

Lipid-metal hybrid nanoparticle-mediated delivery of CRISPR/Cas9 for restructuring of tumor immune microenvironment

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Seoul National University

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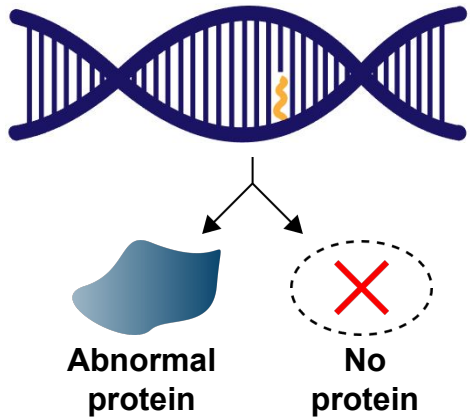
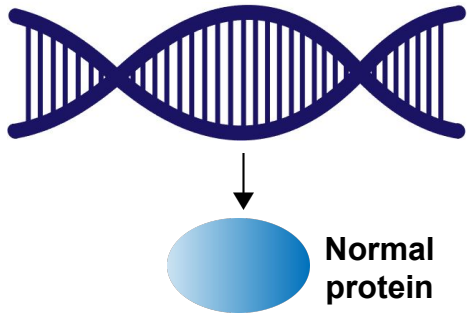
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Advanced Delivery Science

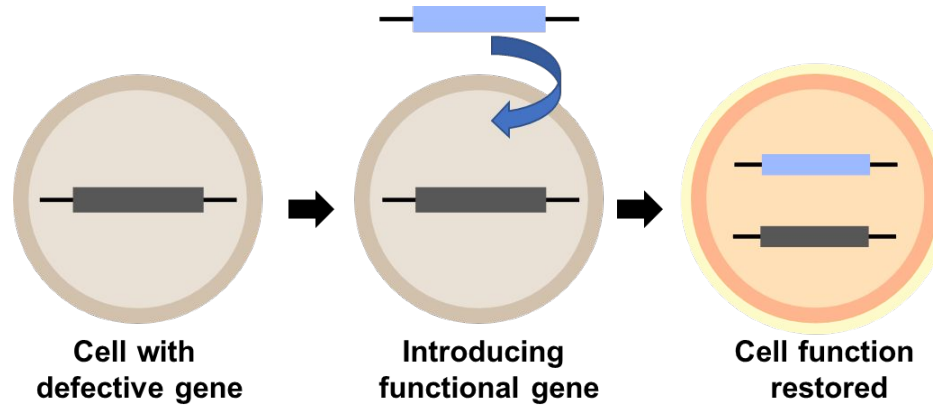


Principle of gene therapy

Genetic mutation



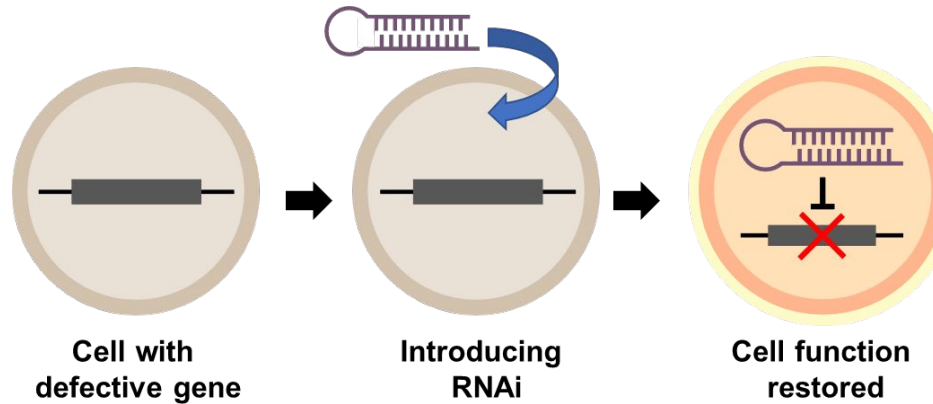
1.



Protein replacement therapy

- Plasmid
- mRNA
- Viral gene transfer

2.

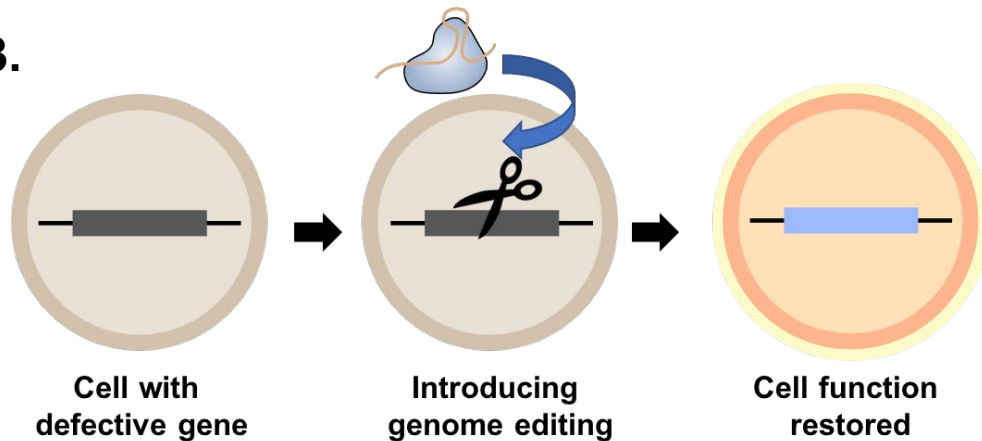


Gene silencing

- siRNA
- Anti-sense oligonucleotide (ASO)

Principle of genome editing

3.

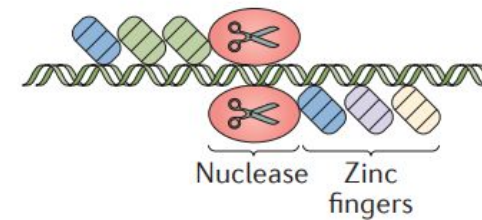


Genome editing

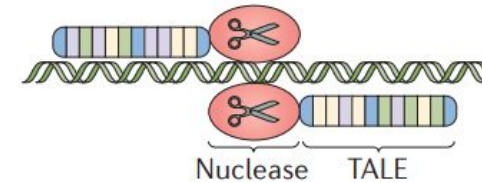
- Zinc finger nuclease (ZFN)
- Transcription activator-like effector nuclease (TALEN)
- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

Genome editing

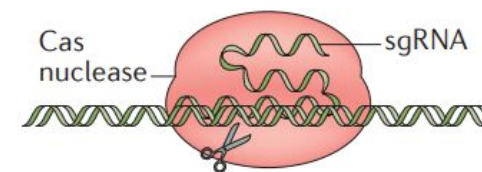
ZFN



TALEN

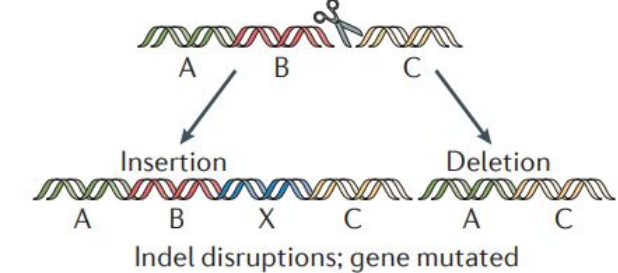


CRISPR-Cas

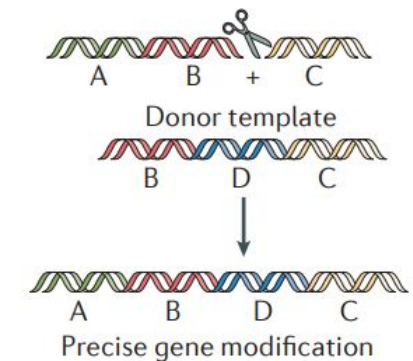


DSB repair

NHEJ



HDR



Yin et al., Nat. Rev. Drug Discov. (2017)

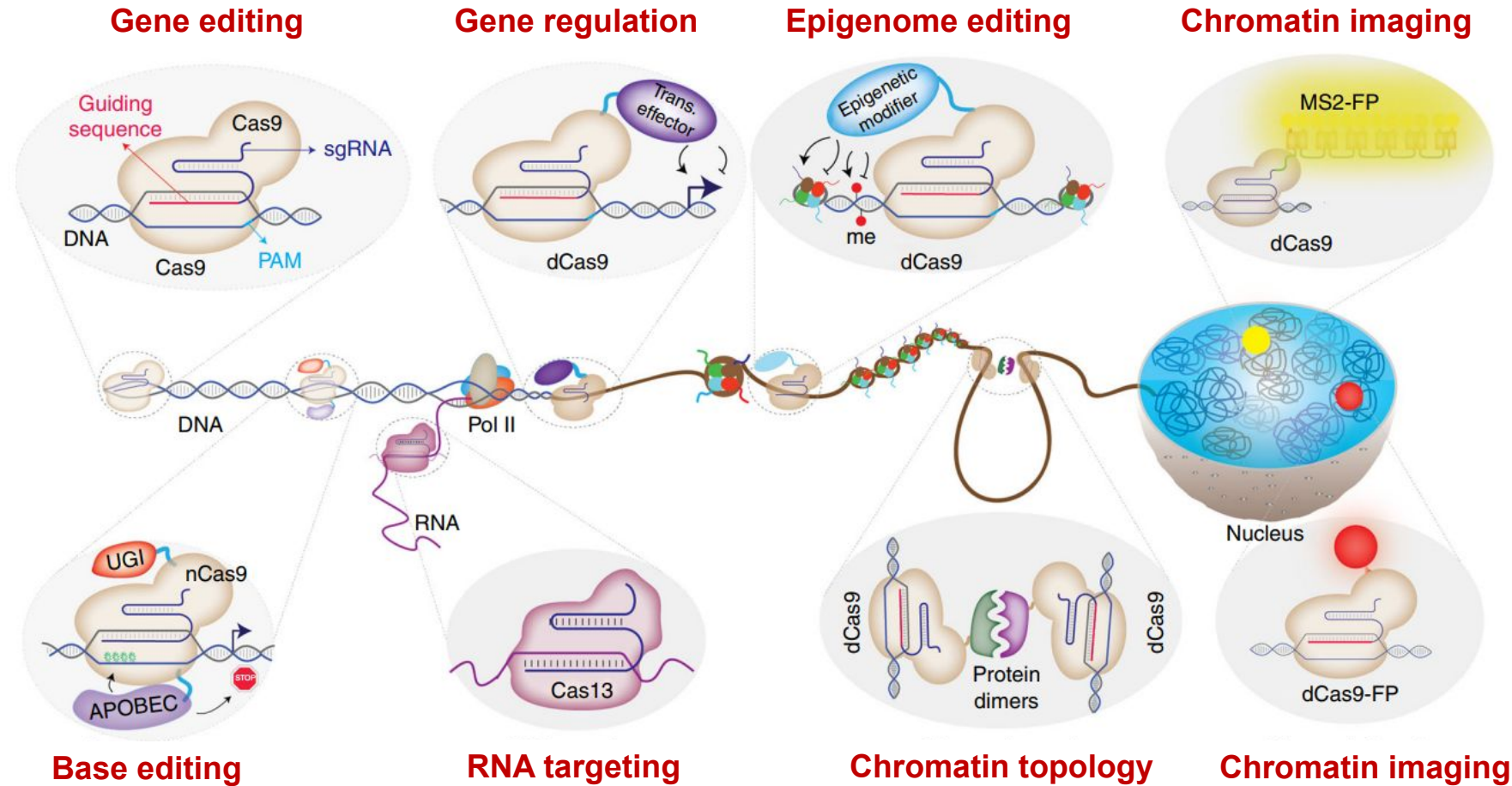


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Major application areas of CRISPR/Cas-based technologies



