ONLINE SUPPLEMENTARY MATERIAL

Cardiac Fibro-Adipocyte Progenitors Express Desmosome Proteins and Preferentially Differentiate to Adipocytes Upon Deletion of the Desmoplakin Gene

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ONLINE MATERIAL AND METHODS

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The Institutional Animal Care and Use Committee approved the studies.

Isolation and culture of FAPs from human hearts: Cardiac myocyte depleted cell fraction was obtained by collagenase type 2 digestion of ~100mg of fresh tissue. In detail, bioptic samples were minced and incubated in a 0.1% collagenase 2 solution (Worthington Biochemical Corp; Lakewood, NJ08701; cat# LS004176) for 45 min at 37 °C under gentle agitation. The collagenase activity was stopped by adding 10 mL of α Modification media (aMEM, Hyclone; cat# SH30265.01) supplemented with 10% stem cell certified Fetal Bovine Serum (ES-FBS, Hyclone; cat# SH30070.03-E) and 1% Antibiotic-Antimycotic solution (Gibco; cat # 15240). The solution was filtered through a 40mm cell strainer (BD Bioscience cat# 352340) and centrifuged for 5 min at 300g. The cell pellet was washed one time with PBS and re-suspended in complete medium, composed by aMEM supplemented with 20% ES-FBS, and 1% Antibiotic-Antimycotic solution, plated in 60mm 0.1%gelatin coated plates and placed in a 5% CO_2 humidified incubator at 37 °C.
After 2 to 3 days in culture, cardiac myocyte-depleted cells were detached by trypsin treatment, resuspended in MACS buffer (Miltenyi Biotec, Bergisch Gladbach, Germany; cat # 130-091-221) and incubated for 45 min at 4 °C in the dark with the following antibodies: anti platelet-derived growth factor receptor A (PDGFRA) (BD Pharmingen, PE Mouse Anti-Human CD140a antibody; cat# 556002), hematopoietic lineage antibody cocktail (BD Pharmingen, FITC Human Lin cocktail; cat #562722), anti thymocyte antigen 1 (THY1) (BD Pharmingen, APC Mouse Anti-Human CD90; cat# 561971) and anti Discoidin domain receptor 2 (DDR2) (Santa Cruz Biotechnology) . Unlabeled cells and cells stained with the appropriate isotype IgG controls were included as controls. Unbound antibody was removed by two washes in 2 mL of MACS buffer and cell suspension was passed

through a 35 μm mesh strainer (BD Bioscience cat# 352235) and sorted through the FACS-Aria flow cytometer (BD Pharmingen, San Diego, CA). A mult-step approach was used (Online Figure I) in order to isolate hematopoietic lineage negative (Lin^{neg}) cells that expressed PDGFRA but not the stem cell and fibroblast marker THY1 or the fibroblast marker DDR2. FACS-isolated $PDGFRA^{pos}$:Lin^{neg}:$THY1^{neg}$:$DDR2^{neg}$ cells were seeded onto 0.1% gelatin coated plates at the density of 2.5 x $10^4/cm^2$ in growth medium (α MEM, supplemented with 10% embryonic stem cell certified ES-FBS and 1% Antibiotic-Antimycotic solution) and incubated at 37 °C in a 5% CO2 humidified incubator. The growth medium was changed every day and cultures were expanded by serial passages. Upon reaching 70% confluency, cells were detached by trypsin digestion and split 1:2 ratio. All the experiments were conducted on cells collected at first to third passages. Cells from the experimental groups were matched by the passage number and number of cells.

Isolation and culture of FAPs from mouse heart: Adult mice (3-7 months old) were euthanized and hearts were explanted, washed with cold sterile PBS, and minced into <2 mm pieces. The pieces were incubated in 3 mL of 0.1% type 2 collagenase in α-MEM medium for 20 min at 37°C with gentle agitation. During the incubation, the minced tissue was gently pipetted up and down with a 5 mL pipette every 5 min. The collagenase activity was stopped and the cell suspension was passed through a 40 μm mesh cell strainer to remove debris and centrifuged at 300 g for 5 min at 4 °C. The supernatant was removed and the cell pellet resuspended in MACS buffer and incubated for 45 min at 4 °C in the dark with the following antibodies: anti PDGFRA (eBioscience, San Diego, CA 92121, APC anti-mouse CD140a antibody; cat# 17-1401-81), mouse lineage antibodies cocktail (BD Pharmingen, BD Horizon™ V450 Mouse Lineage Antibody Cocktail, with Isotype Control, cat #561301), anti THY1 (eBioscience, PE-Cy5 Anti-Mouse CD90.2; cat # 15-0902) and anti DDR2. After staining the cell suspension was sorted using the same multipstep approach utilized for human cells. FACS isolated mouse $PDGFRA^{pos}$:Lin^{neg}:$THY1^{neg}$:$DDR2^{neg}$ cells

were plated onto 0.1% gelatin coated plates in growth medium [α MEM supplemented with 10% ES-FBS, 10 ng/mL mouse basic Fibroblast Growth Factor (bFGF, R&D, Minneapolis, MN; cat #3139-FB), 1000 U/mL of mouse Leukemia Inhibitory Factor (mLIFMillipore; cat # ESG1106) and 1% Antibiotic-Antimycotic solution] and incubated at 37 °C in a 5% CO2 humidified incubator. Cell expansion in culture and experimental conditions were the same used for human cells.

Characterization of cardiac FAPs. Myocyte-depleted cardiac cells were analyzed for co-expression of PDGFRA and the following additional lineage markers: TIE2, a marker for endothelial cells; KIT antigen, a marker for progenitor cells; CD146, a marker for pericytes and PDGFRB, a marker for progenitor cells and pericytes. by flow cytometry and immunostaining. The list of antibodies is provided in Online Table I.

Isolation of mouse adult cardiac myocytes (CMs): Mice were anesthetized by intraperitoneal (I.P.) injection of pentobarbital (62 mg/Kg) and were anti-coagulated with I.P. injection of 200U of heparin. The heart was then harvested and immediately placed in Ca^{2+} free perfusion buffer [120 mM NaCl, 15 mM KCl, 0.6 mM KH2PO4, 0.6 mM Na2HPO4, 1.2 mM MgSO4 7H2O, 30 mM Taurine, 4.6 mM NaHCO3, 10 mM HEPES, 10 mM 2,3-Butanedione monoxime (BDM), and 5.5 mM Glucose; pH 7.0]. Then, ascending aorta was cannulated with a 22 G blunt needle under a magnifying dissection microscope (Leica, S6D). The cannula was positioned above the aortic valve cusps and was connected to a retrograde perfusion system. The heart was perfused for 2 min with the perfusion buffer at a constant rate of 4 mL/min at 37 °C. Upon washing out the blood, the perfusate was switched to a digestion buffer containing 624 U/mL of collagenase II solution (Worthington, Lakewood, NJ). After 2 min of digestion, 10 ul of 100 mM CaCl2 was added to the CM digestion buffer and the digestion was continued until the heart became pale and spongy upon gentle pinching. The atria was then removed, the heart was disconnected from the cannula, and minced into small pieces using a fine scissors in a 60 mm dish containing 2 ml of the

digestion buffer. The mixture was pipetted up and down gently several times with a sterile plastic transfer pipet (2 mm opening) to dissociate the myocytes. After one min, 8 ml of stop buffer (10% calf serum and 12.5 μM CaCl2 in the perfusion buffer) was added to stop the digestion. The cell suspension was filtered through a 100 μm nylon mesh and transferred to a 15 ml polypropylene conical tube. 100 μl of 200 mM ATP were added to the tube and the myocytes were allowed to sediment by gravity for 2 min. CMs were pelleted by centrifugation at 20 g for 3 min. Following removal of the supernatant, cells were subjected to a four-step calcium reintroduction by resuspending the cells in aliquots of 10 ml of stop buffer containing 2 mM ATP and increasing concentration of CaCl2 at 200 μM, 500 μM, 1 mM and 1.5 mM. At each calcium reintroduction step, the pellet was resuspended in the buffer containing the corresponding incremental concentration of CaCl2 and after 2 min at room temperature the preparation was centrifuged at 20 g for 3 min. The final pellet was resuspended in a CM plating media (MEM media, 1% penicillin-streptomyocin, 10% Calf serum, 10 mM BDM, and 2 mM ATP). CMs were plated in culture dishes or cover glasses coated with laminin and incubated immediately in a 2% CO2 incubator at 37 ^{o}C. After 1 hour, the plating medium was replaced with fresh medium to remove the unattached CMs. The cells were collected 2 hour later for RNA or protein extraction or fixed in 4% paraformaldehyde for immunostaining.

Isolation and culture of smooth muscle cells (SMCs), endothelial cells (ECs), and cardiac fibroblasts (CFs). Mouse aortic SMCs were isolated from the mouse aortic tissues and cultured as previously published (1). In brief, mouse aorta was dissected and adventitia and endothelium were removed under a dissecting microscope. The remaining tissue containing the medial muscle cells was digested by incubation with type II collagenases at 37 ^{o}C for about one hour. The digestion was stopped by adding DMEM supplemented with 10% FBS. The cell suspension was centrifuged for 5 min at 300 g and the cell pellet was re-suspended and cultured in DMEM medium.

Mouse primary cardiac microvascular ECs were purchased by Cell-Biologics (2201 West Campbell Park Drive, Chicago, IL 60612; cat# BALB-5024). ECs were grown on cell culture plates pre-coated with gelatin-based coating solution (cat# 6950) in complete mouse endothelial cell growth medium (Cell Biologics, cat# M1168).

Cardiac fibroblasts were isolated as previously published (2). Mouse heart was harvested and immediately washed in cold Base Buffer (1 vial of Krebs-Henseleit buffer powder (Sigma, K3753-10X1L) 0.323 g calcium chloride, 2.02 g sodium bicarbonate, 2.6 g HEPES, and 3.75 g taurine; H2O to final volume of 1L; pH 7.2-7.3). The heart was placed in a sterile dish, covered it with 1 ml Liberase solution [250μL HEPES, 24.15mL HBSS+/+, 500μL Liberase TH Research Grade (Roche, 05401151001), 200μL DNase1 (sigma, D4263)] and quickly minced into small pieces (1 x 1 mm). The minced heart was transferred to a 50 mL tube containing a total of 5 mL of Liberase solution and incubated 5 min at 37°C while stirring at low setting. The suspension was removed from the stirrer and after the tissue settled down, the supernatant was carefully transferred, through a 40 m cell strainer, into a 50 mL tube containing stop buffer (13.5 mL base buffer, 1.5 mL FBS, 120 μL DNase1) an left on ice while digesting the rest of the heart. An aliquot of 5 ml Liberase solution was added to the undigested tissue, and the solution was incubated for 8 min at 37 °C while stirring. The supernatant was then collected in stop buffer and the same procedure was repeated until the heart was completely digested. At the end of digestion, the cell suspension was centrifuged at 300 g for 5 min at 4 °C and the cell pellet was washed one time in 20 mL of cold Base buffer. After the wash, the cells were resuspended in DMEM/F-12 HEPES (Life Technologies, 11330) medium supplemented with 10% of FBS (GE, SH30070.03) and 1x Antibiotic-Antimycotic (Life Technologies, 15240-062), plated in tissue culture plates and incubated in a 5% CO2 incubator at 37 ^{o}C.

Immunofluorescence (IF): Thin myocardial sections or isolated cells were immunostained, as published (3-5). In brief, cardiac cross-sections were placed in the OCT compound (Sakura Finetek USA, Inc. Torrance, CA 90501; cat#4583) and frozen in isopentane (2-Methyl Butane, Sigma-Aldrich St Louis, MO, cat #320404). Human myocardial samples were from 2 controls and 2 patients with advanced AC. Samples were fixed in formalin and paraffin embedded. OCT and paraffin embedded tissues were cut into 5-7 μm thin myocardial sections for IF staining. Human myocardial thin sections were deparaffinazed, rehydrated and boiled for 15 min in 10 mM Sodium Citrate (pH 6.0) for antigen retrieval before blocking. For IF staining cells were grown on cover slips. Thin myocardial section and 70 to 80% confluent cells were fixed in 4% paraformaldehyde for 15 min at room temperature. The samples were washed 3 times in PBS and blocked in 5% donkey serum in PBS containing 0.3% Triton X-100 for 1 hour at room temperature. After blocking, samples were incubated with the primary antibody (list of antibodies is available in Online Table I) in 1% BSA and 0.3% Triton X-100 overnight at 4 °C. After 3 washes, samples were incubated with the corresponding secondary antibody in 1% BSA and 0.3% Triton X-100 for 1 hour at room temperature. After 3 rounds of washing in PBS, fluorescence-labeled secondary antibodies were added for 1 hour at room temperature. After 3 more washes in PBS, the samples were stained with a 0.1 mg/mL of 4′, 6-Diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich St Louis, MO; cat# D8417) for 2 min, mounted in fluorescent mounting medium (Dako North America Inc.6392 Via Real, Carpinteria CA 93013, cat# S3023), and examined under fluorescence microscopy (Zeiss, Axioplan Fluorescence Microscope).

Immunoblotting (IB): IB was performed per conventional methods (3-5). In brief, total cellular proteins were extracted by homogenizing heart tissues or the collected cells in a RIPA lysis buffer [1X formulation: 25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% Nonidet P-40 (NP-40), 1% Sodium deoxycholate and 0.1 % Sodium Dodecyl Sulphate (SDS); Pierce Biotechnology, Rockford,

IL; cat #89901] in the presence of protease and phosphatase inhibitors (complete protease inhibitor cocktail; Roche Diagnostics, GmbH, Mannheim, Germany; cat # 11-697-498-001; Phosphatase Inhibitor Cocktail 2 and 3; Sigma St. Louis, MO 63103; cat# P0044 and P5726). Protein concentration was measured using the Bio-Rad DC Protein Assay Kit (Bio-Rad Laboratories; cat # 5000111). After denaturation in Laemmli loading buffer at 95 °C for 5 min, 30 μg aliquots of each protein extract were loaded onto SDS-polyacrylamide gels (PAGE), subjected to electrophoresis, and transferred to nitrocellulose membranes. The membranes were blocked in 5% non-fat milk for 1 hour at room temperature and incubated with the primary antibody overnight at 4 °C. After 3 washes in TBS, membranes were incubated with the corresponding horseradish peroxidase (HRP) conjugated secondary antibody for 1 hour and the signal was detected by chemiluminescence. The list of primary and secondary antibodies is provided in Online Table 1. Membranes were stripped by incubation in Restore PLUS Western Blot Stripping Buffer (Thermo Scientific, Hudson, New Hampshire, cat #46430) for 10 min at room temperature, washed in TBS for 3 times and probed with an anti α- tubulin antibody as a loading control.

