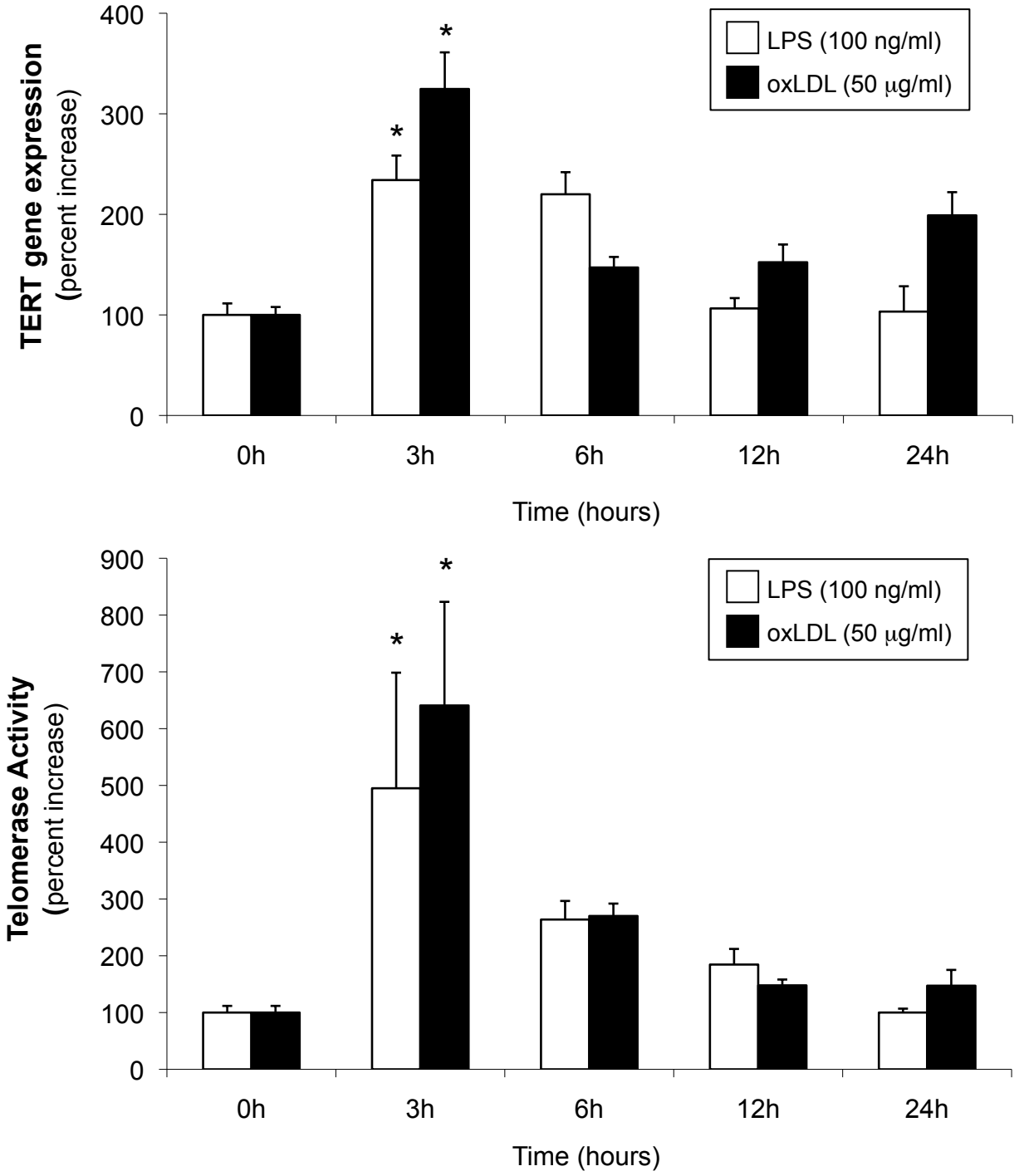
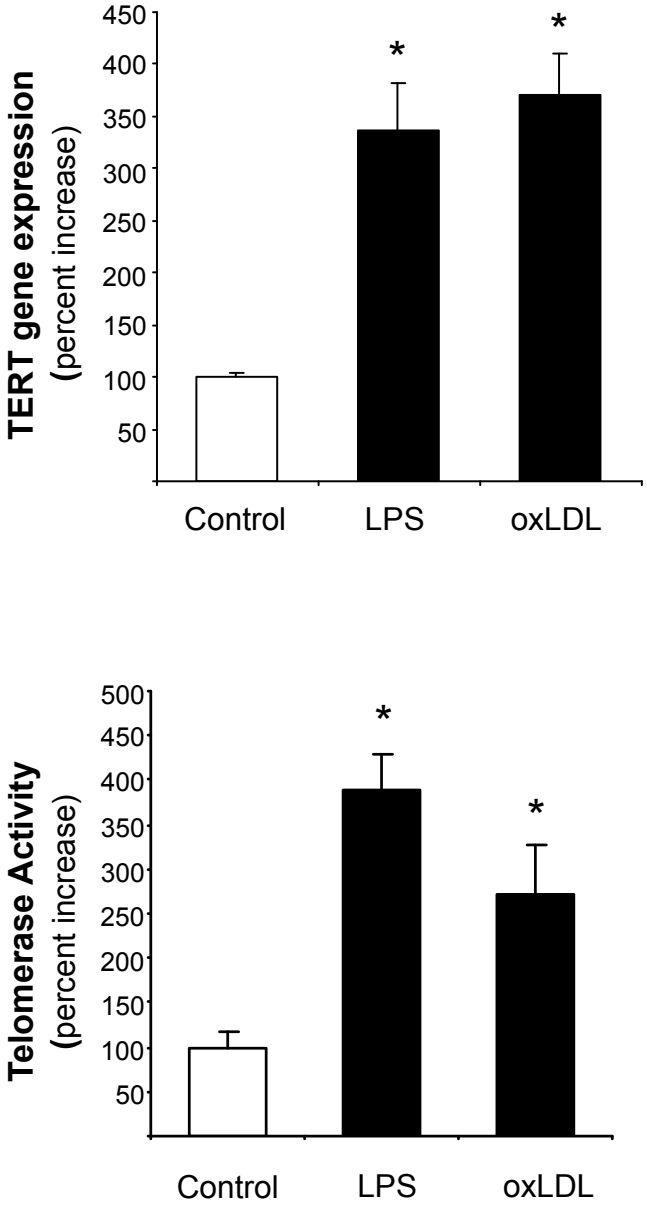
Gizard et al., Supplemental Data File

Name	Use	Sequence
human TERT	Real-time RT-PCR	F : 5'-TTCCTGCACTGGCTGATGAG-3' R: 5'-CCGGTAGAAAAAGAGCCTGTTC-3'
mouse TERT	Real-time RT-PCR	F: 5'-GGCTCTTCTTCTACCGTAAG-3' R: 5'-TGATGCCTGACCTCCTCTTG-3'
mouse p16	Real-time RT-PCR	F: 5'-CTTTGTGTACCGCTGGGAAC-3' R: 5'-CGCTAGCATCGCTAGAAGTG-3'
mouse p21	Real-time RT-PCR	F: 5'-ACCCCATACTTCCCCTTCTG-3' R: 5'-ACCCTAGACCCACAATGCAG-3'
mouse RB	Real-time RT-PCR	F: 5'-CCTTGAACCTGCTTGTCCTC-3' R: 5'-GGGCAAGGGAGGTAGATTTC-3'
human TBP	Real-time RT-PCR	F: 5'-GGAGAGTTCTGGGATTGTACCGC-3' R: 5'-ATATTCGGCGTTTCGGGCAC-3'
mouse TFIIB	Real-time RT-PCR	F: 5'-CTCTCCCAAGAGTCACATGTCC-3' R: 5'-CAATAACTCGGTCCCCTACAAC-3'
mouse 28S rRNA	Real-time RT-PCR	F: 5'-AAACTCTGGTGGAGGTCCGT-3' R: 5'-CTTACCAAAAGTGGCCCACTA-3'
hTERT-NF-κB	ChIP PCR	F: 5'-TCCATTTCCCACCCTTTCTCG-3' R: 5'-AGGACGATTGCTCCCTGGAC-3'
mTERT-NF-κB	ChIP PCR	F: 5'-CAGCAGGCTGGAGCAGTCAG-3' R: 5'-CAGAATGCTTCTCGGGCTTC -3'
mTERT-distal	ChIP PCR	F: 5'-GTGAGTTGAGATGATGCTCTGG-3' R: 5'-CGACCATACACTCAGATCCC-3'
mMCP-1-NF-κB	ChIP PCR	F: 5'-CAGTCCTCACCCCATTACATC-3' R: 5'-TGGAAATTCCCATTCTGAGG-3'
