

APAC, A DUAL ANTIPLATELET AND ANTICOAGULANT HEPARIN PROTEOGLYCAN MIMETIC, INTEGRATES WITH EXTRAVASCULAR MATRIX DURING VASCULAR INJURY

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Introduction

- Mast cell heparin proteoglycans (HEP-PG) inhibit collagen-induced and Von Willebrand (VWF)-mediated platelet thrombosis, but preserve adhesion¹⁻³
- APAC as a HEP-PG mimic contains conjugate of unfractionated heparins (UFH) with a core protein⁴ is designed to be used in association with vascular interventions
- APAC shows dual antiplatelet and anticoagulant activity, inhibits arterial thrombosis (Folts and AV-shunt model in baboons) and reduces fibrin formation and protects from ischemic reperfusion injury in acute kidney injury model⁵

Methods

In vivo we locally, intraluminally applied biotinylated APAC (0.5 mg/mL) on vascular injury site, allowed 2 min exposure and released blood flow. *In vitro* APAC-Biotin was incubated with a scraped artery injury models:

- Arterio-venous fistulae (AVF) of the femorals (n=5). Tissue harvest at 30 min
- Balloon angioplasty denudation of iliac (n=2) OR carotid (n=3) artery. Tissue harvest at 10-30 min.
- Tissues were fixed (10% formalin 4h), and processed for cryosectioning

Imaging

- Samples were immunostained for VWF, PECAM, laminin and Podocalyxin
- APAC-Biotin was detected with streptavidin conjugated to Efluor 660
- Pictures were taken with SP8 leica confocal microscope
- Mander's co-localization coefficients were calculated with ImageJ software

Aims

- APAC's binding and localization to denuded iliac, carotid and femoral artery after local exposure *in vitro* and *in vivo* in a pig model.
- APAC co-localization with Von Willebrand factor (VWF), laminin, podocalyxin, and PECAM.

