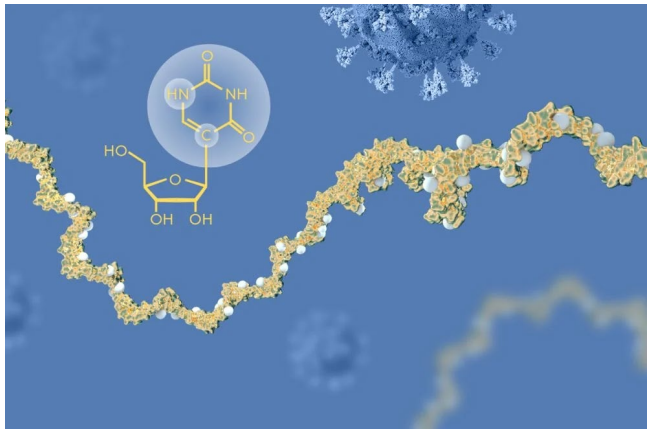
Nobel Prize lecture by 2023 Laureates in Physiology or Medicine at Karolinska

Institutet

Nucleoside Modified mRNA-LNP Therapeutics



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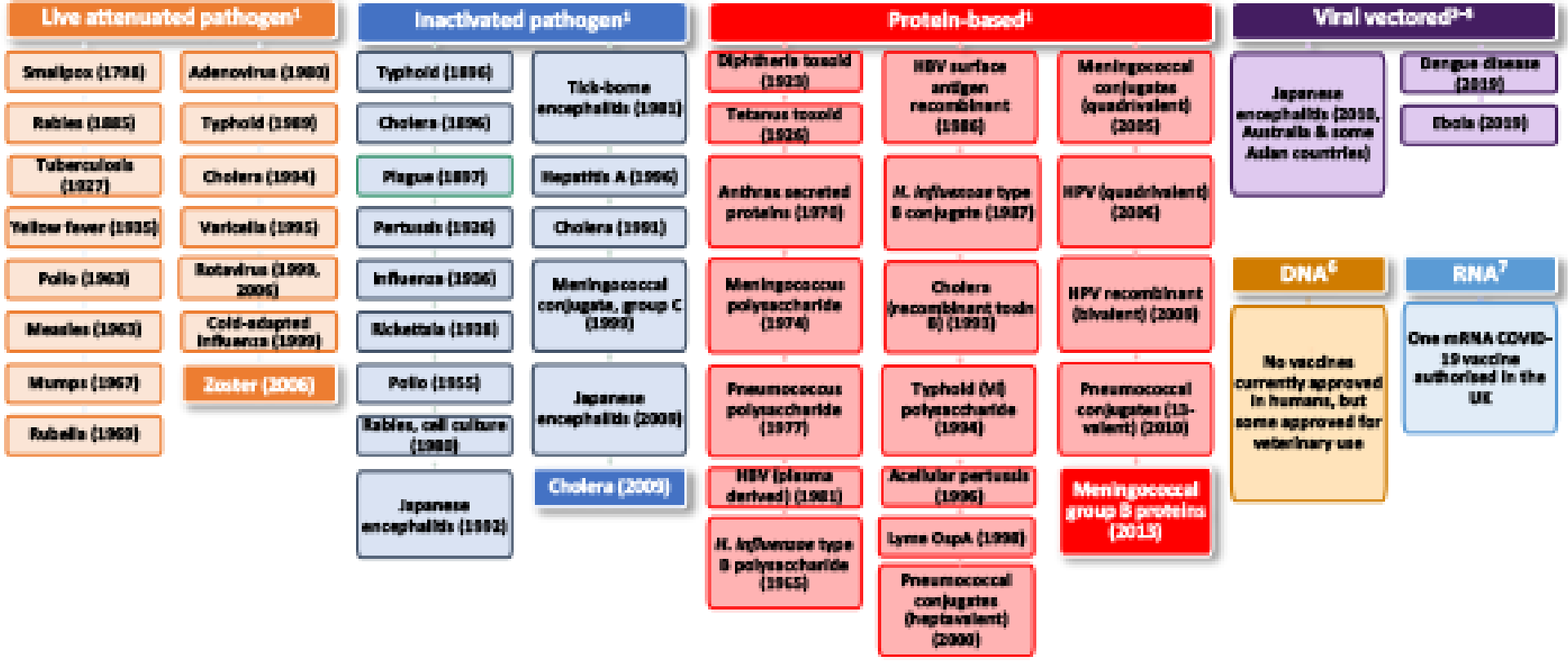
THE NOBEL PRIZE

Niklas Elmehed © Nobel Prize Outreach

Drew Weissman, M.D., Ph.D.
Roberts Family Professor in Vaccine Research
Perelman School of Medicine, University of Pennsylvania

Evolution of Vaccine Platforms



1. Plotkin S. *PNAS*. 2014;111:12283-7; 2. World Health Organization. JE Vaccine rates information sheet. Available at: https://www.who.int/vaccine_safety/initiative/tools/JE_vaccine_rates_information_sheet_Jan_2016.pdf Published January 2016. (Accessed November 2020); 3. Dengvaxia [prescribing information]. Swiftwater, PA, USA: Sanofi Pasteur Inc.; 2019; 4. US Food and Drug Administration. Press Announcement. Available at: https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health. Published December 19, 2019. [Accessed November 2020]; 5. Guy B, et al. *Vaccine*. 2015;33:7100-7111; 6. Kutzler MA, et al. *Nat Rev Genet*. 2008;9:776-88;
7. Gov.uk. Available at: https://www.gov.uk/government/news/uk-authorises-pfizer-biontech-covid-19-vaccine. [Accessed November 2020].

Nucleoside modified mRNA-LNP vaccine platform

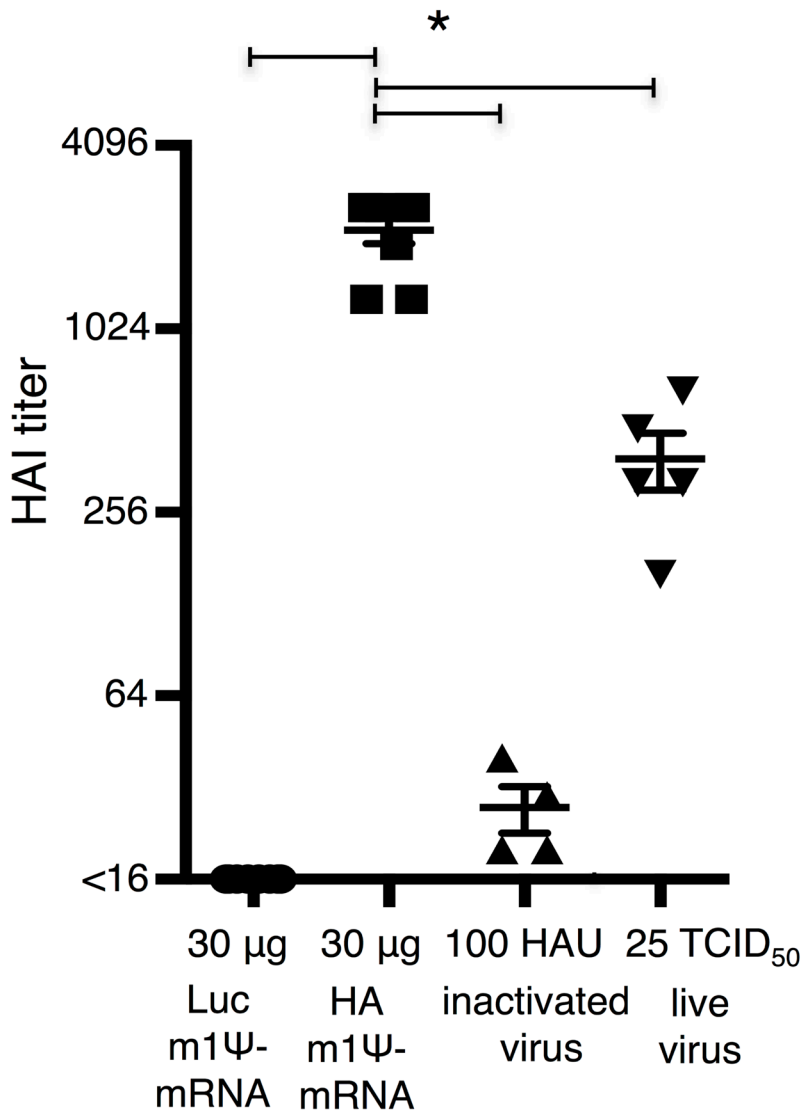
Background

- The number of influenza-associated deaths varies substantially by year, influenza virus type and subtype, and age group.
- In a study of influenza seasons from 76-77 through 06-07 in the US, the estimated number of annual influenza-associated deaths ranged from a low of 3,349 (1985-86 season) to a high of 48,614 (2003-04 season)
- Persons 65 years of age and older account for approximately 90% of deaths attributed to pneumonia and influenza.

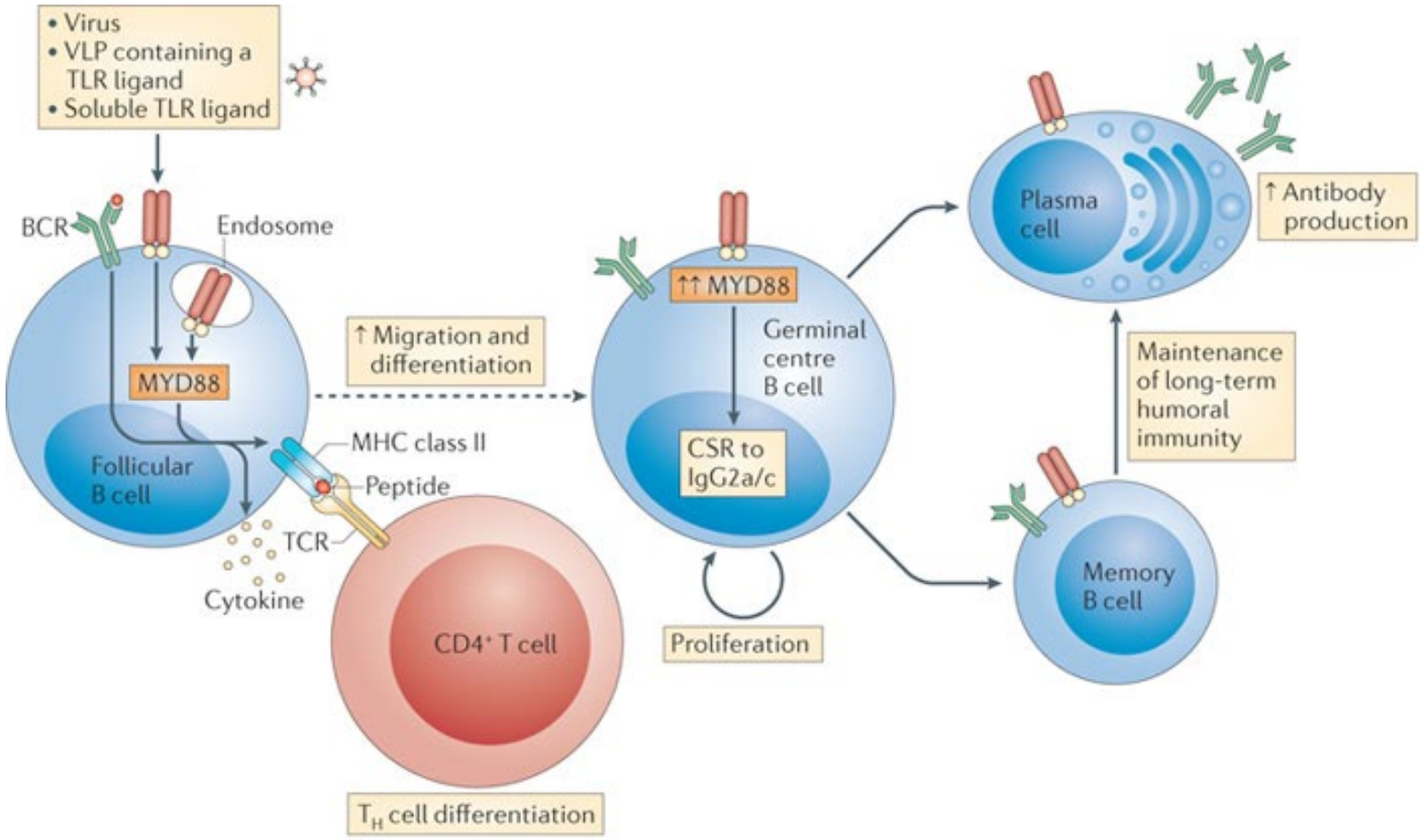
Antibody responses (HAI titers) for various influenza vaccines (H1N1) in mice.

- Intranasal live virus, x 2 vaccinations, (1:128).
- Intramuscular delivery of HA protein, x 2 vaccinations, (1:80).
- Baculovirus derived virus like particle, 2 immunizations, (1:400).
- Intradermal delivery of split virus, x 2 vaccinations, (1:70), response had short duration.

Acute infection with PR8 influenza induces lower levels of neutralization than modified mRNA-LNP vaccination

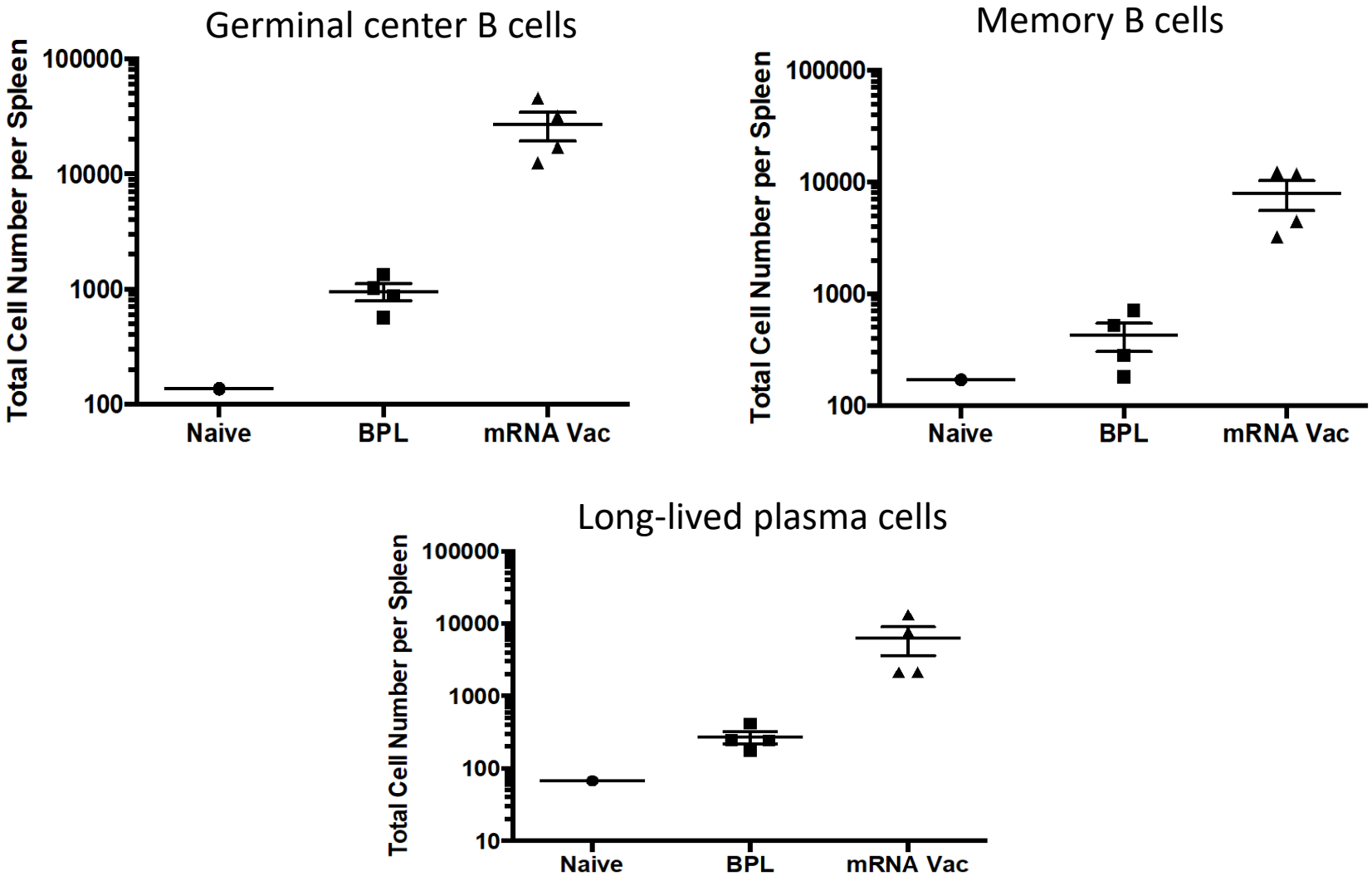


B cell response



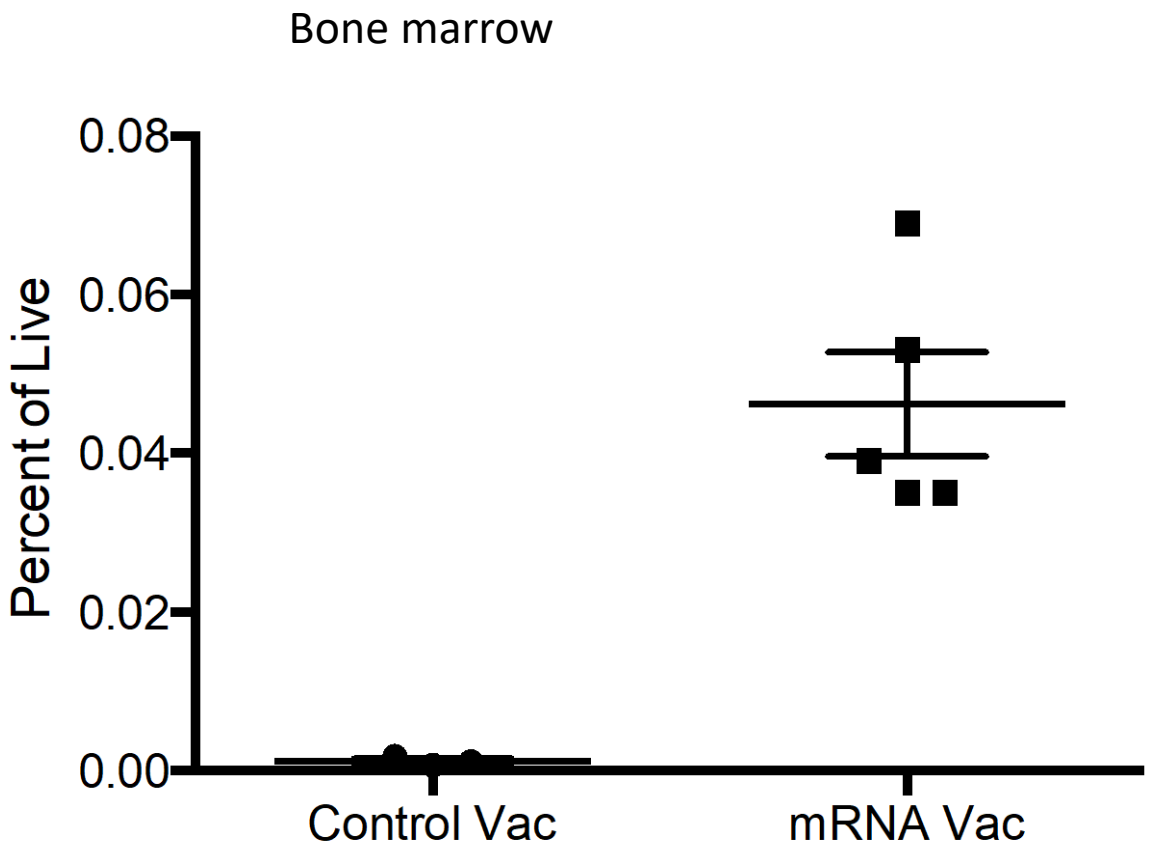
Nature Reviews | Immunology

A single immunization of PR8 HA encoding mRNA-LNPs produces HA-specific germinal center, memory, and long-lived plasma cells



