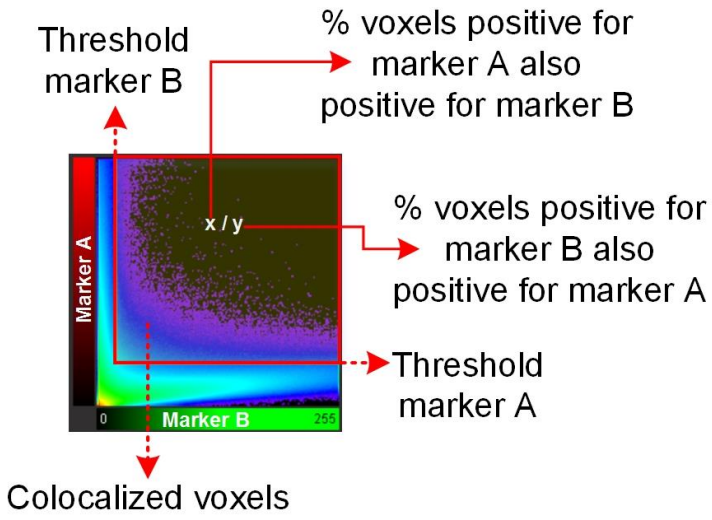
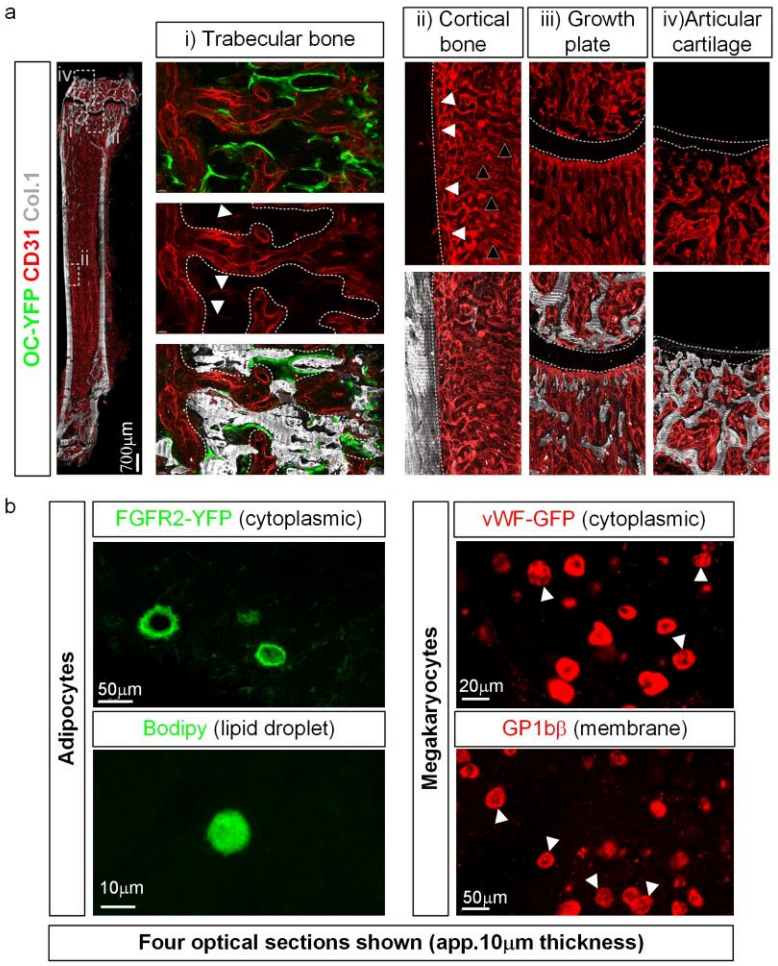


Supplementary Figure 1

Display of colocalization analysis results.

