SUPPLEMENTAL MATERIALS

Mature vascular smooth muscle cells, but not endothelial cells, serve as the major cellular source of intimal hyperplasia in vein grafts

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Running title: The cellular source of vein graft hyperplasia

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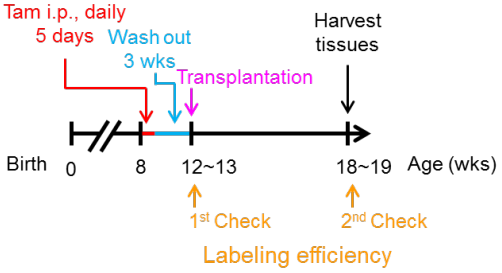
ONLINE SUPPLEMENTAL MATERIALS

I. Full Description of Methods

Reagents
All reagents were purchased from Sigma-Aldrich, USA unless specified. The antibodies used are listed in **Online Table I and Table II**.

Animals
Wild type (WT) C57BL/6J and Rosa26$^{Floxed\text{-}Stop}$eYFP mice were purchased from the Jackson Laboratory. *SM22α* knockin (Ki)-CreER^{T2} mice were kindly provided by Dr. Robert Feil at Technische Universität München, Germany. *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice, *Cdh5*-Cre CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice and *SM22α* (Ki)-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice were generated as previously reported.[1-3] Since *myh11*-CreER^{T2} transgene is integrated on the Y chromosome, only male could carry the *myh11*-CreER^{T2} transgene. Due to the technique limitation, only male mice were used in this study. All animals were housed at the AAALAC-accredited animal facility of University of South Carolina School of Medicine. All animals were treated in compliance with the USA National Institute of Health Guideline for Care and Use of Laboratory Animals. The use of animals and all animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at University of South Carolina.

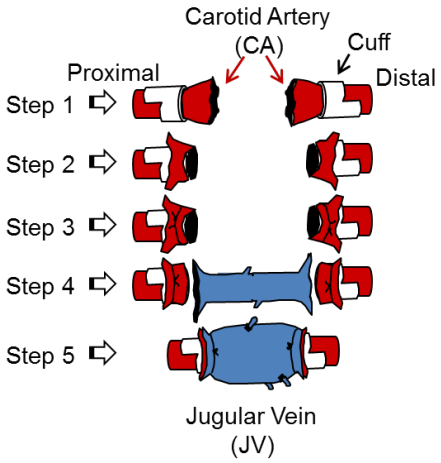
Induction and Quantification of Cre-loxP Recombination by Tamoxifen
Tamoxifen (Cat#: T5648, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in warm sunflower seed oil at a concentration of 10 mg/ml and injected intraperitoneally (i.p.) into the reporter mice, i.e., *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice, *Cdh5*-Cre CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice and *SM22α* knockin (Ki)-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice, at age of 8 wks at 1 mg/day/per mouse for 5 consecutive days as previously reported.[4] After at least 20 days of washout time period, the tamoxifen-induced YFP labeling of mature SMCs and ECs as well as their progeny were first quantified prior to the vein graft transplantation. Moreover, after additional 6 wks, at the time of the experimental end point for lineage tracking, the tamoxifen-induced YFP-labeling efficiencies in the reporter mice without receiving operation were assessed again (**Online Figure I**). The details in morphological analyses of the YFP-labeling cells are described in "**Morphological Analyses**" (see below).



Online Figure I. The scheme of experimental designs for tracking down the fate of mature SMCs and ECs in mouse vein graft remodeling. Tam, tamoxifen; i.p., intraperitoneally. The tamoxifen-induced genetic labeling efficiency was quantified prior to vein graft transplantation (1st check) as well as at experimental end point (2nd check).

Venous Bypass Graft Procedure
Male wild type (WT) or transgenic mice in C57BL/6J genetic background at age of 12 wks were used for reciprocal jugular vein graft transplantation as previously described.[5] Briefly, the mice were anesthetized by intraperitoneal injection of a solution of xylazine (5 mg/kg body weight) with ketamine (80 mg/kg body weight) and fixed on a clean surgical platform in supine position. The operation was performed under a dissecting microscope (KL1500 LCD; Carl Zeiss, Jena, Germany). In anesthetized recipient mice, the right common carotid artery was mobilized and divided. A 1-mm cuff with a 1-mm handle (0.65 mm in diameter outside and 0.5 mm inside) (REF 800/200/100/200, Portex LTD, United Kingdom) was placed on both ends of the artery, and the ends were reverted over the cuff and ligated with an 8-0 silk ligature. The vein segment which was isolated from donor mice (see below) was grafted between the 2 ends of the carotid artery by sleeving the ends of the vein over the artery cuff and ligating them together with the 8-0 suture without changing the direction of blood flow (**Online Figure II**).

For vein graft isolation, a midline incision was made from mandible to clavicle in donor mice. The right external jugular vein was exposed, and 3 branches were ligated with 8-0 silk suture. Right prior to the vein transplantation (see below), a ~1-cm segment of the jugular vein was excised and stored on ice in phosphate-buffered saline (PBS). The ischemia time of vein segments was less than 15 min. Because human vein grafts are nearly always partially denuded prior to grafting, we determined if our procedure may have a similar impact by quantifying the median proportion of EC coverage and variation in donor veins. Immunochemical staining revealed that there are 68.15 ± 22.38 % of vWF positive cells and 88.96 ± 18.72 % of CD31 positive cells in the intima of isolated donor veins right prior to transplantation (3 sections per vein and 12 sections of four jugular veins in total). These results indicate that the donor jugular veins undergo a similar biological process to the human donor veins prior to the transplantation, providing the pathophysiological relevance of this mouse vein graft model.



Online Figure II. The scheme of vein graft transplantation in mice. Step 1: The left common carotid artery was dissected in the middle and passed through the cuffs. Step 2: a segment of the artery was turned inside out with a stent and fine tweezers to cover the cuff body, Step 3: which was fixed to the cuff with a 9-0 silk suture. Step 4: the right external jugular vein (~1 cm), was harvested, Step 5: and grafted between the two ends of the carotid artery by sleeving the ends of the vein over the artery cuff and suturing them together with 8-0 suture ligation.

Tissue Harvest and Immunohistochemical Staining

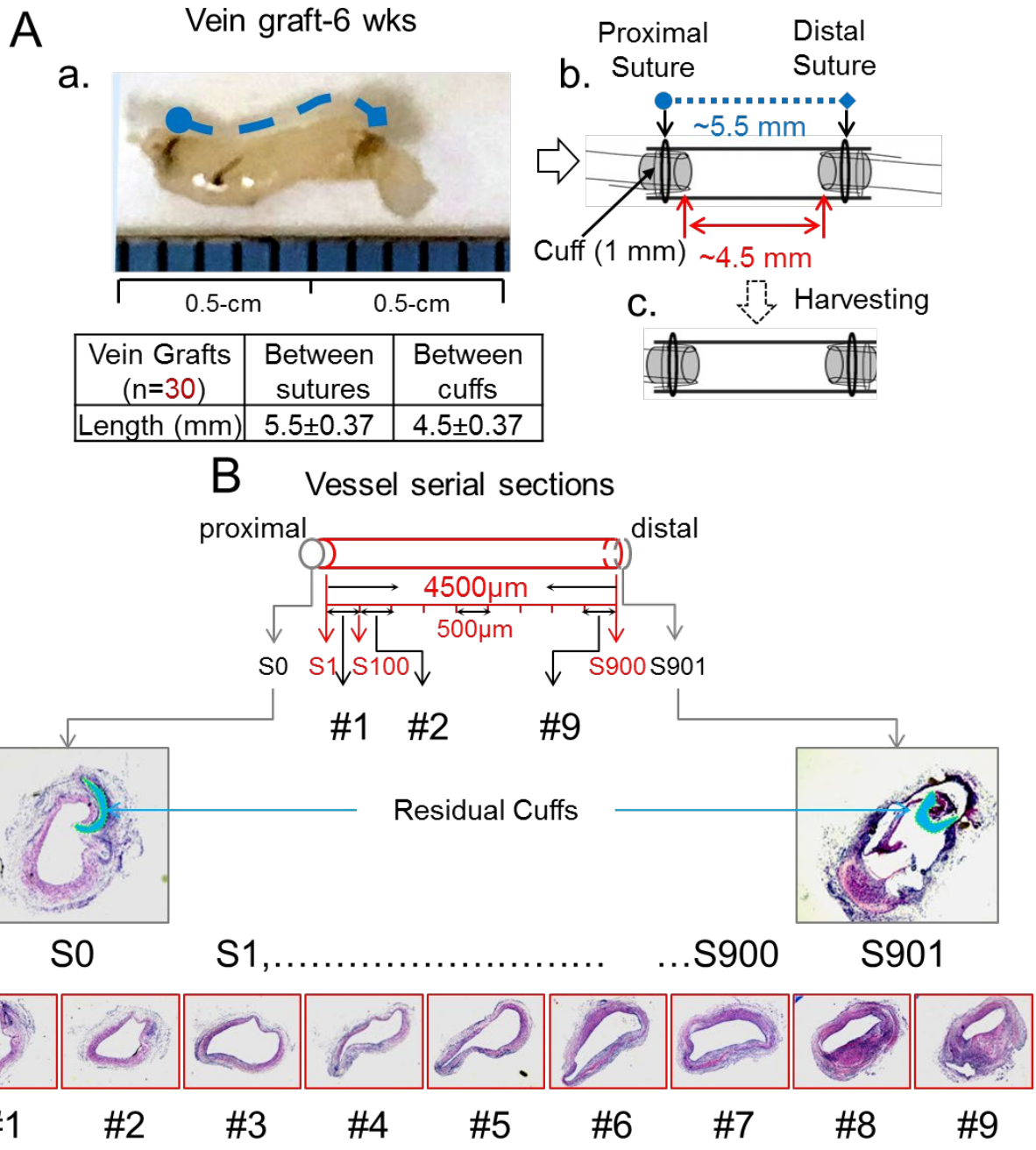
Mice were euthanized with an overdose of pentobarbital (50 mg/kg, i.p., Cat#: 2821, Vortech, USA) or CO_2 inhalation. After washing out blood, the aorta, carotid arteries, jugular veins or vein grafts, as well as the other organs were dissected after perfusion fixation with 4% paraformaldehyde (Cat#: J19943-K2, Thermo Fisher Scientific, USA) for 5 min and further fixed with 4% paraformaldehyde in 4°C overnight, and then embedded using Optimal cutting temperature (O.C.T.) compound (Cat#: 4585, Fisher Health Care, USA) prior to cryosection. The frozen tissue sections with a 5-μm thickness were mounted on standard microscope slides and dried at room temperature for 30 min first, and the washed with 1 ×PBS for 3 times, 15 min for each. To remove out all of the O.C.T. compounds, additional wash was carried out. Thereafter, the tissue sections were incubated in 1% BSA (Cat#: A3912, Sigma-Aldrich, USA) for 1 h in room temperature and then incubated with primary antibodies (**Table S1**) in 1% BSA solution at 4°C for overnight. These sections were washed with 1 ×PBS for 3 times, 30 min for each, and then incubated with secondary antibodies (**Table S2**) in room temperature for 2 h. After the washing with PBS, these sections were further stained with 4',6-diamidino-2-phenylindole (Dapi, Cat#: D1306, Invitrogen, USA). Tunel staining was performed using the in-situ cell death detection kit (Cat#: 12156792910, Roche, USA). Hematoxylin and eosin (HE) staining was carried out with autostainer program of LEICA AUTOSTAINER XL in Instrumentation Resource Facility (IRF) of University of South Carolina School of Medicine.

Confocal Microscopy

All images of Tunel and immunofluorescence staining in vivo were acquired using a Zeiss LSM 510 META confocal scanning laser microscope. A cross-section of VGs was scanned from the right side and along the lumen step by step thereby resulting in 15-40 images to cover the whole cross-section. The image of a whole VG cross-section was integrated by combining these image pieces with a black background using Power Point.

Morphological Analyses

Measurement of Vein Graft Length — Prior to the O.C.T. embedment, the fixed vein grafts were placed on a hard and flat surface for digital imaging with a ruler. Using IPP (Image Pro Plus) analysis software, a curve was draw from the proximal to distal ligated sites by identifying the remaining sutures along one edge of the vein graft body. Since the length of the cuff is 1 mm and the suture is usually located at the middle of the cuff, we defined the length of a vein graft by a

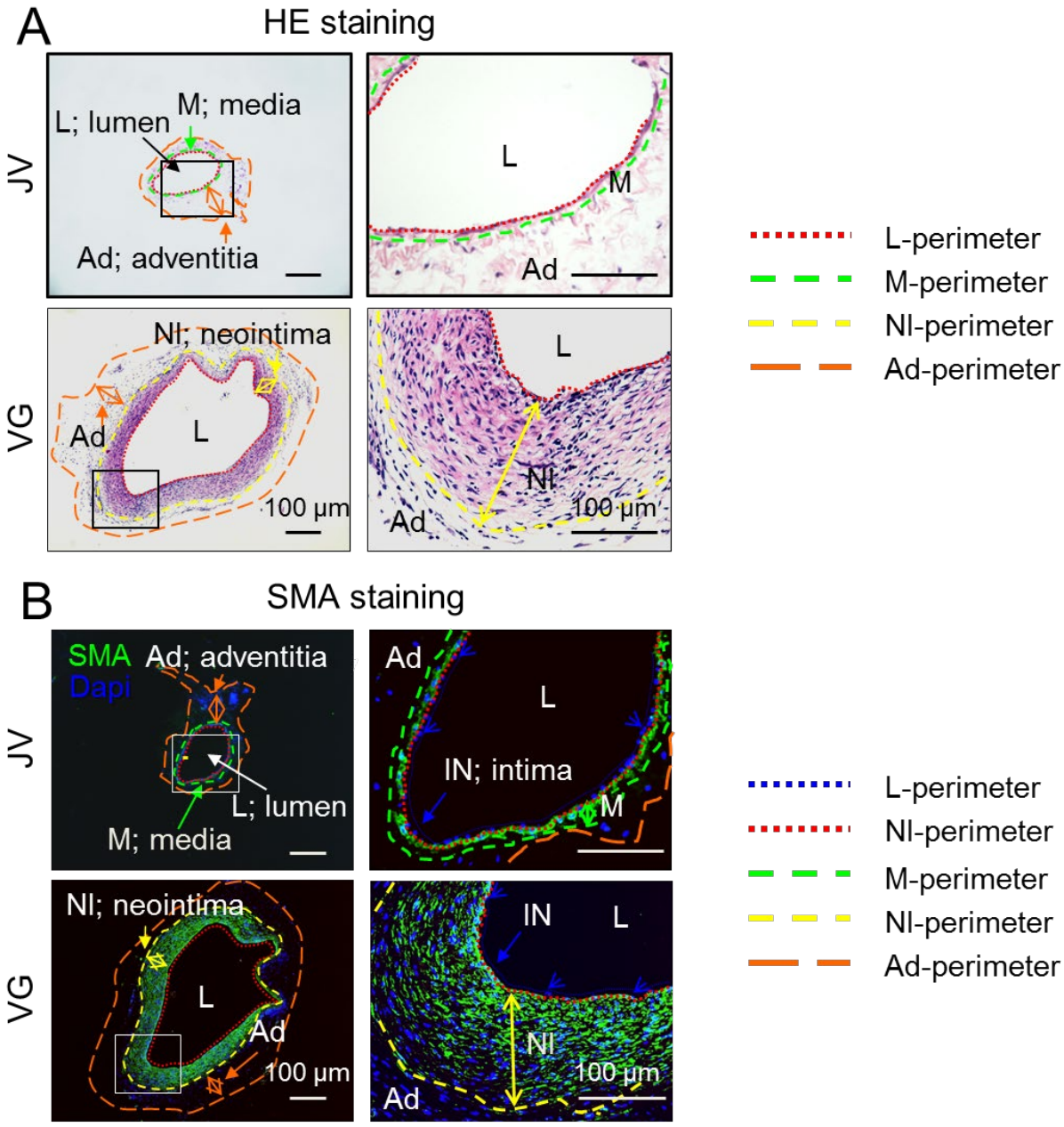


Vein Grafts (n=30)	Between sutures	Between cuffs
Length (mm)	5.5±0.37	4.5±0.37

Online Figure III. Measurement of mouse jugular vein graft (VG) length and consecutive cross-sectioning of mouse jugular (VG)s. VGs were harvested at 6 wks after transplantation between adult male C57BL/6J mice (n=30). (**A**) A curve was draw from the proximal to distal ligated sites by identifying the remaining sutures along one edge of the VG body. The total length of the curve was measure by IPP (Image pro plus) software. **a**, The image of VGs. **b**, **c**, The scheme of whole length of VGs. (**B**) The scheme of consecutive cross-sectioning of VGs and representative HE staining of the cross-section (**S**). **#1** is one of the first 100 cross-sections from the proximal end of the VG; **#9** is one of 100 cross-sections of the distal end of the VG. Blue parts indicated residual cuffs.

formula: the vein graft length = the length between the sutures at proximal and distal ends – 1 mm (0.5 mm × 2) (**Online Figure III. 3A**). We carefully measured the length between the sutures vein grafts (WT to WT transplantation) at 3 wks, 6 wks, 8 wks and 12 wks after transplantation. We found it was around 5.5 mm, which was defined by a large sample size (n=30) at 6 wks after transplantation, i.e., 5.5 ± 0.37 mm (**Online Figure IIIA**). Thus, we postulated the length of vein grafts in this mouse model is 4.5 ± 0.37 mm (**Online Figure IIIA**).

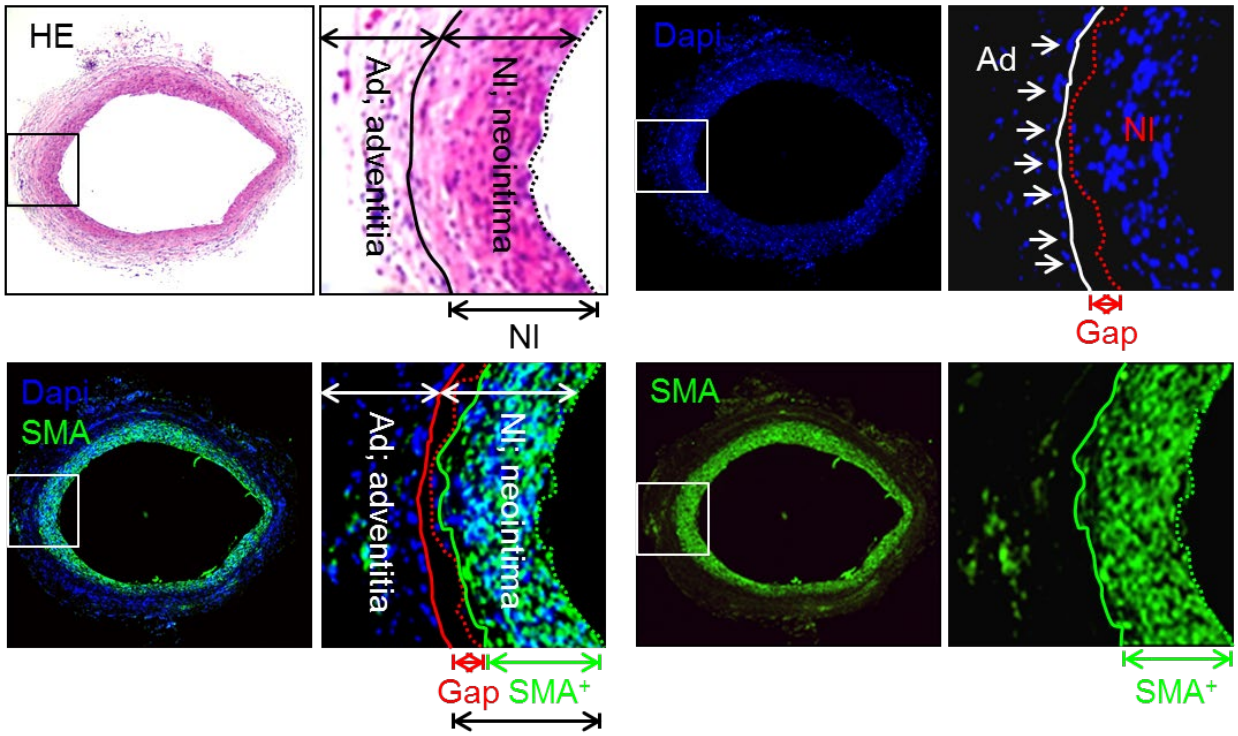
Vein Graft Remodeling — We cut the whole length of vein grafts from the proximal to distal ends (**Online Figure IIIA-c** and **B**). The thickness for each tissue cross-section is 5-μm. The first cross-section (S) of proximal end was defined as the section immediately after disappearance of the



Online Figure IV. HE and SMA staining of normal jugular vein (JV)s and vein graft (VG)s. (**A**) Representative HE staining of normal JVs and VGs of mice. (**B**) Representative Dapi/SMA staining of normal JVs and VGs of mice. Normal JVs were isolated from 12 wks-old male C57BL/6J wild type (WT) mice. VGs were harvested from male WT C57BL/6J WT mice at 6 wks after isologous jugular vein transplantation.

cuff, while the last cross-section of distal end was defined as the section right prior to visualization of the cuff. According, we numbered the last section with the cuff at the proximal end as S0 and the first cross-section of proximal end as S1. Usually, it yielded around 900 sections (900 × 5 μm = 4.5 mm), supporting our postulation of the vein graft length between the cuffs. These tissue sections were uniformly partitioned into 9 segments with the number (#) 1 to 9, which represents each 500 μm long segment from the proximal to distal end per graft (**Online Figure IIIB**).

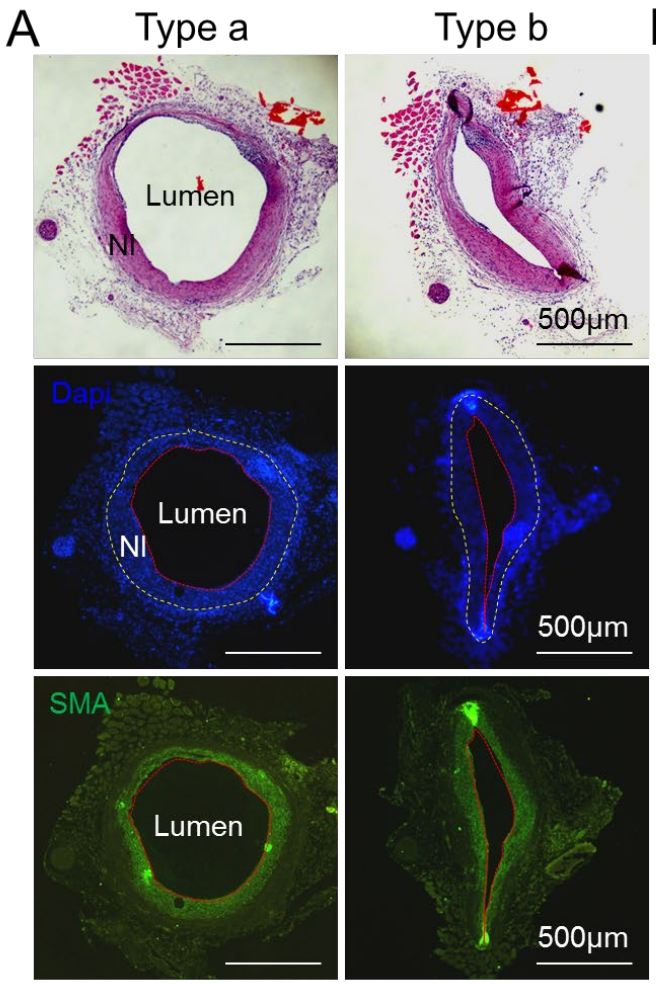
To accurately examine the whole vein graft remodeling, we performed both HE and immunofluorescent SMA (smooth muscle alpha actin) staining on 90 cross-sections (10 sections from each segment), which were consecutively selected from S1 with an interval of 50 μm (**Online Figure IIIB**). Because of the inherently ill-defined nature of the lamina in venous tissues, delineation of the boundary of the media layer in veins is often difficult.[6] We found that hematoxylin and eosin (HE) staining and Masson's trichrome staining are hardly to distinguish the internal elastin lamina (IEL) between the intima and the media, particularly in vein grafts. In addition, even the elastic tissue Van Gieson (EVG) staining proved superior in delineation of the elastic lamina



Online Figure V. A gap between adventitial inner edge and neointima outer edge in Dapi/SMA staining of vein grafts. Representative HE and Dapi/SMA staining of VGs at 6 wks after isologous jugular vein transplantation between adult male C57BL/6J mice. There is a gap between adventitial inner edge (while line toward the lumen, white arrows indicated) and neointimal outer edge (red dotted line toward the adventitia). Black line indicated the boundary between the adventitia (Ad) and neointima (NI) in HE staining. Black dotted line indicated lumen boundary of NI in HE staining. Green line indicated the outside boundary of SMA^+ area. Green dotted line indicated lumen boundary of SMA^+ area.

yet quantification of the intimal and medial layers in vein grafts was often difficult as previously reported.[6] Hence, we carefully carried out morphological analyses of these cross-sections by comparing the HE staining which could highlight nuclei and cytoplasma/connective tissue with the immunofluorescent staining of SMA and Dapi which recognizes SMCs. We found that there is a gap between adventitial inner edge (toward the lumen) and neointimal outer edge (toward the adventitia) (**Online Figure IV** and **Figure V**). Usually, it is easier to find out the gap by the SMA and Dapi staining, compared to the HE staining (**Online Figure V**). Accordingly, we defined the area between the gap and intimal inner edge of the HE or SMA and Dapi staining as the neointimal area and the area between the gap and adventitial outer edge as the adventitial area.

However, we found that the values of lumen and NI areas as well as NI thicknesses varied significantly depending on the methods used, particularly when the vessel structure was

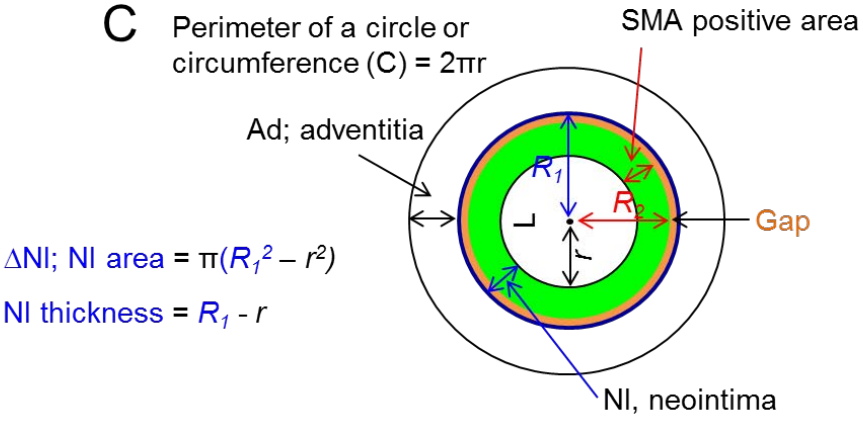


B

	NI area (×10^5 μm^2)	
	Image-ProPlus	C=2πr, Calculated
Type a	3.442566	3.128558
Type b	2.841785	2.995995

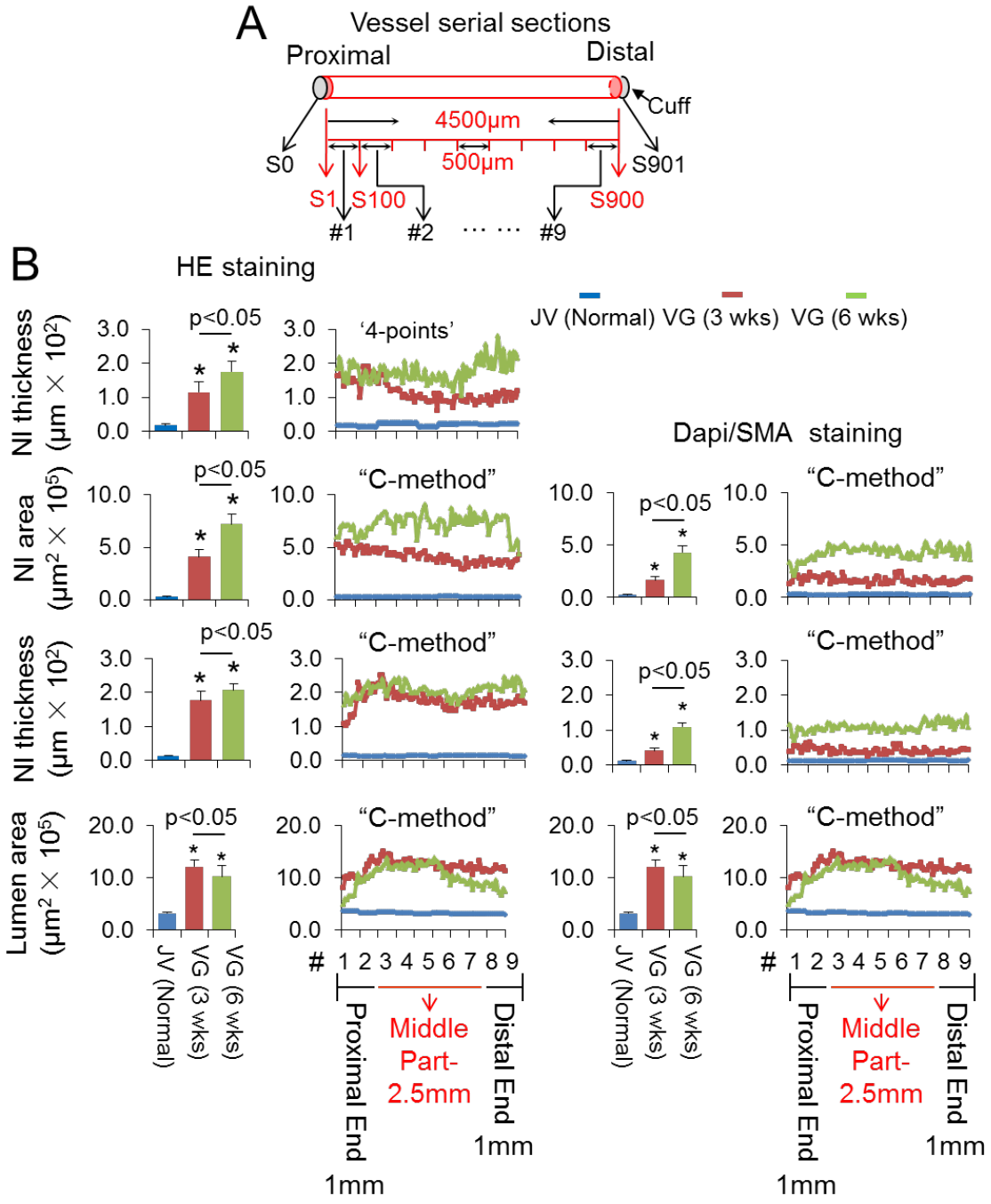
	Lumen area (×10^5 μm^2)	
	Image-ProPlus	C=2πr, Calculated
Type a	4.368795	4.897585
Type b	1.31719	4.507812

	NI thickness (×100 μm)	
	Four-points (Ref.4)	C=2πr, Calculated
Type a	1.2960	1.087392
Type b	1.1475	1.080641



Online Figure VI. The comparison of reported methods and circumference (C)-method for quantifying neointima (NI) area and thickness. VGs were harvested as described in **Online Figure V**. (**A**) Representative HE or Dapi and SMA staining of VG cross-sections in which the vessel structure is regularly configured (type a) or irregularly configured with partly closed lumen (type b). (**B**) Quantified results using indicated methods. (**C**) A scheme of the C-method using Dapi and SMA staining.

irregularly configured with partly closed lumen (**Online Figure VI**). For examples, the lumen and NI areas as well as NI thicknesses of these vessels measured by the most popular method using Image ProPlus software and the 4-point method reported by Zou et al[5] are significantly different



Online Figure VII. Characterization of jugular vein (JV) graft (VG) remodeling in mice. (**A**) A scheme of consecutive cross-sectioning of VGs. S, cross-section. #1 is one of the first 100 Ss from the proximal end of VGs; #9 is one of 100 Ss of the distal end of VGs. (**B**) Neointima (NI) areas and thicknesses, and lumen areas of VGs measured by different methods as indicated. Male 12-wks old C57BL/6J WT mice were subject to isologous VG transplantation (WT to WT, 270 cross-sections of 3 VGs). The isografts at 3 and 6 wks were sectioned as indicated in (**A**) and 90 consecutive Ss from the proximal and distal ends were subject to HE or Dapi and SMA staining for quantifying the NI area and thickness as well as the lumen area as described in "Methods". The left panel is the average values at each anatomical location along the VG and the right panel shows the overall averages. *p<0.05 vs. normal JV group.

(**Online Figure VIA** and **VIB**). Therefore, we tried to calculate these area sizes and NI thicknesses by the mathematics formulas: the perimeter of a circle or the circumference = π2r adius) and the circle's area = πr^2 (**Online Figure VIC**). The underlying rationale is that we found; (1) the vessel structure in vivo is always well configured in an open tubular shape, and (2) the perimeters or circumferences of the lumen, intima or NI, and adventitia in a vessel are usually independent of the vessel shape. Accordingly, we used the circumference measured by Image ProPlus software and calculated the lumen and NI areas as well as the NI thickness and named it as C-method. We found that the calculated values by C-method more accurately reflect the vessel remodeling independent of the vessel shape (**Online Figure IVB**). Accordingly, we used this C-method to quantify the lumen size and the NI area as well as the NI thickness using the cross-sections with HE and SMA & Dapi staining. We found that the results are quite similar, but the variations of results via SMA and Dapi staining are much less than that via HE staining (**Online Figure VII**). Therefore, we used C-method to analyze vein graft remodeling utilizing the cross-sections stained with Dapi and SMA throughout this study unless specified. Minimal numbers of tissue cross-section per VG for analyzing each biomarker was 3, representing the proximal end, the body, and the distal end. However, as a standard analysis, 9 consecutive tissue cross-sections (#1~#9) representing 9 segments of each VG form the proximal end to distal end were analyzed for every parameter throughout this study unless specified.

