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INTRODUCTION

- Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease.
- Genome-wide association studies (GWAS) have fine mapped genetic polymorphisms in *UBE2L3* gene to confer disease risk in SLE and other autoimmune diseases (Fig 2,3).
- UBE2L3* encodes UbCh7 protein, an E2 conjugating enzyme, involved in the ubiquitin pathway (Fig 1).
- GWAS have been highly successful in identifying susceptibility genes associated with SLE but have failed to precisely identify the causal variants responsible for these statistically significant associations.

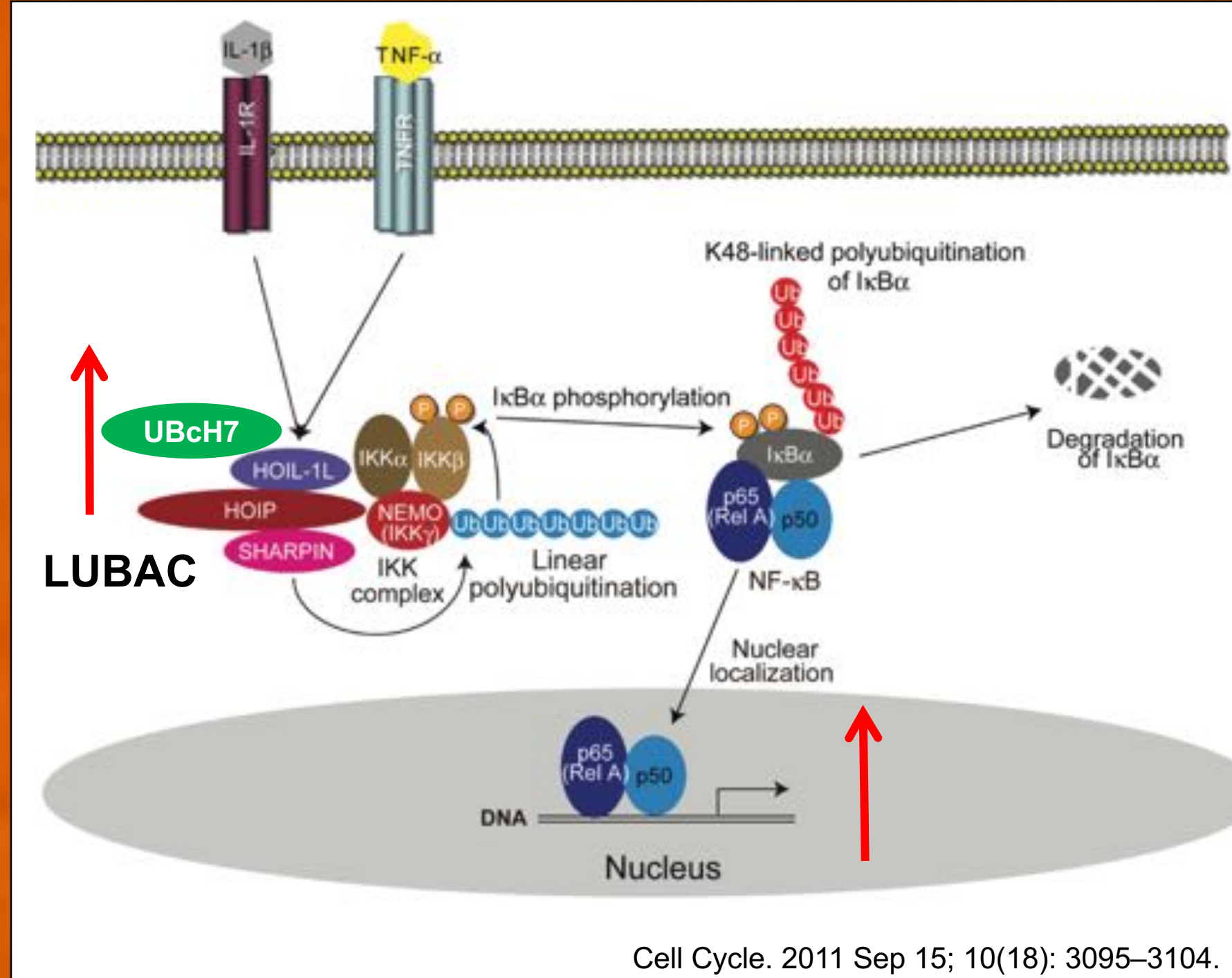


Figure 1. UbCh7, together with HOIL-1, HOIP and Sharpin form the components of Linear Ubiquitin Chain Assembly Complex (LUBAC). LUBAC can activate NF-κB signaling pathways. Cell Cycle. 2011 Sep 15; 10(18): 3095–3104.

