
Supplementary information

Beyond chronological age: Sex and IL-6 are associated with natural killer cell and monocyte counts in a Sicilian cohort spanning adulthood to extreme longevity

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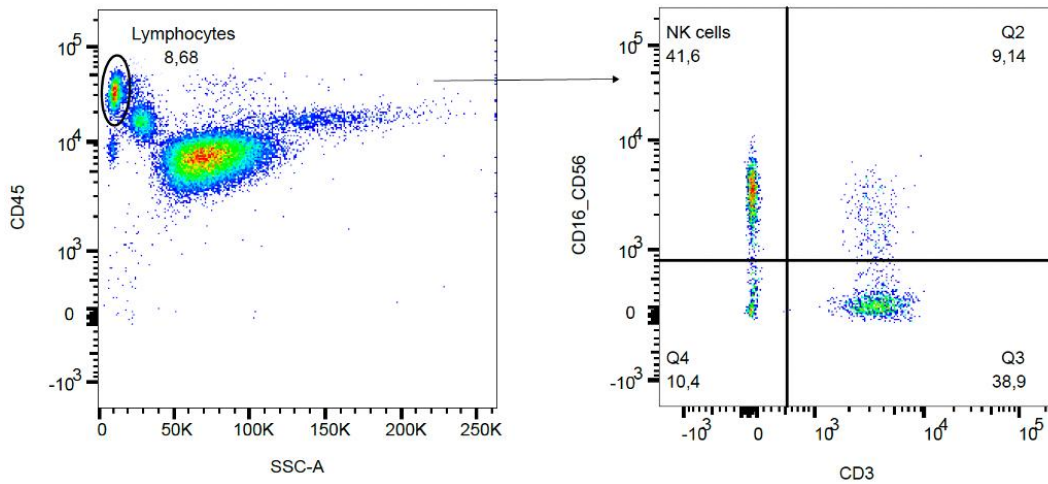


Figure S1. Gating strategy for the identification of NK lymphocytes ($CD16^+CD56^+CD3^-$). For explanation, see text. NK: natural killer.

