SUPPLEMENTAL MATERIAL

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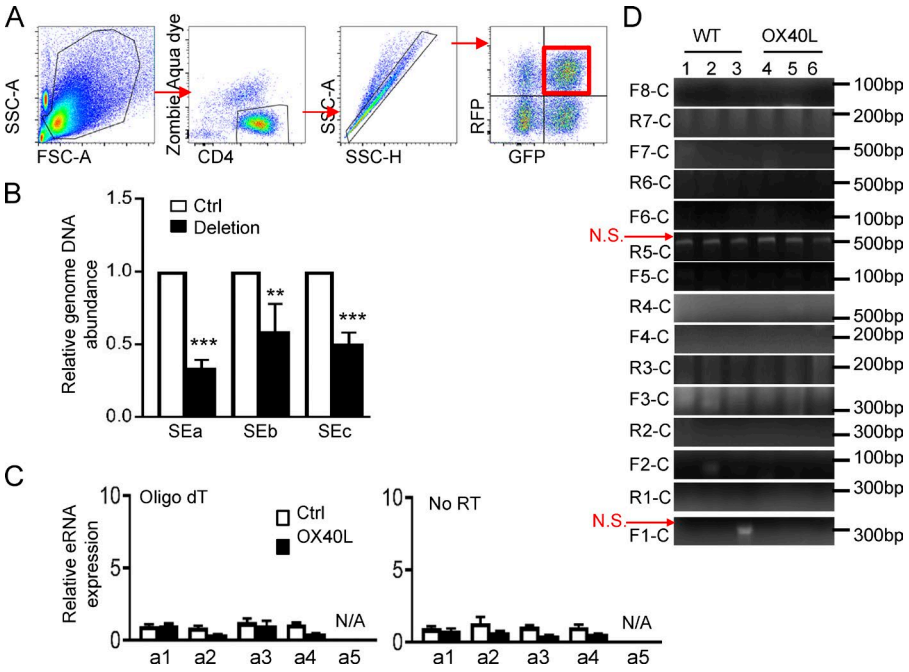

Figure S1. **CRISPR/Cas9 strategy in deletion of Il9 SE in activated CD4⁺ T cells. (A)** Gating strategy for FACS analysis after transduction of activated Cas9 transgenic CD4⁺ T cells with a pair of sgRNA flanking a designated SE region for deletion. The retroviral vectors carrying the sgRNA are marked by GFP and RFP, respectively, so live CD4⁺ T cells expressing both GFP and RFP markers were selectively gated for further analysis. **(B)** Deletion efficiency by the Cas9/sgRNA method in primary T cells, normalized against the Il9 coding region. Data are pooled from four independent experiments (mean and SD of $n = 4$). **, $P < 0.01$; and ***, $P < 0.001$. **(C)** Quantitative real-time PCR analysis of eRNA expression from the Il9 SEa region using different primer sets. Left: The oligo dT primer (specific for mRNA) was used for reverse transcription. Right: No reverse transcription of RNA to cDNA. Data shown are mean ± SEM of three independent experiments with triplicate cultures. **(D)** 3C assays measuring chromatin loop formations using all primer combinations as indicated in Fig. 4 E, except the R8 and C primer set. N.S., nonspecific. Data are representative of three independent experiments.

