Supplementary materials for:

Age but not sex modifies lymphoid immune responses in murine sepsis

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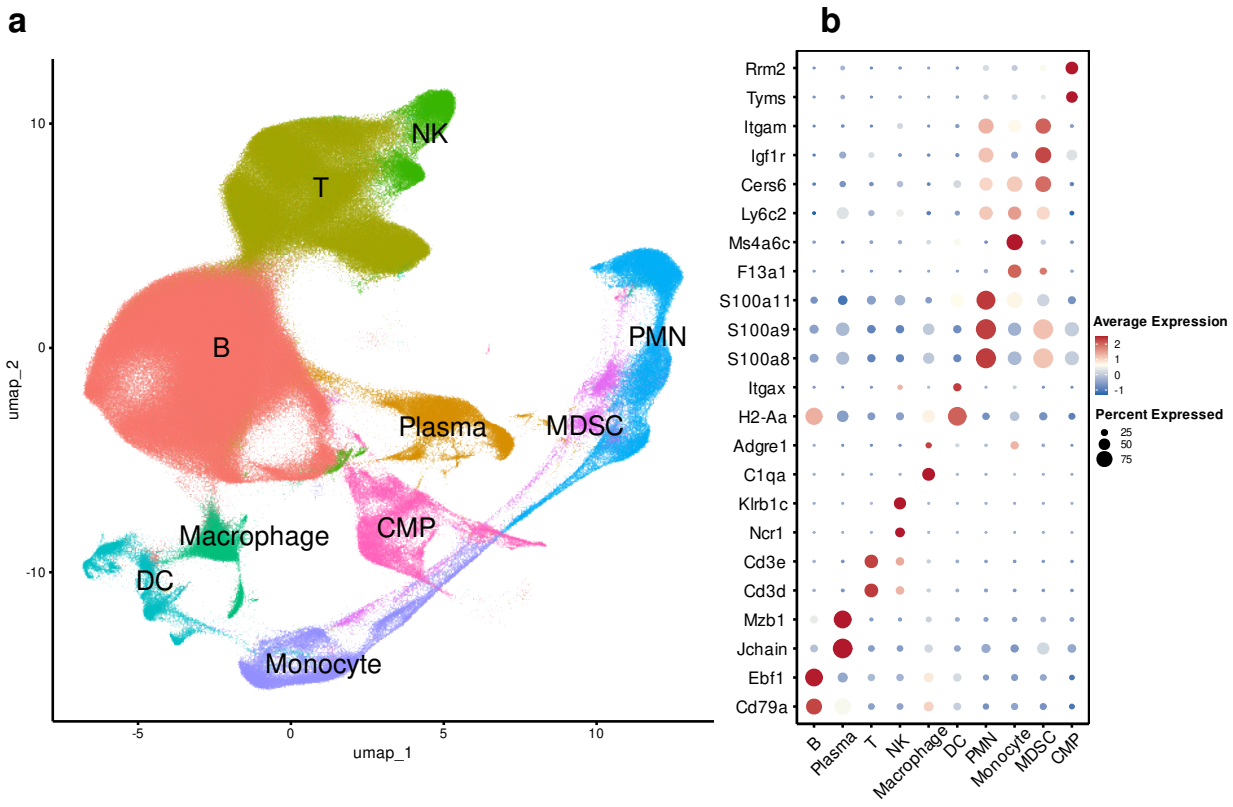
Supplementary Table 1. Summary of Murine Experimental Cohorts.

