CFU assay

Colony-forming unit granulocyte-macrophage (CFU-GM), burst-forming unit erythroid cell (BFU-E) and multipotential (CFU-GEMM or CFU-Mix) progenitors were assayed in methylcellulose cultures using the specialized MethoCult® H4434 Classic medium (StemCell Technologies)[1], in the presence of: GM-CSF (10 ng/mL), IL-3 (10 ng/mL), erythropoietin (3 U/mL), SCF (50 ng/mL), which was further supplemented with G-CSF (20 ng/mL) and IL-6 (20 ng/mL) (PeproTech). CFU-C were scored after 7–14 days.

1. Kobari L, Giarratana MC, Poloni A, et al. Flt3 ligand, MGDF, Epo and G-CSF enhance *ex vivo* expansion of hematopoietic cell compartments in presence of SCF, IL-3 and IL-6. *Bone Marrow Transplant*. 1998;21(8):759–767.

REFERENCE

Limiting dilution assay

1. Fazekas de St Groth. The evaluation of limiting dilution assays. *J Immunol Methods*. 1982;49(2):R11–23.

Table S1. Pool of lineage-specific PE-labeled mAbs

Species	Class	Name	Clonality	Vendor
Mouse	IgG1	Anti-CD2	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD3	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD14	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD16	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD19	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD24	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD56	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-glycophorine A	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD11c	Monoclonal	Miltenyi Biotec Auburn,CA
Mouse	IgG1	Anti-CD66b	Monoclonal	Miltenyi Biotec Auburn,CA

Table S2. Antibodies for the detection of cell surface antigen of cell obtained in LTC-HE and LTC-IC-HE cultures

Species	Class	Name	Clonality	Vendor
Mouse	IgG1 k	Anti–CD45-FITC	Monoclonal	BD Bioscience, San Jose,CA
Mouse	IgG1 k	Anti–CD33-PE	Monoclonal	BD Bioscience, San Jose,CA
Mouse	IgG1 k	Anti–CD56-PE	Monoclonal	BD Bioscience, San Jose,CA
Mouse	IgG1 k	Anti–CD105-PE	Monoclonal	BD Bioscience, San Jose,CA
Mouse	IgG1 k	Anti–c-kit-PE	Monoclonal	BD Bioscience, San Jose,CA
Mouse	IgG1 k	Anti–CXCR4-PE	Monoclonal	BD Bioscience, San Jose,CA
Mouse	IgG1	Anti–KDR-PE	Monoclonal	Sigma-Aldrich Milan, Italy
Mouse	IgG1 k	Anti–VE-Cadherin-PE	Monoclonal	BD Bioscience San Jose, CA
Mouse	IgG1 k	Anti–VE-Cadherin-Purified	Monoclonal	eBioscience San Diego, CA
Mouse	IgG1 k	Anti–CD45-ECD-PETR	Monoclonal	Immunotech Marseille,France
Mouse	IgG1 k	Anti–Nanog	Monoclonal	BD Bioscience San Jose, CA
Mouse	IgG	Anti–OCT4	Polyclonal	Abcam, Cambridge, MA

Table S3. RT-PCR oligonucleotides

Product	GeneBank number	Foward primer Sequence 5′ → 3′	Reverse primer Sequence 5′ → 3′	Optimal Temperature	Size (bp)
Notch1	NM 017617	ACTGTGAGGACCTGGTGGAC	TTGTAGGTGTTGGGGAGGTC	56	196
Wnt5A [2]	NM 003392	CAAGGTGGGTGATGCCCTGAAGGAG	CGTCTGCACGGTCTTGAACTGGTCGTA	64	276
B-Catenin	NM 001904	TGCAGTTCGCCTTCACTATG	CTGCACAAACAATGGAATGG	56	355
Bmi-1	NM 005180	AATCCCCACCTGATGTGTGT	CATTTTTGAAAAGCCCTGGA	56	232
Stat3	NM 139276 NM 003150 NM 213662	GGAGGAGTTGCAGCAAAAAG	TGTGTTTGTGCCCAGAATGT	56	322
Src	NM 005417 NM 198291	CTGTTCGGAGGCTTCAACTC	TGAGAGGCAGTAGGCACCTT	58	384
LMO2 [3]	NM 005574	TACTGGCACGAGGACTGC	CTTTCACCCGCATTGTCAT	56	190
Gata2	NM 032638	AAGGCTCGTTCCTGTTCAGA	GCCCCTTTCTTGCTCTTCTT	56	380
Gata3	NM 002295 NM 002051	CTCATTAAGCCCAAGCGAAG	TTTTTCGGTTTCTGGTCTGG	58	205
Gp130	NM 175767	TGGCCTAATGTTCCAGATCC	GGACTGACGGAACTTGGTGT	56	370
SLAM (CD150)	NM 003037	TGTCGTCACAATGGCAAAAT	CCCAGTATCAAGGTGCAGGT	56	303
Podocalyxin	NM 005397	CCGTGGTCGTCAAAGAAATC	GTCGTCCTTGGTCAGGTTGT	54	428
Vegfr1 [4]	NM 002019	ATTCTGACGGTTTCTACAAGGAG	TCCTGTCAGTATGGCATTGATTG	62	580
Vegfr2	NM 002253	TGATCGGAAATGACACTGGA	TGCTTCACAGAAGACCATGC	58	329
Tie2	NM 000459	CCAAACGTGATTGACACTGG	TCCGAGCTTGGAAATATTGG	56	362
Tie1	NM 005424	GTGCCAGTGTCAGAATGGTG	GGGCACTTTCACATTGACCT	58	385
EphB4[5]	NM 004444	CACAAAATTGGAAACTGCTGAT	GCTGGGCGCACTTTTTGTAGAA	60	532
EphB2[5]	NM 004442	GGAAGAGGTGAGTGGCTACGAT	TACATCCACCTCTTCCGCATTG	60	577
EphrinB2[5]	NM 004093	GAAAATACCCCTCTCCTCAACT	CTTGGACCGAGGATGTTGTTC	55	394

