



# ADTKD-*UMOD*: New Therapeutic Approaches

*Prof. Dr. med. Olivier Devuyst*

# Autosomal dominant tubulointerstitial kidney disease

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PRIMER

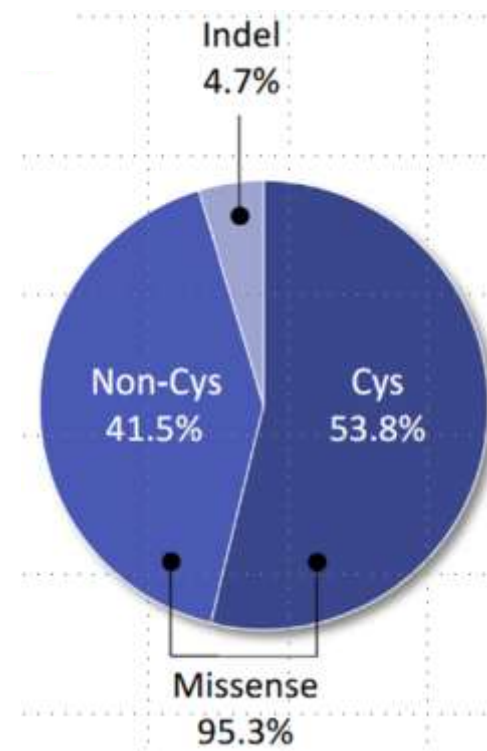
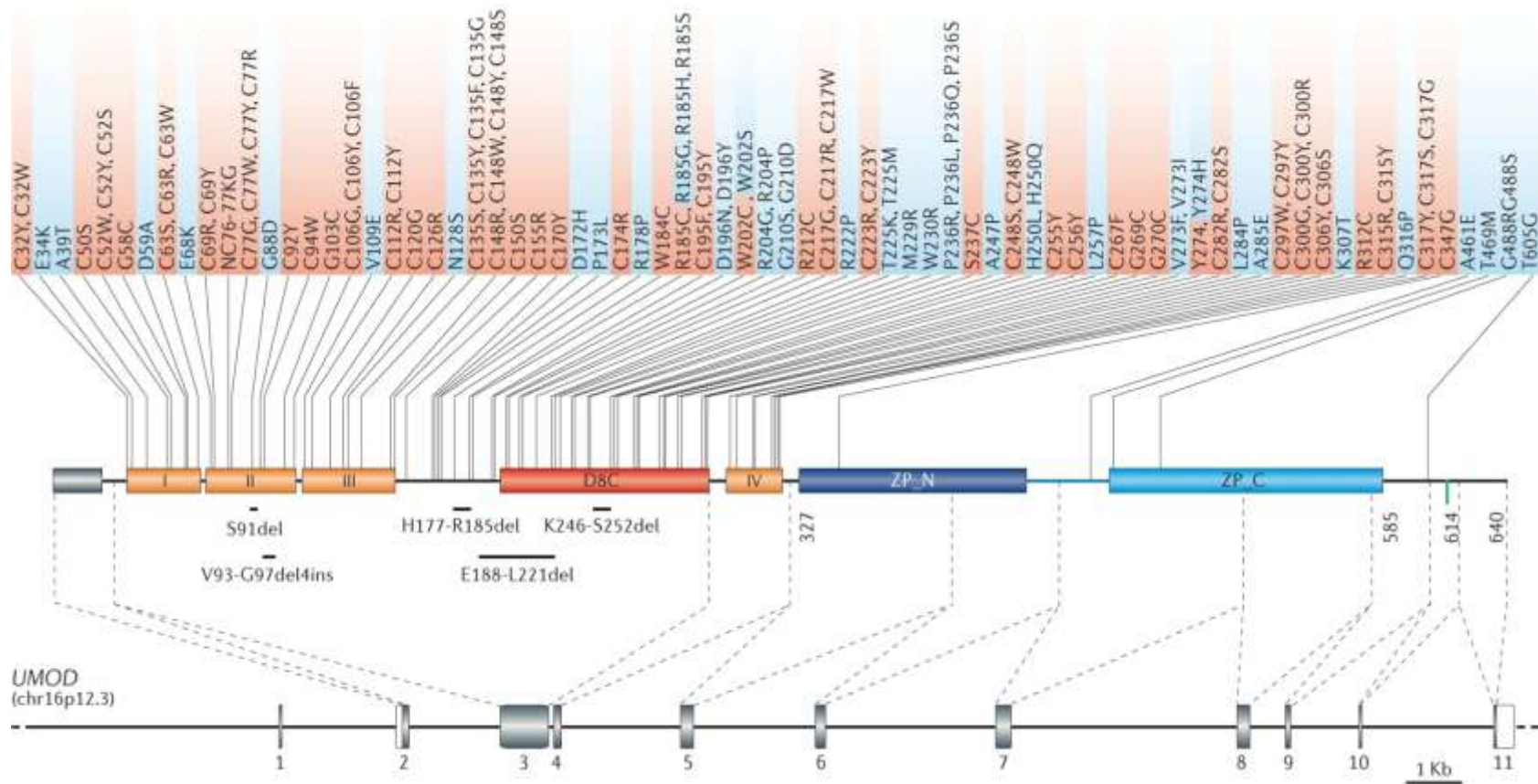
## ADTKD-Gene: Replacing FJHN, MCKD

Affected Gene (OMIM ID; chromosome)	Terminology <sup>a</sup>	Protein	Expression (distribution)	Protein function(s)
<i>UMOD</i> (*191845; 16q12)	ADTKD- <i>UMOD</i>	Uromodulin	Kidney (TAL and DCT segments)	<ul style="list-style-type: none"> <li>Regulates transport, blood pressure and urinary concentration</li> <li>Protection against kidney stones</li> <li>Protection against urinary tract infections</li> <li>Regulation of innate immunity</li> </ul>
<i>MUC1</i> (*158340; 1q22)	ADTKD- <i>MUC1</i>	Mucin 1	Secretory epithelia (for example, lungs, stomach, intestine and kidney)	<ul style="list-style-type: none"> <li>Protection of epithelial mucus barrier</li> <li>Immunomodulatory properties</li> <li>Signal transduction</li> </ul>
<i>HNF1B</i> (*189907; 17q12)	ADTKD- <i>HNF1B</i>	Hepatocyte nuclear factor 1B	Kidney, pancreas, liver, lung, intestine and urogenital tract	<ul style="list-style-type: none"> <li>Transcription factor involved in the (early) development of neural tube, pancreas, gut, liver, lung, kidney and</li> </ul>
<i>SEC61A1</i> (*609213; 3q21.3)	ADTKD- <i>SEC61A1</i>	$\alpha 1$ subunit of SEC61	Ubiquitous	<ul style="list-style-type: none"> <li>Component of SEC61 channel-forming translocon complex that mediates transport of signal peptide-containing precursor polypeptides across the ER</li> </ul>

1% of patients with CKD stages 3–5 and 2% of patients with ESRD had ADTKD- *UMOD*

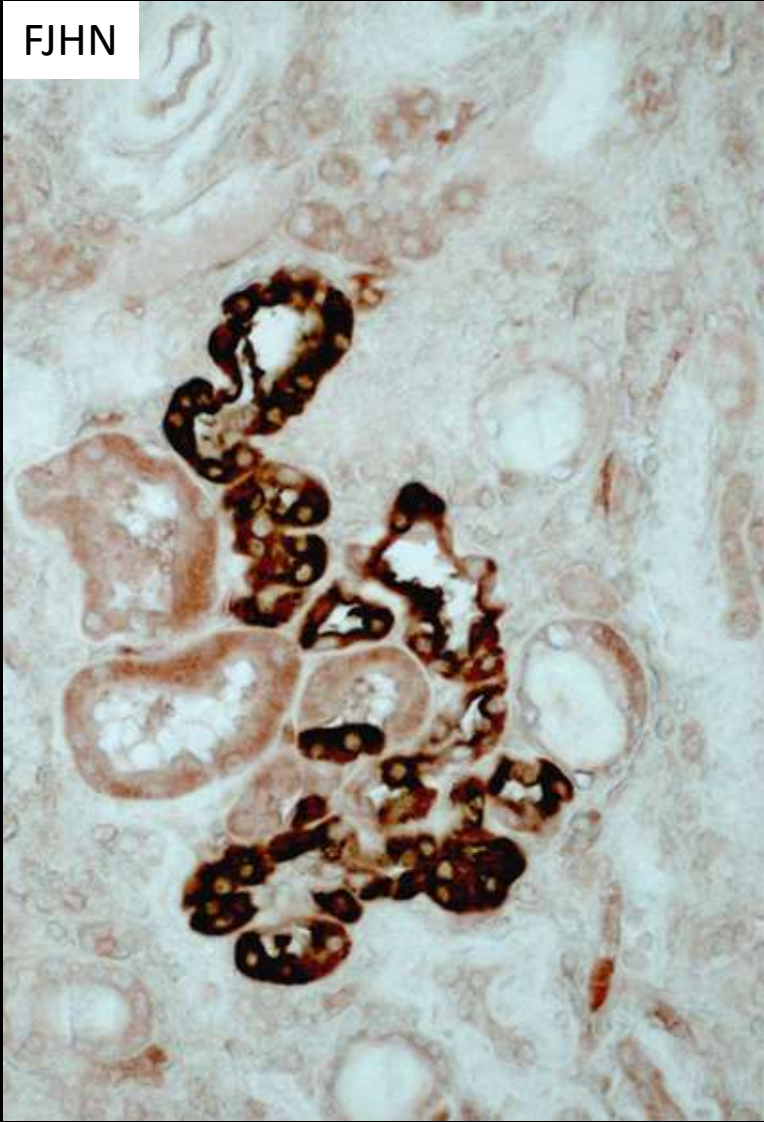
→ **Most common monogenic kidney disease** after collagen IV mutations and ADPKD

# Landscape of *UMOD* Mutations in ADTKD

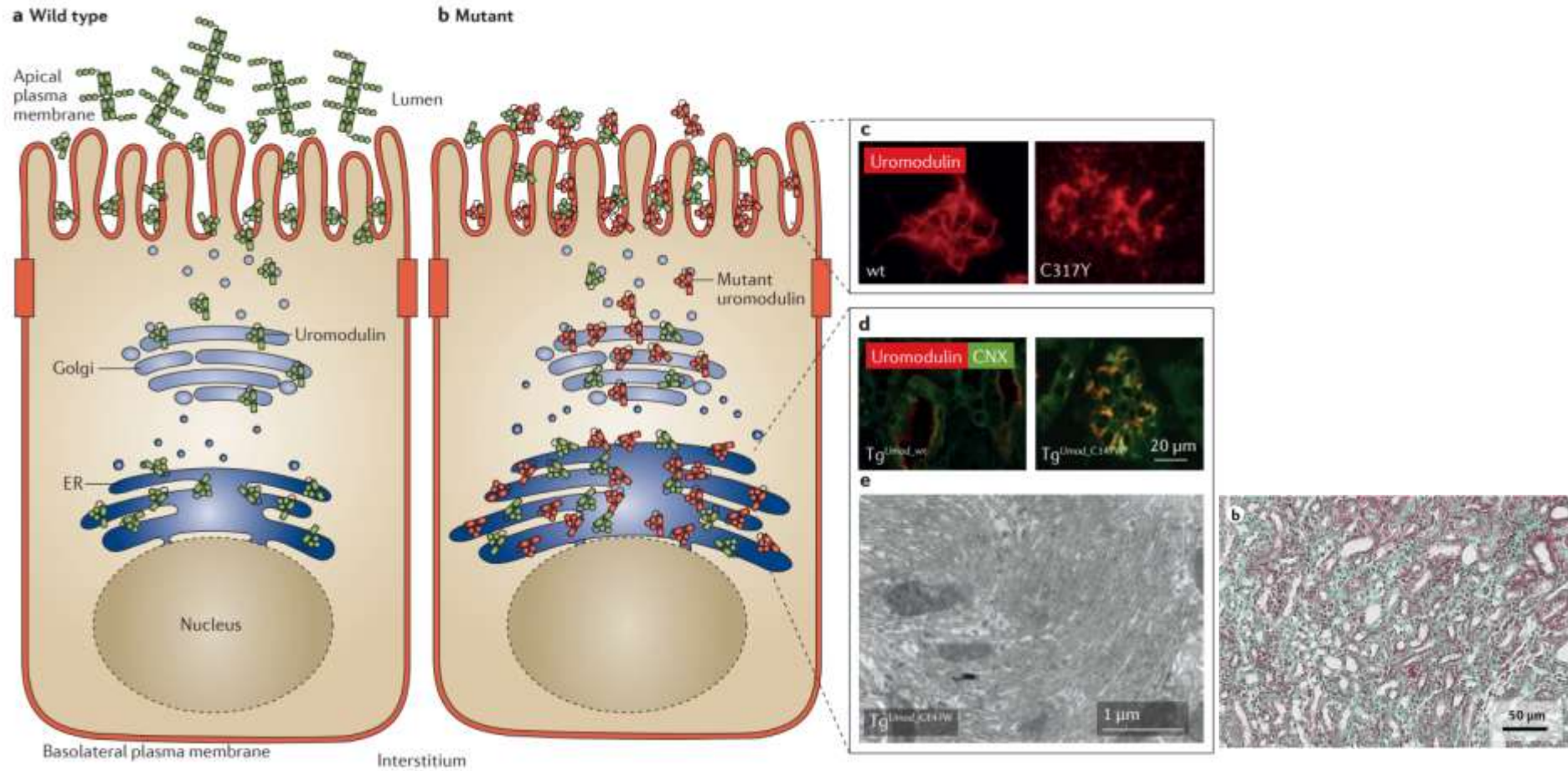


→ >150 mutations, 95% cluster in exons 3 and 4  
 → 96% missense mutations, 4 in-frame deletions  
 → Cysteine residues (>50%)

# Accumulation of Mutant Uromodulin in Kidney Tubules: Storage Disease



# ADTKD-UMOD – Typical Proteinopathy

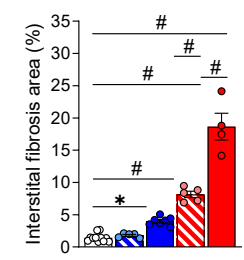
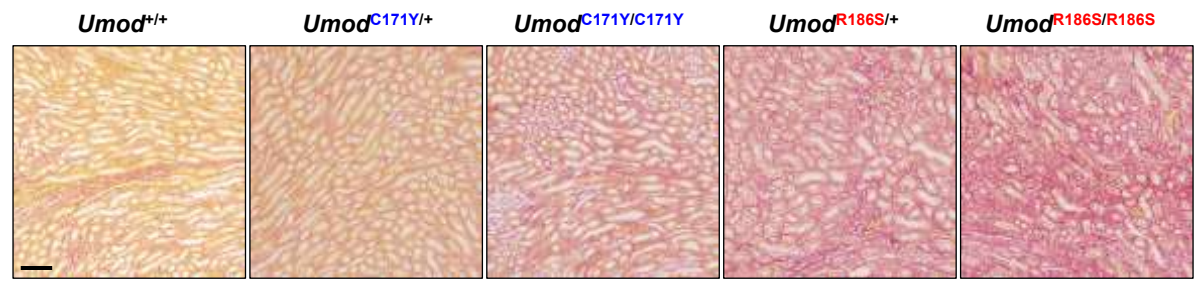
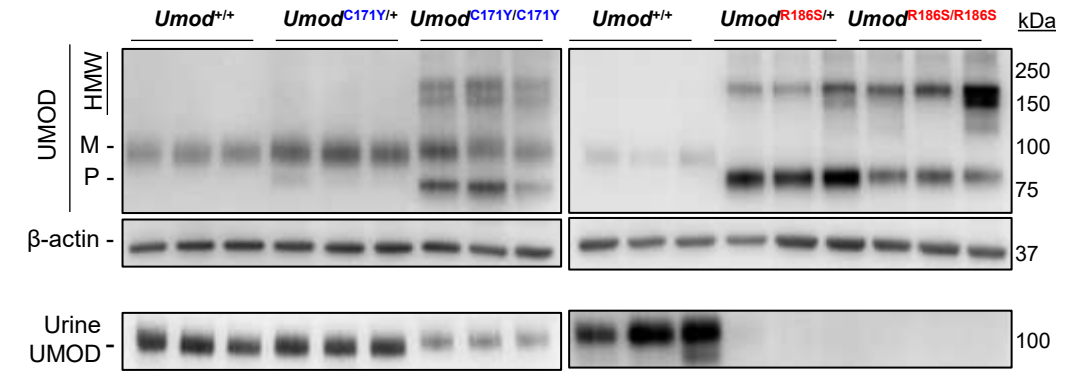
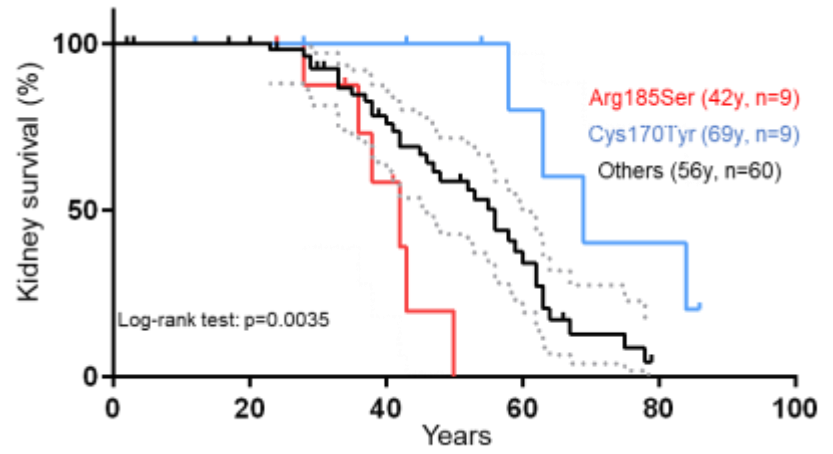
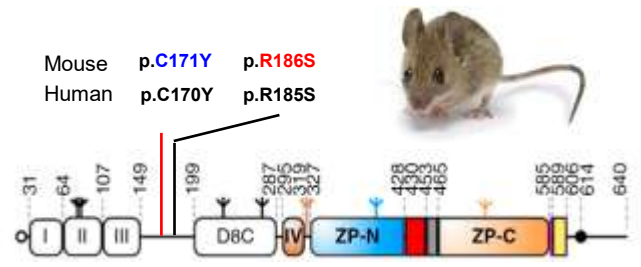


- Mutant uromodulin is misfolded and accumulates in the ER – Toxic aggregates - ER stress
- **Kidney damage: infiltrate – fibrosis – CKD progression**

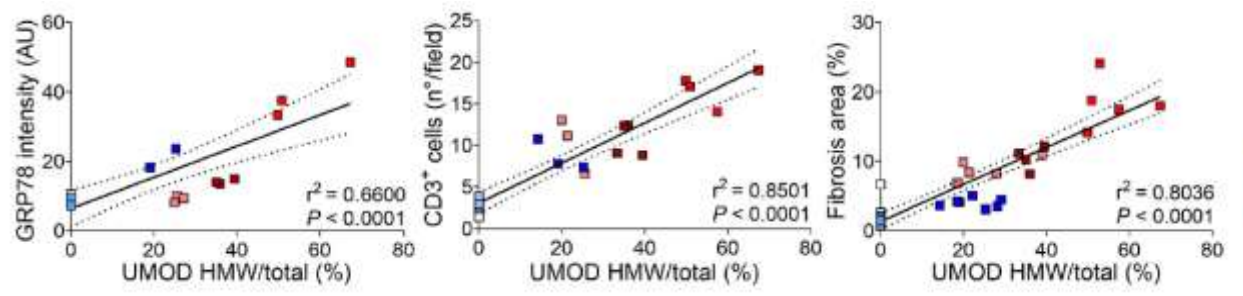
## ADTKD-*UMOD*: What's new - Therapeutic perspectives ?

