Article

Cell

A Quantitative Tissue-Specific Landscape of Protein Redox Regulation during Aging

Xiao et al, *(2020)*

Presented

by

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10-11-2021

Introduction

- Mammalian tissues control distinct physiological processes despite all sharing substantially overlapping transcriptomes and proteomes
- Much of this specialized physiology is controlled by PTMs
- Majority of PTMs occur through production of ROS and related species
- Dysregulation of ROS and redox signaling induce pathology by damaging lipids, proteins and DNA

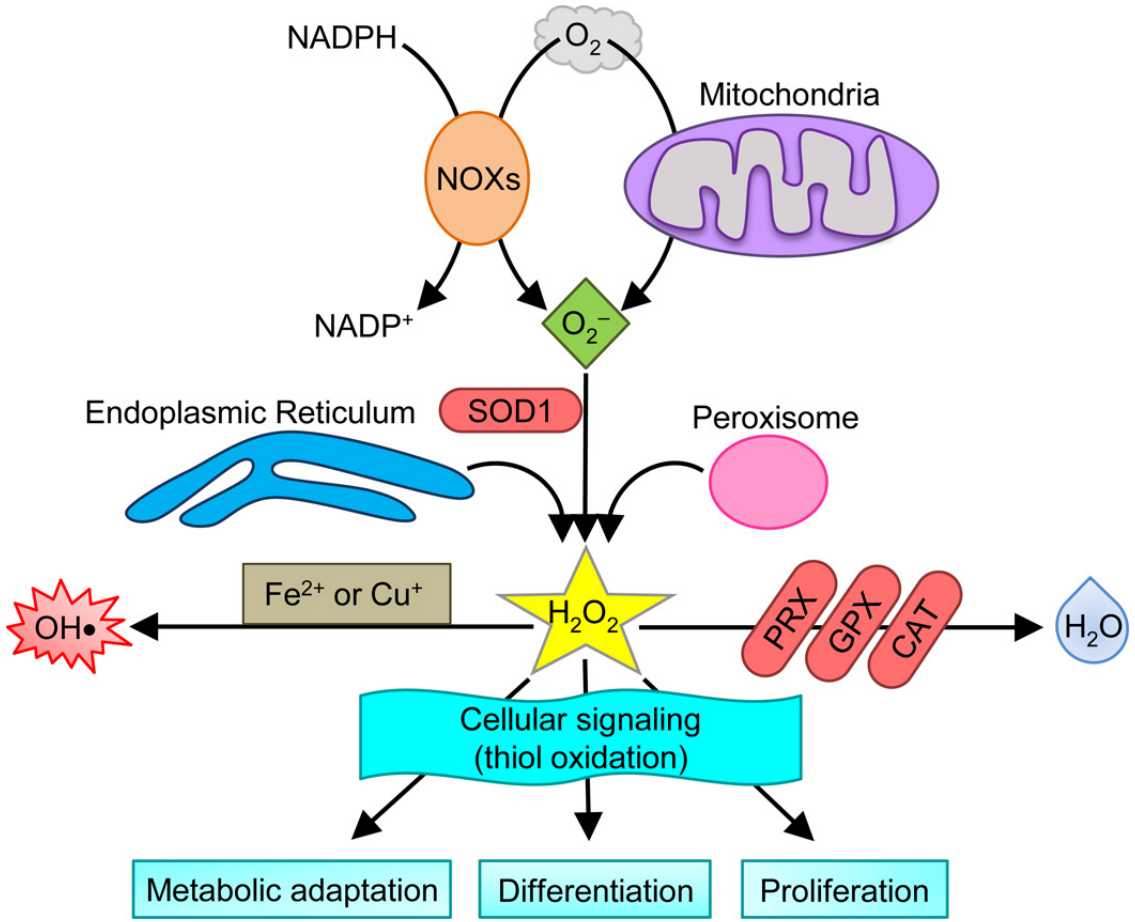


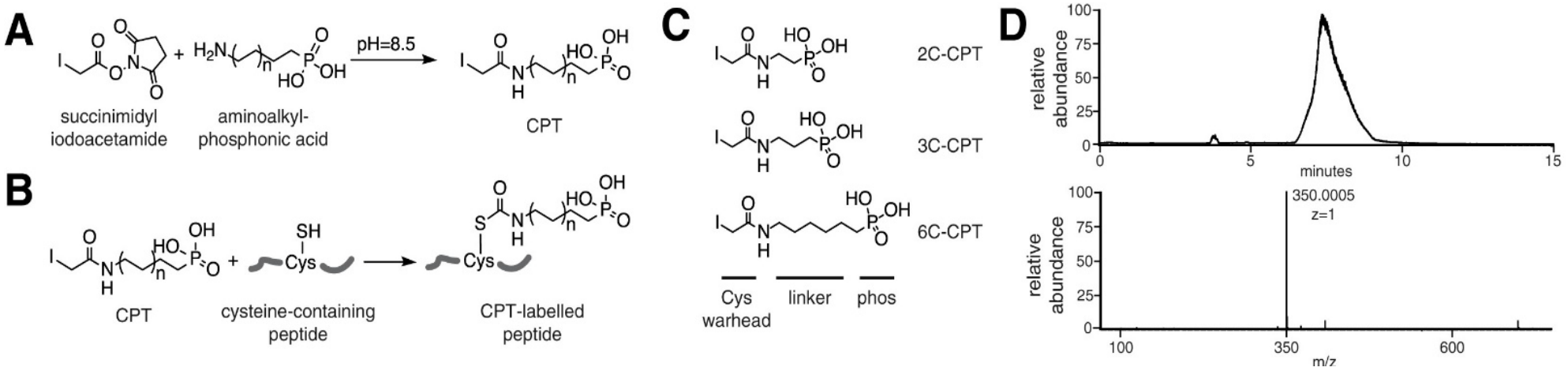
Fig: Endogenous sources of ROS signal

Intracellular ROS is primarily produced by NADPH oxidase enzymes (NOXs), the mitochondria, the endoplasmic reticulum, and the peroxisome. Cytosolic superoxide (O_2^-) is rapidly converted into hydrogen peroxide (H_2O_2) by superoxide dismutase 1 (SOD1). H_2O_2 can either act as a signaling molecule by oxidizing critical thiols within proteins to regulate numerous biological processes, including metabolic adaptation, differentiation, and proliferation or be detoxified to water (H_2O) by the scavenging enzymes peroxiredoxin (PRX), glutathione peroxidase (GPX), and catalase (CAT). In addition, H_2O_2 can react with metal cations (Fe^{2+} or Cu^+) to generate the hydroxyl radical (OH•), which causes irreversible oxidative damage to lipids, proteins, and DNA.