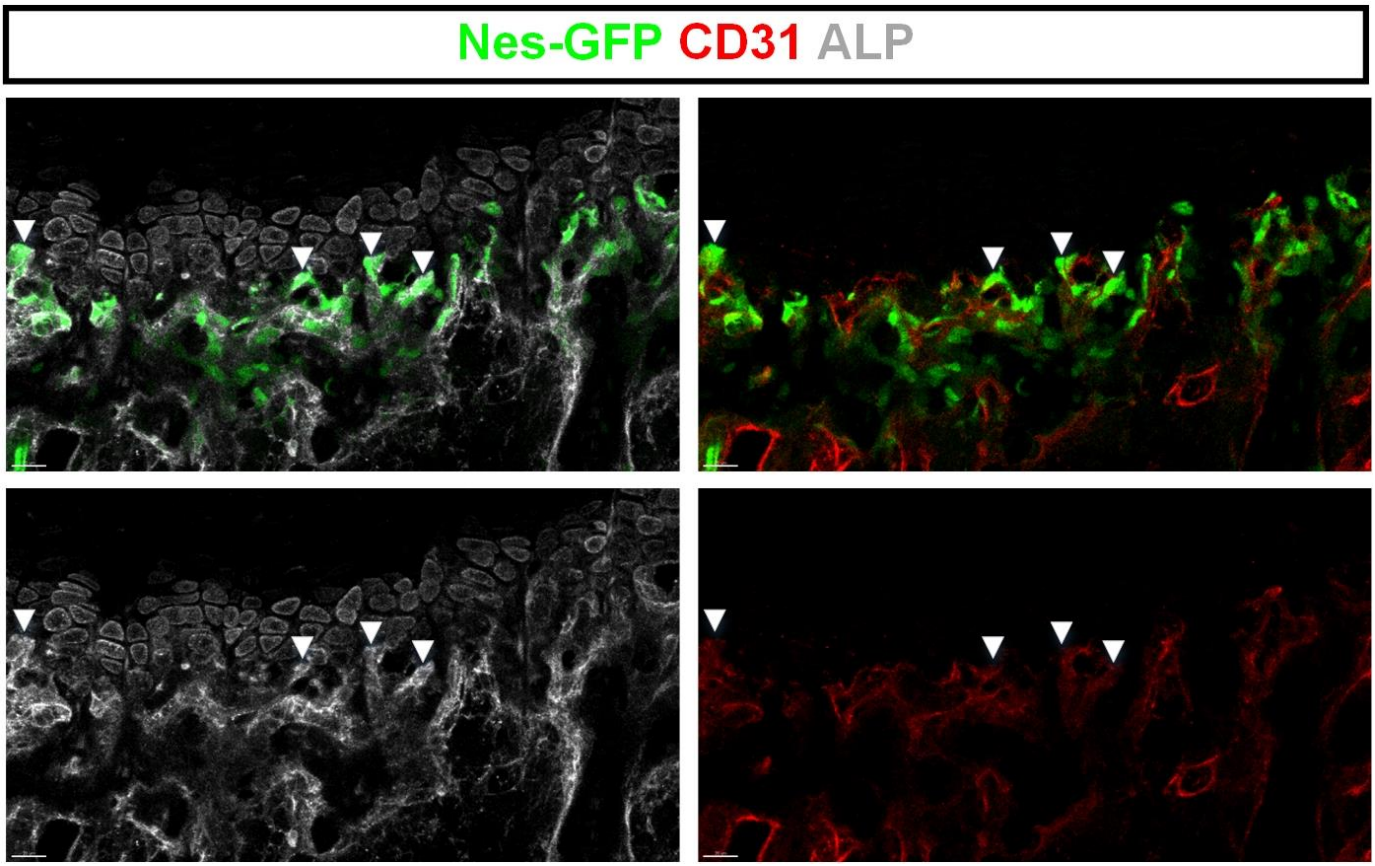
The Coloc module of Imaris was used for voxel colocalization analyses. The software tools were used to select thresholds for two given markers (marker A and B here), which are indicated by the red box. The value given by x represents the percentage of voxels with a fluorescence intensity for marker A above the threshold that also have a fluorescence intensity for marker B above threshold. Inversely, the value given by y represents the percentage of voxels with a fluorescence intensity for marker B above the threshold that also have a fluorescence intensity for marker A above threshold. The colocalization data presented is derived from the full or partial image (see figure legends for details) shown in the same figure panel.



Supplementary Figure 2

Identification of distinct anatomical locations and cell types in adult mouse femurs without landmark staining.

a) Examples showing how to identify trabecular (i) and cortical (ii) bone surfaces, as well as growth plate (iii) and articular (iv) cartilage using only a vascular marker (collagen 1 is here shown to validate proper identification). i) Trabecular bone in the metaphyseal area can be identified by the complete absence of vascular sinusoids (dotted lines) although it can be irrigated by rare arterioles or capillaries (arrowheads). ii) Similarly, cortical bone shows a complete absence of sinusoids but is traversed by some arterioles (not shown). At the bone surface (dotted line), vasculature consists of capillaries running parallel to the long axis of the bone (arrowheads), as opposed to the central marrow where mainly sinusoids are present and radiate axially from the center of the marrow cavity. iii, iv) Growth plate and articular cartilage show a complete absence of vasculature and are located in very specific anatomical locations. b) Adipocytes and megakaryocytes can be difficult to distinguish by inexperienced researchers without specific staining. However, adipocytes are typically bigger and rounder than megakaryocytes. Also, a cytoplasmic staining in adipocytes clearly shows a lack of staining in the large lipid droplet (upper left) whereas a lipid droplet staining shows a smaller spherical staining (lower left). Cytoplasmic (upper right) or membrane (lower right) stainings in megakaryocytes shows cells with irregular shapes, in many of which we can observe multiple/complex nuclei (arrowheads).

