UNIVERSIDADE NOVA DE LISBOA

NOVA MEDICAL SCHOOL

HEALTH FOR THE FUTURE

SINCE 1977

Lysosome Exocytosis as a Therapeutic Target in Cancer Progression

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NOVA Medical School

NOVA University of Lisbon

17th November 2025

Disclosures

Duarte Barral's group receives grant support from Sea4Us

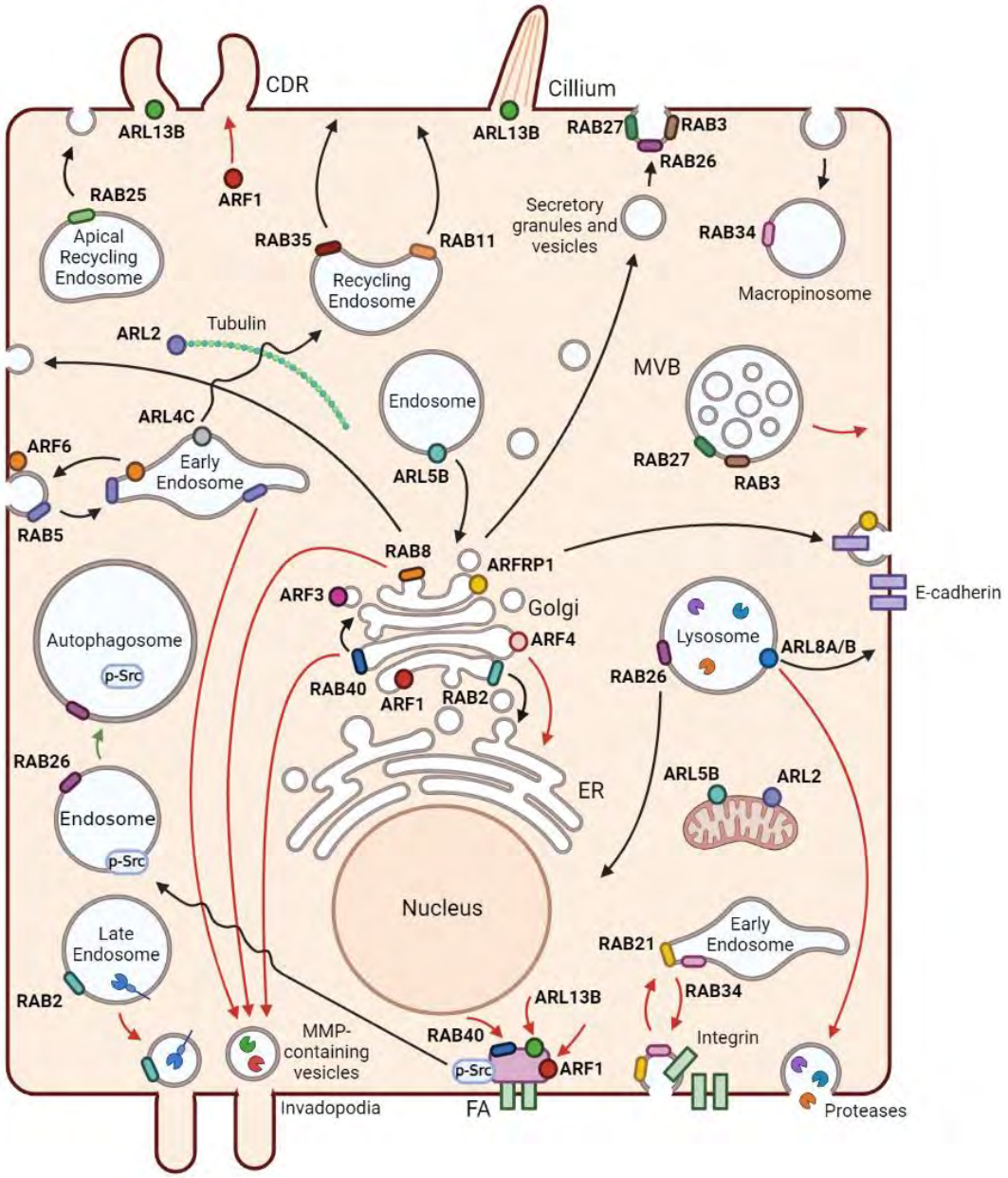
This continuing education activity is provided by AffinityCE, The Lysosomal and Rare Disorders Research and Treatment Center (LDRTC), and CheckRare CE. AffinityCE, CheckRare CE and LDRTC staff, planners, and reviewers, have no relevant financial relationships with ineligible companies to disclose. AffinityCE adheres to the ACCME's Standards for Integrity and Independence in Accredited Continuing Education. Any individuals in a position to control the content of a CME activity, including faculty, planners, reviewers, or others, are required to disclose all relevant financial relationships with ineligible companies. All relevant financial relationships when present, have been mitigated by the peer review of content by non-conflicted reviewers prior to the commencement of the activity.

Learning Objectives

At the conclusion of this activity, participants will be able to:

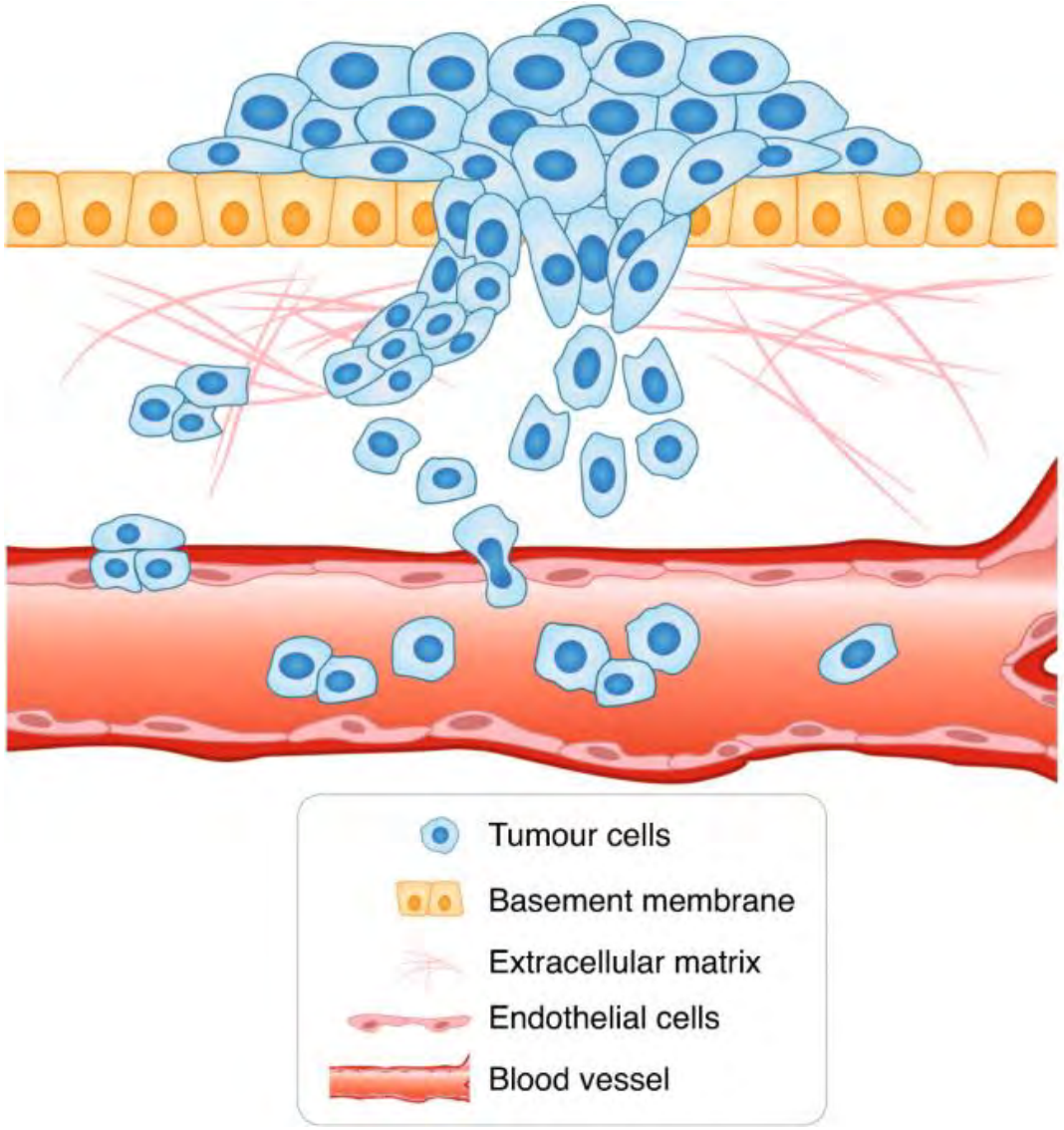
1. Understand the process of lysosome exocytosis;
2. Recognize the importance of lysosome exocytosis in cancer progression;
3. Identify lysosome exocytosis as a therapeutic target.

Rabs and Arfs in Breast Cancer



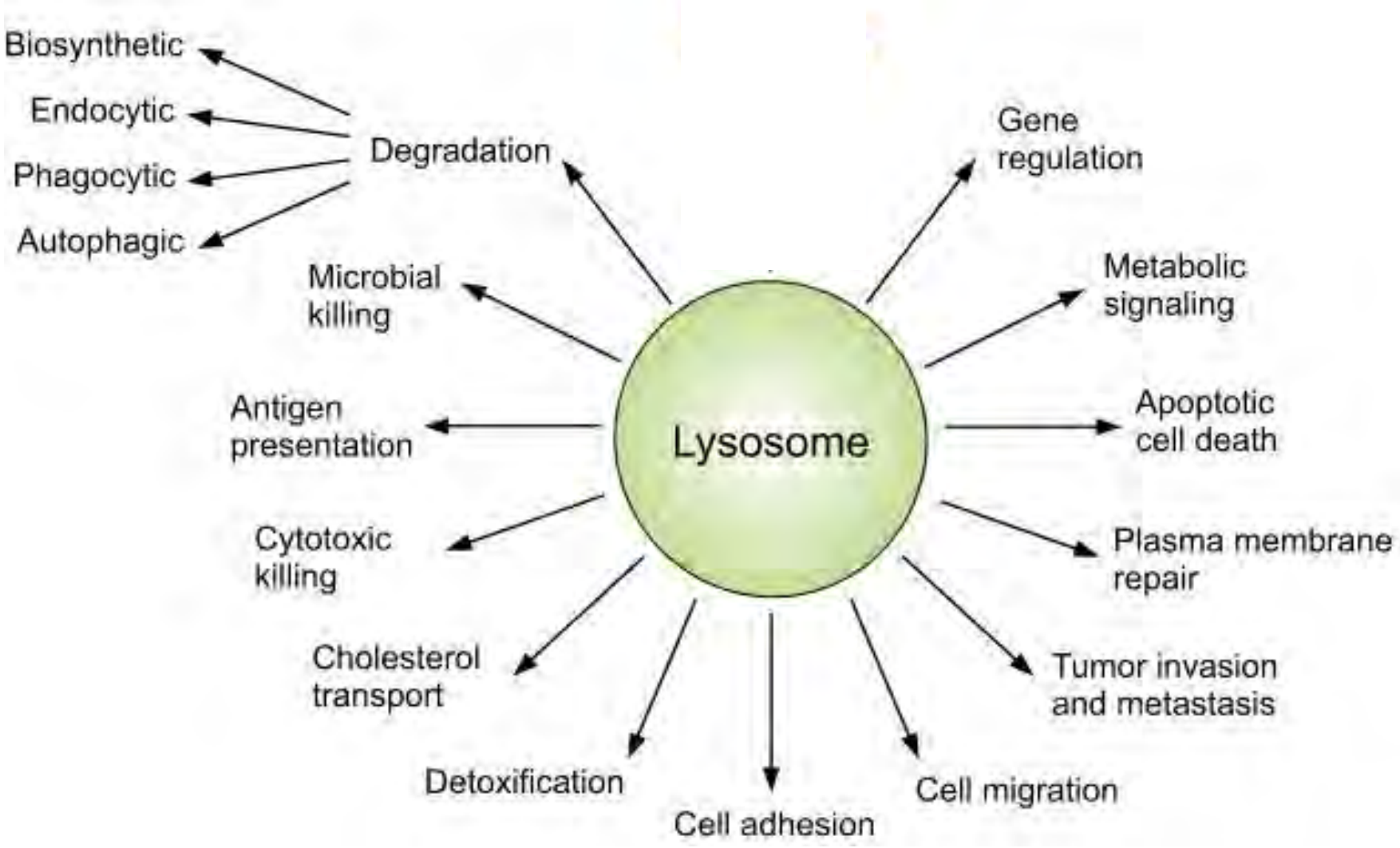
Ferreira, Castanheira et al., *Front. Cell Dev. Biol.*, 2024

Cancer Progression



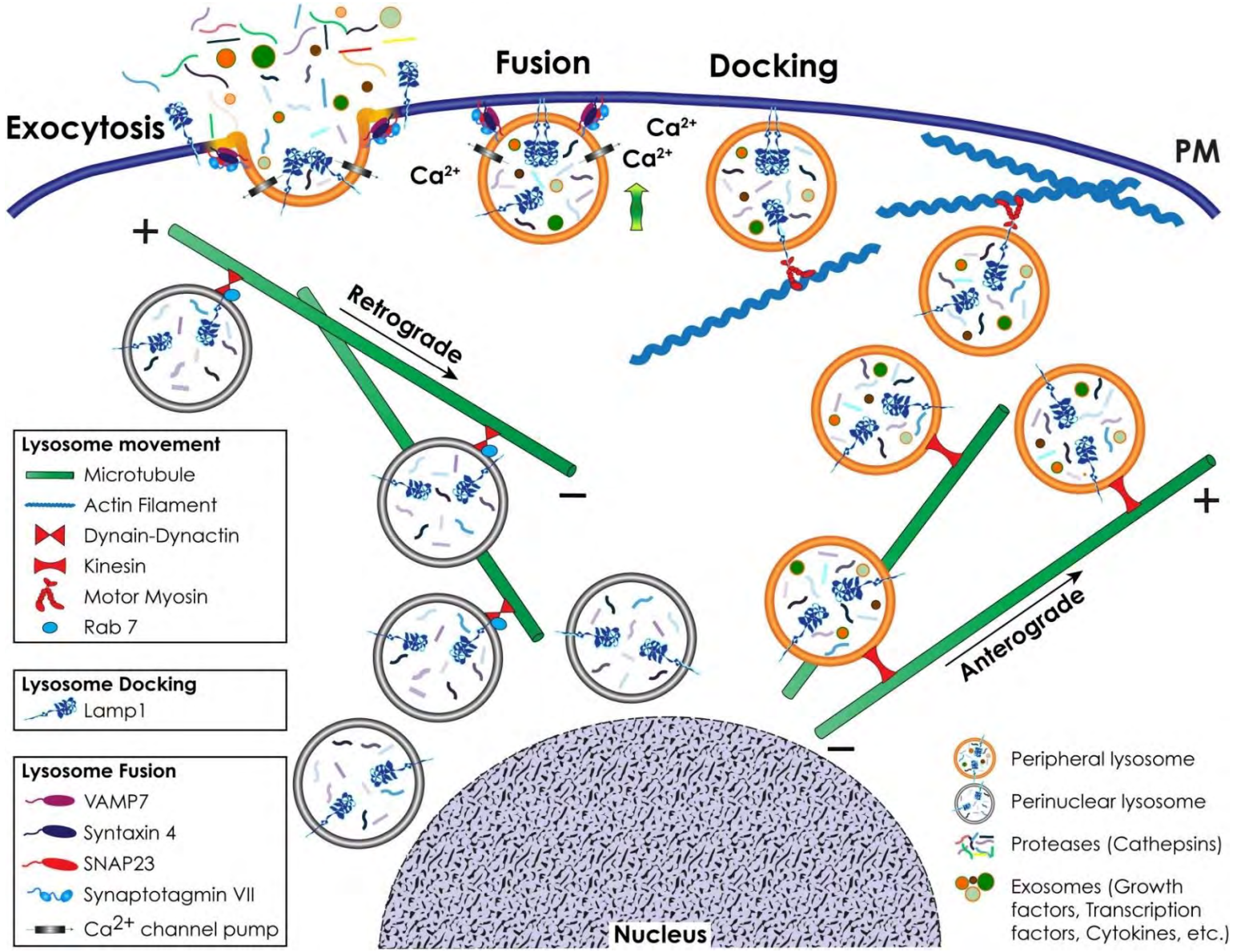
Novikov *et al.*, *Br. J. Cancer*, 2020

Lysosome Functions



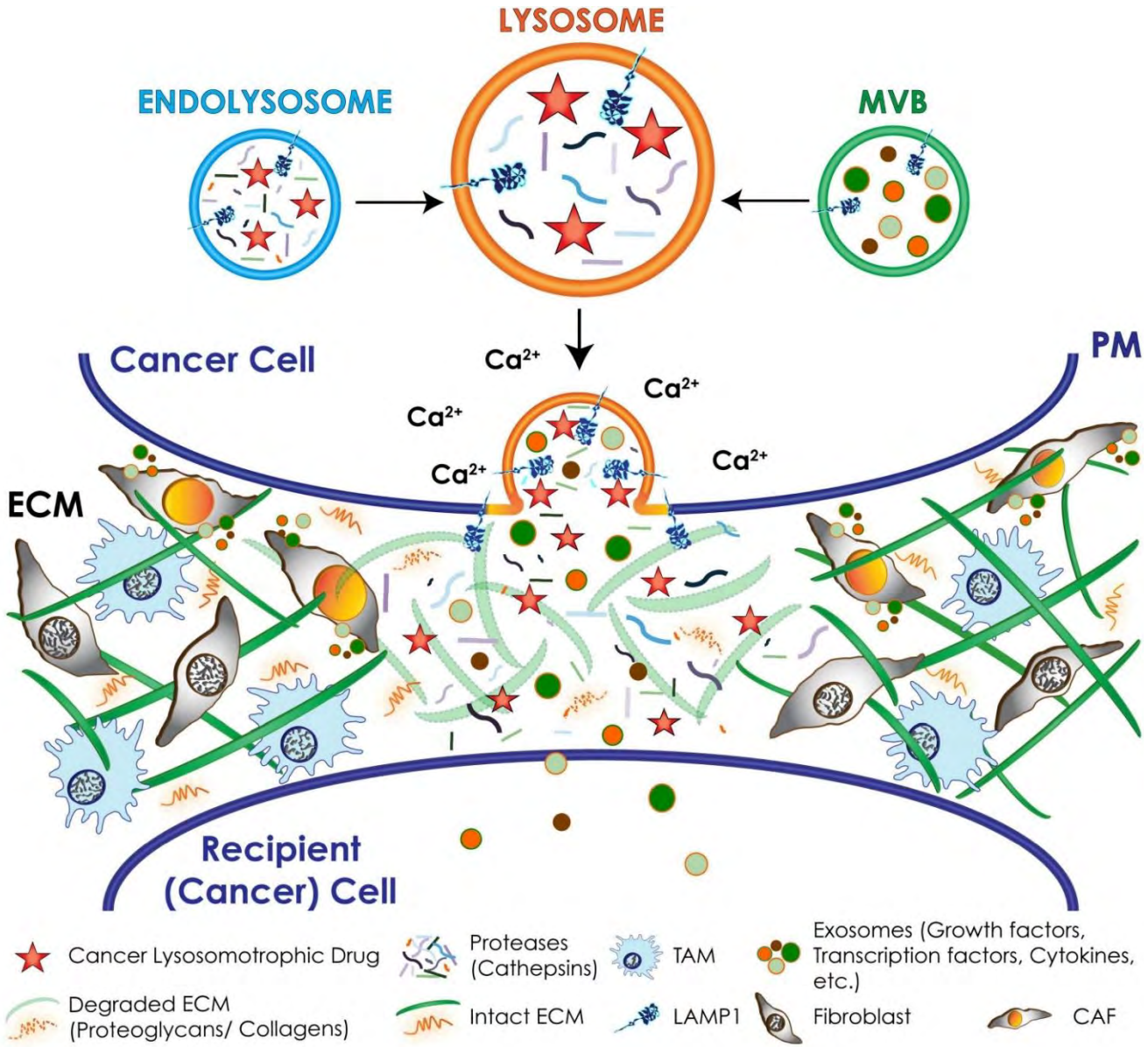
Adapted from Pu *et al.*, *J. Cell Sci.*, 2016

Lysosome Exocytosis in Cancer



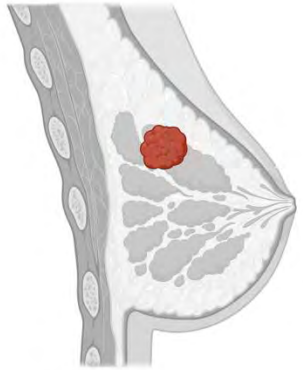
Machado *et al.*, *Front. Cell Dev. Biol.*, 2021

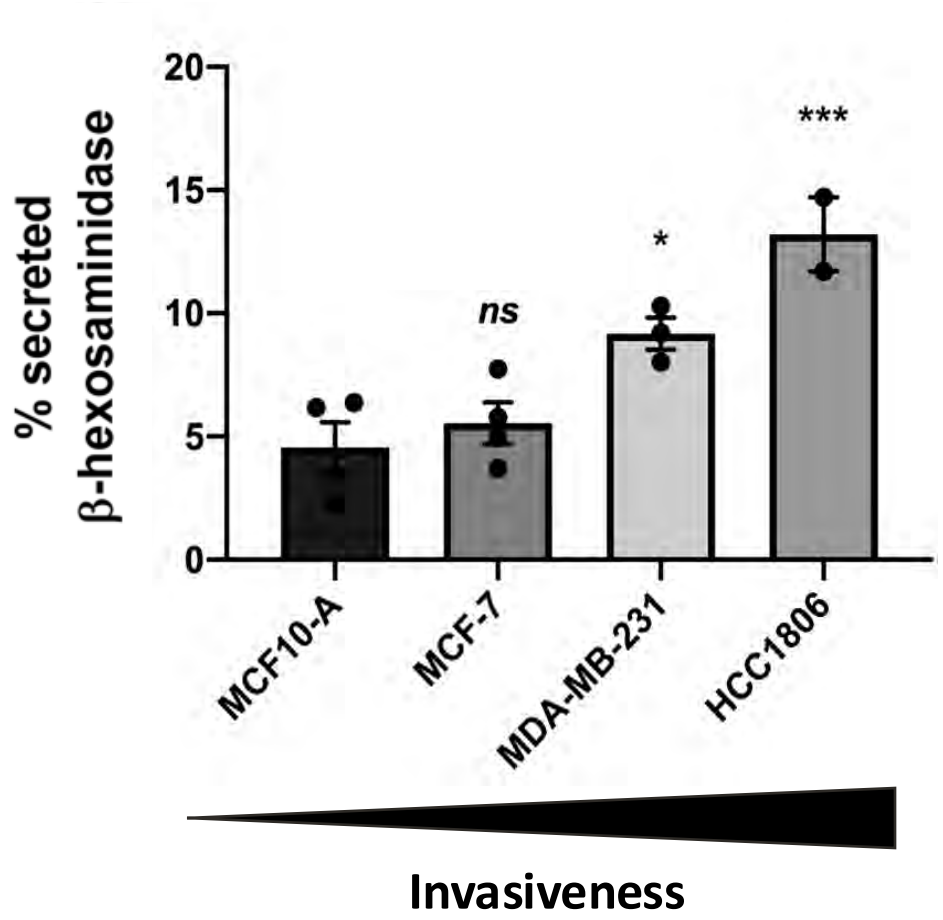
Lysosome Exocytosis in Cancer



Machado *et al.*, *Front. Cell Dev. Biol.*, 2021

Lysosome Exocytosis is Increased in Highly Invasive Breast Cancer Cells





MCF10-A – non tumorigenic breast cells
MCF-7 – poorly invasive luminal A breast cancer cells
MDA-MB-231 and **HCC1806** – highly invasive TNBC cells

Cristina Escrevente, unpublished

Can Lysosome Exocytosis Serve as a Therapeutic Target to Inhibit Cancer Cell Invasion?

Lysosome Exocytosis as a Therapeutic Target in Lysosome Storage Disorders

Drug induced exocytosis of glycogen in Pompe disease

Christopher T. Turner [a], Maria Fuller [b], John J. Hopwood [c], Peter J. Meikle [d], Doug A. Brooks [e, *]

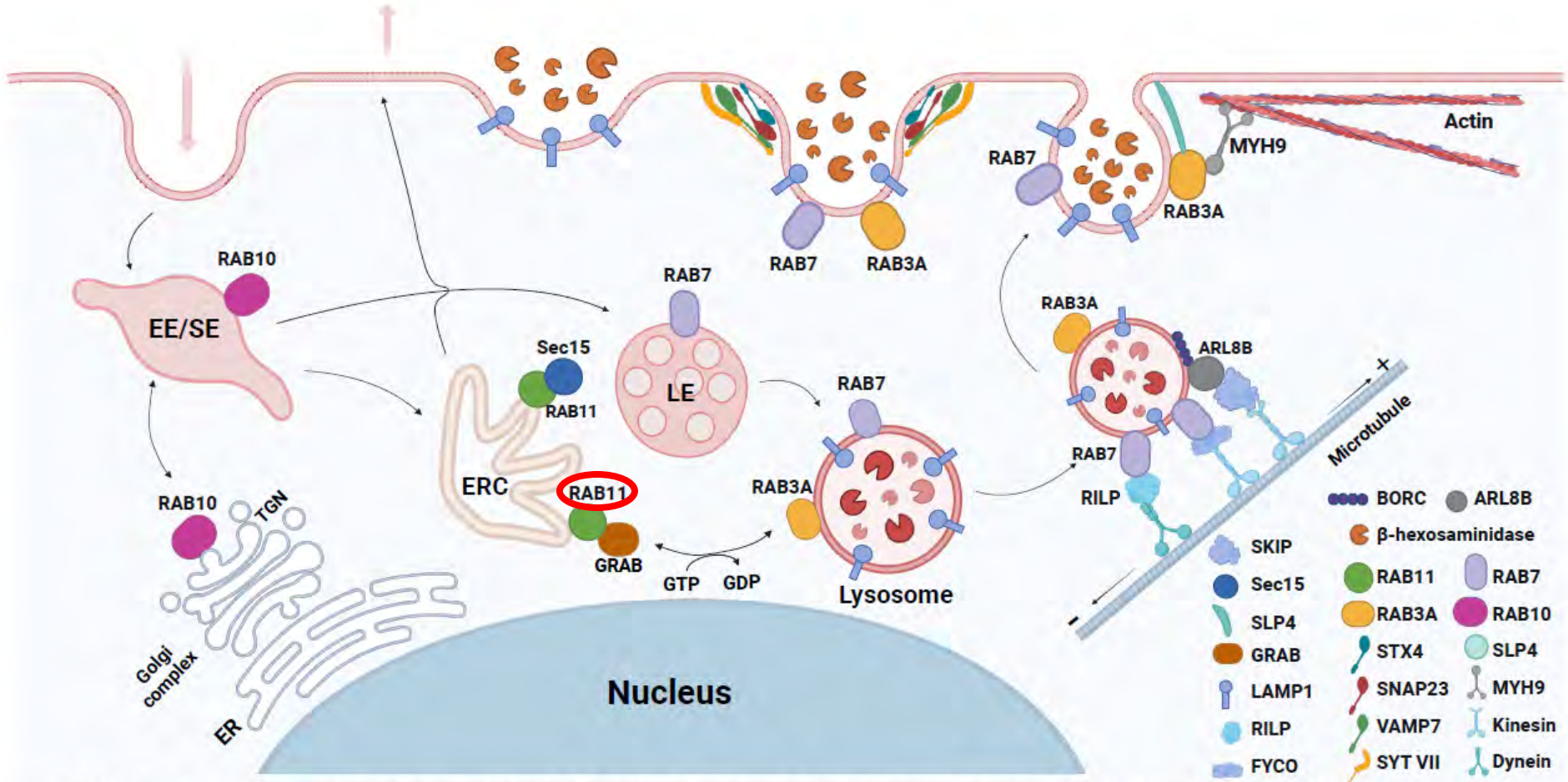
Biochemical and Biophysical Research Communications 2016

Glycogen Exocytosis from Cultured Pompe Skin Fibroblasts

Christopher T Turner[1], Maria Fuller[2], John J Hopwood[3], Peter J Meikle[4] and Doug A Brooks[5]

Translational Biomedicine 2015
Vol. 6 No. 2:17

Lysosome Exocytosis Regulation



EE/SE - Early endosome/Sorting endosome; **LE**- Late endosome; **ERC** - Endocytic recycling compartment; **TGN** - *trans*-Golgi network; **ER** - Endoplasmic reticulum

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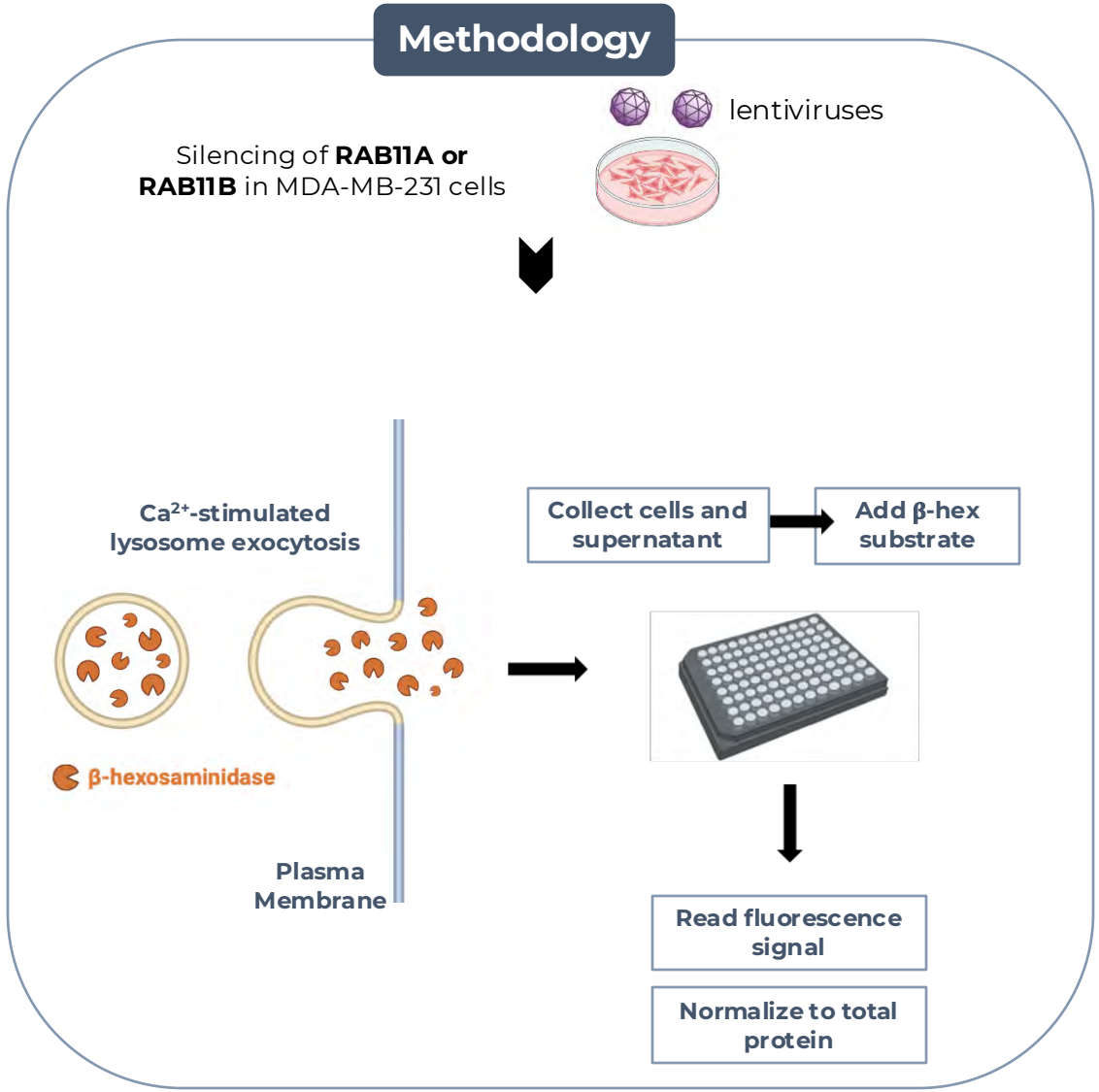
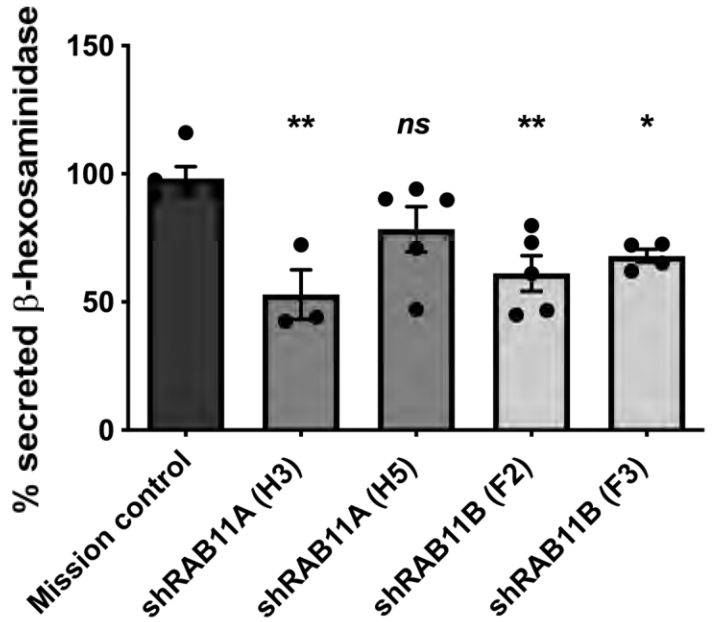
RESEARCH ARTICLE

Rab11 is required for lysosome exocytosis through the interaction with Rab3a, Sec15 and GRAB

Cristina Escrevente*, Liliana Bento-Lopes*, José S. Ramalho and Duarte C. Barral‡

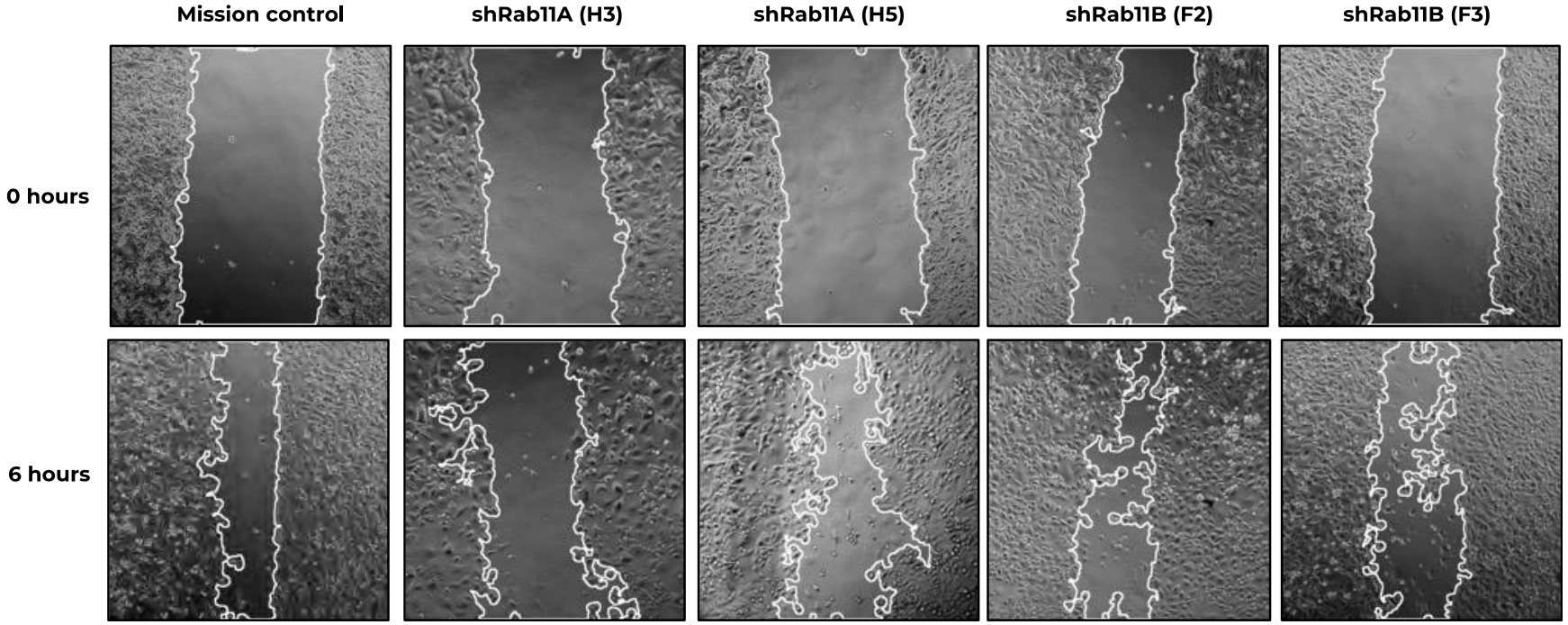
RAB11A or RAB11B Silencing Impairs Lysosome Exocytosis in Breast Cancer Cells

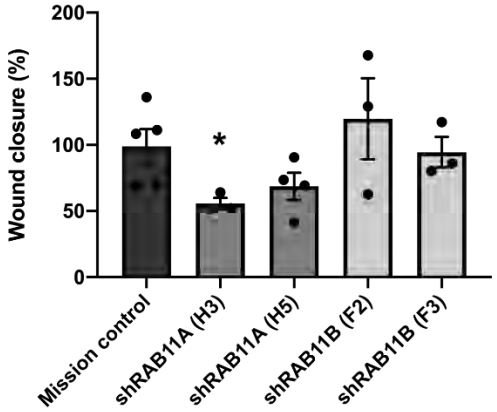




Cristina Escrevente, unpublished

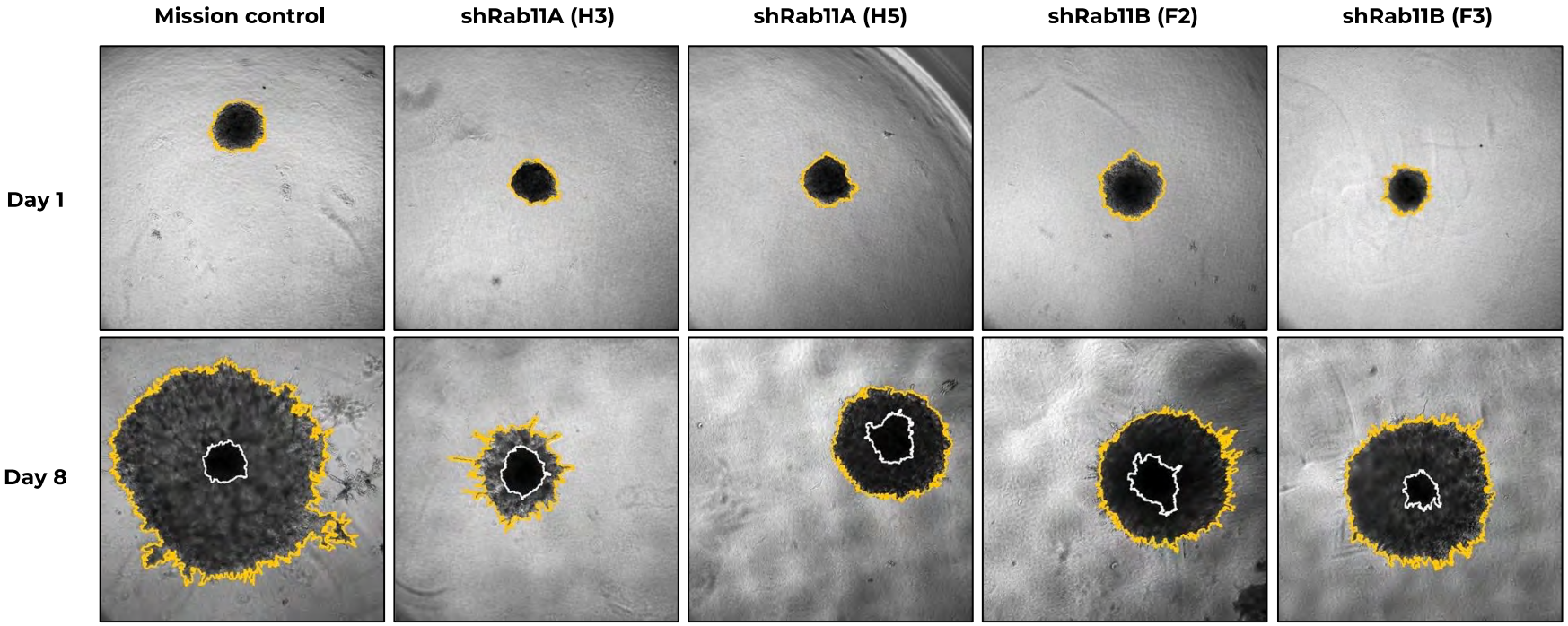
RAB11A (but not RAB11B) Silencing Impairs Breast Cancer Cell Migration

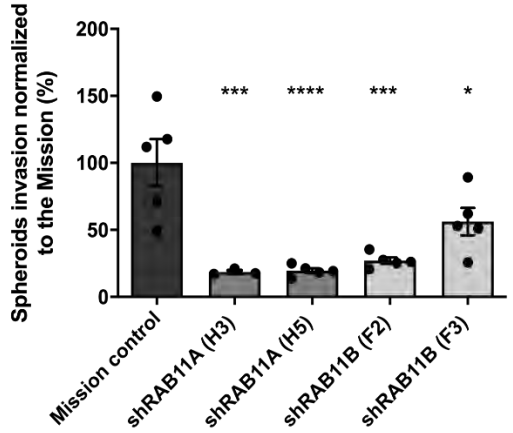




Cristina Escrevente and Isabel Sesifredo, unpublished

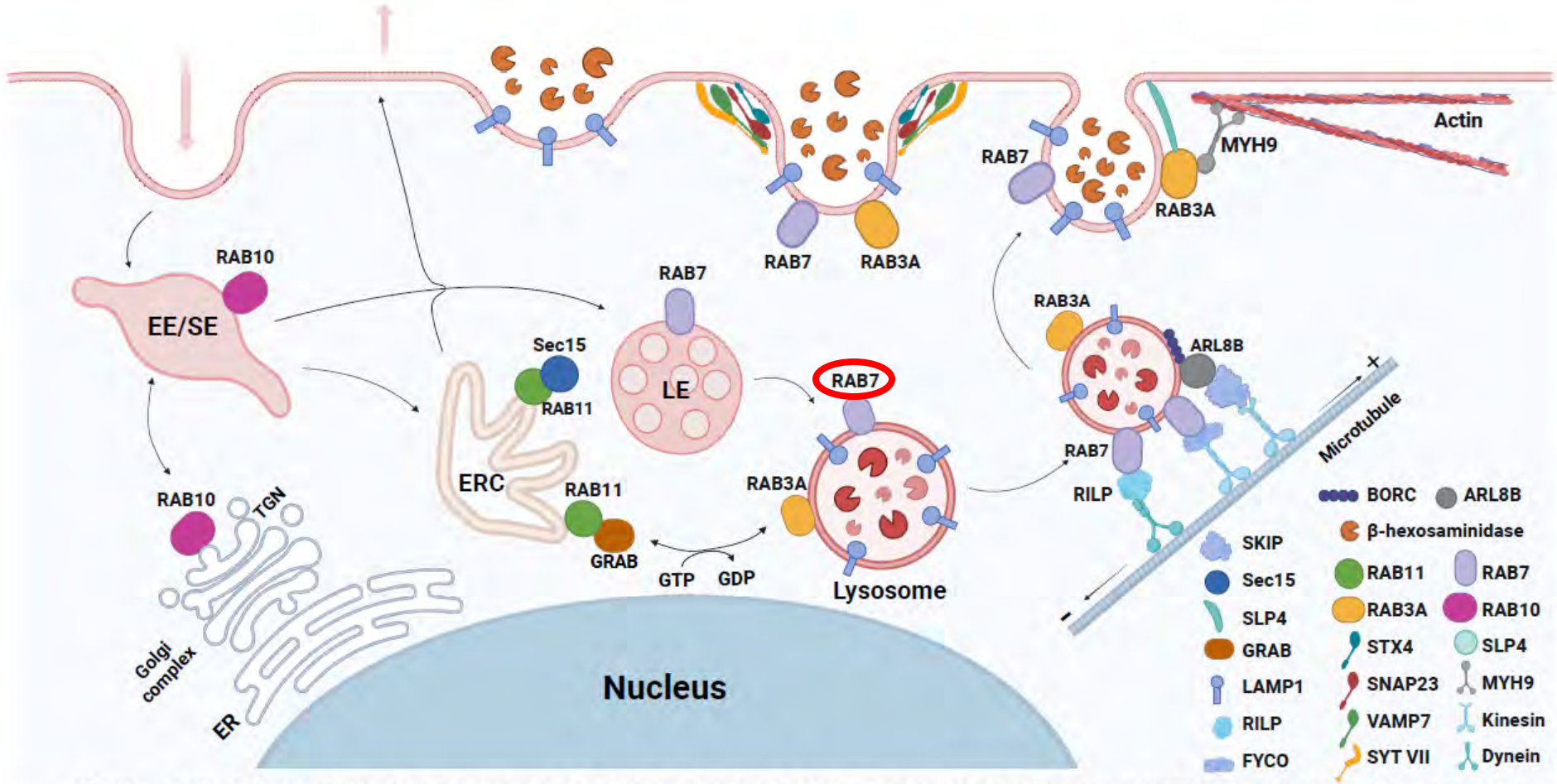
RAB11A or RAB11B Silencing Impairs Breast Cancer Cell Invasion





