The systemic immune response to viral infection is shaped by master transcription factors, such as NF-κB, STAT1, or PU.1. Although long noncoding RNAs (lncRNAs) have been suggested as important regulators of transcription factor activity, their contributions to the systemic immunopathologies observed during SARS-CoV-2 infection have remained unknown. Here, we employed a targeted single-cell RNA sequencing approach to reveal lncRNAs differentially expressed in blood leukocytes during severe COVID-19. Our results uncover the lncRNA PIRAT (PU.1-induced regulator of alarmin transcription) as a major PU.1 feedback-regulator in monocytes, governing the production of the alarmins S100A8/A9, key drivers of COVID-19 pathogenesis. Knockout and transgene expression, combined with chromatin-occupancy profiling, characterized PIRAT as a nuclear decoy RNA, keeping PU.1 from binding to alarmin promoters and promoting its binding to pseudogenes in naïve monocytes. NF-κB-dependent PIRAT down-regulation during COVID-19 consequently releases a transcriptional brake, fueling alarmin production. Alarmin expression is additionally enhanced by the up-regulation of the lncRNA LUCAT1, which promotes NF-κB–dependent gene expression at the expense of targets of the JAK-STAT pathway. Our results suggest a major role of nuclear noncoding RNA networks in systemic antiviral responses to SARS-CoV-2 in humans.

Significance

SARS-CoV-2–infected patients often display characteristic changes in the production of immune mediators that trigger life-threatening courses of COVID-19. The underlying molecular mechanisms are not yet fully understood. Here, we used single-cell RNA sequencing to investigate the involvement of the emerging class of long regulatory RNA in COVID-19. Our data reveal that a previously unknown regulatory RNA in the nucleus of immune cells is altered after SARS-CoV-2 infection. The degradation of this RNA removes a natural brake on the production of critical immune mediators that can promote the development of severe COVID-19. We believe that therapeutic intervention in this nuclear RNA circuit could counteract the overproduction of disease-causing immune mediators and protect against severe COVID-19.
long-noncoding RNAs (lncRNAs) are increasingly recognized as regulators of mammalian immune responses. Defined as ncoding transcripts > 200 nts, lncRNAs constitute a heterogeneous category of RNA, participating in protein complex assembly, disintegration, and turnover (12–14). So far, only a minor fraction of the ~20,000 human lncRNAs has been characterized and their roles in the human immune system are only beginning to be explored (14). Among the few characterized lncRNAs in this context is MalL1, which associates with the ubiquitin-reader OPTN to promote TBK1–dependent IRF3 phosphorylation, and thus type I IFN immunity (14). GPR38C, PACER, and CARLR regulate proinflammatory gene expression by adjusting NF-kB p50/p65 expression and activity (15–17). Despite the emerging roles of noncoding RNAs in immunity, however (18), the exploration of lncRNA mechanisms contributing to severe COVID-19 has lagged behind.

Here, we used scRNA-seq to study lncRNAs involved in the systemic immunopathologies during COVID-19. Our results highlight the lncRNA PIRAT (PU.1-induced regulator of alarmin transcription) as a regulator of exacerbated PU.1-dependent alarmin production during SARS-CoV-2 infection. A single nucleotide polymorphism (SNP) in the PIRAT locus has been associated with hematological malignancies (19); the function of PIRAT, however, has remained unknown. We characterize PIRAT as a nuclear RNA primarily expressed in CD14+ monocytes. PIRAT recruits the PU.1 transcription factor to pseudogenes and suppresses PU.1-binding to the S100A8 and S100A9 alarmin promoters. NF-kB–triggered down-regulation of PIRAT in monocytes upon PAMP stimulation or during severe COVID-19 consequently removes a transcriptional break on alarmin production. PIRAT down-regulation is accompanied by the up-regulation of the lncRNA LUCAT1 in monocytes, which propels alarmin induction in an NF-kB–dependent manner at the expense of the JAK-STAT pathway. Up-regulation of LUCAT1 and down-regulation of PIRAT thus alters PU.1, NF-kB, and JAK-STAT–dependent gene-expression in favor of the production of mediators associated with severe COVID-19.

Results
Identification of COVID-19 Relevant Myeloid lncRNA Signatures. To chart candidate long intergenic noncoding RNAs (lincRNAs) relevant to disturbed myeloid immunity in COVID-19, we consolidated RNA-seq data from several sources, followed by in-depth scRNA-seq profiling (Fig. 1A). At first, leukocyte-specific mRNAs and lincRNAs were narrowed down using Illumina Human Bodymap data (Fig. 1A). Confirming successful extraction of leukocyte-specific RNAs from these datasets, pathway analysis revealed an exclusive enrichment of immune-relevant terms, such as “hematopoietic cell lineage,” “cytokine–cytokine receptor interaction,” or “chemokine signaling pathway” (SI Appendix, Fig. S1 A and B). We then charted expression of these transcripts among three publicly available replicates of peripheral blood monocyte, granulocyte, B cell, natural killer (NK) cell, and T cell RNA-seq profiles (20, 21). Principal component analysis (PCA) and hierarchical clustering successfully discriminated the major leukocyte compartments, based on their lincRNA and mRNA profiles, respectively (Fig. 1B and SI Appendix, Fig. S1 C and D). To confirm the cell-type specificity of the interrogated myeloid and lymphoid lincRNAs (SI Appendix, Table S1), we studied their expression in blood-derived macrophages, dendritic cells, monocytes, granulocytes, NK cells, B cells, and naïve (CD45RO+ or memory (CD45RO+) T cells. qRT-PCR confirmed preferential expression of LINC00211 (henceforth PIRAT), LUCAT1, and AC064805.1 in myeloid cells, whereas LINCO0295, LINCO2446, and LINCO0861 were confirmed as lymphoid transcripts (Fig. 1C and SI Appendix, Fig. S1 E–J). Among the lymphoid lincRNAs, LINCO2446 was particularly abundant in CD8+/CD45RO+ T cells, indicating a specific role in the CD8-memory niche (SI Appendix, Fig. S1 H and J). Among the myeloid lincRNAs, our attention was caught by PIRAT, since a SNP in the PIRAT locus (rs4670221-G, P value 3 × 10–16) had been associated with hematological alterations (19). The function of PIRAT, however, has remained unknown. Besides PIRAT, LUCAT1 was selected as a candidate lncRNA relevant to myeloid immunity in COVID-19 due to its particularly high expression in monocytes and granulocytes.

