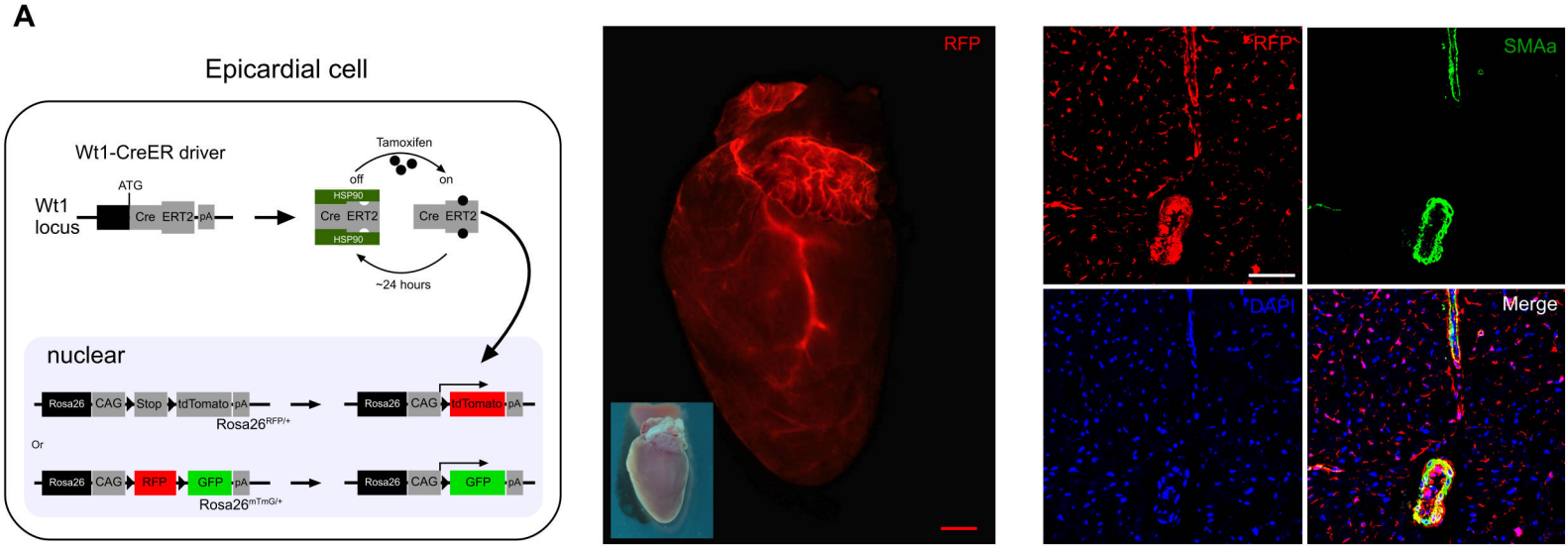
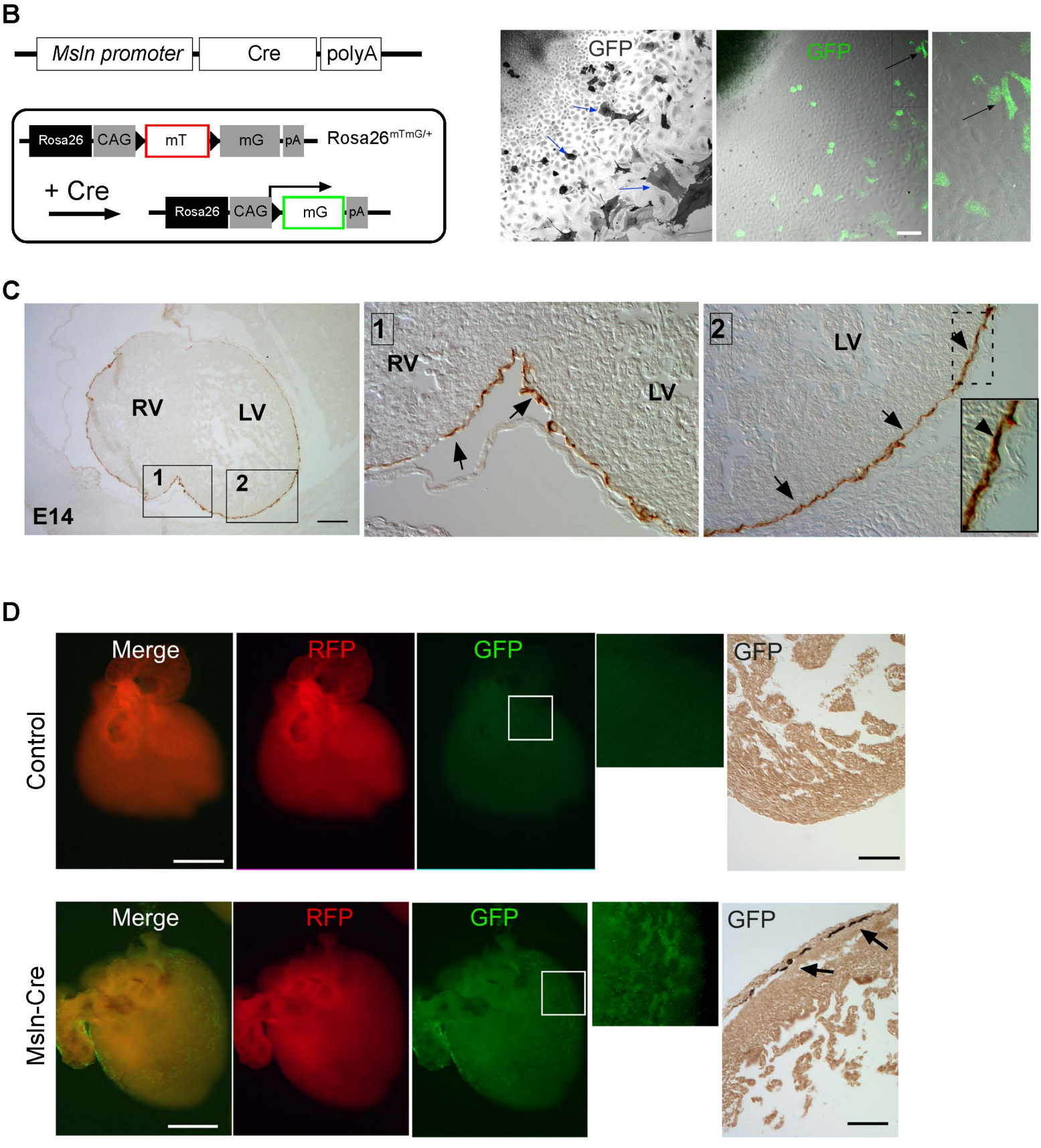
Supplementary Figure 1

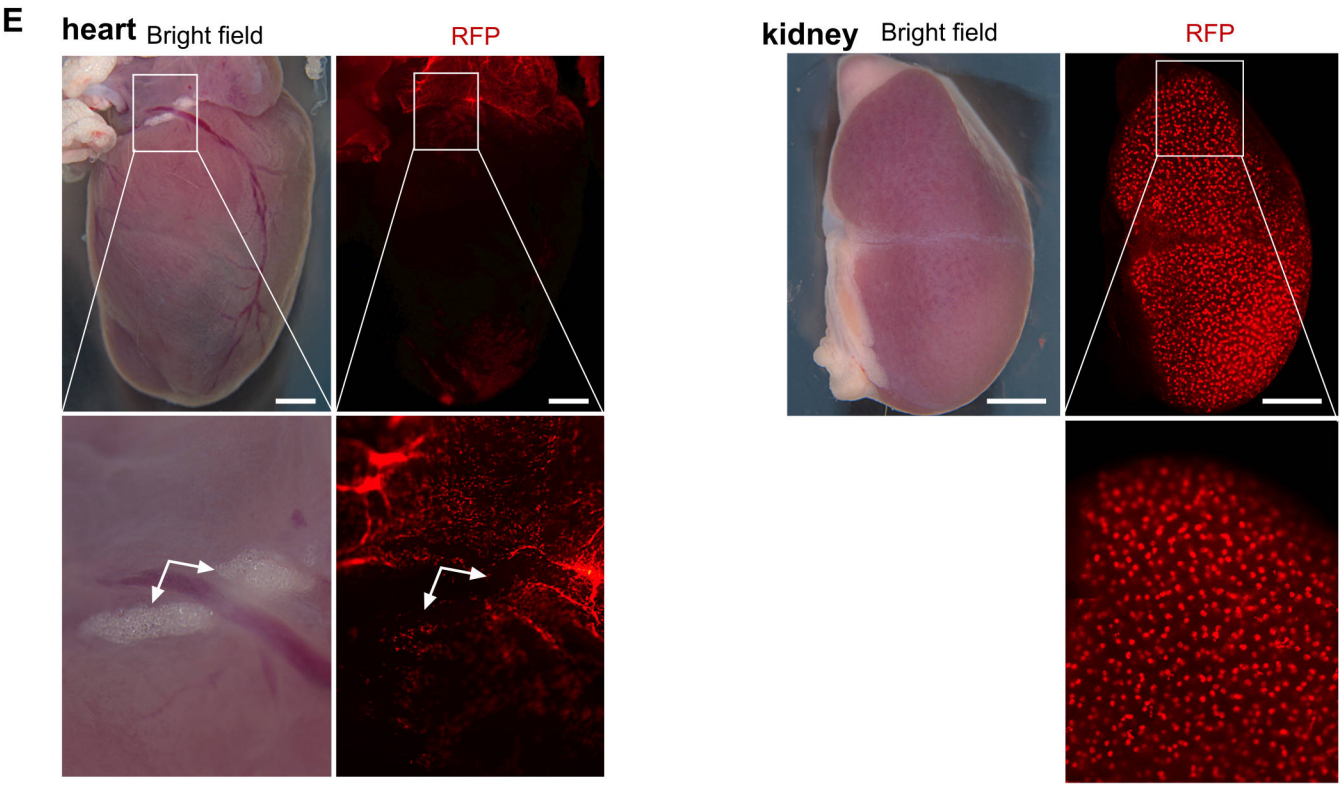
A

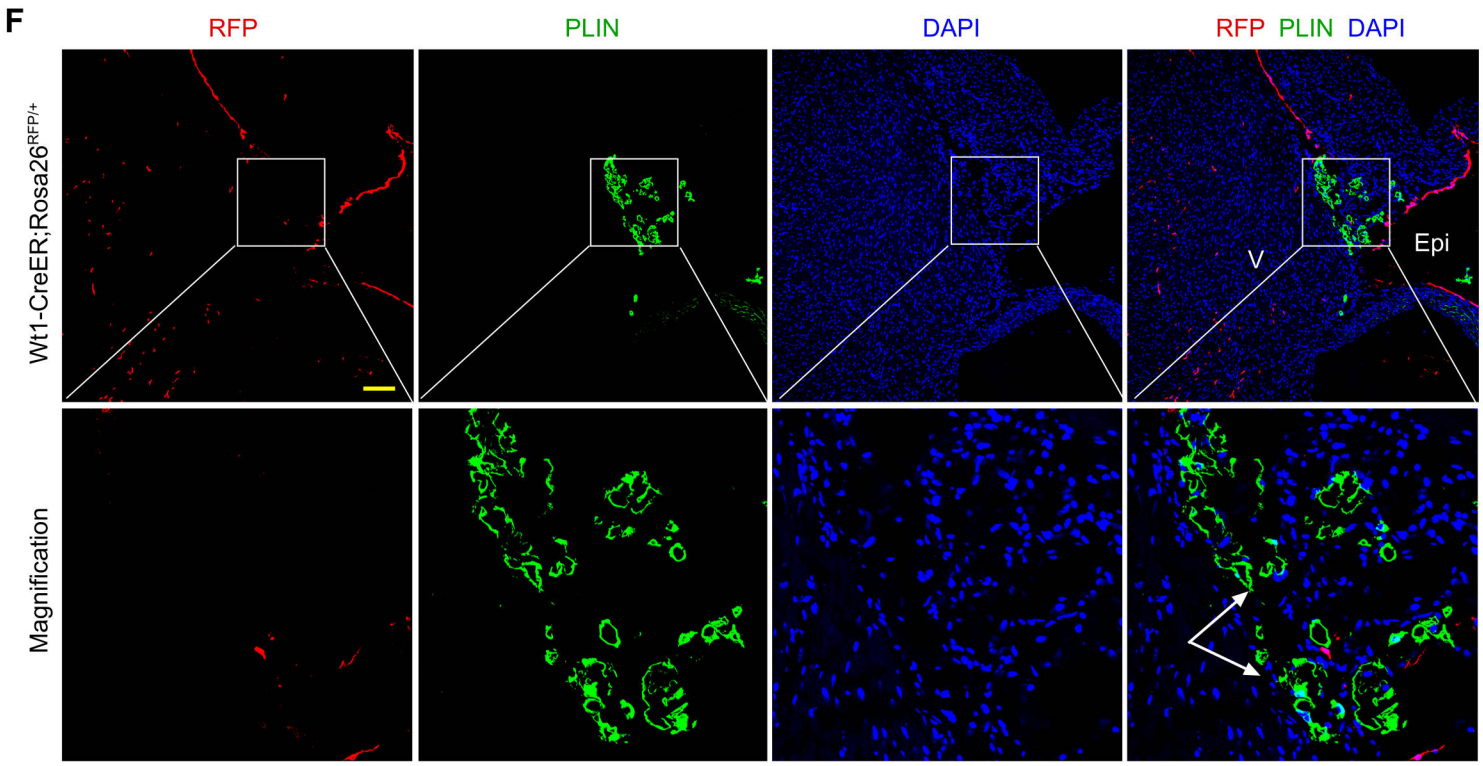


Supplementary information, Figure S1A Wt1-CreER lineage tracing from embryonic stage to adult heart. Schematic figure (left panel) showing inducible lineage tracing of epicardial cells. Whole mount view of P9w Wt1-CreER;Rosa26$^{RFP/+}$ heart (middle panel). Tamoxifen was injected at