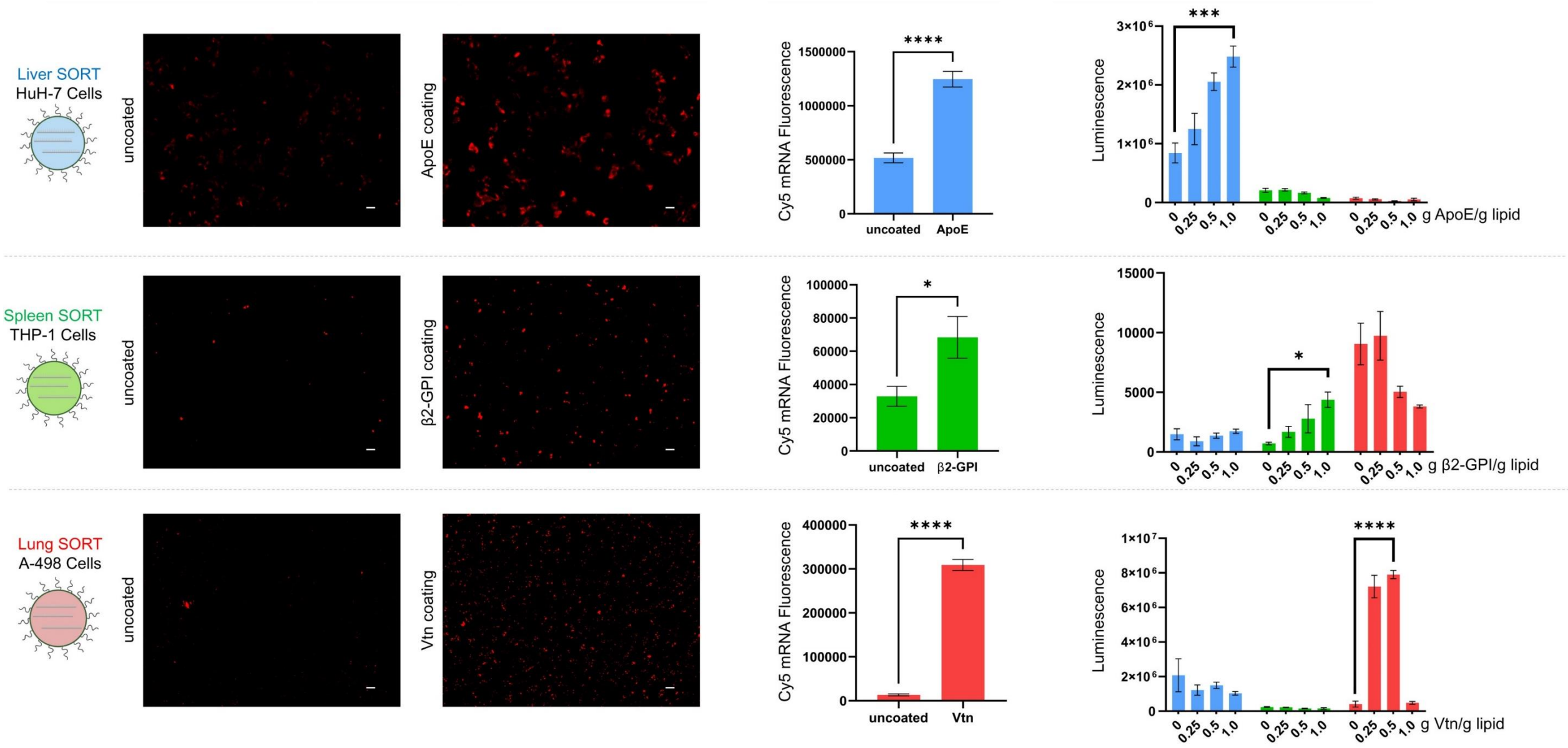
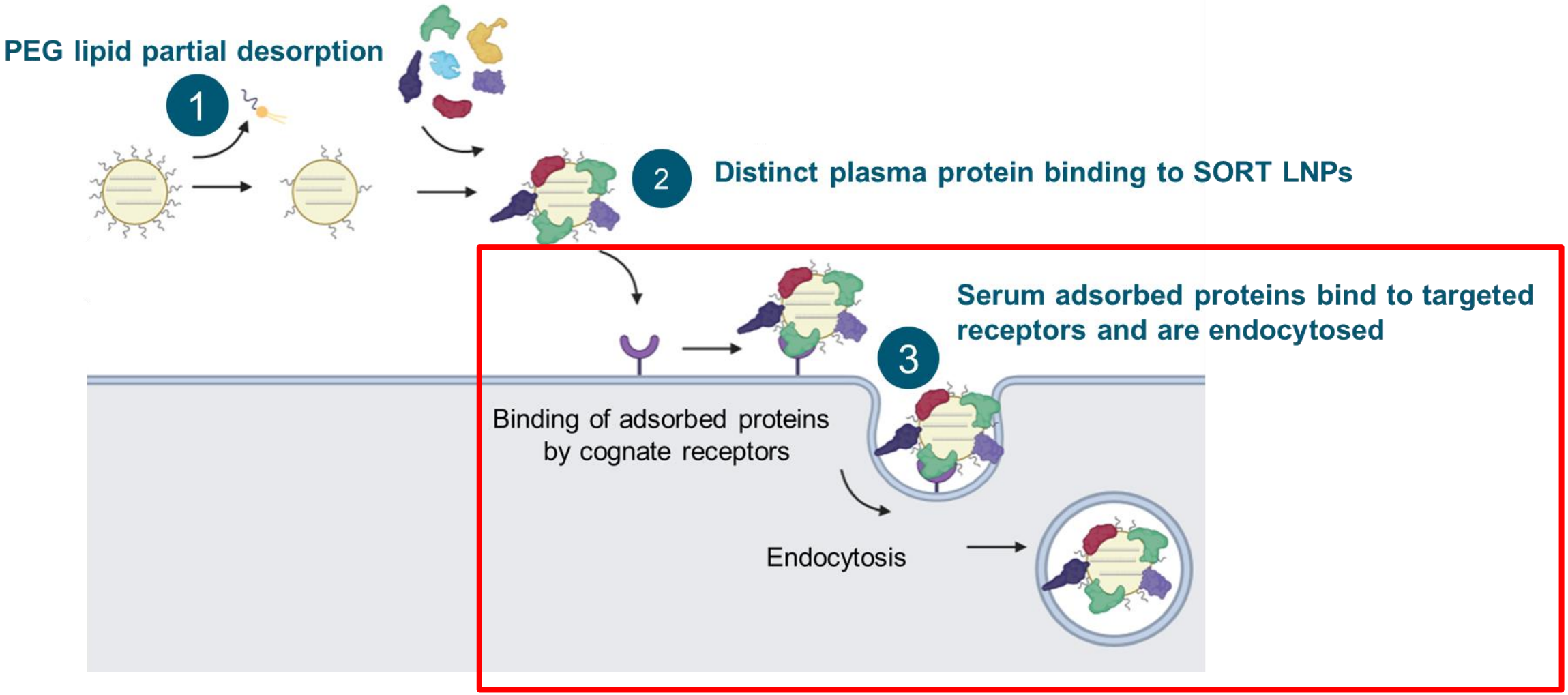
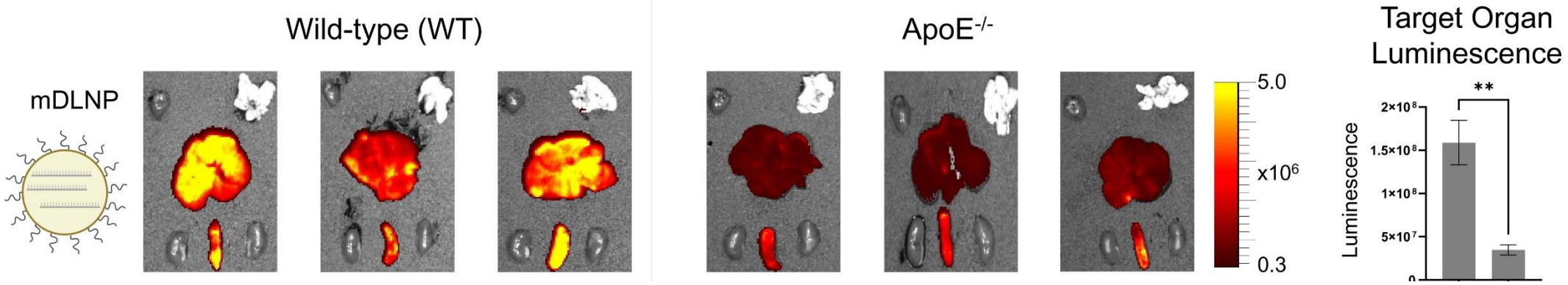


Does the inclusion of a SORT molecule impact ApoE-dependent delivery?



