Supplemental material

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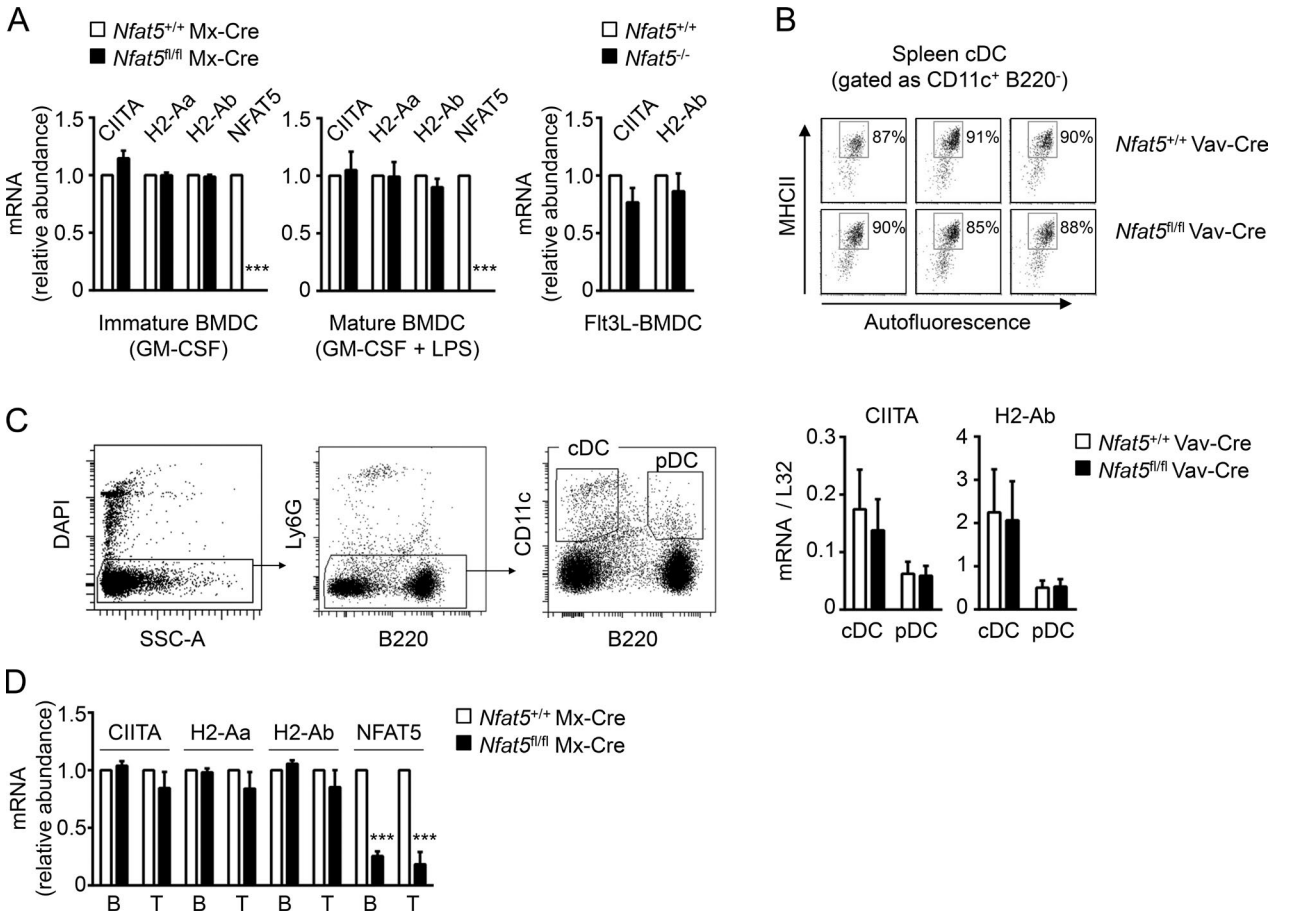