E10.5. Insert is picture of same heart taken under bright field. Red bar = 1mm. Staining of SMAa and RFP shows epicardial cells contribute to smooth muscle cells (right panel). White bar = 100 µm. Representative of 6 individual hearts.

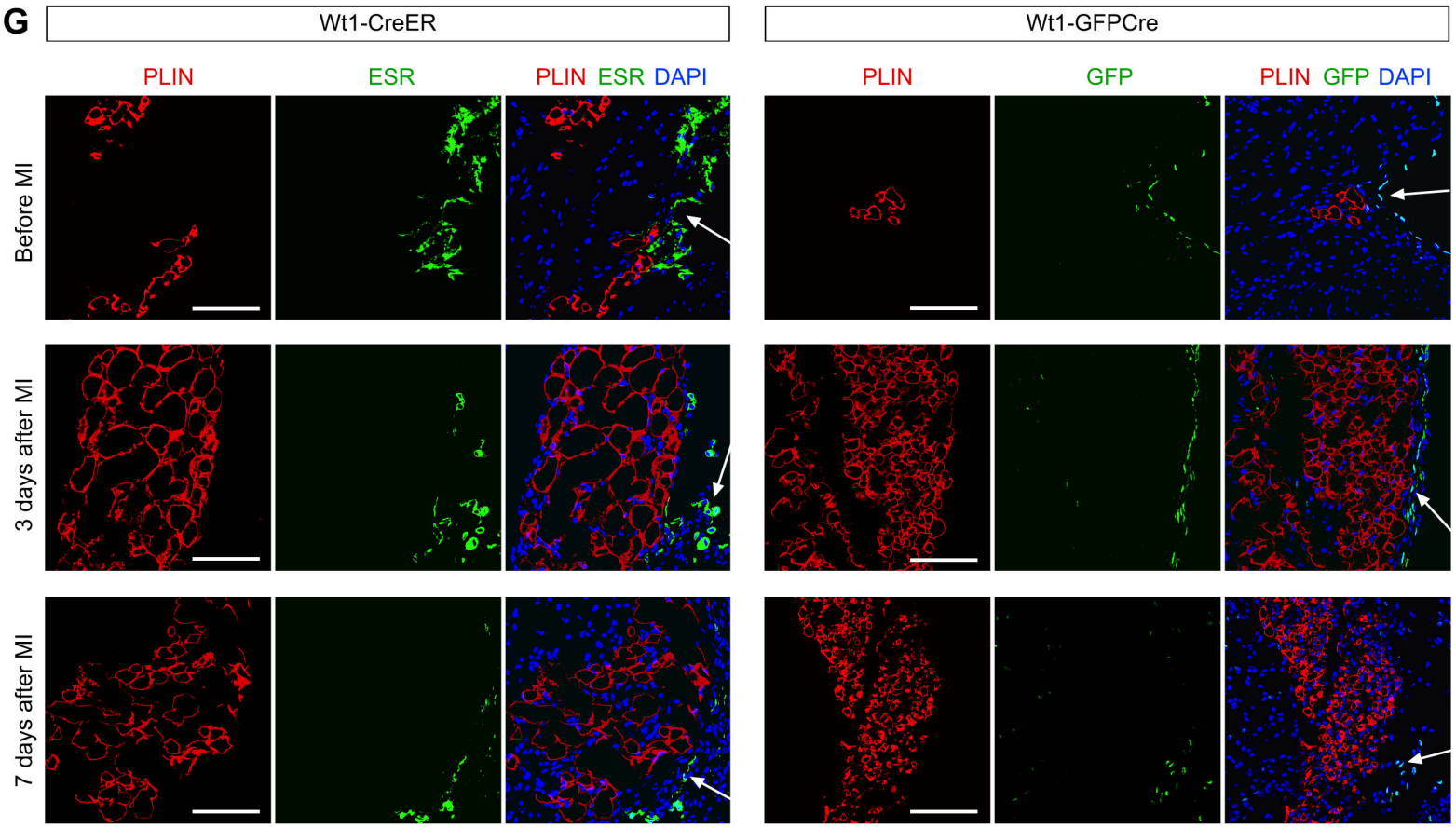


Supplementary information, Figure S1B-D Labeling of embryonic epicardial cells by Msln-Cre adenovirus. **(B)** Schematic figure showing Msln promoter driven Cre adenovirus, and Cre-loxp mediated recombination for lineage tracing. Rosa26$^{mTmG/+}$ epicardial explant transfected with Msln-Cre virus was stained with GFP antibody (blue arrows, DAB; black arrows, FITC). White bar = 50 μm. **(C)** MSLN was specifically expressed in epicardial mesothelial cells (black arrows). Black bar = 200 μm. **(D)** Msln-Cre virus injected into the embryonic heart selectively labeled epicardial cells (black arrows). PBS was injected as control. White bar = 500 μm; black bar = 100 μm.