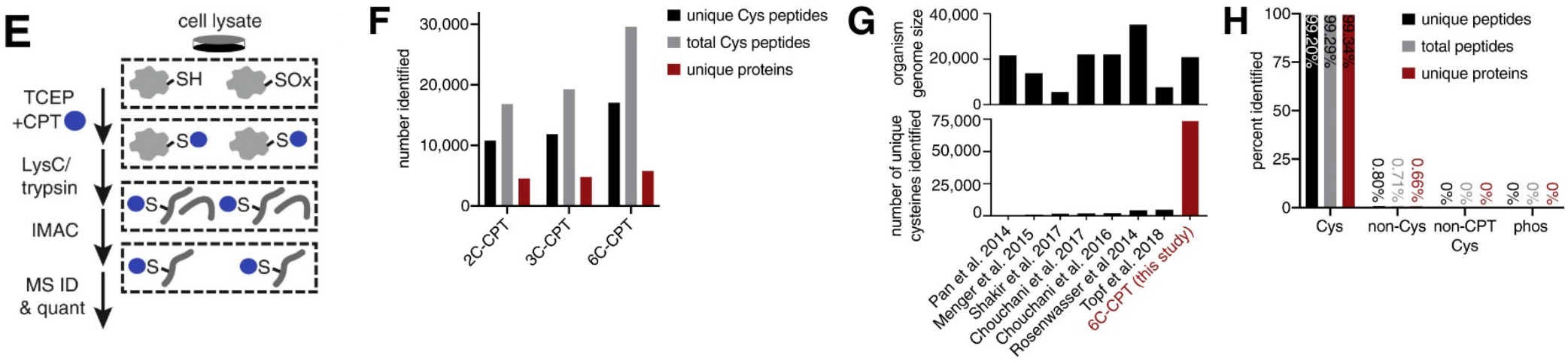
Colleen et al, 2015 Curr Opin Cell Biol

Methodology

- CPT Synthesis, labelling and purification

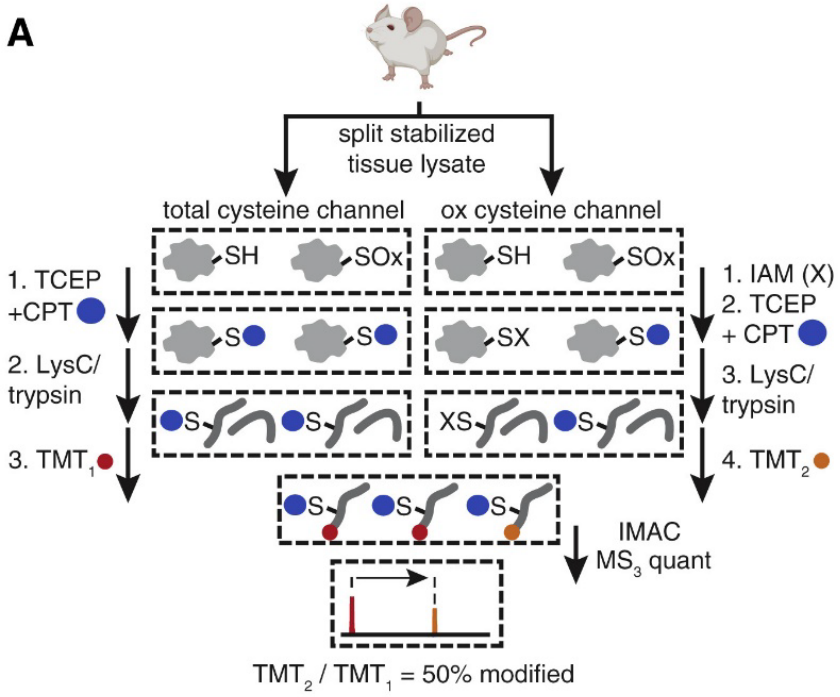


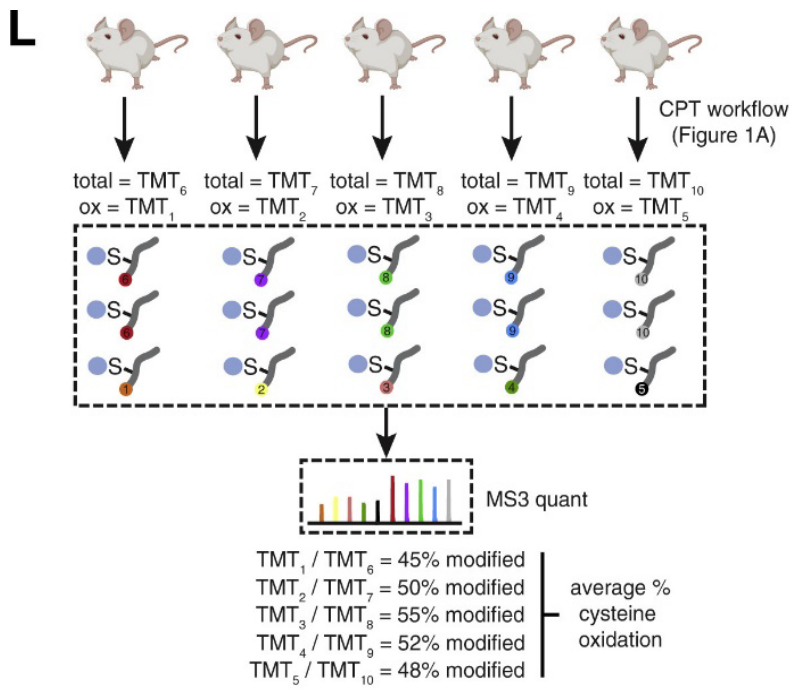
- Work-flow, comparison and validation of CPTs



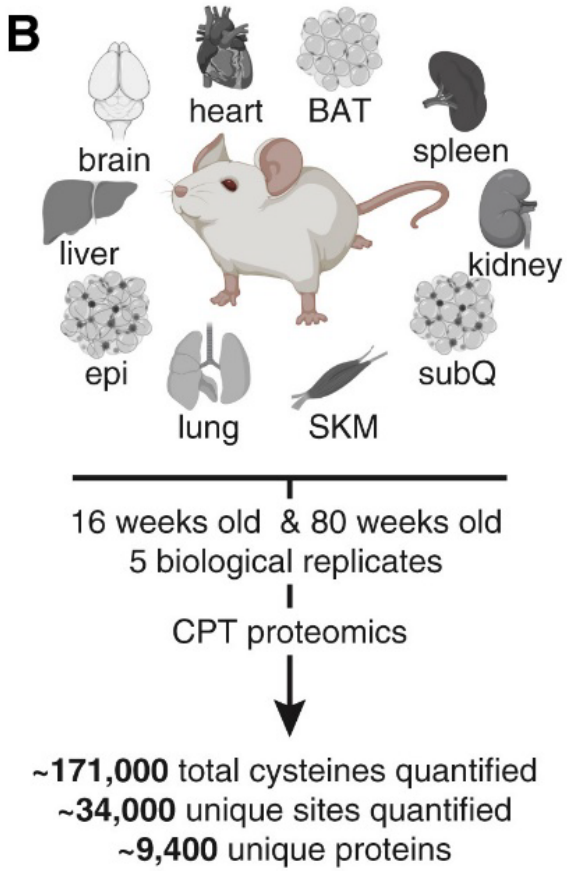
Quantification of reversibly oxidized thiols work-flow

