The environmental stress response as a target for therapeutic intervention

Jennifer Harbottle
Supervisor: Dr Andreas Kolb

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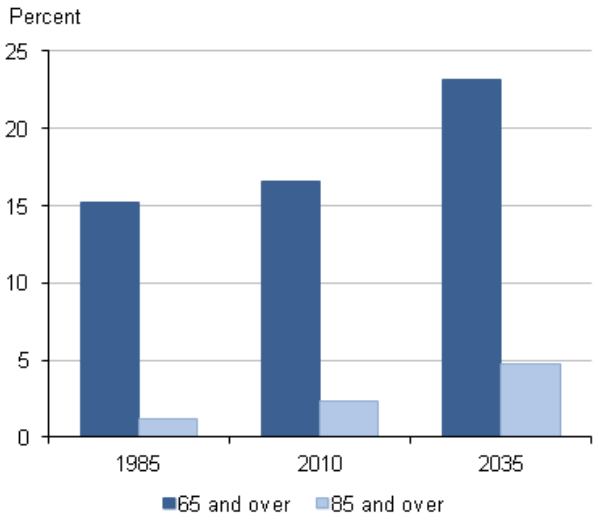
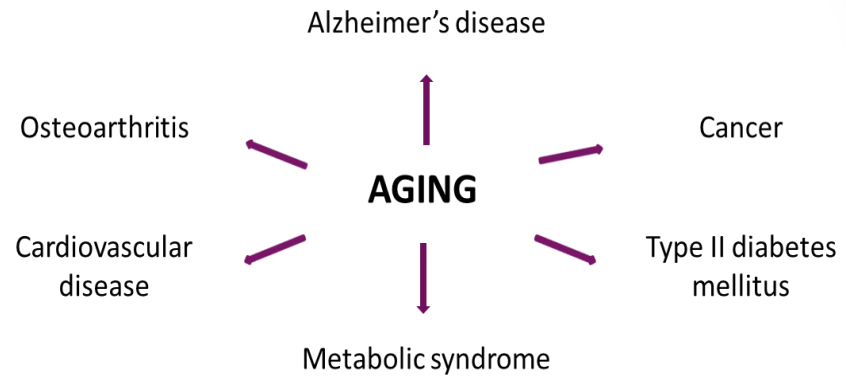
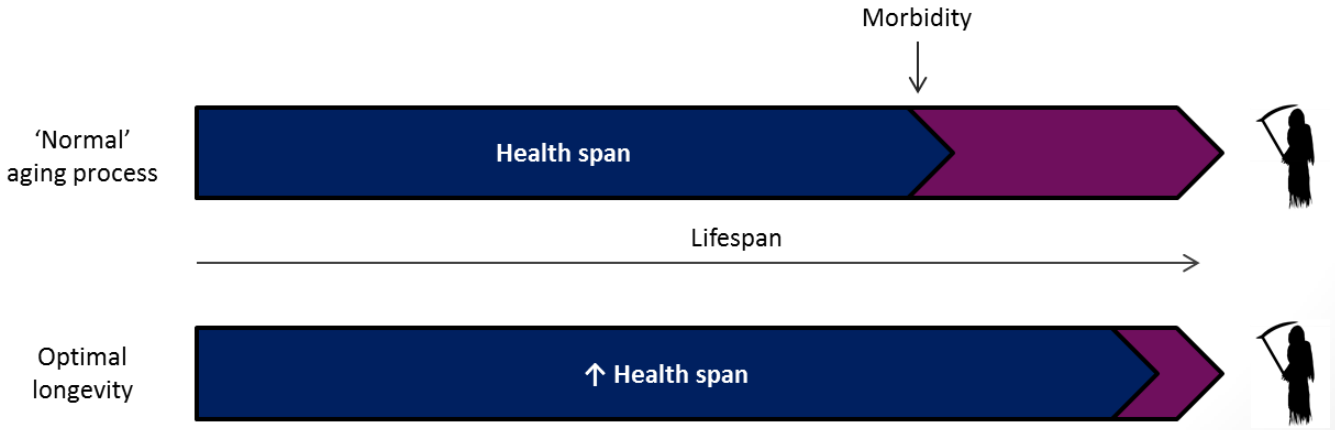
Aging and health span

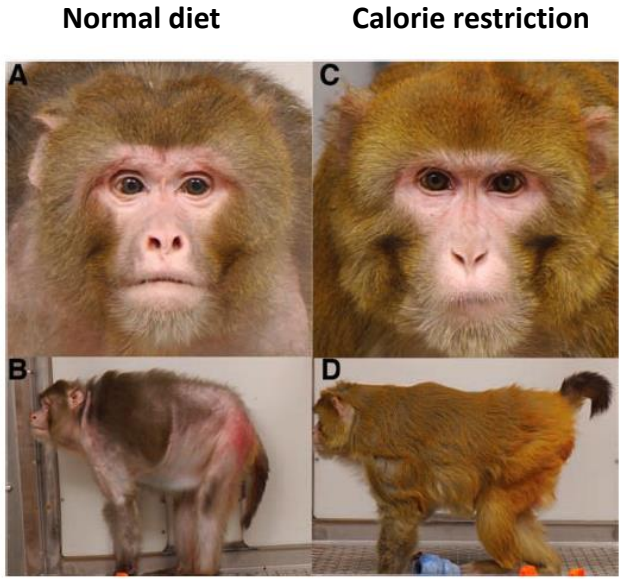


Office for National Statistics, 2012





A common denominator



Stress resistance

Upregulation of the environmental stress response

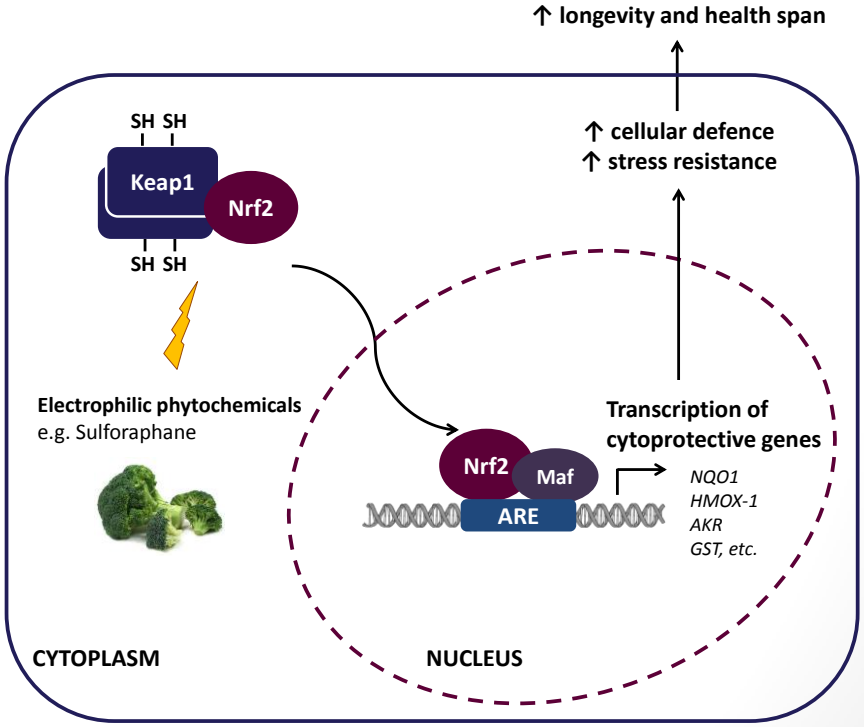
Nrf2 drives the ESR

Nrf2, the “guardian of health span and the gate-keeper of species longevity” (Lewis et al. 2010)