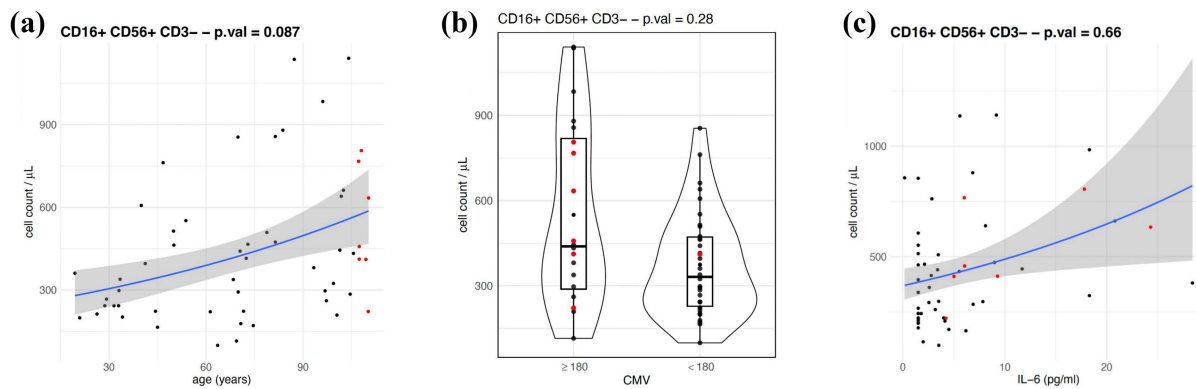


Figure S2. (a) Age-related variation in NK cell counts ($CD3^-CD16^+CD56^+$). Scatter plot showing the relationship between NK cell counts and age in all participants ($n = 54$). Each dot represents an individual healthy donor; red dots indicate the oldest centenarians (≥ 105 years), and black dots indicate younger participants. The solid blue line represents the fitted trend from the negative binomial regression model, and the shaded area denotes the 95% model-based confidence interval. Multivariable regression analysis revealed a non-significant age-related trend ($p = 0.087$); (b) NK cell counts ($CD3^-CD16^+CD56^+$) stratified by anti-CMV antibody titres. Violin plots illustrate the distribution of circulating NK cell counts in participants with anti-CMV titres < 180 and ≥ 180 . Each dot represents an individual healthy donor; red dots indicate the oldest centenarians (≥ 105 years), and black dots indicate younger participants. Multivariable regression analysis showed no statistically significant association. The low anti-CMV group includes individuals with titres < 180 , including CMV-seronegative subjects, whereas the high anti-CMV group includes individuals with titres ≥ 180 ; (c) Association between circulating IL-6 levels and NK cell counts ($CD3^-CD16^+CD56^+$). Scatter plot depicting the relationship between NK cell counts and IL-6 concentrations in all

participants ($n = 54$). Each dot represents an individual healthy donor; red dots indicate the oldest centenarians (≥ 105 years), and black dots indicate younger participants. The solid blue line represents the fitted trend from the negative binomial regression model, and the shaded area indicates the 95% confidence interval. No statistically significant association was observed. NK: natural killer; CMV: cytomegalovirus; IL-6: interleukin 6.

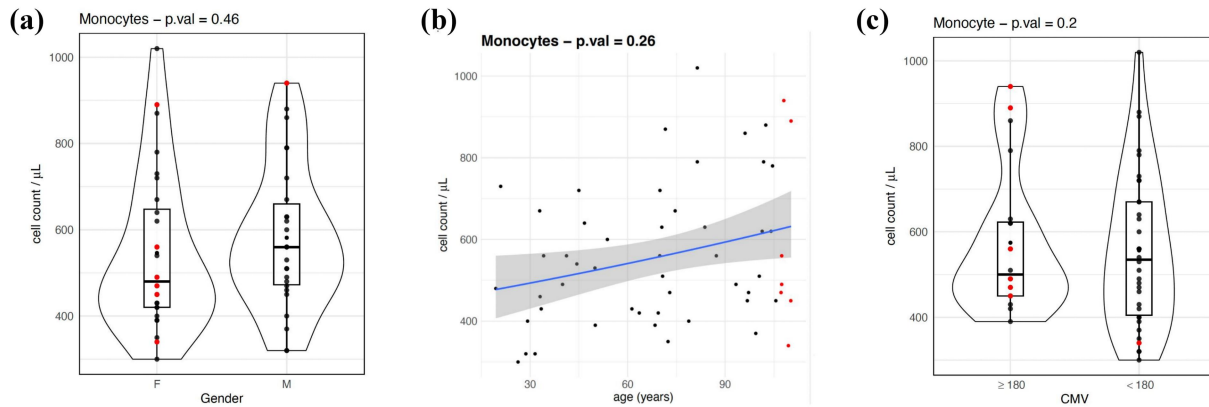


Figure S3. (a) Blood monocyte counts stratified by sex. Violin plots show the distribution of monocyte counts in women and men. Each dot represents an individual healthy donor; red dots indicate the oldest centenarians (≥ 105 years), and black dots indicate younger participants. Multivariable regression analysis revealed no statistically significant sex effect; (b) Age-related variation in blood monocyte counts. Scatter plot showing the relationship between monocyte counts and age in all participants ($n = 54$). Each dot represents an individual healthy donor; red dots indicate the oldest centenarians (≥ 105 years), and black dots indicate younger participants. The solid blue line represents the fitted trend from the negative binomial regression model, and the shaded area denotes the 95% confidence interval. No statistically significant association was detected; (c) Blood monocyte counts stratified by anti-CMV antibody titres. Violin plots illustrate the distribution of circulating monocyte counts in participants with anti-CMV titres < 180 and ≥ 180 . Each dot represents an individual healthy donor; red dots indicate the oldest centenarians (≥ 105 years), and black dots indicate younger participants. Multivariable regression analysis showed no statistically significant association. The low anti-CMV group includes individuals with titres < 180 , including CMV-seronegative subjects, whereas the high anti-CMV group includes individuals with titres ≥ 180 . CMV: cytomegalovirus.