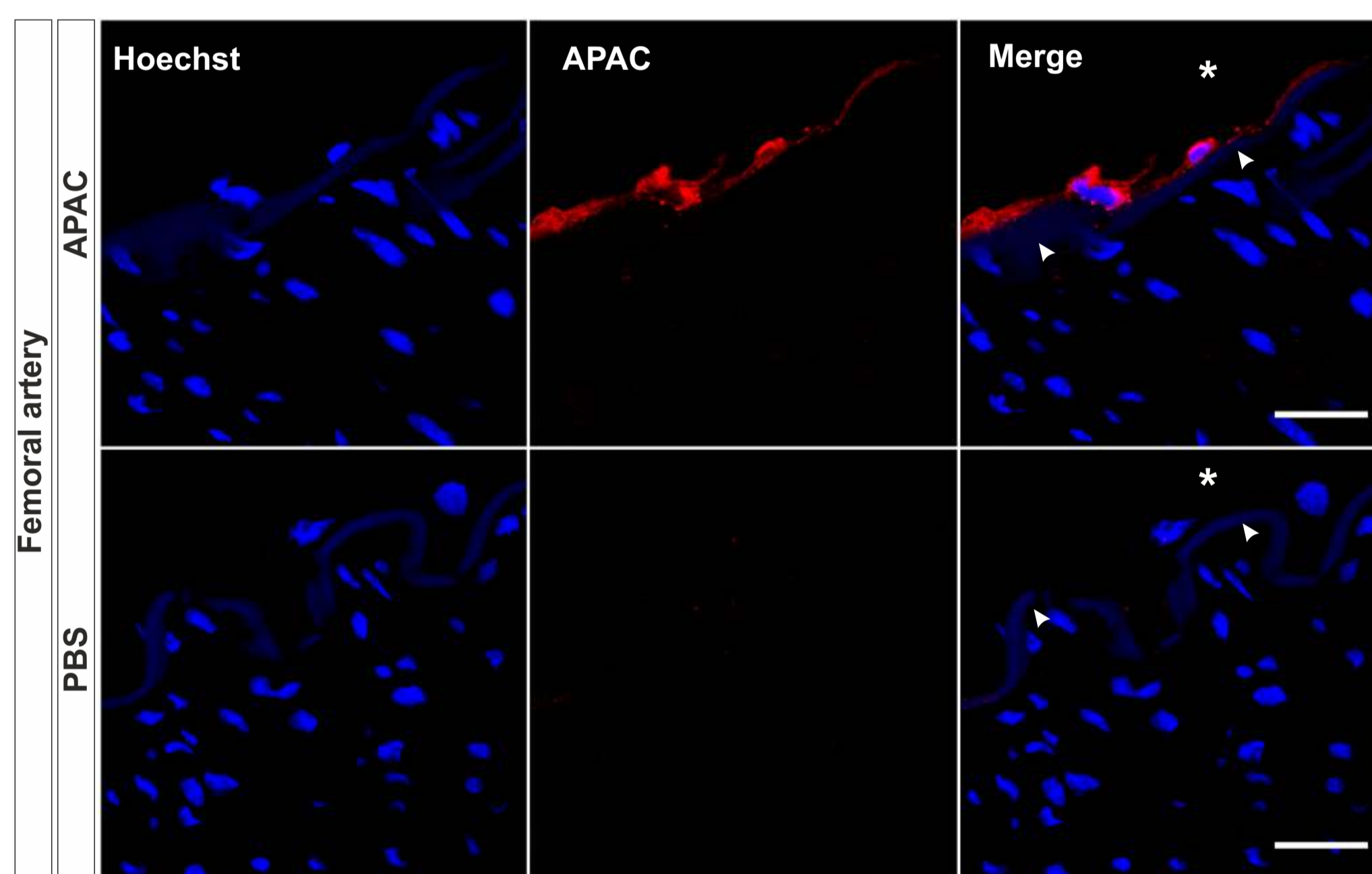
Conclusions

- APAC, developed as a local antithrombotic therapy to be used in association with vascular intervention shows multiple binding sites on vascular injuries
- APAC adheres to vascular injury site of arteries and AVF and co-localizes with VWF and Laminin
- APAC binding and colocalization are strongly reduced in presence of PECAM or Podocalyxin
- APAC, a dual antiplatelet and anticoagulant, binds to site of vascular damage and offers local antithrombotic action

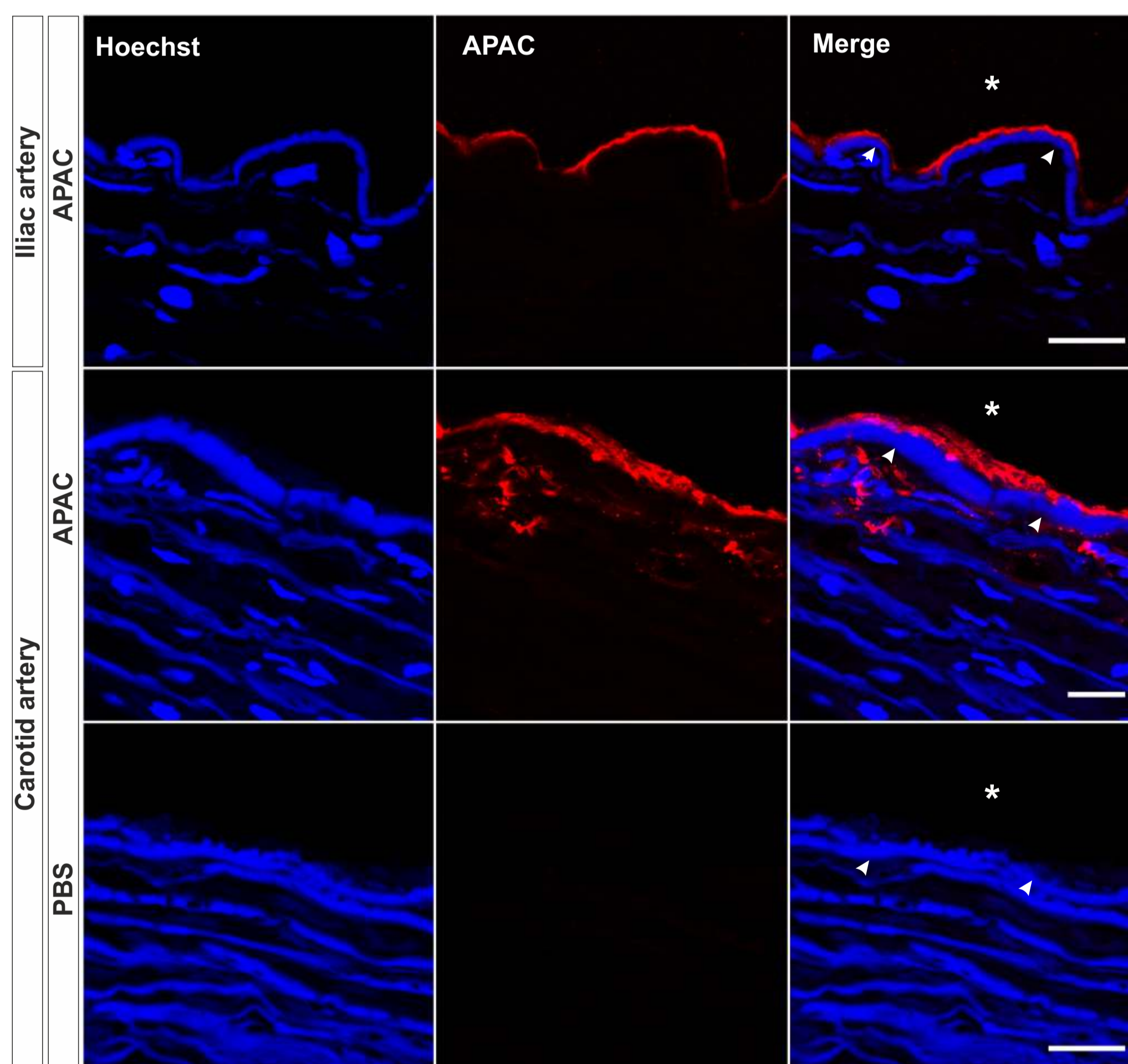
References: 1. Lassila et al., ATVB 1997, 2. Kauhanen et al., ATVB 2000, unpublished Z.Ruggeri, 3. Olsson et al., TH 2002, 4. Lassila & Jouppila, STH 2014, and 5. Tuuminen et al., Clin Exp Nephrol 2016

