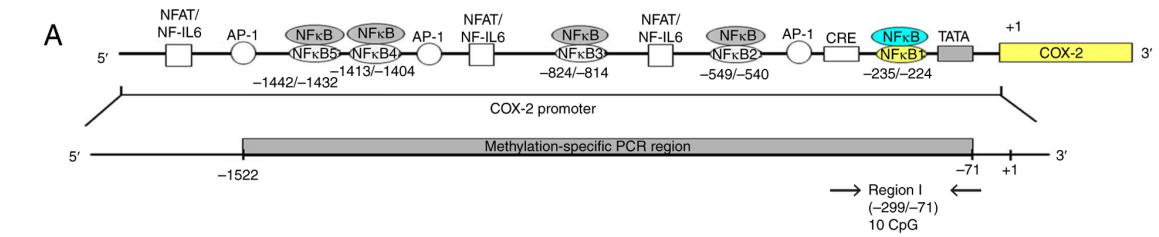
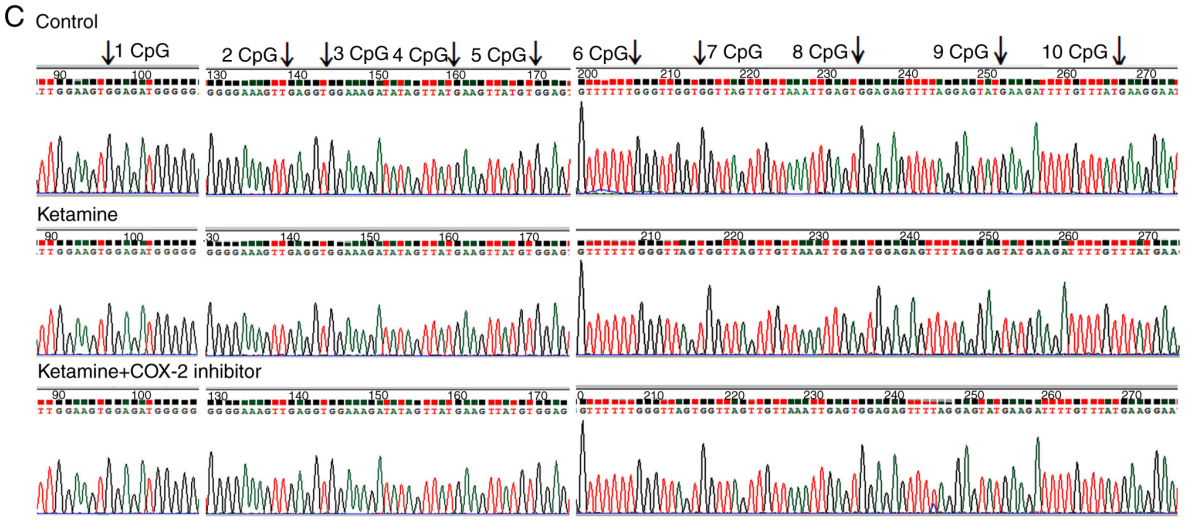
Figure S1. Detailed bisulfite genomic sequencing of the COX-2 promoter region ranging from -299 to -71 bp. (A) Schematic diagram of region I ranging from -299 to -71 bp in the COX-2 promoter for MSP was presented. (B) Bisulfite genomic sequencing data of promoter fragment containing 10 CpG sites were marked with red number. The potential sequence for NF-κB transcriptional factor binding in region I was marked with square box. Bisulfite sequence of mNFκB1 was located at -235 to -224 bp, 5'-GGGGAAAGT CGA-3' (Genomic NF-κB binding site (NFκB1): 5'-GGGGAAAGCCGA-3'). (C) Sequence traces obtained from the PCR products were from bisulfite-treated DNA using primers (blue color in bisulfate sequence). The trace represented an approximation of the 'average' methylation status at each CpG residue. CpG sites were highlighted as arrows. (D) Detailed normal and bisulfite genomic sequencing of region I spanned from -299 to -71 bp in the COX-2 promoter were presented. COX, cyclooxygenase; NF, nuclear factor.