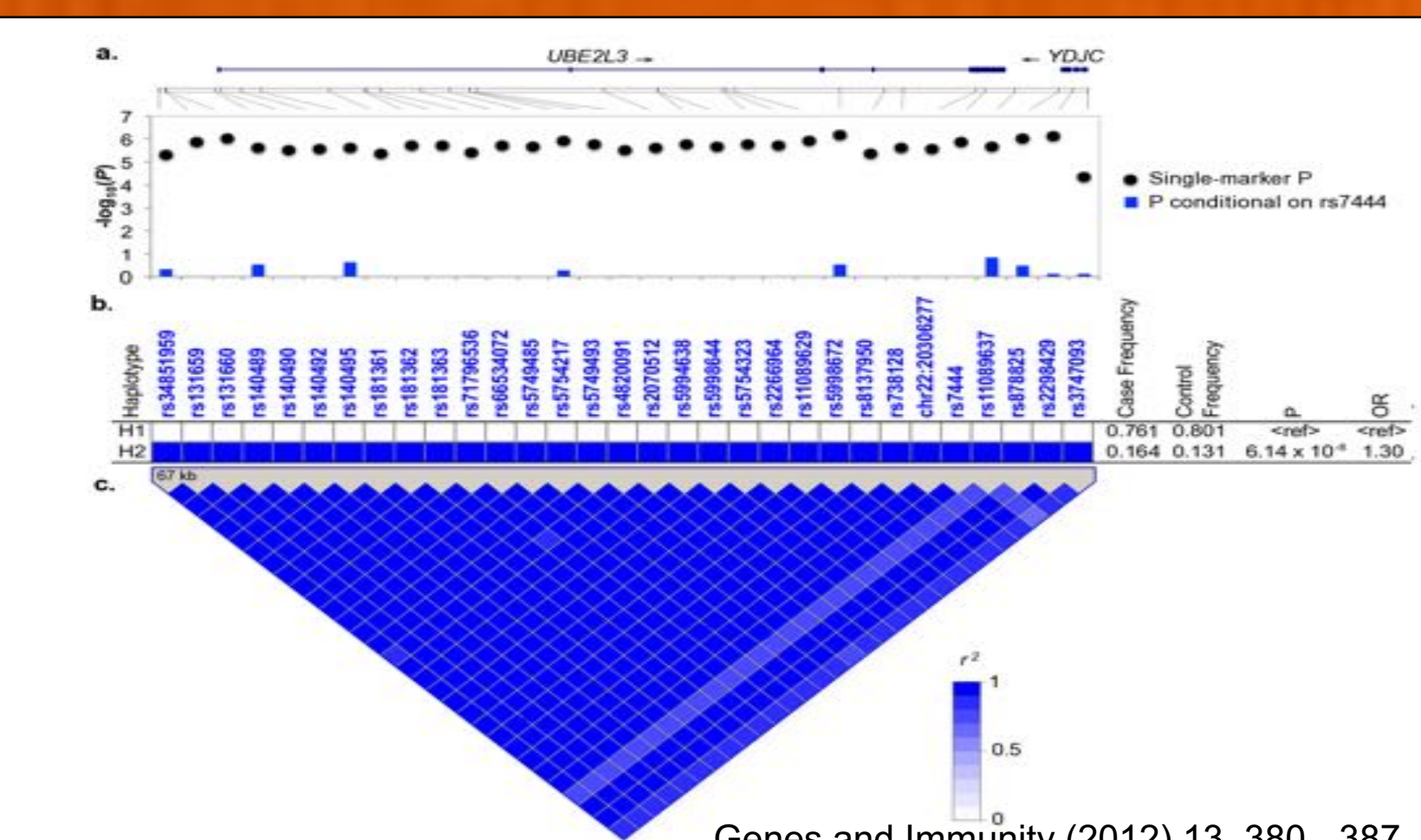


Figure 2. A single 67kb risk haplotype spanning *UBE2L3* in SLE subjects of European ancestry. A. Single marker P values for association. B. Haplotype analysis. C. LD plot demonstrates high LD across the locus. Genes and Immunity (2012) 13, 380 - 387

