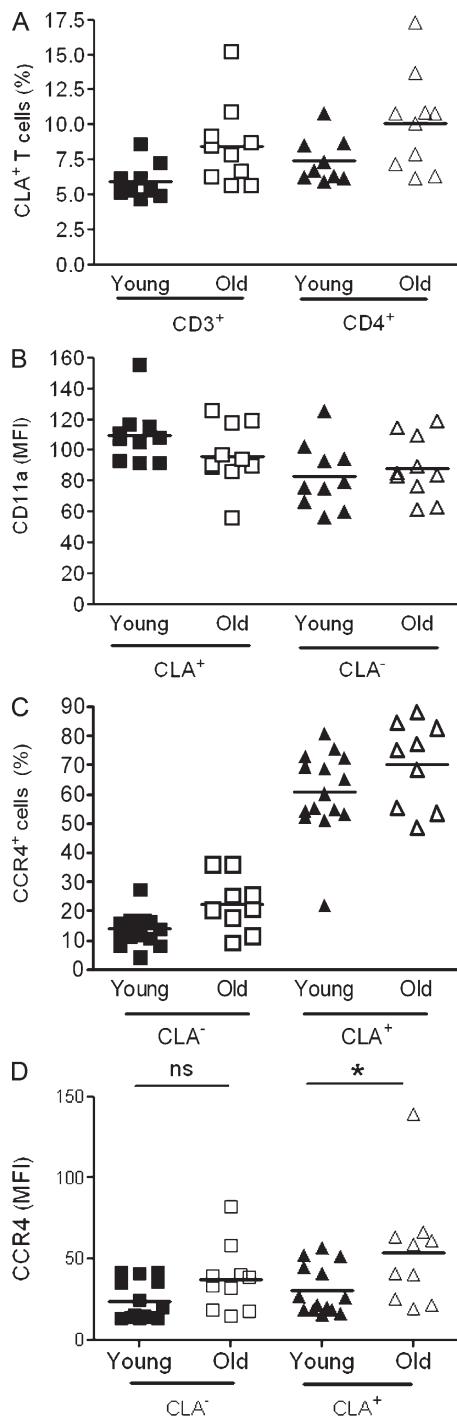
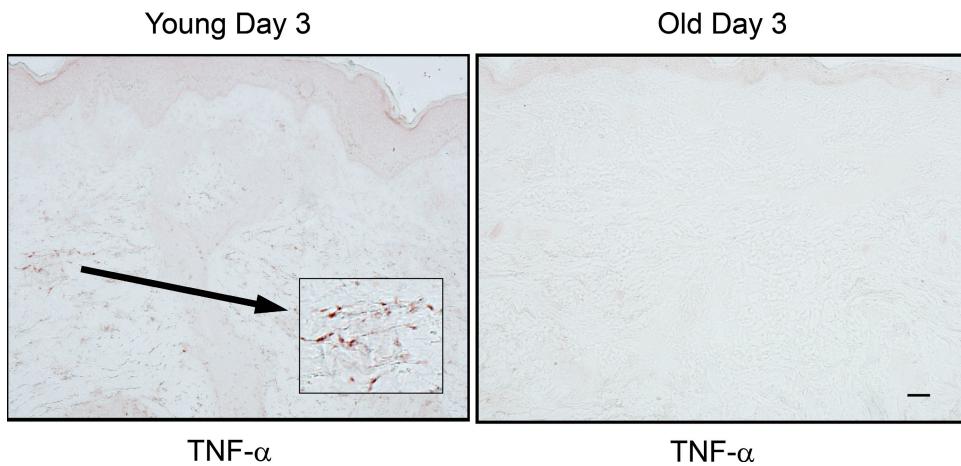


SUPPLEMENTAL MATERIAL

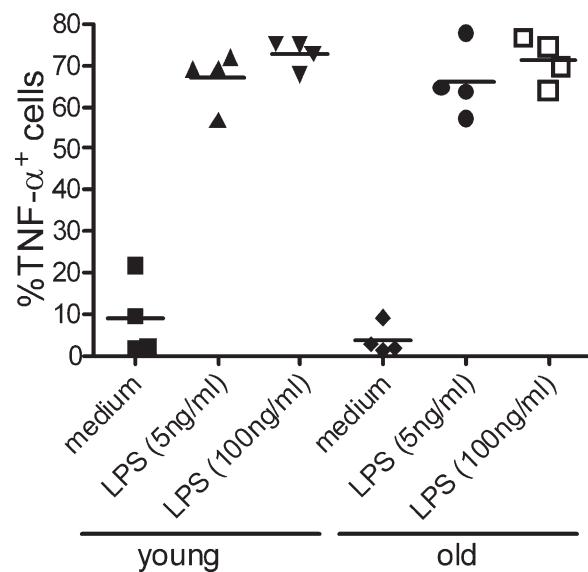
Agius et al., <http://www.jem.org/cgi/content/full/jem.20090896/DC1>



