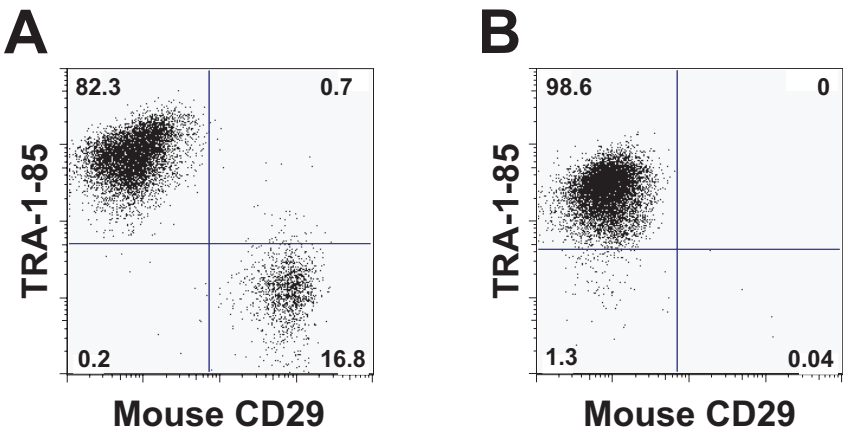
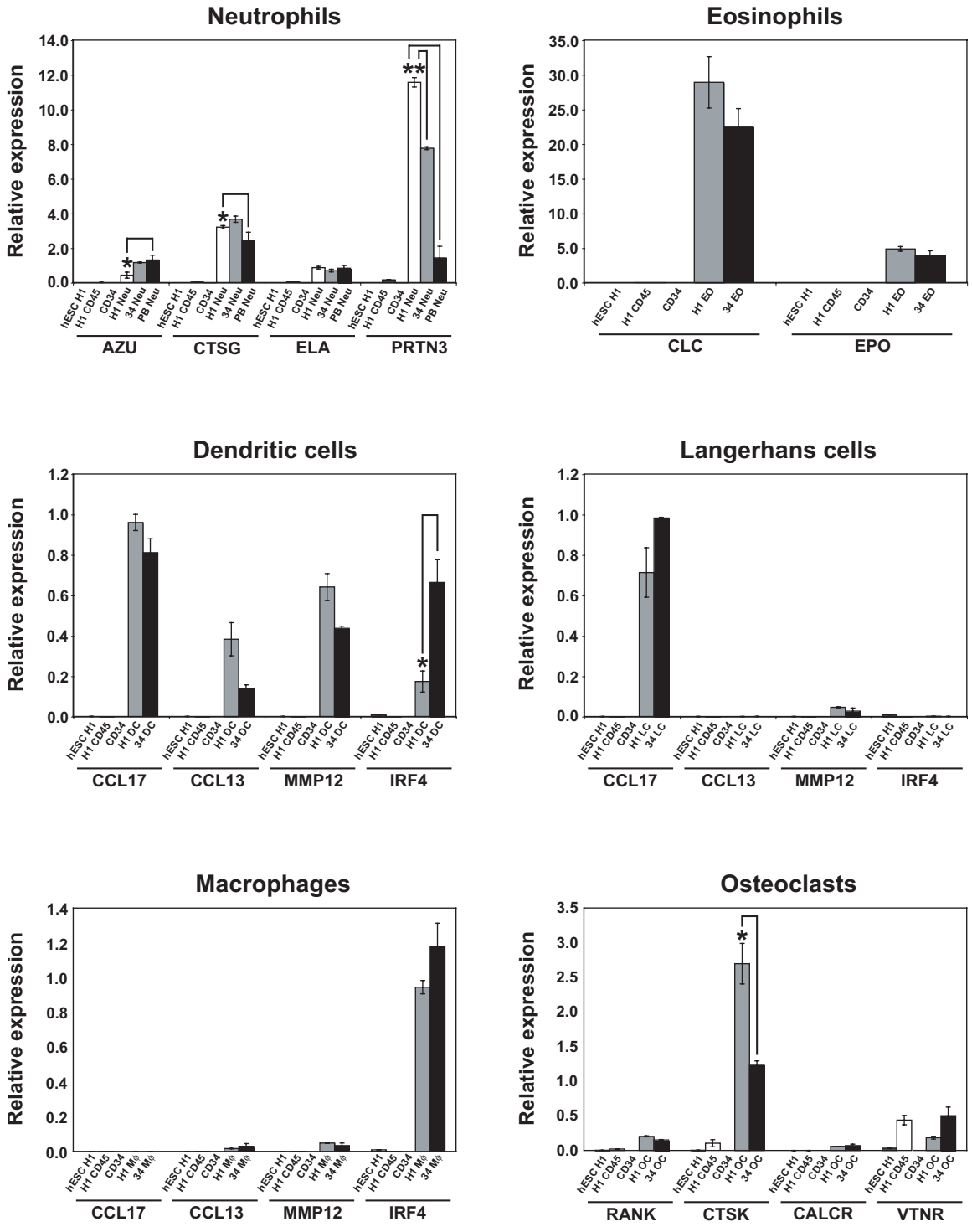
Supplemental Materials