Results

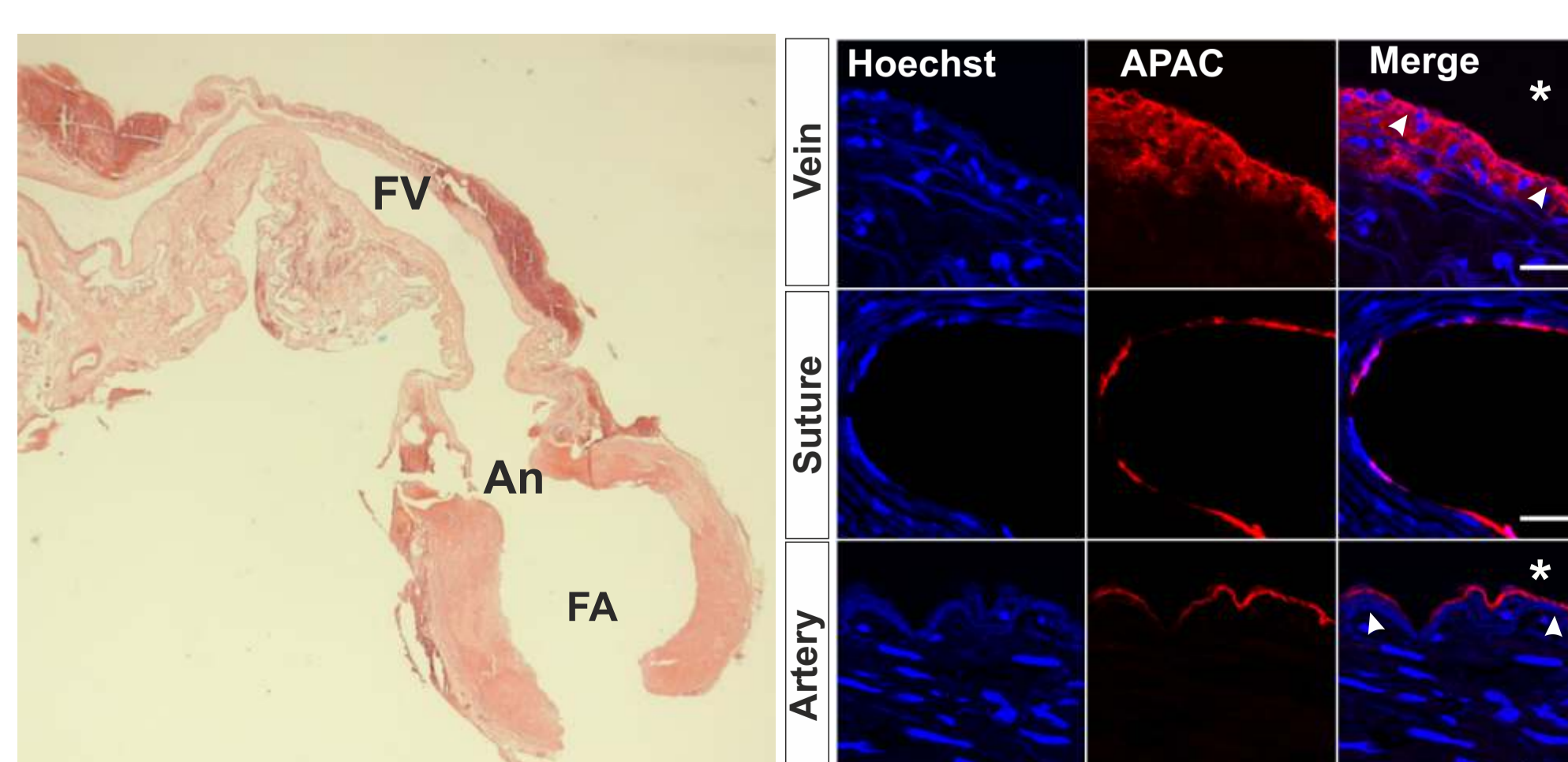
1. APAC binds to *in vitro* denuded arteries



