National Institute of Environmental Health Sciences
Your Environment. Your Health.

The human tumor suppressor p53 links chromosomal stress and immune responses

Daniel Menéndez

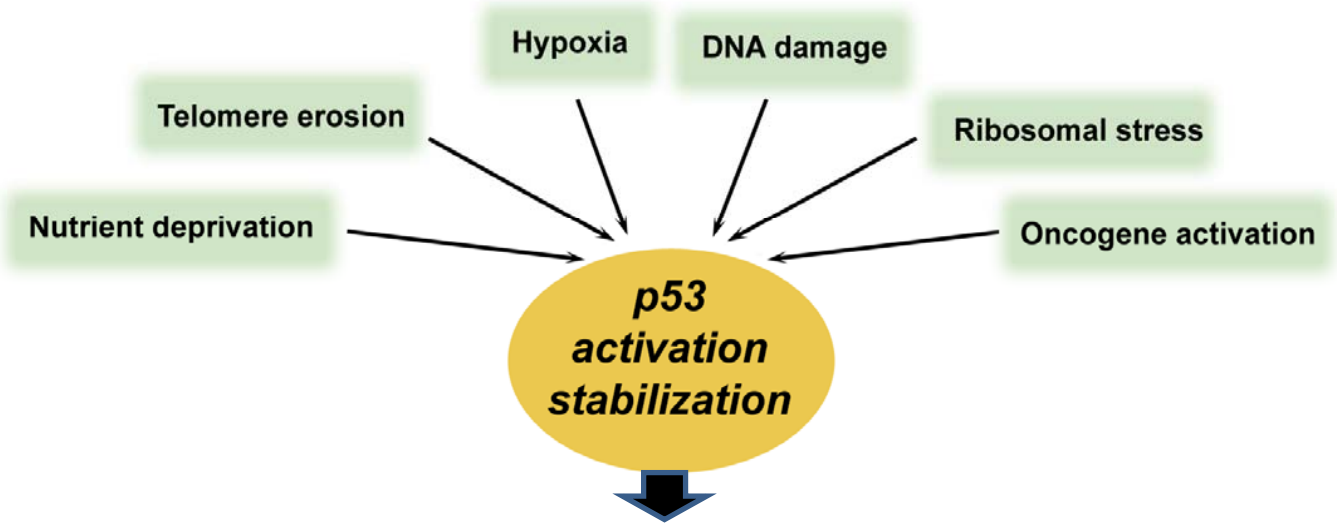
Chromosome Stability Group
Genome Integrity and Structural Biology Laboratory
National Institute of Environmental Health Sciences
Research Triangle Park, NC.

The p53 Tumor Suppressor

- **Guardian of the genome**
- **A transcription factor**
- **Highly conserved in multi-cellular organisms**
- **50% of all cancers have a mutation in the *p53* gene**
- **>90% of all cancers have altered expression of p53**

p53 as integrator in the DNA Damage Response

- **Responds to a wide variety of cellular stresses including DSBs**



Cell cycle arrest **Apoptosis** **DNA repair** **Senescence**

Autophagy **Metabolism** **Differentiation** **Stem cell function** **Immune Response**

Characterization of the human p53 master regulatory network in response to environmental stressors

1) Characterization of p53 transactivation

What constitutes "functional" p53 response elements?

2) Variation and functional conservation in the p53 regulatory network

Diversity of REs within p53 regulatory networks

3) Expansion of the p53 transcriptional network

Changes in p53: effects on network & on biology?

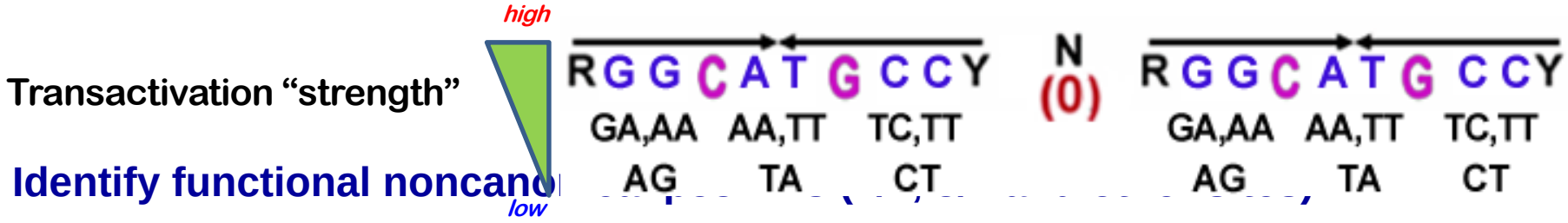
Interacting pathways

p53 sequence-specific transcription factor

Consensus motif for p53-binding site (response element RE)

Developed functionality rules for p53 REs in yeast / human cell systems

MCB 22, (2002); PNAS 105, (2008) ; PLOS Genetics 4, (2008)



Identify functional noncano

PNAS 103 (2006); JBC 284 (2009); PNAS 107 (2010), PLoS Genetics 7 (2011), NAR 41 (2013)

What type of DNA sequences does p53 see in vivo?

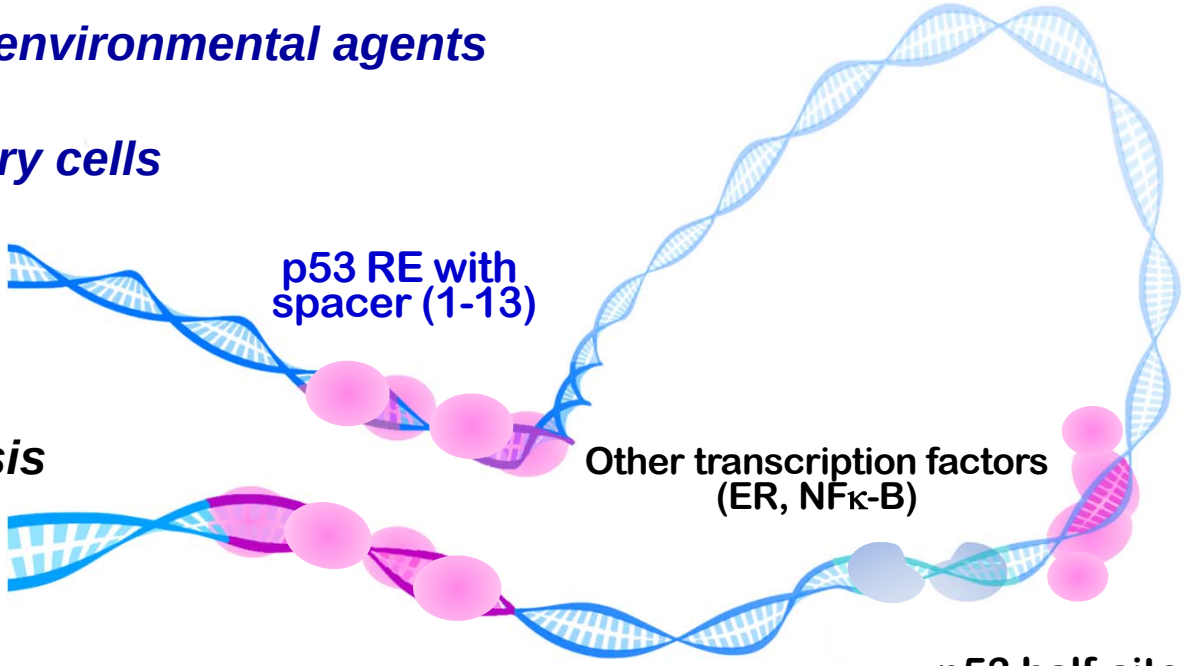
in response to stress / environmental agents

