Epigenetics and Air Pollution?

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Epigenetics and Cumulative Risk Workshop

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Summary of studies linking air pollution to epigenetic changes

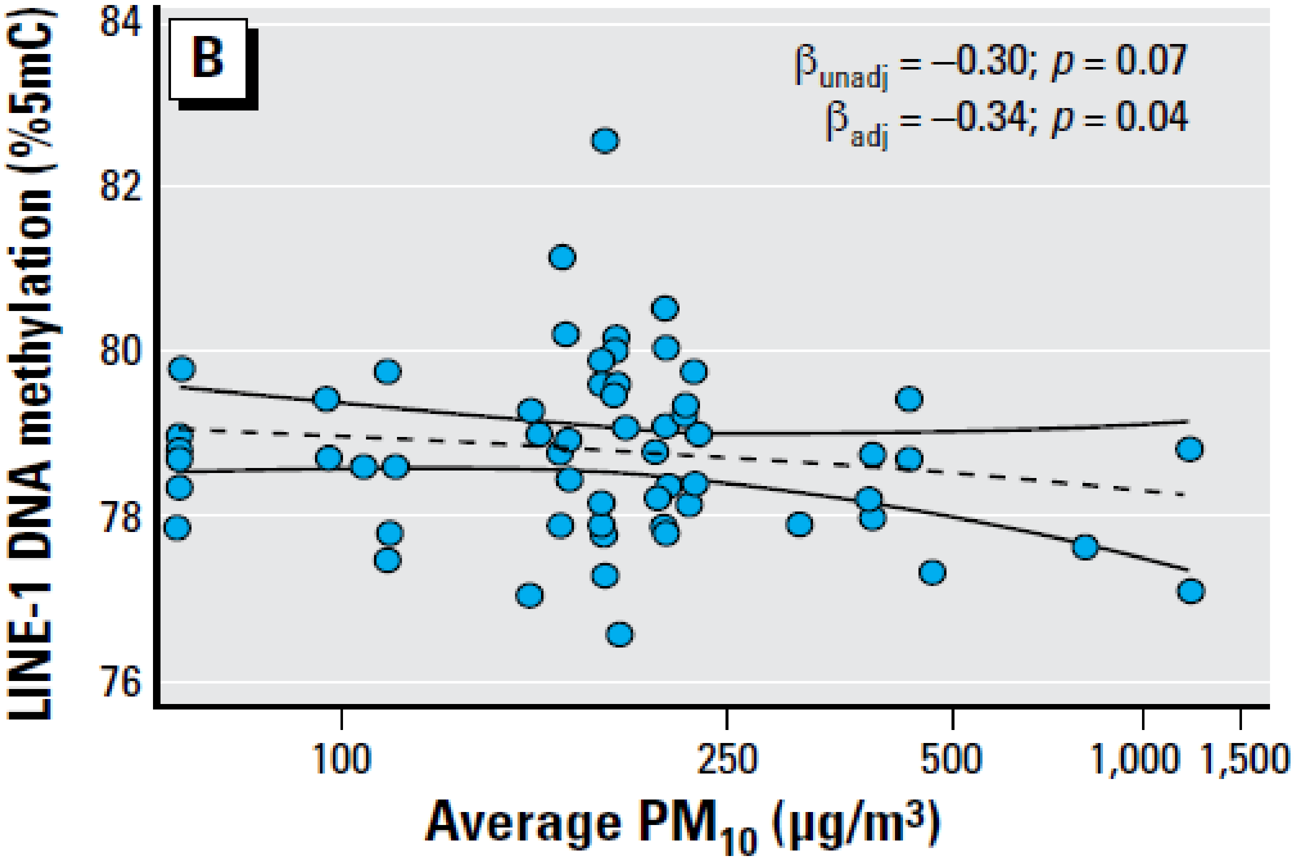
Human Population

$PM_{2.5}$	Hypomethylation of LINE1 linked to exposure of PM	Baccarelli, 2009[52]
PM	microRNA expression changes following occupational exposure	Bollatti, 2010[43]
PM_{10}	Decrease in iNOS promoter methylation following exposure	Tarantini, 2009[53]
ETS	In utero exposure associated with differences in methylation patterns;	Breton, 2009[54]
	Genetic variants in detox genes enhanced association	
Ambient	Foxp3 hypermethylation in asthmatic children	Nadeau 2010
Black C	miRNA processing genes	Wilker 2010
$NO2/PM_{2.5}$	Methylation in iNOS promoter in Childrens Study	Salam 2012
$PM_{2.5}$	Methylation in asthma pathway	Sofer 2013
$PM_{2.5}$	Methylation and blood pressure in controlled exposure	Bellavia 2013
Ozone	Ozone altered microRNAs in the sputum of human subjects	Fry 2014
Traffic	Methylation in normative aging study	Lepeule 2014
$PM_{2.5}$	miRNA in normative aging study	Fossati 2014

Human Cells

DEP	DEP induced up-regulation of Cox2 is due to chromatin modifications	Cao, 2007
DEP	Exposure of ALI grown cells led to changes in miRNA expression	Jardim, 2009
DEP	MicroRNA-375 regulation by diesel exhaust particles in epithelial cells	Bleck 2013

Line 1 association with exposure to PM in workers in an electric furnace steel plant



Tarantini et al., EHP 2009

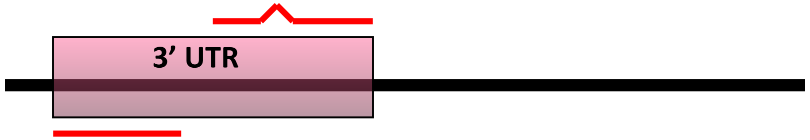
Placental mitochondrial methylation and exposure to airborne particulate matter

	First trimester		Second trimester		Third trimester		Entire pregnancy	
Variable	**β**	**(95% CI)**	**β**	**(95% CI)**	**β**	**(95% CI)**	**β**	**(95% CI)**
mtDNA methylation								
MT-RNR1, %	1.27	(0.23 to 2.32)	0.19	(−0.80 to 1.16)	1.04	(−0.20 to 1.86)	0.91	(0.56 to 4.18)
D-loop, %	0.44	(0.12 to 0.75)	0.09	(−0.22 to 0.39)	0.04	(−0.29 to 0.36)	0.21	(−0.003 to 1.02)
Combined, %	0.75	(0.16 to 1.34)	0.10	(−0.47 to 0.65)	0.46	(−0.23 to 0.96)	0.47	(0.20 to 2.23)
mtDNA content, %	−7.57	(−20.78 to 7.86)	−15.19	(−28.34 to 0.38)	−23.58	(−36.27 to −8.37)	−15.60	(−23.92 to −6.38)

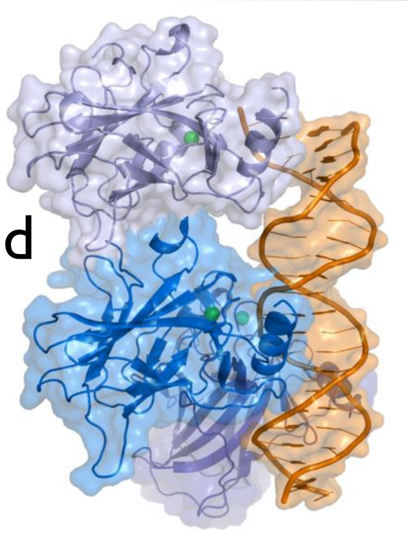
Janssen et al., Epigenetics 2015

microRNAs

- Growing class of small, noncoding RNAs
  - 19-25 nt long
- Regulators of several cellular processes including, differentiation, apoptosis, and growth
- Generally negatively regulate mRNA targets



- One miR, several genes (pathways); one gene, several miRs
- Attenuate cellular signals
  - Regulate methylation of target genes
  - Positive/negative feedback loops
  - Communication between cells

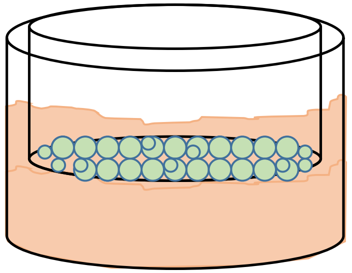


What does miRNA expression tell us about human bronchial epithelial cells response to diesel particles?

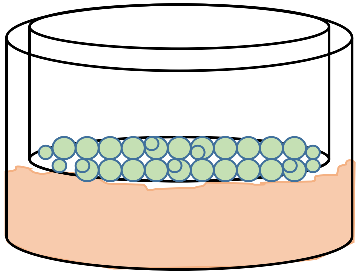
Cell Collection

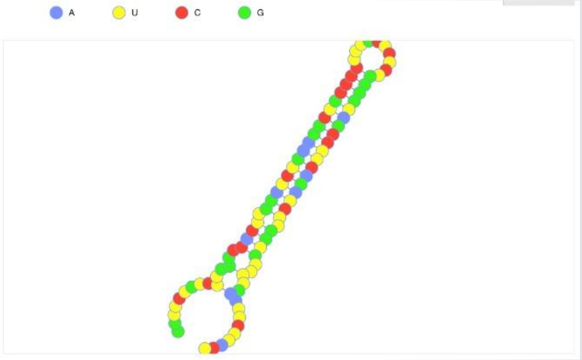
www.olympus-me.com

Plating



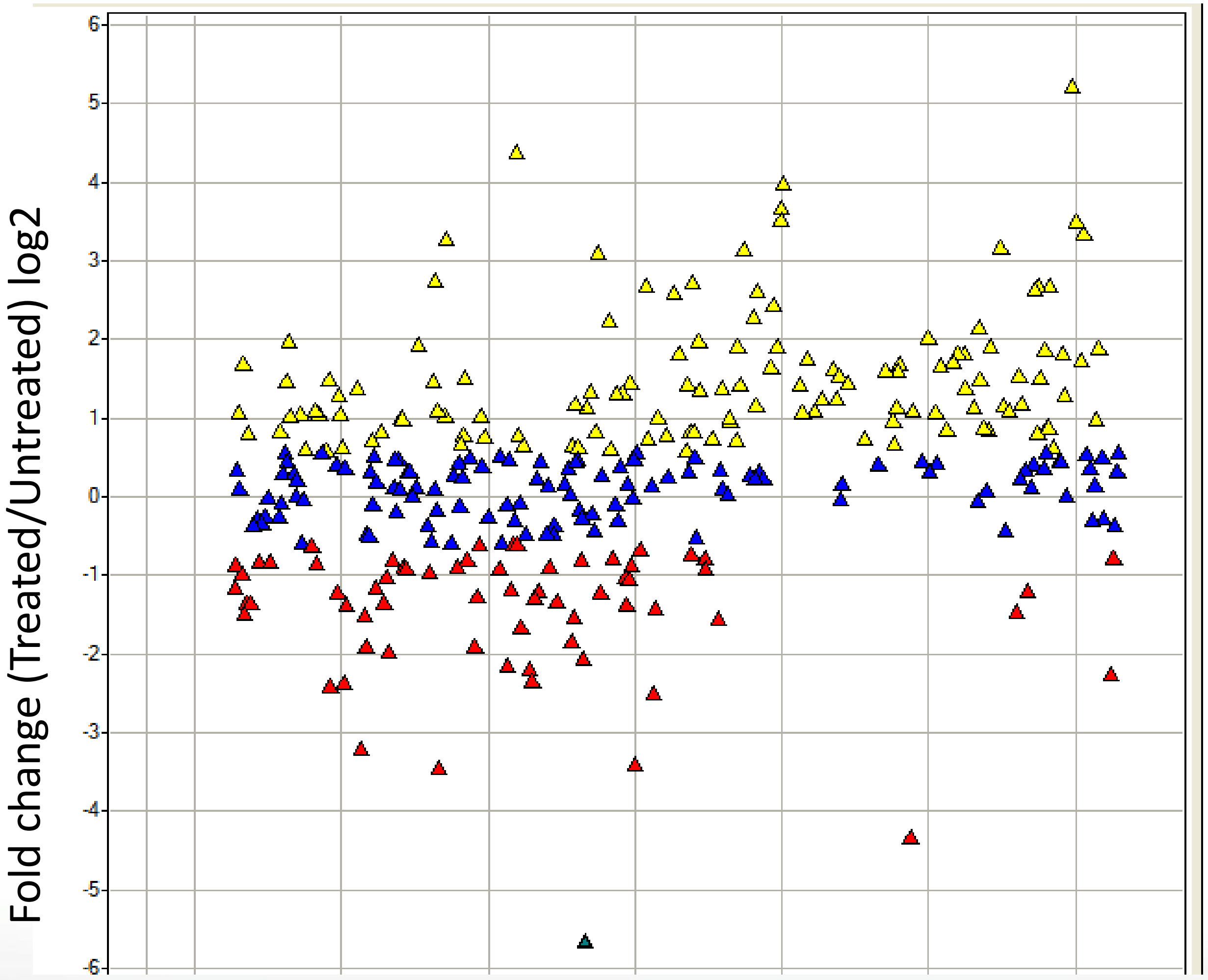
Air-liquid interface and differentiation