Extrahepatic mRNA delivery occurs via an ApoE-independent mechanism



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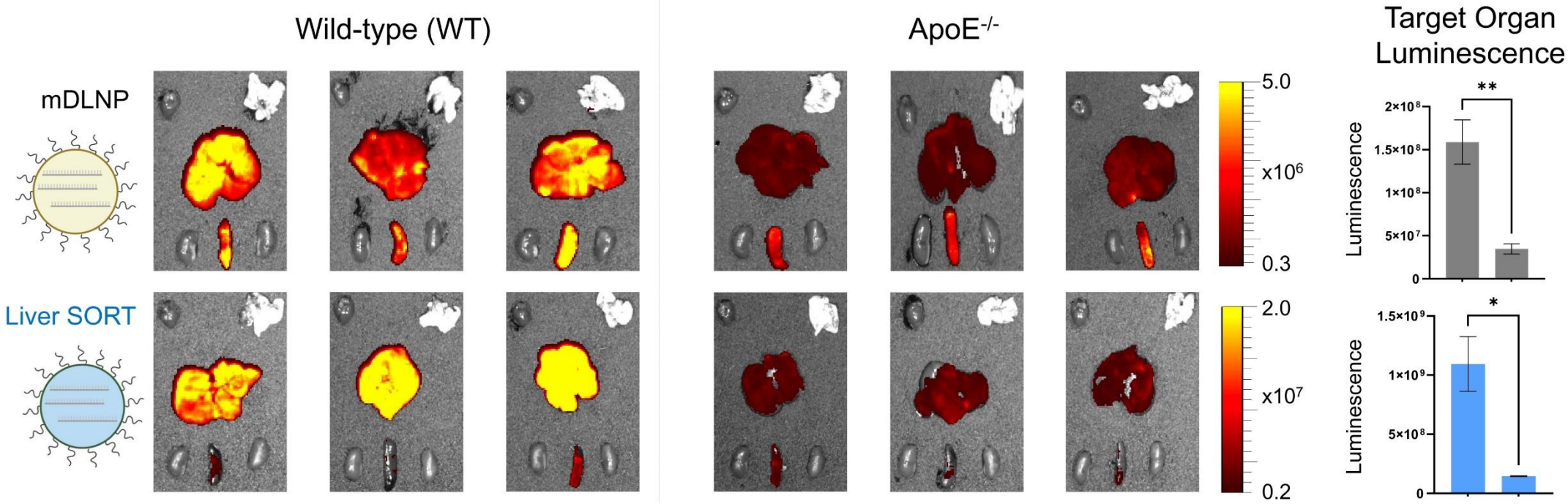

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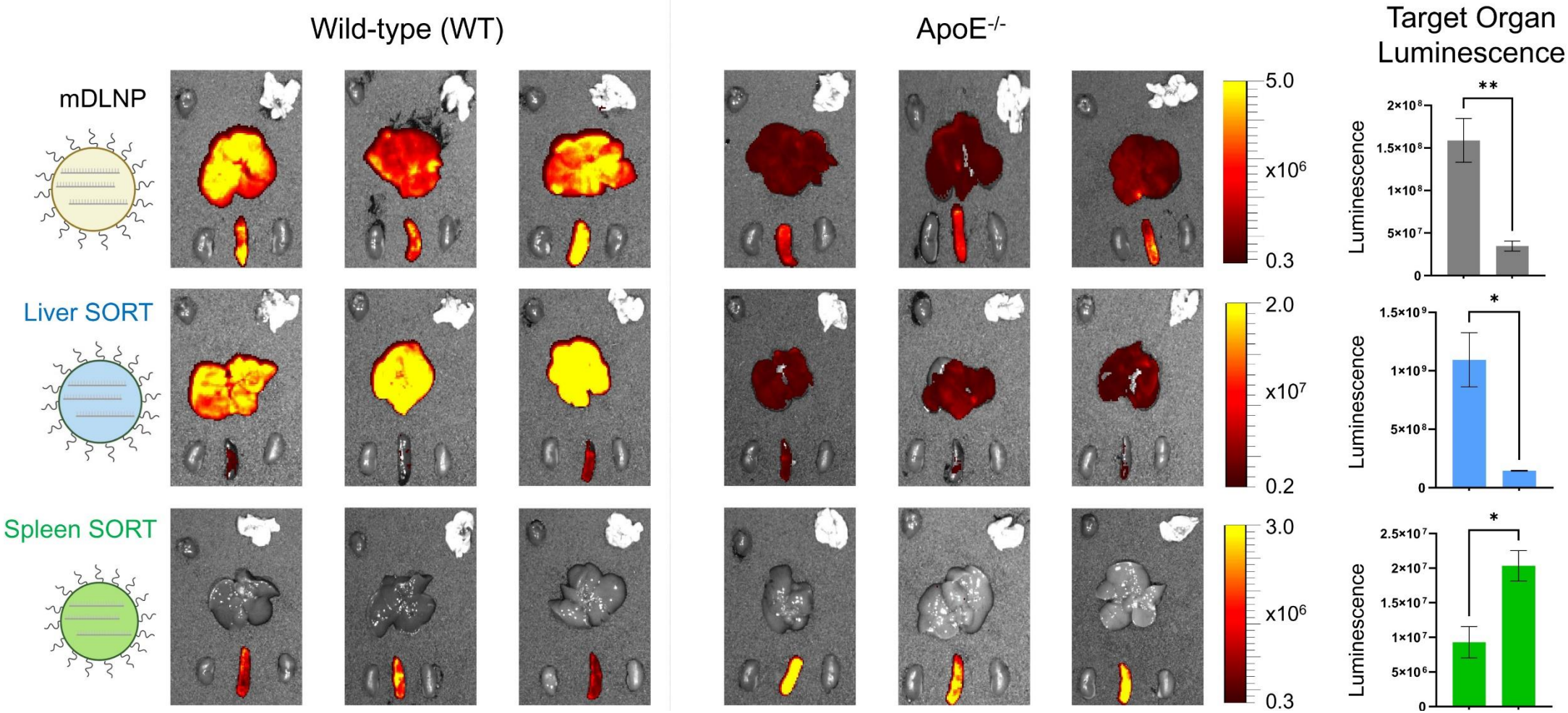

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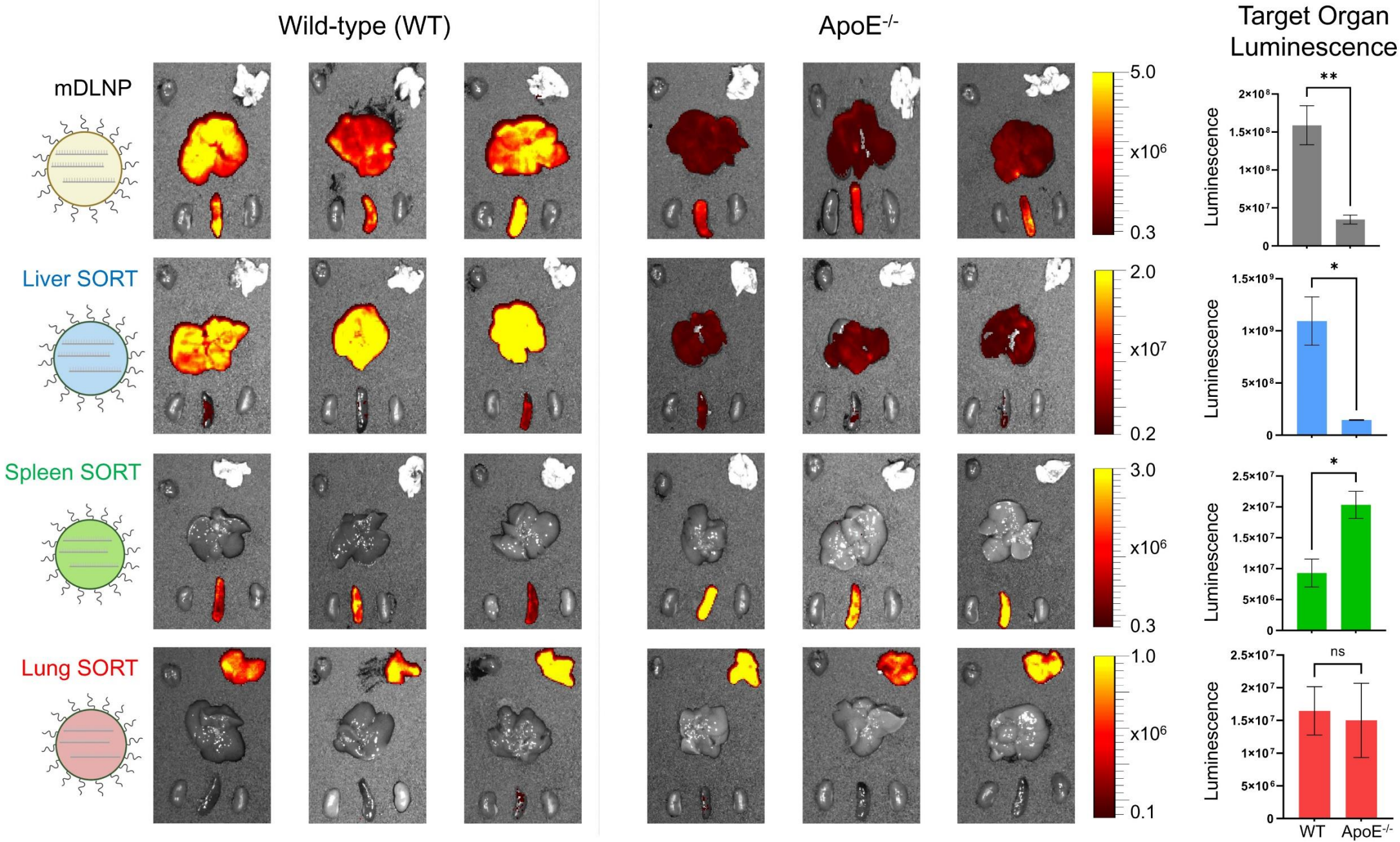
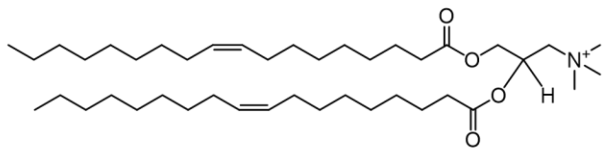


A library of permanently cationic quaternary ammonium lipids were incorporated into LNPs as SORT molecules at 50 mol. %



