

X-linked Alport syndrome: From transcriptome diagnosis to preclinical assessment of splice-switching oligonucleotide therapy

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PPU-Imagine PhD fellow (3rd year)

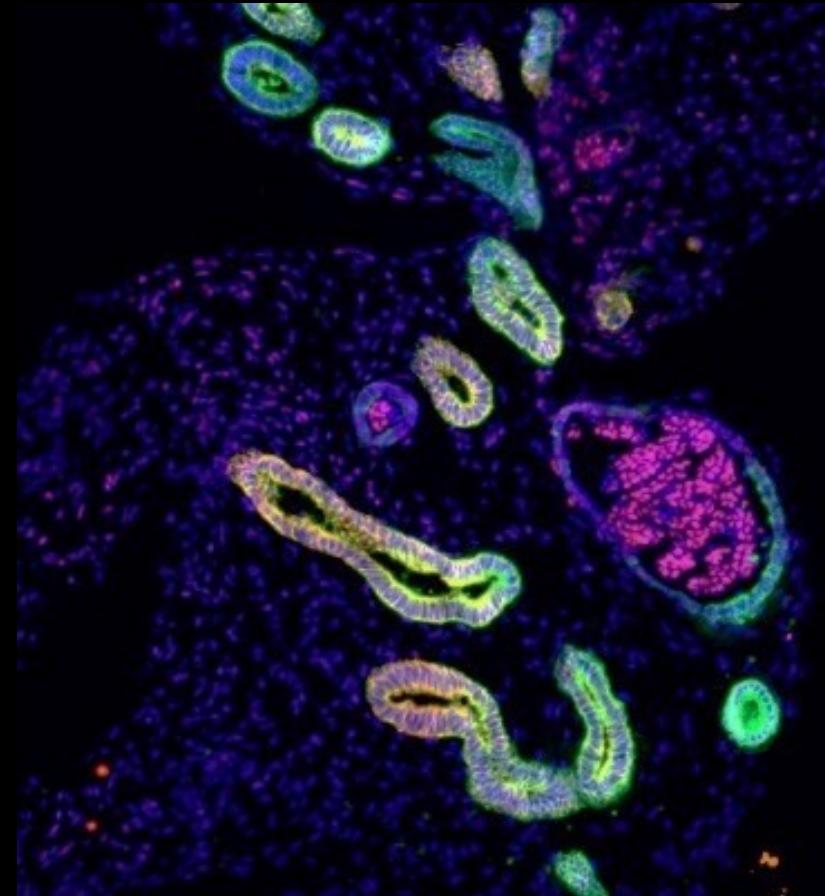
*Laboratory of hereditary kidney diseases
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Imagine Institute*

Supervisors:

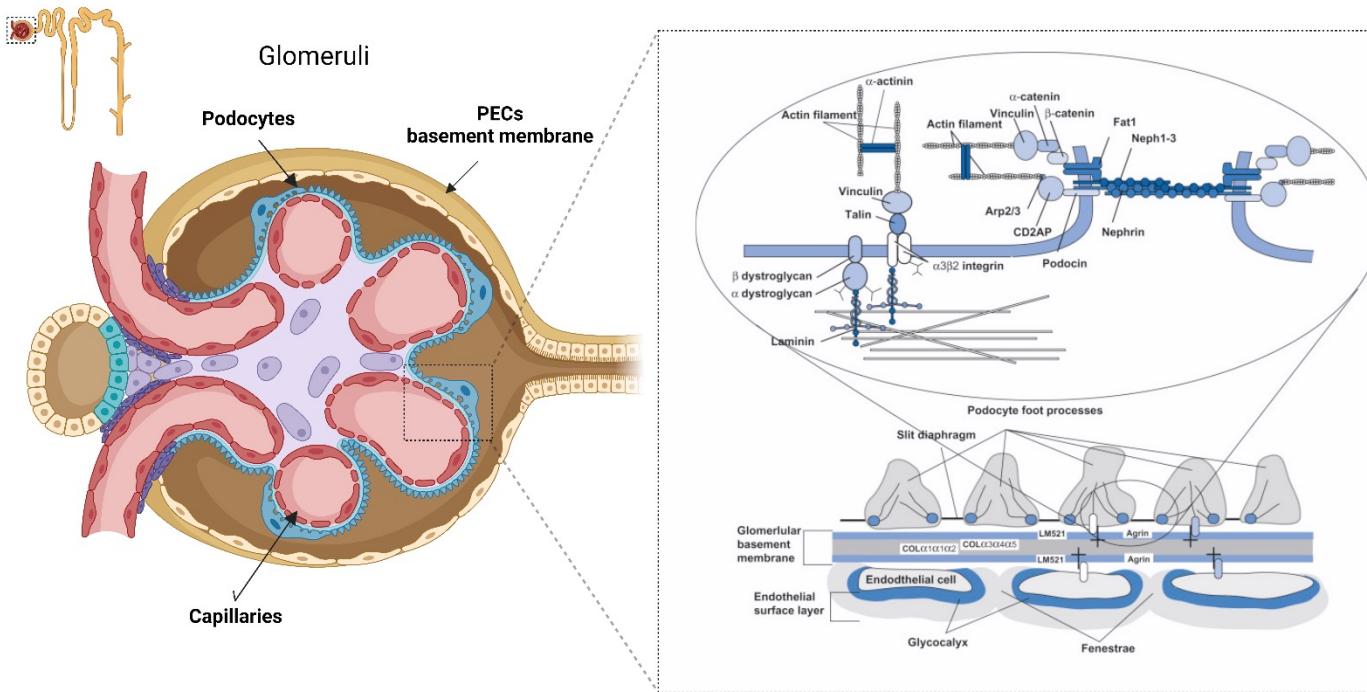
Dr. Géraldine Mollet

Dr. Guillaume Dorval

June 2024



Technical challenges of genetic diagnosis in nephropathies



Missing variants in Alport syndrome?