- Variable disease progression: Allelic effects
- Toxic aggregates drive the disease
- Protective role of wild-type uromodulin
- Therapeutic strategies: now & tomorrow

# ADTKD-UMOD: Allelic and Gene Dosage Effects

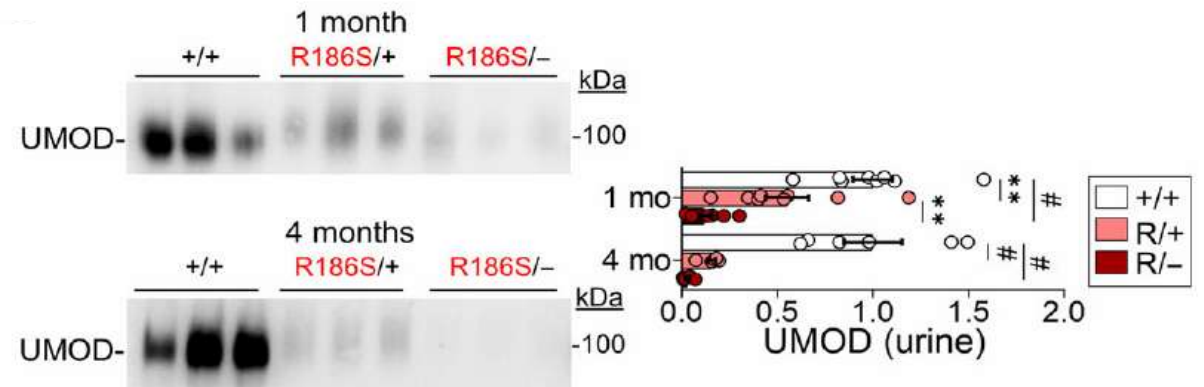
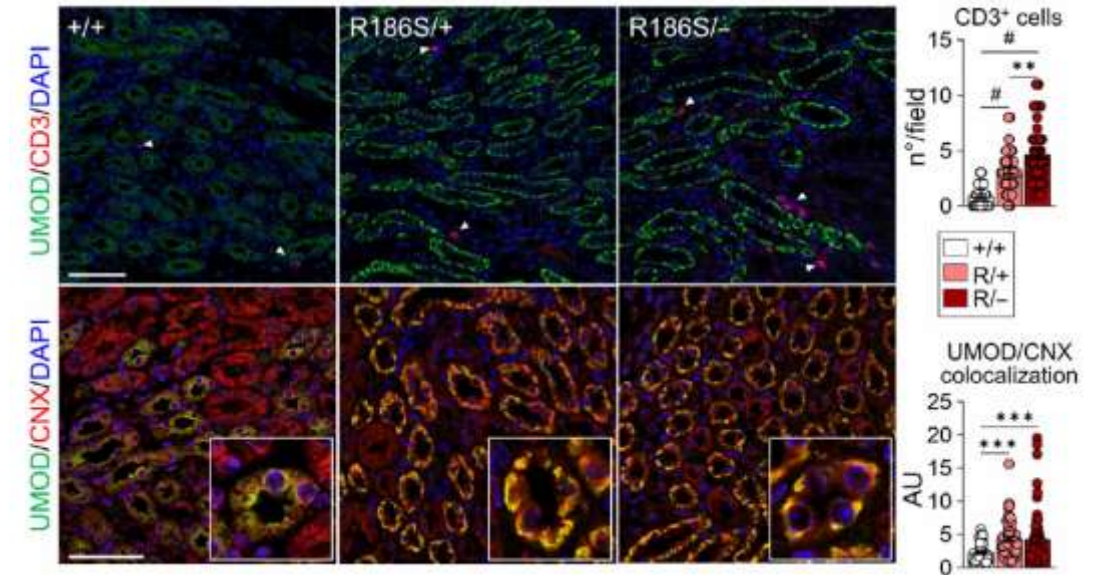
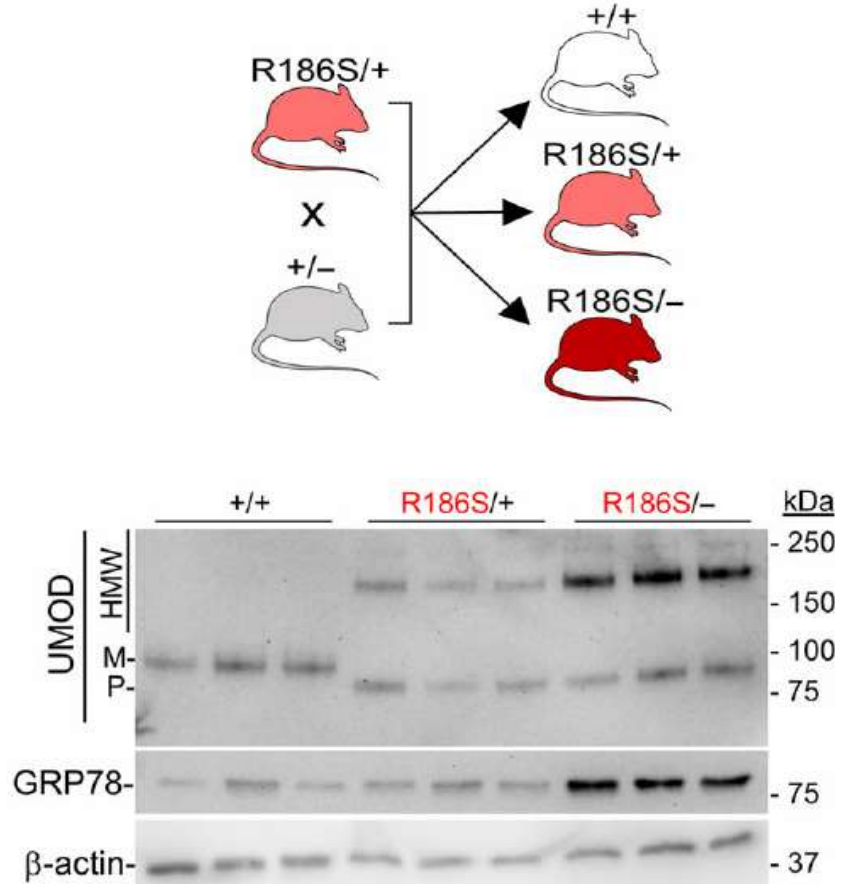


- Defective UMOD processing in kidney
- Formation of HMW aggregates
- Reduced levels in urine



- Progressive kidney damage
- Aggregates / ER stress / Infiltrate / Fibrosis

# Wild-type Uromodulin Protects against Mutant UMOD Aggregation



↑ aggregates // ↑ GRP78 and ER stress // ↑ infiltrates // ↓ UMOD in urine

## Cognitive Dysfunction Precedes Neuropathology and Motor Abnormalities in the YAC128 Mouse Model of Huntington's Disease

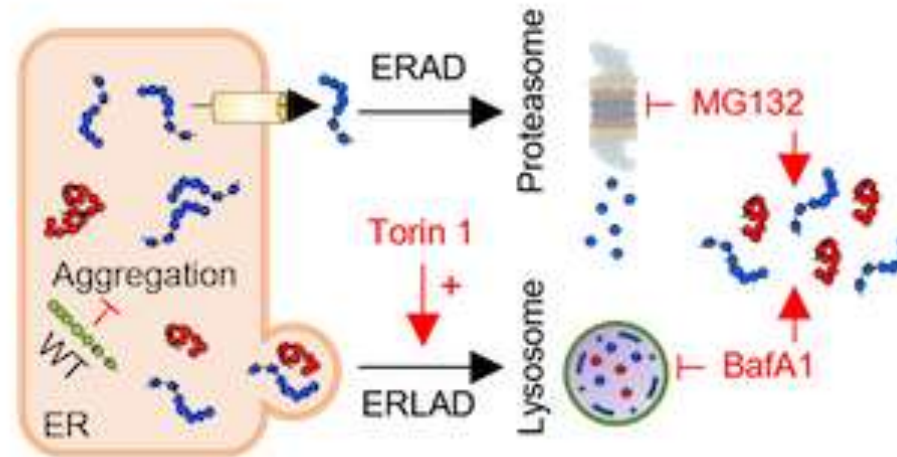
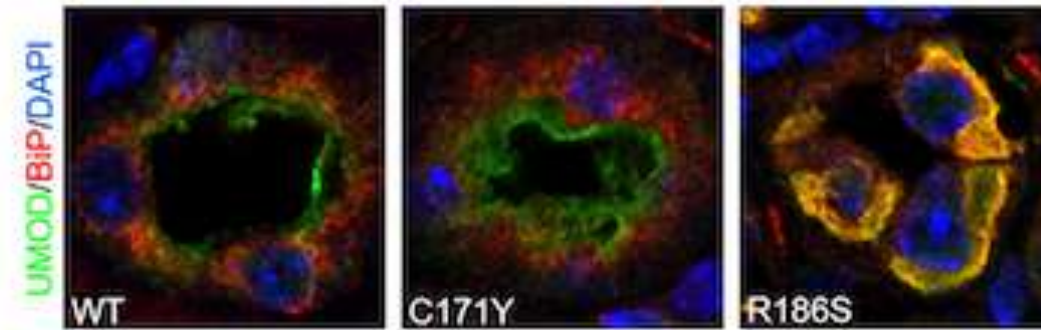
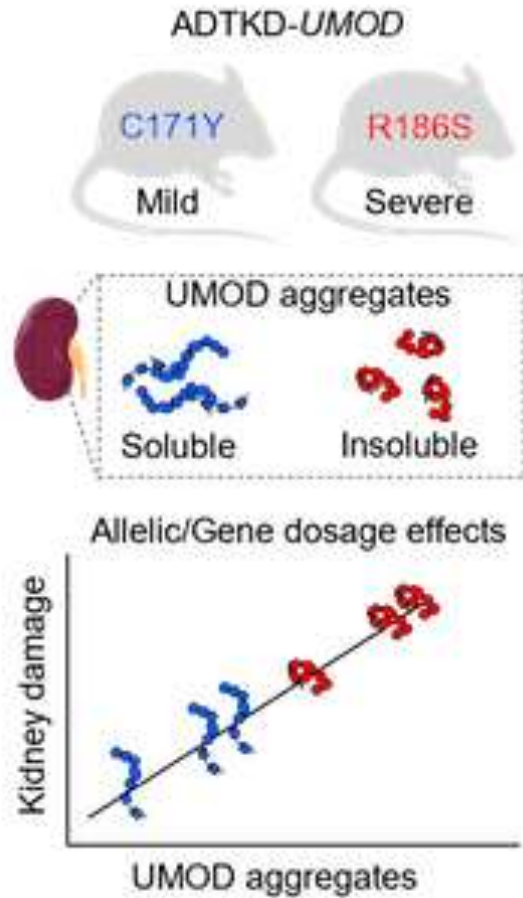
Disease Models & Mechanisms 2, 247–266 (2009) doi:10.1242/dmm.000653

RESEARCH ARTICLE

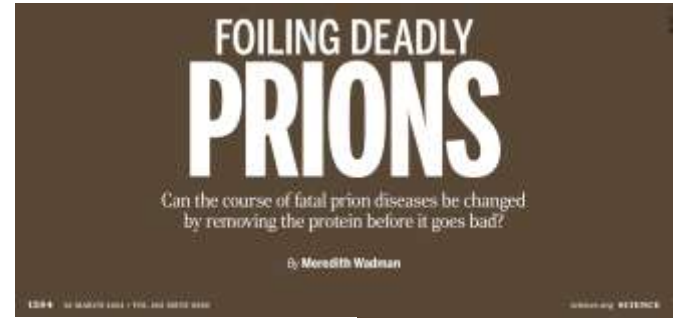
### Inactivation of Drosophila Huntingtin affects long-term adult functioning and the pathogenesis of a Huntington's disease model

*Huntington disease (HD), also characterized by a toxic gain of function, reported a similar protective role of wild-type huntingtin (Htt) protein: the absence of wild-type protein in HD mutant mouse and Drosophila models of Htt toxicity leads to a more severe phenotype (Van Raamsdonk et al, 2005; Zhang et al, 2009). The protective role of the wild-type allele may be to facilitate the trafficking of mutant protein to the plasma membrane or, alternatively, to balance the propensity of mutant uromodulin to form aggregates.*

# Proteinopathies: Not Restricted to Neurodegenerative Diseases

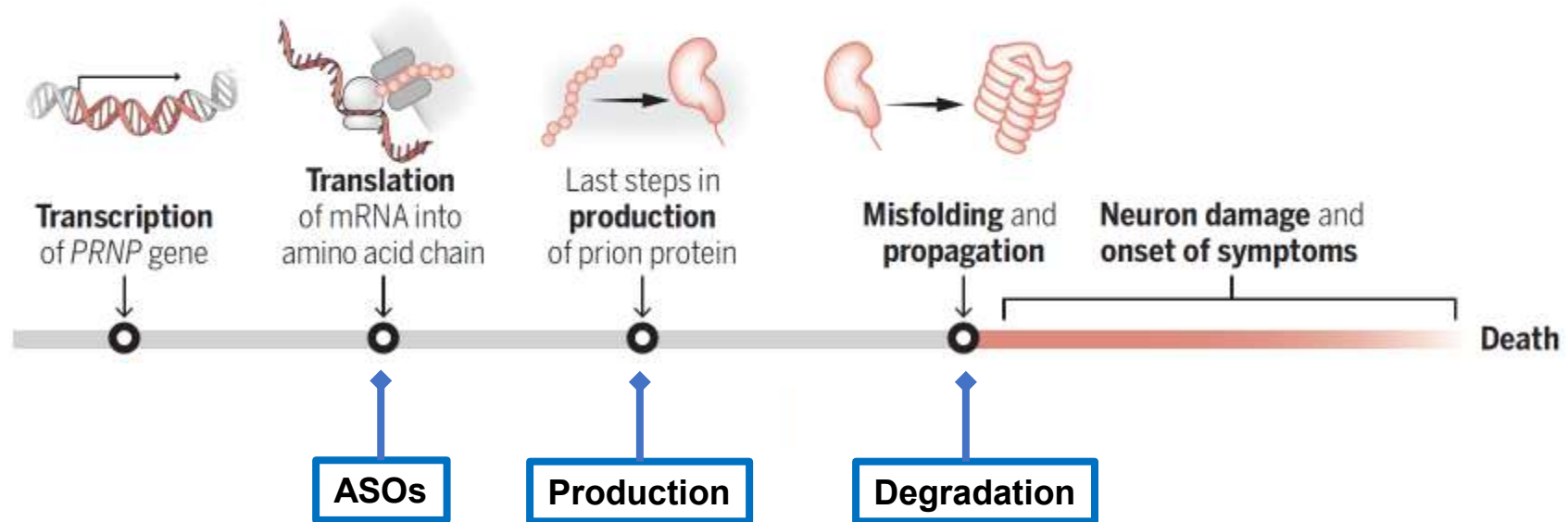


# Proteinopathies: Global Strategy



## Cutting a protein pipeline

For years, scientists have tried unsuccessfully to stymie prion diseases by blocking the conversion of normal prion protein to its pernicious, misfolded form. But a new generation of experimental therapies for the fatal brain diseases has moved upstream, seeking to stop even the production of normal prion protein, encoded by the gene *PRNP*. Three drugs that are in development use different molecules and mechanisms, and take different routes to the brain.



# Rationale to Target ADTKD-UMOD with ASOs

- Mechanism: Gain-of-toxic function - UMOD limited to kidney tubules
- Direct link: defective processing of mutant UMOD - aggregates - disease progression
- Protective effect of wild-type allele
- Available cell and mouse models
- Evidence: Decreasing mutant UMOD/aggregates is protective (lower ER stress)

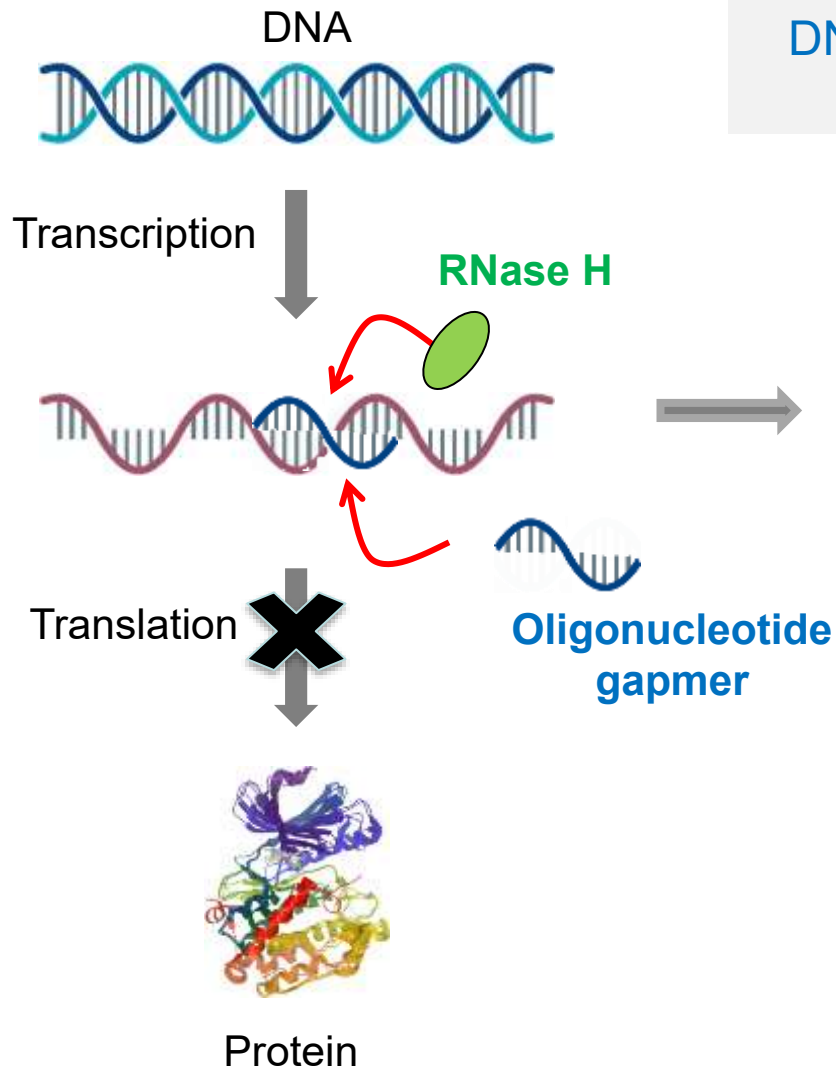
## Thus far, no approved ASOs targeting the kidney

- ASOs tested in ADPKD, Alport syndrome, AKI
- ASOs tested for neurodegenerative proteinopathies (e.g. AD, Huntington)

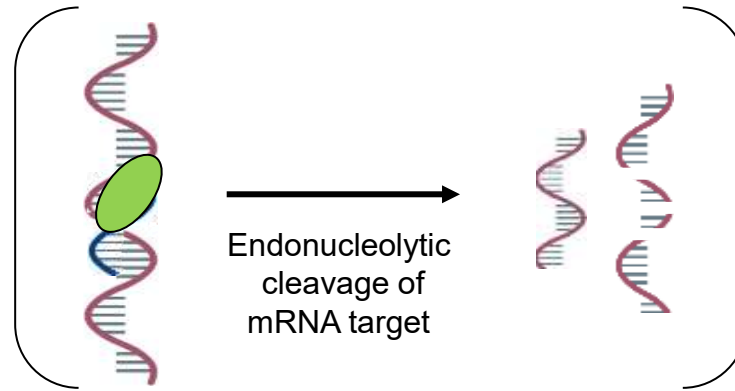
→ *Can we target the TAL ? Be allele-specific ?*

→ *How much suppression of mutant UMOD would be necessary ?*

# ASO: Mechanism of Action and Design

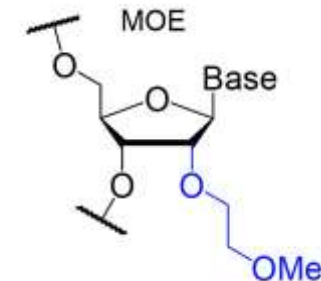
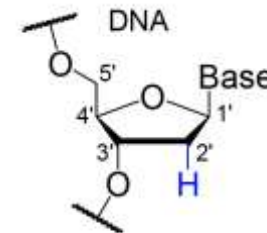


DNA based oligonucleotides induce **RNase H** to cleave the target mRNA strand in nucleus and cytoplasm



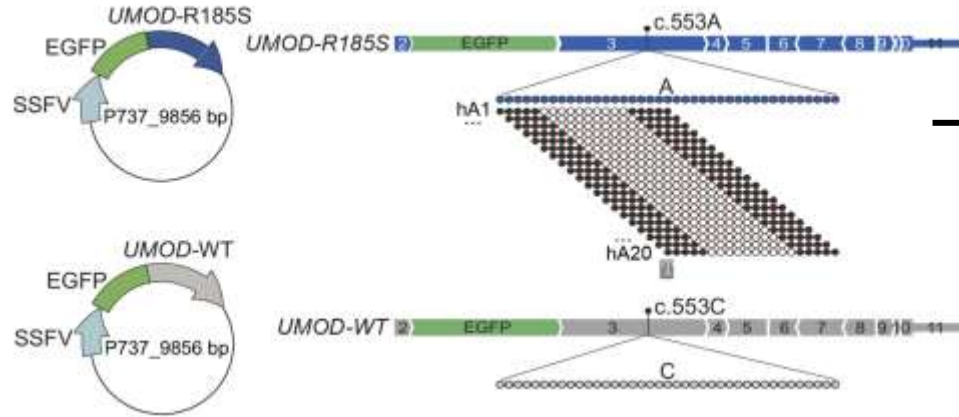
## Gapmer design:

2'-O-Methoxyethyl (MOE)  
5-10-5 (MOE-DNA-MOE)



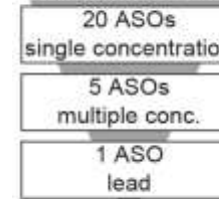
# Identification of Allele-selective ASOs against UMOD

