Apelin is expressed in intimal smooth muscle cells and promotes their phenotypic transition

Supplementary raw data

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Short title: Apelin and smooth muscle cells

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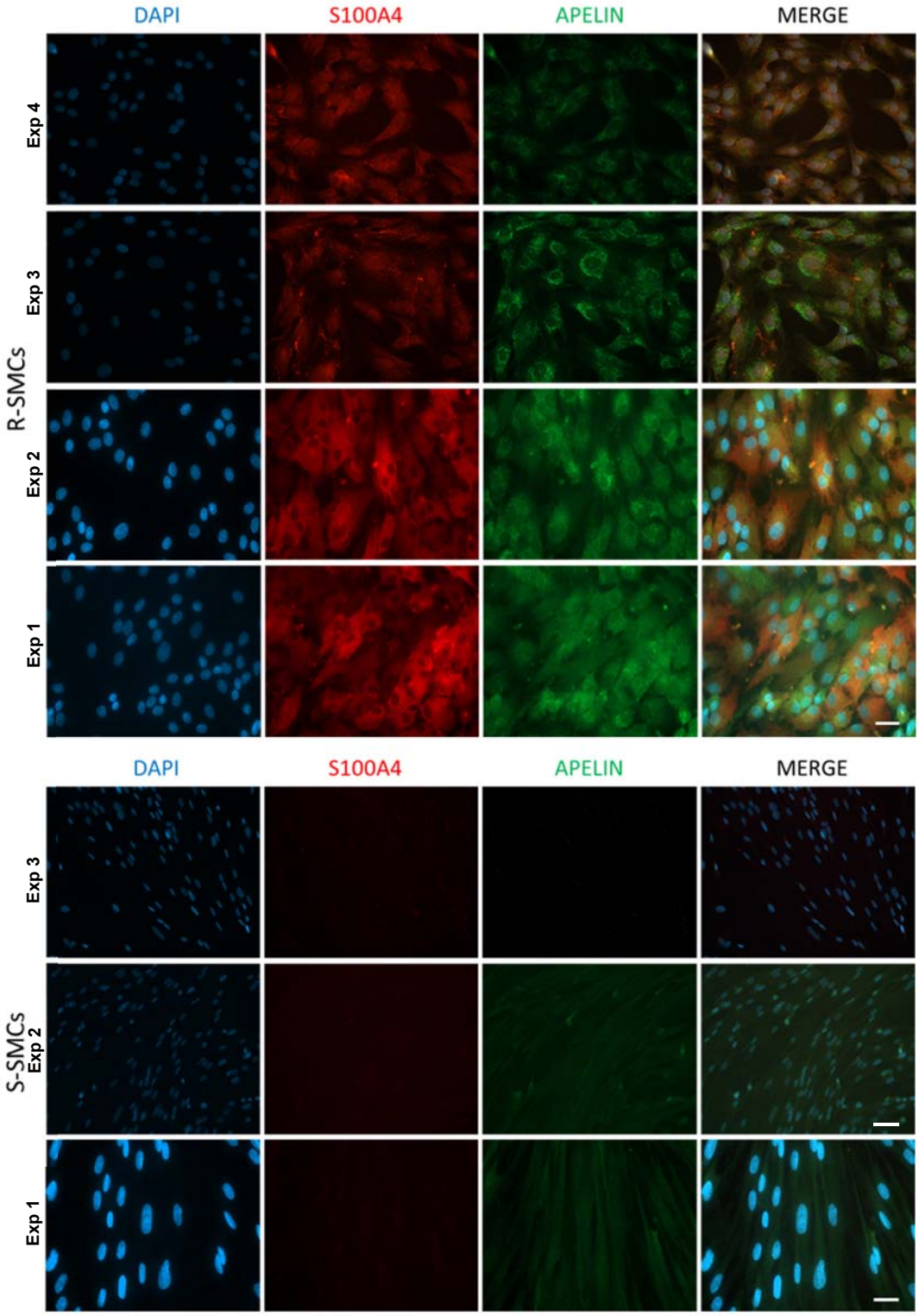
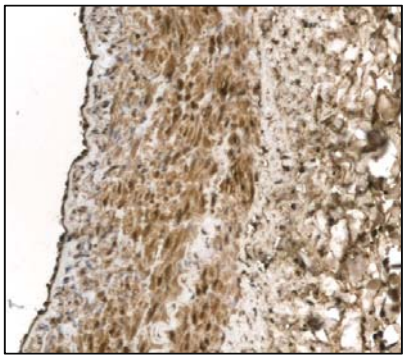
Figure 1A



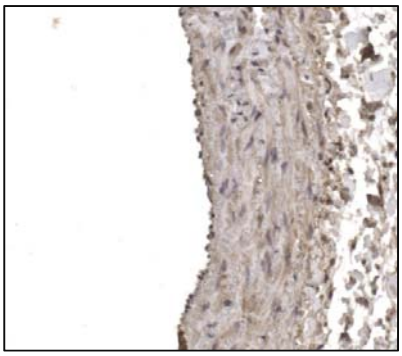
Double immunofluorescence staining showing apelin and S100A4 expression in R-SMCs and S-SMCs from 4 and 3 independent cell cultures respectively, nuclei are stained in blue with DAPI. Scale bar for R-SMCs and S-SMCs Exp 1 30μm, for S-SMCs Exp 2 and Exp 3 60μm.

