Background
Systemic lupus erythematosus is a relapsing autoimmune disease that affects multiple organ systems. T cells play an important role in the pathogenesis of lupus, however, early T cell events triggering disease flares are incompletely understood. We studied DNA methylation in naïve CD4+ T cells from lupus patients to determine if epigenetic remodelling in CD4+ T cells is an early event in lupus flares.

Materials and methods
A total of 74 lupus patients with disease activity ranging from 0–18 as measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) were included in this study. Naïve CD4+ T cells were isolated from peripheral blood samples and DNA extracted for genome-wide methylation assessment. RNA was also extracted from a subset of patients to determine the relationship between epigenetic changes and transcriptional activity using RNA sequencing and miRNA arrays.

Results
We demonstrate that naïve CD4+ T cells in lupus undergo an epigenetic pro-inflammatory shift implicating effector T cell responses in lupus flare. This epigenetic landscape change occurs without expression changes of corresponding genes, and poises naïve CD4+ T cells for Th2, Th17, and Tfh immune responses, and opposes inhibitory TGF-β signalling. Bioinformatics analyses indicate that the epigenetic modulator EZH2 might be playing an important role in shaping the CD4+ T cell responses, and opposes inhibitory TGF-β signalling. Further, the expression of miR26a and miR101, which are positively correlated with disease activity in lupus patients, negatively correlate with EZH2 expression in CD4+ T cells, indicating that EZH2 dysregulation in naïve CD4+ T cells may trigger lupus flares.

Conclusion
An epigenetic landscape shift in naïve CD4+ T cells that favours T cell activation and non-Th1 immune responses precedes disease flare in lupus patients. This epigenetic landscape change might be playing an important role in shaping the CD4+ T cell responses, and opposes inhibitory TGF-β signalling. Further, the expression of miR26a and miR101, which are positively correlated with disease activity in lupus patients, negatively correlate with EZH2 expression in CD4+ T cells, indicating that EZH2 dysregulation in naïve CD4+ T cells may trigger lupus flares.
locus has powerful common variant associations with SLE risk with odds ratio = 1.5 and p < 10^{-20} in all human ancestries and also has an association, though not necessarily identical to SLE, with rheumatoid arthritis, primary biliary cirrhosis, Behcet’s Disease, Sjögren’s syndrome, progressive systemic sclerosis, and type 1 diabetes.

**Methods** Genome-wide association studies of SLE, Bayesian frequentist and fine-mapping methods, DNA affinity purification assays, and electrophoretic mobility shift assays.

**Results** We are attempting to identify the causal variants and determine the mechanism for SLE disease risk at this locus. Our data suggest that the risk haplotype alters the expression of mRNA from both STAT1 and STAT4. Application of frequentist and Bayesian methods restrict the plausibly causal variants to four possibilities in introns 4 and 5 of STAT4 under the assumption that the association observed across human ancestries is being driven by the same causal variants. Three of these four polymorphisms are predicted to alter the binding of a specific transcription factor, leading to the hypothesis that the same transcription factor is operating at multiple sites in a risk haplotype. We have data suggesting differential and allele preferential binding of the transcription factor at one variant with evaluation of the others in process. This may possibly be the first discovered example of the phenomenon of multiple transcription factor binding on multiple variants of a risk haplotype.

**Conclusion** In general, genome-wide association studies (GWASs) provide powerful evidence of the presence of a genetic variation altering phenotype risk without revealing what the specific responsible variant is among those in a statistical dead heat for causation, in which cell type(s) or stage(s) of differentiation in which the risk difference is relevant, or what the molecular mechanism might be. We have work underway to reveal these details for lupus loci, initially concentrating on IRF5, STAT1-STAT4, and ETS1. The STAT1-STAT4 association with SLE can be isolated to involve only a few variants, which are predicted to have curiously similar transcription factor binding behaviour.

**Background** Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with a strong genetic component. Dozens of SLE-associated loci have been identified by genome-wide associated studies (GWAS) and included in the ImmunoChip for fine-mapping.

**Materials and methods** Using ImmunoChip, we assessed case-control subjects including Chinese, European Americans (EA) and African Americans (AA) for association with SLE. Subsequently, we carried out trans-ancestral mapping and resequenced the complex GTF2IRD1-GTF2I-NCF1 region on 7q11.23 rather than SLE-associated GWAS loci. This association was confirmed in EA (OR = 1.37, P = 2.5 × 10^{-5}) but not in AA. By trans-ancestral mapping and sequencing, we identified R90H of NCF1, a neighbouring gene of GTF2I encoding the p47phox subunit of NADPH oxidase, as a highly plausible causal variant. R90H was associated with SLE in East Asians (OR = 3.47, P_{meta} = 3.0 × 10^{-16}), EA (OR = 2.11, P_{meta} = 7.0 × 10^{-8}) and AA (OR = 1.91, P = 7.2 × 10^{-8}), and in conditional test R90H eliminated SLE-associated signals within the GTF2IRD1-GTF2I-NCF1 region including rs73366469. Furthermore, R90H was dose-dependently associated with early age of onset in Korean (P = 0.011) and EA (P = 0.012) patients with SLE. In addition to SLE, R90H was associated with seropositive rheumatoid arthritis (RA) in Koreans (OR = 1.66, P = 1.2 × 10^{-5}) and primary Sjögren’s syndrome (SS) in EA (OR = 1.72, P = 5.8 × 10^{-5}). The conserved arginine 90 to histidine substitution located in the PX-binding domain of p47phox is predicted deleterious, which is supported by a report showing R90H results in reduced reactive oxygen species (ROS) production.

**Conclusions** We identified R90H of NCF1 as a novel risk variant for multiple autoimmune diseases, highlighting the pathogenic role of reduced ROS production in developing autoimmune diseases.

**GG-04** **A MISSENSE MUTATION IN NEUTROPHIL CYTOSOLIC FACTOR 1 (WCF1) IS ASSOCIATED WITH SUSCEPTIBILITY TO MULTIPLE AUTOIMMUNE DISEASES**

**GG-05** **ATAC-SEQ PROFILING REVEALS CELL-TYPE SPECIFIC EPIGENETIC FEATURES OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)**

**Background** Genetics studies have now identified over 80 SLE risk loci that influence predisposition to SLE with the majority of risk variants altering regulatory elements that govern gene expression. Precise understanding of how risk variants in regulatory elements influence gene expression in different cell types and cell states is critical for defining the molecular networks leading to autoimmunity. To begin to address this issue, we profiled the chromatin accessibility landscape of three distinct, albeit heterogeneous, compartments of the immune system across three clinical states.

**Materials and methods** Primary B and T lymphocytes and monocytes from 5 SLE subjects with high disease activity (SLEDAI ≥ 3) and 4 SLE subjects with low disease activity (SLEDAI ≤ 2) and 5 healthy controls were collected and processed for high-throughput open chromatin profiling by ATAC-seq. Reads were aligned to the hg19 genome and regions of enriched