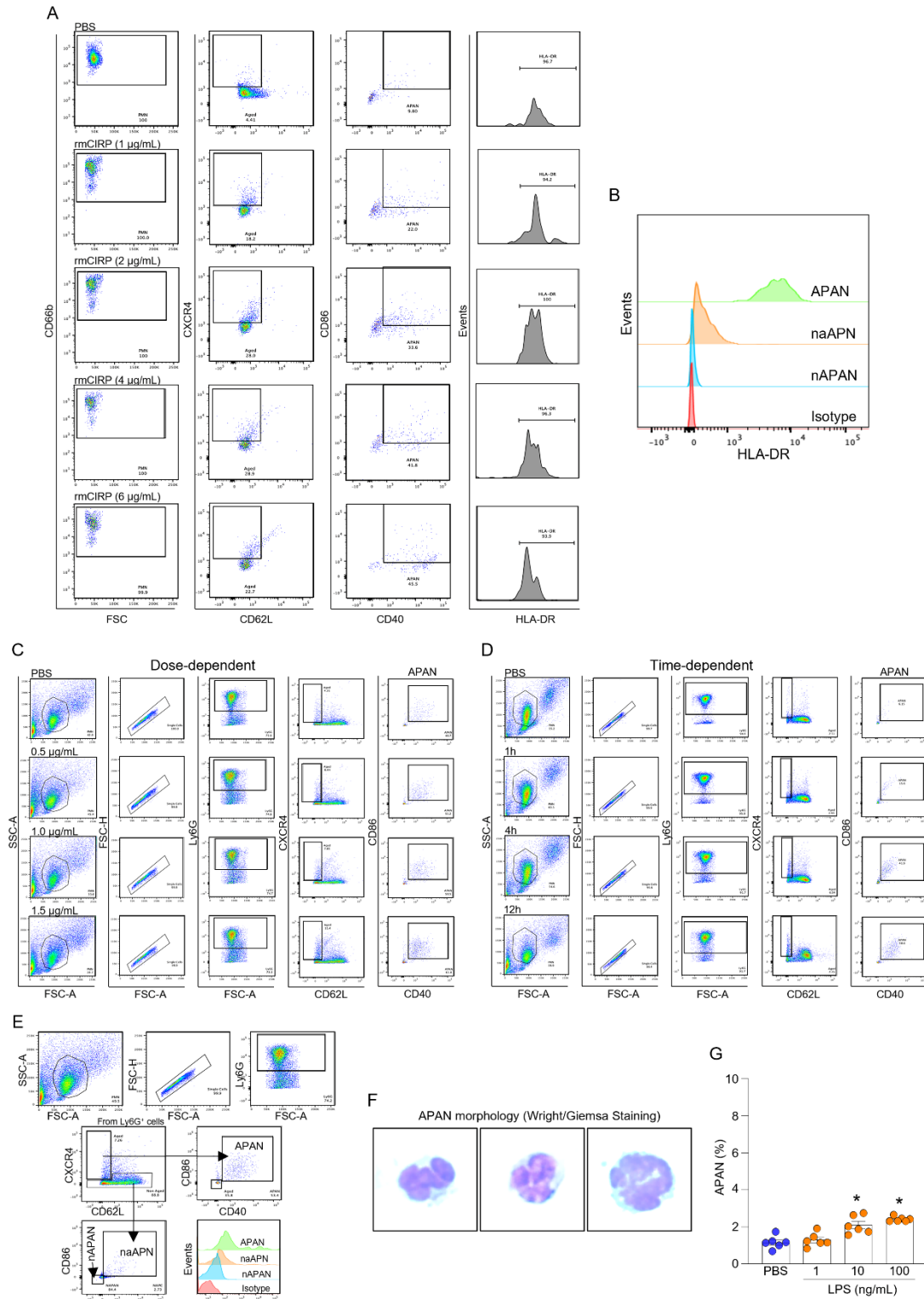


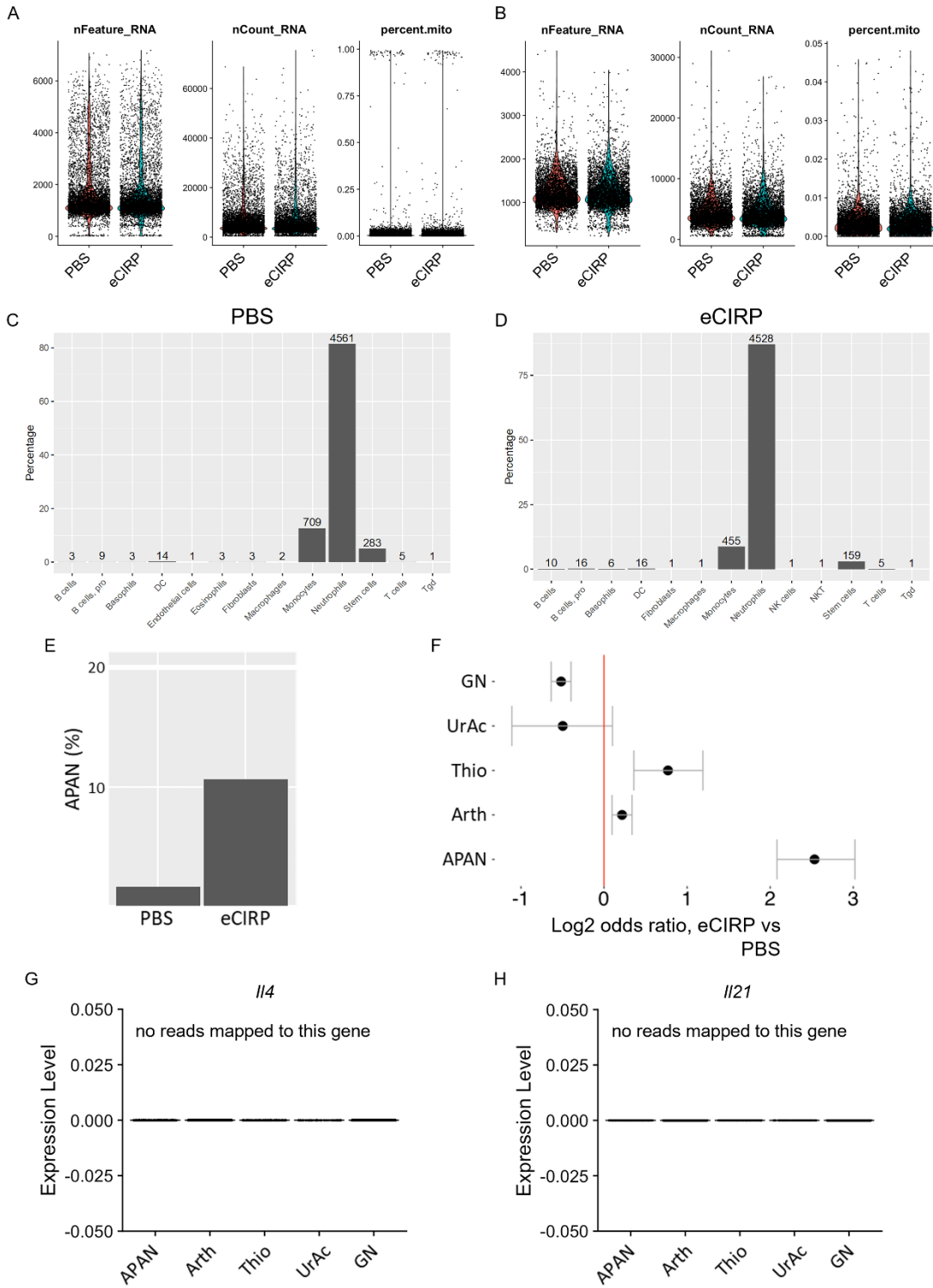
Supplemental Figure 1



**Supplemental Figure 1: Flow cytometry gating strategy for the detection of APANs and the effect of LPS on APAN formation. (A) Human peripheral blood neutrophils ( $1 \times 10^6/\text{mL}$ )**

obtained from healthy volunteers were stimulated with various doses of eCIRP for 4 h. The cells were then stained with anti-CD66b, CXCR4, CD62L, CD86, CD40, HLA-DR, and isotype Ab, followed by the detection of APANs by flow cytometry. Representative images of the gating strategy are shown. **(B)** HLA-DR expression in eCIRP-treated human blood neutrophils was assessed in various neutrophil populations (APANs, naAPNs, and nAPANs) by flow cytometry as presented in the histogram. BMDNs ( $1 \times 10^6$ ) were treated with eCIRP at different **(C)** doses and **(D)** time-points and then the cells were stained with anti-Ly6G, CD40, CD86, CXCR4, and CD62L and subjected to flow cytometry. **(E)** MHC-II expression was assessed in various cell population by flow cytometry as presented in the histogram. **(F)** Morphology of APANs. APANs were sorted from splenic cells of CLP mice, placed on a slide glass, and stained with Wright-Giemsa solution (Sigma-Aldrich). Samples were observed with microscope at  $1000\times$  magnification.  $n=3$  APAN cells are shown as representative images. **(G)** Effect of LPS on APAN formation. Mouse BMDNs ( $1 \times 10^6/\text{mL}$ ) were treated with LPS at various doses for 12 h. The frequency of APANs was then assessed by flow cytometry. Experiments were performed at least 3 times, and all data were analyzed. Data are expressed as mean  $\pm$  SEM and compared by one-way ANOVA and SNK test.  $n=6/\text{group}$ .  $*p<0.05$  vs. PBS. BMDNs, bone marrow-derived neutrophils; APAN, antigen-presenting aged neutrophil.

