frontiers

# *Supplementary Material*

**Systemic lupus erythematosus variants modulate the function of an enhancer upstream of *TNFAIP3***

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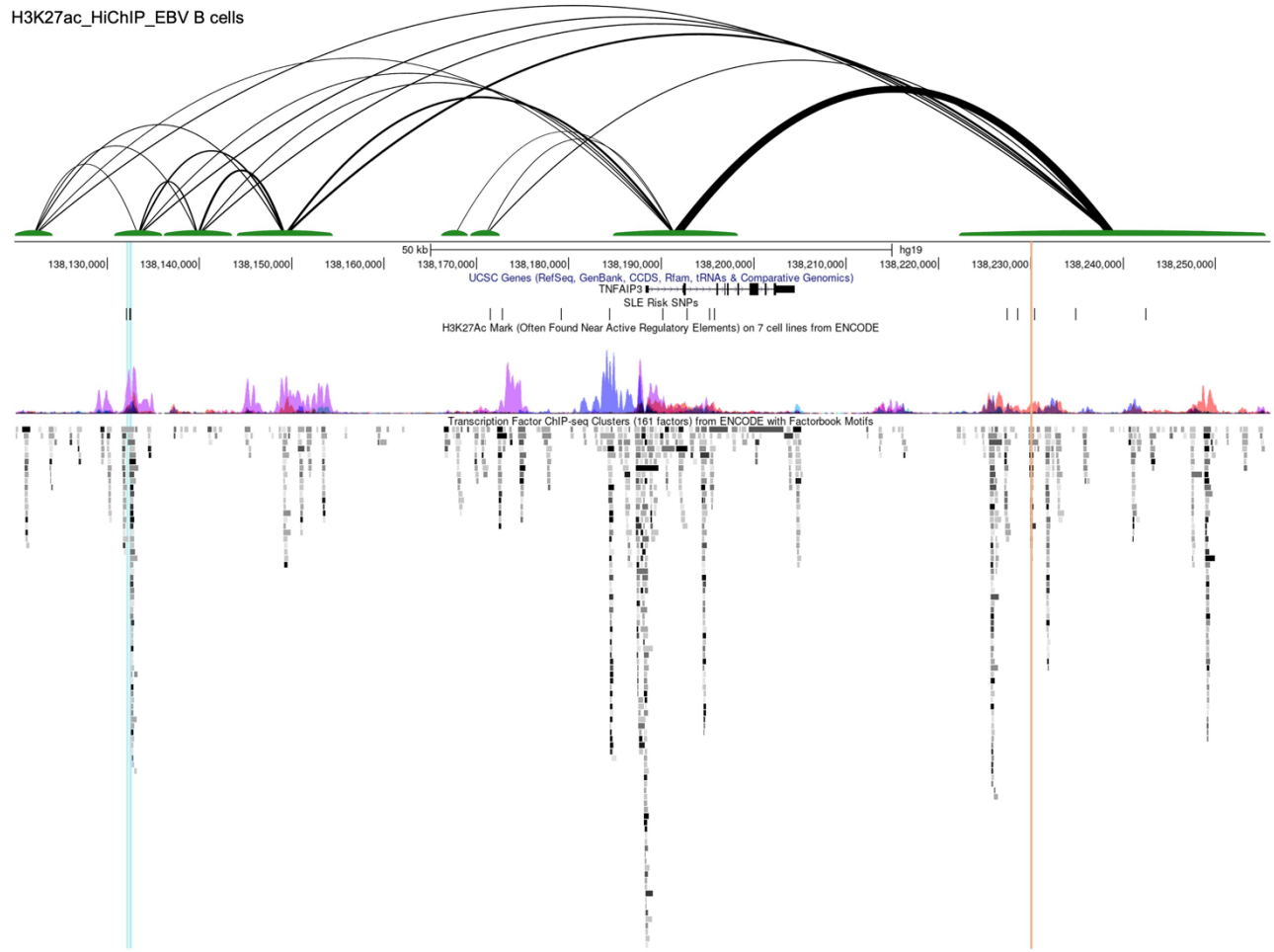
**Keywords: systemic lupus erythematosus, TNFAIP3, IL20RA, IFNGR1, functional genetics**

