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Poster # F.9

The epigenetic landscape of T cell subsets in SLE identifies known and potential novel drivers of the autoimmune response

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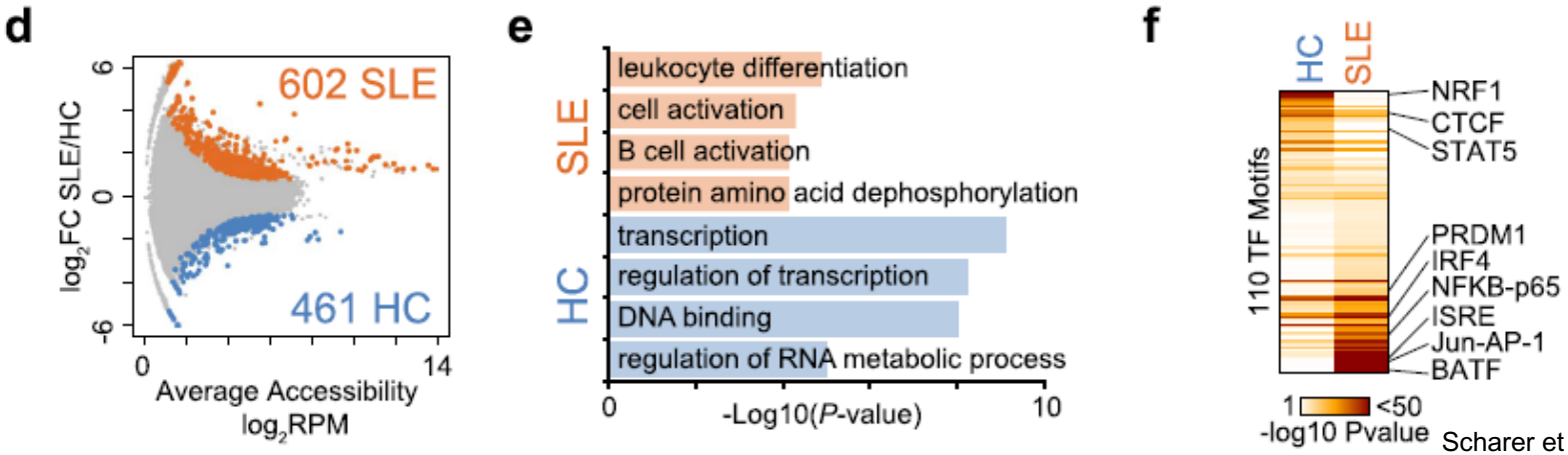
Epigenetic changes are key determinants of immune activation

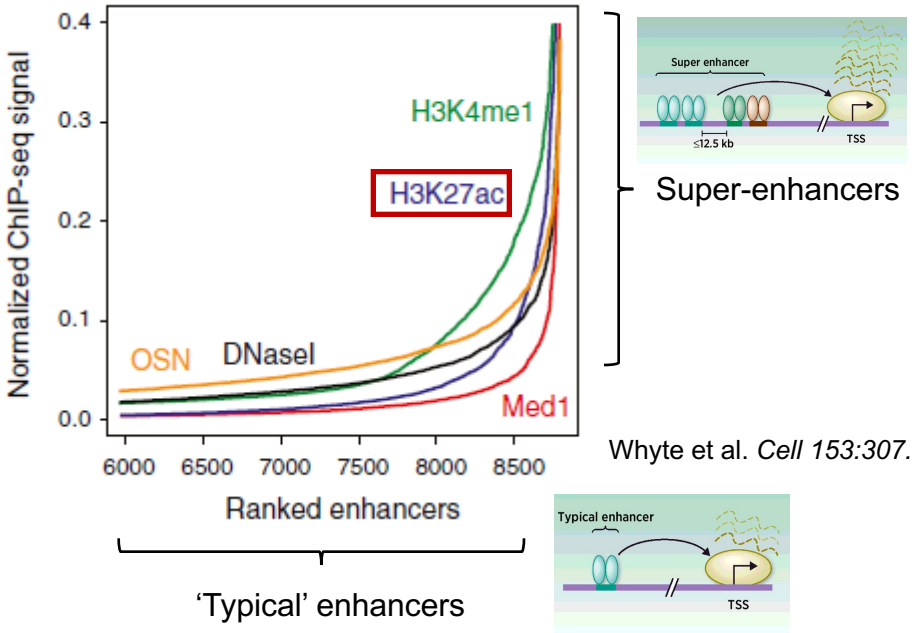
- Epigenetic changes are a key driver of many immune processes (e.g. naïve to memory transition in T and B cells)
- Helper T cells in SLE display profound changes in DNA methylation; however, epigenetic changes beyond methylation have not been characterized in T cells in SLE in detail
- Naïve B cells in SLE display significant changes in open chromatin (see below; Scharer et al. *Sci Reports 6:27030.*)
- Changes in enhancer biology in T cells in SLE have not been addressed
- Super-enhancers (SEs) in T cells may provide important insight into disease pathogenesis



