3ο ΣΧΟΛΕΙΟ ΒΑΣΙΚΗΣ ΑΝΟΣΟΛΟΓΙΑΣ ΓΙΑ ΚΛΙΝΙΚΟΥΣ

FORTH
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

The role of cohesin complex protein SMC1a in determining the sex differences of monocyte biology driving autoimmunity

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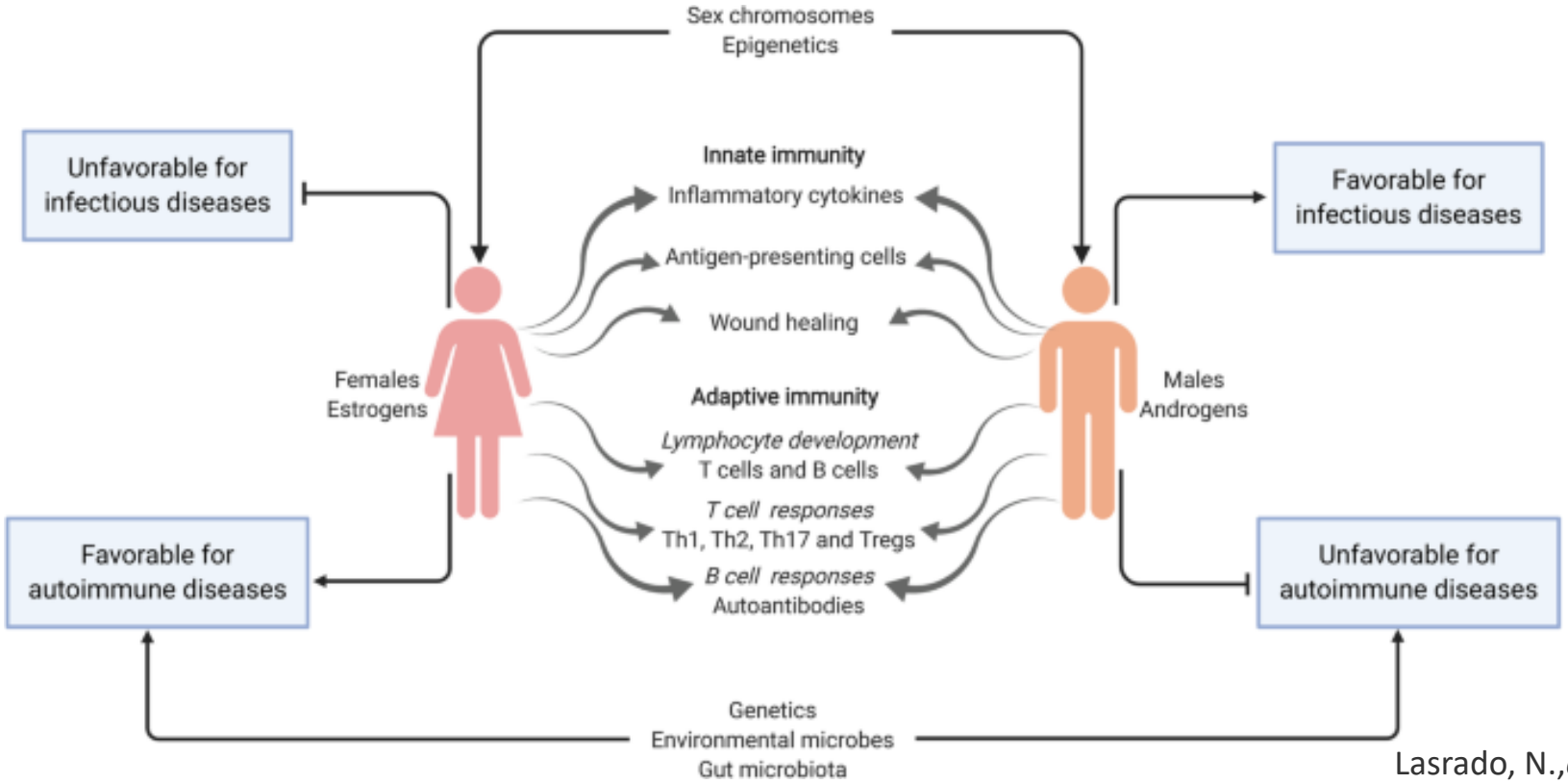
(Special thanks to Chrysoula Stathopoulou, Sofia Papanikolaou, Dimitris Konstantopoulos)

Rheumatology, Autoimmunity and Inflammation Lab
Medical School of Crete
Heraklion, 10/10/2021

Sexual dimorphism in immunity and autoimmunity



Lasrado, N.,*et al., Biol Sex Differ*, 2020

Autoimmune diseases demonstrate greater frequency in females than in males. Males suffer from more severe disease manifestations → sexual dimorphism in autoimmunity

Best example is the case of **Systemic Lupus Erythematosus**.

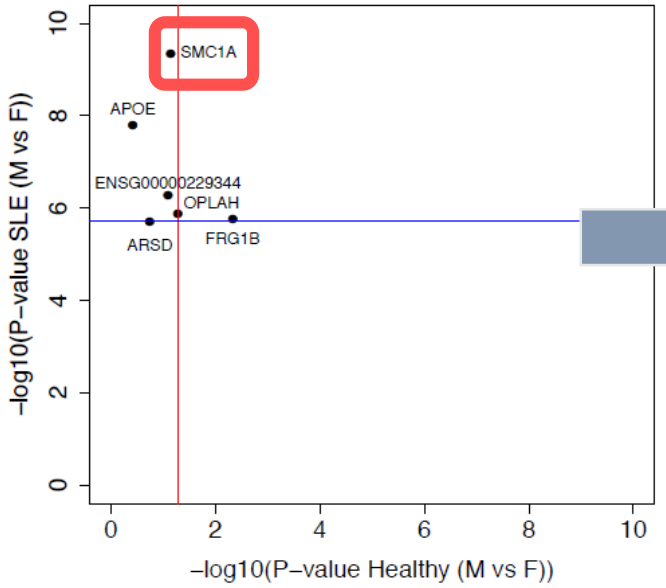
High female-to-male ratio (12:1)

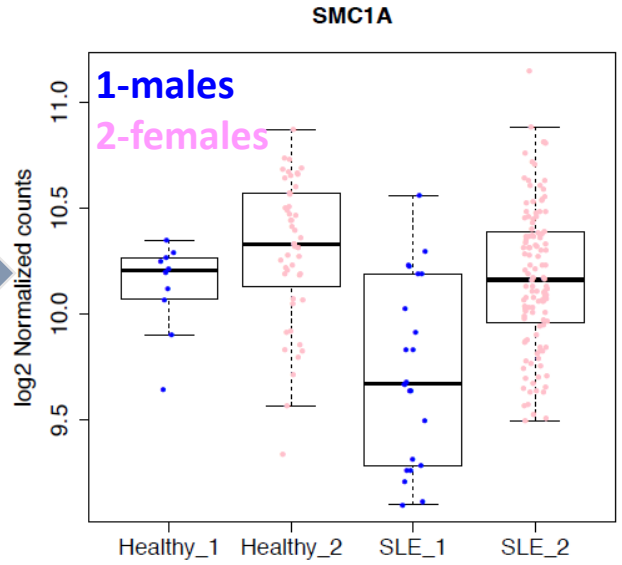
Deciphering the molecular basis of sexual dimorphism in SLE

➢ Unbiased transcriptome analysis in **whole blood** of SLE and healthy individuals

→ **a sex biased molecular signature was unraveled.**

Cohort #1





Confirmed
in an independent validation
Cohort #2 (SYSCID COHORT)

SMC1a:

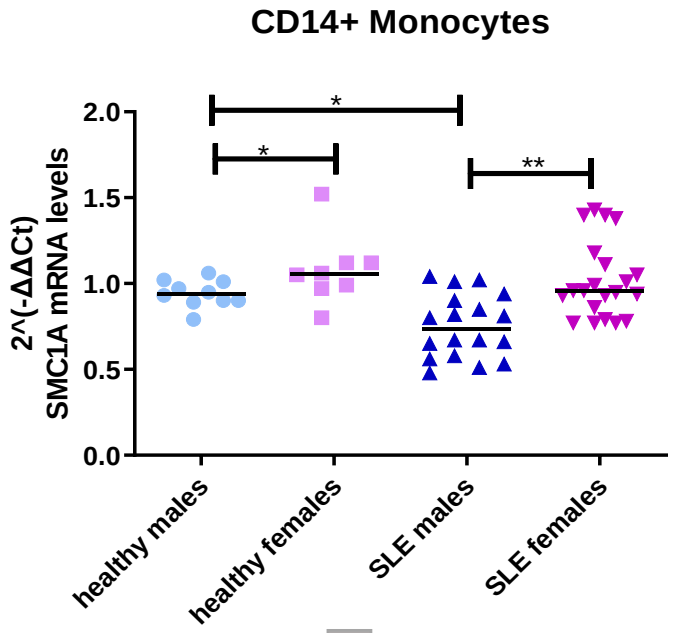
✓ showed **the strongest gender difference.**

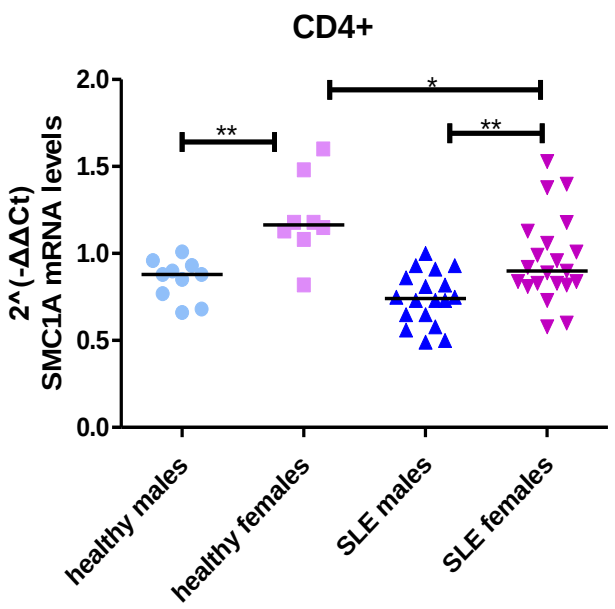
✓ is significantly **reduced** in male SLE versus female SLE as compared to healthy individuals.

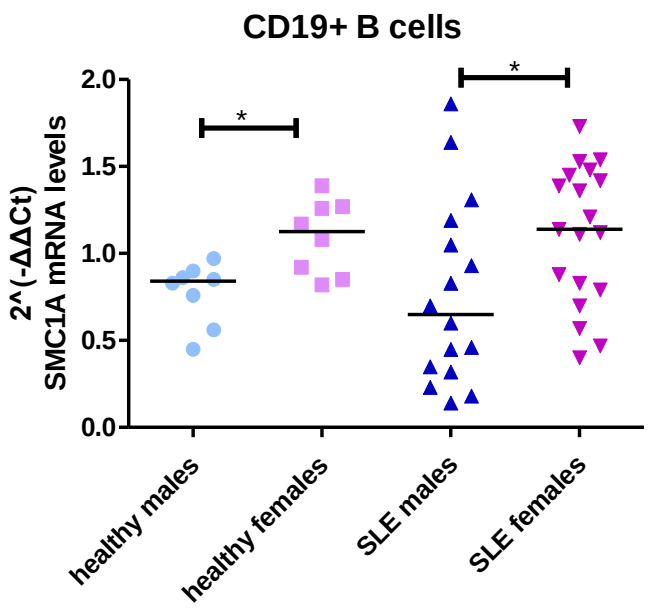
RESULTS

The gender-biased SMC1a expression exists in multiple SLE immune cell subpopulations.

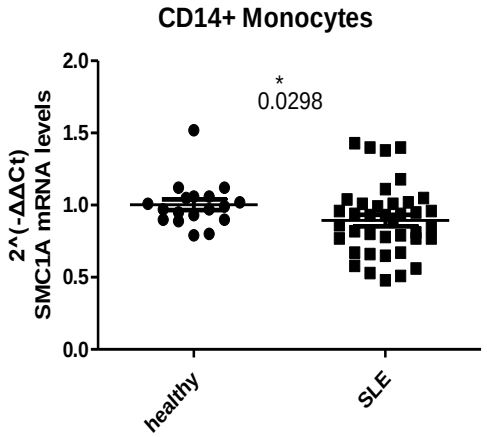
- Taqman PCR for SMC1a in immune cell types from a new, independent cohort of healthy and SLE individuals







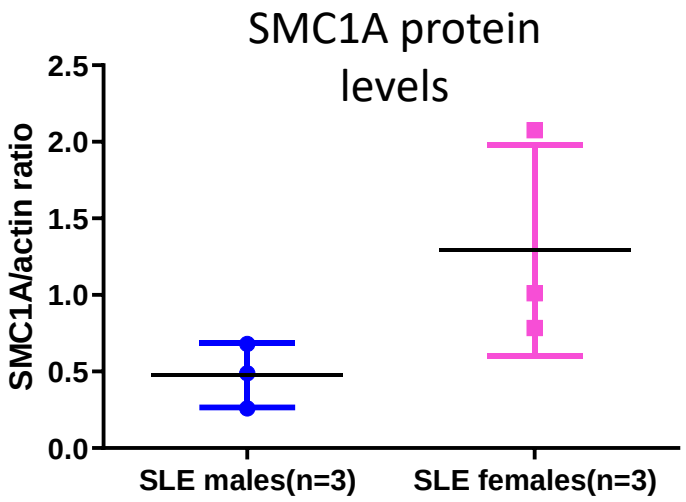
CD14+ Monocytes best recapitulated the gender difference in whole blood SMC1a expression.

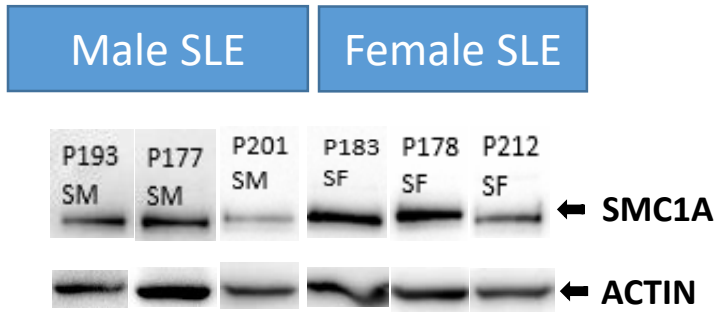


RESULTS

CD14+ monocytes exhibit a gender-biased SMC1a protein expression in SLE patients.

Western blot for SMC1a in CD14+ Monocytes isolated from male and female SLE patients





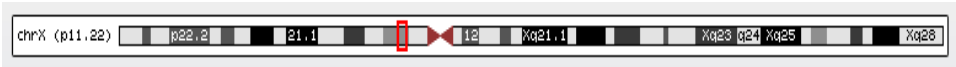
~60% higher expression in SLE females vs males

✓ male SLE monocytes express lower levels of SMC1a compared to female SLE monocytes both at mRNA and protein level.