↓ Nrf2 signaling with age

↓ antioxidant defence
↓ stress resistance

↑ disease susceptibility



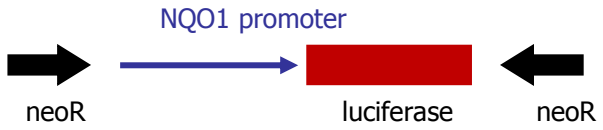
Lewis et al. (2010). *Integr Comp Biol* 50(5):829–843

Phytochemicals activate the ESR

- Aim 1: Identify and characterise novel, natural compounds that induce the ESR.

A cell based assay system for the analysis of phytochemicals activating the ESR

Stable transfection of HepG2 cells & selection of HepG2 C1 clone



↓

Validation of bioluminescent reporter system using sulforaphane

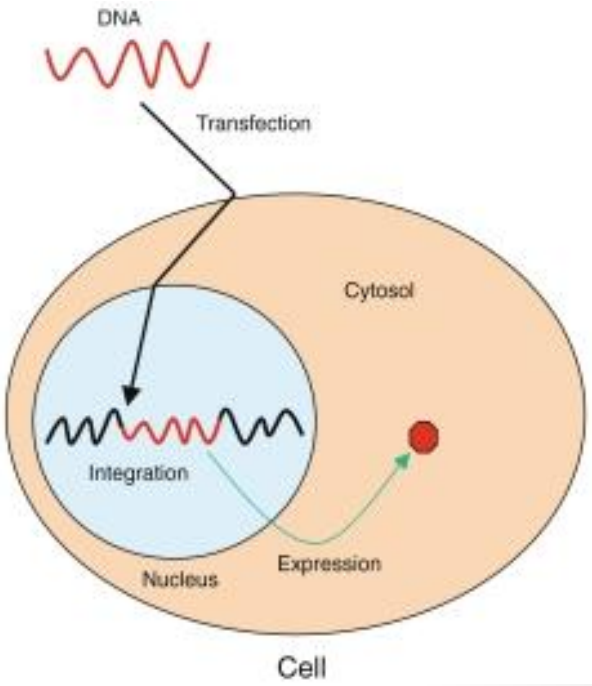
↓

High throughput screening of natural chemical libraries

↓

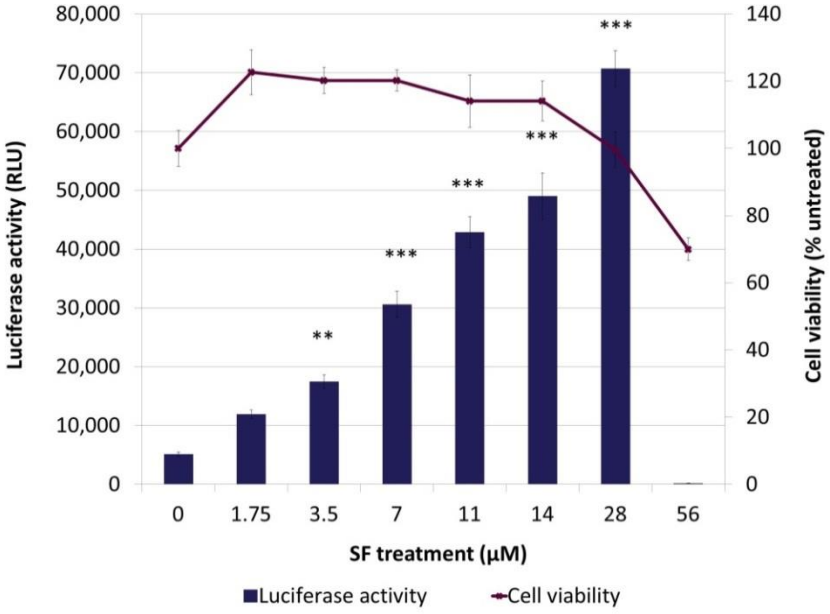
Identification and characterisation of compounds that activate the ESR

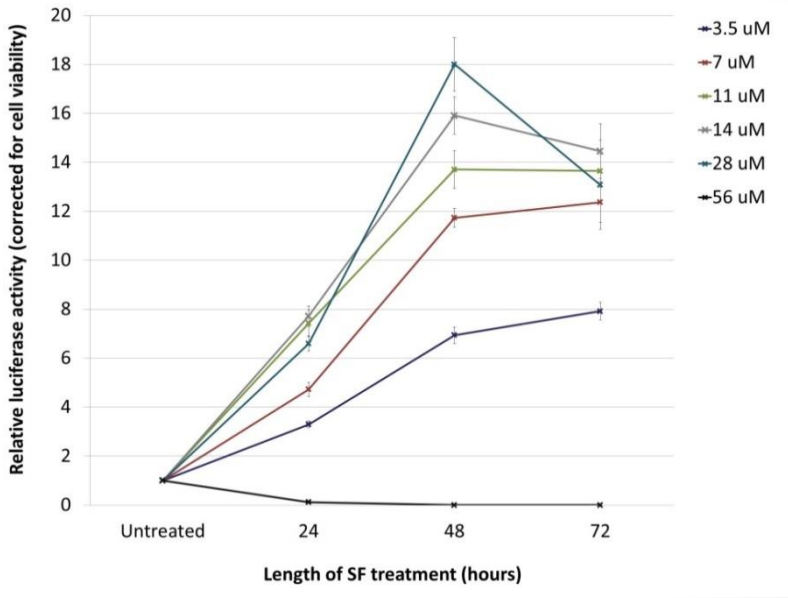
Stable transfection



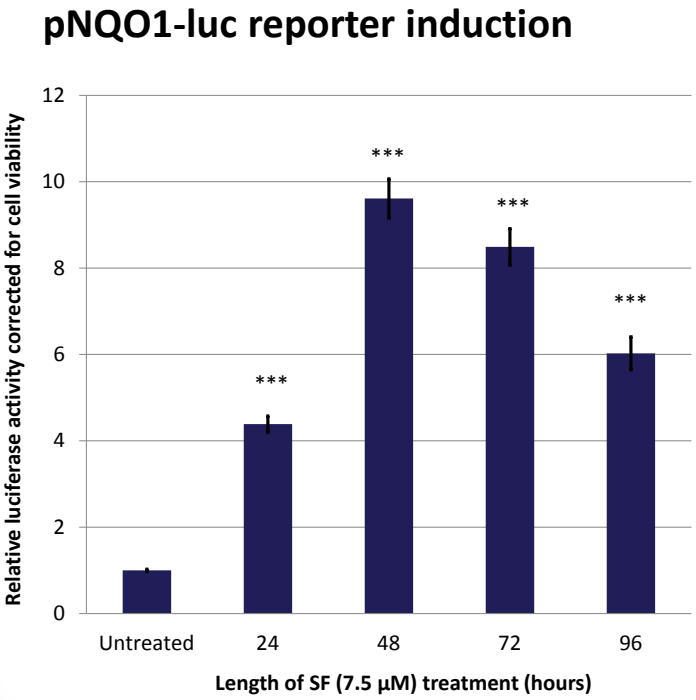
Validation with sulforaphane (1)