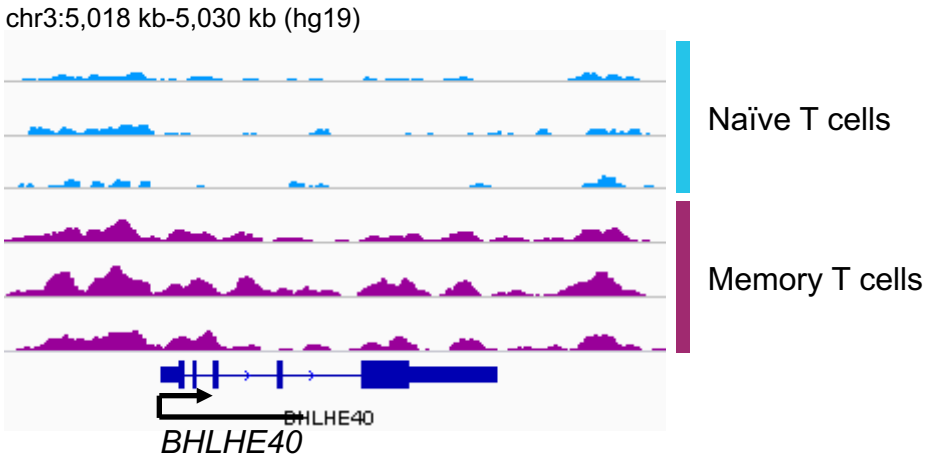
Scharer et al. *Sci Reports 6:27030.*

Super-enhancers regulate transcription of key genes that determine cell fate and function

- **Super-enhancers (SEs)** are regions of the mammalian genome comprising multiple enhancers collectively bound by an array of transcription factor proteins
- SEs are enriched in SNPs associated with autoimmune disease susceptibility
- Genes most likely driven by each SE are linked to SEs based on proximity