Tiled design against  $mut$ UMOD – mIMCD cells



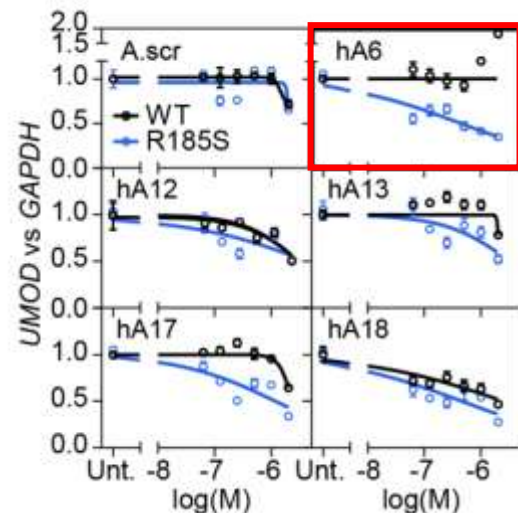
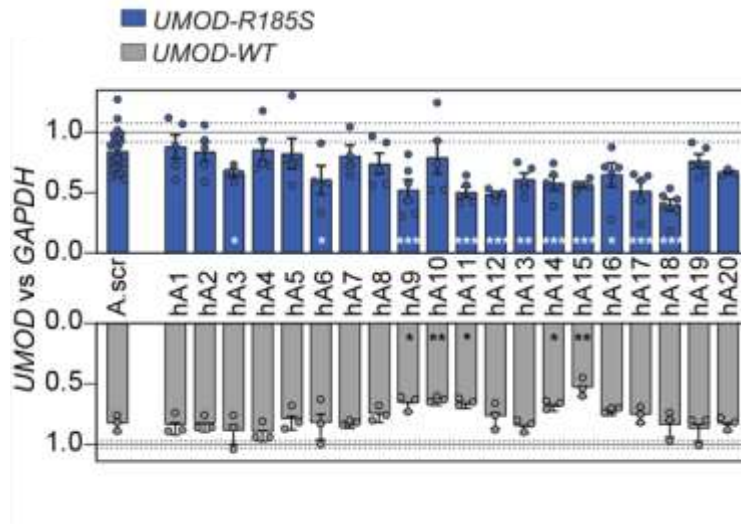
ASO sequence library against  $mut$ UMOD

Selectivity screen

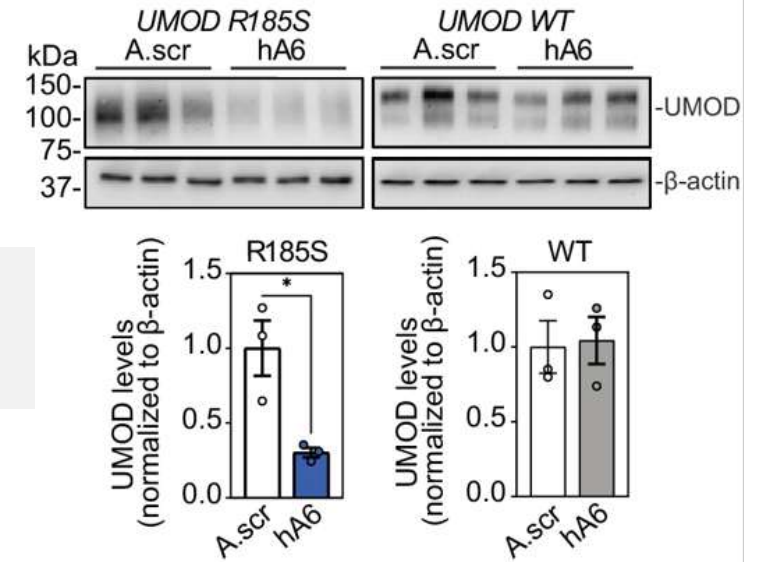


ASO	5'-3' sequence
hA1	CCAGTACTCGTCCAGGGTG
hA2	CCAGTACTCGTCCAGGGT
hA3	GC CCAGTACTCGTCCAGGG
hA4	TGC CCAGTACTCGTCCAGG
hA5	CTGC CCAGTACTCGTCCAG
hA6	GCTGC CCAGTACTCGTCCA
hA7	TGCTGC CCAGTACTCGTCC
hA8	GTGCTGC CCAGTACTCGTC
hA9	GGTGTGC CCAGTACTCGT
hA10	CGGTGTGC CCAGTACTCG
hA11	TGGGTGTGC CCAGTACTC
hA12	CTCGGTGTGC CCAGTACT
hA13	ACTCGGTGTGC CCAGTAC
hA14	TACTCGGTGTGC CCAGTA
hA15	GTACTCGGTGTGC CCAGT
hA16	CGTACTCGGTGTGC CCAG
hA17	CCGTACTCGGTGTGC CCA
hA18	CCCGTACTCGGTGTGC CC
hA19	CCCCGTACTCGGTGTGC C
hA20	TCCCGTACTCGGTGTGC
A.scr	TCACGGGTTTGGACTGGTAG

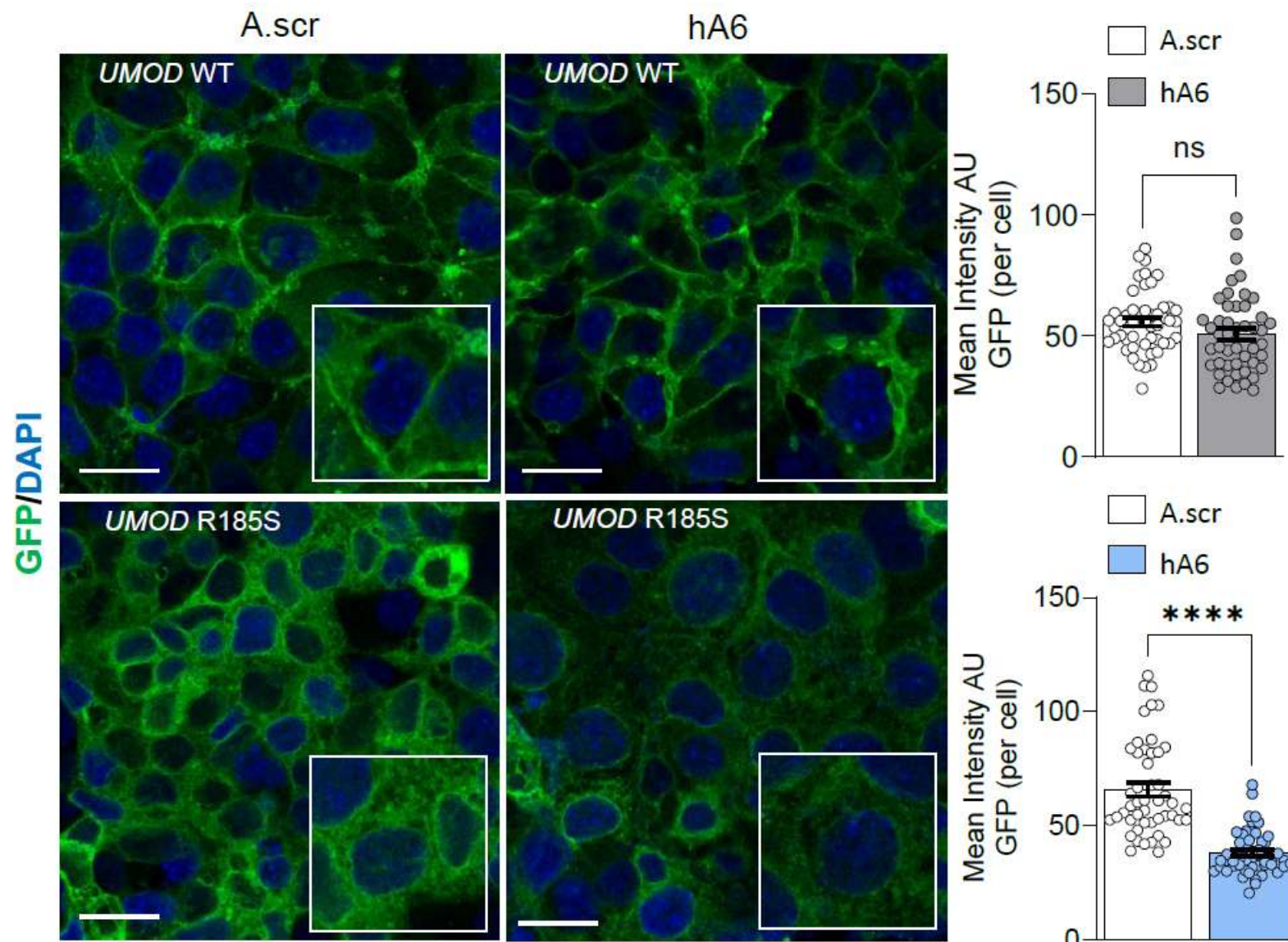
Silencing of UMOD – mIMCD cells (48h, 100 nM ASO)



Validation of hA6 in mIMCD cells (72h, 1.6  $\mu$ M)



# ASOs Suppress Mutant UMOD-EGFP in mIMCD cells



# POC: ASOs Accumulate in Kidney/TAL in *Umod* KI Mice



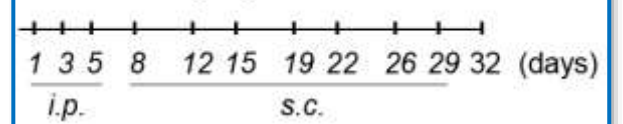
*Umod*<sup>+/+</sup>  
*Umod*<sup>R186S/+</sup>

4 week-old

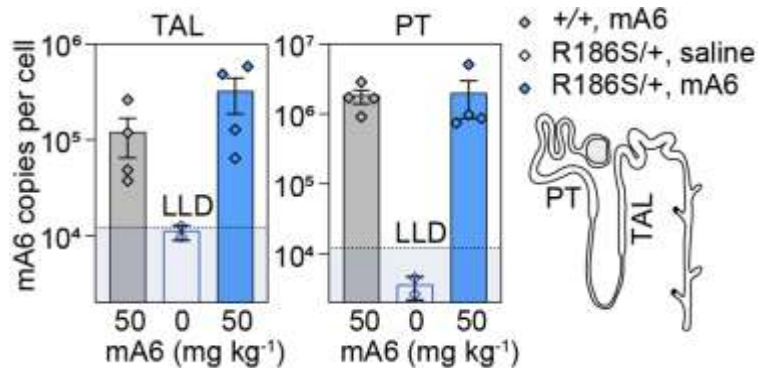
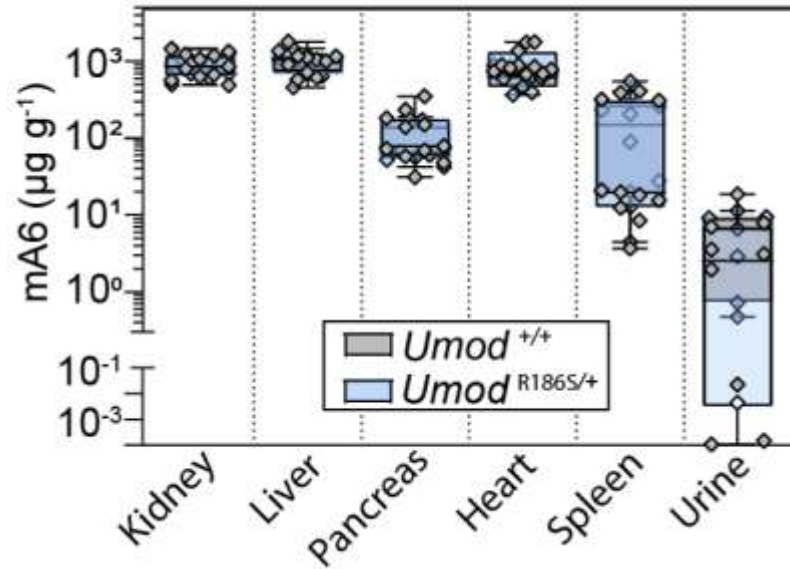
Saline

mA6 50 mg kg<sup>-1</sup>

Sacrifice

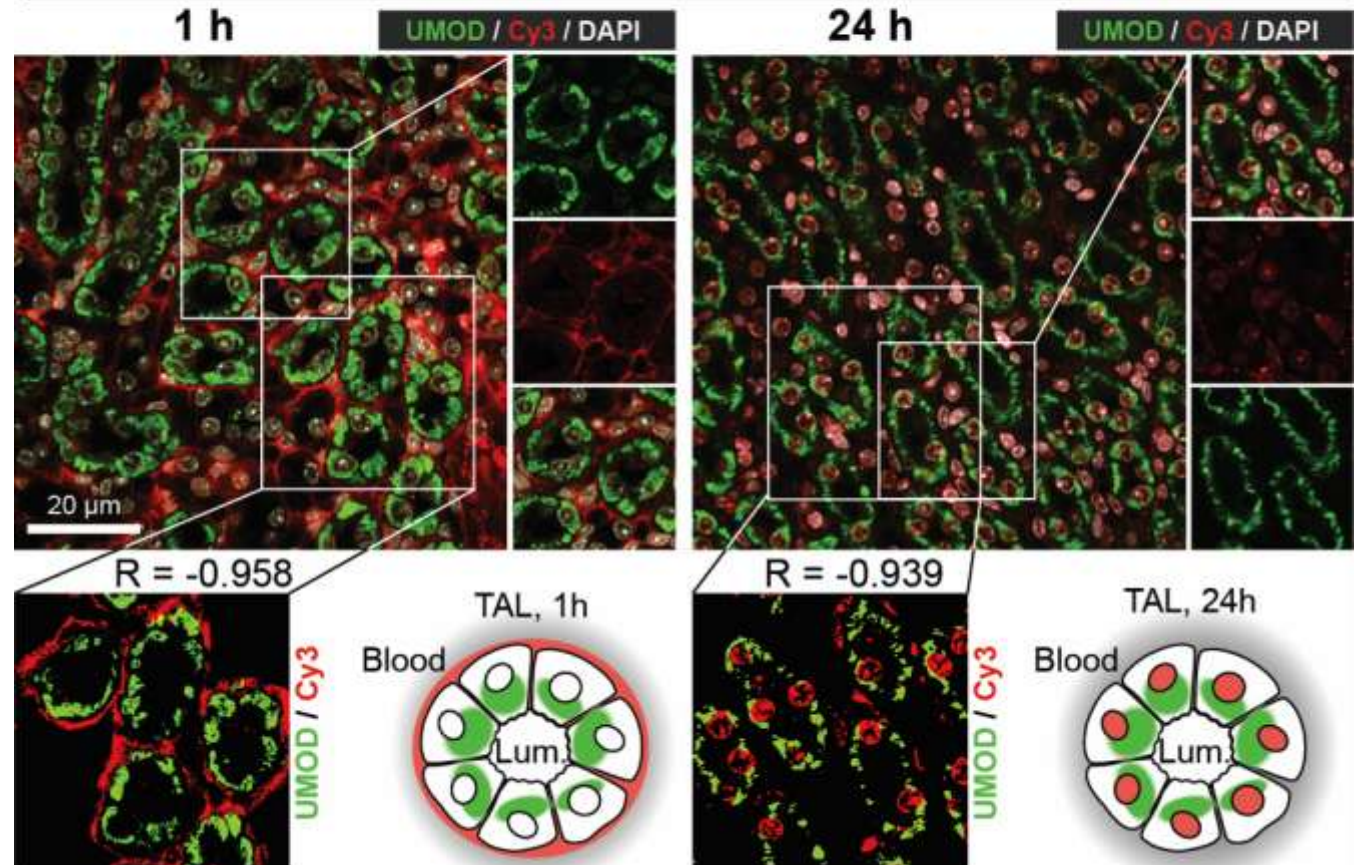


Class-specific accumulation in liver – kidney

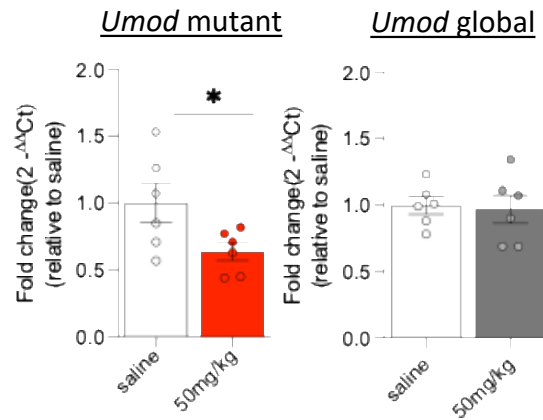
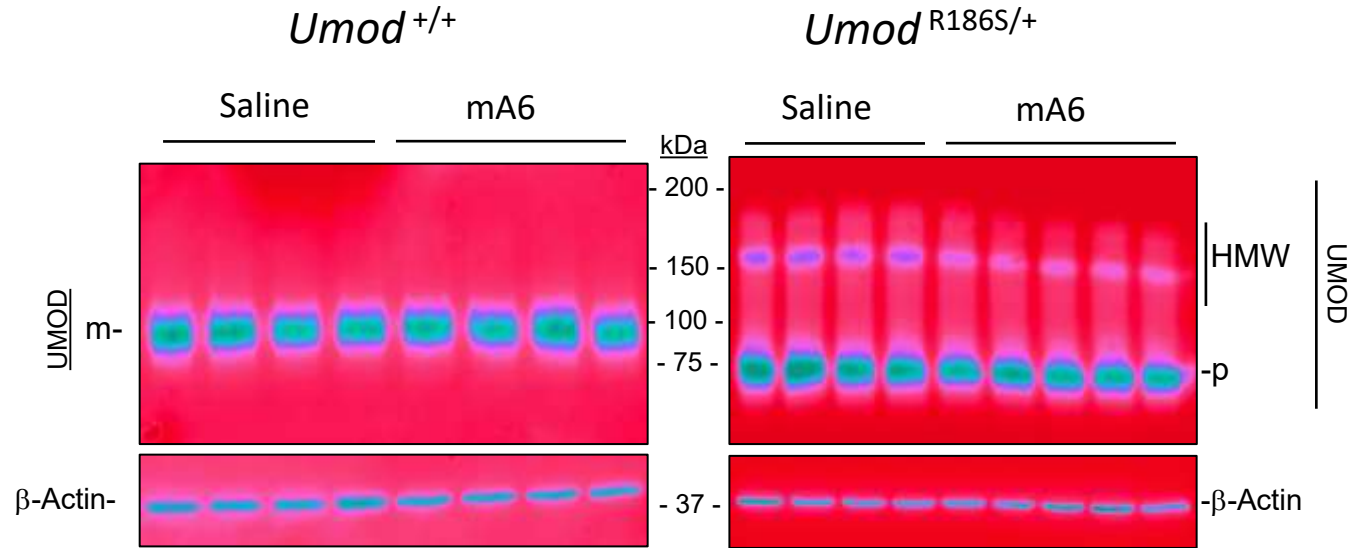
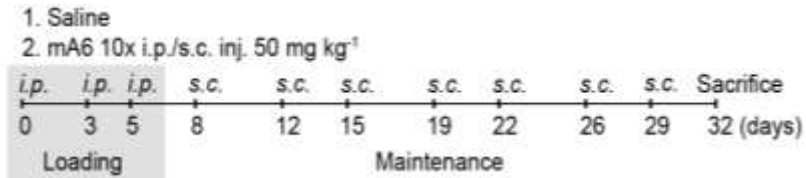


