

# NFκB Inhibition Mitigates Serum Amyloid A-Induced Pro-Atherogenic Responses in Endothelial Cells and Leukocyte Adhesion and Adverse Changes to Endothelium Function in Isolated Aorta

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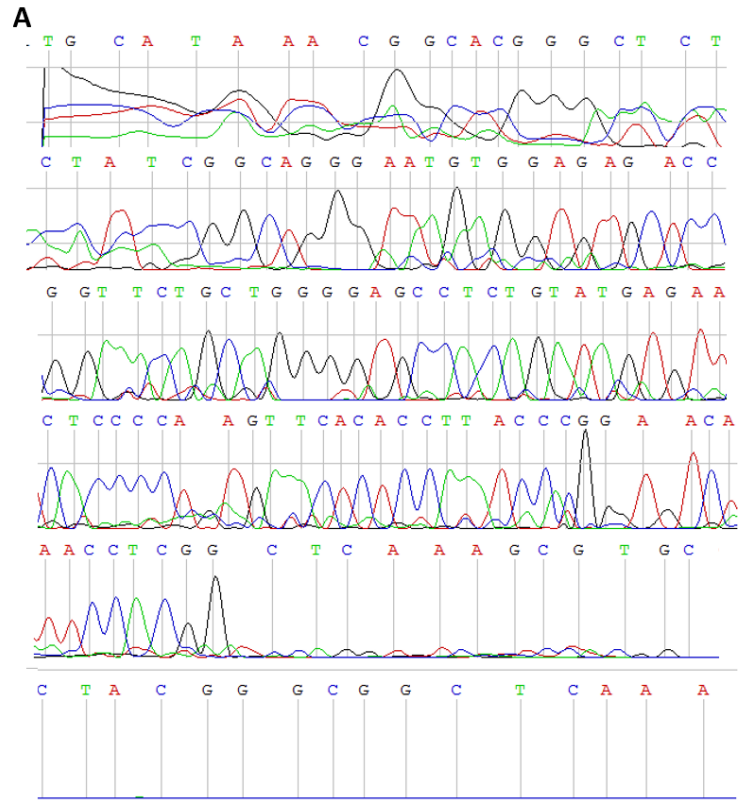
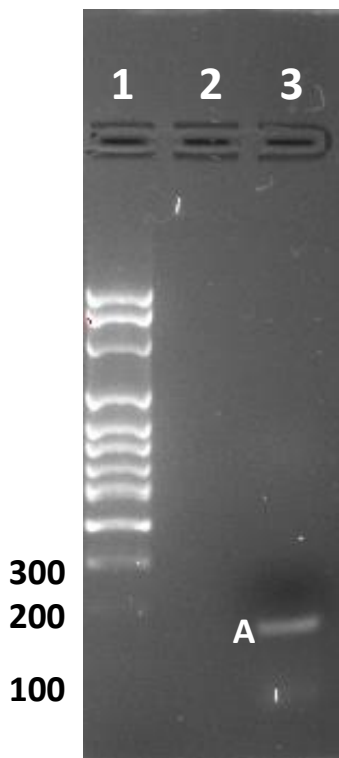
**Supplementary data**

Table S1. HCtAE cell confluency as determined by imaging with an IncuCyte system.

Well	Treatment group	Confluency (%)
A1	1.1 Control	56.85298
B1	1.2 Control	56.13226
A2	1.1 SAA	59.34018
B2	1.2 SAA	64.7347
A3	1.1 BAY11	33.76793
B3	1.2 BAY11	35.37162

<sup>a</sup> High-definition D phase-contrast images were acquired for each well in a 6-well plate using the The IncuCyte Zoom® live cell imaging system (Essen BioScience, Australia). The system software calculated the average confluency of each individual field imaged to obtain the overall mean (n=4) level of confluency for the well expressed as a percentage of the total area imaged (%). Levels of confluency were then used to normalise total secretory IL-6 as determined by ELISA.

**Figure S1. Gene sequencing**



**Figure S1(i). RT-PCR gel for TF expression**

Lane 1. Hyperladder 100 Bp Plus

Lane 2. No sample

Lane 3. HCtAE cDNA

Note Band A corresponds to an amplification product of 157bp

**Figure S1(ii). Sequencing chromatogram for purified bands (Gentle Software)**

DNA from bands noted as A in Figure 1 were extracted from agarose gel, purified and sequenced. Figure shows good quality chromatography data for reliable sequence data.

