Proteomic identification of disease drivers in glomerular disease

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Conflicts of interest

- Research grant, Novo Nordisk A/S

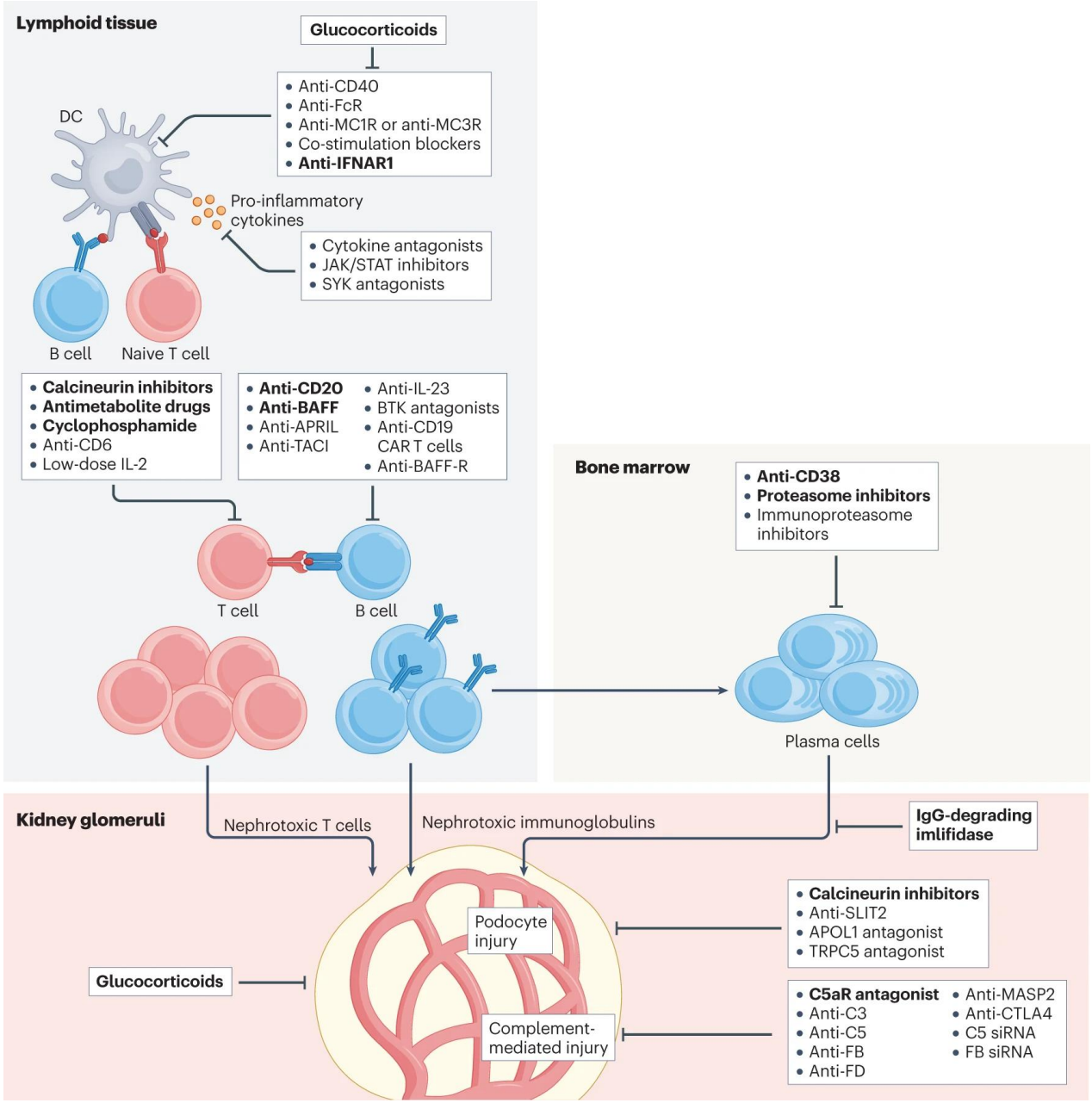
Cagrilintide’s actions in the kidney

- Research grant, Novo Nordisk A/S

Mechanisms of Glp1a in the kidney

- Patent: Endogenous complement inhibitor

Treating glomerular disease: a dynamic clinical landscape



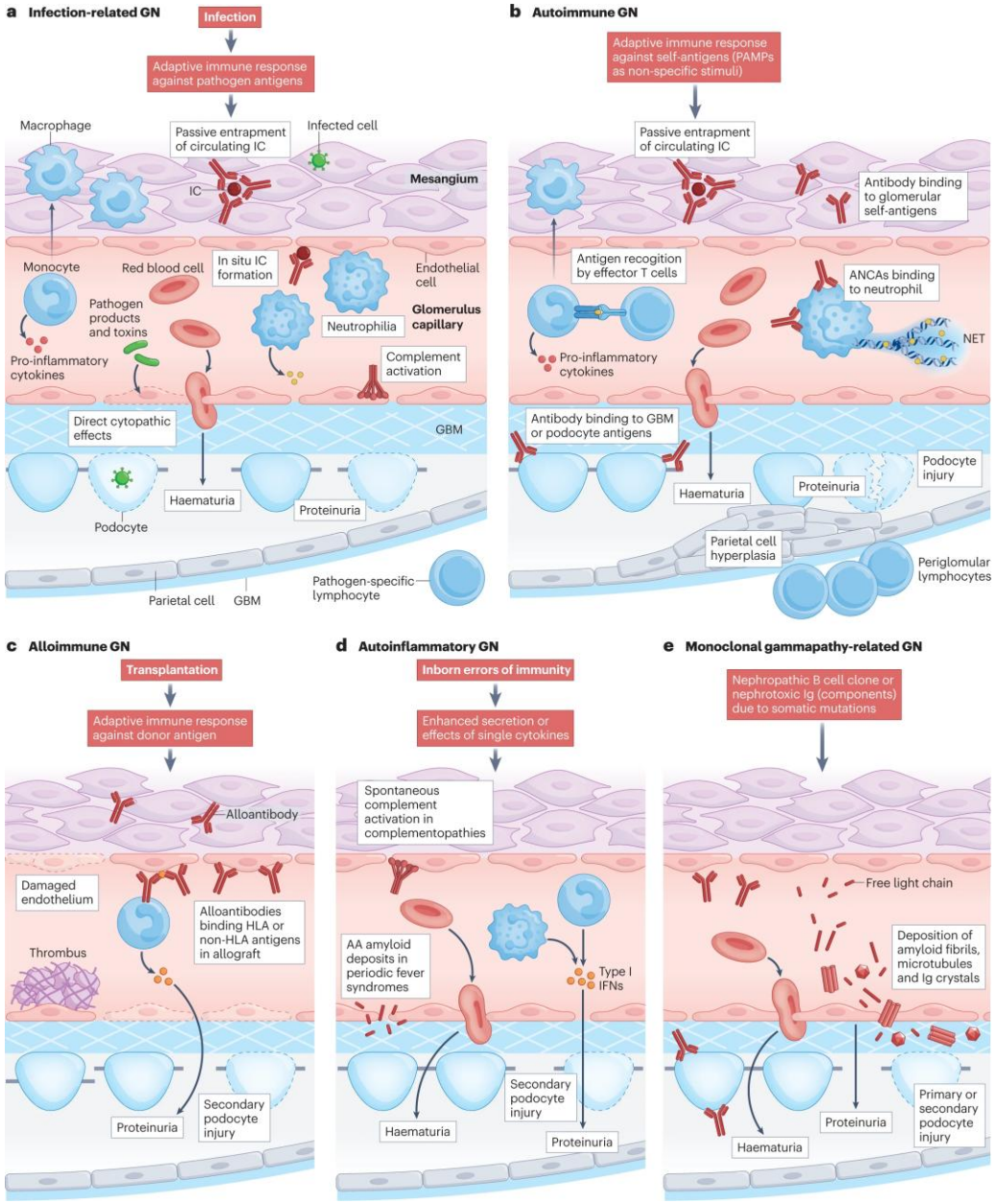
Anders et al. Nat Rev Nephrol 2023

Inflammatory glomerular diseases

- Significant advances to understand pathophysiology

Still challenges in molecular mechanisms

- Identify and monitor disease drivers
- Identify autoantigens in glomerular diseases



Anders et al. Nat Rev Nephrol 2023

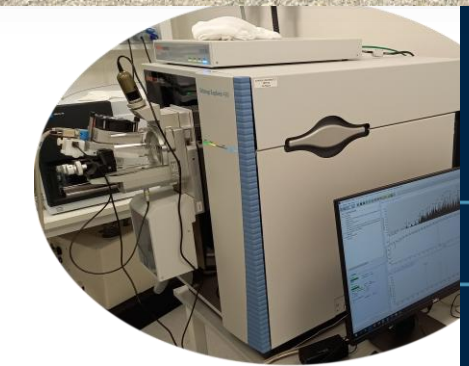
Today, I will tell you about

#1 Proteome-based approaches into identifying glomerular disease drivers in humans

#2 Identification of a novel bioactive complement fragment

… as demonstration how we can study our patients to learn new biology and pathophysiology!