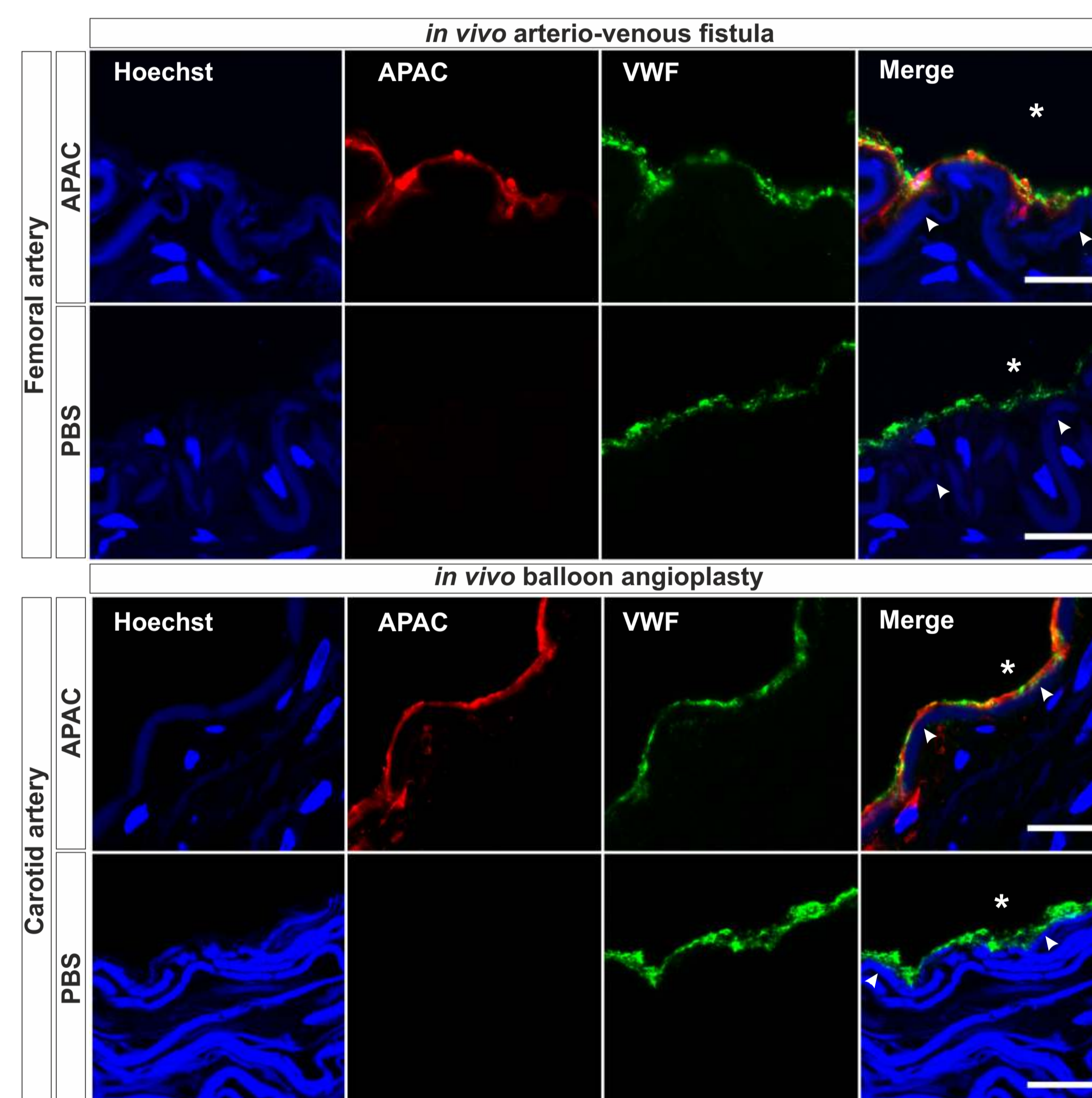
2. APAC binds to *in vivo* to denuded arteries