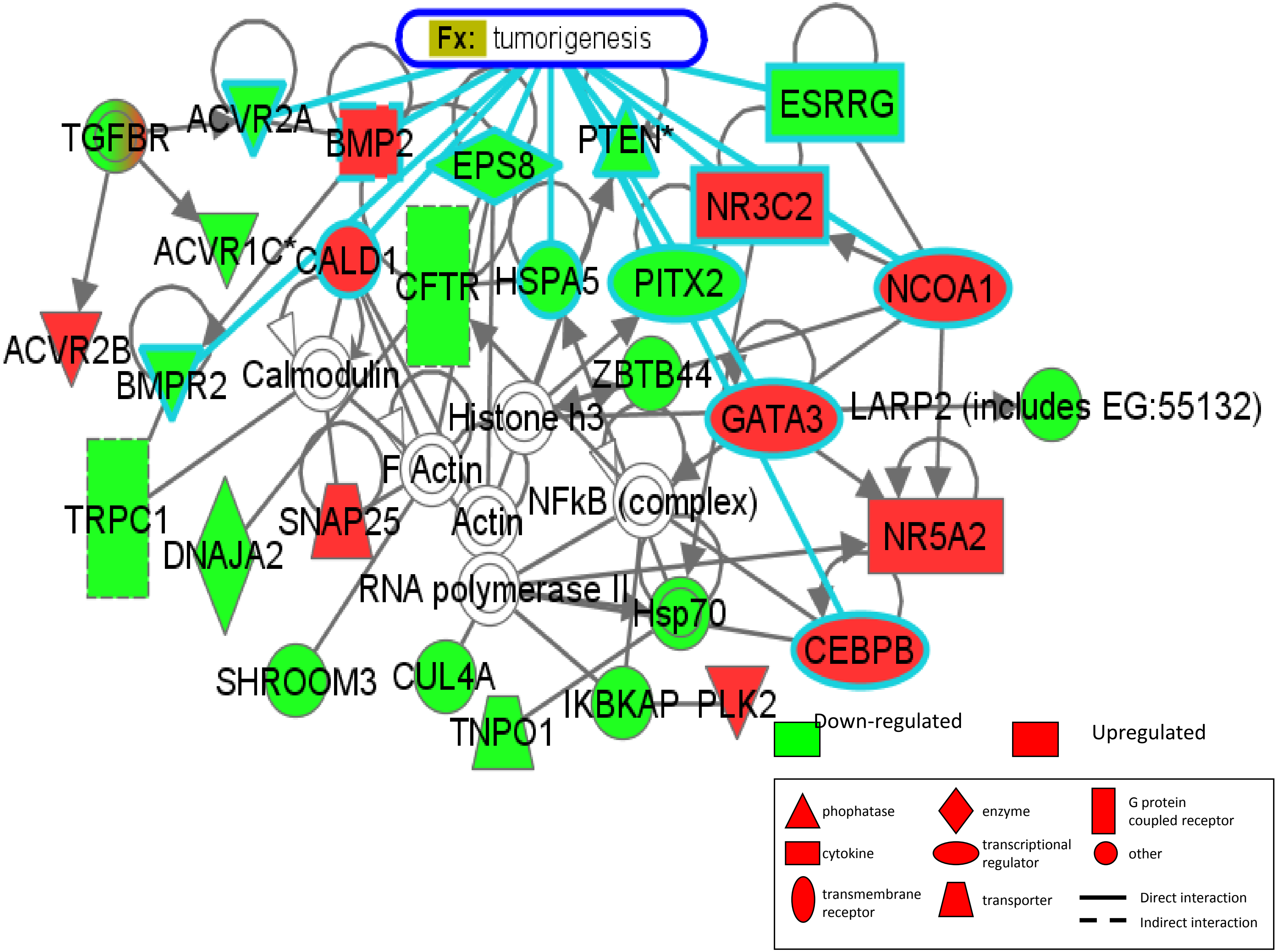


- Cells treated with 10 µg/cm^2 DEP for 24hr
- Isolated RNA for miRNA array (Agilent)
- Confirmation of with qRT-PCR
- miRBase and miRDB to search putative targets of most changed miRNAs
- Ingenuity for further pathway analysis on putative targets

Diesel Exhaust Particles alter microRNA regulation in bronchial epithelial cells



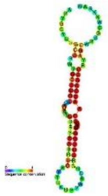
Jardim et al., Environ Health Perspect 117:1745–1751 (2009)

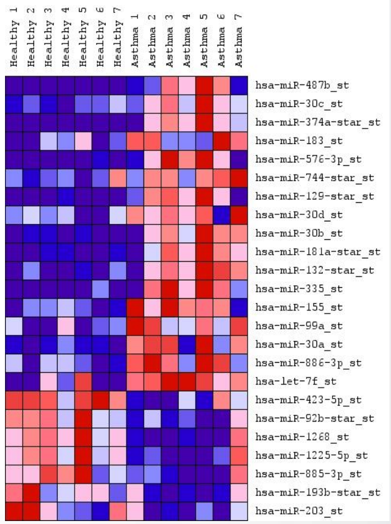


Fx: tumorigenesis
TGFBR
ACVR2A
BMP2
EPS8
PTEN*
ESRRG
NR3C2
ACVR1C*
CALD1
CFTR
HSPA5
PITX2
NCOA1
ACVR2B
BMPR2
Calmodulin
ZBTB44
Histone h3
GATA3
LARP2 (includes EG:55132)
F Actin
TRPC1
SNAP25
Actin
NFkB (complex)
NR5A2
DNAJA2
RNA polymerase II
Hsp70
CEBPB
SHROOM3
CUL4A
IKBKAP
PLK2
TNPO1
Down-regulated
Upregulated
phophatase
enzyme
G protein coupled receptor
cytokine
transcriptional regulator
other
transmembrane receptor
transporter
Direct interaction
Indirect interaction

Comparison of miRNA Profiles in Asthmatic and Healthy Donors: Ozone Exposure

- Bronchial epithelial cells from 16 mild asthmatic and 16 healthy donors were grown on transwells at air-liquid interface
- Exposed cells to 0.4ppm ozone for 4 hours
- miRNA – RNAseq or arrays
- Pathway analysis