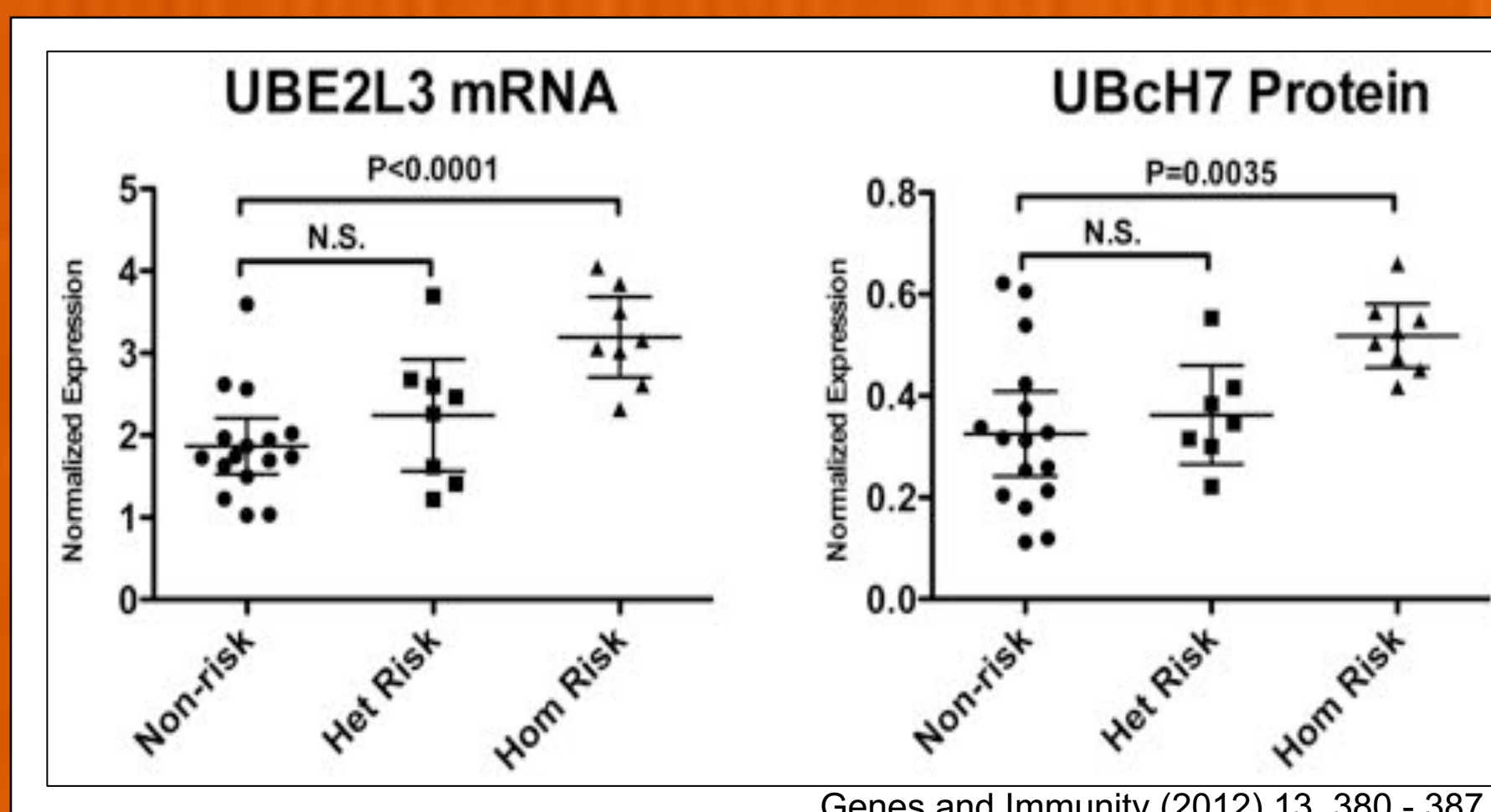


Figure 3. Expression of *UBE2L3* mRNA and UbCh7 protein in EBV transformed B cells carrying 0, 1, or 2 copies of the *UBE2L3* risk haplotype. P values derived from a 2-sided t-test. Error bars show mean and 95% CI. Genes and Immunity (2012) 13, 380 - 387

OBJECTIVE

To identify the potential candidates for SLE associated causal variants among the single nucleotide polymorphisms (SNPs) spanning *UBE2L3* risk haplotype.

