

June 26 - 30, 2023
Smolenice Castle, Slovakia

BenBedPhar Training School 2023

NRF2 in noncommunicable diseases:
From bench to bedside



Nrf2 and inflammation

Albena T. Dinkova-Kostova
University of Dundee

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Discovery of Nrf2 as a regulator of the antioxidant response

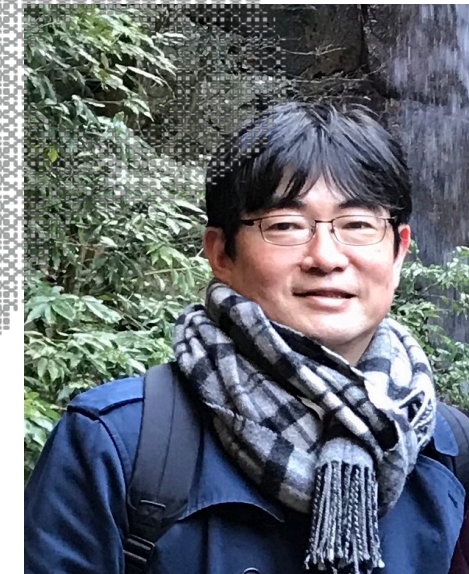
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 236, 313–322 (1997)
ARTICLE NO. RC976943

An Nrf2/Small Maf Heterodimer Mediates the Induction of Phase II Detoxifying Enzyme Genes through Antioxidant Response Elements

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Received June 10, 1997



Ken Itoh



Masayuki Yamamoto

Discovery of Keap1 as the principal regulator of Nrf2

Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain



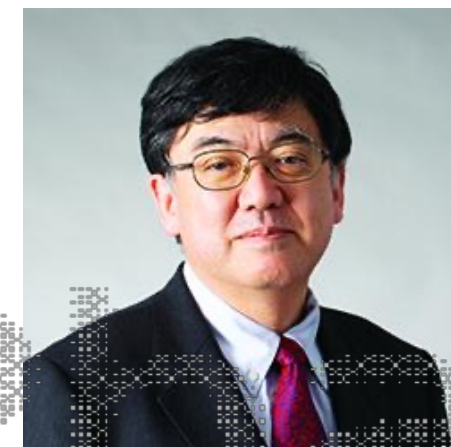
Ken Itoh



Nobunao Wakabayashi

Ken Itoh, Nobunao Wakabayashi, Yasutake Katoh, Tetsuro Ishii, Kazuhiko Igarashi, James Douglas Engel,¹ and Masayuki Yamamoto²

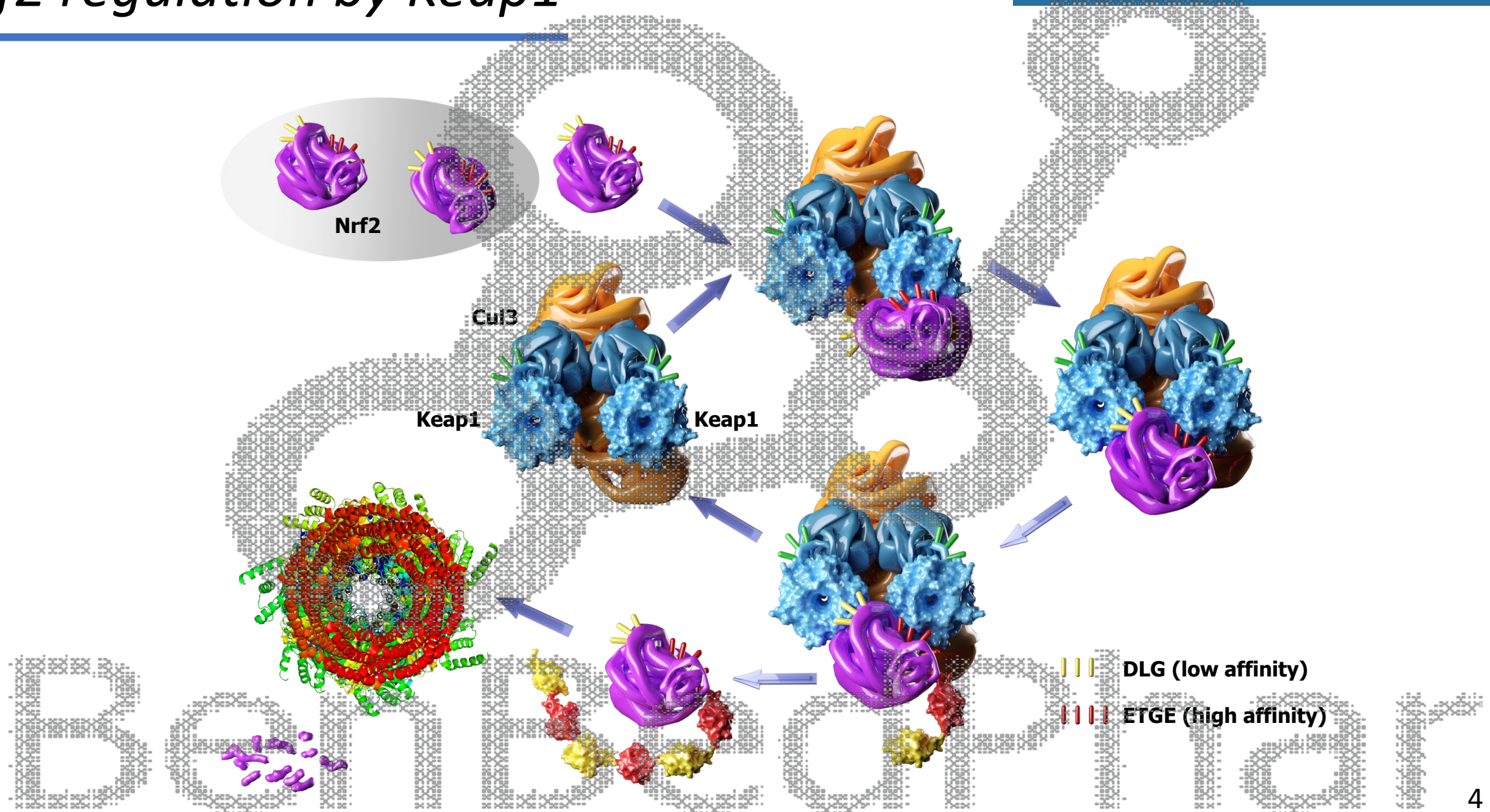
Center for Tsukuba Advanced Research Alliance and Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba 305-8577, Japan



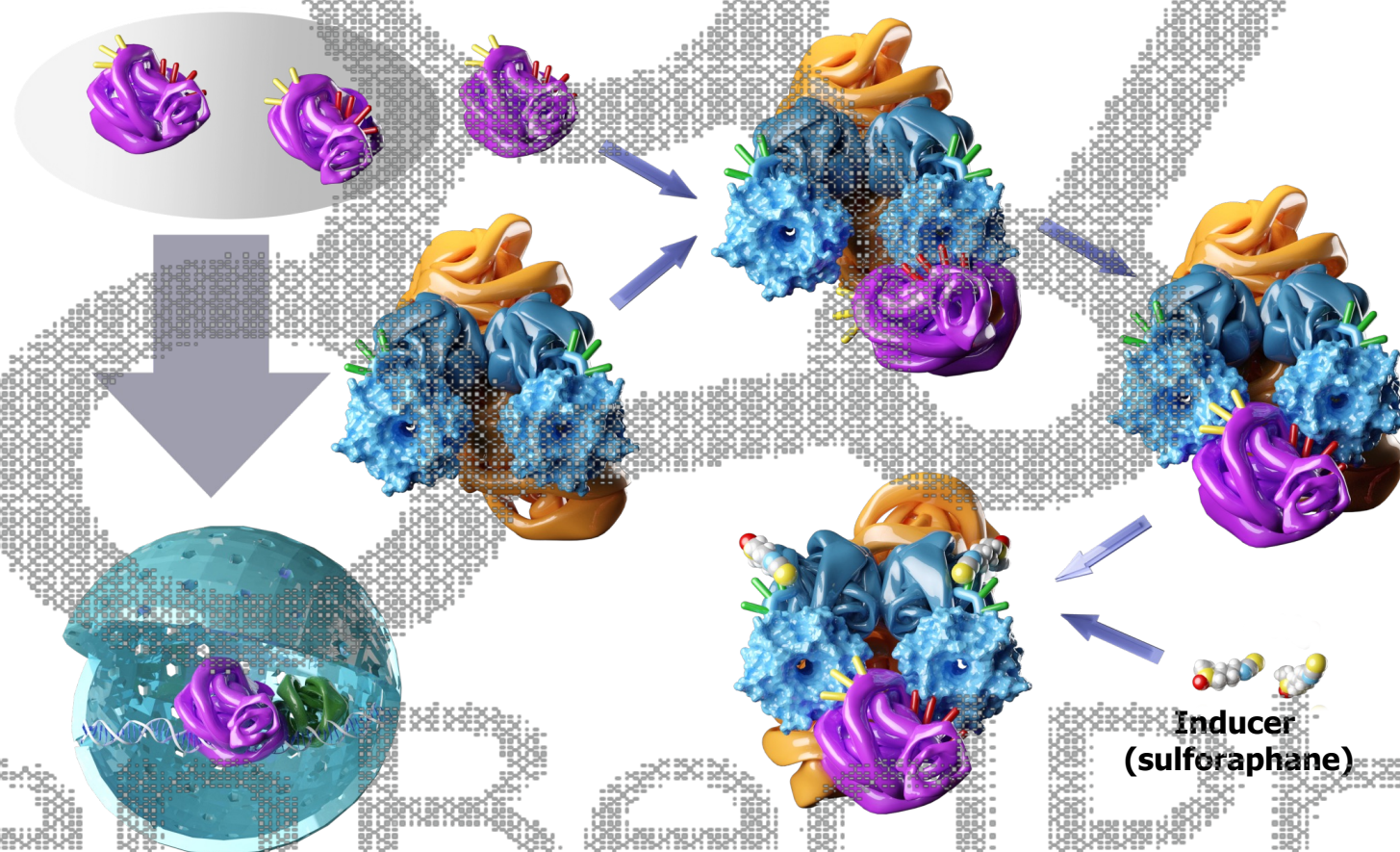
Masayuki Yamamoto

Itoh et al. (1999) Genes Dev 13: 76-86.

Nrf2 regulation by Keap1



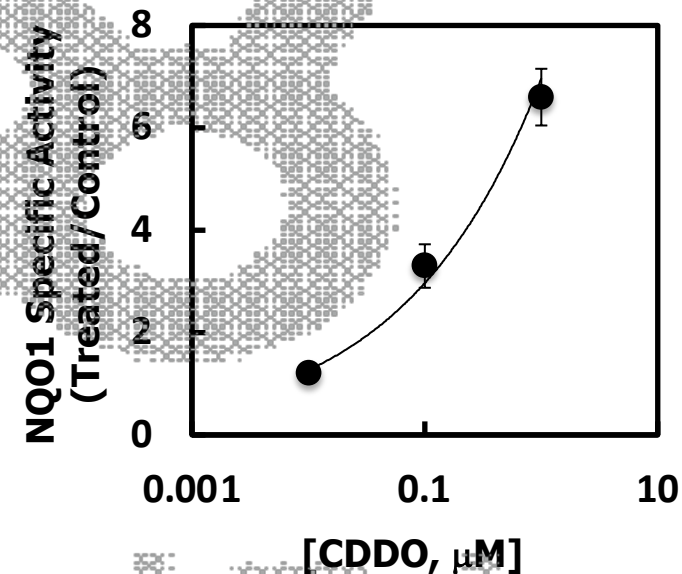
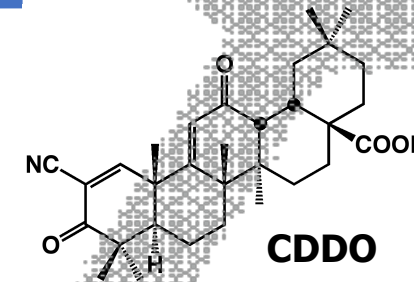
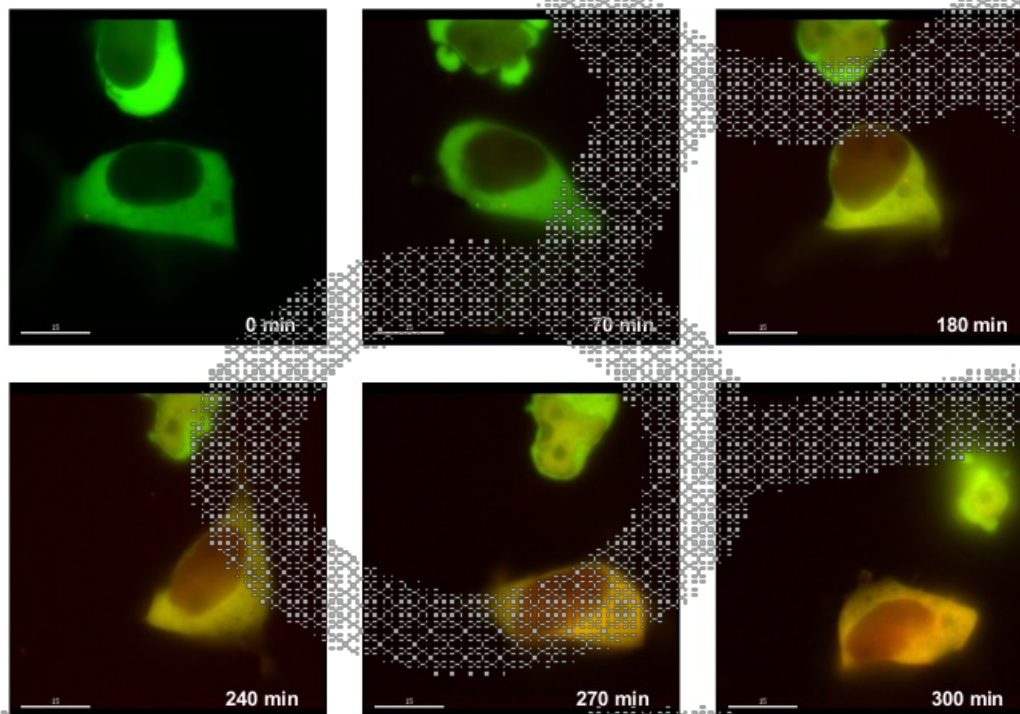
Electrophilic inducers disrupt the cycle by modifying sensor cysteines of Keap1, preventing Nrf2 ubiquitination and Keap1 regeneration



