Supplemental figure legends:

Figure S1: **ImageStream analysis gating steps and ImageStream quantification.** Single cells were imaged using INSPIRE software and image analysis was performed using IDEAS image analysis software version 4.0 (AMNIS Corporation, Seattle USA). A: CD45 and F4/80 negative events of cellular size (using the “area” feature for the bright field channel) were selected. B: From CD45 negative events, round events were selected using the “aspect ratio” and “aspect ratio intensity” features. C: In the next step, cells in focus and with optimal image contrast were selected. D: SPC positive cells were selected and further analyzed for the presence of bright spots (E) in the SPC channel (lamellar bodies) using the “spot count” feature and peak mask. F: From all cells with at least two bright spots in the SPC channel, only cells that did not exhibit co-localization of the SPC signal with the cytokeratin signal (to exclude autofluorescent cells) were selected for further analysis. G: Total number of mice with SPC-positive cells detected (numerator) and total number of mice analyzed (denominator). Total number of SPC-positive cells detected (numerator) and total number of CD45-negative lung cells analyzed (denominator). Percentages of T2 cells derived from donor BM detected by ImageStream calculated based on the number of T2 cells among CD45-negative, round cells in corresponding WT samples, which was set to 100%.

Figure S2: **Sorting of type 2 pneumocytes based on expression of Epcam and CD49f**. A: In SPC-H2B-GFP control mice, about 1% of CD45-CD31-lineage-cells express GFP. GFP+ cells are uniformly positive for both Epcam and CD49f. B: Immunofluorescence for SPC reveals that 90% of sorted cells positive for both Epcam and CD49f express SPC protein.

Figure S3: **Expression of SPC, cytokeratin, CCSP in lung cells and VSELs.** A: Immunofluorescent staining of freshly isolated lung cells or VSELs for SPC (red) and Cytokeratin (green). B-D: Expression of SPC, CCSP, and OCT4 mRNA in lung cells, WBM, HSC and VSELs by RT-qPCR, normalized to expression of the control gene β2-microglobulin. Fold increase of expression is shown compared to WBM, which was set to 1. (Data shown are representative of three independent experiments that gave analogous results.)