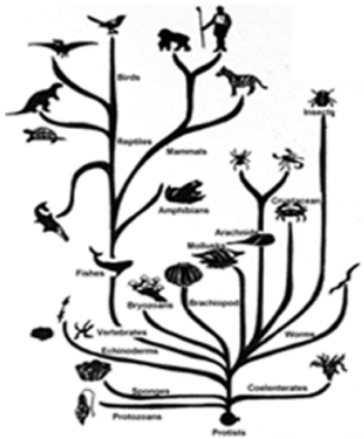
cancer cell lines/ primary cells

ChIPseq

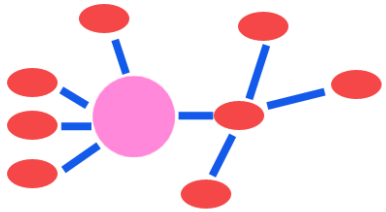
Gene expression analysis

Bioinformatics





Evolution of p53 REs and
transcriptional responses

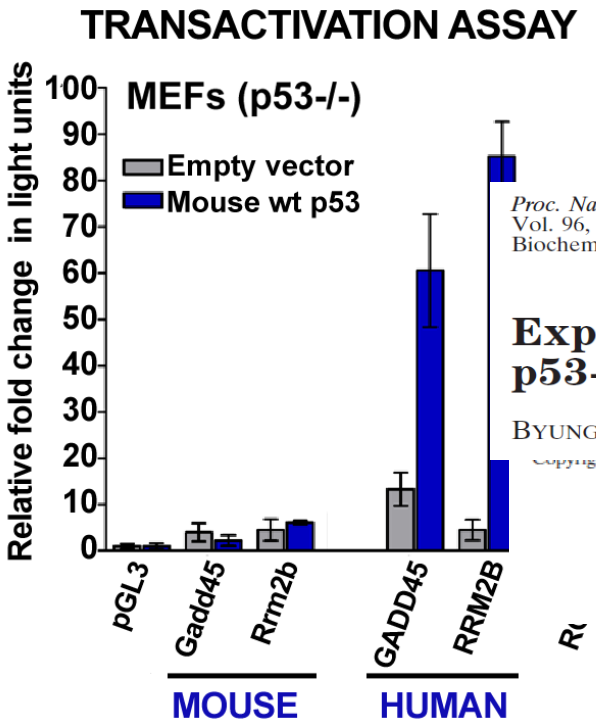


New transcriptional targets and
biological responses
(innate immune response)

Menendez et al., NAR 41 (2013)

Sequence and functional conservation in rodents of p53 target gene REs that have been validated in humans.

BIOLOGICAL PROCESS	TOTAL REs	SEQUENCE CONSERVATION	FUNCTIONAL CONSERVATION
APOPTOSIS	37	8	12
CELL CYCLE SENESCENCE DEVELOPMENT, DIFFERENTIATION	28	14	12
DNA REPAIR	15	3	0
CYTOSKELETON , CELL ADHESION, ANGIOGENESIS, MIGRATION	18	7	5
FEEDBACK AND REGULATION	10	6	5
CYTOKINE AND INFLAMMATION	11	4	5
TRANSCRIPTION, TRANSLATION	14	10	9
VARIOUS	13	6	5
Total of p53 REs analyzed	146		



Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 424–428, January 1999
Biochemistry

Expression of the *p48* xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair

BYUNG JOON HWANG*†, JAMES M. FORD†‡, PHILIP C. HANAWALT‡, AND GILBERT CHU*§

p53 Binds and Activates the Xeroderma Pigmentosum *DDB2* Gene in Humans but Not Mice

Thomas Tan and Gilbert Chu*

Jegga et al., PNAS, 2008; Menendez et al., Nature Cancer Rev, 2010

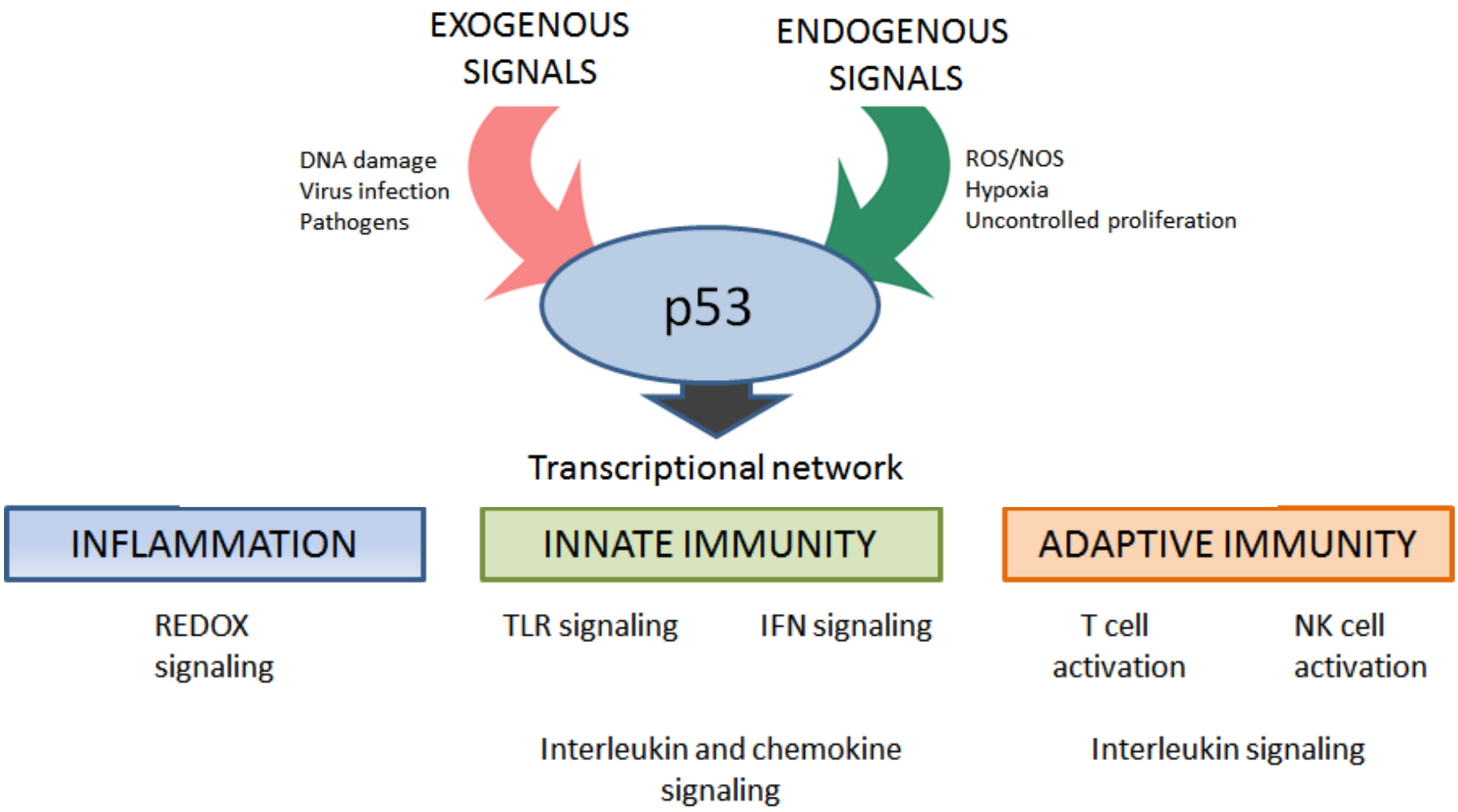
Potential new p53 target genes associated with DNA repair/ metabolism functions

Gene	Full gene name	p53 RE type	p53RE Sequence Conservation Mouse/Rat
ADA	Adenosine deaminase	Spacer 1	NO
LIG3	Ligase III, DNA, ATP-dependent	Half site	NO
MSX1	Msh homeobox 1	Spacer 0	YES
MUTYH	MutY homolog (E. coli)	Half site	NO
POLS	Polymerase (DNA directed) sigma	Spacer 15	YES
RAD23B	RAD23 homolog B (S. cerevisiae)	Half site	NO
RAD51C	RAD51 homolog C (S. cerevisiae)	Spacer 2	NO
REV3L	REV3-like, catalytic subunit of DNA polymerase zeta (yeast)	Spacer 0	NO

Associated p53 binding after stress or p53 activation (ChIP-seq) in U2OS cells (Menendez et al., NAR 41, 2013)

Interactions between p53 and the immune response

Cancer cells **Primary cells**



Julie Lowe et al., BioDiscovery , 2013

Toll-like receptors (TLRs)

