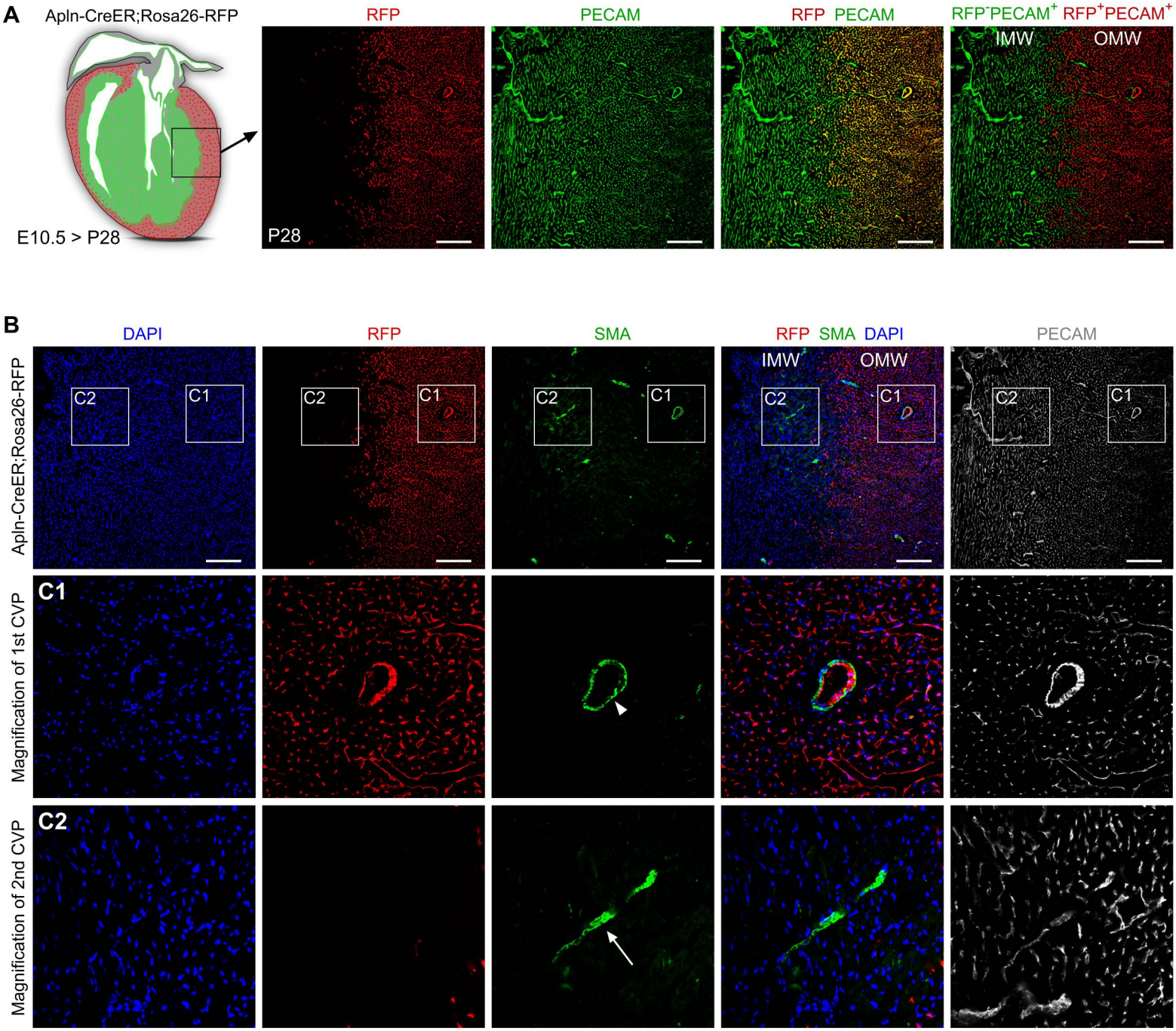

Fig. S1. Smooth muscle of the 2nd CVP in P28 heart. (A) Apln-CreER labels the 1st CVP (RFP^+;$PECAM^+$), but not the 2nd CVP (RFP^-;$PECAM^+$) in the P28 heart. The 1st and 2nd CVP reside in the outer myocardial wall (OMW) and inner myocardial wall (IMW) respectively. Tamoxifen was injected at E10.5 and hearts were collected at P28 (E10.5 > P28). (B,C) SMA^+ smooth muscle cells (arrow) of the 2nd CVP were detected in the IMW (RFP-). C are magnified images of boxed regions in B. The arrowhead points to smooth muscle of 1st CVP, and arrow points to smooth muscle of the 2nd CVP. Images are representative of 4 independent Apln-CreER;Rosa26-RFP hearts. Scale bars, 200 μm.