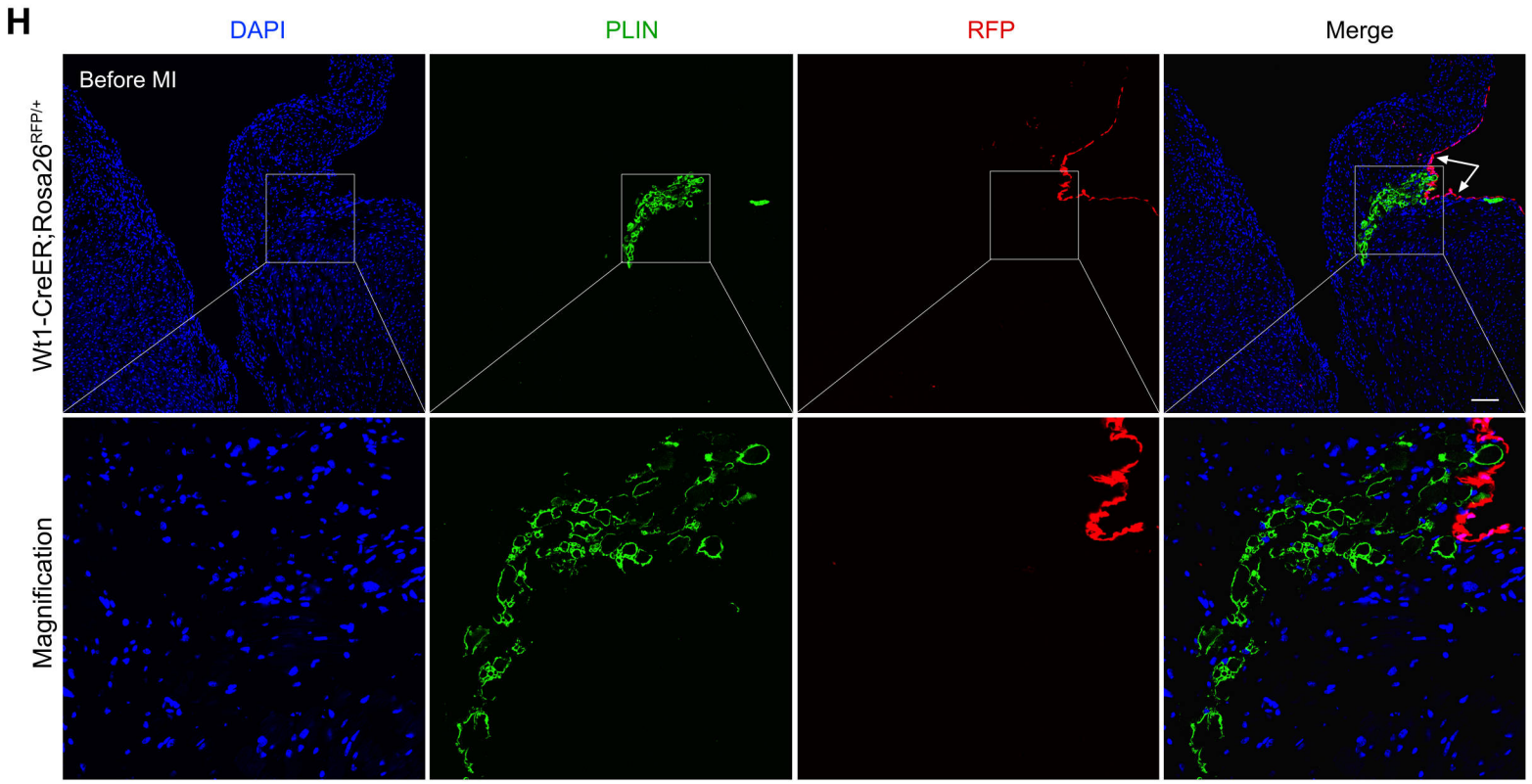




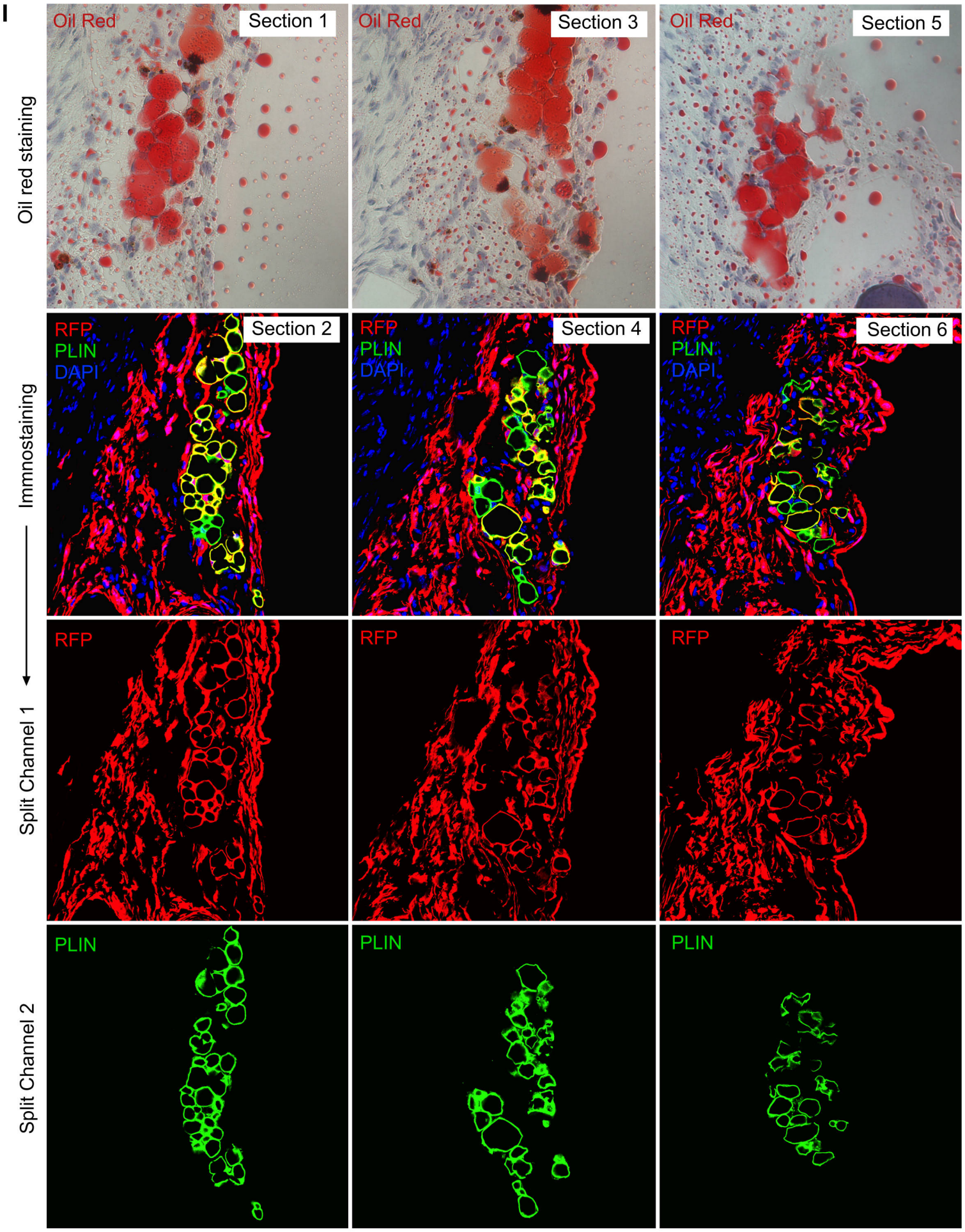
Supplementary information, Figure S1E, F Epicardial cells do not contribute to adipotcytes in adult heart homeostasis. **(E)** Whole mount view of postnatal epicardial cell labeling in Wt1-CreER;Rosa26$^{RFP/+}$ heart (left panel) and kidney (right panel). White arrows indicate epicardial fat on ventricle surface. Tamoxifen was induced in adult mice for 3 weeks and hearts were collected 4 weeks later for analysis. **(F)** Immunostaining of RFP and PLIN on sections