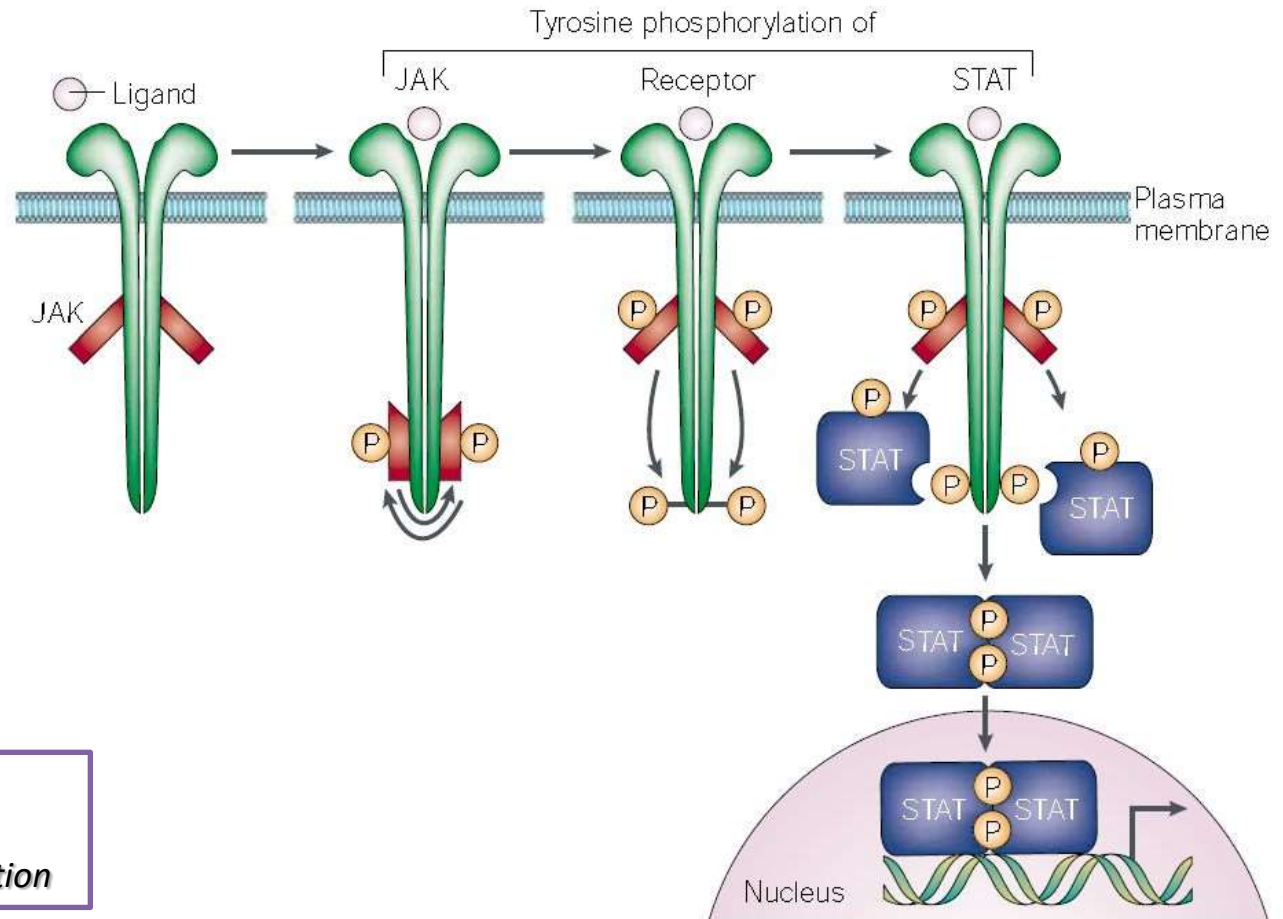


ChIPseq: JAK-STAT signaling

Hans Bluysen
25-11-2020

Canonical JAK-STAT pathway



JAK - Janus Kinase
STAT - Signal Transducer and
 Activator of Transcription

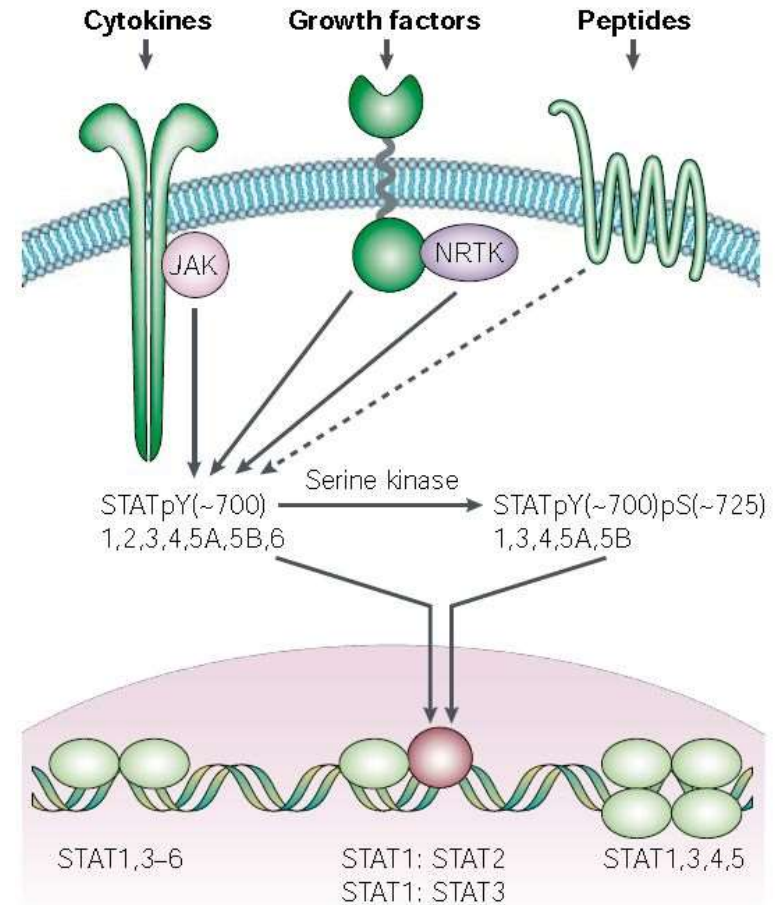
General triggers of JAK-STAT pathway

Cytokines
interferons
interleukines

Growth factors
EGF
PDGF

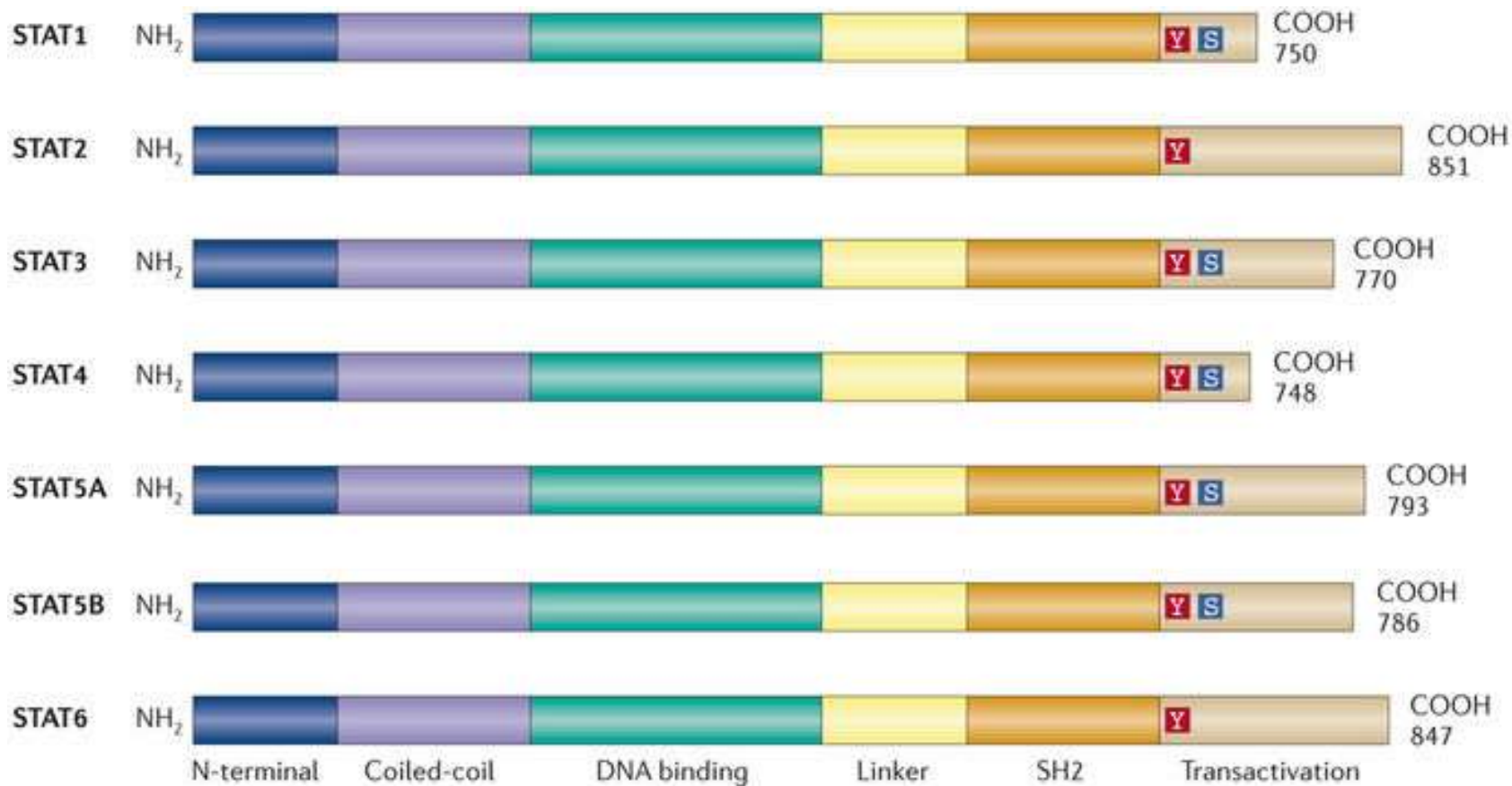
Bacterial molecules
LPS
LTA
dsRNA

Src and Abl kinases
G-coupled receptors
AngII

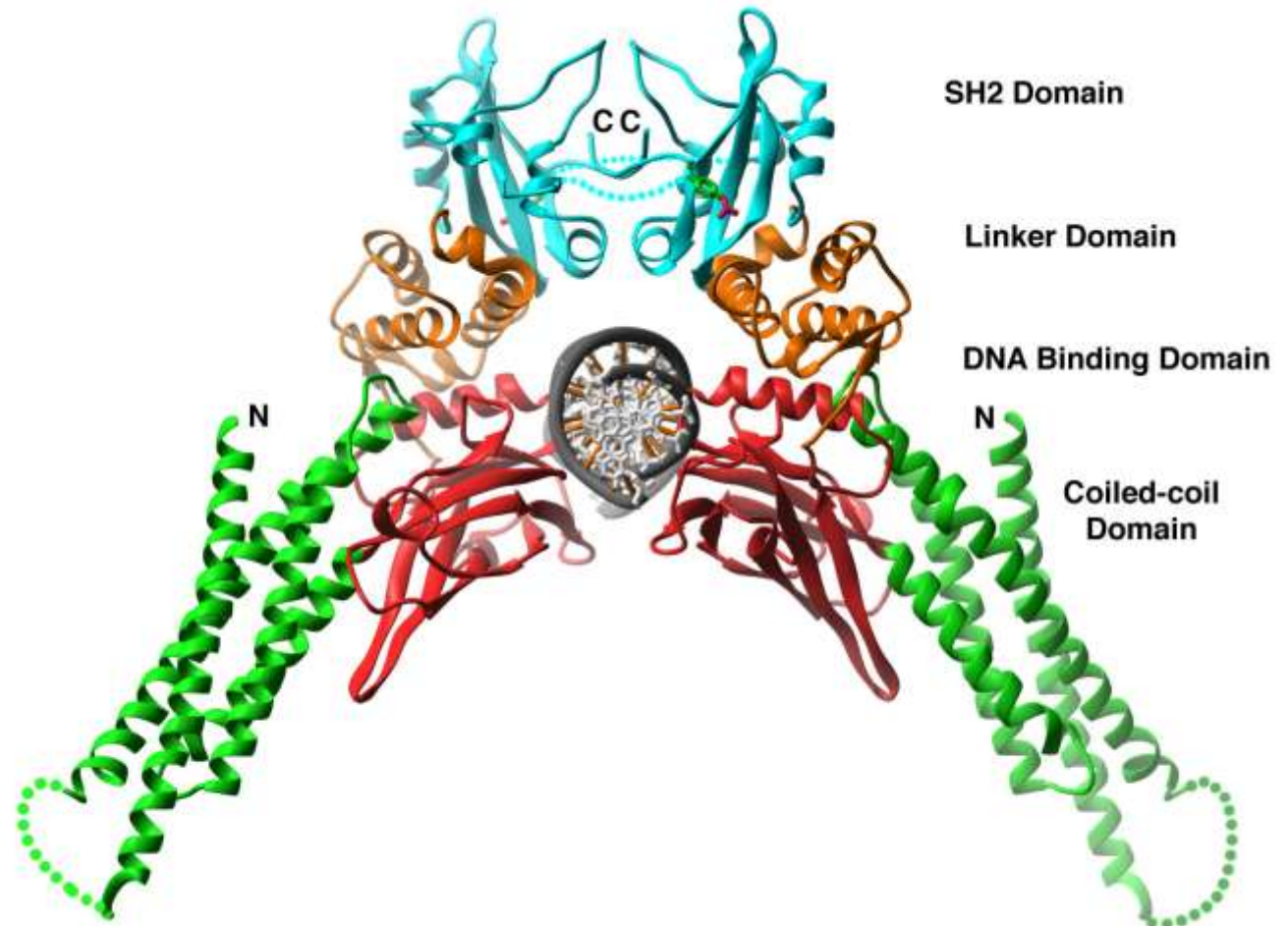




STAT Family: Structure



STAT-DNA Binding





STATs in Health & Disease

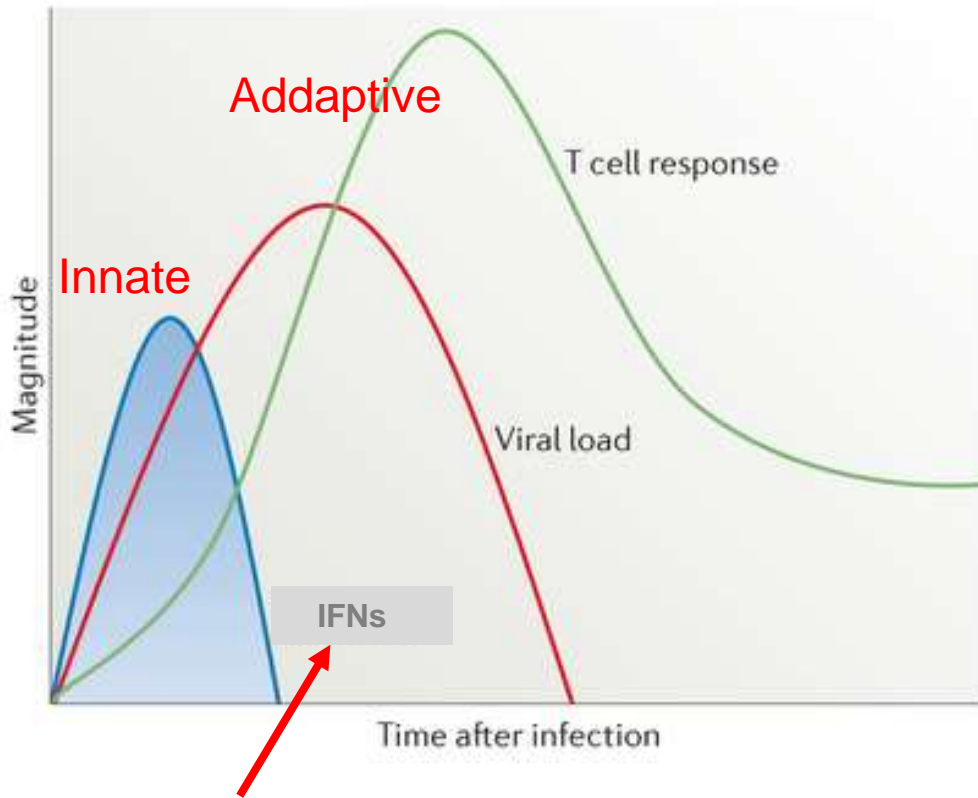
STAT	Cellular functions	Major diseases
1	<ul style="list-style-type: none">• Cell growth and apoptosis• T_H1 cell-specific cytokine production• Antimicrobial defence	<ul style="list-style-type: none">• Atherosclerosis• Infection ←• Immune disorders
2	<ul style="list-style-type: none">• Mediation of IFNα/IFNβ signalling	<ul style="list-style-type: none">• Cancer• Infection ←• Immune disorders
3	<ul style="list-style-type: none">• Cell proliferation and survival• Inflammation• Immune response• Embryonic development• Cell motility	<ul style="list-style-type: none">• Cancer
4	<ul style="list-style-type: none">• T_H1 cell differentiation• Inflammatory responses• Cell proliferation	<ul style="list-style-type: none">• Experimental autoimmune encephalomyelitis (multiple sclerosis)• Systemic lupus erythematosus
5A	<ul style="list-style-type: none">• Cell proliferation and survival• IL-2Rα expression in T lymphocytes• Mammary gland development• Lactogenic signalling	<ul style="list-style-type: none">• Cancer• Chronic myelogenous leukaemia
5B	<ul style="list-style-type: none">• Cell proliferation and survival• IL-2Rα expression in T lymphocytes• Sexual dimorphism of body growth rate• NK cell cytolytic activity	<ul style="list-style-type: none">• Cancer• Chronic myelogenous leukaemia
6	<ul style="list-style-type: none">• Inflammatory and allergic immune response• B cell and T cell proliferation• T_H2 cell differentiation	<ul style="list-style-type: none">• Asthma• Allergy