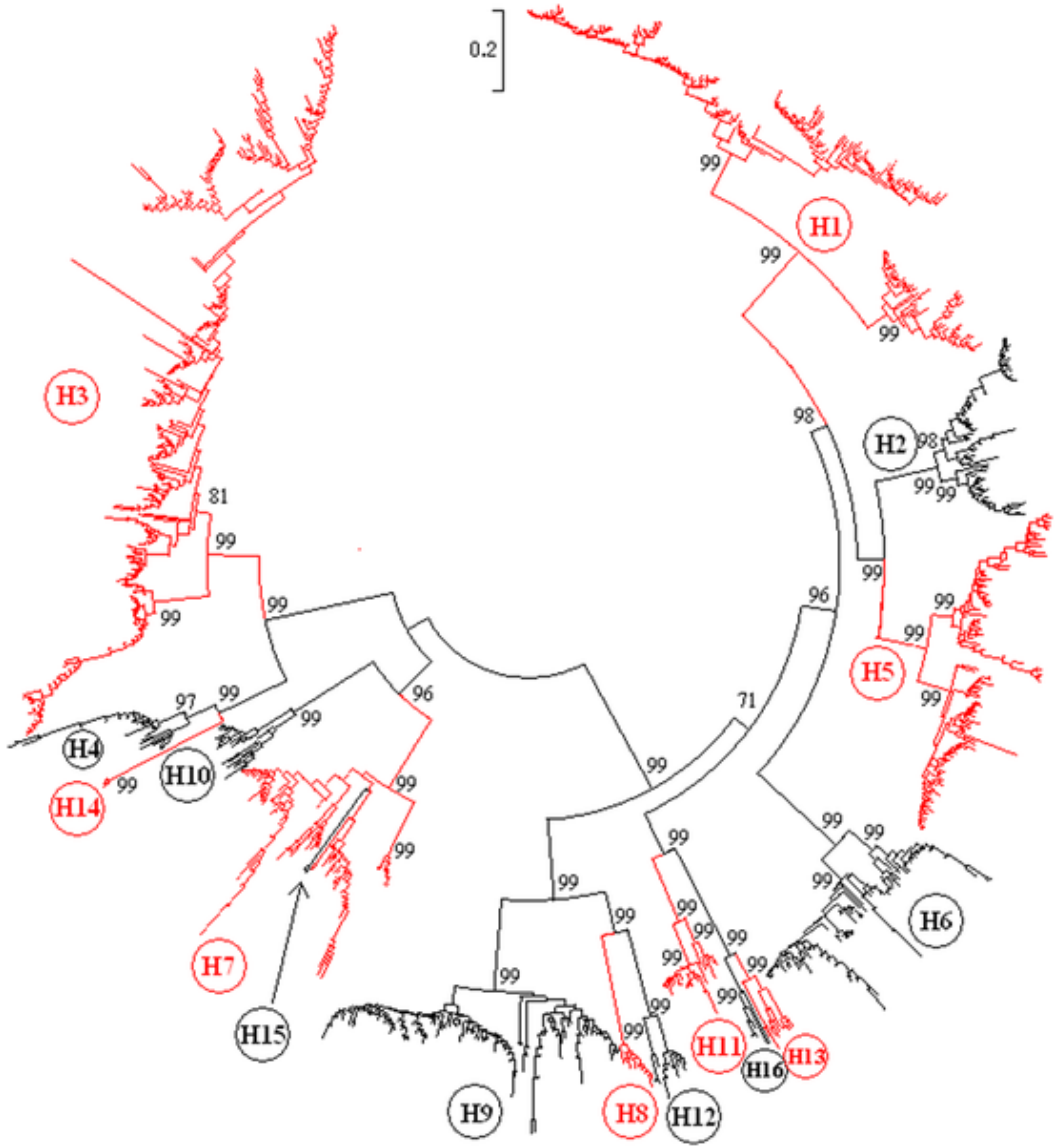
4 weeks after a single immunization with HA mRNA-LNPs

HA-specific bone marrow plasma cells 13 months after a single immunization with modified mRNA-LNPs.

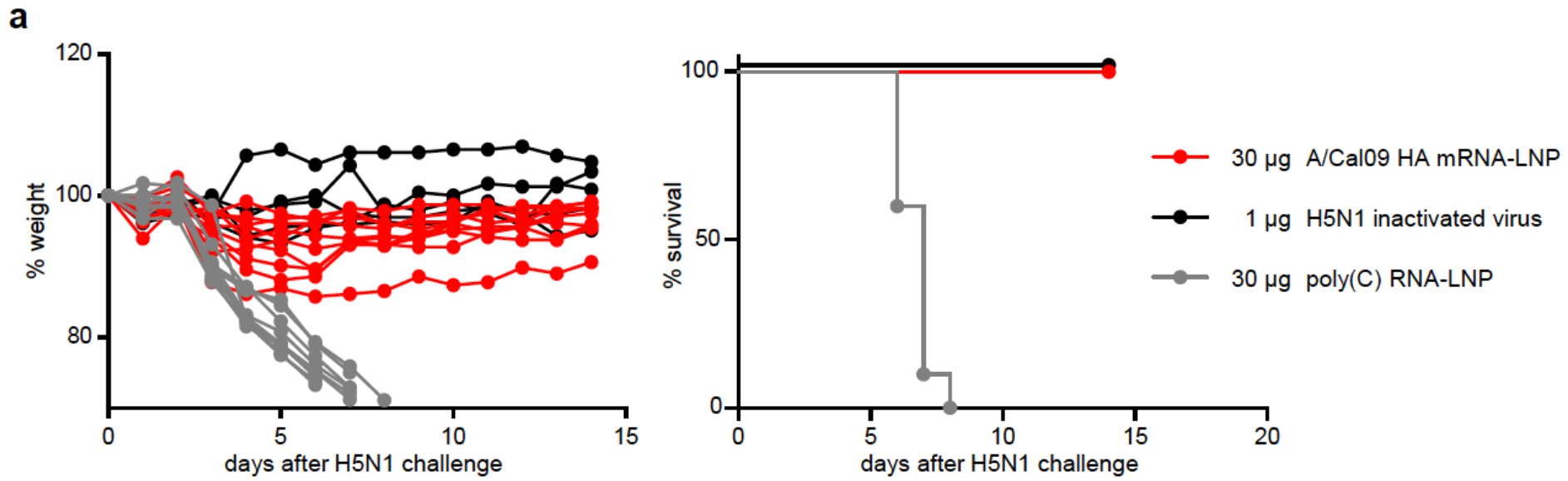


Gated on live, singlets, Dump- (CD4, CD8, F4/80, Ter119), IgD-, B220- that bound fluoresceinated influenza HA.

Influenza subtypes



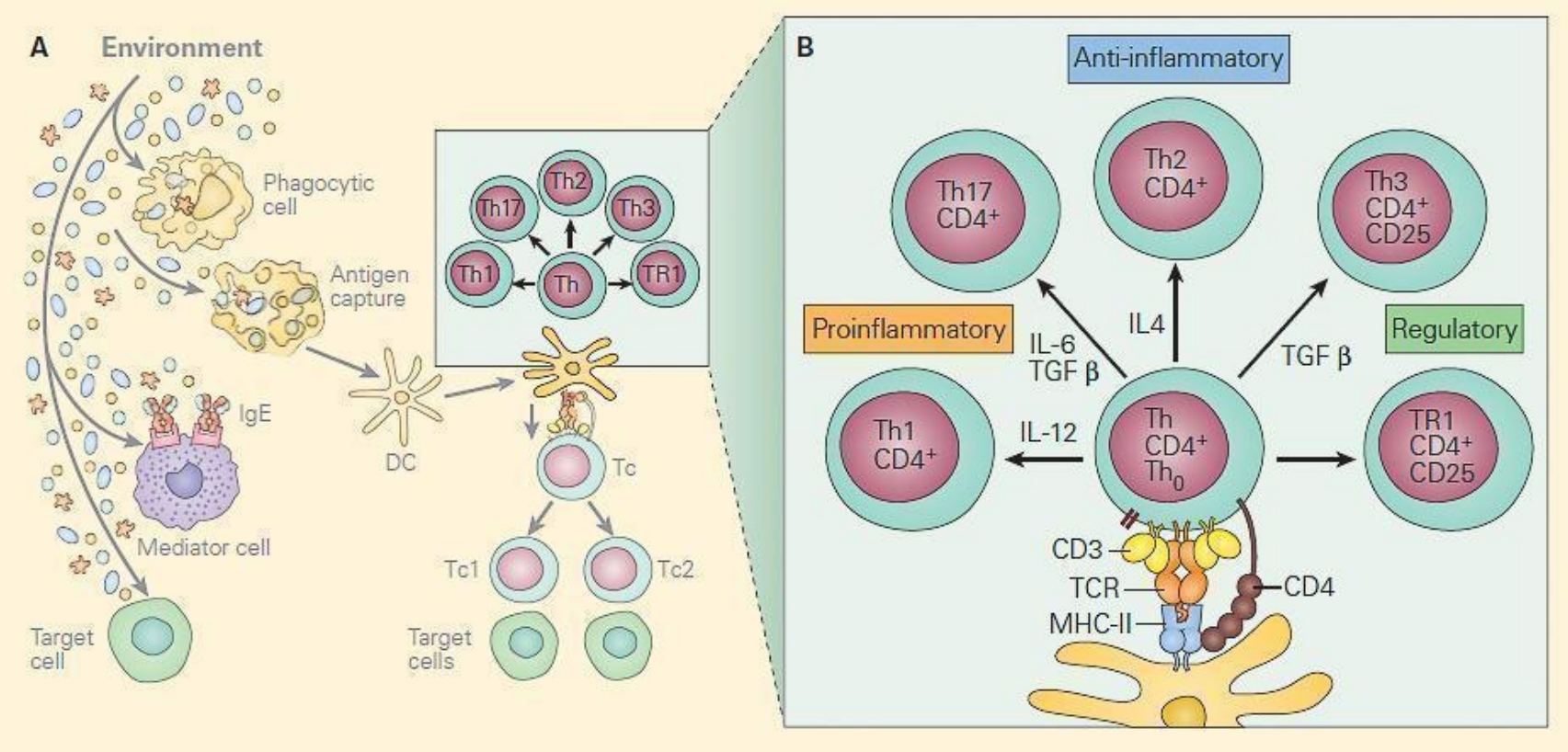
A/Cal/7/2009 H1 cell surface full HA immunized mice were completely protected from H5N1 virus challenge in the absence of neutralization activity.



Vaccines mechanisms

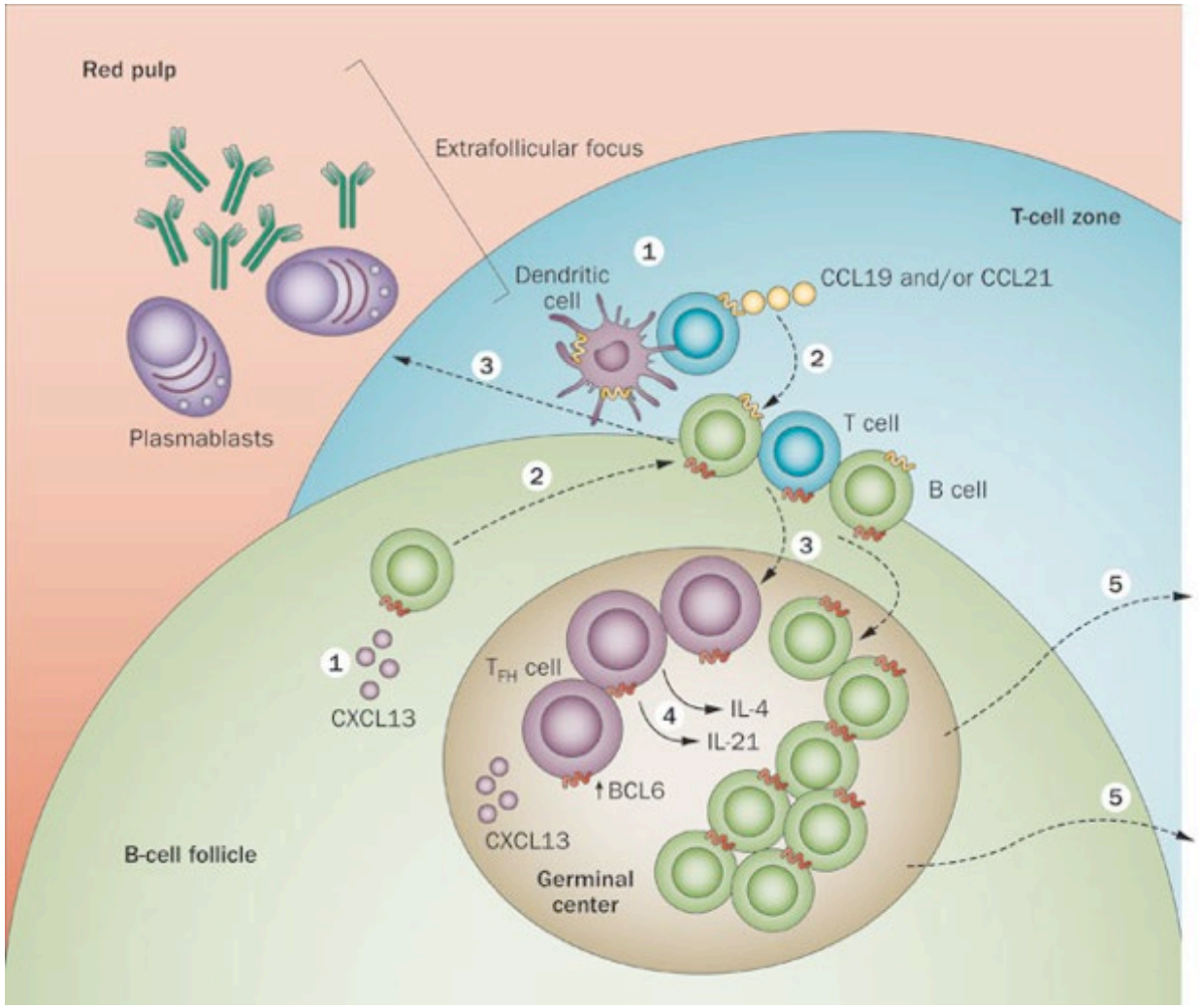
- Vaccines use adjuvants to increase immune responses and reduce amount of immunogen needed.
- All currently developed adjuvants signal innate immune receptors, TLRs, helicases, inflammasomes, to induce inflammation and activate Th1, Th2, or Th17 responses.

T cells develop different effector activities driven by APC interactions.



https://www.immunopaedia.org.za/immunology/basics/5-overview-of-t-cell-subsets/

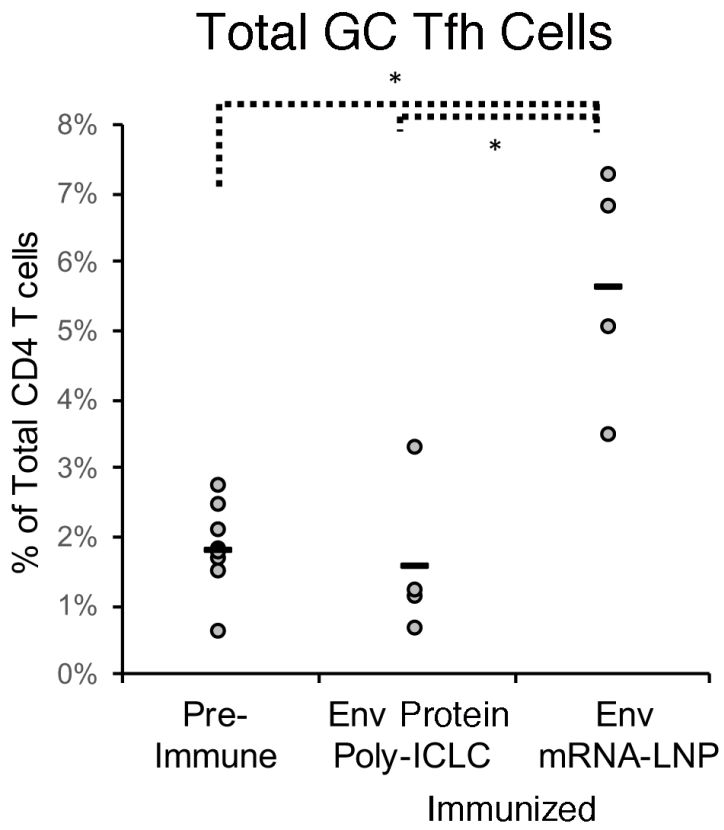
T follicular helper cells

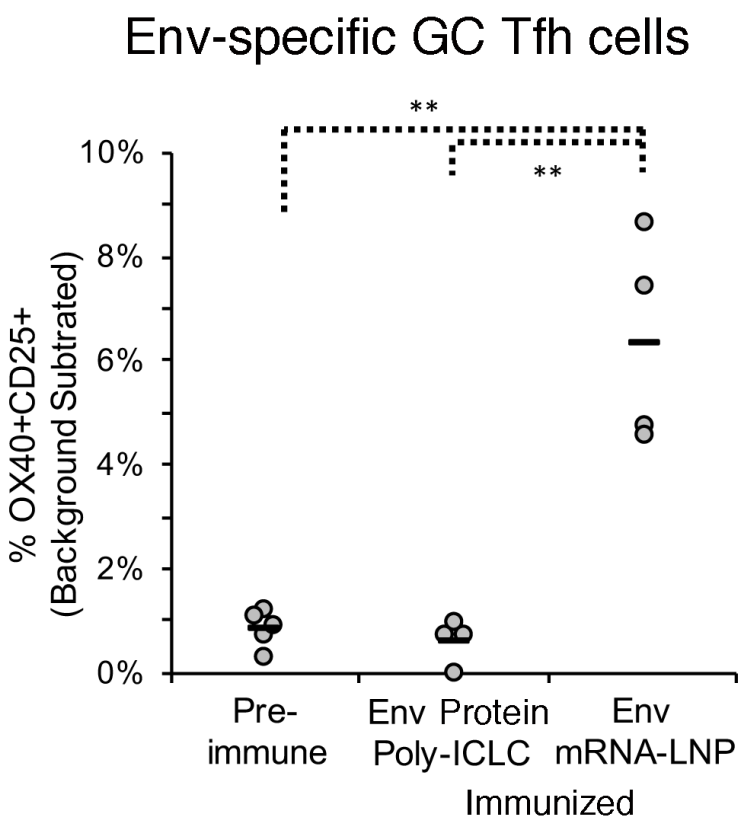


GC formation
Class switch
Affinity maturation
GC B cell proliferation
Long-term memory
Long-lived plasma cells

They are critical in driving B cell responses and memory and are the subject of many vaccine-adjuvant studies.

