Supplemental Information – Sinha et al.

Supplementary Figures:

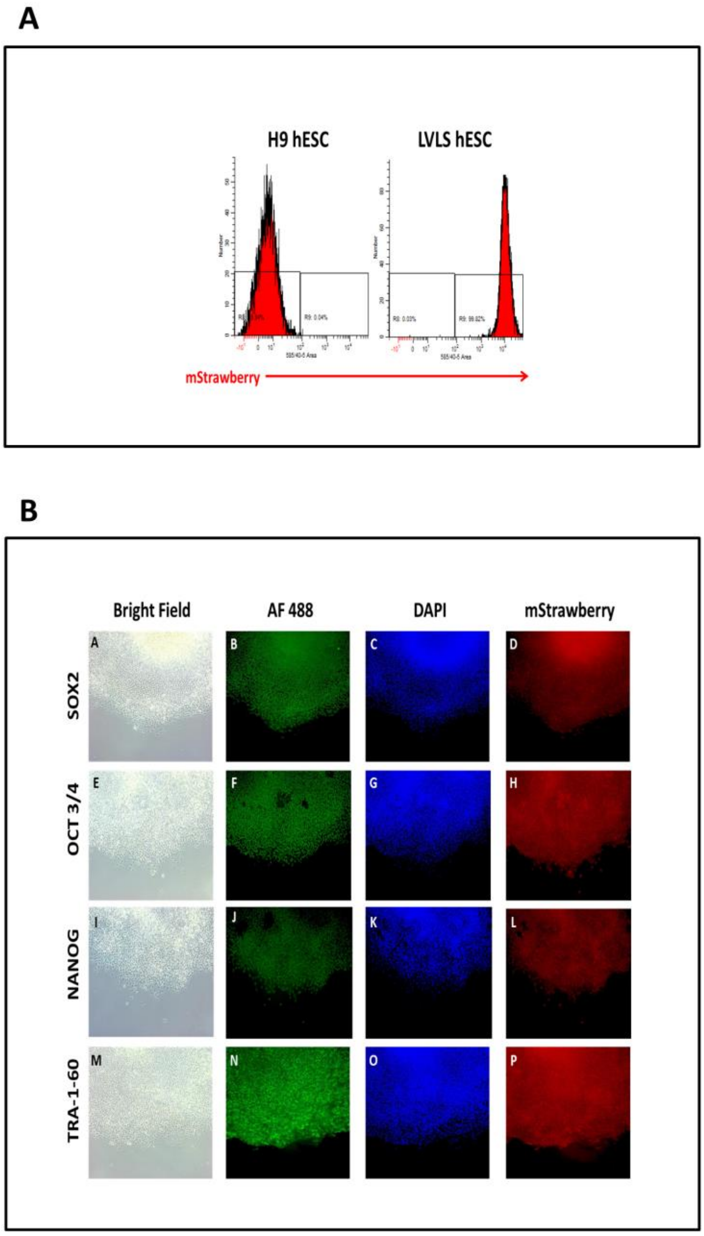

Fig. S1. Generation of mStrawberry-expressing hESCs. (A) Flowcytometry of mStrawberry expressing hESCs. (B) Immunocytochemistry of mStrawberry expressing hESCs.