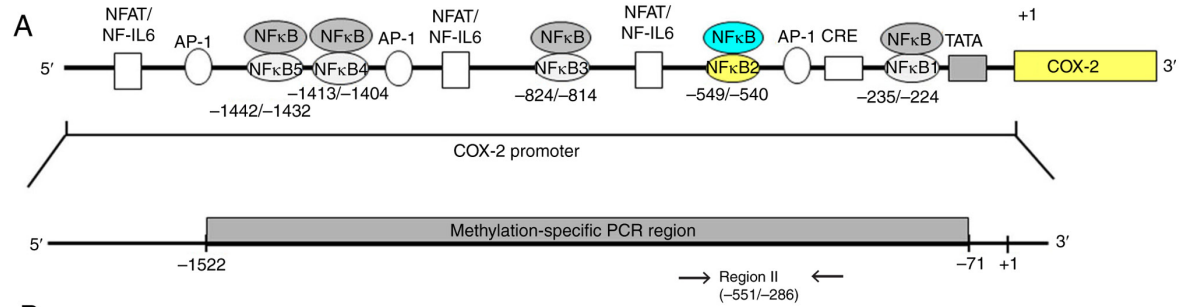


D

	Region I	1	60	120
	Genomic	CTGCCCCTATGGGTATTATGCAATTGGAAGCGGAGATGGGGGAAAGCTGGGGGGGTGGGG	GGGTGGGGAAAGCCGAGGCGGAAAGACACAGTCACGAAGTCACGTGGAGTCCACTTTACT	
	COX2 bisulfite	TTGTTTTTATGGGTATTATGTAATTGGAAGCGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTCGAGGCGGAAAGATATAGTTACGAAGTTACGTGGAGTTTATTTTATTA	
	Control-1	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTACGAAGTTATGTGGAGTTTATTTTATTA	
	Control-2	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Control-3	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Control-4	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Control-5	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Control-6	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine-1	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine-2	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine-3	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine-4	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine-5	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine-6	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine+CI-1	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine+CI-2	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine+CI-3	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine+CI-4	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine+CI-5	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	
	Ketamine+CI-6	TTGTTTTTATGGGTATTATGTAATTGGAAGTGGAGATGGGGGAAAGTTGGGGGGGTGGGG	GGGTGGGGAAAGTTGAGGTGGAAAGATATAGTTATGAAGTTATGTGGAGTTTATTTTATTA	

	Region I	121	180	181	229
	Genomic	AAGATTTAAAAGCAAGGTTCTCCGGGTTAGCGGCCAGTTGTCAAACTGAGCGGAGAGCTTC	AGGAGTACGAAGACCCTGCCTACGAAGGAACTCAGCTCTGTGTCCTGC		
	COX2 bisulfite	AGATTTAAAAGTAAGGTTTTTCGGGTTAGCGGTTAGTTGTTAAATTGAGCGGAGAGTTTTAG	GAGTACGAAGATTTTGTTTACGAAGGAATTTAGTTTTGTGTTTTGT		
	Control-1	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Control-2	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Control-3	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Control-4	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Control-5	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Control-6	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine-1	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine-2	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine-3	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine-4	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine-5	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine-6	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine+CI-1	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine+CI-2	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine+CI-3	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine+CI-4	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine+CI-5	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		
	Ketamine+CI-6	AGATTTAAAAGTAAGGTTTTTTGGGTTAGTGGTTAGTTGTTAAATTGAGTGGAGAGTTTTAG	GAGTATGAAGATTTTGTTTATGAAGGAATTTAGTTTTGTGTTTTGT		

Figure S2. Detailed methylation analysis of region II in the COX-2 promoter. (A) Schematic diagram of region II ranging from -551 to -286 bp in relation to the transcription starting site in the COX-2 promoter was presented. (B) Genomic sequencing data for region II spanned from -299 to -71 bp and contained 10 CpG sites. The promoter fragment of region II contained 10 methylatable CpG sites marked with red numbers. The potential sequence for NF-κB transcriptional factor binding in region II was marked with square box. Bisulfite sequence of mNFκB2 was located at -549 to -540 bp, 5'-GGGGATTTTT-3' (Genomic NFκB2: 5'-GGG GATTCCC-3'). Sequence traces obtained from the PCR products were from bisulfite-treated DNA using primers (blue color in bisulfate sequence). (C) Bisulfite sequencing traces of region II obtained from the PCR products were from bisulfite-treated DNA by using primers, and the CpG sites were highlighted with black arrows. The trace represented an approximation of the 'average' methylation status at each CpG residue.



