Supplementary Figures & Table

Risk Variants with Opposing Functional Effects Result in Hypomorphic Expression of *TNIP1* and Other Genes within a 3D Chromatin Network

Satish Pasula, PhD[1], Kandice L. Tessneer, PhD[1], Yao Fu, PhD[1], Jaanam Gopalakrishnan[1,2], Richard C. Pelikan, PhD[1], Jennifer A. Kelly, MPH[1], Graham B. Wiley, PhD[1], Mandi M. Wiley, PhD[1], and Patrick M. Gaffney, MD[1,2*]

[1]Genes and Human Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA.

[2]Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

***Corresponding Author:** Patrick M. Gaffney, MD, Genes and Human Disease Research Program, Oklahoma Medical Research Foundation; Address: 825 NE 13th St, MS 57, Oklahoma City, OK, USA 73104; Phone: 405-271-2572; Fax: 405-271-2536; Email: patrick-gaffney@omrf.org

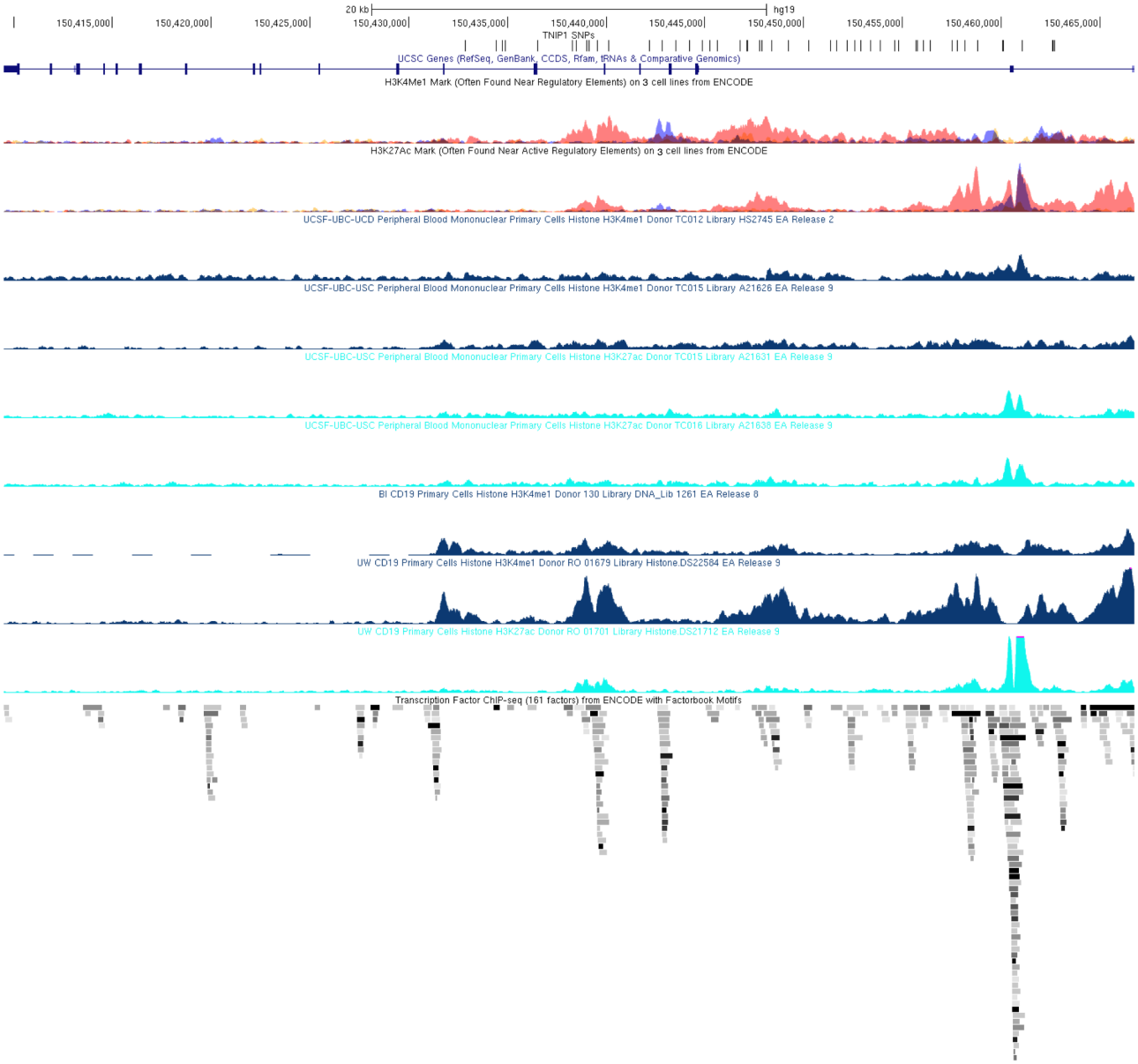
Running Title: *TNIP1* risk variants cumulatively effect gene expression in lupus

Financial Support: Research was supported by National Institutes of Health (NIH) grants: AR063124, AR073606, AR056360, GM110766, and AI082714; as well as the Presbyterian Health Foundation.