INTRODUCTION

SMC1a (Structural Maintenance of Chromosomes Protein 1A)

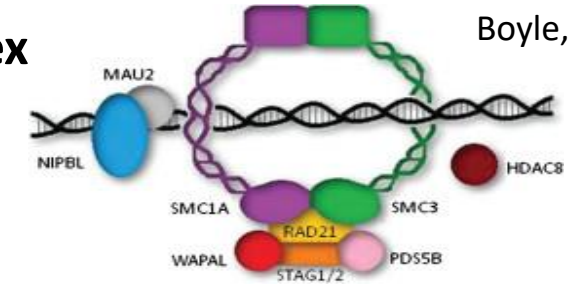
- *Cytogenetic location:* Xp11.22



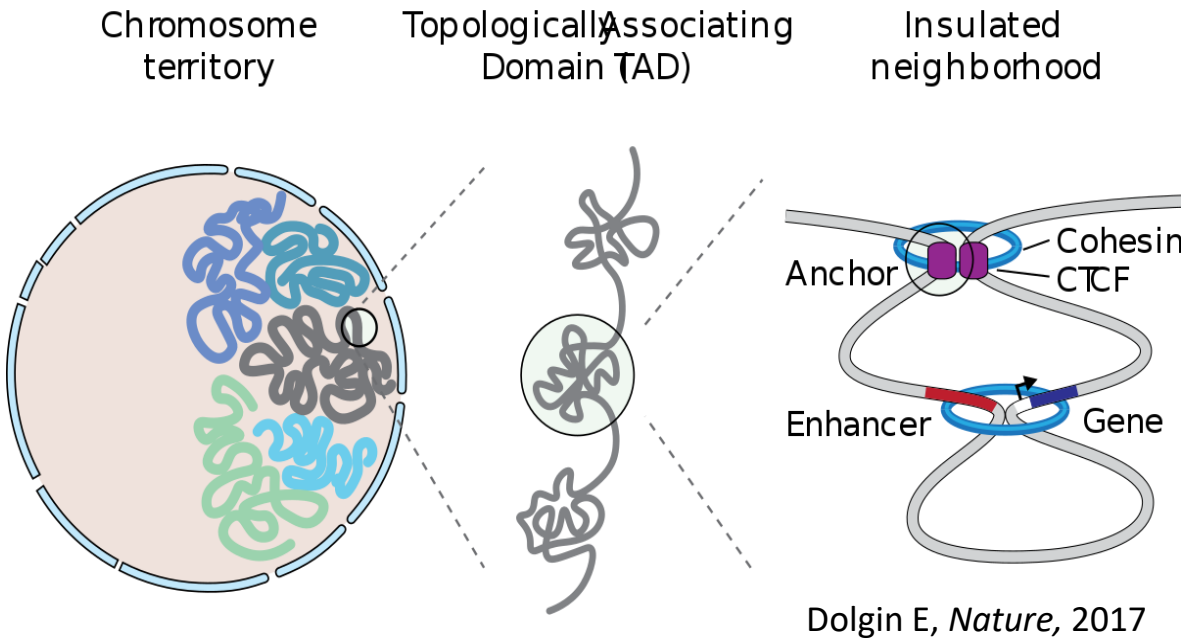
Brown et al, 1995

- Escapes X chromosome inactivation

- Belongs to **cohesin complex**



Boyle, M. I. et al., *Clinical Genetics,* 2014



Dolgin E, *Nature,* 2017

- Functions:

1. sister chromatids cohesion

2. DNA repair

3. gene regulation via chromatin architecture remodeling.

- Cohesin regulate the expression of immune-related genes

(Hadjur et al, Nature, 2009) (Ribeiro de Almeida et al, 2009)

Research Questions

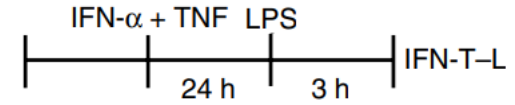
1. **What is the role of SMC1a in regulation of human monocyte biology in the context of lupus inflammation?**

2. **How does gender-biased** expression of SMC1a affect **susceptibility** to and/or **severity of SLE?**

RESULTS

IFNα/TNF/LPS triple combination is the most disease(SLE) relevant stimulus in monocytes in order to study the role of SMC1a

Park, Sung Ho, et al.
Nature immunology (2017)



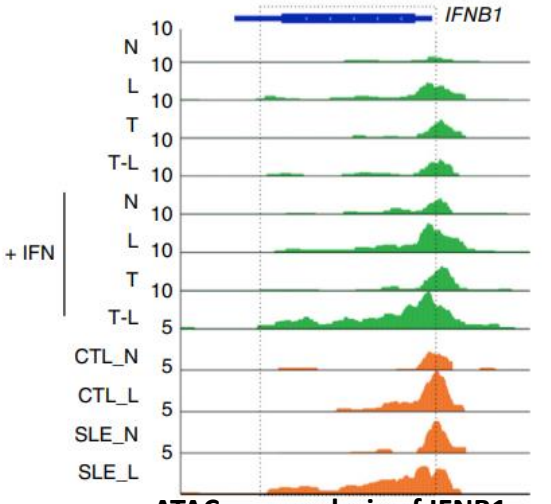
SLE monocytes are exposed in vivo to IFNs, TNF and TLR ligands

In vitro: IFNα and TNF 'primed' chromatin at the promoters of tolerized genes (proinflammatory cytokines, NFκb genes, **IFNb**), facilitating transcriptional responses to weak LPS signals

IFNa has been proposed to contribute to the pathogenesis of SLE by opposing tolerance induction.
Shi, L. et al. *PLoS One*, 2014

The chromatin accessibility profile of SLE monocytes treated with LPS *ex vivo* was closely correlated with the profile of IFNa/TNF/LPS treated cells

This model system mimics aspects of chromatin regulation in an interferon-mediated disease in vivo



ATAC-seq analysis of IFNB1

RESULTS

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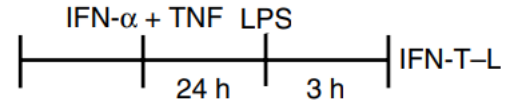
Park, Sung Ho, et al.
Nature immunology (2017)



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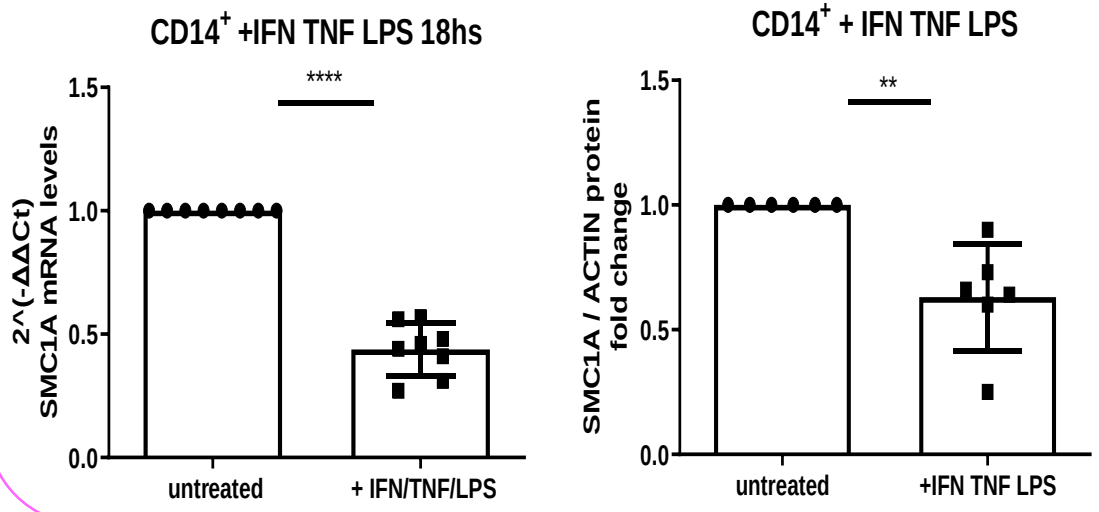
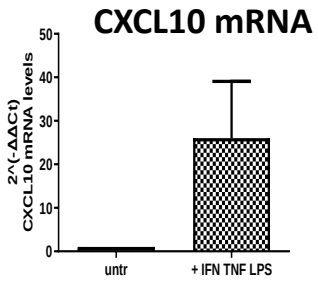
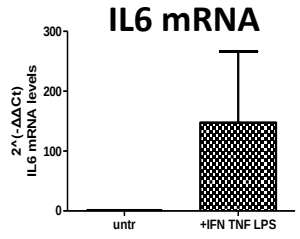






