Supplementary Information for:

High-throughput identification of functional regulatory SNPs in systemic lupus erythematosus

Qiang Wang[1]*, Taehyeung Kim[1]*, Marta Martínez-Bonet[2,3], Vitor R. C. Aguiar[1,4], Sangwan Sim[1], Jing Cui[2], Jeffrey A. Sparks[2], Xiaoting Chen[4], Marc Todd[1], Brian Wauford[1], Miranda C. Marion[5], Carl D. Langefeld[5], Matthew T. Weirauch[6,7,8], Maria Gutierrez-Arcelus[1,4], Peter A. Nigrovic[1,2]

1. Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston MA, USA

2. Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Harvard Medical School, Boston MA, USA

3. Laboratory of Immune-regulation, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid Spain

4. Broad Institute of MIT and Harvard, Cambridge, MA, USA

5. Department of Biostatistics and Data Science, and Center for Precision Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA

6. Center of Autoimmune Genomics and Etiology, Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

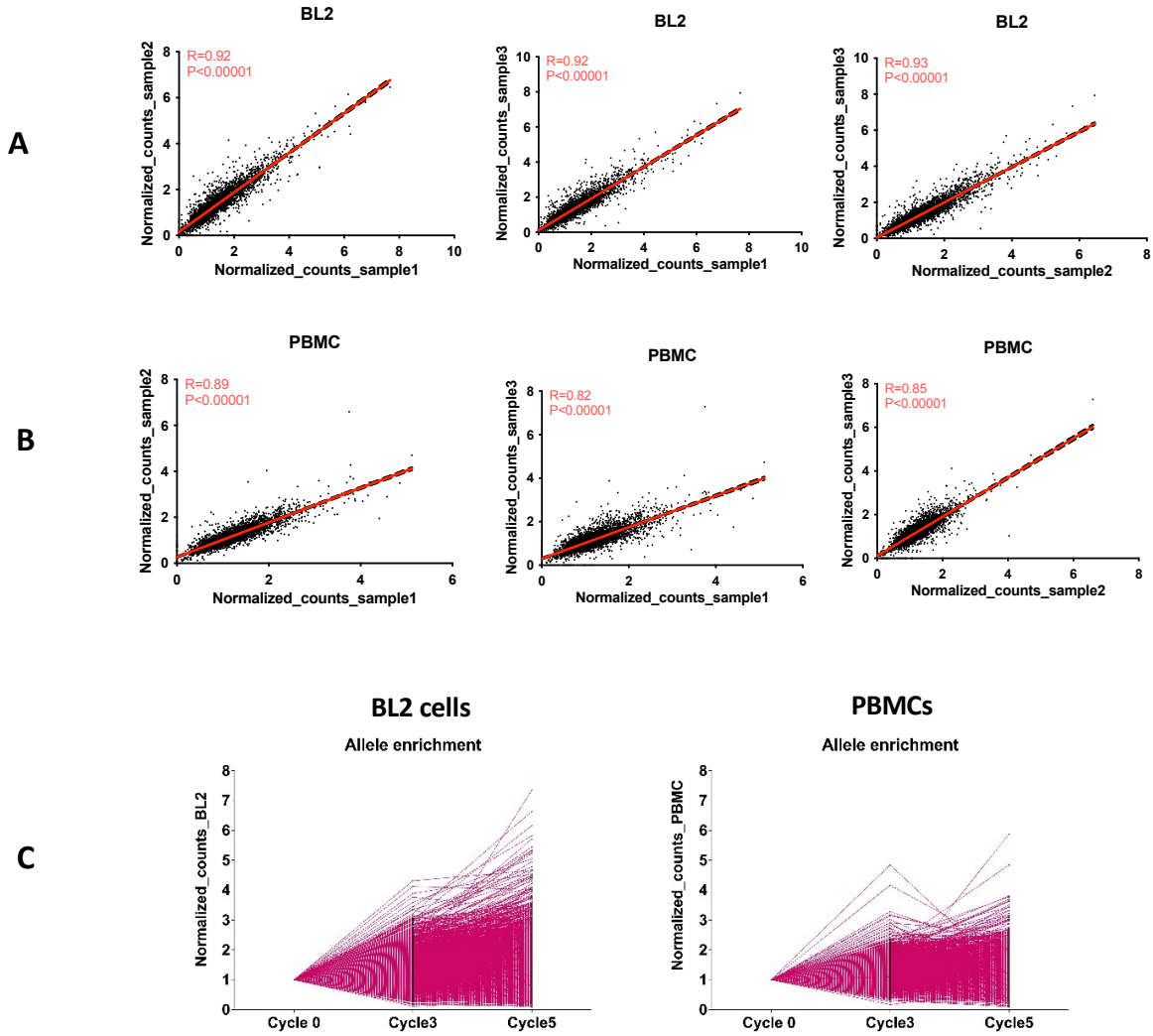
7. Divisions of Human Genetics, Biomedical Informatics, and Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

8. Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

* These authors contributed equally.

