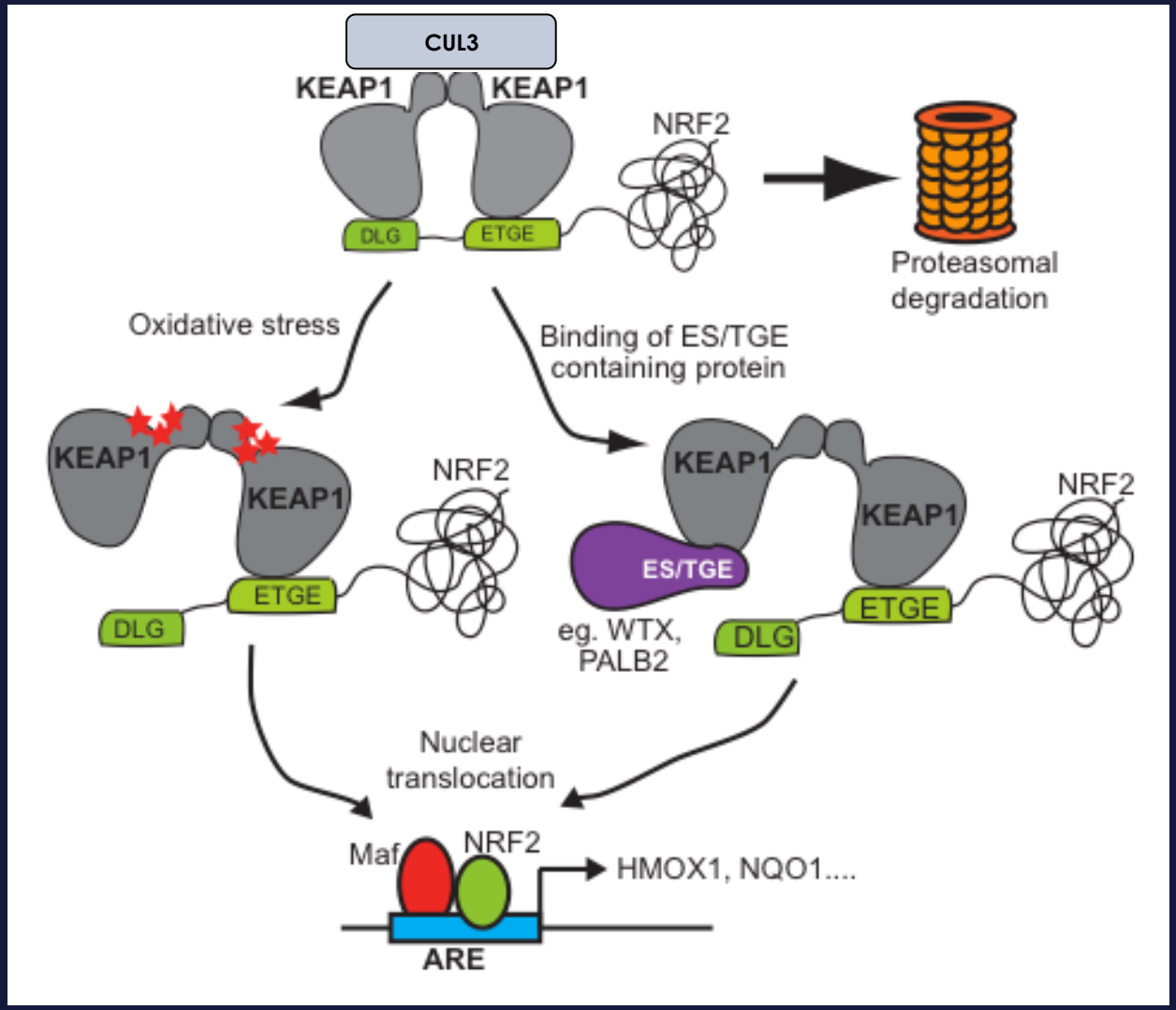
Functional characterization of KEAP1 mutations in lung squamous cell carincoma

Bridgid Hast
Major Lab
UNC Chapel Hill

KEAP1/NRF2 regulates intracellular redox homeostasis



NRF2 activity modulates survival via redox homeostasis

NRF2 target genes

-Heme oxygenase 1 (HMOX1)

-Glutathione synthesis (GCS)

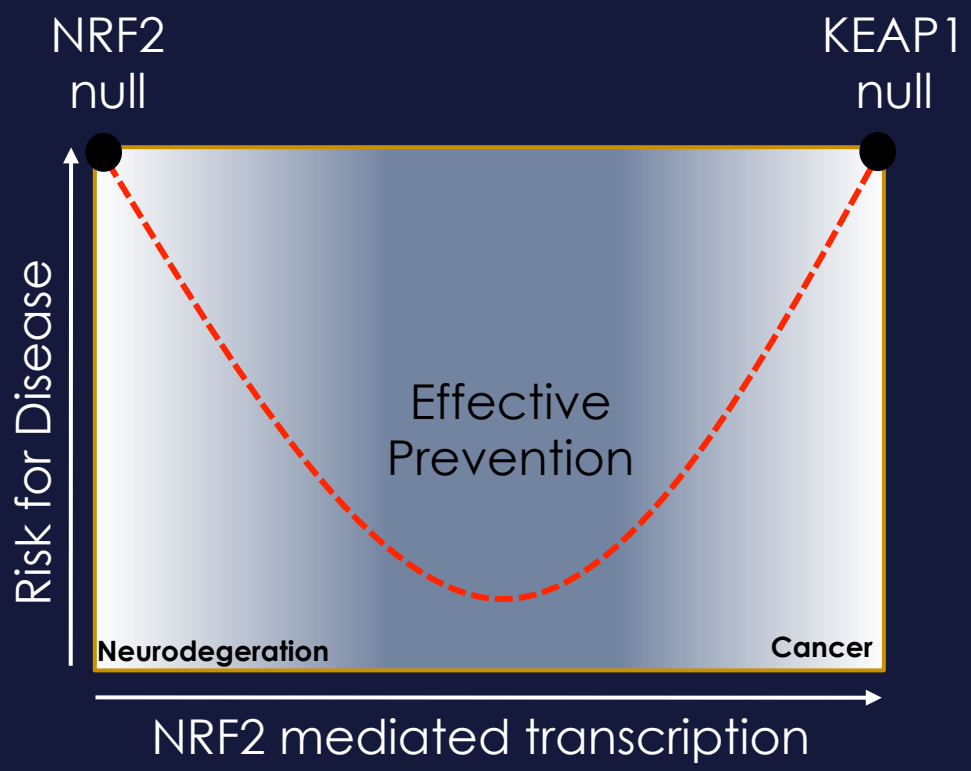
-NADH quinone oxidoreductase 1 (NQO1)

