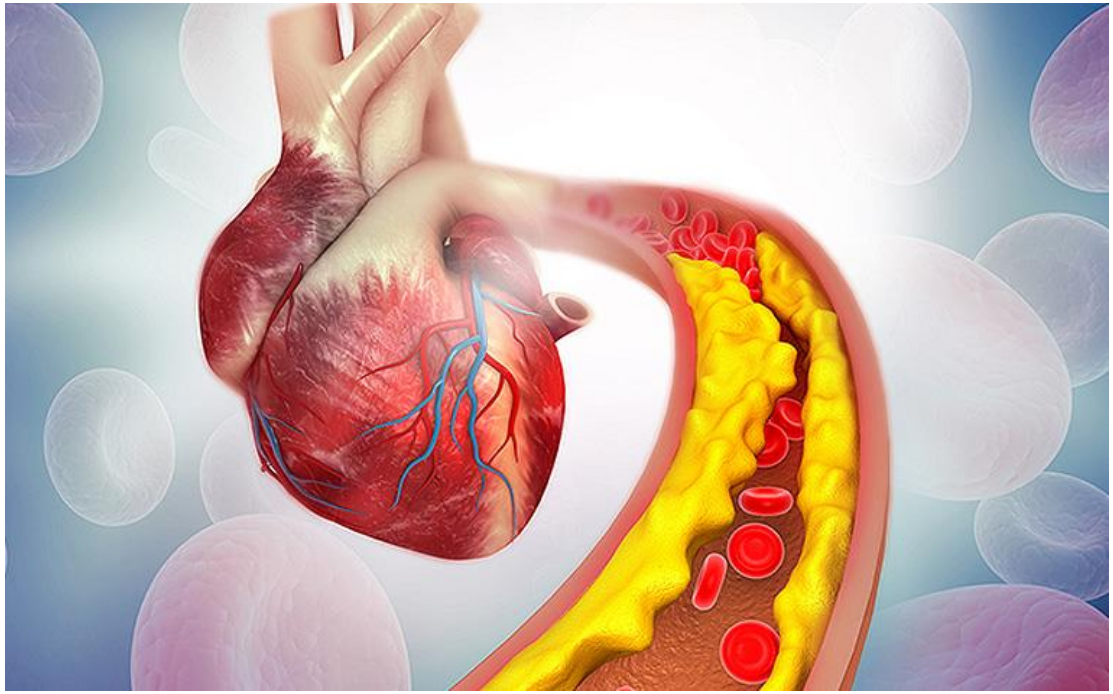
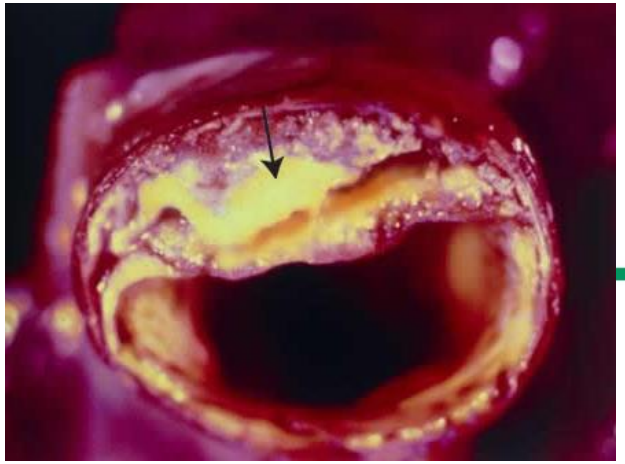
ADAM MICKIEWICZ UNIVERSITY IN POZNAŃ
Faculty of Biology

Diagnostics & Therapeutics of Atherosclerosis



Hans Bluyssen
25-11-2021

Atherosclerosis



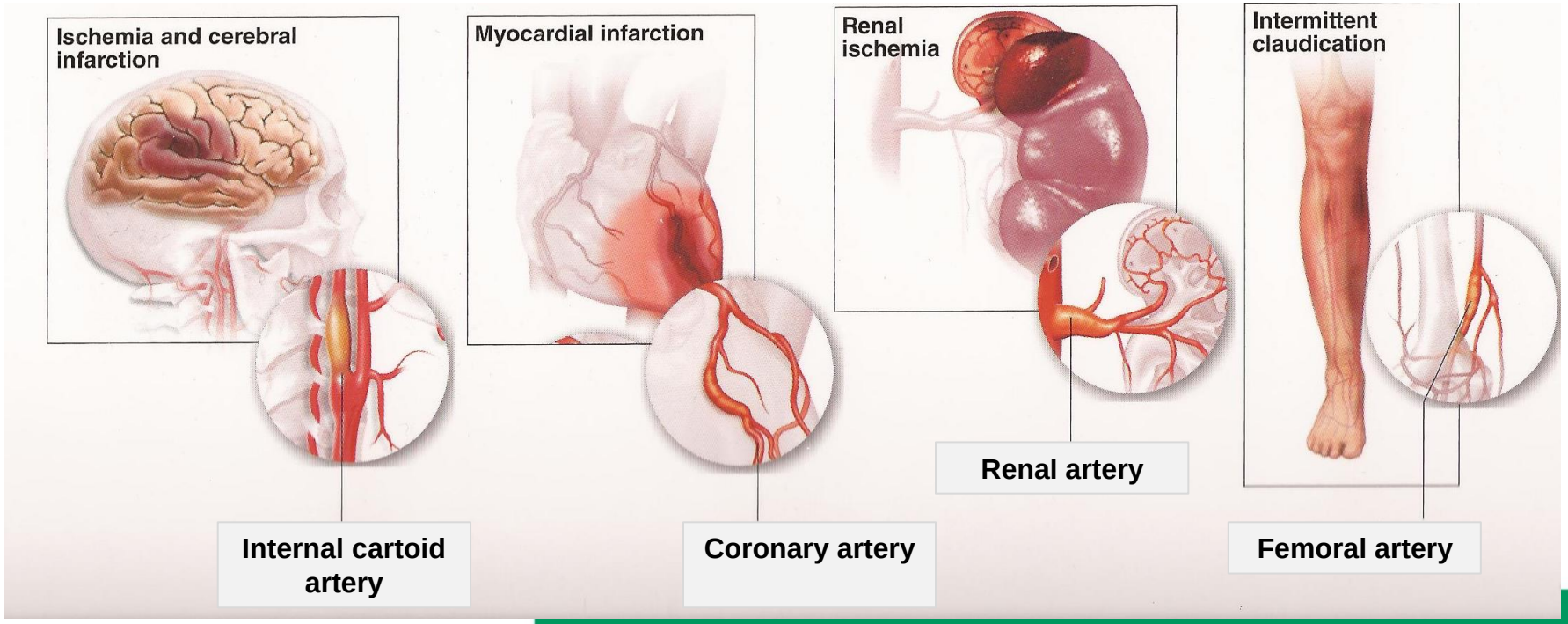
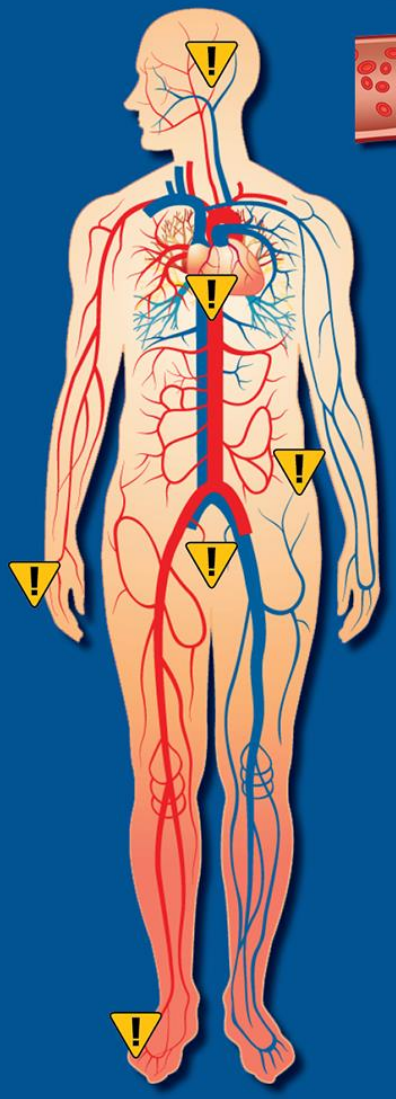
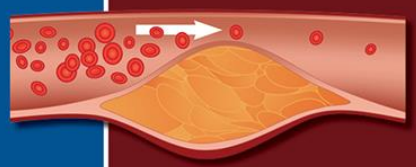


Fig. Atherosclerosis complications. Dr Philip Barlow Mills FCP (SA).

The Warning Signs of Clogged Arteries





When cholesterol particles build up in the arteries, they form plaques that narrow the path for blood flow. Narrowed arteries strike all areas of the body and can lead to pain and discomfort and ultimately result in heart attack.

Stroke:

Plaque that accumulates in the carotid arteries, which carry blood to the brain, can result in stroke.

Fatigue and Dizziness:

Reduced oxygen from poor blood flow can result in dizziness and extreme fatigue, especially in women.

Shortness of Breath:

Reduced blood flow can lead to shortness of breath.

Chest Pain:

Chest pain, or angina, results from reduced blood flow to the heart. Angina can be felt as pressure, numbness, tightness, squeezing, or burning.

Lower Back Pain:

When blood flow to the lower back is reduced, the disks between the vertebrae become fragile, which can result in painful pinched nerves.

Erectile Dysfunction:

Narrowed arteries to the genitals can cause sexual dysfunction.

Painful, Numb, or Cold Hands and Feet:

Plaque in the arteries leading to the arms and legs can result in painful, numb, and cold extremities.

PhysiciansCommittee
for Responsible Medicine

PCRM.org/HeartHealth

Reducing your risk factors for atherosclerosis

What you can't control

Age
The older you are, the more likely you are to develop atherosclerosis.

Gender
Men are more likely to develop atherosclerosis at an earlier age than women, but as women get older they soon catch up with men.

Family history
If your dad or brother developed heart or circulatory disease before they were 55, or your mum or sister before they were 65, then you're at higher risk.

Ethnicity
Some ethnic groups have a different level of risk. For example, South Asian people have a higher risk of developing Type 2 diabetes, which is a risk factor for atherosclerosis.

What you can control

Smoking
If you're a smoker giving up is the single most important thing you can do for your heart health.

Diabetes
Diabetes can damage the walls of your arteries, increasing your risk, so it's important to keep your blood glucose (sugar) well controlled.

High blood pressure
More than 1 in 4 of us has high blood pressure. It's a silent threat as usually there are no symptoms. So get your blood pressure checked by your nurse or GP.

High cholesterol
Too much cholesterol in your blood can increase your risk.

Body weight
Being overweight or obese can increase your risk, particularly carrying too much weight around your middle.

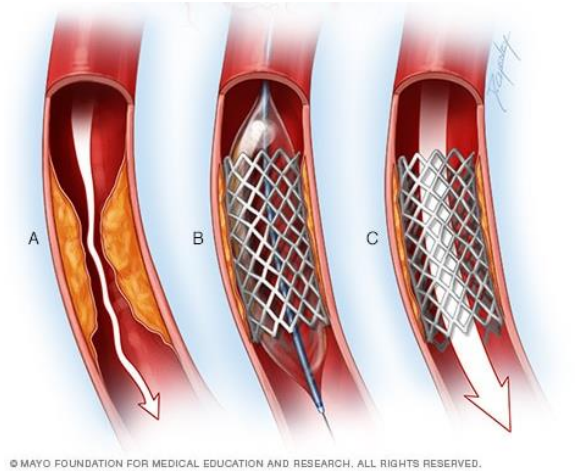
Physical inactivity
Be active every day and aim to build up to a total of 150 minutes' exercise a week.

Atherosclerosis Treatment

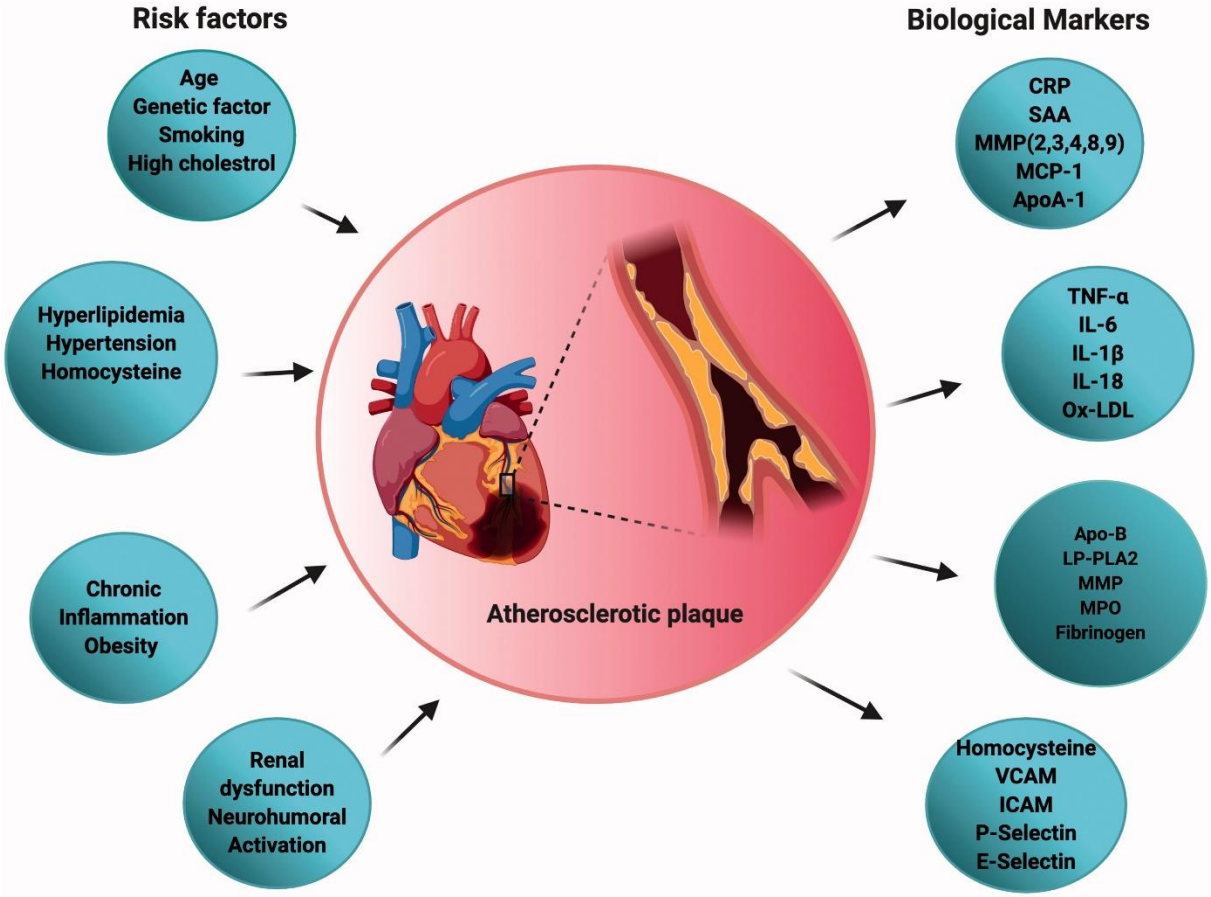
Atherosclerosis
Prevention is generally by eating a healthy diet, exercising, not smoking, and maintaining a normal weight.[4]

Treatment of established disease may include medications to lower cholesterol such as statins, blood pressure medication, or medications that decrease clotting, such as aspirin.[5]

A number of procedures may also be carried out such as percutaneous coronary intervention, coronary artery stent, coronary artery bypass graft, or carotid endarterectomy.[5]



Atherosclerosis – Biomarkers



Inflammation

Sakthi Kumar et al., 2021 Drug Delivery

Atherosclerosis – vascular inflammation

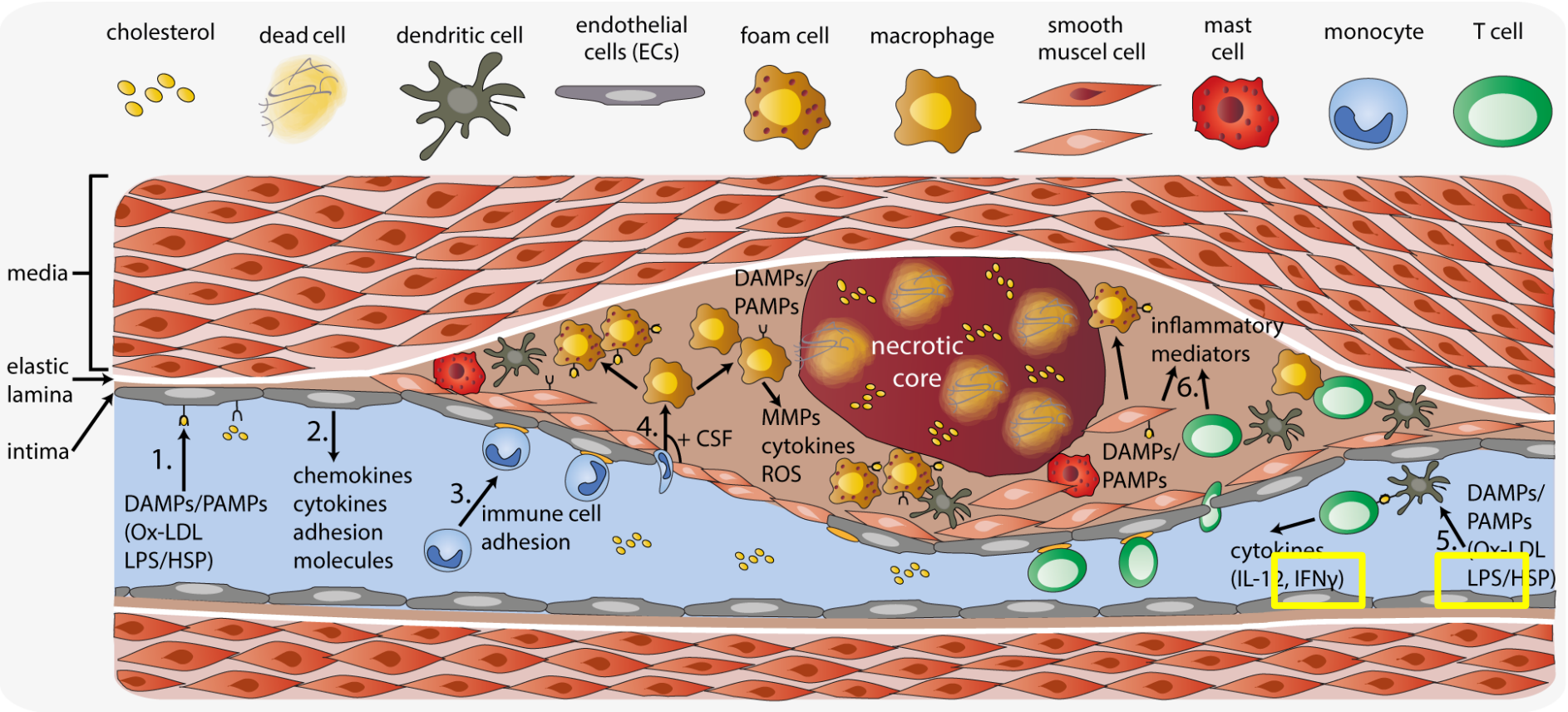
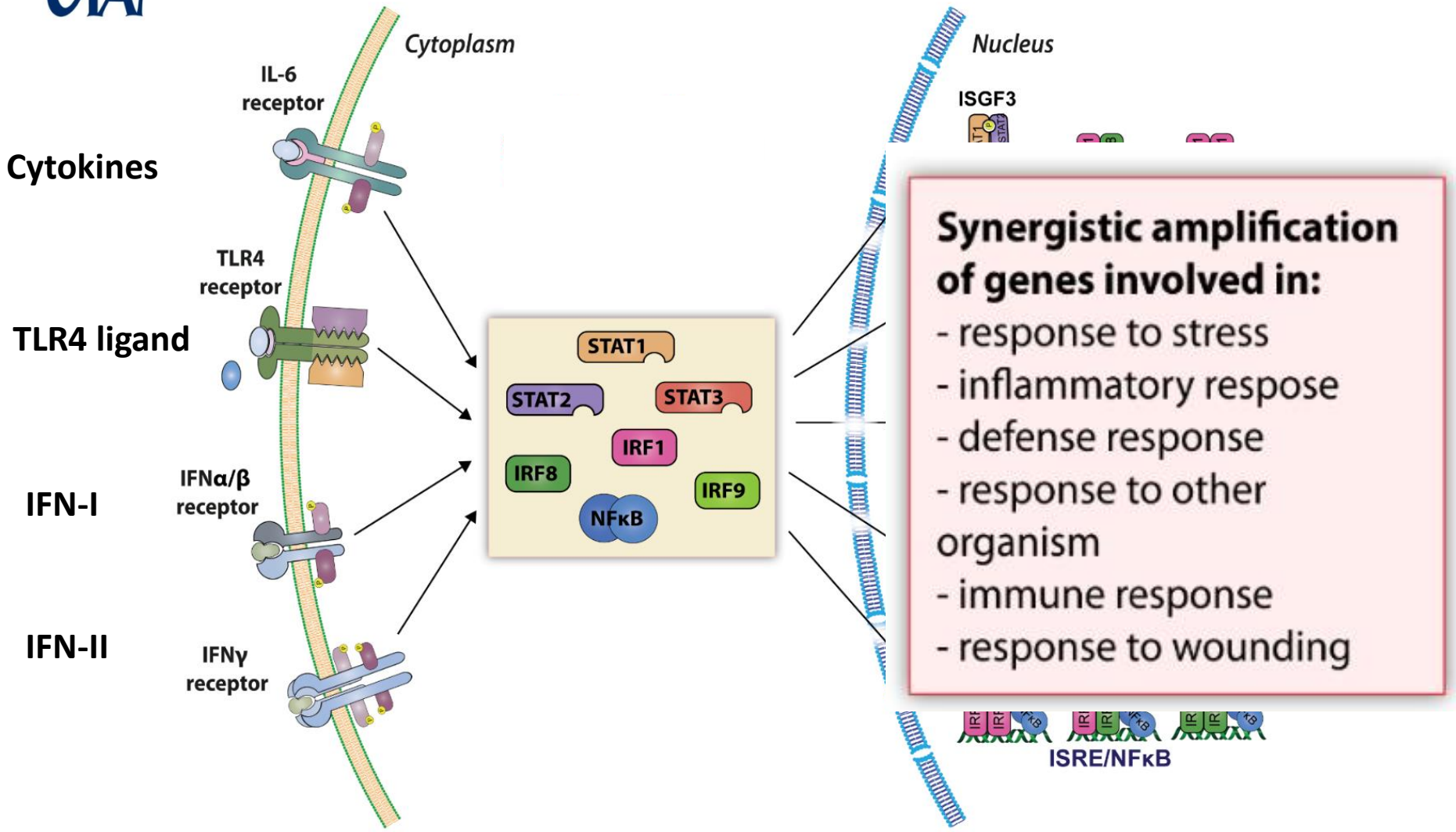