- Pretreatment with Iodoacetamide
- As iodoacetamide blocks unmodified cysteines

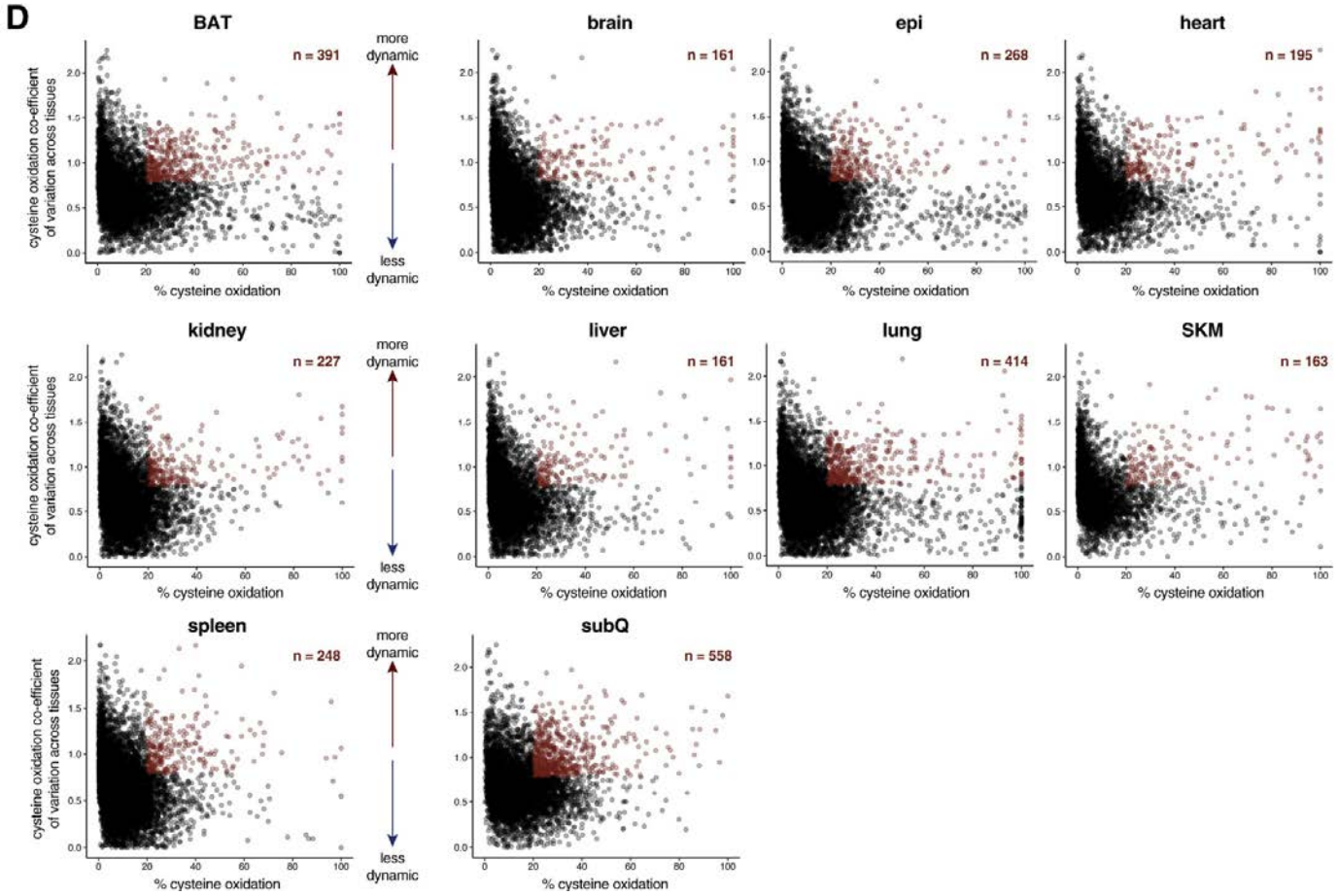




Findings of Oximouse (C57BL/6J) Study



Tissue-Specific Redox Modification Landscape *In Vivo*

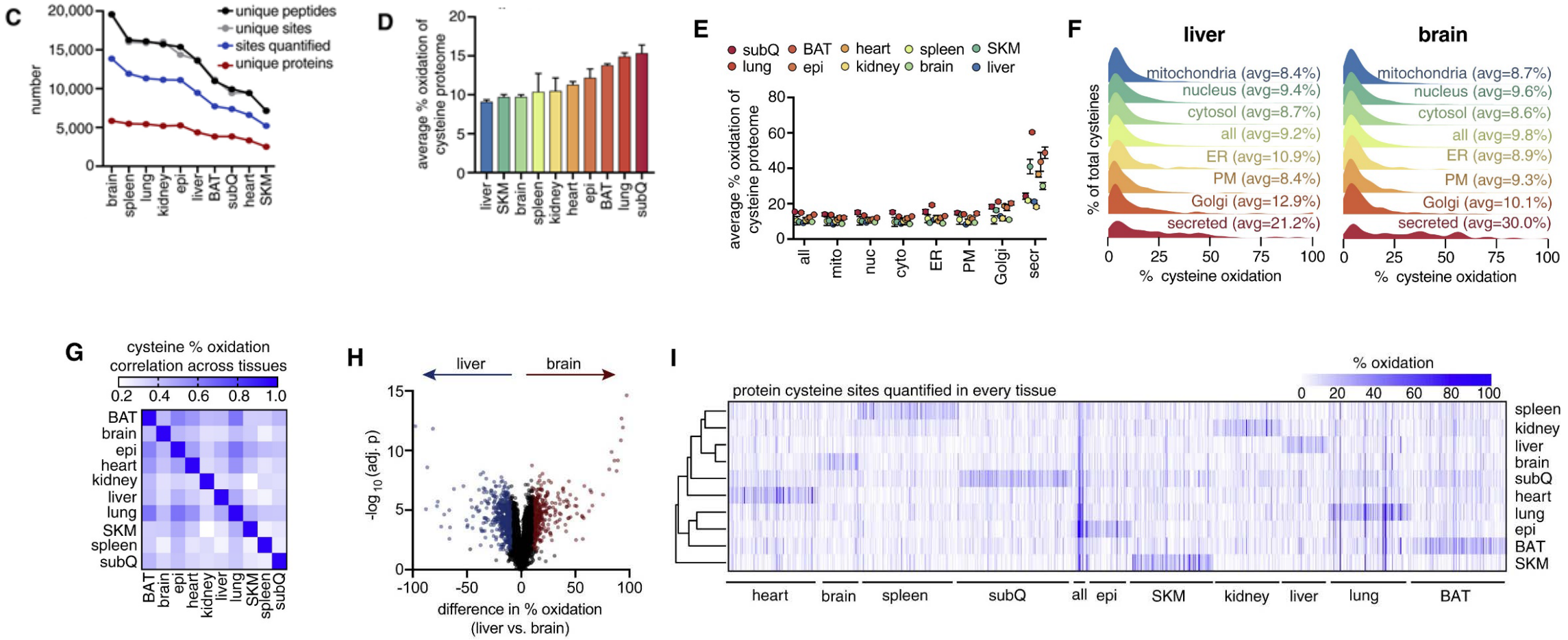


Modification score for all cysteines in the Oximouse dataset across tissues

-Plotted according % oxidation in each tissue against CV across tissues

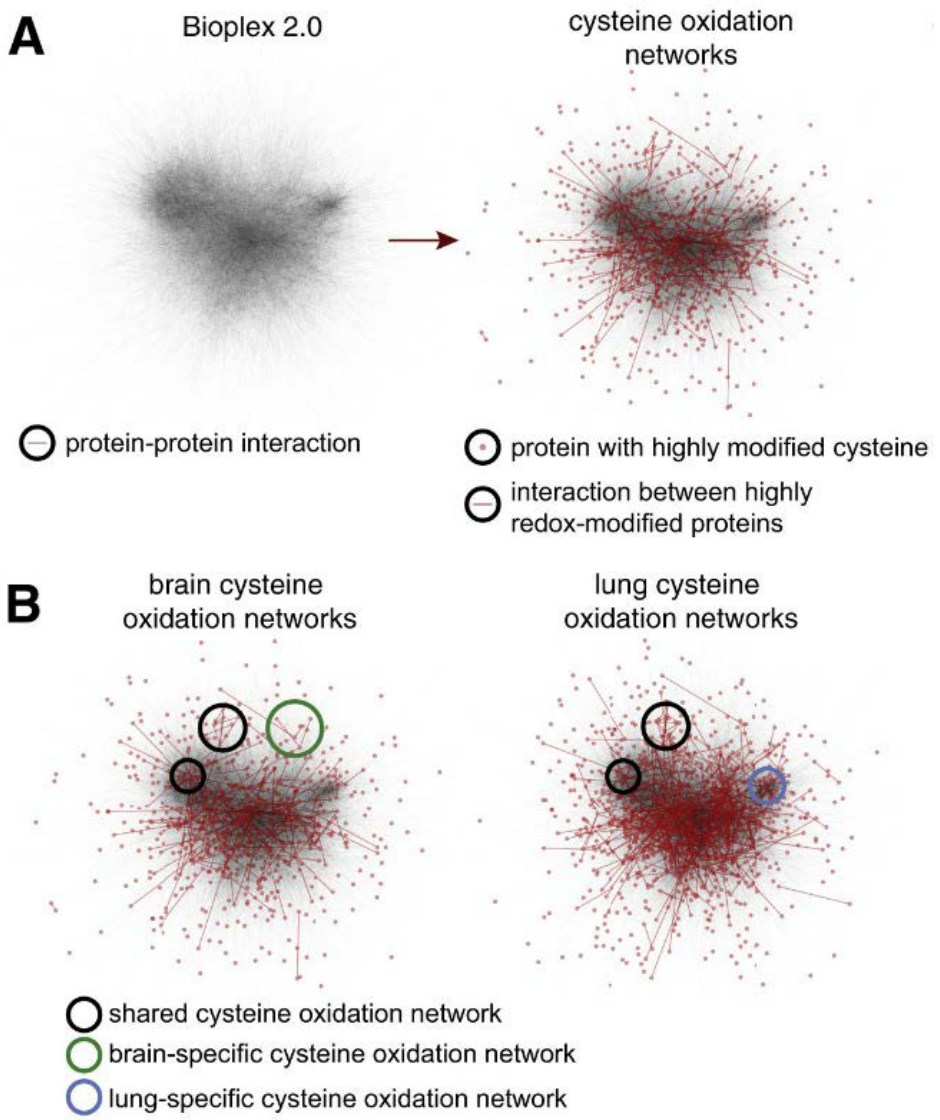
-Higher the CV more dynamical regulation
-High oxidation >20% and high CV are highlighted red

