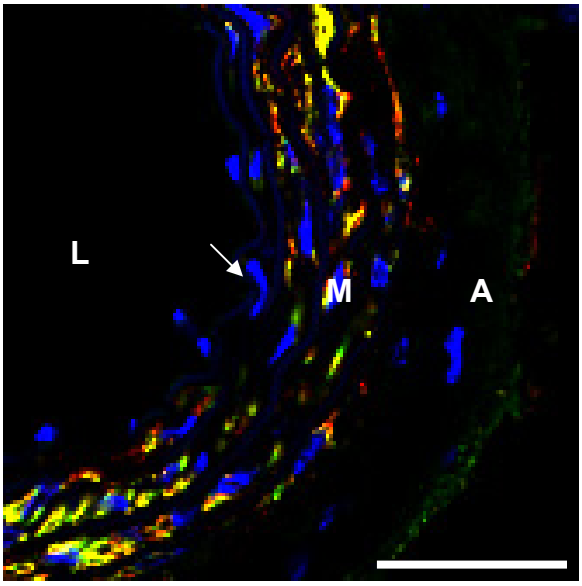
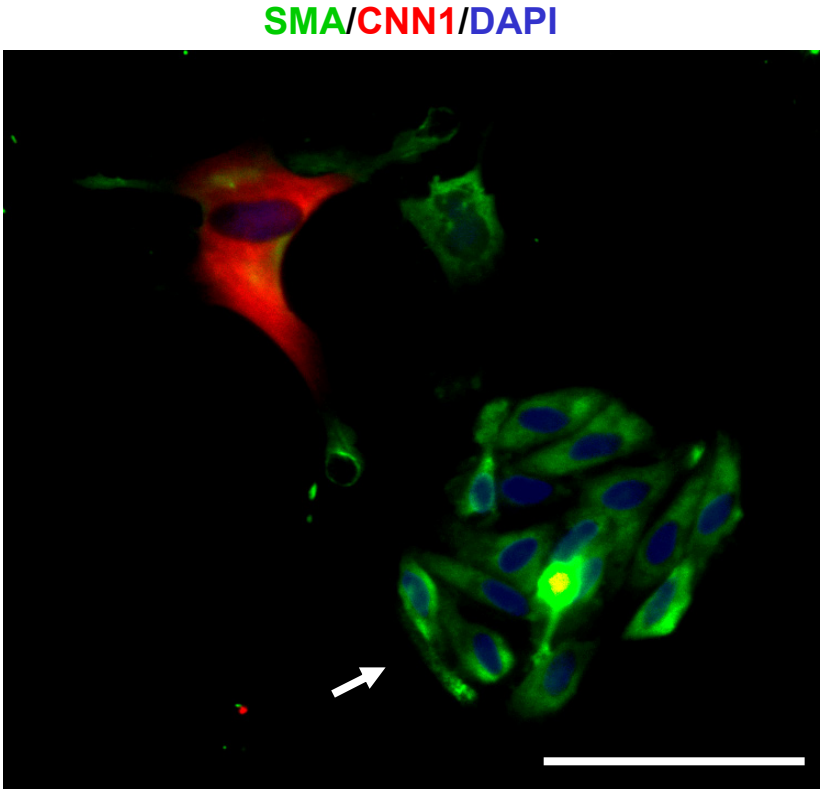
Supplementary Figure S1. Non-SMCs in rat carotid artery.



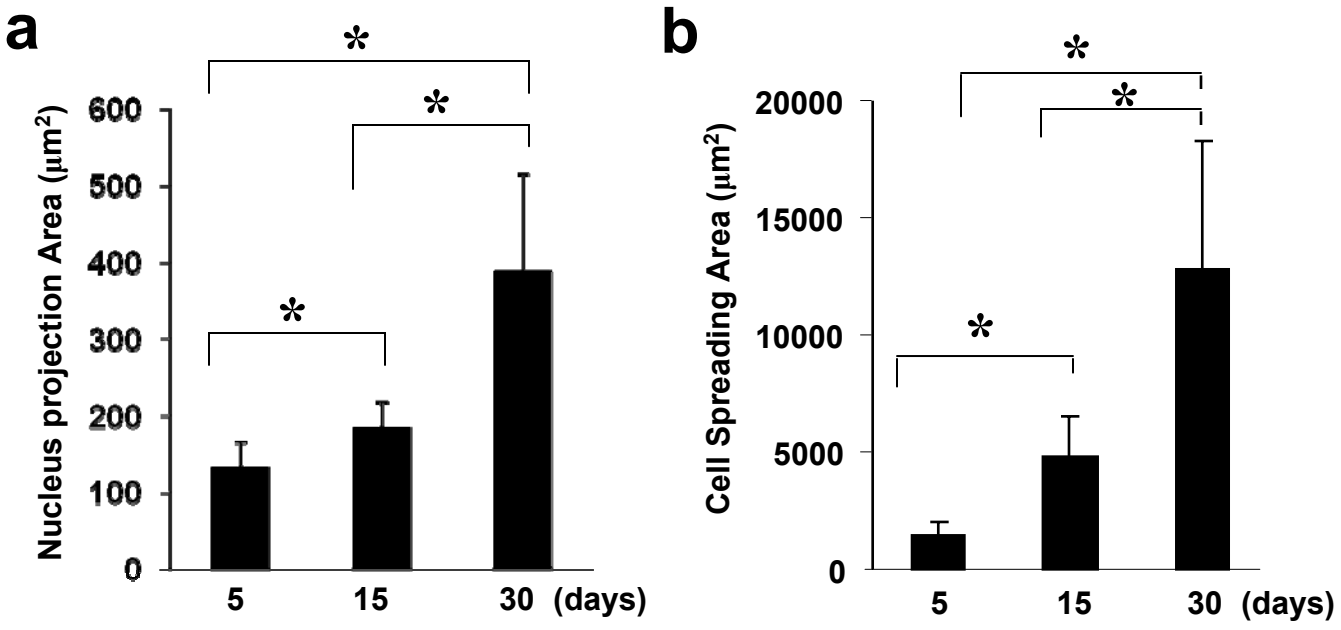
Cross sections of carotid artery were stained for SM-MHC (red) and SMA (Green). Nuclei were stained with DAPI. L: lumen, M: media, A: adventitia. Arrow indicates a SM-MHC$^-$ cell inside tunica media. Scale bar is 50 μm.

Supplementary Figure S2. Non-SMCs proliferated and dominated the culture.