TGFB1 Immunohistochemistry: Expression of transforming growth factor-β1 (TGFB1) was detected by Immunohistochemstry (IHC) as previously published (6) PFA fixed cells and formalin fixed-paraffin embedded thin myocardial sections were incubated with monoclonal mouse anti TGFB1 antibody (R&D System; cat# MAB1835) at the concentration of 10 μg/mL, overnight at 4 °C, followed by staining with an Anti-Mouse HRP-DAB Cell and Tissue Staining Kit (Vector Laboratories, Burlingame, CA 94010; catalog # SK-4100). Signal was detected by peroxidase reaction.

Detection of apoptosis: Apoptosis was detected by TUNEL assay, as previously described (7)by using a commercially available kit (In Situ Cell Death Detection Kit, TMR red; Roche cat# 12156792910), per manufacturer instructions. In brief, formalin fixed-paraffin embedded thin

myocardial sections were permeabilized in 0.3% PBS-T for 10 min at RT and subsequently incubated with the TUNEL reaction mixture. Nuclei were countestained with DAPI solution. After washing, the label incorporated at the damaged sites of the DNA was visualized under fluorescence microscopy.

Lineage tracer mice: *Pdgfra:Egfp* reporter mice were purchased from Jackson Laboratory (stock No: 007669, Bar Harbor, Maine 04609 USA) (6). These mice express the H2B-eGFP fusion protein from the endogenous *Pdgfra* locus, leading to expression of H2B-eGFP mimicking the expression pattern of the endogenous *Pdgfra* gene (6). *Myh6-Cre,* $Dsp^{F/F}$ and *R26-*^{F}*STOP*^{F}*-Eyfp* mice have been published (7-11). *Pdgfra-Cre* BAC transgenic mice (C57BL/6 background) were from Jackson Laboratory (stock No: 013148)(12). The *Pdgfra-Cre* deleter mice were crossed to $Dsp^{F/F}$*:R26-*^{F}*STOP*^{F}*-Eyfp* mice to generate *Pdgfra-Cre:R26-*^{F}*STOP*^{F}*-Eyfp:*$Dsp^{W/F}$ mice (henceforth, *Pdgfra-Cre:Eyfp:*$Dsp^{W/F}$*)*. Expression of the Cre recombinase is expected to delete the floxed exon 2 of *Dsp* gene and the LoxP-flanked STOP sequence upstream of the *Eyfp* gene at the Gt(ROSA)26Sor locus, only in cells that are transcriptionally regulated by the *Pdgfra* locus. Mice were genotyped by PCR of tail DNA. Oligonucleotide primers used in PCR reactions are listed in Online Table I. Control age- and sex-matched wild type mice were included in all experiments.

Echocardiography: Data were obtained and analyzed without knowledge of the genotype. Cardiac structure and function in mice were assessed by B-mode, M-mode and Doppler echocardiography using a HP 5500 Sonos echocardiography unit equipped with a 15-MHz linear transducer, as published (4, 5, 13). Mice were anesthetized by I.P. injection of sodium pentobarbital (60 mg/Kg). Wall thicknesses and left ventricular (LV) dimensions were measured from M-mode images using the leading-edge method on 3 consecutive cardiac cycles. LV fractional shortening and mass were calculated as previously described (4, 5, 13).

Morphometric and histological analyses: Morphometric and histological analyses were performed without knowledge of the genotype or experimental groups. Ventricular/body weight ratio was calculated in age- and sex-matched mice. Myocardial histology was examined by H&E, Masson Trichrome, Picrosirius Red and Oil red O staining of thin myocardial sections, as published (4, 5, 13). Likewise, isolated cardiac FAPs were stained with Oil Red O to detect their differentiation to adipocytes. In brief, for Oil Red O staining, thin myocardial sections or isolated cells were washed one time with PBS and then fixed in 10% formalin for 15 min at room temperature. After 10 min washing under running water, samples were stained in a modified Mayer`s hematoxylin solution (Richard-Allan Scientific, Kalamazoo, MI; cat # 72804) for 2 min. Samples were then washed for 10 min in water, placed in 100% propylene glycol (Poly Scientific, Bay Shore, NY; cat #s264) for 2 min and then placed in Oil Red O 0.5% solution in propylene glycol (Poly Scientific, Bay Shore, NY; cat #s1848) for 5 hour or overnight at room temperature. The Oil Red O solution was removed and the samples were dipped 2 times in 85% propylene glycol (Poly Scientific, Bay Shore, NY; cat #s264A). After 20 min washing the slides were mounted with aqueous mounting medium. Extent of fibrosis was analyzed by quantification of collagen volume fraction (CVF) of Sirius Red stained thin myocardial sections. Images were analyzed using the ImageTool 3.0 analysis software.

Induction of adipogenesis: Adipogenesis was induced as previously published (14) Cardiac FAPs isolated from the wild type and the lineage tracer mice were plated in individual wells in 24-well plates that contained cover glass coated with 0.1% gelatin in proliferating media. Cells were incubated at 37°C in a 5% CO2 humidified incubator. Upon reaching 100% confluence, media was changed to an Adipogenesis Induction Medium [α-MEM supplemented with 10% FBS, 1% Antibiotic-Antimycotic, 10 μg/mL insulin (Sigma-Aldrich, 3050 Spruce St.; St. Louis, MO 63103; cat # I-0516), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich, cat # I-7018), and 1 μM dexamethasone (DXM; Sigma-Aldrich, cat# D-8893)]. The Adipogenesis Induction Medium was

changed every other days for a period ranging from 2 to 7 days. After incubation cells were fixed for Oil Red O staining or IF staining with adipogenic markers. Cells were also collected for RNA extraction and qPCR detection of genes involved in fat metabolism or for protein extraction and IB for selected adipogenic markers.

Quantitative PCR (qPCR): Total RNA was extracted using miRNeasy kit (Qiagen, cat # 271004) and cDNA was synthesized using a high-capacity cDNA Reverse Transcription Kit (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems cat # 4368814). Quantitative PCR was performed using specific TaqMan gene expression assays (Applied Biosystems) per manufacturer's recommendations. *Gapdh* transcript levels were used for normalization. The ΔCt method was used to calculate the normalized gene expression values.

Activation of the canonical Wnt signaling pathway: To activate the canonical Wnt signaling pathway, cells were treated with 2 different concentrations (5 μM, and 10 μM) of 6-bromoindirubin-3'-oxime (BIO; EMD Chemicals-Calbiochem, Gibbstown, NJ 08027; cat # 361550), a known activator of the canonical Wnt signaling, as described (13-15). After 24 hours of incubation with BIO, adipogenesis was induced, as described above, for 7 days in presence of BIO in the culture media. Cells treated with BIO but not subjected to adipogenesis as well as cells induced for adipogenesis but not treated with BIO were included as controls. Media were changed daily. The effects of activation of the canonical Wnt on adipogenesis were determined by quantification the number of cells positive for Oil Red O and CEBPA expression as well as by quantifying transcript levels of the adipogenic genes by qPCR.

Statistical analysis: Statistical analysis has been performed using Graph pad Prism 6 (GraphPad Software, Inc., La Jolla, CA 92037) or SPSS version 20 software (IBM North America, New York, NY 10022). Data are expressed as mean ± SD. Normal distribution of the continuous variables was tested using the 1-sample Kolmogorov-Smirnov test. Differences in the continuous

variables between the two groups were compared by t-test or Mann-Whitney U test and among multiple groups by one-way ANOVA or multivariate analysis of variance (MANOVA). Pairwise comparisons were performed by Bonferroni multiple comparisons test. Differences among the categorical values were compared by Kruskall-Wallis test. A p value< 0.05 was considered significant.

ONLINE FIGURE LEGEND

Online Figure I. **Scheme illustrating the multistep approach used to isolate FAPs from human and mouse hearts:**

Cardiac myocyte-depleted cells were sorted to isolate PDGFRA^{pos}:Lin^{neg} cells. The PDGFRA^{pos}:Lin^{neg} cells were then sorted in order to exclude the THY1^{pos} cells . PDGFRA^{pos}: Lin^{neg}:THY1^{neg} cells were further sorted to exclude the DDR2^{pos} cells. A subset of cardiac PDGFRA^{pos}:Lin^{neg}:THY1^{neg}:DDR2^{neg} cells express only the fibrogenic marker COL1A1, but not the adipogenic transcription factor CEBPA and another subset express CEBPA but not COL1A1. The PDGFRA^{pos}:Lin^{neg}:THY1^{neg}:DDR2^{neg}:COL1A1^{pos} subfraction does not express the desmosome protein desmoplakin (DSP). Only the PDGFRA^{pos}:Lin^{neg}:THY1^{neg}:DDR2^{neg}:CEBPA^{pos} subfraction expresses desmoplakin and hence is susceptible to cre deletion of *Dsp* in the following *in vivo* experiments.

Online Figure II. Characterization of cardiac FAPs:

A. Flow cytometry analysis of myocyte-depleted cardiac cells from wild type mice for co-expression of PDGFRA and additional lineage markers: TIE2, a marker for endothelial cells; KIT antigen, a marker for progenitor cells; CD146, a marker for pericytes; and PDGFRB, a marker for progenitor cells and pericytes. Less than 1% of cardiac PDGFRA^{pos} cells expressed TIE2, KIT, CD146 and PDGFRB. **B.** Immunofluorescence panels showing absence of TIE2, KIT, CD146 and PDGFRB in FACS isolated cardiac FAPs, in accord with the flow cytometry data.

Online Figure III. Assessment of expression of PDGFRA in other cardiac cell types *in vitro*:

A. Immunofluorescence staining of isolated adult cardiac myocytes (CMs), smooth muscle cells (SMCs) and cardiac microvascular endothelial cells (ECs), for PDGFRA and each cell type-specific marker: a-actinin (ACTN2) for CMs, smooth muscle myosin heavy chain II (SM-MHCII) for SMCs

and platelet endothelial cell adhesion molecule-1 (PECAM-1) for ECs, respectively. **B-C.** Absence of expression of PDGFRA in isolated cardiac myocytes as detected by immunoblotting (**B,** upper panel) and qPCR (**C**). Lower immunoblots in **B** depict absence of expression cardiac myocyte sarcomere proteins myosin heavy chain 6 (MYH6) and myosin binding protein C3 (MYBPC3) in FAPs. FACS isolated FAPs were used as positive controls for PDGFRA detection in both experiments. **D-E.** Detection of expression of PDGFRA in isolated cardiac fibroblasts (CFs) identified by the expression of COL1A1 by immunofluorescence (**D**). Quantitative data (**E**) showed that PDGFRA was expressed in 71.1 ± 20.2 % of the isolated CFs. (N=3 independent experiments; ~150 cells counted in each experiment).

Online Figure IV. Assessment of expression of PDGFRA in other cardiac cell types *in vivo*: Co-immunofluorescence of thin myocardial sections from wild type mice for PDGFRA and each specific cell type marker: ACTN2 to identify CMs, COL1A1 for CFs, SM-MHCII for SMCs and PECAM-1 for ECs, respectively. PDGFRA was expressed in CFs but not in CMs, SMCs or ECs, in accordance with the data in isolated cell types.

Online Figure V. Detection of EGFP in cardiac cell types in the *Pdgfra-Egfp* reporter mice:

A. Co-Immunofluorescence staining of thin myocardial sections from *Pdgfra-Egfp* reporter mice for the reporter protein EGFP and the cardiac cell type markers ACTN2, COL1A1, SM-MHCII and PECAM-1 respectively. In this mouse model expression of EGFP is transcriptionally regulated by the Pdgfra locus, hence EGFP is detected in cells expressing PDGFRA. **B.** Quantitative data showing that approximately 51.3 ± 9.8% of CFs was tagged with EGFP while no EGFP expression was detected in CMs, SMCs or ECs. The finding is in accord with the data on isolated cell types and on myocardial

sections from wild type mice after co-immunofluorescence staining for PDGFRA and each cell type marker.

Online Figure VI. Absence of expression of DSP in CFs, SMCs and ECs:

A. Immunofluorescence staining of isolated CFs, SMCs and ECs for PDGFRA and each cell type-specific marker. Isolated CMs are included as positive control, **B.** Detection of DSP by Immunoblot in CFs, SMCs and ECs. Heart lysates are included as positive controls. The data show absence of DSP expression in the cardiac cell types analyzed, except for CMs.

Online Figure VII. *In vivo* conditional deletion of *Dsp* and EYFP labeling of cardiac FAPs:

Dsp$^{F/F}$ and *R26-*^{F}*STOP*^{F}*-Eyfp* mice were repeatedly crossed in order to obtain *Dsp*$^{F/F}$*:R26-*^{F}*STOP*^{F}*-Eyfp* mice. *Dsp*$^{F/F}$*:R26-*^{F}*STOP*^{F}*-Eyfp* mice were crossed with Pdgfra-Cre deleter mice in order to generate *Pdgfra-Cre:R26-*^{F}*STOP*^{F}*-Eyfp:Dsp*$^{W/F}$ lineage tracer mice (*Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$). In the triple transgenic mice, expression of the cre recombinase leads to deletion of the floxed exon 2 of the *Dsp* gene and of the STOP sequence upstream of the *Eyfp* gene, abolishing expression of DSP and inducing expression of EYFP specifically in PDGFRA positive cells.

Online Figure VIII. Detection of DSP levels and expression of EYFP in CMs isolated from the hearts of wild type and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice:

A-C. Detection of the reporter protein EYFP by immunofluorescence in isolated CMs from *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice after staining for EYFP and the CM specific marker ACTN2 (**A**). High magnification panels of selected representative areas are included (**B**). CMs isolated from wild type mice are included as negative controls. **C.** Quantitative data showing that EYFP was expressed in about 18% of the CMs from the lineage tracer mice (N=3, ~1000 cells counted per each experiment

in each group). **D-G.** Exclusion of fortuitous deletion of Dsp in CMs. qPCR (**D**) and Immunoblotting (**E-F**) confirming that mRNA and protein levels of Dsp are not significantly altered in CMs isolated from the lineage tracer mice as compared to control CMs. Lower immunoblot panel in **E** confirms expression of low levels of EYFP in CM- protein extracts from the lineage tracer mice. Likewise, IF staining of CMs isolated from the lineage trace mice (**G**) showed that localization and levels of DSP are not affected in the $EYFP^{pos}$ CM in comparison to the $EYFP^{neg}$ CMs. All the experiments have been repeated in 3 independent CM isolations.