18:1 TAP

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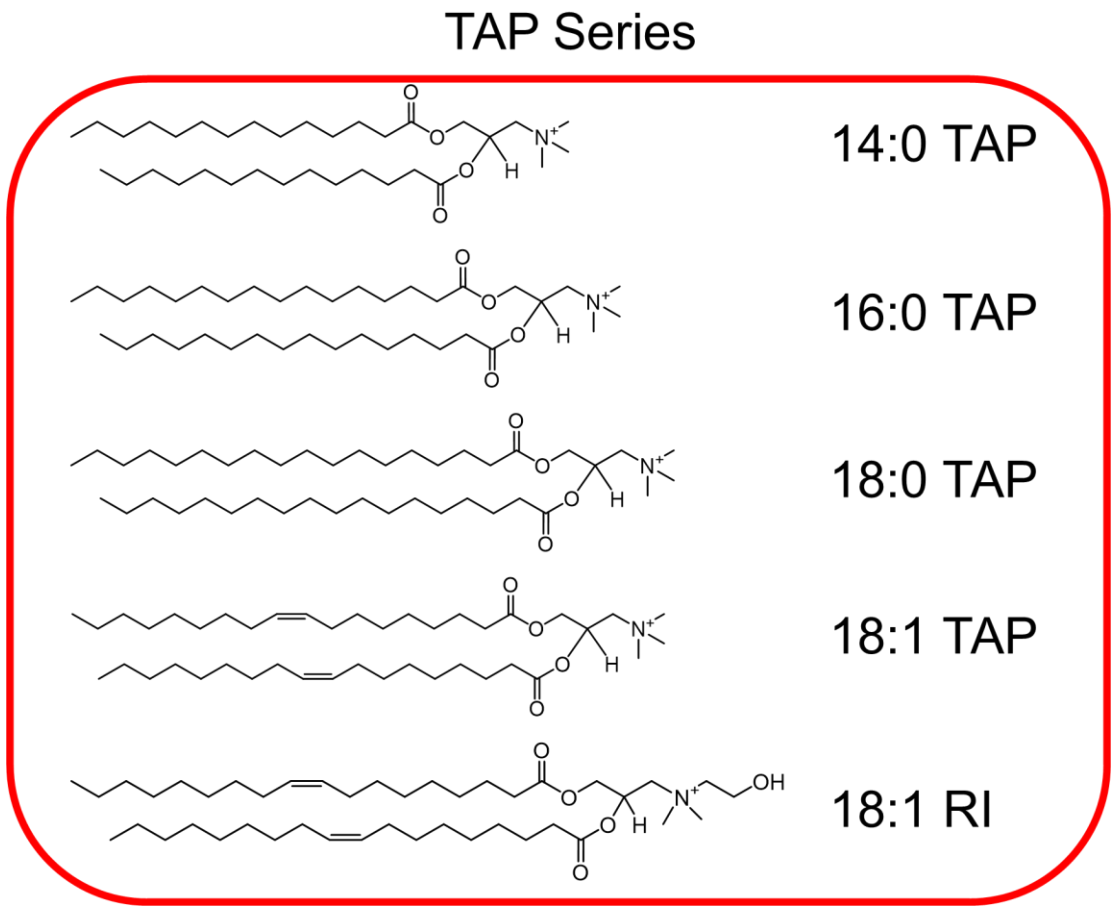

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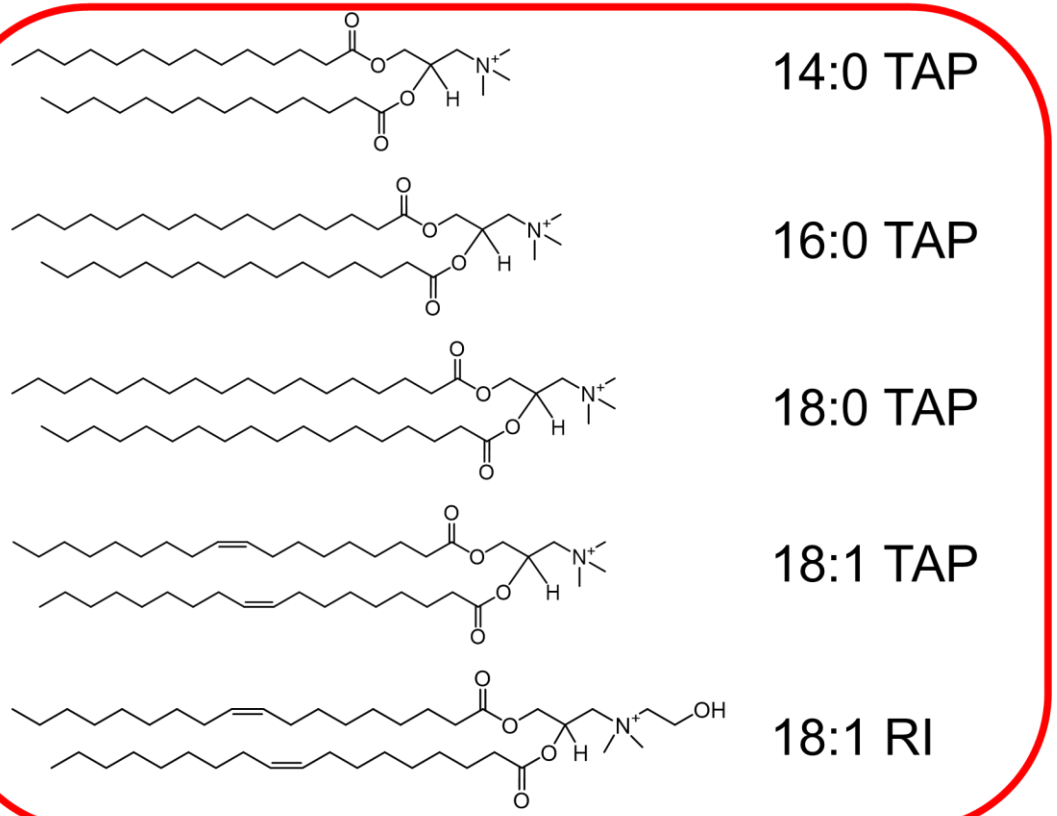
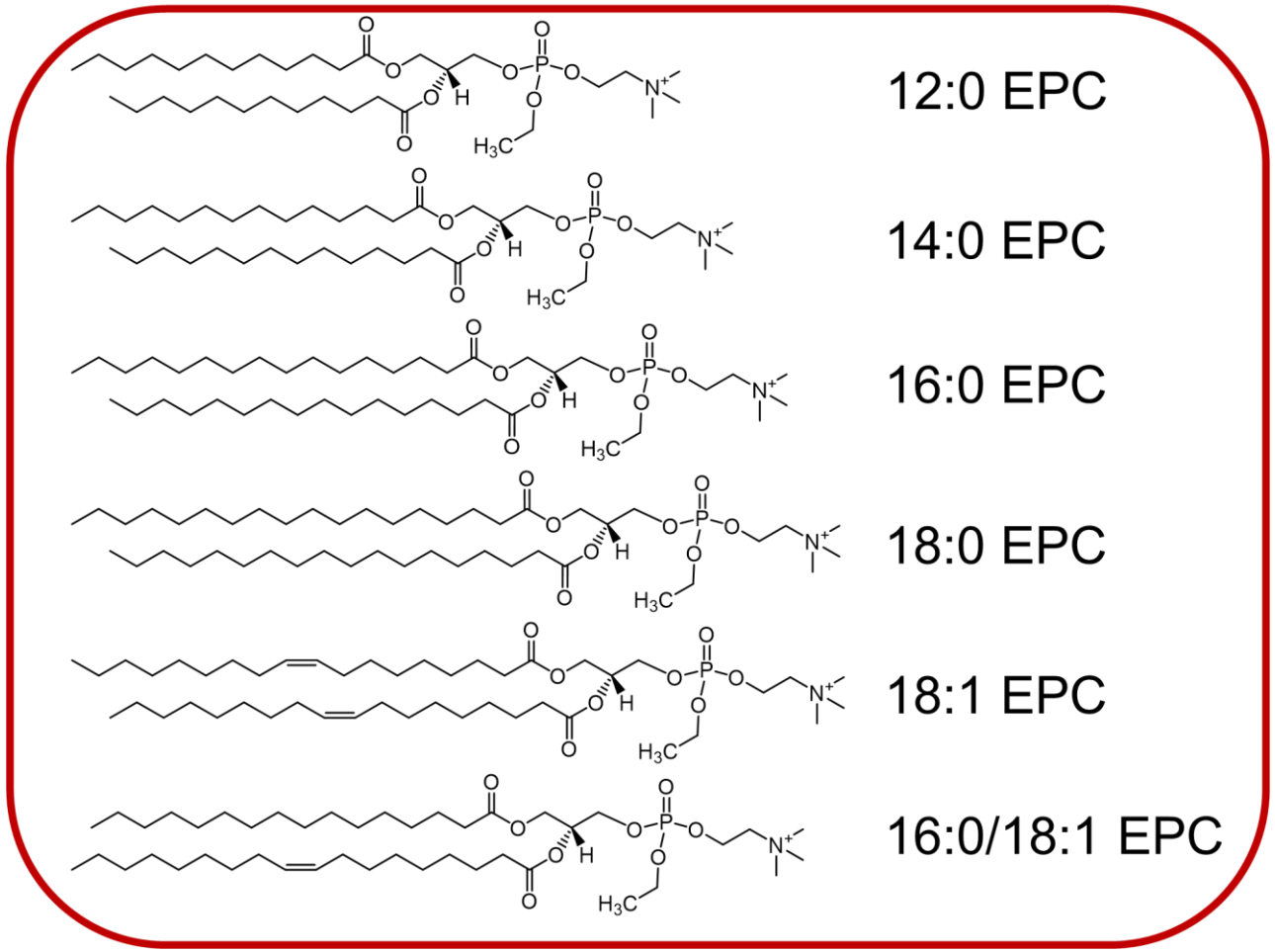
TAP Series



