

### Supplemental Figure Legends

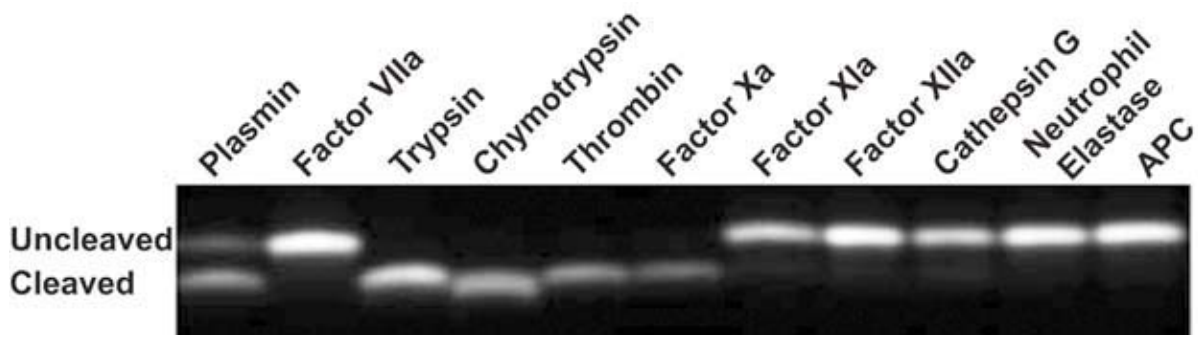
**Fig. S1.** A: Representative polyacrylamide gel showing cleavage of 5  $\mu$ M DPRSFL-ACPP by several enzymes known to cleave the PAR-1 receptor and some additional proteases including plasmin, factor VIIa, trypsin, chymotrypsin, thrombin, factor Xa, factor XIa, factor XIIIa, Cathepsin G, Neutrophil elastase and activate protein C (APC) after one hour incubation in 50nM enzyme. Experiments were done in triplicate. B: Gel quantification as shown in Fig. 1B. After electrophoresis, gels were imaged for 10 secs using a CCD camera. Intensities (I) for the cleaved and uncleaved bands were measured by obtaining the average pixel value within defined areas (yellow and blue rectangles respectively) with Image J. Average intensity of an area away from the samples (red rectangle) was subtracted from individual band intensities to account for camera noise. Percent cleavage was defined as  $(I_{\text{blue}} - I_{\text{red}}) / (I_{\text{blue}} + I_{\text{yellow}} - 2 * I_{\text{red}})$ . To account for minor fluorescence impurities that ran at approximately the level of the cleaved substrate (pink arrow), the % cleavage of uncleaved substrate run in buffer was subtracted from all lanes. C: Plaque intensity was measured by generating a mask over the plaque area using Amira software and then taking the average intensity of that region. Plaque burden was calculated by taking the ratio of the plaque containing region compared to total aorta. D: Quantification of human carotid endarterectomy specimen was done by drawing a region around the entire specimen and taking the average intensity using Image J.

**Fig. S2.** PLGLAG-ACPP uptake is enzyme dependent and correlates with increased plaque burden in atherosclerotic mice. Representative fluorescence images of gross aortas removed from animals six hours after injection with 10 nmol PLGLAG-ACPP showing increased uptake in aortas with higher plaque burden (A) compared to aortas with low plaque burden (B). Aortas from high plaque-burden mice pretreated with MMP inhibitors (C) (SB3CT and GM6001 45 minutes prior to injection of 10nmol PLGLAG-ACPP) or 10 nmol mPEG control ACPP (D) showed reduced uptake compared to similar high plaque-burden mice treated with PLGLAG-ACPP, suggesting that MMP cleavage significantly contributes to PLGLAG-ACPP uptake. E: Scatter plot showing the average fluorescence uptake of PLGLAG-ACPP in ApoE and LDLR deficient mice as a function of total plaque burden.