B Region II-bisulfite sequence:

NFκB (–549/–540)

5′-AAGGGGATTTTTTTAGTTAGGATTTCGGATTTCGGGAGGGGAAGTTGTGATATTTTTTTGTTTTTTCGGTTTTTTAGT

GGATGCGGGATTGGGAGGAAATTCGAGATTTTAAAGAGAGTTAGTTTTGGAGTAGGTATAGCGAATTATAGGGCGTTT

GGAAGGATGTAGAGGGCGGTGTAGTTTTTTTGGTATTATTTTGGGTAGTCGAGGGAAGTTTTTTGGTTTTTTTGGGTTT

ATTTGCGTGAGTAAAGTTTGTTTTTATGGGT-3′

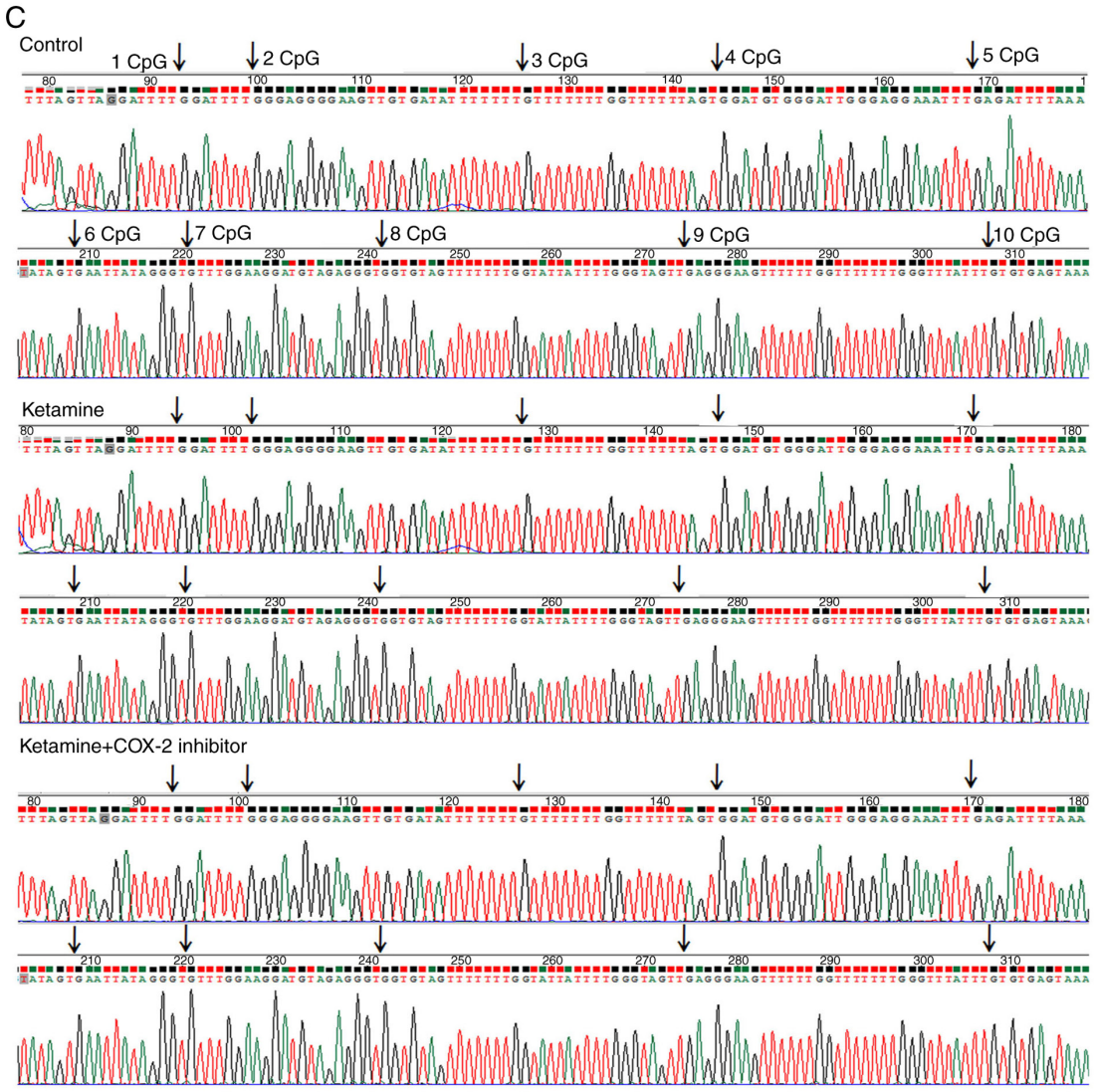


Figure S2. Continued. (D) Detailed normal and bisulfite genomic sequences of region II ranged from -299 to -71 bp in the COX-2 promoter. COX, cyclooxygenase.