↓ SMC1a

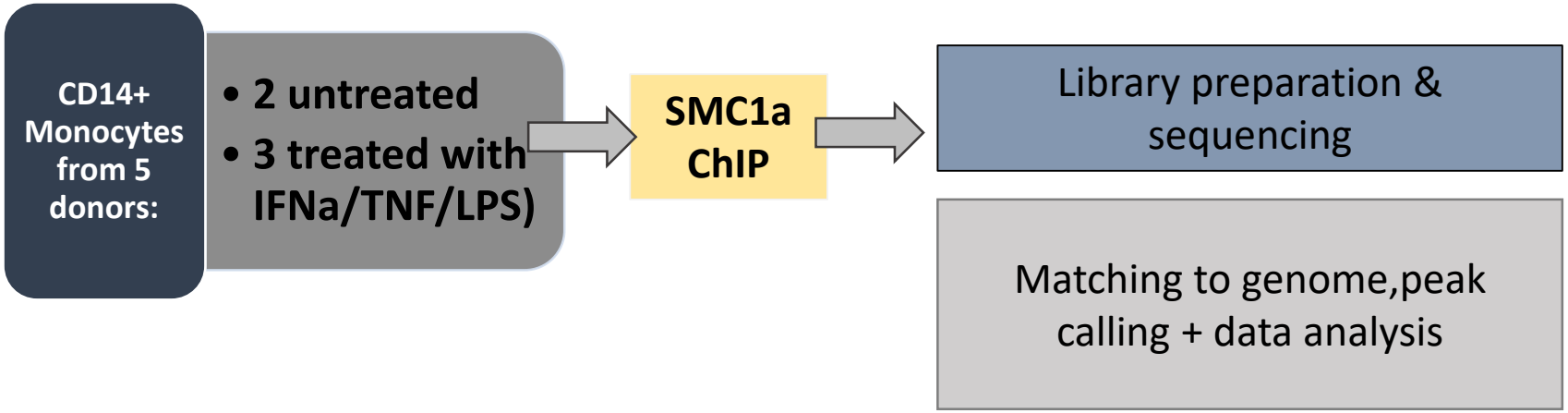
↑ IL6, ISGs(IP-10)

SLE monocyte = IFNα/TNF/LPS monocyte

Experimental set up

ChIPseq experiments based on our in vitro disease associated “set up” to delineate the role of SMC1a as a regulator of gene expression in pathogenic monocytes

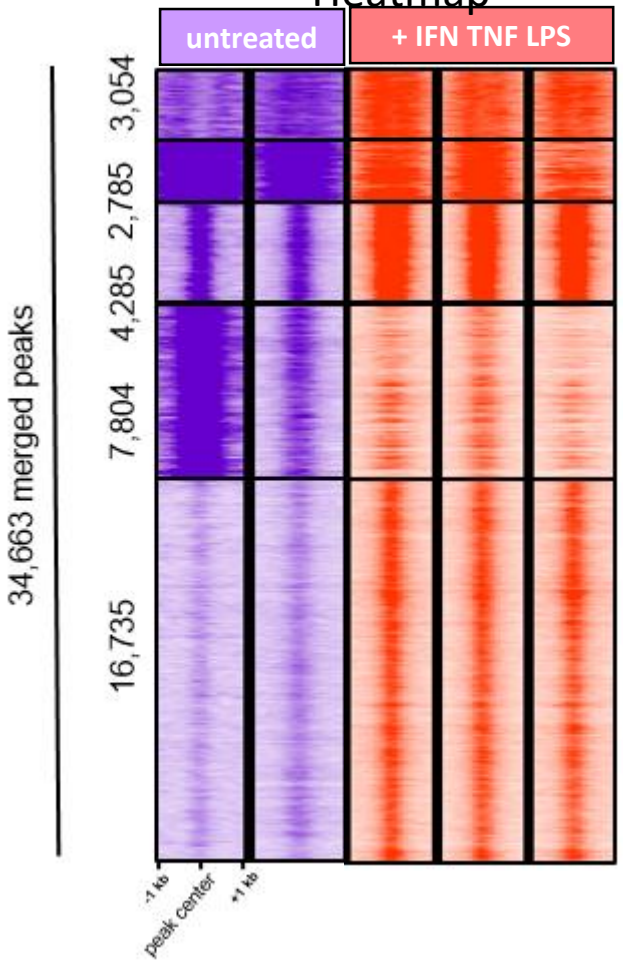
High throughput ChIP sequencing in healthy CD14+ monocytes and IFN/TNF/LPS-treated CD14+ monocytes



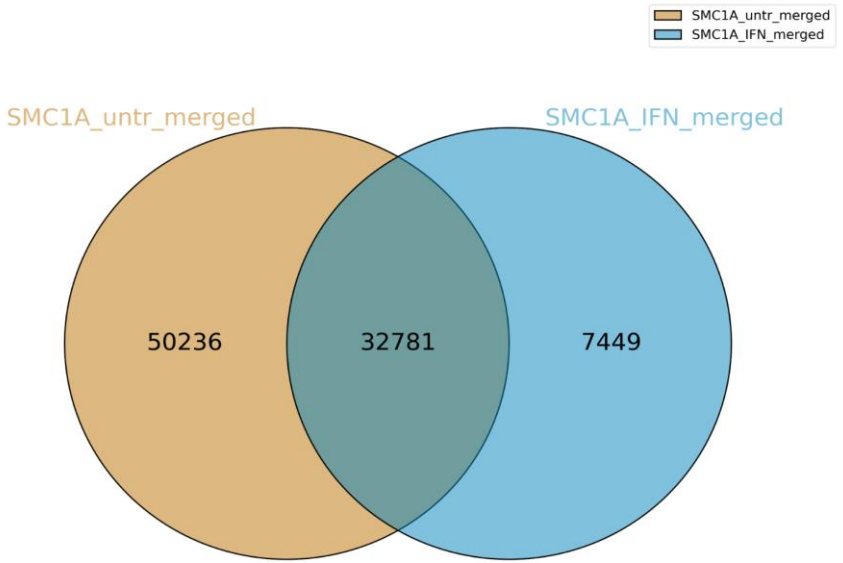
RESULTS

Under lupus-like inflammation, the chromatin occupancy of SMC1a is significantly altered