of Wt1-CreER;Rosa26$^{RFP/+}$ heart treated with tamoxifen from 8 weeks to 11 weeks showed no epicardial cell contribution to adipocytes (white arrows). V, ventricle; Epi, epicardium. Representative figure of 5 individual samples. White bar = 1 mm; yellow bar = 100 μm.



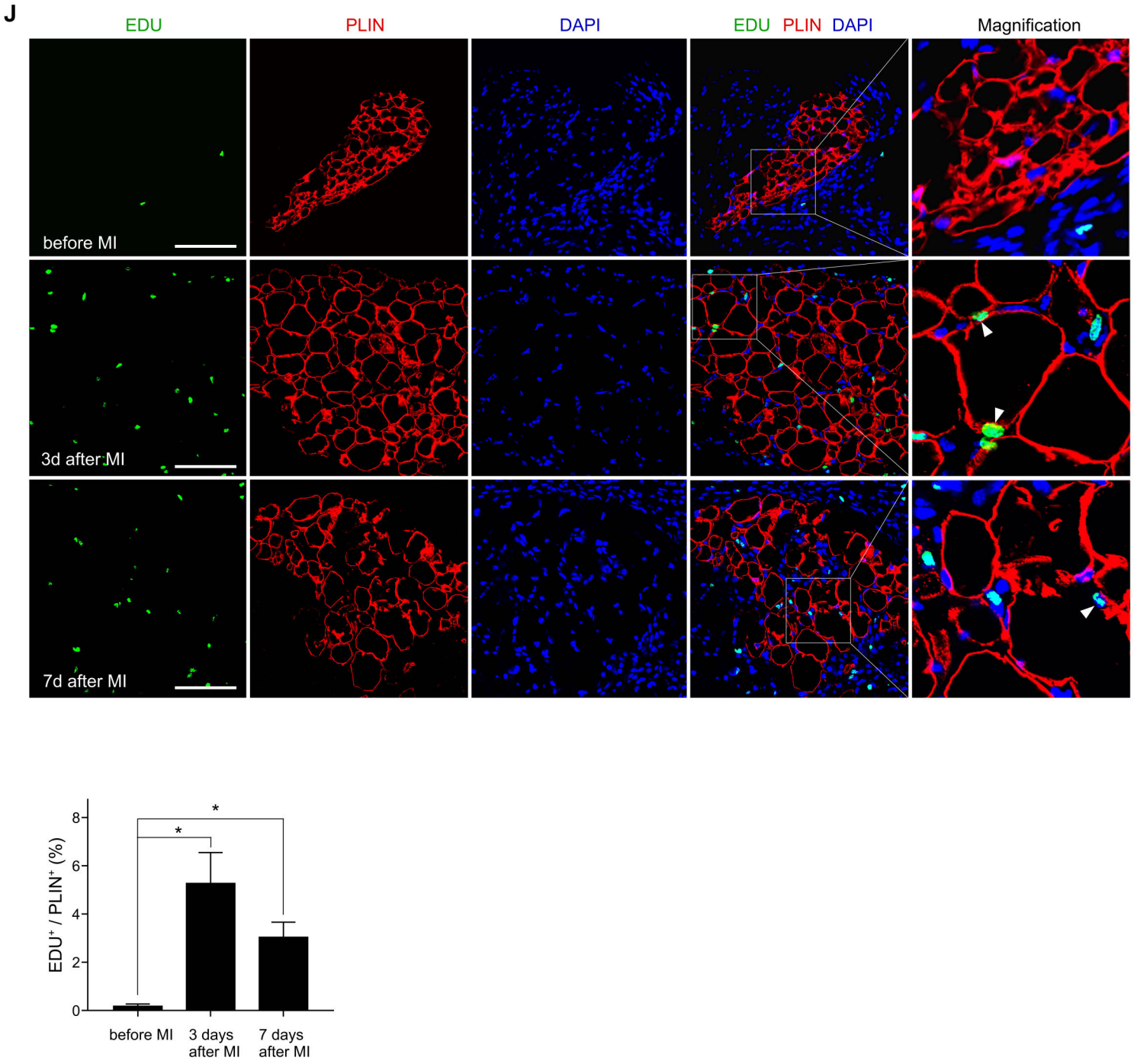
Supplementary information, Figure S1G WT1 is not expressed in epicardial fat cells. Immunostaining of estrogen receptor (ESR for CreER/WT1) or green fluorescence protein (GFP for WT1), PLIN and DAPI on Wt1-CreER or Wt1-GFPCre hearts before and