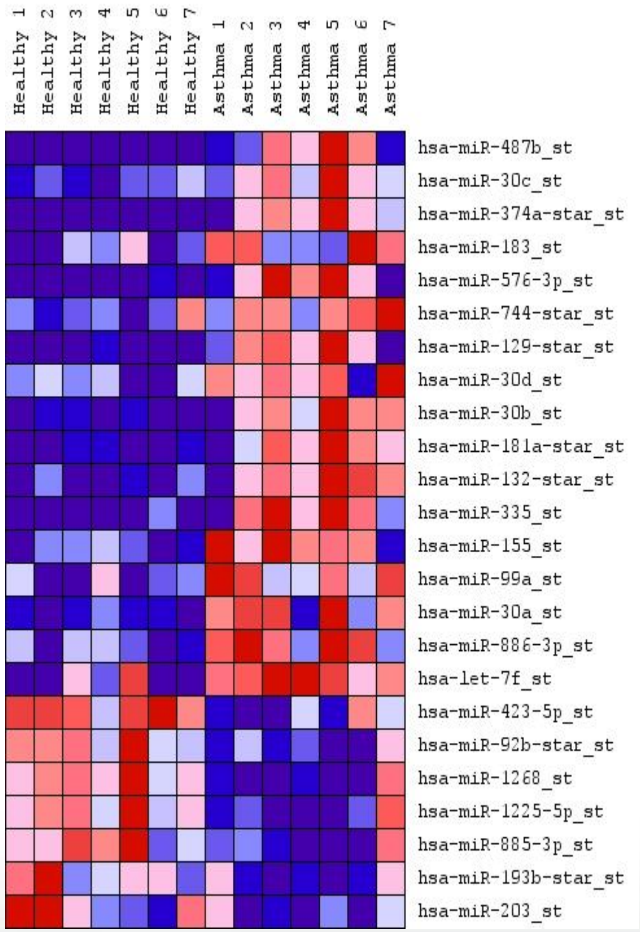




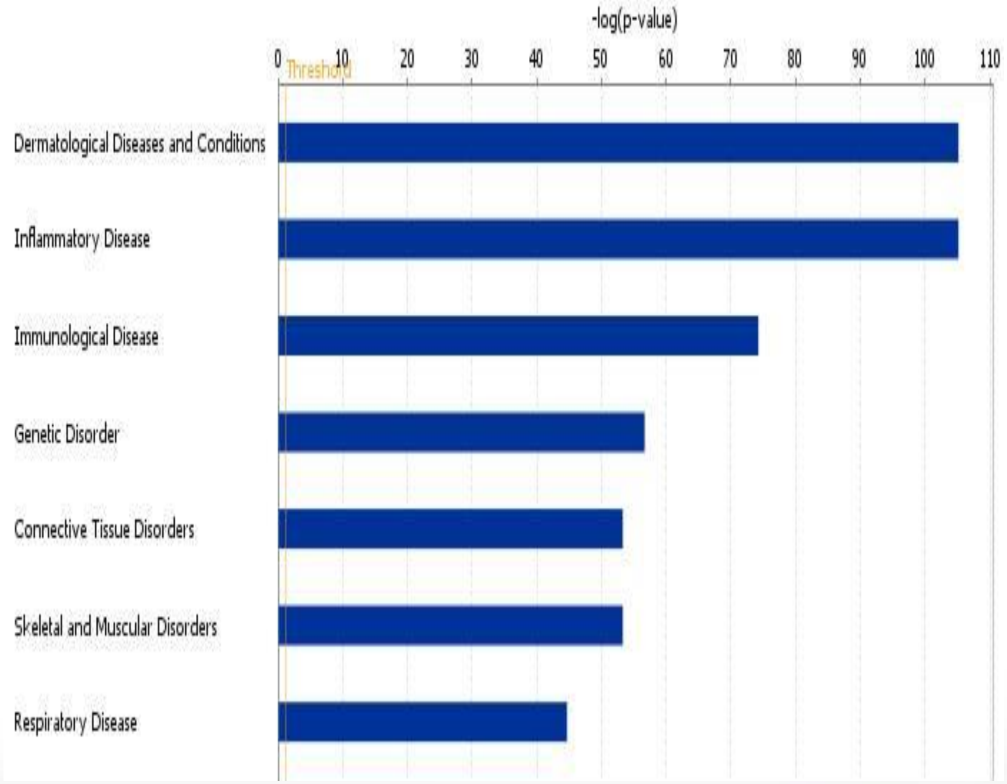
Jardim Am J Respir Cell Mol Biol. 2012

Differential miRNA Expression between Healthy and Asthmatic Donors

A

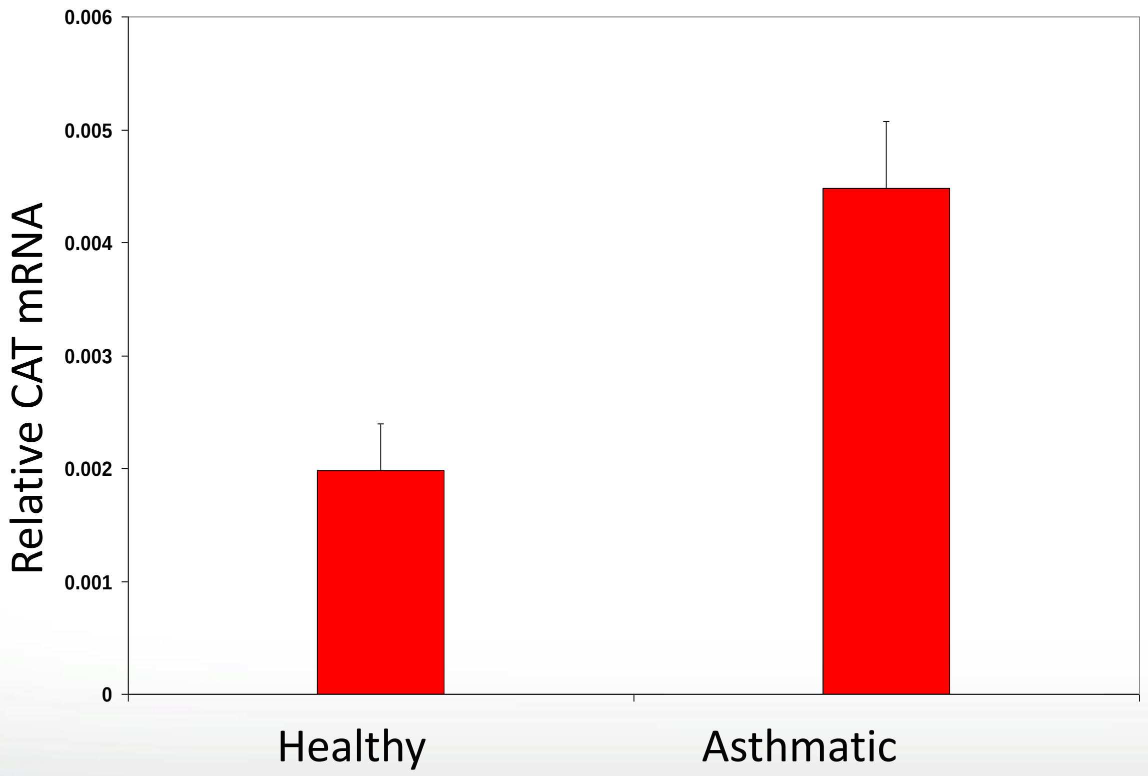


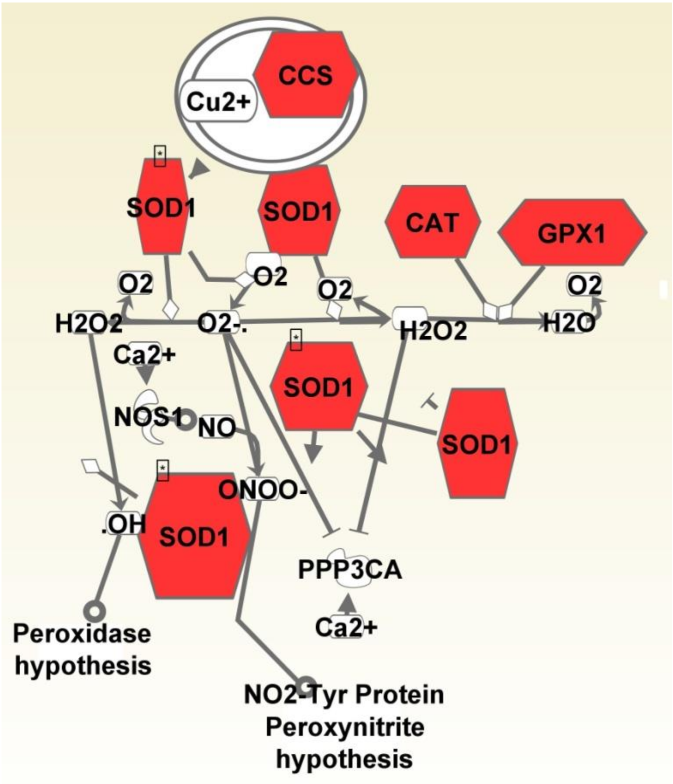
B



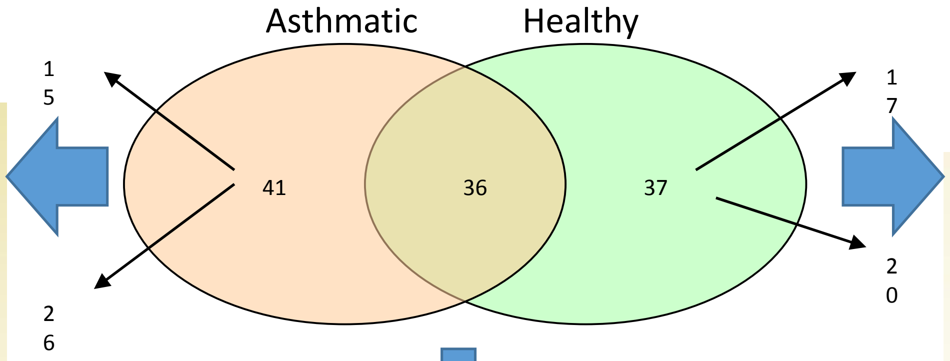
Asthmatics have higher cell stress at baseline

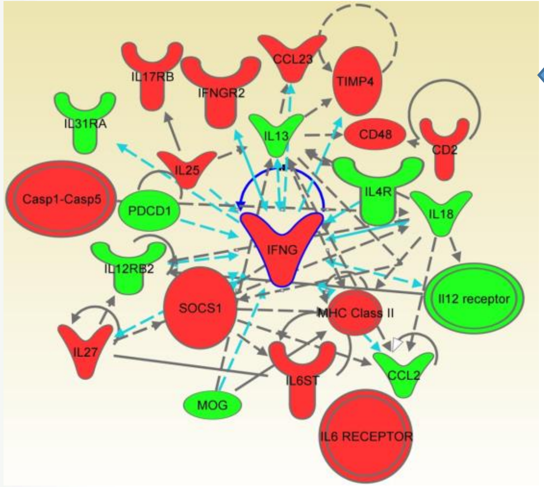
Molecular network analysis suggested that asthmatics have higher baseline levels of cell stress



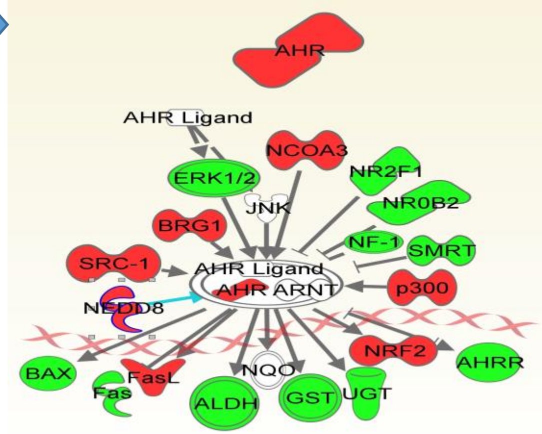


miRNA expression after Ozone exposure predicts Differential Response In Healthy and Asthmatics

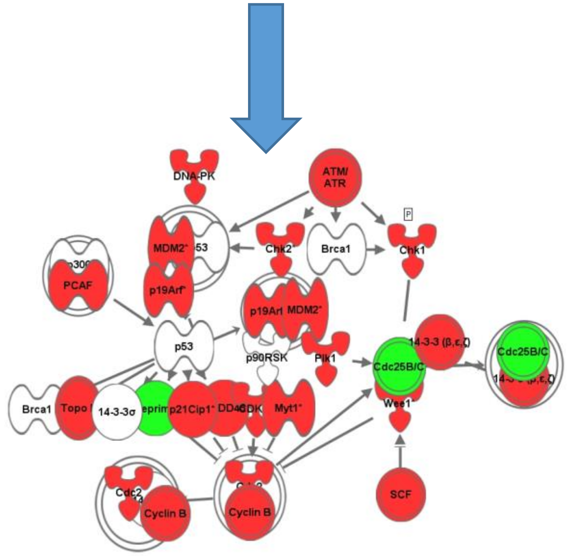




Asthmatic response enriched for immune modulation and inflammation



Healthy response enriched for stress responses



Common miRNA response may regulate DNA damage

EPA Inhalation Facility

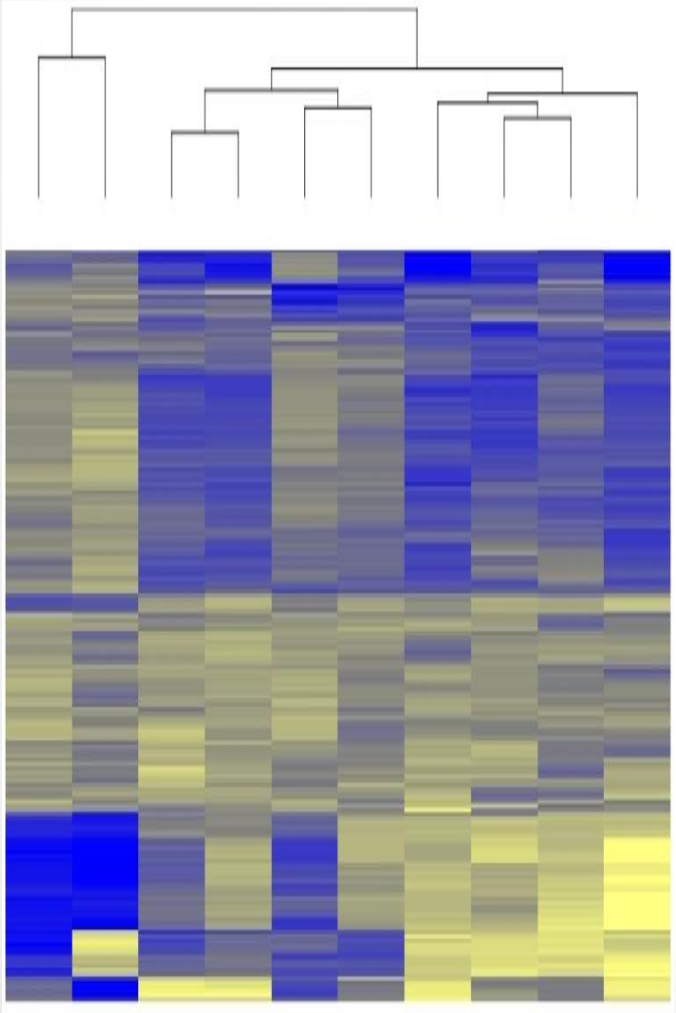
LAMARCK Clinical Study

- 19 Healthy Subjects
- 300 µg/m^3 Diesel /FA/Ozone (0.3ppm)
- 2hr
- Intermittent exercise
- Bronch next day
- Epithelial cells used for mRNA, miRNA, methylation profiles

