



Supplemental figure3. Naged are a unique subset of neutrophils.

(A) RT-PCR verification of the RNA sequencing results for 10 randomly selected genes with increased expression and 10 genes with decreased expression in tumor associated-aged neutrophils compared with inflammation-associated aged neutrophils. (B) RT-PCR verification of RNA sequencing results for the expression of selected genes in tumor-associated aged neutrophils compared to non-aged neutrophils shown in Fig. 3B. (C) The cluster heat map of normalized enrichment scores (NES) for the top 500 differentially expressed mRNAs in tumor-associated aged neutrophils compared with inflammation-associated aged neutrophils (left panel) and tumor-associated aged neutrophils compared with non-aged neutrophils (right panel) according to the enriched KEGG pathways obtained from the RNA sequencing results ($p < 0.0001$). (D) KEGG pathway classification of differentially expressed genes between tumor-associated aged neutrophils and inflammation-associated aged neutrophils (left panel) and between tumor-associated aged neutrophils and non-aged neutrophils (right panel). (E) RT-PCR verification of the RNA sequencing results for changes in the expression of N1-like and N2-like genes in tumor-associated aged neutrophils and non-aged neutrophils shown in Fig. 3C. (F) Flow cytometry analysis of aged neutrophils in HDNs and LDNs. (G) Flow cytometry analysis and quantification of the MFI of CFSE in CD3⁺T cells co-cultured with lung aged, non-aged neutrophils, lung LDNs or HDNs. Neutrophils derived from the BM of tumor-bearing mice served as the positive control. Data are presented as the means \pm SD from one representative experiment. Statistical analysis was performed by one-way ANOVA (G). ns, not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.