Figure 1S. Side and forward light scatter analysis of hMSCs from a monolayer, one (SPH1), two (SPH2) and three (SPH3) day old spheroids. Representative dot plots for forward and side light scatters for hMSCs, SPH1, SPH2 and SPH3 are shown in panel A. Overlays of representative histograms of forward light scatter for hMSCs from a monolayer (black), SPH1 (green), SPH2 (red) and SPH3 (blue) are shown in panel B. Overlays of representative histograms of side light scatter for hMSCs from a monolayer (black), SPH1 (green), SPH2 (red) and SPH3 (blue) are shown in panel C. Data in parentheses represent a mean of 4-7 measurements ± SD. Statistically significant changes (p-value < .01) compared with hMSCs are denoted with asterisks.

Figure 2S. Effects of trypsinization on the expression of cell surface markers by hMSCs. Cells were grown in monolayer, treated with trypsin-EDTA for 5, 30 and 90 min, labeled with FITC- or PE-conjugated monoclonal antibodies specific for proteins indicated in each histogram and analyzed by flow cytometry. Grey filled histograms correspond to hMSCs trypsinized for 5 min. Thin and thick black line histograms represent hMSCs that were trypsinized for 30 and 90 minutes, respectively. Solid black histograms represent isotype controls.

Figure 3S. **Comparison of cell surface marker expression by hMSCs and 3 day old hMSC spheroid cells.** Cells were isolated by trypsinization for 90 min, labeled with FITC- or PE-conjugated monoclonal antibodies and analyzed by flow cytometry. Black and grey filled histograms represent cell from a monolayer of hMSCs and the spheroids, respectively.

Figure 4S. Expression of CD34, CD184, CD49b and CD49d by hMSCs from a monolayer and one, two and three day old hMSC spheroids. Cells were dissociated form a monolayer (hMSCs), one (SPH1), two (SPH2) or three (SPH3) day old hMSC spheroids and stained with CD34-FITC – CD184-PE and CD49b-FITC – CD49d-PE pairs of antibodies. Representative two-color fluorescence dot plots and corresponding isotype controls are shown.
Number

