Supplemental Information

S1P promotes migration, differentiation and immune regulatory activity in amniotic-fluid–derived stem cells

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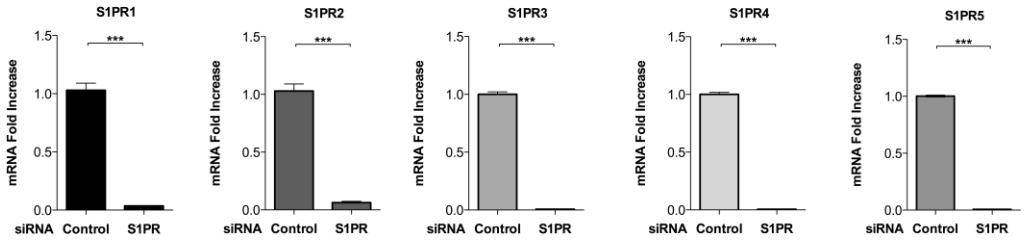
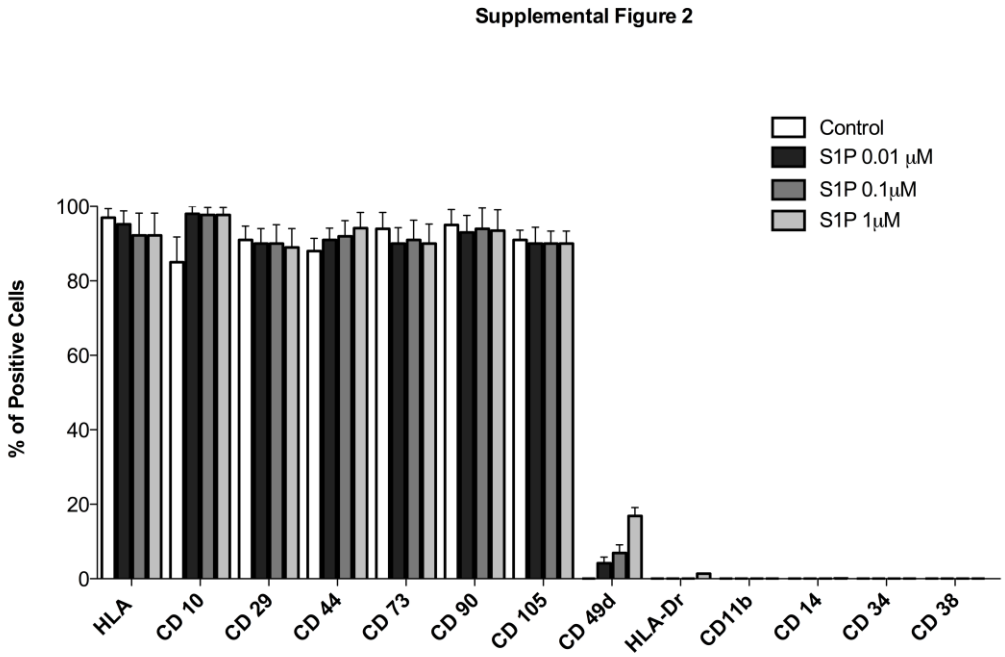
Supplemental Figure 1