Because transplanted human veins undergo both inward and outward remodeling thereby resulting in both focal stenotic lesions often occurring in the perianastomotic regions and the diffused stenosis within the body of vein grafts, a single parameter, such as NI area or thickness, cannot adequately reflect the nature of total wall remodeling; instead the histopathological analysis should also incorporate other parameters such as calculated ideal lumen area and ratios among these parameters.[7, 8] Indeed, in this model, we found that overall NI area and thickness per VG were time-dependently increased; however, overall lumen area per VG was time-dependently decreased (**Online Figure VIIB**). In addition, the lumen area near both the proximal and distal ends was usually smaller than that at the middle portion; whereas, the NI thickness near either the proximal or distal ends was usually larger than that at the middle portion (**Online Figure VIIB**). In addition, the average of initial lumen size was 549106.5±139511.4 μm^2 (n=5) at 4 h after transplantation and the calculated ideal lumen size (the area within the aforementioned gap) was 714986±152430.5 μm^2 (n=6) with actual lumen size of 468217.2±107892.3 μm^2 (n=6) at 42 days after transplantation. These results reveal that like the clinical setting,[7] the transplanted mouse jugular veins over time undergo both inward and outward remodeling thereby leading to both focal stenotic lesions at the perianastomotic regions and the diffused stenosis within the bodies towards VGF.

Quantification of Tamoxifen-induced YFP-labeling SMCs and ECs in vivo—After the washout time period prior to transplantation as well as the time period to reach the experimental end point, the aorta, carotid arteries and jugular veins as well as the other organs including the heart, the esophagus, the skeletal muscle, the lung, the spleen, and the livers or kidneys of the reporter mice that received tamoxifen injection not but vein graft transplantation (n=5) were harvested and processed for immunofluorescent staining of SMC and EC makers and Dapi. To quantify the labeling efficiencies in the vessels, 5 consecutive cross-sections with an interval of 100 μm were selected from each vessel for the analyses. The expression patterns of YFP in SMCs and ECs in the other organs were analyzed via the staining of a few tissue sections (2-3 sections) randomly selected from these organs. The tamoxifen-induced Cre-loxP recombination was also determined by Western blot analysis of YFP protein expression in the aorta.

The fate of YFP-labeled mature SMCs and ECs in vein graft remodeling was quantified at 6 wks after transplantation as described in "**Vein Graft Remodeling**".

Vascular Smooth Muscle Cell (SMC) Culture and SM22α Knockdown

Mouse aortic smooth muscle cells were isolated and cultured as described elsewhere.[9] Briefly, 2-3 month old male mice (n=5) were euthanized by CO_2 overdose inhalation and then the thoracic aortas (the length of each thoracic aorta is ~1-cm) were removed, washed in phosphate-buffered saline (PBS) and incubated in 4.5 g/L glucose Dulbecco's modified Eagle's medium (DMEM) containing 1 mg/ml collagenase type II (Cat#: 17101-015, Worthington Biochemical Corp.) at 37°C for 10-15 min. The surround connective tissue and the adventitia were then removed using a dissection microscope, and the aortas were opened, and ECs were removed by abrasion. The dissected tunica media tissues were chopped into small pieces (~1 mm^2) and digested in 2.5 ml of type II collagenase (2 mg/ml; Cat#: 4177, Worthington Biochemical Corp., USA), soybean trypsin inhibitor (1 mg/ml, Cat#: LS003571, Worthington Biochemical Corp., USA) and elastase III (0.744 units/ml; Cat#: LS002279, Worthington Biochemical Corp., USA) for 1-2 h at 37°C with periodically shaking throughout the incubation. Digestion was stopped by addition of 15 ml of DMEM containing 20%FBS. Finally, the enzymatically isolated single cells (~7000/per aorta, ~35000/~5 aortas) were cultured with high glucose (4.5 g/L) DMEM (Cat#: 11995, Gibco, USA) supplemented with 20%FBS and 100 μg/ml penicillin/streptomycin (Cat#: 30-002-CI, Corning, USA) in 1 well of 6-well plate at 37°C in a 5% CO_2 incubator. The purity of cultured mouse aortic SMCs (mSMCs) was verified by immunohistochemical staining of SMA. The cultured mSMCs between passage (P) 3-10 in which ~99% of cells were positive for αSMA were used in this study.

Mouse aortic SMCs (P8) at 80% confluent state were transfected with Pre-Designed siRNAs, i.e., the control siRNAs (Cat#: AM4611, Ambion, USA) or SM22 siRNAs (Cat#: AM16708, Ambion, USA) using LipofectAMINETM (Cat#: L3000-008, Invitrogen, USA) according to the manufacturer's instruction. At 72 h after transfection, the cells were serum starved for 24h, followed by incubation with or without different doses of H_2O_2 as indicated for 2 h in serum-free DMEM. The viability of cells was determined by measuring propidium iodide (PI, Cat#: AS-83215, aNAspec Inc., USA) uptake. Briefly, at the end of the experiments, the cells were washed twice with 1× PBS, and then were stained with PI (10 μg/ml) at room temperature in the dark for 10 min. The cell death was measured by counting the number of PI positive cells per field under a microscope (Evos FL Auto2, Thermo Fisher Scientific). All experiments (n=3-6 for each group) in vitro were repeated at least three times.

Endothelial Cell (EC) culture and Endothelial Mesenchymal Transition (EndoMT)

Human Umbilical Vein Endothelial Cells (HUVECs) (Cat#: 8000, Sciencell, Carlsbad, CA) were cultured in endothelial culture medium (Cat#: 1001, Sciencell, Carlsbad, CA) consisting of endothelial cell culture basal medium, 5% fetal bovine serum (FBS) (Cat#: 0025, Sciencell, Carlsbad, CA), endothelial cell growth factor supplement (Cat#: 1052, Sciencell, Carlsbad, CA) and penicillin/streptomycin.

For EndoMT,[10] HUVECs were cultured in full endothelial growth medium as above mentioned, and at an 80% of confluent state, were then cultured in human endothelial serum free medium (Cat#: 11111044, Gibco, USA) 24 h. The serum-starved HUVECs were incubated in endothelial cell culture basal medium (Cat#: 1001, Sciencell, Carlsbad, CA) supplemented with endothelial cell growth factor supplement (Cat#: 1052, Sciencell, Carlsbad, CA), 0.25% FBS (Cat#: 0025, Sciencell, Carlsbad, CA), 10 ng ml^{-1} recombinant TGF-β1 (Cat#: 7666-MB, R&D, USA) or BMP4 (Cat:# 314-BP,R&D, USA) for 48 h.

Preparation of Conditioned Medium (CM) of HUVECs

HUVECs at an 80% of confluent state were cultured in human endothelial serum free medium (Cat#: 11111044, Gibco, USA) for 24 h and then cultured in endothelial cell culture basal medium (Cat#: 1001, Sciencell, Carlsbad, CA) supplemented with endothelial cell growth factor supplements (Cat#: 1052, Sciencell, Carlsbad, CA), 0.25% FBS (Cat#: 0025, Sciencell, Carlsbad, CA) alone, or with additional 10 ng ml^{-1} recombinant TGF-β1 (Cat#: 7666-MB, R&D, USA) or BMP4 (Cat:# 314-BP,R&D, USA) for 48 h. These cells were washed three times with PBS, and

the culture media were replaced with endothelial cell culture basal media and incubated for 24 h. Thereafter, the culture media were collected, and filtered by 2.5μM filter (Cat#:6794-2502, whatman, UK), and then stored in -80°C freezer. The conditional medium (CM) from HUVECs without treatment of TGF- β1 or BMP4 was designated as EC CM and conditional media from HUVECs which underwent EndMT in response to TGF-β1 or BMP4 treatment were named as EndMT-T CM or EndMT-B CM, respectively.

Assessments of Vascular SMC Dedifferentiation

Proliferation Assay—Mouse aortic SMCs at passage 10 were seeded into 12-well cell culture plates (1.5 x 10^4 per well) in the different HUVEC-derived conditional media supplemented with 1% FBS (Cat#: FBS-500, X& Cell Culture, USA). The culture media were changed once on day 3 and the cell numbers per well was counted on day 1, day 3, and day 5 after the seeding.

Migration Assay—Mouse aortic SMCs at passage 10 were seeded into 12-well cell culture plates (1.5 x 10^4 per well) in SMC growth medium, i.e., high glucose DMEM (Cat# 11-965-092, Gibco, USA) supplemented with 10% FBS (Cat#: FBS-500, X& Cell Culture, USA). for 48 h thus reaching a confluent state. After serum-starvation for 24 h, a wound on the confluent SMC layer was concreated by a 200 μl tip and then cultured with SMC full growth medium (SMC-M), EC culture basal medium (EC baseM), EndMT-T CM or EndMT-B CM without any supplements for 24 h. The wounds or scratches were captured by a microscope (Nikon E600 Widefield Epifluorescence and Darkfield Microscopy, Nikon, Japan) on day 0 and day 1 after the scratch. The migration rate was expressed as the percentage of area reduction or wound closure. The closure percentage of SMC-M group was set as 100%.

Morphology Measurement—Mouse aortic SMCs at passage 10 were seeded into 12-well cell culture plates (1.5 x 10^4 per well) in SMC full growth medium, EC culture basal medium (EC baseM), and the different HUVEC-derived conditional media without any supplements for 7 days. The images of these cells were captured by a microscope (TMS-F NO.211580 Microscopy, Nikon, Japan) on day 7. The longitudinal length and horizonal width of each cell in each captured image were measured. Usually, 3~5 cells randomly selected in each captured image and 5 images in total were analyzed. The area between the edges of scratch of each experimental group was measured using IPP (Image Pro Plus) analysis software. The percentage changes of these parameters relative to that of SMCs cultured in full growth medium were calculated.

Polymerase Chain Reaction (PCR)

Genomic DNAs were also extracted from mouse tails and subjected to PCR for genotyping of transgene mice. PCR primers and reaction conditions for genotyping are listed in **Online Table III**.

Western Blot Analysis

Harvested tissues were snap-frozen in liquid nitrogen and stored in -80°C. The cultured cells were washed with ice-cold 1x PBS and snap-frozen in liquid nitrogen and stored in -80°C. The frozen tissues were smashed into powder in liquid nitrogen and then lysed in a homogenization buffer (RIPA) contained 150 mM NaCl, 1% NP-40, 50 mM Tris (pH 8.0), 0.5% sodium deoxycholate ($C_{24}H_{39}NaO_4$), 0.1% sodium dodecyl sulfate (SDS), and proteinase and phosphatase inhibitors (Cat#: P8340 and P0044, Sigma-Aldrich, USA). Immunoblotting was carried out as described elsewhere.[11] The primary antibodies used are listed in **Online Table I**. The secondary antibodies used are anti-mouse IgG, HRP-linked Antibody (Cat#: 7076, Cell Signaling, USA) and anti-rabbit IgG, HRP-linked Antibody (Cat#: 7074, Cell Signaling, USA) peroxidase-conjugated AffiniPure rabbit anti-goat IgG (H+L). Images were acquired using KwikQuant Imager (Kindle Biosciences, USA).

Statistics

Data are shown as mean ± SEM if they are not specified. All samples were plotted in figures, showing variations within each mouse and between mice in all samples. To test the difference of experimental groups using biological replicates, we calculated the mean value of each biomarker from each vessel of each mouse and performed statistical test using data from biologically different mice in each group. Thus, we performed the statistical test on the mean of each biomarker from each biologically different mouse (n number is the mouse number) in this study. Differences between 2 groups were evaluated for statistical significance using the Student t test. When differences among > 3 groups were evaluated, results were compared by one-way ANOVA with Bonferroni test for multiple comparisons. Differences were considered significant at $p < 0.05$.

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II. Supplementary Tables, Figures and Legends

Online Tables

Online Table I. Primary antibody Information

Primary Antibodies				
Name	**MW(kDa)**	**Characteristics**	**Sources**	**Dilutions**
Anti-SM22alpha (SM22α)	23	Rabbit polyclonal anti-SM22α	Abcam Cat#: ab14106	IF: 2μg/ml (1:500) WB: 1μg/ml (1:1000)
Anti-Calponin 1 (CNN1)	34	Rabbit monoclonal anti-CNN1 (EP798Y)	Abcam Cat#: ab46794	IF: 0.5μg/ml (1:200) WB: 0.1μg/ml (1:1000)
Anti-smooth muscle α actin (SMA)	42	Mouse monoclonal anti-αSMA	Sigma-Aldrich Cat#: a5228	IF: 0.2μg/ml (1:1000) WB: 0.2μg/ml (1:1000)
Anti-beta Actin (βactin)	43	Mouse monoclonal anti-βactin Clone AC-15	Sigma Aldrich Cat#: a1978	WB: 0.5-1μg/ml (1:2000)
Anti-GAPDH	37	Mouse monoclonal anti-GAPDH (Clone GAPDH-71.1)	Sigma Aldrich Cat#: G8795	WB: 0.5-1μg/ml (1:2000)
Anti-smooth muscle myosin heavy chain 11 (MYH-11)	227	Rabbit polyclonal to MYH-11	Abcam Cat#: ab125884	IF: 0.9μg/ml (1:1000) WB: 0.9μg/ml (1:1000)
Anti-GFP	30.83	Rabbit polyclonal to GFP	Abcam Cat#: ab6556	IF: 0.5μg/ml (1:1000) WB: 0.5μg/ml (1:1000)
Anti-GFP	30.83	Goat polyclonal to GFP	Abcam Cat#: ab6673	IF: 1μg/ml (1:1000) WB: 1μg/ml (1:1000)
Anti-Mouse CD31		Purified Rat Anti-Mouse CD31 Clone MEC 13.3 (RUO)	BD Cat#: 550274	IF: 0.15μg/ml (1:100)
Anti-human Von Willebrand Factor (vWF)		Rabbit polyclonal to VW (antiserum)F	Dako Cat#: A0082	antiserum IF: 1:1000

Anti-smooth muscle α actin-FITC antibody (SMA-FITC)		Mouse monoclonal Anti-αSMA - FITC	Sigma Aldrich Cat#: F3777	IF: 2µg/ml (1:1000)
Anti-CD44 antibody [EPR18668]	82	Rabbit monoclonal [EPR18668] to CD44	Abcam Cat#: ab189524	WB: 0.8µg/ml (1:500)
Purified Rat Anti-Mouse CD45	~200	Rat monoclonal IgG2b, κ	BD Cat#: 550539	IF: 6.25µg/ml (1:1000)
Anti-Human/Mouse Gli1 Antibody	~118	Rat monoclonal IgG2a	R&D Cat#: MAB3324	IF: 8-25µg/ml (1:500)
Anti- Tie-2 (F-6) antibody	140	Mouse monoclonal IgM	Santa Cruz SC-518076	WB: 1µg/ml (1:200)
Anti- Thy-1 (OX7) antibody also referred to as CD90	25-37	Mouse monoclonal antibody anti-CD90	Santa Cruz Cat#: sc-53116	WB: 1µg/ml (1:200)
Anti-Purified mouse Anti-eNOS antibody	140	Mouse monoclonal antibody anti-eNOS	BD Cat#: pS1177	WB: 0.5µg/ml (1:200)
Anti- VE-cadherin (human) monoclonal antibody (BV6)	130-140	Rabbit polyclonal anti-VE-cadherin	Enzo Life Sciences Cat#: ALX-803-305-C100	WB: 0.5µg/ml (1:200)

MW, predicted molecular weight; **IF**, immunofluorescence staining; **WB**, Western blot analysis

Online Table II. Secondary antibody information

Name	Characteristics	Sources	Laser line	Excitation max	Emission max	Dilutions
Alex fluor 488	Donkey anti-rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A21208	488	490	525	1μg/ml (1:1000)
Alex fluor 488	Goat anti-mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A11001	488	490	525	1μg/ml (1:1000)
Alex fluor 488	Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A21206	488	490	525	1μg/ml (1:1000)
Alex fluor 488	Donkey anti-goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A11055	488	490	525	1μg/ml (1:1000)
Alex fluor 546	Donkey anti-mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A10036	488/532	556	573	1μg/ml (1:1000)
Alex fluor 546	Donkey anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A10040	488/532	556	573	1μg/ml (1:1000)
Alex fluor 546	Goat anti rat-IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A11081	488/532	556	573	1μg/ml (1:1000)
Alex fluor 546	Donkey anti-goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Invitrogen Cat#: A11056	488/532	556	573	1μg/ml (1:1000)

Table III. Primer information for genotyping PCR

Mouse strains	Primers	Reaction conditions	Products
Myh11-CreER^{T2} mice	Primer1 (SMWT1): 5'-TGACCCCATCTCTTCACTCC-3' Primer2 (SMWT2): 5'-AACTCCACGACCACCTCATC-3' Primer3 (SMMUT): 5'-AGTCCCTCACATCCTCAGGTT-3'	Cycling: 95°C, 3 min; 95°C, 60 sec; 56°C, 60 sec; 72°C ,1 min, Repeat 40 cyc; 72°C, 6 min, 4°C.	Wild type: 225bp Mutant: 225 bp and 287 bp
Cdh5-CreER^{T2} mice	Primer1 (IMF114): 5'-GCCTGCATTACCGGTCGATGCAACGA-3' Primer2 (IMR114): 5'-GTGGCAGATGGCGCGGCAACGCCATT-3'	Cycling: 94°C, 1 min; 94°C, 30 sec; 70°C, 30 sec; 72°C, 1 min; Repeat 35 cyc; 72°C, 10 min, 4°C.	Wild type: None. Mutant: 720 bp
SM22α (Ki)-CreER^{T2} mice	Primer1 (RF67): 5'-CTCAGAGTGGAAGGCCTGCTT-3' Primer2 (RF90): 5'-CACACCATTCTTCAGCCACA-3' Primer3 (SC135): 5'-GGCGATCCCTGAACATGTCC-3'	Cycling: 95°C, 5 min; 95°C, 30 sec; 58°C, 30 sec; 72°C, 45 sec; Repeat 35 cyc; 72°C, 5 min, 4°C.	WT allele: 276 bp KI allele: 220 bp
Rosa26$^{Floxed\text{-}Stop}$eYFP mice Stock Number: 006148 (JAX)	Primer1 (oIMR8546): 5'-GGAGCGGGAGAAATGGATATG-3' Primer2 (oIMR8545): 5'-AAAGTCGCTCTGAGTTGTTAT-3' Primer3 (oIMR4982): 5'-AAGACCGCGAAGAGTTTGTC-3'	Cycling: 95°C, 5 min; 95°C, 30 sec; 58°C, 30 sec; 72°C, 45 sec; Repeat 35 cyc; 72°C, 5 min, 4°C.	Mutant: 320 bp Heterozygote: 320 bp and 600 bp Wild type: 600 bp

The genotyping of *Myh11*-CreER^{T2} mice: The *Myh11* allele (wild type) was detected by PCR using Primer1 (SMWT1) and Primer2 (SMWT2), which amplified a 276 bp fragment. The *Myh11*-CreER^{T2} allele (mutant) was detected by PCR using Primer2 (SMWT2) and Primer3 (SMMUT), which amplified a 287 bp fragment. **The genotyping of Cdh5-CreER^{T2} mice:** The *Cdh5*-CreER^{T2} allele (mutant) was detected by PCR using Primer1 (IMF114) and Primer2 (IMR114), which amplify a 720 bp fragment. The wild type mice do not have the mutant alleles, thus the PCR using the Primer1 and Primer2 will not produce any products. **The genotyping of SM22α (Ki)-CreER^{T2} mice:** The *SM22α* allele (wild type) was detected by PCR using Primer1 (RF67) and Primer2 (RF90), which amplified a 276 bp fragment. The *SM22α* (Ki)-CreER^{T2} allele (KI allele) was detected by PCR using primers Primer1 (RF67) and Primer3 (SC135), which amplified a 220 bp fragment. **The genotyping of Rosa26$^{Floxed\text{-}Stop}$eYFP mice:** The ROSA26 allele (wild type) was detected by PCR using Primer1 (oIMR8546) and Primer2 (oIMR8545), which amplified a 600 bp fragment. The targeted vector allele (mutant) was detected by PCR using Primer2 (oIMR8545) and Primer3 (oIMR4982), which amplified a 320 bp fragment.

Table IV. The percentages of SMA^+ cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Myh11*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice (Myh11) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Myh11**			**Myh11 to WT**		
SMA^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	58.86±33.46	0.57±0.34	0±0	37.88±53.58	0.37±0.53
Middle	0±0	74.79±5.90	1.46±1.7	0±0	85.11±13.18	0.29±0.21
Dis end	0±0	64.67±10.56	0±0	0±0	83.24±11.8	0.85±1.21
VG-S	**WT to Myh11**			**Myh11 to WT**		
SMA^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	67.83±16.85	0.42±0.39	0±0	61.44±43.49	0.55±0.22
Middle	0±0	73.55±7.55	0.38±0.22	0±0	88.62±3.54	1.58±1.56
Dis end	0±0	73.98±13.81	0.44±1.85	0±0	76.89±8.6	2.13±0.31
VGs (M+S) (All)	**WT to Myh11**			**Myh11 to WT**		
SMA^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	66.03±20.17	0.45±0.38	0±0	56.73±45.51	0.52±0.28
Middle	0±0	73.8±7.42	0.6±0.24	0±0	87.92±4.64	1.32±1.22
Dis end	0±0	72.12±1.66	0.35±0.1	0±0	78.16±4.5	2.6±0.53

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Myh11, transplantation of JVs of wild type mice to CAs of Myh11-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Myh11 to WT, transplantation of JVs of *Myh11*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table V. The percentages of YFP^+ cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Myh11*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice (Myh11) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Myh11**			**Myh11 to WT**		
YFP^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	1.11±1.57	2.99±4.23	1.76±2.49	0.5±0.7	24.35±26.01	4.34±6.14
Middle	0±0	0±0	0.07±0.16	1.14±1.56	44.32±13.97	2.09±1.89
Dis end	2.22±0	32.44±4.91	5.29±1.98	0±0	54.66±15.23	8.05±4.3
VG-S	**WT to Myh11**			**Myh11 to WT**		
YFP^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	2.16±3.06	15.41±21.8	4.15±5.87	0.8±0.41	21±19.49	0.55±0.22
Middle	0±0	0.09±0.2	0.05±0.09	0.46±0.45	73.82±11.62	1.73±1.39
Dis end	5.41±2.94	25.32±11.86	5.74±4.95	0.35±0.5	45.02±32.91	2±0.12
VGs (M+S) (All)	**WT to Myh11**			**Myh11 to WT**		
YFP^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**

Pro end	1.95±2.76	12.93±18.29	3.67±5.19	0.74±0.18	21.67±20.79	4.74±2.25
Middle	0±0	0.07±0.16	0.06±0.08	0.6±0.58	67.92±10.03	2.67±2.31
Dis end	4.77±2.35	26.74±10.47	5.65±3.56	0.28±0.4	46.95±29.38	7.03±1.43

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Myh11, transplantation of JVs of wild type mice to CAs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Myh11 to WT, transplantation of JVs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table VI. The percentages of YFP and SMA double positive (YFP^{+}SMA^{+}) cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (Myh11) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Myh11**			**Myh11 to WT**		
YFP^{+}SMA^{+}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	2.47±2.86	0.16±0.05	0±0	17.63±24.93	0.04±0.05
Middle	0±0	0±0	0.04±0.09	0±0	39.55±14.25	0.06±0.1
Dis end	0±0	21.22±10.24	0.13±0.19	0±0	37.89±22.59	0.32±0.46
VG-S	**WT to Myh11**			**Myh11 to WT**		
YFP^{+}SMA^{+}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	9.14±12.92	0.04±0.06	0±0	17.43±18.42	0.03±0.04
Middle	0±0	0±0	0±0	0±0	69.84±10.8	0.45±0.6
Dis end	0±0	21.9±10.42	0.49±0.44	0±0	30.74±36.67	0.52±0.51
VGs (M+S) (All)	**WT to Myh11**			**Myh11 to WT**		
YFP^{+}SMA^{+}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	7.76±10.98	0.05±0.071	0±0	17.47±19.72	0.03±0.04
Middle	0±0	0±0	0±0.01	0±0	63.78±8.25	0.37±0.5
Dis end	0±0	21.77±10.39	0.42±0.39	0±0	32.17±33.86	0.48±0.5

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Myh11, transplantation of JVs of wild type mice to CAs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Myh11 to WT, transplantation of JVs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table VII. The percentages of YFP positive and SMA negative (YFP^{+}SMA^{-}) cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (Myh11) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Myh11**			**Myh11 to WT**		
YFP^{+}SMA^{-}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	2.61±5.48	0.81±0.85	2±2.79	0.55±1.66	6.72±8.59	4.3±6.53
Middle	0±0	0±0	0.03±0.07	0.4±0.6	1.64±2.51	1.02±1.54
Dis end	2.22±4.96	7.5±7.31	5.15±4.91	0±0	10.5±5.5	9.9±15
VG-S	**WT to Myh11**			**Myh11 to WT**		
YFP^{+}SMA^{-}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	2.5±8	6.37±8.33	4.31±6.15	0.91±2.58	8.59±12.2	4.72±5.47

Middle	0±0	0±0	0.04±0.09	0.49±1.5	2.54±2.4	2.15±3.23
Dis end	6.35±10.9	3.41±5.24	5.25±7.42	0.79±2.38	11.6±15.8	4.58±5.58
VGs (M+S) (All)	**WT to Myh11**			**Myh11 to WT**		
YFP$^+$SMA$^-$/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	2.52±7.4	5.26±6.83	3.85±5.47	0.84±2.4	8.22±11.4	4.63±5.68
Middle	0±0	0±0	0.03±0.09	0.47±1.37	2.36±2.42	1.93±2.89
Dis end	5.52±9.68	4.23±5.65	5.23±6.92	0.63±1.9	11.4±13.7	5.65±7.47

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Myh11, transplantation of JVs of wild type mice to CAs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Myh11 to WT, transplantation of JVs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table VIII. The percentages of YFP$^+$SMA$^-$ cells in total YFP$^+$ cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (Myh11) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Myh11**			**Myh11 to WT**		
YFP$^+$SMA$^-$/YFP$^+$ (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	100	41.66±29.46	70.83±46.54	100	68.1±18.84	99.76±0.52
Middle	0	0	66.66±15.71	100	10.12±12.33	95.83±6.86
Dis end	100	22.04±14.98	96.87±15.03	100	30.86±15.03	90.80±8.33
VG-S	**WT to Myh11**			**Myh11 to WT**		
YFP$^+$SMA$^-$/YFP$^+$ (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	100	43.2±47.53	88.54±8.83	100	32.3±22.06	98.88±2.34
Middle	0	0	83.33±26.21	100	9.94±12.42	94.59±9.35
Dis end	100	10.98±11.14	83.65±28.36	100	54.91±28.09	94.86±8.06
VGs (M+S) (All)	**WT to Myh11**			**Myh11 to WT**		
YFP$^+$SMA$^-$/YFP$^+$ (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	100	42.9±43.91	85±16.38	100	39.46±21.42	99.06±1.97
Middle	0	0	77.77±9.6	100	9.98±11.41	94.84±8.85
Dis end	100	13.46±10.58	86.29±30.23	100	50.1±27.55	94.05±9.13

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Myh11, transplantation of JVs of wild type mice to CAs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Myh11 to WT, transplantation of JVs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table IX. The percentages of $Sca1^+YFP^+SMA^-$, $CD44^+YFP^+SMA^-$, or $CD45^+YFP^+SMA^-$ cells in total DAPI or YFP^+ cells in different layers of jugular vein (JV) grafts (VGs) of *Myh11*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP (Myh11) mice after tamoxifen induction transplanted into carotid arteries (CAs) of wild type (WT) mice.

	Transplantation				**Transplantation**		
All VGs (M+S)	**Myh11 to WT**			**All VGs (M+S)**	**Myh11 to WT**		
$Sca1^+GFP^+SMA^-$ /DAPI (%)	**IN**	**NI**	**Ad**	**$Sca1^+GFP^+SMA^-$ /GFP (%)**	**IN**	**NI**	**Ad**
pro end	0.8±2.4	2.7±2.2	4.5±4.7	pro end	34.5±106	6.0±5.0	78.3±81.5
middle	0.3±1.7	1.3±1.5	2.3±1.5	middle	15.0±72.0	2.9±3.4	39.9±26.5
dis end	0.5±1.9	2.3±1.9	4.0±2.9	dis end	23.0±89.0	5.3±4.3	70±50.1
$CD44^+GFP^+SMA^-$ /DAPI (%)	**IN**	**NI**	**Ad**	**$CD44^+GFP^+SMA^-$ /GFP (%)**	**IN**	**NI**	**Ad**
pro end	0.8±2.4	1.5±1.4	2.6±2.4	pro end	34.5±106	3.4±3.2	45±41.5
middle	0.2±1.5	1.0±1.0	1.3±1.3	middle	10.4±60.0	2.2±2.2	22.2±22.7
dis end	0.4±1.7	2.0±2.0	2.2±1.8	dis end	17.2±77.1	4.6±4.6	38.3±30.6
$CD45^+GFP^+SMA^-$ /DAPI (%)	**IN**	**NI**	**Ad**	**$CD45^+GFP^+SMA^-$ /GFP (%)**	**IN**	**NI**	**Ad**
pro end	0.5±1.9	1.1±1.0	0.8±1.2	pro end	21.6±86.2	2.6±2.1	13.5±21.3
middle	0.3±1.4	0.6±0.7	1.1±1.2	middle	11.5±62.9	1.5±1.7	18.9±19.9
dis end	0.5±2.0	1.5±1.5	1.4±1.3	dis end	23.0±89.0	3.3±3.3	24.4±22.6

IN, the intima; NI, the neointima; Ad, the adventitia. Myh11 to WT, transplantation of JVs of *Myh11*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP after tamoxifen injection into the CAs of WT mice.

Table X. The percentages of SMA^+ cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Cdh5*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice (Cdh5) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Cdh5**			**Cdh5 to WT**		
SMA^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	62.43±22.32	1.7±1.56	0±0	68.6±0.42	2.92±0.7
Middle	0±0	82.11±3.95	0.84±0.53	0±0	60.85±4.71	0.6±0.06
Dis end	0±0	74.27±0.07	0.85±0.75	0±0	61.93±13.83	0.66±0.02
VG-S	**WT to Cdh5**			**Cdh5 to WT**		
SMA^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	62.56±22.9	0.14±0.2	0±0	71.78±15.1	1.6±0.81
Middle	0±0	86.09±3.46	0.73±0.24	0±0	77.48±1.67	1±0.27
Dis end	0±0	85.48±4.6	0.89±0.15	0±0	80.54±3.63	1.81±0.67
VGs (M+S) All	**WT to Cdh5**			**Cdh5 to WT**		
SMA^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	62.53±22.78	0.46±0.16	0±0	71.15±11.99	1.86±0.51
Middle	0±0	85.27±2.58	0.75±0.26	0±0	74.15±2.22	0.92±0.22
Dis end	0±0	83.16±3.66	0.88±0.03	0±0	76.82±0.14	1.58±0.53

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Cdh5, transplantation of JVs of wild type mice to CAs of *Cdh5*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Cdh5 to WT, transplantation of JVs of *Cdh5*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table XI. The percentages of YFP^+ cell in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Cdh5*-$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice (Cdh5) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Cdh5**			**Cdh5 to WT**		
YFP^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	88.11±1.09	0±0	7.58±1.19	8.33±11.78	28.17±9.33	0.27±0.28
Middle	43.64±21.35	0±0	5.57±2.81	1.66±2.28	13.42±11.1	0.04±0.05
Dis end	66.79±19.26	0±0	7.36±2.73	0±0	0.44±0.62	0±0
VG-S	**WT to Cdh5**			**Cdh5 to WT**		
YFP^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	98.5±2.75	0±0	11.09±2.95	0±0	0±0	0±0
Middle	47.57±18.13	0±0	5.78±2.87	0±0	0±0	0±0
Dis end	68.9±28.37	0±0	10.0±0.62	0±0	0±0	0±0
VGs (M+S) All	**WT to Cdh5**			**Cdh5 to WT**		
YFP^+/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	96.41±1.95	0±0	10.37±2.59	1.67±2.36	5.63±1.86	0.05±0.05

Middle	46.76±18.53	0±0	5.74±2.6	0.16±0.37	2.68±2.22	0.00±0.00
Dis end	68.46±26.49	0±0	9.46±1.06	0±0	0.08±0.12	0±0

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Cdh5, transplantation of JVs of wild type mice to CAs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Cdh5 to WT, transplantation of JVs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table XII. The percentages of YFP and SMA double positive (YFP^{+}SMA^{+}) cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (Cdh5) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Cdh5**			**Cdh5 to WT**		
GFP^{+}SMA^{+}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	0±0	0±0	0±0	24.48±9.2	0±0
Middle	0±0	0±0	0±0	0±0	9.02±8.12	0±0
Dis end	0±0	0±0	0±0	0±0	0±0	0±0
VG-S	**WT to Cdh5**			**Cdh5 to WT**		
GFP^{+}SMA^{+}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	0±0	0±0	0±0	0±0	0±0
Middle	0±0	0±0	0±0	0±0	0±0	0±0
Dis end	0±0	0±0	0±0	0±0	0±0	0±0
VGs (M+S) All	**WT to Cdh5**			**Cdh5 to WT**		
GFP^{+}SMA^{+}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	0±0	0±0	0±0	0±0	4.89±1.83	0±0
Middle	0±0	0±0	0±0	0±0	1.8±1.62	0±0
Dis end	0±0	0±0	0±0	0±0	0±0	0±0

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Cdh5, transplantation of JVs of wild type mice to CAs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Cdh5 to WT, transplantation of JVs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table XIII. The percentages of YFP positive SMA negative (YFP^{+}SMA^{-}) cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (Cdh5) after tamoxifen induction.