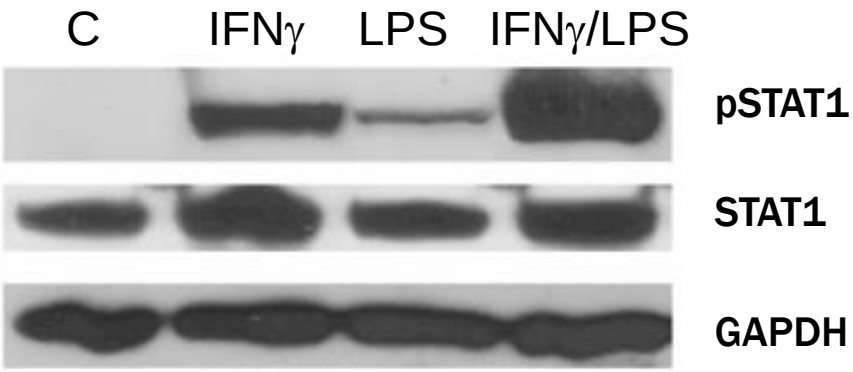
Fig. Atherosclerotic plaque. Chmielewski, Piaszyk-Borychowska et al., Int Rev Immunol, 2016.

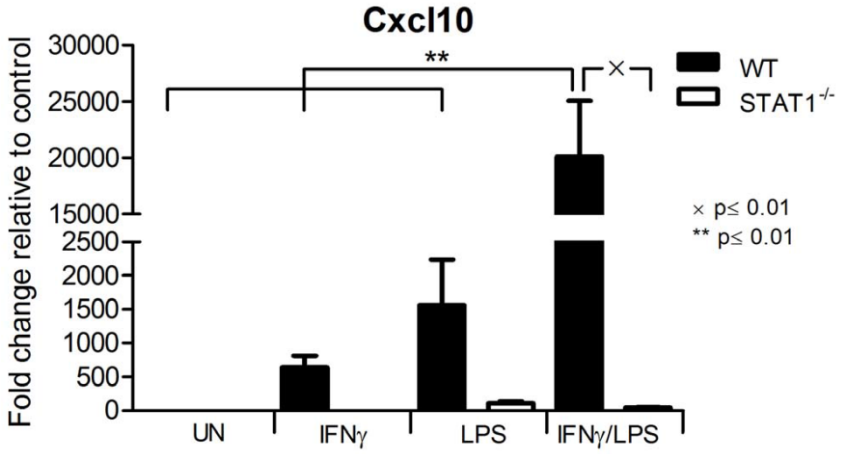
Signal integration in inflammation



Szeląg et al., Oncotarget, 2016

IFNγ Sensitizes Vascular cells for LPS response





HMECs
VSMCs

Sikorski et al., AJP 2011

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

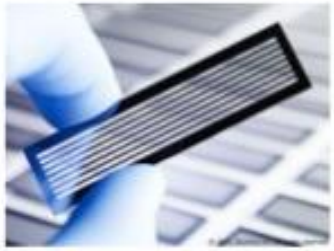
8h IFNγ + 4h LPS

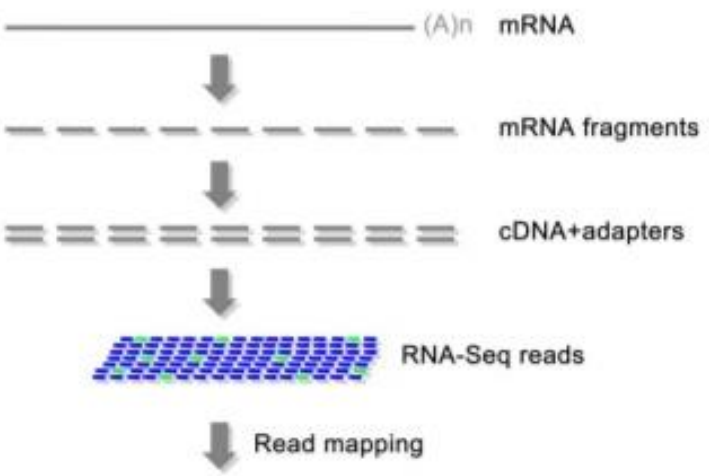
RNA-seq

Piaszyk-Borychowska et al., submitted

RNA-seq Work Flow

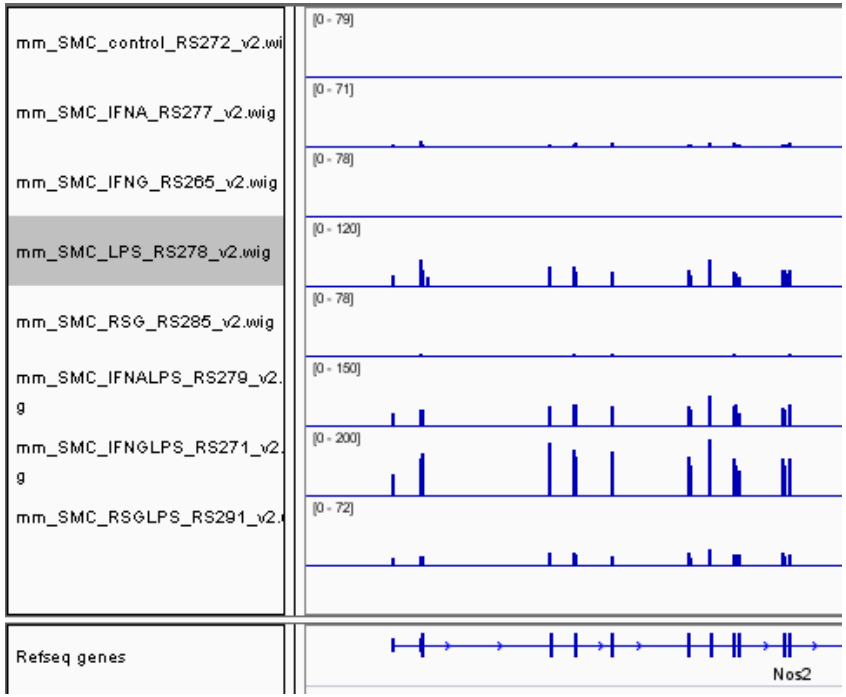
Illumina HiSeq



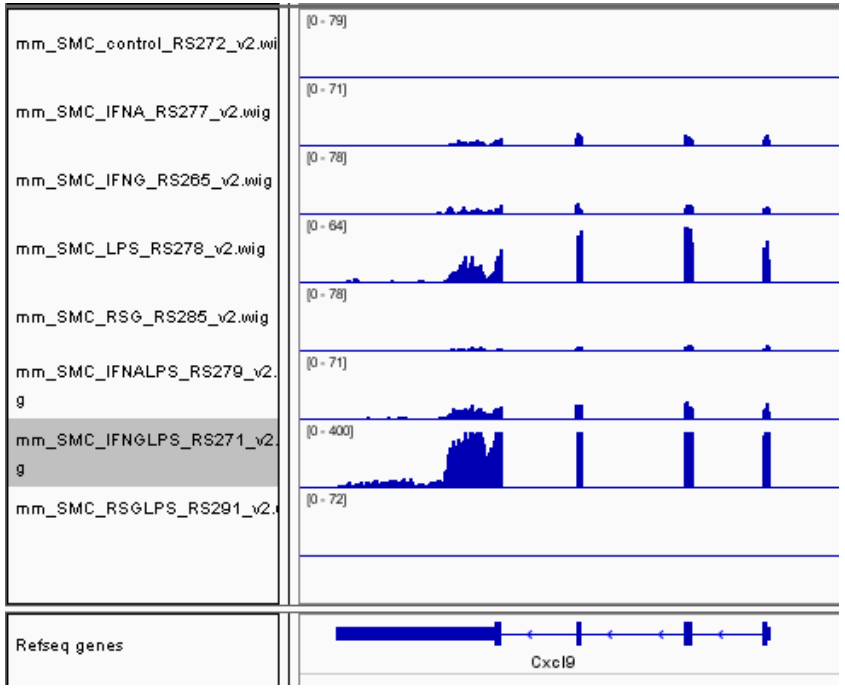


Blencowe B J et al. Genes Dev. 2009;23:1379-1386

IFNγ and TLR Signal integration in SMCs: RNAseq



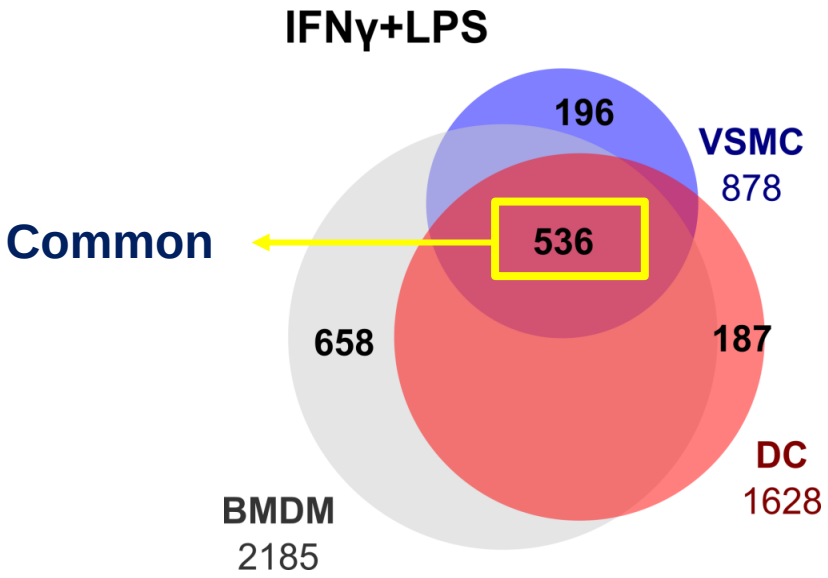
IGV view SMC NOS2 (*.wig files)

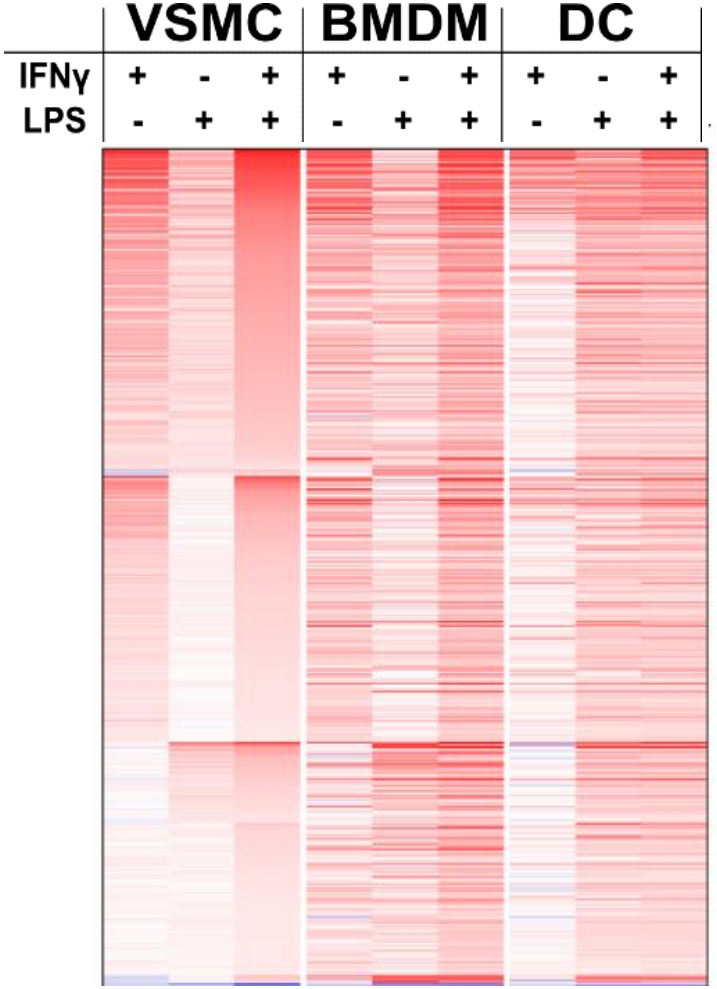


IGV view SMC Cxcl9 (*.wig files)

Piaszyk-Borychowska et al., submitted

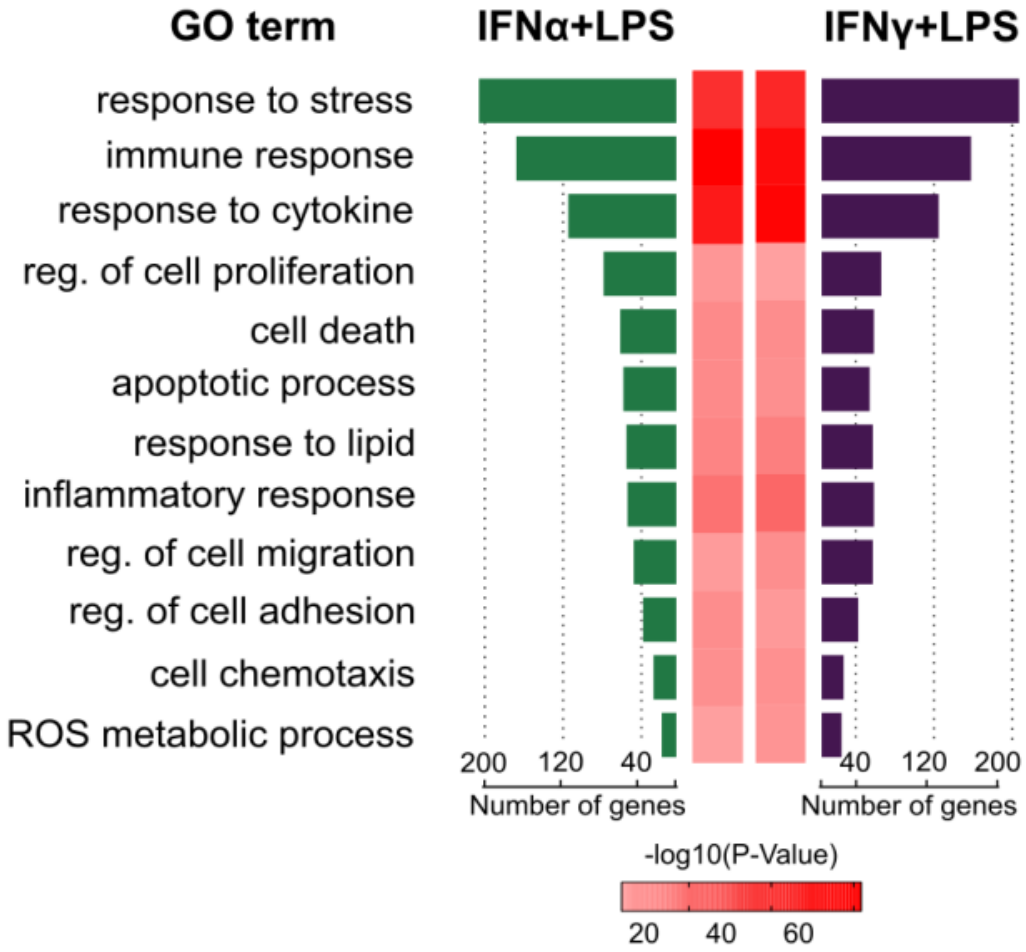
Commonly Up-regulated genes in response to IFNγ and LPS





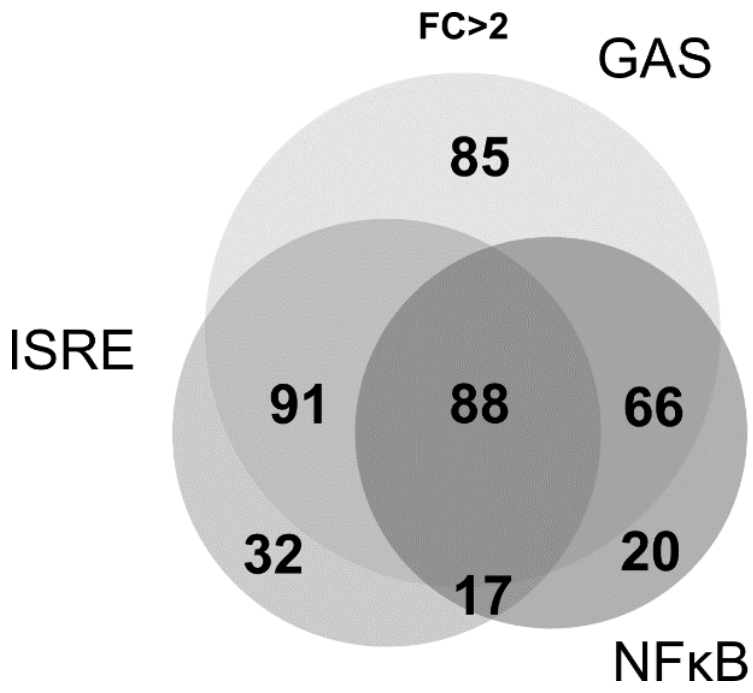
Piaszyk-Borychowska et al., submitted

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

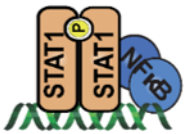


Piaszyk-Borychowska et al., submitted

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



ISRE/NFκB



GAS/NFκB

STAT1 & p65 mediated Signal integration?

