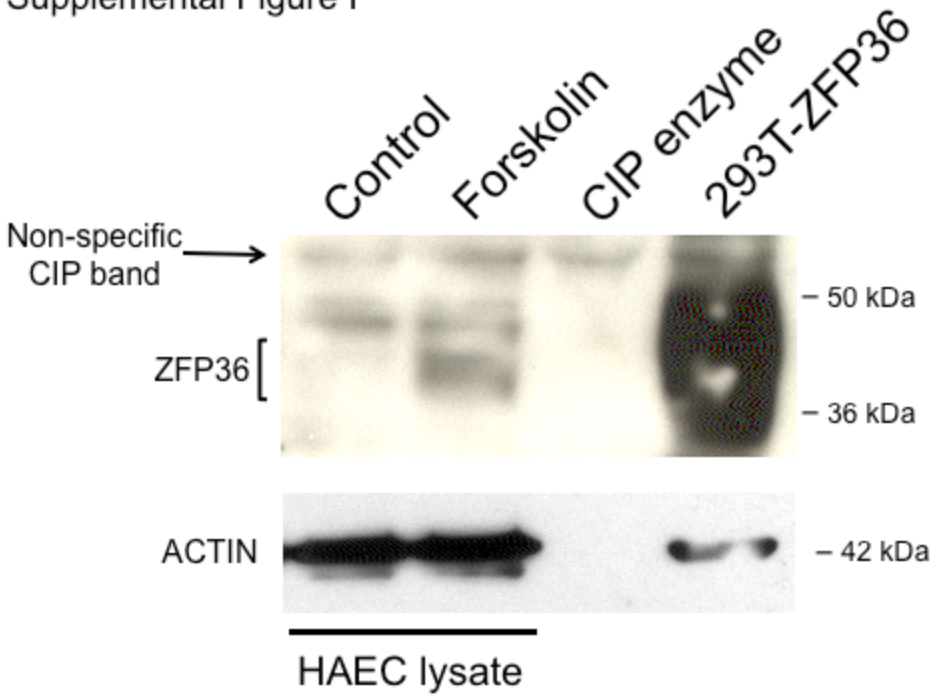
mRNA-binding Protein ZFP36 is Expressed in Atherosclerotic Lesions and Reduces Inflammation in Aortic Endothelial Cells