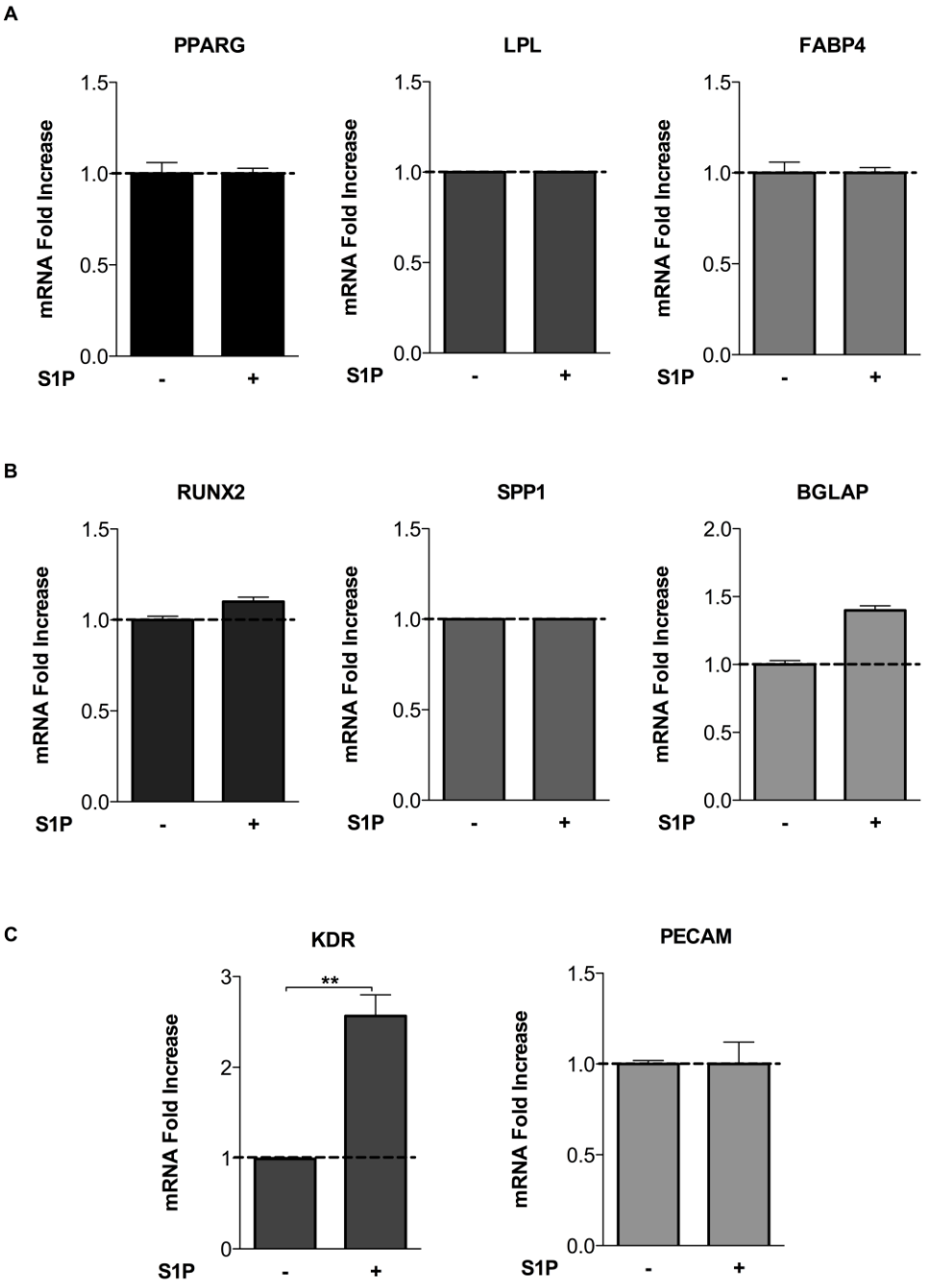
Supplemental figure 1: **Validation of S1PR$_{1\text{-}5}$ silencing by quantitative RT-PCR in fHASCs**. Real Time PCR was performed using 10^6 cells transfected with non-targeting siRNA (control) or with siRNA specific for S1PR1, S1PR2, S1PR3, S1PR4 or S1PR5; the content of housekeeping gene 18S rRNA was analyzed in parallel.

Data (means ± S.D) are presented as normalized transcript expression in the samples relative to normalized transcript expression in cells cultured control medium. ***P < 0.001 by Student's t-test.



Supplemental figure 2. Effects of S1P on expression of stromal markers in fHASCs. Analysis of stromal markers expression by FACS in fHASCs cultured for 24 h in media supplemented with delipidated serum with or without 1 or 0.1 μM S1P. Data are means ± S.D. of three independent experiments, each performed in triplicate.