	Transplantation					
VG-M	**WT to Cdh5**			**Cdh5 to WT**		
YFP^{+}SMA^{-}/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	88.11±1.09	0±0	7.58±1.19	8.33±20.4	3.68±5.13	0.27±0.51
Middle	43.64±21.35	0±0	5.57±2.81	1.66±4.08	4.1±3.14	0.04±0.09

Dis end	66.79±19.26	0±0	7.36±2.73	0±0	0±0	0±0
VG-S	**WT to Cdh5**			**Cdh5 to WT**		
YFP$^+$SMA$^-$/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	98.5±2.75	0±0	11.1±2.95	0±0	0±0	0±0
Middle	47.57±18.13	0±0	5.78±2.87	0±0	0±0	0±0
Dis end	68.9±28.37	0±0	10.01±0.6	0±0	0±0	0±0
VGs (M+S) All	**WT to Cdh5**			**Cdh5 to WT**		
YFP$^+$SMA$^-$/Dapi (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	96.41±1.95	0±0	10.37±2.6	1.66±4.1	0.73±1.0	0.05±0.1
Middle	46.76±18.53	0±0	5.74±2.6	0.33±0.8	0.82±0.6	0.01±0.01
Dis end	68.46±26.49	0±0	9.46±1.06	0±0	0±0	0±0

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Cdh5, transplantation of JVs of wild type mice to CAs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Cdh5 to WT, transplantation of JVs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

Table XIV. The percentages of YFP$^+$SMA$^-$ cells in total YFP$^+$ cells in different layers of reciprocally transplanted vein grafts between wild type mice (WT) and *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (Cdh5) after tamoxifen induction.

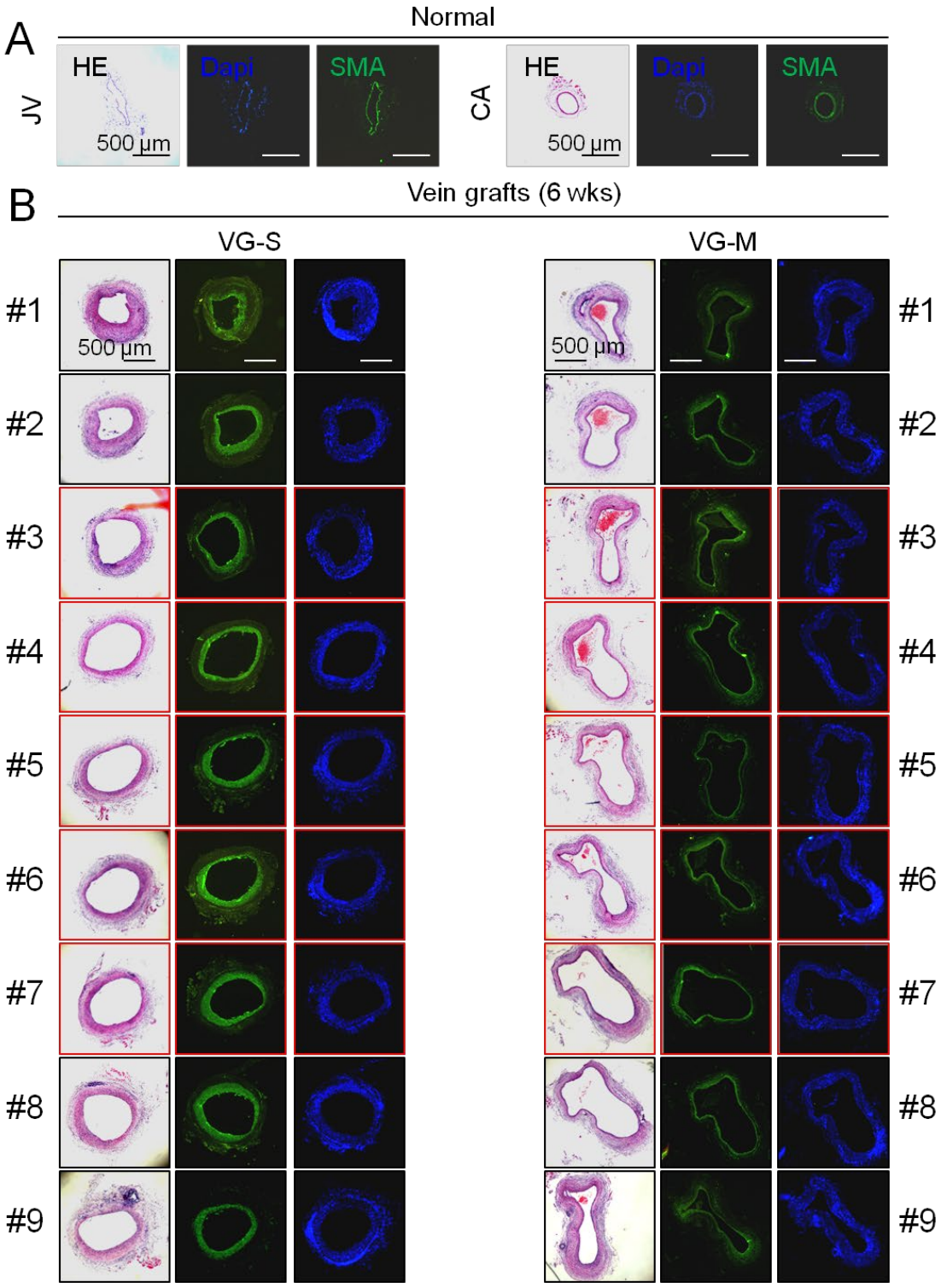
	Transplantation					
VG-M	**WT to Cdh5**			**Cdh5 to WT**		
YFP$^+$SMA$^-$/YFP$^+$ (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	100	0	100	100	9.9±12.4	100
Middle	100	0	100	100	31.4±27	100
Dis end	100	0	100	0	8.3±25.8	0
VG-S	**WT to Cdh5**			**Cdh5 to WT**		
YFP$^+$SMA$^-$/YFP$^+$ (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	100	0	100	0	0	0
Middle	100	0	100	0	0	0
Dis end	100	0	100	0	0	0
VGs (M+S) All	**WT to Cdh5**			**Cdh5 to WT**		
YFP$^+$SMA$^-$/YFP$^+$ (%)	**IN**	**NI**	**Ad**	**IN**	**NI**	**Ad**
Pro end	100	0	100	100	1.97±2.5	100
Middle	100	0	100	100	6.27±5.4	40
Dis end	100	0	100	0	1.66±5.2	0

IN, the intima; NI, the neointima; Ad, the adveintitia. WT to Cdh5, transplantation of JVs of wild type mice to CAs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction; Cdh5 to WT, transplantation of JVs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection to the CAs of WT mice.

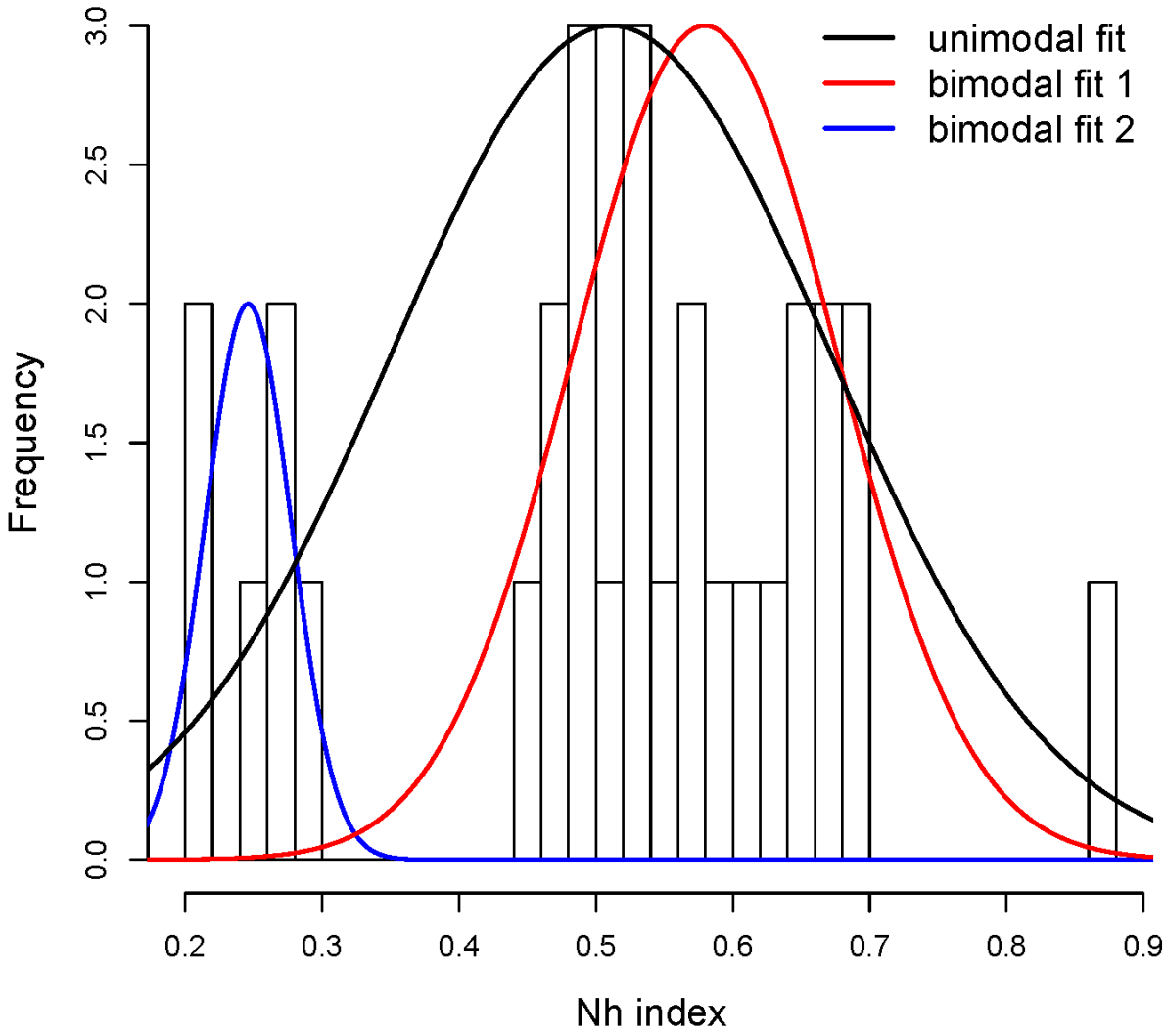
Supplementary Figures and Legends:

Online Figures I-VII
These figures have been inserted into the text.

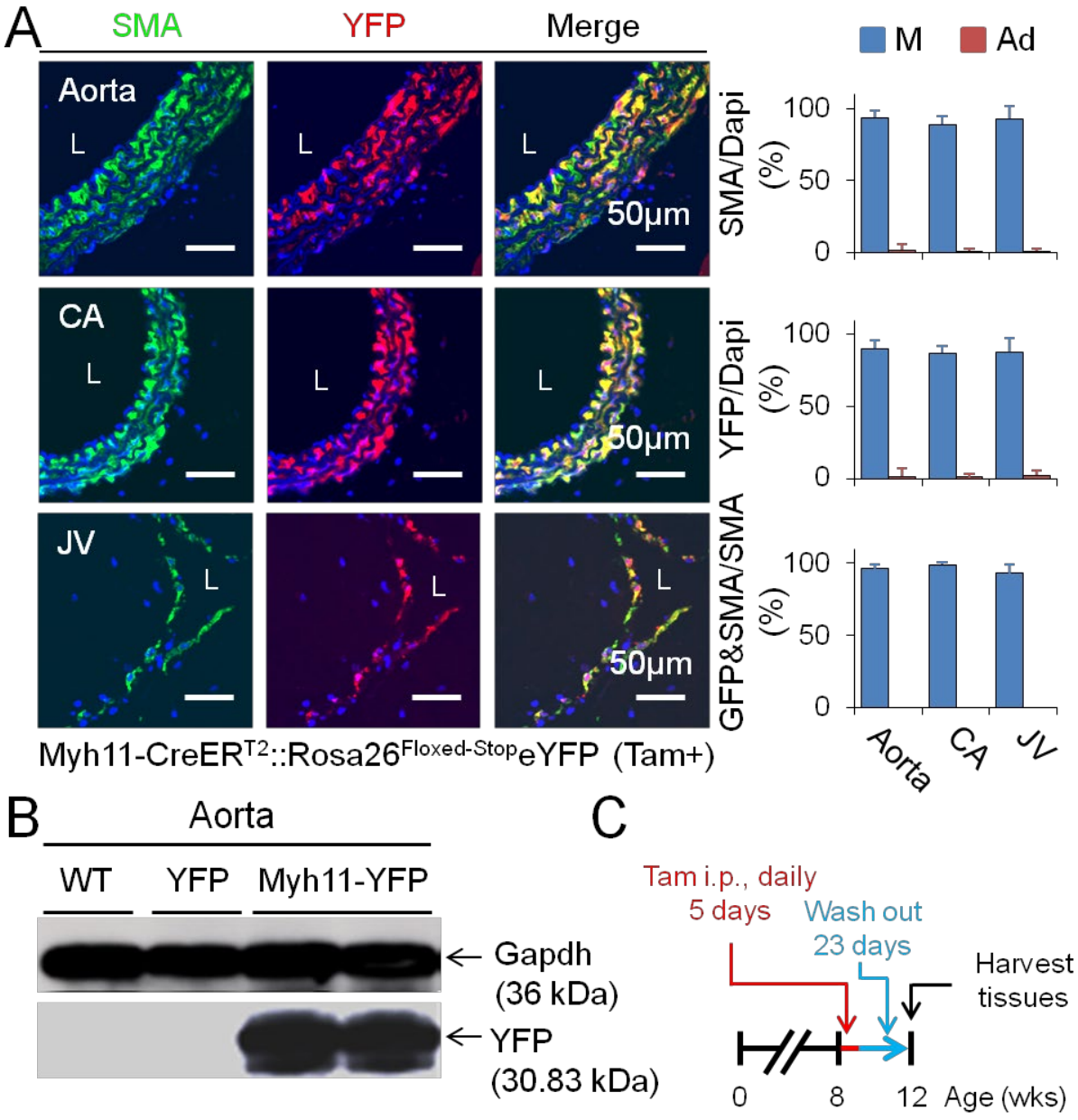
Online Figures VIII-XXXVI



Online Figure VIII. (**A**) The representative HE and SMA staining of normal jugular veins (JVs) and carotid arteries (CAs) of adult male wild type (WT) mice. (**B**) The representative serial HE and SMA staining of vein grafts (VGs) with severe and mild of NI formation in adult male WT mice. Male mice at age of 12 wks were used for these experiments.

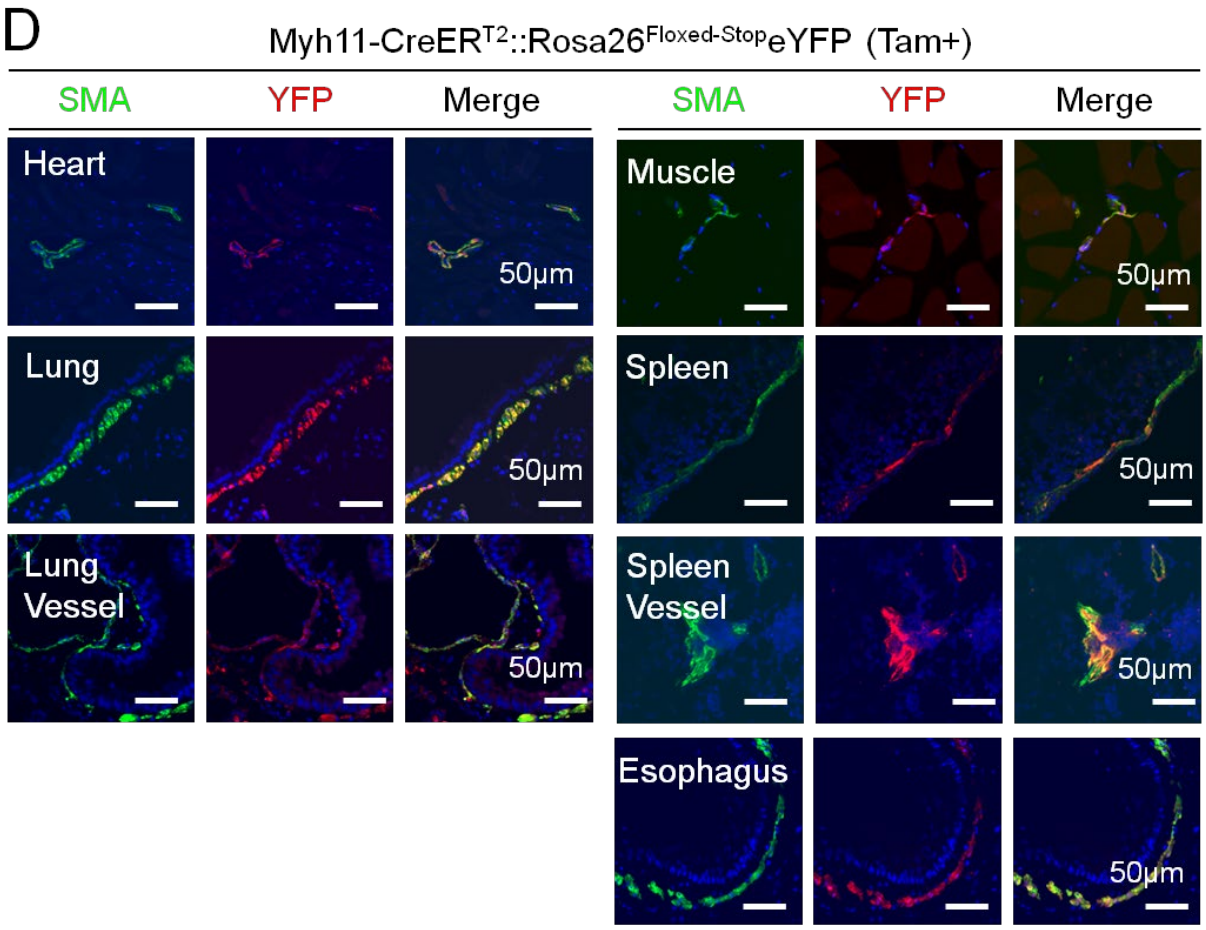


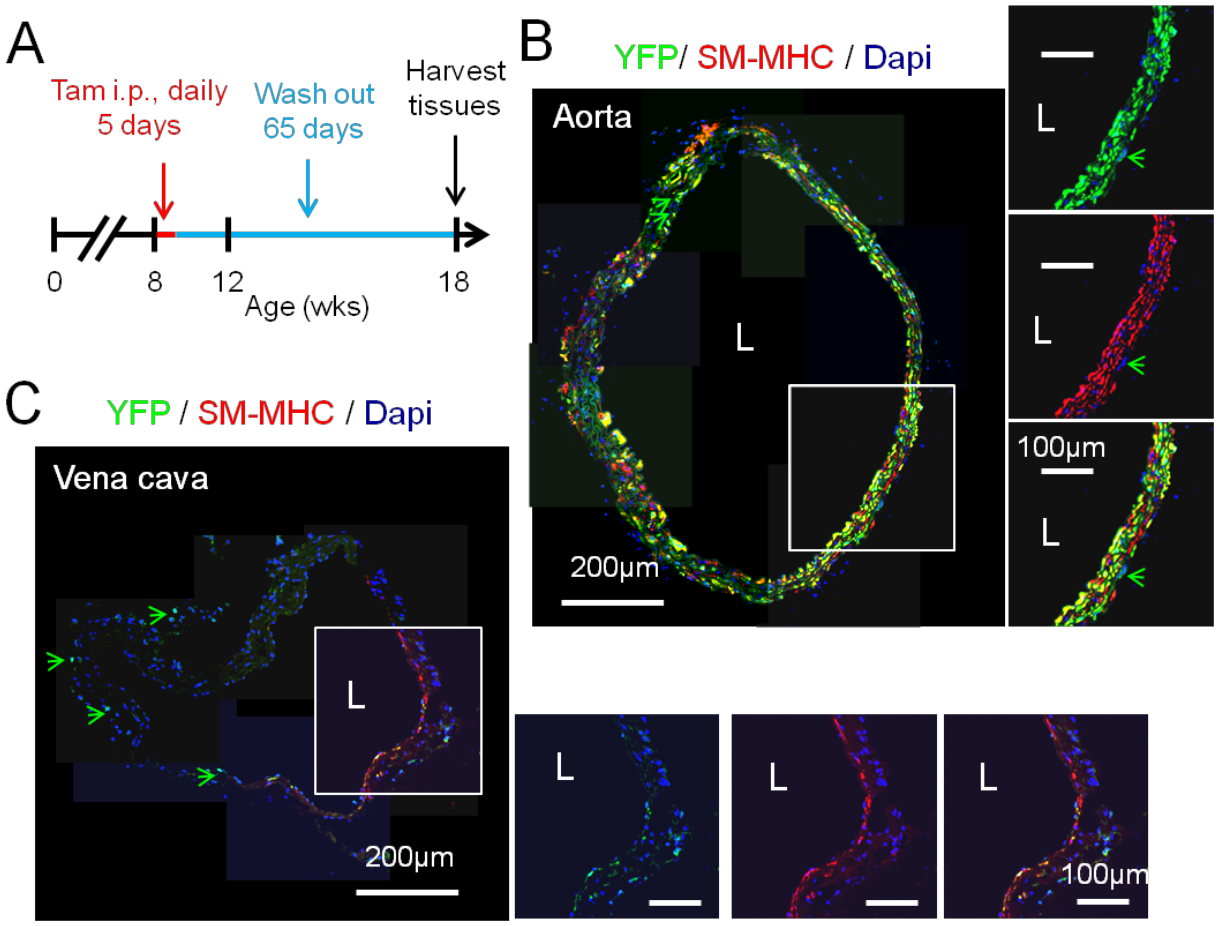
Online Figure IX. Histogram of Nh index of normal JVs and JV isografts at 6 wks after transplantation and fitted distributions of two models. Two models were used to fit the data and show a unimodal fit (black), which assumes data follows a single normal distribution and a bimodal fit, which assumes data follows a mixture of two normal distributions with means at 0.25 (blue) and 0.58 (red) separately. The mixture model fit the data significantly better than the unimodal model (p-value=0.19E-4, log likelihood ratio test).



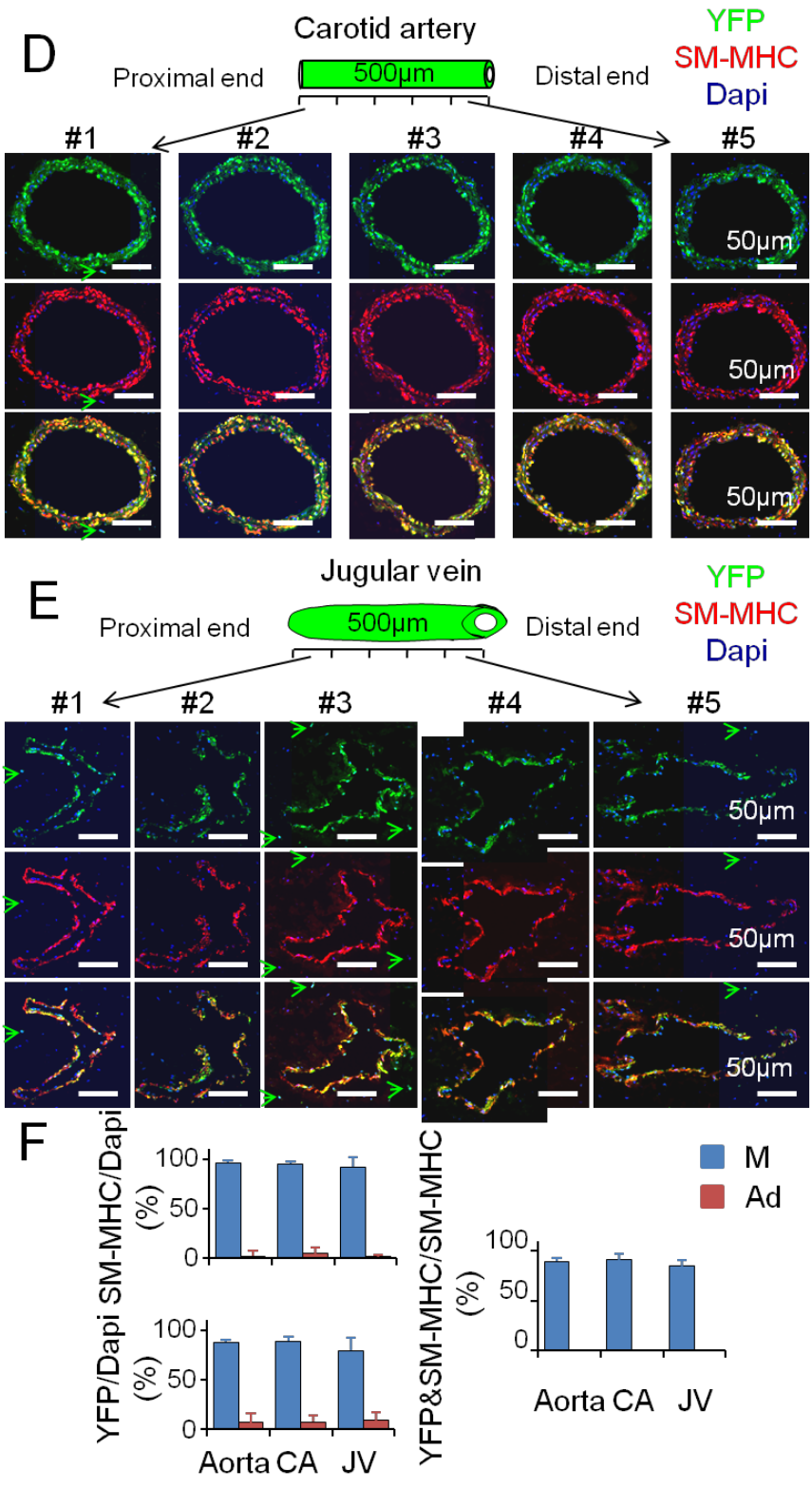
Online Figure X. Tamoxifen-induced YFP-labeling efficiencies in SMCs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice at the baseline. Male *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (n=5) at age of 8 wks were treated with five pulses of tamoxifen and housed for additional at least 20 days without any treatment prior to the tissue harvest as described in Online Figure I. (**A**) Co-staining of YFP (red) and SMA (green) in different vessels including the aorta, the carotid artery (CA) and the jugular vein (JV). The left panel shows the representative images. The right panel shows the quantified percentages of SMA and/or YFP positive cells in the media (M) and the adventitia (Ad). (**B**) Western blot analysis of YFP expression. Whole aortas (n=3) were collect the same time end points as (**A**). The aortas from male and age-matched wild type (WT) and Rosa26$^{Floxed\text{-}Stop}$eYFP mice without tamoxifen treatment were used as the control. L, the lumen. (**C**) The scheme of experimental time periods.

(**D**) Representative images showing YFP labeled of SMCs in different organs (the heart, esophagus, muscles, lungs and spleen as well as the small vessels in these organs).

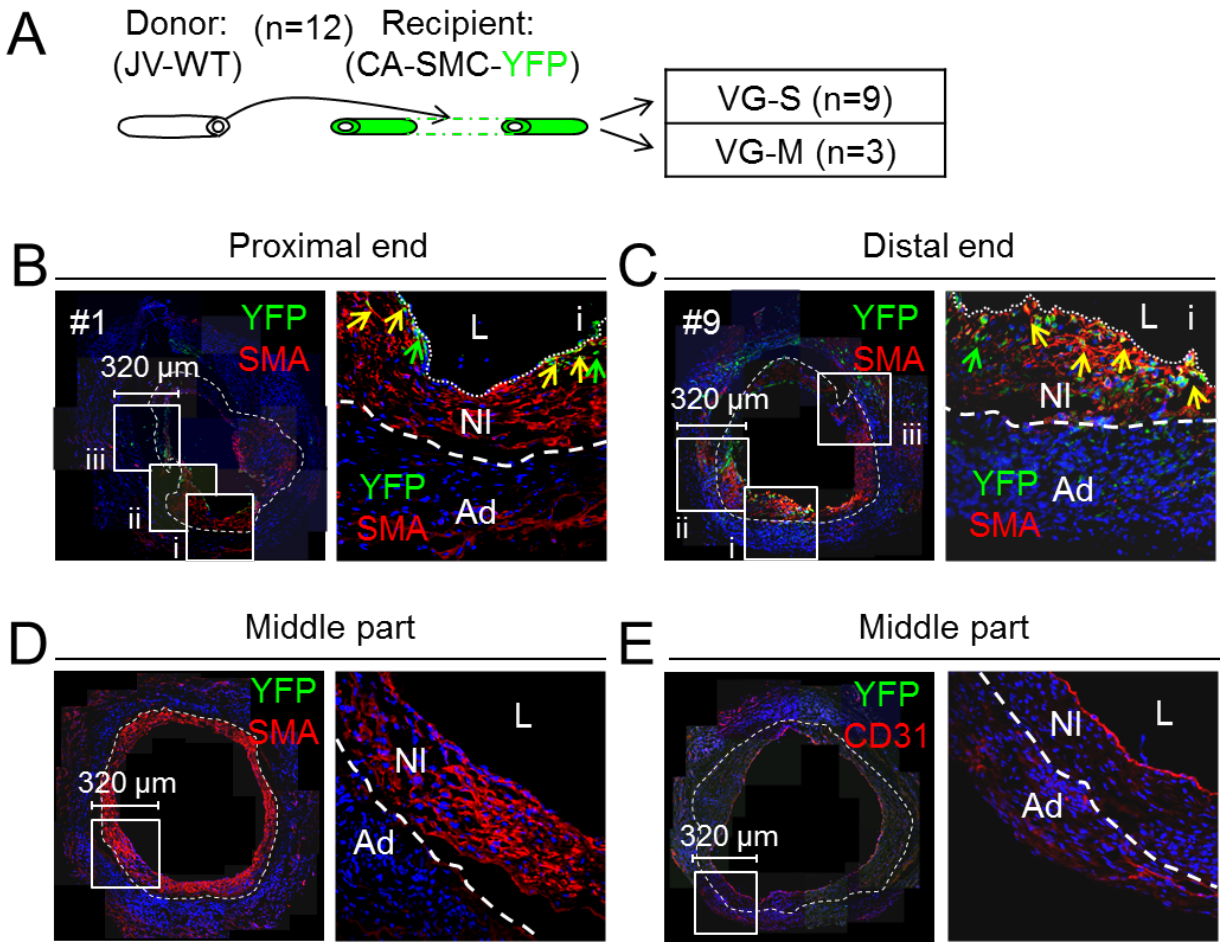




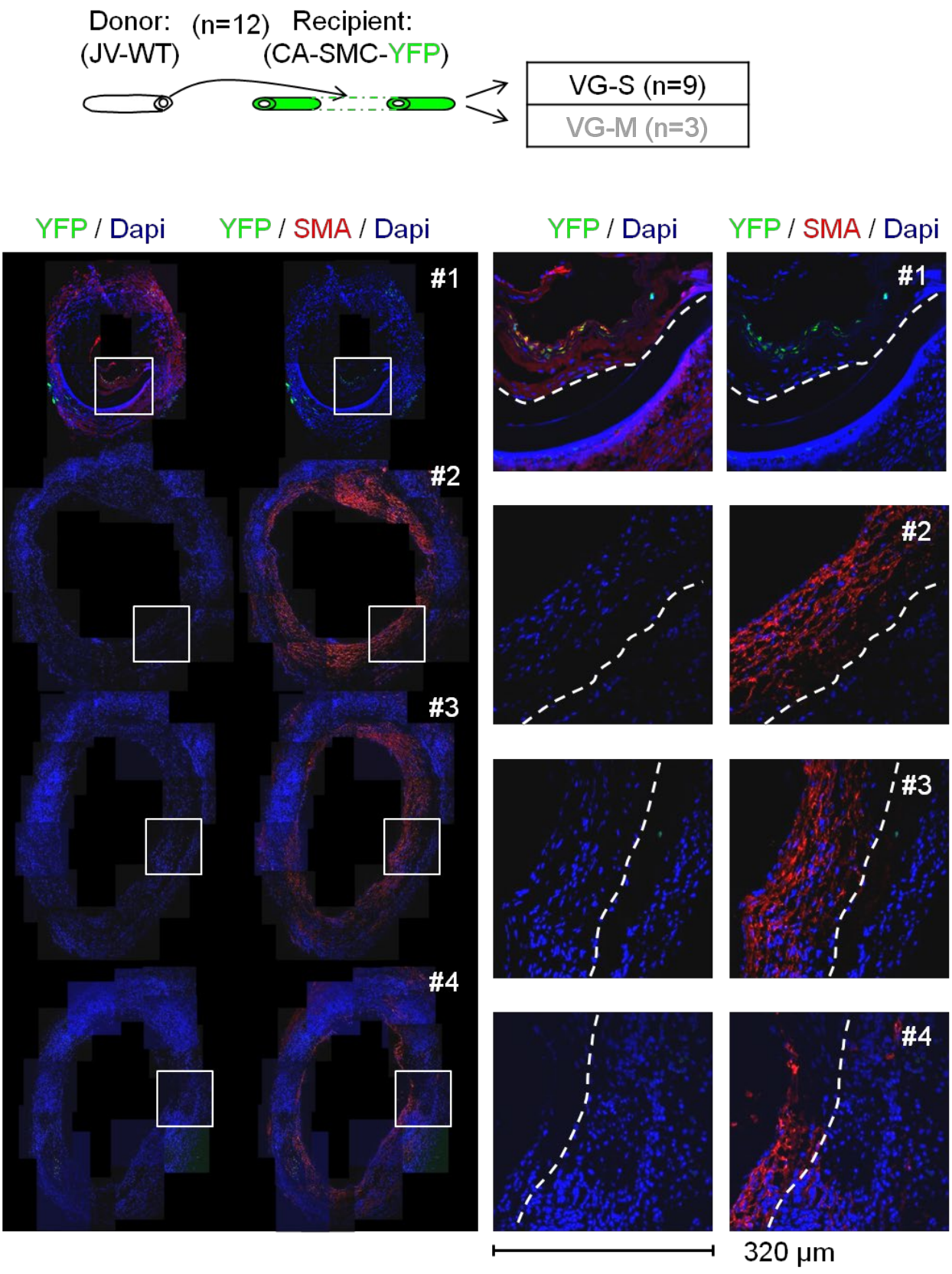
Online Figure XI. Tamoxifen-induced YFP-labeling efficiencies in SMCs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice at the time of experimental end point. Male *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice at age of 8 wks were treated with five pulses of tamoxifen and housed for additional at least 65 days prior to the tissue harvest as described in Online Figure I. (**A**) The scheme of experimental time periods. (**B**, **C**) Representative images of co-staining of YFP (green) and SM-MHC (red) in the aorta and the vena cava. L, the lumen. (**D**, **E**) Representative images of co-staining of YFP (green) and SM-MHC (red) for quantifying the YFP-labeling efficiencies in SMCs in the carotid artery and the jugular vein. Five sections were uniformly selected as indicated from proximal end to distal end. (**F**) The percentages of YFP and/or SM-MHC positive cells in the media (M) and the adventitia (AD) in the carotid arteries or jugular veins were quantified. Data were showed as means ± SD (15 whole vessel cross-sections were analyzed; 5 whole vessel cross-sections per mice, n=3).