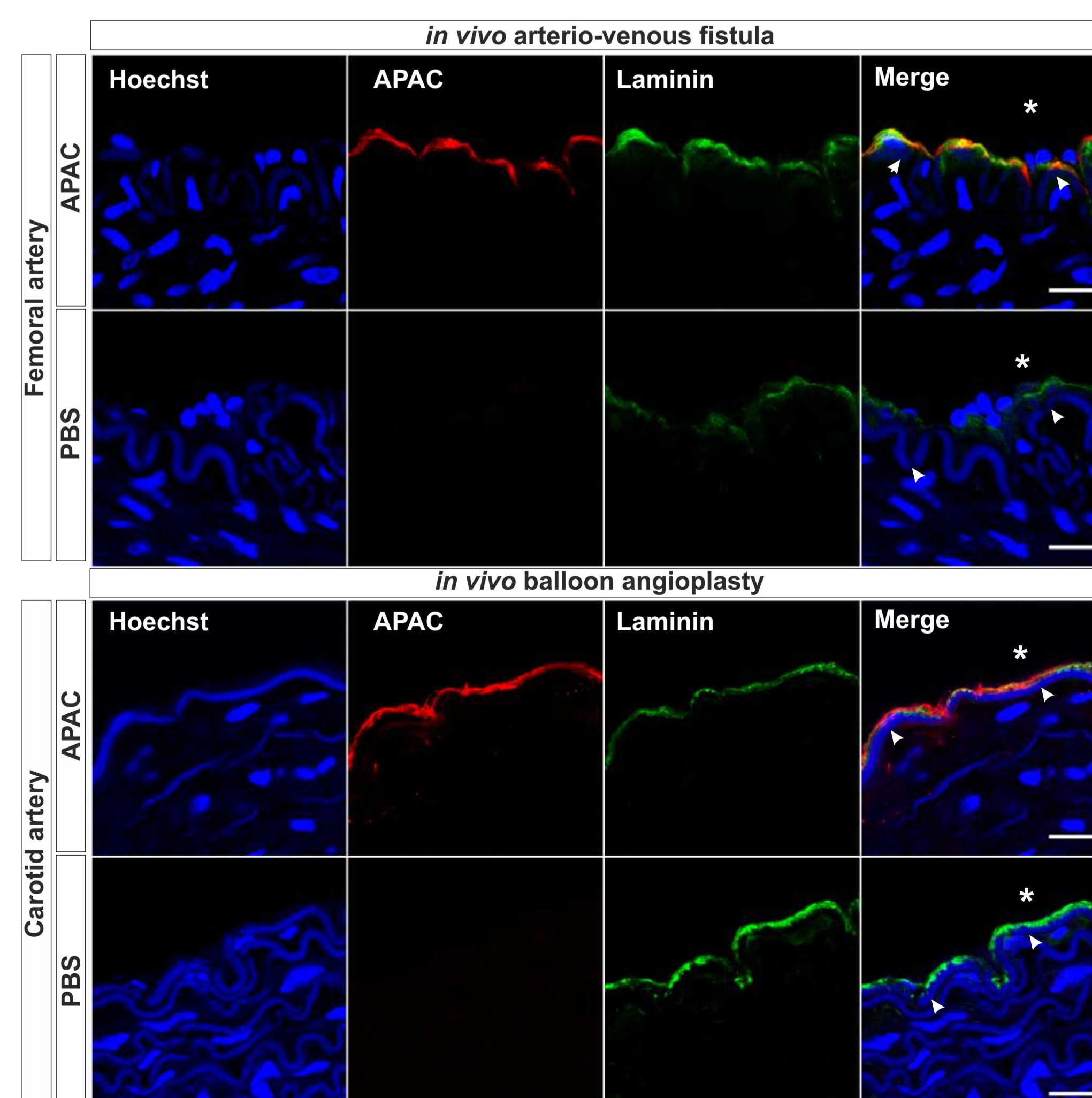
3. APAC binds to *in vivo* arteriovenous fistula wall



