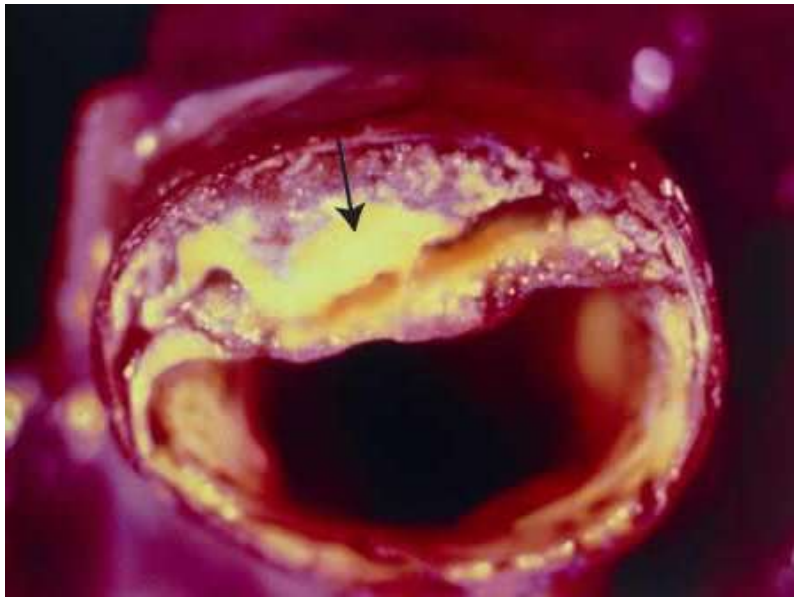
ADAM MICKIEWICZ UNIVERSITY IN POZNAŃ
Faculty of Biology

Diagnostics & Therapeutics of Atherosclerosis



Hans Bluyssen
09-12-2020

Atherosclerosis

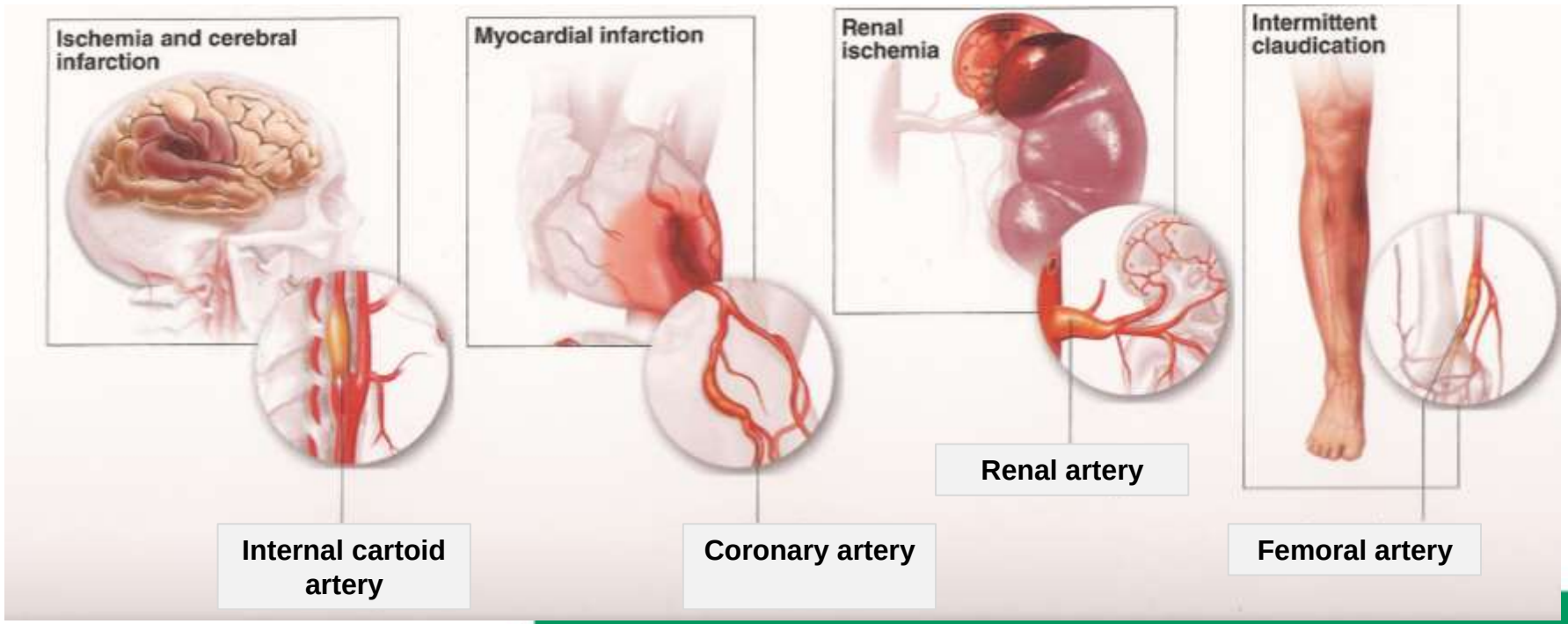


Fig. Atherosclerosis complications. Dr Philip Barlow Mills FCP (SA).

Atherosclerosis – vascular inflammation

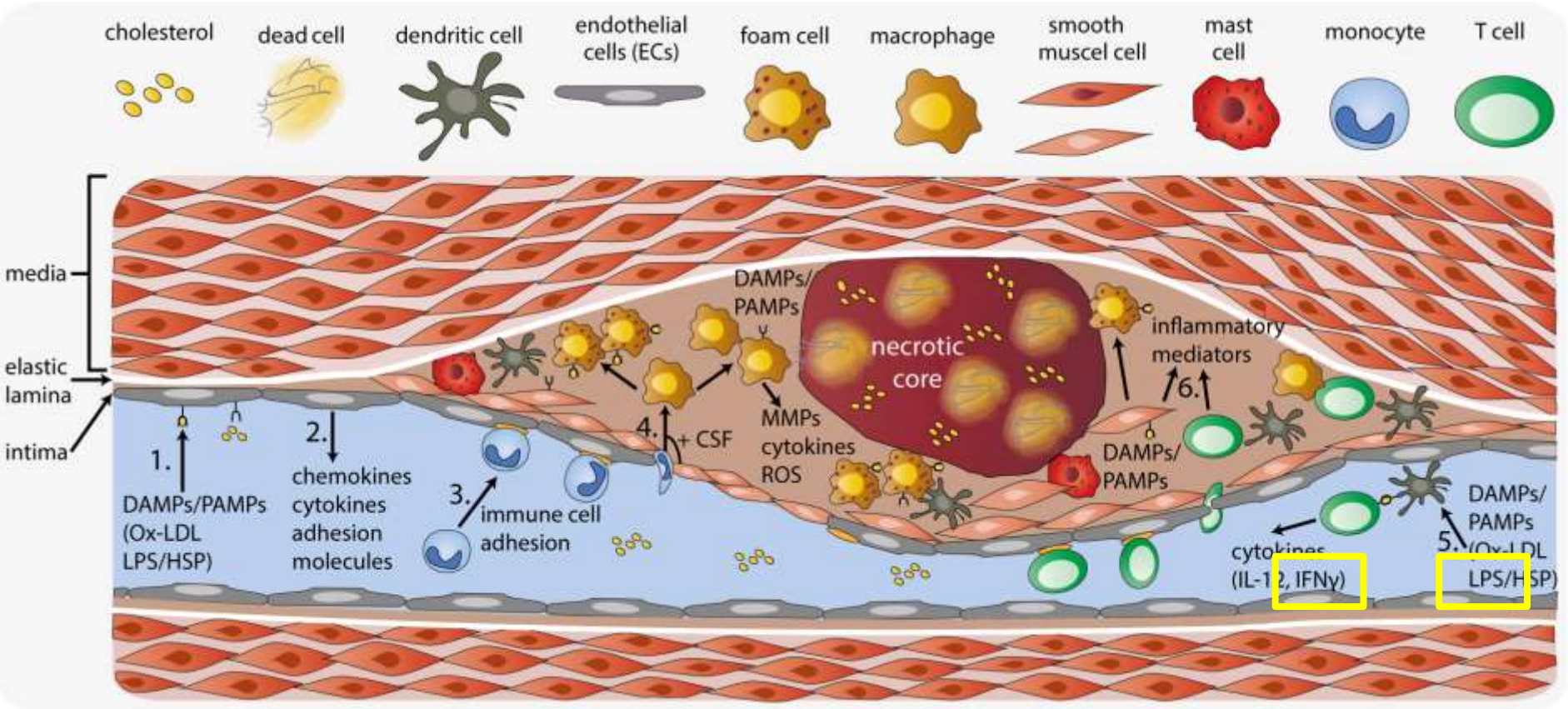
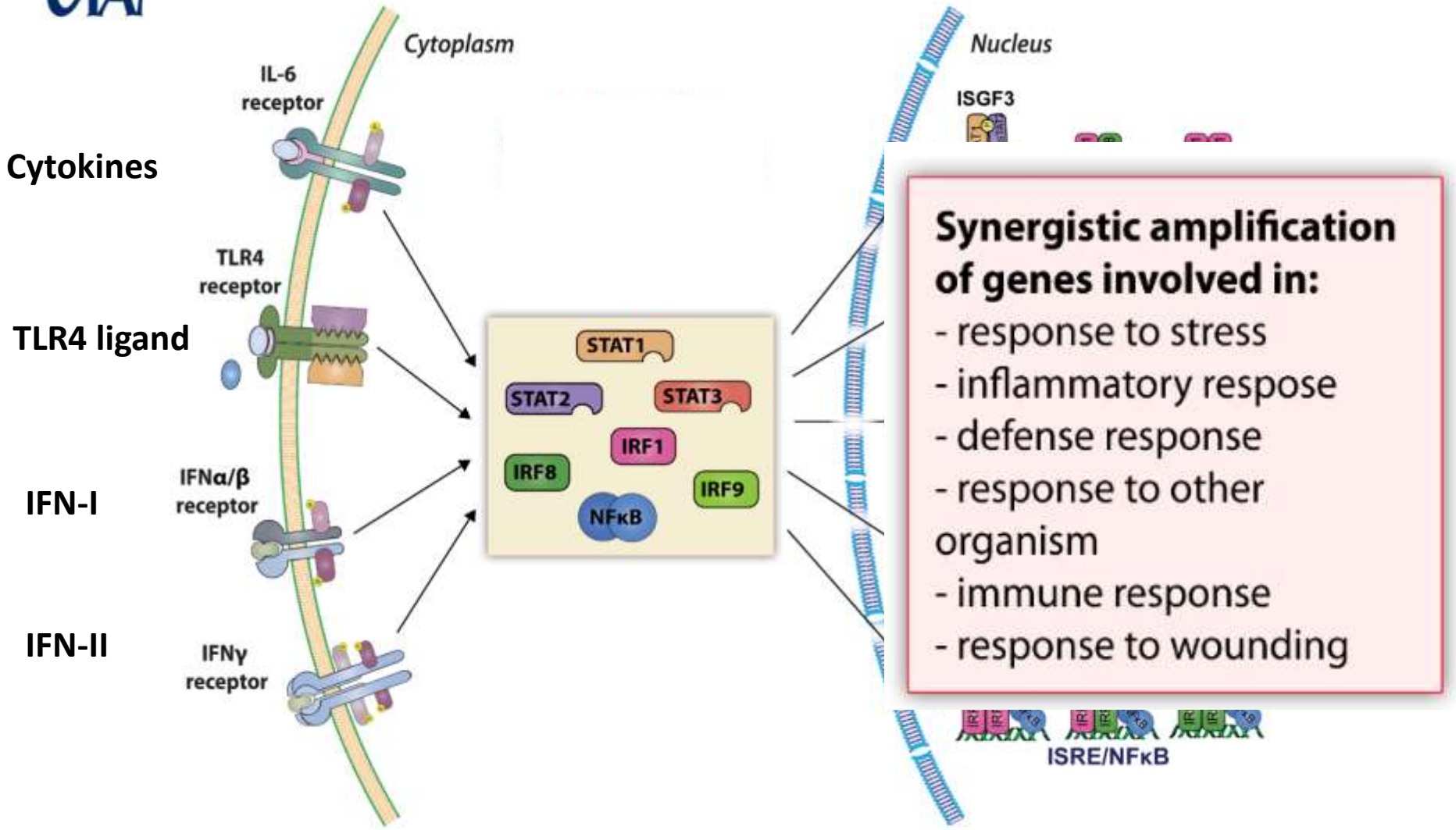


Fig. Atherosclerotic plaque. Chmielewski, Piaszyk-Borychowska et al., Int Rev Immunol, 2016.

STAT-dependent signal integration in inflammation



Cytokines

TLR4 ligand

IFN-I

IFN-II

Szeląg et al., Oncotarget, 2016

How to analyze the transcriptome?

RNA-seq experiment outline

WT C57BL6

Aorta enzymatic digestion

Bone marrow differentation with M-CSF

Bone marrow differentiation with GM-CSF

Vascular smooth muscle cells

Macrophages

Dendritic cells

Treatment (3 replicates):

