

An activator of mTOR inhibits oxLDL -induced autophagy and apoptosis in vascular endothelial cells and restricts atherosclerosis in apolipoprotein E^{-/-} mice

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Supplemental Information

Supplemental Figures

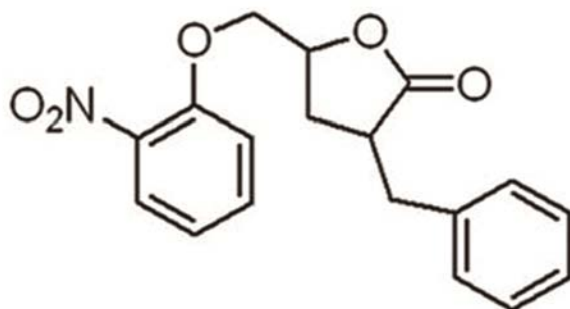


Figure S1. Chemical structure of 3BDO, 3-benzyl-5-((2-nitrophenoxy)methyl)-dihydrofuran-2 (3H)-one.

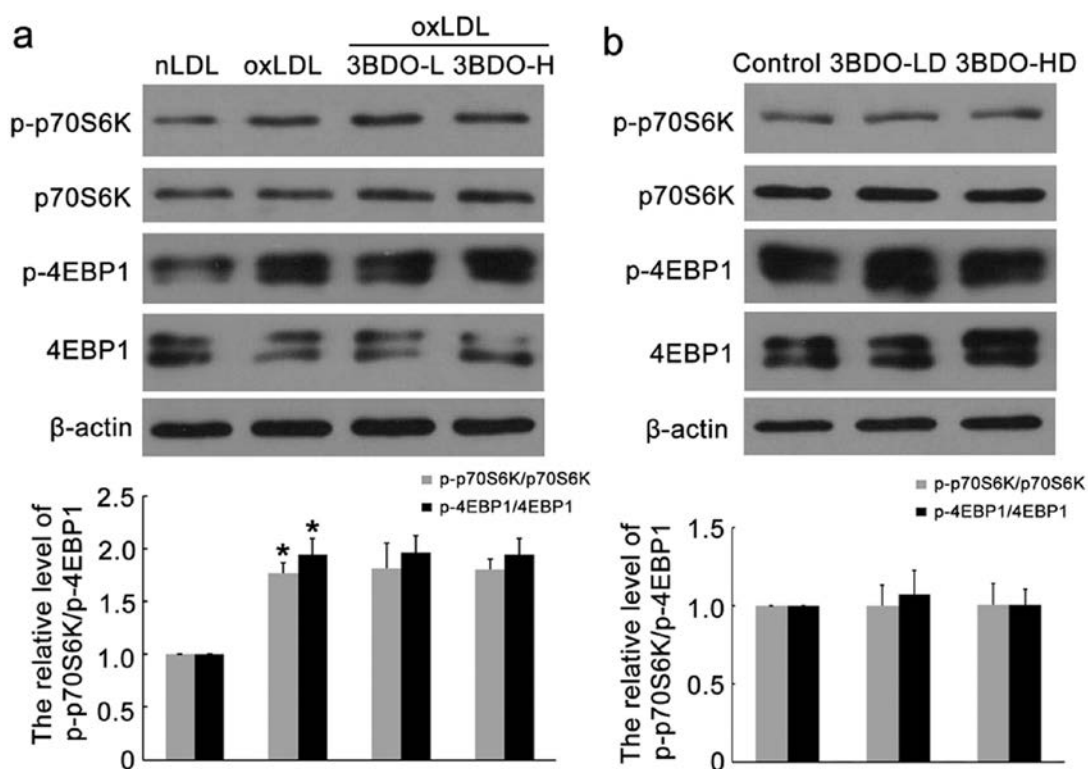


Figure S2. Effect of 3BDO on oxLDL-activated mTOR in macrophage cell line RAW246.7 and vascular smooth muscle cells (VSMCs) of apolipoprotein E-deficient (*apoE*^{-/-}) mice.

a, Western blot analysis of p-p70S6K, p70S6K, p-4EBP1 and 4EBP1 in macrophage cell line RAW246.7. treated with nLDL, 50 μ g/ml; oxLDL, 50 μ g/ml; 3BDO-L, 60 μ M; and 3BDO-H, 120 μ M, for 12 h and densitometry results of ratio of p-p70S6K to total p70S6K and p-4EBP1 to total 4EBP1. Data are mean \pm SEM; * p < 0.05 vs. nLDL, n = 3. **b**, Western blot analysis of p-p70S6K, p70S6K, p-4EBP1 and 4EBP1 in VSMCs of apoE^{-/-} mice and densitometry results of ratio of p-p70S6K to total p70S6K and p-4EBP1 to total 4EBP1. Data are mean \pm SEM. n =6.

