

# The CpG Dinucleotide Adjacent to A $\kappa$ B Site Affects NF- $\kappa$ B Function through Its Methylation

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Table S1:  $\kappa$ B sequences and their bearing genes used in this study.

$\kappa$ b site sequence	Human gene name							
GGGAATTCC	<i>CCL2</i>	<i>NFKB1A</i>	<i>CD40</i>	<i>NOD2</i>	<i>TNFRSF9</i>	<i>PLAU</i>	<i>CXCL5</i>	<i>MUC2</i>
GGGACTTCC	<i>FABP6</i>	<i>CD54</i>	<i>TNFAIP3</i>	<i>LMP2</i>	<i>TICAM1</i>	<i>TP53</i>		
GGGATTCC	<i>VCAM1</i>	<i>APOC3</i>	<i>IL15RA</i>	<i>TNIP3</i>	<i>BCL3</i>	<i>NR4A2</i>		
GGGGTTTCC	<i>CCL20</i>	<i>TRPC1</i>	<i>CD80</i>	<i>RELB</i>	<i>LIPG</i>	<i>TP53</i>		
GGGGATTCC	<i>SELE</i>	<i>TNFAIP3</i>	<i>ELF3</i>	<i>IRF2</i>				
GGGGCTTCC	<i>CD48</i>	<i>VIM</i>	<i>PI3</i>	<i>NFKB2</i>				
GGGATTTCC	<i>BLR1</i>	<i>CD69</i>	<i>IL6</i>					
GGGGAATCC	<i>RELB</i>	<i>TFPI2</i>						
GGGAGTTCC	<i>CCL5</i>	<i>IRF7</i>						
GGGGCTCC	<i>LTA</i>	<i>NFKB1</i>						
GGGAGATCC	<i>FLRG</i>	<i>CCL11</i>						
GGGAATCCTCC	<i>IL2RA</i>	<i>GZMB</i>						
GGGGTTCC	<i>EBI3</i>	<i>BLR1</i>						
GGGGAGCC	<i>CD74</i>	<i>IL1A</i>						
GGGAACTCC	<i>CD40</i>	<i>BLR1</i>						
GGGAATCC	<i>CD40</i>	<i>CCL2</i>						
GGGACCTCC	<i>GSTP1</i>							
GGGGAATCC	<i>IRF1</i>							
GGGGATTCC	<i>PTGS2</i>							
GGGACGTCC	<i>CREB3</i>							
GGGATCCTCC	<i>CD44</i>							
GGGGAATCC	<i>PTX3</i>							
GGGAATCC	<i>NFKB2</i>							
GGGGATCC	<i>CCL5</i>							
GGGGAGTCC	<i>EDN1</i>							
GGGAAATCC	<i>IFNB1</i>							
GGGACATCC	<i>LCN2</i>							
GGGAGCTCC	<i>ADAM19</i>							
GGGAACCTCC	<i>KCNK5</i>							
GGGATCTCC	<i>BTK</i>							
GGGACATCC	<i>ALOX12</i>							

Red, -1C  $\kappa$ b site; blue, -1C or D  $\kappa$ b site; green background,  $\kappa$ b site in CGI.