8h IFNγ

4h LPS

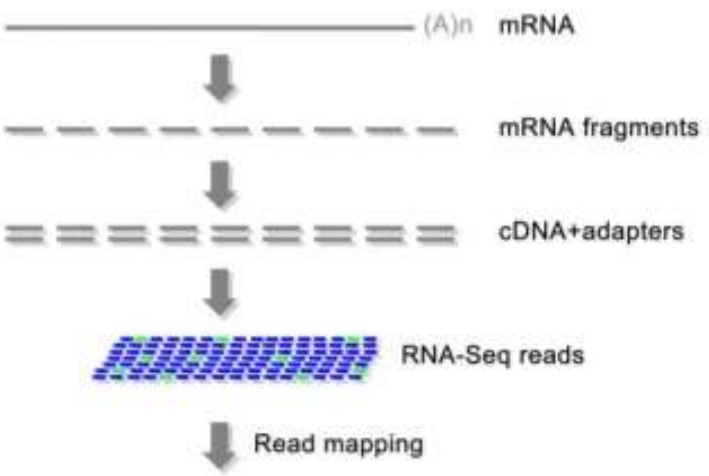
8h IFNγ + 4h LPS

RNA-seq

Piaszyk-Borychowska et al., submitted

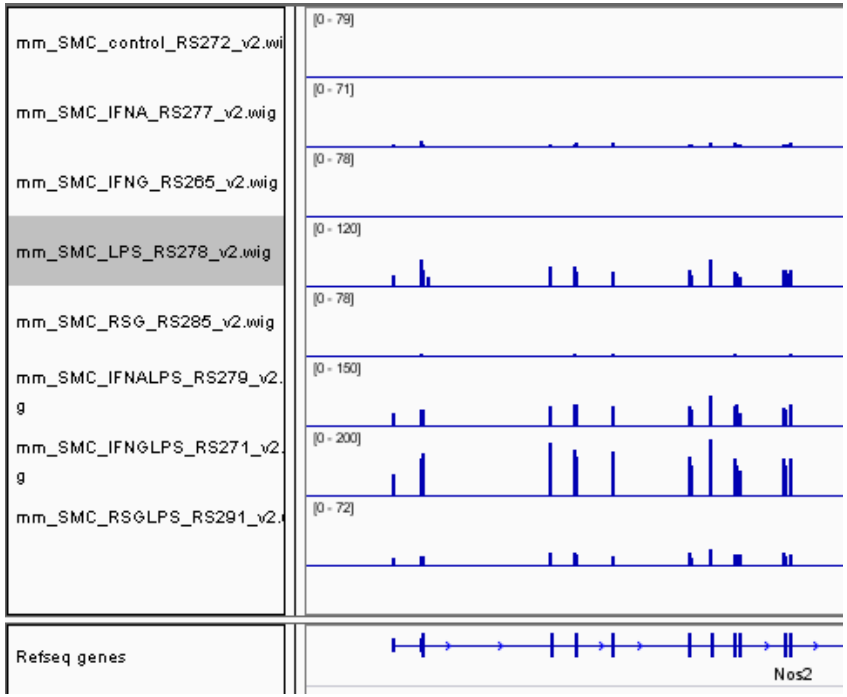
RNA-seq Work Flow

Illumina HiSeq

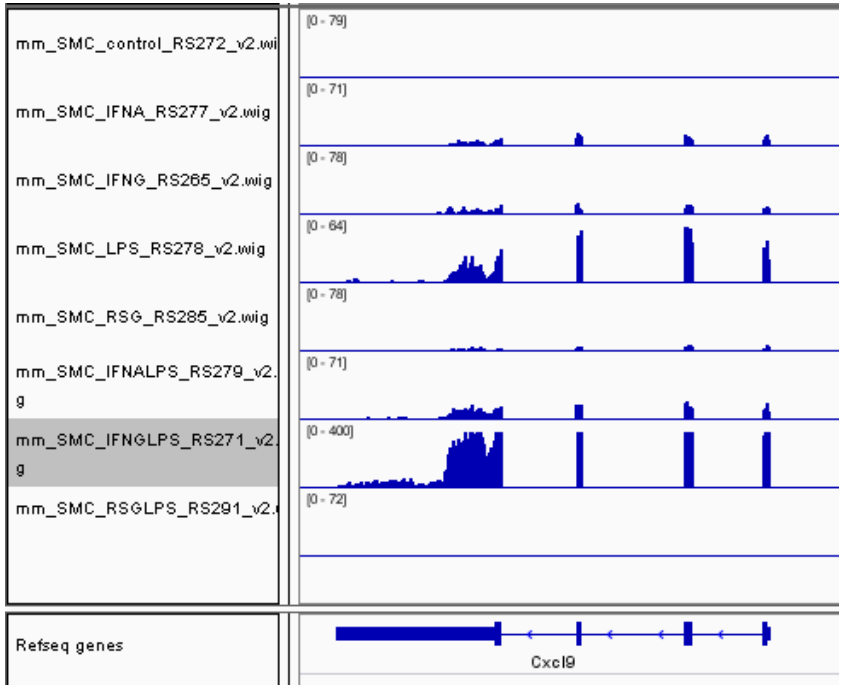


Blencowe B J et al. Genes Dev. 2009;23:1379-1386

IFNγ and TLR Signal integration in SMCs: RNAseq



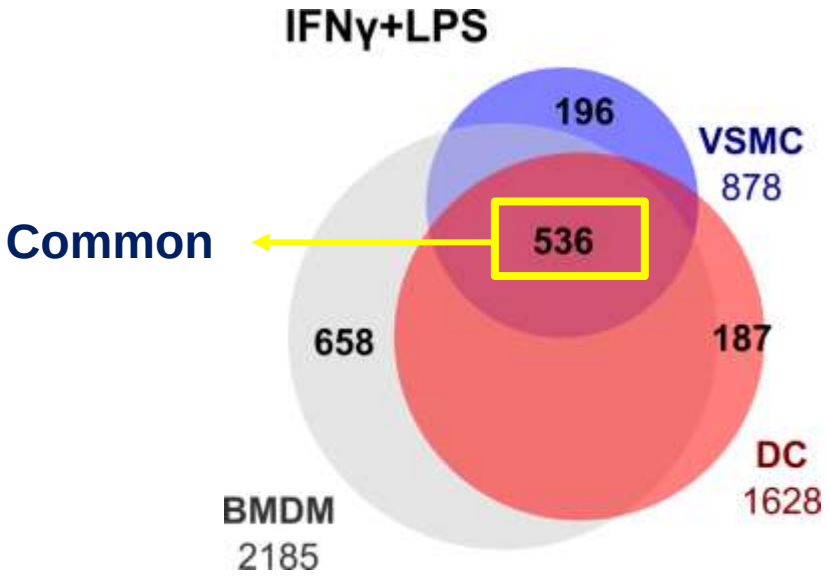
IGV view SMC NOS2 (*.wig files)

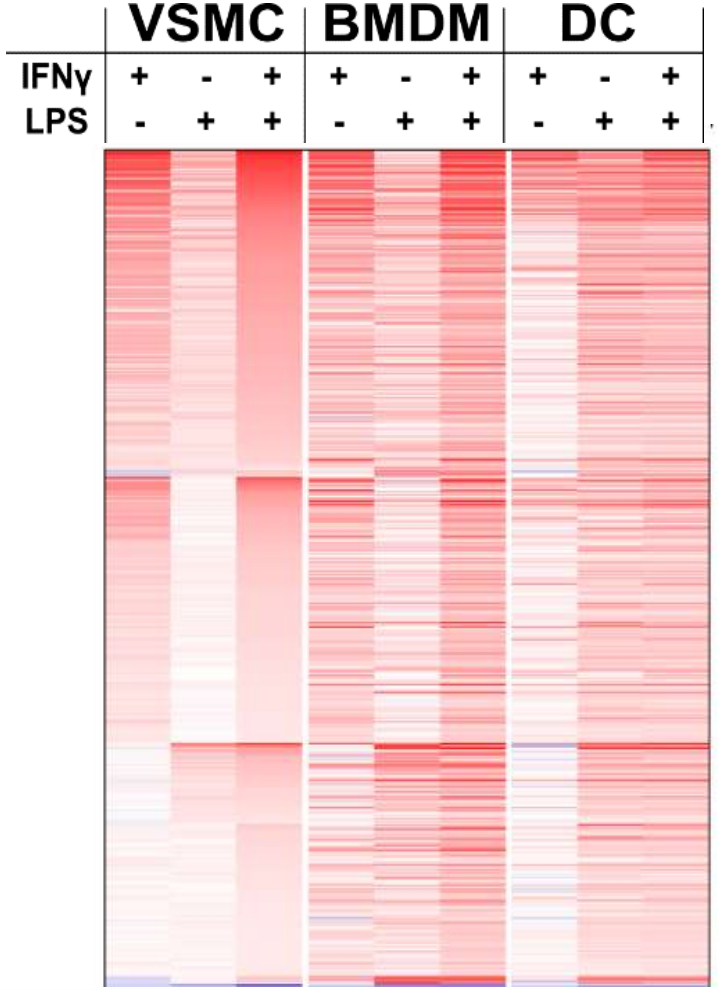


IGV view SMC Cxcl9 (*.wig files)

Piaszyk-Borychowska et al., submitted

Commonly Up-regulated genes in response to IFNγ and LPS





Piaszyk-Borychowska et al., submitted

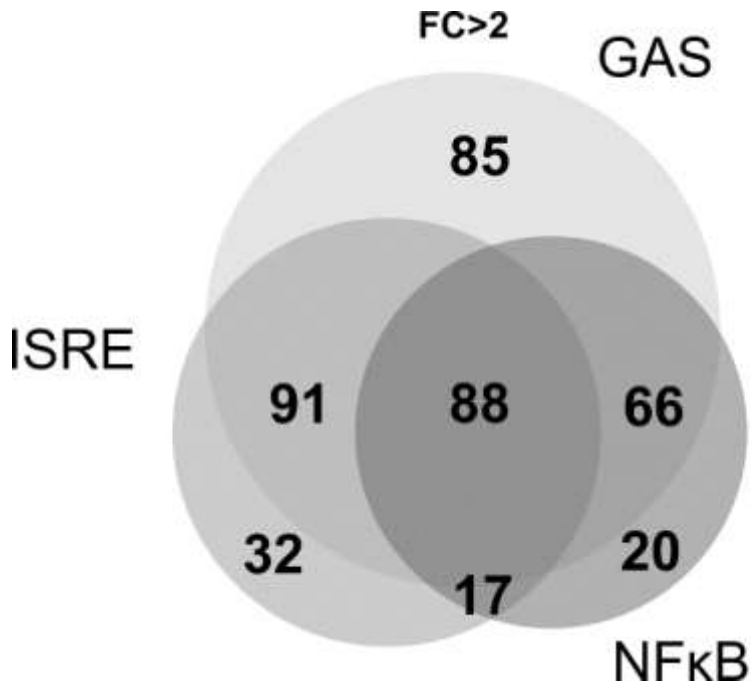
Commonly Up-regulated genes in response to IFNγ and LPS: top 20

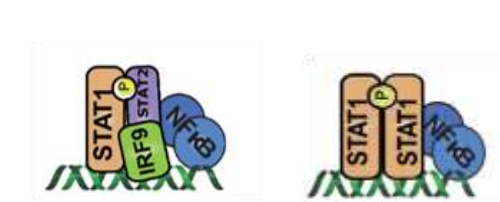
Inflammatory Gene Expression = Amplified

No	Gene symbol	VSMC			BMDM			DC		
		IFNγ	LPS	IFNγLPS	IFNγ	LPS	IFNγLPS	IFNγ	LPS	IFNγLPS
1	Cxcl9	82,2	4,0	2380,5	2401,1	18,7	3915,9	898,0	97,4	2682,0
2	F830016B08Rik	1272,1	6,4	2306,6	191,4	2,1	253,2	88,1	13,6	132,7
3	Gm4841	1087,3	9,0	1650,3	408,2	3,0	569,3	130,5	44,0	215,1
4	Nos2	1,8	90,9	933,3	464,2	60,9	5706,1	39,4	376,3	1809,3
5	BC023105	600,8	19,9	909,4	140,5	6,2	204,7	146,8	87,1	320,2
6	Gbp4	304,3	16,4	795,8	341,8	4,0	648,9	63,2	31,4	69,6
7	Iigp1	687,7	9,2	779,3	521,6	12,9	648,6	590,7	194,7	607,2
8	Ubd	95,6	5,8	655,1	65,9	1,1	124,8	214,5	1,0	258,7
9	Gbp10	315,9	21,1	588,2	200,1	17,7	364,0	33,4	33,6	48,6
10	Gbp9	304,8	22,9	586,1	25,6	1,2	31,6	8,9	13,3	10,3
11	Gbp6	266,1	23,5	555,3	181,8	17,4	332,5	32,2	35,8	49,8
12	Serpina3f	200,1	13,0	529,6	1442,5	16,9	2126,6	533,3	229,0	1133,6
13	Gbp11	302,3	13,6	482,7	149,6	6,7	243,9	40,3	24,2	44,1
14	Gm12250*	502,9	3,6	477,8	31,4	17,0	40,3	13,8	24,9	21,4
15	Gbp8	215,5	12,9	405,2	47,8	1,5	57,8	13,9	3,1	6,2
16	Ciita*	704,4	2,0	376,5	9,4	-2,6	3,6	5,4	1,3	2,5
17	Cxcl10	49,8	4,6	364,9	211,7	179,5	829,0	19,5	580,2	237,5
18	Gbp1	295,0	17,5	364,8	375,8	16,0	428,3	63,8	21,9	56,2
19	Gja4	82,3	1,6	329,9	90,1	1,0	145,3	14,8	6,9	45,9
20	Gm4951	300,1	6,9	327,2	74,9	4,5	74,4	59,5	45,3	80,5

Piaszyk-Borychowska et al., submitted

Commonly Up-regulated genes in response to IFNγ and LPS: promoter analysis





STAT1 & p65 mediated Signal integration?

Promoter region: -950 +50 bp

Piaszyk-Borychowska et al., submitted

STAT1 ChIP-seq experiment outline

WT C57BL6

Aorta enzymatic digestion

Vascular smooth muscle cells

Treatment (2 replicates):

4h LPS

8h IFNγ

4h LPS + 8h IFNγ

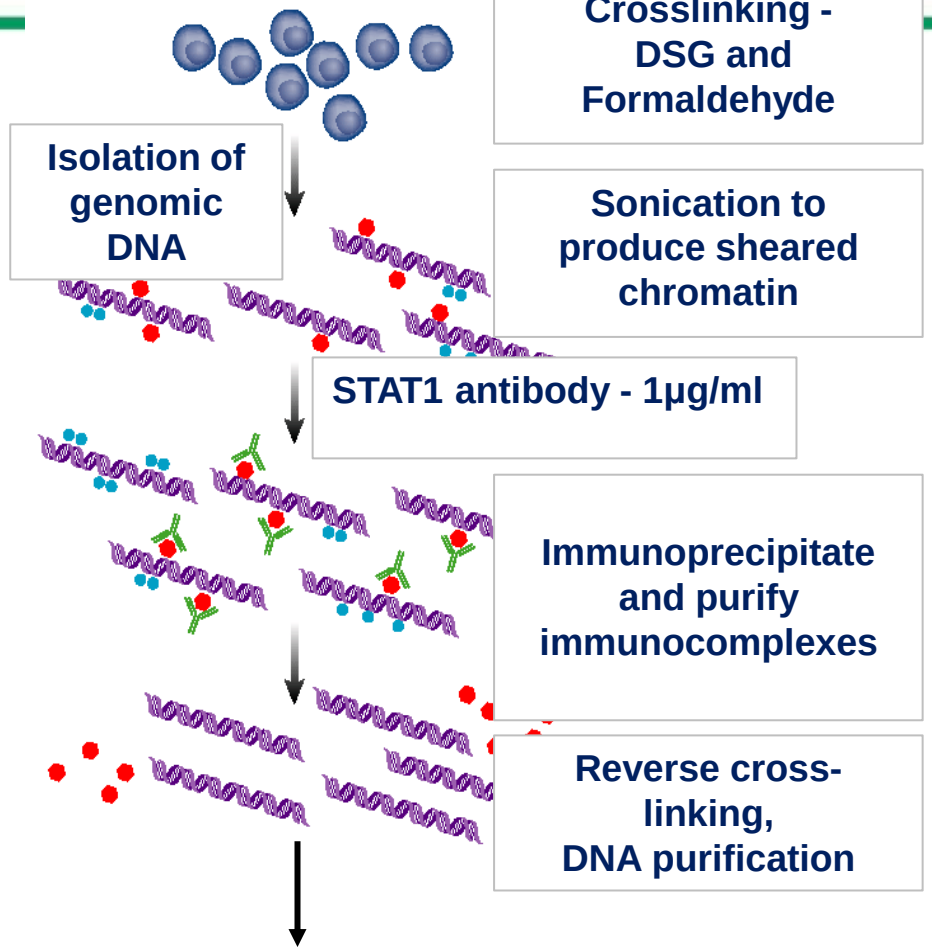
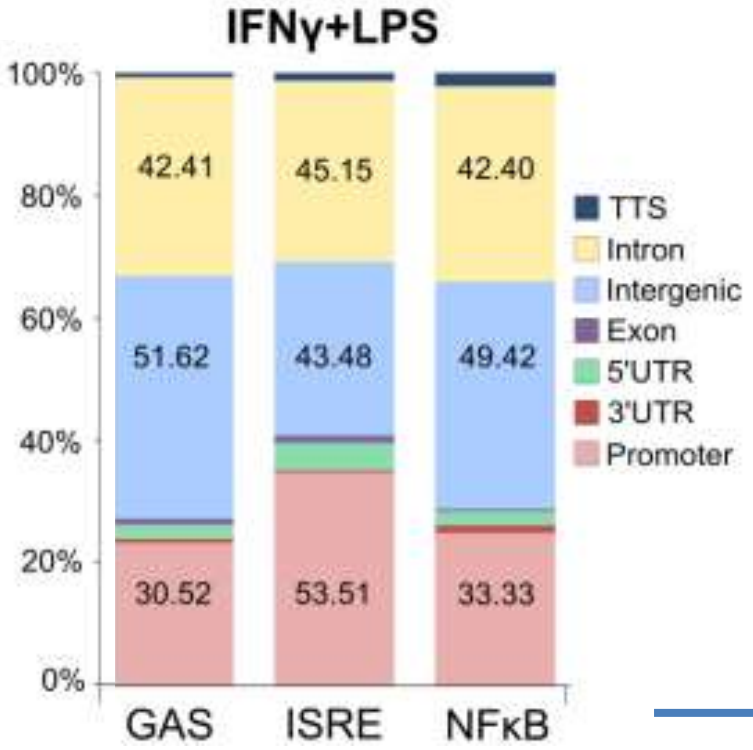


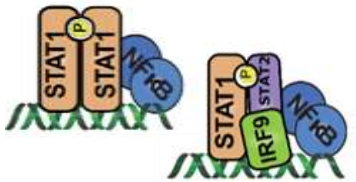
Fig. Nagy Lab modified Mandrup's ChIP protocol. Figure adapted from Nature Methods - 4: 613 – 614. 2007.