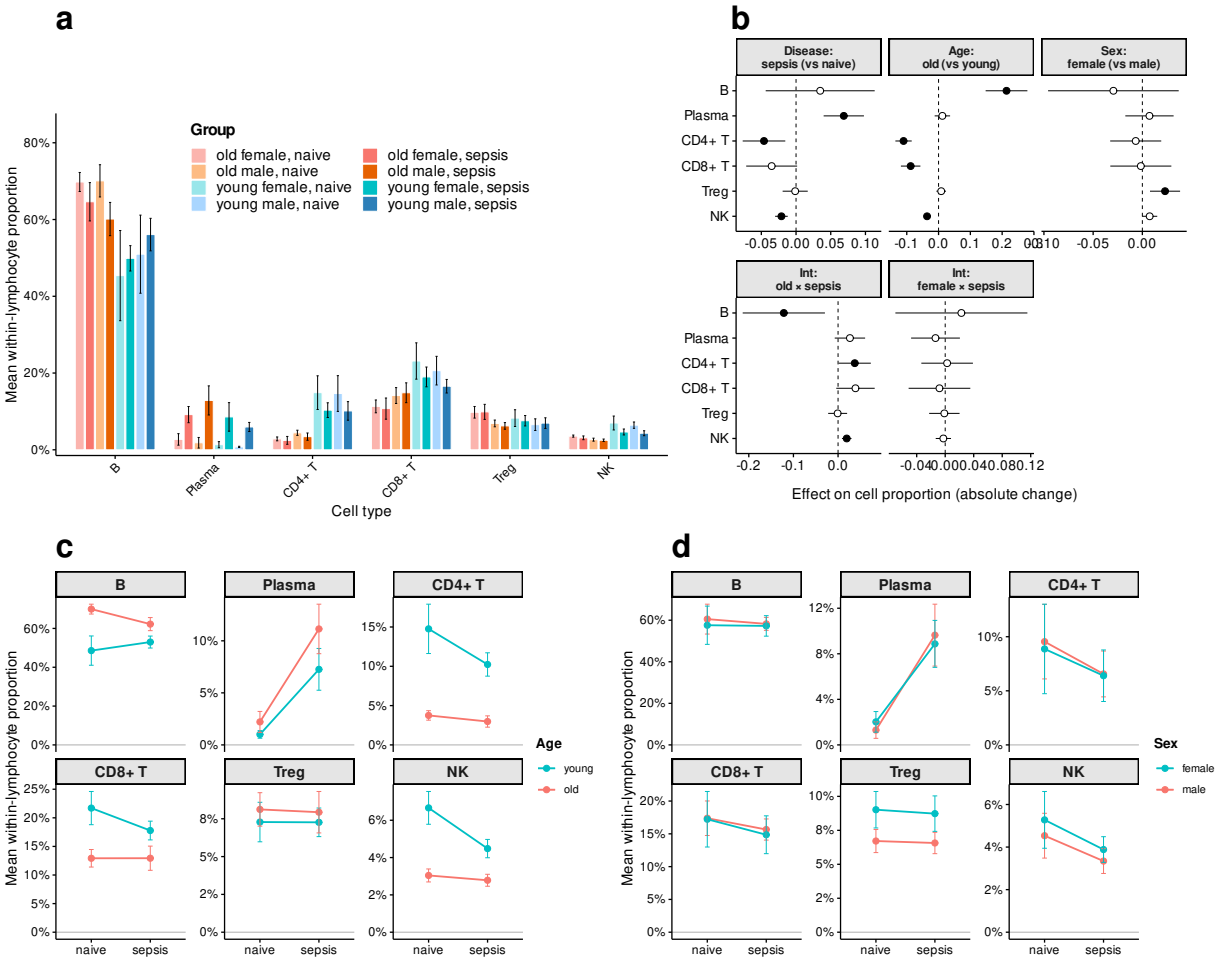
Number of biological replicates (N) included in the single-cell RNA sequencing analysis, stratified by age group (older adult vs. young adult), sex, and disease status (septic vs naive).

Age group	Sex group	Naive	Septic	Total (N)
Young Adult	Male	8	7	15
(3-4 mos)	Female	6	7	13
	Subtotal	14	14	28
Older Adult	Male	8	8	16
(18-24 mos)	Female	6	7	13
	Subtotal	14	15	29
Total		28	29	57