after MI. White arrows indicate epicardial cells. Each figure is representative of 3 - 4 individual samples. White bar = 100 µm.



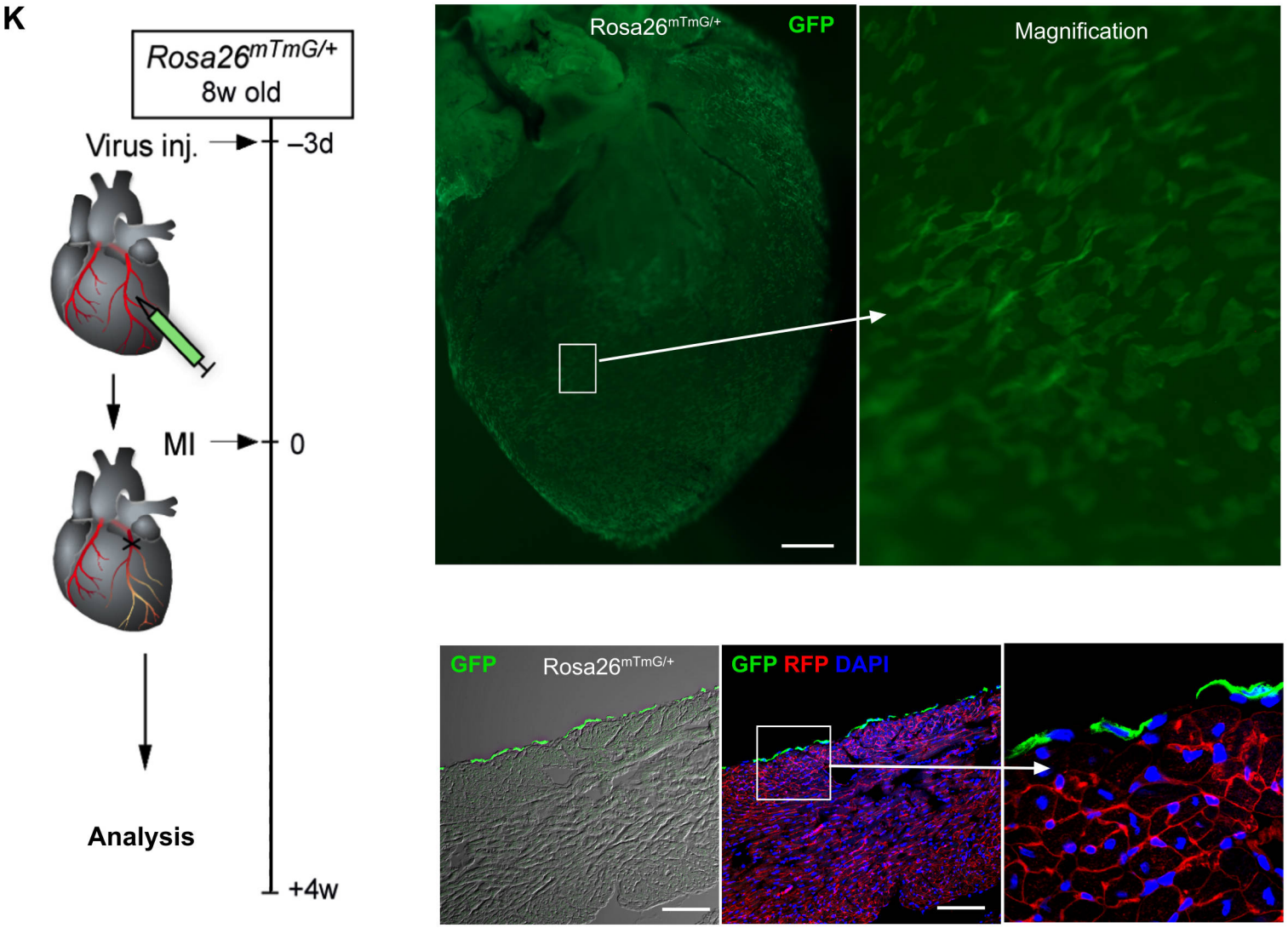
Supplementary information, Figure S1H Wt1-CreER specifically labels epicardium before MI. Immunostaining of RFP, PLIN and DAPI on Wt1-CreER;Rosa26$^{RFP/+}$ heart sections. White arrow indicate specific epicardial labeling before MI. Tamoxifen was induced 4 times between postnatal weeks 8 to 10, and hearts were then collected for analysis. No MI was performed. White bar = 100 μm.