Promoter region: -950 +50 bp

Piaszyk-Borychowska et al., submitted

STAT1+p65 ChIP-seq experiment outline

WT C57BL6

Aorta enzymatic digestion

Vascular smooth muscle cells

Treatment (2 replicates):

4h LPS

8h IFNγ

4h LPS + 8h IFNγ

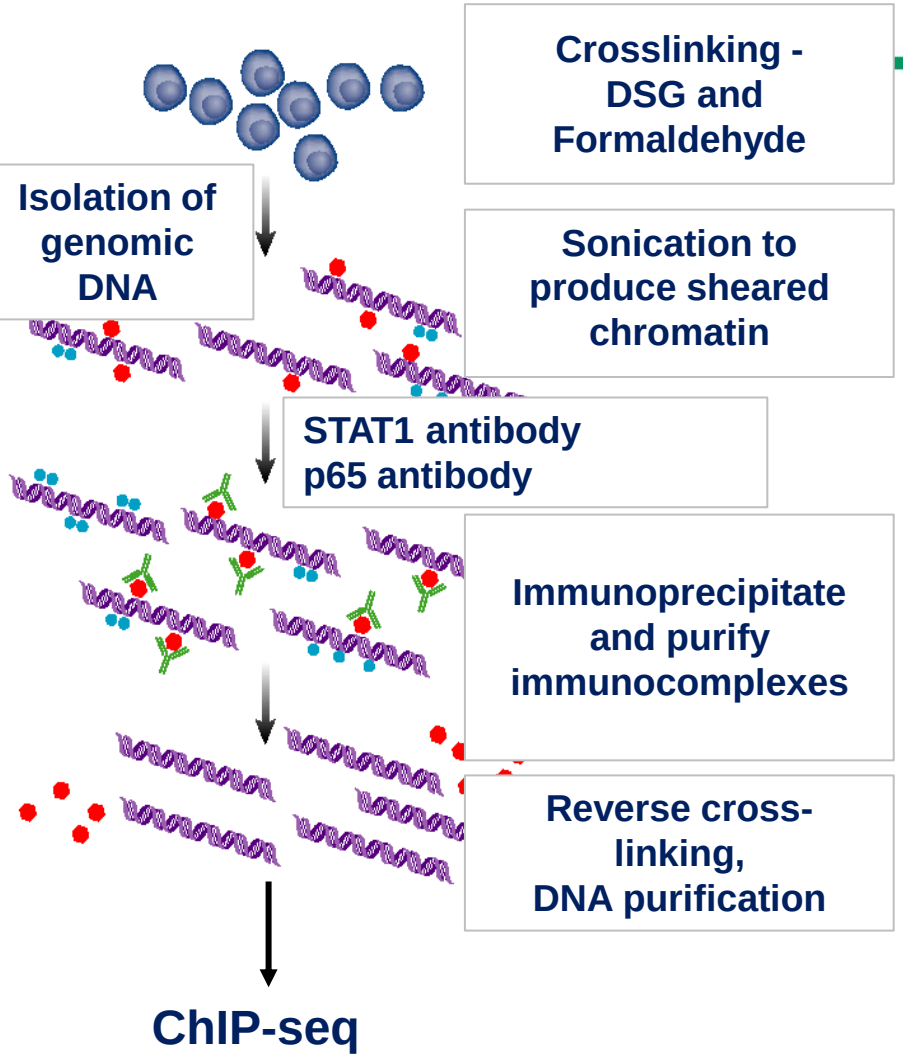
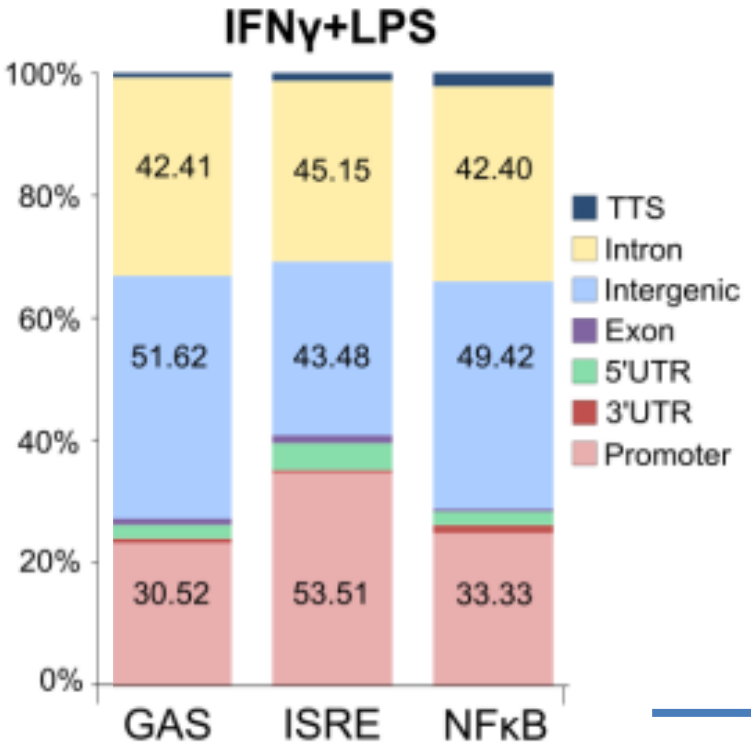


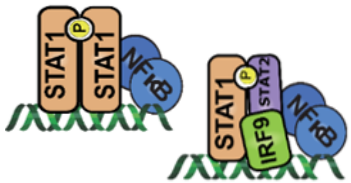
Fig. Nagy Lab modified Mandrup's ChIP protocol. Figure adapted from Nature Methods - 4: 613 – 614. 2007.

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



VSMCs

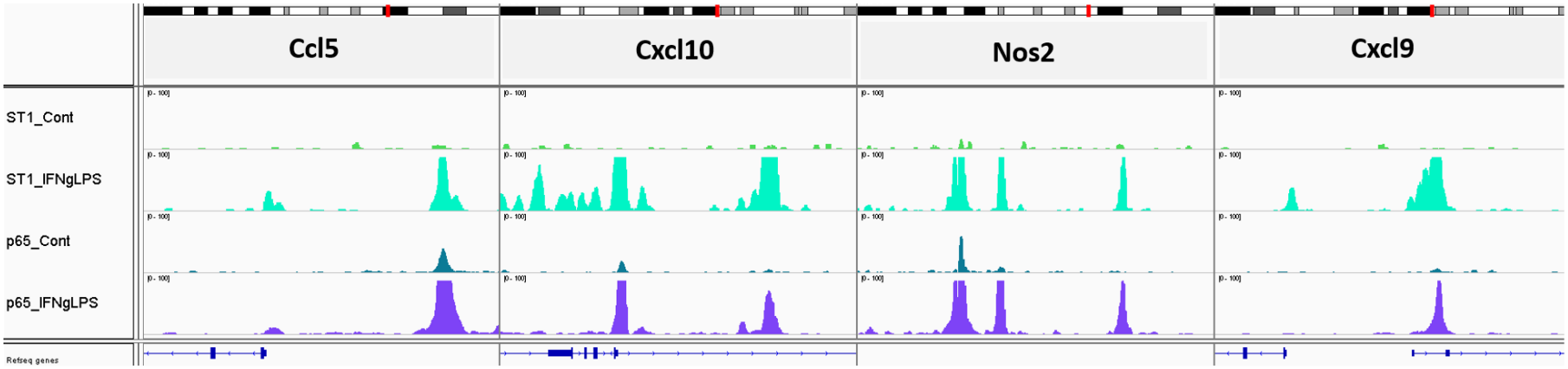
Binding mode	IFNγ+LPS
GAS	17
ISRE	45
NFκB	28
GAS-ISRE	53
GAS-NFκB	40
ISRE-NFκB	59
GAS-ISRE-NFκB	178

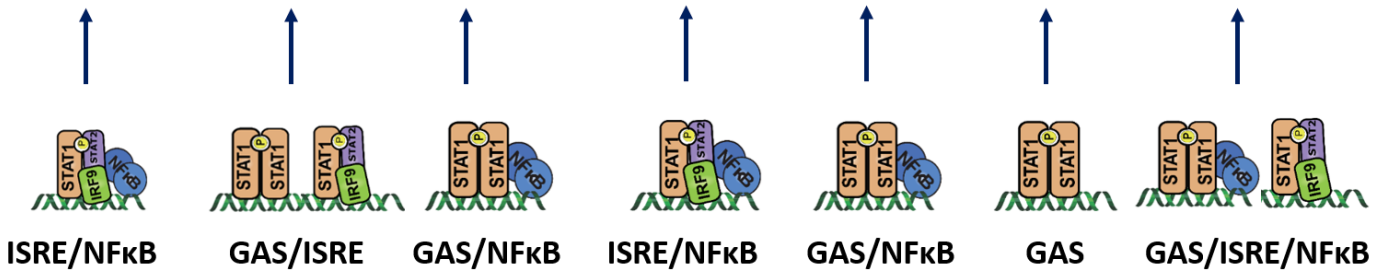


STAT1 & p65 mediated Signal integration?

Piaszyk-Borychowska et al., 2019 Frontiers in Immunology

Commonly IFNγ/LPS upregulated genes: STAT1 + p65 binding

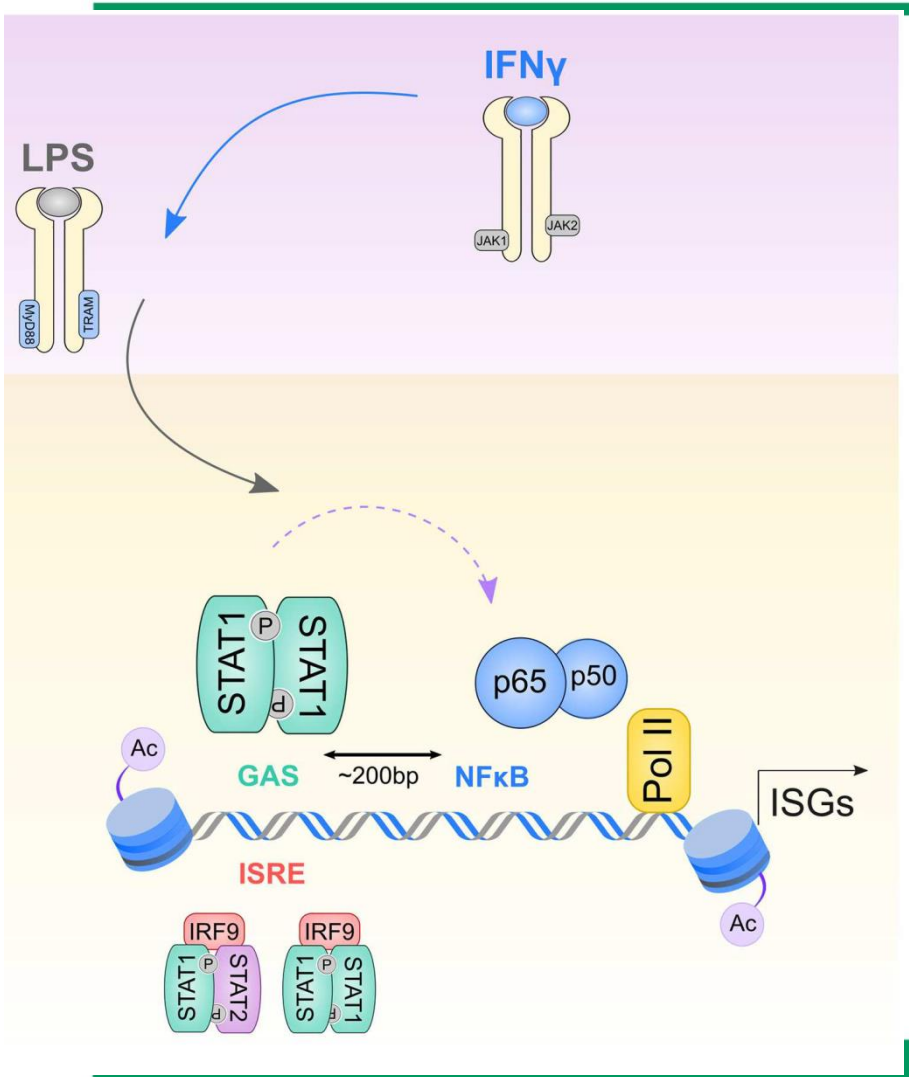




Different "Binding Modes"

