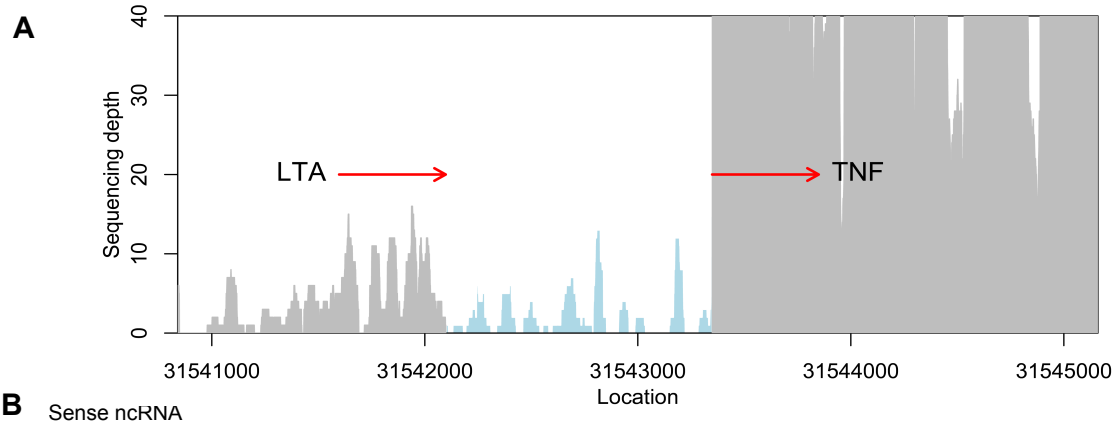
Supplemental Table I.
Primer Sequences

The combinations of primer-probe for detection of TNF upstream ncRNAs and control primers	
TNF1 forward	5'-CTCTCGCCCCAGGGACATAT-3'
TNF1 reverse	5'-ATGTGGCGTCTGAGGGTTGT-3'
TNF1 probe	5'-/56-FAM/CAGAGGACCAGCTAAGAGGGAGAGAAGCAA/36-TAMSp/-3'
TNF2 forward	5'-CGCTTCCTCCAGATGAGCTC-3'
TNF2 reverse	5'-TGC TGTCCT TGCTGAGGGA-3'
TNF2 probe	5'-/56-FAM/CCAAGGAAGTTTTCCGCTGGTTGAATG/36-TAMSp/-3'
TNF3 forward	5'-CCCCCTCGGAATCGGA-3'
TNF3 reverse	5'-GAGCTCATC TGGAGGAAGCG-3'
TNF3 probe	5'-/56-FAM/TGTCCCCAACTTTCCAAATCCCCG/36-TAMSp/-3'
TNF4 forward	5'-CCCAAAAGAAATGGAGGCAAT-3'
TNF4 reverse	5'-AAGCATCAAGGAT ACCCCTCAC-3'
TNF4 probe	5'-/56-FAM/CTACACACAAATCAGTCAGTGGCCCAGAAG/36-TAMSp/-3'
TNF5 forward	5'-CCCCCCCTTAACGAAGACA-3'
TNF5 reverse	5'-AACGTCCCCTGTATTCCATACCT-3'
TNF5 probe	5'-/56-FAM/CCATGTAGAGGGCCC CAGGGAGTG/36-TAMSp/-3'
GAPDH forward	5'-CGG TGC GTG CCC AGT T-3'
GAPDH reverse	5'-CCCTACTTTCTCCCCGCTTT-3'
GAPDH probe	5'-/56-FAM/ACCAGGCGGCTGCGGAAAAAA/36-TAMSp/-3'
Globin forward	5'-AACTCCTAAGCCAGTGCCAGAA-3'
Globin reverse	5'-GTTGTGTCAGAAGCAAATGTAAGC-3'
Globin probe	5'-/56-FAM/TGGCCAATCTACTCCCA/36-TAMSp/-3'
The orientation-specific primers for ncRNA reverse transcription and PCR primers	
Exon1 (for sense)	5'-AAAGTGCAGGCAGAAGAGC-3'
TNF 991(for antisense)	5'-GCTCCTGGGAGATATGGCCAC-3'
-974F	5'-ATGGCCACATGTAGCGGCTCTGA-3'
-606F	5'-TGTCCCAGGCTTGTCCCTGCTA-3'
The primers for RACE sense ncRNA	
S-5 RACE out specific primer	5'-GGTCTTCTGGGCCACTGACTGA-3'
S-5 RACE inner specific primer	5'-GGAGGGAAAAGCTGTGTTGA-3'
S-3 RACE-out specific primer	5'-CCCAAAAGAAATGGAGGCAAT-3' (TNF4F)
S-3 RACE-inner specific primer	5'-CCCCCTCGGAATCGGA-3' (TNF3F)
The primers for RACE anti-sense ncRNA	
AS-5 RACE-out specific primer	5'-CCCAAAAGAAATGGAGGCAAT-3' (TNF4F)
AS-5 RACE-inner specific primer	5'-CCCCCTCGGAATCGGA-3' (TNF3F)
AS-3 RACE-out specific primer	5'-AGGCTTGAGGCCTCAGGAAAG-3'
AS-3 RACE-inner specific primer	5'-GGCCTTCTTCTTTCATTCTGACC-3'
The primers for the sense or antisense ncRNA over-expression vectors	
-674F	5'GGACTAGTCGCGGATCCTTATGAGTCTCCGGGTCAGAAT-3' (SpeI, BamH1)
-674R	5' GAAGATCT GCTCTAGA TGCCAACAACTGCCTTTATATG-3' ((BglII, XbaI)
The primers for making the constructs for ncRNA in vitro transcription	
Vector name	The primers
PGEM-T-674ncRNA	674F/674R
PGEM-T-377ncRNA	TNF4F/TNF2R
PGEM-T-150ncRNA	TNF4F/ TNF4R
PMK- 674ncRNA Sense	674F/674R
PMK- 674ncRNA Antisense	674F/674R
The primers for run on assay	
TNF-exon1-31F	5'-CAGGCGGTGCTTGTTCCT-3'
TNF-exon1-109R	5'-AAAGTGCAGCAGGCAGAAGAG-3'
TNF-exon1-50 probe	5'-/56-FAM/AGCCTCTTCTCCTTCC/36-TAMSp/-3'
CCL20	QT00012971 (QIAGEN)
MMP1	QTOOO14581 (QIAGEN)
Plasminogen	HS01010736 (Applied Biosystems)