Thank you to the team!



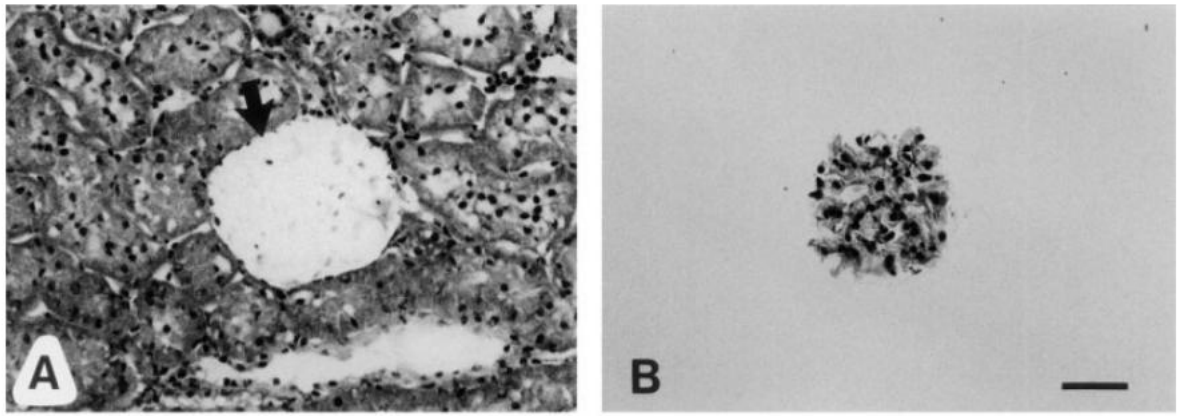
Kidney Omics Lab

Dybbøl Mølle/Dybbeler Schanzen

#1

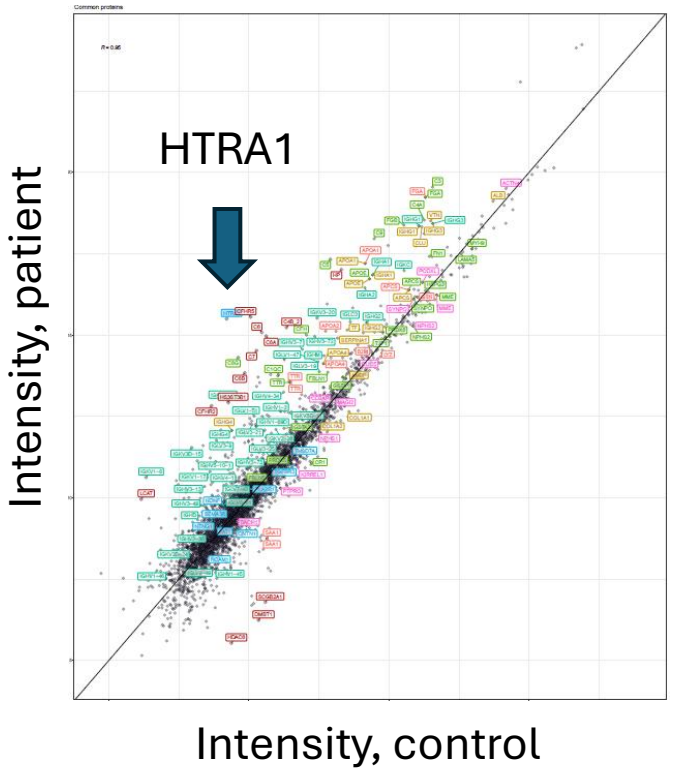
Identify disease drivers in biopsies

- Laser capture microdissection



Kohda et al. KI 2000

- Identification of protein deposits (membranous GN, amyloid, TMA)



Höhne et al. Kidney Int 2018

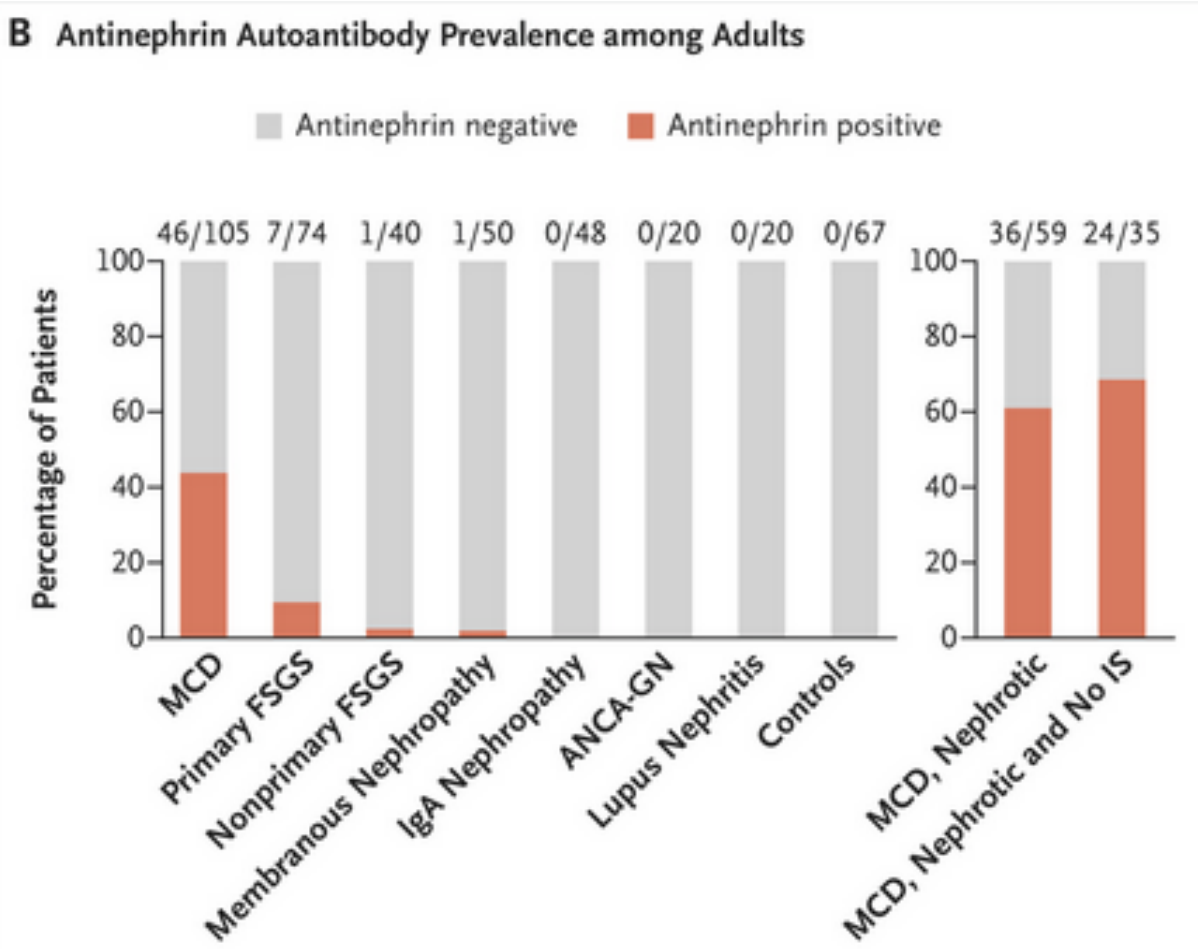
Identify the proteins engaged by patient autoantibodies

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Autoantibodies Targeting Nephrin in Podocytopathies

