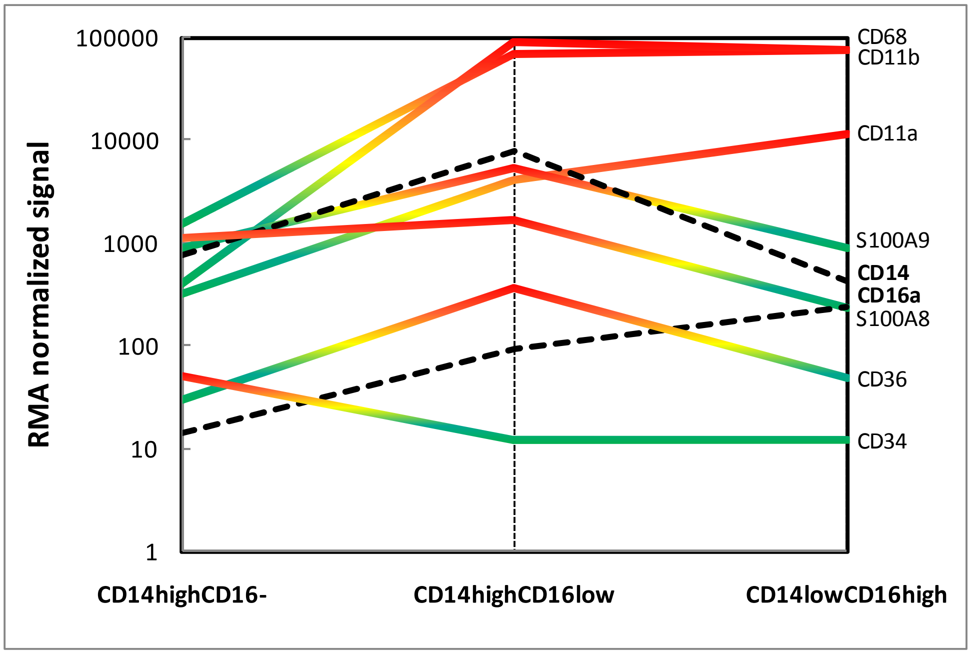
Supplemental Materials and Methods

cDNA microarray and data analysis

The transcriptional profile in three monocyte populations was examined using the Affymetrix GeneChip Rhesus Macaque Genome Array (Affymetrix) containing a total of 47,000 Macaca mulatta transcripts. RNA purification and labeling, hybridization, array scanning, and image quantification were performed according to the manufacturer's instructions. Briefly, FACS-isolated monocyte subpopulations from two healthy uninfected rhesus monkeys were spun down and lysed in Trizol reagents (Invitrogen), and total RNA was prepared using PureLink Micro-to-Midi Total RNA Purification system (Invitrogen). Quality of RNA was determined by 2100 Bioanalyzer RNA LabChip (Agilent Technologies). One hundred ng of high-quality total RNA was subjected to Affymetrix 1-cycle or 2-cycle synthesis amplification, fluorescent labeling, and hybridization to Affymetrix Rhesus Genome Arrays. Expression data was obtained using an Affymetrix GSC3000 scanner and processed by GCOS software (Affymetrix). Partek Genomic Suite System was used for downstream analysis of GCOS processed data. Signals from all probe sets were normalized using Rhesus Array Normalization Controls. The entire data set was deposited at the Gene Expression Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/projects/geo/) with accession number GSE14482.



Supplemental Figure 1. Differential gene expression in monocyte subpopulations. Total cellular RNA was extracted from FACS-sorted monocyte subpopulations, and gene expression profiles were determined by hybridizing Affymetrix rhesus macaque gene arrays. The RMA-normalized gene array signals of the indicated monocyte/macrophage markers are plotted for CD14^{high}CD16^{-}, CD14^{high}CD16^{low}, and CD14^{low}CD16^{high} subpopulations. Phenotypically distinct stages of maturation can be defined. Red and green denote relatively high and low gene expression, respectively.