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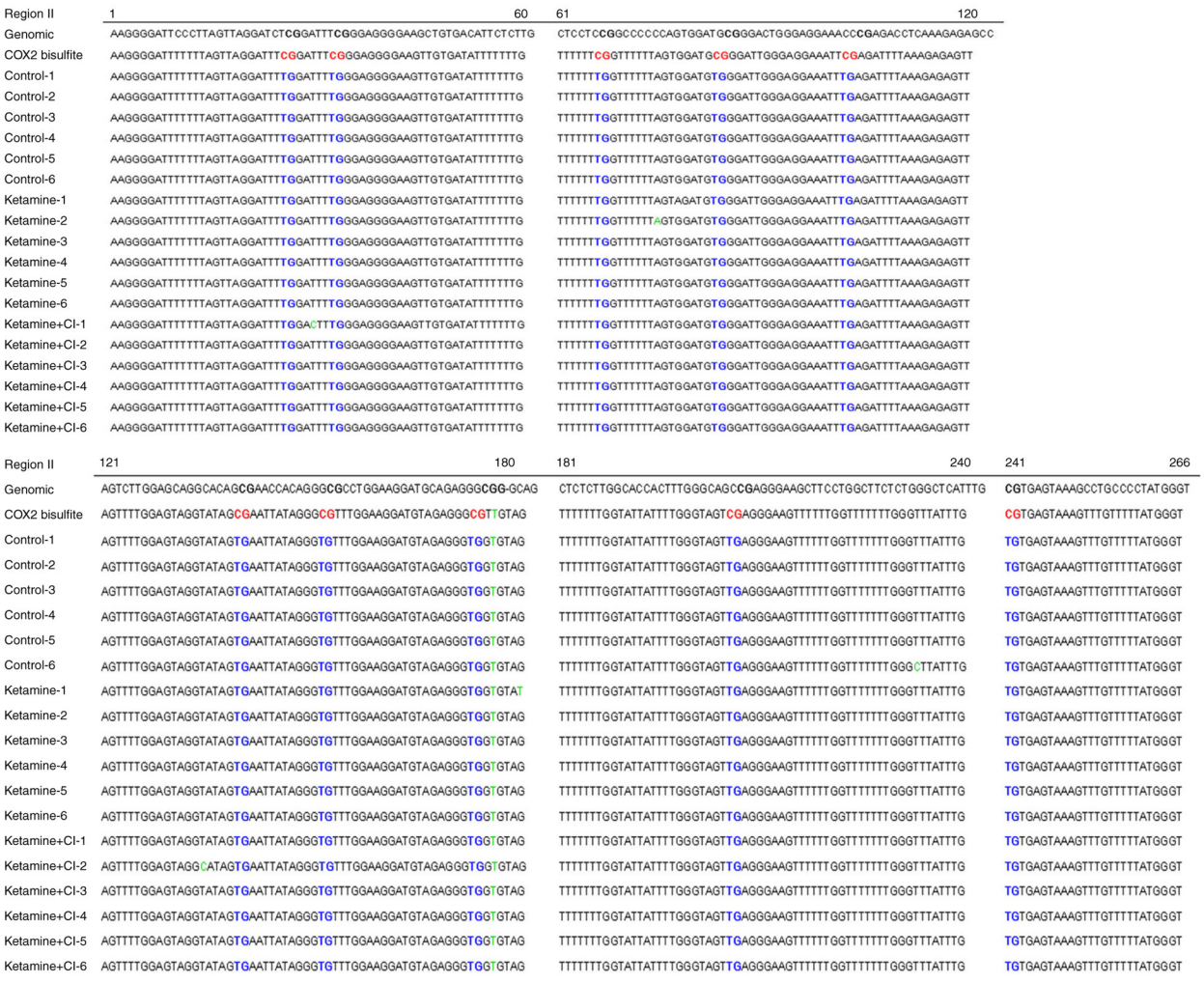

Figure S3. Detailed normal and bisulfite genomic sequences of region III ranging from -830 to -548 bp were shown. n=6.