EPC Series



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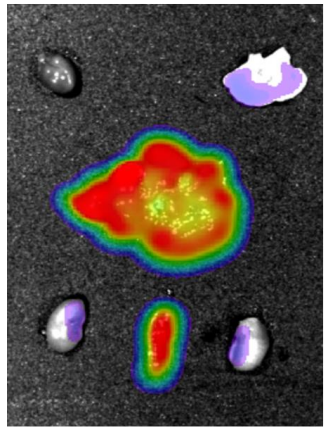
All quaternary ammonium SORT molecules enable mRNA delivery to the lungs via intravenous administration

mDLNP



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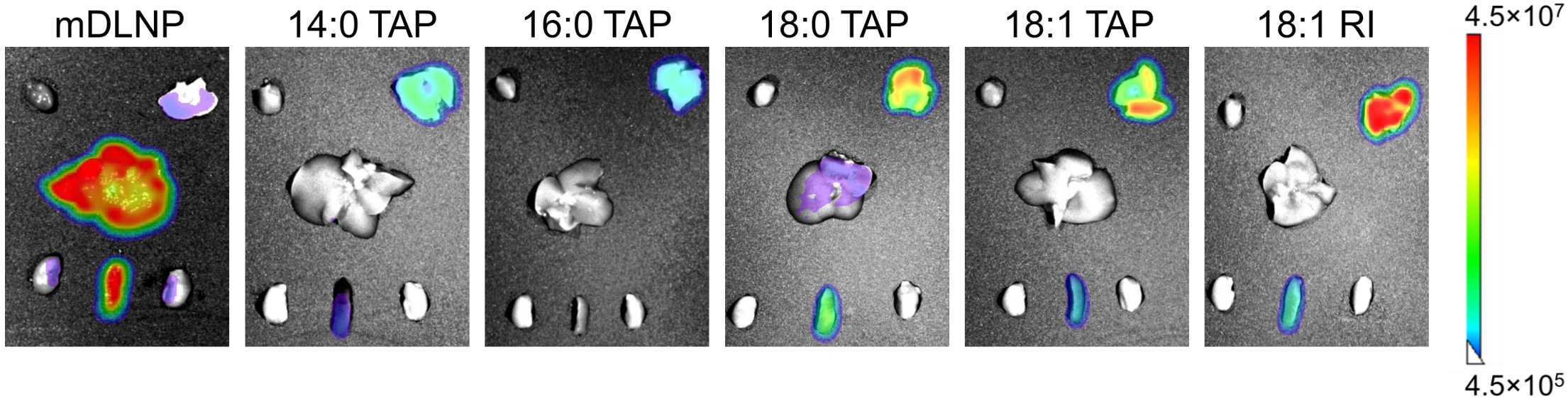

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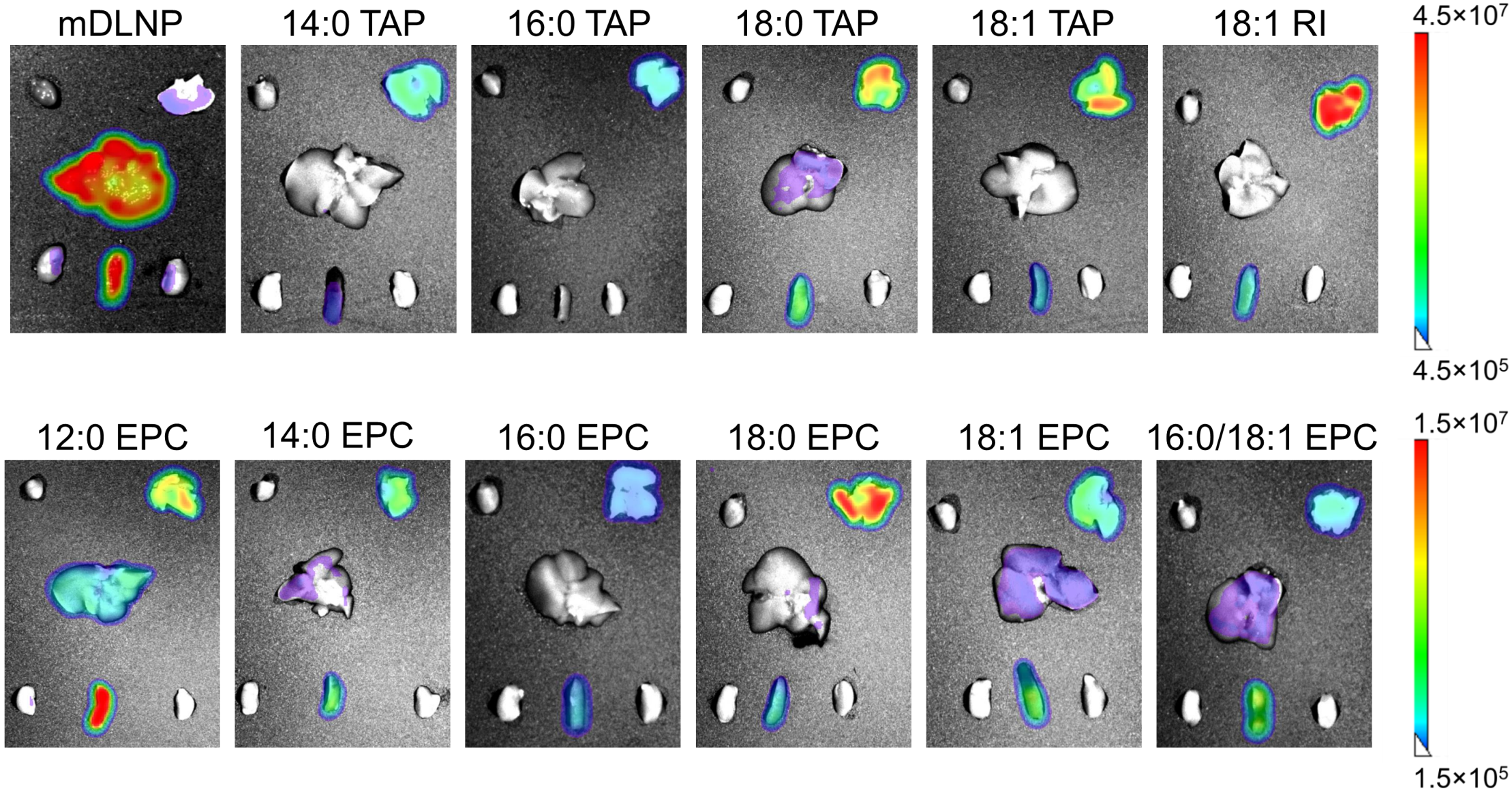
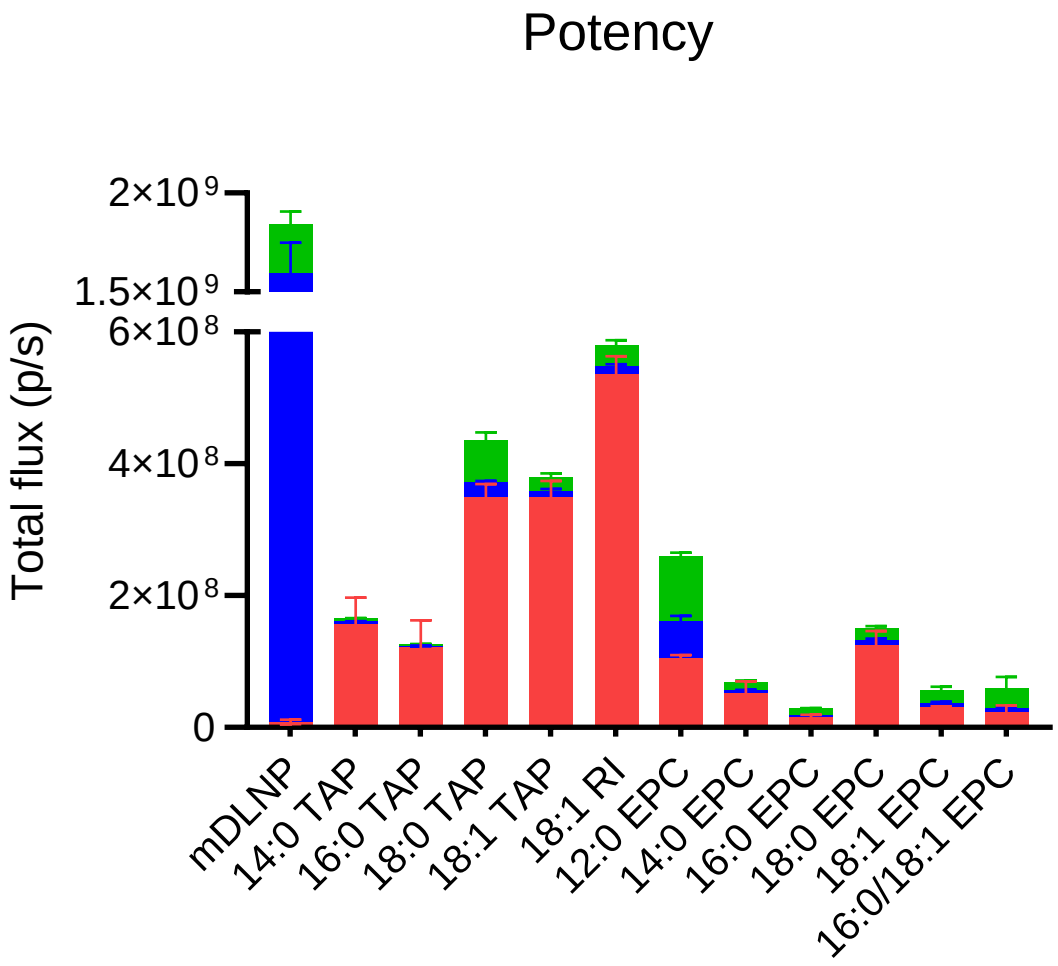