www.kidney-international.org

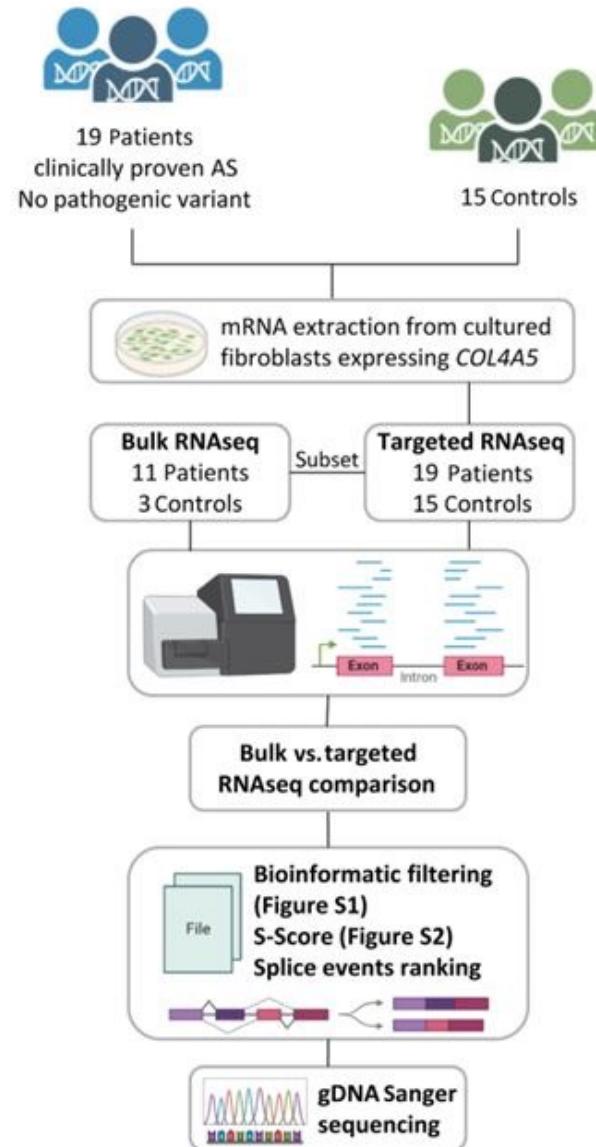
clinical investigation

A wave of deep intronic mutations in X-linked Alport syndrome

Check for updates

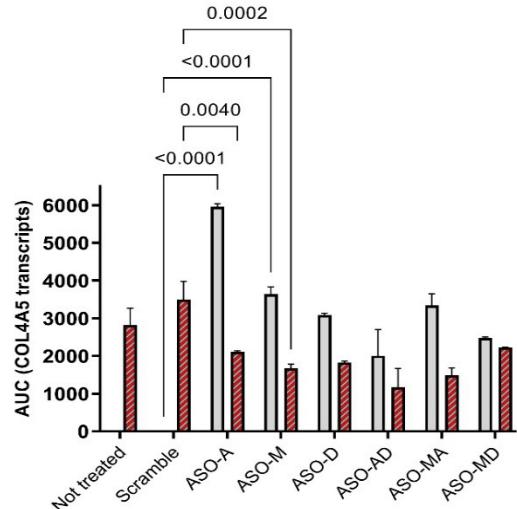
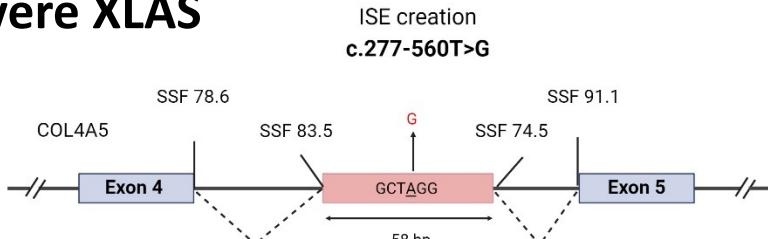
OPEN

Marie Boisson^{1,2}, Christelle Arrondel¹, Nicolas Cagnard³, Vincent Morinière², Zaïna Aït Arkoub², Hassan Saei¹, Laurence Heidet^{1,4}, Jessica Kachmar¹, Aurélie Hummel⁵, Bertrand Knebelmann⁵, Marie-Noëlle Bonnet-Dupeyron⁶, Bertrand Isidor⁷, Hassane Izzedine⁸, Eric Legrand⁹, Philippe Couarch¹⁰, Olivier Gribouval¹, Christine Bole-Feystot¹¹, Mélanie Parisot¹¹, Patrick Nitschke³, Corinne Antignac^{1,2} and Guillaume Dorval^{1,2}

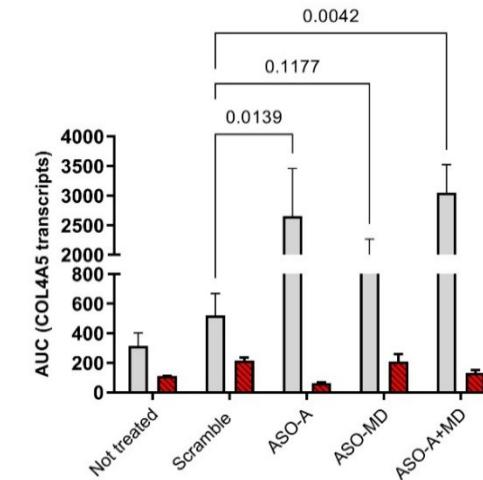
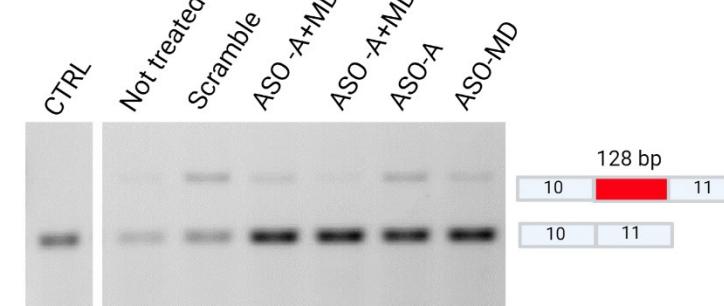
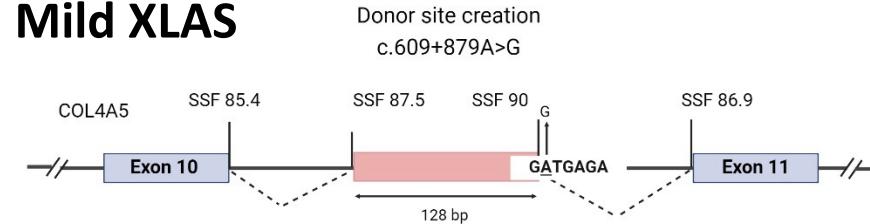


Splicing variants: Intron retention events

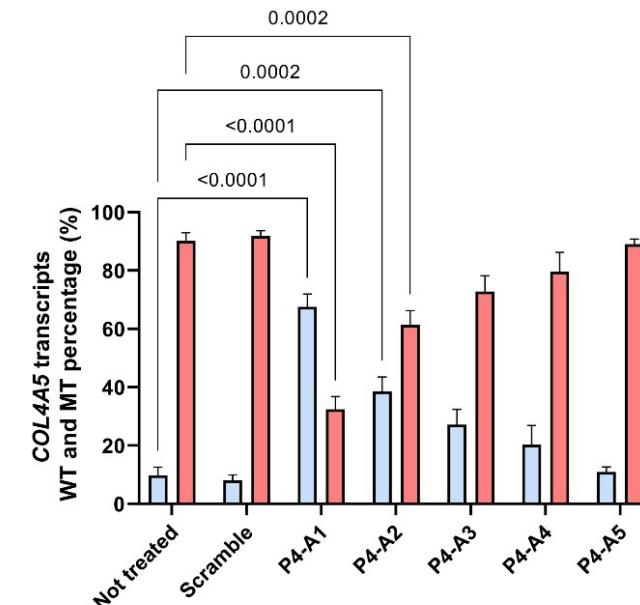
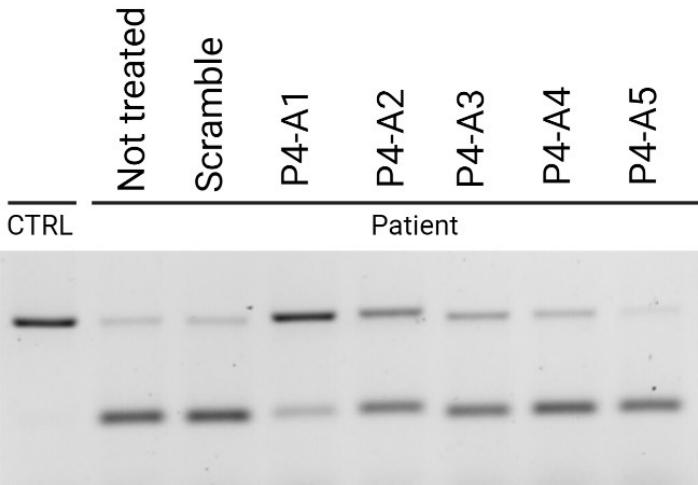
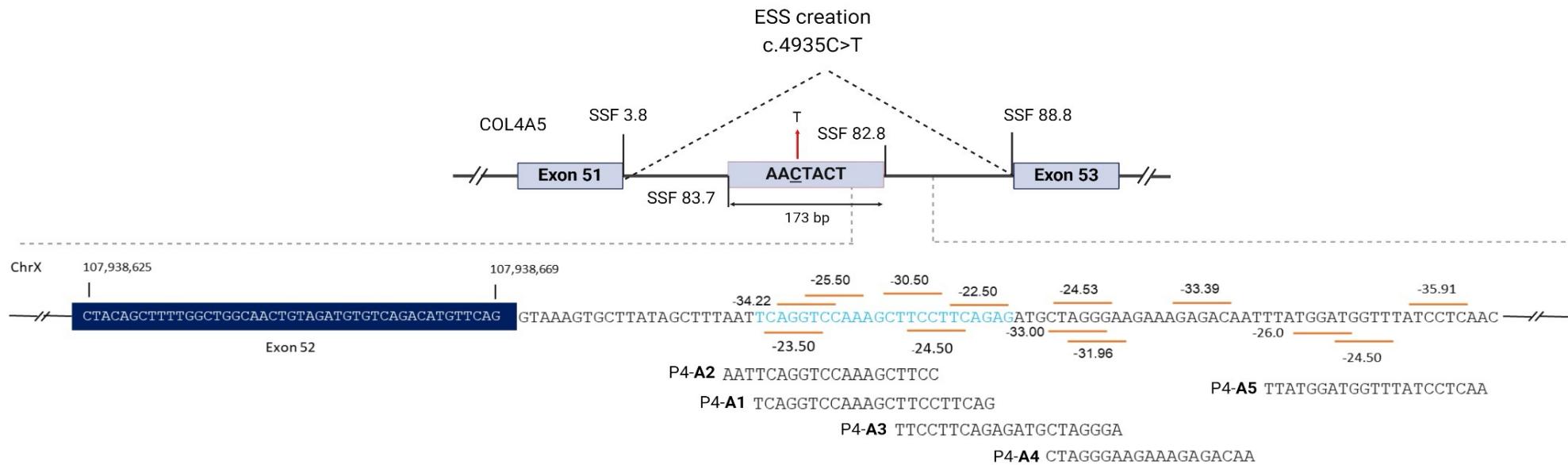
Severe XLAS