Correspondence:
Peter A. Nigrovic, MD
Division of Immunology
Boston Children's Hospital
Karp Family Research Building 10211
One Blackfan Circle
Boston, MA 02115
peter.nigrovic@childrens.harvard.edu

S. Figure 1



S Figure 1. Allele-specific enrichment analysis by SNP-seq. We employed SNP-seq to identify potential regulatory variants from 2180 SNPs utilizing 4363 dsDNA constructs and nuclear extracts from BL2 B cells and PBMCs. **(A, B)** The NGS-generated allele counts normalized to control displayed strong Spearman's correlations among pairs of 3 biological replicates from either BL2 cells or PBMCs. **(C)** The normalized allele1 and allele2 counts ratio for 496 SNPs demonstrated allele-specific enrichment by the protection of nuclear proteins binding across 0-5 cycles. Source data are provided as a Source Data file.

S. Figure 2. Analytical approaches to identify SNPs conferring allele-specific protection. **(A)** Selection of SNPs demonstrating difference in protection between alleles in cycle 5. **(B)** Selection of SNPs demonstrating an accumulated difference in protection between alleles when comparing cycle 3 and 5.

A

cycle 5 of different treatment (Horizontal)
Raw data (read/percentage)

Cycle 5							
	Mean Percentage in Ctrl	Percentage in BL2			Percentage in PBMC		
		Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Allele 1	C1	B1-1	B1-2	B1-3	P1-1	P1-2	P1-3
Allele 2	C2	B2-1	B2-2	B2-3	P2-1	P2-2	P2-3

Sample normalized to control

Cycle 5						
	Normalized Percentage in BL2			Normailized Percentage in PBMC		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Allele 1	B1-1/C1	B1-2/C1	B1-3/C1	P1-1/C1	P1-2/C1	P1-3/C1
Allele 2	B2-1/C2	B2-2/C2	B2-3/C2	P2-1/C2	P2-2/C2	P2-3/C2

Mean Normalized Percentage (MNP)

Cycle 5		
	Mean Normalized Percentage in BL2	Mean Normailized Percentage in PBMC
Allele 1	B1-1+B1-2+B1-3/C1/3	P1-1+P1-2+P1-3/C1/3
Allele 2	B2-1+B2-2+B2-3/C2/3	P2-1+P2-2+P2-3/C2/3

Remove the alleles have a MNP less than 1 in either BL2 or PBMC

Total Alleles	Remaining Alleles
4363	2053

The remaining alleles are divided into two groups:
Group 1: both alleles from one SNPs have a MNP>1;
Group 2: only one allele from one SNPs has a MNP>1.

Both alleles > 1	One allele > 1
1603	450

Group 1

Cycle 5		
	BL2	PBMC
MNP ratio	Allele 1/Allele 2	Allele 1/Allele 2

Group 2
(remove alleles have MNP < 1.2)

Cycle 5	
Allele >1	A1 or A2
450	194 (SNPs)

Remove 0.8<A1/A2 ratio <1.2 in either BL2 and PBMC group

Cycle 5	
Both Alleles >1	A1/A2>1.2 or A1/A2<0.8
1603	258 (SNPs)

S. Figure 2

(continued)

B

cycle 3 and 5 of different treatment (Vertical)

Raw data (read/percentage)

	Mean Percentage in Ctrl	BL2					
		Cycle 3			Cycle 5		
		Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Allele 1	C1	B1.3-1	B1.3-2	B1.3-3	B1.5-1	P1.5-2	P1.5-3
Allele 2	C2	B2.3-1	B2.3-2	B2.3-3	P2.5-1	P2.5-2	P2.5-3

Sample normalized to control, remove the alleles have MNP<1

Total Alleles	Remaining Alleles
4363	1903

The remaining alleles are divided into two groups:
Group 1: both alleles from one SNPs have a MNP>1;
Group 2: only one allele from one SNPs has a MNP>1.