Adli, Nat. Commun. (2018)



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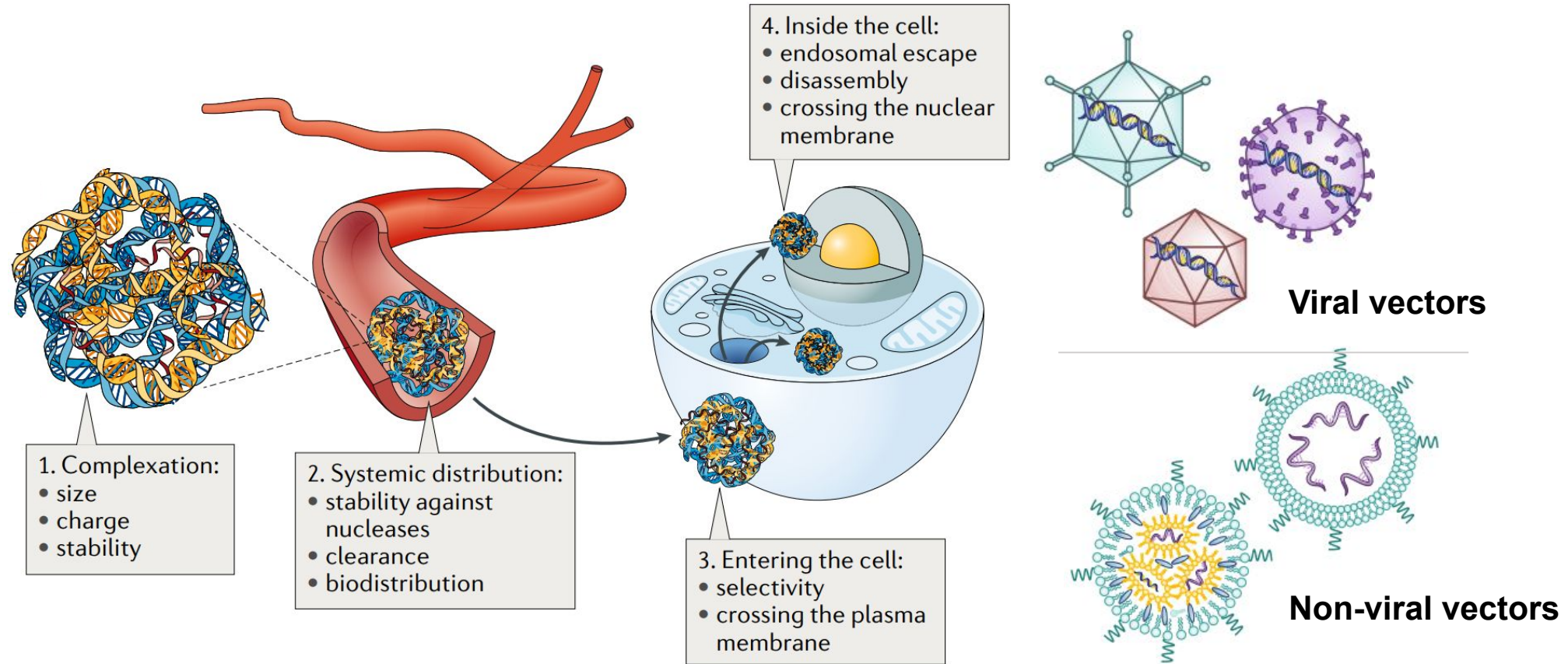
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Challenges in genome editing: “Delivery”

❑ **Low delivery efficiency** hampers the therapeutic application of genome editing

- CRISPR/Cas9 can be delivered as DNA, mRNA or ribonucleoprotein complex



Lostalé-Seijo et al., Nat. Rev. Chem. (2018)



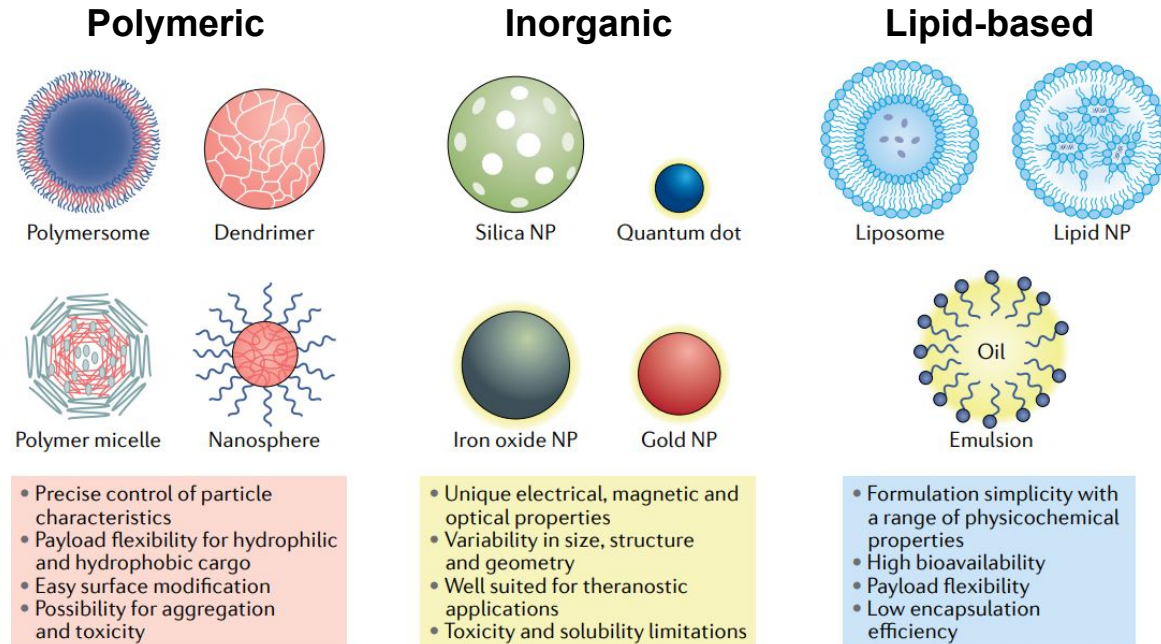
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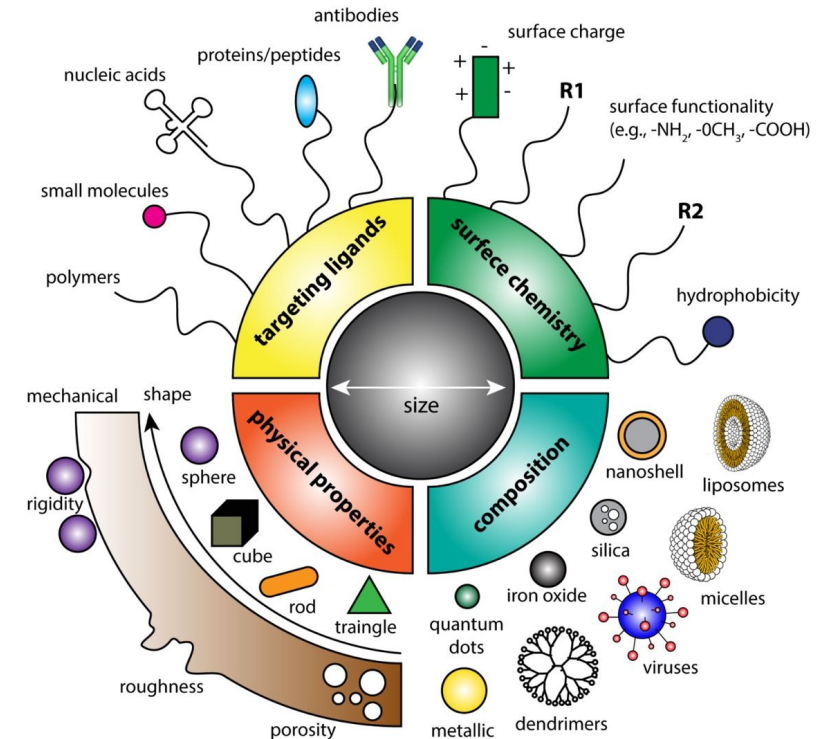
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Potential of nanomedicine for gene delivery

□ Application of nanotechnology in gene delivery



Mitchell et al., Nat. Rev. Drug Discov. (2021)



Shah et al., J. Drug Deliv. Sci. Technol. (2021)

- Improved drug **stability**
- **Functional modification**
- **Tuned pharmacokinetics**
- **Targeted** drug delivery
- **Sustained** delivery

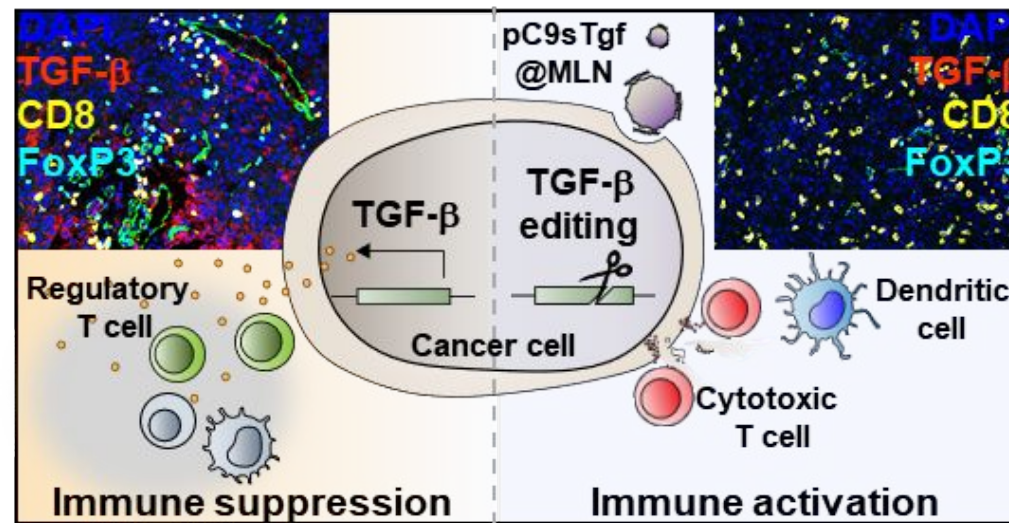


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CRISPR/Cas9 genome editing delivery system for restructuring of tumor immune microenvironment



Kim et al., ACS Nano (2021)