Piaszyk-Borychowska et al., submitted

STAT1-dependent epigenetic changes & nearby NFκB binding

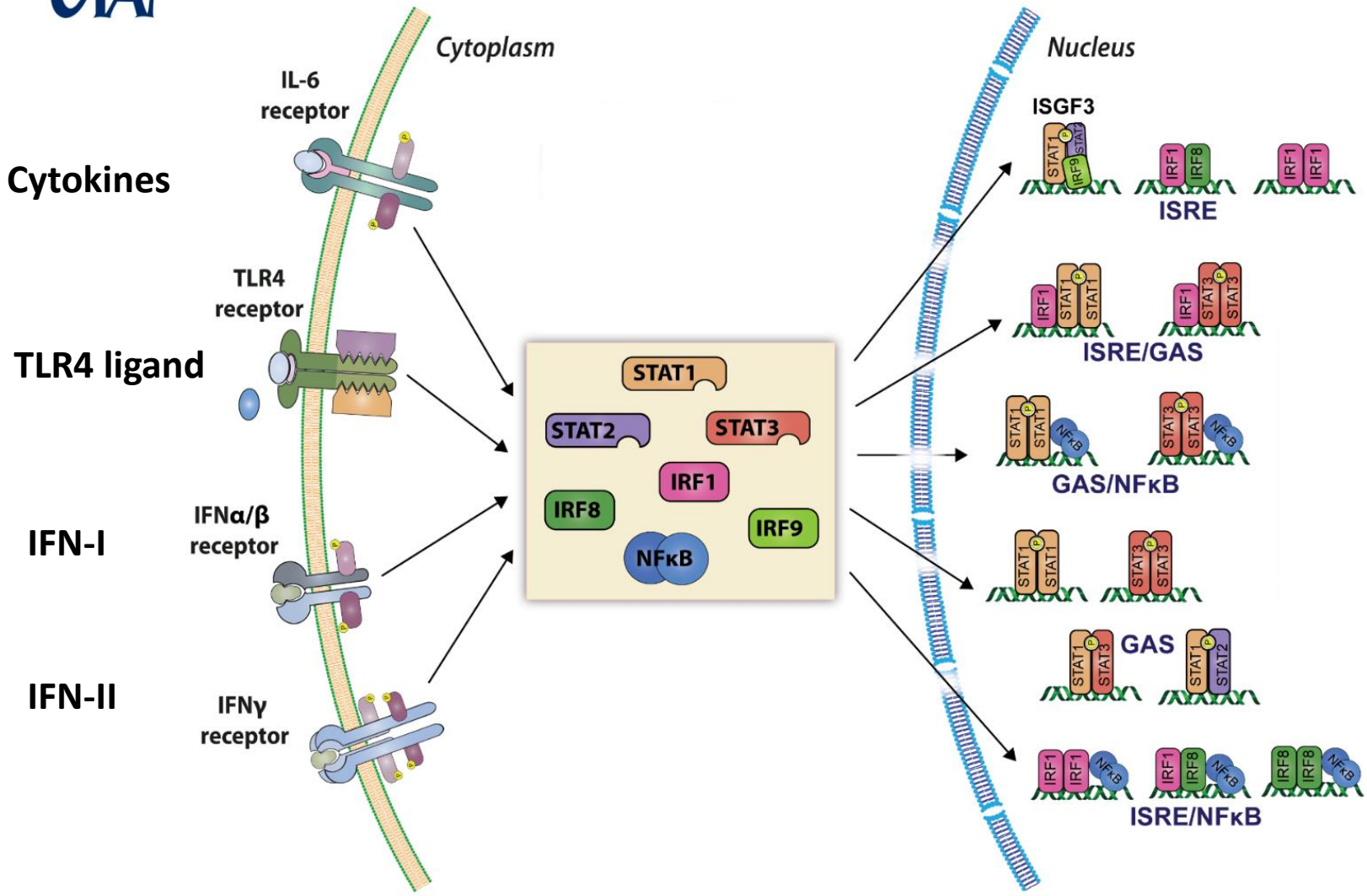


Piaszyk-Borychowska et al., 2019 Frontiers in Immunology

Signal integration in inflammation

Therapeutics

Diagnostics



Szeląg et al., Oncotarget, 2016

Atherosclerosis – vascular inflammation

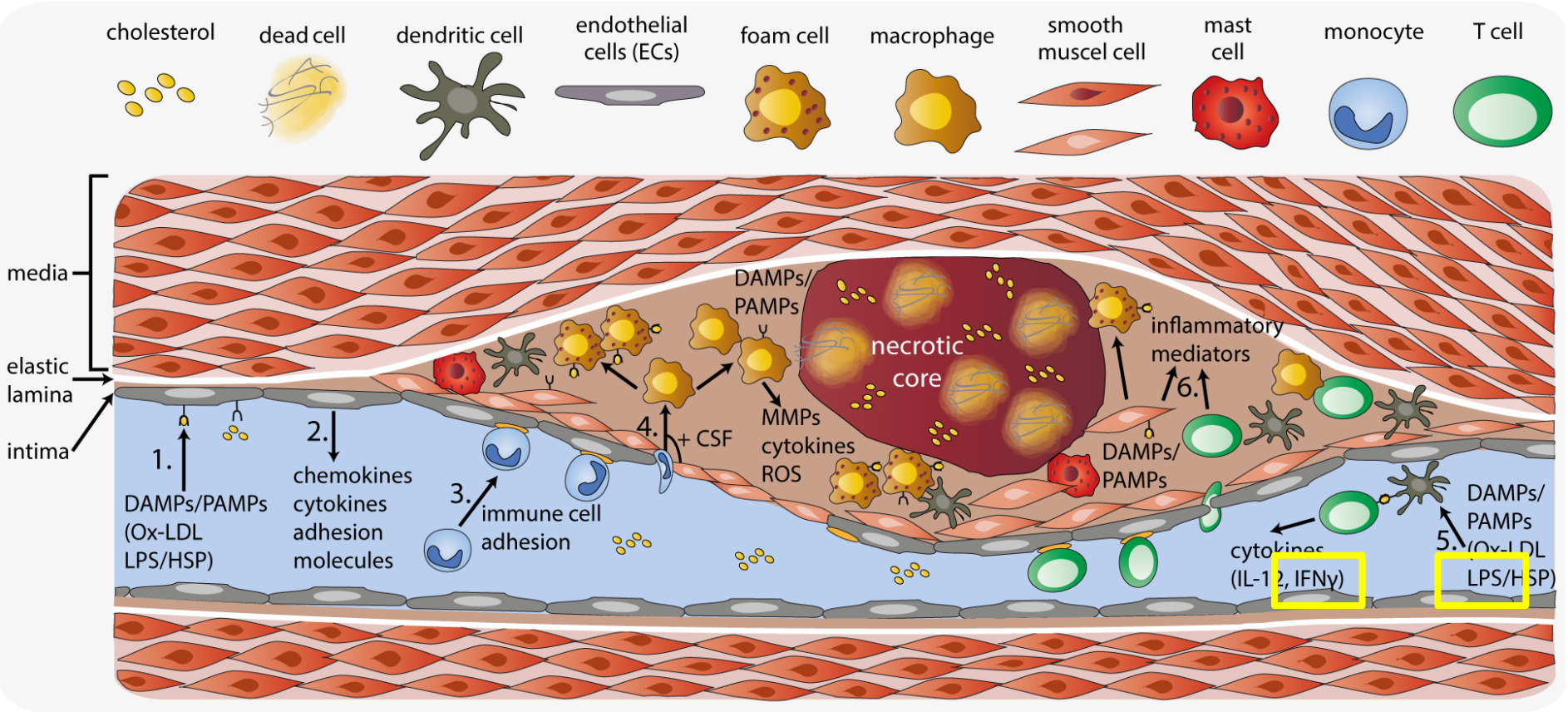
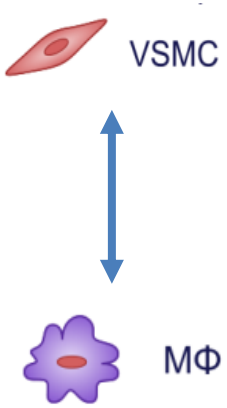
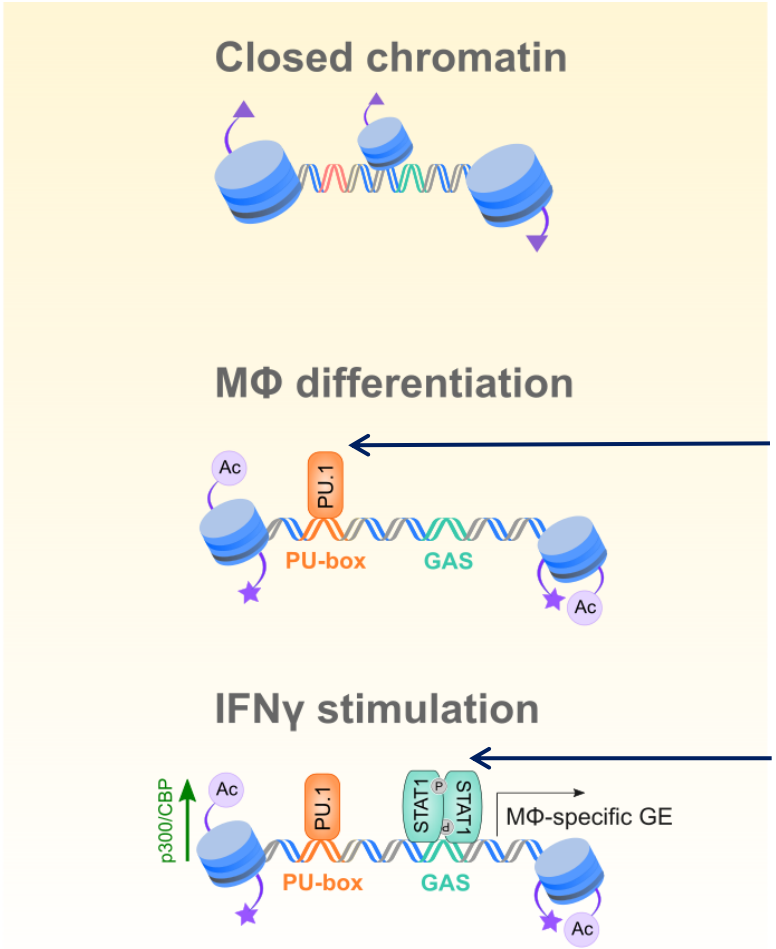


Fig. Atherosclerotic plaque. Chmielewski, Piaszyk-Borychowska et al., Int Rev Immunol, 2016.

Cell type specific gene expression is mediated via collaboration of LDTF and SDTF





Lineage Determining

LDTF (PU.1)

Histone modification (Ac, Me)

SDTF (STAT1)

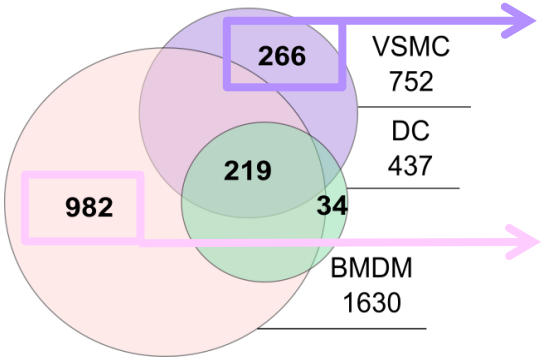
Signal Determining

▲ H3K27me3 ★ H3K4me1 Ac H3K27ac

Source: A Piaszyk-Borychowska own interpretation based on: Cell type specific gene expression. *Heinz et al. Nature reviews Molecular cell biology. 6(3):144-154, 2015*.

Characterization of cell-type specific gene expression in VSMC and MQ in response to IFNγ

A

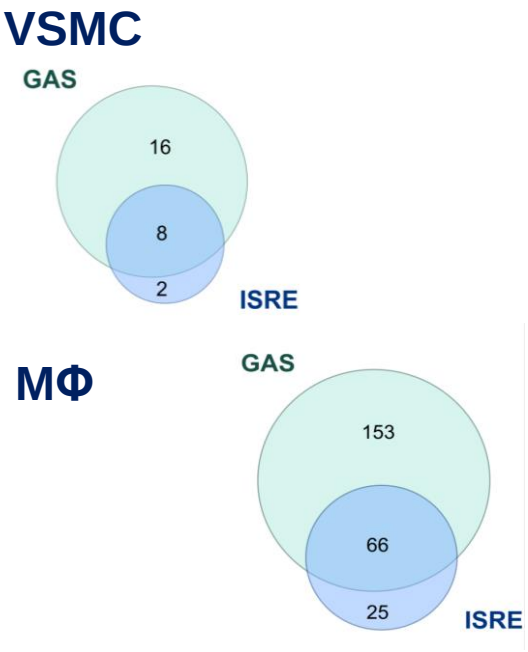


B

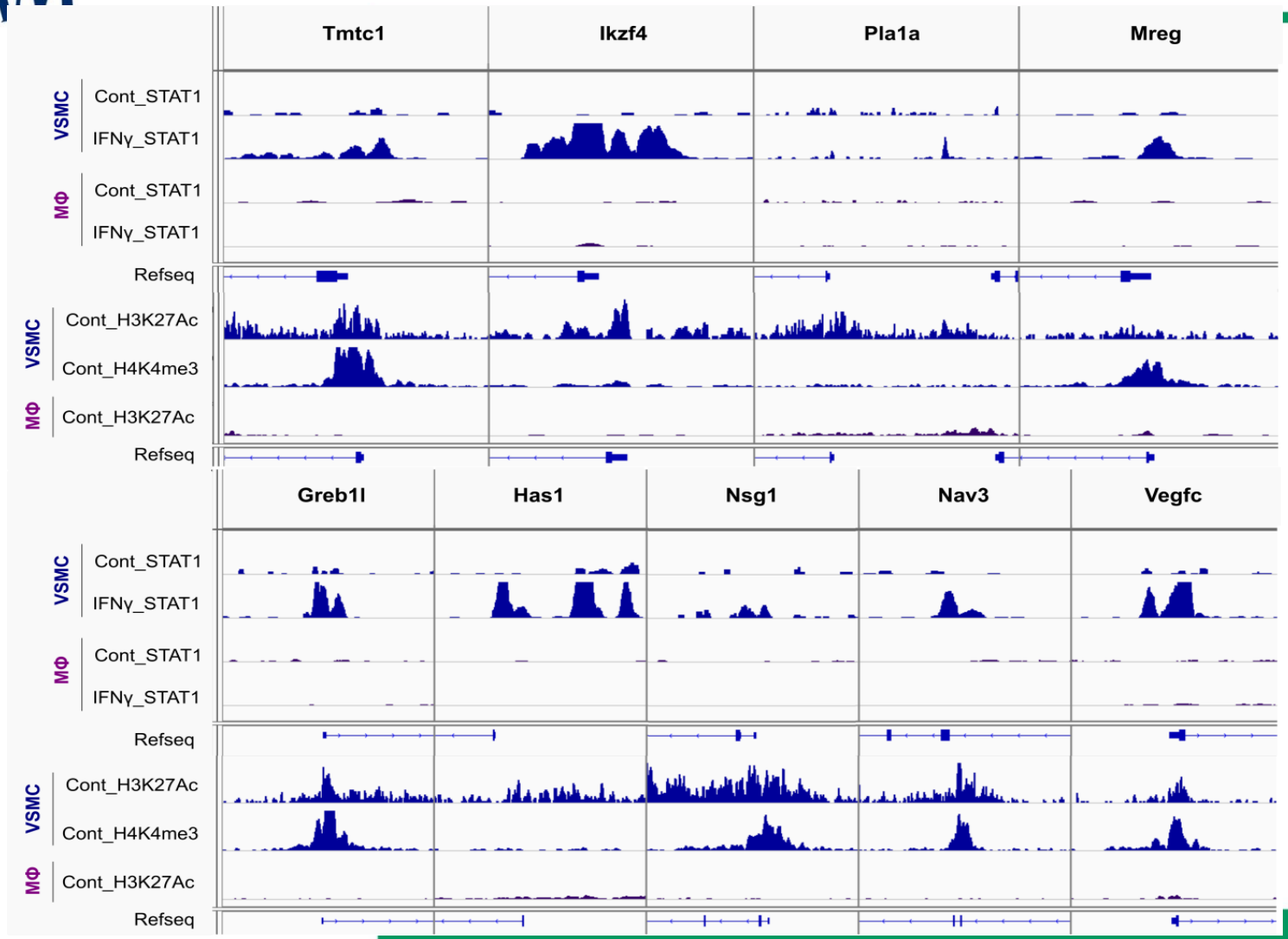
No	Gene name	IFNγ FC	
		VSMC	MΦ
1	Gm6654	270,6	-1,1
2	Chl1	46,6	1,4
3	Mpeg1	34,3	1,4
4	H2-Eb1	16,9	2,0
5	Neurl3	15,8	-1,5
6	Batf3	15,4	1,1
7	Tmtc1	11,8	-1,1
8	Ikzf4	11,7	1,1
9	Mt3	9,3	1,1
10	Trim5	9,2	1,9

No	Gene name	IFNγ FC	
		MΦ	VSMC
1	Clvs1	333,8	1,1
2	Lhx2	190,5	1,0
3	Slc4a11	64,7	-1,1
4	Kdr	59,3	-1,5
5	Prrg4	40,8	1,5
6	Tnfaip8l3	35,3	1,2
7	Kalrn	33,9	-1,2
8	Vcan	31,2	1,4
9	Akap2	25,8	1,5
10	Gm20459	24,1	1,5

C

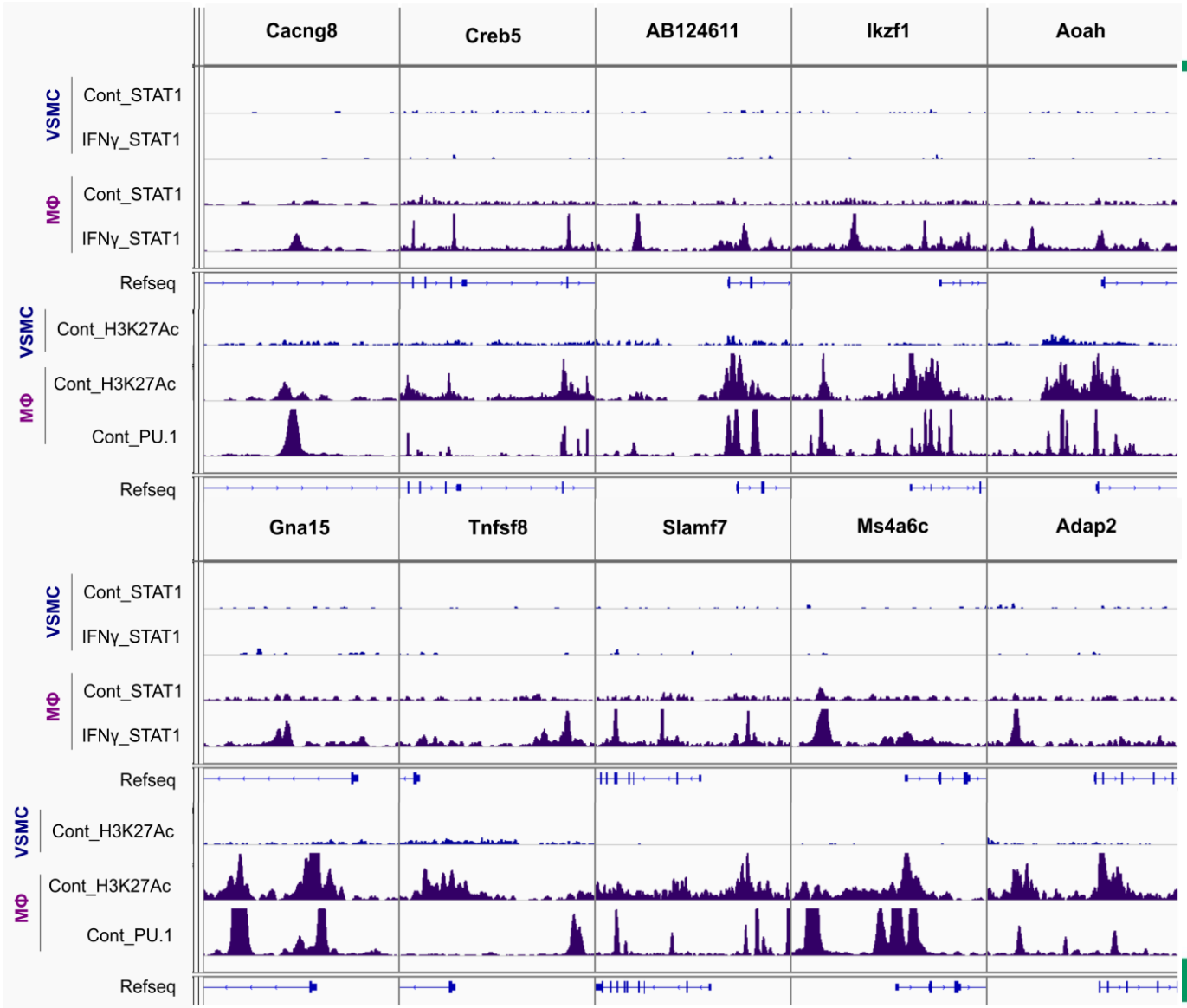


IFNγ activated VSMC-specific transcription: STAT1 binding + epigenetic changes

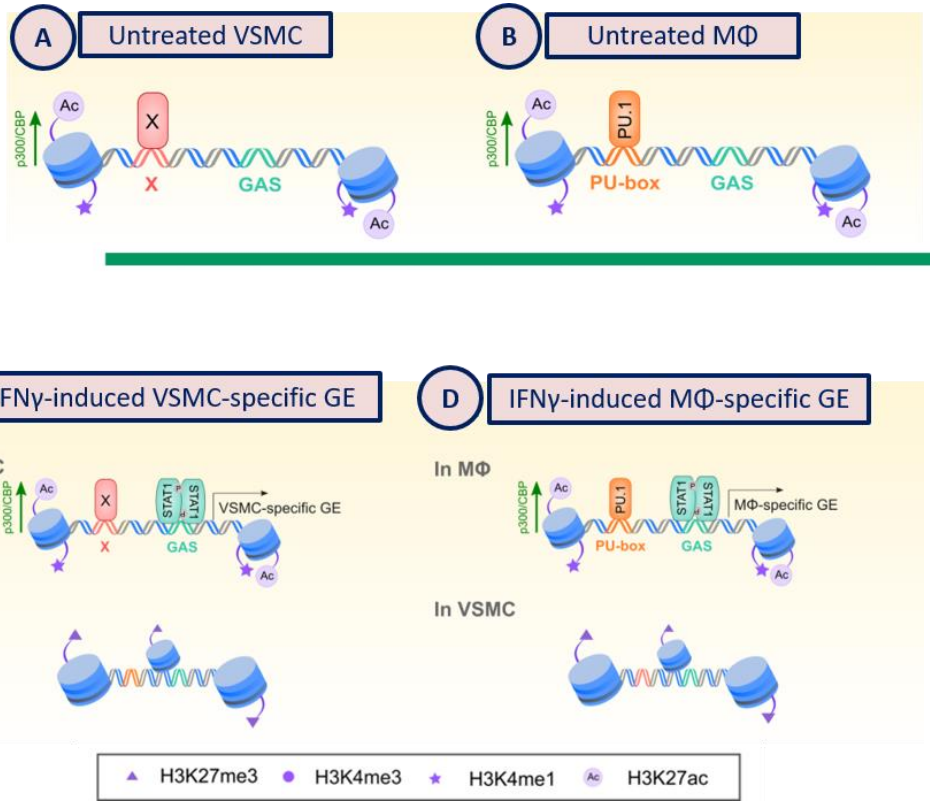


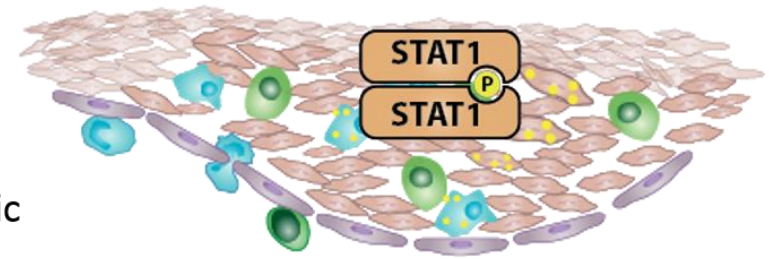
Piaszyk-Borychowska et al., 2019 in preparation

IFNγ activated MQ-specific transcription: STAT1-PU.1 binding + epigenetic changes