Whyte et al. *Cell 153:307.*



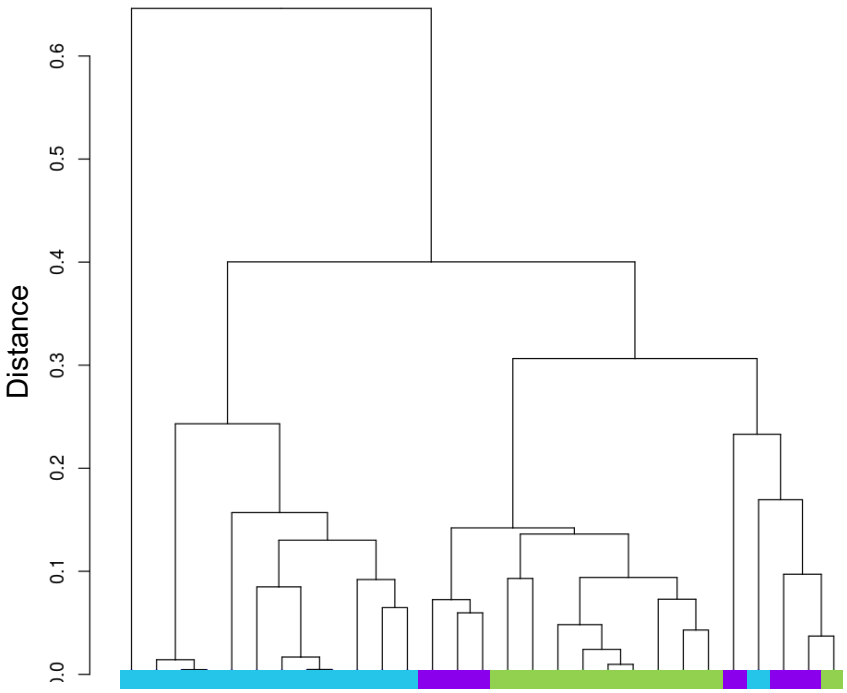
BHLHE40

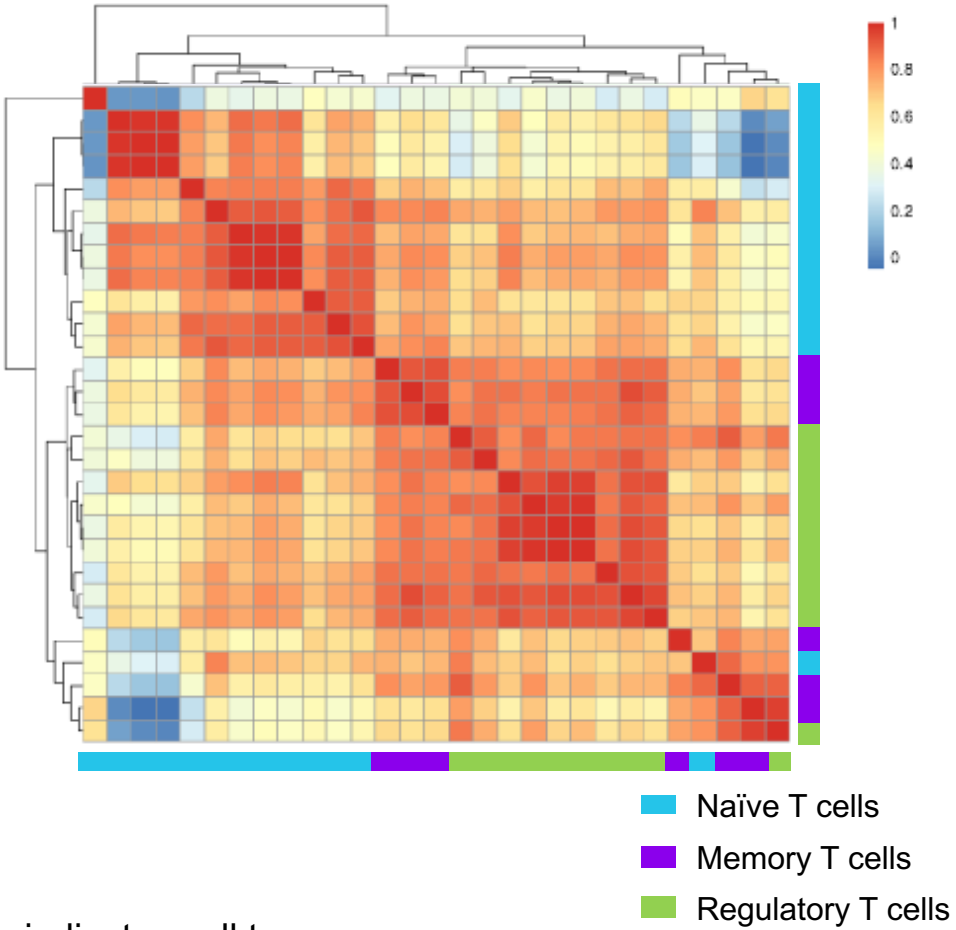
- We have been using chromatin immunoprecipitation-next generation sequencing (ChIP-Seq) for H3K27Ac marks to identify SEs (example of BHLHE40 locus in naïve vs. memory T cells above)

Comparative SE analysis on T cell populations from SLE vs. healthy donors

- Naïve ($CD45RA^+$), memory ($CD45RO^+$) and regulatory ($CD25^+IL7R^-$) $CD4^+$ T cells purified from SLE patients and healthy donors
- Study goals:
  - Establish whether SEs are relevant features to identify T cell subtype biology
  - Analyze SE biology in SLE in each T cell subtype
- Comparison of SE regions using Recomb scores:
  - Generate genome-wide SE maps in T cell populations
  - Calculate total reads over SE regions
  - Calculate SE signal fold change and p value for differentials
  - Further analyze differential SEs using pathway analysis methods