Oligonucleotides used in the RT-PCR analysis
The numbers shown in superscript indicate the reference sources of the oligonucleotide sequences. All other oligonucleotides were designed by our group.

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1. Klimanskaya, I., Chung, Y., Becker, S., Lu, SJ., and Lanza R. (2006). Human embryonic stem cell lines derived from single blastomeres. *Nature* 444, 481–485.
2. Van Den Berg, DJ., Sharma, AK., Bruno, E., and Hoffman R. (1998). Role of Members of the *Wnt* Gene Family in Human Hematopoiesis. *Blood* 92, 3189–3202.
3. Shojaei, F., Gallacher, L., and Bhatia, M. (2004). Differential gene expression of human stem progenitor cells derived from early stages of in utero human hematopoiesis. *Blood* 103, 2530–2540.
4. Bagnard, D., Vaillant, C., Khuth, ST., Dufay, N., Lohrum, M., Puschel, AW., Belin, MF., Bolz, J., and Thomasset, N. (2001). Semaphorin 3A-Vascular Endothelial Growth Factor-165 Balance Mediates Migration and apoptosis of Neural Progenitor Cells by the Recruitment of Shared Receptor. *The Journal of Neuroscience* 21,3332–3341.
5. Salvucci, O., de la Luz Sierra, M., Martina, JA., McCormick, PJ., and Tosato, G. (2006).EphB2 and EphB4 receptors forward signaling promotes SDF-1–induced endothelial cell chemotaxis and branching remodeling. *Blood* 108, 2914–2922.

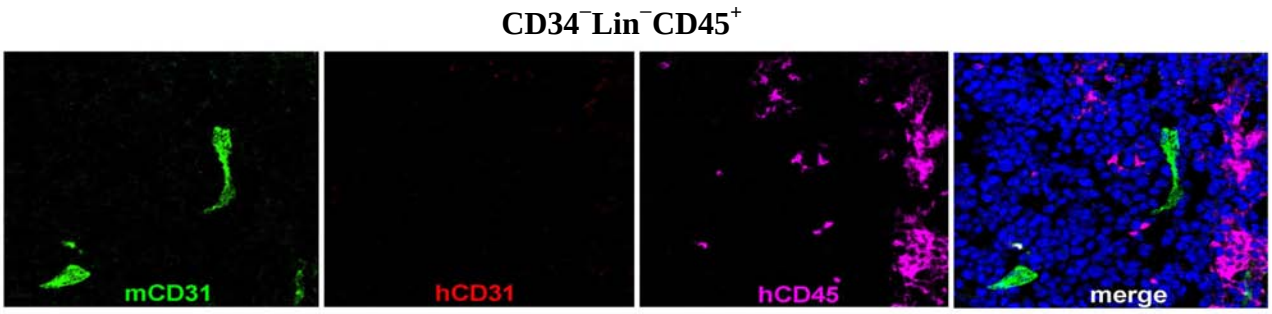

Figure S1. MOPC315 tumor tissue from a mouse injected with $CD34^{-}Lin^{-}CD45^{+}$ cells shows vascular structures expressing mouse, but not human CD31
Clusters of human $CD45^{+}$ cells are observed.