

June 26 - 30, 2023
Smolenice Castle, Slovakia

BenBedPhar Training School 2023

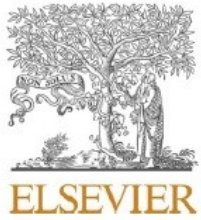
NRF2 in noncommunicable diseases:
From bench to bedside



Tools to study NRF2

Anna Grochot-Przęczek
Department of Medical Biotechnology
Jagiellonian University
Kraków, Poland

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Invited Review Article

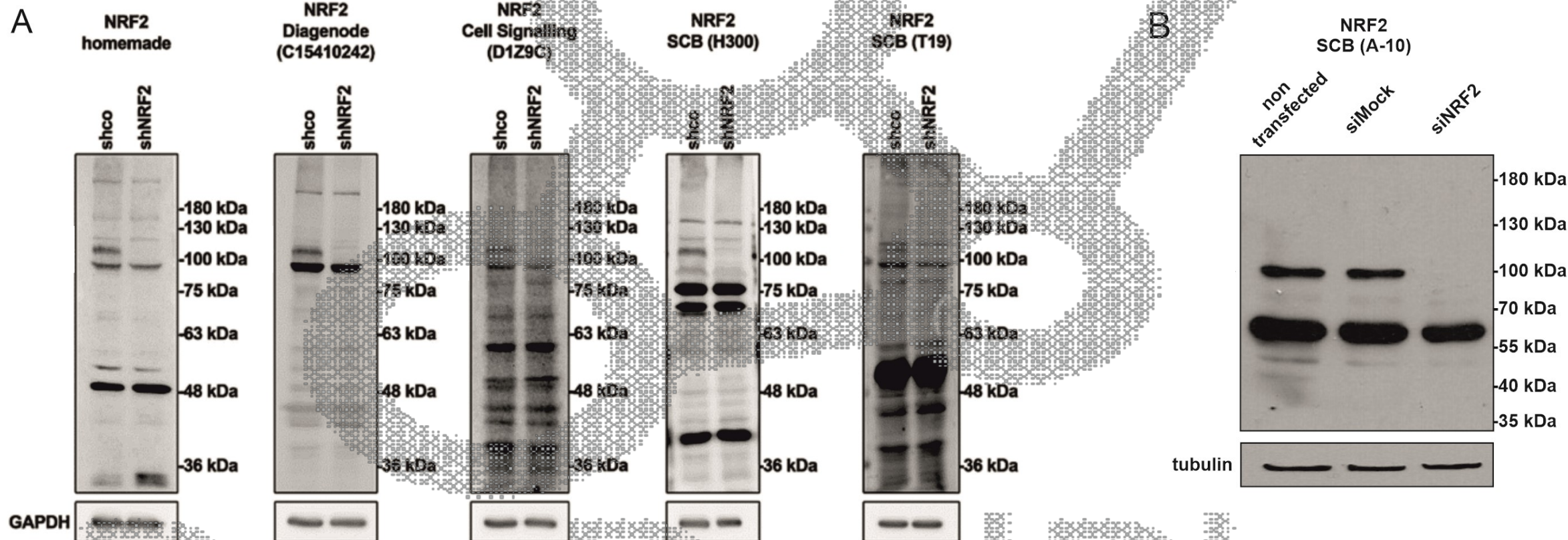
Overlooked and valuable facts to know in the NRF2/KEAP1 field

Aleksandra Kopacz^a, Ana I. Rojo^{b,c,d,e}, Chinmai Patibandla^f, Diego Lastra-Martínez^{b,c,d,e},
Aleksandra Piechota-Polanczyk^a, Damian Kłoska^{a,f}, Alicja Jozkowicz^a, Calum Sutherland^g,
Antonio Cuadrado^{b,c,d,e,**}, Anna Grochot-Przeczek^{a,*}