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Research background

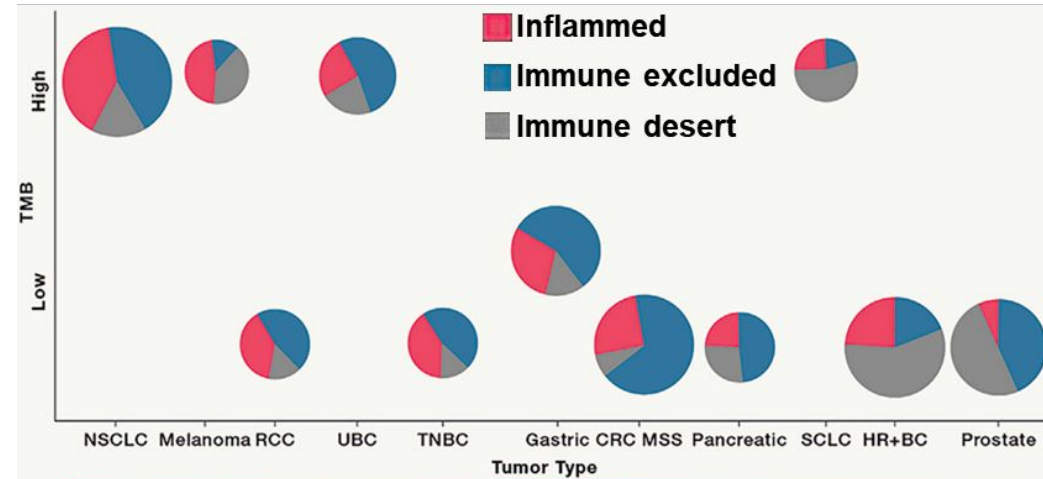
□ Limited efficacy of cancer immunotherapy : Immunosuppressive environment

Pharmaceutical Medicine
LEADING ARTICLE
Addressing Recent Failures in Immuno-Oncology Trials to Guide Novel Immunotherapeutic Treatment Strategies
Shazia K. Nakhoda¹ · Anthony J. Olszanski¹

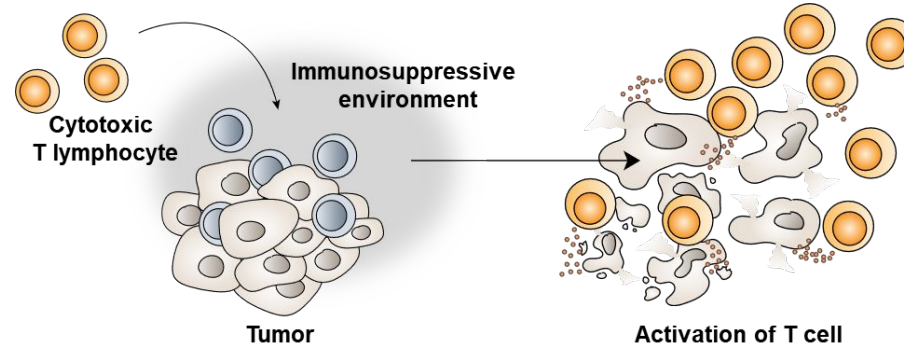
nature medicine LETTERS
<https://doi.org/10.1038/s41591-021-01331-8>
Evolution of delayed resistance to immunotherapy in a melanoma responder
David Liu^{1,2,23}, Jia-Ren Lin^{3,23}, Emily J. Robitschek^{1,2}, Gyulnara G. Kasumova⁴, Alex Heyde^{5,6}, Alvin Shi^{2,7}, Adam Kraya⁸, Gao Zhang^{9,10}, Tabea Moll¹¹, Dennie T. Frederick¹¹, Yu-An Chen³,

REVIEW ARTICLE **nature immunology**
<https://doi.org/10.1038/s41590-019-0433-y>
Mechanisms of immunotherapy resistance: lessons from glioblastoma
Christopher M. Jackson, John Choi and Michael Lim

Immunity **Perspective** **CellPress**
Top 10 Challenges in Cancer Immunotherapy
Priti S. Hegde¹ and Daniel S. Chen^{2,*}



Hegde et al., *Immunity* (2020)



- Overcoming the immunosuppressive environment is the key for therapeutic success

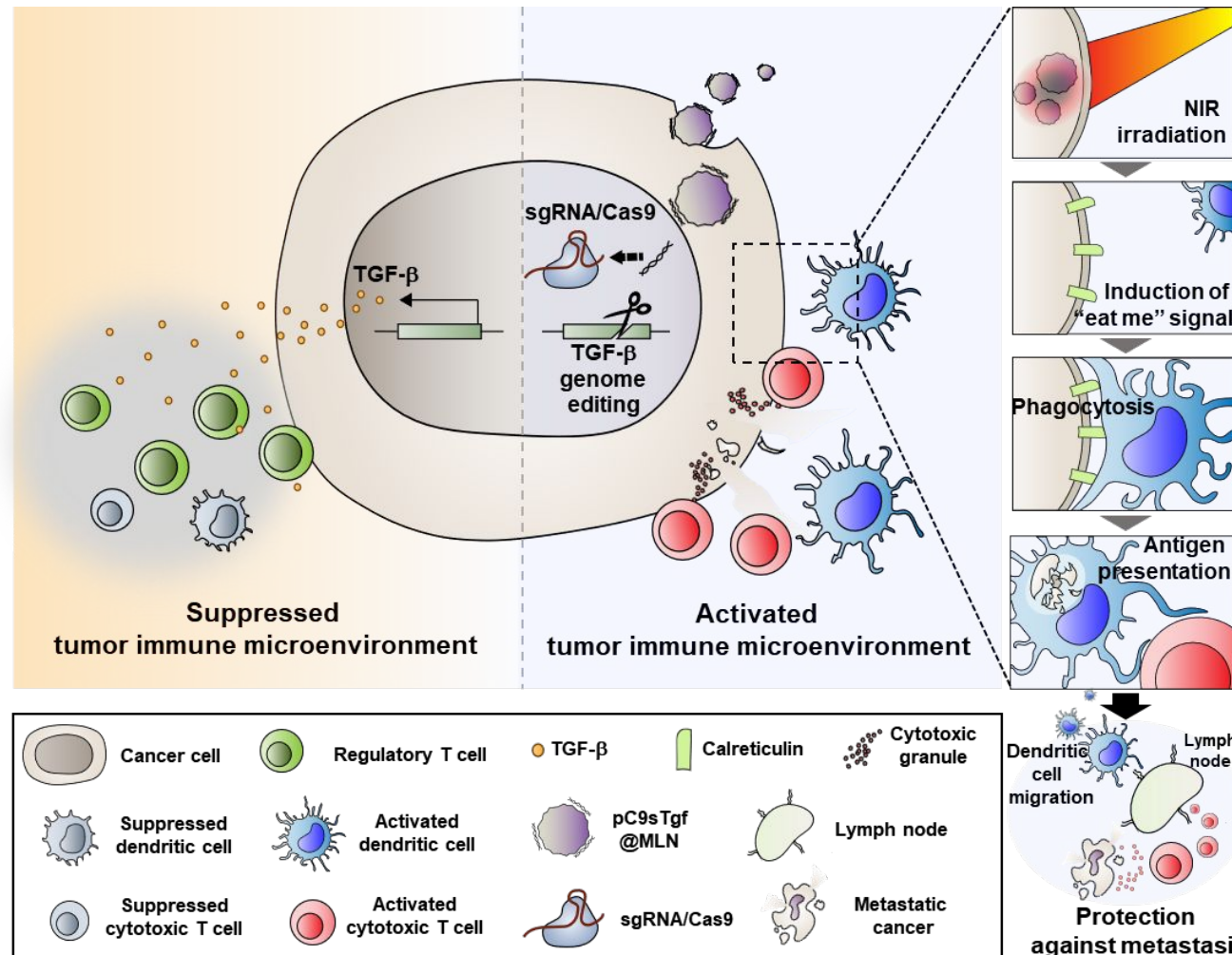


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Restructuring of tumor immune microenvironment



Multifunctional nanoparticle

TGF- β genome editing

Driving tumor immune microenvironment to "activated" state

+

Photothermal effect

Immunogenic cell death

Triggering anti-tumor immune response



Synergistic anti-tumor effect



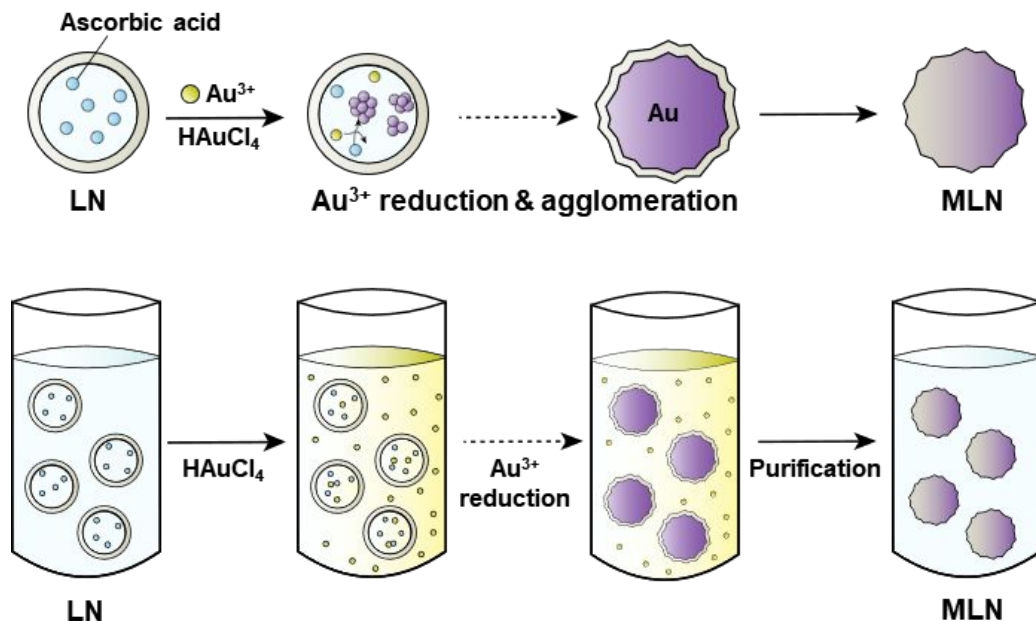
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Construction of MLN for synergistic anti-tumor effect

