From Cellular Genotype to Cigarette Smoke-Induced Phenotype: The Case of Nrf2

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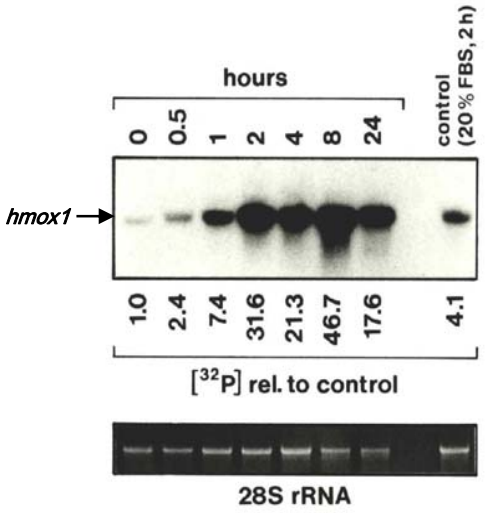
Outline

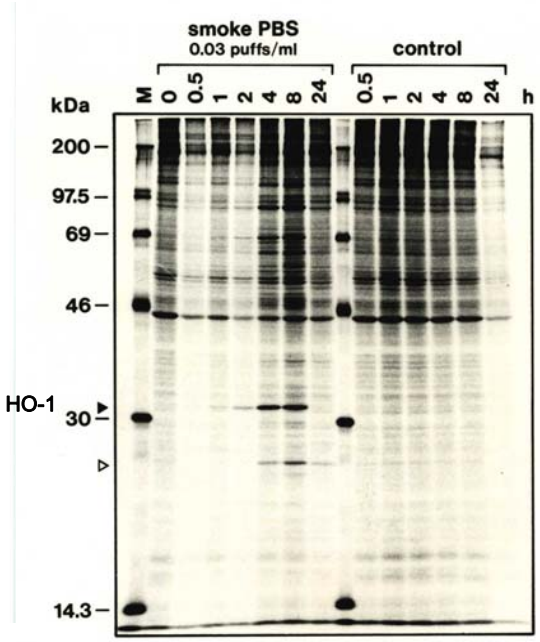
- Introduction
- Nrf2 activation by cigarette smoke *in vitro*
  - Mechanistic investigations using the *hmox1* paradigm
- Nrf2 activation by cigarette smoke *in vivo*
  - The cigarette-smoke-induced *transcriptome* in $Nrf2^{-/-}$ *vs*. $Nrf2^{+/+}$ mice
  - The cigarette-smoke-induced *phenotype* in $Nrf2^{-/-}$ *vs*. $Nrf2^{+/+}$ mice
- Final remarks

Introduction
Cigarette smoke (CS) induces a paramount antioxidant- and Phase II-related response

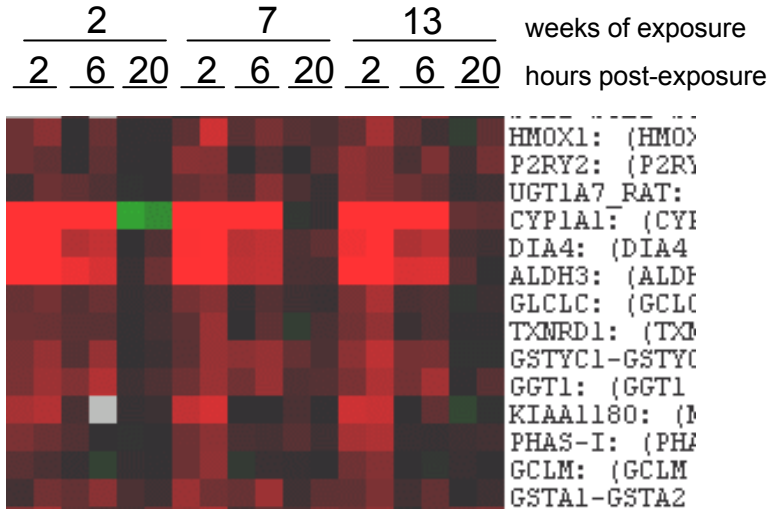
in vitro



(Müller and Gebel, 1994)



in vivo

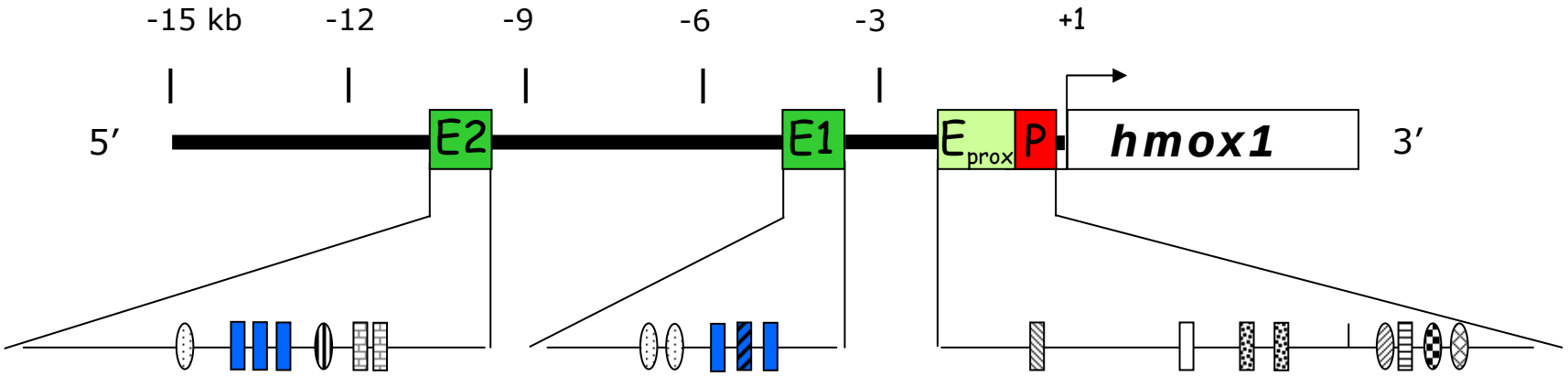


L = 300 µg/l TPM; H = 600 µg/l TPM

Heatmap excerpt (Gebel *et al.*, 2006)

Nrf2 activation by cigarette smoke *in vitro*: The *hmox1* paradigm

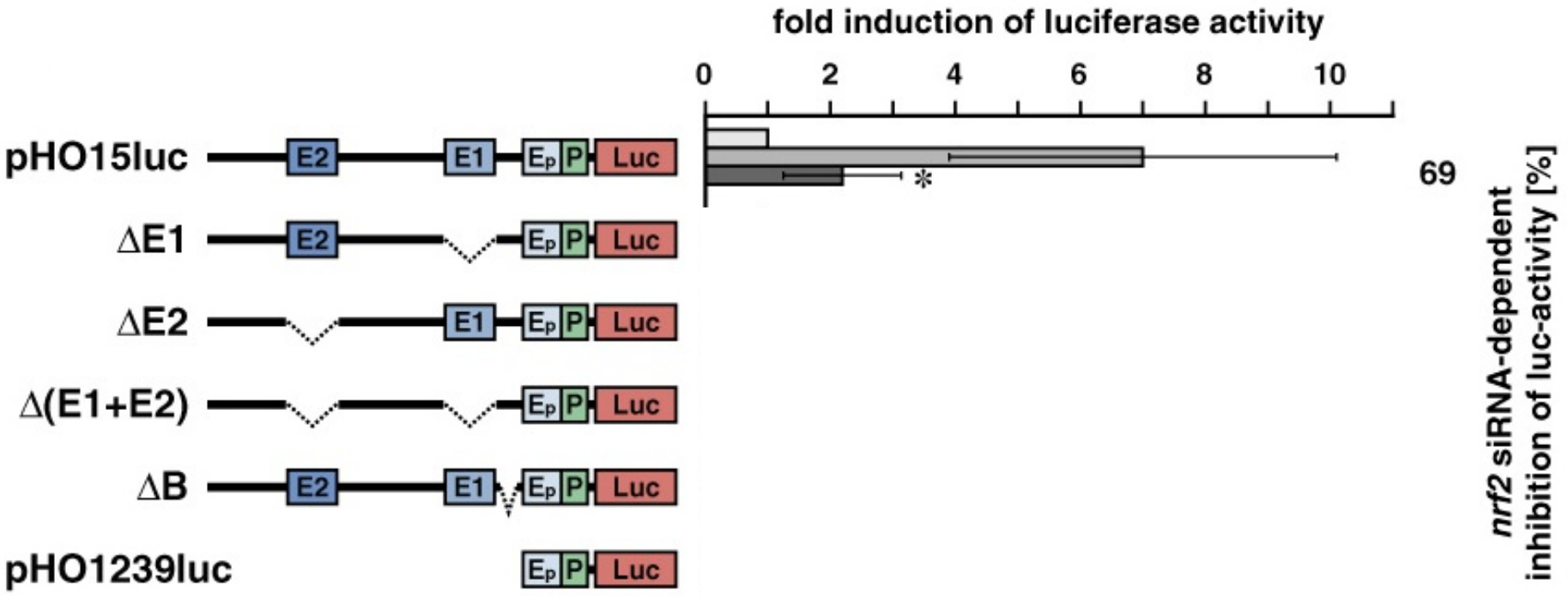
The *hmox1* promoter / enhancer region:



Transcription Factor Binding Sites:

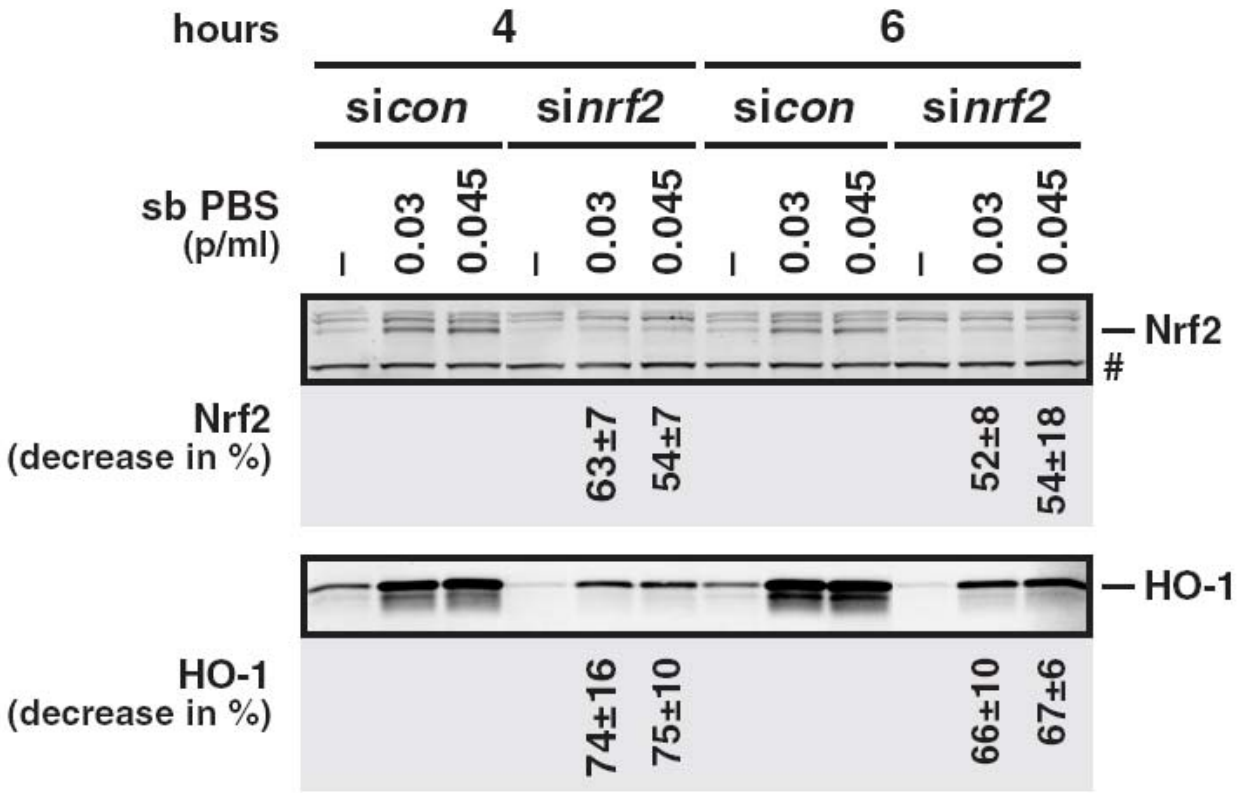
P: Promoter; E_{prox}: proximal Enhancer; E1: Enhancer 1; E2: Enhancer 2

Promoter deletion analysis in the context of Nrf2 down-regulation in NIH3T3 cells by RNAi



(Knörr-Wittmann *et al.*, 2005)

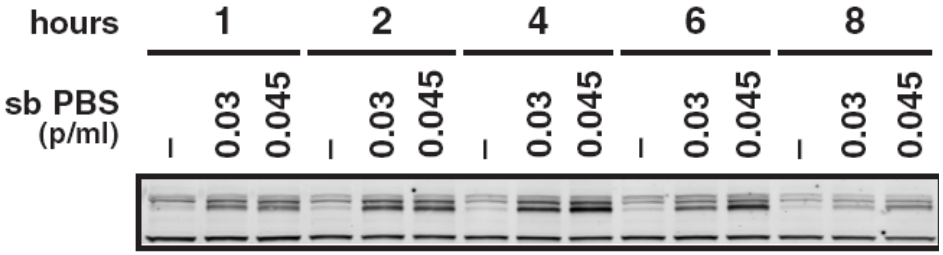
Effect of Nrf2 down-regulation on CS-dependent HO-1 expression



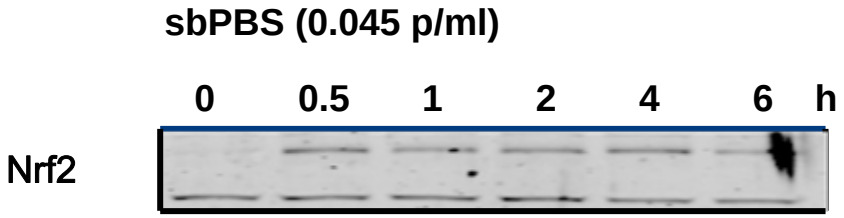
(Knörr-Wittmann *et al.*, 2005)