Isolated tubules: Splint ligation qPCR assay

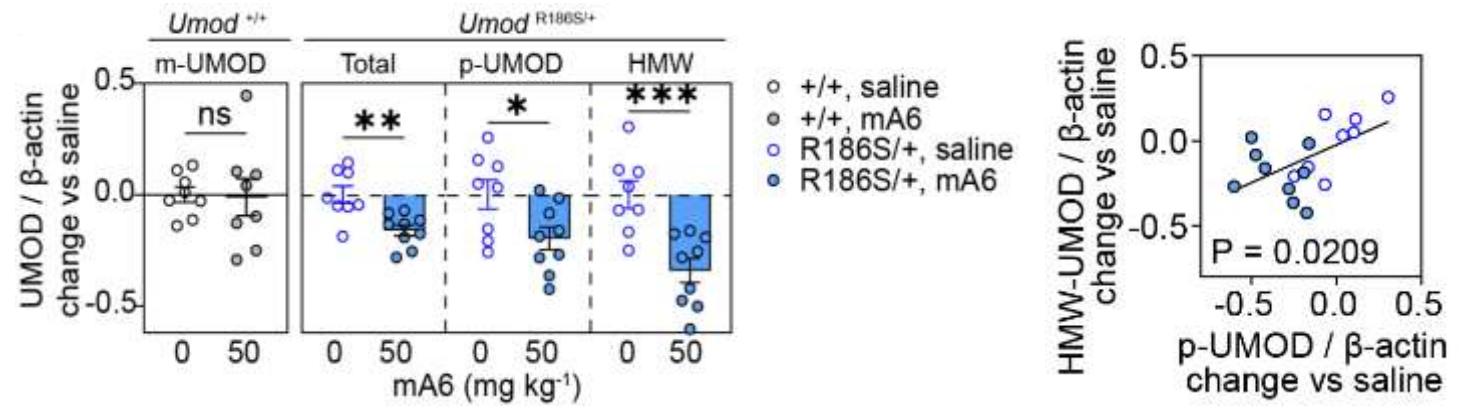
i.p. Cy3-mA6, 50 mg/kg



# POC: Allele-selective ASOs Reduce UMOD Aggregates



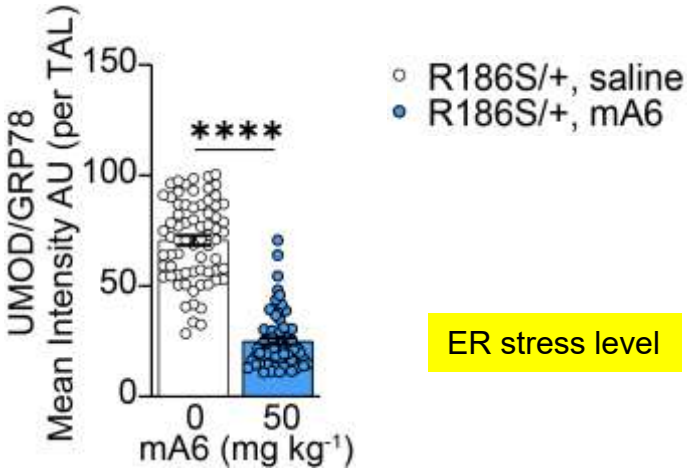
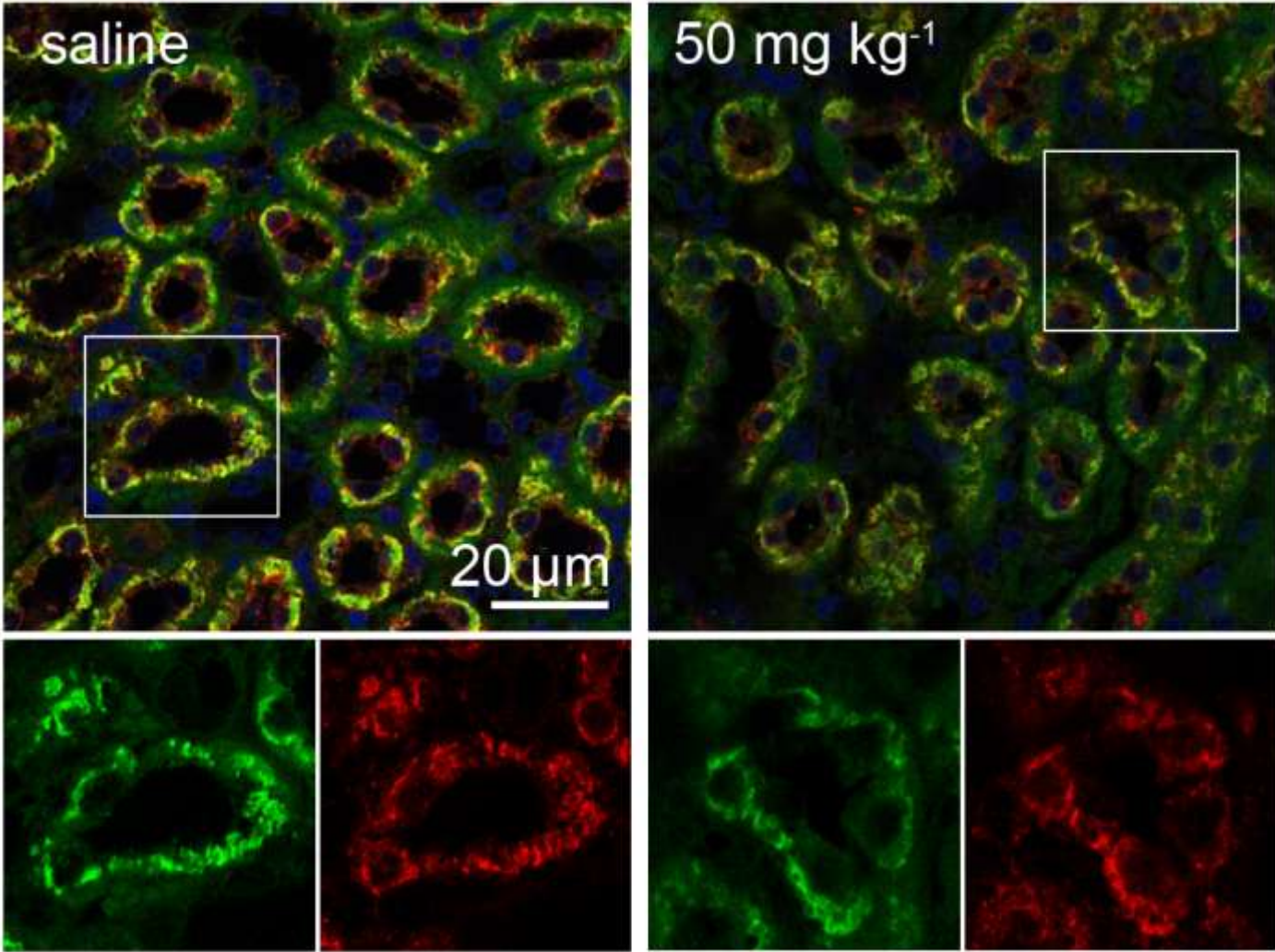
➤ Target engagement



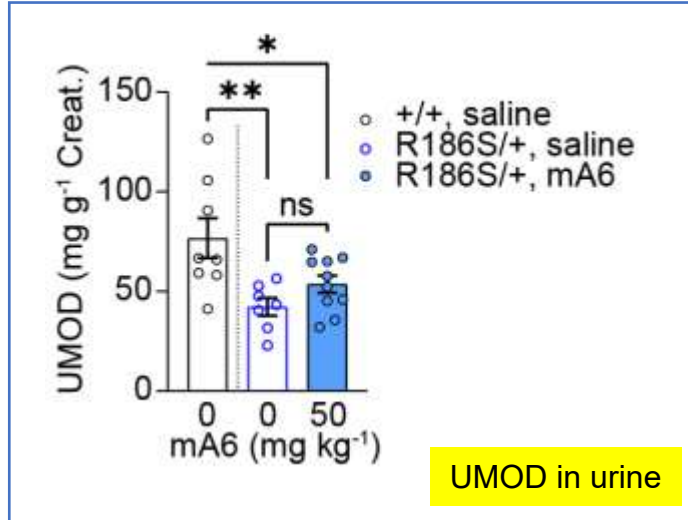
- Reduction of p-UMOD and HMW
- Proportional changes
- No effect in wild-type mice

# Effects of ASOs on UMOD Levels and ER Stress

## UMOD/GRP78



ER stress level



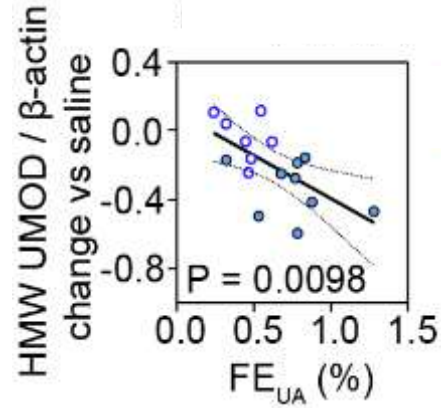
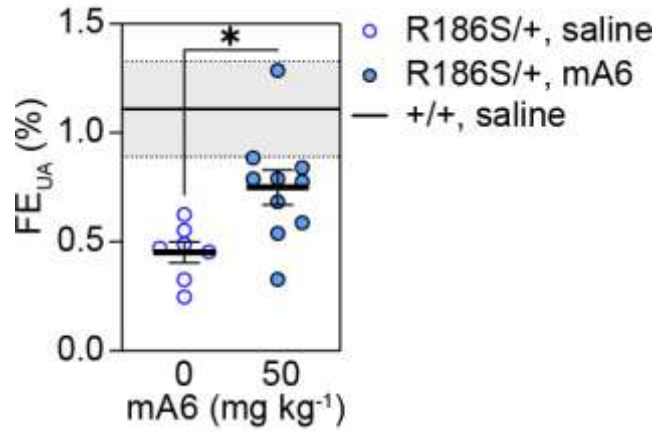
UMOD in urine

# Effects of ASOs on Tubular Function and Kidney Damage



1. Saline  
2. mA6 10x i.p./s.c. inj. 50 mg kg<sup>-1</sup>

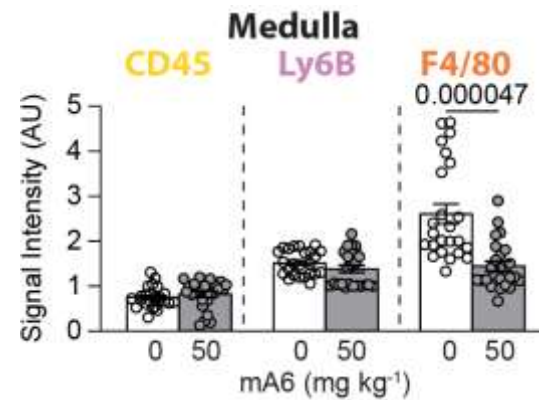
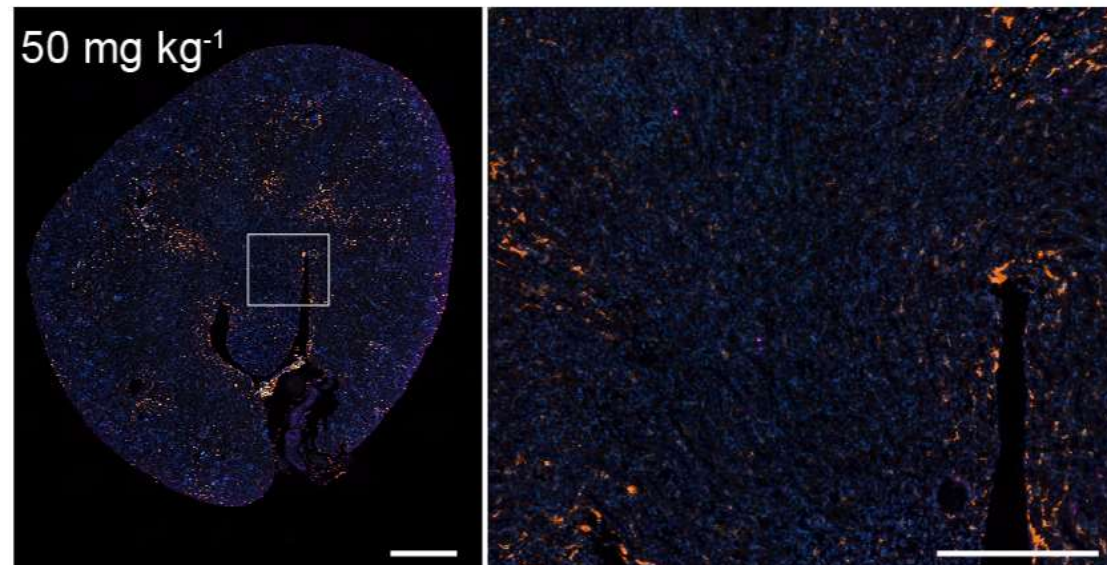
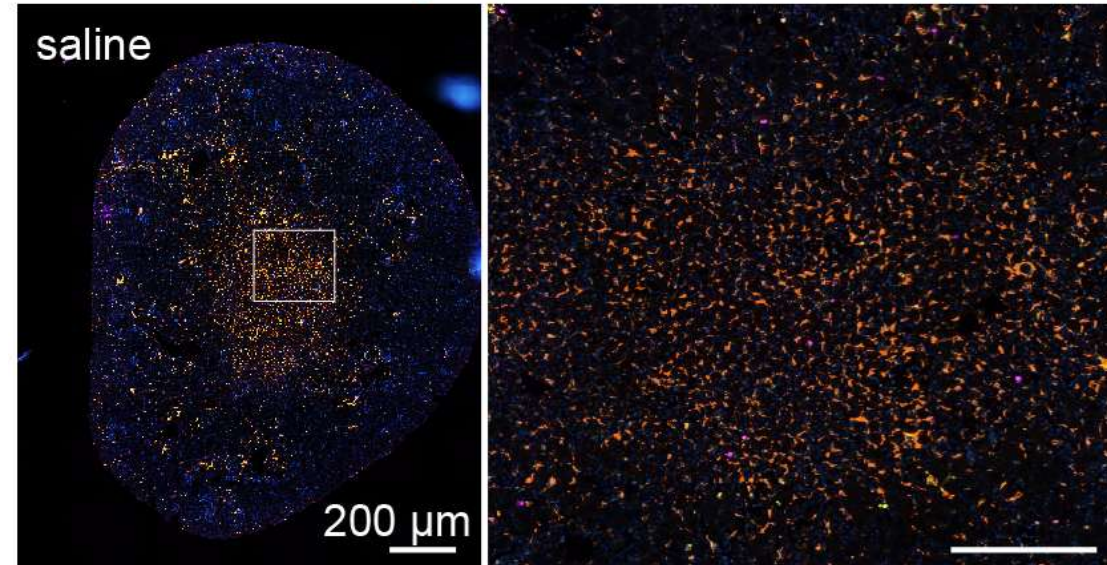
i.p.	i.p.	i.p.	s.c.	s.c.	s.c.	s.c.	s.c.	s.c.	Sacrifice
0	3	5	8	12	15	19	22	26	29
Loading			Maintenance						



- Increased FEurate
- k to reduction in HWM UMOD

- Decreased infiltrate

F4/80/CD45/Ly6B/Hoechst



# Targeting ADTKD-UMOD with ASOs: Summary

- Allele-selective gapmer ASOs were designed against mutated UMOD; characterized in cell systems; visualized in specific tubular cells and quantified using splint ligation qPCR assay.
- Our lead ASO achieves high kidney and TAL targeting, with allele-selective suppression of mutant uromodulin.
- In a POC, 4-week study, ASO treatment reduces mutant UMOD accumulation and mitigates downstream tissue injury in the (severe) *Umod*<sup>R186S/+</sup> mice. The lead ASO showed no effects on wild-type UMOD in *Umod*<sup>+/+</sup> mice.
- Acceptable safety profile: body weight, liver enzymes, tissue distribution.

→ *These findings pave the way for using ASOs to target toxic proteinopathies like ADTKD-UMOD in the kidney*

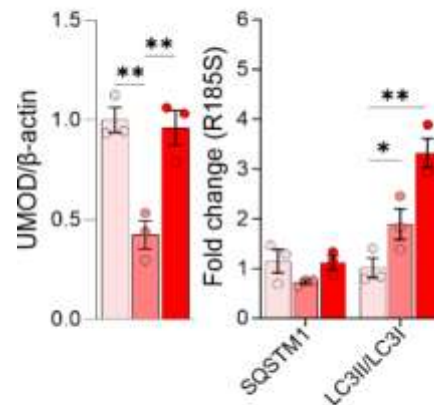
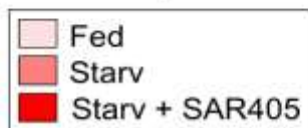
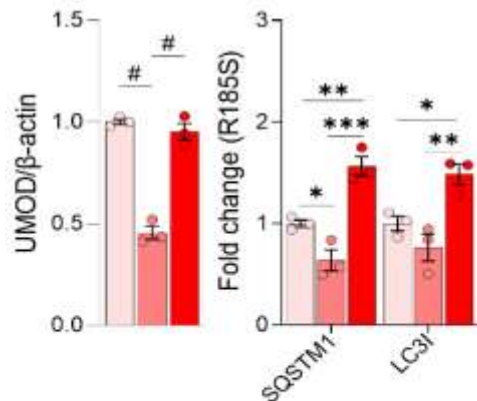
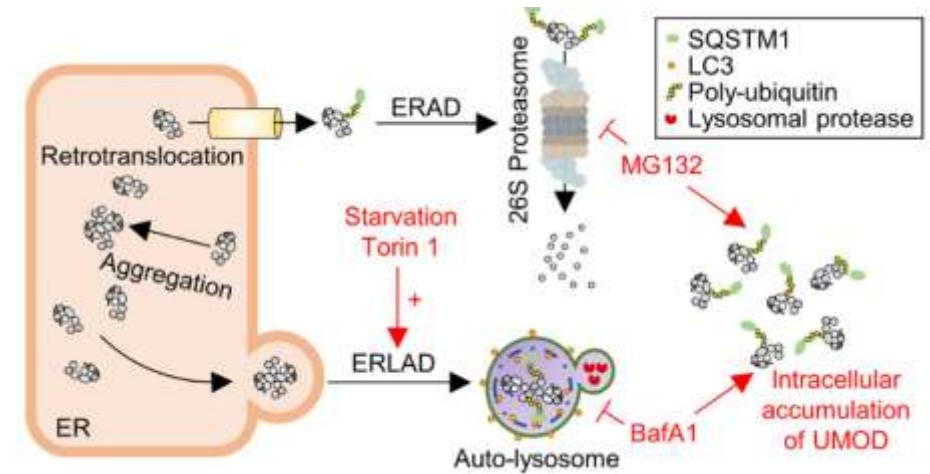
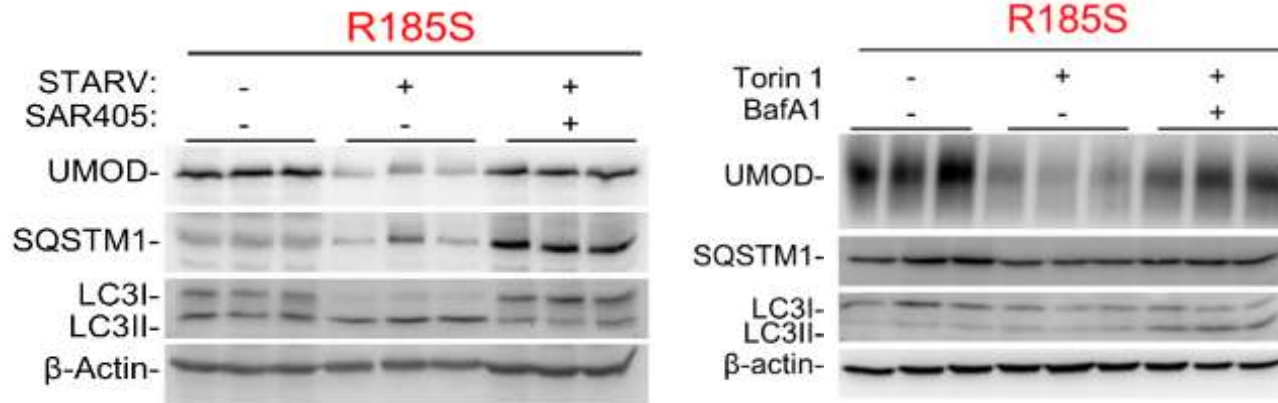
→ *Developments: Reducing dosing (liver toxicity) - increase potency - improve targeting to the TAL*

## Central Translational Issue: Can UMOD Aggregates Be Cleared?

- **ER capacity** is chronically saturated by mutant UMOD aggregates
- **Autophagy** (FAM134B/RETREG1 - ERLAD) is a plausible clearance route
- **Autophagy induced by nutrient deprivation**: mTORC1 suppression + AMPK activation

**Question:** *Can a dietary intervention engage autophagy/ER-phagy to reduce mutant UMOD aggregates in TAL cells?*

# Fasting & Torin1 Clear UMOD Aggregates *in vitro*

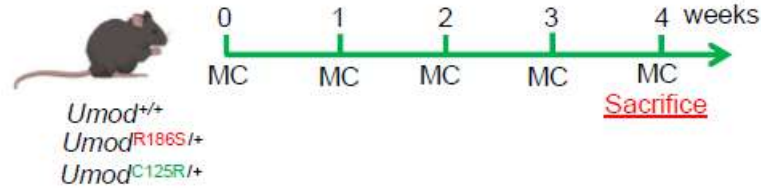


Exposing mIMCD cells to starvation or Torin 1:  
 ↘ aggregates in an autophagy-dependent manner

# Dietary Interventions in *Umod* KI Mouse Models

## Progressive Caloric Restriction (CR)

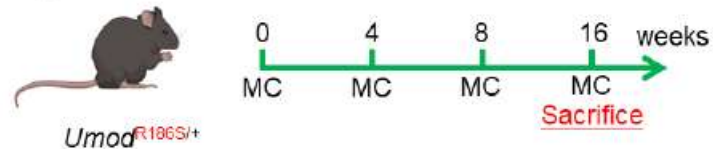
Age: 8 weeks old



Ad libitum (AL)	Free feeding	
(CR)	10-20% caloric restriction	30% caloric restriction

## Methionine restriction (MetR)

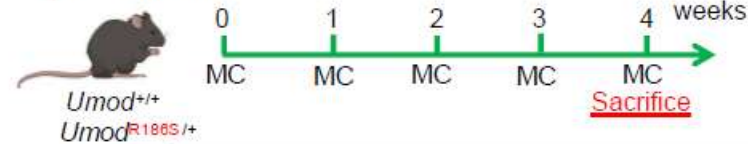
Age: 8 weeks old



Control Fed (CF)	0.86% Methionine
(MetR)	0.12% Methionine

## Intermittent Fasting (IF)

Age: 5 weeks old



Ad libitum (AL)	Free feeding			
IF alternate days (IFA)	Fasting	Feeding	Fasting	Feeding
IF 5:2 days (5:2)	Fasting	Feeding	Feeding	Fasting