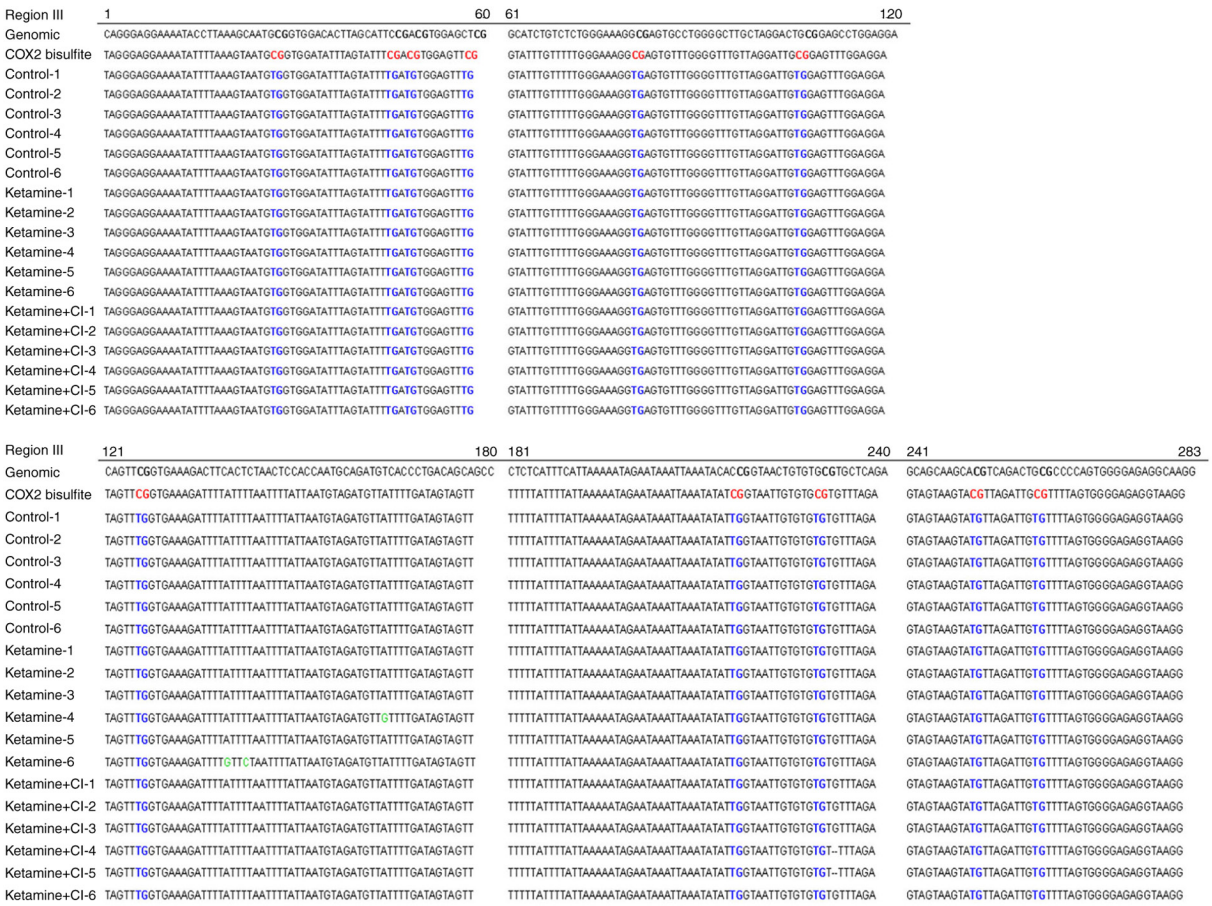

Figure S4. Detailed normal and bisulfite genomic sequences of region IV ranging from -1,195 to -829 bp were determined by bisulfite sequencing analysis. n=6.