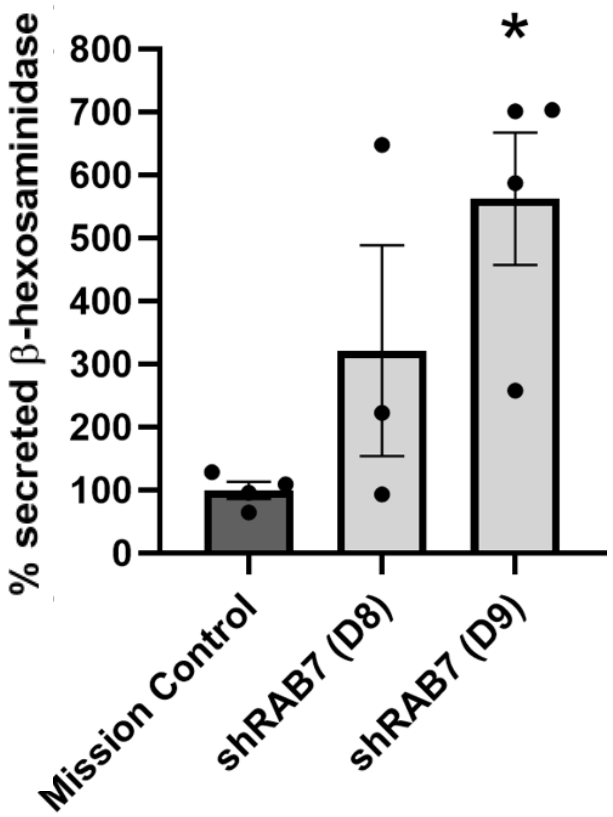
Cristina Escrevente and Isabel Sesifredo, unpublished

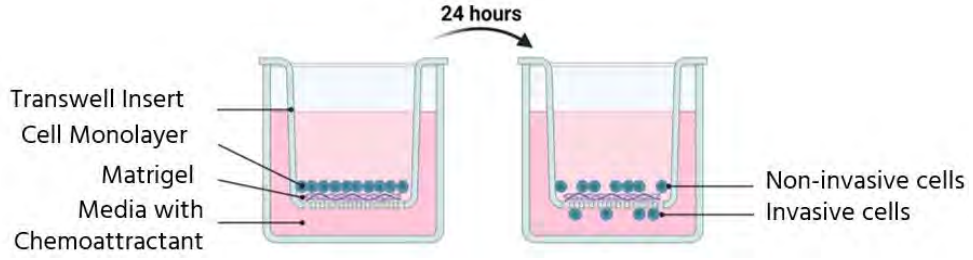
Lysosome Exocytosis Regulation

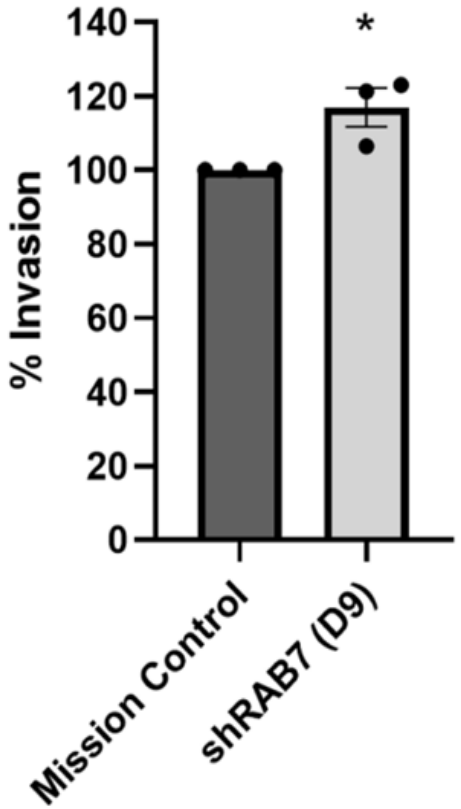


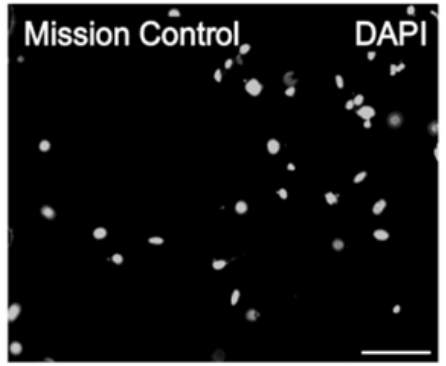
EE/SE - Early endosome/Sorting endosome; **LE**- Late endosome; **ERC** - Endocytic recycling compartment; **TGN** - *trans*-Golgi network; **ER** - Endoplasmic reticulum

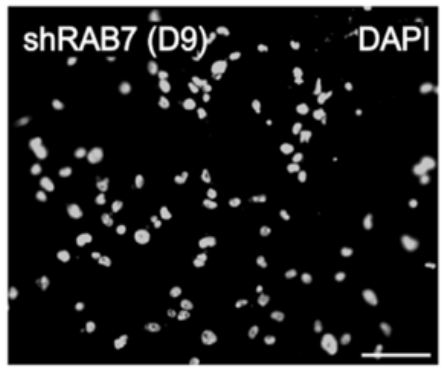
RAB7 Silencing Enhances Lysosome Exocytosis and Invasion of Breast Cancer Cells





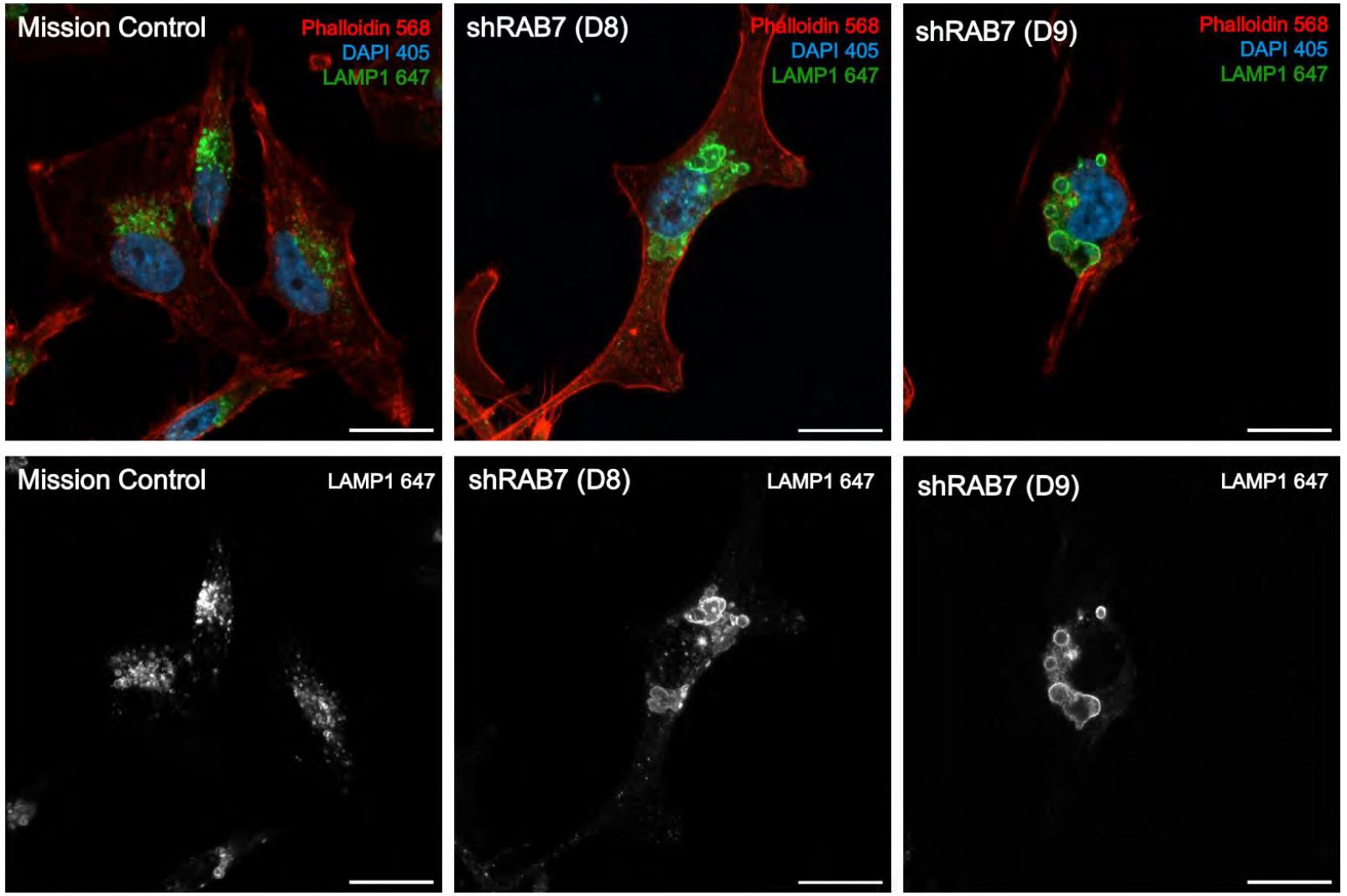






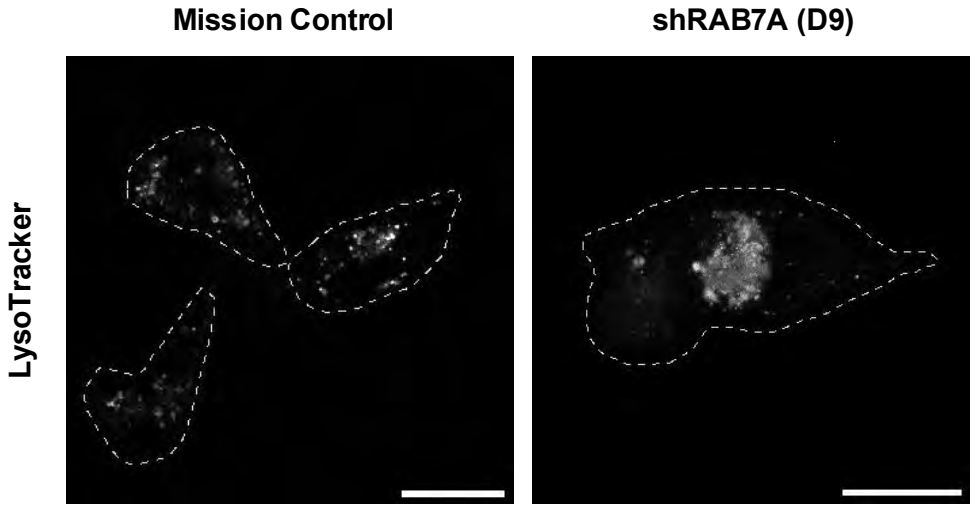
Ana Rita Rodrigues, unpublished

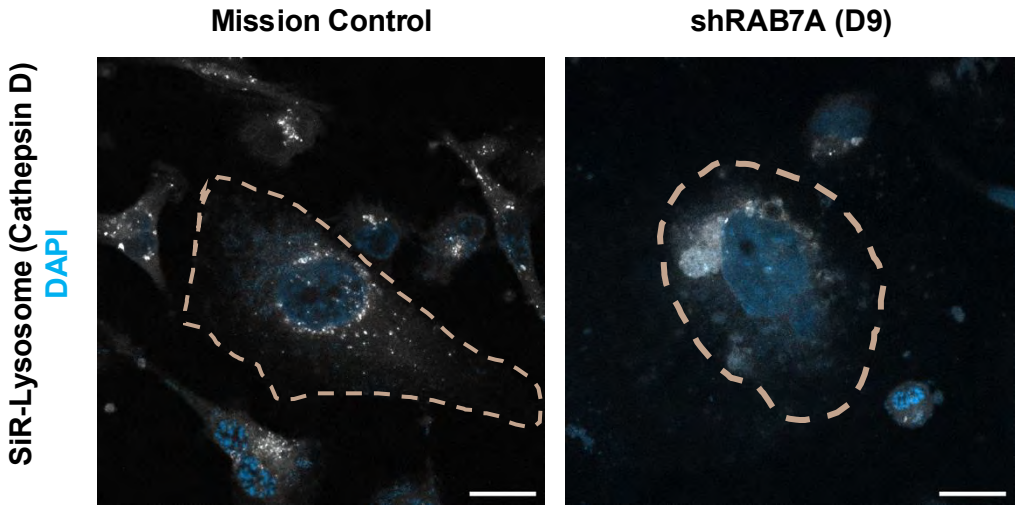
RAB7 Silencing Leads to Enlarged Perinuclear Late Endosomes/Lysosomes

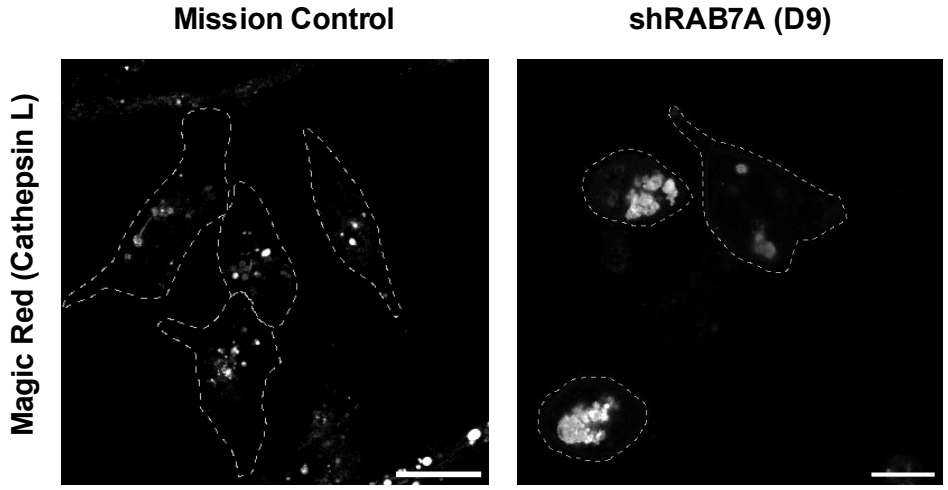


Ana Rita Rodrigues, unpublished

RAB7 Silencing Leads to Enlarged Perinuclear Late Endosomes/Lysosomes

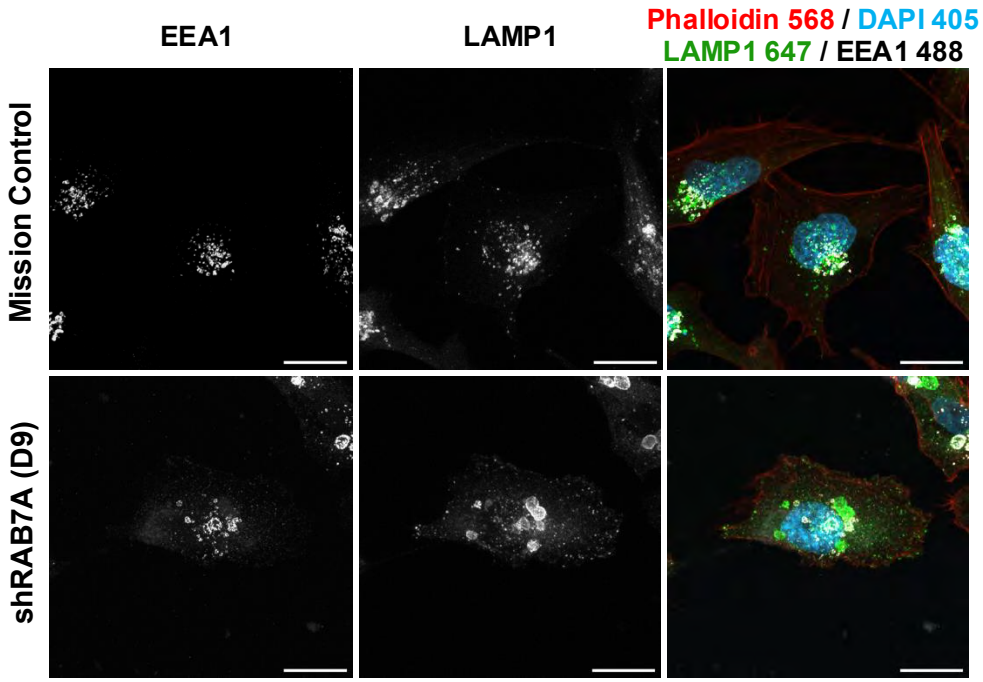


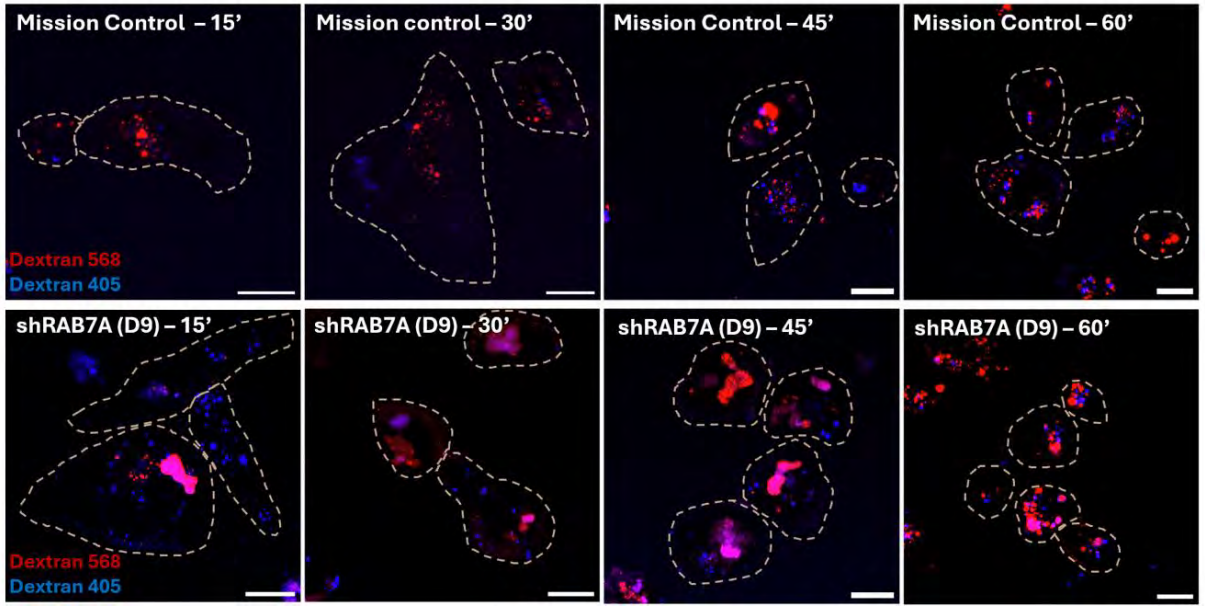


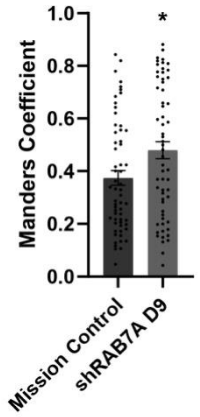


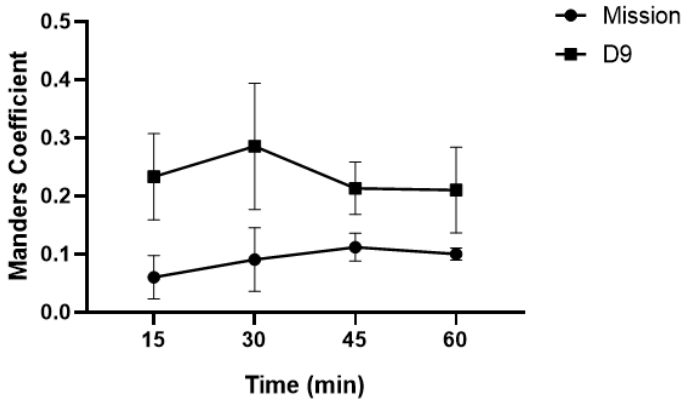
Ana Rita Rodrigues, unpublished

RAB7 Silencing Leads to Enlarged Perinuclear Late Endosomes/Lysosomes

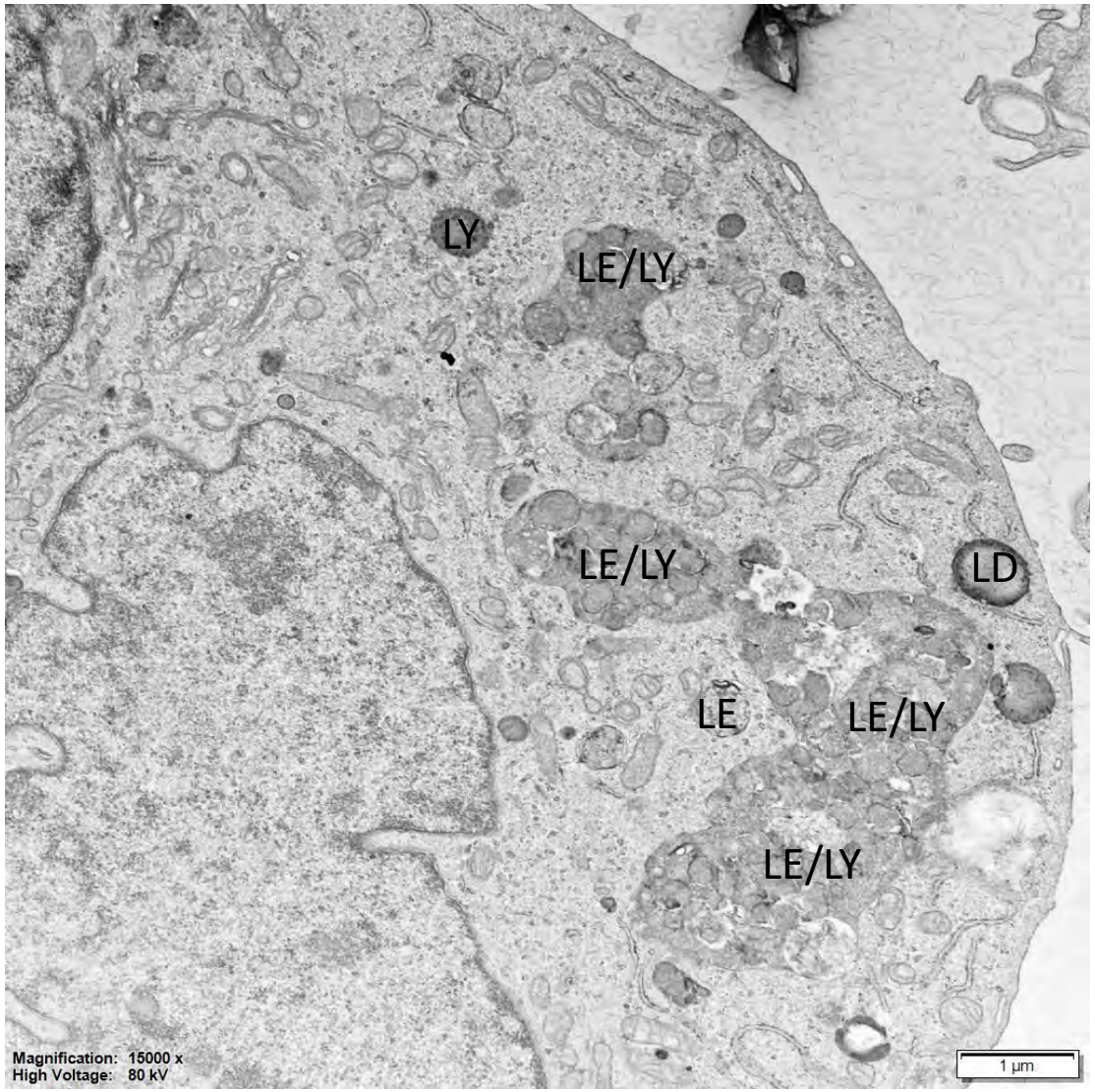


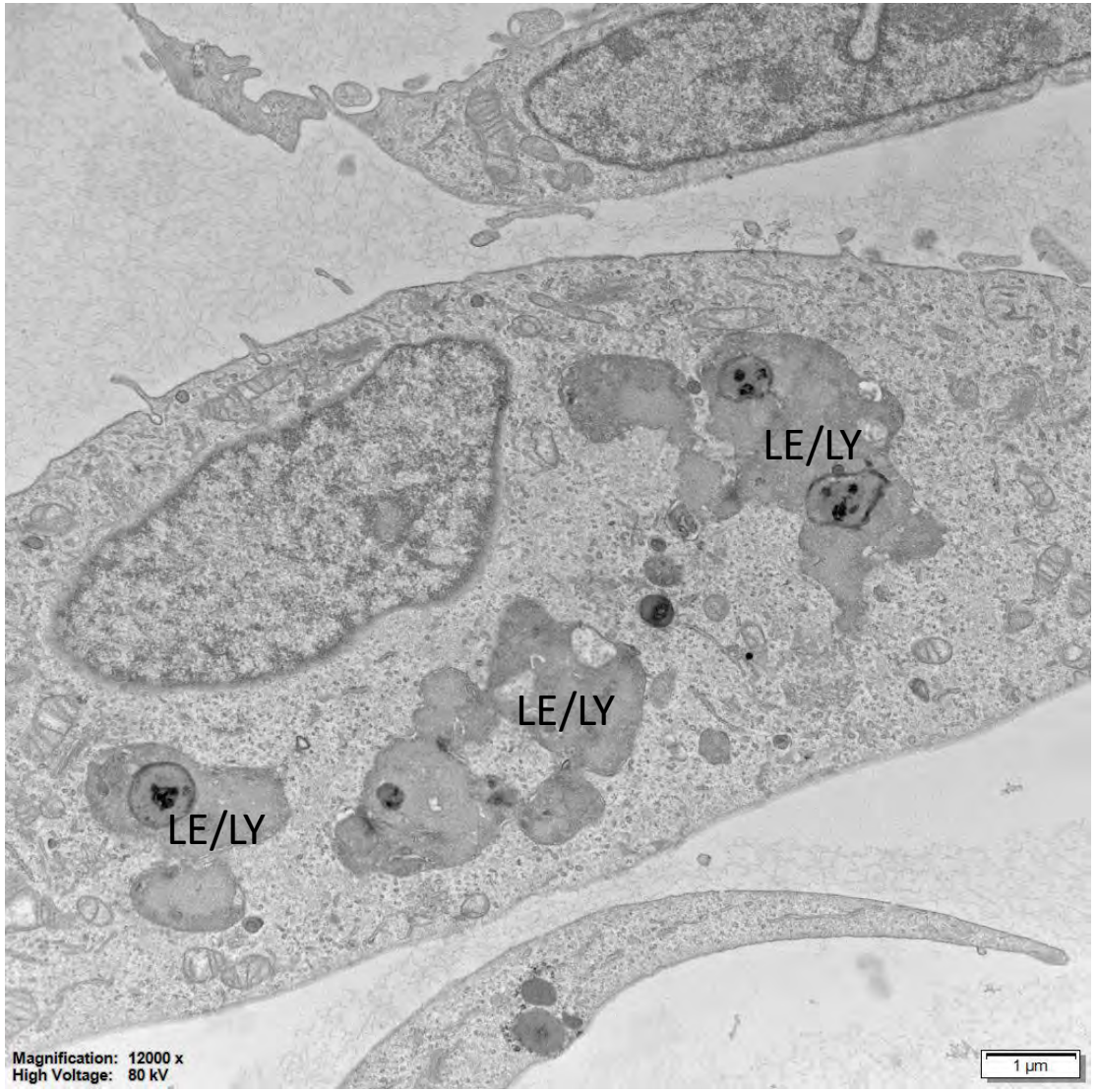






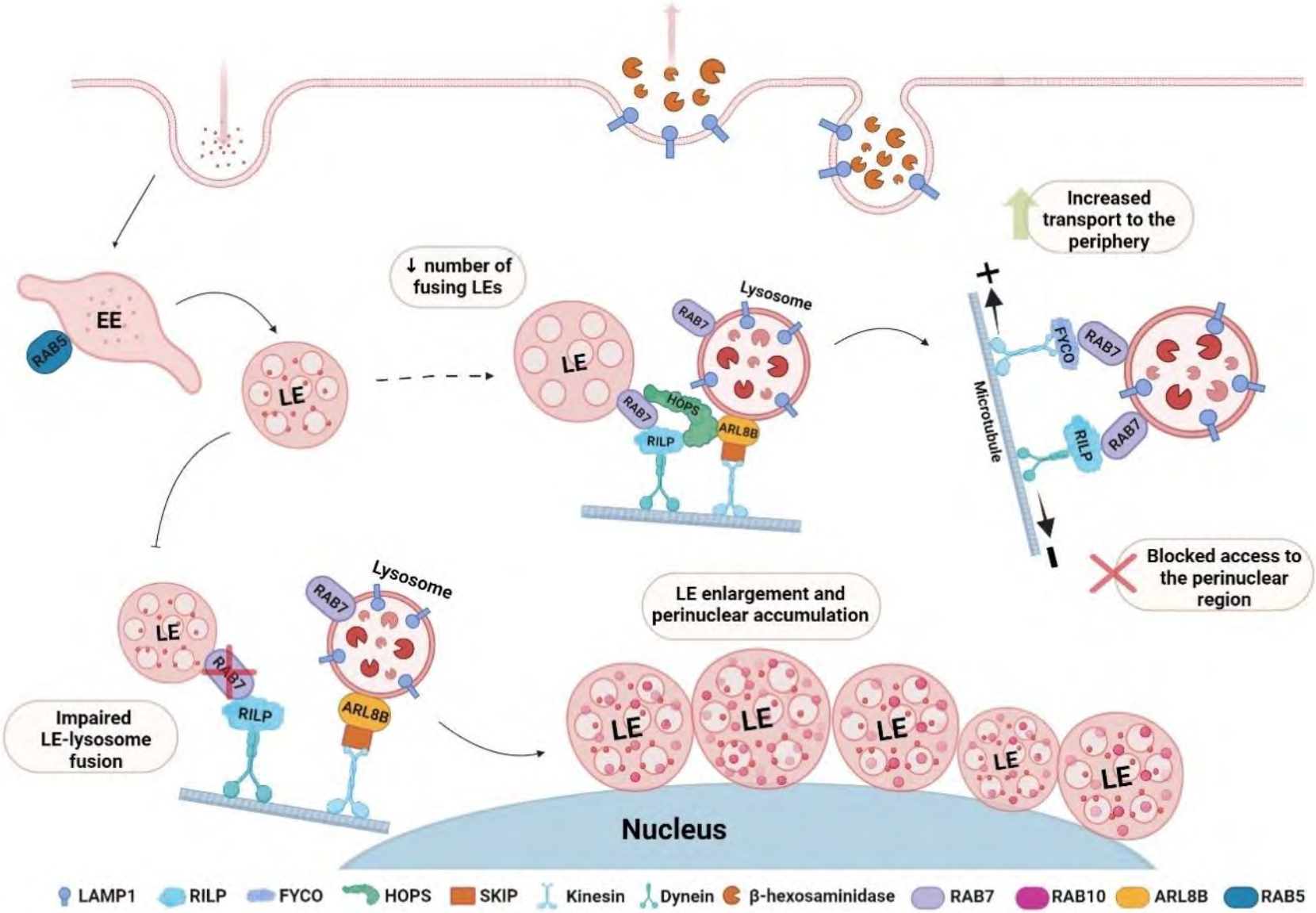
RAB7 Silencing Leads to Enlarged Perinuclear Late Endosomes/Lysosomes





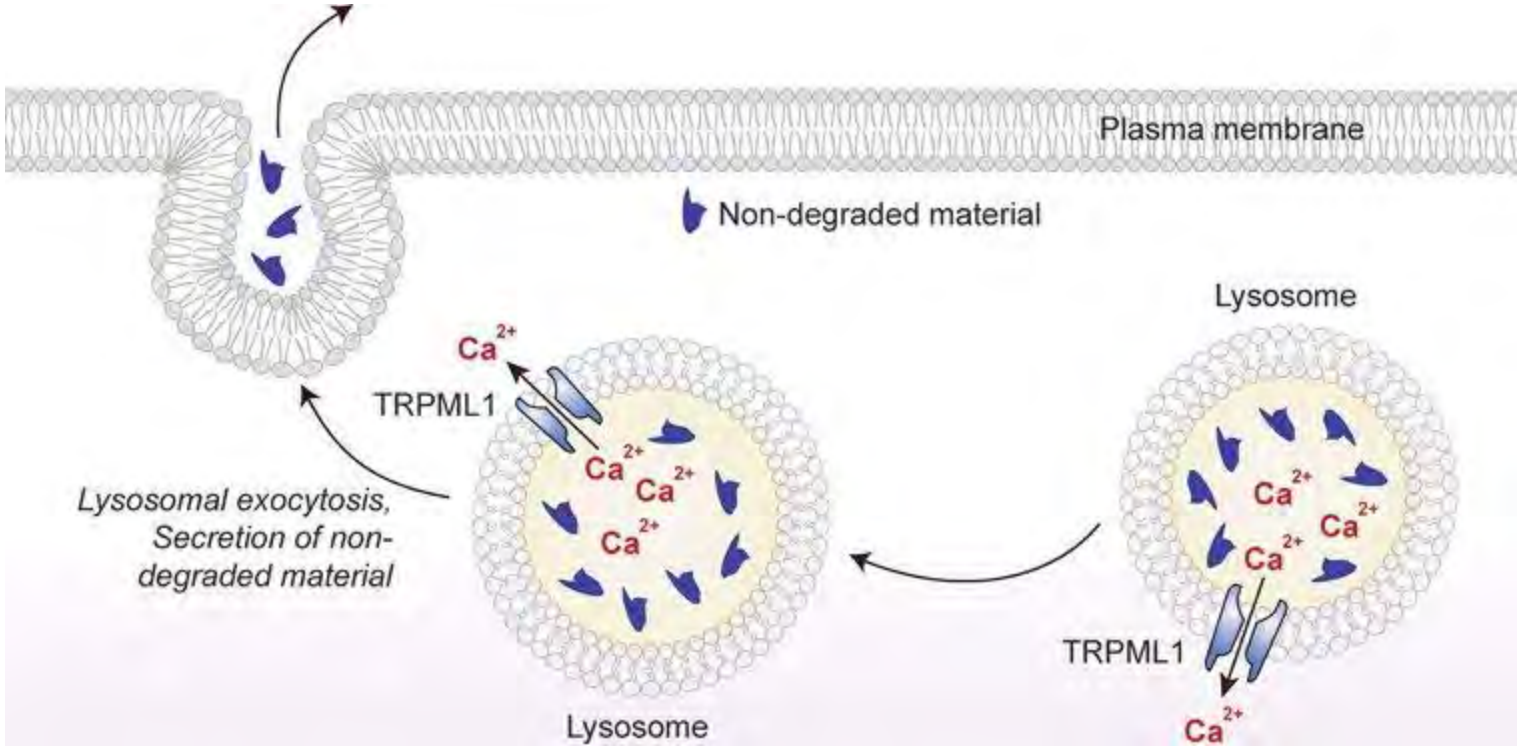
Ana Rita Rodrigues and Nalan Liv, unpublished

Working Model



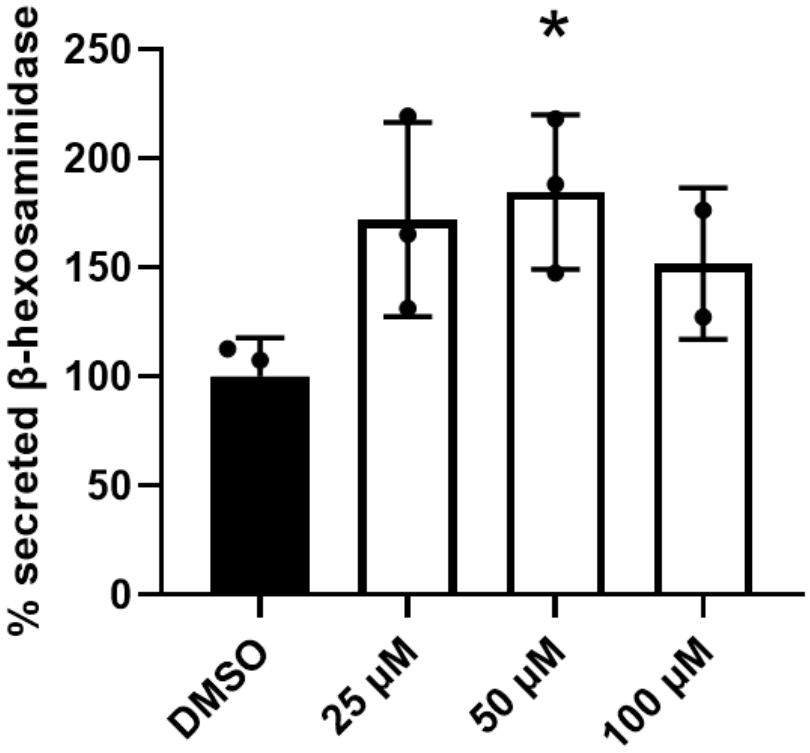
Lysosome Exocytosis Modulation Affects Cancer Cell Invasion ⇒ Lysosome Exocytosis as a Therapeutic Target to Impair Cancer Progression

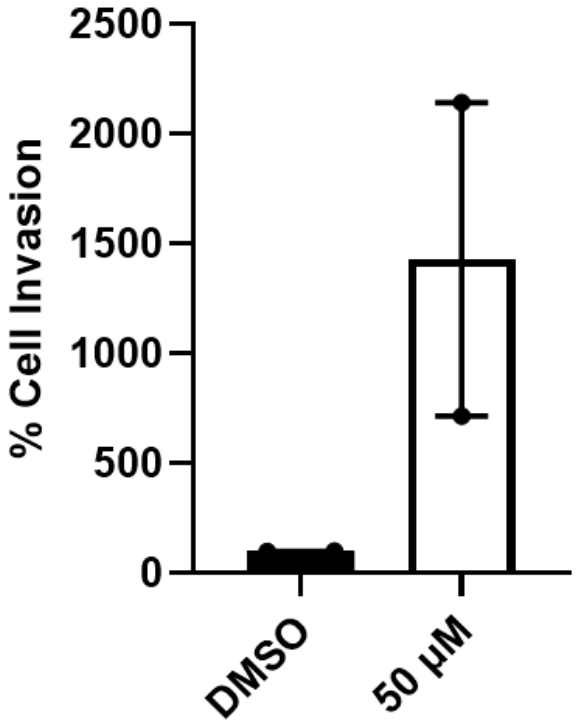
Can We Modulate Lysosome Exocytosis With Drugs and Inhibit Cancer Cell Invasion?

