

YMTHE, Volume 26

Supplemental Information

MicroRNA-532-5p Regulates Pericyte Function

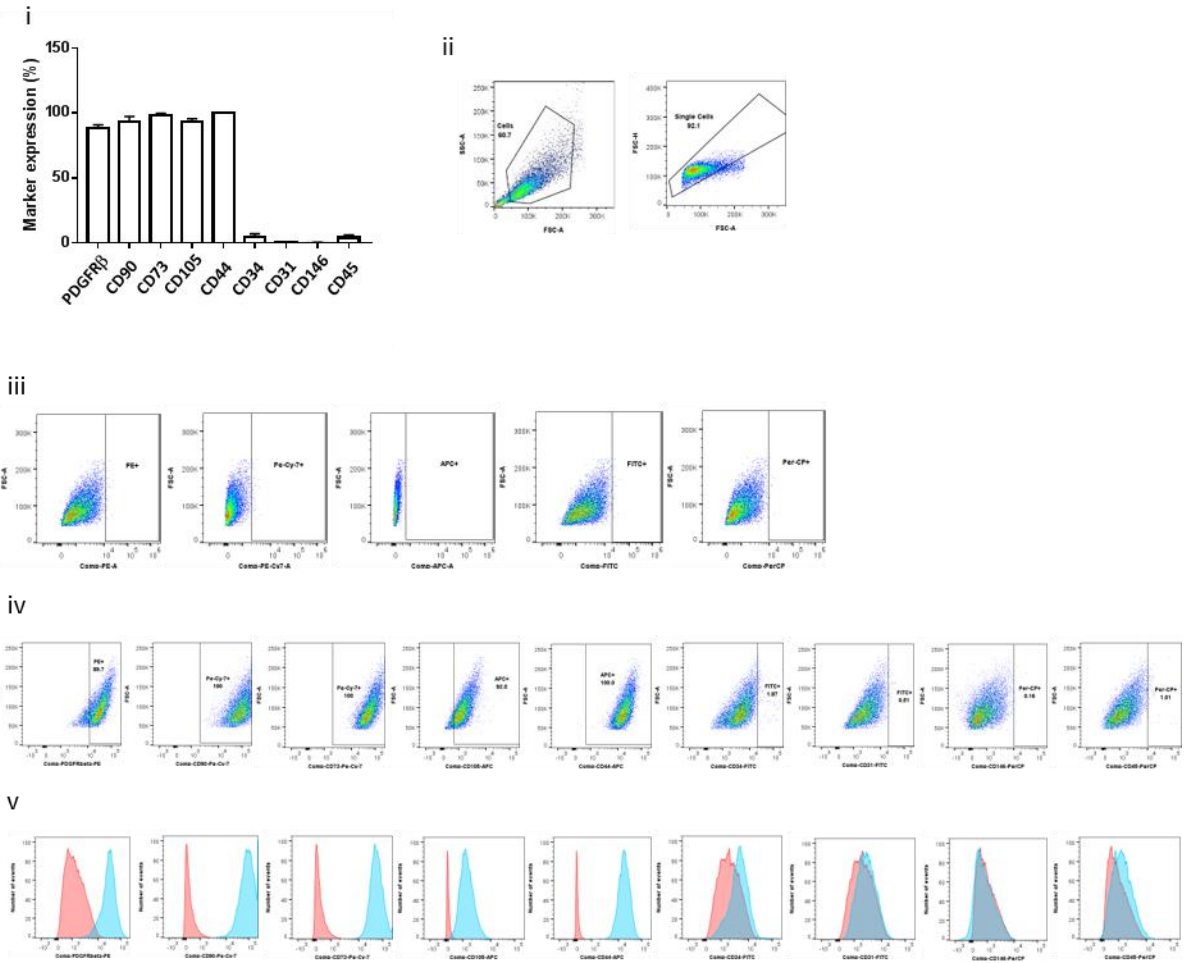
by Targeting the Transcription Regulator