Figure 1B

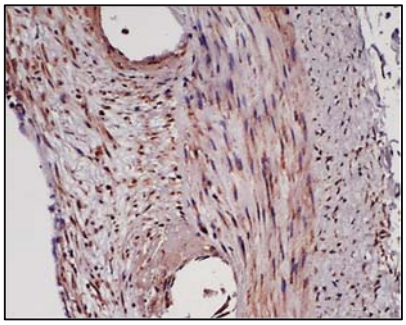
Normal porcine coronary artery



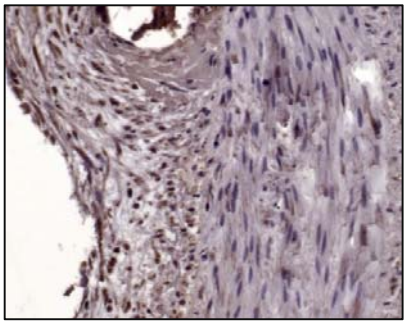
Normal porcine coronary artery



Stented porcine coronary artery

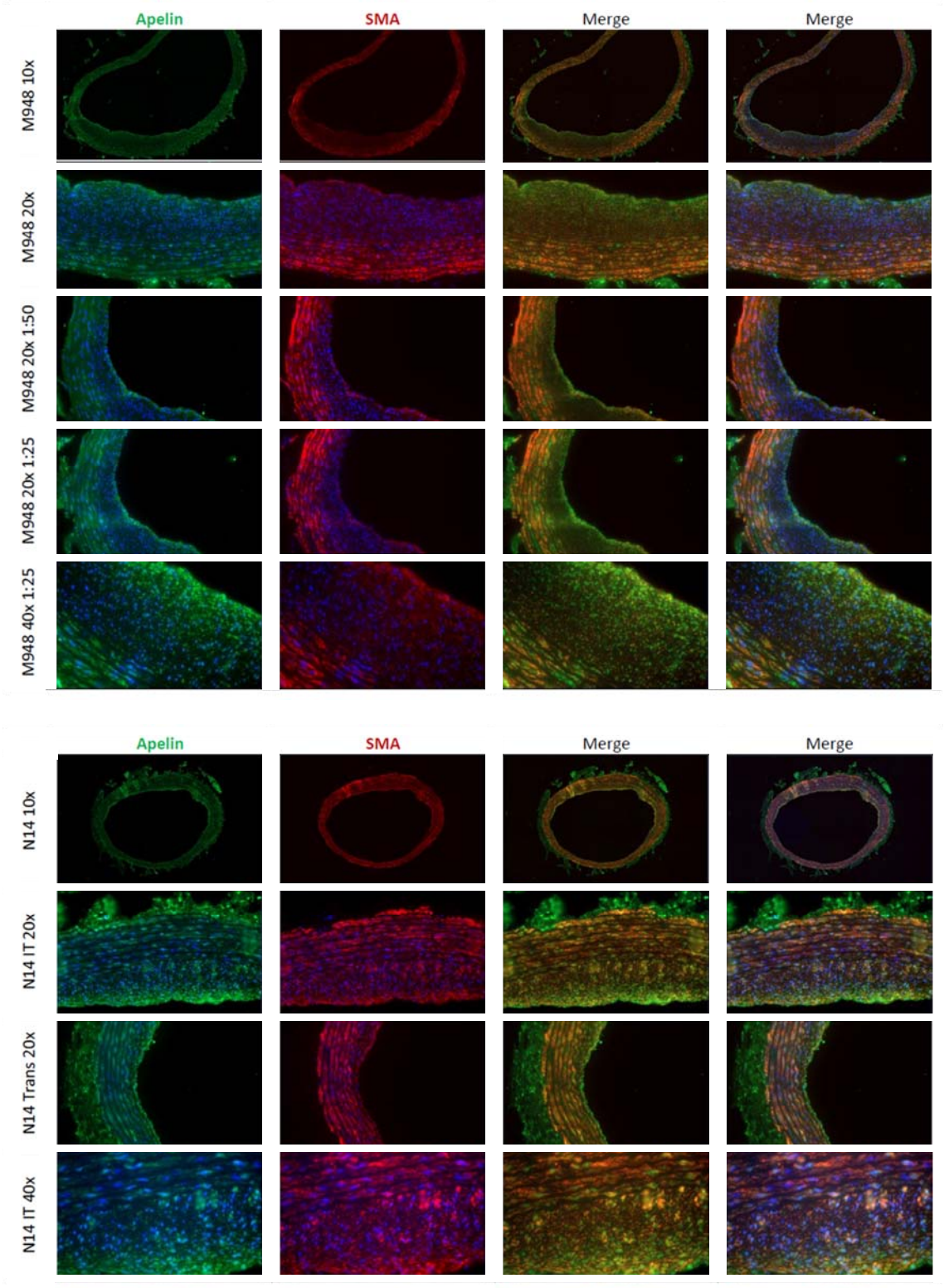


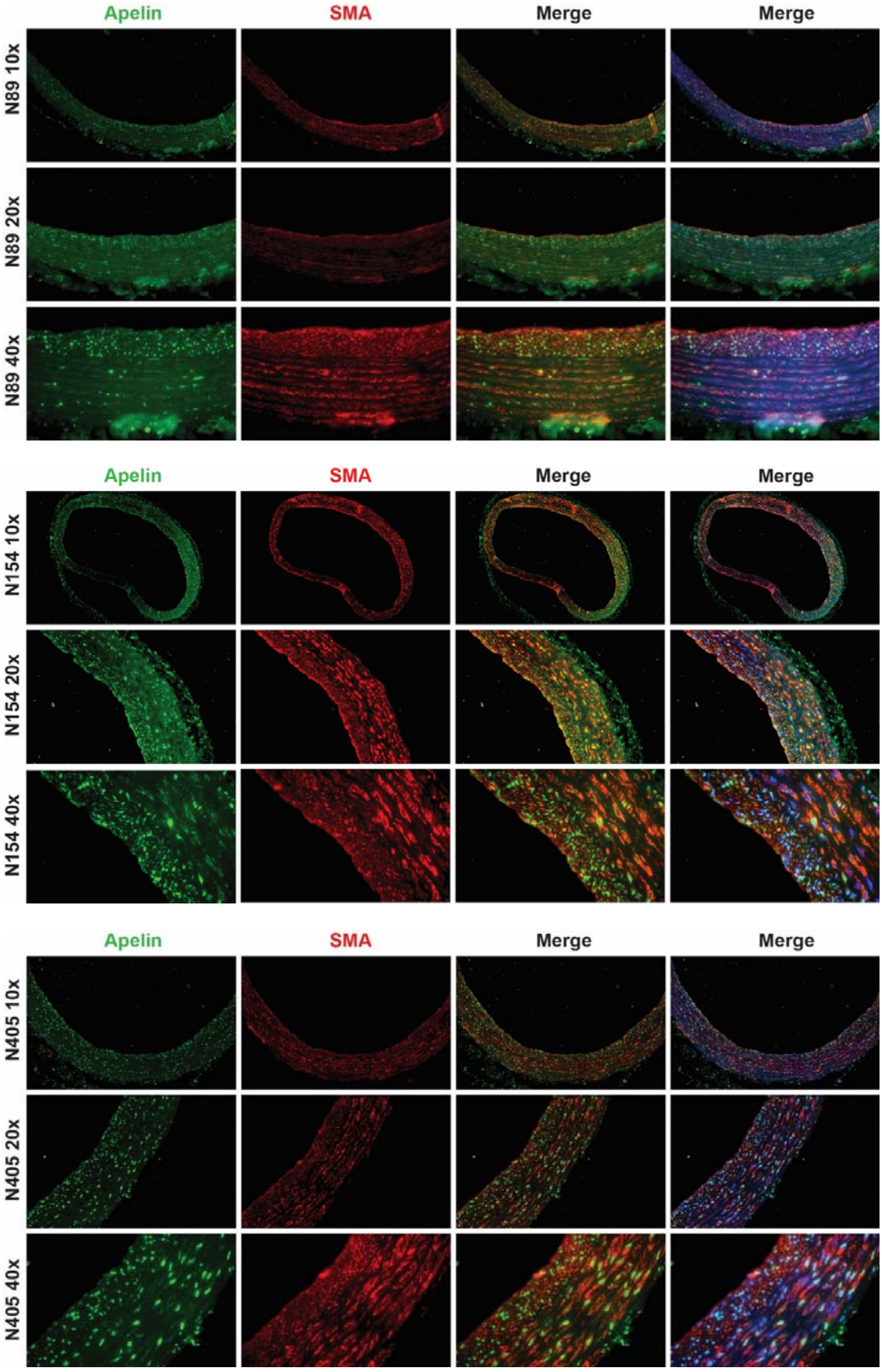
Stented porcine coronary artery