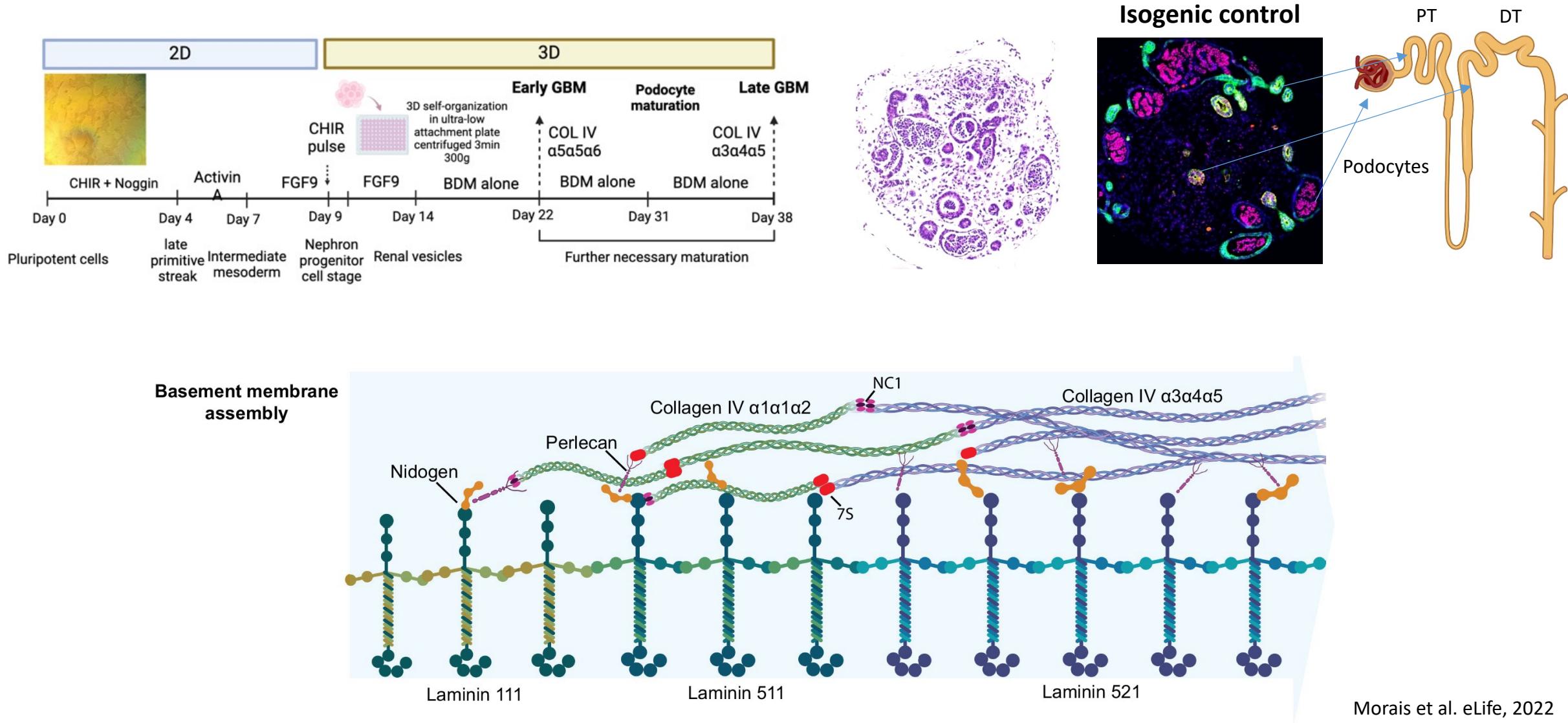
Mild XLAS



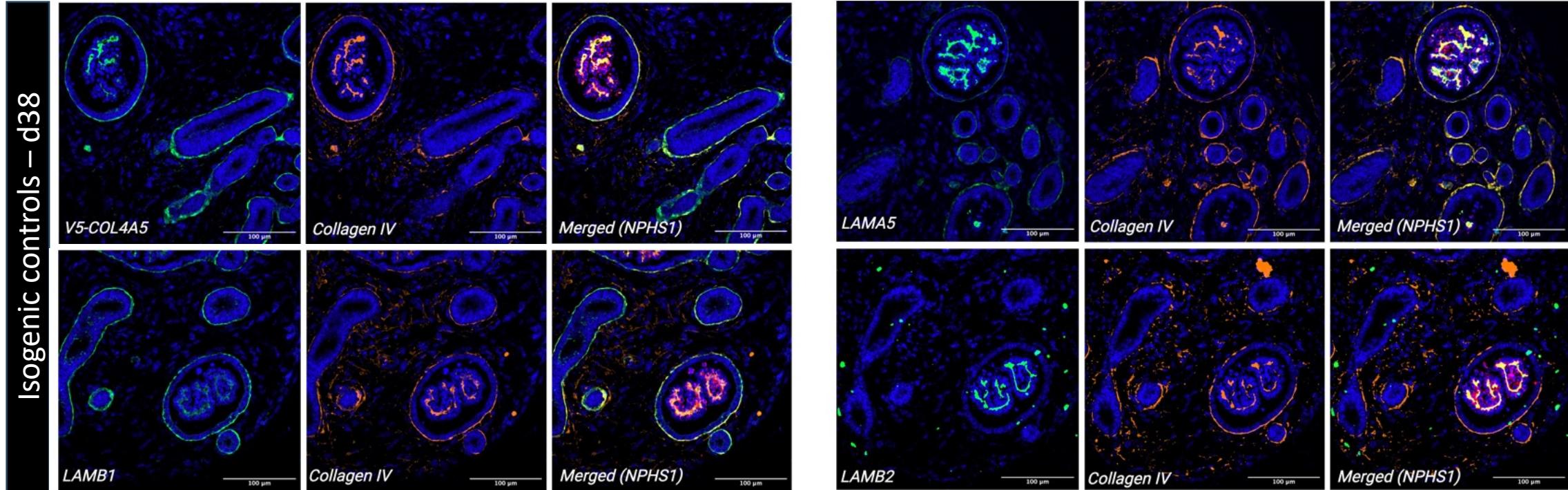
Splicing variants: Exon skipping event