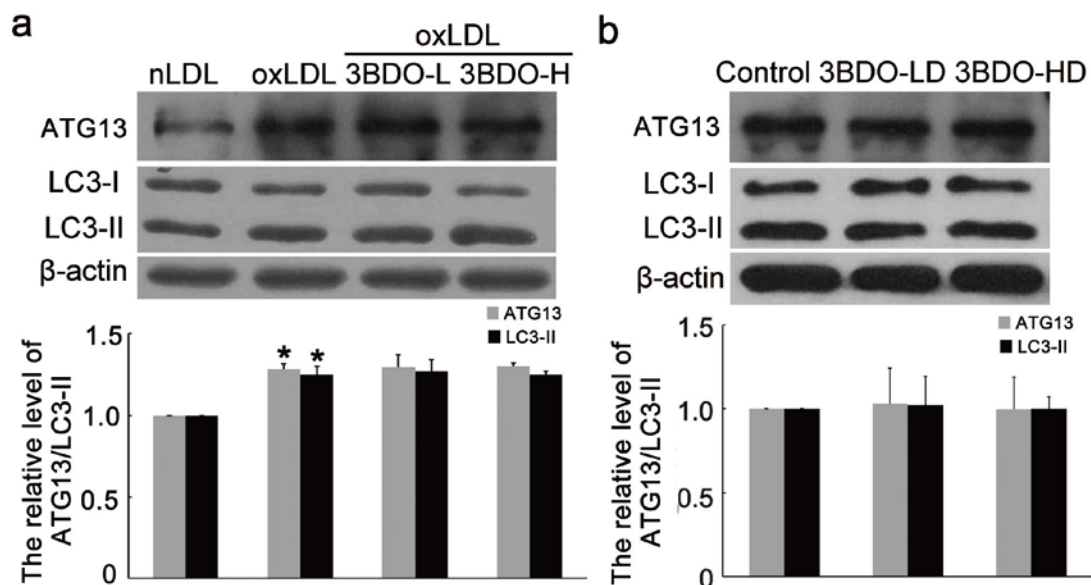


Figure S3. Effect of 3BDO on oxLDL-induced autophagy in macrophage cell line RAW246.7 and vascular smooth muscle cells (VSMCs) of apolipoprotein E-deficient (apoE^{-/-}) mice.

a, Western blot analysis of LC3-II and ATG13 protein level in macrophage cell line RAW246.7 and quantification, with nLDL, 50 μ g/ml; oxLDL, 50 μ g/ml; 3BDO-L, 60 μ M; and 3BDO-H, 120 μ M, for 12 h. Protein levels were normalized to that of β -actin. Data are mean \pm SEM; * p < 0.05 vs. nLDL, n = 3. **b**, Western blot analysis of LC3-II

and ATG13 protein level in VSMCs of apoE^{-/-} mice and quantification. Data are mean ± SEM. n=6.

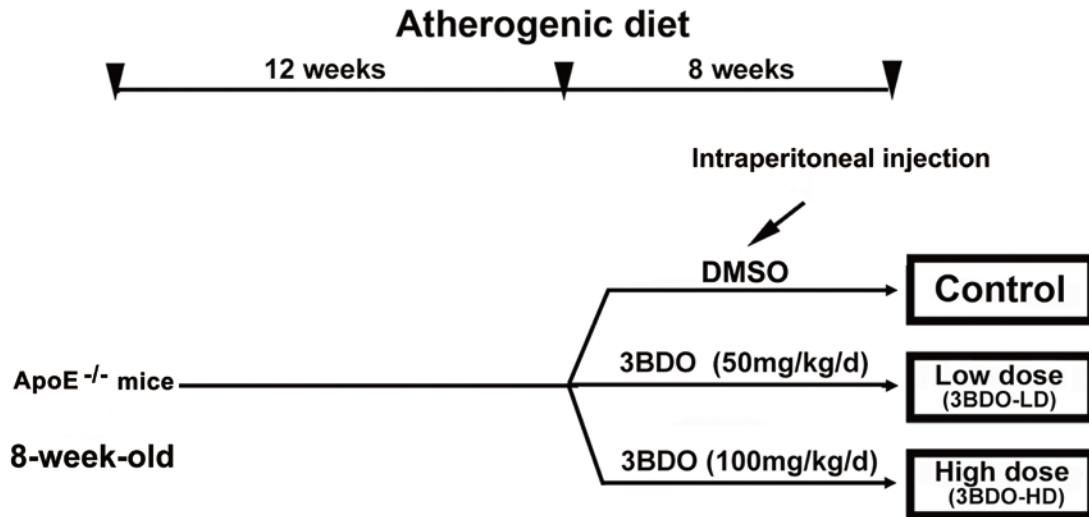


Figure S4. The design of *in vivo* experiment.