mβ–actin-distal	ChIP PCR	F: 5'-AAACTCTCCCTCCTCCTCTTCCT-3' R: 5'-CGAGCCATAAAAGGCAACTTTCG-3'

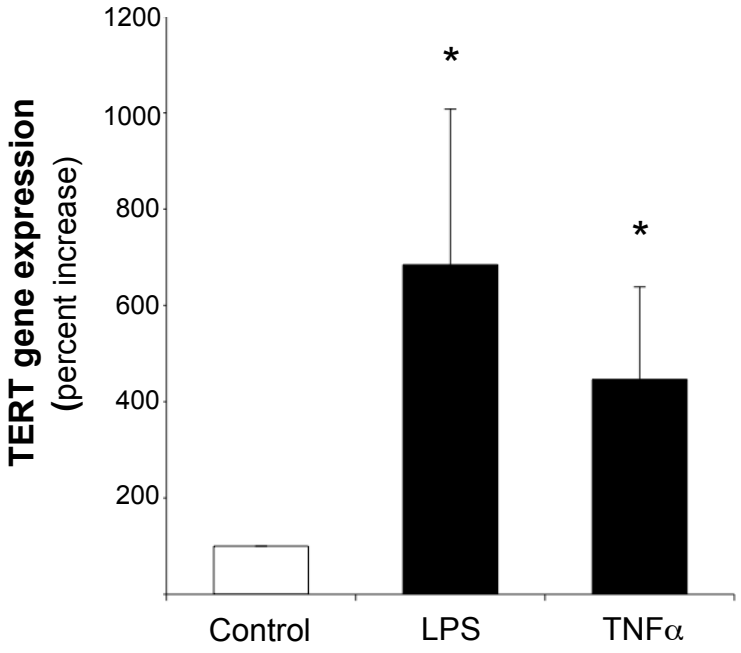
Supplemental Table I: Primer sequences used in this study.



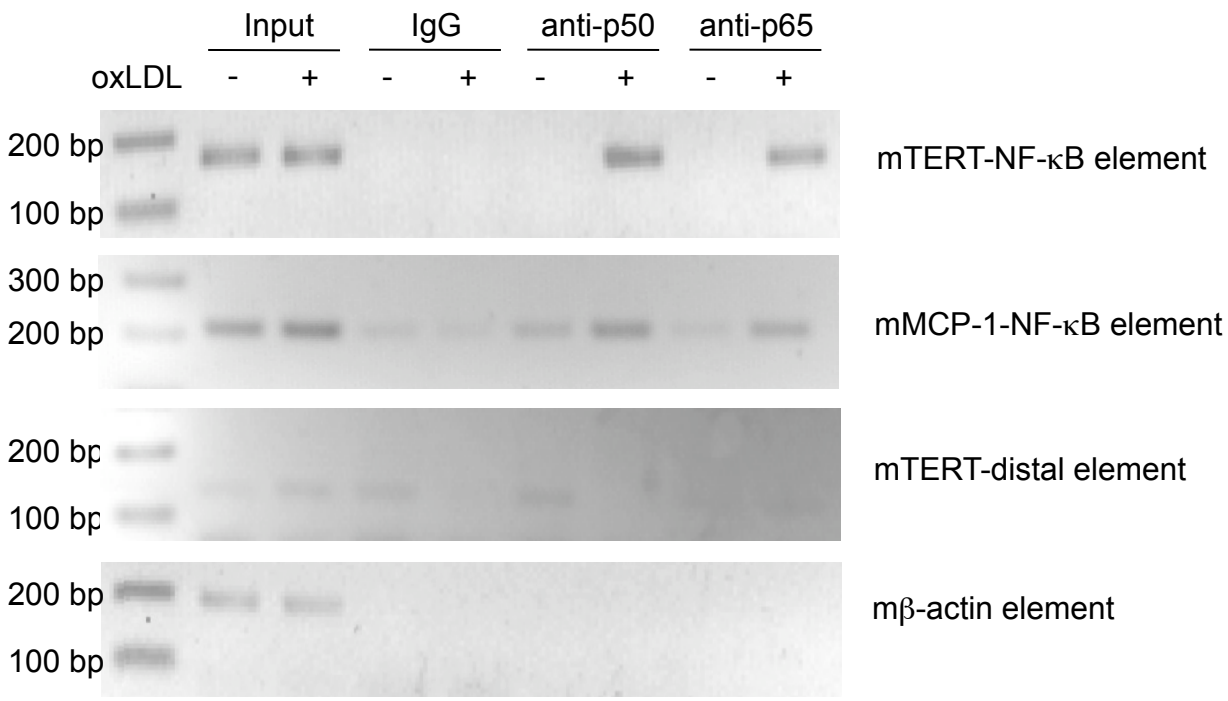
Supplemental Figure I. Time-dependent induction of TERT expression and telomerase activity by oxidized LDL in human macrophages. Differentiated human U937 macrophages were treated with LPS (100 ng/ml) or oxidized LDL (50 μg/ml). At the indicated time points, TERT mRNA expression levels (upper panel) and telomerase activity (lower panel) were analyzed. TERT mRNA expression was quantified by real-time RT-PCR and normalized to transcript levels of the house-keeping gene TBP. Whole cell proteins were analyzed for TERT activity by real-time PCR as described in "Materials and Methods". All data are presented as mean ± SEM percent increase compared to untreated cells (n=3, * P<0.05).



Supplemental Figure II. Induction of TERT expression and telomerase activity by inflammatory stimuli in primary murine macrophages. Mouse peritoneal macrophages were treated with oxidized