## Readouts

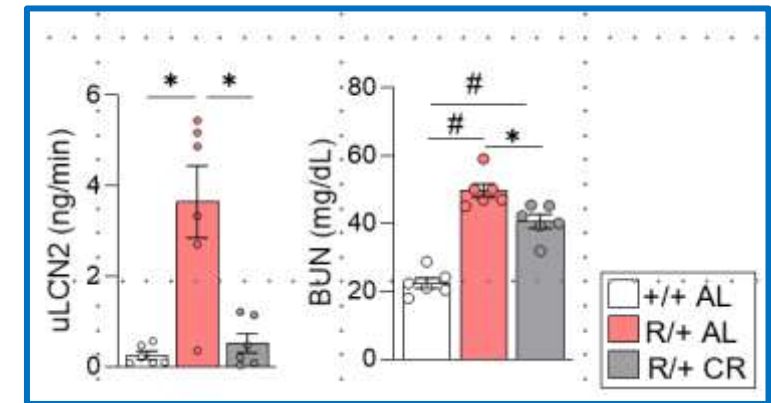
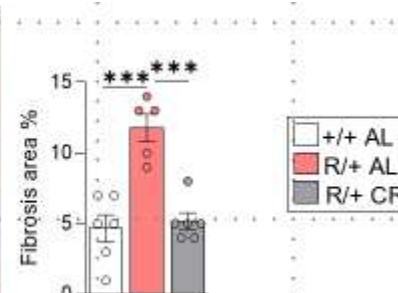
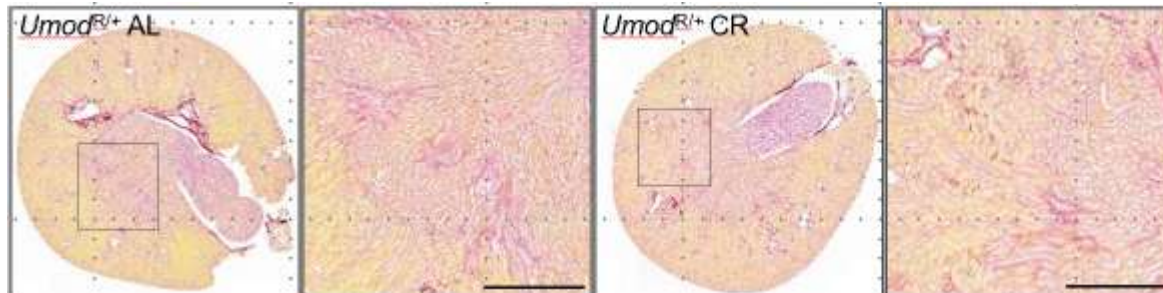
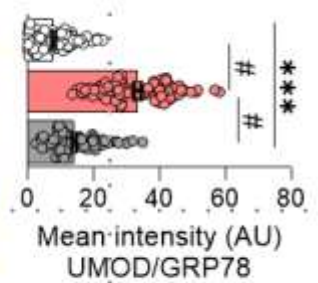
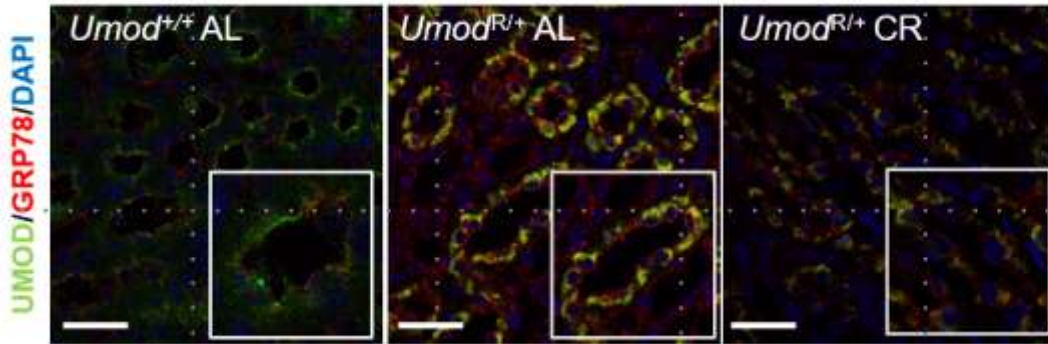
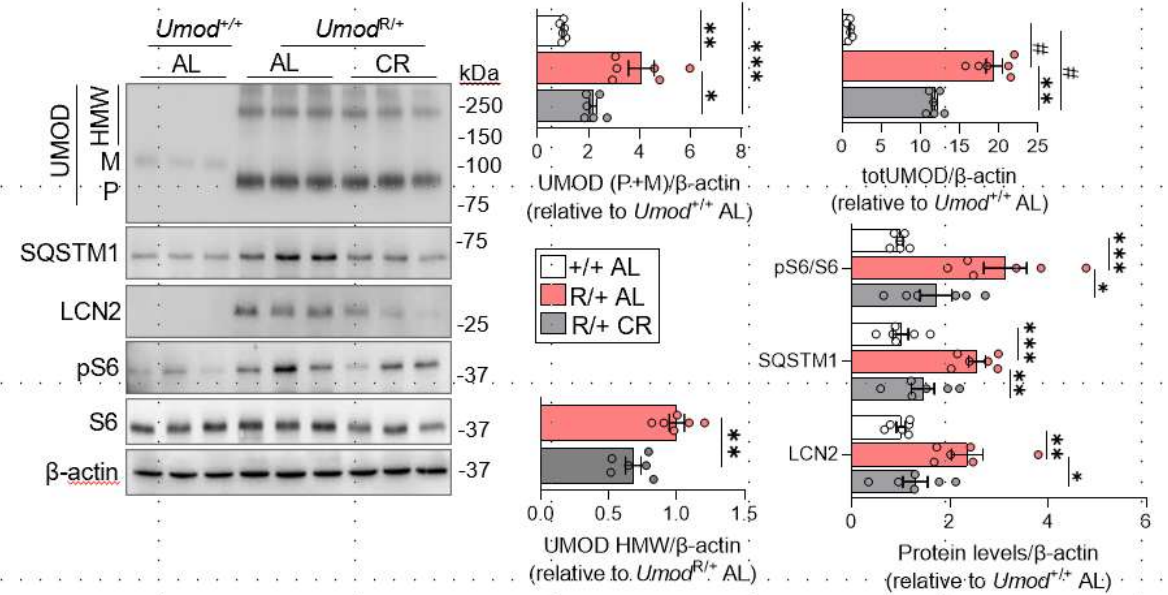
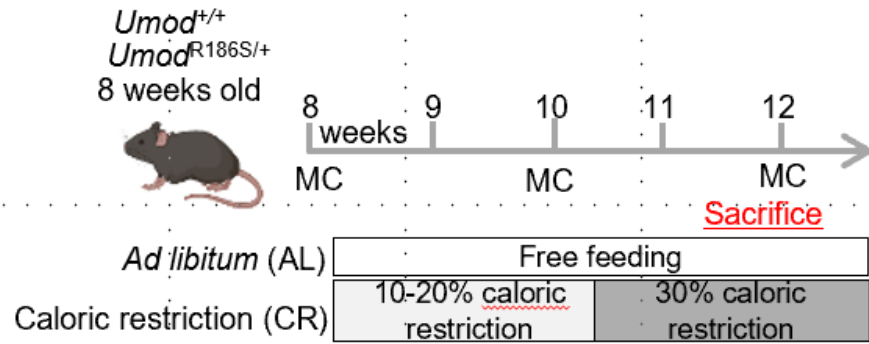


## In vitro mechanistic studies

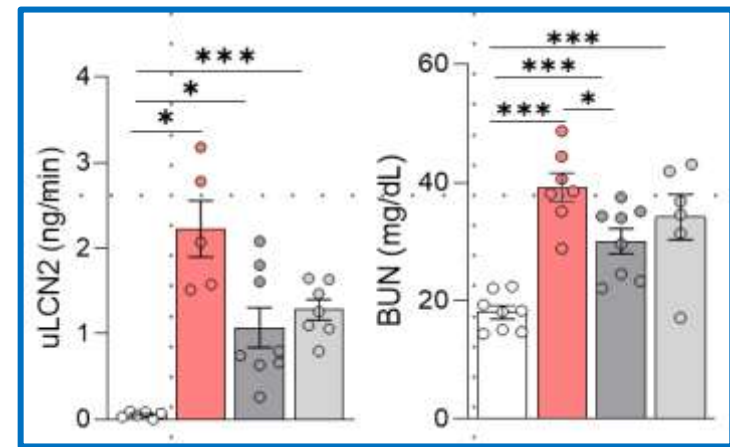
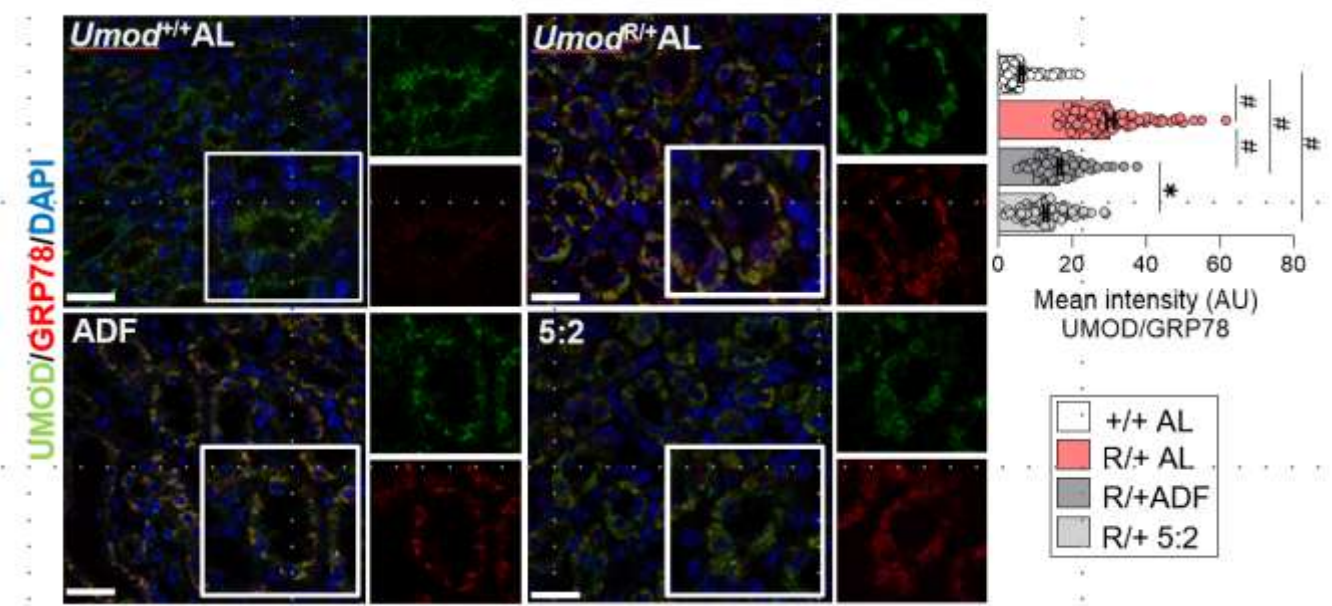
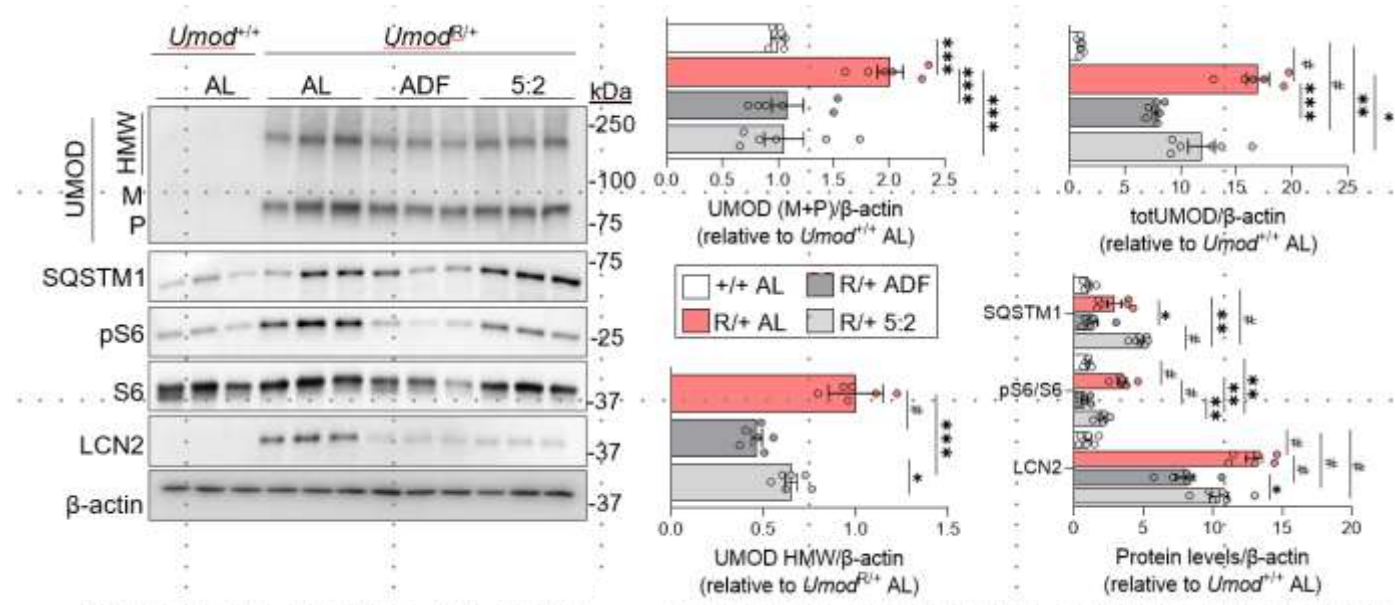
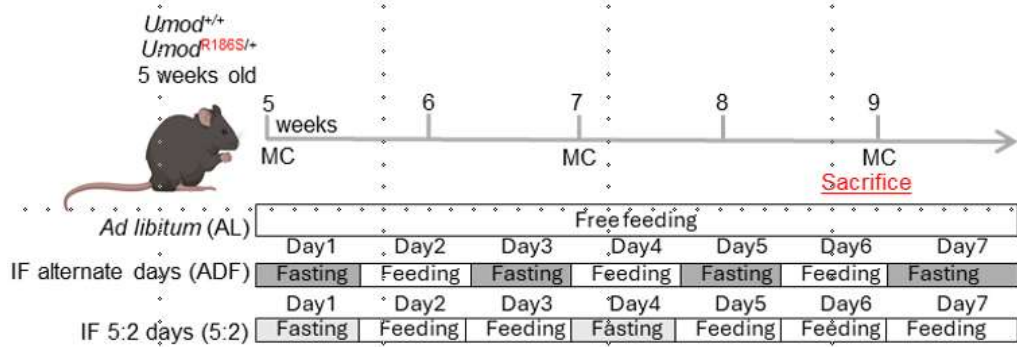


- UMOD/HMW
- ER stress
- Inflammation
- Fibrosis
- Kidney function
- Autophagy
- ER-phagy

# Progressive CR clears mutant UMOD & impacts on disease

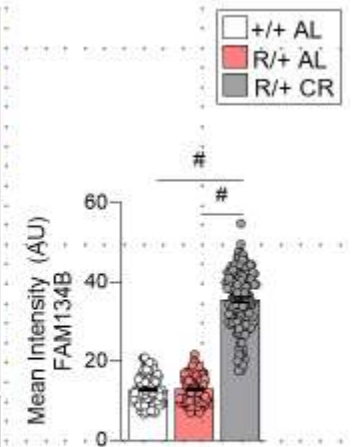
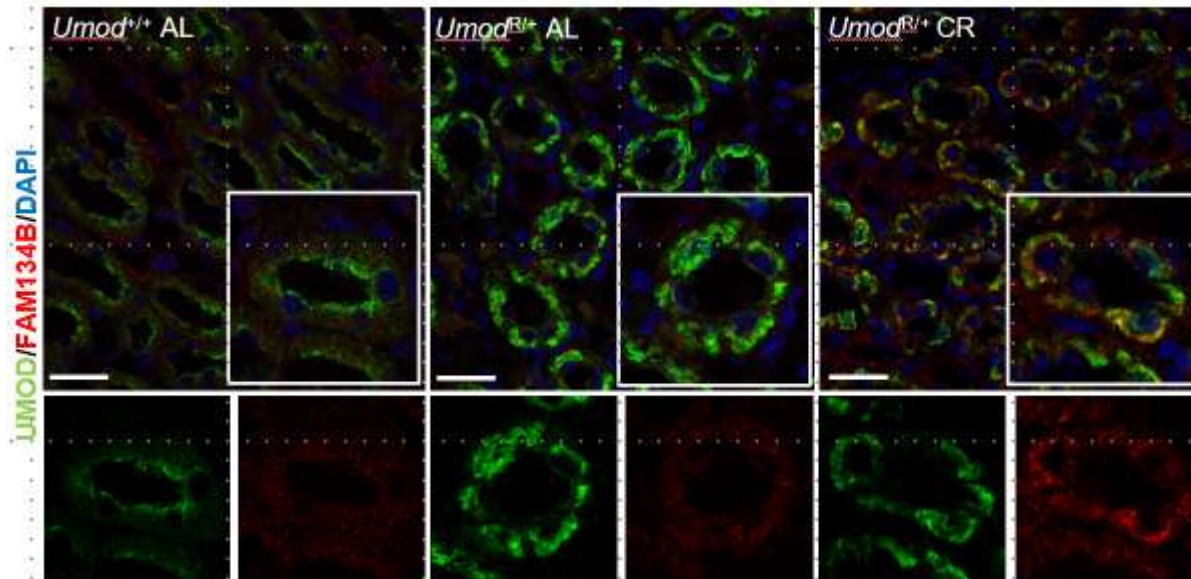
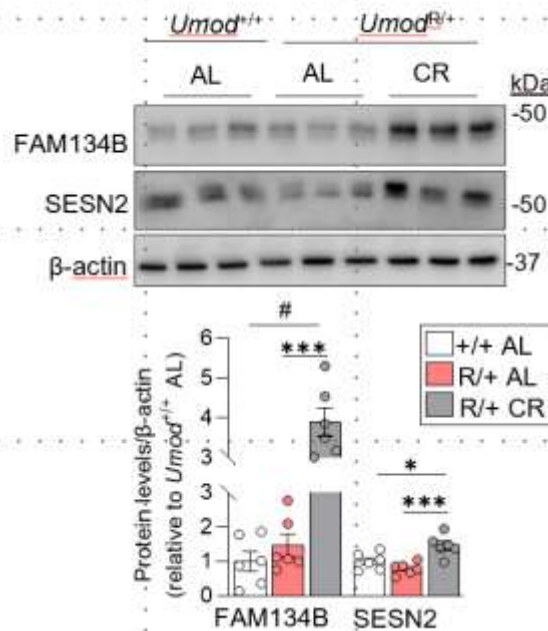
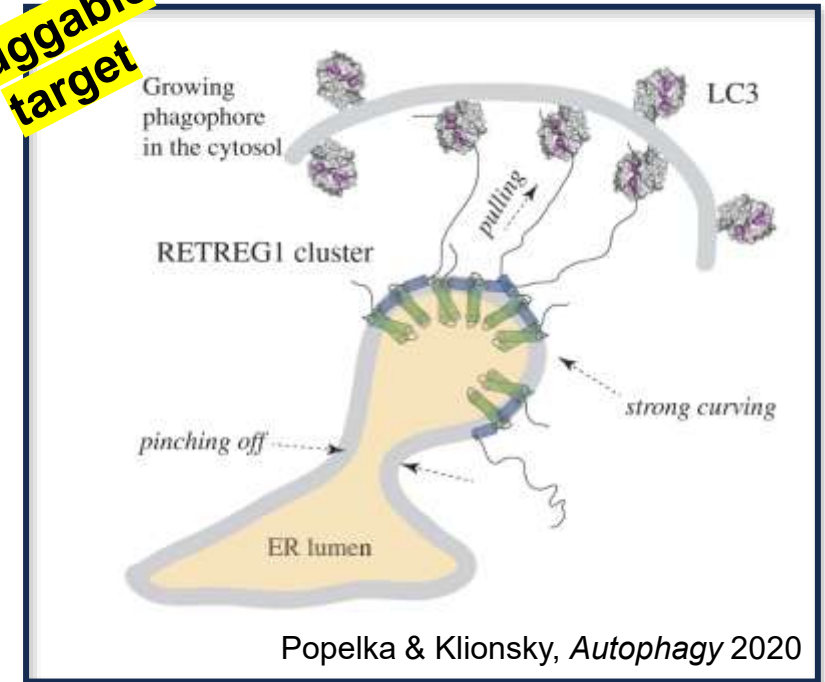
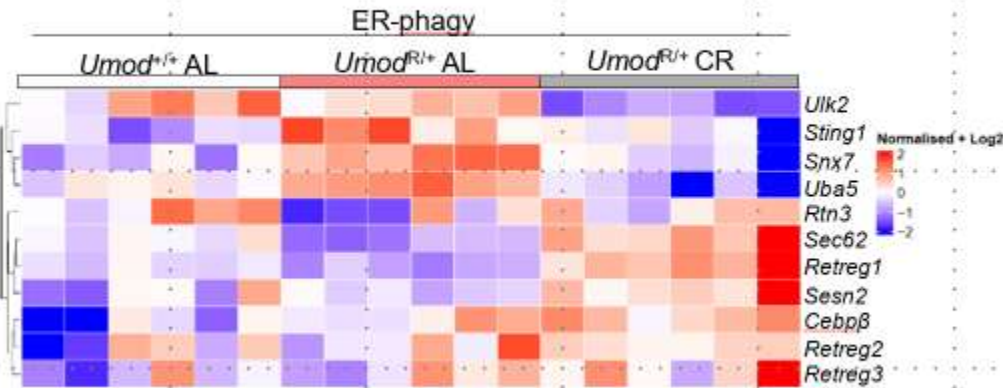
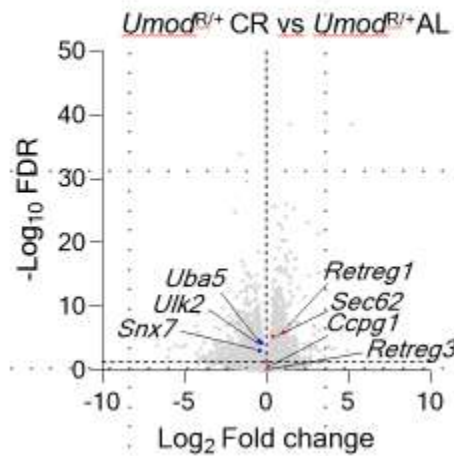


