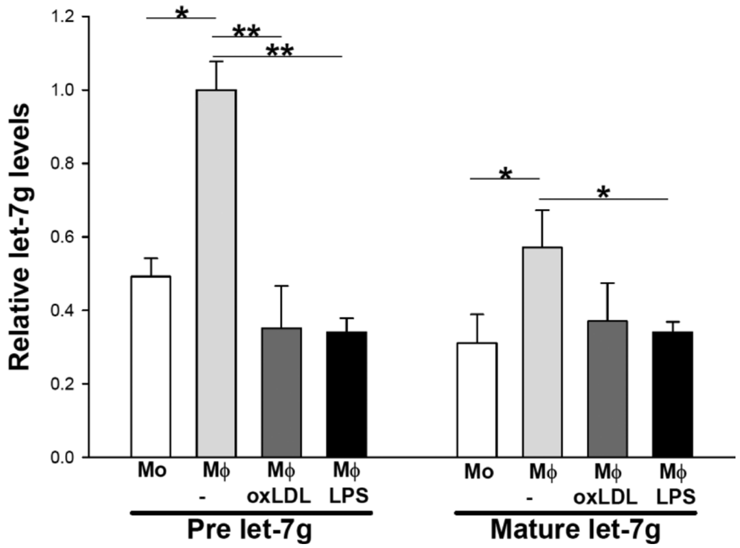
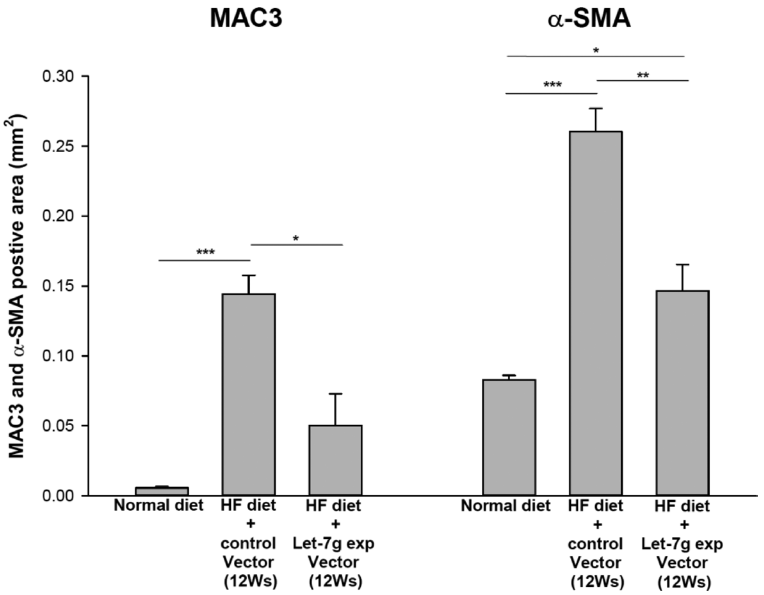
# Let-7g suppresses both canonical and non-canonical NF-κB pathways in macrophages leading to anti-atherosclerosis