B

Sense ncRNA

CCAGGTGAGGCCGCCAGACTGCTGCAGGGGAAGCAAAGGAGAAGCTGAGAAGATGAAGGAAAAGTCAGGGTCTGGAGGGGCGGG
GGTCAGGGAGCTCCTGGGAGATATGGCCACATGTAGCGGCTCTGAGGAATGGGTTACAGGAGACCTCTGGGGAGATGTGACCACAG
CAATGGGTAGGAGAATGTCCAGGGCTATGGAAGTCGAGTATGGGGACCCCCCCTTAACGAAGACAGGGCCATGTAGAGGGCCCCAG
GGAGTGAAAGAGCCTCCAGGACCTCCAGGTATGGAATACAGGGGACGTTTAAGAAGATATGGCCACACACTGGGGCCCTGAGAAGT
GAGAGCTTCATGAAAAAAATCAGGGACCCCAGAGTTCCTTGGAAGCCAAGACTGAAACCAGCATTATGAGTCTCCGGGTCAGAATGA
AAGAAGAAGGCCTGCCCCAGTGGGGTCTGTGAATTCCCGGGGGTGATTTCACTCCCCGGGGCTGTCCCAGGCTTGTCCCTGCTACC
CCCACCCAGCCTTTCCTGAGGCCTCAAGCCTGCCACCAAGCCCCCAGCTCCTTCTCCCCGCAGGGACCC**AAACACAGGCCTCA***G
GACTCAACACAGCTTTTCCCTCCAACCCCGTTTTCTCTCCCTCAAGGACTCAGCTTTCTGAAGCCCCTCCCAGTTCTAGTTCTATCTTT
TTCCTGCATCCTGTCTGGAAGTTAGAAGGAAACAGACCACAGACCTGGTCCCCAAAAGAAATGGAGGCAATAGGTTTTGAGGGGCAT
GAGGACGGGGTTCAGCCTCCAGGGTCCTACACACAAATCAGTCAGTGGCCCAGAAGACCCCCCTCGGAATCGGAGCAGGGAGGAT
GGGGAGTGTGAGGGTATCCTTGATGCTTGTGTGTCCCCAACTTTCCAAATCCCCGCCCCCGCGATGGAGAAGAAACCGAGACAGA
AGGTGCAGGGCCCACTACCGCTTCCTCCAGATGAGCTCATGGGTTTCTCCACCAAGGAAGTTTTCCGCTGGTTGAATGATTCTTTCCC
CGCCCTCCTCTCGCCCCAGGGACATATAAA**GGCAGTTGT***