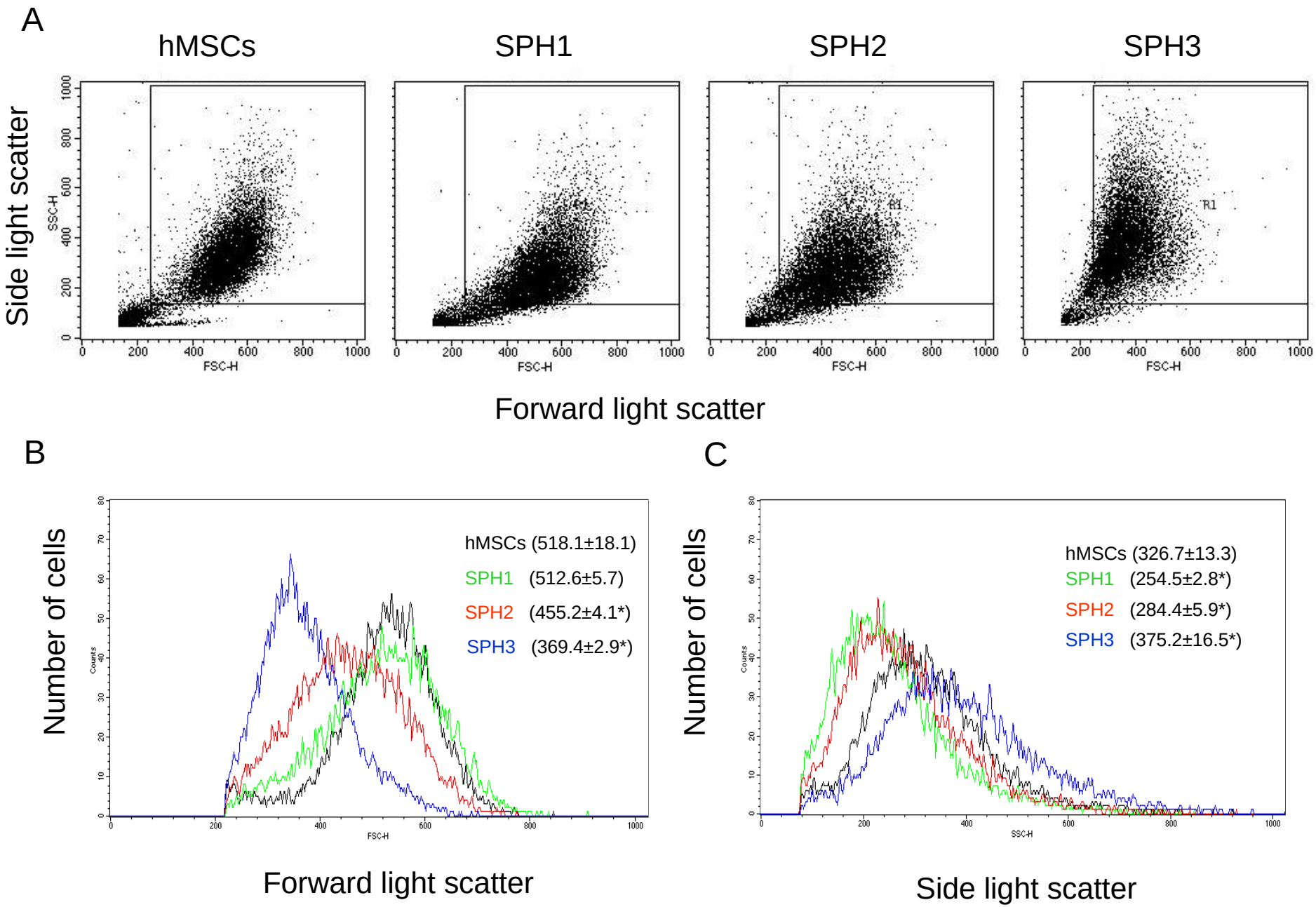
A
hMSCs
SPH1
SPH2
SPH3
Side light scatter
SSC-H
FSC-H
R1
Forward light scatter
B
Number of cells
Counts
hMSCs (518.1±18.1)
SPH1 (512.6±5.7)
SPH2 (455.2±4.1*)
SPH3 (369.4±2.9*)
Forward light scatter
C
Number of cells
hMSCs (326.7±13.3)
SPH1 (254.5±2.8*)
SPH2 (284.4±5.9*)
SPH3 (375.2±16.5*)
Side light scatter