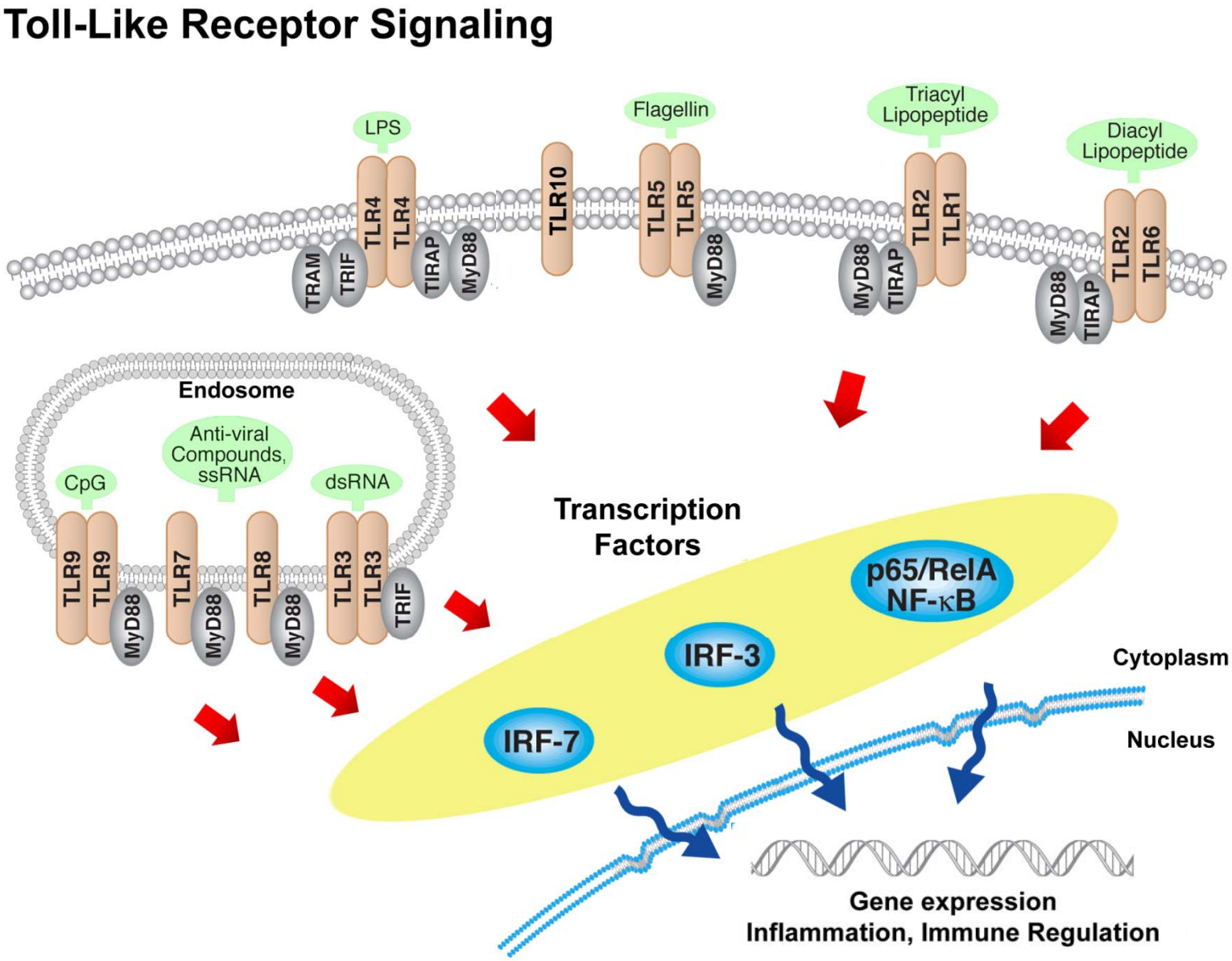
TLRs are the key molecules in innate immune response.

The TLR family comprises membrane glycoproteins that recognize pathogen associated molecular patterns (PAMPs)

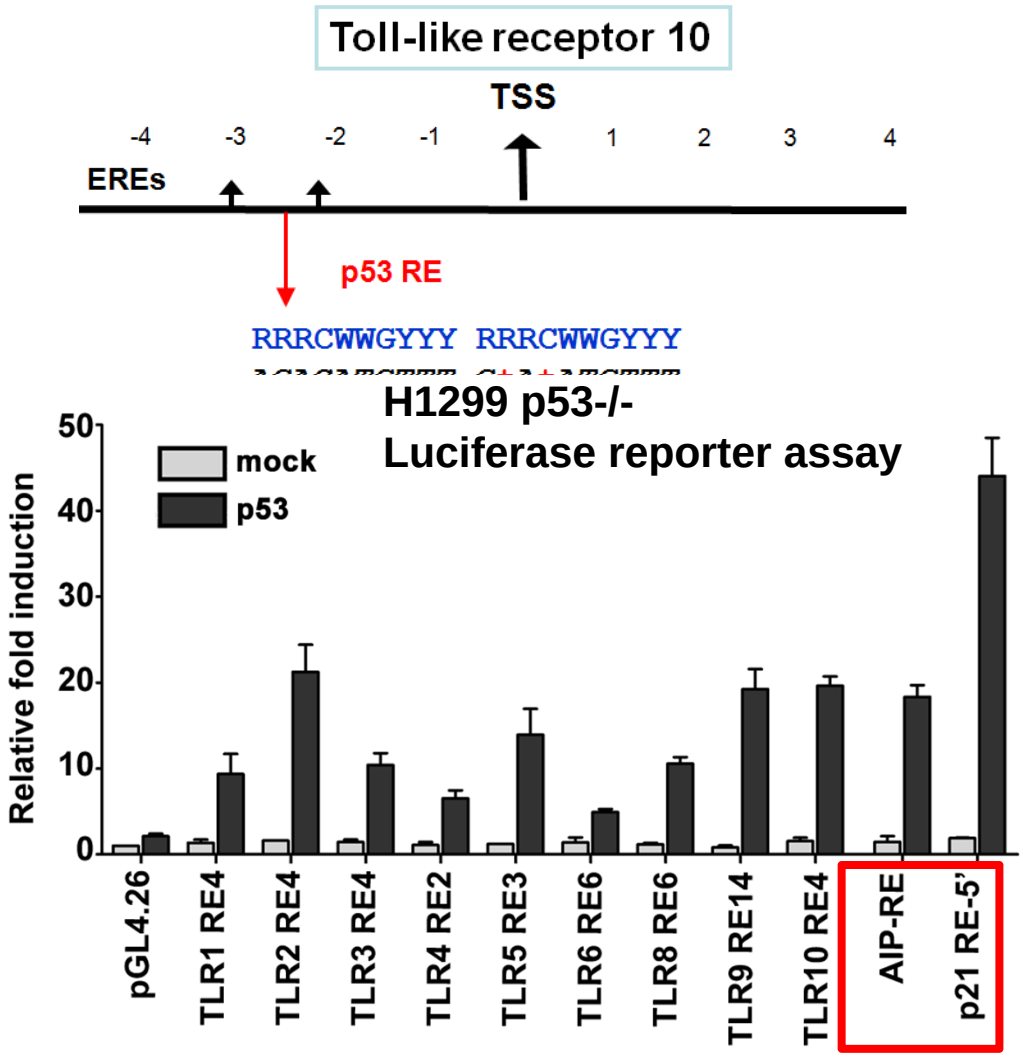
Activation of TLR pathway mediates immune/inflammatory responses.

Ten TLRs are identified in humans.

a "hard-wired" response???



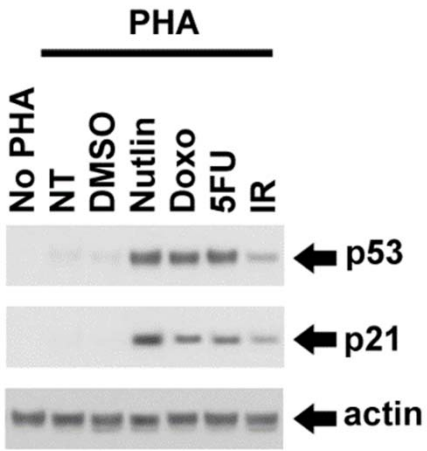
p53 meets the innate immune response via TLRs