-Multidrug resistance proteins (MRP)

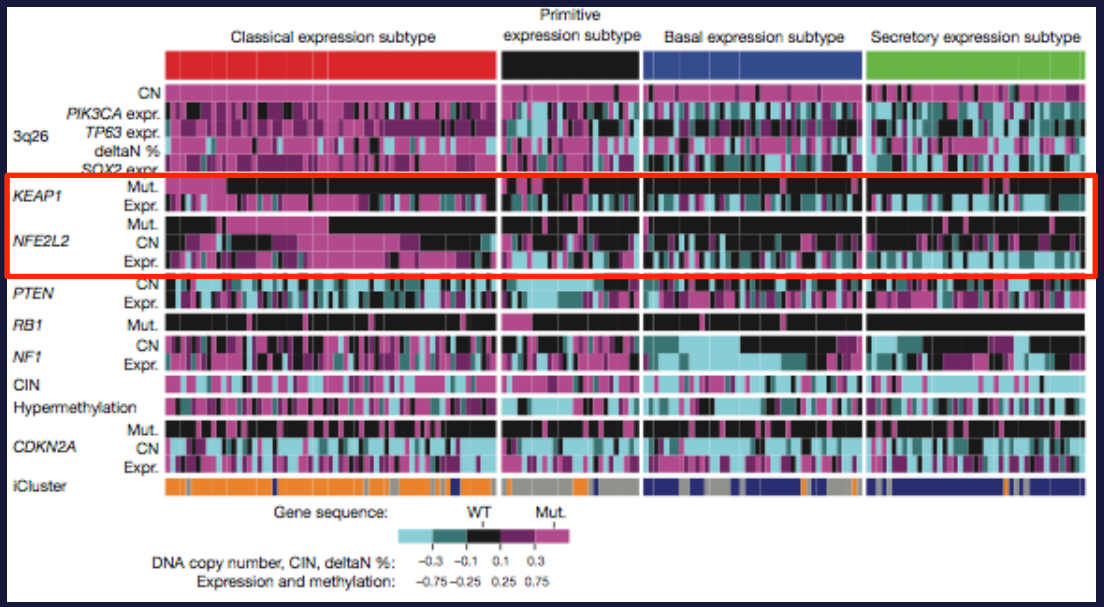
*Mitigate acute spikes in ROS

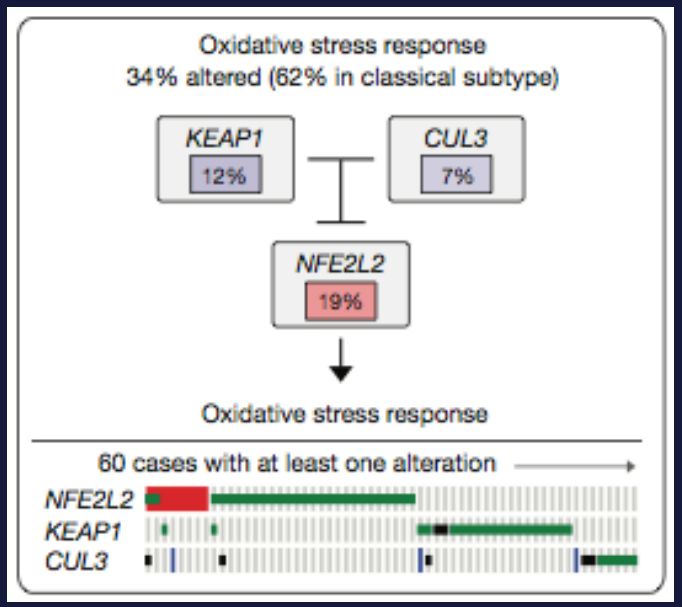
*Chemotherapeutic/xenobiotic clearance

*Control metabolically-derived ROS



Pathway mutations in KEAP1/NRF2 signaling occur in squamous cell lung carcinoma





-178 total squamous cell lung carcinomas analyzed

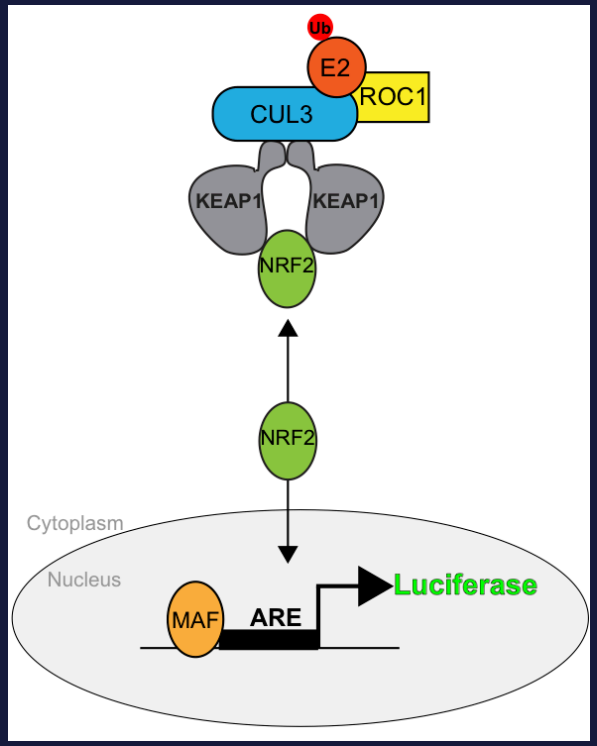
-Mutations in KEAP1 and NRF2 are mutually exclusive