Both alleles > 1	One allele > 1
1474	429

Group 1

BL2		
	Cycle 3	Cycle 5
MNP ratio	Allele 1/Allele 2	Allele 1/Allele 2

Keep the alleles has an increasing or decreasing ratio of A1/A2 from cycle 3 to cycle5

Both alleles > 1	Increasing or Decreasing MNP ratio
1474	297 (SNPs)

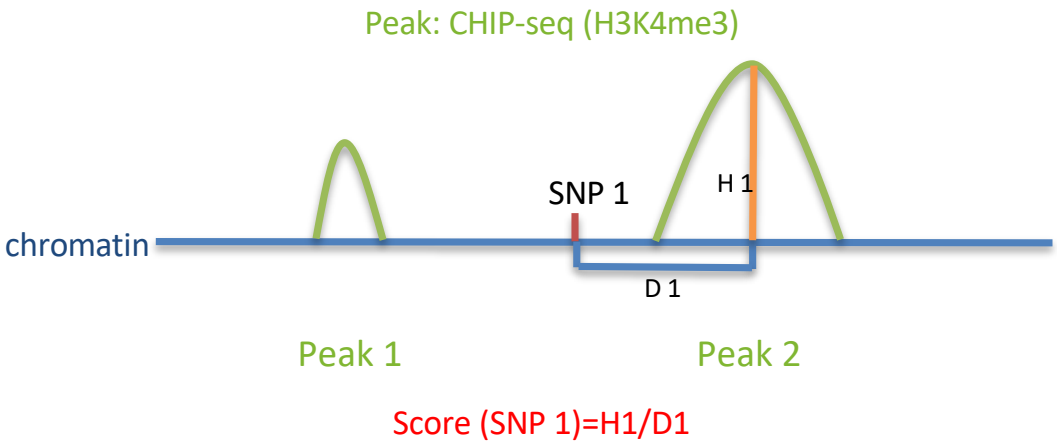
Group 2
(Keep the alleles has increasing MNP)

BL2	
Allele >1	A1 or A2
450	199 (SNPs)

Summary

	Allele Specific Protection	
	Group 1	Group 2
Horizontal	258	194
Vertical	297	199
Overlapping	152	96

S. Figure 3



S. Figure 3. Calculation of H3K4me3 chromatin scores from 5 ChIP-seq studies in human B cells. SNP locus were scored based on the height of the closest H3K4me3 peak divided by the distance from the SNP to that peak, using 5 studies from the Encyclopedia of DNA Elements (ENCODE) database:

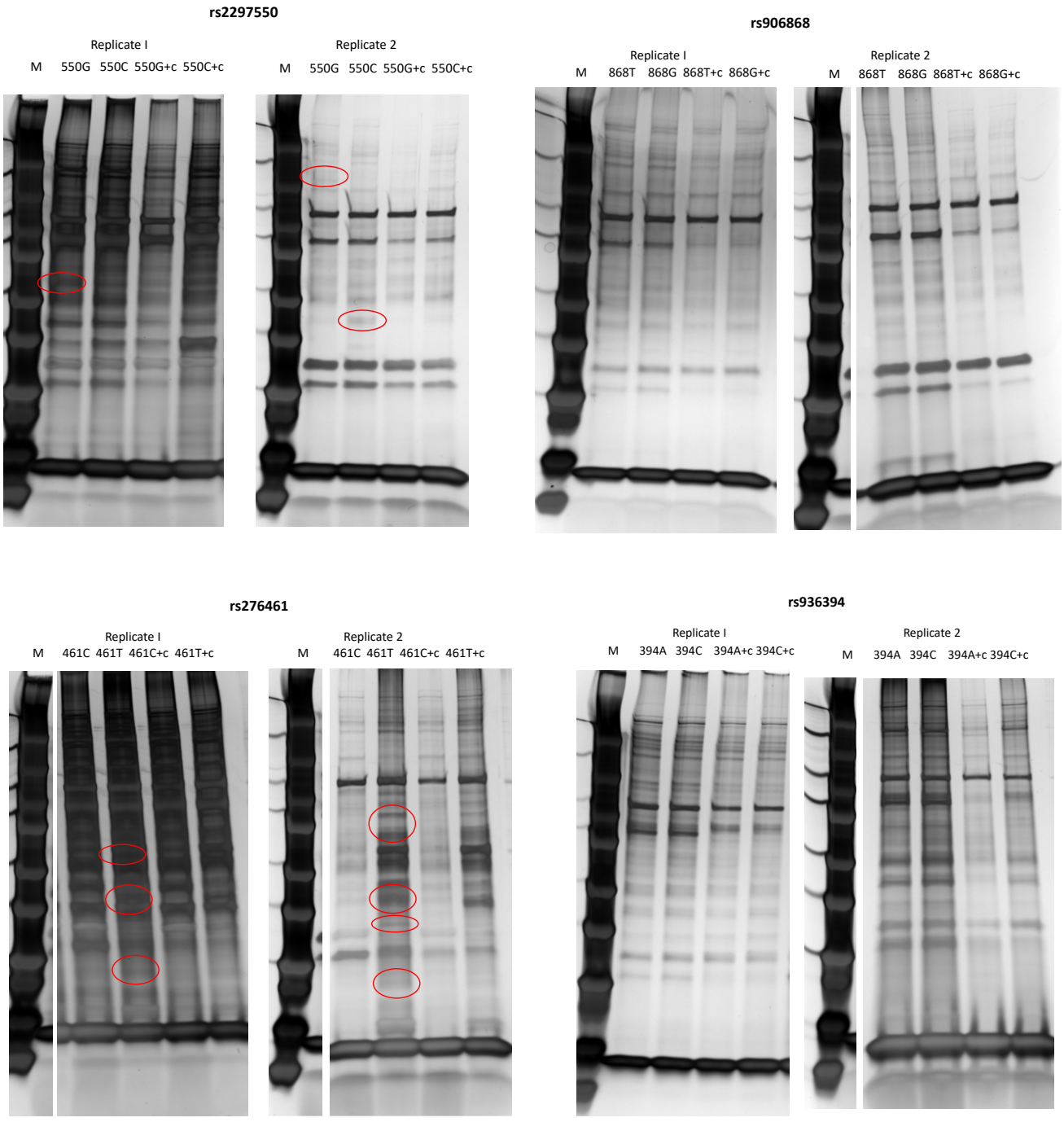
experiment 1 https://www.encodeproject.org/experiments/ENCSR878JSF/
experiment 2 https://www.encodeproject.org/experiments/ENCSR939UQD/
experiment 3 https://www.encodeproject.org/experiments/ENCSR269OVV/
experiment 4 https://www.encodeproject.org/experiments/ENCSR000DQR/
experiment 5 https://www.encodeproject.org/experiments/ENCSR000DQP/

