

SUPPLEMENTARY MATERIAL

Characterization of Carotid Smooth Muscle Cells during Phenotypic Transition

Haize Goikuria ^{1,2}, Maria del Mar Freijo ³, Reyes Vega Manrique ⁴, María Sastre ^{1,2}, Elena Elizagaray ⁵, Ana Lorenzo ³, Koen Vandenbroeck ^{1,2,6} and Iraide Alloza ^{1,2,6,*}

- ¹ Neurogenomiks Neuroscience Department, Faculty of Medicine and Nursing, Basque Country University, 48940 Leioa, Spain; hgoikuria@gmail.com (H.G.); maritxu96@hotmail.com (M.S.); k.vandenbroeck@ikerbasque.org (K.V.)
- ² ACHUCARRO Basque Center for Neuroscience, Basque Country University, 48940 Leioa, Spain
- ³ Neurology Unit, Basurto University Hospital (BUH), 48013 Bilbao, Spain; marimar.freijoguerrero@osakidetza.eus (M.M.F.); ANAMARIA.LORENZOGARCIA@osakidetza.eus (A.L.)
- ⁴ Vascular Surgery and Angiology Unit, BUH, 48013 Bilbao, Spain; mariareyes.vegamanrique@osakidetza.eus
- ⁵ Radiodiagnostic Unit, BUH, 48013 Bilbao, Spain; elizagaray@yahoo.com
- ⁶ IKERBASQUE, Basque Foundation for Science, 48013 Bilbao, Spain
- * Correspondence: iraide.alloza@ehu.eus; Tel.: +34-946-018-292; Fax: +34-946-018-289

Table S1. List of primers used in gene expression analysis for VSMC phenotypic characterization.

Gene Symbol	Gene Name	Accession number	Assay code
<i>ACTA2</i>	α -smooth muscle actin	NM_001613	Hs.PT.56a.2542642
<i>ACTB</i>	Actin β	NM_001101	QT00095431
<i>CALD1</i>	H-caldesmon	NM_033138	QT00997899
<i>CD68</i>	CD68 molecule	NM_001040059	Hs.PT.58.2488447.g
<i>CNN1</i>	calponin 1	NM_001229	Hs.PT.58.38799164
<i>GAPDH</i>	glyceraldehyde-3-phosphate dehydrogenase	NM_002046	Hs.PT.39a.22214836
<i>ICAM1</i>	Intercellular adhesion molecule-1	NM_000201	Hs.PT.56a.4746364
<i>KLF4</i>	Kruppel like factor 4	NM_004235	Hs.PT.58.45542593
<i>KLF5</i>	Kruppel like factor 5	NM_001730	Hs.PT.56a.40282397
<i>LGALS3</i>	Galectin 3	NM_001177388	Hs.PT.58.1435723
<i>MAP1LC3B</i>	Microtubule associated protein 1 light chain 3 beta	NM_022818	QT00055069
<i>MKL2</i>	MKL1/myocardin like 2	NM_002446	Hs.PT.58.19760686
<i>MMP3</i>	Matrix metalloproteinase-3	NM_002422	QT00060025
<i>MMP7</i>	Matrix metalloproteinase-7	NM_002423	QT00001456
<i>MMP9</i>	Matrix metalloproteinase-9	NM_004994	QT00040040
<i>MYH11</i>	Smooth muscle-myosin heavy chain 11	NM_002474	Hs.PT.58.2909933
<i>MYH10</i>	Smooth muscle-myosin heavy chain 10	NM_001256012 NM_001256095 NM_005964	QT00005117
<i>RPL41</i>	Ribosomal protein L41	NM_001035267	Hs.PT.58.38804367
<i>SPPI</i>	Secreted phosphoprotein 1	NM_001040058	Hs.PT.58.19252426
<i>SRF</i>	Serum response factor	NM_003131	Hs.PT.58.4857809
<i>TAGLN</i>	Smooth muscle protein 22- α	NM_001001522 NM_003186	QT01678516
<i>TIMP1</i>	Tissue inhibitor of metalloproteases-1	NM_003254	QT00084168

