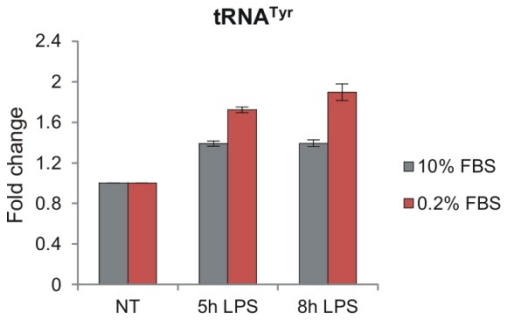
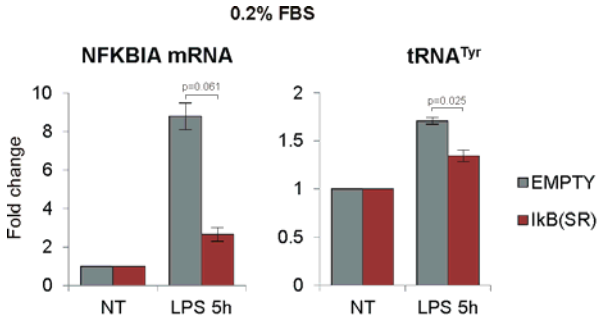
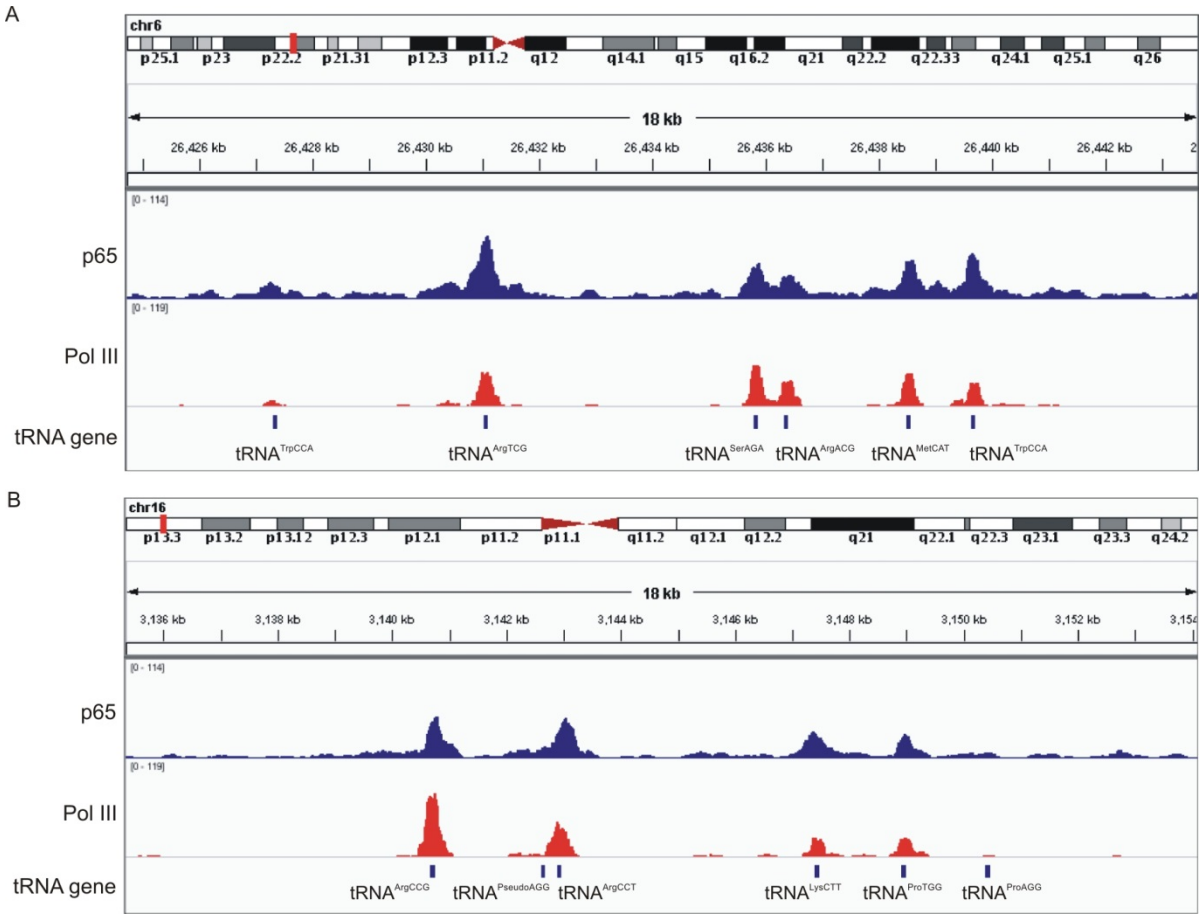