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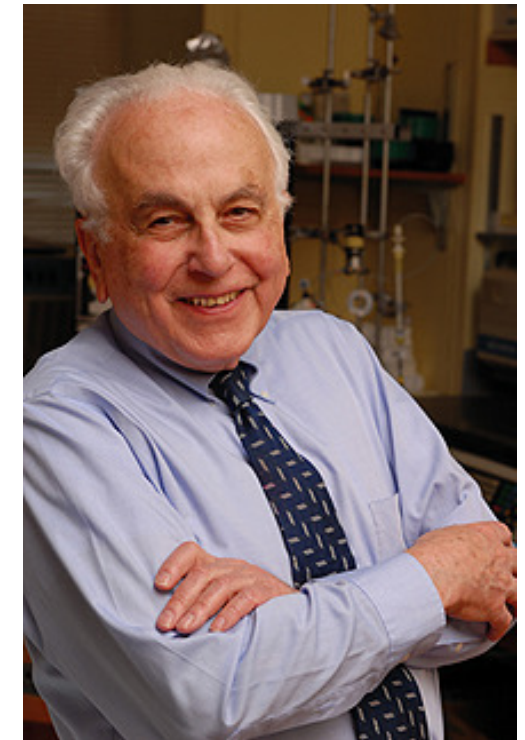
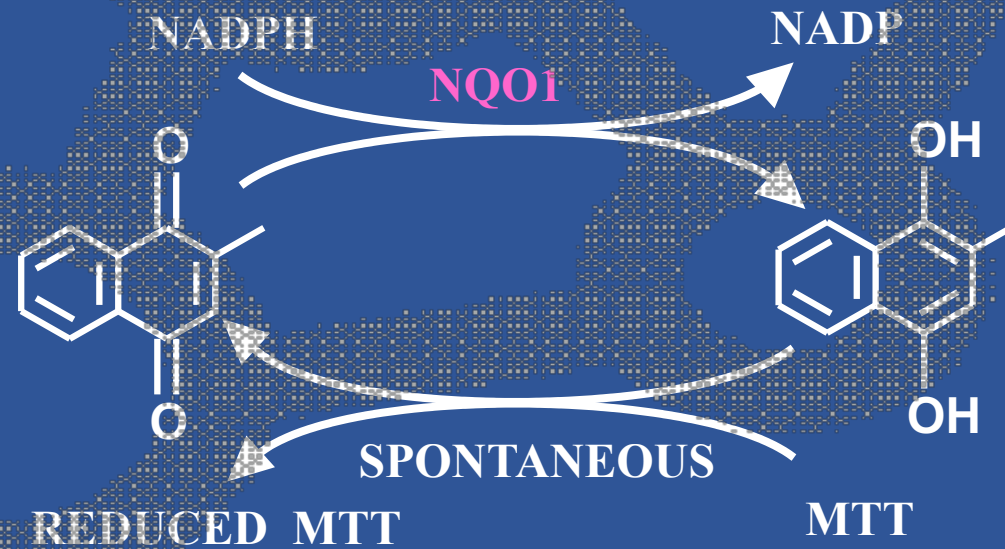
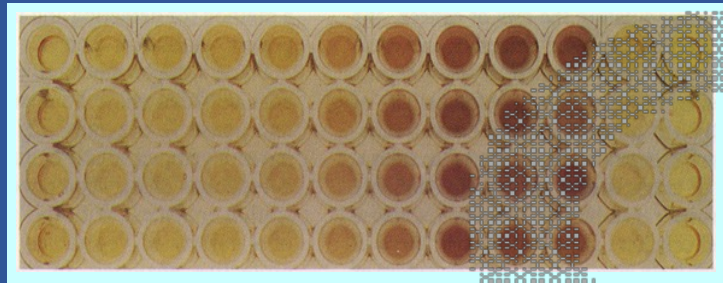
Monitoring the Keap1-Nrf2 interactions in single live cells

EGFP-Keap1 / Nrf2-mCherry



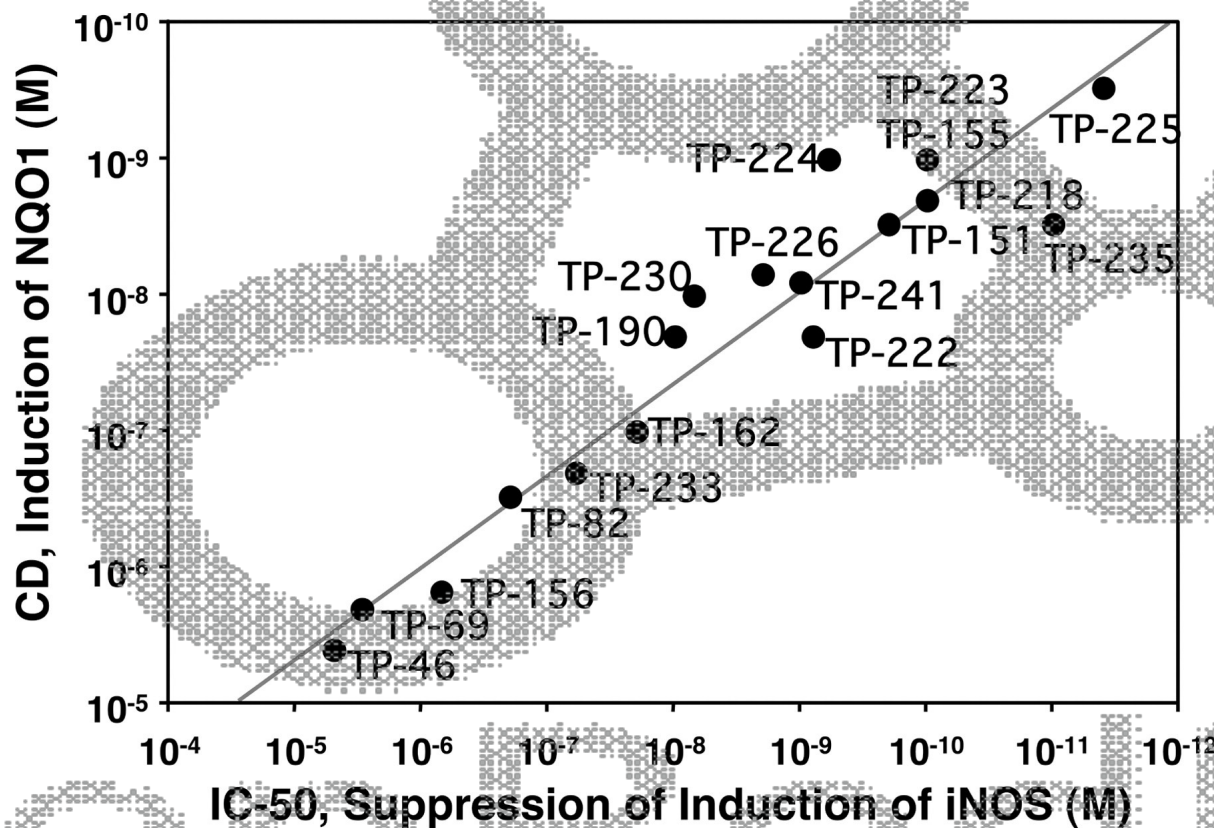
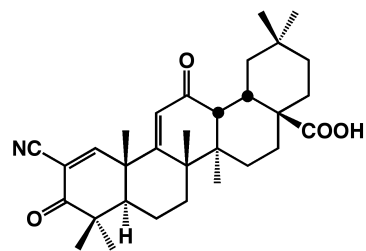
Baird and Dinkova-Kostova (2013) Biochem Biophys Res Commun 433: 58-65.

Principle of the NQO1 assay

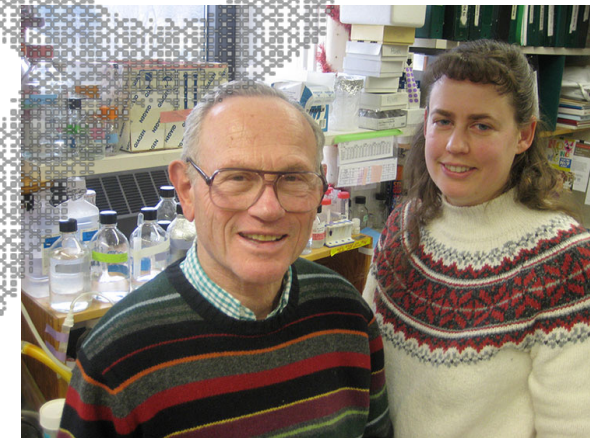


Paul Talalay

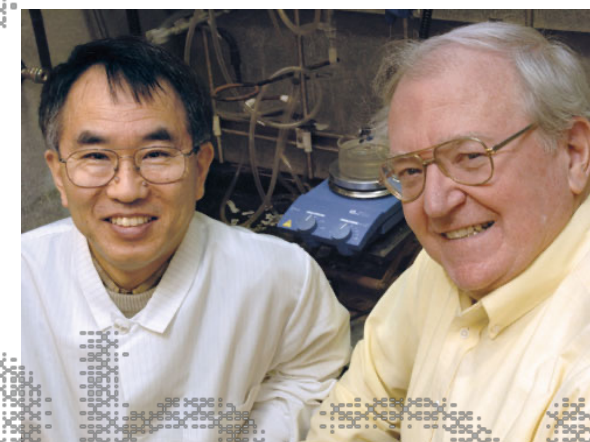
Linear correlation of potencies of pentacyclic cyanoenones as NQO1 inducers and suppressors of iNOS activation by IFN γ



Dinkova-Kostova, Liby et al. (2005) Proc Natl Acad Sci USA 102: 4584-9.

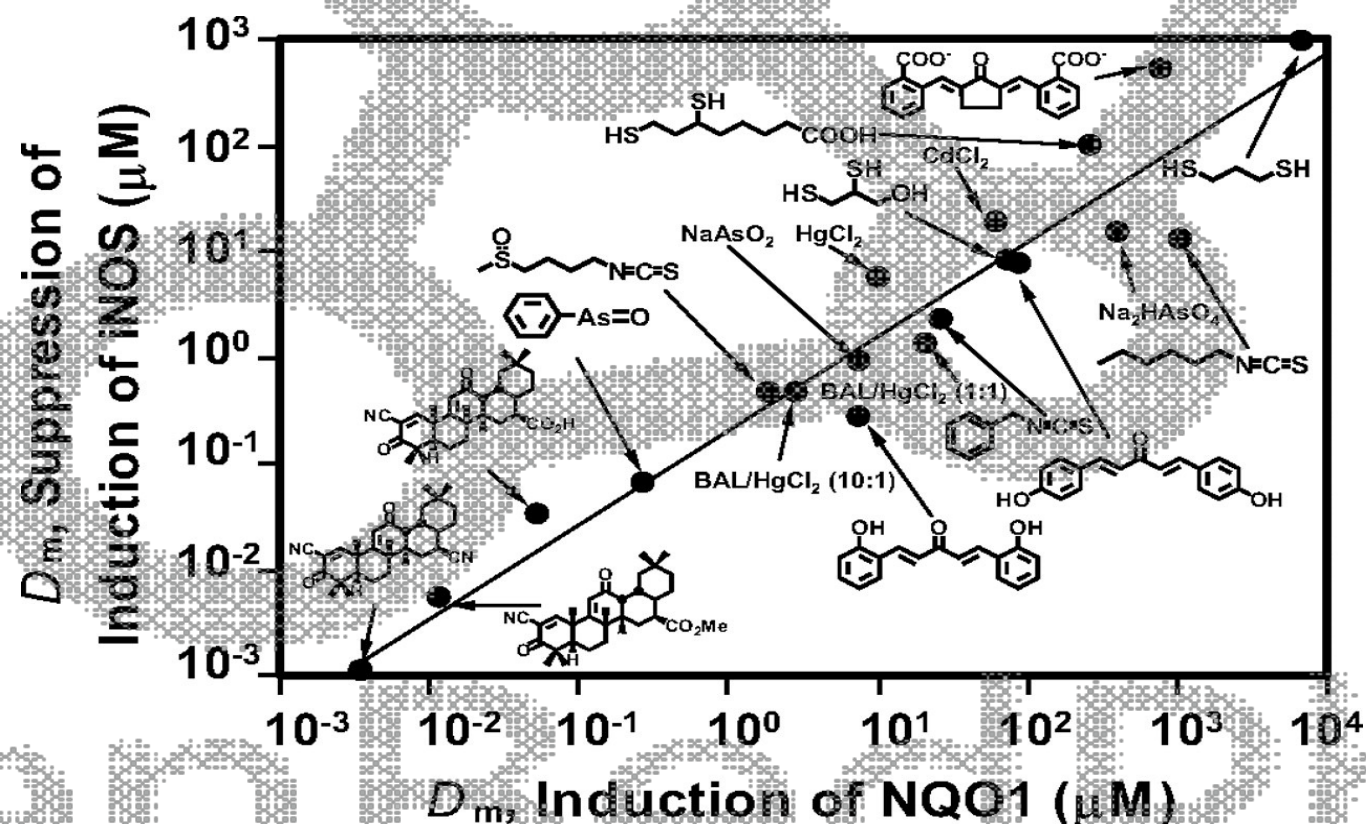


Mike Sporn and Karen Liby



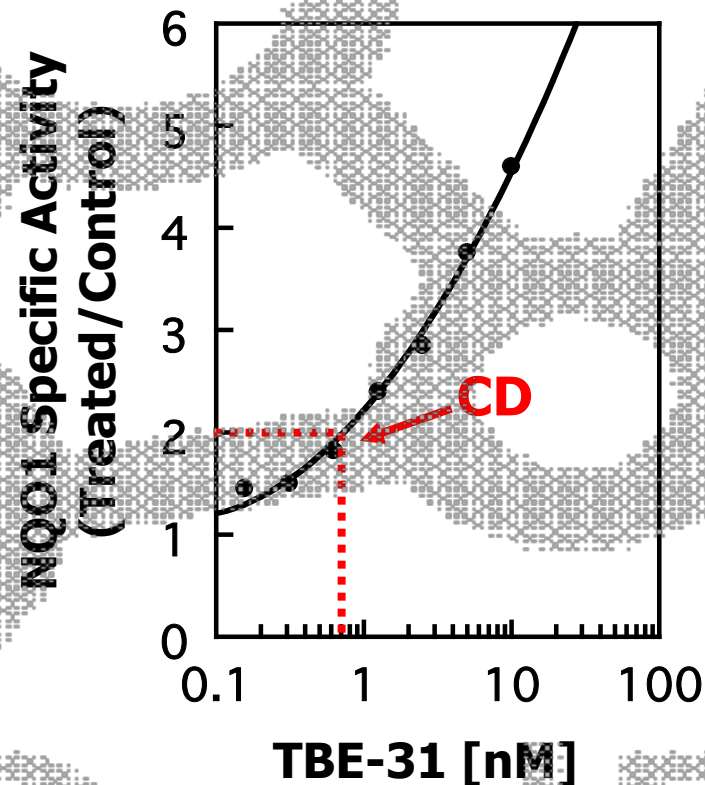
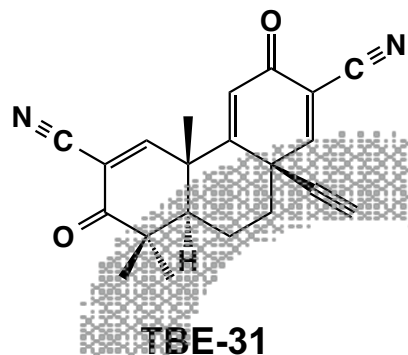
Tadashi Honda and Gordon Gribble