Supplemental Table 1. Genes strongly expressed in both CD14^{high}CD16^{low} and CD14^{low}CD16^{high} versus CD14^{high}CD16^{-}

Gene Name	Probe Set ID	Log$_2$ Ratio CD14^{high}CD16^{low}/ CD14^{high}CD16^{-}	Log$_2$ Ratio CD14^{low}CD16^{high}/ CD14^{high}CD16^{-}
Receptors and cell surface antigens			
CD1d [CD1D]	MmugDNA.14308.1.S1_at	8.86	7.82
CD37 [CD37]	MmugDNA.14342.1.S1_at	4.74	5.67
CD44 [CD44]	MmugDNA.6704.1.S1_at	4.94	3.83
CD63 [CD63]	MmugDNA.14380.1.S1_at	6.95	6.39
CD68 (macrosialin) [CD68]	MmuSTS.3489.1.S1_at	5.55	5.67
CD81 [CD81]	MmugDNA.6428.1.S1_at	6.53	6.87
CD83 [CD83]	MmugDNA.6430.1.S1_at	6.29	6.45
CD86 [CD86]	MmugDNA.32699.1.S1_at	6.98	6.47
CD99 [CD99]	MmugDNA.7402.1.S1_at	5.52	4.56
CD163 [CD163]	MmugDNA.29742.1.S1_at	7.58	5.37
CD164 [CD164]	MmugDNA.19526.1.S1_at	4.42	4.36
CD274 (B7-H1) [CD274]	MmugDNA.20774.1.S1_at	4.63	5.28
colony stimulating factor 2 receptor, β, low-affinity (GM-CSFRβ) [CSF2RB]	MmugDNA.35543.1.S1_at	6.63	6.47
chemokine (C-X-C motif) receptor 4 [CXCR4]	MmuSTS.4017.1.S1_at	6.16	6.90
egf-like module containing, mucin-like, hormone receptor-like 2 [EMR2]	MmugDNA.34795.1.S1_at	2.90	3.35
Fc fragment of IgE, high affinity I, receptor for; γ polypeptide [FCER1G]	MmugDNA.35764.1.S1_s_at	5.30	5.41
Fc fragment of IgG, low affinity IIIa, receptor (CD16a) [FCGR3A]	MmugDNA.19066.1.S1_at	2.70	4.05
Fc fragment of IgG, low affinity IIIb, receptor (CD16b) [FCGR3B]	MmugDNA.7038.1.S1_at	9.03	9.57
major histocompatibility complex, class II, DR α [HLA-DRA]	MmugDNA.1046.1.S1_at	5.93	5.61
Interferon γ receptor 1 [IFNGR1]	MmugDNA.21956.1.S1_at	6.75	6.91
Integrin, α 4 (CD49d, α 4 subunit of VLA-4 receptor) [ITGA4]	MmugDNA.24387.1.S1_at	4.94	5.73
integrin, α L (CD11a, LFA1) [ITGAL]	MmugDNA.22748.1.S1_at	3.65	5.13
integrin, α M (CD11b) [ITGAM]	MmugDNA.22091.1.S1_at	7.82	7.56
integrin, β 2 (CD18, LFA1) [ITGB2]	MmugDNA.11483.1.S1_at	3.33	3.95
lectin, mannose-binding, 1 [LMAN1]	MmugDNA.18553.1.S1_at	3.66	3.98
lectin, mannose-binding, 2 [LMAN2]	MmugDNA.13320.1.S1_at	4.64	4.30
lymphocyte antigen 6 complex, locus E [LY6E]	MmugDNA.40394.1.S1_at	3.66	4.69
monocyte to macrophage differentiation-associated [MMD]	MmugDNA.15918.1.S1_at	4.49	6.03
oxidized low density lipoprotein (lectin-like) receptor 1 [OLR1]	MmugDNA.25334.1.S1_at	3.26	3.04
purinergic receptor P2Y, G-protein coupled, 5 [P2RY5]	MmugDNA.33195.1.S1_at	3.64	2.44
platelet/endothelial cell adhesion molecule (CD31) [PECAM1]	MmugDNA.28182.1.S1_at	5.33	6.14
transforming growth factor, β receptor I [TGFBR1]	MmugDNA.9612.1.S1_s_at	3.72	3.37
toll-like receptor 2 (TLR2)	MmuSTS.1204.1.S1_at	4.29	4.90
Cytokines and extracellular mediators			
apolipoprotein L3 [APOL3]	MmuSTS.2830.1.S1_at	5.46	6.93
chemokine (C-C motif) ligand 3 [CCL3]	MmuSTS.3571.1.S1_s_at	5.19	4.77
interleukin 1, β [IL1B]	MmuSTS.652.1.S1_s_at	5.57	5.01
TIMP metallopeptidase inhibitor 1 [TIMP1]	MmugDNA.5715.1.S1_at	3.71	2.61
tumor necrosis factor (ligand) superfamily, member 13 [TNFSF13]	MmugDNA.24514.1.S1_s_at	4.75	4.97