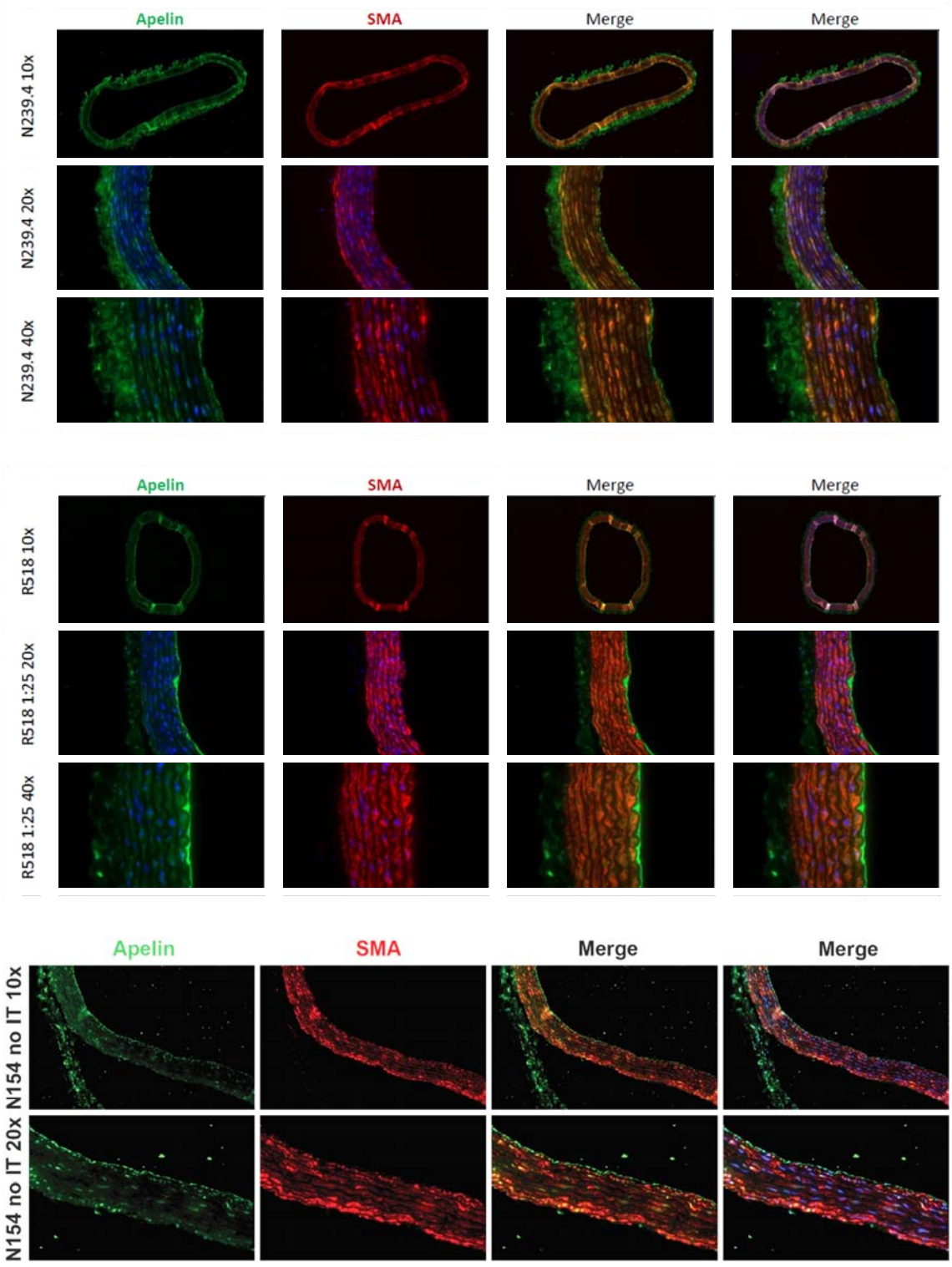


Immunohistochemistry showing expression of apelin in normal and stented porcine coronary arteries. N=2 for each condition

Figure 1 D


Apelin
SMA
Merge
Merge
M948 10x
M948 20x
M948 20x 1:50
M948 20x 1:25
M948 40x 1:25
Apelin
SMA
Merge
Merge
N14 10x
N14 IT 20x
N14 Trans 20x
N14 IT 40x