Activation of Nrf2 in CS-exposed cells

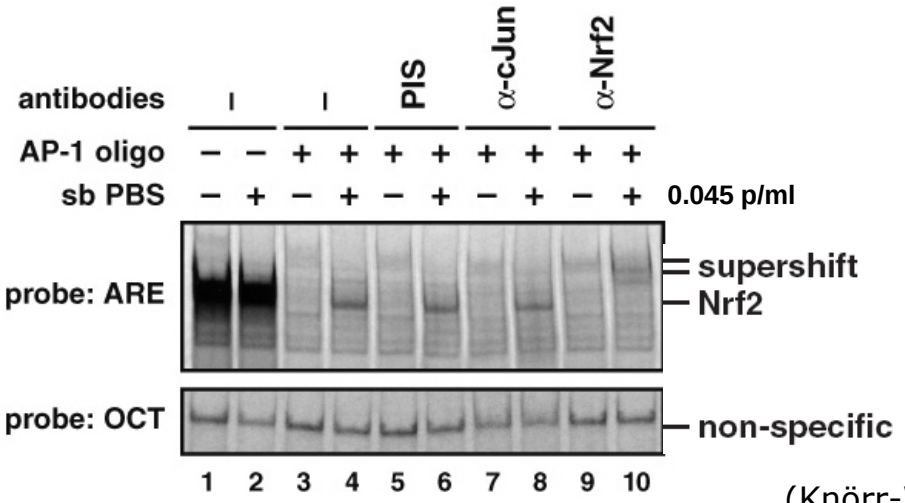
Whole cell extracts



Nuclear extracts

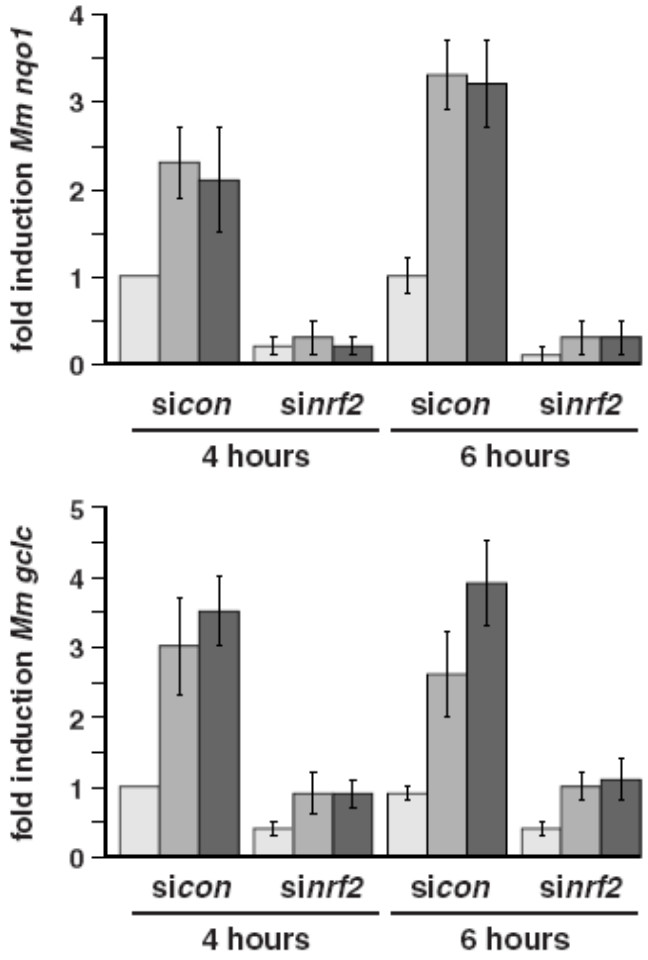


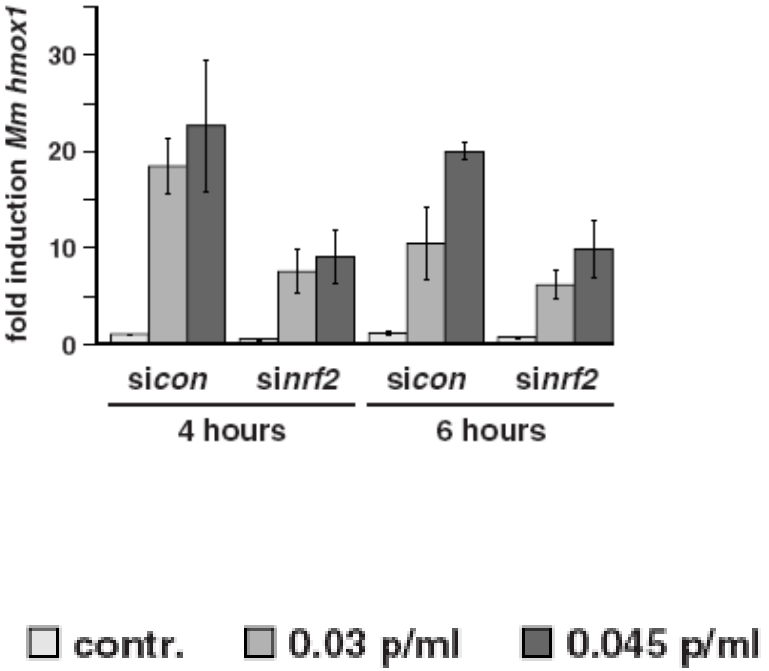
EMSA



(Knörr-Wittmann *et al.*, 2005)

Down-regulation of Nrf2 abrogates CS-dependent induction of Phase II model genes

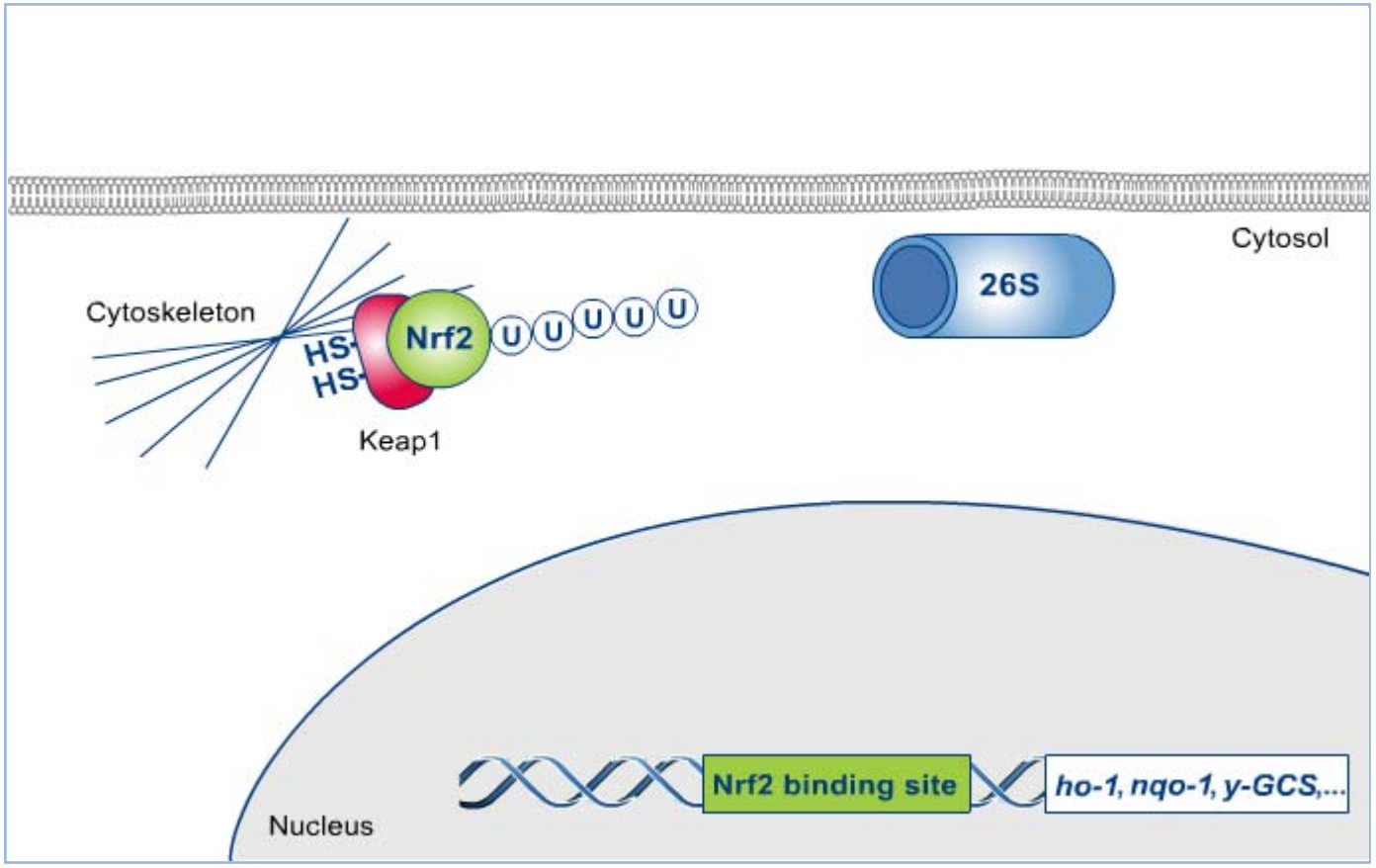




(Knörr-Wittmann *et al.*, 2005)

Conclusion (I)

In vitro, CS induces Nrf2 by a canonical mechanism



Nrf2 activation by cigarette smoke *in vivo*

(Gebel *et al.*, in prep.)

Inhalation Study Design:

Animals: ♀ $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice
Exposure: Whole body; 2, 3, or 4 h/d; 5 d/wk
CS concentration: 125 (single) or 750 µg TPM/l x h

exposure	post-exposure	sham*	single (375 µg)	low (1500 µg)	medium (2250 µg)	high (3000 µg)
1 day	-	X	X	-	-	-
2 months	-	X	-	-	X	-
5 months	-	X	-	X	X	X
5 months	1 days	X	-	X	X	X
5 months	13 days	X	-	-	X	-

*fresh air exposure

Nrf2 activation by cigarette smoke *in vivo*

Inhalation Study Endpoints:

Transcriptome:

- Genome-wide Affymetrix-based analysis of all groups

Phenotype:

- In-life observations: body weight development
- Pathology
- Inflammation/BAL fluid
- Functional respiratory changes (forced pulmonary maneuvers)