Figure S1. **Expression of MHCII-related genes in NFAT5-deficient DCs and B and T lymphocytes. (A)** CIITA and MHCII mRNA expression in wild-type and NFAT5-deficient BMDCs with GM-CSF or Flt3L. Immature BMDCs were derived by culture with GM-CSF, and mature BMDCs were induced from immature cells by treatment with LPS. Results are the mean ± SEM of three independent experiments for GM-CSF–induced BMDCs and another three for Flt3L-induced cells, each comparing one wild-type and one NFAT5-deficient littermate. ***, P < 0.001. **(B)** MHCII surface expression in conventional myeloid DCs (cDCs) isolated from spleens of mice lacking NFAT5 in hematopoietic lineages (*Nfat5*$^{fl/fl}$ Vav-Cre) and control mice (*Nfat5*$^{+/+}$ Vav-Cre). Results from three mice of each genotype are shown. **(C)** Sorting strategy and analysis of CIITA and MHCII mRNA levels in cDCs and plasmacytoid DCs (pDCs) isolated from the spleen of mice lacking NFAT5 in blood lineages (*Nfat5*$^{fl/fl}$ Vav-Cre) and control mice (*Nfat5*$^{+/+}$ Vav-Cre). mRNA analysis (mean ± SEM) shows the result of three independent sorting experiments, each comprising one wild-type mouse and one *Nfat5*$^{fl/fl}$ Vav-Cre littermate. **(D)** CIITA and MHCII mRNA expression in splenic B and CD4$^+$ T lymphocytes (T) from *Nfat5*$^{fl/fl}$ Mx-Cre and control mice. Results are the mean ± SEM of three independent experiments, each comparing B and T lymphocytes from one NFAT5-deficient mouse and one wild-type littermate. Data for each respective mRNA in A and B are shown normalized to wild-type cells, which were a given a value of 1. Statistical significance was determined with a one-sample *t* test.