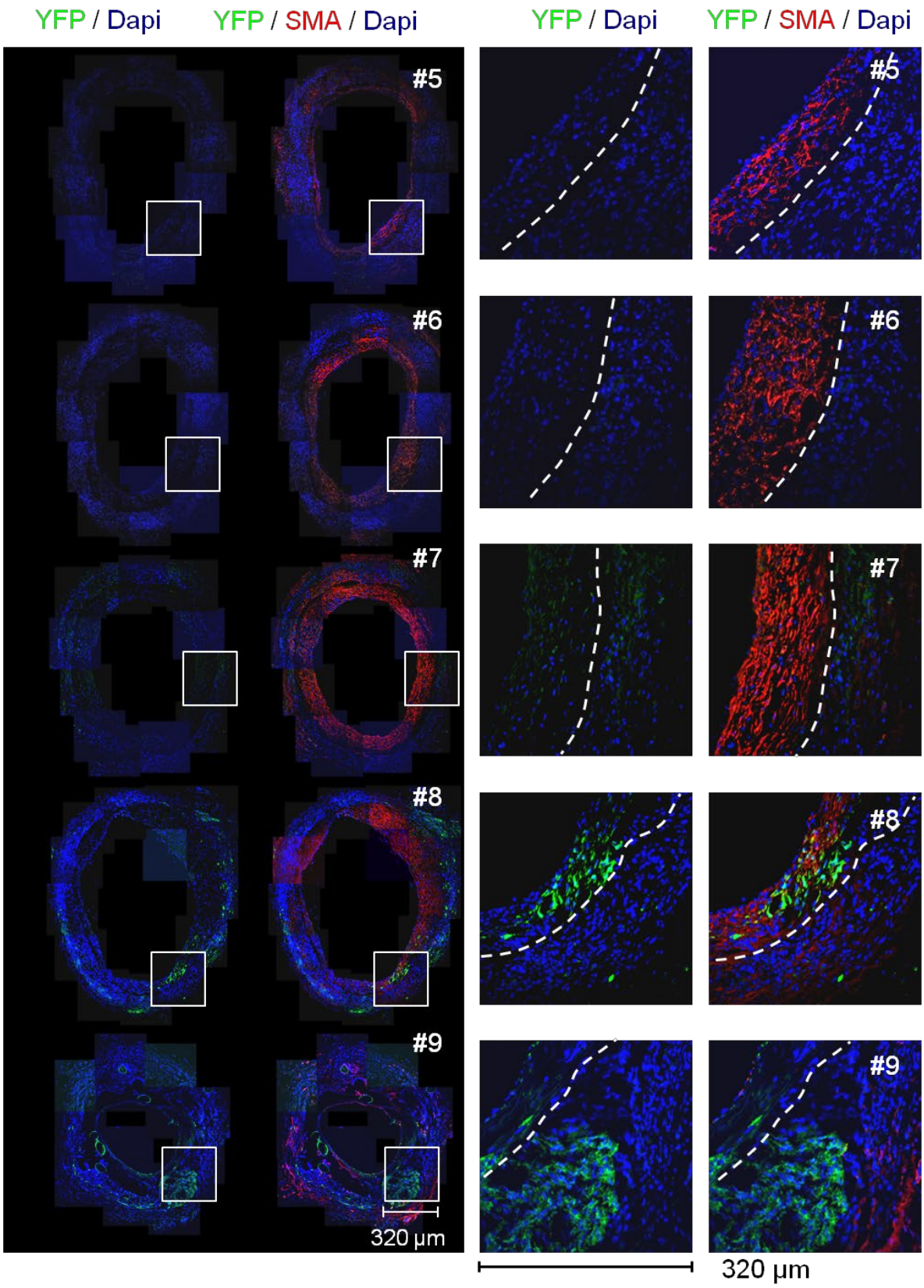


Online Figure XI (continued). (**D-F**)

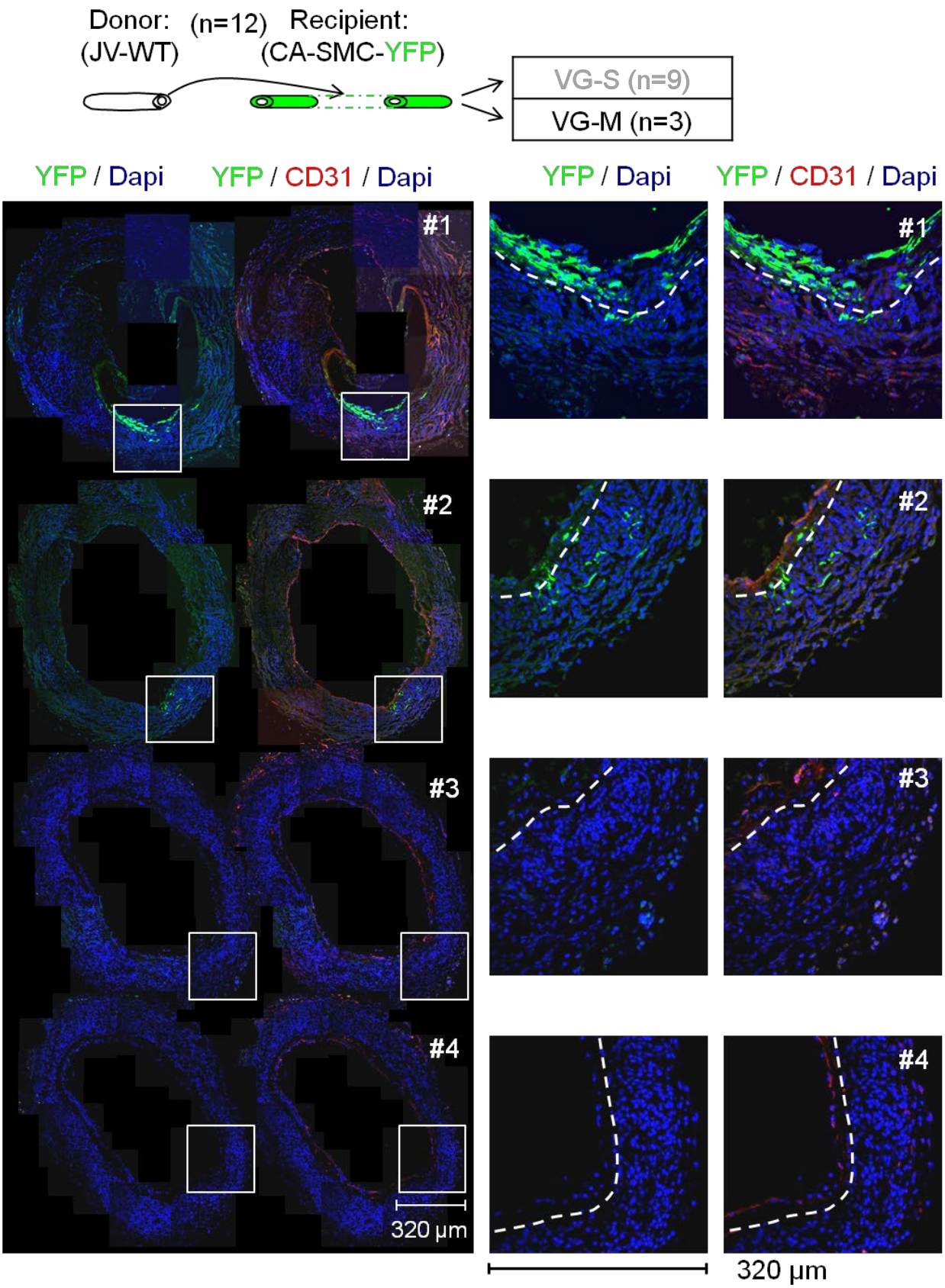


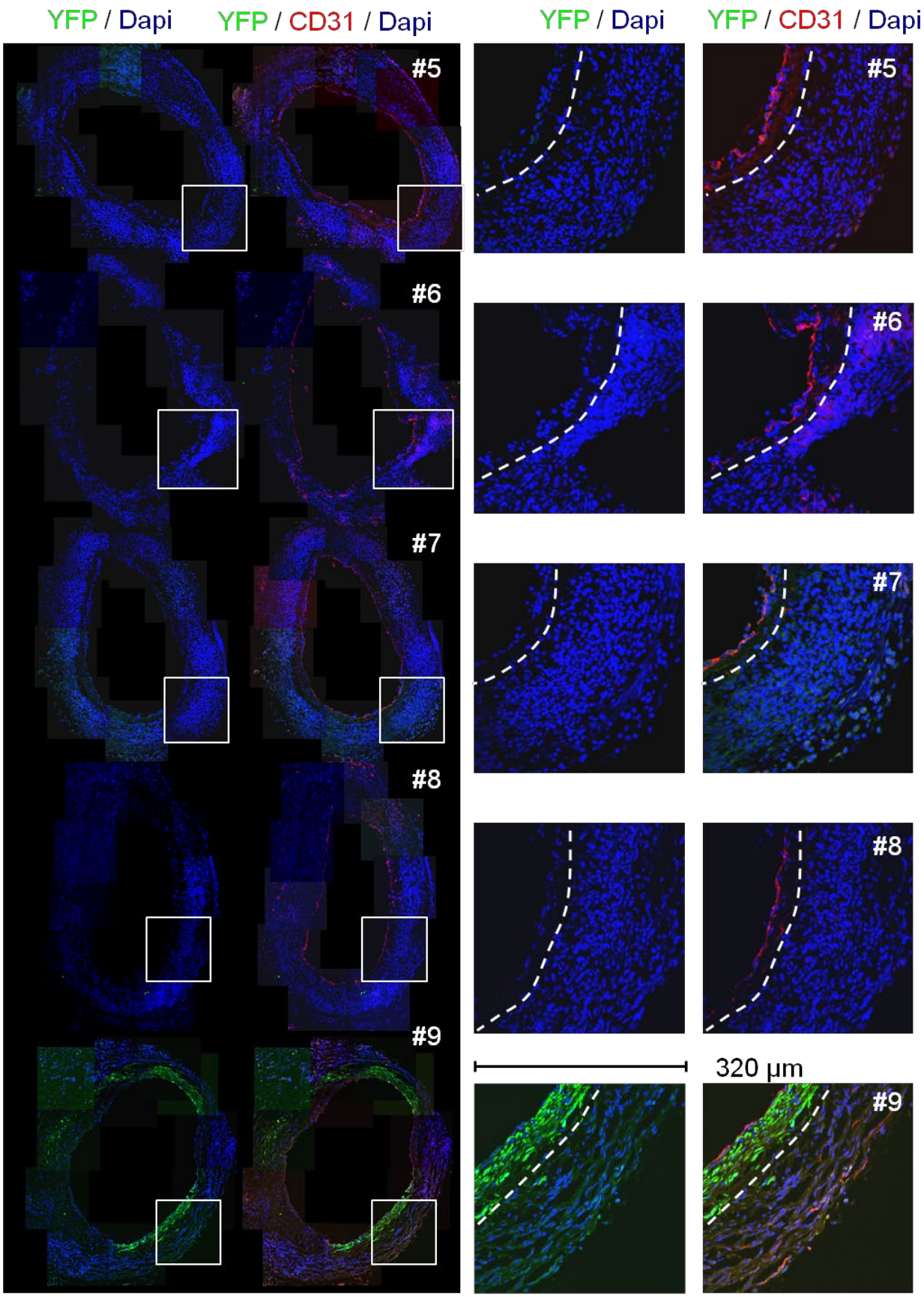
Online Figure XII. The representative images of co-staining of YFP with SMA or CD31 in whole cross-sections of wild type jugular vein (JV) grafts transplanted to carotid arteries (CAs) in which SMCs were genetically labeled with YFP for **Figure 2**. (**A**) Schematic presentation of transplantation of wild type JVs to CAs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection. Total number of VGs is 12. (**B**) The representative images of co-staining of YFP and SMA in whole cross-sections of #1 segment at the proximal end of vein grafts (VGs). (**C**) The representative images of co-staining of YFP and SMA in whole cross-sections of #9 segment at the distal end of vein grafts (VGs). (**D**, **E**) The representative images of co-staining of YFP with SMA or CD31 in whole cross-sections of #3 - #7 segments at the middle part of vein grafts (VGs). YFP is green; SMA and CD31 are red. L, the lumen; IN, the intima; NI, the neointima; Ad, the adventitia. White dotted line separates the NI and the Ad layers.


Donor: (n=12) Recipient:
(JV-WT) (CA-SMC-YFP)
VG-S (n=9)
VG-M (n=3)
YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#1
#2
#3
#4
#1
#2
#3
#4
320 μm

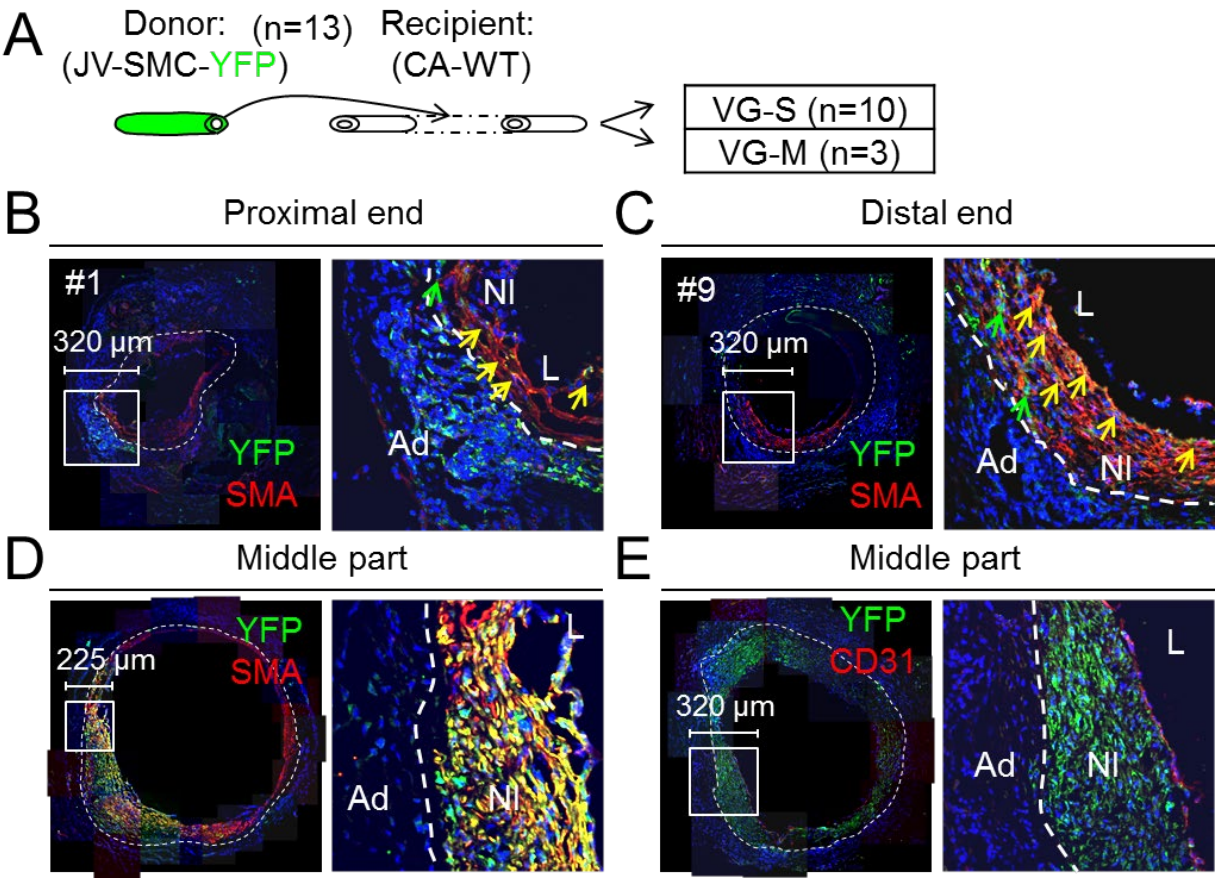

YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#5
#6
#7
#8
#9
320 μm
320 μm

Online Figure XIII. The representative serial immunofluorescence staining of YFP and SMA in consecutive cross-sections of WT jugular veins (JVs) grafted into carotid arteries (CAs) of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection for 6 wks. Vein grafts (VGs) with severe NI formation (n=9) were analyzed as in Online Figure VIIA. Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or SMA across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8, #9) are shown. YFP is green; SMA is red.

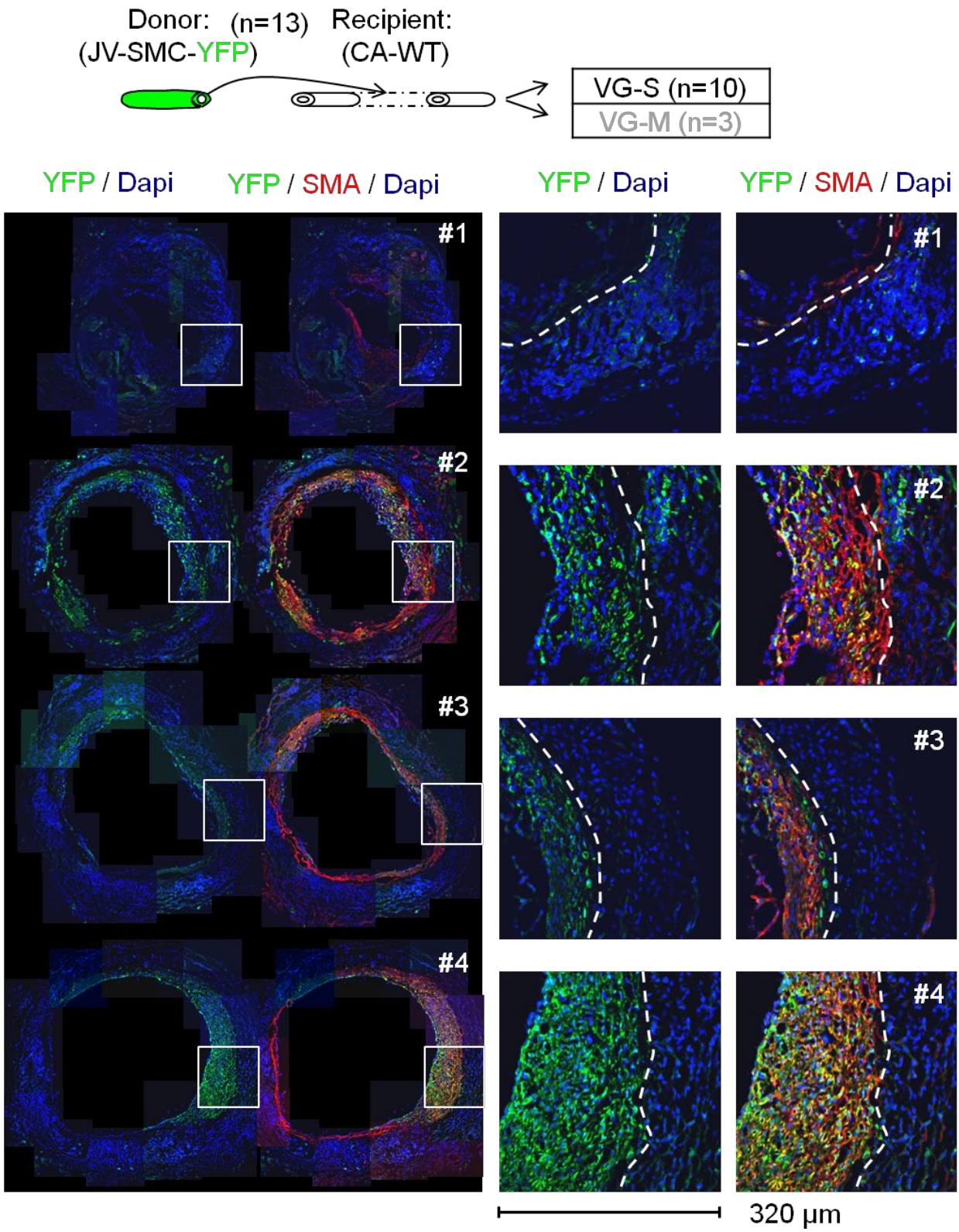

Donor: (n=12) Recipient:
(JV-WT) (CA-SMC-YFP)
VG-S (n=9)
VG-M (n=3)
YFP / Dapi
YFP / CD31 / Dapi
YFP / Dapi
YFP / CD31 / Dapi
#1
#2
#3
#4
320 μm
320 μm

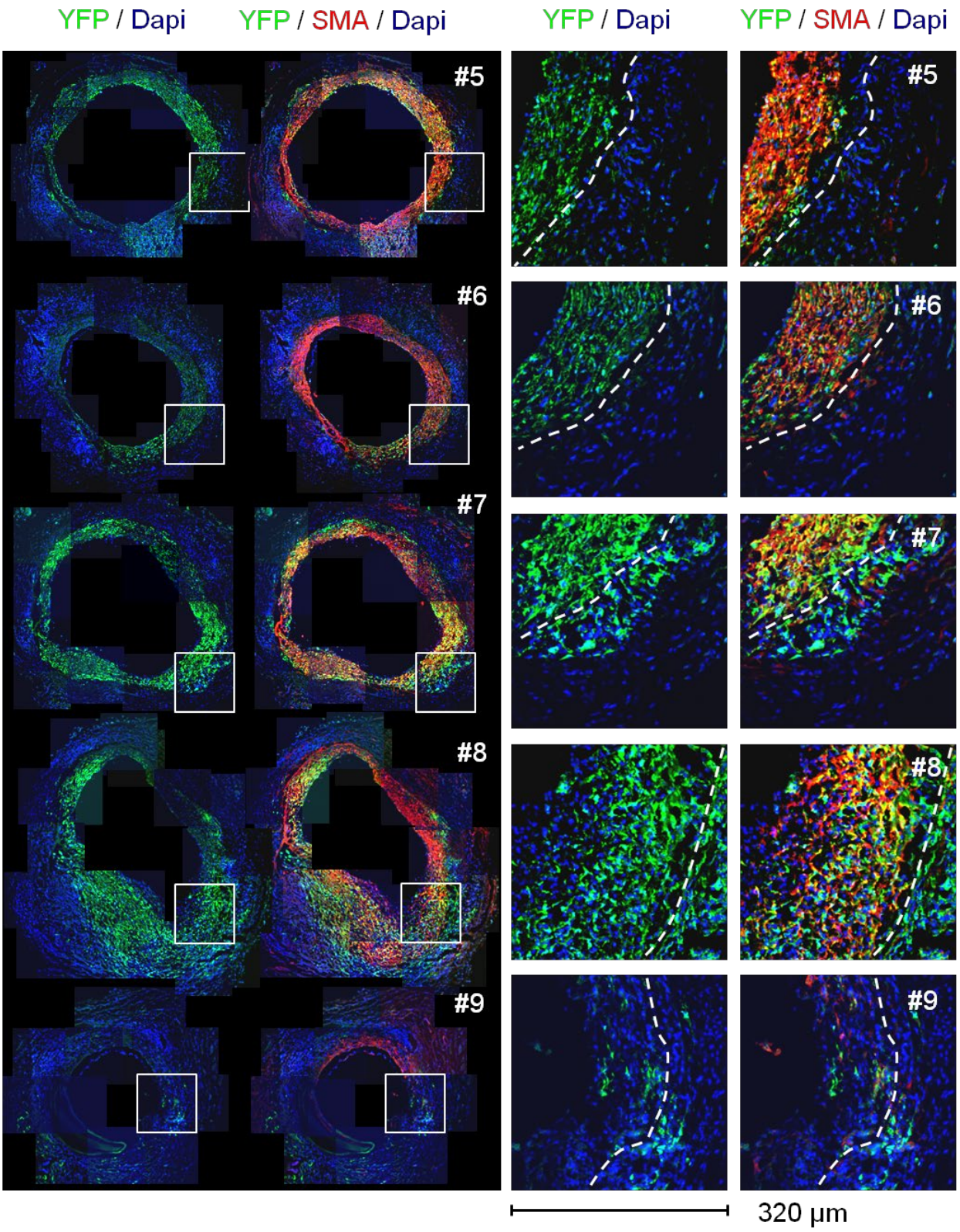

YFP / Dapi
YFP / CD31 / Dapi
YFP / Dapi
YFP / CD31 / Dapi
#5
#6
#7
#8
#9
320 μm

Online Figure XIV. The representative serial immunofluorescence staining of YFP and CD31 in consecutive cross-sections of WT jugular veins (JVs) grafted into carotid arteries (CAs) of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection for 6 wks. Vein grafts (VGs) with mild NI formation (n=3) were analyzed as in Online Figure VIIA. Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or CD31 across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8, #9) are shown. YFP is green; CD31 is red.



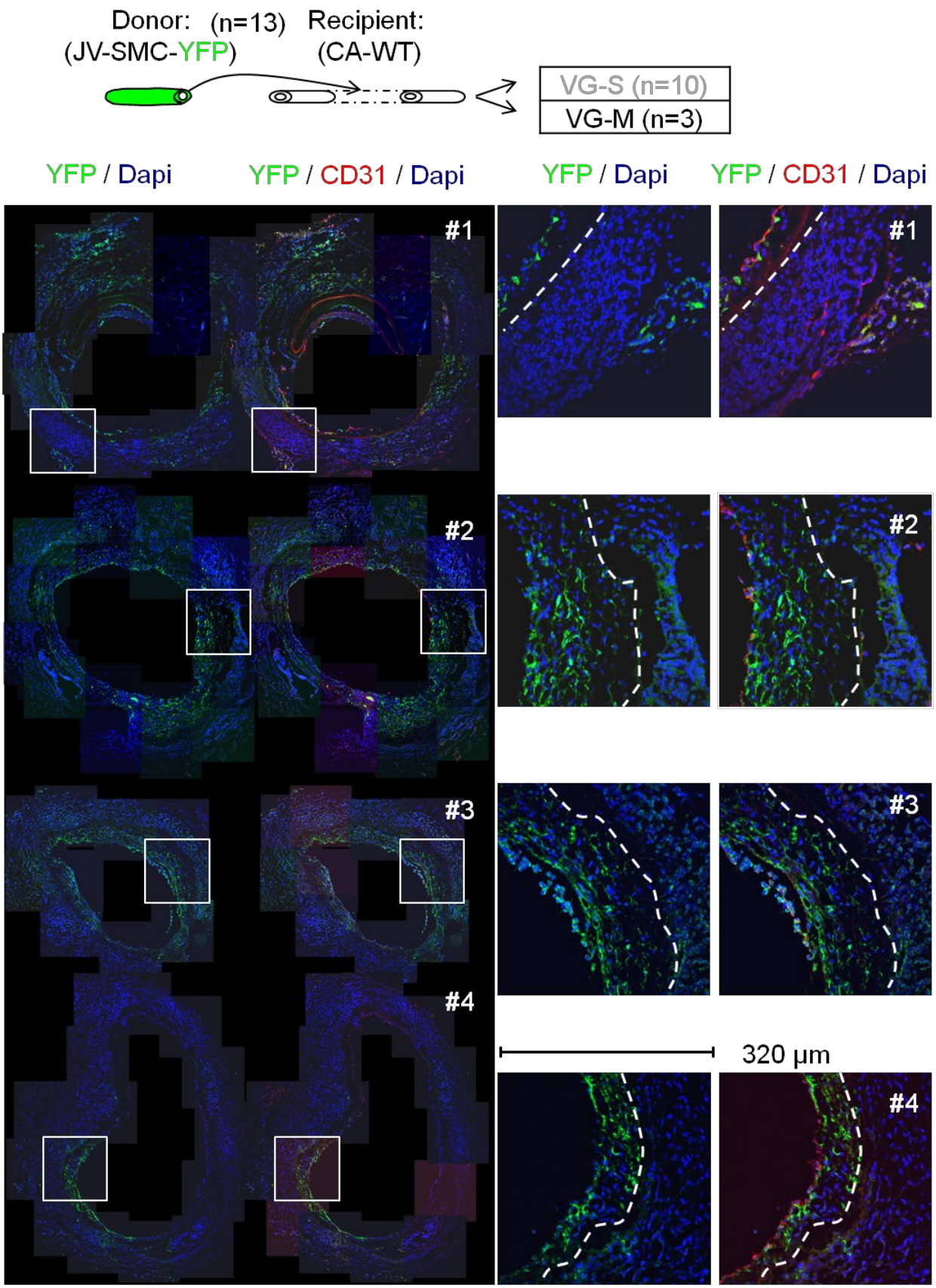
Online Figure XV. The representative images of co-staining of YFP with SMA or CD31 in whole cross-section of jugular vein (JV) grafts in which SMCs were genetically labeled with YFP transplanted to carotid arteries (CAs) of wild type (WT) mice for **Figure 3**. (**A**) Schematic presentation of transplantation of JVs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection to CAs of WT mice. Total number of VGs is 13. (**B**) The representative images of co-staing of YFP and SMA in whole cross-sections of #1 segment at the proximal end of vein grafts (VGs). (**C**) The representative images of co-staining of YFP and SMA in whole cross-sections of #9 segment at the distal end of vein grafts (VGs). (**D**, **E**) The representative images of co-staining of YFP with SMA or CD31 in whole cross-sections of #3 - #7 segments at the middle part of vein grafts (VGs). YFP is green; SMA and CD31 are red. L, the lumen; IN, the intima; NI, the neointima; Ad, the adventitia. White dotted lines separate the NI and the Ad layers.