The CS-induced *transcriptome* in $Nrf2^{-/-}$ vs. $Nrf2^{+/+}$ mice

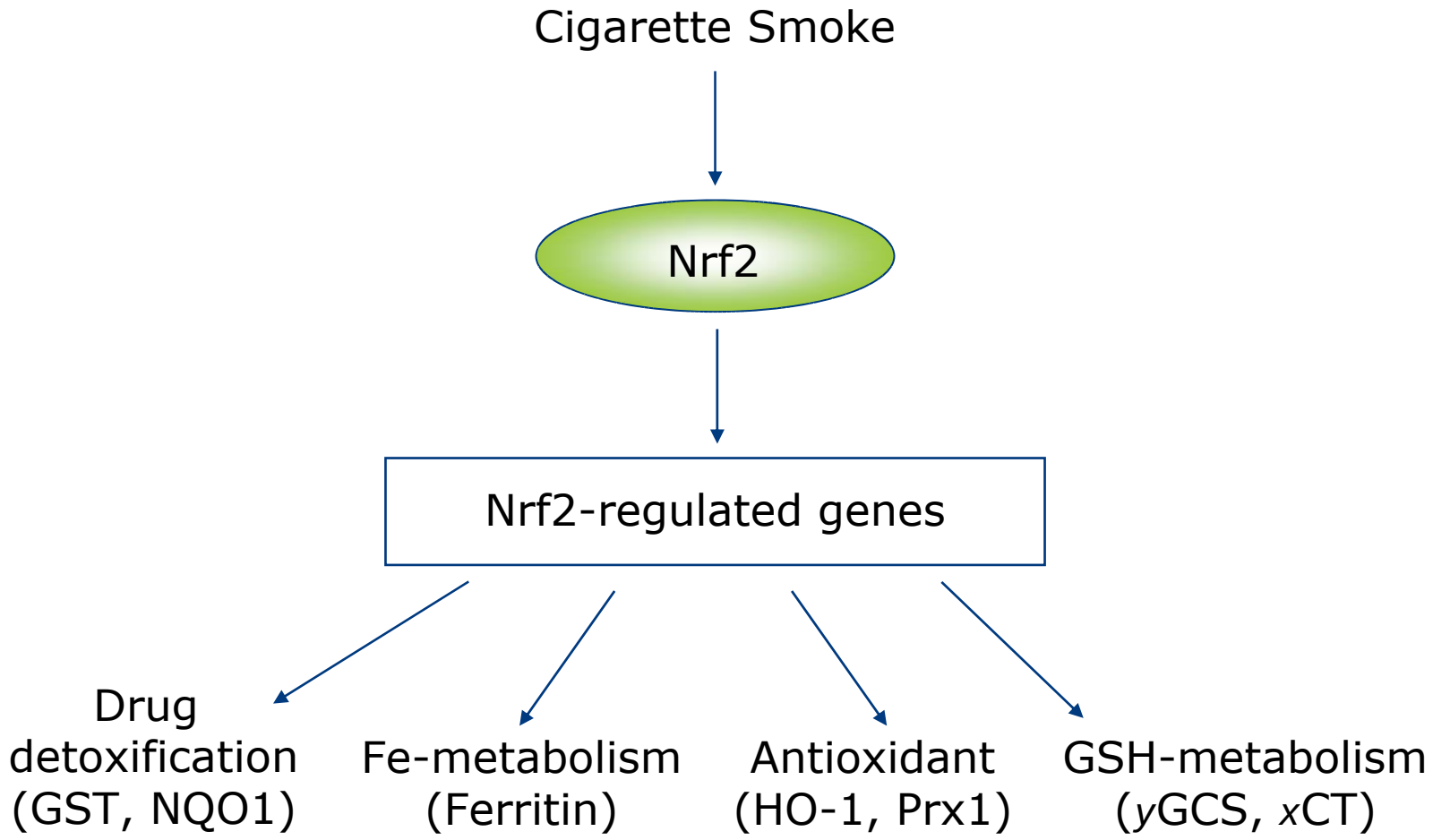
Genes coding for antioxidant and Phase I/II xenobiotic-metabolizing enzymes

Exposure	1 d	1 d	2 m	2 m	5 m	5 m	5 m	5 m	5 m	5 m
Post exp.	-	-	-	-	-	-	1 d	1 d	13 d	13 d
	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
ftl2	1.3		—		—		—		1.7	
gclc	2.4		2.4		2.1		—		—	
gclm	2.4		2.9		2.2		—		—	
gpx2	2.1		3.6		2.3		1.1		1.2	
gsr	2.0		2.1		2.0		—		—	
hmox1	2.2		2.8		3.1		2.1		2.0	
nqo1	14.2		8.9		12.7		1.5		1.3	
txnrd1	2.0		2.3		2.3		—		—	
adh7	2.2		4.2		2.8		—		—	
aldh3A1	5.0		7.9		4.3		—		—	
akr1B8	3.9		4.9		4.5		1.8		1.5	
cyp1A1	62.1		108.8		78.4		—		—	
cyp1B1	5.2		14.4		23.9		3.1		2.9	
gsta1	3.8		4.6		3.5		—		—	
gsta2	2.3		2.8		2.5		—		—	
	Nrf2									

Genes involved in the inflammatory response

Exposure		1 d	1 d	2 m	2 m	5 m	5 m	5 m	5 m	5 m	5 m
Post exp.		-	-	-	-	-	-	1d	1 d	13 d	13 d
gene	alias	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
ccl2	mcp-1	—		5.5		4.0		10.7		5.6	
ccl3	mip1α	—		11.5		8.5		12.3		13.3	
ccl6	mrp-1	—		4.0		3.4		3.7		4.0	
ccl20	mip3α	2.0		16.0		3.5		2.9		3.8	
ccl5	rantes	—		-1.3		-2.3		-3.3		-1.1	
cxcl1	gro-α, kc	2.9		18.2		9.9		15.2		8.4	
cxcl5	ena-78	—		154.1		23.8		29.4		10.5	
cxcl9	mig	—		6.7		7.8		9.0		6.2	
cxcl10	IP-10	—		2.2		3.0		11.0		3.7	
saa3		2.3		53.3		25.0		55.4		40.8	
orm2		3.5		17.8		35.6		24.9		4.9	
cd68		—		3.5		4.4		5.4		4.8	
msr		—		5.8		9.8		10.7		7.9	
mmp12		—		20.8		19.4		14.5		19.6	
timp1		2.8		3.0		3.1		3.5		3.0	
slpi		—		—		1.4		3.2		2.8	
ctsk		—		5.9		19.3		16.3		19.6	
ctss		—		2.1		2.7		2.6		2.7	
		Nrf2									

Nrf2 induces a transcriptional response aimed at counteracting insults from CS-dependent stress



(after Ishii *et al*. 2002)

CS exposure creates a complex pattern of gene down- and up-regulation which is both Nrf2- and dose-dependent

(K. Taguchi and M. Yamamoto, pers. comm.)

Exposure	1 day				2 months				5 months																			
Post exp.	—				—				—								1 day								13 days			
Nrf2	WT		KO		WT		KO		WT				KO				WT				KO				WT		KO	
CS dose	–	++	–	++	–	++	–	++	–	+	++	+++	–	+	++	+++	–	+	++	+++	–	+	++	+++	–	++	–	++
Tnnc2	1.00	0.30	0.32	0.68	1.00	0.50	0.49	0.60	1.00	0.45	0.45	0.99	0.45	0.45	0.45	0.45	1.00	0.99	0.99	0.99	0.99	0.99	0.98	0.99	1.00	1.01	1.59	2.15
Tnni1	1.00	0.68	0.69	1.11	1.00	0.80	0.80	0.80	1.00	0.61	0.61	0.76	0.61	0.61	0.61	0.60	1.00	1.00	0.99	1.00	1.00	0.99	0.99	1.00	1.00	1.02	1.01	1.35
Tnni2	1.00	0.49	0.49	0.77	1.00	0.77	0.70	0.85	1.00	0.65	0.64	0.99	0.66	0.73	0.64	0.68	1.00	1.06	1.06	1.11	1.00	1.11	1.05	1.06	1.00	1.39	1.08	1.85
Tnni2	1.00	0.40	0.43	0.74	1.00	0.64	0.59	0.80	1.00	0.59	0.61	1.10	0.54	0.67	0.58	0.74	1.00	1.03	1.12	1.16	0.94	1.05	1.15	1.14	1.00	1.30	1.31	2.13
Tnnt3	1.00	0.38	0.41	0.75	1.00	0.60	0.59	0.79	1.00	0.53	0.53	0.99	0.55	0.53	0.54	0.56	1.00	0.92	0.92	0.94	0.92	1.03	0.91	0.94	1.00	1.02	1.32	1.80

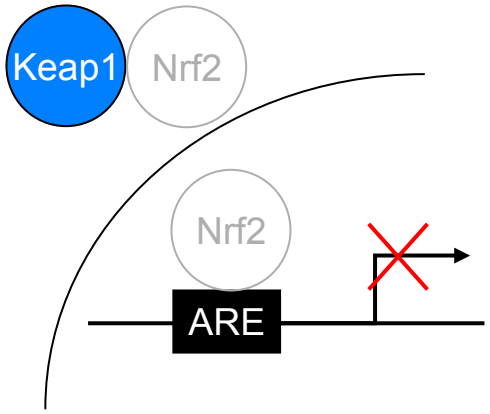
- In $Nrf2^{+/+}$ mice, numerous genes are down-regulated after exposure to low and medium doses of CS
- Some genes show attenuated expression in $Nrf2^{-/-}$ mice exposed to fresh air, but an "$Nrf2^{+/+}$-like" expression profile when exposed to CS (also seen in $Nrf2^{+/+}$ mice at the highest dose)
- The effect of CS exposure is abrogated after exposure is discontinued (1 day)
- In $Nrf2^{-/-}$ mice, some genes become induced only after CS exposure is discontinued (13 days)

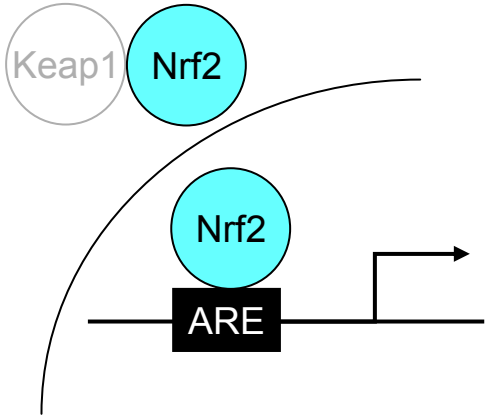
Comparison of gene expression rates from sham Nrf2 $^{+/+}$ and Nrf2 $^{-/-}$ mice *vs.* unexposed Keap1 cond. KO mice…

(K. Taguchi and M. Yamamoto, pers. comm.)