Supplementary Figure S1. **Serum starvation potentiates the effect of LPS treatment in RAW264 macrophages.** RAW264 mouse macrophages were cultured in media containing either 10% or 0.2% of serum for 16 hours. Then LPS was added to the final concentration 1 μg/ml and cells were harvested at the indicated time points. RNA was isolated from cells and transcripts levels of indicated genes were assessed using RT-qPCR. RNA levels were normalized to GAPDH and NT (non-treated) samples set to 1. Error bars represent SEM. (N=3).



Supplementary Figure S2. Inhibition of the NF-kB pathway partially precludes Pol III activation upon LPS treatment. RAW264 control cells or stably expressing IkB super-repressor were cultured in media containing 0.2% of serum for 16 hours then treated with 1 µg/ml LPS for 5 hours. RNA was isolated from cells and transcript levels of the indicated genes were assessed using RT-qPCR. RNA levels were normalized to GAPDH mRNA and NT (non-treated) samples set to 1. Error bars represent SEM. (N=3).



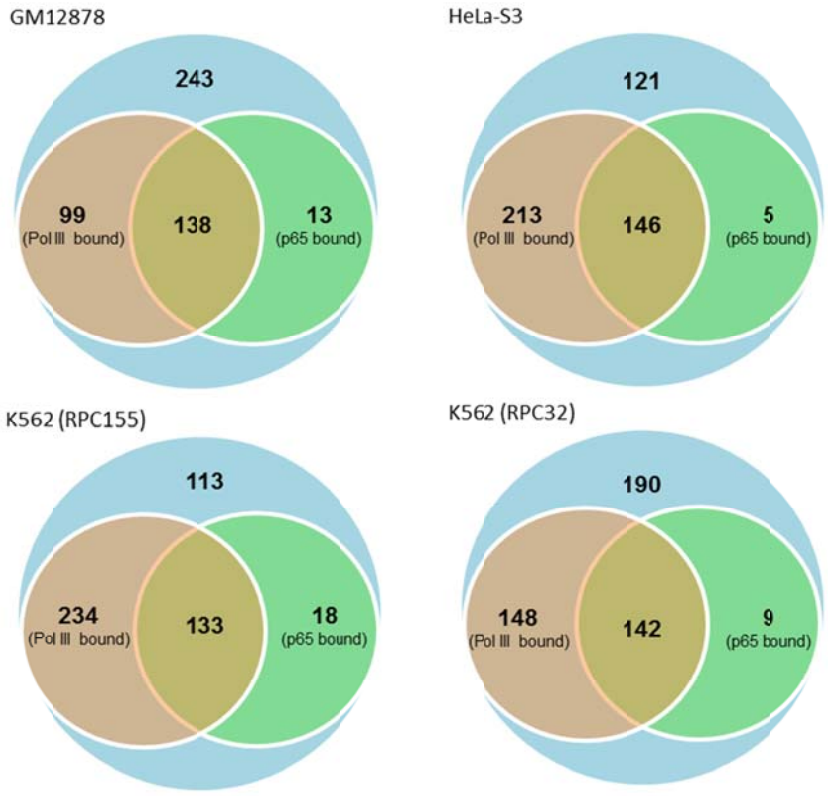
Supplementary Figure S3. NF-κB is present at tRNA genes in lymhoblastoid cells.

Genomic tracks display ChIP-seq data for p65 and Pol III across 18-kb region of chromosome 6 (A) and 16 (B) in human GM12878 lymhoblastoid cells. ChIP-seq data were downloaded from UCSC genome browser and plotted using Integrative Genomic Viewer using hg18 human genome compilation. The p65 ChIP-seq data can be found here:

http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeYaleChIPseq/wgEncodeYaleChIPseqSignalGm12878Pol3.bedGraph4.gz

The Pol III ChIP-seq data can be found here:

http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeYaleChIPseq/wgEncodeYaleChIPseqSignalGm12878NfkbTnfa.bedGraph4.gz



Supplemantary Figure S4. p65 binding overlaps with Pol III binding at tRNA genes.

