Table S1. Sequence of staining with antibodies and dyes

Table S2. List of primary antibodies

Table S3. List of secondary antibodies and dyes

Table S4. Patient characteristics

Table S5. Comparison of cell counts from 3D images and flow cytometry analysis

Figure S1. Visualization of normal human BM
BM wholemount specimens from human healthy donors were fluorescently labeled with various combinations of two lineage-specific antibodies and DAPI (nuclei). Each confocal image is shown with a higher magnification view in the inset. (A) $CD3^+$ T cells (red) in lower number than $CD20^+$ B cells (green) appear scattered in the tissue. (B) $CD33^+$ myeloid cells (red) in high number are dispersed together with $CD20^+$ B cells. (C) Single, large megakaryocytes (green) are surrounded by numerous small platelets (green), both expressing CD41. (D) GPA^+ erythrocytes and erythroid precursors (green) form small clusters dispersed throughout the BM. (E) $CD34^+$ cells lined blood vessels (green) but also appear singly, scattered in the tissue, some co-expressing CD20 (yellow). Scale bars in μm.

Figure S2. Autofluorescence and control experiments
BM tissue exhibits low autofluorescence at 488-nm excitation (A) or 361-nm (B) respectively. The visible signal comes from the outer bone structure; becomes detectable only under much stronger laser illumination and higher voltage-values applied to the imaging detectors compared to instrument settings used for imaging fluorescently-stained samples. Isotype control image (C) with the corresponding DAPI image (D) was captured at instrument settings identical to those used for the specific stained sample.

Figure S3. Bone structures visualized in brightfield images
Panels (A–D) illustrate brightfield images of the mouse (A–B) and human (C–D) tissue; to aid visualization of the bone, the hematopoietic tissue was outlined and bone structures identified by asterisks (*). Panels E and F from fluorescently labeled samples have bone area demarcated by hashed lines.

Table S1. Sequence of staining with antibodies and dyes

Fig No.		1st Step	2nd Step	3rd Step	4th Step
2	B/C	perlecan + DAR-FITC	APC-CD45R	DAPI	
	D/E	perlecan + DAR-FITC	APC-CD45R	DAPI	
3	A/B/C	CollagenIV+DARb-FITC	DAPI		
	E	CollagenIV+DARb-FITC	Biotin-CD34 + Str-Rhodamine RedX	DAPI	
4	A	CD3e + GAAH-FITC	APC-CD45R		
	B	CD45R + DAR-FITC	APC-CD11b		
	C	CD41 + DAR-FITC	APC-CD45R		
	D	TER-119 + DAR-FITC	APC-CD45R		
	E	perlecan + DAR-FITC	APC-CD45R		
	F	Biotin-cKit + Str-Rhodamine RedX	FITC-Sca1		
5	A	Biotin-perlecan + Str-APC	FITC-CD8a	DAPI	
	C	Biotin-perlecan + Str-APC	DAPI	BODIPY	
6	A	perlecan + DAR-Rhodamine RedX	Hoechst33342		
	C	perlecan + DAR-Rhodamine RedX	Hoechst33342		
7	A	CD146 + DAM-Rhodamine RedX	Biotin-CD34 + Str-APC	DAPI	BODIPY
	B	CD146 + DAM-Rhodamine RedX	DAPI		
	C	CD34 + DAM-Rhodamine RedX	FITC-CD33	DAPI	
	D	CD33 + DAM-FITC	Biotin-CD34 + Str-Rhodamine RedX	DAPI	
	E	CD20 + DAM-FITC	APC-CD38	DAPI	
	F	CD3 + DAM-Rhodamine RedX	Biotin-CD8 + Str-APC	FITC-CD20	DAPI
8	A/C	CD146 + DAM-Rhodamine RedX	Biotin-CD34 + Str-APC	DAPI	BODIPY

"+", indirectly conjugated; "-", directly conjugated; "DAR", donkey anti-rat; "DAM", donkey anti-mouse "DARb", Donkey anti-rabbit; "GAAH", goat anti-Armenian hamster; "Str", streptavidin.

