

CD127 imprints functional heterogeneity to diversify monocyte responses in human inflammatory diseases

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32 **Abstract:**

33 Studies on human monocytes historically focused on characterization of bulk responses, whereas functional
34 heterogeneity is largely unknown. Here, we identified an inducible population of CD127-expressing human
35 monocytes under inflammatory conditions and named the subset M127. M127 is nearly absent in healthy
36 individuals yet abundantly present in patients with infectious and inflammatory conditions such as COVID-
37 19 and rheumatoid arthritis. Multiple genomic and functional approaches revealed unique gene signatures of
38 M127 and unified anti-inflammatory properties imposed by the CD127-STAT5 axis. M127 expansion
39 correlated with mild COVID-19 disease outcomes. Thereby, we phenotypically and molecularly
40 characterized a human monocyte subset marked by CD127 that retained anti-inflammatory properties within
41 the pro-inflammatory environments, uncovering remarkable functional diversity among monocytes and
42 signifying M127 as a potential therapeutic target for human inflammatory disorders.

43

44 **Main Text:**

45 Human monocytes and macrophages are considered major mediators of inflammation in a plethora of disease
46 settings including infectious diseases such as COVID-19 and chronic inflammatory diseases such as
47 rheumatoid arthritis (RA)¹⁻³. During the pathological processes, inflammatory monocytes from peripheral
48 blood origin accumulate at the sites of infection and/or inflammation and produce large quantities of pro-
49 inflammatory mediators including cytokines and chemokines⁴, exacerbating disease outcomes by promoting
50 the vicious inflammation cycle^{5,6}. Under homeostasis, human monocyte heterogeneity has been
51 conventionally defined by bimodal expression of CD14 and CD16^{7,8}. However, understanding of functional
52 heterogeneity of human monocytes under inflammatory conditions is limited, which imposes conceptual and
53 technical barriers of therapeutically targeting human inflammatory diseases⁹, a particularly prominent issue
54 amid the global menace of COVID-19¹⁰. Here, through multiomics analyses of human samples including
55 extensive profiling at the single cell level, we defined a subset of human inflammatory monocytes uniquely
56 marked by the expression of CD127 (thus termed M127) that were abundantly present in inflamed tissues of
57 COVID-19 and RA patients. Mechanistic investigations and integrative computational approaches further
58 revealed common molecular and functional features of M127 across multiple inflammatory disease
59 conditions.

60

61 CD127, encoded by *IL7R*, is generally considered a lymphoid lineage marker that is predominantly
62 expressed and functional on T cells and innate lymphoid cells¹¹. Immunohistochemical analyses of
63 pulmonary autopsy samples revealed minimal CD127 expression in alveoli from individuals who deceased
64 due to non-infectious causes yet robust staining in patients succumbing to SARS-CoV-2 infection (Fig. 1a).
65 Unexpectedly, in SARS-CoV-2 infected lung tissues, CD127 signals appeared in the regions of CD68
66 positivity (Fig. 1a), implying plausible expression of CD127 in monocytes/macrophages, which was
67 confirmed by co-localization of CD127 and CD68 signals on immunofluorescently stained sections of
68 autopsied COVID-19 lung tissues (Fig. 1b). Monocytes/macrophage (CD14^{high} CD68^{high}) expression of
69 CD127 was further corroborated at the transcriptome level by single cell RNA sequencing (scRNA-seq)
70 analyses of bronchoalveolar lavage fluid (BALF) from nine COVID-19 patients with clinical manifestations
71 ranging from mild (n = 3) to severe (n = 6) (Extended Data Fig. 1a). Strikingly, a distinct *IL7R*⁺ population
72 (Fig. 1c,d) was revealed to constitute 21% of BALF monocytes/macrophages (Fig. 1e). Moreover, in contrast
73 to the predicted dominance by lymphoid cells, the majority (64%) of *IL7R*⁺ cells in COVID-19 BALF were
74 of monocyte/macrophage lineage (Fig. 1f).

75

76 To validate whether CD127 expression on monocytes/macrophages can be generalized to other human
77 inflammatory conditions such as RA, peripheral blood mononuclear cells (PBMCs) from 16 anti-
78 inflammatory treatment naïve RA patients were analyzed for CD127 expression. Compared with the minimal
79 levels of CD127 in CD14⁺ monocytes from healthy donors, all RA patients examined displayed markedly
80 elevated expression of CD127 on blood monocytes at the protein and mRNA levels (Fig. 1g,h). In RA blood

81 monocytes, *IL7R* expression correlated with the expression of a major pathogenic factor, TNF (Fig. 1h,i)¹².
82 To probe CD127 at the primary sites of inflammation, RA synovial tissue scRNA-seq data sets from 18
83 patients¹³ (Extended Data Fig. 1b) were analyzed for *IL7R* expression, which showed strong positivity in a
84 distinct population (Fig. 1j,k) that represented nearly 22% of synovial monocytes (Fig. 1l). Taken together,
85 expression of CD127 on a subset of inflammatory monocytes/macrophages is likely a hallmark of human
86 inflammatory conditions testified in multiple disease settings (COVID-19 and RA) and multiple tissues
87 (infected lungs, peripheral blood and inflamed joints).

88
89 In order to pursue in-depth investigation of CD127⁺ monocytes/macrophages, we wished to recapitulate
90 such phenotypes *in vitro* using infectious and inflammatory stimuli. Stimulation of human PBMCs from
91 healthy donors with toll-like receptor (TLR) ligands led to drastic increase of monocytic CD127 at the protein
92 and mRNA levels, with the exception of TLR3 agonist poly(I:C) (Fig. 2a,b). Upregulation of CD127 was
93 dynamic, peaking around 6 h post LPS stimulation (Fig. 2c,d), dependent on canonical TLR signaling
94 modules such as IKK and p38 (Extended Data Fig. 2a-e), and observed in all three currently defined human
95 monocyte subpopulations (Fig. 2e and Extended Data Fig. 2f,g). In contrast to human monocytes, LPS failed
96 to upregulate CD127 in murine peripheral blood monocytes (Extended Data Fig. 3a-c) and macrophages from
97 multiple tissue sources (data not shown), suggesting human-specific nature of CD127 induction. In addition
98 to TLR stimulations that mimicked infectious conditions, we wished to identify factors that led to CD127
99 expression in chronic inflammatory diseases and pursued TNF as a plausible candidate as its levels correlated
100 with CD127 expression (Fig. 1i). TNF treatment consistently upregulated CD127 in monocytes, albeit to a
101 lesser extent than LPS (Fig. 2f,g). Importantly, clinically applied TNF blockade treatment significantly
102 reduced *IL7R* expression in RA monocytes (Fig. 2h), further solidifying a role for TNF in upregulation of
103 CD127 *in vivo*. Next, we wished to investigate whether CD127 in activated monocytes was functional given
104 that another subunit of IL-7 receptor, the *IL2RG*-encoding common gamma chain, was constitutively
105 expressed in human monocytes (Extended Data Fig. 3d). IL-7 treatment robustly induced STAT5 tyrosine
106 phosphorylation in LPS-activated monocytes but not in resting monocytes (Fig. 2i) with T cells serving as
107 positive controls (Extended Data Fig. 3e), indicating that activated human monocytes were competent for IL-
108 7 receptor signaling.