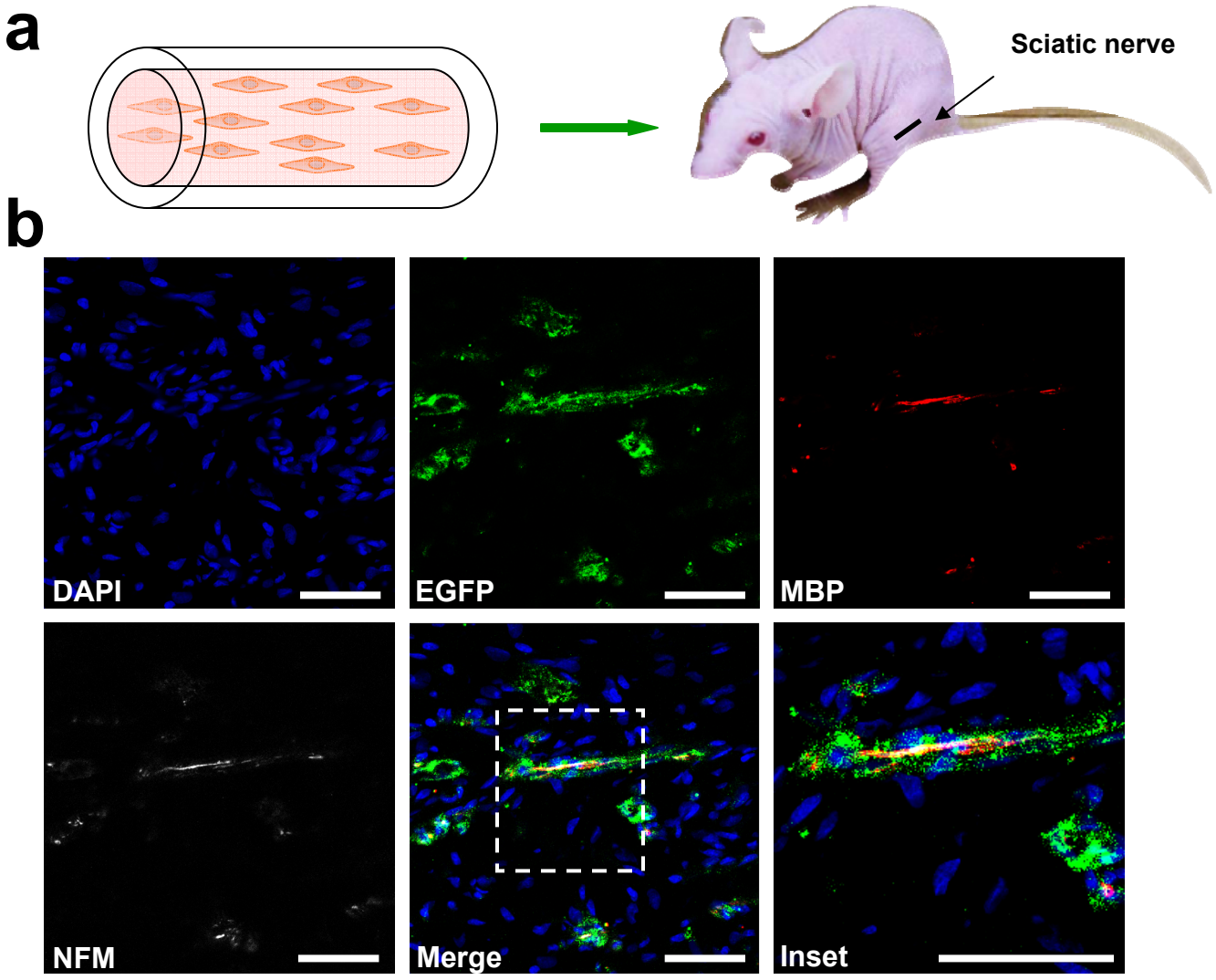
The cells isolated by using enzymatic digestion were cultured for 3 days in DMEM with 10% FBS. Immunostaining for SMA and CNN1 showed that only the $SMA^{low}/CNN1^{-}$ population could expand and form colonies. Scale bar is 100 μm.

Supplementary Figure S3. Increased cell spreading and nuclei projection areas of non-SMCs in culture.



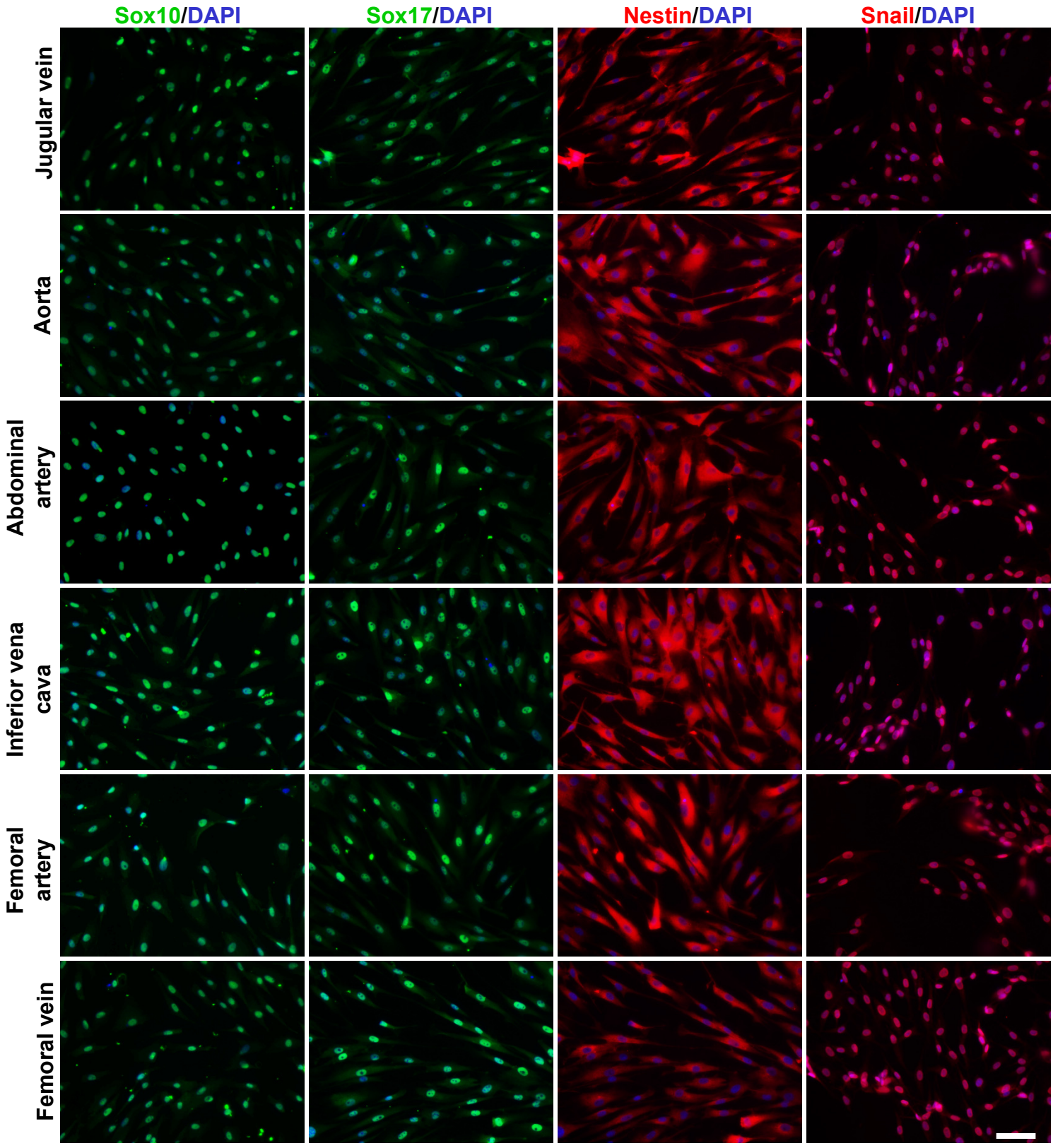
SM-MHC$^-$ cells were isolated from carotid arteries by using tissue explant culture method and cultured for 5, 15 and 30 days in DMEM with 10% FBS. (a) Increase of nucleus projection area during spontaneous differentiation. (b) Increase of cell spreading area during spontaneous differentiation. Data were shown as average±standard deviation (n=3). Holm's t-test was used to calculate the p values. * indicates significant difference ($p<0.05$).

Supplementary Figure S4. *In vivo* differentiation of MVSCs into Schwann Cells.