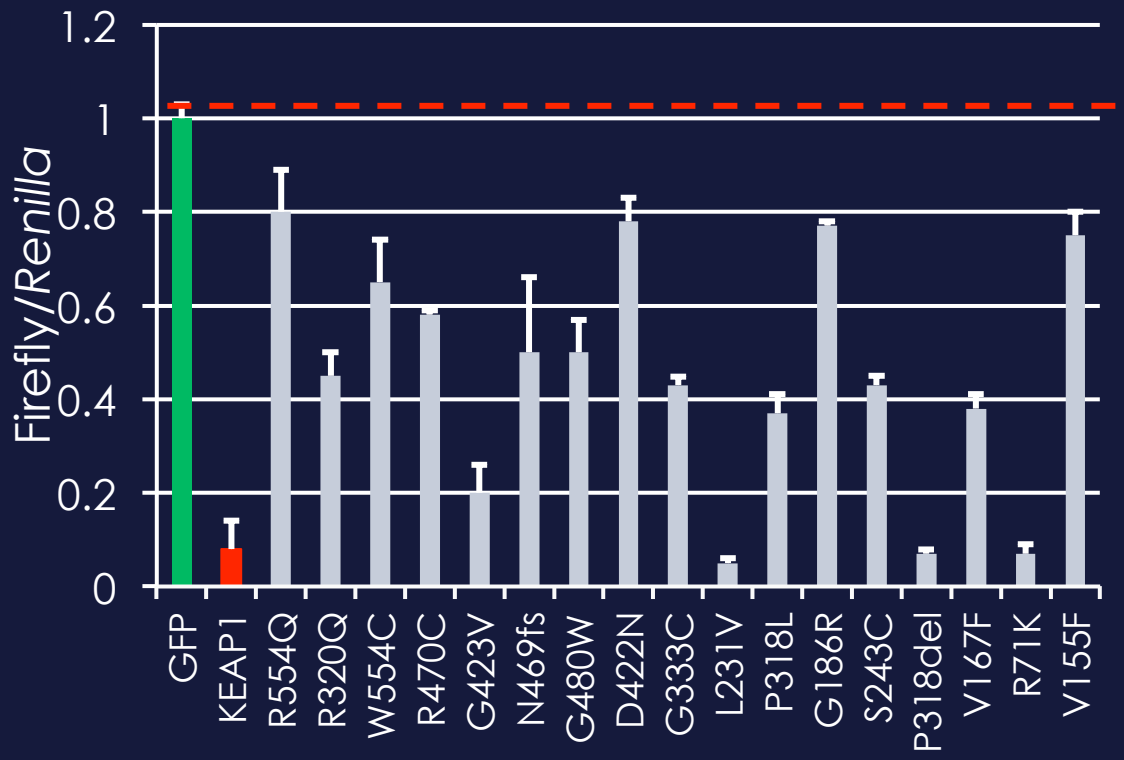
-Primarily in classical subtype

-Collectively KEAP1, NRF2, and CUL3 mutations are altered in 34% of total samples

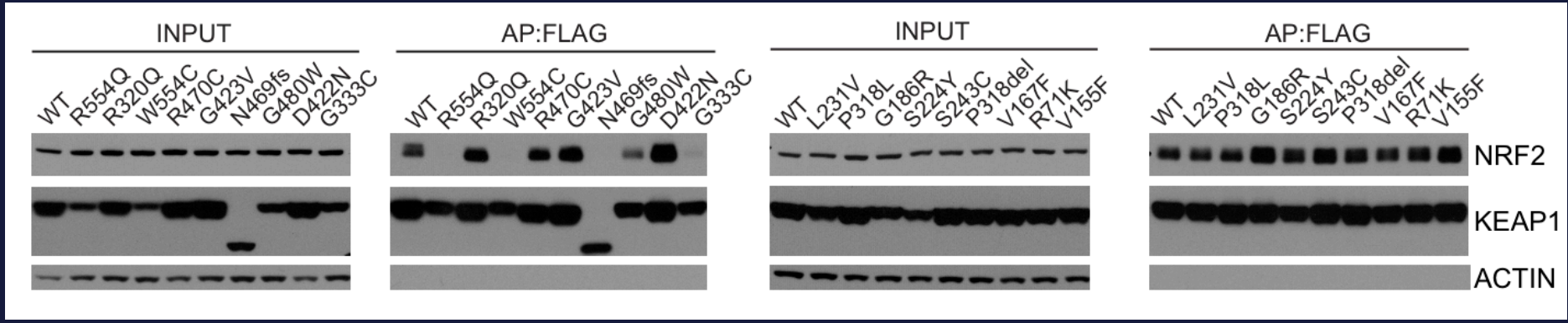
The Cancer Genome Atlas Research Network, *Nature* 2012