Antisense ncRNA

TCTTCTCCATCGCGGGGGCGGGGATTTGGAAAGTTGGGGACACACAAGCATCAAGGATACCCCTCACACTCCCCATCCTCCCTGC
TCCGATTCCGAGGGGGGTCTTCTGGGCCACTGACTGATTTGTGTGTAGGACCCTGGAGGCTGAACCCCGTCCTCATGCCCCTCAA
AACCTATTGCCTCCATTTCTTTTGGGGACCAGGTCTGTGGTCTGTTTCCTTCTAACTTCCAGACAGGATGCAGGAAAAAGATAGAAC
TAGAACTGGGAGGGGCTTCAGAAAGCTGAGTCCTTGAGGGAGAGAAAACGGGGTTGGAGGGAAAAGCTGTGTTGAGTCCTGAGG
CCTGTGTTTGGGTCCCTGCGGGGAGAAGGAGCTGGGGGCTTGGTGGCAGGCTTGAGGCCTCAGGAAAGGCTGGGTGGGGGTAG
CAGGGACAAGCCTGGGACAGCCCCGGGGAGTGAAATCACCCCCGGGAATTCACAGACCCCACTGGGGCAGGCCTTCTTCTTTCA
TTCTGACCCGGAGACTCATAATGCTGGTTTCAGTCTTGGCTTCCAAGGAACTCTGGGGTCCCTGATTTTTTTCATGAAGCTCTCACT
TCTCAGGGCCCCAG**TGTGTGGCCATATCT***TCTTAAACGTCCCCTGTATTCCATACCTGGAGGTCCTGGAGGCTCTTTCACTCCCTG
GGGCCCTCTACATGGCCCTGTCTT**CGTTAAGGGGGGG***TCCCCATACTCGACTTCCATAGCCCTGGACATTCTCCTACCCATTGCTG
TGGTCACATCTCCCCAGAGGTCTCCTGTAACCCATTCCTCAGAGCCGCTACATGTGGC**CATATCTCCCAGGAGC***

FIGURE S1. RNA is expressed between *TNF* and *LTA*. A) A primary monocyte RNA-seq library was prepared with the SOLiD™ whole transcriptome analysis kit (Applied Biosystems, Foster City, CA). Low levels of intergenic transcripts were seen. B) Sense and antisense ncRNA RACE results are shown. The arrows and arrowheads correspond to the Transcription Start Sites (TSSs) mapped by 5' RACE. The asterisks represent the the 3' termini by 3' race. The size of arrow or asterisk indicates the relative frequencies of the recovered clones.