Table S2. Oligonucleotides used for DAPA

Oligonucleotide	Sequence (5'→3')
<i>FABP6</i> kb site -1A	Biotin-CCTCTTCAAAGGGACTTTCCTACAAGGGACTTTCCTTCCCGTCTA TAGACGGGAAGGAAAGTCCCTTGTAGGAAAGTCCCTTTGAAGAGG
<i>FABP6</i> kb site -1C	Biotin-CCTCTTCAAACGGGACTTTCCTACACGGGACTTTCCTTCCCGTCTA TAGACGGGAAGGAAAGTCCCGTGTAGGAAAGTCCCGTTGAAGAGG
<i>FABP6</i> kb site -1 5mC	Biotin-CCTCTTCAA/5mC/GGGACTTTCCTACA/5mC/GGGACTTTCCTTCCCGTCTA TAGACGGGAAGGAAAGTCC/5mC/GTGTAGGAAAGTCC/5mC/GTTGAAGAGG
<i>CCL2</i> kb site -1A	Biotin-CCTCTTCAAAGGGAACTTTCCTACAAGGGAATTCCTTCCCGTCTA TAGACGGGAAGGAAATTCCTTGTAGGAAAGTCCCTTTGAAGAGG
<i>CCL2</i> kb site -1C	Biotin-CCTCTTCAAACGGGAACTTTCCTACACGGGAAATTCCTTCCCGTCTA TAGACGGGAAGGAAATTCCTCGTGTAGGAAAGTCCCGTTGAAGAGG
<i>CCL2</i> kb site -1 5mC	Biotin-CCTCTTCAA/5mC/GGGAATTCCTACA/5mC/GGGAATTCCTTCCCGTCTA TAGACGGGAAGGAAATTC/5mC/GTGTAGGAAAGTTC/5mC/GTTGAAGAGG
<i>PTX3</i> kb site -1C	Biotin-CCTCTTCAAAGGGAACTCCCGTACAAGGGAACTCCCGTTCCCGTCTA TAGACGGGAACGGGAGTTCCCTTGTACGGGAGTCCCTTTGAAGAGG
<i>PTX3</i> kb site -1 5mC	Biotin-CCTCTTCAAAGGGAACTCC/5mC/GTACAAGGGAACTCC/5mC/GTTCCCGTCTA TAGACGGGAA/5mC/GGGAGTTCCCTTGT/5mC/GGGAGTCCCTTTGAAGAGG
<i>FLRG</i> kb site -1C	Biotin-CCTCTTCAAAGGGAGATTCCCGTACAAGGGAGATTCCCGTTCCCGTCTA TAGACGGGAACGGGAATTCCTTGTACGGGAATTCCTTTGAAGAGG
<i>FLRG</i> kb site -1 5mC	Biotin-CCTCTTCAAAGGGAGATTCC/5mC/GTACAAGGGAGATTCC/5mC/GTTCCCGTCTA TAGACGGGAA/5mC/GGGAATTCCTTGT/5mC/GGGAATTCCTTTGAAGAGG
NC	Biotin-CCTCTTCAAATTGAACTTACTACAATTGAACTTACTTCCCGTCTA TAGACGGGAAGTAAGTTCAATTGTAGTAAGTTCAATTTGAAGAGG

Table S3. Primers used for Plasmids construction and site-directed mutagenesis

Primer	Sequence (5'→3')
FABP6- f	CGCGTCTCTTCAAAGGGACTTTCCTACAAGGGACTTTCCTTCCCGTCTAC
FABP6- r	TCGAGTAGACGGGAAGGAAAGTCCCTTGTAGGAAAGTCCCTTTGAAGAGGA
FABP6-M-f	CGCGTCTCTTCAAACGGGACTTTCCTACACGGGACTTTCCTTCCCGTCTAC
FABP6-M-r	TCGAGTAGACGGGAAGGAAAGTCCCGTGTAGGAAAGTCCCGTTGAAGAGGA
CCL2-E-f	ACGTGGTACCGTGTGTCCCAAGCGAG
CCL2-E-r	GATCGTAGCTGCATCCTTACCATGAACT
CCL2-P-f	GATCGTAGCAATTCAGTTCAATGTTTACA
CCL2-P-r	AGTCTCGAGGCTGGAGGCGAGAGTGCGAG
CCL2-Mut1-f	GCATTCTTCTACGGGATCCGGAACTTCCAAAGCTGC
CCL2-Mut1-r	GCAGTTTGGAAAGTCCCGGATCCCGTAGAAGAGAATGC
CCL2-Mut2-f	CAAAGCTGCCTCCTCAGAGCGGGAATTCCTACTACTTC
CCL2-Mut2-r	GAAAGTGTAGTGGAAATTCCTCGTCTGAGGAGGCAGCTTG

Table S4. Primers used for BSP

Primers used for BSP	
Primer	Sequence (5'→3')
GSTP1-M-f	TTTGTTGTTTGTATTTTTTAGGTTT
GSTP1-M-r	ATACTAAAACTCTAAACCCCATCC
RelB-M-f	GTTTAAGTTTATTGGGAGATTAAAG
RelB-M-r	ACCCAAAACTAACCCAAACC
IRF7-M-f	GGGTTTTAGTATTTGGGTGTTAGAG
IRF7-M-r	AAACTATAATAAAATAACTCCATCTC
TNFIP3-M-f	GYGGGGTAGGGAAAGG
TNFIP3-M-r	AACTCCAACTCRCTTAACC
TFPI2-M-f	TTTTATGTTTTTAAGAGGTGGATT
TFPI2-M-r	AAACCTAAAAATAACTAATTCATACAC
PTX3-M-f	GATTTTTTTTTAATTAATTTGATTGTAG
PTX3-M-r	AAATCCTTAATAAATACTAAAAAAACC
FLRG-M-f	TTTTGTGTTTGTATTTTTTTAAGT
FLRG-M-r	CCTAACCTTAACCTCTAACATTTC
CCL2-M-f	TTTTATAGTTTTTTGGGGTTTT
CCL2-M-r	ATCTACCTCCACTCTACTCTATCAA