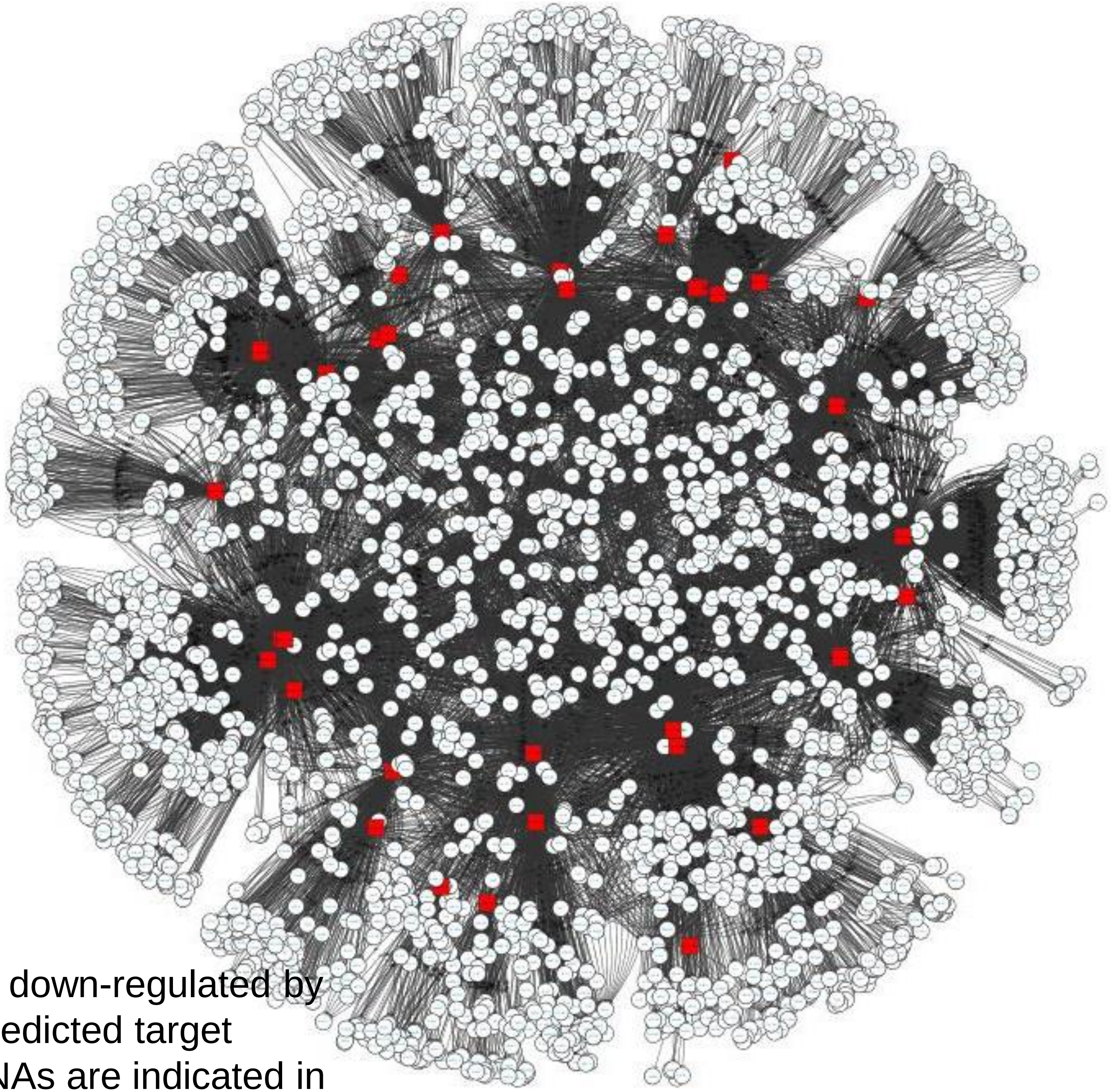
LAMARCK - miRNA

	Ozone
No. of Subjects (M/F)	10 (10/0)
Age	25.90±1.33
Race (C/H/AA)	9/0/1
Height (cm)	182.40±1.53
Weight (kg)	85.01±3.43
BSA (m^2)	2.06±0.05
BMI (kg/m^2)	25.49±0.81
baseline FEV1 (liters)	4.70±0.18
baseline FVC (liters)	5.83±0.18
baseline FEV1/FVC	81.05±1.71

Unsupervised hierarchical clustering of subjects' ranked fold-change after ozone challenge



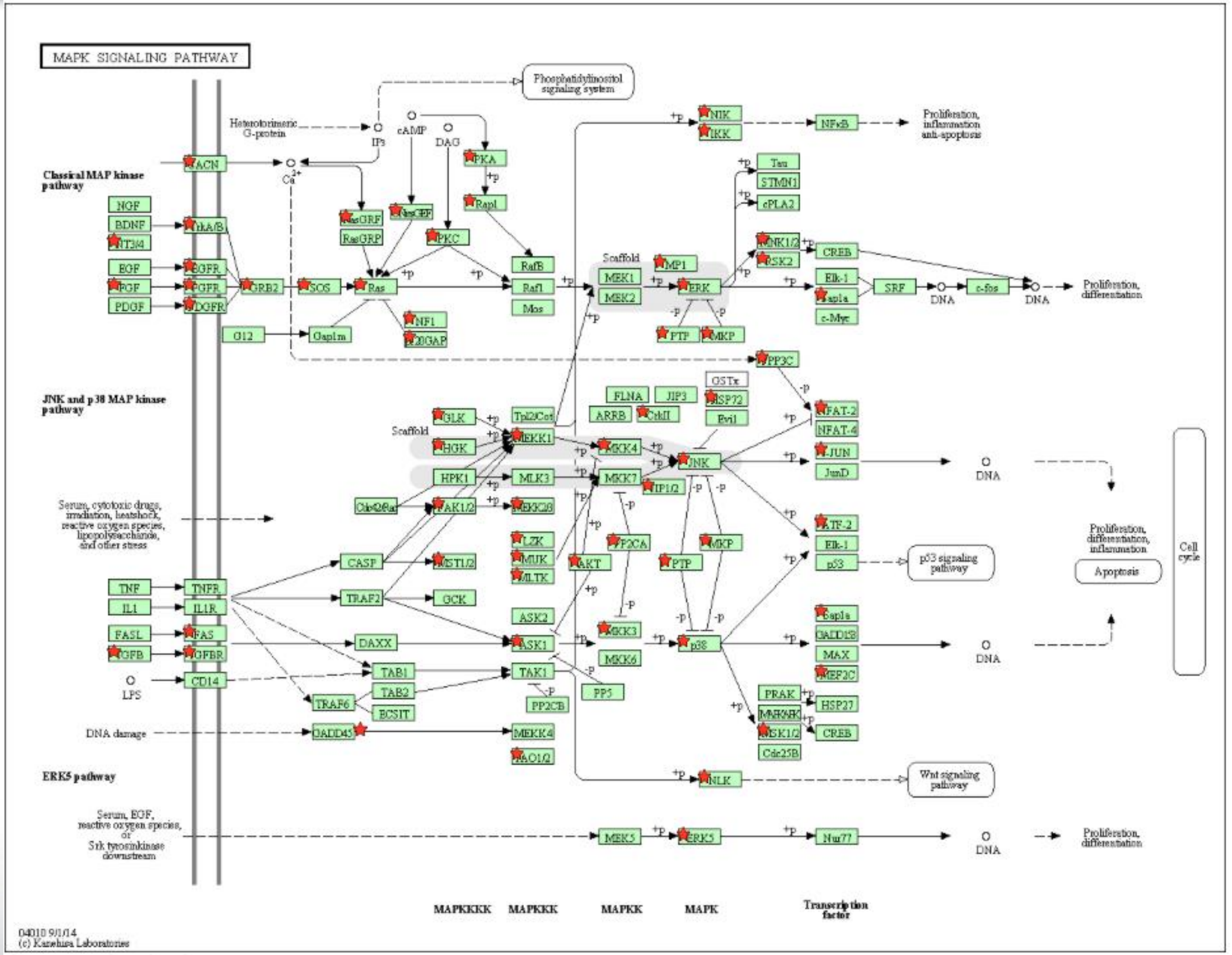
Down after O_3	Up after O_3
hsa-miR-451a	hsa-miR-638
hsa-miR-449a	hsa-miR-1202
hsa-miR-449b-5p	hsa-miR-486-5p
hsa-let-7e-5p	hsa-miR-494-3p
hsa-let-7a-5p	hsa-miR-144-3p

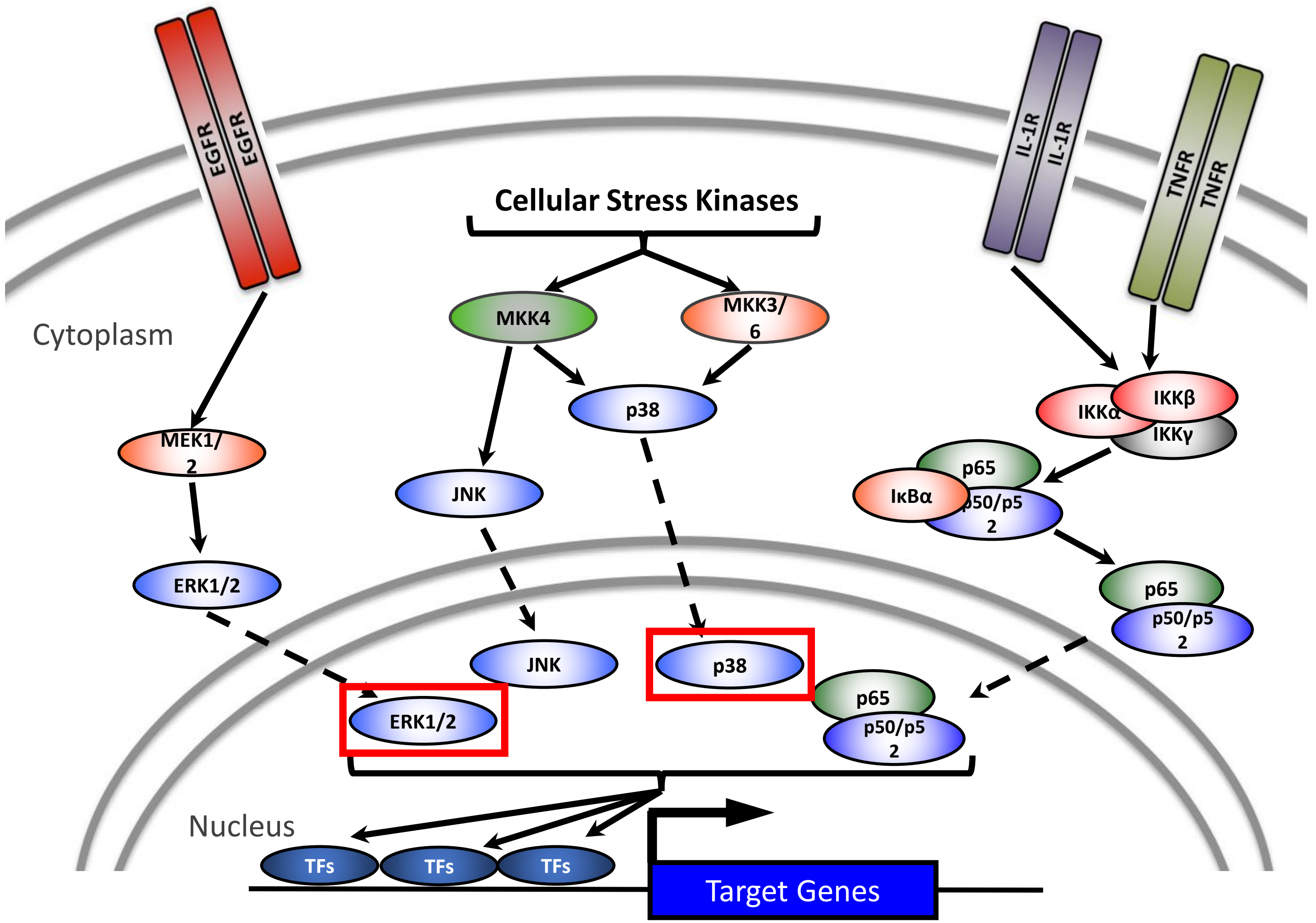


Network of miRNAs that are down-regulated by ozone exposure and their predicted target mRNA using mirDBv5. miRNAs are indicated in red