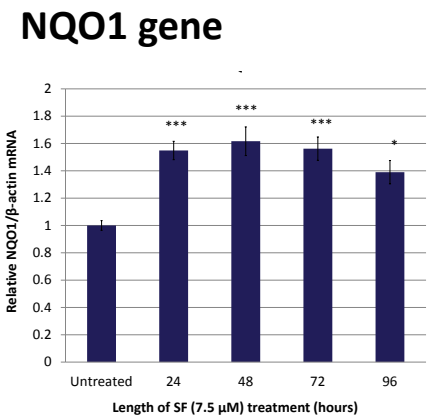
pNQO1-luc reporter induction

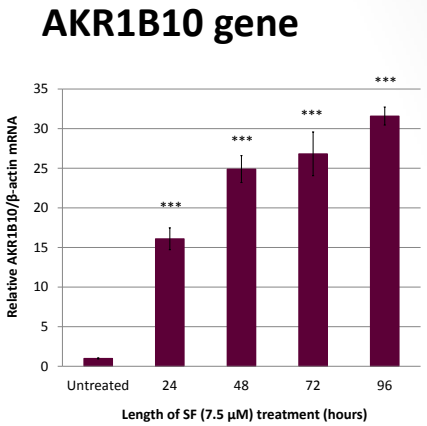


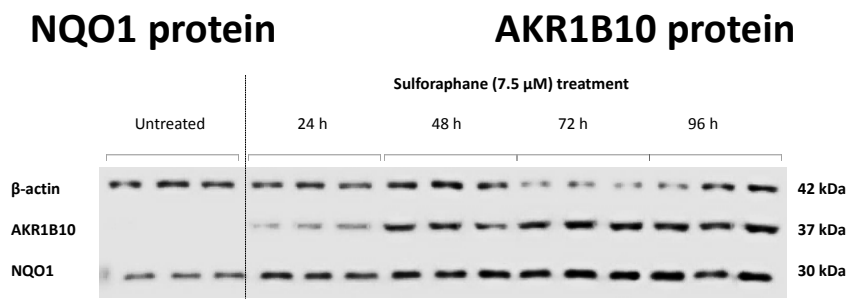


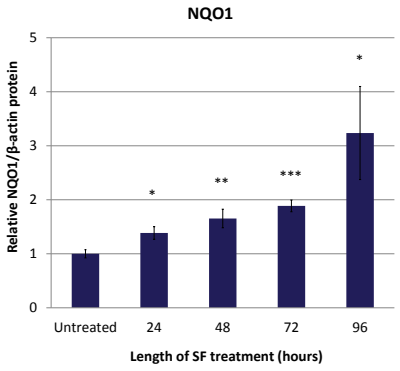
Validation with sulforaphane (2)

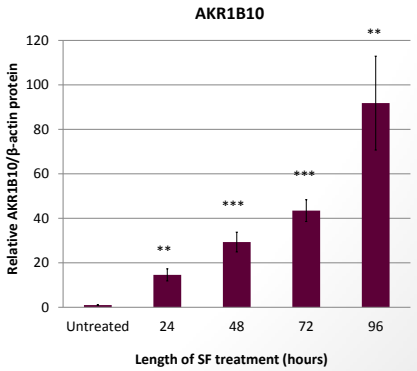






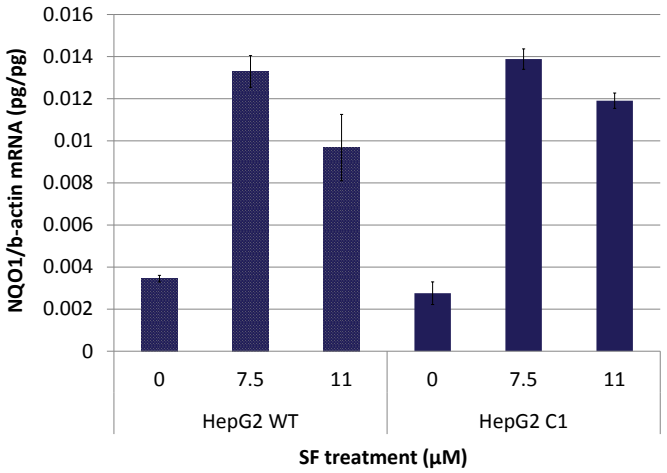






Validation with sulforaphane (3)