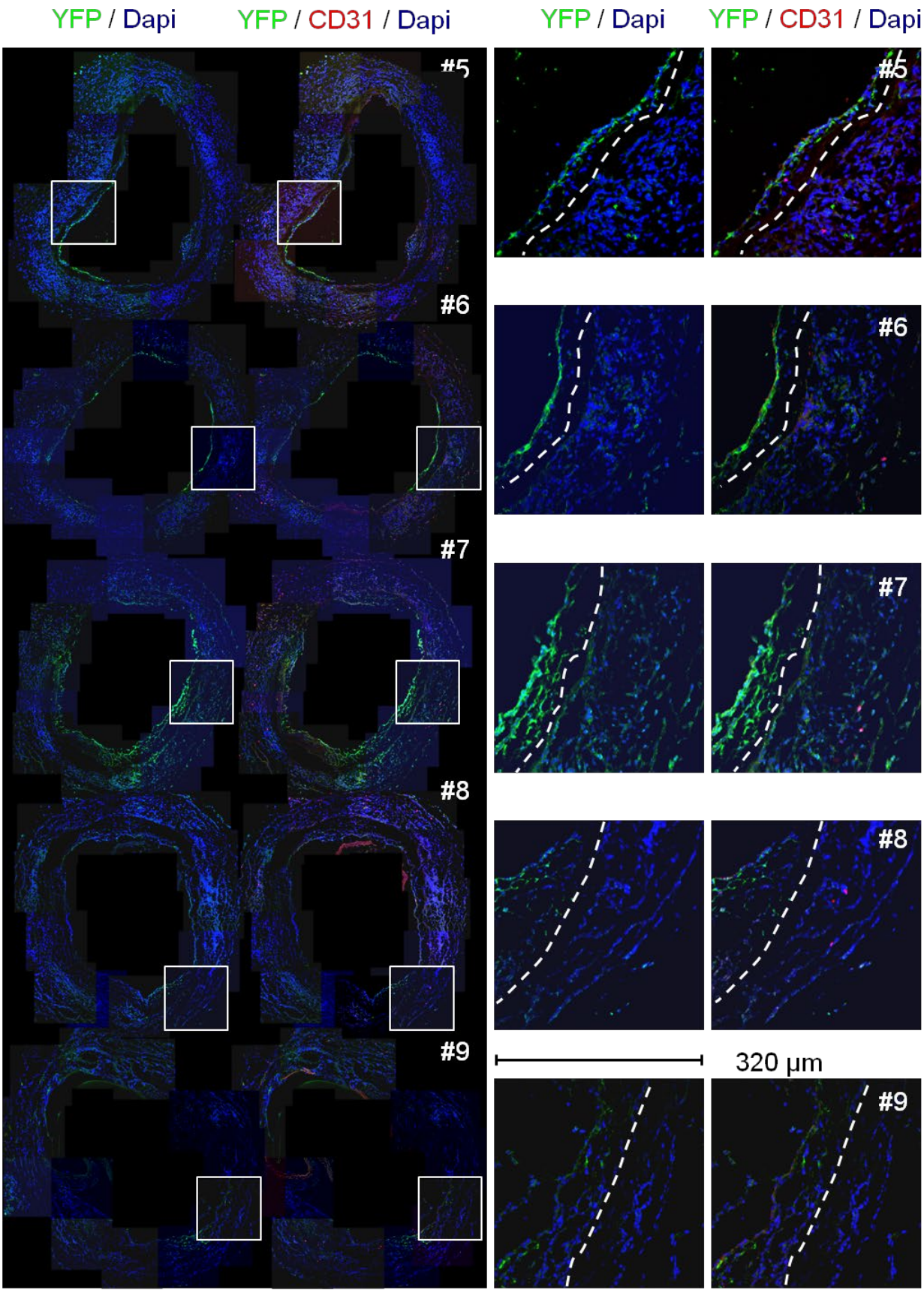

Donor: (n=13)
(JV-SMC-YFP)
Recipient:
(CA-WT)
VG-S (n=10)
VG-M (n=3)
YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#1
#2
#3
#4
#1
#2
#3
#4
320 μm



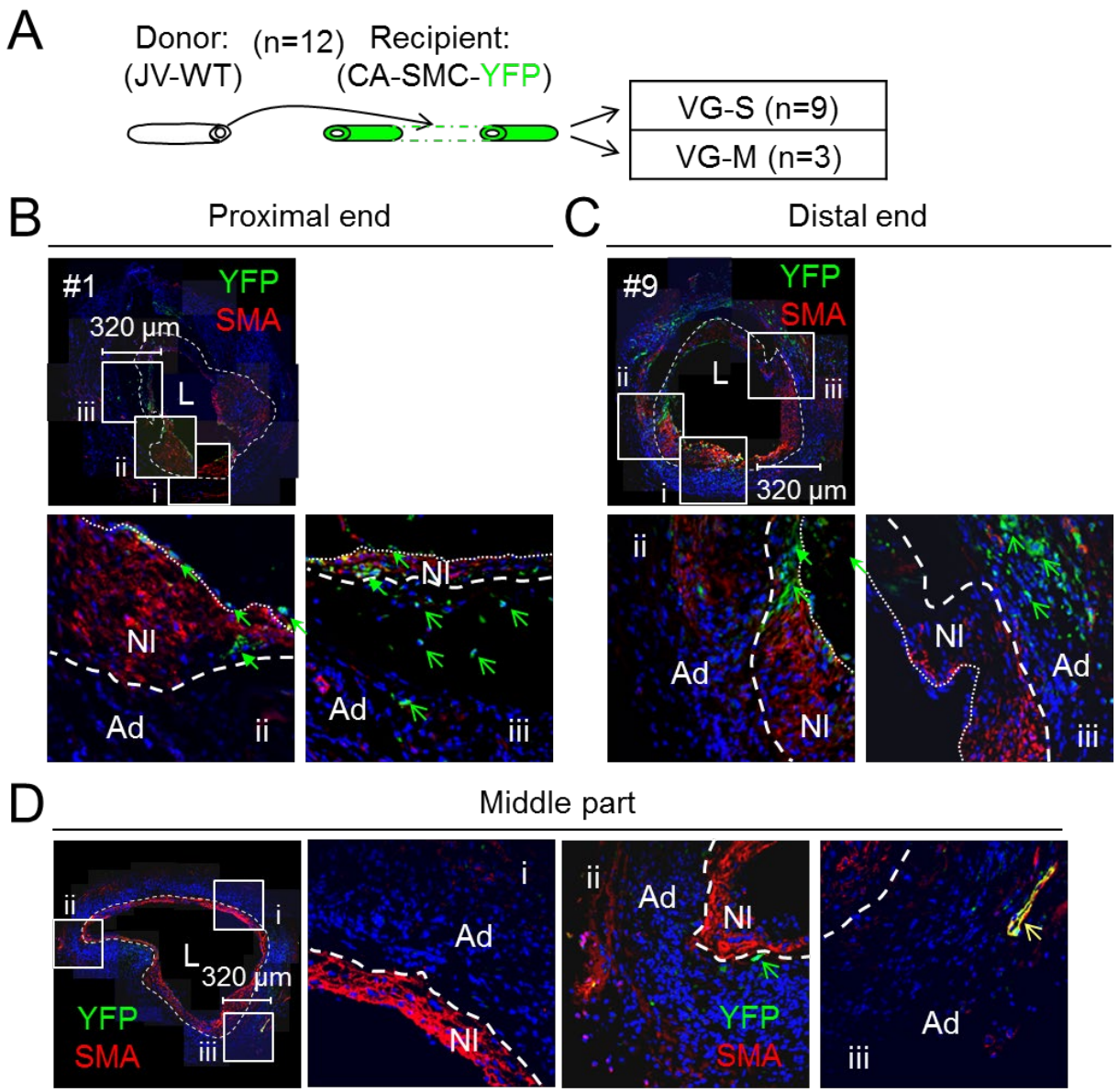
Online Figure XVI. The representative serial immunofluorescence staining of YFP and SMA in consecutive cross-sections of jugular veins (JVs) of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice

after tamoxifen injection grafted into carotid arteries (CAs) of WT mice for 6 wks. Vein grafts (VGs) with severe NI formation (n=10) were analyzed as described in “Methods”. Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or SMA across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8, #9) are shown. YFP is green; SMA is red.

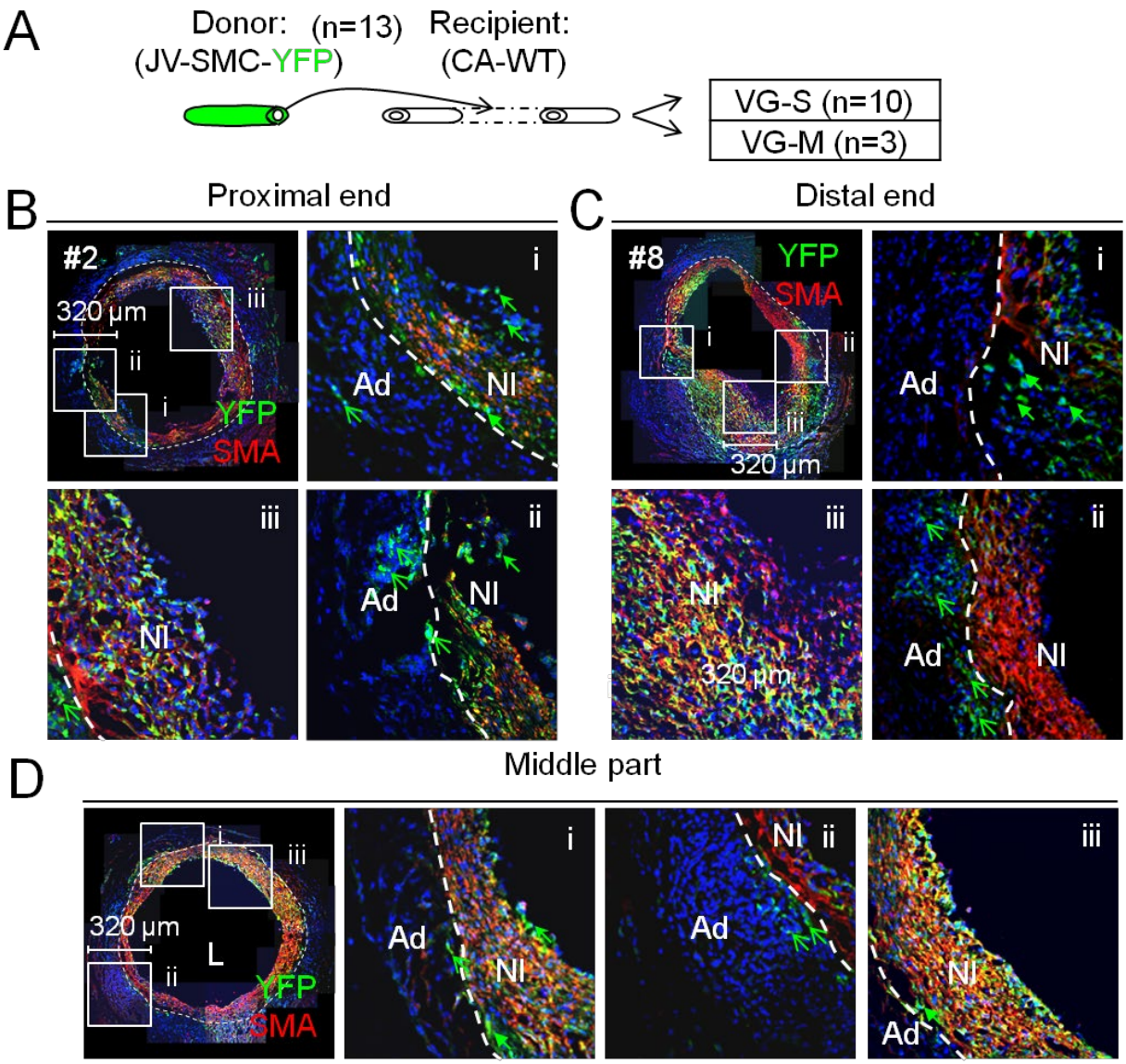

Donor: (n=13) Recipient:
(JV-SMC-YFP) (CA-WT)
VG-S (n=10)
VG-M (n=3)
YFP / Dapi
YFP / CD31 / Dapi
YFP / Dapi
YFP / CD31 / Dapi
#1
#2
#3
#4
#1
#2
#3
#4
320 μm


YFP / Dapi
YFP / CD31 / Dapi
YFP / Dapi
YFP / CD31 / Dapi
#5
#6
#7
#8
#9
320 μm

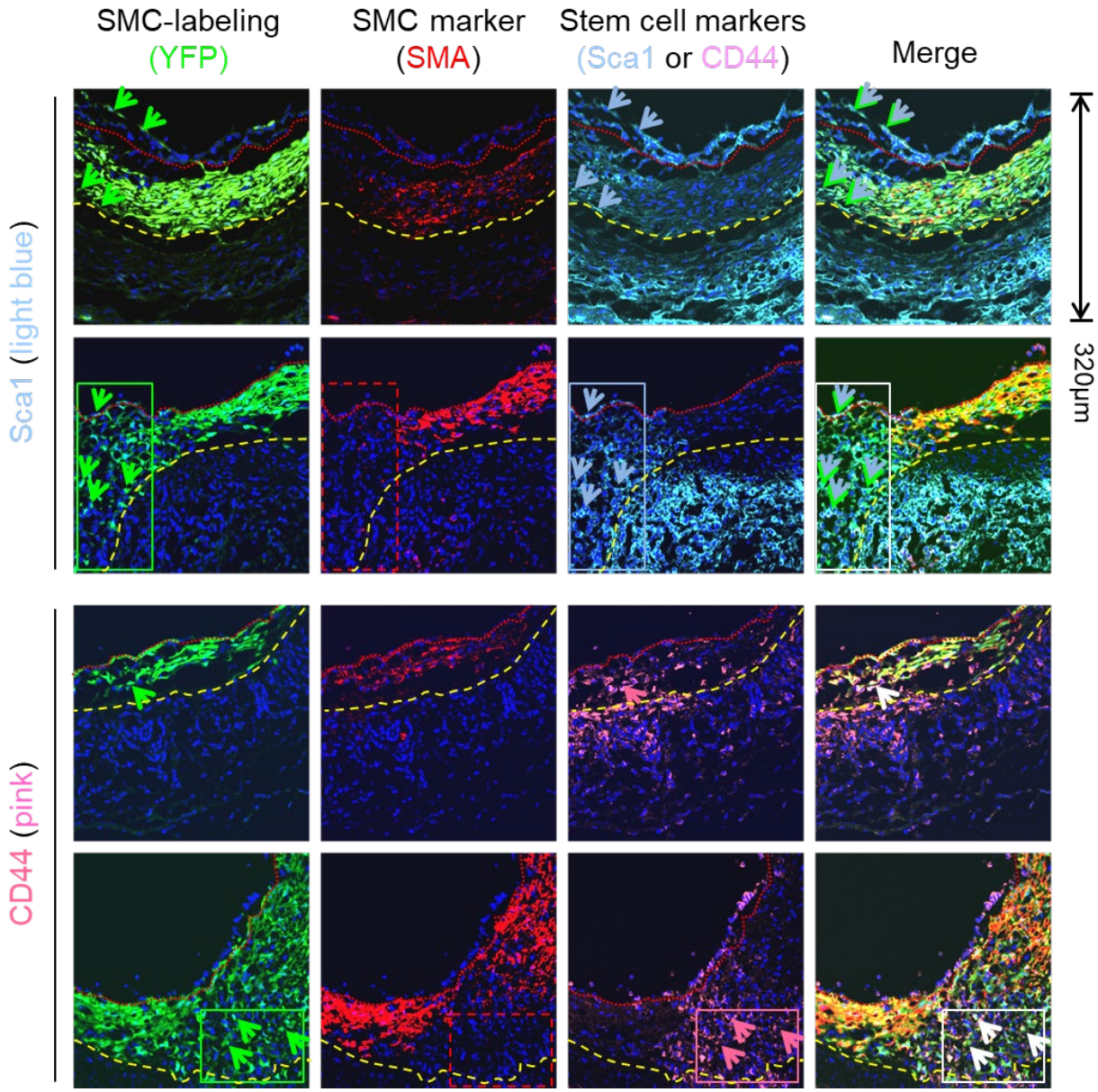
Online Figure XVII. The representative serial immunofluorescence staining of YFP and CD31 in consecutive cross-sections of jugular veins (JVs) of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection grafted into carotid arteries (CAs) of WT mice for 6 wks. Vein grafts (VGs) with mild NI formation (9 sections per VG; 27 sections in total; n=3) were analyzed as described in “Methods”. Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or CD31 across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8, #9) are shown. YFP is green; CD31 is red.



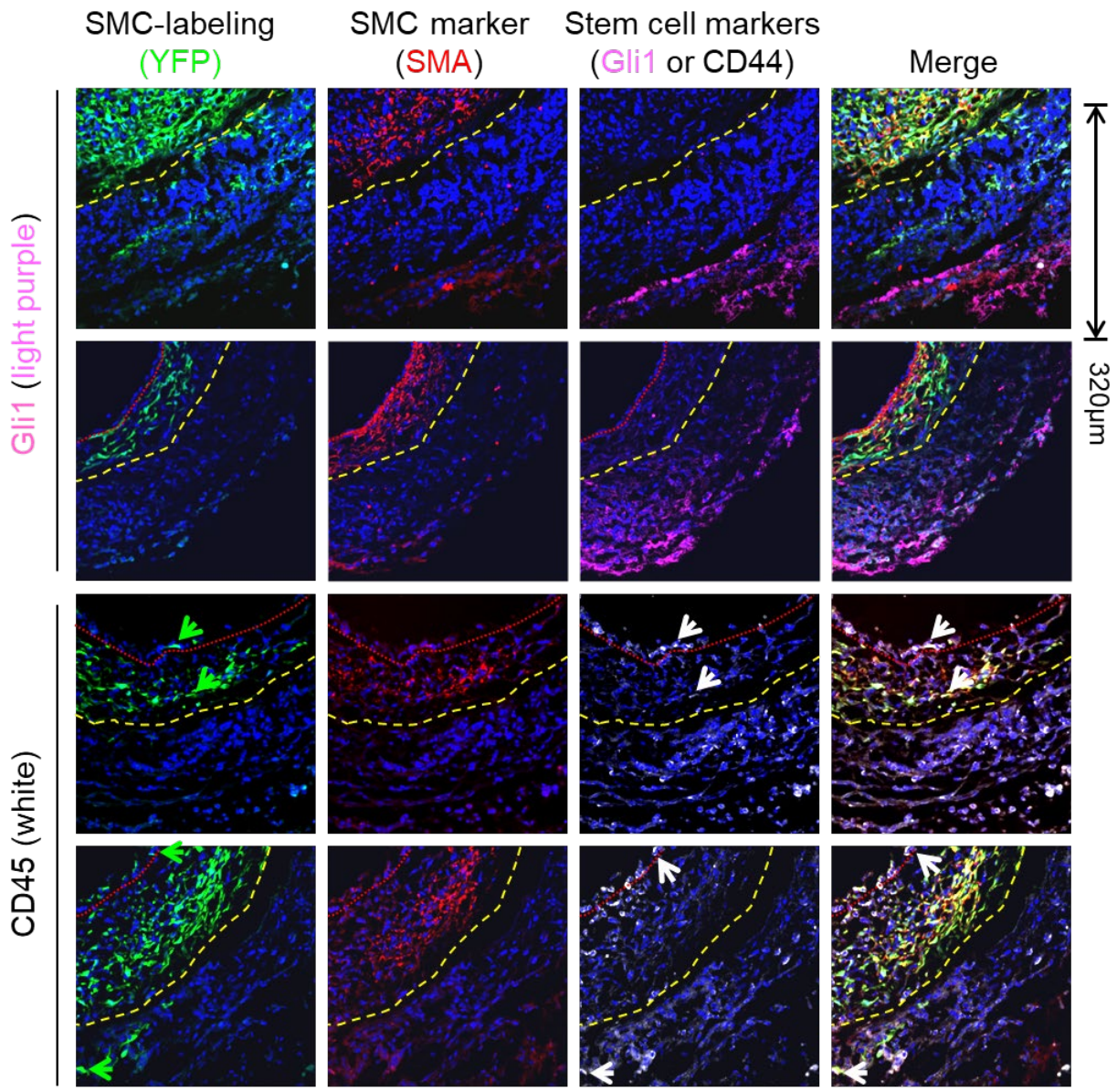
Online Figure XVIII. The representative images of co-staining of YFP with SMA in whole cross-sections of wild type (WT) jugular vein (JV) grafts transplanted to carotid arteries (CAs) of reporter mice in which SMCs were genetically labeled with YFP for **Figure 4**. (**A**) Schematic presentation of transplantation of JVs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection to CAs of WT mice. Total number of VGs is 13. (**B**) The representative images of co-staining of YFP and SMA in whole cross-sections of #2 segment at the proximal end of vein grafts (VGs). (**C**) The representative images of co-staining of YFP and SMA in whole cross-sections of #8 segment at the distal end of vein grafts (VGs). The images (**B**, **C**) of ii and iii at high magnifications highlight the non-SMC cells derived from mature SMCs in VGs. (**D**) The representative images of co-staining of YFP with SMA in whole cross-sections of #3 - #7 segments at the middle part of vein grafts (VGs). The images of i to iii at high magnifications highlight the non-SMC cells derived from mature SMCs in VGs. YFP is green; SMA is red. L, the lumen; IN, the intima; NI, the neointima; Ad, the adventitia. White dotted line separates the NI and the Ad layers.



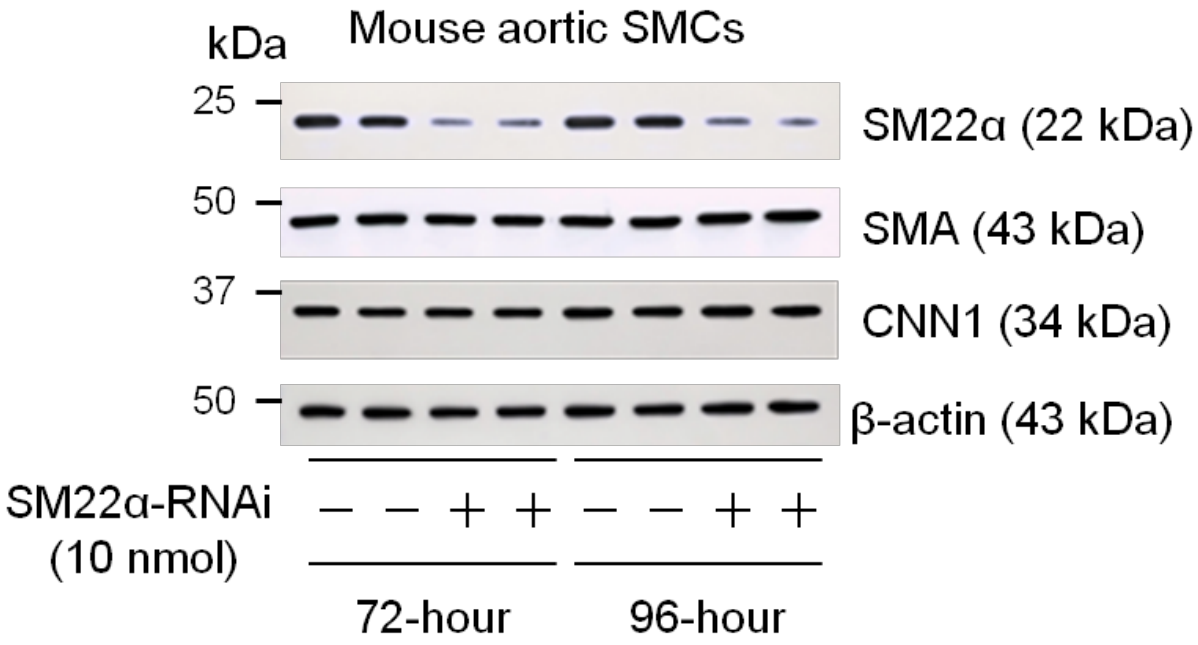
Online Figure XIX. The representative images of co-staining of YFP with SMA in whole cross-sections of) jugular vein (JV) grafts of reporter mice in which SMCs were genetically labeled with YFP transplanted to carotid arteries (CAs) of wild type (WT) mice for **Figure 4**. (**A**) Schematic presentation of transplantation of wild type JVs to CAs of *Myh11*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection. Total number of VGs is 12. (**B**) The representative images of co-staining of YFP and SMA in whole cross-sections of #1 segment at the proximal end of vein grafts (VGs). (**C**) The representative images of co-staining of YFP and SMA in whole cross-sections of #9 segment at the distal end of vein grafts (VGs). (**D**) The representative images of co-staining of YFP with SMA in whole cross-sections of #3 - #7 segments at the middle part of vein grafts (VGs). The images (**B**-**D**) of i to iii at high magnifications highlight the non-SMC cells derived from mature SMCs in VGs. YFP is green; SMA is red. L, the lumen; IN, the intima; NI, the neointima; Ad, the adventitia. White dotted line separates the NI and the Ad layers.



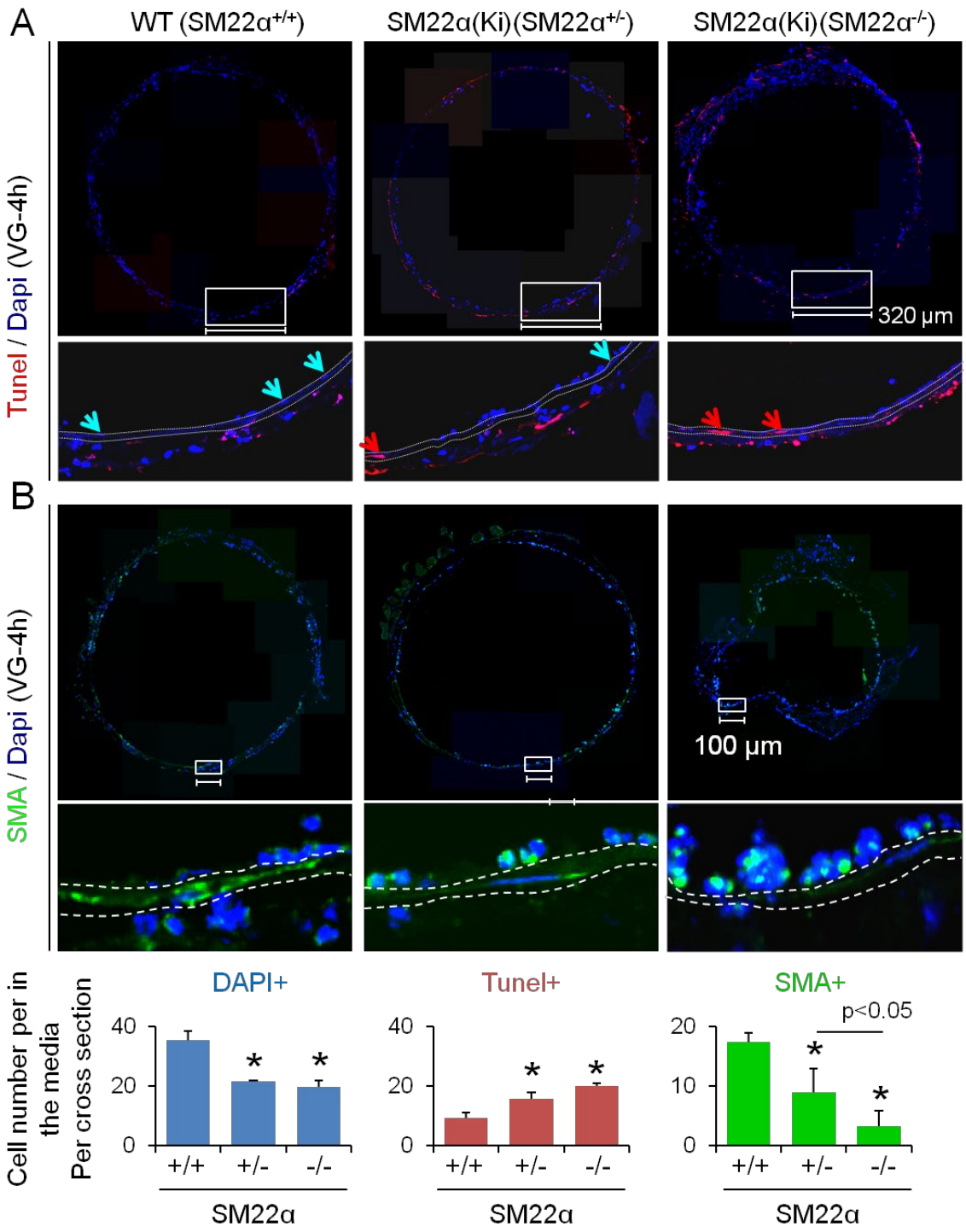
Online Figure XX. The expression of Sca1, a vascular stem cell maker, and CD44, a mesenchymal stem cell marker in VGs 42 days after transplantation. Jugular vein s (JVs) of 3-month old mice in which SMCs were genetically labeled with YFP were transplanted into carotid arteries of 3-month old wild type mice for 42 days. Tissue cross-sections of the 42-day VGs (n=13) were randomly selected for immunofluorescence staining of Sca1 or CD44 with YFP and SMA. The results are representative co-staining of Sca1 or CD44 with YFP and SMA. Arrows indicate the expression of Sca1 (light blue) or CD44 (pink) in YFP+SMA- cells.



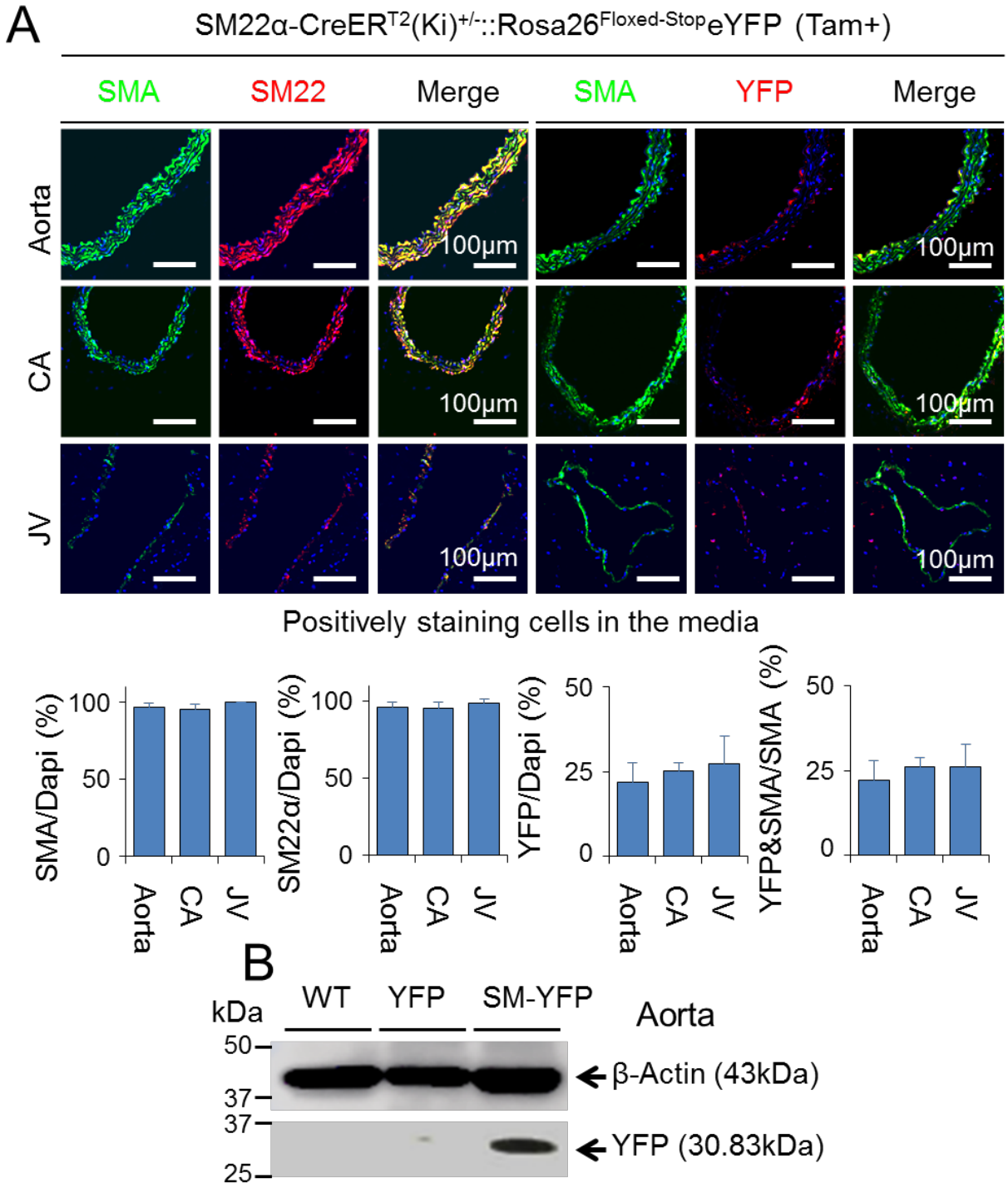
Online Figure XXI. The expression of Gli1, a vascular stem cell maker, and CD45, a macrophage progenitor marker in VGs 42 days after transplantation. Jugular vein s (JVs) of 3-month old mice in which SMCs were genetically labeled with YFP were transplanted into carotid arteries of 3-month old wild type mice for 42 days. Tissue cross-sections of the 42-day VGs (n=13) were randomly selected for immunofluorescence staining of Gli11 or CD45 with YFP and SMA. The results are representative co-staining of Gli1 or CD44 with YFP and SMA. Arrows indicate the expression of CD45 (white) in YFP+SMA- cells.

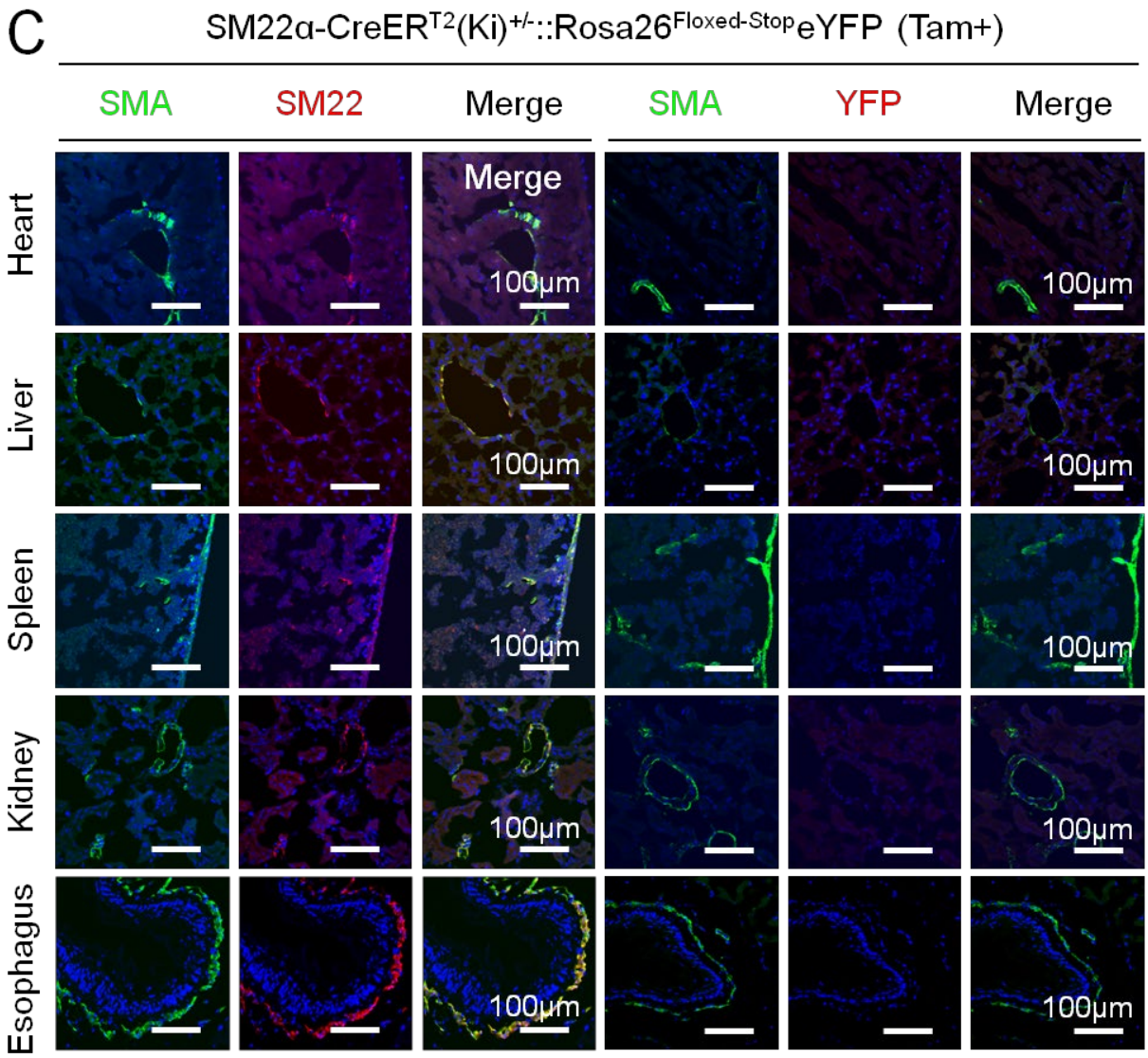


Online Figure XXII. The impact of SM22α knockdown on SMC marker expression. Cultured mouse aortic SMCs were transfected with scramble control RNAi sequences (si-Ctl) and the SM22α siRNAs as described in "Methods". At 72 and 96 h after transfection, the efficacy of SM22α knockdown and the impact of SM22α downregulation on SMA and CNN1 expression were determined by Western blot analysis. The representative results from 3 separated experiments are shown.