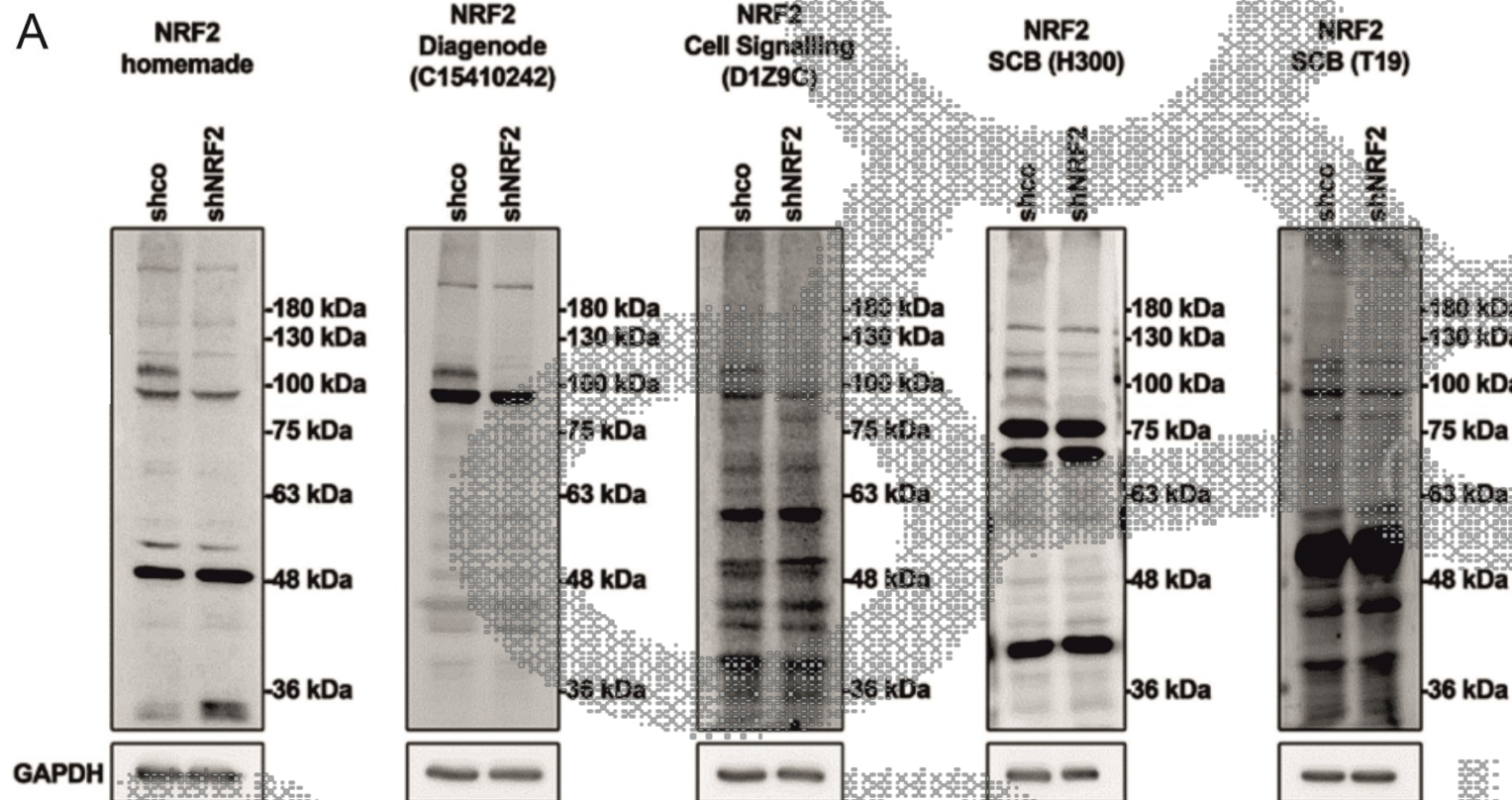


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Anti-NRF2 antibodies

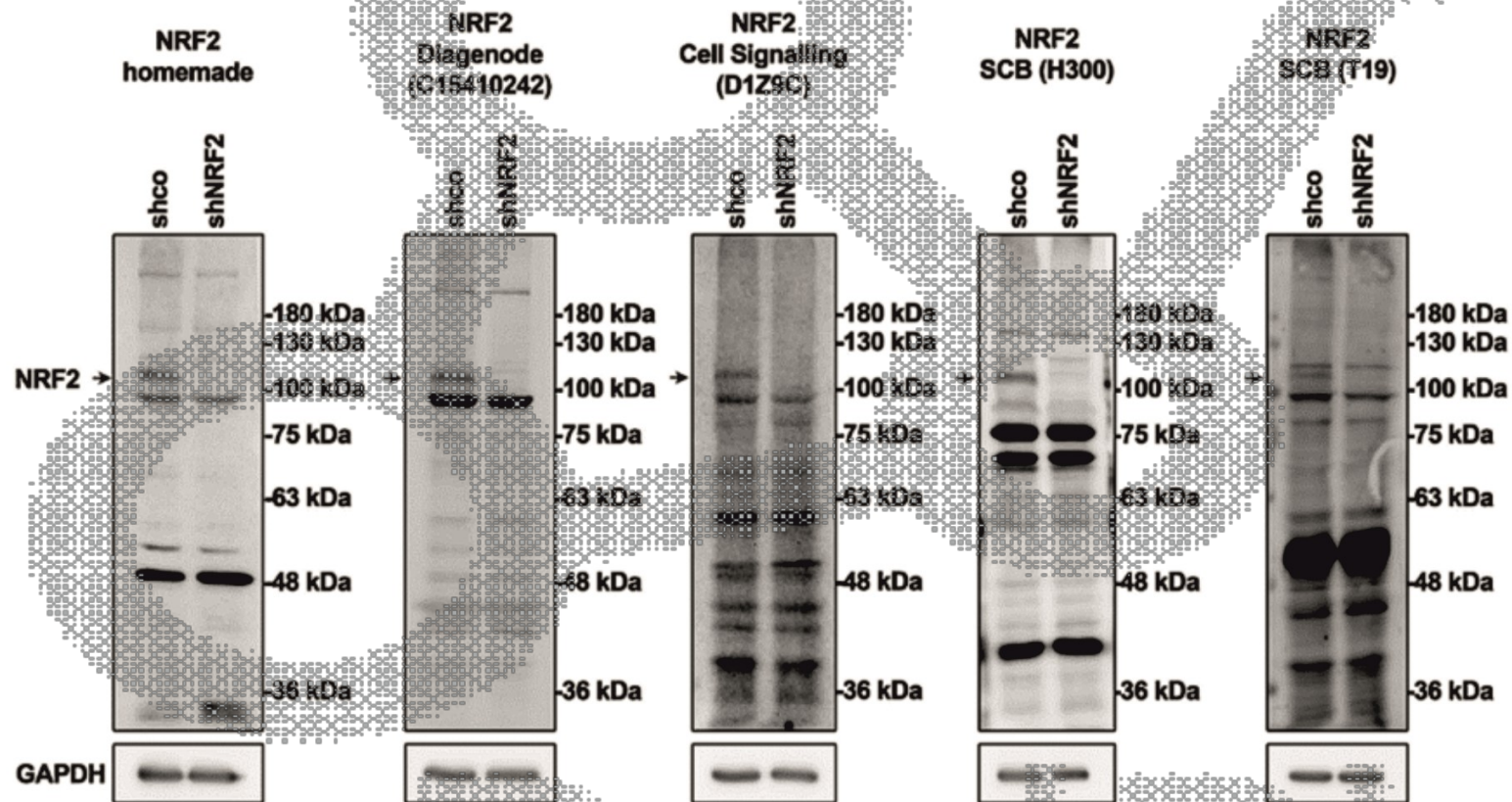


Anti-NRF2 antibodies



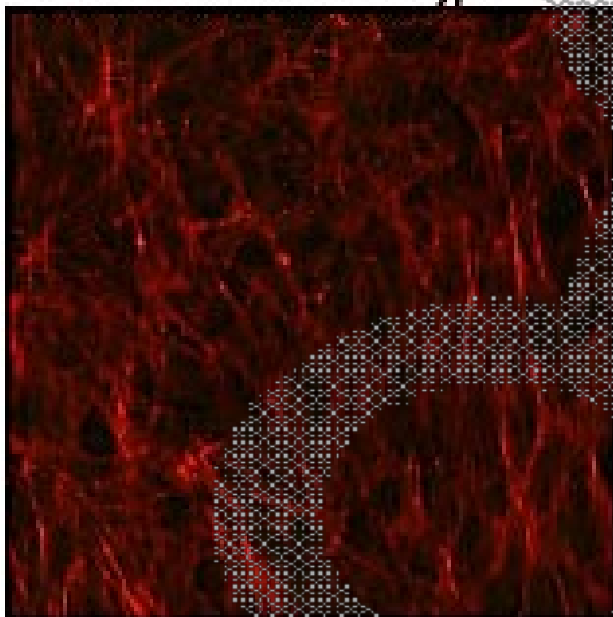
- 1) Antibody validation
- 2) NRF2 in SDS-PAGE
- 3) Epitopes