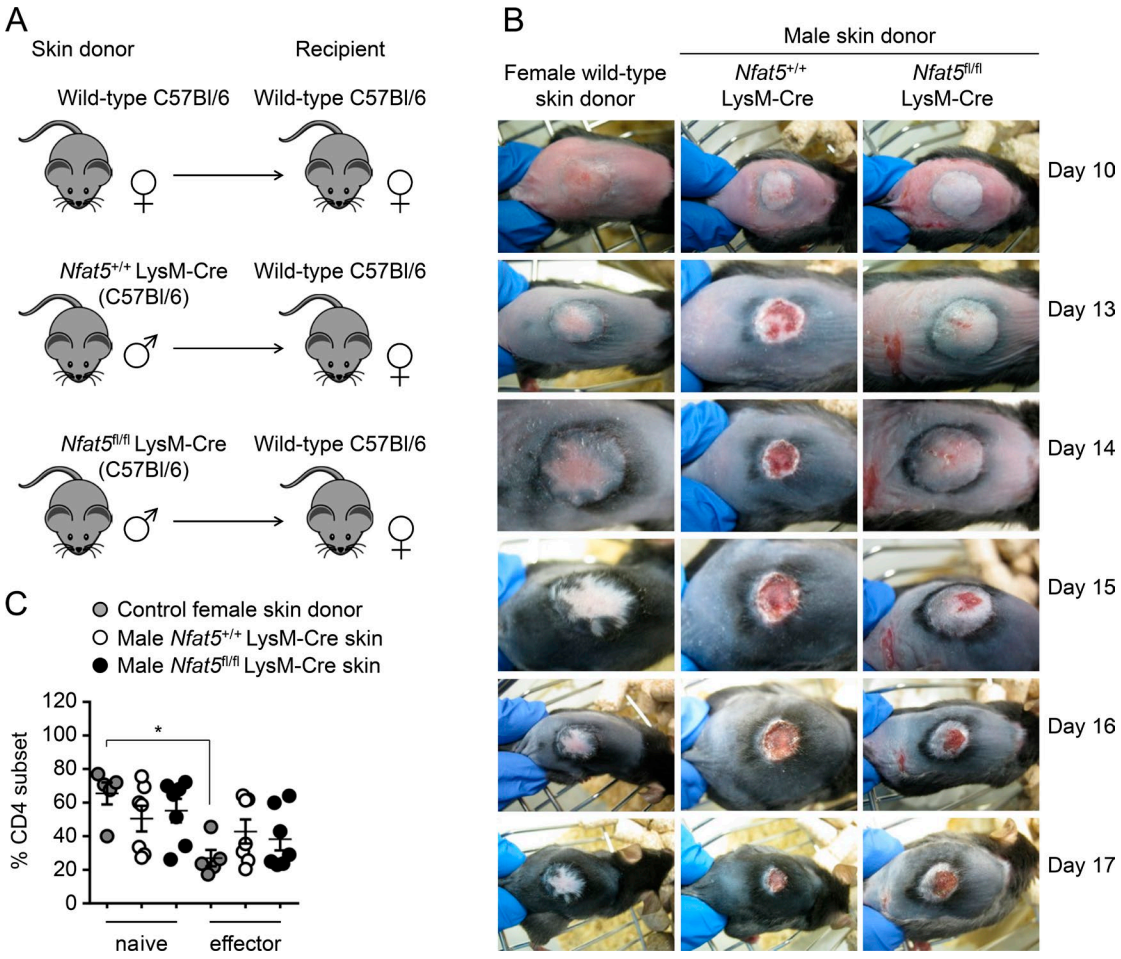


Figure S2. **Rejection of myeloid-specific NFAT5-deficient skin transplants. (A)** Diagram of the skin transplant approach. **(B)** Pictures show the time course (between days 10 and 17 after transplant) of skin rejection in female recipient mice transplanted with skin of female wild-type, male *Nfat5*$^{+/+}$ LysM-Cre, or *Nfat5*$^{fl/fl}$ LysM-Cre donors. Survival data for skin grafts of each mouse genotype are shown in Fig. 3 B. **(C)** Proportion of naive and effector CD4$^+$ T cells in the spleen of transplanted mice. CD4$^+$ T cells were analyzed in five independent transplant experiments, each of them including skin from *Nfat5*$^{+/+}$ LysM-Cre male (wild-type) and *Nfat5*$^{fl/fl}$ LysM-Cre male donors transplanted in female recipients and a control transplant of wild-type female skin to female recipient, as shown in Fig. 3 C. Each group of recipient mice was sacrificed on the day when clear rejection was observed for the wild-type skin graft (between days 12 and 16 after transplant). Results in the graphics are the mean ± SEM. Statistical significance was determined by a Mann–Whitney test. *, $P < 0.05$.