Eight-week-old apoE^{-/-} mice were fed an atherogenic diet for 12 weeks and divided into 3 groups for treatment: control (DMSO), low-dose 3BDO (3BDO-LD, 50 mg/kg/d) and high-dose 3BDO (3BDO-HD, 100 mg/kg/d). N.P. drew this image by using Adobe Photoshop (Adobe, San Jose, USA).

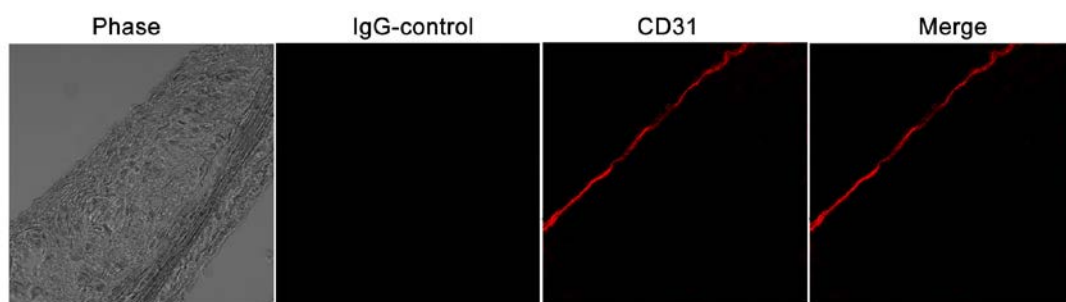


Figure S5. The normal rabbit IgG demonstrated the specificity of the antibody anti-p-70S6K, LC3, α -actin, Mac-3 and ATG13.