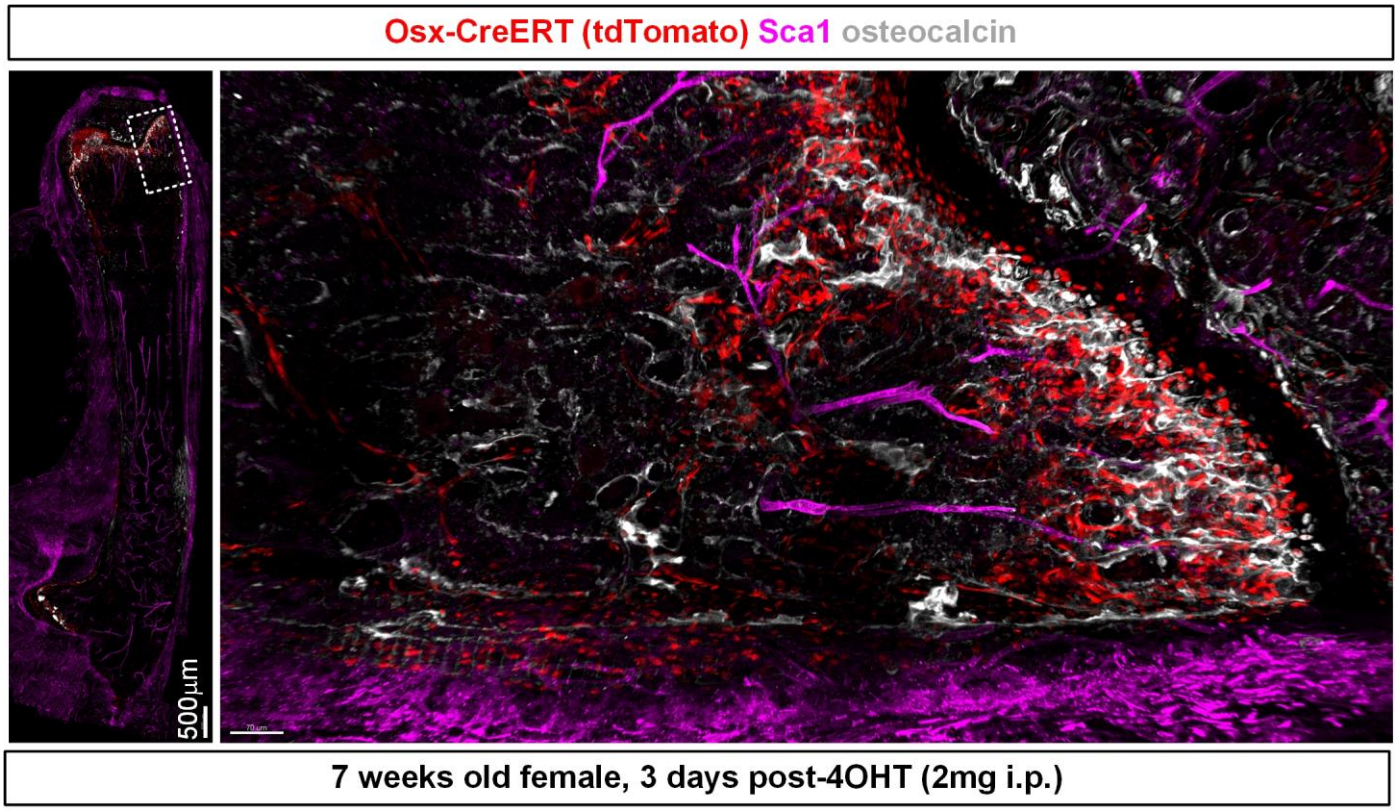


Supplementary Figure 3

Some Nes-GFP-expressing cells are $ALP^{+}CD31^{-}$ osteoblastic cells.