Tissue-Specific Redox Modification Landscape *In Vivo*

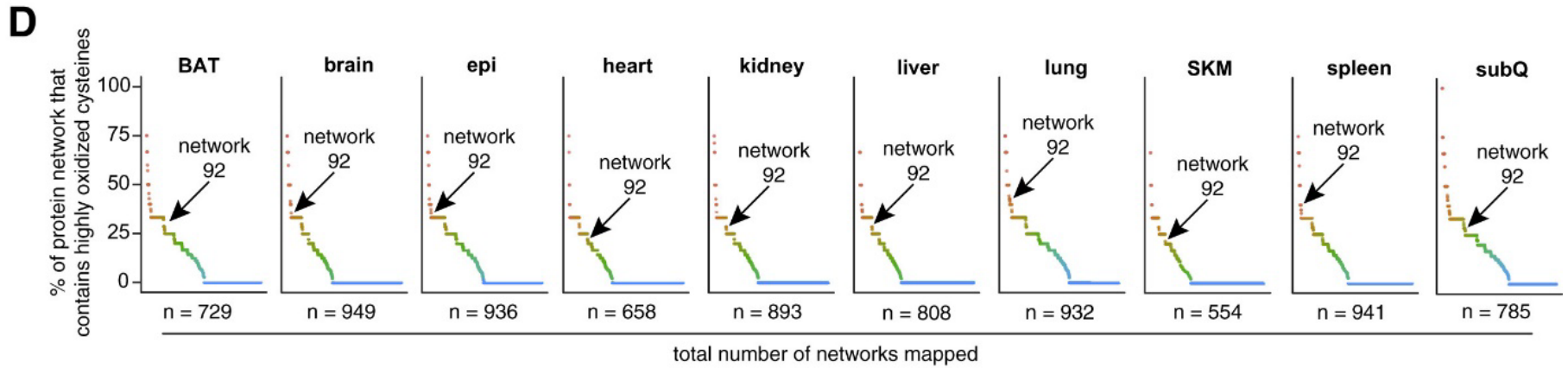


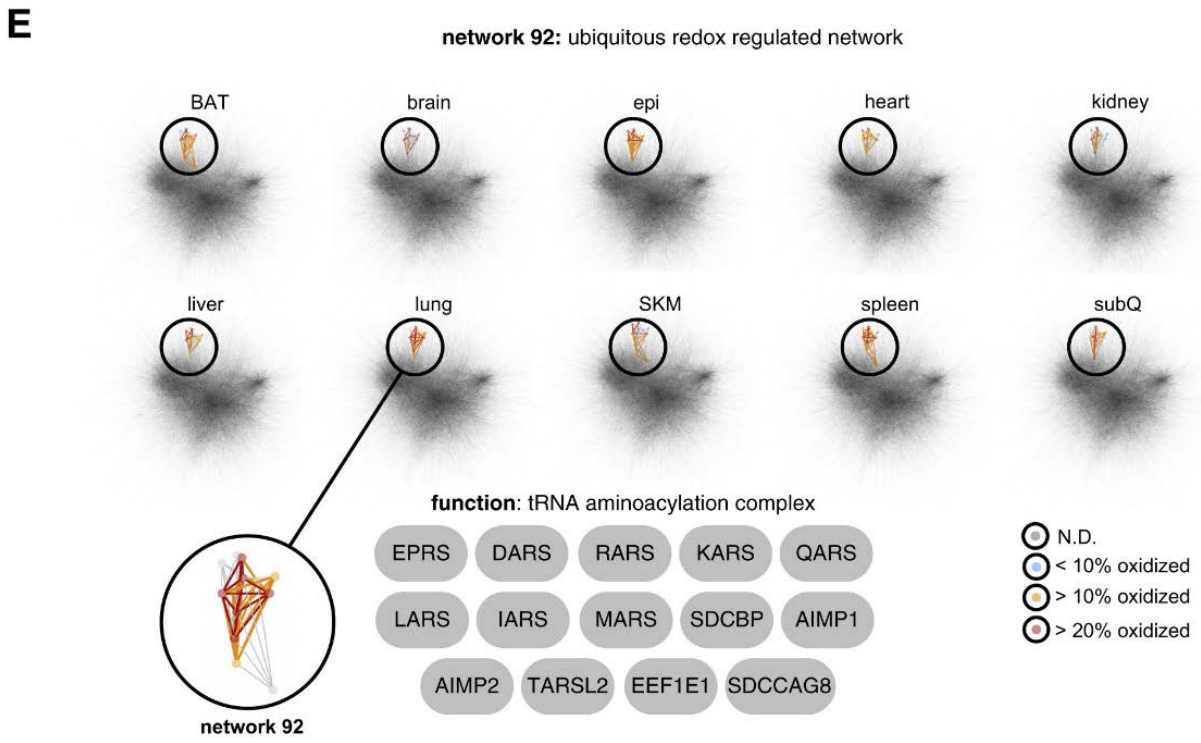
Systematic identification of Redox-modified Protein Networks *In Vivo*

- **Combined Oximouse data with Bioplex 2.0**
- **Visualization of Bioplex 2.0, which includes 56,553 interactions (black lines) across 10,961 proteins (black dots).**
- **Cysteine oxidation networks are determined by mapping proteins with highly modified cysteines (defined as >20% oxidized; red dots) that interact with each other (red lines).**
- **Cysteine oxidation networks in brain and lung identify redox-regulated networks shared across both tissues and redox network-specific to each tissue.**



Tissue ubiquitous Redox-Regulated Protein Networks *In Vivo*





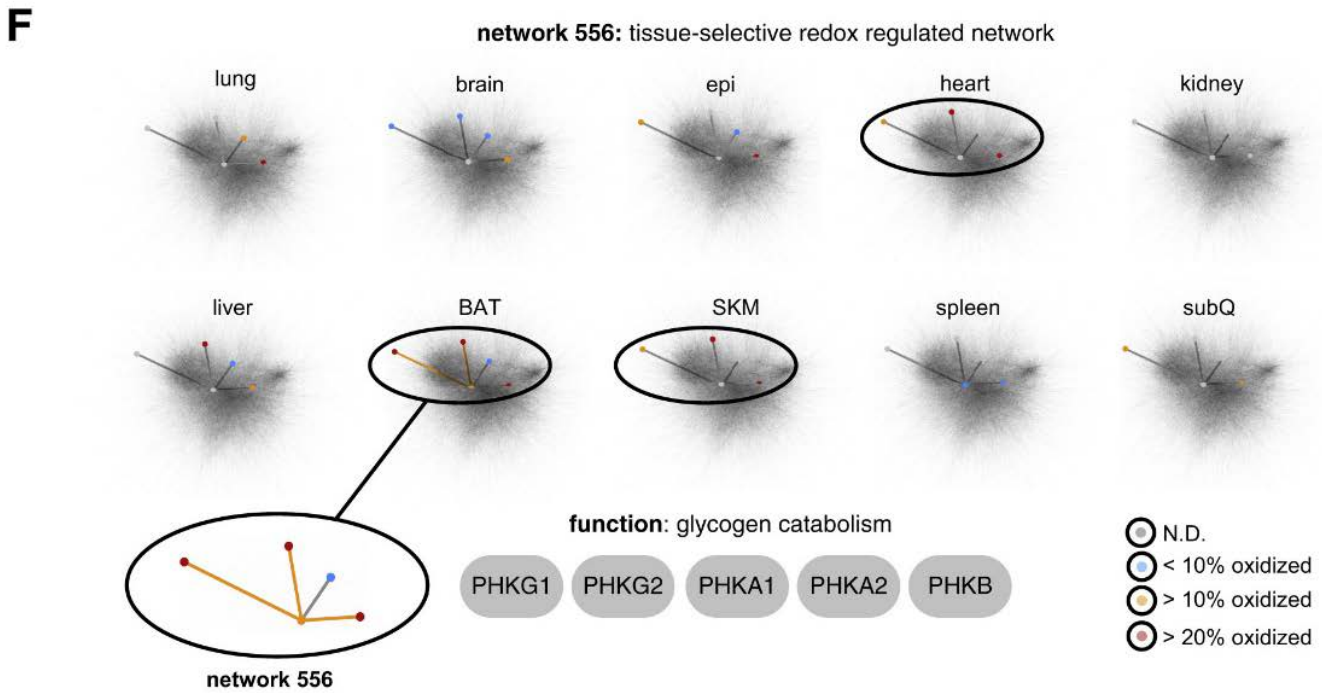
-(D)Number of redox modification of protein networks in each mouse tissue.

-Network 92 highlighted as an example of a ubiquitously redox modified network.

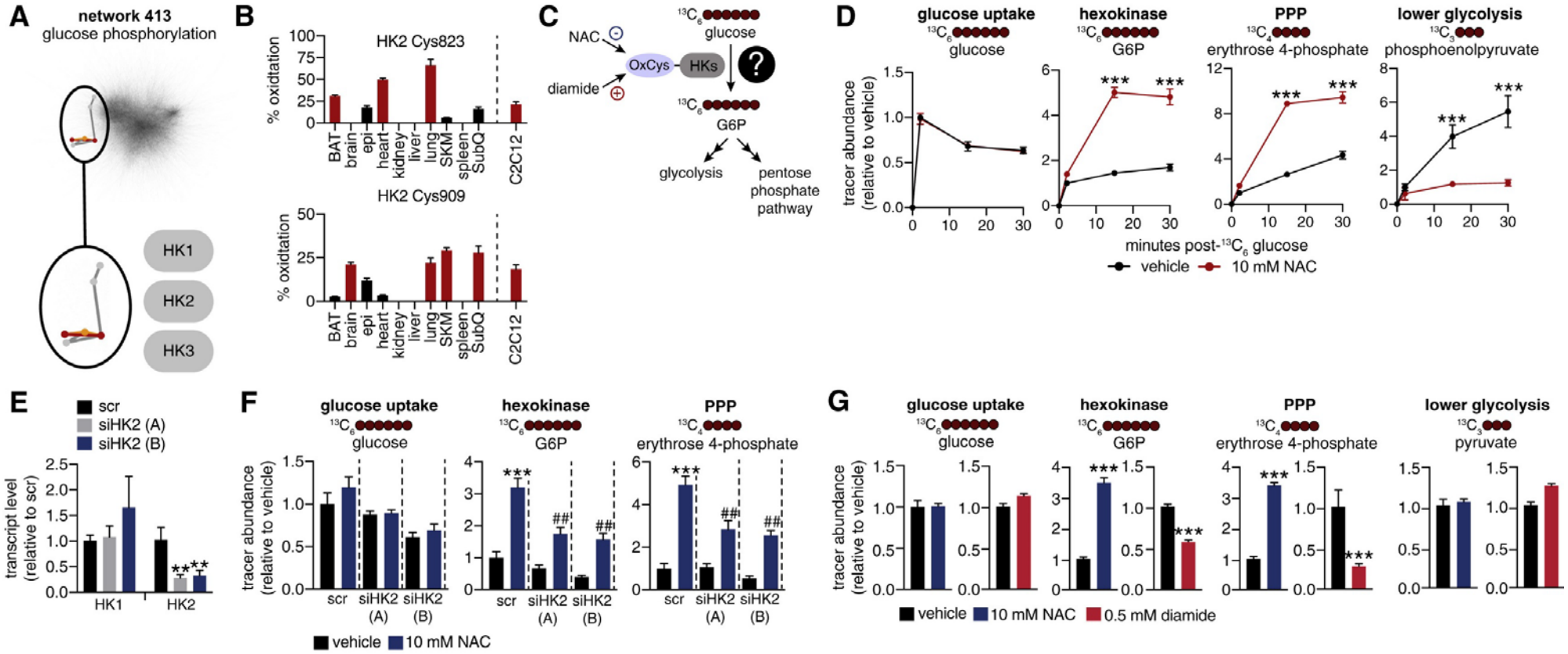
-(E) Network of proteins involved in tRNA aminoacylation are coordinately redox modified in every tissue.