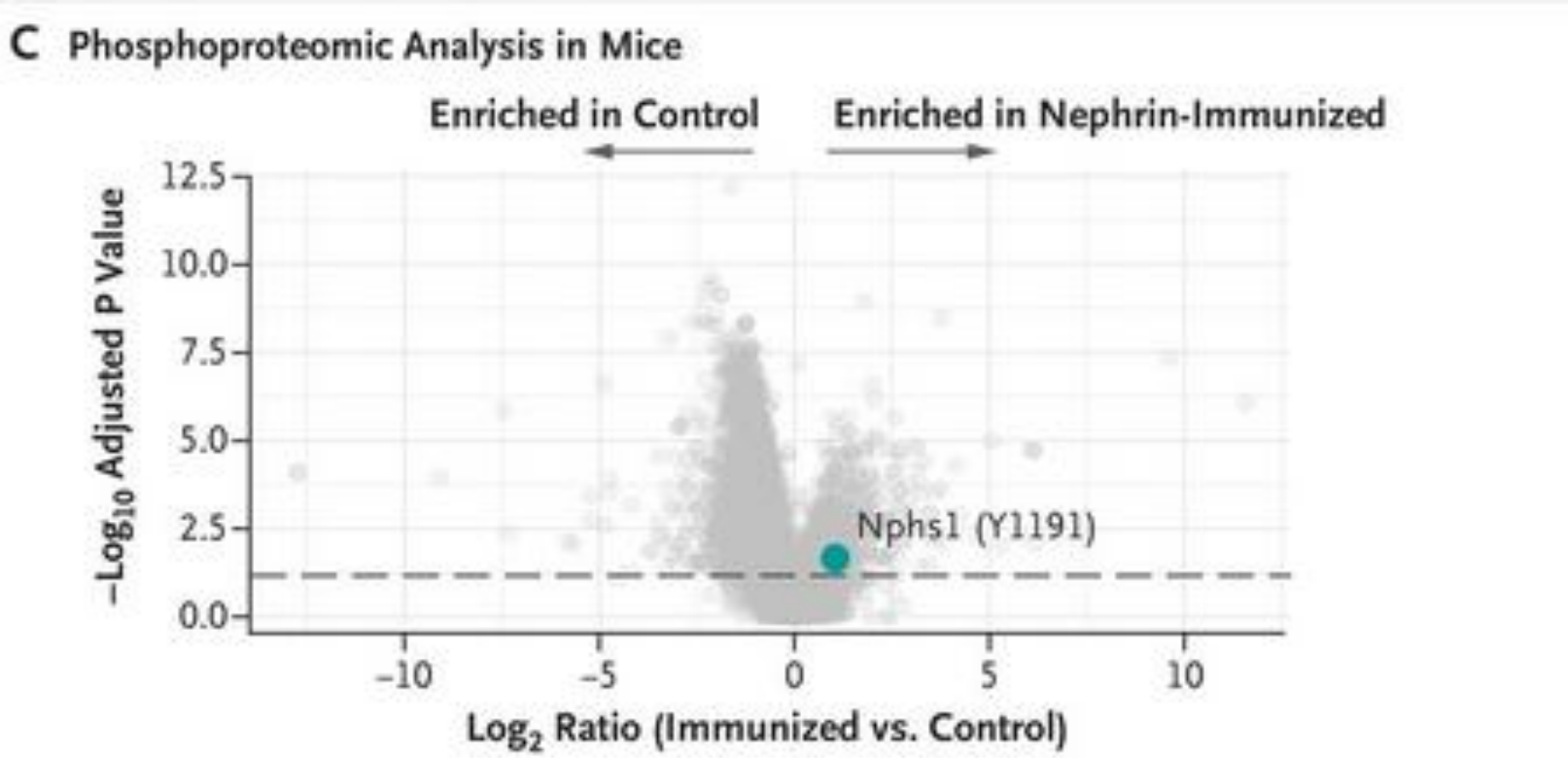
F.E. Hengel, S. Dehde, M. Lassé, G. Zahner, L. Seifert, A. Schnarre, O. Kretz, F. Demir, H.O. Pinnschmidt, F. Grahammer, R. Lucas, L.M. Mehner, T. Zimmermann, A.M. Billing, J. Oh, A. Mitrotti, P. Pontrelli, H. Debiec, C. Dossier, M. Colucci, F. Emma, W.E. Smoyer, A. Weins, F. Schaefer, N. Alachkar, A. Diemert, J. Hogan, E. Hoxha, T. Wiech, M.M. Rinschen, P. Ronco, M. Vivarelli, L. Gesualdo, N.M. Tomas, and T.B. Huber, for the International Society of Glomerular Disease



Hengel et al. NEJM 2024

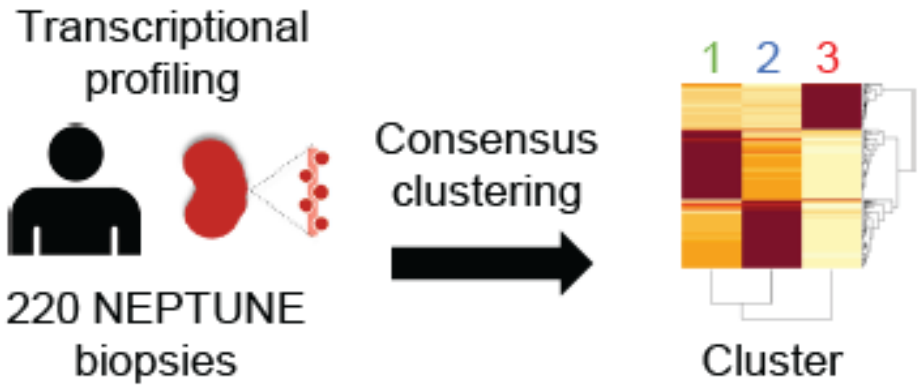
Monitor signaling induced by molecules

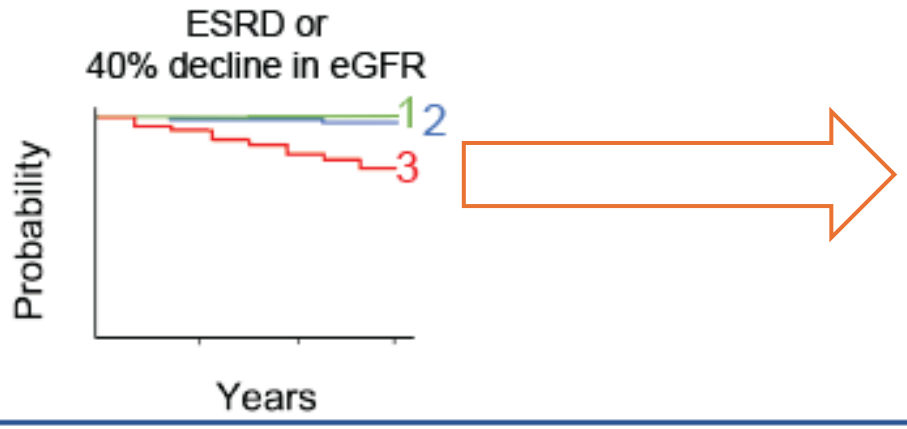
Mouse in vivo – Nephrin antibodies – glomerular phosphoproteomics