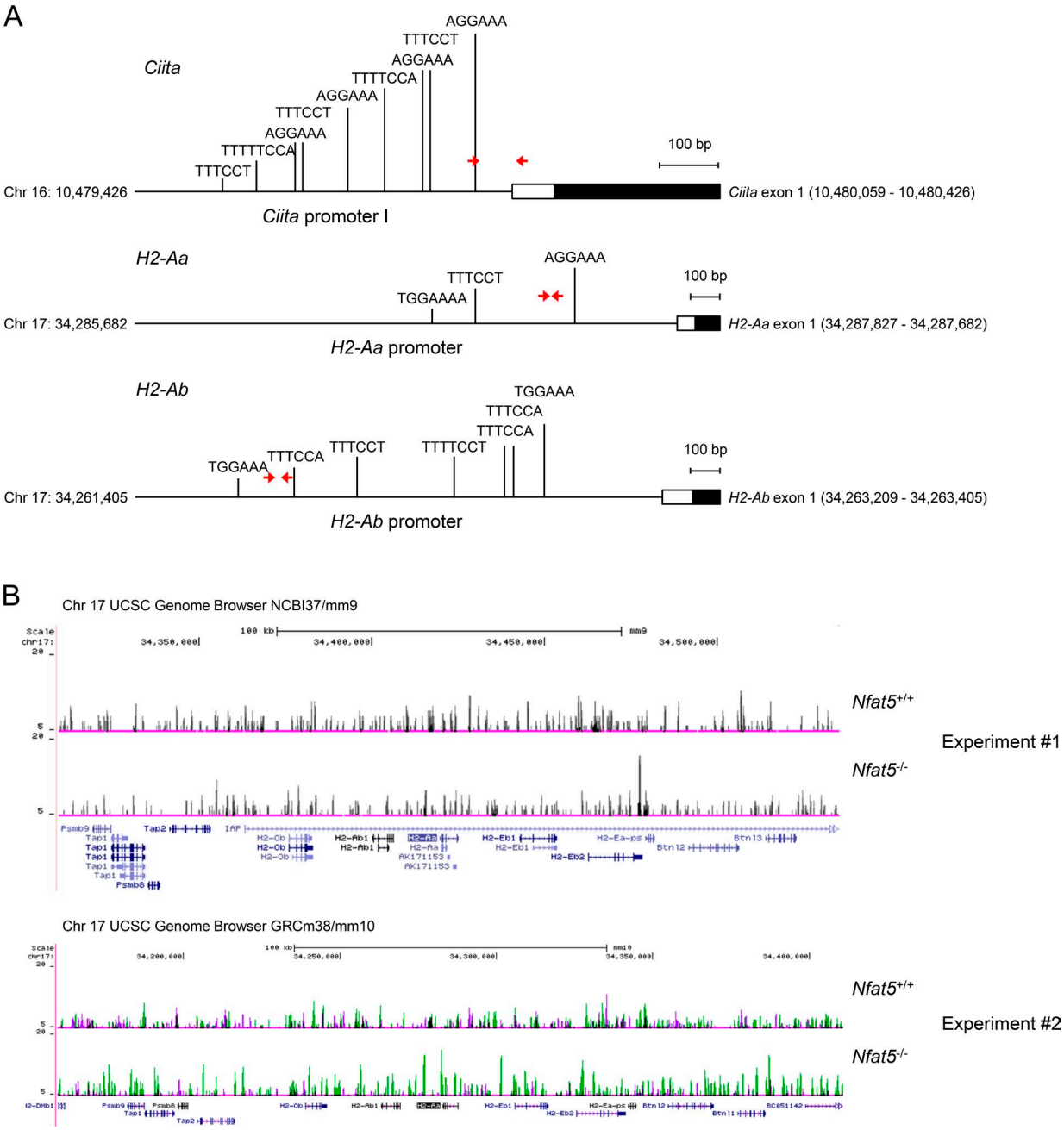
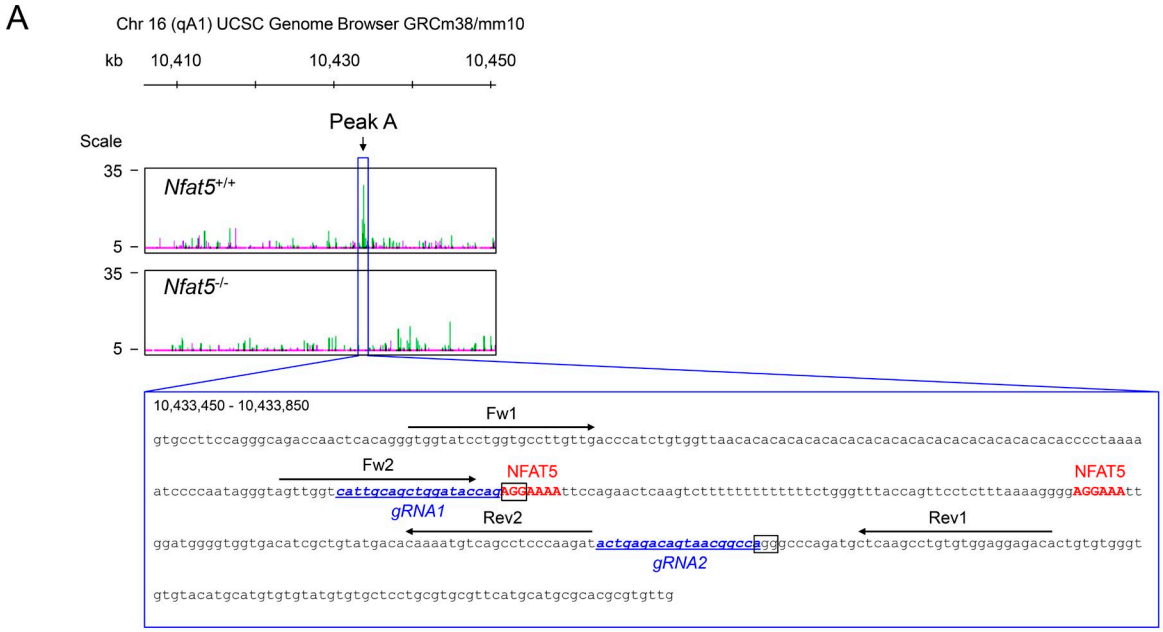