***Experimentally determined Sequence***

TGCATAAACGGCACGGGCTCTCTATCGGCAGGGAATGTGGAGAGACCGGTTCTGCTGGGGAGCCTCTGTATGAGAACTCCCAAGTTCACACCTTACCCGGAACAAACCTCGGCTCAAAGCGTGCCTACGGGCGGCTCAAATTATTGCATCTTTGCTGGGGGTCCCCCGGCGGGGGGGGGGGAGGGCCAAAAGGAAATATTGCACG

### Figure S1(iii). Tissue factor sequencing result

Homo sapiens coagulation factor III (thromboplastin, tissue factor) mRNA, complete cds

Sequence ID: [BT019808.1](#) Length: 888 Number of Matches: 1

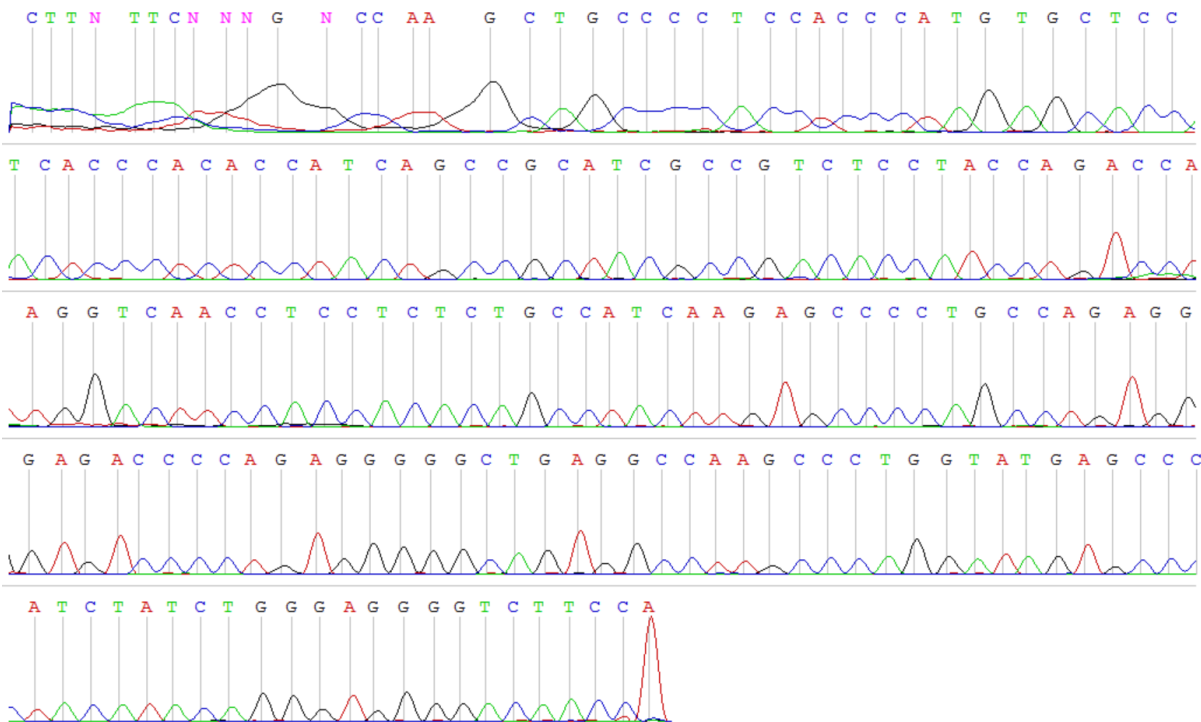
[▶ See 1 more title\(s\)](#)

Range 1: 312 to 422 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
148 bits(80)	3e-32	102/111(92%)	7/111(6%)	Plus/Plus
Query 10	GGCACGGG-C-TCT-CTA-TCGGCAGGGAATGTGGAGAG-ACCGGTTCTGCTGGGGAGCC			64
Sbjct 312	GGCACGGGCTTCTCTCTACCCGGCAGGGAATGTGGAGAGCACCAGGTTCTGCTGGGGAGCC			371
Query 65	TCTGTATGAGAACTCCCCA-AGTTCACACCTTACCCGGA-ACAAACCTCGG			113
Sbjct 372	TCTGTATGAGAACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGG			422