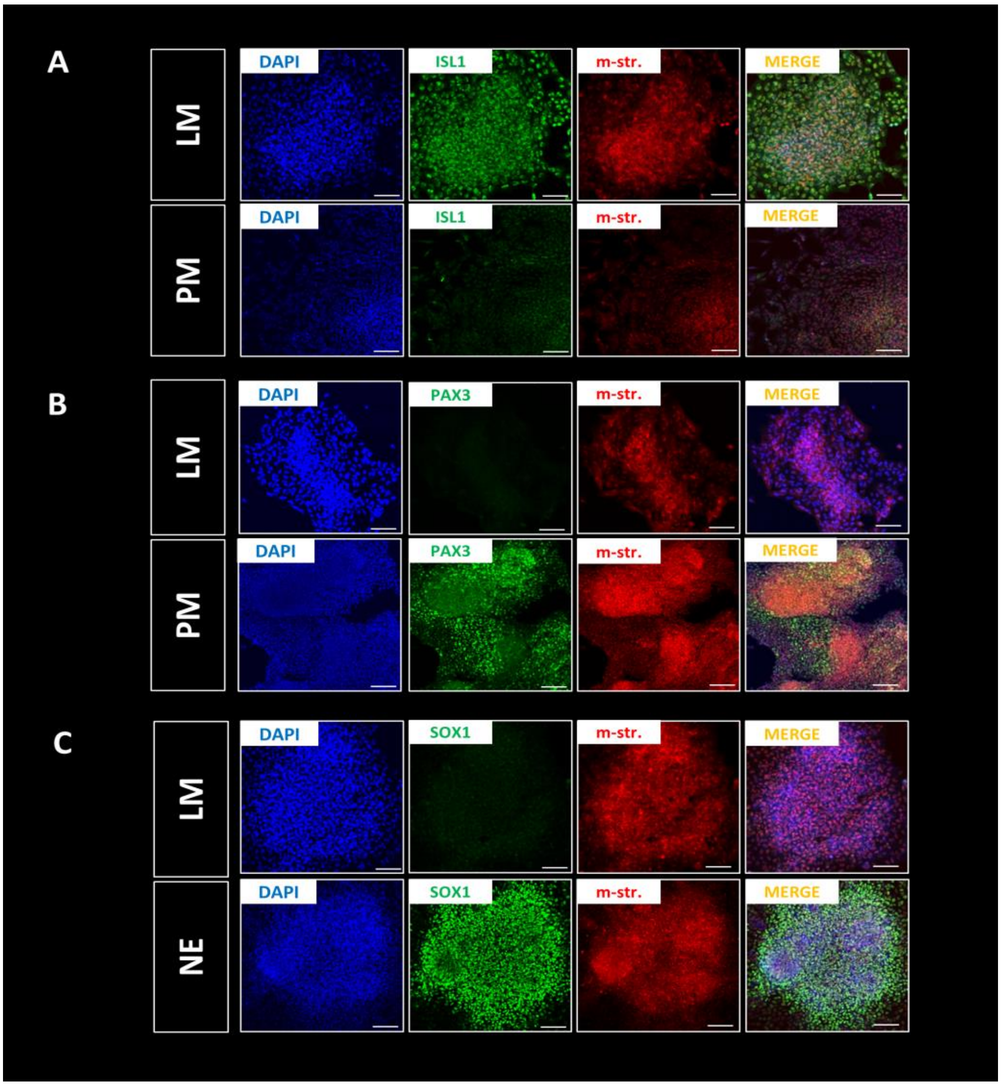

Fig. S2. Generation of embryonic origin specific SMC populations for a 3D co-culture model. *(A-C)* Immunocytochemistry of the LM, PM and NE intermediate lineages derived from an m-Strawberry expressing H9 line (**p*<0,05, ***p*<0,01, ****p*<0,001, *n*=3 independent biological replicates, scale bars 100 μm).

