Supplemental material

Raz et al., https://doi.org/10.1084/jem.20180818

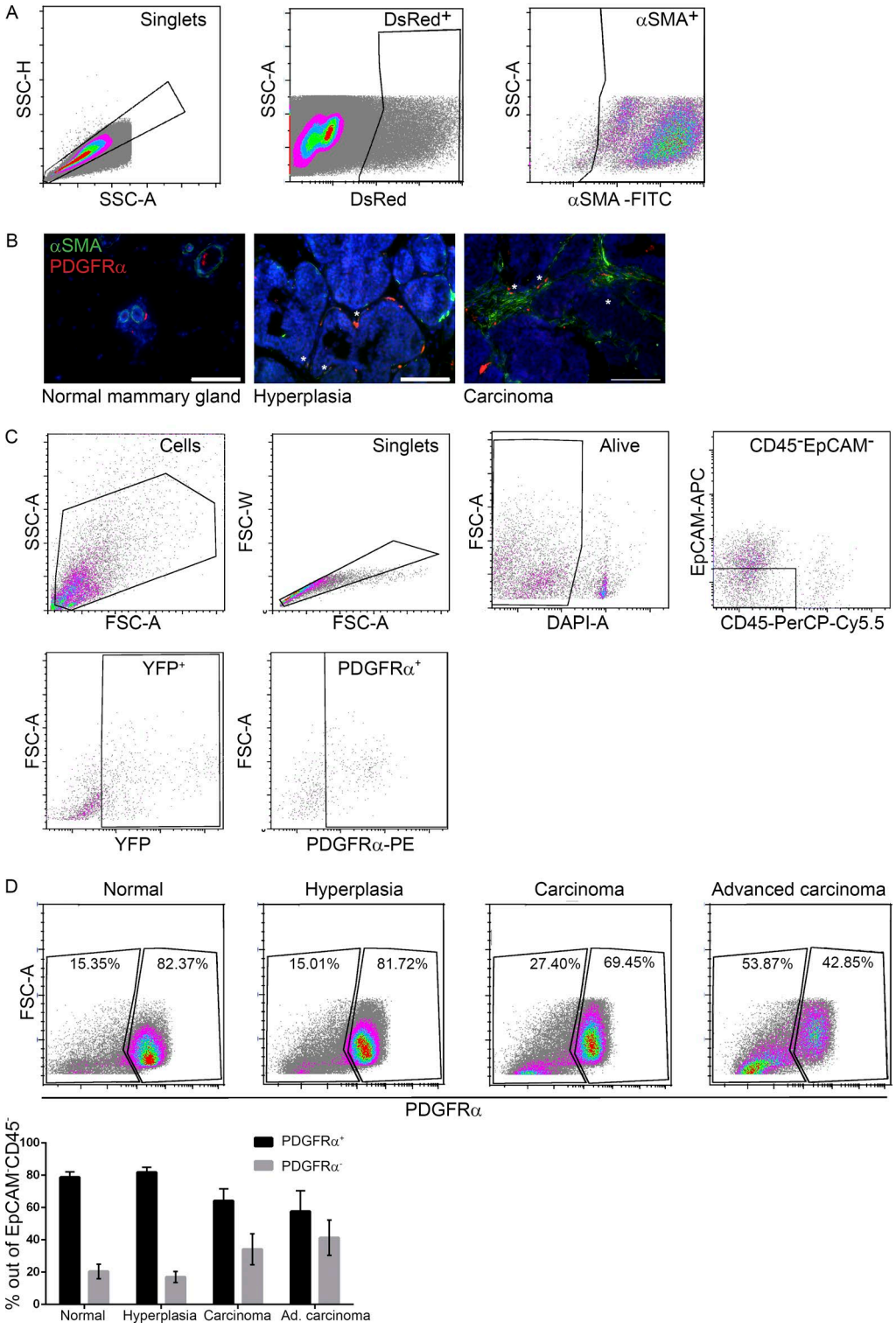


Figure S1. **The percentage of PDGFRα⁺ fibroblasts in mammary tumors decreases with tumor progression. (A)** Gating strategy for flow cytometry presented in Fig. 1 C. Data were analyzed using Kaluza 1.5 Flow Analysis software (Beckman Coulter, Inc). **(B)** Co-staining of αSMA and PDGFRα in normal mammary gland, hyperplasia, or carcinoma. *n* = 4 mice at each stage; four sections/mouse were analyzed. Bars, 100 µm. Asterisks indicate double-positive cells. **(C)** Gating strategy for flow cytometry presented in Fig. 1 O. Data were analyzed using Kaluza 1.5 Flow Analysis software (Beckman Coulter, Inc). **(D)** FACS analysis of PDGFRα in normal mammary glands, hyperplasia, and mammary tumors of MMTV-PyMT mice. Representative of three independent experiments. Results show mean ± SD of biological repeats.