Commonly IFNγ/LPS Up-regulated genes: STAT1 & p65 binding



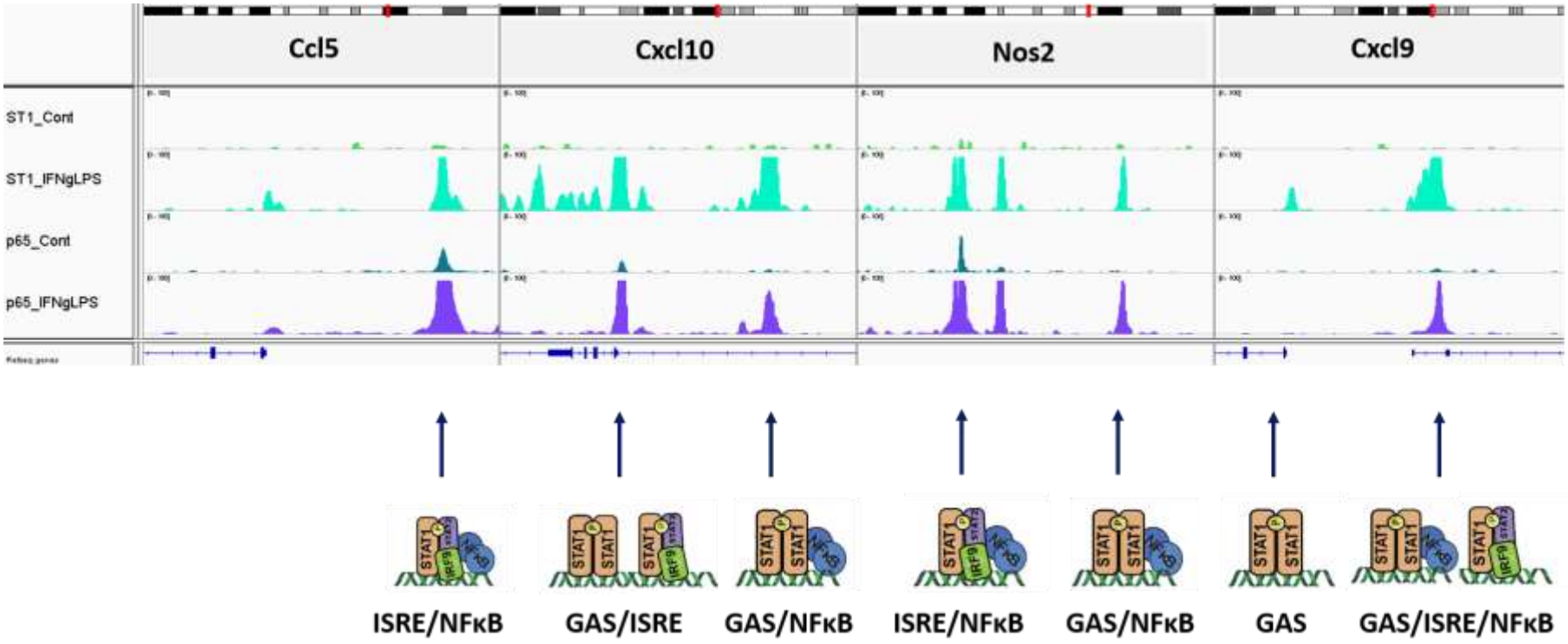
VSMCs

Binding mode	IFNγ+LPS
GAS	17
ISRE	45
NFκB	28
GAS-ISRE	53
GAS-NFκB	40
ISRE-NFκB	59
GAS-ISRE-NFκB	178



Piaszyk-Borychowska et al., submitted

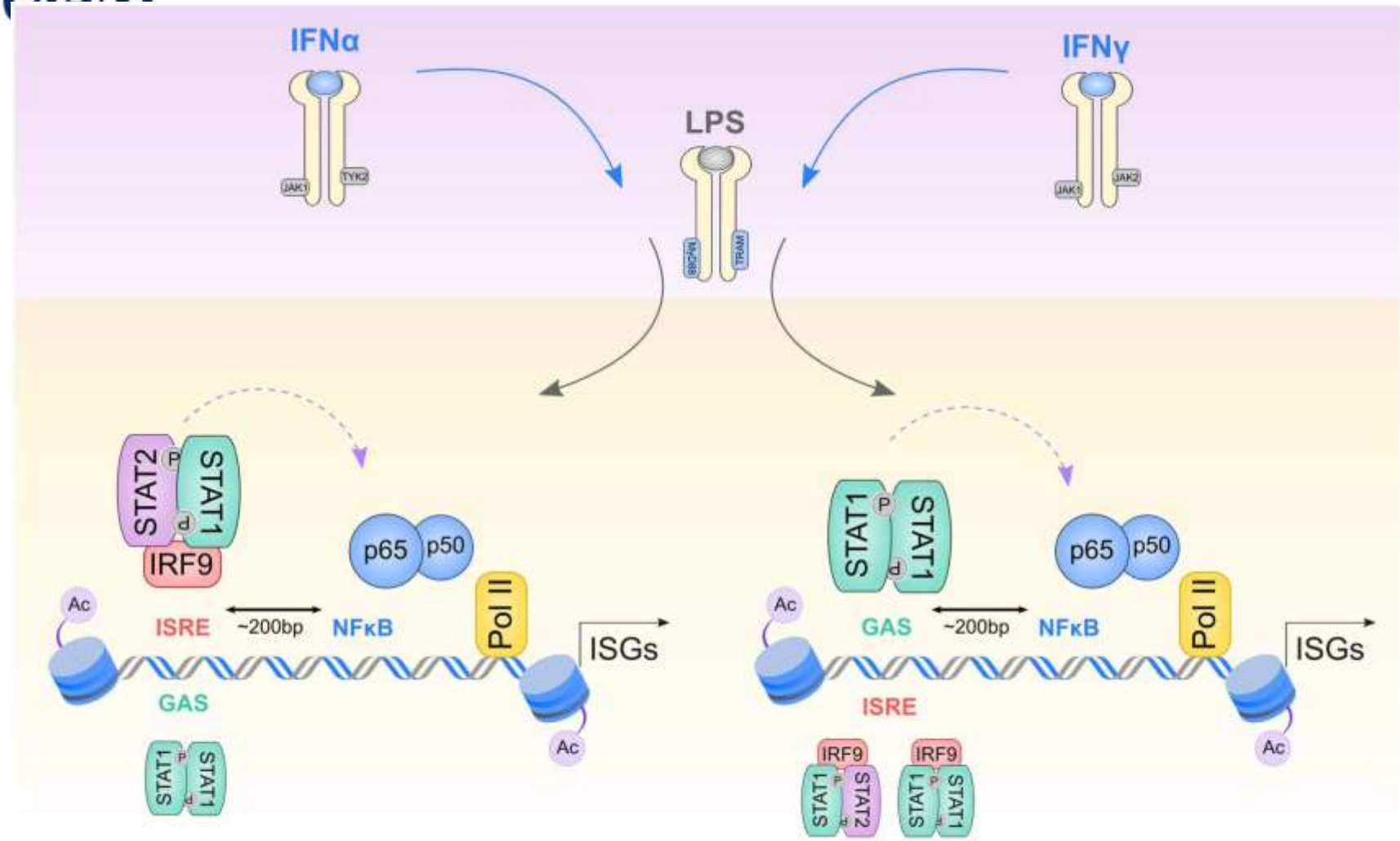
Commonly IFNγ/LPS upregulated genes: STAT1 + p65 binding



Different "Binding Modes"

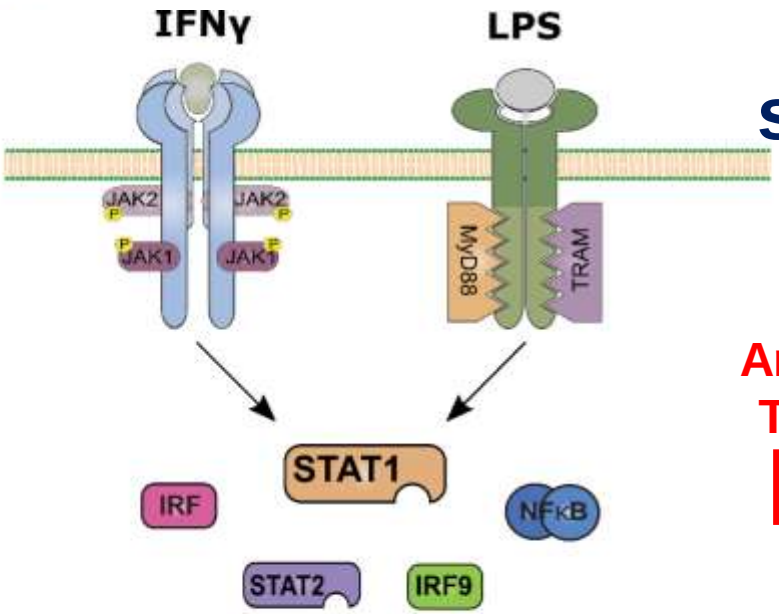
Piaszyk-Borychowska et al., submitted

STAT1-dependent epigenetic changes & nearby NFκB binding

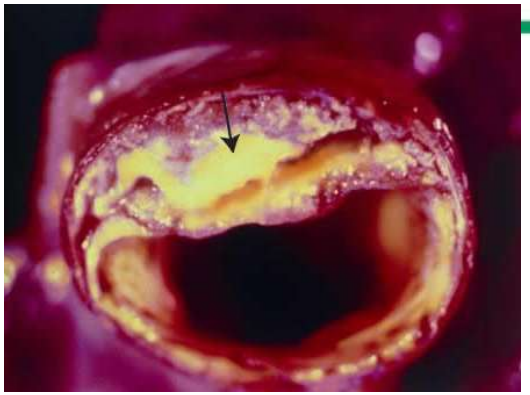


Piaszyk-Borychowska et al., 2019 Frontiers in Immunology

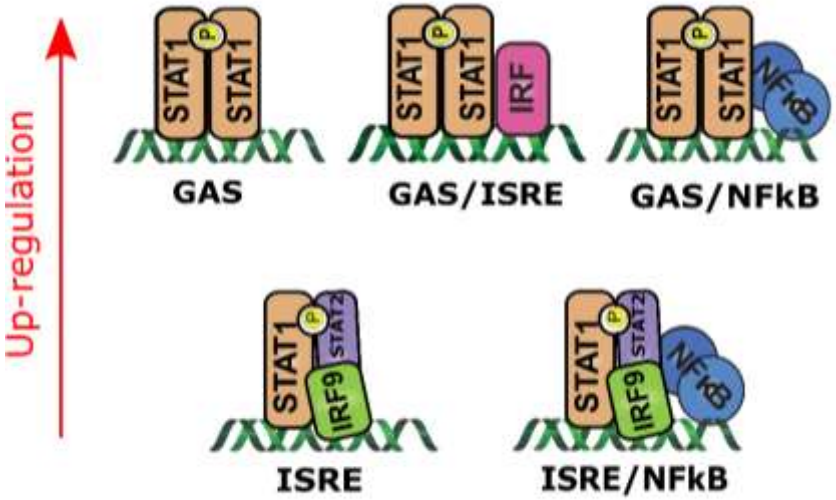
IFNγ/LPS mediated signal integration in CVD



Anti-CVD Therapy



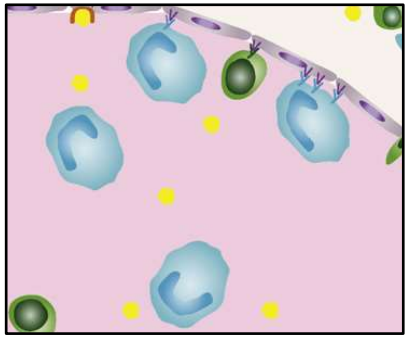
Nucleus



Synergistic amplification of genes involved in:

- response to stress
- inflammatory respose
- defense response
- response to other organism
- immune response
- response to wounding

Piaszyk-Borychowska et al., submitted

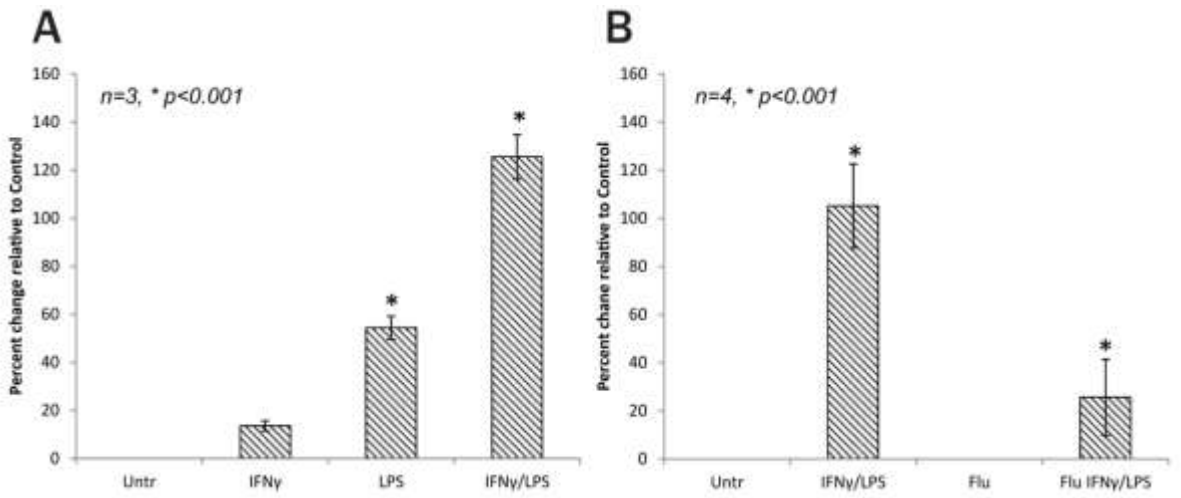


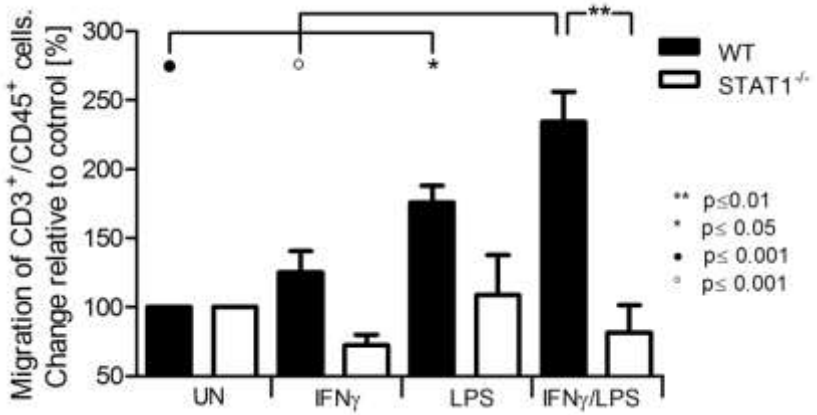
Crosstalk results in increased monocyte adhesion and splenocytes migration

Monocyte-EC adhesion

Hallmarks of Vascular inflammation

Splenocyte migration



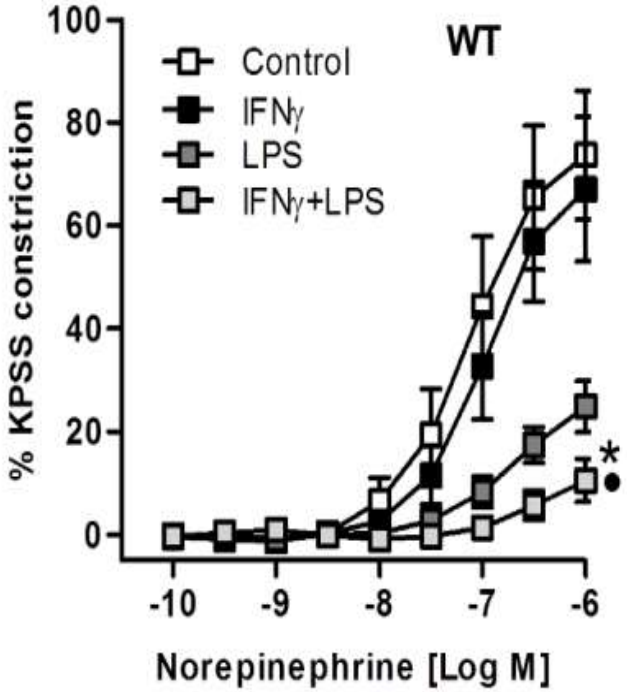


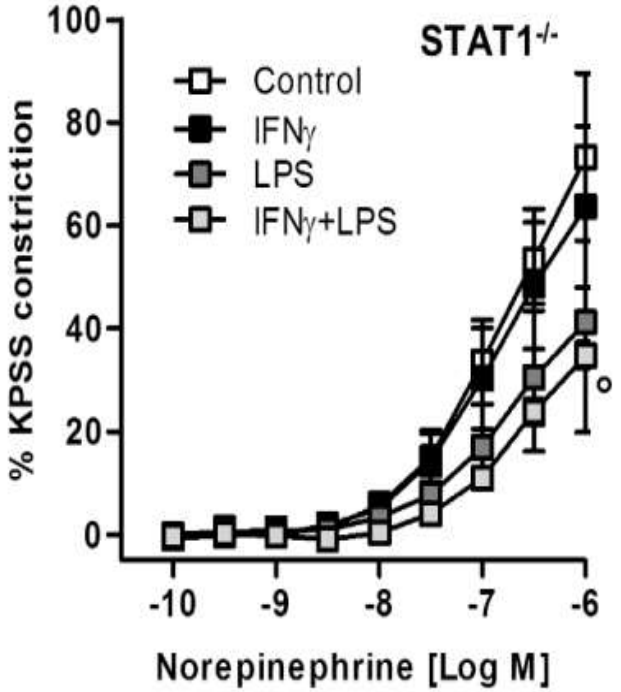
Sikorski, Chmielewski et al., AJP 2011; Chmielewski et al., Plos One 2014

Abolished response to norepinephrine in aortic rings stimulated with IFNγ and LPS

