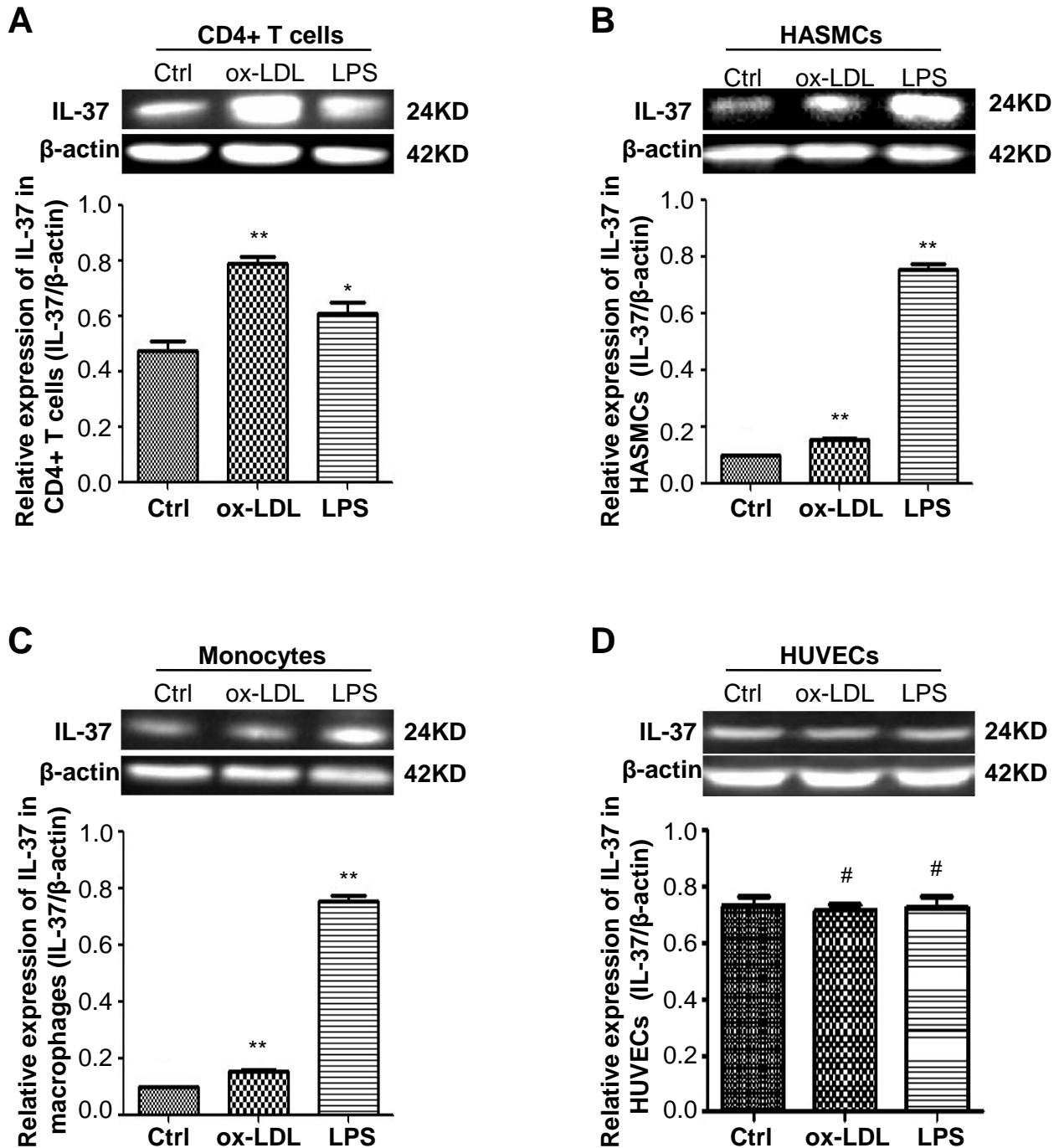