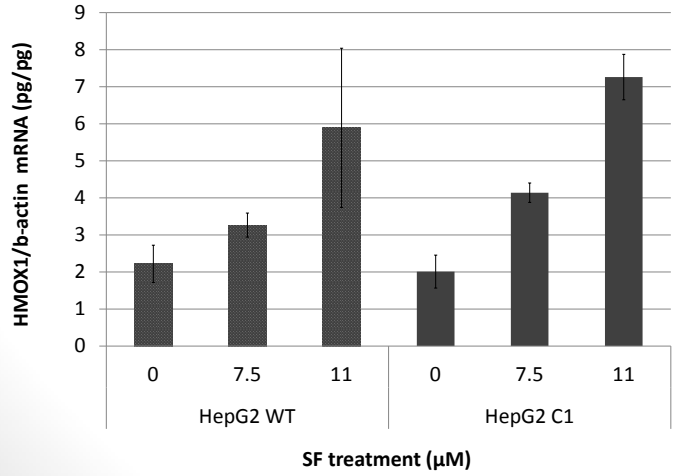
NQO1 gene



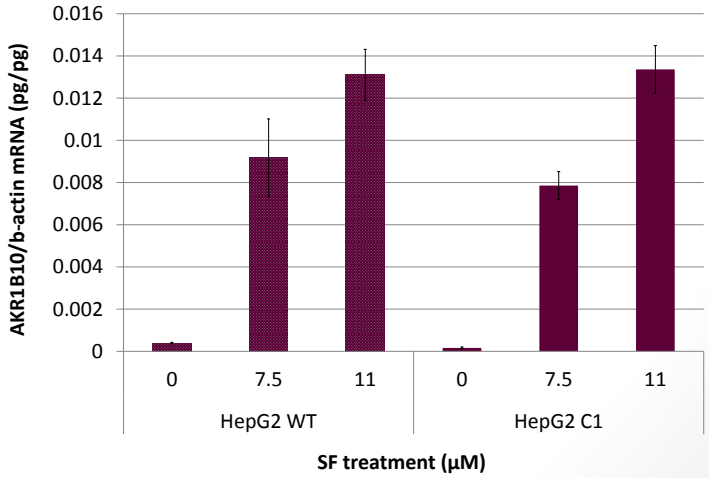
Endogenous ESR gene expression

in HepG2 WT ‘vs’ HepG2 C1

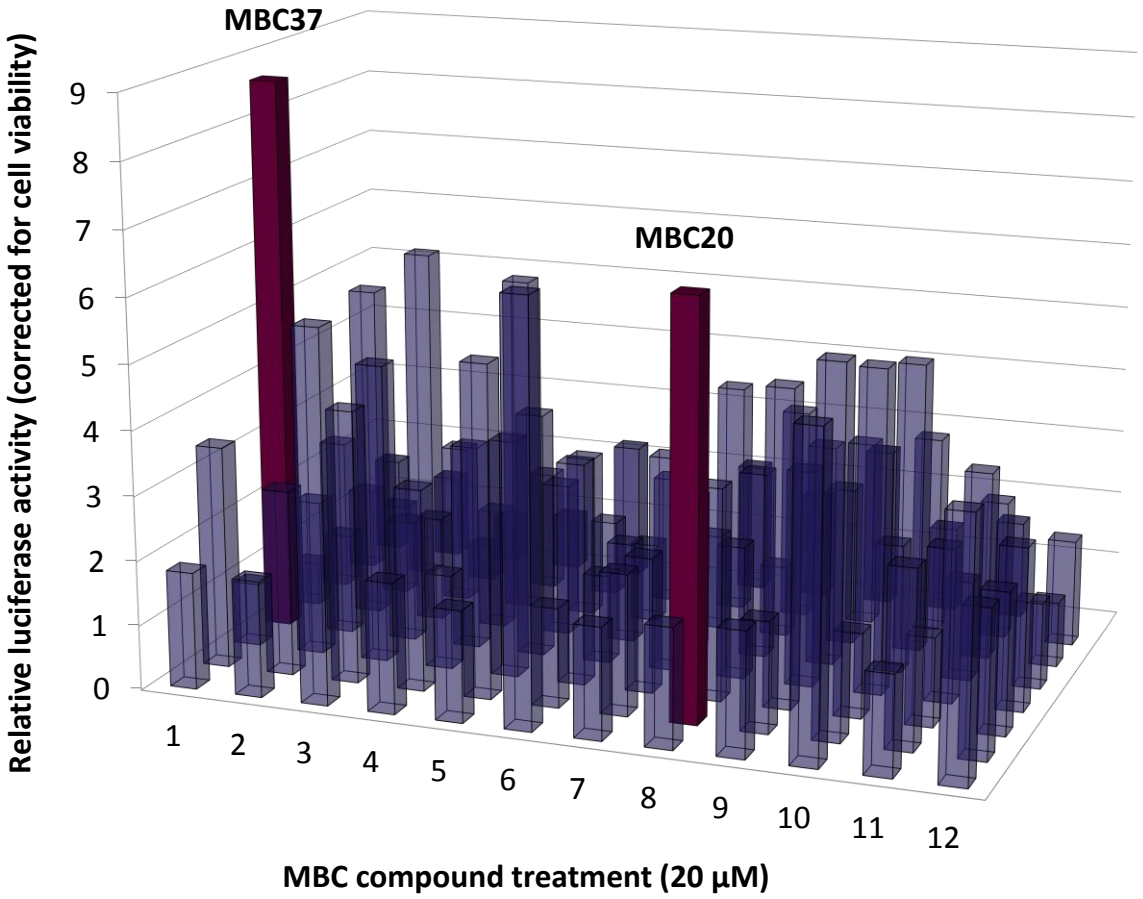
HMOX1 gene



AKR1B10 gene

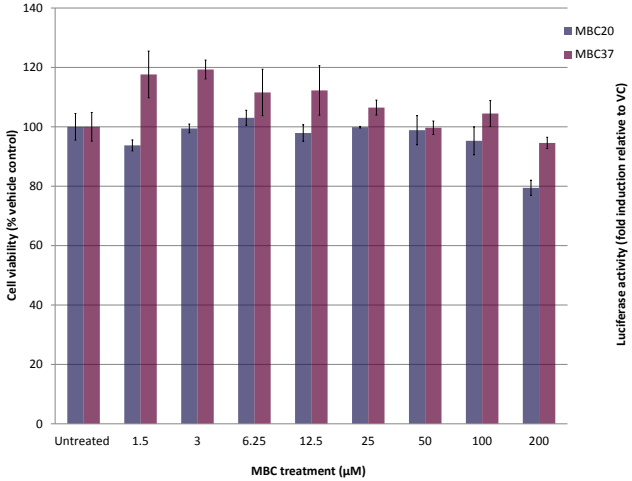


MBC library screening

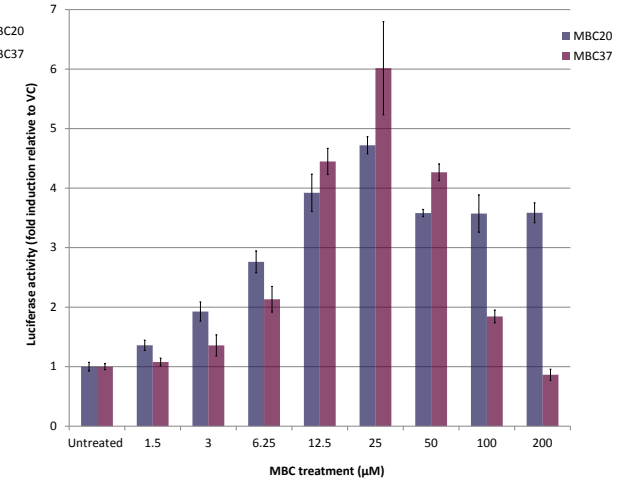


MBC20 and MBC37

Cell viability (0-200 µM)

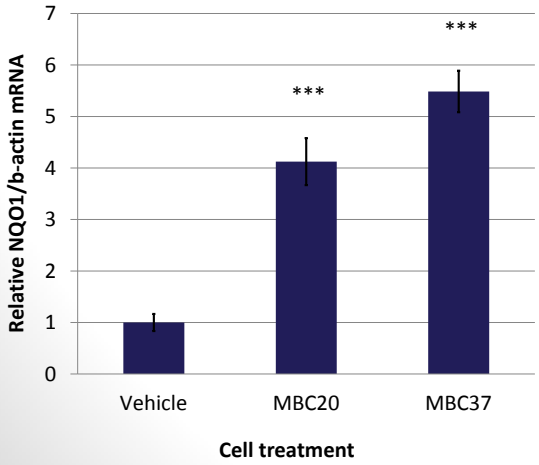


Reporter induction (0-200 µM)