# Intermittent fasting clears mutant UMOD & impacts on disease

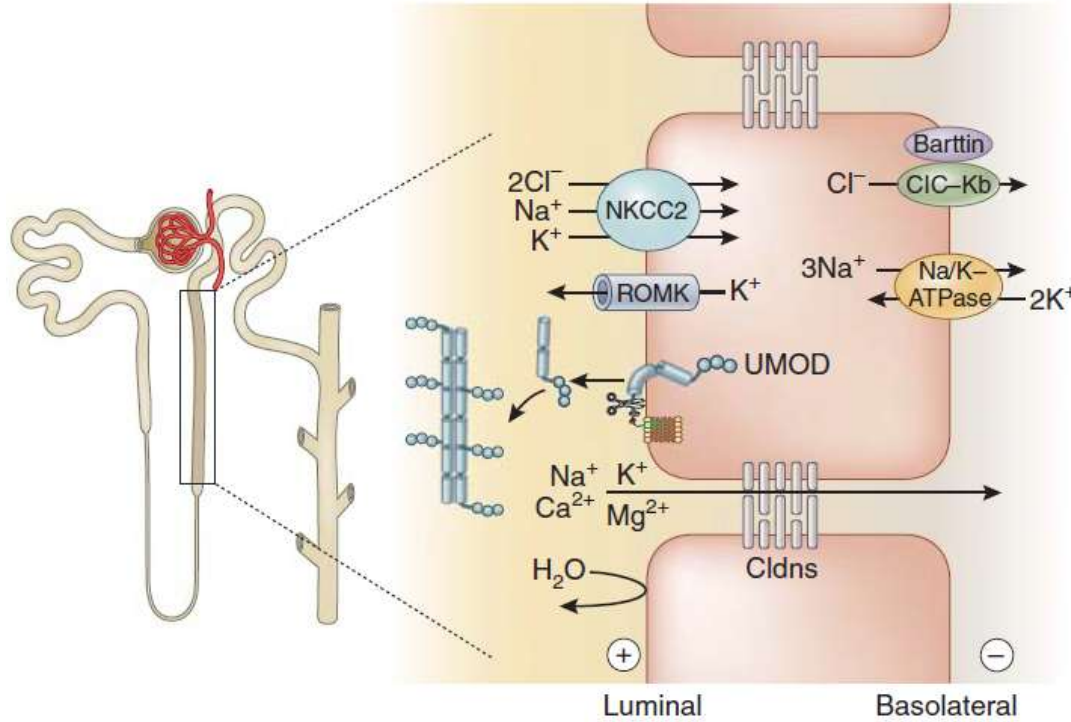


# Dietary interventions increase Retreg1/FAM134B – ER-Phagy gatekeeper

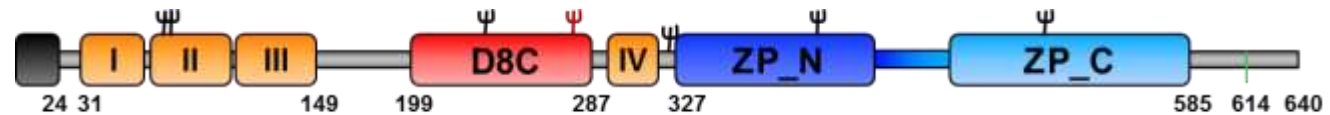
**Druggable target**



# Uromodulin Production and Biochemistry

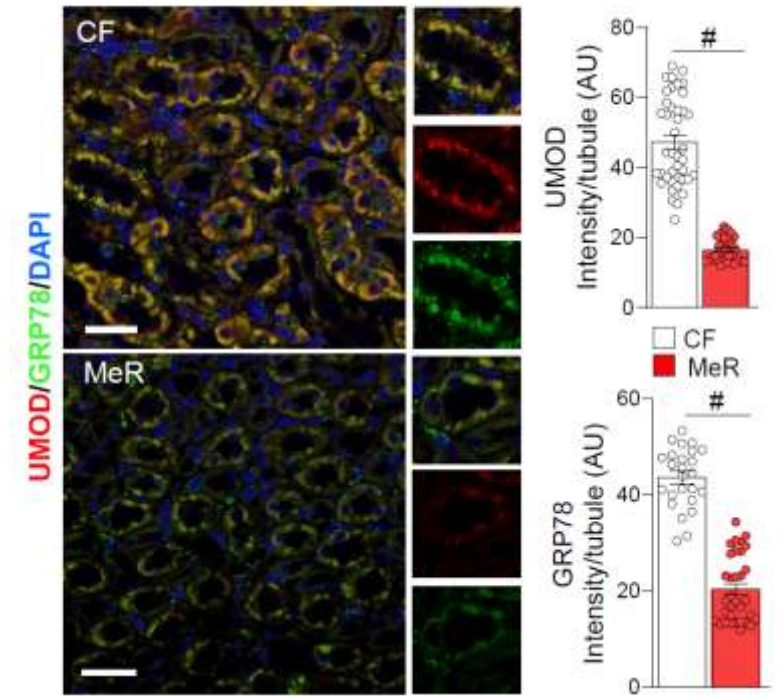
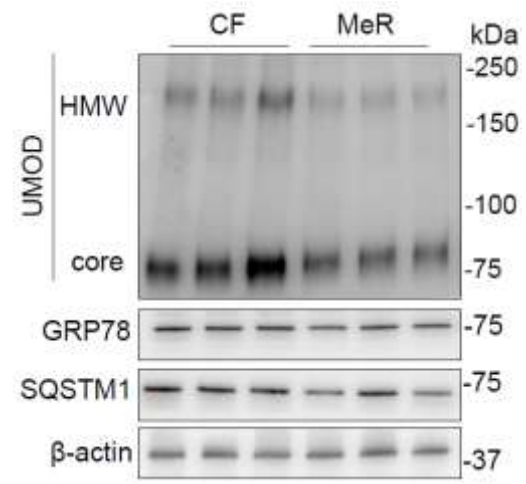
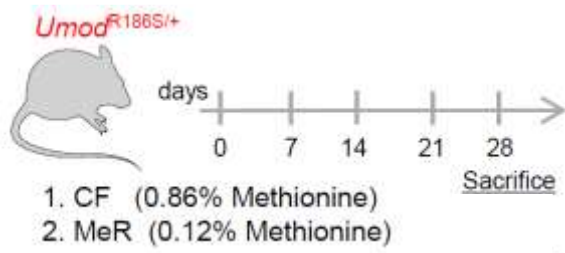
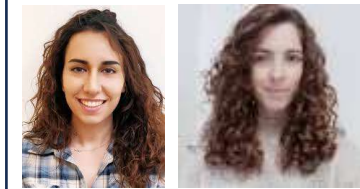
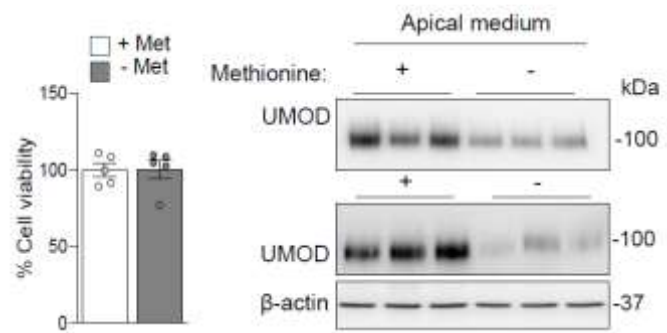
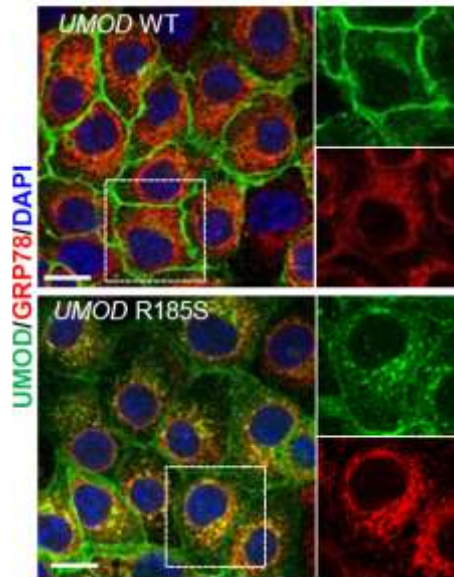
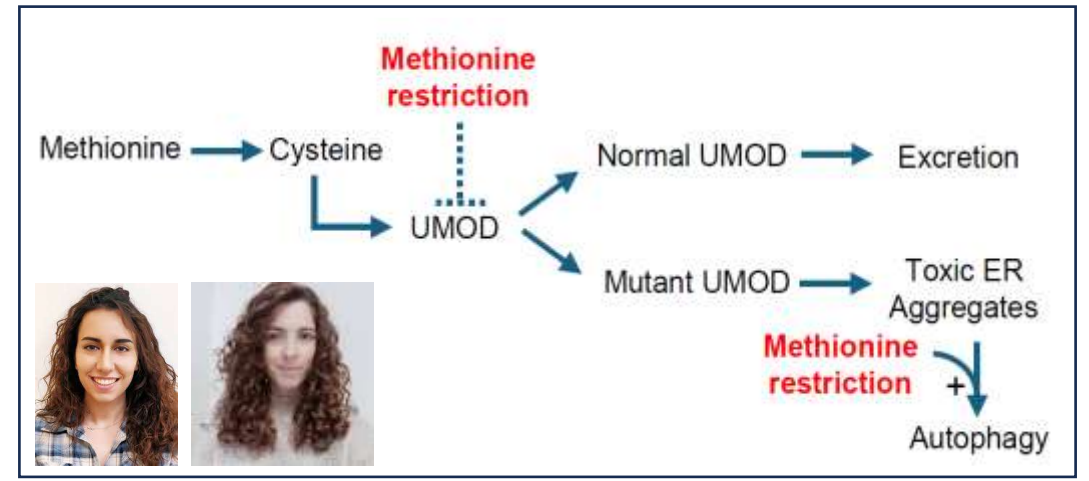


- Handling of NaCl:
  - Blood pressure
  - Urinary concentration
  - Loop diuretics
- Handling of Ca<sup>2+</sup> & Mg<sup>2+</sup>



- 640 AA, 48 cysteines (24 S-S), 7 N-glycosylation (25-30% carbohydrate content)
- GPI - Proteolytic cleavage → urine excretion & polymerisation → filaments

# Methionine Restriction to Modulate UMOD Production



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# Calorie Restriction Leads to Degradation of Mutant Uromodulin and Ameliorates Inflammation and Fibrosis in *UMOD*-Related Kidney Disease

Mariapia Giuditta Cratere <sup>1,2</sup>, Benedetta Perrone,<sup>1,2</sup> Barbara Canciani <sup>3</sup>, Céline Schaeffer <sup>1,2</sup> and Luca Rampoldi <sup>1,2</sup>

BASIC RESEARCH

[www.jasn.org](http://www.jasn.org)

## Food Restriction Ameliorates the Development of Polycystic Kidney Disease

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

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KIDNEY DISEASE

A kidney-specific fasting-mimicking diet induces podocyte reprogramming and restores renal function in glomerulopathy

Cell Reports  
**Medicine**

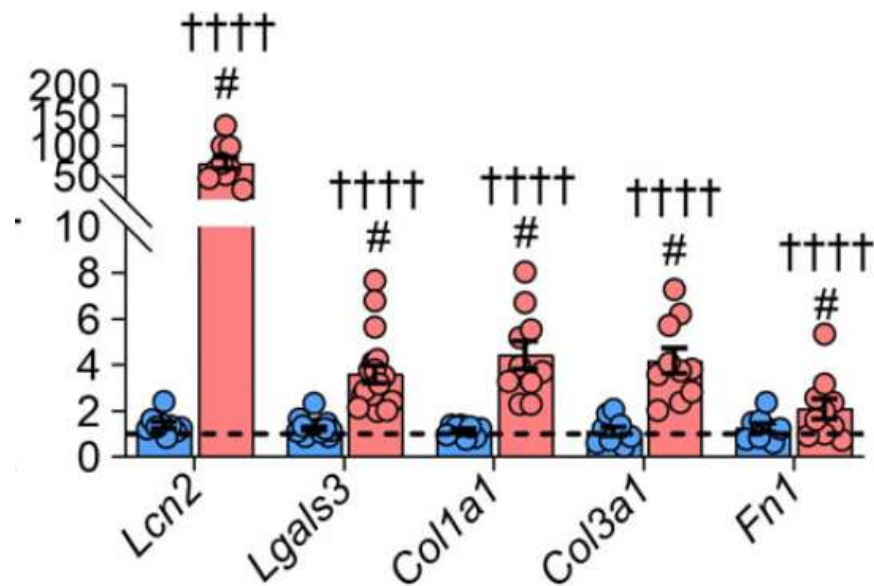
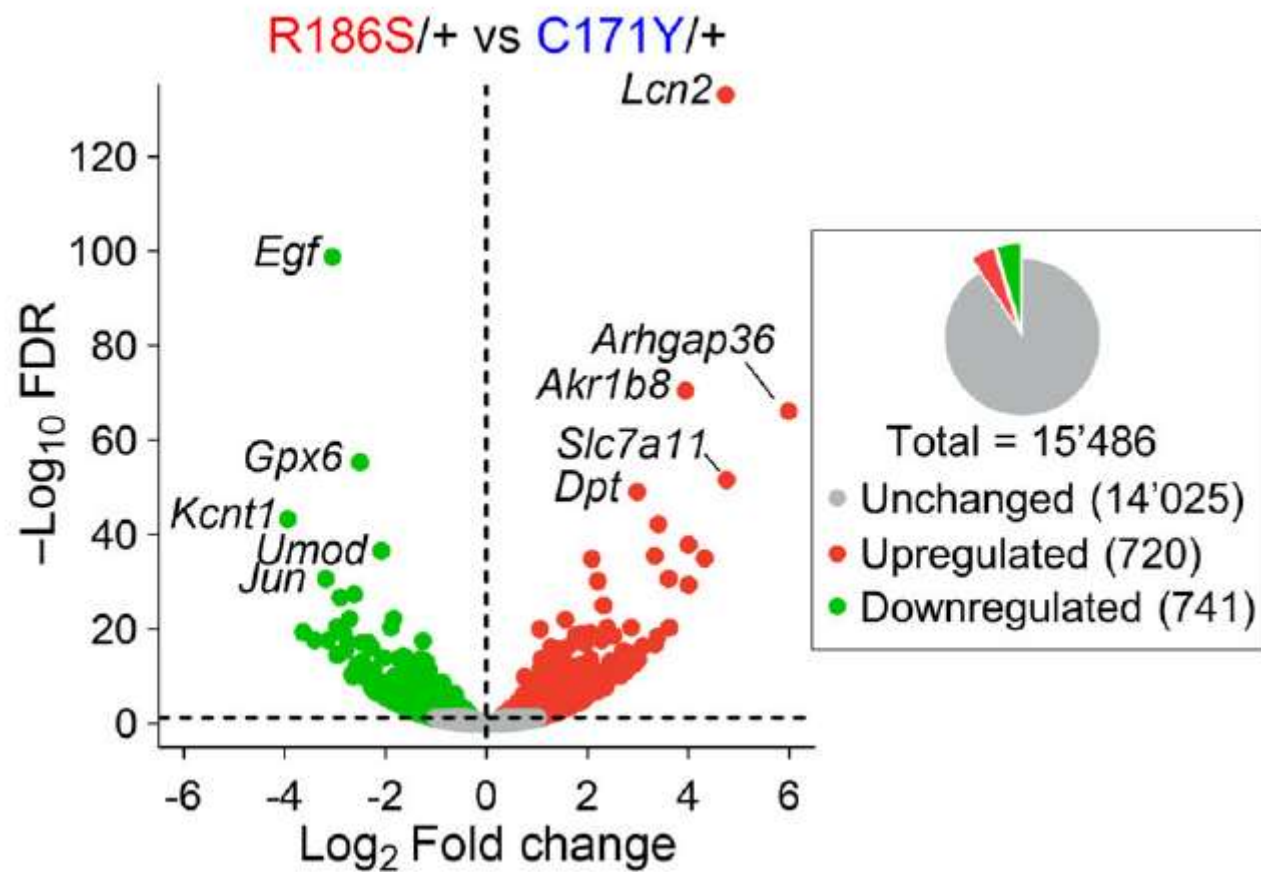
Article

**Feasibility and impact of ketogenic dietary interventions in polycystic kidney disease: KETO-ADPKD—a randomized controlled trial**

## ADTKD-UMOD: Other Therapeutic Perspectives

- Lipocalin-2 ?
- Structural biology: targeted degradation
- Cell biology: Screening program

# Lipocalin-2: Most upregulated transcript in *Umod* KI kidneys





ARTICLE

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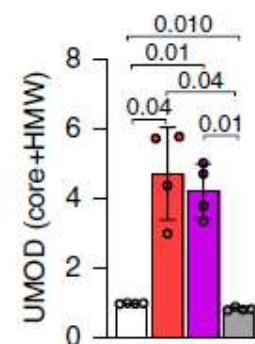
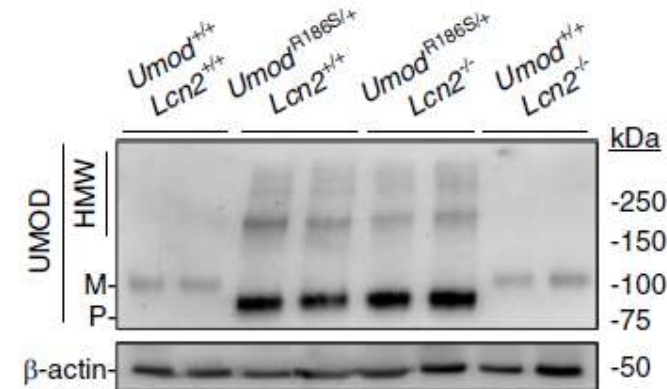
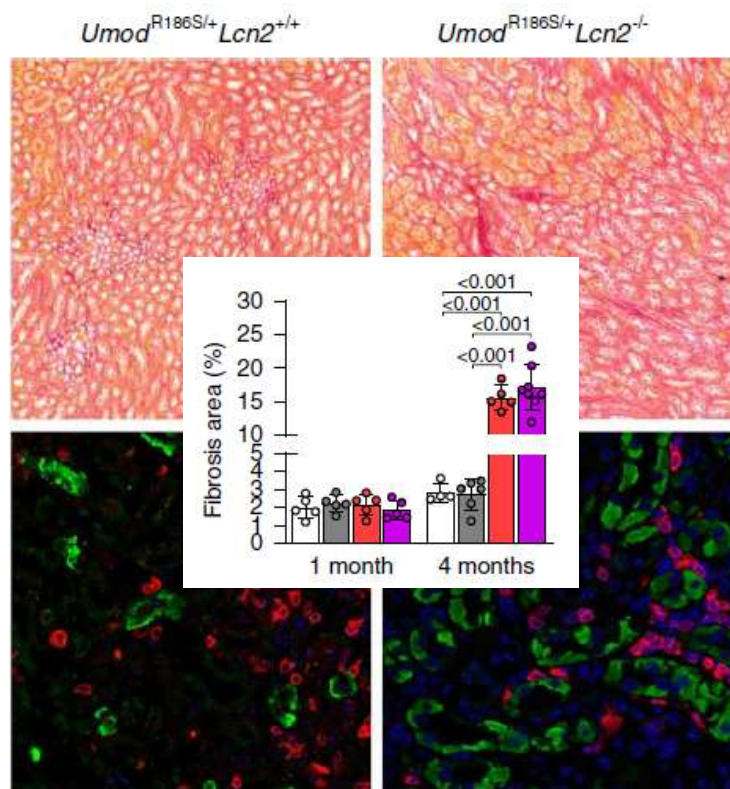
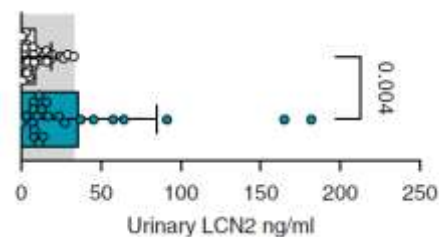
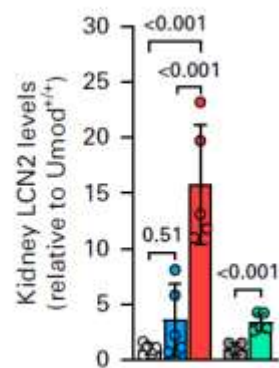
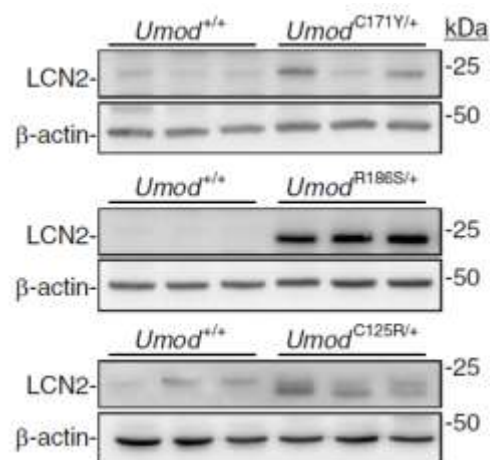
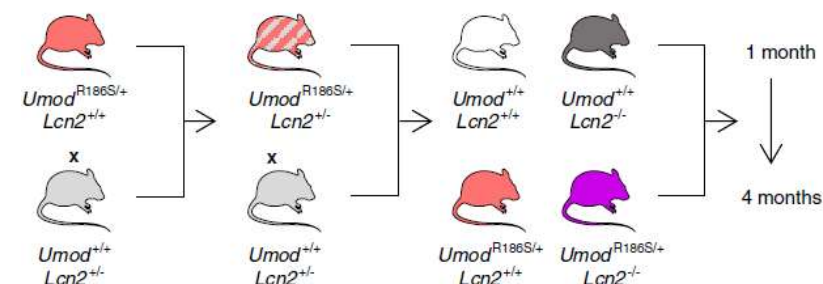
OPEN