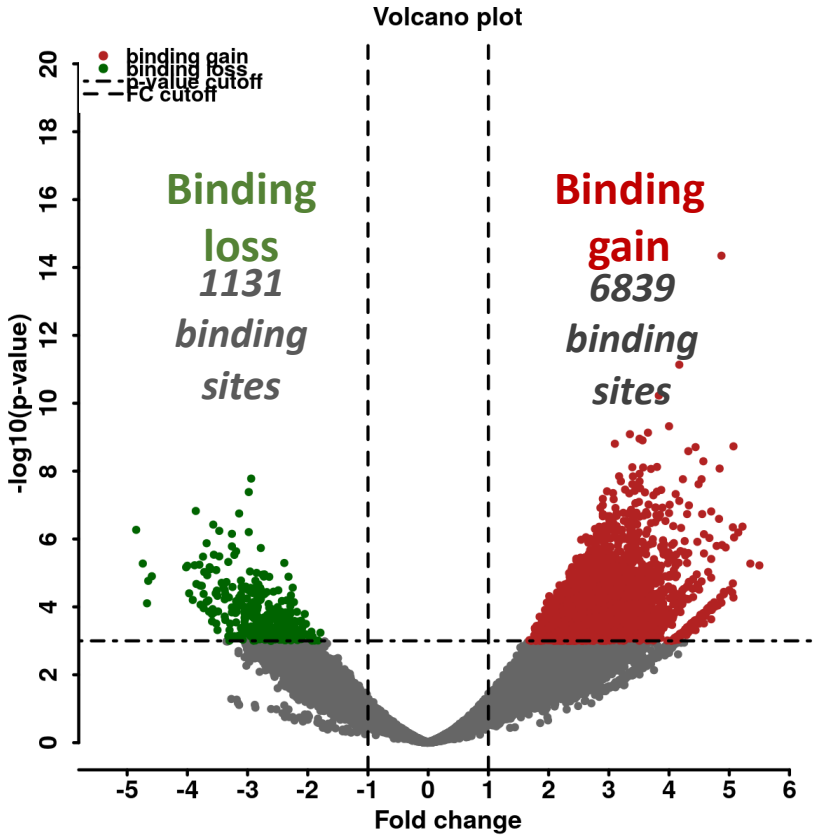
Heatmap



Peak Overlap

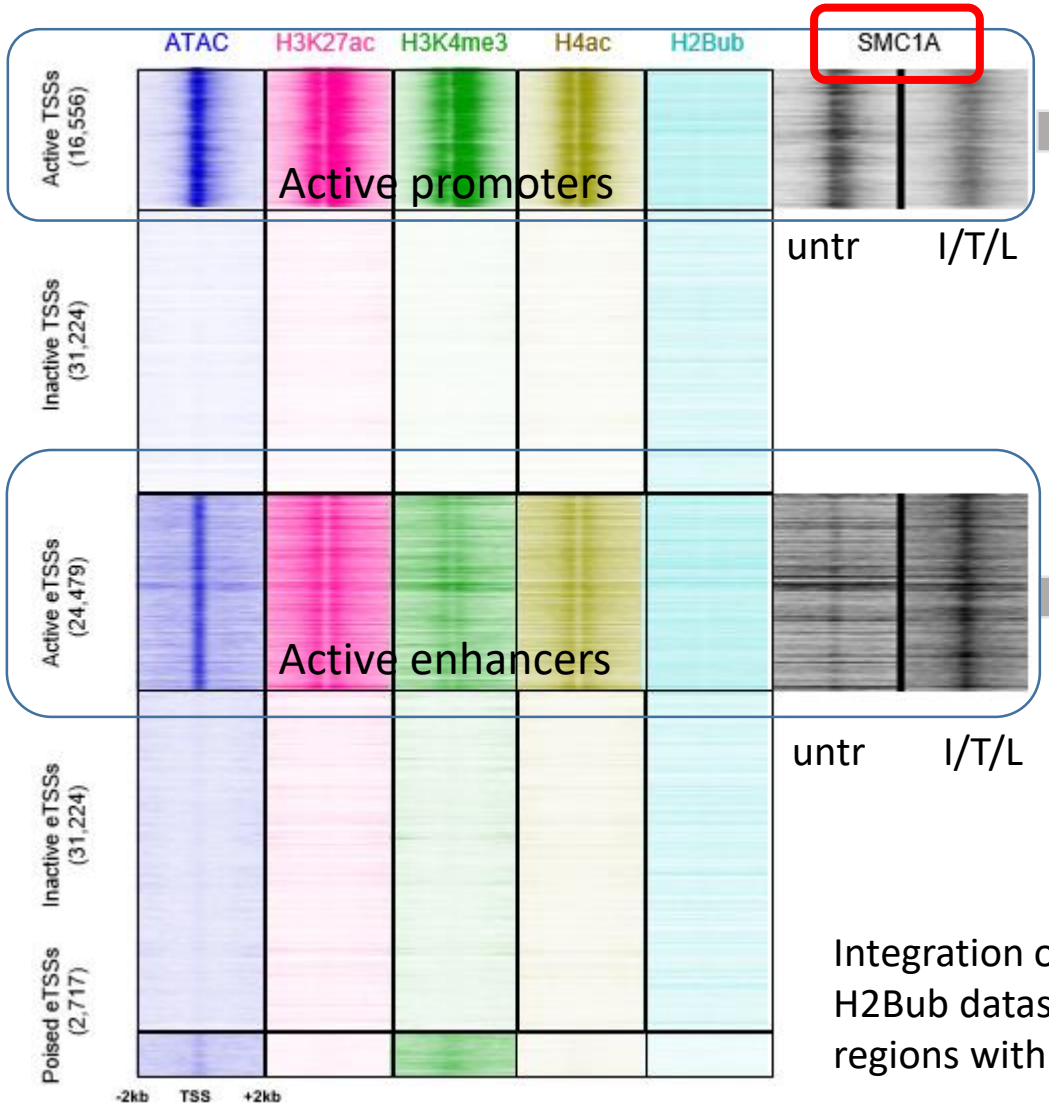


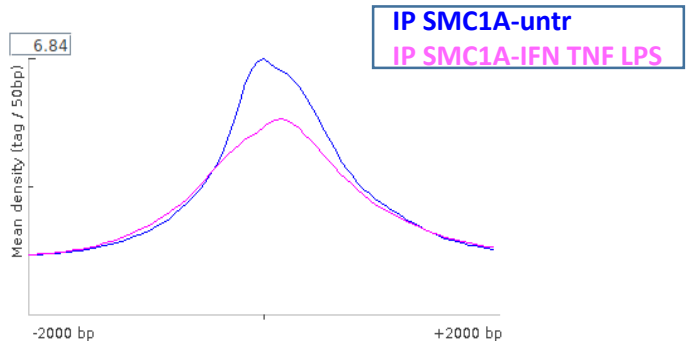
Differential binding

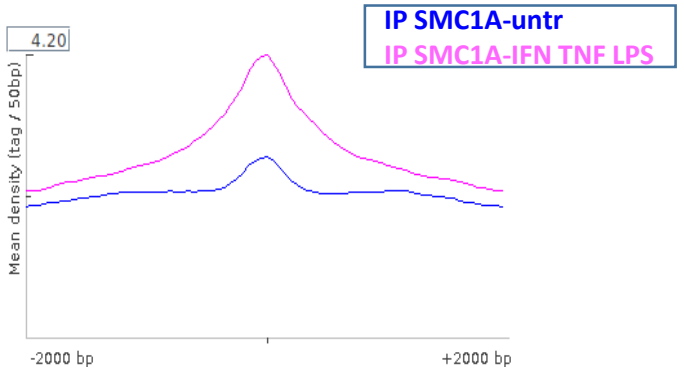


RESULTS

In stimulated (lupus-like) monocytes, SMC1a binding is increased in enhancer regions suggesting a broader role in gene expression regulation