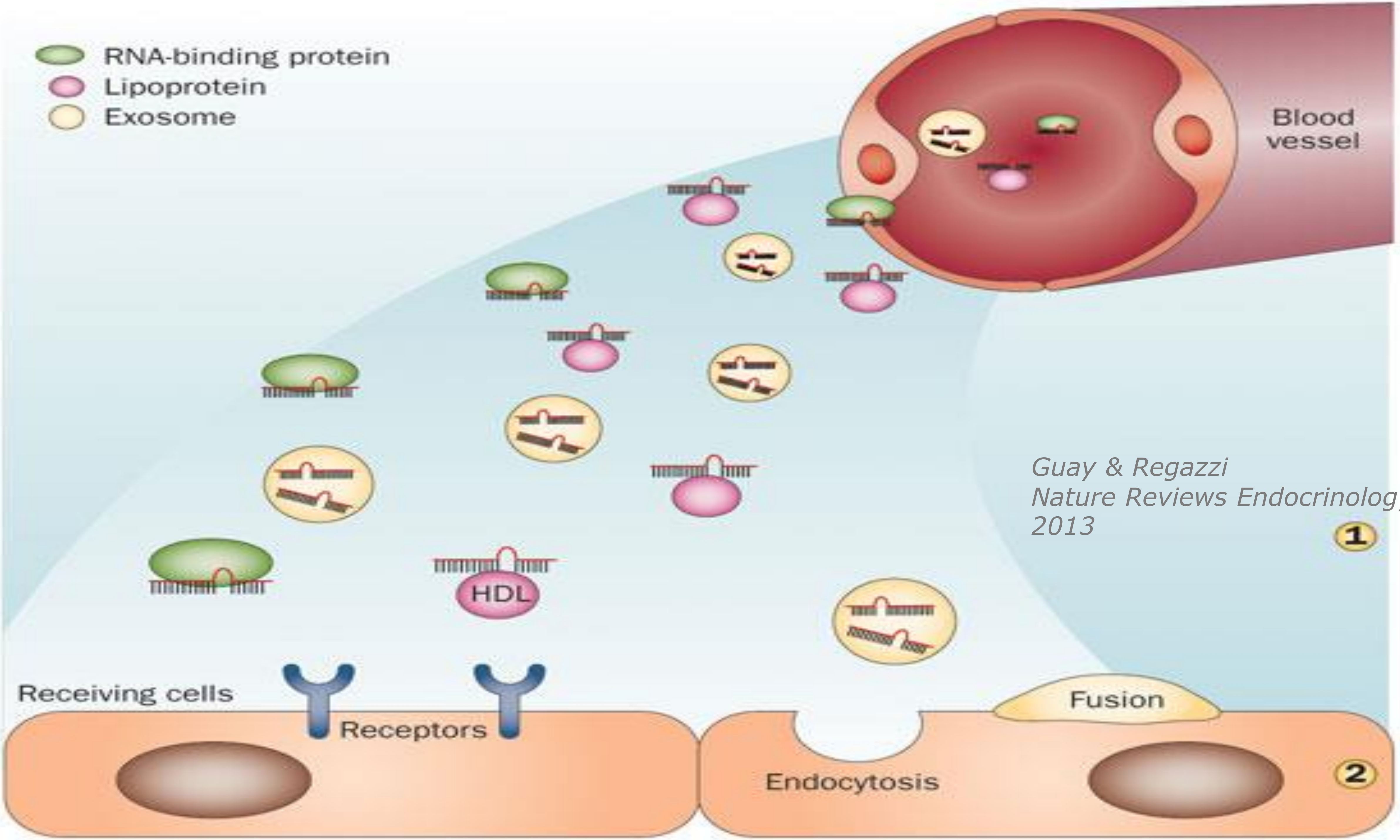
Use of miRNAs to predict Ozone effects

- **The predicted targets of bronchial epithelial miRNAs down-regulated after O_3 exposure *in vivo* are significantly (q=0.0098) overrepresented in the KEGG MAPK signaling pathway**





McCullough et al; Am J Respir Cell Mol Biol. 2014



Guay & Regazzi
Nature Reviews Endocrinology
2013

Top miRNA altered by exposure found in blood

	miRNA	FC	p-value
BECs			
DE	hsa-miR-1202	**-1.74**	0.0329
	hsa-miR-1246	**-1.50**	0.0231
	hsa-miR-638	**-1.55**	0.0352
	hsa-miR-762	**-1.54**	0.0356
O_3	hsa-let-7a-5p	**-1.58**	0.0263
	hsa-miR-21-5p	**-1.52**	0.0122
	hsa-miR-449a	**-2.16**	0.0005
	hsa-miR-449b-5p	**-1.75**	0.0026