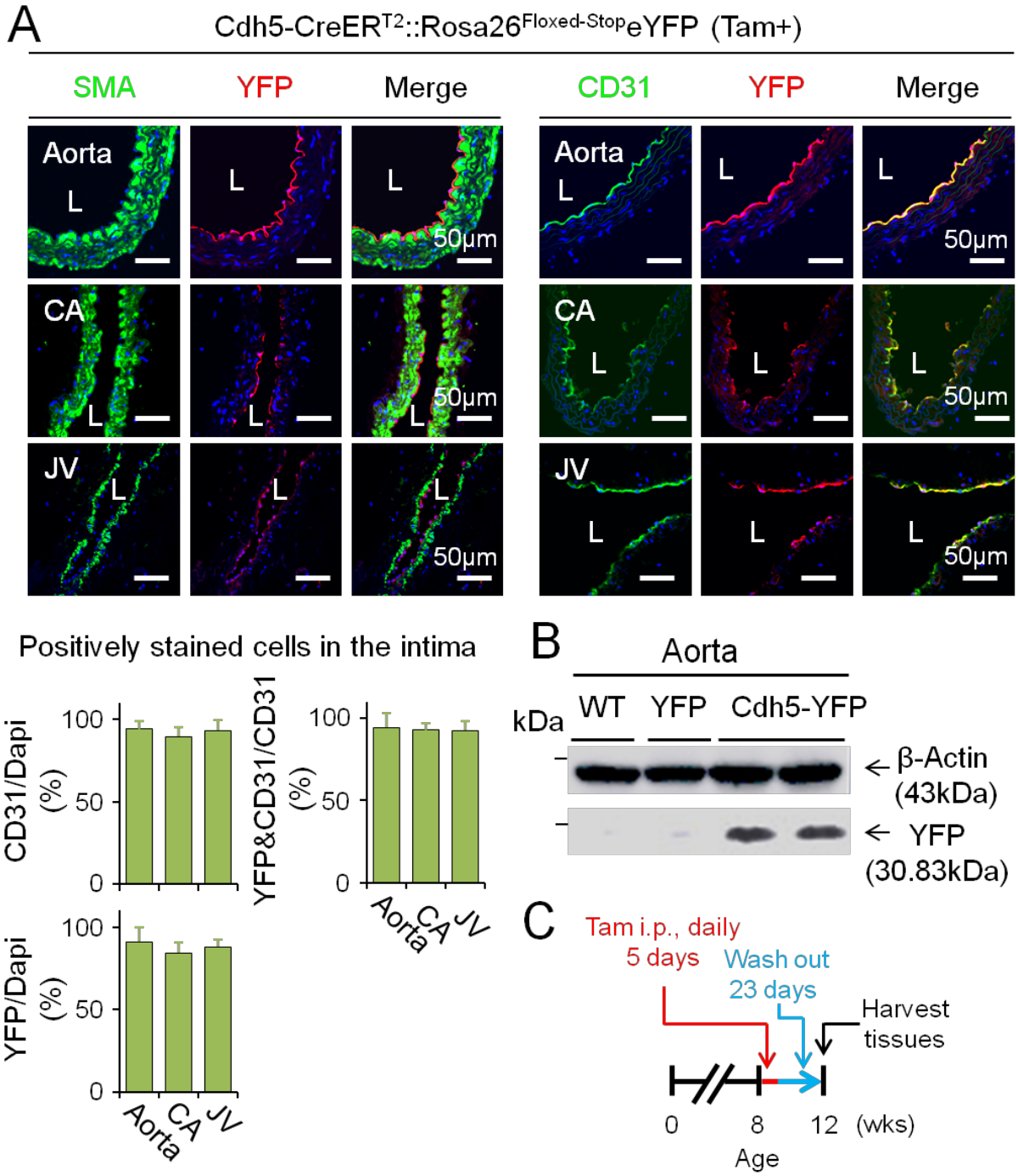


Online Figure XXIII. Downregulation of SM22α expression in vascular SMCs promotes SMC death in VGs. Jugular veins (JVs) of WT, SM22α(Ki)$^{+/-}$, and SM22α(Ki)$^{-/-}$ mice at age of 3 months were transplanted into WT carotid arteries (n=4) for 4 hours. (**A**) Cell death in the media of these VGs was determined by Tunel staining (The whole cross section of Figure V-B). (**B**) Representative picture for SMA and Dapi staining. SMA^{+} and Dapi^{+} cells were determined in the media. The results are means ± SD. *p<0.01 vs the WT (SM22α+/+) group.


A
SM22α-CreER^{T2}(Ki)$^{+/-}$::Rosa26$^{Floxed\text{-}Stop}$eYFP (Tam+)
SMA
SM22
Merge
SMA
YFP
Merge
Aorta
CA
JV
100μm
Positively staining cells in the media
SMA/Dapi (%)
SM22α/Dapi (%)
YFP/Dapi (%)
YFP&SMA/SMA (%)
Aorta
CA
JV
B
kDa
WT
YFP
SM-YFP
Aorta
50
37
37
25
β-Actin (43kDa)
YFP (30.83kDa)

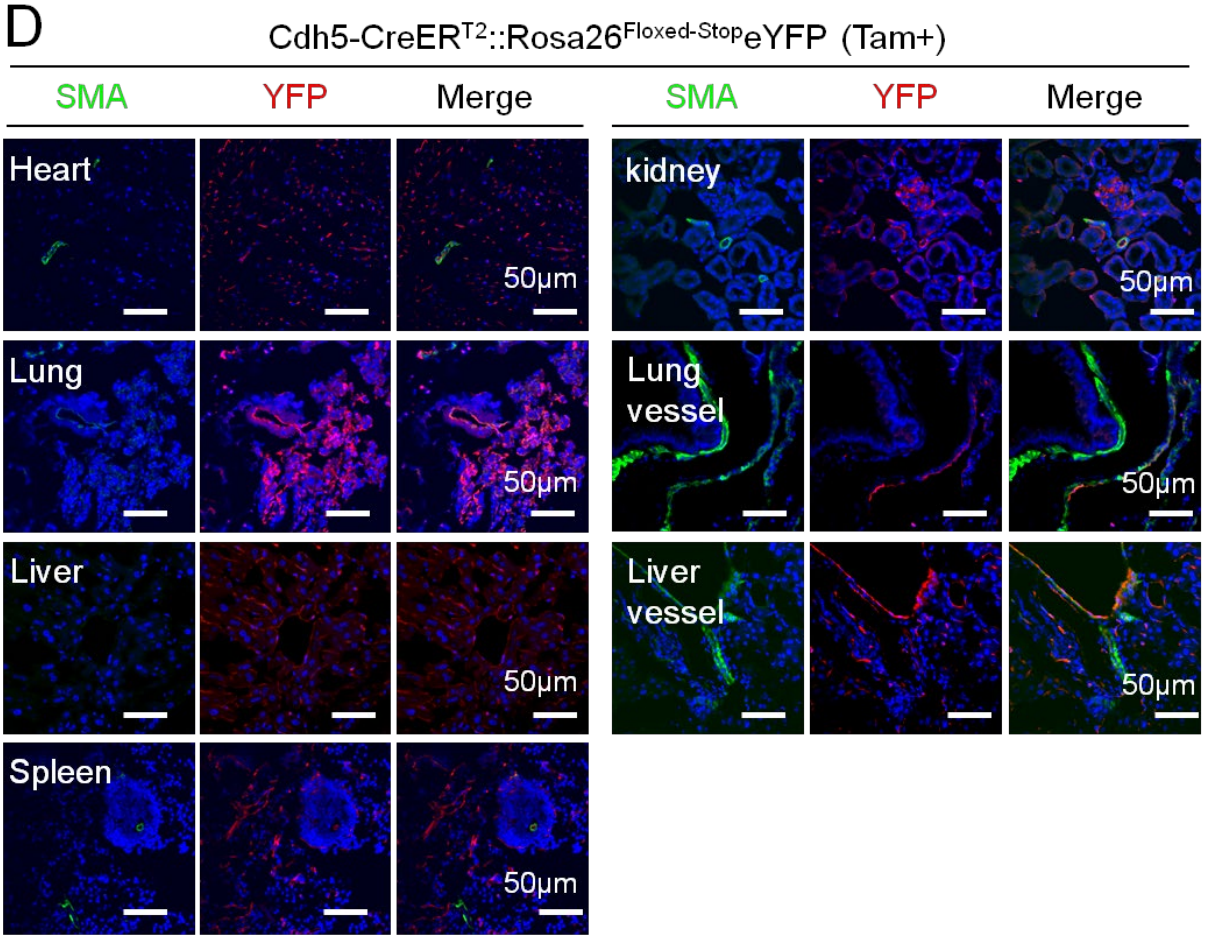


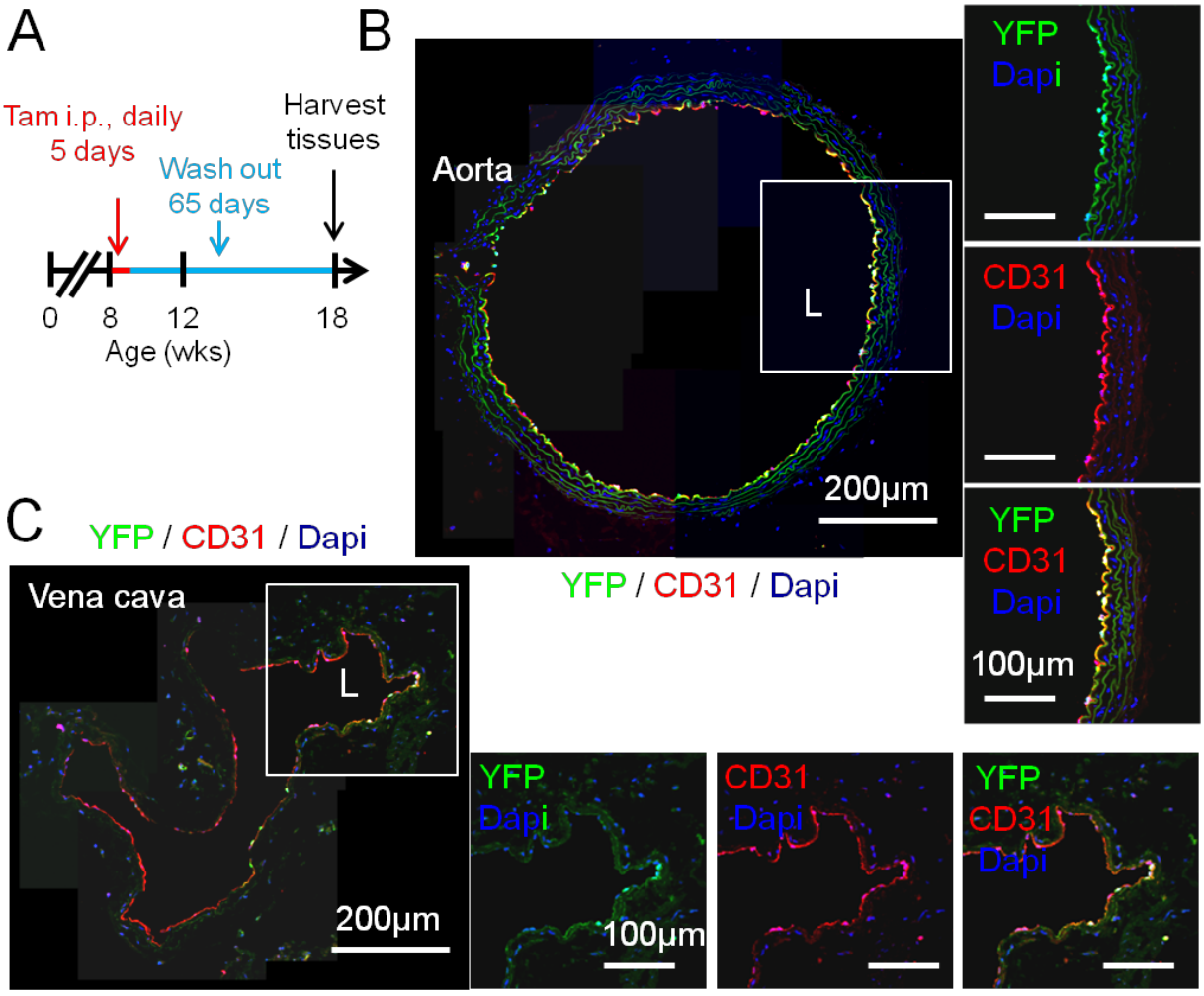
Online Figure XXIV. Characterization of genetically YFP-labeled SMCs in *SM22α* (Ki)-CreER^{T2} ::Rosa26$^{Floxed-Stop}$eYFP mice after tamoxifen injection (Tam+). *SM22α* (Ki)-CreER^{T2} ::Rosa26$^{Floxed-Stop}$eYFP mice (n=5) at age of 2 months were injected with tamoxifen as described in Online Figure I and then housed without any treatment for additional 1 month prior to the tissue harvest for immunochemical staining of SMA, SM22α and YFP. (**A**) Quantified cell numbers positive for SMA, SM22α and YFP as well as the number positive for both SMA and YFP in SMA positive cells in the tunica media of various vessels as indicated. The top panel shows the representative images of SMA, SM22 and YFP in the aorta, carotid artery (CA) and jugular vein (JV). The biomarker analyses were carried out as described in "Methods". (**B**) Western blot analysis of YFP expression in the aorta of wild type (WT), Rosa26$^{Floxed-Stop}$eYFP (YFP) and *SM22α* (Ki)-CreER^{T2} ::Rosa26$^{Floxed-Stop}$eYFP mice after tamoxifen injection (SM-YFP). Whole aortas (n=3) were collect the same time end points as (**A**). The aortas from male and age-matched wild type (WT) and Rosa26$^{Floxed-Stop}$eYFP mice without tamoxifen treatment were used as the control. (**C**) Representative images showing YFP labeled of SMCs in different organs including the heart, the esophagus, the muscles, the lungs and the spleen.



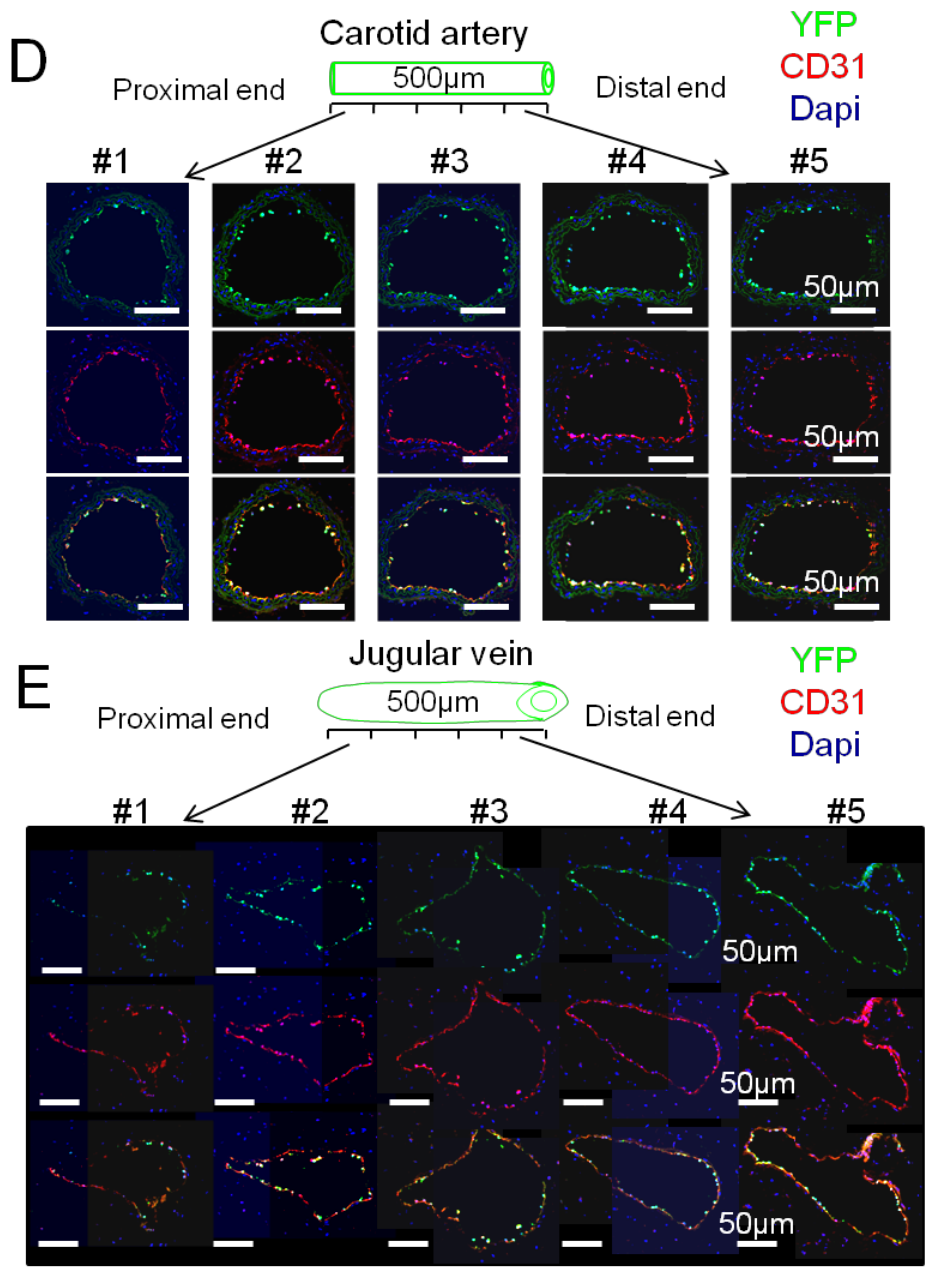
Online Figure XXV. Tamoxifen-induced YFP-labeling efficiencies in ECs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice at the baseline. Male *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (n=5) were treated with five pulses of tamoxifen at age of 8 wks and then housed for additional 23 days without any treatment prior to the tissue harvest as indicated in (**C**). (**A**) Co-staining of YFP (green) with SMA or CD31 in the aorta, carotid artery (CA) and jugular vein (JV). The top panel shows the representative images and the lower shows quantified percentages of CD31 or YFP positive cells as well as YFP and CD31 double positive cells in CD31 positive cells in the intima of these vessels. (**B**) Western blot analysis of YFP expression in the aorta. Whole aortas of Cdh5-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection (Tam+)

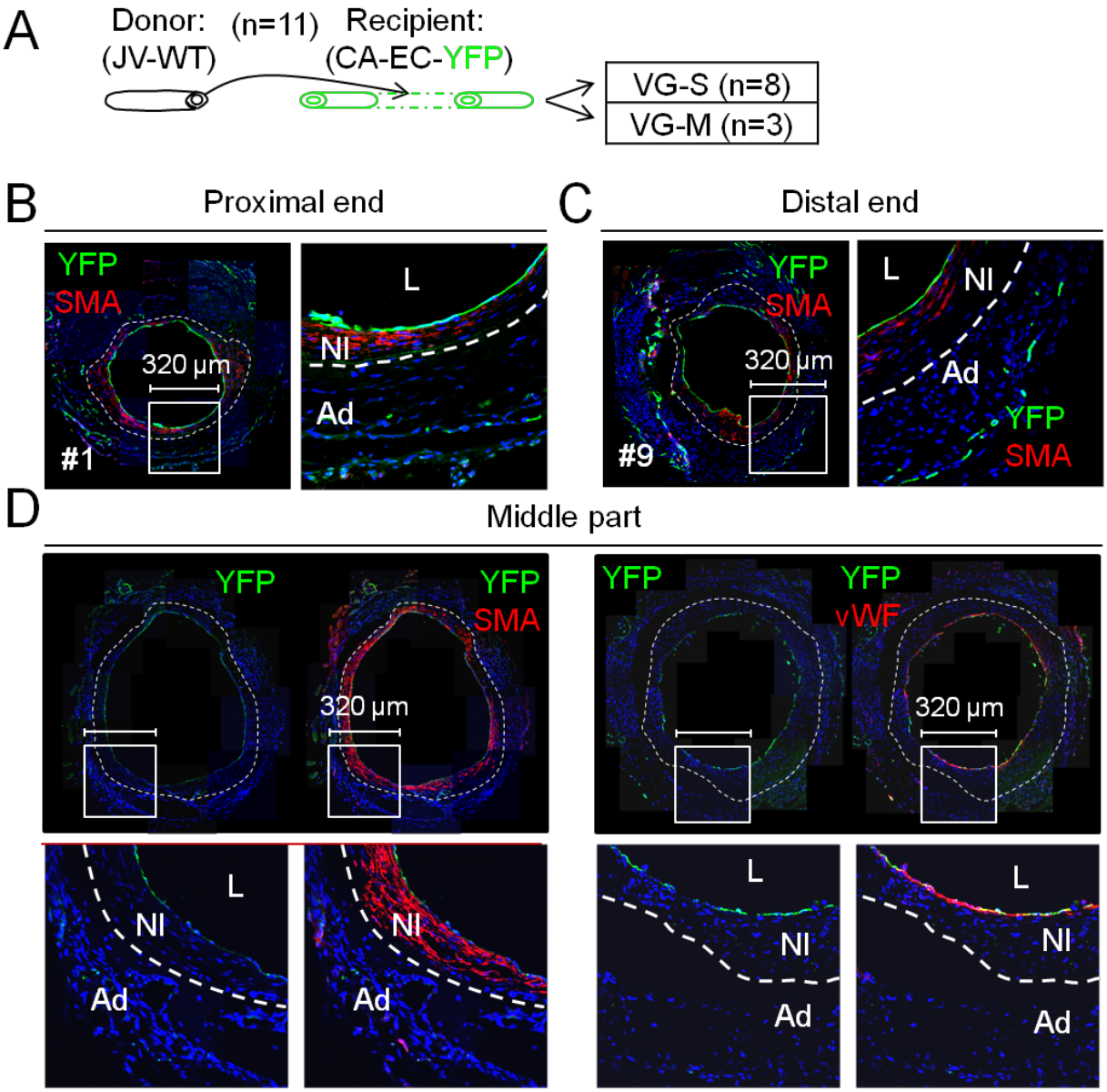
(Cdh5-YFP) were collected at the experimental end point as indicated in (**C**). The aortas of wild type (WT) mice (n=3) and Rosa26$^{Floxed\text{-}Stop}$eYFP (YFP) mice with the same age to the Cdh5-YFP mice were harvested as the control for the Western blot analysis of YFP expression. The typical immunoblots of YFP expression are shown. (**D**) Representative images showing YFP labeled cells in different organs including the heart, the liver, the spleen, the lungs and the kidneys.



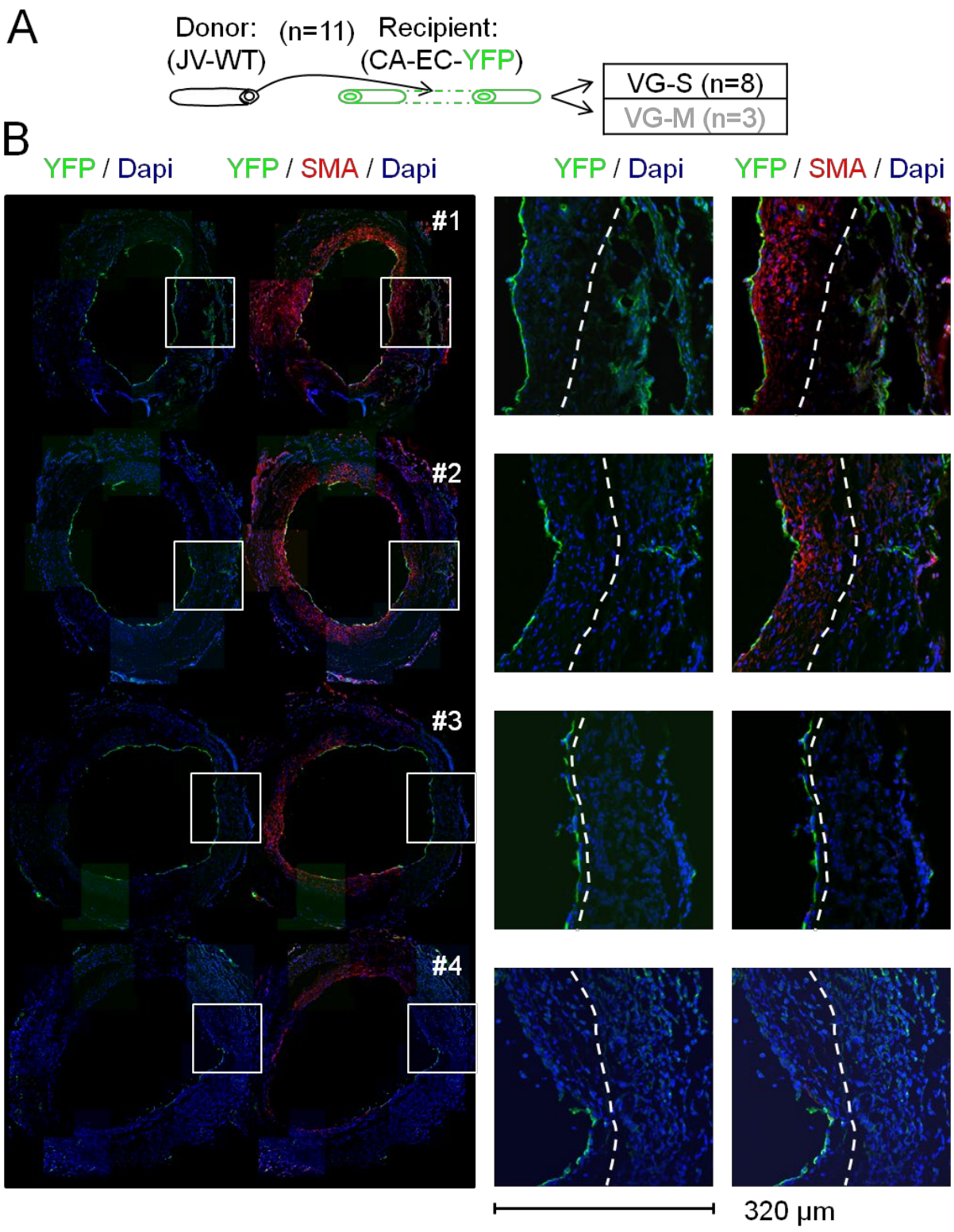


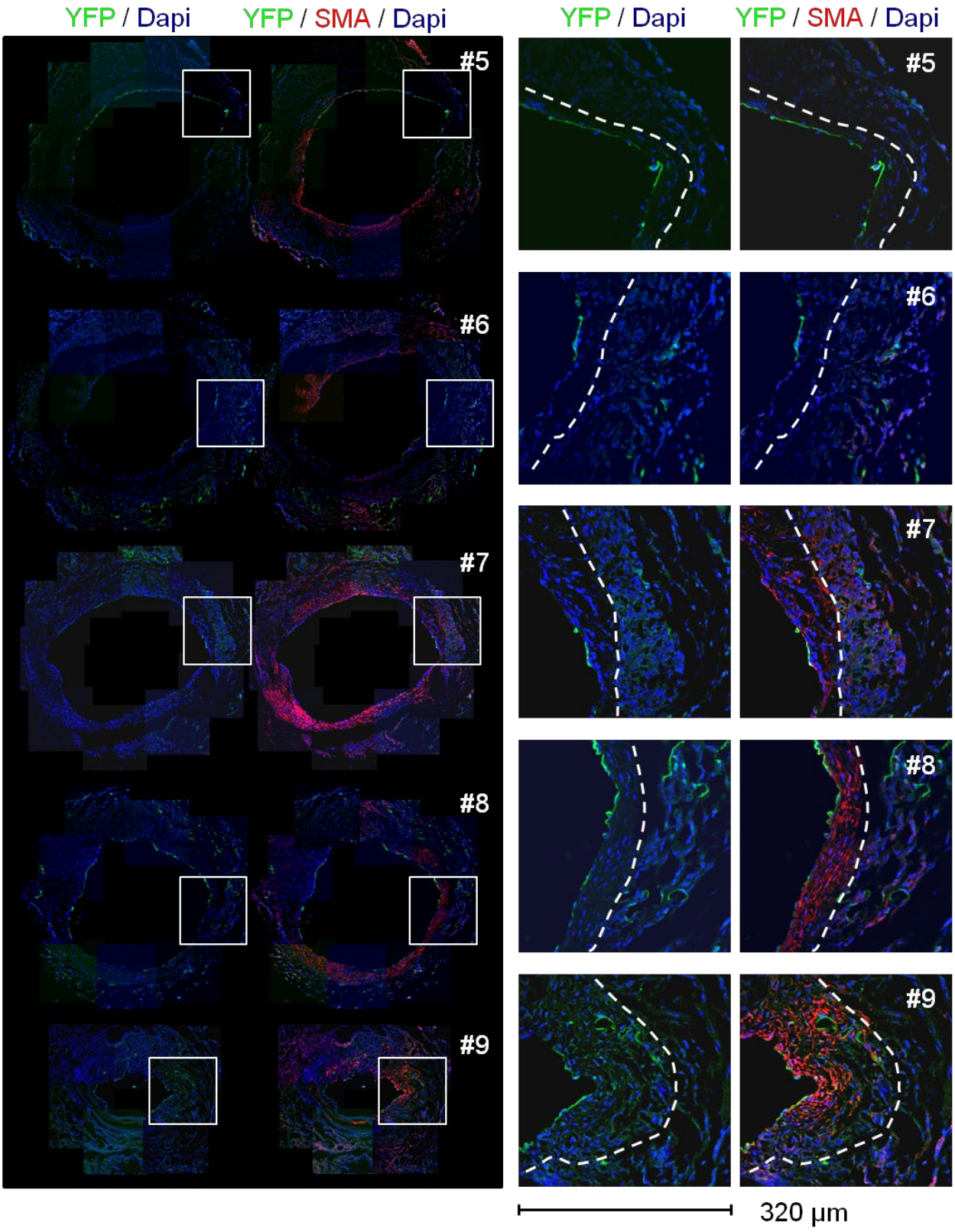
Online Figure XXVI. Tamoxifen-induced YFP-labeling efficiencies in ECs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice at the time of experimental end point. Male *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice (n=5) were treated with five pulses of tamoxifen at age of 8 wks and housed without any treatment for additional 65 days prior to the tissue harvest as indicated in (**A**). (**B**, **C**) Representative images of co-staining of YFP and CD31 in the aorta and the vena cava. (**D**, **E**, **F**) Quantified YFP-labeling efficiency in ECs of the aorta, the carotid artery and the jugular vein. (**D**, **E**) Representative images of co-staining of YFP (green) and CD31 (red) in the whole vessel cross-sections across a randomly selected 500-µm length of carotid arteries and jugular veins. (**F**) Quantified percentages of YFP and/or CD31 positive cells in the intima. Data are showed as means ± SD (15 cross-sections in total, 5 sections per mice, n=3 mice).