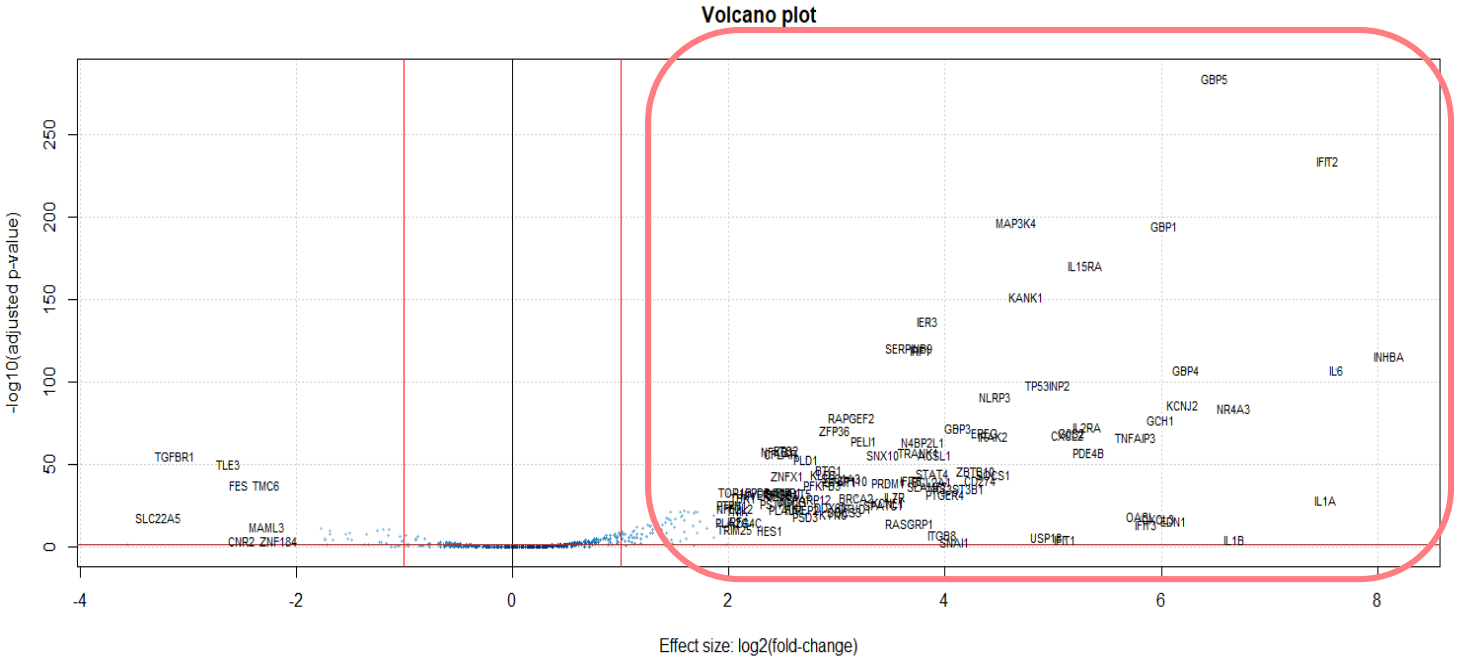


Integration of SMC1a ChIPseq with published ATACseq, H3K27ac, H3K4me3, H4ac, H2Bub datasets for human CD14+ treated with IFN/TNF/LPS to determined genome regions with **active transcription**

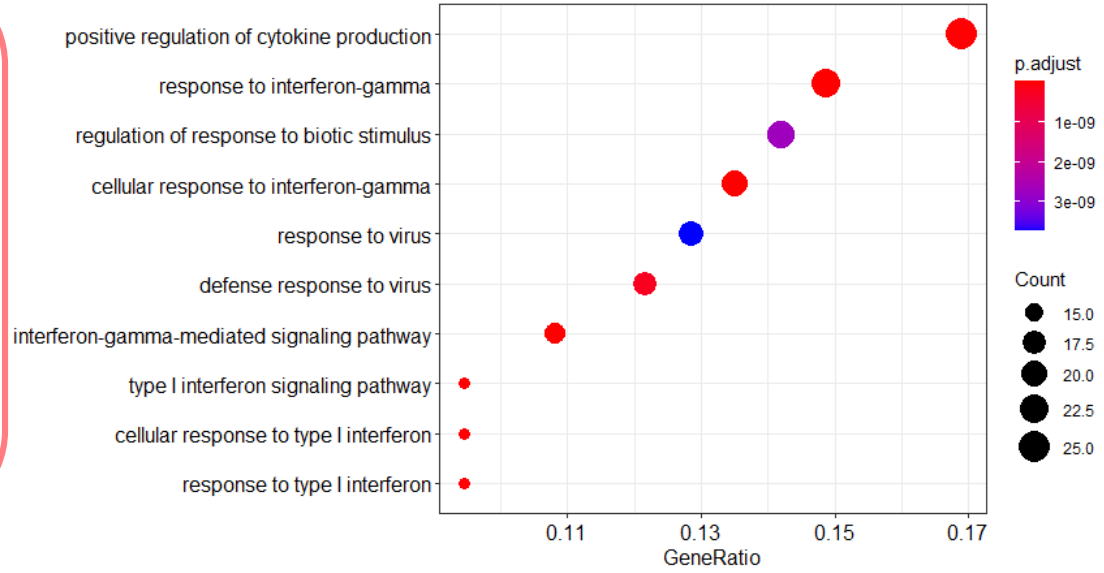
RESULTS

SMC1a mediates the transcription of inflammatory genes in lupus-like monocytes

Genes with **increased binding of SMC1a** at their active **enhancer** and active **promoter: 584 → 162** which are **actively transcribed** **(integration with our RNA-seq data)**



Over-representation analysis



Genes with **increased binding of SMC1a** at their **active promoters: 155**

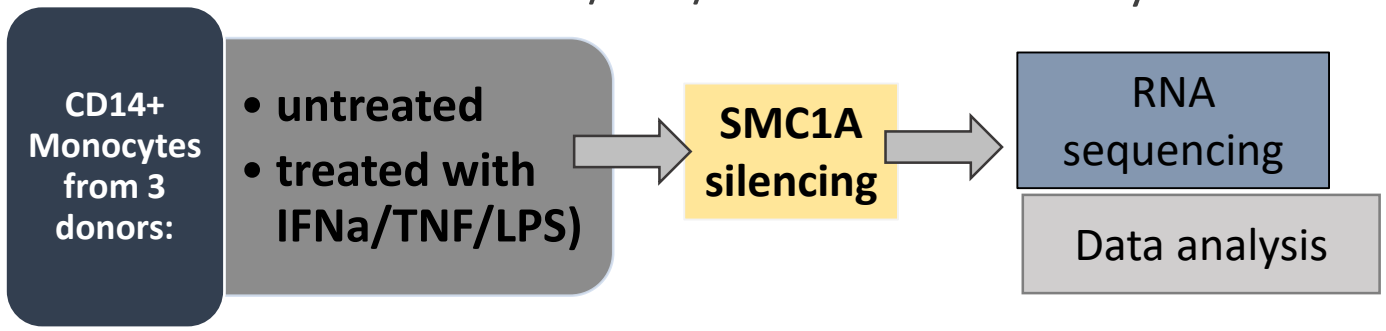
→ **major pathways:**

positive regulation of cytokines, regulation of inflammatory response, T cell activation, regulation of IFNa production, regulation of NFkb signaling

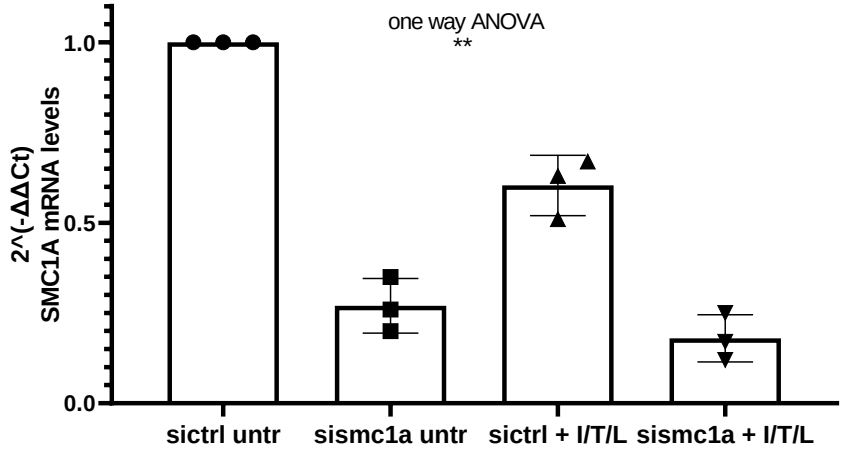
Experimental set up

To address the effect of differential SMC1a expression in the context of lupus-like monocyte, we performed gene silencing assays

Nucleofection of CD14+ Monocytes with siRNA against SMC1a in healthy monocytes and IFN/TNF/LPS-treated monocytes