Nanoparticle synthesis

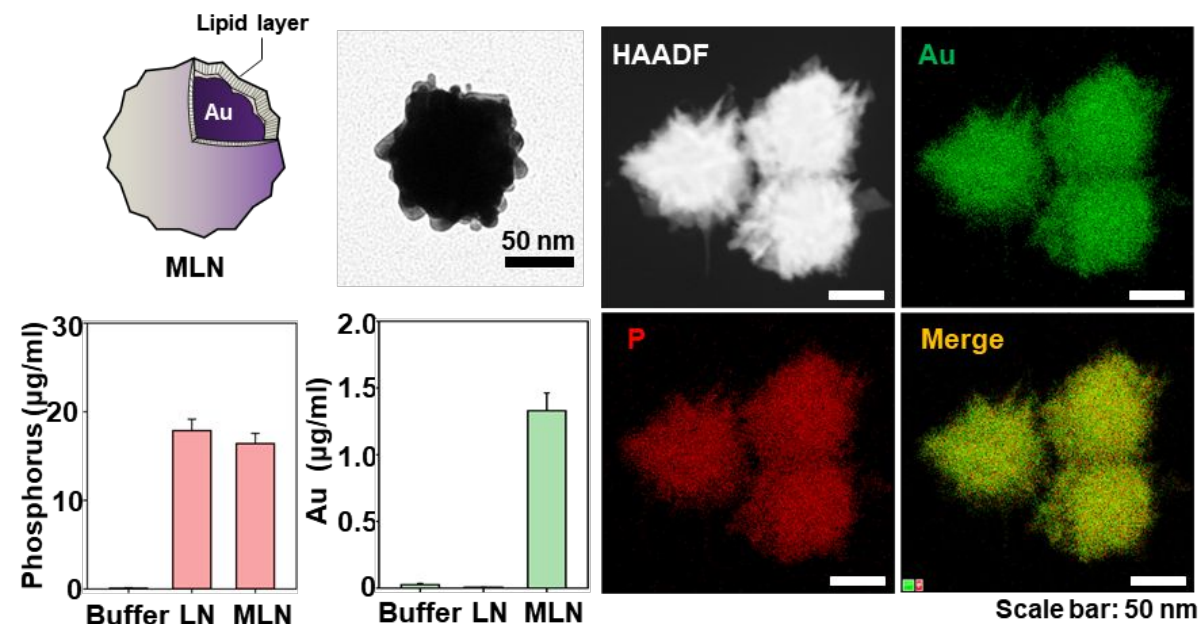


LN: Lipid-based nanoparticle

MLN: Metal-lipid hybrid nanoparticle

Lipid: EDOPC, DC-Chol, DPhPE

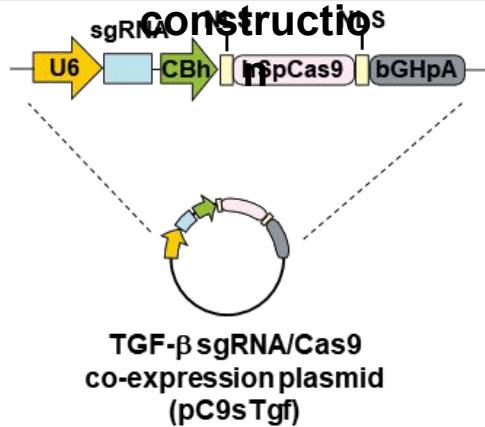
Morphological and elemental analysis



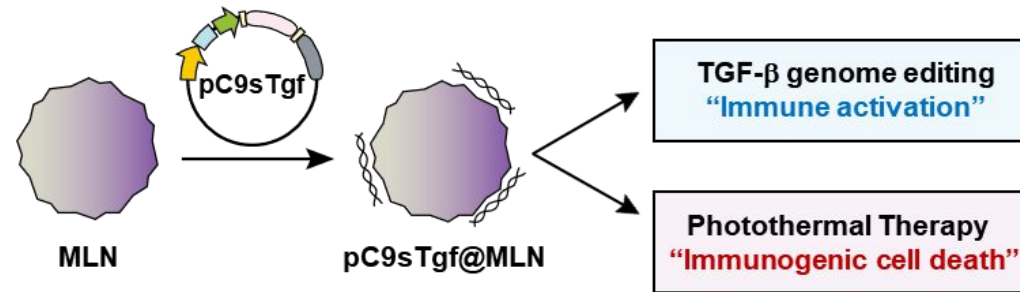
Characteristic multi-rounded shape filled with gold metal

Construction of MLN for synergistic anti-tumor effect

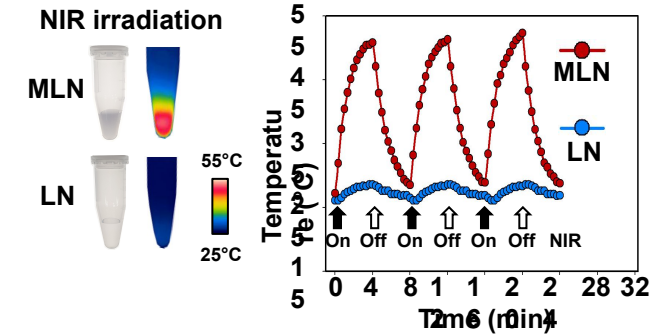
Plasmid construction



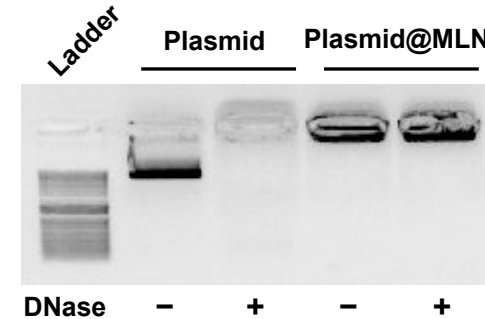
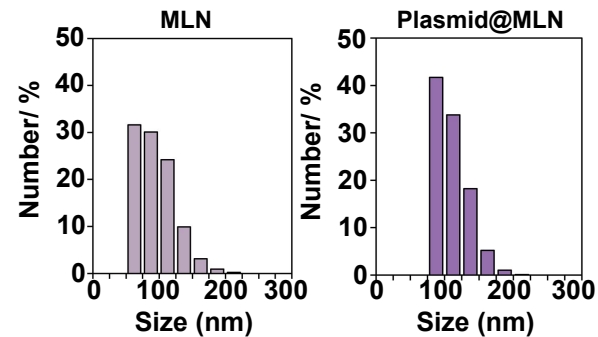
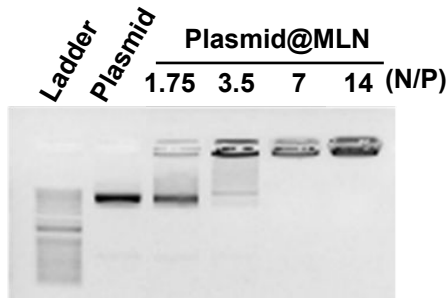
MLN-mediated synergistic anti-tumor effect



Photothermal effect



DNA loading and protection

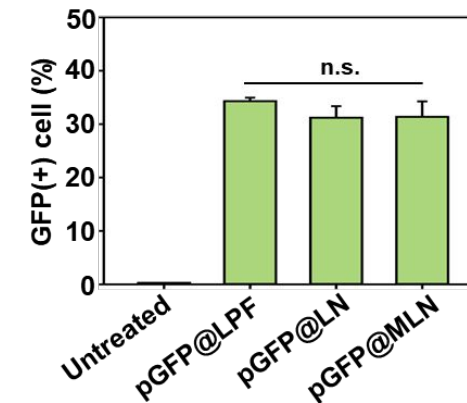
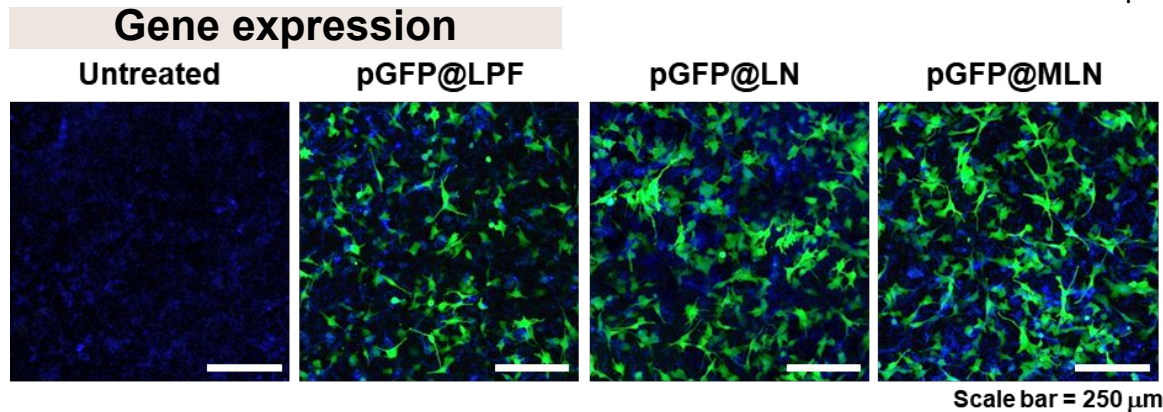
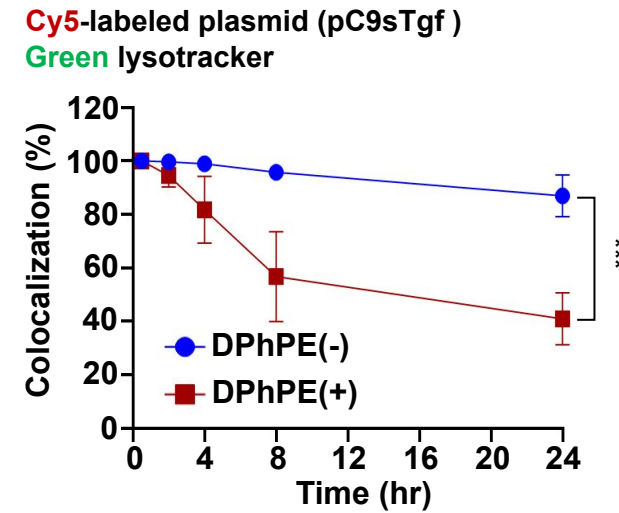
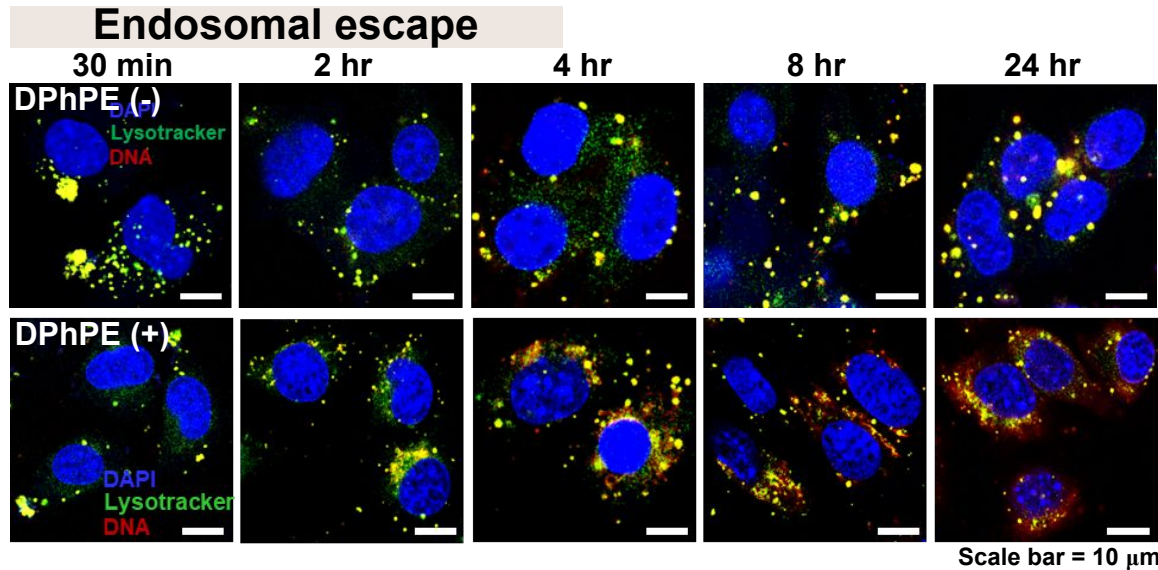