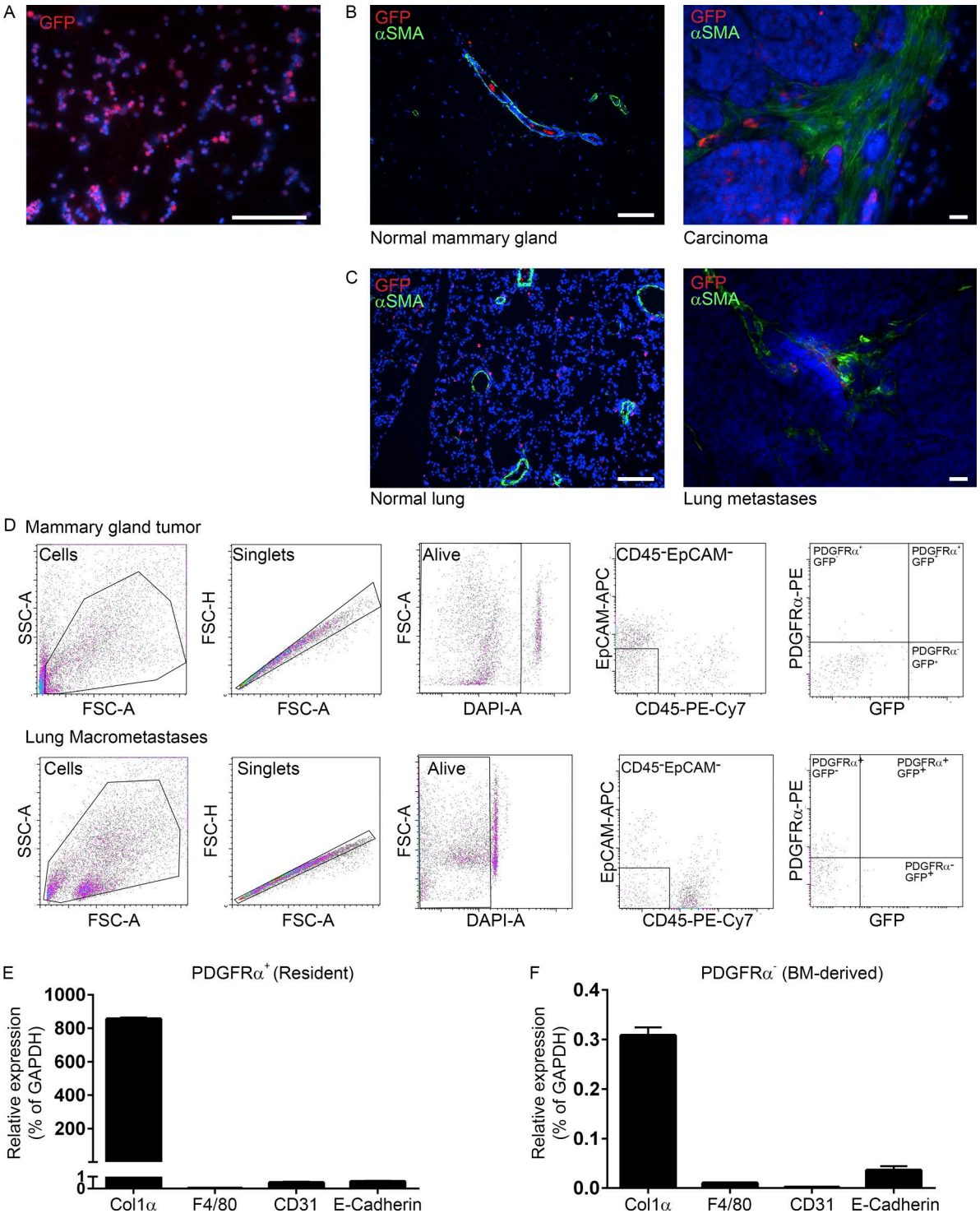


Figure S2. **A subpopulation of CAFs in mammary tumors and lung metastases are BM-derived. (A)** Immunofluorescent staining of GFP in BM smears of donor mice. BM smears from MMTV-PyMT and FVB/n recipient mice were fixed and stained. Representative image out of 18 BM smears analyzed from two separate transplantations. Cell nuclei, DAPI; GFP, Rhodamine. Bar, 100 µm. **(B)** Co-staining of αSMA and GFP in normal mammary glands from FVB/n recipients (n = 5) or in mammary tumors from PyMT recipients (n = 5). Bars, 100 µm. **(C)** Co-staining as above in normal lungs from FVB/n recipients (n = 5) and in lung macrometastases in PyMT recipients (n = 7). Bars, 100 µm. **(D)** FACS gating strategy for flow cytometry presented in Fig. 2 (B and E). Data were analyzed using Kaluza 1.5 Flow Analysis Software (Beckman Coulter, Inc). **(E and F)** qRT-PCR analysis of characteristic cell markers for macrophages (F4/80), epithelial cells (E-Cadherin), and endothelial cells (CD31) was performed on resident PDGFRα$^+$GFP$^-$ (E) and BM-derived PDGFRα$^-$GFP$^+$ (F) cell populations. Results were normalized to GAPDH. Error bars represent SD of technical repeats. n = 9.