D
Carotid artery
Proximal end
500μm
Distal end
YFP
CD31
Dapi
#1
#2
#3
#4
#5
50μm
50μm
50μm
E
Jugular vein
Proximal end
500μm
Distal end
YFP
CD31
Dapi
#1
#2
#3
#4
#5
50μm
50μm
50μm
F
Positively stained cells in the intima
CD31/Dapi (%)
YFP/Dapi (%)
100
50
0
Aorta CA JV
YFP&CD31/CD31 (%)
100
50
0
Aorta CA JV



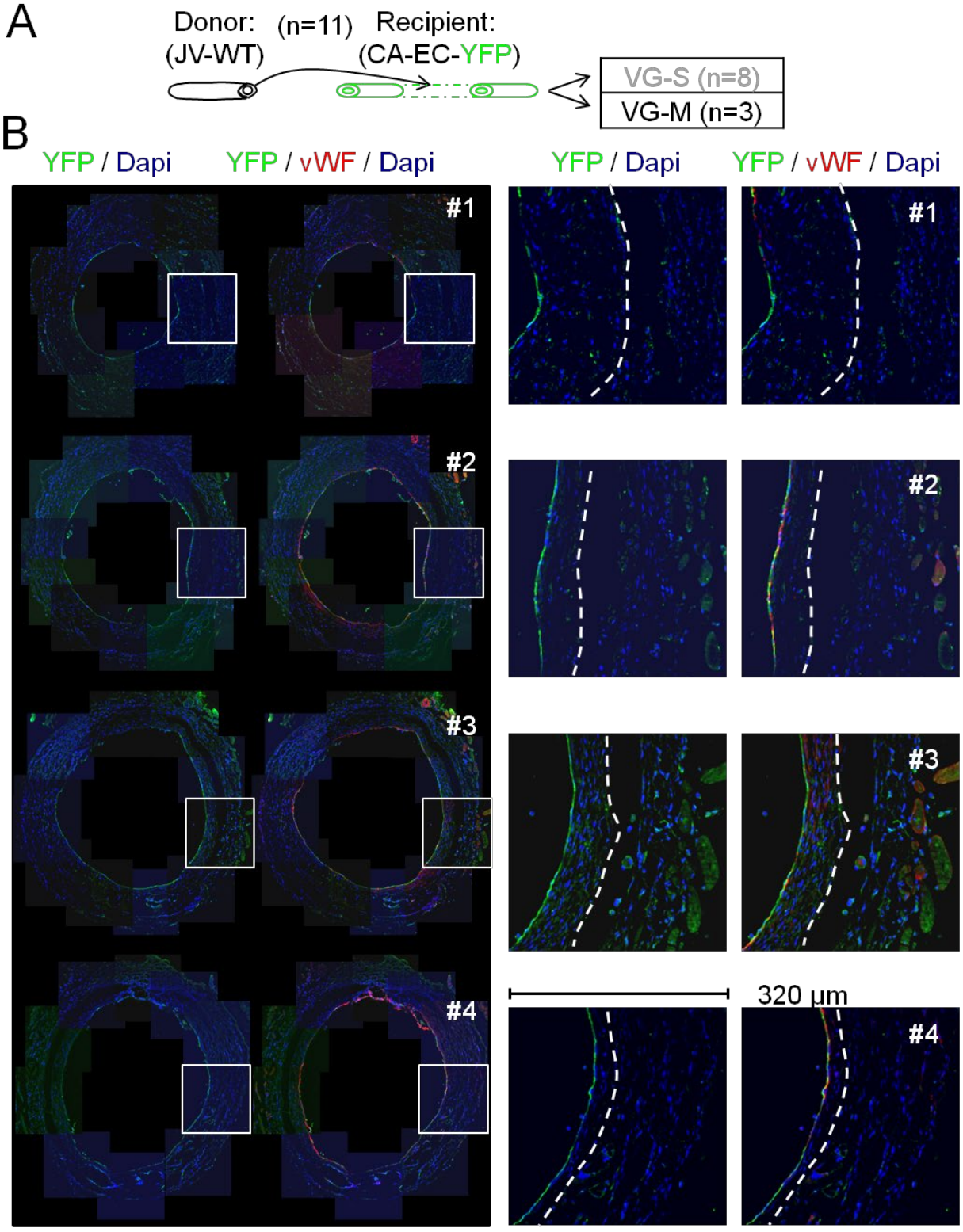
Online Figure XXVII. The representative images of co-staining of YFP and/or SMA or vWF in whole vessel cross-sections of wild type (WT) jugular veins (JVs) grafted to carotid arteries (CAs) of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen inject (for **Figure 6**). (**A**) Schematic presentation of transplantation of WT JVs to CAs of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection. Total number of VGs is 11. (**B**) The representative YFP and SMA staining in whole vessel cross-sections of #1 segments at the proximal end of VGs. (**C**) The representative YFP and SMA staining in whole vessel cross-sections of #9 segments at thediatal end of VGs. (**D**) The representative YFP and SMA staining or YFP and vWF staining in whole vessel cross-sections of middle parts of VGs. YFP is green; SMA and vWF are red. White dotted lines separate the neointima (NI) and the adventitia (Ad) layers of VG.

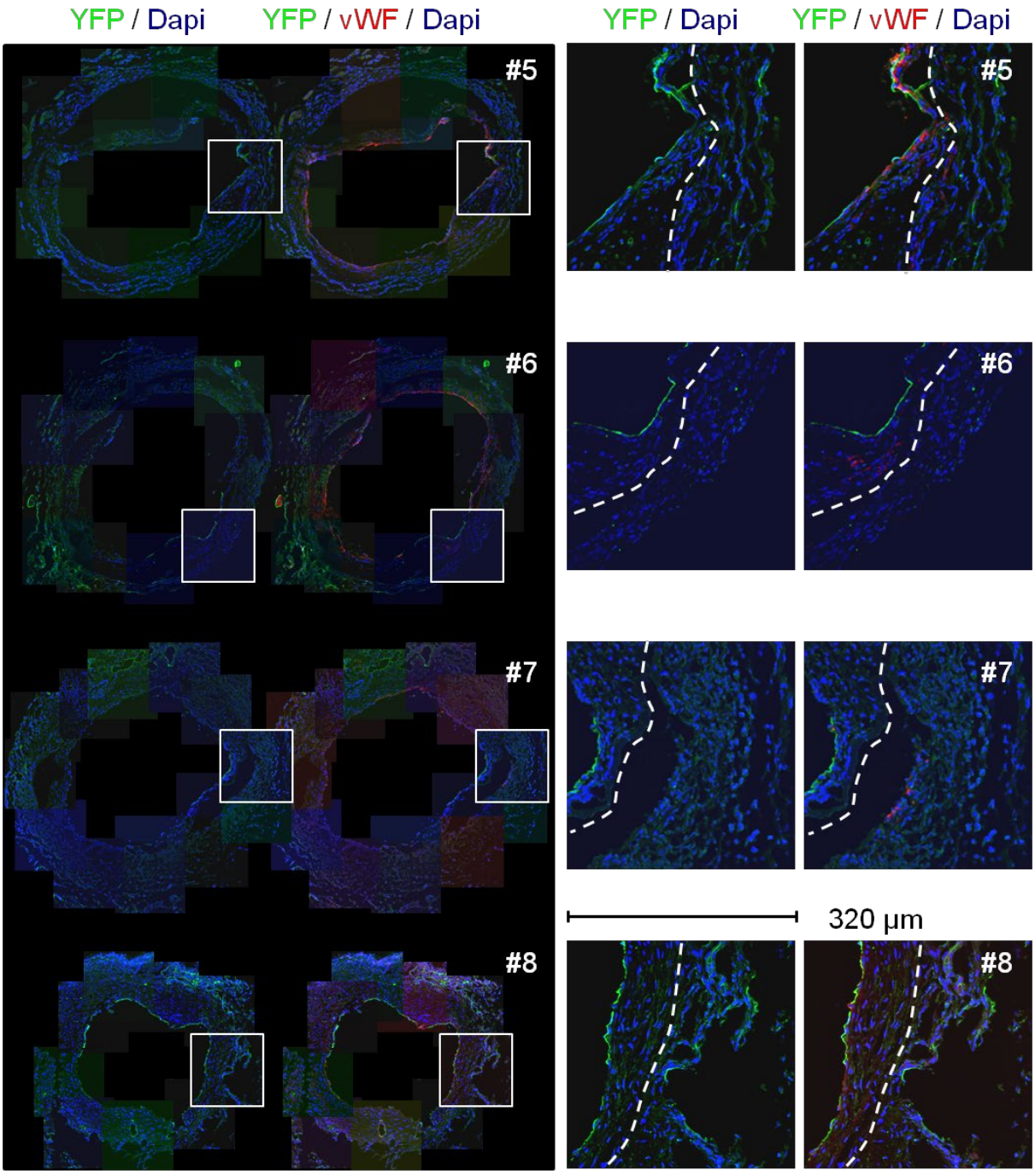

A
Donor:
(JV-WT)
(n=11)
Recipient:
(CA-EC-YFP)
VG-S (n=8)
VG-M (n=3)
B
YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#1
#2
#3
#4
320 µm



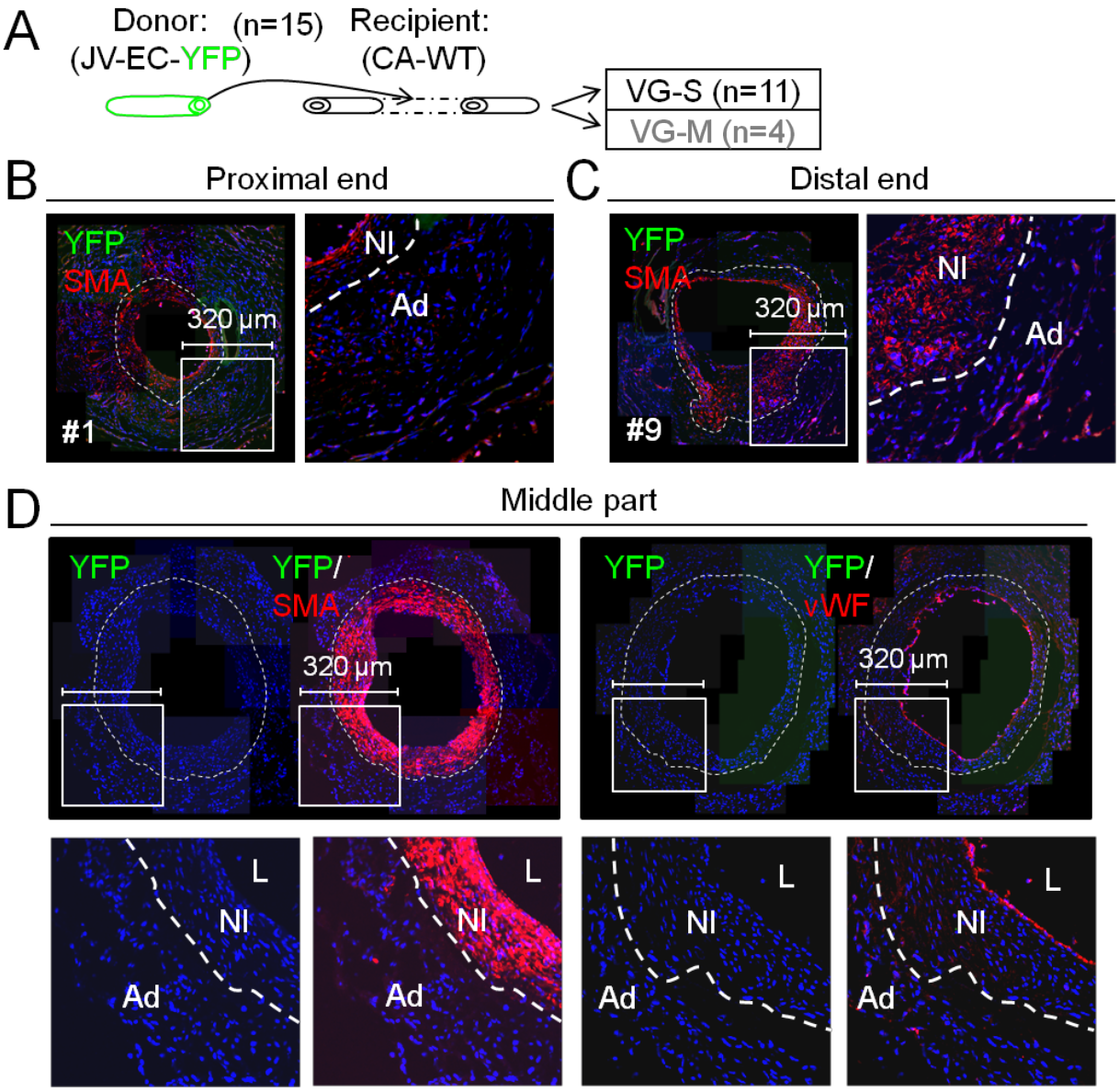
Online Figure XXVIII. The representative serial immunofluorescence staining of YFP and SMA in consecutive cross-sections of WT jugular veins (JVs) grafted into *Cdh5*-

$CreER^{T2}$::$Rosa26^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction. (**A**) Schematic presentation of transplantation of WT JVs to CAs of the reporter mice in which mature ECs were genetically labeled with YFP for 6 wks. Vein grafts (VGs) with severe NI formation (n=3) were analyzed as described in “Methods”. (**B**) Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or SMA across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8, #9) are shown. YFP is green; SMA is red.

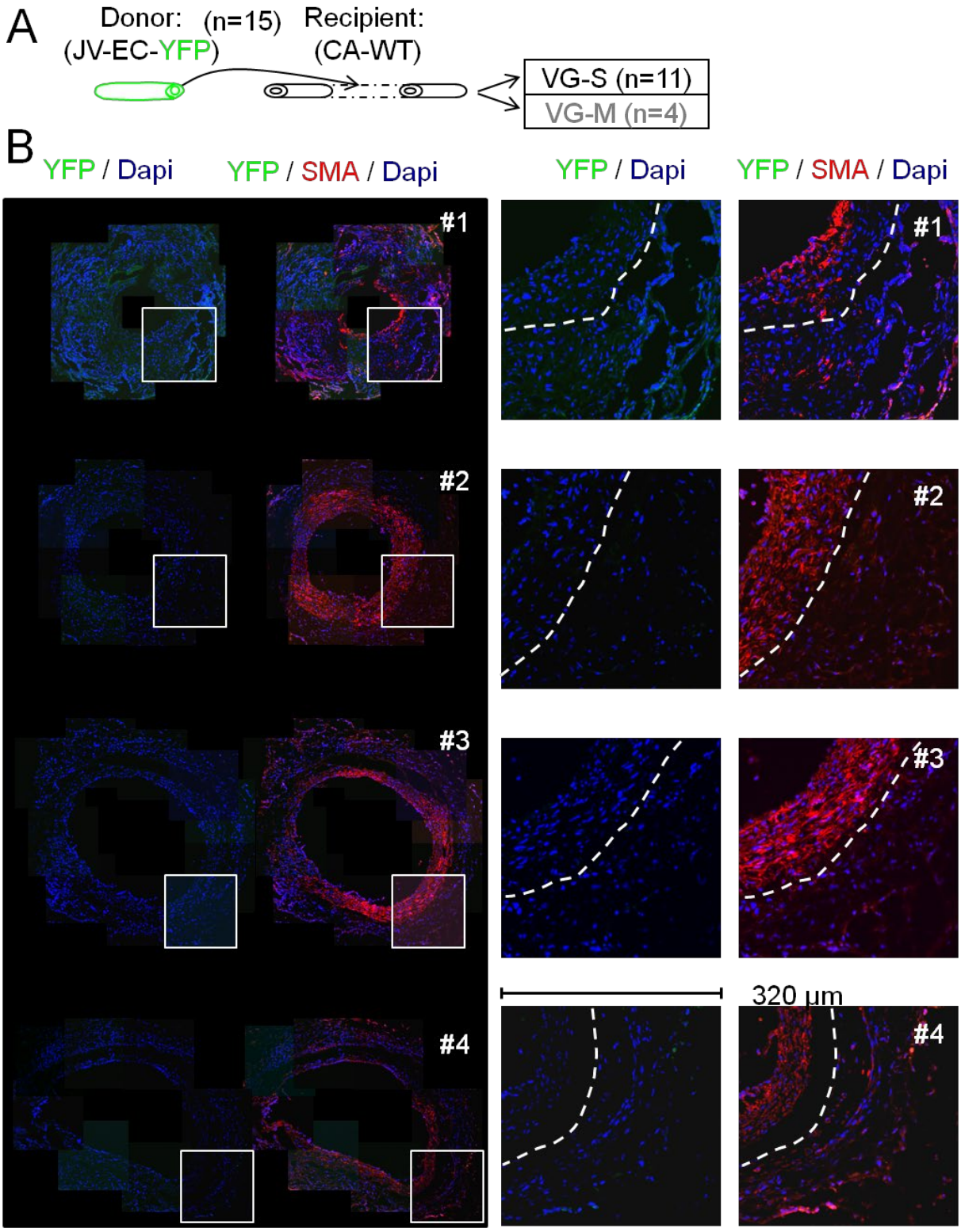

A
Donor:
(JV-WT)
(n=11)
Recipient:
(CA-EC-YFP)
VG-S (n=8)
VG-M (n=3)
B
YFP / Dapi
YFP / vWF / Dapi
YFP / Dapi
YFP / vWF / Dapi
#1
#2
#3
#4
320 μm

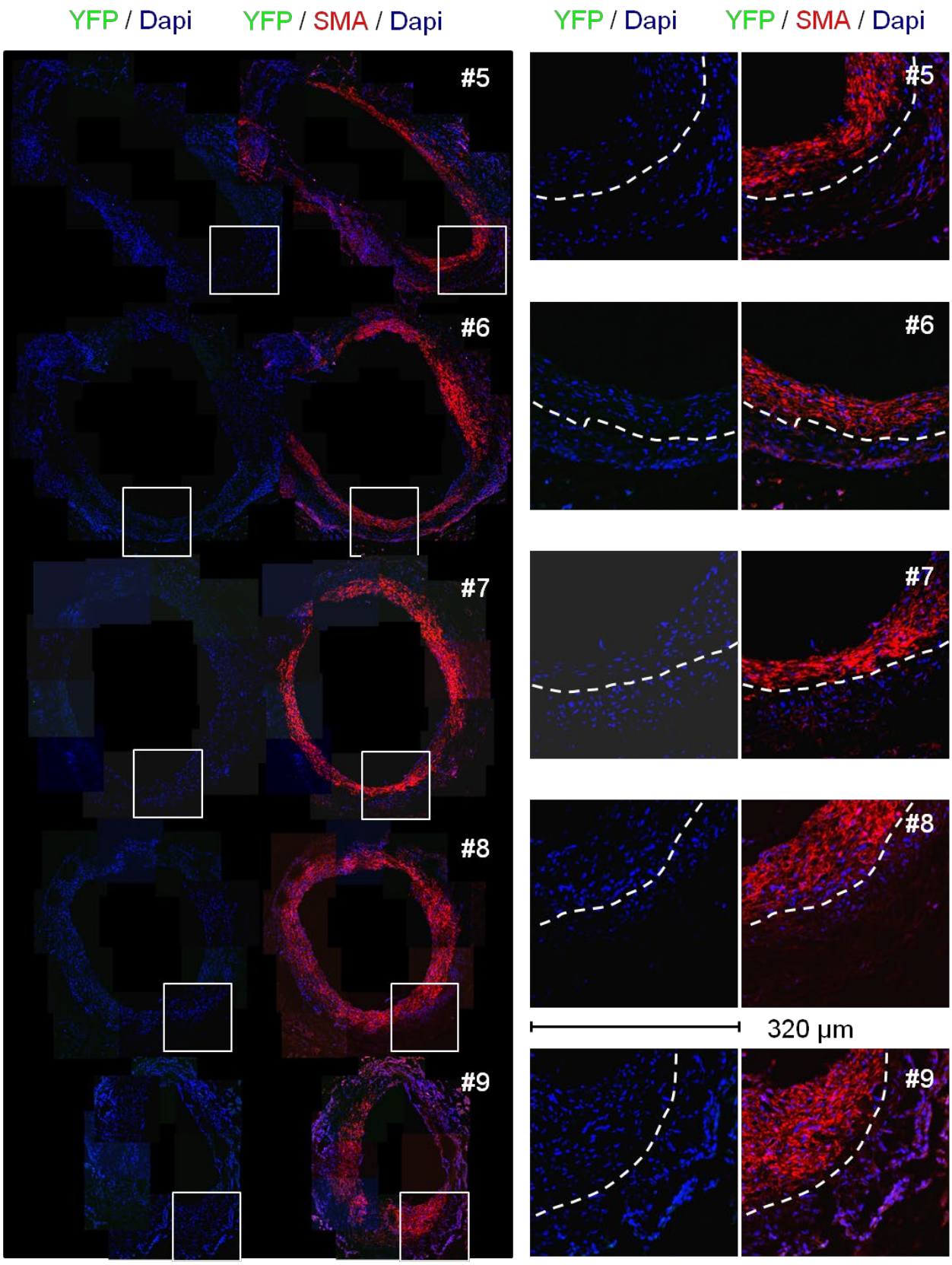


Online Figure XXIX. The representative serial immunofluorescence staining of YFP and vWF in consecutive cross-sections of WT jugular veins (JVs) grafted into *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen induction. (**A**) Schematic presentation of transplantation of WT JVs to CAs of the reporter mice in which mature ECs were genetically labeled with YFP for 6 wks. Vein grafts (VGs) with mild NI formation (n=3) were analyzed as described in "Methods". (**B**) Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or SMA across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8) are shown. YFP is green; SMA is red.

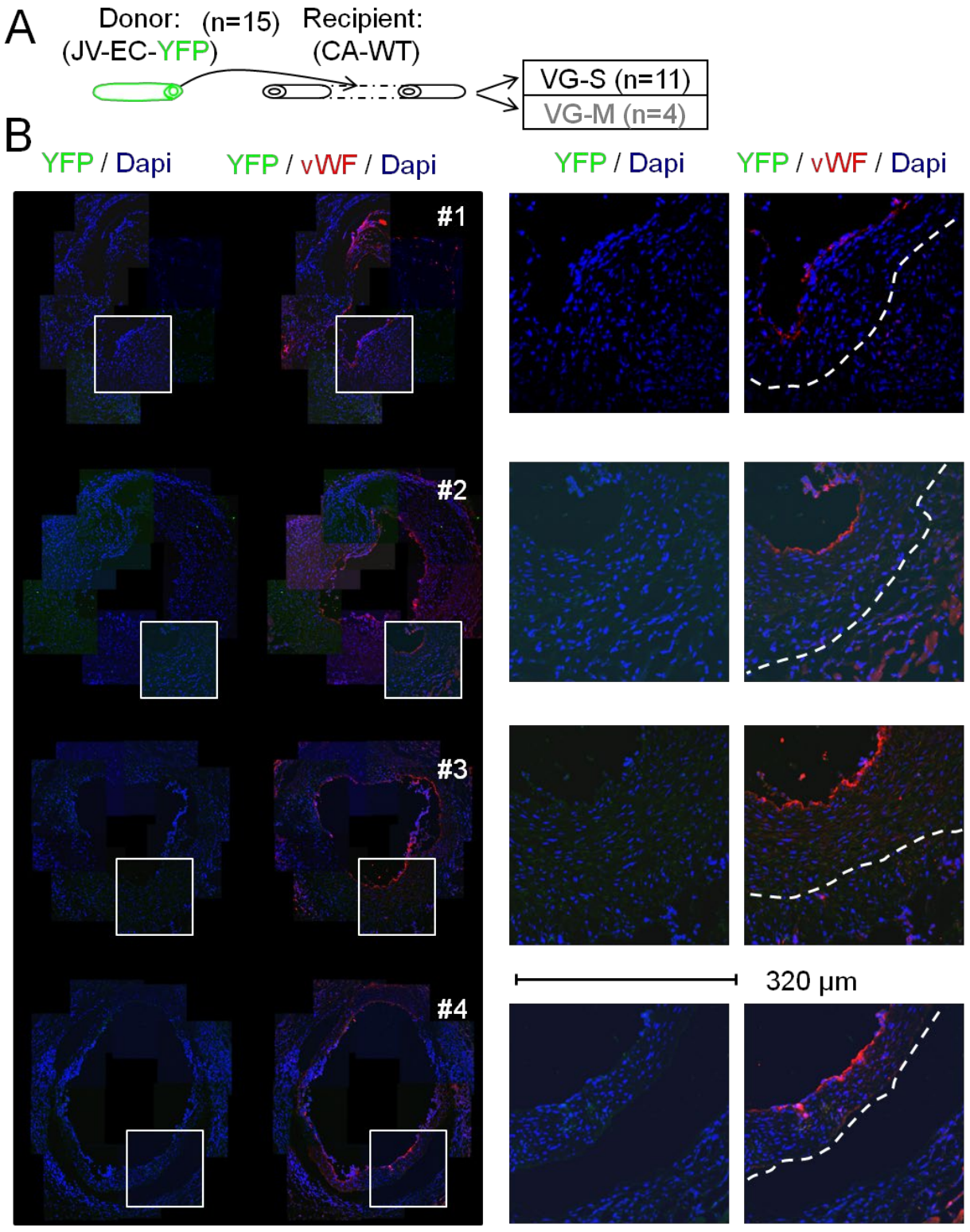


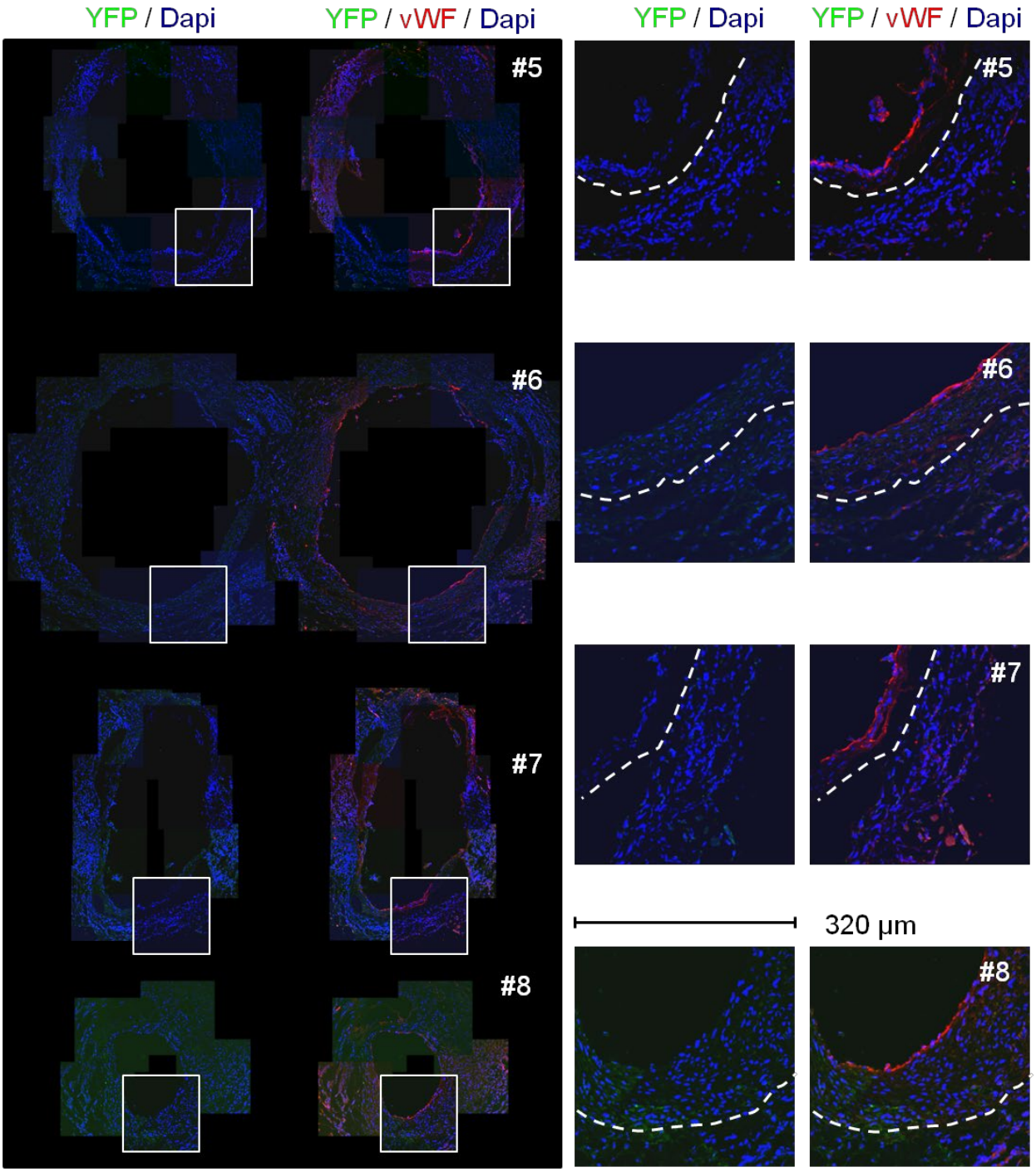
Online Figure XXX. The representative serial immunofluorescence staining of YFP and vWF in whole vessel cross-sections of jugular veins (JVs) of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP after tamoxifen injection grafted to carotid arteries (CAs) of wild type (WT) mice (for **Figure 7**). (**A**) Schematic presentation of JVs of the reporter mice in which ECs were genetically labeled with YFP grafted to CA of wild type mice for 6 wks. (**B**) The representative YFP and SMA staining in whole vessel cross-sections of #1 segments at the proximal end of VGs. (**C**) The representative YFP and SMA staining in whole vessel cross-sections of #9 segments at thediatal end of VGs. (**D**) The representative YFP and SMA staining or YFP and vWF staining in whole vessel cross-sections of middle parts of VGs. YFP is green; SMA and vWF are red. White dotted lines separate the neointima (NI) and the adventitia (Ad) layers of VG.


A
Donor: (n=15)
(JV-EC-YFP)
Recipient:
(CA-WT)
VG-S (n=11)
VG-M (n=4)
B
YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#1
#2
#3
#4
320 μm

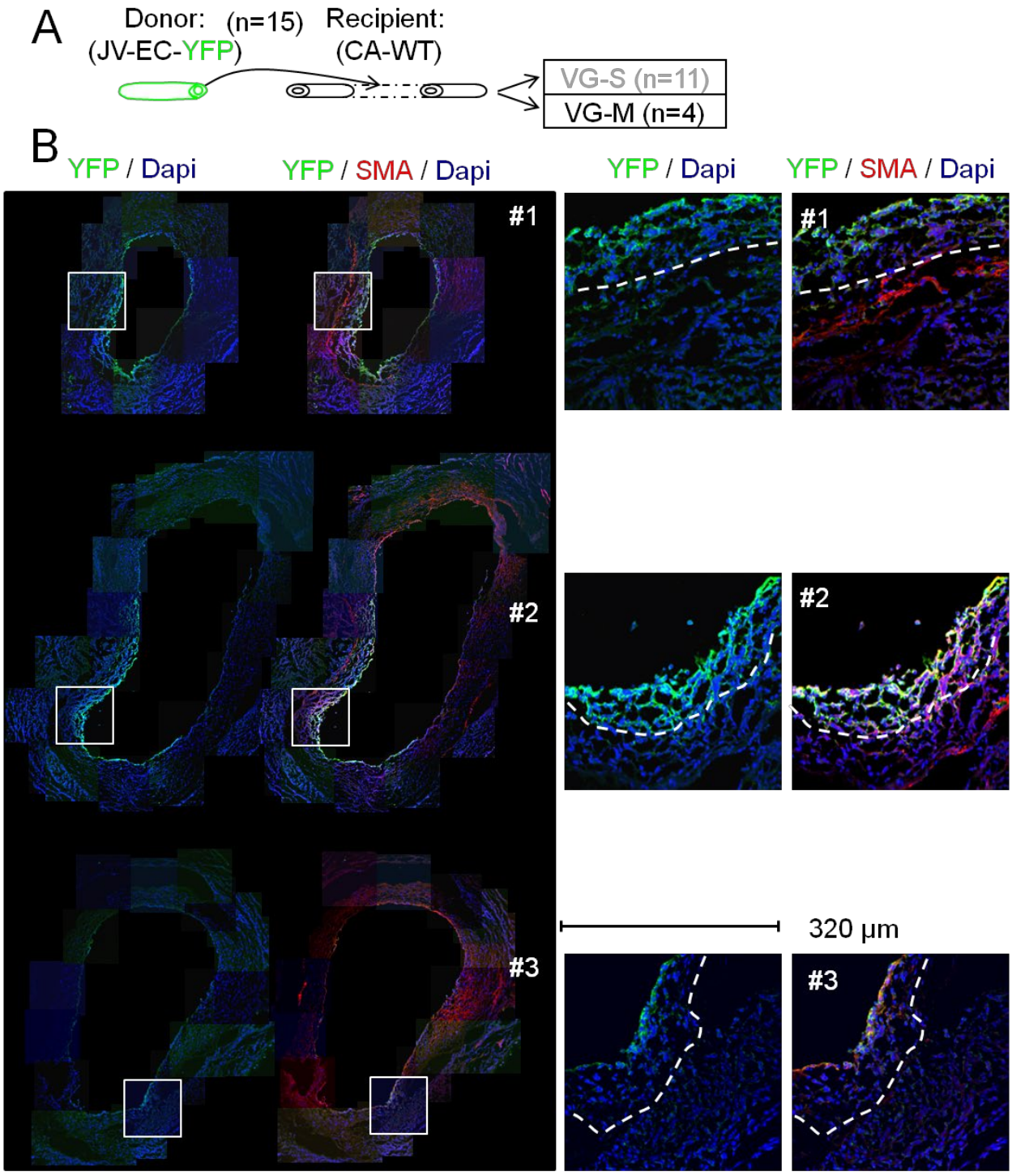

YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#5
#6
#7
#8
#9
#5
#6
#7
#8
#9
320 µm

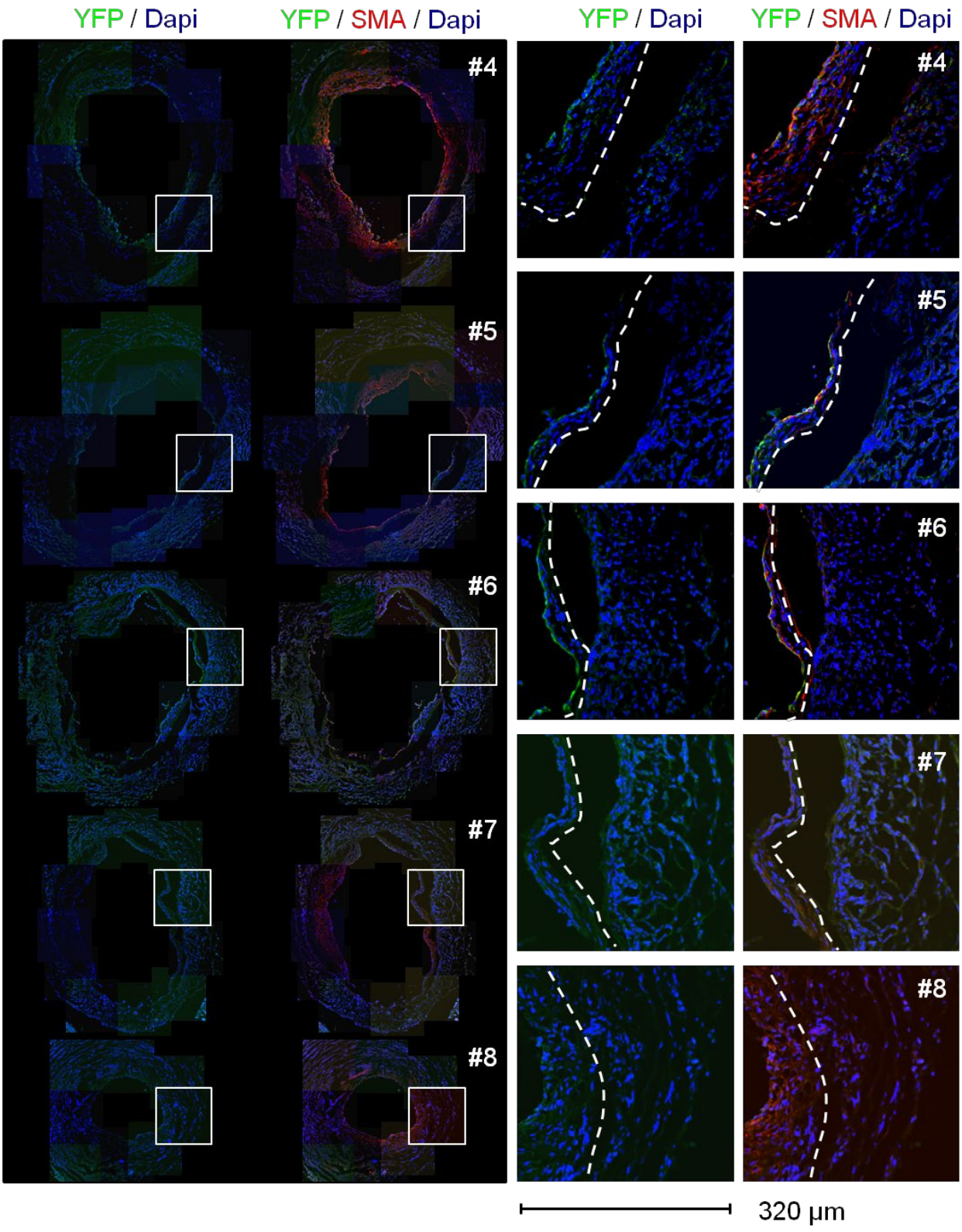
Online Figure XXXI. The representative serial immunofluorescence staining of YFP and SMA in consecutive cross-sections of jugular veins (JVs) of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection grafted into carotid arteries (CAs) of WT mice for 6 wks. Vein grafts (VGs) with severe NI formation (9 sections per VG; 27 sections in total; n=3) were analyzed as described in “Methods”. (**B**) Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or SMA across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8, #9) are shown. YFP is green; SMA is red.