Viral Infection: Influenza

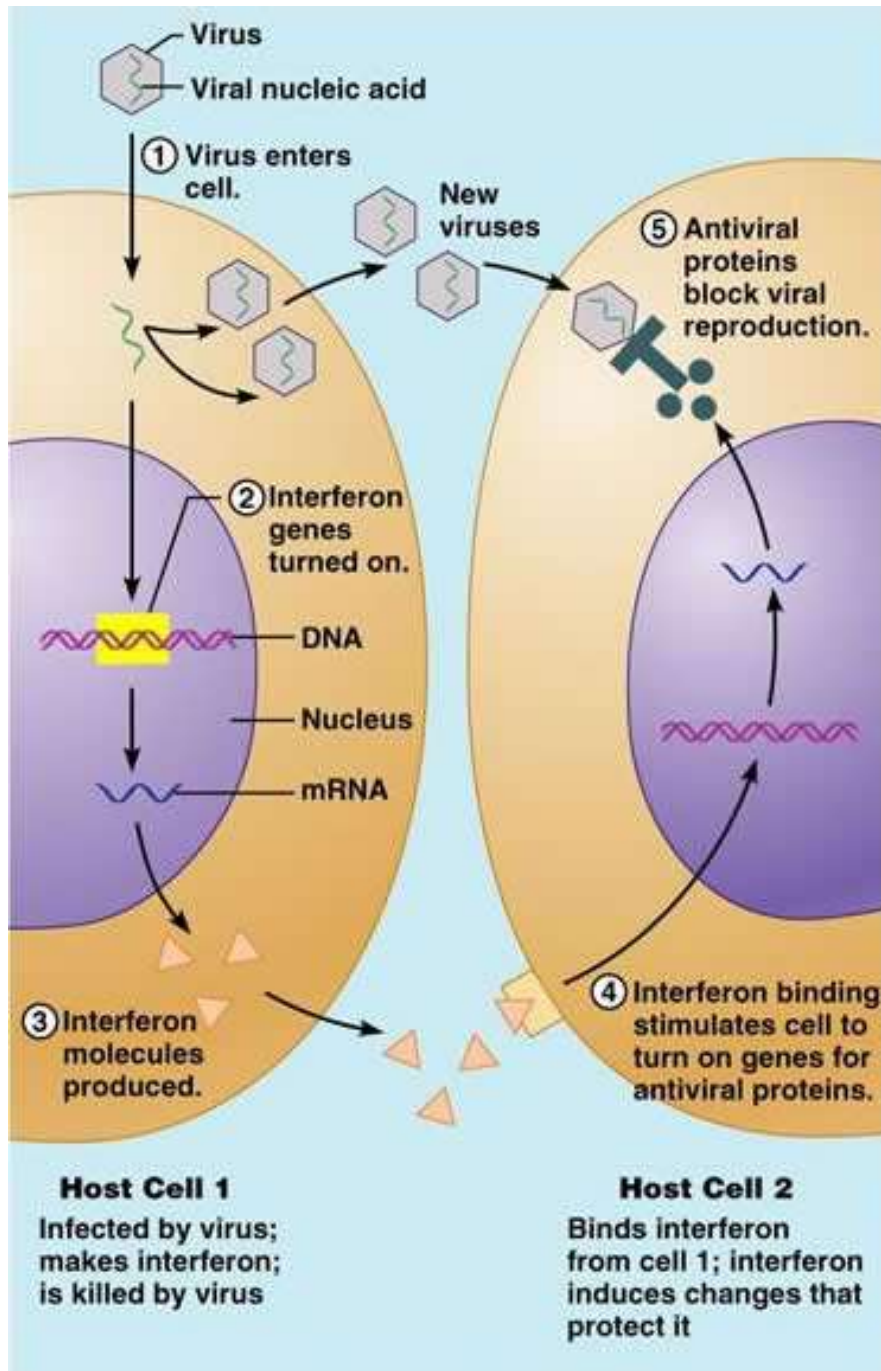


**How
Long?**

Anti-viral Response & IFNs



IFN Production & action



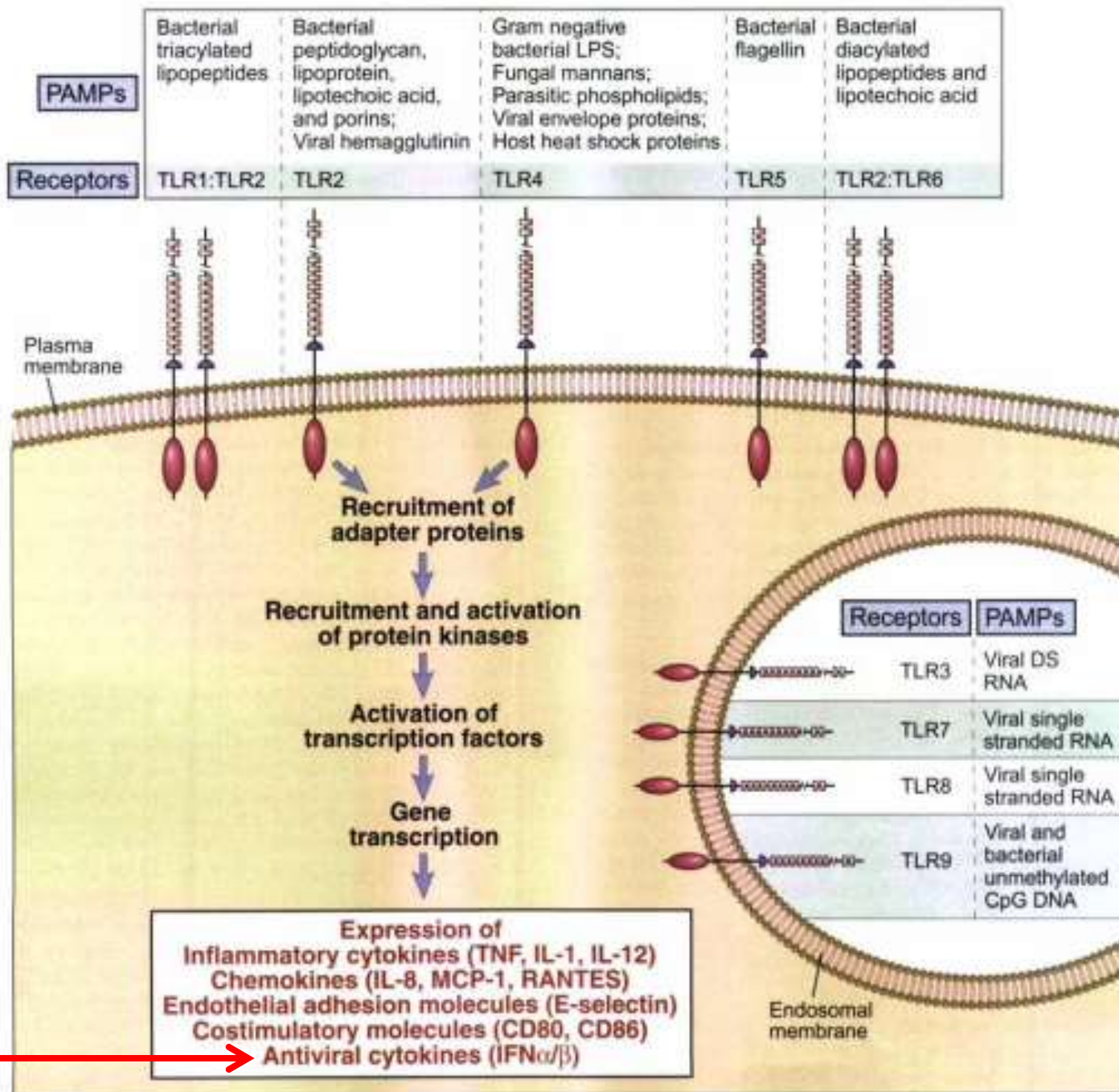
IFN

Inhibition viral replication

Inhibition cell growth

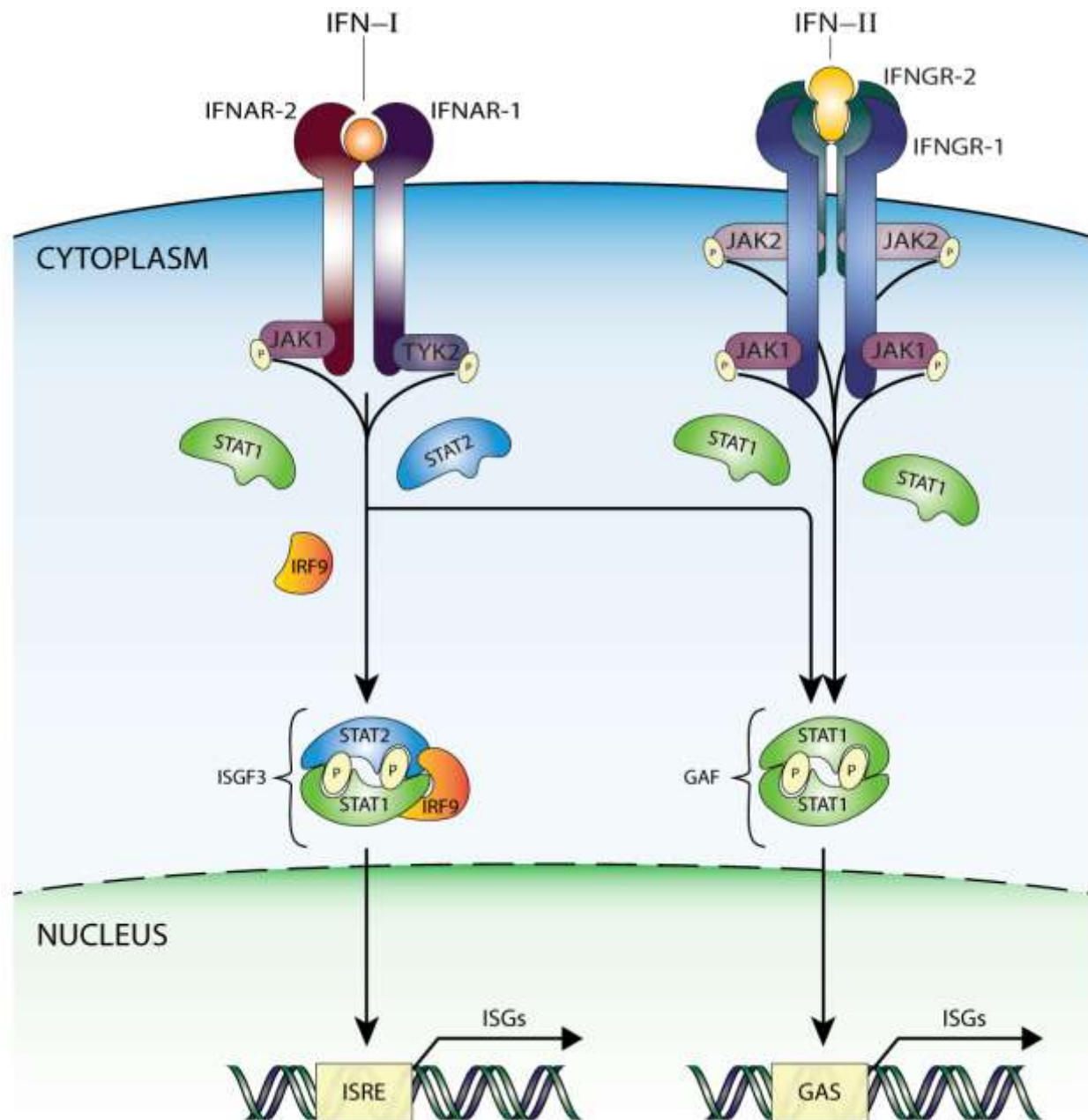
Activation immune system

Anti-viral State
Adaptive immune response



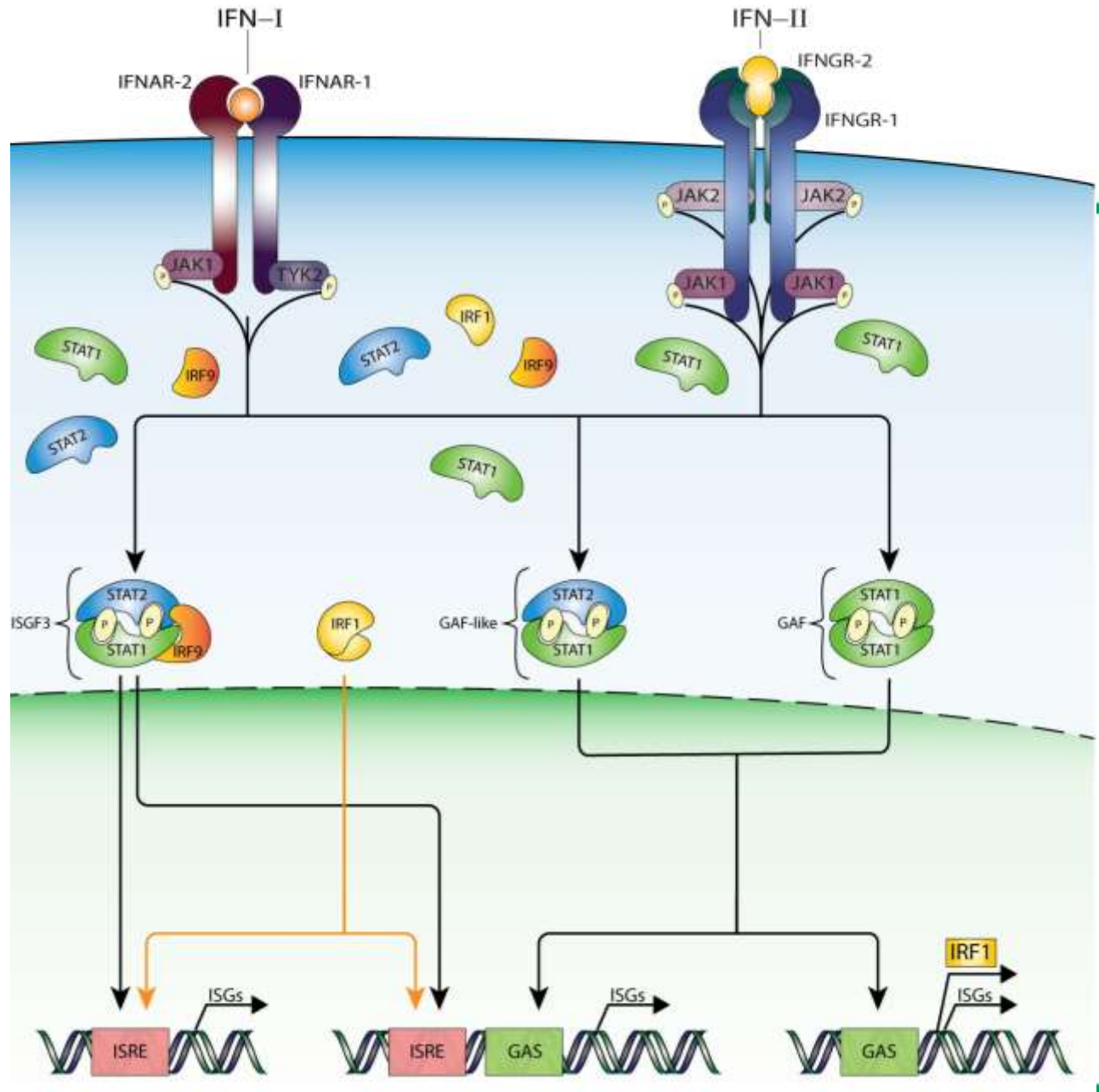


Canonical IFN- signaling (1990)





Canonical IFN- signaling (2020)

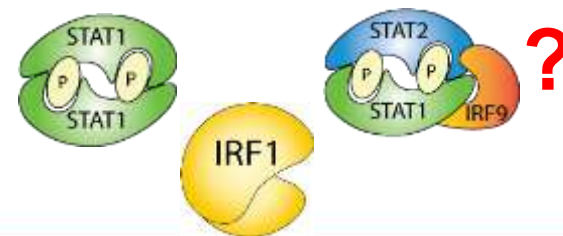
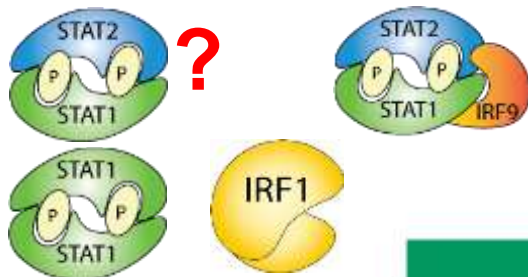
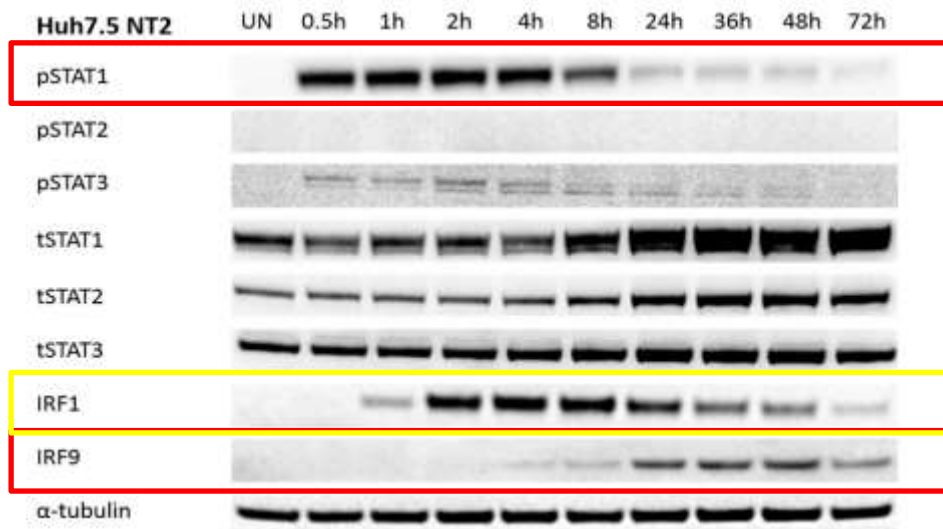
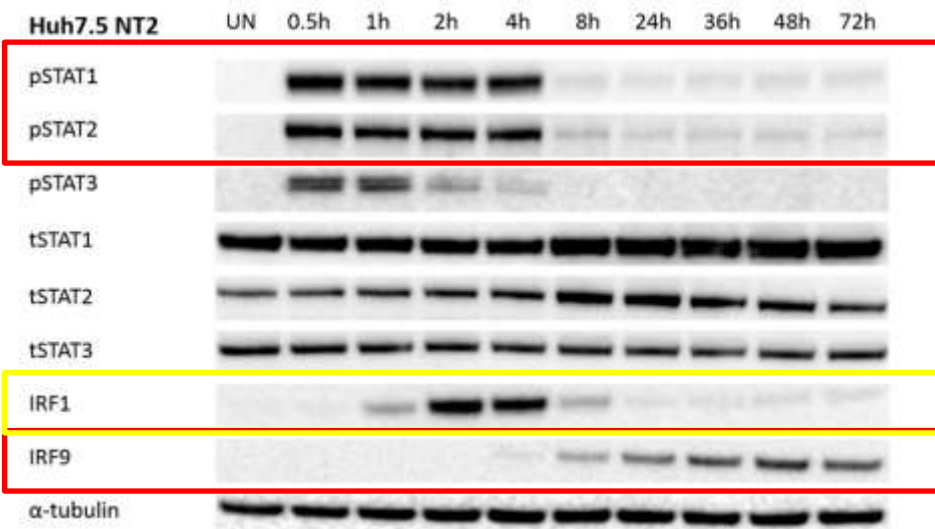