Images show a zoom of data presented in Figure 2b and only four optical sections (total thickness 9.96μm). Near the distal growth plate of the femur, Nes-GFP expressing cells (green, white arrowheads) are closely associated with CD31+ blood vessels (red), but are osteoblastic cells expressing ALP (grey). Scale bars: 20μm

Nature Biotechnology: doi:10.1038/nbt.4006

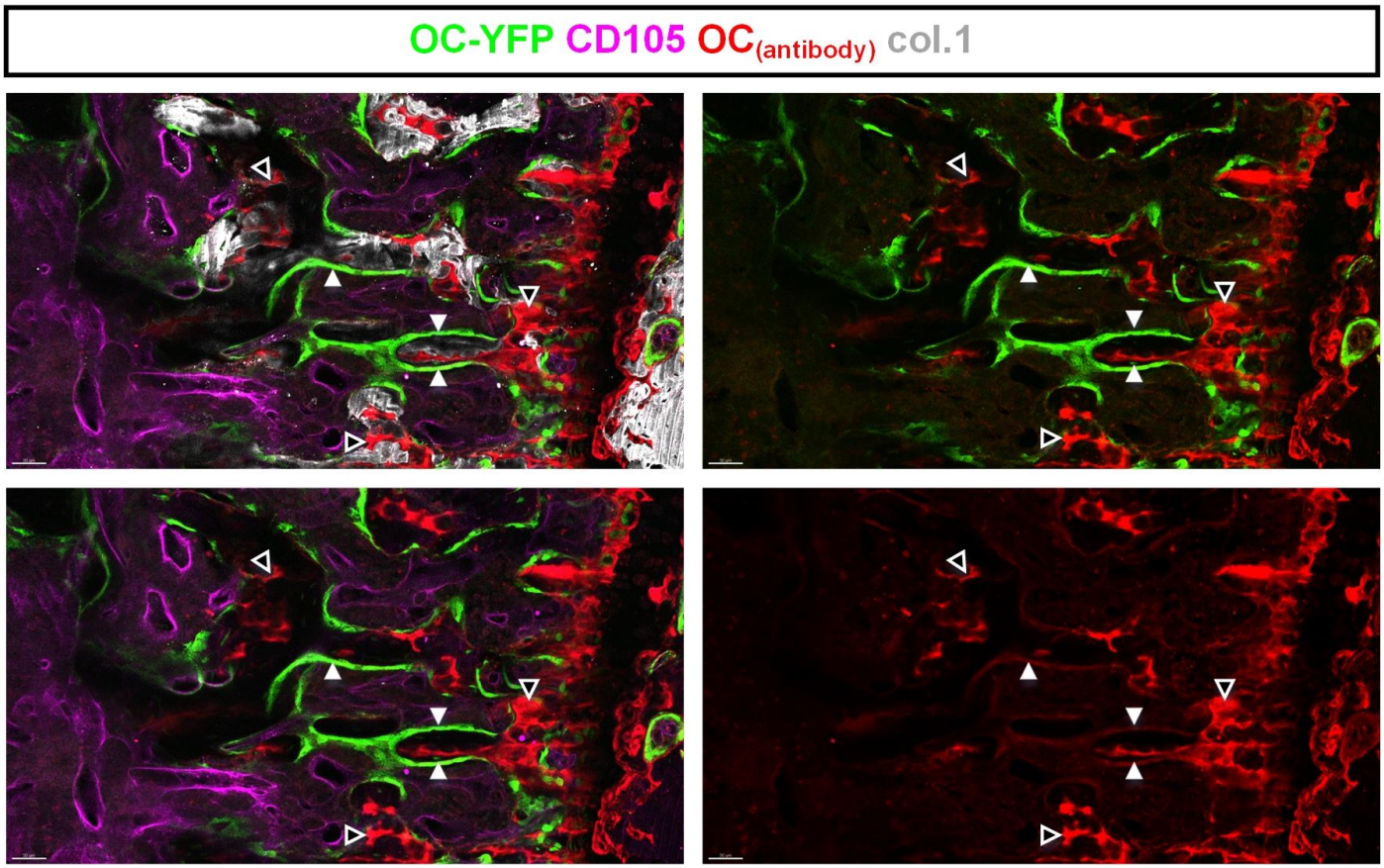


Supplementary Figure 4

Osx-CreERT (tdTomato) reporter shows a similar expression pattern to that of the Osx-GFP reporter.

7 weeks old female Osx-CreERT mice received 2mg 4-hydroxytamoxifen intraperitoneally and femurs were harvested three days later. Expression of the tdTomato reporter at day 3 post-4OHT recapitulates that of the Osx-GFP reporter (see figure 2c). Scale bar of detail 70µm.

