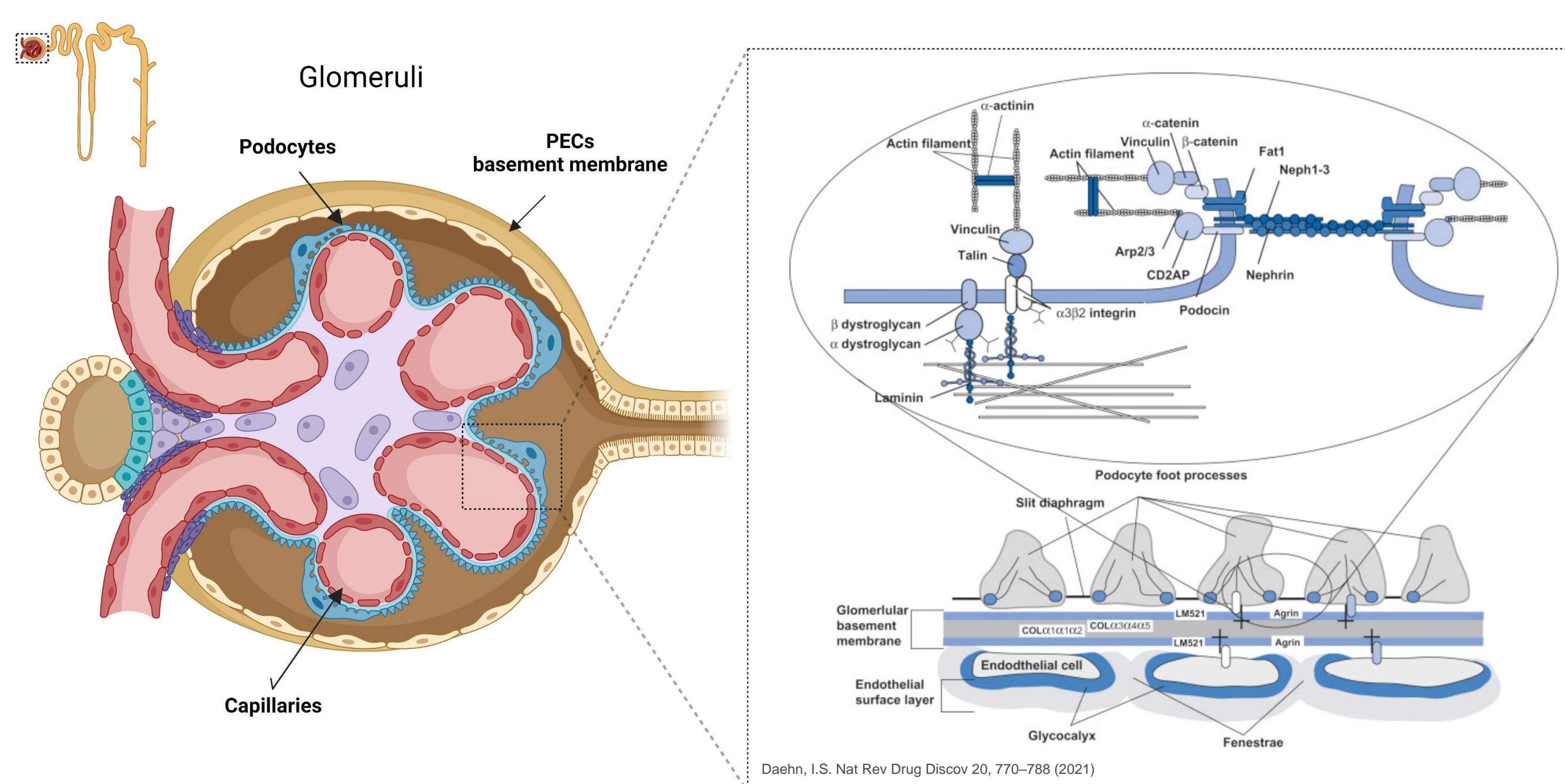


INTRODUCTION AND OBJECTIVE

X-linked Alport syndrome (XLAS) is a hereditary glomerulopathy arising from genetic mutations in the *COL4A5* gene, encoding the $\alpha 5$ chain of the collagen IV [$\alpha 5(\text{IV})$] in the glomerular basement membrane (GBM).