109
110 Given the heterogeneity of CD127 expression, we reasoned that activated human monocytes may
111 display functional diversity and subjected LPS-activated monocytes to single cell expression profiling.
112 Unsupervised hierarchical clustering revealed four groups of cells with differential gene expression patterns
113 (Fig. 3a,b). Interestingly, *IL7R* exhibited a gradient pattern among four clusters, with the highest expression
114 in cluster 1 and the lowest in cluster 4 (Fig. 3c). To quantitatively assess the inflammatory phenotypes of
115 these cells, we devised a numeric index ‘inflammatory score’ based on an algorithm¹⁴ reflecting expression
116 levels of 8 representative prototypical inflammatory genes (see ‘Methods’ for details). Inflammatory score
117 inversely correlated with the expression of *IL7R*, with the highest level shown for cluster 4 that exhibited the

118 lowest level of *IL7R* (Fig. 3d), a trend that could also be clearly visualized for individual inflammatory genes
119 (Fig. 3e). To validate the differences of inflammatory responses observed from single cell analyses, we sorted
120 CD127^{high} and CD127^{low} LPS-activated monocytes (Fig. 3f). Consistent with the single cell results,
121 CD127(*IL7R*)^{low} monocytes produced significantly higher levels of inflammatory mediators such as IL-6 and
122 TNF (Fig. 3g,h), demonstrating that within a highly defined system consisting of purified monocytes and a
123 single stimulus, human monocytes displayed the remarkably diverse range of inflammatory responses.

124

125 Having observed the inverse correlation between CD127 and inflammatory responses, we wished to
126 examine whether CD127 was causally related to inflammation by knocking down *IL7R* in monocytes with
127 RNA interference. Downregulation of *IL7R* resulted in markedly upregulated expression of *IL6* and *TNF*
128 (Fig. 3i), implicating a negative role for monocytic CD127 in inflammation. To characterize the chromatin
129 accessibility landscape of CD127^{high} and CD127^{low} monocytes, we subjected two populations to ATAC-seq
130 (Extended Data Fig. 4a) and identified a highly specified fraction of open chromatin regions in CD127^{high}
131 monocytes (Fig. 3j,k) that displayed enhancer-like features marked by H3K27ac and H3K3me1
132 modifications¹⁵ (Extended Data Fig. 4b,c) and enriched in binding motifs for several transcription factors
133 with STAT5 being the most prominent (Fig. 3l). Pharmacological inhibition of STAT5 led to upregulation
134 of *IL6* and *TNF* expression (Fig. 3m), in line with the effects of *IL7R* abrogation. To elucidate the mechanisms
135 underlying CD127-STAT5-mediated effects, CD127^{high} and CD127^{low} population were profiled by RNA-seq
136 (Extended Data Fig. 5a,b), revealing that transcription factor c-Maf, encoded by the *MAF* gene, was relatively
137 highly expressed in CD127^{high} cells in a STAT5-dependent manner (Extended Data Fig. 5c,d). Knocking
138 down *MAF* expression resulted in upregulation of *IL6* and *TNF* (Extended Data Fig. 5e,f). Of note, *MAF* is a
139 direct STAT5 target gene as shown by occupancy of STAT5 at a consensus binding site upstream of the *MAF*
140 transcription start site (Extended Data Fig. 5g). Together, the above results implicated that the CD127-
141 STAT5-c-Maf axis exerted anti-inflammatory effects, contributing to the functional heterogeneity in human
142 inflammatory monocytes.

143

144 Upon characterizing the hypo-inflammatory phenotypes of CD127^{high} cells *in vitro*, we next wished to
145 validate these findings *in vivo* in human disease settings. In BALF monocytes/macrophages from COVID-
146 19 patients, *IL7R*⁺ population did not extensively overlap with the highly inflammatory cells and exhibited
147 minimal inflammatory properties (Fig. 4a,b). Consistent with the *in vitro* observations, *IL7R*⁺ cells expressed
148 heightened levels of *MAF* relative to the highly inflammatory monocytes (Extended Data Fig. 6a).
149 Importantly, in mild COVID-19 patients, *IL7R*⁺ cells constituted 48% of all BALF monocytes/macrophages
150 yet in stark contrast, such percentage was merely 17% in severe COVID-19 patients (Fig. 4c). The differential
151 *IL7R* patterns in monocytes were not due to the global differences in expression levels as lymphocytic *IL7R*
152 did not significantly differ between mild and severe patients (Extended Data Fig. 6b). These results indicated
153 that CD127 indeed marked a population of hypo-inflammatory monocytes/macrophages *in vivo* and
154 suggested that the prevalence of *IL7R*⁺ monocytes/macrophages likely correlated with subdued inflammation

155 and favorable disease outcomes. In addition to COVID-19, *IL7R*⁺ population in RA synovial monocytes and
156 peripheral blood monocytes were also largely non-overlapping with the highly inflammatory counterparts
157 and displayed minimal inflammatory features (Fig. 4d,e and Extended Data Fig. 6c,d).

158

159 To extrapolate common features of *IL7R*⁺ monocytes/macrophages from various disease conditions, a
160 recently developed bioinformatics method specializing in incorporating single cell sequencing data sets from
161 multiple sources¹⁶ was used to run integrated analyses of three data sets: COVID-19 BALF
162 monocytes/macrophages, RA synovial monocytes and *in vitro* LPS-activated monocytes. Unsupervised
163 clustering identified 10 cellular subsets, with the cluster 10 classified as tissue resident alveolar macrophages
164 from the COVID-19 samples (Extended Data Fig. 6e-g) and thus being excluded from the subsequent
165 analyses intending to discover common monocyte characteristics regardless of tissue origins. The remaining
166 9 clusters (1-9) represented integrated monocyte subsets present in all three tissue sources (Fig. 4f), with
167 cluster 2 prominently featured by high *IL7R* expression (Fig. 4g,h) and sharing high degree of similarity
168 among three conditions (Fig. 4i). Common signature genes of cluster 2 revealed a profile that was distinct
169 from any of the currently known monocyte/macrophage subsets, with *IL7R* unambiguously identified as the
170 top marker gene (Fig. 4j). Of note, cluster 2 phenotypically differed from the ‘M2-like’ cells defined by
171 markers such as CD163 (Extended Data Fig. 6h).

172

173 In summary, we identified a subset of monocytes marked by CD127 in human infectious and
174 inflammatory diseases but not in mice, and named this subset M127 (Extended Data Fig. 7). We further
175 characterized the inflammatory signals that induced M127 and recapitulated M127 phenotypes with an *in*
176 *vitro* system. CD127 did not only serve as a surface marker for this population but also actively transmitted
177 local IL-7 cues¹⁷ to promote a STAT5-coordinated anti-inflammatory program, resulting in hypo-
178 inflammatory phenotypes amid the overall inflammatory tissue environments. As of the knowledge of the
179 current study, M127 from multiple disease conditions and multiple tissues such as COVID-19 lungs and RA
180 joints shared common functional features and gene signatures, albeit it would be interesting and desirable to
181 assess whether the depicted M127 phenotypes could be observed in additional human disease settings. Given
182 the unique presence of this population in human inflammatory diseases, especially the correlation of M127
183 expansion with favorable disease outcomes in COVID-19, it is highly conceivable to propose M127 as a
184 potential therapeutic target for inflammatory disorders.