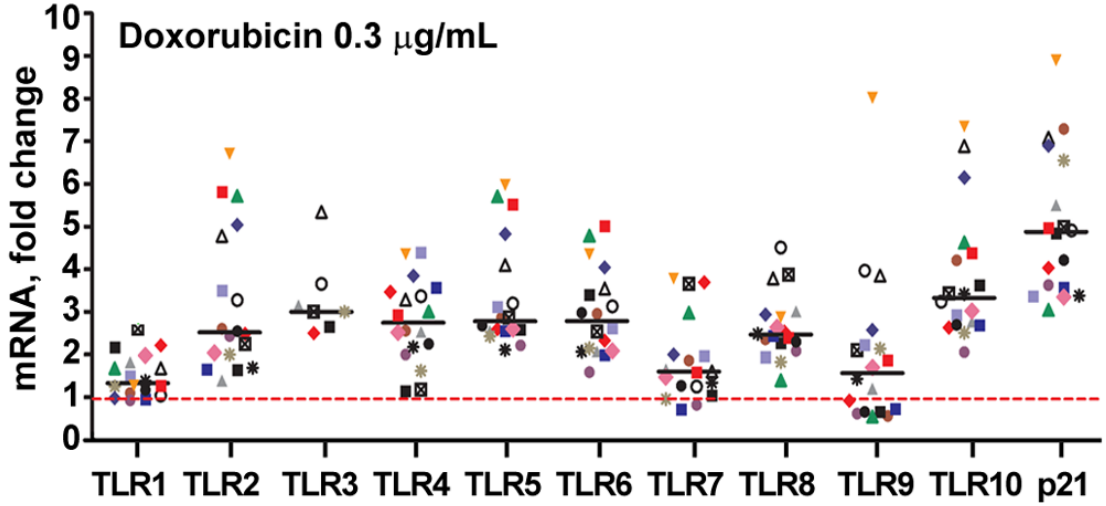


Menendez et al, PLoS Genetics, 2011

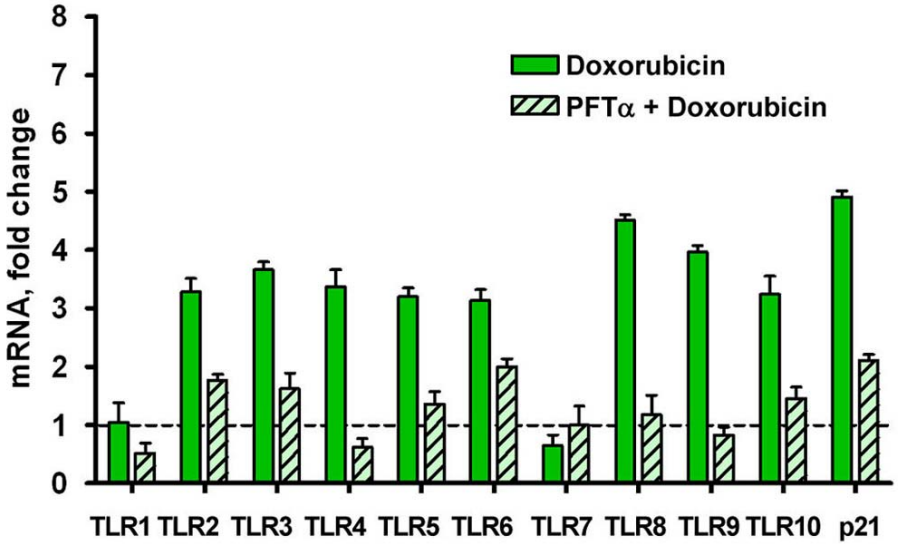
DNA stressors and p53 activation induce expression of the Toll-like receptor (TLR) gene family in human immune cells

- **lymphocytes**



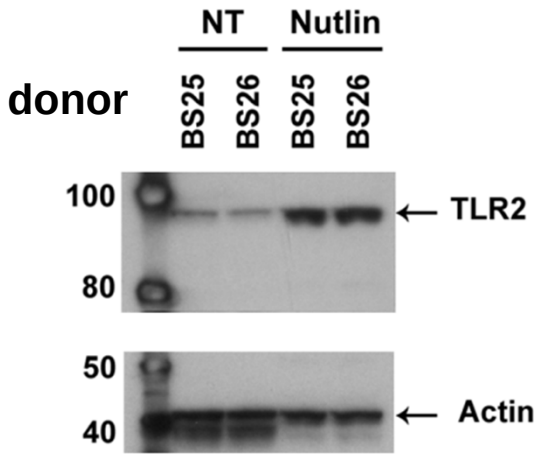


Similar in alveolar macrophages

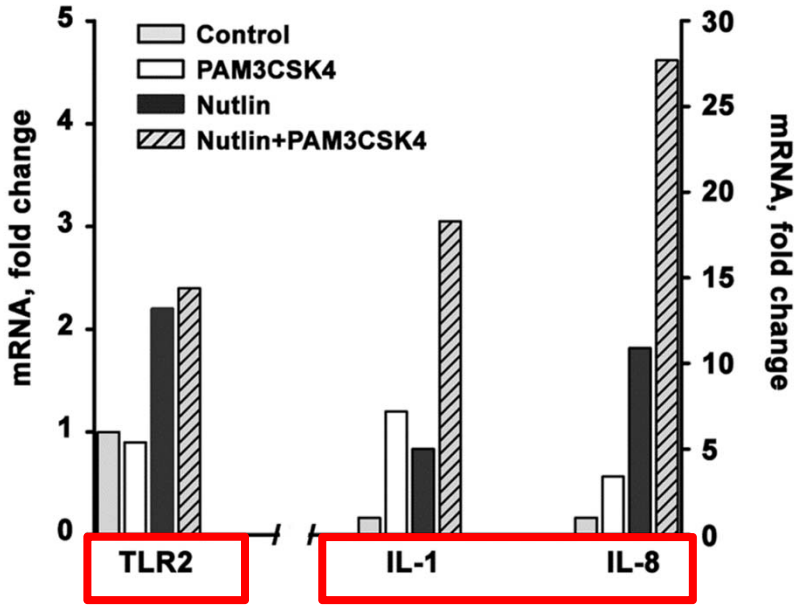


Menendez et al, PLoS Genetics, 2011

p53 regulation of TLRs genes enhances the inflammatory response (cytokine production) in presence of TLRs agonists



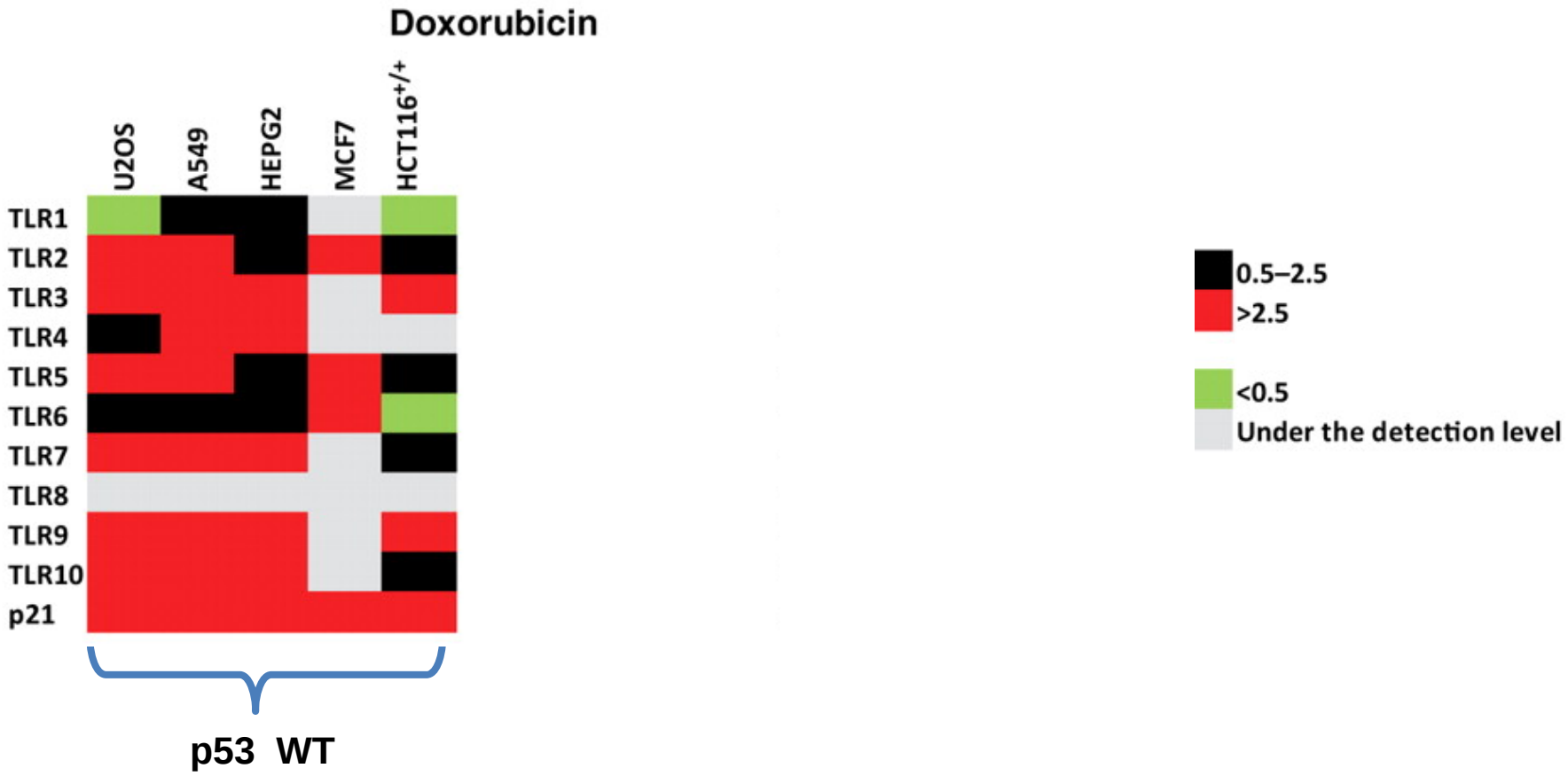
CD3+ lymphocytes.