Table S2. List of primary antibodies

Antigen	Type	Clone	Description	Manufacture
Human				
CD3	MM	HIT3a	T cells, thymocytes	BD Pharmingen
CD5	MM	4C7	Thymocytes, mature T cells, subset of B cells	abcam
CD8a	MM	HIT8a	Subpopulation of mature T cells, NK cells, thymocytes	eBiosciences
CD20	MM	2H7	Developing B cells, mature B cells (not plasma cells)	eBiosciences
CD33	MM	CLB-MD33.6	Myeloid cells, macrophage precursors	abcam Inc
CD34	MM	QBEnd-10	Hematopoietic projenitors, endothelial cells	Beckman Coulter
CD36	MM	FA6-152	Collagen, phospholipids, erythroid cells	abcam
CD38	MM	HIT2	Thymocytes, activated T cells, terminally differenciated B-cells (plasma cells)	BD Pharmingen
CD41	MM	HIP8	Megakaryocytes, platelets	eBiosciences
CD146	MM	NCL-146	Stromal cells, fibroblasts, endothelial cells	Leica Microsystems
GPA	RM	YTH89.1	Erythroid cells	Santa Cruz
Mouse				
CD3e	AHM	145-2C11	Mature T cells, thymocytes, NK cells	BD Pharmingen
CD4	RM	RM4-5	Subpopulation of mature T cells, thymocytes, subset of NK cells	BD Pharmingen
CD8a	RM	53-6.7	Subpopulation of mature T cells, thymocytes	eBiosciences
CD11b	RM	M1/70	granulocytes, macrophages, NK cells, activated lymphocytes	eBiosciences
CD16/32	RM	2.4G2	NK cells, monocytes, macrophages, dendritic cells, Kupffer cells, granulocytes, mast cells, B cells, immature thymocytes, activated mature T cells	BD Pharmingen
CD34	RM	RAM34	Capillary endothelial cells, bone marrow stroma, a small subpopulation of mouse bone marrow cells	eBiosciences
CD41	RM	MWReg30	Megakaryocytes, platelets	abcam
CD45R/B220	RM	RA3-6B2	B-cells, NK cell precursors	BD Pharmingen
Sca1	RM	E13-161.7	Hematopoietic progenitors, B cells, myeloid cells	BD Pharmingen
cKit	RM	2B8	Hematopoietic progenitors, mast cells	eBiosciences
Gr-1	RM	RB6-8C5	Granulocytes, monocytes	BD Pharmingen
Ter-119	RM	Ter-119	Erythroid cells	BD Pharmingen
HSP2/perlecan	RM	A7L6	Basement membranes, endothelial cells	abcam
Collagen IV	RbP	polyclonal	Basement membranes	AbD Serotec

MM, mouse monoclonal; RM, rat monoclonal; AHM, Armenian hamster monoclonal; RbP, rabbit polyclonal.

Table S3. List of secondary antibodies and dyes

Species	Type	Fluorescence	Description	Manufacture
DAM	F(ab')2 fragment	FITC		Jackson ImmunoResearch Laboratories
DAM	F(ab')2 fragment	Rhodamine Red-X		Jackson ImmunoResearch Laboratories
DAM	F(ab')2 fragment	Cy5		Jackson ImmunoResearch Laboratories
DAR	F(ab')2 fragment	FITC		Jackson ImmunoResearch Laboratories
DAR	F(ab')2 fragment	Rhodamine Red-X		Jackson ImmunoResearch Laboratories
DAR	F(ab')2 fragment	Cy5		Jackson ImmunoResearch Laboratories
DARb	F(ab')2 fragment	FITC		Jackson ImmunoResearch Laboratories
GAAH	F(ab')2 fragment	FITC		Jackson ImmunoResearch Laboratories
	Streptavidin	DTAF		Jackson ImmunoResearch Laboratories
	Streptavidin	Rhodamine Red-X		Jackson ImmunoResearch Laboratories
	Streptavidin	APC		Jackson ImmunoResearch Laboratories
		DAPI	Nuclei	Invitrogen Corporation, Carlsbad, CA
		Hoechst33342	Nuclei	Invitrogen Corporation, Carlsbad, CA
		BODIPY	Lipid	Invitrogen Corporation, Carlsbad, CA

DAM, donkey anti-mouse IgG; DAR, donkey anti-rat IgG; DARb, donkey anti-rabbit IgG; GAAH, goat anti-Armenian hamster IgG.