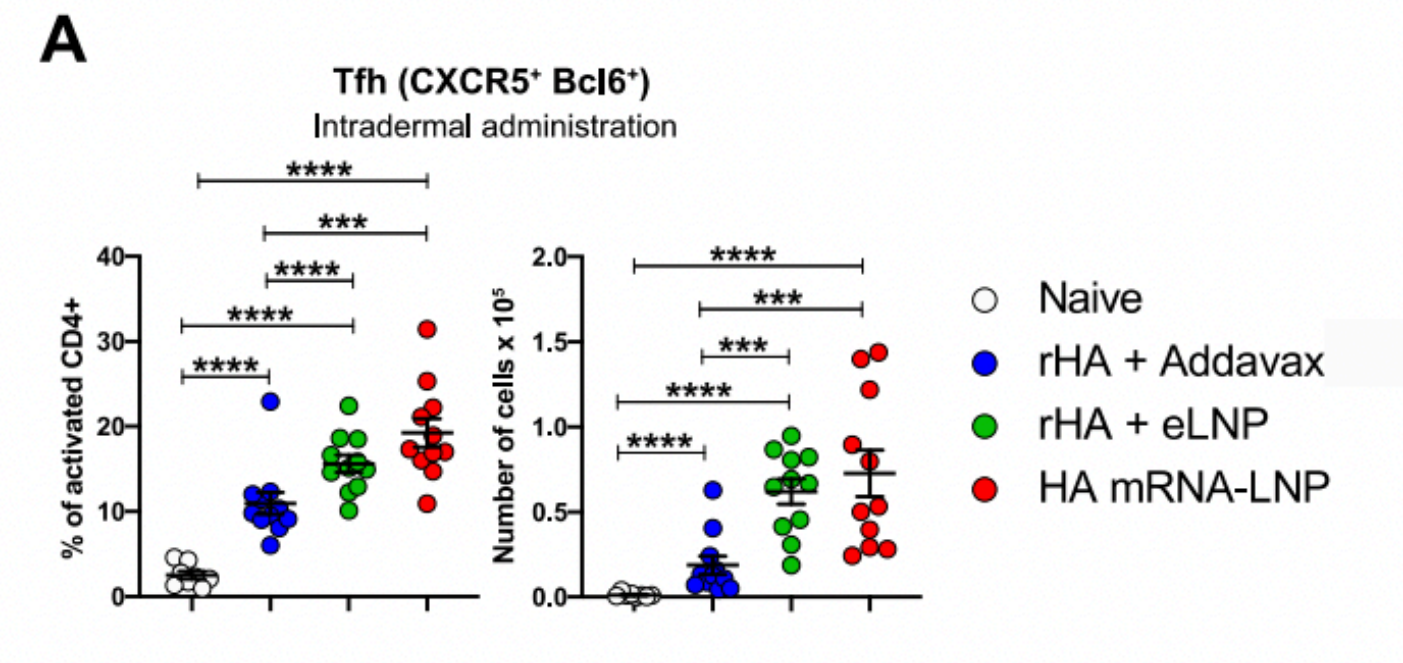
Frequencies of total and Env-specific germinal center (GC) T follicular helper (Tfh) cells in CH505 T/F immunized macaque lymph nodes



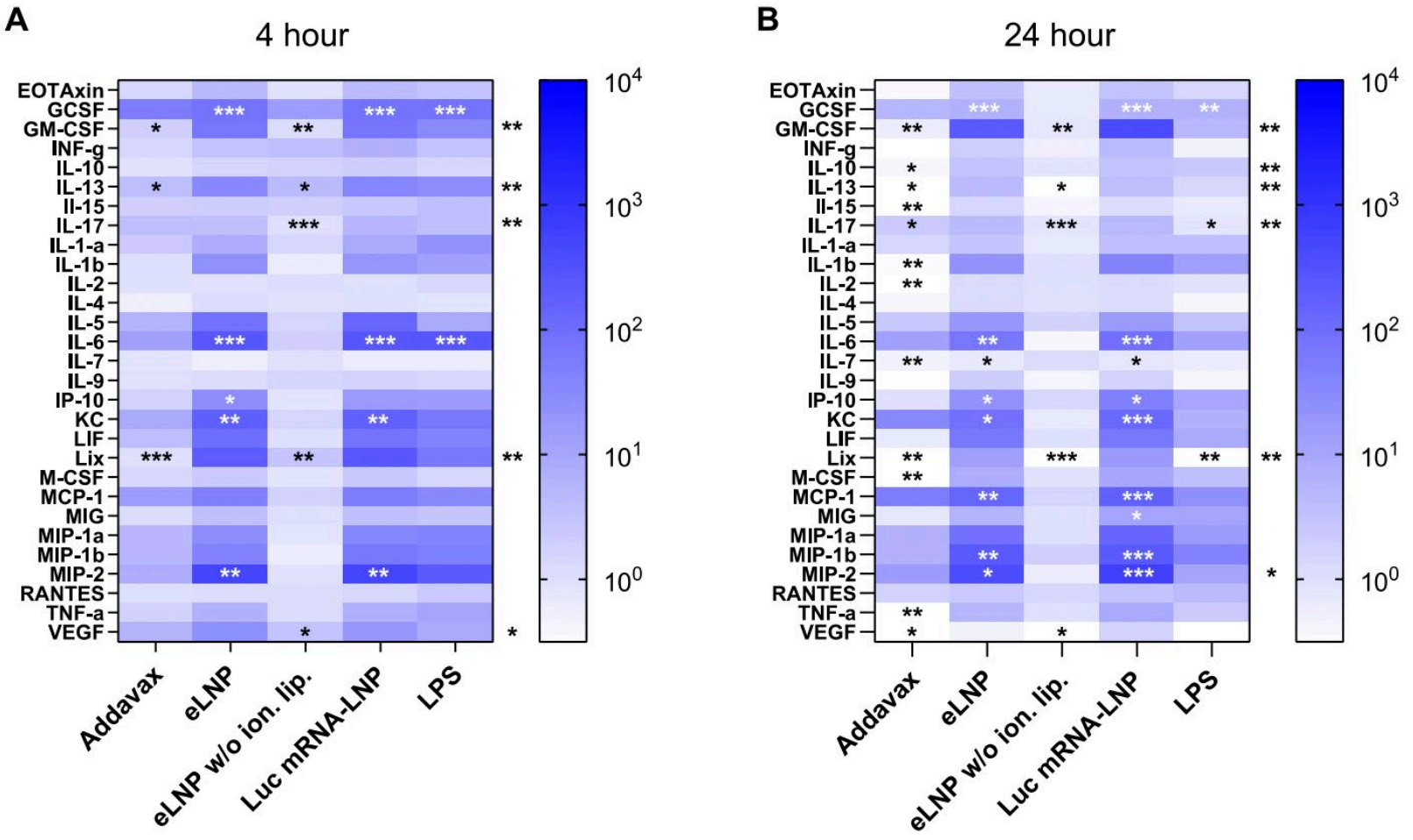


CHAVI-ID
Duke Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery

LNPs are an adjuvant that induces Tfh cells.



LNPs induce IL-6 and chemokines and no type 1 IFNS.



Approach to a universal influenza vaccine

Science 2022 Nov 25;378(6622):899-904. doi: 10.1126/science.abm0271. Epub 2022 Nov 24.

A multivalent nucleoside-modified mRNA vaccine against all known influenza virus subtypes

Claudia P Arevalo [1], Marcus J Bolton [1], Valerie Le Sage [2], Naiqing Ye [1], Colleen Furey [1], Hiromi Muramatsu [1], Mohamad-Gabriel Alameh [3], Norbert Pardi [1], Elizabeth M Drapeau [1], Kaela Parkhouse [1], Tyler Garretson [1], Jeffrey S Morris [4], Louise H Moncla [5], Ying K Tam [6], Steven H Y Fan [6], Seema S Lakdawala [2 7], Drew Weissman [3], Scott E Hensley [1]

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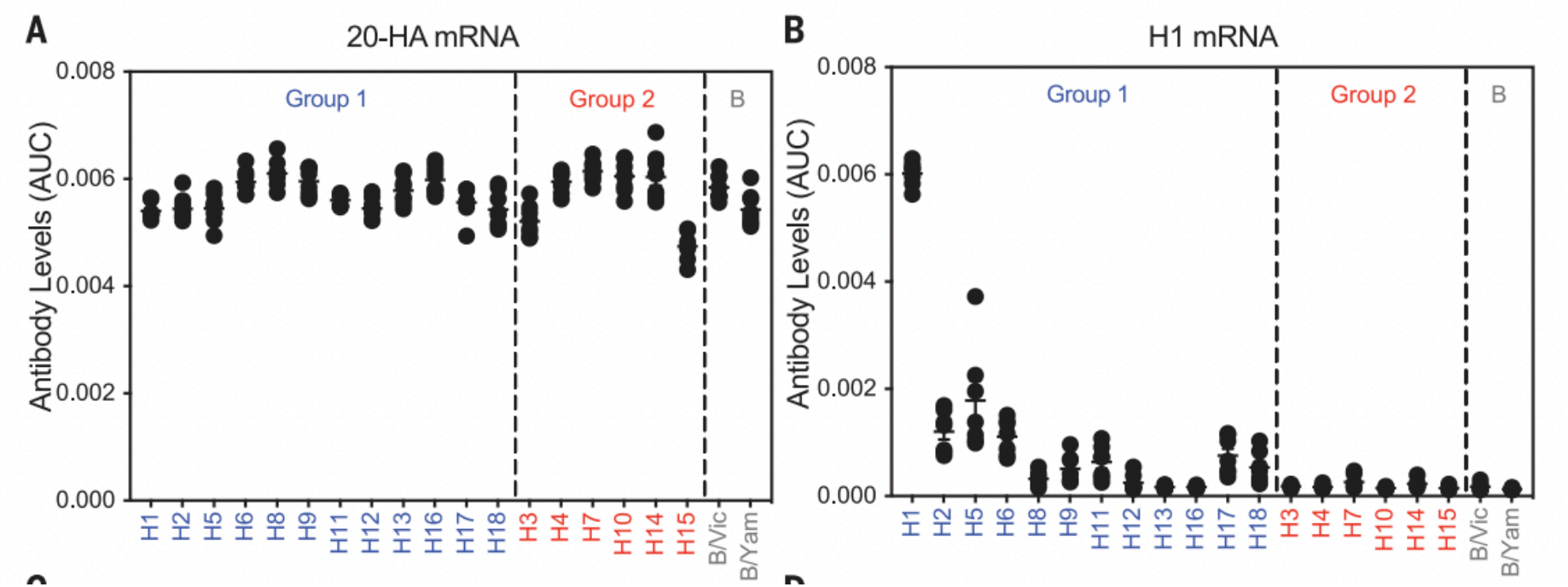
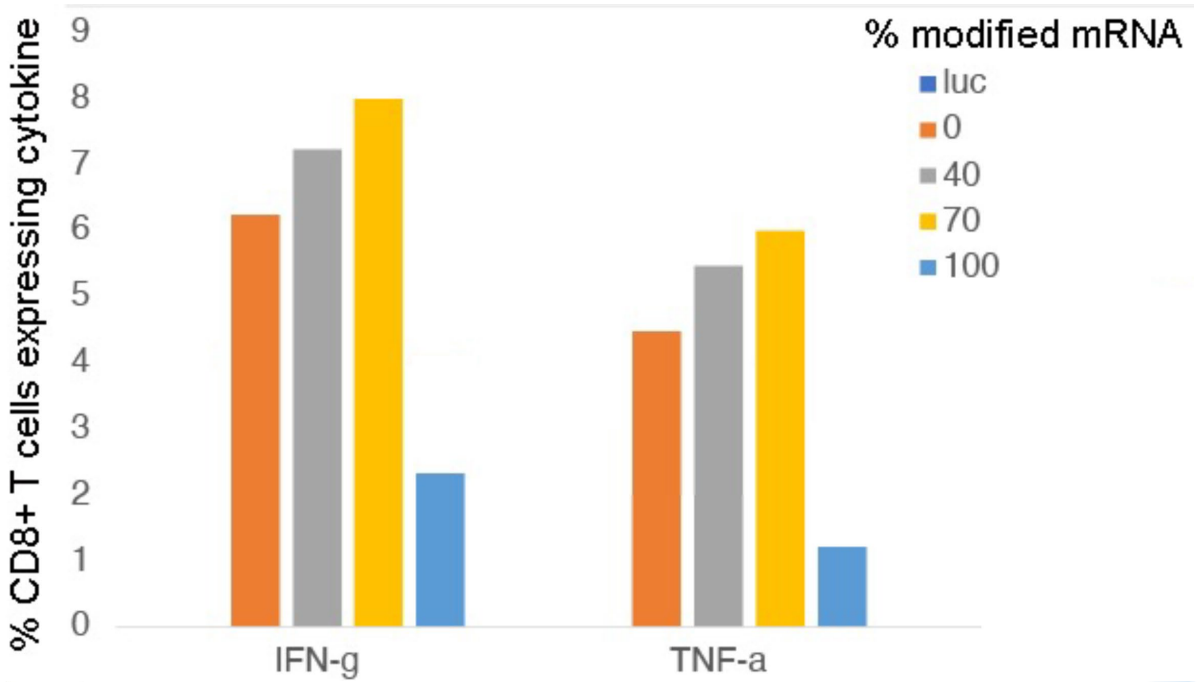


Science. 2022 378:899-904

Influenza conclusions

- The clear superiority of the modified mRNA-LNP vaccine for influenza is demonstrated. Titers after a single immunization were almost 5 times higher than the gold-standard, pathogen infection.
- The most important finding is that only a **single** immunization in a naïve host is needed for complete protection against influenza.
- The potent antibody response is due to the Tfh response that makes up half of the CD4 helper response.
- This is the first report that we know of where immunization with a non-replicating vaccine containing whole HA immunogen is capable of inducing a high titer IgG response and over a quarter of the response is directed at the stalk and mice are protected in the absence of HAI activity.

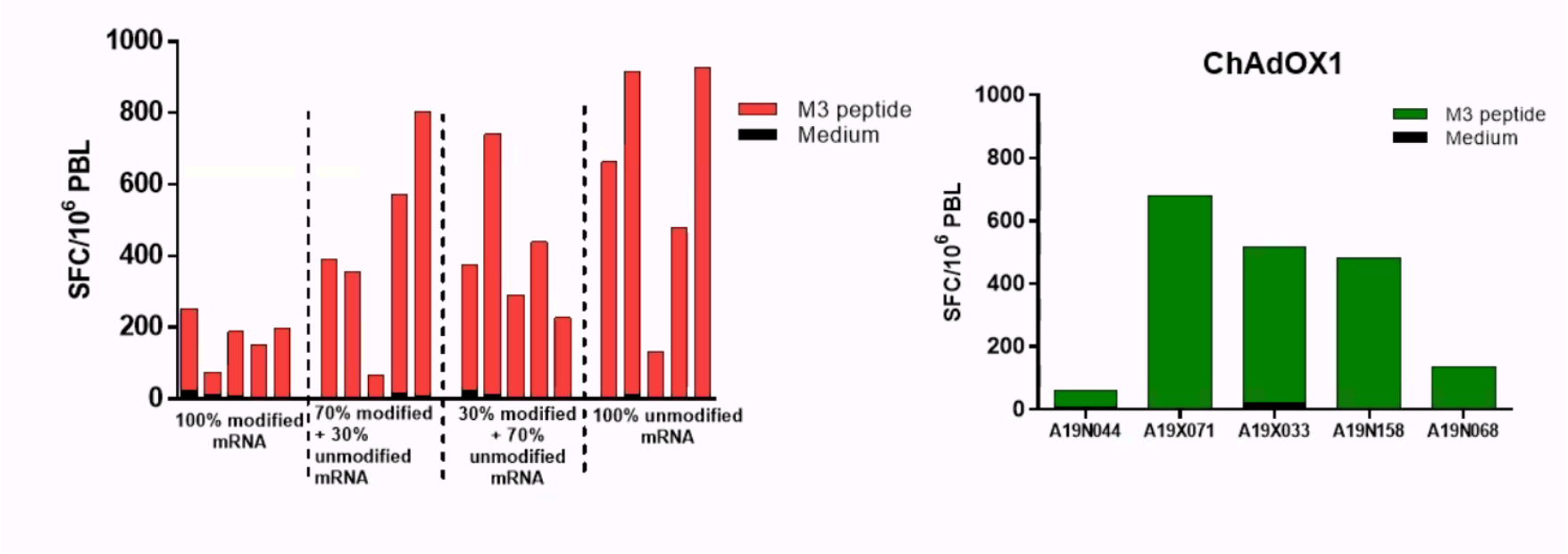
Inclusion of nonmodified bases improves the magnitude of elicited CD8 responses in a mouse OT-1 passive transfer model



National Institute of Allergy and Infectious Diseases

CHAVD
Duke Consortia for HIV/AIDS Vaccine Development

Inclusion of nonmodified bases improves the magnitude of elicited CD8 responses in a SIV vaccine model



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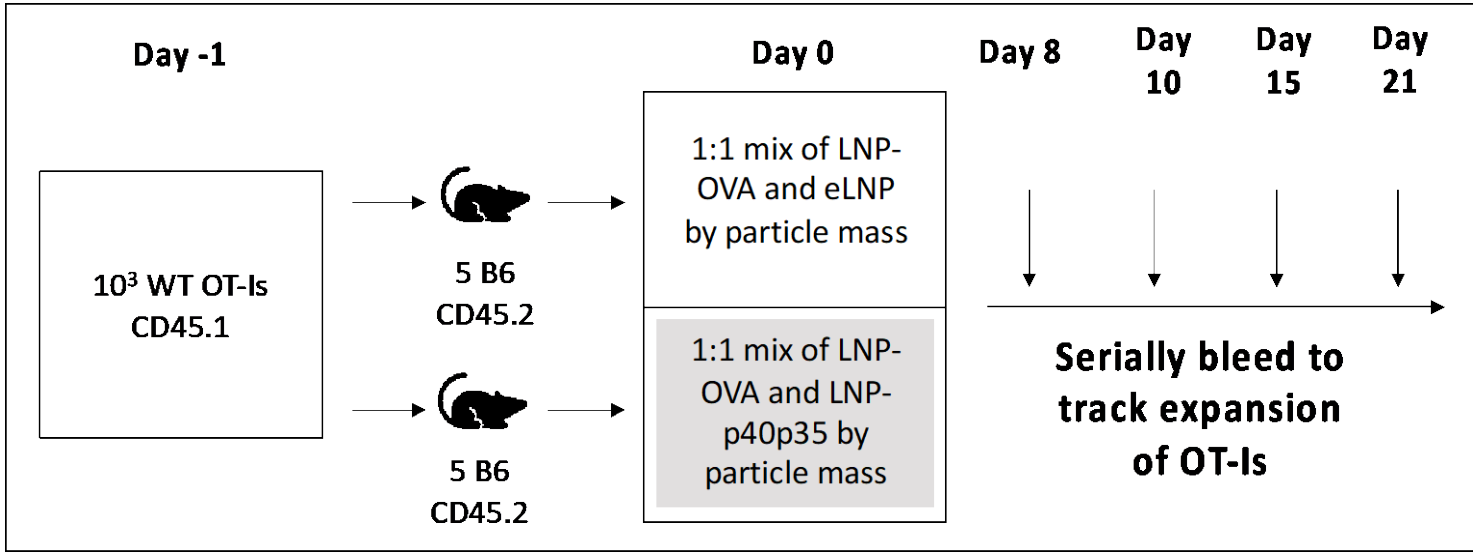
Can administration of mRNA-encoded cytokines, e.g. IL-12, with vaccination improve immunogenicity?