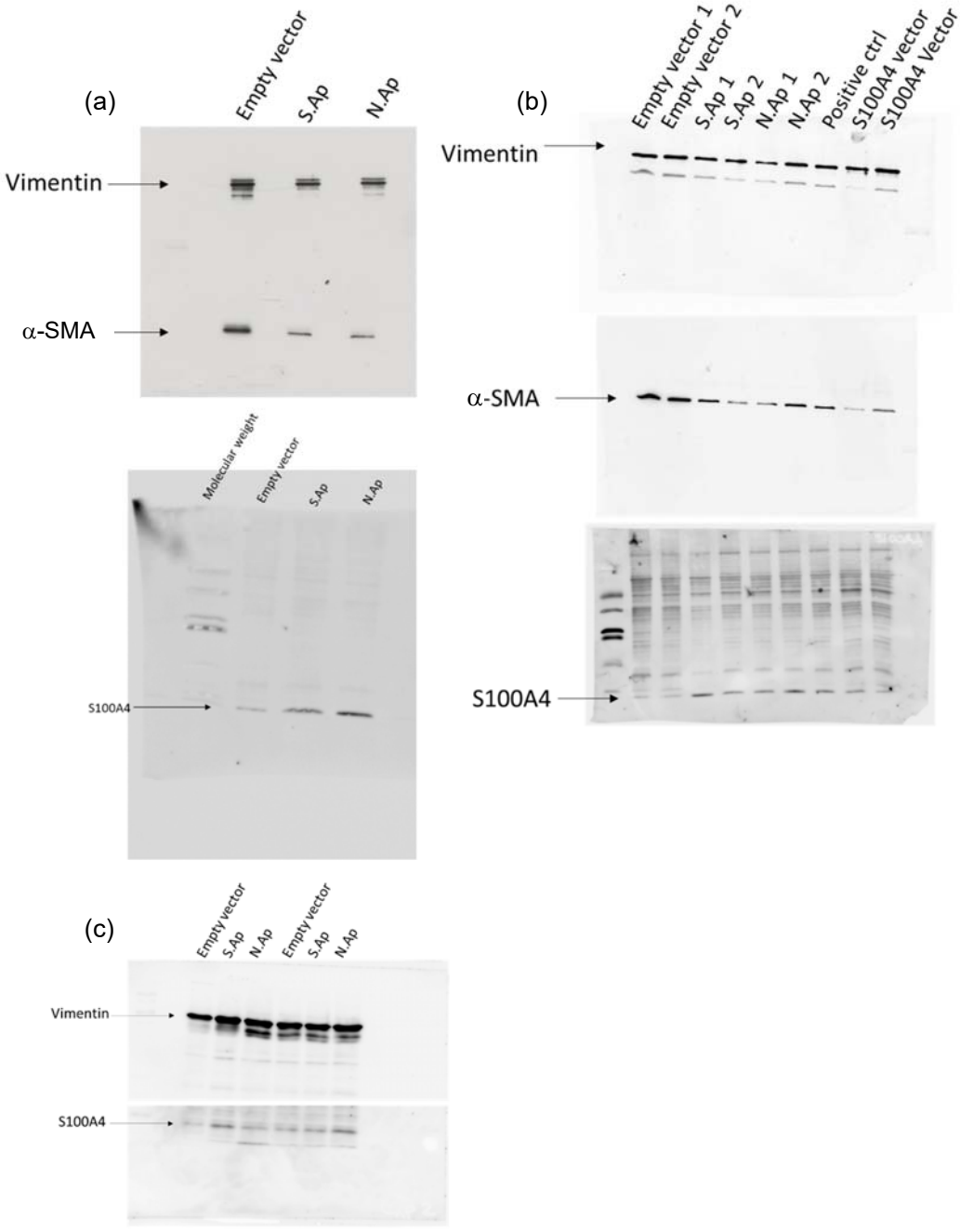

Apelin
SMA
Merge
Merge
N89 10x
N89 20x
N89 40x
Apelin
SMA
Merge
Merge
N154 10x
N154 20x
N154 40x
Apelin
SMA
Merge
Merge
N405 10x
N405 20x
N405 40x

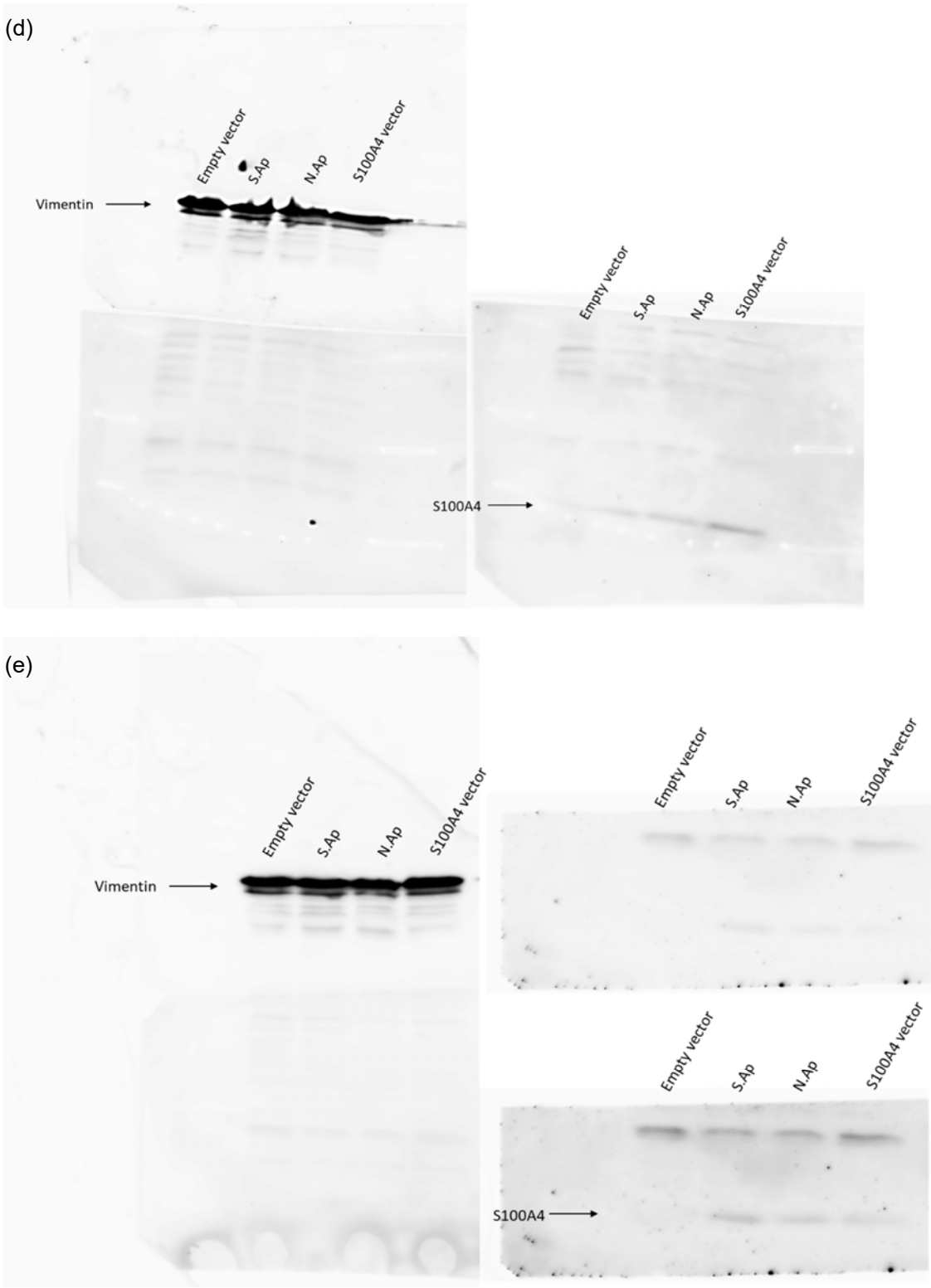


Double immunofluorescence staining showing expression of α-SMA (red) and apelin (green) in normal and balloon-induced intimal thickening of rat aorta. M914, N14, N89, N154 and N405 are samples from 20 days induced intimal thickening of rat aortas and N239.4 and R518 are normal rat aortas. N154 no IT (no intimal thickening) sample represents a non-lesional area of the N154 rat aorta used as a control. 1:25 and 1:50 represent the antibody dilutions used.

