
Supplementary information

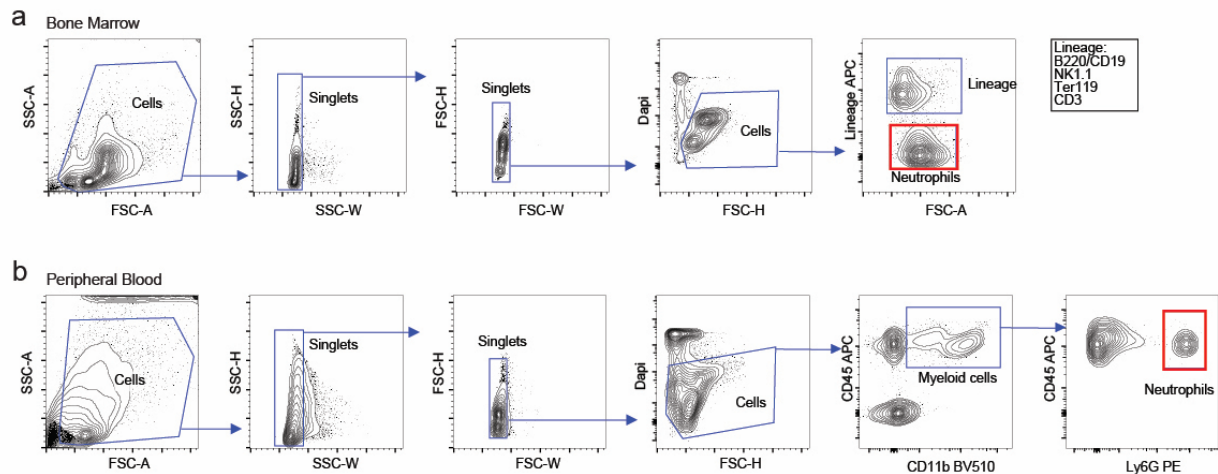
Architecture of the neutrophil compartment

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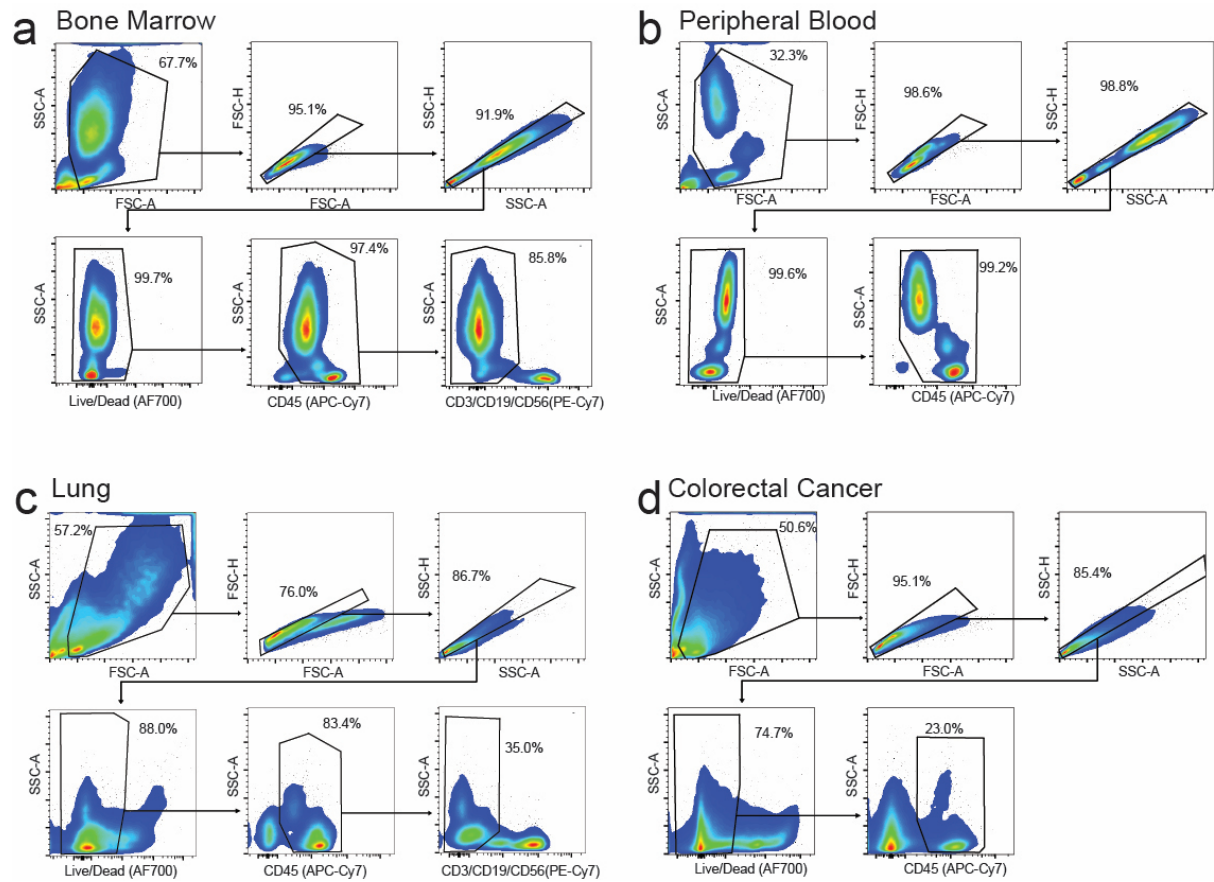
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Architecture of the neutrophil compartment

