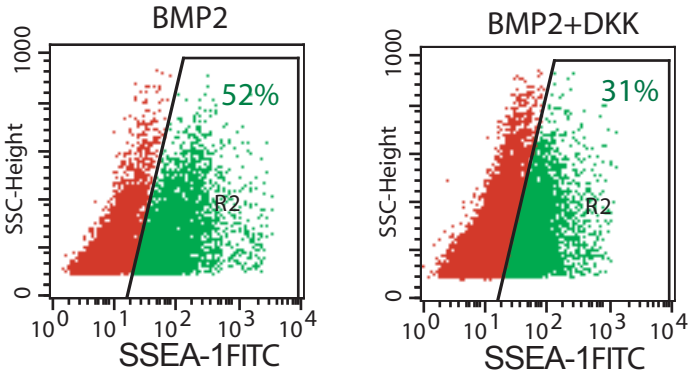
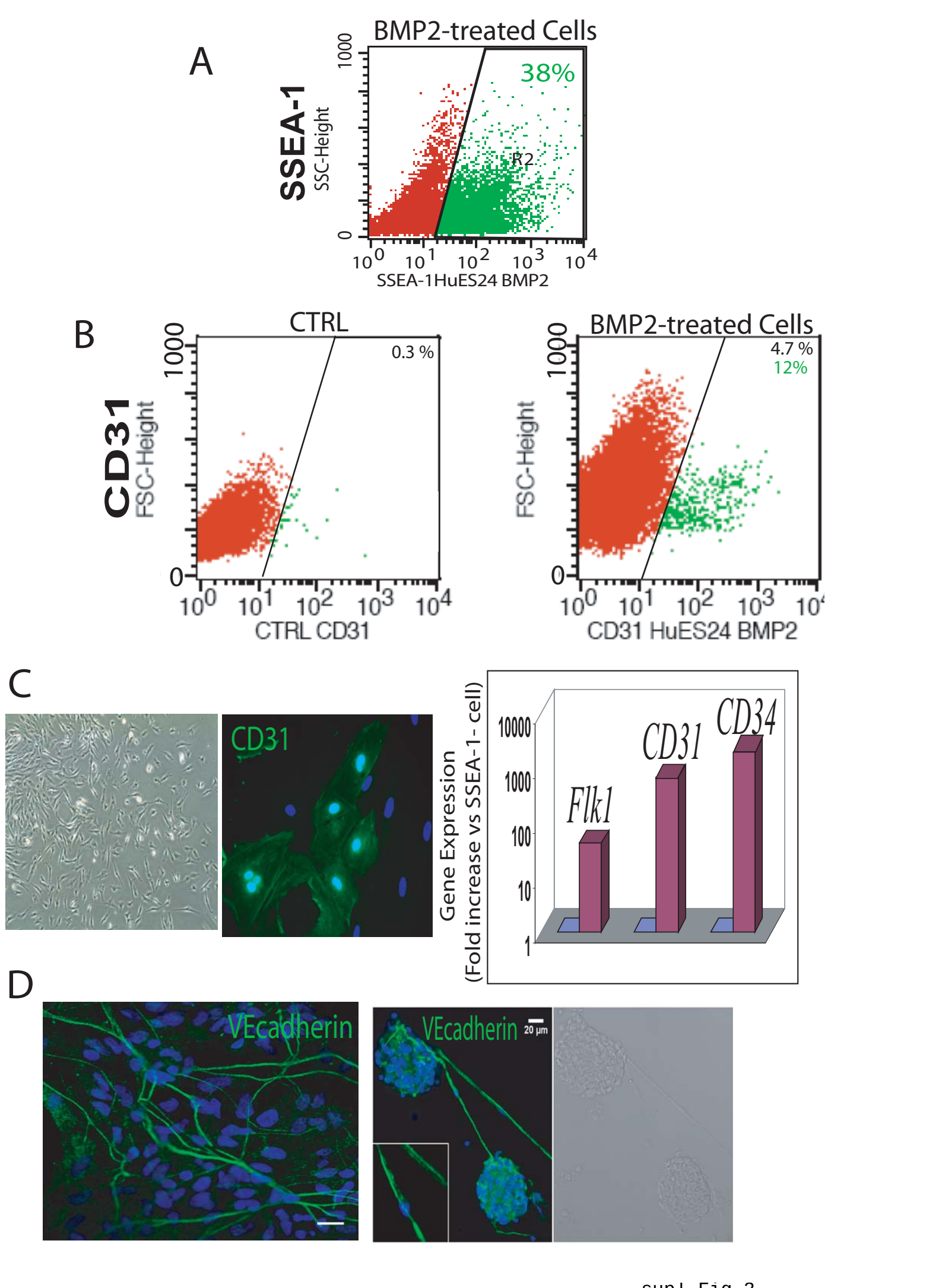