To determine at which stages of myeloid ontogeny both lincRNAs become relevant, we traced their expression from hematopoietic stem cells (HSCs) to mature leukocytes, using Blueprint RNA-seq profiles (22). Expression of PIRAT declined upon HSC differentiation into multipotent progenitors and, similar to LUCAT1, remained low during the common myeloid and granulocyte/monocyte progenitor stages (Fig. 1D). Expression of both lincRNAs strongly increased in mature monocytes and neutrophils (Fig. 1D). Coexpression analysis using RNA-seq data from Fig. 1B suggested PIRAT to depend on a network driven by the myeloid master transcription factor PU.1 (Fig. 1E and SI Appendix, Fig. S2 A and B). Among the PIRAT-coexpressed genes were the PU.1-dependent alarmins S100A8 and S100A9 (Fig. 1E and SI Appendix, Fig. S2B), which play a key role in COVID-19 (5–7, 23, 24). Dependence of PIRAT but not LUCAT1 on PU.1 was confirmed by PU.1 knock-down in THP1 monocytes (Fig. 1F). Further underscoring their differential dependence on myeloid expression programs, PIRAT was down- and LUCAT1 was up-regulated in an NF-kB–dependent manner upon monocyte immune-activation (Fig. 1F and G and SI Appendix, Fig. S2C). Thus, PIRAT and LUCAT1 are myeloid signature lncRNAs, activated during late hematopoiesis and differentially depending on PU.1 and NF-kB.

Single-Cell Resolved Myeloid lincRNA Responses to SARS-CoV-2 Infection. Recent scRNA-seq studies have revealed profound changes in myeloid coding gene-expression in severe COVID-19. To dissect the contributions of myeloid lincRNAs, such as PIRAT and LUCAT1 to these alterations, we performed BD Rhapsody scRNA-seq of PBMCs from control and severe COVID-19 patients (World Health Organization [WHO] grade 4) using an immune-response panel combined with a custom lncRNA panel (Fig. 2A) (patients listed in SI Appendix, Table S2). For qRT-PCR–based validation, we included PBMC samples from a second cohort without WHO grades available (SI Appendix, Table S3). qRT-PCR confirmed the expected induction of immune-response markers CXCL2 and IL-6 in COVID-19 patients from this cohort (Fig. 2B). scRNA-seq analysis of PBMCs from two control and two COVID-19 patients (WHO-graded cohort) (SI Appendix, Table S2) charted all expected myeloid and lymphoid populations and discriminated four monocyte populations along the CD14–, CD16–, and HLA-expression scheme (Fig. 2 C and D and SI Appendix, Fig. S3). FACS confirmed the reported increase in immature CD15+/CD45RO– neutrophils and the reduction of CD14+/CD45RO+ classic monocytes during severe COVID-19 (WHO grade 4), indicative of myeloid exhaustion (5) (Fig. 2E and SI Appendix, Table S2). Differential gene expression and Reactome pathway analysis confirmed the proinflammatory activation of classic, nonclassic, and intermediate monocytes during COVID-19 (Fig. 2F and SI Appendix, Fig. S4).
Analysis of lincRNA scRNA-seq profiles confirmed the abundance of B cell proliferation promoting lincRNA BIC (25) in B lymphocytes. Furthermore, BIC was up-regulated in dendritic cells during COVID-19, in line with its role in antigen-presenting cell activation (Fig. 2G and SI Appendix, Fig. S5A) (26). Moreover, we observed the expected induction of type I IFN-inducing lincRNA MaIL1 (14) in all monocyte populations, but also in B cells from infected patients (Fig. 2G and SI Appendix, Fig. S5B).

scRNA-seq also confirmed the strict myeloid expression of LUCAT1 and PIRAT and suggested preferential expression in CD14+ monocytes (Fig. 2G and SI Appendix, Fig. S5C and D). Similarly, S100A8 and A9, which were coexpressed with PIRAT (Fig. 1E), were particularly highly expressed in myeloid cells (SI Appendix, Figs. S3D and S5E and F). Unlike in classic and intermediate monocytes, LUCAT1 and PIRAT expression remained low in nonclassic CD16+ monocytes (Fig. 2G).

Whereas LUCAT1 expression was up-regulated in classic and intermediate monocytes during COVID-19, PIRAT was down-regulated, reminiscent of the differential regulation of both lincRNAs in response to immune agonists (Fig. 2G compared to Fig. 1F and G). Preferential expression of both lincRNAs in classic monocytes and opposite regulation during COVID-19 was confirmed in qRT-PCR experiments (Fig. 2H and I). These results confirm an imbalanced myeloid compartment during severe COVID-19 and reveal LUCAT1 and PIRAT as CD14+ monocyte-specific lincRNAs, up- and down-regulated upon SARS-CoV-2 infection, respectively.

LUCAT1 Attenuates STAT-Target Expression in Favor of Proinflammatory Genes in COVID-19. While our manuscript was in preparation, LUCAT1 was reported to act as a negative feedback regulator of JAK-STAT–dependent IFN immunity (27). LUCAT1 is a
massively alternatively spliced lincRNA encoded on chromosome 5 (Fig. 3A) (27). Subcellular fractionation and qRT-PCR, based on the first and most frequently used exon, indicated a primarily nuclear localization in CD14+ monocytes (Fig. 3B and SI Appendix, Fig. S6A). To study its role in COVID-19, we silenced LUCAT1 in THP1 monocytes using CRISPR-interference (CRISPRi), followed by RNA-seq analysis and compared the results to patient scRNA-seq data. In line with our primary cell data (Fig. 1F), LUCAT1 expression increased in THP1 cells upon 4- or 16-h treatment with viral RNA analog polyI:C and bacterial LPS. In LUCAT1-CRISPRi cells, LUCAT1 expression was blunted under all conditions (Fig. 3C). Since LUCAT1 up-regulation was most pronounced after 4-h double-stimulation with polyI:C and LPS, this broad immune-activatory condition was selected for RNA-seq analysis. 114 mRNAs were up- and 229 were down-regulated ≥10-fold in PAMP-activated LUCAT1-deficient compared to control cells (Fig. 3D).