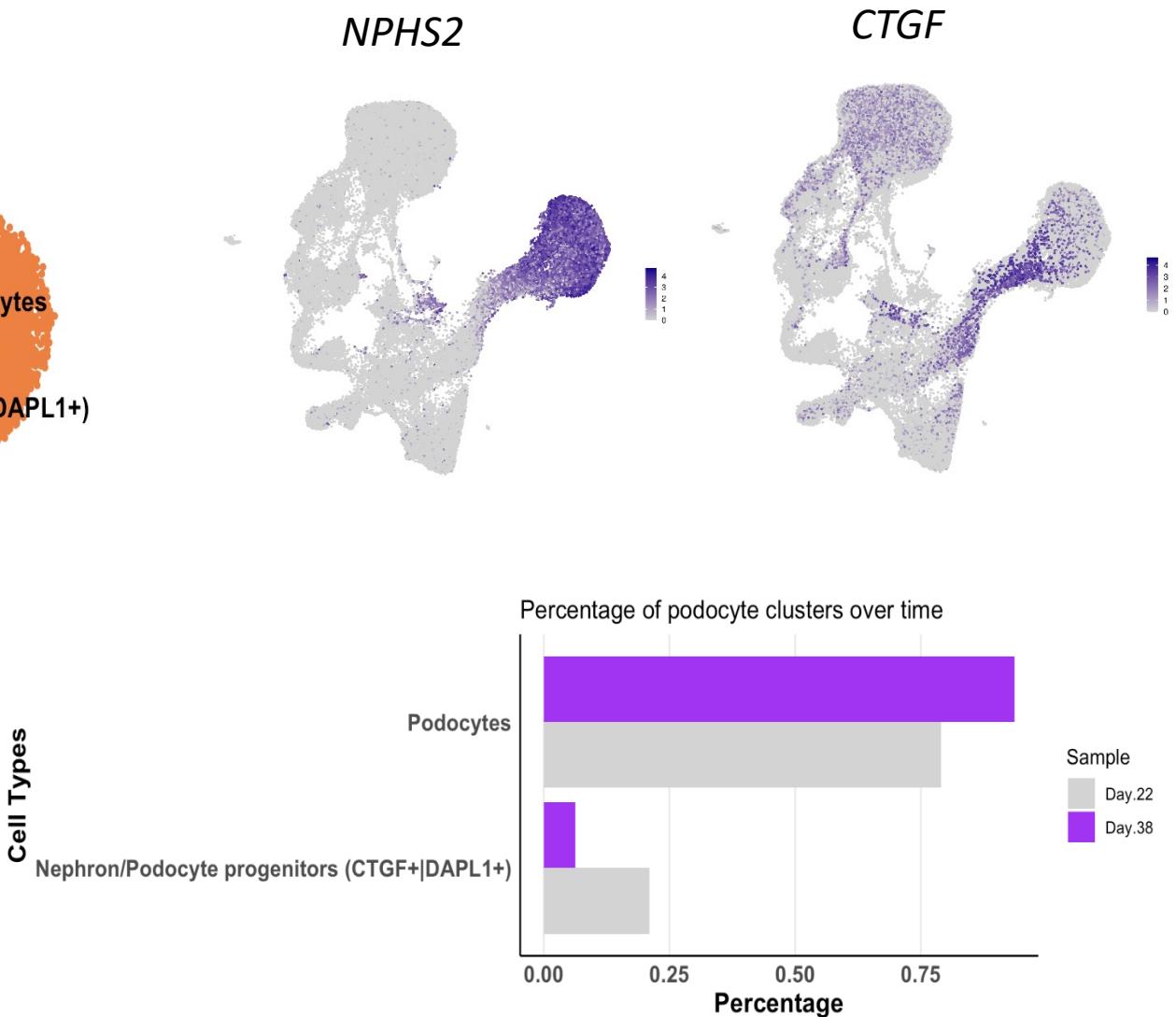
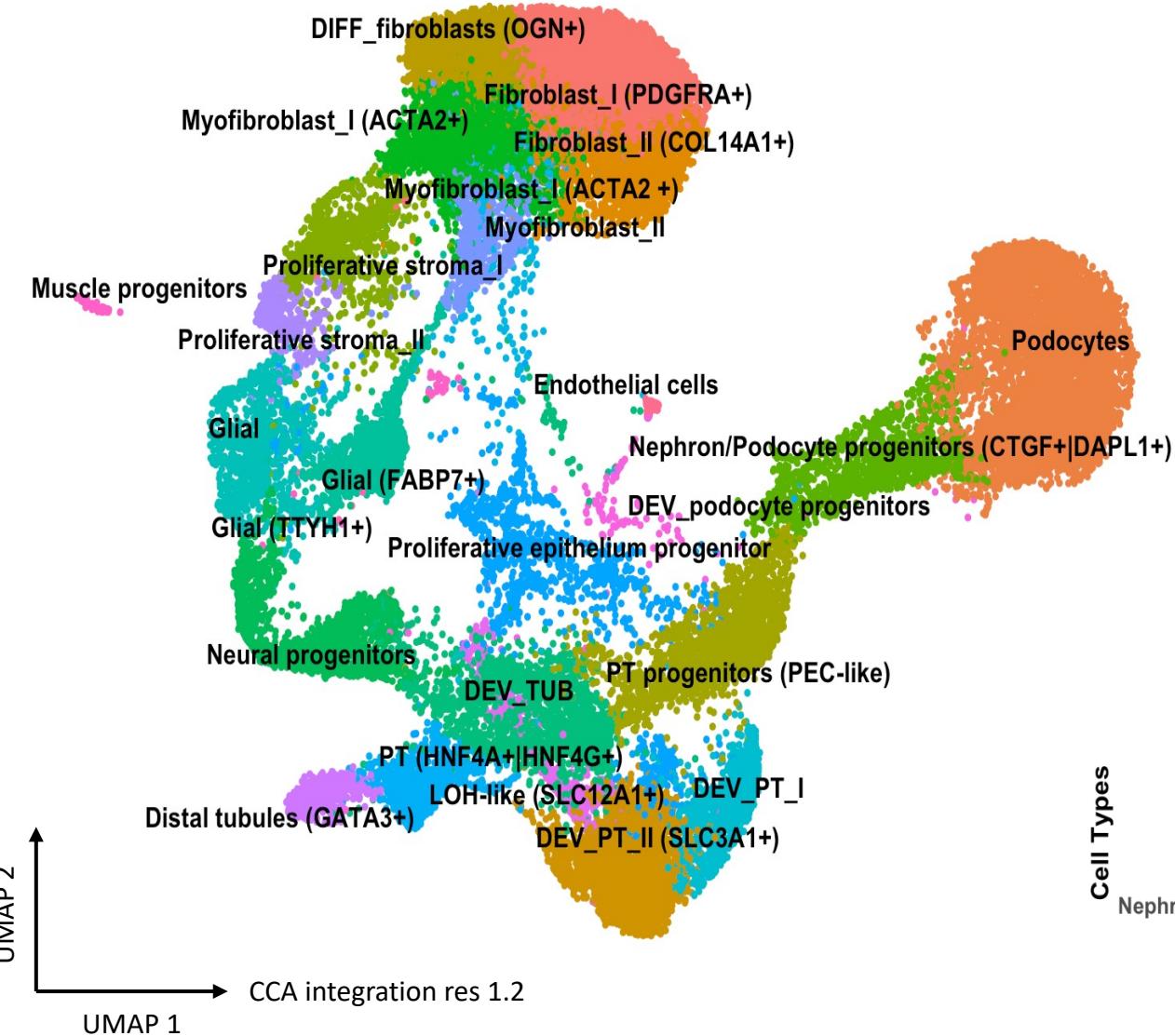
XLAS kidney organoid model development