Depression

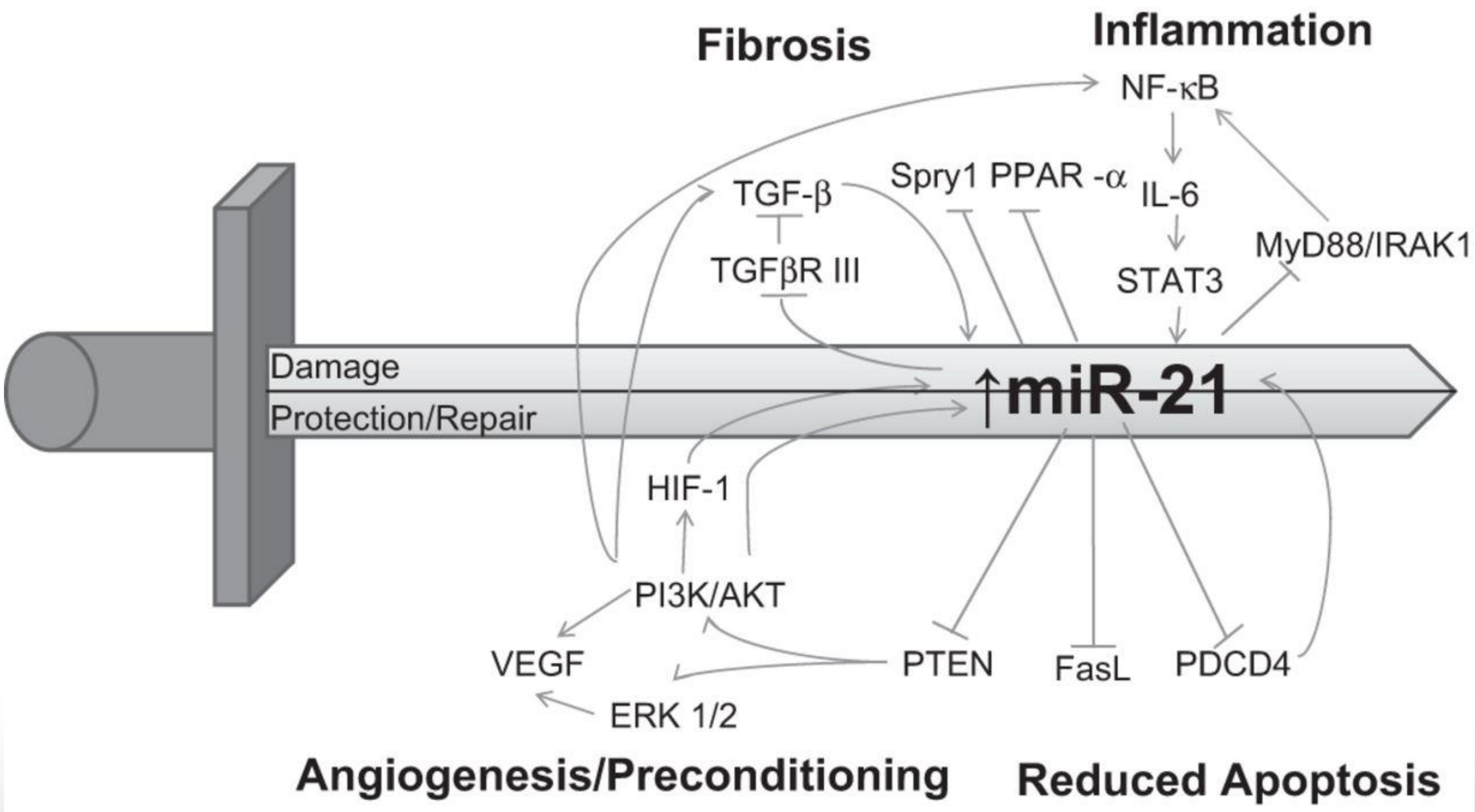
Adenocarcinoma

Cancer

Cardiac

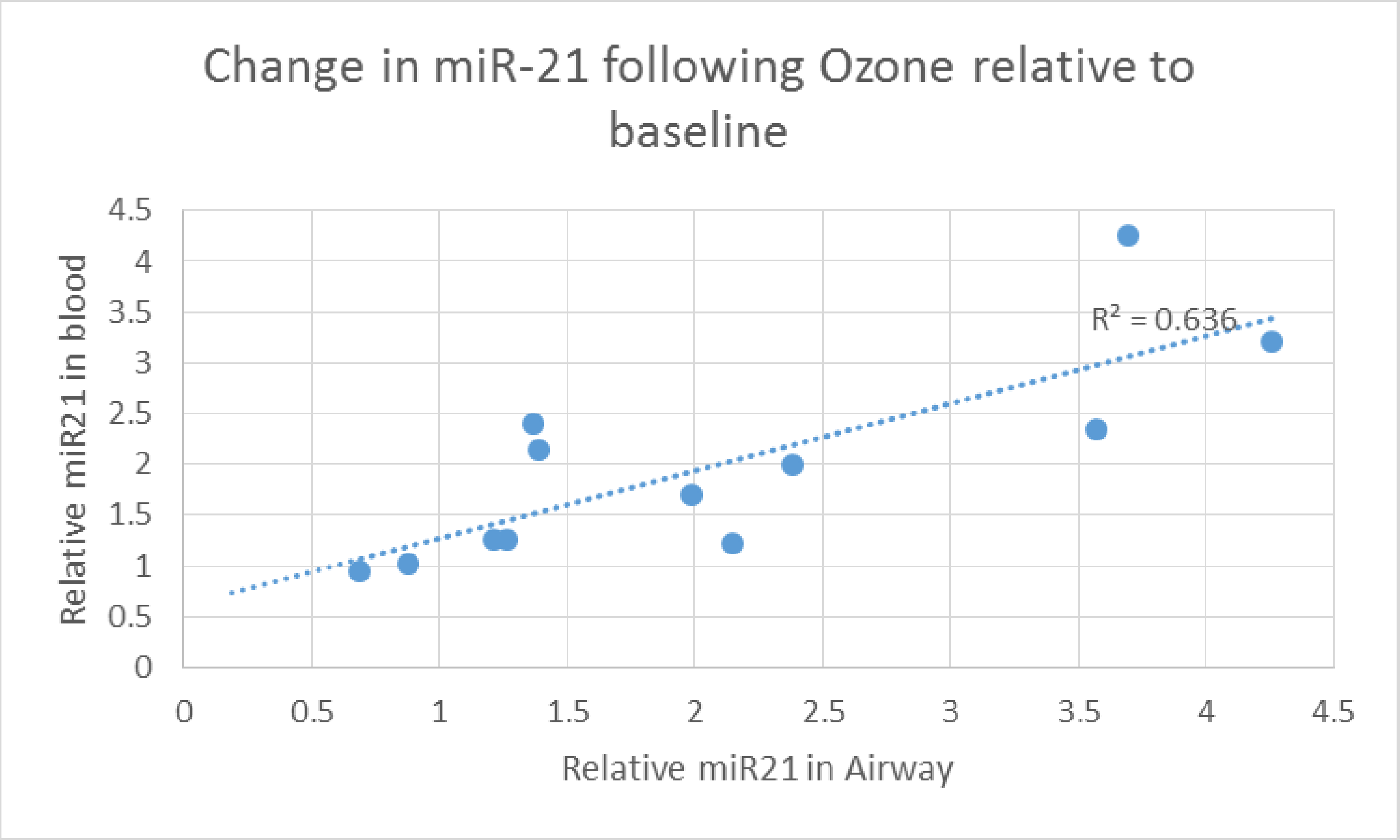
Schizophrenia

miR-21 in ischemia/reperfusion injury



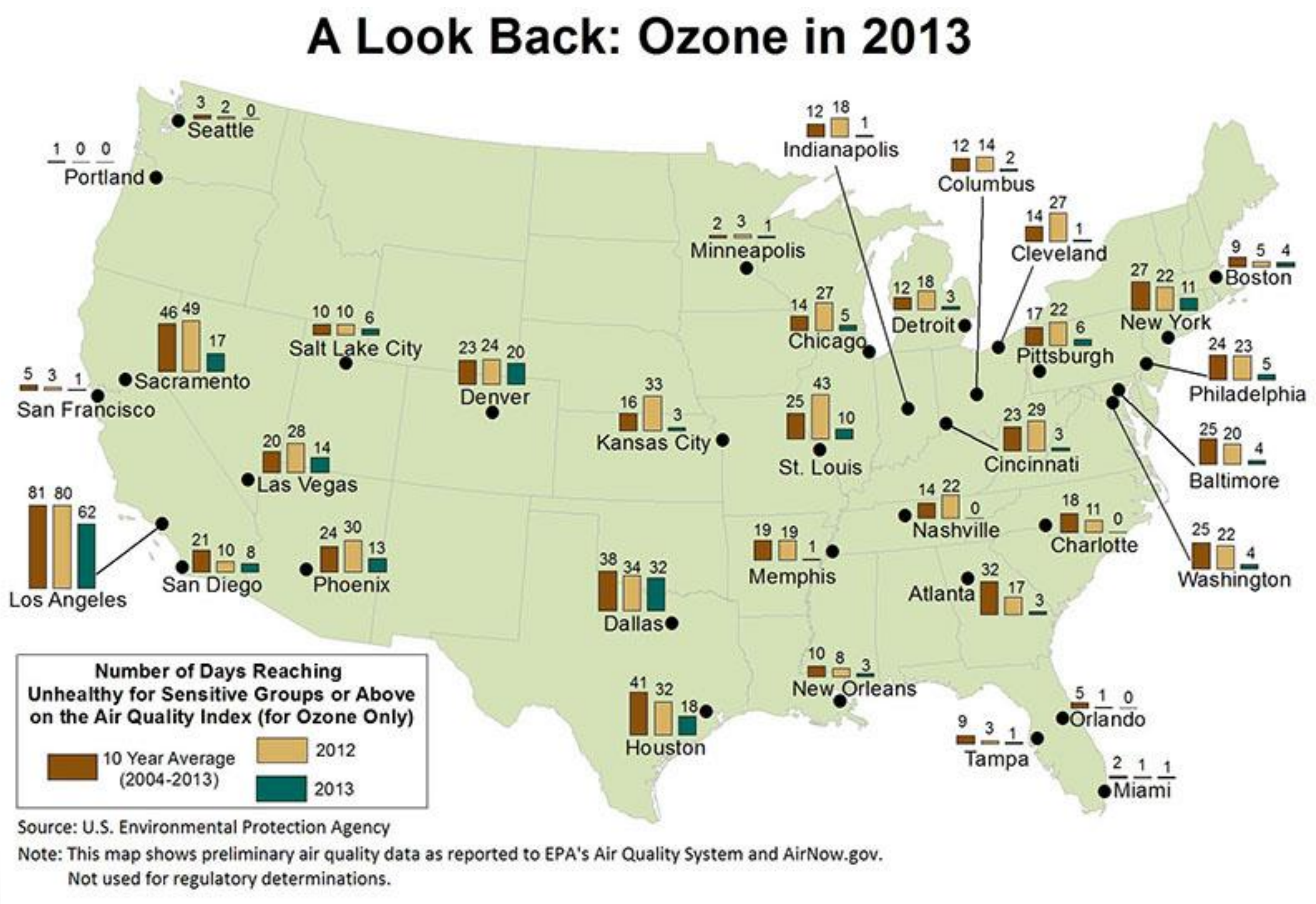
miRNA as a mechanism for extrapulmonary effects of ozone?

- Human in vivo exposure to ozone alters miRNA-21 in lung epithelial cells
- miR-21 is also elevated in blood following ozone exposure
- Changes in blood and airway related
- miR-21 exosomes may be key regulators of cardiac hypertrophy
- Need to be verified in human population studies



Ozone and Epigenetics - a new solution to an old problem?

How do responses differ between populations that experience high ozone levels frequently (daily) versus intermittently?



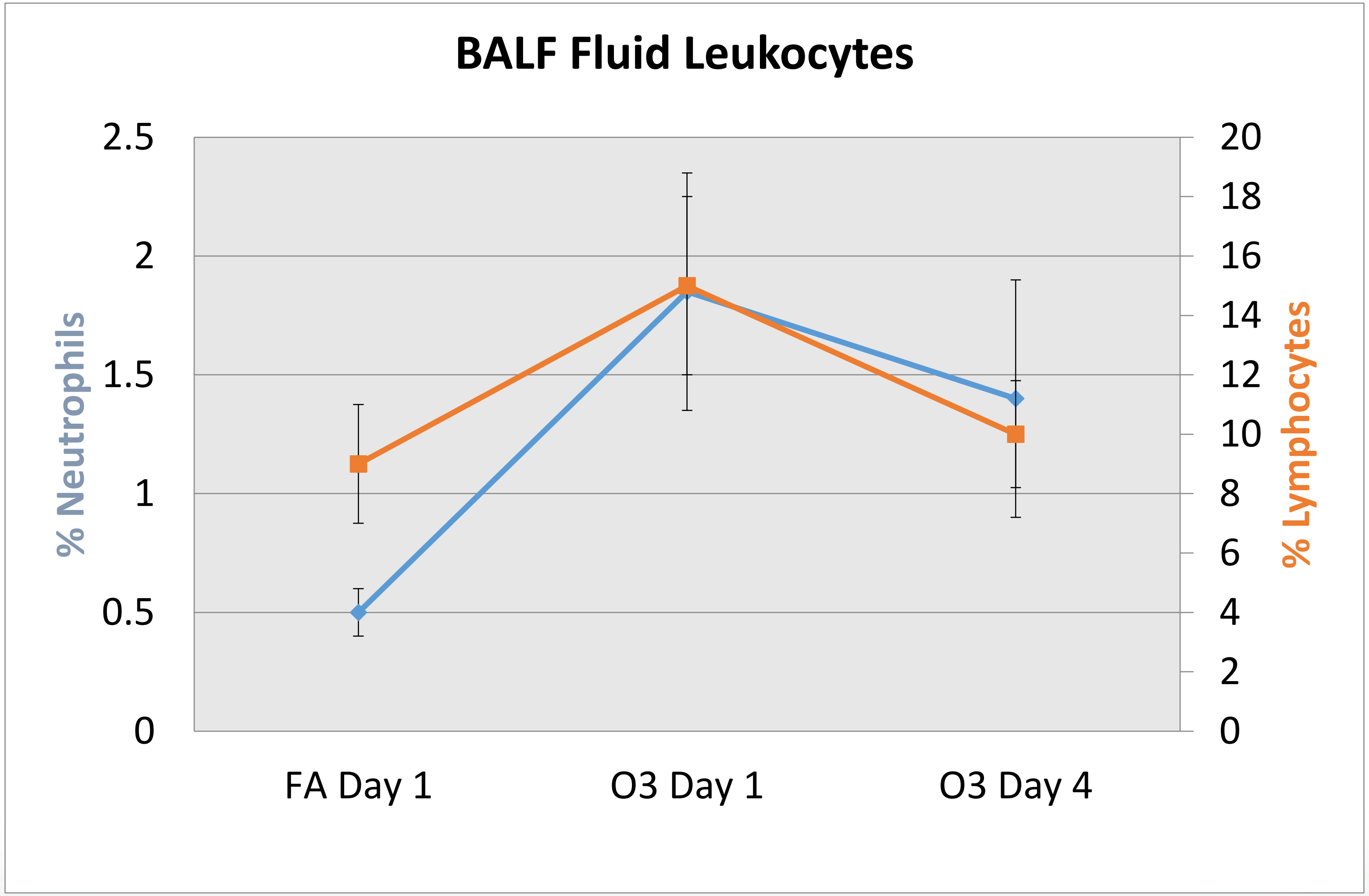
Environmental Health Perspectives
Vol. 18, pp. 141-146, 1976

Studies in Adaption to Ambient Oxidant Air Pollution: Effects of Ozone Exposure in Los Angeles Residents vs. New Arrivals

by Jack D. Hackney,* William S. Linn,* Ramon D. Buckley,* and Helen J. Hislop†

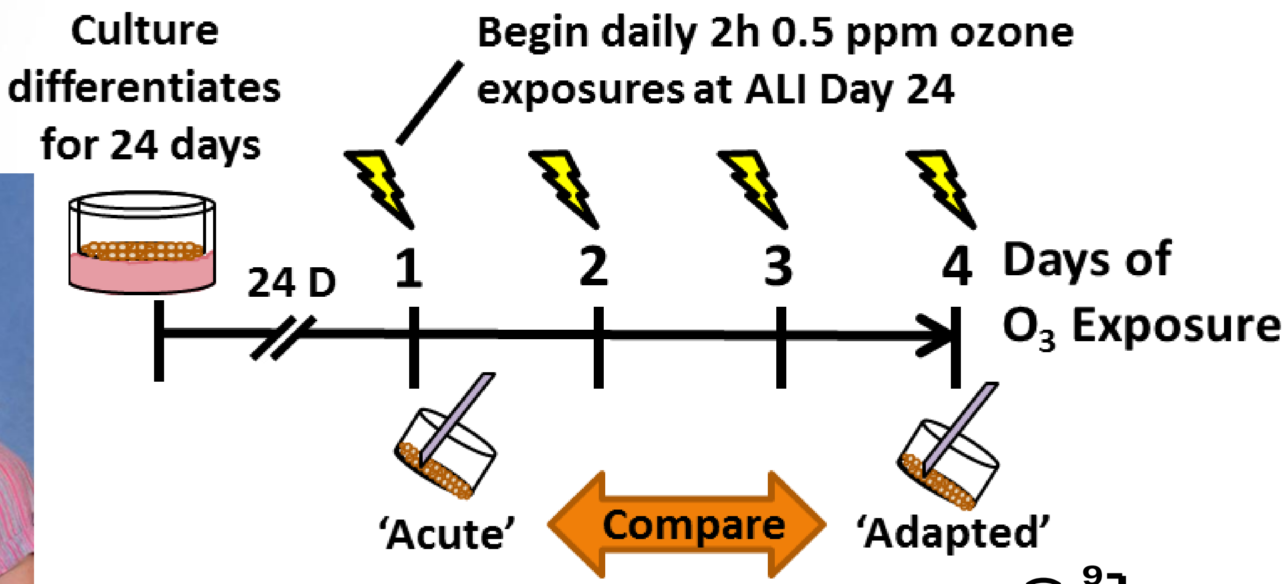
To test the hypothesis that adaptation protecting against acute effects of ambient ozone (O_3) exposures develops in Los Angeles residents, human volunteers were exposed to 0.4 ppm O_3 under conditions simulating ambient pollution exposures. Blood biochemical, pulmonary physiological, and clinical responses were assessed. Los Angeles residents ($N = 6$) showed only minimal clinical or physiological response to O_3, while new arrivals ($N = 9$) showed significant losses in pulmonary function and a tendency toward increased symptoms. Most biochemical responses did not differ significantly between residents and new arrivals. These results agree with others in suggesting that exposures to elevated ambient concentrations of O_3 produce adaptation in at least some residents of photochemical pollution areas. The underlying mechanisms and long-term consequences of such adaptation are unknown.

