Supplementary Data

Additional Methods

Flow cytometry analyses and cell sorting

Except for floating cells, all cells prepared for flow cytometry analysis or sorting were harvested using enzymatic treatment (as described for EB and attached cultures). All cells were extensively washed, filtered through 70 μm Nitex, and suspended in Staining Media (SM) containing 2% Newborn Calf Serum, 1X HBSS, and 1mM Hepes. Prior to antibody staining, cell pellets were blocked with a solution of 0.1 mg/ml mouse IgG (Sigma -I08765) for 10 min and then stained at 1 million cells per 100 μl antibody cocktail. The volume of antibody was increased proportionally to cell number. Monoclonal antibodies directly conjugated to fluorochromes were used in cocktails with up to 6 antibodies. All antibodies were purchased from eBioscience and BioLegend and titrated for optimal dilutions prior to use. The specificities of each antibody can be found in Table S1. All stainings were done on ice in the dark for 45 mins. Cells were then washed with 3 mls of SM and finally resuspended in SM containing 1 μg/ml of Propidium Iodide (PI). Flow cytometric analysis and sorting was done using a BD-FACS ARIA II (BD Biosciences; San Jose, CA, USA) equipped with 5 lasers, 18 fluorescence detectors and FACS DIVA software. Voltages were set with unstained sample and compensations were established with positive signals for each channel. Gating strategies for monocyte/osteoclast progenitors derived from hESCs (Figure S1) and for human PB (Figure S3) can be found in the supplemental material. Cell sorting was done using a 100 μM nozzle and sorted into SM supplemented with 20% FBS. All sorts were reanalyzed for purity greater than 95% or they were not used in experiments. For experiments requiring very small populations or more than 4 populations to be sorted, cells were sorted multiple times. FACS data was analyzed using BD CellQuest Pro™ Software.

Myeloid gene expression

RNA was extracted using the RNAqueous®-4PCR Kit (Ambion®; Foster City, CA, USA) per manufacturer's instructions for the following cell types: Undiffernetiated hESCs, EBs prior to

cytokine induction, EBs with 22 days of hematopoietic induction with bmp4, GCSF, SCF, FLT3L, IL-3, IL-6, myeloid progenitors cultured for 15 days in SCF, FL3L and MCSF and multinuclear osteoclasts cultured for 15 days in SCF, FL3L and MCSF with addition of RANKL the last 6 days. EBs and hESCs grown on Matrigel™ were first enzymatically digested with trypsin and then washed. Myeloid progenitors required trituration to harvest and multinuclear osteoclasts were lysed directly on the TC plate. Prior to reverse transcription, RNA was DNAse I treated, quantified and evaluated for purity using nanodrop 260/280 absorbances. Next, 1 ug of RNA was reverse transcribed into cDNA using SuperScript III (Invitrogen™ 18080; Life Technologies, Grand Island, NY, USA) per manufacturer's guidelines. QPCR using Taq®Man Universal PCR Master Mix (Applied Biosystems, ABI®; Foster City, CA, USA) along with TaqMan® Gene Expression Assays for the following genes: *ACP5, CSF1R, CTSK, MMP9, OSCAR,* and *TNFR11A* (details of assays can be found in Table S2) was performed in triplicate on an ABI® 7900-HT Fast Real-Time System. Samples were normalized to the endogenous control *GAPDH* and then relative gene expression was calculated based on undifferentiated hESCs using the software provided.

Table S1: Antibodies used to define cell populations

Antibody	Clone	Company
CD11b APC	ICRF44	BioLegend
CD11b PE/Cy7	ICRF44	BioLegend
CD11c PerCP/Cy5.5	Bu15	BioLegend
CD11c Alexa Fluor®700	3.9	eBioscience
CD14 FITC	61D3	eBioscience
CD14 PE	61D3	eBioscience
CD14 eFluor®450	61D3	eBioscience
CD14 APC eFluor®780	61D3	eBioscience
CD15 FITC	HI98	eBioscience
CD16 eFluor®450	CB16	eBioscience
CD19 APCe780	HIB19	eBioscience
CD19 Alexa Fluor®700	HIB19	eBioscience
CD34 FITC	581	BioLegend
CD34 Pacific Blue®	581	BioLegend
CD40 FITC	5C3	eBioscience
CD45 FITC	2D1	eBioscience
CD45 eFluor®450	HI30	eBioscience
CD45 APC eFluor®780	HI30	eBioscience
CD56 Alexa Fluor®700	HCD56	BioLegend
CD80 PE	2D10	eBioscience
CD83 APC	HB15e	eBioscience
CD86 PE/Cy7	IT2.2	eBioscience
CD115 PE	9-4D2-1E4	BioLegend
CD115 PE	12-3A3-1B10	eBioscience
CD169 PE	7-239	eBioscience
CD209 PE/Cy7	H209	eBioscience
CD235ab APC	HIR2	BioLegend
CX3CR1 APC	2A9-1	BioLegend
CX3CR1 FITC	2A9-1	BioLegend
HLA-DR eFluor®450	L243	eBioscience