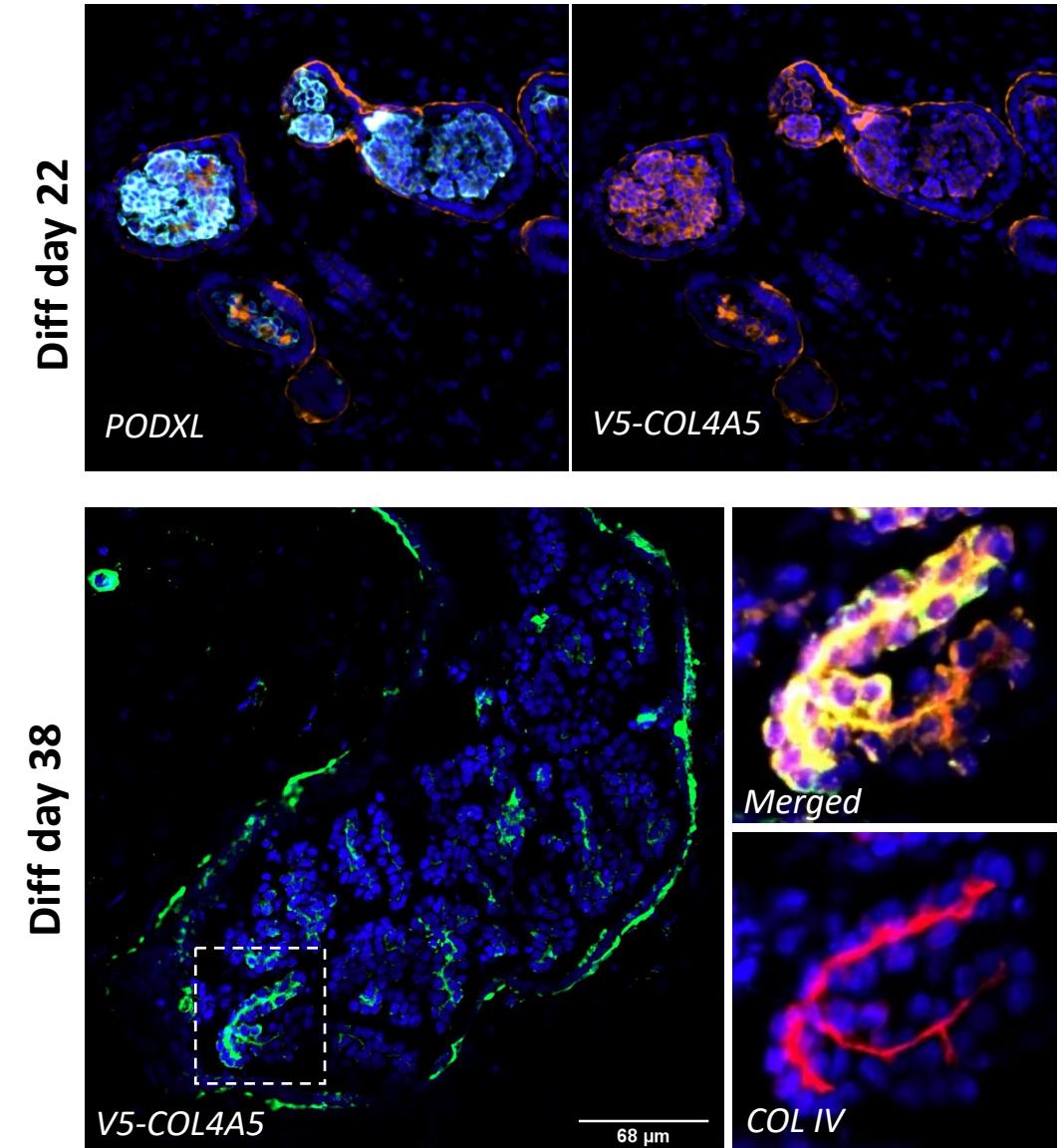
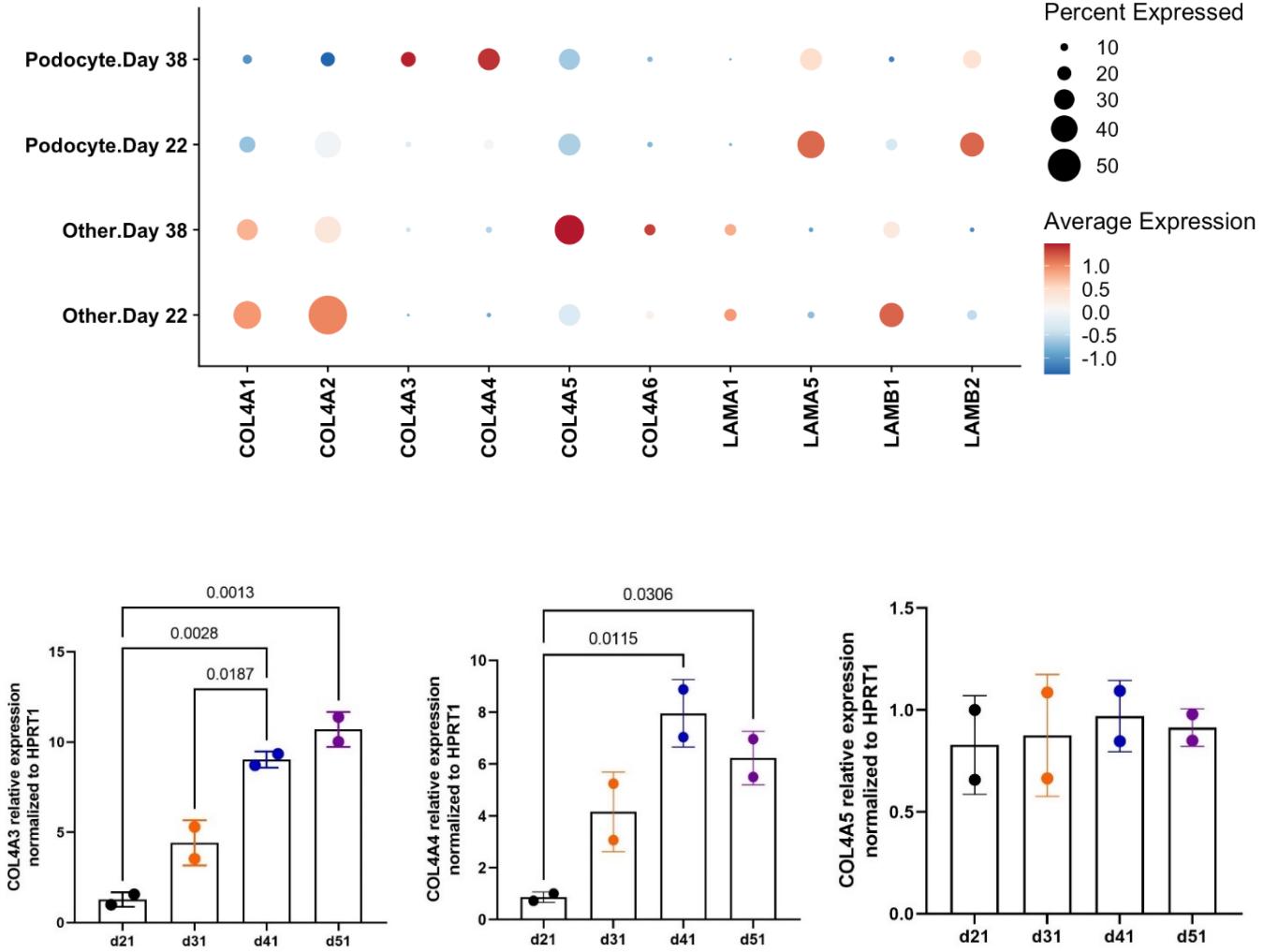
Basement membranes in kidney organoids



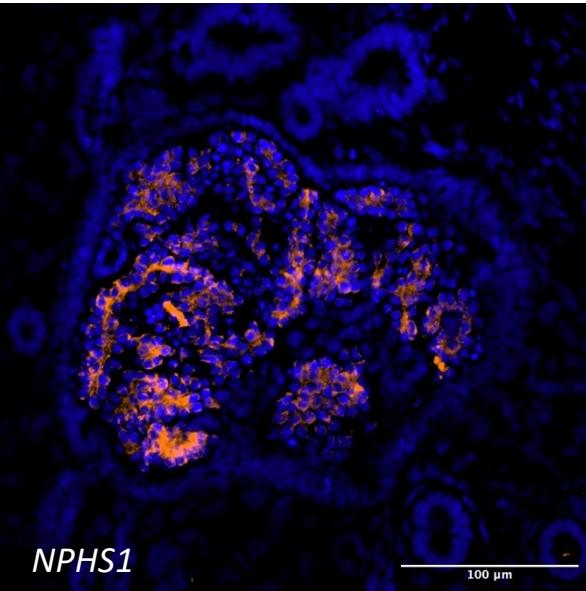
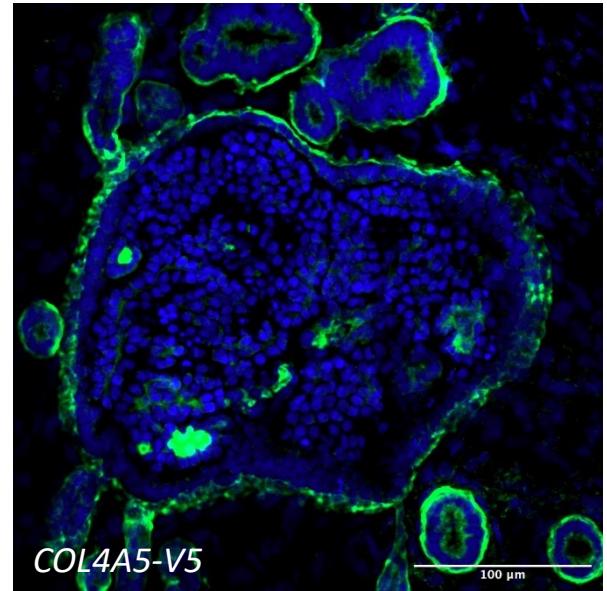
Single cell RNA Sequencing on kidney organoids (Day 22 vs 38)