Anti-NRF2 antibody validation is a must

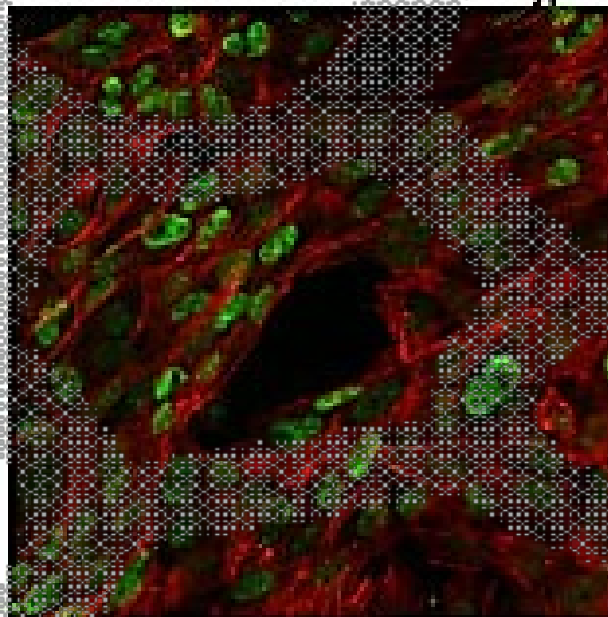


Anti-NRF2 antibody validation is a must

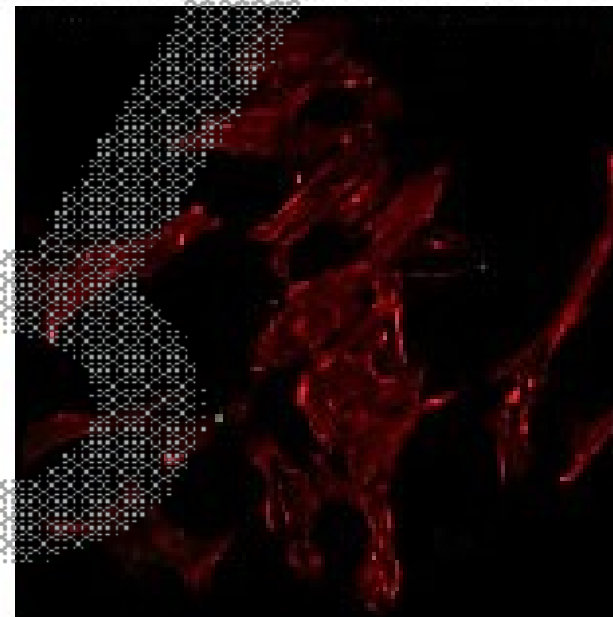
Untreated MEF NRF2 wild-type



MG-132-treated MEF NRF2 wild-type



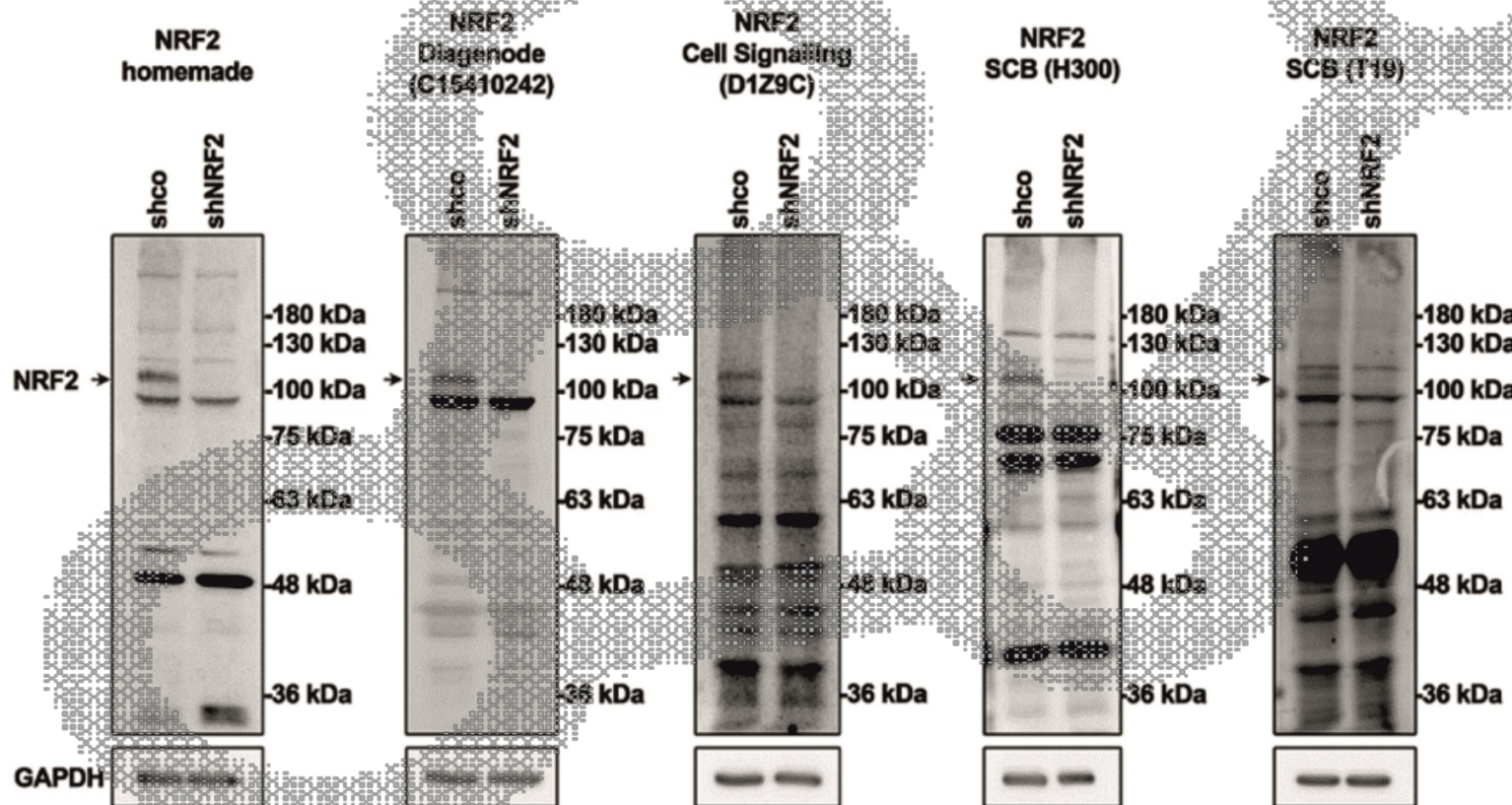