T cells from **healthy donors** display highly distinct SE profiles depending on cell state

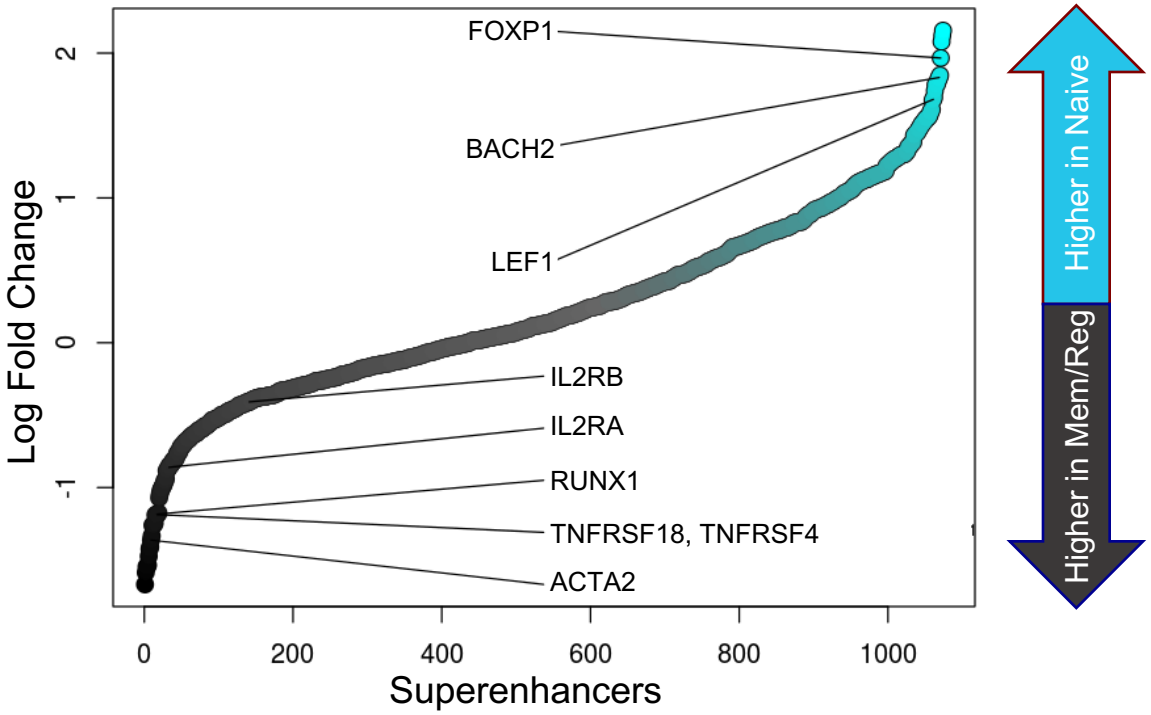




- Samples were clustered based on SE similarity. Bar indicates cell type
- Naïve, antigen-inexperienced T cells show a high degree of similarity
- T cell populations with previous antigen exposure show greater heterogeneity (memory T cells)
- GSEA analysis of cell type-specific SEs indicates enrichment of cell type-specific genes

SEs identify biologically relevant genes in **naïve T cells** from healthy donors