Fig. S1ABC

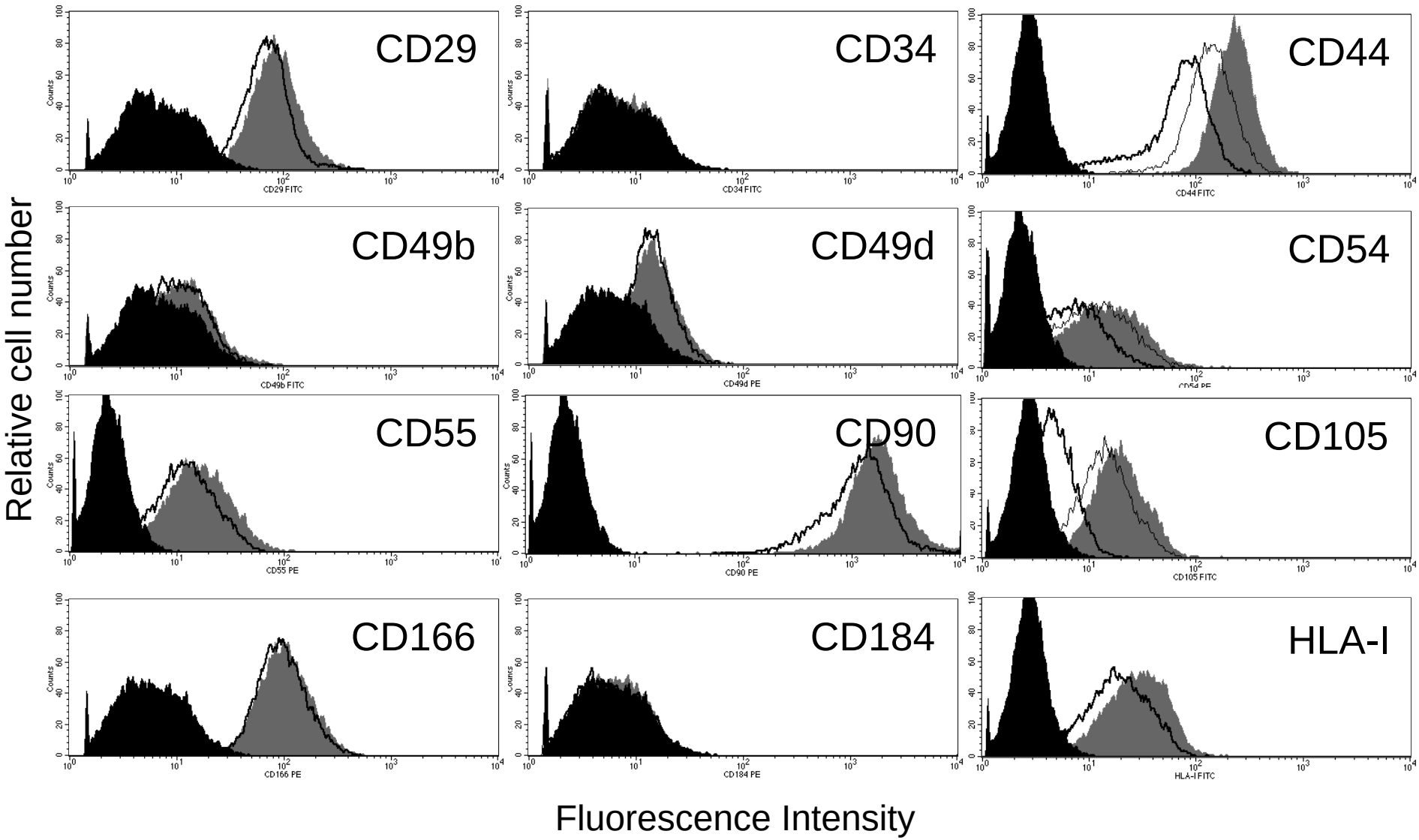
CD29
CD34
CD44
CD49b
CD49d
CD54
CD55
CD90
CD105
CD166
CD184
HLA-I
Relative cell number
Fluorescence Intensity