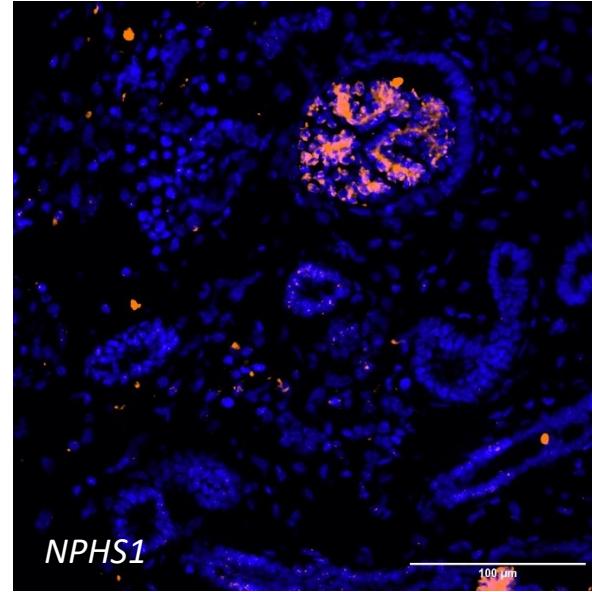
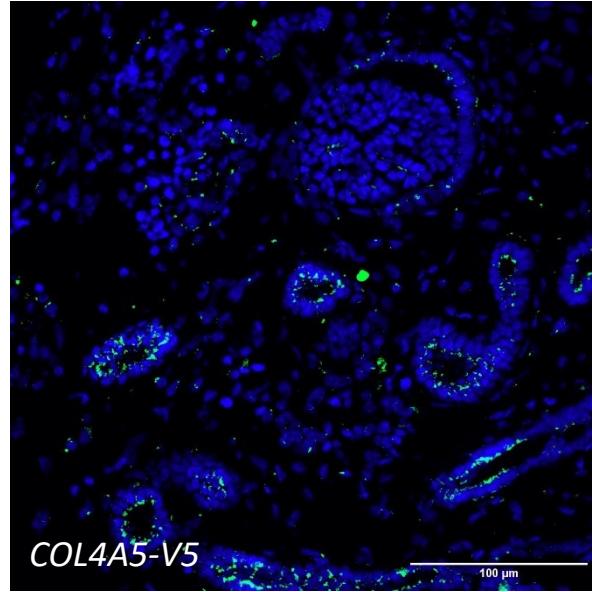
Prolonged organoid culture is necessary for BM maturation



Kidney organoids as a robust XLAS model



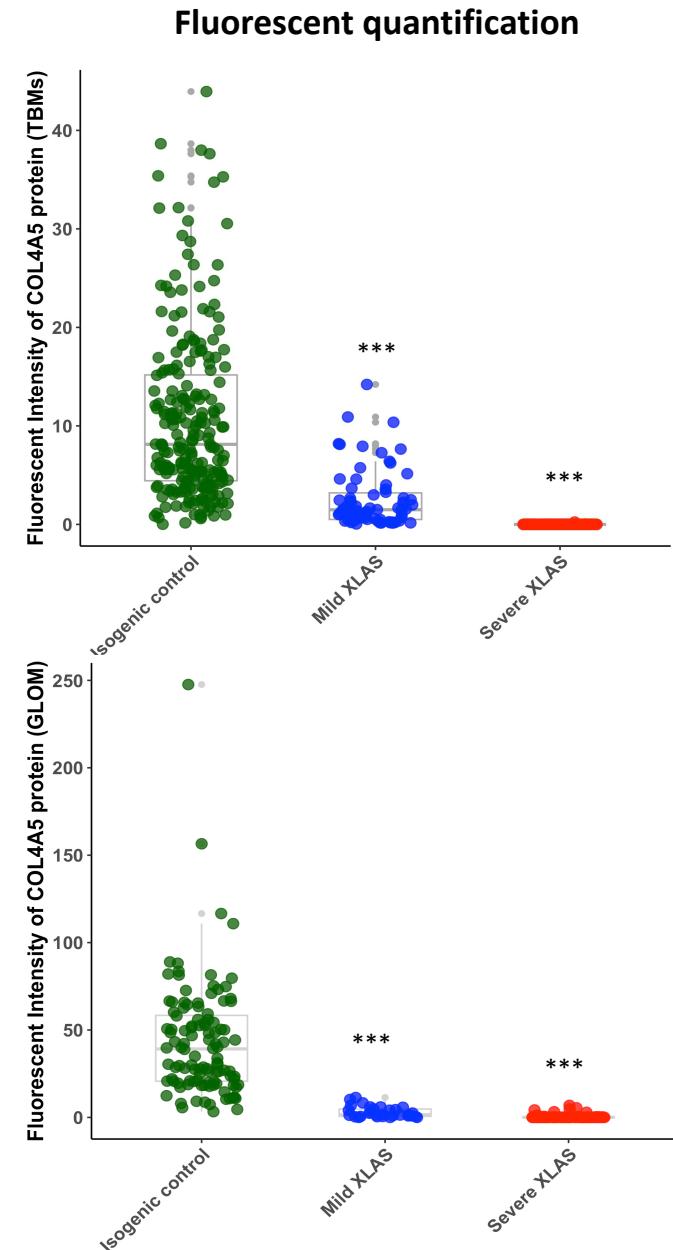
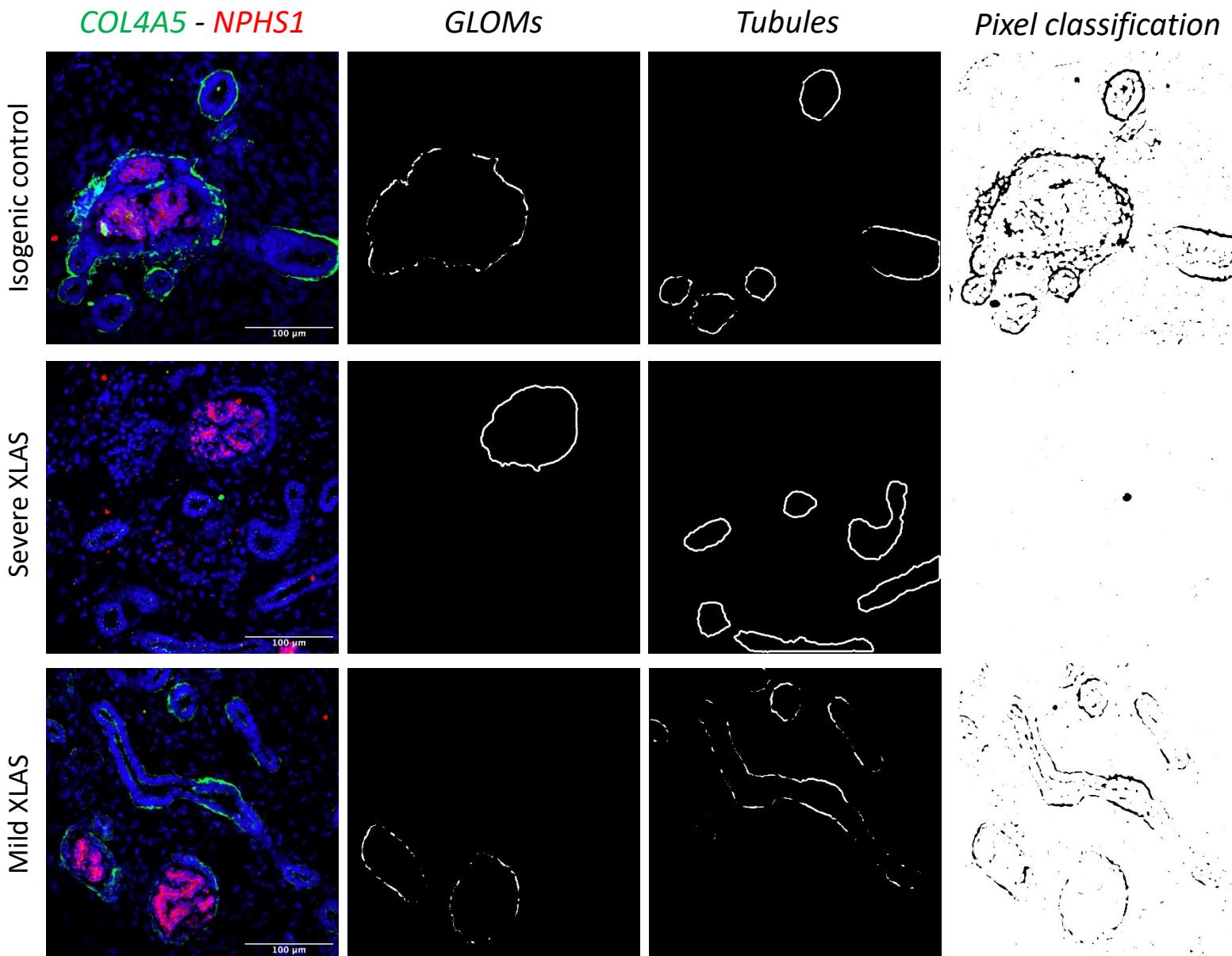
Severe model