S. Figure 4. Summary table of EMSA validation experiments for 52 candidate SNPs. 52 SNPs were initially assessed through EMSA using BL2 nuclear extract. Among them, 21 SNPs showed consistent allelic differential binding across both technical and biological replicates. 9 SNPs were further confirmed using nuclear extract from PBMCs**.** Lead SNPs identified as SLE GWAS hits were marked in red. NE; nuclear extract. Please see Suppl. Fig. 4 for plots.

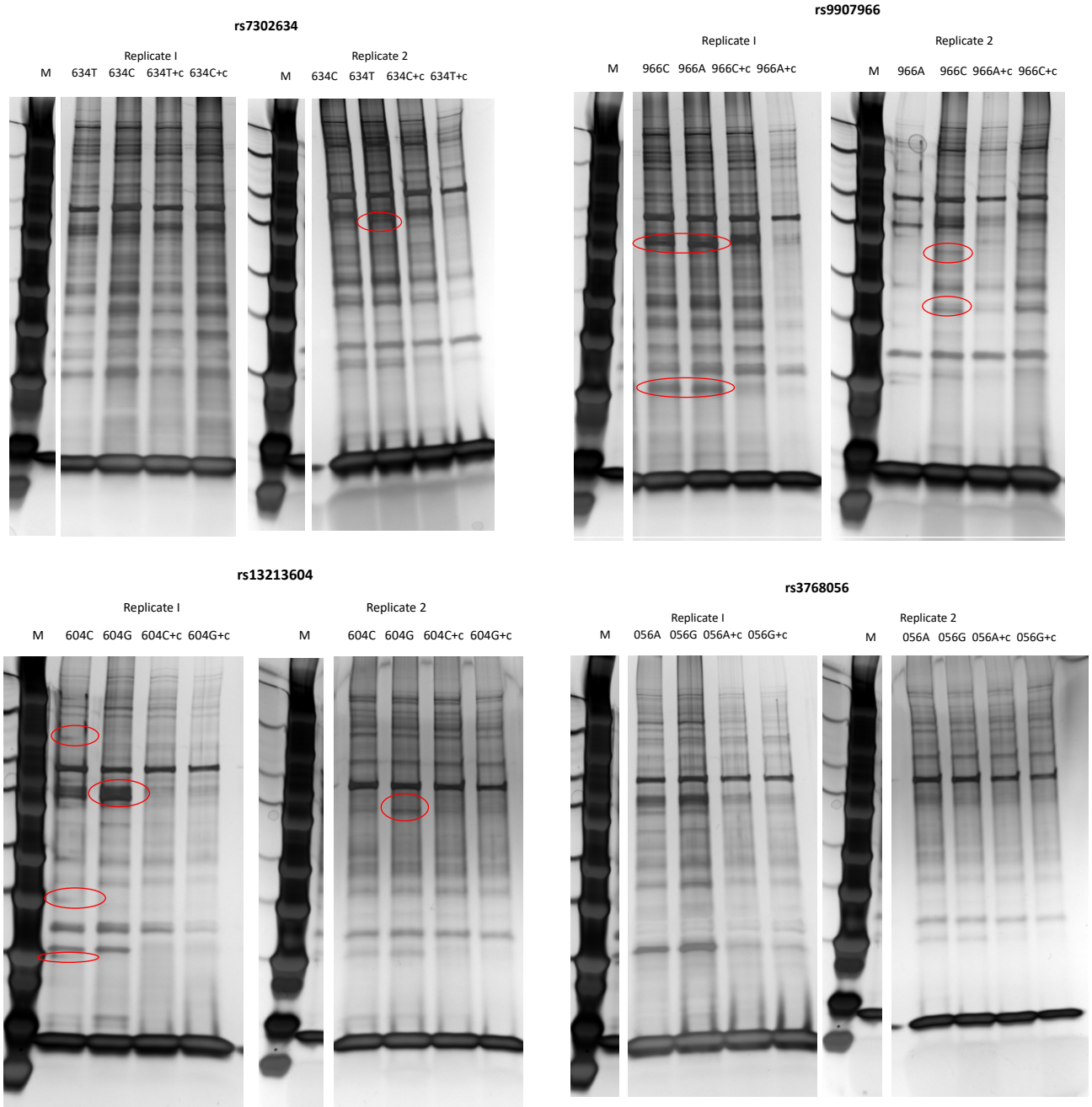
	Allele specific binding			Allele specific binding	Allele specific binding
SNPs	BL2 NE from Sept.		BL2 NE from Oct.	(summary for BL2)	PBMC NE from Sept.
	Exp. 1	Exp.2	Exp.3		
rs2297550	√	√	√	√	√
rs56741490	√	√	√	√	
rs61823882	√	√	×	√	
rs4952115	×	×	×	×	
rs906868	√	√	√	√	√
rs1132200	√	√	√	√	
rs157042	×	×	×	×	
rs7769961	×	√	√	√	
rs13205210	×	×	×	×	
rs6934662	×	×	×	×	
rs13215181	×	×	×	×	
rs10761604	×	×	×	×	
rs28364617	×	×	×	×	
rs11227302	×	×	×	×	