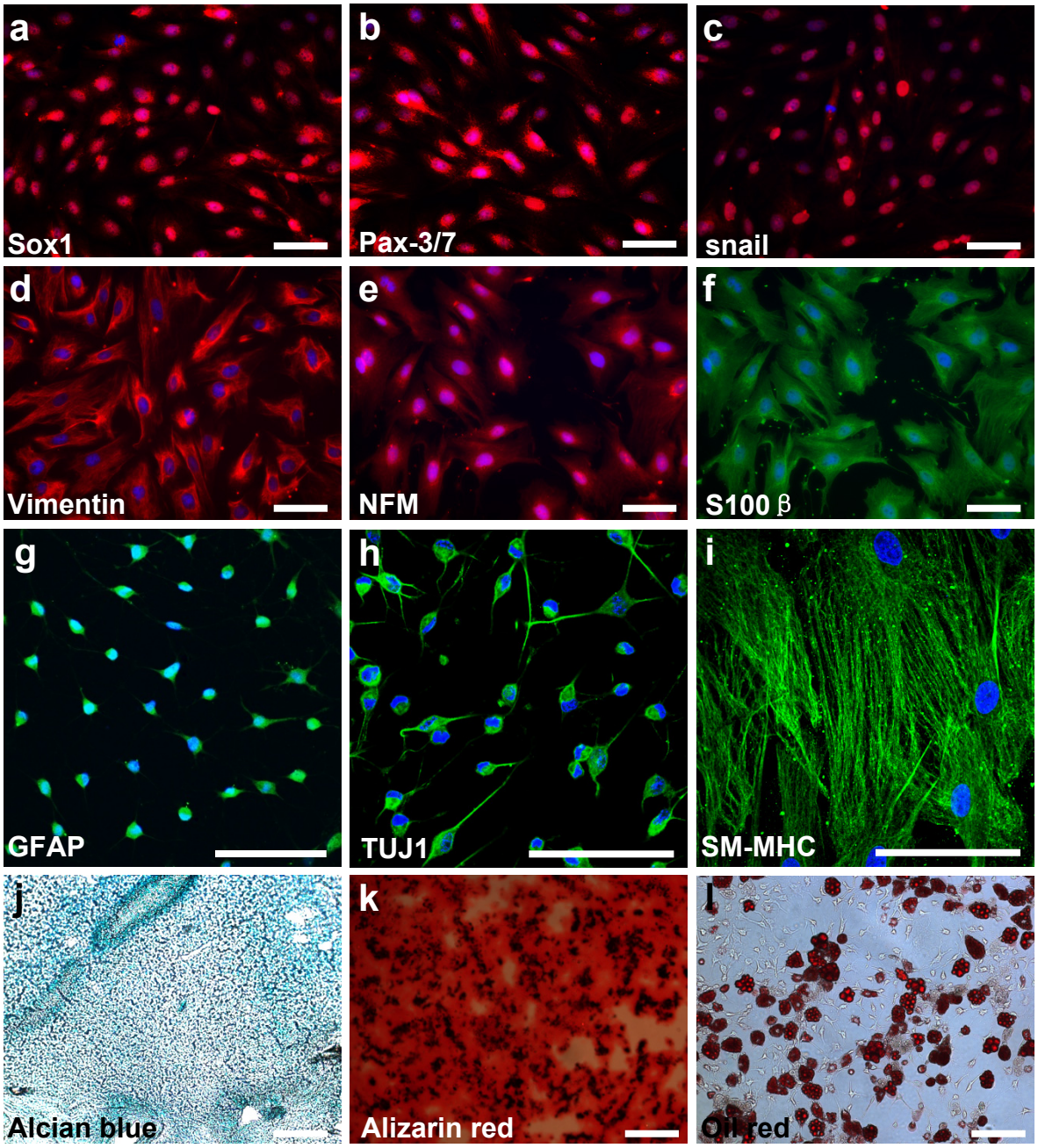
(a) MVSCs isolated from EGFP rats were embedded in matrigel inside the nerve conduit, and the nerve conduit was used to bridge the transected sciatic nerve in an athymic rat. (b) Staining of longitudinal-sections of a nerve conduit at 30 days after transplantation with antibodies against EGFP, MBP and NFM. Nuclei were stained with DAPI. Scale bars are 50 μm.

Supplementary Figure S5. Characterization of MVSCs isolated from different blood vessels of rat.



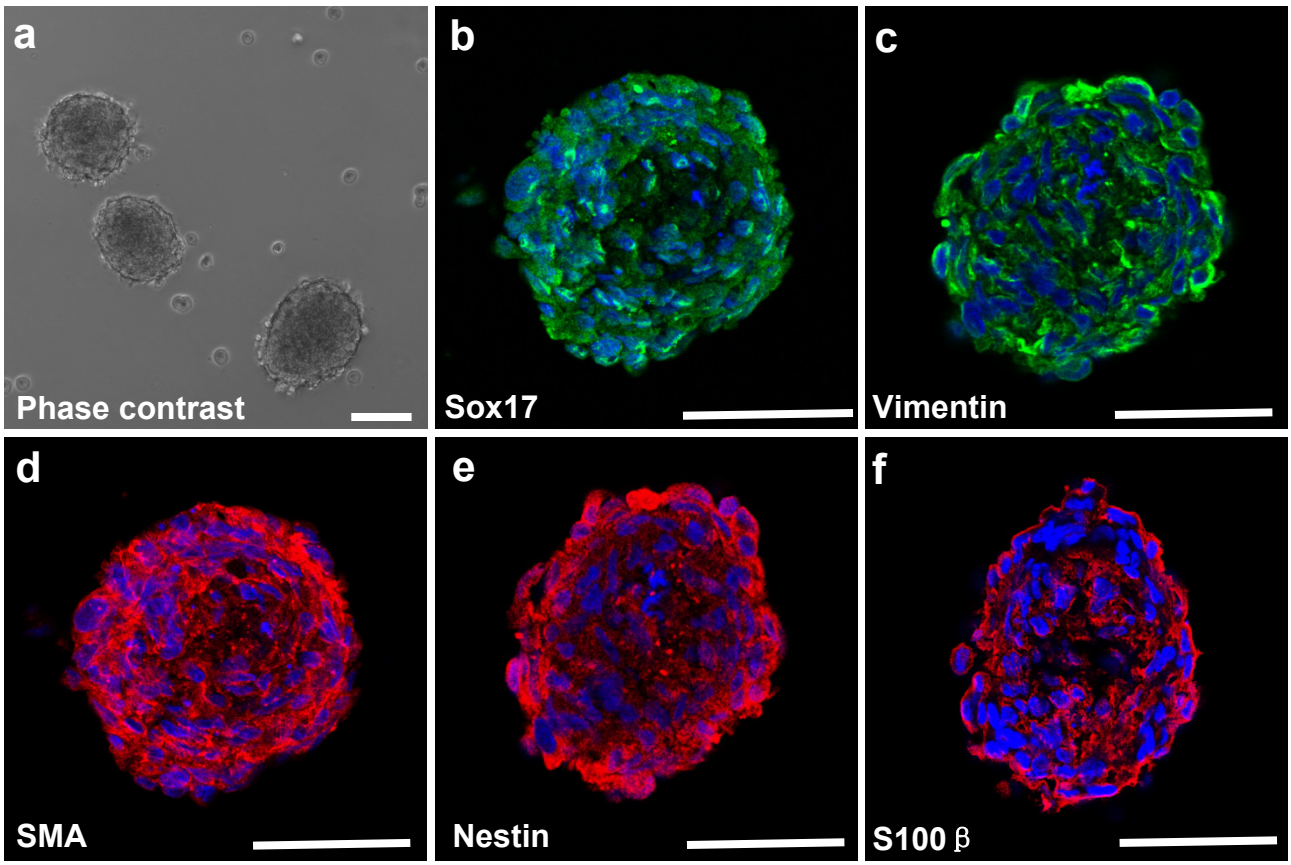
Immunostaining of MVSCs derived from jugular vein, aorta, abdominal artery, inferior vena cava, femoral artery and femoral vein with antibodies against Sox10, Sox17, nestin and snail. Nuclei were stained with DAPI. Scale bar is 100 μm.

Supplementary Figure S6. Characterization of single cell cloned MVSCs.