MG-132-treated MEF NRF2 knock-out



NRF2, actin

CST: NRF2 (D9J1B) Rat mAb, A200

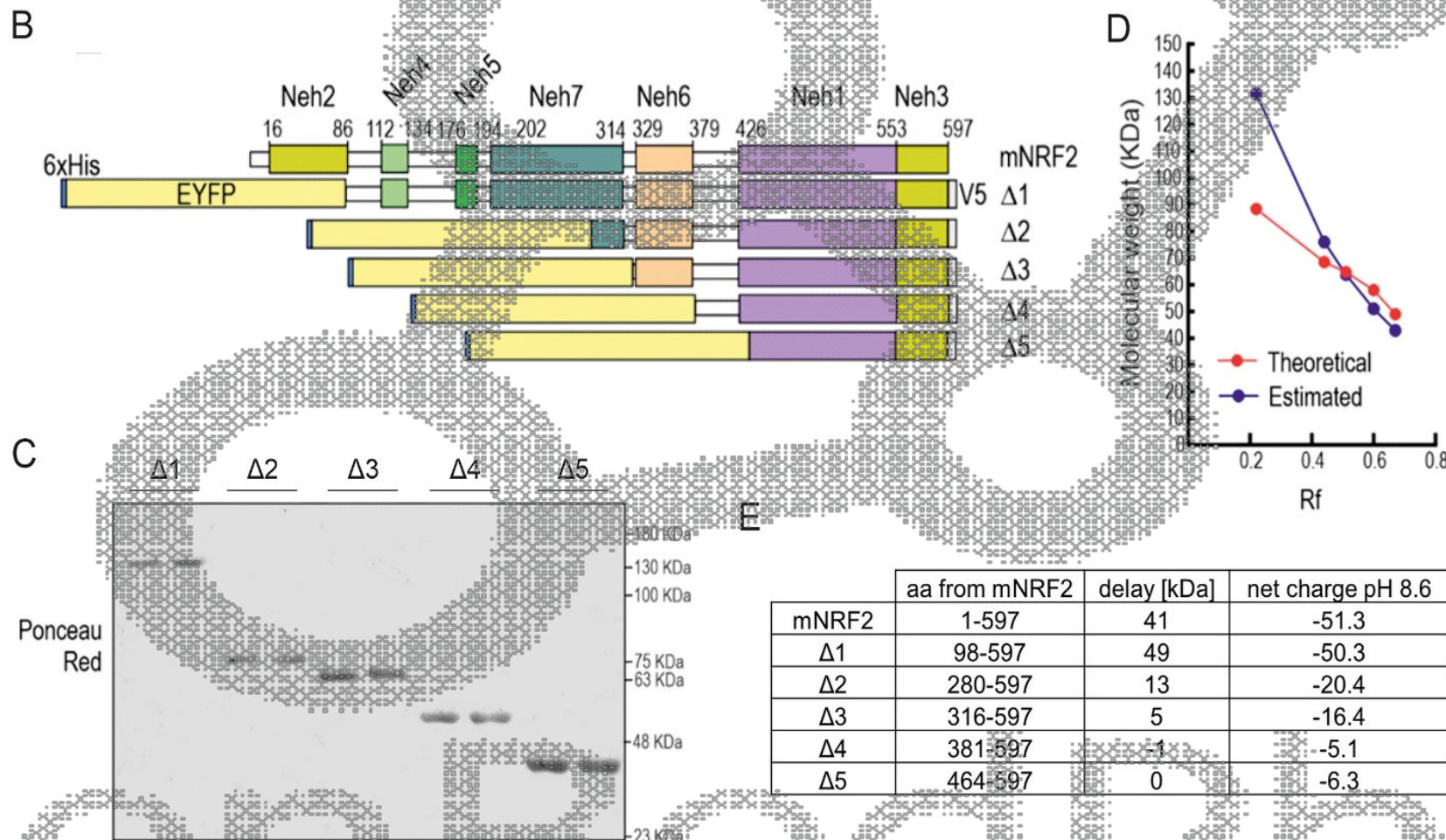
NRF2 in SDS-PAGE



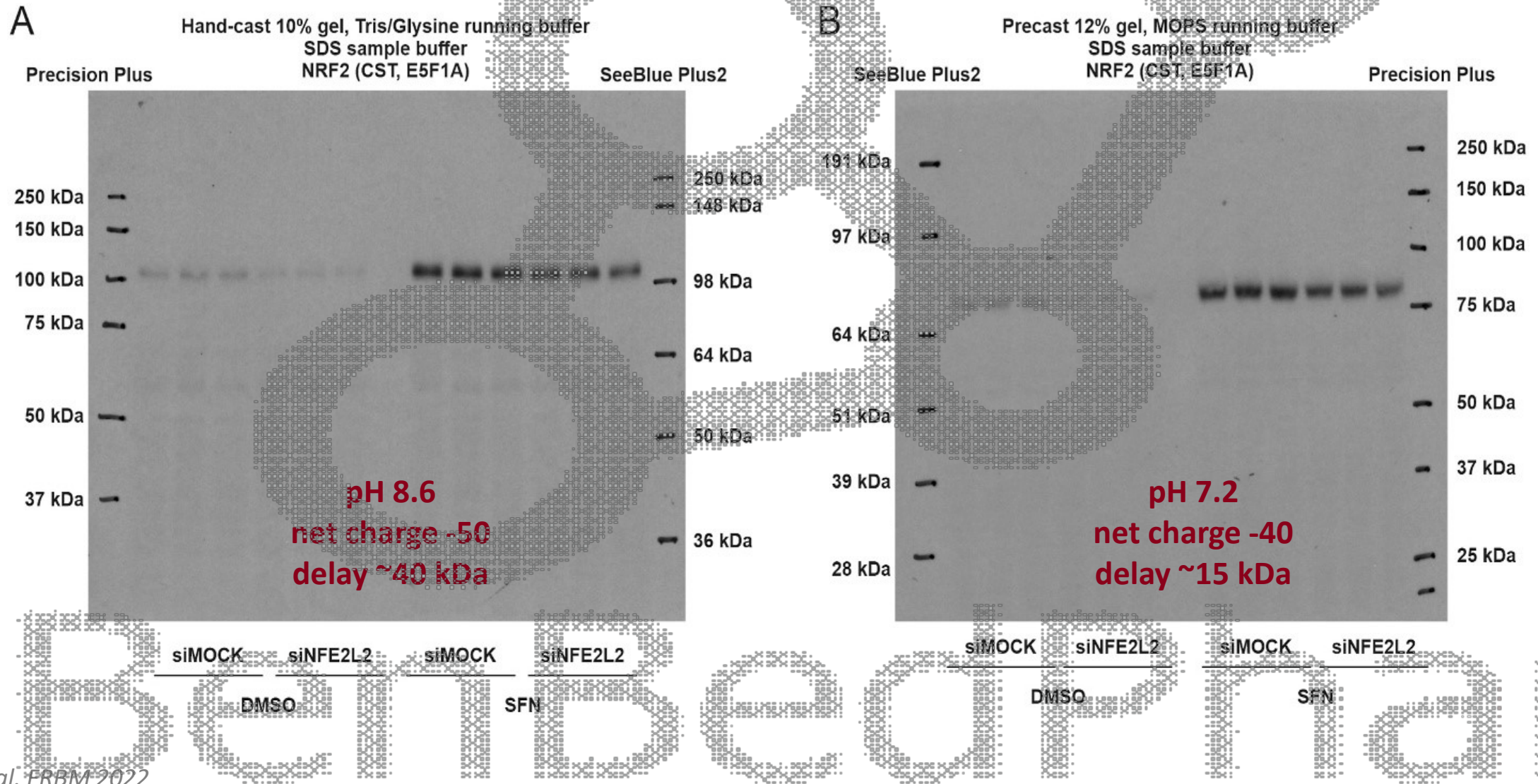
NRF2 predicted molecular weight is 67.8 kDa (human) and 66.9 kDa (mouse)