In agreement with previous reports (27), pathways relating to JAK-STAT–dependent receptors (e.g., IL-9R, IL-15R, or IL-2R) were enriched upon LUCAT1 knockdown (Fig. 3D and SI Appendix, Fig. S6B). mRNAs down-regulated upon LUCAT1 knockdown were associated with proinflammatory pathways, such as “TLR-signaling,” “chemokine receptor,” or “NF-kB signaling” (SI Appendix, Fig. S6C). To investigate the relevance of LUCAT1 in the context of SARS-CoV-2 infections, we compared mRNAs regulated twofold or greater (up or down) upon LUCAT1 knockdown in THP1 cells with mRNAs regulated twofold or greater (up or down) in classic and intermediate monocytes during COVID-19 (scRNA-seq data). Both datasets were reduced to mRNAs detected in both the THP1 CRISPRi and the scRNA-seq experiments (Fig. 3E); 50.8% of the mRNAs regulated in monocytes during COVID-19 were affected by LUCAT1 silencing (Fig. 3F). These mRNAs were associated with pathway terms, such as “rheumatoid arthritis,” “immune system,” or “NF-kB signaling” (SI Appendix, Fig. S6D and E), indicating a broad influence of LUCAT1 on peripheral immunity during infection. When restricting the analysis to mRNAs up-regulated twofold or greater in monocytes during COVID-19 (Figs. 2B and 3F–H and SI Appendix, Fig. S6F), the same dichotomy as in Fig. 3D was observed, with LUCAT1 deficiency...
lifting the expression of STAT-downstream genes (e.g., CXCR4 and NAMPT) and reducing classic proinflammatory marker expression (e.g., CXCL2 and CXCL8) (Fig. 3F and SI Appendix, Fig. S7A).

These results were confirmed in a second LUCAT1-CRISPRi cell line, using an independent guide RNA (gRNA) design (SI Appendix, Fig. S7A). BAY-11-7082 and Ruxolitinib inhibitor experiments confirmed the dependence of LUCAT1-controlled proinflammatory markers CXCL2 and CXCL8 on the NF-κB but not the JAK-STAT pathway, whereas CXCR4 and NAMPT were JAK-STAT-dependent (Fig. 3J and SI Appendix, Fig. S7B). LUCAT1 itself was found to depend both on the NF-κB and the JAK-STAT pathway (SI Appendix, Fig. S7B and Fig. 1G). Thus, LUCAT1 up-regulation during monocyte activation in COVID-19 likely restrains JAK-STAT signaling, in favor of NF-κB-dependent immunity. Interestingly, treatment of monocytes with the STAT-inhibitor and COVID-19 drug (28) Ruxolitinib not only reduced the expression of STAT-targets CXCR4 and NAMPT, but also increased the expression of proinflammatory markers CXCL8 and CXCL2 (Fig. 3J and SI Appendix, Fig. S7A). This suggests that STAT inhibition by LUCAT1 not only restrains STAT-target expression but also eliminates a STAT-dependent break on NF-κB target genes (Fig. 3J).

**COVID-Suppressed lincRNA PIRAT Antagonizes Alarmin Expression in Monocytes.** We next deciphered the function of the uncharacterized lincRNA PIRAT in human monocytes and the reasons

---

*Fig. 3. Role of LUCAT1 in monocytes. (A) ENSEMBL-annotated LUCAT1 isoforms. (B) LUCAT1 subcellular localization (qRT-PCR). (C) LUCAT1 expression in control and knockdown THP1 monocytes (qRT-PCR, relative to unstimulated control). (D) RNA-seq analysis (transcripts regulated ≥10-fold, Kyoto Encyclopedia of Genes and Genomes/Reactome pathways) of LUCAT1 knockdown versus control THP1 monocytes, activated for 4 h with poly:C and LPS. R1 and R2 = replicates 1 and 2. (E) Overlap of gene regulations twofold or greater (up or down) in monocytes during COVID-19 (scRNA-seq populations 0, 3, and 9) (Fig. 2C) and upon LUCAT1 knockdown in THP1 cells. (F, Left) COVID-19-induced (twofold or greater) mRNA_ in monocyte populations from Fig. 2C. (Right) Regulation of the same mRNAs in dataset from D. (G) Expression of LUCAT1-controlled mRNAs in scRNA-seq data. (H) Same as Fig. 2B, but for CXCL8 and NAMPT. (I) Ruxolitinib and BAY-11-7082 sensitivity of selected mRNAs (monocytes; PAMP = 4 h LPS + poly:C). Fold-changes relative to unstimulated control. (J) Model of LUCAT1 function. (K) Rescue of CXCL8 dysregulation in LUCAT1-deficient THP1 cells upon 2-h Ruxolitinib pretreatment. (C, I, and K) One-way ANOVA, three independent experiments. (H) Two-tailed Student’s t test. *p ≤ 0.05; **p ≤ 0.01.*
for its opposite regulation compared to LUCAT1 in COVID-19. First, we mapped the exact PIRAT architecture by RACE-PCR. Deviating from the GENCODE annotation, 5’ and 3’ RACE revealed a two-exon structure in primary monocytes (Fig. 4A and SI Appendix, Fig. S8). ENCODE mono-mRNA RNA-seq, DNAseq, and chromatin immunoprecipitation-sequencing (ChiP-seq) data confirmed a DNasel hypersensitive site at the mapped PIRAT 5’-end and H3K4 trimethylation and RNA-seq coverage across the RACE-defined gene body, hallmarks of transcriptionally active regions (Fig. 4A). The CPC2 algorithm confirmed low coding potential of the defined PIRAT sequence, similar to the noncoding RNAs XIST and HOTAIR, and different from mRNAs (ACTB, GAPDH, IL1B) (Fig. 4B). Copy-number enumeration by absolute quantification qRT-PCR indicated ~40 to 60 PIRAT copies per primary CD14⁺ monocyte (Fig. 4C and SI Appendix, Fig. S9 A-Δ), similar to other functional lncRNAs (14, 29). Subcellular fractionation characterized PIRAT as a nuclear-retained lncRNA (Fig. 4D), which was further corroborated by RNA-FISH (SI Appendix, Fig. S9F). PIRAT sequence conservation exceeded 90% in the genomes of catarrhine primates but dropped to 33.5% in mice (Fig. 4E and SI Appendix, Fig. S9G). Thus, PIRAT is a two-exon nuclear lncRNA, stably maintained during higher primate evolution.