Fig. S2

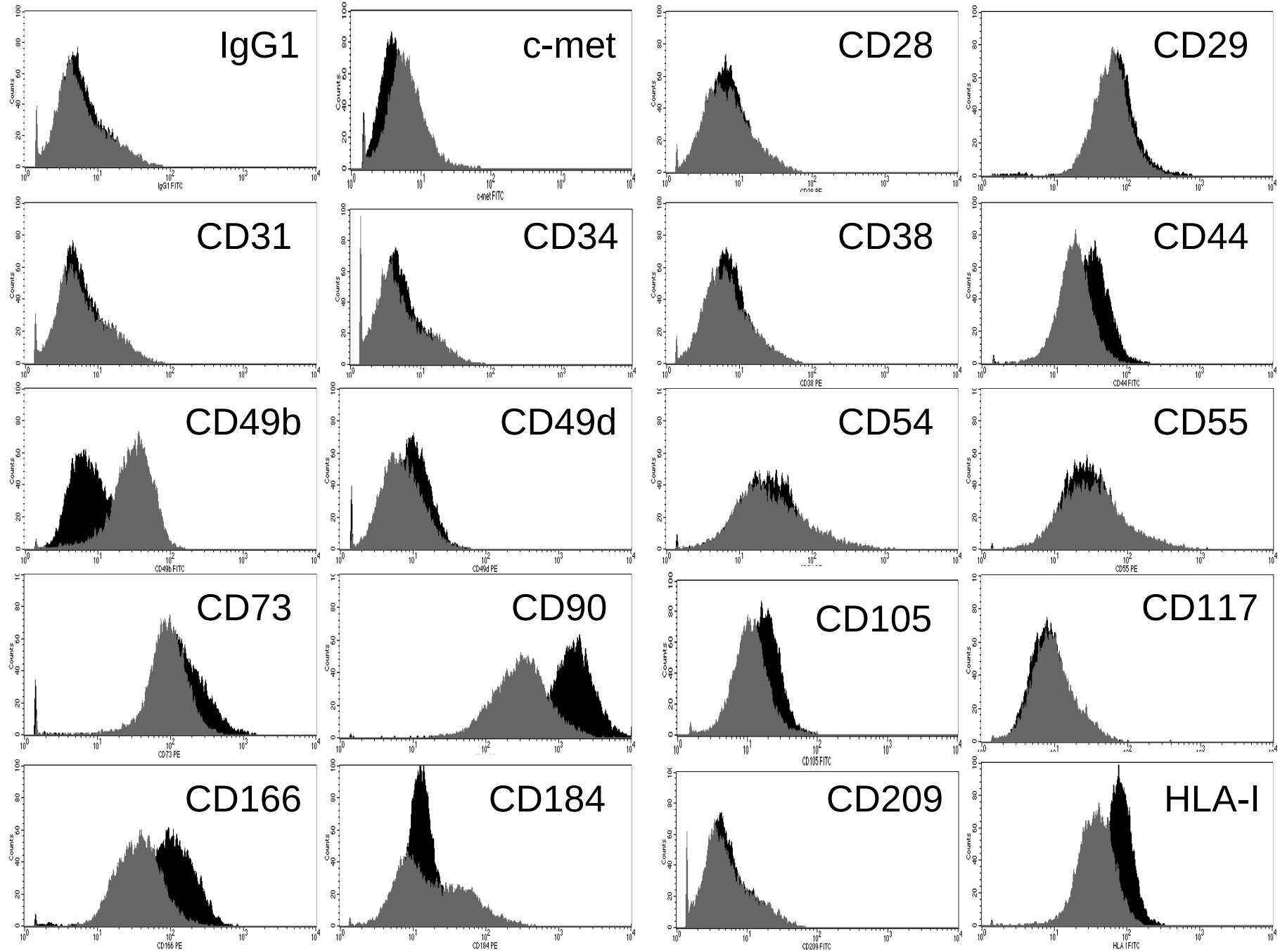
IgG1
c-met
CD28
CD29
CD31
CD34
CD38
CD44
CD49b
CD49d
CD54
CD55
CD73
CD90
CD105
CD117
CD166
CD184
CD209
HLA-I
Relative cell number
Fluorescence Intensity

