



Suppl. Fig. 1. Characterization of MSS31 cells. (A) RT-PCR analyses of the expression of PECAM1 and VE-cadherin mRNAs. Expression of PECAM1 and VE-cadherin mRNAs was analyzed by RT-PCR, using the total RNA of MSS31 cells and specific primers for PECAM1 (5'-GACCCAGCAACATTCACAGAT-3' and 5'-TCTTTCACAGAGCACCGAAGT-3') or VE-cadherin (5'-CCCGTCTTTACTCAATCCACA-3' and 5'-GGGTTTGATGATACCCTCGTT-3'). (B) Immunoblot analyses of the expression of VEGFR-1 (Flt-1), VEGFR-2 (Flk-1), VEGFR-3 (Flt-4), and LYVE1 proteins in MSS31 cells and HUVEC. Cell lysates were immunoprecipitated (IP) and analyzed by immunoblotting (IB) with their specific antibodies. Antibodies against VEGFR-1 (C-17), VEGFR-2 (C-1158), and VEGFR-3 (C-20) were purchased from Santa Cruz. Antibodies against LYVE-1 and β -actin (AC-15) were from Upstate and Sigma, respectively. The levels of expression of VEGFR-1 or VEGFR-2 were similar in MSS31 cells and HUVEC. Expression of VEGFR-3 and LYVE1 (lymphatic endothelial cell markers) was scarcely detected in both cells after the longer exposure. (C) Anchorage dependency of growth of MSS31 cells. MSS31 cells and MCF-7 breast carcinoma cells (about 1,000 cells) suspended in 0.33% soft agar were piled up on 0.5% soft agar and cultured for 1 and 6 days. MCF-7 cells, but not MSS31 cells, grew to form colonies in soft agar. Bar, 100 μ m.