185 **Methods**

186 Cell culture and reagents

187 PBMCs of anonymous healthy donors were isolated from buffy coats purchased from the Beijing Red Cross
188 Blood Center using density gradient cell separation by Ficoll (Lymphoprep™, STEMCELL Technologies)
189 following the protocol approved by the Institutional Review Board of School of Medicine, Tsinghua
190 University. The private information of anonymous blood donors was inaccessible to investigators. PBMCs

191 of RA patients were obtained from Peking Union Medical College Hospital using the protocol that was
192 approved by the Institutional Review Board of Peking Union Medical College Hospital. CD14⁺ Monocytes
193 were further isolated from PBMCs using anti-CD14 magnetic beads (130-050-201, Miltenyi Biotec). CD14⁺
194 monocytes were cultured in RPMI 1640 medium (10040CM, Corning) supplemented with 10% (vol/vol)
195 fetal bovine serum (FBS) (Gibco) and human recombinant M-CSF (300-25, Peprotech) (10 ng/ml). LPS
196 (*Escherichia coli* O127:B8, Sigma-Aldrich), human recombinant IL-7 (200-07, Peprotech), human
197 recombinant TNF (H8916, Sigma-Aldrich) or chemical inhibitors (SB203580 from Selleck, STAT5 Inhibitor
198 from Santa Cruz and Bay 11-7082 from Sigma-Aldrich) were used as indicated for various experiments.

199
200 Collection of lung tissues and immunohistochemistry

201 Two cases of uninfected lung tissues and 3 cases of COVID-19 lung tissues were from Biobank of Southwest
202 Hospital, Third Military Medical University (Army Medical University). COVID-19 lung tissues were
203 obtained during autopsy of the patients succumbing to SARS-CoV-2 infection. Pathologically normal lung
204 tissues from pulmonary bulla patients were used as uninfected controls. Tissue collection and the following
205 histological analyses were approved by the ethics committee of Southwest Hospital, Third Military Medical
206 University (Army Medical University), and were in accordance with regulations issued by the National
207 Health Commission of China and the Helsinki Declaration. Lung tissue sections were stained with
208 hematoxylin for assessment of pulmonary architecture, and anti-CD68 (ab201340, abcam) and anti-CD127
209 (PA5-97870, Invitrogen) antibodies were used for immunohistochemistry. Specifically, lung sections were
210 deparaffinized and rehydrated. Antigen retrieval was performed with the Improved Citrate Antigen Retrieval
211 Solution (Beyotime) and incubated with H₂O₂ in dark for 15 min to block endogenous peroxidase activity.
212 Slides were blocked with 10% goat serum in TBS for 30 min at room temperature and stained with primary
213 antibodies overnight at 4°C. Slides were washed three times with 0.1% TBS-Tween before incubation with
214 HRP-conjugated secondary antibodies. Stained slides were washed again in PBS and stained with DAB
215 (TIANGEN) in conjunction with a hematoxylin counterstain (Solarbio). After dehydration, sections were
216 mounted in neutral balsam.

217
218 Immunofluorescence histology

219 COVID-19 lung tissues were collected as described above, and were washed and fixed overnight at 4°C in a
220 solution of 1% paraformaldehyde in PBS. The tissues were incubated in a solution of 30% sucrose in PBS
221 and the mixture of 30% sucrose and OCT compound 4583 (Sakura Finetek) separately at 4°C overnight. The
222 samples were then embedded in OCT, frozen in a bath of ethanol cooled with liquid nitrogen and stocked at
223 -80°C. Frozen samples were cut at 10-µm thickness and collected onto slides. Slides were dried at 50°C for
224 30 min and fixed in 1% paraformaldehyde for 10 min and processed for staining. The tissues were
225 permeabilized in PBS/0.3% Triton X-100/0.3 M glycine at 37°C for 30 min and blocked in PBS/5% goat
226 serum at room temperature for 1 h. The tissues were then incubated with indicated primary antibodies diluted
227 (anti-CD68, 1:100; anti-CD127, 1:500) in PBS/5% goat serum at 4°C overnight, and washed in PBS/0.2%

228 Tween-20 at room temperature for 30 min three times. The tissues were incubated with Alexa dye-conjugated
229 secondary antibodies (Alexa Fluor™ 488 goat anti-mouse IgG, 1:500, B40941, Life Technologies; Alexa
230 Fluor™ 555 goat anti-rabbit IgG, 1:500, A27039, Invitrogen) and DAPI (1:200, C0060-1, Solarbio) in
231 PBS/0.5% BSA at room temperature for 2 h and washed in PBS/0.2% Tween-20 at room temperature for 1
232 h five times before mounting with SlowFade Diamond Antifade Mountant (S36963, Life Technologies).

233

234 Bronchoalveolar lavage fluid (BALF) collection for single cell RNA sequencing (scRNA-seq)
235 Nine COVID-19 patients were enrolled from the Shenzhen Third People's Hospital. BALF collection from
236 COVID-19 patients and healthy donors and following studies were conducted according to the principles
237 expressed in the Declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee
238 of Shenzhen Third People's Hospital (2020-112). Diagnosis of COVID-19 was based on clinical symptoms,
239 exposure history, chest radiography and SARS-CoV-2 RNA positivity. Disease severity was defined as
240 moderate, severe and critical, according to the 'Diagnosis and Treatment Protocol of COVID-19' by the
241 National Health Commission of China. Approximately 20 ml of BALF was obtained for each patient. BALF
242 was directly processed within 2 h and all operations were performed in a BSL-3 laboratory. BALF cells were
243 collected, counted, re-suspended, and subsequently processed for scRNA-seq library construction as
244 described in our previous study¹⁸. According to the clinical diagnosis, nine enrolled patients included three
245 moderate cases, one severe case and five critical cases. For the subsequent analyses of scRNA-seq data, given
246 that there was only one clinically defined severe case, the patients were stratified into mild (n = 3) and severe
247 (n = 6 including both severe and critical cases) groups.

248

249 Mice

250 The laboratory animal facility at Tsinghua University has been accredited by AAALAC (Association for
251 Assessment and Accreditation of Laboratory Animal Care International), and the IACUC (Institutional
252 Animal Care and Use Committee) of Tsinghua University approved the protocol used in this study for blood
253 collection from mouse cheeks. C57BL/6J mice were bred and housed in isolated ventilated cages (maxima
254 six mice per cage) at the specific pathogen free facility at Tsinghua University. The mice were maintained
255 on a 12/12-h light/dark cycle, 22–26 °C, 40–70% humidity with sterile pellet food and water ad libitum.