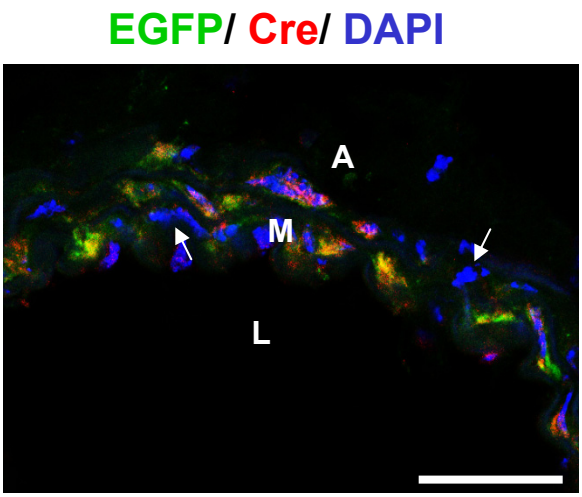
(a-f) Staining of MVSCs derived from single cell cloning with antibodies against Sox1 (a), Pax-3/7 (b), snail (c), vimentin (d), NFM (e) and S100β (f). (g-l) Staining of differentiated cell derived from cloned MVSCs: Schwann cells were stained for GFAP (g), neurons were stained for TUJ1 (h), SMCs were stained for SM-MHC (i), chondrocytes were stained for aggrecan by using alcian blue (j), osteoblasts were stained for calcified matrix by using alizarin red (k), and adipocytes were stained for oil droplets by using oil red (f). Scale bars are 100 μm.

Supplementary Figure S7. Neural sphere culture of single cell cloned MVSCs.



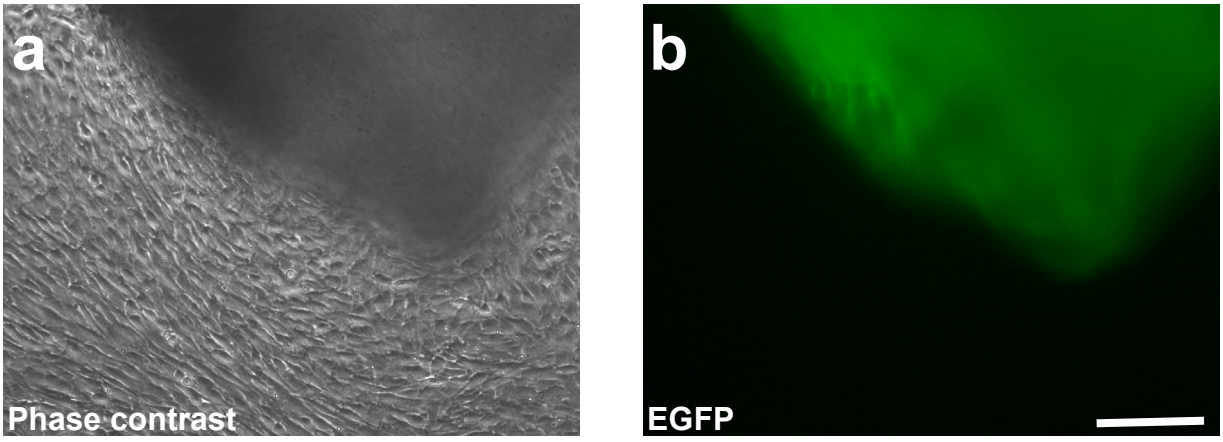
(a) Phase contrast image of neural spheres formed by cloned MVSCs. (b-f) Immunostaining of cross sections of neural spheres derived from MVSCs for MVSC markers including Sox17 (b), vimentin (c), SMA (d), nestin (e) and S100 β (f). The nuclei were stained with DAPI. Scale bars are 50 μm.

Supplementary Figure S8. Non-SMCs in carotid artery of SM-MHC-Cre/LoxP-EGFP mouse.



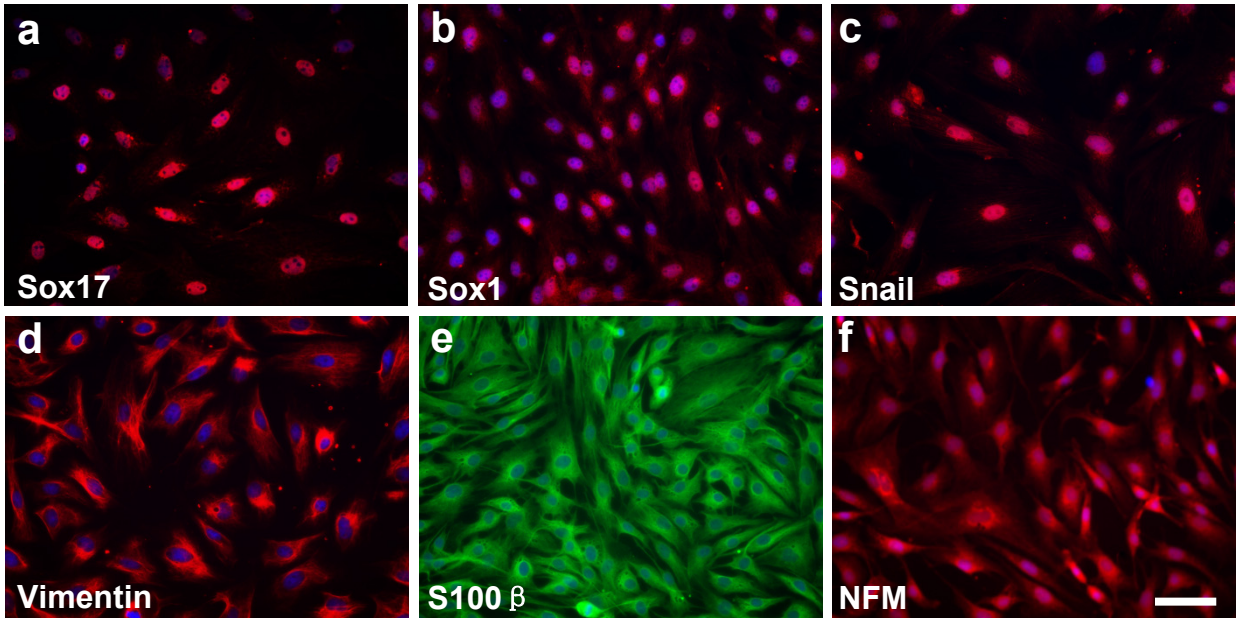
Staining of a cross-section of native carotid artery from a SM-MHC-Cre/LoxP-EGFP mouse with antibodies against EGFP (green) and Cre (red). Arrows indicate non-SMCs in tunica media which do not express GFP or Cre. Nuclei were stained with DAPI. Scale bar is 50 μm. L: Lumen. M: media layer. A: Adventitia.

Supplementary Figure S9. MVSC isolation from lineage tracing mice with β -actin promoter.



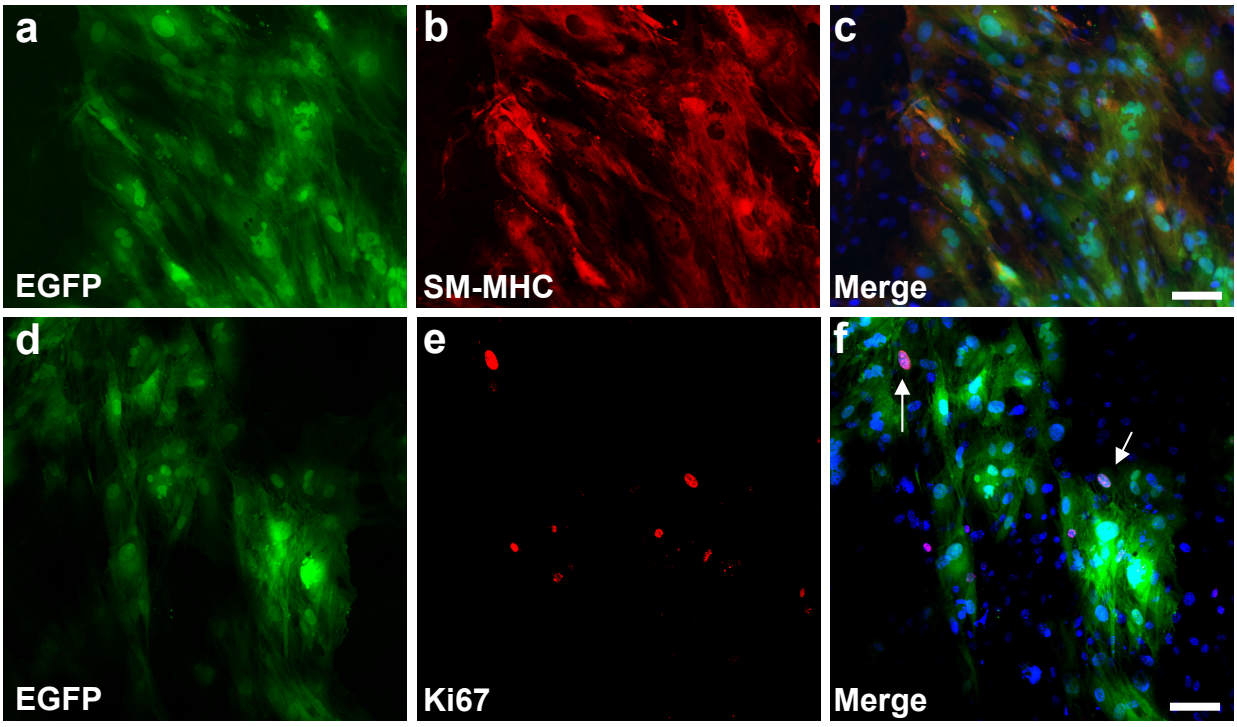
MVSCs were derived from the carotid arteries of SM-MHC-Cre/Actin-LoxP-EGFP mice by using tissue explant culture method. (a) Phase contrast image of MVSCs and the tissue chunk from which the cells migrated from. (b) FITC channel was used to detect the EGFP signal. Scale bar is 100 μm.