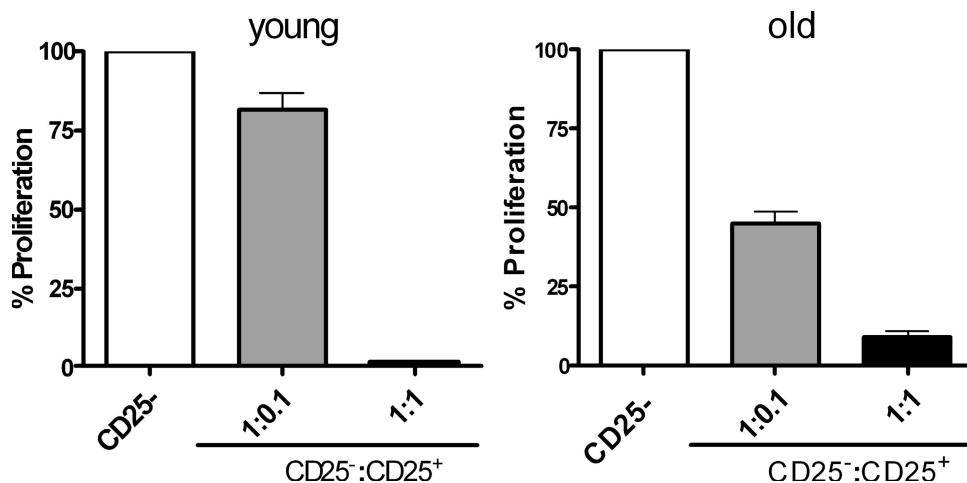
**Figure S1. Expression of skin homing receptors is not reduced in old peripheral blood leukocytes.** Immunostaining of peripheral blood mononuclear cells from old and young with antibodies to CLA, CD11a, and CCR4 was analyzed by flow cytometry. (A) The percentage CLA expression on CD3 and CD4 T cells was similar for young and old ( $n = 9$  young, 10 old). (B) No difference was found for CD11a expression (mean fluorescence intensity) between young and old CLA-positive and -negative CD4 T cells ( $n = 10$  young, 10 old). (C) CCR4 expression was compared on both CLA<sup>+</sup> and CLA<sup>-</sup> CD4<sup>+</sup> T lymphocytes isolated from the peripheral blood in the young and old donors. In both groups, CCR4 expression was found to be significantly higher on the CLA<sup>+</sup> T cells. No difference was found for percentage CCR4<sup>+</sup> cells between the young and old groups ( $n = 15$  young, 9 old). (D) Mean fluorescence intensity of CCR4 expression was also compared and was not different between young and old CD4<sup>+</sup> CLA<sup>-</sup> cells, but slightly higher on CD4<sup>+</sup> CLA<sup>+</sup> T cells in the old ( $n = 14$  young, 10 old).



**Figure S2.** Representative immunohistochemical staining of TNF- $\alpha$ -positive cells in biopsies taken on day 3 after *C. albicans* injection ( $n = 3$  young, 3 old). Inset shows high power magnification of TNF- $\alpha$ -positive cells. Bars, 100  $\mu$ m



**Figure S3.** Macrophages from old donors secrete similar levels of TNF- $\alpha$  in response to low-intensity TLR signals in vitro. Graph shows percentage of TNF- $\alpha$ -secreting CD14 $^{+}$  cells after in vitro stimulation of PBMCs with LPS (100 ng/ml or 5 ng/ml;  $n = 4$  young, 4 old).



**Figure S4.** Purified CD4+CD25<sup>-</sup> T cells were stimulated with *C. albicans* antigen, in the presence of autologous irradiated PBMCs as APCs (white bars). CD4+CD25<sup>+</sup> T cells were added at ratios of 1:0.1 (gray bars) and 1:1 (black bars). Proliferation was measured by [<sup>3</sup>H]thymidine incorporation on day 6 and results were expressed as mean  $\pm$  SEM of triplicate wells. Representative results from 1 of 4 experiments performed are shown. Percentage of proliferation was calculated in comparison to the proliferation of CD4+CD25<sup>-</sup> subset alone (100%).