Nature Biotechnology: doi:10.1038/nbt.4006

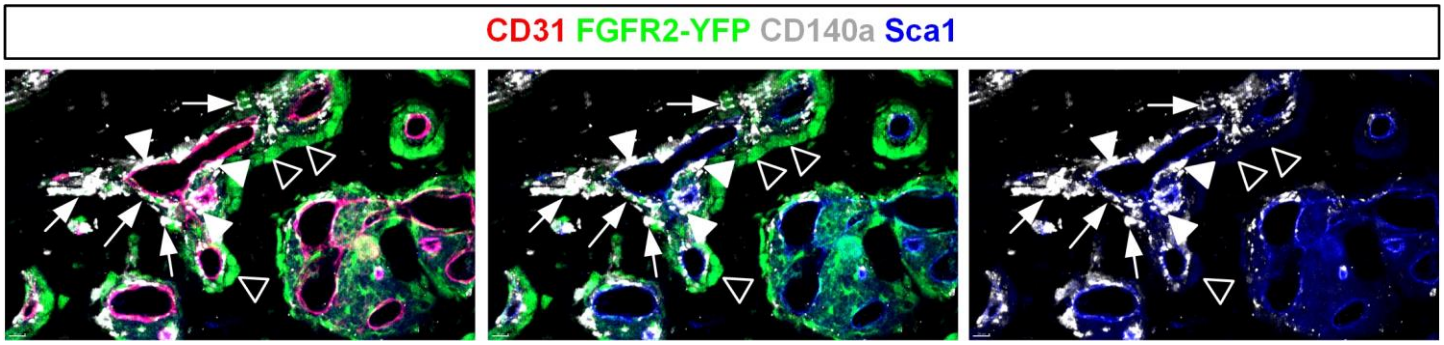


Supplementary Figure 5

Antibody staining for osteocalcin partially overlaps with OC-YFP expression.

Images show a zoom of data presented in Figure 2e and only four optical sections (total thickness 9.96μm). Near the distal growth plate of the femur, OC-YFP expressing cells (green, white arrowheads) line collagen 1+ (grey) trabecular bone surfaces and stain positive for osteocalcin antibody (red). Osteocalcin antibody also detects OC+ matrix away from YFP+ cells in trabecular bone and adjacent to the growth plate (black arrowheads). Scale bars: 30μm

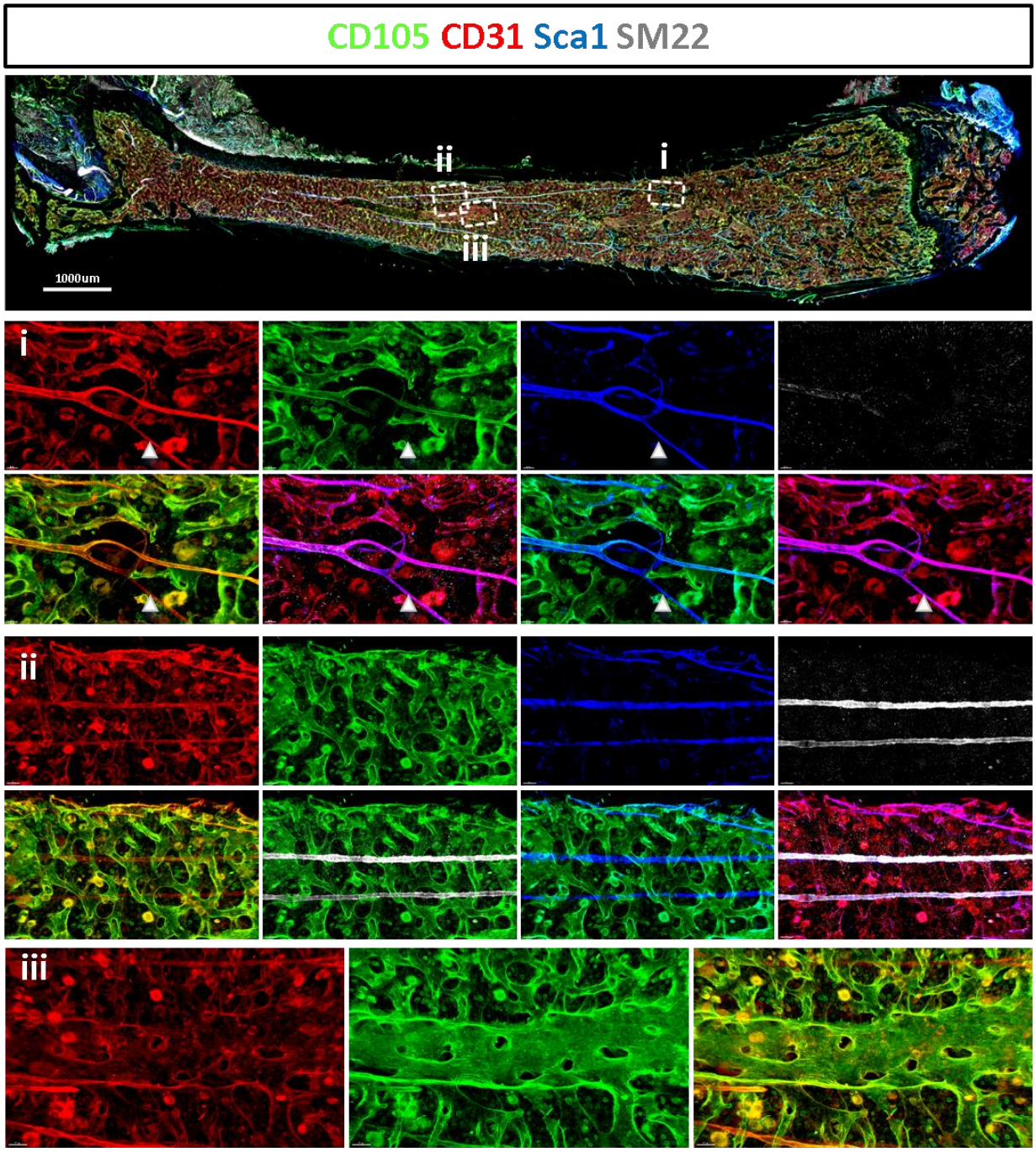
Nature Biotechnology: doi:10.1038/nbt.4006