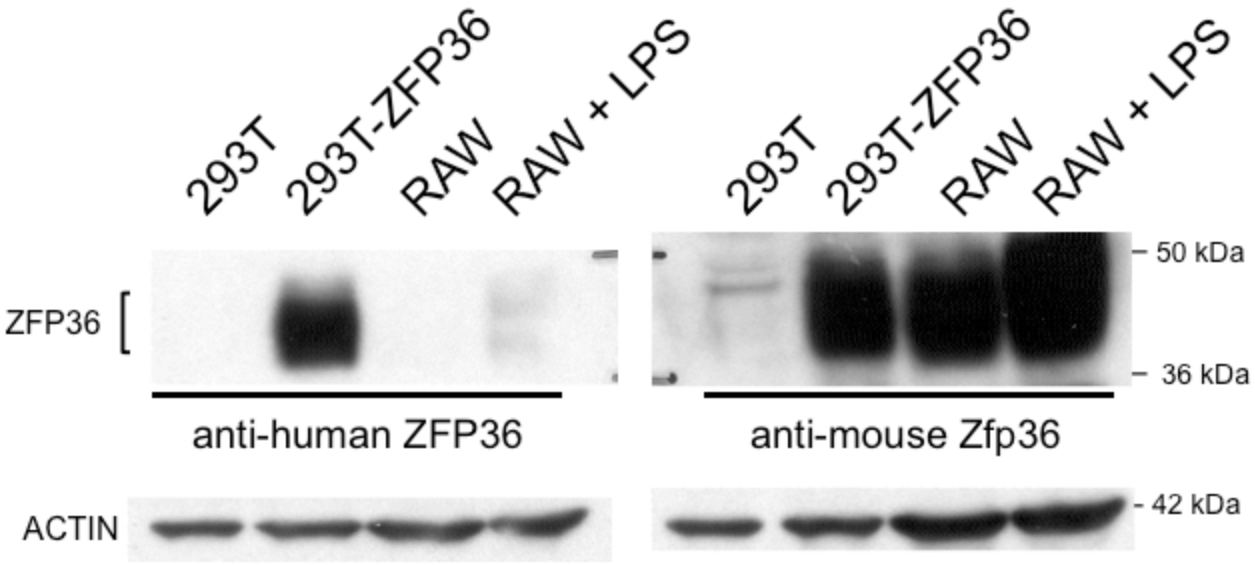
-Supplementary Figures

-Supplementary Figure Legends

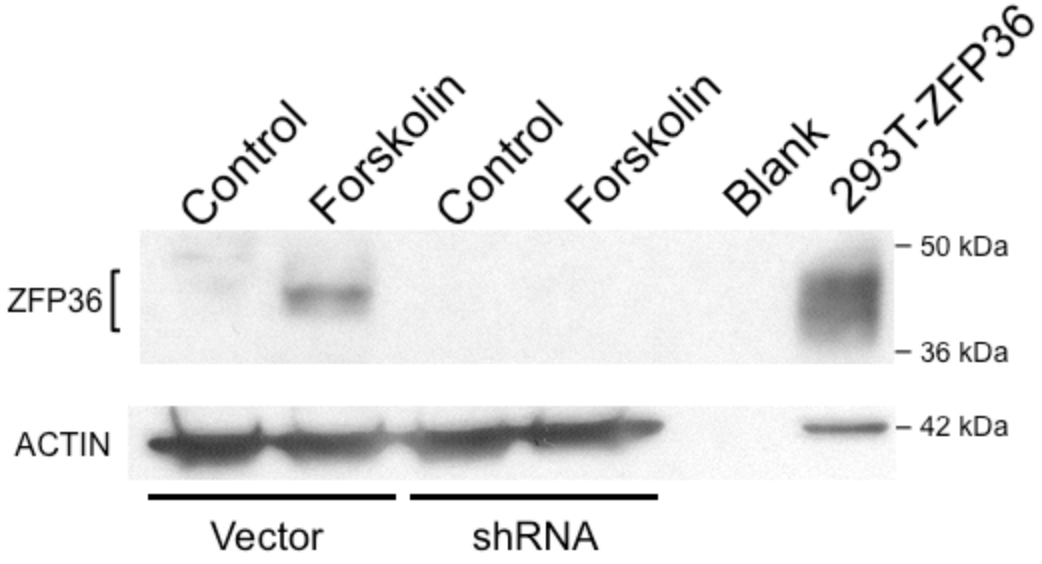
Supplemental Figure I



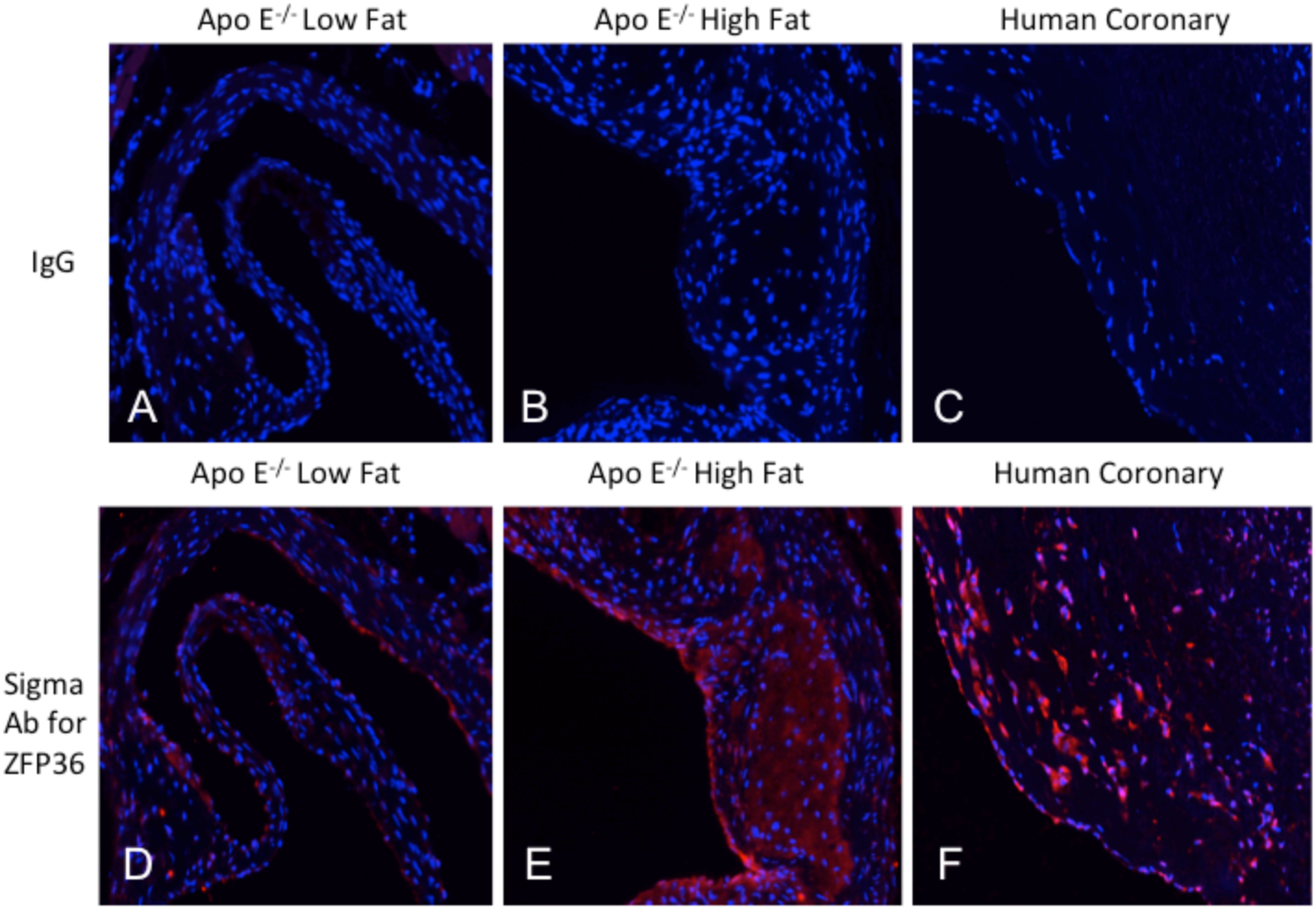
Supplemental Figure II



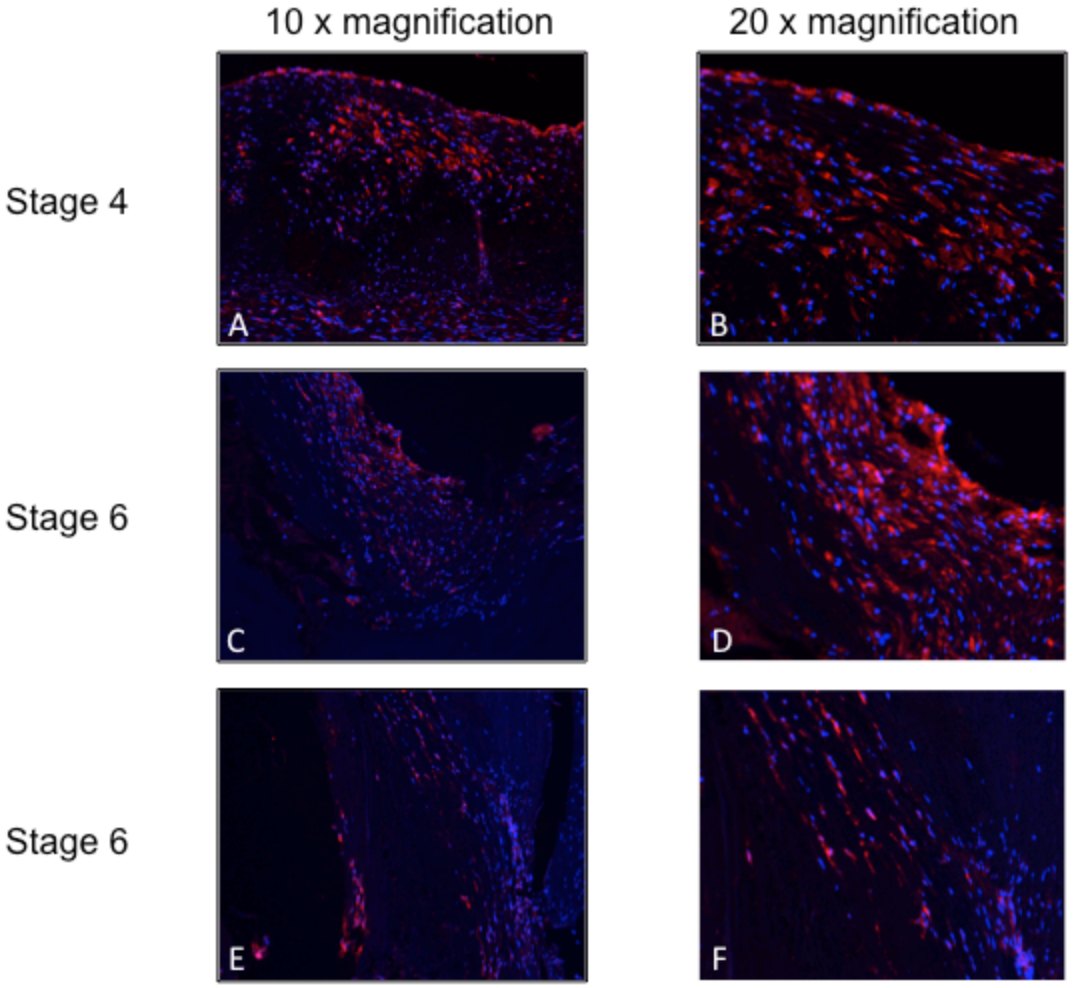
Supplemental Figure III



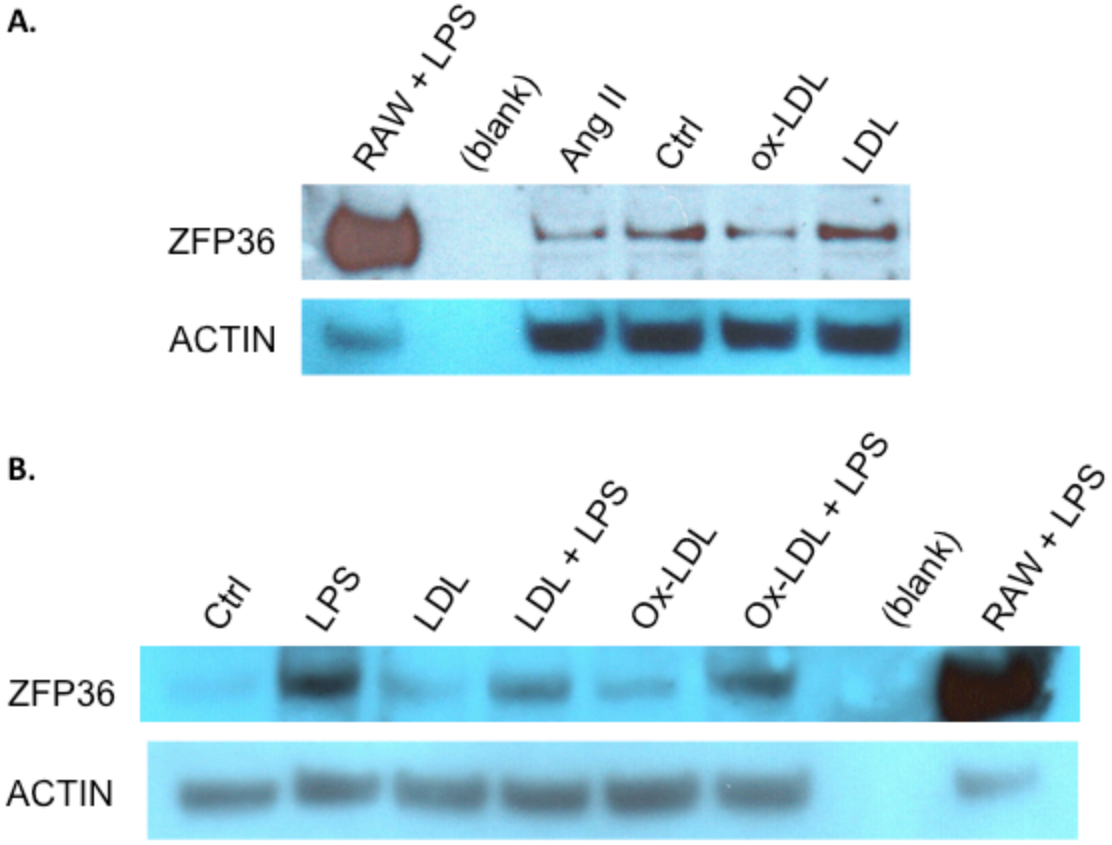
Supplemental Figure IV



Supplemental Figure V



Supplemental Figure VI



Supplemental Figure I. The CIP enzyme produces a non-specific band detected with the anti-human ZFP36 antiserum. HAEC cells were stimulated for four hours with vehicle control or forskolin (10 uM). Lysates (40 ug/lane) were treated with CIP enzyme to reduce the number of phosphorylated residues on the protein, generating a tighter band on WB. In the third lane, CIP enzyme alone was run on the WB and in lane four a positive control lysate was loaded (293T-ZFP36, 5 ug/lane). ACTIN loading control is also shown. A non-specific band representing CIP enzyme (estimated molecular weight ~69 kDa) is noted (arrow). Data are representative of an experiment performed twice.