Hengel et al. NEJM 2024

Nominate disease drivers and test responses





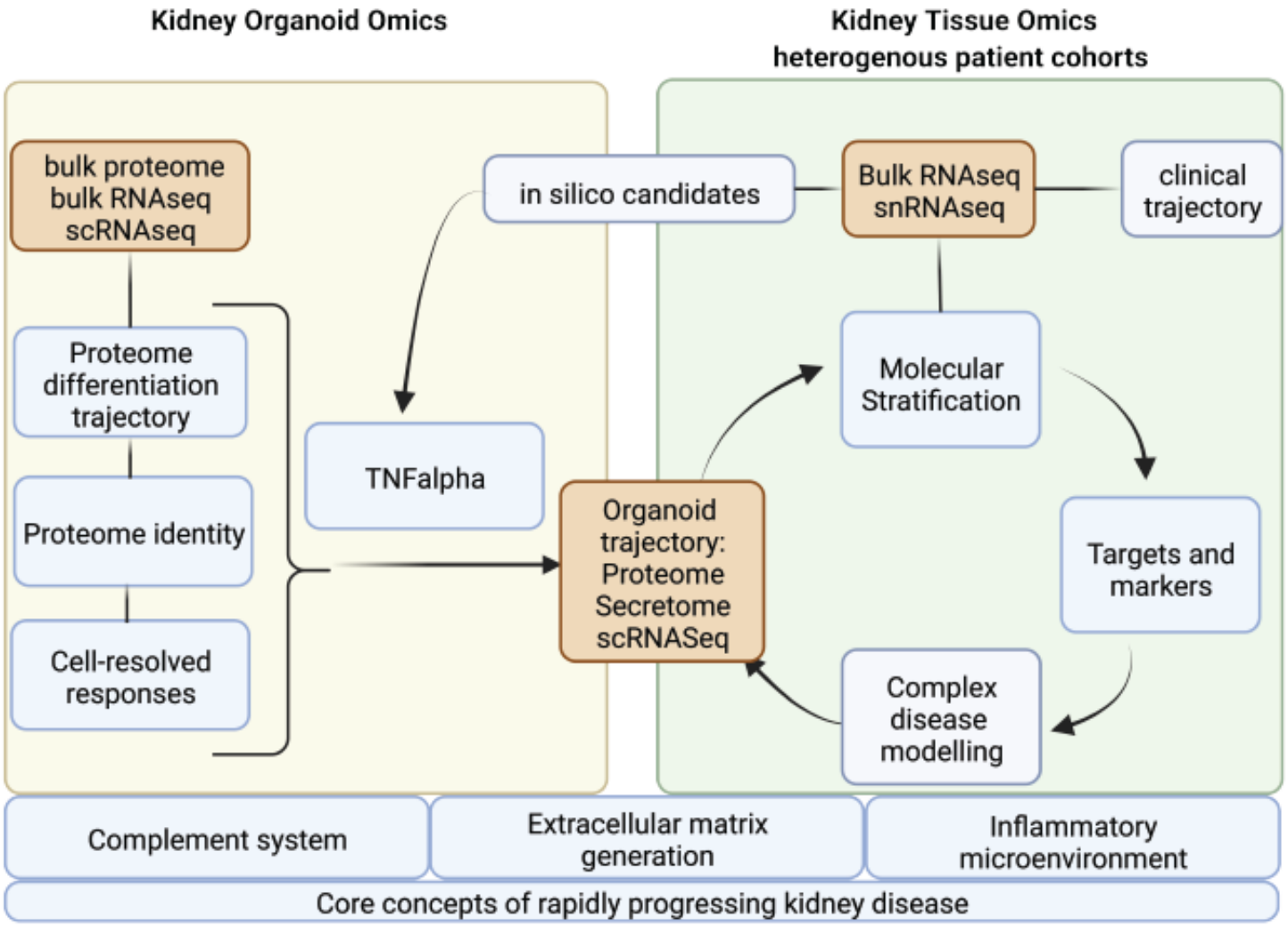
Cluster 1 +2: Slow progressors

Cluster 3: Fast progressors

Tissue omics data available

Lassé et al. Nat Comm 2023

Monitor signaling induced by molecules



Lassé et al. Nat Comm 2023

Conclusions part 1: Identify disease drivers

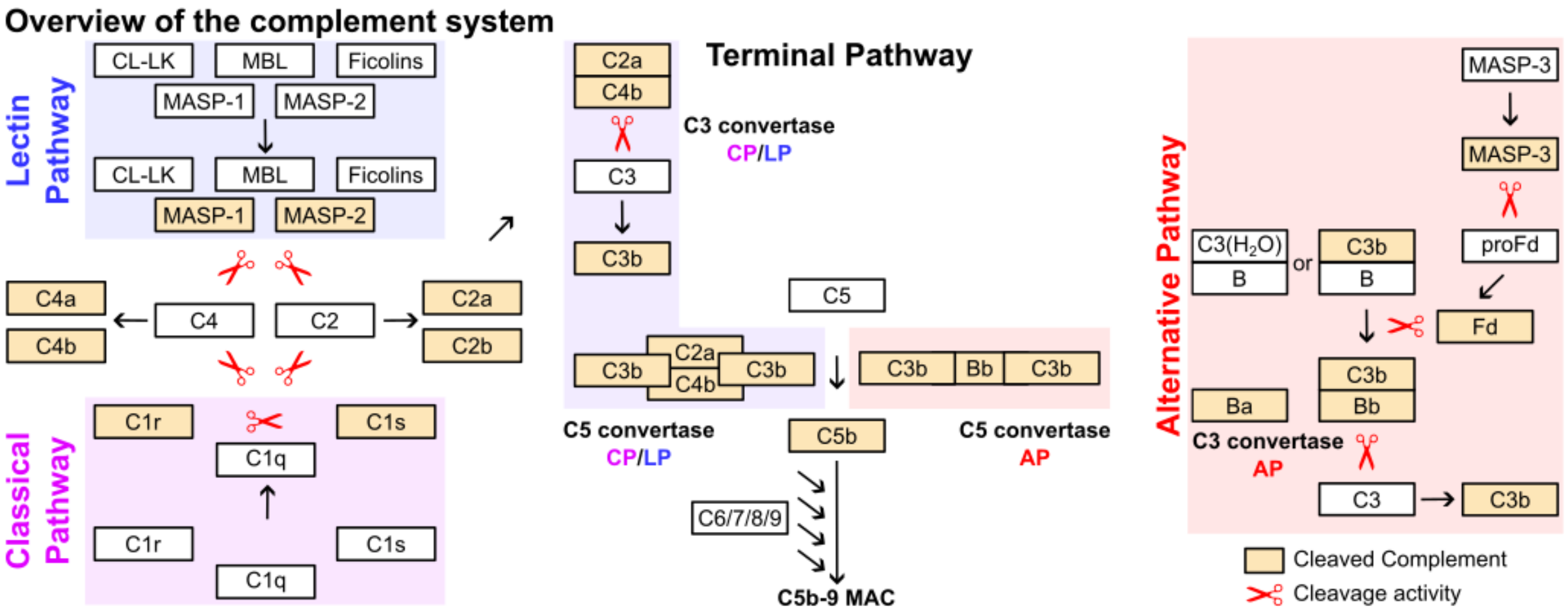
The tool box of glomerular proteome analysis covers markers, auto-antigens, signaling processes

- protein deposition in biopsies
- to understand affinity of circulating molecules
- to discover target engagement

#2

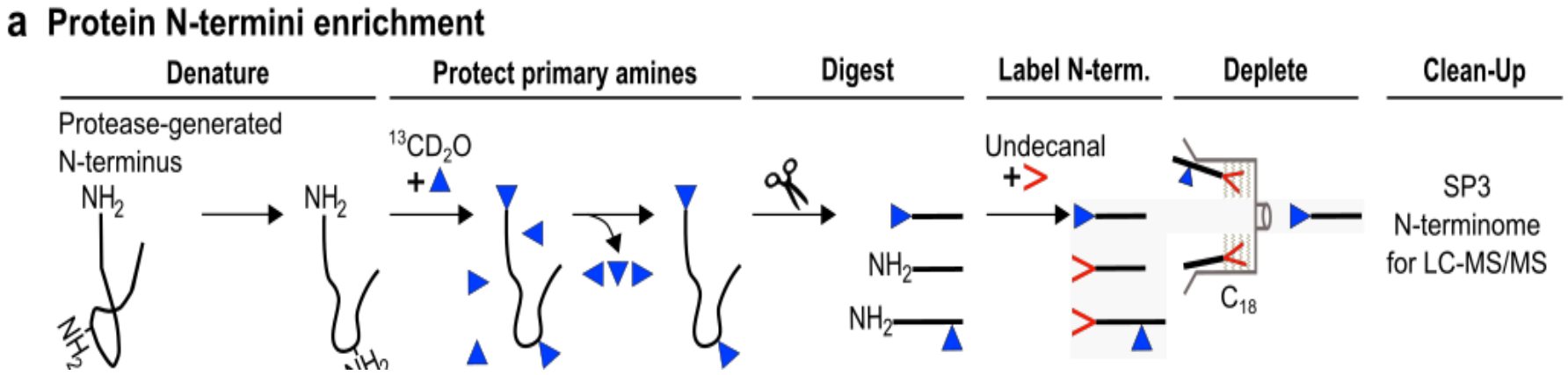
Identification of a potential circulating disease driver.