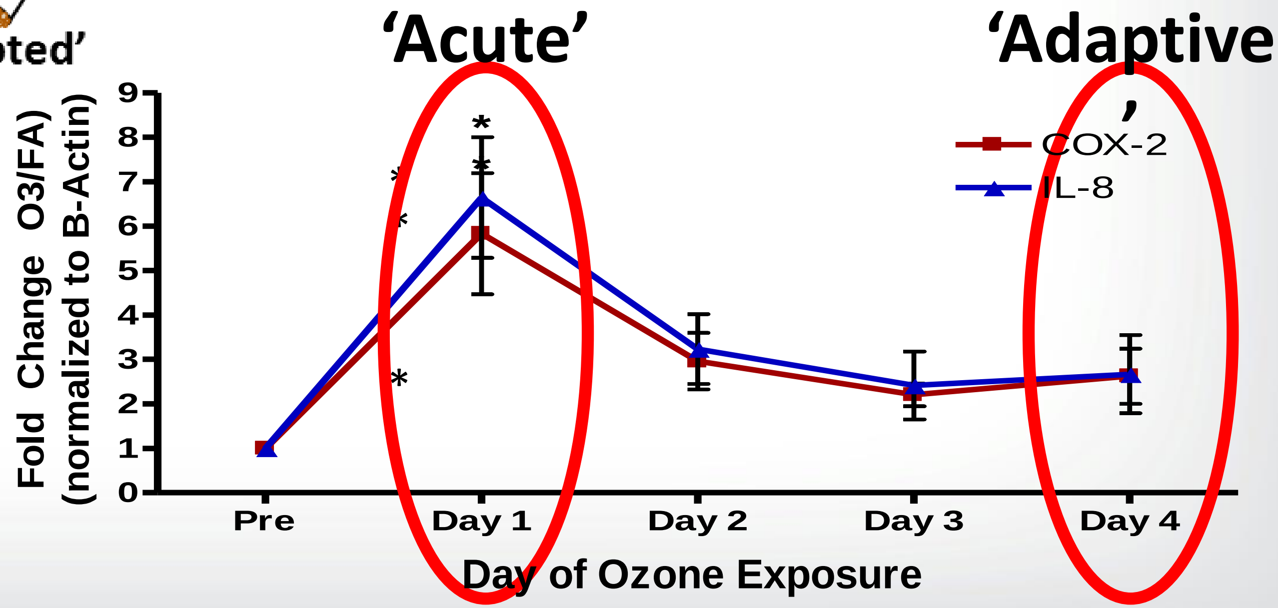
Inflammatory response is suppressed in repeated ozone exposure



Redrawn from Am J of Resp and Crit Care Med, Vol. 161, No. 6 (2000), pp. 1855-1861