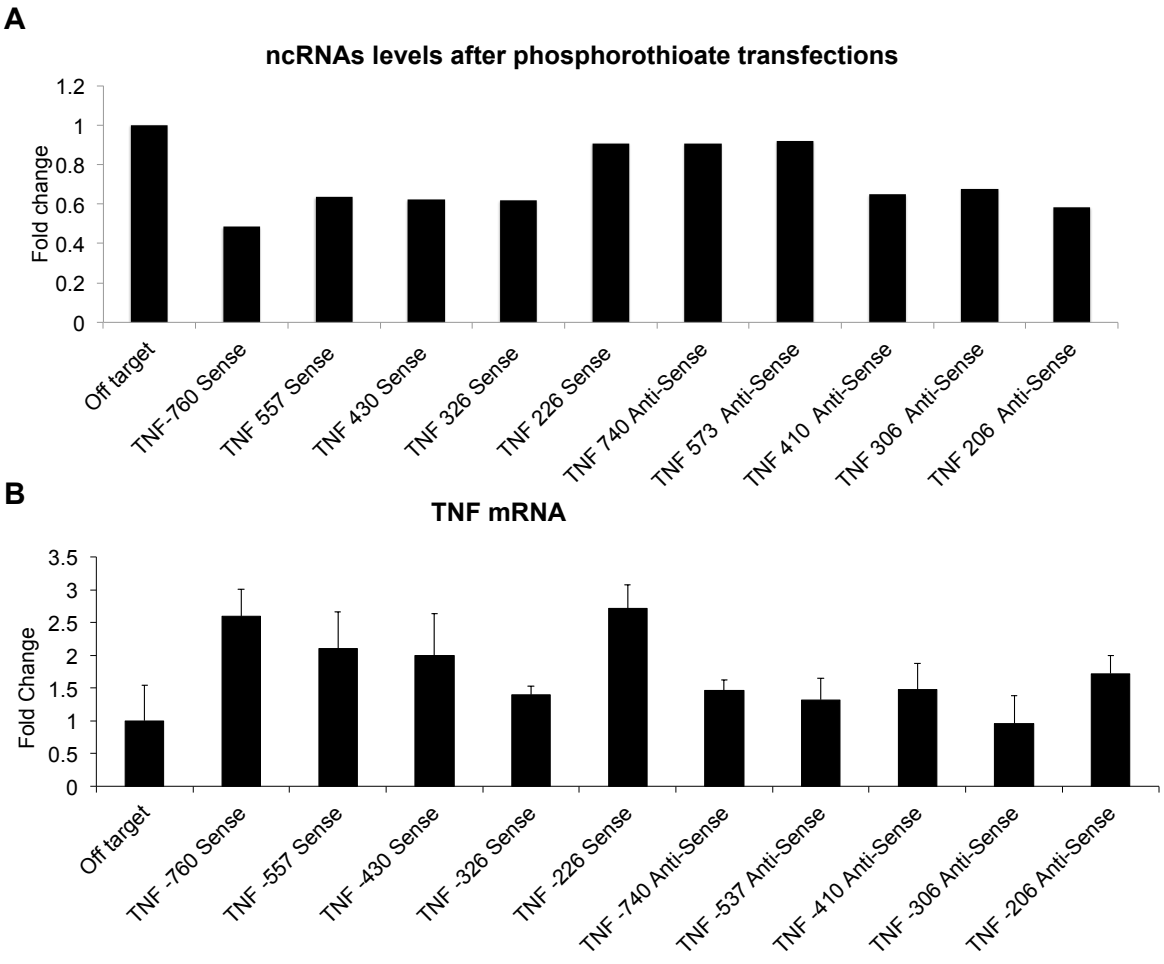


FIGURE S2. Survey of knockdown oligonucleotides. K562 cells were transfected with the indicated 20bp phosphorothioate oligonucleotides which knocked down either the sense or the antisense transcripts. The ncRNA (TNF1-5 averaged) signal (A) and the TNF mRNA levels (B) were measured by qRT-PCR. The qRT-PCR signal was normalized to control transfected cells.