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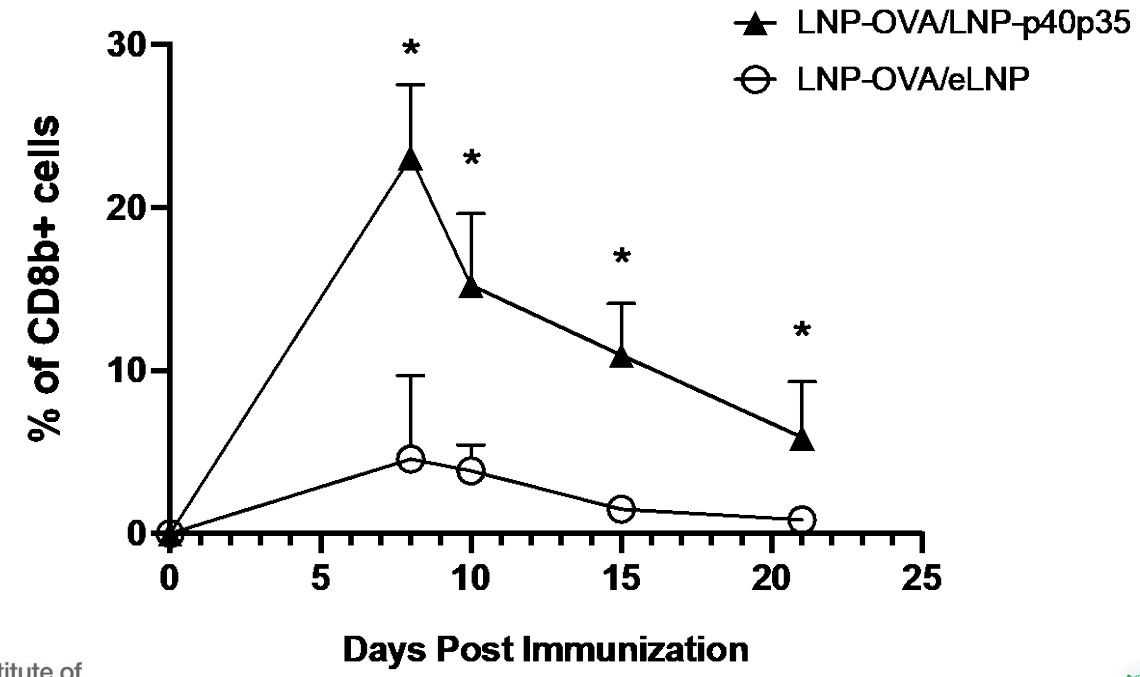
Addition of LNP-IL-12 amplifies expansion of OT-I CD8 T cells in response to vaccination



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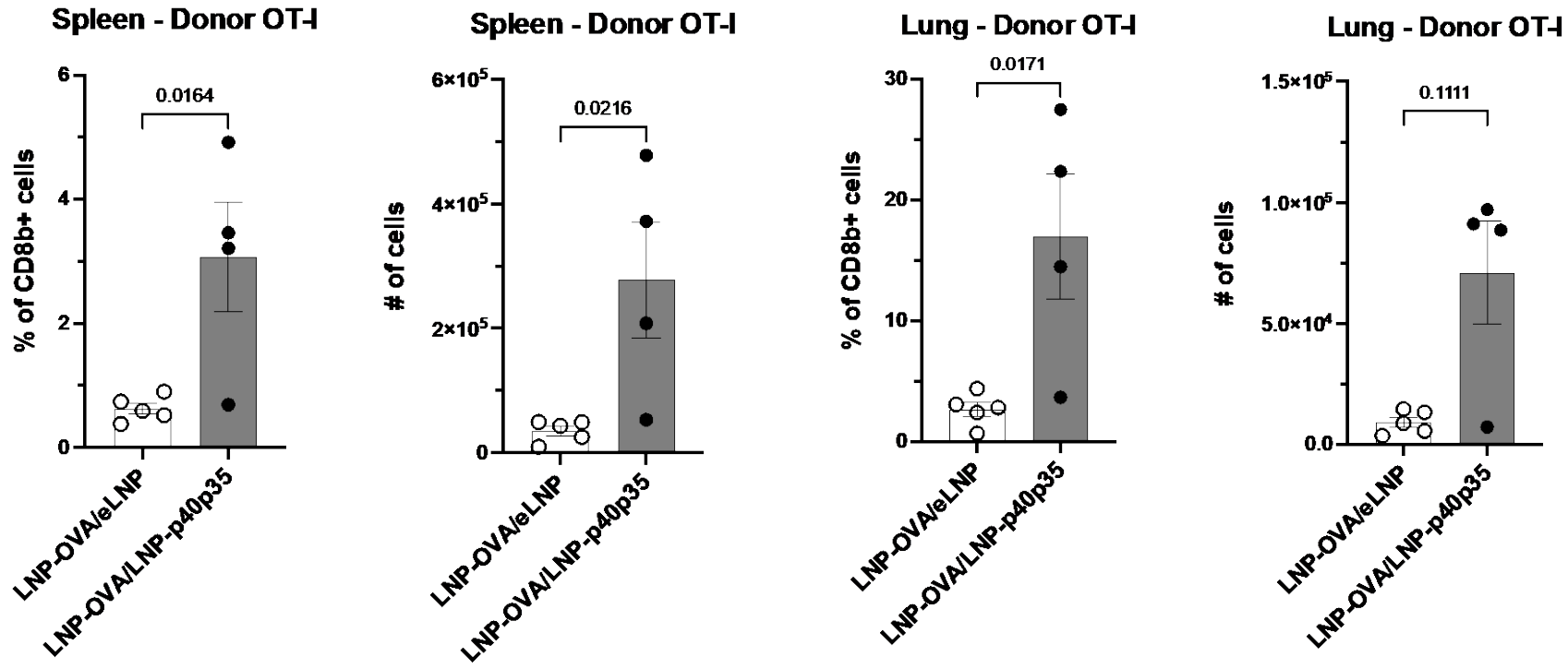
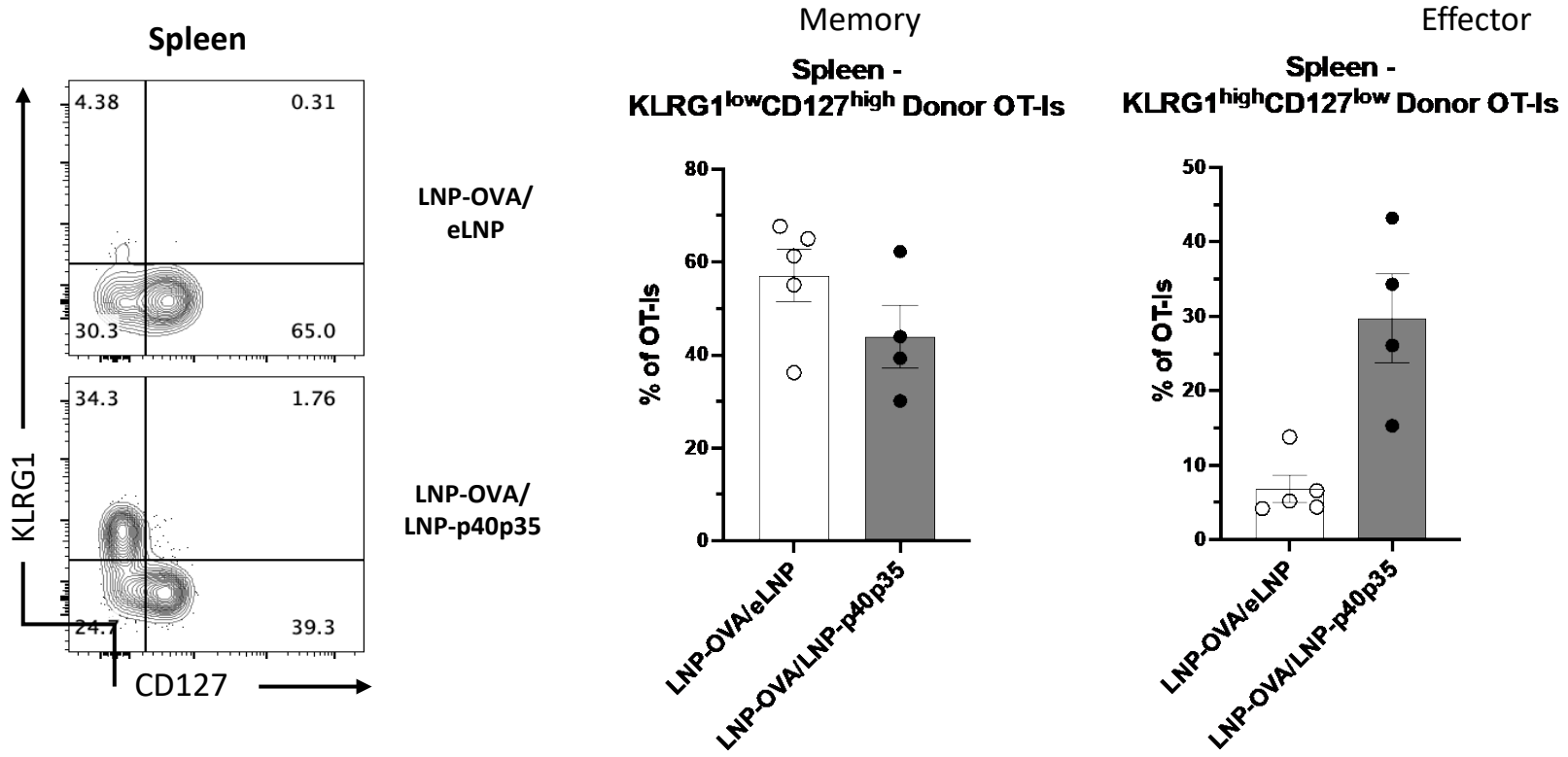
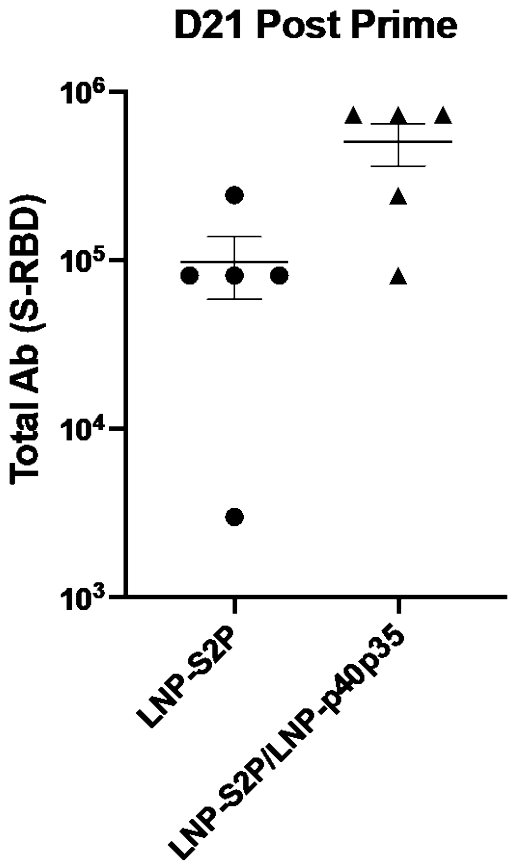
LNP-IL-12 adjuvants expand tissue OT-I CD8 T cells in response to vaccination



LNP-IL-12 adjuvants alter the differentiation of CD8 T cells following vaccination by inducing effector cells



IL-12-adjuvanted vaccines elicit higher antibody titers in aged mice



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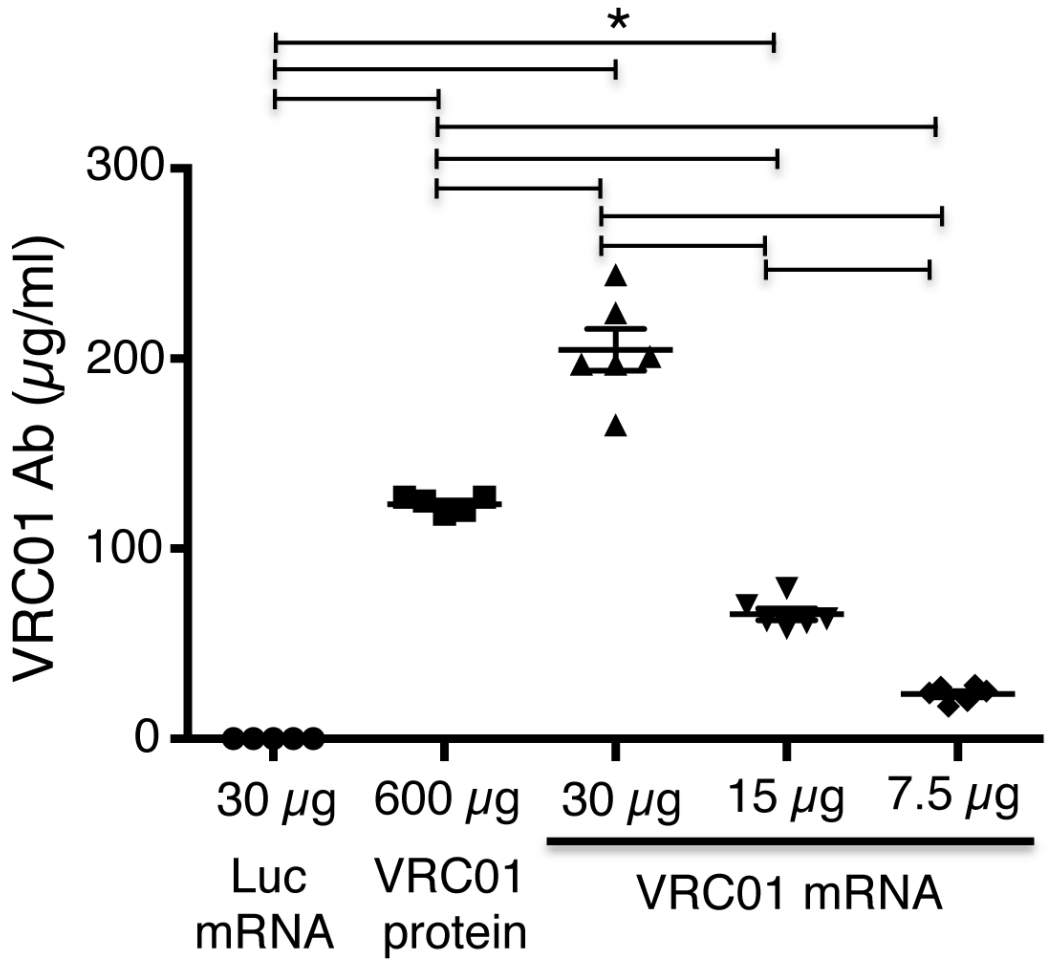
Various vaccines are in development at the Institute of RNA Innovation.

- Many pathogens – HIV, HCV, HSV, norovirus, C. Diff, Malaria, TB, CMV, EBV, Influenza (seasonal and universal), pancoronavirus, and many more
- Allergy vaccine for foods and environmental allergens – peanuts, dust mites.
- Vaccines for autoimmune diseases
- Cancer vaccines

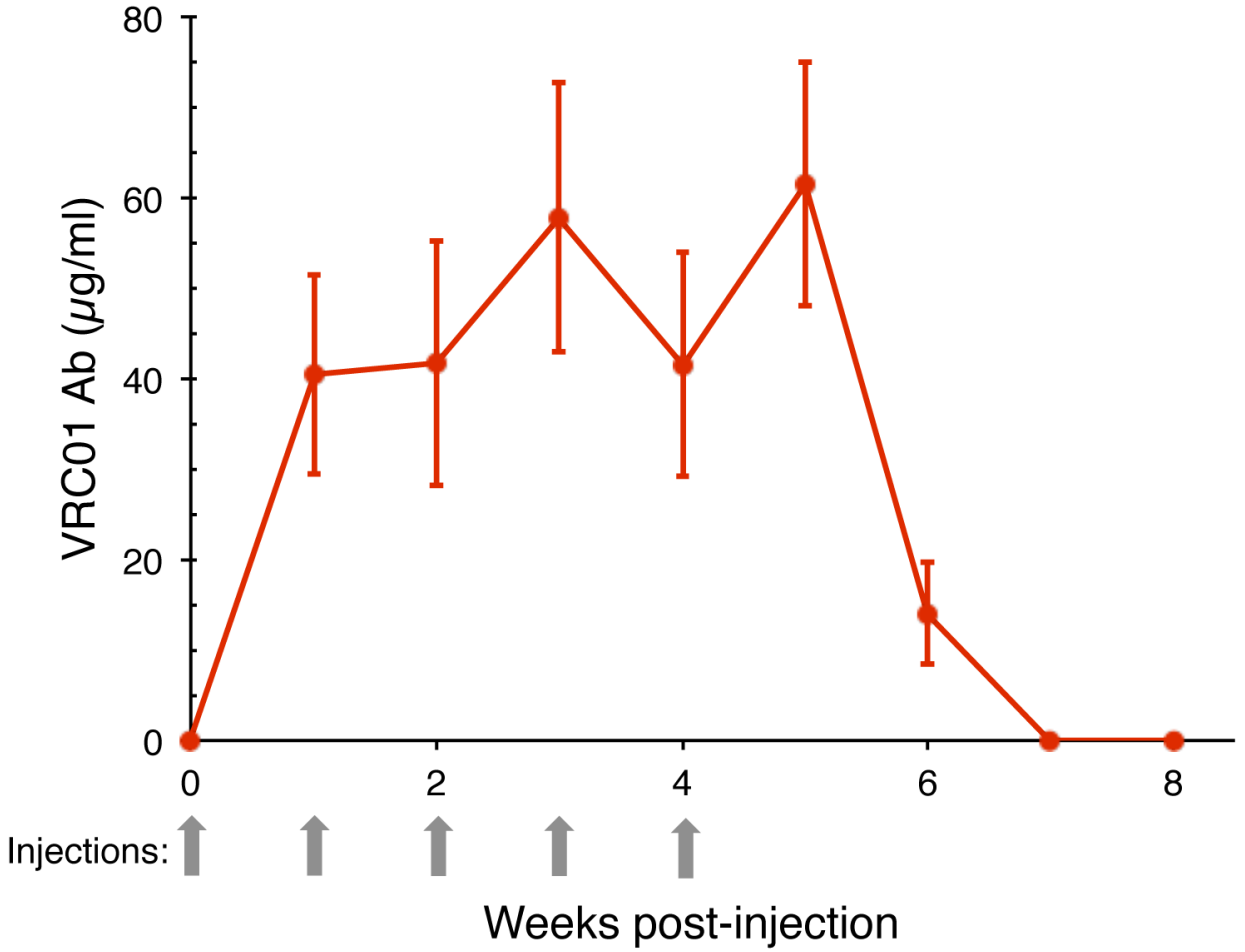
Delivery of therapeutic monoclonal antibodies

- The VRC01 human monoclonal Ab neutralizes 93% of HIV strains worldwide.
- Nucleoside modified mRNA encoding the heavy and light chains were made, complexed to lipid nanoparticles and delivered to humanized mice.
- VRC01 levels were measured and mice were challenged with HIV.