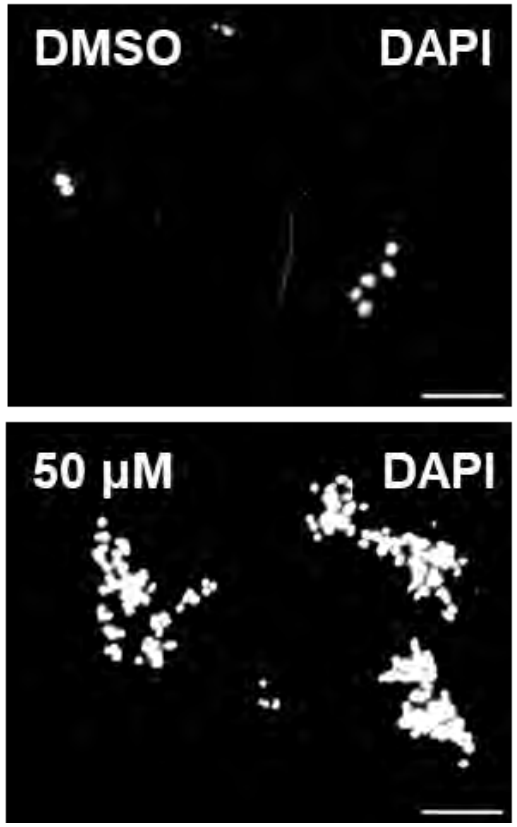


Seranova *et al.*, *Essays Biochem.*, 2017

TRPML1 Agonist ML-SA1 Increases Lysosome Exocytosis and Invasion in Breast Cancer Cells

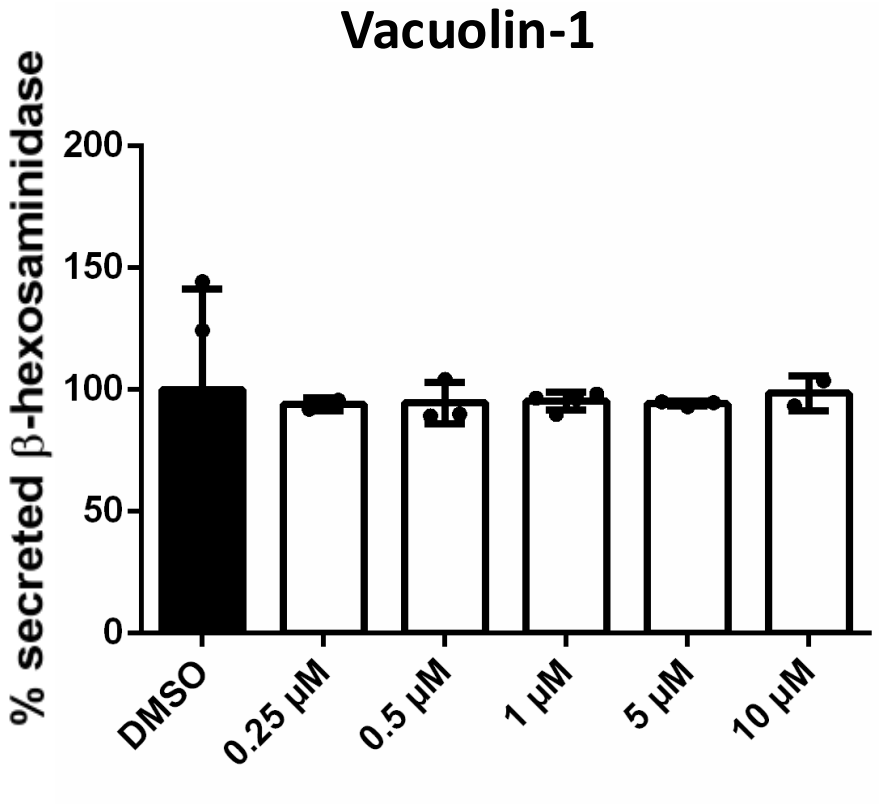


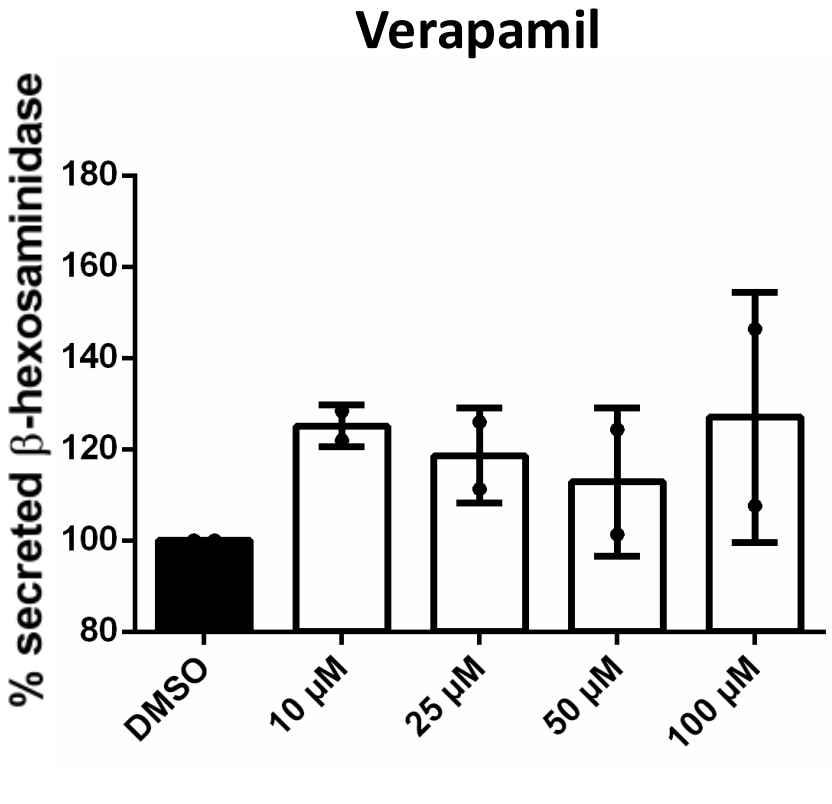




Ana Rita Rodrigues, unpublished

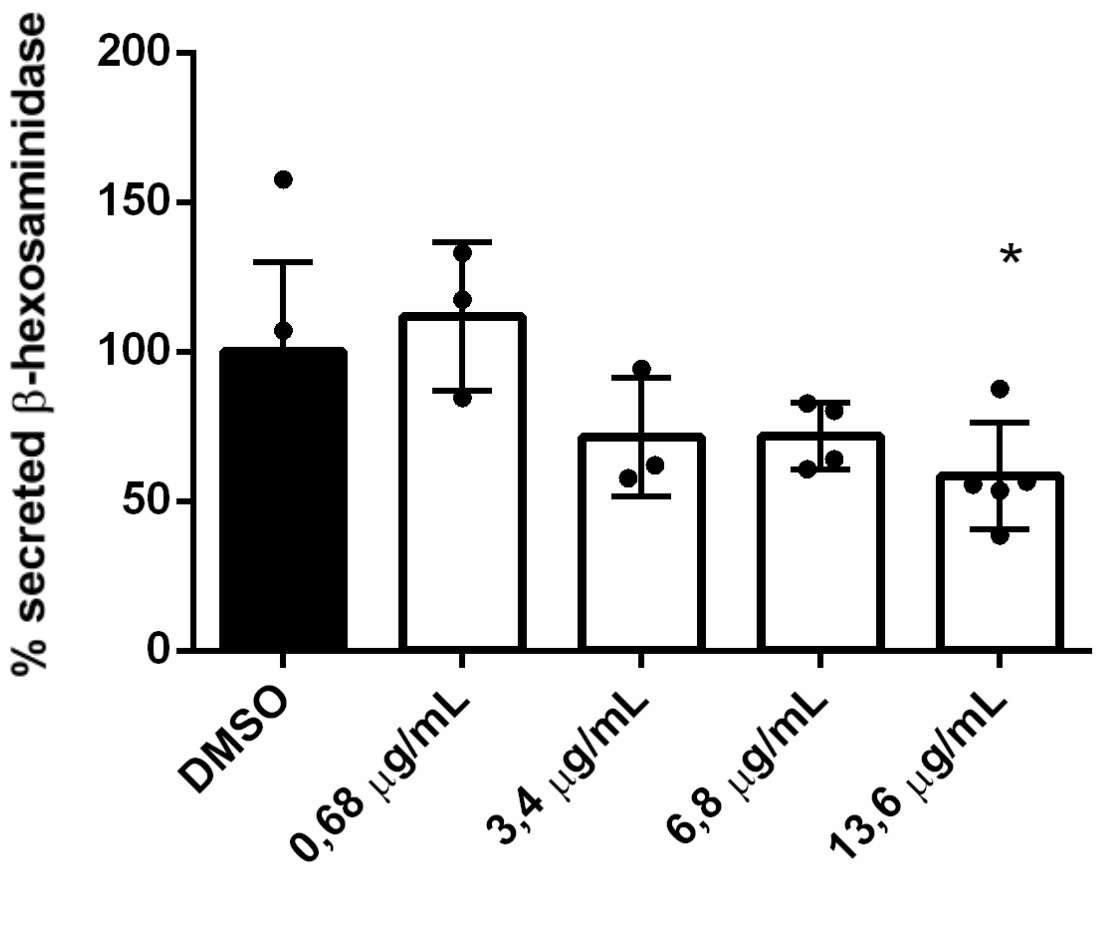
Vacuolin-1 and Verapamil Do Not Affect Lysosome Exocytosis in Breast Cancer Cells





Cristina Escrevente, unpublished

Compound #29 Inhibits Lysosome Exocytosis in Breast Cancer Cells



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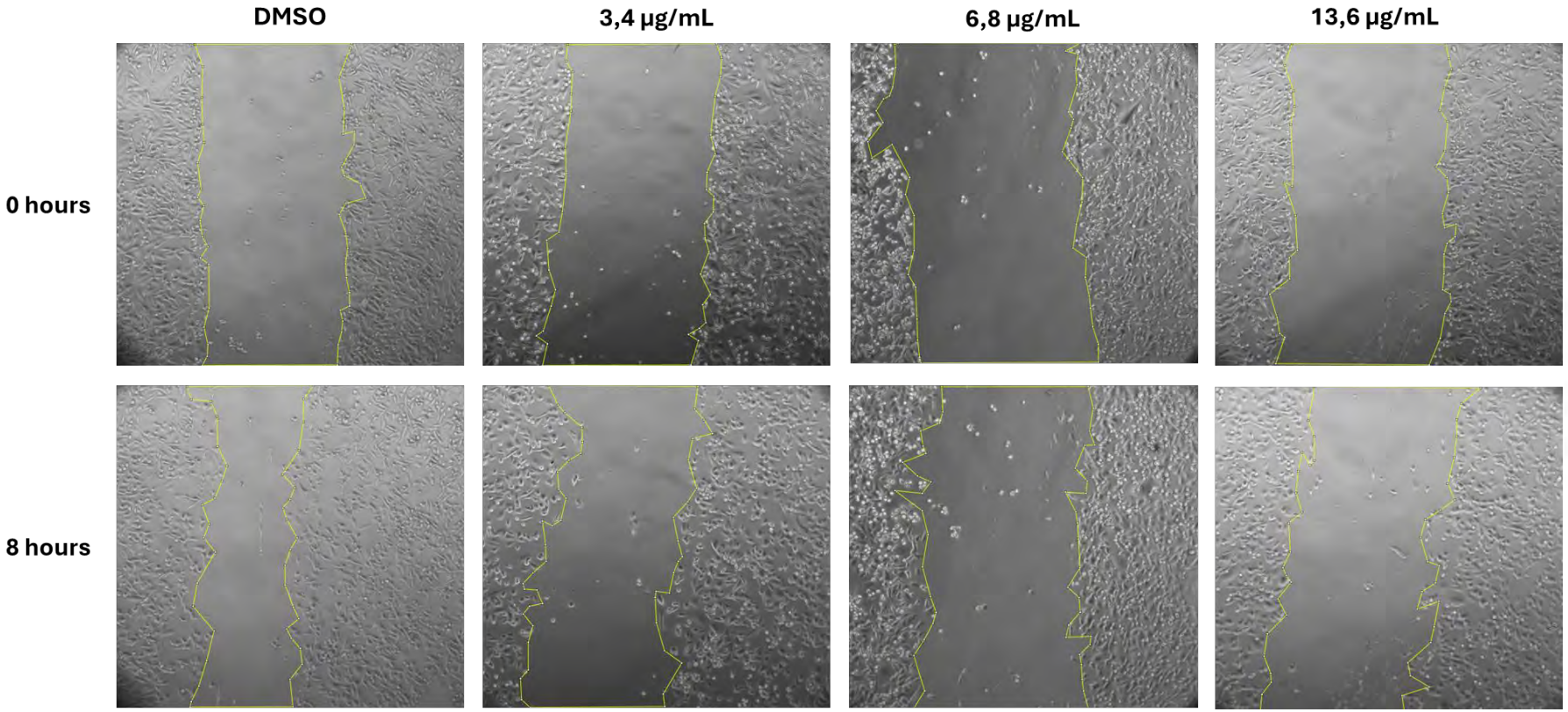
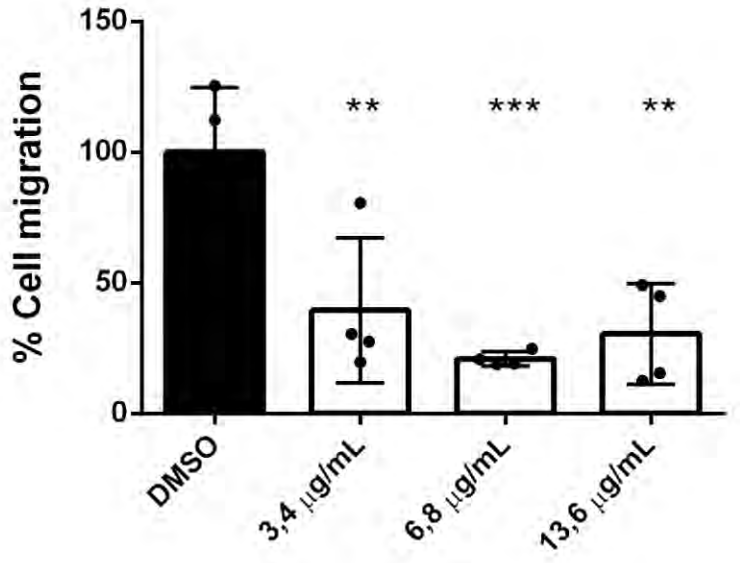
Cristina Escrevente, unpublished

Compound #29 Inhibits Cell Migration in Breast Cancer Cells

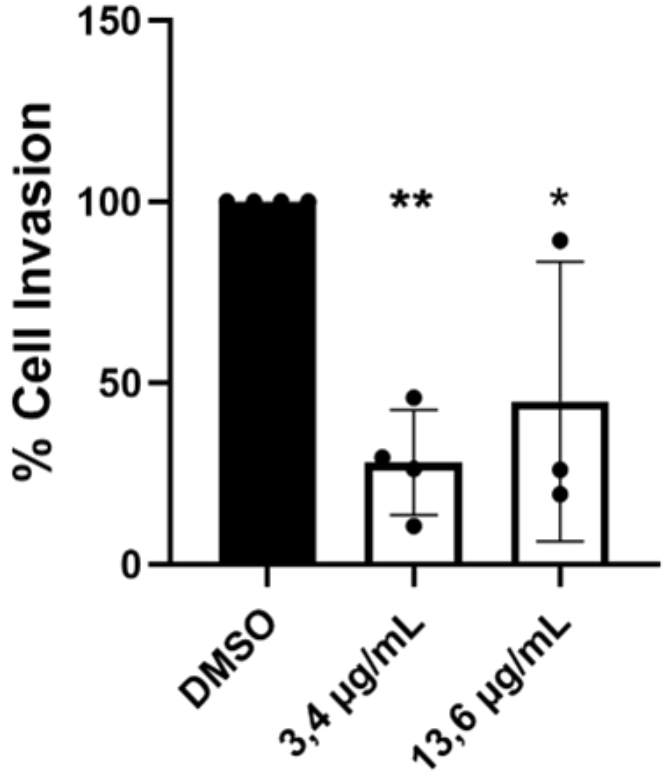


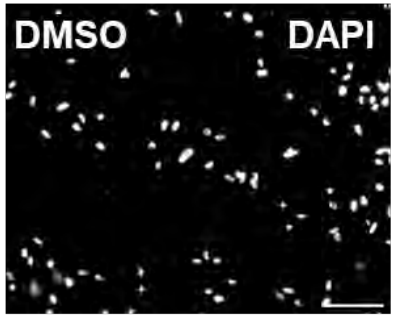


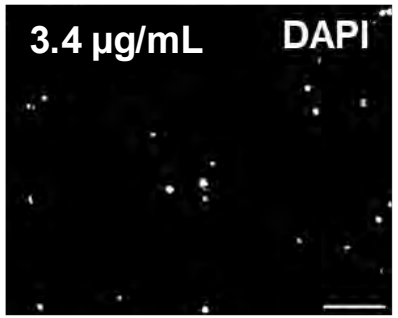
Sea4Us

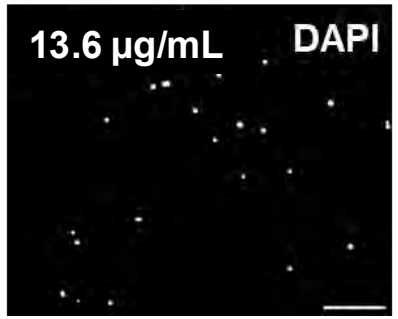
Cristina Escrevente and Ana Rita Rodrigues, unpublished

Compound #29 Inhibits Invasion in Breast Cancer Cells







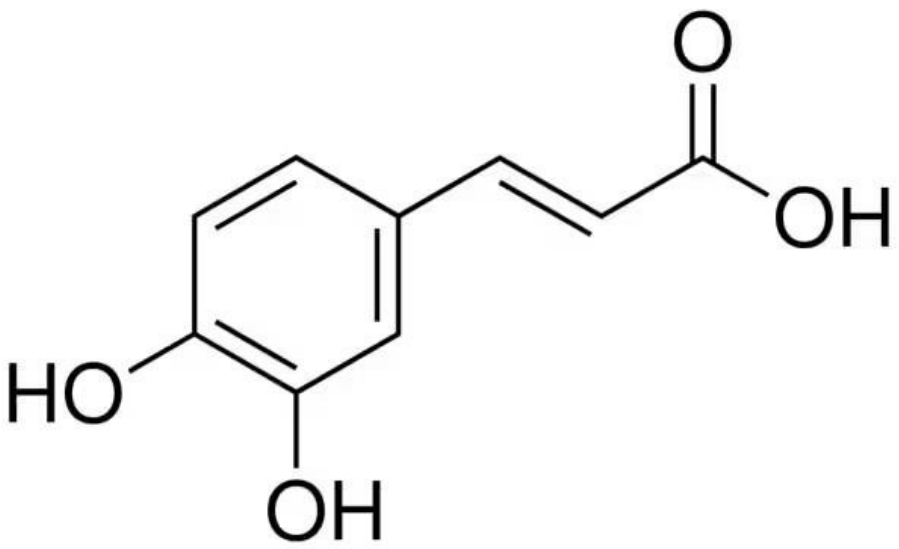


Sea4Us

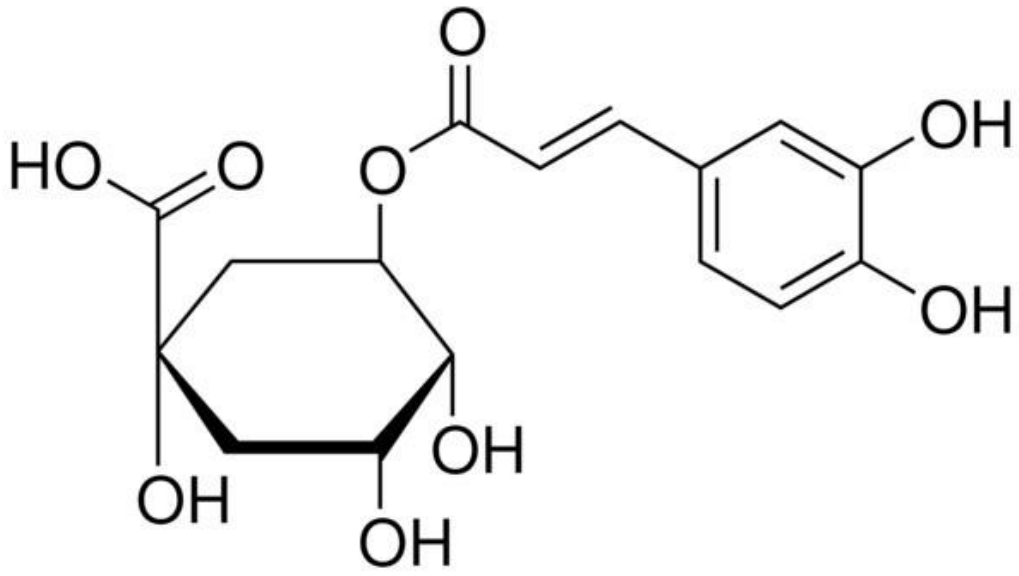
Cristina Escrevente and Ana Rita Rodrigues, unpublished

Phenolic Compounds

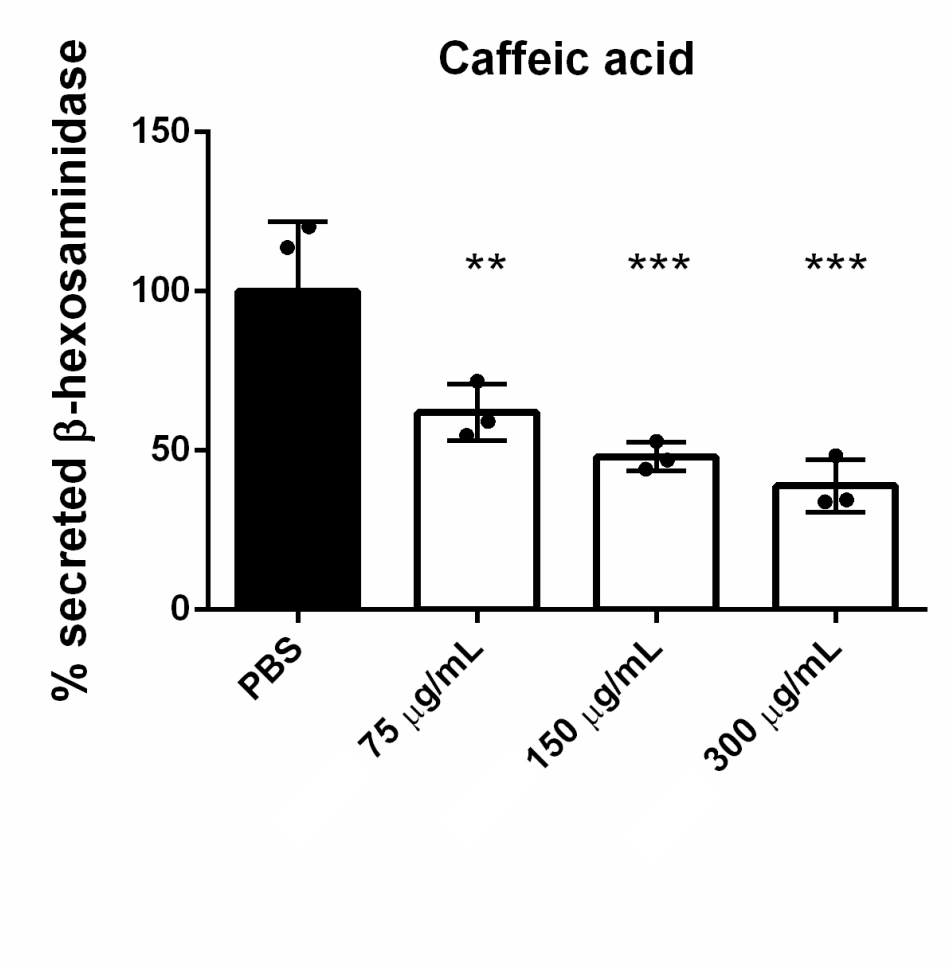
Caffeic acid

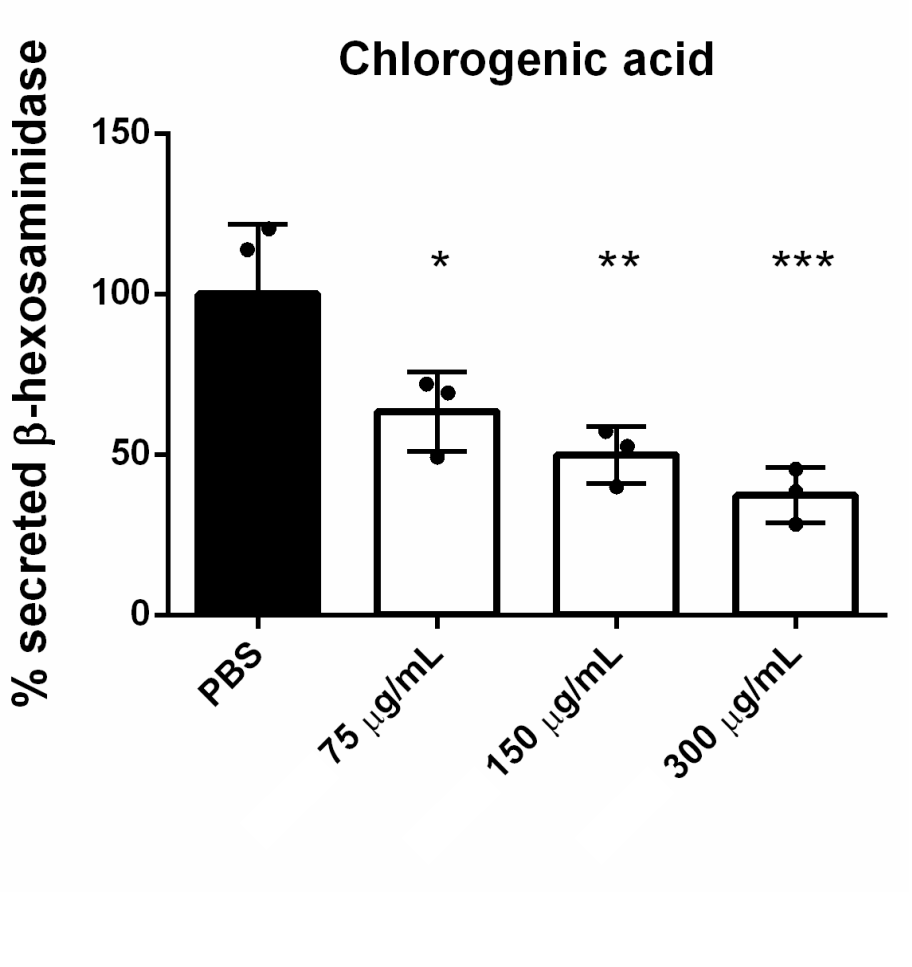


Chlorogenic acid



Caffeic Acid and Chlorogenic Acid Inhibit Lysosome Exocytosis in Breast Cancer Cells

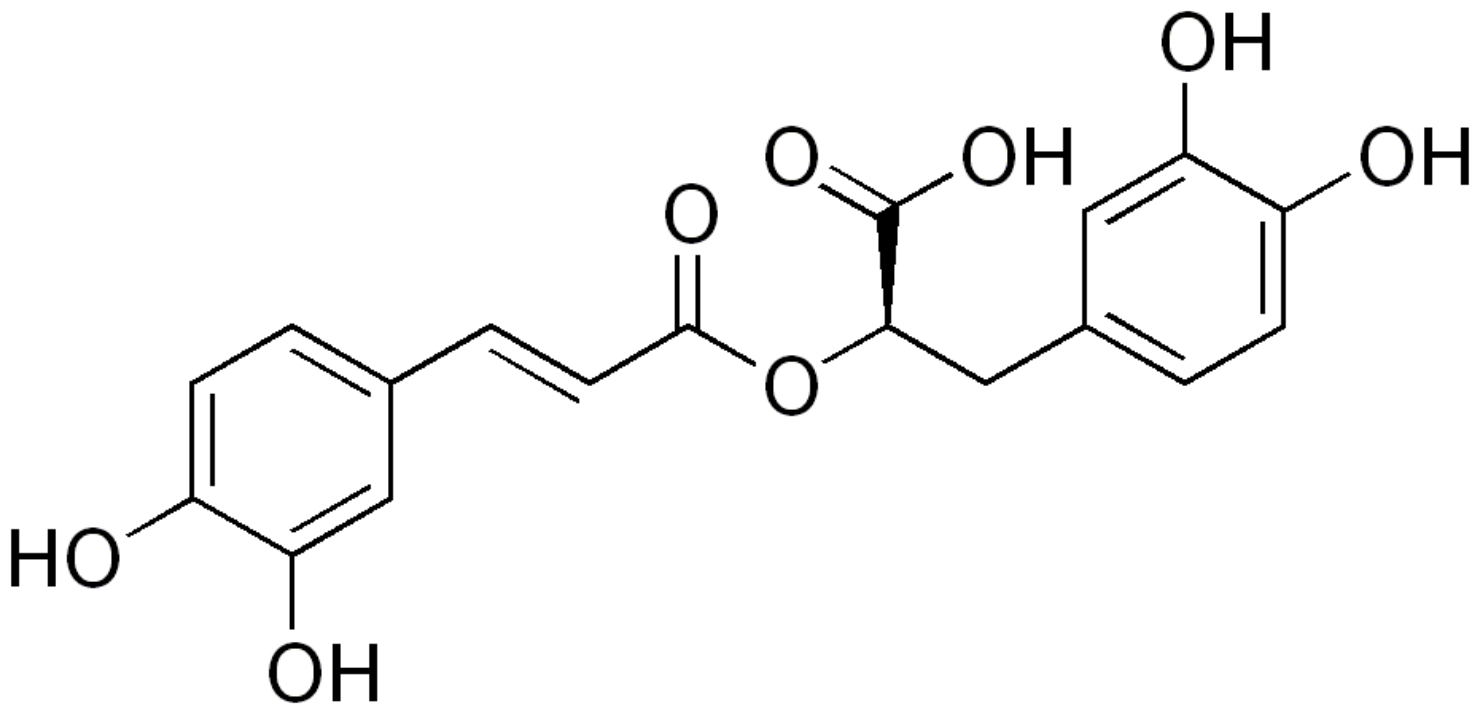




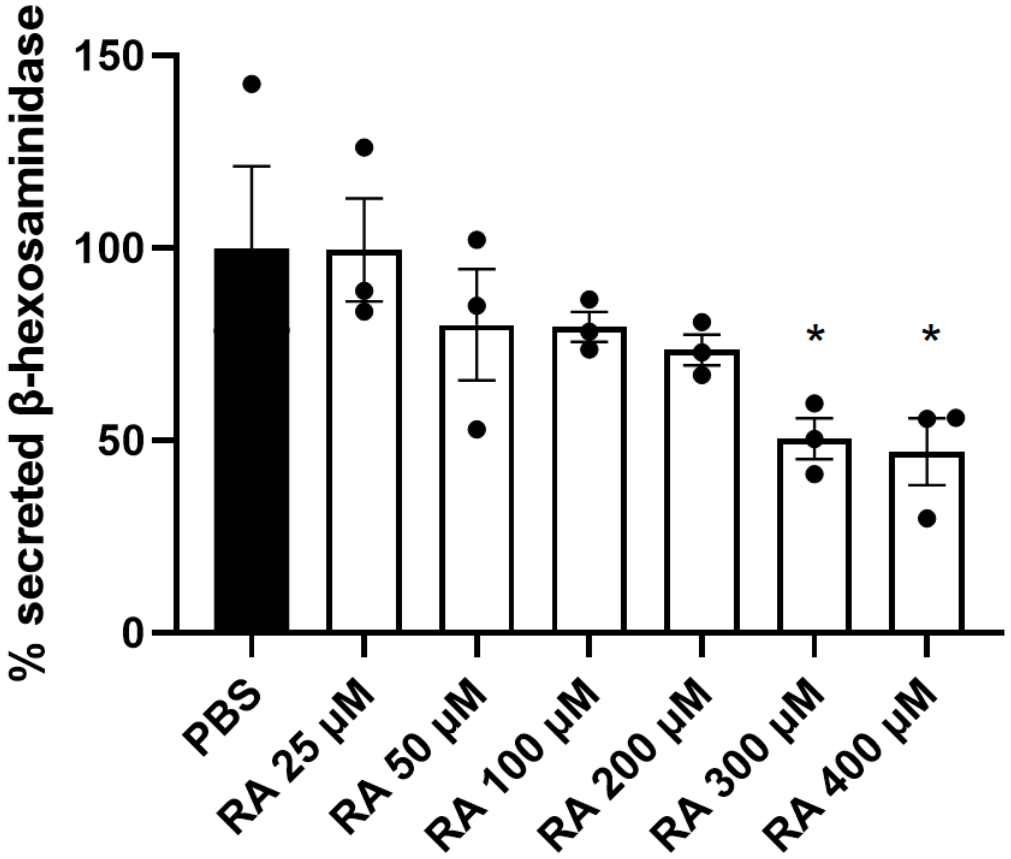
Cristina Escrevente, unpublished

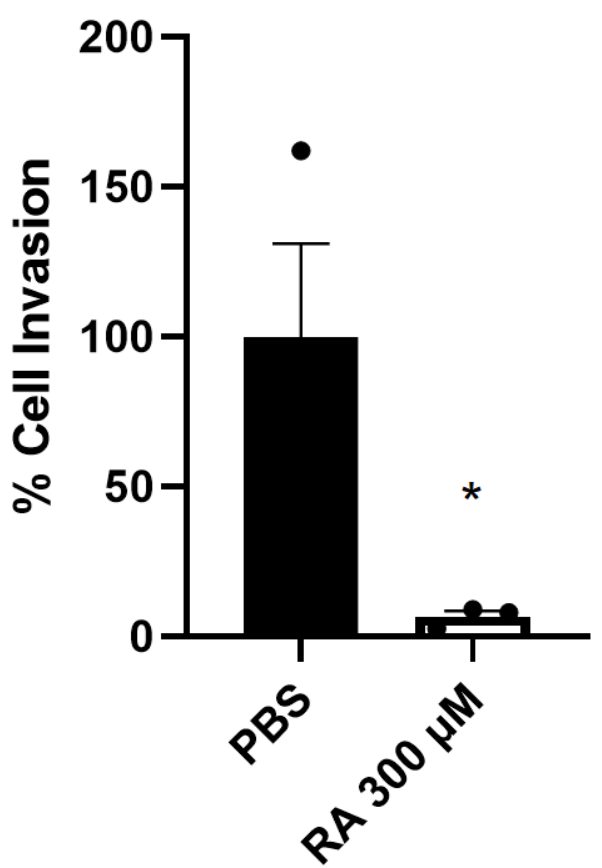
Phenolic Compounds

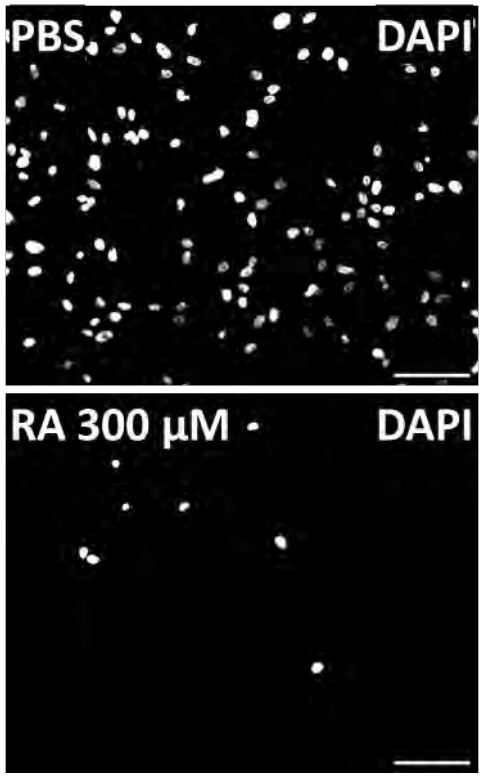
Rosmarinic acid



Rosmarinic Acid Inhibit Lysosome Exocytosis and Invasion in Breast Cancer Cells







Cristina Escrevente and Ana Rita Rodrigues, unpublished

Conclusions

- Lysosome exocytosis directly correlates with cancer cell invasive capacity
- Lysosome exocytosis stimulation enhances cancer cell invasion
- Lysosome exocytosis inhibition impairs cancer cell invasion
- Lysosome exocytosis can serve as a therapeutic target to inhibit cancer cell invasion
- Lysosome exocytosis inhibitory drugs can be used to impair cancer progression and metastasis

Acknowledgements

Former lab members:

Andreia Ferreira

Pedro Castanheira

Isabel Sesifredo

Collaborators:

Miguel Seabra (NMS-UNL)

José Ramalho (NMS-UNL)

Jacinta Serpa (NMS-UNL)

Saudade André (NMS-UNL)

Marta Pojo (IPOLFG)

Cláudia Santos /Diogo Carregosa (NIMSB)

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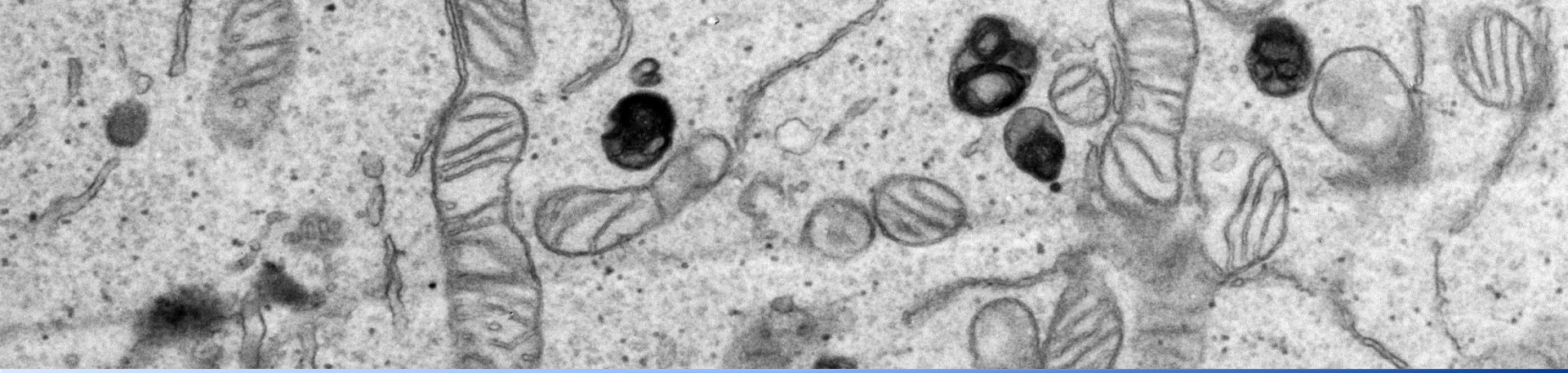

inter-organelle communication driving pathological mechanisms in Batten disease

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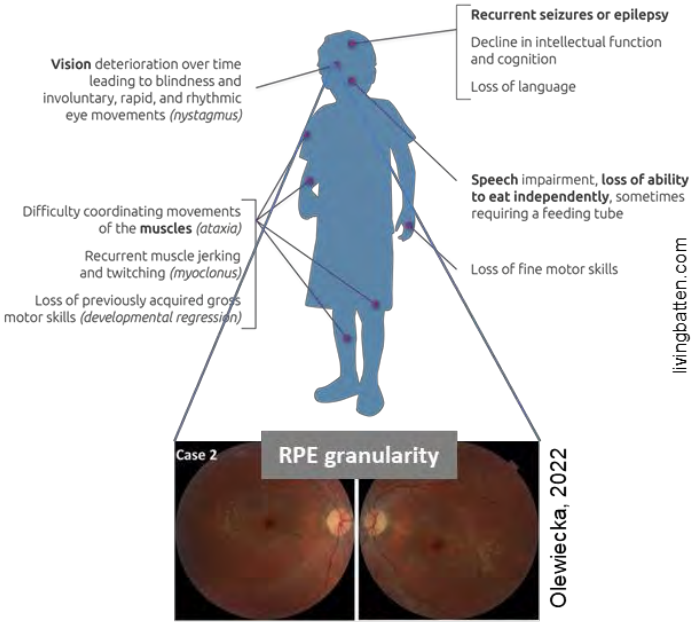
No conflict of interest to disclose.

Batten disease

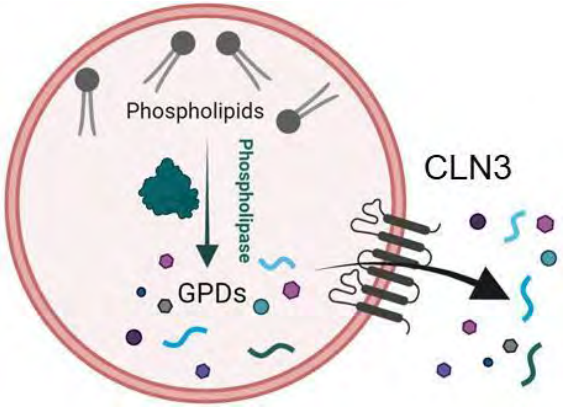
- Neuronal Ceroid Lipofuscinoses (NCL)
- Autosomal recessive, neurodegenerative, lysosomal storage disease
- Affect up to 1 in 25,000 births: fatal by 20–30 years of life
- Mutations in 14 different genes that encode distinct CLN proteins
- No disease-modifying treatments



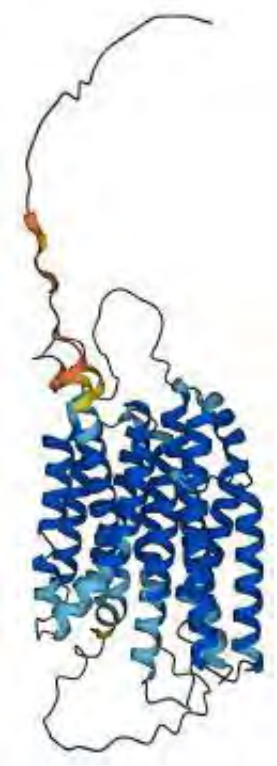
Deteriorating vision is often the first sign of **Batten disease**

Poorly understood disease
in terms of underlying **molecular mechanism**

ceroid lipofuscinosis neuronal 3 (CLN3) Protein



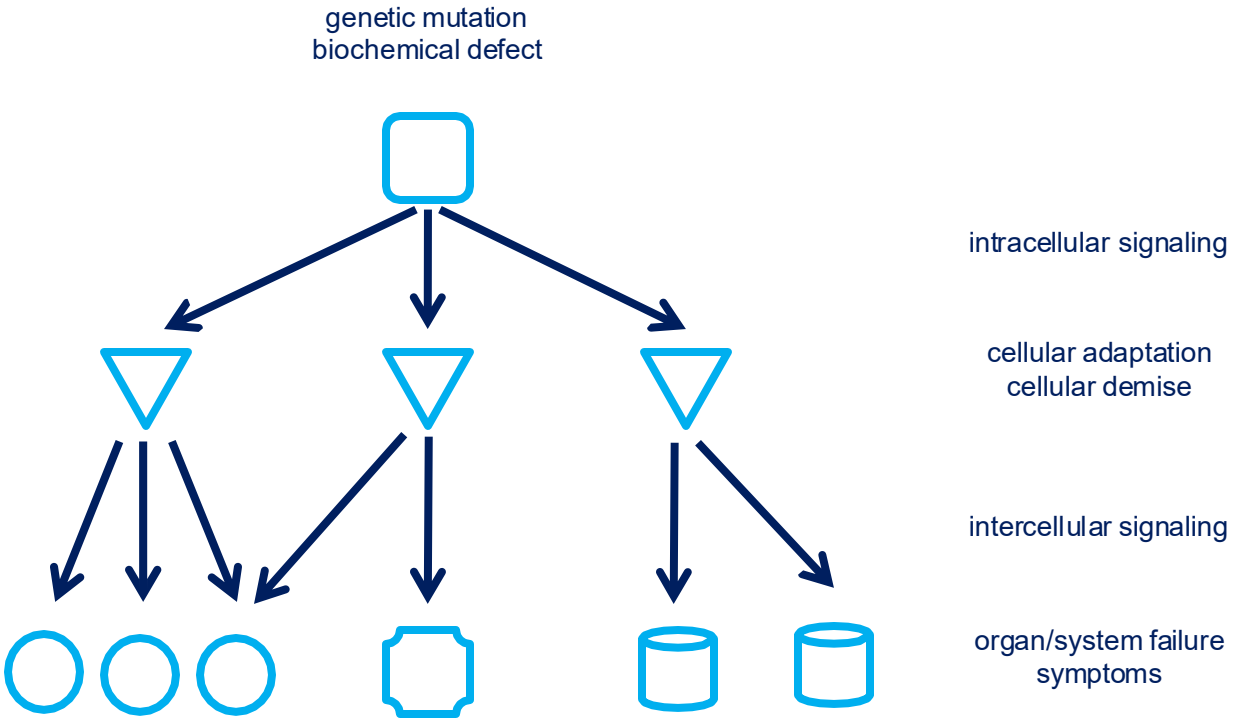
End products of
glycerophospholipid catabolism



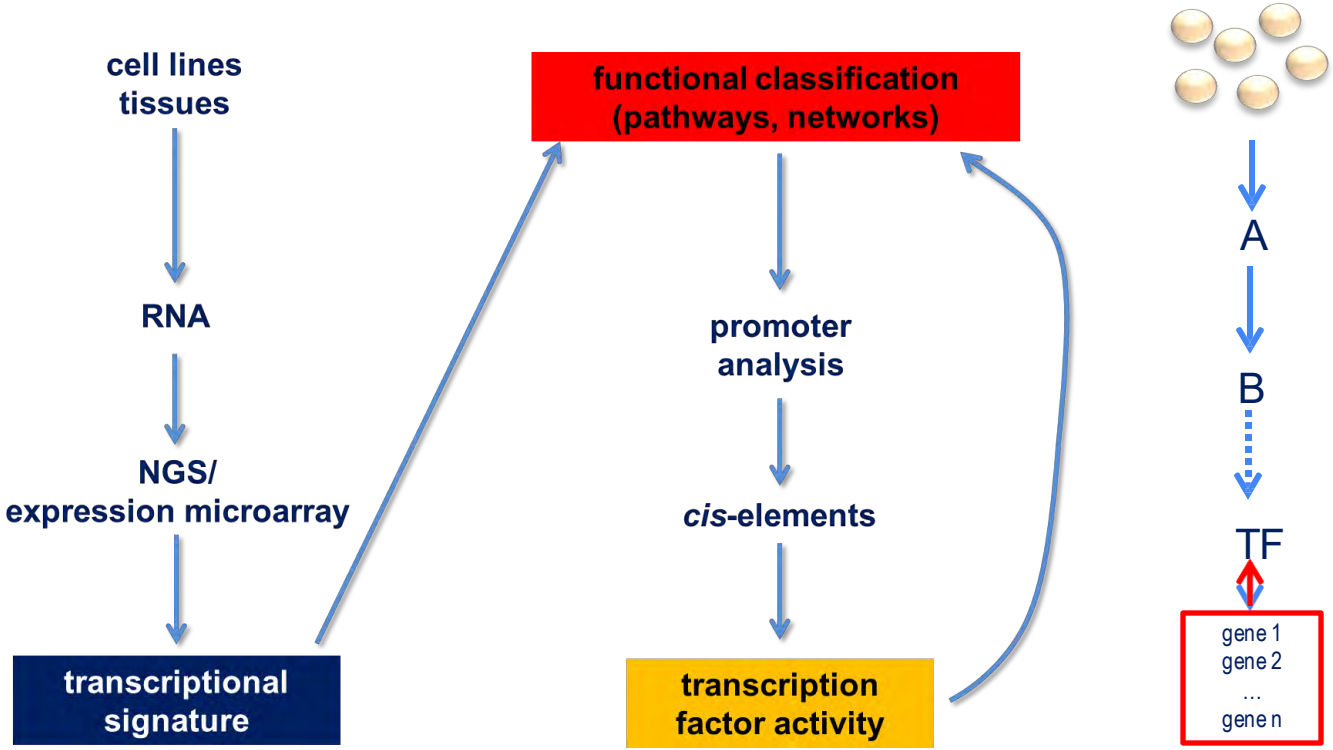
Genecards

CLN3 is required for the clearance of glycerophosphodiesters from lysosomes (Lagtom, Nature, 2022)