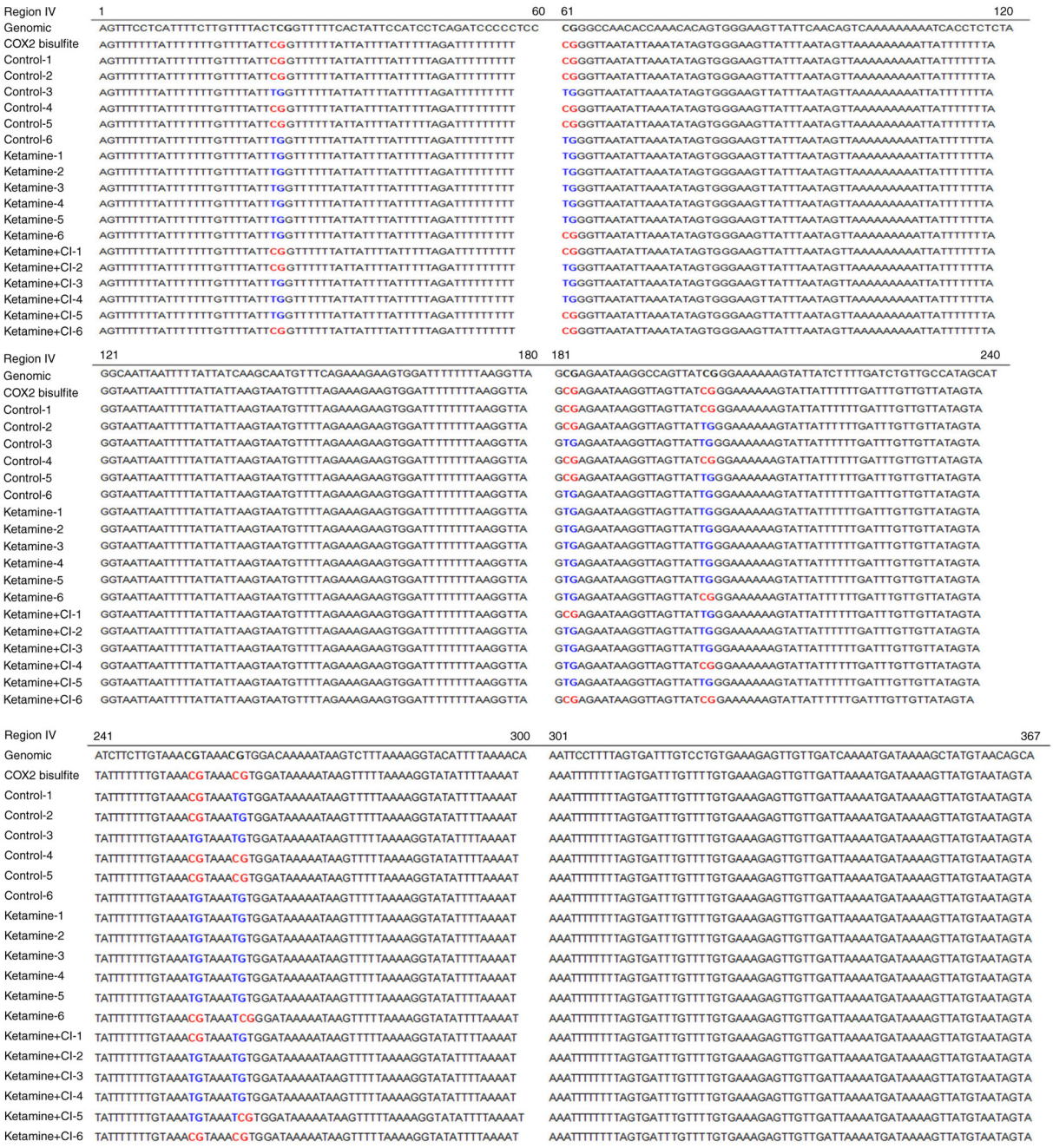

Figure S5. Detailed normal and bisulfite genomic sequences of region V ranging from -1,522 to -1,181 bp were determined by bisulfite sequencing analysis. n=6.

