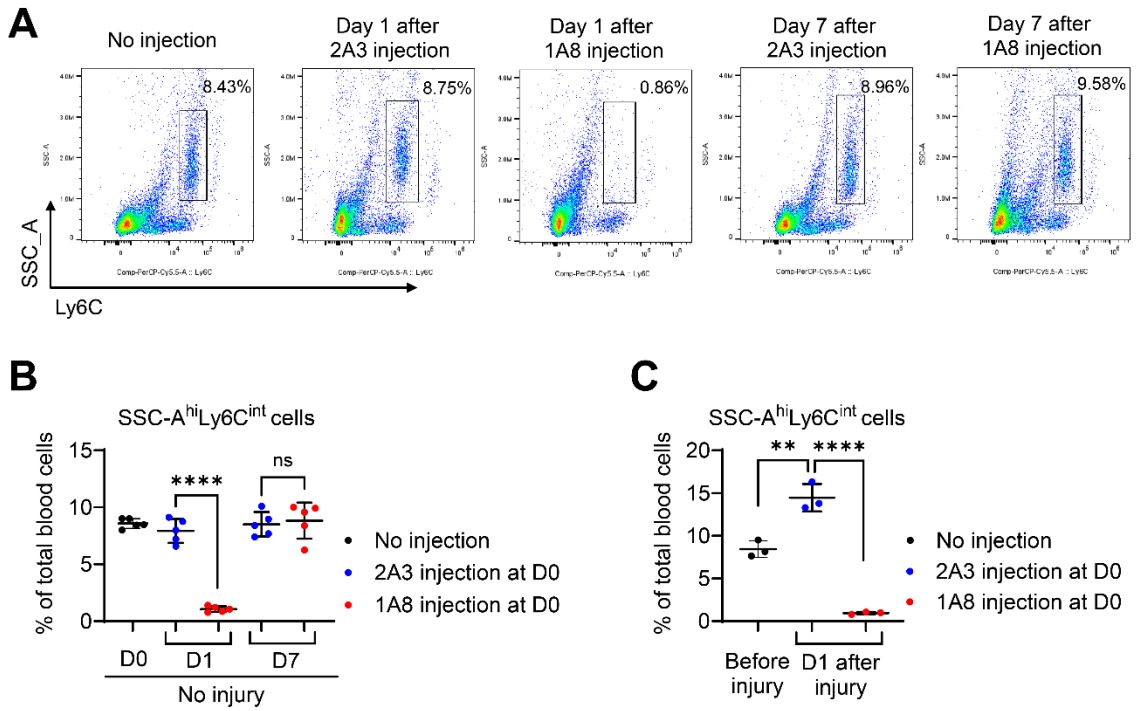
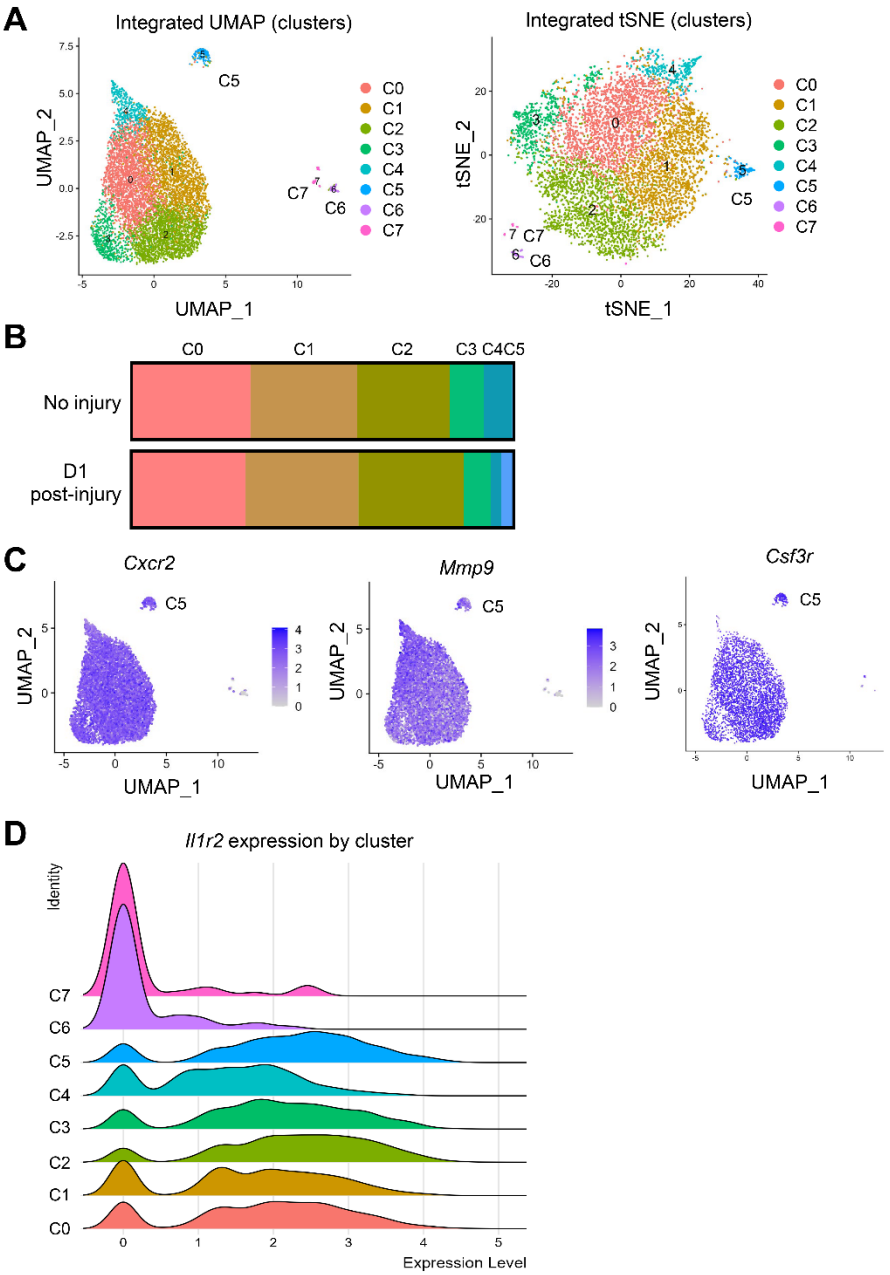
Supplemental Data