Linear correlation of potencies of structurally diverse Nrf2 activators as NQO1 inducers and suppressors of iNOS activation by IFN γ



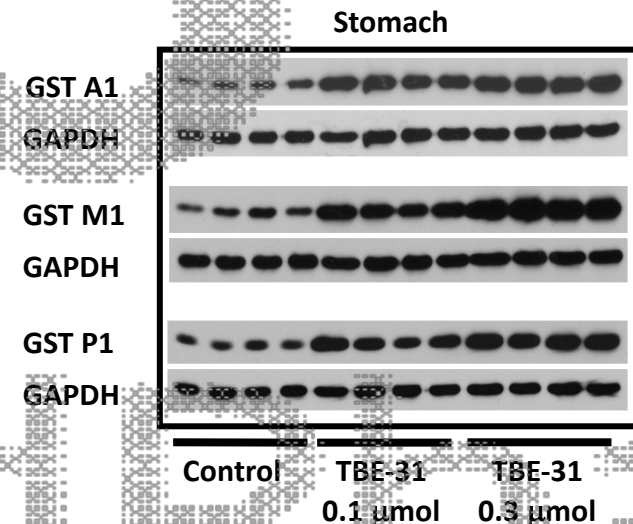
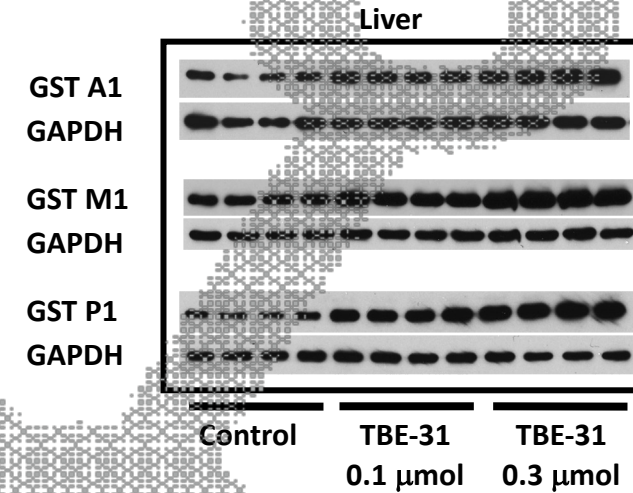
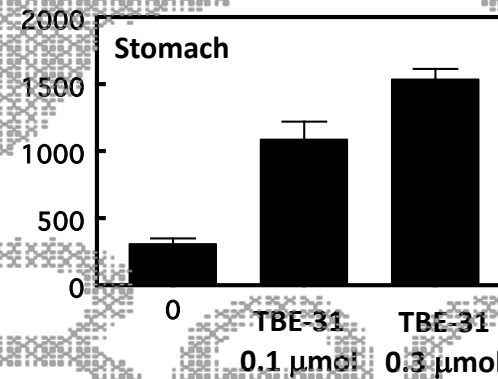
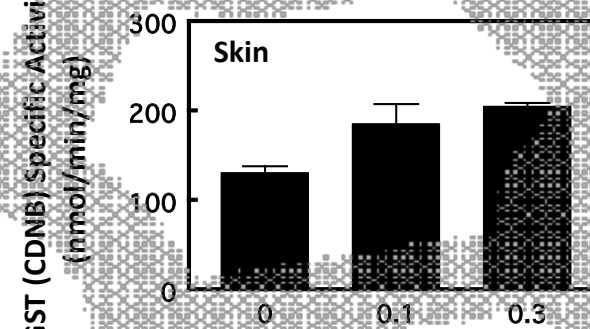
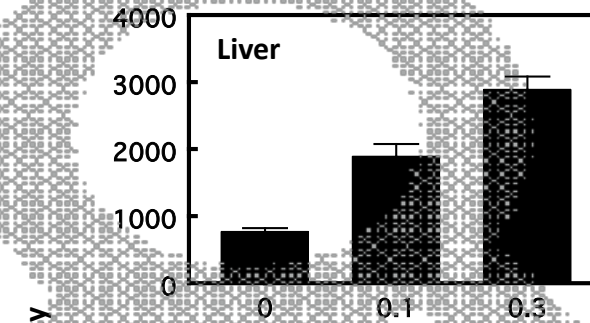
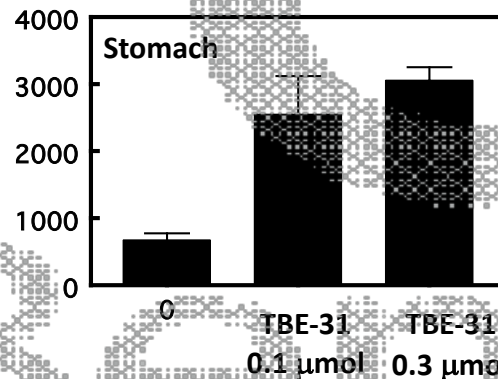
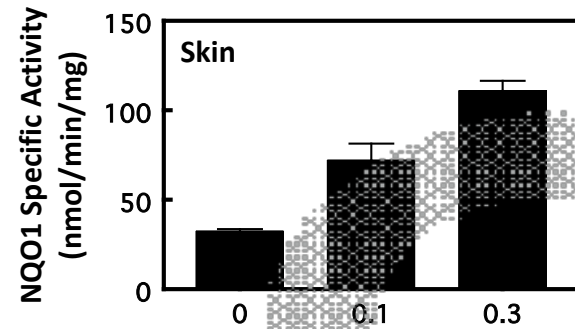
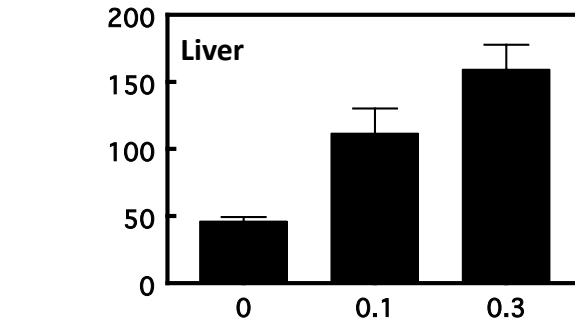
Liu et al. (2008) Proc Natl Acad Sci USA 105: 15926-31.

The tricyclic bis(cyanoenone) TBE-31 is a potent Nrf2 activator in cells

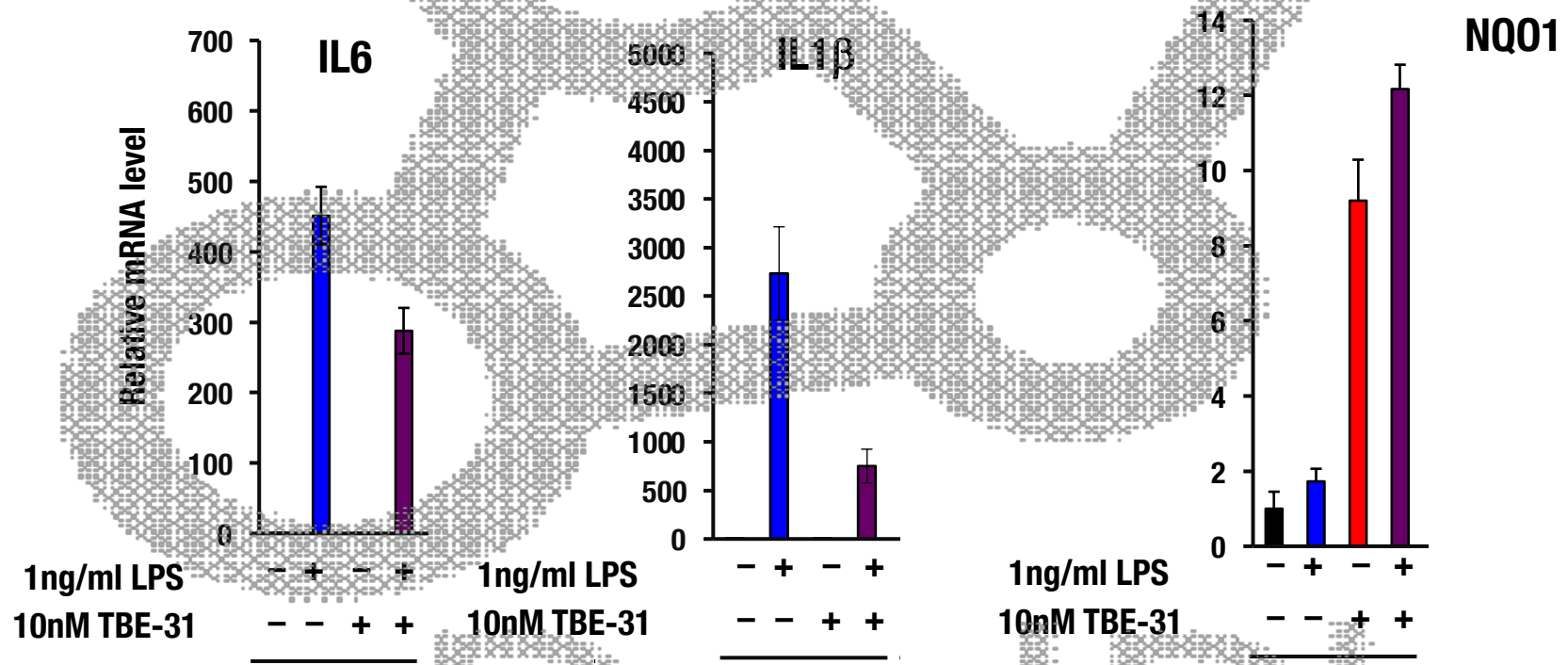


Liby et al. (2008) Cancer Res 68: 6727-33.

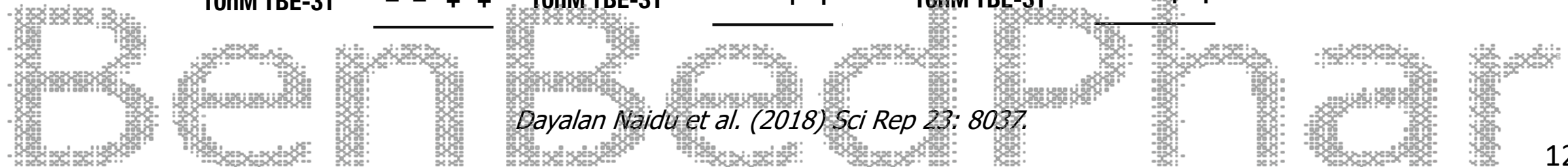
TBE-31 is a potent Nrf2 activator in mice



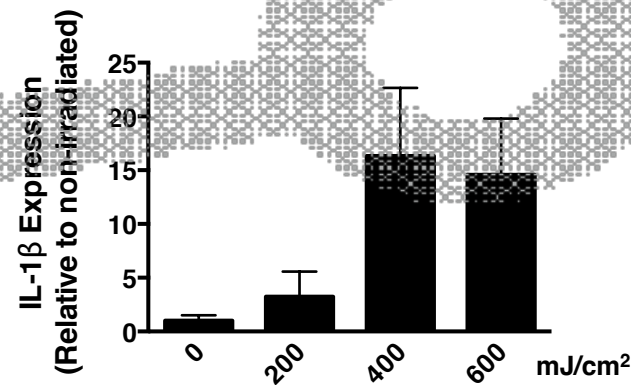
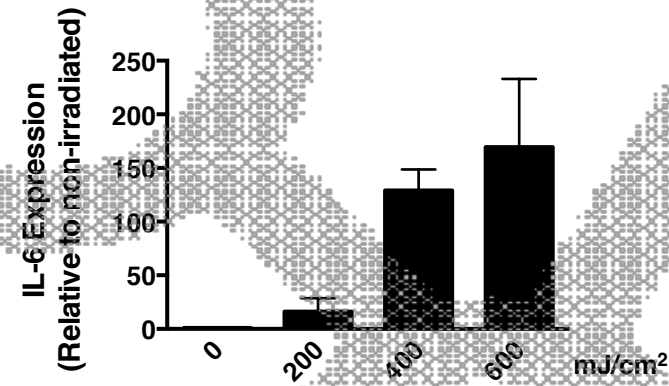
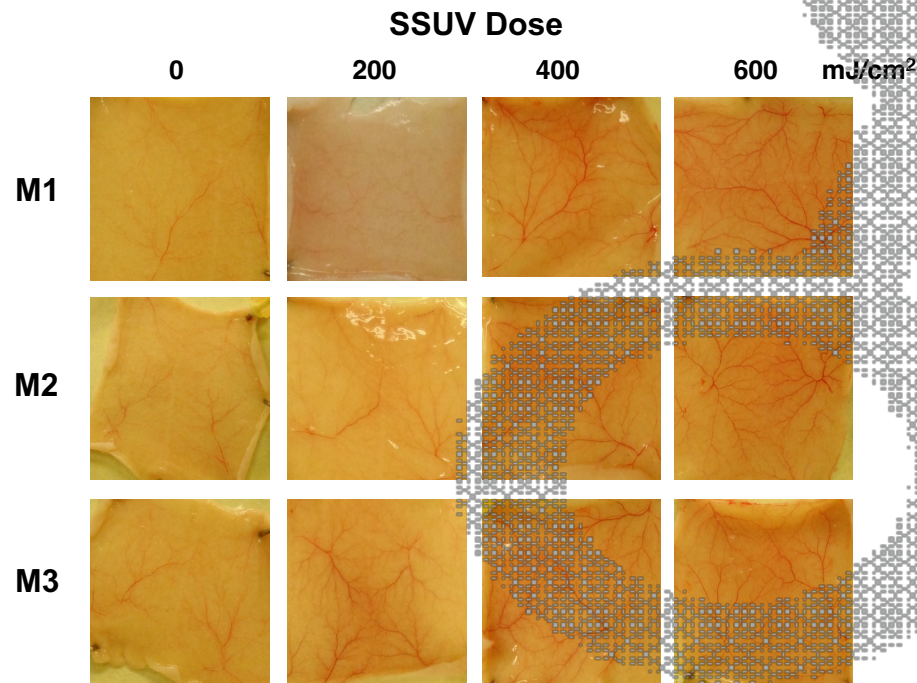
TBE-31 activates Nrf2 and inhibits LPS-mediated pro-inflammatory responses in primary murine peritoneal macrophages



Dayalan Naidu et al. (2018) *Sci Rep* 23: 8037.