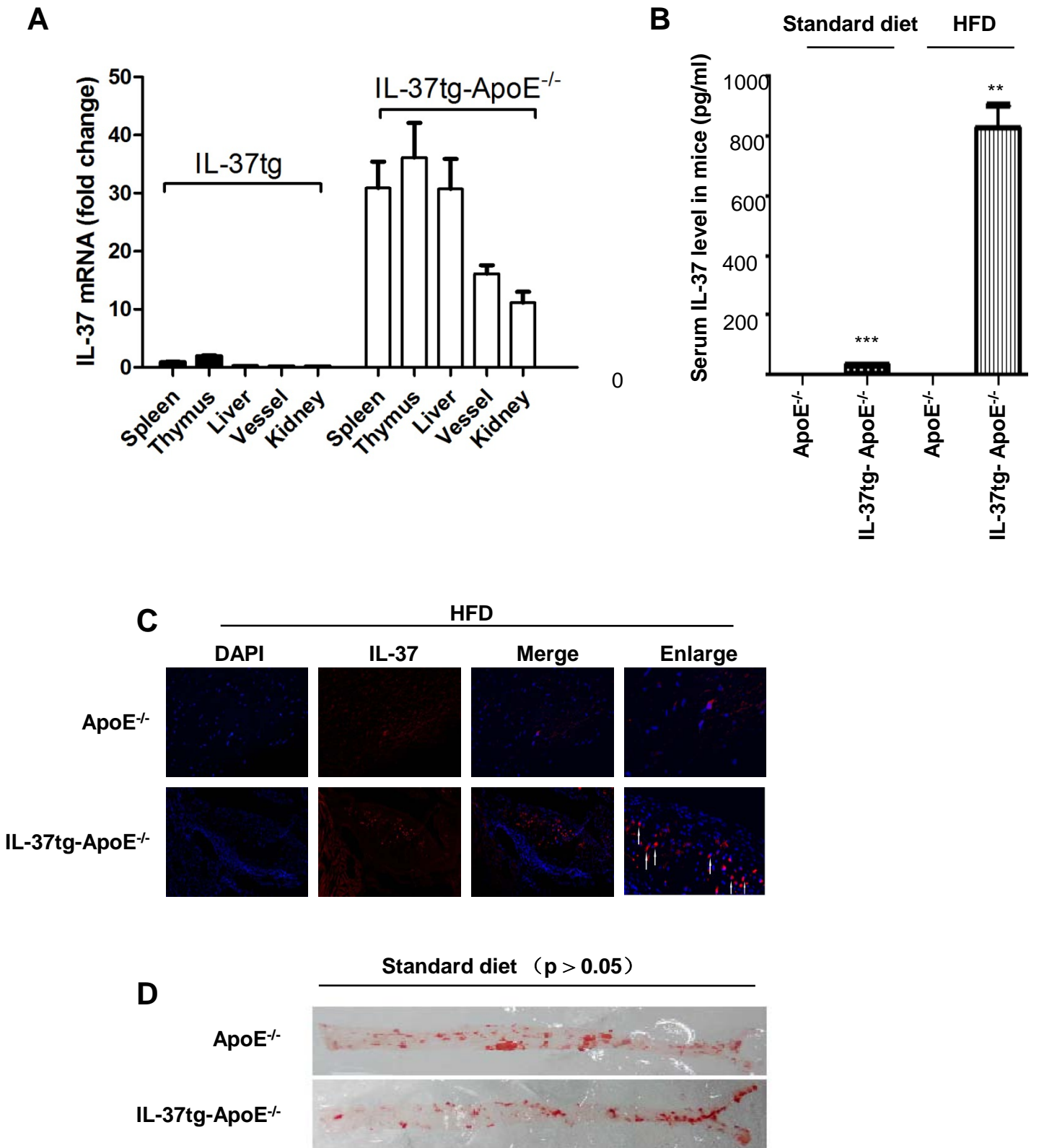