Supplemental Figure 1



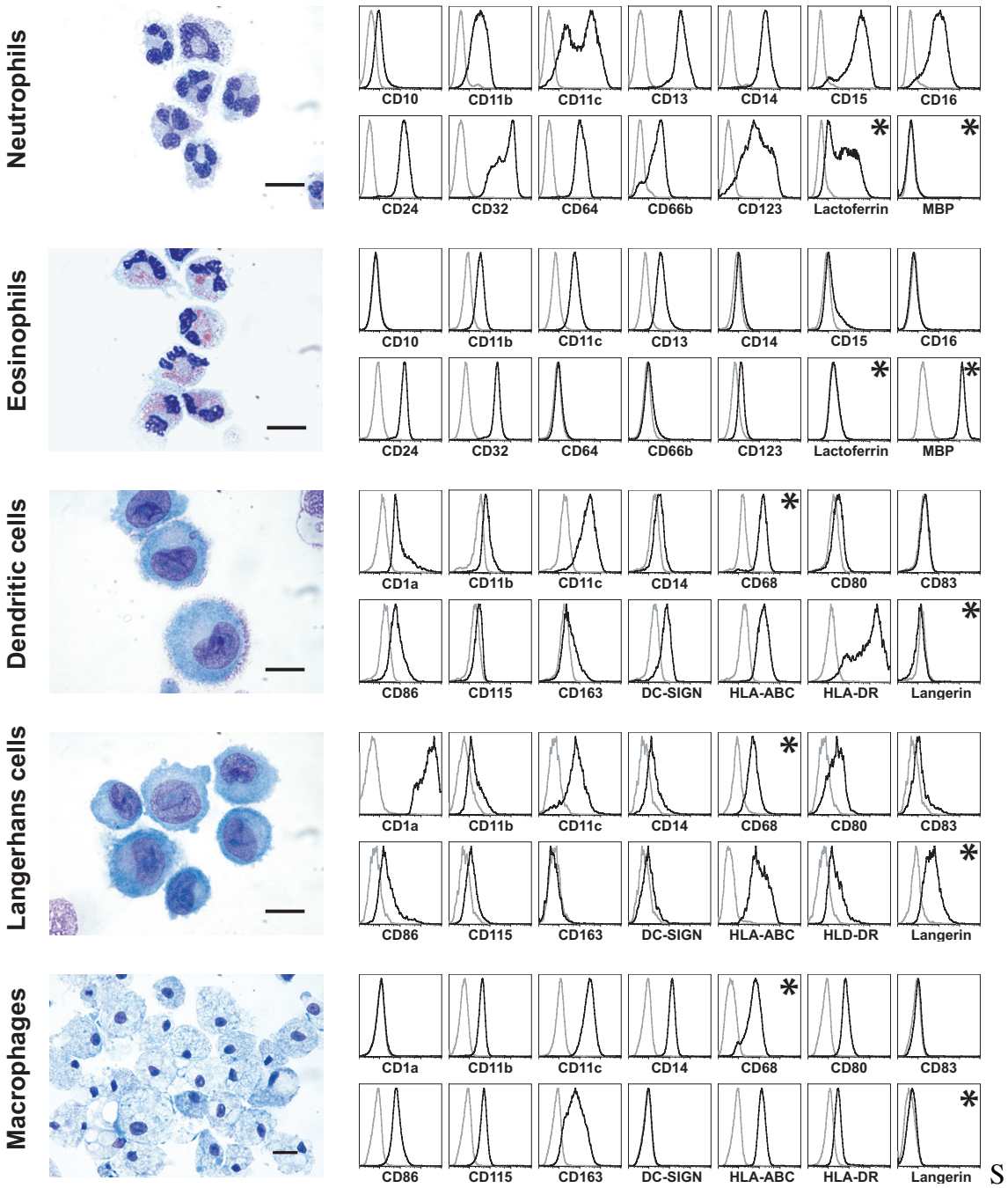
Supplemental Figure 1.
Evaluation of content of human and mouse cells in day 9 hESC/OP9 cocultures (A) and Percoll-isolated cells collected after expansion of coculuture cells with GM-CSF for two days (B). Human cells were identified using TRA-1-85-APC monoclonal antibody (R&D Systems Inc., Minneapolis, MN) which detects the OK, a blood group antigen expressed by virtually all human cells (Williams BP, Daniels GL, Pym B et al. Biochemical and genetic analysis of the OKa blood group antigen. Immunogenetics 1988;27: 322–329). Mouse stromal cells were detected with mouse-specific CD29 antibodies (Serotec, Oxford, UK).