IFN-signaling In Time: pSTAT1, pSTAT2, IRF9 & IRF1

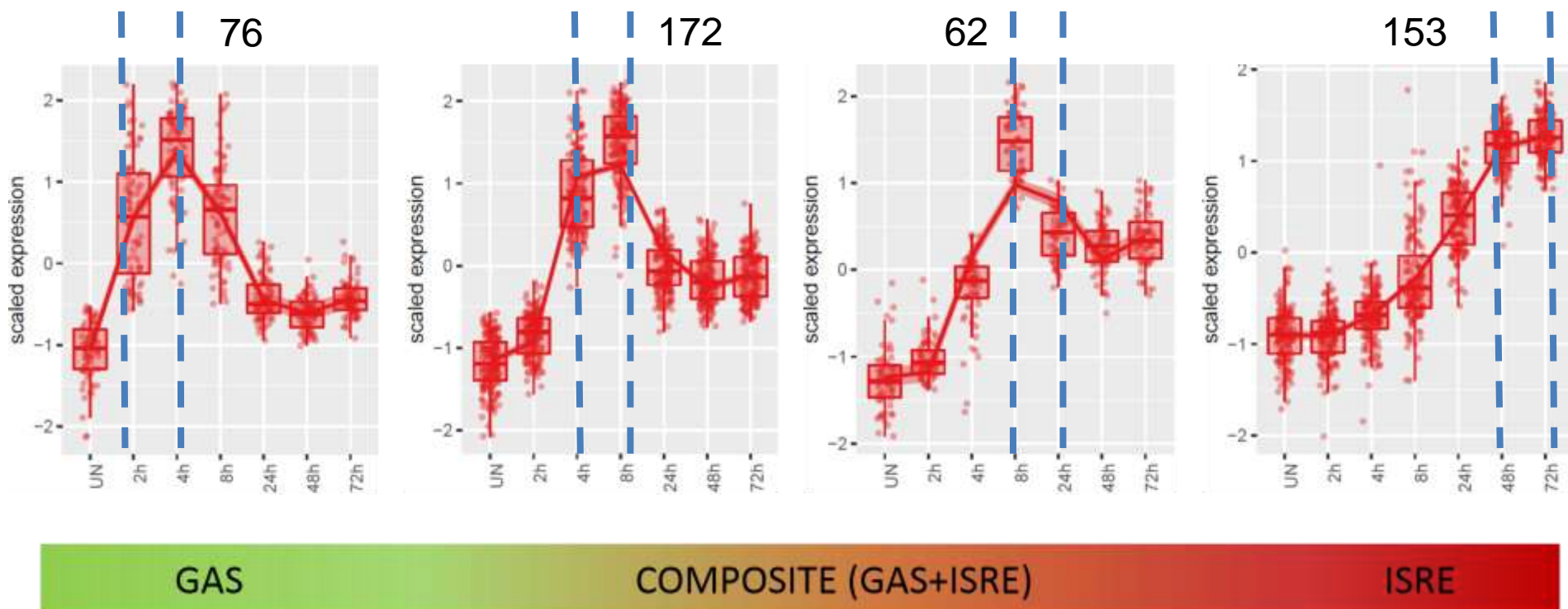
IFN-I

IFN-II



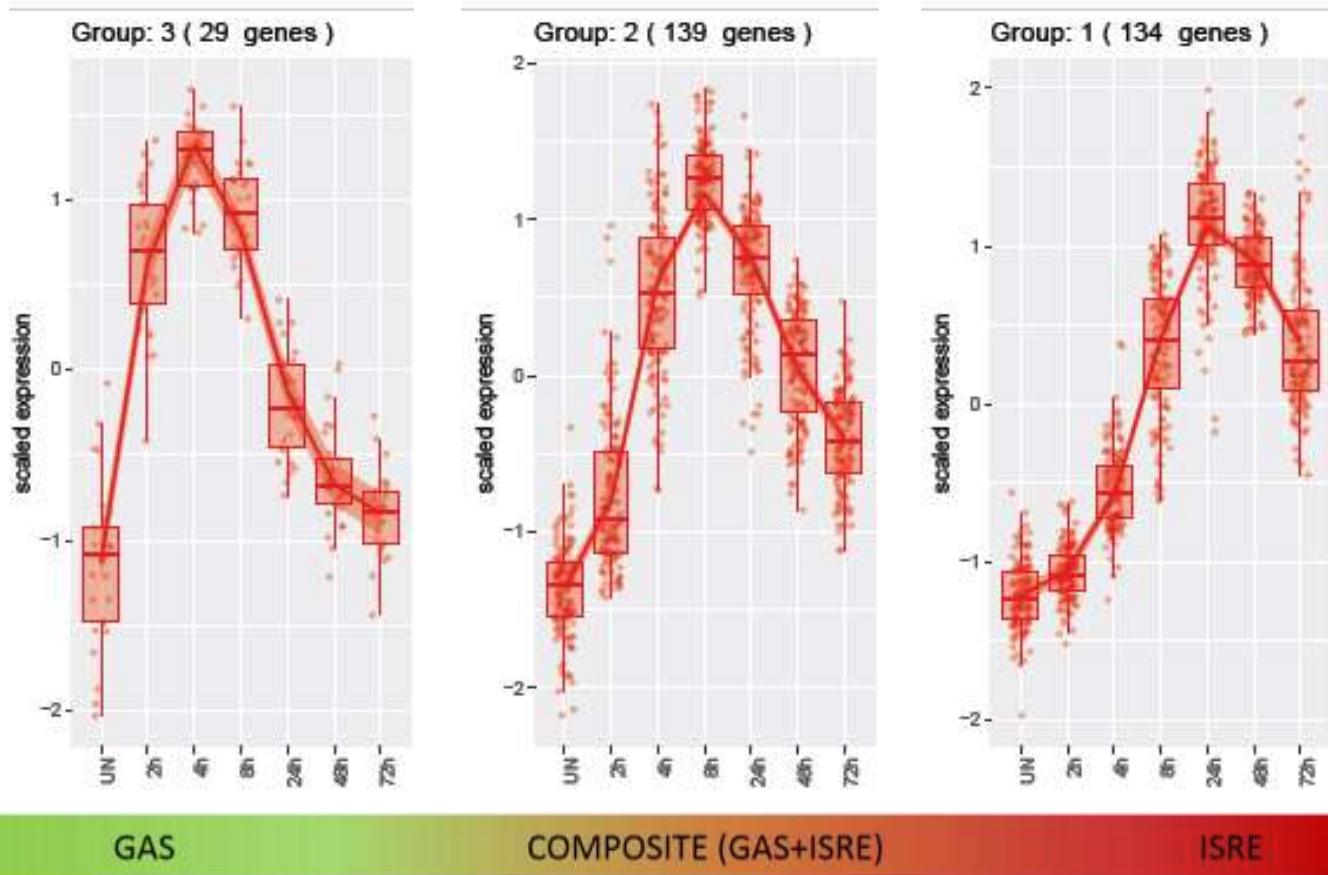


IFN-I Signaling: ISG expression ~ binding-site composition





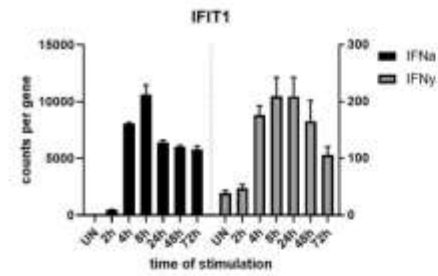
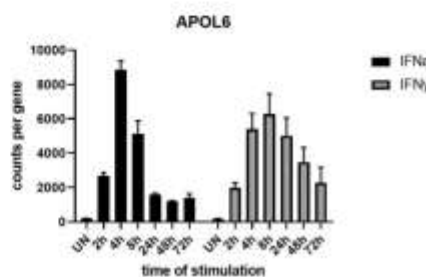
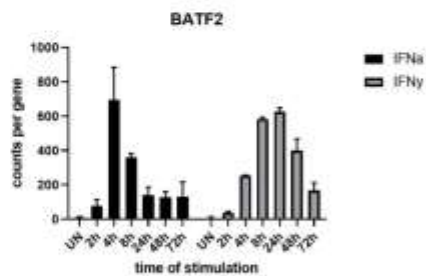
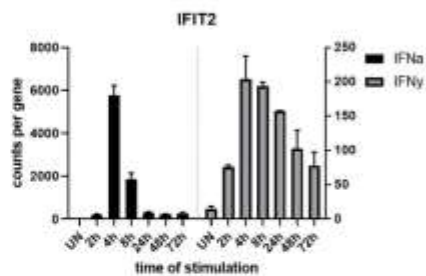
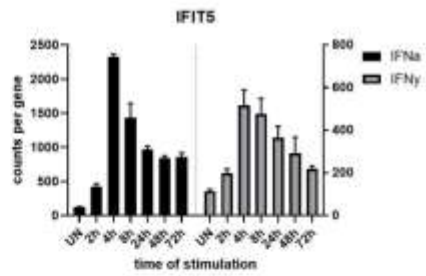
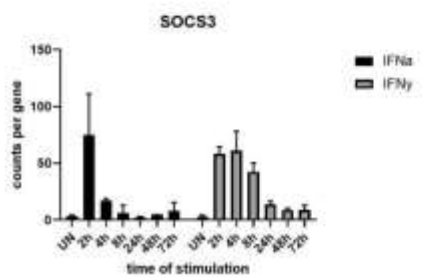
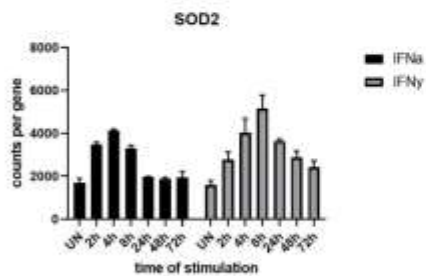
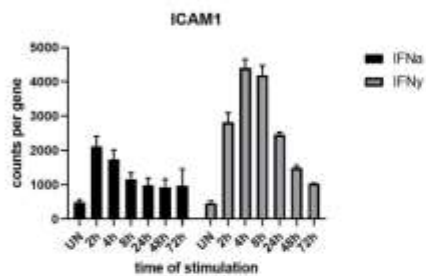
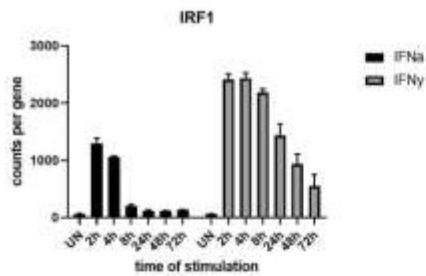
IFN-II Signaling: ISG expression ~ binding-site composition





ISRE-GAS composite sites: > 50 genes

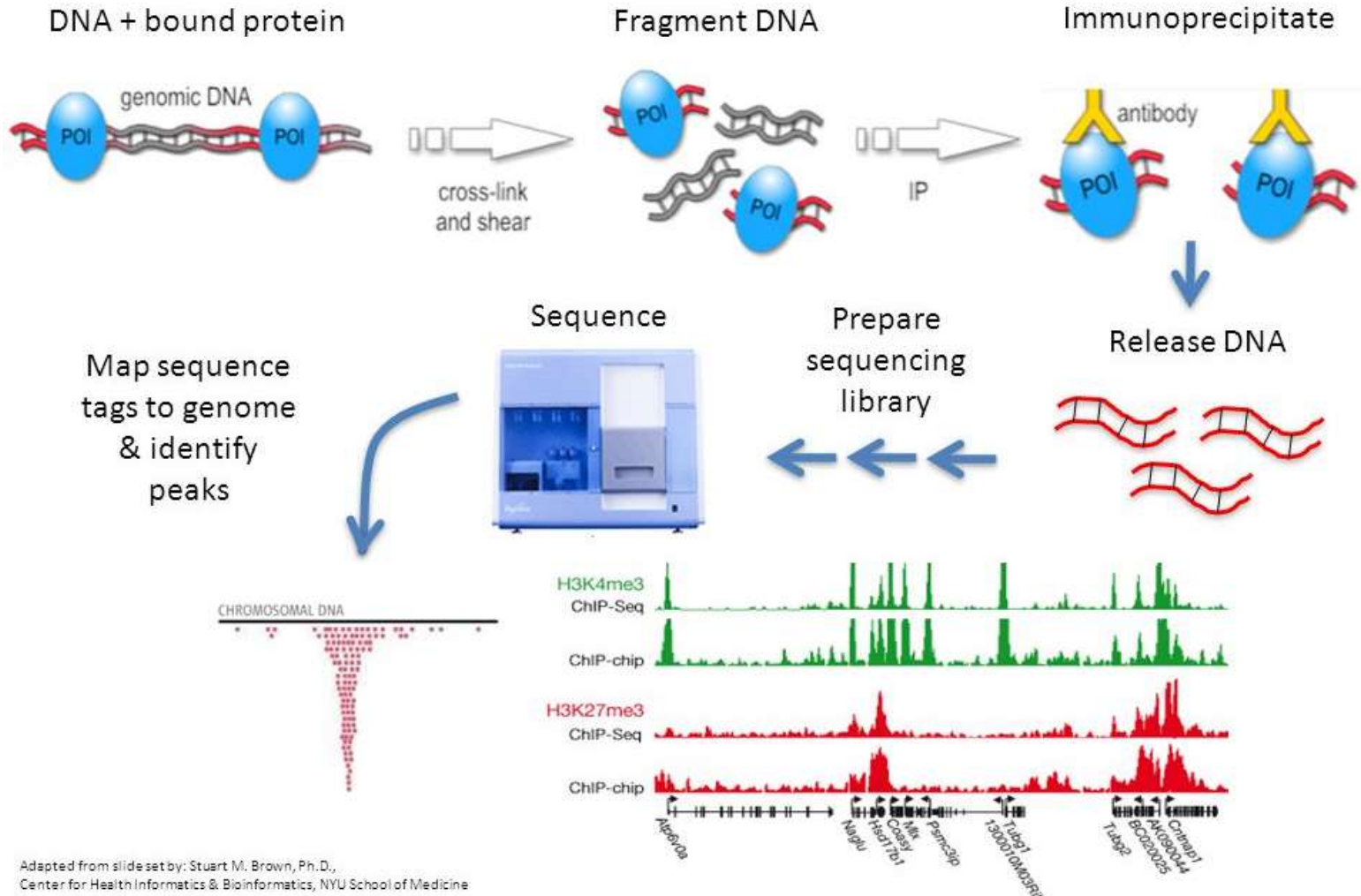
GENE	ISRE	Linker	GAS
GBP5	GG-TTT-CTA-TTT-CC	106nt	TTTC-TAG-GAAA
PARP14	TC-TTT-CGC-TTT-CG GG-TTT-CCT-TTT-GC	overlap 12nt	TTTC-CTG-GAAA
IFITM1	AG-TTT-CTA-TTT-CC	16nt	TTTC-TCA-GAA
IFI27	AG-TTT-CGG-TTT-CC	overlap	TTTC-CTG-GAAA
BATF2	AG-TTT-CAG-TTT-CT	overlap	TTTC-TCC-TAAA