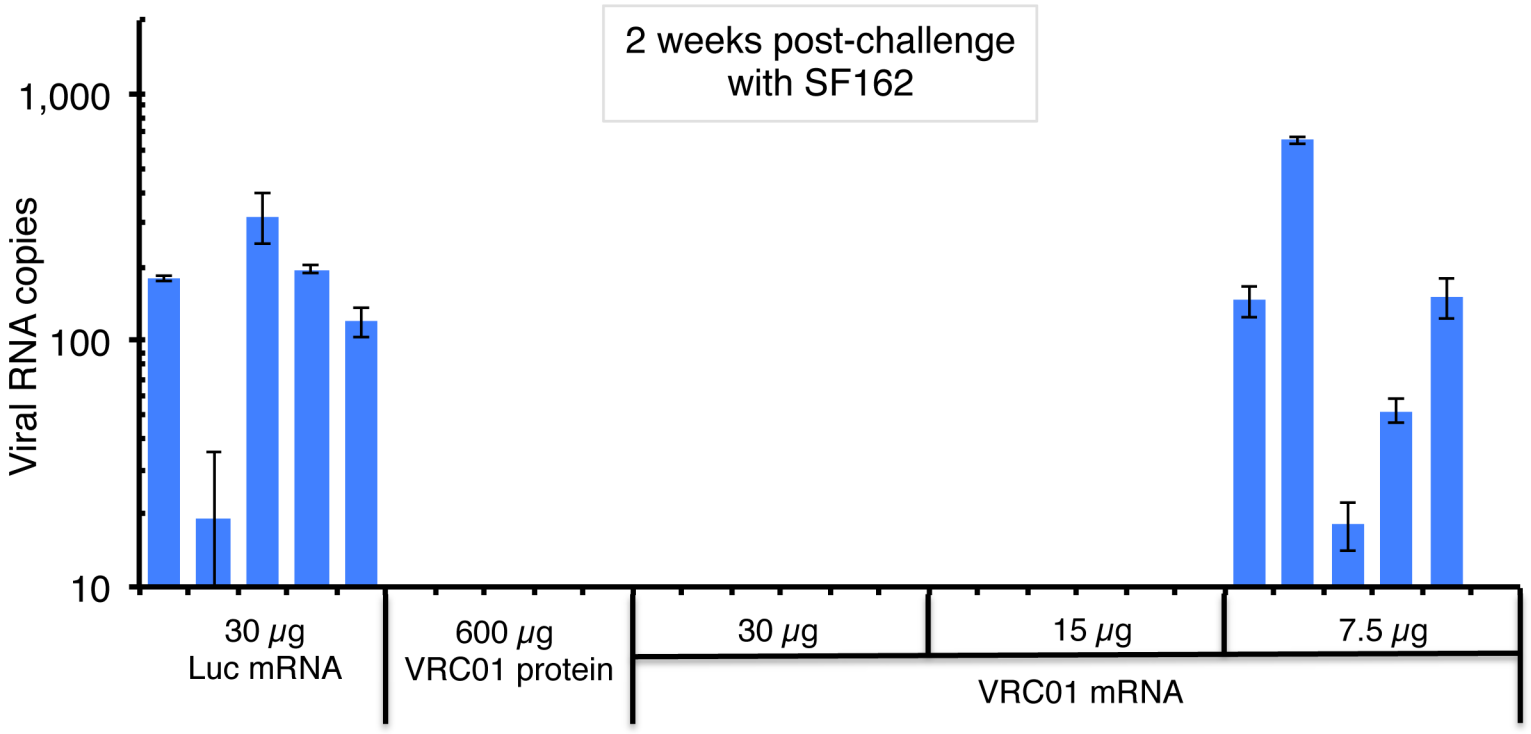
Circulating levels of functional VRC01 mAb after encoding mRNA-LNP injection



Repeated delivery of VRC01 mRNA results in sustained levels of mAb

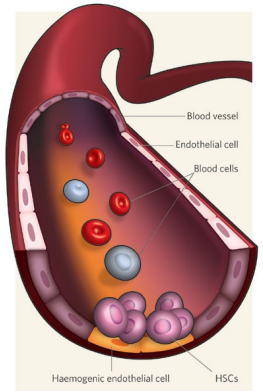


VRC01 encoding mRNA completely protects humanized mice from HIV challenge



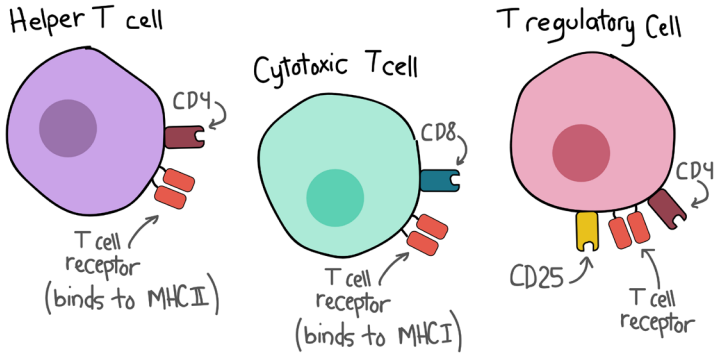
Penn Medicine

Endothelial targeting



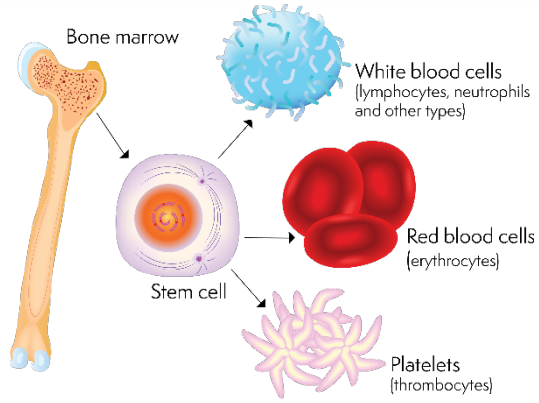
https://cellapplications.com/endothelial

T cell targeting

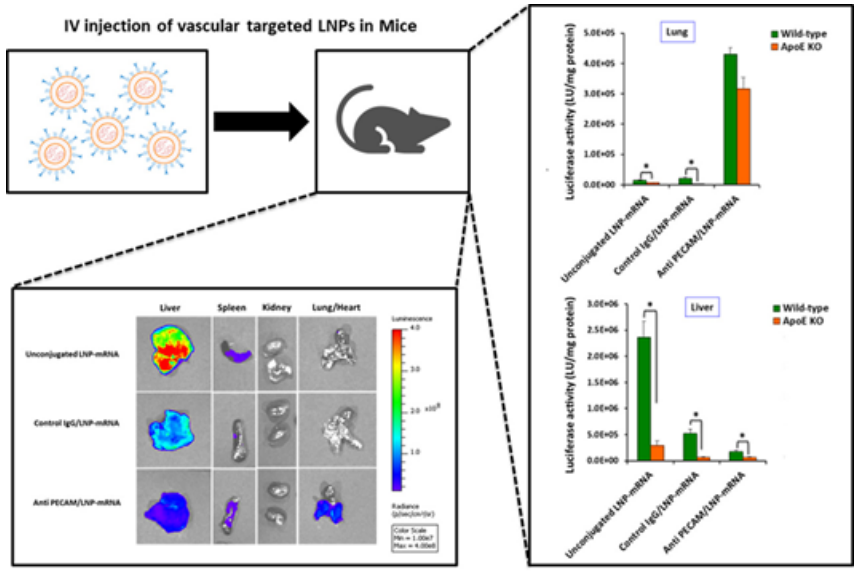


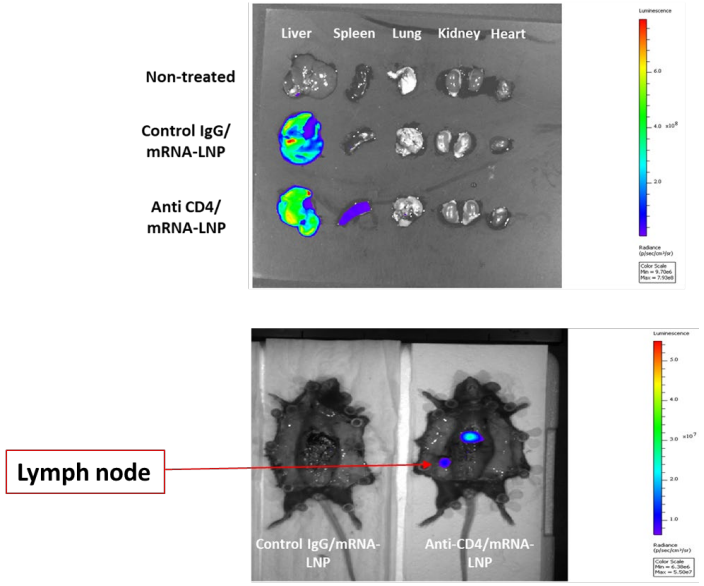
https://cdn.kastatic.org/ka-perseus-images/95c73f88aa68932b0637224f6670208006c5e079.svg

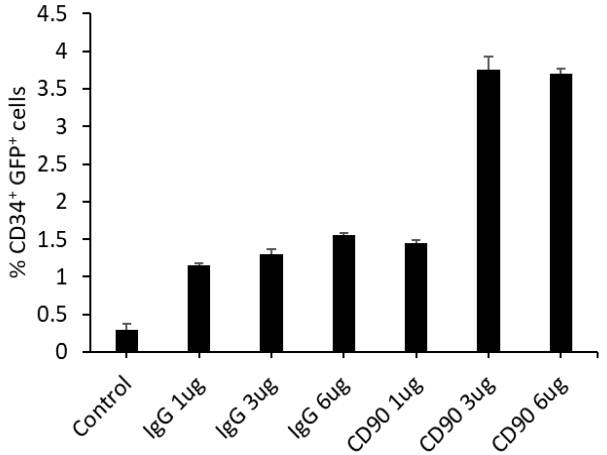
Stem cell targeting



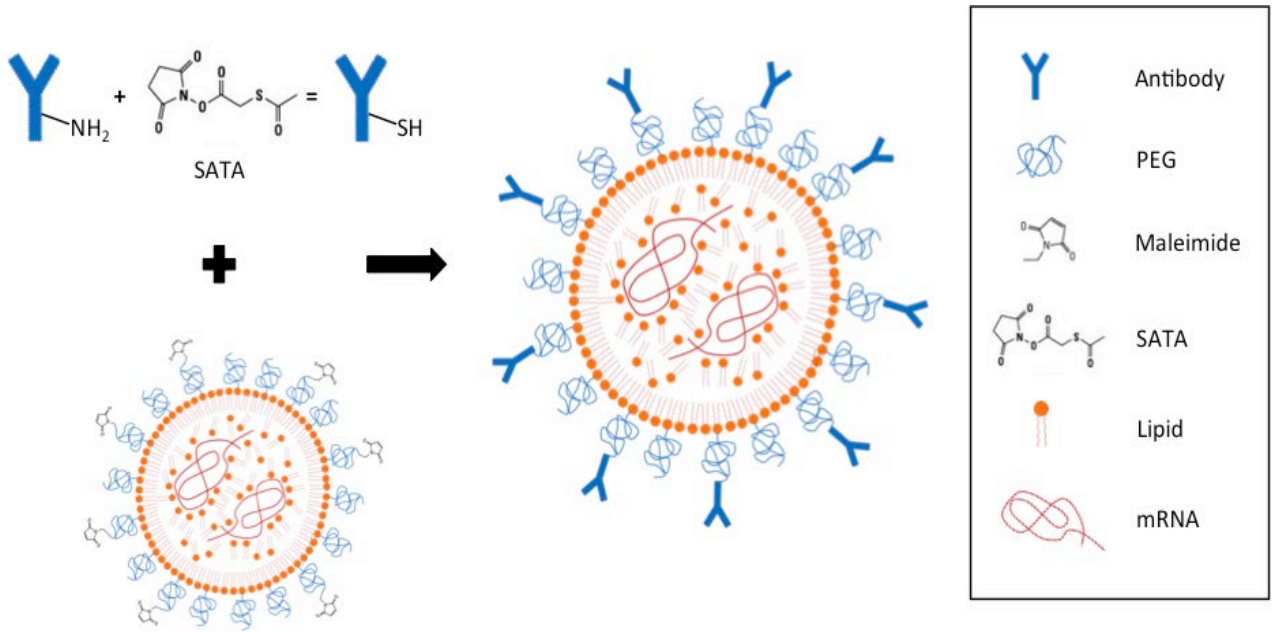
https://lymphoma-action.org.uk/about-lymphoma-treatment-lymphoma/stem-cell-transplants







Addition of targeting molecules to the surface of LNPs



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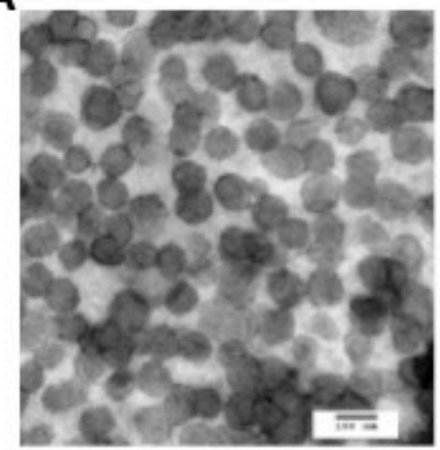
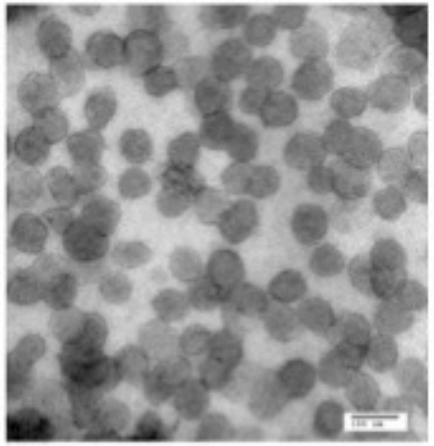
Physicochemical characterization of nanoparticles.

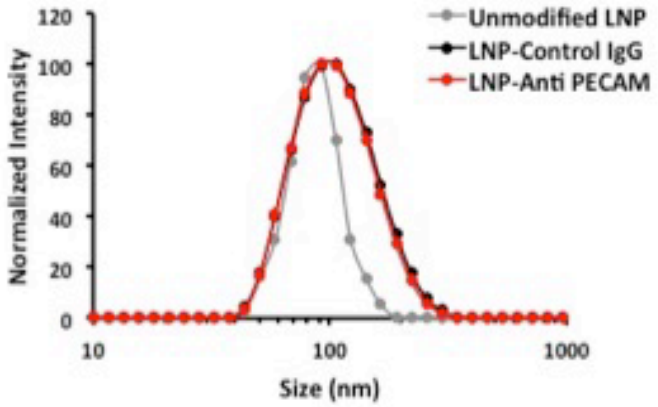
A Unmodified



B antibody-modified



C



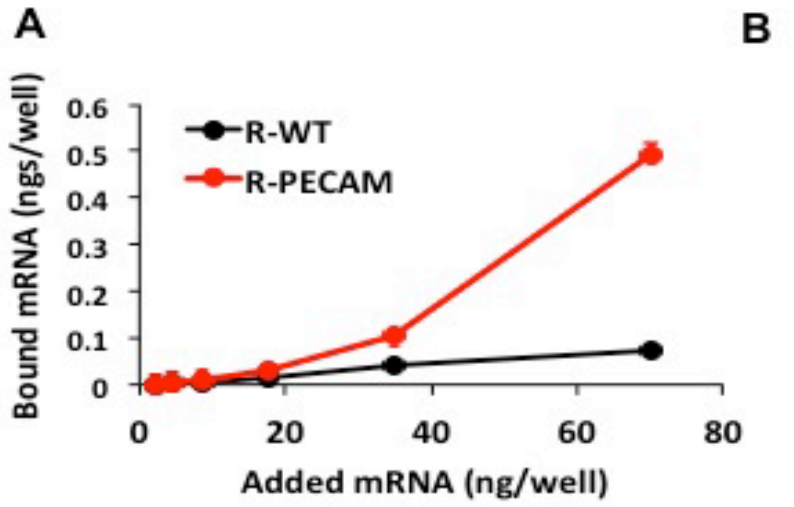
D

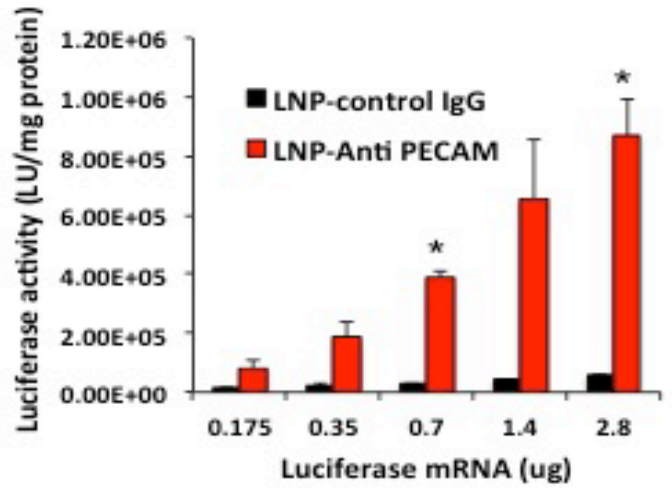
Formulation	Hydrodynamic diameter Z-average(nm)±SEM	Polydispersity index	Surface charge Zeta potential (mv)±SEM
Unmodified LNP	82.5±1.8	0.062	-6.49±0.2
LNP-Control IgG	101.9±0.73	0.197	-6.3±0.9
LNP-Anti PECAM	103.3±0.18	0.195	-4.12±0.1

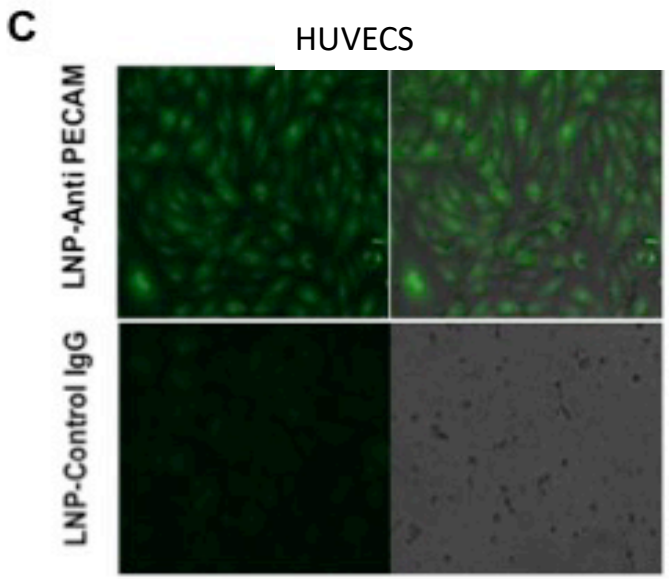
Penn Medicine

J Control Release, 2018 Dec 10;291:106-115.doi: 10.1016/j.jconrel.2018.10.015.