### Figure S1(iv). Band A NCBI Nucleotide Blast result



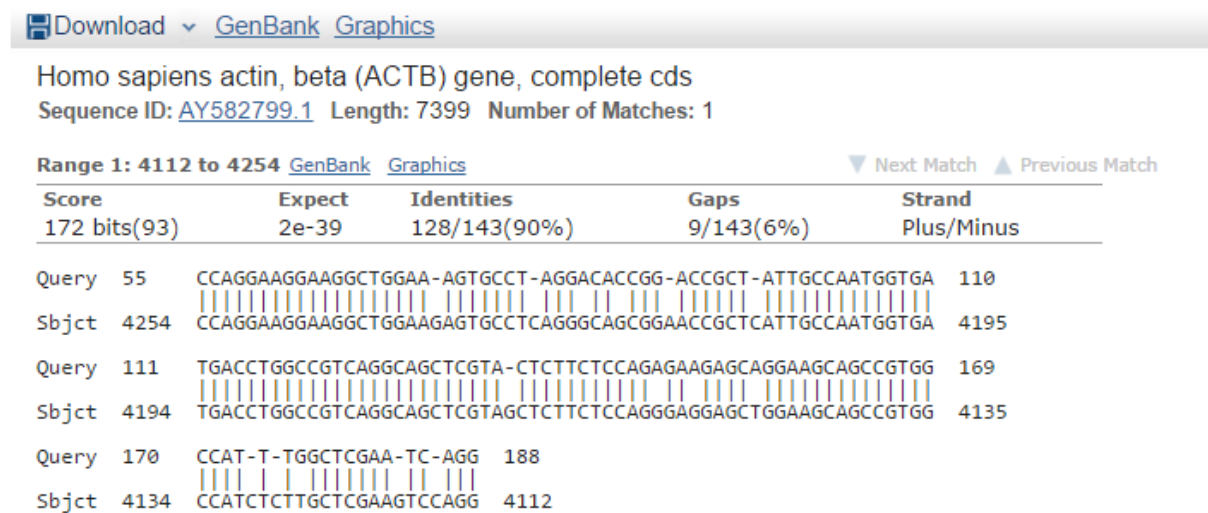
### Figure S1(i-iv). Sequencing Chromatogram for purified bands (Gentle Software)

DNA from bands noted A Figure 1 were extracted from agarose gel, purified and sequenced. Figure shows good quality chromatography data for reliable sequence data. Percentage match obtained was 92% identity.

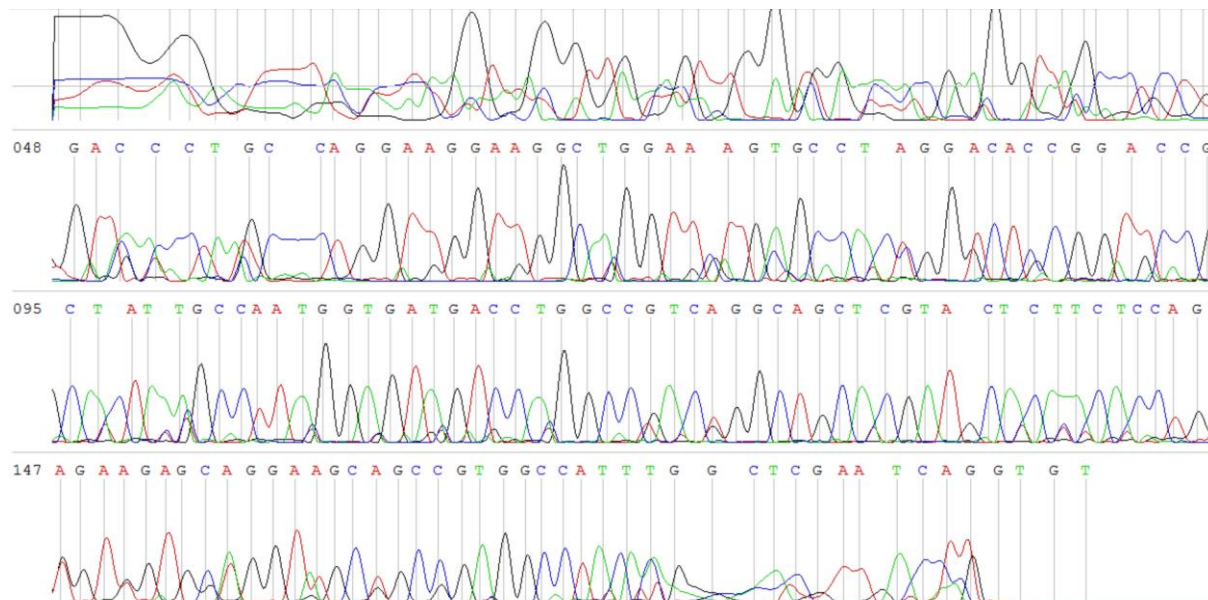
## Figure S2(i). Beta actin house keeping gene