Supplemental Figure 2



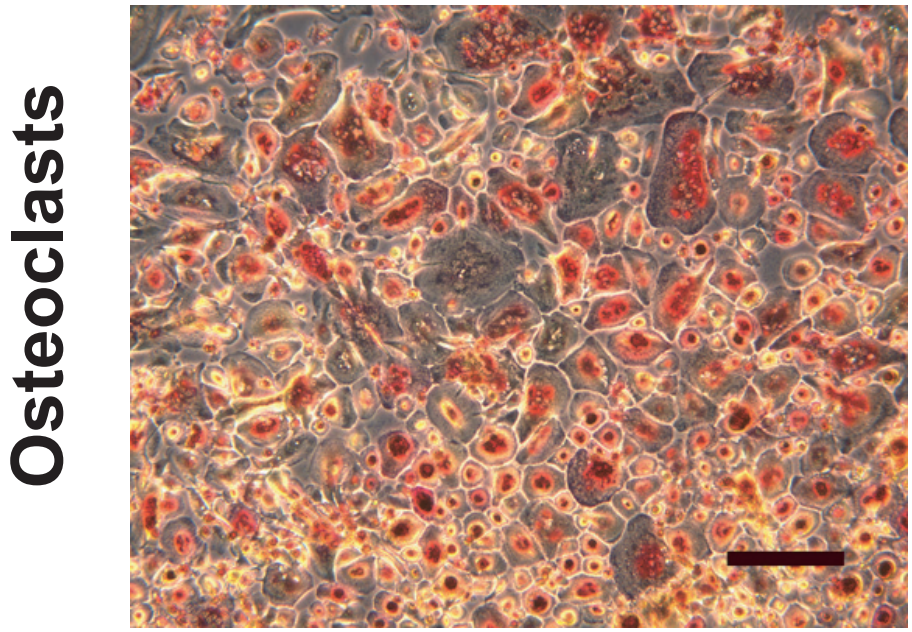
Supplemental Figure 2.
Quantitative real time PCR analysis of expression of indicated genes in undifferentiated hESCs (hES H1), CD235a/CD41a^{-}CD45^{+} cells derived from hESCs after 2 days expansion with GM-CSF (H1 CD45), somatic CD34^{+} cells (CD34), and neutrophils (Neu), eosinophils (EO), dendritic cells (DC), Langerhans cells (LC), macrophages (Mϕ), and osteoclasts (OC) derived from hESC (H1), somatic CD34^{+} cells (CD34), or peripheral blood (PB). Results are mean $\pm$ SE of two experiments performed triplicates; * $p<0.05$, ** $p<0.01$.