BACH1 and Angiopoietin-1

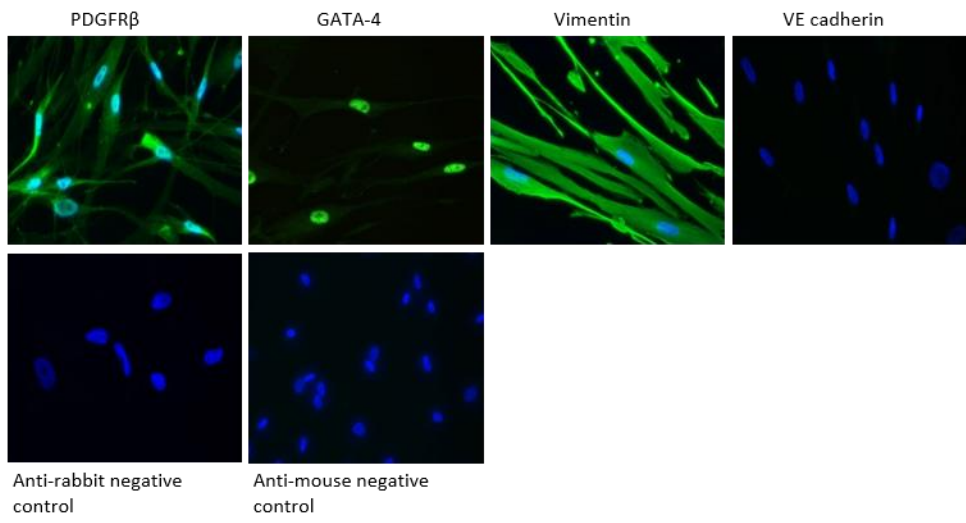
Sadie C. Slater, Eva Jover, Andrea Martello, Tijana Mitić, Iker Rodriguez-Arabaolaza, Rosa Vono, Valeria V. Alvino, Simon C. Satchell, Gaia Spinetti, Andrea Caporali, and Paolo Madeddu

Figure S1

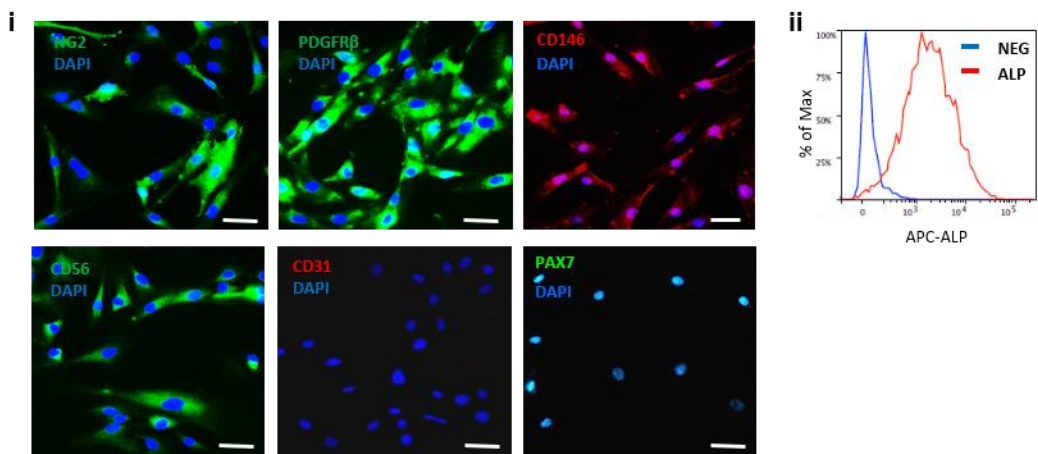
A FACS demonstrating human APC antigenic profile