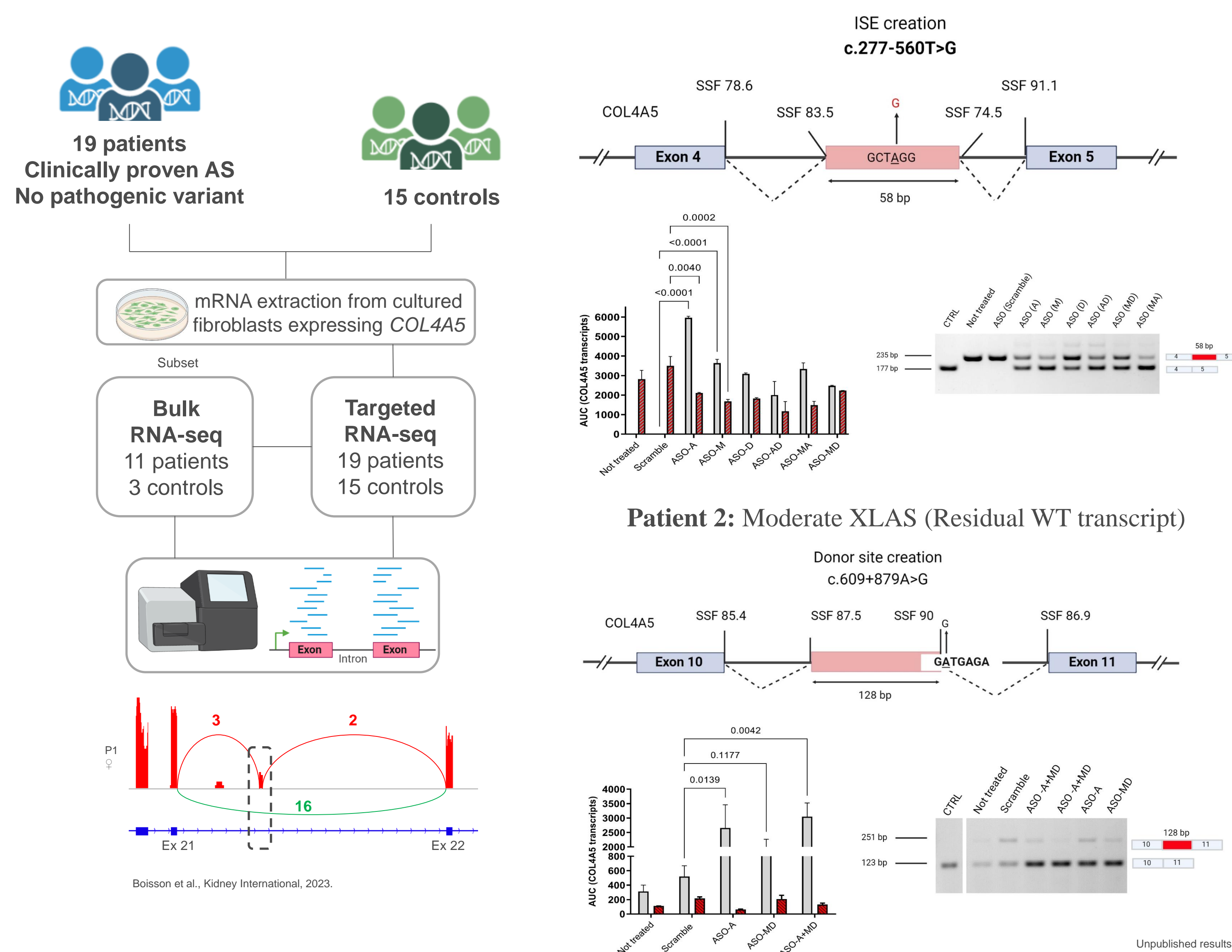
In a study on a cohort of 19 patients with clinically proven XLAS, we identified deep-intronic variants responsible for the aberrant splicing events (17/19) using a targeted RNA sequencing approach.

The objective of this study is to develop a robust *in vitro* model for XLAS to characterize the disease and to test different therapeutic approaches including ASO therapy.