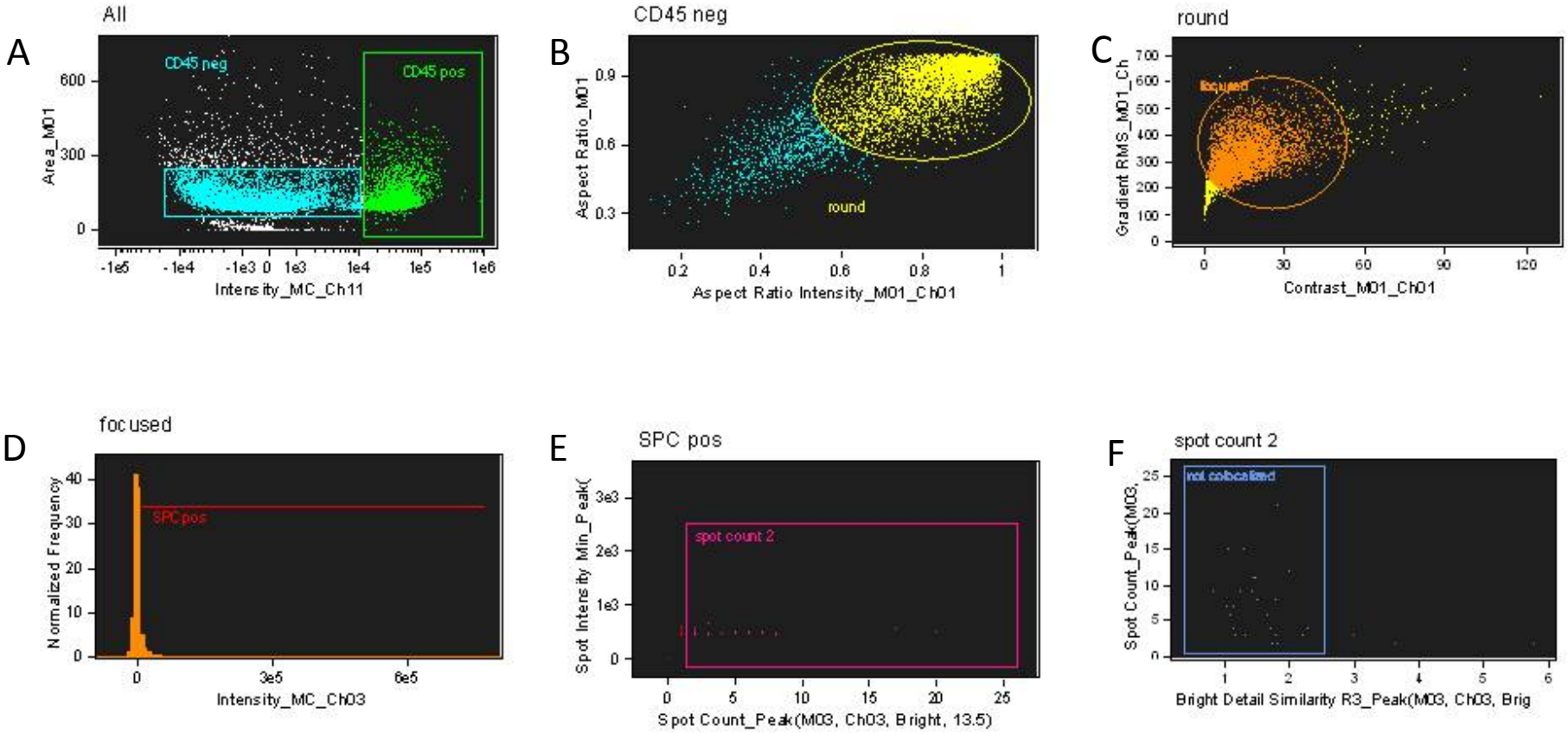


Image Stream Quantification

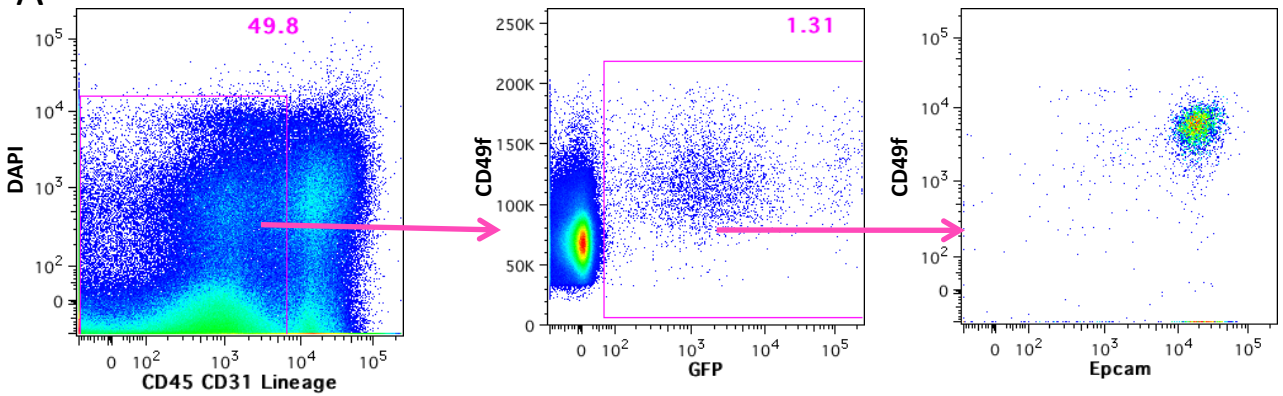
G

	Mice with SPC positive cells/ # of mice analyzed	SPC+ cells/ CD45-	% SPC+/ CD45-
VSEL	27/28	259/166738	0.15
Non-VSEL	12/21	18/87478	0.02
HSPC (Lin- Sca-1+ CD45+)	0/7	0/15,973	0
Wild type	5/5	1920/60.000	3.2