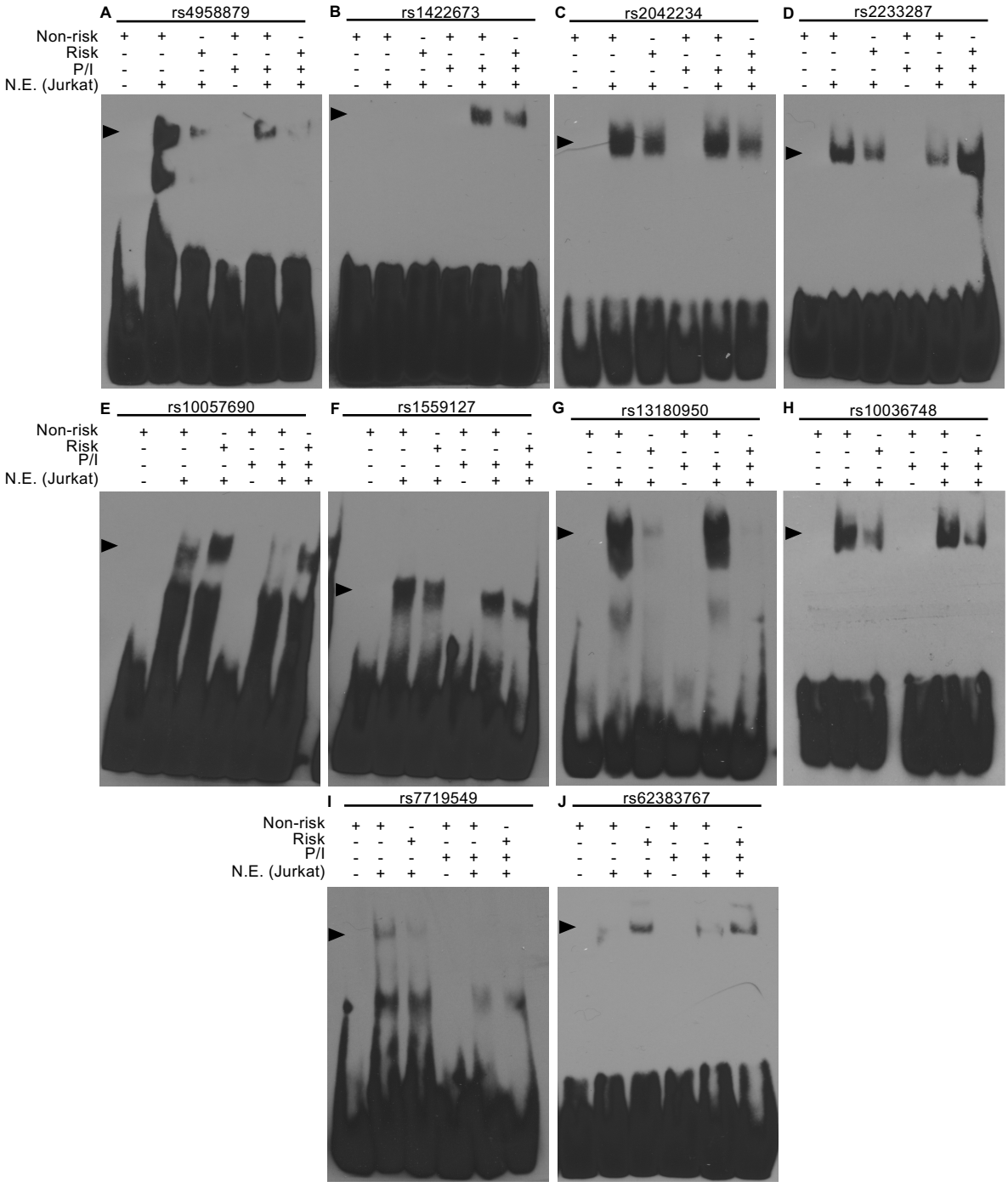
COI Disclosures: None

Supplementary Figures: 7

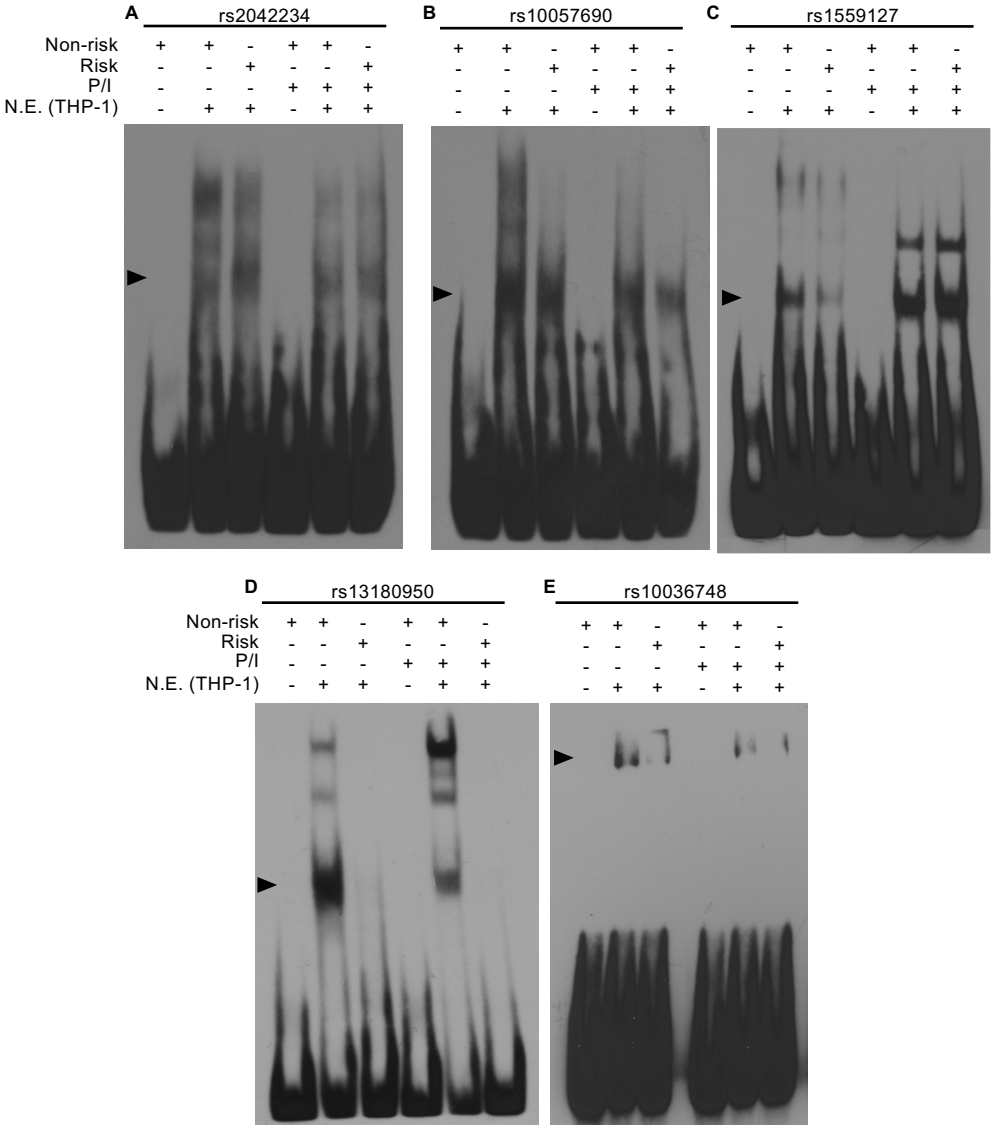
Supplementary Tables: 2



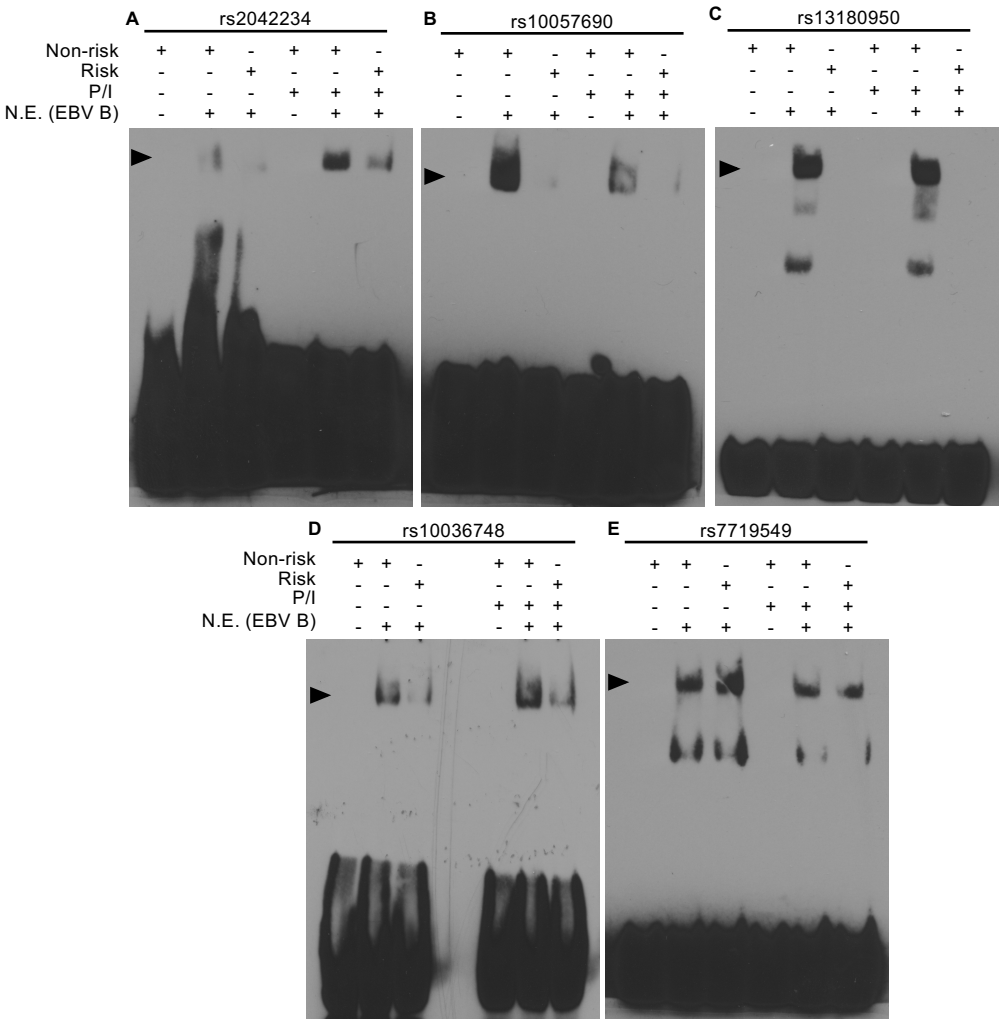
Supplementary Figure 1. Bioinformatic analysis of 11 SLE risk variants located in regulatory elements of the *TNIP1* locus. Eleven of the fifty non-protein-coding SLE risk variants on the *TNIP1* locus were predicted by RegulomeDB to have regulatory functions, and