SORT molecule chemical structure impacts both the potency and tissue-selectivity of mRNA delivery



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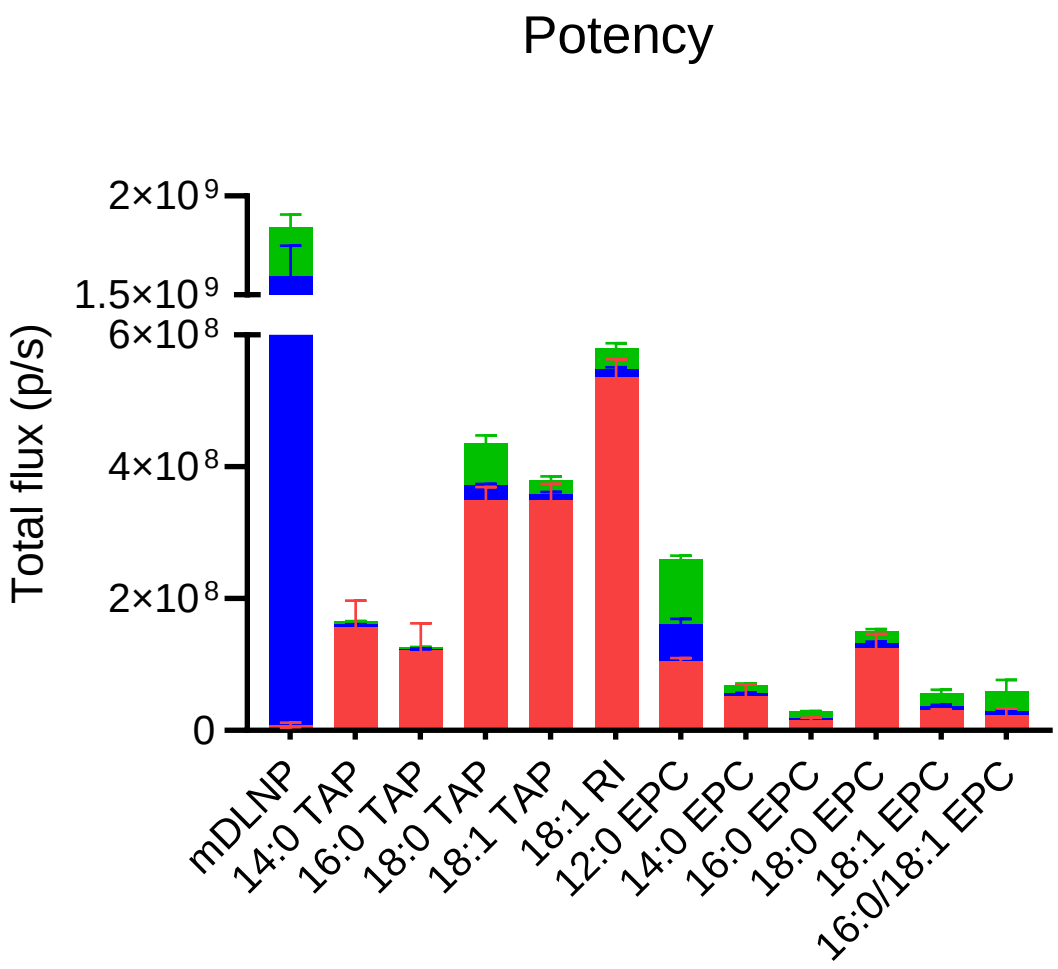
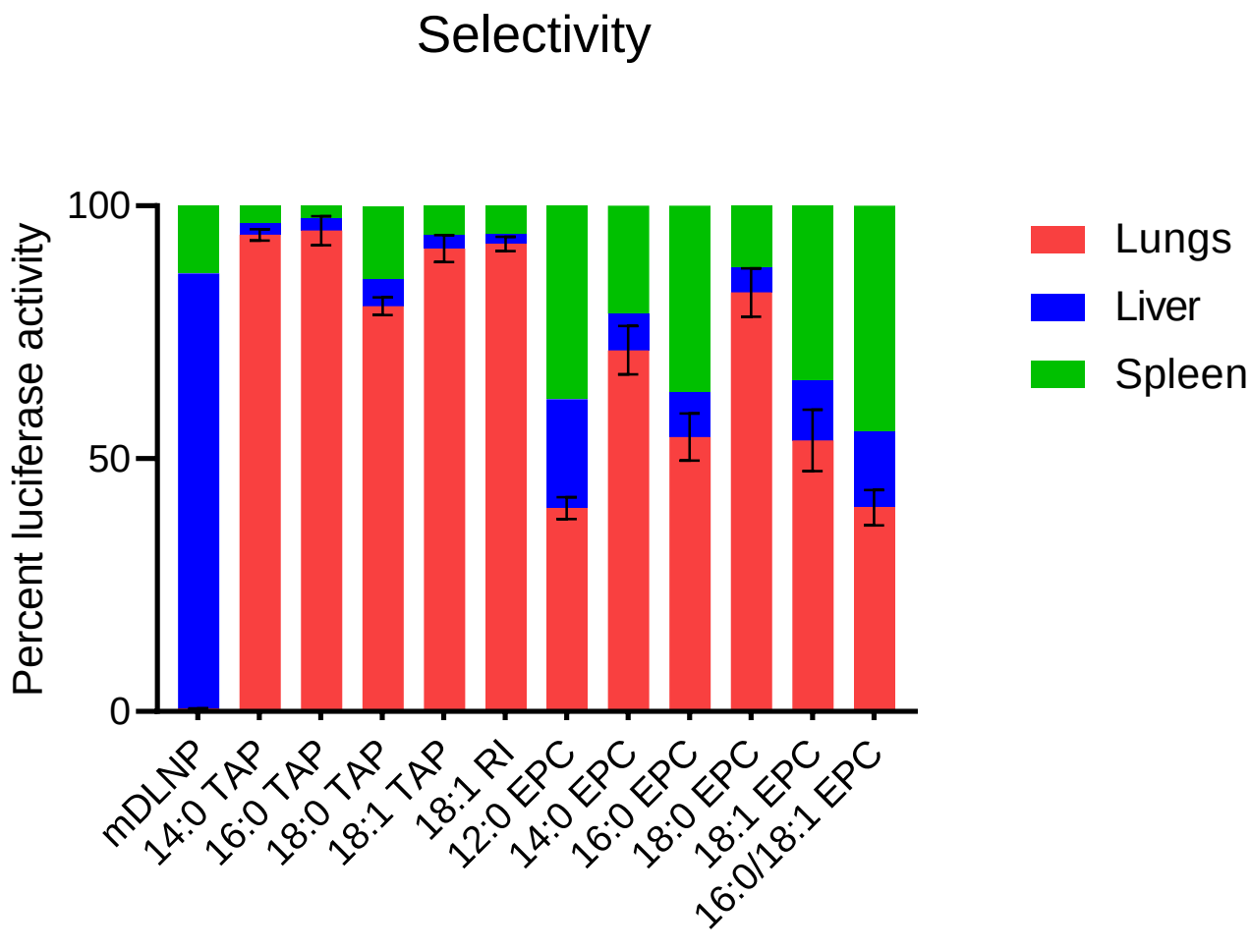
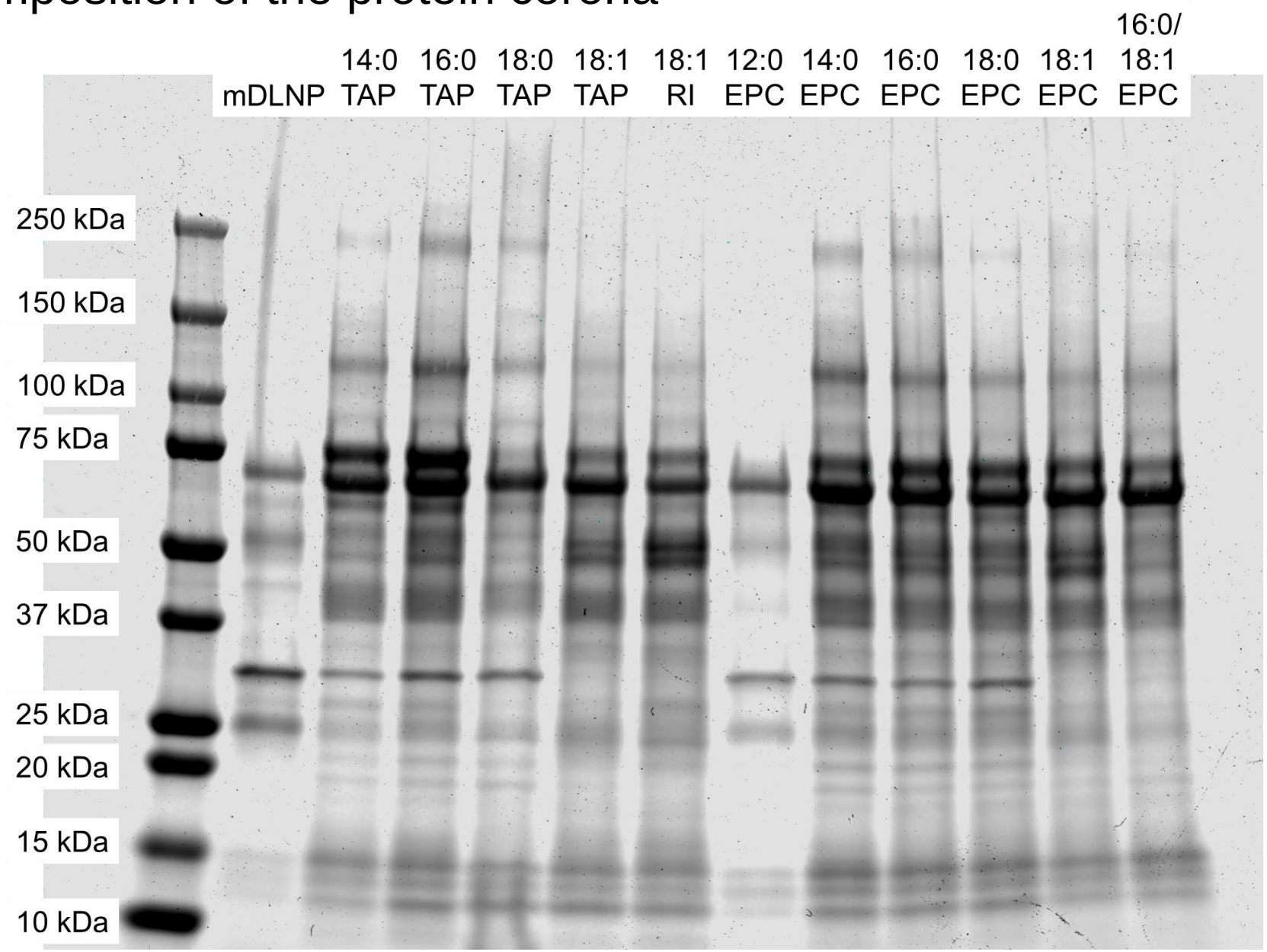
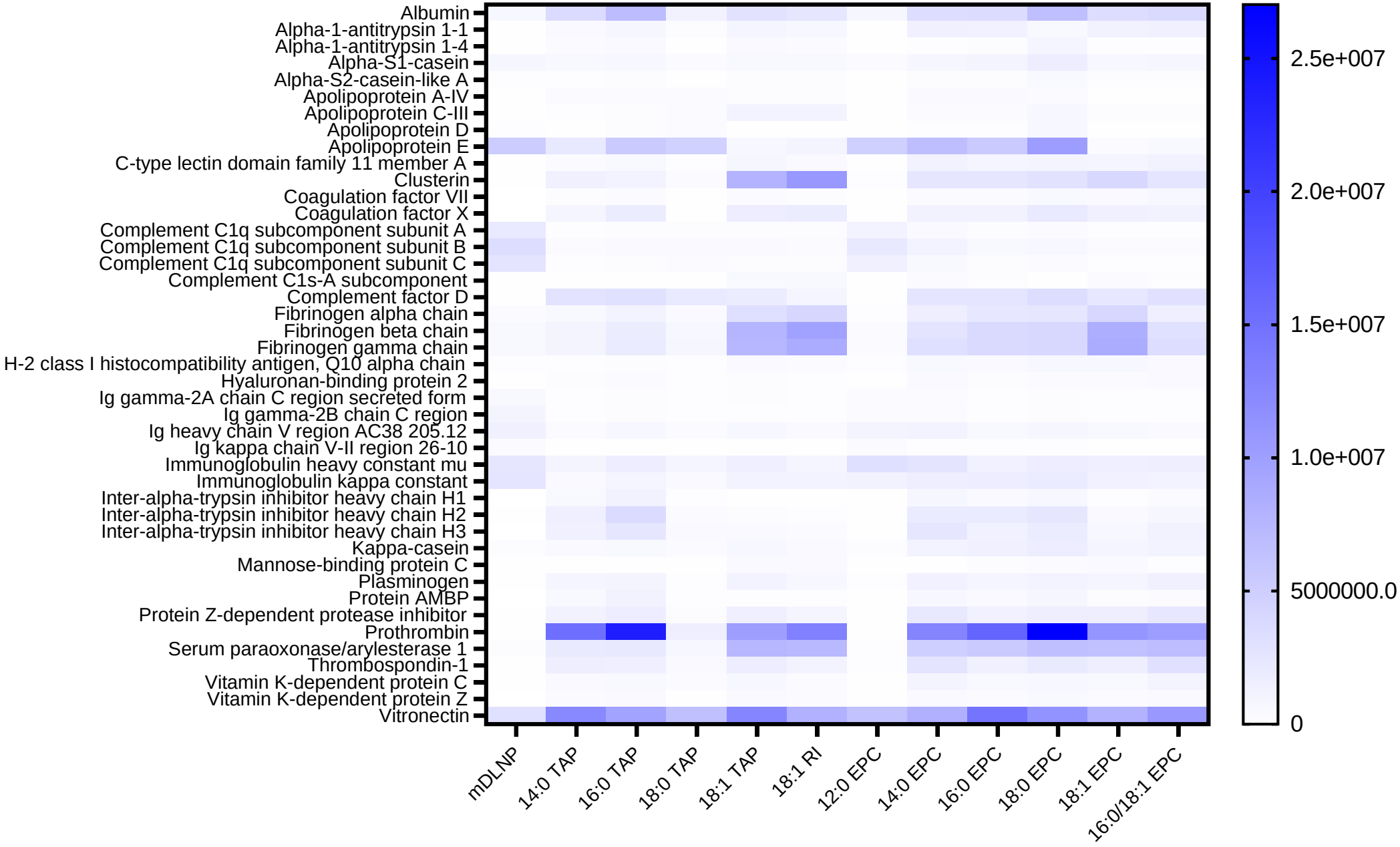