Nutlin activation of p53 increasesTLR2 response to PAM3CSK4

Menendez et al, PLoS Genetics, 2011

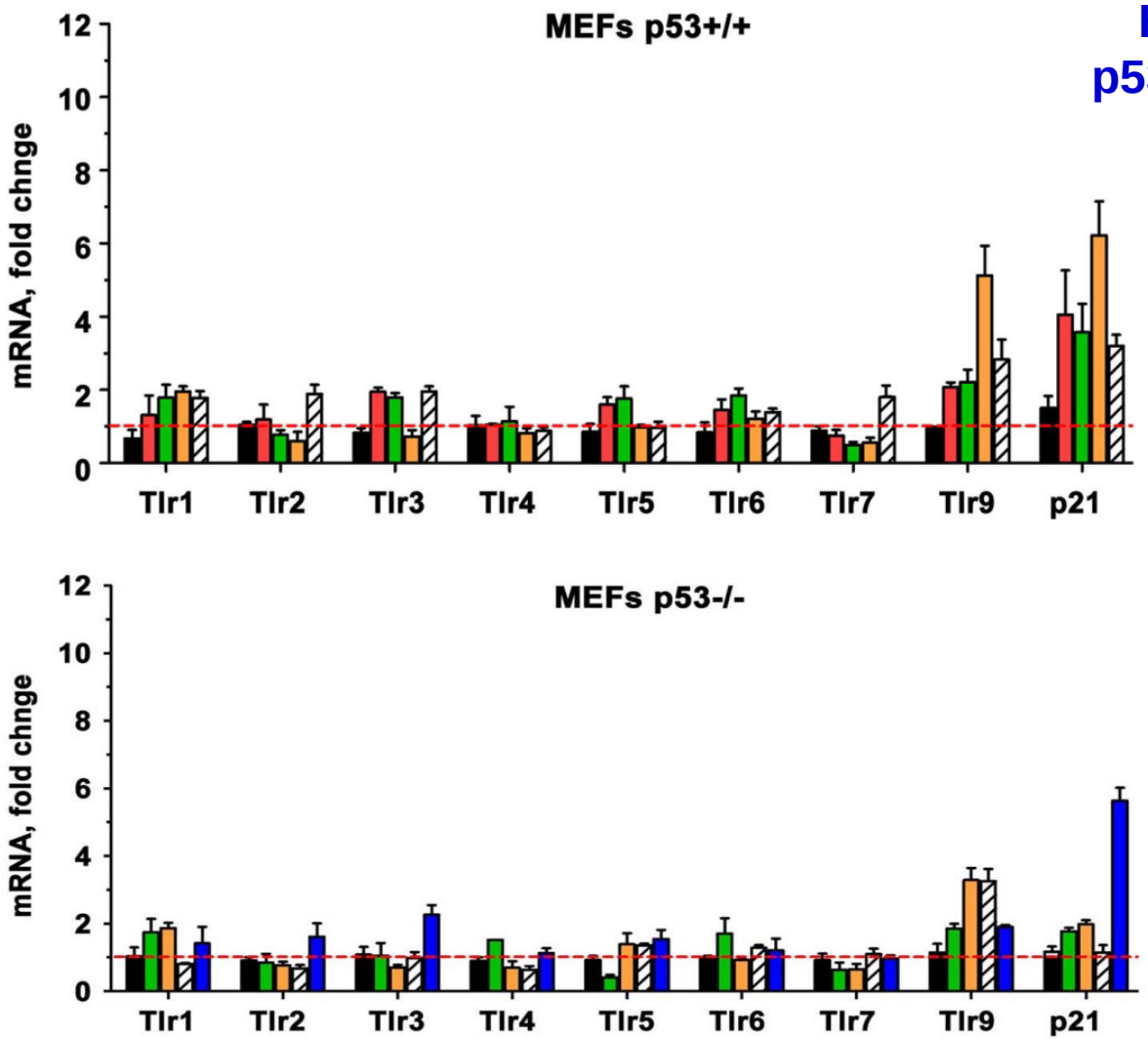
DNA damage and p53 activation induce expression of TLRs in human cancer cell lines



Doxorubicin-mediated increase in TLR5 enhances flagelin-induced IL-6 and IL-8 expression in MCF7 cells

Shatz et.al, Cancer Research, 2012

The TLR gene family in mouse cells is NOT responsive to p53 activation



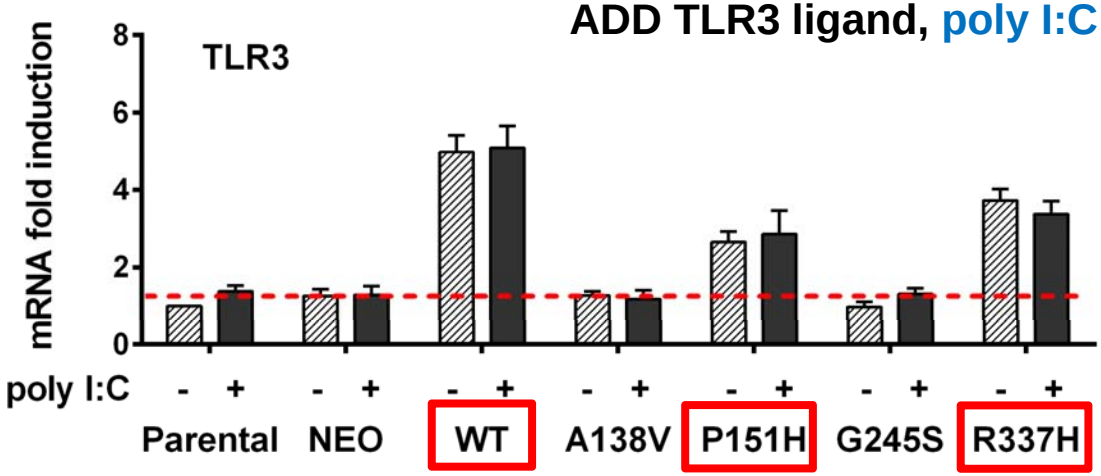
Human TLR-associated p53REs are NOT conserved in rodents!!

Menendez et.al, PLoS Genetics, 2011

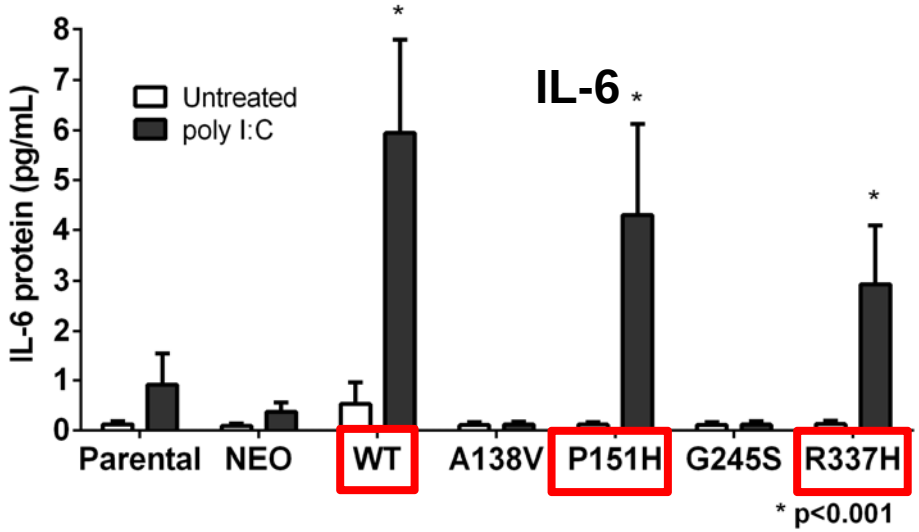
TLR3 expression is affected differently by cancer associated p53 mutants…