Figure 3B

S100A4 and α-SMA Western Blots:



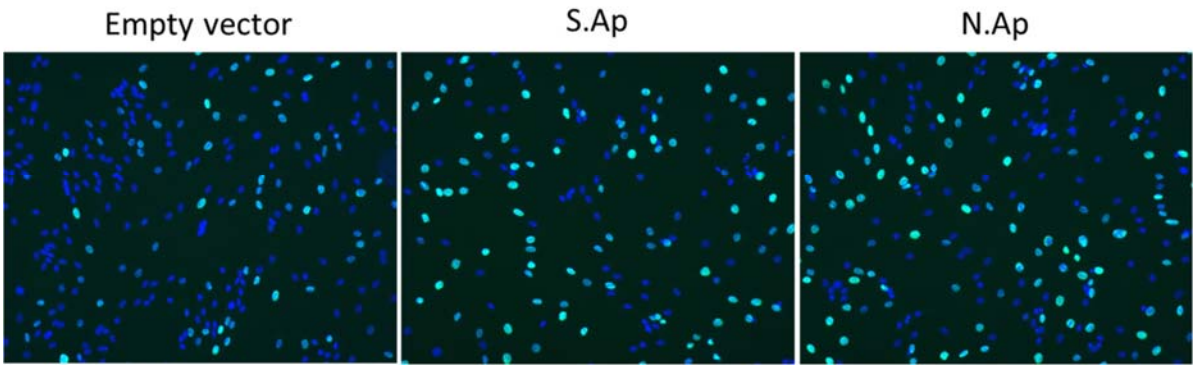


Raw data of western blot showing α-SMA and S100A4 expression in transfected S-SMCs 96 hours after transfection with empty vector, S. Ap, and N. Ap. Positive control represents rhomboid smooth muscle cells. S100A4 vector represents SMCs transfected with an S100A4 expressing vector.

In some experiments, membranes were cut horizontally between 25kDa and 35kDa and incubated with anti-α-SMA or anti-vimentin (upper part of the membrane) or anti-S100A4 (lower part of the membrane) which explains the lack of full-length blots. All corresponding samples (α-SMA, vimentin or S100A4) derive from the same experiment and were processed in parallel.
(a,b,c) Membranes were incubated with the homemade mouse monoclonal IgM antibody recognizing S100A4. (d,e) Membranes were incubated with the mouse monoclonal IgG1 anti-S100A4 antibody AMAB90596, Sigma-Aldrich. In (e), the right panels show two different exposition times.

Figure 3C

BrdU Staining:



BrdU positive cell percentage quantification:

	E vector	S. Ap	N. Ap
Exp 1	32.25	53.35	48.5
Exp 2	31.2	54.54	58.62
Exp 3	39	58.26	52.53

Immunofluorescence staining showing BrdU-positive cells in transfected S-SMCs 96 hours after transfection with empty vector, S. Ap, and N. Ap and quantification of BrdU-positive cells percentage from 3 independent experiments.