Vessel Contraction

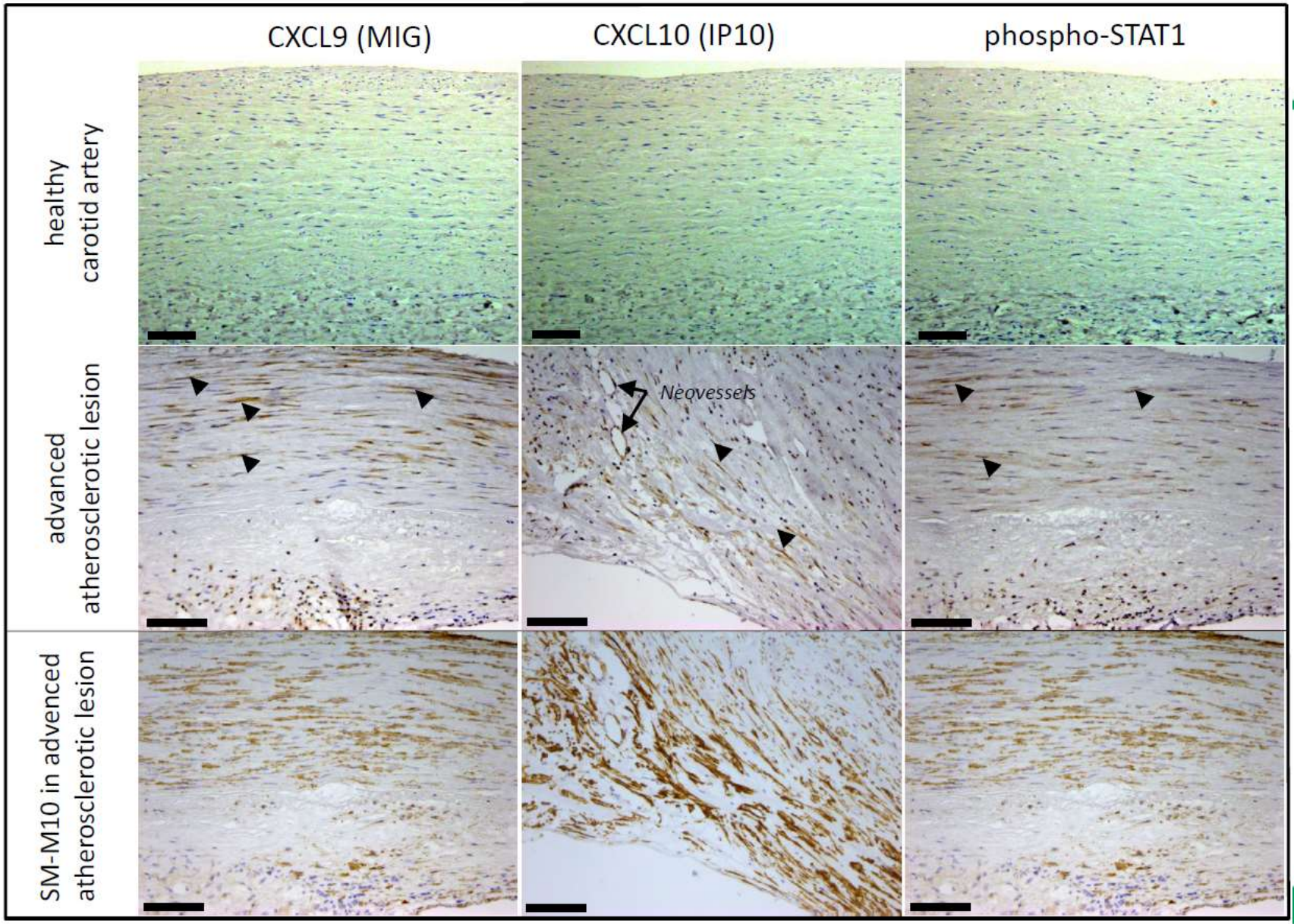
Mesenteric arteries





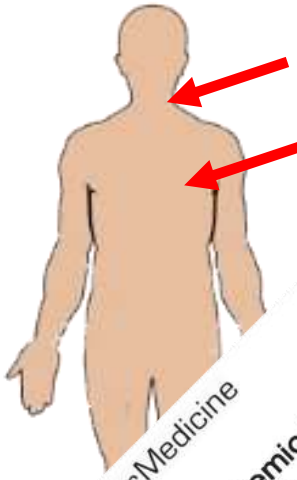
Chmielewski et al., Plos One 2014

pSTAT1, CXCL9 and -10 in human carotid plaque SMCs



Chmielewski et al., PlosOne 2014

Atherosclerotic plaque transcriptomes: Data Mining



Molecular Medicine

Prediction of Ischemic Events on the Basis of Transcriptomic and Genomic Profiling in Patients Undergoing Carotid Endarterectomy

Lasse Folkersen,[1] Jonas Persson,[1,2] Johan Ekstrand,[3] Hanna E Agardh,[1] Göran K Hansson,[1] Anders Gabrielsen,[3] Ulf Hedin,[3] and Gabrielle Paulsson-Berne[1]

OPEN ACCESS Freely available online

PLoS GENETICS

Multi-Organ Expression Profiling Uncovers a Gene Module in Coronary Artery Disease Involving Transendothelial Migration of Leukocytes and LIM Domain Binding 2: The Stockholm Atherosclerosis Gene Expression (STAGE) Study

Sara Hägg[1,2,3*], Josefin Skogsberg[1,3*], Jesper Lundström[1,2,3*], Peri Noori[1,2], Roland Nilsson[2,3], Hua Zhong[4], Shohreh Maleki[1], Ming-Mei Shang[1,3], Björn Brinne[2], Maria Bradshaw[1,2,3], Vladimir B. Bajic[5,6], Ann Samnegård[7], Angela Silveira[8], Lee M. Kaplan[9], Bruna Gigante[10], Karin Leander[10], Ulf de Faire[10], Stefan Rosfors[11], Ulf Lockowandt[12,13], Jan Liska[12,13], Peter Konrad[14], Rabbe Takolander[14], Anders Franco-Cereceda[12,13], Eric E. Schadt[4], Torbjörn Ivert[12,13], Anders Hamsten[8], Jesper Tegnér[1,2,3], Johan Björkegren[1,2,3*]

Carotid n=124
Coronary n=80
Controls n=80

Normalization
Statistical testing

CXCL10
VCAM1
CCL5
.....

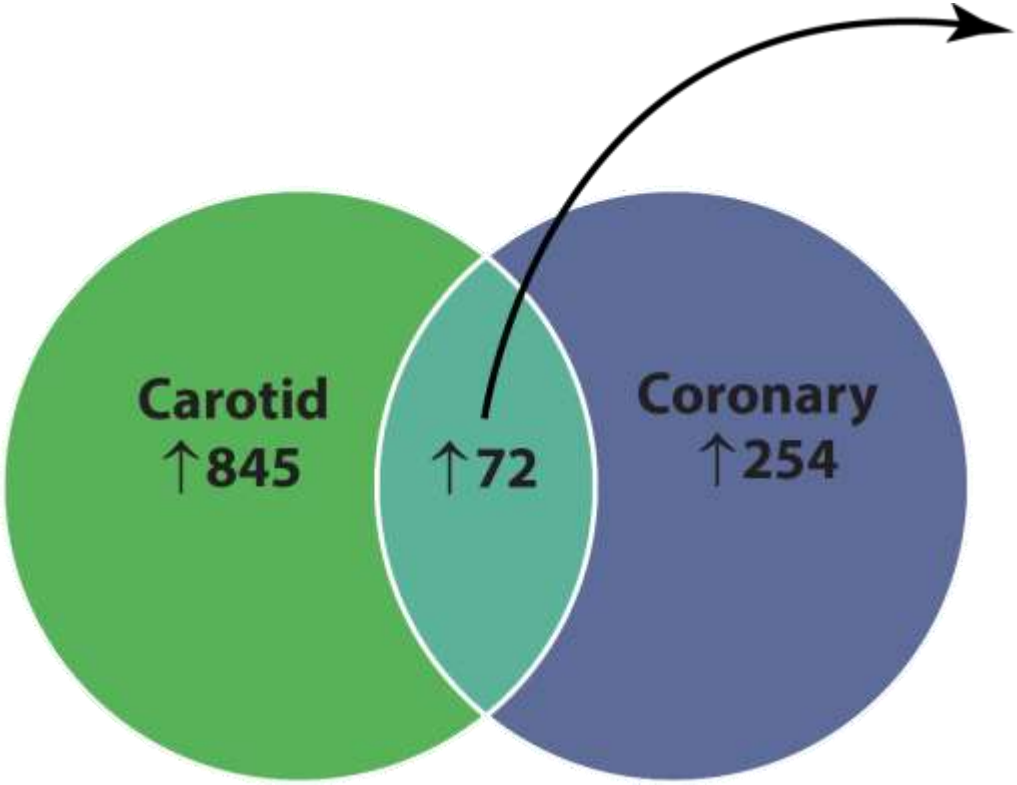
Analysis of promoters and functions

GO terms
functions
pathways
transcription factor binding sites

Sikorski et al., Int J Mol Sci 2014

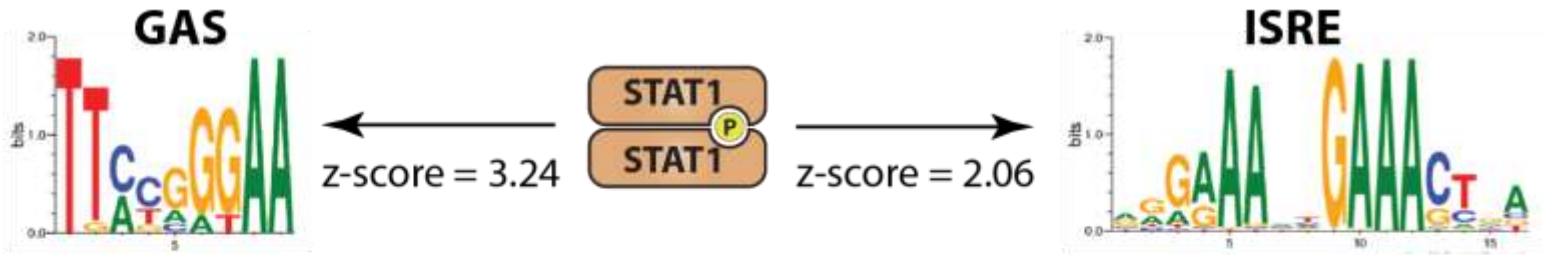
Chmielewski et al., PlosOne 2014

Carotid and coronary plaques share a gene signature

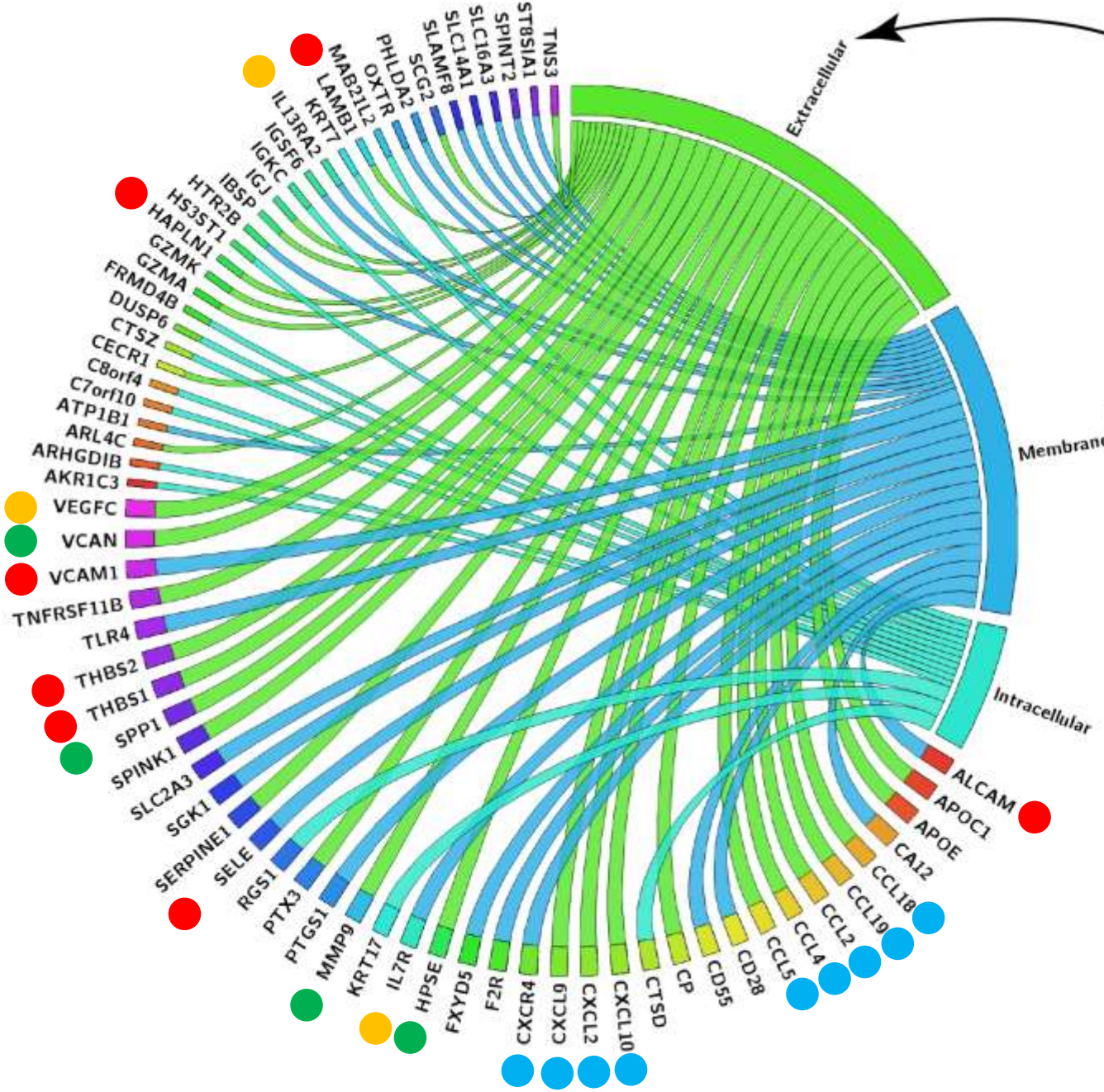


GO term	GO ID	p-value
cell chemotaxis	GO:0060326	2.75E-08
locomotion	GO:0040011	8.40E-08
leukocyte chemotaxis	GO:0030595	1.32E-07
chemotaxis	GO:0006935	1.45E-07
taxis	GO:0042330	1.45E-07
leukocyte migration	GO:0050900	1.80E-07
immune system process	GO:0002376	2.63E-07
cell migration	GO:0016477	4.33E-07
immune response	GO:0006955	7.70E-07
cell motility	GO:0048870	1.06E-06
cellular extravasation	GO:0045123	6.57E-05
cellular response to lipoprotein particle stimulus	GO:0071402	8.46E-05
cellular response to lipopolysaccharide	GO:0071222	1.67E-04
cellular response to interferon-gamma	GO:0071346	5.61E-03
response to interferon-gamma	GO:0034341	8.41E-03

Inflammatory genes upregulated in plaques can be regulated by STAT1



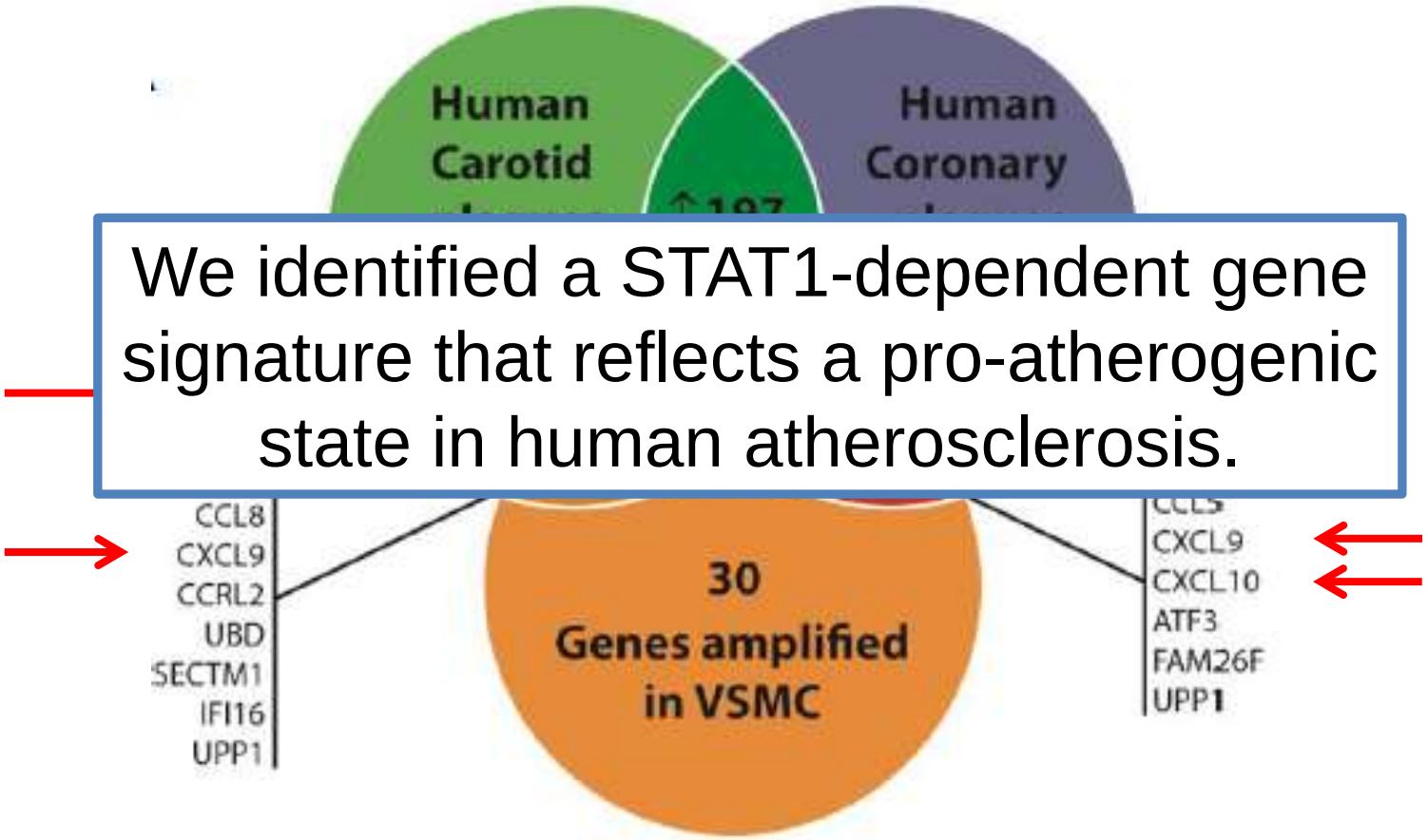
Genomatix



Potential diagnostic markers of plaque development in the patient

Prepared with Circos

IFNγ/LPS STAT1-target genes: Biomarkers in CAD?