are positioned in enhancer regions identified by enrichment of H3K4me1 and H3K27ac chromatin marks and ChIP-seq transcription factor binding. Variant positions are indicated on the UCSC Genome Browser tracks: Gene Symbol, custom track of the *TNIP1* SLE risk SNPs, ENCODE H3K4me1 and H3K27Ac chromatin marks for GM12878, H1-hESC, and K562 cell lines, NIH Roadmap Epigenomics H3K4me1 and H3K27Ac chromatin marks for peripheral mononuclear blood cells and primary CD19+ B cells, and ENCODE ChIP-seq transcription factor enrichment.



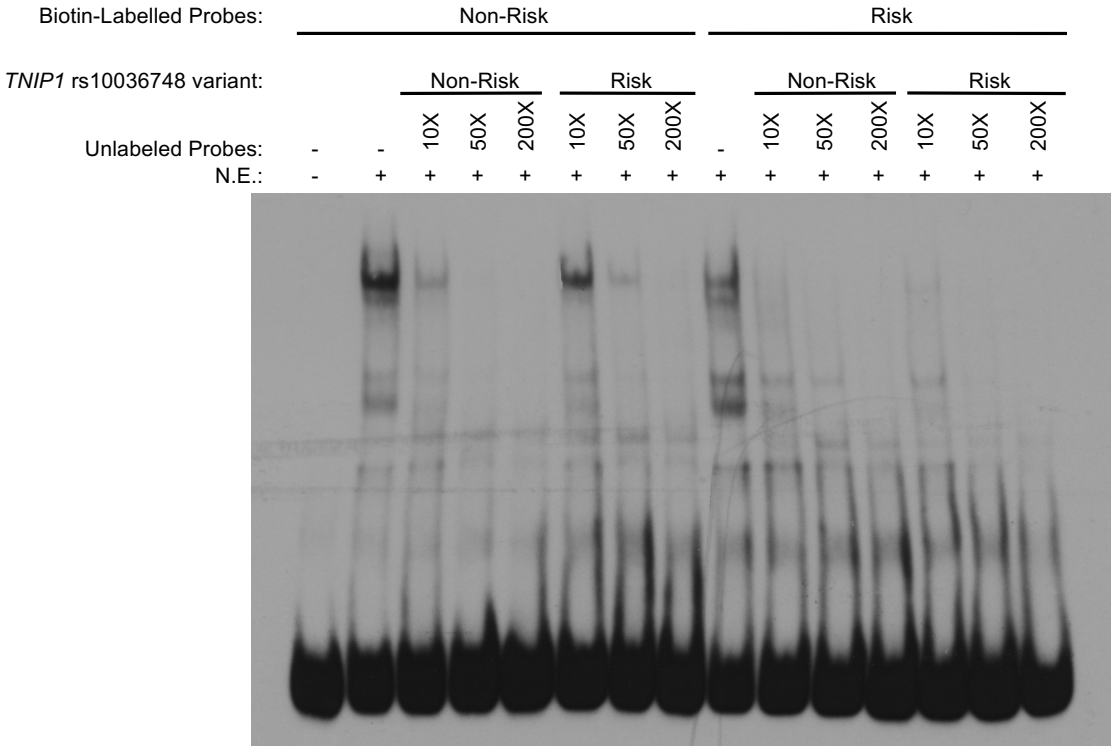
Supplementary Figure 2. *TNIP1* SLE risk alleles differentially effect nuclear factor binding in Jurkat cells. EMSAs were performed using biotinylated oligonucleotides containing the non-risk or risk alleles of the indicated variants. Nuclear extracts (N.E.) were from Jurkat cells at rest or stimulated with P/I for 2 h. Representative image of n>3.