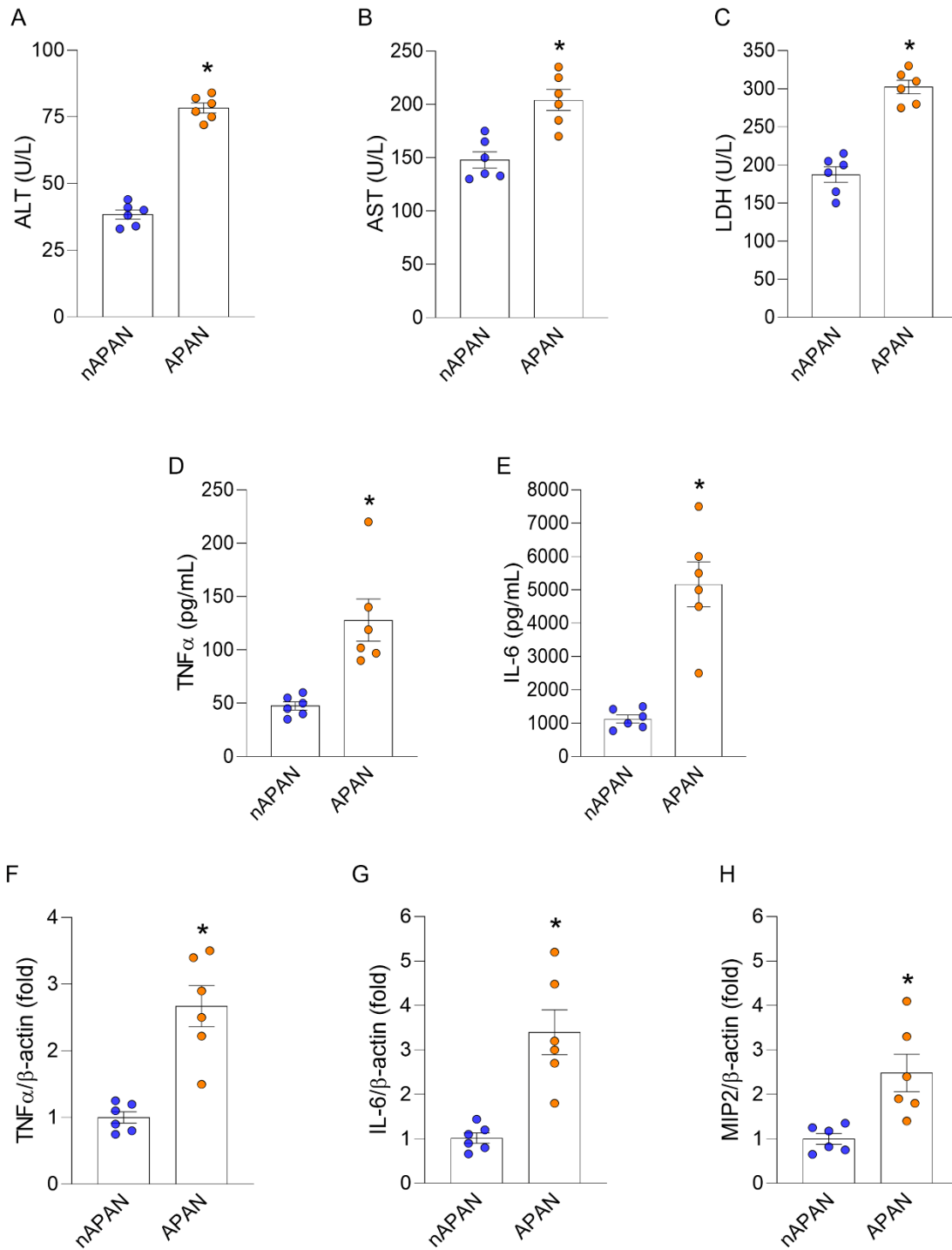
Supplemental Figure 2



**Supplemental Figure 2: Quality control data of single-cell RNA sequencing. (A)** Plots showing the number of genes detected in each cell (nFeature\_RNA), the number of molecules