Constitutive Nrf2 activation in Clara cells in the lung

♀	Nrf2 $^{-/-}$	Nrf2 $^{+/+}$	Keap1 CCSP-CKO
	wk 12; 20; 33-35		12 wk
Nrf2 target genes:	± or ↓	±	↑





… identifies new and confirms known Nrf2 target genes with some genes showing age-related adaptation

(K. Taguchi and M. Yamamoto, pers. comm.)

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Age	12wk		20wk		33wk		33wk		35wk	
Exposure	1d	1d	2m	2m	5m	5m	5m	5m	5m	5m
Post exp.	-	-	-	-	-	-	1d	1d	13d	13d
Gene										
Nfe2l2		1.00	0.20	0.99	0.19	1.00	0.20	0.98	0.21	0.99	0.20
Nqo1	14.8	1.00	0.63	0.91	0.55	0.93	0.60	0.74	0.64	0.84	0.63
Gpx2	4.0	1.00	0.72	0.96	0.63	1.00	0.64	0.92	0.71	0.90	0.70
Aldh3a1	12.8	1.00	0.80	0.87	0.69	0.91	0.77	0.85	0.81	0.79	0.68
Aox1	2.5	1.00	0.74	0.99	0.76	1.02	0.69	0.96	0.79	0.99	0.62
Ces1	13.3-13.5	1.00	0.42	0.88	0.41	0.92	0.40	0.86	0.41	0.89	0.42
Dsc2	3.0-4.2	1.00	0.68	0.81	0.59	0.81	0.79	0.76	0.66	0.67	0.68
Pp11r	2.3	1.00	0.76	0.83	0.72	0.86	0.78	0.84	0.77	0.95	1.21
Tmem40	1.5-1.6	1.00	0.69	0.81	0.71	0.94	0.87	0.90	0.80	0.86	0.78
Tmem45b	2.3	1.00	0.78	0.98	0.63	0.92	0.77	0.90	0.65	0.57	0.60
Trex2	3.6	1.00	0.74	0.83	0.74	0.97	0.74	0.75	0.74	0.74	0.75
Tnni1	1.1-1.9	1.00	0.69	0.84	0.68	1.11	0.68	0.69	0.69	0.67	0.68
1110032A04Rik	2.1	1.00	0.56	0.85	0.68	0.95	0.54	0.67	0.73	0.82	0.68
Anxa9		1.00	0.77	0.89	0.72	0.84	0.67	0.74	0.64	0.78	0.70
AU018778		1.00	0.47	1.11	0.39	1.05	0.44	1.03	0.40	1.06	0.39
A030009H04Rik		1.00	0.68	0.88	0.76	0.95	0.74	0.80	0.73	0.82	0.74
Car3		1.00	0.77	0.92	0.55	0.77	0.68	0.82	0.66	0.52	0.63
Car3		1.00	0.78	0.97	0.57	0.90	0.81	0.93	0.65	0.60	0.65
Cd109		1.00	0.75	0.84	0.74	0.87	0.78	0.78	0.83	0.71	0.79
Cyp1b1		1.00	0.77	0.97	0.58	0.79	0.64	0.92	0.73	0.86	0.75
Ddit4l		1.00	0.72	0.85	0.74	0.93	0.81	0.76	0.84	0.78	0.79
Itgb6		1.00	0.74	1.16	1.06	1.21	0.87	1.12	1.09	1.21	0.86
Lad1		1.00	0.71	0.86	0.80	1.02	0.81	0.87	0.78	0.90	0.74
Rapgef4		1.00	0.77	0.88	0.80	0.87	0.68	0.82	0.74	0.82	0.67
Slc5a12		1.00	0.74	0.92	0.73	1.06	0.70	0.98	0.71	1.05	0.71
Slc5a12		1.00	0.75	1.33	0.74	1.14	0.74	0.82	0.74	1.05	0.74
Slc25a12		1.00	0.72	1.00	0.72	1.27	0.80	1.00	0.82	1.19	0.75
Snx6		1.00	0.74	0.73	0.85	0.93	0.74	1.03	1.32	1.26	1.23
S100a14		1.00	0.79	0.82	0.75	0.87	0.75	0.77	0.78	0.75	0.72
1700012B18Rik		1.00	0.75	0.98	0.82	1.02	0.80	1.02	0.79	0.97	0.74
CKO		+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
KEAP1		Nrf2									

♀	Nrf2 -/-	Nrf2 +/+	Keap1 CCSP-CKO
	wk 12; 20; 33-35		12 wk
Nrf2 target genes:	↓	±	↑

Nrf2 itself
Nfe2l2

Typical Nrf2-target genes

Nqo1	: NAD(P)H: quinone oxidoreductase 1
Gpx2	: Glutathione peroxidase 2
Aldh3a1	: Aldehyde hydrogenase 3a1
Aox1	: Aldehyde oxidase 1
Car3	: Carbonic anhydrase 3
Ces1	: Carboxyesterase 1
Dsc2	: Desmocollin2
Pp11r	: Placental protein 11 related
Rapgef4	: Rap guanine nucleotide exchange factor
Tmem	: Transmembrane protein
Trex2	: Three prime repair exonuclease 2
Tnni1	: Troponin 1

… identifies new and confirms known Nrf2 target genes with some genes showing age-related adaptation

(K. Taguchi and M. Yamamoto, pers. comm.)

= ↑
= ↓

Gene	Age	12wk		20wk		33wk		33wk		35wk	
	Exposure	1d	1d	2m	2m	5m	5m	5m	5m	5m	5m
	Post exp.	-	-	-	-	-	-	1d	1d	13d	13d
Dsc1	18.8	1.00	0.60	0.67	0.57	0.78	0.58	0.59	0.59	0.58	0.59
Dsc2	3.8 - 4.2	1.00	0.64	0.69	0.64	0.85	0.61	0.71	0.73	0.64	0.64
Dsc2		1.00	0.68	0.81	0.59	0.81	0.79	0.76	0.66	0.67	0.68
Gsdm1	3.7	1.00	0.50	0.60	0.49	0.71	0.49	0.51	0.50	0.48	0.50
Krtdap	2.9 - 3.1	1.00	0.28	0.58	0.28	0.64	0.28	0.28	0.28	0.28	0.28
Krt5		1.00	0.54	0.65	0.51	0.79	0.56	0.64	0.55	0.50	0.53
Krt14	11.1	1.00	0.40	0.53	0.40	0.68	0.40	0.41	0.40	0.40	0.40
Krt14		1.00	0.48	0.52	0.47	0.67	0.48	0.48	0.48	0.47	0.48
Mt4	1.6 - 1.7	1.00	0.35	0.58	0.34	0.66	0.35	0.36	0.35	0.34	0.35
Lor		1.00	0.79	0.78	0.78	0.78	0.79	0.80	0.79	0.78	0.79
Lor	1.3	1.00	0.27	0.57	0.27	0.65	0.27	0.28	0.28	0.27	0.28
Lor		1.00	0.35	0.58	0.32	0.65	0.39	0.34	0.34	0.33	0.33
Nmu	1.2	1.00	0.63	0.63	0.64	0.74	0.63	0.64	0.64	0.63	0.64
Otop3	2.5	1.00	0.60	0.60	0.60	0.77	0.60	0.61	0.61	0.60	0.60
Serpinb11	3.2	1.00	0.37	0.55	0.36	0.67	0.37	0.37	0.37	0.36	0.37
Serpinb12	2.7 - 4.4	1.00	0.36	0.59	0.35	0.65	0.35	0.36	0.36	0.35	0.36
Serpinb2	3.2	1.00	0.41	0.46	0.41	0.65	0.41	0.42	0.54	0.46	0.79
Serpinb3a		1.00	0.51	0.65	0.50	0.73	0.50	0.51	0.52	0.50	0.51
Serpinb3a	3.5 - 4.1	1.00	0.35	0.56	0.35	0.63	0.35	0.36	0.35	0.35	0.35
Serpinb3a		1.00	0.35	0.56	0.34	0.63	0.35	0.36	0.35	0.34	0.35
Serpinb5	3.4	1.00	0.54	0.54	0.54	0.70	0.54	0.55	0.54	0.54	0.54
Serpinb5		1.00	0.50	0.61	0.50	0.69	0.50	0.51	0.50	0.50	0.50
Serpinb7	1.2	1.00	0.50	0.51	0.50	0.72	0.50	0.51	0.50	0.50	0.50
Spink5	4.0	1.00	0.45	0.47	0.43	0.65	0.43	0.44	0.43	0.42	0.43
Sprr1a	15.8	1.00	0.44	0.66	0.46	0.64	0.44	0.45	0.45	0.43	0.44
Sprr1b	15.8	1.00	0.48	0.60	0.47	0.59	0.47	0.49	0.48	0.47	0.48
Sprr2a	1.0 / 8.1	1.00	0.28	0.50	0.28	0.61	0.28	0.28	0.28	0.28	0.28
Sprr3	4.0	1.00	0.28	0.57	0.27	0.62	0.29	0.28	0.28	0.28	0.28
Sprrl1	2.3 - 2.4	1.00	0.45	0.65	0.44	0.71	0.45	0.45	0.45	0.44	0.44
Sprrl2	1.5	1.00	0.34	0.56	0.34	0.65	0.34	0.35	0.34	0.34	0.34
Sprrl3	2.4	1.00	0.46	0.62	0.45	0.68	0.45	0.47	0.47	0.45	0.46
Sprrl3		1.00	0.45	0.60	0.44	0.65	0.44	0.45	0.45	0.43	0.44
Sprrl5	1.6 - 1.7	1.00	0.42	0.61	0.41	0.70	0.42	0.43	0.42	0.41	0.42
Sprrl7	1.8	1.00	0.46	0.63	0.43	0.74	0.45	0.46	0.45	0.43	0.44
Sprrl9	3.2	1.00	0.48	0.63	0.47	0.70	0.54	0.49	0.48	0.48	0.53
Tgm3	2.3	1.00	0.46	0.53	0.45	0.77	0.46	0.48	0.46	0.46	0.47
Tgm3		1.00	0.38	0.53	0.38	0.73	0.38	0.39	0.38	0.38	0.38
	CKO	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
	KEAP1	Nrf2									

