

## Supplementary Material

### Context-dependent Cooperation Between NF- $\kappa$ B and the Glucocorticoid Receptor at a TNFAIP3 enhancer: A Mechanism to Maintain Negative Feedback Control of Inflammation

Mohammed O. Altonsy, Sarah K. Sasse, Tzu L. Phang and Anthony N. Gerber

**Table S1**

<b>qPCR primers</b>	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
IL1 $\alpha$	CAT CCT CCA CAA TAG CAG ACA G	GAG TTT CCT GGC TAT GGG ATA AG
IL1 $\beta$	CAAAGGCGGCCAGGATATAA	CTAGGGATTGAGTCCACATTGAG
IL6	TGA CCC AAC CAC AAA TGC	AGG AAC TCC TTA AAG CTG CG
IL8	CTT GGC AGC CTT CCT GAT TT	GGG TGG AAA GGT TTG GAG TAT G
TNF	AGAGGGAGAGAAGCAACTACA	GGGTCAGTATGTGAGAGGAAGA
HBEGF	AGC TCT TTC TGG CTG CAG TTC TCT	TCC AGA TCT GCC TCT TGC AAG TCA
TNFAIP3	AGTGTTCCTCCAGGTGGCCTTAGAAA	TCTCAGCCAAGACGATGAAGCAGT
TNIP1	AACAAGCAGTGGGACCAGCATTTC	TGCTTCTGCAAATCAGCCAGCTTC
TNIP2	TCTGCCTCGAACCAGTCAAATGGA	AACTTCCGGCTGGATGACAAATGC
NFKBIA	GAG TTA CCT ACC AGG GCT ATT C	CTC TCC TCA TCC TCA CTC TCT
DUSP1	ATGCTCCTTGAGAGGAGAAATG	GAGGAACTCGGGTGAAGTTAAA

**Table S2**

<b>ChIP Primers &amp; Sequence location</b>	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
FKBP5 (+134724 / +134941)	TAA CCA CAT CAA GCG AGC TG	GCA TGG TTT AGG GGT TCT TG
IL8 (-65 /+15)	GAGCACTCCATAAGGCACAA	TTCCTTCCGGTGGTTTCTTC
IL1 $\beta$ (-468/-346)	TACAGACAGGGAGGGCTATT	GTGGGACAAAGTGAAGACA
IL1 $\beta$ (+24420/+24505)	GATGGAGGCTCAGGTCTAATG	CGTGTCTCAGGCTCTCATTT
TNFAIP3 (+49/+135)	GTC TAC CTG GCG TTG GTT T	CTC AAC TCC CAG CAC TTC AA
TNFAIP3 (+5936/+6047)	GCTGTTGCTCAATTGCTAGTC	CTTCTTGTGCTTACTTTTCAGTTCT T
NFKBIA (-306/-189)	CTTGACAGAGGGACAGGATTAC	GTCACGGACAGGGAACTTT
NFKBIA (+1033/+1129)	CCTTCCTCAACTTCCAGAACA	GTGACTCTGCTACATCAGCTAC

**Table S3**

Cloning Primers	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
TNFAIP3I2	GAGGGTTCTTCAAAGGCAATATC	GCCATTAATCTGTGACCTTTCC

**Table S4**

SDM Primers	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
TNFAIP3I2 (mut GBS)	GGTGCACCTTCGCAGTTTAAATG TCTACACACCATCACATATGGT CCGATAGCTGTTGCTCAATTGC TAGTCAAATAACTT	AAGTTATTTGACTAGCAATTGAGCAA CAGCTATCGGACCATATGTGATGGT GTGTAGACATTTAAACTGCGAAGTGC ACC
TNFAIP3I2/F KBP5 GBS- swap	CACTTCGCAGTTTAAATGTCTAC ACACCAGAACAGGGTGTCTAT AGCTGTTGCTCAATTGCTAGTC AAATAACTTA	TAAGTTATTTGACTAGCAATTGAGCA ACAGCTATAGAACACCCTGTTCTGGT GTGTAGACATTTAAACTGCGAAGTG