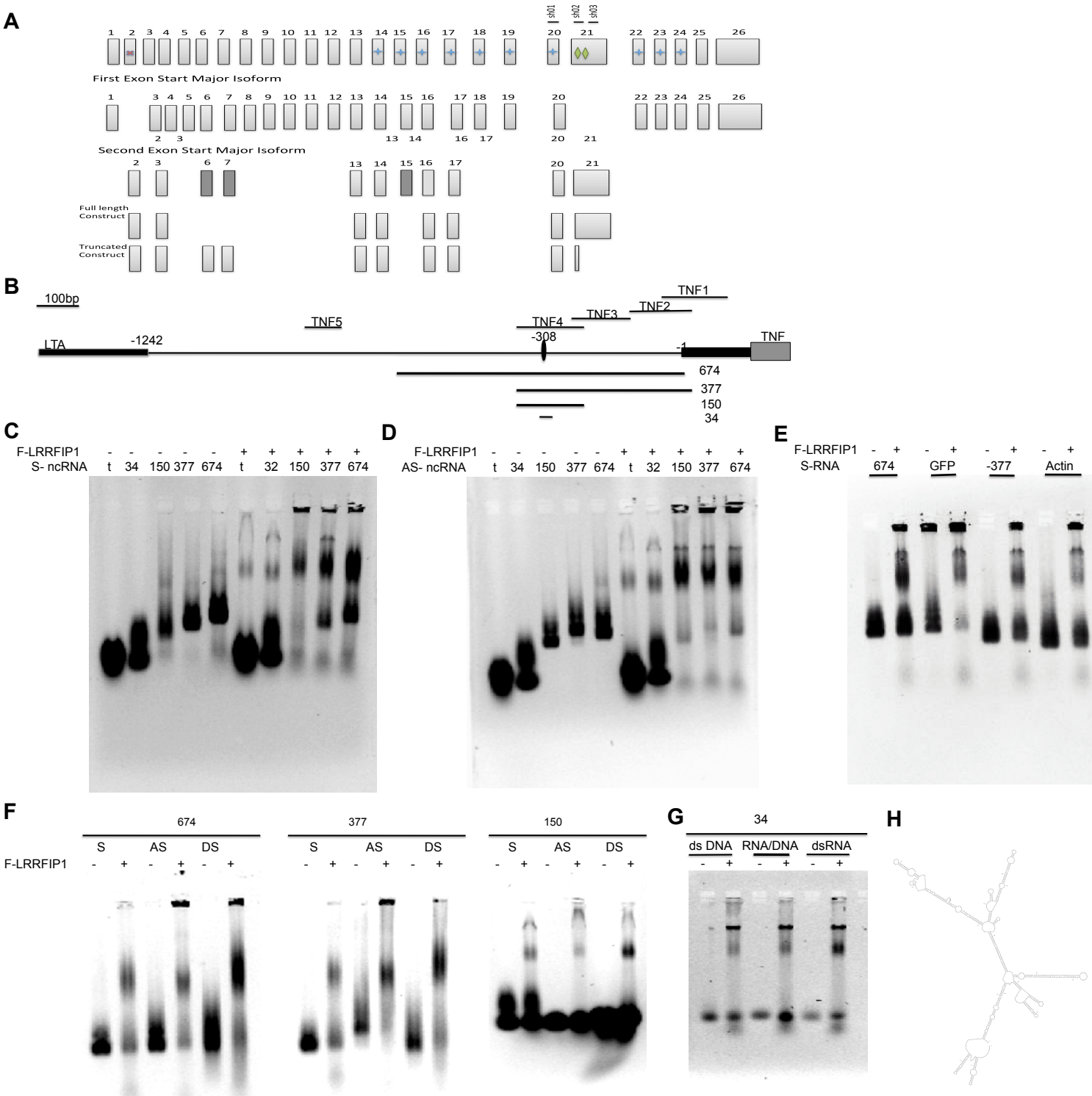

A
sh01
sh02
sh03
First Exon Start Major Isoform
Second Exon Start Major Isoform
Full length Construct
Truncated Construct
B
100bp
TNF1
TNF2
TNF3
TNF4
TNF5
LTA
-1242
-308
TNF
674
377
150
34
C
F-LRRFIP1
S- ncRNA
t 34 150 377 674 t 32 150 377 674
D
F-LRRFIP1
AS- ncRNA
t 34 150 377 674 t 32 150 377 674
E
F-LRRFIP1
S-RNA
674
GFP
-377
Actin
F
674
377
150
S
AS
DS
F-LRRFIP1
G
34
ds DNA
RNA/DNA
dsRNA
H

FIGURE S3. LRRFIP target characteristics. A) Full length and truncated LRRFIP1 cDNAs were ligated into pGEX-5X-1 vector for GST-fusion protein expression. Protein was purified according to the manufacturer's instructions. The full-length LRRFIP1 construct started with exon 2 and expressed a 160kD LRRFIP1 protein. The truncated LRRFIP1 construct lacked the entire DNA-binding region and half of the RNA-binding domain. DNA binding is indicated with diamonds and RNA binding is indicated with four-point stars. The full length and truncated LRRFIP1 were codon-optimized to improve expression (GeneArt, Burlington, Calif.). B) Three different sizes of ncRNAs were ligated into the pGEM-T Easy vector and sense or antisense ncRNAs were transcribed using T7 or SP6 promoter. The map indicates the locations of the constructs. The 34bp nucleic acid structures were annealed oligonucleotides. C) Sense or D) antisense ncRNAs were incubated with or without LRRFIP1 and the mixture was run on a 0.5% agarose gel. t = tRNA. LRRFIP1 bound more of the longer ncRNA. E) LRRFIP1 bound to RNA species of various sequence. The 674 and 377 ncRNAs were derived from the TNF upstream region while the GFP and actin RNAs were of similar length and were produced in vitro from the same vector. LRRFIP1 prefered the TNF 674 RNA but demonstrated binding to the GFP and actin RNAs. F) LRRFIP1 bound to dsRNA better than ssRNAs. The same RNAs were prepared as sense (S), antisense (AS), or double stranded (DS) structures. G) LRRFIP1 showed higher affinity for dsRNA compared with dsDNA and RNA/DNA mixture. Oligonucleotides of different biochemical structure were synthesized and annealed as indicated. H) The proposed highly folded structure of the 674bp sense ncRNA. The structure was predicted based on physiologic salt and temperature by mFOLD.