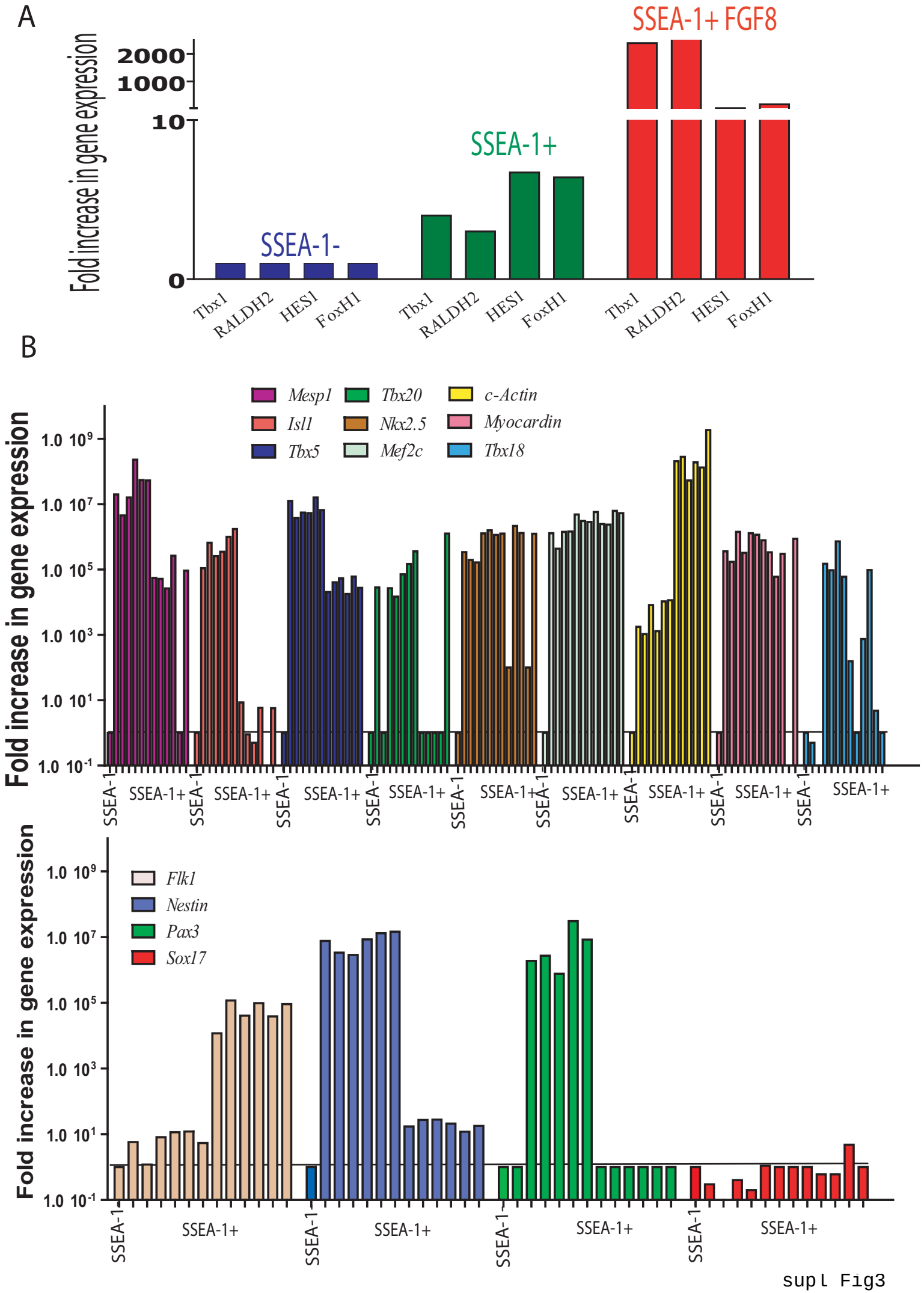