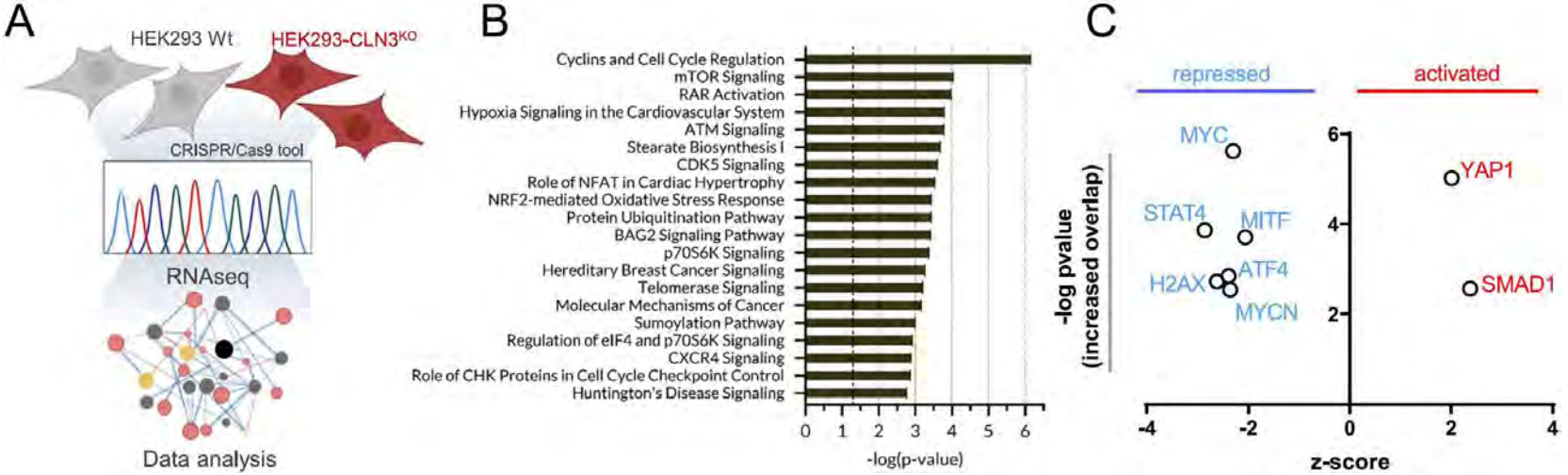
hierarchical signaling in disease mechanisms



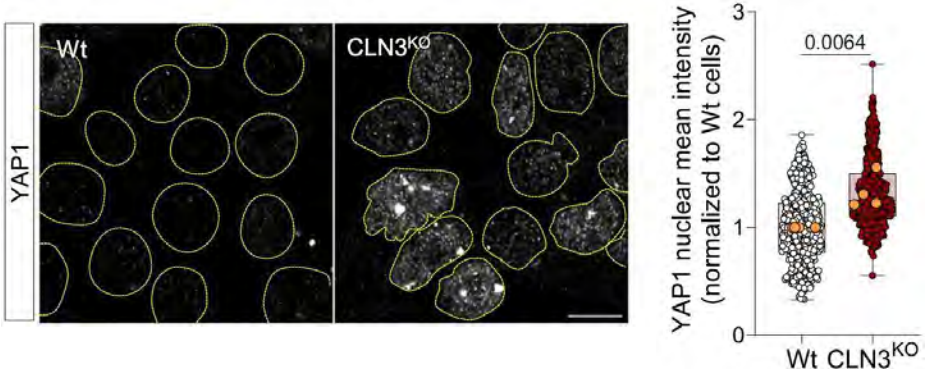
signaling pathways inferred from transcriptome datasets



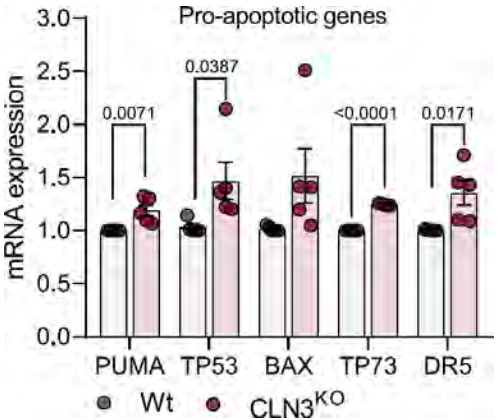
CLN3-KO transcroptome reveals hyperactive YAP1 signaling



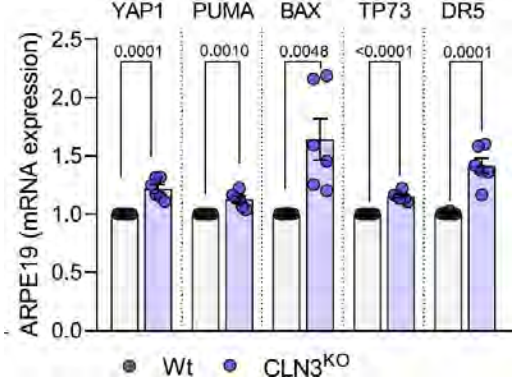
YAP1 transcriptional activity increased in CLN3-KO cells



HEK cells

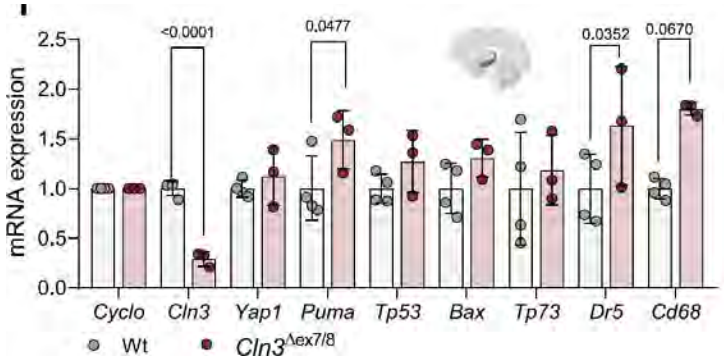


ARPE cells

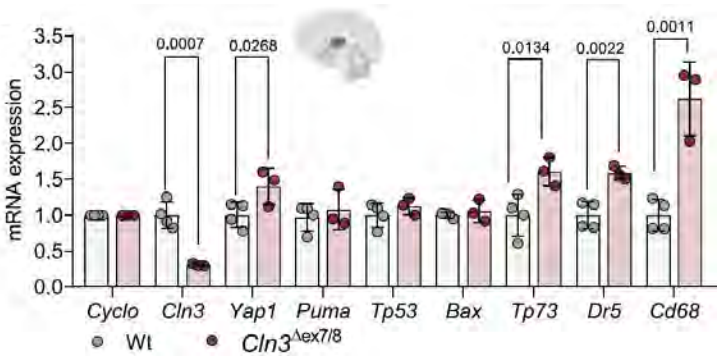


YAP1 transcriptional activity increased in CLN3-KO mouse tissues

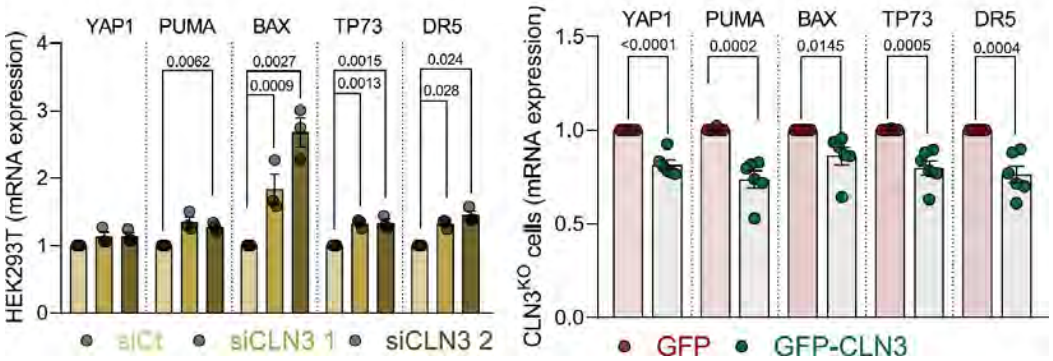
hippocampus



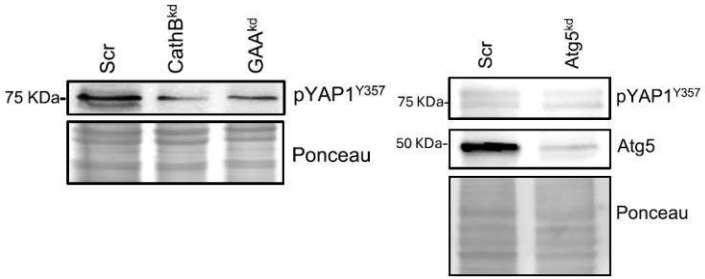
thalamus

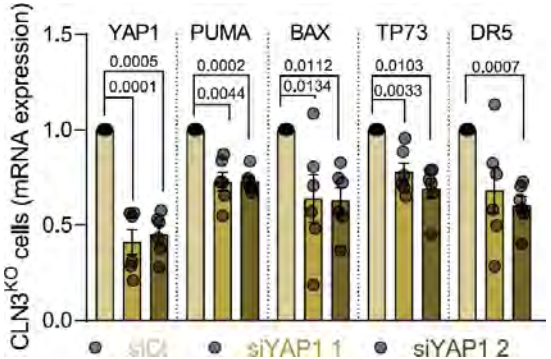


YAP1-specific response to loss of CLN3

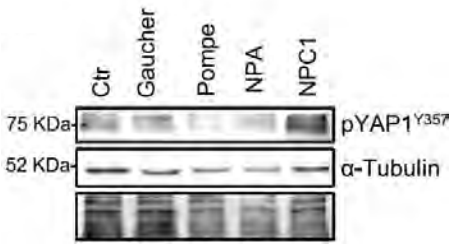


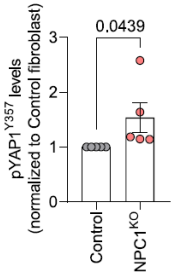
HeLa cells



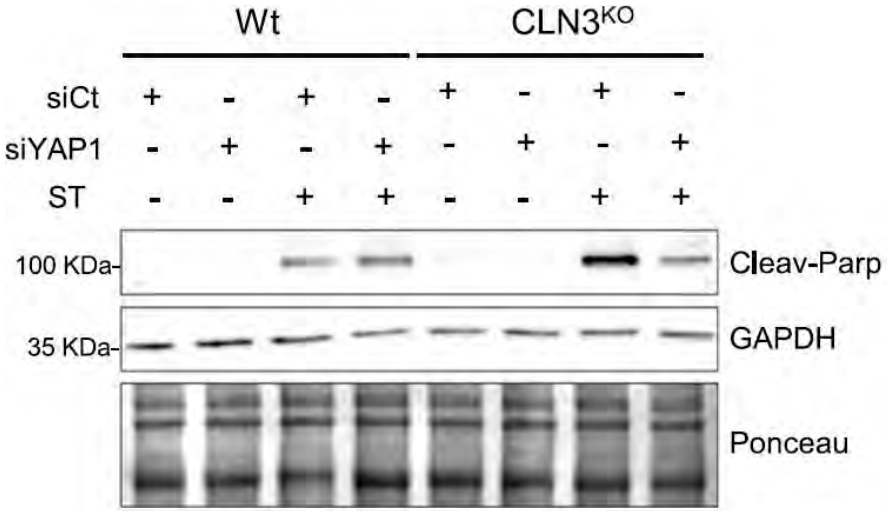


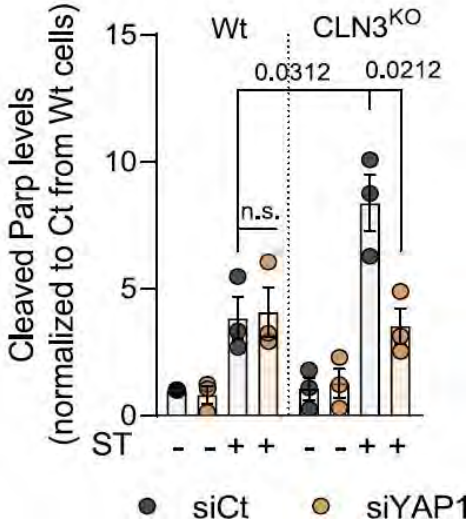
patient fibroblasts



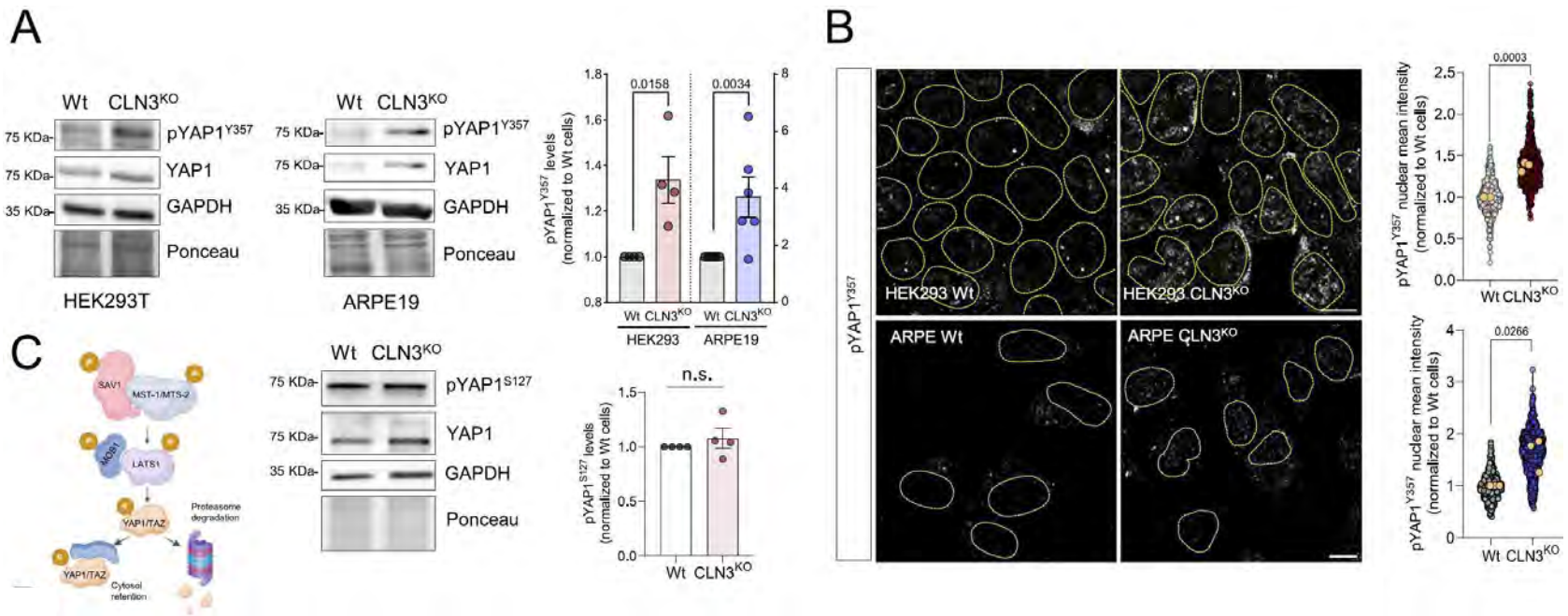


YAP1 signaling increases apoptosis susceptibility in CLN3-KO cells

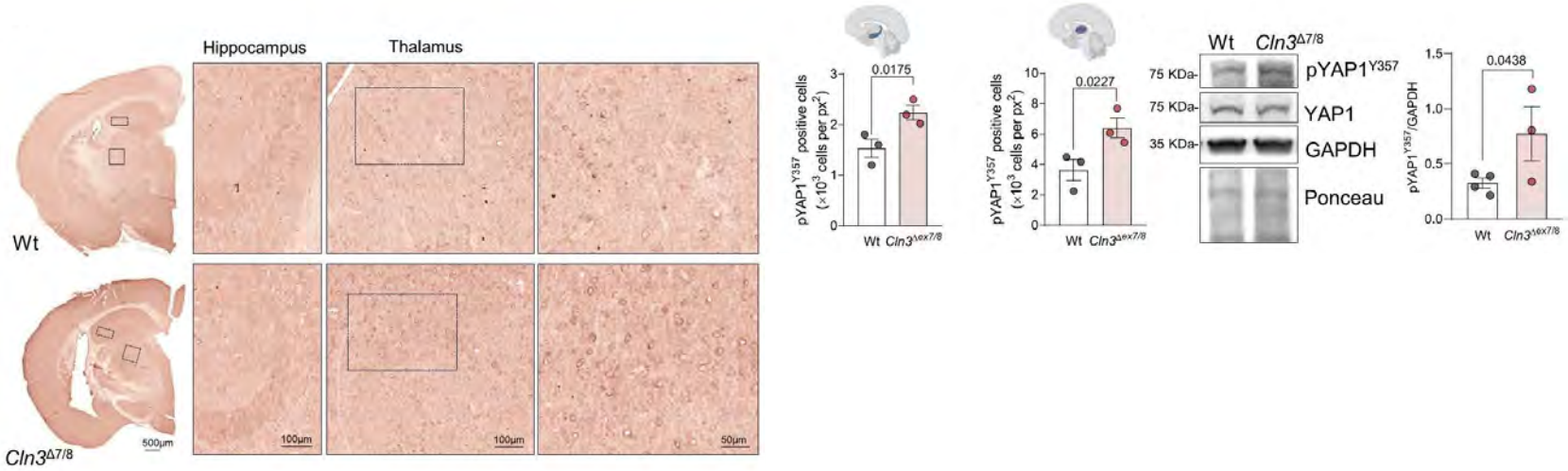




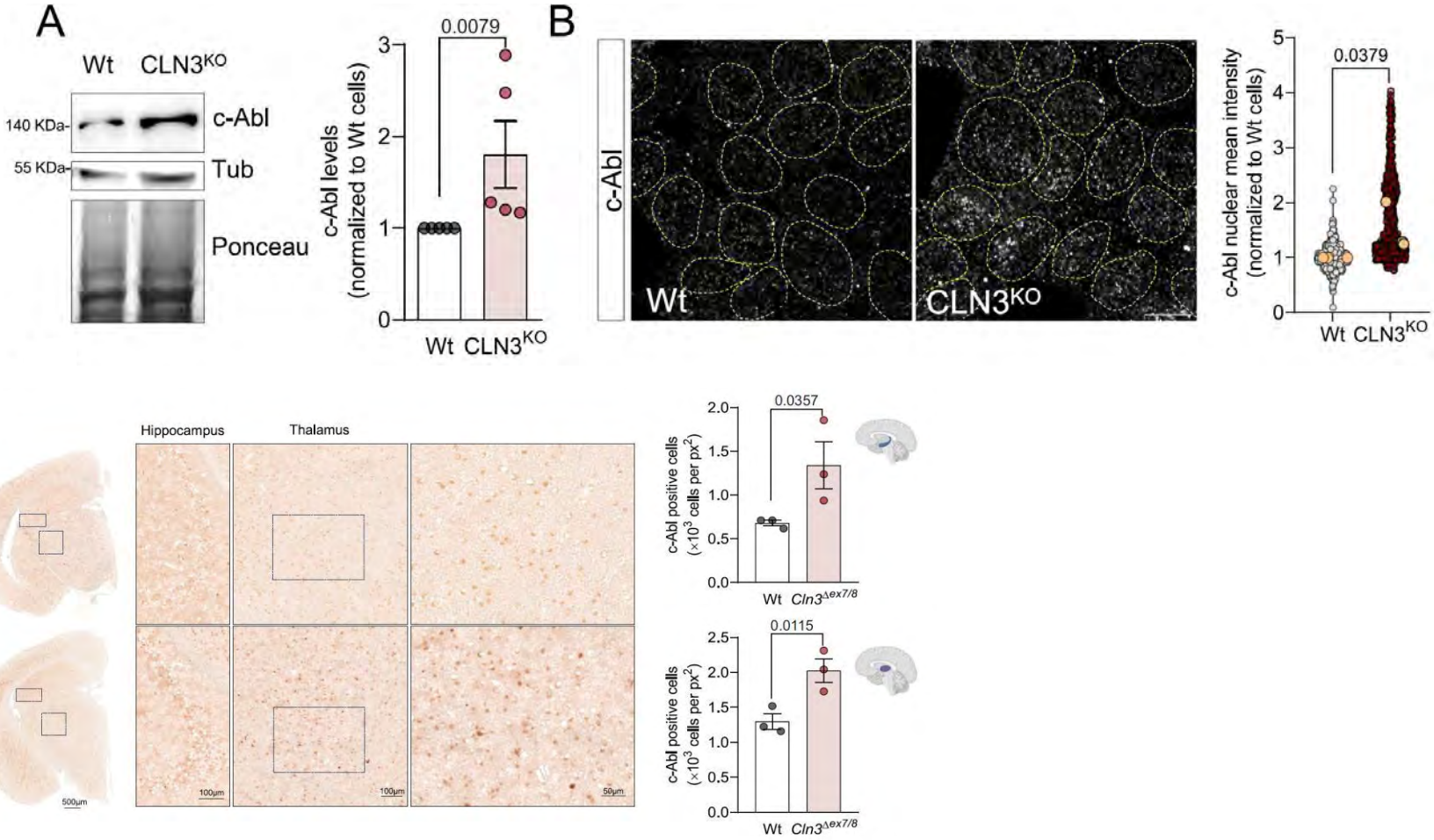
YAP1 transcriptional activity associated with Y357 phosphorylation in cells…



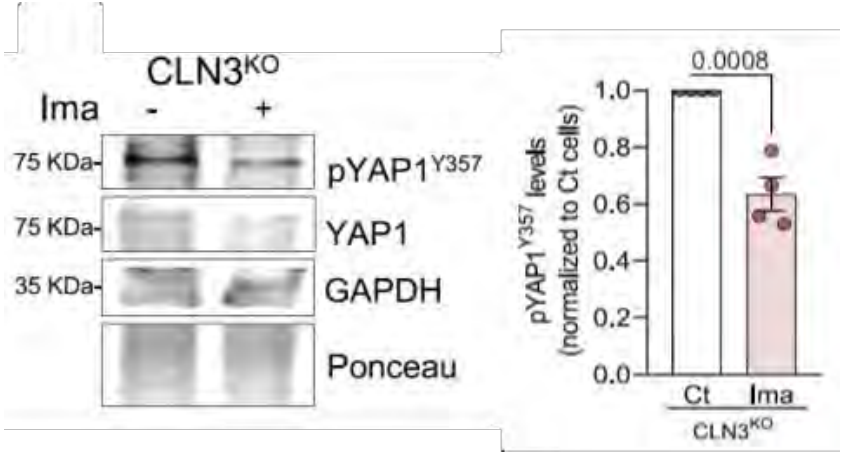
… *and* in vivo


Hippocampus
Thalamus
Wt
Cln3Δ7/8
500µm
100µm
100µm
50µm
pYAP1Y357 positive cells (×10³ cells per px²)
0.0175
Wt
Cln3Δex7/8
0.0227
Wt
Cln3Δex7/8
Wt
Cln3Δ7/8
75 KDa
pYAP1Y357
75 KDa
YAP1
35 KDa
GAPDH
Ponceau
pYAP1Y357/GAPDH
0.0438
Wt
Cln3Δex7/8

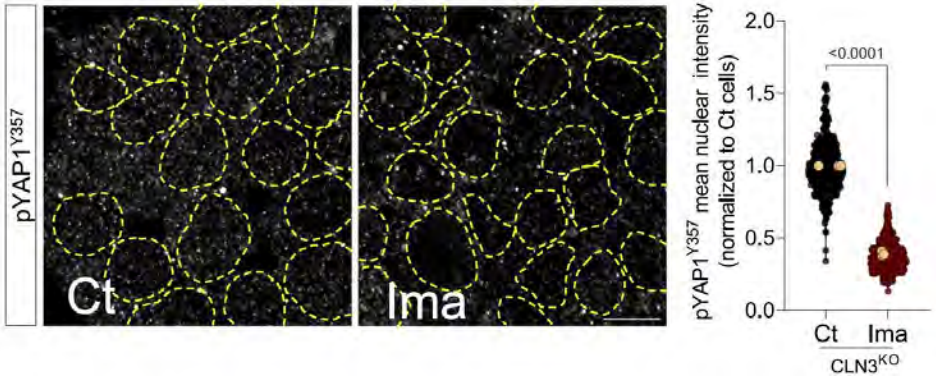
YAP1-Y357 is phosphorylated by c-Abl kinase



inhibition of c-Abl kinase ablates YAP1 signaling in CLN3-KO cells



E



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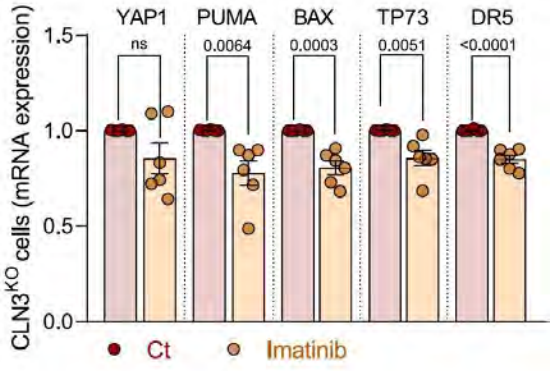
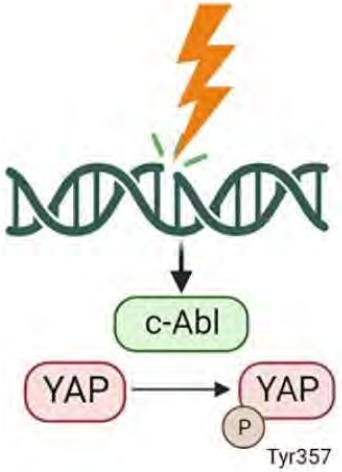
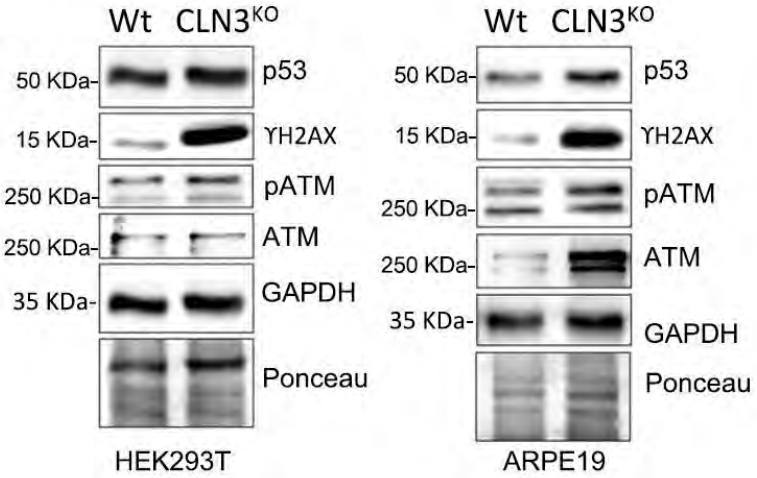
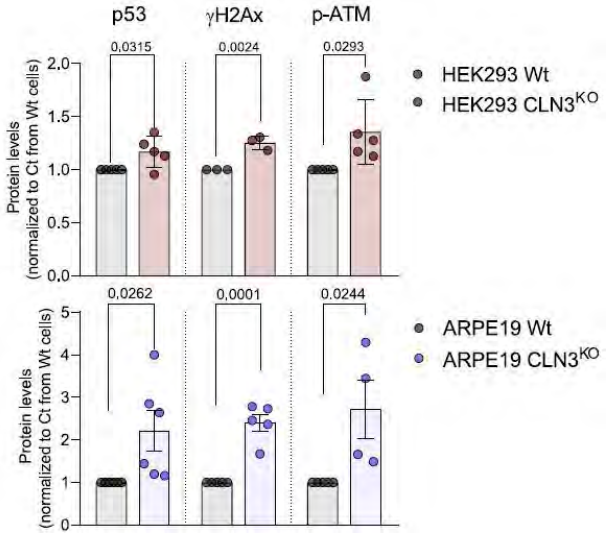


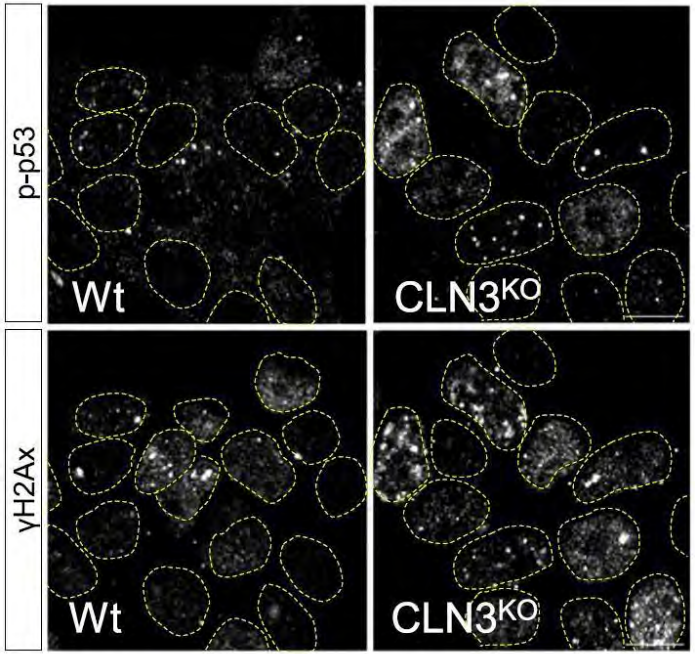
DNA damage is the canonical activator of c-Abl kinase

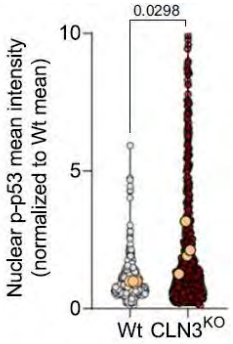




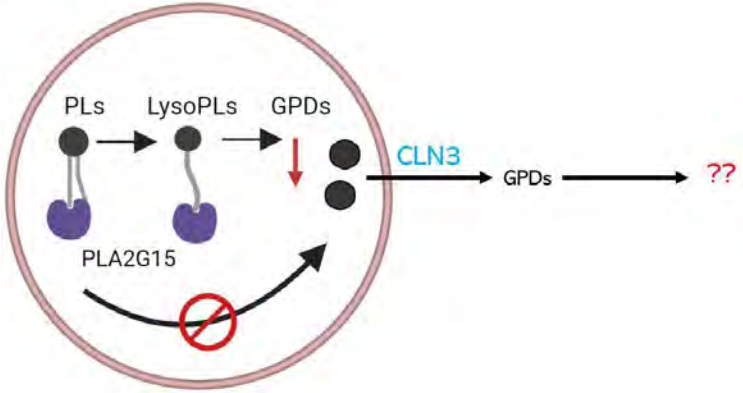


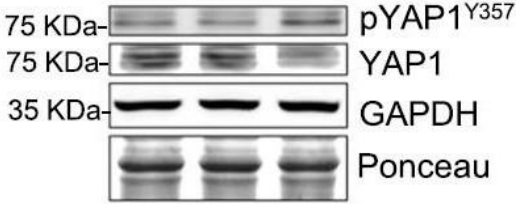
loss of CLN3 leads to accumulation of DNA damage

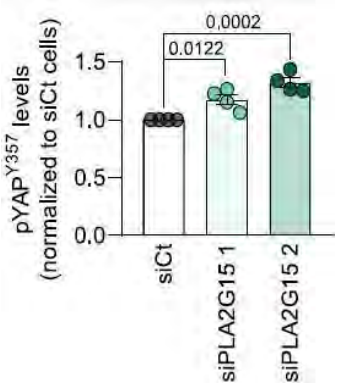


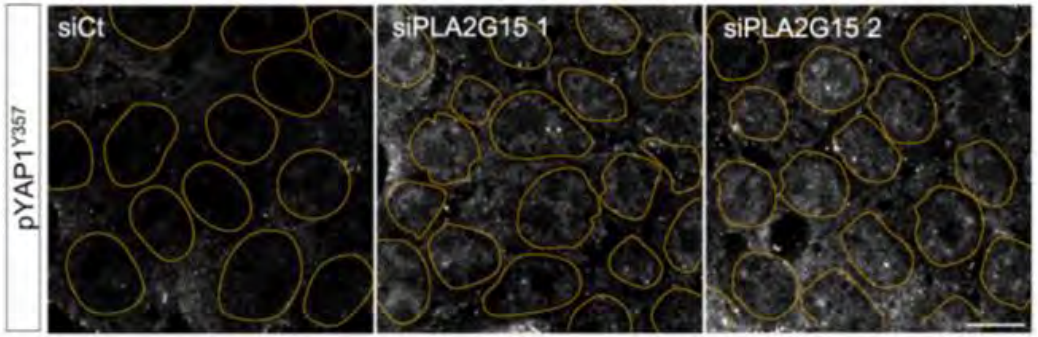


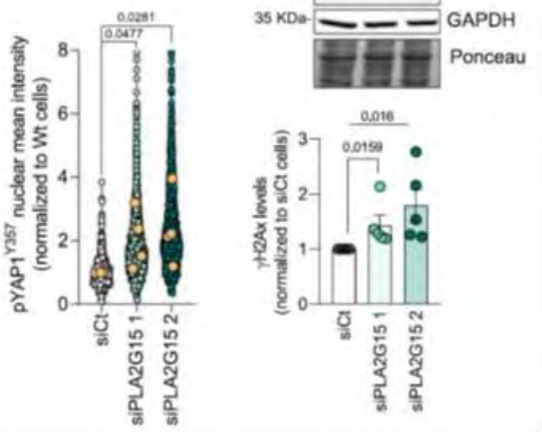
blocking lysosomal lipid catabolism upstream of CLN3 leads to DNA damage and YAP1 activation



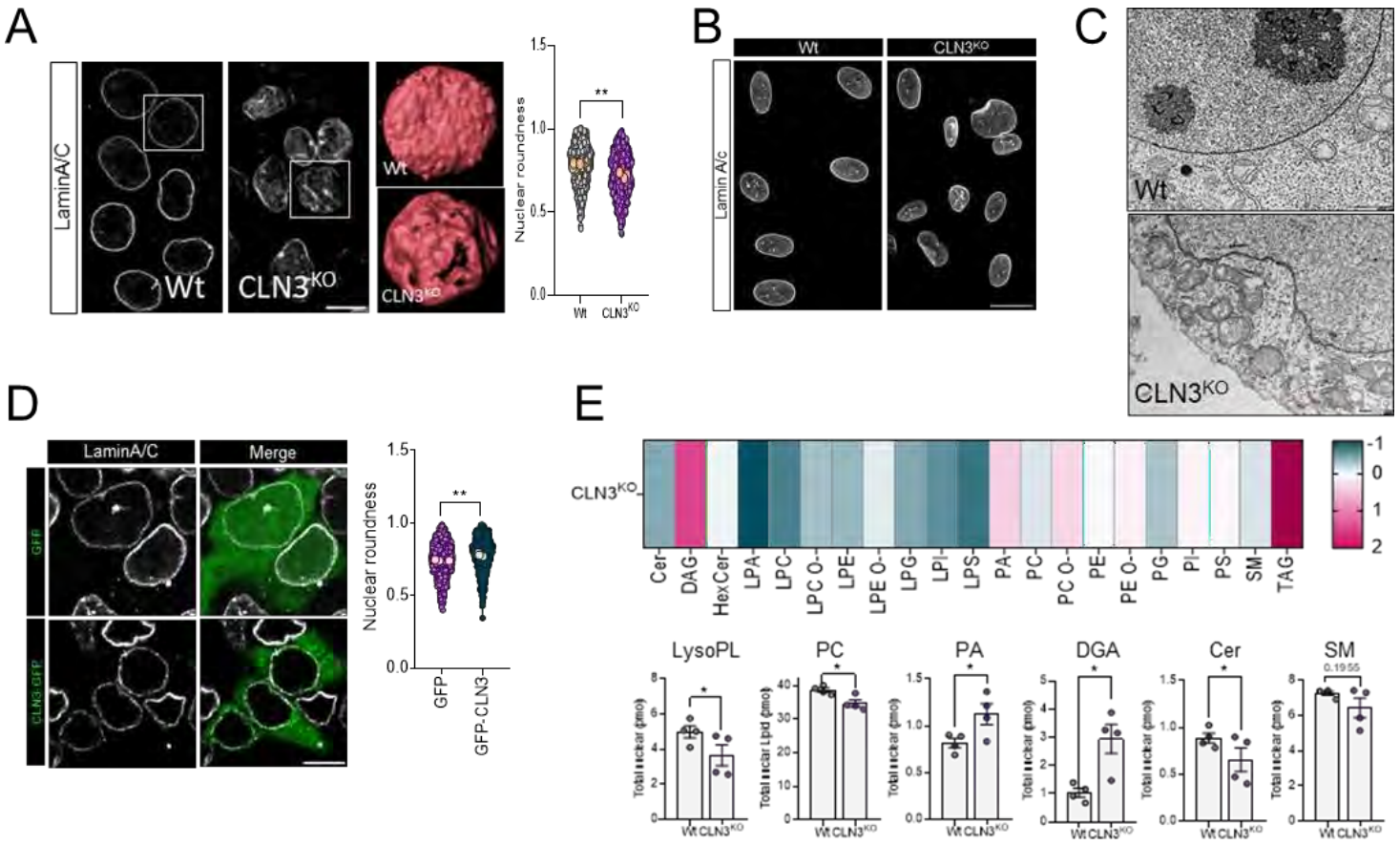




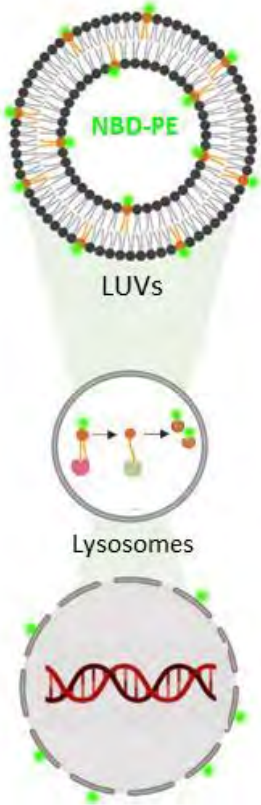


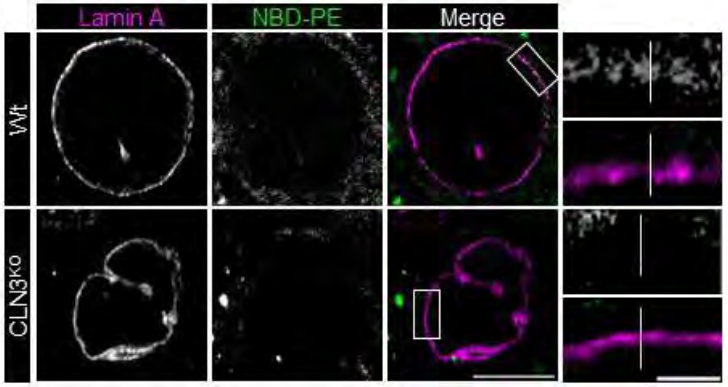


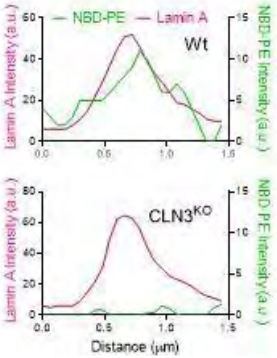
release of GPDs from lysosomes is necessary to maintain nuclear envelope homeostasis

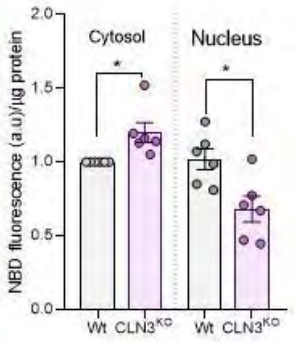


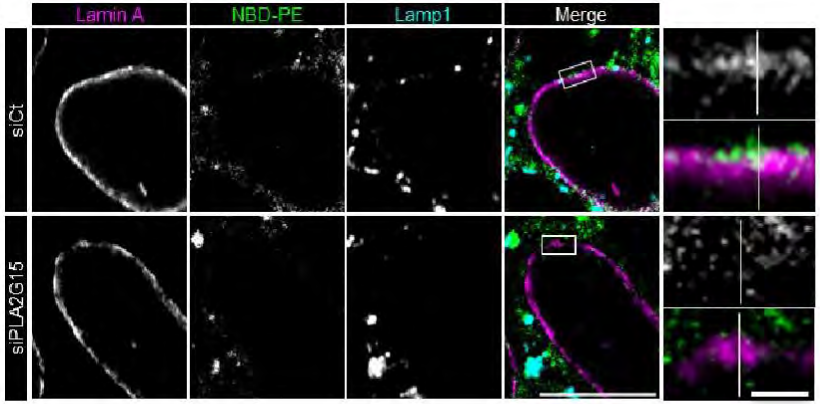
transfer of GPDs from lysosomes to the nuclear envelope requires both PLA2G15 and CLN3

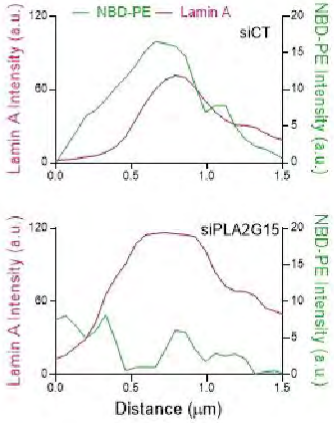




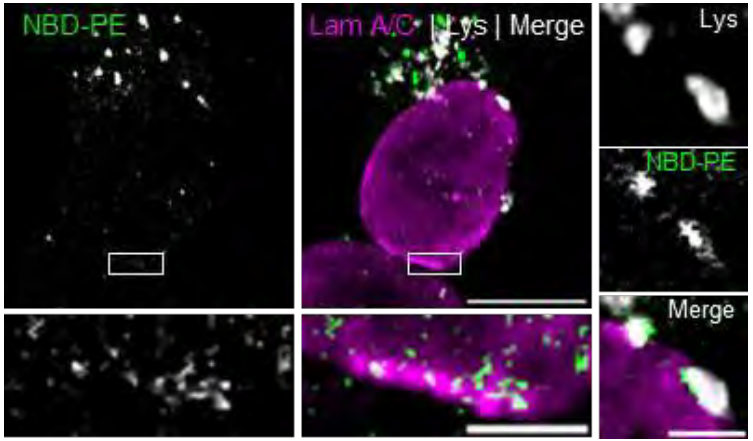


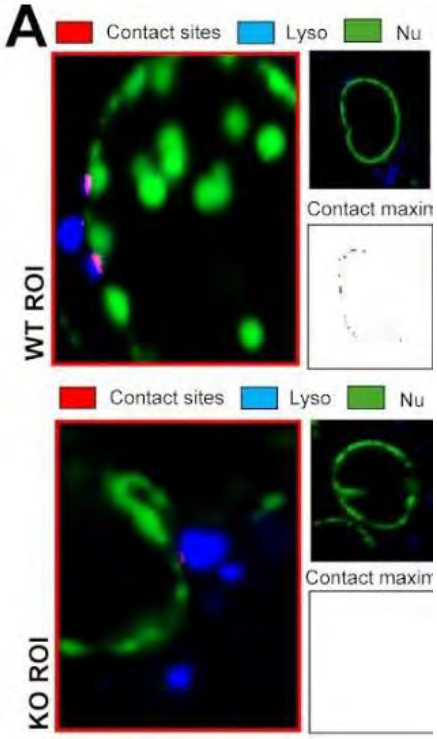


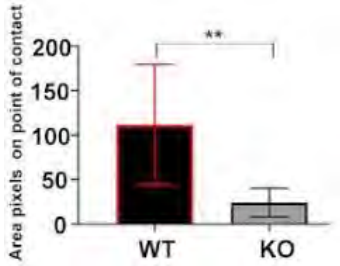


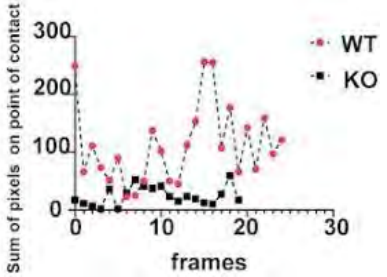


transfer of GPDs from lysosomes to NE via ”kiss-and-run” events









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TARGETING MECHANISMS FOR BONE-DIRECTED THERAPIES

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POSTDOCTORAL FELLOW,

NEMOURS CHILDREN'S HEALTH USA

DISCLOSURES

Betul Celik has no relevant financial relationships with ineligible companies to disclose.