PARP9, DTX3L	AG-TTT-CAG-TTT-CG	1nt	-TTC-CCT-GGA
DDX60	AG-TTT-CGG-TTT-CC	60nt	TTTC-GTG-GAAA
PLSCR1	GG-TTT-CCT-TTT-CC	17nt	TTTC-CT--GAA
BST2	GG-TTT-TCAGTTT-CA	1nt	TTTC-CCA-GAAA
AIM2	AC-TTT-CGC--TT-GG	149nt	TTTC-TGG-GAAA
GMPR	AG-TTT-CA--TTT-CC	overlap	-TTC-CCT-GAAA
IFI35	AC-TTT-CA--TTT-CC	overlap	TTTC-CGT-GAAA
APOL6	AC-TTT-CAG-TTT-CC	18nt	TTTC-CTG-GAA
UBE2L6	AC-TTT-CAT-TTT-CT	19nt	-TAC-TAG-GAAA
HLA-E	CT-TTT-AGG-TTT-CG	15nt	TTGC-TGG-GAAA
ADAR	AA-TTT-CGC-TTT-CG	overlap	TTTC-CTCGGAAA
PSMB8	GC-TTT-CGC-TTT-CA	48nt	TTTC-TCG-GAAA
KLHDC7B	GT-TTT-CCA-TTT-AG	24nt	TTTC-TGA-TAAA
C1S	AC-TT--GAC-TTT-CC	2nt	TTTC-CCA-GAAA
TMEM50A	GG-TTT-CTG-TTT-CC	13nt	TTTC-CTG-GAA
NAPA	GG-TTT-CCT-TTT-CC	overlap	TTTG-TGG-GAAA
WARS	GC-TTT-TGTCTTT-CG	48nt	-TTC-TCA-GAAA
ZC3HAV1	GC-TTT-TAG-TTT-CT	95nt	-TTC-CCG-GAAA
TRIM69	GG-TTT-CTC-TTT-CT	14nt	TTTC-CGA-GAAA
IRF2	AA-TTT-CAT-TTT-CG	3nt	TTTC-TCG-GAAA
NEURL3	AG-TTT-CGC-TTT-CC	overlap	-TTC-TAG-GAAA





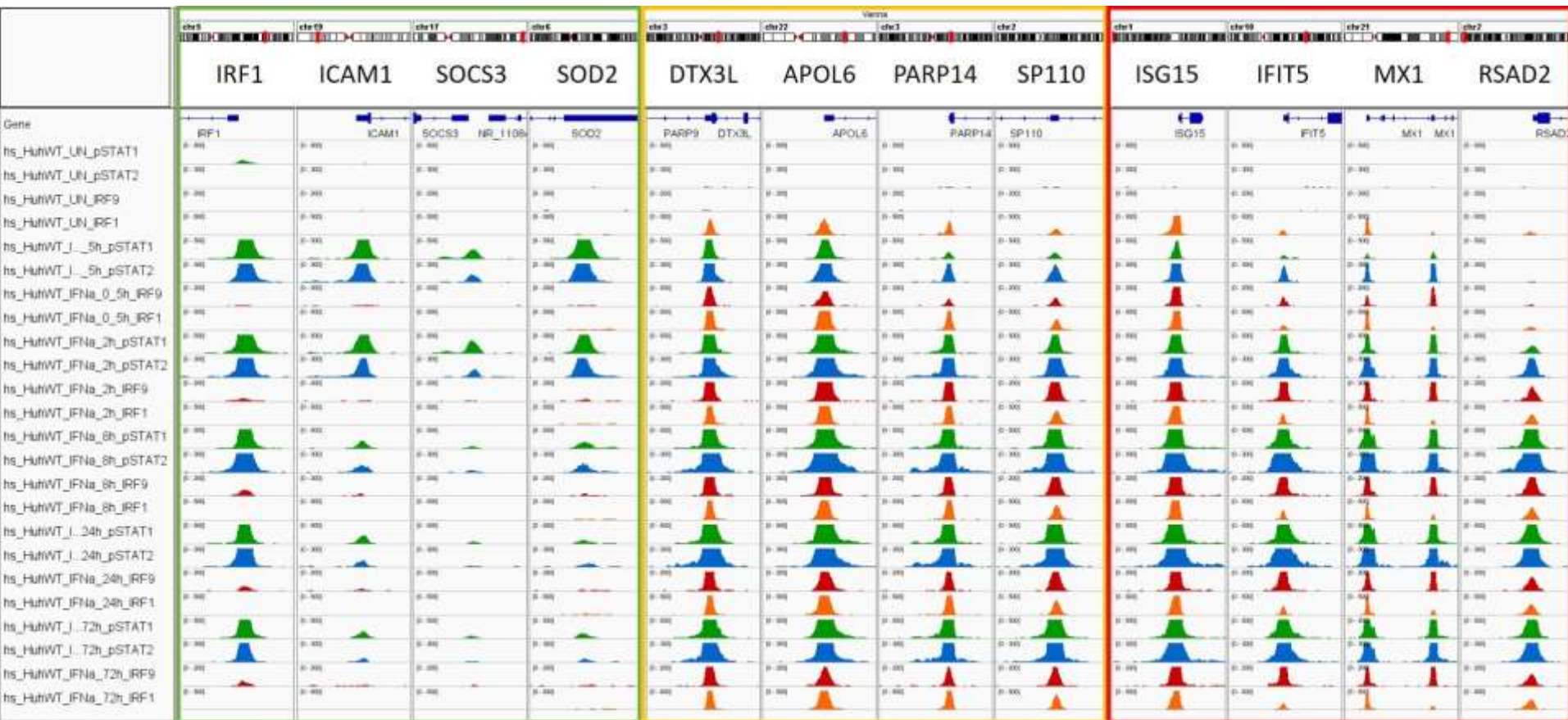
How to analyze chromatin interactions?