Online Figure IX. Detection of EYFP in other cardiac cell types in the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice:

A-B. Co-Immunofluorescence staining of thin myocardial sections from wild type (**A**) *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice (**B**) for the reporter protein EYFP and the cardiac cell type markers ACTN2, COL1A1, SM-MHCII and PECAM-1, respectively. As expected, only CFs but not SMCs or ECs expressed EYFP in the heart of the lineage tracer mice.

Online Figure X. Cardiac phenotype of the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ lineage tracer mice:

A. Ventricular/body weight ratio in wild type, *Pdgfra-Cre* and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice. Ventricular/body weight ratio was modestly increased in the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice as compared to the other 2 groups (N=22 wild type, N=4 *Pdgfra-Cre*, N=11 *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice; *$p< 0.05$ as compared with wild type and *Pdgfra-Cre*). **B.** Picro-Sirius Red stained thin myocardial sections showing increased interstitial fibrosis in the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mouse hearts, as compared to wild type and *Pdgfra-Cre* deleter mice. Quantitative data are shown in panel **E** (N=5 wild type and *Pdgfra-Cre*, N=7 *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice; *$p< 0.05$ as compared with wild type and *Pdgfra-Cre*). **C.** ORO stained thin myocardial sections showing increased number of adipocytes in the heart of the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$

mice. Quantitative data are shown in panel **F** (N=5 wild type, N=4 *Pdgfra-Cre*, N=11 *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice; *p< 0.05 as compared with wild type and *Pdgfra-Cre*). **D.** Immunofluorescence staining of thin myocardial sections showing increased number of cells expressing the adipogenic transcription factor CEBPA in the hearts of *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice as compared to the control groups. Quantitative data are shown in panel **G** (N=5 wild type, N=4 *Pdgfra-Cre*, N=4 *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice; *p< 0.05 as compared with wild type and *Pdgfra-Cre*).

Online Figure XI. Localization of fibroadiposis in the heart of *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mouse hearts:, Low magnification pictures of thin myocardial sections from wild type, *Pdgfra-Cre* and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice after staining with Sirius Red (SR) for detection of fibrosis and with ORO and an anti CEBPA specific antibody for detection of adipocytes. The lineage tracer mice showed patchy areas of fibrosis, while adipocytes were scattered in the myocardial wall of both left and right ventricles.

Online Figure XII. Detection of TGFB1 expression in the myocardium and in isolated FAPs:

A-C. Detection of TGFB1 expression in isolated cardiac FAPs (**A**) and in thin myocardial sections from wild type and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice by immunohistochemistry. High magnification panels of selected representative myocardial areas are included (**C**).TGFB1 expression levels were increased in isolated FAPs and myocardial sections from the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice.

Online Figure XIII. Detection of apoptosis in the myocardium of the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice :

A. TUNEL staining to detect apoptotic cells in thin myocardial sections from wild type and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice. **B**. Quantitative data showing that the number of cells stained positive in the

TUNEL assay was not significantly different between the wild type and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice (N=3 mice per group, ~1200 nuclei analyzed per each mouse).

Online Figure XIV. Assessment of adipocyte proliferation in the myocardium of the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice :

A. Immunofluorescence co-staining of thin myocardial sections from wild type and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice for the expression of CEBPA, to mark adipocytes, and Ki67 to mark proliferating cells. **B**. Quantitative data showing that percent of adipocytes that were stained positive for the proliferation marker did not differ significantly between the wild type and lineage tracer mice, indicating that number of adipocytes in the heart of the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice was not increased because of proliferation of existing adipocytes (N=3 mice per group, ~200 CEBPA^{pos} cells analyzed for ki67 expression per each mouse).

Online Figure XV. Increased number of perilipin expressing cells in cardiac FAPs isolated from *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice :

A-B. low (10X, **A**) and higher (63x, **B**) magnification pictures at of cardiac FAPs from wild type and *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ mice after immuofluorescence staining for perilipin, a marker of mature adipocytes after 4 days of adipogenesis induction. **C**. Quantitative data showing that the number of cells expressing perilipin was significantly increased in the *Pdgfra-Cre:Eyfp:Dsp*$^{W/F}$ as compared to wild type mice (N=3, ~200 cells counted per each group in each experiment).

Online Figure XVI. Cardiac phenotype of the *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ mice:

A. Increased ventricular/body weight ratio in *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ mice as compared with wild type and *Pdgfra-Egfp* mice (N=10 wild type, N=7 *Pdgfra-Egfp*, N=13 *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ mice;

*p< 0.05 as compared with wild type and *Pdgfra-Egfp* mice). **B.** Masson trichrome (MT) and ORO staining showing enhanced fibro-adipogenesis in the *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ mice as compared with the control groups. Quantitative data are shown in panels **C** and **D** (N=4-6 mice per each group; *p< 0.05 as compared with wild type and *Pdgfra-Egfp* mice).

Online Figure XVII. Exclusion of a paracrine mechanism for differentiation of FAPs to adipocytes:

A. Co-expression of the EGFP reporter protein and the adipogenic transcription factor CEBPA in thin myocardial sections obtained from wild type, *Pdgfra-Egfp* reporter, and *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ lineage tracer mice. High magnification inserts are also shown in **B. C-D.** Quantitative data. Panel **C** shows increased number of CEBPA^{pos} adipocytes in the heart of *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ mice as compared to the control groups. Quantitative data in panel **D** show that the percentage of adipocytes expressing EGFP in the control *Pdgfra-Egfp* and in the *Pdgfra-Egfp:Myh6-Cre:Dsp*$^{W/F}$ mice was not significantly different (N= 5 animals per group. Wild type animal have been included as a negative control for EGFP expression).

ONLINE SUPPLEMENTARY TABLE I

A. Antibodies

Antibody	Company	Catalog #	Working dilution
FITC Human Lin cocktail	BD Pharmingen	562722	20ml:10^6cells(FC)
V450 Mouse Lineage Antibody Cocktail, with Isotype Control	BD Pharmingen	561301	20ml:10^6cells(FC)
PE Mouse Anti-Human CD140a	BD Pharmingen	556002	1mg:10^6cells(FC)
APC Anti-Mouse CD140a (PDGFRA)	eBioscience	17-1401-81	1mg:10^6cells(FC)
APC Mouse Anti-Human CD90 (Thy1)	BD Pharmingen	561971	1mg:10^6cells(FC)
PE-Cy5 Anti-Mouse CD90.2 (Thy1.2)	eBioscience	15-0902	1mg:10^6cells(FC)
DDR2	Santa Cruz Biotechnology	sc-7555	1mg:10^6cells(FC), 1:200(IF)
PE Rat Anti-Mouse CD140a (PDGFRA)	BD Pharmingen	562776	1mg:10^6cells(FC)
APC Rat Anti-Mouse CD117 (cKit)	BD Pharmingen	553356	1mg:10^6cells(FC)
AF488 Rat Anti-Mouse CD146	BD Pharmingen	562229	1mg:10^6cells(FC); 5mg/ml (IF)
PE Rat Anti-Mouse CD140b (PDGFRB)	eBioscience	12-1402-81	1mg:10^6cells(FC); 5mg/ml (IF)
Tie2	Abcam	24859	1:500(IF)1mg:10^6cells(FC)
PDGFRA	R&D Systems	AF1062	1:100(IF)
PDGFRA	R&D Systems	AF-307	1:100(IF)
PDGFRA	R&D Systems	AF1062	1:100(IF)
Rat anti mouse CD90.2 (Thy 1.2)	BD Pharmingen	550543	1:200(IF)
Rabbit anti human CD90	Abbiotec	251285	1:200 (IF)
DSP1/2	BioRad	2722-5204	1:1000 (IB) 1:100 (IF)
DSP1/2	Progen	65146	1:1000(IB) 1:1000(IF)
JUP	Invitrogen	13-8500	1:500(IF) 1:1000(IB)
PKP2	Progen	651101	1:300 (IF)
DSG2	Progen	61002	1:50(IF) 1:100(IB)
PECAM1	BD Pharmingen	550274	1:500(IF)

SM-MHCII	Biomedical Technologies	BT-562	1:500(IF)
COL1A1	Abcam	ab 88147	1:300(IF)
COL1A1	Abcam	ab 21286	1:500(IF)
COL1A1	Abcam	ab 138492	1:1000(IF)
ACTN2	Sigma	A7811	1:1000(IF)
MYH6	Developmental Studies Hybridoma		1:800(IB)
MYBPC3	Santa Cruz	sc-166403	1:300(IB)
GFP	Abcam	ab-6673	1:2000(IF)
GFP	Abcam	ab-290	1:1000(IB)
α-Tubulin	Cell Signaling	2125	1:1000 (IB)
PPARγ	Cell Signaling	2435	1:500(IF) 1:1000(IB)
CEBPA	Cell Signaling	8178	1:500(IF) 1:1000(IB)
FABP4	Cell Signaling	3544	1:1000(IB) 1:1000(IF)
Perilipin	Cell signaling	9349	1:200 (IF)
Ki67	eBioscience	14-5698-82	1:100 (IF)
TGF beta	R&D System	MAB1835	1: 500 (IHC/ICC)
Anti-Rabbit IgG, Alexa Fluor 594	Life technology	21207	1:1000(IF) 1mg/mL(FC)
Anti-Rabbit IgG, Alexa Fluor 488	Life technology	21206	1:1000(IF) 1mg/mL(FC)
Anti-Mouse IgG, Alexa Fluor 594	Life technology	21203	1:1000(IF)
Anti-Mouse IgG, Alexa Fluor 488	Life technology	21202	1:1000(IF)
Anti-goat IgG, Alexa Fluor 488	Life technology	11055	1:1000(IF) 1mg/mL(FC)
Anti-goat IgG, Alexa Fluor 594	Life technology	11058	1:1000(IF)
Anti-rat IgG, Alexa Fluor 594	Life technology	11007	1:1000(IF)
Anti-rat IgG, Alexa Fluor 488	Life technology	21208	1:1000(IF)
Anti-goat IgG, Alexa Fluor 790	Life technology	11370	1mg/mL(FC)
APC Rat IgG2a K Isotype Control	eBioscience	17-4321	1mg:10^6cells(FC)
PE Rat IgG2a, κ Isotype Control	BD Pharmingen	555844	1mg:10^6cells(FC)
APC Rat IgG2b, κ Isotype Control	BD Pharmingen	553991	1mg:10^6cells(FC)
AF488 Rat IgG2a, κ Isotype Control	BD Pharmingen	557676	1mg:10^6cells(FC)

PE Rat IgG2a K Isotype Control	eBioscience	12-4321	1mg:10^6cells(FC)
PE-Cy5 Rat IgG2a K Isotype Control	eBioscience	15-4321	1mg:10^6cells(FC)
Anti-Rabbit IgG, HRP linked	Cell Signaling	7074	1:3000(IB)
Anti-Mouse IgG, HRP linked	Cell Signaling	7076	1:4000(IB)

B. Taqman gene expression probes (from life technology)

Pdgfra Mm004340701_m1

CyclinD1 Mm00432359_m1

Ctgf Mm01192932_g1

Serpine1 Mm00435860_m1

Ankrd1 Mm00496512_m1

Inhba Mm00434339_m1

Jag1 Mm00496902_m1

Hes1 Mm01342805_m1

Fabp4 Mm00445878_m1

Cebpa Mm00514283_s1

Pparg Mm01184322_m1

Dgat1 Mm00515643_m1

Dgat2 Mm00499536_m1

Gapdh Mm99999915_g1

Dsp Mm01351876_m1

Tgfb Mm01178820_m1

C. PCR oligonucleotide primers for mouse genotyping:

Pdgfra:Egfp:

Wild type Forward: CCC TTG TGG TCA TGC CAA AC

Wild type Reverse: GCT TTT GCC TCC ATT ACA CTG G

Mutant Reverse: ACG AAG TTA TTA GGT CCC TCG AC

Floxed Dsp:

Forward: TAAGCTCCCCTCACTTCTCCAG

Reverse: TTCTCTTTGTCTGTTGCCATGT

R26-FSTOPF-Eyfp:

Rosa26R-1 AAAGTCGCTCTGAGTTGTTAT

Rosa26R-2 GCGAAGAGTTTGTCCTCAACC

Rosa26R-3 GGAGCGGGAGAAATGGATATG

Pdgfra-Cre:

Forward: GCG GTC TGG CAG TAA AAA CTA TC

Reverse: GTG AAA CAG CAT TGC TGT CAC TT

Internal Positive Control Forward: CTA GGC CAC AGA ATT GAA AGA TCT

Internal Positive Control Reverse: GTA GGT GGA AAT TCT AGC ATC ATC C

Myh6-Cre:

Forward: CCACACCAGAAATGACAGACAGA

Reverse: CGCATAACCAGTGAAACAGCAT

ONLINE TABLE II

Echocardiographic findings in WT, *Pdgfra-Cre* and *Pdgfra-Cre: Eyfp: Dsp*$^{W/F}$ mice

Abbreviations: HR, heart rate; bpm, beats per minutes; IVST, interventricular septal thickness; PWT, posterior wall thickness; LVEDD, left ventricular end diastolic diameter; LVEDDi, LVEDD divided for the body weight; LVESD, left ventricular end systolic diameter; FS, fractional shortening; LVMass, left ventricular mass; LVMi, LVMass divided for the body weight.