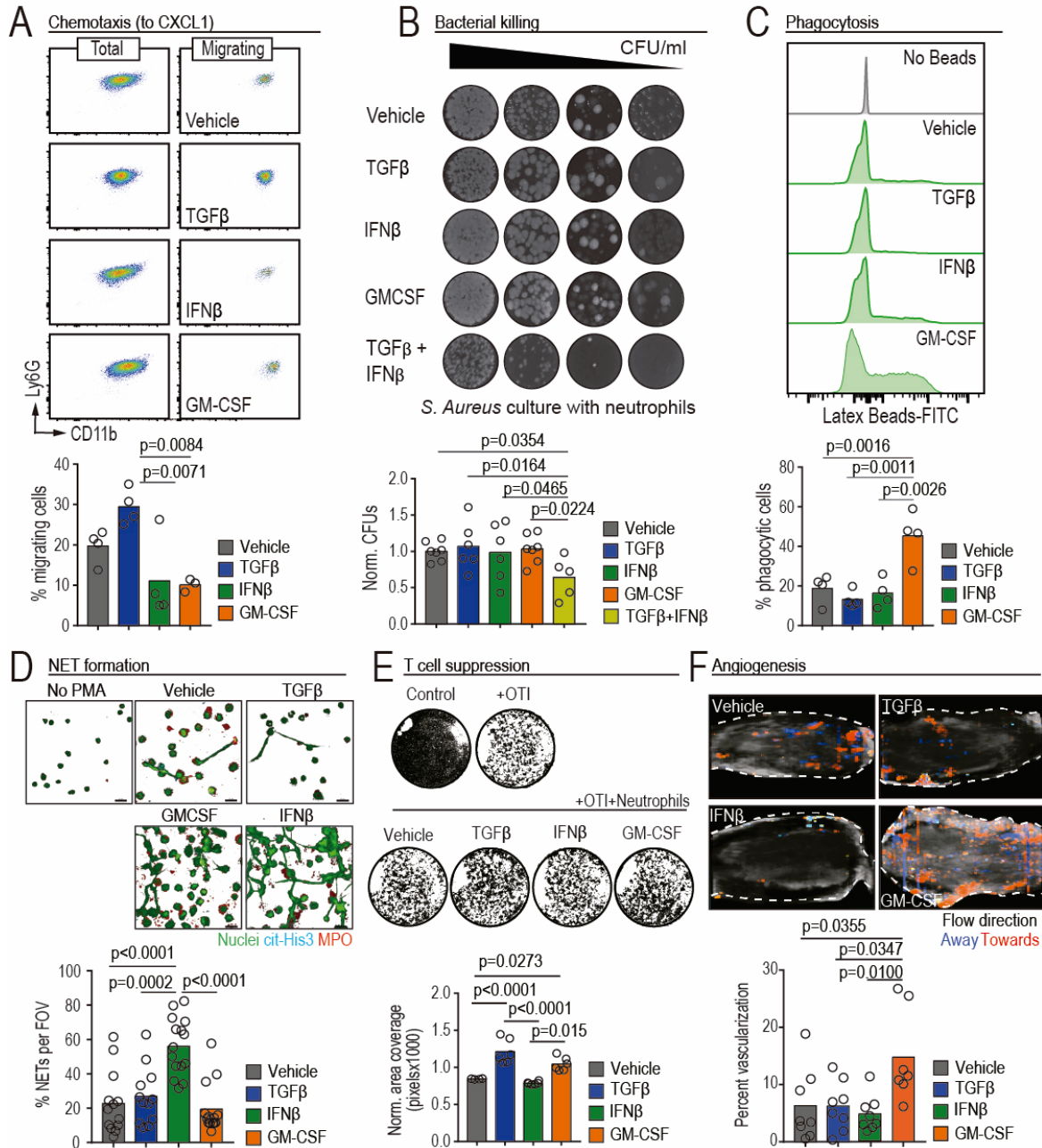
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Supplementary Figure 1. Gating strategy for sorting neutrophils from mouse bone marrow and peripheral blood/ tissues used to generate the mouse NeuMAP. (a) Representative flow cytometry gating strategy for bone marrow cells. Total cells were first gated based on forward (FSC-A) and side (SSC-A) scatter properties, followed by singlet discrimination (SSC-H vs. SSC-W and FSC-H vs. FSC-W). Live nucleated cells were identified as DAPI⁻ and subsequently gated as lineage⁻ (B220/CD19, NK1.1, Ter119, and CD3) myeloid cells. Neutrophils were defined and sorted as Lin⁻CD11b⁺ cells (red gate). (b) Representative gating strategy for peripheral blood/ tissue cells. After gating for total cells (FSC-A vs. SSC-A) and singlets, live DAPI⁻ cells were selected. Myeloid cells were identified as CD45⁺CD11b⁺, and neutrophils were gated as CD45⁺CD11b⁺Ly6G⁺ cells (red gate). Cells were subsequently gated and purified by fluorescence-activated cell sorting (FACS) for downstream single-cell RNA sequencing to generate the mouse NeuMAP reference.