B Immunofluorescent staining demonstrating human APC antigenic profile



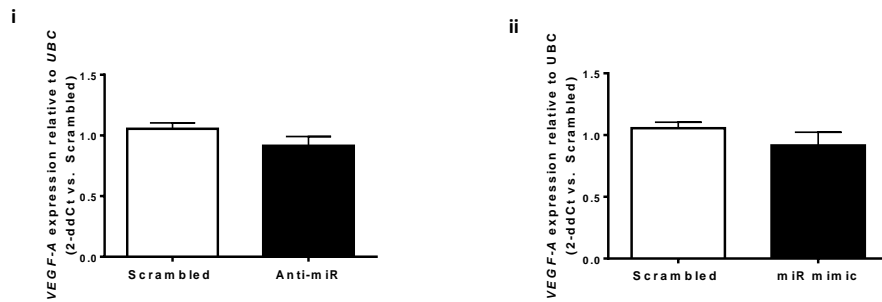
C Muscle pericytes characterization



Supplemental Figure 1. S1A: Flow cytometry analyses of adventitial pericytes isolated from human saphenous veins. (i). Bar graphs show the mean \pm SE values of three human adventitial pericytes (APC) lines. Representative images of flow cytometry gating procedure for a single APC line at P5 are shown. (ii) Total cell populations and the single cells (singlets), were gated according to FSC-A vs. SSC-A and FSC-A vs. FSC-H parameters. Cells were further gated for negative (iii) and positive controls (iv) of selected antigens. Pericyte, mesenchymal, endothelial, and hematopoietic markers were studied. (v) Flow cytometry histograms for each surface marker in representative human APC line. Negative control staining profiles are shown by the red histograms, whereas specific antibody staining profiles are shown by light blue histograms. **S1B: Immunofluorescent analyses of APCs isolated from human saphenous veins.** Representative images from a single APC line stained for PDGFR β , GATA-4, vimentin and VE-cadherin. x40 magnification. Blue staining: DAPI, green staining: protein of interest. **Figure S1C: Characterization of muscular pericytes** Skeletal muscle pericytes (MPCs) were isolated from human sartorius muscle biopsies. The phenotypical characterization was carried out by immunofluorescence staining (i) for the typical pericyte markers NG2, CD146 and PDGFR β . In addition, MPCs show positivity for the muscle marker CD56 and negativity for the expression of the endothelial (CD31) and satellite cell (PAX7) markers. (ii) FACS analysis shows that MPCs express alkaline fosfatase (ALP- red line). The blue line shows the negative control (NEG) as a reference.

Figure S2

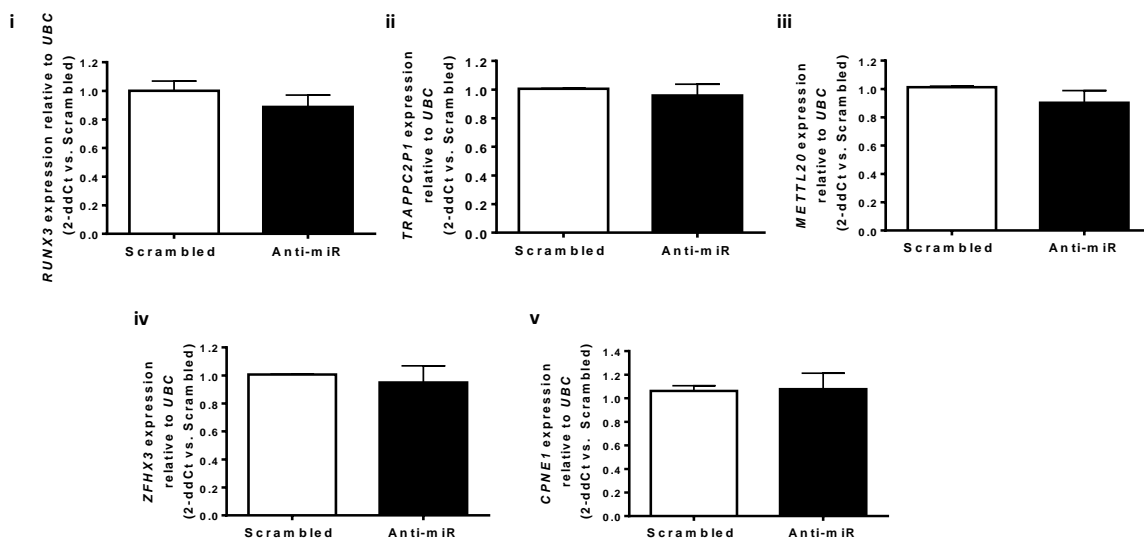
Effect of miR-532-5p modulation on VEGF-A gene expression