Supplementary Figure S10. Characterization of EGFP$^-$ cells isolated from carotid artery of lineage tracing mouse.



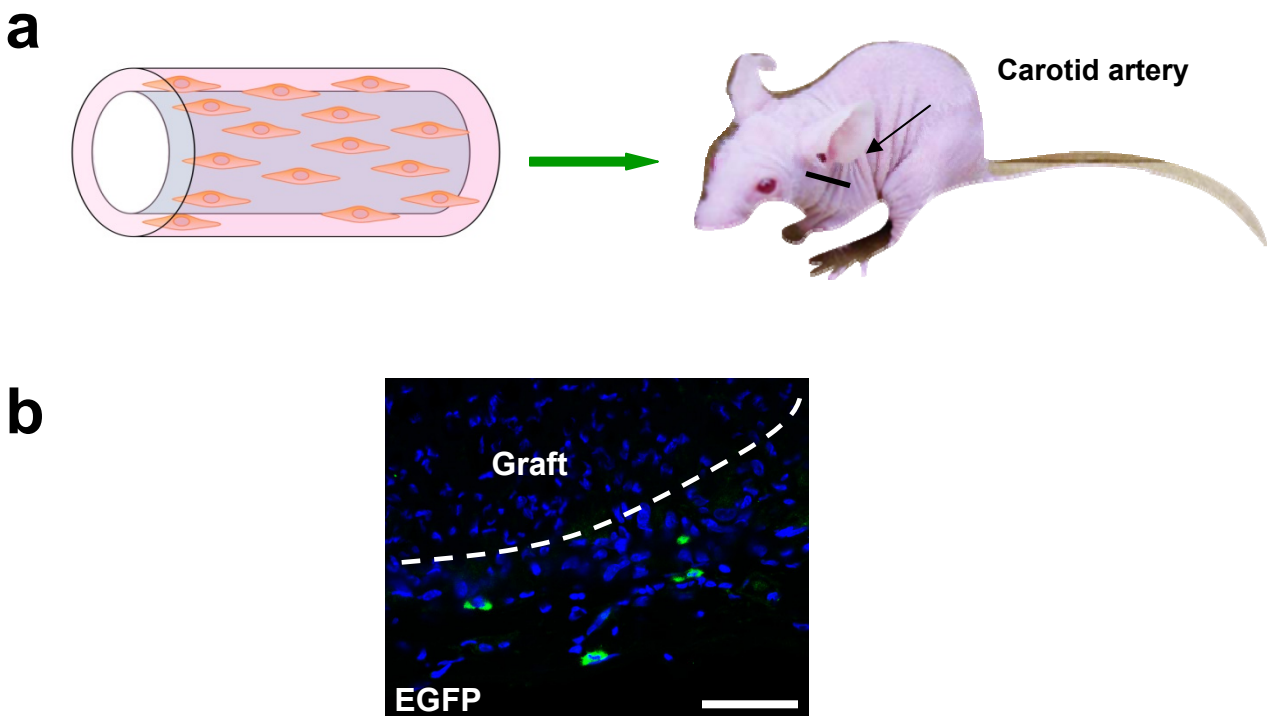
(a-f) Staining of EGFP$^-$ cells with antibodies against Sox17 (a), Sox1 (b), snail (c), vimentin (d), NFM (e) and S100β (f). Scale bar is 100 μm.

Supplementary Figure S11. EGFP expression after MVSCs differentiated into SMCs.



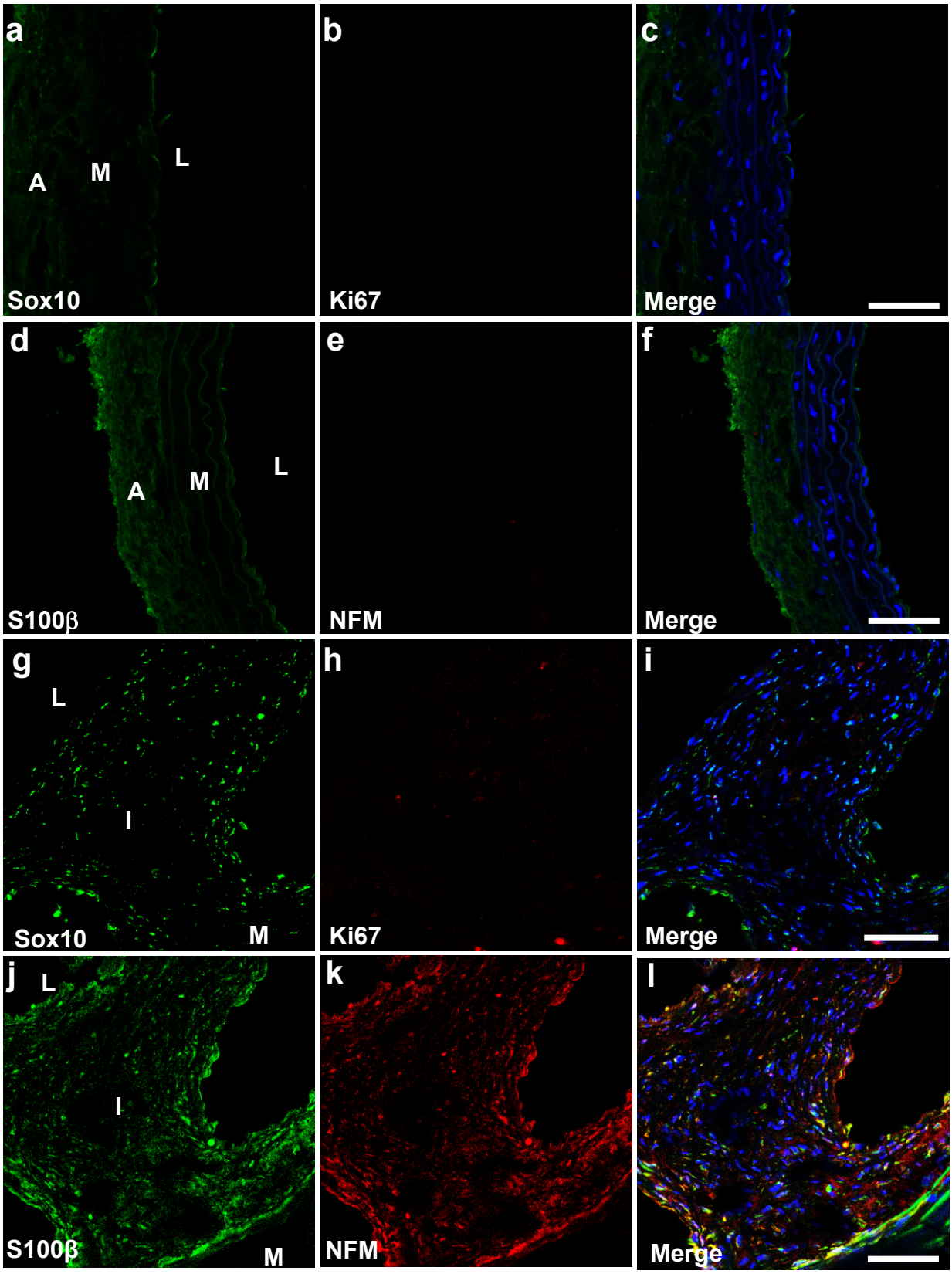
EGFP$^-$ MVSCs were derived from SM-MHC-Cre/LoxP-EGFP mice, co-cultured with OP9-Delta1 feeder cells for 2 weeks, and became EGFP$^+$ cells. (a-c) EGFP$^+$ cells were immunostained with an antibody against SM-MHC. (d-f) EGFP$^+$ cells were immunostained with an antibody against Ki67. Arrows indicate proliferating EGFP$^+$ cells. Nuclei were stained with DAPI. Scale bars are 100 μm.