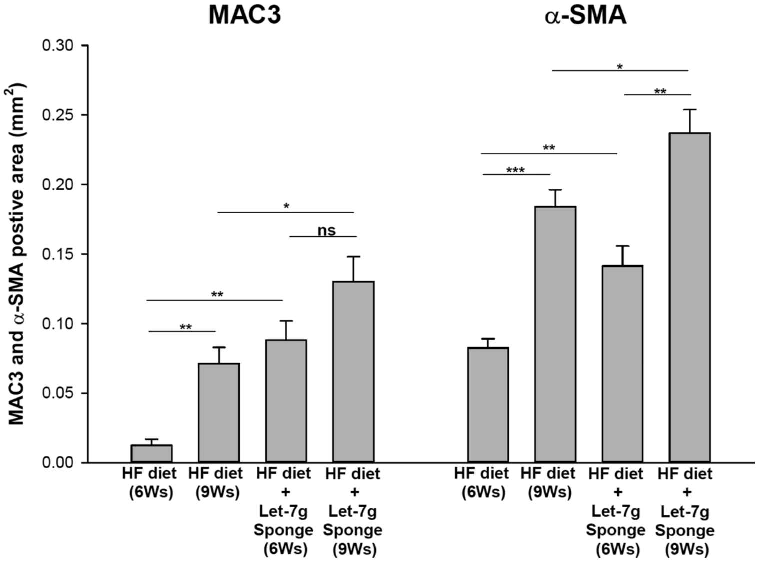
## SUPPLEMENTARY MATERIALS



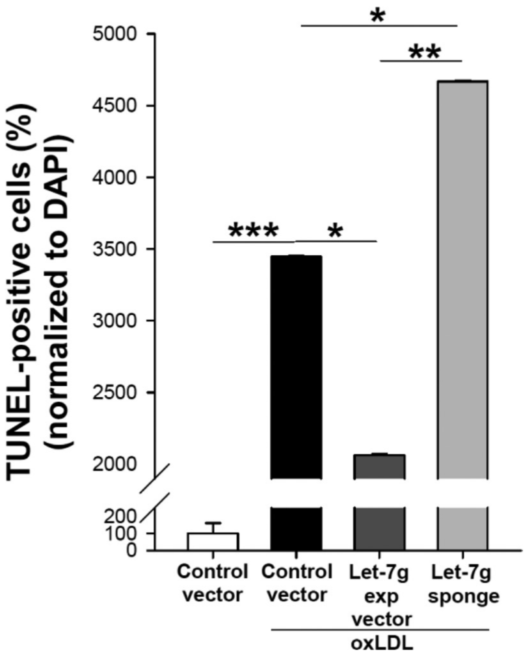
**Supplementary Figure 1: Quantitative analysis of northern blot for biotin labeled mature and pre-microRNA let-7g in monocyte or macrophage treated with oxLDL or LPS.** The amount of RNA loaded in each well was normalized to the amount of U6. Data are presented as mean ± SEM. $^{*}$< 0.05; $^{**}$< 0.01.



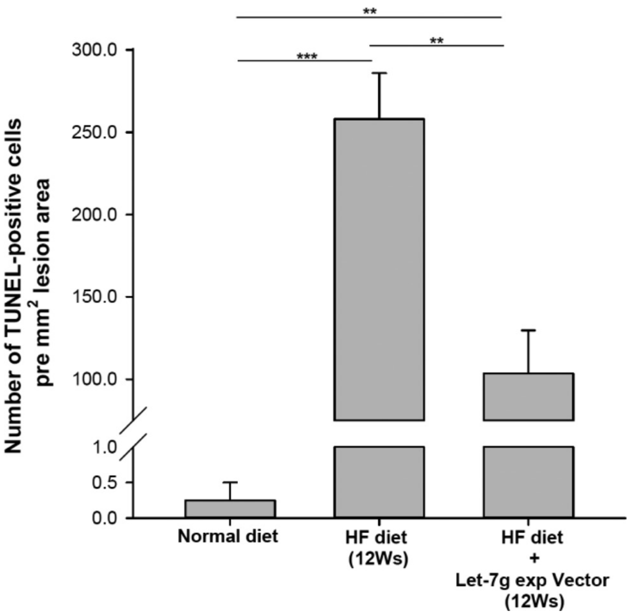
**Supplementary Figure 2: Quantitative analysis of fluorescently labeled MAC3 and α-SMA of let-7g's effect in apoE KO mice under a HF diet for 12 weeks.** Data are presented as mean ± SEM from three independent experiments. $^{*}$< 0.05; $^{**}$< 0.01; $^{***}$< 0.001.



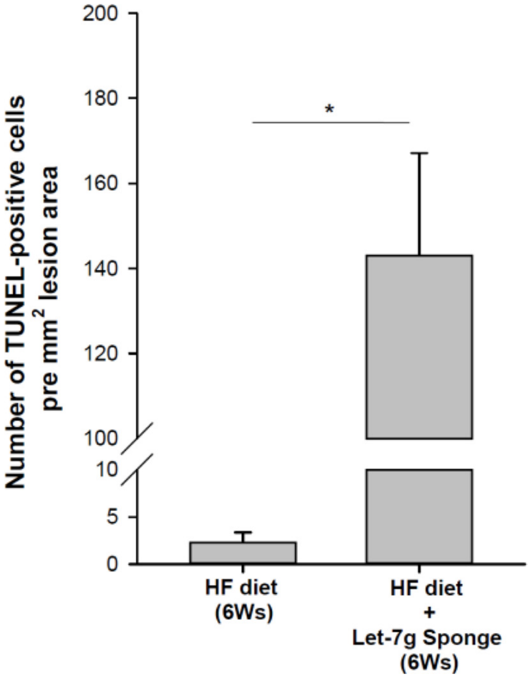
**Supplementary Figure 3: Quantitative analysis of fluorescence labeled MAC3 and α-SMA of let-7g's knock-out effect in apoE KO mice under a HF diet for 6 or 9 weeks.** Data are presented as mean ± SEM from three independent experiments. $^{*}$< 0.05; $^{**}$< 0.01; $^{***}$< 0.001.