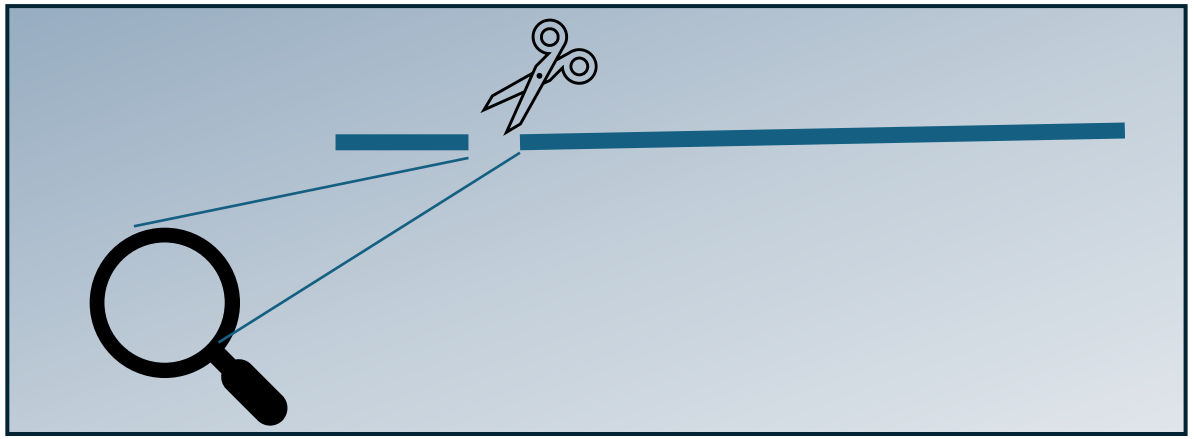
Proteolytic activation of the human complement system



Can we measure fragments directly with mass spectrometry?

Improving N-terminal analyses for cohorts



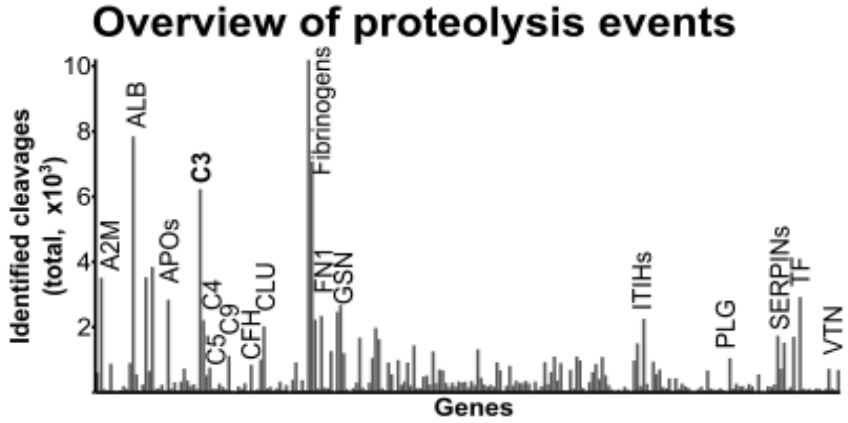


Scalable through automation

Heterogeneity of Lupus patients

Cross-sectional setup

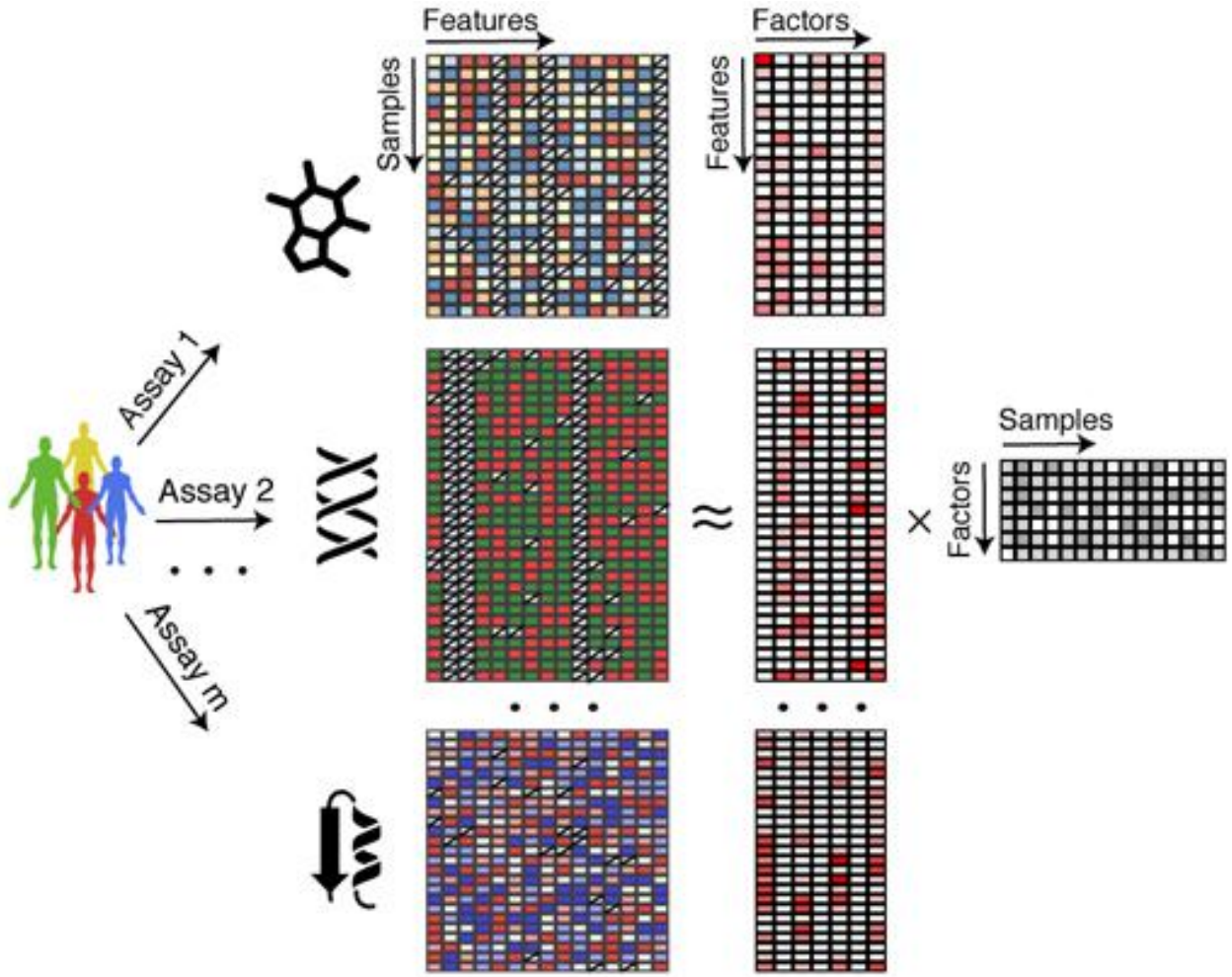
- n=143 patients
- 91.6% female
- mean age 45.3 ± 14.5
- mean SLEDAI disease score 3.8 ± 2.1
- 32.9% lupus nephritis



- More than 10 000 proteolytic events
- EDTA-stabilized plasma ----- <u>never thawed before!</u>

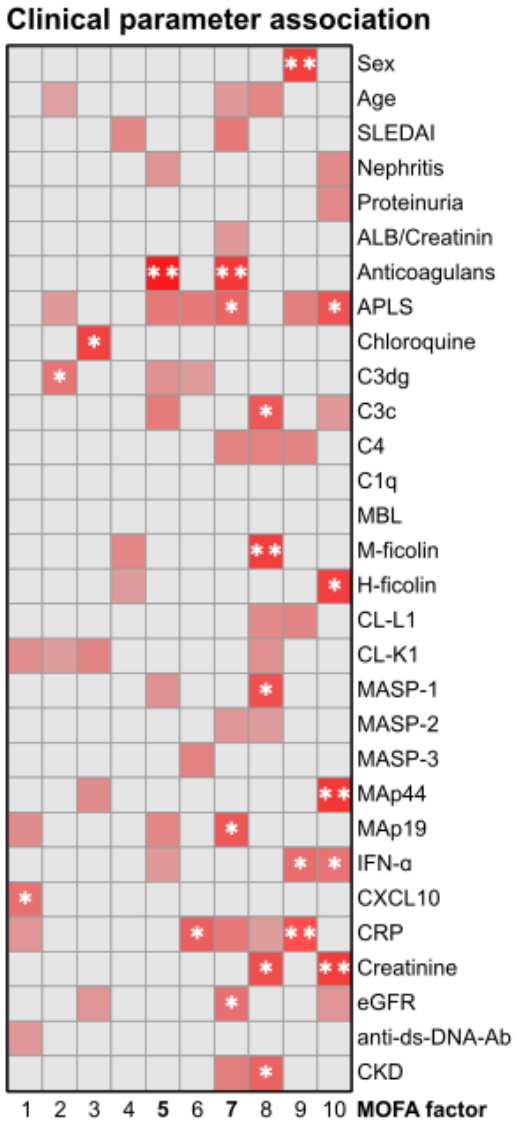
Can we take advantage of the patient heterogeneity?