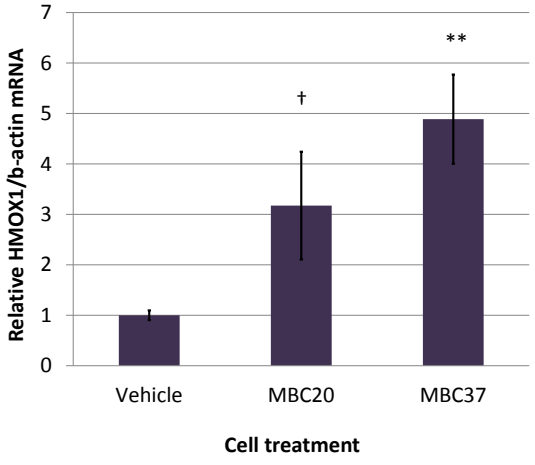


- No apparent cytotoxic effect
- Dose-dependent reporter activation

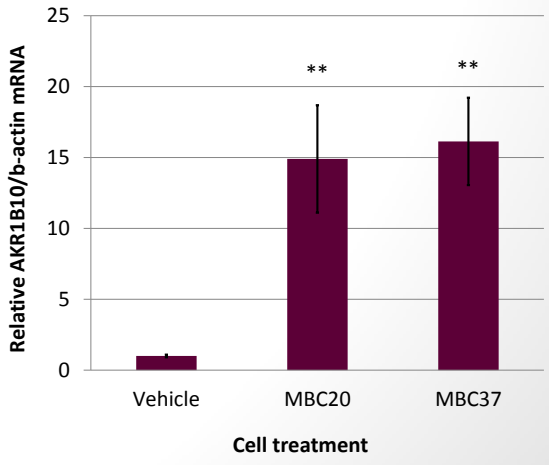
NQO1 gene



HMOX1 gene

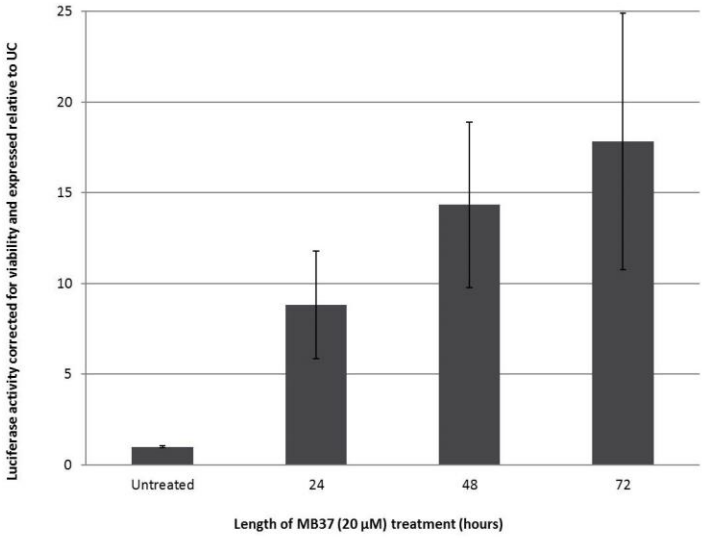


AKR1B10 gene



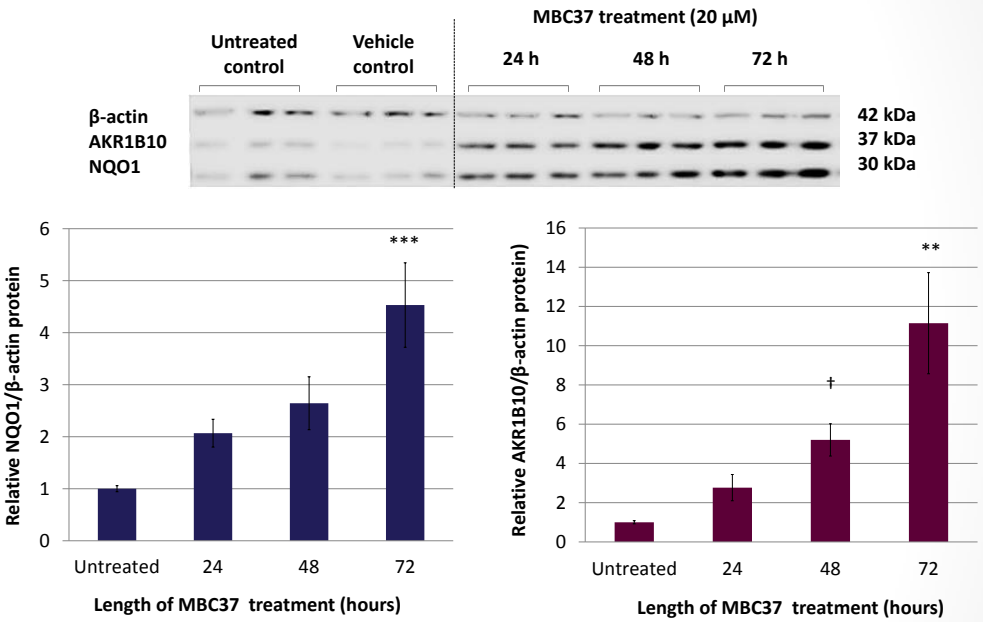
MBC37 (0-72 h)

Reporter induction



NQO1 protein

AKR1B10 protein



- MBC37 ↑ ESR protein expression over time
- ↑ biopotency and ↓ cytotoxicity compared with SF

→ Successful screening

What now?

- Further characterisation of MBC20 and MBC37
  → Bioavailability, absorption, metabolism

- PhytoQuest library screening (MRCT)
  → Stereochemistry and ASR.

Limitations…

- **… Due to the nature of the transfection method.**

  - Random integration site;
  - Variable transgene copy number.