# Endoplasmic reticulum stress drives proteinuria-induced kidney lesions via Lipocalin 2

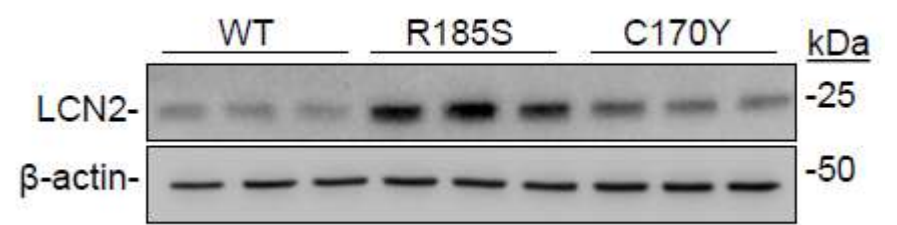
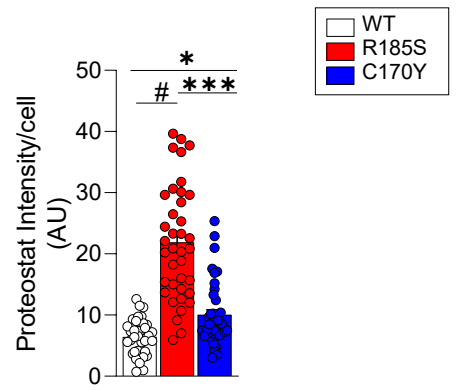
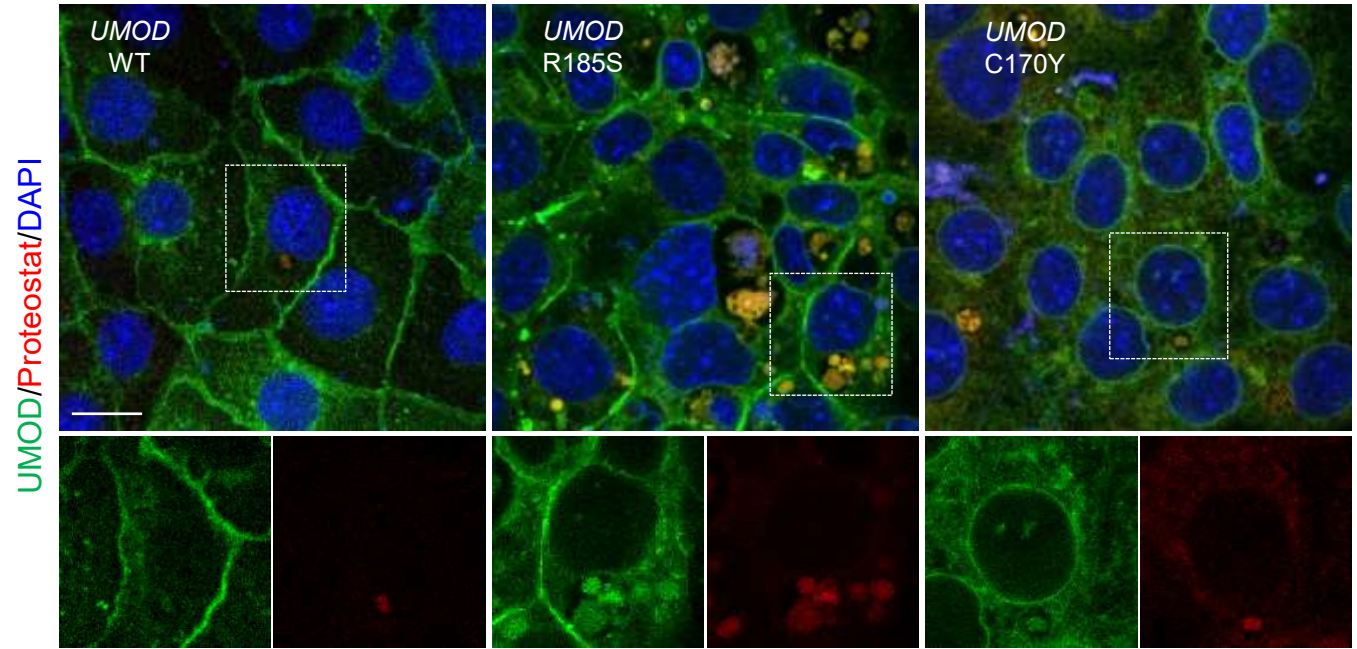
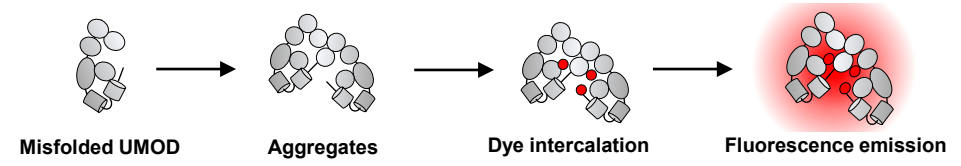
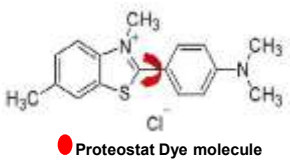
Khalil El Karoui<sup>1,2</sup>, Amandine Viau<sup>1</sup>, Olivier Dellis<sup>3</sup>, Alessia Bagattin<sup>4</sup>, Clément Nguyen<sup>1</sup>, William Baron<sup>1</sup>, Martine Burtin<sup>1</sup>, Mélanie Broueilh<sup>1</sup>, Laurence Heidet<sup>5</sup>, Géraldine Mollet<sup>5</sup>, Anne Druilhe<sup>1</sup>, Corinne Antignac<sup>5</sup>, Bertrand Knebelmann<sup>1</sup>, Gérard Friedlander<sup>1,2</sup>, Frank Bienaimé<sup>1,2</sup>, Morgan Gallazzini<sup>1,\*</sup> & Fabiola Terzi<sup>1,\*</sup>

# Lipocalin-2 Is Induced by Uromodulin Aggregates without Affecting Kidney Disease Progression in Autosomal Dominant Tubulointerstitial Kidney Disease–*UMOD*

Jennifer Lake <sup>1,2</sup>, Marta Mariniello <sup>1</sup>, Guglielmo Schiano <sup>1</sup>, Qin Guo,<sup>1</sup> Holly Mabillard,<sup>3</sup> John A. Sayer <sup>3,4,5</sup>, Eric Olinger <sup>6</sup>, Fabiola Terzi <sup>2</sup>, and Olivier Devuyst <sup>1,7</sup>



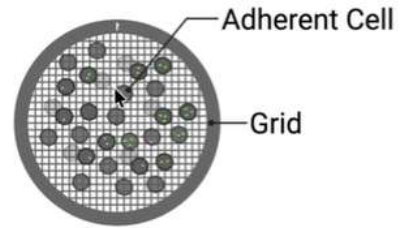
# Uromodulin Aggregates and ER Stress in mIMCD Cells



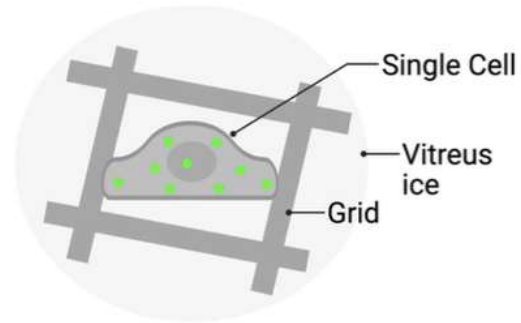
- Mutant uromodulin is detected in Proteostat<sup>+</sup> structures
- The Proteostat signal correlates with severity of epithelial damage

# *In situ LM-correlated cryo-ET workflow*

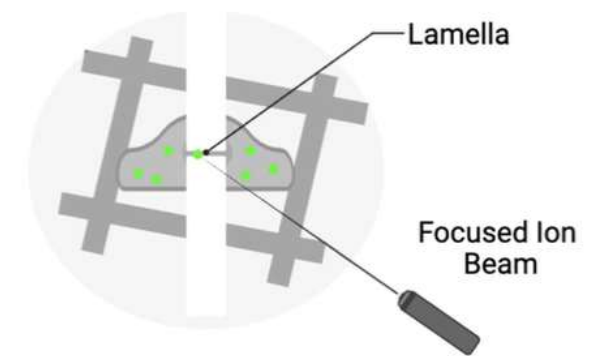
## 1.) Growing the cells on grids



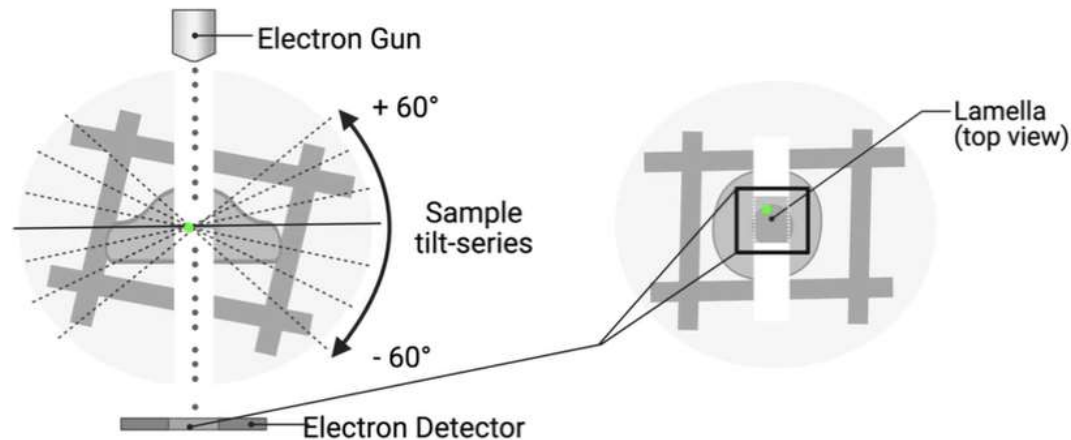
## 2.) Plunge freezing



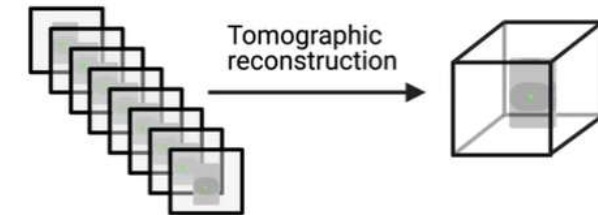
## 3.) FIB-milling



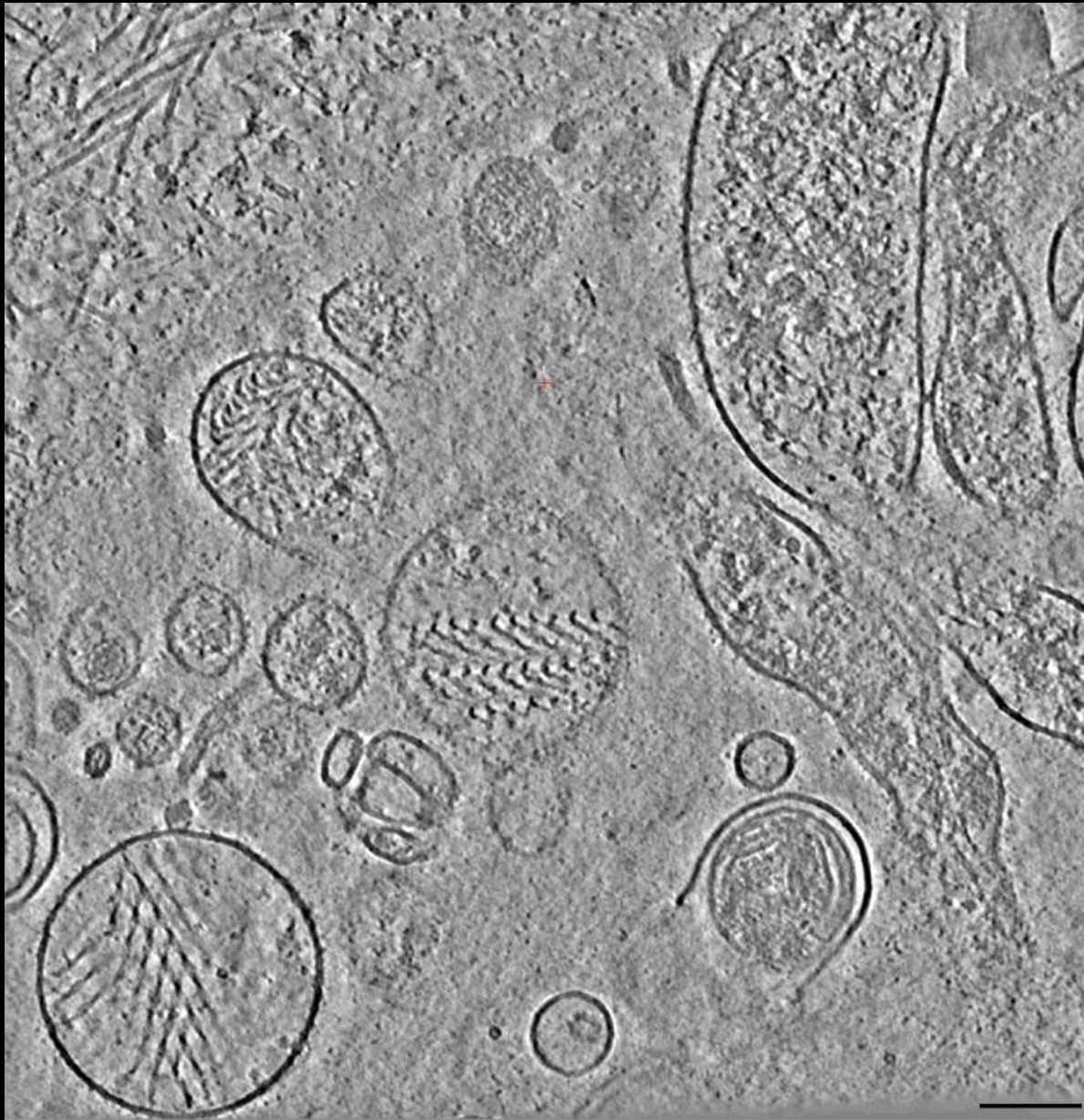
## 4.) Cryo-Electron Tomography



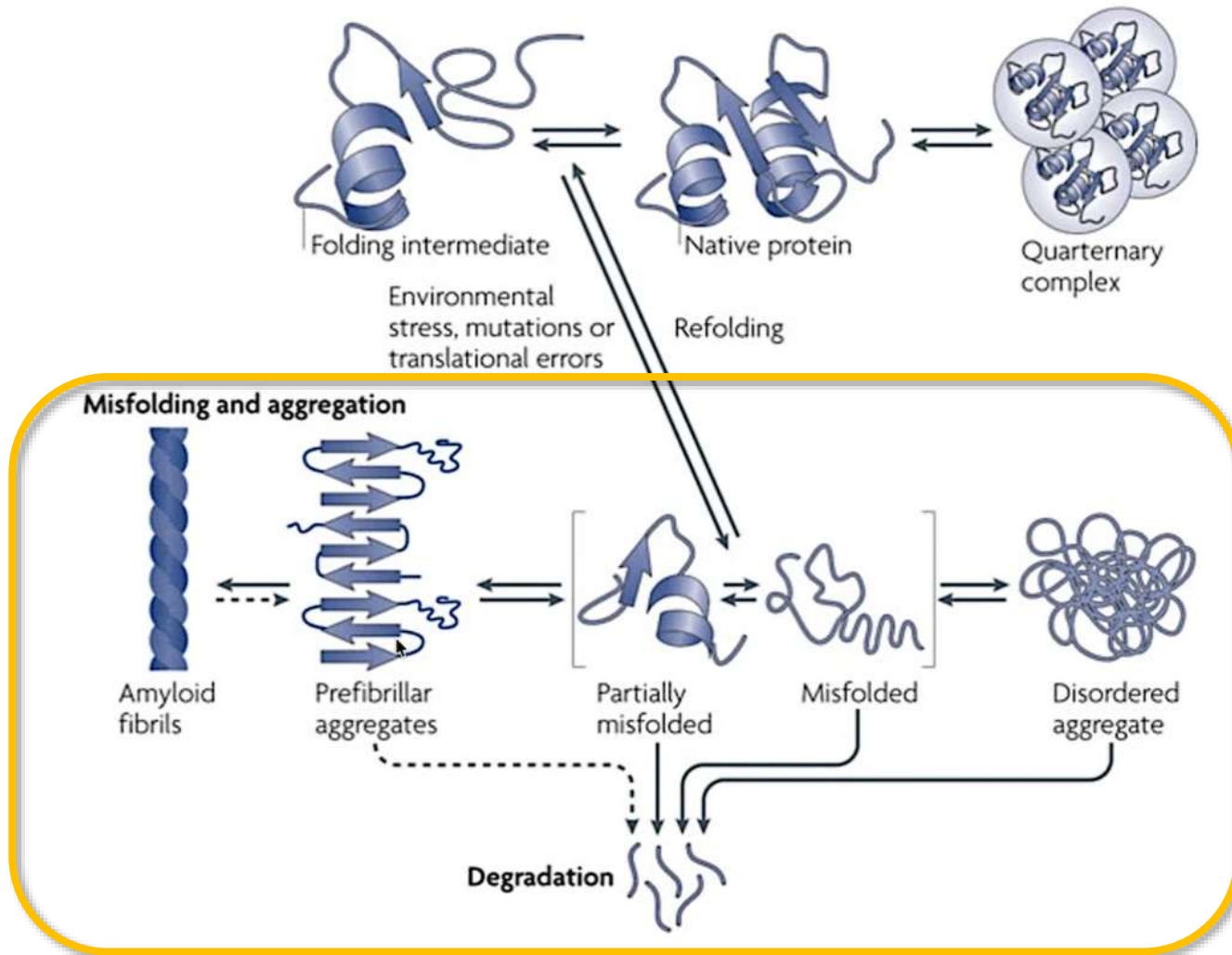
## 5.) Tomogram reconstruction



Tomograms: crystalline-like aggregates in vesicles - mIMCD



# Protein folding, misfolding and aggregation

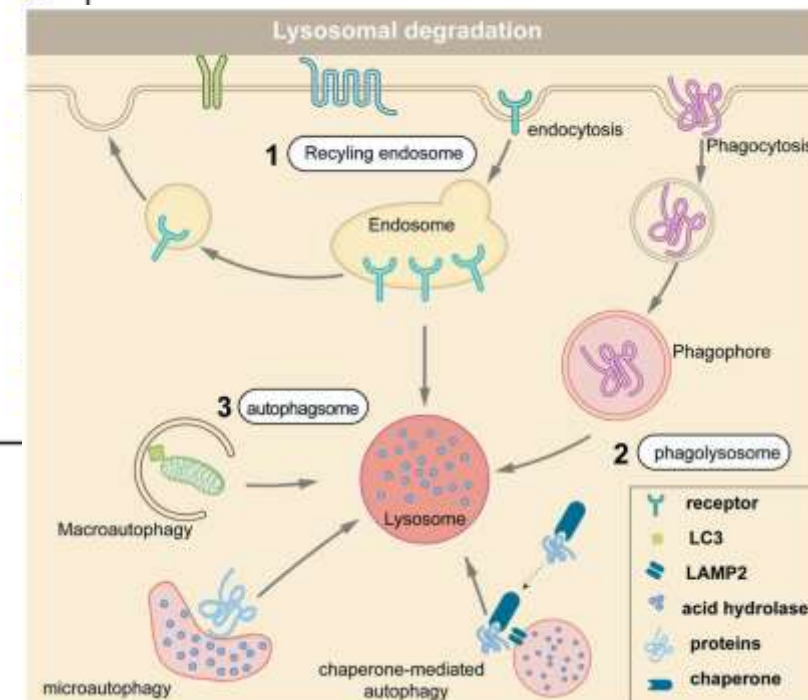
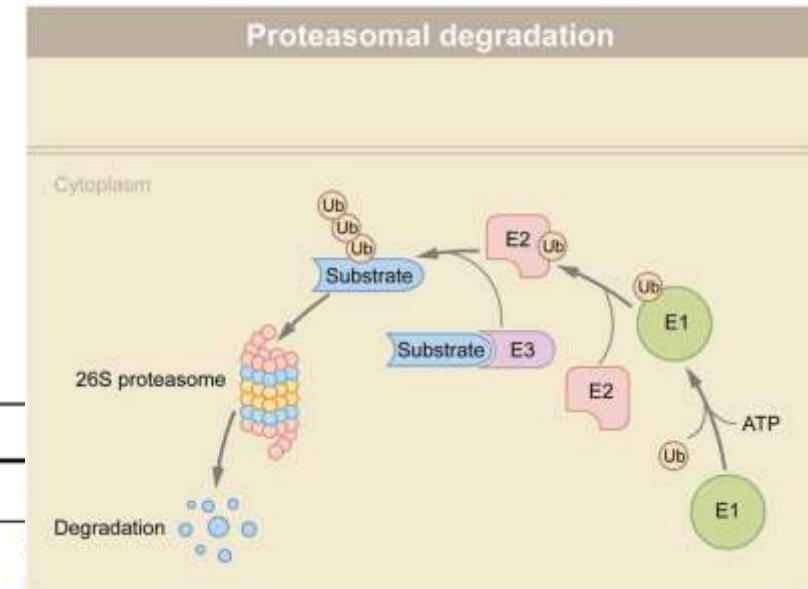