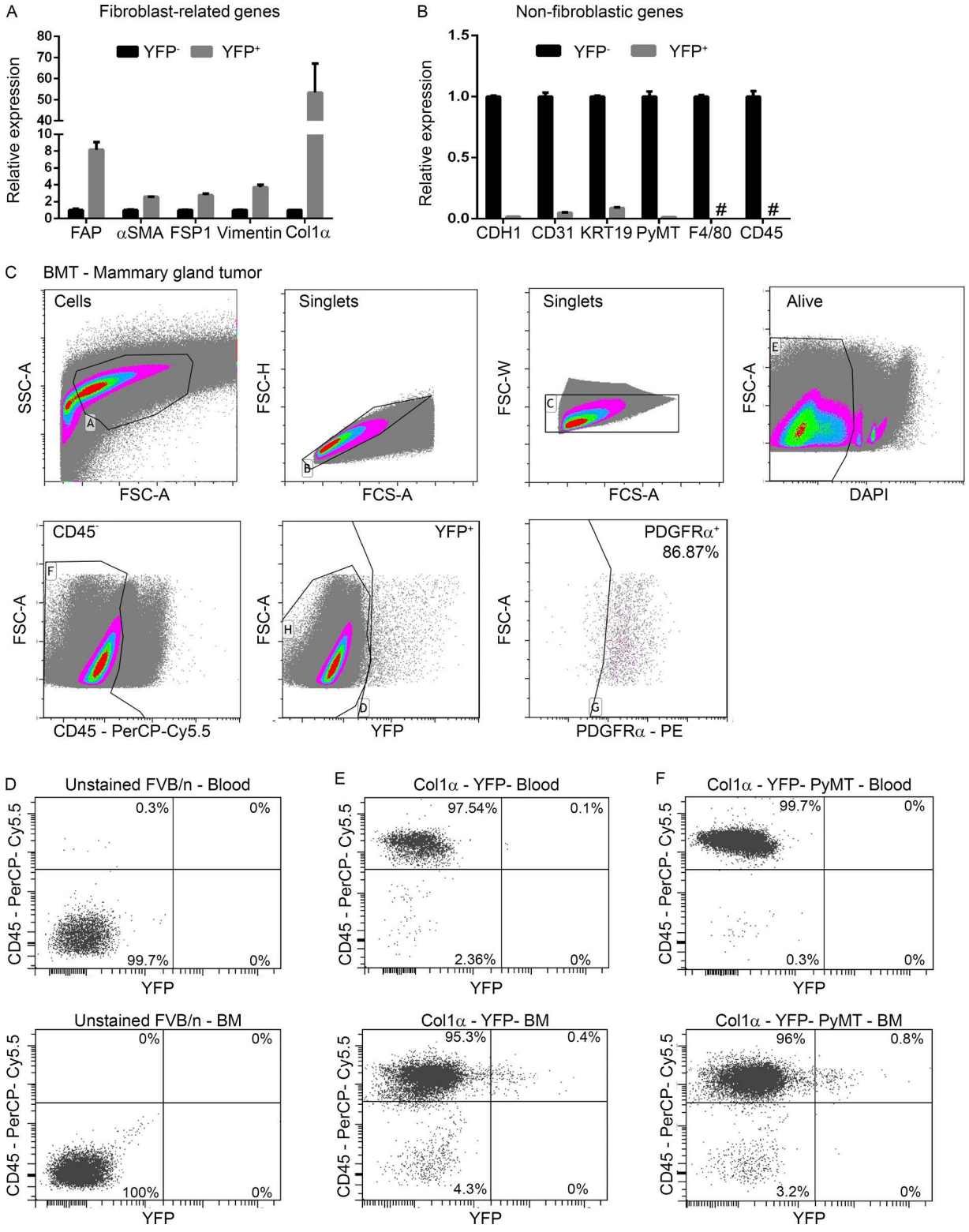


Figure S3. **Col1α labels all fibroblasts, while PDGFRα is specific for resident fibroblasts. (A and B)** qRT-PCR expression analysis of fibroblastic (A) and nonfibroblastic (B) markers in EpCAM⁻CD45⁻ cells isolated from mammary tumors of female PyMT;Col1α-YFP mice (n = 2). Error bars represent SD of technical repeats. #, undetected. **(C)** FACS analysis and gating strategy of PDGFRα in resident (YFP⁺) CAFs in the BM transplantation described in Fig. 2 H. **(D–F)** Col1α⁺ nonhematopoietic cells are not detected in peripheral blood or in the BM. FACS analysis of peripheral blood (upper panel) and BM (lower panel) of unstained control (D), *Col1a*-YFP (E), and PyMT;*Col1a*-YFP (F) female mice. Representative of four mice analyzed from two independent experiments.