Binding and functional activity of PECAM-targeting LNPs.







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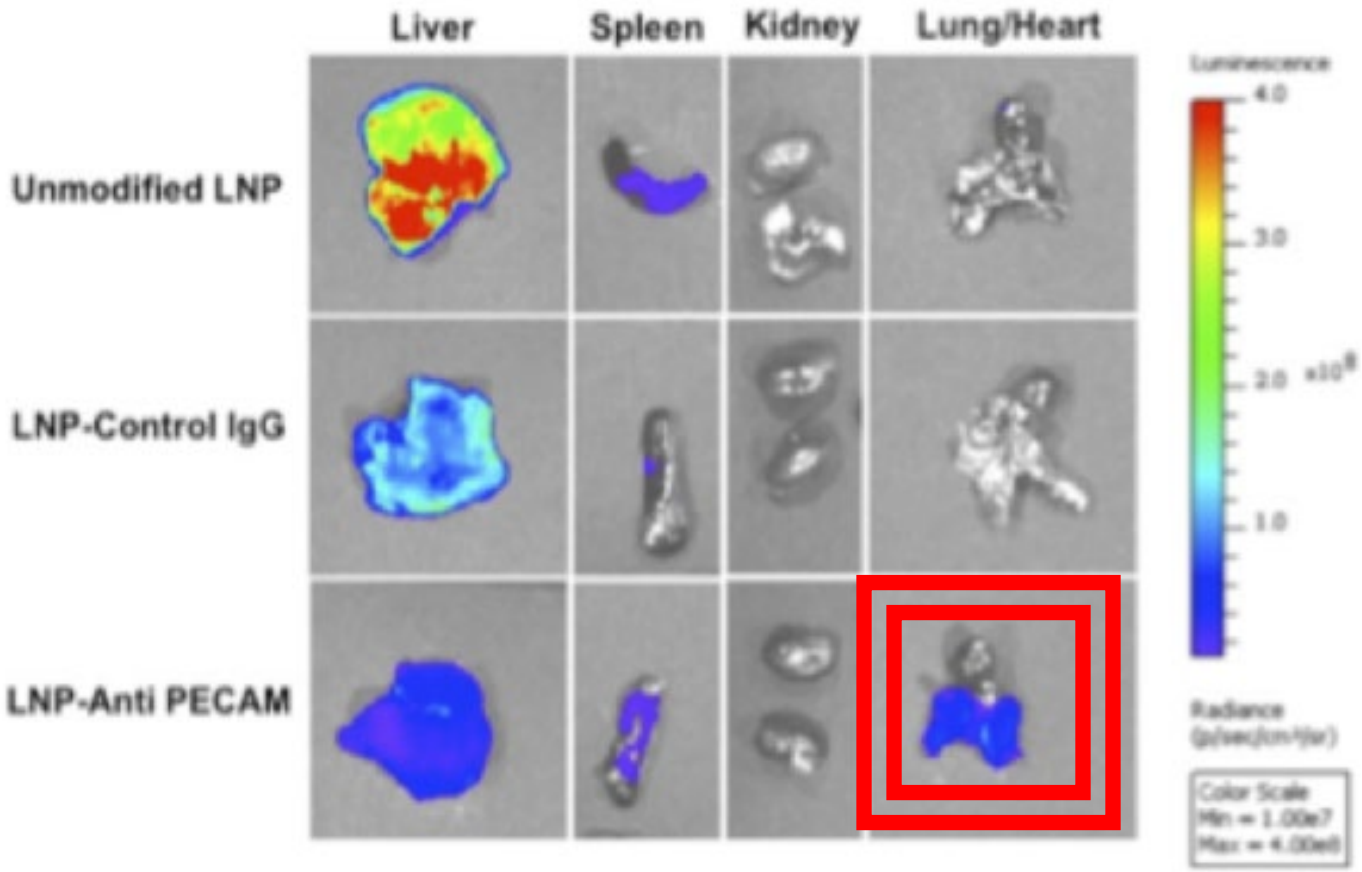
J Control Release, 2018 Dec 10;291:106-115.doi: 10.1016/j.jconrel.2018.10.015.

Functional activity of targeted luciferase mRNA-loaded nanoparticles to PECAM-1 *in vivo*.



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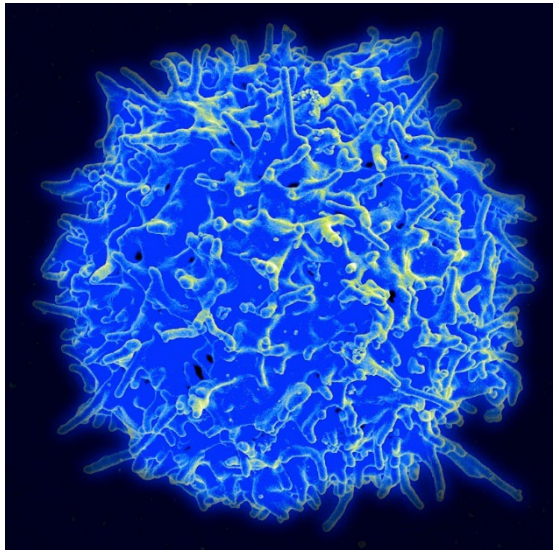
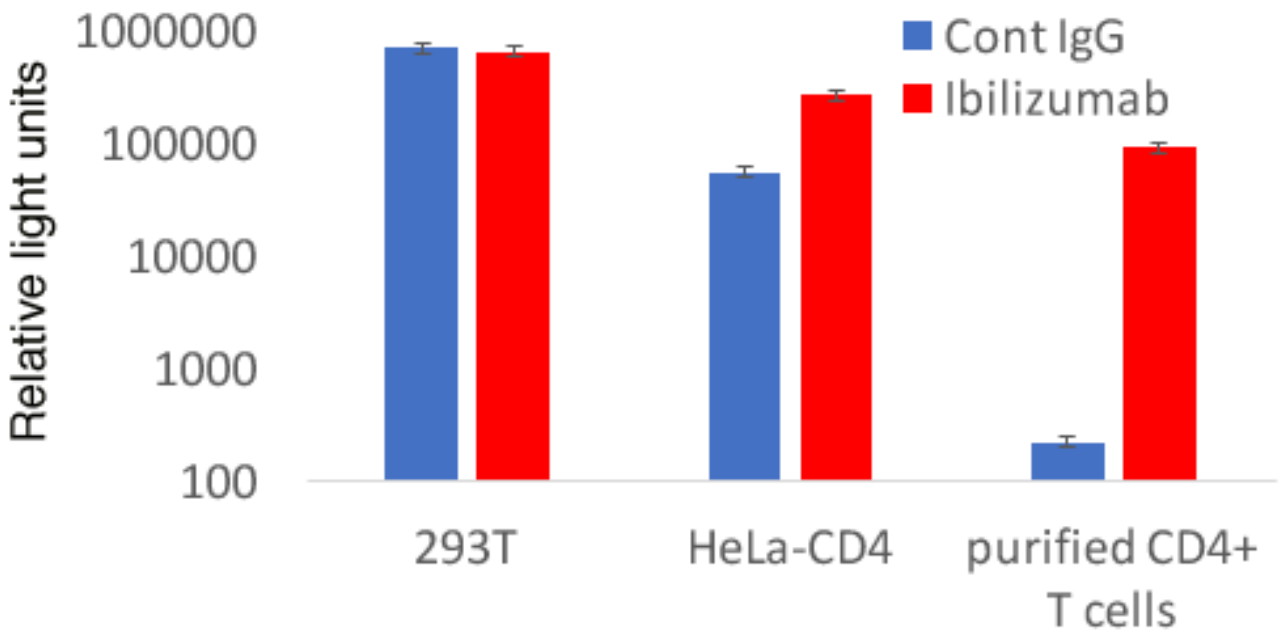
Targeted mRNA Therapeutics for T cell Modification



- Targeted delivery of mRNA-based therapeutics for modulation of specific immune cells, such as T lymphocytes, could be used for development of superior immunotherapeutics for cancer, infectious diseases, and other applications.
- To date, T cell modification has required extraction of autologous T cells, expansion, and genomic editing ex vivo, which is expensive and time-consuming.
- The key challenge, however, has been the lack of an efficient delivery platform, as T cells are known to be hard to transfect.

- We developed a T cell targeting platform which has great potential in many in vivo T cell manipulation-based applications by making T cell targeted therapeutic mRNA delivery possible. T cell targeting can be used for a variety of applications such as CAR-T cell therapy and viral eradication treatments.

Ibilizamab targeted LNPs deliver luciferase mRNA to CD4+ cells



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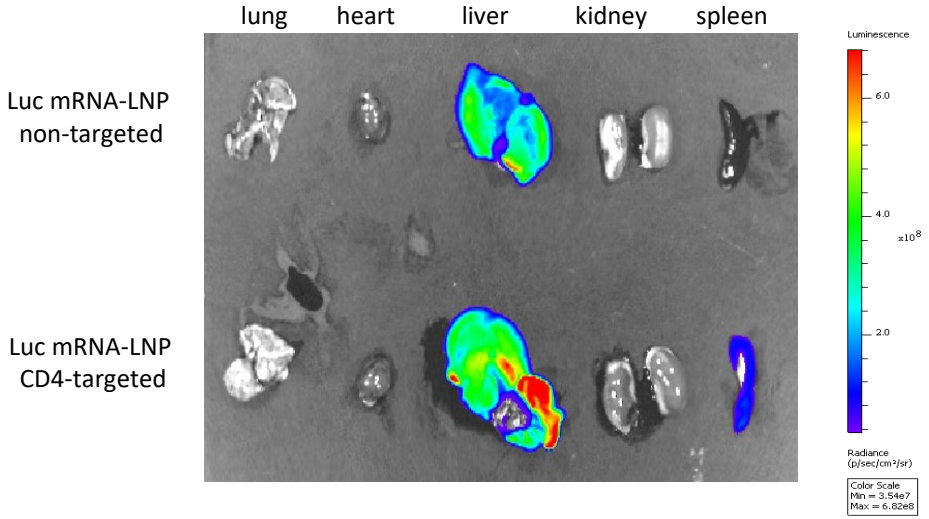
Mol Ther. 2021 Nov 3;29(11):3293-3304. doi:10.1016/j.ymthe.2021.06.004.

Organ imaging after intravenous mRNA-LNP injection of WT mice.



Animals were i.v. injected with 7 µg of mRNA-LNPs and luminescence was measured 4 hours later.
Notice splenic translation of CD4 targeted LNPs

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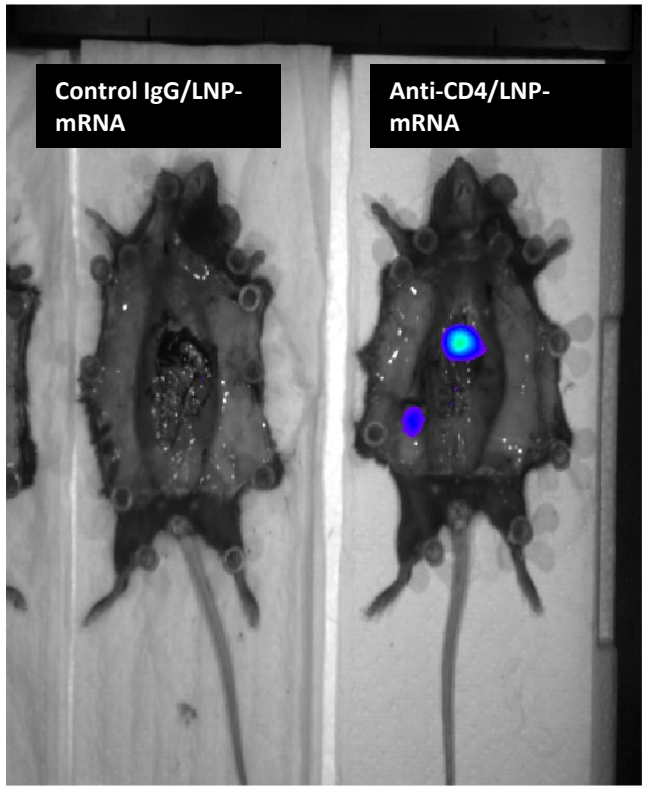
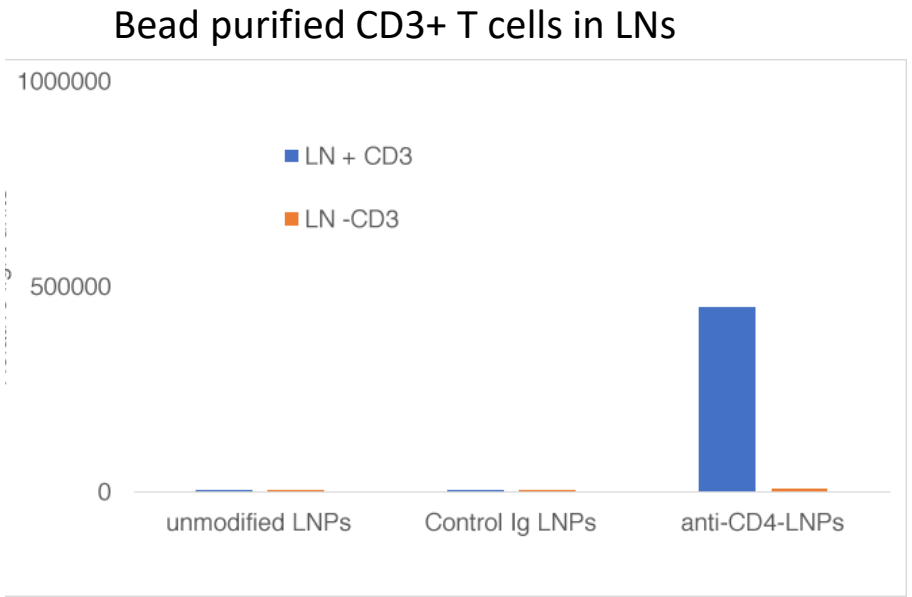
Mol Ther. 2021 Nov 3;29(11):3293-3304. doi:10.1016/j.ymthe.2021.06.004.

Lymph nodes express the LNP delivered luciferase mRNA with anti-CD4 targeting, organs removed.





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Mol Ther. 2021 Nov 3;29(11):3293-3304. doi:10.1016/j.ymthe.2021.06.004.

Delivery of LNP-complexed Cre-mRNA to Ai6 mice in vitro and in vivo

- Ai6 mice carry a genetic element encoding ZsGreen reporter gene regulated by a STOP cassette
- STOP cassette is flanked by loxP sites
- Cre excises the STOP cassette, allowing ZsGreen expression
- Green cells can be analysed with flow cytometry

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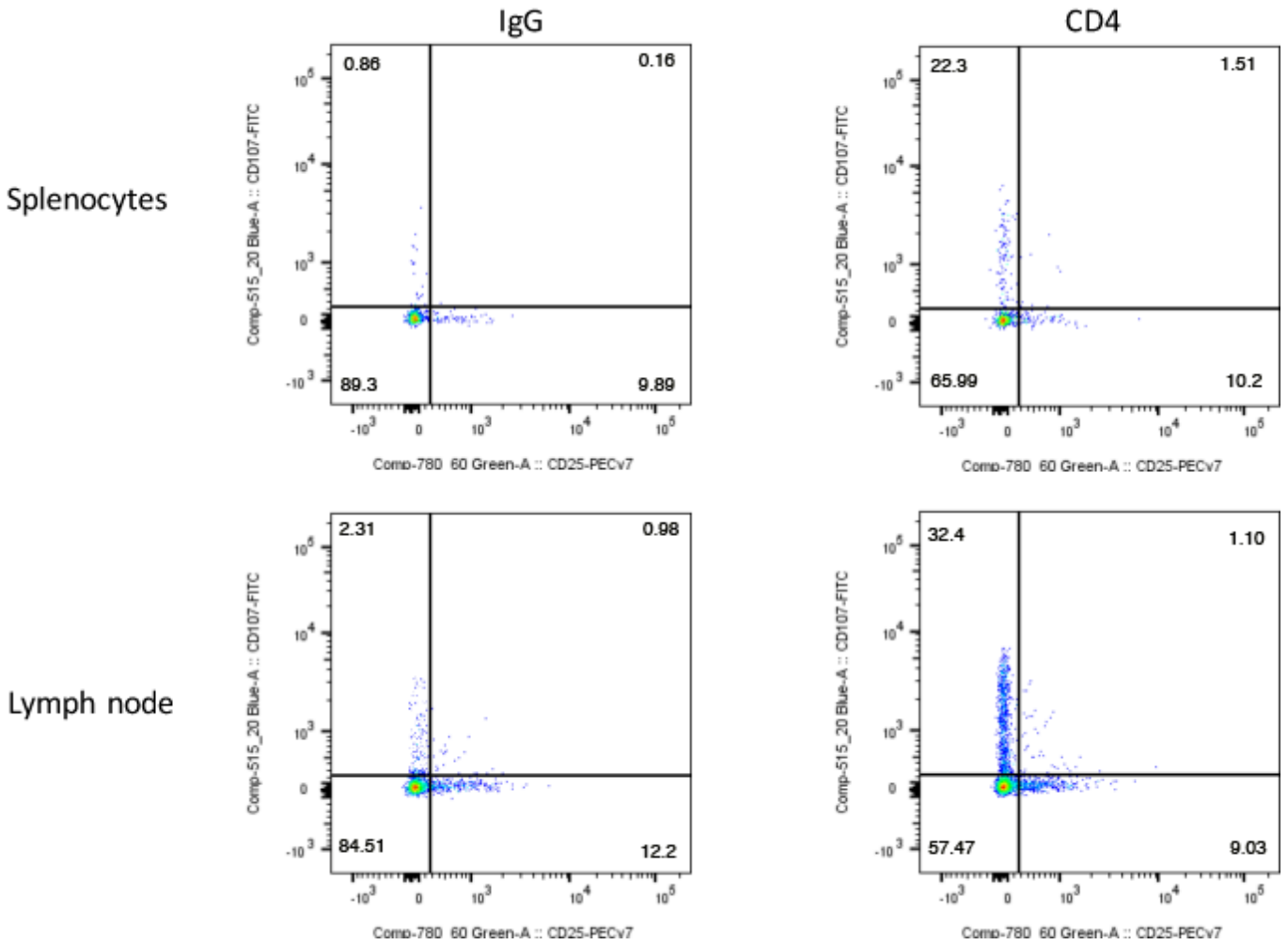
Mol Ther. 2021 Nov 3;29(11):3293-3304. doi:10.1016/j.ymthe.2021.06.004.