BMP2
SSC-Height
1000
0
52%
R2
10^0 10^1 10^2 10^3 10^4
SSEA-1FITC
BMP2+DKK
SSC-Height
1000
0
31%
R2
10^0 10^1 10^2 10^3 10^4
SSEA-1FITC

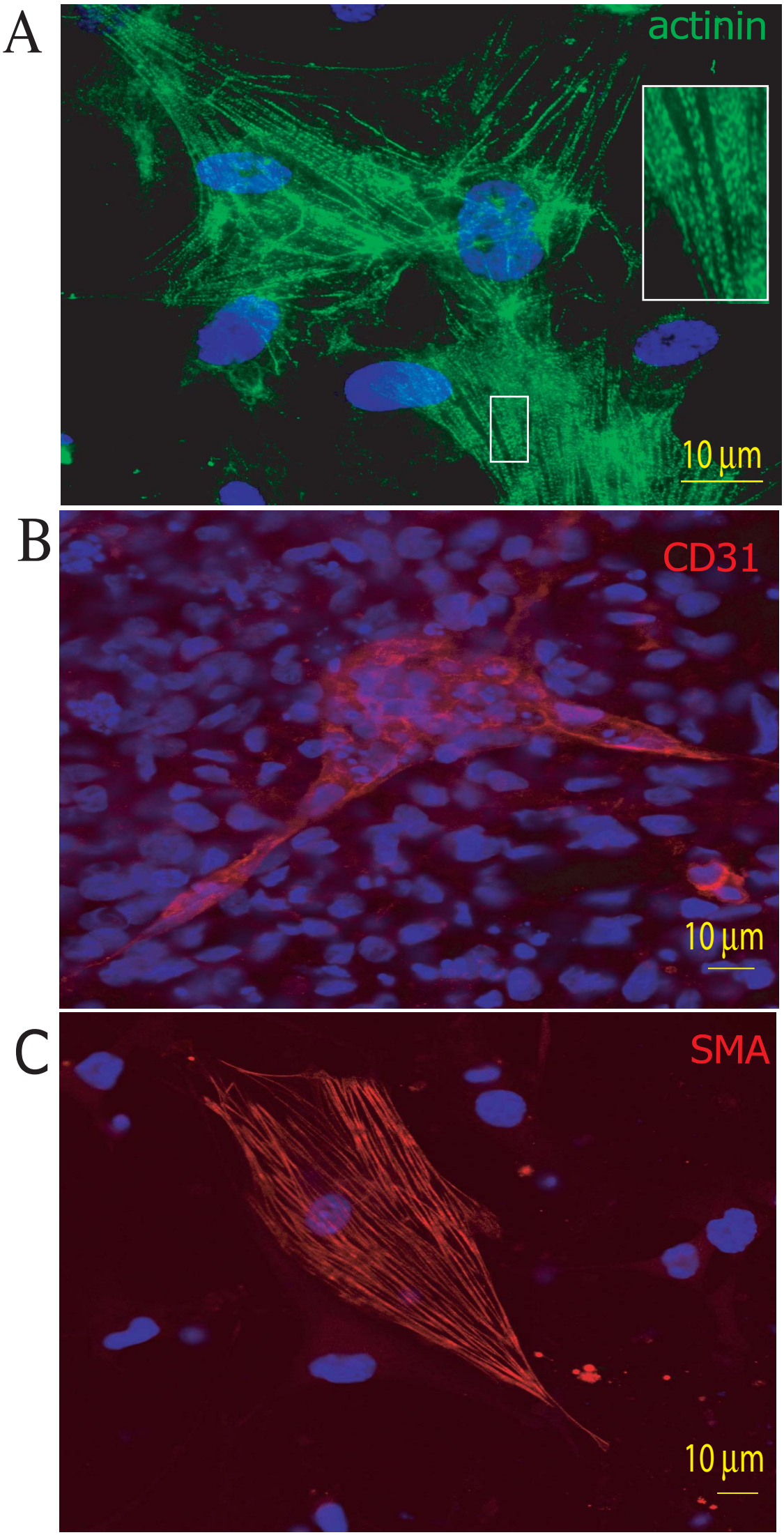
supl Fig1


A
BMP2-treated Cells
SSEA-1
SSC-Height
1000
0
38%
R2
10^0 10^1 10^2 10^3 10^4
SSEA-1HuES24 BMP2
B
CTRL
CD31
FSC-Height
1000
0
0.3 %
10^0 10^1 10^2 10^3 10^4
CTRL CD31
BMP2-treated Cells
FSC-Height
1000
0
4.7 %
12%
10^0 10^1 10^2 10^3
CD31 HuES24 BMP2
C
CD31