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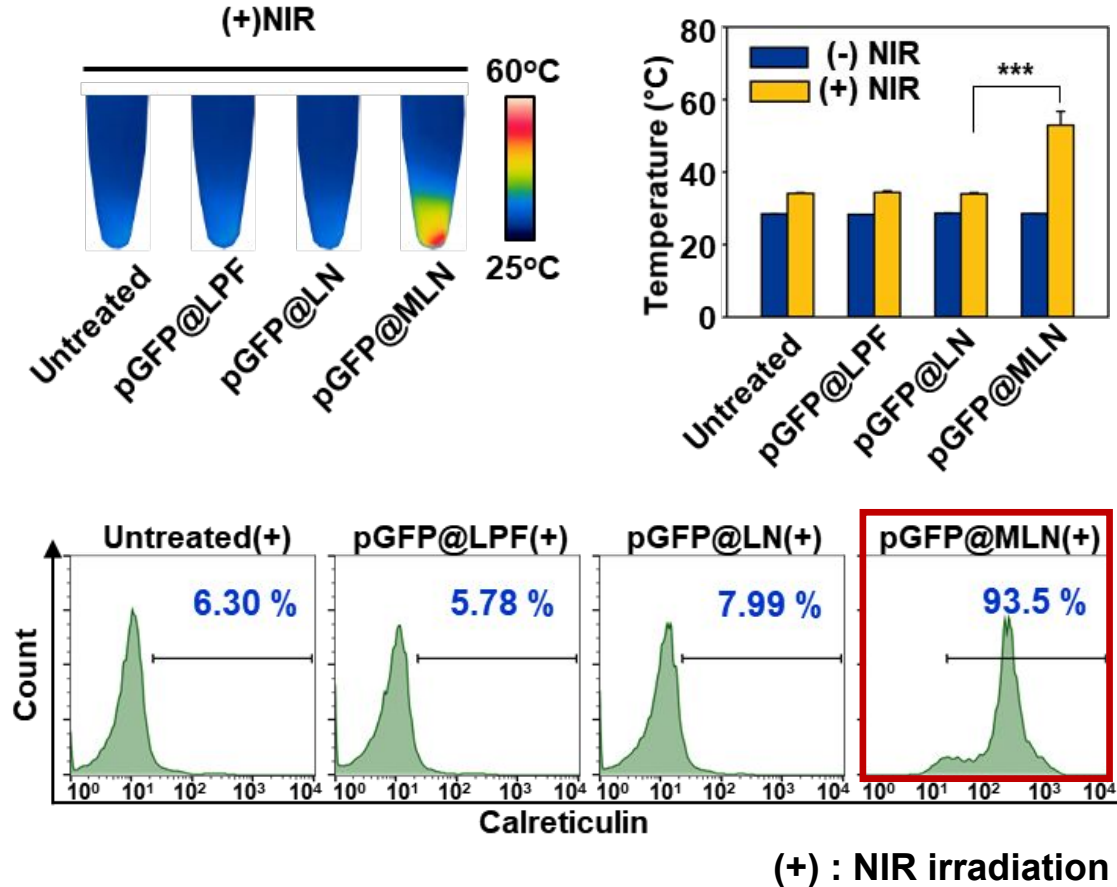
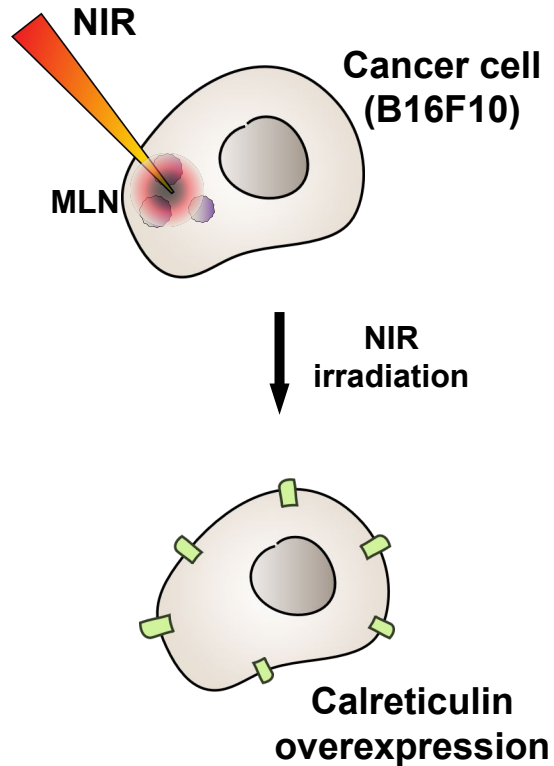
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MLN-mediated plasmid transfection



MLN-mediated immunogenic cell death

Photothermal effect mediated
calreticulin exposure



NIR irradiation
to MLN treated cells



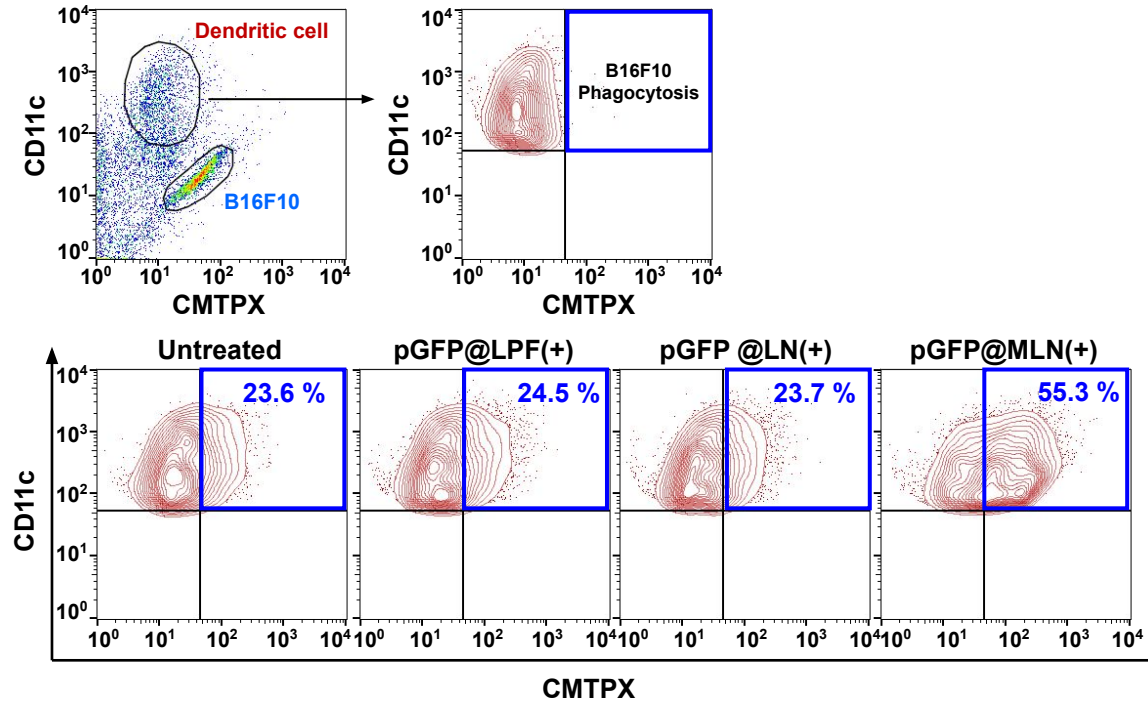
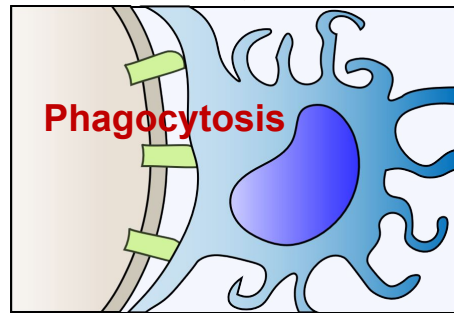
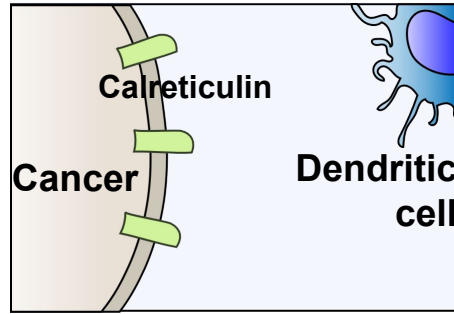
Photothermal effect
(Heat generation)



Calreticulin expression
(Danger signal)

MLN-mediated immunogenic cell death

Calreticulin-mediated cancer cell phagocytosis by dendritic cell



(+) : NIR irradiation

Calreticulin exposure
“Eat me” signal



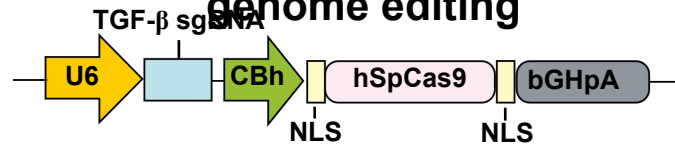
Dendritic cell
phagocytosis



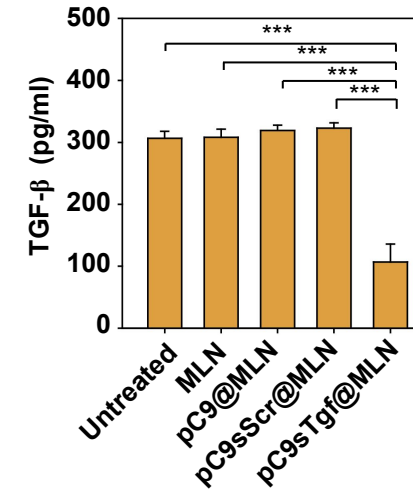
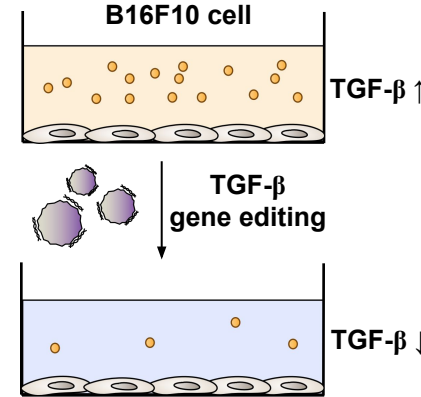
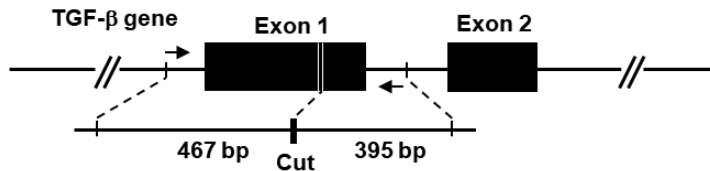
Anti-tumor
immune response