Identification of tissue selective Redox-Regulated Protein Networks *In Vivo*

- **Network 556 coordinately modified in heart, BAT and SKM**
- **Involve in breakdown of glycogen to feed glycolysis and PPP**

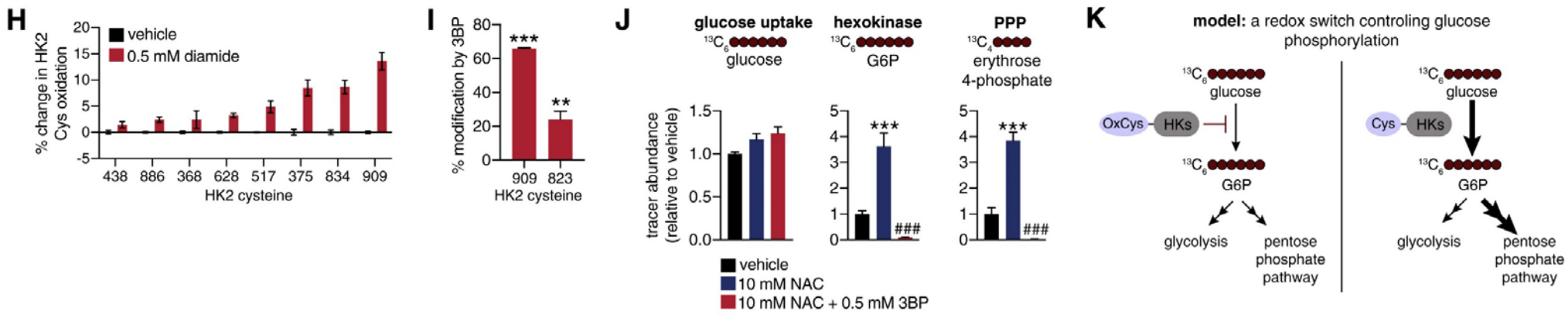


Identification of New Pathways of Redox Regulation using Oximouse



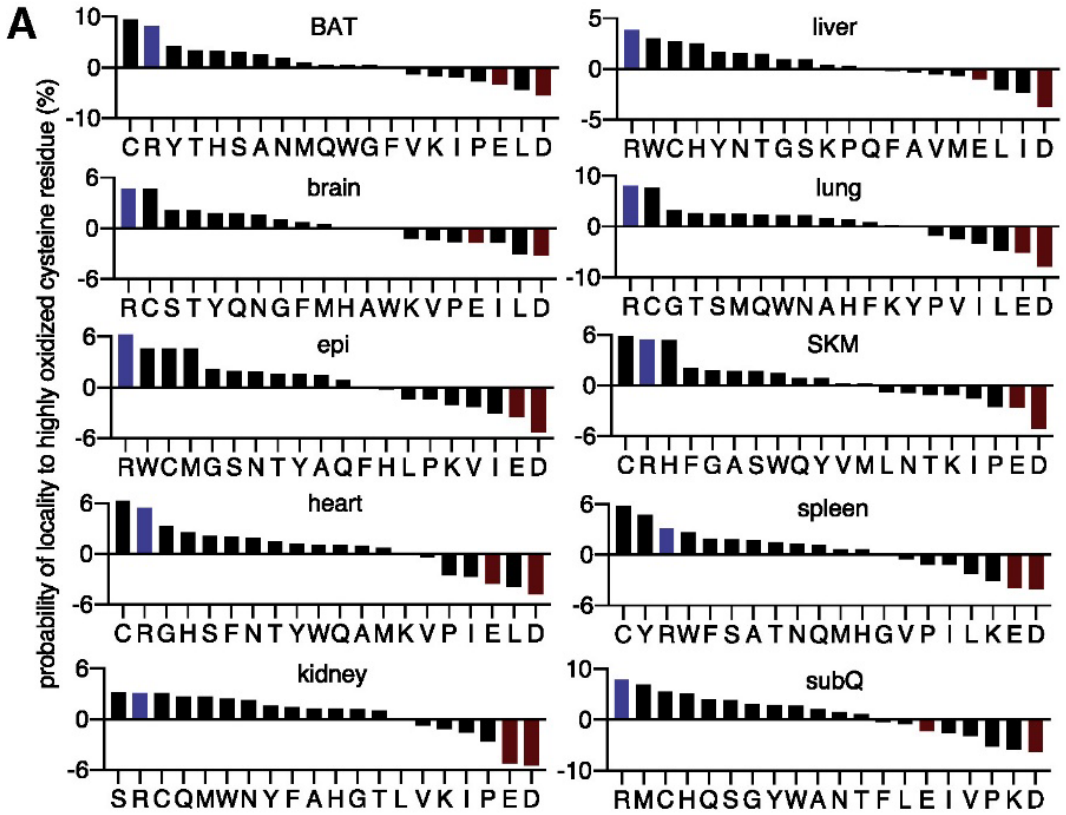
*Oximouse redox-network 413 suggests a node of redox regulation exists for glucose phosphorylation by HKs

Identification of New Pathways of Redox Regulation using Oximouse

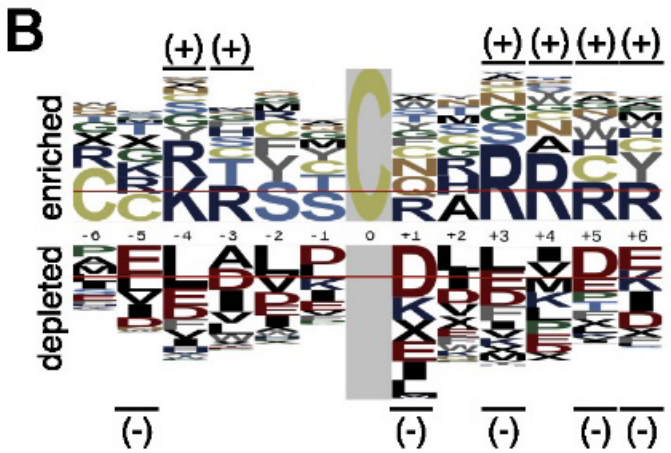


(H) 15 min diamide treatment increases HK2 Cys909 oxidation over 10%. n = 6.
(I) 3BP covalently modifies HK2 Cys909 and Cy823. n = 3.
(J) Inhibition of HK2 activity by 3BP abrogates NAC stimulated G6P production through HK2. n = 6.
(K) Model for cysteine redox node controlling glucose phosphorylation to G6P.

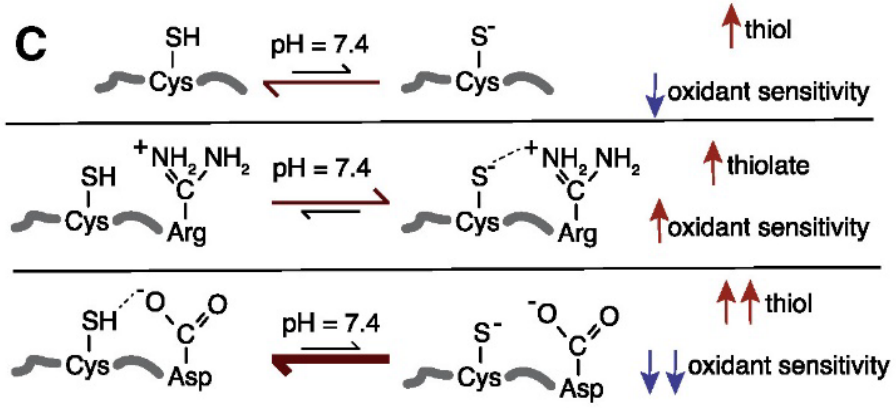
Cysteine Thiol Redox Sensitivity Is Encoded by Local Electrostatic Effects



(A) In every tissue, significant enrichment of arginine, and selection against aspartic acid and glutamic acid, proximal (±four positions) to highly (>20%) redox modified cysteine residues.



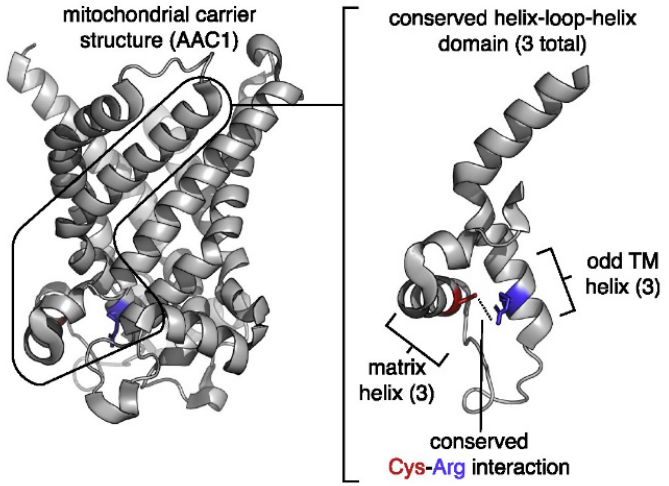
(B) Consensus motif for highly modified (>20%) cysteine residues illustrates significant enrichment of arginine and selection against aspartic acid and glutamic acid across a range of proximal positions.