Table S2: Genes considered for defining of osteoclast lineage progression

Gene Symbol	Assay ID	Gene name
ACP5	Hs00356261_m1	Acid phosphatase 5, tartrate resistant (TRAP)
CSF1R	Hs00911250_m1	Colony stimulating factor 1 receptor (C-FMS)
CTSK	Hs00166156_m1	Cathepsin K (CATK)
GAPDH	Hs99999905_m1	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
MMP9	Hs00234579_m1	Matrix metallopeptidase 9 (MMP9)
OSCAR	Hs01100185_m1	Osteoclast associated immunoglobulin-like receptor (OSCAR)
RN18S1	Hs03928985_g1	RNA, 18 S ribosomal 1 (R18S)
TNFRSF11A	Hs00187192_m1	Tumor necrosis factor superfamily, member 11a (RANK)

Supplemental Figure 1

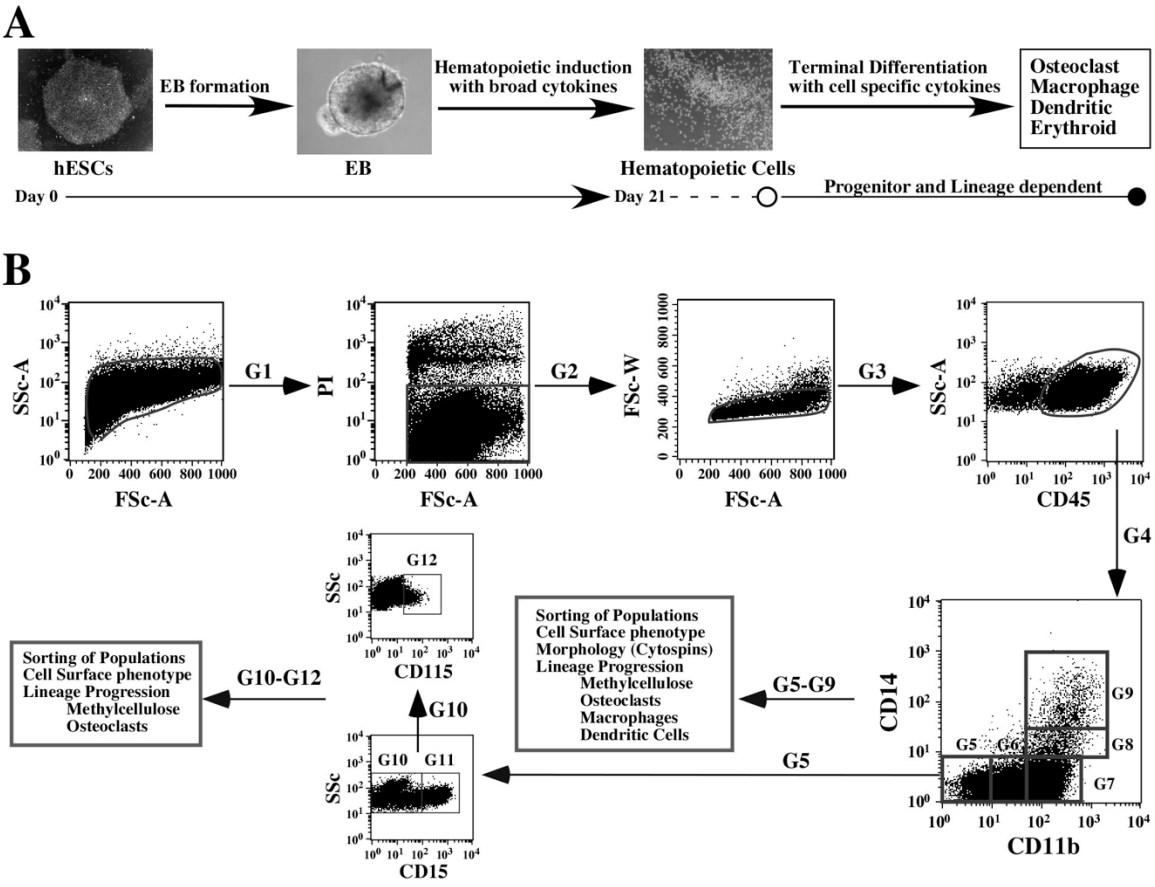

Figure S1. Generation of monocyte progenitors from hESCs. A) Undifferentiated hESC colonies were grown until 90% confluent and then enzymatically digested to recover multicellular aggregates. Aggregates were placed in low attachment flasks and allowed to form EBs overnight. A differentiation media containing six hematopoietic inducing cytokines (bmp4, GCSF, SCF, FLT3L, IL-3, IL-6) was added the following day and cultures were maintained under these conditions until at least day 21 when the emergence of large amounts of $CD45^+$ cells were visible. Cultures were either continued in order to increase numbers of hematopoietic progenitors or they were sorted and placed into lineage specific inducing conditions MCSF for macrophages, MCSF and RANKL for osteoclasts, GMCSF and IL4 for dendritic cells, or myeloid-erythroid methylcellulose for CFU experiments. **B)** Flow cytometry gating strategy to purify hematopoietic cells with myeloid potential included exclusion of dead cells with