Supplemental Figure 3



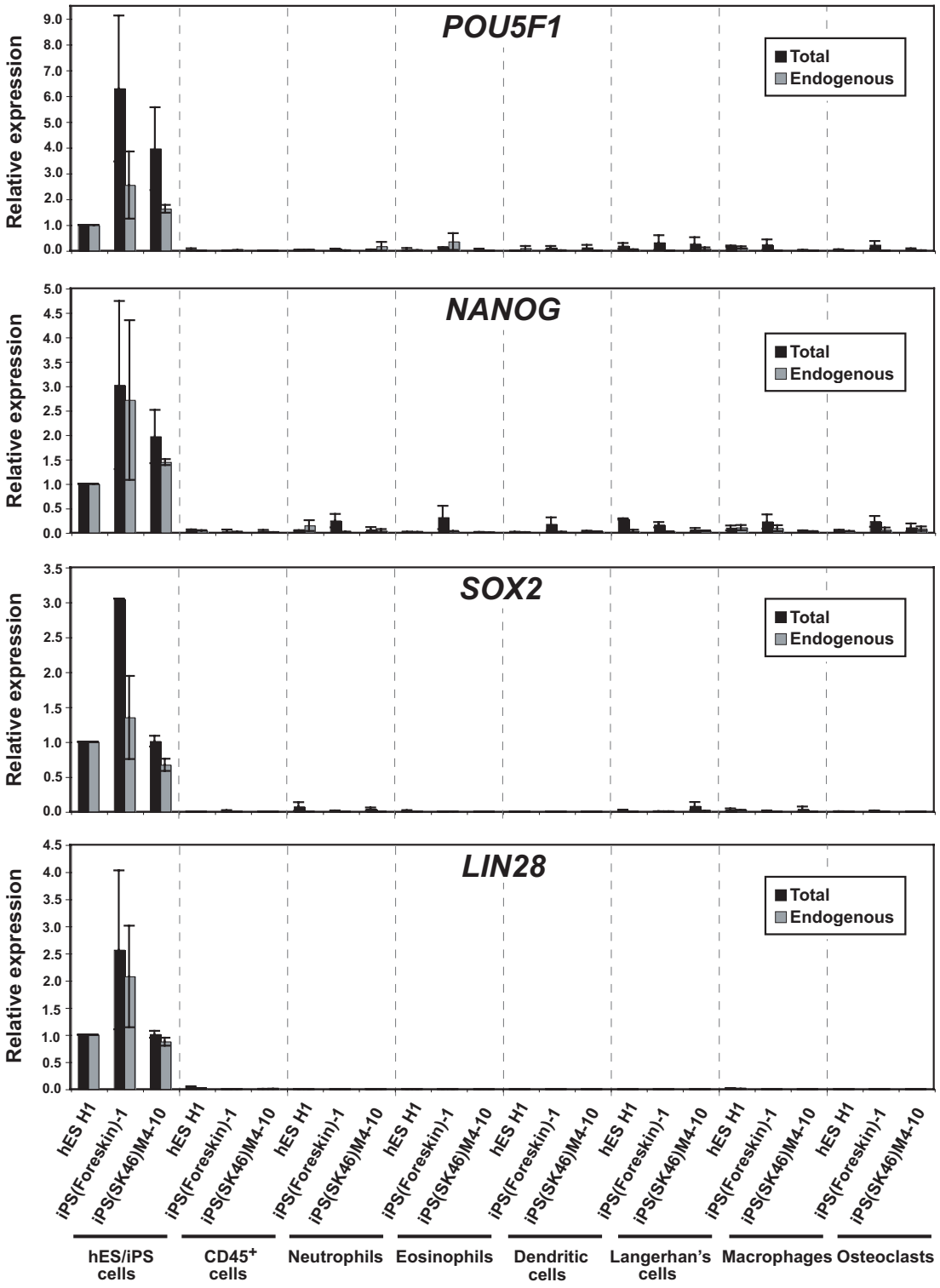
Supplemental Figure 3.
Morphology and phenotypic analysis of neutrophils, eosinophils, LCs and DCs obtained from iPS(foreskin)-1 line. Left panels show Wright stained cytospins of corresponding cells; scale bar is 10 μm (neutrophils, eosinophils, DCs and LCs) and 200 μm (macrophages). Right panels show FACS analysis of phenotype of hiPSC-derived neutrophils, eosinophils, DCs and LCs. Plots show isotype control (open gray) and specific antibody (open black) histograms; * indicates intracellular staining.