To study the function of PIRAT, we generated PIRAT promoter-deficient THP1 cells using CRISPR/Cas9 (Fig. 4F and SI Appendix, Fig. S9H) and cells overexpressing PIRAT from a lentiviral backbone (Fig. 4F). RNA-seq uncovered dozens of mRNAs regulated (twofold or greater) into opposite directions upon PIRAT knockout and overexpression, respectively (Fig. 4G). Among the top 10 PIRAT-suppressed genes were the PU.1-dependent alarmins S100A8 and S100A9 (Fig. 4G). S100A8 and A9 form a heterodimer, referred to as calprotectin, which plays important roles in the immune system, ranging from promyelopoietic to immunomodulatory and metabolic functions, relevant to a wide range of diseases (30), including COVID-19 (5, 7, 23, 24). S100A8 and S100A9 are coexpressed with PIRAT at the PBMC whole-population level (Fig. 1E) but negatively correlate with PIRAT expression at the single-cell level (SI Appendix, Fig. S10A). This further hints at a role of PIRAT as an intrinsic negative regulator of a PU.1-driven module, driving S100A8, S100A9, and PIRAT expression in myeloid cells. Beyond S100A8/A9, the suppressive effect of PIRAT extended to other PU.1-driven genes (Fig. 4H and SI Appendix, Fig. S10B and Table S4). Reciprocally, genes suppressed by PU.1, such as ITGAX (CD11c) or CHI3L1 (31–33), were derepressed upon PIRAT knockout (Fig. 4H). Thus, PIRAT is a myeloid nuclear RNA, restraining the expression of PU.1-driven genes, such as S100A8 and S100A9.

Next, we overlaid the RNA-seq profiles of PIRAT-manipulated cell lines with the scRNA-seq profiles of COVID-19 and control patient PBMCs. Among all mRNAs up- or down-regulated twofold or greater during COVID-19 (scRNA-seq data, classic

![Fig. 4. Role of PIRAT in human monocytes.](https://www.pnas.org)
monocytes) or upon PIRAT expression-manipulation (THP1 monocytes), 33 were detected in both datasets. The overlap of mRNAs regulated twofold or greater in both datasets was 12.1% (four mRNAs) (Fig. 5A and SI Appendix, Fig. S11A), and these mRNAs fell into immune-relevant categories, such as “Toll-like receptor cascades” (SI Appendix, Fig. S11B). Similarly, COVID- and PIRAT-specific regulations, respectively, were associated with immune- and infection-specific terms (SI Appendix, Fig. S11 C and D). Among the mRNAs up-regulated during COVID-19 (scRNA-seq data), S100A8 and S100A9 experienced the strongest derepression upon PIRAT knockout (Fig. 5 B and C). Vice versa, genes down-regulated in CD14+ monocytes during severe COVID-19 were under significant positive influence by PIRAT, headed by the PU.1-suppressed genes IRF5 and ITGAX (Fig. 5 B and C). ITGAX (CD11c) is a cell surface integrin of inflammatory monocytes, elevated in mild courses of COVID-19 (5). IRF5 is a transcription factor involved in the production of type I IFN and other immune mediators and has been suggested as a therapy-relevant COVID-19 marker (34, 35). Thus, disease-relevant genes activated and suppressed by PIRAT are reciprocally regulated by PU.1 and in COVID-19. This notion was further corroborated in qRT-PCR and FACS validations, which confirmed the control of S100A8, S100A9, ITGAX, and IRF5 by PIRAT and regulation of these factors during COVID-19 (Fig. 5 D–F and G and SI Appendix, Fig. S11 E and F). Finally, knockdown of PU.1 in THP1 monocytes using CRISPR interference verified the dependence not only of PIRAT, but also of S100A8 and S100A9 on this transcription factor (Fig. 1E and SI Appendix, Fig. S11G). These data suggest PIRAT as a negative feedback regulator of PU.1, limiting S100A8 and A9 alarmin expression in monocytes at baseline. NF-κB–dependent down-regulation of PIRAT (Fig. 1G) consequently removes a molecular break on the production of alarmins.

To determine the reason for the opposite regulation of PIRAT and LUCAT1 during COVID-19, we compared the influence of both lincRNAs on genes regulated in CD14+ monocytes in patients. Comparison of all mRNAs regulated twofold or greater (up or down) during COVID-19 in CD14+ monocytes and upon silencing of either lincRNA in THP1 cells (overlaps from Venn diagrams in Figs. 3E and 5A), suggested only a small overlap in the regulatory networks of PIRAT and LUCAT1 (Fig. 5H). In line with our assumptions (Figs. 3 H–K and J, ENRICHR transcription factor analysis predicted COVID-relevant genes up- and down-regulated upon LUCAT1-loss to depend on STAT and NF-κB (ReA), respectively, whereas PIRAT-controlled genes were predicted to depend on IRF8 (rank 1) and PU.1 (＝ SP1I, rank 2) (SI Appendix, Fig. S12A). Among the few mRNAs influenced by both lincRNAs was S100A8, which is up-regulated upon loss of PIRAT in naïve cells, and down-regulated upon LUCAT1 silencing in PAMP-challenged cells (Fig. 5 H and J, S100A9 was down-regulated in only one RNA-seq replicate after LUCAT1 silencing (0.509- and 1.614-fold); qRT-PCR, however, confirmed a significant reduction of S100A9 expression in LUCAT1-deficient cells, similar to S100A8 (Fig. 5J and SI Appendix, Fig. S12B). Up-regulation of both alarmins upon PAMP-stimulation was NF-κB–dependent (Fig. 5J), in line with the elimination of STAT-dependent NF-κB target suppression by LUCAT1 (Fig. 3) and in line with the NF-κB–dependent down-regulation of PIRAT during monocyte activation (Fig. 1G).

Of note, STAT-inhibition in LUCAT1-deficient cells partially restored S100A8 and A9 expression (SI Appendix, Fig. S12C). IRF5, a PIRAT target, predicted by our RNA-seq data not to be influenced by LUCAT1, was confirmed to remain unaffected by LUCAT1-silencing or JAK-STAT inhibition (Fig. 5 H–J). Taken together, our results suggest PIRAT and LUCAT1 to regulate largely discrete sets of genes in CD14+ monocytes during COVID-19, with LUCAT1 inhibiting STAT and promoting NF-κB target gene expression and PIRAT serving as a withdrawable inhibitor of PU.1-dependent programs. S100A8 and S100A9 depend both on the NF-κB pathway, promoted by LUCAT1, and the PU.1-pathway, suppressed by PIRAT. As a result, the opposite regulation of both lincRNAs in COVID-19 likely supports the production of these critical alarmins.