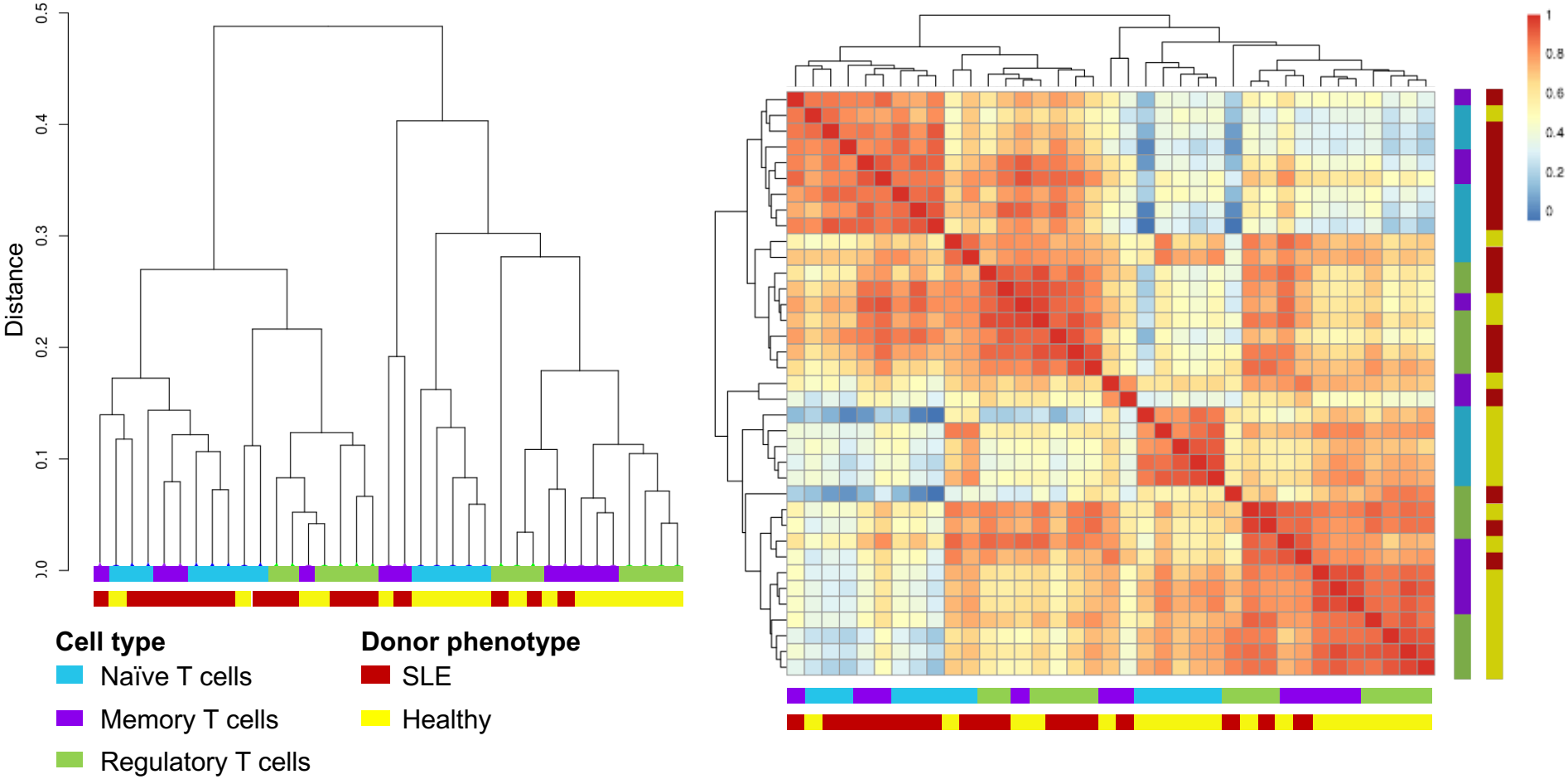
Differential SEs between healthy **naïve T cells vs. memory/regulatory T cells**



- SEs identify genes within pathways specific to naïve T cells
- Similar results were obtained in memory and regulatory T cells

Top 5 enriched GSEA signatures in SEs upregulated in naïve T cells from healthy donors	Significant? (FWER<0.05)
NAIVE_VS_CENT_MEMORY_CD4_TCELL_UP	Y
RESTING_VS_BYSTANDER_ACTIVATED_CD4_TCELL_UP	Y
NAIVE_VS_EFF_MEMORY_CD4_TCELL_UP	Y
NAIVE_VS_CENT_MEMORY_CD4_TCELL_UP	Y
EFF_MEM_VS_CENT_MEM_CD4_TCELL_UP	Y