A
Donor: (n=15)
(JV-EC-YFP)
Recipient:
(CA-WT)
VG-S (n=11)
VG-M (n=4)
B
YFP / Dapi
YFP / vWF / Dapi
YFP / Dapi
YFP / vWF / Dapi
#1
#2
#3
#4
320 µm



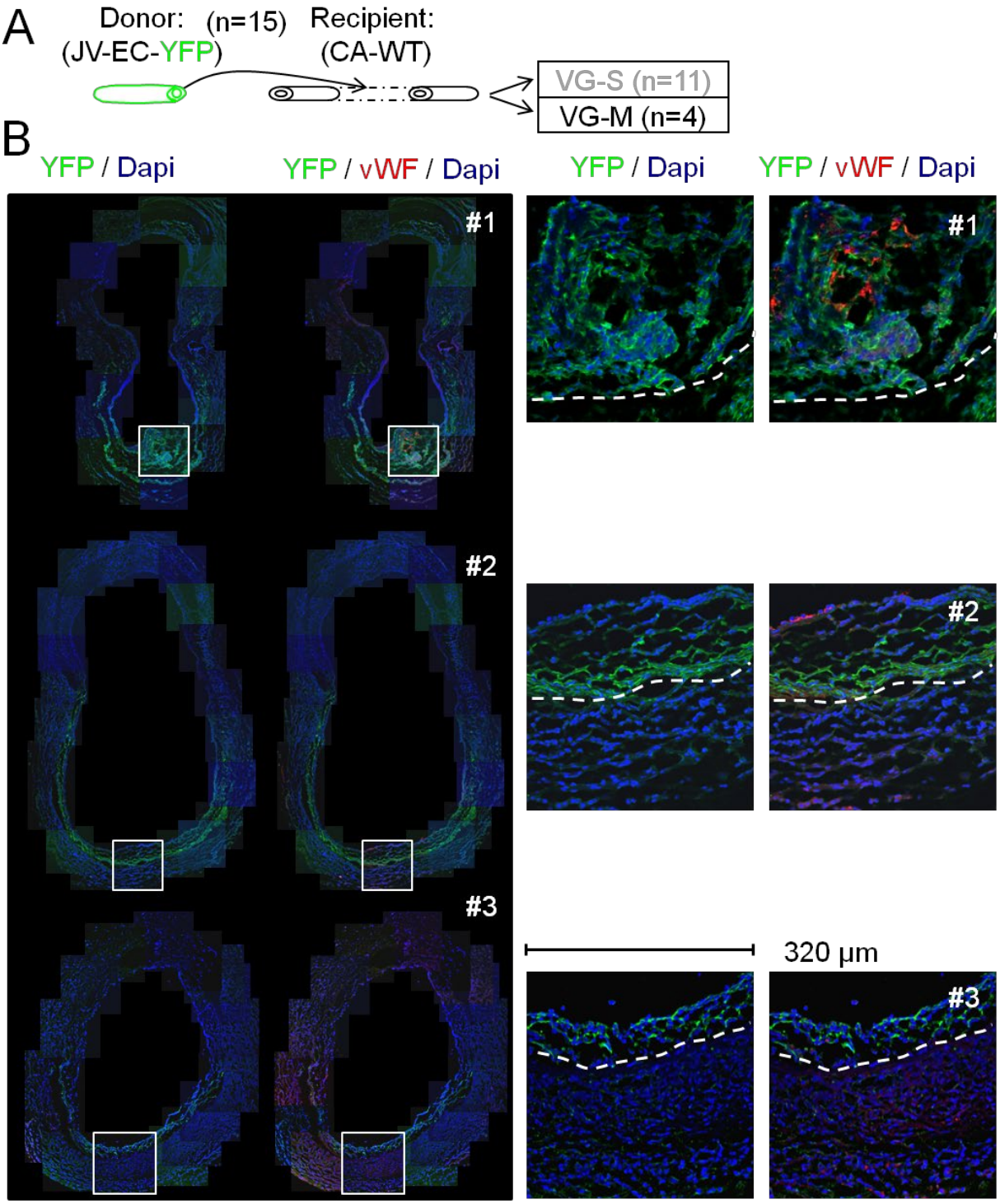
Online Figure XXXII. The representative serial immunofluorescence staining of YFP and vWF in consecutive cross-sections of jugular veins (JVs) of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection grafted into carotid arteries (CAs) of WT mice for 6 wks. Vein grafts (VGs) with severe NI formation (9 sections per VG; 27 sections in total; n=3 VGs) were analyzed as described in “Methods”. (**B**) Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or vWF across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8) are shown. YFP is green; vWF is red.

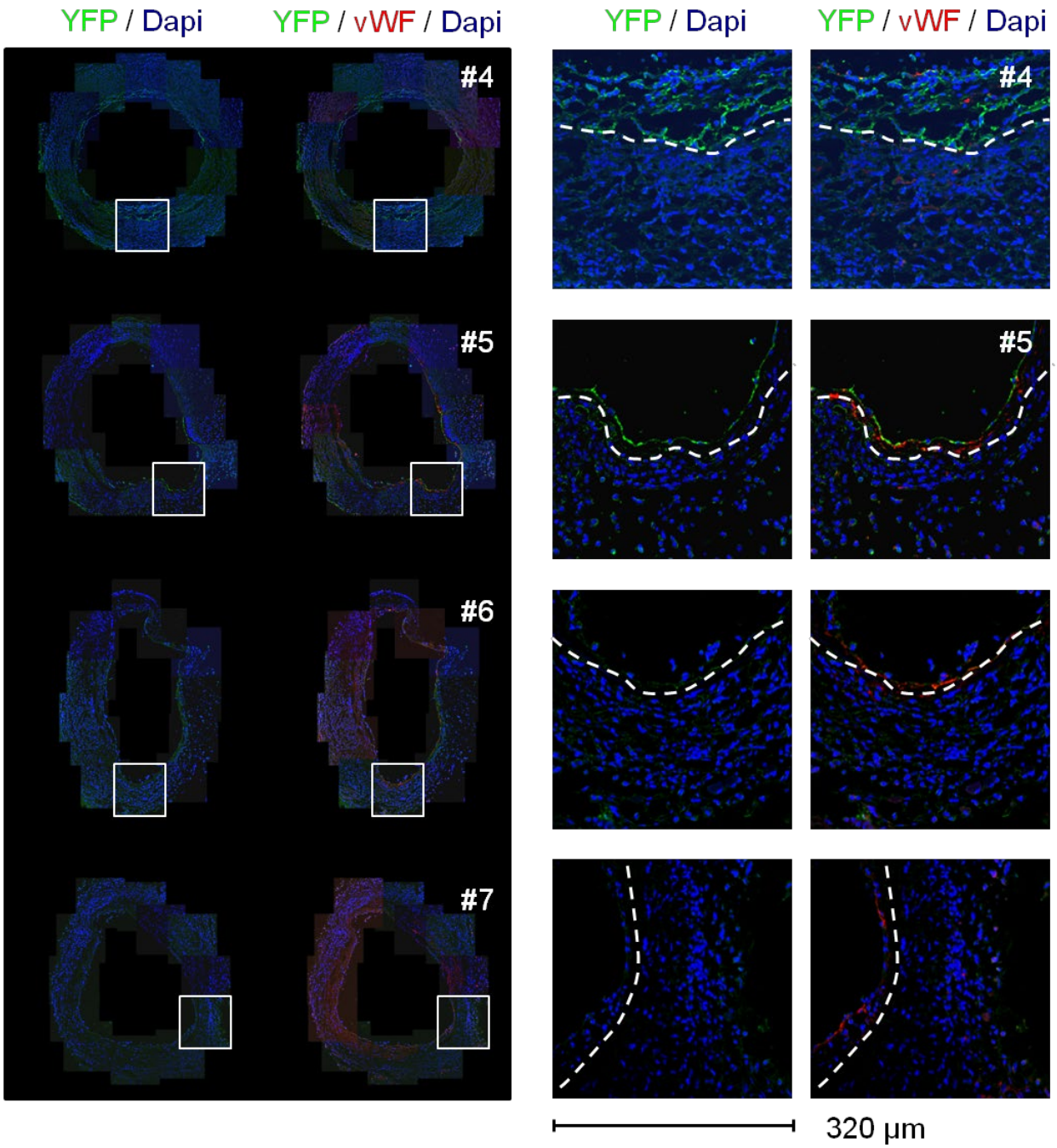

A
Donor: (n=15)
(JV-EC-YFP)
Recipient:
(CA-WT)
VG-S (n=11)
VG-M (n=4)
B
YFP / Dapi
YFP / SMA / Dapi
YFP / Dapi
YFP / SMA / Dapi
#1
#2
#3
320 μm



Online Figure XXXIII. The representative serial immunofluorescence staining of YFP and SMA in consecutive cross-sections of jugular veins (JVs) of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP

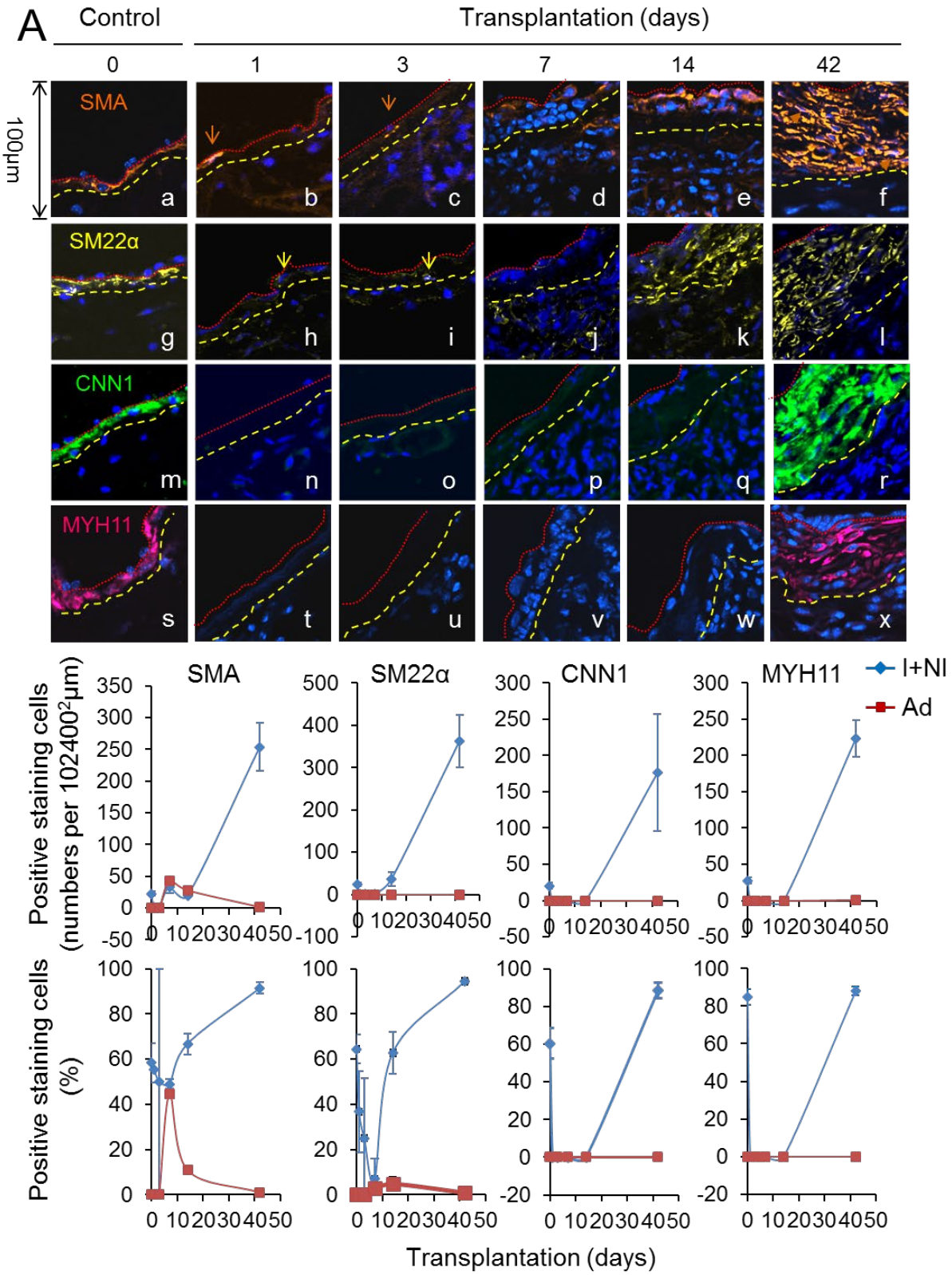
mice after tamoxifen injection grafted into carotid arteries (CAs) of WT mice for 6 wks. Vein grafts (VGs) with mild NI formation (9 sections per VG; 27 sections in total; n=3 VGs) were analyzed as described in “Methods”. (**B**) Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or SMA across the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8) are shown. YFP is green; SMA is red.


A
Donor: (n=15)
(JV-EC-YFP)
Recipient:
(CA-WT)
VG-S (n=11)
VG-M (n=4)
B
YFP / Dapi
YFP / vWF / Dapi
YFP / Dapi
YFP / vWF / Dapi
#1
#2
#3
#1
#2
#3
320 μm



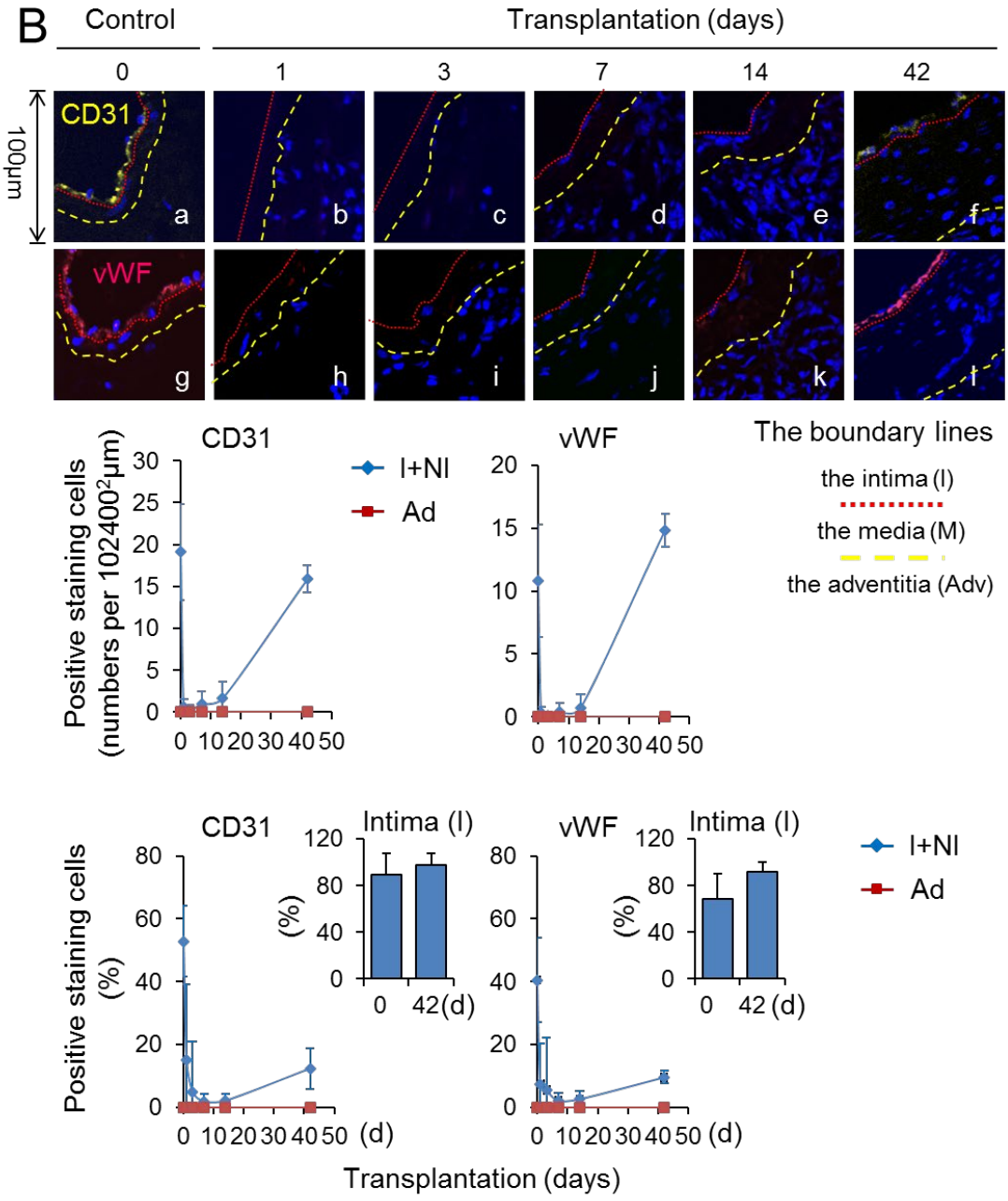
Online Figure XXXIV. The representative serial immunofluorescence staining of YFP and vWF in consecutive cross-sections of jugular veins (JVs) of *Cdh5*-CreER^{T2}::Rosa26$^{Floxed\text{-}Stop}$eYFP mice after tamoxifen injection grafted into carotid arteries (CAs) of WT mice for 6 wks. Vein grafts (VGs) with mild NI formation (9 sections per VG; 27 sections in total; n=3 VGs) were analyzed as described in “Methods”. (**B**) Typical images of whole cross-sections of VGs reflecting the expression of YFP and/or vWFacross the VGs from the proximal end (#1, #2), the middle part (#3 - #7) to the distal end (#8) are shown. YFP is green; vWF is red.

Online Figure XXXV. The cellular dynamics of SMCs and ECs during VG remodeling in

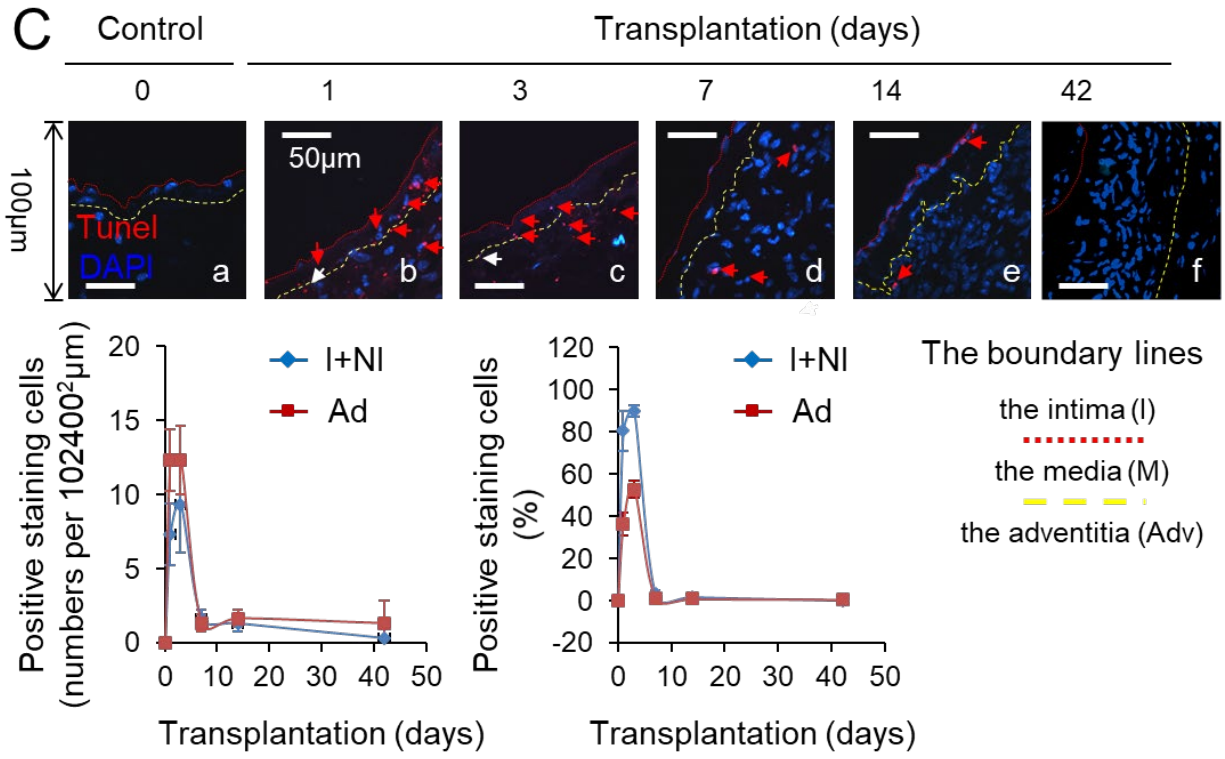


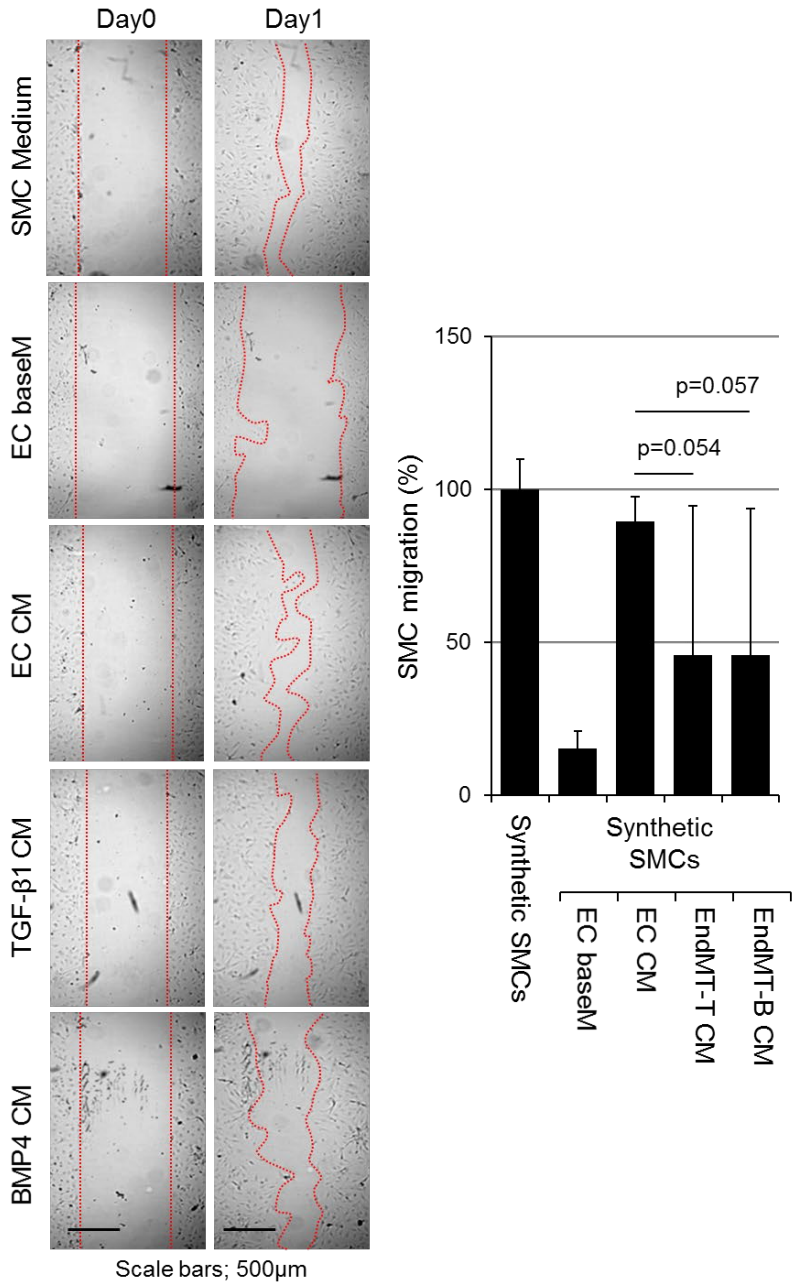
mice. Isograft transplantation of jugular veins (JVs) was performed in male 3-month old wild

type mice in a C57BL/6J genetic background. Normal JVs and JV isografts were harvested at 0, 1, 3, 7, 14 and 42 days after transplantation. (**A**) SMCs were identified by immunofluorescence staining of SMA, SM22α, CNN1 and SM-MHC on the tissue cross-sections of normal JVs and JV grafts. SMA is labeled with orange, SM22α with yellow, CNN1 with green, and SM-MHC with purple. Nuclei are labeled with DAPI (blue). The arrows indicate the representative positive cells. The boundary between the intima (I) and the media (M) is red dotted line; The boundary between the media (M) and the adventitia (Ad) is yellow dotted line. The cell numbers per field (102400 μm^2) and percentages of the cells stained positive for SMC markers were quantified (low panel). Three cross-sections per vein graft were randomly chosen at each time point (18 sections in total; n=6 mice) for the immunofluorescence staining analysis. (**B**) ECs were visualized by immunofluorescence staining of CD31 and vWF in the same ways as described in **A**. CD31 is labeled with yellow, vWF with purple, and nuclei are labeled with DAPI (blue).



(**C**) Cell death in VGs was determined in the same way as described in **A**. Tunel positive is red and DAPI is blue.





Online Figure XXXVI. The effects of conditional media from human umbilical vein endothelial cells (HUVECs) with or without EndMT on vascular SMC migration in vitro. Wounds were created on monolayers of mouse aortic SMCs, and then cultured with the SMC full growth medium (synthetic SMC group), EC culture basal medium without any supplements (EC baseM group), conditional medium (CM) from ECs without EndMT (EC CM group), CM from ECs with EndMT induced by TGF-β1 (EndMT-T CM group), or CM from ECs with EndMT induced by BMP4 (EndMT-B CM group) for one day as described in “Methods”. Images of wound closure were captured at 0 and 1 day after the treatment. Left panel: Representative images of SMC migration. Right panel: Quantified percentages of wound closure relative to that of SMCs cultured in SMC full growth medium. Cultured mouse aortic SMCs are synthetic SMCs.

III. Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Jackson laboratory	C57BL/6J	Male	https://www.jax.org/strain/000664

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	*Myh11*-CreER^{T2} mice	Mary C.M. Weiser-Evans,	C57BL/6J	Mary.weiser@ucdenver.edu	N/A
Parent - Male	*Cdh5*-CreER^{T2} mice	Mohamad Azhar	C57BL/6J	Mohamad Azhar@uscmed.sc.edu	N/A
Parent - Male	*SM22α* (Ki)-CreER^{T2} mice	Robert Feil	C57BL/6J	robert.feil@uni-tuebingen.de	N/A
Parent - Female	Rosa26$^{Floxed-Stop}$eYFP mice	Jackson lab	C57BL/6J	Stock Number: 006148	https://www.jax.org/strain/006148

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Anti-SM22alpha (SM22α)	Abcam	Cat#: ab14106	IF: 2µg/ml (1:500) WB: 1µg/ml (1:1000)		https://www.abcam.com/taglntransgelin-antibody-ab14106.html
Anti-Calponin 1 (CNN1)	Abcam	Cat#: ab46794	IF: 0.5µg/ml (1:200) WB: 0.1µg/ml (1:1000)		https://www.abcam.com/calponin-1-antibody-ep798y-ab46794.html
Anti-smooth muscle α actin (SMA)	Sigma-Aldrich	Cat#: a5228	IF: 0.2µg/ml (1:1000) WB: 0.2µg/ml (1:1000)		https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/6/a5228dat.pdf

Anti-beta Actin (βactin)	Sigma Aldrich	Cat#: a1978	WB: 0.5-1µg/ml (1:2000)		https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/6/a1978dat.pdf
Anti-GAPDH	Sigma Aldrich	Cat#: G8795	WB: 0.5-1µg/ml (1:2000)		https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/7/g8795dat.pdf
Anti-smooth muscle myosin heavy chain 11 (MYH-11)	Abcam	Cat#: ab125884	IF: 0.9µg/ml (1:1000) WB: 0.9µg/ml (1:1000)		https://www.abcam.com/smooth-muscle-Myosin-heavy-chain-11-antibody-ab125884.html
Anti-GFP	Abcam	Cat#: ab6556	IF: 0.5µg/ml (1:1000) WB: 0.5µg/ml (1:1000)		https://www.abcam.com/gfp-antibody-ab6556.html
Anti-GFP	Abcam	Cat#: ab6673	IF: 1µg/ml (1:1000) WB: 1µg/ml (1:1000)		https://www.abcam.com/gfp-antibody-ab6673.html
Anti-Mouse CD31	BD	Cat#: 550274	IF: 0.15µg/ml (1:100)		https://www.bdbiosciences.com/ds/pm/tds/550274.pdf
Anti-human Von Willebrand Factor (VWF)	Dako	Cat#: A0082	Antiserum IF: (1:1000)		https://www.agilent.com/cs/library/packageinsert/public/SSA0082IVD-US_01.pdf
Anti-smooth muscle α actin-FITC antibody (SMA-FITC)	Sigma Aldrich	Cat#: F3777	2µg/ml IF: 1:1000		https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/7/f3777dat.pdf
Anti-CD44 antibody [EPR18668]	Abcam	Cat#: ab189524	WB: 0.8µg/ml (1:500)		https://www.abcam.com/cd44-antibody-epr18668-ab189524.html
Purified Rat Anti-Mouse CD45	BD	Cat#: 550539	IF: 6.25µg/ml (1:1000)		https://www.bdbiosciences.com/ds/pm/tds/550539.pdf
Anti-Sca1	BD	Cat#: 55333	IF: 2.5 µg/ml (1:200)		https://www.bdbiosciences.com/us/applications/research/stem-cell-research/hematopoietic-stem-cell-markers/mouse/positive-markers/purified-rat-anti-mouse-ly-6ae-e13-1617/p/553333
Anti-Human/Mouse Gli1 antibody	R&D	Cat#: MAB3324	IF: 8-25µg/ml (1:500)		https://resources.rndsystems.com/pdfs/datasheets/mab3324.pdf

Anti-Tie-2 (F-6) antibody	Santa Cruz	Cat#: sc-518076	WB: 1µg/ml (1:200)		https://datasheets.scbt.com/sc-518076.pdf
Anti-Thy-1 (OX7) antibody also referred to as CD90	Santa Cruz	Cat#: sc-53116	WB: 1µg/ml (1:200)		https://www.scbt.com/p/thy-1-antibody-ox7
Anti-Purified mouse Anti-eNOS antibody	BD	Cat#: pS1177	WB: 0.5µg/ml (1:200)		https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/cell-biology-reagents/cell-biology-antibodies/purified-mouse-anti-enos-ps1177-19enoss1177/p/612392
Anti- VE-cadherin (human) monoclonal antibody (BV6)	Enzo Life Sciences	Cat#: ALX-803-305-C100	WB: 0.5µg/ml (1:200)		https://www.enzolifesciences.com/ALX-803-305/ve-cadherin-human-monoclonal-antibody-bv6/
Alex fluor 488	Invitrogen	Cat#: A21208	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208
Alex fluor 488	Invitrogen	Cat#: A11001	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001
Alex fluor 488	Invitrogen	Cat#: A21206	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206
Alex fluor 488	Invitrogen	Cat#: A11055	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11055
Alex fluor 546	Invitrogen	Cat#: A10036	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10036
Alex fluor 546	Invitrogen	Cat#: A10040	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10040
Alex fluor 546	Invitrogen	Cat#: A11081	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11081
Alex fluor 546	Invitrogen	Cat#: A11056	1µg/ml		https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11056

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL

None			

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Smooth muscle cell (SMC)	N/A	Male	N/A
Human Umbilical Vein Endothelial Cells (HUVEC)	Sciencell Cat#: 8000	unknown	https://www.sciencellonline.com/PS/8000.pdf

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
None		

Other

Description	Source / Repository	Persistent ID / URL
None		