Table S4. Patient characteristics

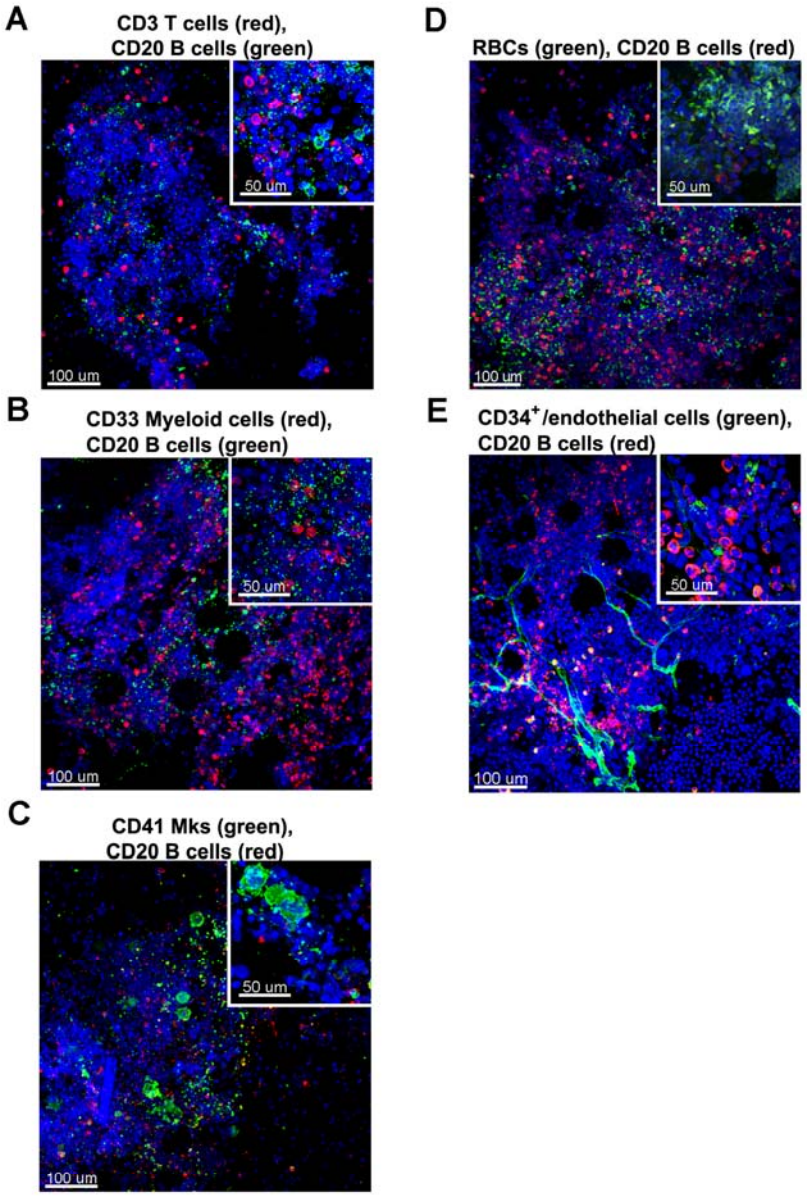
Fig No.	Diagnosis	Age	Sex	Status	Pathological Reading
7 A/B	Severe aplastic anemia (SAA)	24	M	Pre-treatment	Markedly hypocellular bone marrow with severe trilineage hypoplasia.
7C	Acute myeloid leukemia (AML)	75	M	Pre-treatment	CD34 stain highlights frequent CD34-positive cells approximately 20-25% of marrow cells.
7 D	Chronic myeloid leukemia blast crisis (CML-BC)	48	F	Post-treatment	Marrow heavily infiltrated with pleomorphic myeloid blasts.
7 E	Multiple myeloma (MM)	43	F	Pre-treatment	There are numerous clusters of mature plasma cells present throughout the marrow. The plasma cells comprise approximately 30% of marrow cells.
7 F	T-cell Large granular lymphocyte leukemia (T-LGL)	82	M	Pre-treatment	There are modelate numbers of CD3 positive cells without abnormal collections and similar numbers of CD8 positive cells are present. There were only rare CD20 positive
8 A	Severe aplastic anemia (SAA)	67	F	Post-treatment	The sample varied focally from 5-50% in cellularity with overall cellularity 20%.
8 D	Severe aplastic anemia (SAA)	28	M	Post-treatment	Marrow of variable cellularity with decreased megakaryocytes.