Propidium Iodide (PI) staining, doublets (FSc-W and FSC-A), and non-hematopoietic components by gating for only $CD45^+$ cells. Cells within these gates were used to dissect myeloid populations using the myeloid markers CD14 and CD11b which were analyzed in the context of other markers, their ability to progress to terminally differentiated myeloid cells and for their progenitor activity. Finer dissections within the identified progenitor population were analyzed in the same fashion using CD15 and CD115 antibodies.

Supplemental Figure 2

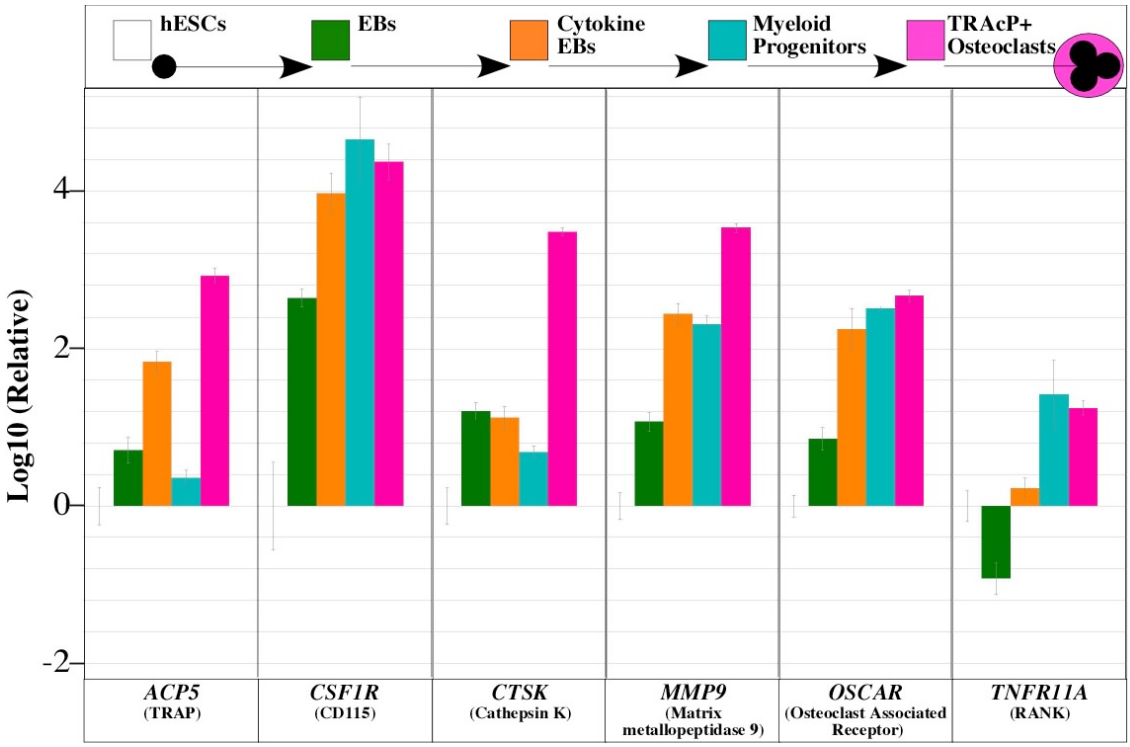

Figure S2. Upregulation of myeloid and osteoclastic gene expression. Relative gene expression of myeloid and osteoclast specific genes compared to undifferentiated hESCs throughout the different stages of commitment towards osteoclast generation. Osteoclast specific transcripts TRAP, CatK, and MMP9 are preferentially expressed in the mature osteoclast fraction.

EBs represent bulk culture prior to cytokine addition; ***Cytokine EBs*** represent cultures for 22 days in Cyto6 cocktail (see methods); Dissociated Day 22 Cytokine EBs were then cultured for 15 days in SCF, FL3L and MCSF representing ***Myeloid progenitors*** or cultured for 15 days in SCF, FL3L and MCSF with addition of RANKL the last 6 days to represent multinuclear ***Osteoclasts***.