Supplemental Figure 1. Clone-independent confirmation of neutrophil depletion by anti-Ly6G (1A8) treatment.



(**A**) Representative flow cytometry plots showing SSC-A^{hi}Ly6C^{int} neutrophils in peripheral blood without injection or on days 1 and 7 after 2A3 or 1A8 injection into normal mice without injury. Because the 1A8 clone used for depletion is also used for Ly6G detection, neutrophils were identified as SSC-A^{hi}Ly6C^{int} cells to avoid epitope masking. Numbers indicate the percentage of gated cells. Plots are representative of the quantification in (B). (**B**) Quantification of SSC-A^{hi}Ly6C^{int} cells in uninjured mice on days 0, 1, and 7 after 2A3 or 1A8 injection, showing effective depletion on day 1 but not on day 7. (**C**) Quantification of SSC-A^{hi}Ly6C^{int} cells before injury and on day 1 after injury in 2A3- or 1A8-injected mice. Circulating neutrophils increased on day 1 after injury, which was completely prevented by 1A8 injection. Data are presented as mean ± SD, with each dot representing an individual mouse (n = 3–5 per group). $^{**}P < 0.01$, $^{****}P < 0.0001$; ns, not significant; by 2-tailed Student's t test (B) or 1-way ANOVA with Tukey's multiple-comparisons test (C).

Supplemental Figure 2. Cluster composition and expression of *Il1r2* and canonical neutrophil markers across clusters in the integrated dataset.



(**A**) Integrated UMAP and t-SNE projections of the combined control and day 1 post-injury dataset, showing all eight clusters (C0–C7). Clusters C6 and C7 represent low-abundance contaminating populations (monocytes/macrophages and lymphocytes, respectively) and were excluded from downstream neutrophil analyses. (**B**) Relative proportions of the six neutrophil clusters (C0–C5) in the no-injury and day 1 post-injury conditions, showing expansion of C2 and C5 and contraction of C3 and C4 after injury. (**C**) Feature plots of the canonical neutrophil markers *Cxcr2*, *Mmp9*, and *Csf3r* projected onto the integrated UMAP, confirming that cluster C5 retains neutrophil identity. (**D**) Ridge plots of *Il1r2* expression across clusters (C0–C7), showing high expression in clusters C2 and C5 and low expression in C4.

Supplemental Table 1. Top 10 differentially expressed marker genes for each cluster identified by single-cell RNA sequencing.

Marker genes were identified by comparing each cluster against all other clusters using the Wilcoxon rank-sum test in Seurat (FindAllMarkers), and the top 10 genes per cluster ranked by average $\log_2$ fold change are shown. Clusters C0–C5 correspond to neutrophil clusters, whereas C6 (monocytes/macrophages) and C7 (lymphocytes) represent low-abundance contaminating populations that were excluded from downstream neutrophil analyses.