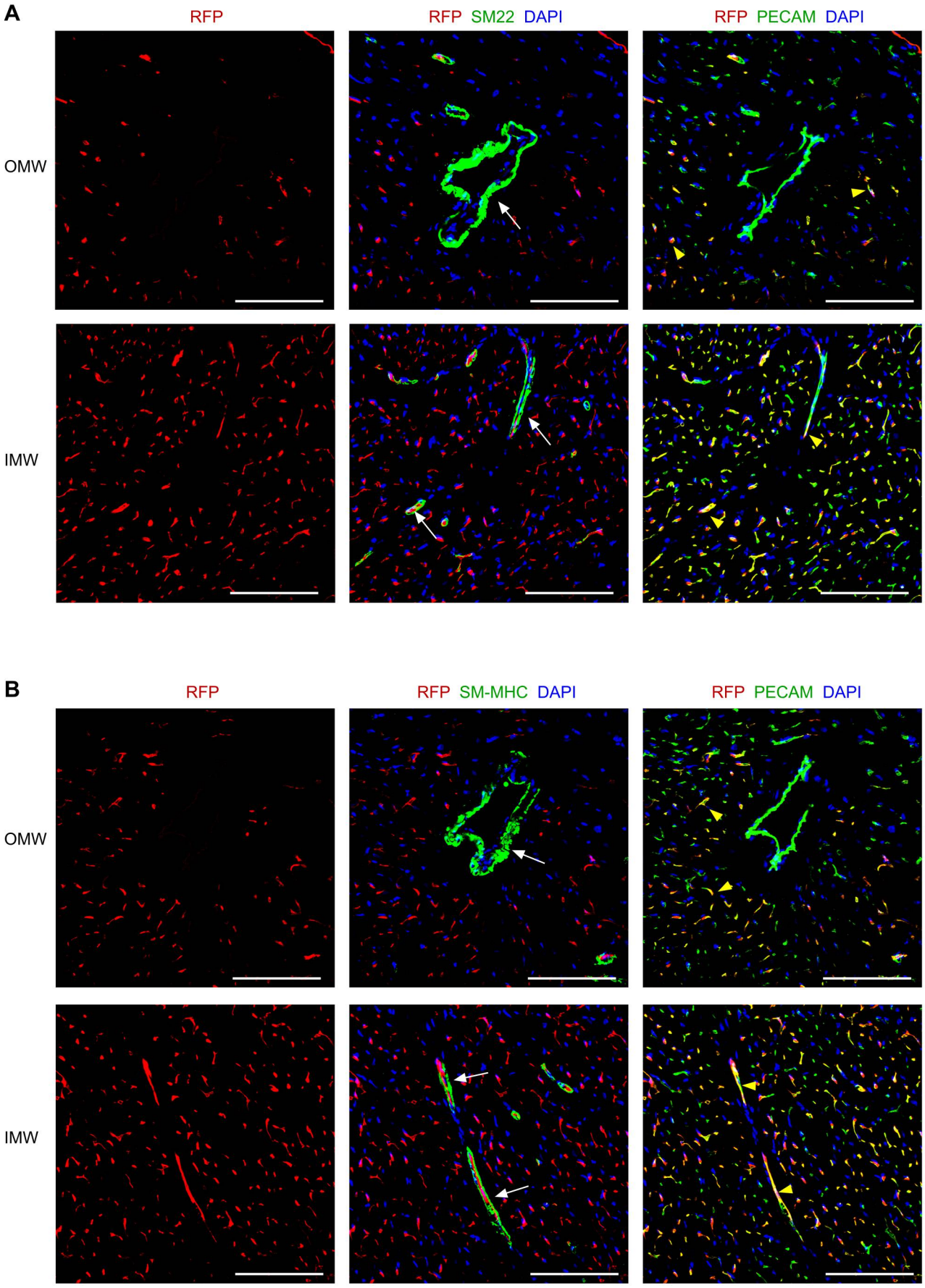

Fig. S2. Epicardial cells labeled at P1 do not contribute to smooth muscle cells in OMW and IMW. (A) Immunostaining for RFP and SM22 on adult Wt1-CreER;Rosa26-RFP heart sections showed Wt1-CreER labeled cells do not contribute to SM22+ smooth muscle cells (white arrows), but label a substantial number of coronary endothelial cells (yellow arrowheads). Tamoxifen was administered at P1, and hearts were collected at postnatal 10 weeks. (B) Immunostaining