ChIPseq Work Flow



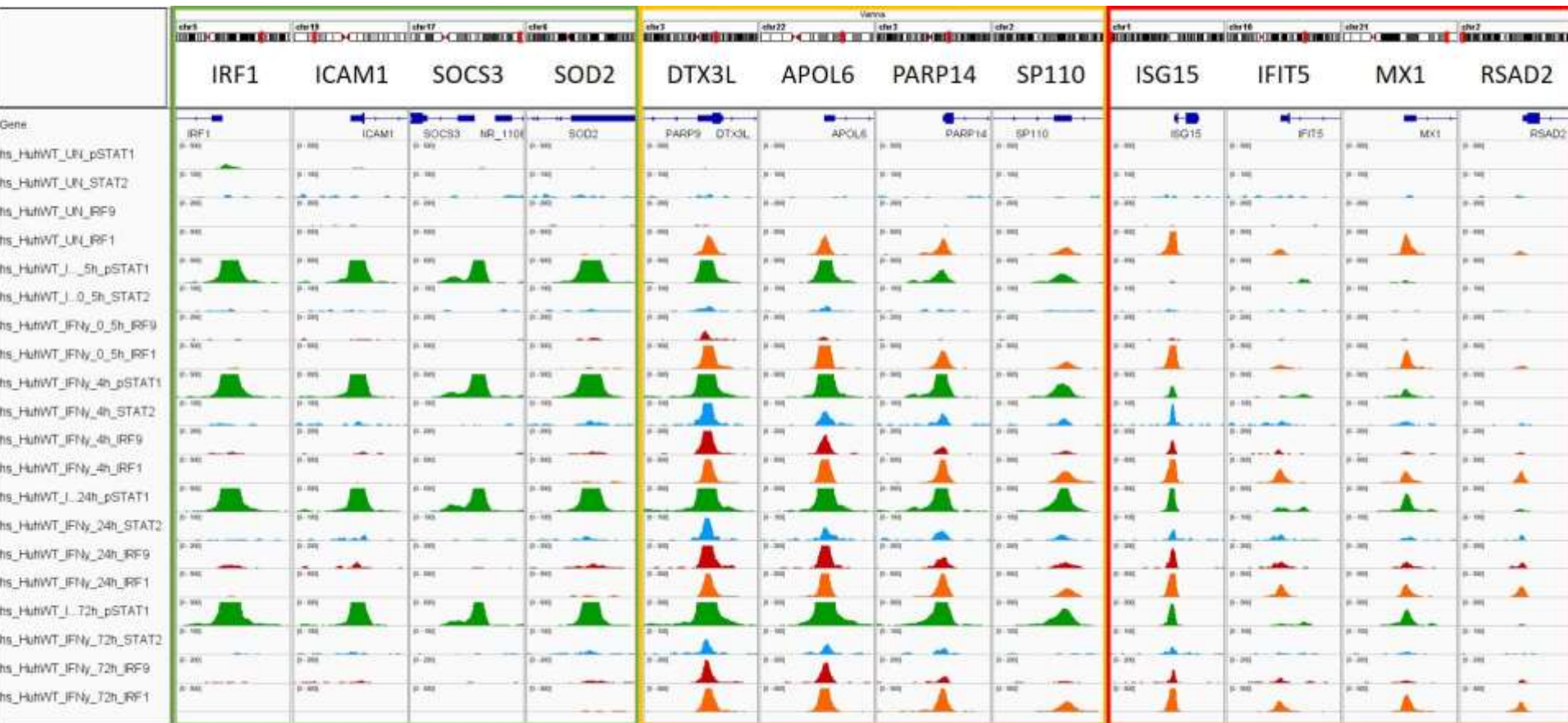


IFN-I signaling: pSTAT1, pSTAT2, IRF9 & IRF1 DNA-binding



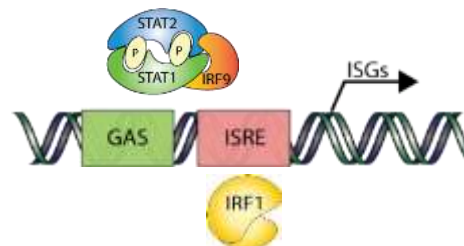
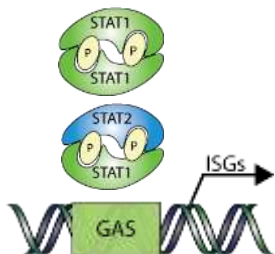
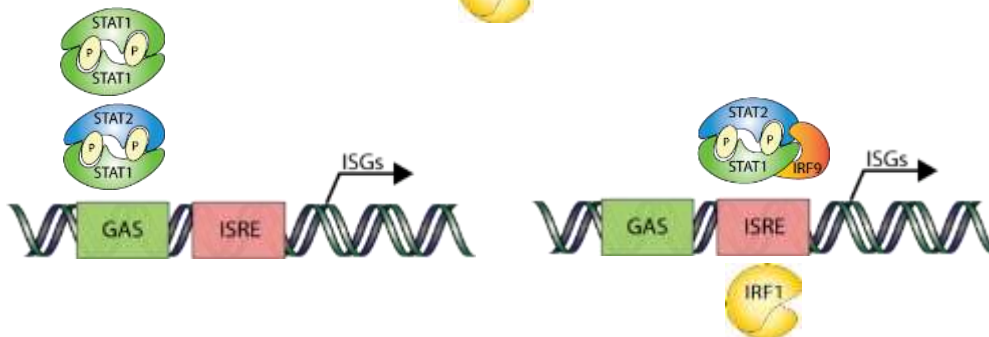
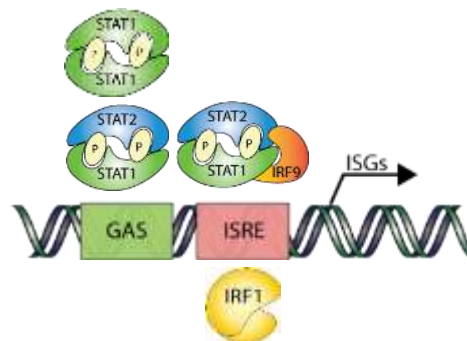
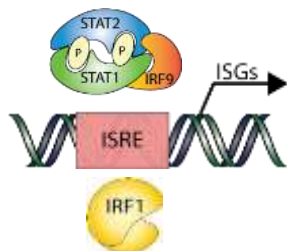


IFN-II signaling: pSTAT1, STAT2, IRF9 & IRF1 DNA-binding





Novel STAT1, STAT2, IRF9 & IRF1 DNA Binding Modes



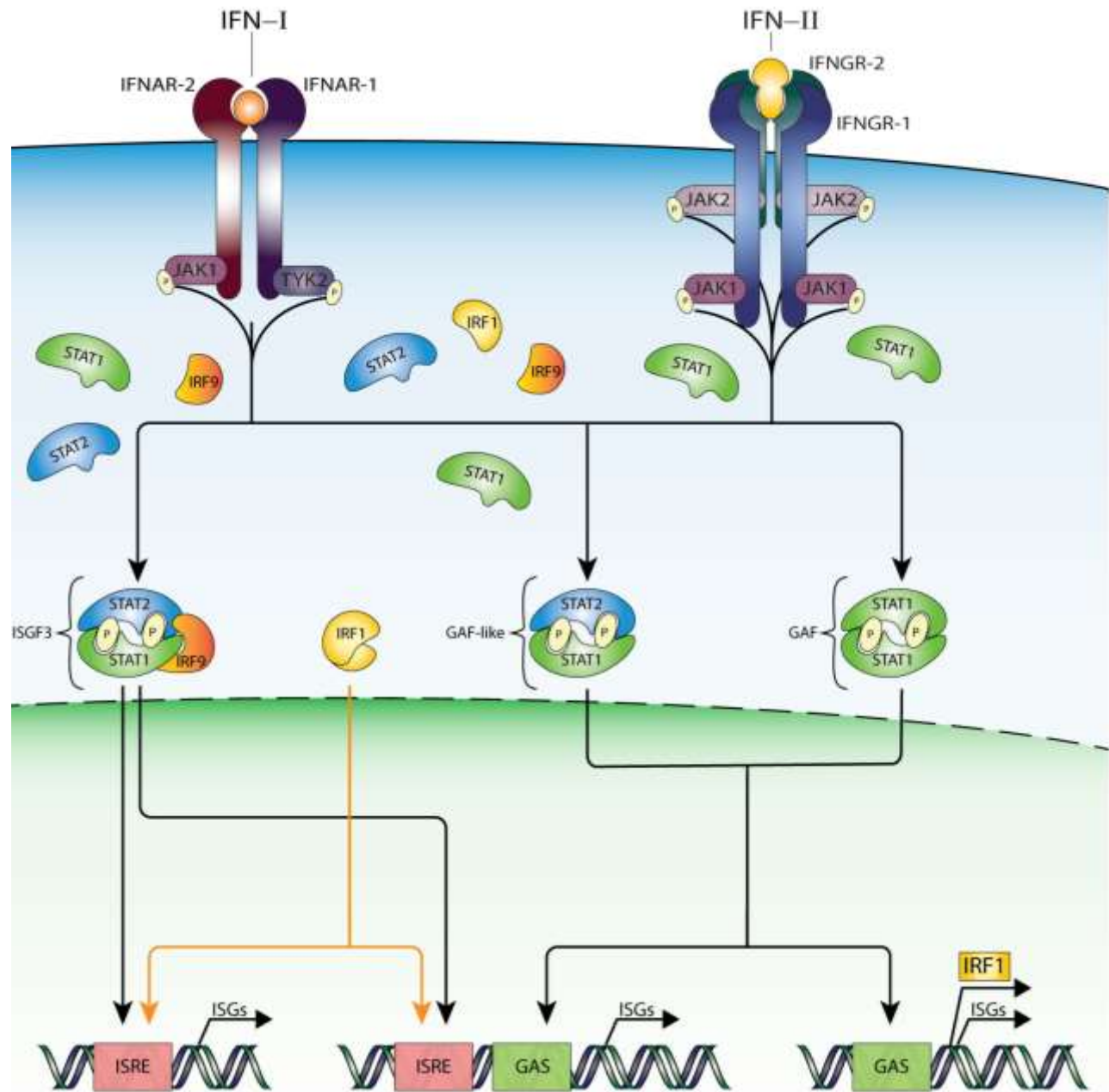


ISGF3, GAF
and IRF1

ISRE-GAS
Single or
Composite sites



Increased Complexity
Alternative solutions





IFN-signaling:

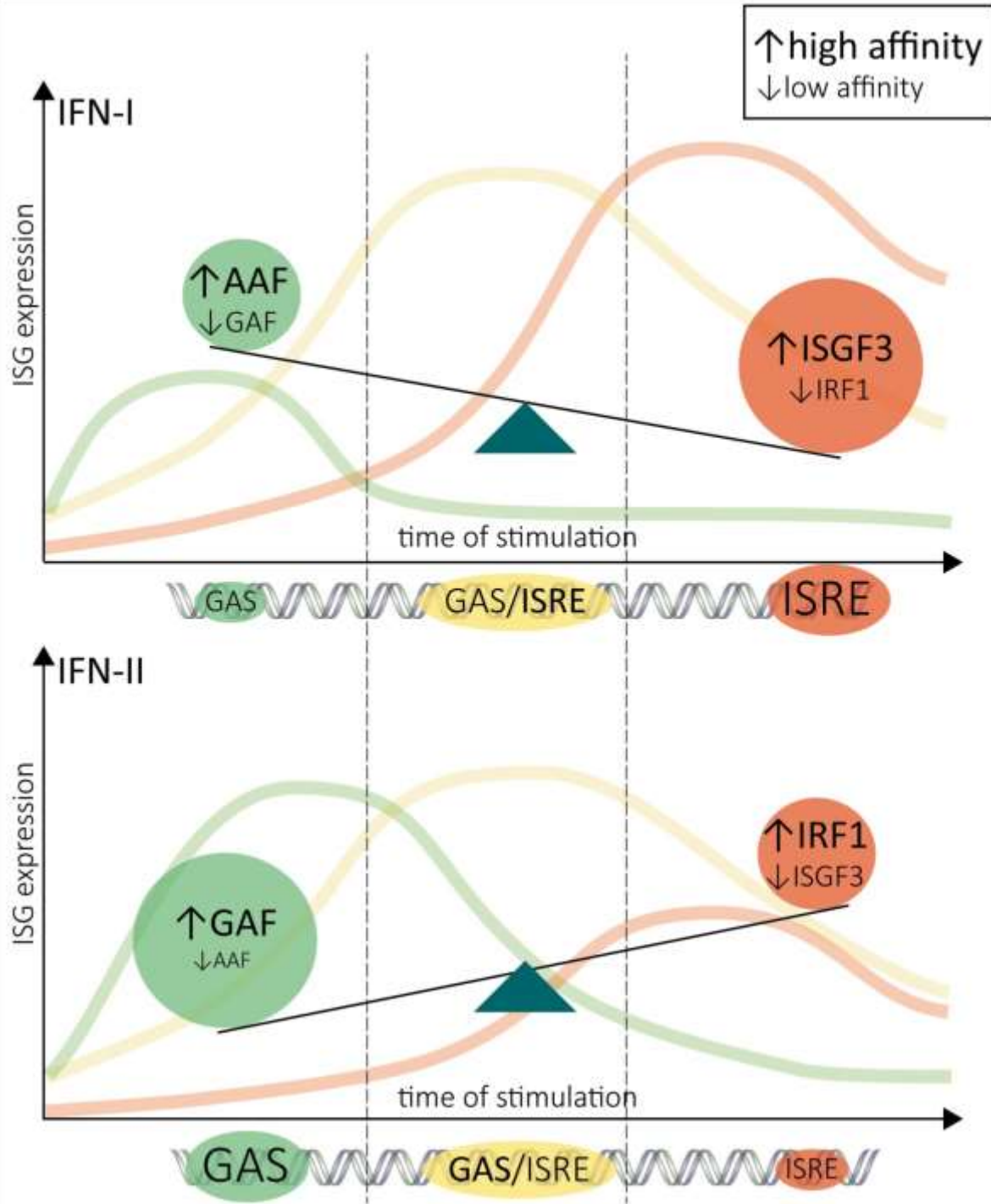
- Increased complexity
- Overlap

Time-dependent

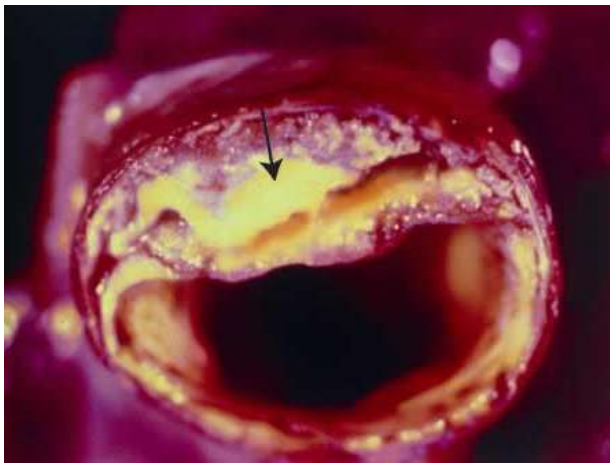
- Complex formation
- DNA-binding &
- ISG expression



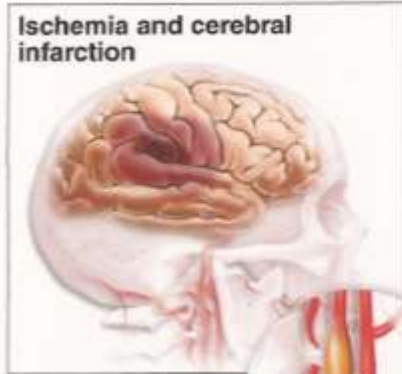
Biological Function?