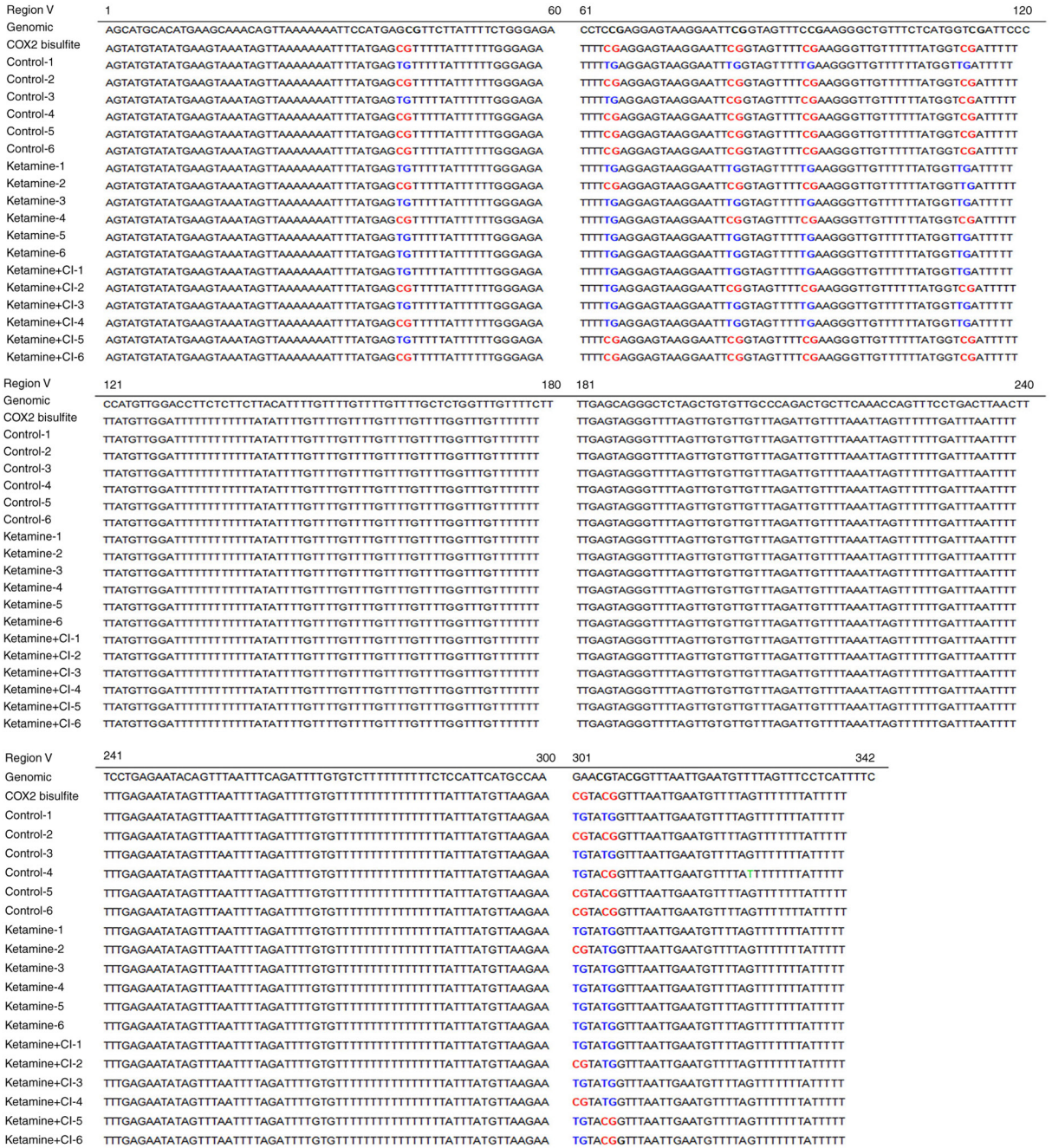

Figure S6. The NF-κB binding sites and the potential methylation of CpG sites in the COX-2 promoter region were compared between rat and human species. (A) Schematic diagram of human COX-2 promoter spanning the region from -2,000 to -1 bp with respect to the ATG start site (+1) (translation initiation site). Additionally, seven potential NF-κB DNA binding sites in the region were identified by TFBIND software. Two potential NF-κB binding sequences for methylation analysis were denoted. (B) Schematic diagram of rat COX-2 promoter spanning the region from -1,522 to -71 bp with respect to the ATG start site (+1). Five putative NF-κB DNA binding sites in the region were identified. Additionally, five putative NF-κB binding sequences for methylation analysis were denoted. NF, nuclear factor, COX, cyclooxygenase.