Supplementary Figure S12. *In vivo* differentiation of MVSCs into SMCs.



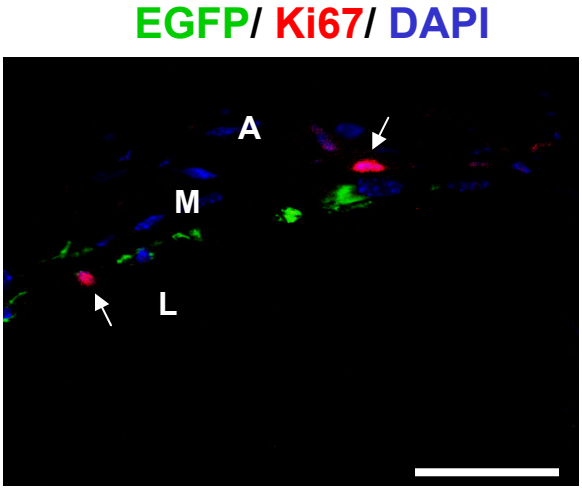
(a) MVSCs derived from SM-MHC-Cre/LoxP-EGFP mice were embedded in collagen gel on the outer surface of a nanofibrous poly(l-lactic acid) vascular graft. The construct was transplanted into an athymic rat by performing carotid artery anastomosis. (b) Staining of cross-sections of a vascular graft with an antibody against EGFP at 1 month after transplantation. Nuclei were stained with DAPI. Scale bar is 50 μm. Dashed line indicates the boundary of the graft and collagen gel. $EGFP^+$ cells indicate the differentiation of MVSCs into SM-MHC expressing SMCs.

Supplementary Figure S13. Expression of MVSC markers in normal and injured carotid arteries of rat.



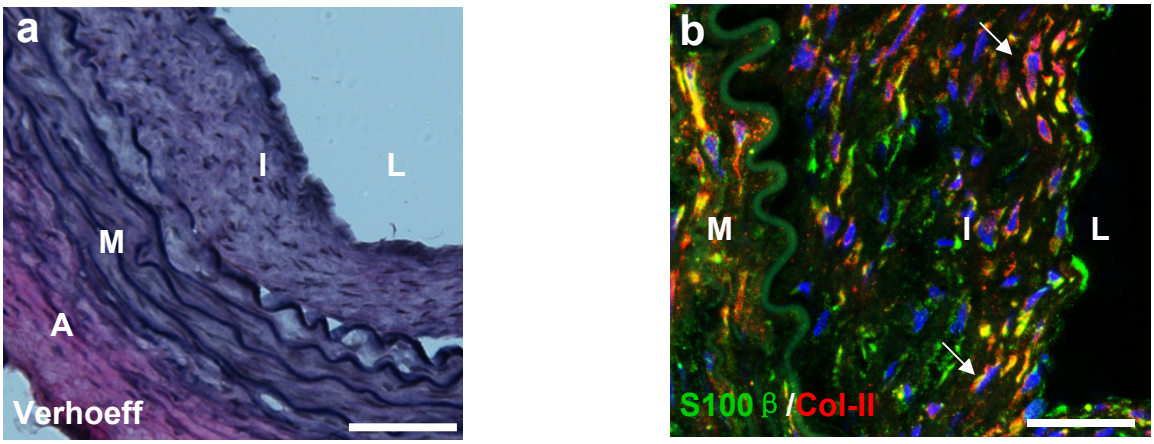
(a-f) Immunostaining of the cross-sections of native carotid artery with antibodies against Sox10, Ki67, S100β and NFM. (g-l) Immunostaining of cross-sections of injured carotid arteries (day 30) with antibodies against Sox10, Ki67, S100β and NFM. Scale bars are 50 μm. A: adventitia, M: media layer, I: intima, L: lumen. Nuclei were stained with DAPI.

Supplementary Figure S14. SMCs cannot proliferate after vascular injury.



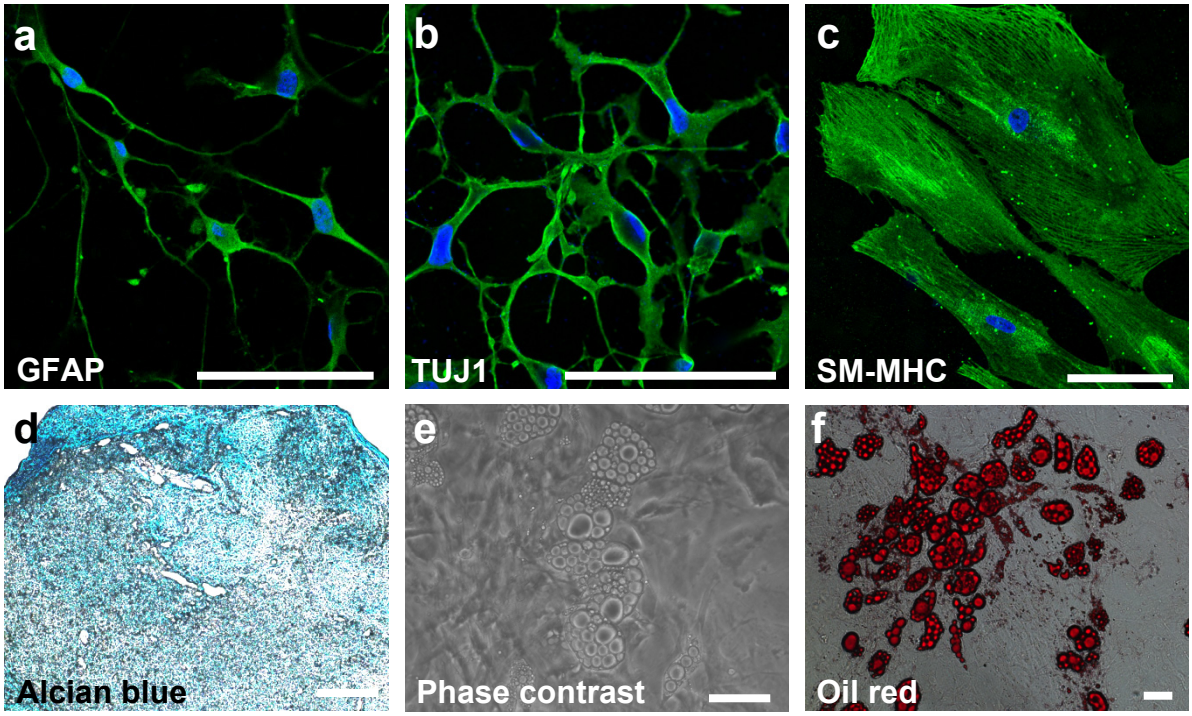
Staining of a cross-section of carotid artery of SM-MHC-Cre/LoxP-EGFP mouse at day 5 after injury with antibodies against EGFP (green) and ki67 (red). Nuclei were stained with DAPI. Arrows indicate proliferating non-SMCs. Scale bar is 50 μm. L: Lumen. M: media layer. A: Adventitia.