Supplementary Figurers and Table

Transgenic overexpression of IL-37 protects against atherosclerosis and strengthens plaque stability

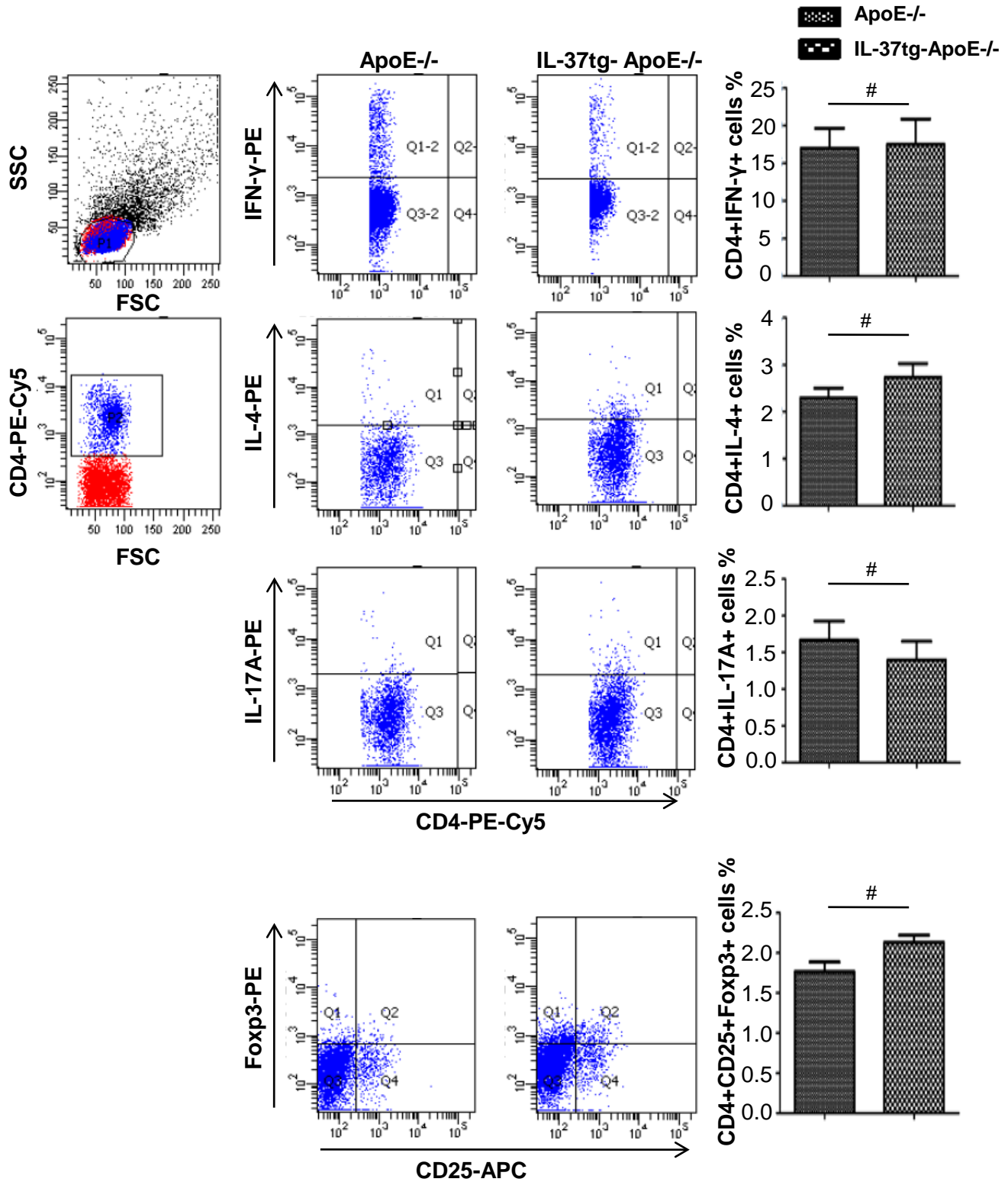


Supplementary Fig 1. IL-37 protein expression is up-regulated in human CD4⁺ T lymphocytes, HASMCs and monocytes, but not HUVECs by LPS or ox-LDL. Freshly isolated human CD4⁺ T lymphocytes (A), monocytes (C), human aortic smooth muscle cell line (HASMCs, B), and human umbilical vein endothelial cell line (HUVECs, D) were stimulated with LPS (1μg/ml) for 12 h or ox-LDL (40ug/ml) for 24h. Statistic analysis were executed by 5-6 independent experiments. Data are presented as means ± SD, *p<0.05, **p<0.01, #p > 0.05 vs Control group (Ctrl).



