# SUPPLEMENTAL MATERIAL

### SUPPLEMENTAL FIGURE I



Supplemental Figure I. Study protocol for all *in vivo* and histological studies.

## SUPPLEMENTAL FIGURE II



**Supplemental Figure II.** Creation of AVF in mice. **A.** AVFs were created in C57BL/6 mice by mobilizing the carotid artery and then anastomosing it end-toside to the ipsilateral jugular vein (SJV: superior jugular vein; IJV: inferior jugular vein; CA: carotid artery. The dotted box shows the centered imaging field during IVM studies. **B.** The carotid artery blood flow measured by Doppler ultrasound in mice (n=8) increased 7-fold after AVF creation (\*p < 0.01). Scale bar, 1 mm. Error bars depict the S.E.M.

## SUPPLEMENTAL FIGURE III



**Supplemental Figure III.** Representative axial intravital microscopy (IVM) images of day 14 murine AVF. **A.** The section was 120µm away from the anastomosis. CLIO-VT680 (red) localized below adventitial arterial wall collagen (SHG, Blue). **B.** FITC-dextran, a blood pool agent outlined the arterial lumen (green). **C.** Fusion image demonstrates that CLIO-VT680 signals are within the imaging illumination field on confocal microscopy. Pulsatile motion artifact likely explains why CLIO-VT680 appears to colocalize with the lumen (green), while in reality localizing to the intima as shown in figure 2. Scale bar, 100µm. SHG, second harmonic generation.

#### SUPPLEMENTAL FIGURE IV



**Supplemental Figure IV.** Additional examples of week 2 CLIO endothelial IVM signals and the corresponding week 6 neointimal hyperplasia (NH) from matched sections. The confocal IVM images assess approximately the top 1/3<sup>rd</sup> of the vessel. (A, B) AVF examples show that the week 2 IVM CLIO signals and week 6 neointimal patterns are roughly similarly asymmetric. (C) An AVF example with more symmetric week 2 IVM CLIO signals and week 6 neointimal hyperplasia. Red=CLIO-VT680 (pathological endothelium); blue=second harmonic generation signal (SHG, type I collagen). IVM images were processed and windowed identically. IVM Scale Bar: 100µm.