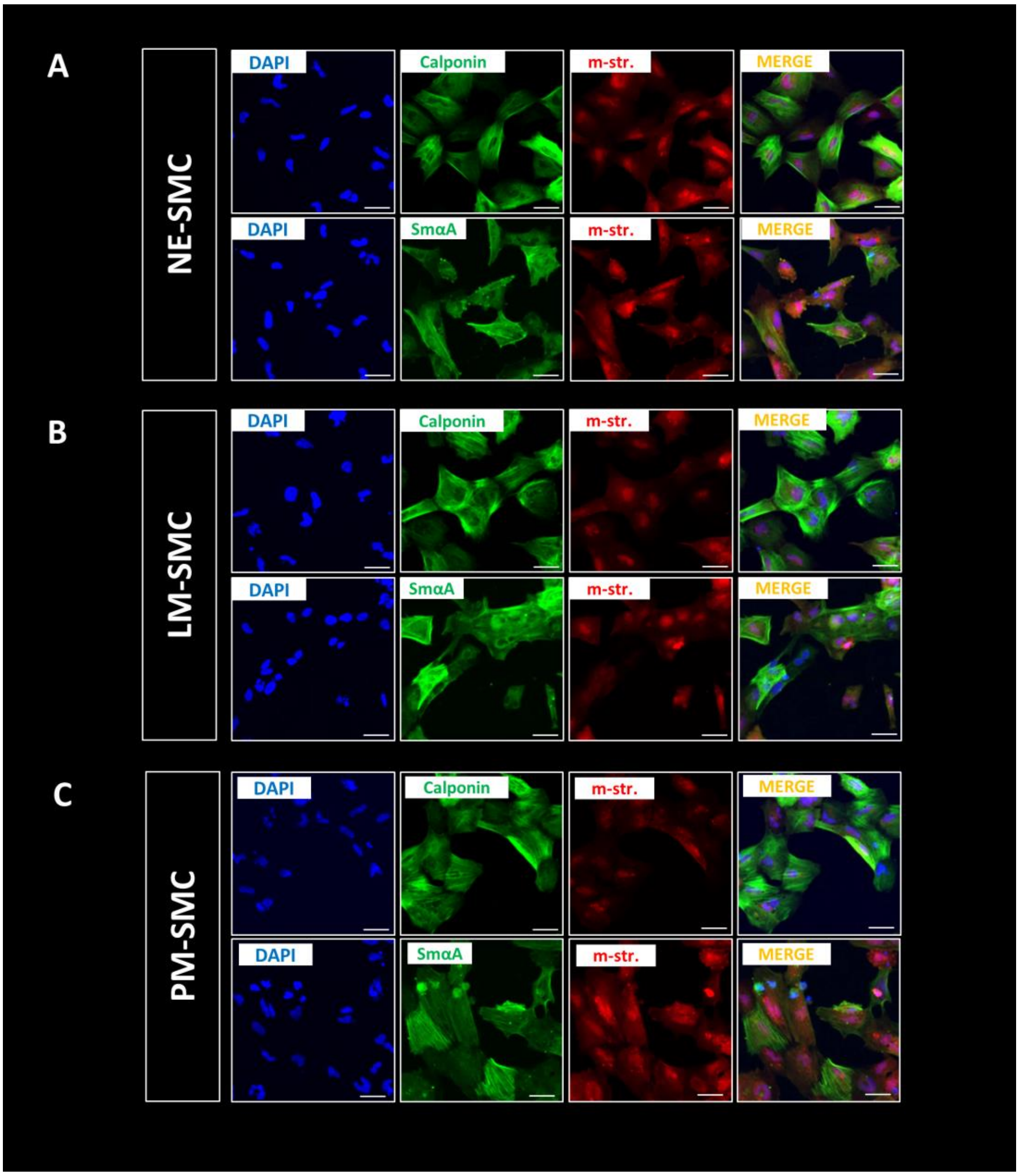

Fig. S3. Lineage specific SMC derivation. *(A-C)* Immunocytochemistry of lineage-specific SMCs of an m-Strawberry expressing H9 line, derived from NE-, LM- and PM-lineage respectively (**p*<0,05, ***p*<0,01, ****p*<0,001, *n*=3 independent biological replicates, scale bars 50 µm).