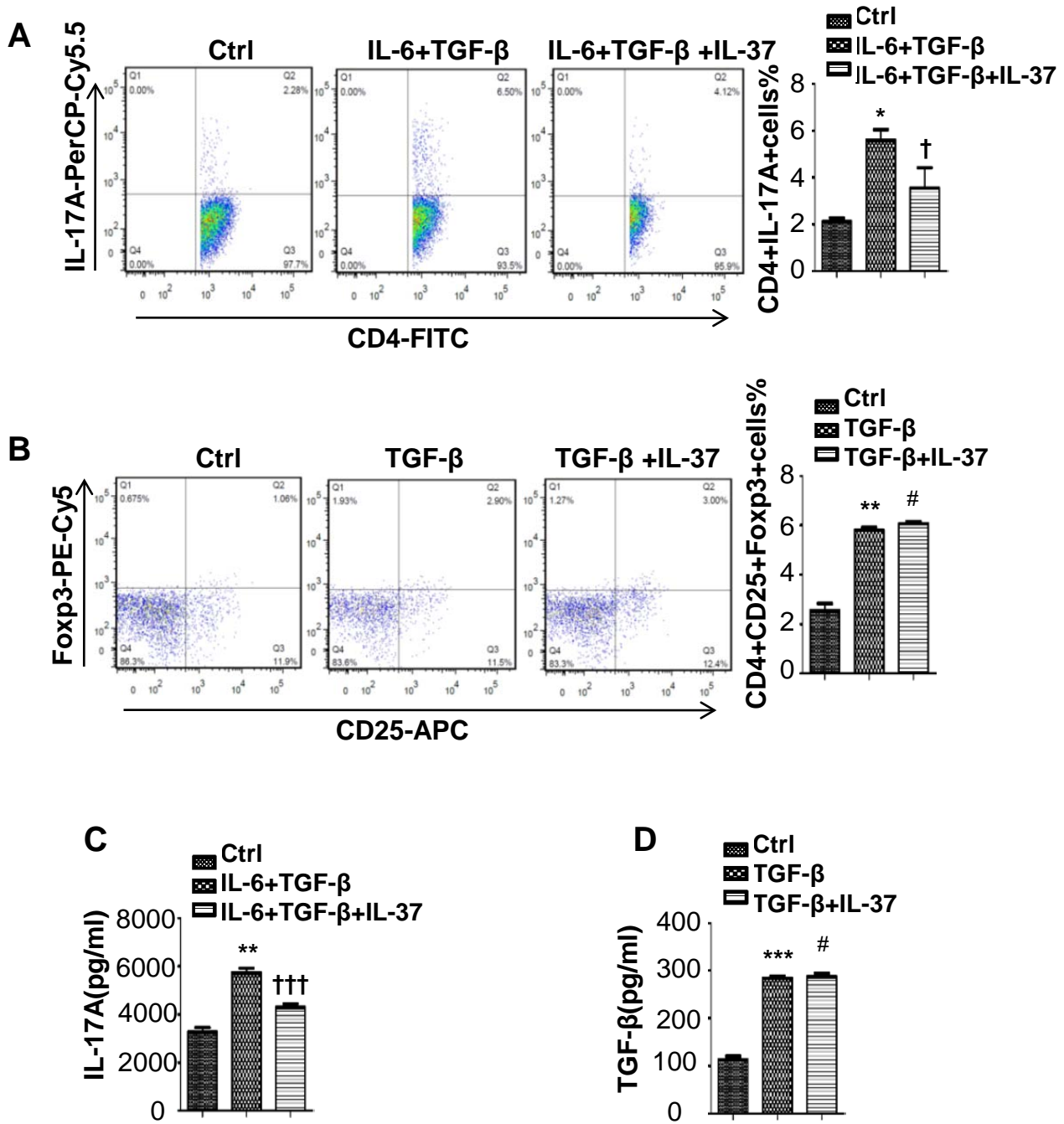
Supplementary Fig 2. IL-37 is increased in IL-37tg-ApoE^{-/-} mice after a high fat diet (HFD).

(A) IL-37 mRNA expression in IL-37tg and IL-37tg-ApoE^{-/-} mice fed a HFD for 12 weeks. (B) Concentration of serum IL-37 in ApoE^{-/-} and IL-37tg-ApoE^{-/-} mice (both with standard diet or HFD) was measured by ELISA test (n=5). (C) Frozen sections of aortic root of ApoE^{-/-} (n=6) and IL-37tg-ApoE^{-/-} (n=6) mice were stained with anti-IL-37 mAb (red) and DAPI (blue) after a HFD by immunofluorescence staining. (D) Representative images of oil red O staining in sections showing the aortic root in different groups. Data are presented as means±SD, **p<0.01, ***p<0.001, p > 0.05 vs ApoE^{-/-} mice group.



Supplementary Fig 3. IL-37 did not regulate CD4+ T cell subsets in spleen in vivo.

Representative FACS plots of Th1 (CD4+IFN-γ+), Th2 (CD4+IL-4+), Th17 (CD4+IL-17A+), Treg (CD4+CD25+Foxp3+) cells in spleen in ApoE^{-/-} and IL-37tg-ApoE^{-/-} mice groups. Statistic analysis were executed by 5-6 independent experiments. Data are presented as means ± SD, #p > 0.05.