NRF2 in SDS-PAGE



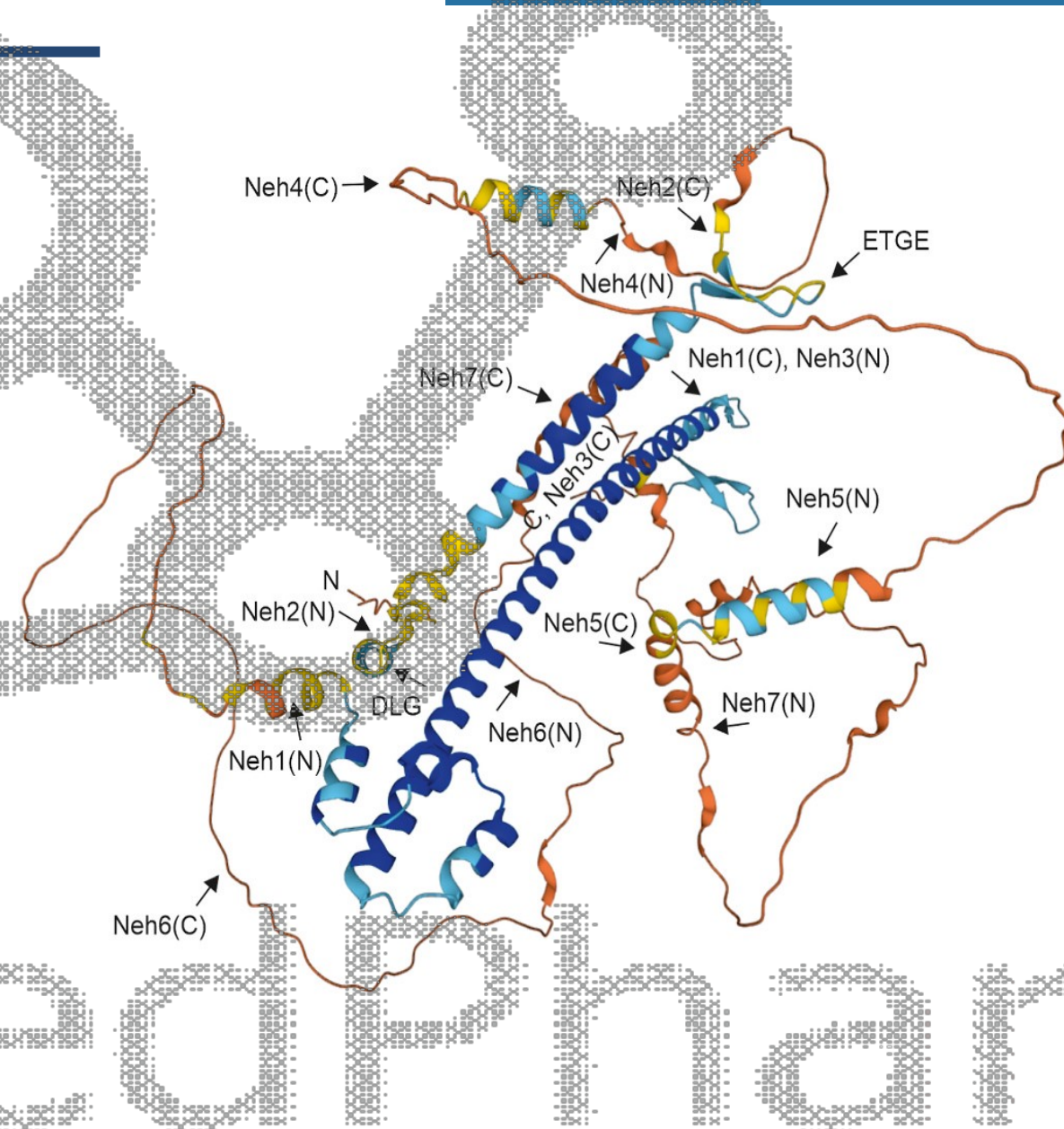
NRF2 in SDS-PAGE



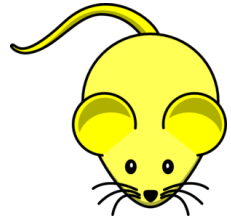
NRF2 in SDS-PAGE

Intrinsically disordered proteins (IDPs)

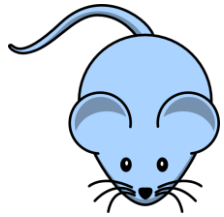
- The **high net charge** and **anomalous mobility in SDS-PAGE** are characteristic features of IDPs
- The apparent **molecular weight** of IDPs is usually **overestimated based on SDS-PAGE** by 1.2–1.8 times (1.6 for NRF2)
- More than half of eukaryotic transcription factors are estimated to possess unstructured domains, and many present abnormal mobility in SDS-PAGE