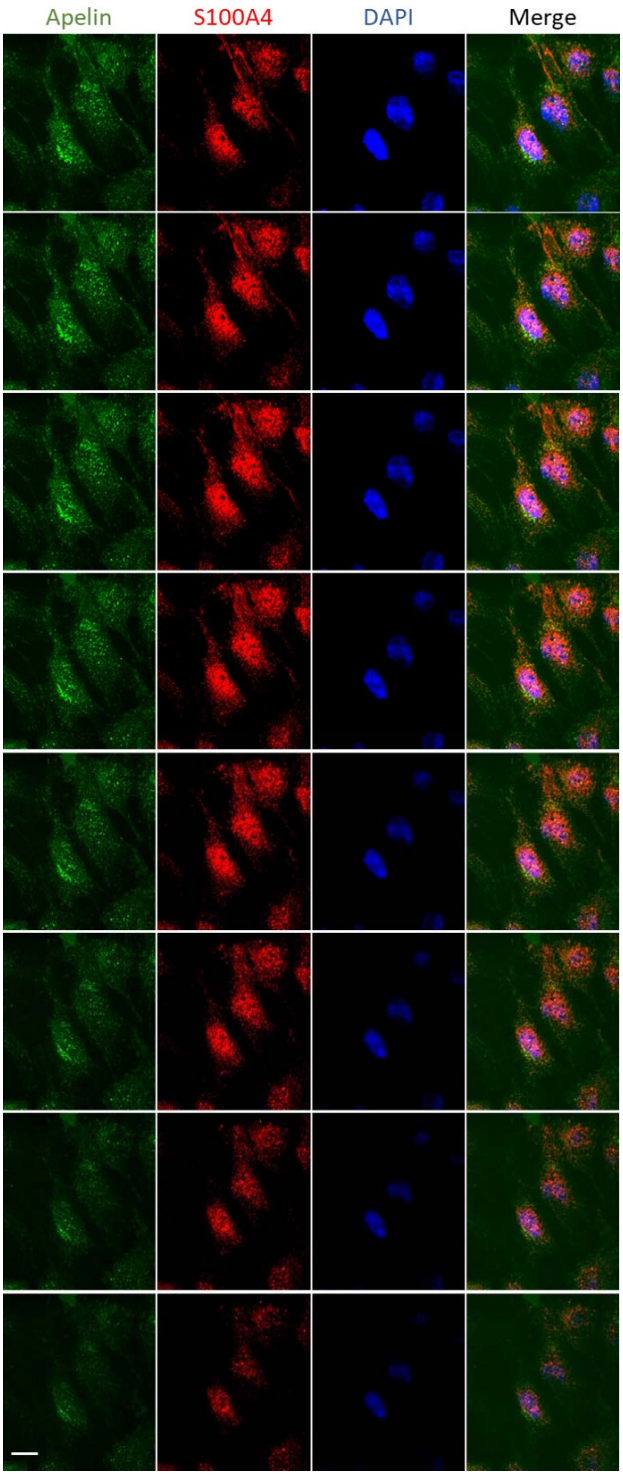
Figure 3D

S100A4 ELISA:

Table representing experimental Optical density values and concentration of extracellular S100A4 detected by competitive ELISA assay in S-SMC supernatants after transfection with empty vector, S. Ap, and N. Ap from 6 independent experiments.

n=6	OD1	OD2	OD3	OD4	OD5	OD6	OD Mean	Conc [nM]	Conc [pM]	Nb cells	concen/1000 cell	Normalized data
Empty vector	0.131	0.126	0.137				0.131	0.632	632	990000	0.638383838	1
S.Ap	0.102	0.079	0.137				0.106	1.191	1191	990000	1.203030303	1.884493671
N.Ap	0.1	0.097	0.127				0.108	1.133	1133	1026000	1.104288499	1.72981901
Empty vector	0.311	0.326	0.317				0.318	0.427	427.415	1038000	0.411767757	1
S.Ap	0.267	0.271	0.26				0.266	0.819	818.731	840000	0.974679468	2.367061167
N.Ap	0.227	0.261	0.276				0.255	0.943	943.335	852000	1.107201232	2.688897353
Empty vector	0.279	0.276	0.261				0.272	0.760	759.572	990000	0.767244569	1
S.Ap	0.214	0.233	0.238				0.228	1.311	1311.057	906000	1.447082277	1.886076924
N.Ap	0.222	0.219	0.297				0.221	1.446	1445.926	1026000	1.409284675	1.83681284
Empty vector	0.289	0.278	0.294	0.286	0.268	0.295	0.285	0.235	234.570	990000	0.237	1
S.Ap	0.251	0.249	0.251	0.257	0.253	0.233	0.249	0.427	427.415	906000	0.471	1.987341772
N.Ap	0.238	0.256	0.268	0.246	0.247	0.25	0.251	0.415	414.553	1026000	0.404	1.70464135
Empty vector	0.157	0.241	0.182				0.170	0.209	209	855000	0.245	1
S.Ap	0.162	0.14	0.154				0.152	0.328	328	848300	0.386	1.578673711
N.Ap	0.139	0.127	0.176				0.147	0.369	369	902000	0.410	1.673409258
Empty vector	0.152	0.186	0.167				0.168	0.216	216	883000	0.244	1
S.Ap	0.15	0.134	0.122				0.135	0.503	503	967500	0.519	2.127108662
N.Ap	0.122	0.147	0.136				0.135	0.507	507	1020400	0.497	2.034145988

Figure 4A



Z-stack of confocal imaging of PDGF-BB stimulated SMCs showing apelin and S100A4 nuclear localization.