rs11606631	×	×	×	×	
rs1728769	√	√	×	×	
rs1728770	×	×	×	×	
rs58363746	√	√	√	√	
rs936394	√	√	√	√	√
rs113370572	√	×	×	×	
rs180769054	√	√	×	×	
rs118126443	×	×	×	×	
rs11089620	×	×	×	×	
rs2298428	×	×	×	×	
Red: Lead SNPs					

	Allele specific binding			Allele specific binding	Allele specific binding
SNPs	BL2 NE from Oct.		BL2 NE from Sept.	(summary for BL2)	PBMC NE from Sept.
	Exp. 1	Exp.2	Exp.3		
rs17321999	×	√	×	×	
rs1040029	√	√	√	√	
rs528765	√	√	√	√	
rs13360613	√	√	√	√	
rs276461	√	√	√	√	√
rs13213604	√	√	√	√	√
rs1645935	×	×	×	×	
rs6501784	√	√	×	×	
rs9894009	×	×	×	×	
rs4660115	√	√	√	√	
rs3768056	√	√	√	√	√
rs574808	×	×	×	×	
rs6937876	√	√	√	√	
rs2814955	√	√	√	√	√
rs7745097	×	×	×	×	
rs205286	×	×	×	×	
rs9469877	×	×	×	×	
rs7302634	√	√	√	√	√
rs56928975	×	×	×	×	
rs9913957	√	√	√	√	
rs73304123	√	×	×	×	
rs9907966	√	√	√	√	√
rs9907564	√	√	√	√	
rs112561568	×	×	×	×	
rs200906779	×	×	×	×	
rs61134033	√	×	×	×	
rs131664	×	×	×	×	
rs5754467	×	×	×	×	
Red: Lead SNPs					