Supplementary Figure 6

Expression of mesenchymal-progenitor-cell markers near the distal growth plate.

Images show a zoom of data presented in Figure 2f and only four optical sections (total thickness 9.96μm). We can observe CD140a+Sca1+ cells lining CD31+ blood vessels (white arrowheads), whereas bone lining cells are either FGFR2+ (black arrowheads) or FGFR2+CD140a+ (white arrows). Scale bars 20 μm.



Supplementary Figure 7

CD105 is not a panendothelial marker.

(i) Most arterioles do not express CD105, but only CD31 and Sca1 (white arrowheads) potentially marking distinct arteriolar sub-types. (ii) Diaphyseal arteries marked by SM22 expression are CD105-CD31+Sca1+. (iii) Strong CD105 expression in the endothelial wall of the central sinus.

Supplementary Table 1. Antibodies tested and optimized in this study.

Antibody	Alternative names	Clone	Species	Company	Catalog number	Working	Requires streptavidin amplification
Osteocalcin	Ocn, bone Gla protein	poly	goat	AbD serotec	7060-1815	Yes	No
CD271	p75 LNGF	poly	rabbit	Abcam	ab8874	Yes	No
c-Kit	CD117, SCF-R	poly	goat	R&D systems	AF1356	Yes	No
		ACK2	rat	eBioscience	14-1172-82	Yes	No
		poly	rabbit	Novus Biologicals	NBP1-19865	No	Not applicable
CD105	Endoglin	MJ7/18	rat	eBioscience	14-1051-82	Yes	No
		poly	goat	R&D systems	AF1320	Yes	No
		M-20	goat	Santa Cruz	sc-19793	No	Not applicable
GFAP	Glial fibrillary acidic protein	poly	rabbit	Thermo Scientific	PA1-10019	Yes	No
		poly	chicken	Aves Labs	GFAP	No	Not applicable
CD44	hyaluronic acid receptor	IM7	rat	eBioscience	14-0441-82	Yes	No
Alkaline phosphatase	ALP	poly	goat	R&D systems	AF2910	Yes	No
		EPR4477	rabbit	Novus Biologicals	NBP1-95392	Yes	Yes
Collagen 1	Col.1	poly	rabbit	Cedarlane	CL50151AP	Yes	No
CD31	PECAM1	poly	goat	R&D systems	AF3628	Yes	No
		ER-MP12	rat	Santa Cruz	sc-52713	No	Not applicable
		poly	rabbit	Novus Biologicals	NB100-2284	No	Not applicable
		SP38	rabbit	Thermo Scientific	MA5-16337	No	Not applicable
NG2	chondroitin sulfate proteoglycan	poly	rabbit	EMD Millipore	AB5320	Yes	Yes
		poly	goat	Novus Biologicals	NBP-46332	No	Not applicable
CD73	NT5E, ecto-5'-nucleotidase	ebioTy/11.8	rat	eBioscience	14-0731-82	Yes	No
		poly	rabbit	Thermo Scientific	PA5-11871	No	Not applicable
		D7F9A	rabbit	Cell Signaling Technology	13160	No	Not applicable
CD140a	PDGFRa	poly	rabbit	Novus Biologicals	NBP1-67666	Yes	Yes
		951	rabbit	Santa Cruz	sc-431	No	Not applicable
		958	rabbit	Santa Cruz	sc-432	No	Not applicable