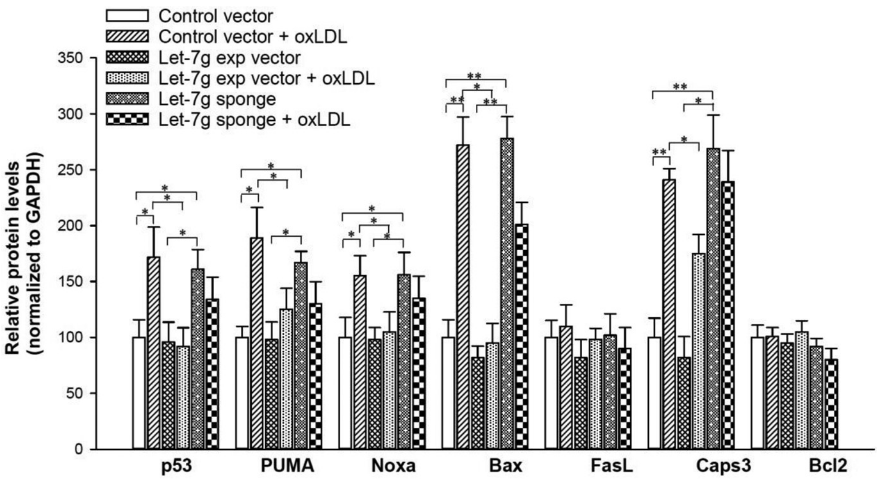
**Supplementary Figure 4: Quantitative analysis of TUNEL-positive macrophages of let-7g's effect.** Data are presented as mean ± SEM from three independent experiments. $^{*}$< 0.05; $^{**}$< 0.01; $^{***}$< 0.001.



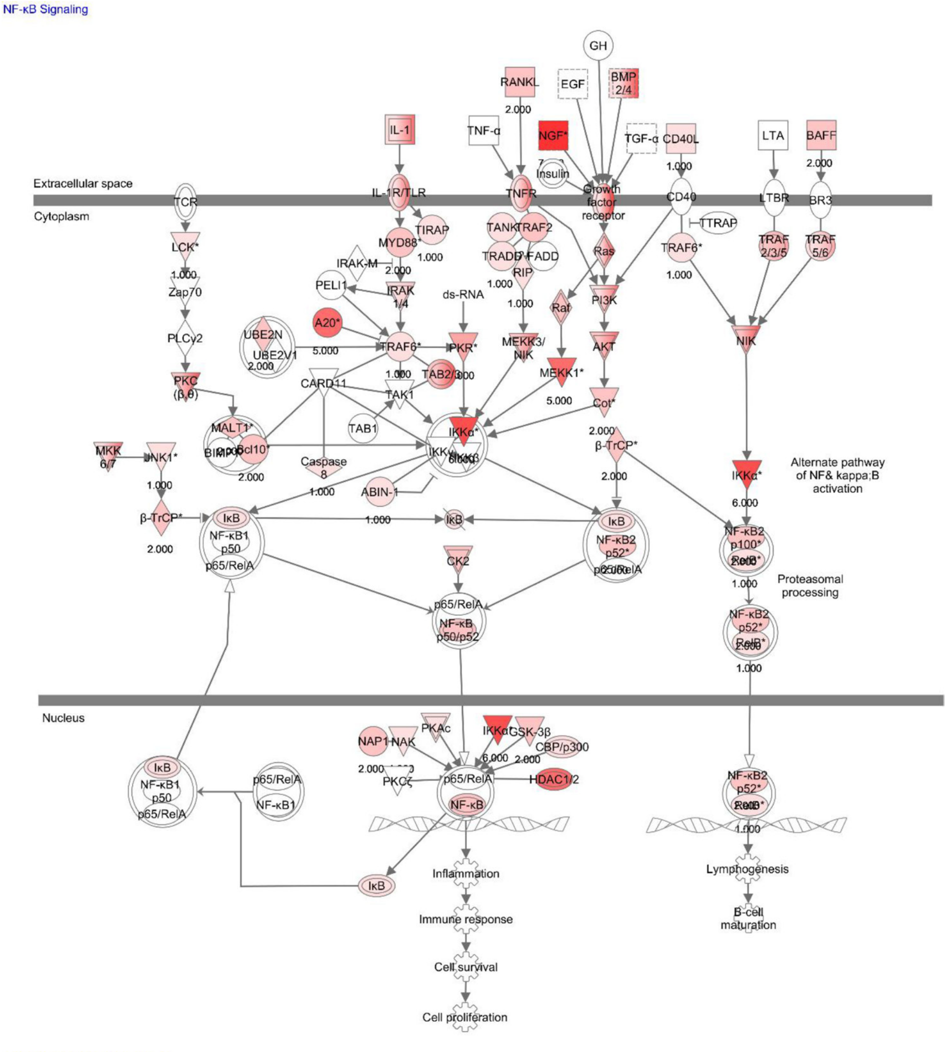
**Supplementary Figure 5: Quantitative analysis of TUNEL-positive cells in apoE KO mice aortic sections of let-7g's effect.** Data are presented as mean ± SEM from three independent experiments. $^{*}< 0.05$; $^{**}< 0.01$; $^{***}< 0.001$.