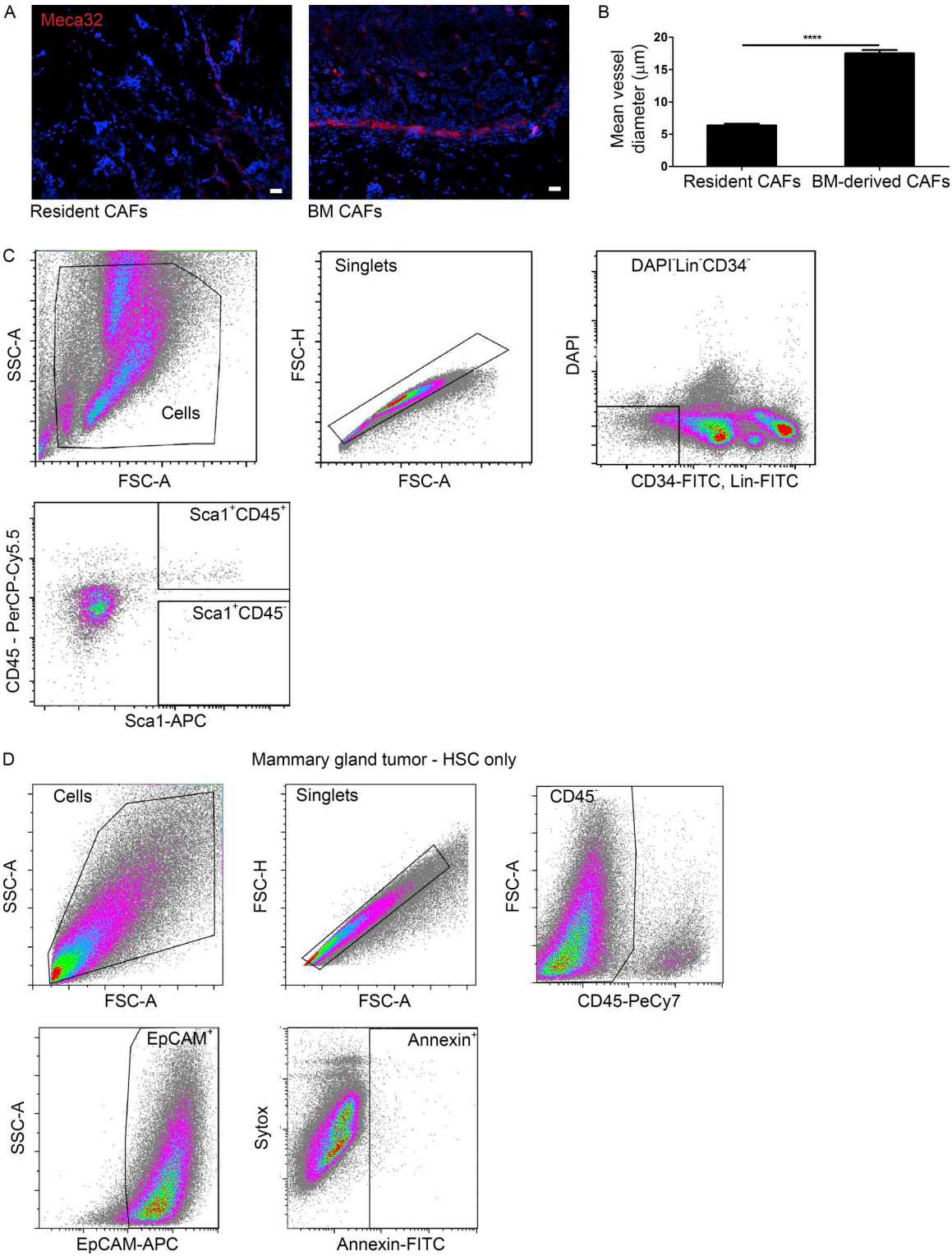