PIRAT Suppresses PU.1 Binding to Alarmin Promoters and Fosters Its Association with Pseudogenes. To interrogate the molecular mechanism of alarmin control by PIRAT, we investigated the interaction of this lincRNA with chromatin and PU.1. Antisense-purification of PIRAT-occupied chromatin from primary monocytes by chromatin isolation by RNA purification (ChIRP) (Fig. 6A) recovered PIRAT RNA and verified cross-linking of PIRAT to its own site of transcription (Fig. 6B and C). Refusing a model where the lincRNA controls PU.1 directly at its target gene promoters, PIRAT did not bind to the PU.1 occupied region upstream of the S100A8 gene (Fig. 6D and SI Appendix, Fig. S13A). In search of alternative explanations, we recorded the genome occupancy profile of PIRAT in CD14+ monocytes using ChIRP-seq. Peak-calling, comparing PIRAT ChIRP-seq signals to a control ChIRP-seq library, revealed PIRAT to occupy multiple sites along the uncharacterized REXO1L pseudogene array at chromosome 8q21.2 (Fig. 6E and SI Appendix, Table S5). Comparison to matched ENCODE CD14+ monocyte ChIP-seq data uncovered a repetitive pattern of alternating PIRAT and PU.1 binding sites along the entire open chromatin of the REXO1LP repeat (Fig. 6F and SI Appendix, Fig. S13B). The identified PIRAT occupied sequences in this locus only differ at single nucleotide positions (SI Appendix, Fig. S14A). The same is true for the PU.1 sites in this locus (SI Appendix, Fig. S14B). ChIRP– and ChIP–qRT-PCR, using alternative primer pairs directed against the concatenated, REXO1LP-specific PIRAT- and PU.1-peak sequences confirmed PIRAT and PU.1 interaction with REXO1LP repeat DNA (Fig. 6 G and H and SI Appendix, Fig. S15A).

Subcloning of qRT-PCR products from PIRAT ChIRP eluates (SI Appendix, Fig. S15A), followed by Sanger sequencing, discriminated at least four PIRAT binding sites, differing at single nucleotide positions, respectively (SI Appendix, Fig. S15 B and C). These nucleotide variations are not annotated in the current GENCODE GRCh38 human reference genome, potentially, due to the difficulty of repeat sequence reconstructions (36). All obtained Sanger sequences exclusively mapped to the REXO1LP locus (allowing up to 10 mismatches) (SI Appendix, Fig. S15D), underscoring their origin from this locus. Notwithstanding possible uncertainties in REXO1LP locus annotation, these data support the possibility of PIRAT-mediated redirection of PU.1 from alarmin promoters to REXO1LP sites (Fig. 6D). In line with such a decoy function, PIRAT interacted with PU.1 in primary monocytes in UV-CLIP experiments (~12-fold enrichment) (Fig. 7A and SI Appendix, Fig. S16A). ChIP confirmed PU.1 binding to the promoters of S100A8 and A9 in primary monocytes (Fig. 7B and SI Appendix, Fig. S13A), which was enhanced in PIRAT-deficient compared to wild-type THP1 monocytes (Fig. 7C). Concurrently, PU.1-binding to the repeated REXO1LP sites was diluted in the absence of PIRAT (Fig. 7C). This supports the hypothesis, that PIRAT dampens alarmin expression in naïve monocytes by redirecting PU.1 from
alarmin promoters to pseudogene binding sites. To verify that the increase in alarmin expression upon PIRAT knockout is PU.1-dependent, we treated PIRAT-deficient cells with the small molecule DB2313, which inhibits chromatin-binding of PU.1 (37). PU.1 inhibitor treatment not only reduced PIRAT expression in wild-type THP1 cells, but also counteracted the increased S100A8 and S100A9 expression in PIRAT-deficient cells in a dose-dependent manner (Fig. 7D and SI Appendix, Fig. S16B). Thus, PIRAT inhibits alarmin expression as a negative feedback regulator of PU.1 in the nucleus of human monocytes.

**PIRAT is a Myeloid Marker with Clinical Utility beyond COVID-19.**

Given the specific myeloid expression and function of PIRAT, we predicted its utility as a marker of myeloid cell abundance and tissue infiltration in infectious and inflammatory diseases. Indeed, expression of PIRAT in PBMC samples from control...
and COVID-19 patients correlated with the relative abundance of CD24^+ neutrophils and classic monocytes, but not with nonclassic monocytes (Fig. 7E), in agreement with little expression of PIRAT in the latter (Fig. 2). Beyond COVID-19, PIRAT levels correlated with the percentage of infiltrating myeloid cells (granulocytes; \( R^2 = 0.82 \)) in bronchoalveolar lavage fluid (BALF) from patients with bronchopulmonary infection (Fig. 7F). To test the utility of PIRAT as a myeloid infiltration marker in noninfectious lung diseases, we measured PIRAT in idiopathic pulmonary fibrosis (IPF) tissue. Neutrophils play an important role in IPF tissue remodeling and elevated migration of these cells into IPF tissue has been associated with early mortality (38). PIRAT levels significantly correlated with the percentage of neutrophils in IPF tissue (\( R^2 = 0.83 \)) but not with NK cells (\( R^2 = 0.14 \)) (SI Appendix, Fig. S16C). Thus, PIRAT is a suitable marker for myeloid cell abundance in patient biomaterial, in line with its important role in the myeloid system.

In summary, our results suggest a vital role of lncRNAs as regulators of immune mediator production in the myeloid lineage during COVID-19. Activation of PIRAT upon differentiation of myeloid precursors in the bone marrow likely establishes a break on PU.1-dependent S100A8 and A9 expression by repressing PU.1 to pseudogenes. NF-κB–dependent downregulation of PIRAT during infection enhances PU.1 binding to the S100A8 and A9 promoters. Simultaneously, the NF-κB and JAK-STAT pathways promote the expression of LUCAT1, which further propels the production of alarmins and classic NF-κB responsive factors, such as CXCL8, at the expense of STAT-dependent immunity. Collectively, PIRAT downregulation and LUCAT1 upregulation in monocytes in this model fuels the expression of S100A8 and S100A9, which contribute to myeloid imbalances during severe COVID-19 (Fig. 7G).

**Discussion**

Besides characteristic cellular changes, indicative of emergency myelopoiesis, severe COVID-19 entails systemic inflammatory components also registered in other difficult to treat infectious disease trajectories (1, 2). A better understanding of the underlying molecular circuits is urgently needed to improve the outcome of infections with SARS-CoV-2 and other potentially pandemic agents. Several recent studies have employed scRNA-seq to dissect peripheral immune alterations in COVID-19 (3, 5, 7). So far, however, the noncoding RNA layer has been neglected.