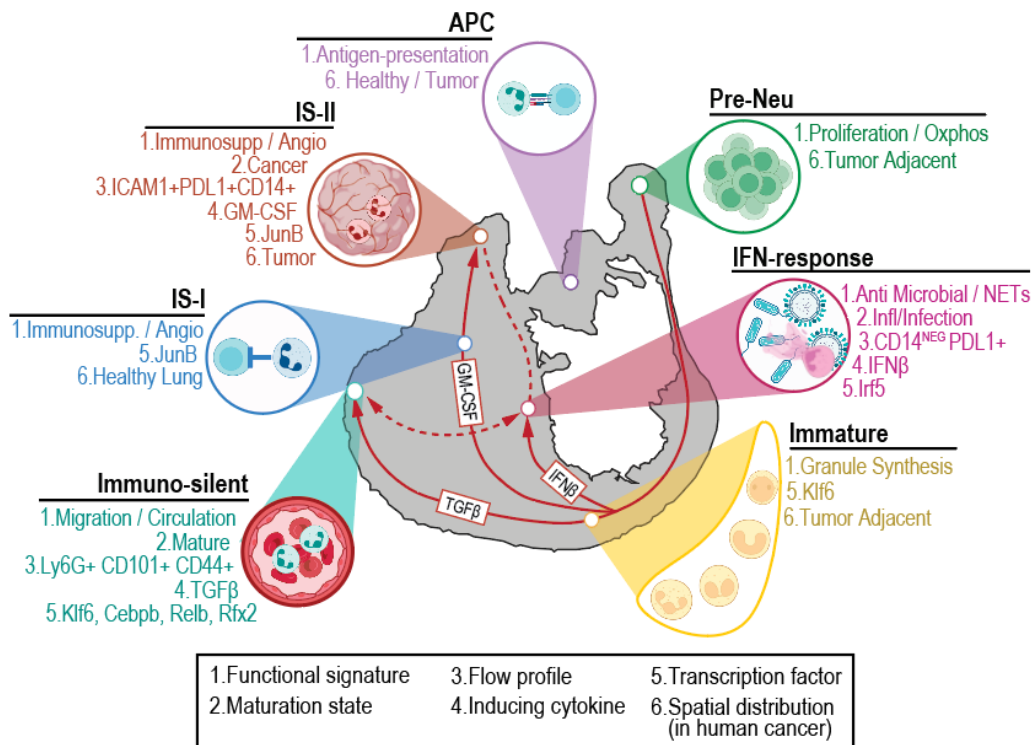


Supplementary Figure 2. Gating strategy for sorting human neutrophils from bone marrow, peripheral blood, lung, and colorectal cancer tissues used to generate the human NeuMAP. (a–d) Representative flow cytometry gating strategies used to isolate viable neutrophils from human (a) bone marrow, (b) peripheral blood, (c) lung, and (d) colorectal cancer samples. For each tissue, total cells were first gated based on forward (FSC-A) and side (SSC-A) scatter properties, followed by singlet discrimination (FSC-H vs. FSC-A and SSC-H vs. SSC-A). Live cells were identified as Live/Dead– (AF700–), and leukocytes were selected as CD45+ (APC-Cy7+). Lineage-positive cells (CD3, CD19, CD56; PE-Cy7) were excluded to enrich for myeloid populations in bone marrow and tissues. Cells were subsequently gated and purified by fluorescence-activated cell sorting (FACS) for downstream single-cell RNA sequencing to generate the human NeuMAP reference.



Supplementary Figure 3. Functional properties of cytokine-treated neutrophils. BM-derived neutrophils were treated with vehicle, TGFβ, IFNβ, or GM-CSF for 48 h before functional assays (A) Migration to CXCL1 in Boyden chambers, quantified by flow cytometry. Data are mean ± SEM from n=3-4 biologically independent samples per group. One-way ANOVA with Tukey's post-test. (B) Bacterial killing assay. *S. aureus* incubated with cytokine-treated neutrophils. Data are mean ± SEM from n=6-7 biologically independent samples per group, shown as fold-change in CFUs relative