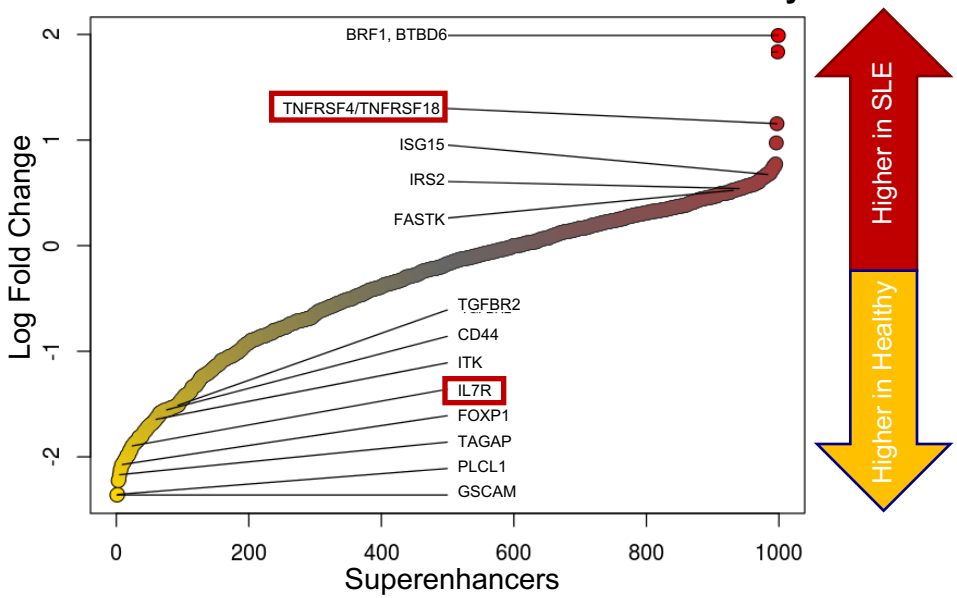
All three T cell subpopulations show different epigenetic profiles between SLE and healthy



- Samples were clustered based on SE similarity. Bars indicate cell type (upper bar) or phenotype (lower bar)
- Samples from SLE and healthy donors generally cluster together regardless of T cell subpopulation
- Within either SLE or healthy, clustering on SE profiles generally groups T cells by subtype
- SLE memory T cells are highly heterogeneous

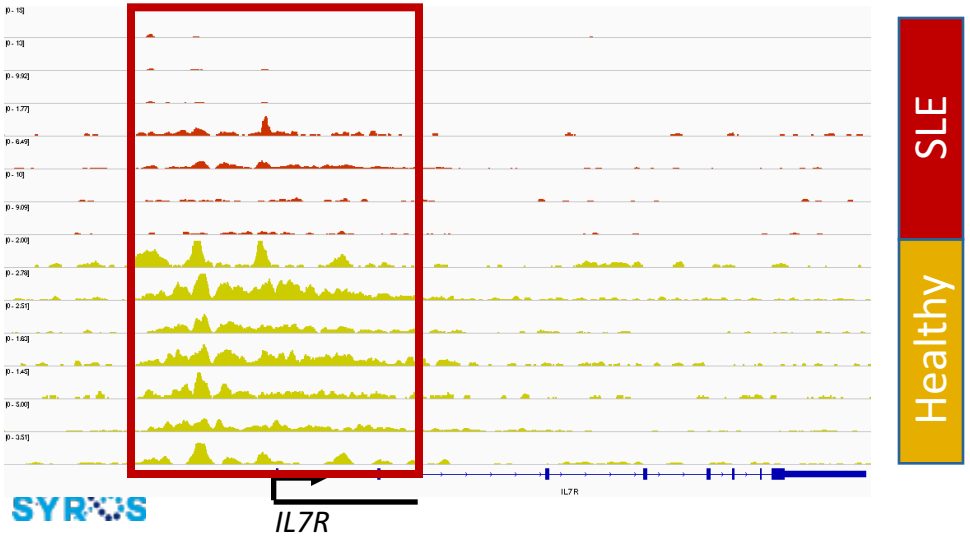
Differential SE-linked genes point to key changes in **naïve T cell** biology in SLE