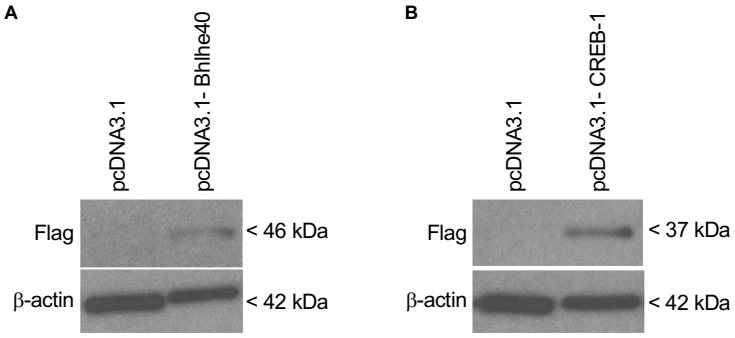
Supplementary Figure 3. *TNIP1* SLE risk alleles differentially effect nuclear factor binding in THP-1 cells. EMSAs were performed using biotinylated oligonucleotides containing the non-risk or risk alleles of the indicated variants. Nuclear extracts (N.E.) were from THP-1 cells at rest or stimulated with P/I for 2 h. Representative image of n>3.



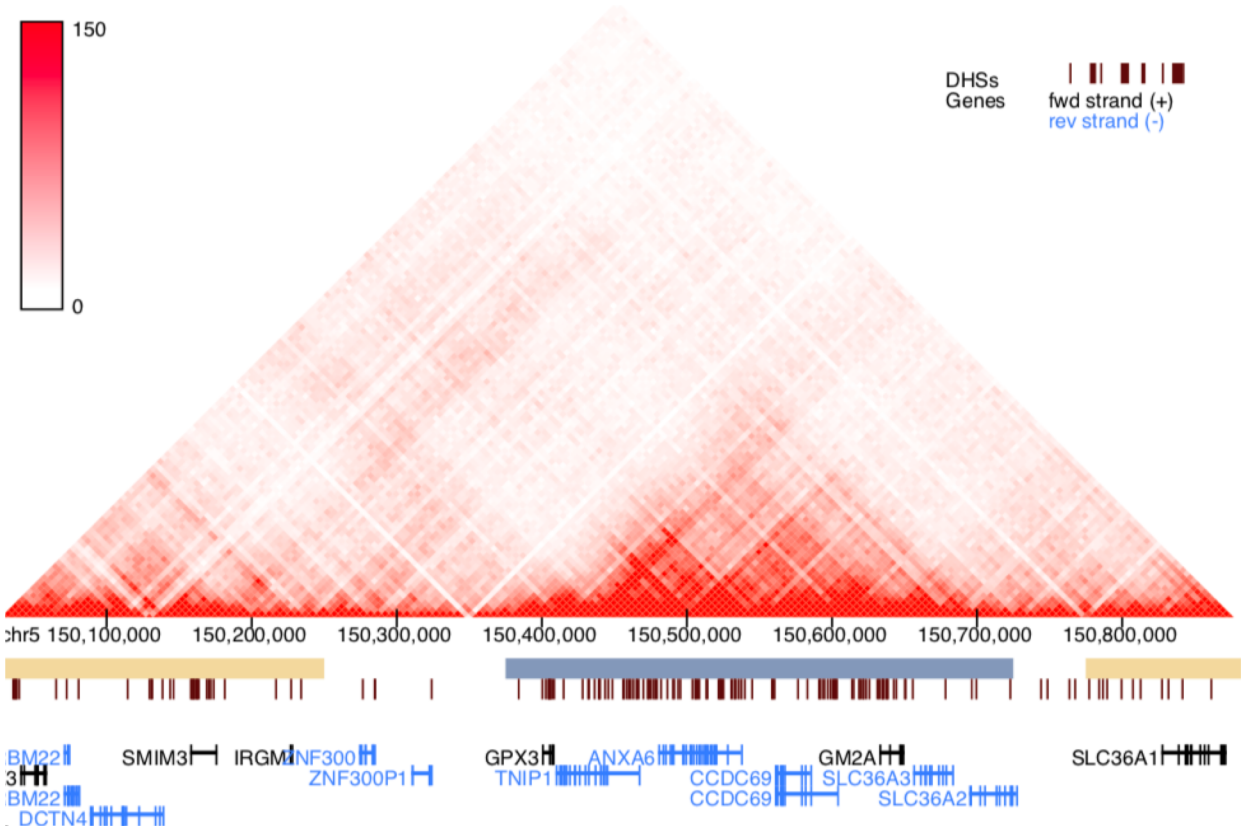
Supplementary Figure 4. *TNIP1* SLE risk alleles differentially effect nuclear factor binding in EBV B cells. EMSAs were performed using biotinylated oligonucleotides containing the non-risk or risk alleles of the indicated variants. Nuclear extracts (N.E.) were derived from EBV B cells at rest or stimulated with P/I for 2 h. Representative image of n>3.