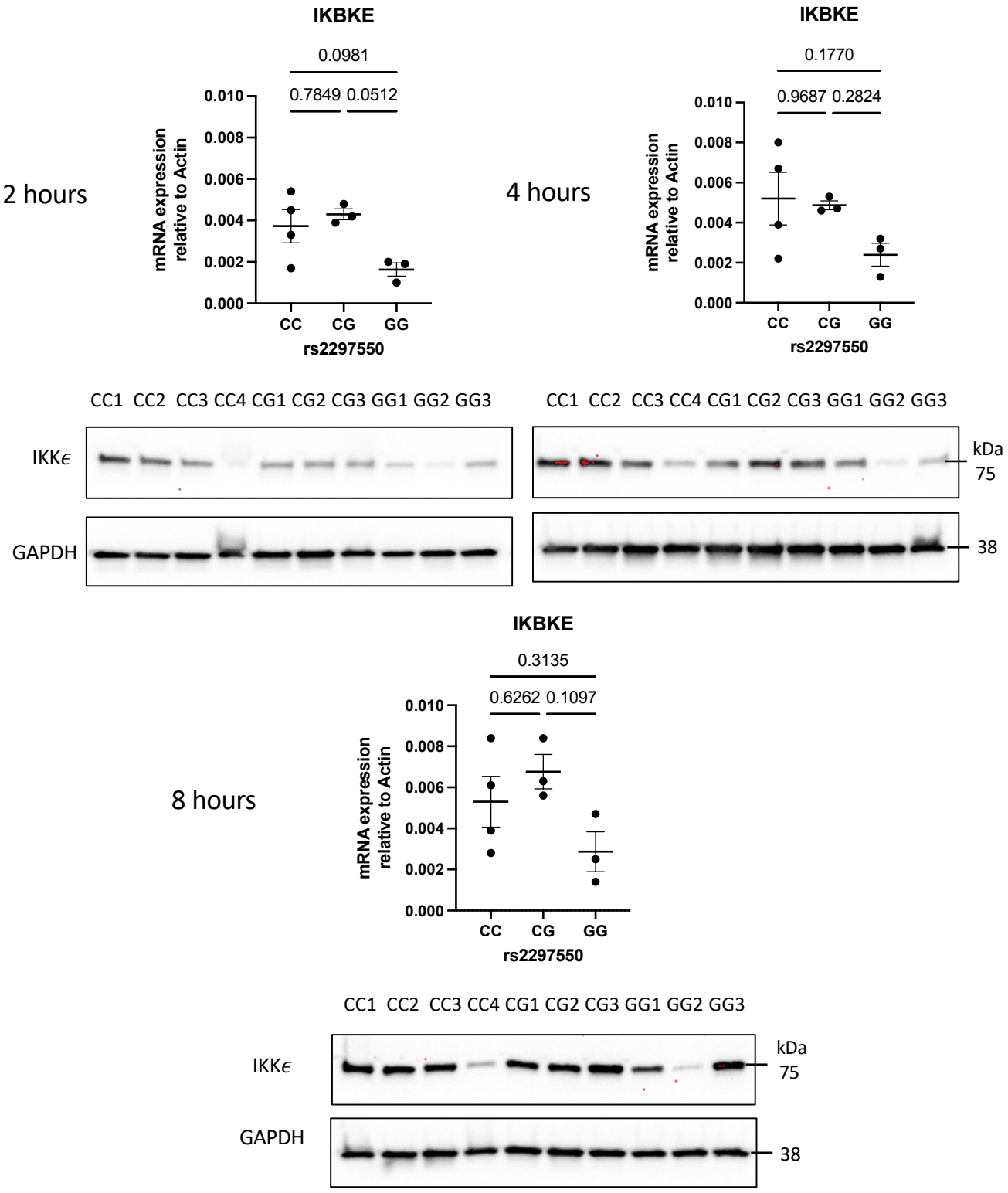
S. Figure 5. Pulldown and silver staining of 8 EMSA-validated SNPs. We performed pulldown assays using 31bp oligonucleotides for each allele of the 8 SNPs using nuclear extract from Daudi cells, with or without an excess of non-biotinylated competitor. Silver staining was employed to visualize the allele-specific binding of eluted proteins that were separated by gel electrophoresis. In two biological replicates, four variants showing differential binding were indicated with red circles.