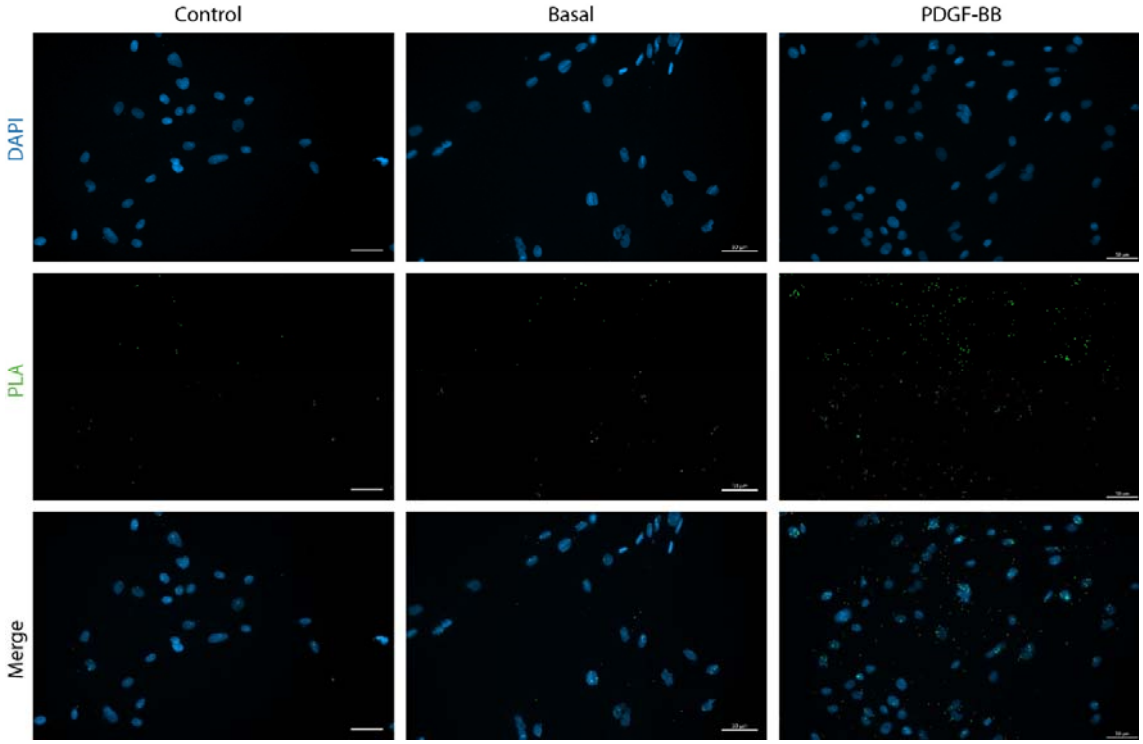
Figure 4B and C

		PDGF-BB treated SMCs					Control SMCs			
	Cell number	**Nuc Apelin**	**Nuc S100A4**	**Cyto Apelin**	**Cyto S100A4**	Cell number	**NucApelin**	**Nuc S100A4**	**Cyto Apelin**	**Cyto S100A4**
Values of CCD Unit/5sec	1	474	120	264	127	1	172	121	188	117
	2	262	130	285	128	2	140	113	168	101
	3	263	163	296	128	3	204	138	189	123
	4	244	114	270	129	4	183	113	216	122
	5	243	196	231	123	5	170	101	207	111
	6	194	128	185	125	6	138	97	185	109
	7	215	161	222	123	7	143	109	179	107
	8	150	101	195	105	8	152	127	143	121
	9	162	103	140	100	9	111	112	116	103
	10	303	174	339	130	10	137	110	163	118
	11	272	127	312	137	11	119	104	180	165
	12	324	194	313	127	12	136	108	135	120
	13	312	259	265	332	13	188	100	194	99
	14	419	148	241	110	14	216	103	254	110
	15	245	154	215	134	15	181	105	222	112
	16	170	134	177	122	16	211	113	251	117
	17	172	110	181	104	17	166	106	152	100
	18	151	199	150	135	18	189	129	230	138
	19	200	223	204	141	19	156	134	224	150
	20	207	246	209	136	20	182	119	222	123
	21	106	107	107	107	21	311	176	361	198
	22	161	144	169	124	22	270	179	362	164
	23	130	125	129	120	23	274	149	307	145
	24	116	116	122	107	24	352	158	292	127
	25	103	100	122	105	25	686	161	419	156
	26	358	501	307	435	26	533	147	475	147
	27	323	108	337	164	27	332	143	501	146
	28	458	262	402	167	28	149	128	286	139
	29	442	205	320	162	29	253	126	241	122
	30	208	165	253	128	30	216	104	191	110
	31	381	315	353	193	31	256	137	398	185
	32	278	329	358	159	32	242	184	270	167
	33	374	318	356	174	33	221	115	137	119
	34	396	284	367	184					
	35	309	320	358	182					
	36	229	197	294	146					
	37	266	169	299	153					
	38	573	187	428	184					
	39	553	178	397	196					
	40	229	178	287	153					

Table representing CCD unit values of Nuclear apelin, nuclear S100A4, cytoplasmic apelin, cytoplasmic S100A4 for each analysed cell treated with PDGF-BB vs Control.

Figure 4D and E

Proximity Ligation Assay:

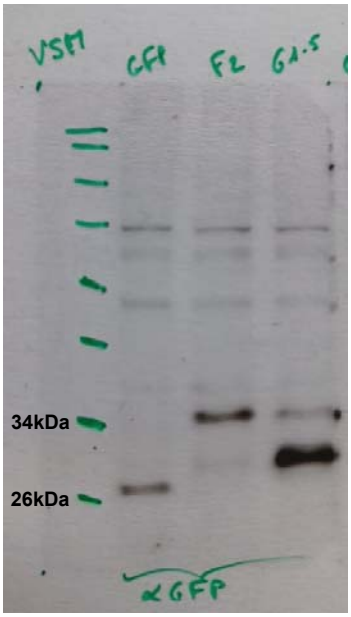


n=4	Number of Cells	Dots	Dots/cell
Ctrl	262	182	0.6946565
Basal	275	480	1.7454545
PDGF-BB	108	518	4.7962963
Ctrl	192	96	0.5
Basal	304	586	1.9276316
PDGF-BB	52	227	4.3653846
Ctrl	257	95	0.3696498
Basal	61	151	2.4754098
PDGF-BB	79	297	3.7594937
Ctrl	108	73	0.6759259
Basal	36	55	1.5277778
PDGF-BB	164	796	4.8536585

Proximity ligation assay showing the interaction between apelin and S100A4 upon PDGF-BB stimulation. Representative field image with quantification of PLA dots/cell from 4 independent biological replicates

Figure 5C

Green Fluorescent Protein (GFP) Western Blots:



Raw data of western blot showing GFP expression in transfected S-SMCs 24 hours after transfection with empty GFP vector (pEGFP-N1, GFP), S. Ap (F2), and N. Ap (G1.5).