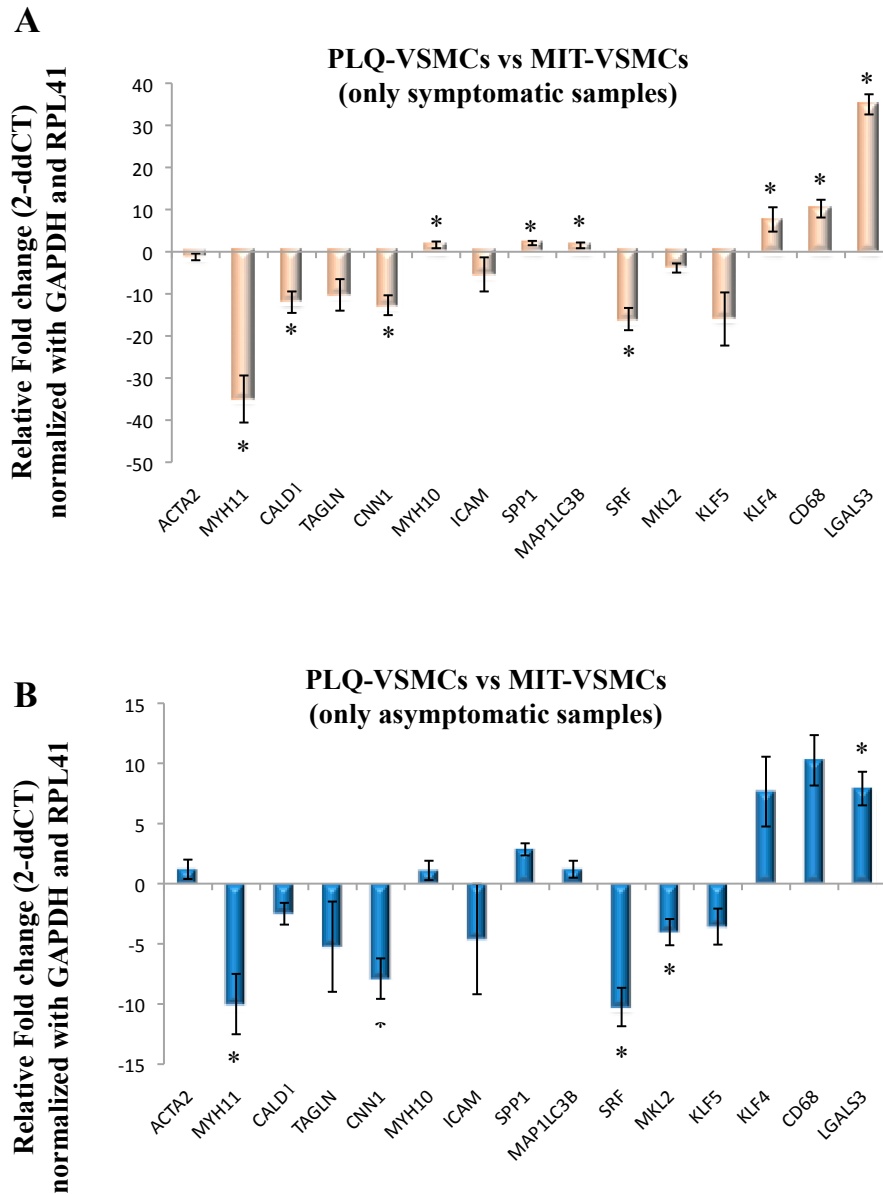


Figure S1. Fold change differences of contractile and synthetic markers in plaque VSMCs (PLQ-VSMCs) versus healthy area VSMCs (MIT-VSMCs) analyzed by quantitative PCR. **(A)** 20 PLQ-VSMCs versus MIT-VSMCs. **(B)** 19 PLQ-VSMCs versus 19 MIT-VSMCs (error bars represent $\pm$ SEM, $n=20$ symptomatic and $n=19$ asymptomatic). Wilcoxon matched-pairs signed rank test ($p<0.05^*$).

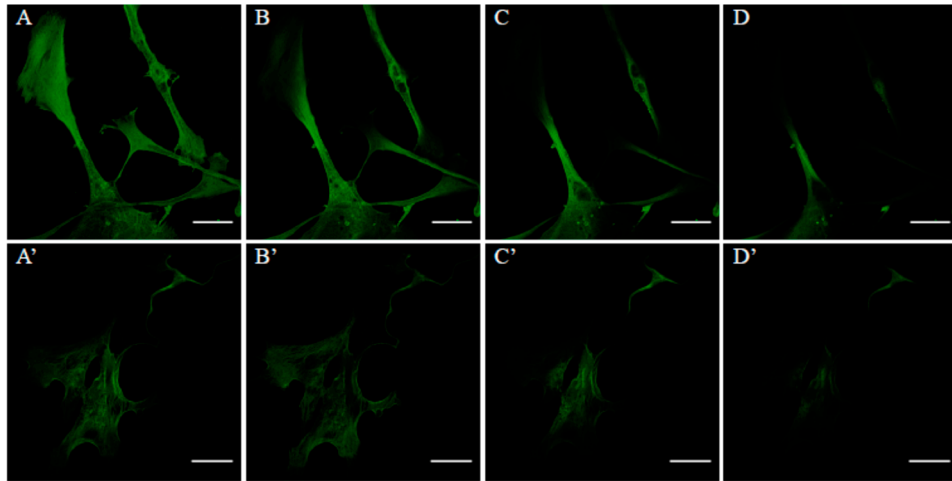


Figure S2. MYH11 staining in VSMCs. **A, B, C** and **D** VSMCs from adjacent site. 0,9 μm z-stacks from the base o the top of the cells. MYH11 staining appear in perinuclear region in the upper planes of the cell foring thick myofilaments. **A', B', C'** and **D'** VSMCs from atheroma plaque. 0,9 μm z-stacks from the base to the top of the cells, where there is almost lack of MYH11staining and myofilaments appear as thin fibers. Scale bar 50 μm .