Gene Expression
(Fold increase vs SSEA-1- cell)
10000
1000
100
10
1
Flk1
CD31
CD34
D
VEcadherin
VEcadherin
20 µm

supl Fig 2

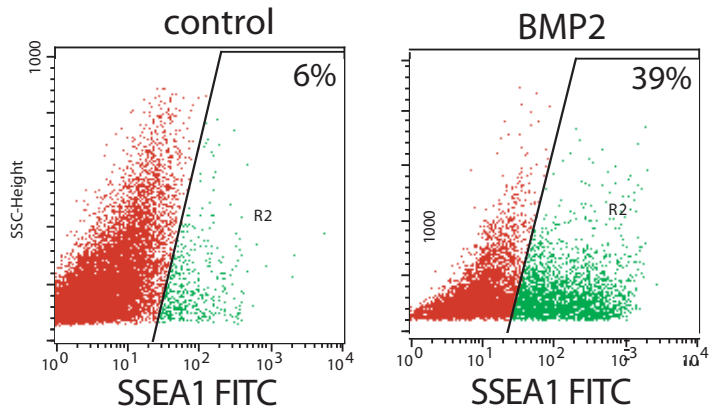

A
Fold increase in gene expression
2000
1000
10
0
SSEA-1-
SSEA-1+
SSEA-1+ FGF8
Tbx1
RALDH2
HES1
FoxH1
B
Fold increase in gene expression
Mesp1
Isl1
Tbx5
Tbx20
Nkx2.5
Mef2c
c-Actin
Myocardin
Tbx18
1.0 10^9
1.0 10^7
1.0 10^5
1.0 10^3
1.0 10^1
1.0 10^-1
SSEA-1-
SSEA-1+
Flk1
Nestin
Pax3
Sox17