♀	Nrf2 -/-	Nrf2 +/+	Keap1 CCSP-CKO
	wk 12; 20; 33-35		12 wk
Nrf2 target genes:	↓	±	↑

Dsc1/2	: Desmocollin 1/2
Dsg	: Desmoglein
Gsdm1	: Gasdermin 1 (skin and digestive tract epithelial-specific)
Krtdap	: Keratinocyte differentiation associated protein
Krt	: Keratin
Lor	: Loricrin
Nmu	: Neuromedin U
Sprr	: Small proline-rich protein
Sprrl	: Small proline rich-like protein
Tgm3	: Transglutaminase
Mt4	: Metallothionein 4
Serpinb	: Serine (or Cysteine) peptidase inhibitor, clade B
Spink5	: Serine peptidase inhibitor, kazal type 5
Them5	: Thioesterase superfamily member 5

Gene	Age	13wk		20wk		33wk		33wk		35wk	
	Exposure	1d	1d	2m	2m	5m	5m	5m	5m	5m	5m
	Post exp.	-	-	-	-	-	-	1d	1d	13d	13d
Dsg1a	1.2	1.00	0.54	0.60	0.52	0.72	0.53	0.54	0.54	0.52	0.53
Dsg1b	0.8 / 1.7 – 3.9	1.00	0.63	0.62	0.59	0.72	0.60	0.62	0.61	0.59	0.62
Dsg3	1.1 / 3.4	1.00	0.63	0.67	0.62	0.78	0.66	0.61	0.63	0.59	0.62
Krt13		1.00	0.35	0.40	0.34	0.67	0.35	0.35	0.35	0.35	0.35
Krt13	0.9 / 1.7	1.00	0.28	0.57	0.28	0.63	0.28	0.28	0.28	0.28	0.28
Krt13		1.00	0.36	0.50	0.36	0.62	0.36	0.37	0.36	0.36	0.36
	CKO	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
	KEAP1					Nrf2					

Conclusion (II)

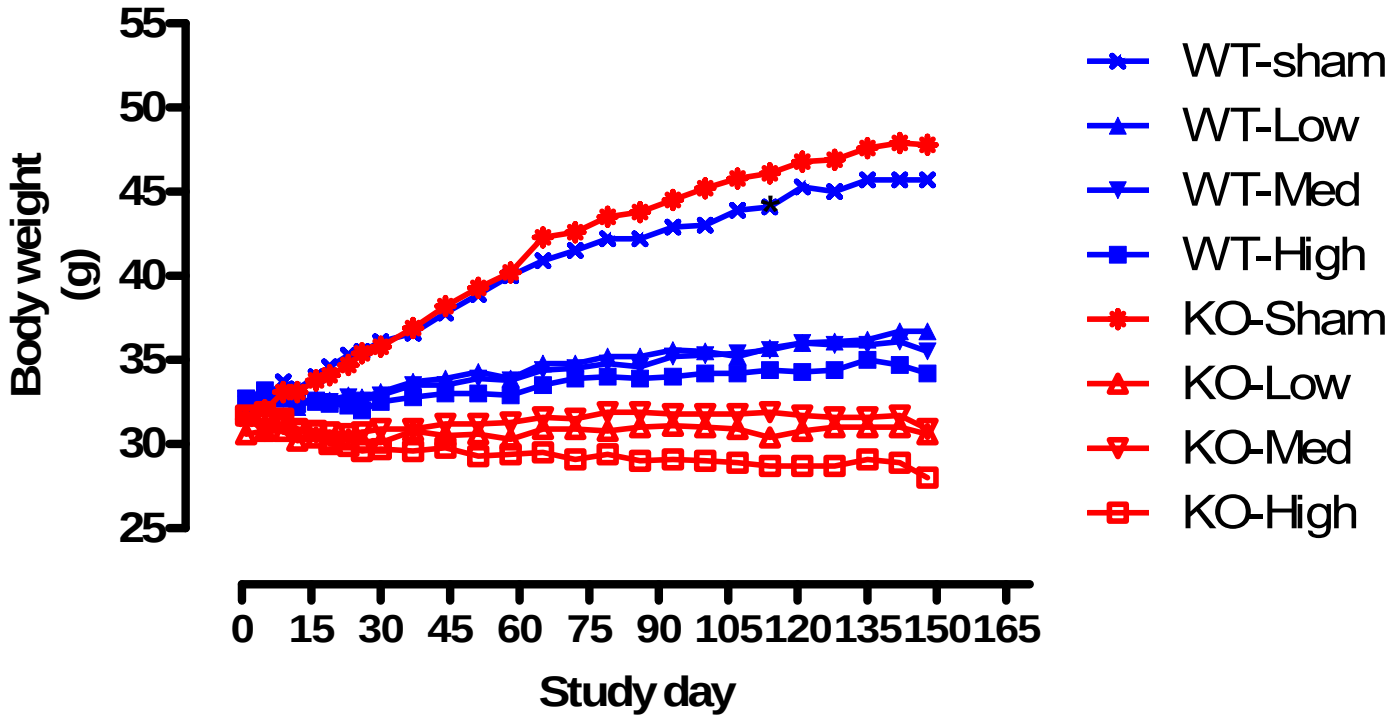
$Nrf2^{-/-}$ mice confirm the central role of Nrf2 in the cell's strategy to combat CS-induced damage and disclose new Nrf2 functions

Genome-wide effects on the *transcriptome* in lungs of $Nrf2^{+/+}$ *vs*. $Nrf2^{-/-}$ mice in the context of CS-exposure and age:

- CS-exposed $Nrf2^{-/-}$ mice are compromised in the expression of a distinct spectrum of antioxidant and Phase II-related genes
- CS-exposed $Nrf2^{-/-}$ mice compensate somewhat for the lack of Nrf2 during chronic exposure (other transcription factors)
- The acute (single exposure) response to CS inhalation is controlled exclusively by Nrf2 (as concluded from the profile seen in Keap1 CKO mice)
- Genotype-dependent dose effects widely determine CS-dependent gene expression in a complex manner
- Aging $Nrf2^{+/+}$ mice show age-related adaptation to various Nrf2 target genes

The CS-induced *phenotype* in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice: Body weight development

- CS exposure generally reduces body weight development; the effect is most pronounced in in CS-exposed Nrf2$^{-/-}$ mice