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

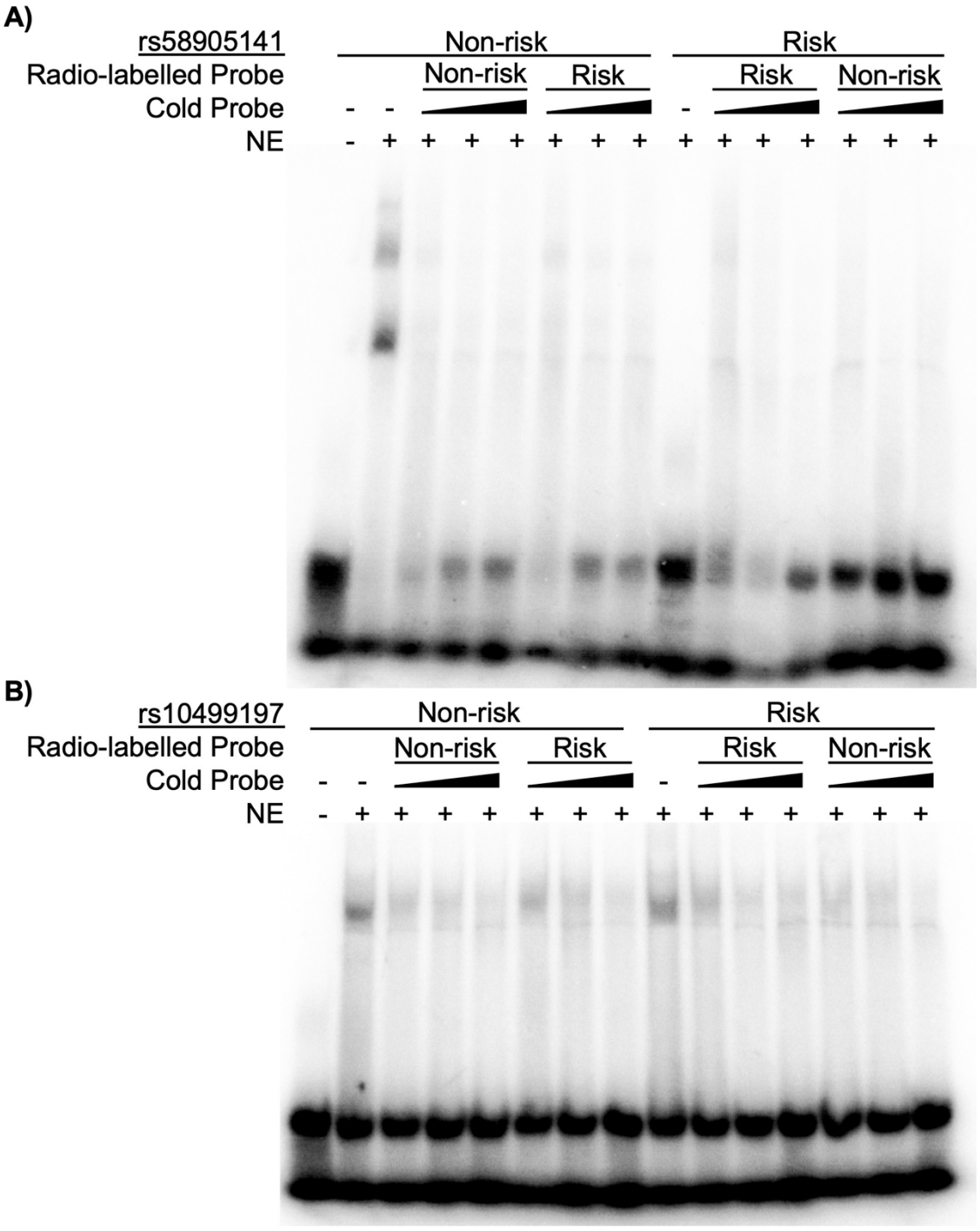
**Funding:** Research reported in this publication was supported by the National Institute of General Medical Sciences, the National Institute of Arthritis and Musculoskeletal and Skin Diseases, and the National Institutes of Allergy and Infectious Diseases of the National Institutes of Health (NIH) grants: R01AR063124, R01AR073606, R01AR056360, P30GM110766, U19AI082714, R01AI156724; as well as the Presbyterian Health Foundation. The ORDRCC (source of EBV B cell lines) is supported by the National Institutes of Health grants U54GM104938, P30AR073750, P30GM103510 and P20RR020143. The content of this publication is solely the responsibility of the authors and does not represent the official views of the funding agencies.