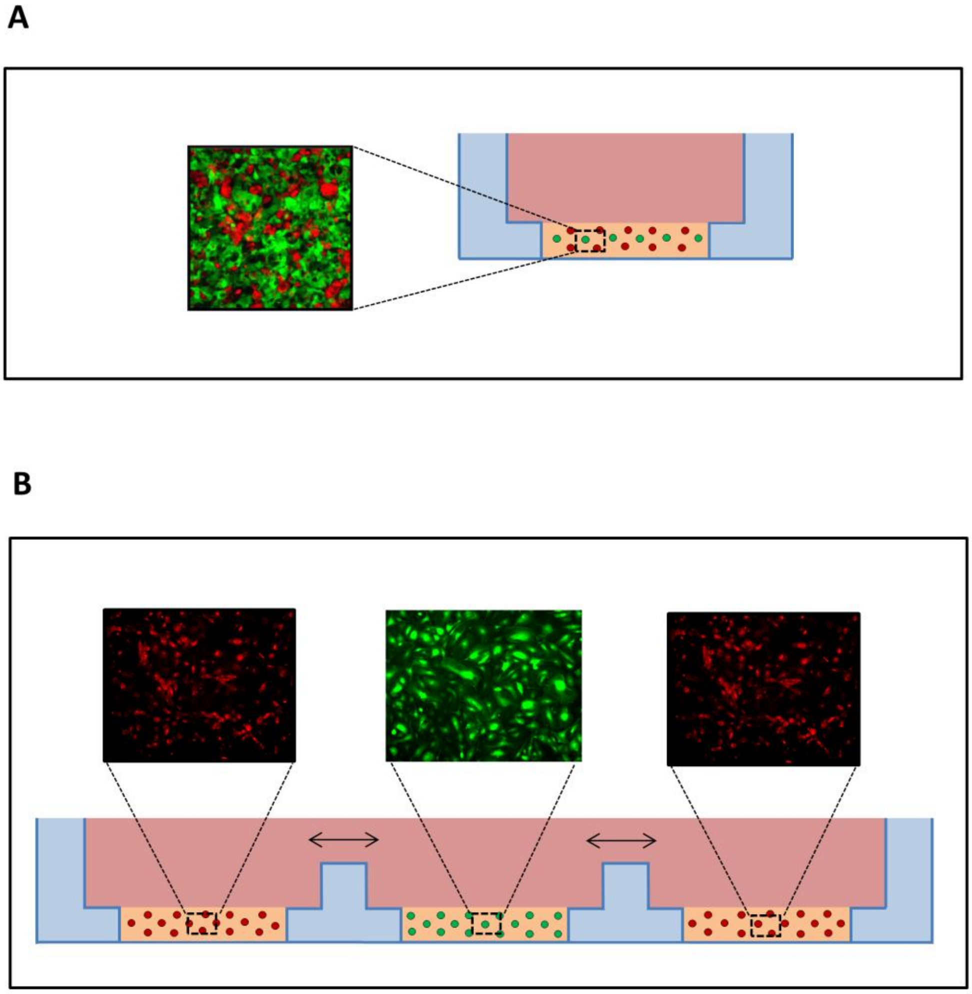

Fig. S4. Schematic of 3D co-culture and 3D paracrine assay. *(A)* Schematic cross section of a 3D co-culture of HUVECS (green) and embryonic origin specific SMCs (red). *(B)* Schematic representation of a 3D paracrine assay containing HUVECs in the central well and embryonic origin specific SMCs in the surrounding wells, allowing for exchange of the supernatant but not for cell-cell contact.