supl Fig3

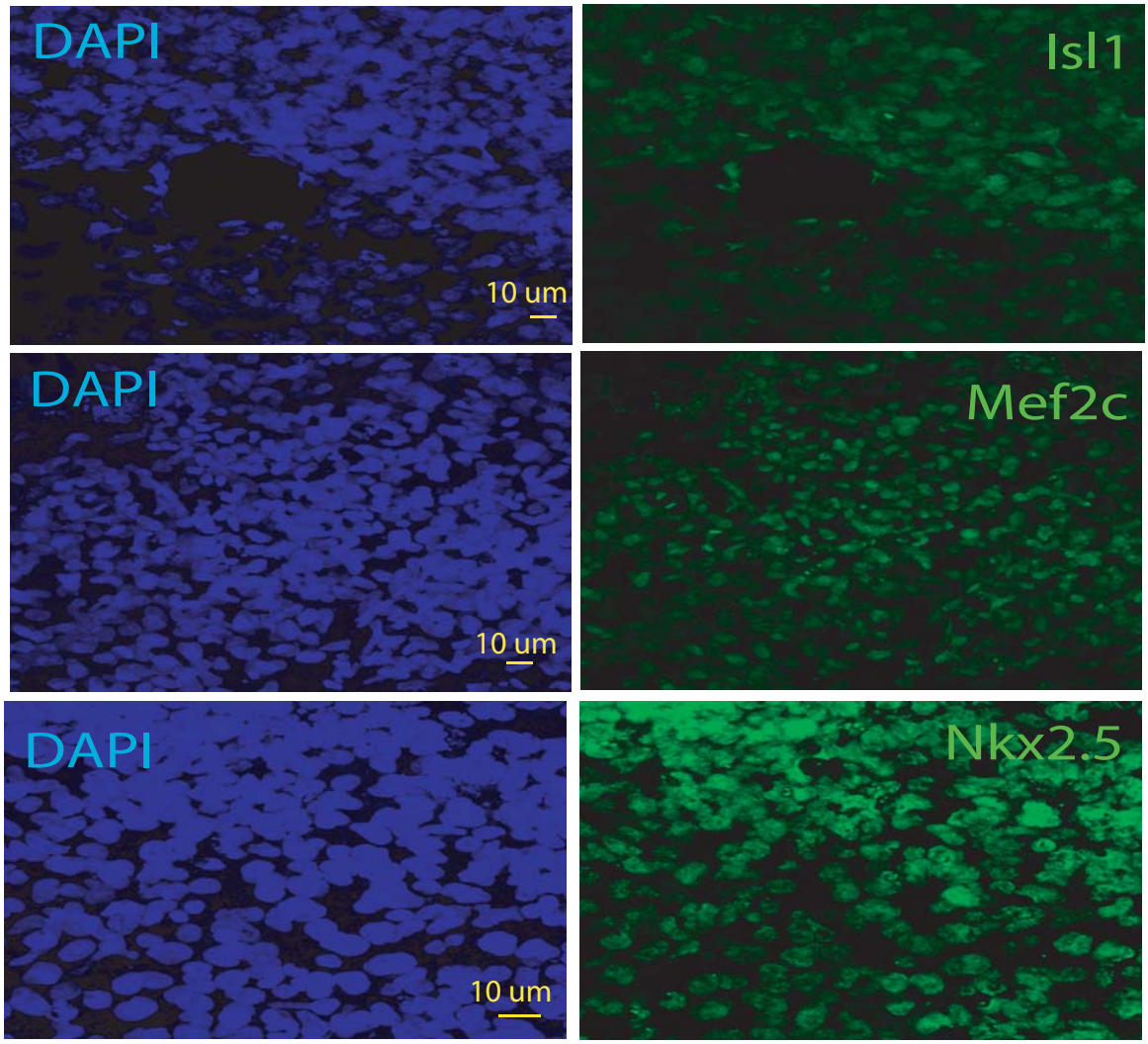


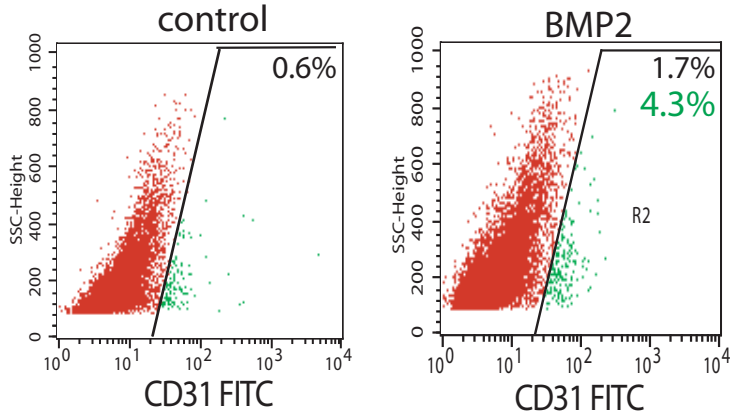
supl Fig 4

A


control
6%
R2
SSC-Height
SSEA1 FITC
BMP2
39%
R2
SSEA1 FITC

B


DAPI
10 um
Isl1
DAPI
10 um
Mef2c
DAPI
10 um
Nkx2.5


control
0.6%
SSC-Height
CD31 FITC
BMP2
1.7%
4.3%
R2
SSC-Height
CD31 FITC

Supl Fig 5

Supplementary Figure 1: DKK1 prevents BMP2-induced generation of SSEA-1$^+$ cardiac progenitors. Human ESCs were treated for 4 days with BMP2 alone or together with DKK and monitored by flow cytometry using the anti-FITC/SSEA-1 antibody. The experiment was performed in duplicate.

Supplementary Figure 2: Flow cytometry analysis of SSEA-1$^+$ cells (**A**) using an anti-CD31 antibody (**B**); gates were set as a function of control undifferentiated (SSEA-1 negative) cells. The percentage in green indicates the percentage of positive cells normalised to the percentage of SSEA-1+ cells. (**C**) SSEA-1$^+$ sorted cells were cultured for one week on MEF and then plated for 10 days on collagen-I-coated dishes and treated with VEGF (50ng/ml). The images show the morphology of cells and an anti-CD31 immunostaining and the graph, the real time PCR quantitation of Flk1, CD31 and CD34 mRNAs levels normalised to HUES SSEA-1 negative cells. (**D**) SSEA-1$^+$ sorted cells were plated for 10 days on collagen IV and challenged by VEGF. Formation of capillaries (left image) is evidenced by anti-VE cadherin immunostaing and cell morphology (right images).