4. APAC co-localizes *in vivo* with VWF in both balloon injury and AVF



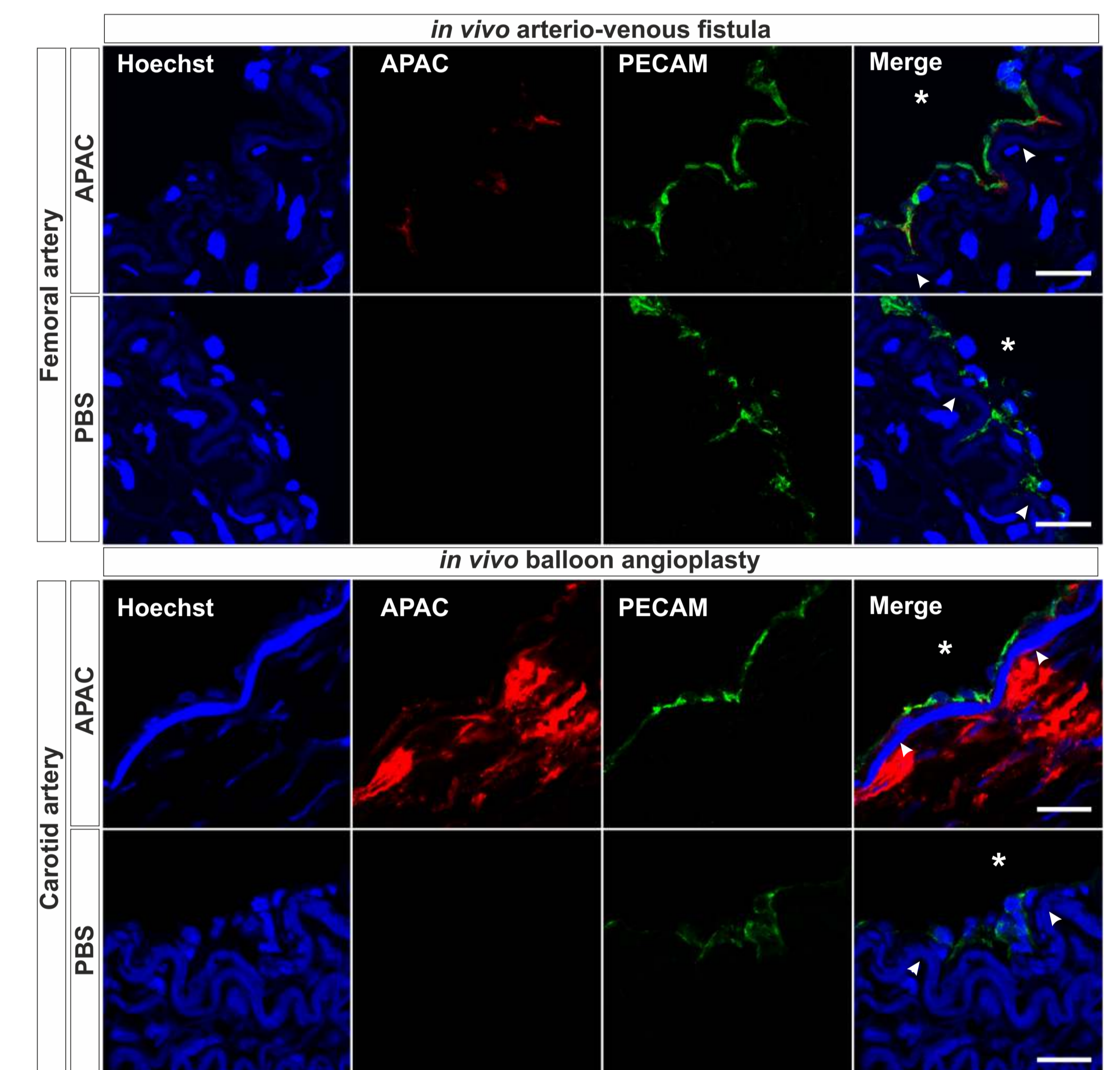
5. APAC co-localizes *in vivo* with Laminin in both balloon injury and AVF