for RFP and smooth muscle myosin heavy chain (SM-MHC) showed Wt1-CreER labeled cells do not contribute to SM-MHC+ smooth muscle cells (white arrows), but label coronary endothelial cells (yellow arrowheads). Scale bars, 100 μm.

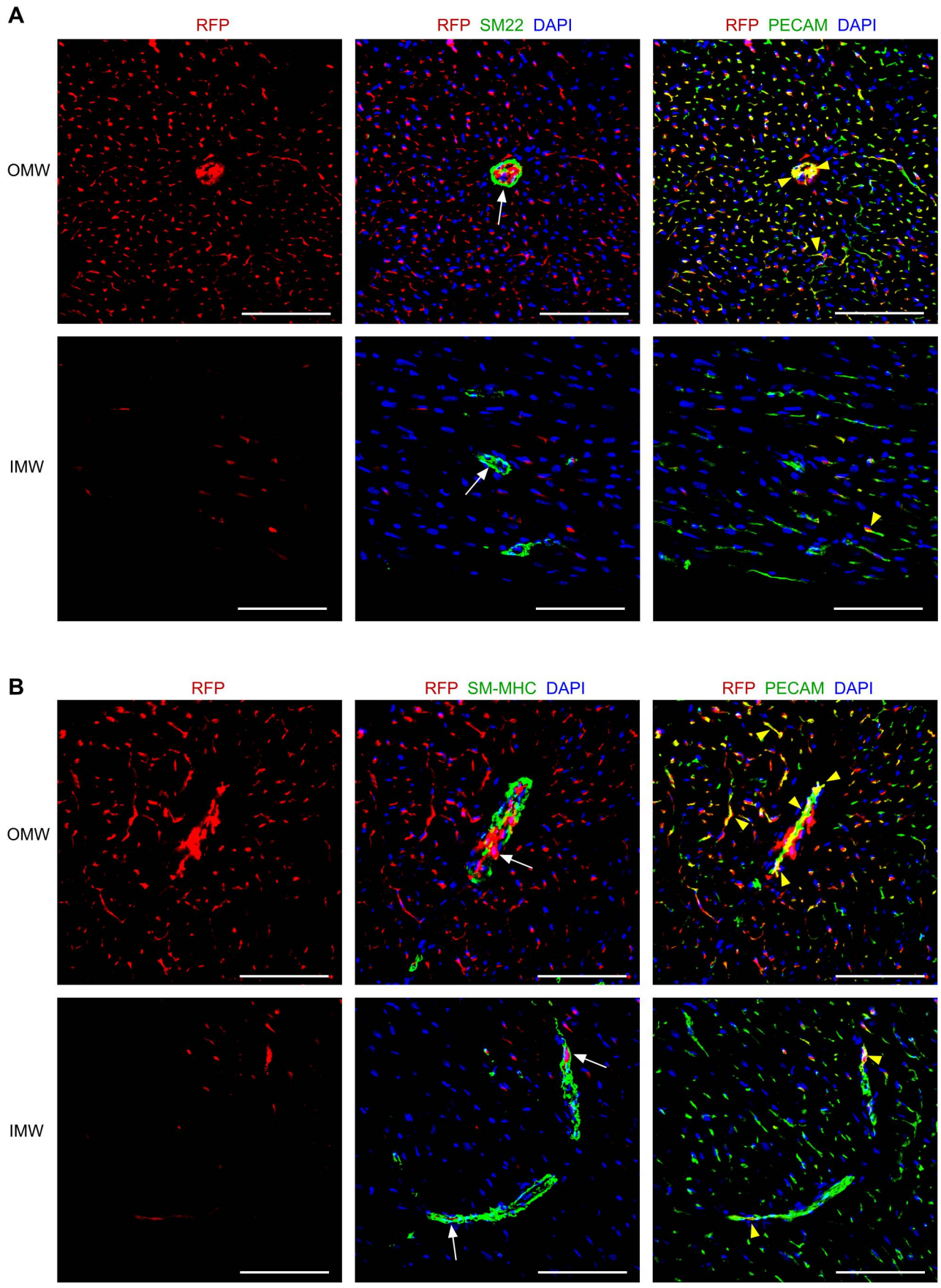

Fig. S3. Epicardial cells labeled at E14.5 contribute to few smooth muscle cells in OMW and IMW. (A) Immunostaining for RFP and SM22 on adult Wt1-CreER;Rosa26-RFP heart sections showed Wt1-CreER labeled cells contribute to