# 1. Supplementary Figures and Tables

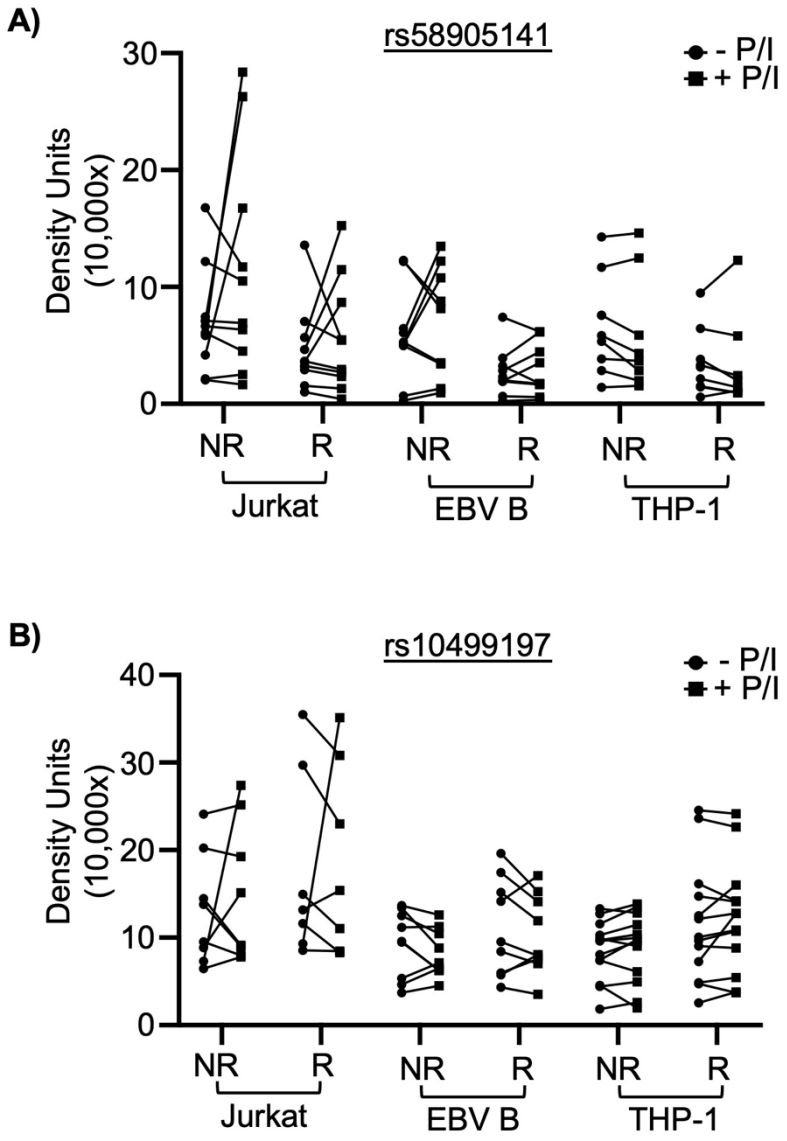
## 1.1 Supplementary Figures



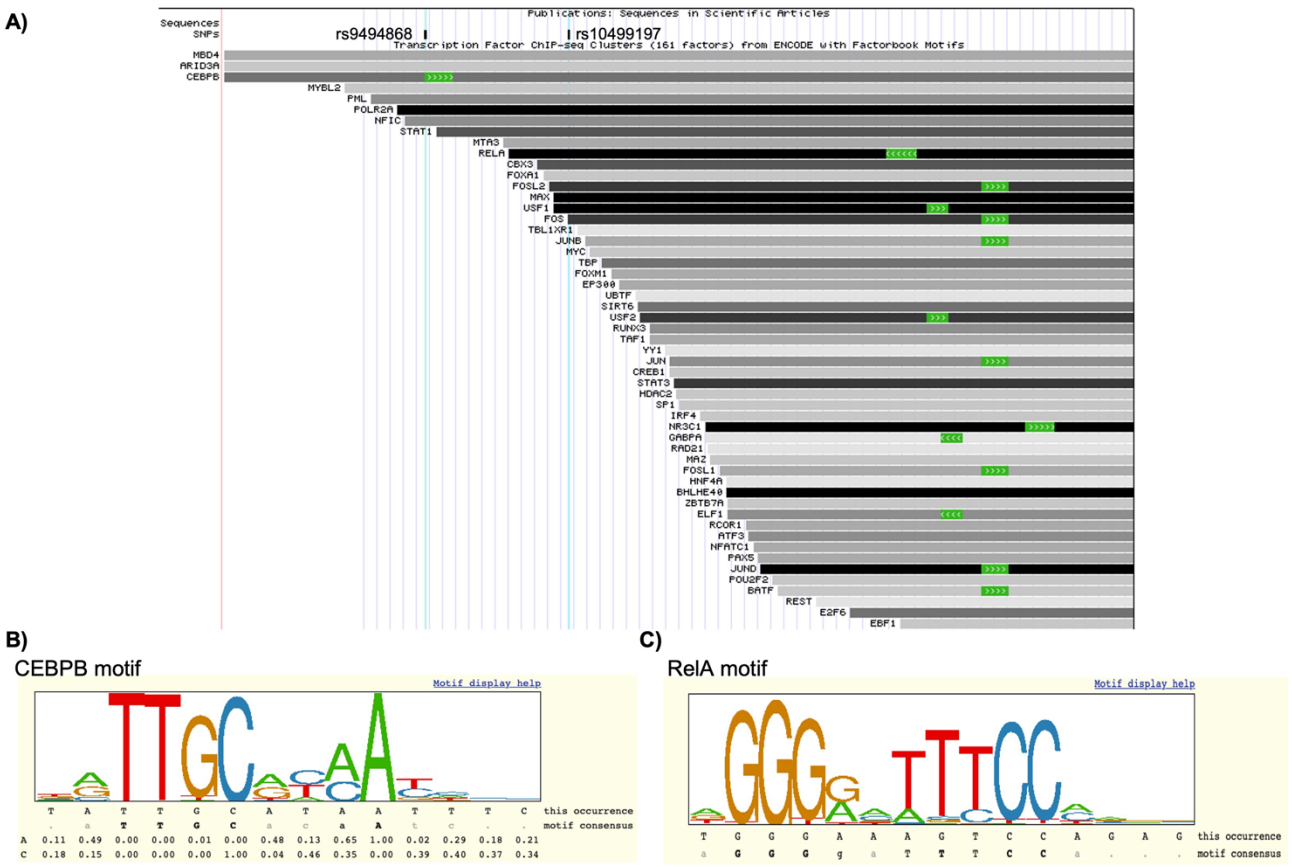

**Supplementary Figure S1. SLE risk variants, rs10499197, rs58905141 are positioned in an enhancer with complex 3D chromatin structure ~55 kb upstream of *TNFAIP3*.** Three-dimensional organization of H3K27ac HiChIP looping interactions at the *TNFAIP3* locus is visualized as a two-dimensional looping diagram. HiChIP revealed interactions between the *TNFAIP3* gene body and the well-characterized TT>A enhancer (rs14314164, rs200820567; orange line) downstream of *TNFAIP3*, as well as an enhancer at position 138.14 (~55 kb upstream of the *TNFAIP3* promoter) that overlaps two SLE risk alleles, rs58905141 and rs10499197 (blue lines). Identified enhancers are consistent with ENCODE H3K27ac ChIPseq peaks for GM12878 EBV B, K562 lymphoblastoid, and H1-hESC cell lines, and transcription factor ChIP-seq clusters from ENCODE.