256

257 RNA extraction and quantitative PCR.

258 Total RNA was extracted from cells using TRIzol® Reagent according to the manufacturer's procedure, and
259 total RNA was reverse-transcribed to cDNA with Moloney Murine Leukemia Virus Reverse Transcriptase
260 (2641B, TAKARA). Real-time quantitative PCR (qPCR) was performed in duplicates with SYBR Green
261 Master Mix (A25742, Applied Biosystems) on StepOnePlus thermal cycler (Applied Biosystems). Primer
262 sequences are listed in the Extended Data Table 1.

263

264 Flow cytometry

265 Upon indicated treatment, cells were collected and washed with staining buffer (PBS with 0.5% BSA and 2
266 mM EDTA). Then, the surface markers were stained with the indicated fluorochrome-conjugated antibodies
267 in 1:400 dilution for 30 min on ice in the dark. After staining, cells were washed three times with staining
268 buffer and re-suspended in PBS for analysis in BD FACSFortessa or for fluorescence-activated cell sorting
269 (FACS) in BD FACSAria III. Further data analysis was implemented using Flowjo software (Tree star). For
270 intracellular staining, cells were treated with Golgistop (554724, BD Biosciences) for 4–5 h before collection.
271 The routine stainings for surface markers were performed, after which the cells were fixed with 100 μ l/tube
272 Fixation Buffer (420801, Biolegend) for 25 minutes at room temperature, and the fixed cells were
273 permeabilized and stained in 1 \times Permeabilization Wash Buffer with fluorochrome-conjugated antibodies for
274 30 minutes on ice in the dark. The fixed and intracellularly stained cells were washed twice with 1 \times
275 Permeabilization Wash Buffer and suspended in PBS for analysis. The fluorochrome-conjugated antibodies
276 for targets of interest and fluorochrome-conjugated isotype control antibodies are listed in Extended Data
277 Table 2.

278

279 Immunoblotting

280 Whole-cell lysates were prepared by direct lysis in sodium dodecyl sulfate (SDS) loading buffer. All samples
281 for immunoblotting were denatured at 95 °C for 10 min. For immunoblotting analysis, denatured cell lysates
282 were separated by 10% SDS polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride
283 membrane (Millipore) for probing with specific primary antibodies and HRP-conjugated secondary
284 antibodies. SuperSignal™ West Pico Chemiluminescent Substrate (34580, Thermo Fisher Scientific) was
285 used for detection. Relative density of blotting bands was quantified using Image J (v1.52a). Antibodies used
286 for probing proteins of interest are listed in Extended Data Table 2.

287

288 RNA interference.

289 Immediately after isolation, primary human monocytes were nucleofected with On-Target plus SMARTpool
290 siRNA purchased from Dharmacon Inc. specific for *IL7R*, *MAF* or *MAP3K3*. Non-targeting siRNA from
291 GenePharma was used as control. Human Monocyte Nucleofector buffer (V4XP-3024, Lonza) and the Lonza
292 4D-Nucleofector™ platform were used according to the manufacturers' instructions with human monocytes
293 nucleofection program. The nucleofected monocytes were cultured in RPMI 1640 medium (Corning)
294 supplemented with 10% (vol/vol) FBS (Gibco) and human recombinant M-CSF (PeproTech) (20 ng/ml) for
295 48 h before the following experiments.

296

297 Chromatin immunoprecipitation (ChIP) assay

298 For STAT5 ChIP assays, THP-1 cells were stimulated with LPS (100 ng/ml) for 6 h and subsequently with
299 IL-7 (10 ng/ml) for 30 min. 10–20 $\times 10^6$ cells per condition were fixed in 1% methanol-free formaldehyde
300 (Thermo Scientific) for 8 min at room temperature followed by quenching with 125 mM glycine for another
301 5 min. ChIP assay was performed using the SimpleChIP enzymatic ChIP kit (Cell Signaling Technology)

302 according to the manufacturer's instructions. The DNA-protein complexes were immunoprecipitated using
303 5.0 µl per sample of STAT5 antibody (9363S, Cell Signaling Technology), and IgG (2729P, Cell Signaling
304 Technology) control was performed in an equally allocated DNA-protein complexes fraction as STAT5 ChIP
305 samples. The immunoprecipitated DNA fragments were extracted with QIAquick PCR purification kit
306 (QIAGEN) and subjected to qPCR assay for enrichment detection in *MAF* transcription start site (TSS)
307 upstream GAS motif with primer pair, forward-AAGTGCAGTGCTATAAAGTTGTTT and reverse-
308 ATGTTCAAGACGCTGGCTTA.

309 RNA-seq

310 Human CD14⁺ monocytes were stimulated with LPS 10 ng/ml for 6 h, and CD127^{high} and CD127^{low}
311 populations for each donor were sorted by FACS. Total RNA was extracted from CD127^{high} and CD127^{low}
312 cells using TRIzol[®] Reagent (Thermo Fisher Scientific) according to the manufacturer's procedure, and RNA
313 samples were processed for library construction with TruSeq mRNA-seq Sample Preparation Kit (Illumina)
314 and sequencing in BGI Genomics Co., Ltd. on a BGISEQ-500RS platform. Three independent sets of paired
315 samples collected from three healthy donors were subjected to RNA-seq and the subsequent bioinformatics
316 analyses.
317

318 Single cell RNA sequencing for RA PBMCs and LPS-activated monocytes

319 After the isolation or treatment, cells were frozen in FBS + 10% DMSO for preservation in liquid nitrogen.
320 The frozen cells were processed for scRNA-seq in BGI Genomics Co., Ltd. Single-cell capturing and
321 downstream library constructions were performed using Chromium Single Cell 3' Reagent kits (10x
322 Genomics) according to the manufacturer's protocol. The constructed libraries were sequenced on a BGI
323 MGISEQ2000 platform.
324

325 Assay for transposase-accessible chromatin coupled with high-throughput sequencing (ATAC-seq)

326 Human CD14⁺ monocytes from healthy donors were stimulated with 10 ng/ml of LPS for 6 h, and CD127^{high}
327 and CD127^{low} populations for each donor were sorted by FACS. Cells were pelleted by centrifugation for 10
328 min at 500 g 4°C using a swing rotor with low acceleration and brake settings. Cell pellets were washed once
329 with 1 × PBS and cells were pelleted again by centrifugation using the previous settings. Cell pellets were
330 re-suspended in 50 µl of lysis buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3.0 mM MgCl₂, 0.5% NP-40)
331 and nuclei were pelleted by centrifugation for 30 min at 500 g, 4°C using a swing rotor with low acceleration
332 and brake settings. Supernatant was discarded and nuclei were re-suspended in 50 µl reaction buffer
333 containing 5.0 µl Tn5 transposase and 10 µl of 5 × TTBL buffer (TruePrep[™] DNA Library Prep Kit V2 for
334 Illumina, Vazyme Biotech). The reaction was incubated at 37°C for 30 min. After the tagmentation, the
335 transposed DNA fragments were purified by 1 × AMPure XP beads (Beckman Coulter). PCR was performed
336 to amplify the libraries for 9 cycles using the following PCR conditions: 72°C for 3min; 98°C for 30 s; and
337 thermocycling at 98°C for 15 s, 60°C for 30 s and 72°C for 3 min; following by 72°C 5 min. After the PCR
338

339 reaction, libraries were purified with the $0.5 \times$ and $1.2 \times$ AMPure XP beads. DNA concentrations were
340 measured with StepOnePlus™ Real-Time PCR System (Life Technologies) and library sizes were
341 determined using Agilent 2100 Bioanalyzer. Libraries were sequenced on an Illumina HiSeq X-ten platform
342 for an average of 20 million unique reads per sample. Three independent sets of paired samples collected
343 from three healthy donors were subjected to ATAC-seq and the subsequent bioinformatics analyses.