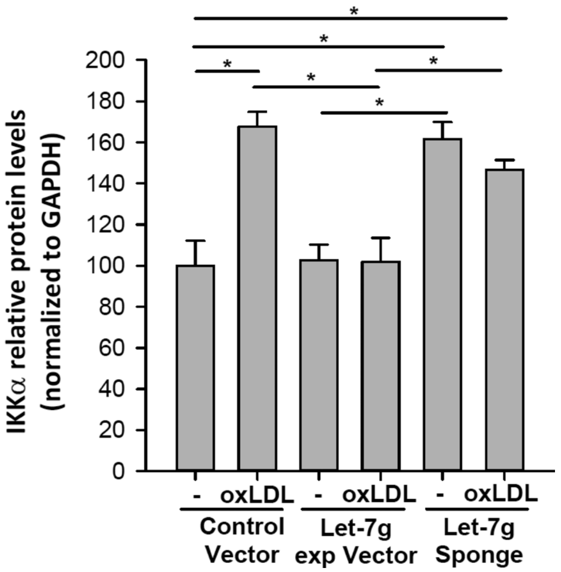
**Supplementary Figure 6: Quantitative analysis of TUNEL-positive cells in apoE KO mice aortic sections of let-7g's knock-out effect.** Data are presented as mean ± SEM from three independent experiments. $^{*}< 0.05$; $^{**}< 0.01$; $^{***}< 0.001$.



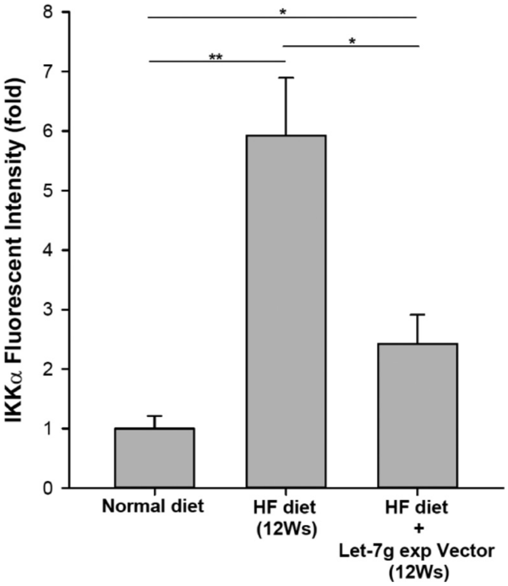
**Supplementary Figure 7: Semi-quantitative western blot data of let-7g's effect on the p53-signaling in oxLDL-treated macrophages.**



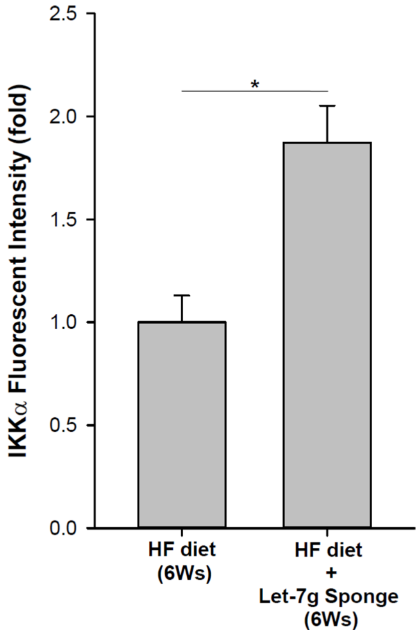
**Supplementary Figure 8: Potential let-7g target genes involved in the NF-κB signaling pathway by ingenuity pathway analysis (IPA).**



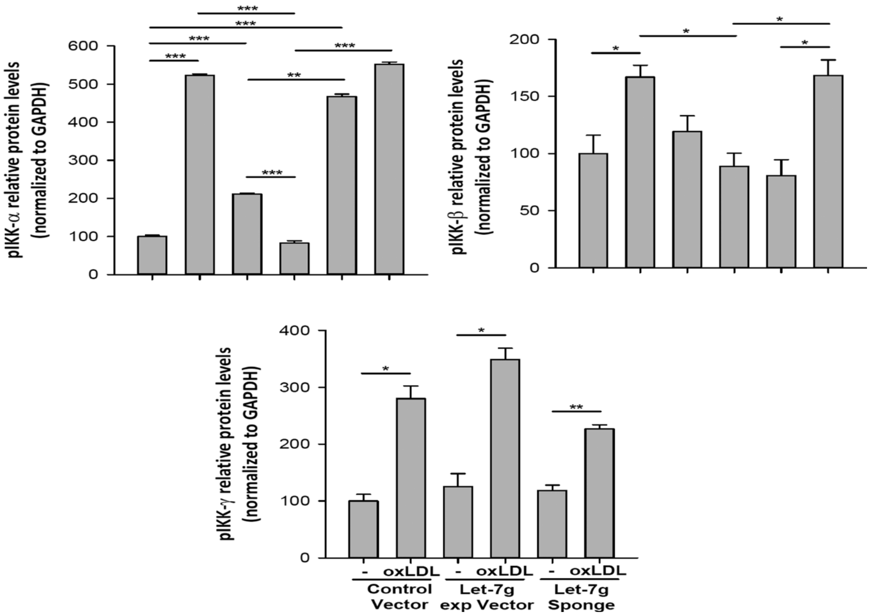
**Supplementary Figure 9: Semi-quantitative western blot data of let-7g's effect on IKKα relative proteins expression in macrophages.** Data are presented as mean ± SE from 3 independent experiments. $^{*}< 0.05$; $^{**}< 0.01$; $^{***}< 0.001$.