Supplementary Fig 4. IL-37 did not regulate Th17 and Treg cell subsets in human CD4⁺ T cells in vivo. (A)-(B) Representative FACS plots of Th17 (CD4⁺IL-17A⁺), Treg (CD4⁺CD25⁺Foxp3⁺) cells human CD4⁺ T cells. (C)-(D) ELISA test of IL-17A and TGF-β. Statistic analysis were executed by 3-4 independent experiments. Data are presented as means ± SD, *p<0.05, **p<0.01, ***p<0.0001, #p > 0.05.

Supplementary Table: Primers used for PCR.

| <i>Gene</i> | <i>Forward (5'-3')</i> | <i>Reverse (5'-3')</i> |
|------------------|--------------------------|----------------------------|
| H GAPDH | GGAGCGAGATCCCTCCAA AAT | GGCTGTTGTCATACTTCTCATGG |
| H IL-37 | GCCCTGAGCAAAGACCCCAA | TTTTCACTGCATTCTAGTTGTGG |
| H T-bet | TTGAGGTGAACGACGGAGAG | CCAAGGAATTGACAGTTGGGT |
| H IFN- γ | ACCACTCCATTGTACCAACTCA | TGTGCTTCTCCACTCATCTGT |
| H GATA3 | GCCCCTCATTAAGCCCAAG | TTGTGGTGGTCTGACAGTTTCG |
| H IL-4 | CCAAGTGTCTCCCTCTG | TCTGTTACGGTCAACTCGGTG |
| H ROR γ t | GTGGGGACAAGTCGTCTGG | AGTGCTGGCATCGGTTTCG |
| H IL-17A | CCCACGAAATCCAGGATGC | GGATGTTGAGTTGACCATCAC |
| H Foxp3 | GTGGCCCGGATGTGAGAAG | GGAGCCCTTGTCCGATGATG |
| H TGF- β | TGAGGGCTTTTCGCCTTAGC | CGGTAGTGAACCCGTTGATGT |
| M β -actin | GTGACGTTGACATCCGTAAAGA | GCCGGACTCATCGTACTCC |
| M T-bet | AGCAAGGACGGCGAATGTT | GTGGACATATAAGCGGTTCCC |
| M IFN- γ | GCCACGGCACAGTCATTGA | TGCTGATGGCCTGATTGTCTT |
| M GATA3 | AAGCTCAGTATCCGCTGACG | GTTTCCGTAGTAGGACGGGAC |
| M IL-4 | GGTCTCAACCCCAAGCTAGT | GCCGATGATCTCTCTCAAGTGAT |
| M ROR γ t | TCCACTACGGGGTTATCACCT | AGTAGGCCACATTACACTGCT |
| M IL-17A | GGCCCTCAGACTACCTCAAC | TCTCGACCCTGAAAGTGAAGG |
| M Foxp3 | ACCATTGGTTTACTCGCATGT | TCCACTCGCACAAAGCACTT |
| M TGF- β | CCACCTGCAAGACCATCGAC | CTGGCGAGCCTTAGTTTGGAC |
| M Collagen I | AATTCGGACTAGACATTGGCCCTG | GGTTGTTTCTGTCTGTTTCCAGGGTT |
| M Collagen III | TGAAGGCGAATTCAAGGCTGAAGG | AGGGCCAATGTCCACACCAAATTC |
| M CollagenVIII | ACTCTGTCAGACTCATTGAGGC | CCCAAAGGCATGTGAGGGAC |
| M MMP-1 | TGCAACTCTGACGTTGATCCCAGA | ACTGCACATGTGTTCTTGAGCTGC |
| M MMP-2 | ACCTGAACACTTTCTATGGCTG | CTTCCGCATGGTCTCGATG |
| M MMP-9 | CTGGACAGCCAGACACTAAAG | CTCGCGGCAAGTCTTCAGAG |
| M MMP-13 | CTAGGTCTGGATCACTCCAAGG | CTGGTAATGGCATCAAGGGATA |
| M VCAM-1 | TACCAGCTCCCAAATCCTG | TCTGCTAATTCCAGCCTCGT |
| M VCAM-1 | GACTGAGGAGTTCGACAGAACC | AGGACCGGAGCTGAAAAGTT |
| M E-selectin | CAAATCCCAGTCTGCAAAGC | CAACTGGACCCATTTTGGAA |
| M P-selectin | CCCTGGCAACAGCCTTCAG | CAACTGGACCCATTTTGGAA |

H means human, M means mouse.