Here, we employed an lncRNA-centric approach to dissect mechanisms underlying immune-alterations in COVID-19 at the single-cell level. Our results reveal the lncRNA PIRAT to be primarily expressed in monocytes, a critical source of peripheral immune mediators, such as S100A8 and A9 in COVID-19 (7). We also find PIRAT to be expressed in granulocytes (Fig. 1C) and to correlate with granulocyte counts in biomaterial from diseased tissue (Fig. 7F and SI Appendix, Fig. S16C). As granulocytes are major sources of alarmins (6), expression of which is restrained by PIRAT, it would be worthwhile to investigate the role of PIRAT in this cell type. Upon their release from monocytes and granulocytes, S100A8 and S100A9 form the calprotectin complex, which has intricate pro- and antiinflammatory functions and influences cellular metabolism and cytoskeletal processes in various cell types (30, 39, 40). Furthermore, S100A8 and S100A9 expression may be uncoupled. S-nitrosylated S100A8 has, for example, been reported to suppress mast cell degranulation (30, 39, 41). In line with their pleiotropic functions, S100A8 and S100A9 have been implicated in a variety of diseases, ranging from arthritis to diabetes and cardiovascular diseases (30, 39, 40). Therefore, PIRAT, as a regulator of these alarmins, could be involved in other diseases beyond COVID-19.

Upstream of S100A8 and A9, PIRAT controls PU.1 as a negative feedback regulator. Feedback control constitutes a universal regulatory principle, conferring stability to cellular circuits (42). Mechanistically, PIRAT inhibits PU.1 association with alarmin promoters and fosters PU.1 binding to the REXO1LP locus, which suggests a novel function of pseudogenes as nuclear caches for transcription factors. An open question concerns how PU.1 recruitment to the REXO1LP locus by PIRAT is achieved mechanistically. Despite the alternating binding pattern, PIRAT- and PU.1-occupied regions in this locus do not overlap. The physical association of PIRAT with PU.1 (Fig. 7A) suggests that chromatin loops may occur, bringing PIRAT and PU.1 binding sites into spatial proximity at the REXO1LP locus. This could result in a condensed, PU.1-inhibiting chromatin focus that is maintained by PIRAT. Further experiments, for example using single-molecule RNA-FISH with PU.1 containing or PU.1 ChIA-PET experiments, could further narrow down the mechanism of PIRAT-dependent PU.1 recruitment into this locus. Since PU.1 is a master-regulator of myelopoiesis, PIRAT might also contribute to the imbalanced myeloid differentiation trajectories seen in severe COVID-19, independent of S100A8 and A9. The PU.1 dose, for example, decides over the commitment to the macrophage and granulocyte differentiation paths, respectively (43, 44).
Furthermore, reduction of PU.1 levels is required for megakaryocyte differentiation and thus platelet production (45). This might also explain the association of a SNP in the PIRAT locus with altered platelet volume (19). Granulocyte and platelet differentiation trajectories again are disturbed in COVID-19 (5, 46). In vivo studies could further clarify the role of PIRAT in myeloid cell differentiation and activation. The low sequence conservation of PIRAT in rodents (Fig. 4i), however, calls into question the possibility of such investigations.

Besides PIRAT, other lncRNAs have been reported to act in the myeloid niche. Schwarzer et al. (47) identified LINC00173 as a regulator of myeloid progenitor proliferation, contributing to granulopoiesis, probably through PRC2 complex-dependent modifications at HOX-gene loci. Similarly, the lncRNA Hotairm1 was found to regulate granulocytic differentiation and HOX gene expression through a yet unknown mechanism (48, 49). During terminal myeloid differentiation, PU.1-induced lncRNA Inc-MC was reported to promote monocyte-to-macrophage differentiation (50). These seminal studies support the notion that lncRNAs critically contribute to the timing of myelopoietic programs and suggest that PIRAT is embedded into a larger regulatory RNA network in myeloid cells.

Due to their important roles in the immune system, lncRNAs such as PIRAT should be considered potential pharmacological targets. Recent successes in antisense-directed therapies (51) and antisense-manipulation of myeloid RNA-circuits (52) make PIRAT targeting therapeutics seem feasible. Further immune-regulatory lncRNAs, such as Mal1L, GAPLINC, PACER, or CARLR, could become relevant in this context as well. Mal1L, for example, supports type I IFN immunity, which in turn is counter-regulated by LUCAT1 (14, 27). Both lincRNAs are up-regulated during COVID-19 (Fig. 2G). IFN-STAT pathway inhibition by Ruxolitinib has been reported to prevent the progression of COVID-19 with systemic hyperinflammation into multiorgan-failure (28). Thus, whereas Mal1L could nurture COVID-19 pathogenesis, LUCAT1 might adopt a protective function, preventing excessive IFN-STAT-driven immune responses. Importantly, however, LUCAT1 seems to contribute to the production of alarmins, associated with severe courses of COVID-19. This suggests that pharmacological intervention in lncRNA circuits needs to be considered with similar care as the use of conventional pathway inhibitors, such as Ruxolitinib.

In summary, our results suggest a multistaged model of immunoregulation in COVID-19 and other infectious diseases, in which lncRNAs occupy a central position. In the myeloid system, lncRNAs such as PIRAT and LUCAT1 control the activity of immune master-transcription factors such as PU.1 and STAT1 via complex feedback mechanisms. Negative feedback between PU.1 and PIRAT in resting cells ensures that downstream production of the critical alarmins S100A8/A9 is kept within narrow limits. Under inflammatory conditions, PIRAT-dependent alarmin suppression is lifted and alarmin production is further promoted by LUCAT1, which ties JAK/STAT inhibition to NF-kB-dependent gene expression. Correspondingly, malfunctions at the lincRNA level are anticipated to have a decisive influence on the transcription factor networks determining the course of COVID-19 and other immune-associated diseases.

Materials and Methods

Cell Culture and Human Biomaterial. Buffy coats were obtained from the transfusion medicine department, University Hospital of Giessen and Marburg, Giessen, and deidentified prior to use. THP1 and Hek293T cells were obtained from ATCC. All cells were cultured at 37 °C in a humidified atmosphere with 5% CO2. Cell purification, culture and stimulation conditions are further specified in SI Appendix, Supplementary Methods.