detected in each cell (nCount\_RNA), and percent mitochondrial genes (percent.mito) prior to and (B) after filtering. (C) Plots showing the percentage and counts of each cell type in the PBS group and (D) in the eCIRP-stimulated group. (E) Increase in the percentage of APANs after stimulation with eCIRP. (F) eCIRP-induced changes in the percentage of cells matching ImmGen mouse neutrophil transcriptomes. (G) Graphs showing differential expression of *Il4* and (H) *Il21* across different neutrophil transcriptomes. No reads were detected to these genes. BMDN, bone marrow-derived neutrophils; APANs, antigen-presenting aged neutrophils; eCIRP, extracellular cold-inducible RNA-binding protein; Immunological Genome Project, ImmGen; unstimulated circulating neutrophils, GN; arthritic mouse neutrophils, Arth; thioglycolate-simulated peritoneal neutrophils, Thio; uric acid-simulated peritoneal neutrophils, UrAc. APAN, antigen-presenting aged neutrophil; eCIRP, extracellular cold-inducible RNA-binding protein.

Supplemental Figure 3



**Supplemental Figure 3:** BMDNs were isolated from WT mice and stimulated with eCIRP for 6 h and after that the cells were stained with anti-Ly6G, CXCR4, CD62L, CD40, and CD86 Ab and then APANs and nAPANs were sorted by FACS. A total of  $1 \times 10^6$  each of APANs, and

nAPANs were adoptively transferred into neutropenic mice (PMN<sup>DTR</sup> mice) via retro-orbital injection at the time of CLP. At 20 h later, blood and lungs were collected to assess several parameters. Serum **(A)** ALT, **(B)** AST, and **(C)** LDH were determined using specific colorimetric enzymatic assays. Serum **(D)** TNF $\alpha$ , and **(E)** IL-6 levels were measured by ELISA. Lung mRNA levels of **(F)** TNF $\alpha$ , **(G)** IL-6, and **(H)** MIP2 were measured by real-time PCR. Data are expressed as means  $\pm$  SE (n=6 mice/group) and compared by one-way ANOVA and SNK method (\*p<0.05 vs. CLP+nAPAN-injected mice). APAN, antigen-presenting aged neutrophil; nAPAN, non-antigen-presenting aged neutrophil; CLP, cecal ligation puncture; ALT, alanine aminotransferase; AST, aspartate amino transferase; LDH, lactate dehydrogenase; IL, interleukin; TNF, tumor necrosis factor; MIP2, macrophage chemoattractant protein 2.

Supplemental Table 1: Clinical parameters of patients with sepsis

Patient	Age	Sex	SOFA*	APACHE-II*	Hours <sup>#</sup>	Initiating Clinical Events
1	65	F	7	26	21	Iatrogenic injury of transverse colon with associated feculent peritonitis
2	71	F	5	14	20	Perforated ischemic bowel secondary to parastomal hernia with associated feculent peritonitis, incidentally COVID-19 <sup>+</sup>
3	66	M	6	12	22	Perforated appendicitis
4	69	F	8	19	19	Deep surgical site infection after incarcerated hernia repair

\*SOFA and APACHE-II scores upon presentation; <sup>#</sup>Time from identification of sepsis to blood sample collection, hours

Supplemental Table 2: WBC and neutrophil counts in WT and PMN<sup>DTR</sup> mice (MRP8-Cre<sup>+</sup> × ROSA-iDTR<sup>K1</sup>)

Mice strains	WBCs (x10 <sup>3</sup> cells/μL)	Neutrophils (x10 <sup>3</sup> cells/μL)
WT	7.58 ± 3.79	2.93 ± 1.46
PMN <sup>DTR</sup> mice (MRP8-Cre <sup>+</sup> × ROSA-iDTR <sup>K1</sup> )	4.12 ± 2.06*	0.76 ± 0.38*

N=3/5 mice/group; Student's T-Test; \*p<0.05 vs WT mice

Supplemental Table 3: Mouse primer sequences

Gene	Forward (5'-3')	Reverse (5'-3')
TNF $\alpha$	AGACCCTCACACTCAGATCATCTTC	TTGCTACGACGTGGGCTACA
IL-6	CCGGAGAGGAGACTTCACAG	CAGAATTGCCATTGCACAAC
KC	GCTGGGATTCACCTCAAGAA	ACAGGTGCCATCAGAGCAGT
MIP2	CCCTGGTTCAGAAAATCATCCA	GCTCCTCCTTTCCAGGTCAGT
$\beta$ -actin	CGTGAAAAGATGACCCAGATCA	TGGTACGACCAGAGGCATACAG