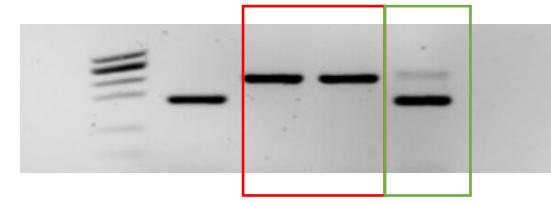
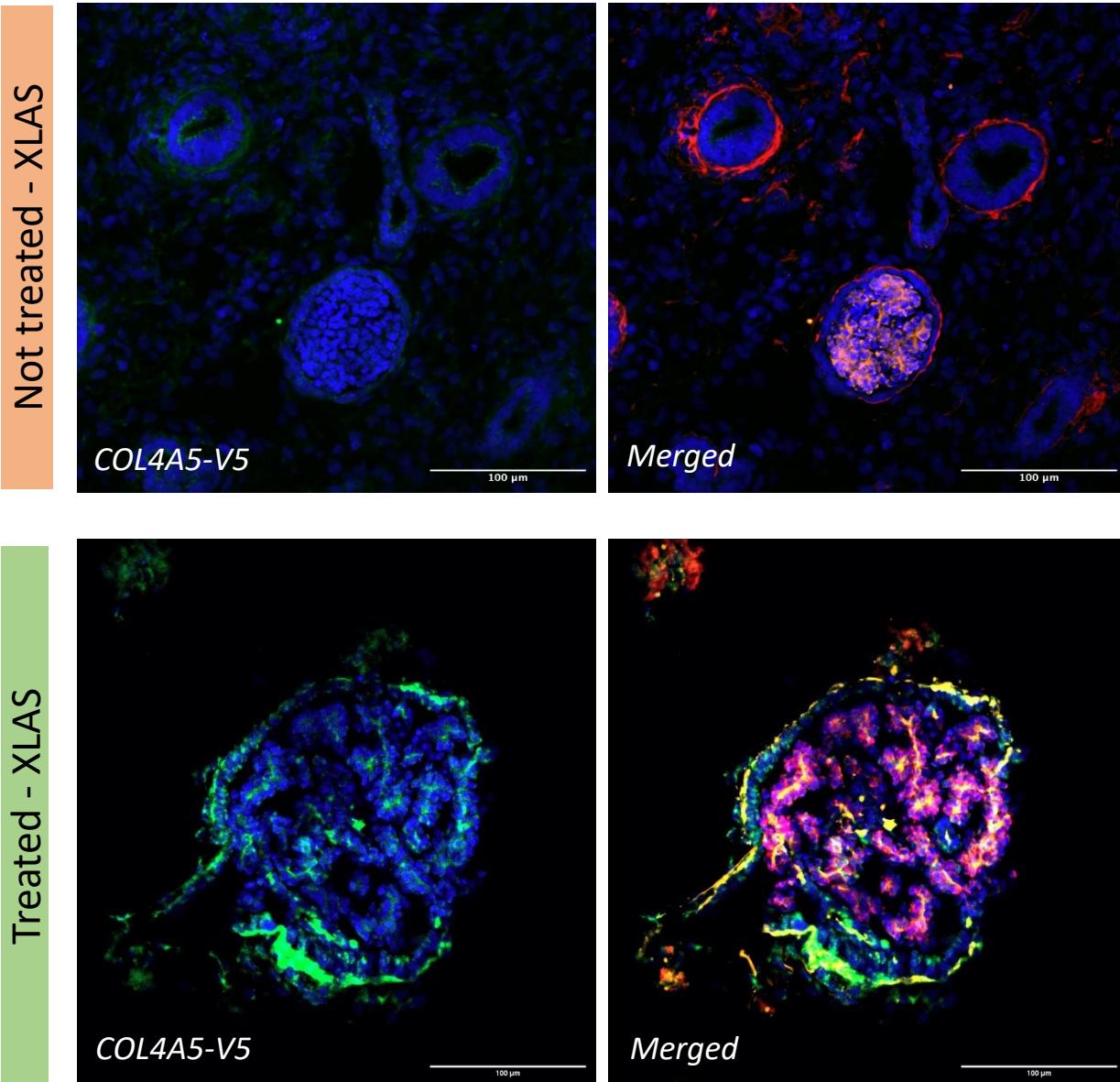
Mild model

- ✓ No COL4A5 protein in the basement membranes in the severe model
- ✓ Signs of mutant protein retention inside cells!

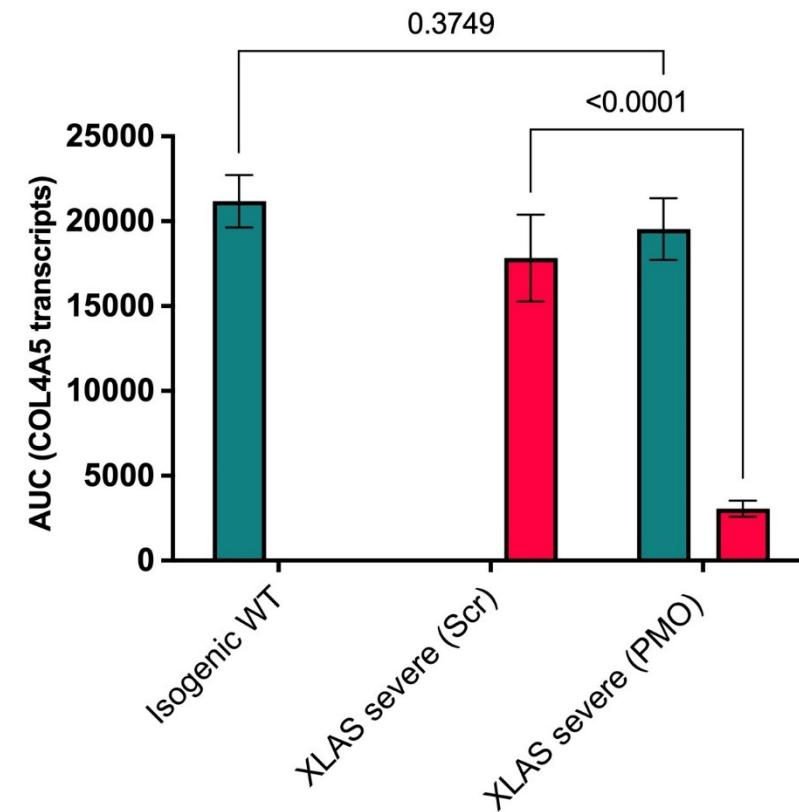
AI-powered macro for Basement membrane quantification