- 143 lupus patients



Multi-omics factor analysis: 143 lupus patients

N-terminome associated with kidney parameters

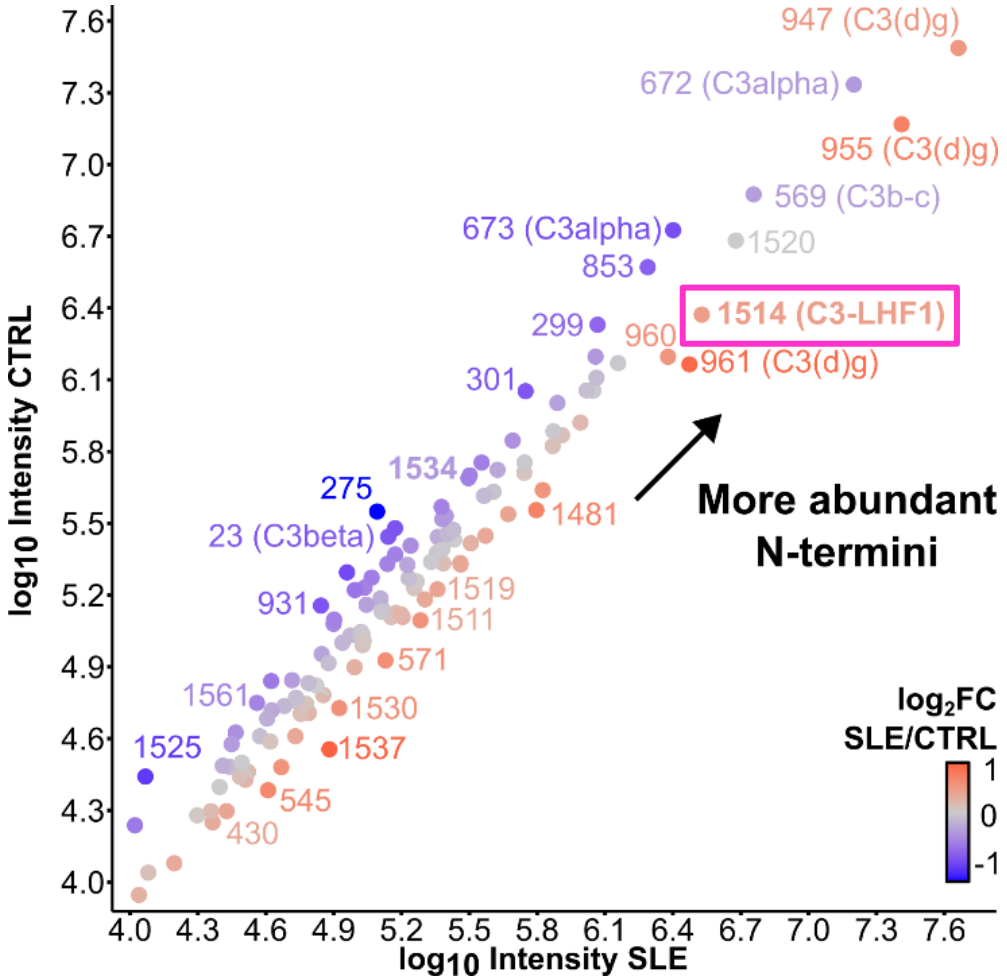


N-terminome driven factors:

Factor 3,5,7

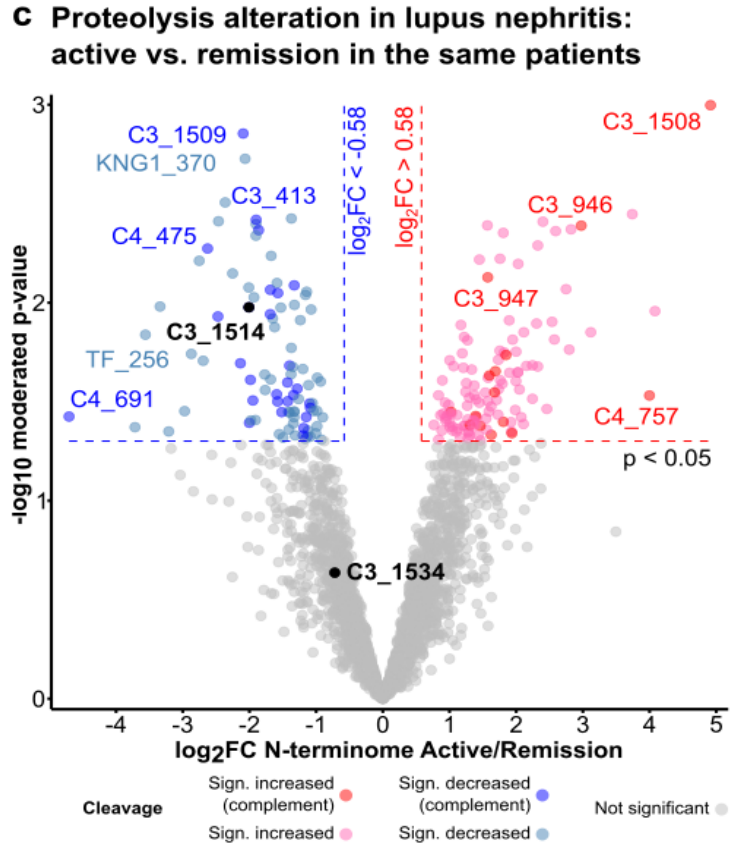
- Associate with GFR, proteinuria, nephritis status
- One of the termini: C3-1514