Piaszyk-Borychowska et al., 2019 in preparation

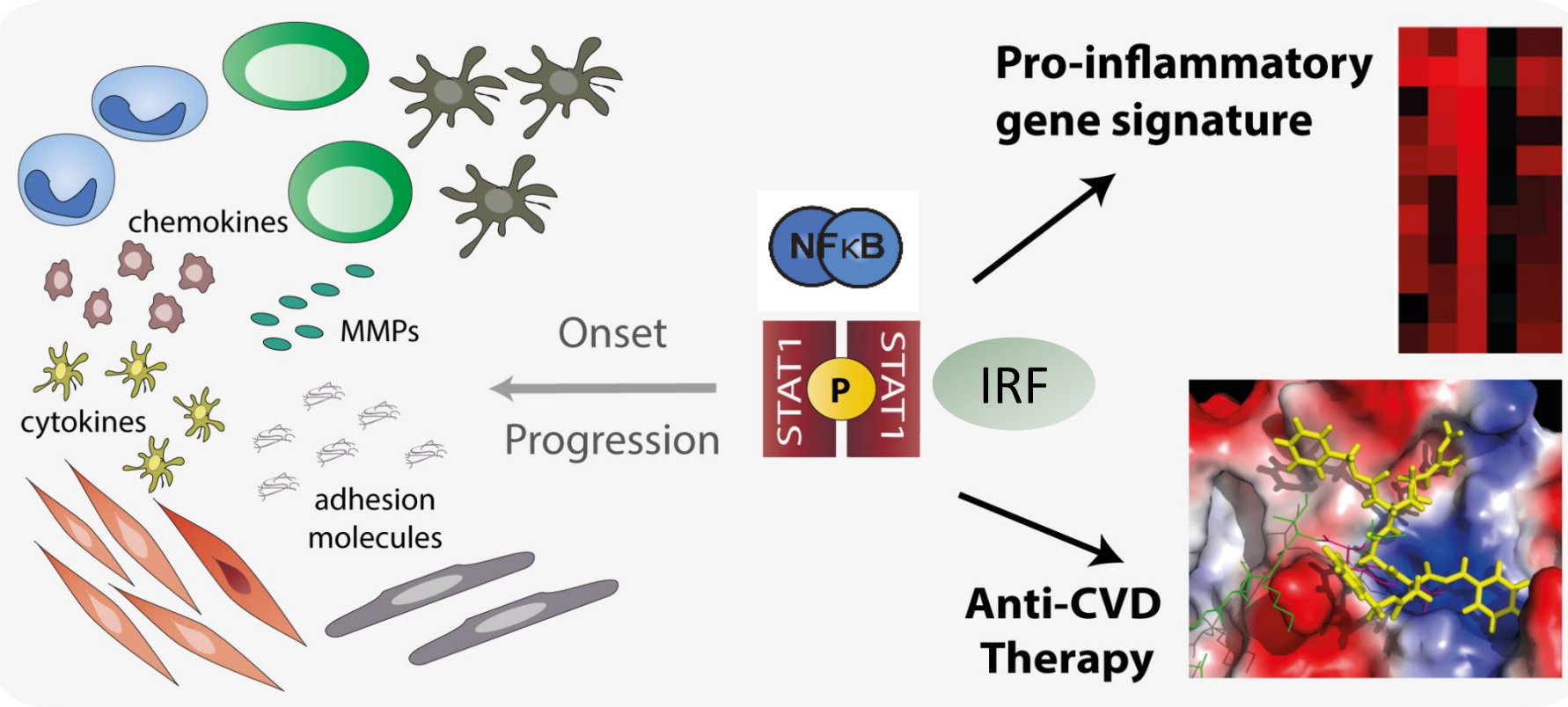




Cell-type specific Diagnostics

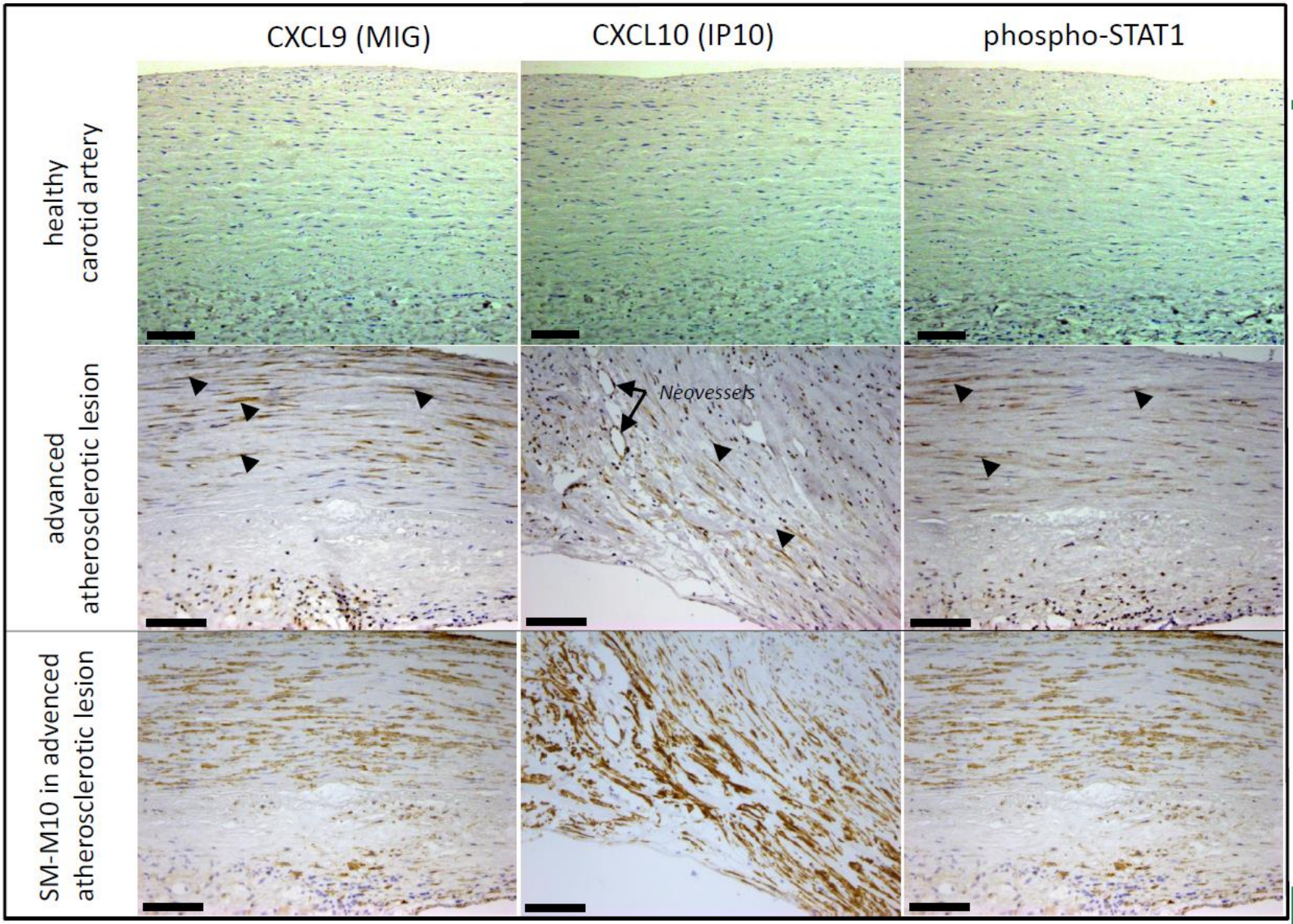
Targeted Therapeutics

STAT1, NF-kB & IRFs in vascular disease



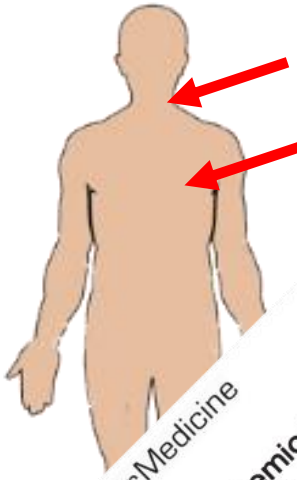
Chmielewski, Piaszyk-Borychowska et al., IRI, 2015

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,[1] 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
.....

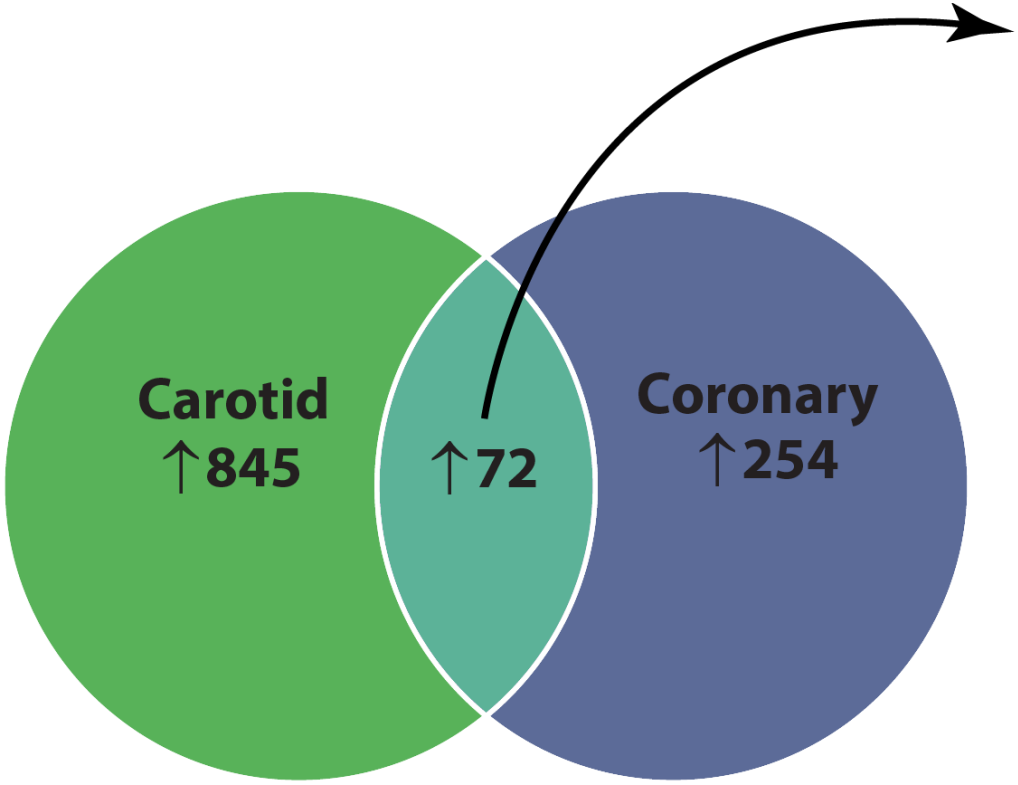
Analysis of promoters and functions

GO terms
functions
pathways
transcription factor binding sites

Sikorski et al., Int J Mol Sci 2014

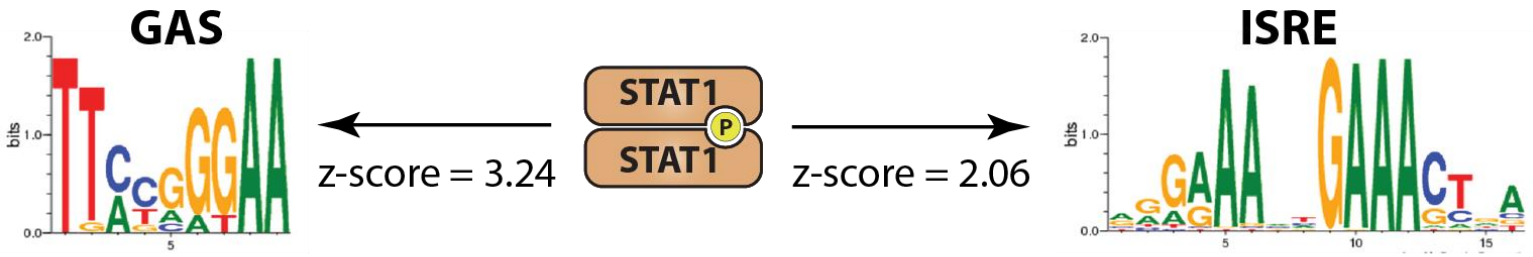
Chmielewski et al., PlosOne 2014

Carotid and coronary plaques share a gene signature

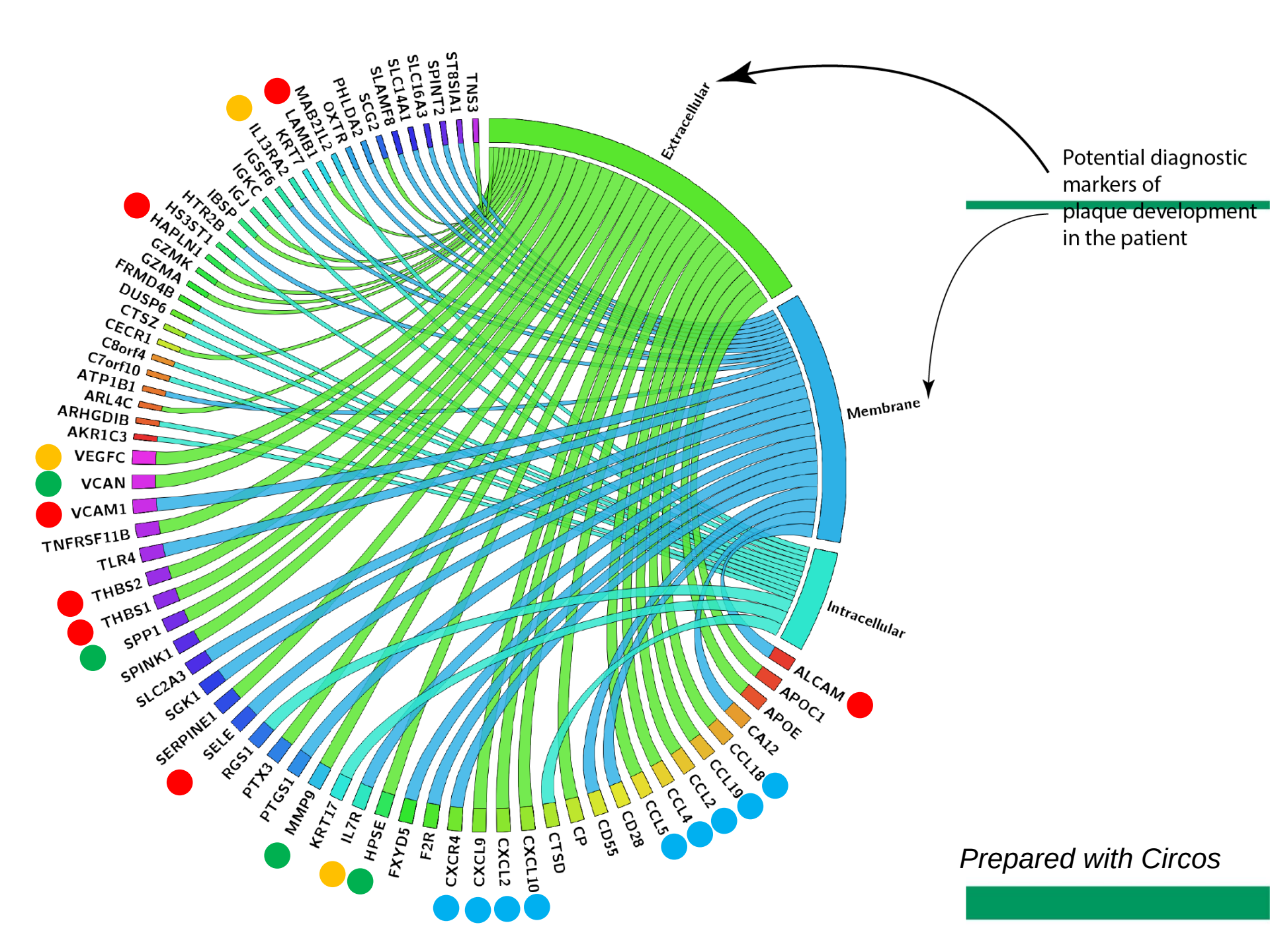


GO term	GO ID	p-value
cell chemotaxis	GO:0060326	2.75E-08
locomotion	GO:0040011	8.40E-08
leukocyte chemotaxis	GO:0030595	1.32E-07
chemotaxis	GO:0006935	1.45E-07
taxis	GO:0042330	1.45E-07
leukocyte migration	GO:0050900	1.80E-07
immune system process	GO:0002376	2.63E-07
cell migration	GO:0016477	4.33E-07
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

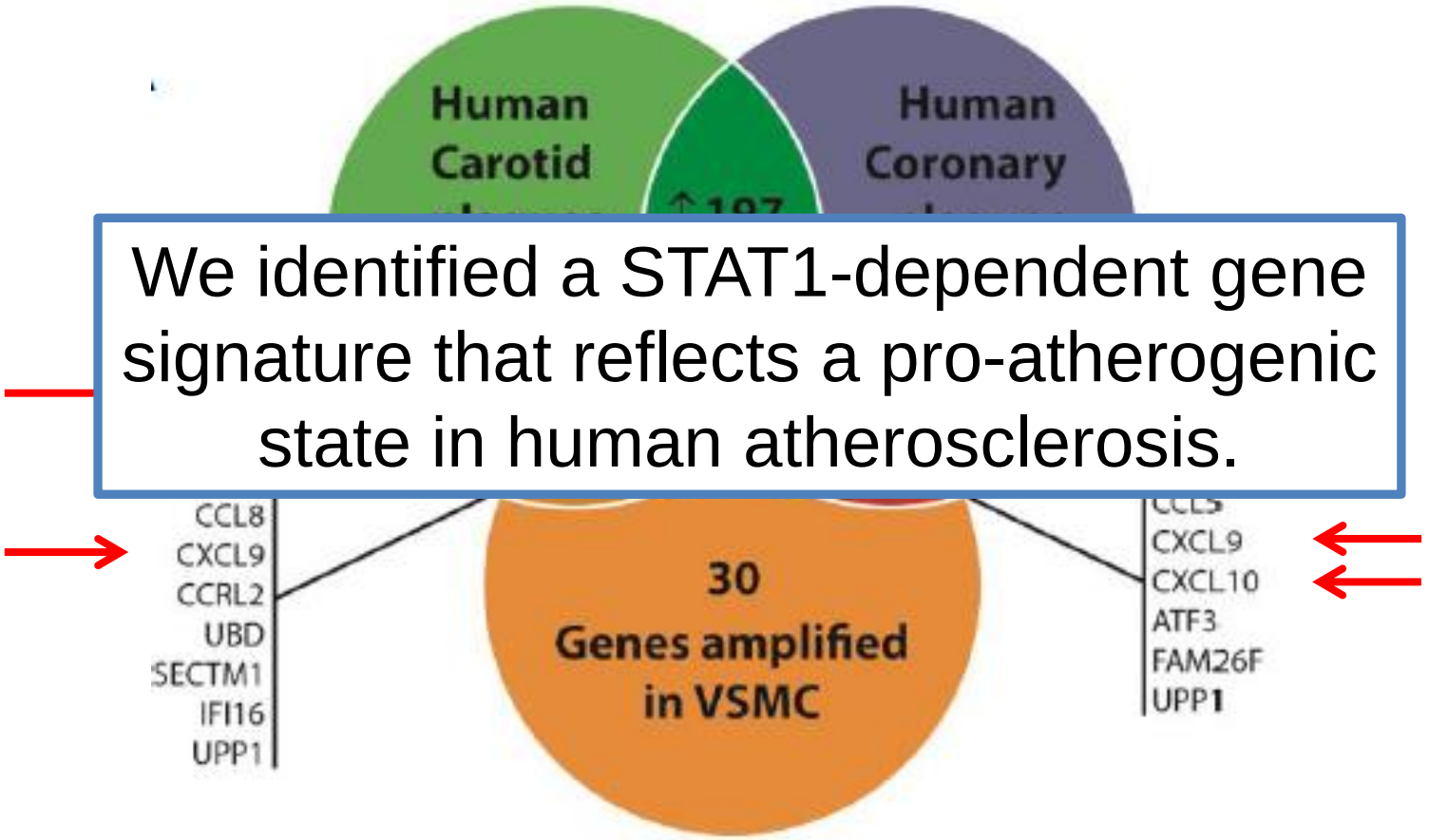
Inflammatory genes upregulated in plaques can be regulated by STAT1