Cluster	Annotation	Gene	Avg $\log_2$FC	Adjusted *P* value
C0	Baseline/ metabolically active	*Msra*	0.753	2.14×10^{-82}
		Zfp516	0.751	4.30×10^{-22}
		Nampt	0.735	7.73×10^{-15}
		Lrrk2	0.712	7.20×10^{-36}
		Clasp1	0.673	1.22×10^{-11}
		9830107B12Rik	0.652	1.56×10^{-11}
		Rdh12	0.644	4.34×10^{-26}
		Prkar2b	0.641	1.77×10^{-9}
		Atp11a	0.632	9.66×10^{-19}
		Mctp1	0.627	8.11×10^{-72}
C1	Granule-rich/ alarmin-expressing	*Uba52*	0.547	8.98×10^{-5}
		Ifitm1	0.488	5.41×10^{-15}
		Gapdh	0.455	7.76×10^{-36}
		Wfdc17	0.413	2.13×10^{-10}
		Ifitm2	0.409	1.81×10^{-76}
		Stfa2l1	0.400	3.01×10^{-7}
		S100a8	0.395	2.11×10^{-87}
		Lrg1	0.394	1.69×10^{-36}
		Eno1	0.393	7.11×10^{-5}
		Eif4ebp1	0.386	1.21×10^{-18}
C2	NF-κB–activated/ immediate-early	*Csf1*	1.731	1.23×10^{-142}
		Ppp1r15a	1.578	2.71×10^{-97}
		Tnfaip3	1.453	4.15×10^{-49}
		Atf3	1.416	8.67×10^{-81}
		Ptpn22	1.379	4.67×10^{-55}
		Ccrl2	1.214	1.13×10^{-105}

Cluster	Annotation	Gene	Avg $\log_2$FC	Adjusted *P* value
		Mapre2	1.204	2.56×10^{-71}
		Ptgs2	1.169	7.27×10^{-37}
		Gm6209	1.169	1.14×10^{-39}
		Plxnc1	1.156	4.30×10^{-57}
C3	Interferon-stimulated gene (ISG)–high	*Ifit3b*	4.456	$< 1 \times 10^{-300}$
		Herc6	4.216	2.56×10^{-217}
		Ifit1	4.216	2.26×10^{-298}
		Usp18	4.014	8.06×10^{-251}
		Rsad2	3.880	$< 1 \times 10^{-300}$
		Cmpk2	3.617	2.94×10^{-227}
		Slfn5	3.562	2.79×10^{-314}
		Oasl2	3.120	9.26×10^{-125}
		Isg15	2.976	1.91×10^{-293}
		Rnf213	2.700	7.48×10^{-125}
C4	Mature granule–high	*Ngp*	5.985	2.52×10^{-260}
		Stfa3	3.872	1.42×10^{-112}
		Anxa3	3.808	1.03×10^{-187}
		Gpr27	3.684	2.71×10^{-209}
		Serpinb1a	3.649	2.02×10^{-123}
		Mgst2	3.462	2.18×10^{-151}
		Ly6g	3.347	3.22×10^{-225}
		Adpgk	3.341	1.70×10^{-247}
		Stfa2	3.150	1.89×10^{-91}
		Ifitm6	3.070	7.76×10^{-174}
C5	Neutrophil–platelet aggregates	*Ppbp*	8.932	$< 1 \times 10^{-300}$
		Nrgn	8.740	$< 1 \times 10^{-300}$
		Pf4	8.459	$< 1 \times 10^{-300}$
		Tsc22d1	7.952	$< 1 \times 10^{-300}$
		Ctla2a	7.905	$< 1 \times 10^{-300}$
		Gng11	7.839	$< 1 \times 10^{-300}$
		Lsm12	1.798	1.97×10^{-9}
		March2	1.219	4.25×10^{-2}
		1810058I24Rik	1.050	$< 1 \times 10^{-100}$
		Fkbp5	0.759	1.88×10^{-3}

Cluster	Annotation	Gene	Avg log_2FC	Adjusted *P* value
C6	Monocytes/macrophages (excluded)	*Plxnb2*	13.585	$< 1 \times 10^{-300}$
		Apba1	13.263	$< 1 \times 10^{-300}$
		Ms4a6d	12.891	$< 1 \times 10^{-300}$
		Ms4a4a	12.589	$< 1 \times 10^{-300}$
		Ptpro	12.482	$< 1 \times 10^{-300}$
		Stxbp6	12.355	$< 1 \times 10^{-300}$
		Cfh	12.185	$< 1 \times 10^{-300}$
		Ms4a8a	12.102	$< 1 \times 10^{-300}$
		Fn1	11.029	$< 1 \times 10^{-300}$
		Arhgef37	11.006	$< 1 \times 10^{-300}$
C7	Lymphocytes (excluded)	*Ccr7*	13.792	$< 1 \times 10^{-300}$
		Il7r	13.741	$< 1 \times 10^{-300}$
		Trbc1	13.062	$< 1 \times 10^{-300}$
		Trbc2	12.694	$< 1 \times 10^{-300}$
		Aff3	12.571	$< 1 \times 10^{-300}$
		Themis	12.511	$< 1 \times 10^{-300}$
		Ebf1	12.493	$< 1 \times 10^{-300}$
		Thy1	12.394	$< 1 \times 10^{-300}$
		Pax5	12.333	$< 1 \times 10^{-300}$
		Lax1	12.071	$< 1 \times 10^{-300}$