RESULTS

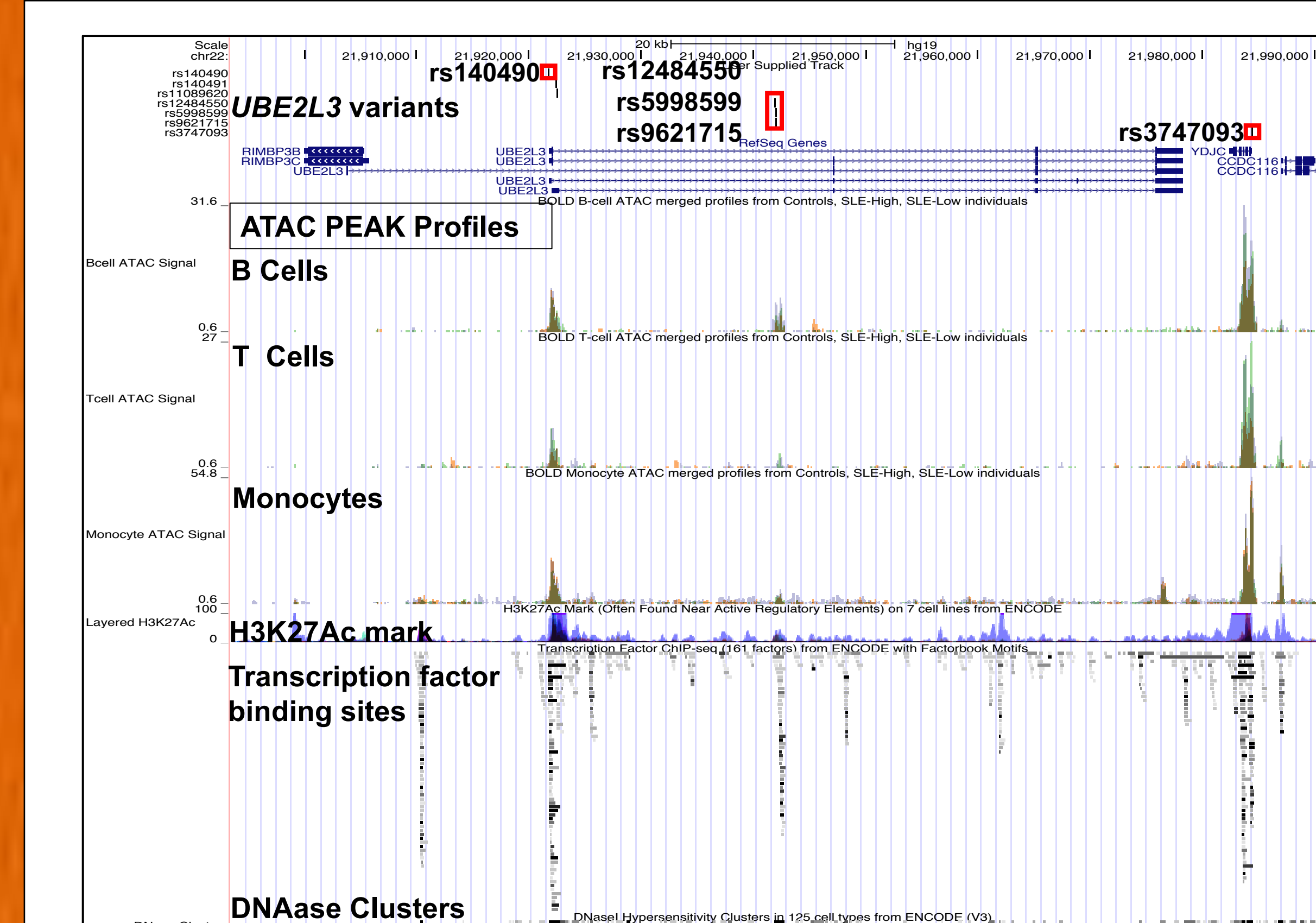


Figure 4. *UBE2L3* risk haplotype SNPs that overlap with the ATAC peak profile from T, B lymphocytes and monocytes of SLE patients were selected as causal variant candidates for the study. Image - UCSC genome browser.