Cellular Adaptation to Ozone

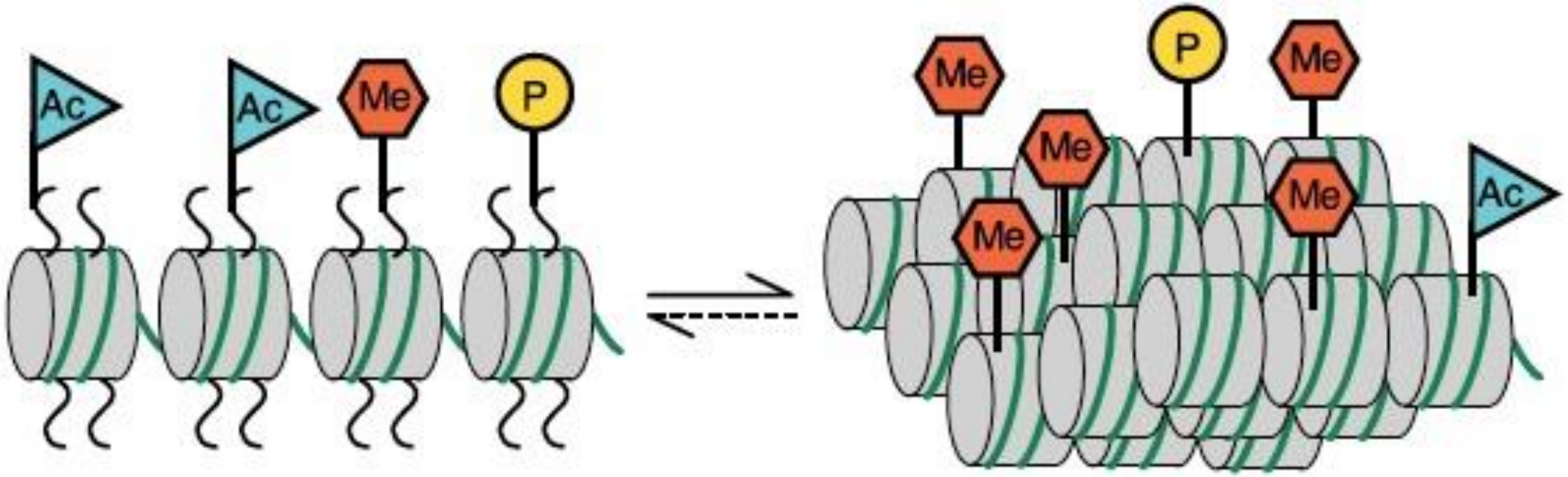




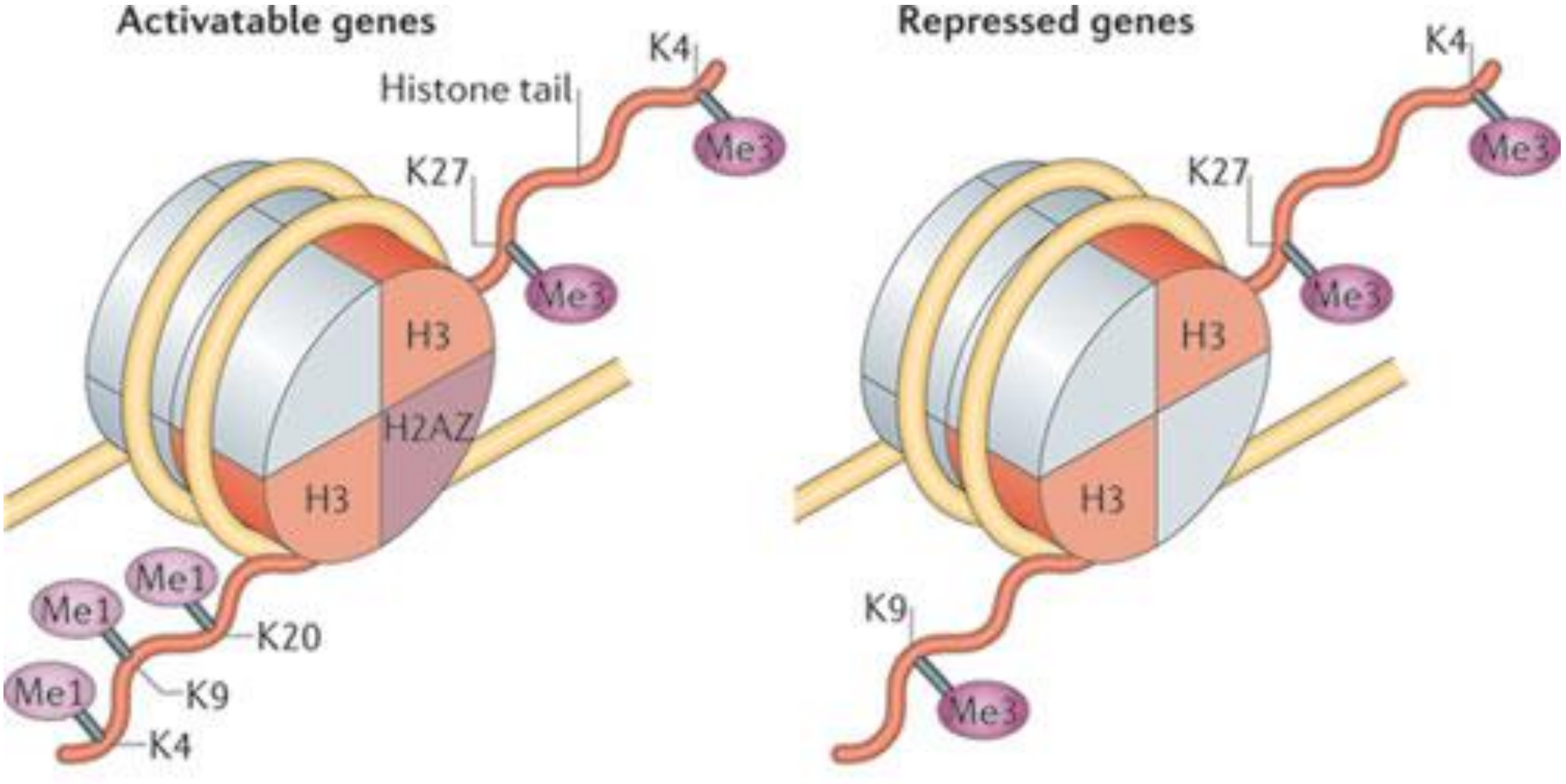
Chromatin States

"Open" = Active

"Closed" = Repressive

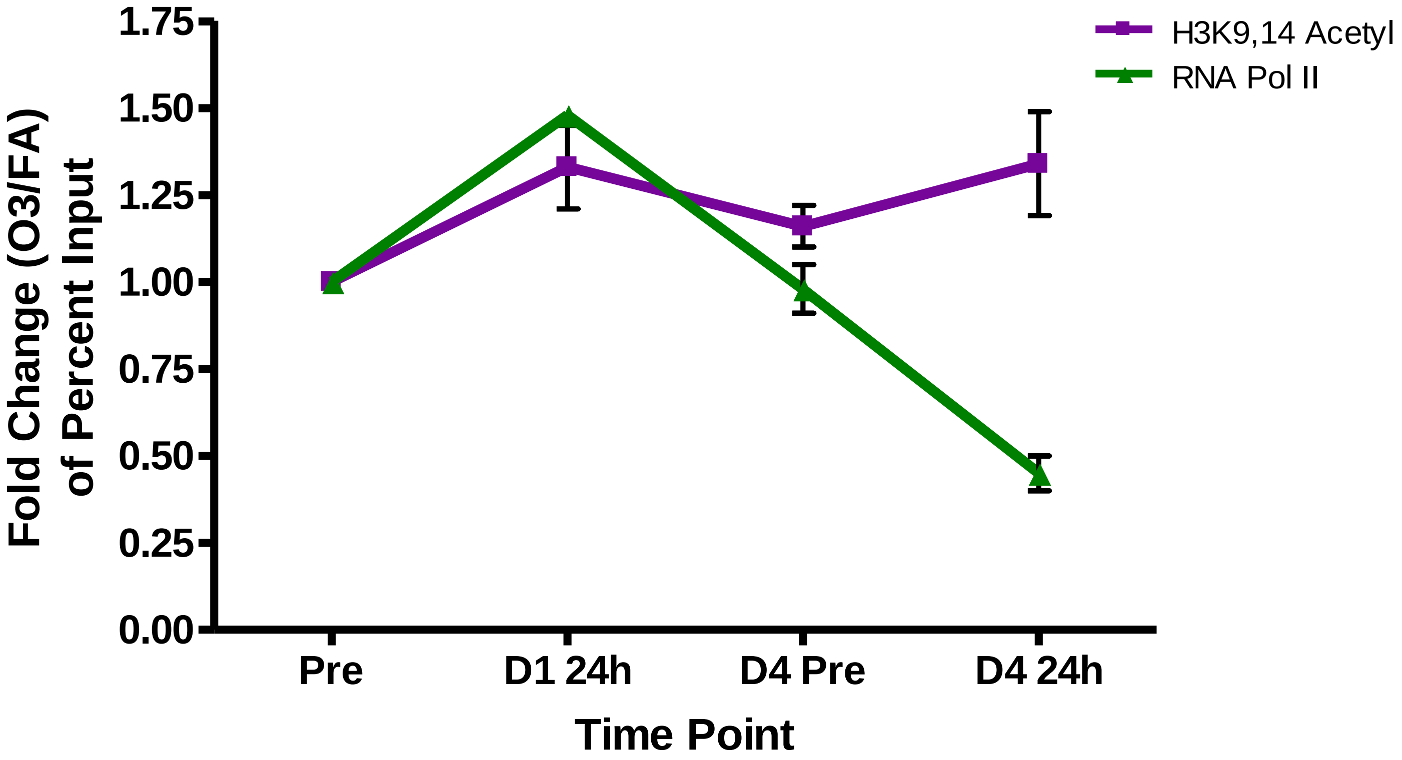


Jenuwein and Allis (2001) *Science*. **293:** 1074-1080

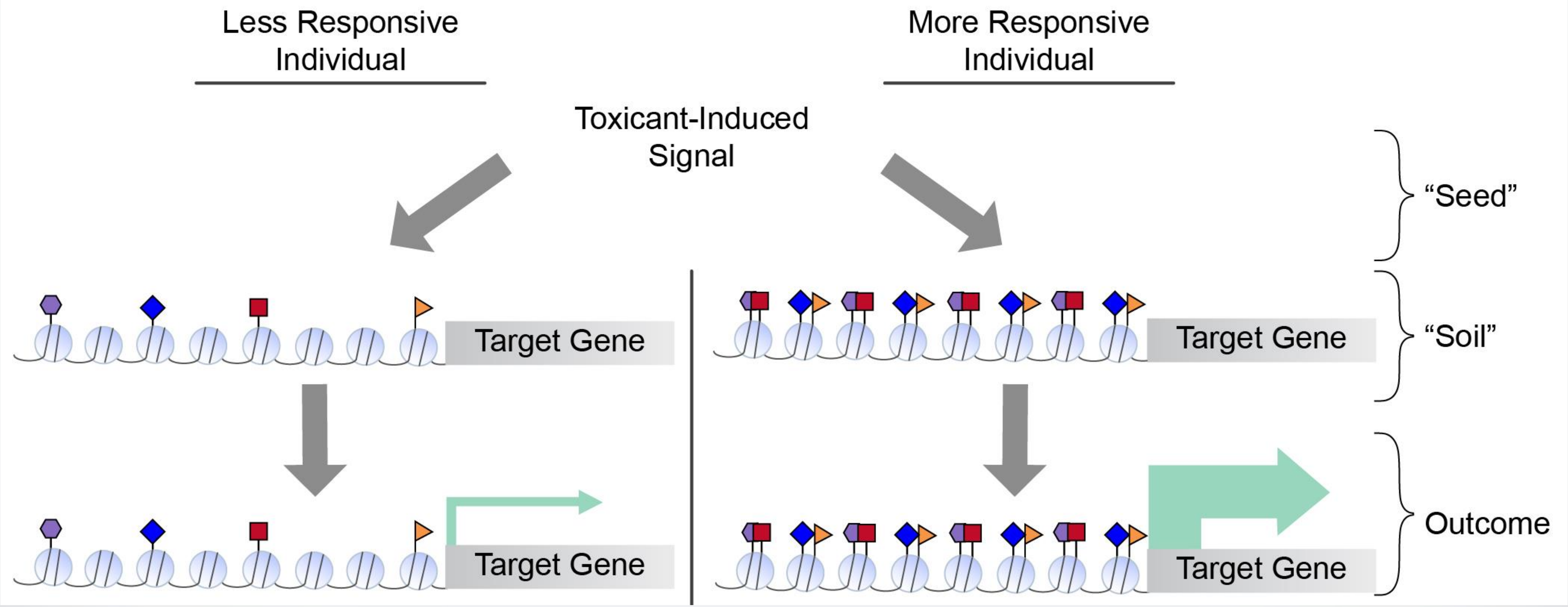


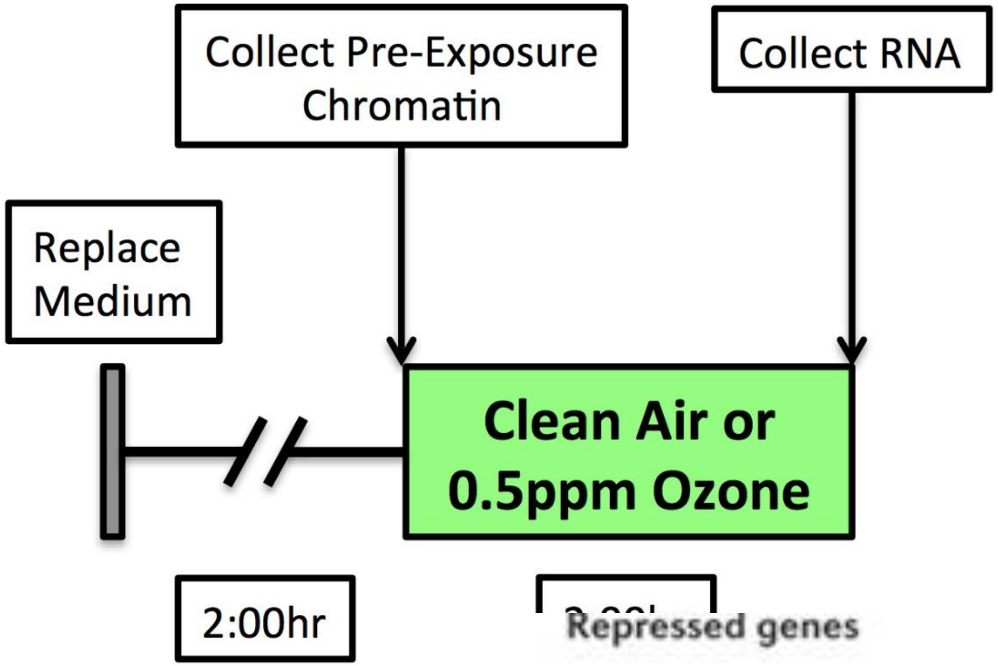
Nature Reviews | Immunology

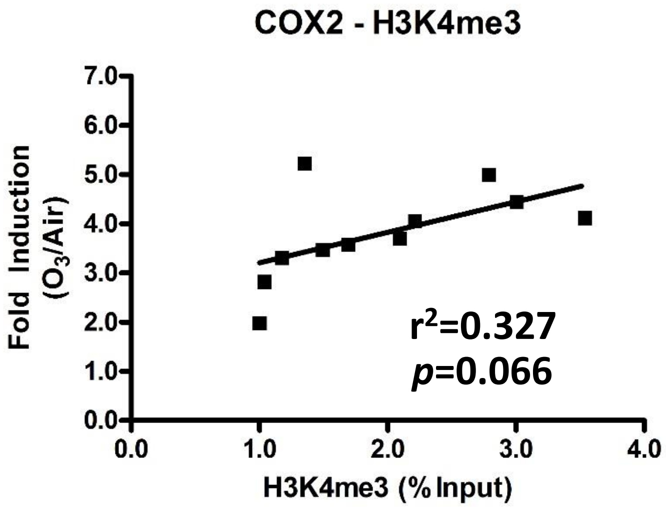
Chromatin state differs between single vs. multiple ozone exposure

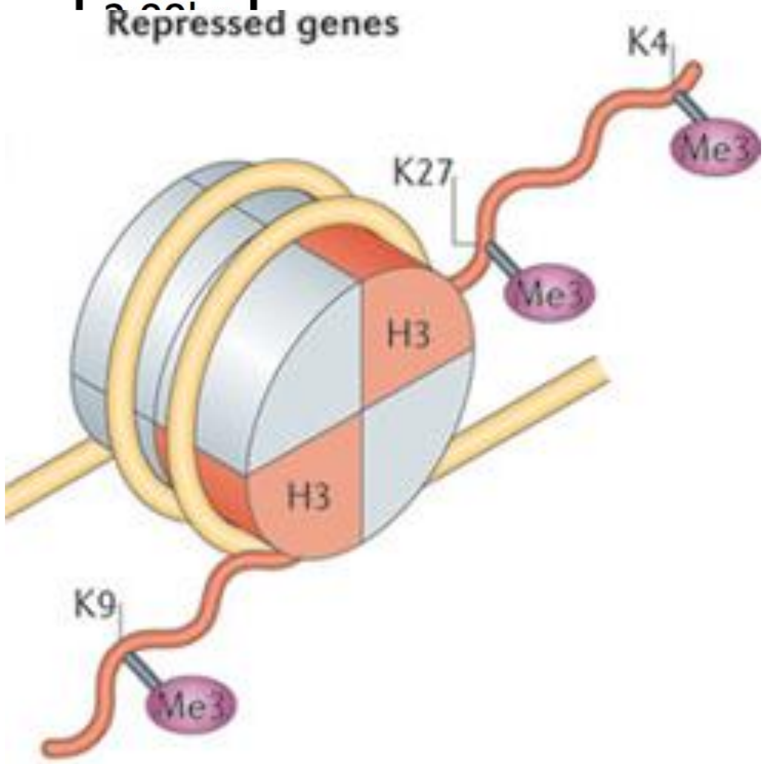


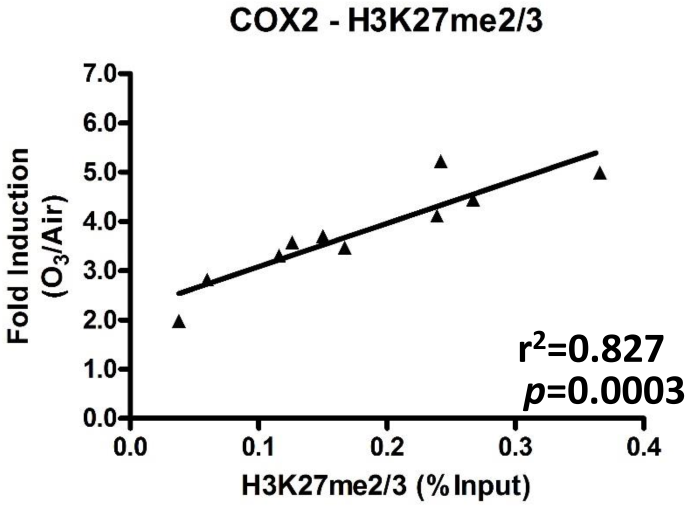
Can Baseline Chromatin predict Responsiveness?




Collect Pre-Exposure Chromatin
Collect RNA
Replace Medium
Clean Air or 0.5ppm Ozone
2:00hr


COX2 - H3K4me3
Fold Induction (O_3/Air)
H3K4me3 (% Input)
r^2=0.327
p=0.066
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0


Repressed genes
K4
Me3
K27
Me3
H3
H3
K9
Me3


COX2 - H3K27me2/3
Fold Induction (O_3/Air)
H3K27me2/3 (% Input)
r^2=0.827
p=0.0003
0.0
0.1
0.2
0.3
0.4
1.0
2.0
3.0
4.0
5.0
6.0
7.0

- markers of exposure
- markers of response
- indicate adverse outcome pathways
- provide biological plausibility
- predict risk