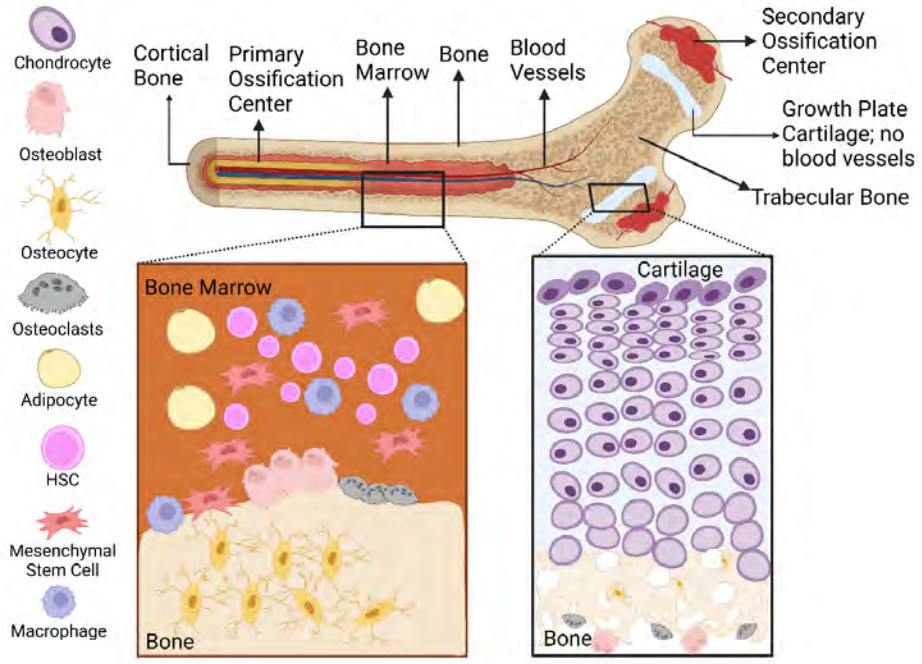
LEARNING OBJECTIVES

At the conclusion of this activity, participants will be able to:

- Explain the biological challenges of targeting bone tissue
- Describe key targeting strategies used in bone-directed therapies
- Understand the role of glycosaminoglycans (GAGs) in bone-targeted delivery
- Evaluate the limitations of conventional therapies in lysosomal storage diseases (LSDs)
- Assess emerging strategies for improving skeletal outcomes in LSDs
- Interpret schematic models and comparative data on targeting efficacy
- Identify future directions for bone-targeted therapeutics

WHY ARE BONE-TARGETED THERAPIES NEEDED?

Bone Biology and ECM composition



Organic and Inorganic Compositions of ECM

Collagens, proteoglycans, glycoproteins, growth factors, and hydroxyapatite and other ions (magnesium, sodium, potassium, bicarbonate)

Challenges

Avascularity
Dense ECM
Poor drug penetration

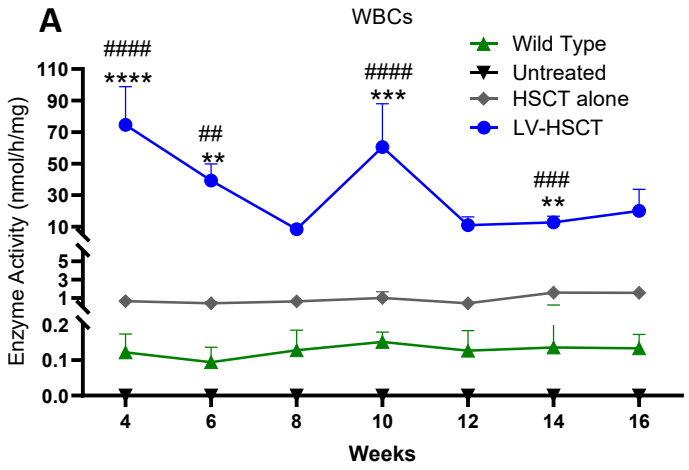
Clinical Relevance

Skeletal Diseases
LSDs (MPS IV, VII, MLD, Gaucher, etc)
Metastasis

Fig. Characterization of long bone components. *Manuscript in preparation*.

WHAT IF MORE ENZYMES WERE PRODUCED IN THE BM?

Case: Morquio A syndrome and LV-mediated ex vivo HSCT



No improvement in pathology!

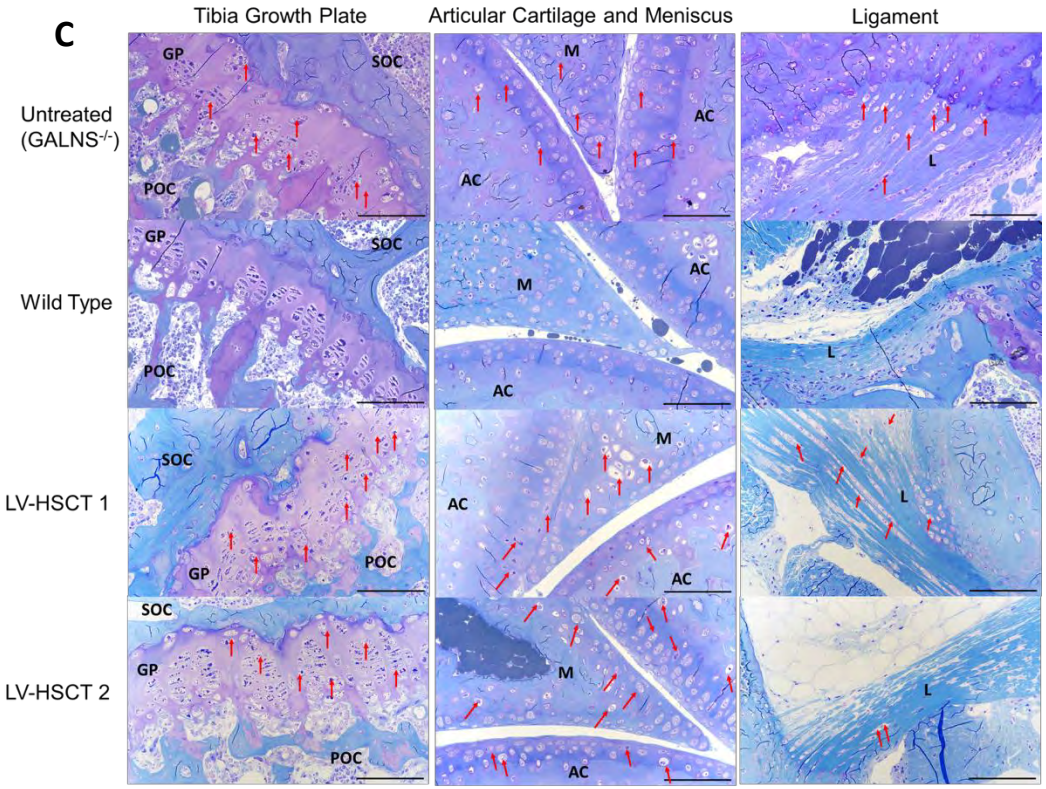


Fig. Ex vivo LV-HSCT in Morquio mouse model. **A-B.** GALNS enzyme activity in WBCs and bone after ex vivo LV-HSCT. **C.** Bone pathology

(Celik et al, 2024, Human Gene Therapy)

HOW ABOUT OTHER LSDS AND THEIR IMPACT ON SKELETON?

Are these the same “Bone growth, Bone formation, Bone resorption, bone homeostasis”?

LSD Type	Affected Skeletal Cells	Cellular Impact
MPS I (Hurler, Scheie)	Chondrocytes, Osteoblasts	Impaired ECM production, inflammation via TLR4, reduced osteogenesis
MPS II (Hunter)	Chondrocytes, Osteoblasts	Similar to MPS I; lysosomal overload, joint stiffness, skeletal dysplasia
MPS III (Sanfilippo A–D)	Chondrocytes (minor), Neural cells	Primarily neurodegenerative; minimal direct skeletal impact
MPS IV A/B (Morquio A/B)	Chondrocytes, Growth plate cells	Severe cartilage dysplasia, disorganized growth plate, apoptosis
MPS VI (Maroteaux-Lamy)	Chondrocytes, Osteoblasts	Swollen chondrocytes, disrupted BMP/TGF-β signaling, poor cartilage response to ERT
MPS VII (Sly)	Chondrocytes, Osteoblasts	Enlarged/disorganized growth plate, delayed differentiation, PTHrP/Wnt5a dysregulation
MPS IX (Hyaluronidase deficiency)	Synoviocytes, Chondrocytes	Joint effusions, mild skeletal symptoms; rare and less characterized
Gaucher Disease (Types I–III)	Osteoblasts, Osteoclasts	Impaired osteoblast function, increased osteoclast activity, bone pain, osteonecrosis
Fabry Disease	Osteocytes, Endothelial cells	Vascular dysfunction in bone, osteopenia, pain due to small fiber neuropathy
Pompe Disease	Skeletal muscle cells	Muscle weakness, impaired bone remodeling due to reduced mechanical loading
Mucolipidosis II/III	Chondrocytes, Osteoblasts	Severe skeletal dysplasia, abnormal cartilage and bone matrix, poor growth
Niemann-Pick Disease (Types A/B)	Osteoclasts, Macrophages	Foam cell infiltration, osteopenia, hepatosplenomegaly affecting bone marrow
Cystinosis	Osteoblasts, Renal tubular cells	Rickets-like bone changes due to Fanconi syndrome, impaired mineralization
Danon Disease	Skeletal muscle cells	Muscle weakness, secondary bone effects from reduced mobility
Sialidosis (Mucolipidosis I)	Chondrocytes, Osteoblasts	Mild skeletal abnormalities, joint stiffness, short stature
GM1 Gangliosidosis	Chondrocytes, Osteoblasts	Dysostosis multiplex, growth plate disruption, vertebral anomalies
GM2 Gangliosidosis (Tay-Sachs, Sandhoff)	Neural cells (primary), minor skeletal involvement	Neurological focus; skeletal effects secondary to motor decline

Bone growth - Chondrocytes – Originated from MSCs
Bone formation - Osteoblasts – Originated from MSCs
Bone resorption - Osteoclasts – Originated from HSCs
Maintaining bone tissue - Osteocytes – Originated from Osteoblasts

→ **Bone Homeostasis**

Affected cell types and accumulated materials determine the skeletal condition.

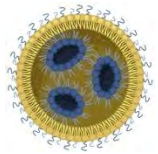
Manuscript in preparation

TARGETING STRATEGIES

Passive Targeting
Utilizing physiological features like leaky vasculature
!! Low specificity, high off-target accumulation (liver, spleen), and ineffective in non-leaky tissues like bone or CNS

Active Targeting
Involves functionalizing drug carriers with ligands (e.g., antibodies, peptides, aptamers) that bind to specific receptors on target cells
!! High specificity and cellular uptake, and reduced systemic toxicity

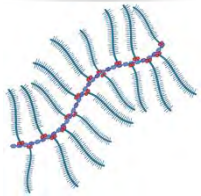
Key Platforms



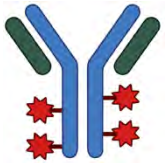
Nanoparticles

Peptides

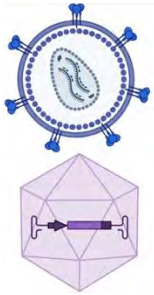
GAGs



Antibodies

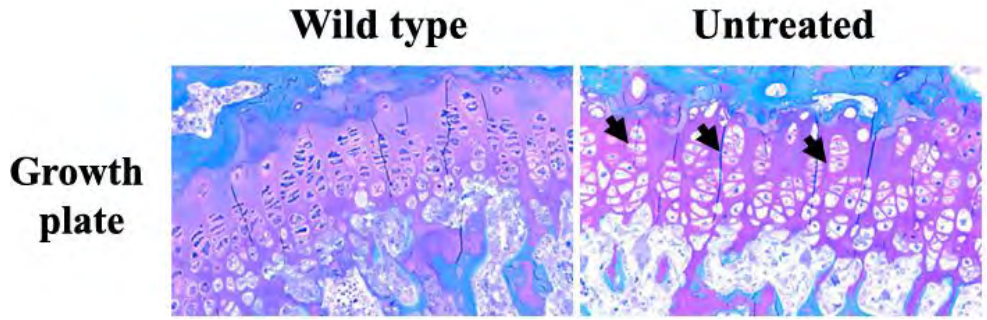


Viral vectors

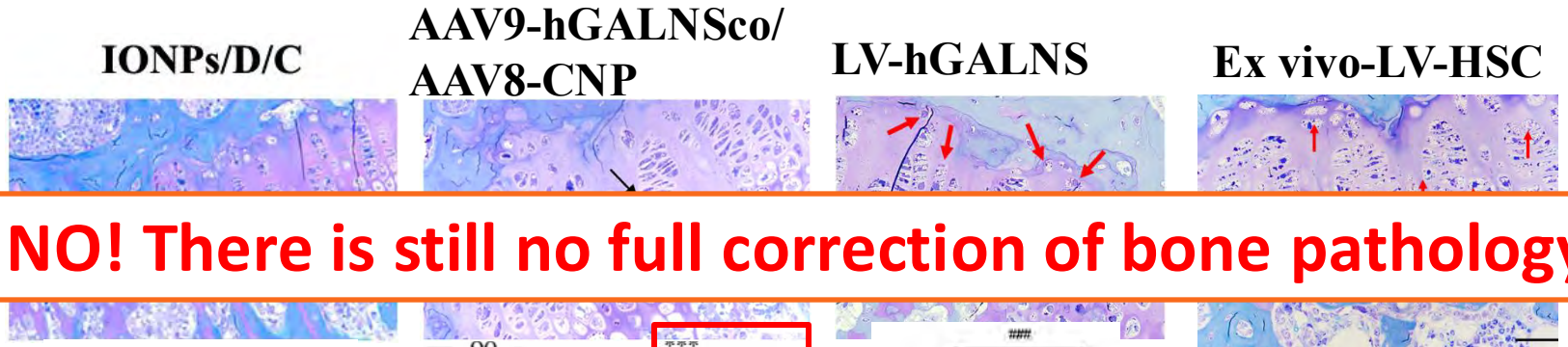


ARE THESE STRATEGIES CORRECTING BONE PATHOLOGY?

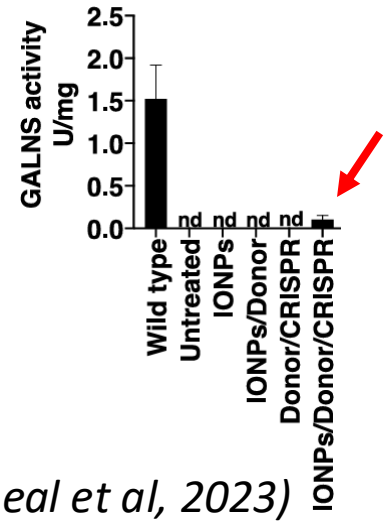
Case:
Morquio A



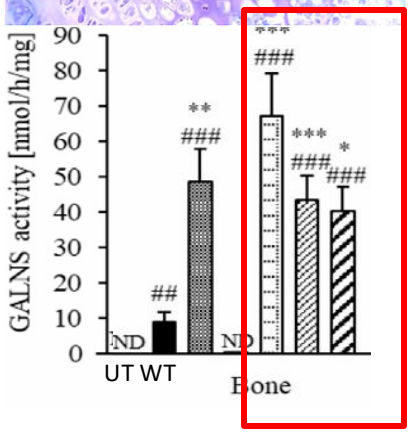
All alone or together???



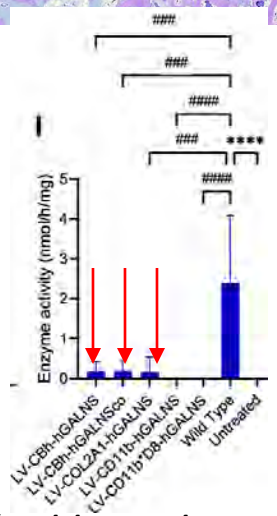
NO! There is still no full correction of bone pathology!



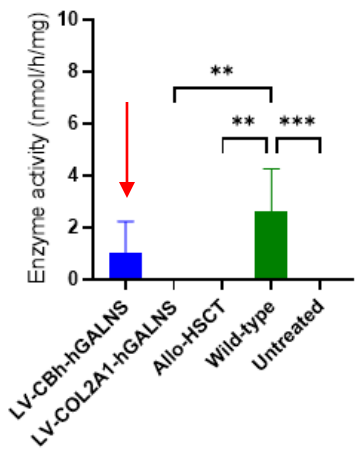
(Leal et al, 2023)



(Rintz et al, 2024)



(Celik et al, 2025)



(Celik et al, 2024)

POTENTIAL TARGETS FOR BONE AND CARTILAGE

Collagens

Main Type I, II, IX, XI; Minor type III, IV, V, VI, X; Bind to the DDR-1 and DDR-2 receptors; Utilizing the ECM-collagen matrix, abundant in hyaline cartilage
Mechanism: chondrocyte targeting, differentiation, skeletal development

Proteoglycans

Decorin, biglycan, aggrecan, syndecan, KS, CS, HA, vitronectin
GAG-mediated ECM interactions; regulating mineralization and collagen fibril formation; Pan-RTK inhibitions

GPCRs

Transmembrane receptors responding to extracellular signals; bone/cartilage formation, resorption, and maintenance

Membrane adhesion

Maintaining tissue integrity, cell behavior; integrins, cadherins, connexins

Gla proteins

Ca binding, membrane interactions, and ECM anchoring

Inorganic Compounds

Direct binding to bone mineral phase; enhances retention in bone

Ion Channels

Bone metabolism and crystal formation

Small leucin-rich proteoglycans (SLRPs)

ECM remodelling, osteogenesis, inflammation regulation, RTK/TLR signaling

Glycoproteins

Calcium/hydroxyapatite binding; Integrin signaling, mineralization, osteoblast differentiation, and regeneration; osteonection/calcin, bone-sialoproteins

GAG-BASED TARGETING MECHANISMS

Types of GAGs and their bone affinity

Chondroitin sulfate (CS) – *high affinity* - Major component of cartilage ECM; binds collagen and hydroxyapatite; supports mineralization (Periosteal bone)

Heparan sulfate (HS) – *high affinity* - Regulates growth factor signaling (e.g., BMPs, FGFs); involved in osteoblast differentiation

Dermatan sulfate (DS) – *high affinity* - Found in periosteum and connective tissue; modulates collagen fibrillogenesis

Keratan sulfate (KS) – *moderate affinity* - Present in cartilage; contributes to hydration and load-bearing

Hyaluronic acid (HA) – *low direct affinity* - Lubricates joints; scaffolding for cell migration

Hyaluronan – *moderate affinity* - Limited role in bone; may influence osteoclast activity and angiogenesis

****Sulfation pattern specifically affects the stemness of different stem cells*

****GAG-regulated signal transduction affects the self-renewal of stem cells*

(Celik, 2024; Orlińska et al, 2023; Chen et al, 2021)

GAG-BASED TARGETING MECHANISMS

1. Electrostatic interactions

- Negatively charged functional groups:
  - Sulfate groups ($–SO_3^-$)
  - Carboxyl groups ($–COO^-$)
- Strong polyanionic character to interact with calcium ions, collagen fibrils, hydroxyapatite crystals, and positively charged proteins and growth factors (BMP, FGF, etc.)
- Sulfation pattern, chain length determine binding strength

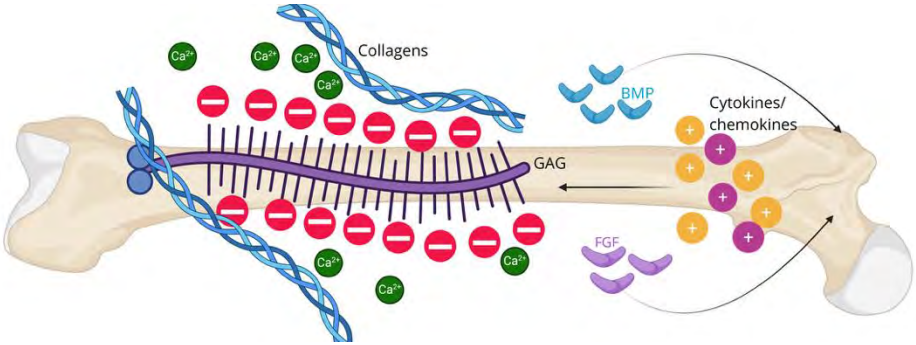
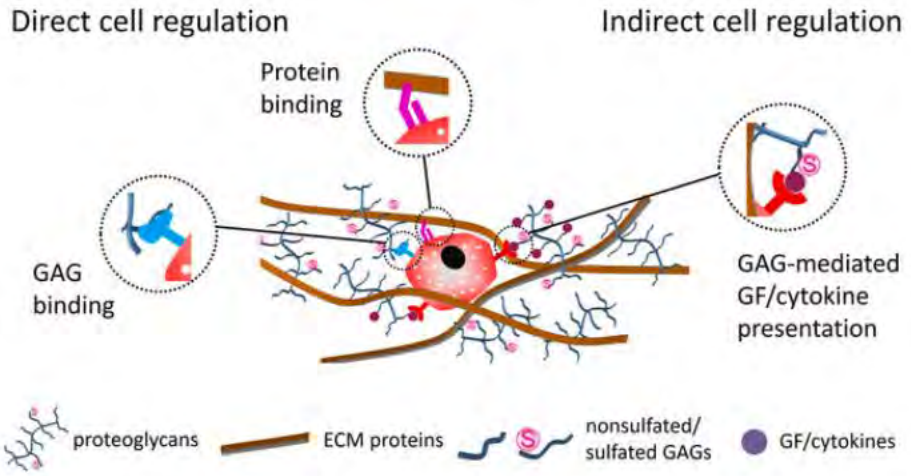


Fig. Charge interactions.
Manuscript in preparation.

2. Ligand-receptor binding



(Torregrossa et al, 2021)

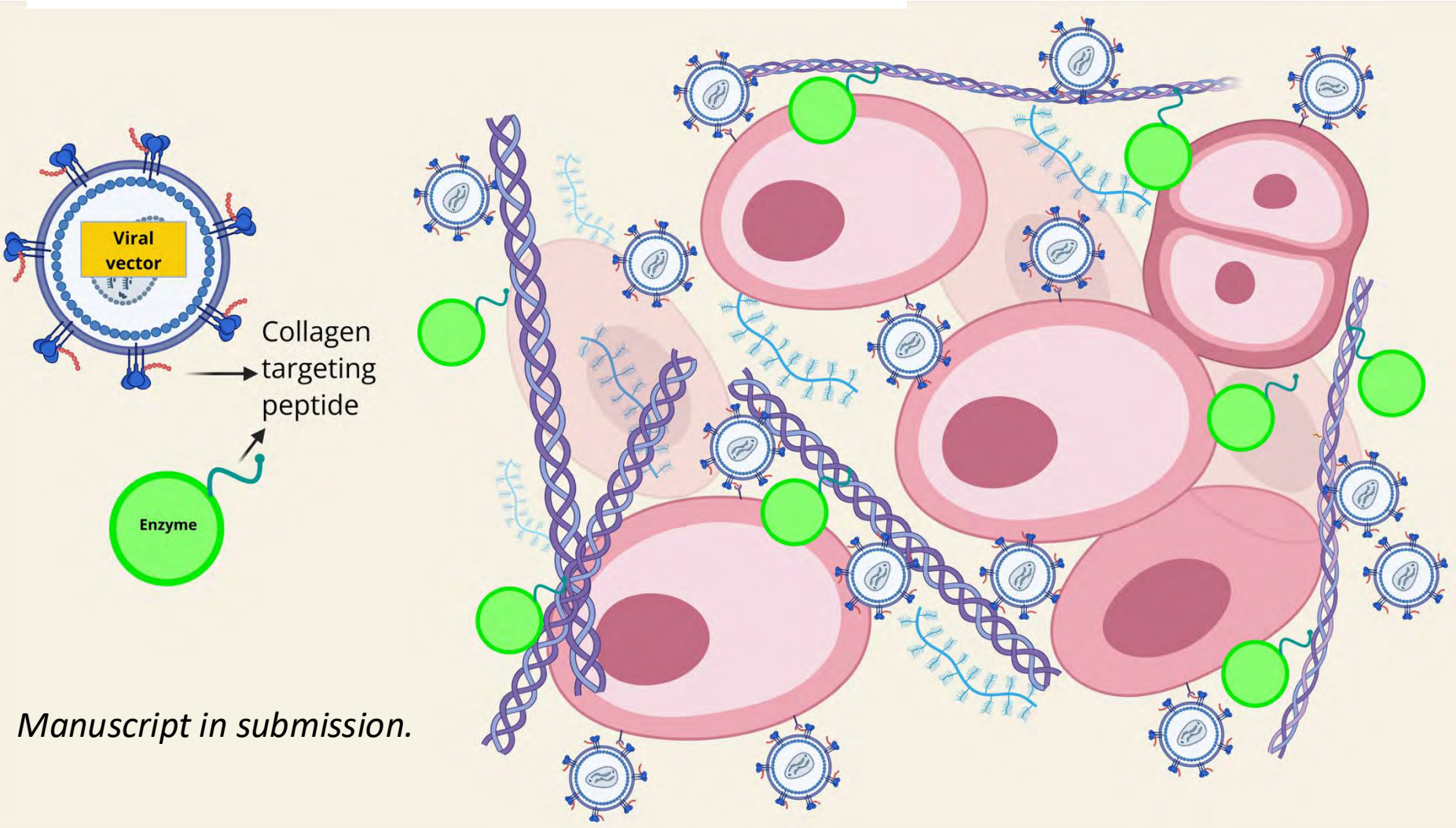
3. ECM retention

Sulfated HA extends the retention time of delivered growth factors through electrostatic interactions, thus contributing to the residence and bioavailability of protein-based drug formulations and promoting hMSC chondrogenesis in the osteoarthritis model. *(Feng et al, 2017)*

COLLAGEN-BASED TARGETING MECHANISMS

Targeting collagen receptors or specific collagens in the ECM?

2. ECM Collagen Targeting



Manuscript in submission.

APPLICATIONS IN LYSOSOMAL STORAGE DISORDERS

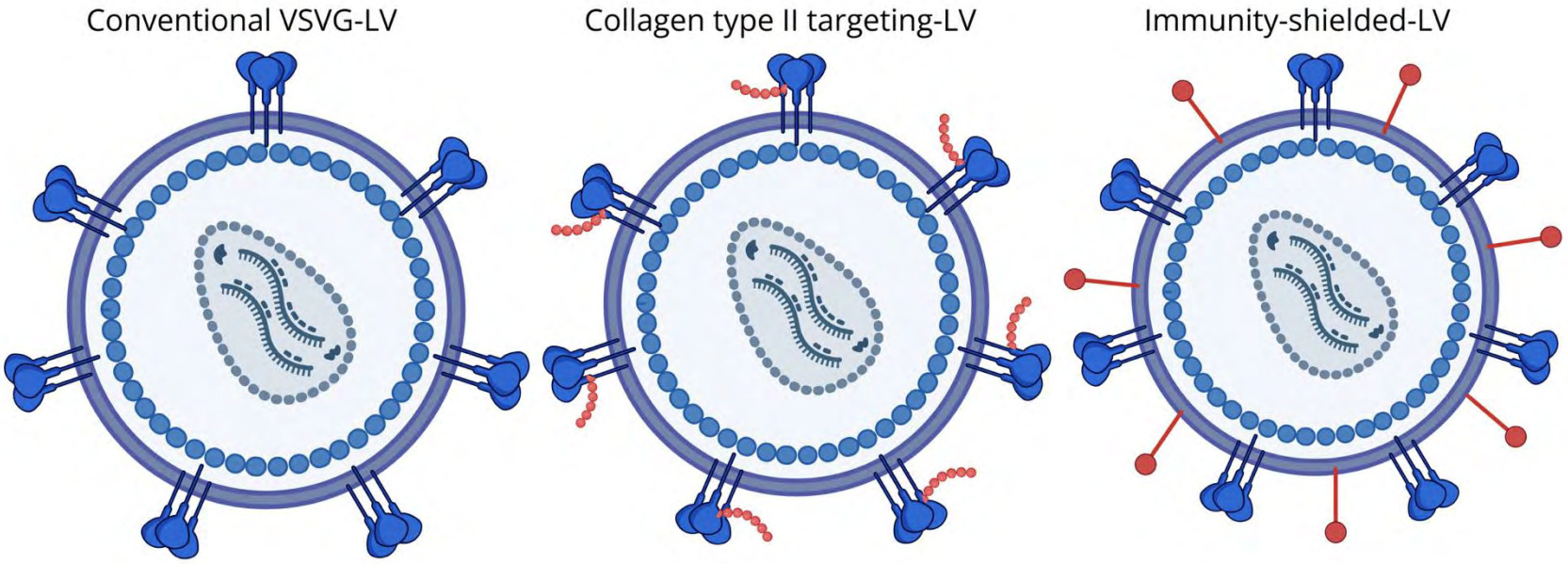
- LSDs like MPS I, MPS IVA, and MPS VII involve skeletal abnormalities due to the enzyme deficiency
- Conventional ERT is ineffective in bone and cartilage, while HSCT just ameliorates (but has side effects)
- Enhanced delivery of enzymes (e.g., BBB-targeting via transferrin) is required
- Acidic oligopeptides (e.g., AspSerSer), Hydroxyapatite-binding domains, and Gene therapy vectors with bone tropism are some other alternatives

Several therapies have been implemented: AAV, LV, CRISPR/Cas9, cell therapies, substrate reduction, chaperone, etc.

Which one is better for skeletal dysplasia?

WE STILL DON'T KNOW!

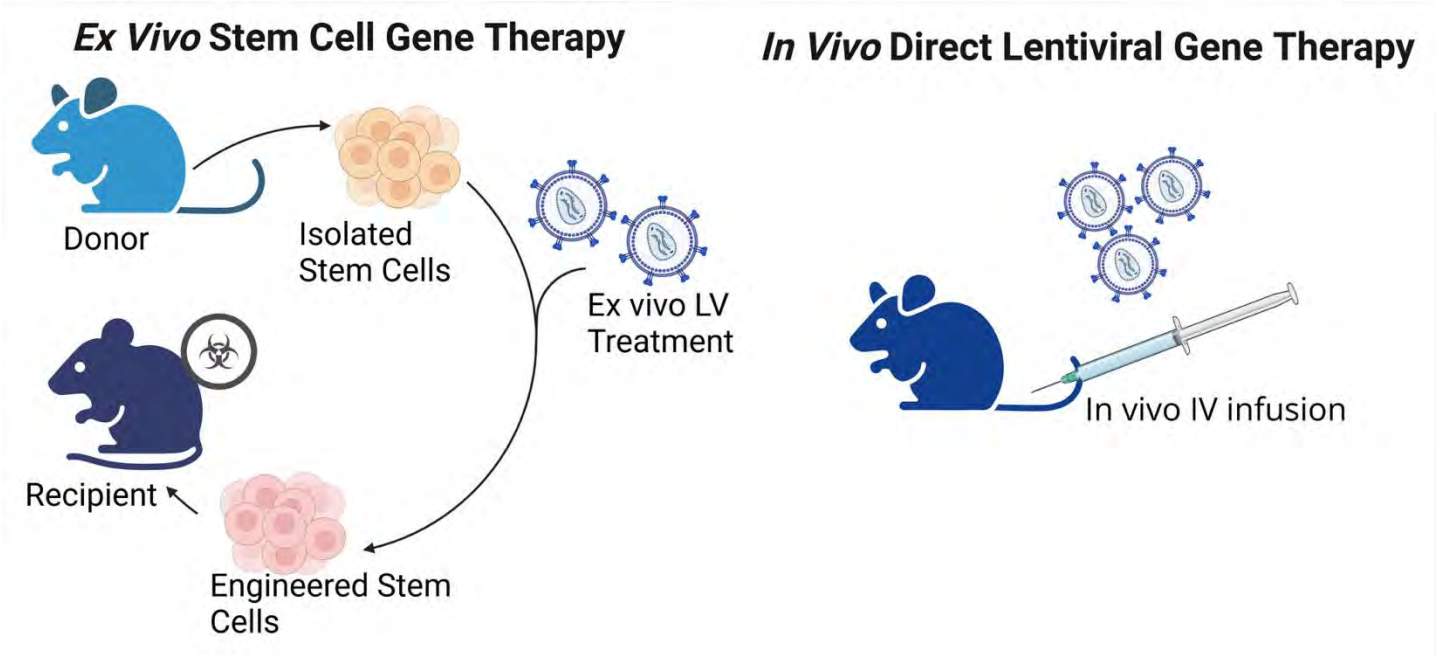
LENTIVIRAL VECTOR-MEDIATED GENE AND CELL THERAPY APPROACHES



(Celik et al, 2025) *Manuscript in submission* *Manuscript in preparation*

Designed a variety of LVs and gene expression cassettes.

LENTIVIRAL VECTOR-MEDIATED GENE AND CELL THERAPY APPROACHES



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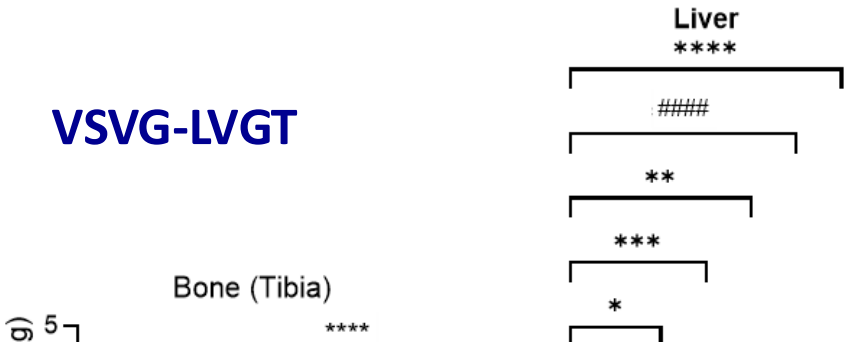
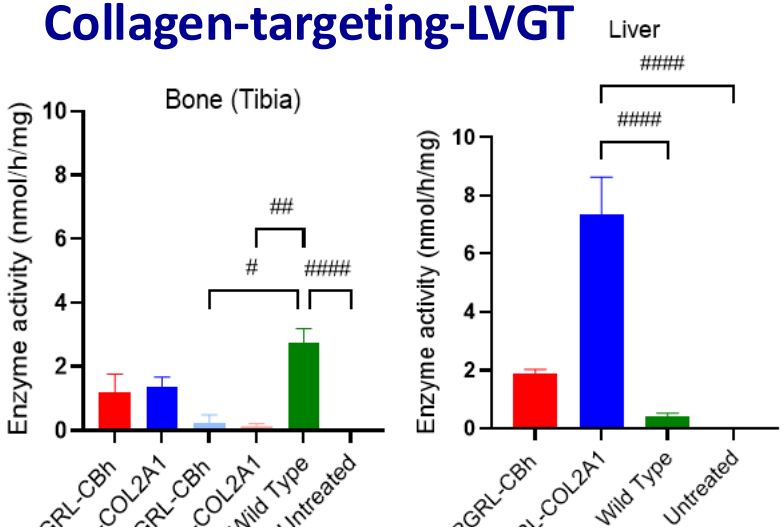
WHAT WE FOUND WAS…

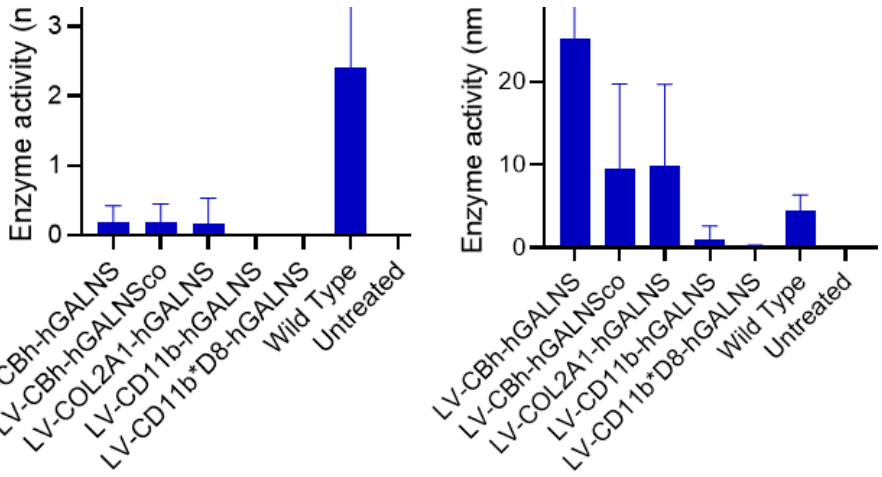
VSVG-LVGT



Collagen-targeting-LVGT



Fewer enzymes in bone, → incomplete pathological correction!



Immunity-shielded-LVGT

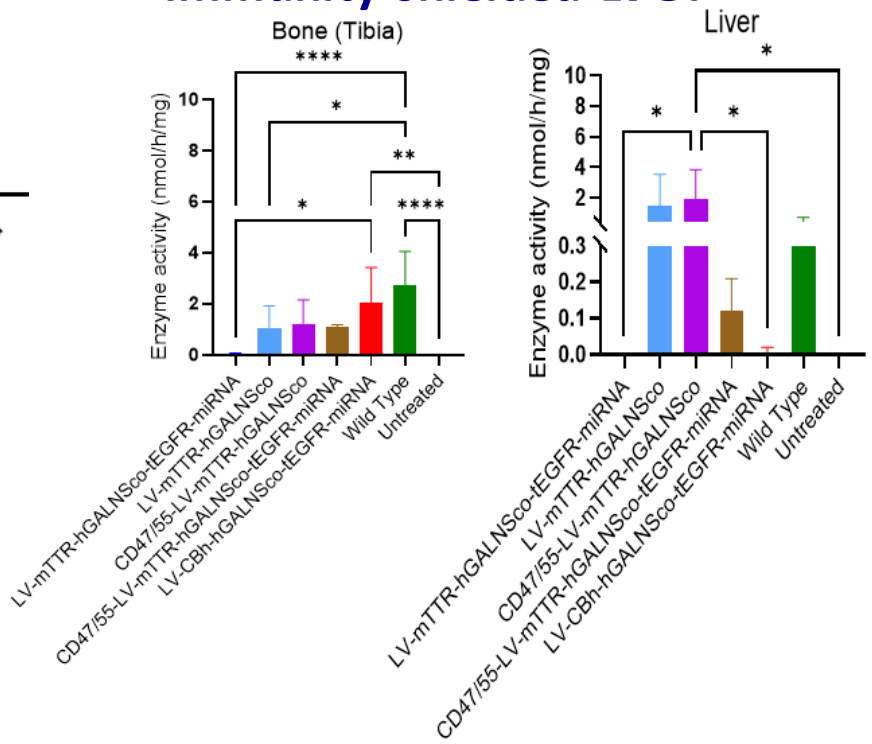
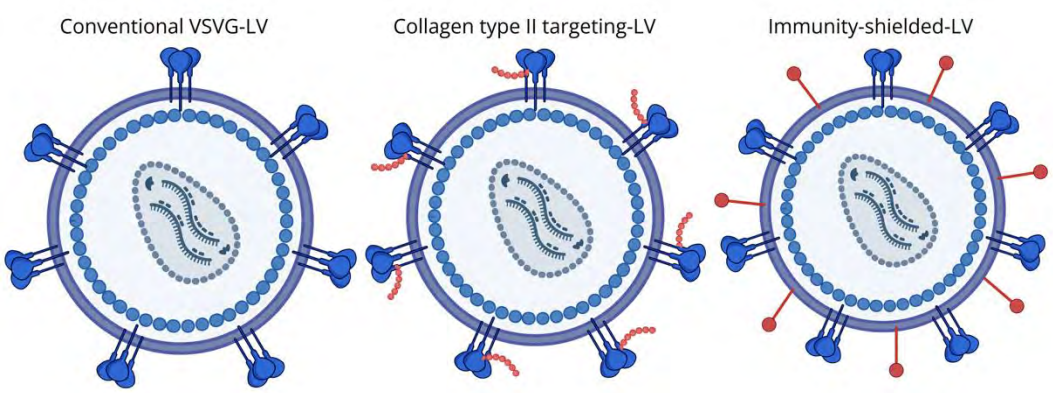


Fig. GALNS enzyme activity in bone and liver after IV infusion of VSVG-LV, collagen-targeting LV, immunity-shielded-LV

(Celik et al, 2025)

WHY?

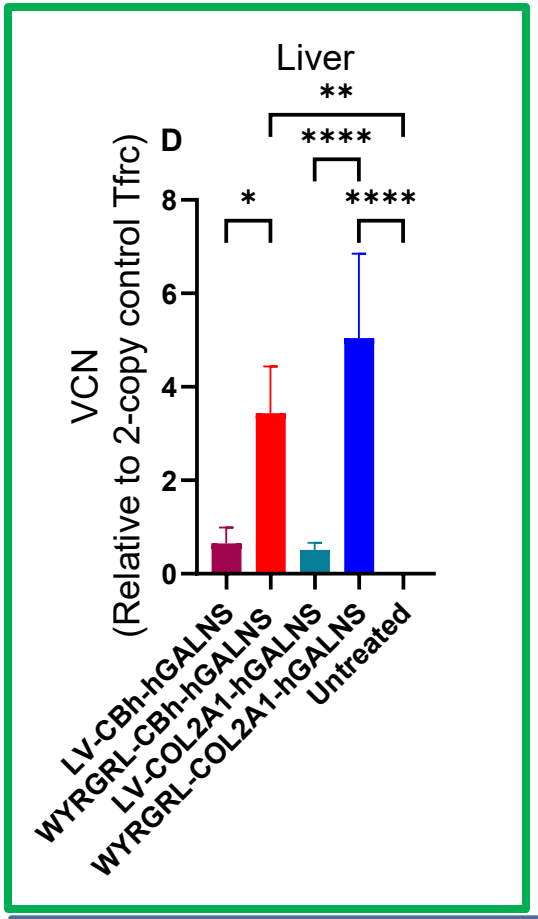
Established LVs – Hypothetically work, but not experimentally??



(Celik et al, 2025) *Manuscript in submission* *Manuscript in preparation*

We proved that such peptides can increase the uptake of virus (VCN), but result in

- Lowering of enzyme activities in the liver and other tissues
- Not all the viruses are going to bone; stuck in the liver or spleen
- Changes in viral tropism
- Such a high level of gene uptake by the cell (liver in this case) might have a detrimental effect on the gene expression
    - A high number of viral integrations harms the gene expression



WYRGRL peptide increased VCN of LV-CBh by 5.3-fold and LV-COL2A1 by 9.8-fold

Manuscript in submission

CHALLENGES AND FUTURE DIRECTIONS

Biological barriers

Immunogenicity and off-target effects

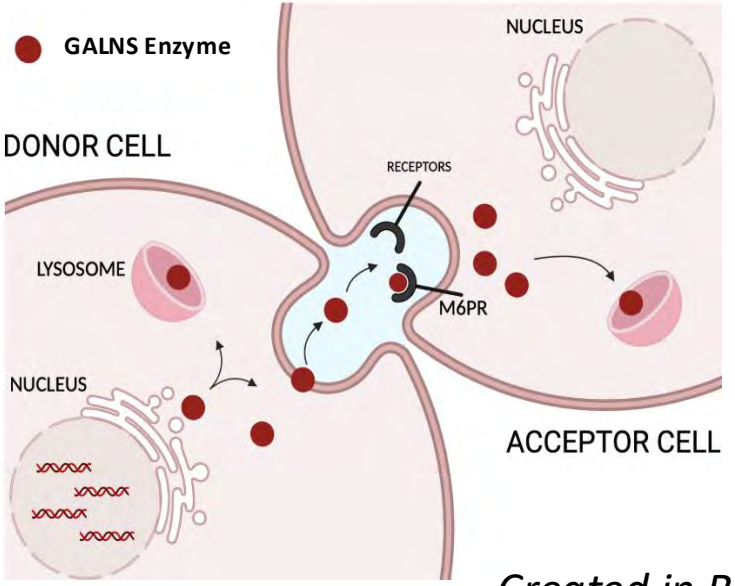
Clearance mechanisms of the lymphoid system, liver, spleen, and kidney; anti-transgene/viral antibody

Not many enzyme/LVs/HSCs reach the bone

The complexity of the gene expression cassette results in poor production of proteins

Distribution issues and the cross-correction, tissue-related, cartilage avascularity

Delivery route



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CONCLUSIONS

None of these preclinical studies fully corrected bone pathology, but other tissues; molecular mechanisms behind the cartilage and bone development should be deeply analyzed

Ligands should be selected based on cross-reactivity, distinguished-unique sequences, and tested in systemic delivery

Gene or cell therapies may not be sufficient to improve the disease alone. They should be combined with ligands, hormones, antibodies, and other similar compounds.

ACKNOWLEDGEMENT

Prof. Shunji Tomatsu
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Sampurna Saikia (PhD candidate)
Nidhi FNU (PhD candidate)
Angelica Herreno Pacheco (PhD candidate)
Amali Karanuthilaka (PhD candidate)
Georgina Baya (PhD candidate)
Krishna Sai Musini (Research Assistant)
Dione Holder (Master's student)

NIH grants
1R01 HD102545-01A1
1R01HD104814-01A1
4R44HD102242-02

THANKS!

Base editing and prime editing to address genetic disease

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ASSISTANT PROFESSOR, DEPARTMENT OF GENETIC MEDICINE, JOHNS HOPKINS SCHOOL OF MEDICINE

Disclosures

Gregory Newby has no relevant financial relationships with ineligible companies to disclose.