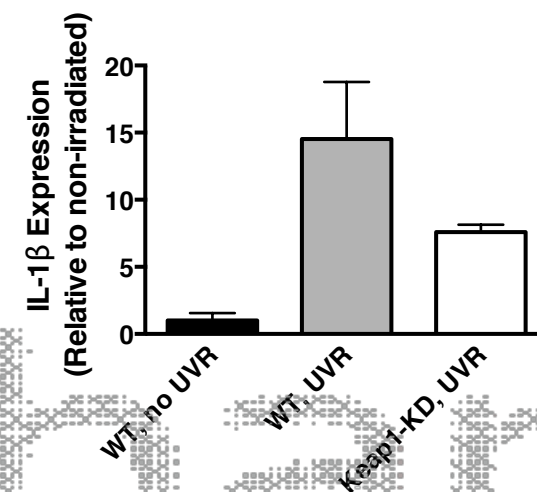
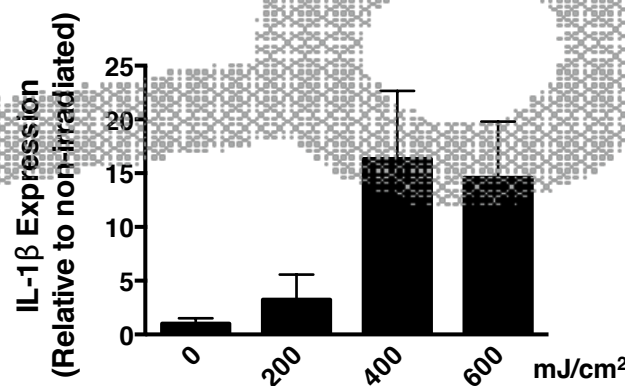
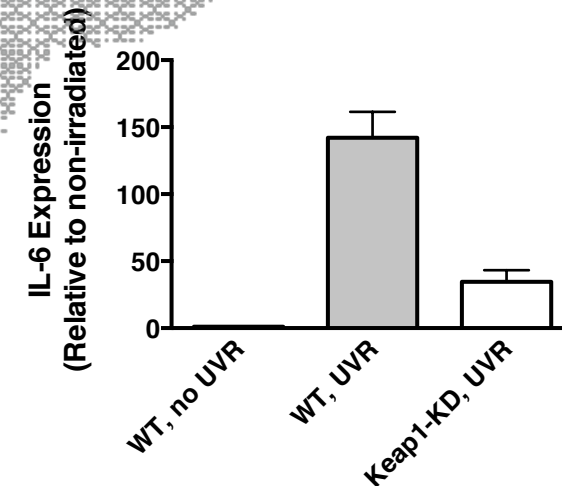
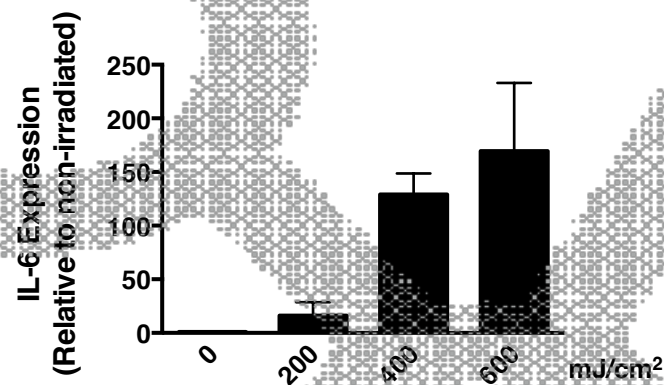
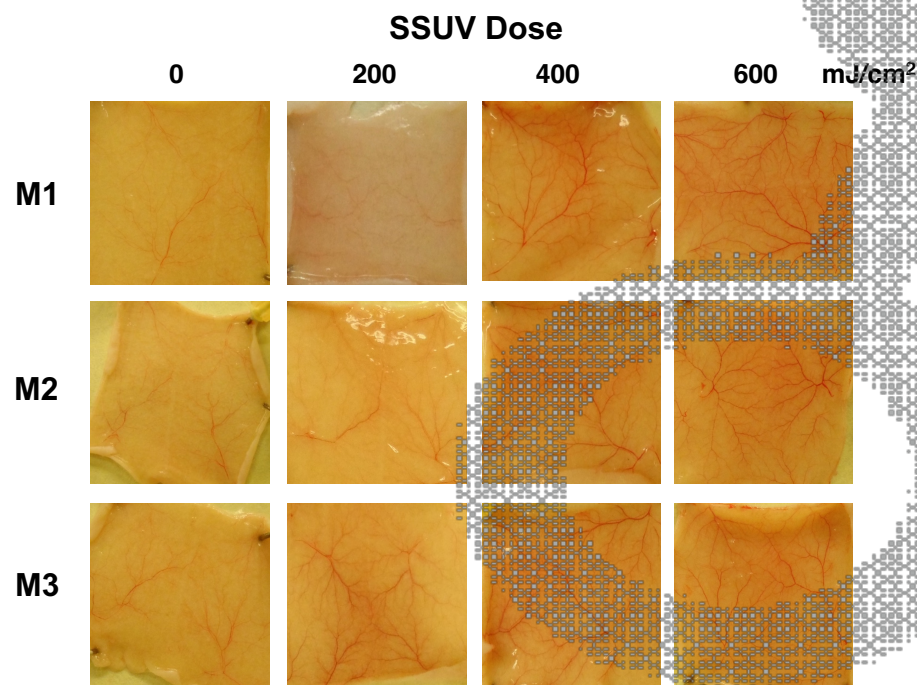


Genetic activation of Nrf2 protects against UV radiation-mediated inflammation



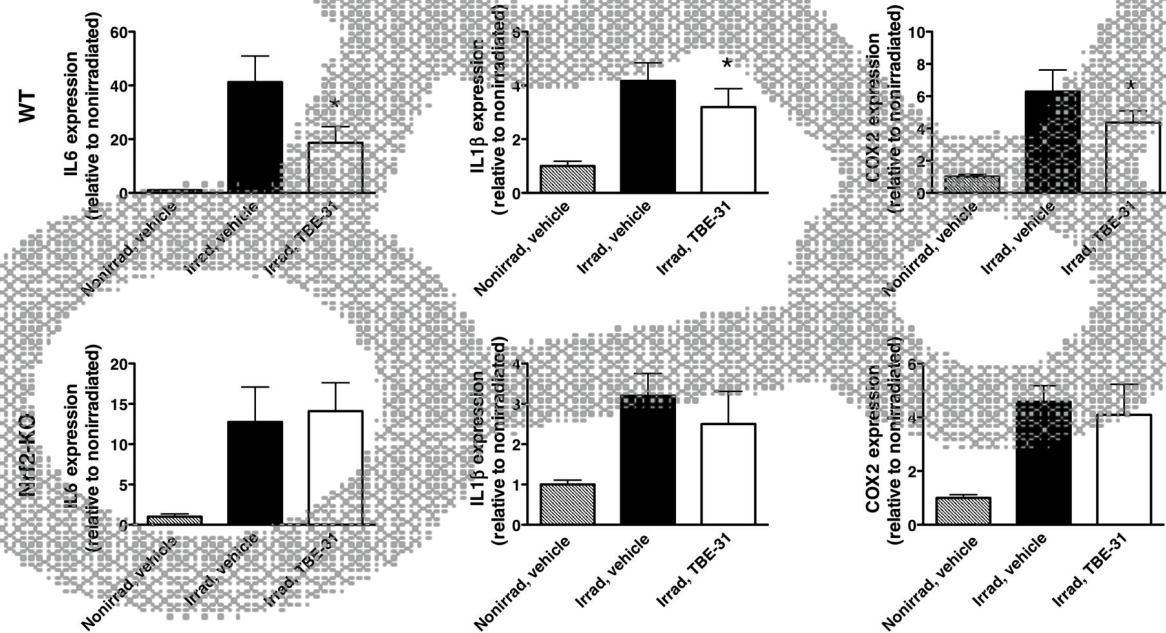
Knatko et al. (2015) Cancer Prev Res 8: 475-86.

Genetic activation of Nrf2 protects against UV radiation-mediated inflammation



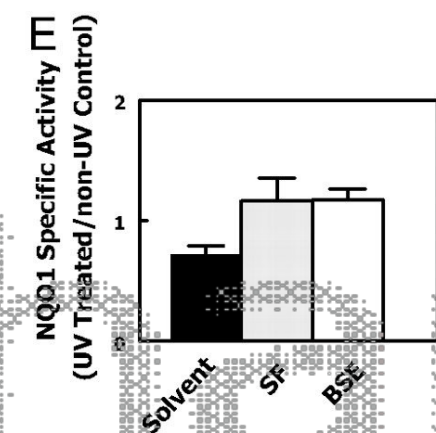
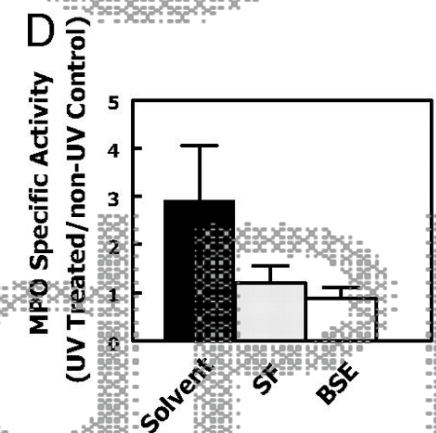
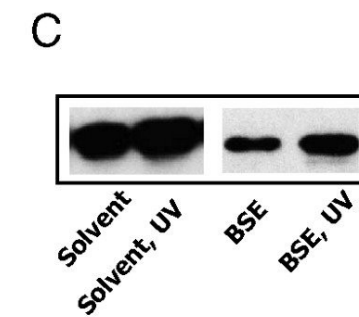
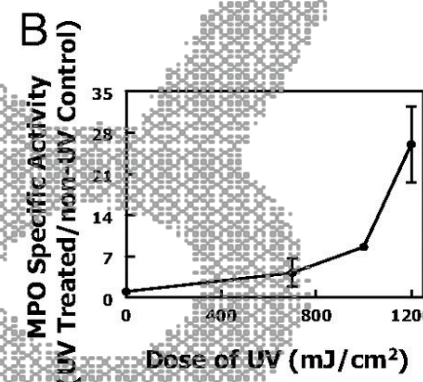
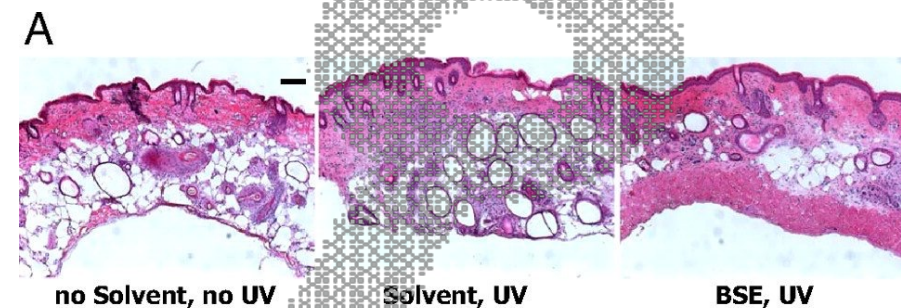
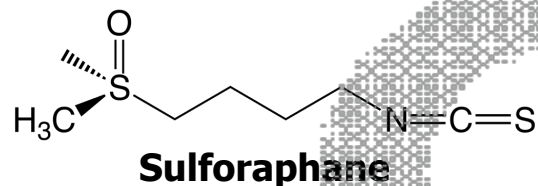
Knatko et al. (2015) Cancer Prev Res 8: 475-86.

TBE-31 inhibits UV radiation-mediated pro-inflammatory responses in the skin of wild-type, but not Nrf2-knockout mice



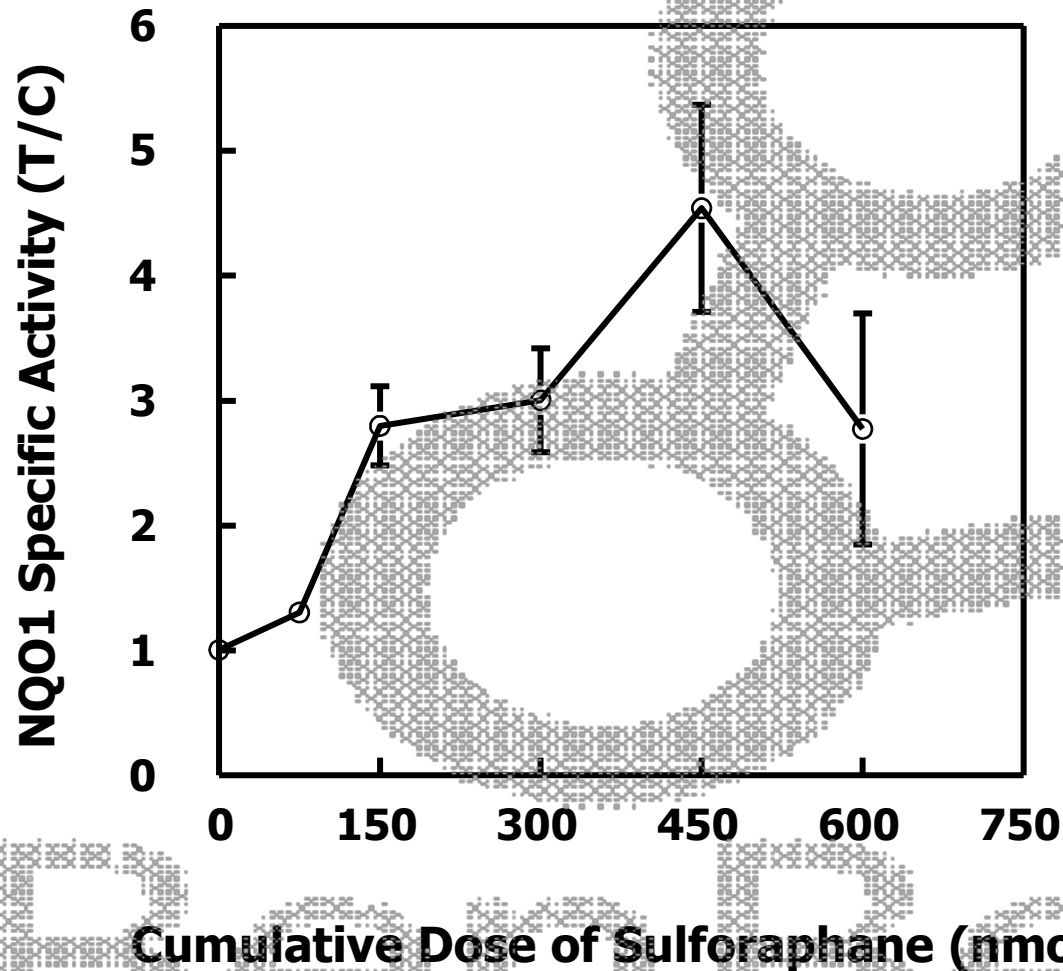
Knatko et al. (2015) Cancer Prev Res 8: 475-86.