344
345 Next-generation sequencing (NGS) data alignment

346 ATAC-seq pair-end reads were collected. Adapter sequences were trimmed from the ends of reads by
347 Cutadapt (v1.14), and the reads that failed to pass the quality control ($Q > 10$) were discarded. H3K27ac and
348 H3K4me1 ChIP-seq data sets were downloaded from NCBI GEO DataSet under the GEO accessions:
349 GSE85245¹⁹. SRA files were converted to fastq files using fastq-dump included in SRA toolkit. Pair-end
350 ATAC-seq reads were aligned to human genome (UCSC hg38) using Bowtie2 (v2.2.5)²⁰ to generate
351 alignment files of uniquely mapped pair-end fragments with maximum length in 1000 bp and no more than
352 one mismatch for each alignment seed with 15 bp in length. ChIP-seq reads in fastq files were aligned to
353 human genome (UCSC hg38) using Bowtie (v1.1.2)²¹ to generate alignment files of uniquely mapped reads
354 with maximum allowed mismatch of 2 (-m 1 -n 2) for each alignment seed. ChIP-seq reads aligned to genome
355 were extended to 150 bp from their 3' end for further analysis. RNA-seq data were collected and single-end
356 reads were aligned to human genome hg38 using TopHat (v2.1.0)²² with the parameters --min-segment-intron
357 50 --no-novel-indels --no-coverage-search, and only uniquely mapped reads were preserved.

358
359 Identification of opened chromatin regions (OCR) by ATAC-seq

360 The ATAC-seq alignment files for CD127^{high} and CD127^{low} monocytes from three donors were used to call
361 peaks for significantly opened chromatin regions using MACS2 (v2.1.1) (FDR < 0.05). The peaks from six
362 samples were merged as total OCRs in LPS treated monocytes. To identify the differentially opened
363 chromatin regions between CD127^{high} and CD127^{low} monocytes, the ATAC-seq fragments were counted in
364 each OCR for each sample by FeatureCounts (v1.5.0)²³. Subsequently, the fragments count was normalized
365 to count per million mapped fragment for each sample. The normalized fragments count was used to identify
366 differentially opened chromatin regions by edgeR (v3.28.1)²⁴. Mean values of (fragments count+1) fold
367 change (CD127^{high}/CD127^{low}) among three donors were log₂ transformed, and differentially opened
368 chromatin regions were identified by log₂ transformed fold changes (CD127^{high}/CD127^{low}) ≥ 1 or ≤ -1 for
369 CD127^{high} monocytes feature OCRs or CD127^{low} monocytes feature OCRs with cutoff of p-value < 0.05. To
370 visualize ATAC-seq and ChIP-seq signals around open chromatin regions of interest, we firstly counted
371 ATAC-seq fragments (-fragLength given) and ChIP-seq extended reads (-fragLength 150) every 10 bp from
372 the center of OCR to ± 2.5 kb regions for each OCR by using annotatePeaks.pl program in HOMER (v4.7.2)²⁵.
373 The output counting matrices were used to generate average signals around OCRs by calculating the average
374 fragments/reads count per bin (10 bp) per OCR.

375

376 RNA-seq data analyses

377 For coverage of mapped RNA-seq reads in transcripts, the expression level of each gene transcript was
378 calculated as normalized reads count per kilobase of transcript per million mapped reads (FPKM) using
379 Cufflinks (v2.2.1)²⁶. Differential gene expression between CD127^{high} and CD127^{low} monocytes from three
380 donors was identified using DESeq2 (v1.27.9)²⁷. Genes with p-value < 0.05 and mean (FPKM+1) fold
381 changes (CD127^{high}/CD127^{low}) ≥ 1.5 or ≤ 0.67 among three donors were defined as highly expressed genes
382 in CD127^{high} monocytes or highly expressed genes in CD127^{low} monocytes, respectively.

383
384 scRNA-seq data analyses

385 For scRNA-seq of COVID-19 patients' BALF cells, the Cell Ranger Software Suite (v.3.1.0) was used to
386 perform sample de-multiplexing, barcode processing and single-cell 5' unique molecular identifier (UMI)
387 counting. Specifically, splicing-aware aligner STAR was used in FASTQs alignment. Cell barcodes were
388 then determined based on the distribution of UMI counts automatically, and the gene-barcode matrices were
389 saved for downstream analysis. In addition, one additional healthy control was acquired from the GEO
390 database under accession number GSE128033. All samples were loaded as Seurat objects by using Seurat
391 (v3.2.1)¹⁴, quality control for each cell were done with criteria as following: gene number between 200 and
392 6,000, UMI count > 1,000 and mitochondrial gene percentage < 0.1. All samples were further integrated to
393 remove the batch effects with the parameter settings of the first 50 dimensions of canonical correlation
394 analysis (CCA) and principal-component analysis (PCA). Integrated Seurat project was first normalized, and
395 top 2,000 variable genes were then identified by using the Seurat analysis pipeline that has been described in
396 our previous studies. Gene expression scaling and PCA was performed using the top 2,000 variable genes.
397 Then UMAP was performed on the top 50 principal components for visualization, and graph-based clustering
398 was simultaneously performed on the PCA-reduced data with the 1.2 resolution setting. According to the
399 clustering result, 32 clusters were identified, and the annotations for each cluster were implemented based on
400 the expression of marker genes that were used in our previous study¹⁸. Monocytes/macrophages (CD14^{high}
401 CD68^{high}) were extracted from the total BALF cells after annotation, and re-clustering (PCA and UMAP)
402 was performed. The clusters showing expression of both monocyte/macrophage marker genes and T cell
403 marker genes were excluded as doublets. Only the cells from COVID-19 patients were used for downstream
404 analyses on both total BALF cells and monocytes/macrophages.

405 RA synovial scRNA-seq data sets¹³ were downloaded from ImmPort with the study accession code of
406 SDY998. The reduction and clustering result from the original study were used. The monocytes clusters were
407 extracted for downstream analysis.