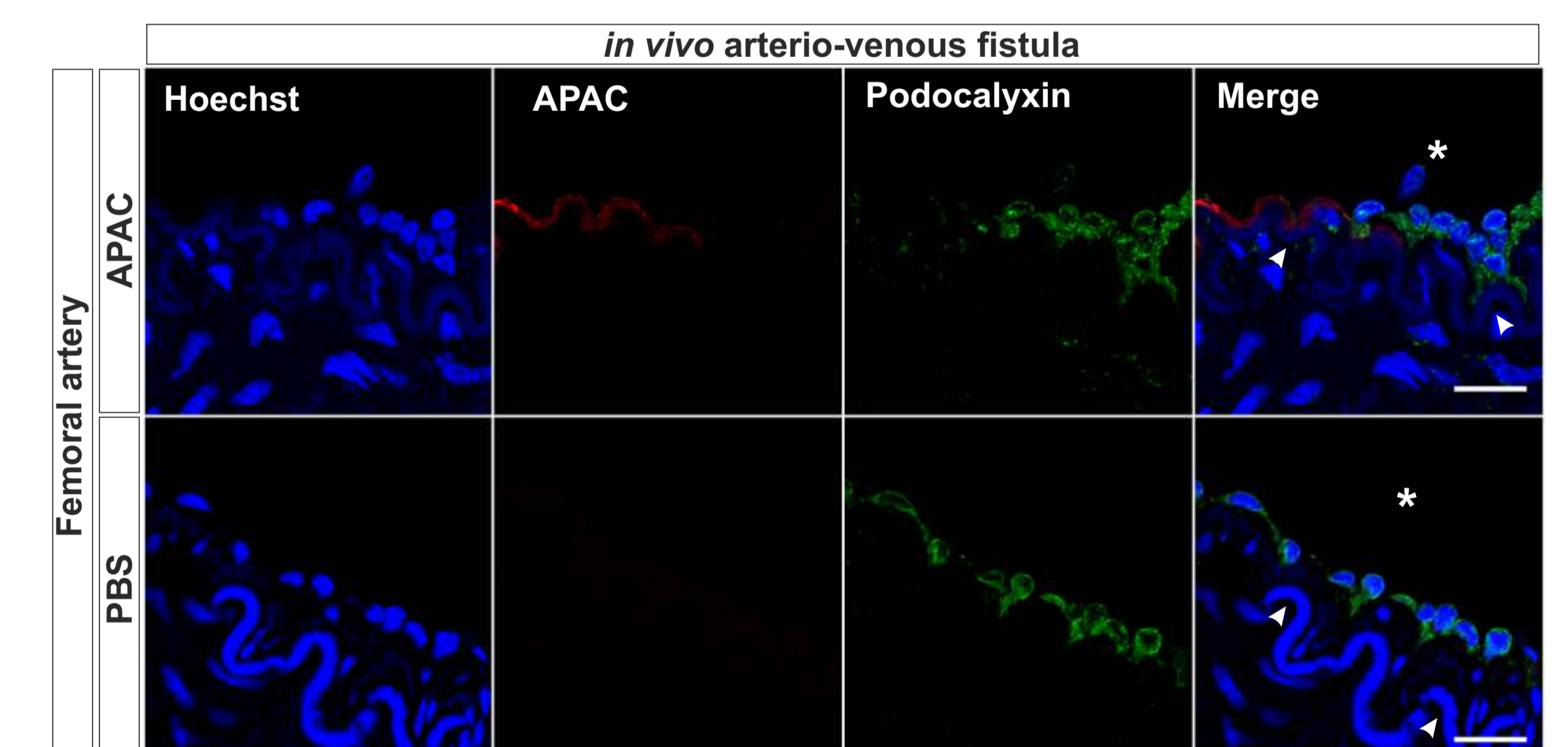
Abbreviations

*: Lumen; Arrowheads: Internal elastic lamina; FV: femoral vein; An: anastomosis; FA: femoral artery; scale bar: 20 μm