The Nrf2 activator sulforaphane inhibits UV radiation-mediated pro-inflammatory responses in mouse skin

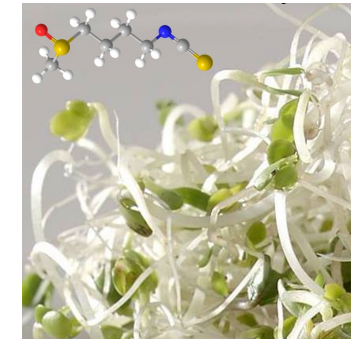
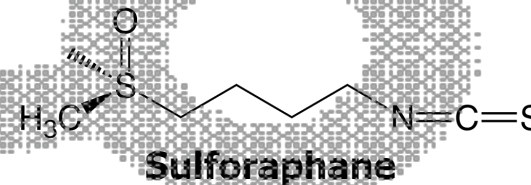


Talalay et al. (2007) Proc Natl Acad Sci USA 104: 17500-5

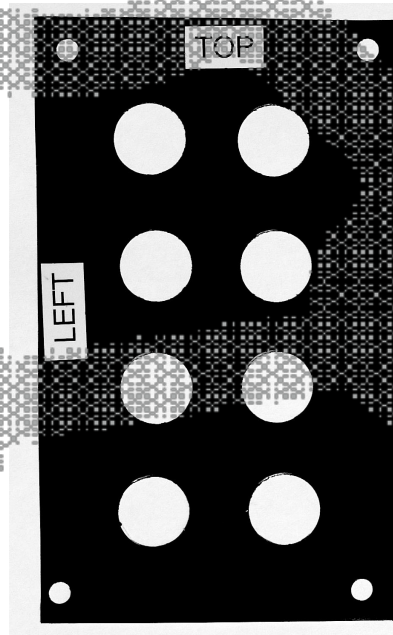
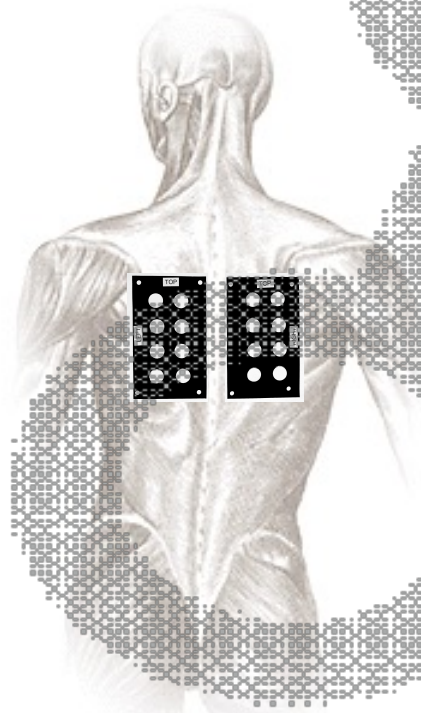
Induction of NQO1 by the Nrf2 activator sulforaphane in human skin



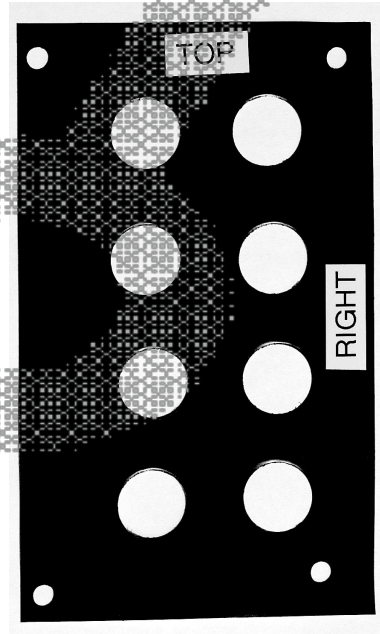
Three applications,
every 24 h



Template design for topical application of sulforaphane and UV irradiation of human skin

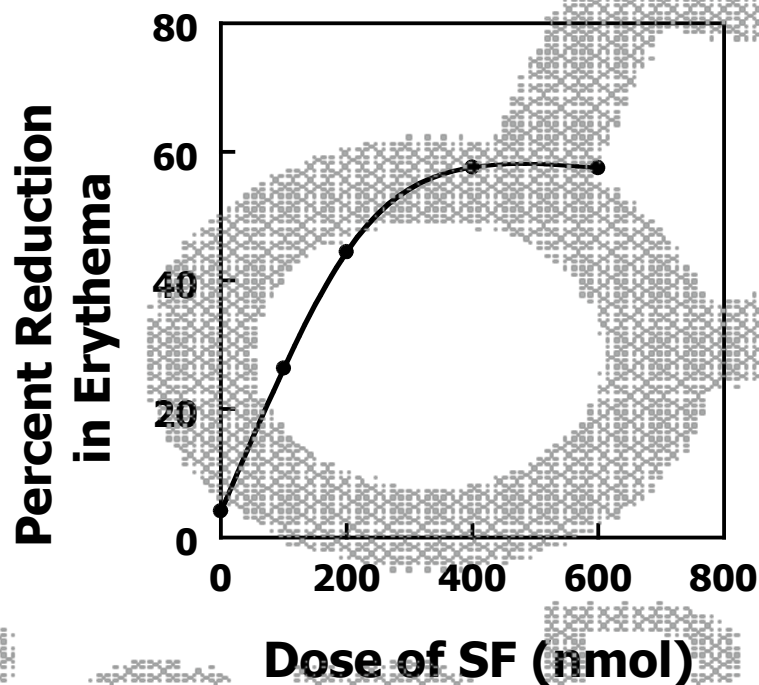


..... Spine



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Inhibition of UV-induced skin erythema development by topical treatment with sulforaphane-rich broccoli extracts



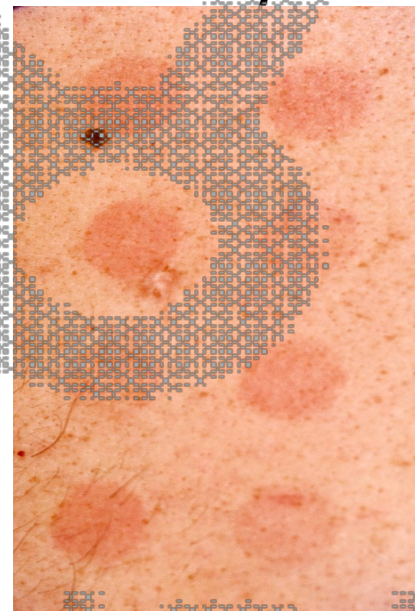
SF 100

Solvent

SF 400

Solvent

500 mJ/cm²



Solvent

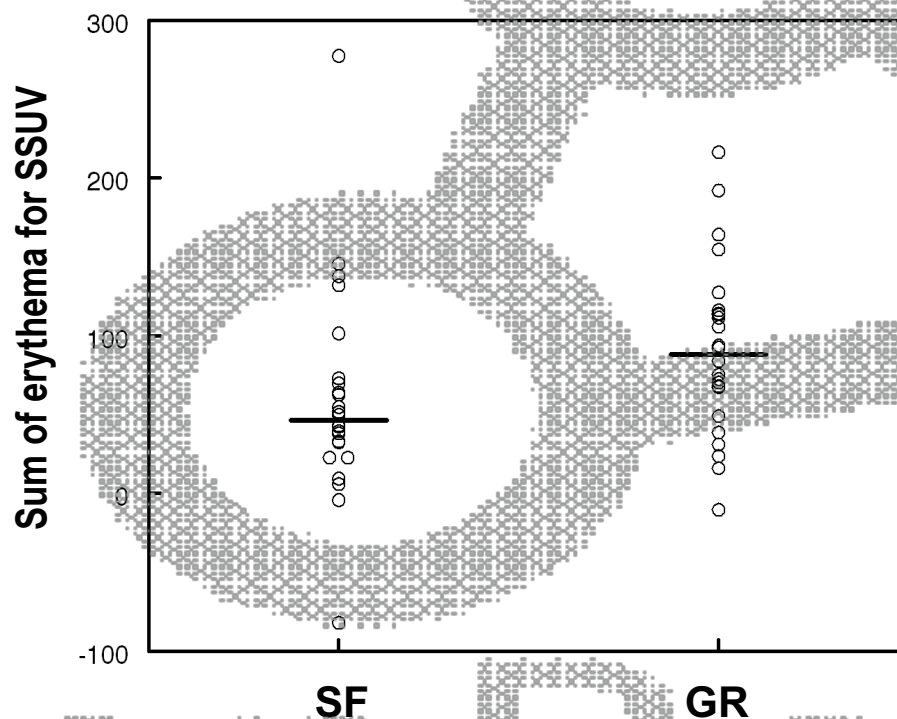
SF 200

Solvent

SF 600

Talalay et al. (2007) Proc Natl Acad Sci USA 104: 17500-5.

Photoprotective effect of sulforaphane in acute SSUV radiation: A randomised double blind placebo controlled study in healthy human subjects



Before study:

- 1 week wash-out (no cruciferous veg or psoralen-containing foods)
- Baseline UV sensitivity testing to establish MED

Day 1 - Day 3:

- 3 x topical SF and GR extract application, 24-hr apart

Day 4:

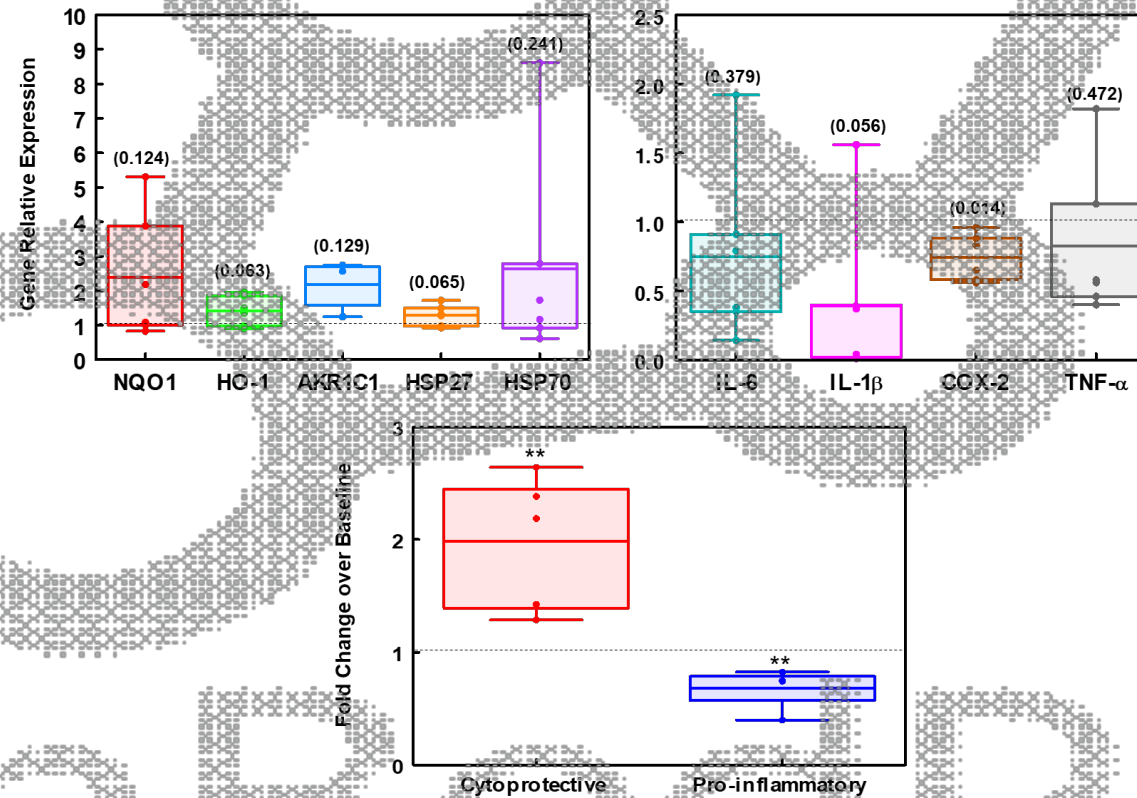
- SS-UV exposure (range of doses calculated on the basis of MED)

Day 5:

- Erythema measurements

Knatko et al. (2015) Cancer Prev Res 8: 475-486.

Gene expression level changes in PBMCs from human subjects after daily intervention with sulforaphane for 2 weeks show a decrease in inflammatory markers



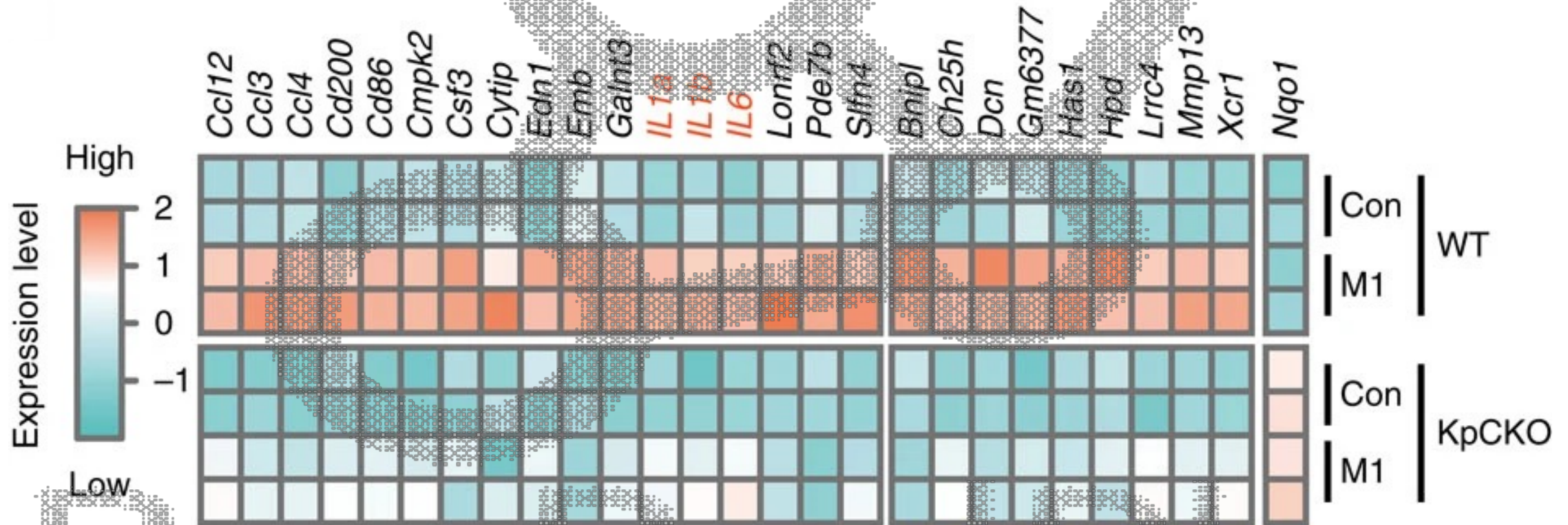
Liu et al. (2020) Sci Rep 10: 5822.