In vivo delivery of IgG and anti-CD4 conjugated LNPs.



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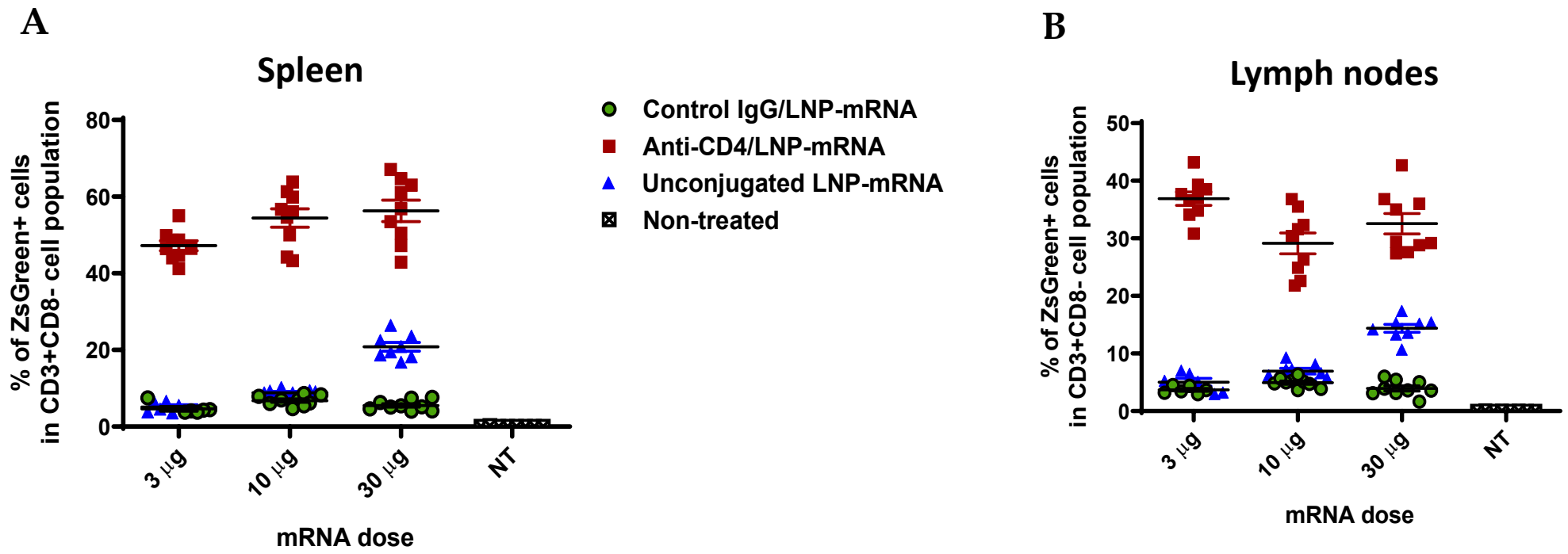
Mol Ther. 2021 Nov 3;29(11):3293-3304. doi:10.1016/j.ymthe.2021.06.004.

CD4+ T cell Targeting of mRNA-LNP
(CD4 targeting of Cre recombinase mRNA-LNP)



Ai6 mice received Cre mRNA-LNPs at doses of 3, 10, and 30 µg via IV administration. Spleens and lymph nodes were harvested at 24h post treatment and % of ZsGreen1+ cells in the CD3+CD8- cell population were determined in splenic (A) and lymph node (B) single cell suspensions using flow cytometry.

Mol Ther. 2021 Nov 3;29(11):3293-3304. doi:10.1016/j.ymthe.2021.06.004.

Conclusions.

- CD4-targeting allows uptake and translation of mRNA.
- Anti-CD4-targeted LNPs are specifically delivered to and translated by $CD4^+$ T cells in lymph nodes and spleen.
- The delivery of cre mRNA to LoxP fluorescent protein mice demonstrates targeting of CD4+ T cells and gene modifying activity in targeted cells in vivo.
- We propose to use cas9 or CPF1 gene editors to knock out HIV provirus in latently infected cells after in vivo treatment, to insert bnAbs by homologous recombination into hepatocytes, and CAR-Ts to T cells or bone marrow stem cells in vivo.
- We have developed similar targeting of lung, heart, brain, all T cells, bone marrow stem cells.

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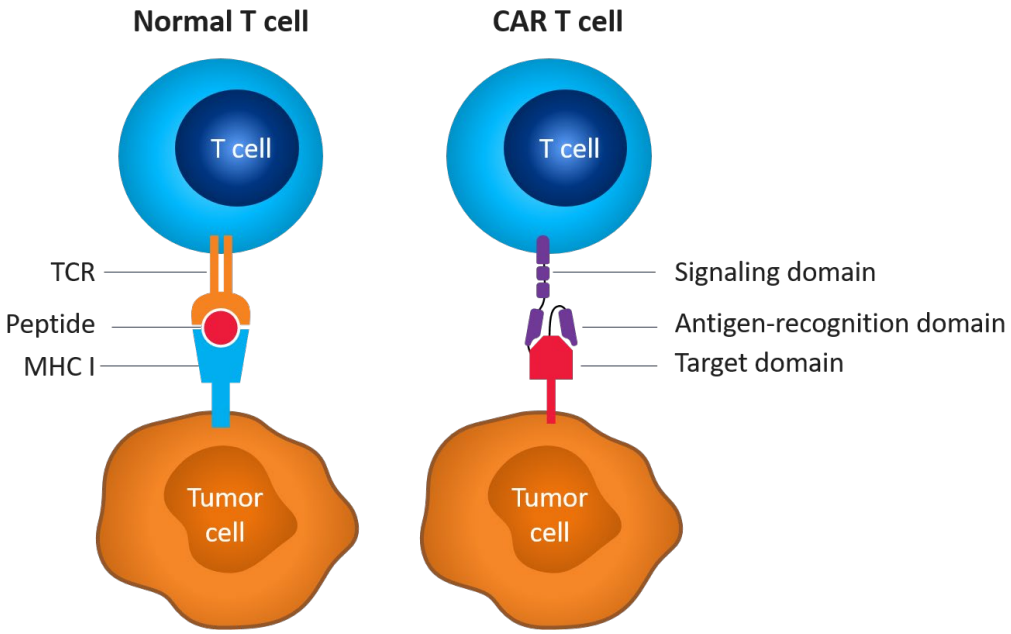
T cell targeting for *in vivo* generation of CAR T cells



- One of the most relevant applications of cancer immunotherapeutics are CAR T cell therapies.
- Currently, CAR T cells are generated ex vivo, which is costly and takes a long time. Additionally, it is not a treatment option for patients with highly malignant cancers or with very low T cell counts.
- There is a vital need for development of in vivo T cell-targeted mRNA delivery systems for robust and rapid generation of CAR T cells.
- mRNA-based CAR T cell therapeutics could also provide a safer platform by reducing the risk of CAR T cell-induced toxicities because of their transient nature, as well as avoiding the risk of genomic integration.

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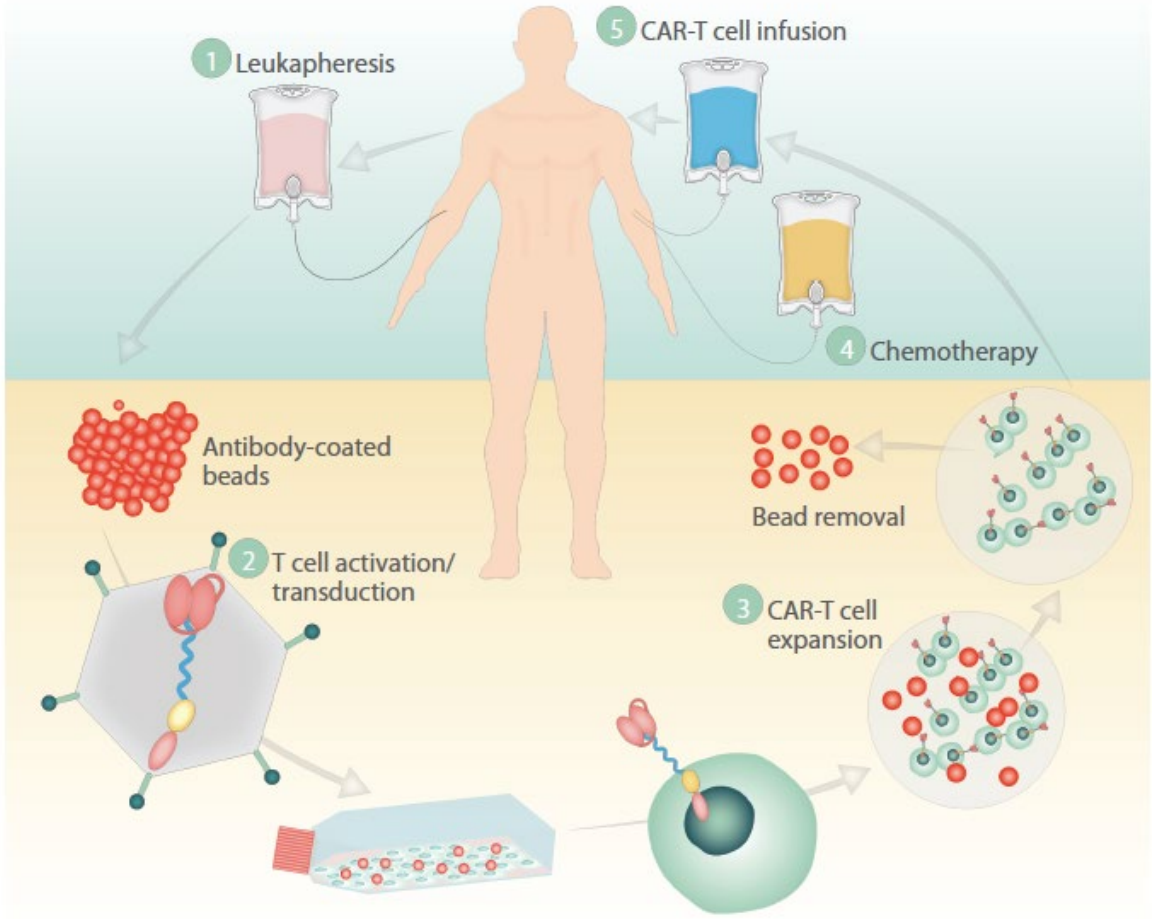
Current chimeric antigen receptor (CAR)-T cell therapy

- T cells are removed from the patient's blood
- Engineered to express a chimeric antigen receptor
- Reprograms T cells to target tumor cells



Citation: Buechner J, Kersten MJ, Fuchs M, Salmon F, Jäger U. Chimeric Antigen Receptor-T Cell Therapy: Practical Considerations for Implementation in Europe. *HemaSphere*, 2018;2:1. http://dx.doi.org/10.1097/HS9.0000000000000018

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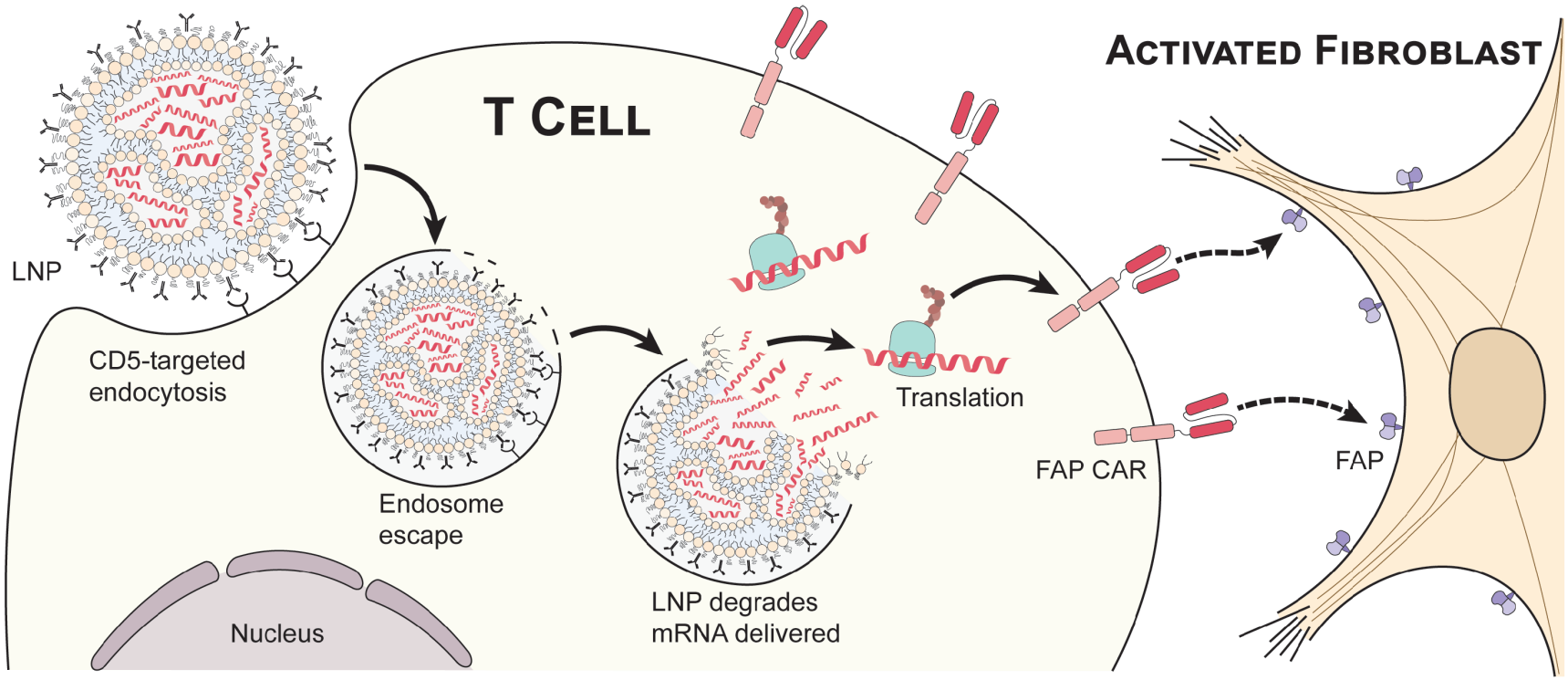
In vivo CAR T cell therapy



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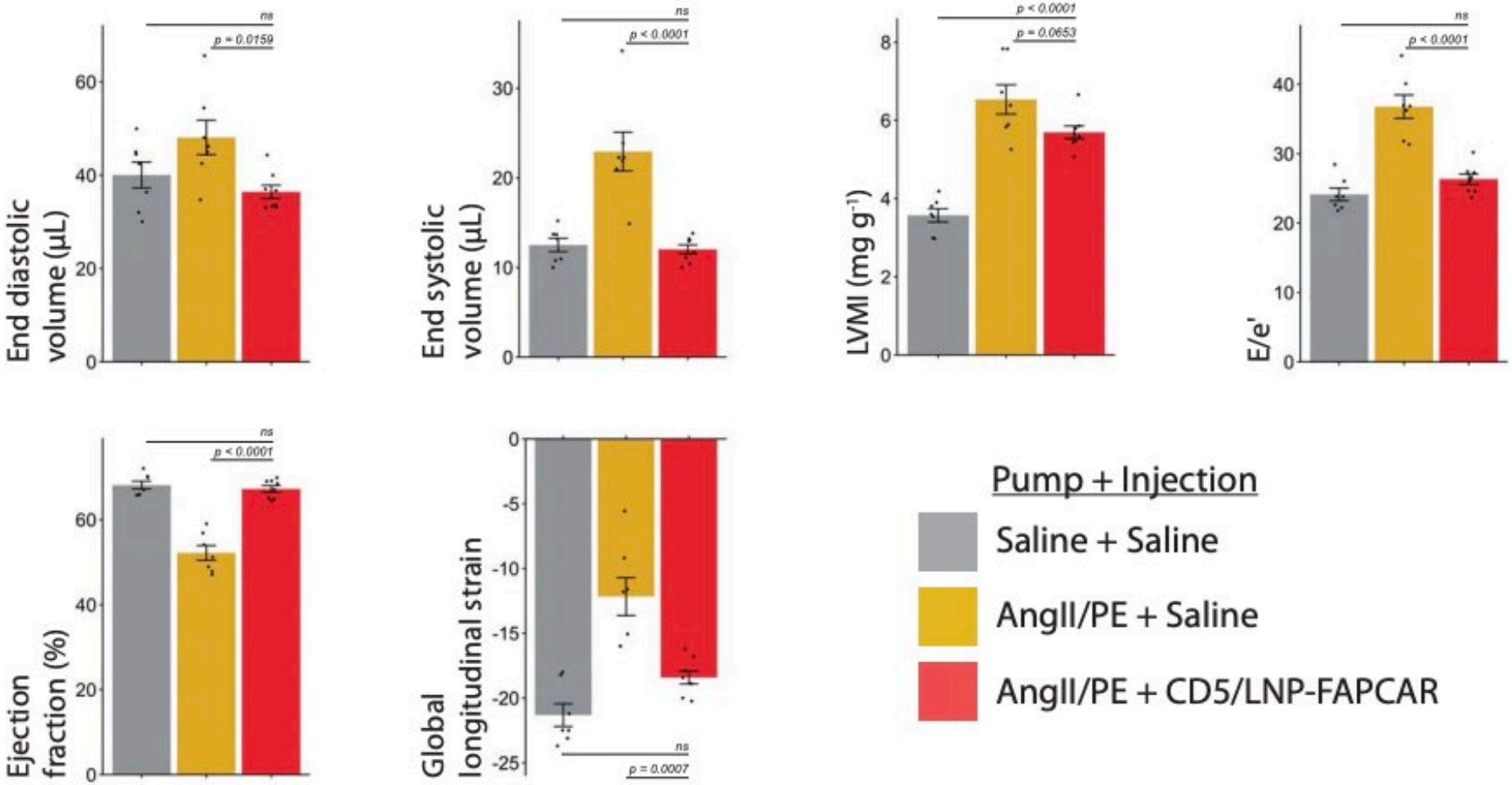
Science. 2022 Jan 7;375(6576):91-96. doi: 10.1126/science.abm0594.