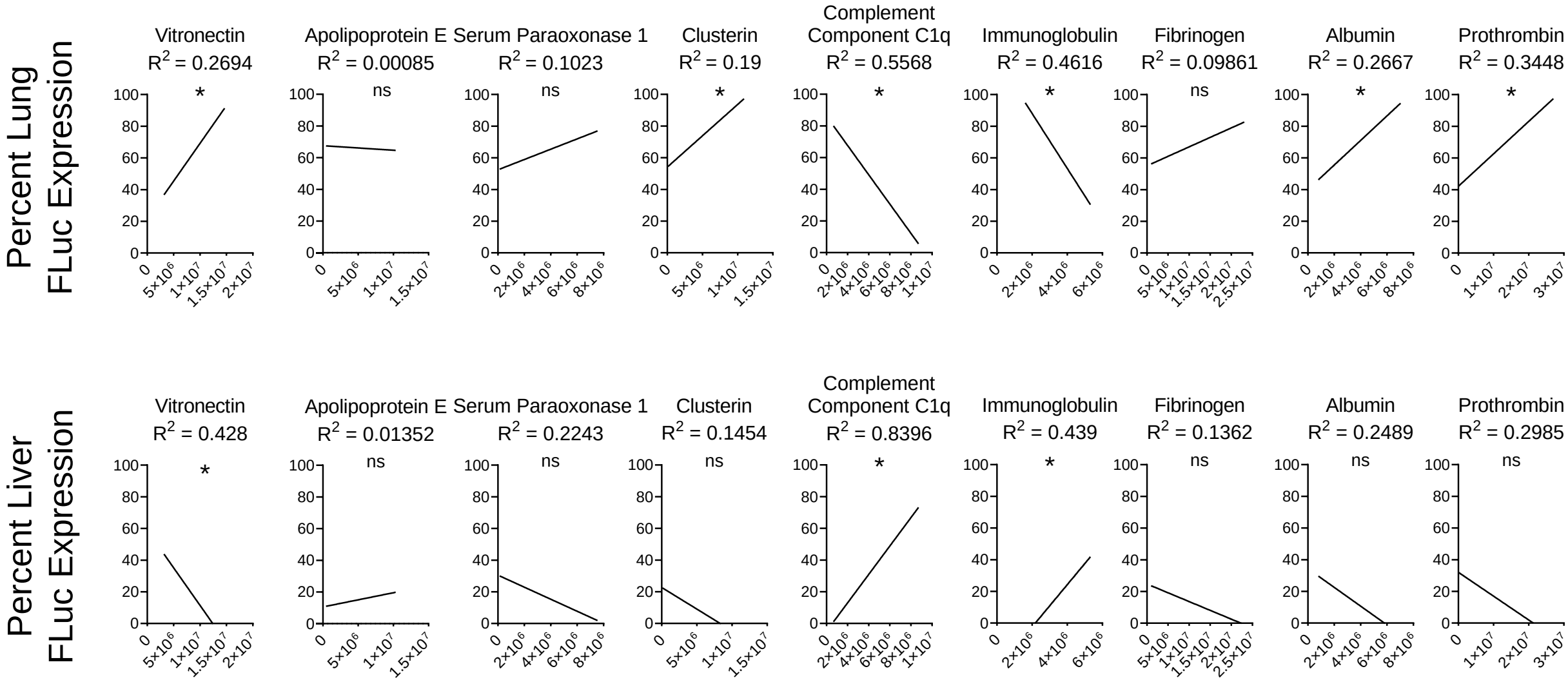
Differences in SORT molecule chemical structure within the same biophysical class can impact the composition of the protein corona