Epitopes



x



NRF2^{fl/fl}

Cre recombinase



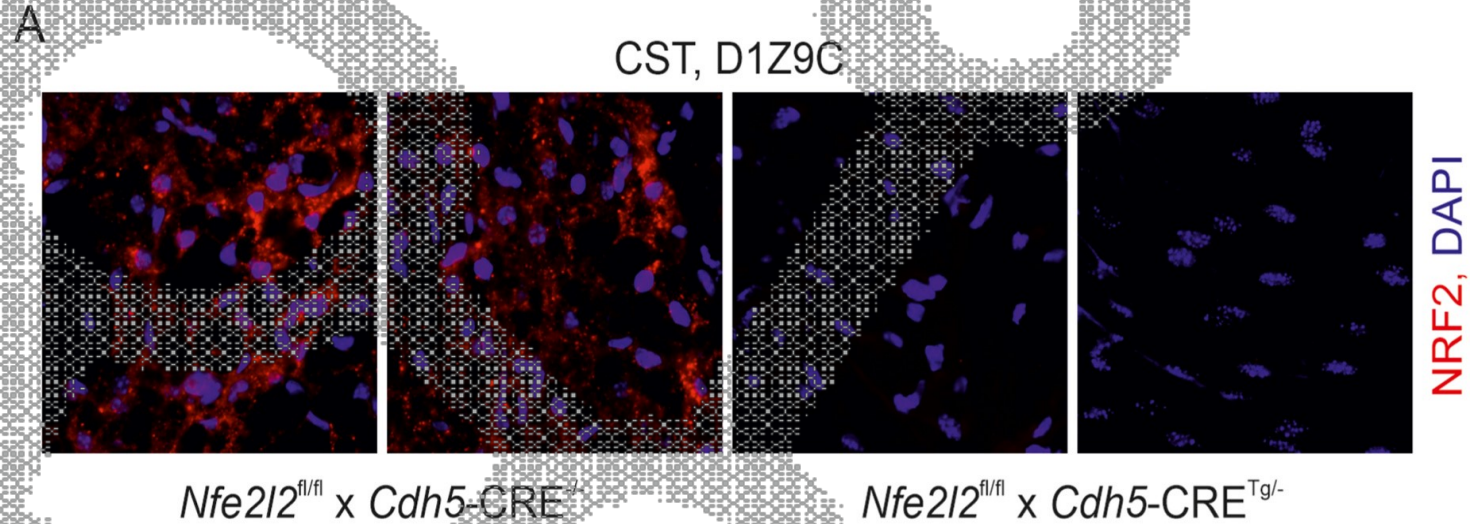
loxP

loxP

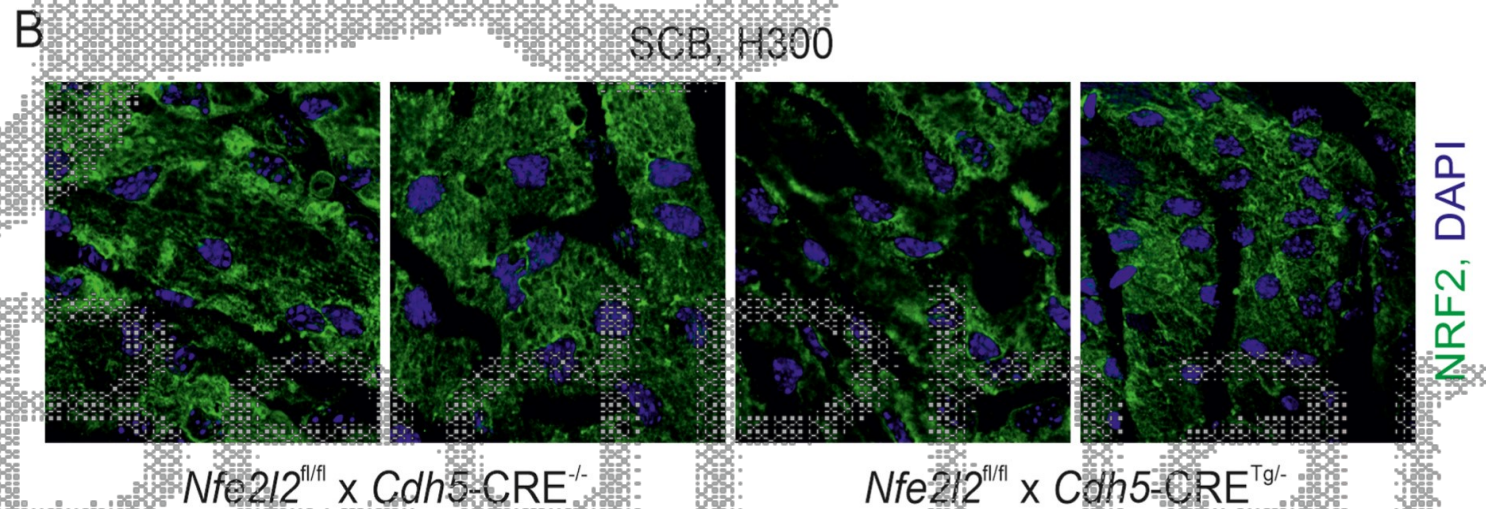


Epitopes

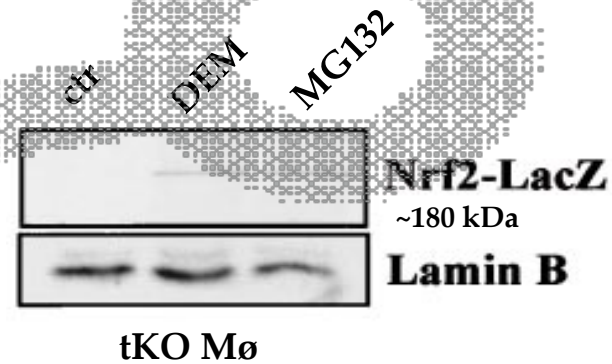
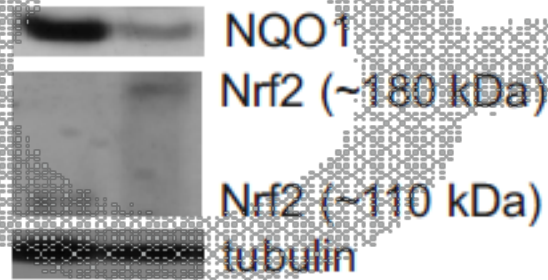
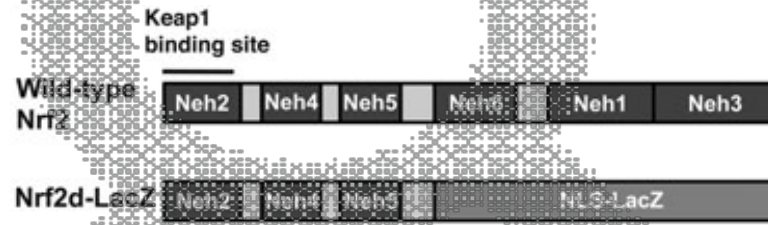
monoclonal
residues surrounding 275 aa



polyclonal
the epitope mapping to 37–336 aa

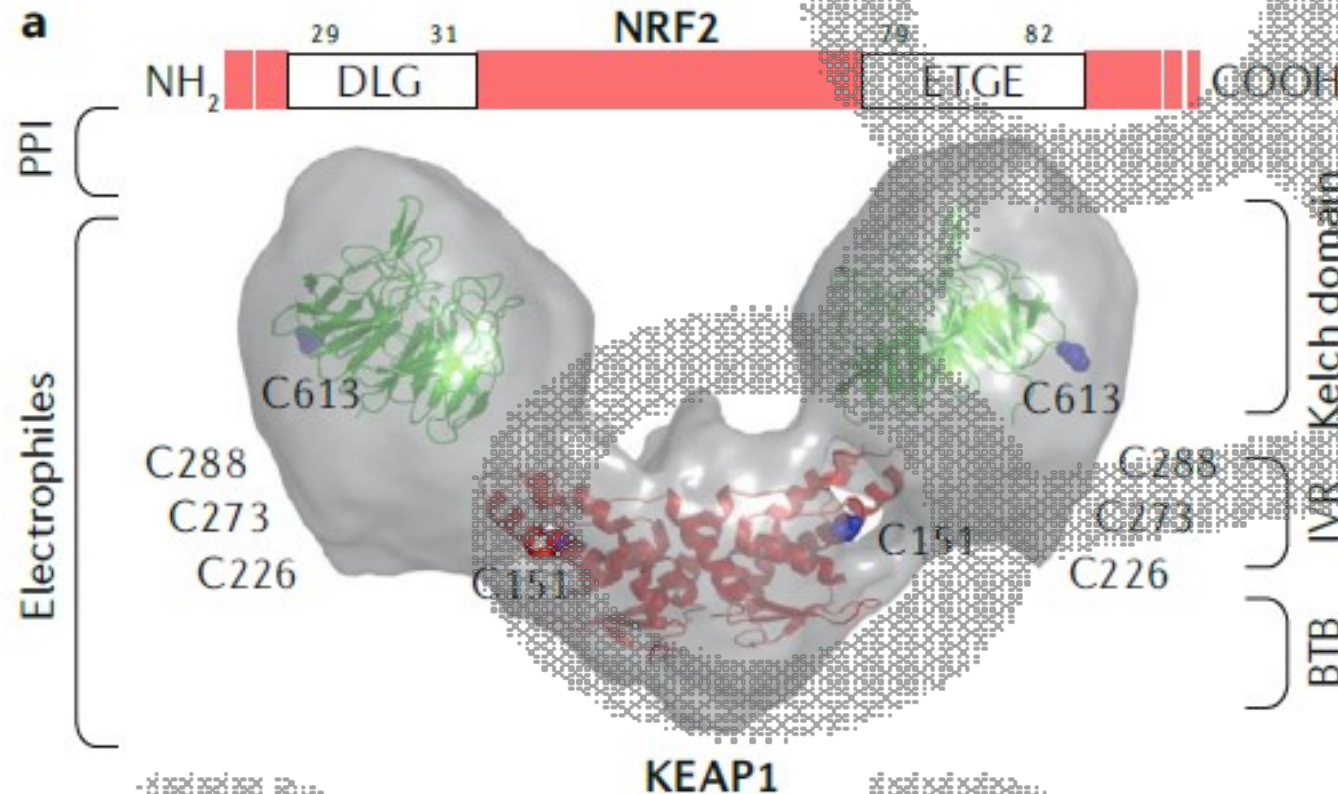


N-terminal domains of NRF2 in KO mice



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NRF2 activators have off-targets