Atherosclerosis

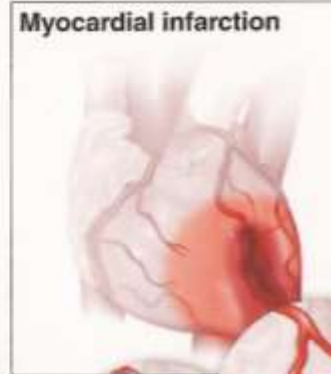


Atherosclerotic plaque



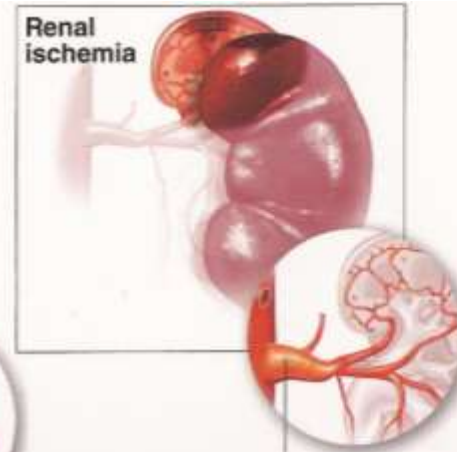
Ischemia and cerebral infarction

Internal carotid artery



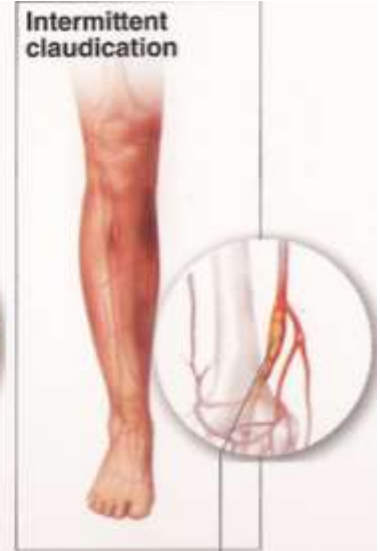
Myocardial infarction

Coronary artery



Renal ischemia

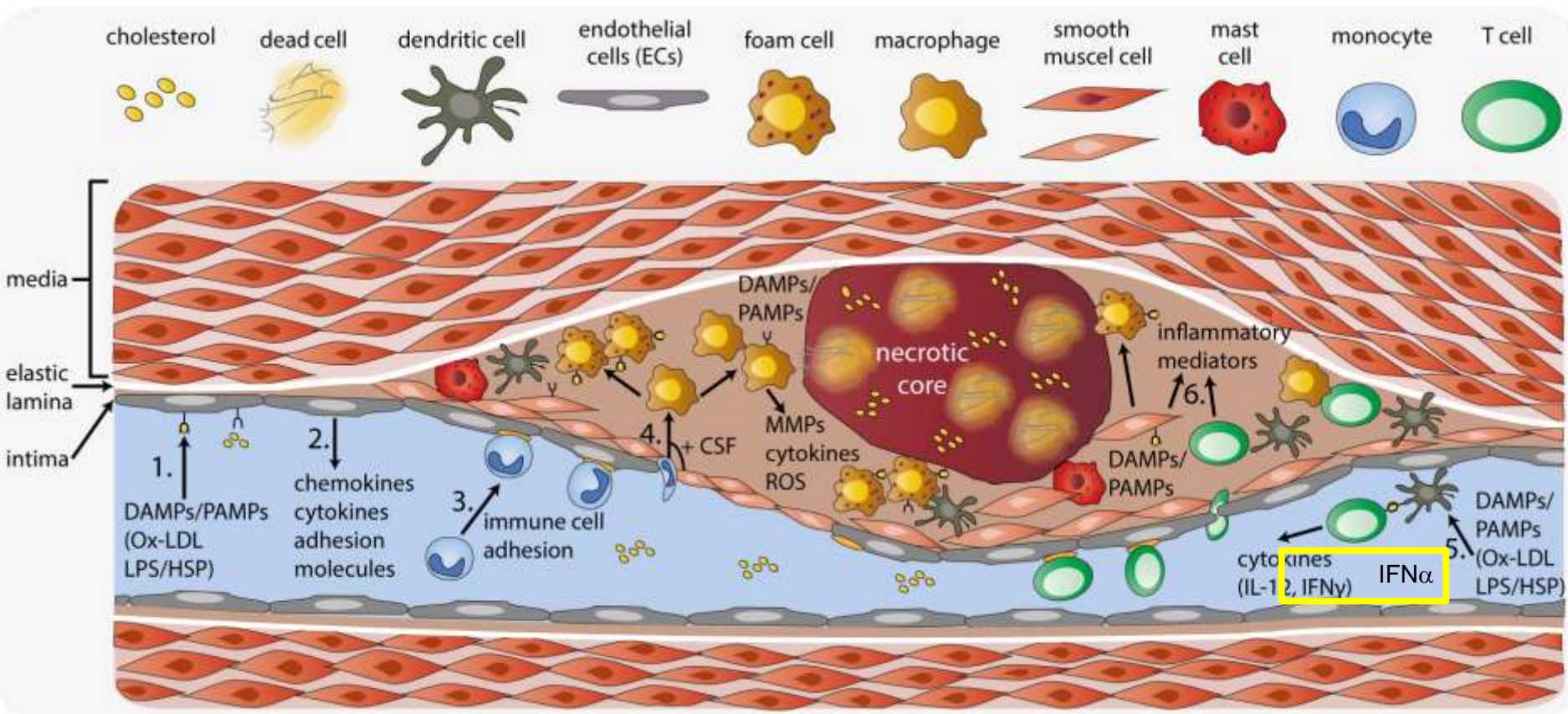
Renal artery



Intermittent claudication

Femoral artery

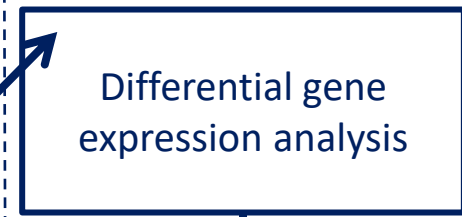
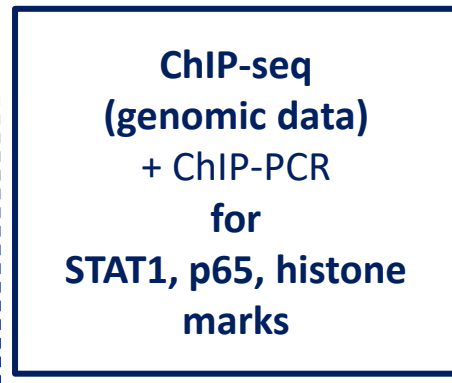
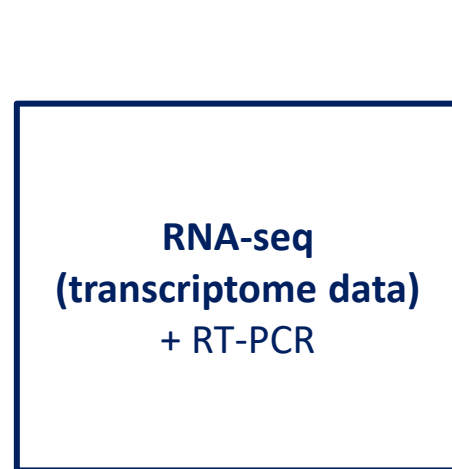
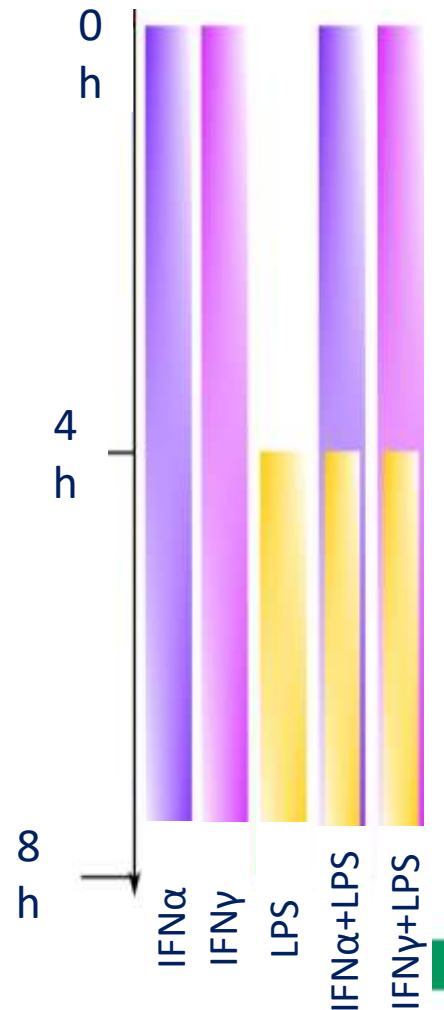
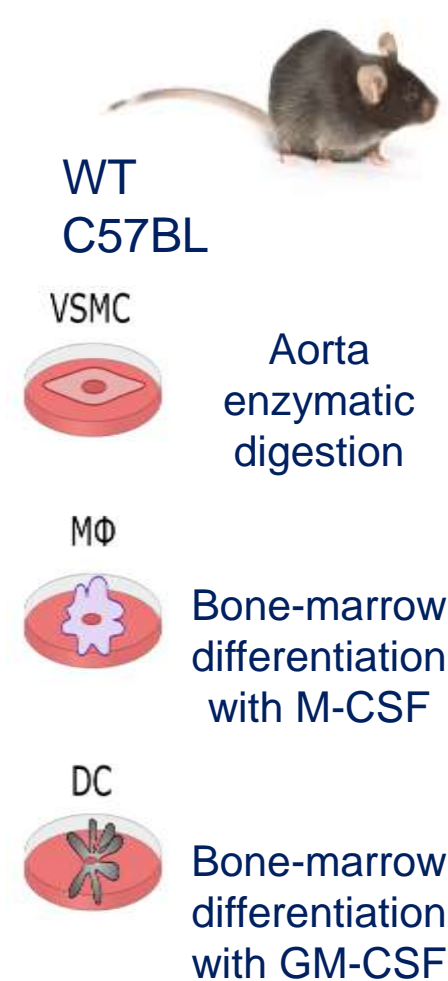
Atherosclerosis – vascular inflammation



Atherosclerotic plaque

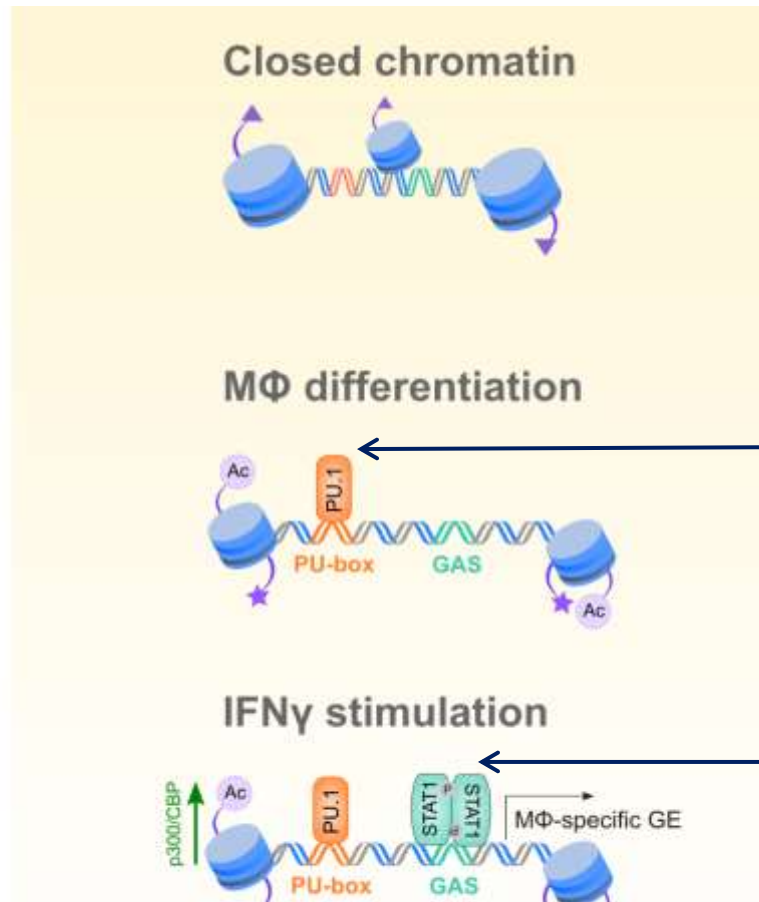
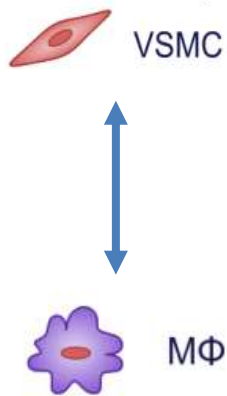