MLN-mediate genome editing

In vitro TGF- β genome editing



- pC9: **Cas9** expression plasmid
- pC9sScr: **Cas9** expression + **Scrambled sgRNA** expression plasmid
- pC9sTgf: **Cas9** expression + **TGF- β sgRNA** expression plasmid



Indel

analysisTarget PAM

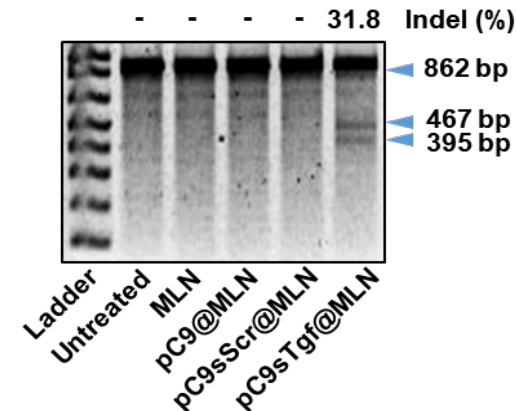
WT 5'-TCGCTTTGTACAACAGCACCCGCGACC**GGG**TGGCAGGCCGA-3'

#1 TCGCTTTGTACAACAGCACCCGC-ACC**GGG**TGGCAGGCCGA

#2 TCGCTTTGTACAACAGCACCC--**A**GACC**GGG**TGGCAGGCCGA

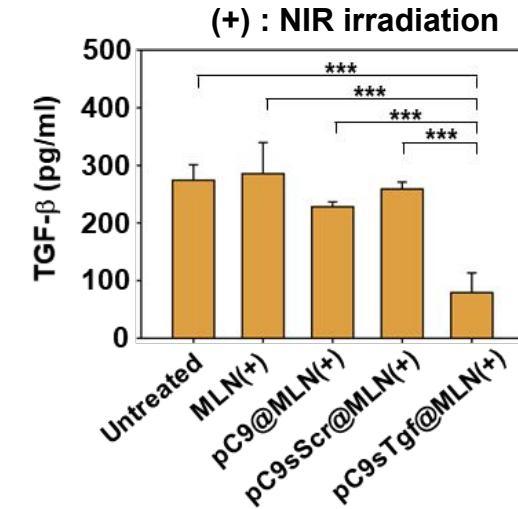
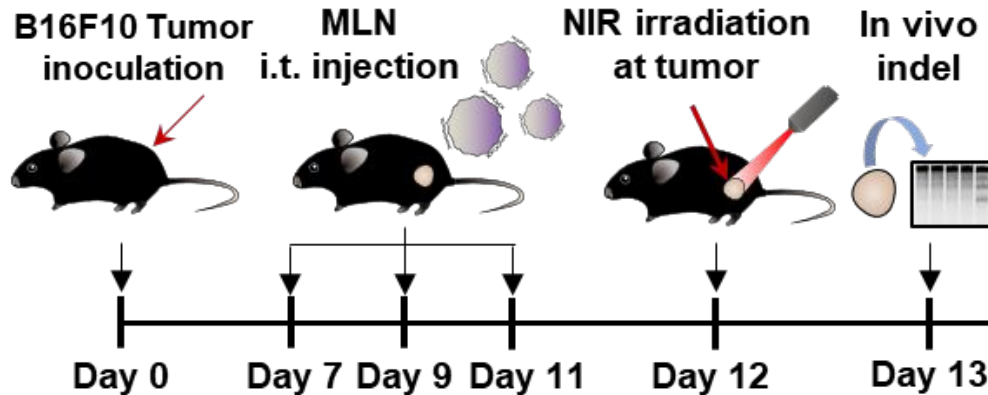
#3 -----ACC**GGG**TGGCAGGCCGA

#4 TCGCTTTGTACAACAGCACCCGC-----



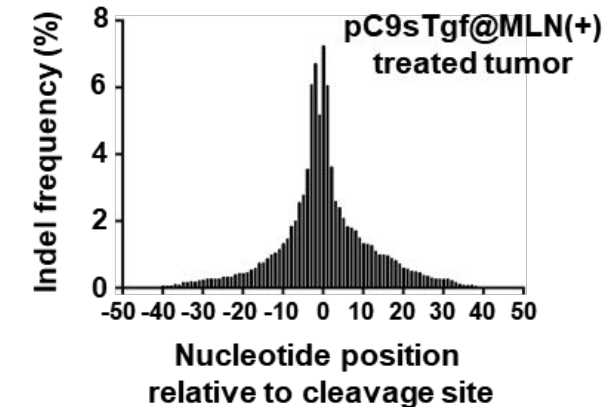
MLN-mediate genome editing

In vivo TGF- β genome editing



	Target	PAM
WT	5'-TCGCTTTGTACAACAGCACCCGCG	ACC GGG TGGCAGGCGA -3'
#1	TCGCTTTGTACAACAGCAC-----	-CC GGG TGGCAGGCGA
#2	TCGCTTTGTACAACAGC-----	ACC GGG TGGCAGGCGA
#3	TCGCTTTGTACAACAGCACCCGCG	G ACC GGG TGGCAGGCGA
#4	TCGCTTTGTACAA-----	-----CAGGCGA

Insertion/deletion (indel) detection by NGS

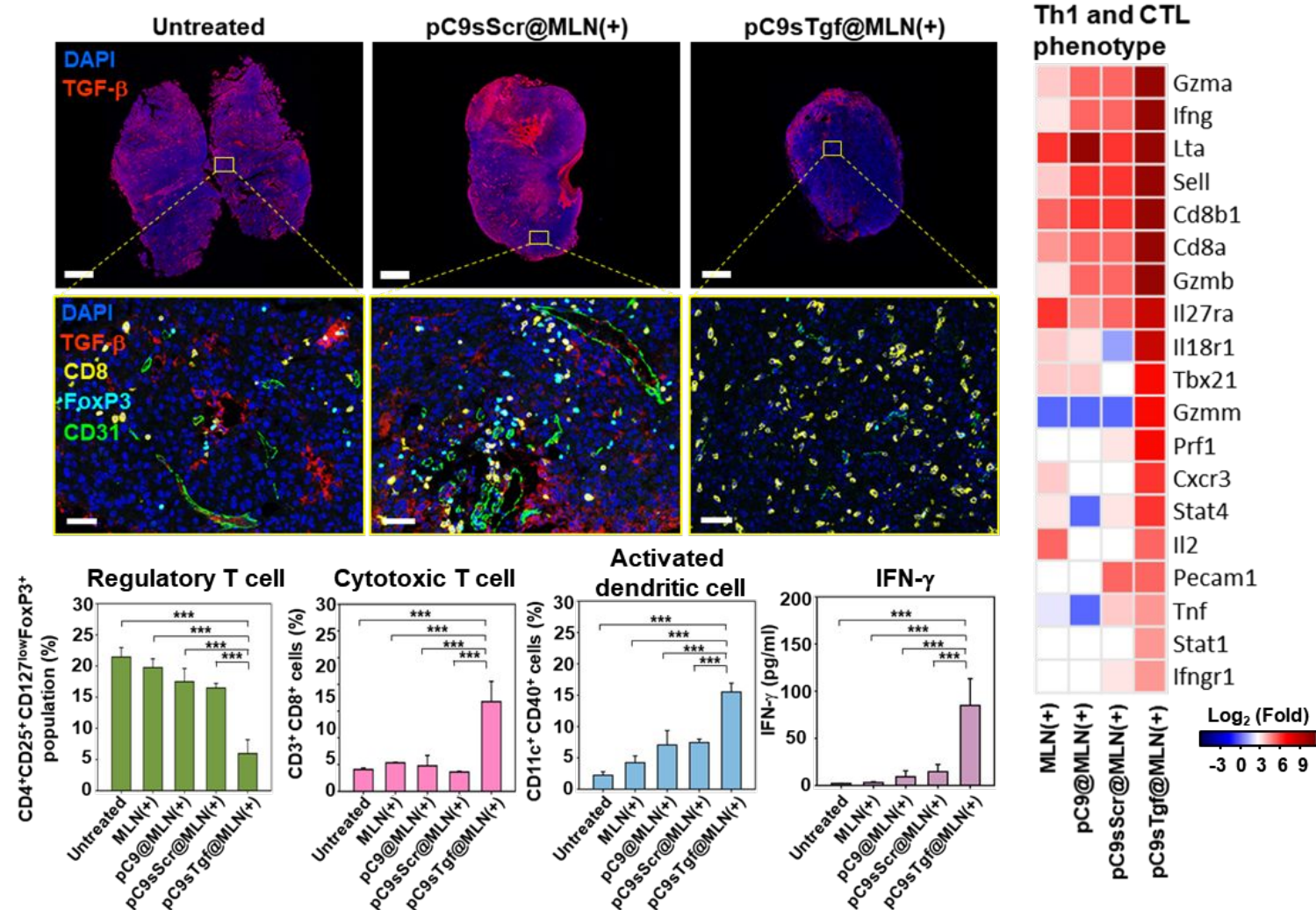


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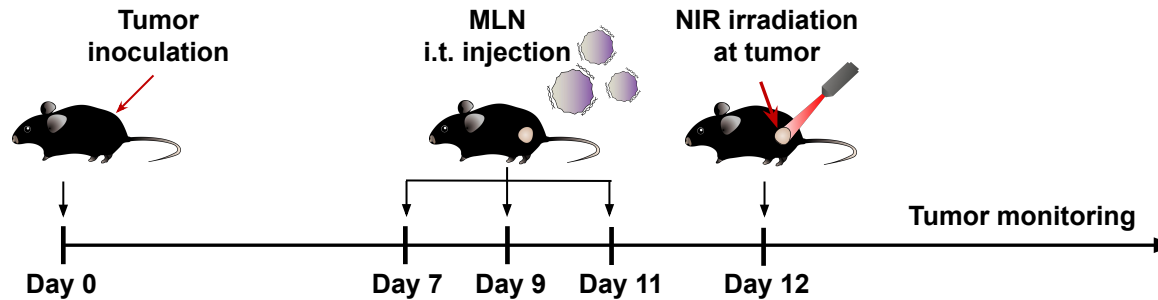
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Modulation of tumor immune microenvironment