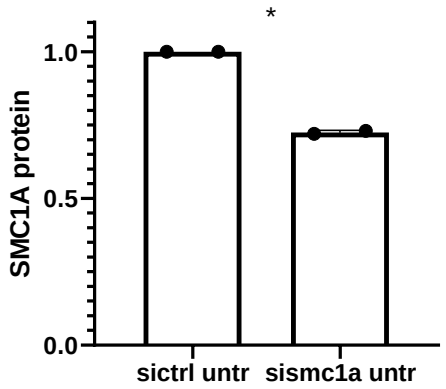


SMC1A mRNA reduction



~70% reduction of SMC1a mRNA in untreated CD14+

SMC1A protein reduction

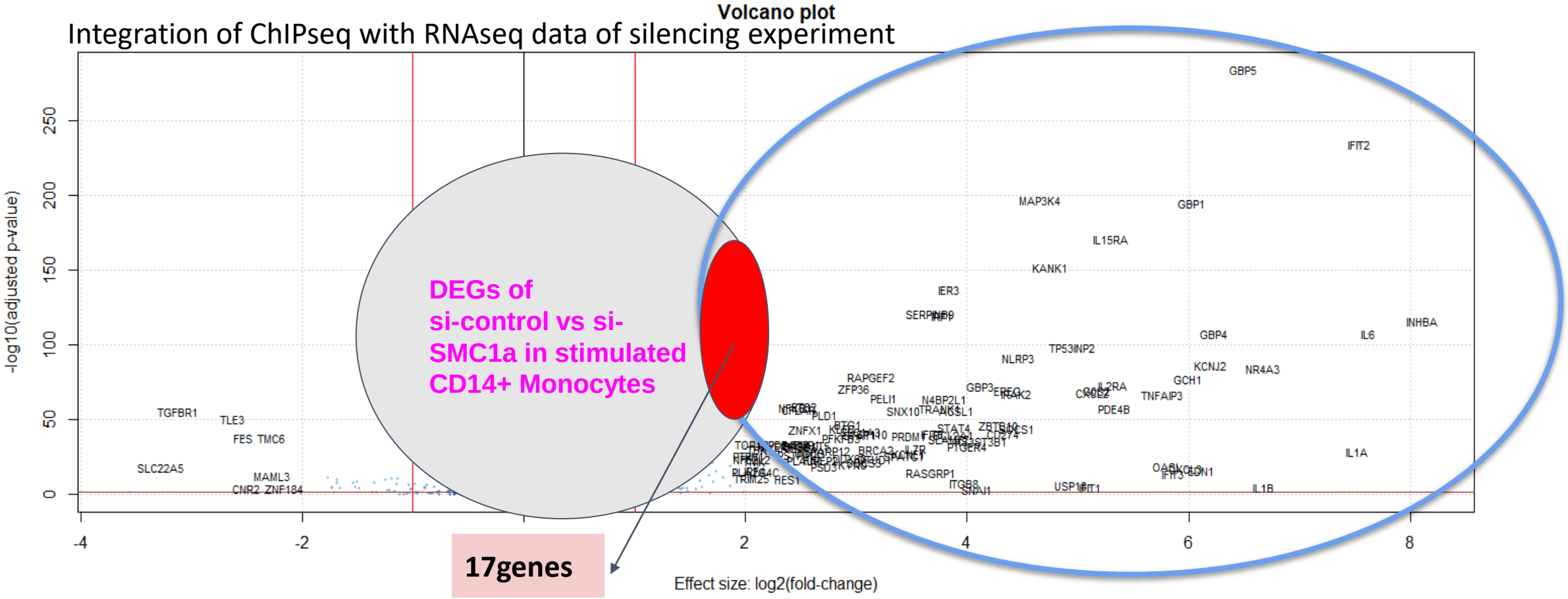


~30% reduction of SMC1a protein in untreated CD14+ Monocytes

RESULTS

Bioinformatics analysis revealed 15 gene-targets of SMC1a in lupus-like monocytes

Integration of ChIPseq with RNAseq data of silencing experiment



162 genes with increased binding of SMC1a at their active transcription sites

17 genes are statistically significant differentially expressed upon SMC1a silencing in stimulated cells

15 genes are statistically significant downregulated upon SMC1a silencing in stimulated cells

GBP5, N4BP2L1, TRIM25, PSTPIP2, PELI1, IL1A, TP53INP2, ADA, SLC9A8, TRANK1, AGPAT4, ITGB8, IL6, INHBA, SPATC1

RESULTS

The 15 SMC1a target-genes participate mainly in immune related pathways

g Profiler

GO:BP	stats		GBP5	N4BP2L1	TRIM25	PSTPIP2	PELI1	IL1A	TP53INP2	ADA	SLC9A8	TRANK1	AGPAT4	ITGB8	IL6	INHBA	SPATC1
Term name	p_{adj}	$-\log_{10}(p_{adj})$ 0 ≤16															
regulation of cytokine production	4.140×10^{-4}		■		■		■	■						■	■	■	
cytokine production	4.378×10^{-4}		■		■		■	■						■	■	■	
positive regulation of lymphocyte proliferation	2.238×10^{-3}						■	■		■					■		
positive regulation of mononuclear cell proliferation	2.302×10^{-3}						■	■		■					■		
positive regulation of leukocyte proliferation	3.264×10^{-3}						■	■		■					■		
mononuclear cell differentiation	7.667×10^{-3}							■		■				■	■	■	
regulation of B cell activation	8.462×10^{-3}						■			■					■	■	
germinal center B cell differentiation	1.517×10^{-2}									■					■		
regulation of lymphocyte proliferation	1.615×10^{-2}						■	■		■					■		
regulation of mononuclear cell proliferation	1.671×10^{-2}						■	■		■					■		
regulation of lymphocyte activation	1.777×10^{-2}						■	■		■					■	■	
positive regulation of multicellular organismal process	1.910×10^{-2}		■				■	■		■				■	■	■	
regulation of leukocyte proliferation	2.272×10^{-2}						■	■		■					■		
leukocyte differentiation	2.604×10^{-2}							■		■				■	■	■	
regulation of leukocyte activation	3.948×10^{-2}						■	■		■					■	■	
lymphocyte proliferation	4.206×10^{-2}						■	■		■					■		
mononuclear cell proliferation	4.376×10^{-2}						■	■		■					■		

RESULTS

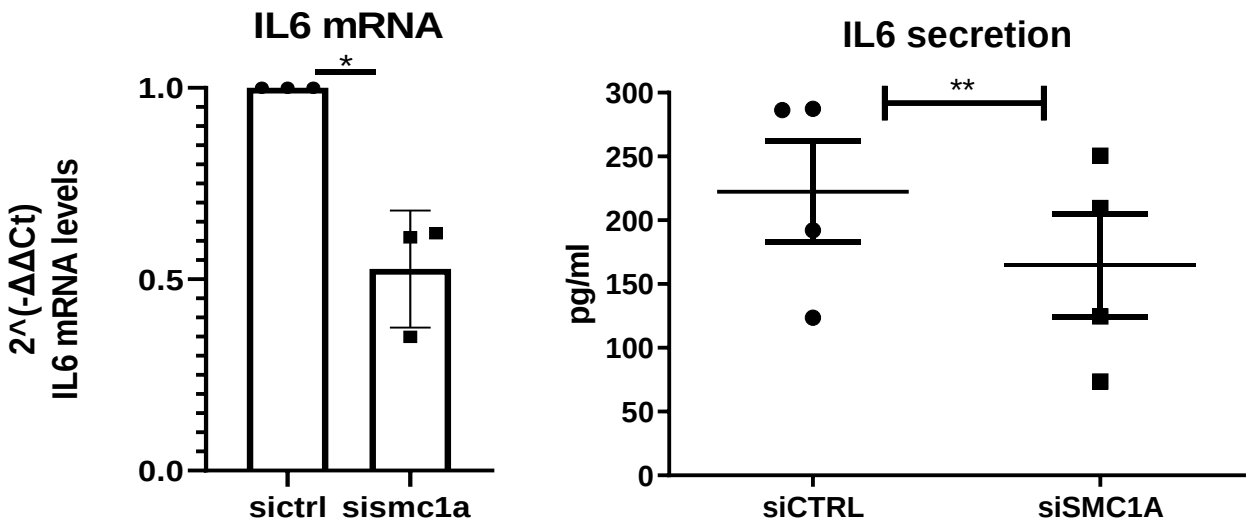
The majority of SMC1a target-genes constitute lupus DEGs and some of them display gender biased expression in human immune cells