**TNFAIP3I2 NF $\kappa$ B-BS1, NF $\kappa$ B-BS2 and GR-BS Sequence (5'→3')**

GAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTTGAGGGT  
TCTTCAAAGGCAATATCTGTTTGGGGAGGGGGTGATTCACAAGTTTGCATTTGAGG  
ATATGAGACTGCGGTGAATGAGGAAGGGAGTAGGTGGCATAAAATTGAACTTCAT  
GTGAAAAATTCCAGCGTCTATTGGCTGCAGCGATTTCACTTGTATAATGAGTAGCC  
CTATTCCAGCTCACCCCTGACCACACCCA **CTTGGAAGTCCAGG** GTGCACTTCGCA  
NF $\kappa$ B-BS1 5872-5887

GTTTAAATGTCTACACA **CCAGAACA AAAAGTACAAT** AGCTGTTGCTCAATTGCTAG  
GR-BS 5916-5935

TCAAATAACTTAGCA **CTGGGGAATTCCAGA** TGTTACTTAGGGAATTTTATACTGGT  
NF $\kappa$ B-BS2 5970-5985

CATCTCAATAAAGA ACTGAAAGTAAGCACAAGAAGAAAAAAGCCTTATCTTTGCTC  
TAGATTTTGCAAAGGGGAAATTTCAACAGAACGCAATCATTGCTACACGTCTGCCA  
AGACACAAGGCTTGGGCGATCTTTTTTTGTTCAATTTGTTTGAATACTTAGCTAGTTTTT  
CTAAATGTATACATTGAAGGAATACTGGCTGTGGAAGTATATTTGAGGGTTTTTTGG  
TTTTCTTTACAGTTTGACTGTAATTACAATTTGATAAAATTTCAAATAGCTCTATTGCC  
CTACTTATAGAGTAATGTAAGAAACAATGTAACTTTTTCATTATAAAAATTATTTTGCA  
CTTGCCAAAGGAGATTAAGGAGTTAACTTTTTTTGTTTTAAACATTCACCCCAAATAT  
TTATCGTTTGGGGGTTGAAAAAATGCAGCCATTGAATTGTGAGTTTTTAACTGGA  
AAGGTCACAGATTAATGGCAAGGGCGAATTCTGCAGATATCCATCACACTGGCGG  
CCG

Supplemental Figure 1

	NF- $\kappa$ B binding site 1 <u>cttgaaagtccagg</u>
Human	ca <b>cttgaaagtccagg</b> gtg
Rhesus	ca <b>cttgaaagtccagg</b> gtg
Tarsier	ca <b>cttgaaagtccagg</b> gtg
Mouse	ca <b>cttgaaagtccagg</b> gtg
Dog	ca <b>cttgaaagtccagg</b> gcg
Opossum	ca <b>cttgaaagtccag</b> tatg
Platypus	ca <b>cttgaaagtccag</b> tatg
Chicken	ccc- <b>gggaaagtccg</b> aggga
Lizard	cact <b>tgaaagtccag</b> tatg

  

	GR binding site 1 <u>agaacaaaagtaca</u>
Human	cc <b>agaacaaaagtaca</b>
Rhesus	cc <b>agaacaaaagtaca</b>
Tarsier	cc <b>agaacaaaagtaca</b>
Mouse	cc <b>agaacaaaacgtaca</b>
Dog	cc <b>agaacaaaagtaca</b>
Opossum	cc <b>agaacaaaagtaca</b>
Platypus	cc <b>agaacaaaagtaca</b>
Chicken	gc <b>agaacaaaagtacga</b>
Lizard	tc <b>agaacaagaagtaca</b>

  

	NF- $\kappa$ B binding site 2 <u>ctggggaattccaga</u>
Human	gc <b>ctggggaattccag</b> atgt
Rhesus	gc <b>ctggggaattccag</b> atgt
Tarsier	gc <b>ctggggaattccag</b> atgt
Mouse	gc <b>ctggggaattcca</b> atgt
Dog	gc <b>ctggggaattccag</b> atgt
Opossum	gc <b>ctggggaattcca</b> agtgt
Platypus	aca <b>ctggggaattccag</b> atgt
Chicken	g <b>ctggggaattcca</b> agtgt
Lizard	gt <b>ctggggaattcca</b> agtgt

**Figure S1. Cross-species conservation of a GR binding site and two NF- $\kappa$ B sites within TNFAIP3 intron 2.** Sequence alignments extracted from the UCSC genome browser for the indicated binding sites within the second intron of TNFAIP3. Conserved residues within each binding motif are shown in red.