Supplemental Figure 2. Effect of miR-532-5p modulation on VEGF-A expression: (i) VEGFA mRNA levels in APCs transfected with scrambled sequence or miR-532-5p anti-miR. (ii) VEGFA mRNA levels in APCs transfected with scrambled sequence or miR-532-5p mimic; N=5 biological replicates per group. P=N.S. for both comparisons. All values are means±SE.

Figure S3

qPCR validation of predicted miR-532-5p target genes

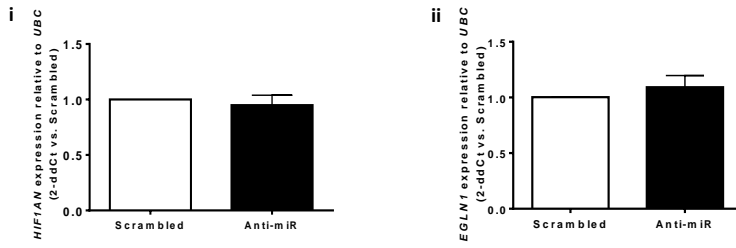


Supplemental Figure 3. Inhibition of miR-532-5p does not affect the expression of genes predicted by in silico analysis: (i) RUNX3, (ii) TRAPPC2P1, (iii) METTL20, (iv) ZFH3 or (v) CPNE1 expression remain unaltered upon miR-532-5p inhibition. P=N.S. for any of the tested genes; N=6-8 biological replicates. All values are means±SE.

Figure S4

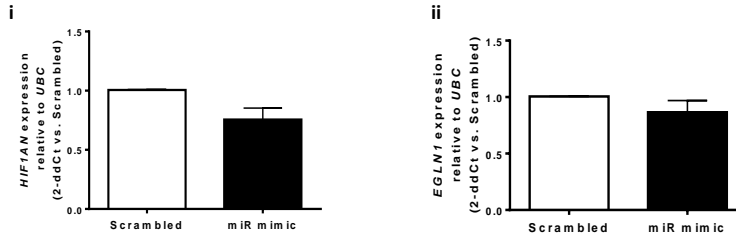
A

Effects of miR-532-5p inhibition on HIF1AN and EGLN1 expression



B

Effects of miR-532-5p overexpression on HIF1AN and EGLN1 expression



Supplemental Figure 4. Validation of *in silico* predicted targets for the miR-532/Ang-1 axis. *In silico* analysis identified BACH1, HIF1AN and EGLN1 as possible regulators. qPCR demonstrated (A) inhibition of miR-532 had no effect on *HIF1AN* (i) or *EGLN1* (ii) expression. (B) Likewise, overexpression of miR-532 had no effect on *HIF1AN* (i) or *EGLN1* (ii) expression; N=3 replicates. All values are means±SE