	Wild type	*Pdgfra-Cre*	*Pdgfra-Cre: Eyfp: Dsp*$^{W/F}$	**p**
N	21	15	23	
Male/Female	12/9	8/7	13/10	0.972
Age (months)	9.7±2.5	9.9±3.1	9.2±2.6	0.703
Body weight (g)	31.3±3.6	33.0± 5.0	33.5± 5.7	0.299
HR (bpm)	563 ± 71	589 ±65	562 ±81	0.493
IVST (mm)	0.97 ±0.09	0.94± 0.10	0.73± 0.09*	<0.001
PWT (mm)	0.99± 0.10	0.91± 0.14	0.73 ±0.11*	<0.001
LVEDD (mm)	2.82±0.25	3.17±0.14#	3.81± 0.31*	<0.001
LVEDDi (mm/g)	0.09±0.01	0.10±0.01	0.12± 0.02*	<0.001
LVESD (mm)	0.99±0.21	1.07± 0.14	1.78± 0.47 *	<0.001
FS (%)	65±6	66±5	54±10*	<0.001
LVM (mg)	86±22	96±17	92±20	0.331
LVMi (mg/g)	2.8±0.7	2.9±0.5	2.8±0.6	0.716

* p≤0.01 vs WT and vs Pdgfra-Cre, # p ≤ 0.01 vs WT by Bonferroni pairwise comparison

ONLINE TABLE III

Echocardiographic findings in the wild type, *Pdgfra-Egfp, Myh6-Cre:Dsp$^{W/F}$*, and *Pdgfra-Egfp:Myh6-Cre:Dsp$^{W/F}$* mice

	Wild type	*Pdgfra-Egfp*	*Myh6-Cre:Dsp$^{W/F}$*	*Pdgfra-Egfp: Myh6-Cre:Dsp$^{W/F}$*	**p**
N	9	7	6	9	
Male/Female	5/4	3/4	3/3	5/4	0.954
Age (months)	8.3±1.2	8.4±0.3	8.4±0.4	8.3±0.3	0.997
Body weight (g)	31.8±3.7	37.4± 8.2	34.0± 4.5	34.5± 6.7	0.318
HR (bpm)	546± 73	563±56	567 ±41	599 ±56	0.309
IVST (mm)	0.99 ±0.05	0.96± 0.08	0.78± 0.09*	0.71± 0.07*	<0.001
PWT (mm)	1.00± 0.09	0.94± 0.01	0.71 ±0.04*	0.74±0.08*	<0.001
LVEDD (mm)	2.8±0.1	3.2±0.2	3.7± 0.3*	3.9± 0.4*	<0.001
LVEDDi (mm/g)	0.09±0.01	0.08±0.01	0.11± 0.01*	0.11± 0.02*	0.006
LVESD (mm)	1.0±0.2	1.2±0.2	2.1±0.6*	2.1± 0.6*	<0.001
FS (%)	63±5	63±6	44±10*	47±11*	<0.001
LVM (mg)	91.8±8.4	98.9±15.6	91.2±18.2	96.3±16.5	0.729
LVMi (mg/g)	3.0±0.5	2.7±0.7	2.7±0.4	2.8±0.4	0.725

Abbreviations: HR, heart rate; bpm, beats per minutes; IVST, interventricular septal thickness; PWT, posterior wall thickness; LVEDD, left ventricular end diastolic diameter; LVEDDi, LVEDD divided for the body weight; LVESD, left ventricular end systolic diameter; FS, fractional shortening; LVMass, left ventricular mass; LVMi, LVMass divided for the body weight.

* $p \leq 0.05$ vs WT and vs Pdgfra-Egfp, by Bonferroni pairwise comparison.

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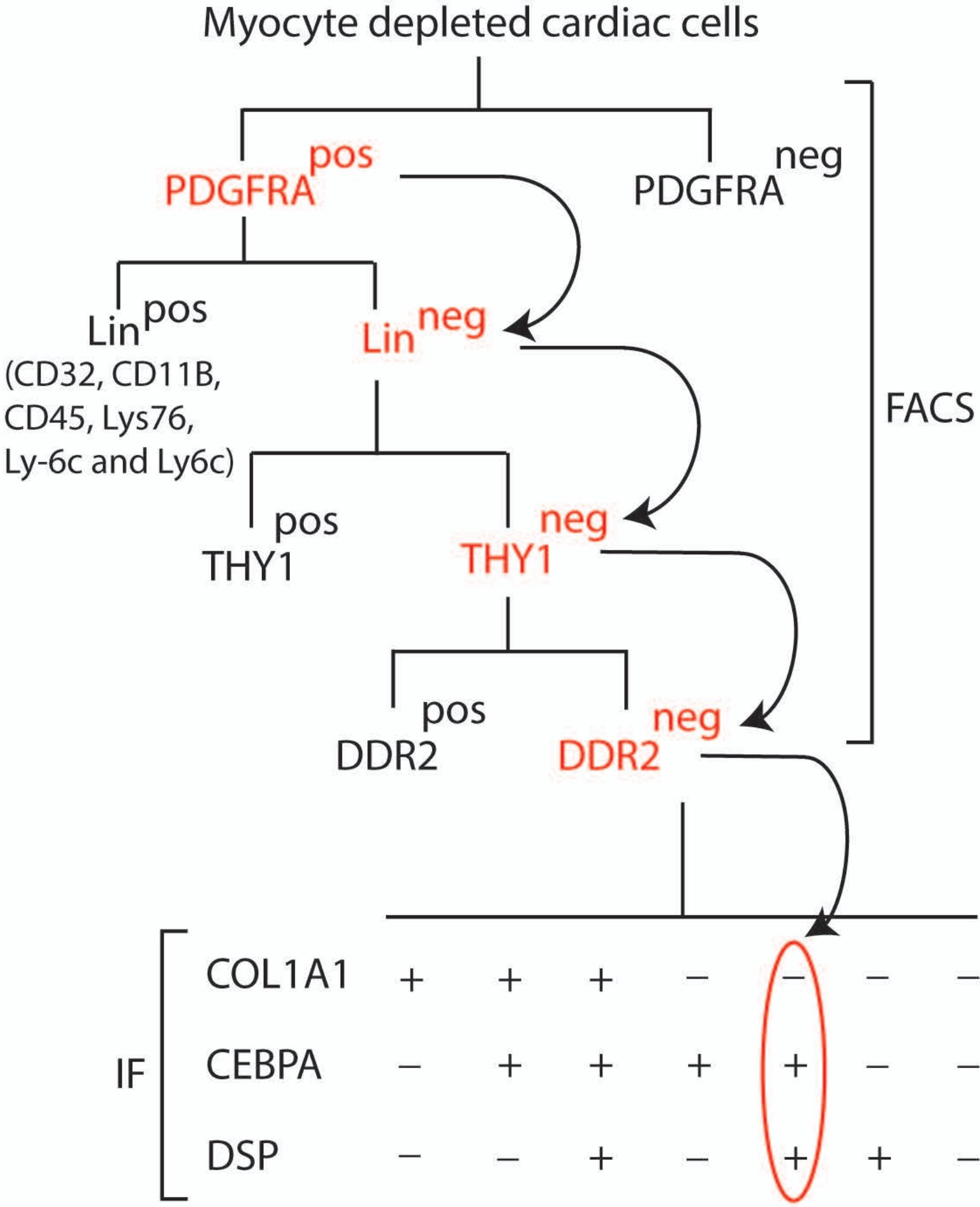

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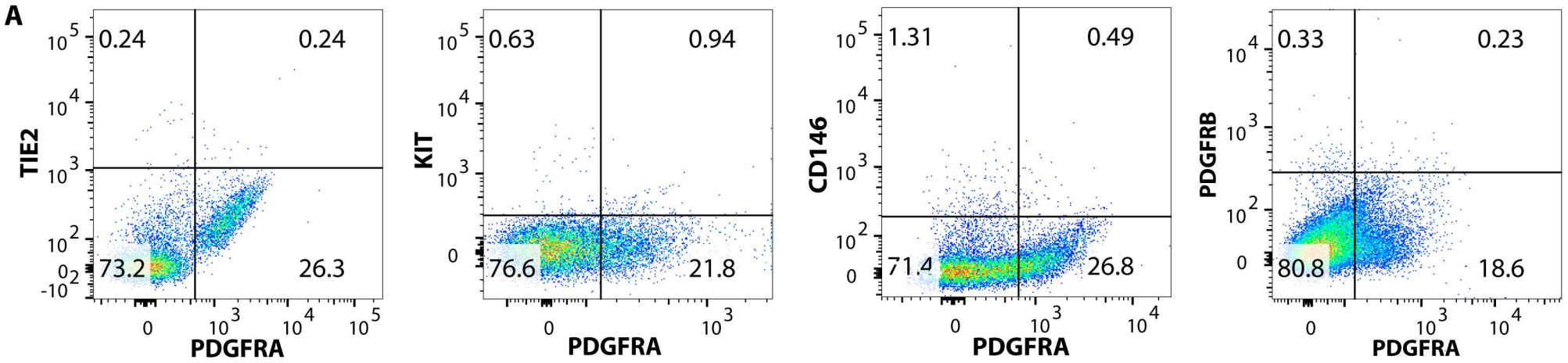

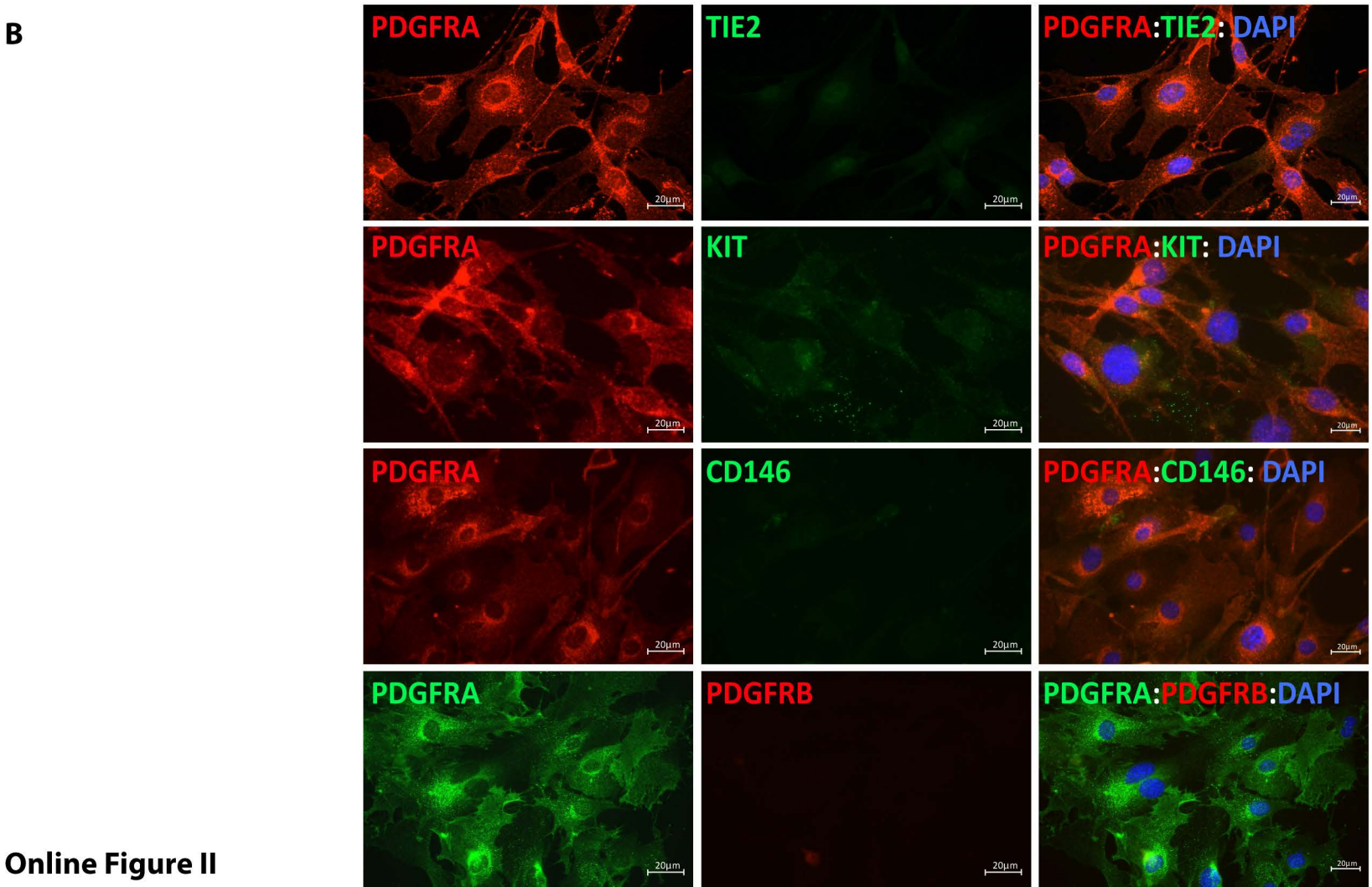

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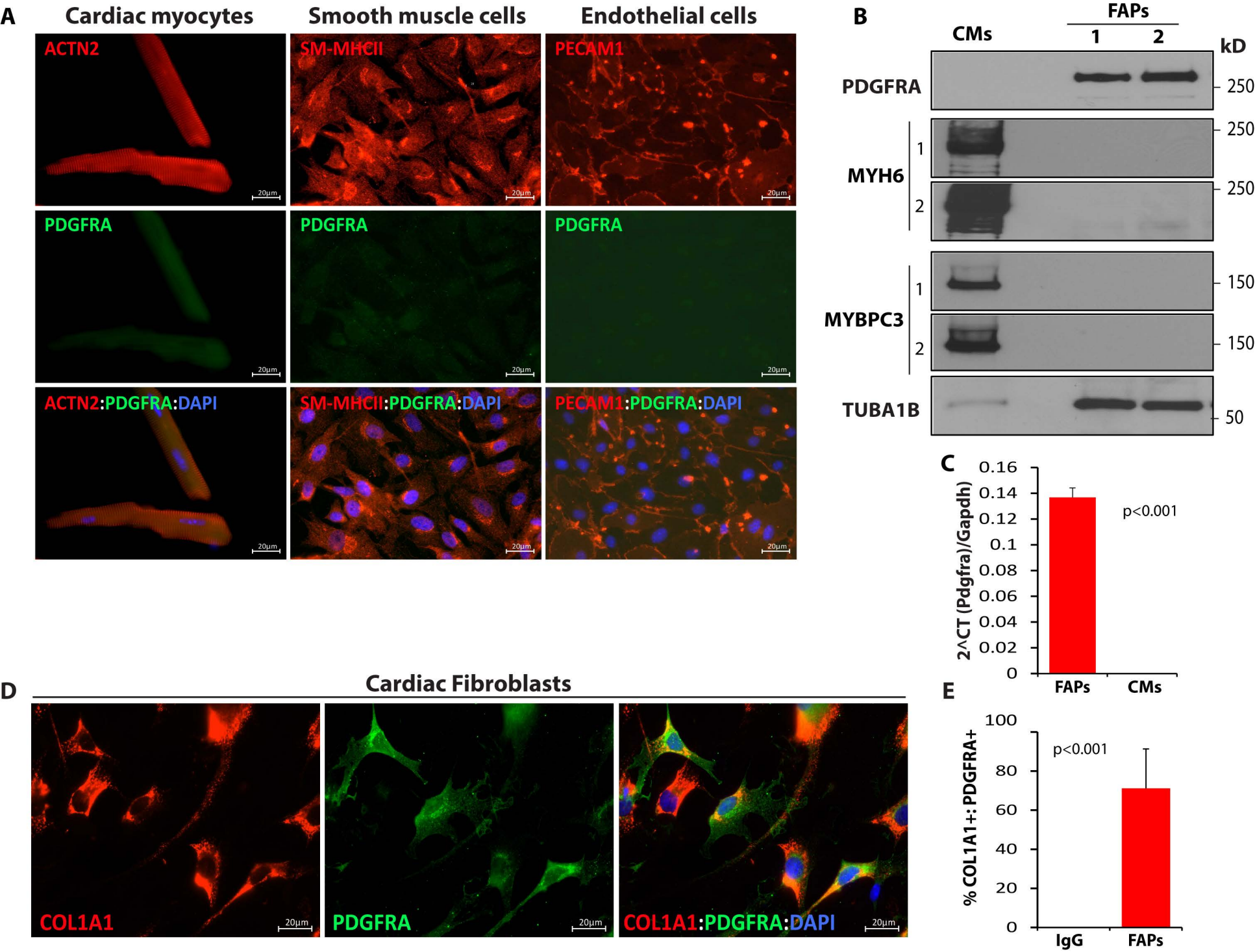