Summary (I)

- **Nrf2 activation linearly correlates with inhibition of pro-inflammatory responses**
- **Nrf2 activation protects against UV radiation-mediated inflammation in mice and humans**

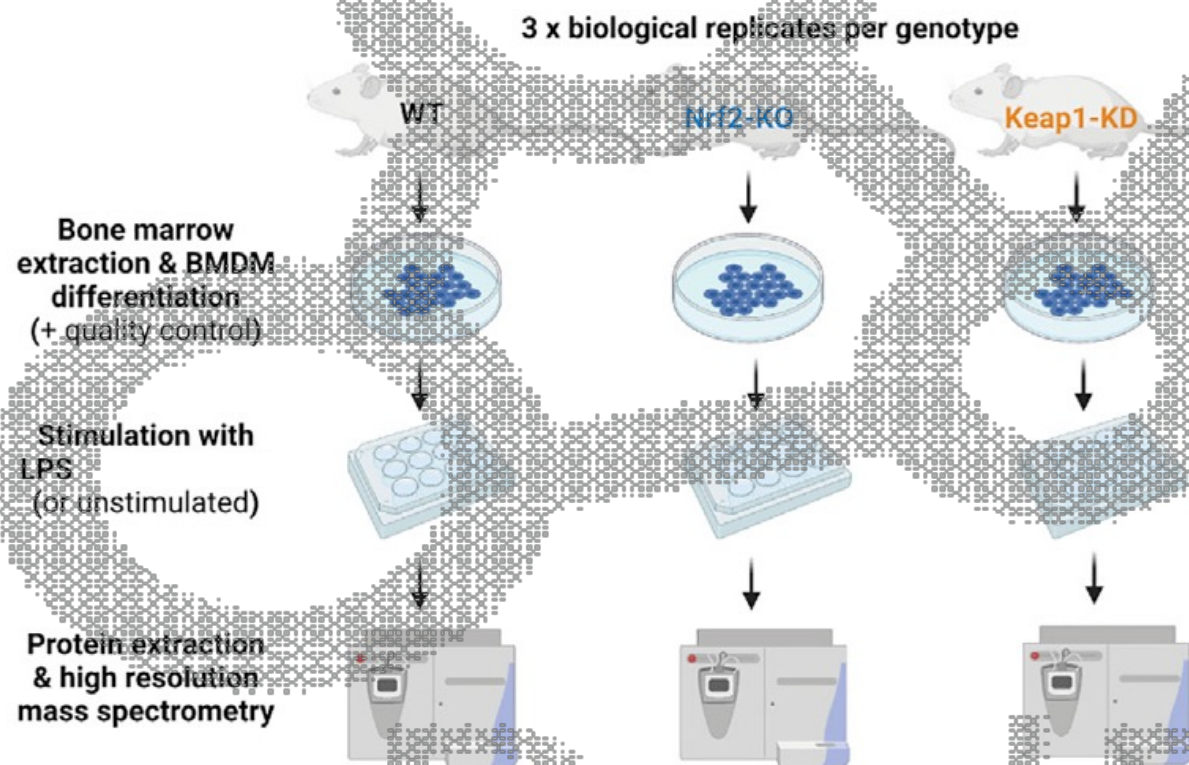
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Nrf2 suppresses macrophage inflammatory response by blocking pro-inflammatory cytokine transcription



Kobayashi et al. (2016) Nat Commun 7: 11624.

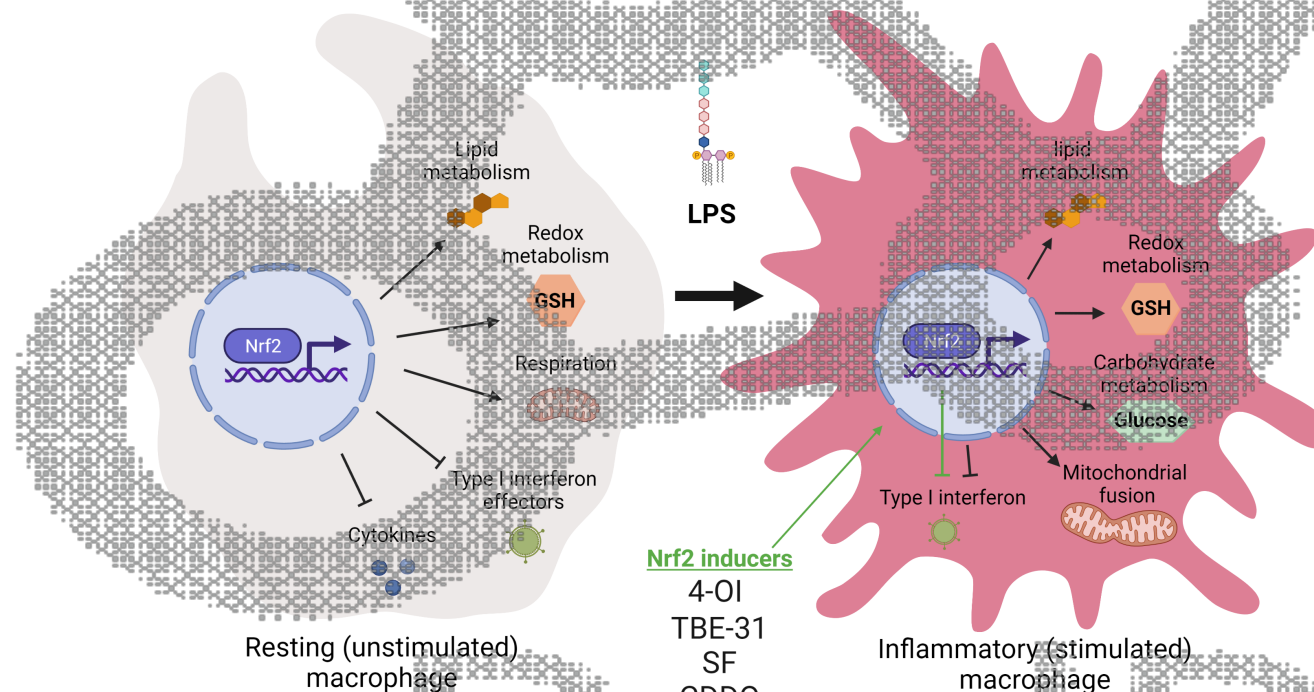
High-resolution proteomics to investigate the effects of Nrf2 on the response to LPS in bone marrow-derived macrophages (BMDM)



Ryan et al. (2022) *iScience*; 25: 103827.

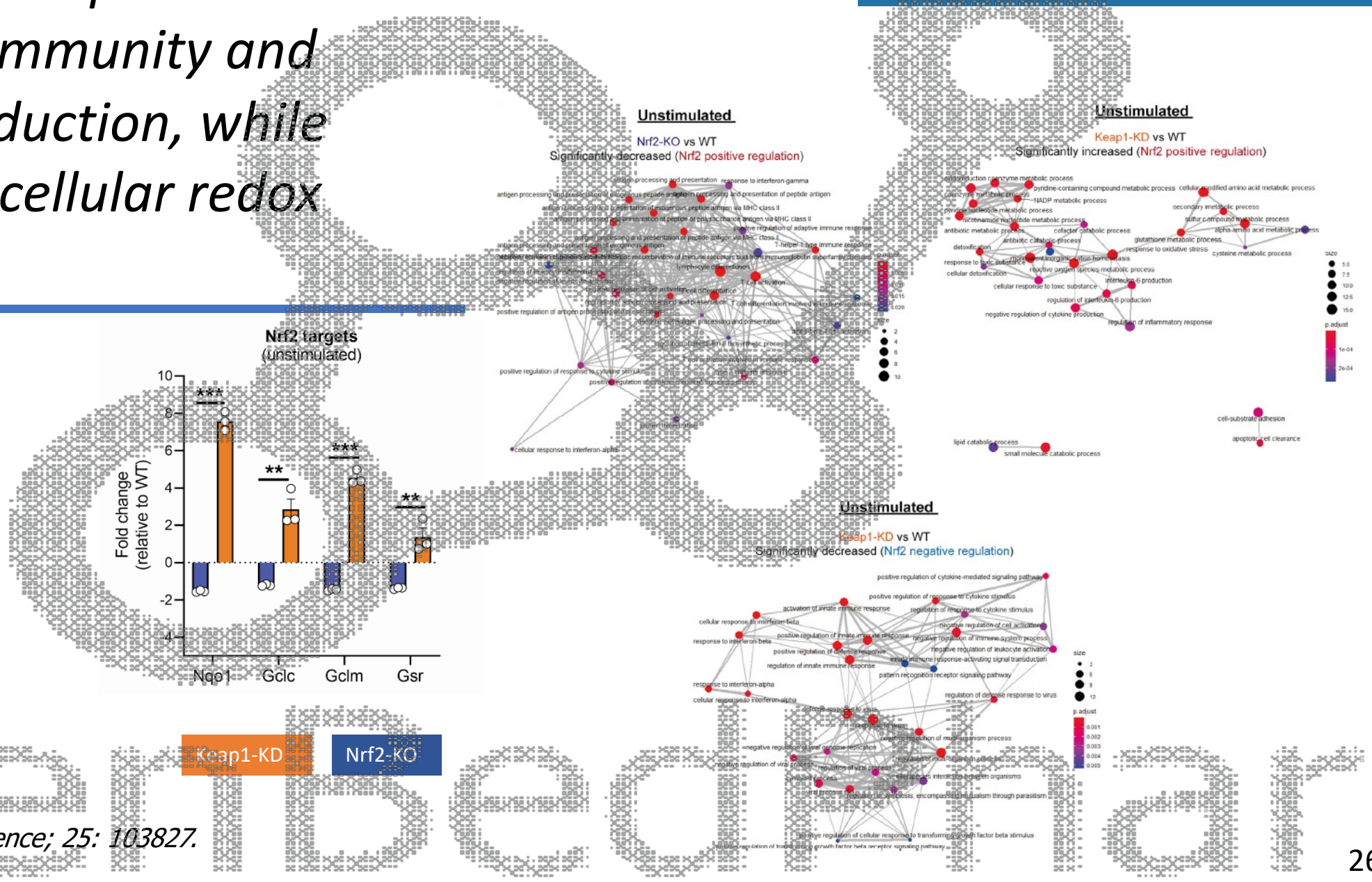
Nrf2 regulates multiple biological processes in resting and stimulated macrophages and suppresses the type 1 interferon response

Key biological processes regulated by Nrf2



Ryan et al. (2022) *iScience*; 25: 103827.

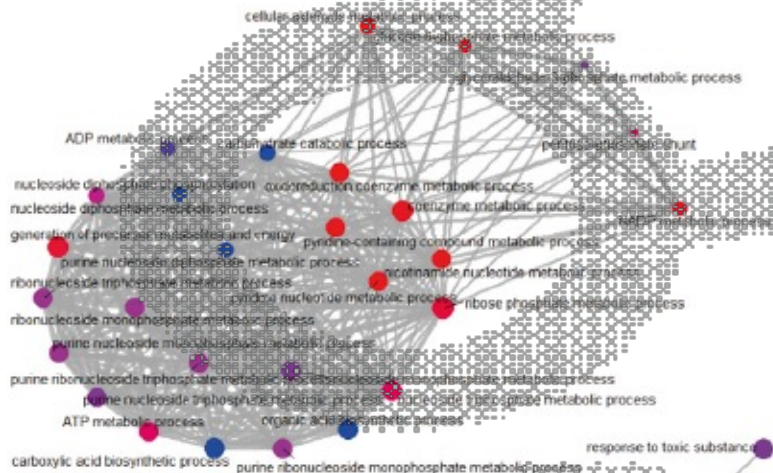
Nrf2 suppresses proteins involved in anti-viral immunity and cytokine production, while maintaining cellular redox metabolism



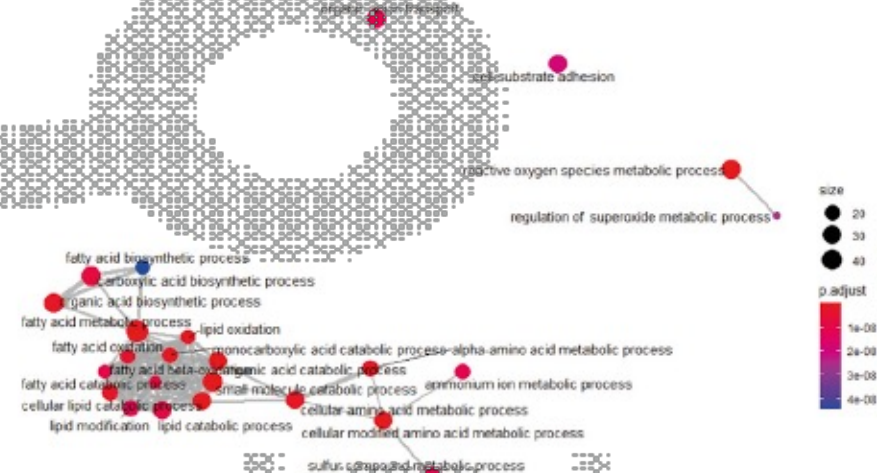
Ryan et al. (2022) *iScience*; 25: 103827.