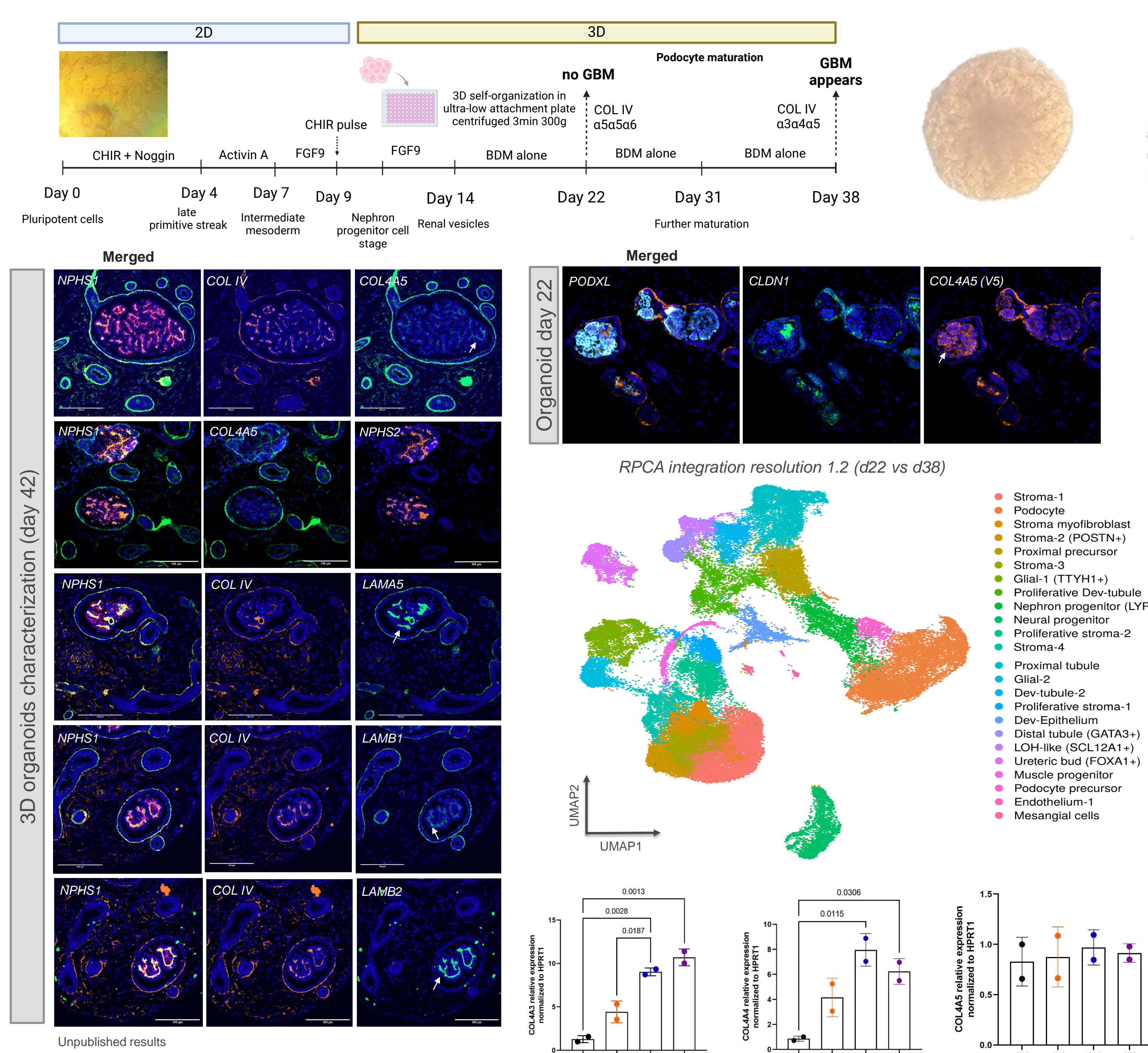


GENETIC DIAGNOSIS – SPLICING VARIANTS

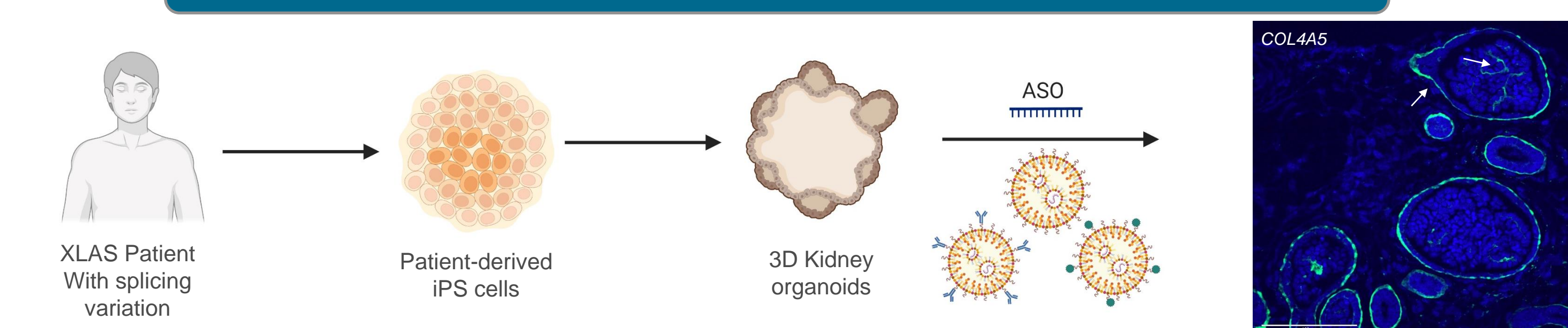
Patient 1: Severe XLAS (No WT transcript)



KIDNEY ORGANOID MODEL (XLAS)



ORGANOID MODEL FOR THERAPY DEVELOPMENT



- We are optimizing the antisense-oligo (ASO) treatment in the kidney organoid model to restore splicing.
- There are still some challenges regarding drug delivery in *in vitro* models due to the lack of proper vascularization. However, if the ASO could penetrate and restore splicing back to normal with promising efficiency and minimal side effects, we can expedite the transition to clinical trials.

HIGHLIGHTS AND CONCLUSION

- We improved the genetic diagnosis of patients with XLAS with targeted RNA sequencing on patient-derived fibroblasts. Different independent patients have identified with the same intronic variation, which supports the importance of analyzing intronic sequences in the *COL4A5* gene.
- We developed the XLAS organoid model for variant characterization and testing different therapeutic approaches including ASO therapy.
- Single-cell RNA sequencing identified different cell types and validated kidney organoids as a robust in vitro model, despite not being fully mature.
- Single-cell RNA sequencing on 3D organoids at two time points (d22 vs d38) confirmed podocyte maturation and the need for prolonged culture to detect “mature” glomerular basement membrane.