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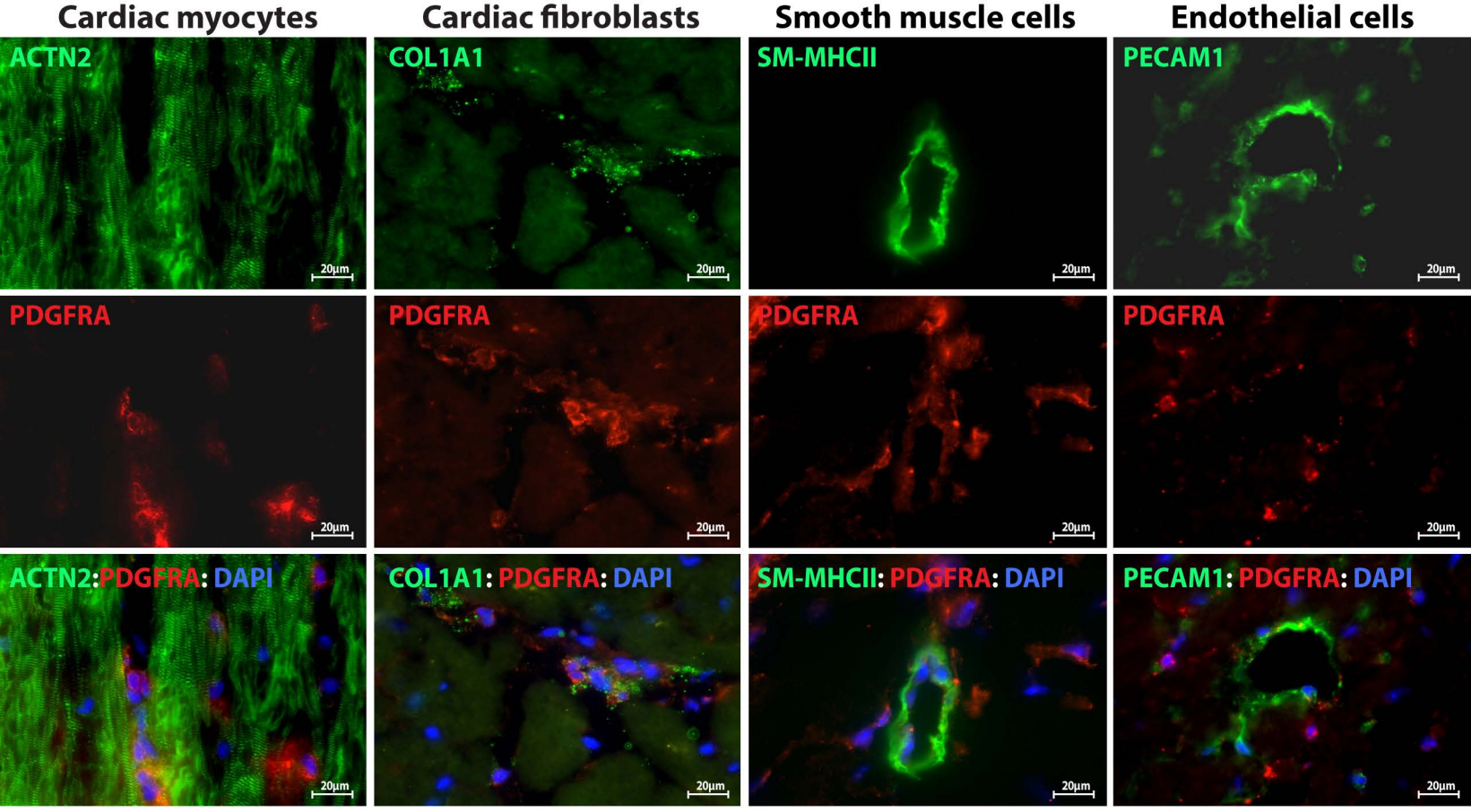

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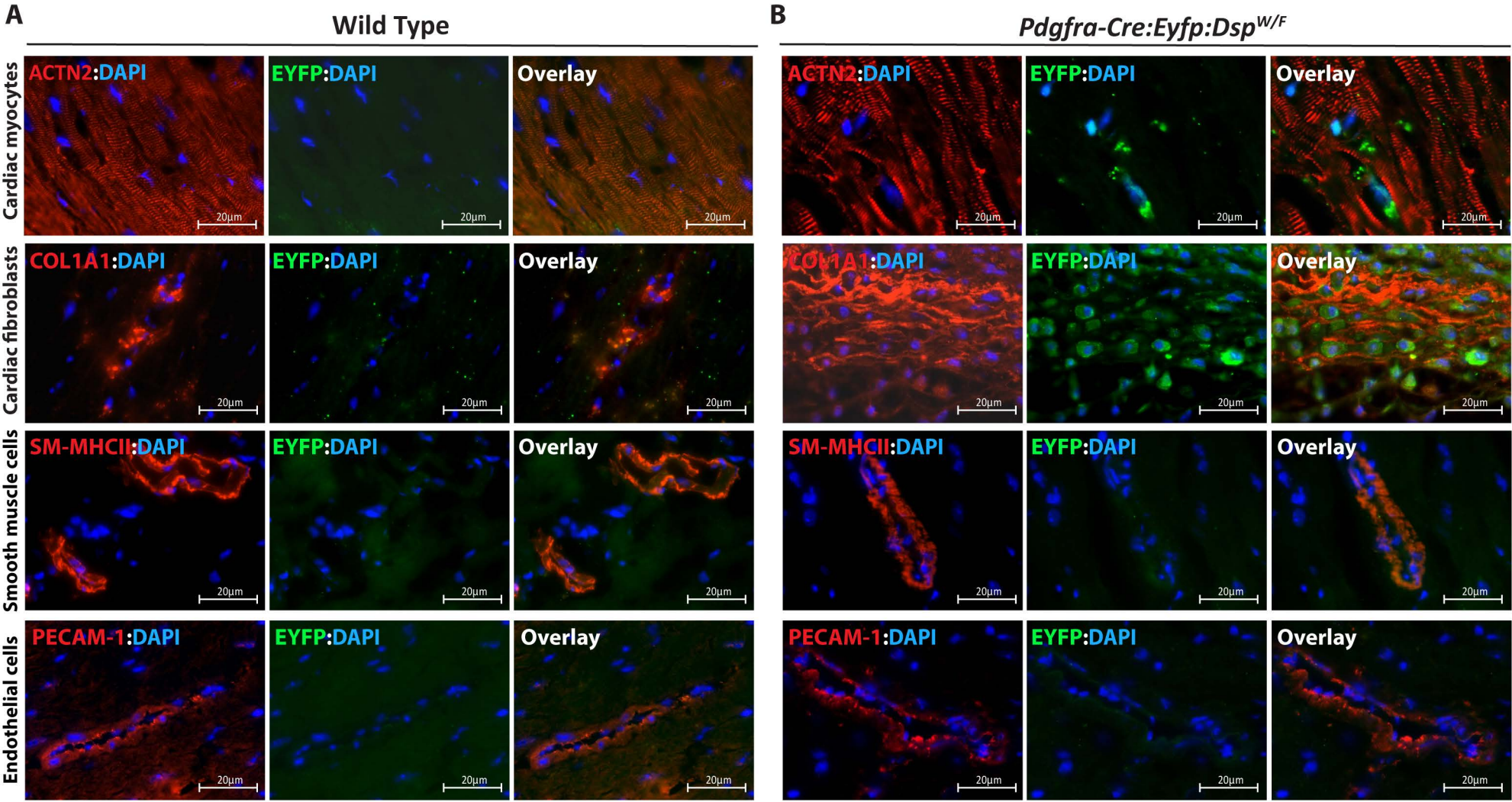

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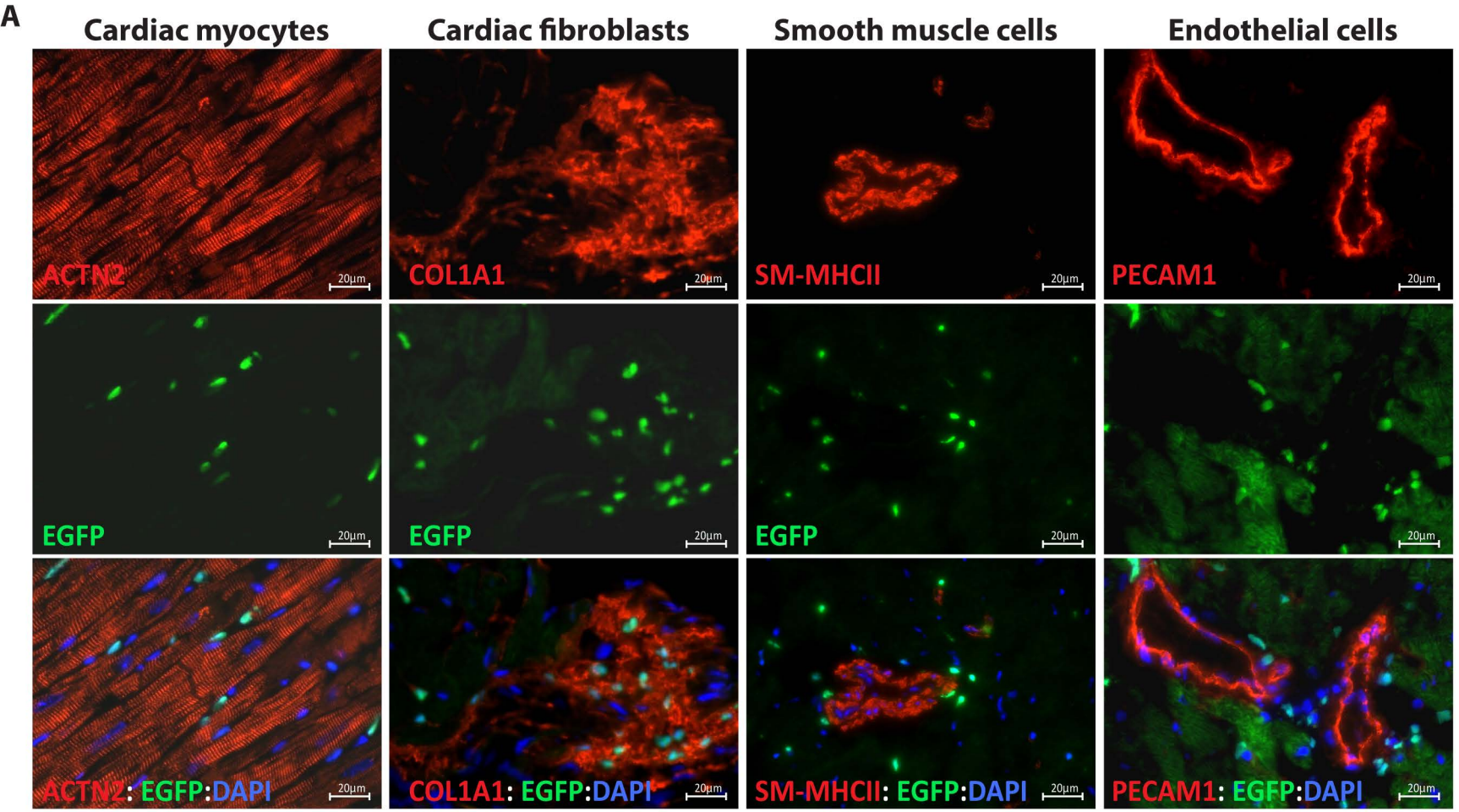