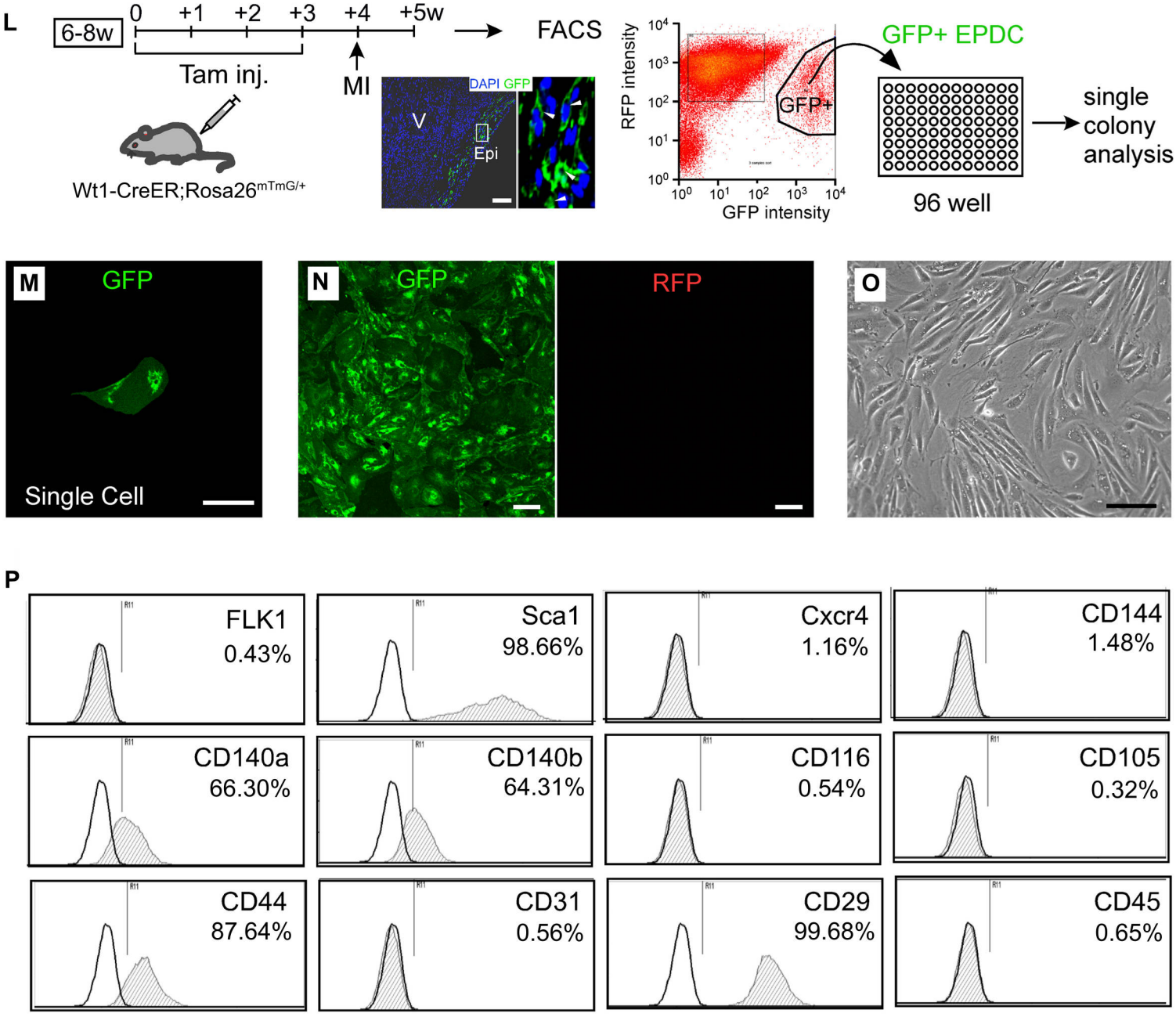
Supplementary information, Figure S1I Oil red staining and immunostaining of MI heart sections. Sections 1 - 6 are consecutive sections of Wt1-CreER;Rosa26$^{RFP/+}$ MI hearts. White bar = 500 µm. Figures are representative of 4 individual hearts.