Venn diagrams showing the number of human tRNA genes that are bound by either p65 or Pol III or tRNA genes bound by both p65 and Pol III in three different human cell lines: GM12878 (Rpc32 antibody), HeLa-S3 (Rpc155), K562 (Rpc155 and Rpc32 antibodies). p65 ChIP-seq data are from GM12878 cells treated with TNFα. ChIP-seq data were downloaded from UCSC genome browser. The p65 ChIP-seq data can be found here:

http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeYaleChIPseq/wgEncodeYaleChIPseqPeaksGm12878NfkbTnfa.narrowPeak.gz

The Pol III ChIP-seq data can be found here:

http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeYaleChIPseq/wgEncodeYaleChIPseqPeaksGm12878Pol3.narrowPeak.gz
http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeYaleChIPseq/wgEncodeYaleChIPseqPeaksHelas3Rpc155.narrowPeak.gz
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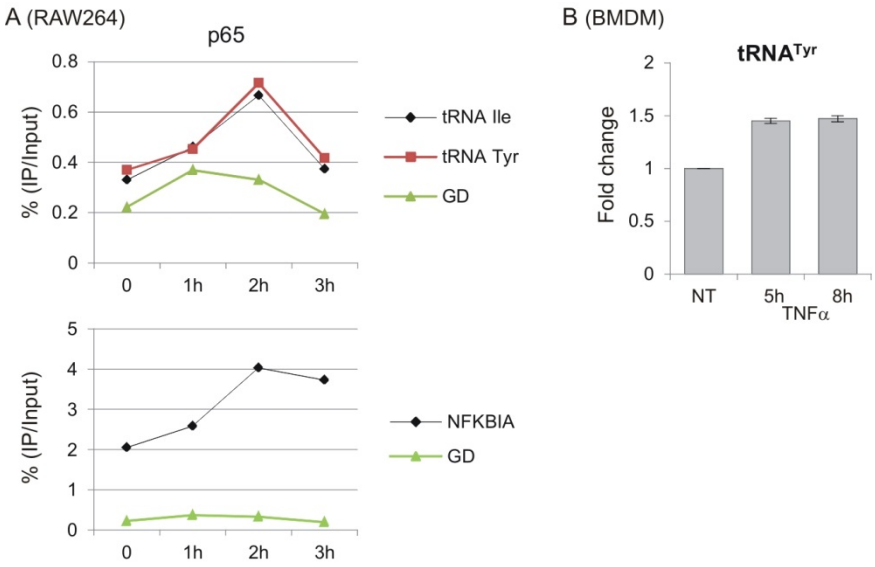
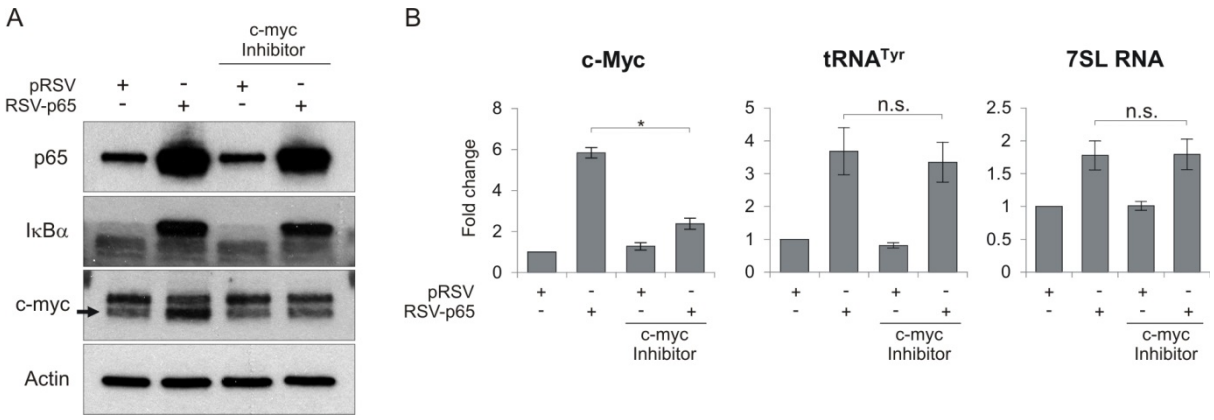
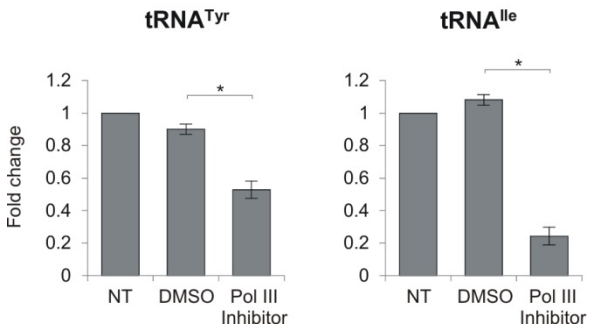