Supplemental Figure 4



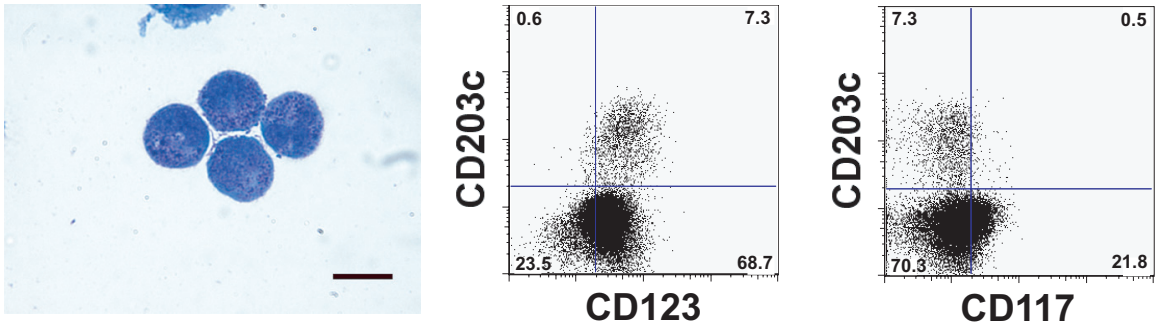
Supplemental Figure 4.
Cytochemical staining for TRAP of iPS(foreskin)-1 derived osteoclasts (scale bar is 200 μm).

Supplemental Figure 5



Supplemental Figure 5.
Quantitative real time PCR analysis of total and endogenous expression of key pluripotency genes in undifferentiated hiPSCs obtained through reprogramming of human foreskin (iPS(Foreskin)-1) and adult (iPS(SK46)M4-10) fibroblasts and hiPSC-derived CD235a/CD41a^{-}CD45^{+} cells expanded with GM-CSF for 2 days (CD45+), mature neutrophils, eosinophils, dendritic and Langerhans cells, macrophages and osteoclasts. PCR amplification was performed using primers to amplify endogenous and total POU5F1, NANOG, SOX2, or LIN28 mRNA. The PCR products were normalized with GAPDH of the same samples and compared with hESC H1 as relative standard. Primers sequences and details of PCR procedure have been previously described (Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–1920).

Supplemental Figure 6



Supplemental Figure 6.
Differentiation of peripheral blood $CD34^+$ cells into basophils. Peripheral blood $CD34^+$ cells obtained from healthy adult donor were cultured at $5x10^4$/ml for 14 days in IMDM (Invitrogen) supplemented with 20% of heat-inactivated FBS (HyClone) in the presence of 10 ng/ml IL-3 and 10 ng/ml of SCF, with a half-medium change every third day. In this culture, cells with typical basophil morphology were generated (left panel shows Wright stain of cytospins, bar is 10 μm). Flow cytometric analysis depicted on the right demonstrated that approximately 7% of the cells generated in this condition had typical mature basophil phenotype $CD203c^+CD123^+CD117^-$.

Supplemental Table 1. Generation of CD235a/41a$^-$CD45$^+$ cells and differentiated myeloid cells from H9 hESCs, and iPS(foreskin)-1 and iPS(SK46)M4-10 hiPSCs.

Cell type	Yield 10^6*		
	H9	iPS(foreskin)-1	iPS(SK46)M4-10
GM-CSF expanded CD235a/41a$^-$CD45$^+$ cells	0.3 ± 0.01	1.5 ± 0.1	0.9 ± 0.1
Neutrophils	6.8 ± 0.9	63.0 ± 8.4	61.3 ± 14.0
Eosinophils	46.6 ± 4.1	455.3 ± 65.8	312.8 ± 18.2
Dendritic cells	0.6 ± 0.01	6.4 ± 1.1	4.7 ± 0.9
Langerhans cells	0.3 ± 0.01	0.9 ± 0.1	0.6 ± 0.1
Macrophages	1.5 ± 0.2	14.9 ± 1.5	9.0 ± 1.0
Osteoclasts	2.7 ± 0.2	17.2 ± 1.7	5.7 ± 0.6