Figure S3. **Potential NFAT5 binding sites in the promoters of *Ciita* and MHCII genes and NFAT5 ChIP-seq analysis in the MHCII locus region. (A)** Diagram illustrating the presence of potential NFAT5 consensus binding sites ([A/T]GGAAA) in promoter regions of *Ciita* and MHCII genes *H2-Aa* and *H2-Ab*. Exon 1 of each gene is depicted. Sequences were obtained from Ensembl (NM_001243760.2 for *Ciita*, ENSMUST00000040655.12 for *H2-Aa*, and ENSMUST00000040828.5 for *H2-Ab*). Red arrows indicate the positions of primers used to amplify the immunoprecipitated chromatin in Fig. 4 A. **(B)** ChIP-seq analysis of NFAT5 binding to the MHCII locus region in wild-type and NFAT5-deficient BMDMs. Graphics correspond to the two independent ChIP-seq experiments shown in Fig. 4 B. The bottom panel includes the density of sequencing reads for whole chromatin (peaks in purple) before ChIP with anti-NFAT5 antibodies (green peaks). These experiments show no evident binding of NFAT5 in the chromatin regions analyzed. The accession numbers for these datasets are GSE107948 (upper panel) and GSE107950 (lower panel).



B

Expected size of PCR products with forward (Fw) and reverse (Rev) diagnostic primer pairs

	Control	Δ145
Fw1-Rev1:	301 bp	156 bp
Fw1-Rev2:	250 bp	no product
Fw2-Rev1:	202 bp	57 bp

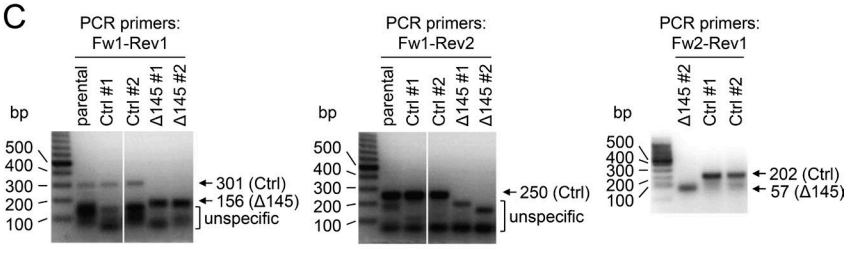


Figure S4. **CRISPR-Cas9 gene editing strategy for deletion of peak A in the distal *Ciita* enhancer. (A)** DNA sequence of the region comprising peak A is shown with the positions of gRNA1 and gRNA2 used for CRISPR-Cas9–mediated deletion of a 145-bp region in *Ciita* peak A and the primers used to screen iBMDM clones. NFAT5 consensus binding sites are shown in capital letters, and boxed sequences mark the expected cut sites. **(B)** Table indicating the predicted length of PCR products amplified from genomic DNA of control clones and clones with a deletion of peak A (Δ145) with the sets of diagnostic primers indicated in A. **(C)** PCR analysis with diagnostic primers in the parental iBMDMs, control clones (Ctrl), and Δ145 clones (Δ145).