KEAP1 mutations exhibit differential suppression of NRF2-mediated transcription

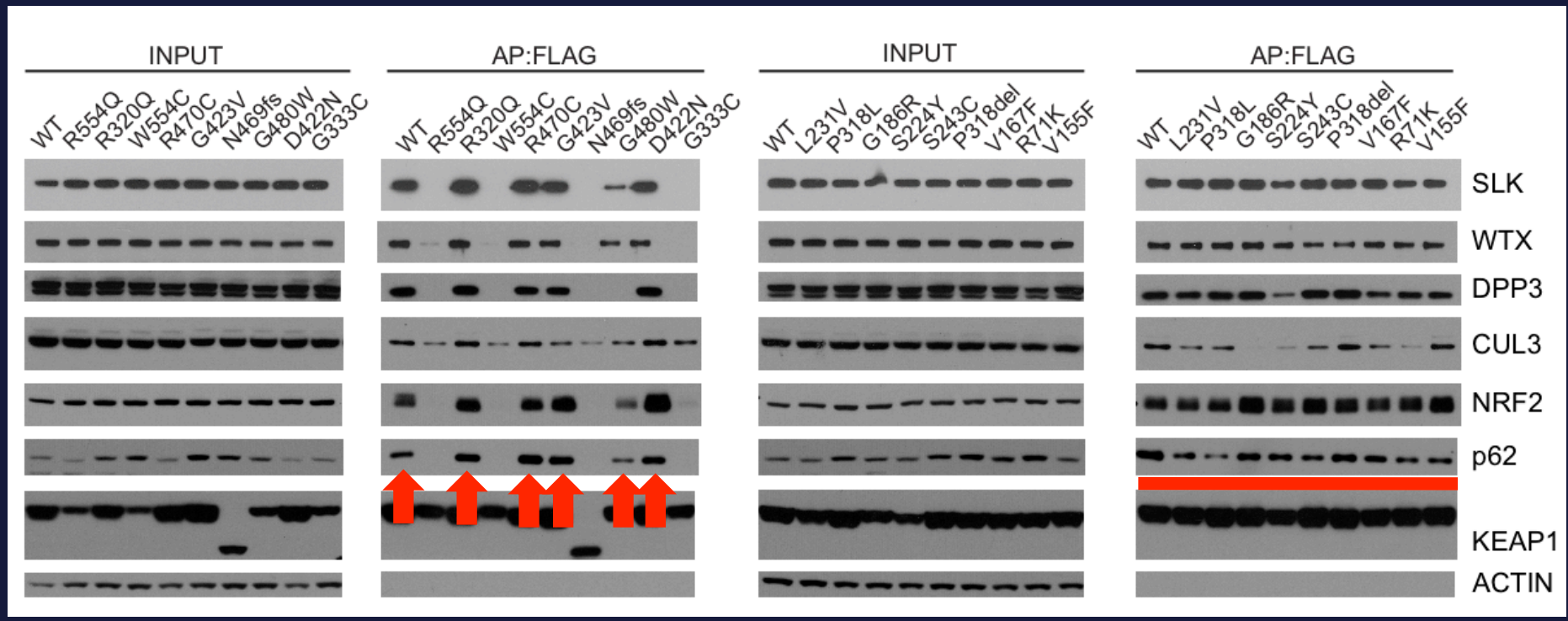




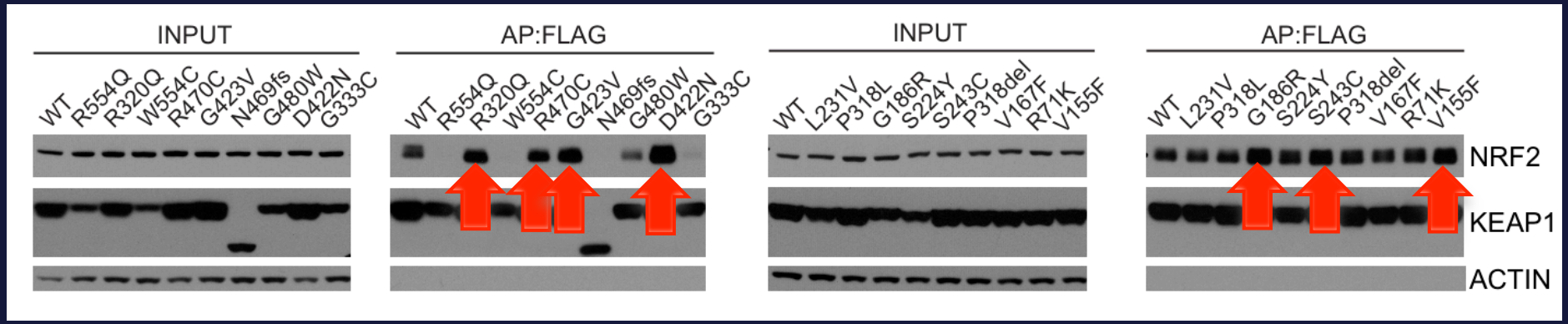
KEAP1 mutants differentially bind to interacting proteins



KEAP1 mutants differentially bind to interacting proteins

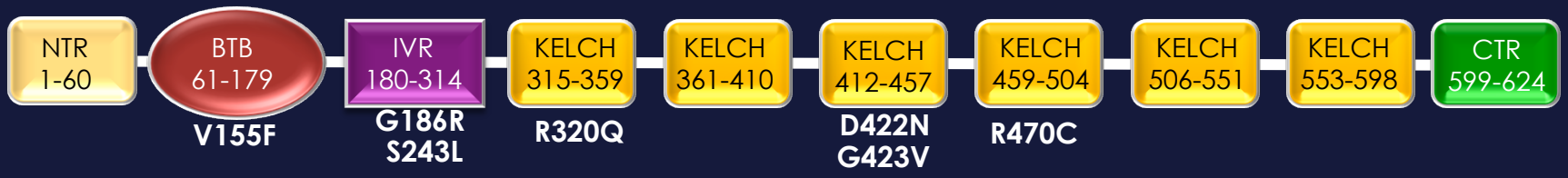


KEAP1 mutants differentially bind to interacting proteins



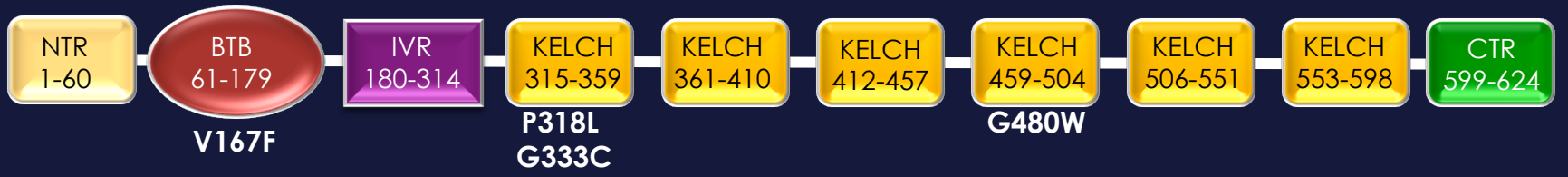
The KEAP1 mutants cluster into four classes