Supplemental Figure 3

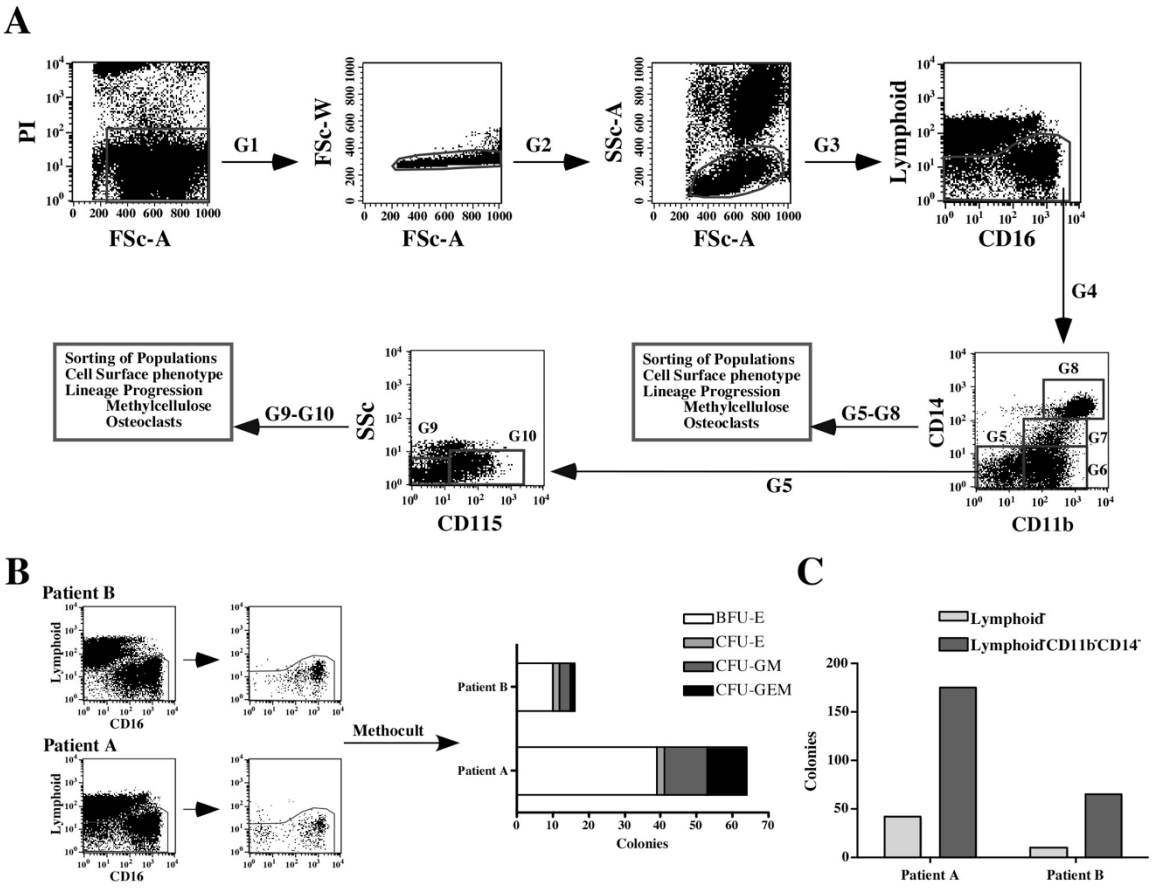

Figure S3. Purification of human peripheral blood monocyte progenitors by flow cytometry. A) Human peripheral blood (PB) mononuclear cells were obtained after dextran gradient and RBC lysis. Gating strategy to enrich for PB monocytes includes removal of dead cells with Propidium Iodide (PI) staining, doublets (FSc-W and FSC-A), granulocytes (SSc-A high) and lymphoid cells (using CD3, CD19, and CD56 antibodies). Cells within these gates were used to dissect myeloid populations using the myeloid markers CD14 and CD11b which were analyzed in the context of other markers, their ability to progress to terminally differentiated myeloid cells and for their progenitor activity. Finer dissections within the identified progenitor population were analyzed in the same fashion using SSc and CD115

antibodies. **B) and C)** Lymphoid negative cells, from two healthy representative individuals, were sorted and plated at equal numbers into methylcellulose and colonies enumerated for BFU-E, CFU-E, CFU-GM and CFU-GEM. **C)** The progenitor activity within the lymphoid negative compartment was further enriched by sorting for CD11bCD14 DN cells. Equal numbers of cells were plated for each population into methylcellulose and total colonies enumerated at day 10 of culture.

BFU-E, burst-forming unit-erythroid; CFU- colony forming unit; E, erythrocyte; G, granulocyte; GEM, granulocyte, erythrocyte, and monocyte; GM, granulocyte and monocyte; and M, monocyte.