### Experimentally determined Sequence

TGGTCTCATATTCATTGAAGGAGCGAAGGTGTCTCGTGGATGCCCAGGACCCTGCCA  
 GGAAGGAAGGCTGGAAAGTGCCTAGGACACCGGACCGCTATTGCCAATGGTGTATGA  
 CCTGGCCGTCAGGCAGCTCGTACTCTTCTCCAGAGAAGAGCAGGAAGCAGCCGTGG  
 CCATTTGGCTCGAATCAGGTGT



## Figure S2(ii). Beta actin Nucleotide Blast



## Figure S2(iii). Beta actin Sequencing Chromatogram

DNA from a single band was extracted from agarose gel (as per figure S2(i) above), purified and sequenced. Figure shows good quality chromatography data for reliable sequence data. Percentage match obtained was 90% identity.

## Figure S3(i). TNF gene sequencing

### Experimentally determined sequence

CTTNTTCNNGNCCAAGCTGCCCTCCACCCATGTGCTCCTCACCCACACCCATCAGC  
CGCATCGCCGTCTCTACCAGACCAAGGTCAACCTCCTCTCTGCCATCAAGAGCCCC  
TGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTATGAGCCCATCTA  
TCTGGGAGGGGTCTTCCA

Homo sapiens isolate RSH TNF gene, complete cds

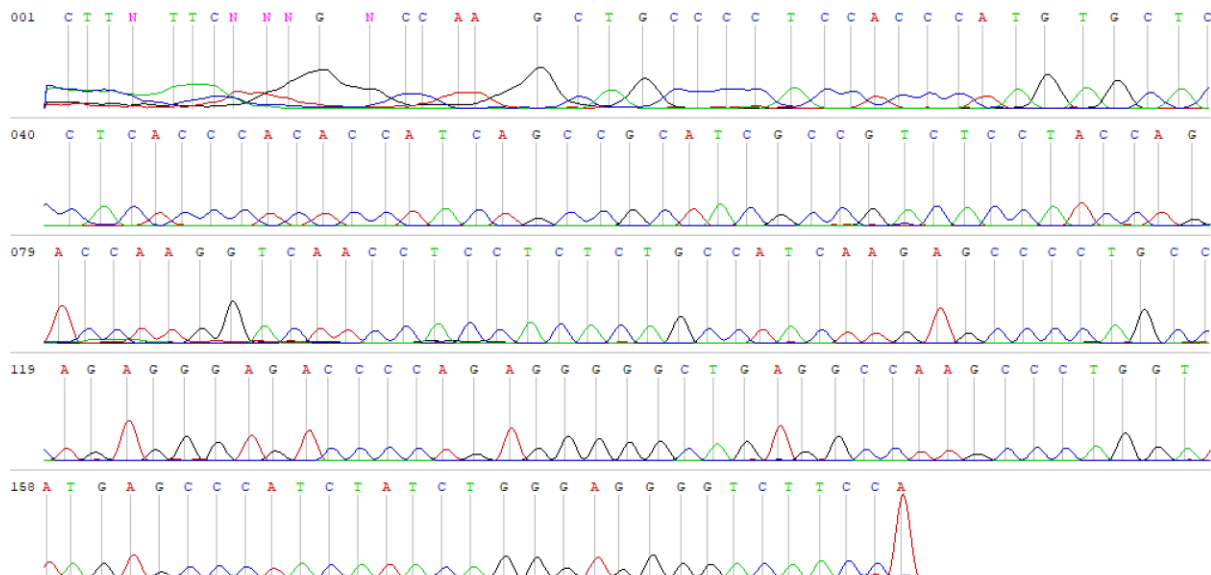
Sequence ID: [KY500678.2](#) Length: 1796 Number of Matches: 1