Class I: Strong binders of NRF2 but cannot suppress NRF2-mediated transcription

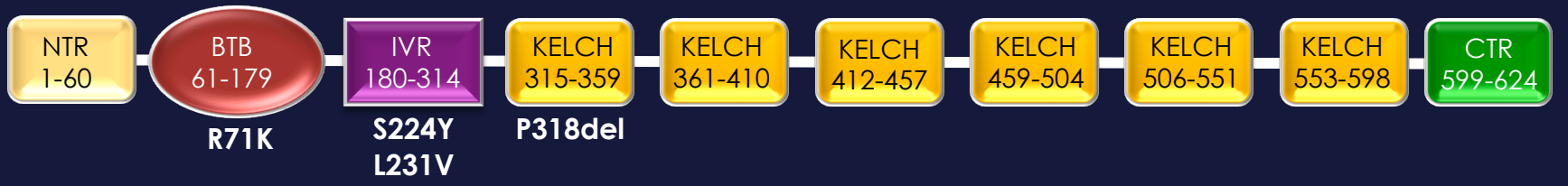


Class II: Do not bind NRF2 and cannot suppress NRF2

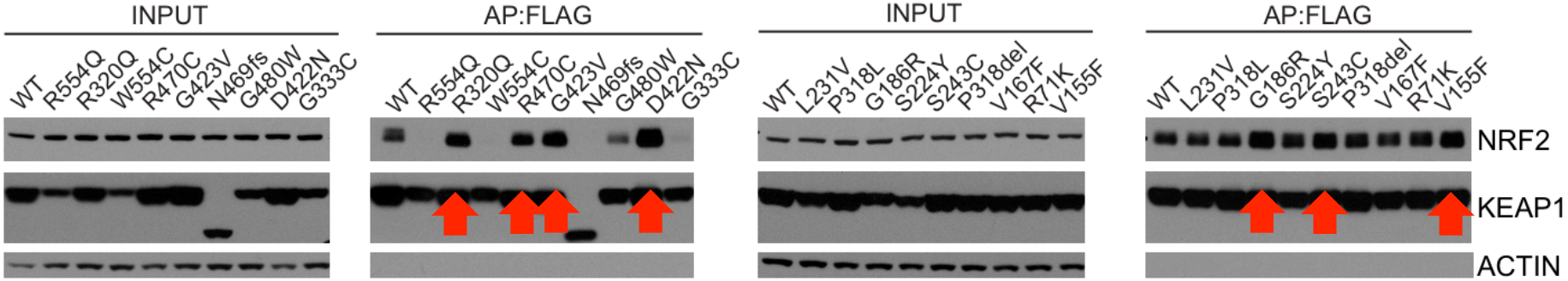
Class III: Weakly bind NRF2 and cannot suppress NRF2

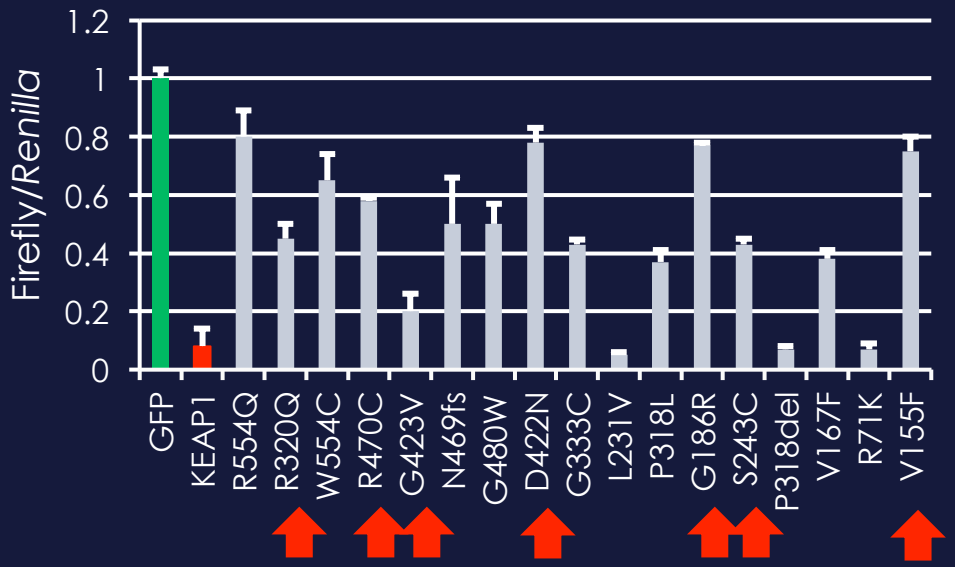


Class IV: Behave like wildtype



KEAP1 mutants differentially bind to interacting proteins





-"Superbinders" only bind more NRF2

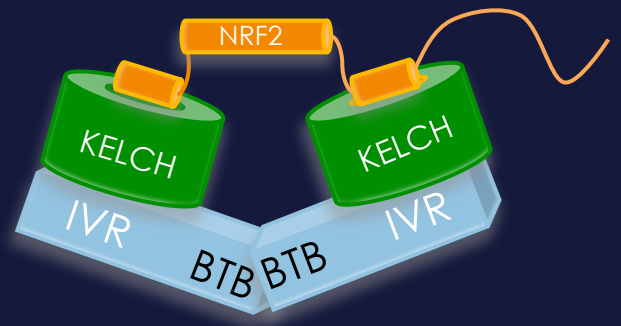
-Cannot suppress NRF2-mediated transcription