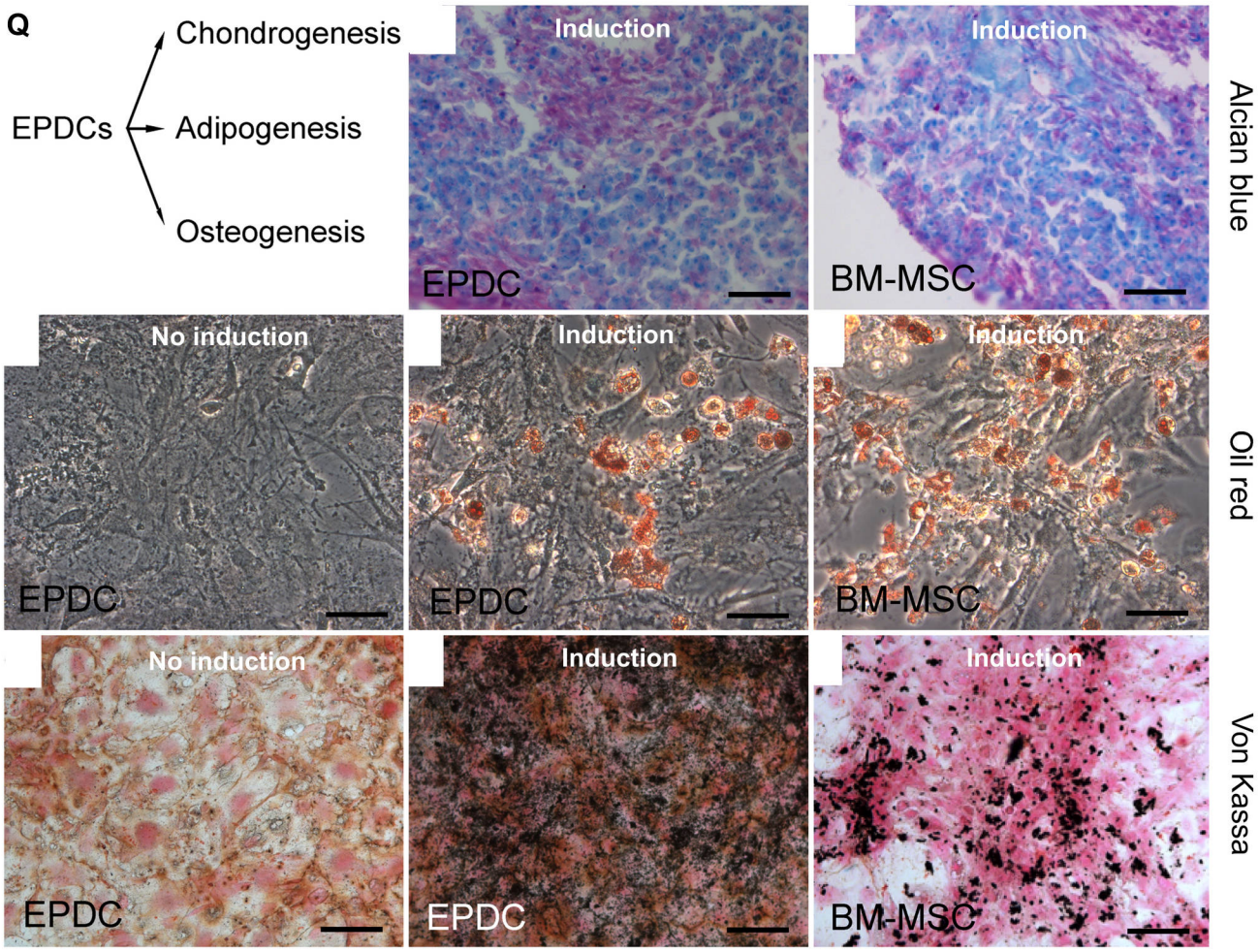
Supplementary information, Figure S1J Epicardial fat cell turn over before and after MI. Immunostaining of EDU, PLIN and DAPI on normal and MI heart sections (upper panel). MI hearts were collected 3 or 7 days after MI. EDU was injected 24 hours before mice were sacrificed. White arrowheads indicate EDU$^+$ fat cells. Each figure is representative of 3 individual heart samples. White bar = 100 μm. Quantification of percentage of EDU$^+$PLIN$^+$ cells in PLIN$^+$ fat cells (lower panel). *$P < 0.05$; n = 3.



Supplementary information, Figure S1K Specific labeling of adult epicardium by Msln-Cre virus injection before MI. **(K)** Schematic figure showing the experimental outline. Msln-Cre adenovirus was injected into hearts of Rosa26$^{mTmG/+}$ mice before myocardial infarction (left panel). Whole mount view of Rosa26$^{mTmG/+}$ heart after Msln-Cre virus injection (right upper panel). White bar = 1 mm. Immunofluorescence of sections shows epicardial cell labeling (GFP^{+}) by Msln-Cre virus (right lower panel). White bar = 100 μm.



Supplementary information, Figure S1L-P Isolation and characterization of single-cell clone of EPDC from MI heart. (**L**) Schematic figure showing the procedures of obtaining EPDC clonal outgrowths. EPDCs were isolated by FACS from dissociated 7 day post-MI Wt1-CreER;Rosa26$^{mTmG/+}$ hearts and individual cells were deposited into 96 well dishes. The EPDCs were clonally expanded. V, ventricle; Epi, epicardium layer; white arrowheads indicate GFP^{+} EPDCs. (**M**) Single GFP^{+} cell deposited in a well. Out of 247 wells, 13 grew out. 10 were successfully expanded into 24 well dishes, and 3 were used for multi-potency assays. (**N**) Cells were cultured for 12 weeks and expanded to over 10^{7} cells. The cells retained a GFP^{+}RFP^{-} signature excluding expansion of non-EPDCs. (**O**) EPDC morphology after 12 weeks of passage resembled mesenchymal stem cells (MSC). (**P**) FACS analysis of EPDCs was consistent with an MSC immunophenotype. n = 3. Y-axis indicates cell counts, and X-axis is the APC intensity. Bar = 100 μm.



Supplementary information, Figure S1Q Multipotency of epicardium derived progenitor cells (EPDCs). (**Q)** Clonal EPDCs outgrowths were cultured in chondrogenic, adipogenic, or osteogenic differentiation conditions.EPDCs differentiated into chondrocyte (Alcian blue), adipocyte (Oil red), and osteoblast (von Kossa). EPDC differentiation into chondrocytes or osteoblasts was not observed in the absence of inducing conditions. Bone marrow derived mesenchymal stem cells (BM-MSC) were used as positive controls for staining. Black Bar = 100 µm.