Range 1: 1520 to 1696 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

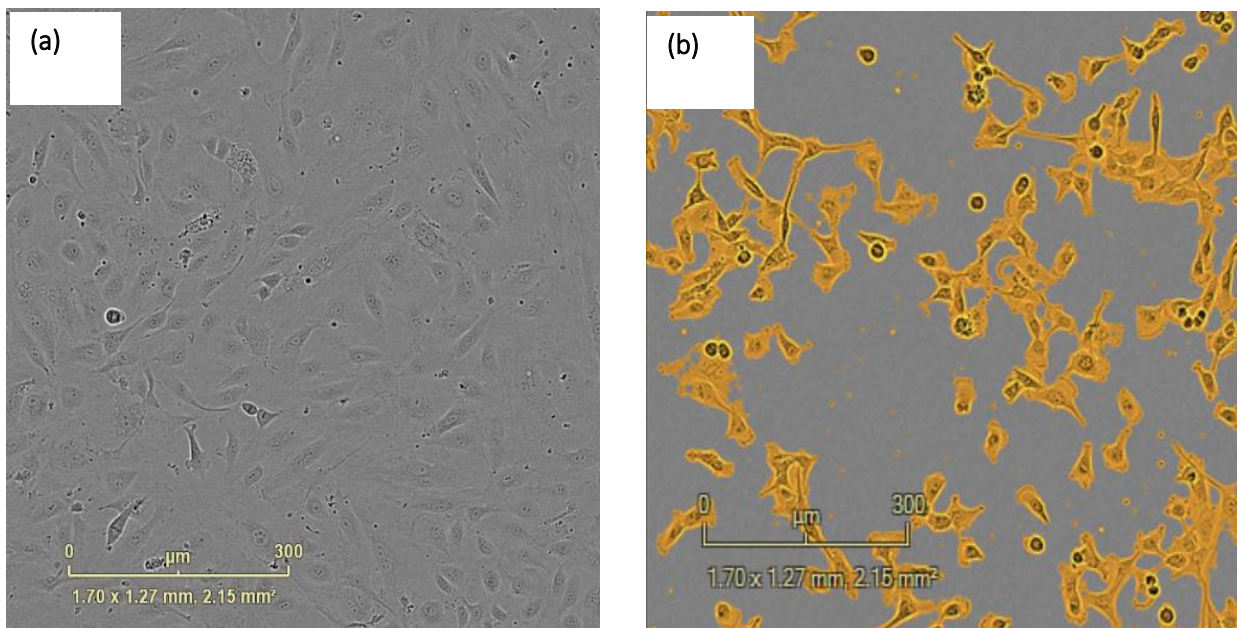
Score	Expect	Identities	Gaps	Strand
320 bits(173)	9e-84	176/177(99%)	1/177(0%)	Plus/Plus
Query 13	CCAA-GCTGCCCTCCACCCATGTGCTCCTCACCCACACCCATCAGCCGCATCGCCGTCTC			71
Sbjct 1520	CCAAGGTGTCCTCCACCCATGTGCTCCTCACCCACACCCATCAGCCGCATCGCCGTCTC			1579
Query 72	CTACCAGACCAAGGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCC			131
Sbjct 1580	CTACCAGACCAAGGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCC			1639
Query 132	AGAGGGGGCTGAGGCCAAGCCCTGGTATGAGCCCATCTATCTGGGAGGGGTCTTCCA			188
Sbjct 1640	AGAGGGGGCTGAGGCCAAGCCCTGGTATGAGCCCATCTATCTGGGAGGGGTCTTCCA			1696

## Figure S3(ii). TNF Nucleotide Blast



## Figure S3(iii). TNF Sequencing Chromatogram

DNA from a single band was extracted from agarose gel (as per figure S2(i) above), purified and sequenced. Figure shows good quality chromatography data for reliable sequence data. Percentage match obtained was 99% identity.



**Figure S4. Cultured HCtAE cell confluence as assessed with an IncuCyte imaging system.**

The IncuCyte Zoom® live cell imaging system (Essen BioScience, Australia) was used to measure the level of cell confluency (expressed as a percentage of the total area imaged) immediately prior to the time of harvest. (a) Representative field image of well A1 (*Table S1*); showing areal coverage at the 56% confluency level. (b) Representative field image of well B3 (*Table S1*) showing fewer cells in the same areal field (35%) – orange highlight shows confluence mask employed by the software to identify cells). Images were taken using a fixed 10x objective lens (Nikon, Australia).