Supplementary Figure 5. Competition EMSA assay demonstrates nuclear factor binding specificity to rs10036748. Competition EMSA assay was performed using increasing concentrations of unlabeled oligonucleotides containing the non-risk (G) or risk (A) allele of rs10036748 to compete against biotinylated oligonucleotides containing the non-risk (G) or risk (A) allele of rs10036748. Nuclear extracts (N.E.) were derived from EBV B cells at rest. Representative image of n=3.



Supplementary Figure 6. Bhlhe40/DEC1 and CREB-1 overexpression in EBV B cells. EBV B cells were transiently transfected with empty pcDNA3.1 **(A,B)**, pcDNA3.1-Bhlhe40/DEC1 **(A)**, or pcDNA3.1-CREB-1 **(B)**. Whole cell lysates were separated by SDS-PAGE. Overexpressed flag-tagged Bhlhe40/DEC1 **(A)** or CREB-1 **(B)** was detected by Western blot using an antibody specific against Flag. β–actin was used as a loading control. Images are representative of n=3.



Supplementary Figure 7. The *TNIP1* locus has a complex chromatin architecture. Hi-C chromatin looping events at the TNIP1 locus in the EBV B cell line, GM12878, were visualized as a heatmap. The heatmap was adapted from the Penn State Computational and Functional Genomics/Epigenomics Browser.