Supplementary Figure 3: (A) Real time PCR monitoring of gene expression in SSEA-1$^-$ and SSEA-1$^+$ sorted cells and in SSEA-1$^+$ cells stimulated for 4 days with FGF8 **(B)** Real time PCR monitoring of gene expression in single colonies. SSEA-1$^+$ single colonies cultured for one week on MEF were picked-up randomly and RNA extracted. Expression of genes was monitored by real-time PCR. Results are normalised to GAPDH expression and SSEA-1$^-$ cells. The first bar from each gene indicates gene expression in SSEA1$^-$ cells and the following are SSEA-1$^+$ colonies from 1 to 12 in the same order for all genes.

Supplementary Figure 4 : a single clone of SSEA-1$^+$ cardiovascular progenitors give rise to cardiomyocytes (upper panel) when cultured on human fibroblasts (inset: magnification of sarcomeres), endothelial CD31$^+$ cells (middle panel) when cultured on collagen 1 and treated for one week with VEGF and SMA$^+$ smooth muscle cell (bottom panel) when cultured on collagen 1 and treated with PDGF. This experiment was repeated with 5 separate clones.

Supplementary Figure 5: iPS cells give rise to SSEA-1$^+$ cardiovascular progenitors.

iPS cells challenged with BMP2 expressed SSEA-1 (**A**) and CD31 (**B**) monitored by flow cytometry as well as 3 cardiac-restricted markers (Isl1, Mef2c, Nkx2.5) following one week of culture on MEF. In the CD31 panel, the percentage in green indicates the percentage of positive cells normalised to the percentage of SSEA-1$^+$ cells. The scale bars indicate 10 µm.