(C) A model for electrostatic gating of cysteine thiol redox sensitivity.

Cysteine Thiol Redox Sensitivity Is Encoded by Local Electrostatic Effects

D

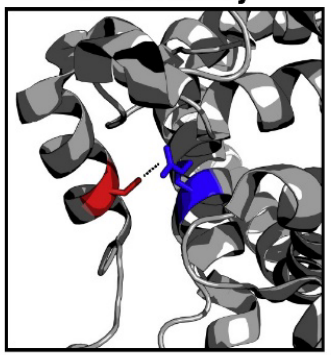


E

mitochondrial carriers	matrix helix			max % oxidation			odd TM helix		
	1	2	3	1	2	3	1	2	3
AAC1	C	C	C	21	22	18	R	R	R
AAC2	C		C	20		17	R		R
UCP1			C			24			R
UCP3			C			7			R
SLC25A1	C		C	24		16	R		R
SLC25A10			C			24			R
SLC25A20	C	C		16	14		R	R	
SLC25A22	C		C	50		30	R		R
SLC25A34			C			16			R
SLC25A36	C	C		24	21		R	R	
SLC25A45	C	C	C	68	20	43	R	R	R
MTCH1			C			10			L
average % oxidation (carrier C-R sites)				25***					
average % oxidation (mitochondria)				10					

F

SLC25 family



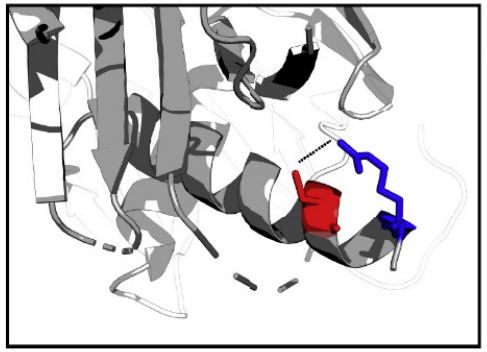
Protein: AAC1, AAC2, UCP1

Cys-Arg pair:
Cys56-Arg31 (AAC1 & AAC2)
Cys159-Arg138 (AAC1)
Cys257-Arg237 (AAC1 & AAC2)
Cys253-Ar239 (UCP1)

Consequence of Cys mod:
i. inhibition of transport (McStay et al., 2002)
ii. proton leak (AAC1 & AAC2) (Bertholet et al., 2019)
iii. proton leak (UCP1) (Chouchani et al., 2016)

G

hexokinase 2



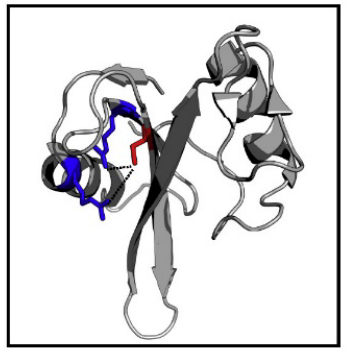
Protein: hexokinase 2

Cys-Arg pair:
Cys909-Arg912

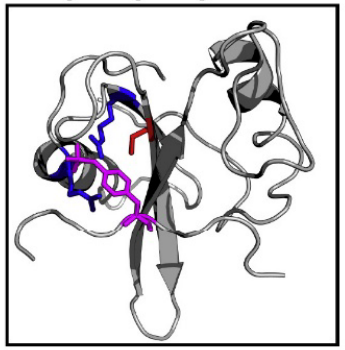
Consequence of Cys mod:
i. inhibition of G6P production (Figure 3)

H

Src kinase



phosphorylated

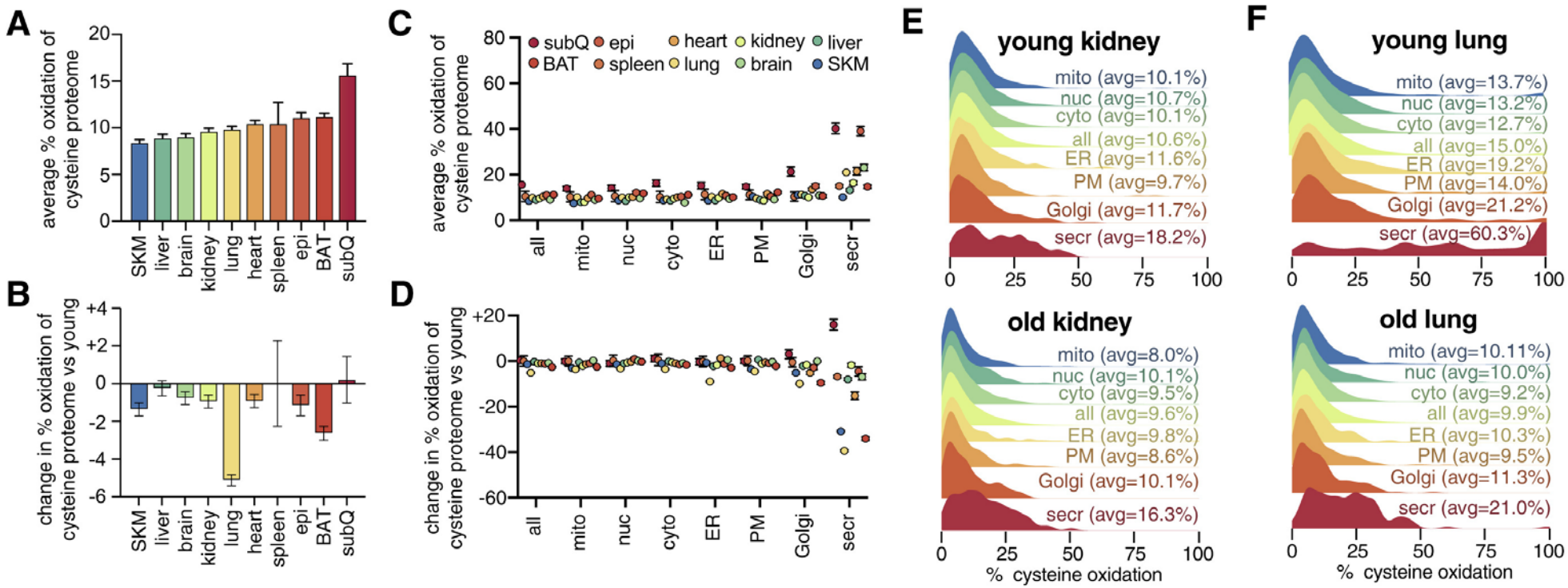


Protein: Src kinase

Cys-Arg pair:
Cys185-Arg175 (Src)
Cys185-Arg155 (Src)

Consequence of Cys mod:
i. release from auto-inhibited state & activation (SH2 domain)
ii. antagonized by **phosphorylation**
(Heppner 2018)

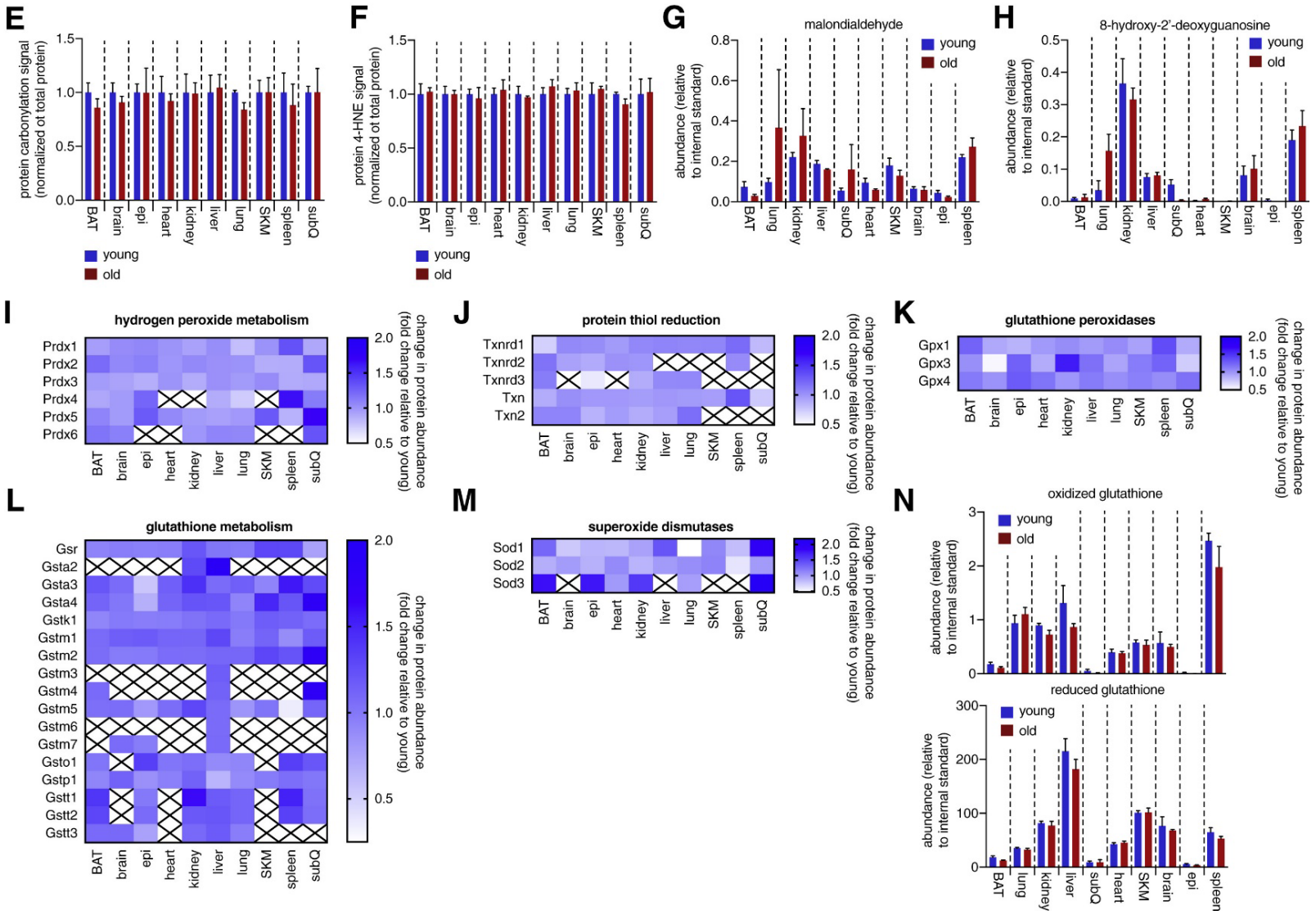
Aging Globally Remodels Tissue Specific Cysteine Oxidation Networks