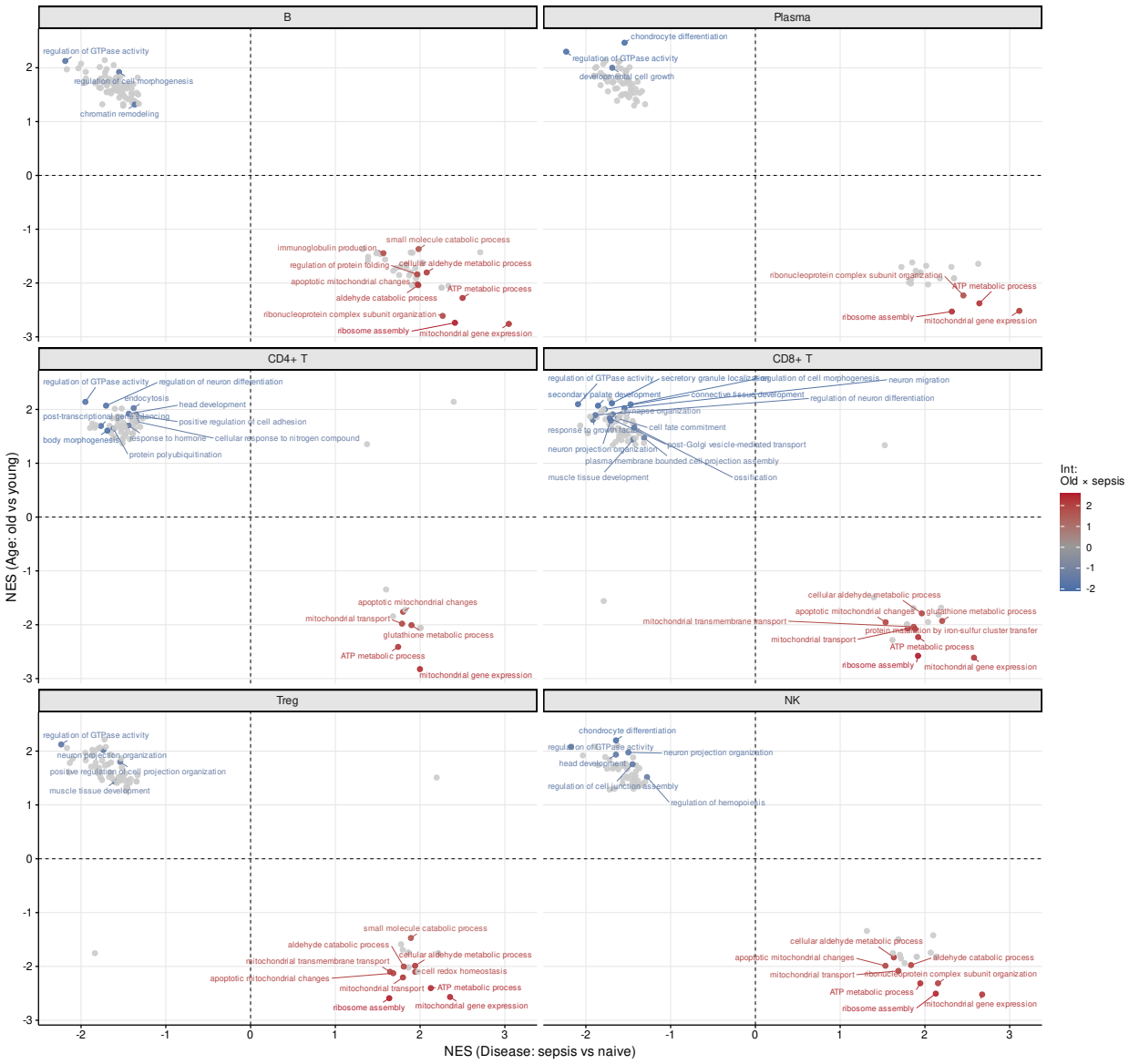
Supplementary Figure 1. UMAP visualization of the global splenic immune cell atlas and marker gene validation.

(a) UMAP projection of all single cells showing the major immune cell populations identified in the dataset, including B cells, plasma cells, T cells, NK cells, macrophages, dendritic cells (DC), polymorphonuclear cells (PMN), monocytes, myeloid-derived suppressor cells (MDSC), and common myeloid progenitors (CMP). Cell identities were assigned based on canonical marker gene expression and clustering patterns. **(b)** Dot plot showing expression of representative marker genes used for cell type annotation across the identified clusters. Dot size indicates the proportion of cells expressing the gene and color intensity indicates the average expression level.