few SM22+ smooth muscle cells (white arrows), but label coronary endothelial cells (yellow arrowheads). Tamoxifen was administered at E14.5, and hearts were collected at postnatal 10 weeks. (B) Immunostaining for RFP and smooth muscle myosin heavy chain (SM-MHC) showed Wt1-CreER labeled cells contribute to few SM-MHC+ smooth muscle cells (white arrows), but label coronary endothelial cells (yellow arrowheads). Scale bars, 100 μm.

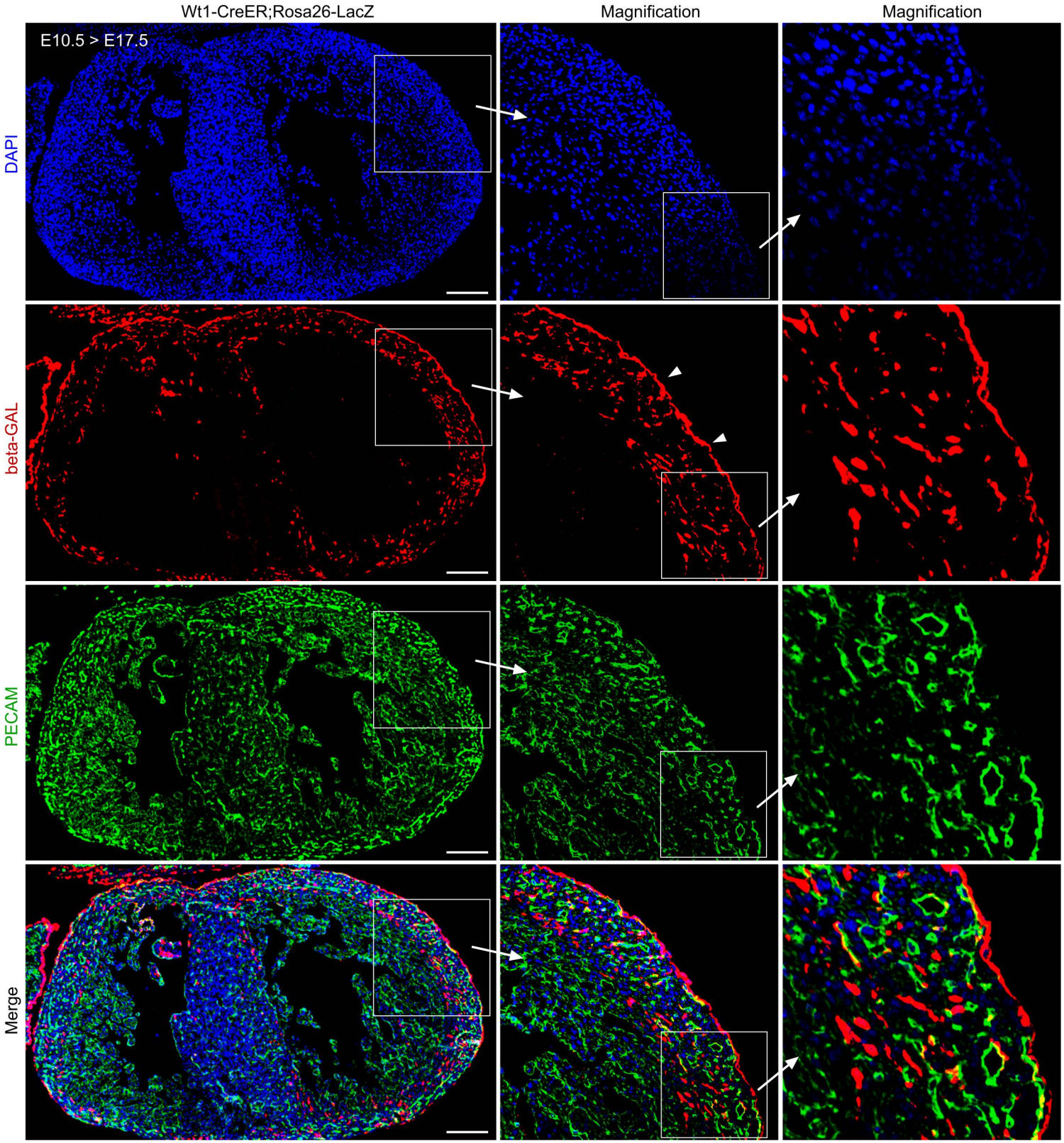

Fig. S4. The early epicardium contributes to EPDCs that reside in the OMW at later embryonic stages. Immunostaining for beta-galactosidase (beta-GAL), PECAM and DAPI on sections of E17.5 Wt-CreER;Rosa26-LacZ hearts. Tamoxifen was administered at E10.5 and hearts were collected at E17.5 (E10.5 > E17.5). EPDCs (beta-GAL^{+}) were detected in the compact myocardium wall (OMW), with relatively fewer EPDCs in the trabecular or inner myocardium. Images are representative of 3 individual hearts. Scale bars, 200 μm.