Data analysis pipeline applied in the PhD Thesis





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



Lineage Determining

LDTF

SDTF

Signal Determining

▲ H3K27me3 ★ H3K4me1 ● Ac H3K27ac



IFN γ activates VSMC and M Φ -specific gene expression

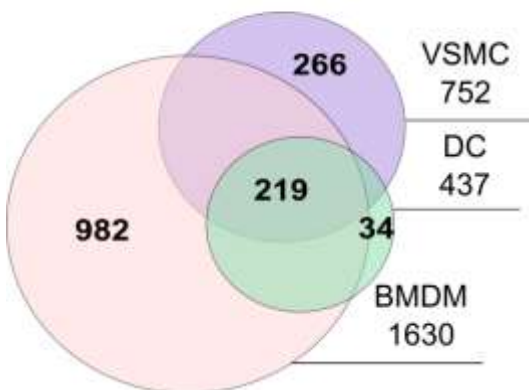


VSMC



M Φ

IFN γ

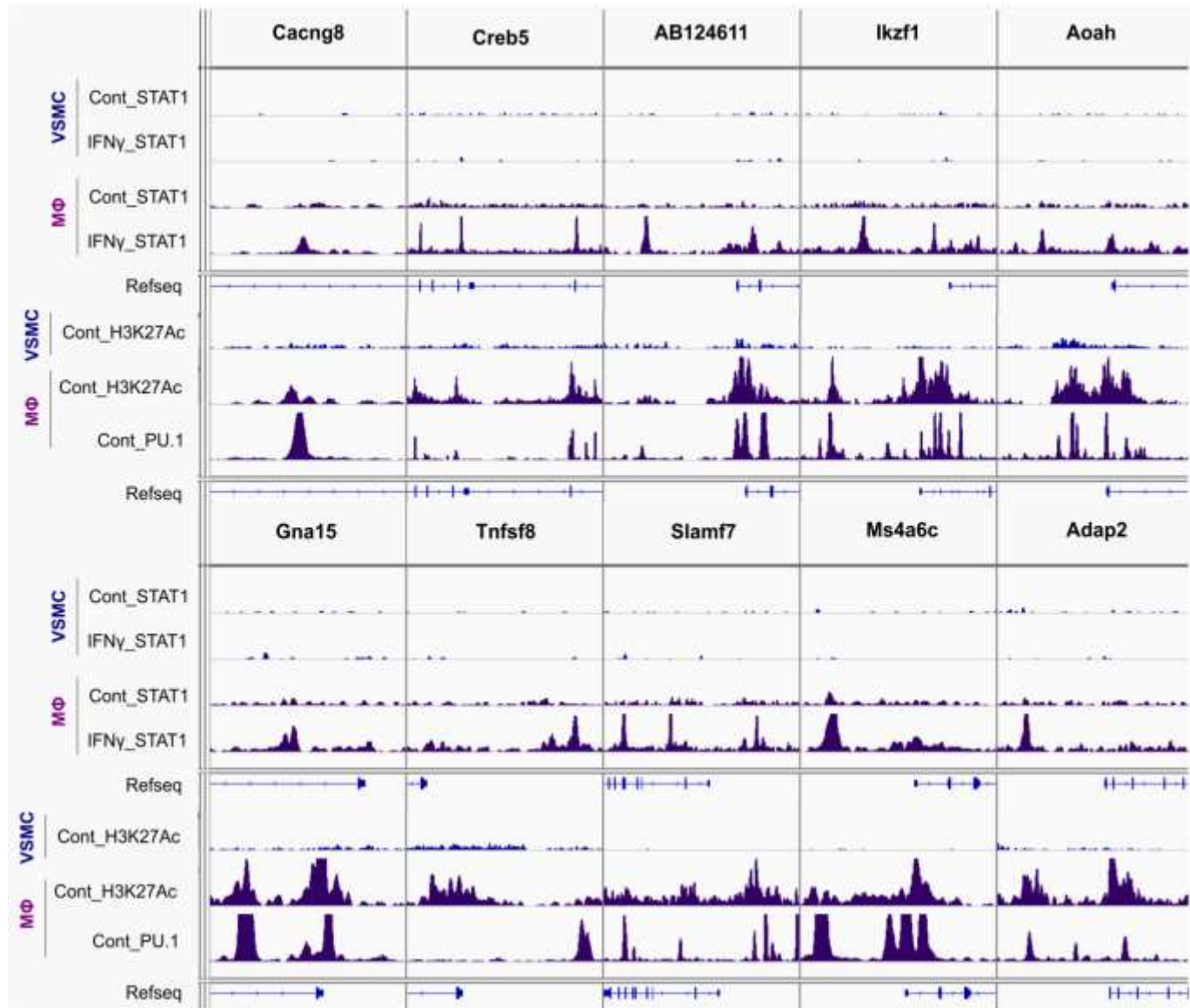
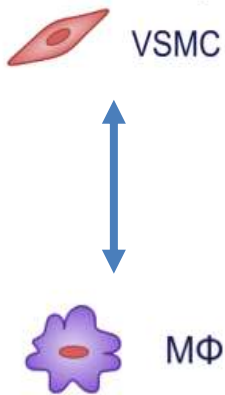


No	Gene name	IFN γ FC	
		VSMC	M Φ
1	Gm6654	270,6	-1,1
2	Ch11	46,6	1,4
3	Mpeg1	34,3	1,4
4	H2-Eb1	16,9	2,0
5	Neur13	15,8	-1,5
6	Batf3	15,4	1,1
7	Tmtc1	11,8	-1,1
8	Ikzf4	11,7	1,1
9	Mt3	9,3	1,1
10	Trim5	9,2	1,9
11	Lmo2	8,2	-1,5
12	Pla1a	7,3	1,7
13	Ccr1	7,2	-1,9
14	Mreg	6,7	1,8
15	Csf2rb2	6,0	1,9
16	Nuak2	5,7	1,9
17	H2-Q2	5,3	2,0
18	Gm9574	5,2	2,0
19	H2-M3	5,1	1,7
20	H2-Q7	4,9	1,8

No	Gene name	IFN γ FC	
		M Φ	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
11	Spsb1	22,4	1,9
12	Bcl2a1b	21,6	-1,1
13	Mycl1	19,3	1,0
14	Gm20547	19,2	1,8
15	Hbegf	19,2	-2,0
16	Csf1	19,1	-1,1
17	Slc2a6	18,8	1,0
18	Bcl2a1c	18,3	1,1
19	Rap1gap2	18,2	1,4
20	Stk39	18,2	1,0

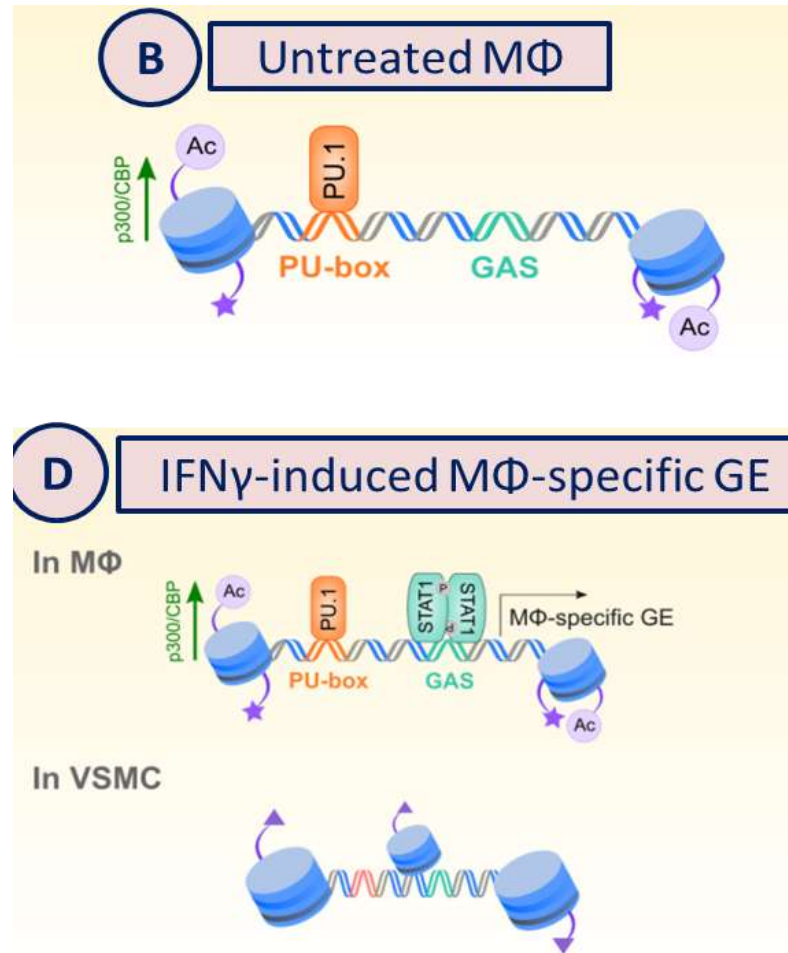
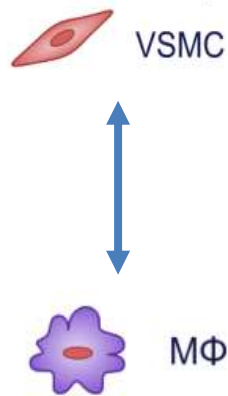


IFN γ activated MQ-specific transcription: STAT1-PU.1 binding + epigenetic changes



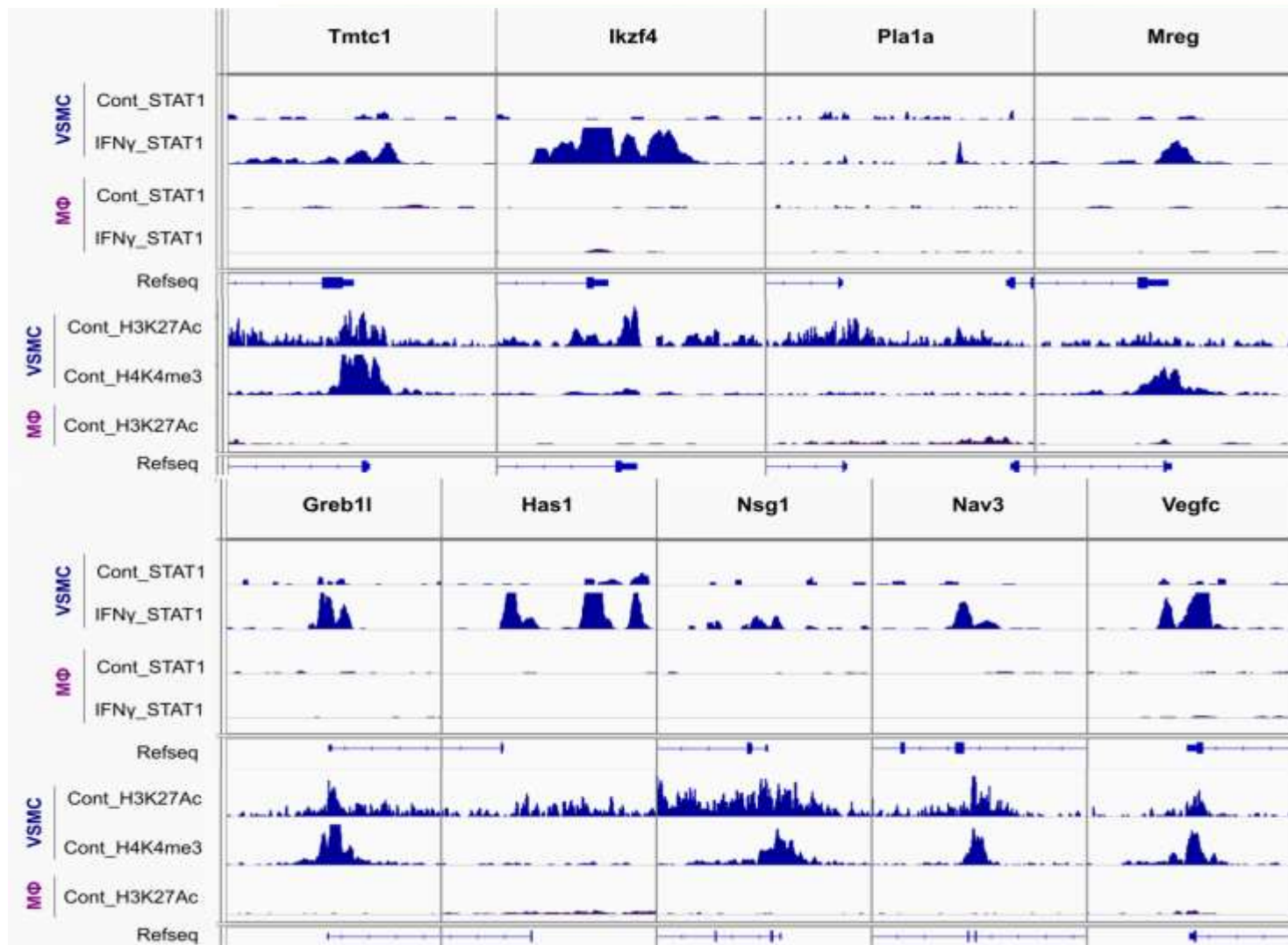
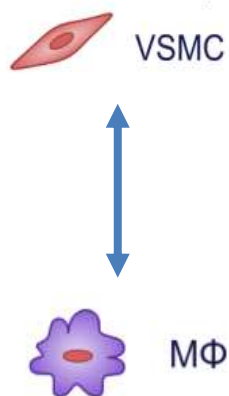


MΦ-specific IFN γ -induced gene expression involves collaboration between STAT1 and PU.1