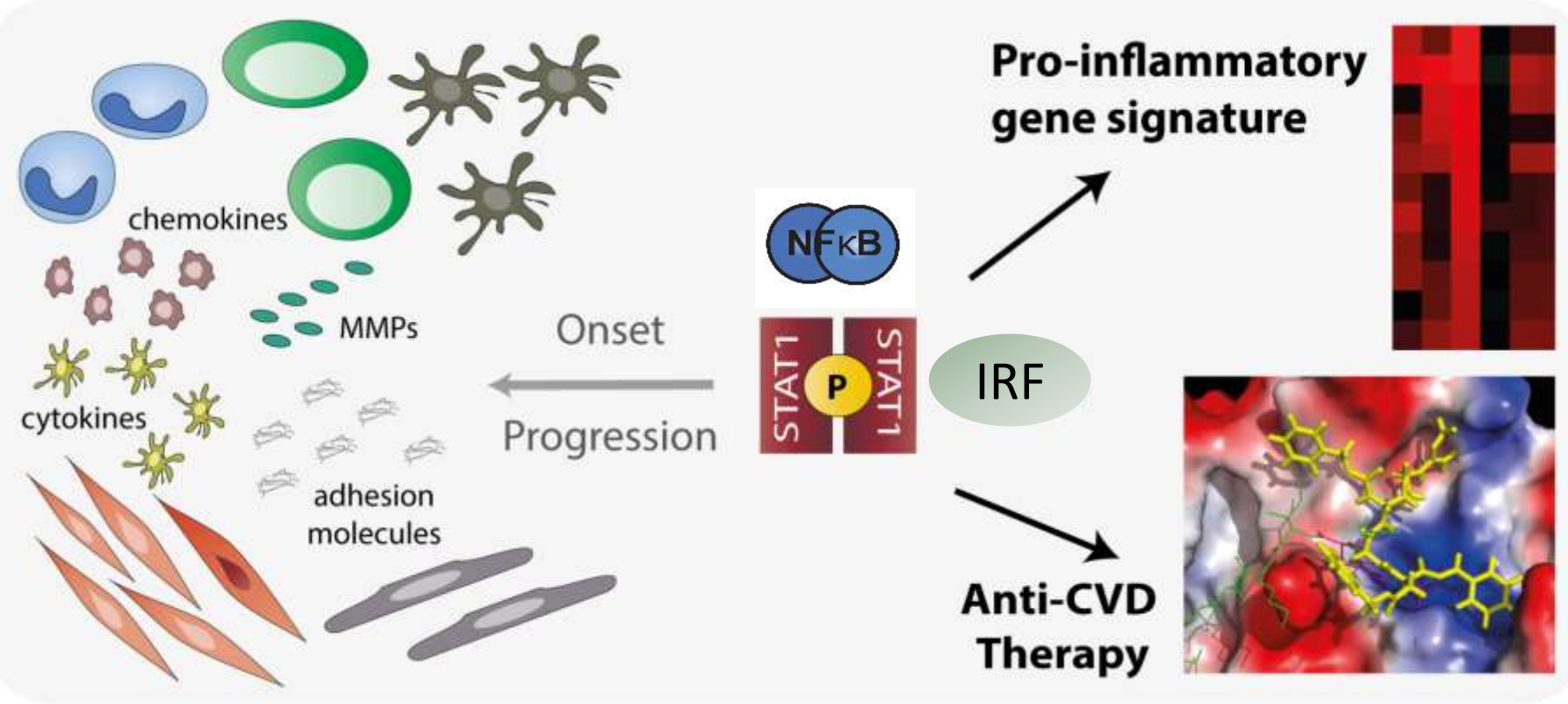


We identified a STAT1-dependent gene signature that reflects a pro-atherogenic state in human atherosclerosis.

Sikorski et al., Int J Mol Sci 2014

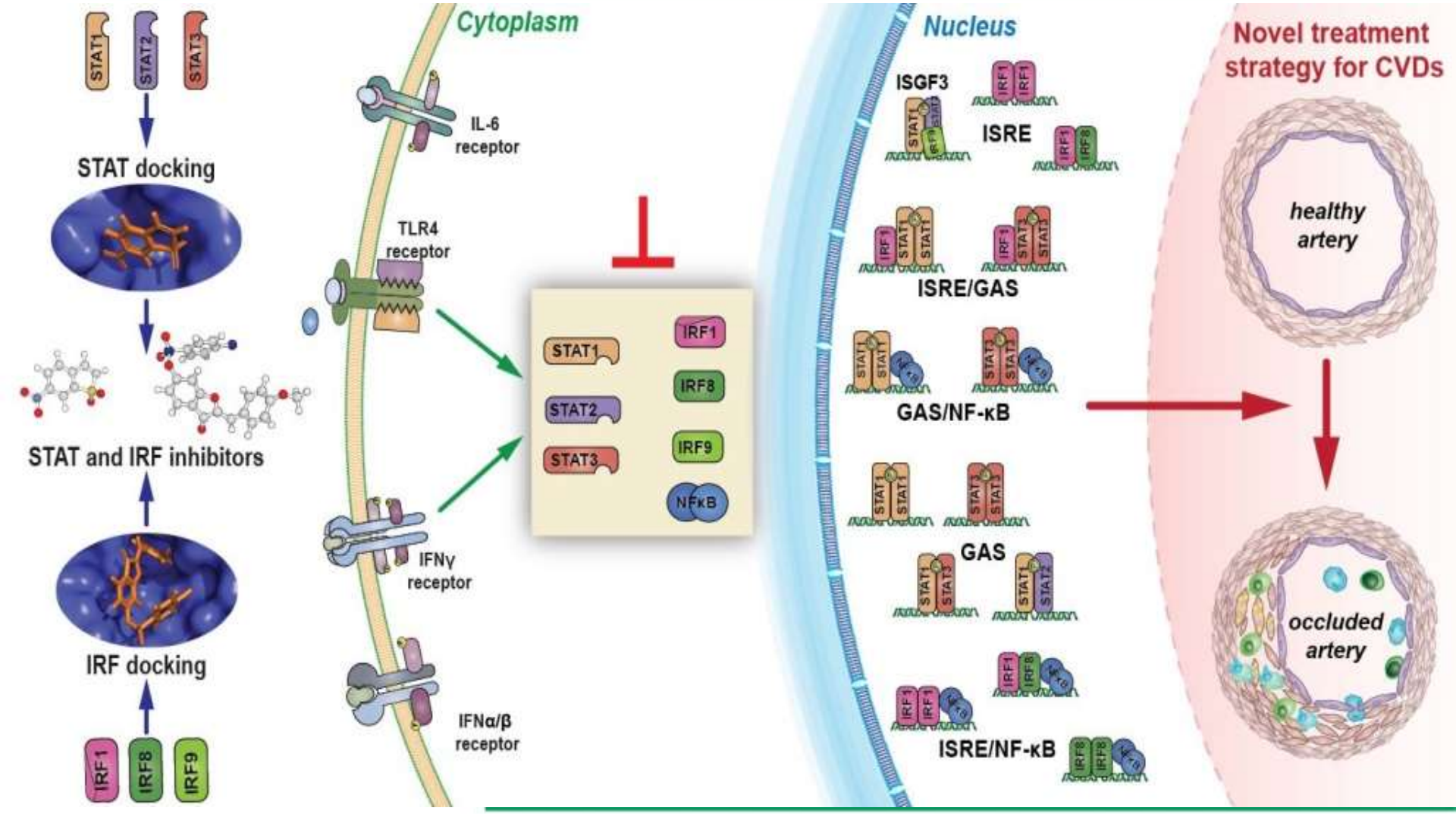
Chmielewski et al., PlosOne 2014

STAT1, NF-kB & IRFs in vascular disease

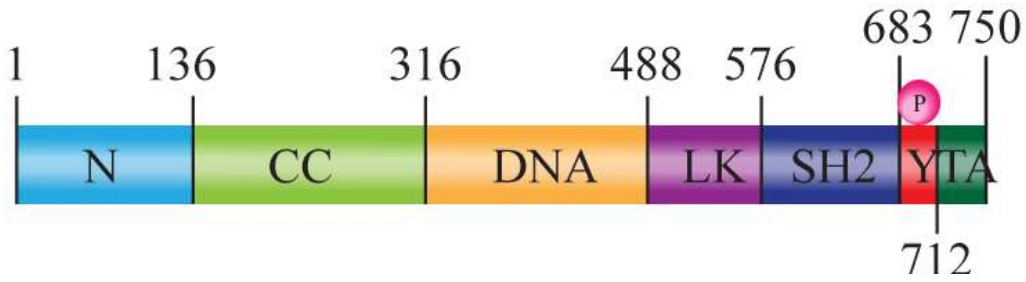


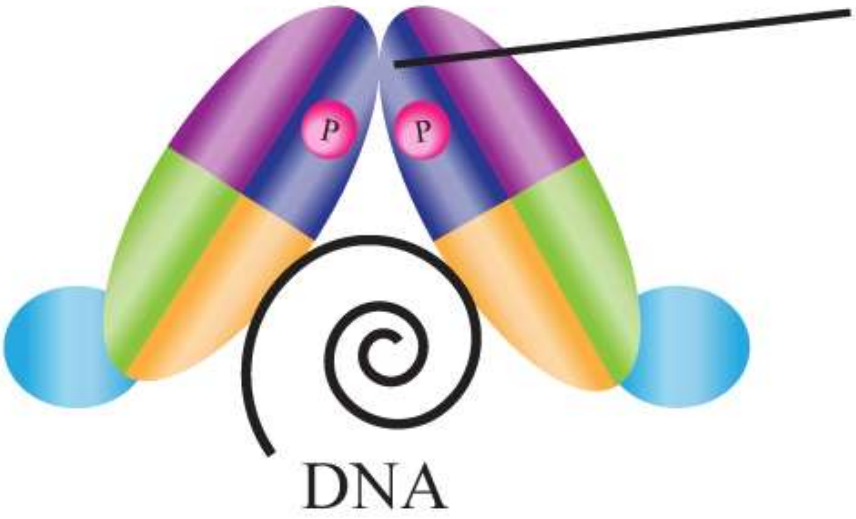
Chmielewski, Piaszyk-Borychowska et al., IRI, 2015