Supplemental table 1

Patient ID	Age	Gender	Pathology									
			Smoker	DM	MI	HPT	HPC	CPD	CHF	AF	PCI	
APC												
5.4.13H		M										
15.4.13		M	Yes	Yes	No	Yes	No	No	No	No	No	No
29.5.13	85	M	Ex >1 month	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No
12.6.13	71	M	Ex >1 month	No	No	Yes	No	No	No	No	No	No
19.6.13		M	Ex >1 month	No	Yes	Yes	Yes	Yes	No	No	No	No
9.7.13D	59	M	Ex >1 month	Yes	Yes	Yes	Yes	No	No	No	No	No
15.11.13C		M	Ex >1 month	No	Yes	Yes	Yes	No	No	No	No	No
15.12.13B		M										
8.5.14D	40	M	Yes	No	Yes	Yes	Yes	No	No	No	No	No
30.4.14C	75	M	No	No	No	Yes	Yes	No	No	No	No	No
30.4.14G	63	M	Ex >1 month	No	No	Yes	Yes	No	No	No	No	No
8.7.14A	61	M	Ex >1 month	No	Yes	Yes	Yes	No	No	No	No	No
16.10.14A	67	M	Ex >1 month	No	No	Yes	Yes	No	No	No	No	No
28.10.14C	82	M	No	No	No	Yes	Yes	No	No	No	No	No
9.2.15B	82	M	No	No	Yes	Yes	Yes	No	No	No	No	No
20.2.15B	67	M	Yes	No	Yes	Yes	Yes	Yes	No	No	No	No
13.3.15A	56	M	Yes	No	Yes	Yes	No	No	No	No	No	No
30.3.15A	79	M	Ex >1 month	No	Yes	No	Yes	No	No	No	No	No
29.4.15C	63	M	No	No	Yes	Yes	Yes	No	No	No	No	Yes
10.6.15B	69	M	Ex >1 month	No	Yes	Yes	Yes	No	No	No	No	No
15.9.15B	64	M	Ex >1 month	No	Yes	Yes	Yes	No	No	No	No	No
15.9.15C	41	M	No	No	No	No	Yes	No	No	No	No	No
17.11.15B	67	M	Yes	No	Yes	No	Yes	No	No	No	No	No
13.10.15E	72	M	Ex >1 month	No	Yes	Yes	Yes	No	No	No	No	No
12.1.16A	80	M	Ex >1 month	Yes	No	Yes	Yes	No	No	No	No	Yes
9.2.16B	73	M	Ex >1 month	No	Yes	Yes	Yes	No	No	No	No	No
9.2.16C	57	M	No	Yes	Yes	Yes	Yes	No	No	No	No	No
5.3.16A	64	M	Yes	No	No	Yes	Yes	No	No	No	No	No
1.4.16B	80	M	Yes	No	No	Yes	Yes	No	No	No	No	No
5.4.16A	71	M	No	No	No	Yes	Yes	No	No	No	No	No
4.10.16B	75	M	Ex	No	Yes	Yes	Yes	No	No	No	No	No
4.10.16C	57	M	Yes	No	Yes	Yes	Yes	No	No	No	No	Yes
Muscle pericytes donors												
Patient ID	Age	Gender	Pathology									
MP36	34	M	CTRL									
MP37	53	F	CTRL									
MP40	44	M	CTRL									
MP 20	58	F	CTRL									
MP 46	46	M	CTRL									
MP57	57	F	CTRL									
MP 67	67	F	CTRL									
MP 73	73	F	CTRL									
MP 7	51	M	T2D+CLI									
MP 10	76	M	T2D+CLI									
MP12	84	F	T2D+CLI									
MP18	67	F	T2D+CLI									
MP22	86	M	T2D+CLI									
MP14	80	M	CLI									
MP23	77	M	CLI									
MP24	85	F	CLI									

Table S1. Clinical characteristics of patient donors. APCs and MPCs were isolated as previously described. *Abbreviations:* Ex = Ex-smoker (patient that stopped smoking at least one month before the surgery); DM = Diabetes Mellitus; MI = Myocardial Infarction; HPT = Hypertension; HPC = Hypercholesterolemia; CPD = Chronic Pulmonary Disease; CHF = Congestive Heart Failure; AF = Atrial Fibrillation; PCI = Percutaneous Coronary Intervention; CTRL = no pathology; CLI = critical limb ischemia; T2D+CLI = type 2 diabetes + CLI.