Different SORT molecules alter the identities and quantities of corona proteins



Alterations in protein corona are correlative with delivery outcomes



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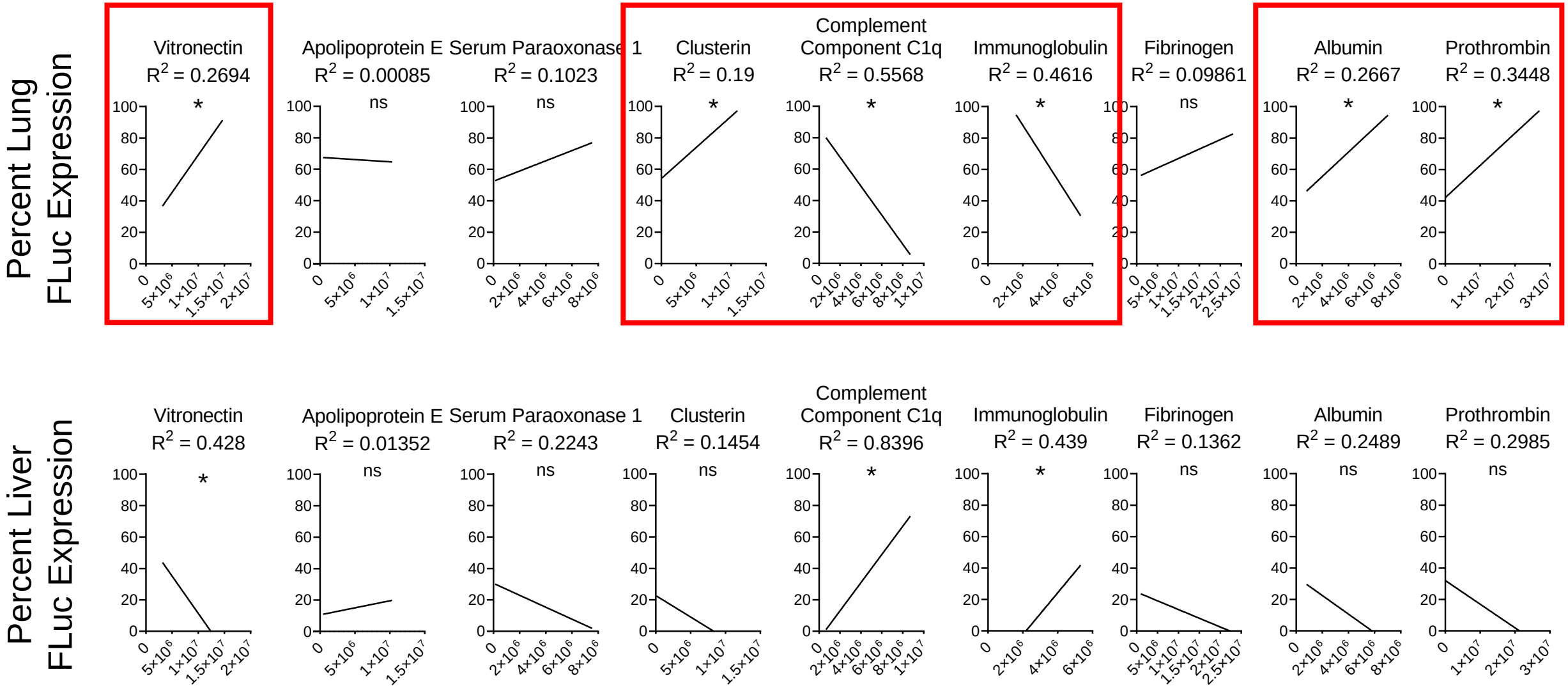

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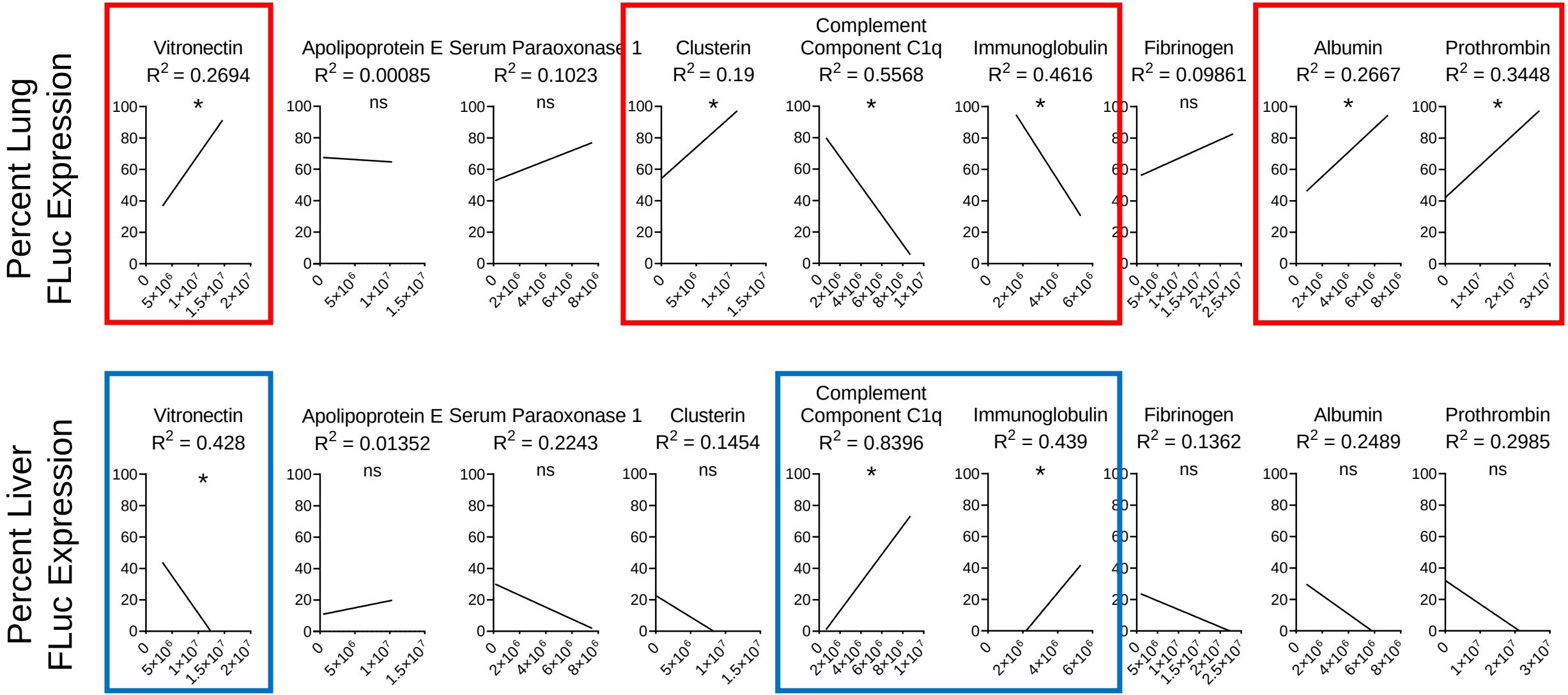

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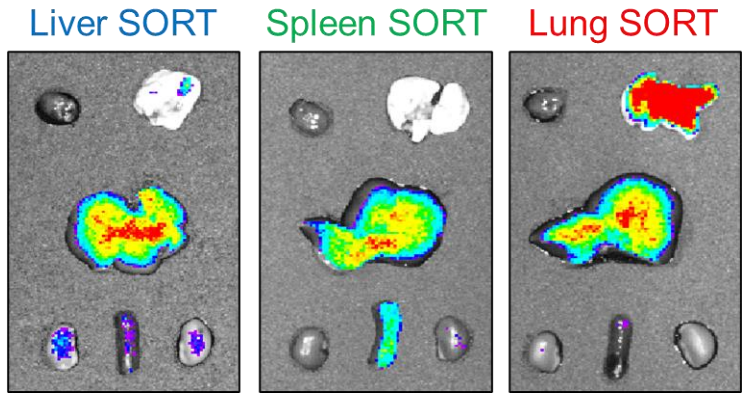
Key findings:

SORT molecules shift LNP biodistribution to the target organ



Sean Dilliard et al. **PNAS** *52,* e2109256118 (2021).

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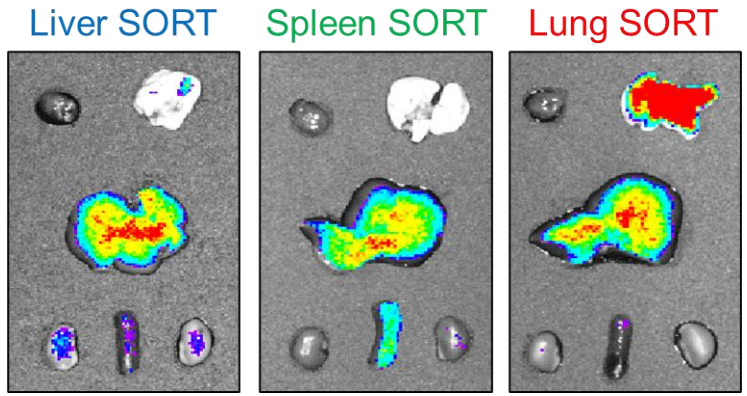
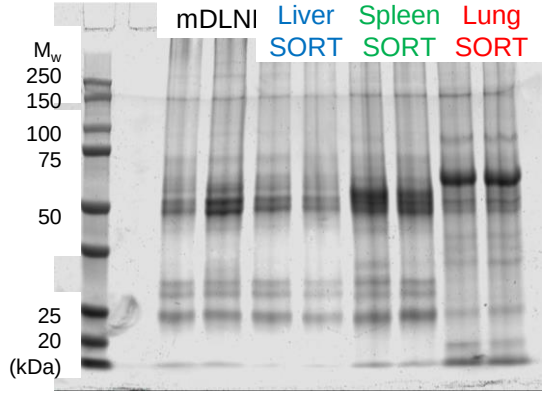