A new C3 fragment is highly abundant



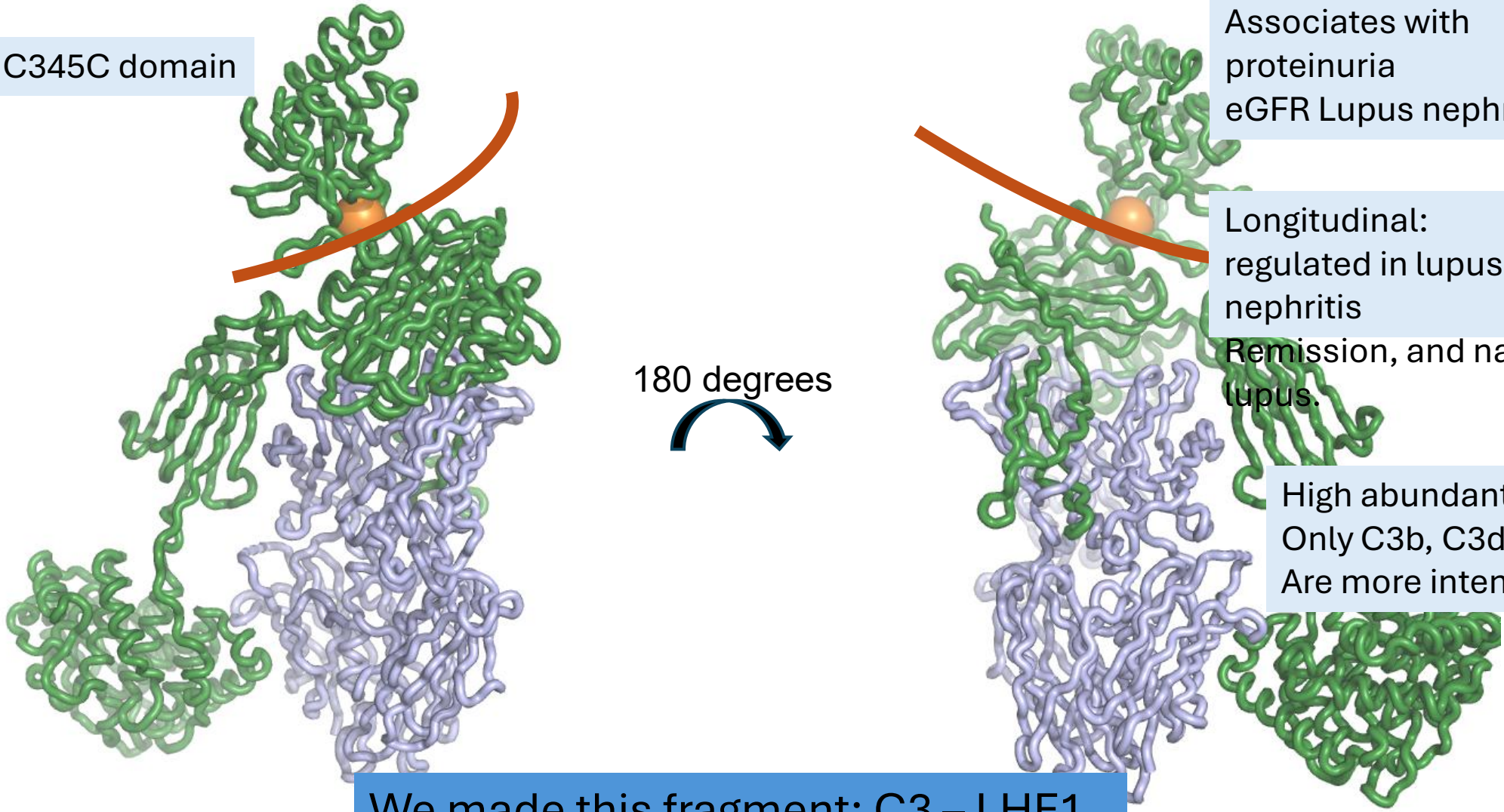
Lupus nephritis vs remission

- Plasma from patients with active LN
- Same patients with remission (KDIGO)
- C3-1514 was regulated in remission



Simon Melderis, Oliver Steinmetz

A prioritized cleavage site in C3: C3-1514



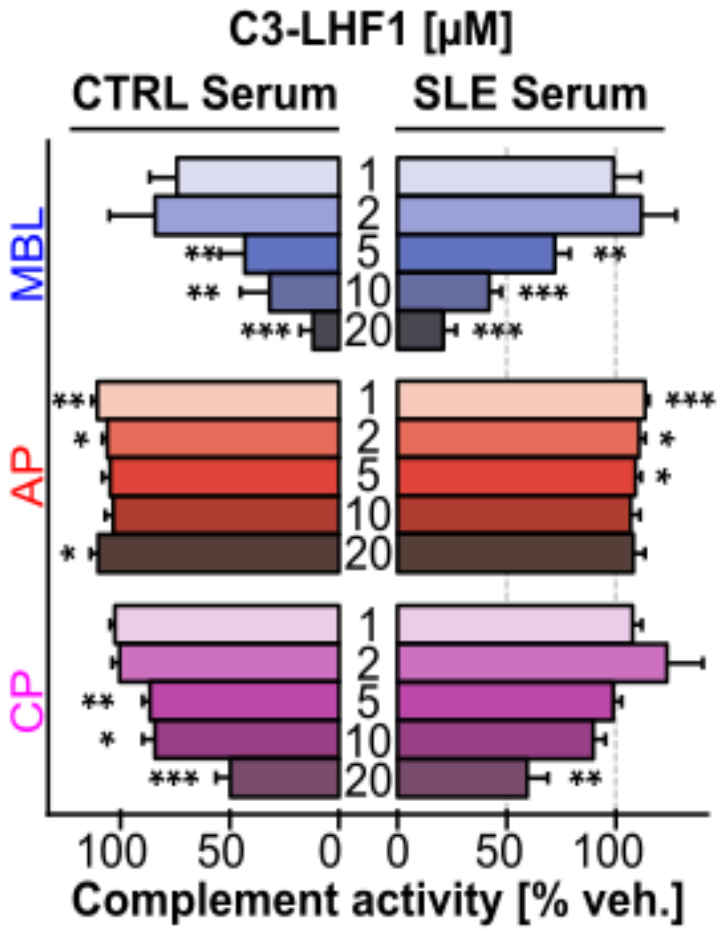
Cross-sectional:
Associates with proteinuria
eGFR Lupus nephritis

Longitudinal:
regulated in lupus nephritis
Remission, and naïve lupus.

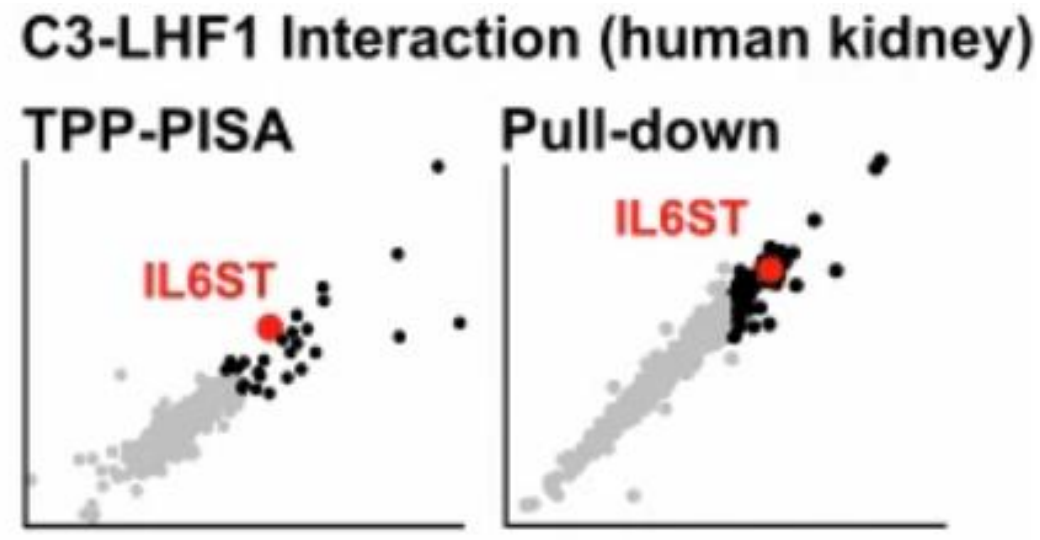
High abundant
Only C3b, C3dg and C3a
Are more intense