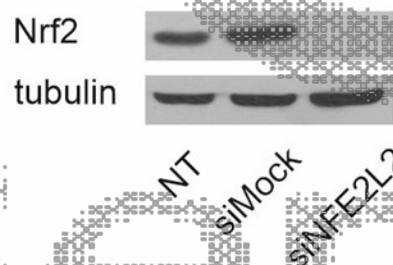
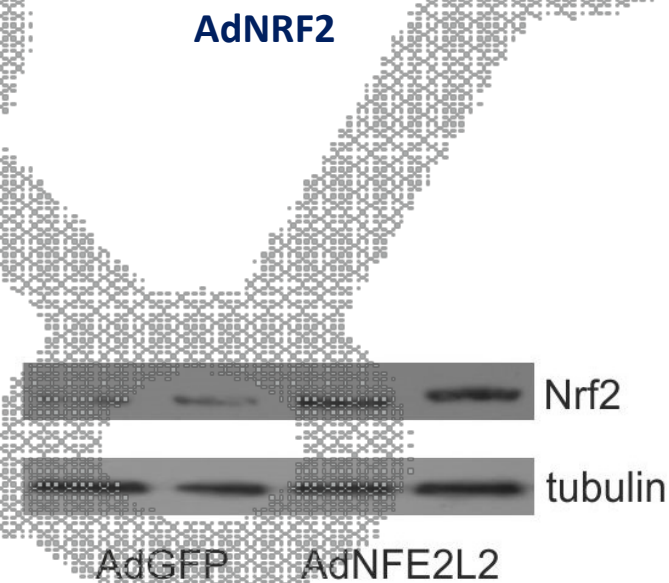
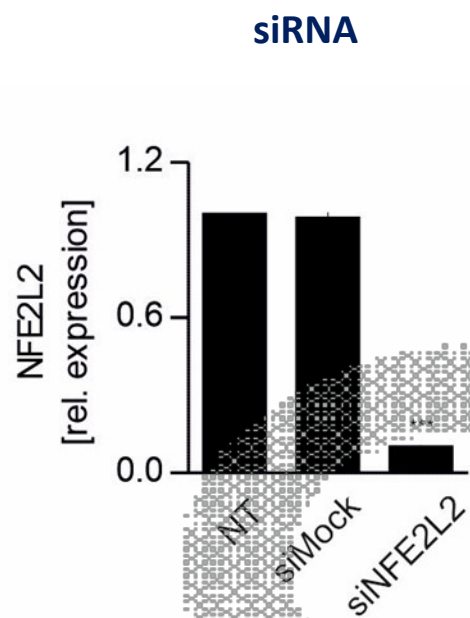
KEAP1, a cysteine-based sensor and a drug target for the prevention and treatment of chronic disease

Sharadha Dayalan Naidu¹ and Alben T. Dinkova-Kostova^{1,2}

DMF – 24 proteins (Piroli et al.)
SFN - >500 proteins (Clulow et al.)
CDDO-Im – 576 putative proteins (Yore et al..)

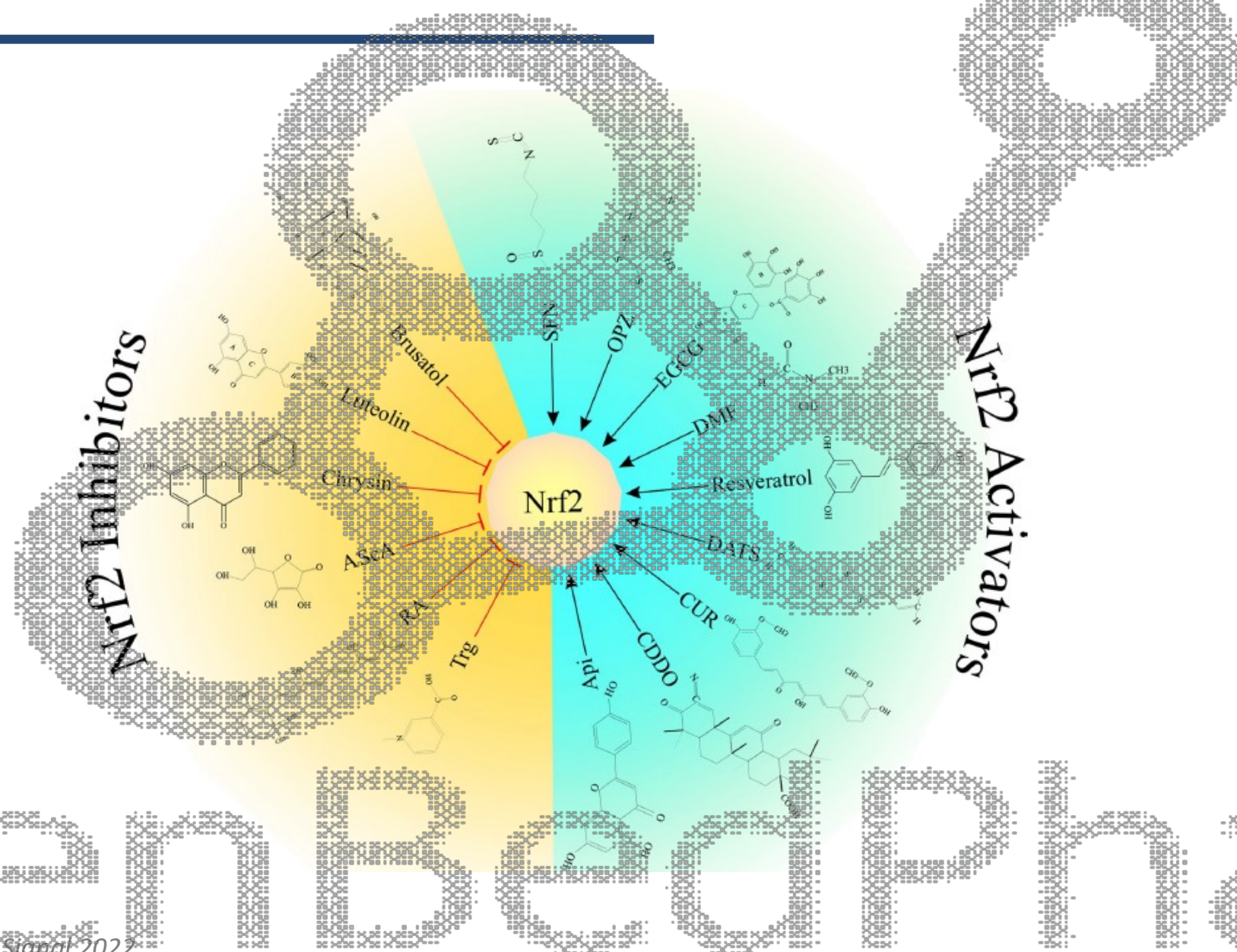
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NRF2 knock-down and overexpression



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NRF2 inhibitors



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Stapled Peptides as Direct Inhibitors of Nrf2-sMAF Transcription Factors

Ramya Modi, Nick McKee, Ning Zhang, Amir Alwali, Samantha Nelson, Aditi Lohar, Raluca Ostafe, Donna D. Zhang, and Elizabeth I. Parkinson*