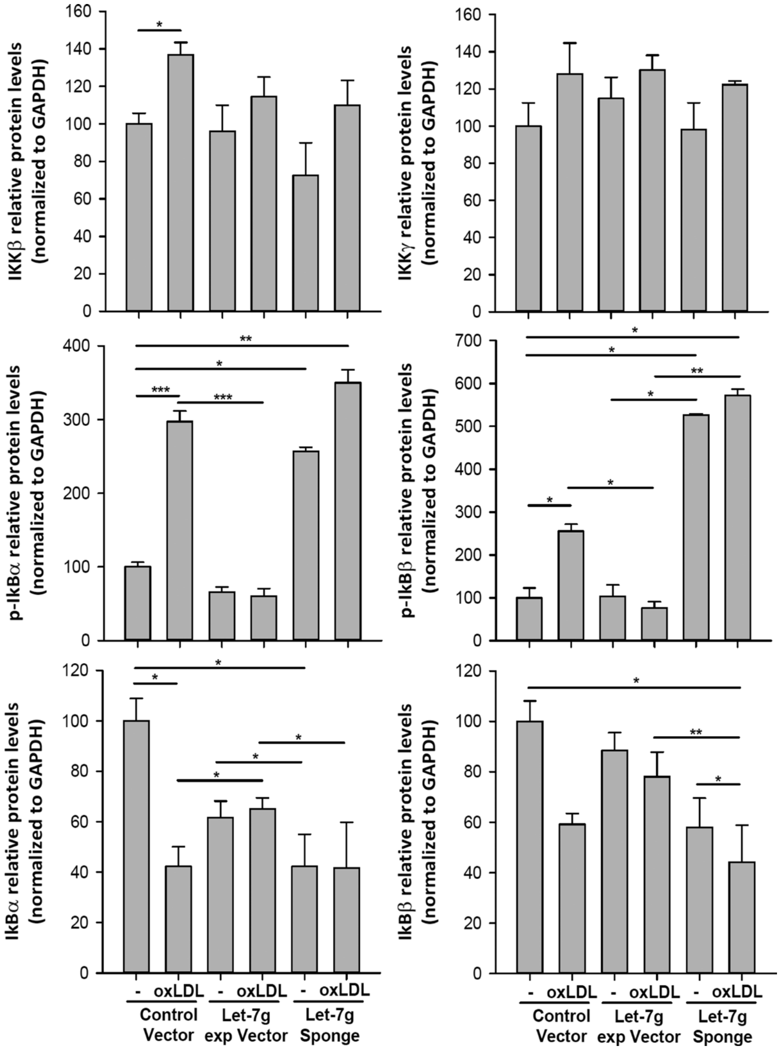
**Supplementary Figure 10: Quantitative analysis of fluorescence labeled plaques of let-7g's effect on IKKα expression in apoE KO mice under a HF diet for 12 weeks.** Data are presented as mean ± SEM from three independent experiments. $^{*}< 0.05$; $^{**}< 0.01$.



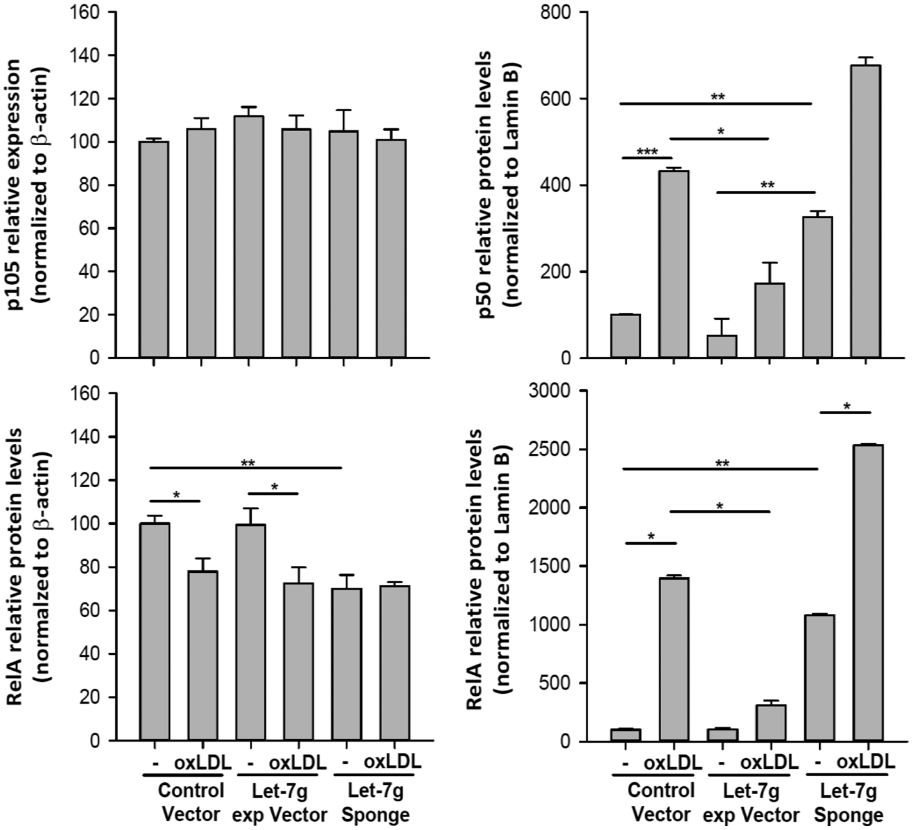
**Supplementary Figure 11: Quantitative analysis of fluorescence labeled plaques of let-7g's knock-out effect on IKKα expression in apoE KO mice under a HF diet for 6 weeks.** Data are presented as mean ± SEM from three independent experiments. $^{*}< 0.05$