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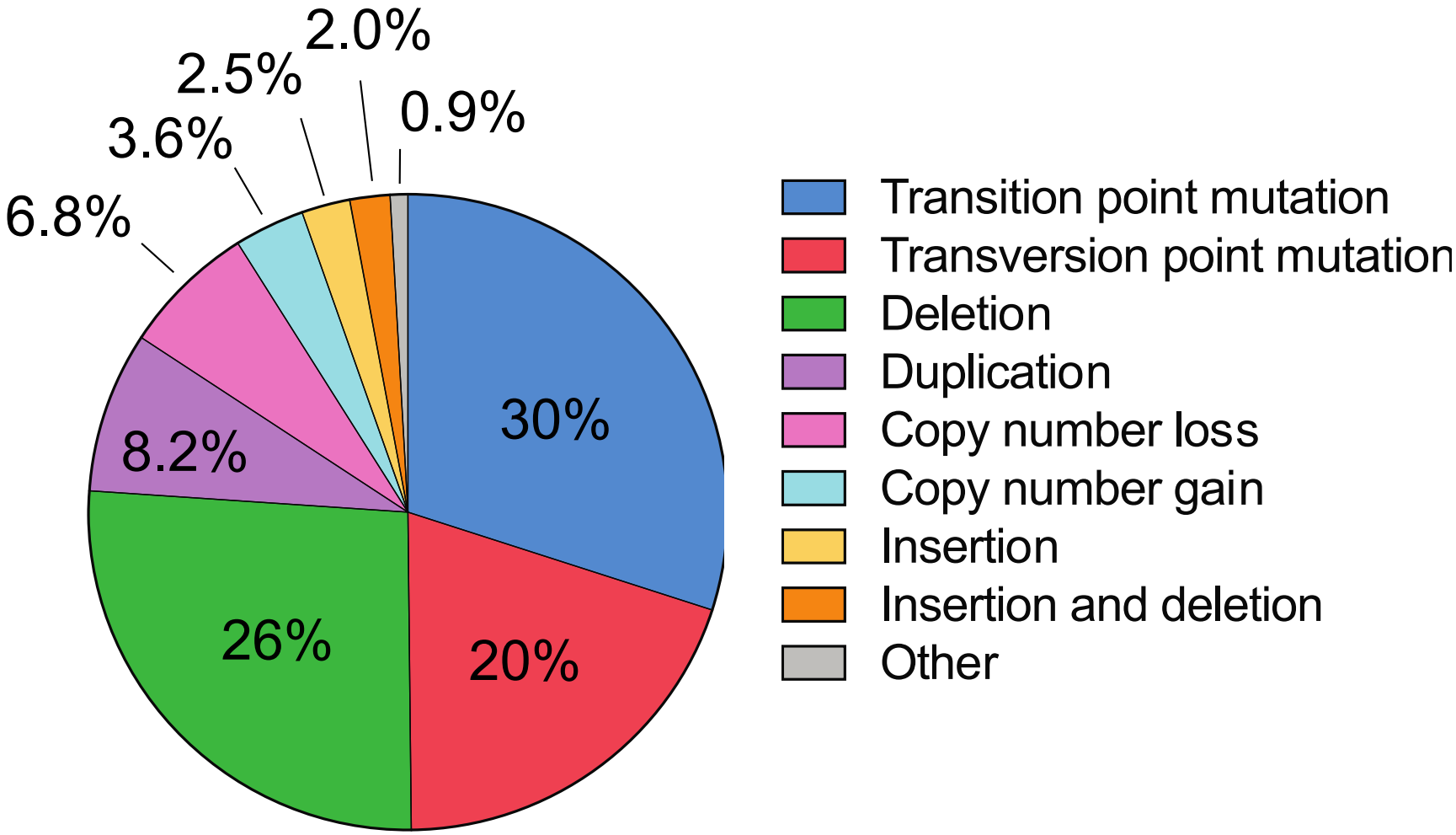
Learning OBJECTIVES

At the conclusion of this activity, participants will be able to:

1. Differentiate genome editing through nucleases, base editors, and prime editors
2. Identify delivery tools that have been successful in enabling in vivo genome editing in mice
3. Evaluate the prospects of editing to treat genetic diseases

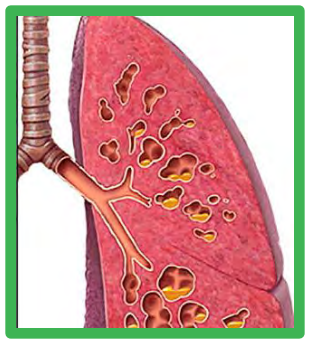
Pathogenic Human Genetic Variants



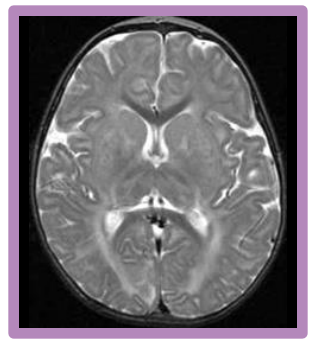
LMNA c.1824C→T

HBB E6V(A→T)

CFTR ΔF508



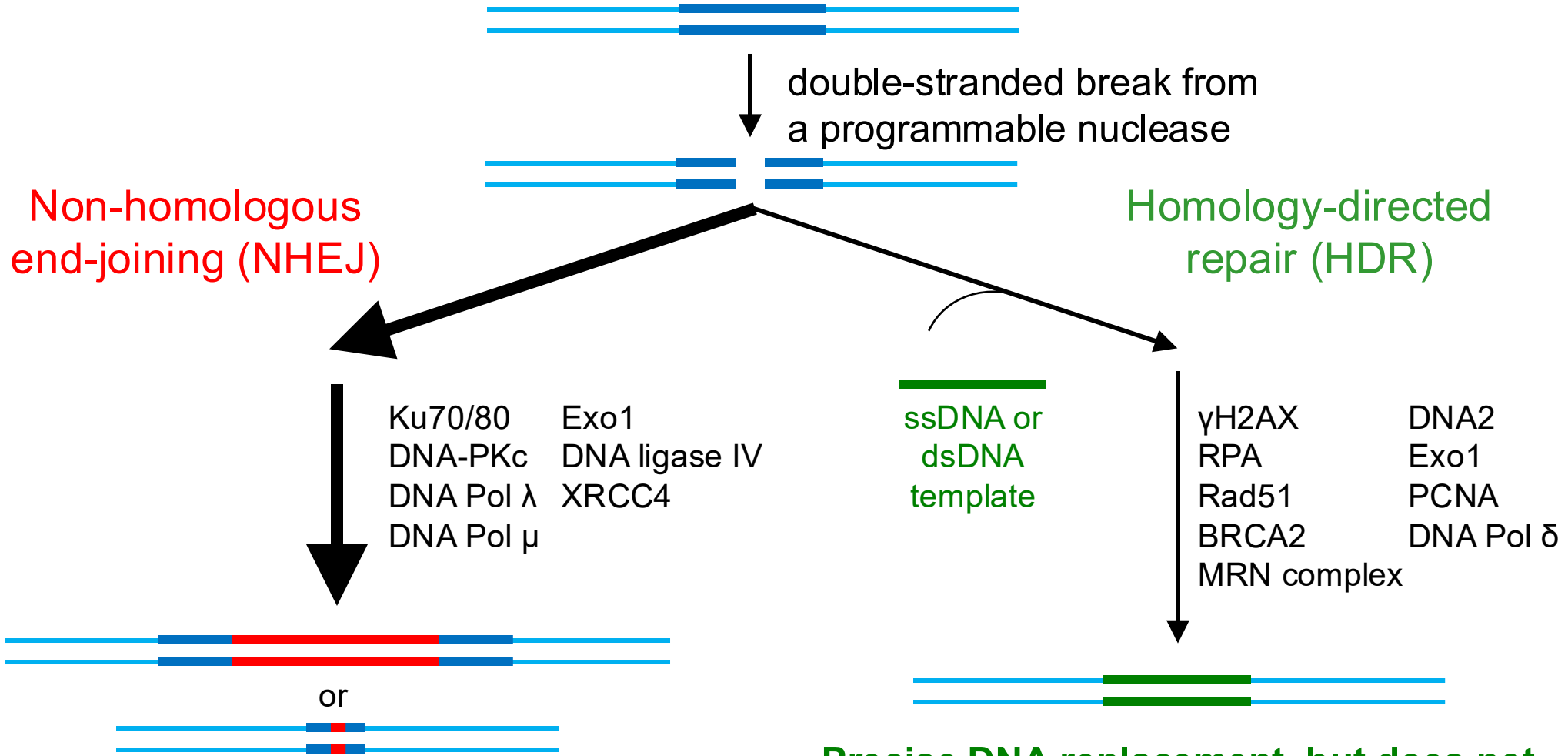
HEXA c.1278+TATC



>250,000 pathogenic or likely pathogenic variants

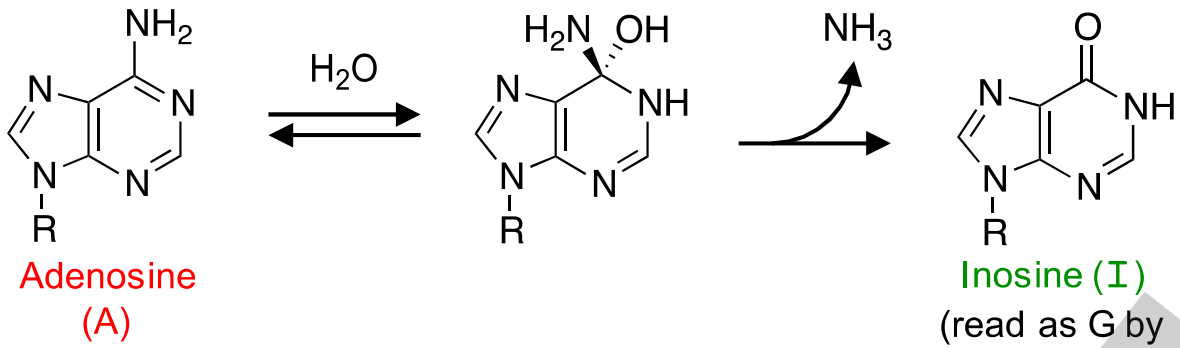
ClinVar database: Landrum et al., *Nucl. Acids Res.* **44**, D862 (2016), pie chart data accessed July, 2019

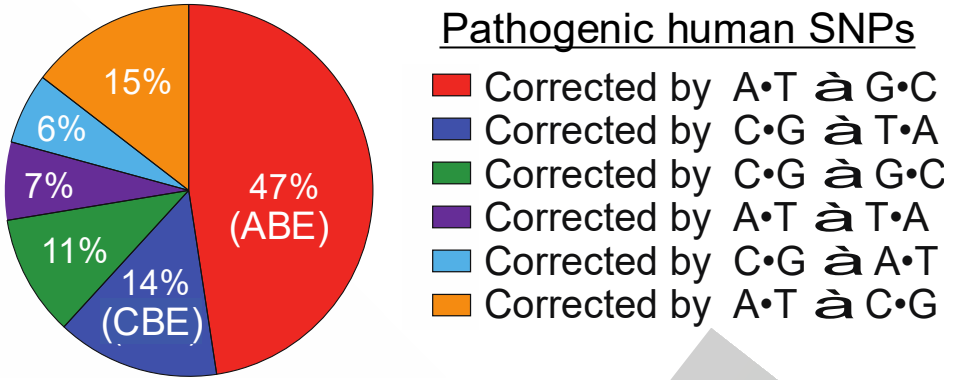
Genome Editing Using Double-Stranded DNA Breaks

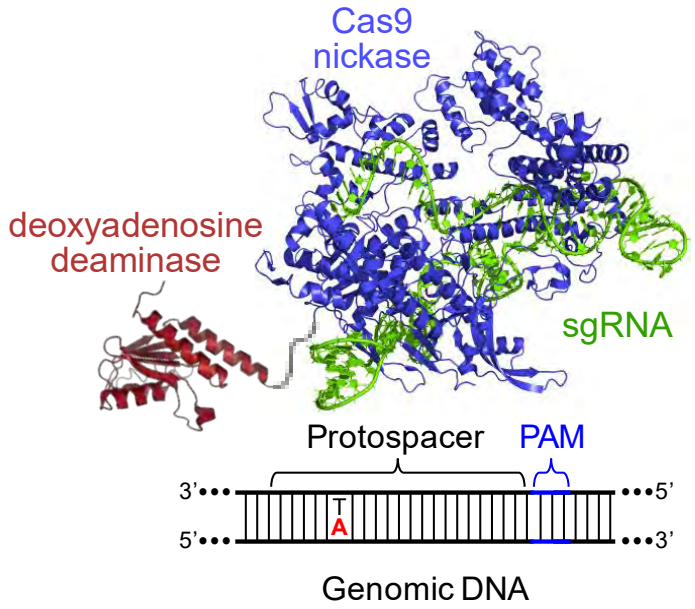


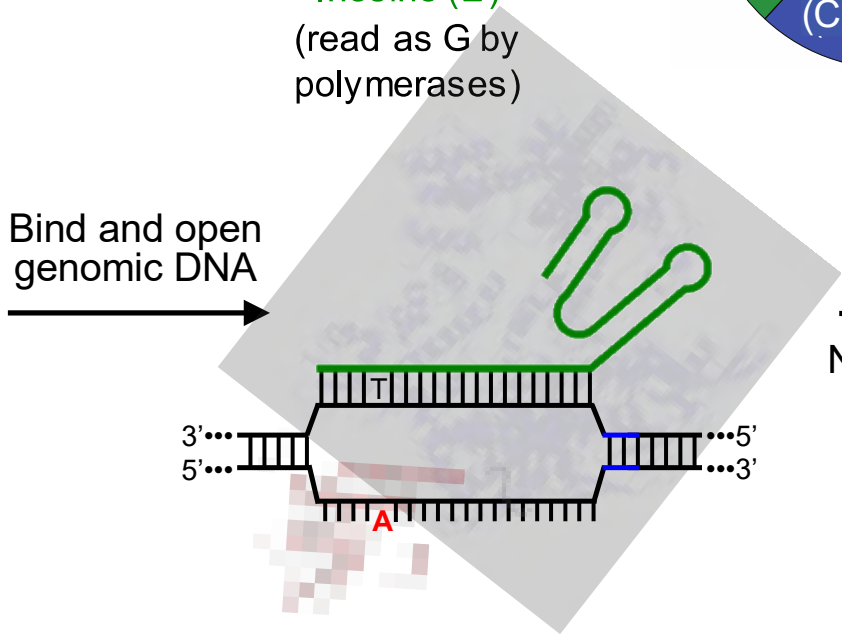
Choulika, Perrin, et al., *Mol. Cell. Biol.* **15**, 1968 (1995)
Rouet, Smih, & Jasin, *Mol. Cell. Biol.* **14**, 8096 (1994); Lukacsovich, Yang, et al., *Nucl. Acids Res.* **22**, 5649 (1994)

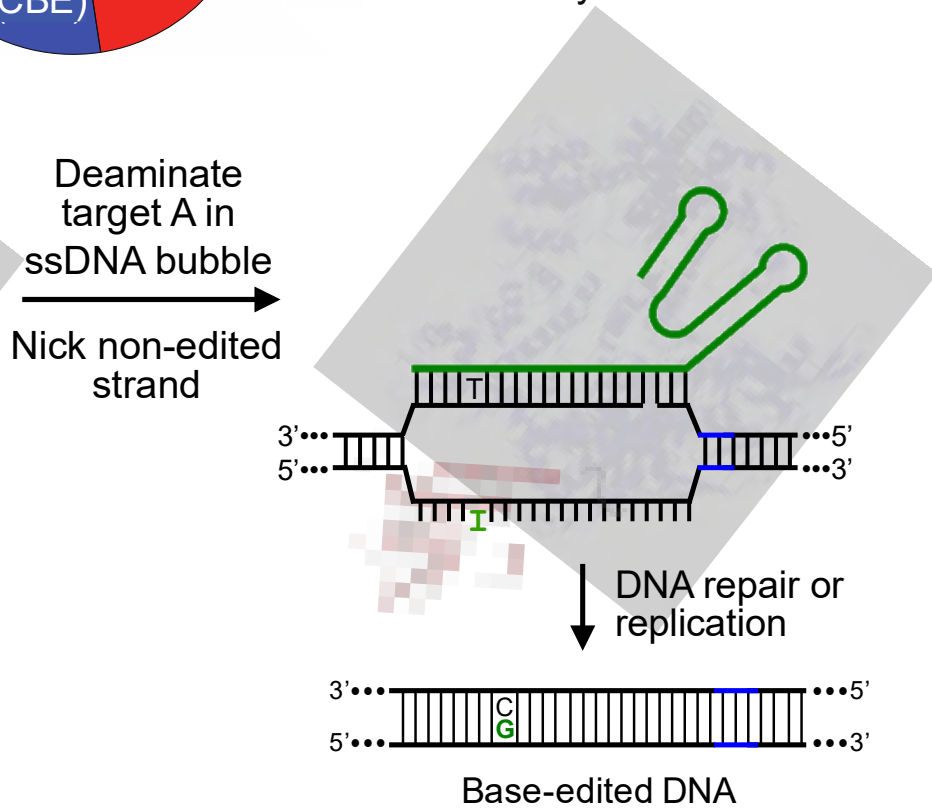
Development of the Adenine Base Editor (ABE)







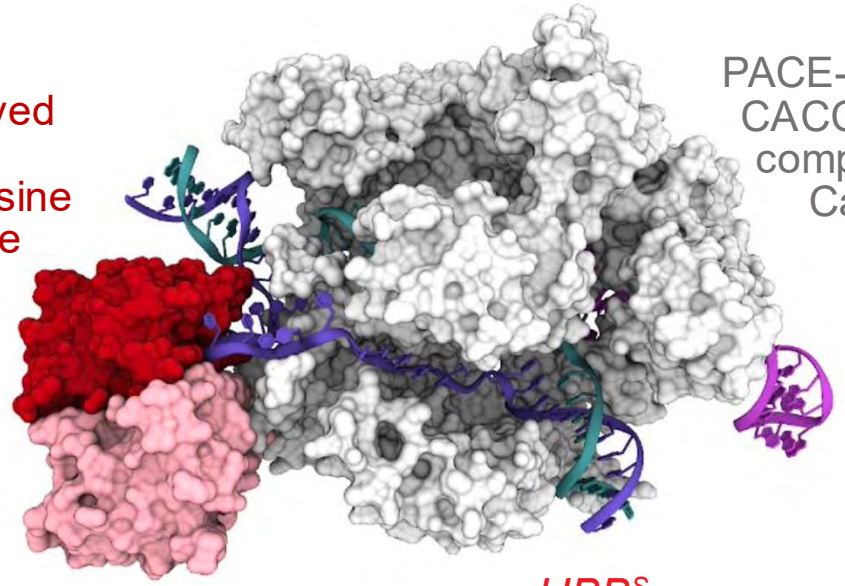




Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu *Nature* **551**, 464 (2017)

Richter, Zhao, Eton, Lapinaite, Newby, Thuronyi, Liu, *et al.* , *Nature Biotechnology* **38**(7), 883-891 (2020)

ABE Converts *HBB*^{S} to Benign *HBB*^{G} in SCD Patient HSPCs



HBB^{S}

	Met	Val	His	Leu	Thr	Pro	Val	Glu	Lys	
5′	ATG	GTG	CAC	CTG	ACT	CCT	GTG	GAG	AAG	3′
3′	TAC	CAC	GTG	GAC	TGA	GGA	CAC	CTC	TTC	5′

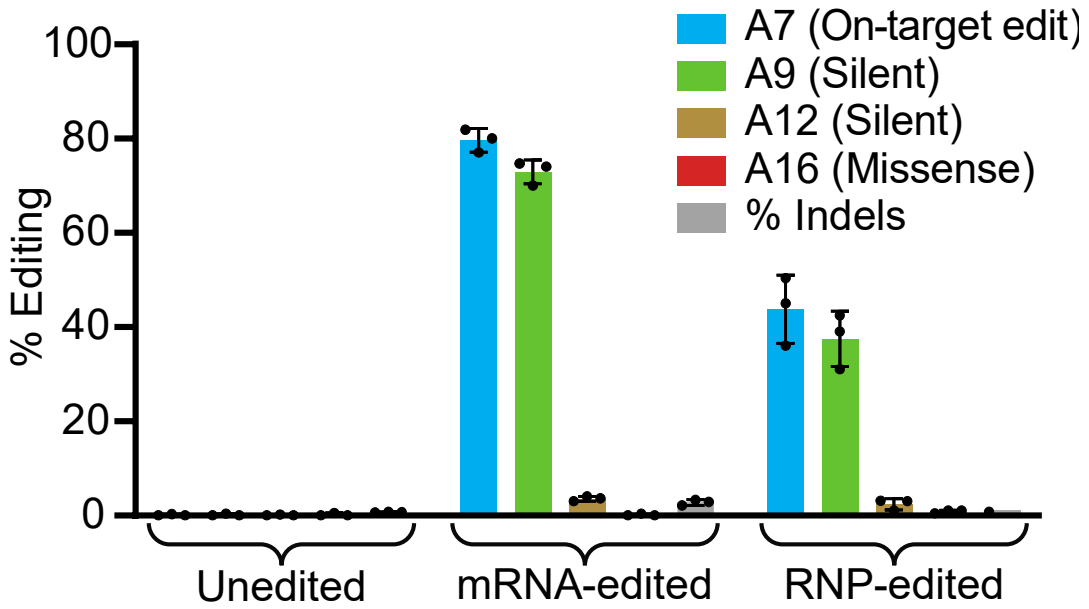
PAM (5′-CACC)

A16 A12 A9 A7 ↓

Protospacer for ABE8e-NRCH

	GGC	TGG	GGG	CGC
	Pro	Thr	Pro	Ala

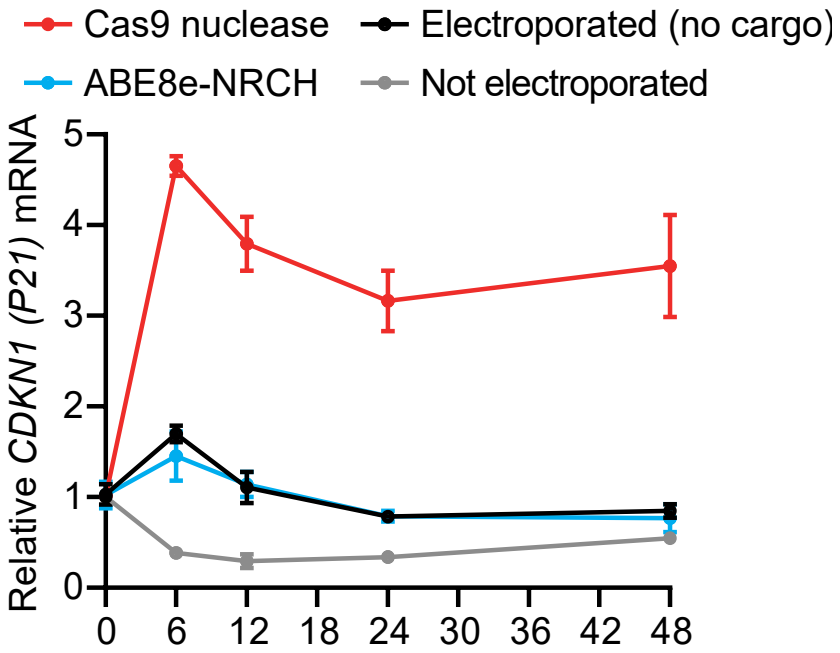
HBB^{G} (Makassar)

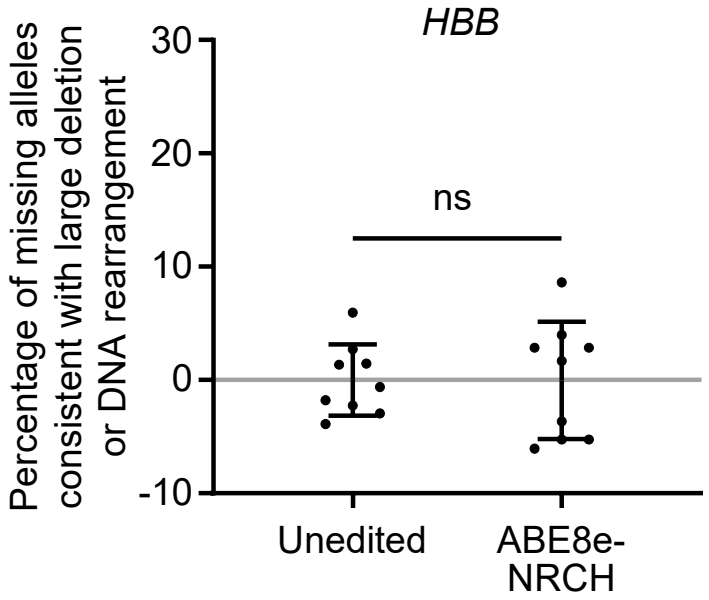


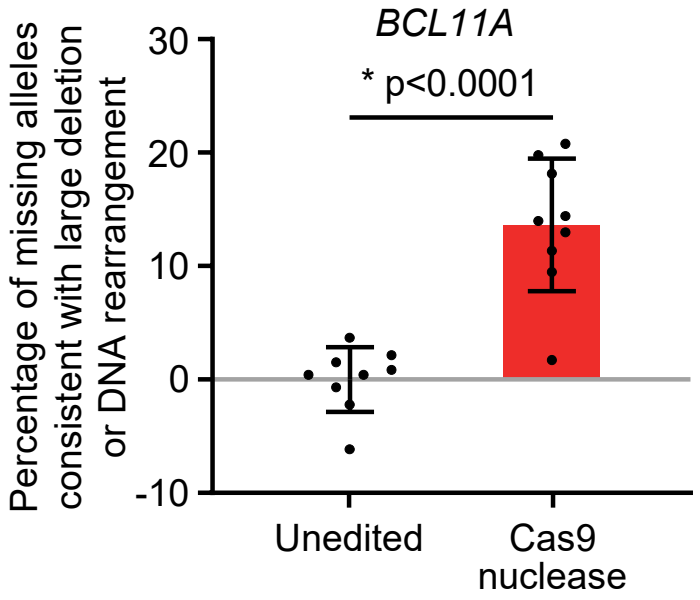
- ABE8e-NRCH mRNA or RNP electroporated *ex vivo* into human SCD patient hematopoietic stem and progenitor cells converts *HBB*^{S} to naturally occurring non-pathogenic *HBB*^{G}
- 80% editing efficiency, <3% indels or missense

Newby, Yen, Woodard, Mayuranathan, Weiss, Liu *et al. Nature* **595,** 295-302 (2021); ABE8e structure: Lapinaite, Knott, Beal, Liu, Doudna *Science* **369**, 566 (2020)
see also Chu, Packer, Rees, Lam, Yu, Gaudelli, Ciaramella, Slaymaker *et al. CRISPR J.* **4**, 169 (2021)

DNA Damage Response and Target Site Disruption Following Cas9 Nuclease vs. ABE Treatment



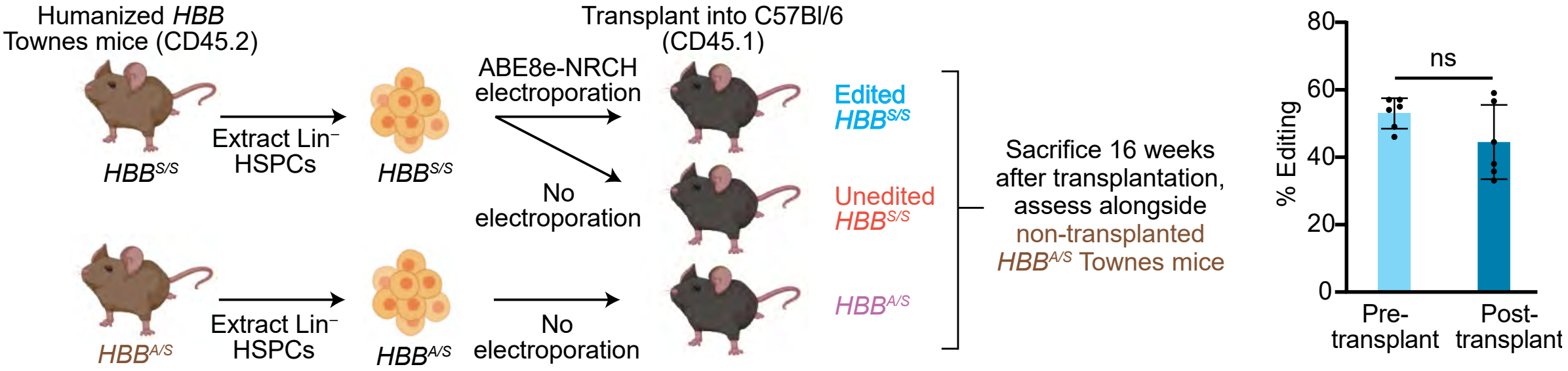


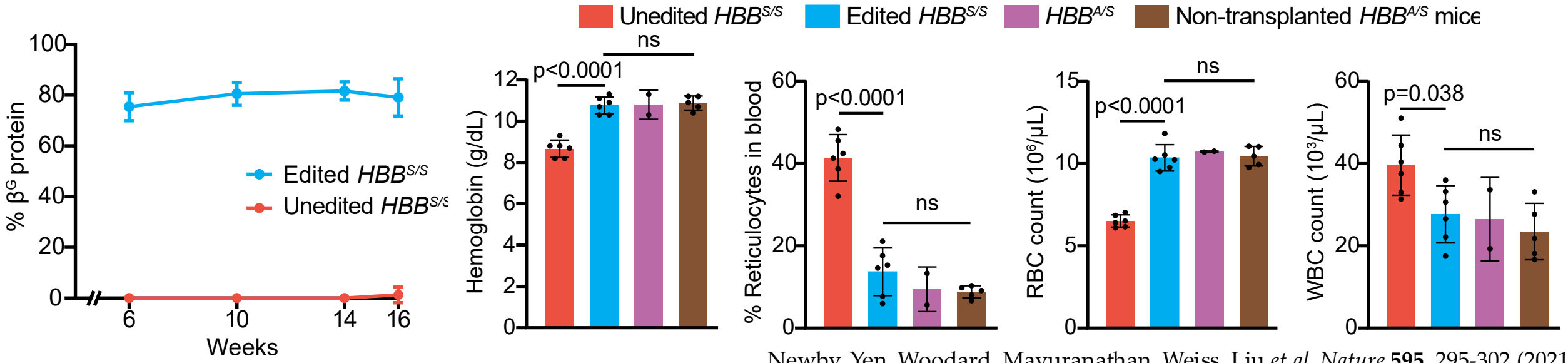


- *CDKN1* (*P21*) RT-ddPCR shows 2.7- to 4.2-fold higher p53 DNA damage response in human HSPCs from treatment with *BCL11A* enhancer-targeted Cas9, but no change for *HBB*-targeted ABE8e-NRCH
- Droplet digital PCR quantification of the target locus relative to a non-targeted control locus shows 14% allele loss from *BCL11A* enhancer-targeted Cas9, but no loss for *HBB*-targeted ABE8e-NRCH

Newby, Yen, Woodard, Mayuranathan, Weiss, Liu *et al. Nature* **595,** 295-302 (2021); see also Song *et al. Mol. Ther. Nucl. Acids* **21**, 523 (2020); Kosicki *et al. Nat. Biotechnol.* **36**, 765 (2018); Haapaniemi *et al. Nat. Med.* **24**, 927 (2018); Ihry *et al. Nat. Med.* **24**, 939 (2018); Schiroli *et al. Cell Stem Cell. 24(4)*,551-565 (2019)

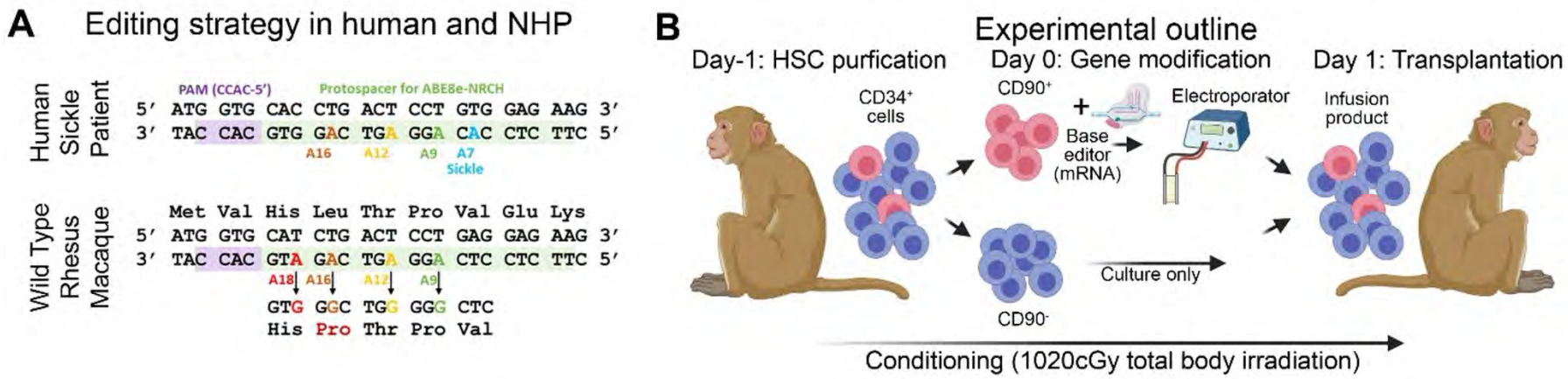
Transplantation of Edited SCD Mouse HSPCs into Mice Rescues Hematological Defects

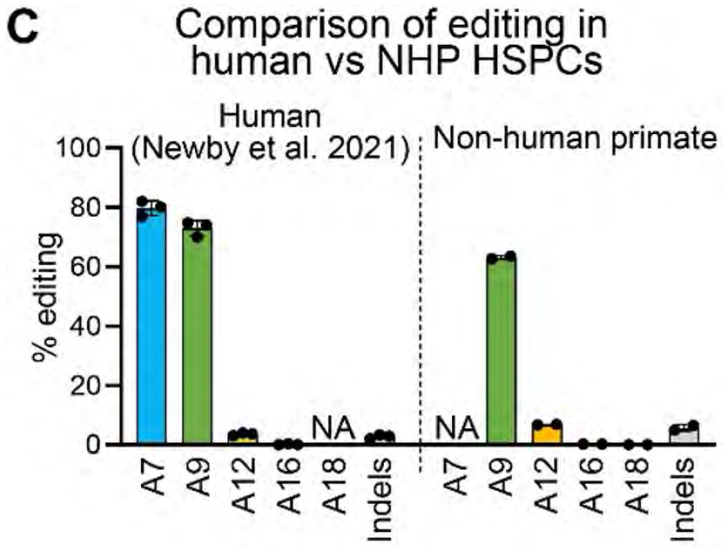


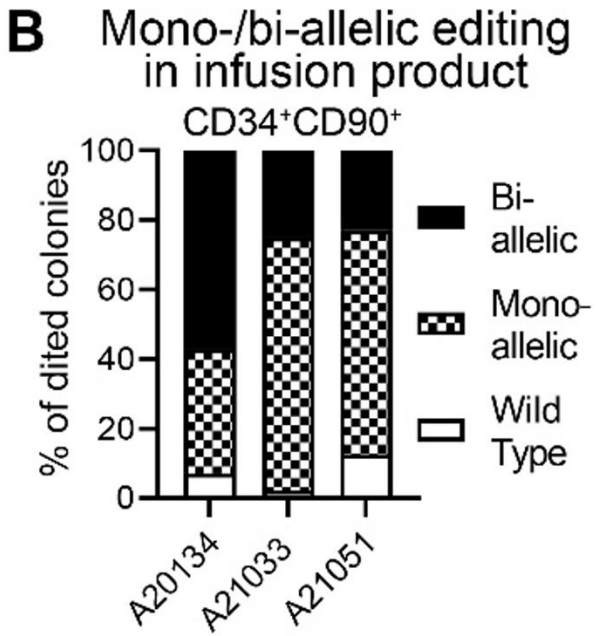


Newby, Yen, Woodard, Mayuranathan, Weiss, Liu *et al. Nature* **595,** 295-302 (2021)

Autologous transplantation into Rhesus macaques



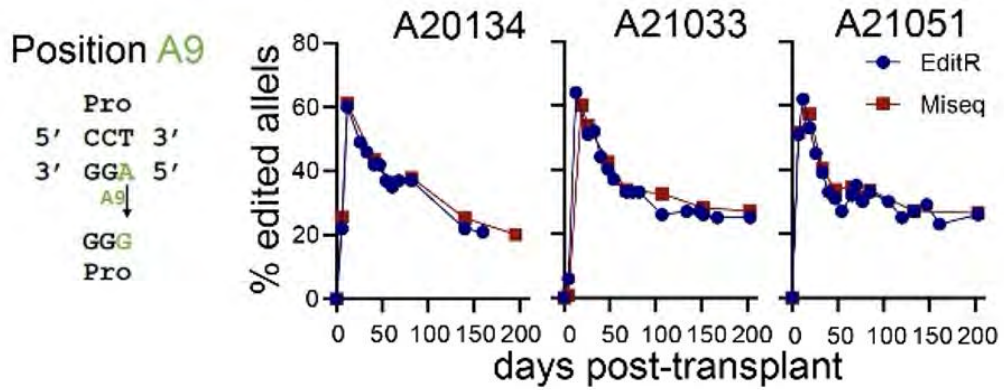


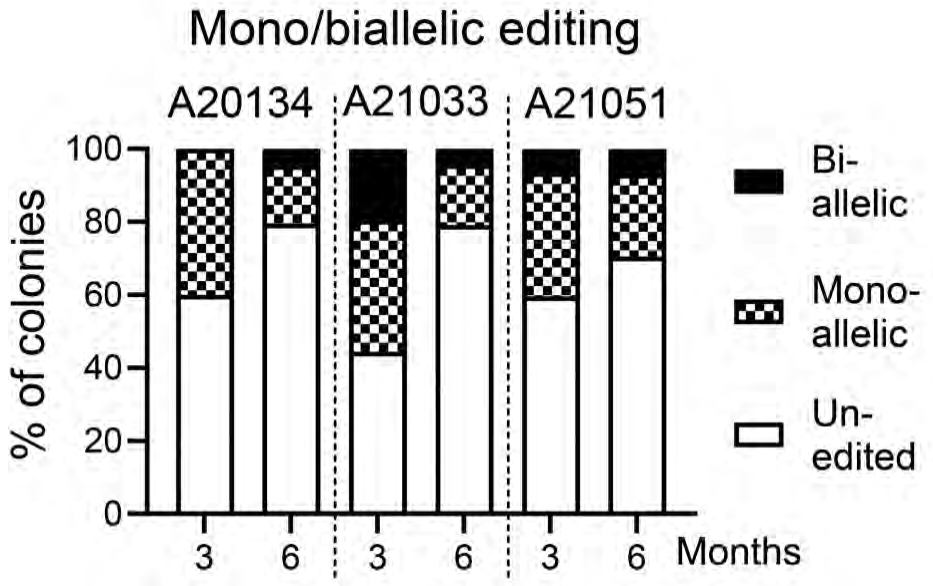


Radtke, Fields, Swing Kanestrom, Yen, Pande, Weiss, Liu, Newby, Kiem, *et al. Science Translational Medicine* 17(811) 2025.

Autologous transplantation into Rhesus macaques

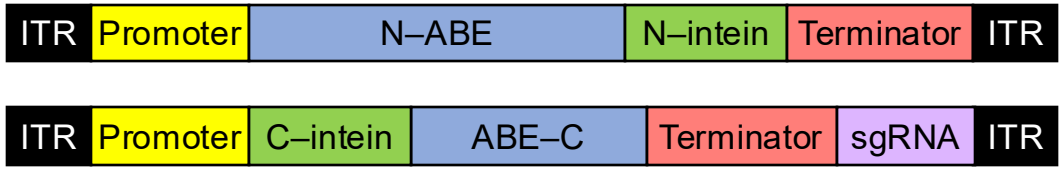
Editing over 6 months following infusion of edited cells



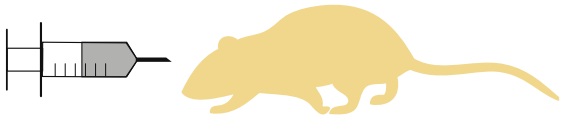


Radtke, Fields, Swing Kanestrom, Yen, Pande, Weiss, Liu, Newby, Kiem, *et al. Science Translational Medicine* 17(811) 2025.