✓ **Cite this:** *J. Med. Chem.* 2023, 66, 9, 6184–6192

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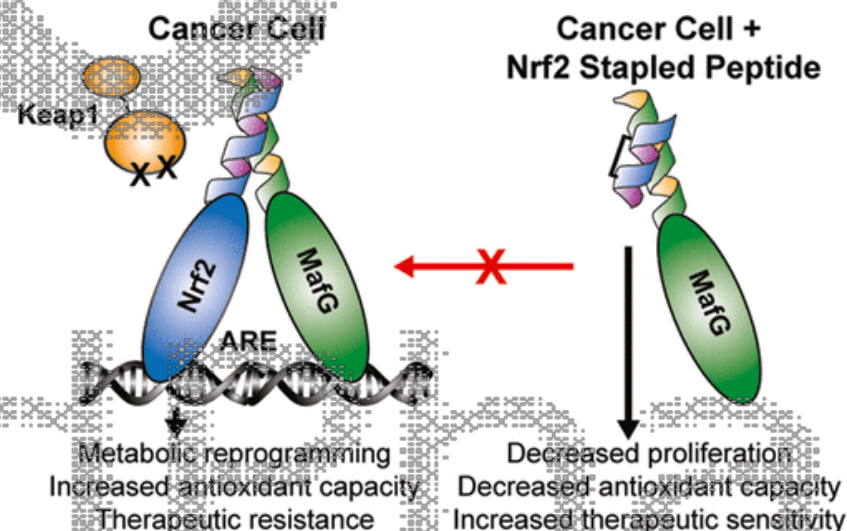
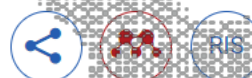
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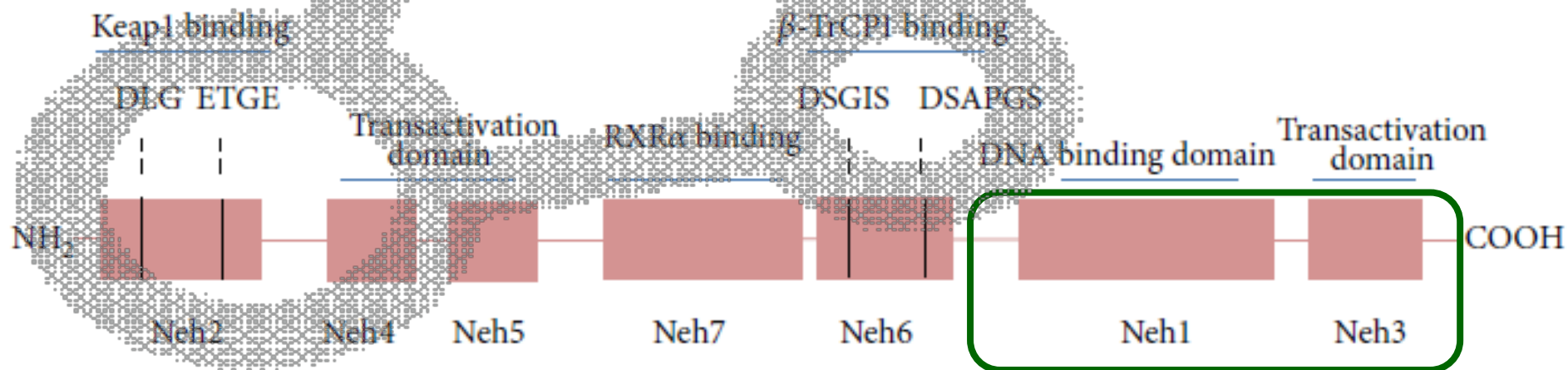
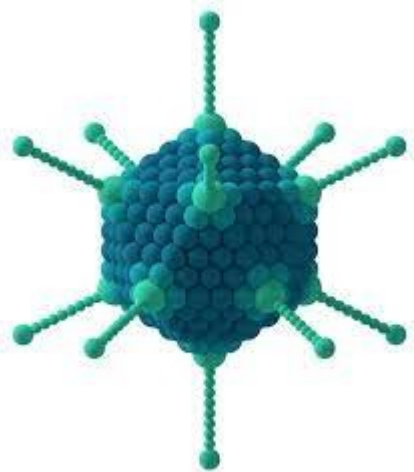
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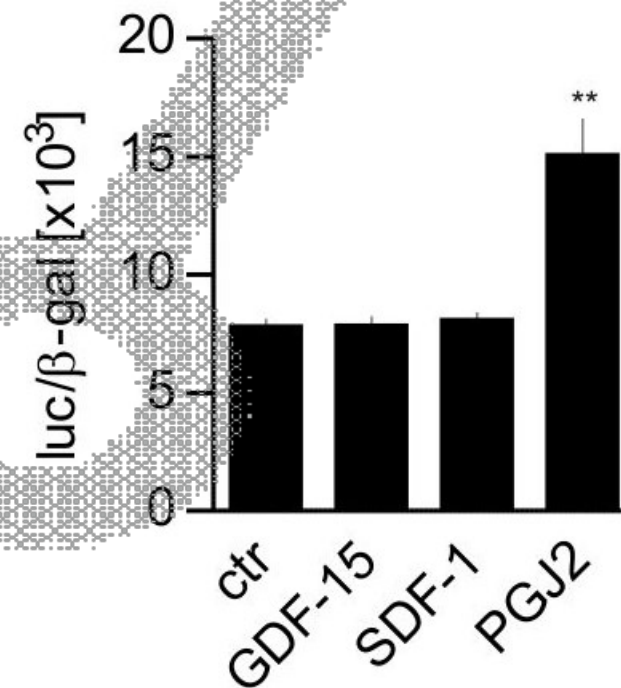
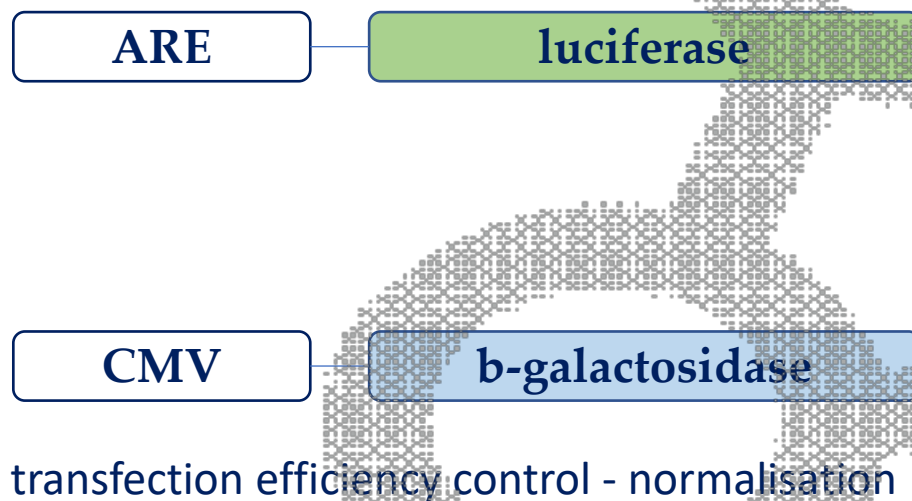


dominant negative NRF2 (AdNRF2^{DN})



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ARE-luc reporter plasmid



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Good luck!

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