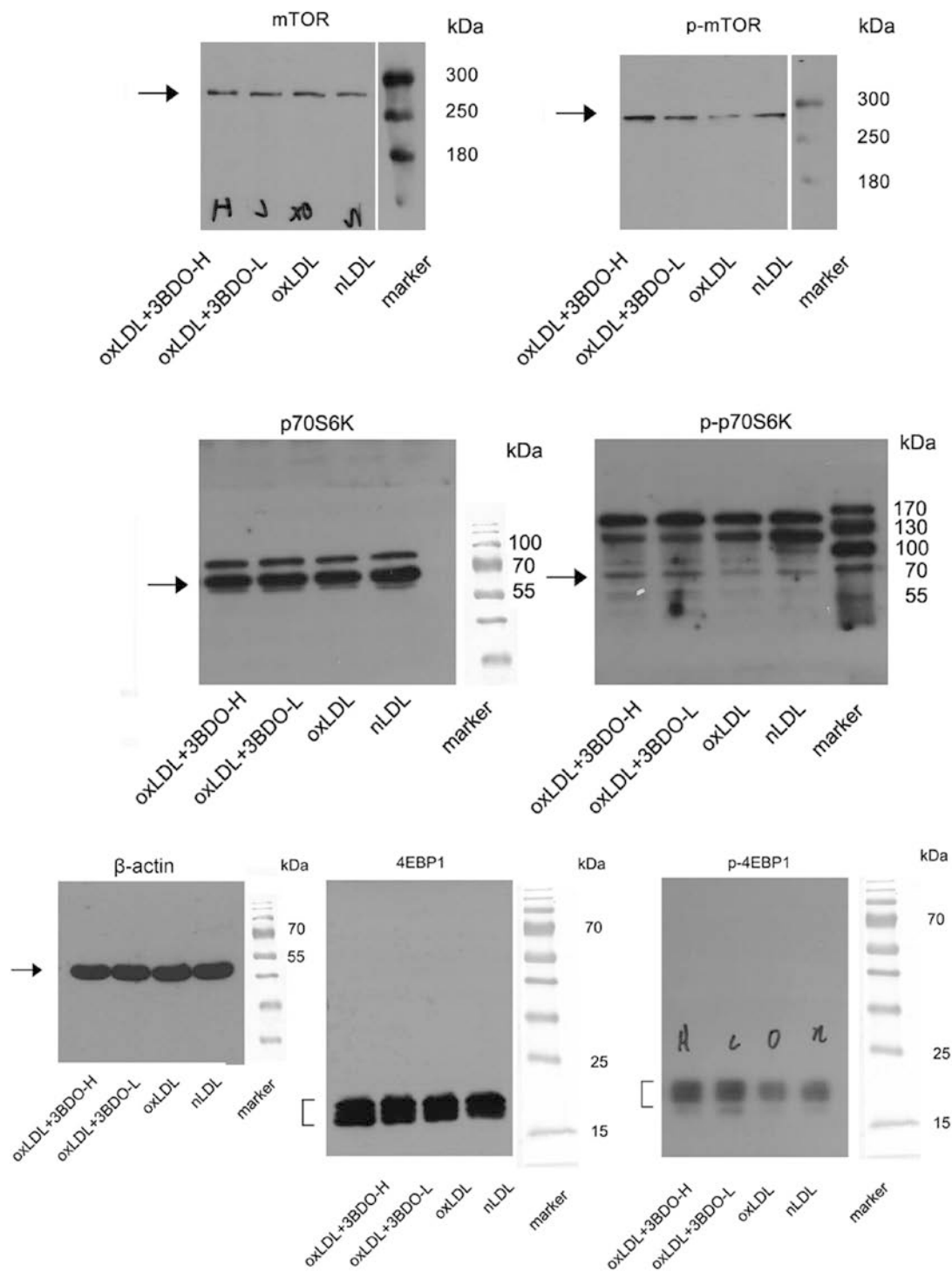


Figure S6. Uncropped blots probed with p-mTOR, mTOR, p-p70S6K, p70S6K , p-4EBP1, 4EBP1 and β -actin.

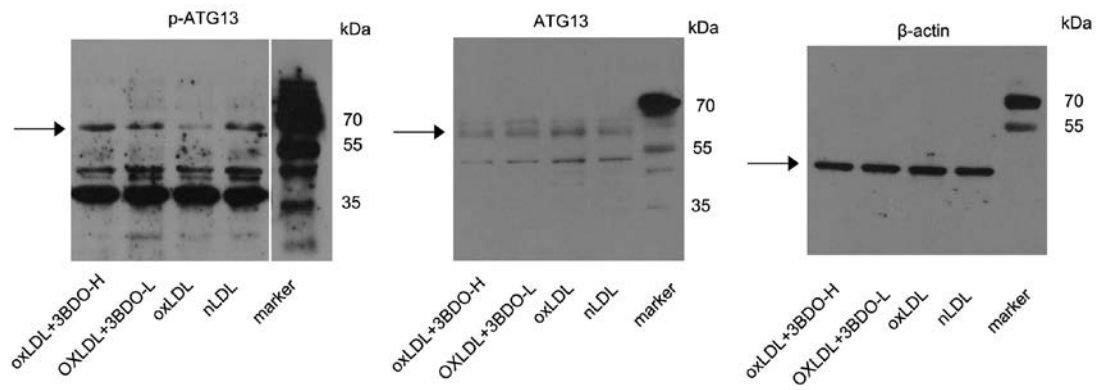


Figure S7. Uncropped blots probed with p-ATG13, ATG13 and β -actin.

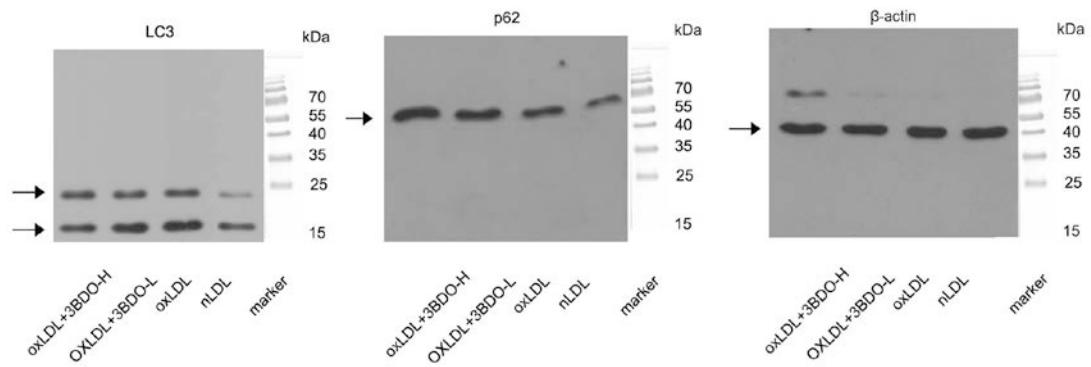


Figure S8. Uncropped blots probed with LC3, p62 and β -actin.