408 The Cell Ranger Software Suite (v.3.1.0) was used to perform sample de-multiplexing, barcode
409 processing and single-cell 5' unique molecular identifier (UMI) counting, and gene-barcode matrices were
410 generated for LPS-treated human CD14⁺ monocytes and RA patient's PBMCs. The gene-barcode matrices
411 were loaded as Seurat objects, and quality control for each cell was performed with criteria for LPS-treated
412 human CD14⁺ monocytes (gene number between 200 and 4,500, UMI count > 1,000 and mitochondrial gene

413 percentage < 0.15) and RA PBMCs (gene number between 200 and 6,000, UMI count > 1,000 and
414 mitochondrial gene percentage < 0.1). After quality control, top 2,000 variable genes were identified, and
415 gene expression scaling, PCA and UMAP clustering were performed for each data set. Marker genes for each
416 cluster in each scRNA-seq data set were identified by using FindAllMarkers function in Seurat. According
417 to the expression of well-studied PBMC marker genes, each cluster of RA PBMCs was annotated with certain
418 cell type, and monocyte clusters were extracted for downstream analysis.

419 *IL7R*⁺ cells were identified based on the normalized expression (> 0) of *IL7R* for each cells in each
420 scRNA-seq dataset. Inflammatory score was calculated based on the normalized average expression of eight
421 inflammatory genes: *TNF*, *IL6*, *IL8*, *CCL2*, *CCL3*, *CCL4*, *CCL8* and *CXCL10*, which is implemented by
422 AddModuleScore function in Seurat with 100 control for each inflammatory gene.

423 Integration of COVID-19 monocytes/macrophages, RA synovial monocytes and LPS-treated
424 monocytes was implemented by a recently developed SCALEX method based on the original SCALE
425 method¹⁶. Leiden clustering and UMAP visualization were performed based on the features extracted by
426 SCALEX for integrated monocytes/macrophages, and marker genes for each cluster were identified.
427 Correlation between clusters among three datasets were calculated as Pearson correlation coefficient, and the
428 negative values were normalized to zero for heat map presentation.

429

430 Statistical analysis

431 Types of statistical tests are indicated in figure legends. Statistical analyses were performed using GraphPad
432 Prism Software (GraphPad Software Inc., La Jolla, CA, USA) for Student's *t* test, and Wilcoxon rank-sum
433 test was implemented using R (v.4.0.2). A value of *P* < 0.05 was considered statistically significant.

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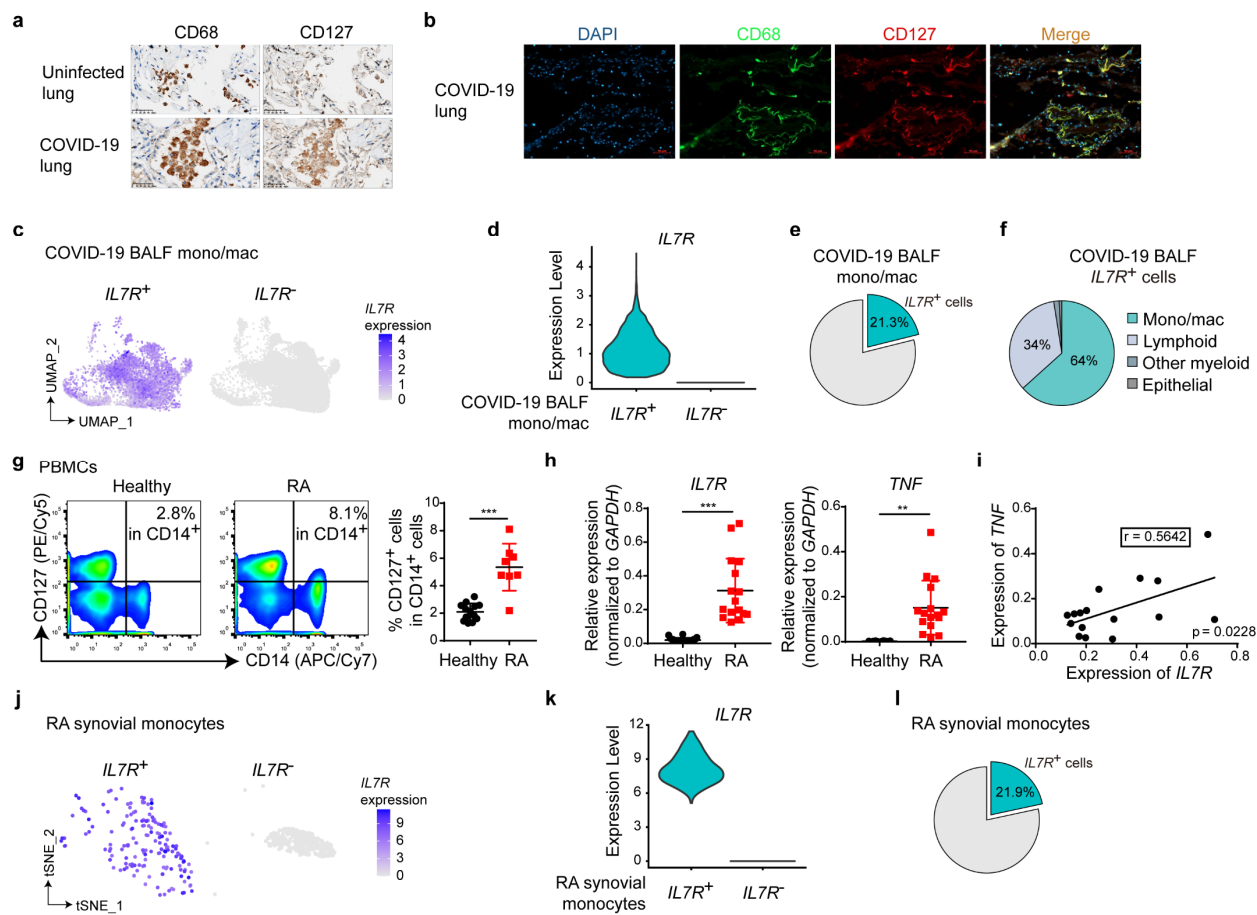
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499 performed some of the monocyte experiments and assisted with single cell RNA-seq data analyses. Jiuliang
500 Zhao, Hui Jiang, and Can Li assisted with collection of rheumatoid arthritis patient samples. Yunqi Liu
501 performed STAT5 ChIP experiments. Xindong Liu, Haofei Liu, and Yi-Fang Ping performed pathological
502 analyses of COVID-19 patient tissue samples. Qiangfeng Cliff Zhang directed the integration of single cell
503 RNA-seq datasets and provided advices on bioinformatics. Zheng Zhang provided COVID-19 BALF single
504 cell RNA-seq data sets and valuable advices. Xiu-Wu Bian directed pathological analyses of COVID-19
505 patient tissue samples. Yan Zhao provided rheumatoid arthritis patient samples and advices on the related
506 experiments. Xiaoyu Hu conceptualized the project, supervised experiments, analyzed and interpreted data,
507 and wrote the manuscript.

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509 **Materials & Correspondence:** Correspondence and requests for materials should be addressed to X.H.

510 **Data availability:** Sequencing data sets are deposited in the Genome Expression Omnibus with assigned
511 accession numbers as follows: RNA-seq and ATAC-seq in GSE159118, healthy donor scRNA-seq in
512 GSE159113, RA scRNA-seq in GSE159117 and COVID-19 BALF scRNA-seq in GSE145926.