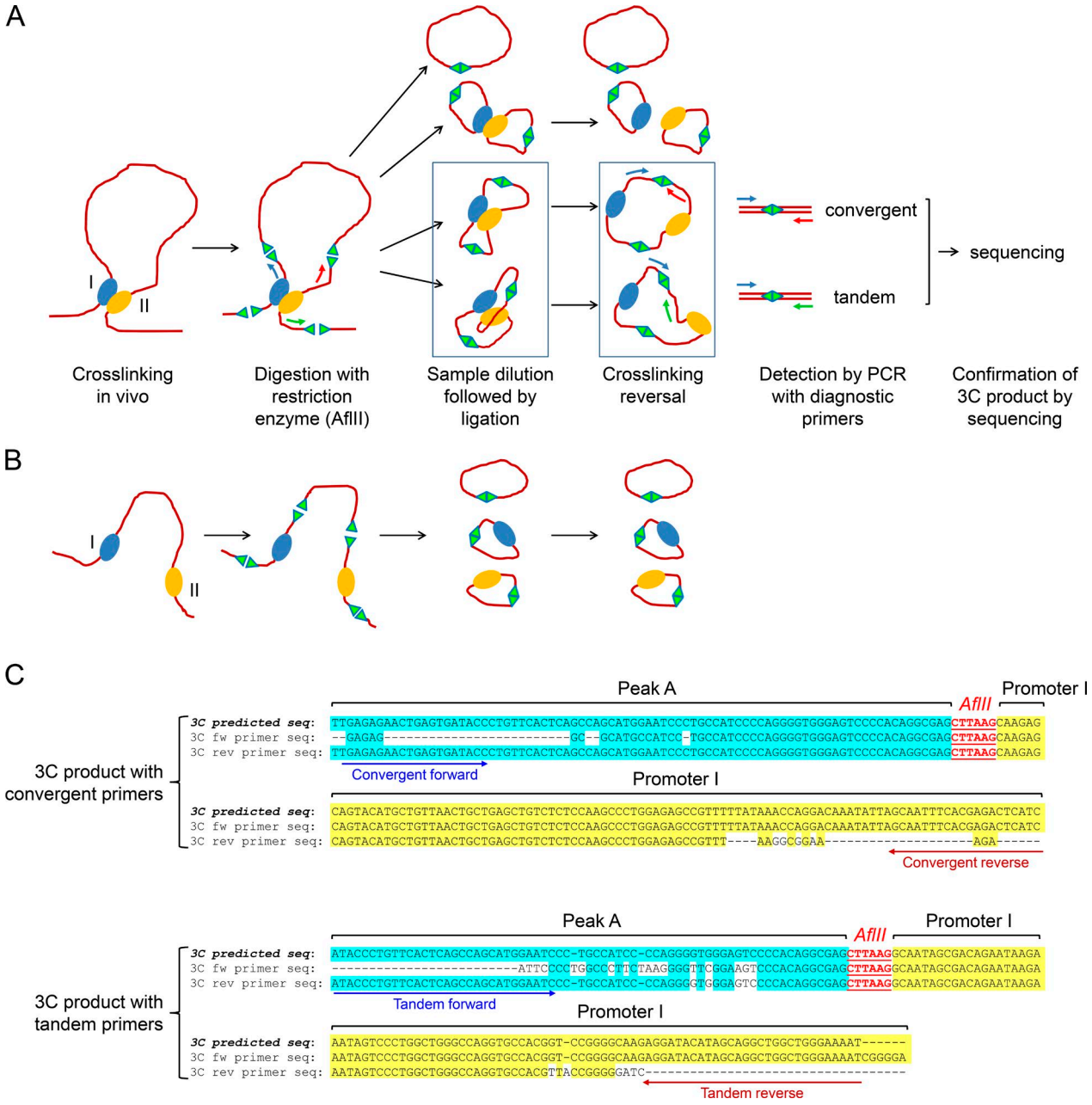


Figure S5. **3C assay between peak A and promoter I. (A and B)** Schematic diagram of the 3C assay. In A, genomic regions I and II (such as peak A enhancer and *Ciita* promoter I) are brought together in vivo by the action of chromatin regulators or transcription factors, such as NFAT5. Upon cross-linking, digestion with a specific restriction enzyme (in this case AflII, indicated by small triangles), and ligation, they will yield a mixture of ligated products, some of which would occur only if regions I and II were initially juxtaposed (represented in the boxes). These can be identified by PCR with diagnostic convergent or tandem primers and confirmed by sequencing. In B, regions I and II are not interacting, and the 3C assay will not produce ligated products amplifiable with the diagnostic primers. **(C)** Comparison of the predicted sequence of the expected 3C product and the sequence of the PCR products obtained with convergent or tandem primers in the 3C assay shown in Fig. 5 D with wild-type BMDMs. Sequences of 3C products with both forward and reverse primers show extensive alignment with peak A (blue overlay) and *Ciita* promoter I (yellow overlay) regions, respectively upstream and downstream of the *AflII* site (marked in red underlined). Positions of forward and reverse sequencing primers are indicated with arrows.

Table S1. **Microarray analysis of mRNA expression of histocompatibility and T cell costimulation genes in wild-type and NFAT5-deficient BMDMs**

Gene name	Fold change	P value	Adjusted P value
MHCII-related			
Ciita	**0.272**	**0.000004**	**0.0100**
Cd74	**0.386**	**0.000010**	**0.0180**
H2-Aa	**0.206**	**0.000004**	**0.0104**
H2-Ab1	**0.285**	**0.000007**	**0.0138**
H2-Eb1	**0.244**	**0.000016**	**0.0247**