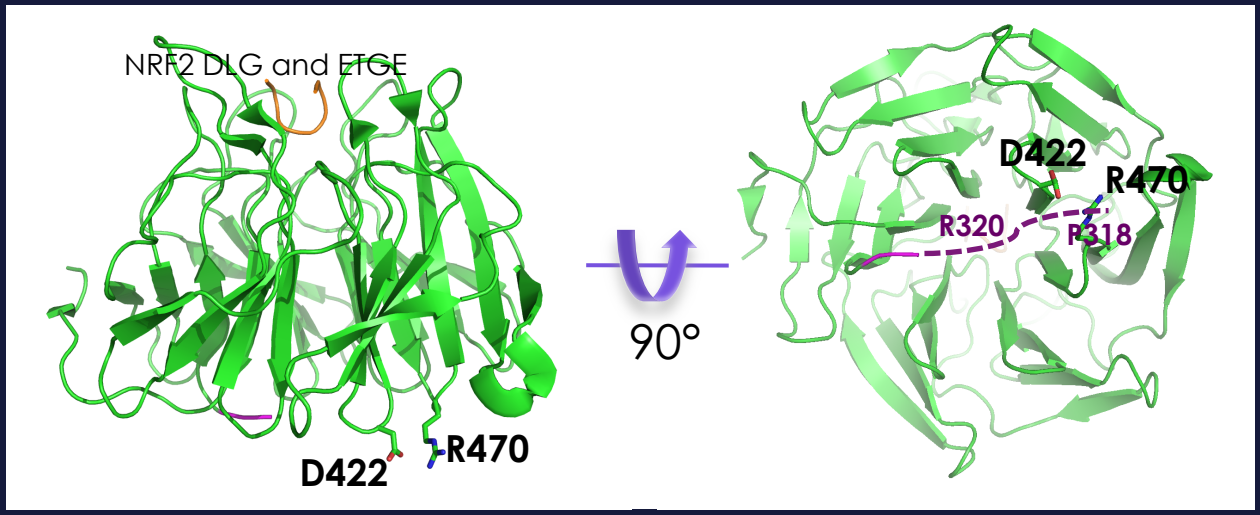
-Exhibit increased NRF2 half-life

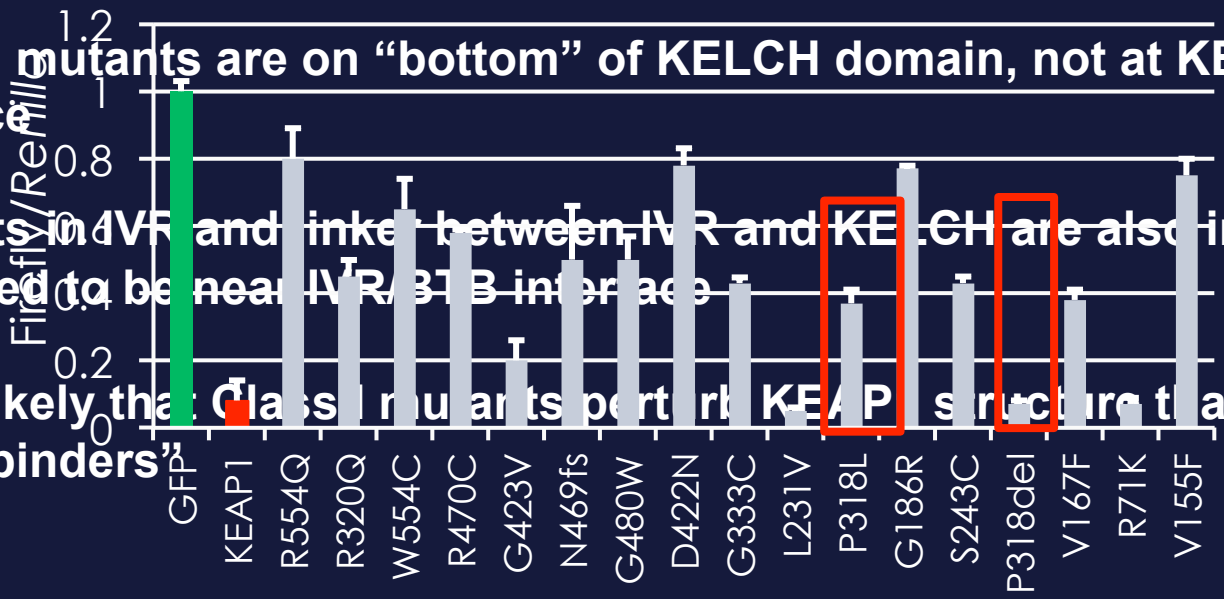
-Have enhanced cell viability in response to chemotherapeutic insult

Mechanism?

“Superbinders”: slow cyclers or subpar structures?







-Class I mutants are on “bottom” of KELCH domain, not at KELCH/NRF2 interface

-Mutants in IVR and linker between IVR and KELCH are also in Class I. Predicted to be near IVR/BTB interface

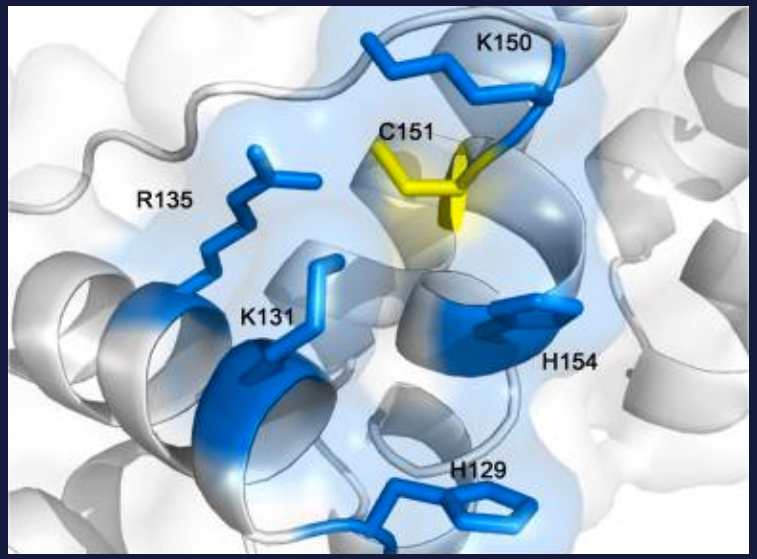
-More likely that Class I mutants perturb KEAP1 structure than act as “superbinders”