Genomatix


Extracellular
Membrane
Intracellular
TNS3
ST8SIA1
SPINT2
SLC16A3
SLC14A1
SLAMF8
SCG2
PHLDA2
OXTR
MAB21L2
LAMB1
KRT7
IL13RA2
IGSF6
IGKC
IGJ
IBSP
HTR2B
HS3ST1
HAPLN1
GZMK
GZMA
FRMD4B
DUSP6
CTSZ
CECR1
C8orf4
C7orf10
ATP1B1
ARL4C
ARHGDIB
AKR1C3
VEGFC
VCAN
VCAM1
TNFRSF11B
TLR4
THBS2
THBS1
SPP1
SPINK1
SLC2A3
SGK1
SERPINE1
SELE
RGS1
PTX3
PTGS1
MMP9
KRT17
IL7R
HPSE
FXYD5
F2R
CXCR4
CXCL9
CXCL2
CXCL10
CTSD
CP
CD55
CD28
CCL5
CCL4
CCL2
CCL19
CCL18
CA12
APOE
APOC1
ALCAM
Potential diagnostic markers of plaque development in the patient
Prepared with Circos

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

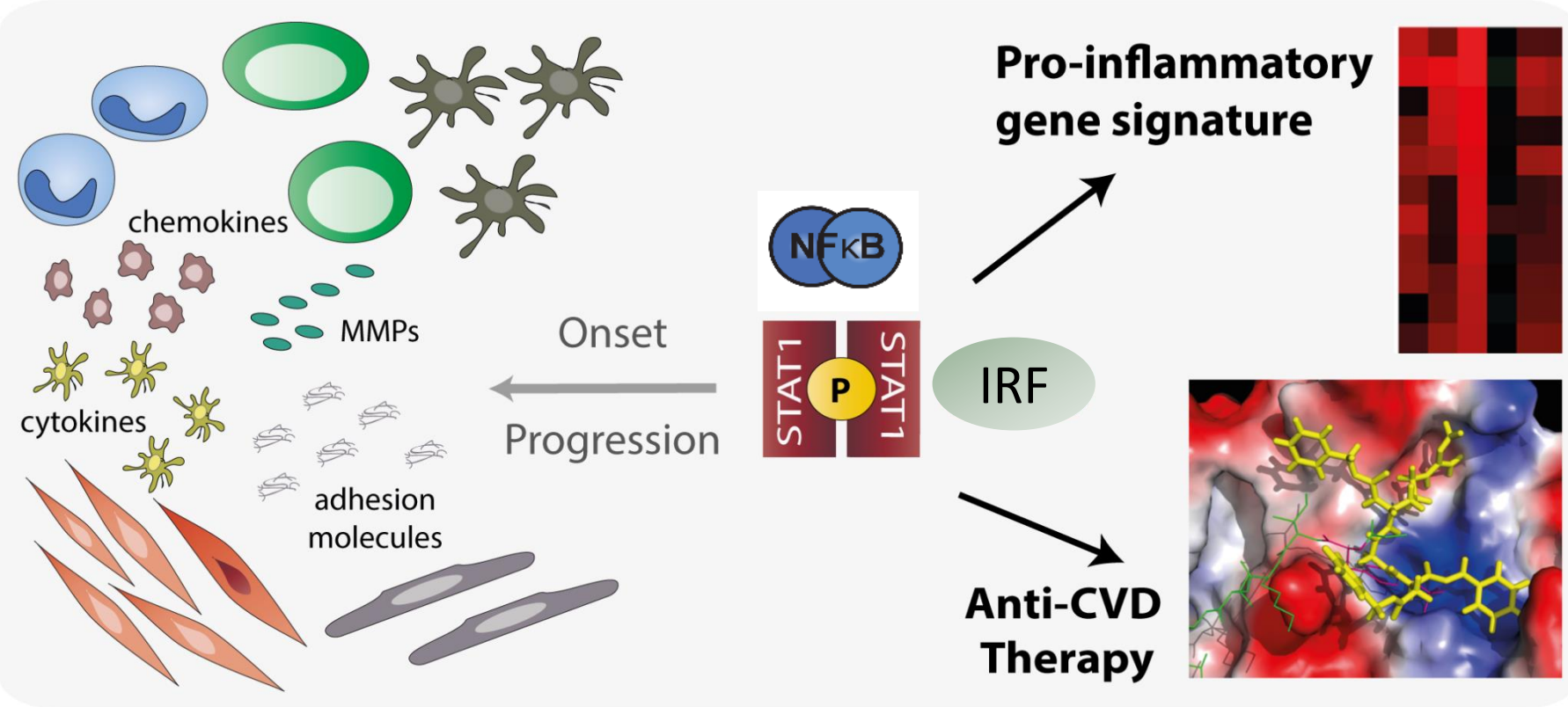


We identified a STAT1-dependent gene signature that reflects a pro-atherogenic state in human atherosclerosis.

Sikorski et al., Int J Mol Sci 2014

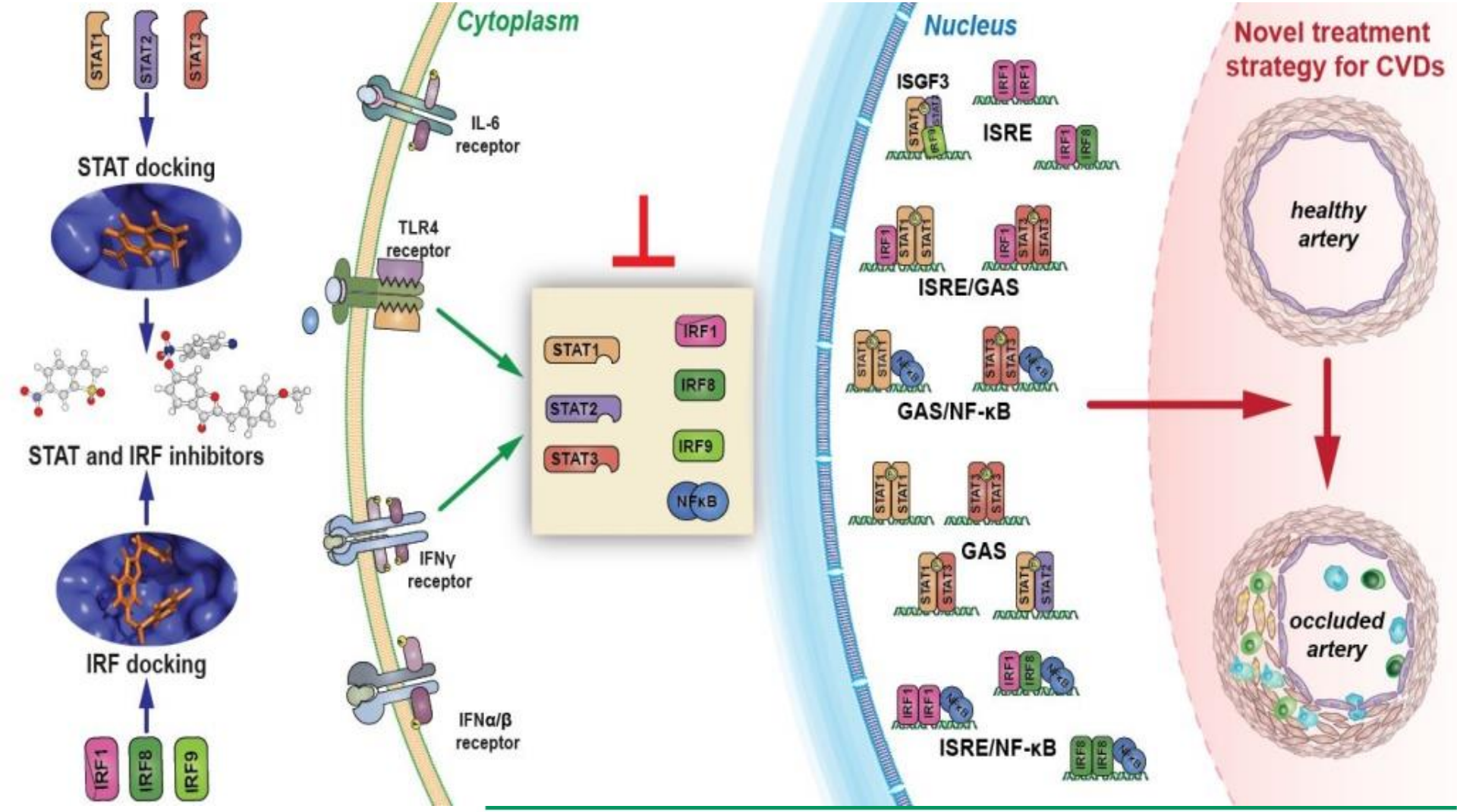
Chmielewski et al., PlosOne 2014

STAT1, NF-kB & IRFs in vascular disease

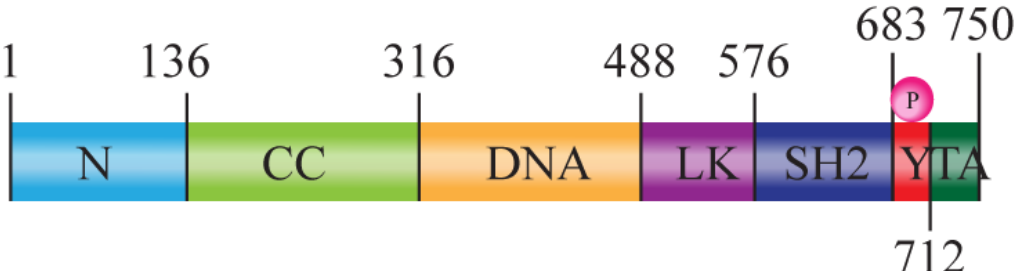


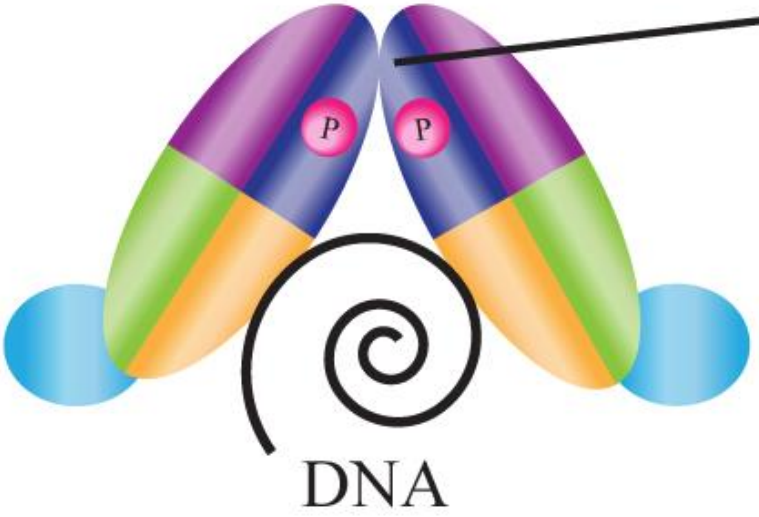
Chmielewski, Piaszyk-Borychowska et al., IRI, 2015