Enzymes			
adenosine deaminase [ADA]	MmugDNA.17218.1.S1_at	5.46	6.80
caspase 3, apoptosis-related cysteine peptidase [CASP3]	MmugDNA.6342.1.S1_at	6.23	8.06
cathepsin C [CTSC]	MmugDNA.38162.1.S1_at	6.08	5.75
cathepsin D (lysosomal aspartyl peptidase) [CTSD]	MmugDNA.35516.1.S1_at	3.66	4.44
cathepsin S [CTSS]	MmugDNA.24863.1.S1_at	6.33	6.68
indoleamine-pyrrole 2,3 dioxygenase [INDO]	MmugDNA.18432.1.S1_at	7.95	8.16
lysozyme [LYZ]	MmugDNA.22297.1.S1_at	8.79	8.48
phosphodiesterase 4B [PDE4B]	MmugDNA.22024.1.S1_at	2.76	2.72
superoxide dismutase 2, mitochondrial [SOD2]	MmugDNA.24030.1.S1_at	6.70	6.78
Signaling molecules and transcription factors			
CCAAT/enhancer binding protein (C/EBP), β [CEBPB]	MmugDNA.9809.1.S1_at	6.22	6.09
forkhead box O1A [FOXO1A]	MmugDNA.41363.1.S1_at	4.38	4.27
forkhead box O3A [FOXO3A]	MmugDNA.42021.1.S1_at	6.60	6.85
forkhead box P1 [FOXP1]	MmugDNA.27089.1.S1_at	3.35	4.36
interferon, α-inducible protein (clone IFI-15K) [G1P2]	MmugDNA.17118.1.S1_at	4.93	4.95
growth arrest and DNA-damage-inducible, β [GADD45B]	MmugDNA.30178.1.S1_s_at	7.75	7.30
hypoxia-inducible factor 1, α subunit [HIF1A]	MmugDNA.17998.1.S1_at	6.25	5.01
interferon regulatory factor 8 [IRF8]	MmugDNA.8826.1.S1_at	4.91	5.47
interferon-stimulated transcription factor 3, γ [ISGF3G]	MmugDNA.36516.1.S1_at	3.65	3.47
v-*maf* musculoaponeurotic fibrosarcoma oncogene homolog B [MAFB]	MmugDNA.32362.1.S1_at	4.83	4.44
myxovirus (influenza virus) resistance 1 [MX1]	MmuSTS.3006.1.S1_at	4.86	6.27
myeloid differentiation primary response gene (88) [MYD88]	MmugDNA.10008.1.S1_at	5.67	6.15
nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α [NFKBIA]	MmugDNA.23424.1.S1_at	5.93	6.46
proliferating cell nuclear antigen [PCNA]	MmugDNA.865.1.S1_at	4.07	5.40
signal transducer and activator of transcription 1 [STAT1]	MmugDNA.23497.1.S1_at	4.60	4.84
tumor necrosis factor, α-induced protein 3 [TNFAIP3]	MmugDNA.40138.1.S1_at	7.46	7.85

The table lists a selection of genes specifically associated with both CD16$^+$ monocyte subpopulations. Specific genes are defined as genes that were expressed >4 (2log$_2$) fold greater with $P<0.05$ than those in the CD14^{high}CD16$^-$ subpopulation.