Distinct protein coronas enable endogenous targeting of specific organs



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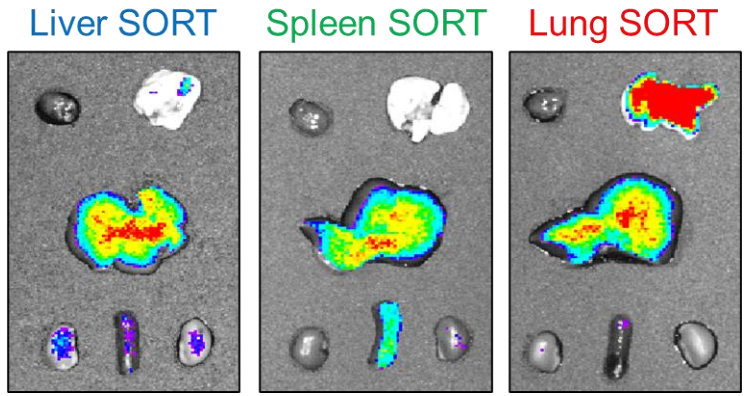

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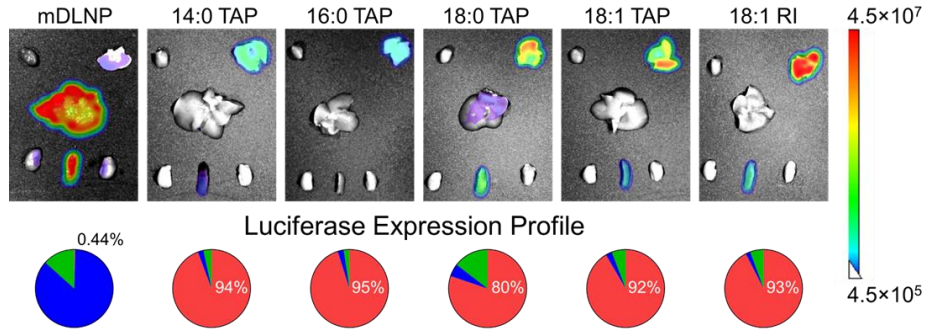

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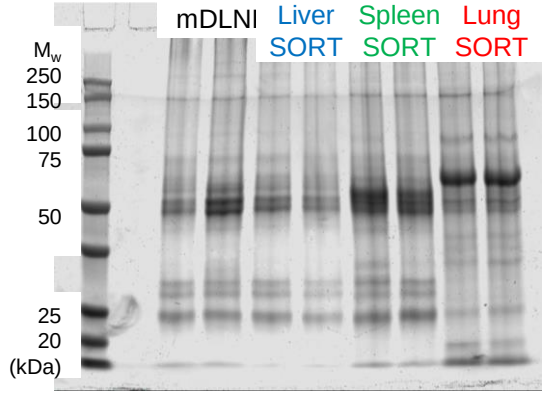

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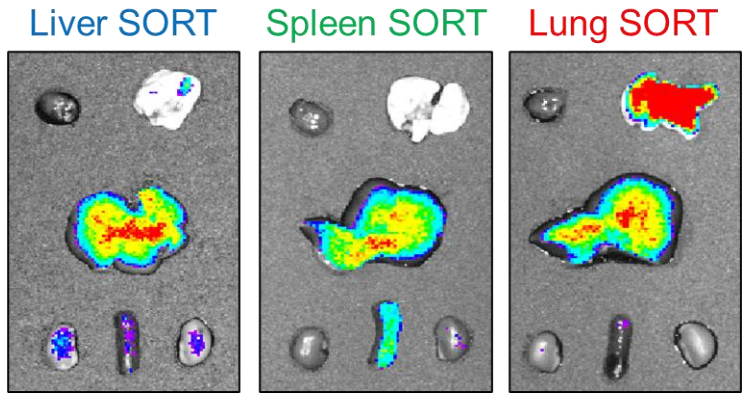

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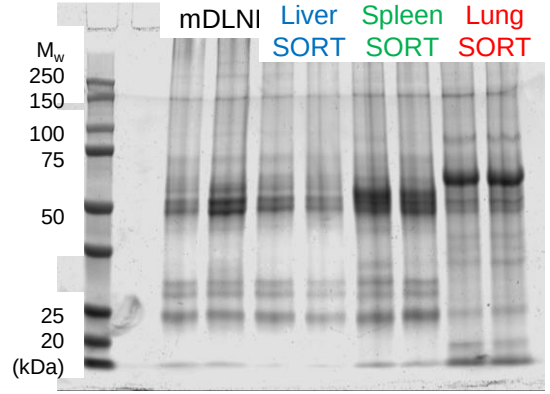

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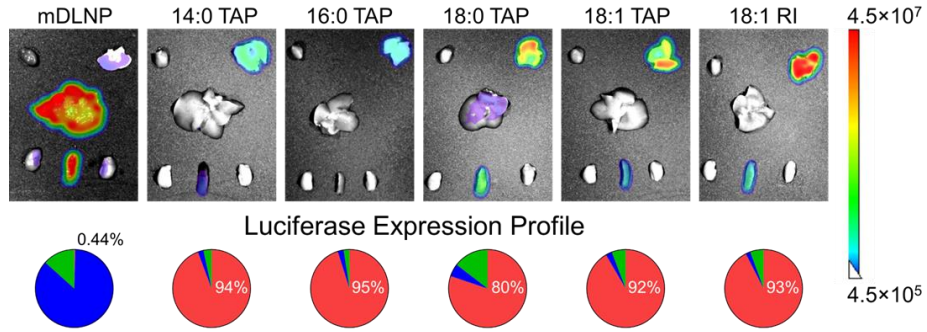
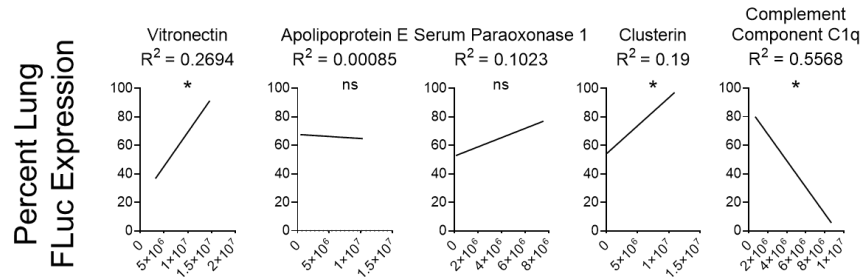


Multiple proteins appear to modulate delivery outcomes by affecting targeting of the lungs or de-targeting of the liver



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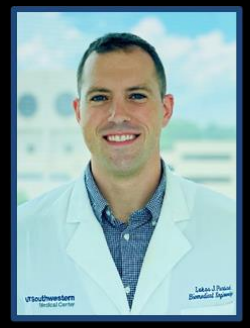
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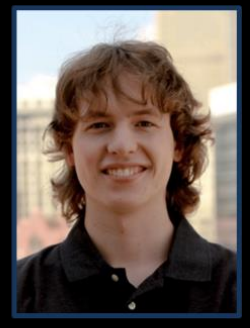
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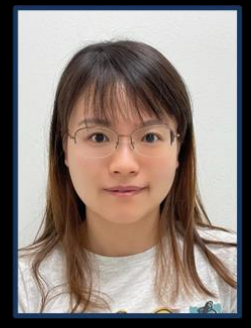
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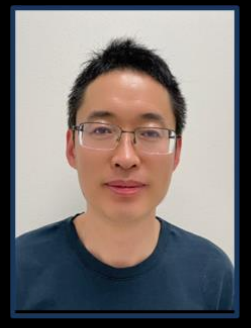
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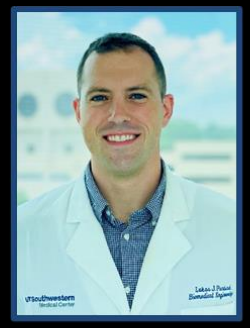
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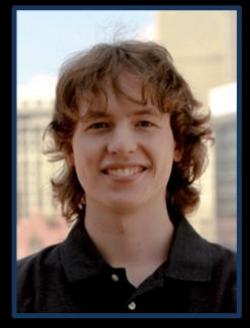
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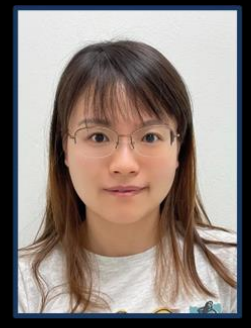
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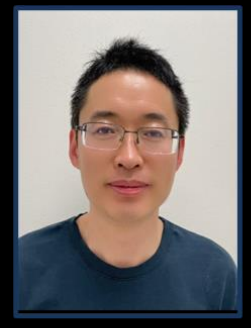
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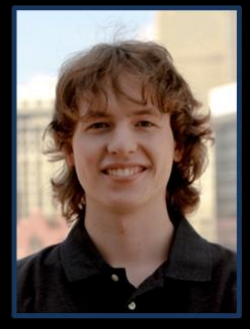
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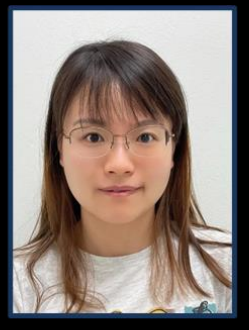
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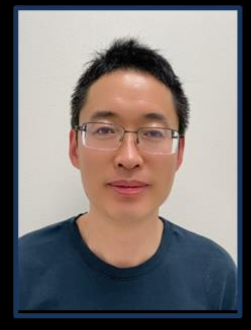
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