KEAP1 cysteine residues are stress-specific

-C151 forms adducts with electrophiles
  -H129, K131, R135, K150, and H154 comprise microenvironment that alters reactivity of C151

-H225/C226 and C613 are reactive to heavy metals

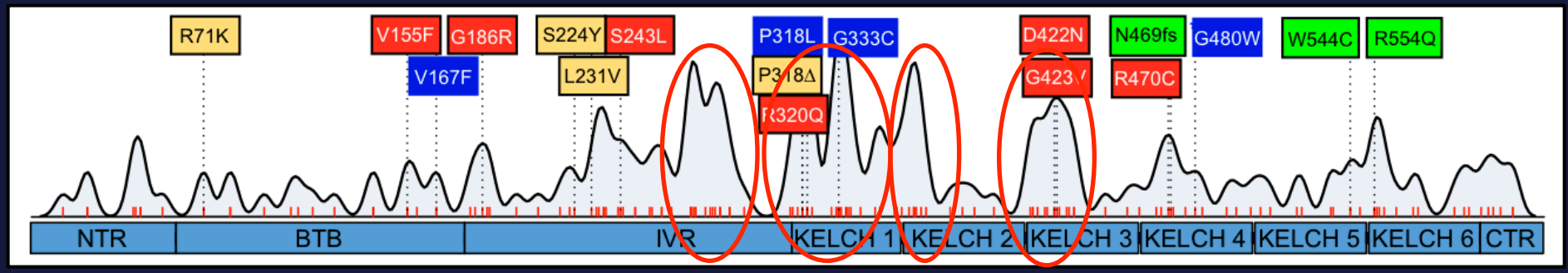
-C288 specific reactivity to alkenals



Is cysteine reactivity in KEAP1 altered in cancer?

McMahon *et al, PNAS* 2010

KEAP1 mutations cluster



Class I: binds NRF2 strongly, does not inhibit

Class II: does not bind NRF2, does not inhibit

Class III: weakly binds NRF2, does not inhibit

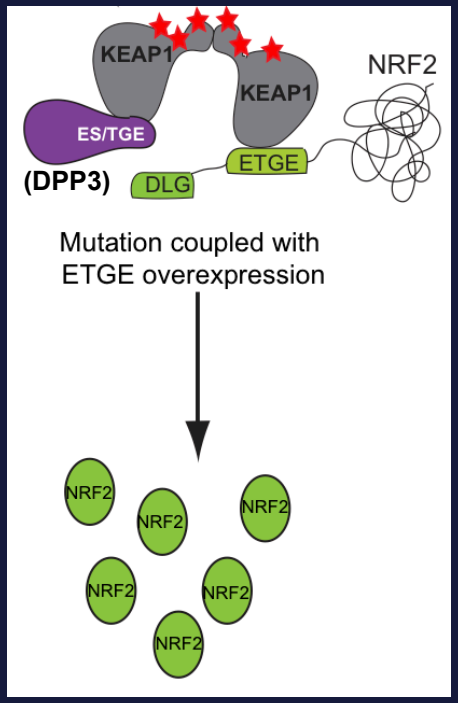
Class IV: behaves like WT

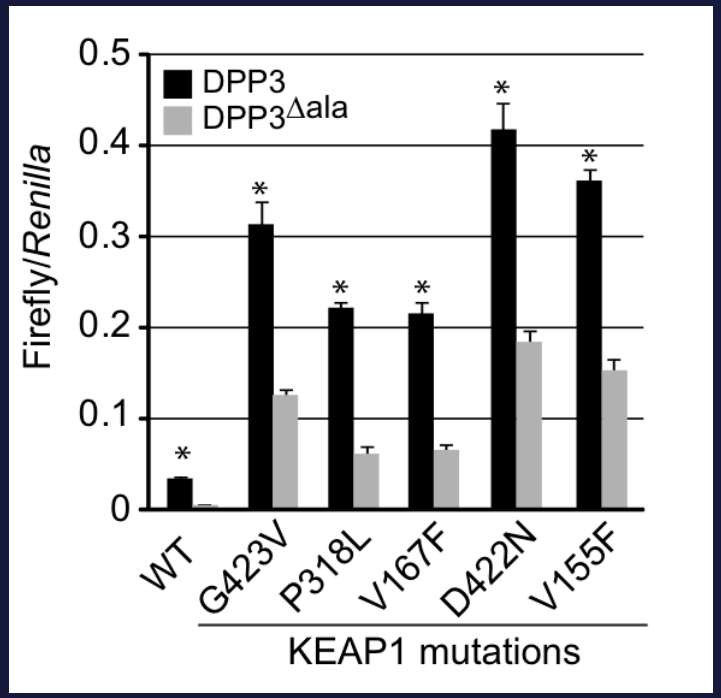
240 320-350 430 Approximate Residue

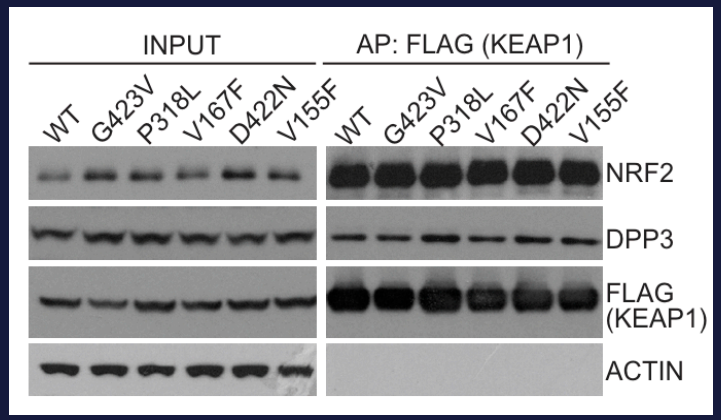
Cys 241, 249, 319, 368, 434, 489 have been shown to react with electrophilic fatty acids as well as sulforaphane

Are clustered mutations "pointing" to important regions of KEAP1?