Supplementary Figure S15. Matrix synthesis by MVSCs in neointima.



(a) A cross-section of carotid artery at 5 weeks after injury were subjected to Verhoeff's staining. (b) Immunostaining of a cross-section of injured carotid artery with antibodies against S100β (green) and collagen II (red). Arrows indicate cells expressing both S100β and collagen II. A: adventitia. M: media. I: intima. L: lumen. Scale bars are 50 μm.

Supplementary Figure S16. Multipotency of MVSCs isolated from human carotid arteries.



Human MVSCs derived Schwann cells were stained with an antibody against GFAP (a), neurons were stained with an antibody against TUJ1 (b), SMCs were stained with an antibody against SM-MHC (c), chondrocytes were stained for aggrecan by using alcian blue (d), phase contrast picture of oil drops (e), adipocytes were stained for oil droplets by using oil red staining (f). Scale bars are 100 μm.

Supplementary Table S1. The marker expression in MVSCs.

Antibody	MVSC	Company	Dilution/Application	Catalog #
SMA	low	Sigma	1: 400/IF	A2547
CNN1	-	Epitomics	1:200/IF	1806-1
SM-22α	low	Abcam	1:100/IF	Ab14106
NM-MHC	+	Abcam	1:100/IF	Ab684
SM-MHC	-	Santa Cruz	1:50/IF	Sc-79079
Myocardin	-	Santa Cruz	1:50/IF	Sc-34238
Sox10	+	R&D	1:100/IF	MAB2864
Slug	-	Santa Cruz	1:50/IF	Sc-166476
Snail	+	Santa Cruz	1:50/IF	Sc-28199
Pax-3/7	+	Santa Cruz	1:50/IF	Sc-25409
Sox1	+	Chemicon	1:100/IF	AB15766
Sox17	+	R&D	1:100/IF	MAB1924
Nestin	+	Abcam	1:100/IF	ab5968
AP-2α	-	DSHB	1:50/IF	3b5
AP-2β	-	Santa Cruz	1:50/IF	sc-6310
Vimentin	+	DAKO	1:100/IF	M0725
Musashi	-	Chemicon	1:100/IF	AB5977
p75	-	Abcam	1:100/IF and FC	ab8874
HNK1	-	Sigma	1:200/IF and FC	C-6680
NFM	+	Sigma	1:200/IF	N4142
TUJ1	-	Chemicon	1:300/IF	MAB1637
Peripherin	+	Chemicon	1:200/IF	AB1530
Brn3a	+	Millipore	1:200/IF	AB5945
Phox2b	+	Santa Cruz	1:50/IF	Sc-13224
GFAP	-	Millipore	1:200/IF	AB5804

Supplementary Table S1 (Continued)

Antibody	MVSC	Company	Dilution/Application	Catalog #
S100β	+	Sigma	1:200/IF	S2532
Oct4	-	Santa Cruz	1:50/IF	Sc-5279
Nanog	-	Chemicon	1:100/IF	AB9220
Sox2	-	Millipore	1:100/IF	AB5603
Pax6	-	Covance	1:300/IF	PRB-278P
O4	-	Millipore	1:200/IF	MAB345
CD44	+	BD Phar	1:100/FC	550974
CD29	+	BD Phar	1:100/FC	555005
CD146	-	R&D	1:100/FC	FAB3250F
PDX1	-	Millipore	1:100/IF	AB3505
AFP	-	R&D	1:100/IF	MAB-1368
CD133	-	Abcam	1:100/IF	Ab19898
CD31	-	Chemicon	1:50/IF	MAB1393
CD34	-	Santa Cruz	1:50/IF	Sc-7045
C-kit	-	BD Phar	1:50/FC	561680
Flk-1	-	BD Phar	1:50/FC	561025
Sca-1	-	BD Phar	1:100/FC	562059
P63	-	Abcam	1:100/IF	ab59691
CK14	-	Millipore	1:200/IF	MAB3232
Alkaline phosphatase (ALP)	-	DSHB	1:50/IF	B4-78
Collagen II	-	Chemicon	1:100/IF	MAB8887
Sox9	-	Abcam	1:100/IF	ab3697
CD14	-	Santa Cruz	1:50/IF and FC	Sc-1182

Supplementary Table S2. Differentially expressed genes in MVSCs derived from carotid arteries and jugular veins. Expression levels and ratio were presented in log_2 scale. A: carotid artery-derived MVSCs. V: jugular vein-derived MVSCs.

Gene title	A (average)	V (average)	Ratio(A/V)
lipoprotein lipase	8.031393	12.7333	-4.7019
dermatopontin	6.666412	10.91014	-4.24373
complement factor D (adipsin)	6.968016	10.74751	-3.7795
secreted and transmembrane 1B	6.980182	10.71332	-3.73313
leucine rich repeat containing 17	6.780308	10.25873	-3.47843
placenta-specific 8	9.917507	13.37598	-3.45847
retinoic acid receptor responder (tazarotene induced) 2	9.282749	12.70668	-3.42393
T-box 15	6.942331	10.2196	-3.27727
ADP-ribosyltransferase 3	6.982144	10.21136	-3.22922
olfactomedin 1	7.493702	10.6807	-3.187
proenkephalin	7.637622	10.78178	-3.14416
pleiomorphic adenoma gene-like 1	7.130618	10.2124	-3.08179
chemokine (C-X-C motif) ligand 13	10.07631	13.1315	-3.05518
carboxypeptidase X (M14 family), member 1	10.13501	13.18826	-3.05326
cellular retinoic acid binding protein 1	7.703065	10.73564	-3.03258
homeo box D9	6.473827	9.45664	-2.98281
complement component 2	6.333837	9.301748	-2.96791
thrombospondin 2	6.855276	9.751714	-2.89644
growth hormone receptor	7.287906	10.1836	-2.89569
BMP-binding endothelial regulator	6.865798	9.729056	-2.86326
complement component 2	7.188658	9.979958	-2.7913
H19, imprinted maternally expressed transcript	6.517743	9.287932	-2.77019
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1	6.860742	9.560874	-2.70013
transforming growth factor, beta induced	6.978212	9.634068	-2.65586
hyaluronan synthase 2	6.977956	9.633457	-2.6555
Eyes absent homolog 2 (Drosophila)	6.389388	9.037509	-2.64812
complement factor I	6.317993	8.954533	-2.63654
proprotein convertase subtilisin/kexin type 6	6.878133	9.419053	-2.54092
amine oxidase, copper containing 3 (vascular adhesion protein 1)	9.070738	11.59163	-2.5209
chemokine (C-C motif) ligand 11	9.043801	11.56433	-2.52053
LRRN4 C-terminal like	6.890534	9.349794	-2.45926
matrix metallopeptidase 12	8.132424	10.57703	-2.4446
angiopoietin 4	6.820709	9.238427	-2.41772
quinolinate phosphoribosyltransferase	8.164757	10.54528	-2.38052
complement component 4, gene 2 /// complement component 4B (Chido blood group)	9.732406	12.06506	-2.33265