In vivo produced FAPCAR T cells improve cardiac fibrosis after injury



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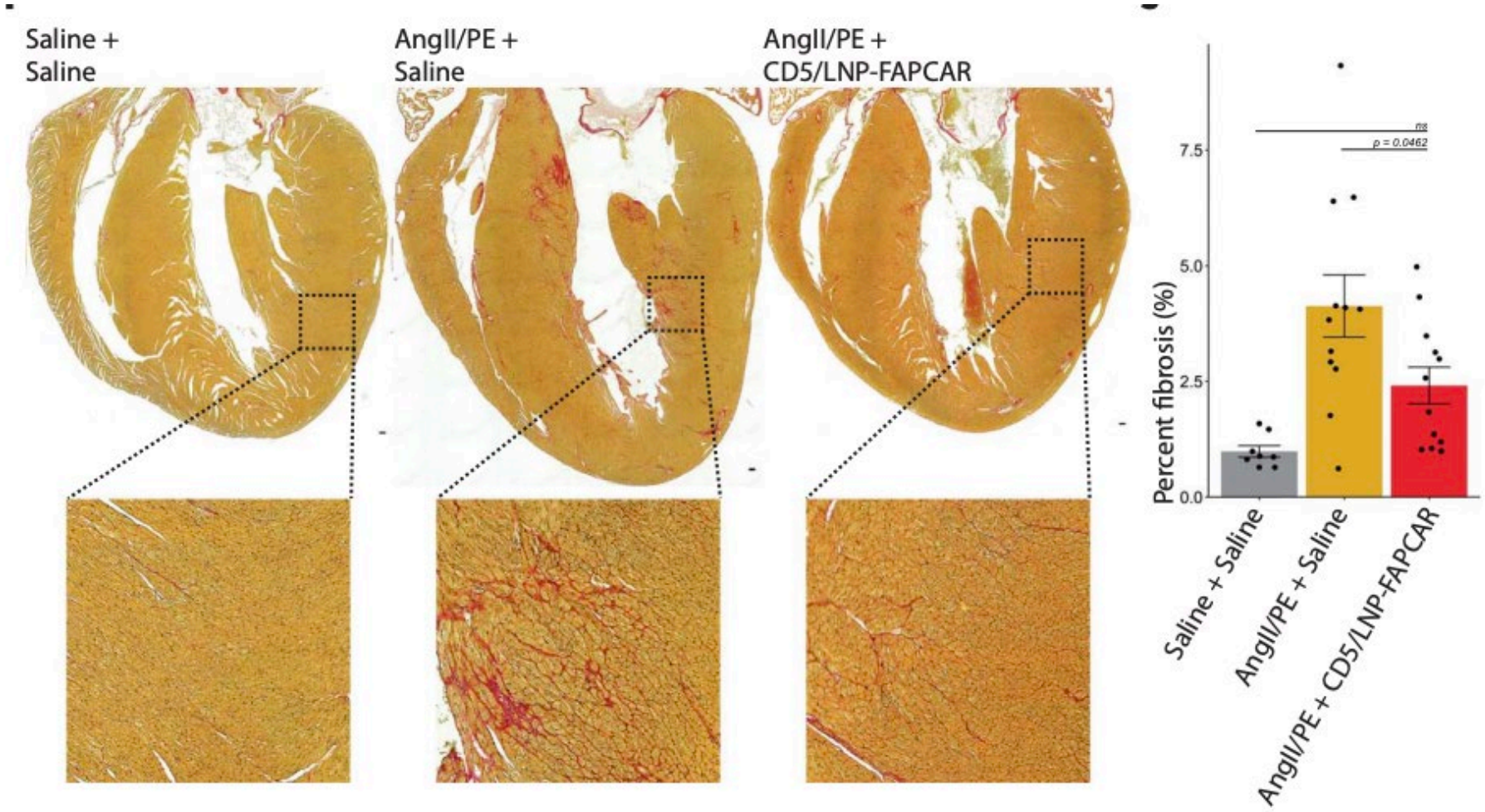
Science. 2022 Jan 7;375(6576):91-96. doi: 10.1126/science.abm0594.

CD5-LNP FAP CARs reduce cardiac fibrosis



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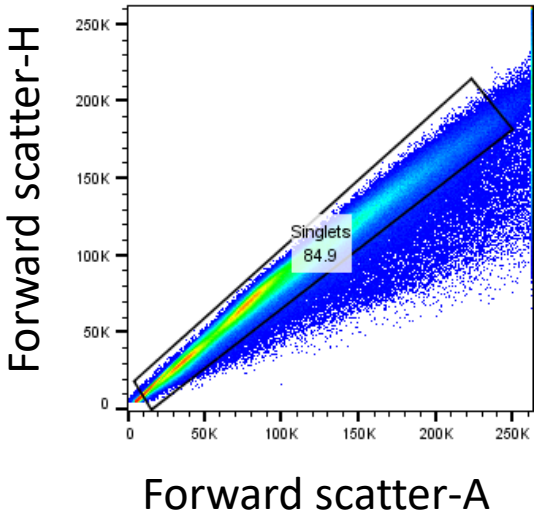
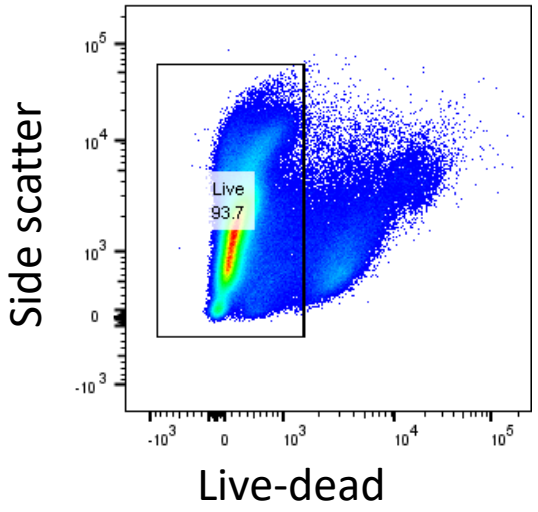
Science. 2022 Jan 7;375(6576):91-96. doi: 10.1126/science.abm0594.

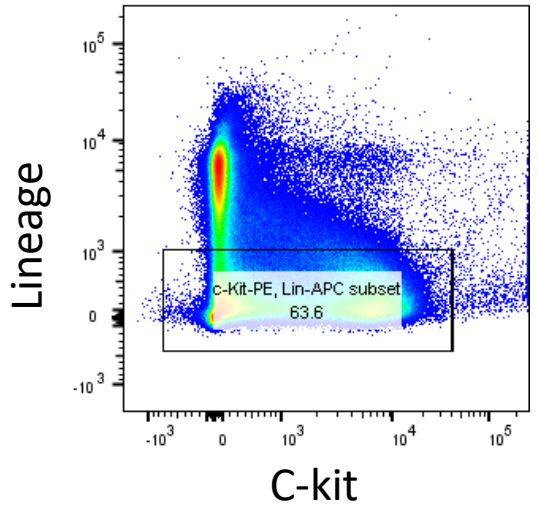
Bone marrow stem cell targeting

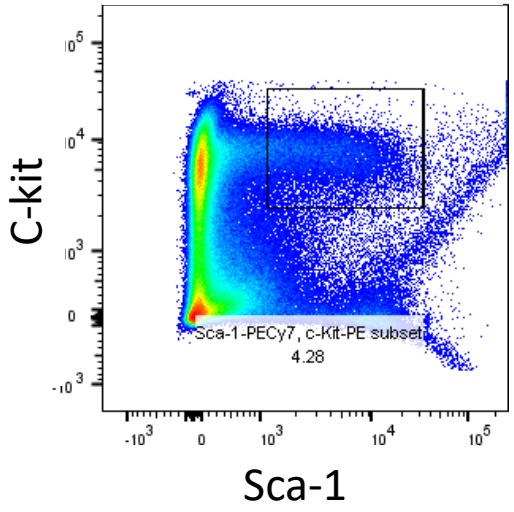
- Many bone marrow stem cell congenital defects have been identified, which continues to expand, some currently treated with bone marrow transplant or trials of ex vivo bone marrow stem cell gene therapy.

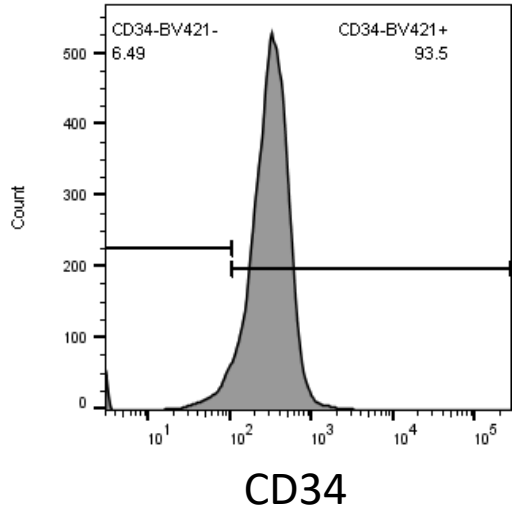
Stem cell targeting





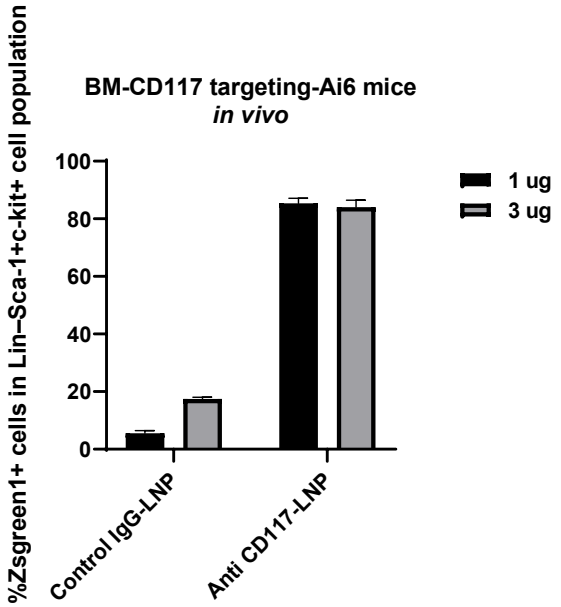


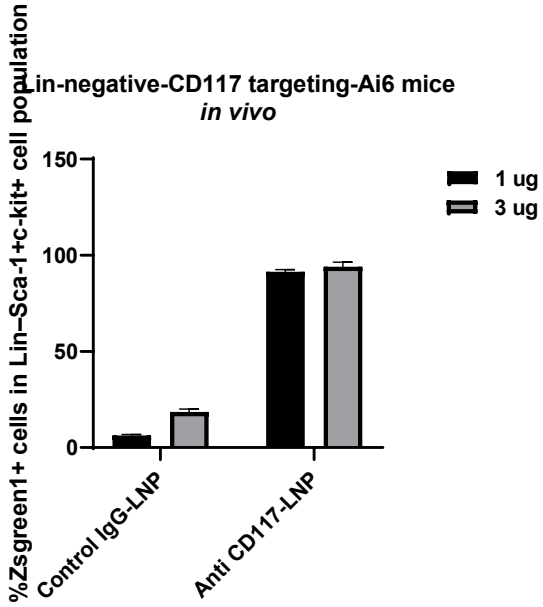




Total # of cells analyzed	Total # of CD34 positive (stem cells+progenitor cells)	Total # of CD34 positive stem cells
689595	14918	968

CD117 targeting-whole bone marrow and Lineage negative-enriched prep-*in vivo*





LNP-Cre mRNA was injected IV and ZsGreen signal was tracked with flow cytometry in Hematopoietic stem and progenitor cells (LSK, $Lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$).

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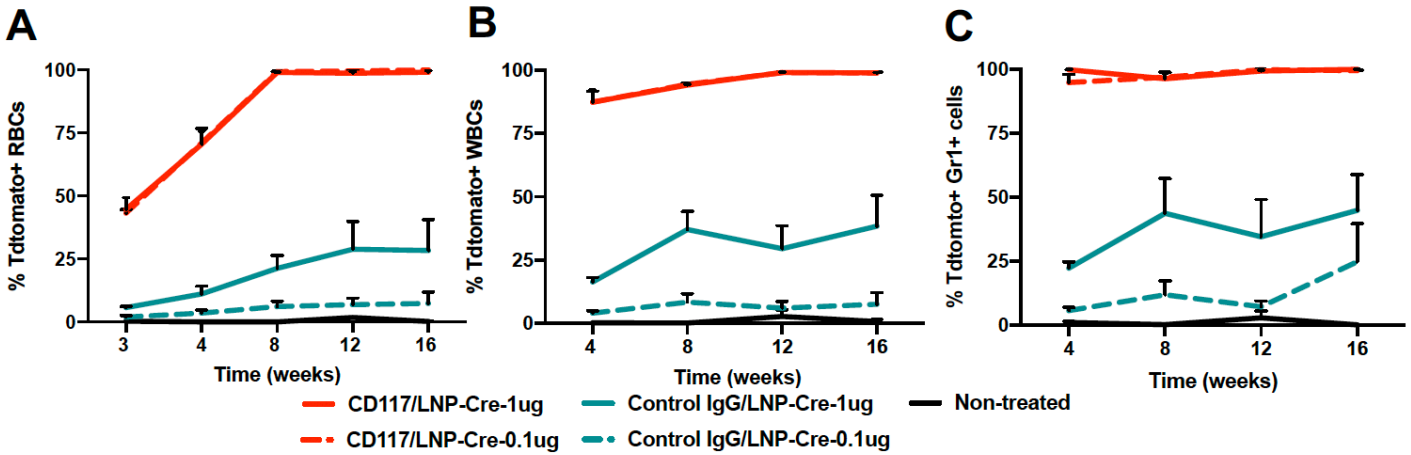
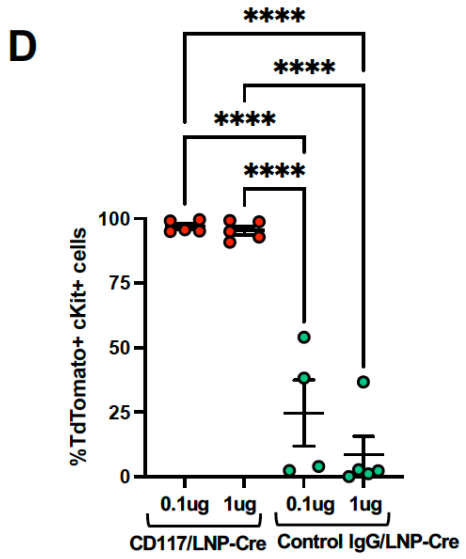
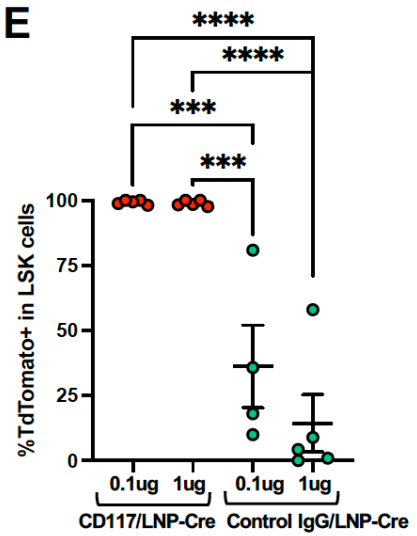
Nearly all bone marrow stem cells are gene edited by a single treatment of targeted LNPs







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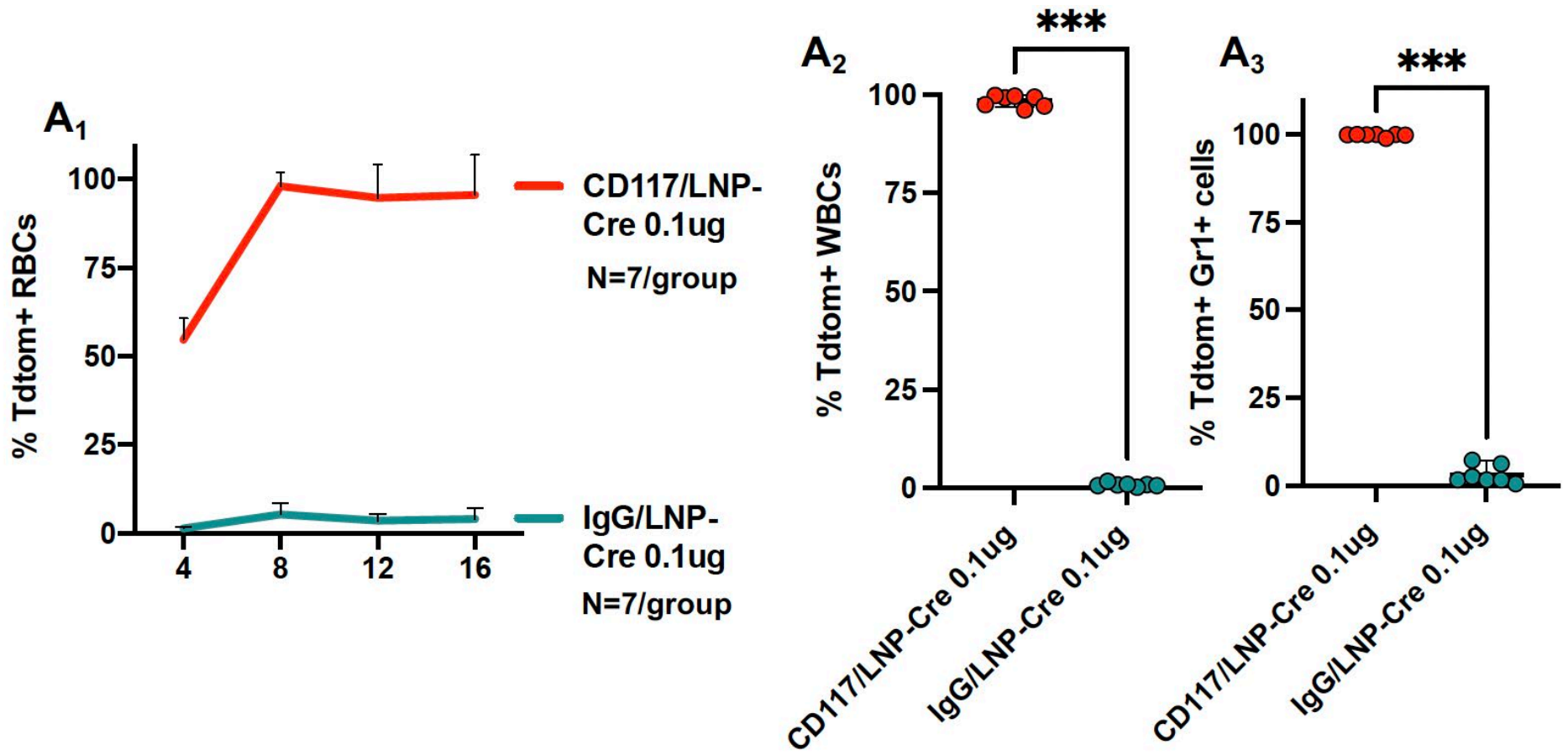
All repopulating bone marrow stem cells are gene edited by a single treatment of targeted LNPs as determined by secondary transplant.



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Potential uses of BM targeting

- Delivery of gene editing modalities to treat diseases, including sickle cell anemia, SCID, etc.
- Delivery of toxic genes to selectively deplete bone marrow stem cells for subsequent transplant.
- Delivery of congenitally absent proteins
- Delivery of therapeutic proteins

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