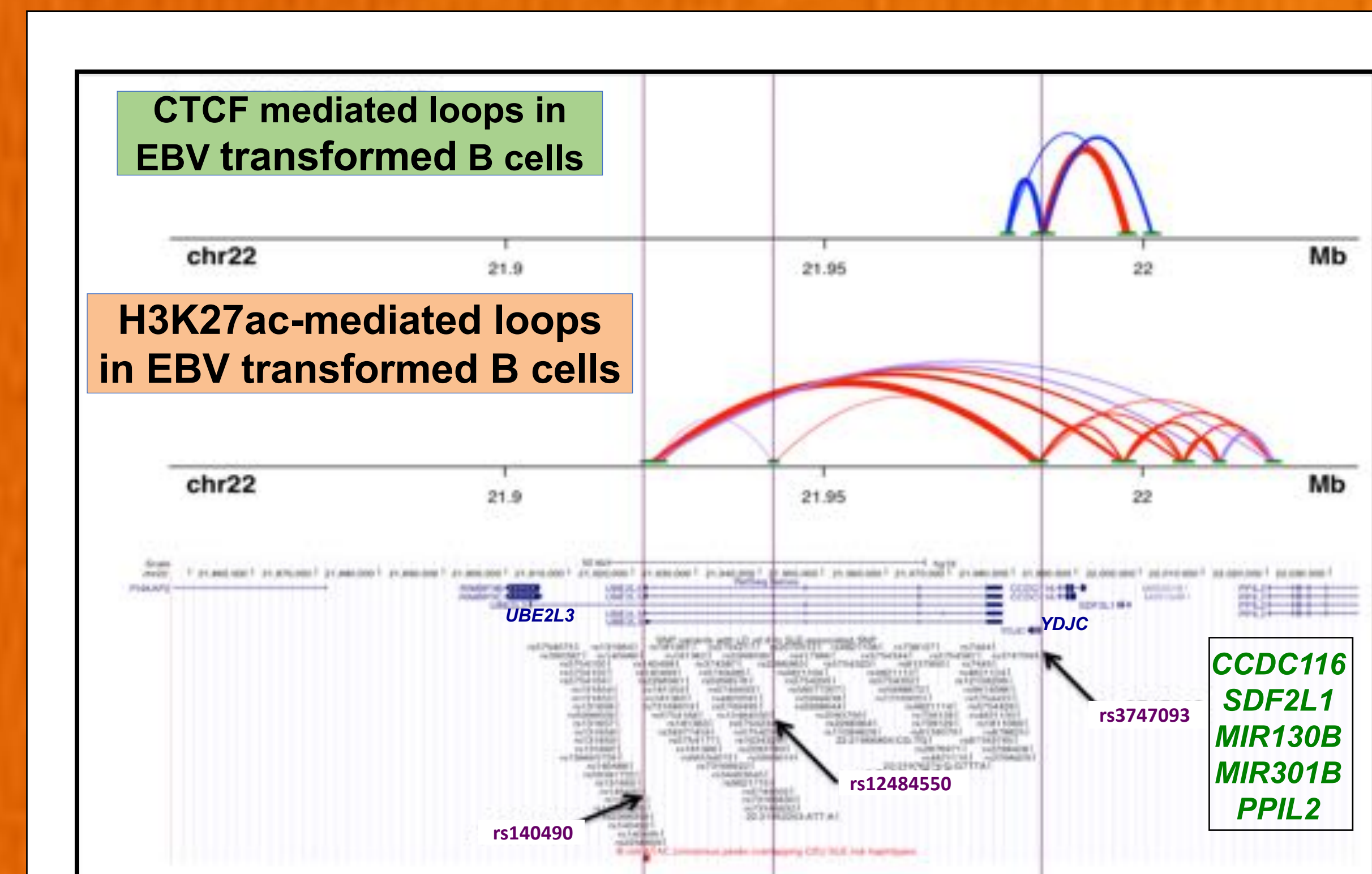


Figure 7. HiChIP assays were performed to map the DNA looping patterns in EBV-transformed B cell lines. 3D DNA contacts mediated by an CTCF and histone 3 lysine 27 acetylation (H3K27ac) were measured. Three dimensional chromatin interactions with *UBE2L3* promoter were observed. Image – DNA Landscaper