KEAP1 mutations are hypomorphic and can be further inactivated by interacting proteins







-Overexpression of the ETGE-containing protein DPP3 further activates NRF2 signaling in a KEAP1 mutant background

-DPP3 is overexpressed in tumor verses normal lung squamous cell carcinoma (p=4.6e-14)

Summary

-Mutations in KEAP1 from lung squamous cell carcinoma can be grouped into four phenotypic classes

-The “superbinder” class exhibits enhanced NRF2 activity and stability, and is likely a result of structural changes in the KEAP1 homodimer

-KEAP1 mutations in cancer cluster around cysteines with reactivity to electrophilic compounds

-Overexpression of ETGE-containing proteins can further activate NRF2 activity in a KEAP1 mutant background

Major Lab
Ben Major
Erica Cloer

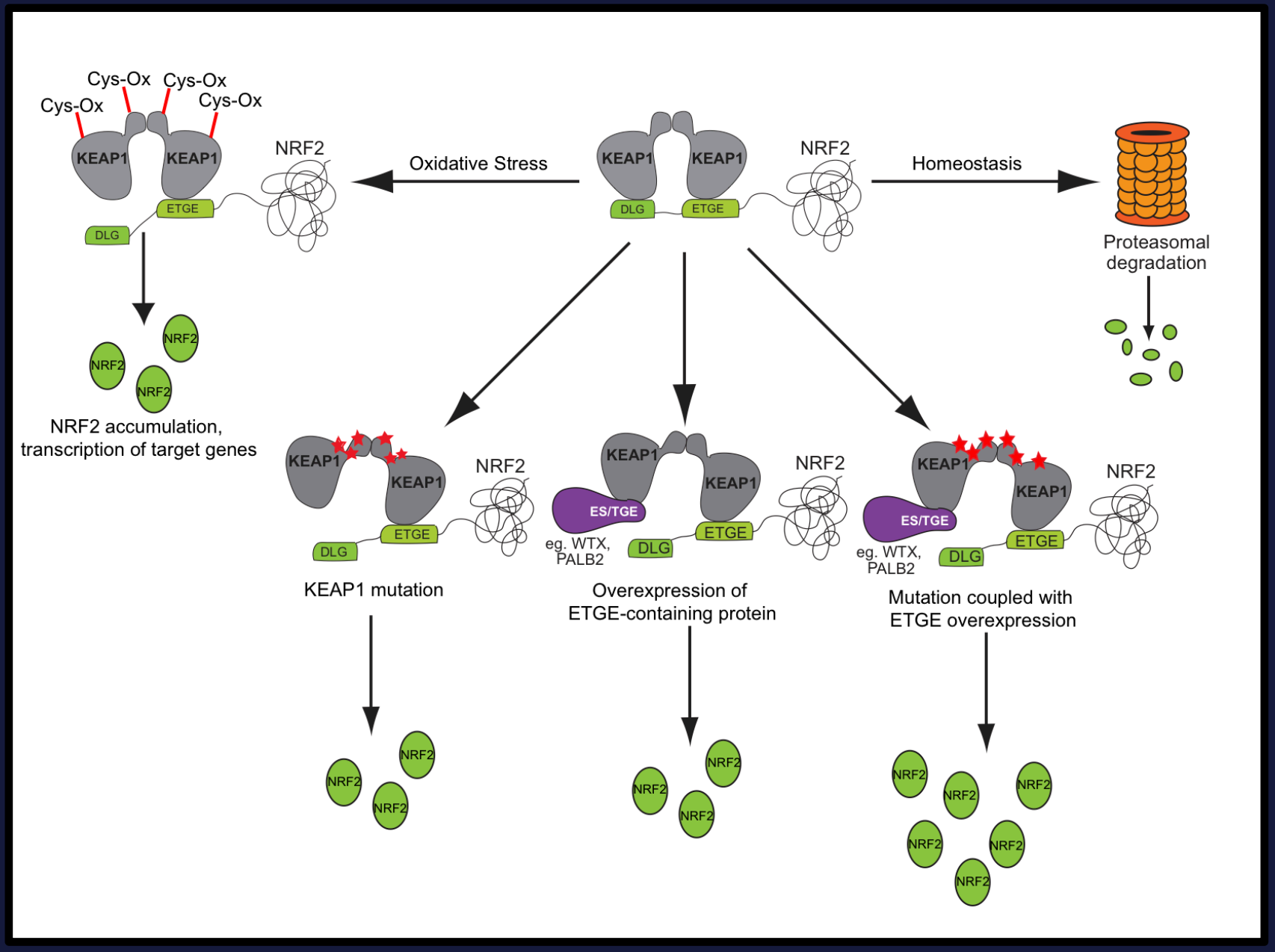
Kathleen Mulvaney
Dennis Goldfarb
Priscila Siesser
Matt Walker
Feng Yan
Alex Rabinowitz

Hayes Lab
Neil Hayes
Matt Wilkerson

Ning Zheng (U. Washington)

TCGA Research Network

Questions?


Cys-Ox
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KEAP1
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NRF2
ETGE
DLG
Oxidative Stress
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NRF2
DLG
ETGE
Homeostasis
Proteasomal degradation
NRF2
NRF2
NRF2
NRF2 accumulation, transcription of target genes
KEAP1
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Mutation coupled with ETGE overexpression