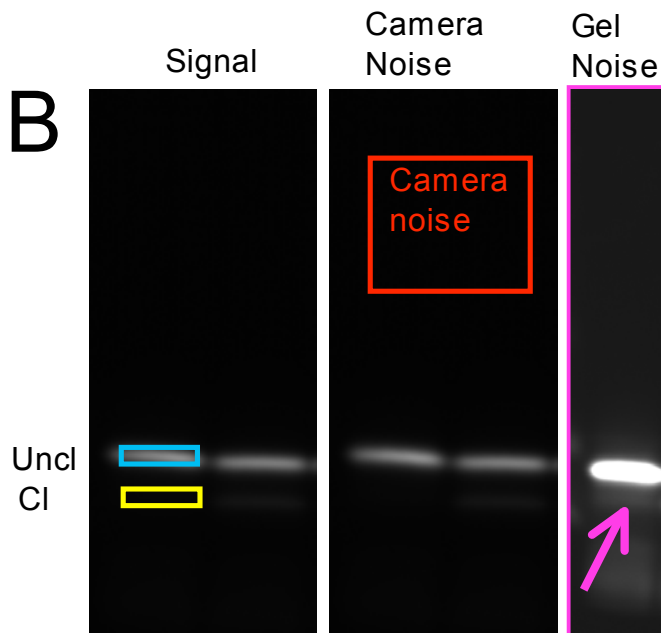
**Fig. S3.** DPRSFL-ACPP highlights coronary artery plaques. Whole mount fluorescence photomicrographs from a two year old LDLR deficient mouse 16 hours after dual injection with rhodamine-DPRSFL-ACPP and Cy5-PLGLAG-ACPP showing higher fluorescence uptake of the coronary artery with DPRSFL-ACPP (A) compared to PLGLAG-ACPP (B). Fluorescence photomicrographs of 10 $\mu$ m sections through the same coronary arteries as in A-B showed much higher fluorescence uptake of DPRSFL-ACPP (C) compared to PLGLAG-ACPP (D). H/E staining was done on the same area at low power (E) and high power (F) after fluorescence histology to verify the presence of atherosclerosis plaques.

**Cover Image:** Carotid artery from an ApoE<sup>-/-</sup> mouse following intravenous injection of DPRSFL-ACPP showing fluorescence accumulation in atherosclerotic plaques at the common carotid just prior to bifurcation.

A

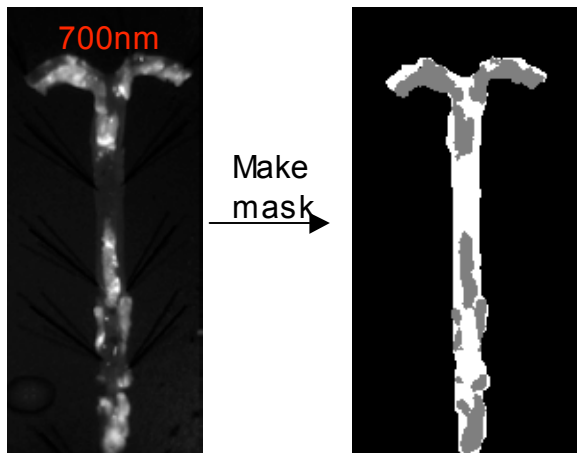


B



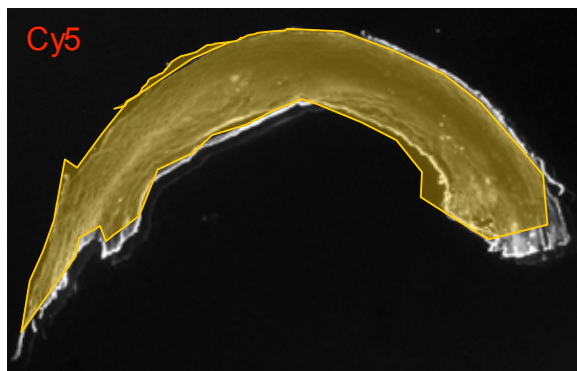
Quantification (average intensity of bands) done with Image J