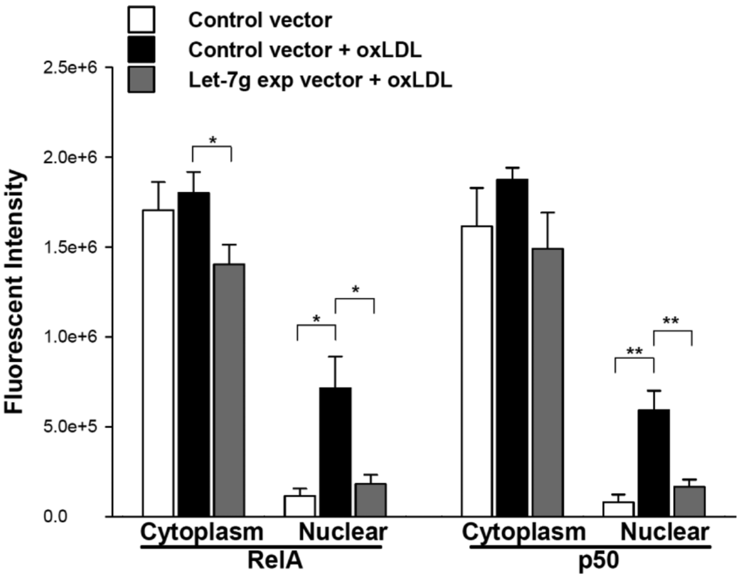
**Supplementary Figure 12: Semi-quantitative western blot data of let-7g's effect on phosphorylated-IKKα, IKKβ and IKKγ relative proteins expression in macrophages.** Data are presented as mean ± SE from 3 independent experiments. $^{*}<0.05$; $^{**}< 0.01$; $^{***}< 0.001$.



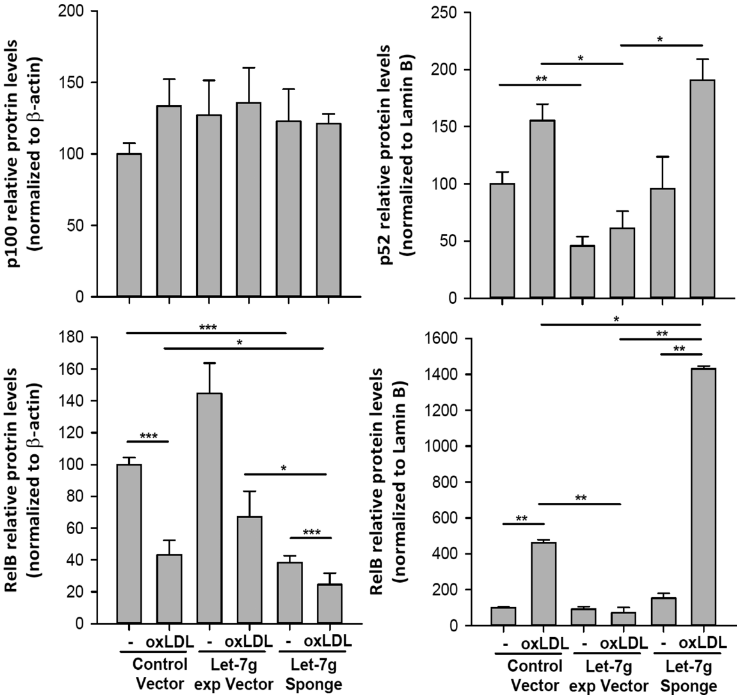
**Supplementary Figure 13: Semi-quantitative western blot data of let-7g's effect on IKKβ, IKKγ, IκBα, IκBβ, phosphorylated-IκBα (p-IκBα) and p-IκBβ relative proteins expression in macrophages.** Data are presented as mean ± SEM from 3 independent experiments. $^{*}< 0.05$; $^{**}< 0.01$; $^{***}< 0.001$.