Supplementary Table 1. Oligonucleotide Sequences

EMSA/Affinity Pulldown Probes	Forward (5'→ 3')	Complement/Reverse (5'→ 3')
rs10036748 NR	CCCCTTTTTTCACTTTCTGTCACGTTACTATTTTATTTTTCTCATA	TATGAGAAAAATAAAATAGTAACGTGACAGAAAGTGAAAAAAGGGG
rs10036748 R	CCCCTTTTTTCACTTTCTGTCACATTACTATTTTATTTTTCTCATA	TATGAGAAAAATAAAATAGTAATGTGACAGAAAGTGAAAAAAGGGG
rs4958879 NR	CCAGGCAGTAAAAATACCCAAGATGCCATGTCTCTGGGC	GCCCAGAGACATGGCATCTTGGGTATTTTTACTGCCTGG
rs4958879 R	CCAGGCAGTAAAAATACCCGAGATGCCATGTCTCTGGGC	GCCCAGAGACATGGCATCTCGGGTATTTTTACTGCCTGG
rs4958435 NR	TTATGGGAATCACAGGACAGCAGAGCTACAGGGAGCCTC	GAGGCTCCCTGTAGCTCTGCTGTCCTGTGATTCCCATAA
rs4958435 R	TTATGGGAATCACAGGACATCAGAGCTACAGGGAGCCTC	GAGGCTCCCTGTAGCTCTGATGTCCTGTGATTCCCATAA
rs1422673 NR	ACAGCCACCTGAGGGGGCCCTGCACTGGGCACTGACAGG	CCTGTCAGTGCCCAGTGCAGGGCCCCCTCAGGTGGCTGT
rs1422673 R	ACAGCCACCTGAGGGGGCCTTGCACTGGGCACTGACAGG	CCTGTCAGTGCCCAGTGCAAGGCCCCCTCAGGTGGCTGT
rs2042234 NR	CGCATAACAACAATTAAAGAAAAAAACTTAAAAGTGGGA	TCCCACTTTTAAGTTTTTTTCTTTAATTGTTGTTATGCG
rs2042234 R	CGCATAACAACAATTAAAGGAAAAAACTTAAAAGTGGGA	TCCCACTTTTAAGTTTTTTCCTTTAATTGTTGTTATGCG
rs2233287 NR	GTTACATATCATCTCACATGAGATCACAAAGCAGGATTC	GAATCCTGCTTTGTGATCTCATGTGAGATGATATGTAAC
rs2233287 R	GTTACATATCATCTCACATAAGATCACAAAGCAGGATTC	GAATCCTGCTTTGTGATCTTATGTGAGATGATATGTAAC
rs10057690 NR	TGAAAGTCTTAAGTTAATTTAGTAATCTTTAAAGTGAAA	TTTCACTTTAAAGATTACTAAATTAACTTAAGACTTTCA
rs10057690 R	TGAAAGTCTTAAGTTAATTCAGTAATCTTTAAAGTGAAA	TTTCACTTTAAAGATTACTGAATTAACTTAAGACTTTCA
rs1559127 NR	TCTACAGCATAGCACAAAGTGCAGATGTTTCCACCGAAC	GTTCGGTGGAAACATCTGCACTTTGTGCTATGCTGTAGA
rs1559127 R	TCTACAGCATAGCACAAAGCGCAGATGTTTCCACCGAAC	GTTCGGTGGAAACATCTGCGCTTTGTGCTATGCTGTAGA
rs13180950 NR	GCACTTTGGGAGGCCGAGGTGGGCGGATCACCTGAGGTC	GACCTCAGGTGATCCGCCCACCTCGGCCTCCCAAAGTGC
rs13180950 R	GCACTTTGGGAGGCCGAGGCGGGCGGATCACCTGAGGTC	GACCTCAGGTGATCCGCCCGCCTCGGCCTCCCAAAGTGC
rs7719549 NR	CAATAAAAATTATTATTTTCCTCCTCGTGACATATGTAC	GTACATATGTCACGAGGAGGAAAATAATAATTTTTATTG
rs7719549 R	CAATAAAAATTATTATTTTTCTCCTCGTGACATATGTAC	GTACATATGTCACGAGGAGAAAAATAATAATTTTTATTG
rs62383767 NR	TAAACACAGGCTATGAATTGAGCTAATGGGATTCCAATC	GATTGGAATCCCATTAGCTCAATTCATAGCCTGTGTTTA
rs62383767 R	TAAACACAGGCTATGAATTAAGCTAATGGGATTCCAATC	GATTGGAATCCCATTAGCTTAATTCATAGCCTGTGTTTA
Scrambled	TTTTTACTCACCCTGTCTCTACTTTTGTATACTTACCTCTTATTTT	AAAATAAGAGGTAAGTATACAAAAGTAGAGACAGGGTGAGTAAAAA
ChIP-qPCR	ACATATGCTTTCTCATTCGCAAA	TACATGCTGTTCCCTCTGCC
Luciferase Cloning Primers		
rs10036748	ACTGGTACCGGACTAAGGAGGTCCCTGCTCA	AGTCTCGAGTTCTCATCCTTAGGCTTTCATA
rs4958879	ACTGGTACCCCCTTAAAAAAAAGAAATCA	AGTCTCGAGATGATTCTATCCATTGGCCGGT
rs4958435	ACTGGTACCCAGTTGGGCTCCCCACTTTA	AGTCTCGAGTTCCTCCAGAGTTCAGAGAAGT
rs1422673	ACTGGTACCTTGCCTCCATGGCCAGCATCTCA	AGTCTCGAGATTCTCCCTTCCTTCCCACTTTTAAGT
rs2042234	ACTGGTACCGCTAGTTAGCCAGACAGCCA	AGTCTCGAGTCCTCATTCCACACCTTACAACACT
rs2233287	ACTGGTACCTCAGGAGTGACCACTTCAAATT	AGTCTCGAGAAAGCATTTAAAAAGTACACG
rs10057690	ACTGGTACCAGGAATCCCATATGTTTTACCCA	AGTCTCGAGGACAGACCATATATGATATGAGA
rs1559127	ACTGGTACCCTTCTCCCAAAGACTGACAGAT	AGTCTCGAGATGCCCGCACCAGTCAGACACAT
rs13180950	ACTGGTACCGTATACATTTGCTTTTCTTACAGCTG	AGTCTCGAGTTACTGCAATCTCCACCTCCCA
rs7719549	ACTGGTACCCTTTGCAACTCACAGCTGTGT	AGTCTCGAGTGCGCGGGGTAGCACGCCT
rs62383767	ACTGGTACCAAAGGACTTACTCTAGGCCACA	AGTCTCGAGTCTTGAATGCCTACTGTGTGCT