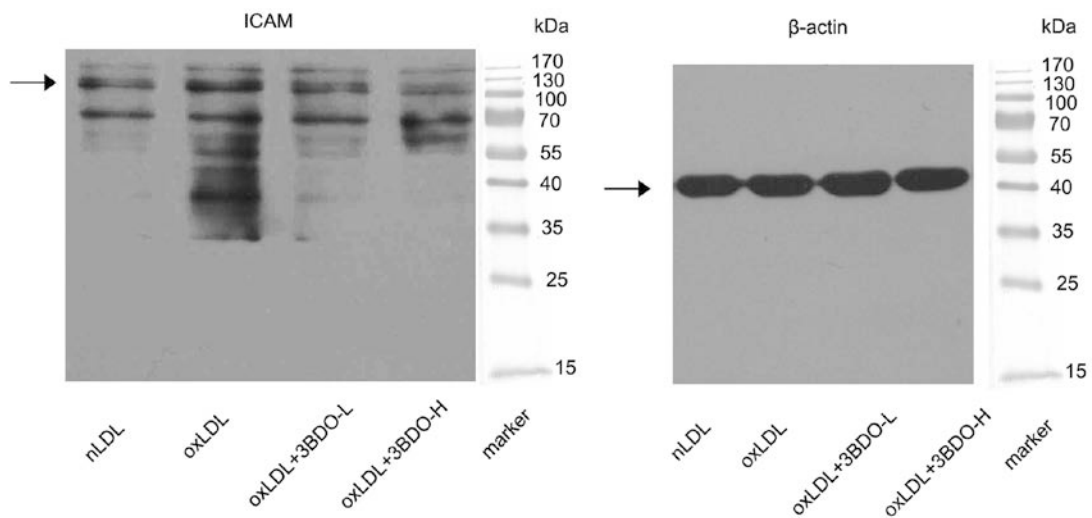


Figure S9. Uncropped blots probed with ICAM-1 and β -actin.

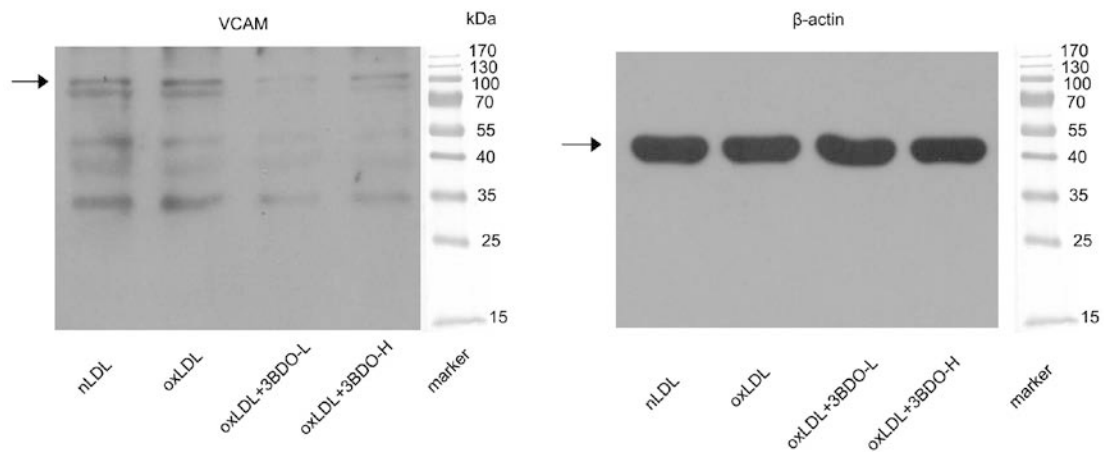


Figure S10. Uncropped blots probed with VCAM-1 and β -actin.

Table S1. Measurement of organ coefficients of mice after treatment with 3BDO.

| Groups | Heart (%) | Liver (%) | Spleen (%) | Lung (%) | Kidney (%) |
|---------|-----------|-----------|------------|-----------|------------|
| Control | 0.40±0.02 | 4.62±0.51 | 0.49±0.03 | 0.53±0.05 | 1.19±0.14 |
| 3BDO-LD | 0.39±0.03 | 4.59±0.53 | 0.48±0.04 | 0.52±0.06 | 1.23±0.15 |
| 3BDO-HD | 0.41±0.03 | 4.66±0.61 | 0.50±0.02 | 0.50±0.03 | 1.15±0.12 |