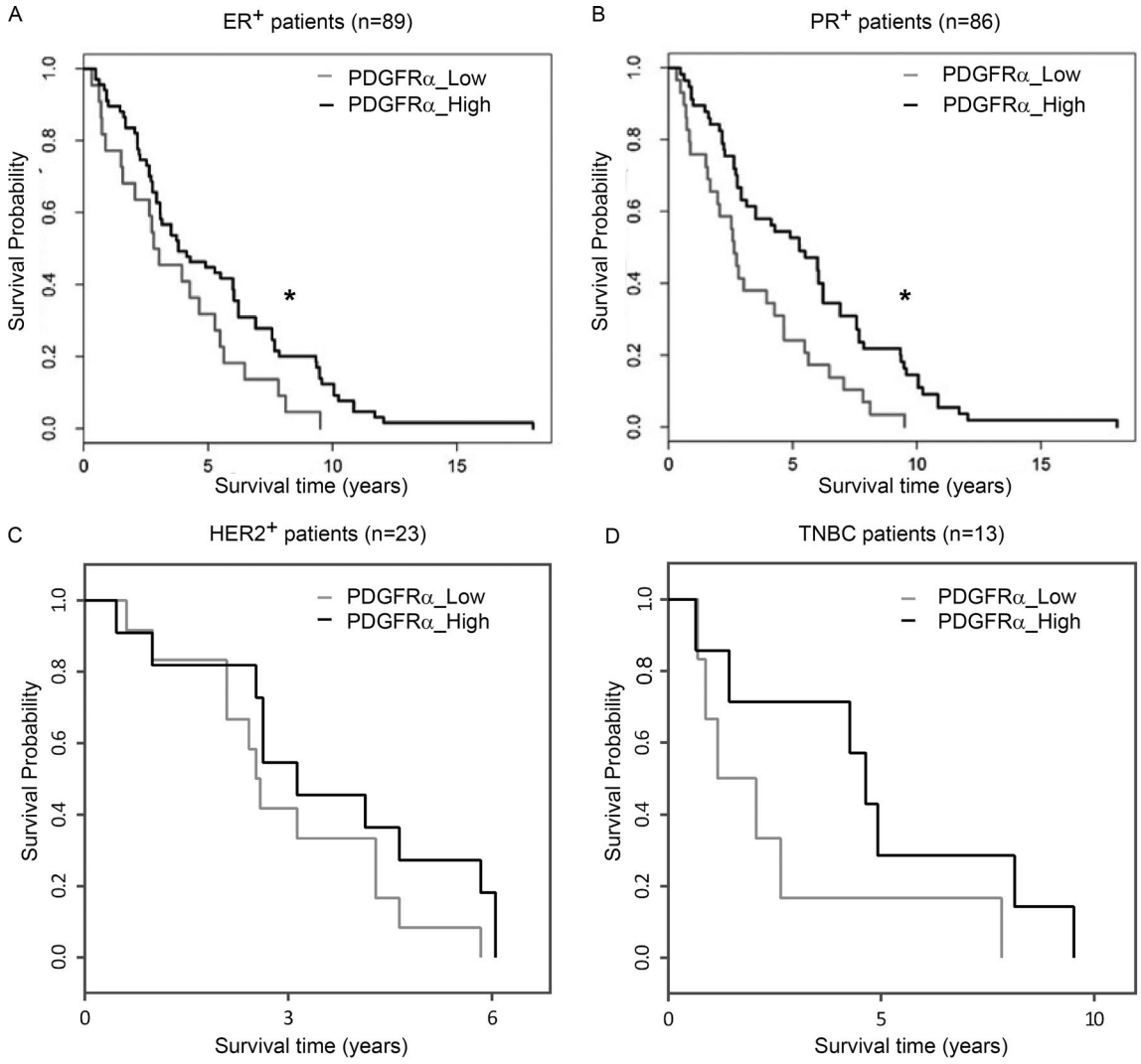