In Vivo Dual-AAV Delivery of Base Editors to Correct Progeria

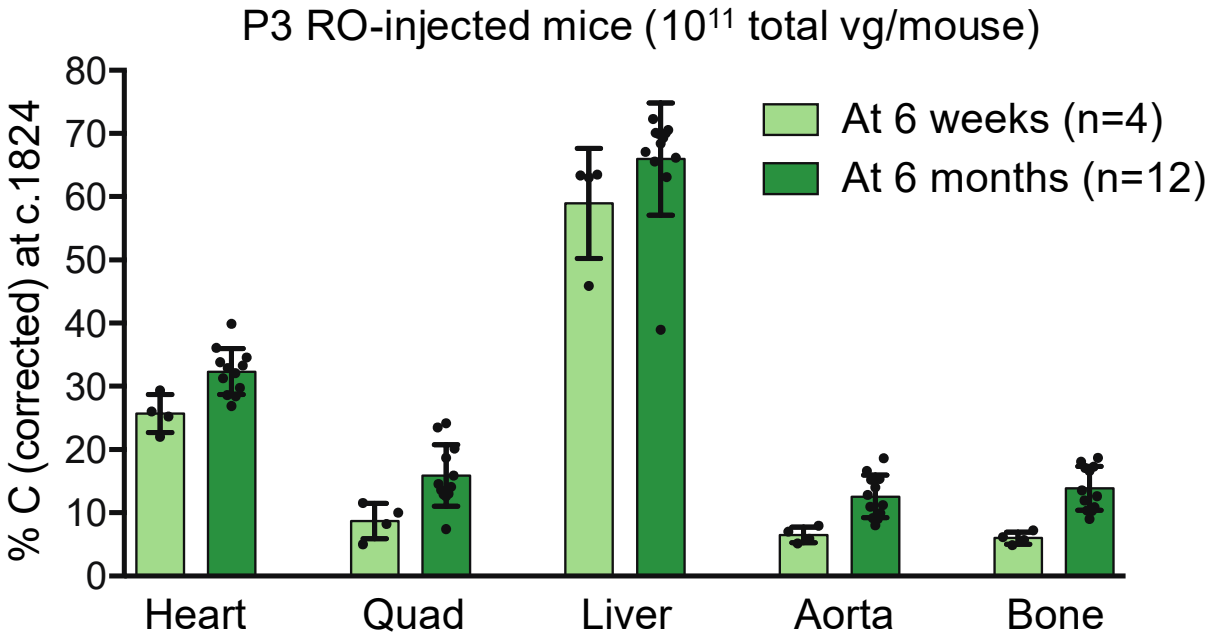


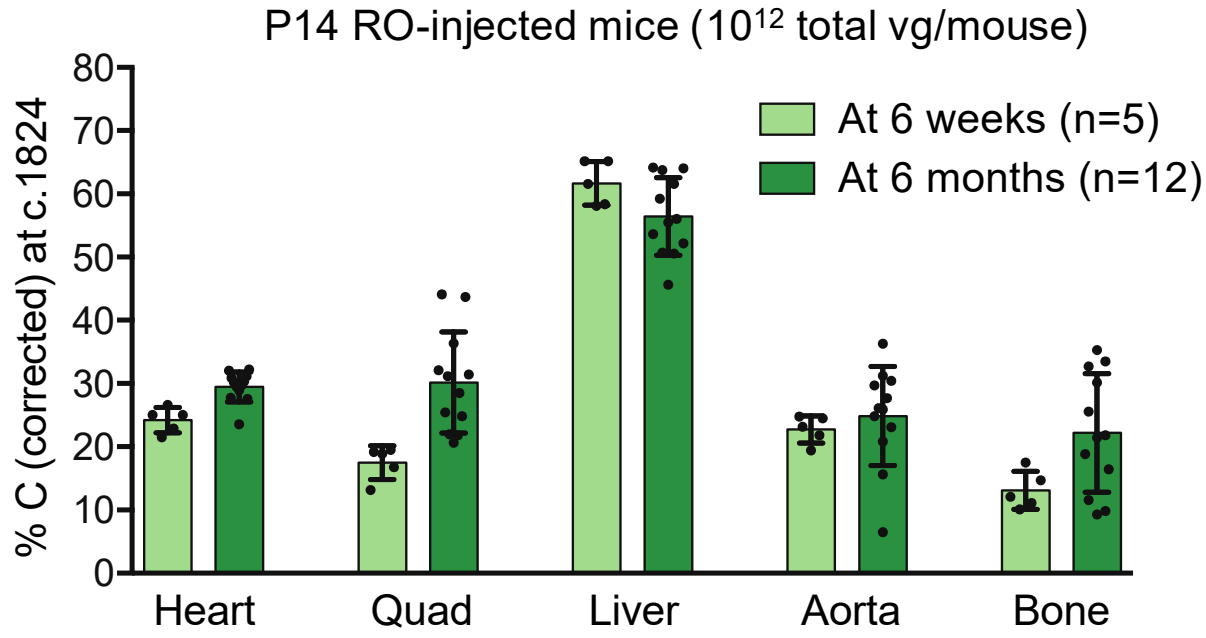
Optimized v5 split-intein ABE dual AAV9



Dual-copy human *LMNA*
c.1824 C>T (progerin) mice

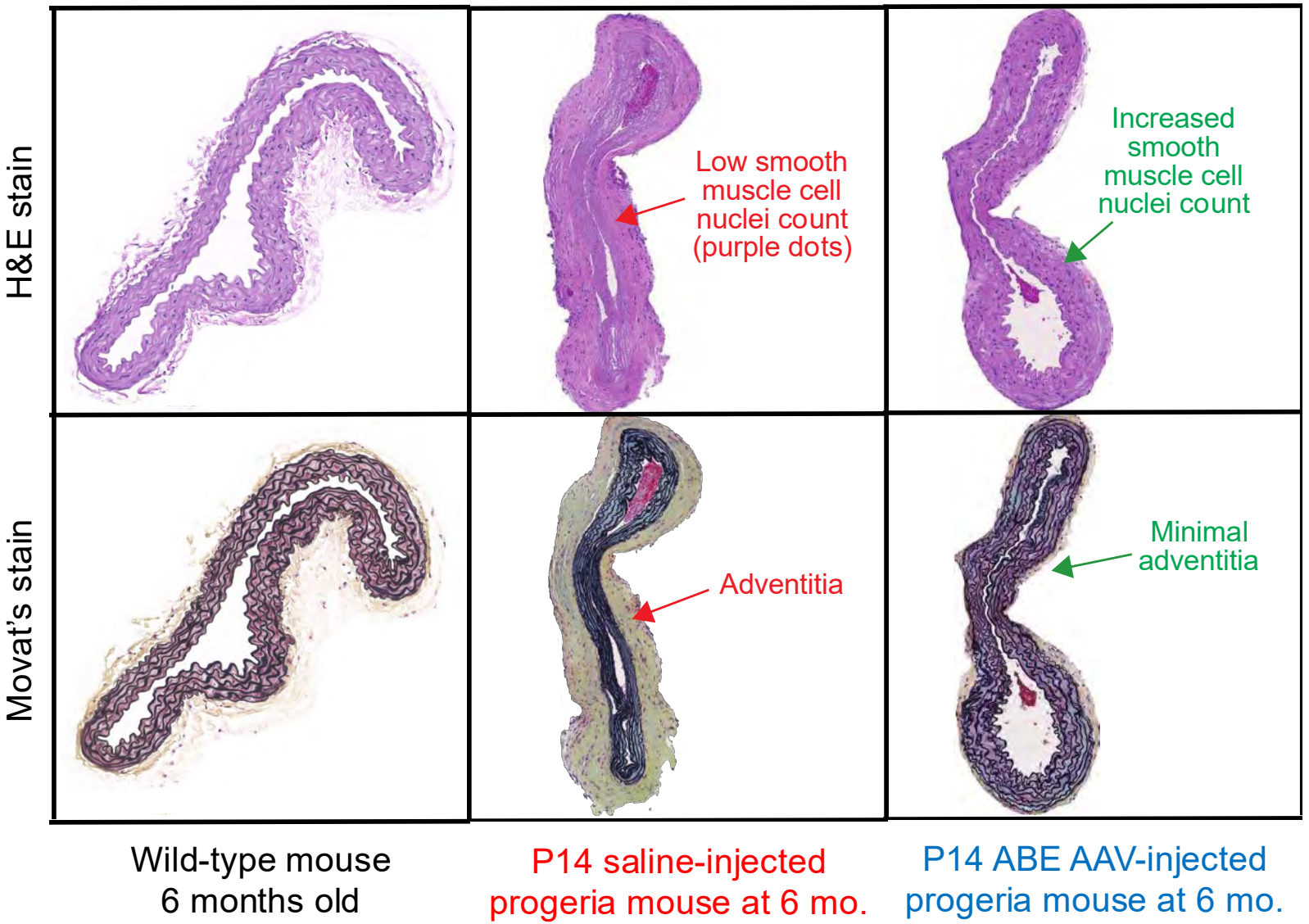
ABE AAV9 injection routes
10^{11} total vg P3 retro-orbital
10^{12} total vg P14 retro-orbital
10^{12} total vg P14 intraperitoneal

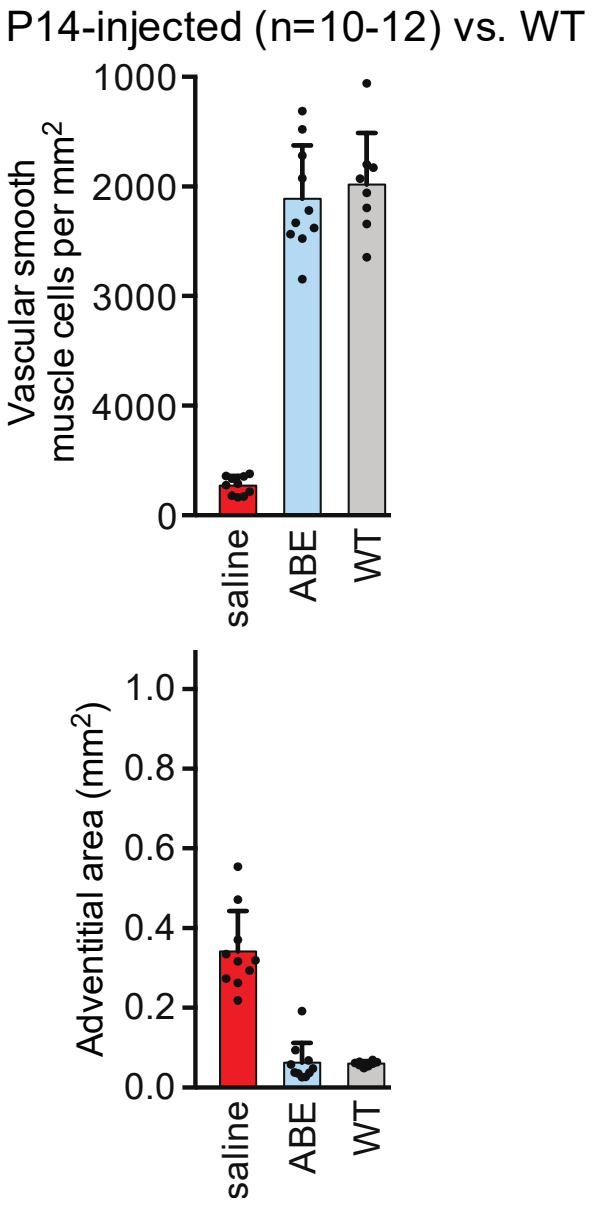




Koblan, Erdos, Wilson, Cabral, Levy, Xiong, Tavaraz, Davison, Gete, Mao, Newby, Lin, Gordon, Cao, Collins, Brown, Liu *et al. Nature* **589**, 608 (2021)
Dual AAV BE delivery system: Levy, Yeh, Pendse, Davis, Liu *et al. Nat. Biomed. Eng.* **4**, 97 (2020); single-copy HGPS mouse: Varga, Collins *et al. PNAS* **103**, 3250 (2006)

Single-Dose *In Vivo* ABE Treatment Improves Aorta Pathology





Koblan, Erdos, Wilson, Cabral, Levy, Xiong, Lin, Gordon, Newby, Cao, Collins, Brown, Liu *et al. Nature* **589**, 608 (2021)

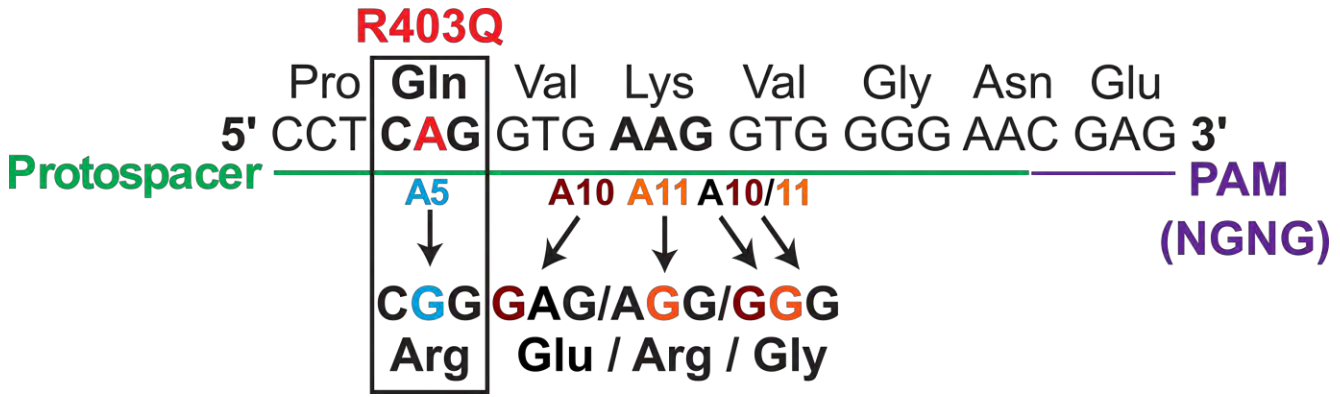
ABE Treatment Rescues Progeria Animal Function and Vitality

Untreated progeria mouse
at 7.5 months old (end of life)

P14-injected ABE-treated progeria
mice at ~11 months old

Koblan, Erdos, Wilson, Cabral, Levy, Xiong, Tavaraz, Davison, Gete, Mao, Newby, Lin, Gordon, Cao, Collins, Brown, Liu *et al. Nature* **589**, 608 (2021)

Base editing to correct a hypertrophic cardiomyopathy mutation in *MYH7*



N-terminal AAV Vector Architecture

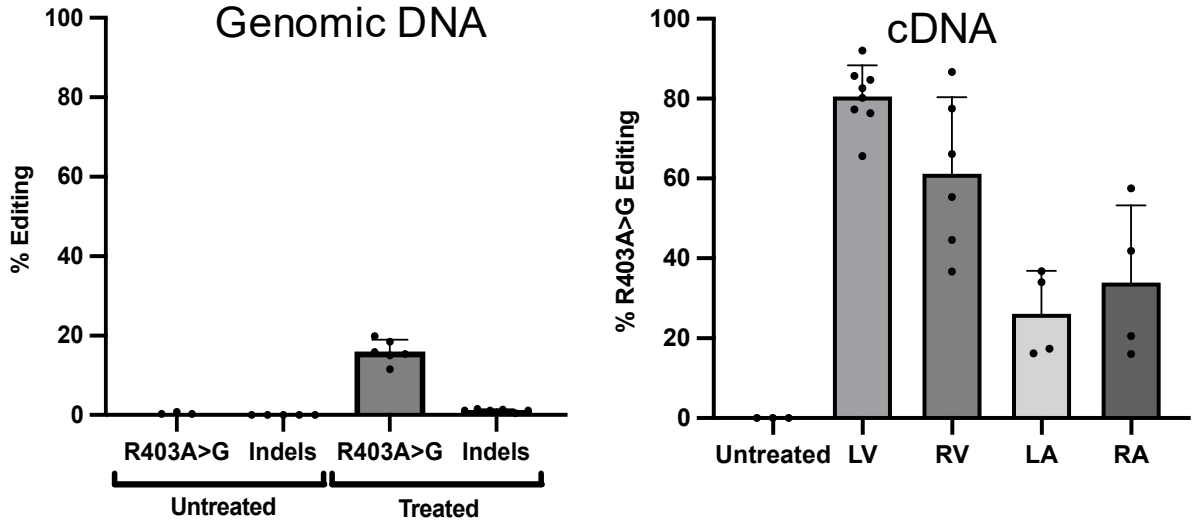
C-terminal AAV Vector Architecture

Reichart, Newby, Wakimoto, Liu, Seidman *et al. Nature Medicine* **29**, 412-421 (2023)

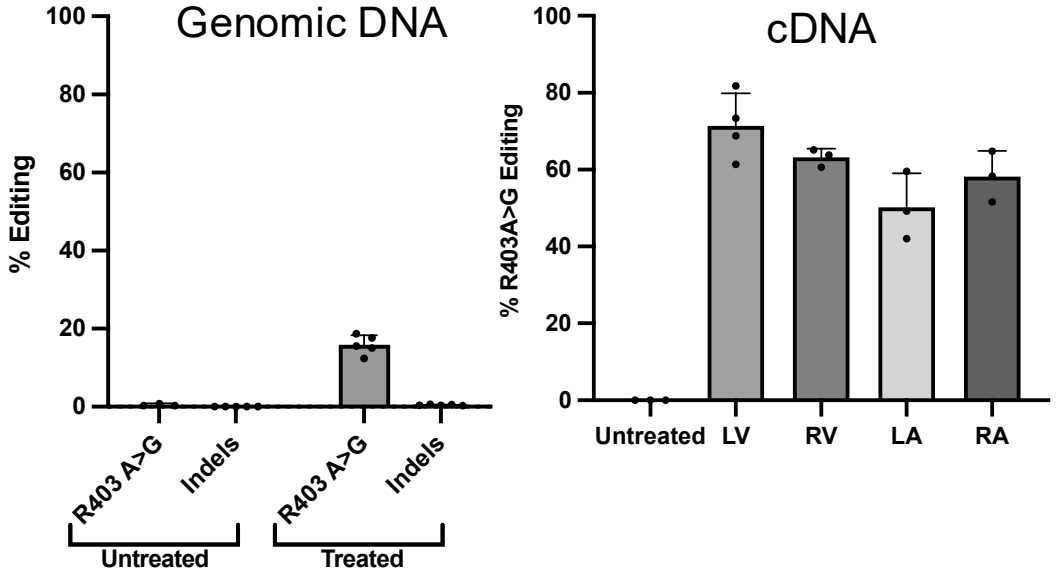
Levy, Yeh, Pendse, Davis, Liu, Liu *et al. Nature Biomedical Engineering* **4**(1):97-110. (2020)

Two doses lead to substantially more editing in the atria

One dose



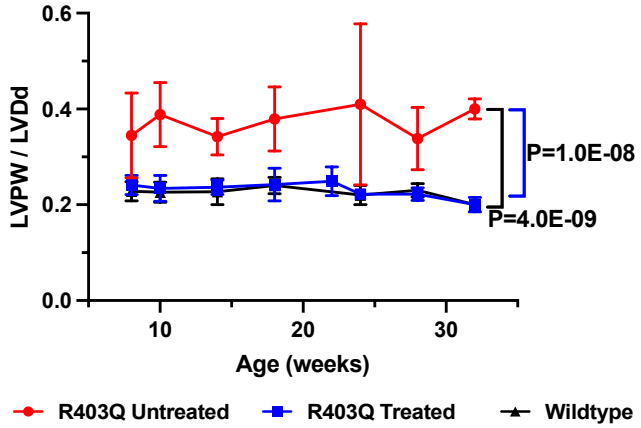
Two doses

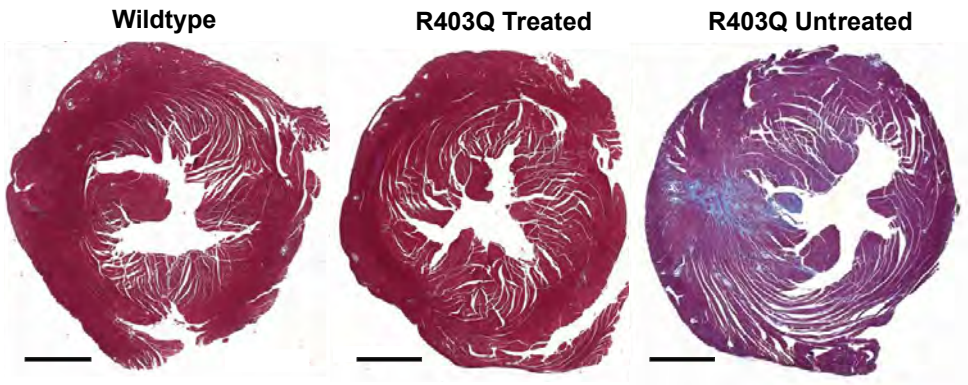


- Mice dosed at 2 weeks of age, editing quantified at 30 weeks. 2.5×10^{13} vg/kg dosed intrathoracically

Reichart, Newby, Wakimoto, Liu, Seidman *et al. Nature Medicine* **29**, 412-421 (2023)

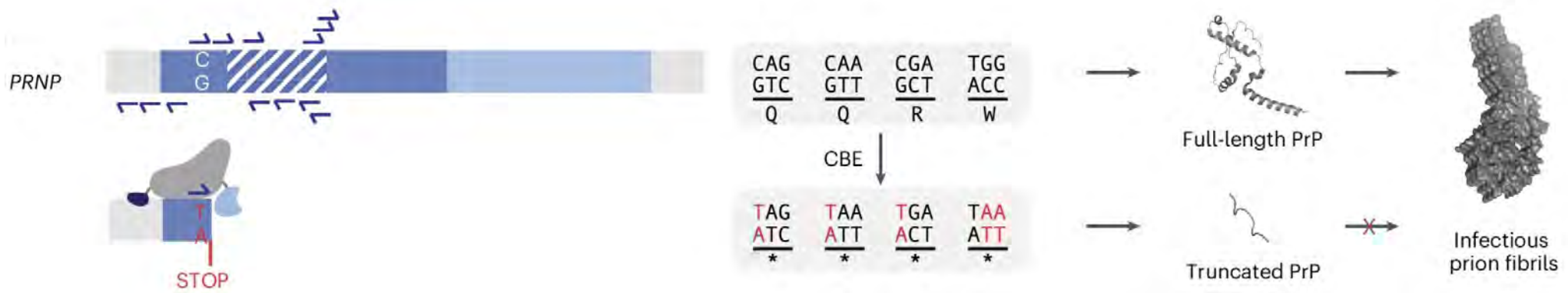
One or two doses fully prevent hypertrophy and fibrosis

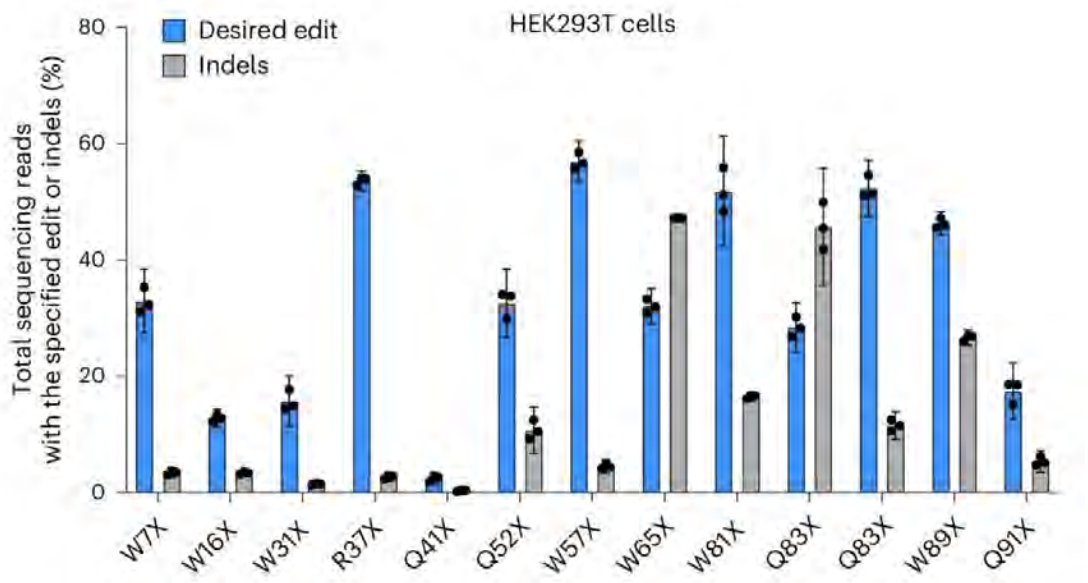


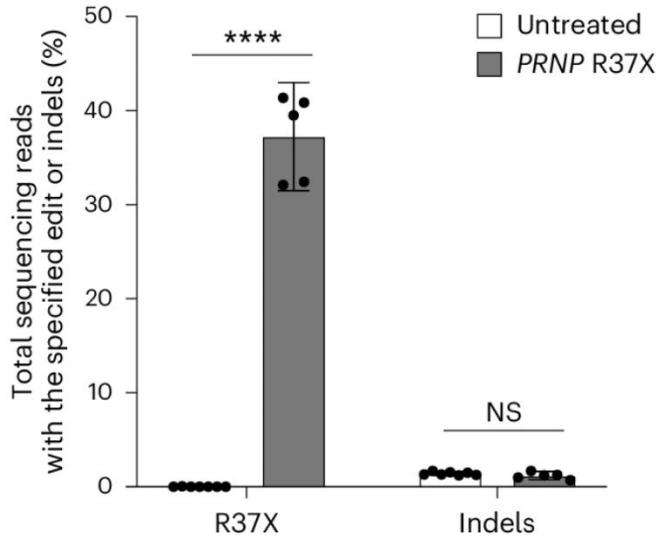


Reichart, Newby, Wakimoto, Liu, Seidman *et al. Nature Medicine* **29**, 412-421 (2023)

Base editor AAVs disrupt the prion protein and prolong life

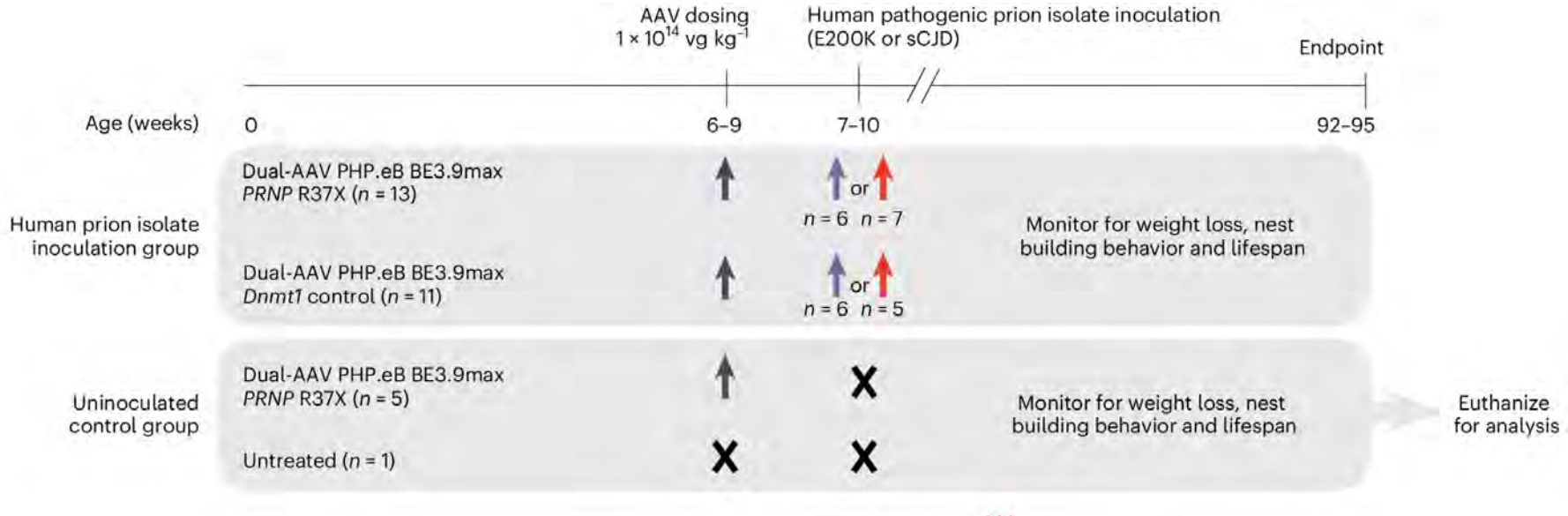






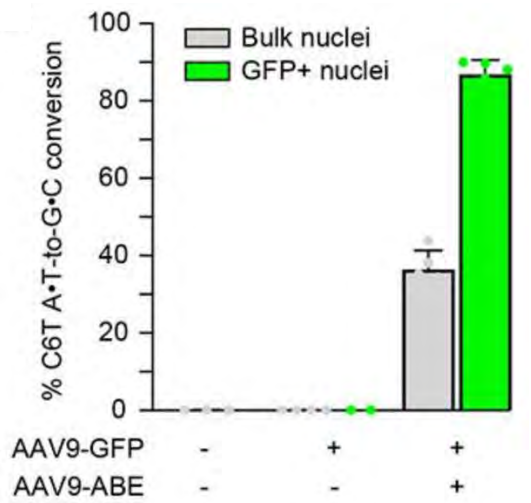
An, Davis, Levy, Newby, Deverman, Minikel, Vallabh, Liu, and coworkers *Nature Medicine*. (2025).

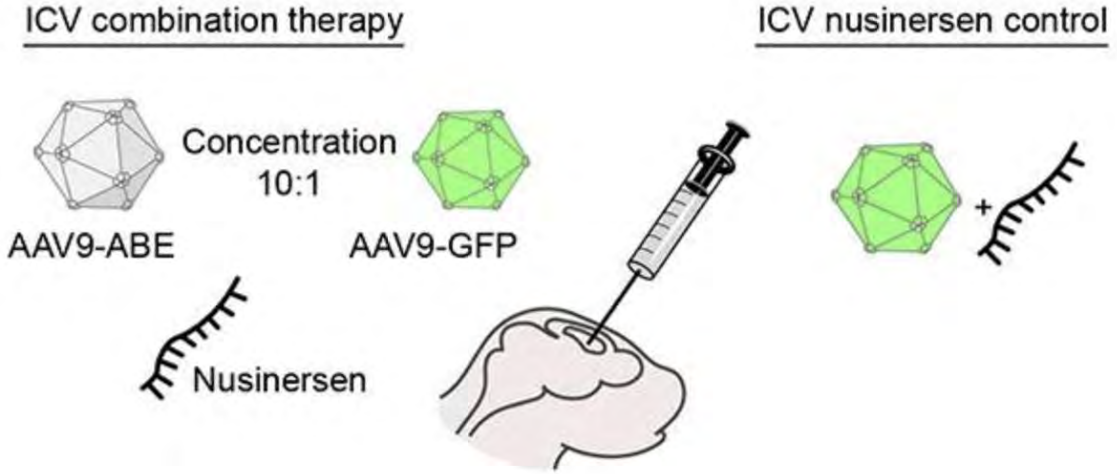
Base editor AAVs disrupt the prion protein and prolong life

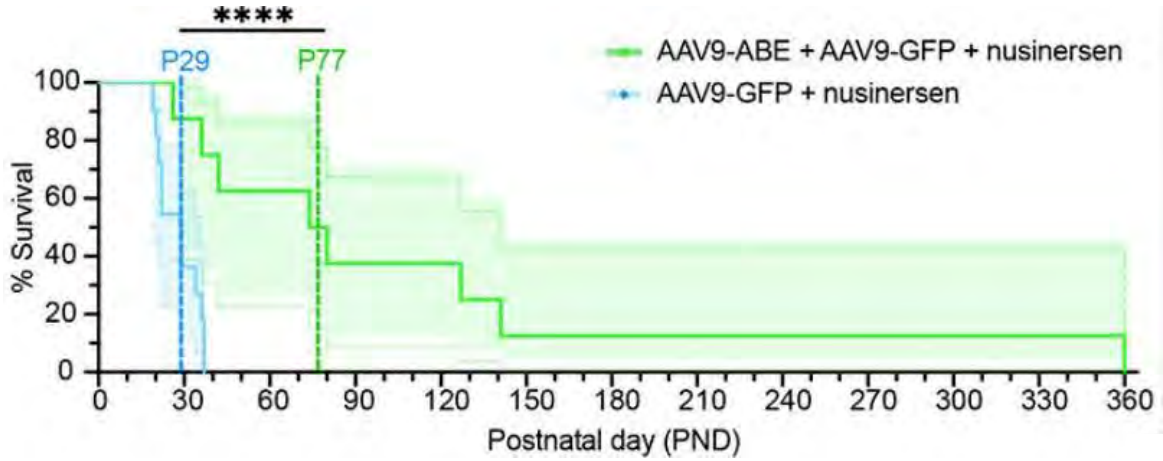


An, Davis, Levy, Newby, Deverman, Minikel, Vallabh, Liu, and coworkers *Nature Medicine*. (2025).

Base editing to restore *SMN2* splicing to treat SMA

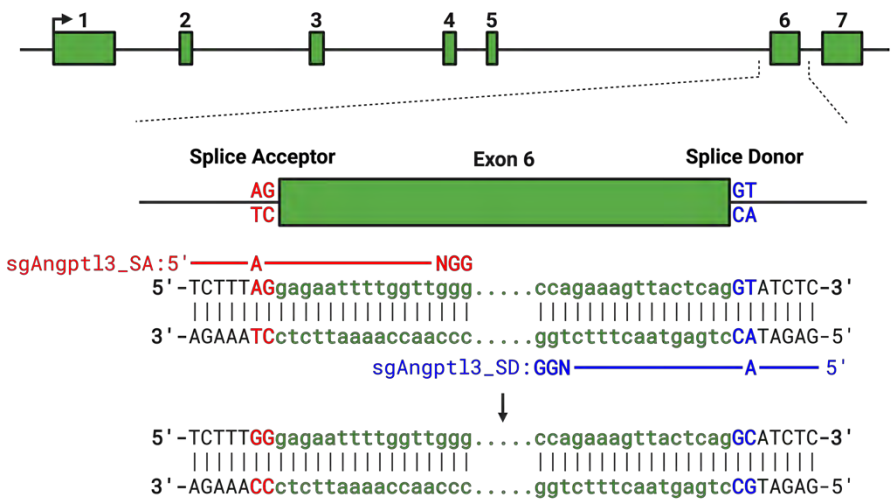


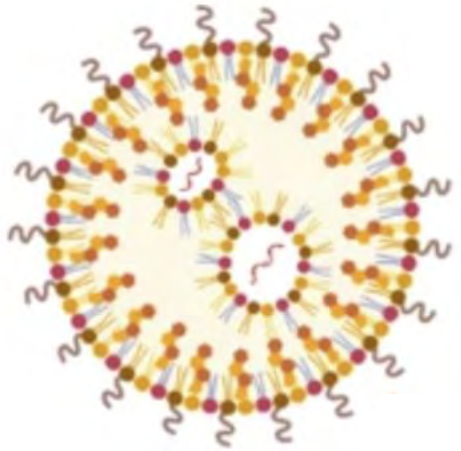


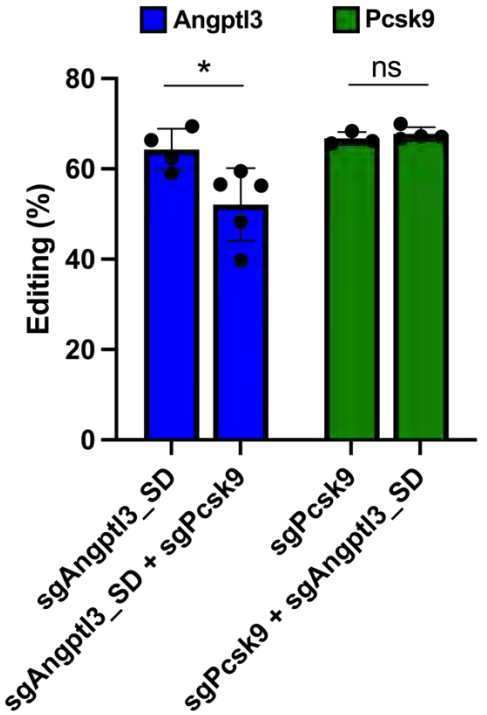


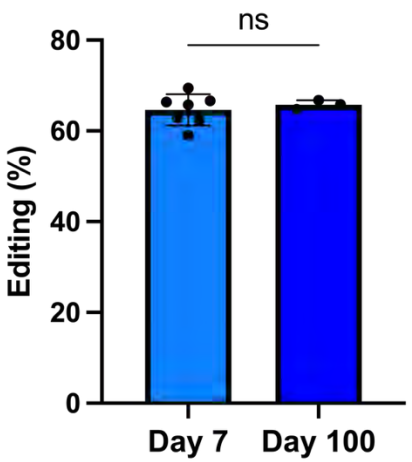
Arbab, Matuszek, Kray, Du, Newby, Liu, *et al. Science.* **380** (2023)

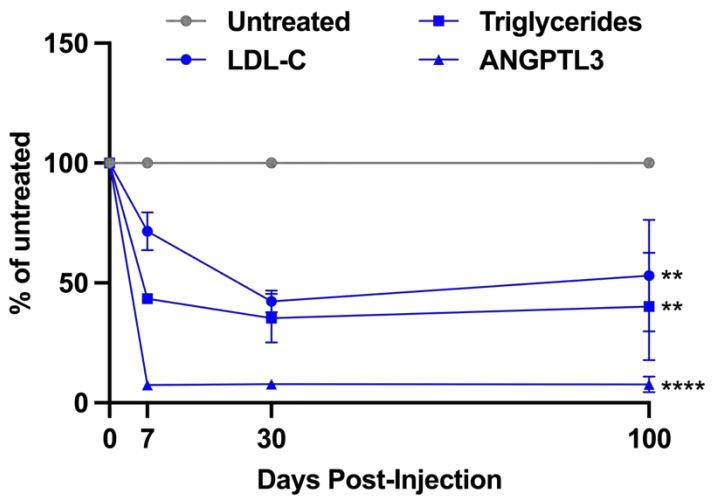
Lipid nanoparticles efficiently edit the mouse liver





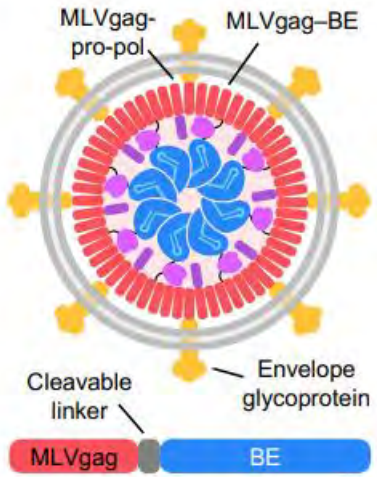


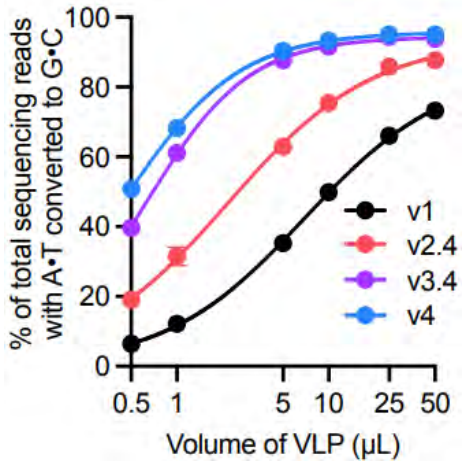


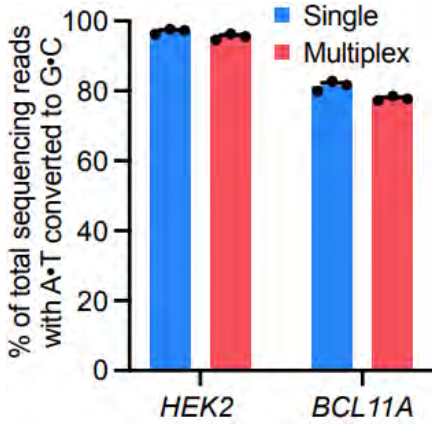


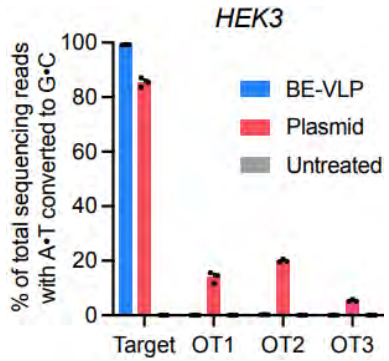
Khirallah, Bloomer, Newby, Liu, Xu *et al. Mol Ther Nuc Acids* Accepted

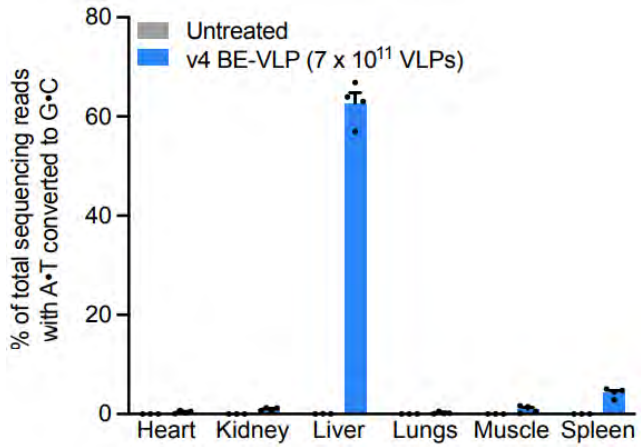
Virus-like particles efficiently delivery ABE RNPs

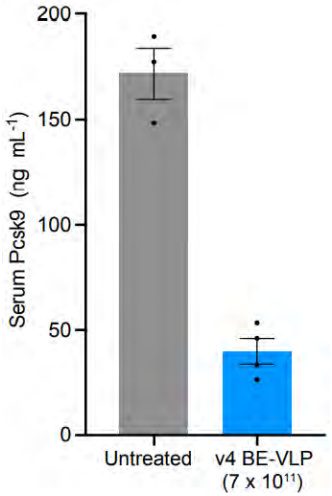


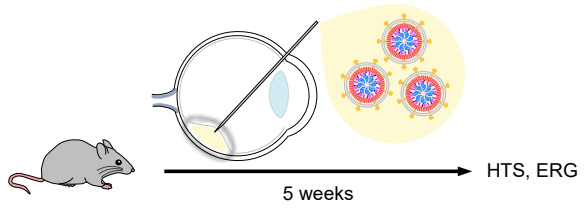


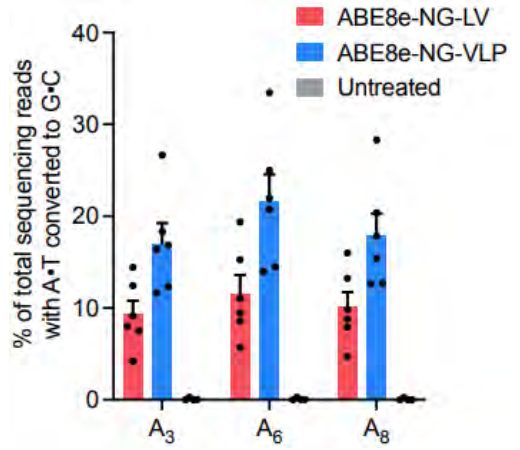






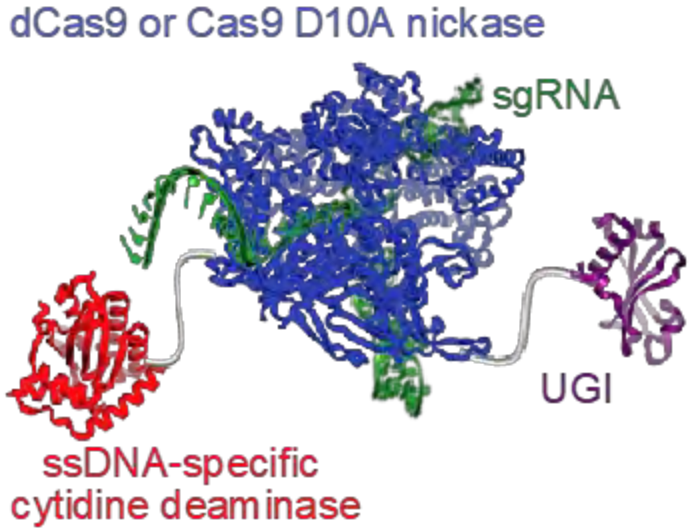






Banskota, Raguram, Newby, Liu *et al. Cell.* **185**. 250-265. (2022).

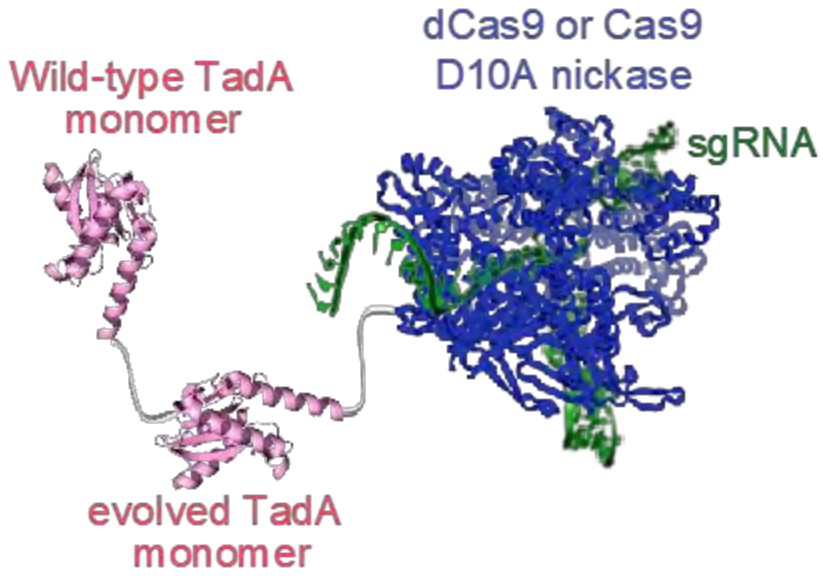
What about the other types of pathogenic mutations?