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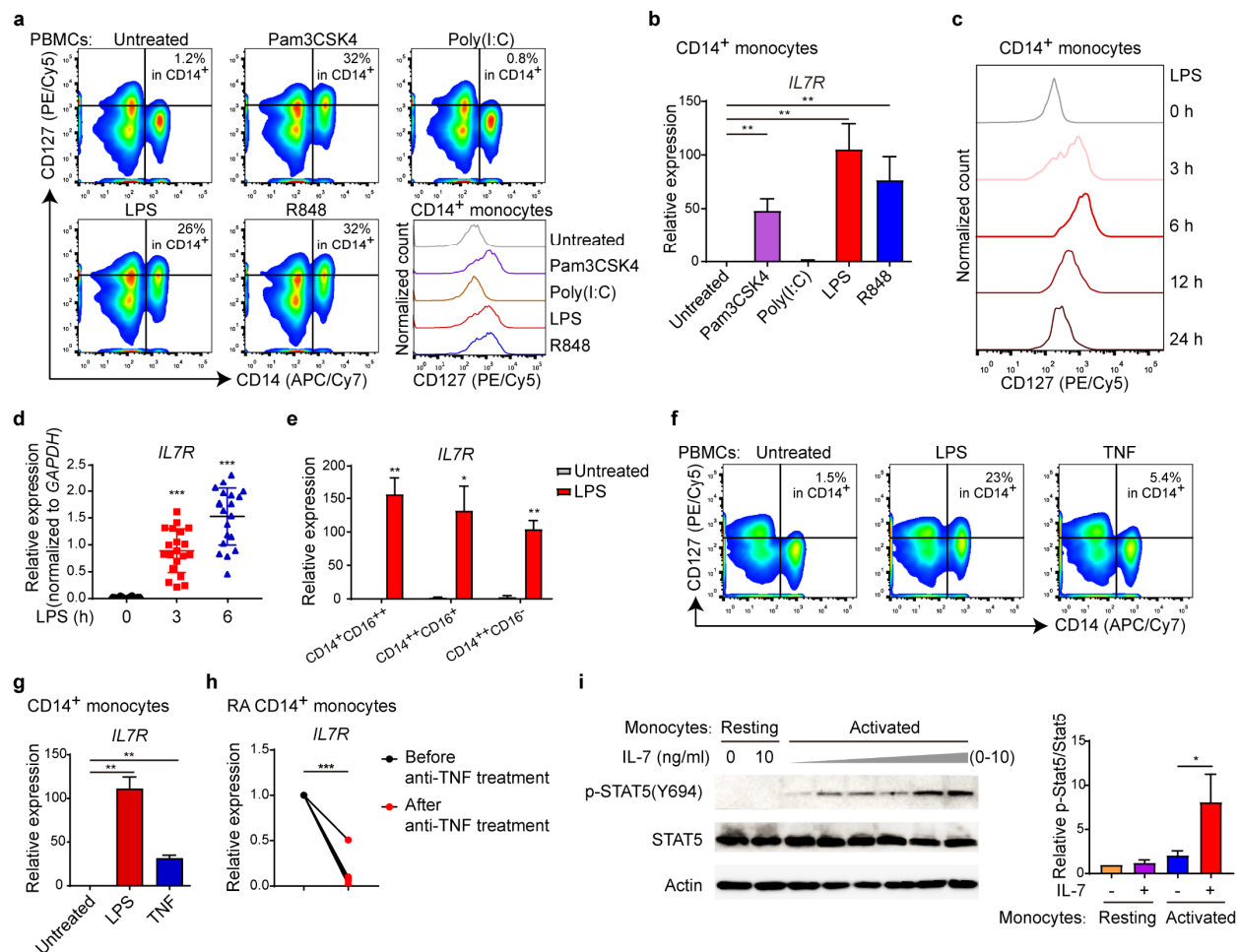
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Figure 1. CD127^{high} monocytes/macrophages are present in human inflammatory conditions

(a) Immunohistochemical analysis of CD68 and CD127 expression in lung tissues sections. Uninfected lung tissues and COVID-19 lung tissues were obtained during autopsy following the protocol described in Methods. One representative result from tissue sections of three COVID-19 cases is shown. Scale bars represent 50 μ m. (b) Immunofluorescence staining for DAPI (blue), CD68 (green) and CD127 (red) in sections from COVID-19 lung tissues. One representative result from tissue sections of three COVID-19 cases is shown. Scale bars represent 50 μ m. (c) UMAP projection of *IL7R*⁺ and *IL7R*⁻ monocytes/macrophages (mono/mac) in broncho-alveolar lavage fluid (BALF) from COVID-19 patients. *IL7R* expression among cells was shown by color as indicated. (d) Violin plot shows the expression of *IL7R* in *IL7R*⁺ and *IL7R*⁻ BALF mono/mac from COVID-19 patients. (e) Pie graph shows the percentage of *IL7R*⁺ cells in BALF mono/mac from COVID-19 patients. (f) Pie graph shows the percentages of each cell type in total *IL7R*⁺ BALF cells from COVID-19 patients. (g) PBMCs were isolated from the peripheral blood of healthy donors (n = 13) and rheumatoid arthritis (RA) patients (n = 8), and CD127 expression was measured by flow cytometry analysis (FACS). Representative FACS plot (left) and cumulative percentages (right) of CD127⁺ population are shown. ****P* < 0.001 by unpaired Student's *t* test. Error bars indicate means \pm SD. (h, i) CD14⁺ monocytes were isolated from PBMCs of healthy donors and RA patients, and mRNA of *IL7R* and *TNF* was measured using quantitative PCR (qPCR) (h). Relative expression was normalized to internal control (*GAPDH*). Linear regression analysis was used to compare the expression of *TNF* and *IL7R* in CD14⁺