In stimulated macrophages, Nrf2 affects positively carbohydrate, cofactor, and energy metabolism, and cellular response to oxidative stress

LPS-stimulated
Nrf2-KO vs WT
 Significantly decreased (Nrf2 positive regulation)

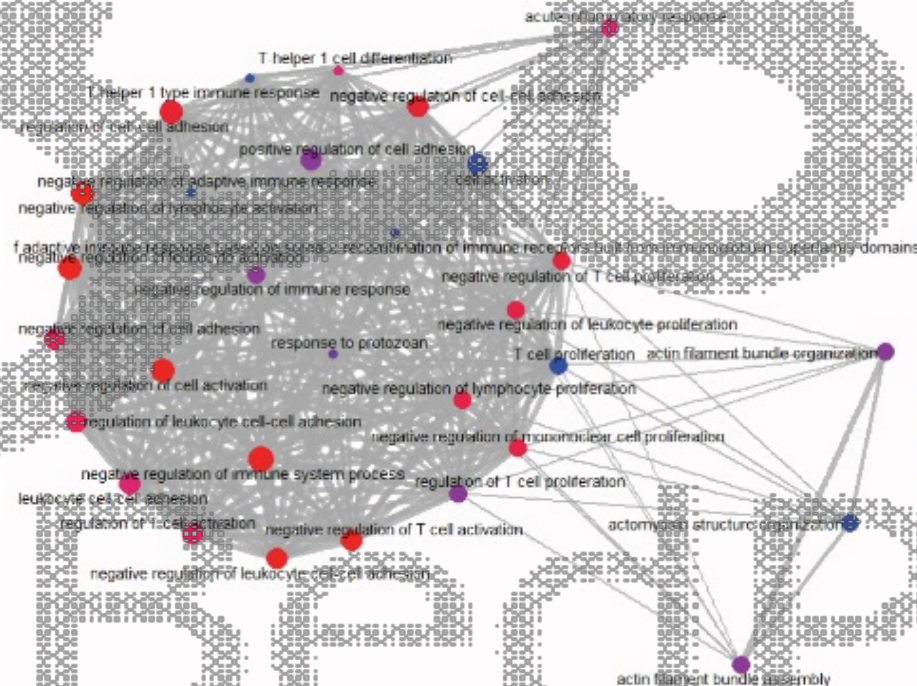


LPS-stimulated
Keap1-KO vs WT
 Significantly increased (Nrf2 positive regulation)



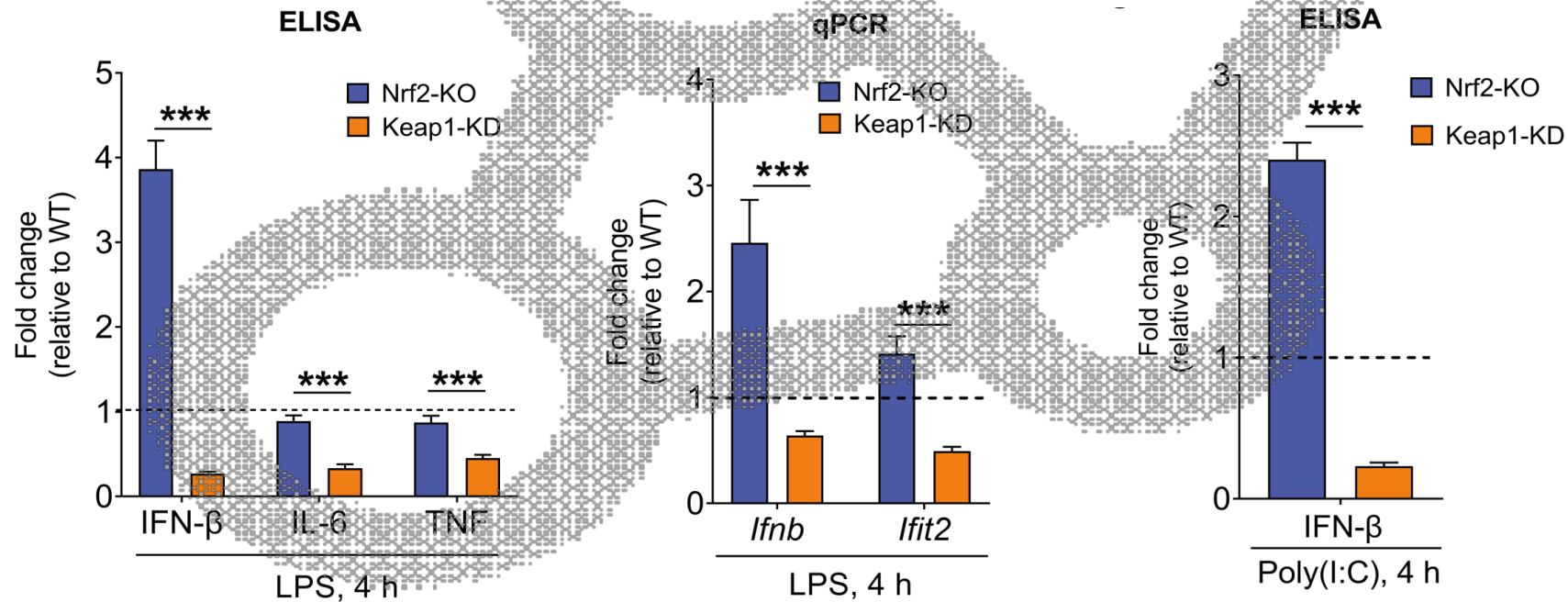
In stimulated macrophages, Nrf2 affects negatively T cell and leukocyte activation, adhesion, and proliferation

LPS-stimulated
Keap1-KD vs WT
Significantly decreased (Nrf2 negative regulation)



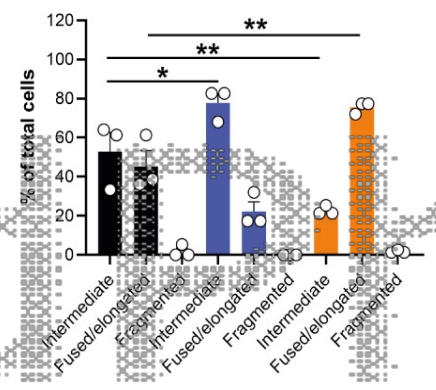
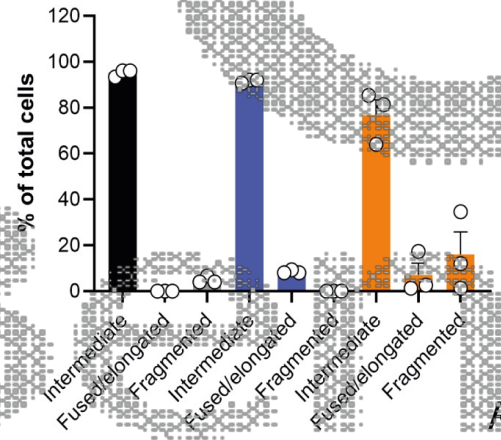
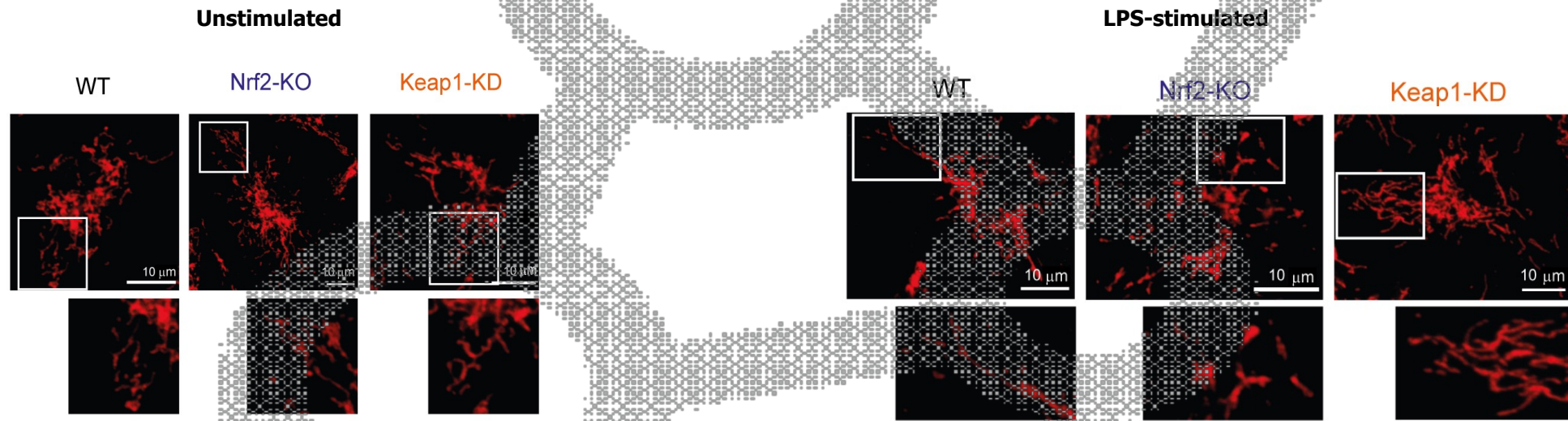
Ryan et al. (2022) *iScience*; 25: 103827.

In stimulated macrophages, genetic *Nrf2* activation suppresses *Ifnb* expression, whereas *Nrf2* depletion enhances it



Ryan et al. (2022) *iScience*; 25: 103827.

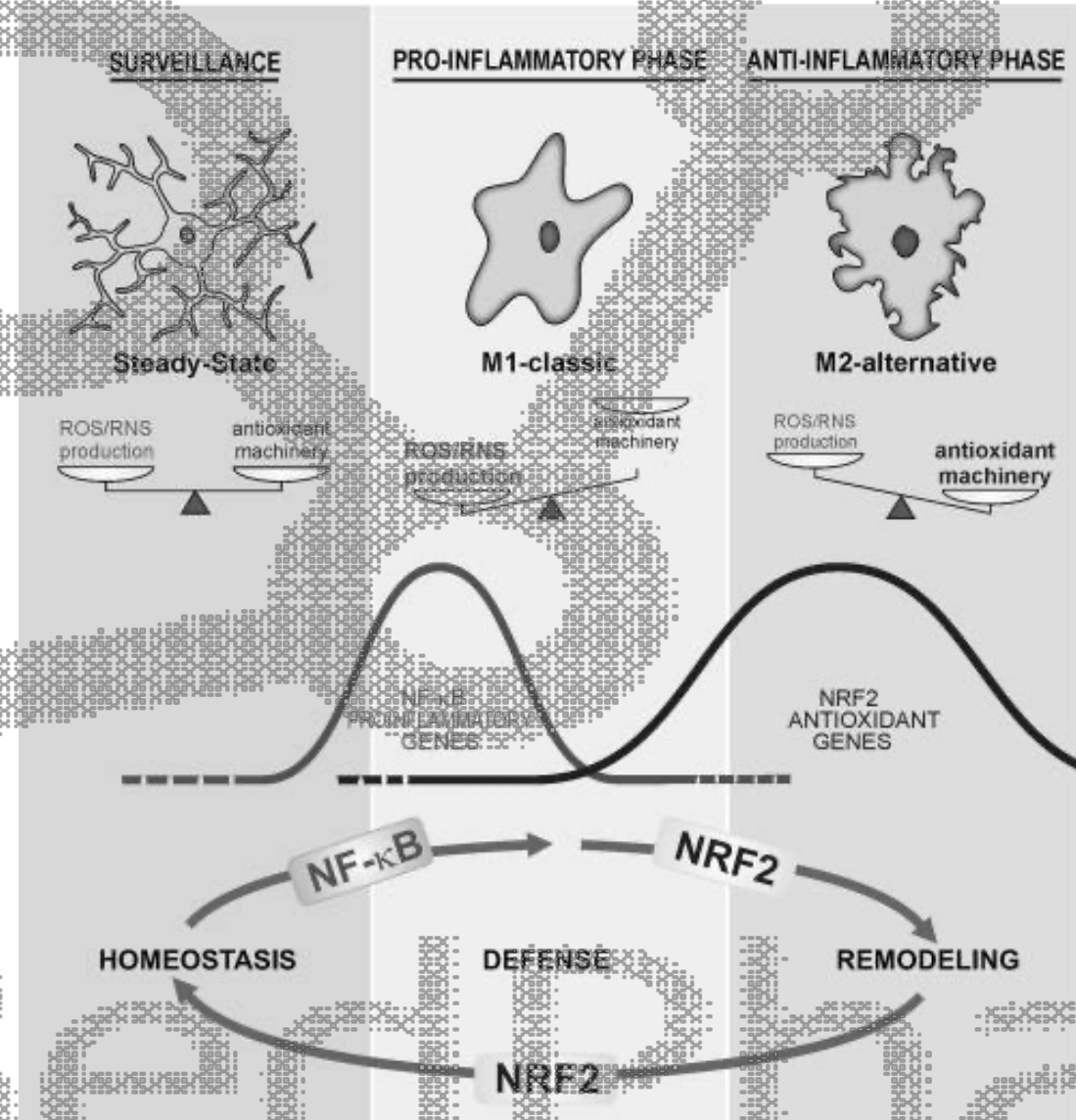
LPS stimulation promotes mitochondrial fusion, which is enhanced by Nrf2 activation and inhibited by Nrf2 disruption



Ryan et al. (2022) *iScience*; 25: 103827.

Nrf2 activation promotes resolution of inflammation and is protective in models of neurodegenerative diseases

Rojo et al. (2014) Antioxid Redox Signal 21: 1766-801.
Innamorato et al. (2008) J Immunol 181: 680-9.
Rojo et al. (2010) Glia 58: 588-98.
Rojo et al. (2018) Redox Biol 8: 173-180.
Pajares et al. (2022) Cells 9: 1687.