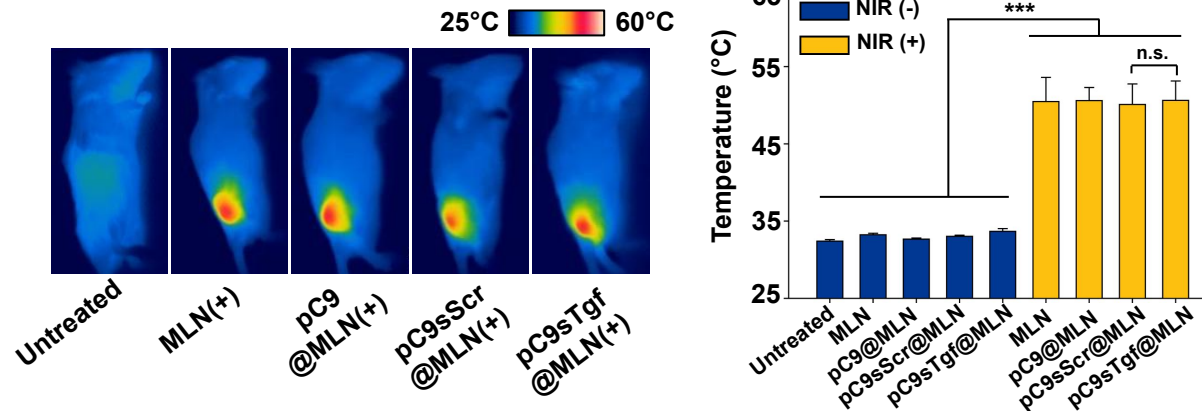


Anti-tumor efficacy against primary tumor

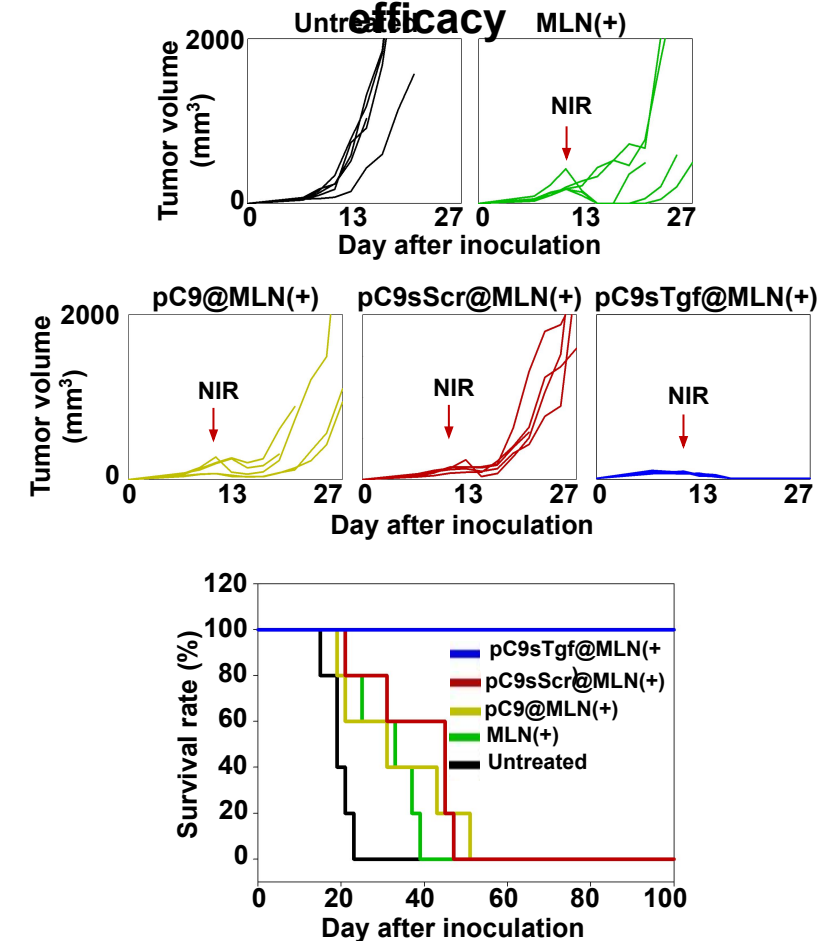
In vivo experiment schedule



Photothermal effect

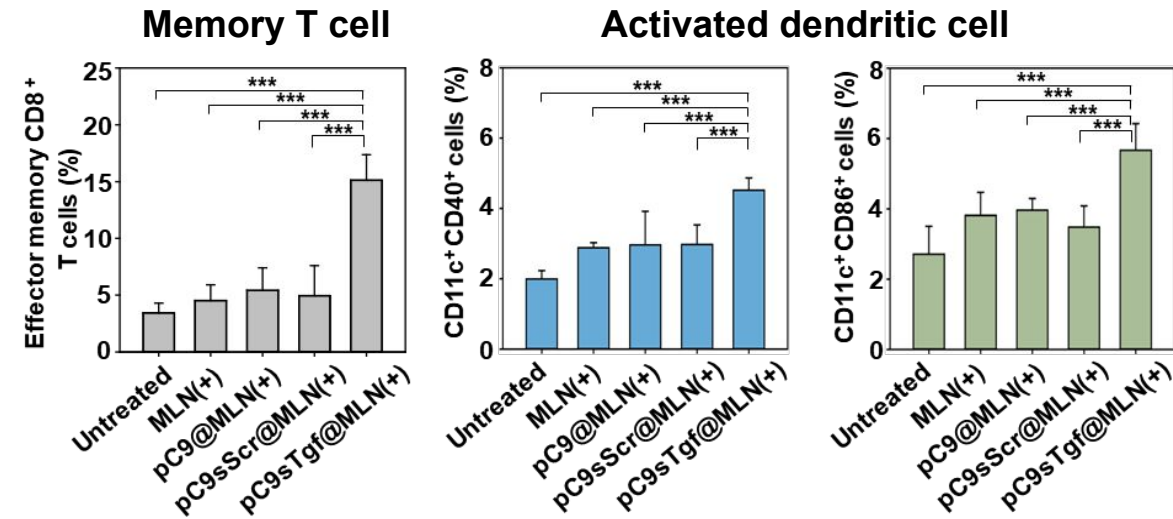
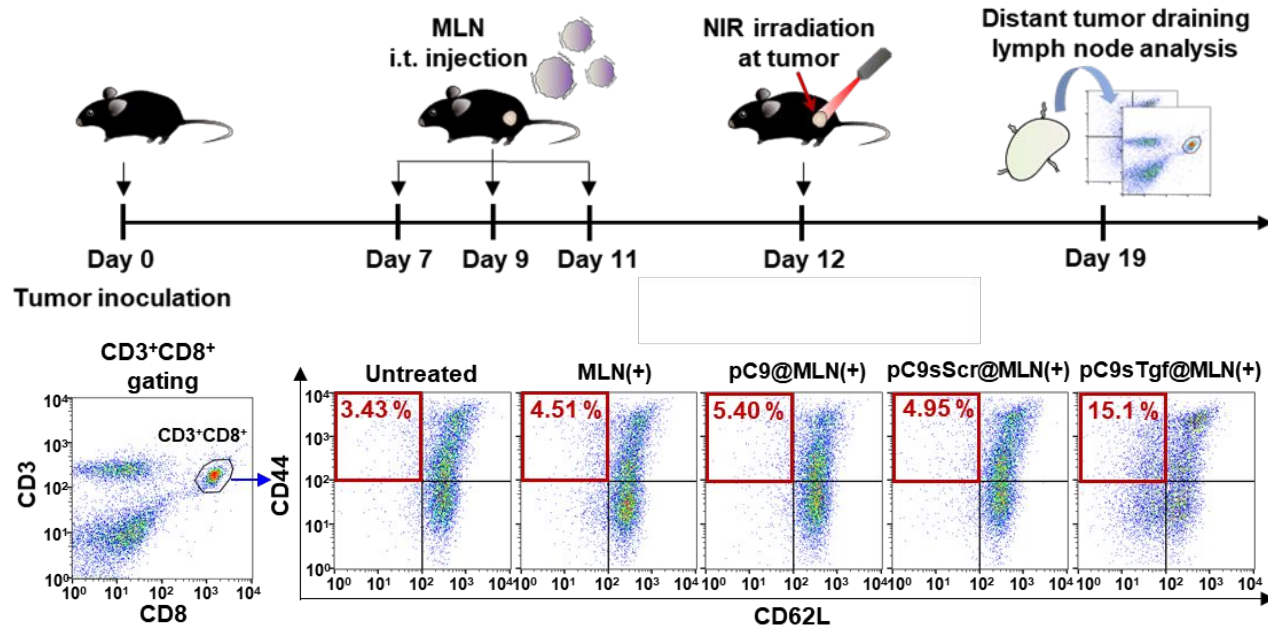


Anti-tumor efficacy



Systemic immunity against tumor

Immune cell analysis in tumor draining lymph nodes



- Genome editing-mediated immune activation
- Photothermal effect-mediated Immunogenic cell death