COVID-19 patients (SI Appendix, Tables S2 and S3) were tested positive for SARS-CoV-2 RNA in nasopharyngeal swabs. The Bioinflame study was approved by the ethics committee of the Charité-Universitätsmedizin Berlin (EA2/030/09) and the University Medical Center Marburg (55/17). BALF (Fig. 7F) was obtained at the University Clinics Giessen and Marburg (ethics approval Marburg: 87/12) or
at Charité, Berlin (ethics approval EA2/086/16). Late stage IPF tissue was obtained from the UGMMLC Giessen Biobank/euIPF registry biobank, member of the DLZ Platform Biobanking, on approval by ethics committee (Az 58/15 and 111/08). Patient characteristics are listed in SI Appendix, Table S6. BAL procedure, study design, and patient characteristics are further detailed in SI Appendix, Supplementary Methods.

Cell Manipulation. For gene silencing the pX458 vector system (53) (SI Appendix, Fig. S9H) or a lentiviral CRISPR interference vector (54) was used. (Addgene #71237). gRNA sequences are provided in SI Appendix, Table S7.

For PIRAT overexpression, the SparQ lentivector (Systembio, # QM511B-1) was used. Detailed procedures are provided in the SI Appendix, Supplementary Methods.

PCR and Cloning. DNA from PIRAT ChIRP elutions was amplified using Advantage 2 polymerase (Takara) and subcloned using the Stratagene TA PCR cloning kit (Agilent), followed by Sanger sequencing (SeqLab GmbH).

RACE-PCR was performed using the SMARTer 5′/3′ RACE kit (Clontech) and products were subcloned and sequenced as above. For detailed procedures see SI Appendix, Supplementary Methods.

Copy Number Enumeration. PIRAT copy number was determined by qRT-PCR reactive to a synthesized PIRAT RNA standard as described in SI Appendix, Supplementary Methods.

Subcellular Fractionation. Cytoplasm and nucleus were separated by differential centrifugation, followed by RNA extraction, as detailed in SI Appendix, Supplementary Methods.

Nucleic Acid and Protein Detection. For RNA and DNA detection by quantitative PCR, the High-Capacity CDNA Reverse Transcription Kit and PowerUP SYBR Green Master Mix (Thermo Fisher) or the Power SYBR RNA-to-Ct 1-Step Kit (Thermo Fisher) was used. Expression changes were calculated using the 2−ΔΔCT method. RNA-FISH was performed using the ViewRNA IMISH Tissue 1-plex Assay (Alymentix) (14). For Western Blot, 10% polyacrylamide SDS PAGE gels, nitrocellulose membranes and a Chemostar Imager (INTAS Science Imaging) were used. For details, see SI Appendix, Supplementary Methods.

Flow Cytometry. Cells were stained with fluorochrome-coupled antibodies and analysed using a Guava EasyCyte (Millipore) instrument. For details, see SI Appendix, Supplementary Methods.

Chromatin and Protein Affinity Purification. ChIP was performed by coupling magnetic beads to PLU C1 plus A7 antibody or FLAG antibody (SI Appendix, Table S8), as described by Tawk et al. (55). SI Appendix, Supplementary Methods.

ChIP-seq was performed using 3′ monobiotinylated antisense DNA probes (SI Appendix, Table S9) as described previously (56).

For co-ip, the procedure published by Tawk et al. (55) was used with minor modifications using antibodies listed in SI Appendix, Table S8. For details, see SI Appendix, Supplementary Methods.

Single-Cell RNA-Sequencing Analysis. Single-cell multiplex was performed using the BD Rhapsody system and the Human Immune Response Panel supplemented with custom-made primers for additional genes. For details see SI Appendix, Supplementary Methods.

Bulk Sequencing and Bioinformatics Analysis. Illumina TruSeq mRNA libraries and Cross-linking immunoprecipitation (CLIP)-seq and ChIRP-seq libraries (VeriTech Biotech AG) were sequenced on a HiSeq 1500 or a NEXTSEQ500 machine. Further sequencing data were obtained through public sequence read archives (see SI Appendix, Supplementary Methods).

For RNA-seq and ChIP-seq data analysis and visualization tools and strategies see SI Appendix, Supplementary Methods.

Statistical Analysis. Statistical analysis was performed based on at least three independent experiments, except for srqRNA-seq experiments. Test details can be found in the figure legends and methods details. If not specified differently, GraphPad Prism software was used for two-tailed Student’s t test and ANOVA analysis. Differences between two or more compared conditions were regarded significant when P values were ≤ 0.05. Where possible, P values are shown in the respective figure panels.

Data, Materials, and Software Availability. Bulk and srqRNA-seq data have been deposited in the NCBI GEO database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE142503) (58) and are publicly available as of the date of publication.

ACKNOWLEDGMENTS. We thank Kerstin Hoffmann, Kathleen Stabila and Jennifer Kremer for assisting in patient sample collection and cell culture. This study makes use of data generated by the Blueprint Consortium. A full list of the investigators who contributed to the generation of the data are available from www.blueprint-epigenome.eu. Funding for the project was provided by the European Union’s Seventh Framework Programme (FP7/2007-2013) under Grant agreement 282510–BLUEPRINT. This work was funded by Deutsche Forschungsgemeinschaft SFB-TR84 “innate immunity of the lung”, projects C10 (to L.N.S. and L.N.S.), C1 (to B.S.), and Z1 (to A.D.G.); the Hessian Ministry of Higher Education, Research, Science and the Arts (LOEWE Diffusible Signals) (to B.S. and L.N.S.); and von Behring Röntgen Stiftung Project 63-0036 (to L.N.S.). S.M.V. was supported by the Jürgen Manchot Foundation (Doctoral Research Fellowship). Y.L. was supported by a European Research Council Starting Grant (948207) and the Radboud University Medical Centre Hypatia Grant (2018) for Scientific Research. C.S. is supported by the UGMLC, the DZK UKG research funding according to article 2, section 3 of the cooperation agreement, the Deutsche Forschungsgemeinschaft SFB 1021 (Project-ID 197785649), KFO 309 (Project ID 284237345), and SK 317/1-1 (Project-ID 428518790), and the Foundation for Pathobiomeohysics and Molecular Diagnostics.