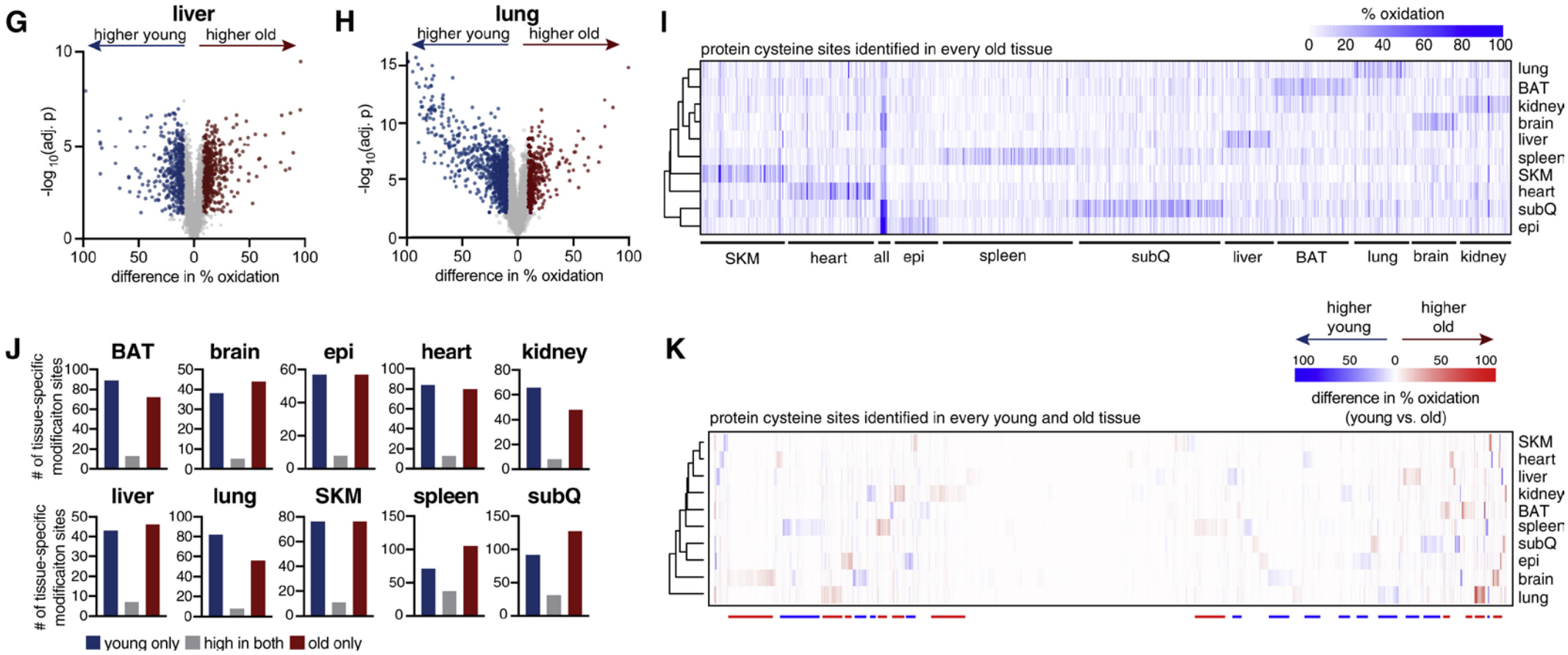
Aging Globally Remodels Tissue Specific Cysteine Oxidation Networks

-Quantification of change in abundance in mouse tissues (young vs old)

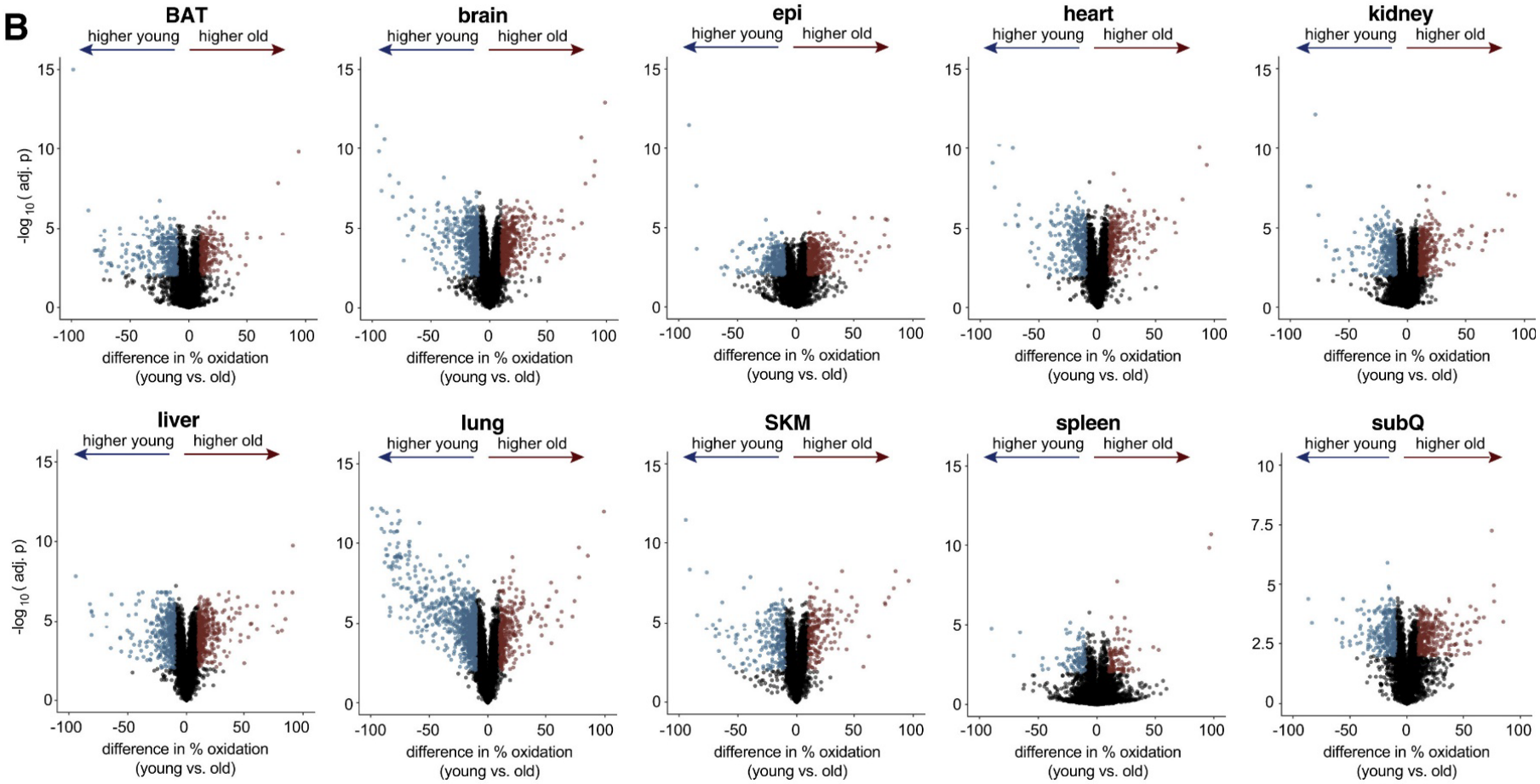
- I-K fold change in protein abundance (relative to young)