Denominator: intact CD45- cells

Figure S1

A



SPC-H2B-GFP-positive cells are Epcam and CD49f positive

B

Epcam CD49f + cells: 90% SPC pos

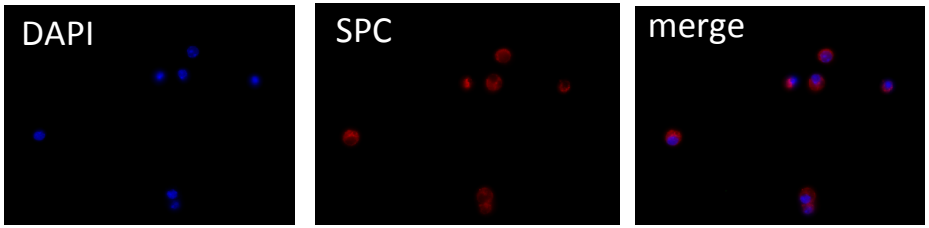


Figure S2

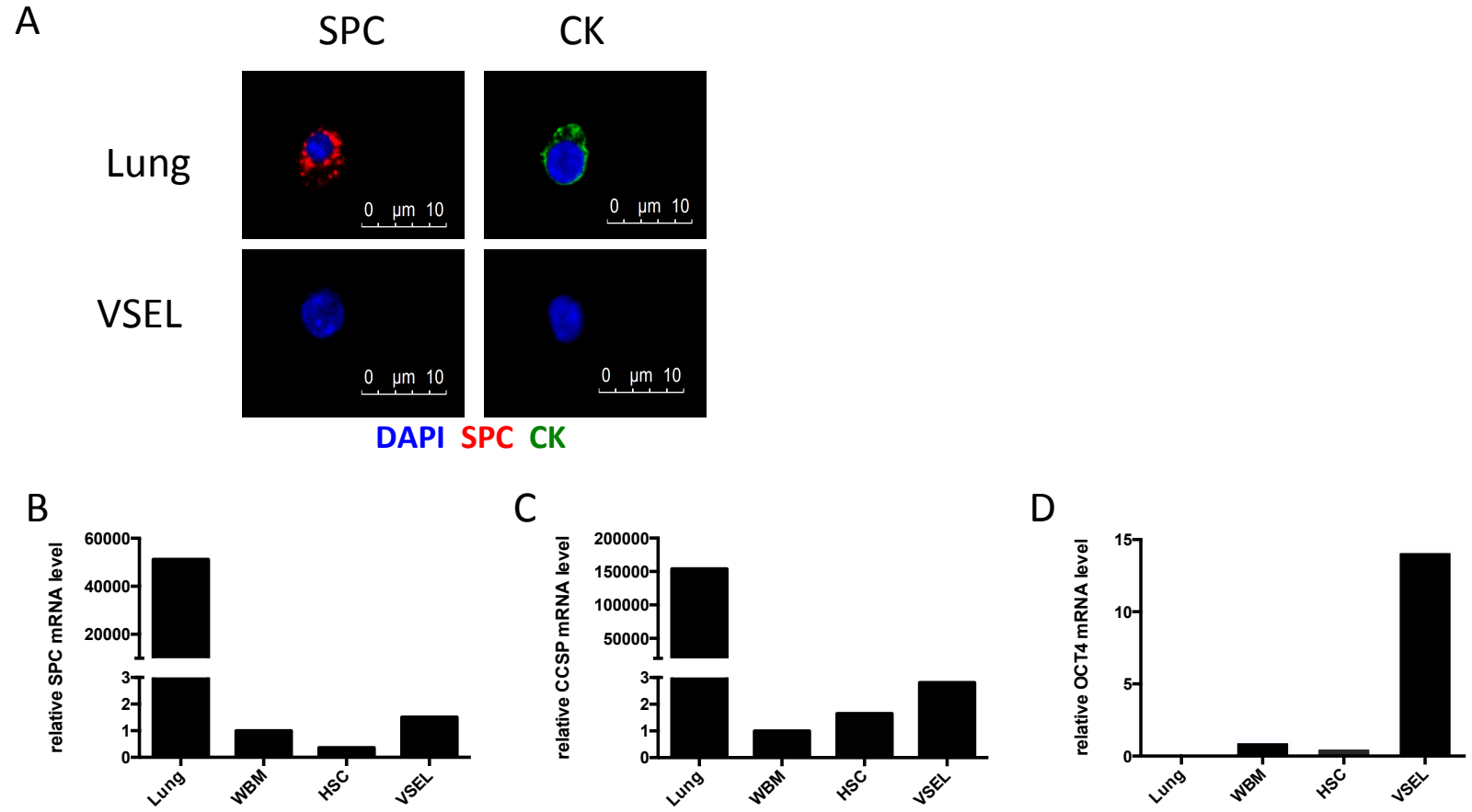

A
SPC
CK
Lung
VSEL
0 μm 10
DAPI SPC CK
B
relative SPC mRNA level
60000
40000
20000
3
2
1
0
Lung
WBM
HSC
VSEL
C
relative CCSP mRNA level
200000
150000
100000
50000
D
relative OCT4 mRNA level
15
10
5
E
Lin-Sca1+ CD45-
Lin-Sca1+ CD45+
SS Lin: SS
FS Lin: FS
4000
3000
2000
1000
0

Figure S3