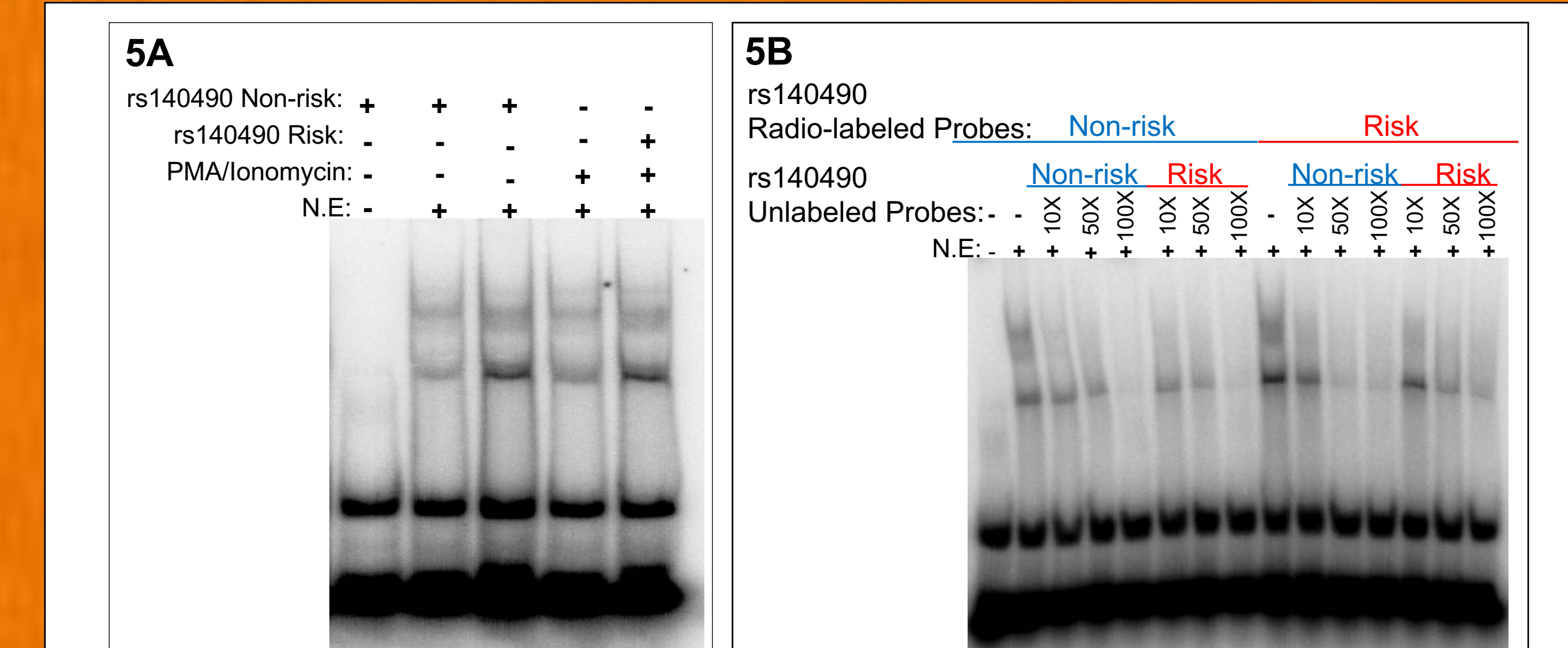


Figure 5. Radioactive Electrophoretic mobility shift assays (EMSA) were performed on non-risk and risk alleles of individual variants. A. Representative EMSA blot for rs140490 with nuclear extracts from THP1 cells with/without PMA/Ionomycin stimulation for 2hrs. B. Competition Assay for non-risk/risk rs140490 probes with nuclear extracts from EBV-B cells. N.E = Nuclear extracts