Cytosine base editors

C → T

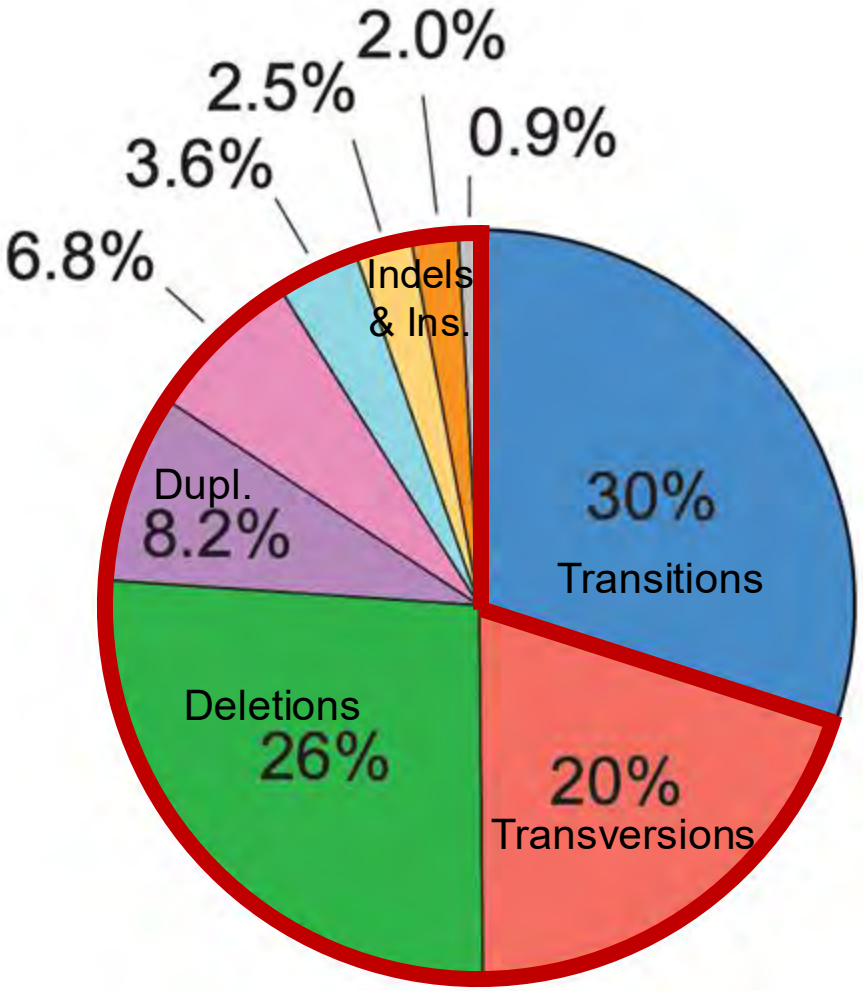
G → A



Adenine base editors

A → G

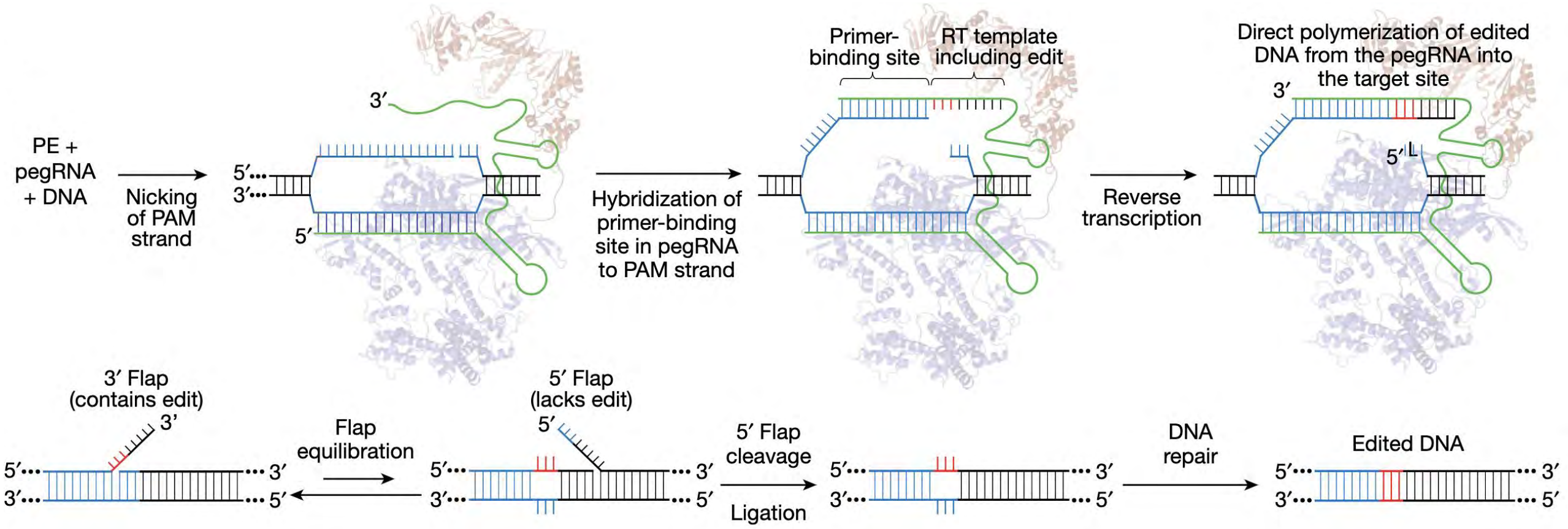
T → C



Total variants > 75,000

Komor, Kim, Packer, Zuris, Liu *Nature* **533**, 420 (2016); Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu *Nature* **551**, 464 (2017); Rees & Liu, *Nat. Rev. Genet.* **19**, 770 (2018)

Prime Editing, a multi-step reaction, expands the targetable pathogenic genetic variants

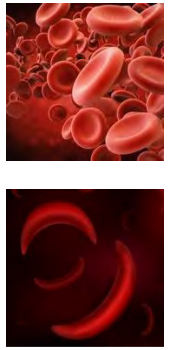


All 4 transition point mutations
All 8 transversion point mutations
Insertions (1 bp to ≥ 44 bp)
Deletions (1 bp to ≥ 80 bp)
Combinations of the above

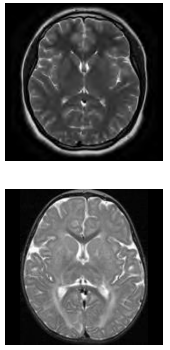
Anzalone, Randolph, Newby, Liu, and co-workers *Nature* **576**, 149 (2019)

Installing or Correcting Pathogenic Alleles and Sequence Tags

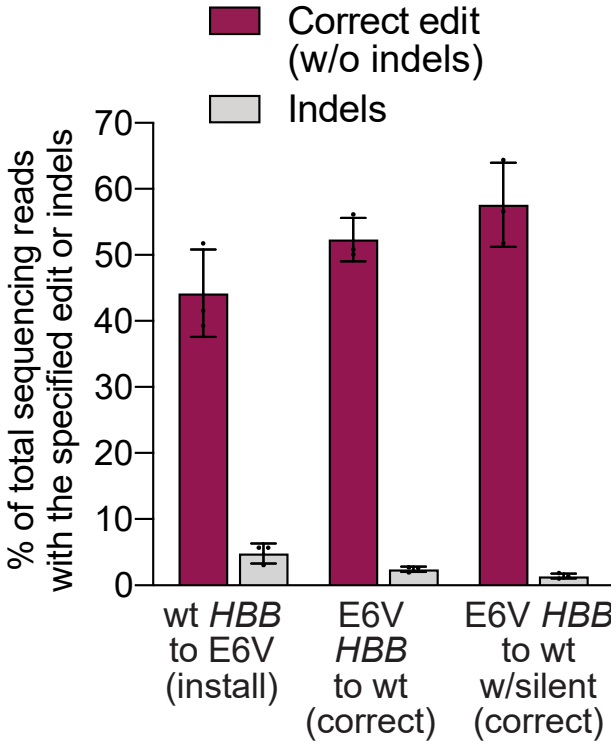
wt *HBB*
Normal
… T G A G G …
PE3 ↓ ↑ PE3
HBB E6V
Sickle cell
… T G T G G …

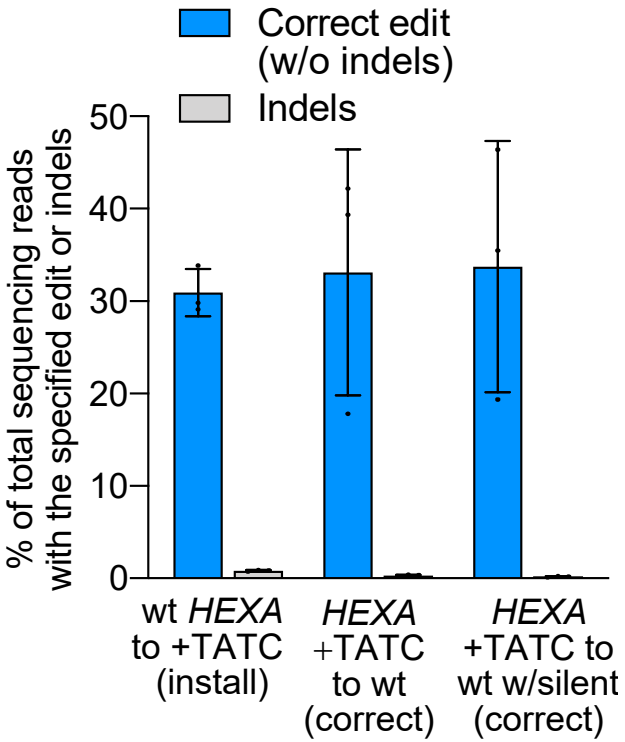


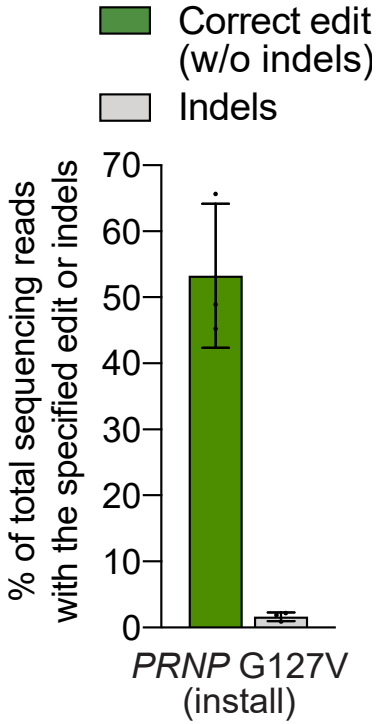
wt *HEXA*
Normal
… A T C C T A …
PE3 ↓ ↑ PE3
*HEXA*1278
+TATC
Tay-Sachs
… A T C T A T C C T A …

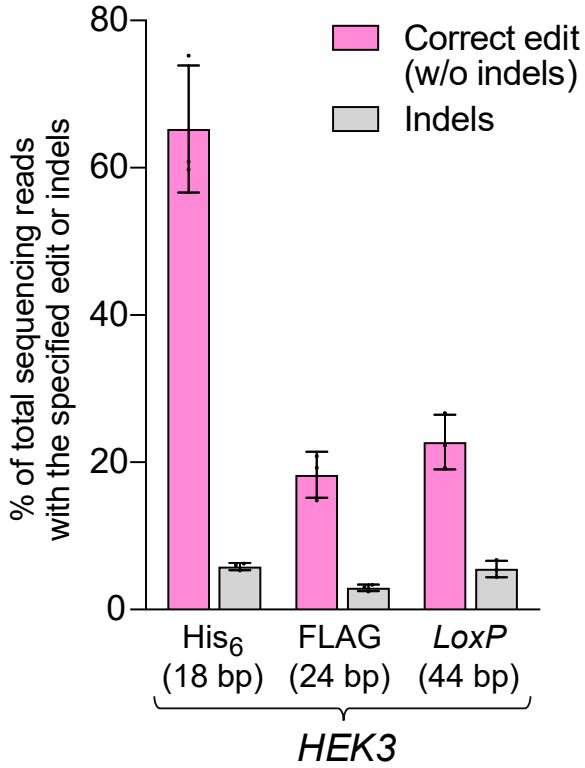


wt *PRNP*
Normal
… C G G C T …
PE3 ↓
PRNP G127V
Prion-resistant
… C G T C T …



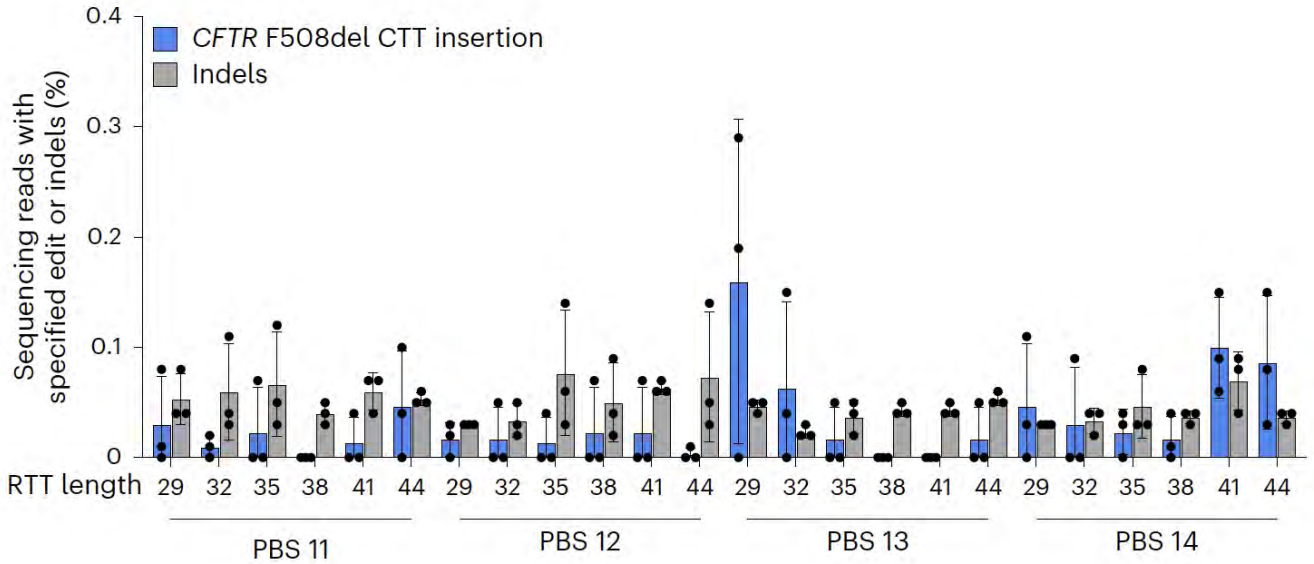


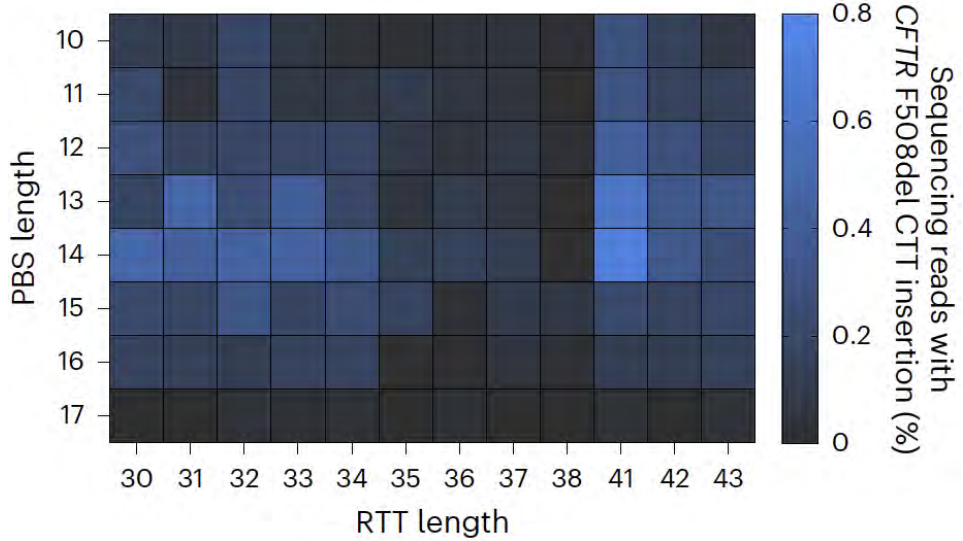


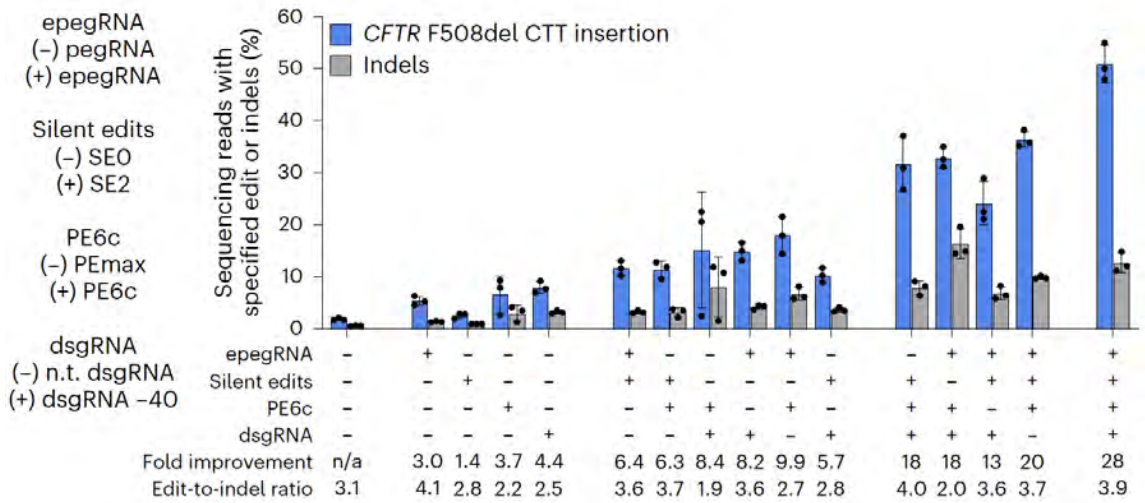


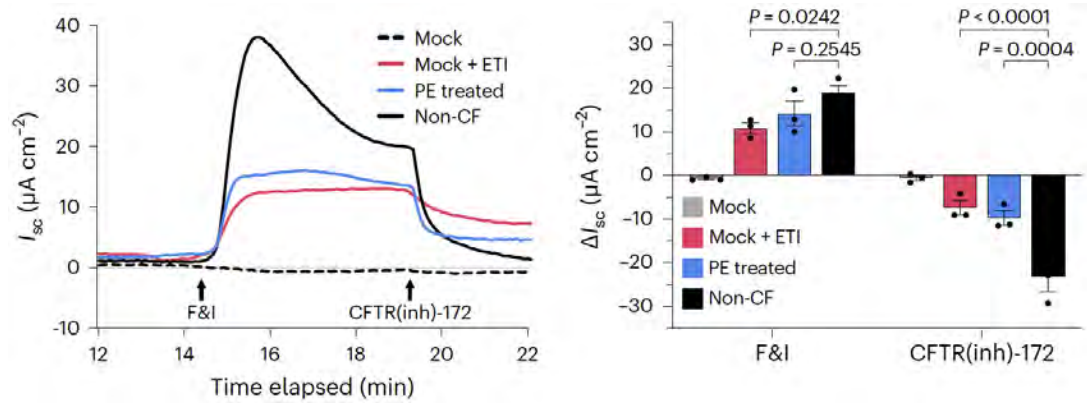
Anzalone, Randolph, Newby, Liu, and co-workers *Nature* **576**, 149 (2019)

Correcting the common Delta508 disease allele of CFTR



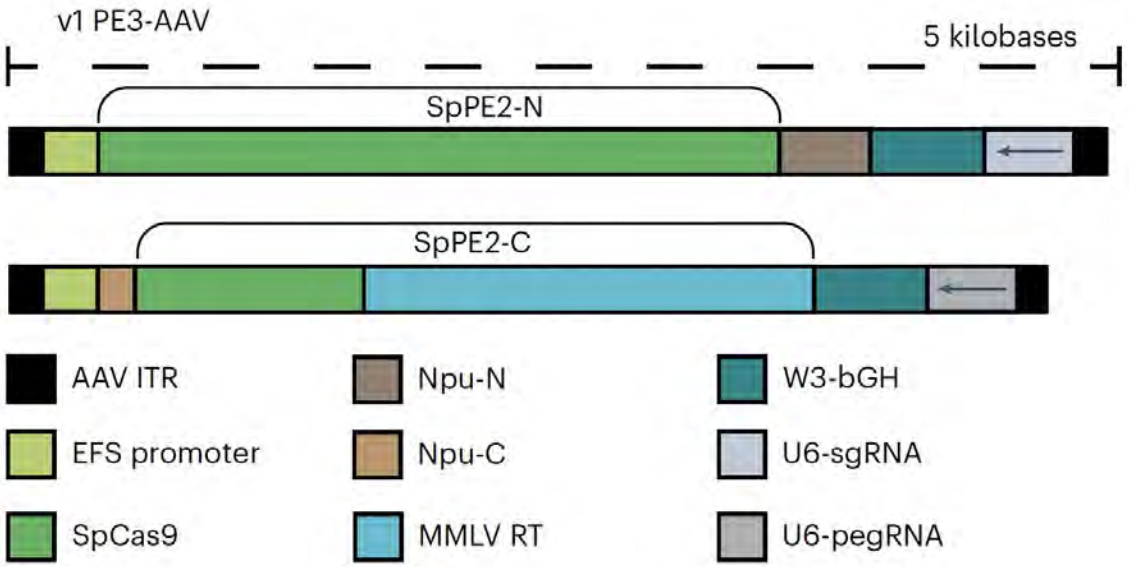


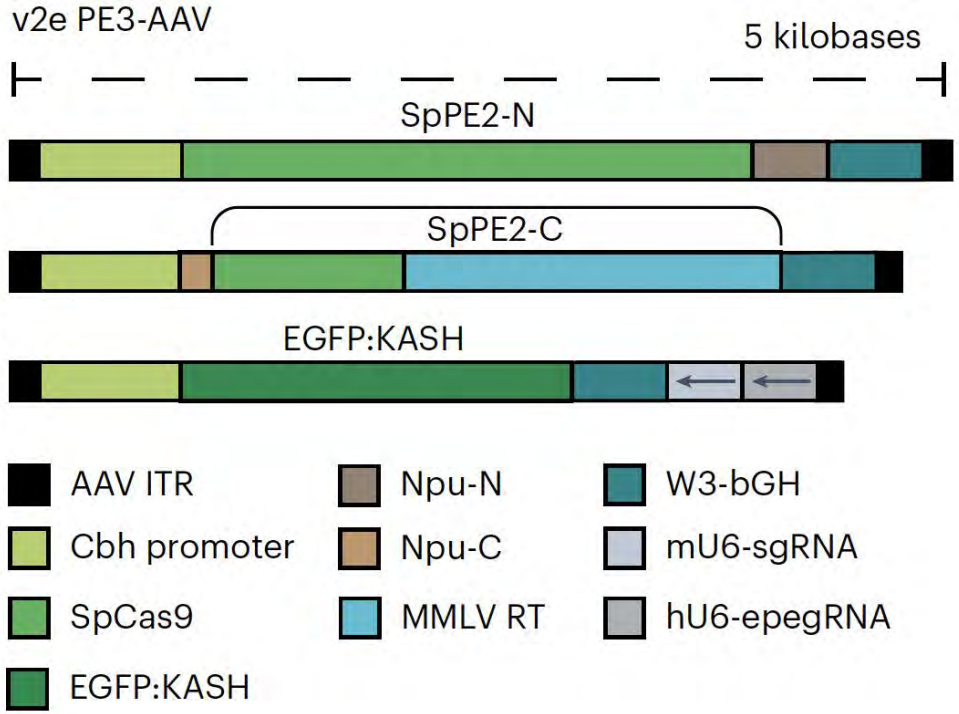


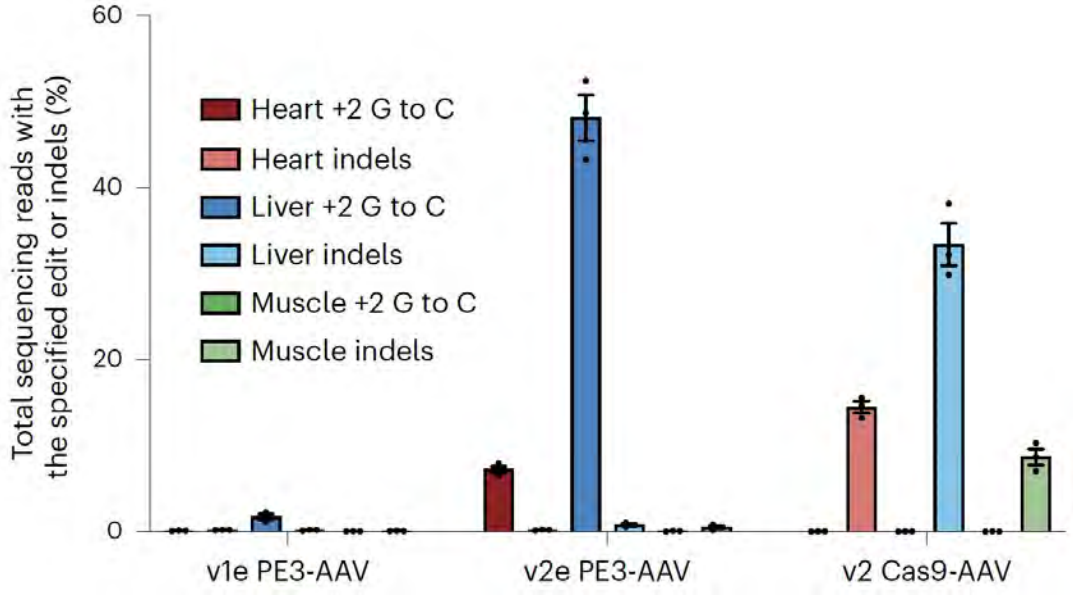


Sousa, Hemez, Newby, McCray, Liu, and co-workers *Nature Biomed Eng* **9**, 7-21(2025)

Prime editor AAVs

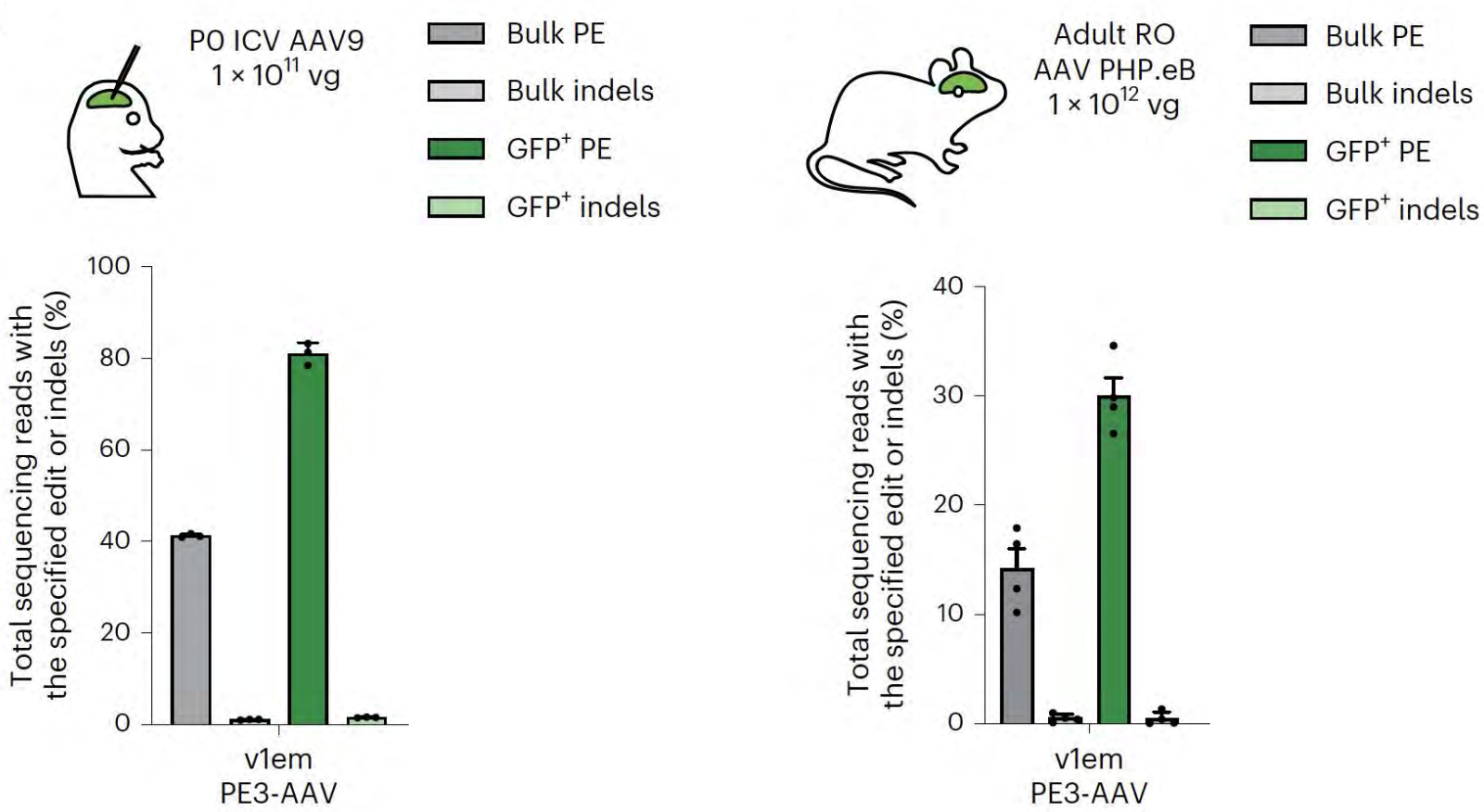






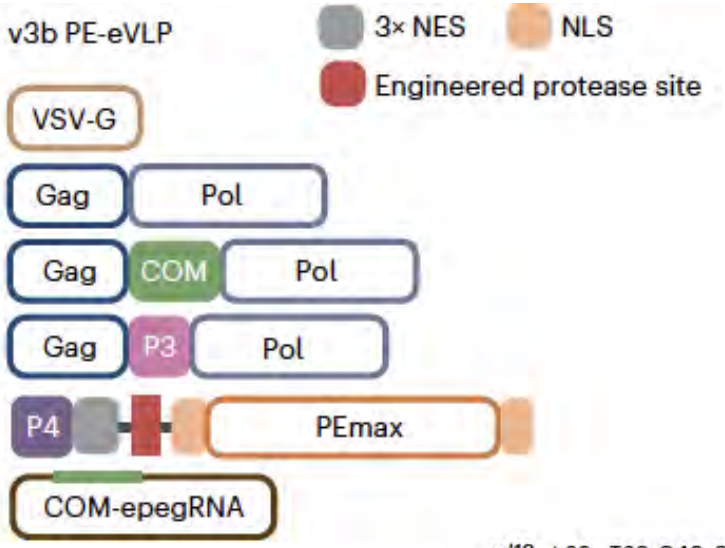
Davis, Banskota, Newby, Liu, and co-workers *Nature Biotechnology* (2023)

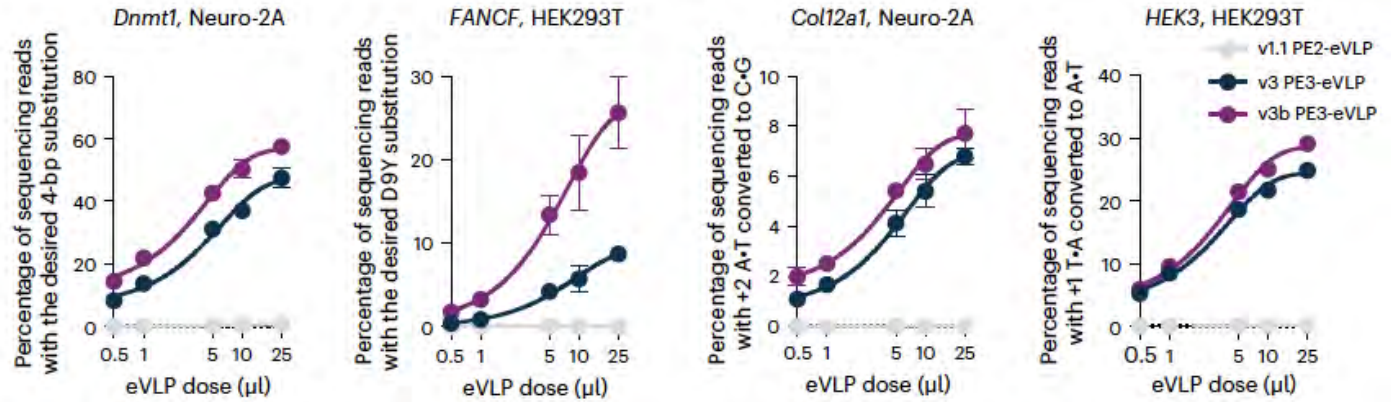
Prime editing in the mouse CNS

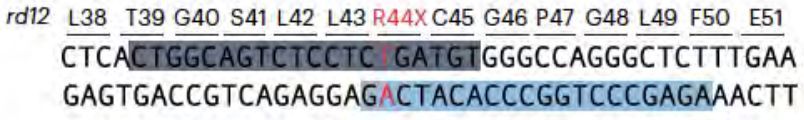


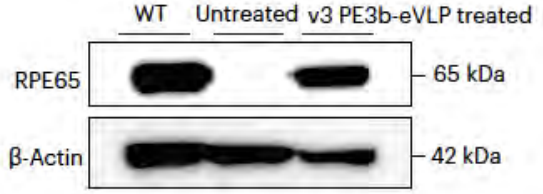
Davis, Banskota, Newby, Liu, and co-workers *Nature Biotechnology* (2023)

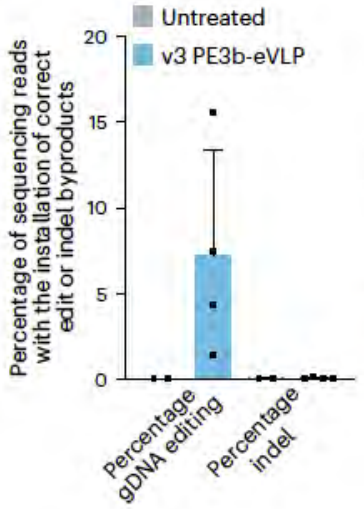
Prime editor eVLPs enable correction of mouse blindness

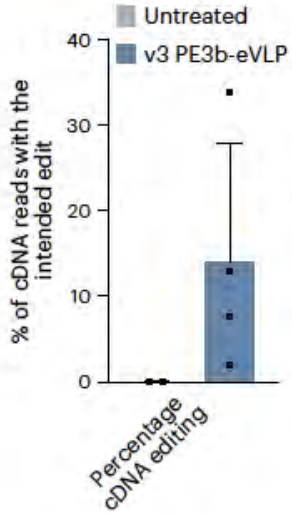


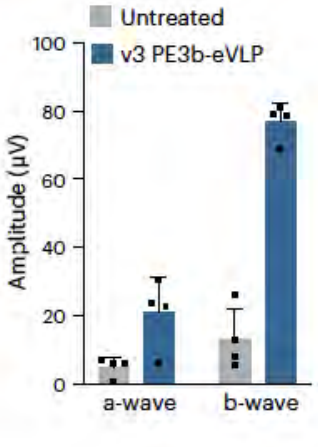


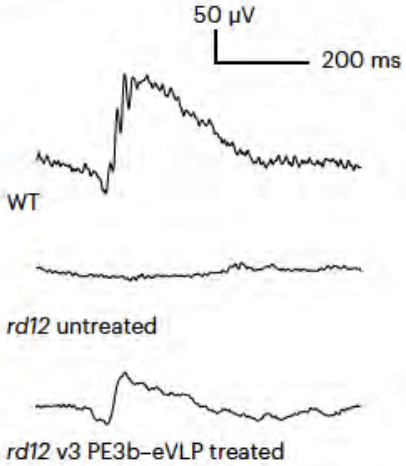






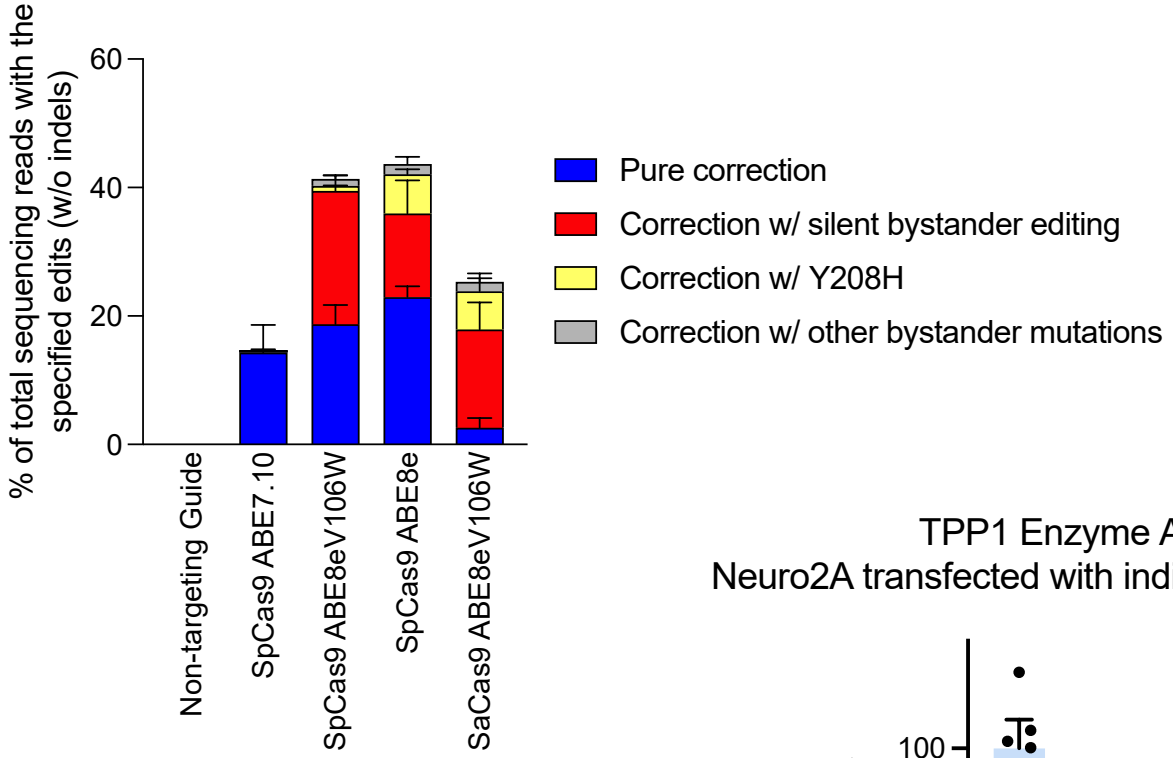


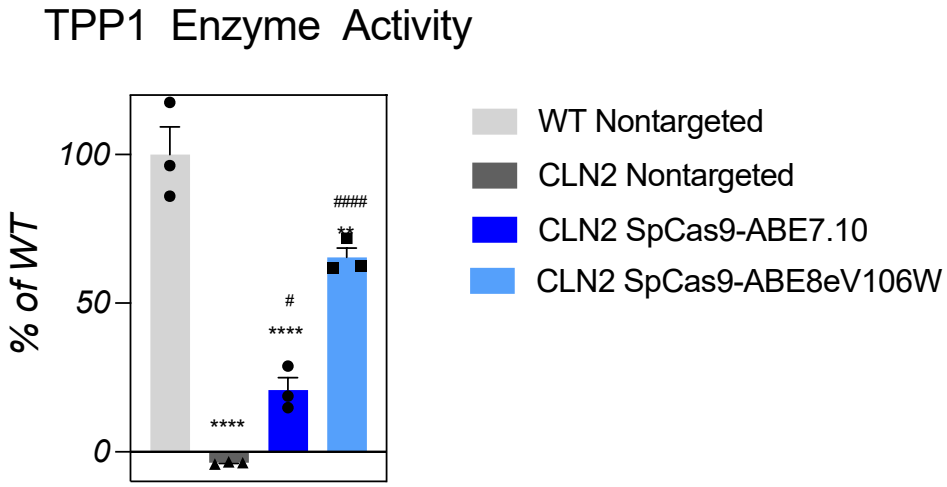


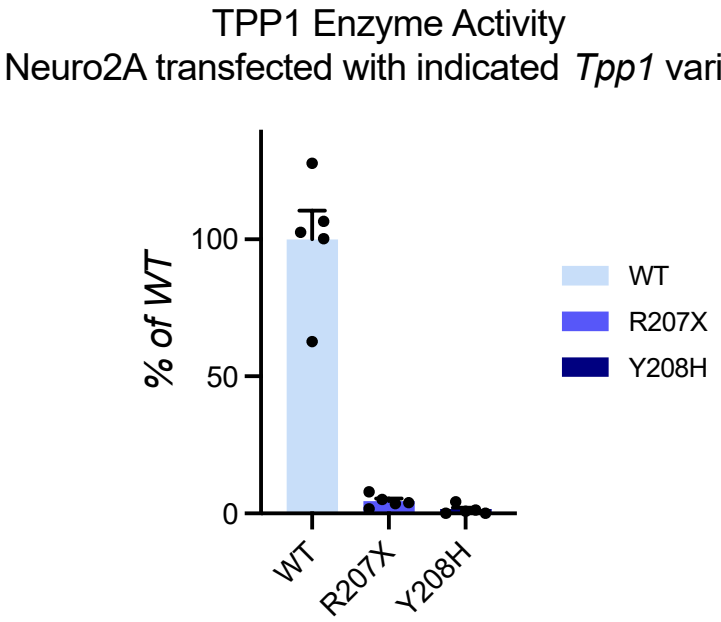


An, Raguram, Du, Newby, Liu, and co-workers.
Nature Biotechnology (2024)

Correcting *CLN2* R207X MEF cells





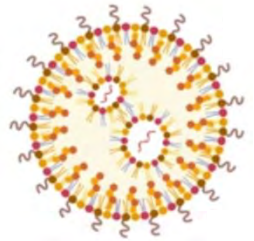


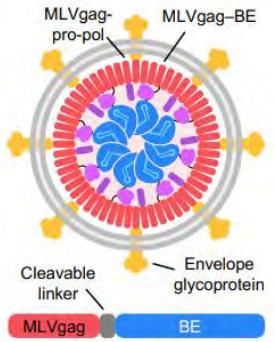
Unpublished.

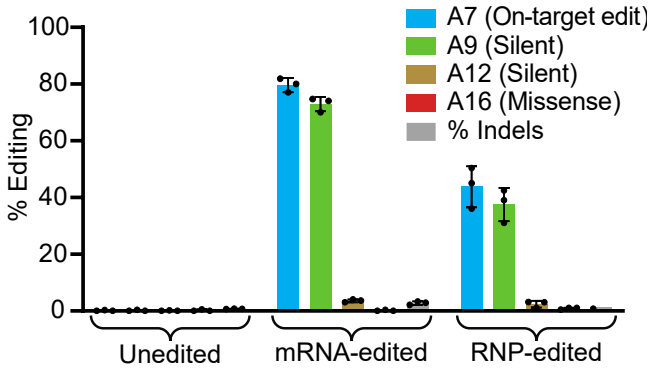
Peyton Randolph
Jill Weimer
David Liu
Melissa Pratt
Joelle Anderson
Mitchell Rechtzigel
Erin Hickey
Steven Ortmeier
Clarissa Booth
Hannah Leppert

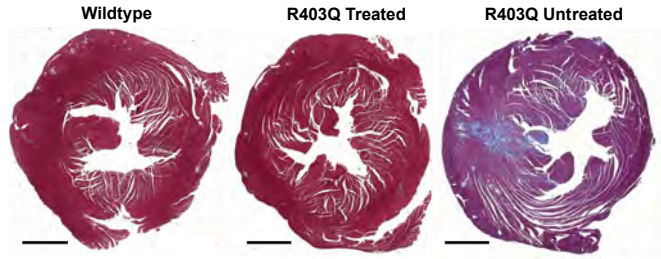
Summary

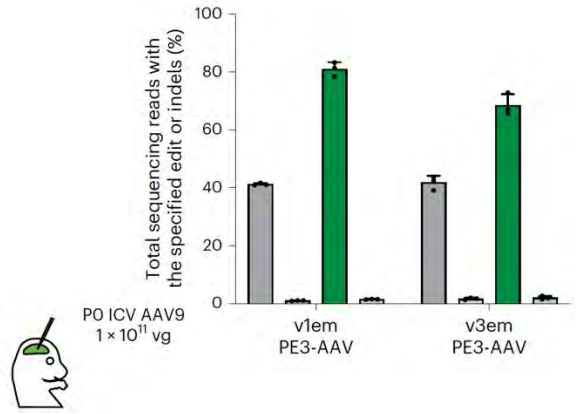
- Base editing and prime editing are compatible with several delivery methods including AAVs, LNPs, and eVLPs to reach mammalian tissues
- Single-dose treatments could treat or prevent genetic disease in the blood, brain, liver, heart, and retina
- These candidate therapies face economic and regulatory challenges. Safely deploying editors to treat neurological conditions in humans has not yet been achieved, but advances in nanoparticle and viral vector technology could permit this in the future.

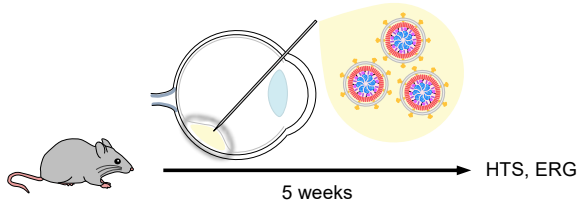












Acknowledgments

Broad Institute of MIT and Harvard:

David Liu
Peyton Randolph
Meirui An
Aditya Raguram
Jessie Davis
Samagya Banskota
Luke Koblan
Andrew Anzalone
Alex Sousa
Colin Hemez
Mandana Arbab
Ben Deverman
Sajeev Kohli
Kelcee Everette

University of Iowa:

Paul McCray
Patrick Sinn
Ashley Cooney

St. Jude Children's Research Hospital:

Mitch Weiss
Shengdar Tsai
Jonathan Yen
Thiyagaraj Mayuranathan
Kaitly Woodard
Cicera Lazzarotto

Harvard Medical School:

Christine Seidman
Jon Seidman
Daniel Reichart
Hiroko Wakimoto
Leslie Gordon

UC Irvine:

Kris Palczewski
Susie Suh
Elliot Choi
Sam Du

Newby Lab:

Emma Burke
Maria Viskadourou
Bradley Yates
Mark Eckley
Johannes Dohr
Jeff Ehmsen
Nivedhitha Devaraju
Noah Workman
Allison Czora
Nick Saba
Patrick Nnoromele
Tyler Lee
Payton Noe
Jonathan Xiao

JHU/KKI:

Ali Fatemi
Christina Mertz
Yousif Almehza
Paul Watkins
Steven Steinberg
Ann Moser
Mike Beer

NIH:

Francis Collins
Michael Erdos
Wayne Cabral
Zheng-Mei Zhong
Urraca Tavarez
Narisu Narisu
Chad Krilow

Vanderbilt:

Jonathan Brown
Sean Doherty
Quanhu Sheng

Sanford Health:

Jill Weimer
Melissa Pratt
Joelle Anderson
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