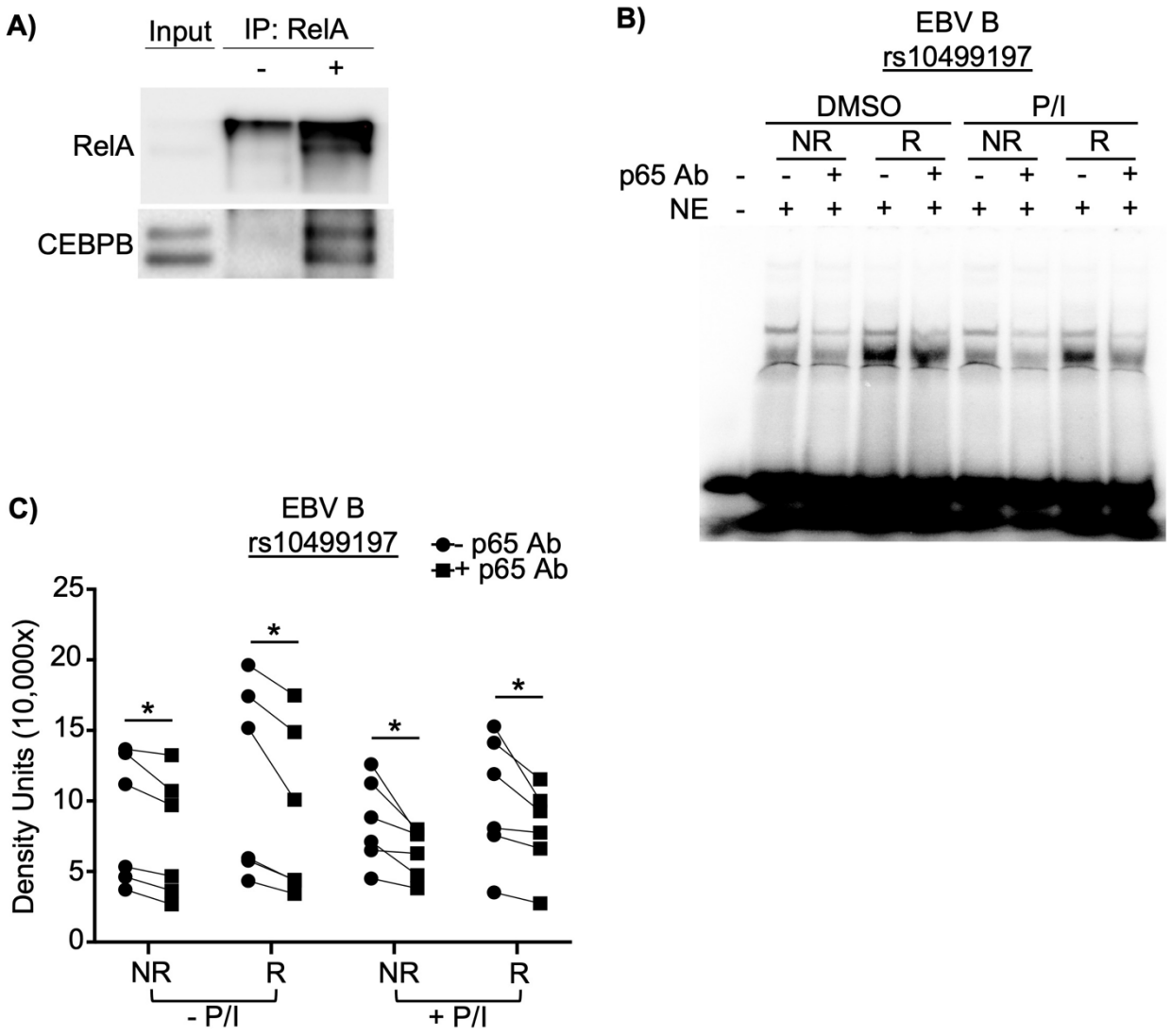

**Supplementary Figure S2. Cold-probe competition assay demonstrates nuclear protein binding specificity.** Cold-probe competition EMSAs using nuclear extracts from THP-1 cells and radiolabeled probes of the non-risk (A) or risk (G) allele of rs58905141 (**A**) or radiolabeled probes of the non-risk (T) or risk (G) allele of rs10499197 (**B**). Cold probes were used at 10X, 50X, or 100X concentration of the cold probe; indicated by black triangle. Images are representative of n>2. (Abbreviations: NE, nuclear extract).