H2-DMa	**0.324**	**0.000101**	**0.0909**
H2-DMb1/2	**0.448**	**0.000511**	**0.2141**
H2-Eb2	0.921	0.458000	0.9993
Btn2a2	1.103	0.317000	0.9993
Creb1	0.982	0.836000	0.9993
Nfya	1.118	0.241000	0.9993
Nfyb	0.833	0.082000	0.9993
Nfyc	0.909	0.282000	0.9993
Rfx1	0.986	0.884000	0.9993
Rfx2	0.942	0.425000	0.9993
Rfx3	0.947	0.678000	0.9993
Rfx4	0.980	0.823000	0.9993
Rfx5	0.931	0.553000	0.9993
Rfx6	1.031	0.787000	0.9993
Rfx7	0.804	0.203100	0.9993
Rfx8	1.011	0.926000	0.9993
Rfxap	0.972	0.629000	0.9993
Rfxank/Nr2c2ap	0.860	0.185000	0.9993
T cell costimulation			
Itgal (CD11a)	0.862	0.333000	0.9993
Itgb2 (CD18)	0.902	0.150000	0.9993
Icam1	0.988	0.916000	0.9993
Icam2	0.984	0.817000	0.9993
Cd40	0.725	0.005820	0.6438
Cd80 (B7.1)	0.836	0.274000	0.9993
Cd86 (B7.2)	0.799	0.217000	0.9993
MHCI-related			
B2m	0.892	0.101000	0.9993
H2-D1	0.930	0.340000	0.9993
H2-K1	0.744	0.002940	0.5052
H2-M1	0.906	0.200000	0.9993
H2-M10.1	0.976	0.753000	0.9993
H2-M10.2	0.945	0.565000	0.9993
H2-M10.3	0.937	0.548000	0.9993
H2-M10.4	0.942	0.751000	0.9993
H2-M10.5	0.964	0.704000	0.9993
H2-M10.6	0.963	0.640000	0.9993
H2-M11	0.867	0.080300	0.9993
H2-M2	0.835	0.196000	0.9993
H2-M3	0.657	0.000645	0.2517
H2-M5	0.914	0.203000	0.9993
H2-M9	0.966	0.666000	0.9993
H2-Q1	0.927	0.340000	0.9993
H2-Q10	0.975	0.760000	0.9993
H2-Q2	0.921	0.517000	0.9993
H2-Q5	0.655	0.047800	0.9993
H2-Q6	0.811	0.029000	0.9993
H2-Q8	0.736	0.023600	0.9912
H2-T10	0.696	0.015100	0.8701
H2-T23	0.714	0.010600	0.7926
H2-T24	0.458	0.001340	0.3670
H2-T3	0.898	0.290000	0.9993
H2-T9	0.802	0.092600	0.9993
Tap1	0.529	0.000526	0.2175
Tap2	0.792	0.013300	0.8454
Tapbp	0.784	0.048200	0.9993

Results correspond to four independent macrophage samples of each mouse genotype. Genes marked in bold showed reduced expression in NFAT5-deficient macrophages. The accession number for this set of data is GSE26343.