Table S5. Comparison of cell counts from 3D images and flow cytometry analysis

Fig No.		Antigen	number from 3D image	% from image	% by FACS
4A		CD3	73	2.5	2.6
		CD45R	685	23.7	22.4
		nuclei	2896		
4B		CD11b	924	26.6	21.9
		nuclei	3743		
4C		CD41	84	2.1	N/A
		nuclei	4080		
4D		Ter119	330	31.3	23.5
		nuclei	1055		
4F		cKit+Sca1	1	0.1	0.1
		nuclei	1019		
5A	day 7	CD8	49	3.1	2.6
		nuclei	1595		
5A	day 10	CD8	620	11	8
		nuclei	5627		
5A	day 17	CD8	1228	24.8	25.2
		nuclei	4946		
6A	day 1	GFP+	95	4.6	5.5
		nuclei	2058		
6A	day 7	GFP+	1437	37.3	38.6
		nuclei	3849		
6A	day 28	GFP+	3537	65.8	72.4
		nuclei	5378		
6C	day 7	GFP+	46	4.3	5.5
		nuclei	1078		
6C	day 14	GFP+	169	17.1	18.1
		nuclei	989		
6C	day 28	GFP+	1240	47.2	48
		nuclei	2624		
S1A		CD3	170	12.6	35.7
		CD20	27	2	1.8
		nuclei	1354		
S1B		CD33	345	18	6.7
		nuclei	1915		
S1E		CD34	17	0.8	1.3
		nuclei	2039		

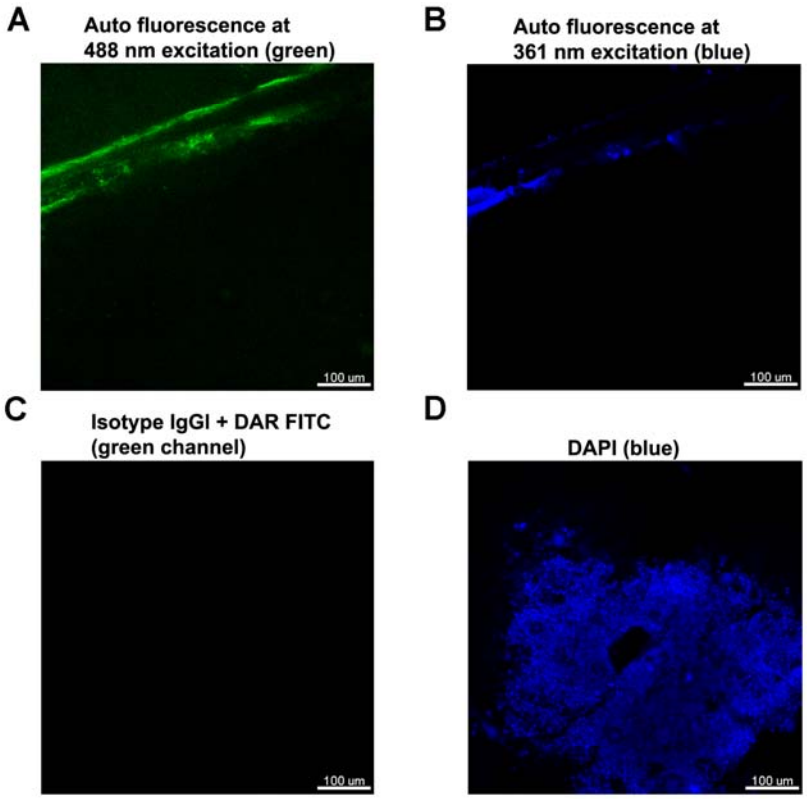
"FACS", Flow cytometry.

Takaku et al., Figure S1



Normal human

Takaku et al., Figure S2



Takaku et al., Figure S3