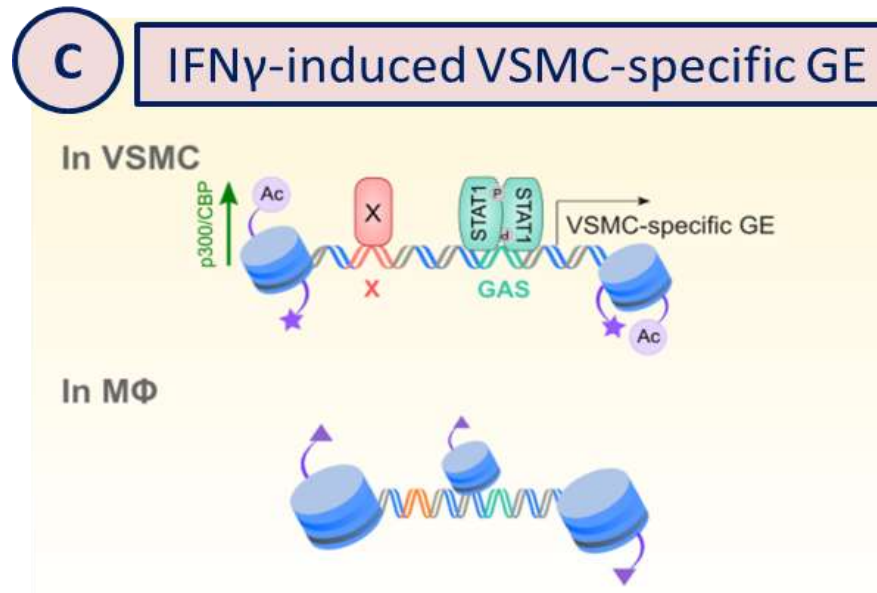
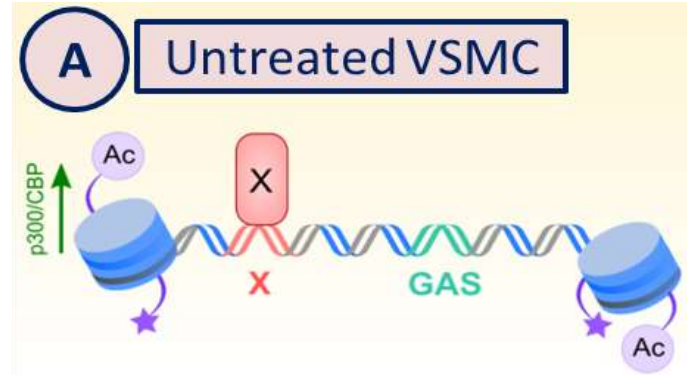
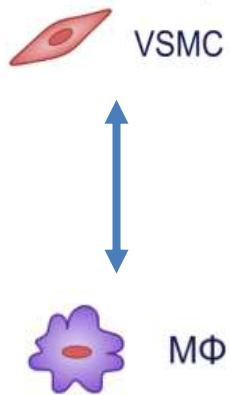


IFN γ activated VSMC-specific transcription: STAT1 binding + epigenetic changes



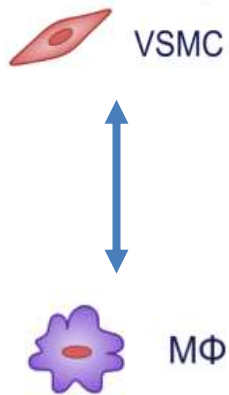


MΦ-specific IFN γ -induced gene expression involves collaboration between STAT1 and LDTF



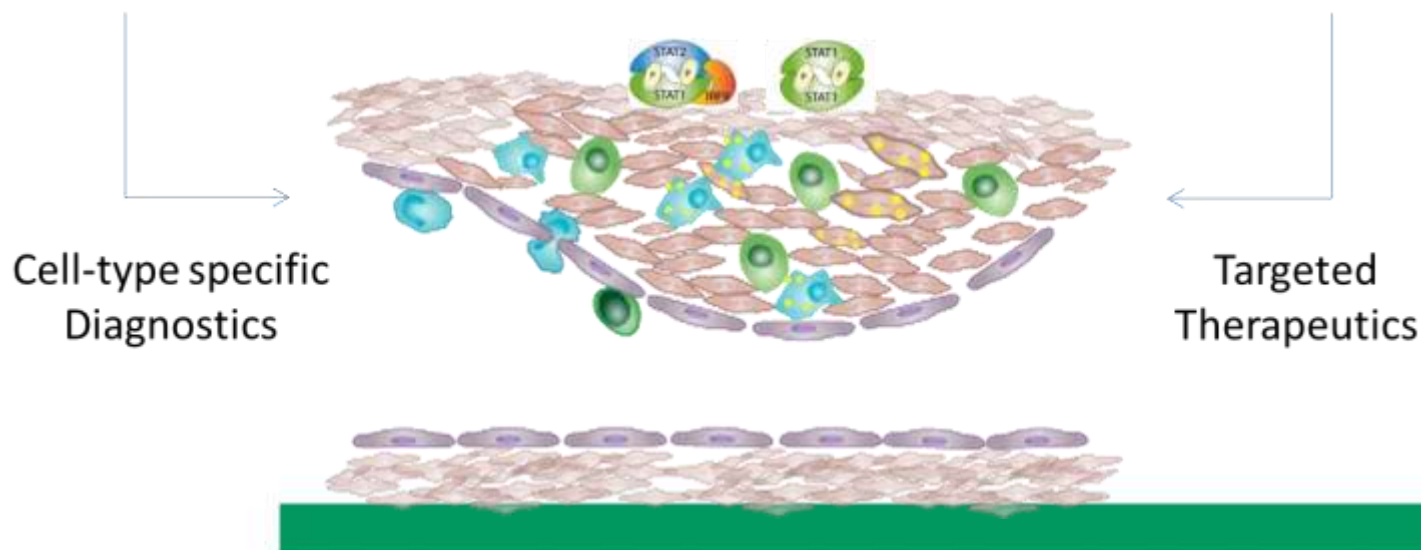
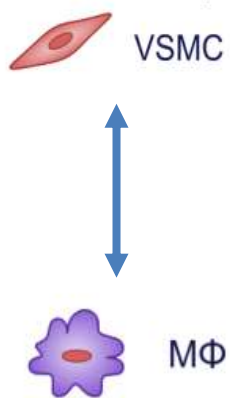
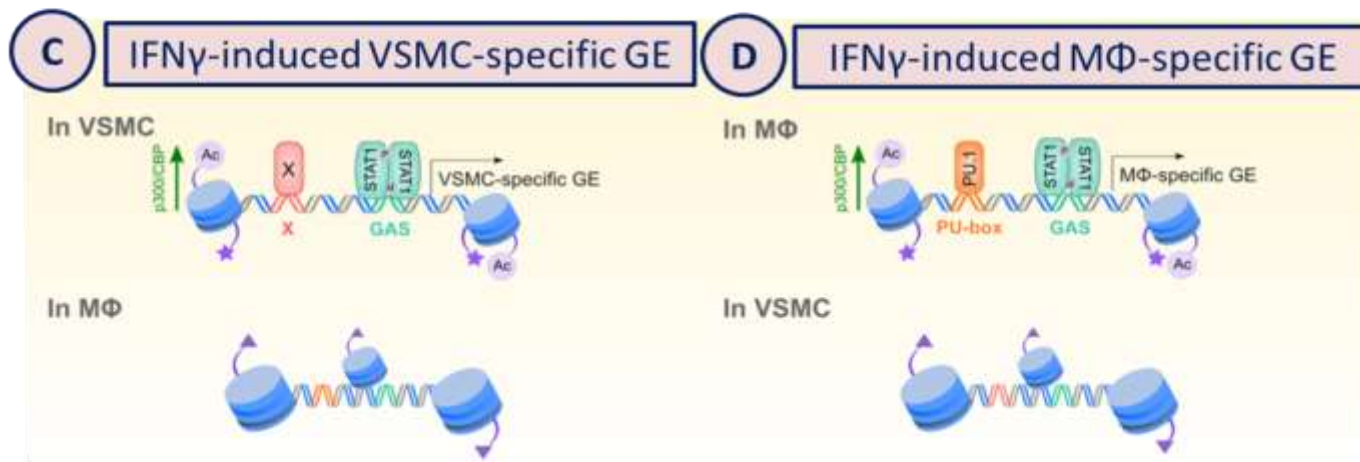
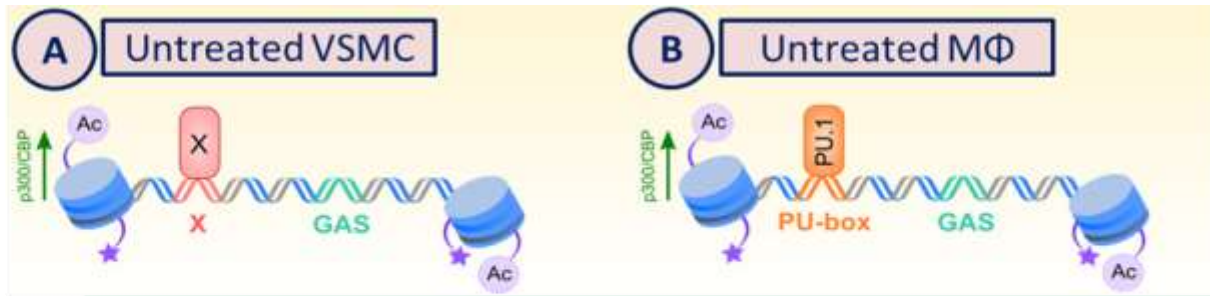


Identification of putative VSMC-LDTF



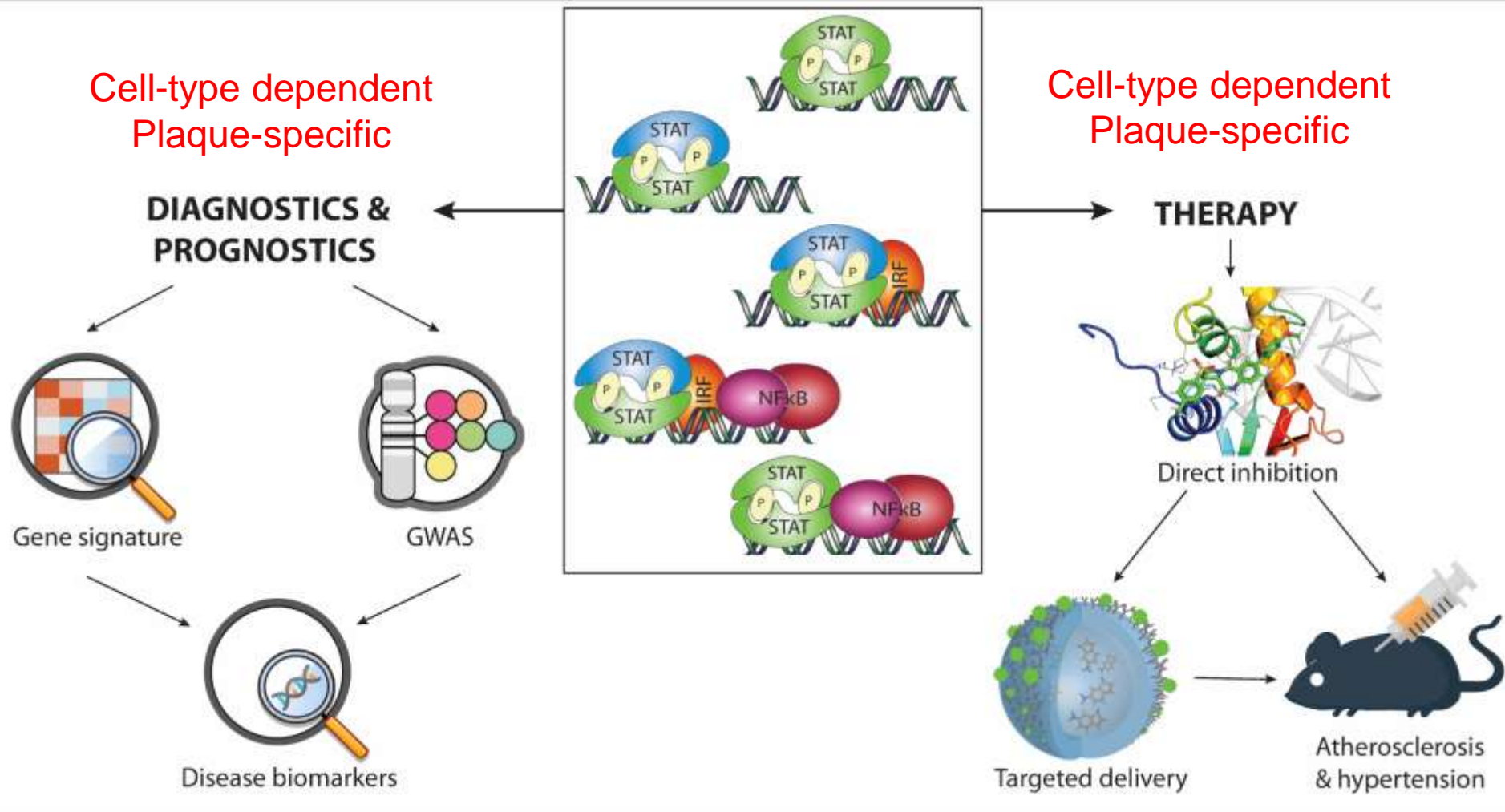
TF name	Raw value in control [RPKM]		Target gene hits	Z-score	Fisher score	
	MΦ	VSMC				
VSMC-specific	Gata6	3,2	3933,6	382	3,9	36,6
	Tead1	4,0	2455,2	444	22,4	83,0
	Zeb1	43,1	1747,8	845	-2,8	84,0
	Glis2	13,6	1681,3	234	9,0	42,4
	Nr2f2	1,2	1323,9	410	8,3	37,8
	Sox12	5,0	1203,9	340	2,3	34,9
	Foxa2	1,4	1166,6	465	-0,5	47,3
	Sox9	2,0	1051,9	726	14,0	70,1
Hoxa5	5,7	910,0	855	25,5	71,4	

Promoter analysis of 892 VSMC-specific genes and the top-50 of the IFN γ dependent VSMC-specific up-regulated genes





STATs in Diagnostics & Therapeutics





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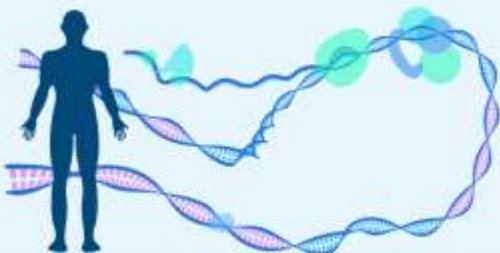
Dr. Balint Laszlo Balint

University of Madrid

Department of Pharmacology

School of Medicine

Prof. Concha Piero



Department of Human Molecular Genetics

Laboratory of High Throughput Technologies

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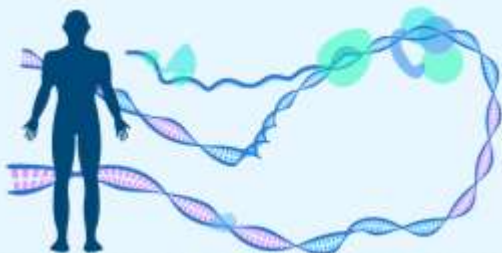
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Department of Human Molecular Genetics
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