S. Figure 5 (continued)

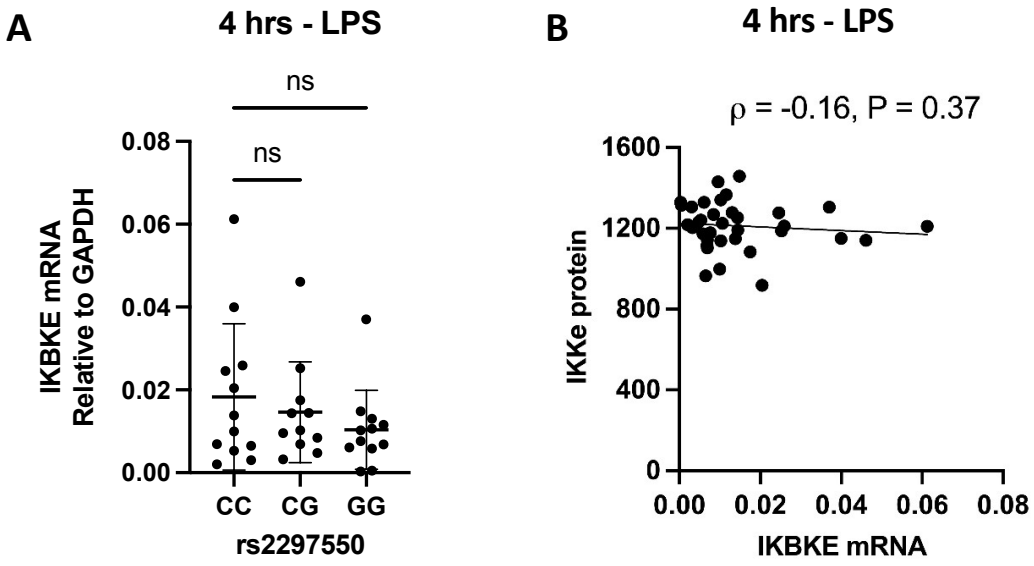


S. Figure 6



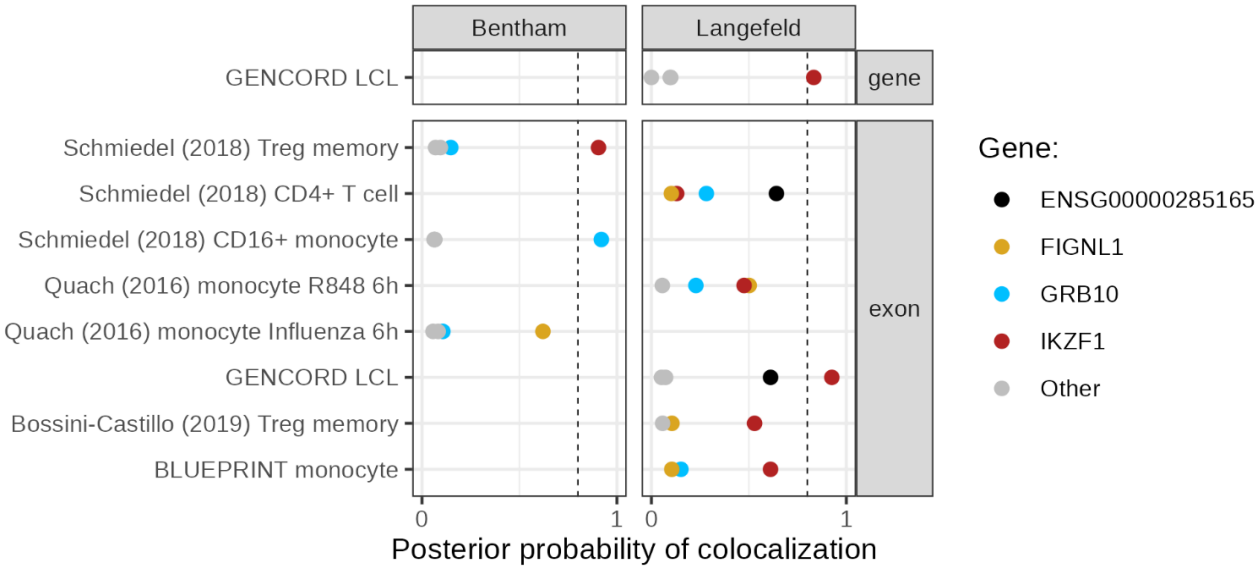
S. Figure 6. Relationship between genotype and IKKε expression in LPS-stimulated Daudi cells. Base-edited Daudi cells from Figure 4 were stimulated with LPS 5 μg/mL for the intervals shown to determine RNA for *IKBKE* and Western blot for IKKε protein. This experiment was performed once. Source data are provided as a Source Data file.

S. Figure 7



S. Figure 7. Genotype at rs2297550 does not correlates with *IKBKE* mRNA expression in PBMCs stimulated with LPS for 4 hours. (A) RNA level of *IKBKE* was determined by RT-qPCR for 35 healthy subjects with C/C (n=12), C/G (n=11), G/G (n=12) genotype at SNP rs2297550. **(B)** Correlation between IKKe protein level (mean fluorescence intensity) and *IKBKE* RNA level (normalized to GAPDH) was determined by Spearman's rank correlation with a two-tailed test. Source data are provided as a Source Data file.