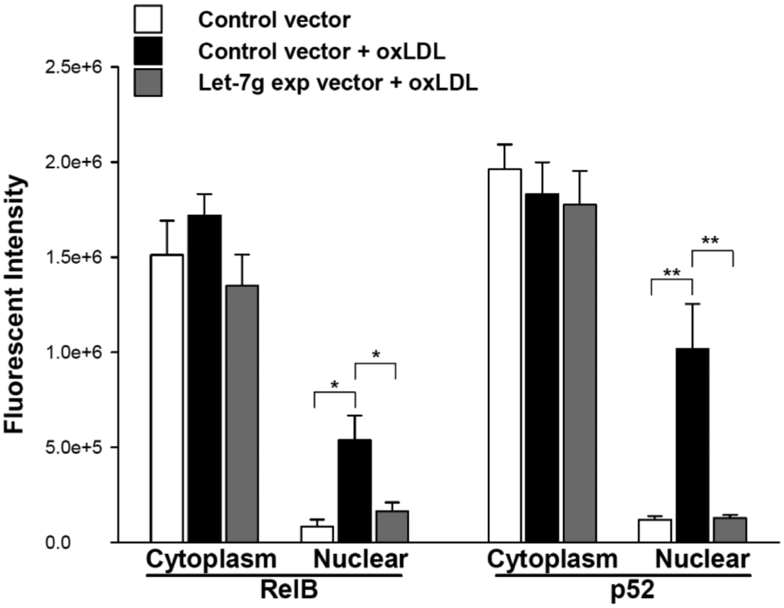
**Supplementary Figure 14: Semi-quantitative western blot data of let-7g's effect on p105, p50 and RelA relative proteins expression in macrophages.** Data are presented as mean ± SEM from 3 independent experiments. $^{*}$< 0.05; $^{**}$< 0.01; $^{***}$< 0.001.



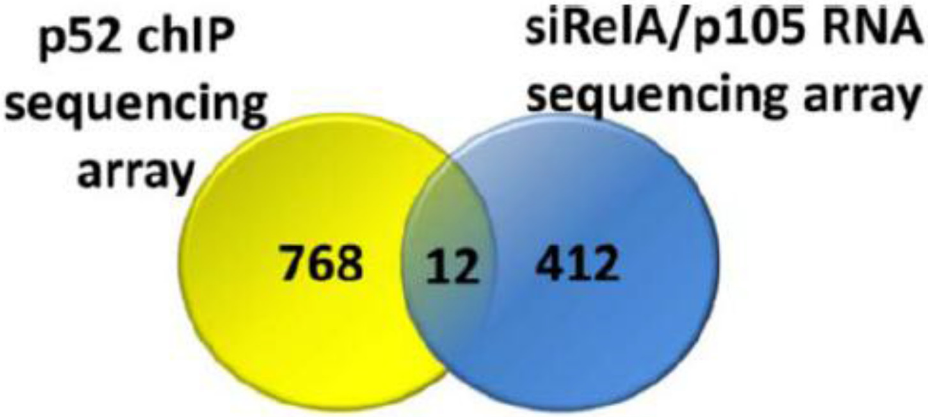
**Supplementary Figure 15: Semi-quantitative analysis of RelA or p50 fluorescence in the oxLDL treated-macrophages treated with control vector or Let-7g exp vector.** Data are presented as mean ± SEM from 3 independent experiments. $^{*}$< 0.05; $^{**}$< 0.01



**Supplementary Figure 16: Semi-quantitative western blot data of let-7g's effect on p100, p52 and RelB relative proteins expression in macrophages.** Data are presented as mean ± SEM from 3 independent experiments. $^{*}$< 0.05; $^{**}$< 0.01; $^{***}$< 0.001.



**Supplementary Figure 17: Semi-quantitative analysis of RelB or p52 fluorescence in the oxLDL traeted-macrophages treated with control vector or Let-7g exp vector.** Data are presented as mean ± SEM from 3 independent experiments. $^{*}$< 0.05; $^{**}$< 0.01.



**Supplementary Figure 18: Venn diagram for Chip-seq and RNA-seq experiments.** Venn diagram shows p52 binding sites identified by p52 ChIP-seq and RNA-seq experiments in macrophages co-transfected with RelA-siRNA and p105-siRNA during foam cell formation. Twelve p52-affected and differentially expressed transcripts in the non-canonical pathway are indicated by the combination of results from ChiP-seq and RNA-seq experiments.