Inhibition of STATs & IRFs in Vascular Disease



STAT Structure & Dimerization



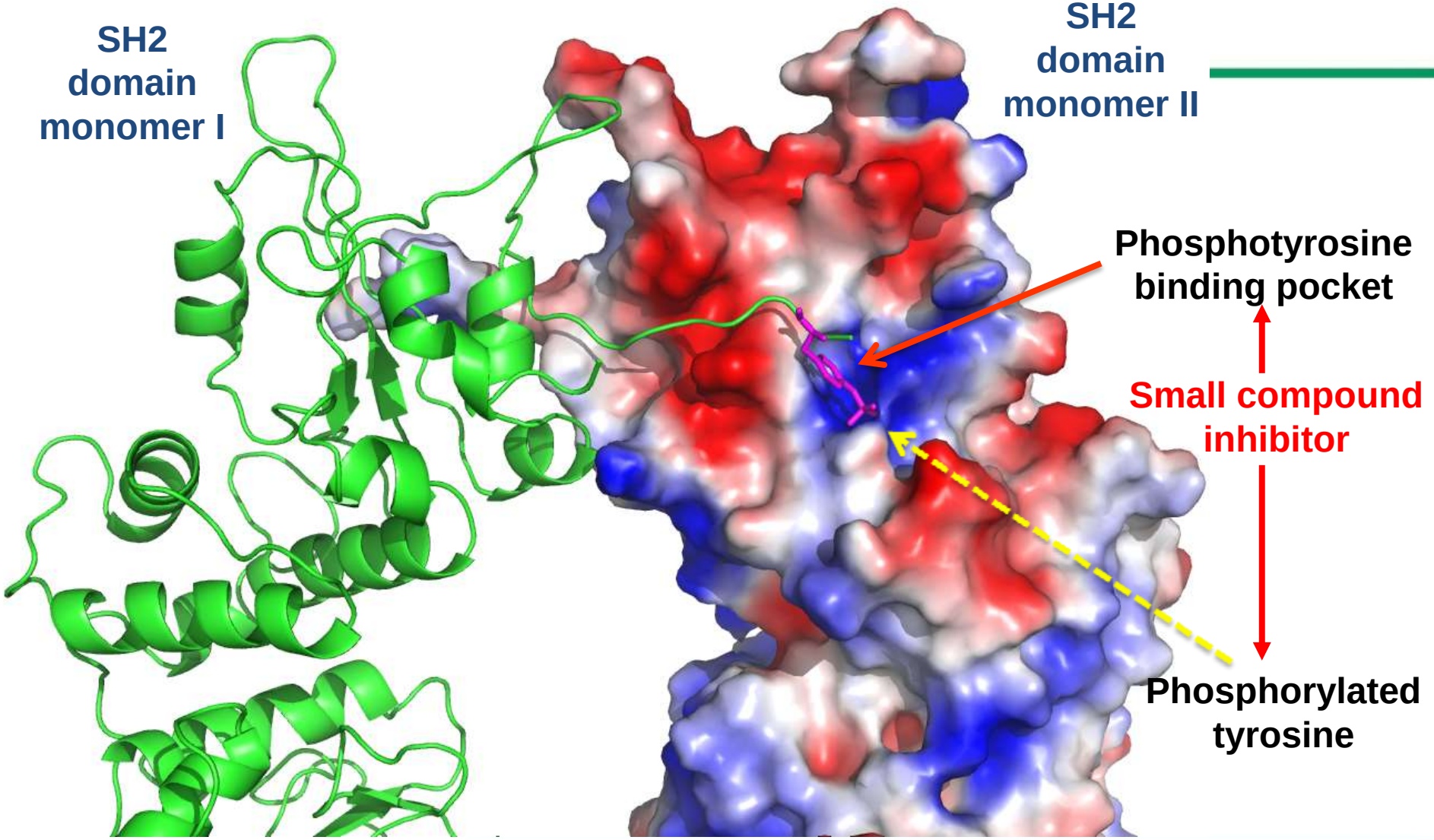


Interacting
SH2-domain

STAT-dimer

Sikorski et al., CGFR 2011

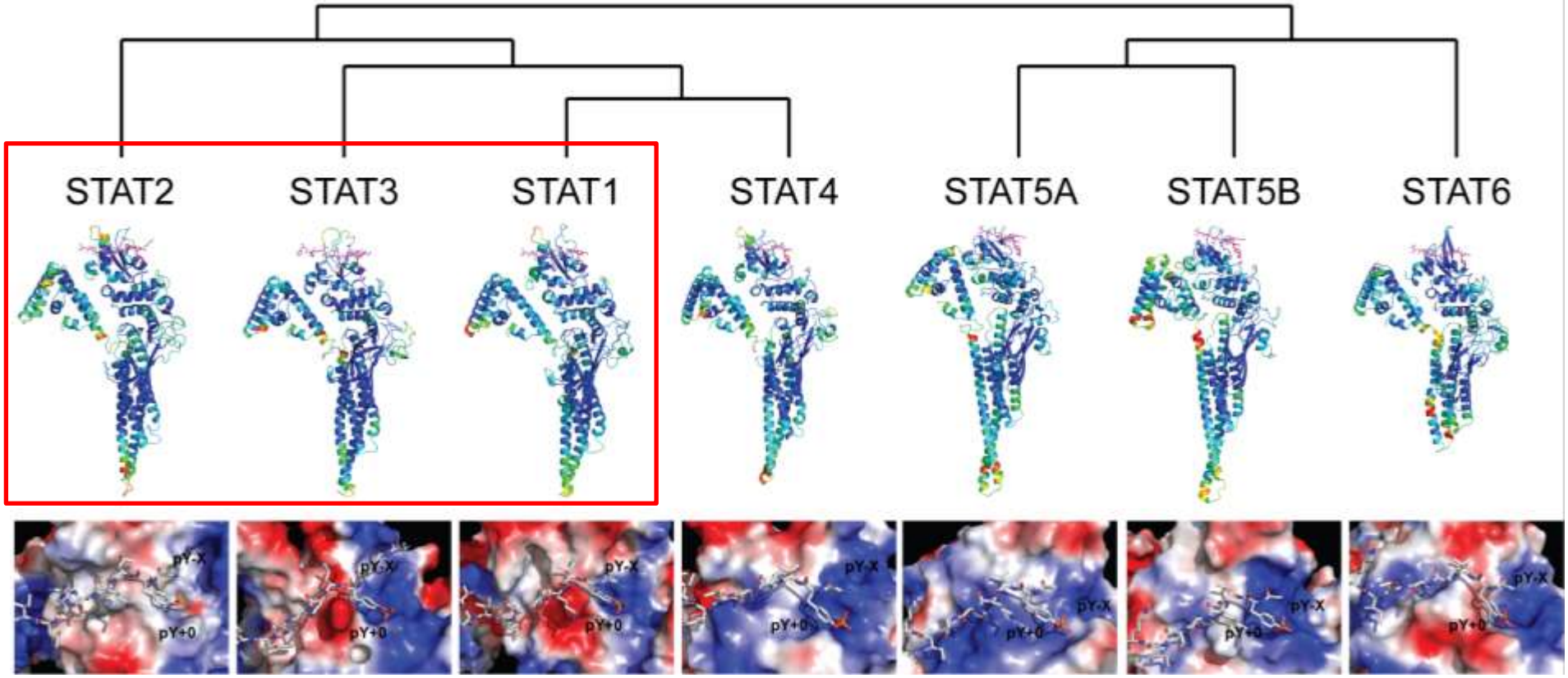
Structural information: STAT1-STAT3



Chen et al., 1998, Cell, 93:827

Becker et al., 1998, Nature, 394:145

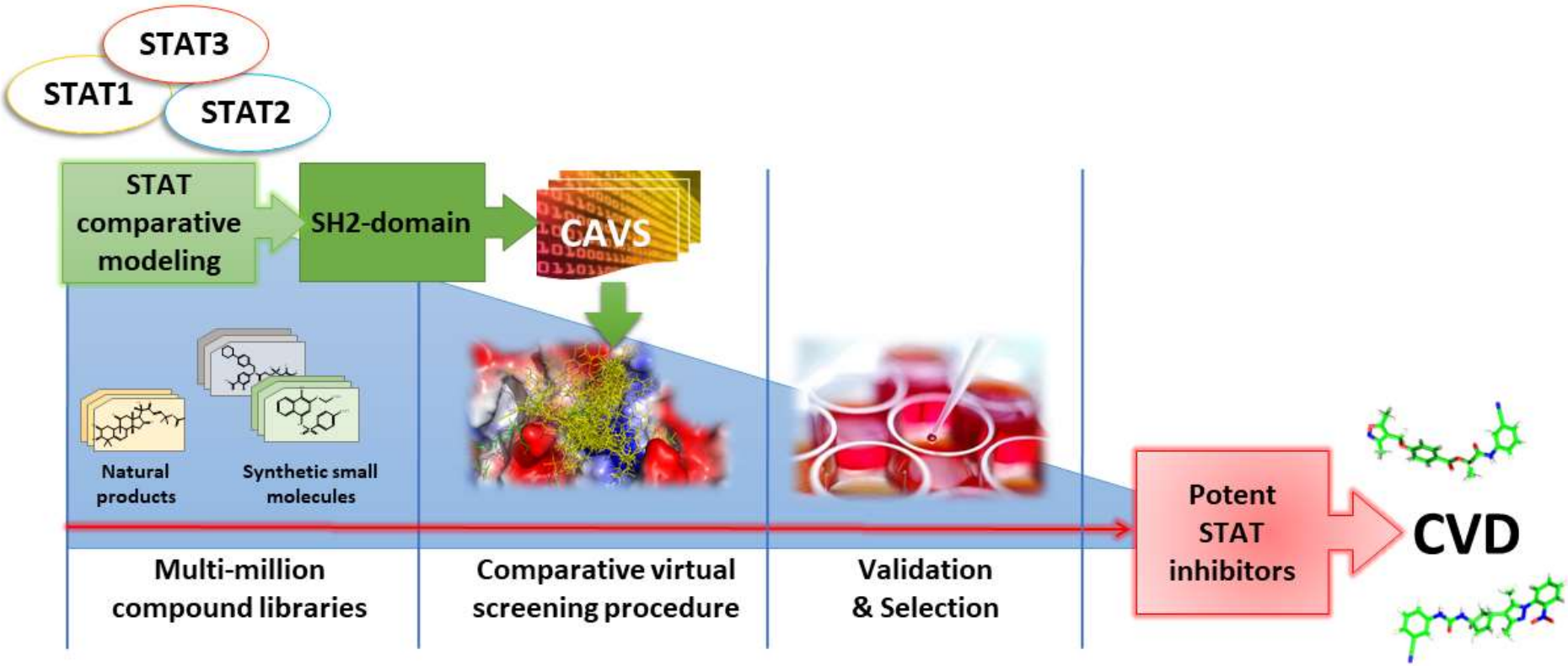
New homology models for all STATs: STAT-SH2 small molecule interactions



High SH2 homology!

Szeląg et al., PlosOne, 2015

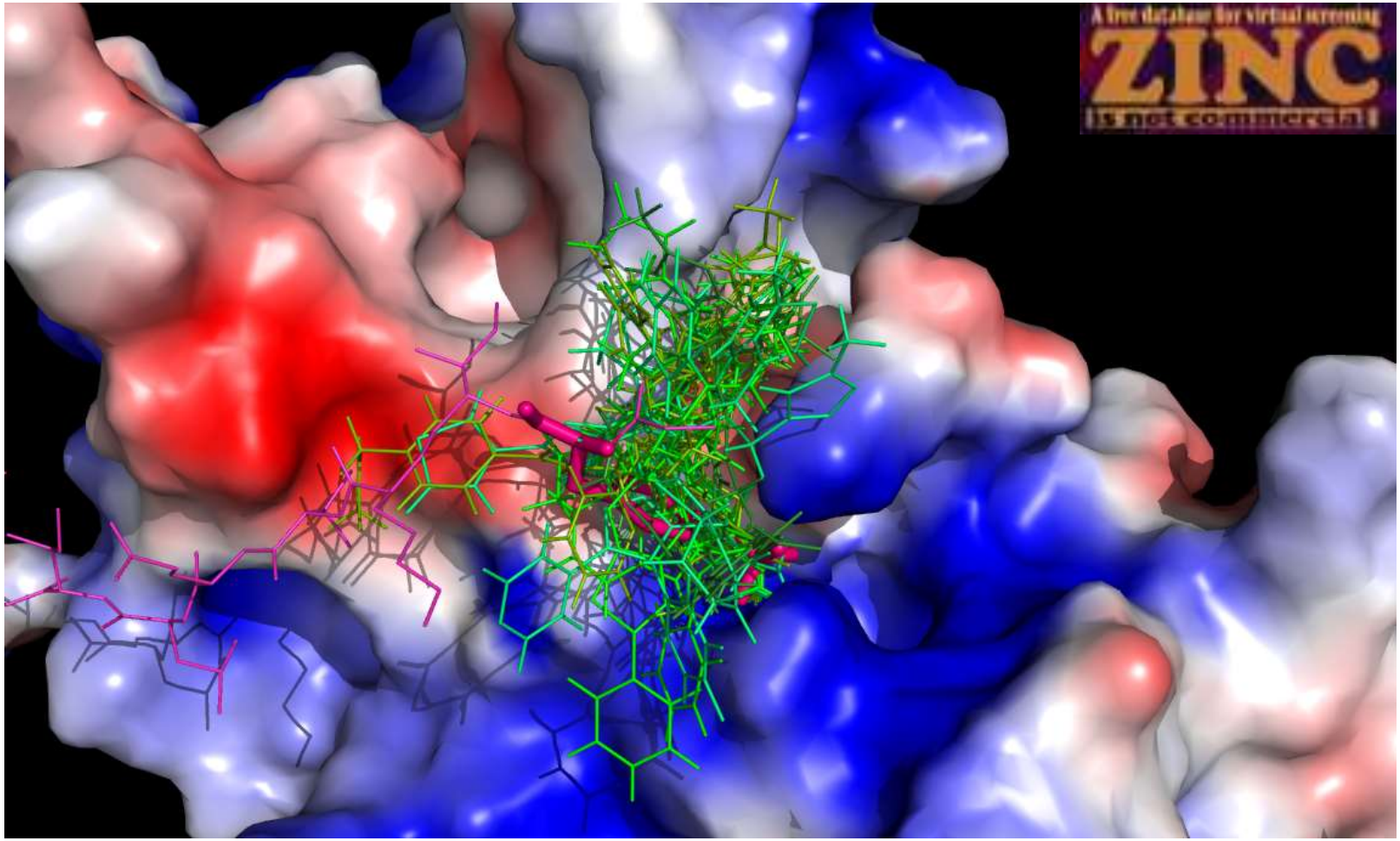
STAT inhibition approach



Szeląg et al., Oncotarget, 2016

Novel STAT Inhibitors: Virtual Screening

HSTAT1-SH2 model



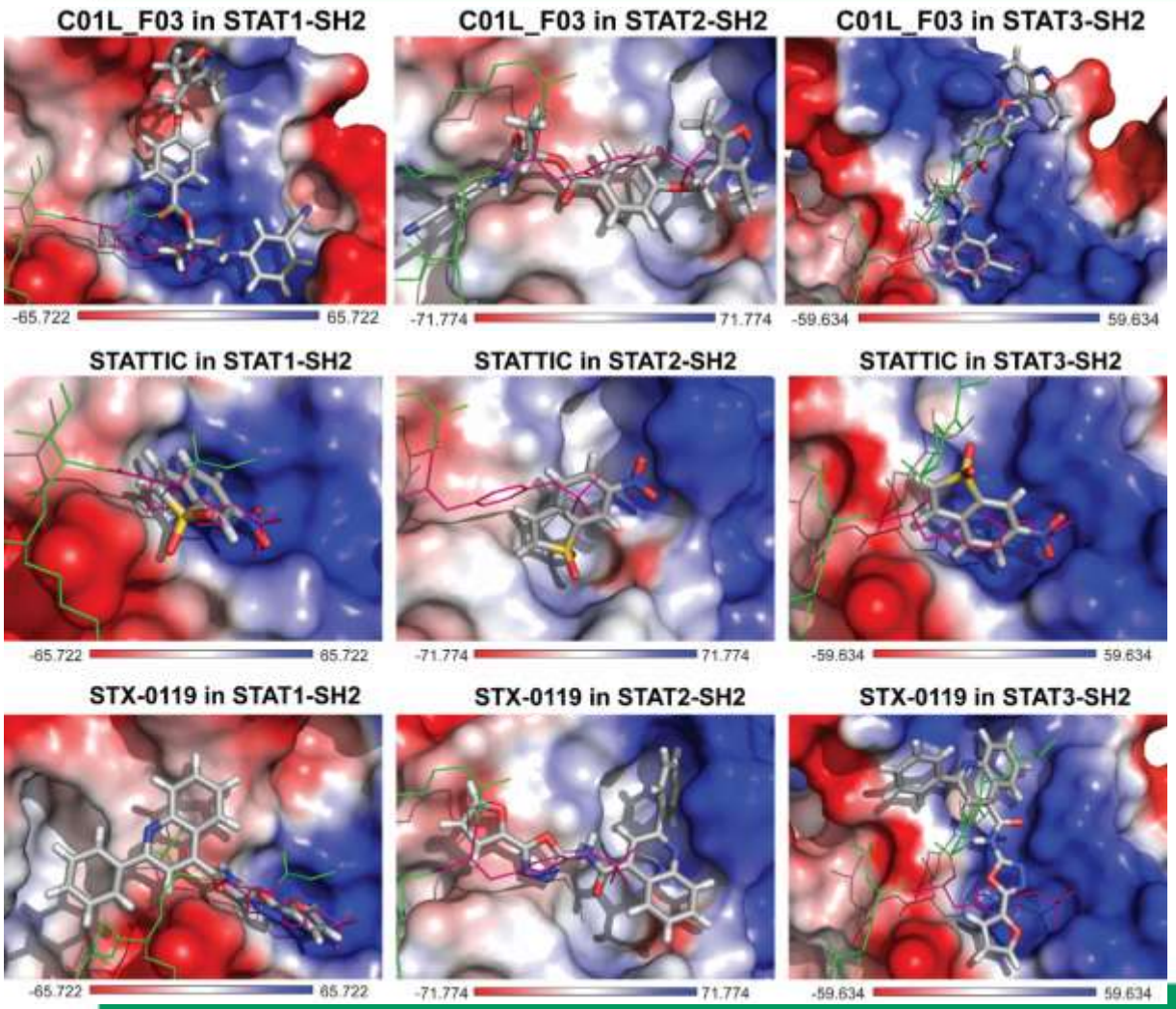
Szeląg and Czerwoniec, 2013

Multi STAT inhibitors bind *in silico* SH2 models of STAT1, 2 and 3

C01L_03

STATTIC

STX-0119



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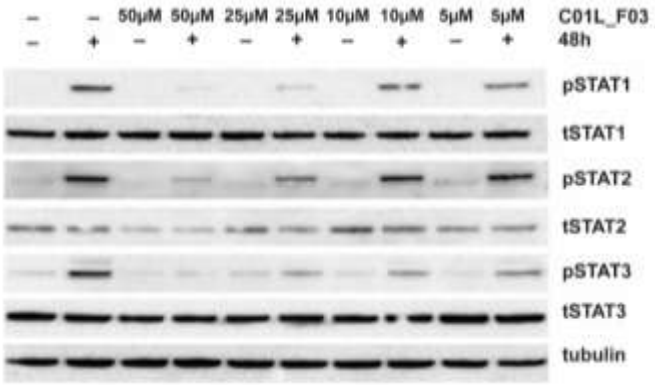
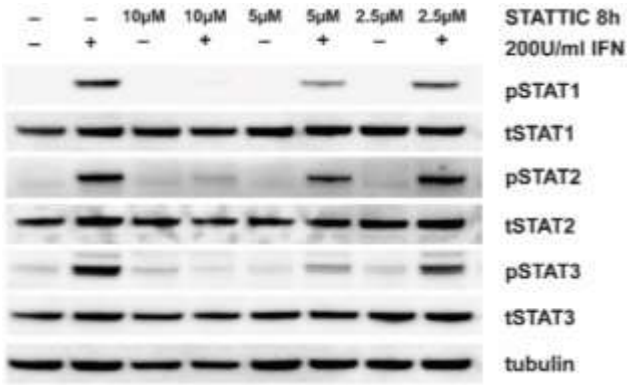
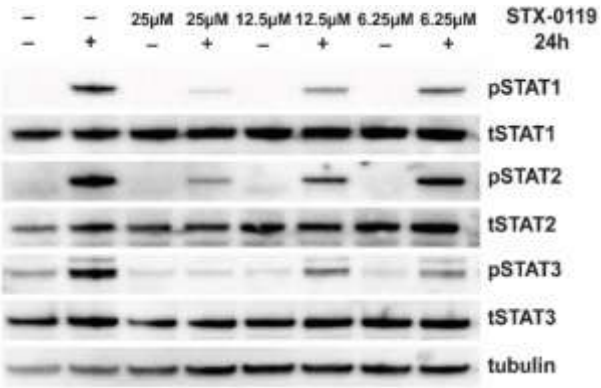
Multi STAT inhibitors Inhibit phosphorylation of STAT1, 2 and 3