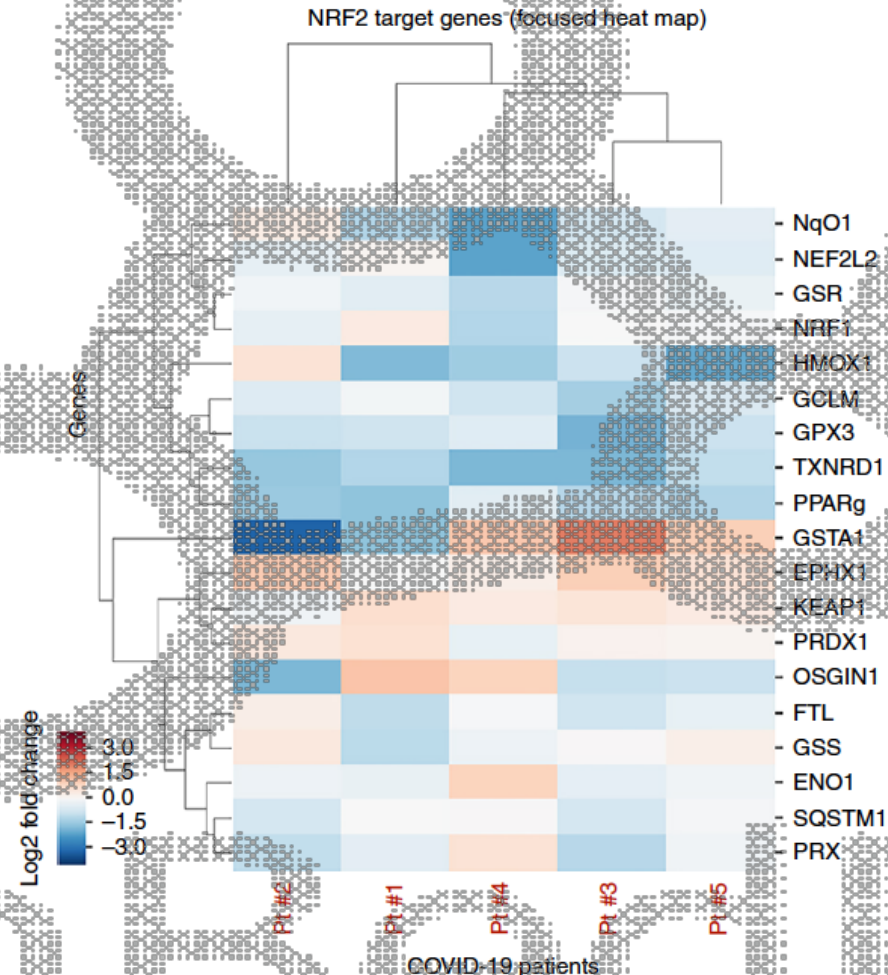
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Summary (II)

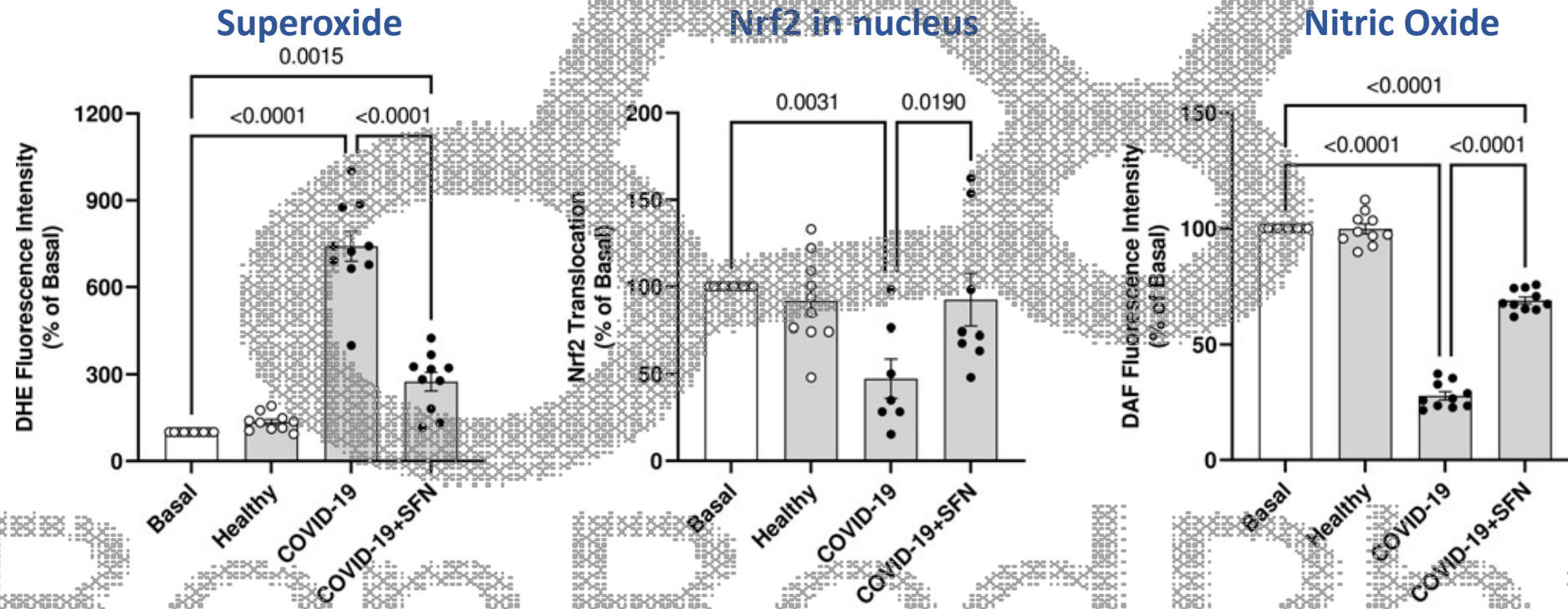
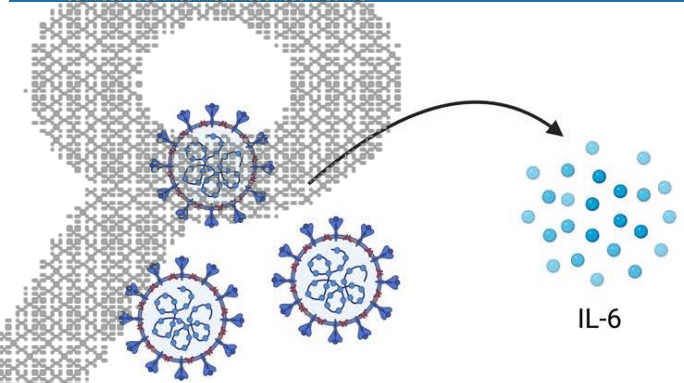
- **In unstimulated macrophages, Nrf2 suppresses proteins involved in anti-viral immunity and cytokine production, while maintaining cellular redox metabolism**
- **LPS stimulation promotes mitochondrial fusion, which is enhanced by Nrf2 activation and inhibited by Nrf2 disruption**
- **In stimulated macrophages, genetic and pharmacologic Nrf2 activation suppresses *Ifnb* expression**
- **Nrf2 activation promotes resolution of inflammation and is protective in models of neurodegenerative diseases**

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Expression of Nrf2-target genes is suppressed in COVID-19 patient biopsies

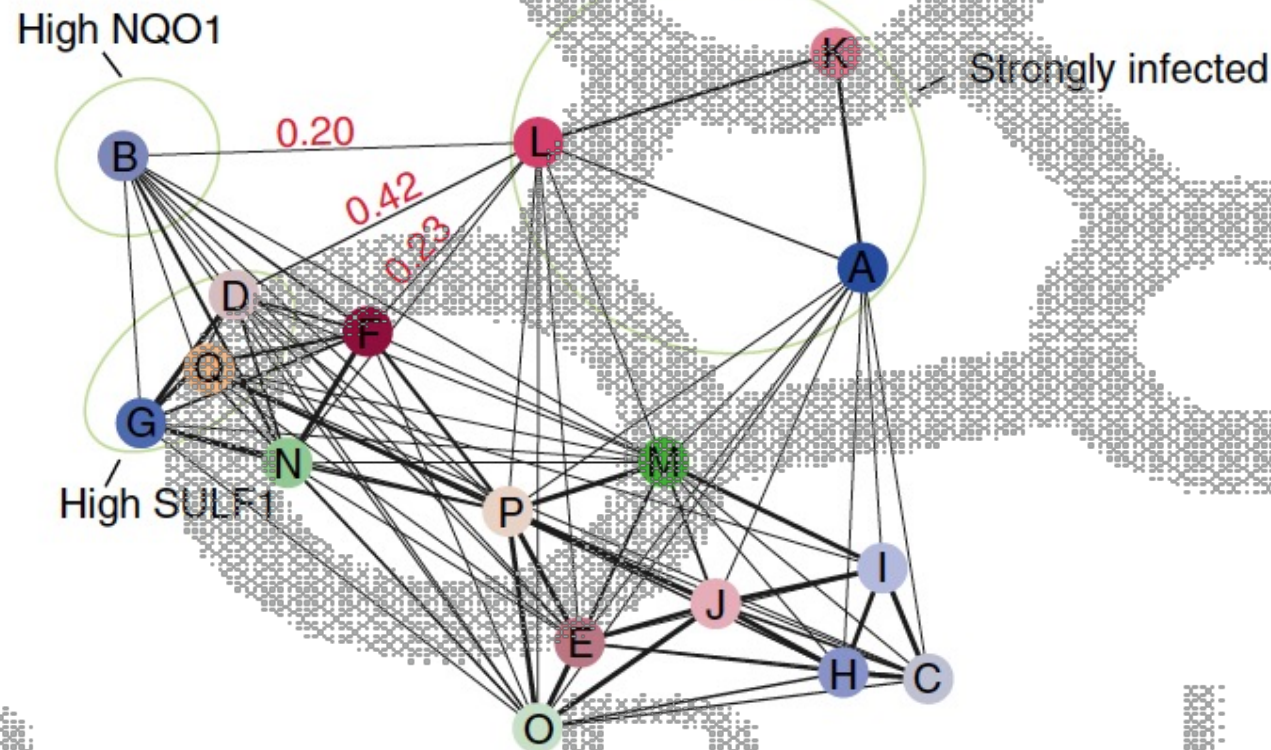


Pharmacological activation of Nrf2 with sulforaphane (SFN) restores redox balance in HUVEC exposed to serum from individuals with severe COVID-19



Rodrigues et al. (2023) Am J Physiol Heart Circ Physiol; in press.

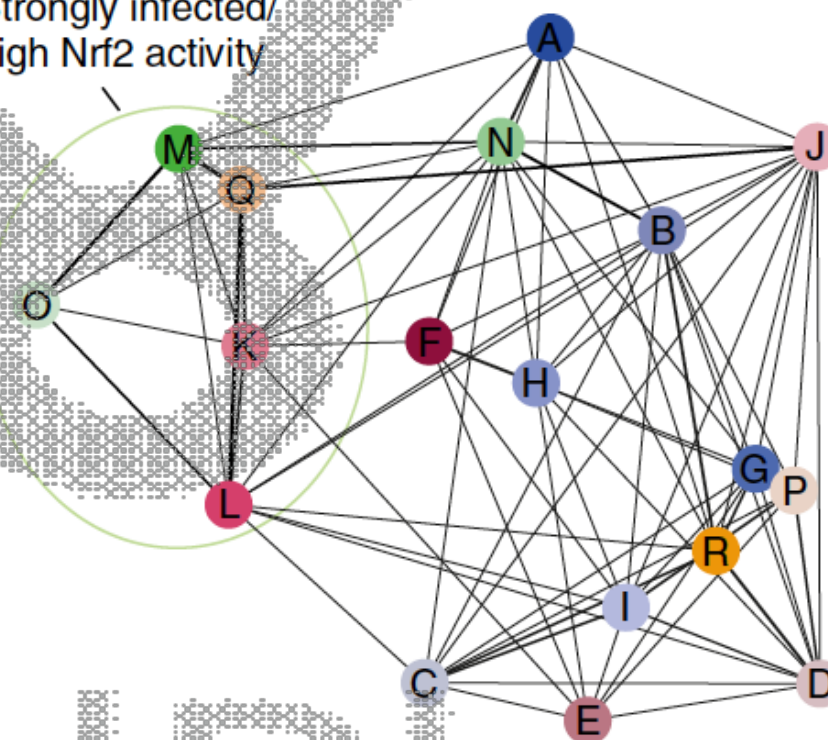
In early stages of HSV1 infection, cells with high Nrf2 activity, have a low transition probability into later stages



Wyller et al. (2019) Nature Commun. 10: 4878.

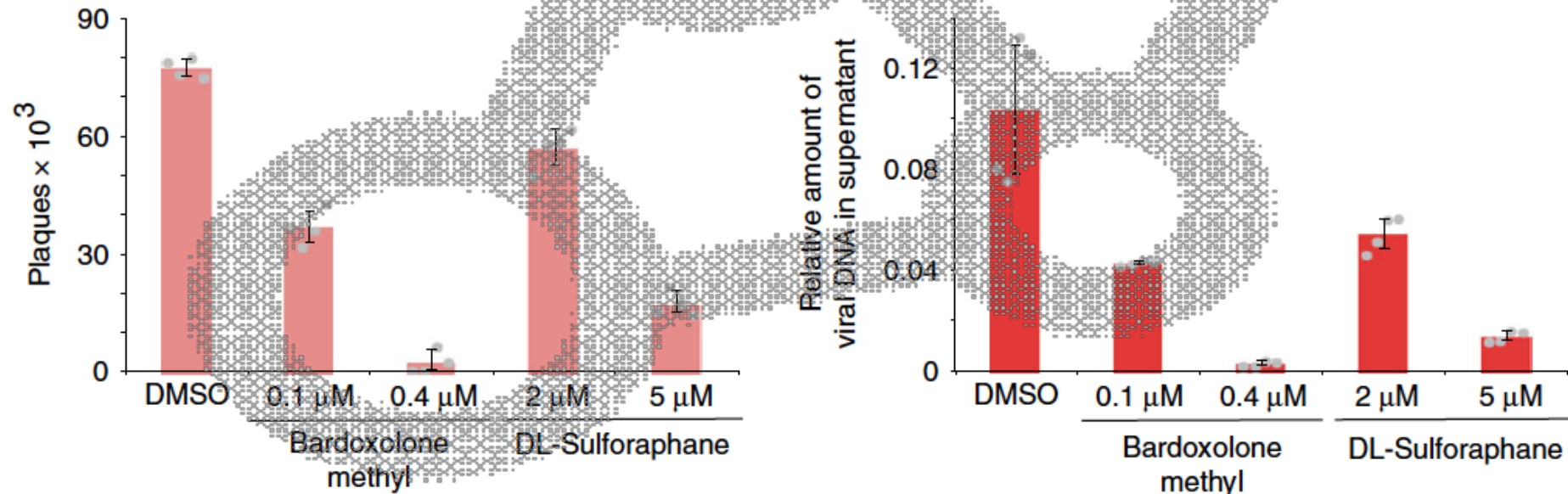
Cells at later stages of infection respond by increasing transcription of Nrf2-target genes, which could reflect a cellular defense mechanism against HSV1 infection

Strongly infected/
high Nrf2 activity



Wyller et al. (2019) Nature Commun. 10: 4878.

Pharmacological activation of Nrf2 with bardoxolone methyl or sulforaphane counteracts HSV1 infection



Wyller et al. (2019) Nature Commun. 10: 4878.

Summary (III)

- **Certain viruses, including HSV1 and SARS-CoV2, inactivate Nrf2**
- **High Nrf2 activity impairs HSV1 infection**
- **Pharmacological activation of Nrf2 with bardoxolone methyl or sulforaphane counteracts HSV1 infection**

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Acknowledgments



Colleagues



Sharadha Dayalan Naidu

Elena Knatko

Maureen Higgins

Oliver Read
Miroslav Novak
Jialin Feng
Louisa Watt



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