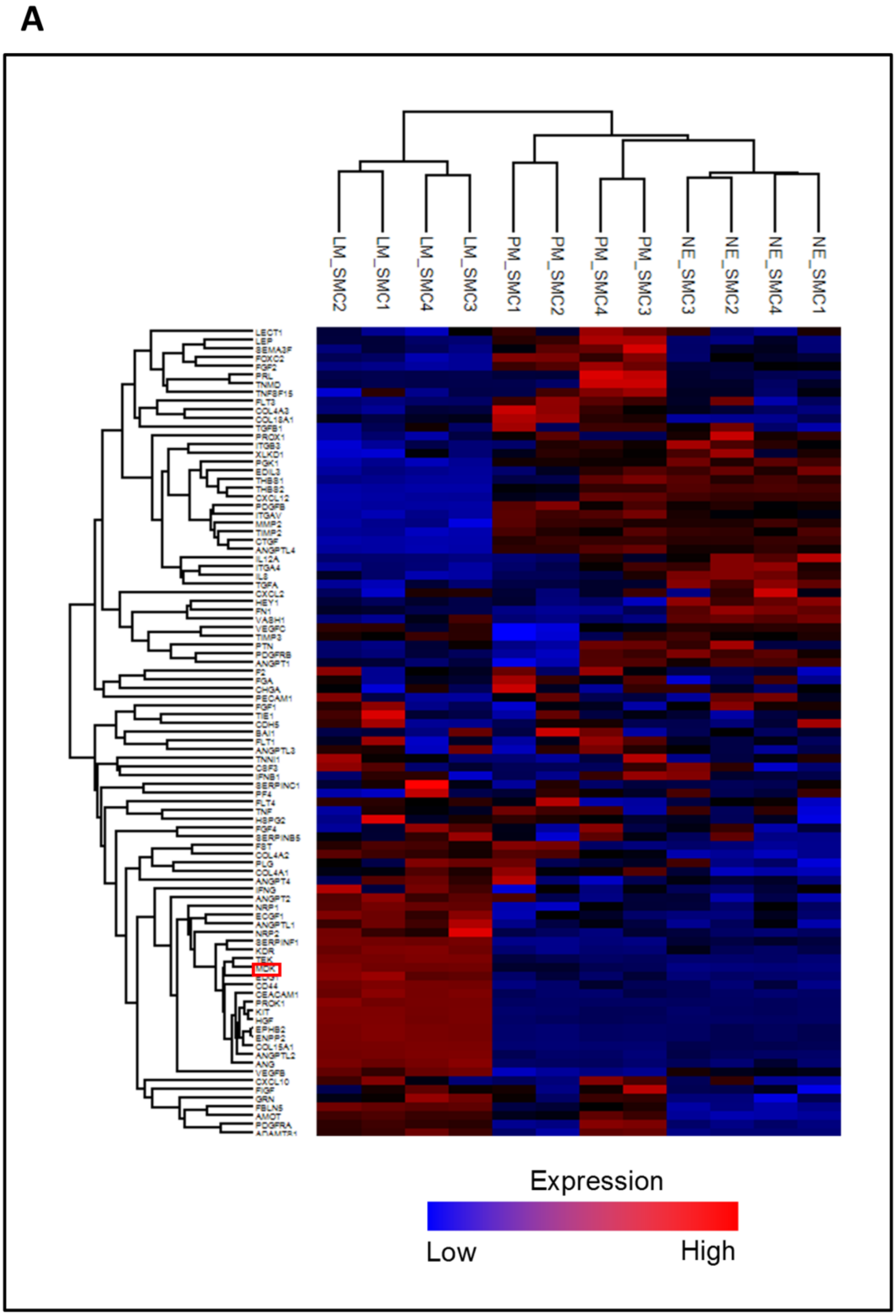

Fig. S5. Microarray analysis. *(A)* Heat map of all angiogenesis genes as expressed by the three embryonic origin specific smooth muscle cells lineages. MDK highlighted in red.