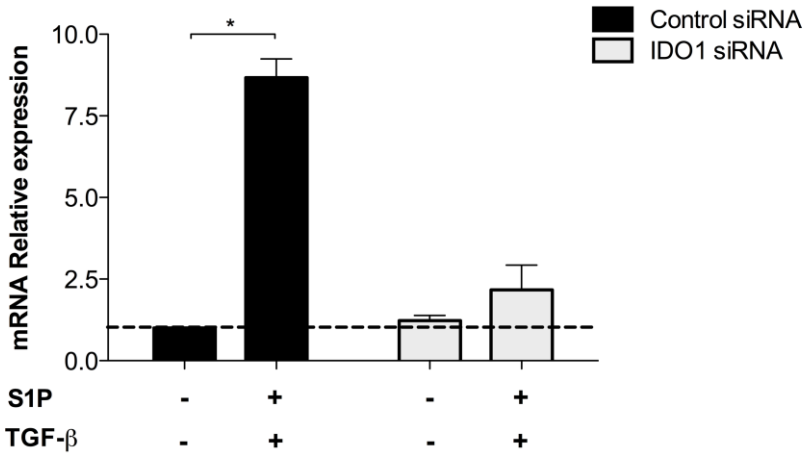
Supplemental Figure 3



Supplemental figure 3. S1P effects on fHASC differentiation potential towards specific cell lineages. fHASCs were cultured in specific differentiation media for adipogenic, osteogenic or endothelial differentiation in combination with 1µM S1P.

A) mRNA levels of specific adipogenic markers. B) mRNA levels of specific osteogenic markers. C) mRNA levels of specific endothelial markers. Data (means ± S.D) are presented as normalized transcript expressions in the samples relative to normalized transcript expression in control medium in one experiment representative of two.

Supplemental Figure 4



Supplemental figure 4. Validation of IDO1 silencing by quantitative RT-PCR in fHASCs. Real Time PCR was performed on 10^6 cells transfected with non-targeting siRNA (control) or with siRNA specific for IDO1. Data are means ± S.D. of three independent experiments performed in triplicate. Data (means ± S.D) are presented as normalized transcript expression in the samples relative to normalized transcript expression in cells cultured with control medium. **P < 0.01 by ANOVA followed by Bonferroni multiple comparison test.

Table 1. Real-time-q- PCR primer sequences

Name	*Accession number*	Forward sequences	Reverse sequences
OCT-4a	**NM_002701**	5'GGGTTGAGTAGTCCCTTCGC3'	5'TAGCCAGGTCCGAGGATCAA3'
OCT-4b	**NM_001285987**	5'ACGACCATCTGCCGCTTTG3'	5'GTTGCCTCTCACTCGGTTCTC3'
NANOG	**NM_024865**	5'CCACCAGTCCCAAAGGCAAAC3'	5'GAGGTTCAGGATGTTGGAGAGTTC3'
SOX2	**NM_003106**	5'AAGTAGTTTGCTGCCTCTTTAAG3'	5'GCTTCCCTCCTCCTCTGG3'
KLF4	**NM_004235**	5'ACGGCTGTGGATGGAAATTC3'	5'ATGTGTAAGGCGAGGTGGTC3'
SPP1	**NM_001251830**	5'GCAGGAGGAGGCAGAGCACAG3'	5'GGTCGGCGTTTGGCTGAGAAGG3'
BGLAP	**NM_199173**	5'CCTCACACTCCTCGCCCTATTGG3'	5'TCGCTGCCCTCCTGCTTGG3'
RUNX2	**NM_001024630**	5'ACTGTCATGGCGGGTAACGATG3'	5'GTGAAGACGGTTATGGTCAAGGTG3'
PPARG	**NM_015869**	5'GGTTGACACAGAGATGCCATTC3'	5'TGGAGTAGAAATGCTGGAGAAGTC3'
LPL	**NM_000237**	5'CCGTGTGGCTCCAGAGTC3'	5'GAATGAGGTGGCAAGTGTCC3'
FABP4	**NM_001442**	5'AGAAGTAGGAGTGGGCTTTGC3'	5'ATCTAAGGTTATGGTGCTCTTGAC3'
PECAM1	**NM_000442**	5'TGACGGAATCCTTCTCTACACC3'	5'TCGCCTTGTCCTTCTGAATTATG3'
KDR	**NM_002253**	5' TTGGATGAACATTGTGAACGACTG 3'	5' AGGCATCTGCTTCAATCACTTGG 3'
ACTB	**NM_001101**	5'CTCTTCCAGCCTTCCTTCCT3'	5'AGCACTGTGTTGGCGTACAG3'