**Supplementary Figure S3. *TNFAIP3* rs58905141 and rs10499197 alleles are not stimuli-responsive.** EMSA using nuclear extracts from Jurkat, EBV B, and THP-1 cells stimulated with or without PMA/Ionomycin for 2 h. Radiolabeled probes of the non-risk (A) or risk (G) allele of rs58905141 (**A**) or non-risk (T) or risk (G) allele of rs10499197 (**B**) were incubated with nuclear extracts as indicated. Representative images shown in Figure 1A and 1C; n>6. Densitometry was used to quantify nuclear protein-bound DNA probe; paired t-test: not significant. (Figure Abbreviations: NE, nuclear extract; NR, non-risk allele; R, risk allele; P/I, PMA/Ionomycin).




**Supplementary Figure S4. RelA/p65 and CEBPB are integral members of the nuclear protein complex associated with the enhancer. (A)** ChIP-seq data visualized in the UCSC Genome Browser revealed two binding motifs of interest: CCAAT/enhancer-binding protein beta (CEBPB) ~70 bp upstream and RelA/p65 ~160 bp downstream of rs1049917. Blue lines indicate the positioning of rs9494868 and rs10499197 variants. (**B-C**) Encode predicted CEBPB (**B**) and RelA (**C**) motif regions aligned with respective motif consensus.




**Supplementary Figure S5.** ***TNFAIP3*** **rs10499197 exhibits allele-specific RelA/p65 binding.** (**A**) Co-immunoprecipitation of CEBPB by RelA in cell extracts from quiescent EBV B cells. Images are representative immunoblots of n=3. (**B-C**) EMSA Supershift assay using nuclear extracts from EBV B cells stimulated with or without PMA/Ionomycin for 2 h. Radiolabeled probes with the non-risk (T) or risk (G) allele of rs10499197 were incubated with nuclear extracts supplemented with or without anti-p65-specific antibody as indicated. (**B**) Image is representative of n>5. (**C**) Densitometry was used to quantify nuclear protein-bound DNA probe; paired t-test: *$p<0.05$. (Figure Abbreviations: Ab, antibody; IP, Immunoprecipitation; NE, nuclear extract; NR, non-risk allele; R, risk allele; P/I, PMA/Ionomycin)

## 1.2 Supplementary Tables

**Supplementary Table S1. EMSA Oligonucleotide Probe Sequences**

| Radiolabeled Probes (Non-Risk or Risk Allele) | Forward Probe | Reverse Probe |
|---|---|---|
| rs58905141 Non-risk (A) | ATCCATCATGTTCTTCCTTGA AACAACTCTTAAACTCTTAC | GTAAGAGTTTAAGAGTTGTTTCAAG GAAGAACATGATGGAT |
| rs58905141 Risk (G) | ATCCATCATGTTCTTCCTTGG AACAACTCTTAAACTCTTAC | GTAAGAGTTTAAGAGTTGTTCCAAG GAAGAACATGATGGAT |
| rs10499197 Non-risk (T) | CTGAGACCTACTTACTTAGCT TCCAAATATGGTAGTAATTA | TAATTACTACCATATTTGGAAGCTA AGTAAGTAGGTCTCAG |
| rs10499197 Risk (G) | CTGAGACCTACTTACTTAGCG TCCAAATATGGTAGTAATTA | TAATTACTACCATATTTGGACGCTA AGTAAGTAGGTCTCAG |