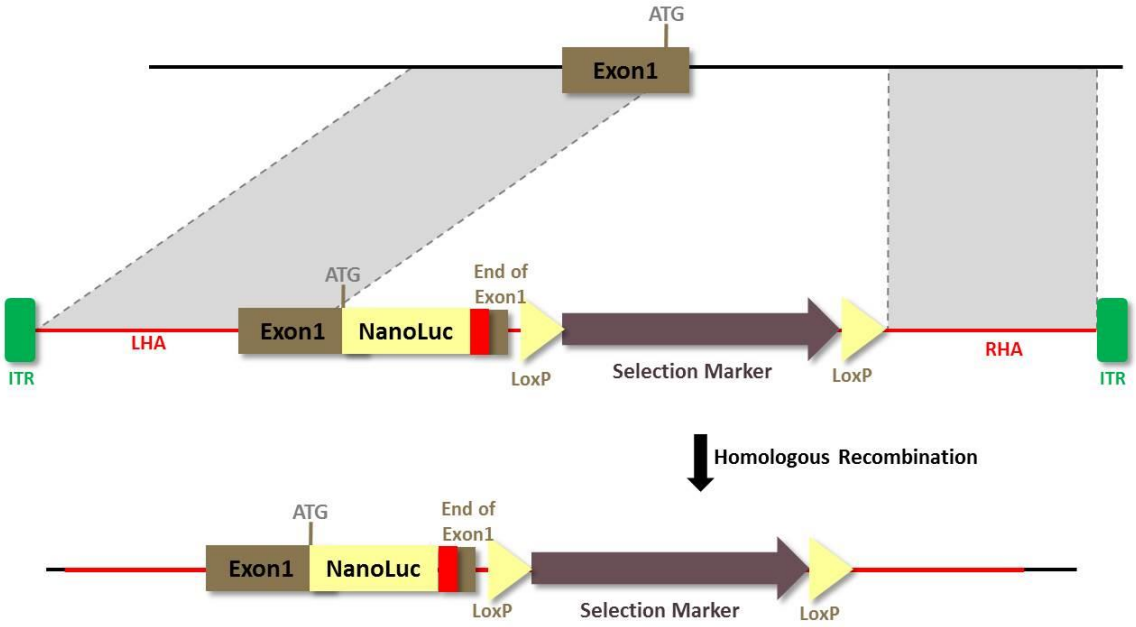
- **… Due to the complexity of mammalian transcription .**

  - Regulatory elements (*cis*-acting enhancers/silencers).

→ Simply inserting a promoter cannot capture the whole picture.

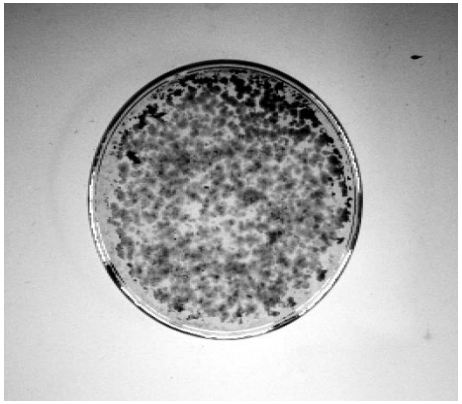
AAV-mediated integration

Aim 2: Site-specific integration of the luciferase reporter gene into the locus of the *HMOX1* gene.

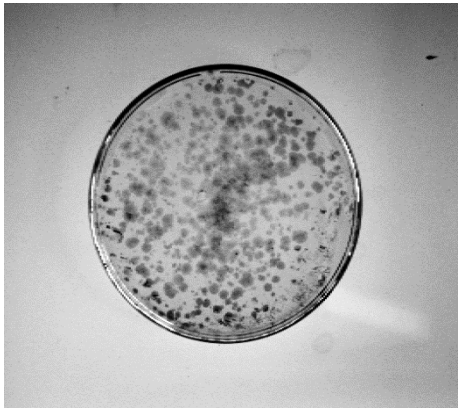


→ The NanoLuc reporter will reflect the expression pattern of endogenous *HMOX1*

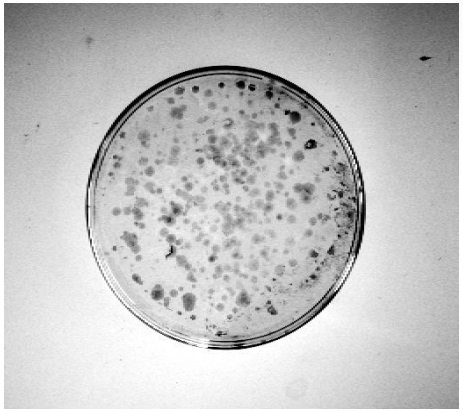
rAAV dilution range



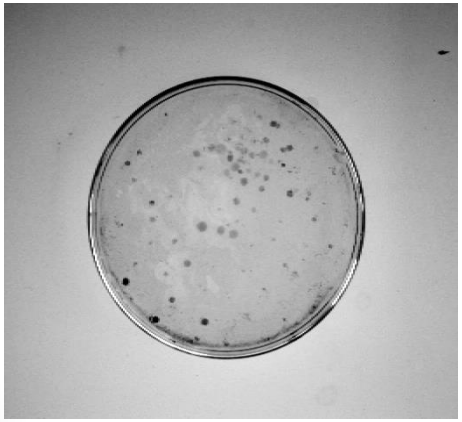
$1x10^5$ copies



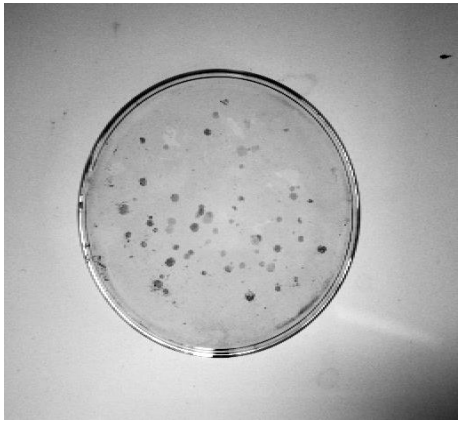
$5x10^4$ copies



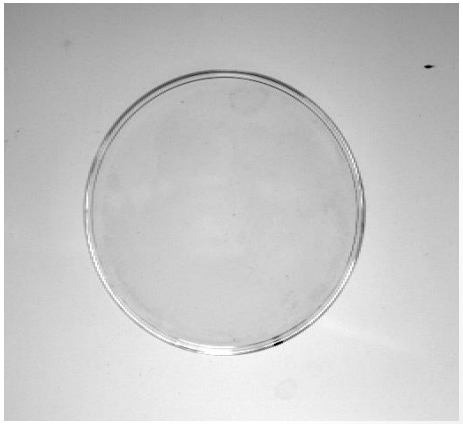
$1x10^4$ copies



$5x10^3$ copies



$1x10^3$ copies



Not infected

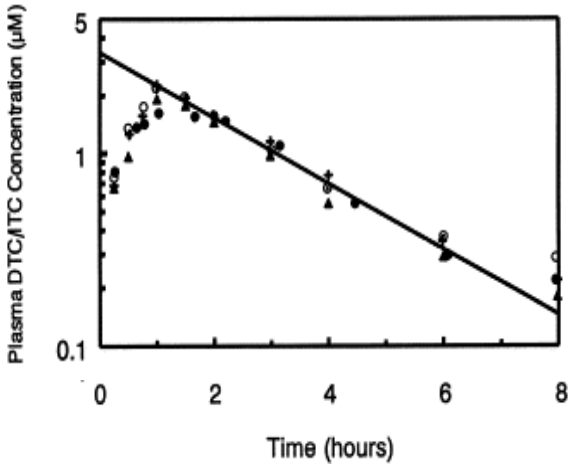
Screening


A1 A2 A3 A4 A5 A7 A8 A9 A10 A11 A12 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 C2 C3
gDNA
Integration
Site-specific
C5 C6 C7 C9 C10 C11 C12 D1 D2 D3 D5 D4 D6 D8 D9 D10 D11 D12 E1 E2 E5 E6 E7 E8
gDNA
Integration
Site-specific
E9 E11 E12 F1 F3 F5 F6 F8 F10 F12 G1 G2 G3 G5 G11 H1 H5 H7 H9 H10 H12
gDNA
Integration
Site-specific