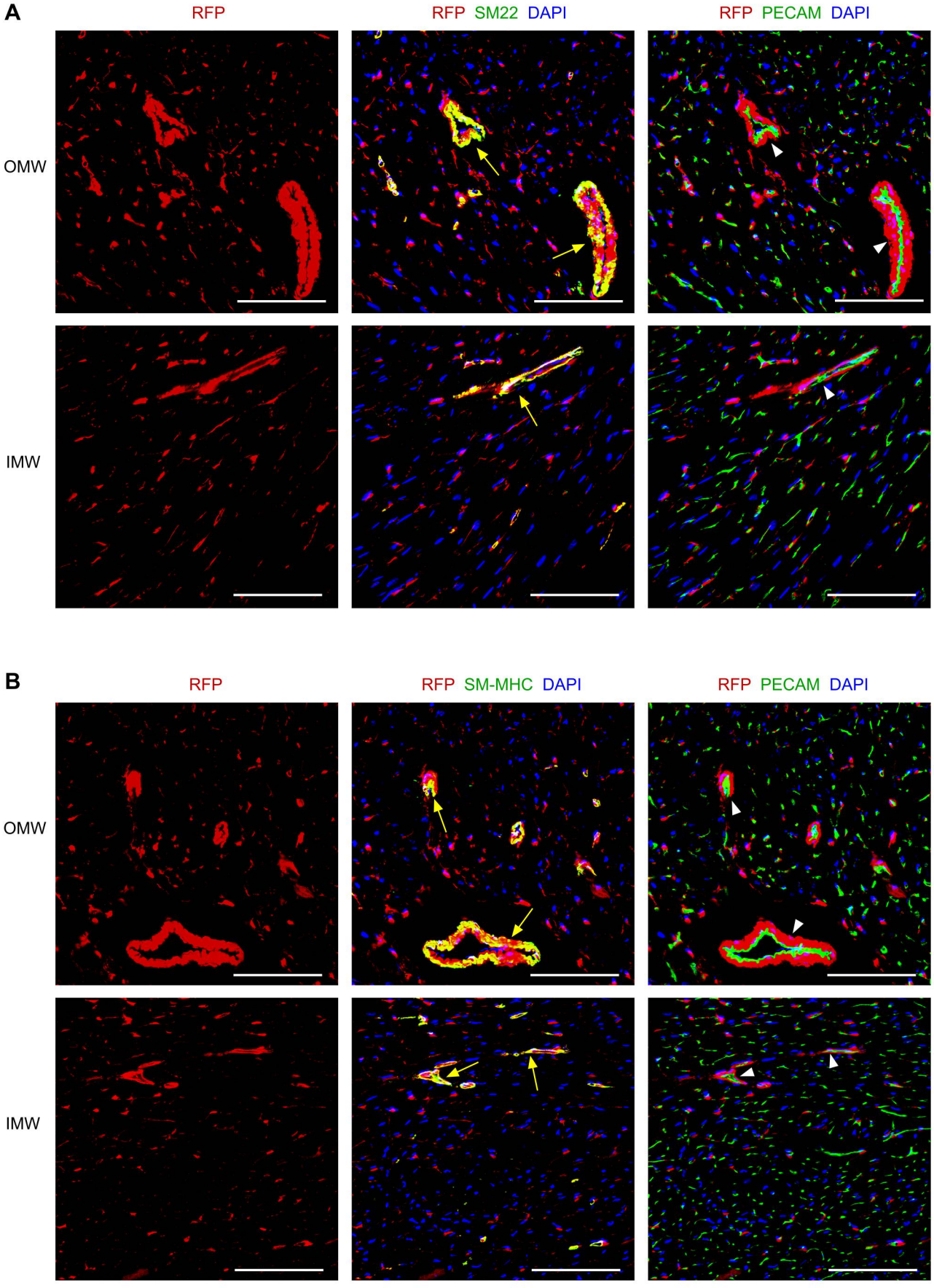

Fig. S5. Epicardial cells labeled at E10.5 give rise to smooth muscle cells in OMW and IMW. (A) Immunostaining for RFP and SM22 on adult Wt1-CreER;Rosa26-RFP heart sections showed epicardial cells contribute to SM22+ smooth muscle cells (yellow arrows) but not endothelial cells (white arrowheads). Tamoxifen was administered at E10.5, and hearts were collected at postnatal 10 weeks. (B) Immunostaining for RFP and smooth muscle myosin heavy chain (SM-MHC) showed epicardial cells contribute to SM-MHC+ smooth muscle cells (yellow arrows) but not endothelial cells (white arrowheads). Scale bars, 100 μm.