S. Figure 8



S. Figure 8. Colocalization between SLE GWAS data and QTLs from the eQTL Catalogue at the *IKZF1* locus. We selected eQTL data on immune cell types from eQTL Catalogue release 6[1], including molecular traits (genes or exons) within 500kb from the risk variant and with at least one QTL variant passing 5% FDR. We obtained GWAS summary statistics from Bentham et al[2] and Langefeld et al[3]. We tested for colocalization with coloc v5.2.3[4]. The x-axis shows posterior probability of hypothesis 4 (PP4) from coloc. These colocalizations would not be observed if more stringent significance thresholds were used, for example, $p < 1x10^{-5}$ for eQTLs and gene-level FDR for exon-QTLs to account for the high number of molecular traits tested per gene, therefore they should be considered only suggestive evidence for colocalization.

S. Table 1

CADD score: PHRED-scaled transformation of the model score, scale range 0 (potentially benign) to 48 (potentially pathogenic).
H3K4me3 score: The height of the closest H3K4me3 peak divided by the distance from the SNP to that peak (S. Figure 3).
Selection Criteria: Prioritization of candidate SNPs by SNP-seq and bioinformatics enrichment (Figure 2).
N/A, Not Applicable

52 plausible functional SNPs	CADD score	H3K4me3 score	Selection Criteria
rs9913957	1.33	N/A	SNP-seq Top20
rs6937876	2.99	N/A	SNP-seq Top20
rs3768056	N/A	N/A	SNP-seq Top20
rs9469877	2.97	N/A	SNP-seq Top20
rs112561568	12.67	N/A	SNP-seq Top20
rs7745097	2.82	N/A	SNP-seq Top20
rs13213604	6.43	N/A	SNP-seq Top20
rs61134033	3.40	N/A	SNP-seq Top20
rs574808	3.12	N/A	SNP-seq Top20
rs528765	6.37	N/A	SNP-seq Top20
rs2814955	0.71	N/A	SNP-seq Top20
rs205286	4.31	N/A	SNP-seq Top20
rs6501784	1.21	N/A	SNP-seq Top20
rs131664	0.52	N/A	SNP-seq Top20
rs276461	2.71	N/A	SNP-seq Top20
rs7302634	0.40	N/A	SNP-seq Top20
rs73304123	2.18	N/A	SNP-seq Top20
rs4660115	0.45	N/A	SNP-seq Top20
rs56928975	0.08	N/A	SNP-seq Top20
rs17321999	6.77	N/A	SNP-seq Top20
rs2298428	24.80	N/A	SNP-seq & CADD Top8
rs61823882	19.79	N/A	SNP-seq & CADD Top8
rs13360613	17.37	N/A	SNP-seq & CADD
rs1040029	16.88	N/A	SNP-seq & CADD
rs200906779	0.02	0.58	SNP-seq & H3K4me3
rs5754467	3.95	0.56	SNP-seq & H3K4me3
rs9907564	4.32	2.01	SNP-seq & H3K4me3
rs9894009	2.86	0.63	SNP-seq & H3K4me3
rs9907966	2.94	1.27	SNP-seq & H3K4me3
rs1645935	2.79	0.98	SNP-seq & H3K4me3
rs13205210	25.40	N/A	CADD Top8
rs1132200	23.10	N/A	CADD Top8
rs4952115	22.30	N/A	CADD Top8
rs157042	20.70	N/A	CADD Top8
rs10761604	20.30	N/A	CADD Top8
rs11227302	18.09	N/A	CADD Top8
rs11606631	6.02	55.68	H3K4me3 Top8
rs906868	5.21	32.88	H3K4me3 Top8
rs1728769	8.82	27.28	H3K4me3 Top8
rs1728770	4.08	11.94	H3K4me3 Top8
rs118126443	6.30	10.86	H3K4me3 Top8
rs6934662	4.89	10.23	H3K4me3 Top8
rs13215181	5.54	8.80	H3K4me3 Top8
rs180769054	5.66	7.66	H3K4me3 Top8
rs113370572	10.60	1.60	CADD&H3K4me3
rs7769961	10.91	8.73	CADD&H3K4me3
rs28364617	15.09	6.07	CADD&H3K4me3
rs11089620	10.64	5.64	CADD&H3K4me3
rs2297550	11.57	2.33	CADD&H3K4me3
rs58363746	11.04	1.45	CADD&H3K4me3
rs936394	10.25	1.28	CADD&H3K4me3
rs56741490	15.82	0.69	CADD&H3K4me3

Supplementary References

1. Kerimov, N. *et al.* A compendium of uniformly processed human gene expression and splicing quantitative trait loci. *Nat. Genet.* **53**, 1290–1299 (2021).
2. Bentham, J. *et al.* Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat. Genet.* **47**, 1457–1464 (2015).
3. Langefeld, C. D. *et al.* Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat. Commun.* **8**, 16021 (2017).
4. Giambartolomei, C. *et al.* Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet.* **10**, e1004383 (2014).