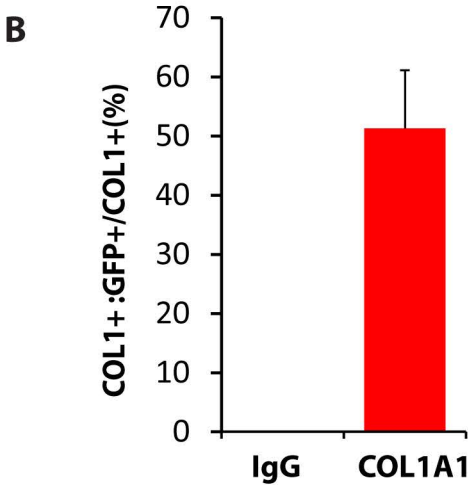

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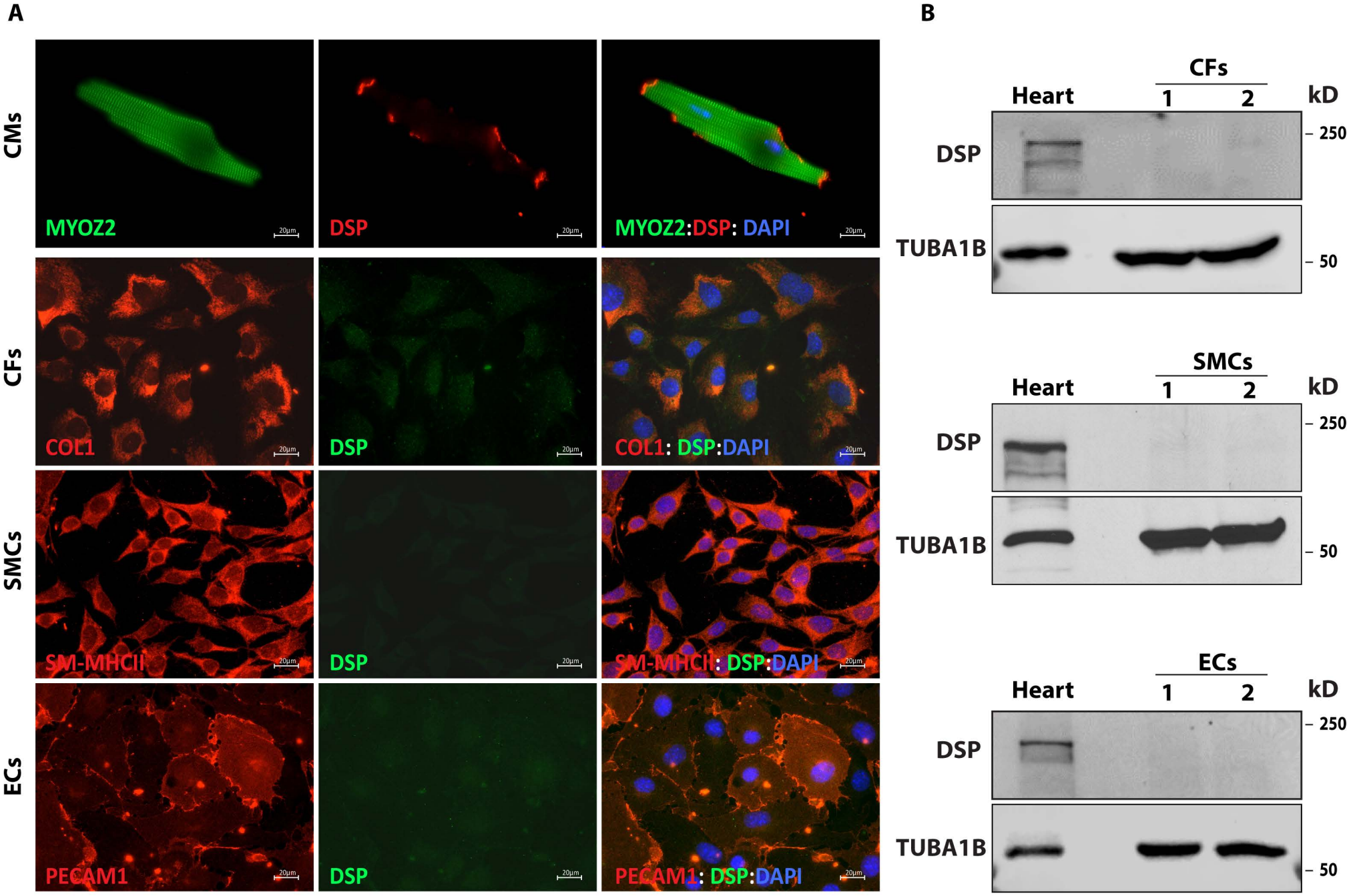

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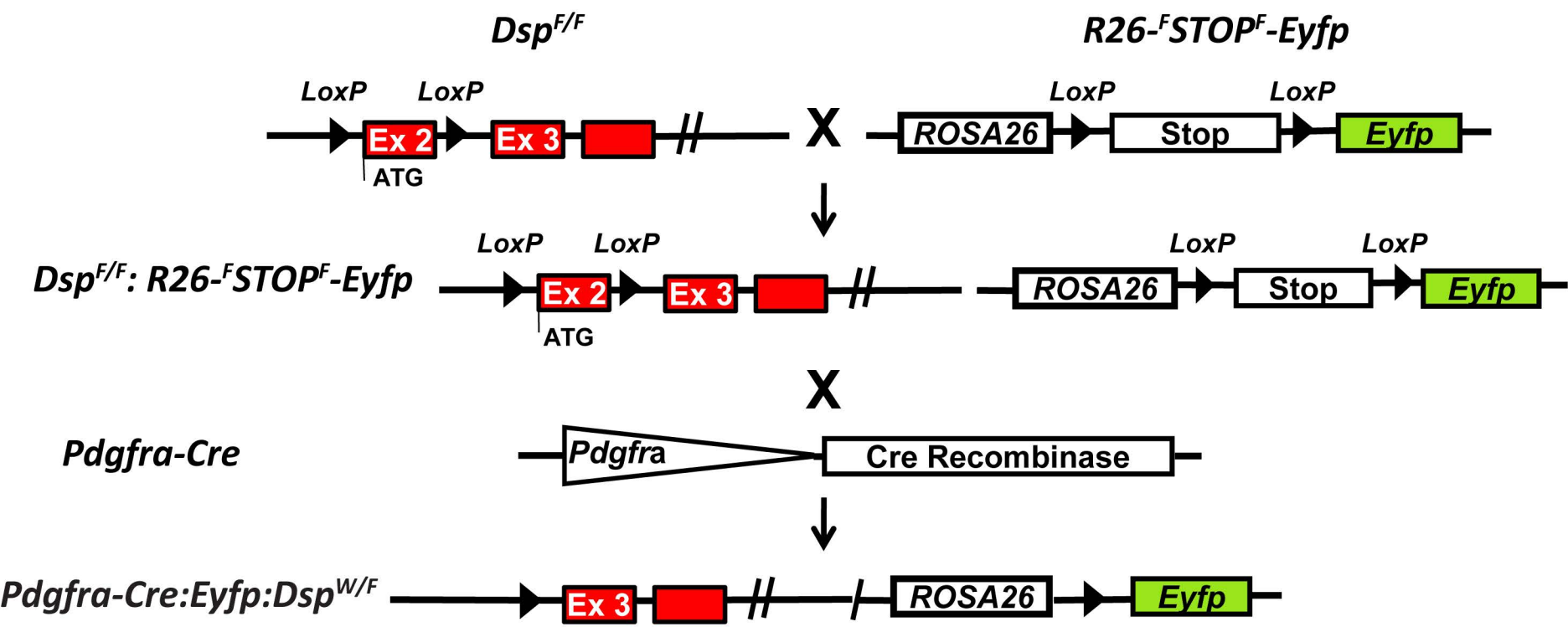

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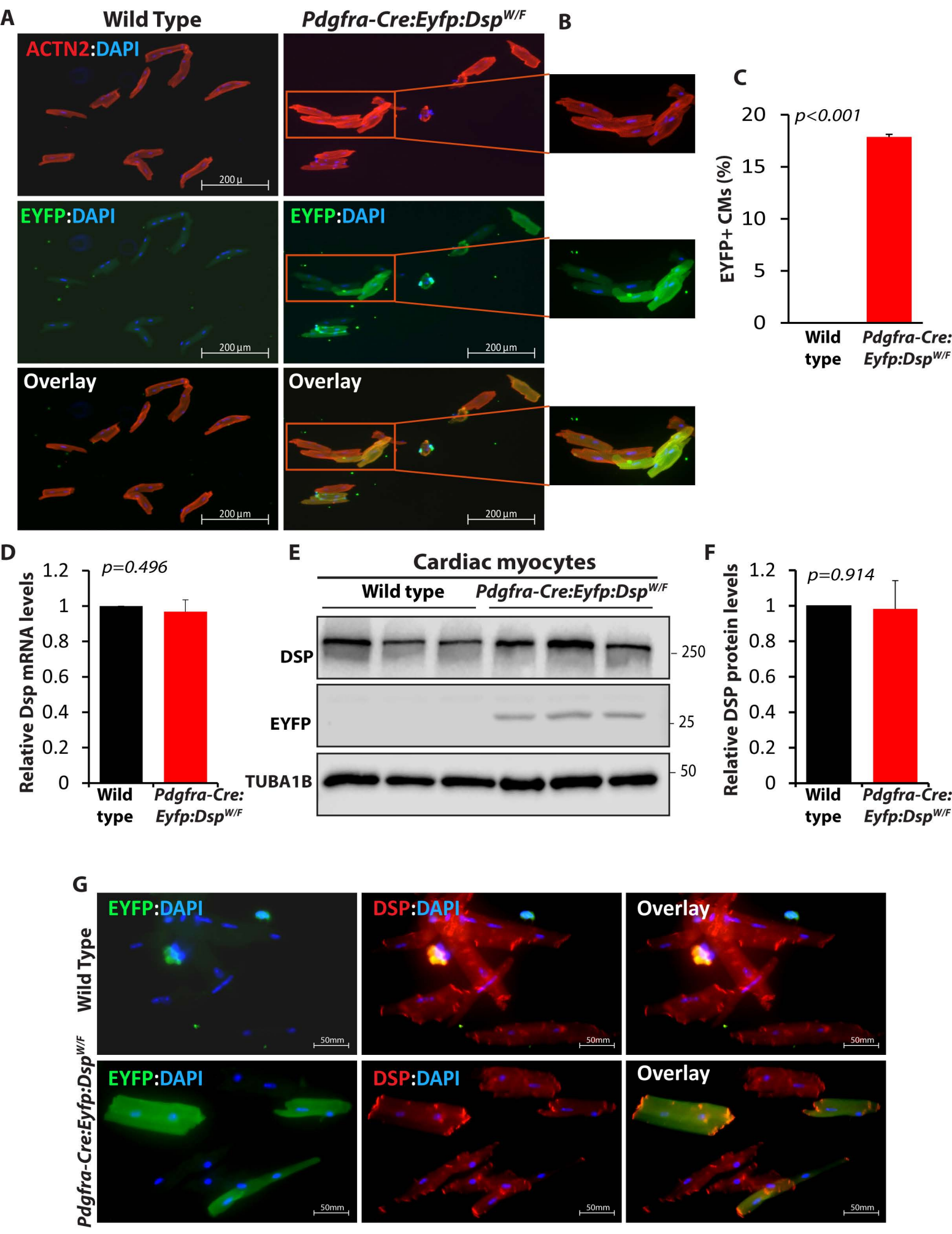

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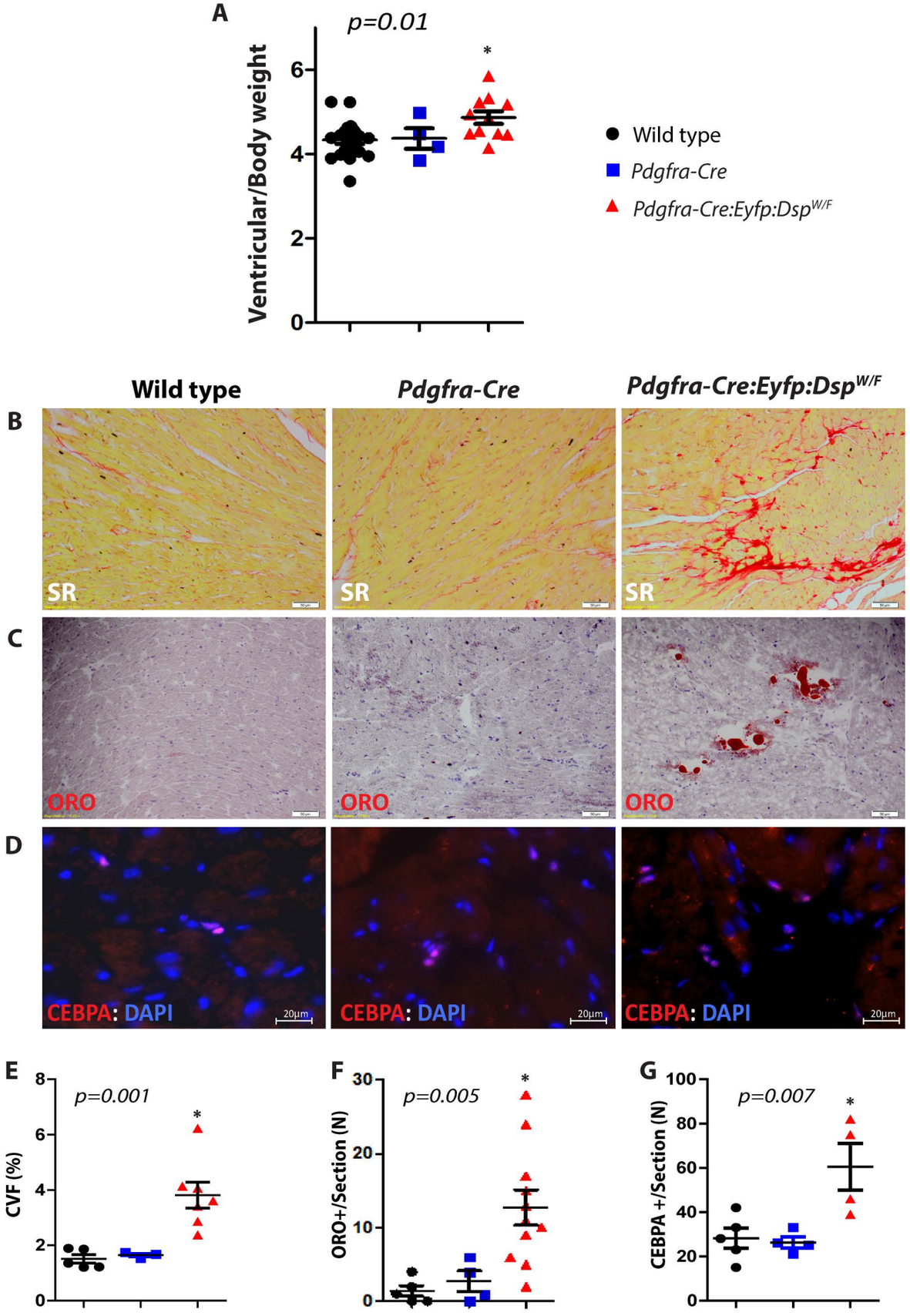