Supplementary Figure S5. TNFα induces p65 binding to tRNA genes and up-regulates tRNA transcription. Mouse RAW264 (A) and murine bone marrow-derived macrophages (B) were treated with 10 ng/ml TNFα and harvested at the indicated time points. (A) ChIP assay showing p65 binding to $tRNA^{Tyr}$, $tRNA^{Ile}$, and NFKBIA promoter region. (B) RT-qPCR analysis of $tRNA^{Tyr}$. RNA levels were normalized to GAPDH and NT (non-treated) sample set to 1. Data shown are means of two experiments performed on macrophages derived from two different mice (N=2).



Supplementary Figure S6. C-myc is not required for p65-induced Pol III activation. U2OS cells were transfected with empty vector or vector encoding p65, as indicated. After 24h, cells were serum deprived (0.2% FBS), cultured for another 24h and then harvested. 6h before harvesting, cells were treated with 10μM CPI-203, an inhibitor of bromodomain-containing proteins. (A) Western blot analysis of c-myc, p65, IκBα, Actin. (B) RT-qPCR analysis of c-myc mRNA and indicated Pol III transcripts. RNA levels were normalized to RPLP0 mRNA and non-treated empty vector samples set to 1. n.s. = non-significant. Error bars represent SEM (N = 3).



Supplementary figure S7. Pol III inhibitor ML-60218 decreases tRNA levels in dTHP-1 cells. PMA-differentiated THP-1 cells were treated exactly as in figure (VI); cells were harvested and total RNA isolated. The levels of indicated tRNAs were analyzed by RT-qPCR. RNA levels were normalized to RPLP0 mRNA and NT (non-treated) sample set to 1. Asterisk indicates $P < 0.05$. Error bars represent SEM ($N = 3$).

Supplementary Table 1. tRNA specific oligonucleotides used for cDNA synthesis

Gene name	Sequence (5’ -> 3’)
Hs $tRNA^{Tyr}$	TCCTTCGAGC
Hs $tRNA^{Leu}$	TGTCAGAAGTG
Hs $tRNA^{Ile}$	TGCTCCAGGTG
Mm $tRNA^{Ile}$	TGCTCCAGGTG
Mm $tRNA^{Tyr}$	TCCTTCGAGCC

Supplementary Table 2. PCR primers used in the study

Gene name	Forward (5’ -> 3’)	Reverse (5’ -> 3’)
Hs $tRNA^{Tyr}$	CCTTCGATAGCTCAGCTGGT	CGACCTAAGGATGTCCACAAA
Hs $tRNA^{Thr}$	AGTGGTAAGGCGTCGGTCTC	CCCTACCTCGGGTCTCAGG
Hs $tRNA^{Ile}$	GTTAGCGCGCGGTACTTATA	AGGCTCGAACTCACAACCTC
Hs 7SL RNA	GTGTCCGCACTAAGTTCGGCA	TATTCACAGGCGCGATCCCACT
Hs RPLP0	GCGACCTGGAAGTCCAACTA	TGTCTGCTCCCACAATGAAA
Mm $tRNA^{Ile}$	GTTAGCGCGCGGTACTTATA	GGATCGAACTCACAACCTCG
Mm $tRNA^{Tyr}$	AGTTGGTAGAGCGGAGGACT	CGAACCAGCGACCTAAGGAT
Mm IL6	GATGGATGCTACCAAACTGG	TCTGAAGGACTCTGGCTTTG
Mm TNFa	GTCCCCAAAGGGATGAGAAG	CACTTGGTGGTTTGCTACGAC
Mm IL10	TGGGTGAGAAGCTGAAGACC	CATGGCCTTGTAGACACCTTG
Mm GAPDH	AAGGGCTCATGACCACAGTC	GGATGACCTTGCCCACAG