HCT116 (p53-/-) transfected with p53 plasmid

ADD TLR3 ligand, poly I:C which mimics dsRNA

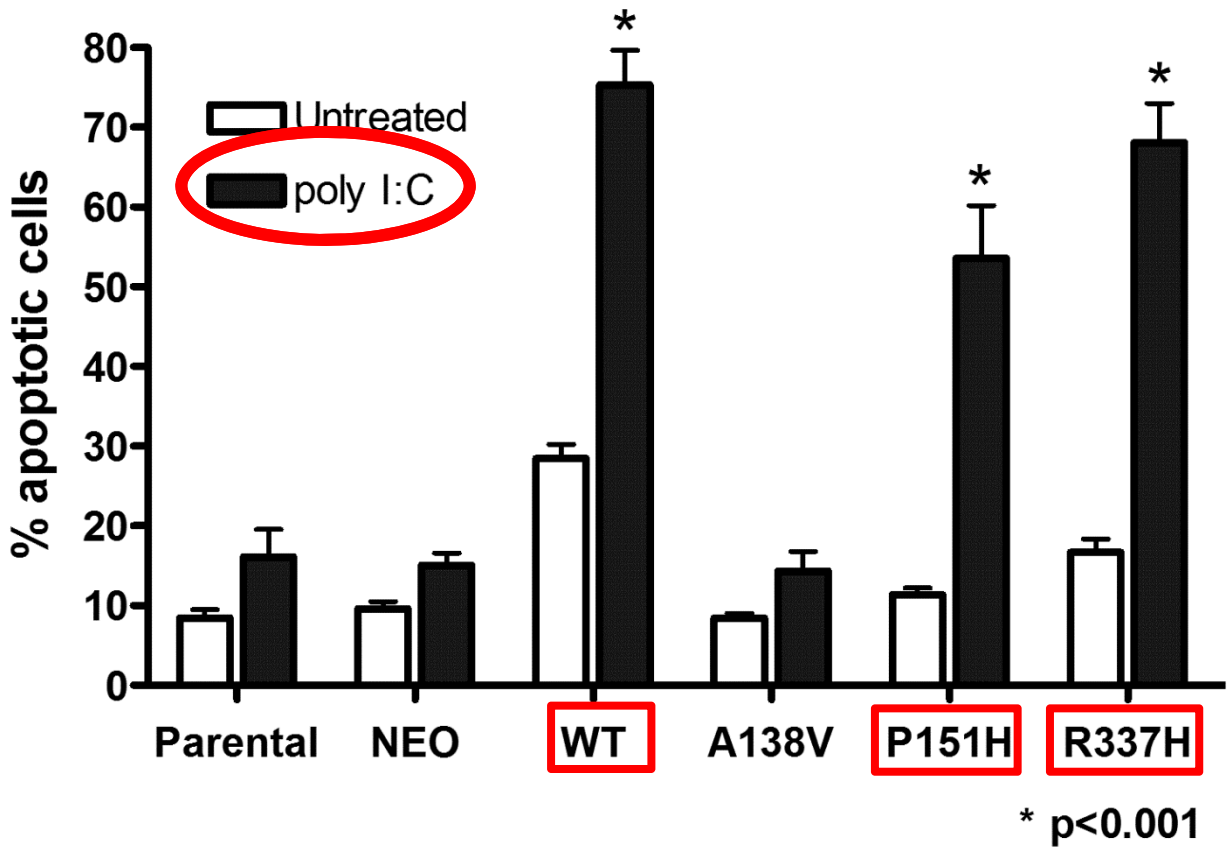


…increased TLR3 amplifies cytokine response to the poly I:C ligand



Enhanced TLR3 signaling also increases apoptosis

HCT116 (p53-/-) transfected cells

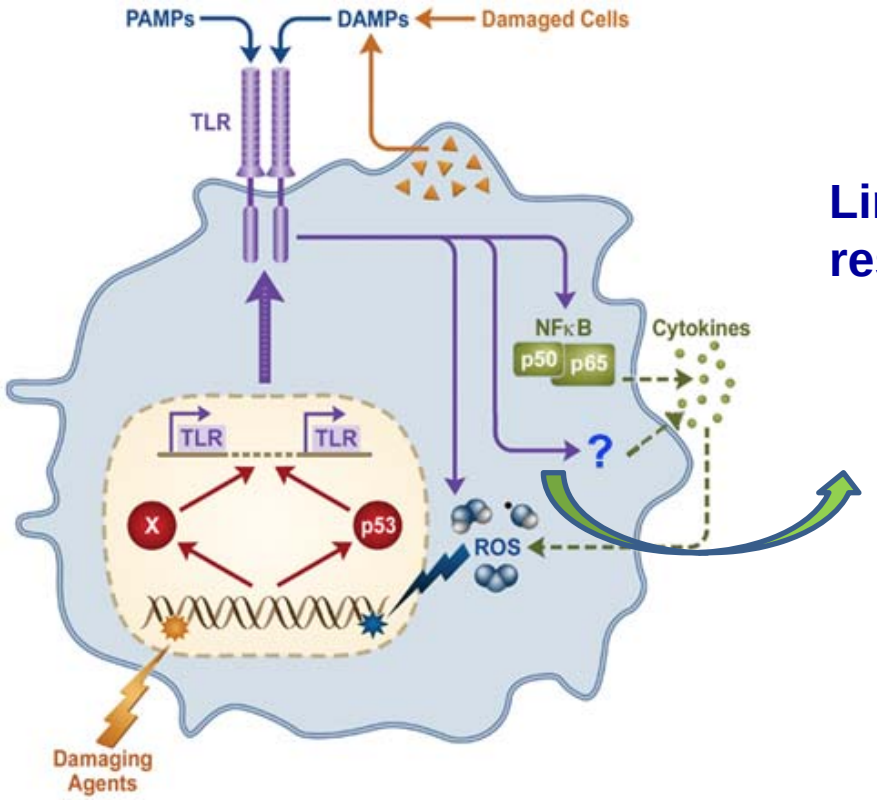


p53 modulates the innate immune response

In response to DNA damage,

- **p53 regulates the expression of the entire family of TLRs in human cells**

- **and modulates the TLR response to its ligands, thereby affecting innate immune responses**



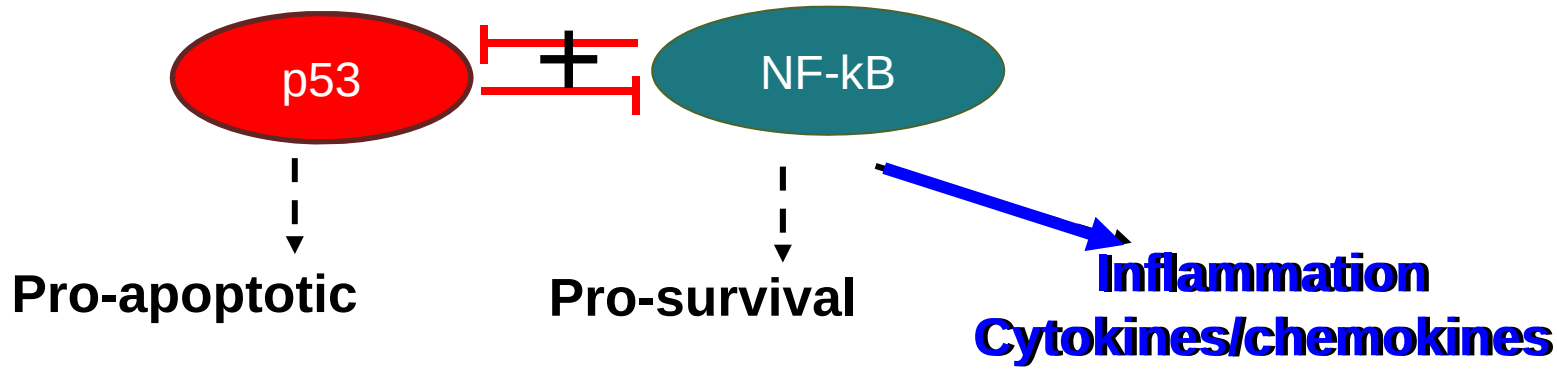
Link between the DNA damage response and the immune response

p38 MAPK (Shatz et al, submitted)

NFκB

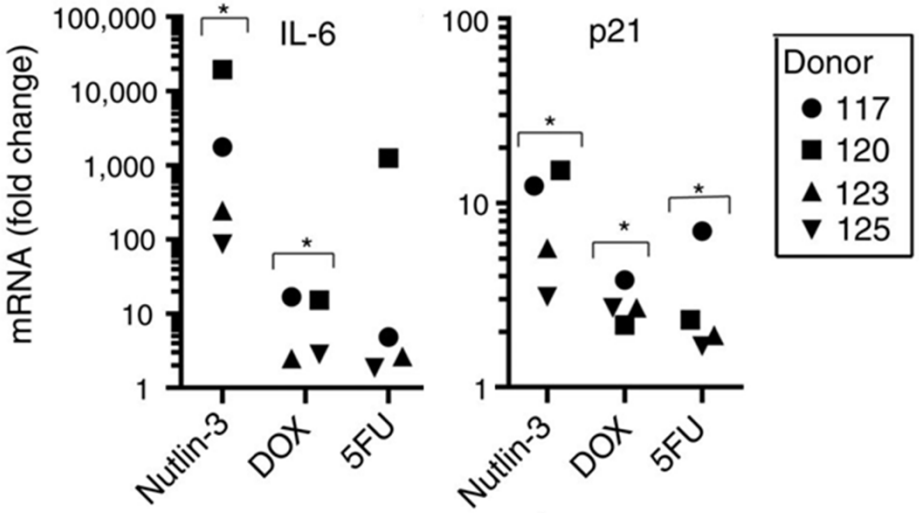
NF-kB and p53 relationship

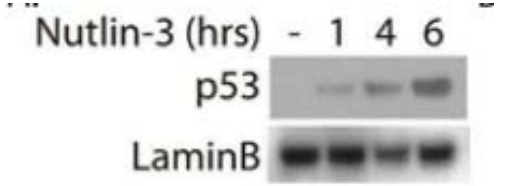
- Key transcription factors in stress responses
- NF-kB often constitutively activate in cancers
- p53 often inactive or altered in cancers