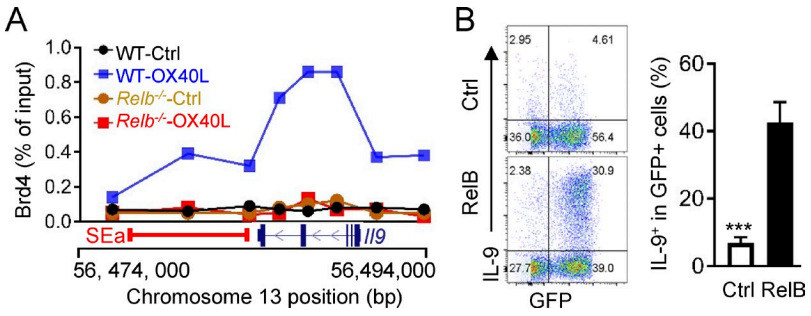

Figure S2. **Role of RelB in Il9 SE formation. (A)** ChIP data showing selective enrichment of Brd4 at the SEa and the Il9 coding region in WT and Relb$^{-/-}$ Th9 cells induced with or without OX40 stimulation. Brd4 failed to bind to such regions in the absence of RelB. Data are representative of two independent experiments. **(B)** FACS plots and quantification of Th9 cells induced from WT CD4⁺ T cells transduced with empty vector (Ctrl) or retroviral vector expressing full-length RelB (RelB), after 3 d of culture with TGF-β and IL-4 (Th9 cell conditions). The retroviral vector also has a GFP marker for identification. Thus IL-9–producing cells in the GFP⁺ fraction were selected for analysis. Graphs depict mean ± SEM of 10 experiments with triplicate cultures. ***, $P < 0.001$.

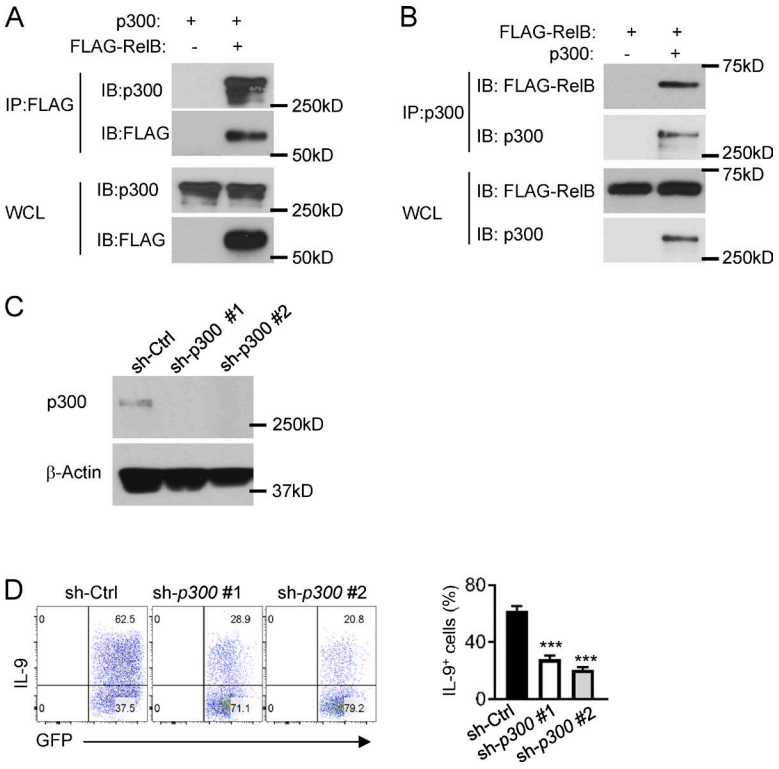

Figure S3. **Critical role of p300 in the formation of Il9 SEs. (A and B)** Coimmunoprecipitation experiments assessing RelB and p300 interactions in 293T cells. FLAG-tagged RelB and p300 were transduced to 293T cells, followed by immunoprecipitation of RelB with anti-FLAG. The immunoblots show the presence of p300 in the RelB immunoprecipitates (A). p300 was immunoprecipitation from 293 T cells using anti-p300, and RelB was detected by immunoblot using anti-FLAG (B). Data are representative of three independent experiments. **(C)** Immunoblot showing the knockdown efficiency of p300 using shRNA specifically targeting p300 in activated $CD4^+$ T cells. β-Actin was used as a housekeeping control. Data are representative of three independent experiments. **(D)** FACS plots showing Th9 cell induction from activated $CD4^+$ T cells transduced with empty vector (sh-Ctrl) or retroviral vector expressing p300 specific shRNA and cultured under Th9 cell–polarizing conditions in the presence of OX40 stimulation. The bar graphs on the right show summary of Th9 cells with or without the p300 knockdown. Graphs depict mean ± SEM of five experiments with triplicate cultures. ***, $P < 0.001$.