LDL (oxLDL, 50 μg/ml) or LPS (100 ng/ml). TERT mRNA expression levels (upper panel) were analyzed by real-time RT-PCR and normalized to transcript levels of 28S rRNA. Telomerase activity (lower panel) was quantified by real-time PCR as described in "Materials and Methods". All data are presented as mean ± SEM percent increase compared to untreated cells (Control), (n=3, * P<0.05).



Supplemental Figure III. Induction of TERT expression by inflammatory stimuli in primary human macrophages. Mononuclear cells were isolated from blood of healthy normolipidemic donors. After Ficoll gradient centrifugation, monocytes were suspended in RPMI 1640 medium containing gentamycin (40 µg/ml), glutamine (0.05%) (Sigma), and 5% pooled human serum. Cells were cultured at a density of 3×10^6 cells/well in 6-well culture dishes. Differentiation of monocytes into macrophages occurred spontaneously by adhesion of cells and culture for 12 days. Mature macrophages were cultured in 0.5 % serum overnight and treated with LPS (100 ng/ml) or TNFα (5 ng/ml). TERT mRNA expression was quantified by real-time RT-PCR and normalized to transcript levels of 28S rRNA. Data are presented as mean ± SEM percent increase compared to untreated cells (Control), (n=3, * $P < 0.05$ vs. Control).



Supplemental Figure IV. Oxidized LDL induces NF-κB binding to the endogenous TERT promoter in primary murine macrophages. Mouse peritoneal macrophages were treated for 2.5 h with oxidized LDL (50 μg/ml). Chromatin immunoprecipitations were performed with antibodies raised against p50 or p65, followed by PCR amplification using primer pairs that cover the proximal NF-κB site in the murine TERT promoter (mTERT-NF-κB element). Controls included non-precipitated genomic DNA (Input) or immunoprecipitations performed with non-immune IgG (IgG). Additional controls for specificity included PCR amplification with primer pairs encompassing the NF-κB site in the murine MCP-1 promoter (mMCP-1-NF-κB element, positive control), a distal unrelated element in the murine TERT promoter (mTERT-distal element, negative control), or the unrelated murine β-actin promoter (mβ-actin element, negative control). All ethidium bromide–stained agarose gels shown are representative of three independently performed experiments.