Table S2. **Primers used**

Gene, gene region, primer, gRNA sequence, or diagnostic primer	Forward (5′–3′)	Reverse (5′–3′)
mRNA analysis by RT-qPCR (gene)		
Nfat5	CAGCCAAAAGGGAACTGGAG	GAAAGCCTTGCTGTGTTCTG
Ciita	AGGCCTATGCCAACATTGCG	CCATAGCATGCTCTTCCGGG
Ciita Exons 16-18	TGCGTGTGATGGATGTCCAG	CCAAAGGGGATAGTGGGTGTC
Ciita-pI	ACAGGGACCATGGAGACCATAG	GGGTCGGCATCACTGTTAAGG
Ciita-pIII	GCCGGAGTTGCAAGACCATAG	GGGTCGGCATCACTGTTAAGG
Ciita-pIV	GAGACTGCATGCAGGCAGCAC	GGGTCGGCATCACTGTTAAGG
Cd74	TTGCTGATGCGTCCAATGTC	GGGTCATGTTGCCGTACTTG
H2-Aa	AGGTGAAGACGACATTGAGG	AACTCAGGAAGCATCCAGAC
H2-Ab	CCATTACCTGTGCCTTAGAG	GAACTGGTACACGAAATGCC
H2-DMb1	AGCCTTCTCCAGCGTTTGC	TTTGGGCTACTCGGACAGATG
H2-K1	CGCTGATCACCAAACACAAGTG	CAGCACCTCAGGGTGACTTTATC
CREB1	TCAAGCCCAGCCACAGATTG	ATTGGGCAGCTGCACTAAGG
NF-YA	AAGTCCAGACCCTCCAGGTAG	GGCACAGCCTGTACCATGATG
Rfx5	GCAGAGCGTCTATGATGCCTATC	GCCTTGATGTCAGGGAAGATCTC
Nubp1	CGTTGGGAAAAGCACGTTCAG	TGGCCCACAGATATCGATGTC
Tvp23a	AGCCAGAAAGGTCTCTGCAAAC	CAGATCACTGGGCAGATGATGAG
ChIP (gene region)		
H2-Aa promoter	AGGTGGATCATCTCACAATTTGG	GCTTGCATGCATCATGAGTTAGC
H2-Ab promoter	AGGCAGAGGCTGCAGATTATTG	AGCAGACAAACATGGCCATTC
Ciita promoter I	CTGCACCGGAATGAGGAAAC	AGCCTTGCAGCATCCAAAAC
Ciita peak A	GGTGGTGACATCGCTGTATGAC	TCTCCTCCACACAGGCTTGAG
Ciita exon 2	AGAGGGCAGCTACCTGGAACTC	GCCAGGTCCATCTGGTCATAG
3C (primer)		
Convergent	TGAGAGAACTGAGTGATACC	GATGAGTCTCGTGAAATTGC
Tandem	ATACCCTGTTCACTCAGCCAGCATGGAATC	ATTTTCCCAGCCAGCCTGCTATGTATCCTC
Ligation control	TGGGTTTGTGCACATGAATGAAGGTGTCTG	CTCACCCGCAATGATGATGAC
Loading control	GGTGGTGACATCGCTGTATGAC	TCTCCTCCACACAGGCTTGAG
CRISPR-Cas9 genome editing		
gRNA sequences	CATTGCAGCTGGATACCAG (gRNA1-target site)	ACTGAGACAGTAACGGCCA (gRNA2-target site)
Diagnostic primers	GTGGTATCCTGGTGCCTTGTTG (Fwd1)	TGTCTCCTCCACACAGGCTTGAG (Rev1)
	AGTTGGTCATTGCAGCTGGATAC (Fwd2)	ATCTTGGGAGGCTGACATTTTG (Rev2)