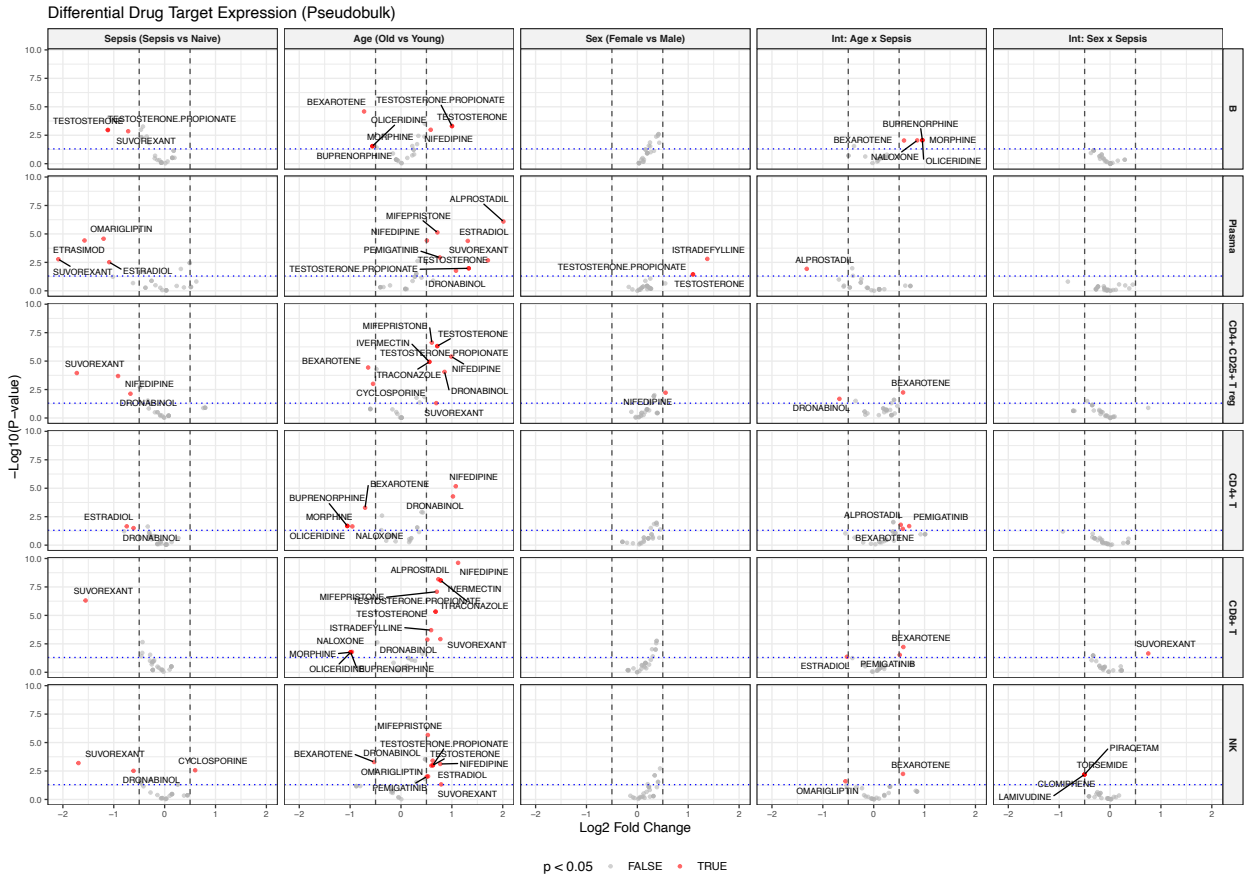
Supplementary Figure 2. Within-lymphocyte proportional analysis confirms age-dependent remodeling patterns.

(a) Stacked bar plots showing the mean proportion of each lymphocyte subset calculated within the lymphocyte compartment only (excluding myeloid cells) across all experimental groups (naive/sepsis, young/old, male/female). **(b)** Forest plots displaying the linear model estimates (β) for the main effects (sepsis, age, sex) as well as interaction terms, with cell type proportions computed within lymphocytes as the denominator. Points represent the coefficient estimate and error bars denote 95% Wald confidence intervals. Filled circles indicate statistically significant effects ($p < 0.05$); open circles indicate non-significant effects. **(c)** Interaction plots illustrating the effect of sepsis across age groups on within-lymphocyte proportions. **(d)** Interaction plots illustrating the effect of sepsis across sex groups on within-lymphocyte proportions.



Supplementary Figure 3. Gene set enrichment analysis of lymphocyte subsets showing sepsis, age, and interaction effects.

Scatter plots summarize pathway enrichment results for each lymphocyte subset, including B cells, plasma cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+CD25^+$ regulatory T cells, and NK cells. Each point represents an enriched biological pathway identified through gene set enrichment analysis. The x-axis indicates normalized enrichment score (NES) for the sepsis effect (sepsis vs naive), and the y-axis indicates the NES for the age effect (old vs young). Point color represents the magnitude and direction of the age × sepsis interaction. Selected pathways are labeled to highlight significant age × sepsis interactions.



Supplementary Figure 4. Differential Drug2Cell signatures across lymphocyte subsets.

Volcano plots showing differential drug-target enrichment derived from Drug2Cell analysis across lymphocyte populations. Each panel corresponds to a specific cell type and model term, including sepsis effect (sepsis vs naive), age effect (old vs young), sex effect (female vs male), and interaction terms (age × sepsis and sex × sepsis). Each point represents a drug-target signature, with the x-axis indicating log2 fold change and the y-axis showing $-log_{10}(p\ value)$. Drug signatures with p < 0.05 are highlighted and labeled for selected compounds.