Inhibition of STATs & IRFs in Vascular Disease



STAT Structure & Dimerization



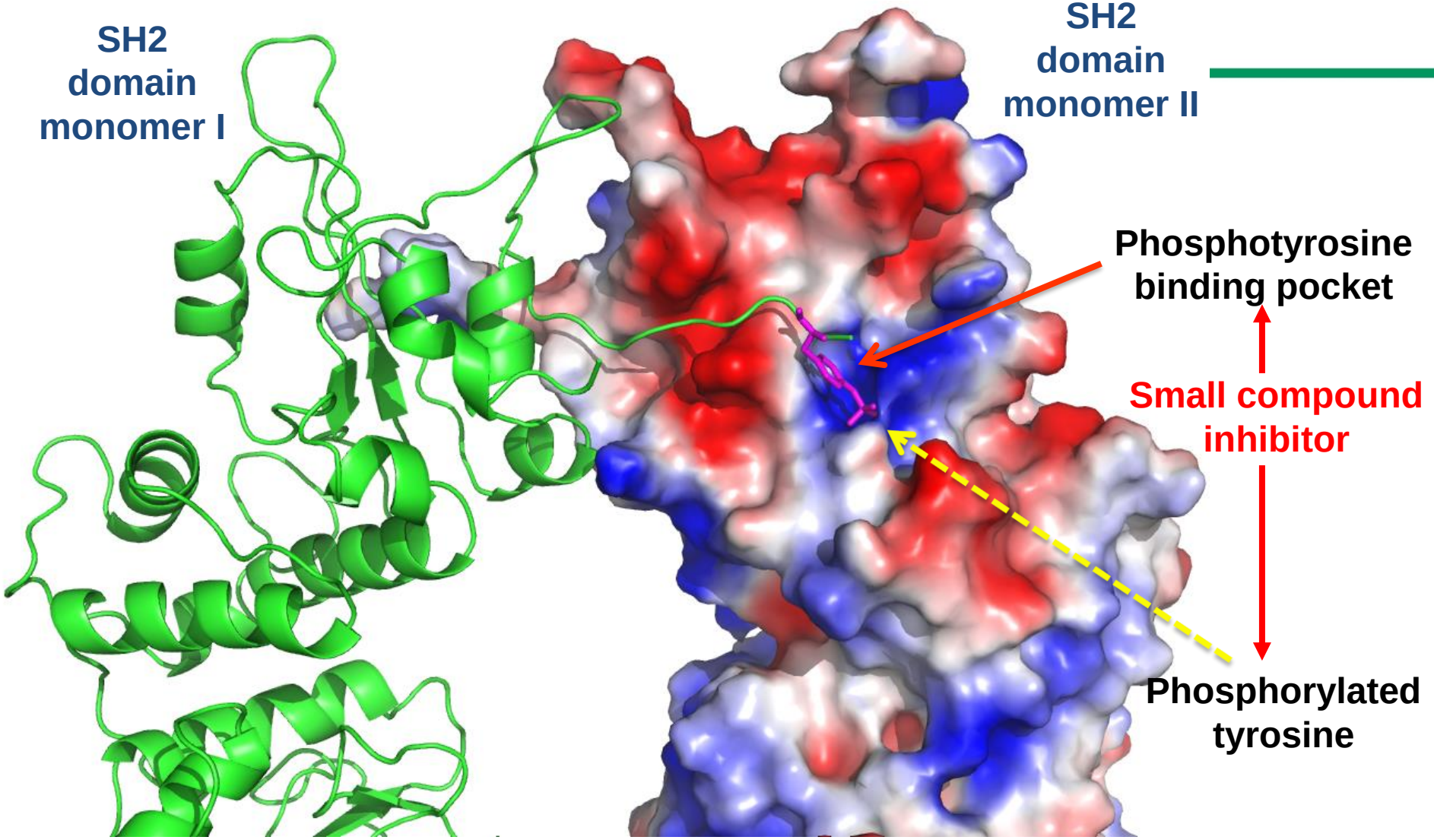


Interacting
SH2-domain

STAT-dimer

Sikorski et al., CGFR 2011

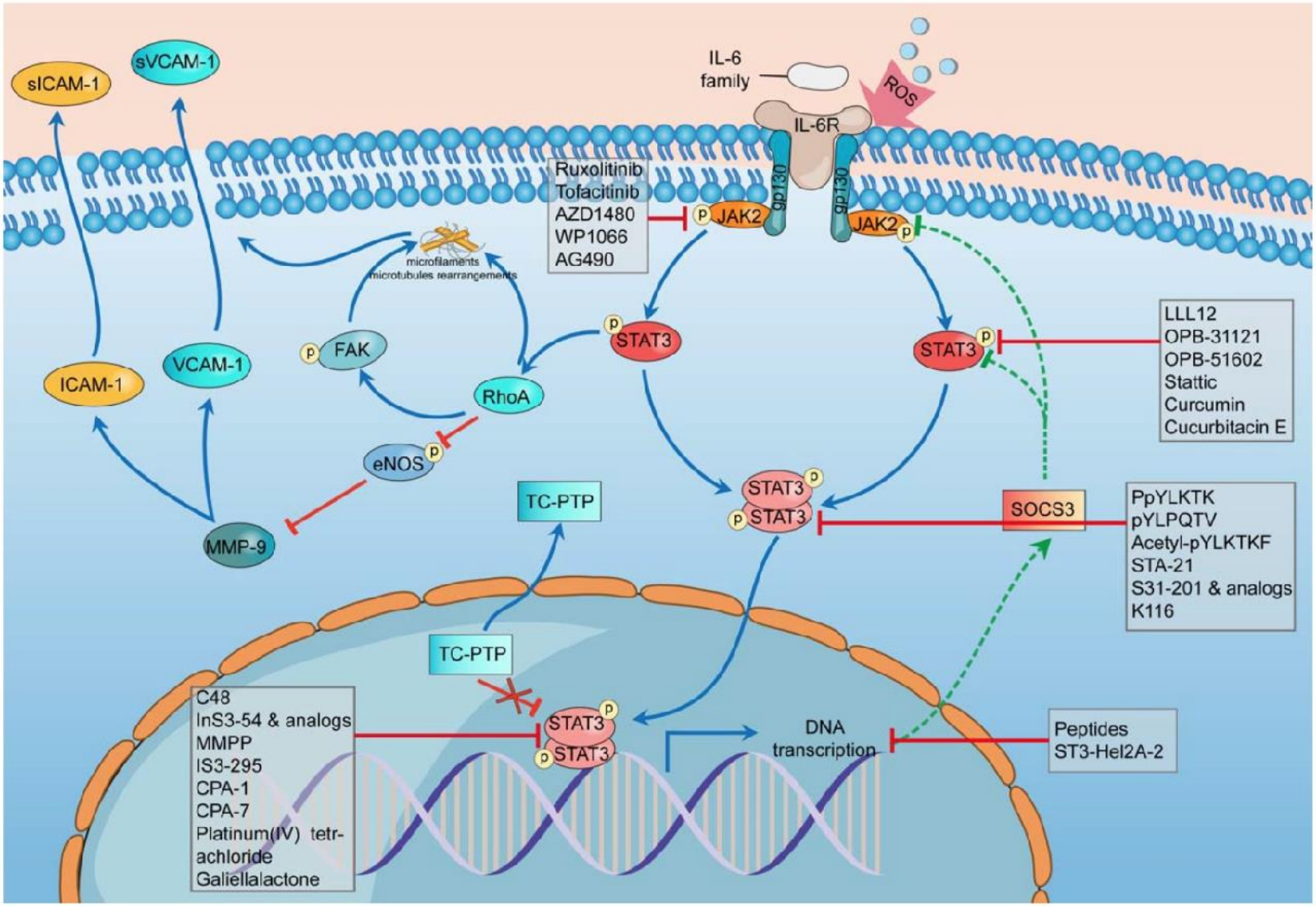
Structural information: STAT1-STAT3



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

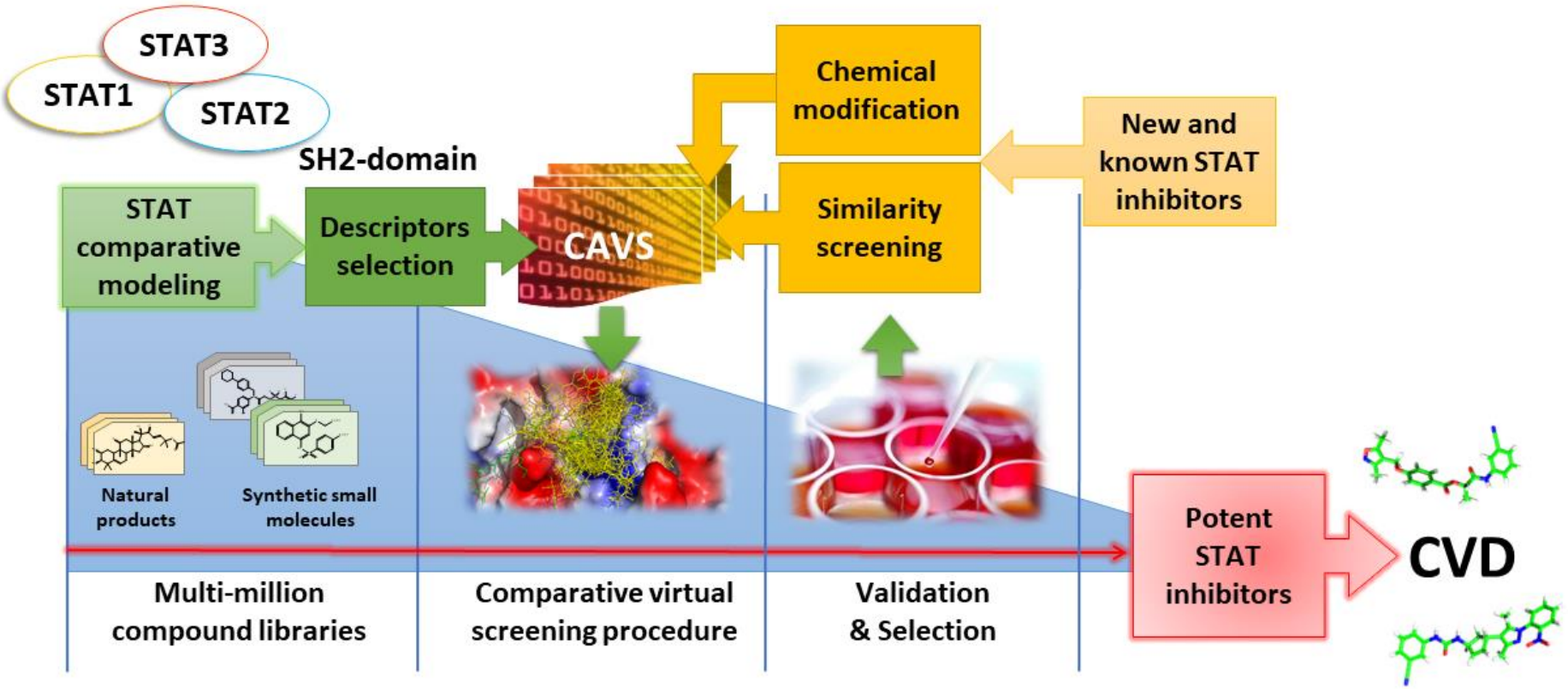
Becker et al., 1998, Nature, 394:145

Known STAT3 Inhibitors



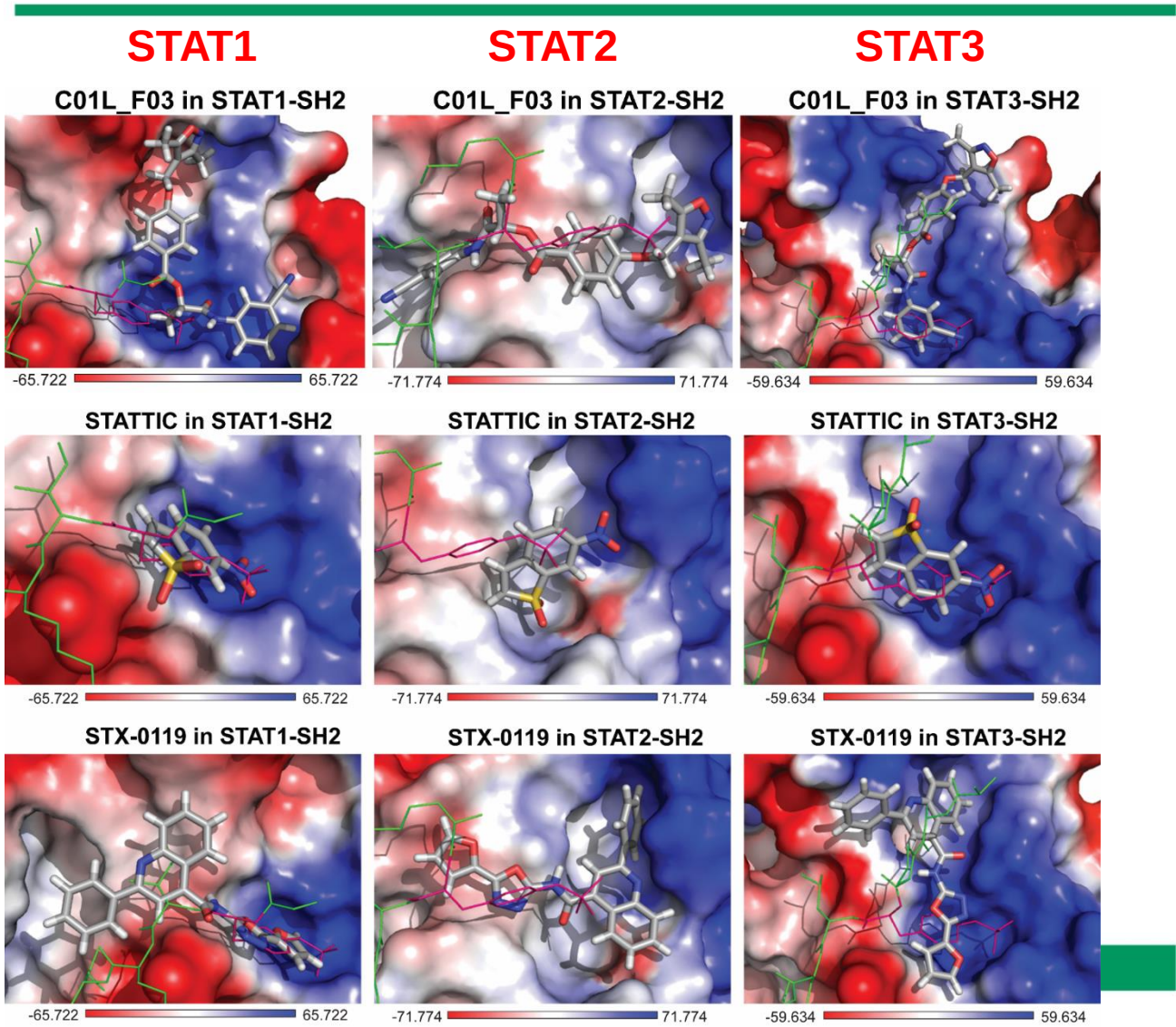
Qi Chen et al. Theranostics, 2019

Pipeline approach to Identify & Validate potent STAT inhibitors



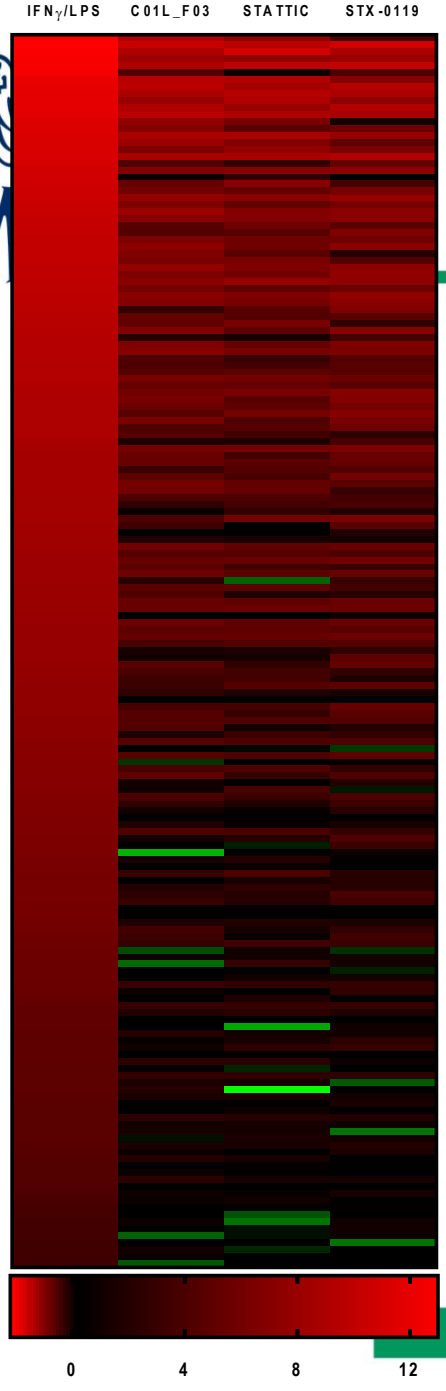
Szeląg et al., Oncotarget, 2016

Multi-STAT inhibitors bind *in silico* STAT-SH2 models



Plens-Gałąska et al. 2018
Frontiers in Immunology

Genome-wide effect of multi-STAT inhibitors



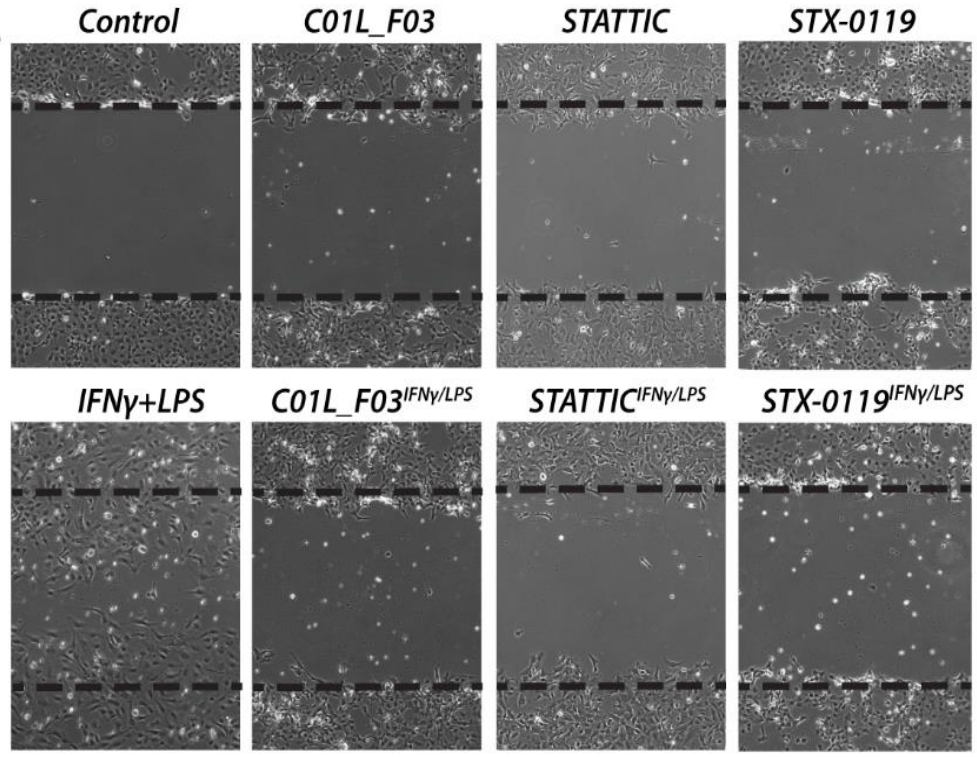
159 genes commonly inhibited by C01L_F03, STATTIC, STX-0119

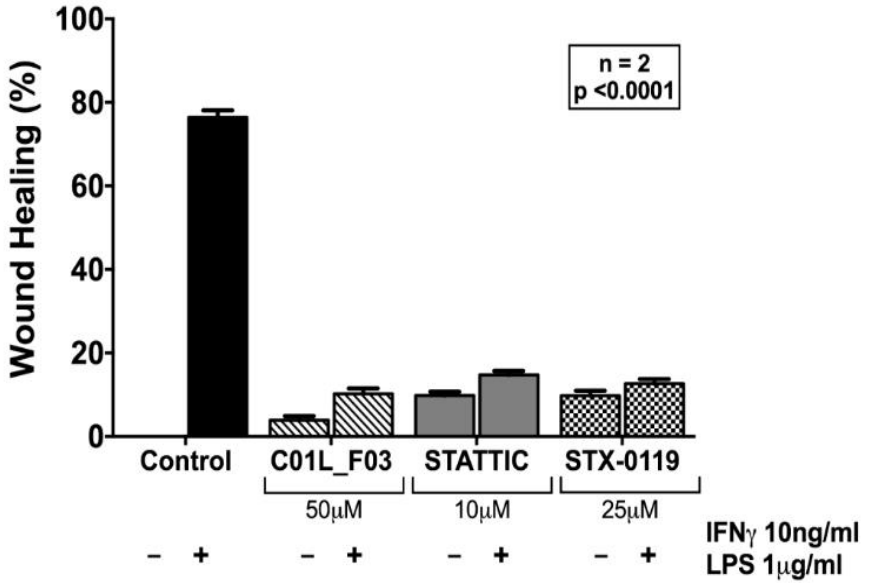
GO term	Biological Process	Fold Enrichment
GO:0043207	response to external biotic stimulus	28.14
GO:0009607	response to biotic stimulus	27.38
GO:0006952	defense response	29.61
GO:0019221	cytokine-mediated signaling pathway	29.62
GO:0002376	immune system process	28.66
GO:0001817	regulation of cytokine 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

Plens-Gałąska et al. 2018 Frontiers in Immunology

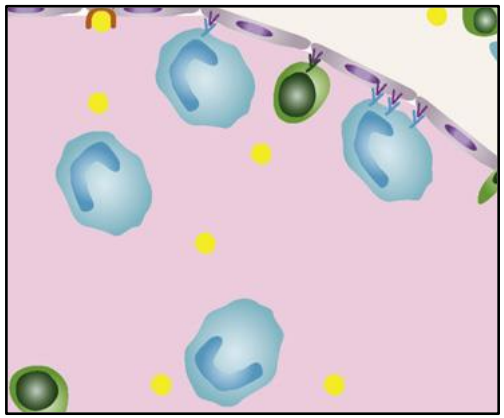
Multi-STAT inhibitors block inflammation induced EC-migration



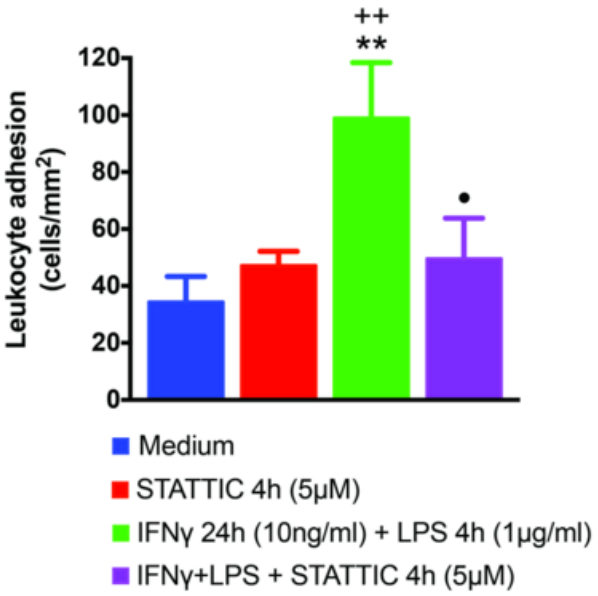


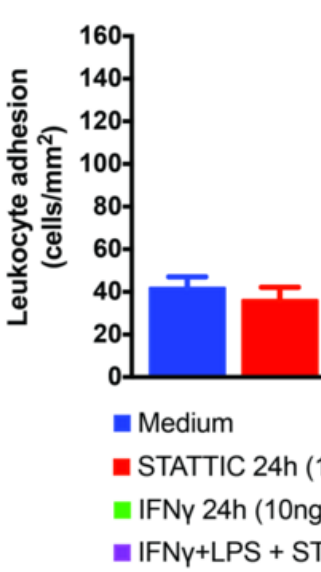
Plens-Gałąska et al. 2018 Frontiers in Immunology

Multi-STAT inhibitors block inflammation induced leukocyte-EC adhesion



STATTIC (1 uM)



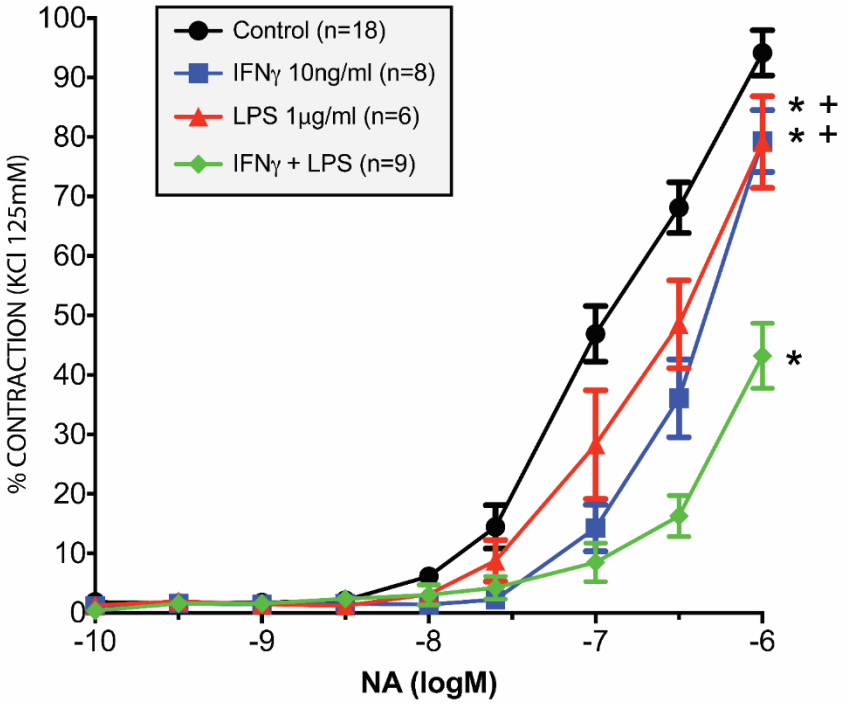


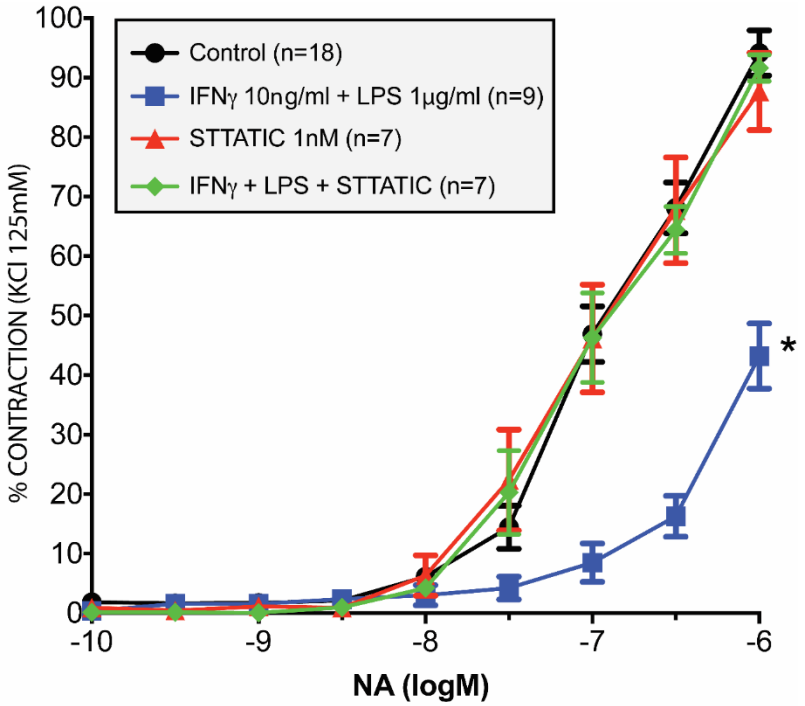
Plens-Gałąska et al. 2018 Frontiers in Immunology

Multi-STAT inhibitors restore inflammation induced impaired arterial contractility

Mesenteric arteries

STATTIC (1 nM)



