

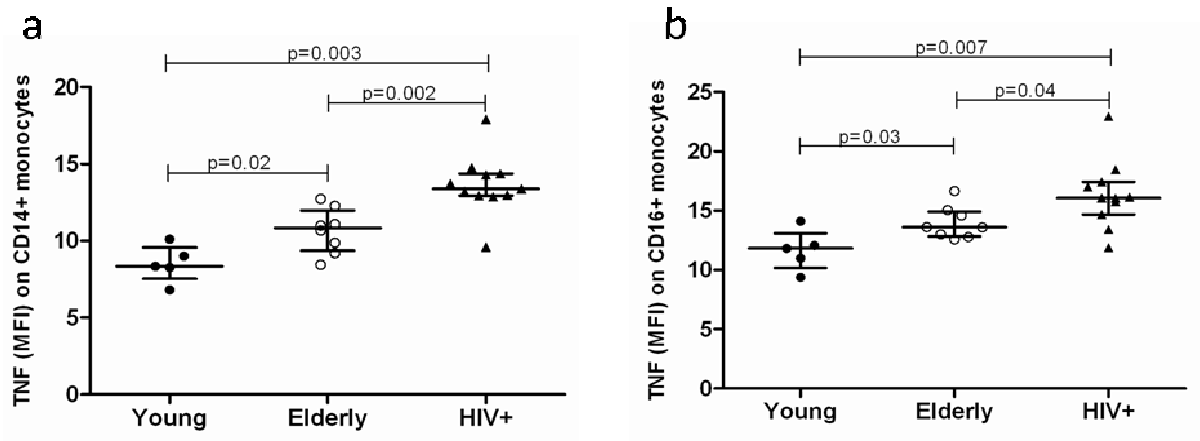
	CD16 ⁺ monocytes			CD14 ⁺ monocytes		
	Young	Elderly	p value	Young	Elderly	p value
HLA-DR	146.9 [97.8-195.2]	153.4 [123.5-193.4]	NS (0.5)	54.3 [34.4-66.6]	51.1 [40.8-66.8]	NS (0.9)
CD38	14.5 [9.4-19.3]	13.2 [6.9-20.0]	NS (0.7)	84.3 [66.1-93.7]	81.8 [69.1-97.9]	NS (0.9)
CD62L	Not determined*			56.5 [49.2-72.9]	45.3 [33.0-59.1]	0.02
CD11b	28.8 [11.3-41.8]	54.1 [36.7-61.0]	0.002	32.5 [17.5-41.8]	47.6 [40.8-53.2]	0.0005
TLR4	3.7 [2.6-4.8]	2.1 [1.4-3.4]	0.007	2.9 [1.5-3.7]	2.3 [1.1-2.7]	NS (0.3)
CD115	20.6 [17.3-30.6]	20.2 [16.0-26.5]	NS (0.7)	13.3 [10.6-16.1]	9.7 [7.7-14.2]	0.03

Supplementary Table 1: Identification of phenotypic markers of monocyte ageing

Expression of phenotypic markers on monocytes from young (median age 36 years, range 20-45 years for HLA-DR, CD38 and CD11b and median age 28 years, range 20-34 years for CD62L, TLR4 and CD115) and elderly (median age 72 years, range 66-84 years for all) healthy HIV-uninfected individuals. Expression levels were measured as Δ MFI (geometric mean fluorescence intensity of stain minus fluorescence of isotype control). Median Δ MFI values and interquartile range (in square brackets) are shown for CD16⁺ and CD14⁺ subsets. P values were determined using Mann-Whitney analysis. NS = not statistically significant.

* CD62L is not significantly expressed on CD16⁺ monocytes and thus CD62L expression was not measured on this subset.

Supplementary Figure 1



One hundred μ l of fresh whole blood (collected into heparin anticoagulant) was washed with FACS wash and stained with anti-CD14-APC and CD16-PE.Cy7 (BD Biosciences) for 30 min on ice in the dark. Red blood cells were lysed with BD FACS lysis solution and the sample fixed with 1% formaldehyde. Cells were permeabilised with Perm/Wash Buffer I and intracellular TNF stained with anti-TNF-PE or an isotype control antibody (all from BD Biosciences) for 30 min in the dark. Stained cells were washed with FACS wash, fixed in 1% formaldehyde and analysed on a dual laser FACS Calibur flow cytometer within 24 hours.

Values shown are mean fluorescence intensity (MFI, geometric mean fluorescence intensity of stain minus isotype control) of intracellular TNF in CD14⁺ (a) and CD16⁺ (b) monocytes from young healthy controls (closed circles, aged 29-45, median 34), elderly healthy controls (open circles, aged 66-72, median 68) and young HIV⁺ individuals (closed triangles, aged 33-49, median 44). Data from viremic and virologically suppressed HIV⁺ individuals were not significantly different and were thus pooled. Median values and interquartile ranges are shown. P values were calculated via Mann-Whitney analysis and are shown for values <0.05.