PMO treatment restored COL4A5 protein in the organoid BMs

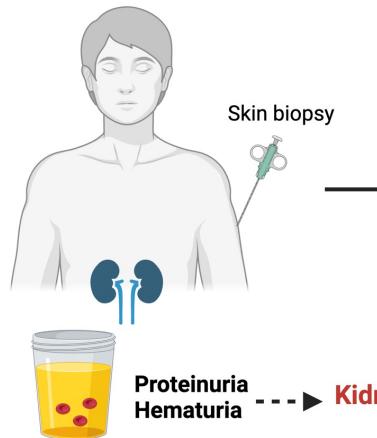


Not treated Treated



Conclusion I

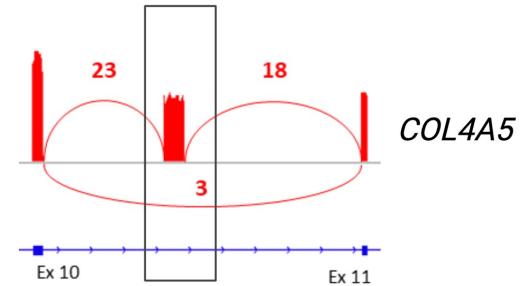
A Patient with X-linked Alport syndrome



Culture skin fibroblasts
Perform Targeted RNAseq
on fibroblast total RNA

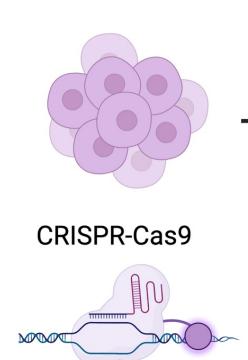
Bioinformatic analysis

Detect missing variants
(Splicing events)



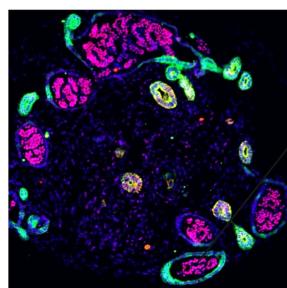
Boisson, M. KI, 2023

B Induce patient mutation in hiPS cells



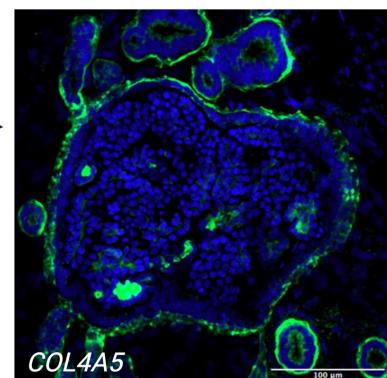
Differentiation
38 days

XLAS Kidney Organoid development

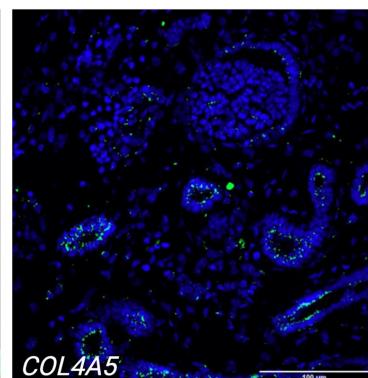


Podocytes (P)
Proximal tubules (PT)
Distal tubules (DT)

Variant characterization and indepth analysis



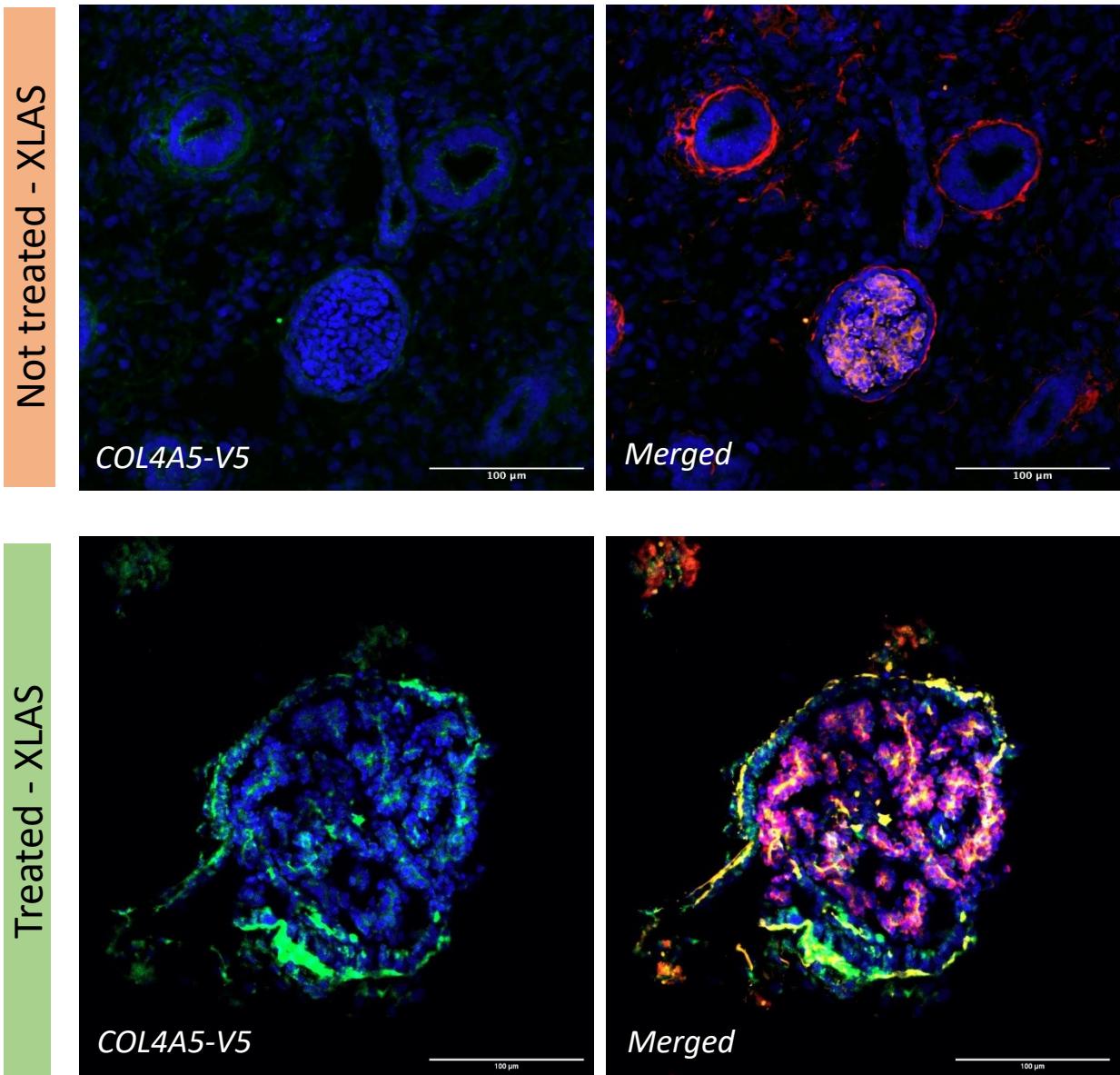
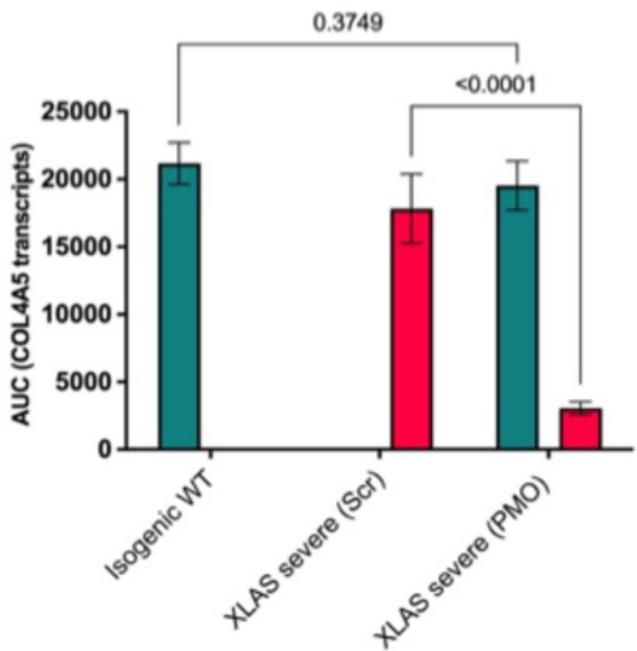
Healthy organoid



Alport organoid

Conclusion II

3D kidney organoids
XLAS model





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ASO

Thank you for your attention!