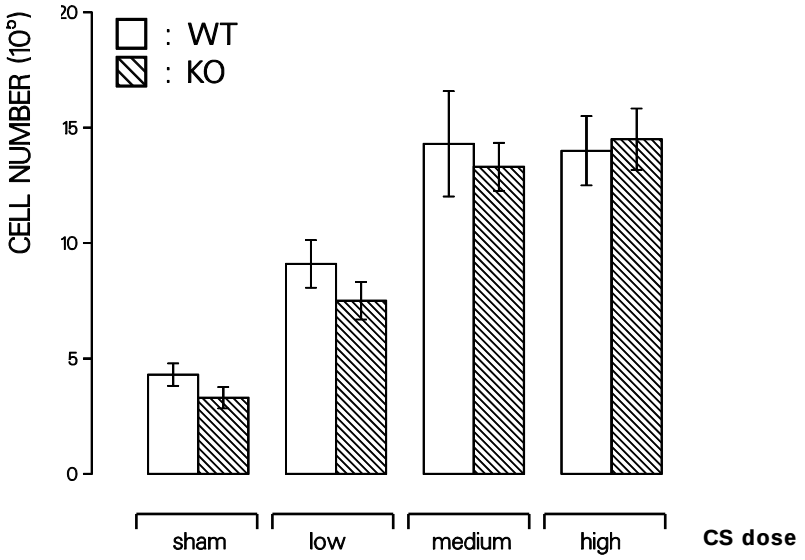


The CS-induced *phenotype* in $Nrf2^{-/-}$ *vs.* $Nrf2^{+/+}$ mice: Pathology (Summary)

- $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice develop lung inflammation as well as alveolar emphysema after 5 months of exposure to CS in a concentration-dependent manner
- Scores for '*mean cord length*', an indicator of emphysema, are significantly elevated in CS-exposed (medium and high) $Nrf2^{-/-}$ mice *vs*. sham
- Scores for '*general lung inflammation*', '*macrophage activation*', '*destructive index of lung tissue*', and '*bronchiolar attachments*' were not found to be significantly different between CS-exposed $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice

The CS-induced *phenotype* in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice: Inflammation/BAL fluid (Summary)

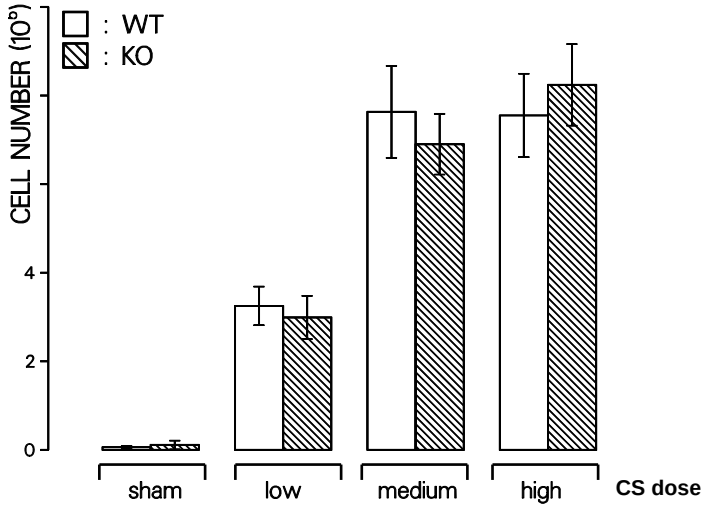
- CS exposure results in a strong increase in free lung cells (FLC) in BAL fluid, which is generally not significantly different between Nrf2$^{+/+}$ and Nrf2$^{-/-}$ mice



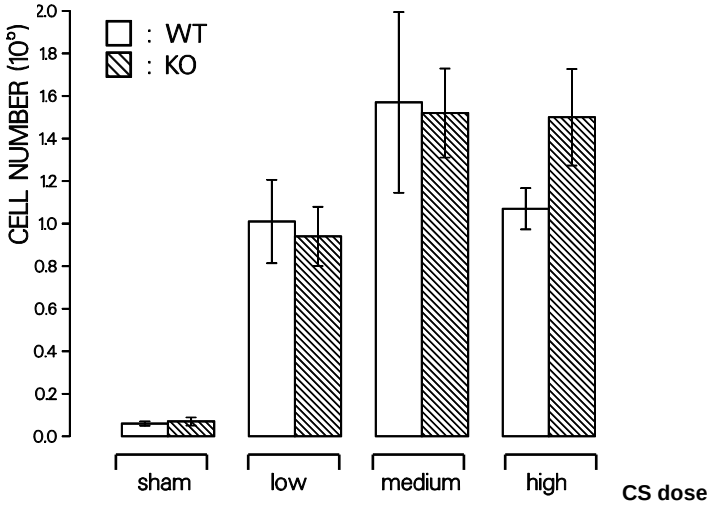
Number of FLC (overall)

The CS-induced *phenotype* in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice: Inflammation/BAL fluid (Summary)

- CS exposure results in a strong increase in free lung cells (FLC) in BAL fluid, which is generally not significantly different between Nrf2$^{+/+}$ and Nrf2$^{-/-}$ mice
- Discrimination of FLC for neutrophils and lymphocytes (CD4, CD8, and B cells) reveals a trend for increased numbers of lymphocytes in CS-exposed Nrf2$^{-/-}$ mice



Neutrophils



Lymphocytes

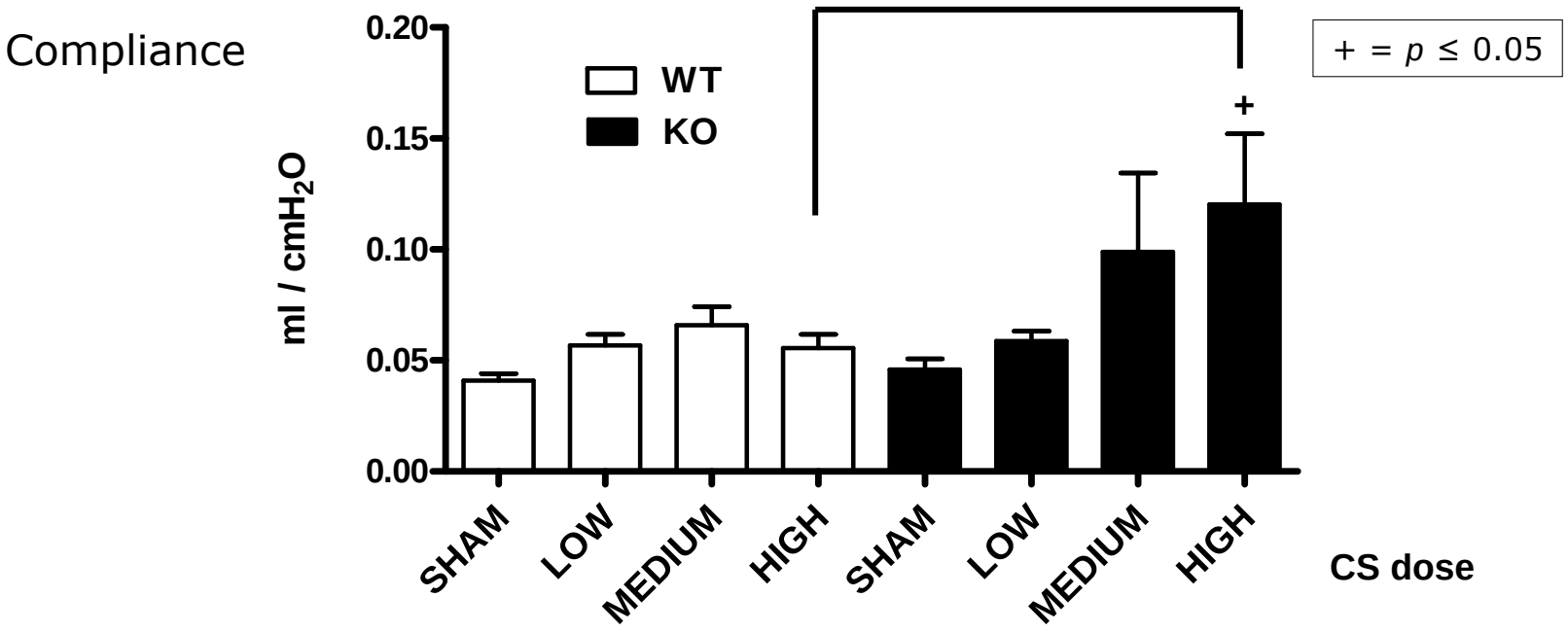
The CS-induced *phenotype* in $Nrf2^{-/-}$ *vs.* $Nrf2^{+/+}$ mice: Inflammation/BAL fluid (Summary)