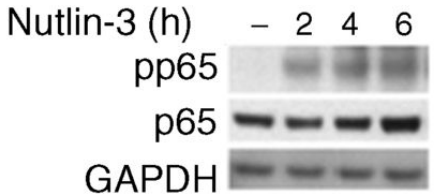


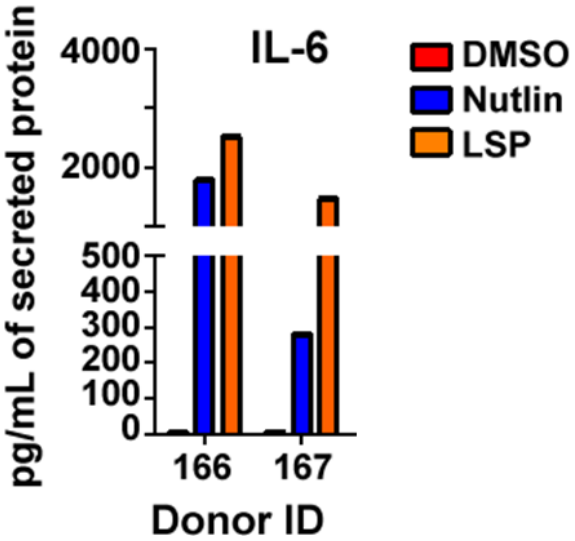
DNA damage and p53 activation in macrophages induces pro-inflammatory genes *independent of a ligand*

Monocyte-derived macrophages



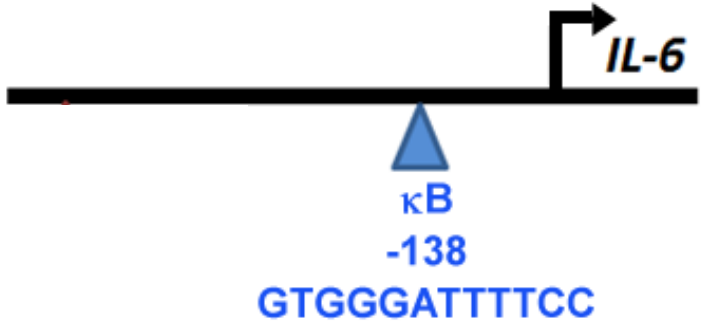






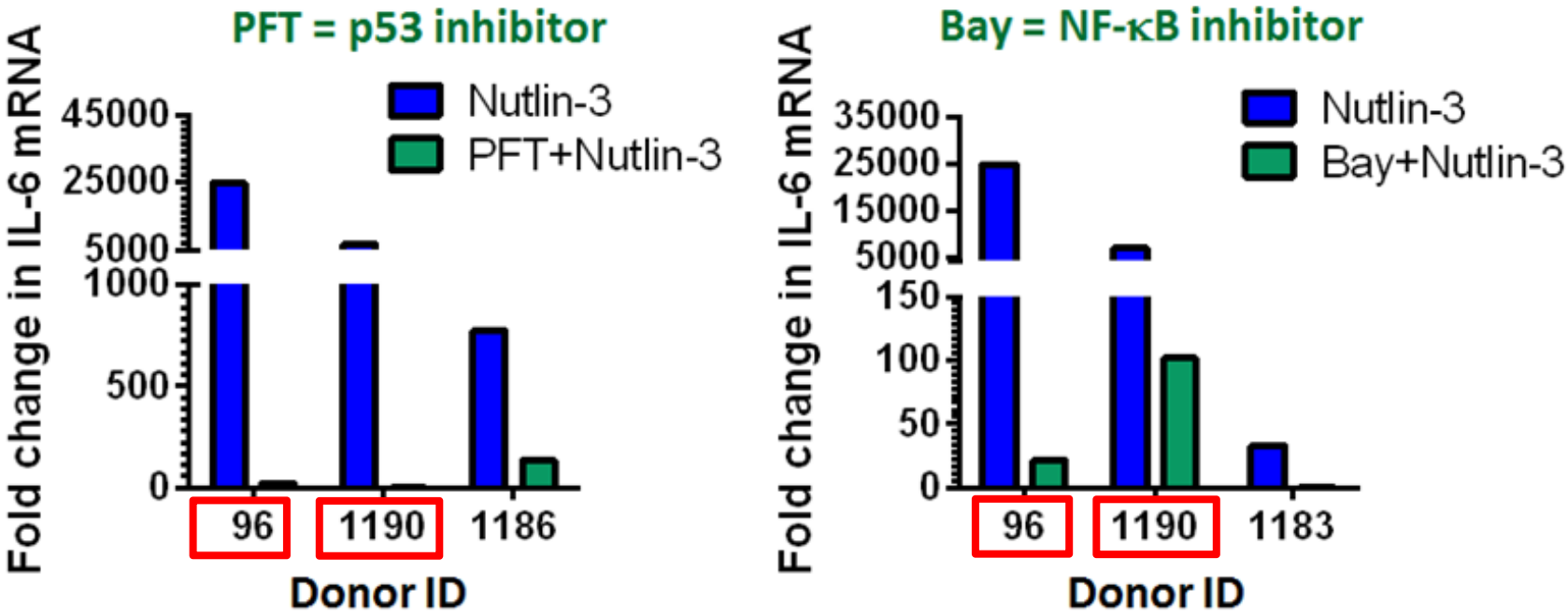
J. Lowe et al, Cancer Res 2014

NF-kB bind to the IL-6 promoter region



J. Lowe et al, Cancer Res 2014

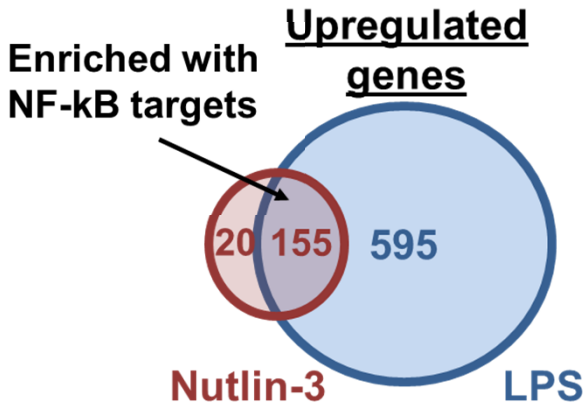
p53 and NF-kB are required for Nutlin-3-induced IL-6 expression



J. Lowe et al, Cancer Res 2014

p53 and NF-kB co-regulate a subset of pro-inflammatory genes in blood derived macrophages