Sca1	Ly6A/E	D7	rat	eBioscience	14-5981-85	Yes	No
		177228	rat	R&D systems	MAB1226	Yes	No
		poly	goat	R&D systems	AF1226	Yes	No
		M-86	rabbit	Santa Cruz	sc-134474	No	Not applicable
SM22	aplha smooth muscle 22	poly	rabbit	Abcam	ab14106	Yes	Yes
CD34	-	RAM34	rat	eBioscience	16-0341-85	Yes	Yes
vWF	von Willebrand Factor	poly	rabbit	Neomarkers	RB-281-A	Yes	No
Vcam1	CD116	429	rat	eBioscience	14-1061-82	Yes	No
		H-276	rabbit	SantaCruz	sc-8304	Yes	No
		429	rat	Novus Biologicals	NB100-77474	Yes	No
Endomucin	-	V.7C7	rat	Santa Cruz	SC-65495	Yes	No
CD90	Thy1	poly	sheep	R&D systems	AF2067	Yes	No
		poly	rabbit	Thermo Scientific	PA5-11917	No	Not applicable
Peripherin	neurofilament 4	poly	rabbit	Biolegend/Covance	PRB-576C	Yes	No
Tyrosine Hydroxylase	TH	poly	rabbit	EMD Millipore	AB152	Yes	No
Neurofilament	-	poly	rabbit	Thermo Scientific	PA3-16721	Yes	No
Fibronectin	-	poly	rabbit	Abcam	ab-23750-100	Yes	No
Laminin	LAMA1	poly	rabbit	Novus Biologicals	NB300-144	Yes	No
Vitronectin	-	347317	rat	R&D systems	MAB38751	Yes	No
Col.III	-	poly	goat	Abcam	ab24823	Yes	No
Col.IV	-	poly	rabbit	ABDserotec	2150-1470	No	Not applicable
		poly	rabbit	Abcam	ab19808	Yes	No
Osteopontin	Opn	poly	goat	R&D systems	AF808	Yes	No
	-	poly	rabbit	Abcam	ab-63856-100	No	Not applicable
Perlecan	Endorepellin, Heparan Sulfate Proteoglycan	A7L6	rat	Novus Biologicals	NB600-583	Yes	No
Periostin	osteoblast-specific factor 2	poly	goat	R&D systems	AF2955	Yes	No
LepR	Leptin receptor, CD295	poly	goat	R&D systems	AF497	Yes	No
		poly	rabbit	Life Span	LS-C385018	No	Not applicable
		poly	rabbit	Bioss	bs-0109R-A488	No	Not applicable
alpha-smooth muscle actin	aSMA	poly	rabbit	Abcam	ab5694	No	Not applicable
beta-III tubulin	-	poly	chicken	Aves Labs	TUJ	No	Not applicable
		poly	rabbit	Abcam	ab18207	No	Not applicable
		poly	rabbit	Thermo Scientific	PA1-46430	No	Not applicable
VE cadherin	CD144	eBioBV13	rat	eBioscience	14-1441-82	No	Not applicable
GFP	-	poly	chicken	Aves Labs	GFP-1020	Yes	No
nestin	-	poly	chicken	Aves Labs	NES	No	Not applicable
		poly G-20	goat	Santa Cruz	sc-21248	No	Not applicable
		poly	rabbit	LifeSpan BioSciences	LS-B656	No	Not applicable
S100 beta	-	poly	rabbit	Abcam	ab14688	No	Not applicable
Doublecortin	-	poly	rabbit	Abcam	ab18723	No	Not applicable
MECA-32	Panendothelial Cell Antigen	MECA-32	rat	Biolegend	120501	No	Not applicable

Supplementary Table 2. Details of the full bone scans available to the community.