- CS exposure results in a strong increase in free lung cells (FLC) in BAL fluid, which is generally not significantly different between $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice
- Discrimination of FLC for neutrophils and lymphocytes (CD4, CD8, and B cells) reveals a trend for increased numbers of lymphocytes in CS-exposed $Nrf2^{-/-}$ mice
- Alveolar macrophages respond to CS exposure with an increased expression of activation markers, i.e., CD11b, CD11c, CD86, CD14, and MHCII, which is not significantly different between $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice
- CS exposure generally results in a strong increase in expression of chemokine and cytokine markers with $Nrf2^{-/-}$ mice showing a trend for an enhanced response in some markers (e.g., CD40,GMCSF, TIMP1, TNFα, VCAM-1, VEGF) and a slightly lower response in others (e.g., IL-1α, IL-1β, Osteopontin)

The CS-induced *phenotype* in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice: Functional respiratory changes (Summary)

'Forced pulmonary maneuvers' revealed:

- Concentration-dependent, statistically significantly compromised 'compliance' (at zero pressure) in Nrf2$^{-/-}$ mice *vs.* Nrf2$^{+/+}$ mice

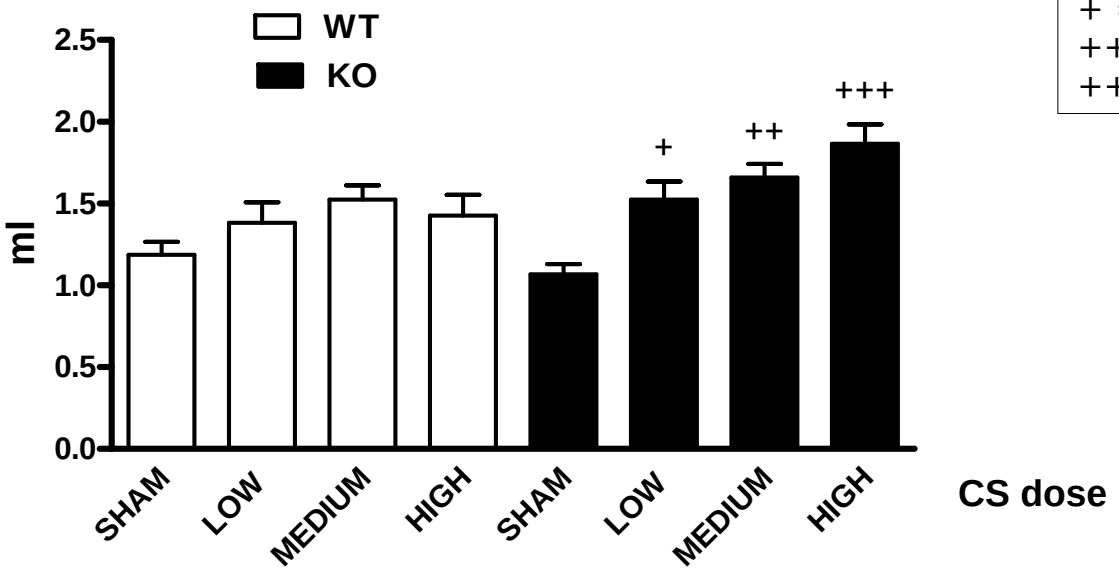


The CS-induced *phenotype* in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice: Functional respiratory changes (Summary)

'Forced pulmonary maneuvers' revealed:

- CS concentration-dependent, statistically significantly compromised 'compliance' (at zero pressure) in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice
- CS concentration-dependent, statistically significantly increased 'total lung capacity' in Nrf2$^{-/-}$ mice

Total lung capacity



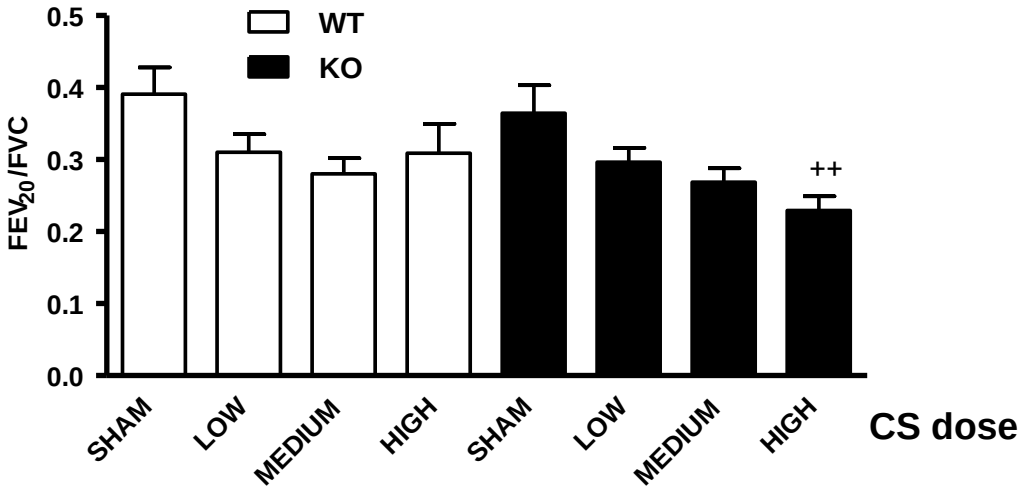
+ = $p \leq 0.05$
++ = $p \leq 0.01$
+++ = $p \leq 0.001$

The CS-induced *phenotype* in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice: Functional respiratory changes (Summary)

'Forced pulmonary maneuvers' revealed:

- CS concentration-dependent, statistically significantly compromised 'compliance' (at zero pressure) in Nrf2$^{-/-}$ *vs.* Nrf2$^{+/+}$ mice
- CS concentration-dependent, statistically significantly increased 'total lung capacity' in Nrf2$^{-/-}$ mice
- CS concentration-dependent, statistically significantly decreased 'FEV_{20}/FVC' in Nrf2$^{-/-}$ mice

FEV_{20}/FVC



++ = $p \leq 0.01$

Conclusion (III)
Compared to CS-exposed $Nrf2^{+/+}$ mice, $Nrf2^{-/-}$ mice show a slightly enhanced pathological phenotype

On the phenotype level:

- Body weight gain in CS-exposed $Nrf2^{-/-}$ mice is significantly attenuated
- Only marginal differences are observed for histopathological parameters (except 'median cord length') between the two genotypes
- Analysis of BAL fluid (free lung cells, chemokines, and cytokines) revealed no significant differences between CS-exposed $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice
- Lung function parameters ('compliance', 'total lung capacity', 'FEV_{20}/FVC) are rather more compromised by CS exposure in $Nrf2^{-/-}$ than in $Nrf2^{+/+}$ mice

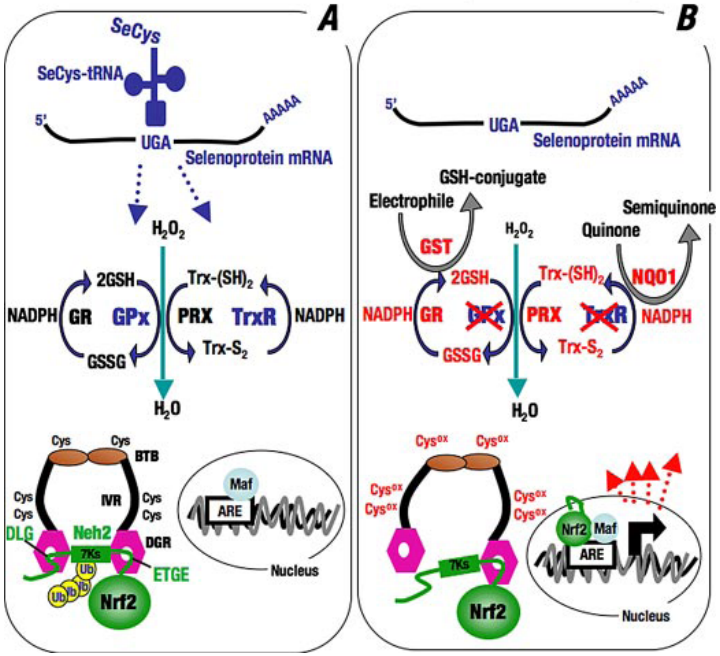
Final remarks

The Nrf2 pathway has been proven a major target of CS exposure *in vitro* and *in vivo*!

- However, in comparison to literature data (*i.e.*, Rangasamy *et al*. 2004), the current study has revealed a less pronounced emphysematous phenotype in the context of CS exposure, *e.g.*, regarding oxidative stress, morphometric, and inflammatory parameters. This may be explained by the fact that the Rangasamy *et al*. study used:
  - a CS mainstream/sidestream mix, which is far more irritating than mainstream smoke only
  - a longer exposure period

Final remarks

- Alternatively, it may be explained by the emerging role of Nrf2 as a second line defense tool acting behind / on top of the GSH/GR – Trx/TrxR barrier analogous to the concept suggested by Suzuki *et al*. (2008):



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