The highest induced genes by Nutlin-3 are immune-related

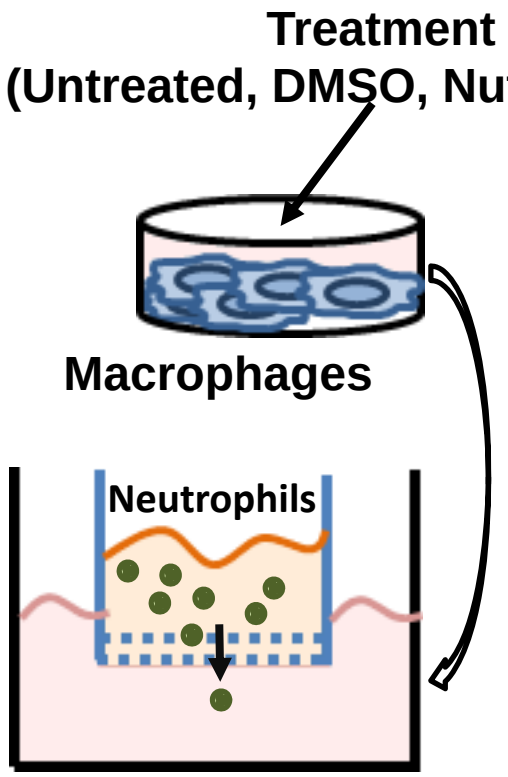


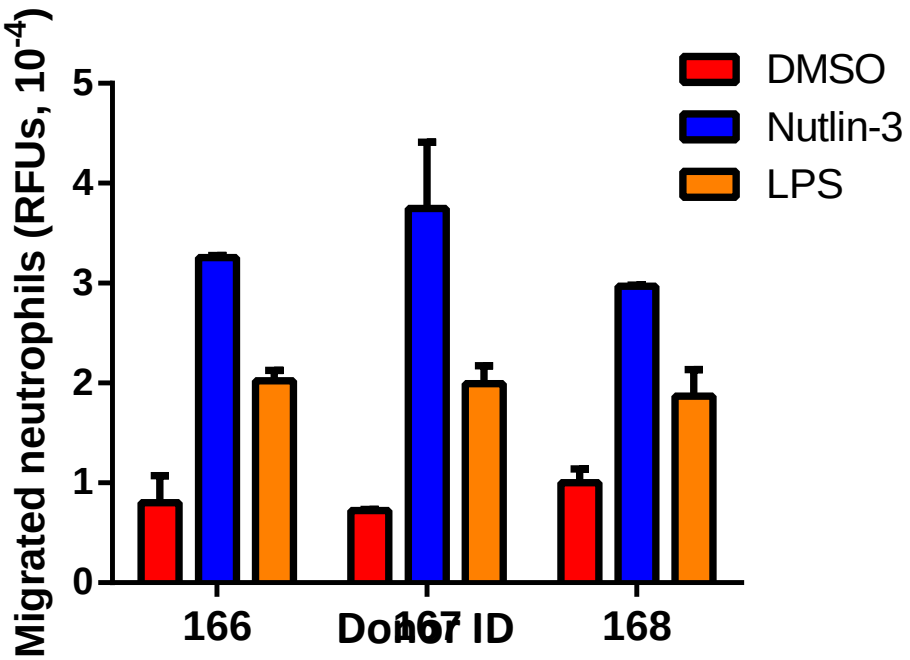
Gene	Function
CXCL1	Chemokine; immune response
CCL20	Chemokine; immune response
IL8	Chemokine; immune response
TNFAIP6	Ser protease inhibitor; inflammation
CCL4	Chemokine; immune response
CXCL3	Chemokine; immune response
PTX3	Phagocytosis; inflammatory response
CXCL2	Chemokine; immune response
IL1B	Cytokine; immune response
HS3ST3B1	Heparan sulfate biosynthetic enzyme
NFKBIZ	NF-kB inhibitor; inflammatory response
TNF	Cytokine; immune response
IL6	cytokine; immune response

*** Most chemokine genes had associated p53REs and kB sites**

J. Lowe et al, Cancer Res 2014

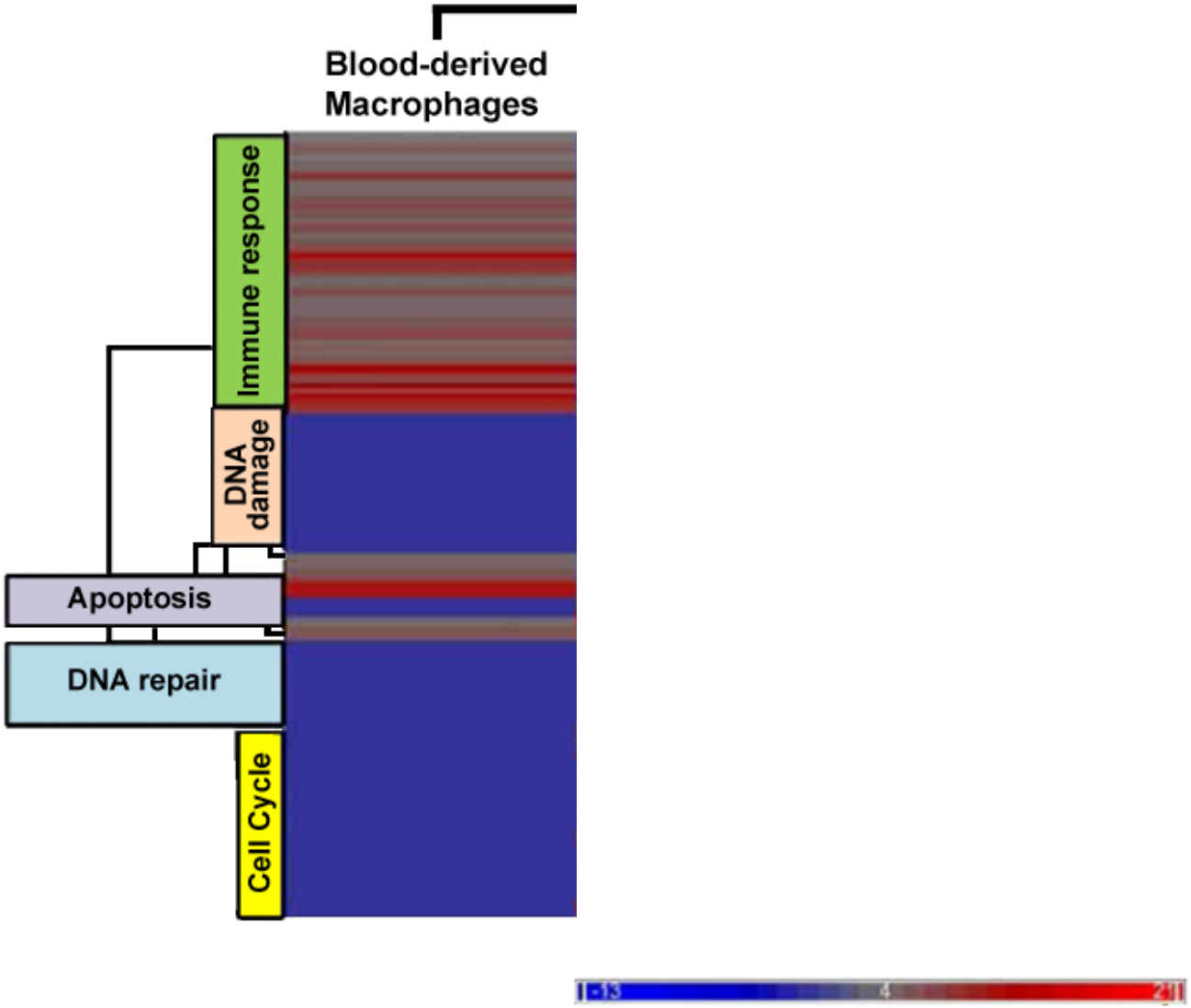
Chemokines secreted by Nutlin-3 treated macrophages enhance neutrophil migration





Lowe et al, Cancer Res 2014

Clustering of physiological processes enriched by p53 induction after nutlin treatment



Nguyen, Lowe, Shatz gene expression data; P. Bushell analysis

The human tumor suppressor p53 links chromosomal stress and immune responses

Overall summary

New roles for p53 "guardianship" in human biology in response to DNA stressors

p53 is an inducer and/or enhancer of immune/inflammatory response

- ***modulate the expression and signaling of Toll-like receptors***
- ***affect cytokines and chemokines production***
- ***can directly target the expression of several immune related genes***

Cell type specificity: macrophages, lymphocytes, cancer cells

p53 impact on immune/inflammatory responses often depends on an interaction with NFκB

Current/ Future directions

- **Modulation of immune responses by WT and mutant p53 in response to environmental stresses**

- **Variability in the p53 transcriptional network in humans**

  p53 ability to drive TLR8 expression in primary human cells depends on SNP in response element of TLR8 promoter
  (NIEHS Environmental Polymorphism Registry study)

- **Characterization of the p53 transcriptional network /p53 cistrome in primary human T lymphocytes (Thuy-Ai Nguyen)**

  Meta-analysis of p53ChIPseq and p53 transcriptome studies

- **Characterization of the transcriptional responses to p53 induction in human primary monocytes and macrophages (Maria Shatz)**

ACKNOWLEDGMENTS

Chromosome Stability Group

Mike A. Resnick
Julie Lowe
Maria Shatz
Thuy-Ai Nguyen
Carl W. Anderson
Joyce Snipe

NIEHS

Michael Fessler (IID)
Stavros Garantziotis (IID)

Raja Jothi (ESB)
Pierre Bushel (Biostatistics)
David Fargo (Bioinformatics)

External Collaborations

University of Trento, Italy
Alberto Inga

NIH Intramural Sequencing Center (NISC)
Robert Blakesley
Alice Young