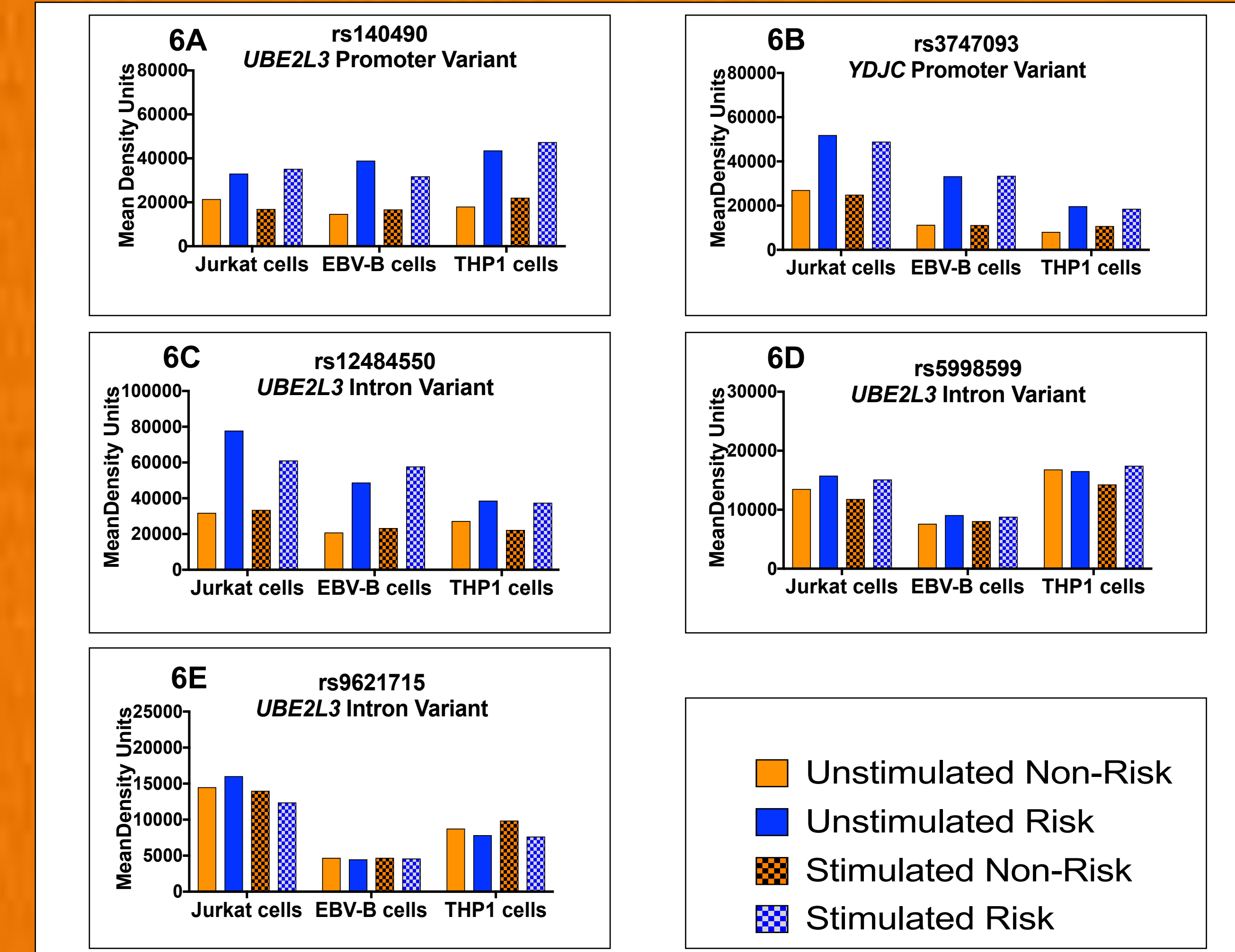


Figure 6. Qualitative densitometry of Radioactive EMSAs performed on selected variants with nuclear extracts from T (Jurkat), B (EBV-B) cells and Monocytes (THP1) with or without PMA/Ionomycin stimulation for 2hrs. A-C. Risk alleles showed increased binding to nuclear protein complexes. D-E. Risk and non-risk alleles did not show a clear difference in binding affinity to nuclear protein complexes.

CONCLUSIONS AND FUTURE DIRECTIONS

CONCLUSIONS:

- Identified **three high quality causal variant candidates**, that have increased binding of nuclear proteins to the risk allele, both in a cell type and stimulation independent manner.
- Enhanced binding of nuclear protein complexes to risk alleles may strengthen long-range DNA looping events, with *UBE2L3* promoter to enhance *UBE2L3* expression associated with SLE risk.
- Identifying and functionally validating the causal variants spanning *UBE2L3* risk haplotype can be helpful in exploring novel therapeutic targets for treatment of SLE.

FUTURE DIRECTIONS:

- Validate the ability of risk and non-risk alleles of a variant to modulate gene expression – Luciferase assay
- Identify transcription factors that have allele specific binding affinities – Chromatin Immunoprecipitation (ChIP) qPCR
- Understand allele specific effects on three dimensional DNA looping patterns – Chromosome Conformation Capture (3C) assay
- Engineer cell line model, to explore the impact of risk alleles individually or in combinations within an isogenic background and outside the haplotype context.