Differential SEs between **SLE naïve T cells vs. healthy naïve T cells**

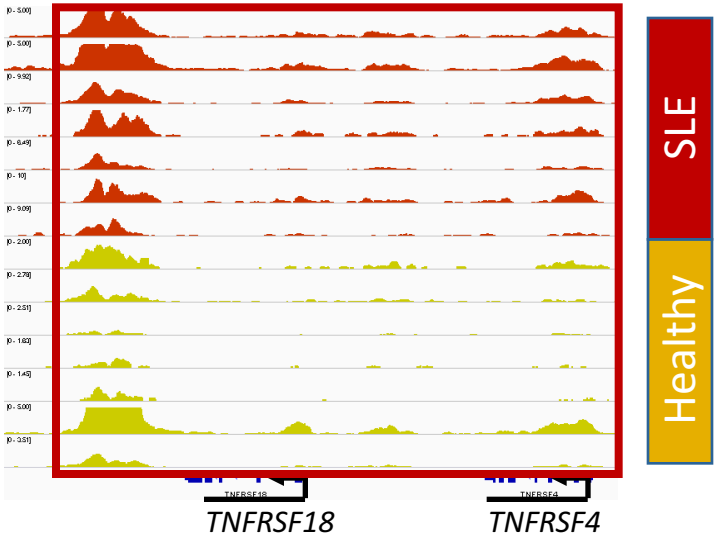


- H3K27Ac tracks from highlighted example genes are shown below
- Naïve T cells show significant changes in SE biology between SLE and healthy
- Results analyzed through Ingenuity Pathway Analysis (IPA) upstream regulator analysis

chr5:35,848 kb-35,880 kb (hg19)



chr1:1,134 kb-1,150 kb (hg19)



Many canonical T cell activation pathways are strongly downregulated in SLE

- IPA generates hypotheses on up- and down-regulated gene expression networks and their potential regulators

Gene networks differentially regulated in **naïve T cells between SLE and healthy**

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
T cell receptor	Inhibited	-2.401	4.86E-15
IL2	Inhibited	-2.475	1.03E-12
IL15	Inhibited	-2.149	3.44E-07
CD40LG	Inhibited	-3.765	1.35E-05
PI3K (family)	Inhibited	-2.468	2.97E-05
EP300	Inhibited	-2.100	3.31E-05
IL7	Inhibited	-3.625	4.81E-05

- This overall inhibition of T cell activation is probably mostly due to SOC taken by these patients that have strong effects on lymphocyte activation
- The obvious questions are: what maintains the activated state of T cells in SLE? What are the pathways missed by the SOC?

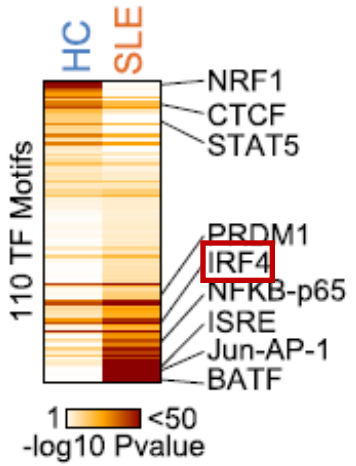
SYK and IRF4 were identified as drivers of the SE landscape in SLE T cells

Gene networks differentially regulated in **naïve T cells between SLE and healthy**

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
SYK	Activated	2.236	4.67E-04
MNT	Activated	2.000	1.76E-02
IRF4	Activated	2.332	2.70E-02
CBL	Activated	2.159	3.11E-02
TAL1	Activated	2.334	4.98E-02

Gene networks differentially regulated in **memory T cells between SLE and healthy**

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
RBM5	Activated	2.598	6.81E-05
PRKAA1	Activated	2.563	6.97E-03
PRKAA2	Activated	2.449	3.96E-02
GFI1	Activated	2.353	3.34E-04
IRF4	Activated	2.213	3.05E-05
Alpha catenin	Activated	2.121	6.16E-03



Scharer et al. *Sci Reports 6:27030.*

- SYK is a key driver of T cell activation in SLE
- IRF4 is a critical driver of both T and B cell activation in SLE
- Naïve T cells (with no previous antigen exposure) already display profound changes in epigenetic landscape

Summary and conclusions

- SE changes between SLE and healthy point to major changes in T cell biology in all subsets of T cells (e.g. IL7R)
- Naïve T cells (without prior antigen exposure) already display profound changes in epigenetic landscape and point to critical transcription factor networks driving disease
- SE analysis in memory T cell population in SLE may provide insight into disease heterogeneity, a major hurdle in SLE drug development
- SE analysis uncovered critical pathways driving disease pathogenesis in SLE that may be unaffected by SOC treatment
- SE analysis may point to key dependencies in cell types driving SLE that can lead to development of novel, more specific therapies in selected subsets of patients with SLE

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