Fig. S3

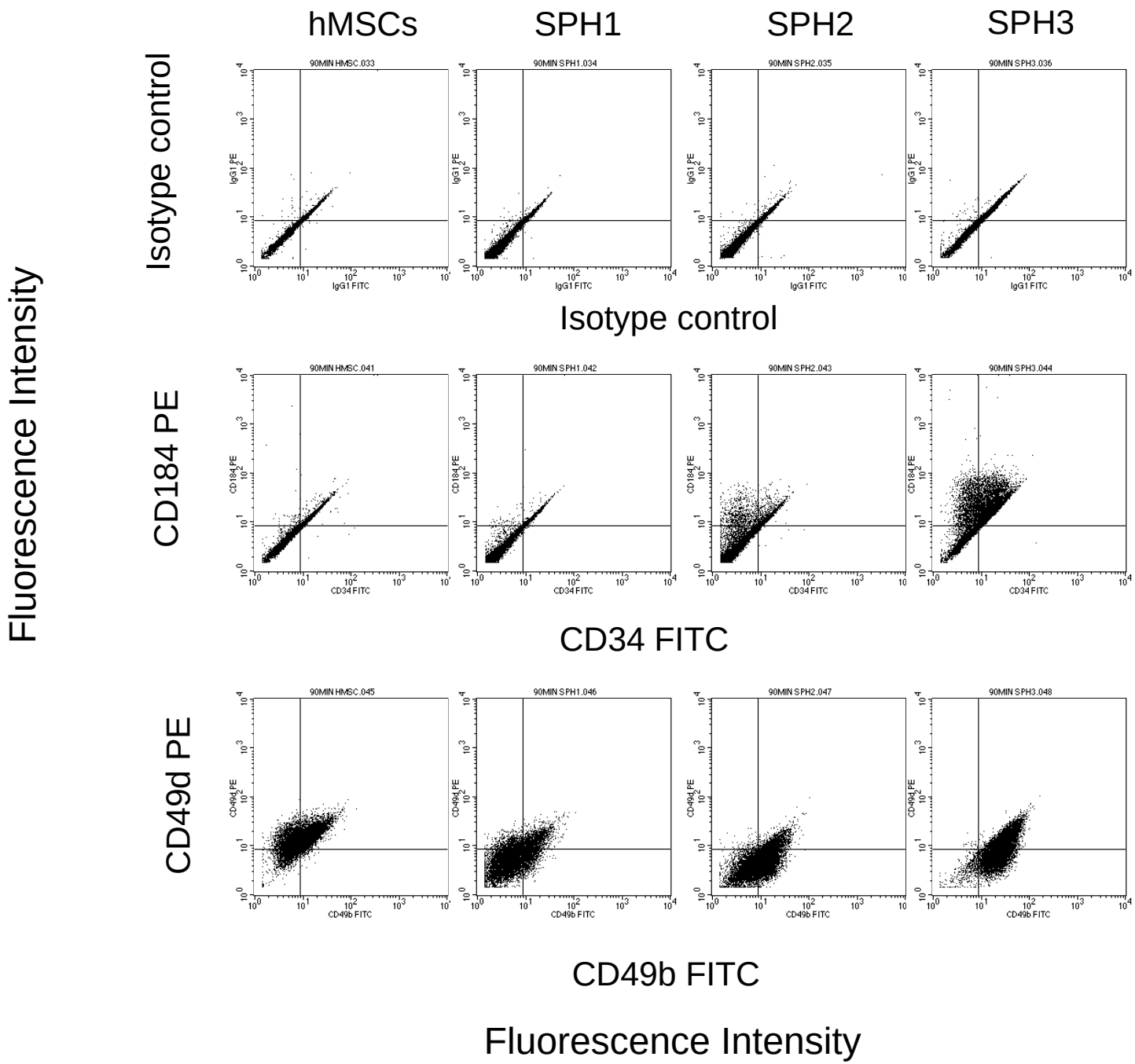
hMSCs
SPH1
SPH2
SPH3
Isotype control
90MIN HMSC.033
90MIN SPH1.034
90MIN SPH2.035
90MIN SPH3.036
IgG1 PE
IgG1 FITC
Isotype control
CD184 PE
90MIN HMSC.041
90MIN SPH1.042
90MIN SPH2.043
90MIN SPH3.044
CD184 PE
CD34 FITC
CD34 FITC
CD49d PE
90MIN HMSC.045
90MIN SPH1.046
90MIN SPH2.047
90MIN SPH3.048
CD49d PE
CD49b FITC
CD49b FITC
Fluorescence Intensity
Fluorescence Intensity

Fig. 3S