Supplementary Table S2 (Continued)

complement component 2	7.120215	9.432509	-2.31229
adenosine A2B receptor	9.371842	11.66264	-2.2908
transglutaminase 1, K polypeptide	6.639287	8.922598	-2.28331
solute carrier family 39 (zinc transporter), member 8	6.470646	8.741465	-2.27082
matrix metallopeptidase 3	9.572365	11.83371	-2.26134
secreted frizzled-related protein 4	10.09367	12.34915	-2.25547
wingless-type MMTV integration site family, member 4	6.328639	8.543901	-2.21526
Hypothetical protein LOC689663	7.251499	9.431611	-2.18011
lecithin-retinol acyltransferase (phosphatidylcholine-retinol-O-acyltransferase)	8.20533	10.38089	-2.17556
early B-cell factor 3	6.910186	9.078814	-2.16863
alpha-2u globulin PGCL4	8.247121	10.36101	-2.11389
complement factor B	9.624536	11.72206	-2.09752
tumor necrosis factor alpha induced protein 6	10.46488	12.55217	-2.08729
nidogen 2	10.66323	12.73784	-2.07461
sushi, nidogen and EGF-like domains 1	10.24981	12.32093	-2.07112
alpha-2u globulin PGCL4	6.114782	8.185016	-2.07023
fibrillin 2	7.539471	9.609447	-2.06998
SH3-domain kinase binding protein 1	6.963905	9.010674	-2.04677
plexin domain containing 2	7.615719	9.656075	-2.04036
poly(rC) binding protein 3	6.611741	8.63729	-2.02555
von Willebrand factor A domain containing 1	6.261853	8.286512	-2.02466
aldo-keto reductase family 1, member C-like 1	8.786936	10.80652	-2.01958
hypothetical protein LOC688459	8.919705	6.411005	2.5087
WNK lysine deficient protein kinase 4	7.499638	4.985299	2.51434
similar to integrin alpha 8	10.09637	7.57154	2.524826
flavin containing monooxygenase 2	8.22844	5.700068	2.528372
family with sequence similarity 107, member B	7.984358	5.44916	2.535198
transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	7.435926	4.871385	2.56454
flavin containing monooxygenase 3	7.15284	4.580658	2.572181
regulator of G-protein signaling 2	11.54102	8.963578	2.577443
growth differentiation factor 10	10.71561	8.088668	2.62694
transforming growth factor alpha	9.81371	7.167007	2.646703
phospholipid scramblase 2	9.492705	6.795921	2.696783
MDS1 and EVI1 complex locus	8.858904	6.157443	2.701462
similar to integrin alpha 8	9.392531	6.682334	2.710197
collagen, type IV, alpha 5	10.27227	7.555335	2.716931
cyclin D2	10.99901	8.27241	2.726598
gap junction protein, beta 2	7.060222	4.330215	2.730008
myosin light chain kinase	10.94207	8.196665	2.745403
serum deprivation response	11.18922	8.433875	2.755343

Supplementary Table S2 (Continued)

ankyrin repeat domain 1 (cardiac muscle)	7.766035	5.00664	2.759396
odz, odd Oz/ten-m homolog 3 (Drosophila)	6.80937	4.040711	2.768659
serum deprivation response	10.21024	7.428725	2.781519
cell adhesion molecule 1	7.885473	5.097356	2.788117
complement component 1, q subcomponent-like 3	8.641059	5.841769	2.799289
LOC363015	8.214709	5.400896	2.813812
paired box 9	7.752265	4.892829	2.859436
acid phosphatase, prostate	7.712842	4.848012	2.864829
cytochrome P450, family 26, subfamily b, polypeptide 1	10.17016	7.255595	2.914562
ceruloplasmin	13.19412	10.26331	2.930816
phospholipid scramblase 1	10.9648	7.997051	2.967751
serglycin	10.91656	7.933558	2.983003
chemokine (C-X3-C motif) ligand 1	9.427428	6.436873	2.990555
similar to SMAD-interacting zinc finger protein 2	6.987849	3.92835	3.059499
laminin, alpha 5	8.94223	5.877273	3.064957
receptor (G protein-coupled) activity modifying protein 1	8.368172	5.287092	3.08108
transmembrane 4 L six family member 1	8.135064	5.048338	3.086727
lipopolysaccharide binding protein	10.51802	7.413863	3.104157
Cd200 molecule	10.95231	7.758638	3.193671
Forkhead box P2	7.578067	4.365891	3.212176
bone morphogenetic protein 2	9.452797	6.233906	3.218891
S100 calcium binding protein G	7.427089	4.164898	3.262191
transmembrane 4 L six family member 1	11.47979	8.214201	3.265586
SRY (sex determining region Y)-box 17	8.001019	4.687529	3.31349
complement component 1, q subcomponent-like 3	8.136158	4.682555	3.453603
bone morphogenetic protein 2	8.888307	5.428536	3.459771
hairy/enhancer-of-split related with YRPW motif 2	8.269008	4.752881	3.516127
gap junction protein, alpha 5	12.03444	8.509872	3.524565
gap junction protein, alpha 4	9.529695	5.969648	3.560048
tumor protein D52-like 1	7.462407	3.88732	3.575087
inhibitor of DNA binding 4	8.387406	4.809774	3.577632
alcohol dehydrogenase 1 (class I)	8.255159	4.669793	3.585366
bone morphogenetic protein 4	7.906448	4.306913	3.599534
growth associated protein 43	8.628532	4.793128	3.835404
growth arrest specific 6	10.61567	6.65438	3.961289
phospholamban	9.051457	5.057065	3.994393
protein tyrosine phosphatase, receptor type, D	9.646863	5.333628	4.313235
aldehyde dehydrogenase 1 family, member A3	10.00725	5.344812	4.662438
solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	9.344938	4.411995	4.932943

Supplementary Table S3. Relative mRNA expression levels of MSC markers in MVSCs derived from carotid arteries and jugular veins. Expression levels were presented in log_2 scale. A: carotid artery-derived MVSCs. V: jugular vein-derived MVSCs.

	A1	A2	A3	V1	V2	V3
CD29	12.68622	12.68937	12.56967	12.72439	12.76024	12.67875
CD54	11.42541	11.04618	11.07476	10.75978	10.71997	10.85471
CD13	12.58192	12.19374	12.55867	12.70696	12.85414	12.889
CD44	10.17512	9.58491	9.86529	9.339053	9.576896	9.890446
CD73	8.407454	8.193264	8.438201	7.98988	8.023622	8.219538
CD90	9.909732	10.10694	9.659861	10.56263	10.51612	10.21175
CD105	9.780092	9.635467	9.681691	9.041794	9.229681	9.735992
CD166	11.60239	11.50017	11.35184	10.81729	11.13338	11.22315
CD106	12.3402	12.69535	13.03897	11.53258	10.66766	11.01302

Supplementary Table S4. Primer sequences used for genotyping.

Transgene	Forward	Reverse
Wnt1-Cre	GCAATTTCGGCTATACGTAACAGGG	GCAAGAACCTGATGGACATGTTCAG
SM-MHC-Cre	GCGGTCTGGCAGTAAAAACTATC	GTGAAACAGCATTGCTGTCACTT
Rosa26-loxP-lacZ	AAAGTCGCTCTGAGTTGTTAT	GCGAAGAGTTTGTCCTCAACC
Rosa26-loxP-EGFP	GAGTTCTCTGCTGCCTCCTG	AAGACCGCGAAGAGTTTGTC
β-actin-LoxP-EGFP	AAGTTCATCTGCACCACCG	TCCTTGAAGAAGATGGTGCG

Supplementary Table S5. Primer sequences used in qPCR.

Gene	Forward	Reverse
Rat Sox10	CTGGAGGTTGCTGAACGAGAGT	GTCCGGATGGTCTTTTTTGTG
Rat Sox17	AGAACCCGGATCTGCACAAC	AGGATTTGCCTAGCATCTTGCT
Rat Aggrecan	CTTCAAGCTGAACTATGACCACTTTACT	CATGGTCTGGAACTTCTTCTGAGA
Rat SMA	TCCTGACCCTGAAGTATCCGATA	GGTGCCAGATCTTTTCCATGTC
Rat CNN1	AGAACAAGCTGGCCCAGAAA	CACCCCTTCGATCCACTCTCT
Rat 18S	GCCGCTAGAGGTGAAATTCTTG	CATTCTTGGCAAATGCTTTCG