C01L_03



STATTIC





STX-0119

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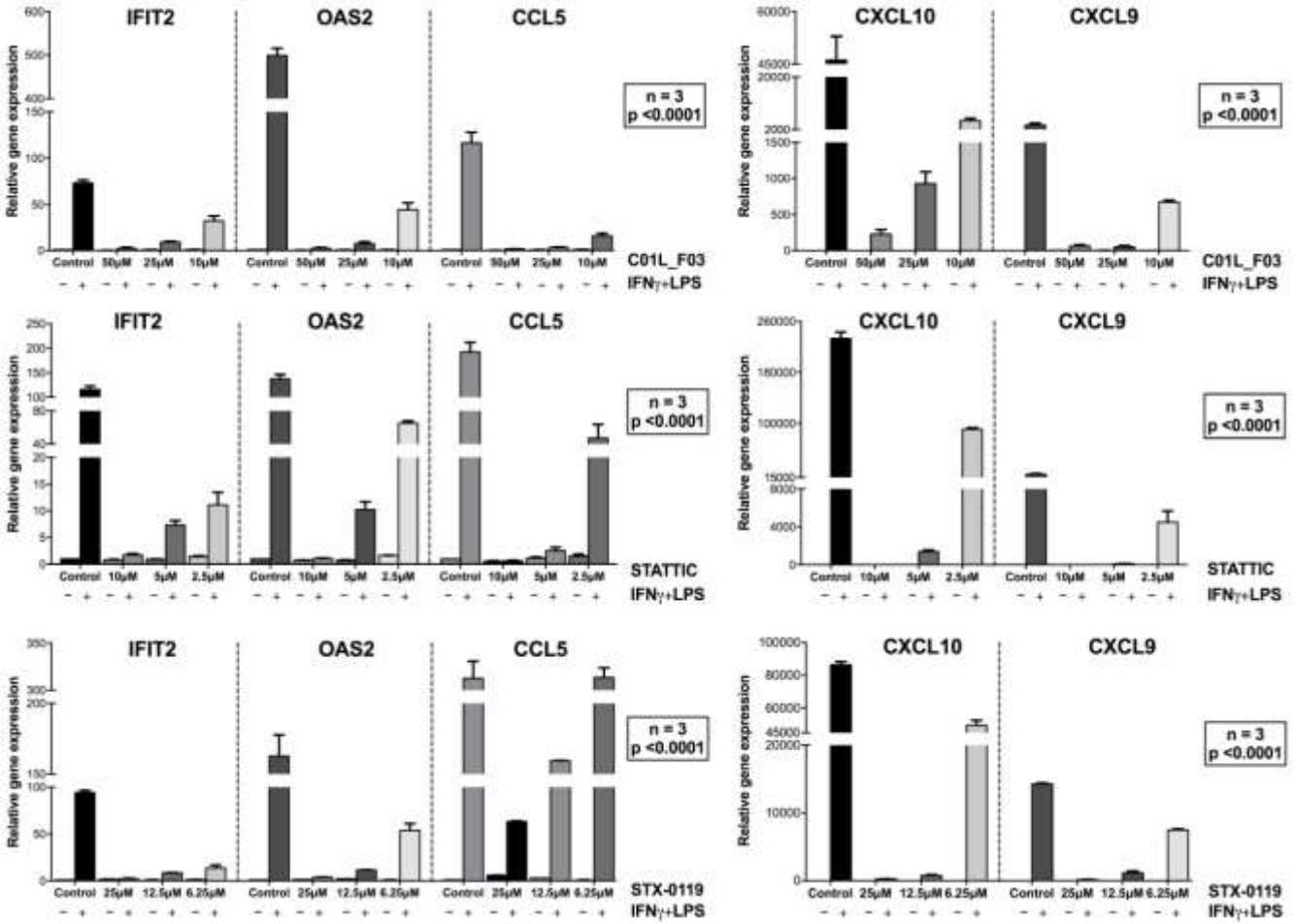
Multi-STAT inhibitors block inflammation induced gene expression

C01L_03

STATTIC

STX-0119



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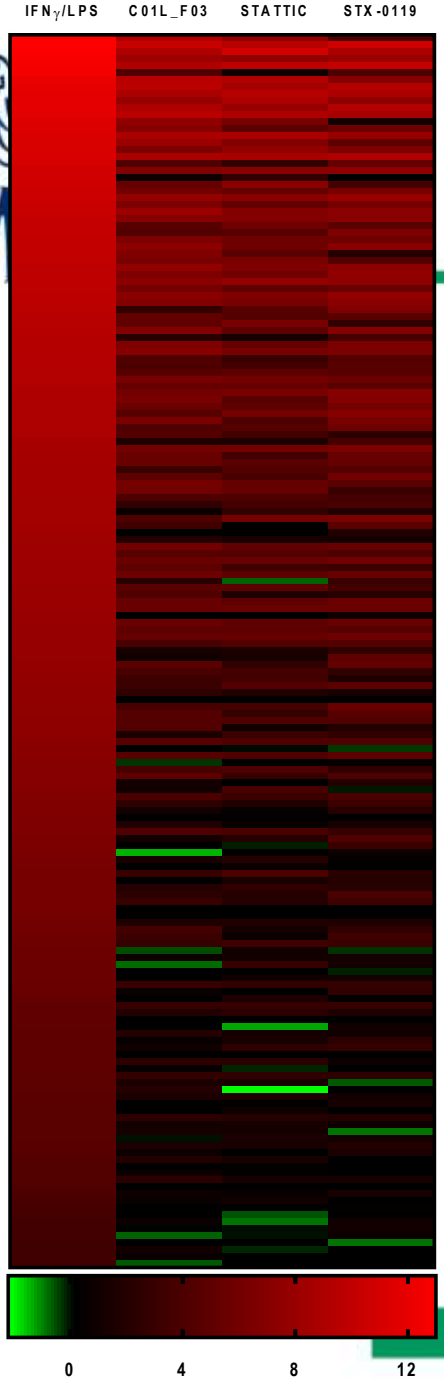
Genome-wide effect of multi-STAT inhibitors



159 genes commonly inhibited by C01L_F03, STATTIC, STX-0119

GO term	Biological Process	Fold Enrichment
GO:0043207	response to external biotic stimulus	28.14
GO:0009607	response to biotic stimulus	27.38
GO:0006952	defense response	29.61
GO:0019221	cytokine-mediated signaling pathway	29.62
GO:0002376	immune system process	28.66
GO:0001817	regulation of cytokine production	10.67
GO:0007166	cell surface receptor signaling pathway	13.89
GO:0006954	inflammatory response	8.28
GO:0042127	regulation of cell proliferation	7.97
GO:0042981	regulation of apoptotic process	4.23
GO:0030334	regulation of cell migration	4.70
GO:0030155	regulation of cell adhesion	9.09

Vascular inflammation

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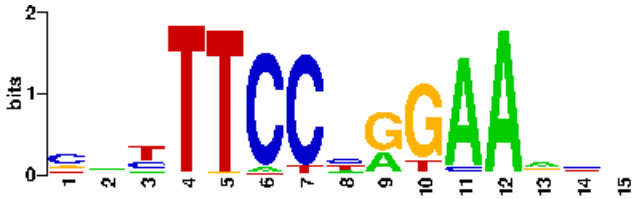
Multi-STAT inhibitors act in a ”STAT-only” manner

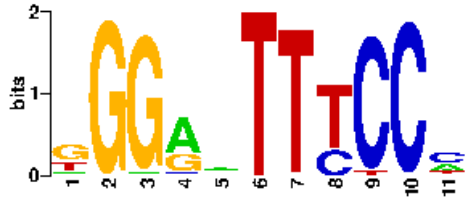
159 commonly Inhibited genes

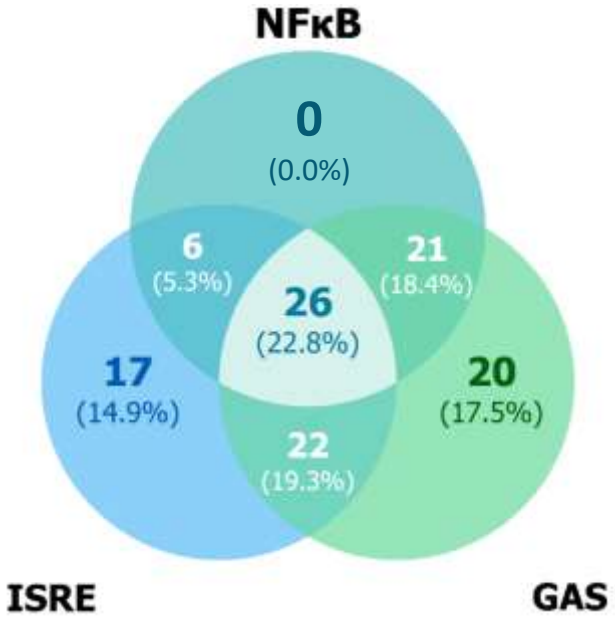
ISRE

GAS



NFκB





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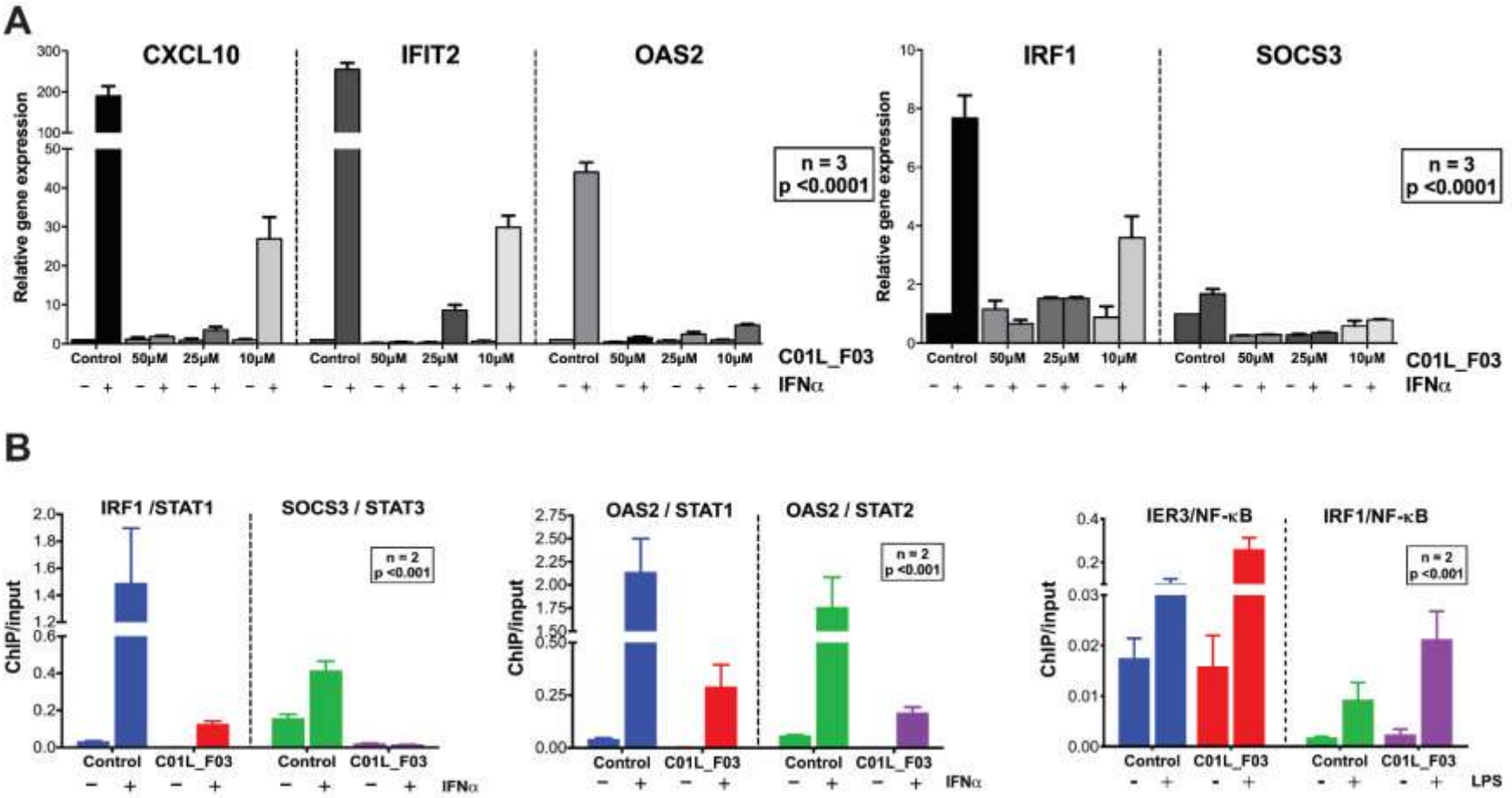
Multi-STAT inhibitors act in a ”STAT-only” manner



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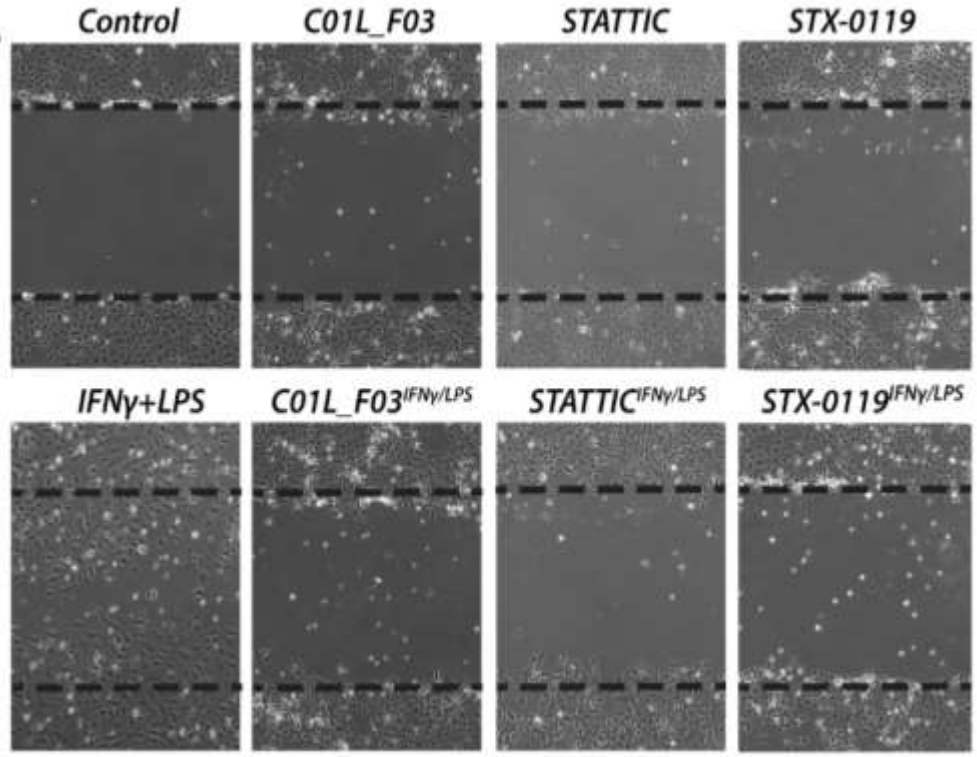
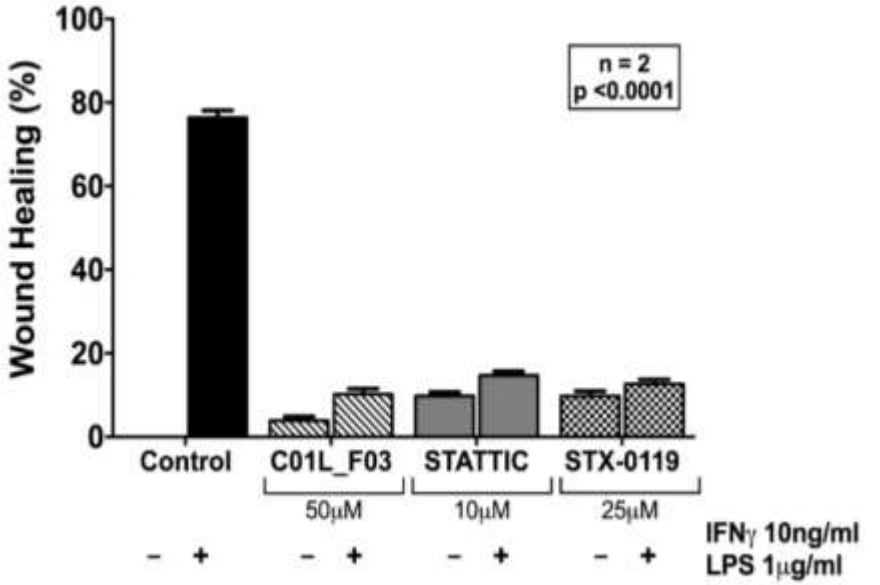
Multi-STAT inhibitors block inflammation induced EC-migration





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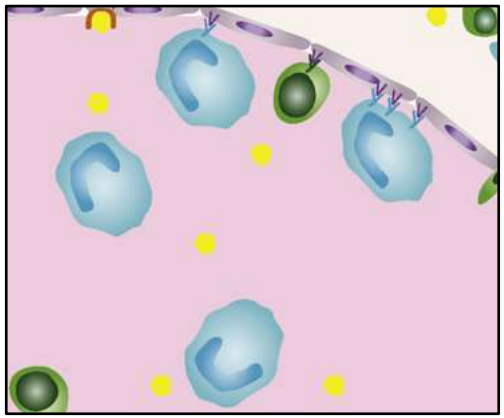
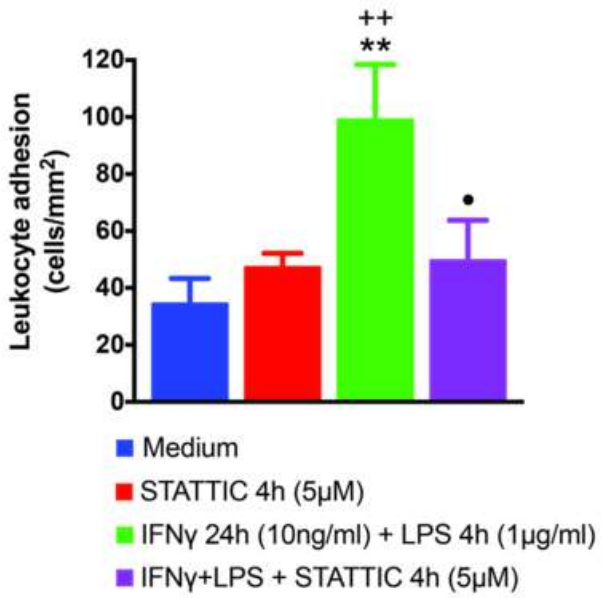
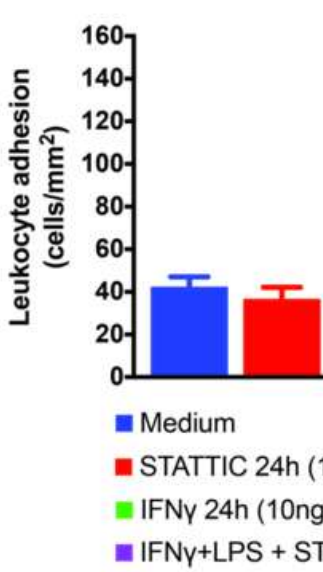
Multi-STAT inhibitors block inflammation induced leukocyte-EC adhesion