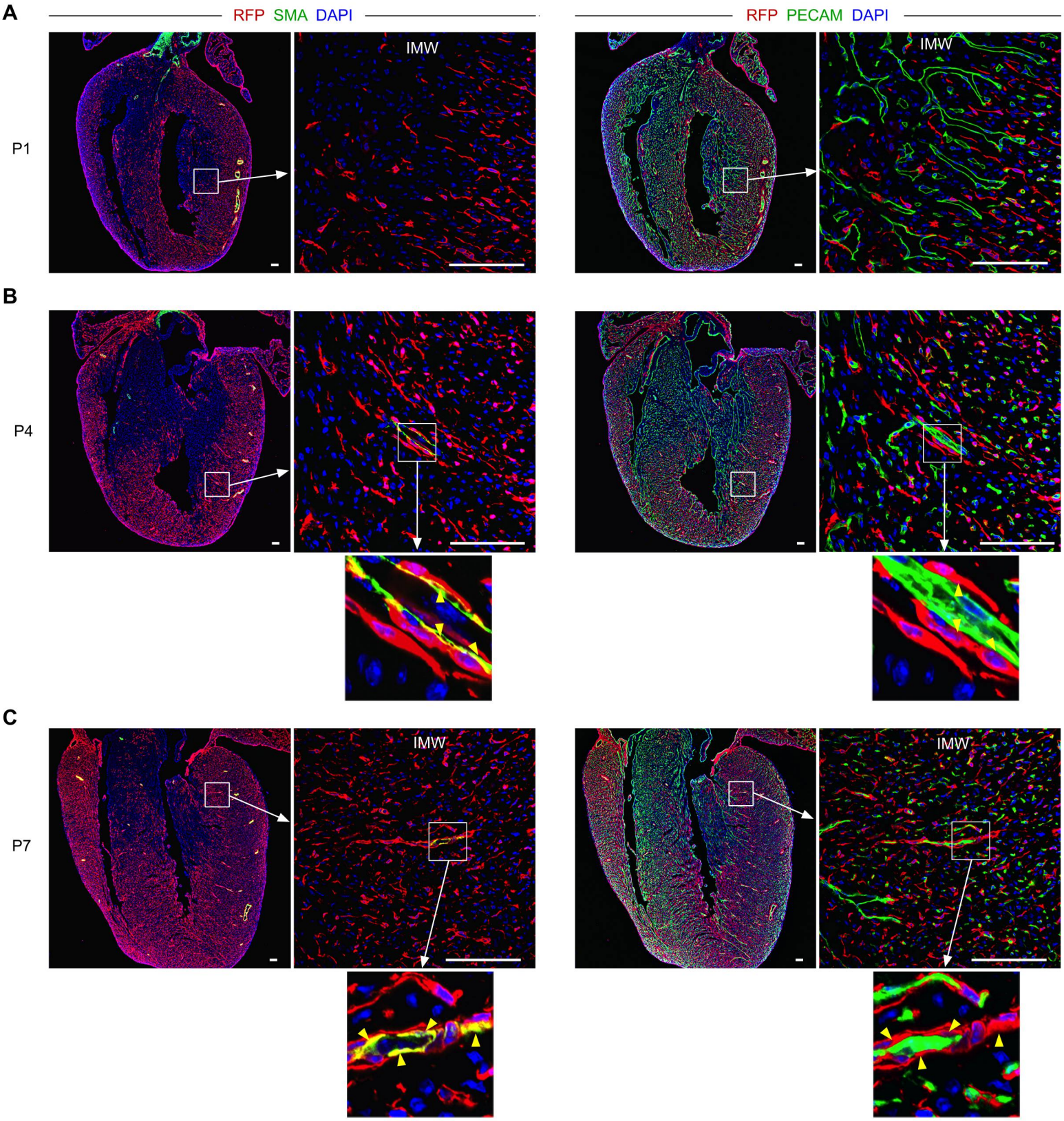

Fig. S6. 2nd CVP SMCs start to form at neonatal stages. (A-C) Immunostaining for RFP, SMA and PECAM on P1, P4, P7 Wt1-CreER;Rosa26-RFP heart sections. Tamoxifen was injected at E10.5. White arrows indicate the magnfication of boxed regions in the images. In P4 and P7 hearts, we detected RFP+SMA+PECAM- cells (yellow arrowheads). IMW, inner myocardial wall. Each image is representative of three individual hearts. Scale bars, 100 μm.