- Disordered aggregates form inclusion bodies (e.g. Lewy bodies)
- Amyloid fibrils vary in structure across disease

# Targeted protein degradation: mechanisms, strategies and application

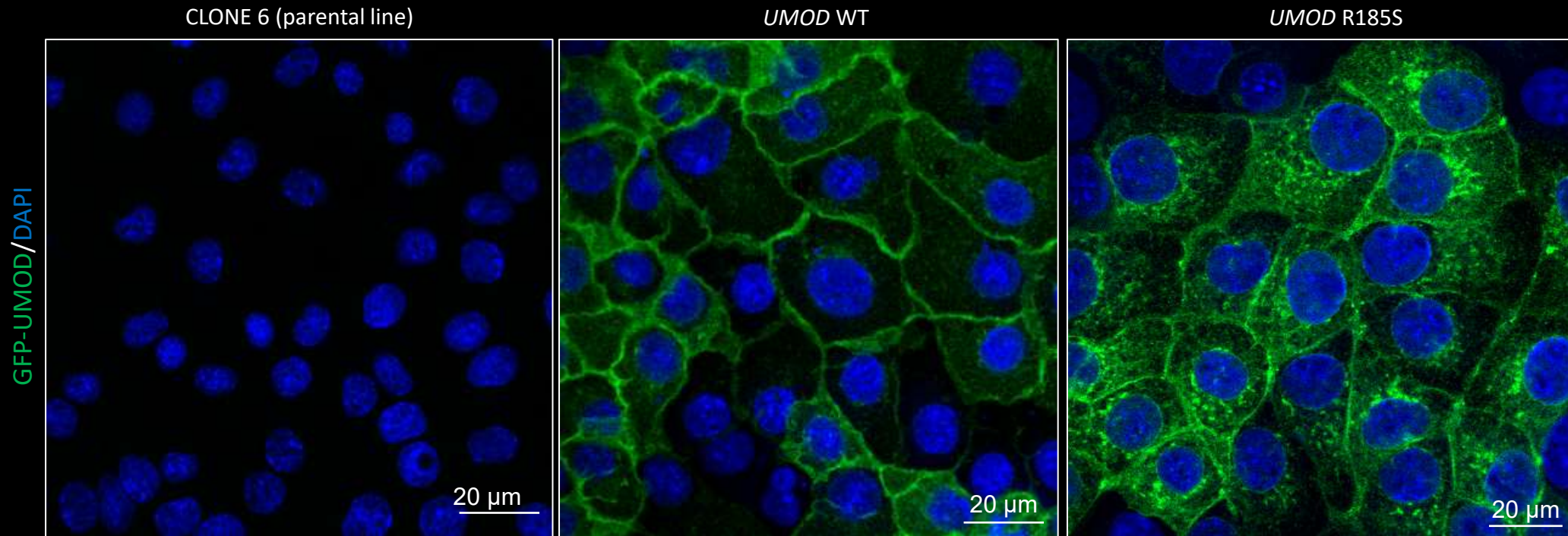
**Table 1.** Different TPD technologies

Degradation pathways	Degradation system	Technologies
Targeted protein degradation via proteasome	ubiquitin–proteasome system (UPS)	PROTAC Molecular glue Double-mechanism degrader PROTAC-based technologies: SARD, HyT, TF-PROTAC, dual-PROTAC, SERD
Targeted protein degradation via lysosome	endosome-lysosome system	LYTAC Bispecific Aptamer Chimera AbTAC GlueTAC
	autophagy-lysosome system	AUTAC ATTEC AUTOTAC CMA-based degrader



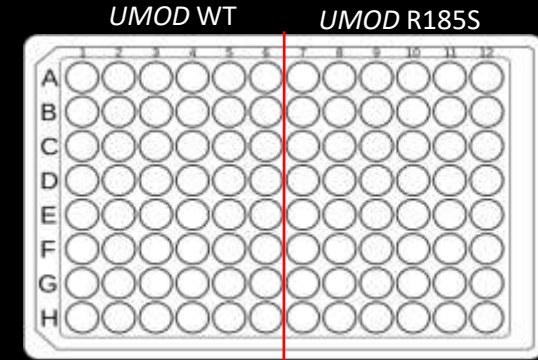
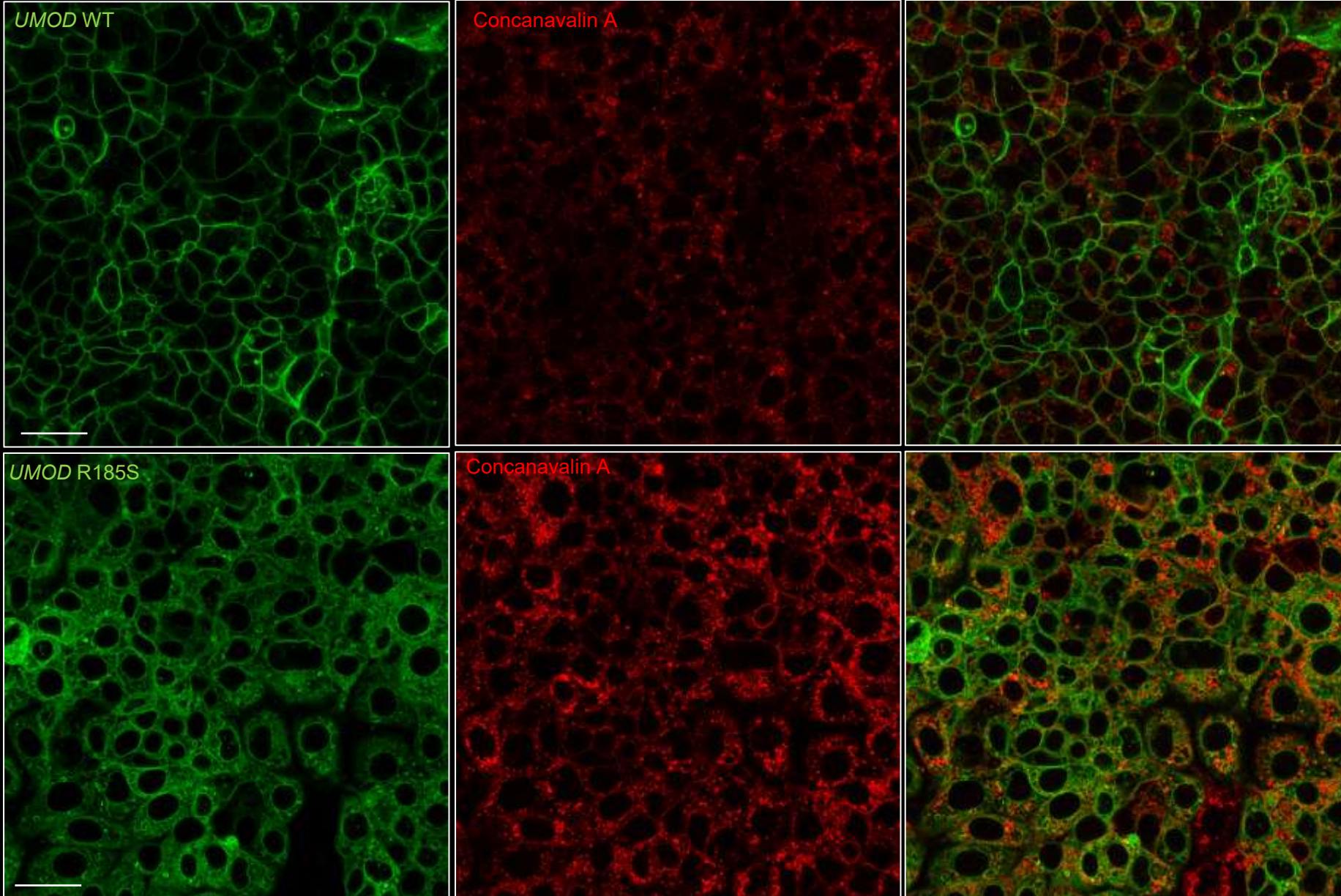
# Stable mIMCD Cell System - Mutant UMOD

- Human *UMOD* transgene
- Lentiviral vector (Lv) expressing SFFV viral promoter for stable transduction
- GFP tag placed at N-term (after the leader peptide) to be fused to UMOD
- UMOD R185S obtained by mutagenesis
- Adherent cells
- Easy to transfect or transduce with viruses- high duplication rate (48h)
- Stable expression of UMOD over time
- Possibility to polarize them on permeable transwell filters - UMOD secretion



# Trafficking defect in mutant UMOD cells

UMOD/CONCAVALIN A



- *UMOD* R185S cells exhibit increased Concanavalin A overlap, consistent with defective trafficking.

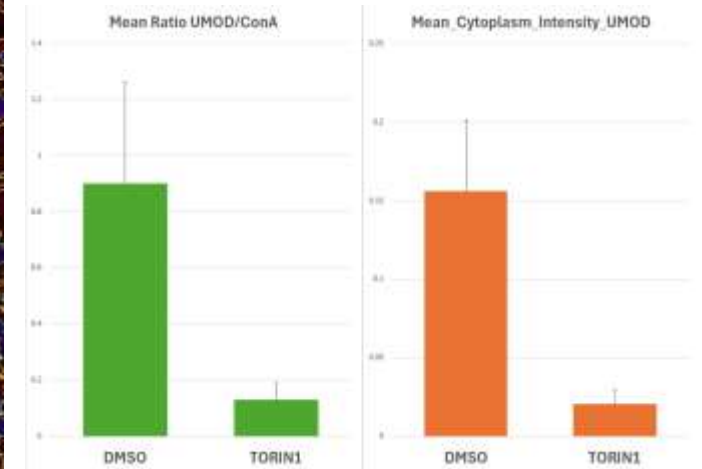
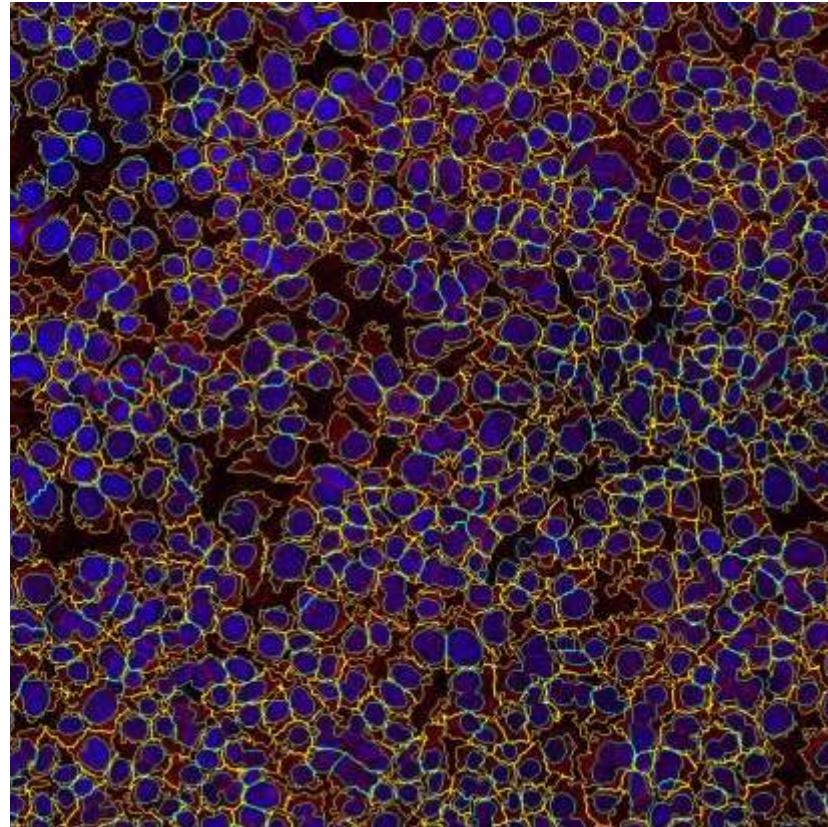
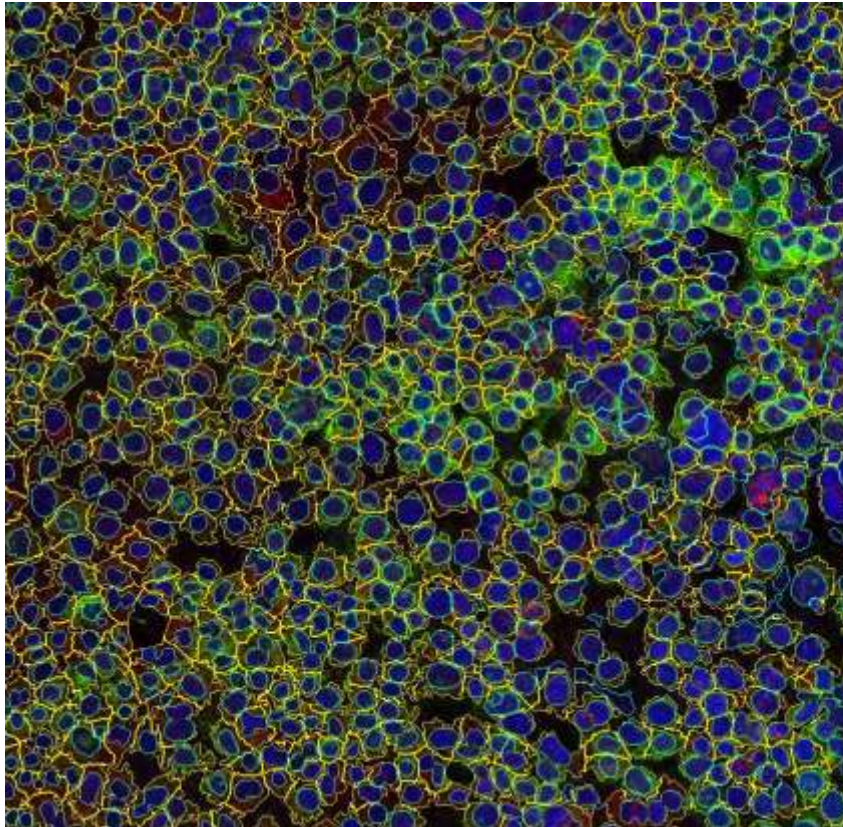
# 384-well Plate Test: Cell Segmentation and Positive Control

UMOD R185S

DMSO

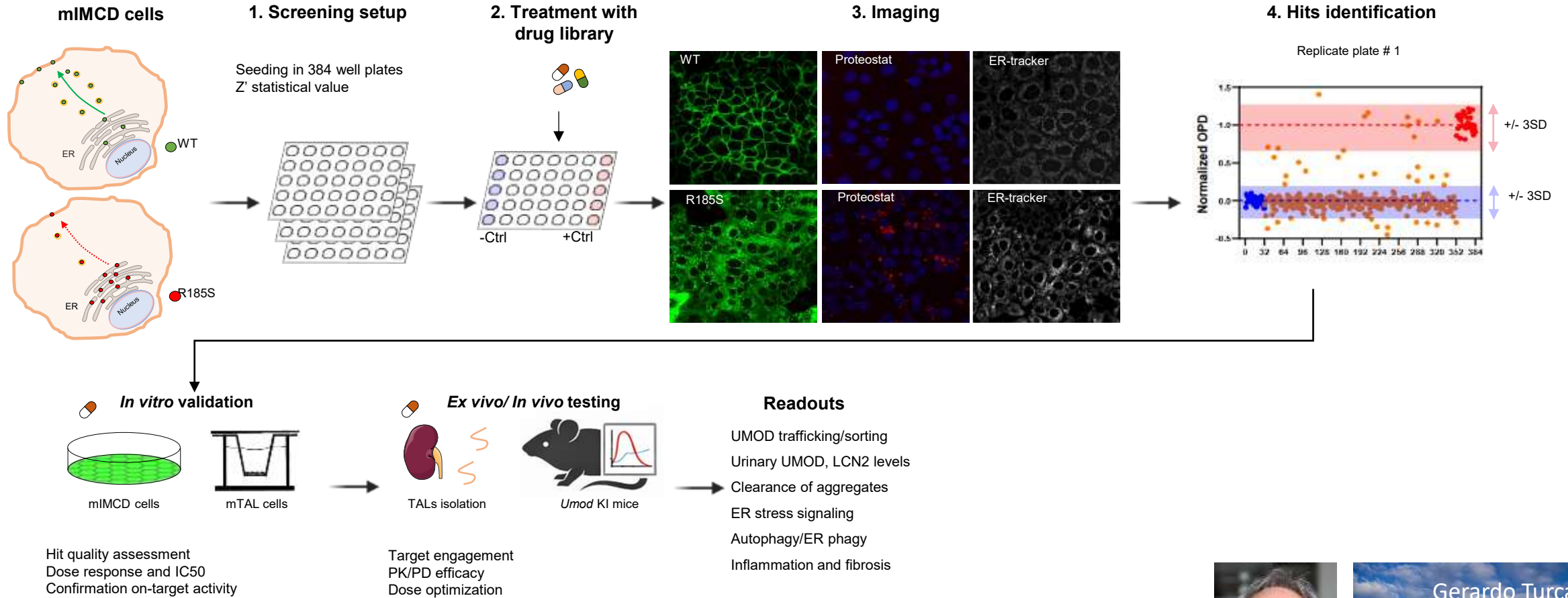
TORIN1

UMOD/CONCAVALIN A/DAPI



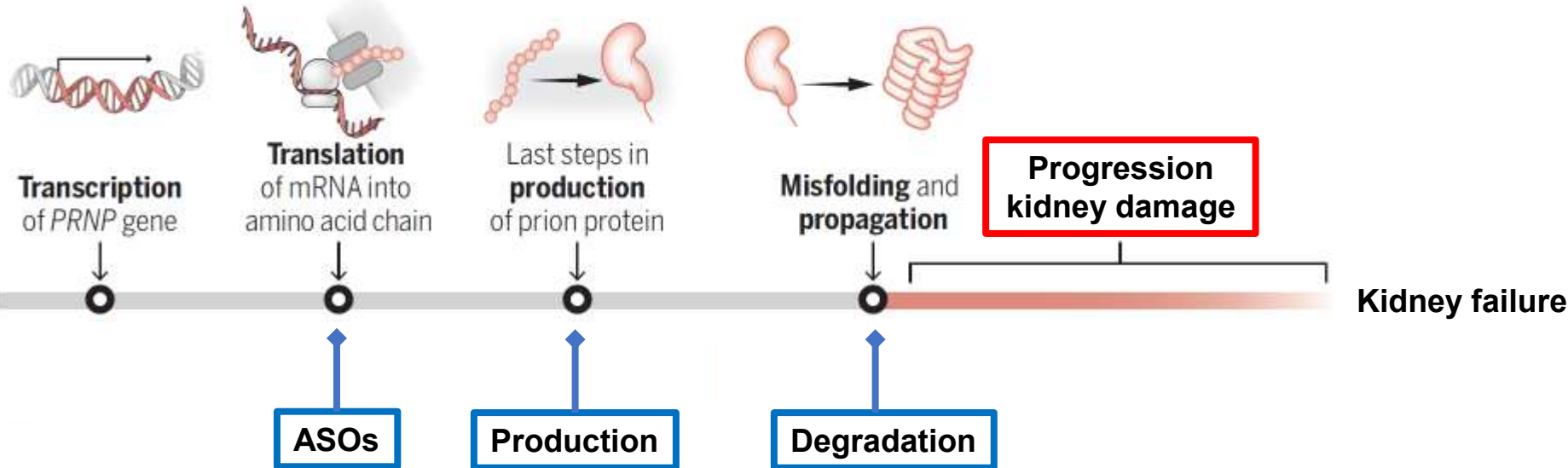
- 384-well plate: Mean Intensity Ratio of UMOD/Concanavalin A and Mean Intensity of UMOD alone.
- TORIN1 (positive control) decreases UMOD and the overlap with Conca.

# High-content Screening Workflow for Mutant UMOD



# ADTKD-UMOD: Global Strategy

## Cutting a protein pipeline





**University of  
Zurich** <sup>UZH</sup>

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