## Supplementary Table 1: Primer sets

| Method | Gene | Primer Sequence | Reference |
|---|---|---|---|
| QRT-PCR | **IKKα** | F: 5′-CAGCCATTTACCTGGCATGAG-3′<br>R: 5′-GAGGGTCCCAATTCAACATCAA-3′ | [1] |
| | **p53** | F: 5′-CCCAAGCAATGGATGATTTGA-3′<br>R: 5′-GGCATTCTGGGAGCTTCATCT-3′ | [2] |
| | **GAPDH** | F: 5′-ACCCACTCCTCCACCTTTGA-3′<br>R: 5′-CTGTTGCTGTAGCCAAATTCGT-3′ | [3] |
| | **SREBF2** | F: 5′-CGAATTGAAAGACCTGGTCATG-3′<br>R: 5′-TCCTCAGAACGCCAGACTTGT-3′ | [4] |
| ChIP Assay | **SREBF2** | | |
| | (-1615 bp region) | F: 5′-CAACTCCTGACCTCAAATGATCTG-3′<br>R: 5′-GCTCTCGGCATAAGCTTTGG-3′ | |
| | (-15 bp region) | F: 5′-CCCATTGACAACAAACAG-3′<br>R: 5′-CCGCCATGTTTGCGTTGC-3′ | |
| Genotyping | **Cre** | | [5] |
| | NLSCre | 5′-CCCAAGAAGAAGAGGAAGGTGTCC-3′ | |
| | Cre8 | 5′-CCCAGAAATGCCAGATTACG-3′ | |
| | **apoE** | | [6] |
| | Primer 1 | 5′-GCCTAGCCGAGGGAGAGCCG-3′ | |
| | Primer 2 | 5′-TGTGACTTGGGAGCTCTGCAGC-3′ | |
| | Primer 3 | 5′-GCCGCCCCGACTGCATCT-3′ | |

## Supplementary Table 2: 29 NF-κB related genes

| let7g and NFkB | DIANAmT | miRanda | miRDB | miRWalk | RNAhybrid | PICTAR5 | PITA | RNA22 | Targetscan | SUM |
|---|---|---|---|---|---|---|---|---|---|---|
| AZI2 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| BCL10 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| BTRC | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| CASP8 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| CD40LG | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| IKKα | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 6 |
| EIF2AK2 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 3 |
| GSK3B | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| LCK | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| MALT1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| MEKK1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 5 |
| MAP3K8 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| MAPK8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| MYD88 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| NFKB2 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| NGF | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 7 |
| RELB | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| RIPK1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| TANK | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TBK1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TIRAP | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| A20 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 5 |
| TNFSF11 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| TNFSF13B | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| TNIP1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TRADD | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| TRAF2 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| TRAF6 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| UBE2N | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |

## Supplementary Table 3: Gene ontology annotation of 12 genes which were directly affected by p52 in the non-canonical pathway

| | Fold Change | Molecule Function | Biological Process | Cellular Component |
|---|---|---|---|---|
| **Foam Cell Formation** | | | | |
| **ATGL** | **2.21** | Protein binding | Lipid catabolic process | Cytosol |
| **MARCO** | **0.33** | Scavenger receptor activity | Receptor-mediated endocytosis | Membrane |
| **Macrophage Movement** | | | | |
| **NPAS3** | **35.83** | Signal transducer activity | Locomotory behavior | Cytoplasm |
| **TNC** | **5.65** | Fibronectin binding | Extracellular matrix organization | Extracellular matrix |
| **DNAH11** | | Microtubule motor activity | Microtubule-based movement | Axonemal dynein complex |
| **Response to Oxidative Stress** | | | | |
| **RRM2B** | **2.33** | Oxidoreductase activity | Response to oxidative stress | Mitochondrion |
| **Cell Apoptosis** | | | | |
| **DAB2IP** | **18.81** | Protein binding | Induction of apoptosis via death domain receptors | Cytoplasm |
| **OBSCN** | **0.19** | Protein binding | Positive regulation of apoptotic process | Cytosol |
| **Others** | | | | |
| **THAP8** | **3.06** | DNA binding | NA | NA |
| **ZNF625** | **2.26** | DNA binding | Regulation of transcription | Nucleus |
| **ATAD3A** | **0.35** | ATP binding | Cell growth | Mitochondrion |
| **NUBPL** | **0.32** | ATP binding | Mitochondrial respiratory chain complex I assembly | Mitochondrion |

## Supplementary Table 4: List of siRNAs used in this study

| siRNA | Accession No. | Sequence |
|---|---|---|
| p105 siRNA-1 | NM_003998 | GGGUAUAGCUUCCCACACU |
| p105 siRNA-2 | | AGUGUGGGAAGCUAUACCC |
| Rel A siRNA-1 | NM_021975 | GGAAUCCAGUGUGUGAAGA |
| Rel A siRNA-2 | | UCUUCACACACUGGAUUCC |
| p100 siRNA-1 | NM_001077493 | CUGUCAAGAUCUGUAACUA |
| p100 siRNA-2 | | UAGUUACAGAUCUUGACAG |
| Rel B siRNA-1 | NM_006509 | GAGAUUGUCGAGCCCGUGA |
| Rel B siRNA-2 | | UCACGGGCUCGACAAUCUC |
| Control siRNA-1 | | GAUCAUACGUGCGAUCAGA |
| Control siRNA-2 | | UCUGAUCGCACGUAUGAUC |

## REFERENCES

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