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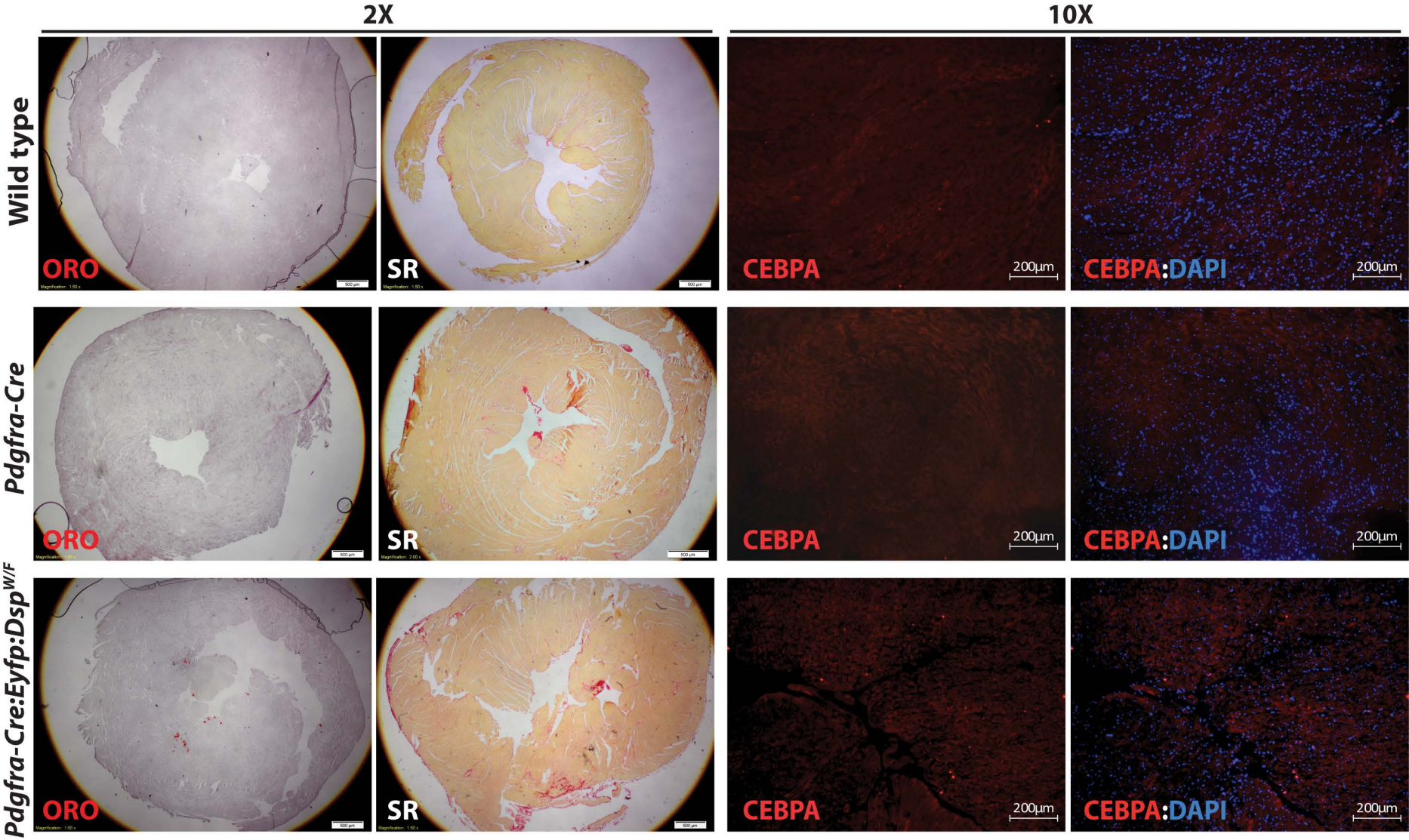

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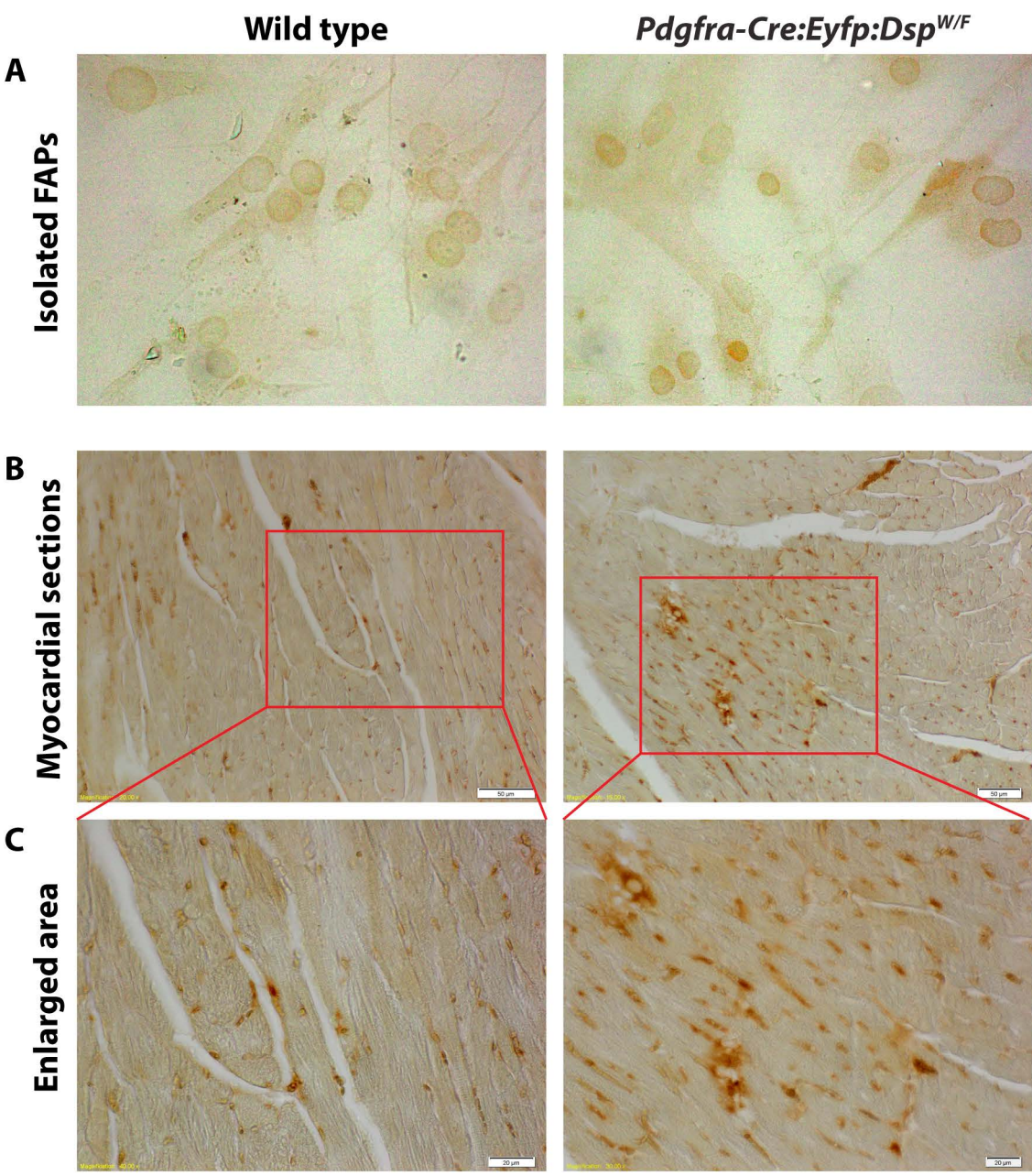
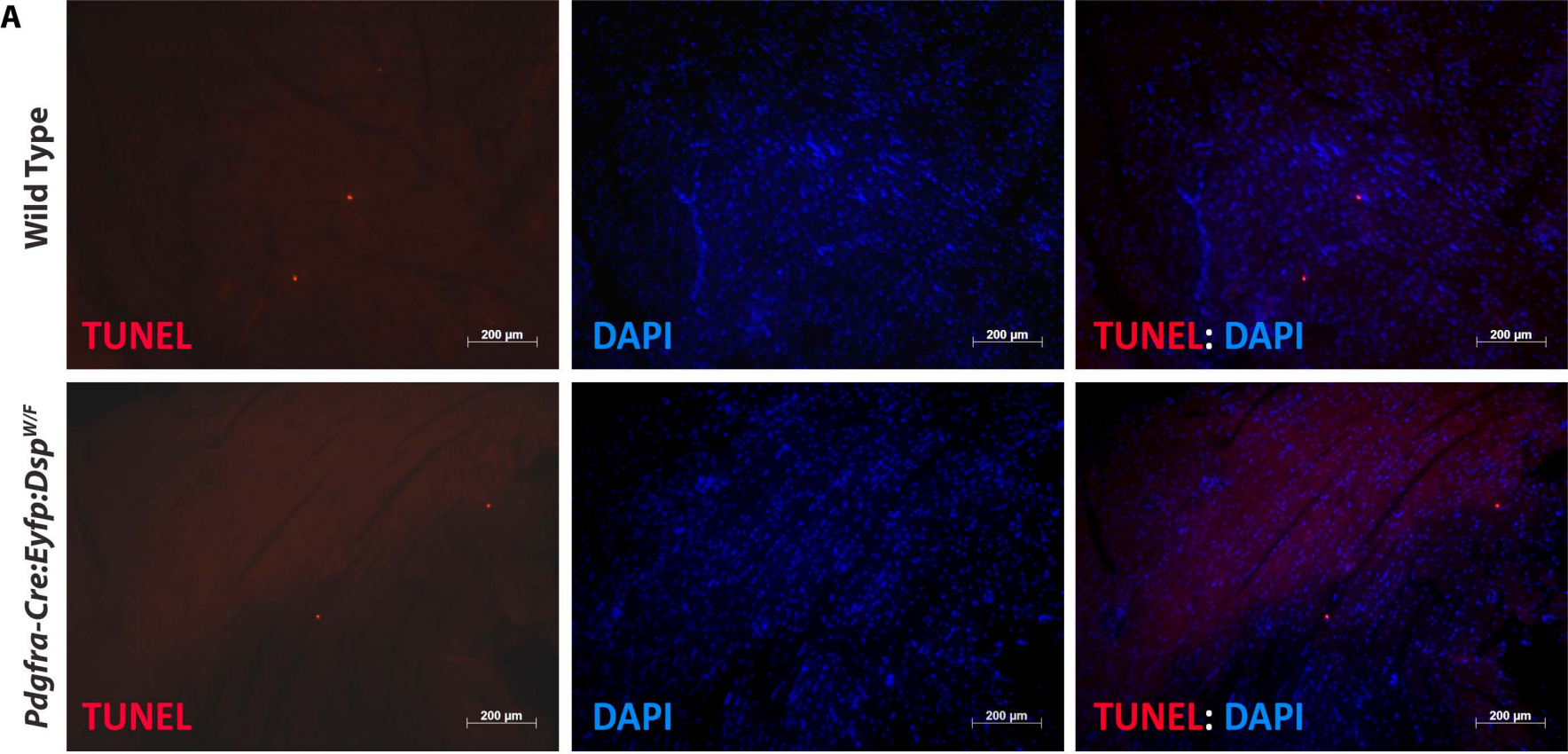
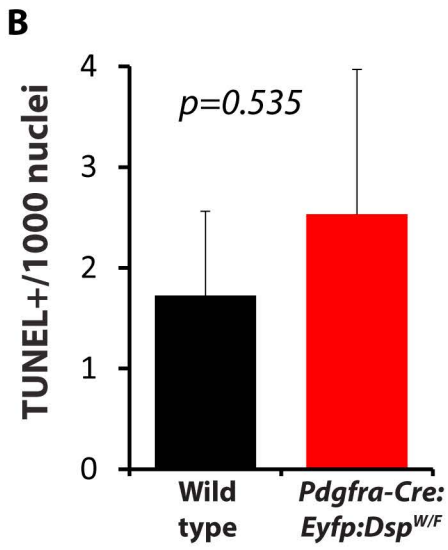
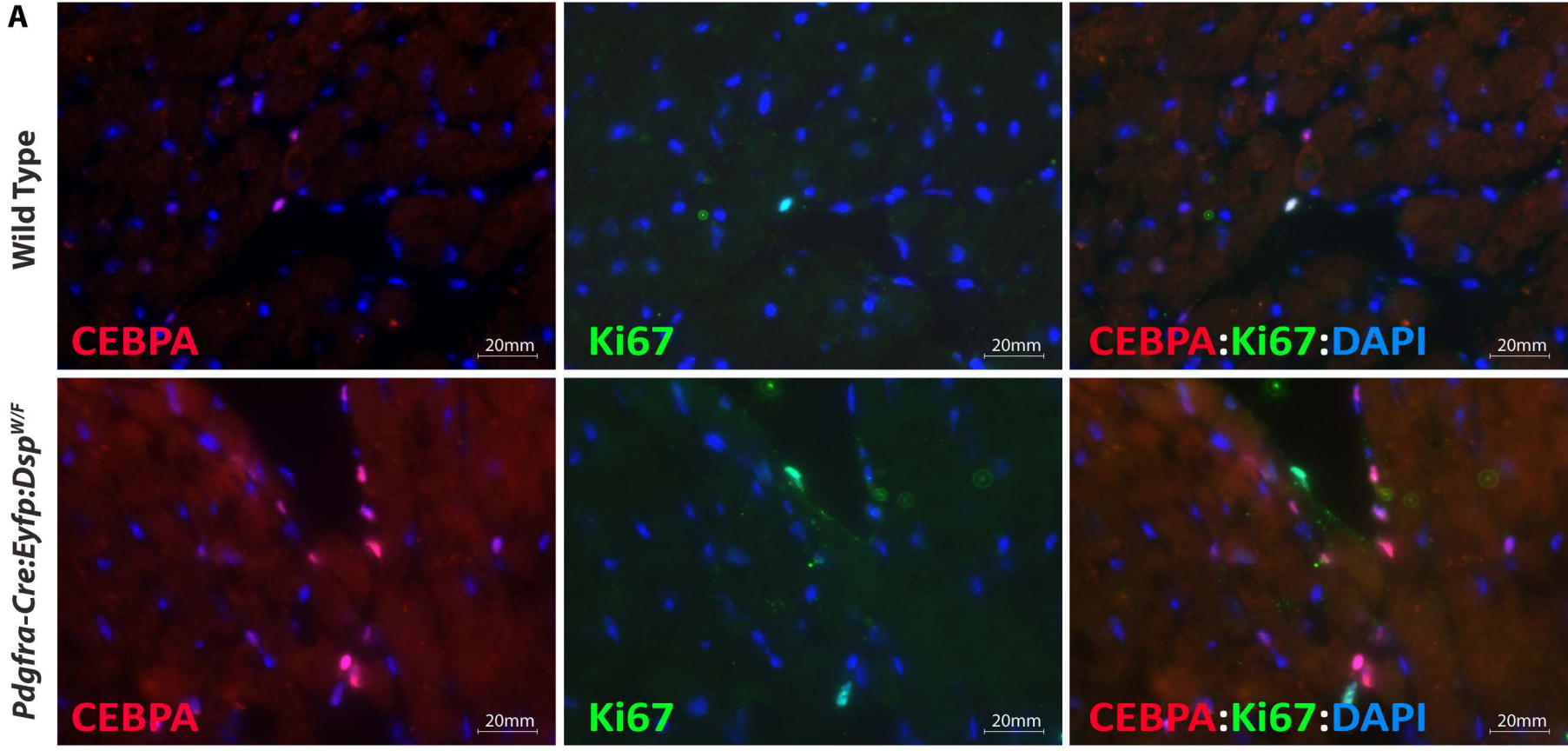