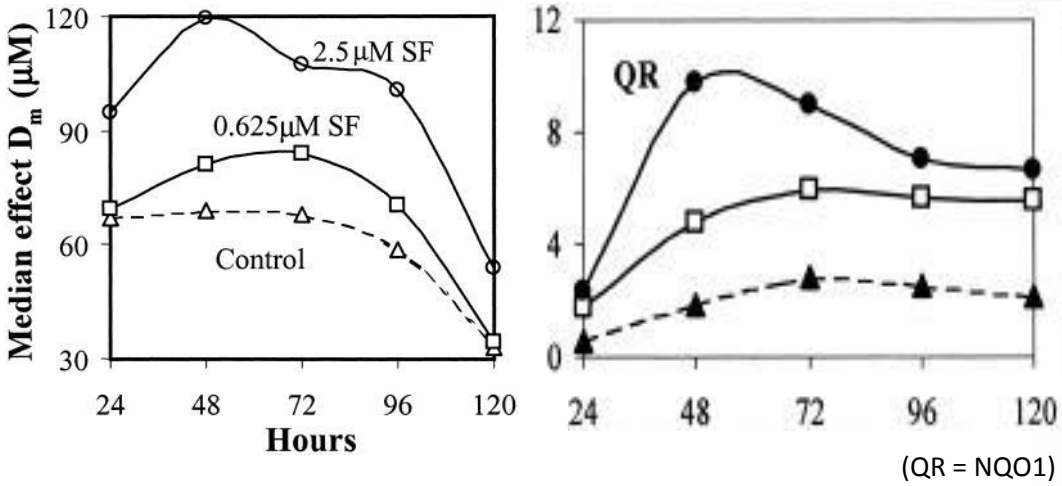

Lindt
LINDOR
BROCCOLI
Lindt
Lindt
Lindt
IRRESISTIBLY SMOOTH
BROCCOLI

The ESR – A long term effect?

Plasma SF

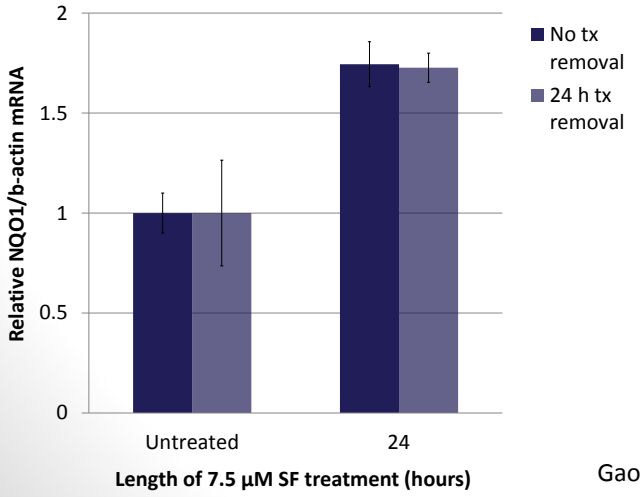


Prolonged protection against menadione



(QR = NQO1)

NQO1 mRNA

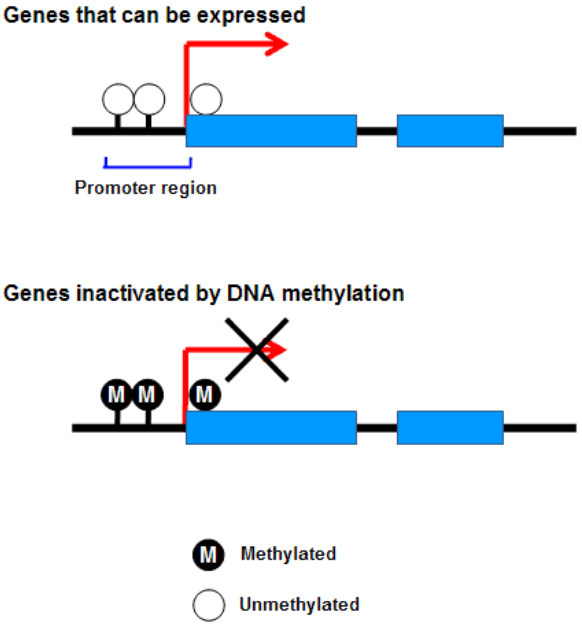


- Does SF evoke an antioxidant response that persists?
- Are epigenetic mechanisms involved?

Gao et al., 2001. Proc Natl Acad Sci U S A. 98(26):15221-6; Ye et al., 2002. *Clin Chim Acta*. 316(1-2):43-53

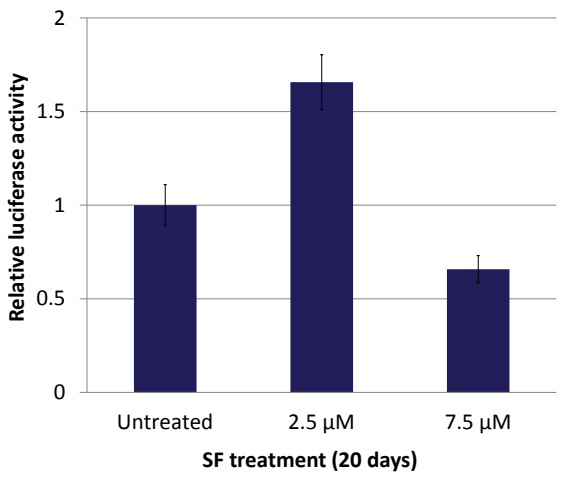
Phytochemicals and epigenetics

- Epigenetic silencing of Nrf2 and NQO1 with ↑ age.
- Phytochemicals upregulate gene promoter demethylation → Facilitate gene transcription.

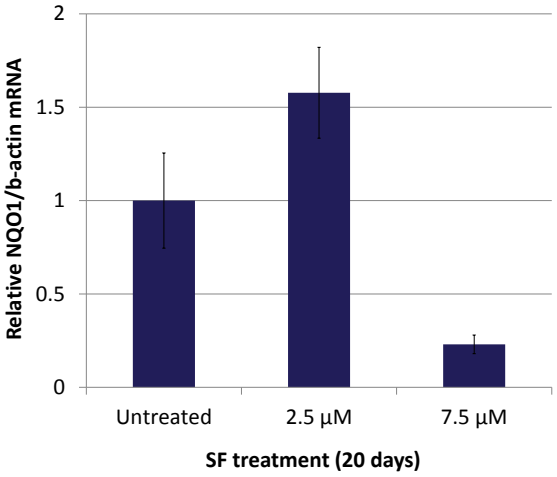


20-day SF treatment

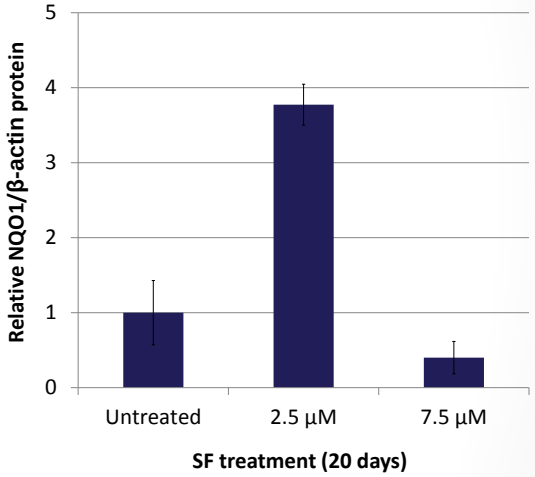
Reporter induction



NQO1 gene



NQO1 protein



Does SF (2.5 µM) treatment demethylate ESR gene promoter?