Table S5. Primers used for RT-PCR

Primer	Sequence (5'→3')
RelB-T-f	GCCTCGTGGGAAAGACT
RelB-T-r	TGTCGAGAGCAAGTAGAGC
TNFAIP3-T-f	ACCCATTGTTCTCGGCTAT
TNFAIP3-T-r	CCCTGCTCGCTGTTTTCC
CCL2-T-f	TAGCAGCCACCTTCATTCC
CCL2-T-r	GCTTGGGGTCAGCACAGAT
IκBα-T-f	AGTACGAGCAGATGGTCAAG
IκBα-T-r	TCATGGATGATGGCCAAGTG
TFPI2-T-f	CCCTACTTCTCCGTTACTACT
TFPI2-T-r	TCTGAAAACCTGTTCTCAAT
IRF7-T-f	CAGATCCAGTCCCAACCAA
IRF7-T-r	GCAGCAGTTCCTCCGTGT
GAPDH-T-f	AGCCACATCGCTCAGACACC
GAPDH-T-r	ACGTACTCAGCGCCAGCATC

U937 *CCL2* κB site (-2822/-2513)

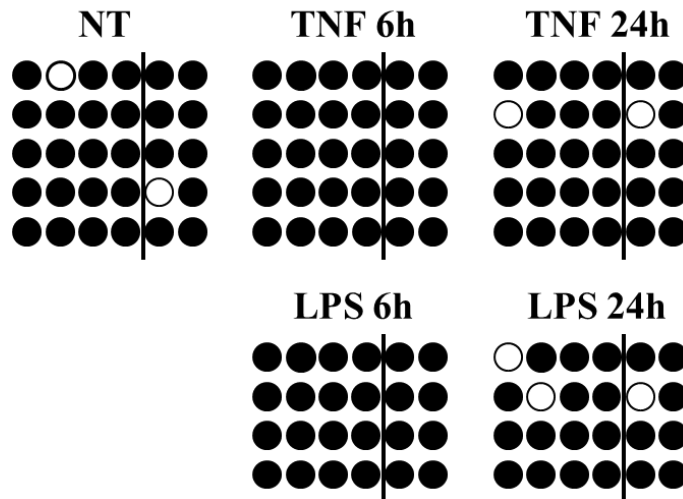


Figure S1. Methylation status around the κB sites of *CCL2* was not changed upon NF-κB activation by TNFα or LPS in U937 cells. Genomic DNAs were isolated from U937 cells treated with TNFα (10 ng/ml) or LPS (1μg/ml) for the indicated time and methylation status was determined by BSP. The κB site was shown as a vertical line.

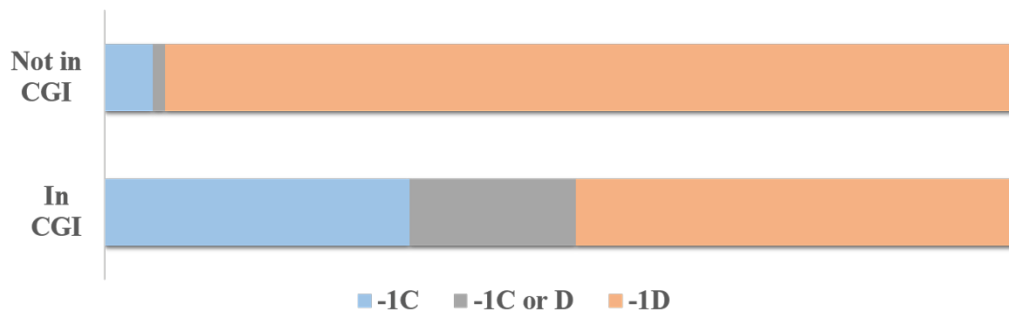


Figure S2. The ratio between -1C κB sites and total κB sites outside CGIs is substantially lower than that inside CGIs in *VCAM*, *CCL2* and *RelB* of multiple vertebrate.