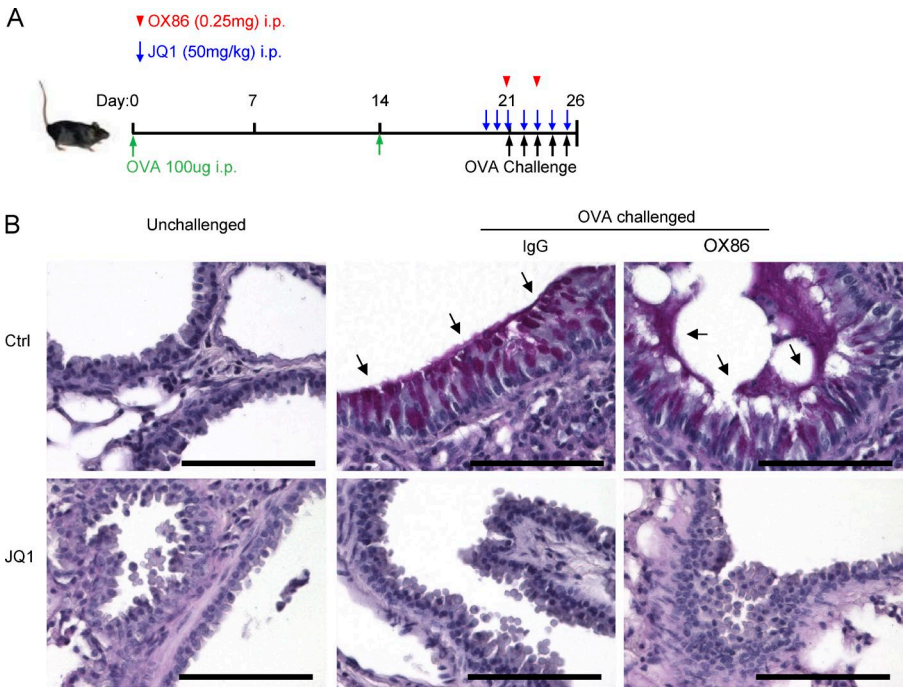

Figure S4. **The role of BET inhibitor JQ1 in allergic airway inflammation in vivo. (A)** Schematic representation of OVA immunization and challenge, as well as timing of treatment with JQ1 and OX86. **(B)** PAS staining of mucin-producing cells in the airway epithelia (arrows) during acute allergic lung inflammation, with or without JQ1 treatment, showing striking reduction of PAS^+ cells after treatment (400×). Bars, 100 µm. Data are representative of two experiments with five to seven mice per group.