If so, how stable is this epigenetic modification?

The big picture

- Identification and characterisation of compounds that elicit cytoprotection
  → Food fortification
  → Production of plants with enhanced nutritional quality
  → Optimise health span

- A better understanding of how food provides a conditioning environment that shapes the activity of the (epi)genome and determines the stress adaptive response.

Acknowledgements

Lab group supervision and support

- Dr Andreas Kolb
- Linda Petrie
- Christopher Knowles
- Patrikas Pultinevicius

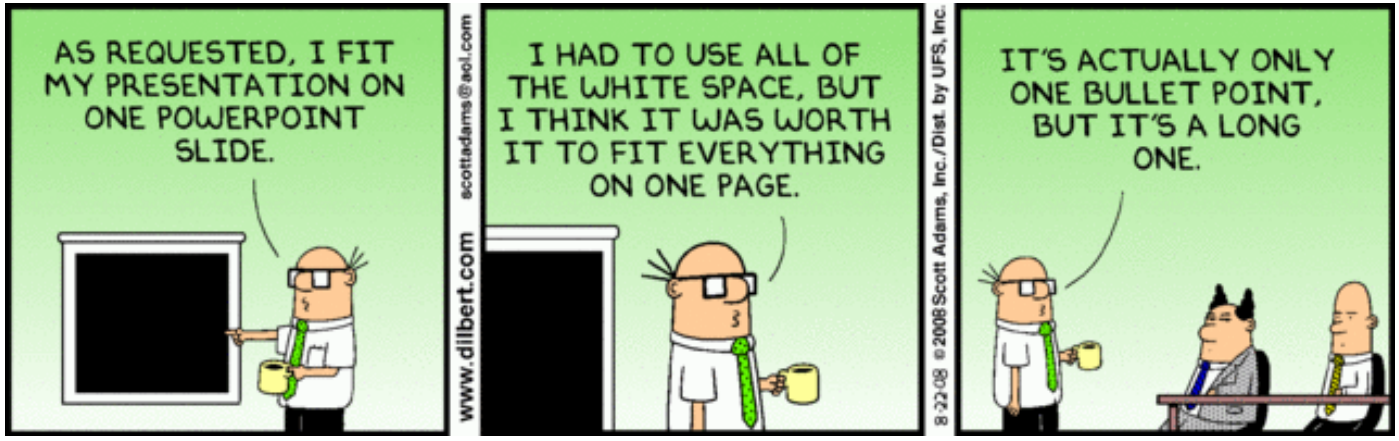
Marine Biodiscovery Centre

- Professor Marcel Jaspars
- Dr Wael Houssen

Genomics

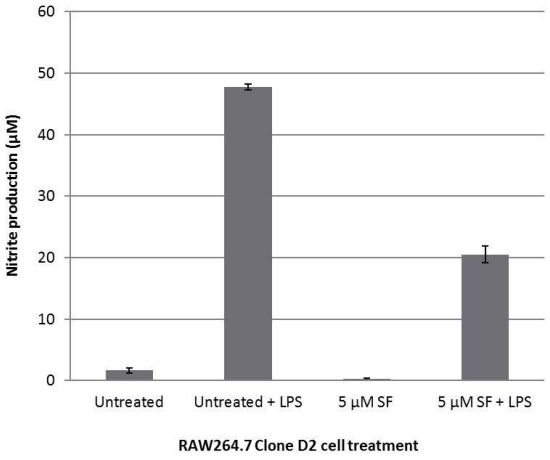
- Pauline Shiach

Thank you for listening

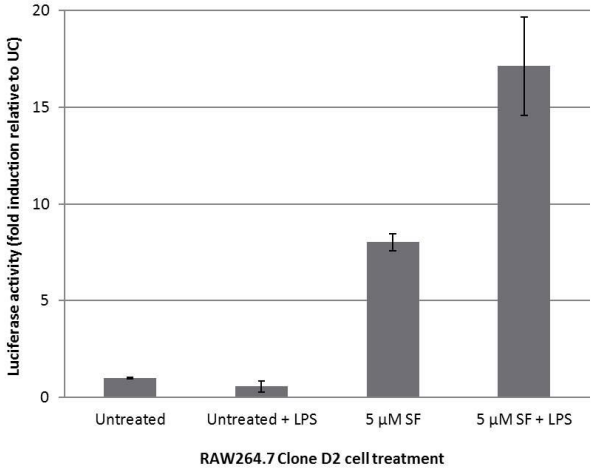


RAW264.7 NQO1-luc D2

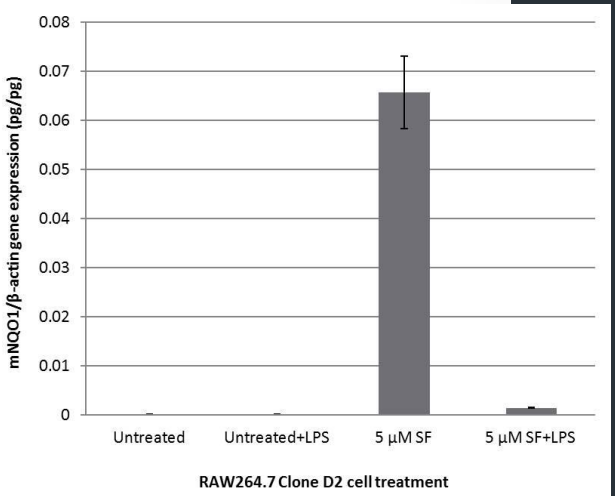
Nitrite production



NQO1-luc reporter induction



NQO1 gene expression



gDNA isolation from HepG2 C1 cells after 20 days treatment with SF (n=3)

↓

Bisulfite conversion of gDNA

↓

PCR with a selection of **Nrf2**-BSP and **NQO1**-BSP primers that span a CpG island within the respective gene promoters

↓ ↘

Unal[illegible] obtain **Nrf2**-BSP amplicon

↓

T[illegible] continued

Obtain 335 bp **NQO1**-BSP amplicon from nested PCR

↓

Purify and clone into pGEMT vector

↓

PCR & Sequence positive clones

↓

Assess methylation status of 24 CpG dinucleotides (> 10 clones)