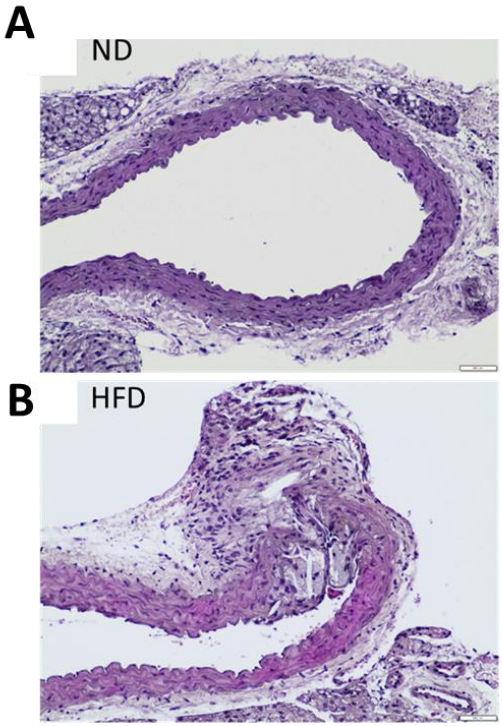


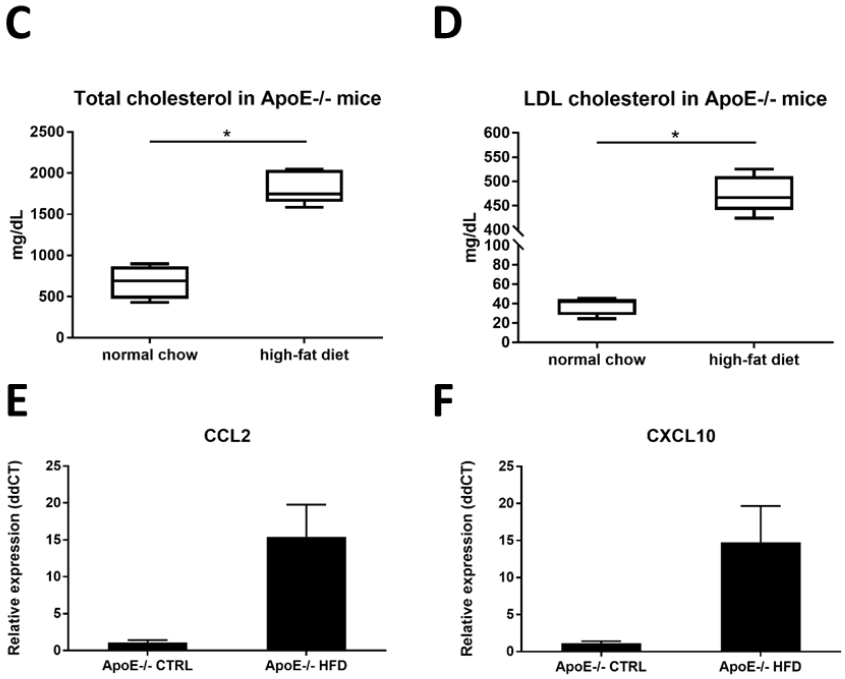
* - relative to Control + - relative to IFNγ+LPS

* - relative to Control

Plens-Gałąska et al. 2018 Frontiers in Immunology

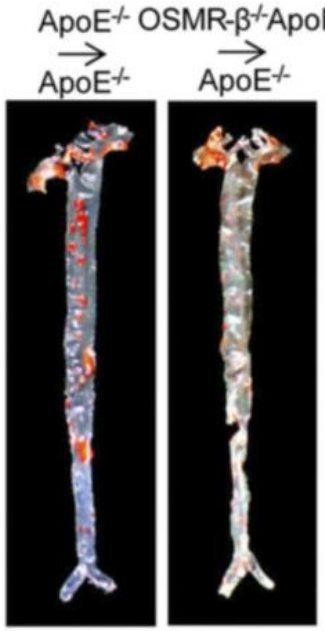
STAT-target gene expression in HFD treated ApoEKO mice

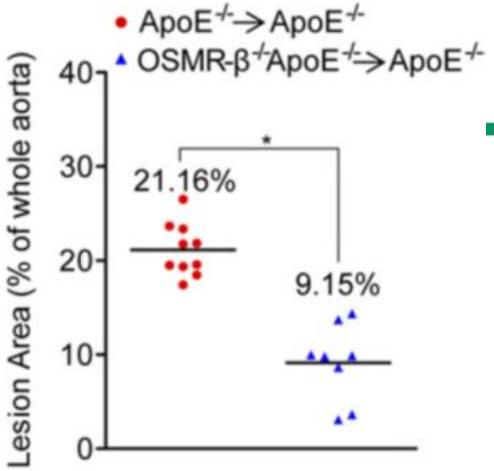


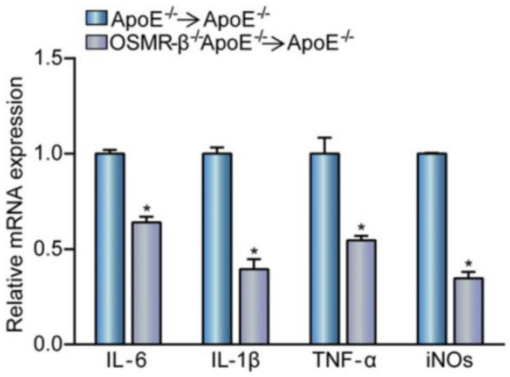


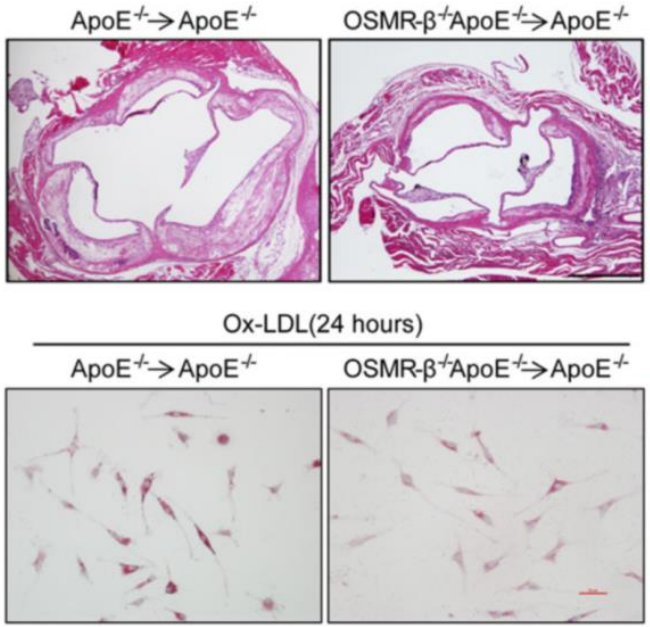
ApoEKO + 10 weeks HFD

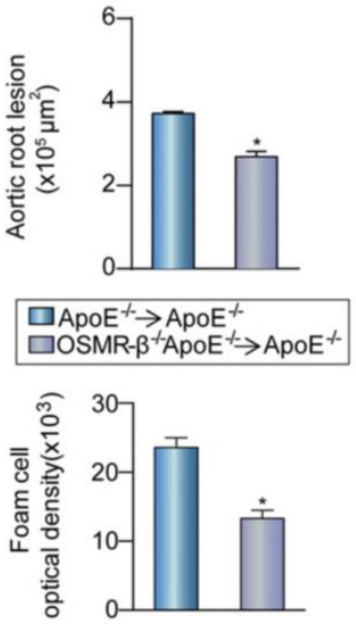
Oncostatin M - STAT3 in Atherosclerosis



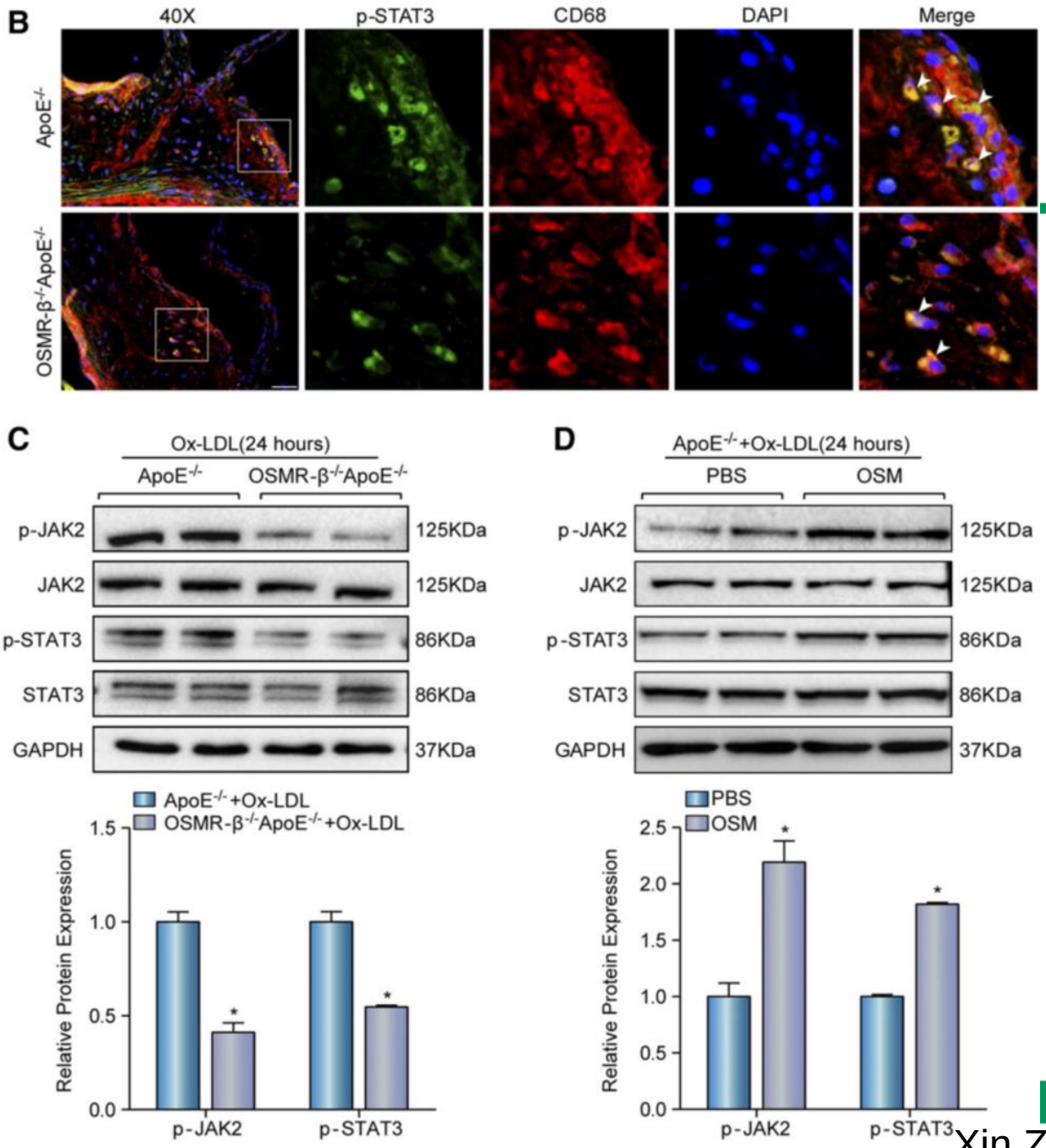






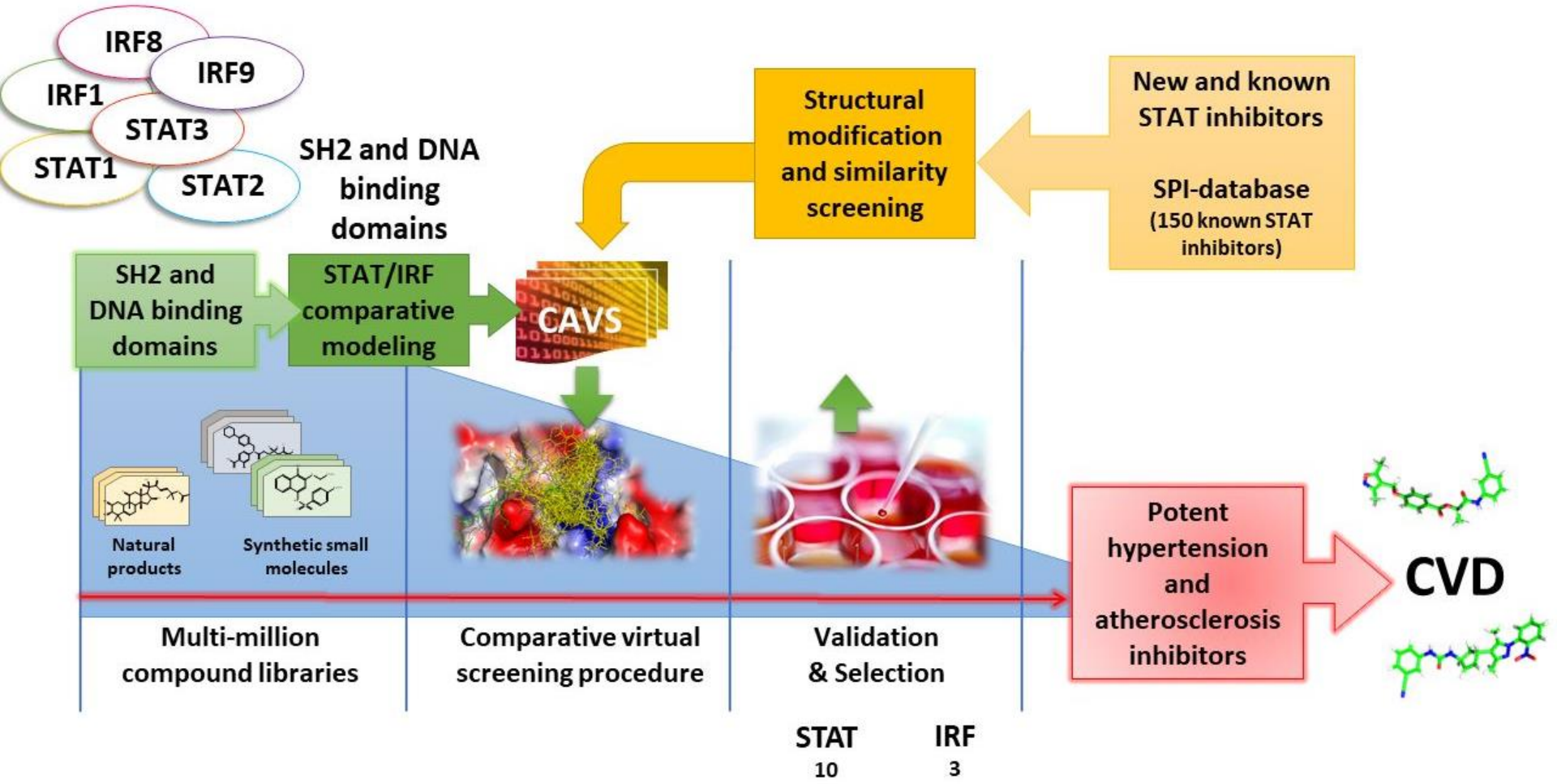


Xin Zhang et al. J Lipid Res, 2017

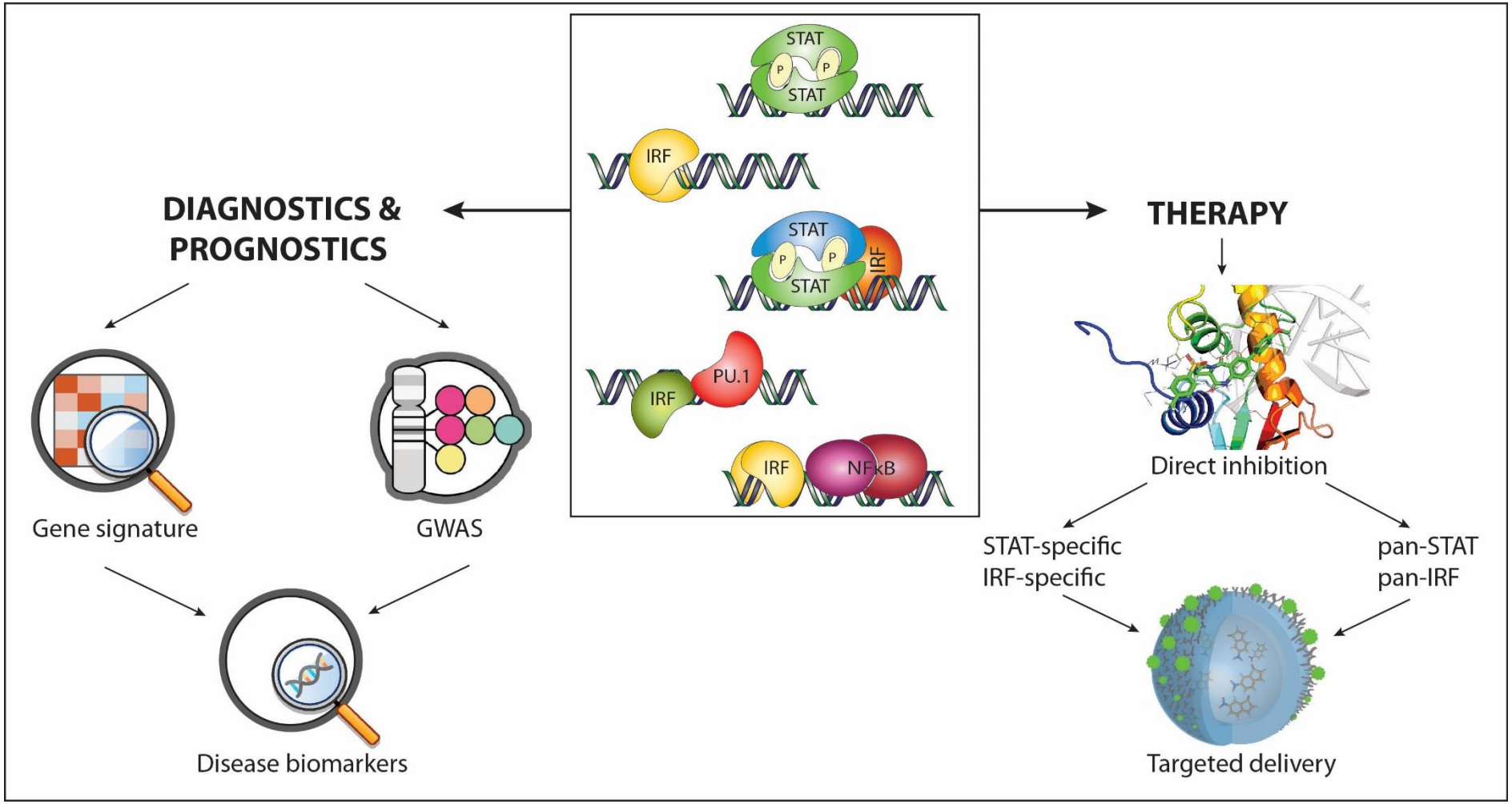


Xin Zhang et al. J Lipid Res, 2017

Pipeline approach to identify potent STAT & IRF inhibitors



STATs & IRFs in Diagnostics & Therapeutics



Antonczyk et al., submitted

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School of Medicine
Prof. Concha Piero

Department of Human Molecular Genetics

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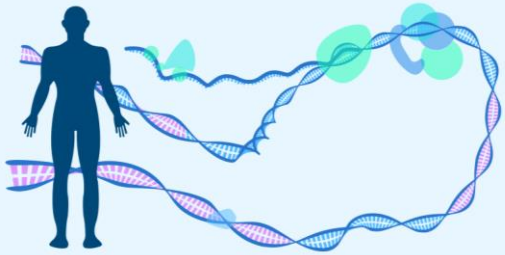

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