Author affiliations: *Institute for Lung Research, Philips University Marburg, 35043 Marburg, Germany; †Department of Computer Science for Individualised Medicine, Centre for Individualised Infection Medicine & TWINCORE, joint ventures between the Helmholtz-Centre for Infection Research and the Hannover Medical School, 30625 Hannover, Germany; ‡Department of Genetics, University of Groningen and University Medical Center Groningen, 9713 AV, Groningen, The Netherlands; §Translational Inflammation Research Division & Core Facility for Single Cell Multimomics, Philips University Marburg, 35043 Marburg, Germany; ¶Institute of Veterinary Pathology, Free University Berlin, 14195 Berlin, Germany; ‖Department of Infectious Diseases and Respiratory Medicine, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, 10117 Berlin, Germany; ‡Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany; ¶Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, GR-70013, Greece; ‖Institute of Laboratory Medicine, Philipps University Marburg, 35043 Marburg, Germany; ††Department of Hematology, Oncology and Immunology, Philipps University Marburg, University Hospital Giessen and Marburg, 35043 Marburg, Germany; ‡‡Universities of Giessen and Marburg Lung Center (UGMLC), Giessen, 35392 Germany; ‡§German Center for Lung Research (DZL), Giessen, 35392 Germany; ‡‖Institute of Virology, University Hospital Giessen and Marburg, 35043 Marburg, Germany; ‡¶Department of Gastroenterology, Endocrinology, Metabolism and Infectiology, University Hospital of Giessen and Marburg, 35043 Marburg, Germany; ‡‖Institute for Computational Biology, Helmholtz Centre, 85764 Munich, Germany; ¶Genomics Core Facility, Philips University Marburg, 35043 Marburg, Germany; ¶Institute of Molecular Oncology, Philips University Marburg, 35043 Marburg, Germany; ¶¶UGMLC Giessen Biobank and European IFP registry (eupIPReg), Giessen, 35392 Germany; ‡§Department of Internal Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands; §Department of Respiratory and Critical Care Medicine, University Medical Center Marburg, 35043 Marburg, Germany; ¶¶Center for Synthetic Microbiology, Philips University Marburg, 35043 Marburg, Germany; †††German Center of Infection Research, 35043 Marburg, Germany.

Author contributions: M. A. M. Azenourov, N. S., E. M., C. S., C. K., C. B., A. M., A. N., T. S., A. D. G., Y. L., H. G., L. E. S., B. S., and L. N. S. designed research; M. A. M. Azenourov, N. S., H. J., Z. Z., K. P., J. B., S. M. V., D. W., P. G., E. N., M. A. Lillaud, M. G., A. M., C. R., and L. N. S. performed research; K. P., S. M. V., D. W., P. G., M. G., E. M., C. S., C. K., C. B., W. B., A. N., T. S., A. D. G., C. R., H. G., L. E. S., and L. N. S. contributed new reagents/analytic tools; M. A. M. Azenourov, N. S., H. J., Z. Z., K. P., J. B., S. M. V., D. W., P. G., E. N., W. B., A. D. G., C. R., Y. L., H. G., B. S., and L. N. S. analyzed data; and M. A. M. Azenourov and L. N. S. wrote the paper.
5. J. Schulte-Schrepping et al., Severe COVID-19 is marked by a dysregulated myeloid cell compartment. Cell 182, 1419–1440.e23 (2020).
6. A. Silvin et al., Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. Cell 182, 1401–1418.e18 (2020).
7. X. Ren et al., COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. Cell 184, 1895–1913.e19 (2021).
8. O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation. Cell 140, 805–820 (2010).
9. L. De Armas et al., Comparative genomewide RNA seq identifies Eif2d2 as a novel regulator of innate immunity. Genetics 197, 440–459 (2016).
10. Z. Li et al., Spliceosome protein Eif2d2 promotes colitis-associated tumorigenesis by modulating inflammatory response of macrophage. Mucosal Immunol. 12, 1164–1173 (2019).
11. C. E. Gleason, A. Ordureau, R. Goulay, J. S. C. Arthur, P. Cullen, Polyubiquitin binding to optineurin is required for optimal activation of TANK-binding kinase 1 and production of interferon β. J. Biol. Chem. 286, 35663–35674 (2011).
12. J. L. Rinn, H. Y. Chang, Long noncoding RNAs: Molecular modalities to organizational functions. Annu. Rev. Biochem. 87, 283–308 (2020).
13. M. Aflalo, I. N. Schulte, Emerging roles of long noncoding RNAs in the cytoplasmic milieu. Noncoding RNA 6, e44 (2020).
14. M. Azaanourova et al., Noncoding RNA MalT1 is an integral component of the TLR4-TRIF pathway. Proc. Natl. Acad. Sci. U.S.A. 117, 9042–9053 (2020).
15. M. Kowczyk, B. M. Emerson, G50-associated COX-2 extragenic RNA (PACER) activates COX-2 gene expression by occluding repressive NF-kB complexes. eLife 3, e01776 (2014).
16. A. C. Vollmer et al., A conserved long non-coding RNA, GAPLINC, modulates the immune response during endotoxic shock. Proc. Natl. Acad. Sci. U.S.A. 118, e201644118 (2021).
17. A. Castellanos-Rubo et al., Cytoplasmic form of Calsc IncRNA facilitates inflammatory gene expression upon NF-κB activation. J. Immunol. 199, 581–588 (2017).
18. K. Walther, L. N. Schulte, Role of long non-coding RNAs in innate immunity and inflammation. RNA Biol. 18, 587–603 (2020).
19. W. J. Aistle et al., The allelic landscape of human blood cell trait variation and links to common complex disease. Cell 167, 1415–1429.e19 (2016).
20. P. S. Limley, N. Speake, E. Whalen, D. Chausabel, Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in COVID-19 patients. Cell. Mol. Immunol. 17, 992–994 (2020).
21. Q. Guo et al., Induction of alarmin S100A8/A9 mediates activation of aberrant neutrophils in the immunopathogenesis of SARS-CoV-2 infection: Implications for the treatment of COVID-19. Front. Immunol. 12, 63844 (2021).
22. F. Alcadi et al., Monocytopenia, monocyte morphological anomalies and hyperinflammation characterize severe COVID-19 in type 2 diabetes. EMBO Mol. Med. 12, e13038 (2020).
23. I. Antony-Deb et al., Pharmacological inhibition of the transcription factor PU.1 in leukemia. J. Clin. Invest. 127, 4297–4313 (2017).
24. J. E. Kienzler et al., Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. Chest 133, 223–232 (2008).
25. M. S. Wang et al., Vascular inflammation in the anticoagulant response to COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. Cell Host Microbe 22, 805–806 (2021).
26. J. Rea et al., Comparative genomewide RNA seq identifies Eif2d2 as a novel regulator of innate immunity. Genetics 197, 440–459 (2016).