* Yield was calculated as a total number of indicated types of cells obtained from one 10 cm dish of hESC/OP9 coculture. To initiate differentiation, hESC H9 ($2.0 \pm 0.2 \times 10^6$ cells), iPS(foreskin)-1 ($1.5 \pm 0.6 \times 10^6$ cells), or iPS(SK46)M4-10 ($1.5 \pm 0.1 \times 10^6$ cells) were collected from one 6 well plate and cocultured with OP9 for 9 days in one 10 cm dish. The $0.03 \pm 0.01 \times 10^6$ (H9), $0.2 \pm 0.1 \times 10^6$ (iPS(foreskin)-1), or $0.2 \pm 0.1 \times 10^6$ (iPS(SK46)M4-10) lin$^-$CD34$^+$CD43$^+$45$^+$ cells were generated at day 9 of differentiation from one 10 cm dish of hESC or iPSC/OP9 coculture, respectively. The absolute number of CD235a/CD41a$^-$CD45$^+$ cells generated after expansion for 2 days with GM-CSF and mature myeloid cells generated from these expanded cells is shown. Results are mean ± SE of 3 experiments.

Supplemental Table 2. Primers used for analysis of hESC-derived myeloid cells

Genes	Symbol	Sequences	PCR product size (bp)
Azurocidin	AZU1	Forward:5'-gac tgg atc gat ggt gtt ctc-3'	188
		Reverse:5'-cag agg aga gat cgg ctt ctt-3'	
Calcitonin receptor	CALCR	Forward:5'-tgc ggt ggt att atc tct tgg -3'	212
		Reverse:5'-ttc cct cat ttt ggt cac aag -3'	
Cathepsin G	CTSG	Forward:5'-ctc aat ata atc agc gga cc-3'	453
		Reverse:5'-cca gca gtt tga agc ttc tc-3'	
Cathepsin K	CTSK	Forward:5'-aag aag aaa act ggc aaa ct-3'	595
		Reverse:5'-atc gtt aca ctg cac cat cg-3'	
Charcot-Leyden crystal protein	CLC	Forward:5'-cta ctg tga caa tca aag ggc gac-3'	308
		Reverse:5'-agc ctc agg ctt gat tct atg gtc-3'	
Chemokine (C-C motif) ligand 13	CCL13	Forward:5'-atg aca gca gct ttc aac ccc-3'	451
		Reverse:5'-ctc caa acc agc aac aag tca at-3'	
Chemokine (C-C mitif) ligand 17	CCL17	Forward:5'-atg gcc cca ctg aag atg ctt-3'	351
		Reverse:5'-tga aca cca acg gtg gag gt-3'	
Elastase 2	ELANE	Forward:5'-atc aac gcc aac gtg cag-3'	252
		Reverse:5'-gat tag ccc gtt gca gac-3'	
Eosinophil peroxidase	EPO	Forward:5'-gca tct gct ccc agc cct-3'	334
		Reverse:5'-gaa ggg tcc gga ccg ctg-3'	
Interferon regulatory factor-4	IRF4	Forward:5'-tcc cca cag agc caa gca taa ggt-3'	436
		Reverse:5'-agg gag cgg ccg tgg tga gca-3'	
Metallo proteinase 12	MMP12	Forward:5'-ttt tgc ccg tgg agc tca t-3'	400
		Reverse:5'-ttc cca cgg tag tga cag ca-3'	
Proteinase-3	PRTN3	Forward:5'-ctt gat cca ccc cag ctt cgt g-3'	228
		Reverse:5'-gca gaa gaa ggt gac cac ggt gac-3'	
Receptor activator NFκB	RANK	Forward:5'-tta agc cag tgc ttc acg gg-3'	497
		Reverse:5'-acg tag acc acg atg atg tcg c-3'	
Ribosomal protein L13A	RPL13A	Forward:5'-cct gga gga gaa gag gaa aga ga-3'	126
		Reverse:5'-ttg agg acc tct gtg tat ttg tca a-3'	
Vitronectin receptor	VTNR	Forward:5'-gtt ggg aga tta gac aga gg-3'	440
		Reverse:5'-cta gtg ggt caa gat gta gc-3'	