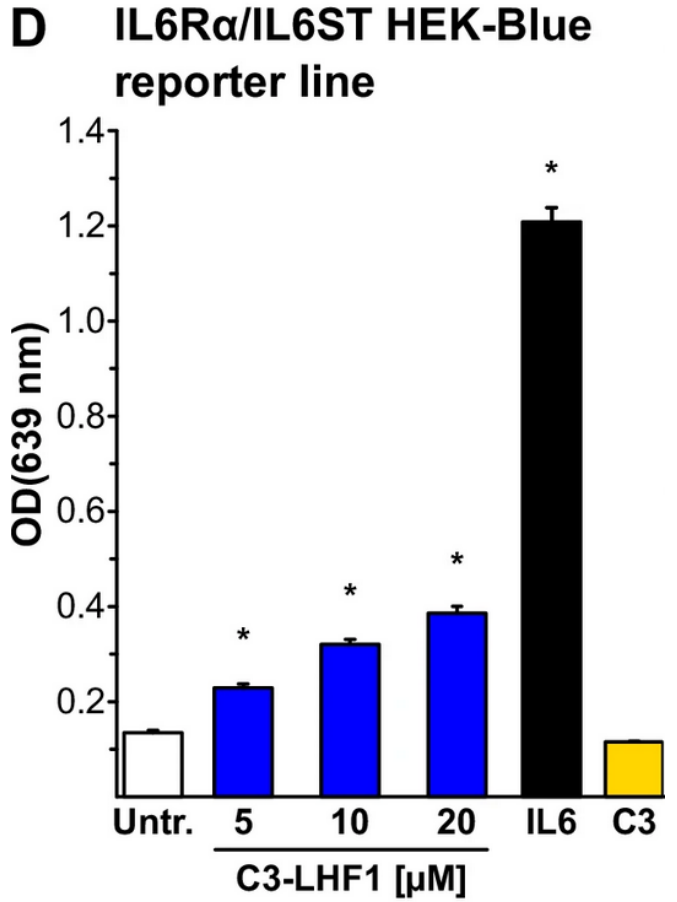
We made this fragment: C3 – LHF1

C3-LHF1 inhibits complement mannose binding lectin (MBL) pathway



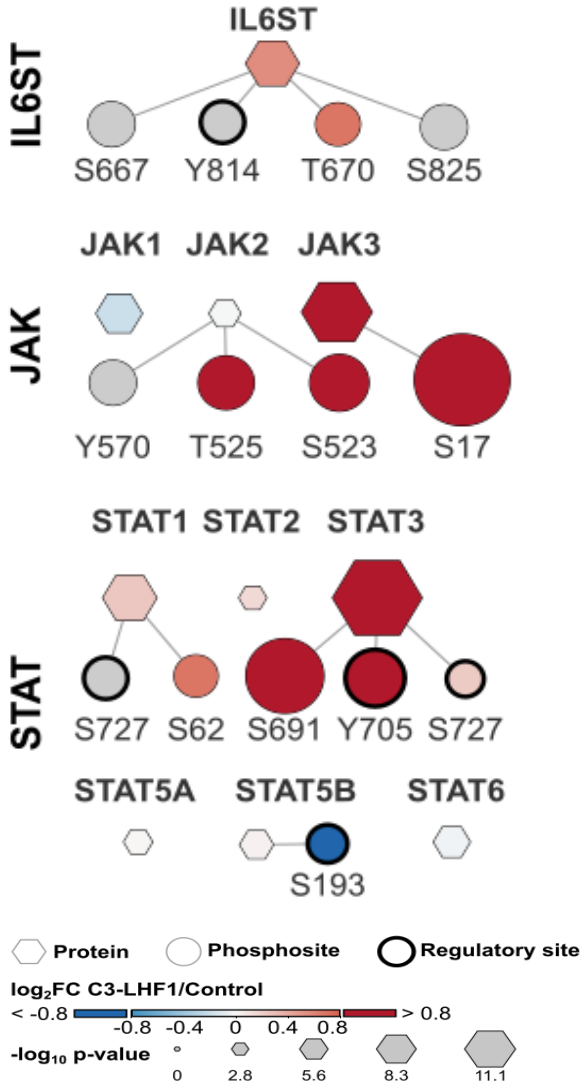
C3-LHF1: affinity in human glomeruli

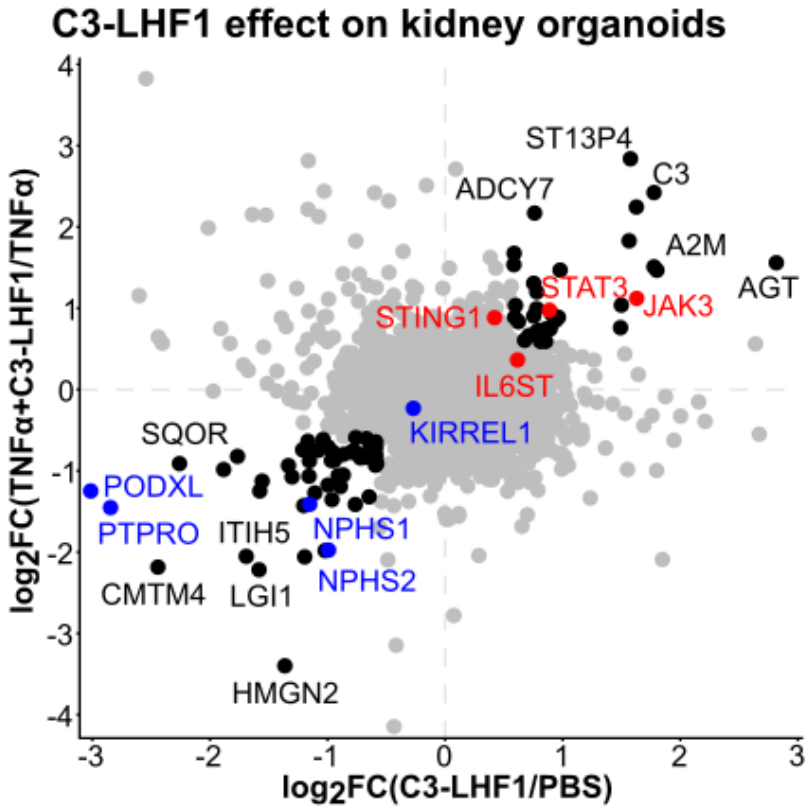




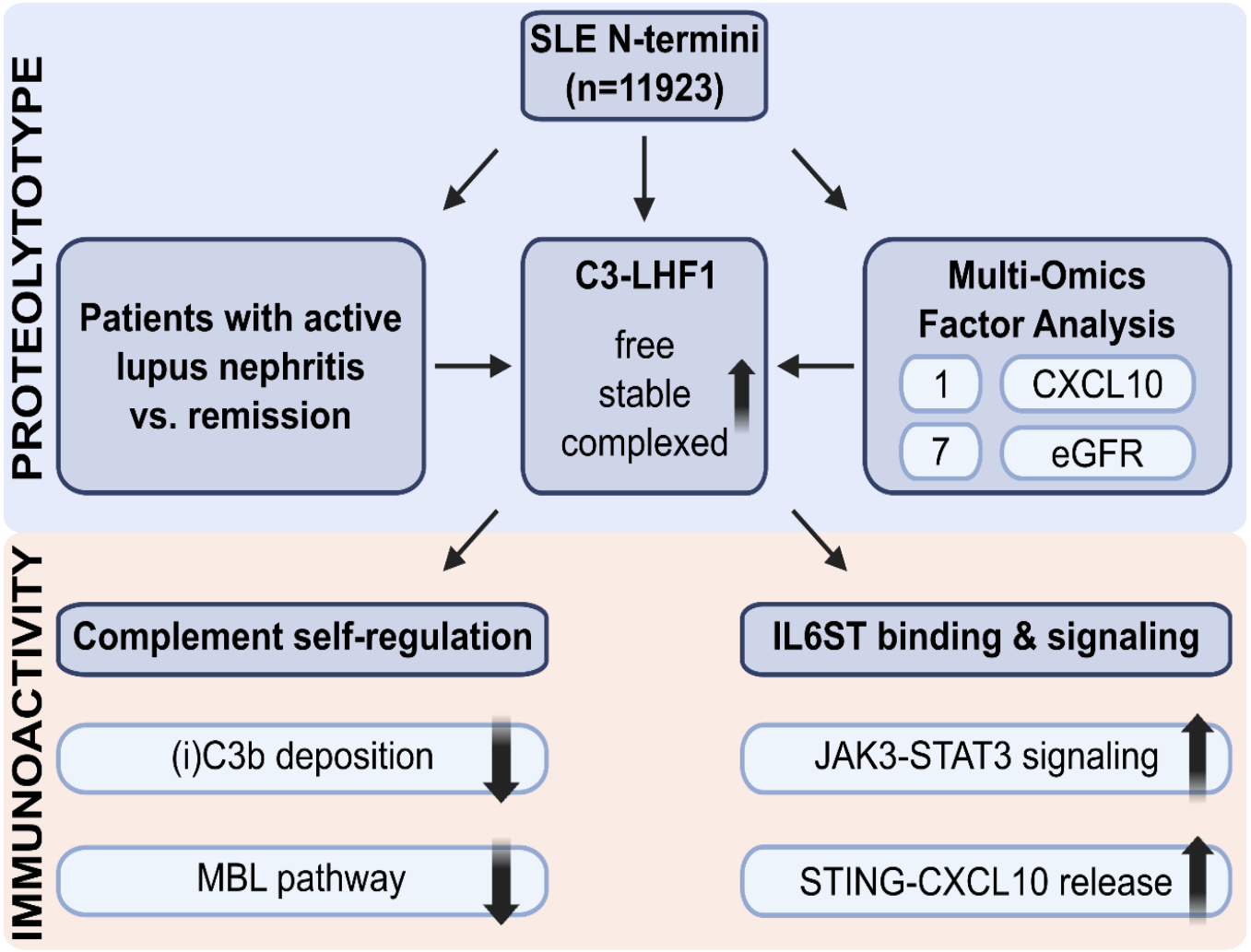
Demir et al. EMBOJ 2025

C3-LHF1 effects in organoids are consistent with IL6ST signaling





Demir et al. EMBOJ 2025



Plus resource: ProteleolySee shiny app....

Demir et al. EMBOJ 2025

Conclusion: disease drivers in glomerular disease

The tool box of glomerular proteome analysis covers markers, auto-antigens, signaling processes
- protein deposition in biopsies
- to understand affinity of circulating molecules
- to discover target engagement

Proteolytic profiling reveals a new disease-associated C3 fragment in SLE
- **Take advantage** of patient heterogeneity
- atypical complement inhibition
- IL6ST (gp130) modulation
- JAK/STAT signaling.

Demir et al., EMBO J 2025

Thank you very much!

Aarhus University

Fatih Demir

Anne Troldborg

Steffen Thiel

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Thomas Poulsen

Hamburg University

Elion Hoxha

Simon Melderis

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Immune-Mediated Glomerular Diseases

FOR 2743

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Danish Diabetes and Endocrine Academy