**Supplementary Table S2. Dual Luciferase Assay Primer Sequences**

| Cloning Primers | Forward Primer | Reverse Primer |
|---|---|---|
| rs58905141 (383bp) | ACTGGTACCGATAACTGATTT GGACTTTGCT | AGTCTCGAGCAGAGGTTTAGG GAGAGAAAT |
| rs10499197 (337bp) | AGCAGGTACCCCACACTGACA AAGCCAATGT | TCTACTCGAGGTCCAGAGTAG ACTGAACGTACT |
| 330bp ΔRelA | AGCAGGTACCCCACACTGACA AAGCCAATGT | AGTCTCGAGACTGAACGTACT TTTTAAGAAAAGGA |
| 150bp ΔRelA | ACTGGTACCTGATTATTGCATA ATTTCATCCT | AGTCTCGAGTTTGATAGTTCT GTGCAAGGCA |
| 203bp ΔCEBPB | AGTGGTACCTGCTCTTTATTTA ATGACTGAGA | TCTACTCGAGGTCCAGAGTAG ACTGAACGTACT |
| 267bp ΔCEBPB | AGTGGTACCTAATTTATTATTT AACCAAAG | TCTACTCGAGGTCCAGAGTAG ACTGAACGTACT |
| **Site-Directed Mutagenesis Primers** | **Forward Primer** | **Reverse Primer** |
| rs10499197 (G>T) | CCTACTTACTTAGCTTCCAAAT ATGGTAG | CTACCATATTTGGAAGCTAAG TAAGTAGG |
| rs9494868 (G>T) | CCAAAGCCAGTGATTATTGCA TAATTTCATCC | GGATGAAATTATGCAATAATC ACTGGCTTTGG |

**Supplementary Table S3. 3C-qPCR Oligonucleotide Primer Sequences**

| Primer | Sequence | Start Position | End Position | Cutting Site |
|---|---|---|---|---|
| Anchor | AGAGGAAAGGACAGACTTGAG | 138132791 | 138132811 | 138132883 |
| U1 | CAGGGCTTGACAAGACATTTCTC | 137377272 | 137377295 | 137377334 |
| U2 | TCCGAAGAGCTTTGTTTGTGG | 137381301 | 137381322 | 137381475 |
| U3 | TGCTGCCCAGACATAGGAAA | 137406792 | 137406812 | 137407040 |
| U4 | TGCCATTCTAGCCCTTCCAG | 137423110 | 137423130 | 137423210 |
| U5 | GCCAAGTGCCTATTACTCCAT | 137544290 | 137544311 | 137544439 |
| U6 | TCTACTGGTGCACATTTCCT | 137555115 | 137555135 | 137555184 |
| U7 | CAAGGCAAGGTGGTGGTTTT | 137583168 | 137583188 | 137583223 |
| D1 | GTGACAGAGTGAGACCTTGT | 138180102 | 138180121 | 138180192 |
| D2 | TAACCCAACACTTAGGCTCA | 138182379 | 138182399 | 138182573 |
| D3 | TGGGTTTCTCCATTCAGTCC | 138184563 | 138184582 | 138184707 |
| D4 | AGACTGGTCATTATGGGCTT | 138186764 | 138186783 | 138186854 |
| D5 | GTTTTGTCCTCAGTTTCGGG | 138192529 | 138192548 | 138192635 |
| D6 | TAGGTTCTATTGCCTATGCCC | 138193187 | 138193207 | 138193357 |
| D7 | TTGTAGAGTGATGTCAGAATGA | 138196743 | 138196764 | 138196796 |
| NCR | ACAGGCAGTGGTATGTTGGA | 137833999 | 137834018 | 137834160 |
| RLC | CTTGACACTGATCCCTCCAAT | 138126775 | 138126795 | 138126865 |

**Supplementary Table S4. ChIP-qPCR Primer Sequences**

| | Forward Primer | Reverse Primer |
|---|---|---|
| RelA/p65 | TAACCTTGTGACACAGCCCT | GGTCAGCTAACAGAGCAGGAA |
| CEBPB | GTTCTGCTTCACAATTCGGCA | AAGAGCAGCATTCTTGATTGGAA |