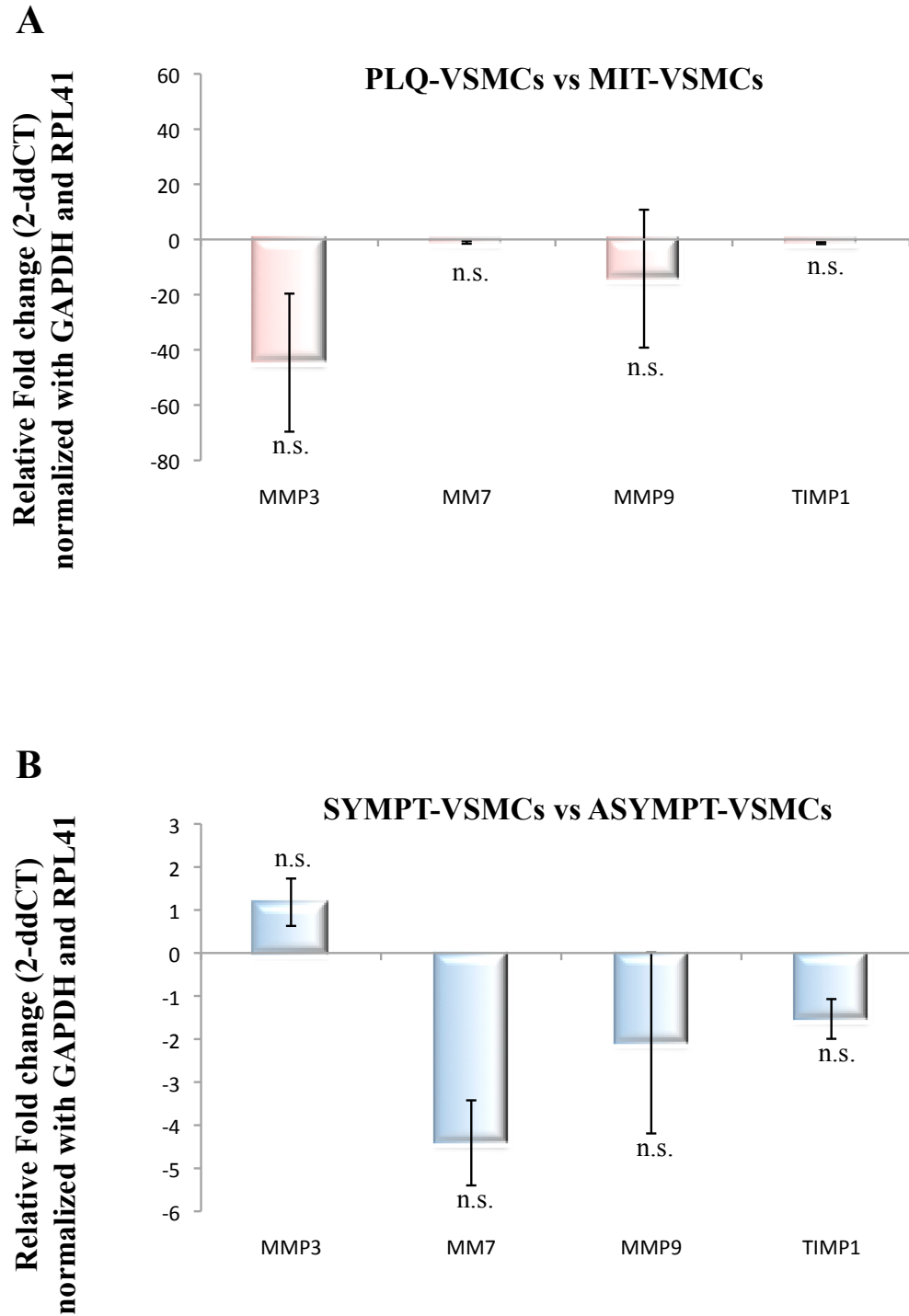


Figure S3. MMMP3, 7, 9, tissue inhibitor MMP 1 mRNA expression quantified by qPCR in plaque VSMCs and adjacent site VSMCs. **(A)** PLQ-VSMCs versus MIT-VSMCs. **(B)** SYMPT-VSMCs versus ASYMPT-VSMCs. Wilcoxon matched-pairs signed rank test was used ($p < 0.05$ was considered statistically significant). (n.s. no significant)