Figure & panel	Markers	.lif file size (KB)	.ims file size (KB)	Total samples analyzed for each marker
Fig.2a	OC-YFP	72 423 340	7 861 110	26
	CD44			3
	ALP			92
	Col.1			56
Fig.2c	Osx-GFP	60 397 117	4 884 944	14
	ALP			92
	CD105			163
	Col.1			56
Fig.2d	FGFR2-YFP	46 239 342	10 970 778	19
	NG2			11
	CD73			12
	CD31			218
Fig.2e	Col.1	61 955 683	9 560 231	56
	OC-YFP			26
	Oc(antibody)			48
	CD105*			163
Fig.2f	FGFR2-YFP	18 967 505	1 487 583	19
	CD31			218
	CD140a			10
	Sca1			192
Fig.3a	CD105	78 325 924	6 331 948	163
	Sca1			192
	CD31			218
	SM22			27
Fig.3b	VCAM1	80 348 571	4 578 352	9
	CD31			218
	FGFR2-YFP*			19
	Laminin*			98
Fig.3c	Endomucin	59 522 632	17 348 505	16
	CD31			218
	Col.IV*			4
	Cxcl12-GFP*			53
Fig.3d	CD34	46 191 630	12 657 026	10
	CD31			218
	Col.1			56
Fig.3e	vWF	43 040 065	6 557 380	16
	Sca1			192
	CD31			218
Fig.3f	Nes-GFP	60 377 292	9 723 916	39
	Sca1			192
	CD90			22
	SM22**			27
Fig.4a	GFAP	80 808 235	15 239 660	107
	Sca1			192
	CD31			218
	TH			10
Fig.4c	NF	29 053 651	6 033 094	4
	Sca1			192
	CD31			218
Fig.5a	OC	35 748 863	2 749 095	48
	Fibronectin			11
Fig.5b	Laminin	54 379 213	9 507 438	98
	CD105			163
	OC*			48
Fig.5c	Cxcl12-GFP*	42 284 586	8 914 924	53
	Vitronectin			8
	Col.III			4
	Fn			11
Fig.5d	Col.IV	59 522 632	17 348 505	4
	CD31			218
	Cxcl12-GFP*			53
	Endomucin*			16
Fig.5e	Cxcl12-GFP	44 026 791	11 079 527	53
	CD105			163
	Col.1			56
	OPN			9
Fig.5f	Perlecan	38 946 822	8 446 520	3
	Periostin			3
	Col.I*			56
Fig.6a	Nes-GFP	64 783 309	7 197 368	39
	CD271			35
	ALP			92
	VCAM1**			9
Fig.6b	FGFR2-YFP	80 348 571	4 579 352	19
	CD31			218
	Laminin*			98
	VCAM1*			9
Fig.6c	BODIPY	55 136 504	20 341 492	4
	CD31			218
	B220*			77
	DAPI*			-
Fig.6d	Cxcl12-GFP	50 798 977	13 231 698	53
	LepR			20
	Col.1			56
	CD105			163

*: Not shown in article; **: immunostaining not working

Supplementary Table 3. Location of stromal cell markers in adult mouse femurs and expression in cellular subtypes.

Marker	Epiphysis	Metaphysis	Diaphysis	Stroma	Osteoblasts/ osteocytes	Periarteriolar	Perisinusoidal	Endothelium	Adipocytes
CD271	Low	Low	High	Yes	No	No	No	No	No
FGFR2	Low	High	Low	Yes	Yes	No	Yes	No	Yes
Bopidy	High	High	Low	No	No	No	No	No	Yes
Cxcl12-GFP	High	High	High	Yes	No	Yes	Yes	No	No
LepR	High	High	High	Yes	No	No	Yes	Yes	No
ALP	Low	High	Low	Yes	Osteoblasts	No	No	No	No
Nestin-GFP	Low	High	Medium	No	Osteoblasts	Yes, enriched in metaphysis	No	Yes	No
CD105	High	High	High	No	No	No	No	Sinusoids only	No
CD73	Low	Low	No	No	Chondrocytes	No	No	No	No
Osteocalcin-YFP	Low	High	Low	No	Osteoblasts	No	No	No	No
CD140a	Low	Medium	Low	No	Osteoblasts	Yes, enriched in metaphysis	No	No	No
Sca1	Low	Medium	High	No	Putative pre-osteoblasts	Yes, throughout bone marrow	No	Low	No
CD90	Low	High	Medium	No	Putative pre-osteoblasts	Yes, enriched in metaphysis	No	No	No
Osterix-GFP	Low	High	Low	No	Osteoblasts	No	No	No	No