Figure S4. **BM-derived PDGFRα⁻ CAFs induce the formation of larger blood vessels as compared with PDGFRα⁺ resident CAFs. (A)** BM-derived PDGFRα⁻ CAFs induce the formation of larger blood vessels as compared with PDGFRα⁺ resident CAFs. Immunostaining of Meca32 in plugs described in Fig. 4 D. Experiments were repeated twice. *n* = 4. 5 sections per plug were stained and 5 fields per section were analyzed using the ImageJ software for a total of 100 fields per cell type. Bars, 30 µm. **(B)** Quantification of staining presented in A, performed with ImageJ software. Error bars represent SEM. ****, P < 0.00001; two-tailed Mann-Whitney test. **(C)** Gating strategy for isolation of HSCs and MSCs used for BMT described in Fig. 5 A. **(D)** Gating strategy for flow cytometry presented in Fig. 5 E. Data were analyzed using Kaluza 1.5 Flow Analysis Software (Beckman Coulter, Inc).



E Details of cancer data sets

	Short name	Cancer name	Normal samples (n)	Cancer samples (n)	Total samples (n)
1	Bladder	Bladder urothelial Ca	16	182	198
2	Breast	Breast invasive Ca	79	649	728
3	Colon	Colon adenoCa	10	230	240
4	Head&Neck	Head&Neck SCC	20	303	323
5	Kidney	Kidney papillary cell Ca	23	125	148
6	Lung	Lung SCC	8	354	362
7	Prostate	Prostate adenoCa	30	195	225
8	Rectal	Rectal adenoCa	1	82	83
9	Thyroid	Thyroid Ca	49	486	535
10	Uterine	Uterine corpus endometroid Ca	17	118	135

Ca - carcinoma. AdenoCa - Adenocarcinoma. SCC - Squamous cell carcinoma.

Figure S5. **Decreased PDGFRα in human tumors correlates with worse prognosis in different subtypes of human breast cancer. (A–D)** Kaplan-Meier plot for survival rates at high and low expression levels of PDGFRα compared with the median expression (black and gray curves, respectively). Pre-processed and normalized RNA-seq gene expression data from the new TCGA were analyzed (n = 89 for PR$^+$ patients [A]; n = 86 for ER$^+$ [B]; n = 13 for TNBC [C]; n = 23 for HER2$^+$ patients [D]). *, $P < 0.05$; χ^2 test. ER, estrogen receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer. **(E)** Details of cancer datasets. All datasets were derived from TCGA database.