STATTIC (1 uM)





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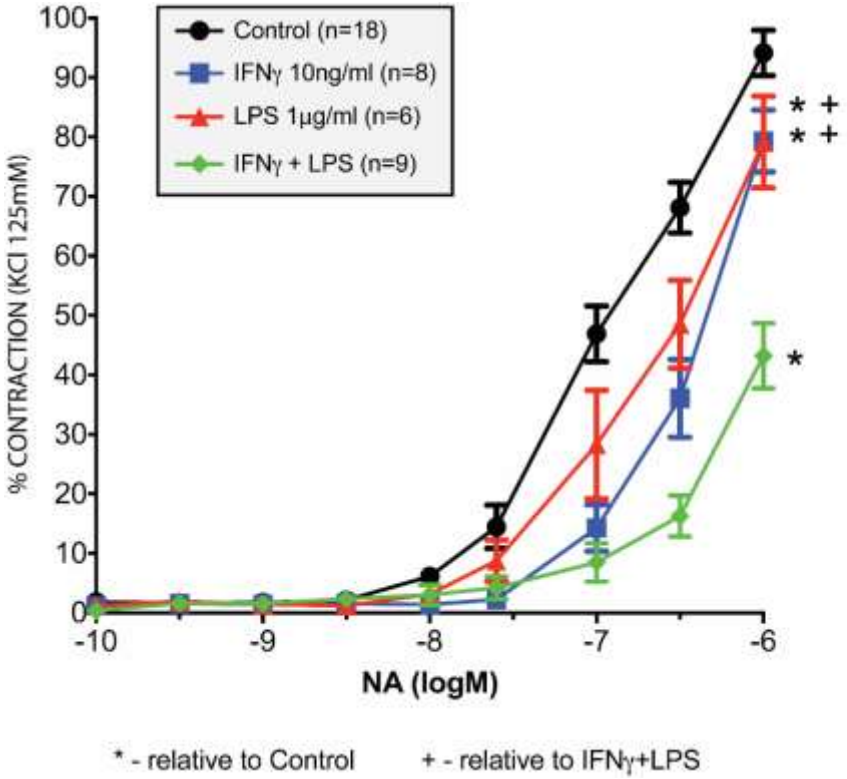
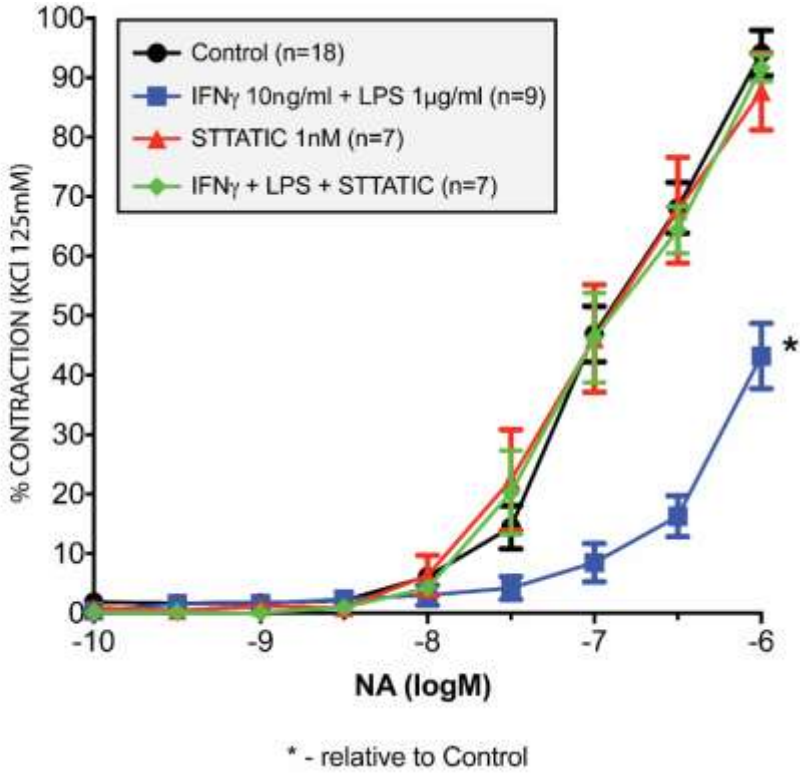
Multi-STAT inhibitors restore inflammation induced impaired arterial contractility

Mesenteric arteries

STATTIC (1 nM)





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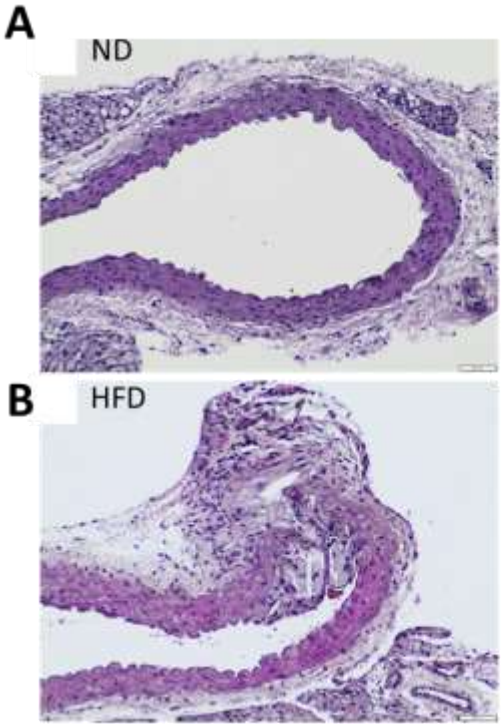
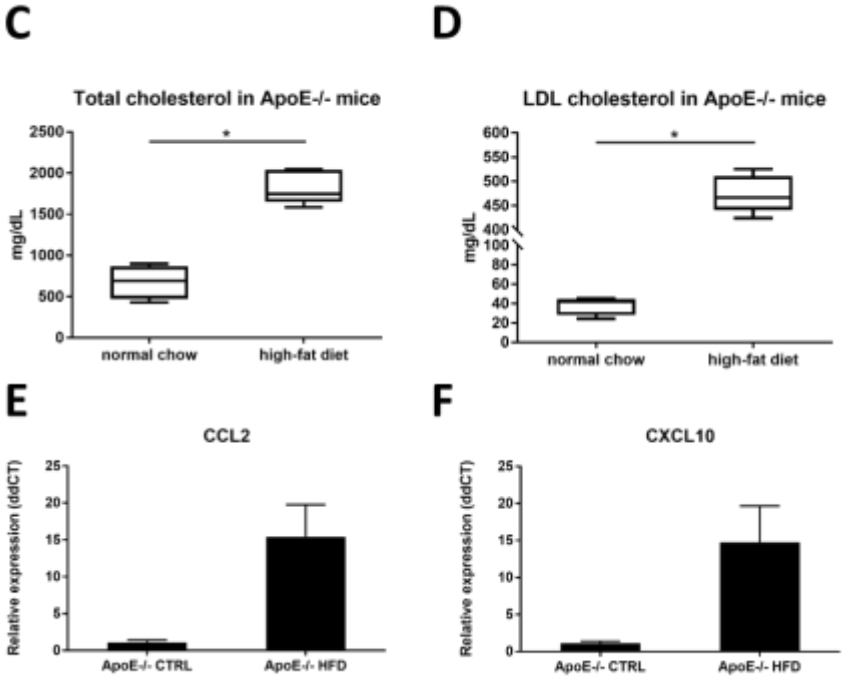
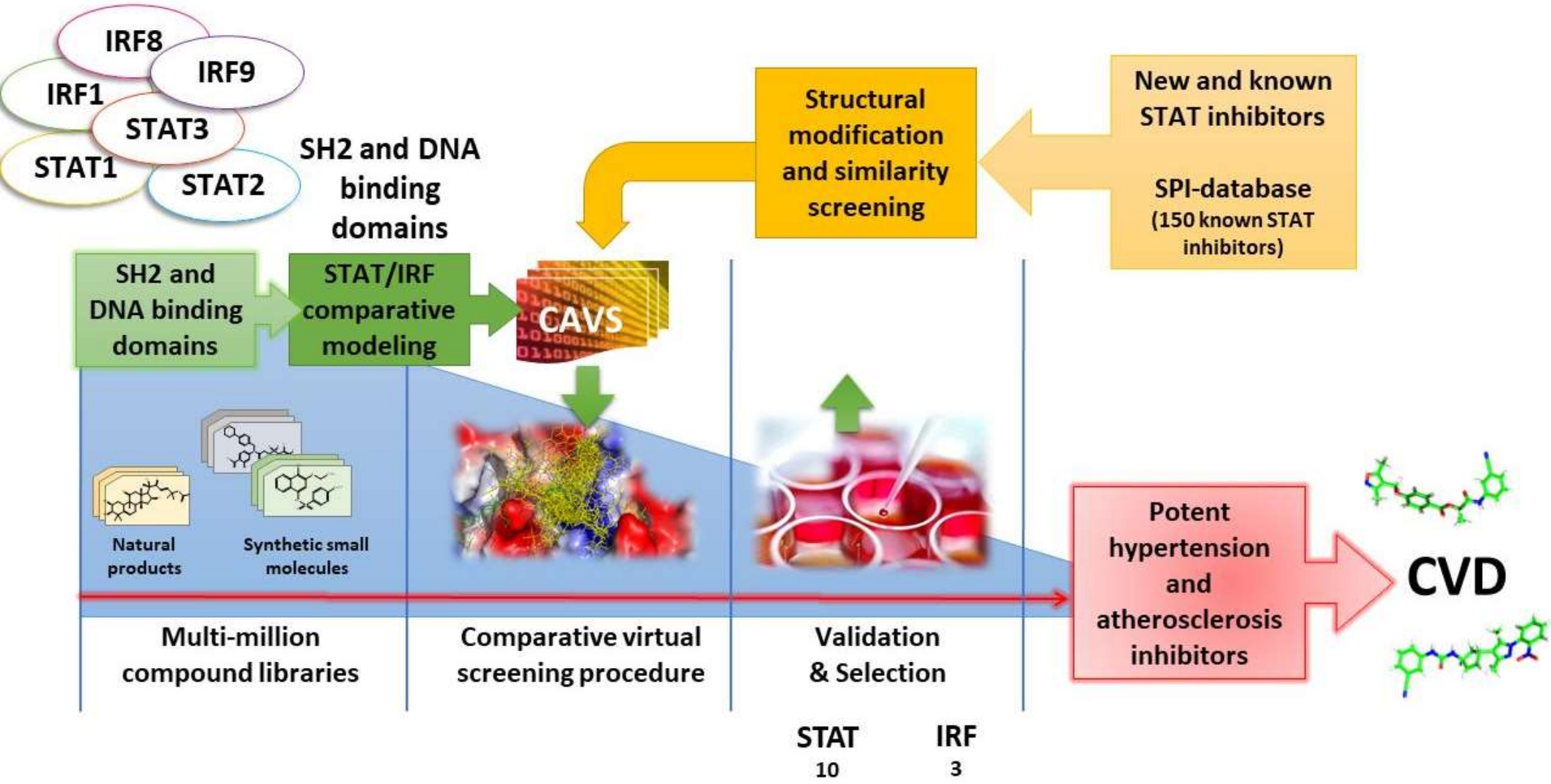
STAT-target gene expression in HFD treated ApoEKO mice



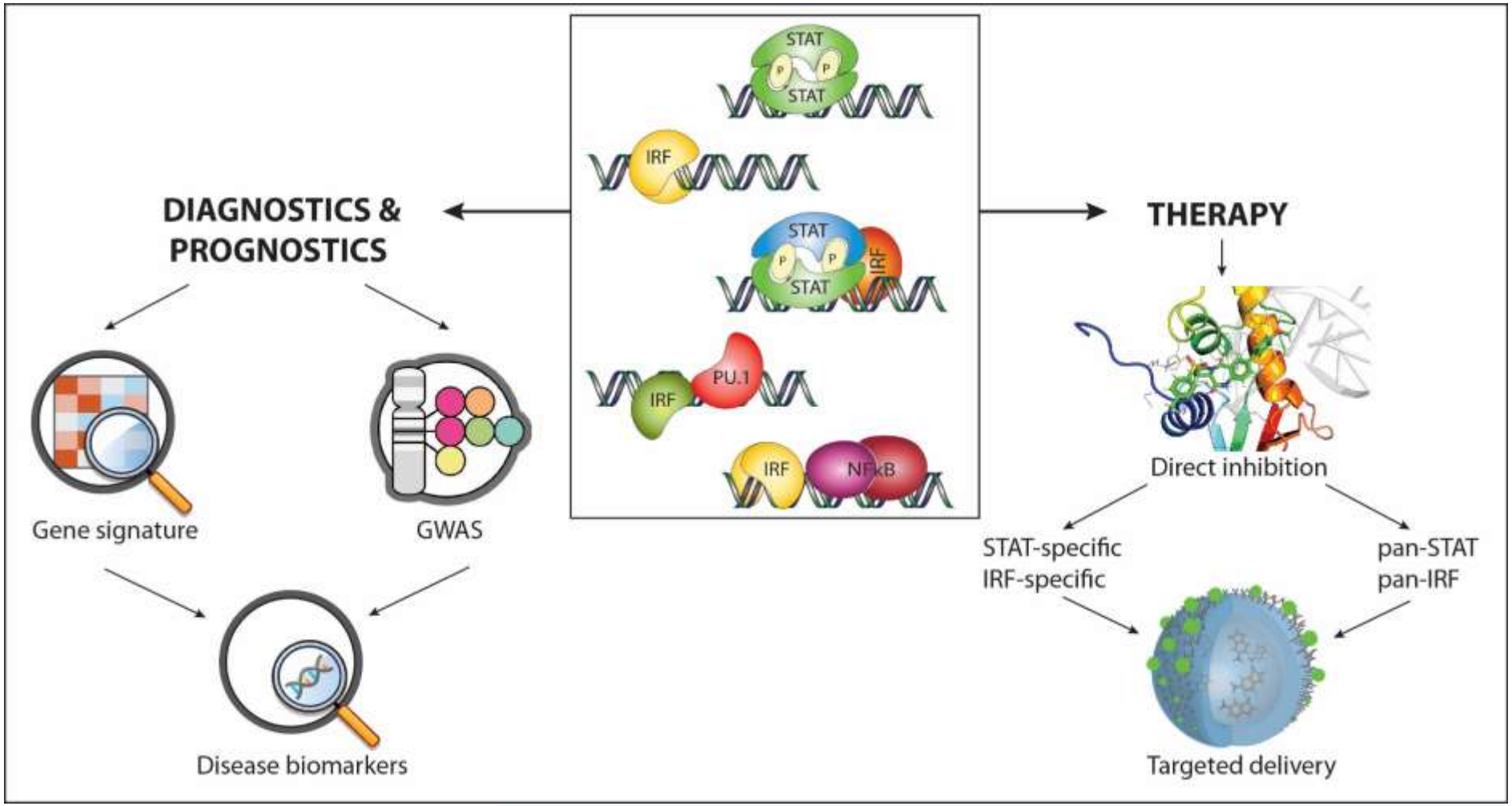


ApoEKO + 10 weeks HFD

Pipeline approach to identify potent STAT & IRF inhibitors



STATs & IRFs in Diagnostics & Therapeutics



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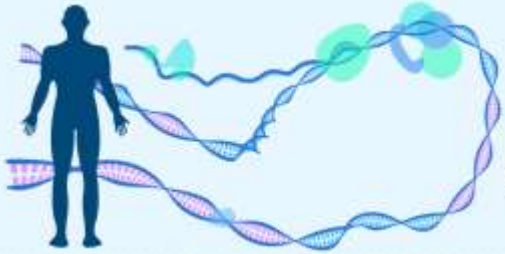

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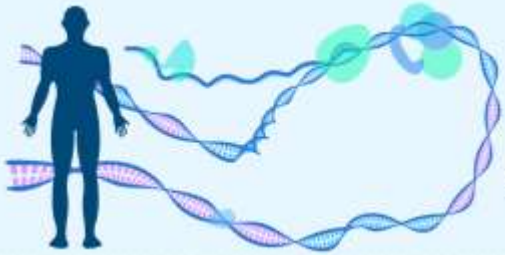

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