Abbreviations: NR, non-risk; R, risk

Supplementary Table 2. RegulomeDB Scores of Screened *TNIP1* SNPs

SNP ID	Chr	Base pair position	Allele	Function[1]	RegulomeDB Score[2]	eQTL - Whole Blood[3]
rs2001542	chr5	150432859	C/T	Intron Variant	No Score	-
rs4958879	**chr5**	**150434422**	**A/G**	**Intron Variant**	**2b**	**-**
rs3792794	chr5	150434722	C/T	Intron Variant	No Score	-
rs6579837	chr5	150434894	G/T	Intron Variant	5	-
rs2233290	chr5	150436503	G/C	Missense Variant	5	-
rs4958435	**chr5**	**150438284**	**G/T**	**Intron Variant**	**1f**	**-**
rs4958880	chr5	150438477	C/A	Intron Variant	4	-
rs1422673	**chr5**	**150438988**	**C/T**	**Intron Variant**	**2f**	**-**
rs2042234	**chr5**	**150439131**	**A/G**	**Intron Variant**	**2b**	**-**
rs3805431	chr5	150439539	G/A	Intron Variant	4	-
rs2233287	**chr5**	**150440097**	**G/A**	**Intron Variant**	**2b**	**-**
rs3792790	chr5	150442171	C/A	Intron Variant	4	YES
rs4958436	chr5	150442829	T/C	Intron Variant	4	-
rs17111708	chr5	150443507	G/A	Intron Variant	5	-
rs7732451	chr5	150444212	A/G	Intron Variant	No Score	-
rs73272818	chr5	150444843	T/C	Intron Variant	No Score	-
rs10057690	**chr5**	**150445215**	**T/C**	**Intron Variant**	**2b**	**-**
rs1422674	chr5	150445609	T/G	Intron Variant	5	-
rs1559127	**chr5**	**150446753**	**T/C**	**Intron Variant**	**2b**	**-**
rs6880110	chr5	150447090	A/G	Intron Variant	5	-
rs6861227	chr5	150447128	T/G	Intron Variant	5	-
rs59926079	chr5	150447743	A/G	Intron Variant	4	-
rs58474444	chr5	150447880	T/G	Intron Variant	4	-
rs1862364	chr5	150448376	A/G	Intron Variant	4	-
rs73272828	chr5	150449220	C/T	Intron Variant	5	-
rs4958881	chr5	150450236	T/C	Intron Variant	4	-
rs10700649	chr5	150451348	C/CCT	Intron Variant	5	-
rs3792785	chr5	150451650	T/C	Intron Variant	4	-
rs13160369	chr5	150452196	C/G	Intron Variant	4	-
rs13180950	**chr5**	**150452553**	**T/C**	**Intron Variant**	**2b**	**-**
rs6869605	chr5	150452866	A/C	Intron Variant	5	-
rs73272841	chr5	150453384	C/T	Intron Variant	No Score	-
rs73272842	chr5	150453888	G/A	Intron Variant	5	-
rs1107239	chr5	150454606	T/A	Intron Variant	4	-
rs4958882	chr5	150454787	C/G	Intron Variant	No Score	-
rs3792784	chr5	150455672	A/G	Intron Variant	4	-
rs3792783	chr5	150455732	A/G	Intron Variant	4	-
rs5872188	chr5	150456054	CAG/C	Intron Variant	4	-
rs7731150	chr5	150456392	G/A	Intron Variant	4	-
rs7708392	chr5	150457485	G/C	Intron Variant	5	-
rs6889239	chr5	150457771	T/C	Intron Variant	4	-
rs10036748	**chr5**	**150458146**	**C/T**	**Intron Variant**	**3a**	**-**
rs918498	chr5	150458788	C/T	Intron Variant	4	-
rs7719549	**chr5**	**150460047**	**C/T**	**Intron Variant**	**3a**	**-**
rs33934794	chr5	150460089	GA/G	Intron Variant	4	-

rs960709	chr5	150461049	A/G	Intron Variant	4	-
rs78717966	chr5	150462574	GGC/G	Intron Variant	N/A	-
rs62383766	chr5	150462576	C/A	Intron Variant	4	-
rs13168551	chr5	150462638	T/C	Intron Variant	4	-
rs62383767	**chr5**	**150462705**	**G/A**	**Intron Variant**	**3a**	**-**

[1]Variant functional prediction was determined from ENCODE data visualized in UCSC Genome Browser.
[2]RegulomeDB scores were determined by importing variant dbSNP IDs into http://www.regulomedb.org/.
[3]Whole blood eQTL data was obtained from the GTEx Portal (https://gtexportal.org/home/)
Abbreviations: Chr, chromosome; eQTL, expression quantitative trait loci