Supplemental Table 2. Genes strongly expressed in CD14^{high}CD16^{low} versus both CD14^{high}CD16^{-} and CD14^{low}CD16^{high}

Gene Name	Probe Set ID	Log$_2$ Ratio CD14^{high}CD16^{low}/ CD14^{high}CD16^{-}	Log$_2$ Ratio CD14^{high}CD16^{low}/ CD14^{low}CD16^{high}
adenosine A2b receptor [ADORA2B]	MmugDNA.35810.1.S1_at	2.61	3.06
chemokine (C-C motif) receptor 1 [CCR1]	MmugDNA.20995.1.S1_at	6.47	2.82
chemokine (C-C motif) receptor 2 [CCR2]	MmugDNA.36749.1.S1_at	5.72	3.87
chemokine (C-C motif) receptor 5 [CCR5]	MmuSTS.1981.1.S1_at	2.29	2.24
CD36 [CD36]	MmugDNA.21439.1.S1_at	3.58	2.88
chemokine (C-X-C motif) ligand 9 [CXCL9]	MmuSTS.4003.1.S1_at	2.37	2.75
Fc fragment of IgG, low affinity IIa, receptor (CD32) [FCGR2A]	MmugDNA.35261.1.S1_at	2.56	2.29
Fc fragment of IgG, low affinity IIc, receptor (CD32) [FCGR2C]	MmugDNA.23544.1.S1_s_at	6.87	2.63
fibronectin 1 [FN1]	MmugDNA.29987.1.S1_at	3.22	4.13
interleukin 13 receptor, α 1 [IL13RA1]	MmugDNA.28810.1.S1_at	5.10	2.35
interleukin 1 receptor, type II [IL1R2]	MmugDNA.28534.1.S1_at	5.03	3.11
matrix metallopeptidase 1 [MMP1]	MmuSTS.921.1.S1_at	5.50	6.04
macrophage scavenger receptor 1 [MSR1]	MmugDNA.42499.1.S1_at	2.93	2.18
runt-related transcription factor 2 [RUNX2]	MmugDNA.9398.1.S1_at	3.97	2.48
S100 calcium-binding protein A9 (myeloid related protein14, MAC387) [S100A9]	MmuSTS.2130.1.S1_s_at	2.61	2.56
syndecan 2 [SDC2]	MmugDNA.22062.1.S1_at	7.48	3.04
serpin peptidase inhibitor, clade B, member 2 [SERPINB2]	MmugDNA.334.1.S1_at	2.39	5.69
triggering receptor expressed on myeloid cells 1 [TREM1]	MmugDNA.9703.1.S1_at	4.07	5.45

The table shows 18 unique known genes that were strongly, specifically expressed in the CD14^{high}CD16^{low} subpopulation.

Supplemental Table 3. Genes strongly expressed in CD14^{low}CD16^{high} versus both CD14^{high}CD16^{-} and CD14^{high}CD16^{low}

Gene Name	Probe Set ID	Log$_2$ Ratio CD14^{low}CD16^{high}/ CD14^{high}CD16^{-}	Log$_2$ Ratio CD14^{low}CD16^{high}/ CD14^{high}CD16^{low}
chemokine (C-C motif) ligand 5 [CCL5]	MmugDNA.43376.1.S1_s_at	5.39	4.86
CD47 [CD47]	MmugDNA.36982.1.S1_at	2.18	2.07
cystatin F [CST7]	MmugDNA.6381.1.S1_at	6.25	4.52
chemokine (C-X3-C motif) receptor 1 [CX3CR1]	MmugDNA.6875.1.S1_at	6.35	4.69
granzyme A [GZMA]	MmugDNA.36434.1.S1_at	5.30	6.59
granzyme B [GZMB]	MmugDNA.14204.1.S1_at	3.62	6.74
granzyme H [GZMH]	MmugDNA.2086.1.S1_at	3.91	6.28
granzyme K [GZMK]	MmugDNA.17084.1.S1_at	5.87	5.62
interferon induced transmembrane protein 1 (9-27) [IFITM1]	MmugDNA.5697.1.S1_s_at	9.28	5.56
interleukin 2 receptor, β [IL2RB]	MmugDNA.20381.1.S1_at	4.06	3.82
lymphotoxin β [LTB]	MmugDNA.40364.1.S1_at	6.60	2.49
perforin 1 [PRF1]	MmugDNA.14985.1.S1_at	3.11	2.97
tumor necrosis factor ligand superfamily, member 10 (TRAIL) [TNFSF10]	MmugDNA.30129.1.S1_at	2.57	2.00

The table shows 13 unique known genes that were strongly, specifically expressed in the CD14^{low}CD16^{high} subpopulation.