Establishment of **immunological memory** against tumor



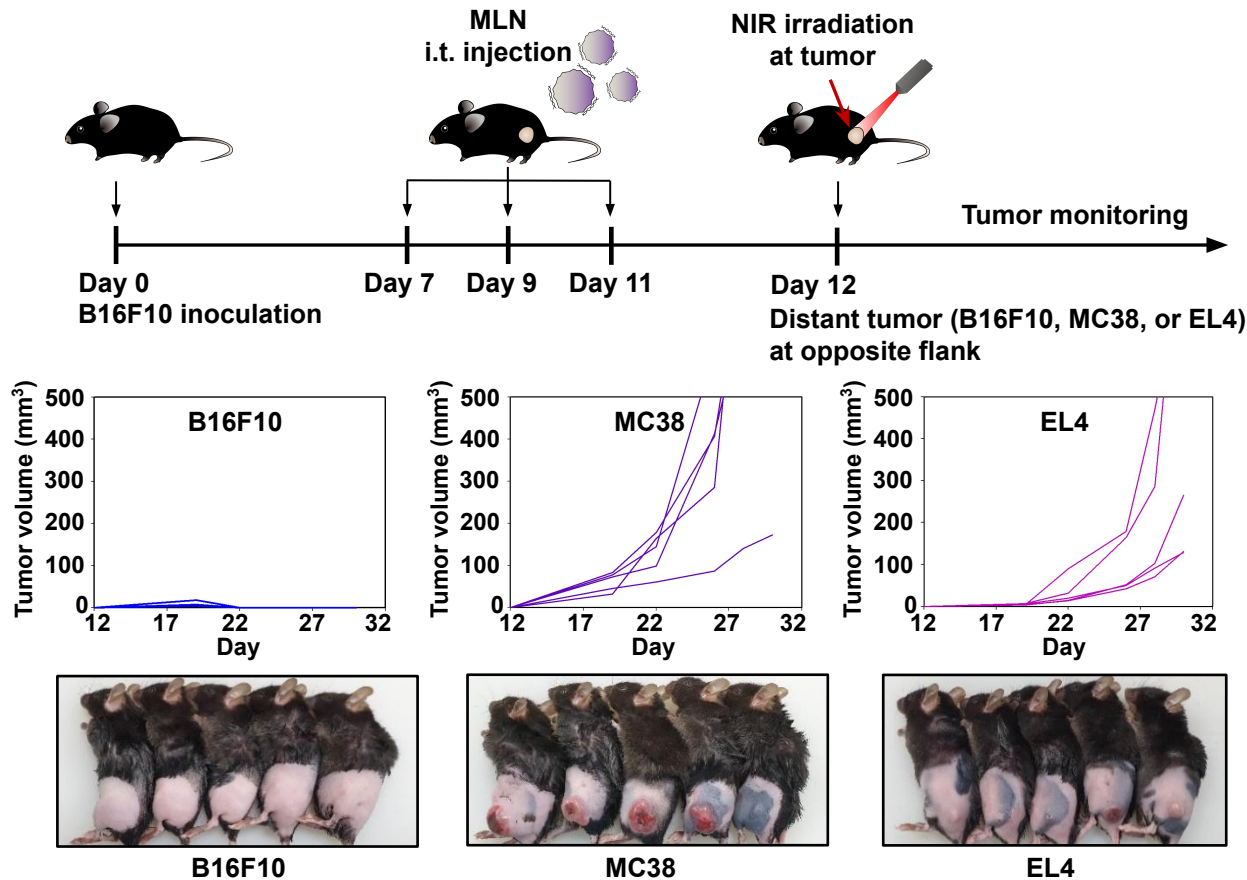
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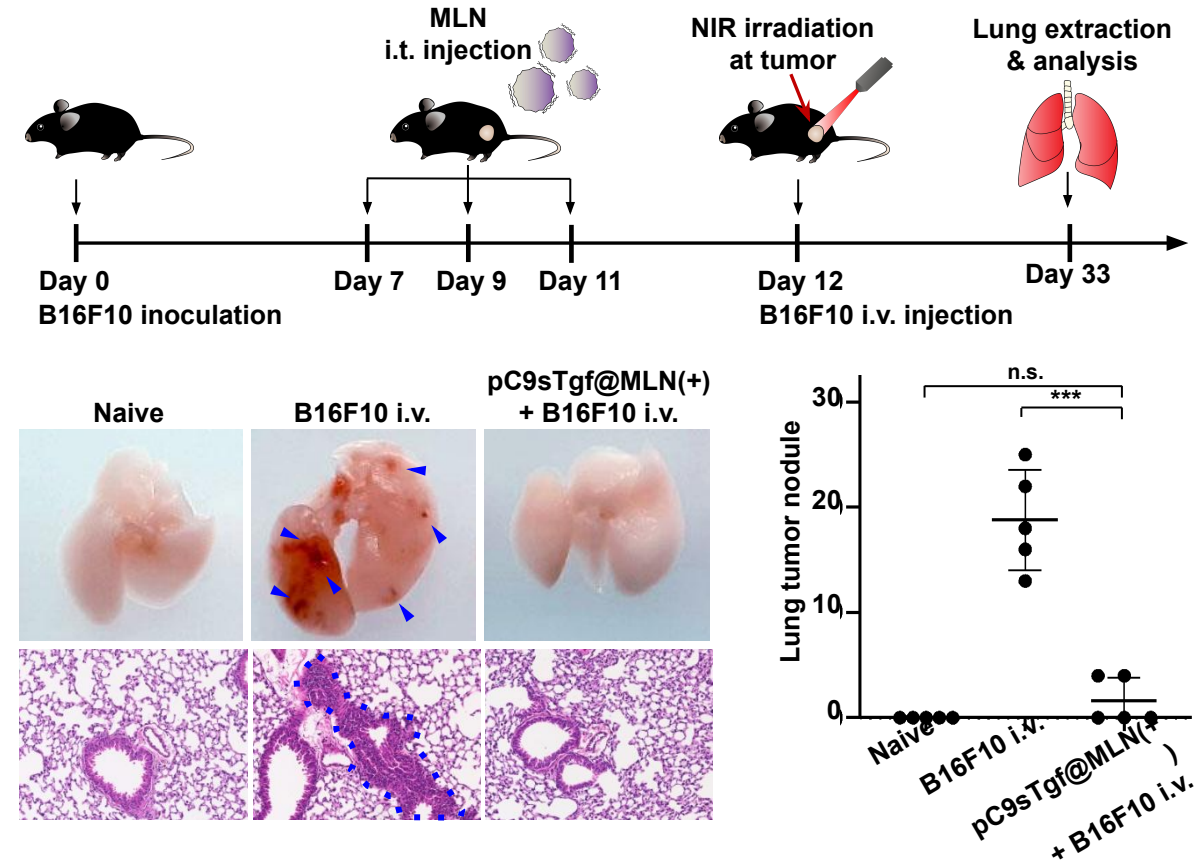
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Anti-tumor efficacy against distant tumor

Cell-type dependent prevention of distant tumor

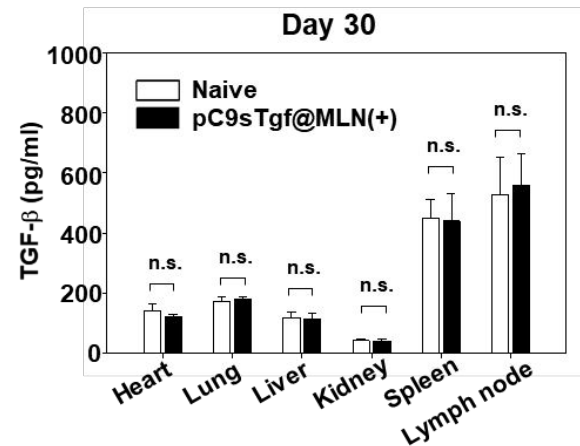
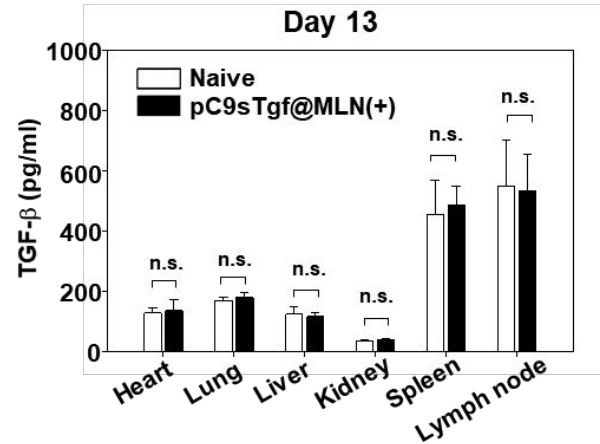


Prevention of tumor metastasis

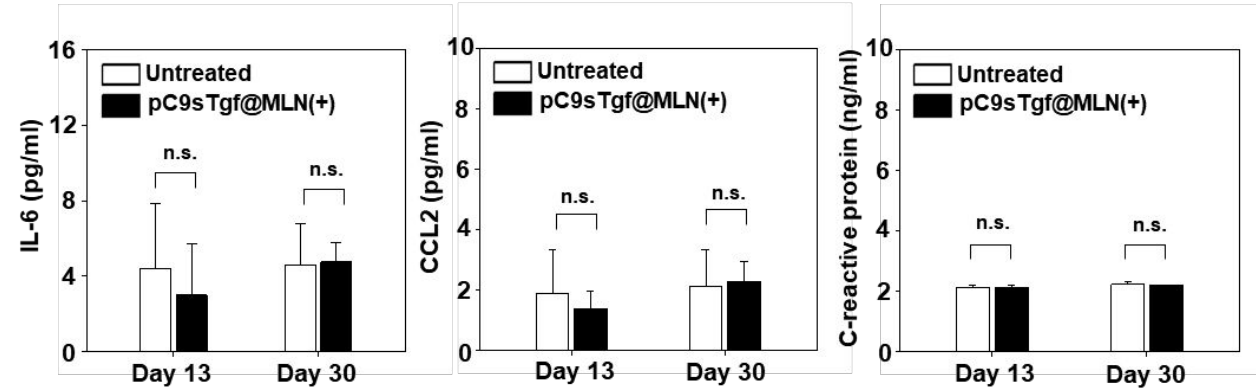


Lack of autoimmune response

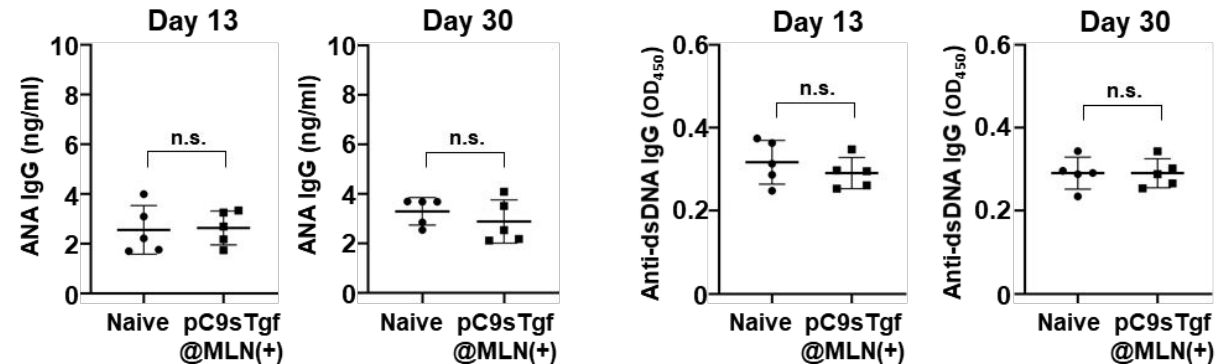
TGF- β level in major organs



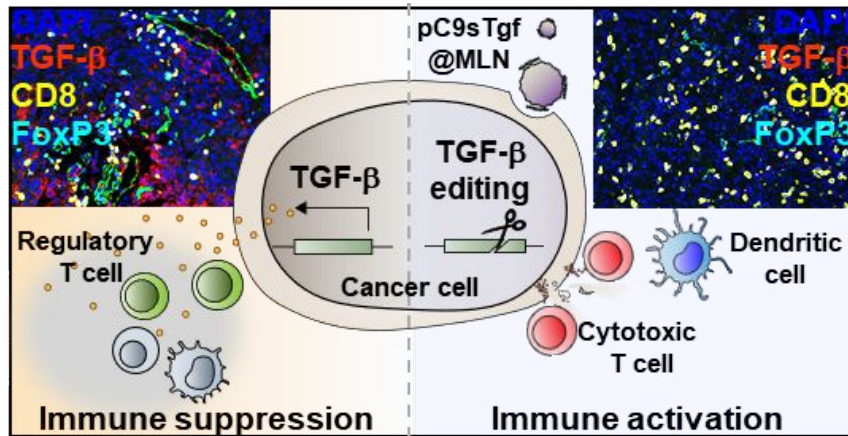
Inflammatory cytokine release



Production of autoimmune antibodies



Summary



- Metal lipid hybrid nanoparticle (MLN) was developed for inducing synergistic effect between genome editing and photothermal therapy
- pC9sTgf@MLN(+) resulted in TGF-β genome editing in tumor tissue, thereby restructuring of tumor immune microenvironment
- The tumor immune microenvironment became favorable environment for anti-tumor immune response
- The established anti-tumor immune response could also induce systemic protection from tumor recurrence and metastasis

Acknowledgements



Professor

Yu-Kyoung Oh, Ph.D.

Lab members

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Jaehyun Choi

Hyemin Ju

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Dong-Un Jin

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Yong-Hyun Han, Ph. D.



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Thank you for your attention !



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