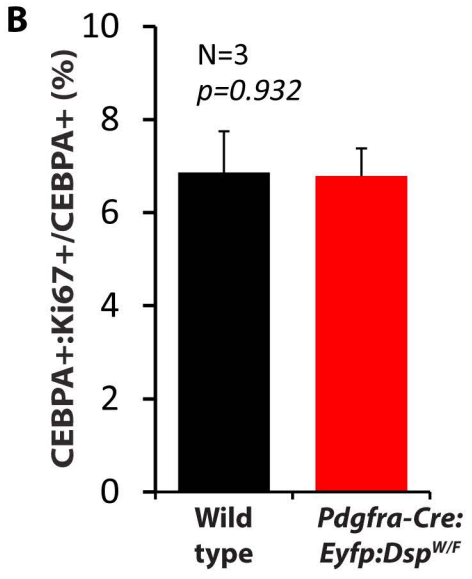
Online Figure XII



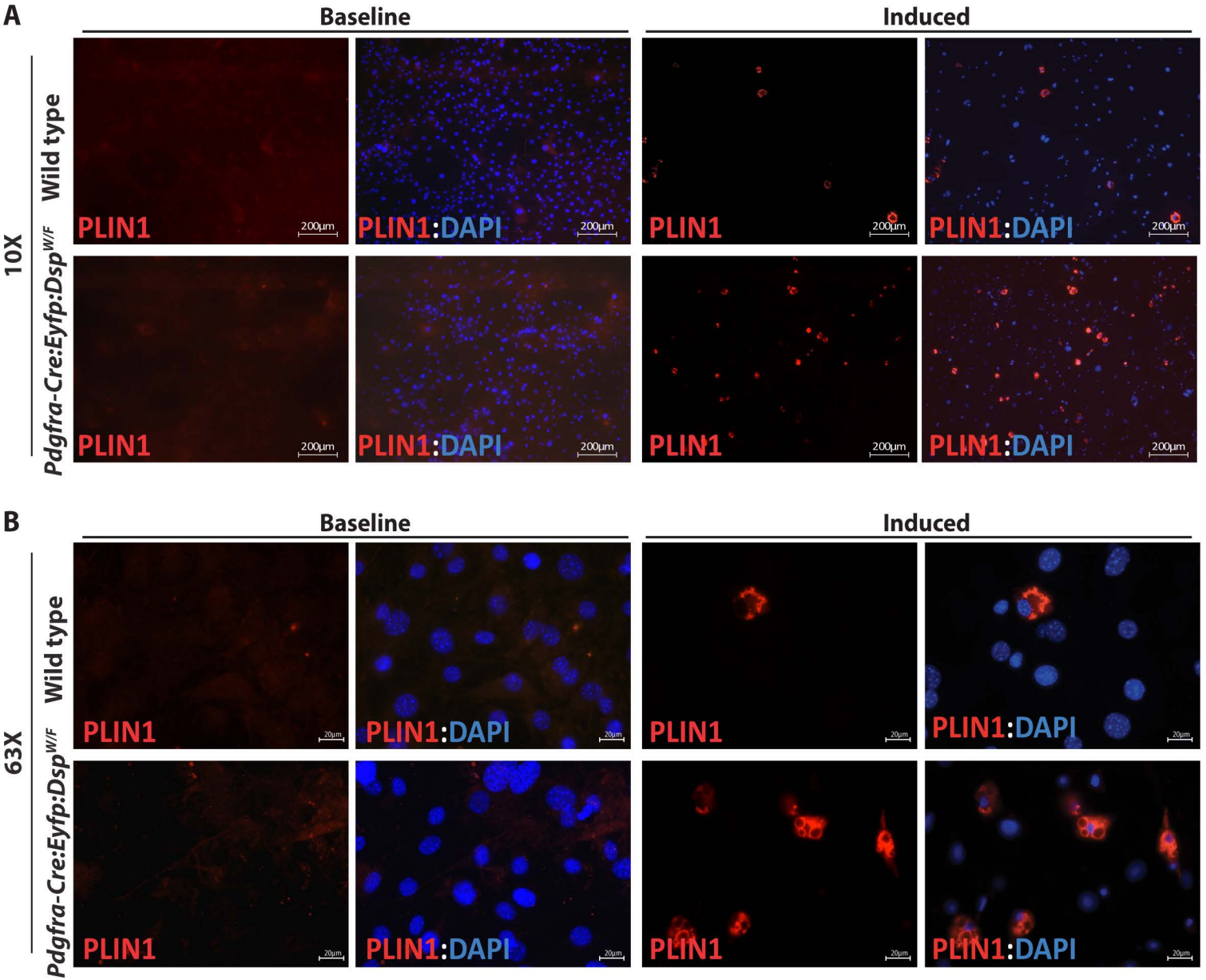


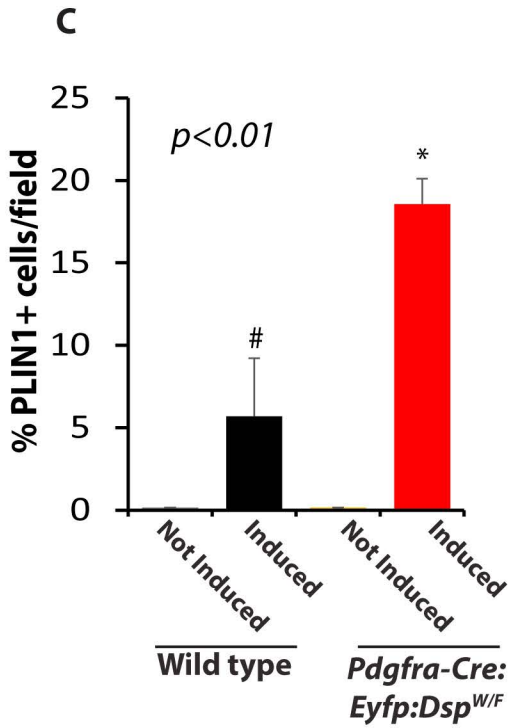
Online Figure XIII



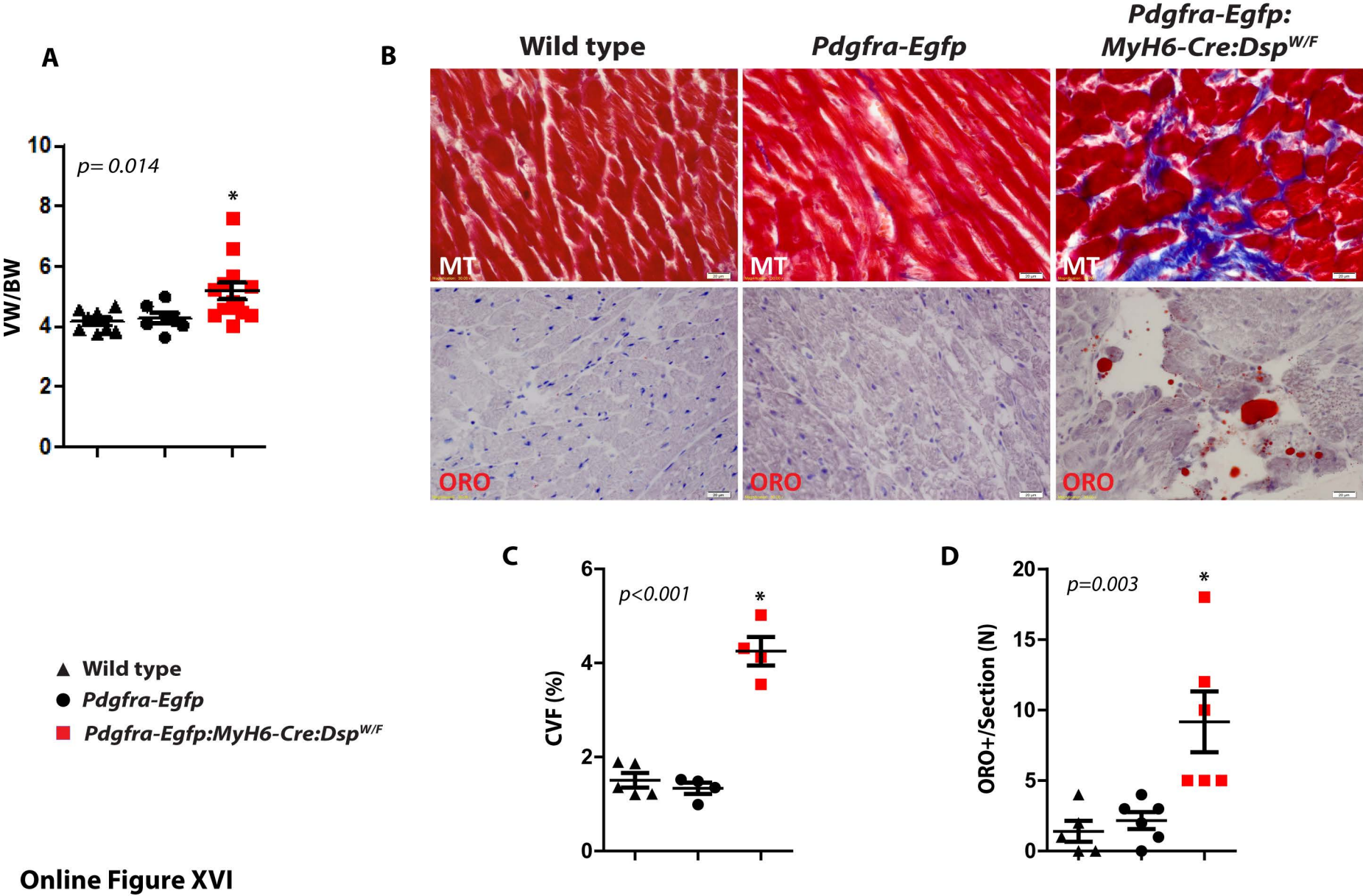


Online Figure XIV

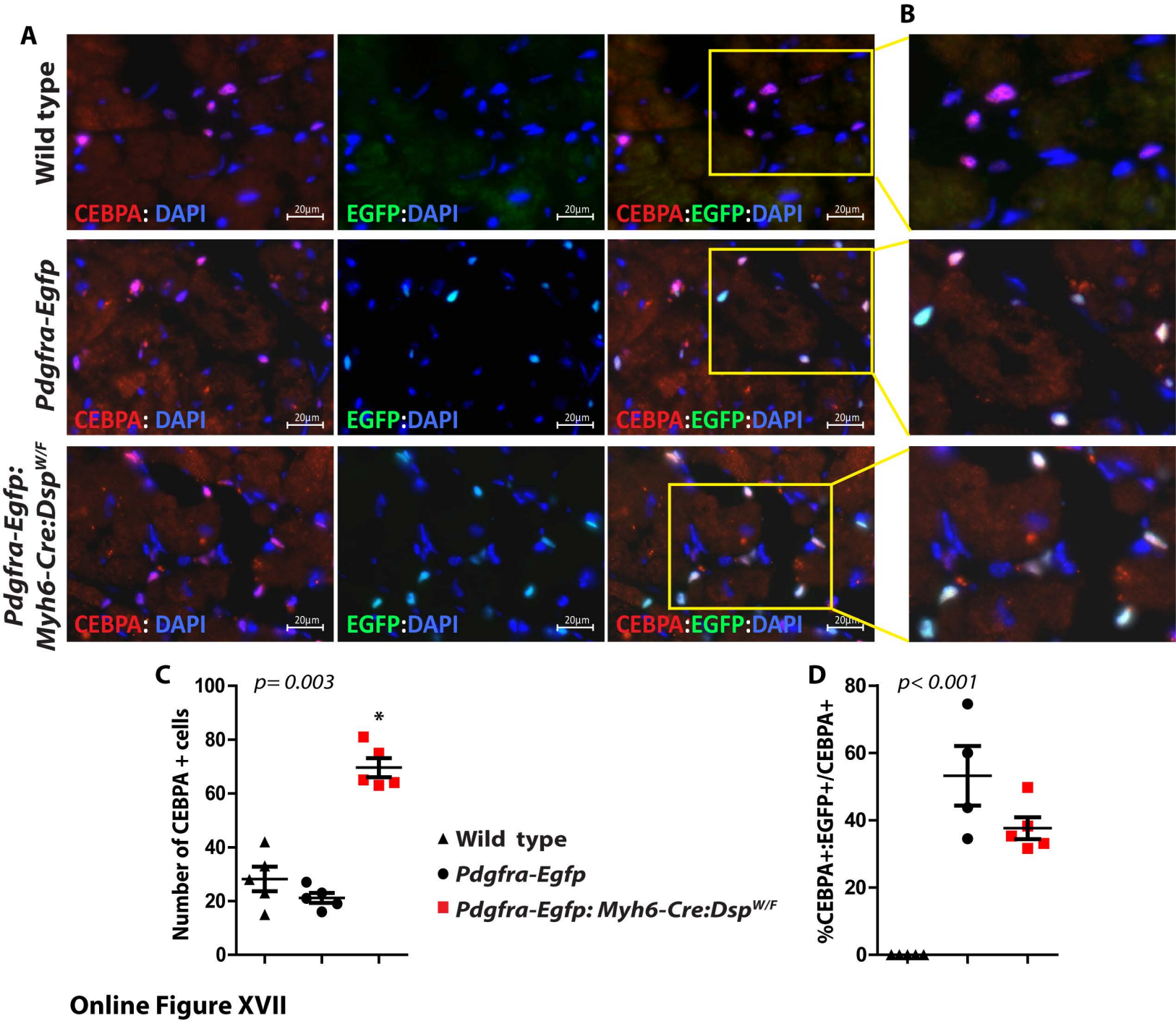




Online Figure XV



Online Figure XVI



Online Figure XVII