Aging Globally Remodels Tissue Specific Cysteine Oxidation Networks

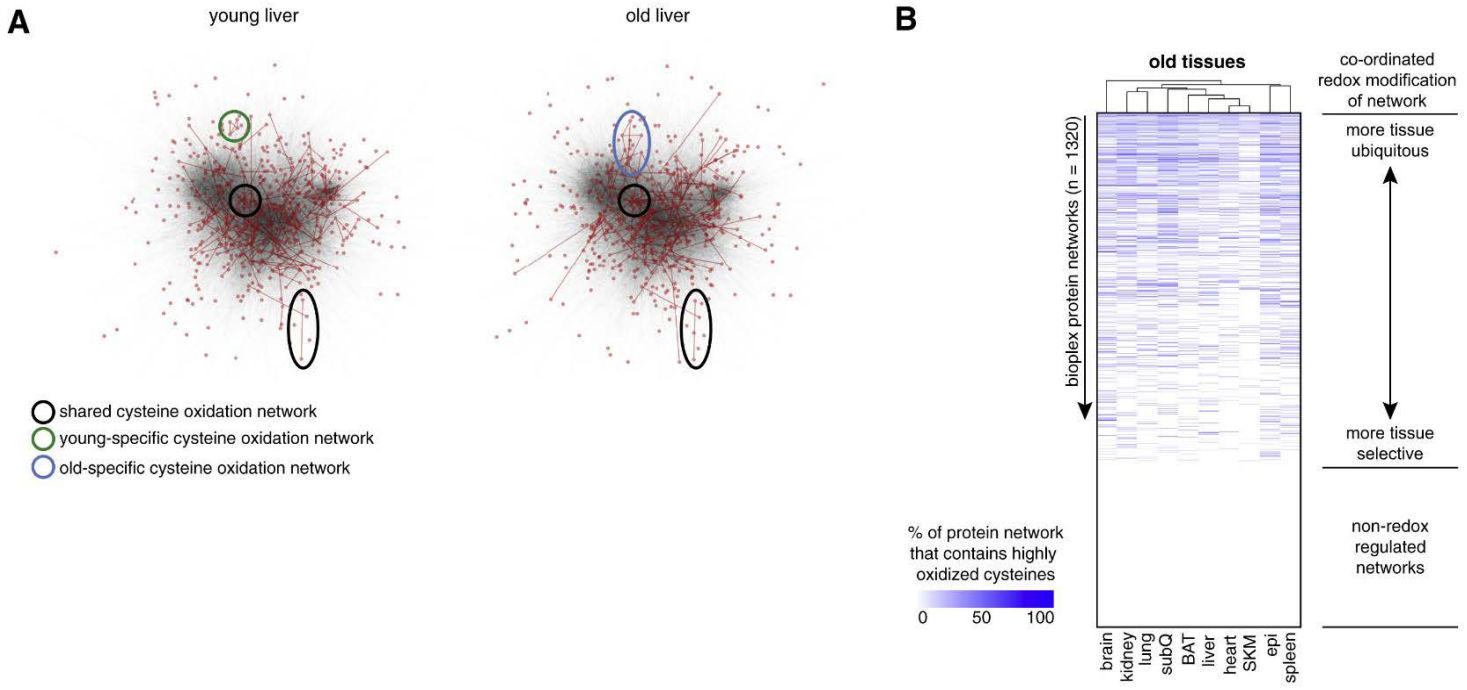


Pairwise comparison of change in % oxidation of individual cysteines between young and old tissues.



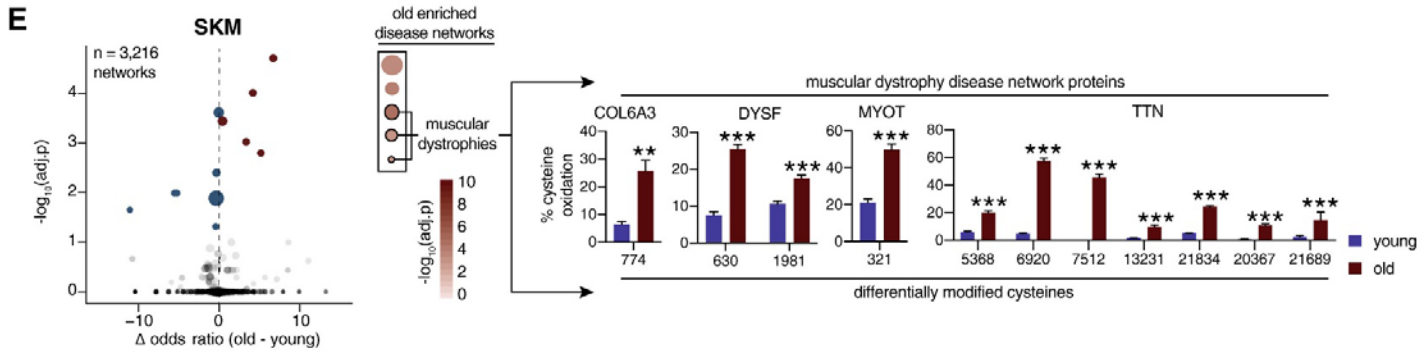
(B) Pairwise comparison of change in % oxidation of individual cysteines between old and young tissues. % oxidation value change of more than ± 10% and p value < 0.01 are highlighted. n = 5.

Age Depended Redox Regulation Of Protein Disease Networks

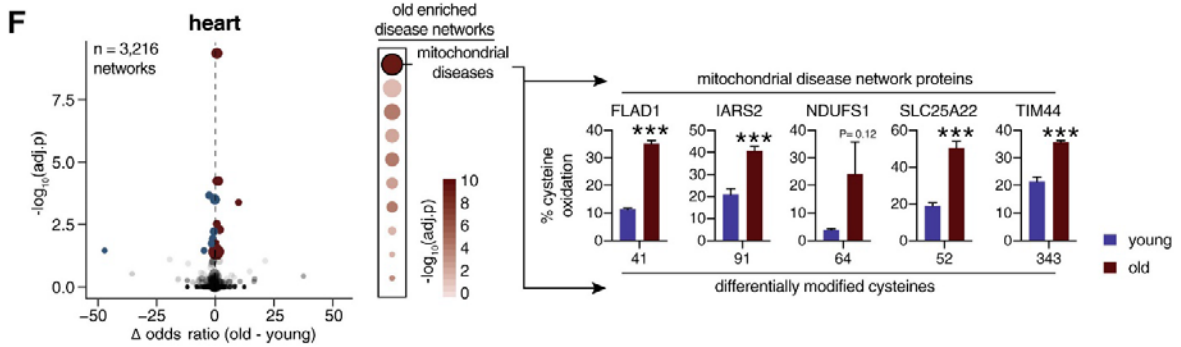


(A) Redox-regulated networks that are maintained with age (e.g., black highlight) and age-dependent loss and gain of redox-regulated networks (green and blue, respectively).
(B) Clustering of protein networks on the basis of extent of coordinated redox modification in aged tissues. Highly oxidized cysteines defined as >20% oxidized.
(C) Change in extent of redox modification of protein networks with age. Distinct protein networks are subject to coordinated redox modification in young and old tissues. Number of young (blue) and old (red) enriched redox networks are indicated for each tissue.

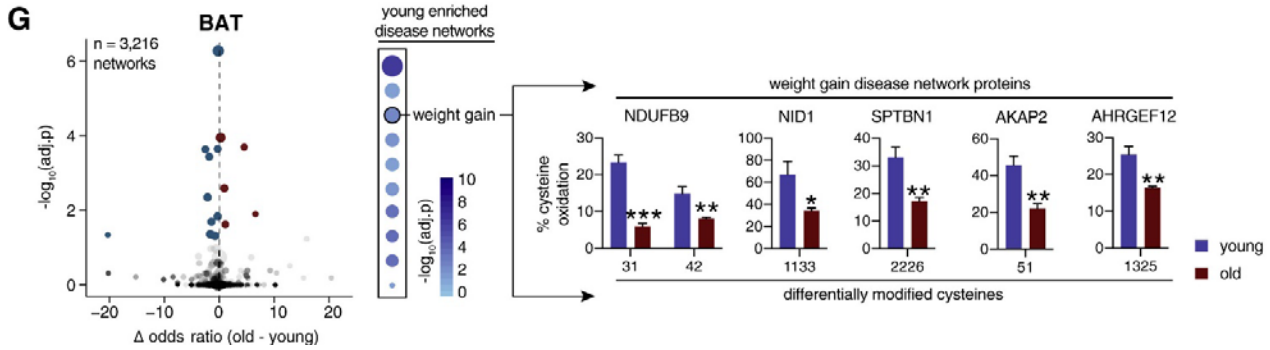
Disease Network Analysis in SKM, Heart and BAT



(E) Protein drivers of muscular dystrophies as selectively redox modified in **aged muscle**.



(F) Protein drivers of mitochondrial dysfunction as selectively redox modified in **aged heart**.



(G) Proteins involved in weight gain as selectively redox modified in **young BAT**. **p < 0.01; ***p < 0.001. All data are presented as mean ± SE.

Summary

- **A new method for deep quantitative analysis of cysteine proteome**

- Identification of 171000 total cysteines, 34000 unique sites, 9400 unique proteins

- Many redox proteins are tissue specific

- Identification of coordinated protein networks

- Highly modified cysteines are enriched in regions with R (+ve) residues while E and D regions are deriched

- Mapped aged specific and disease related redox networks in aged and young tissues

- A comprehensive source for future targeted research