C

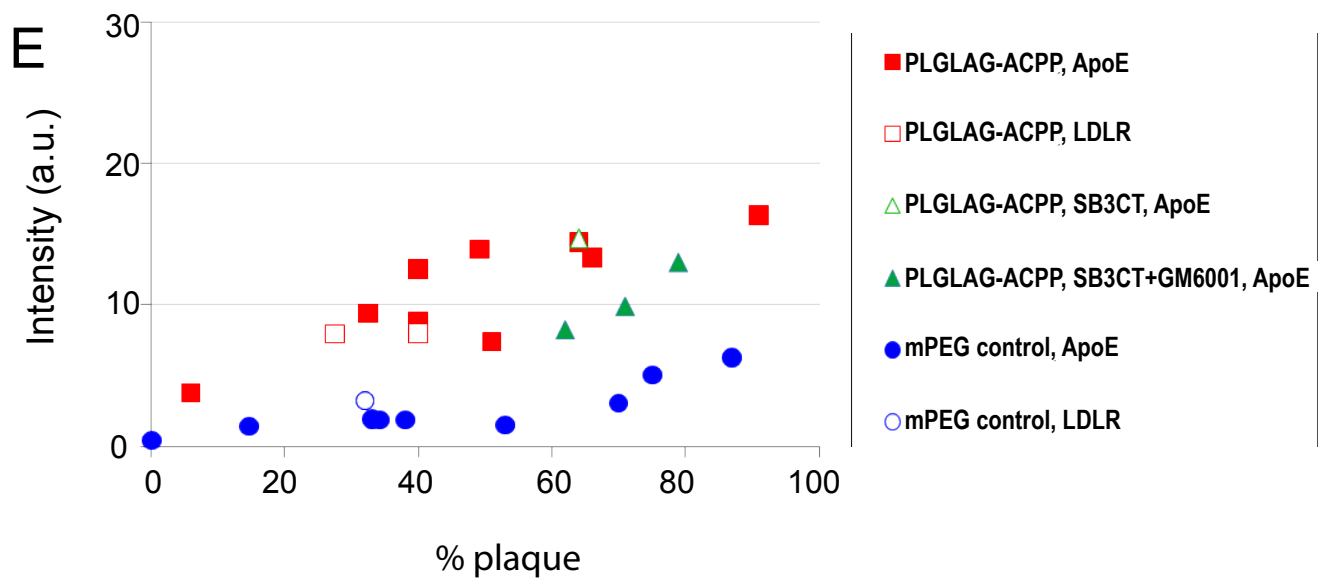
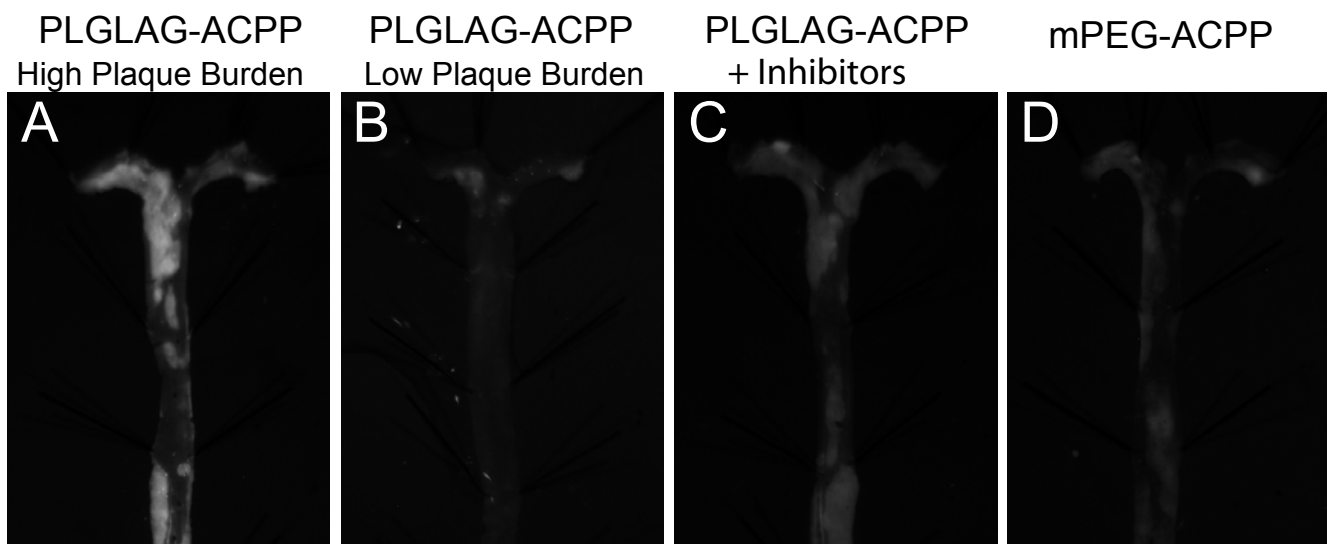


Quantification (average intensity and area of plaque) done with Amira

D



Quantification (average intensity of region) done with Image J



# Coronary atherosclerosis