Table S1. **qPCR primers and oligos used in this study**

Name	Forward	Reverse
qPCR primers used for eRNA detection		
*Il9*SEa1	AGACAGATGCTATTTATTCT	TCCCTCCCCATAGTTCCAAA
*Il9*SEa2	ATAGGTAGGATGGAGCAAAG	TGTCTCCCTCATGCTGTTCT
*Il9*SEa3	AGTGGTTACAGCACTTGCTG	CACATGACTGCAGGTGCCCT
*Il9*SEa4	GCCTATTTACCTGGAACCTG	ATGGTTGCATCCGCATTCTG
*Il9*SEa5	GATGACTGGTCTCCAGGGTG	CACCCTGGAGACCAGTCATC
*Il9*SEb1	GCAGATGATTAACTAAGGTCT	GTGTGCTCAGTTGCAGTGCTC
*Il9*SEb2	GATGGAGTTCTCACACTCTAC	TTTCTGAGCCATGTGGATATC
*Il9*SEc1	GGCACTGCCTACAATCCTGAT	GTGAAAGTGAGAAGTCAGTTC
*Il9*SEc2	GACATCAGAGAGGGTACACAG	GCTCTGTGTGAAGCTAGAGTA
*Il9*SEc3	GCTGAGCACTGTCTGTCTGGA	AGTACACTAAATCCTGAAGGC
*Ill9*SEc4	GAGTCAAGTTGACAAGCAGGA	TTTACTCAGACATGCTGCTAC
*Il9*SEc5	GGACCACATAGATAGCCAGCA	GCTTCTGAGTCAAGGATCTTC
*Il9*SEc6	GGCAGTGCTGCGATGTGCAAT	TGTTAGCATCAACCTGCCATG
*Il9*SEc7	GACCTGTCATAAGCAGTTCAT	GAGGCTTGTCTGCCAAGCAGC
ChIP-qPCR primers		
MIL9-1	CCATCCTCCACAGCTCTTGGA	TAGAATGAAAACTCTGCTGGG
MIL9-2	GCTGGAGTAAAGCTGTCTTGT	CACCCATAACAGGACCTGGGG
MIL9-3	ATAGGTAGGATGGAGCAAAG	TGTCTCCCTCATGCTGTTCT
MIL9-4	TGACAGTGTGTTGCCTGCCAT	TCAACTGGCCACCAGGTTTGA
MIL9-5	AGTGGCACCTGCCTTGGCTA	CCAGGGTTTAAGTCACAGCG
MIL9-6	TCTCTCATTTGCTTGGATGTG	CTGACCAATGCCACACAGAAA
MIL9-8	AGCTAGACTGGAAGATGCTG	GGTAATTGGTGTCTCTGATG
MIL9-9	AGTCGGGTTCTGAAATACTAA	CCTGTAACTCACTGTCTATCA
MIL9-10	CACTCTCAGAATTTGGCTGTA	ATAGATTAGGAGGCCATCAGC
PCR primers for Cas9-mediated gene deletion efficiency		
Il9-SEa	GCCTATTTACCTGGAACCTG	ATGGTTGCATCCGCATTCTG
Il9-SEb	AAGTCATCTCCTCACTCCTCAAATG	CAACACATCATTCCCAGCAGAT
Il9-SEc	GAGAGGGTACACAGCTTGCAT	GACGCCAAGTGTGACTTTGAG
Il9-coding	CTCAATTGGCCTCAACTTACA	CCCTTTGCCATCCTCCAGCAG
PCR primers used for 3C assays		
1	GAAGTAAAAGGTATCTGGAA	TTCCAGATACCTTTTACTTC
2	ATAGGTAGGATGGAGCAAAG	CTTTGCTCCATCCTACCTAT
3	AGTTCCCAACACCTTTGGCA	TGCCAAAGGTGTTGGGAACT
4	AGGGCACCTGCAGTCATGTG	CACATGACTGCAGGTGCCCT
5	TCTCTGCAACACCTCCAGGC	GCCTGGAGGTGTTGCAGAGA
6	GTCTTCATGCATGAAGGTTCC	GGAACCTTCATGCATGAAGAC
7	GAGATGCTGAGGGAGTGCCAC	GTGGCACTCCCTCAGCATCTC
8	GGTTGGAGACGTTCCCAAGAG	CTCTTGGGAACGTCTCCAACC
C	CATCAGAGACACCAATTACC	
shRNA oligos used for target gene knockdown		
Genes	**Target sequence**	
Brd4 1	GCCGCTCTTTATAAGTGTTCG	
Brd4 2	GACAGACGTCCTACTACATGC	
Med1 1	GCTCTCAAAGTAACATCTTTG	
Med1 2	GCCCTTTAGAAAGGCAGAACT	
P300 1	GGATACTGTTGTGGCAGAAAG	
P300 2	GCACCAGATCTGTGTCCTTCA	
shRNA oligos Cas9-mediated target gene deletion		
Name	**Target sequence**	
*Il9*SEa-1	GGAATAAAGATCCACGGGG	
*Il9*SEa-2	GCACTGTTTCTGTTTGTACC	
*Il9*SEb-1	GGCATATTAAATAGGAAGAG	
*Il9*SEb-2	GTGTTTCGAGACGCCTCACA	
*Il9*SEc-1	GAGTAATCTTGCTGAAGCAC	