Supplemental Figure II. Comparison of anti-human ZFP36 (Kallen) and anti-mouse ZFP36 (Blackshear) antibodies by Western Blot. Identical lysates (40 ug/lane) were run on a Western Blot that was then divided. These parallel blots were probed with our anti- human ZFP36 antiserum or an anti-mouse Zfp36 antiserum. Both antiserum detected human and mouse ZFP36 protein, although the mouse antiserum was more sensitive for mouse protein than the human antiserum (see RAW and RAW + LPS lanes). ACTIN loading controls are also shown. Data are representative of an experiment performed twice.

Supplemental Figure III. Specificity of anti-human ZFP36 antiserum for ZFP36 protein. HAEC cells were transduced with empty vector containing lentiviral particles (control) or anti-ZFP36-shRNA-expressing lentiviral particles (as previously described[19]) to knockdown expression of ZFP36 protein. Cells were then treated with vehicle or forskolin (10 uM) for 4h to induce endogenous expression of ZFP36. Lysates (40

ug/lane) were analyzed by Western blot. Also shown is positive control 293T cells transfected with ZFP36 expressing plasmid (5 ug protein/lane). ACTIN loading control is also shown. Data are representative of an experiment performed twice.

Supplemental Figure IV. Confirmation of vascular ZFP36 expression using a third antibody. We employed a rabbit anti-human ZFP36 antibody (Sigma-T5242, affinity purified rabbit anti-human ZFP36, amino acids 270-284) to assess ZFP36 expression in mouse and human vasculature. Compared non-immune IgG (S4A), the Sigma antibody demonstrated modest endothelial expression of ZFP36 in healthy mouse endothelium (S4D). Endothelial cell and neo-intimal ZFP36 expression was high in mouse atherosclerosis lesions (S4E) compared to IgG (S4B). Similarly, endothelial cell and neo-intimal ZFP36 expression was high in human atherosclerosis lesions (S4F) compared to IgG (S4C). These data provide validation of our presented data using an independent antibody. The data demonstrate that vascular endothelial cells and neo-intimal cells express high levels of ZFP36 in both mouse and human atherosclerosis.

Supplemental Figure V. ZFP36 Expression in Human Coronary Arteries in Advanced Stages of Atherosclerosis. ZFP36 (red, anti-human ZFP36 antiserum) and DAPI-nuclei (blue) staining of representative sections of stage 4 and stage 6 atherosclerosis (at 10x and 20x magnifications).

Supplemental Figure VI. ZFP36 Expression in Primary HAEC cells and THP-1 Macrophages in Response to Atherogenic Stimuli. **A.** HAEC cells were stimulated for

four hours with Angiotensin II (Ang II, 100 nM), oxidized LDL (Ox-LDL, 50 ng/ml), or native LDL (LDL, 50 ng/ml) and cell lysates were tested for ZFP36 expression (70 µg protein/lane). Also shown are positive control RAW cell lysate after LPS treatment (15 µg/lane) and ACTIN loading control. **B.** THP-1 macrophages were were cultured for 48 hours with or without native LDL (50 ng/ml) or Ox-LDL (50 ng/ml), then cells were treated with or without LPS for an additional four hours. Cell lysates were tested for ZFP36 expression (50 µg protein/lane). Also shown are positive control RAW cell lysate after LPS treatment (15 µg/lane) and ACTIN loading control.