to vehicle. One-way ANOVA with Tukey's post-test. (C) Phagocytosis of fluorescent latex beads measured by flow cytometry. Representative histograms (top) and percentage of phagocytic neutrophils (bottom). Data are mean ± SEM from n=4 biologically independent samples per group. One-way ANOVA with Tukey's post-test. (D) NET formation after PMA stimulation. NETs identified by MPO and citrullinated histone 3 (cit-H3) staining and quantified as % field of view (FOV). Data are mean ± SEM from n=3-4

biologically independent samples per group, each with 3–5 replicates. One-way ANOVA with Tukey's post-test. **(E)** T cell immunosuppression assay. Neutrophils co-incubated with OT-I T cells before killing assays on B16F10-OVA cells. Data are mean \pm SEM from n=4–6 biologically independent samples per group. One-way ANOVA with Tukey's post-test. **(F)** Angiogenesis assay. Cytokine-treated neutrophils co-injected subcutaneously with LLC cells; vascularization of tumors measured 15 d later by color Doppler ultrasound. Data are mean \pm SEM from n=7–8 biologically independent mice per group. One-way ANOVA with Tukey's post-test.



Supplementary Figure 4. Integrated transcriptional and functional map of the neutrophil compartment in mice. The scheme highlights the general structure and dynamics within the NeuMap, including cytokines driving the main trajectories, associated functions, maturation stages, cytometric profiles, transcription factors, and the spatial distribution (in human lung specimens) of neutrophils associated with each hub.