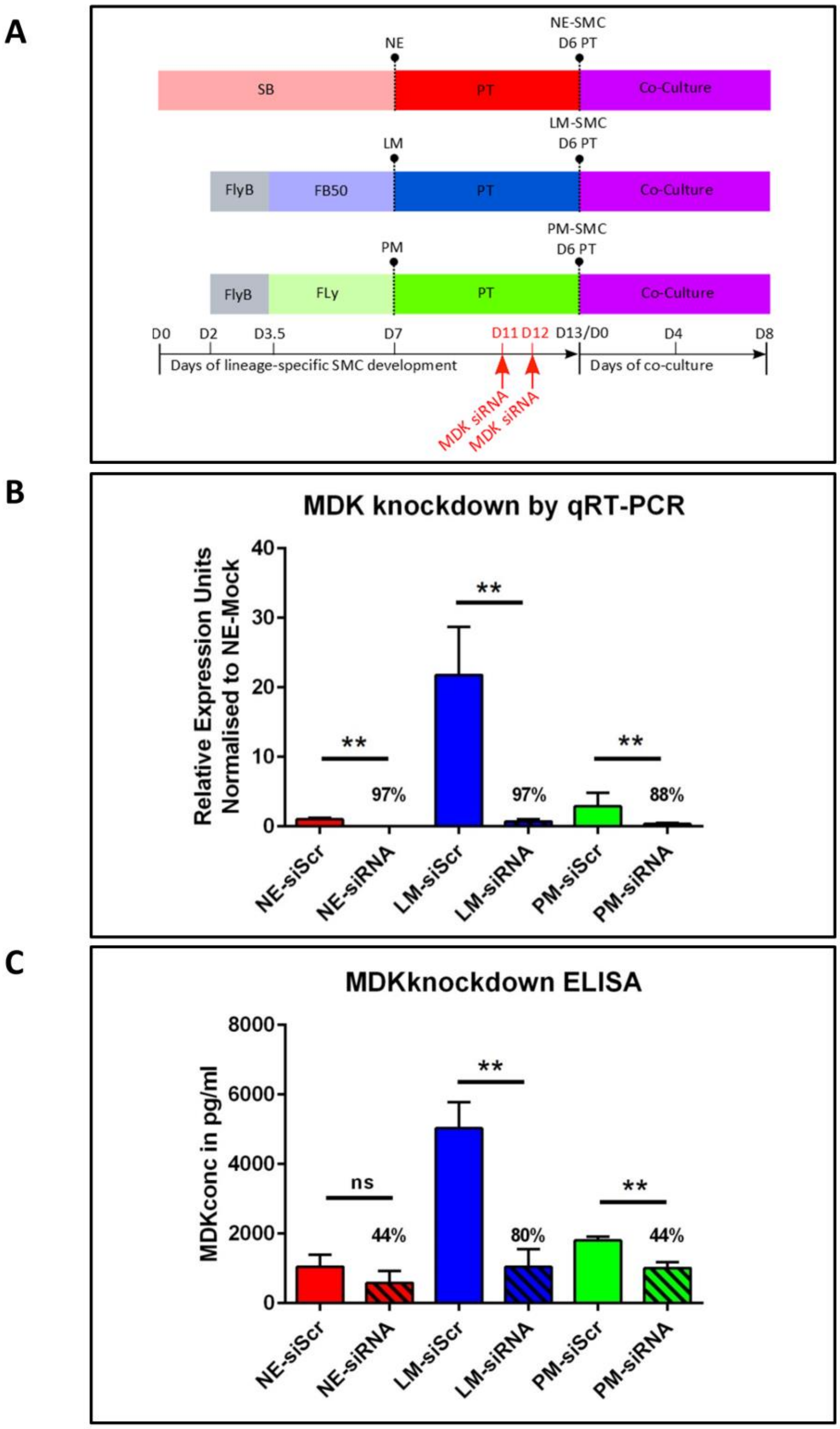

Fig. S6. Confirmation of siRNA-mediated knockdown of MDK in embryonic origin specific SMCs. *(A)* Schematic of SMC differentiation and timing of MDK siRNA intervention. *(B)* Confirmation of effective knockdown of MDK by qRT-PCR. *(C)* Confirmation of effective knockdown of MDK by ELISA (**p*<0,05, ***p*<0,01, ****p*<0,001, *n*=3 independent biological replicates).

Supplementary tables

Sanjay Sinha, Table S1

Gene	Species	Direction and Sequence
GAPDH	Human	Forward AACAGCCTCAAGATCATCAGC Reverse GGATGATGTTCTGGAGAGCC
PBGD	Human	Forward GGAGCCATGTCTGGTAACGG Reverse CCACGCGAATCACTCTCATCT
SOX1	Human	Forward GGTCAAACGGCCCATGAAC Reverse GCTTGCTGATCTCCGAGTTG
GBX2	Human	Forward CAGGCTTCGCTCGTCGG Reverse GGGTCTTCCTCCTTGTGAGC
PAX6	Human	Forward CTTTGCTTGGGAAATCCGAG Reverse AGCCAGGTTGCGAAGAACTC
ISL1	Human	Forward GCAAATGGCAGCGGAGCCCA Reverse AGCAGGTCCGCAAGGTGTGC
NKX2.5	Human	Forward AGCCGAAAAGAAAGAGCTGTGCG Reverse GACCTGCGCCTGCGAGAAGAG
PAX3	Human	Forward CGCCTGACGTGGAGAAGAAA Reverse TGATGGAACTCACTGACGGC
TCF15	Human	Forward GCACCTTCTGCCTCAGCAACCAGC Reverse GGTCCCCCGGTCCCTACACAA
MEOX1	Human	Forward AAAGTGTCCCCTGCATTCTG Reverse CACTCCAGGGTTCCACATCT
CNN1	Human	Forward GTCCACCCTCCTGGCTTT Reverse AAACTTGTTGGTGCCCATCT
ACTA2	Human	Forward CACTGTCAGGAATCCTGTGA Reverse CAAAGCCGGCCTTACAGA
MDK	Human	Forward CCTGCAACTGGAAGAAGGAG Reverse CTTTCCCTTCCCTTTCTTGG

Sanjay Sinha, Table S2

Marker	Application	Dilution	Manufacturer (Cat. #)
SOX1	ICC	1:100	R&D Systems (AF3369)
ISL1	ICC	1:200	Abcam (ab86472)
PAX3	ICC	1:50	Developmental Studies Hybridoma Bank (monoclonal)
CNN1	ICC	1:15000	Sigma (C2687)
SMαA	ICC	1:400	Sigma (F3777)