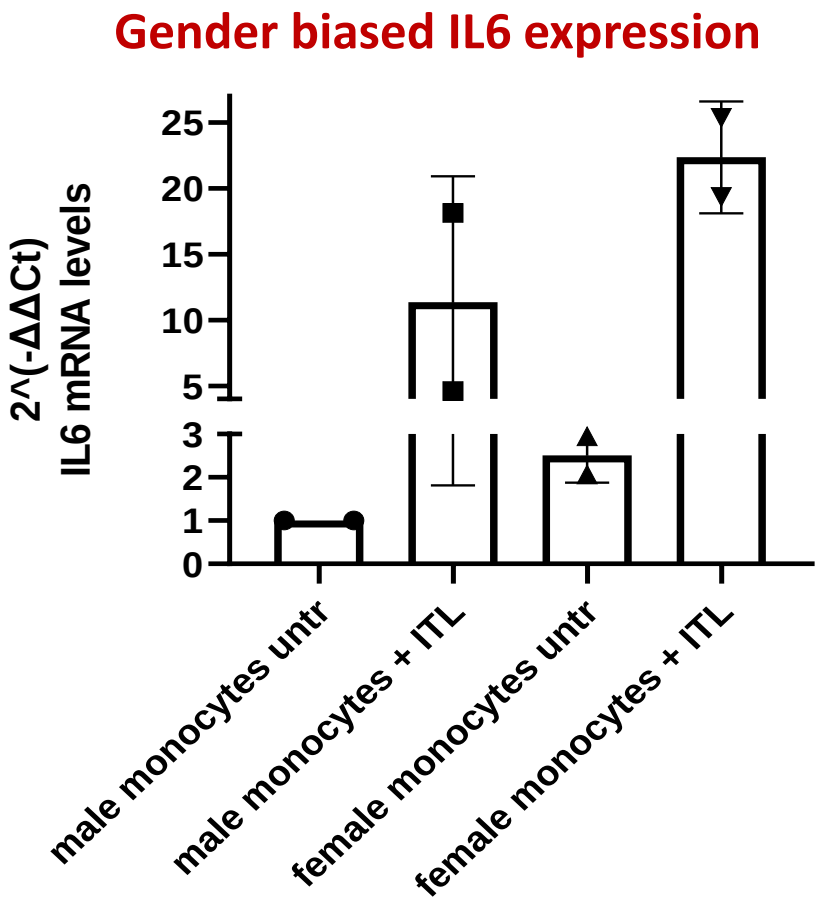
SMC1A TARGET GENES	LUPUS DEG	GENDER-BIASED
GBP5	YES	YES(MONOCYTES)
N4BP2L1	NO	YES (TH1)
TRIM25	YES	YES(TH2,TREG)
PSTPIP2	NO	YES(CD8)
PELI1	NO	NO
IL1A	YES	NO
TP53INP2	NO	NO
ADA	YES	NO
SLC9A8	YES	NO
TRANK1	YES	NO
AGPAT4	NO	NO
ITGB8	YES	NO
IL6	YES	YES (MONOCYTES)
INHBA	NO	NO
SPATC1	NO	NO

Benjamin J Schmiedel et al., Cell, 2018

RESULTS

Gender-biased SMC1a controls IL6 leading to greater induction in female monocytes.





Future Experiments

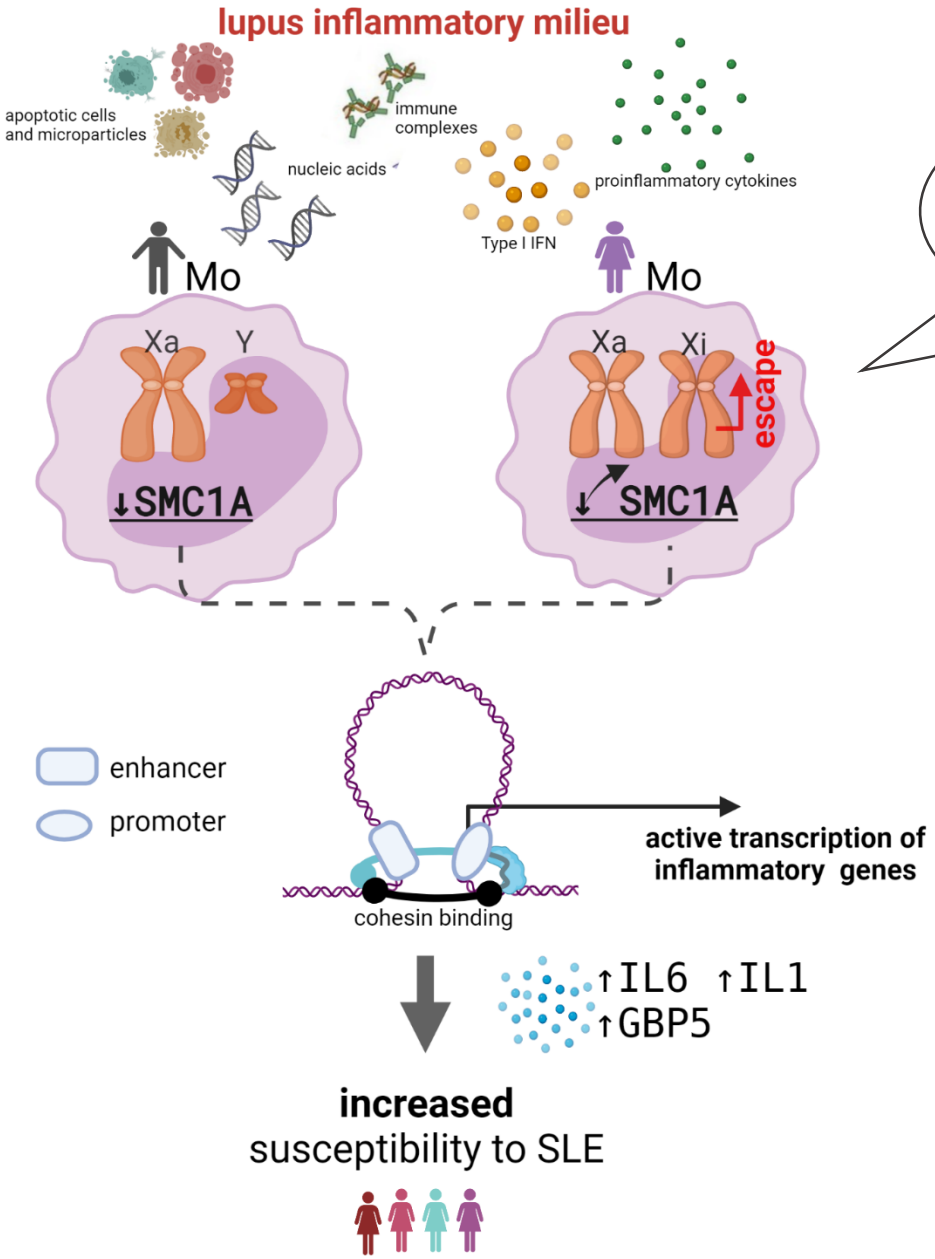
1. Integrate available Hi-C data of healthy monocytes to focus on specific chromatin interactions affecting gene "communities" regulated by SMC1a. Zhipeng Zhang et al., *Acta Biochimica et Biophysica Sinica*, 2020

2. Analyse the 'enrichment' of DEGs in SLE vs. healthy monocytes with the SMC1a-regulated genes

3. Functional assays (e.g. flow cytometry, secreted cytokines etc) in male/female SLE or lupus-induced (triple combination) monocytes (+/- after SMC1a silencing)

4. RNA FISH for Xist and SMC1a to study the X inactivation escape of SMC1a in SLE female monocytes.

Graphical Abstract



SMC1a escape of X chromosome inactivation remains to be elucidated

Thank you!