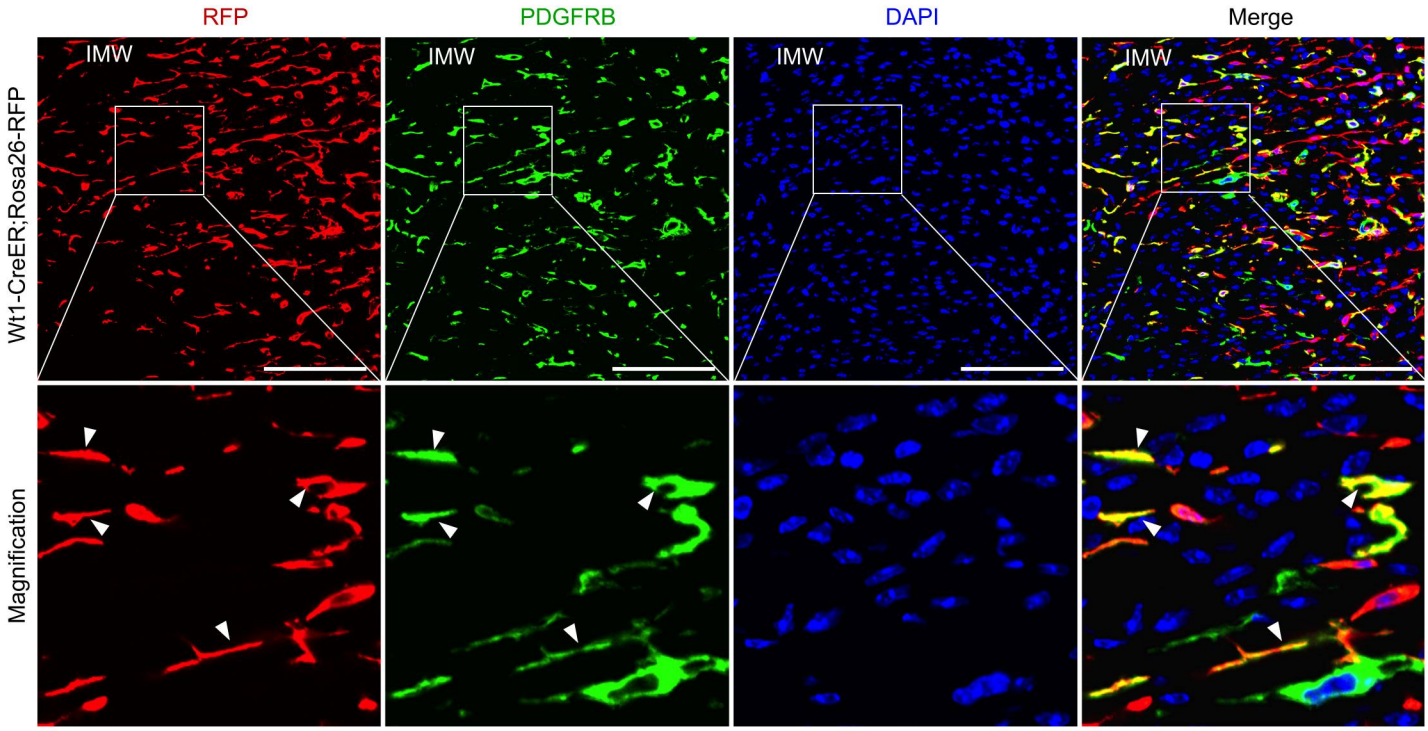

Fig. S7. Wt1-derived EPDCs express PDGFRB before differentiation into smooth muscle cells of 2nd CVP. Immunostaining for RFP and PDGFPRB on P1 Wt1-CreER;Rosa26-RFP heart sections. A subset of EPDCs express PDGFRB (arrowheads). Tamoxifen was injected at E10.5. Each image is representative of 3 individual samples. IMW, inner myocardial wall. Scale bars, 100 μm.