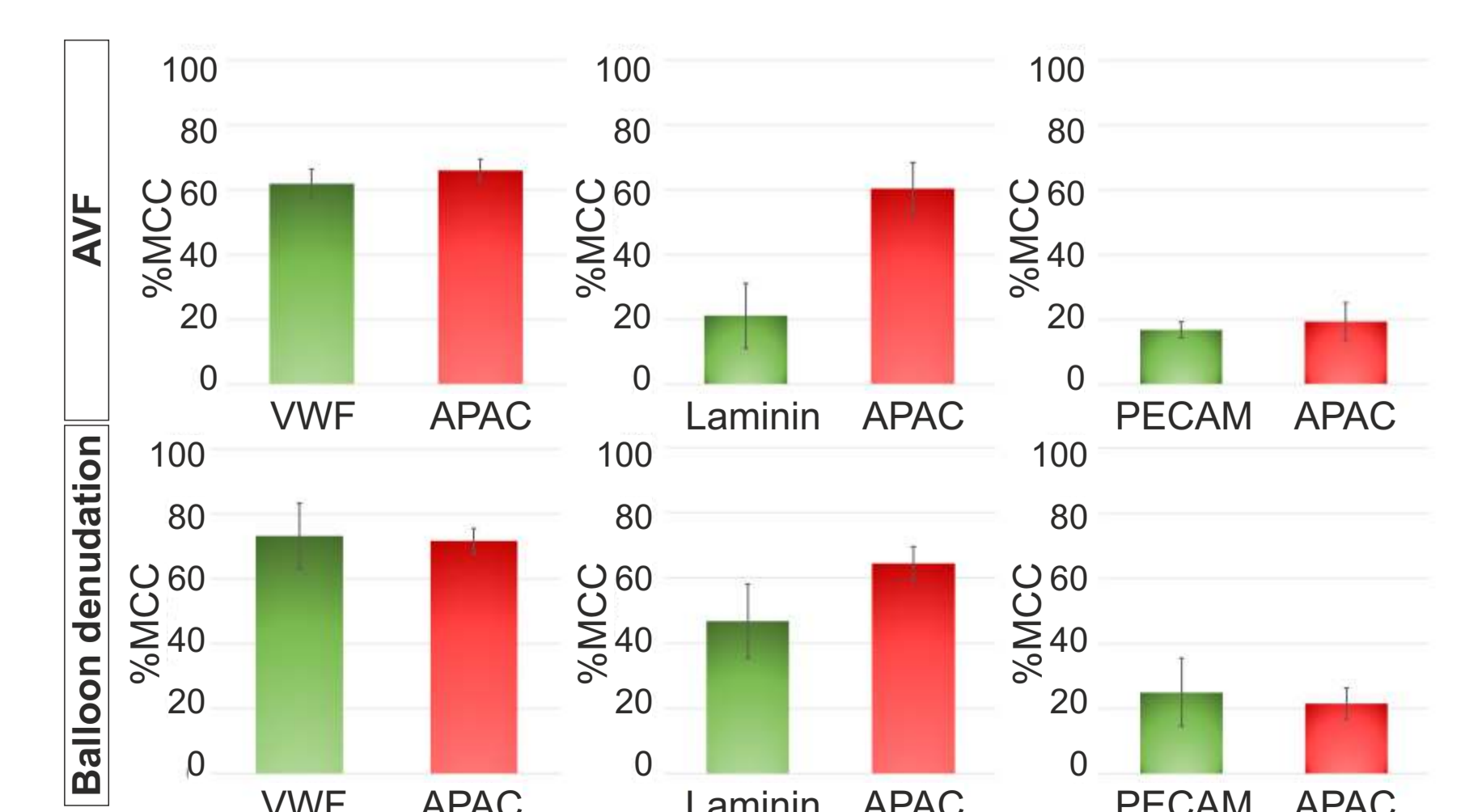
6. APAC shows limited co-localization with PECAM



7. APAC binding is reduced in presence of Podocalyxin



8. Manders' co-localization coefficients quantified the vascular co-localization of APAC



Acknowledgments