532 monocytes from RA patients **(i)**. Correlation coefficient (r) and p-value for coefficient are shown in the panel.
533 ****** $P < 0.01$, ******* $P < 0.001$ by unpaired Student's t test. Error bars indicate means \pm SD. (*IL7R*, Healthy $n = 20$
534 and RA $n = 16$; *TNF*, Healthy $n = 8$ and RA $n = 16$) **(j)** t-SNE projection of *IL7R*⁺ and *IL7R*⁻ cells in RA
535 synovial monocytes. *IL7R* expression among cells was shown by the indicated color. **(k)** Violin plot shows
536 the expression of *IL7R* in *IL7R*⁺ and *IL7R*⁻ RA synovial monocytes. **(l)** Pie graph shows the percentage of
537 *IL7R*⁺ cells in RA synovial monocytes.
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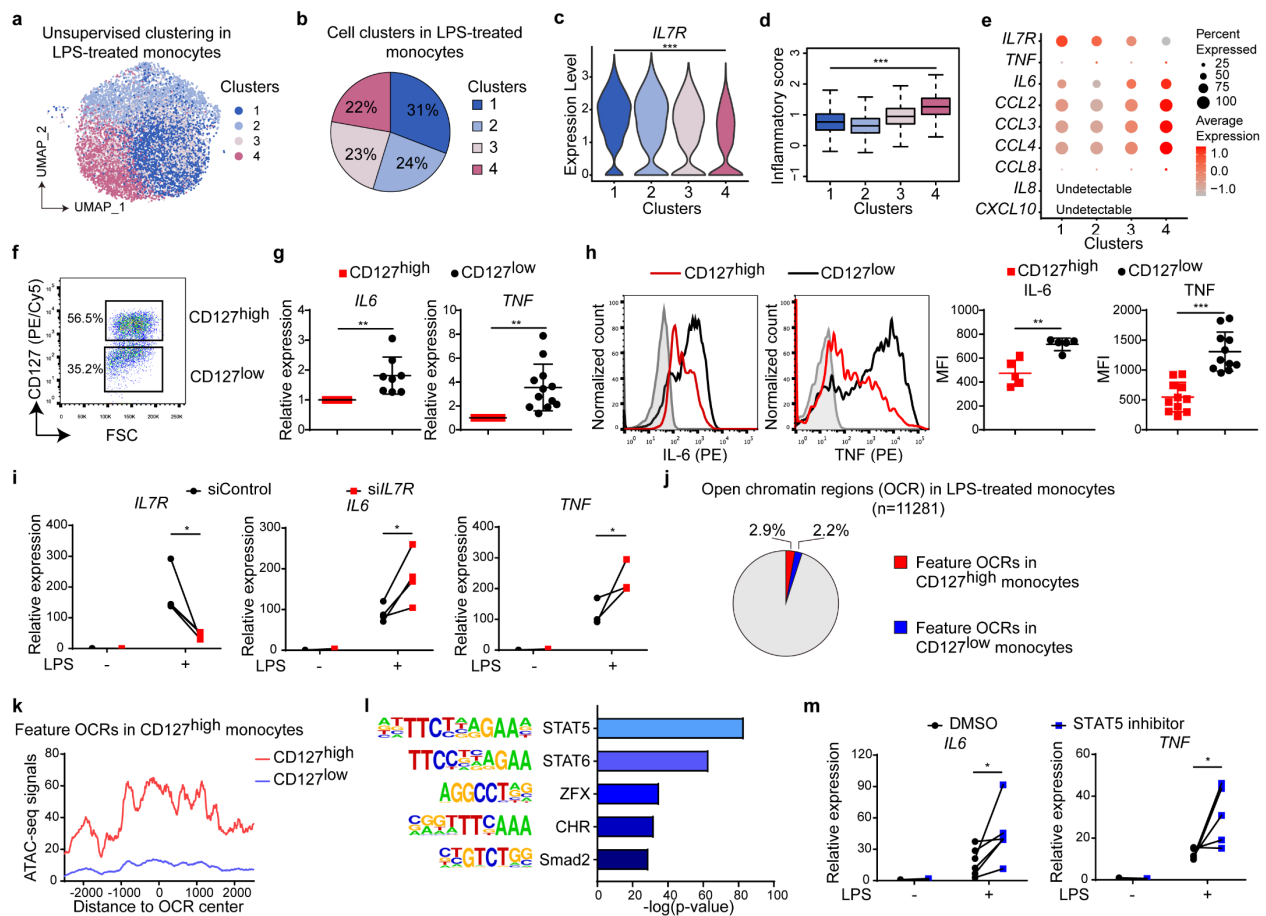


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540 **Figure 2. CD127^{high} monocytes are inducible by inflammatory stimuli and competent for CD127-**
 541 **STAT5 signaling**

542 (a) PBMCs from healthy blood donors were stimulated with Pam3CSK4 (100 ng/ml), Poly(I:C) (1 μg/ml),
 543 LPS (10 ng/ml) or R848 (1 μg/ml) for 6 h, and CD127 expression was measured by FACS. Histograms in
 544 bottom right shows the CD127 staining signal in CD14⁺ cells under each condition as indicated. One
 545 representative FACS result from three biological replicates is shown. (b) CD14⁺ monocytes from healthy
 546 donors' PBMCs were stimulated with Pam3CSK4 (100 ng/ml), Poly(I:C) (1 μg/ml), LPS (10 ng/ml) or R848
 547 (1 μg/ml) for 3 h. The mRNA of *IL7R* was measured by qPCR. Relative expression was normalized to internal
 548 control (*GAPDH*) and expressed relative to untreated sample. ***P*<0.01 by paired Student's *t* test. Data are
 549 shown as means ± SD of four independent experiments with one healthy donor for each data set. (c) CD14⁺
 550 monocytes from healthy donor's PBMCs were treated with 10 ng/ml LPS for various time points as indicated,
 551 and the expression of CD127 was measured by FACS. (d) CD14⁺ monocytes were treated with 10 ng/ml LPS
 552 for 3 h and 6 h, the mRNA of *IL7R* was measured by real time qPCR. Relative expression was normalized to
 553 *GAPDH* as internal control. Each data point represented an independent experiment from one healthy donor.
 554 ****P*<0.001 by unpaired Student's *t* test. Error bars indicate means ± SD of 20 independent experiments. (e)
 555 Three different monocyte subsets were FACS-sorted from PBMCs as shown in Extended Data Fig. 2f. The

556 sorted cells were treated with or without 10 ng/ml LPS for 3 h. The expression of *IL7R* was measured by q-
557 PCR. Relative expression was normalized to internal control (*GAPDH*) and expressed relative to LPS-
558 untreated CD14⁺CD16⁺⁺ sample. ***P*<0.01, **P*<0.05 by unpaired Student's *t* test. Data are shown as means
559 ± SD of three independent experiments with one healthy donor for each data set. **(f, g)** PBMCs from healthy
560 donor were treated with LPS (10 ng/ml) or recombinant human TNF (100 ng/ml). Upon 6 h LPS or TNF
561 treatment, CD127 expression was measured by FACS. Representative FACS distribution is shown **(f)**.
562 mRNA of *IL7R* was measured in 3 h LPS or TNF treated monocytes by qPCR **(g)**. Relative expression was
563 normalized to internal control (*GAPDH*) and expressed relative to untreated sample. ***P*<0.01 by paired
564 Student's *t* test. Data are shown as means ± SD of three independent experiments with one healthy donor for
565 each data set. **(h)** mRNA level of *IL7R* was measured by real time qPCR in monocytes from RA patients (n
566 = 5) before and after Etanercept anti-TNF treatment for 2 months. Relative expression was normalized to
567 internal control (*GAPDH*). ****P*<0.001 by paired Student's *t* test. **(i)** CD14⁺ monocytes were pre-treated with
568 or without LPS for 6 h, then followed by various doses of recombinant human IL-7 (from 1 pg/ml to 10 ng/ml)
569 for 30 min. STAT5 activation was detected by western blotting. Actin was used as loading control. One
570 representative experiment out of three biological replicates is shown (left). The protein level of p-
571 STAT5(Y694) was quantified by densitometry, normalized to total STAT5 protein and expressed relative to
572 untreated (without LPS and IL-7) sample (right). **P*<0.05 by paired Student's *t* test. Data were expressed as
573 mean ±SD of three independent experiments with one healthy donor for each data set.
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Figure 3. CD127 imposes heterogeneity in monocyte inflammatory responses mediated by CD127-STAT5 axis

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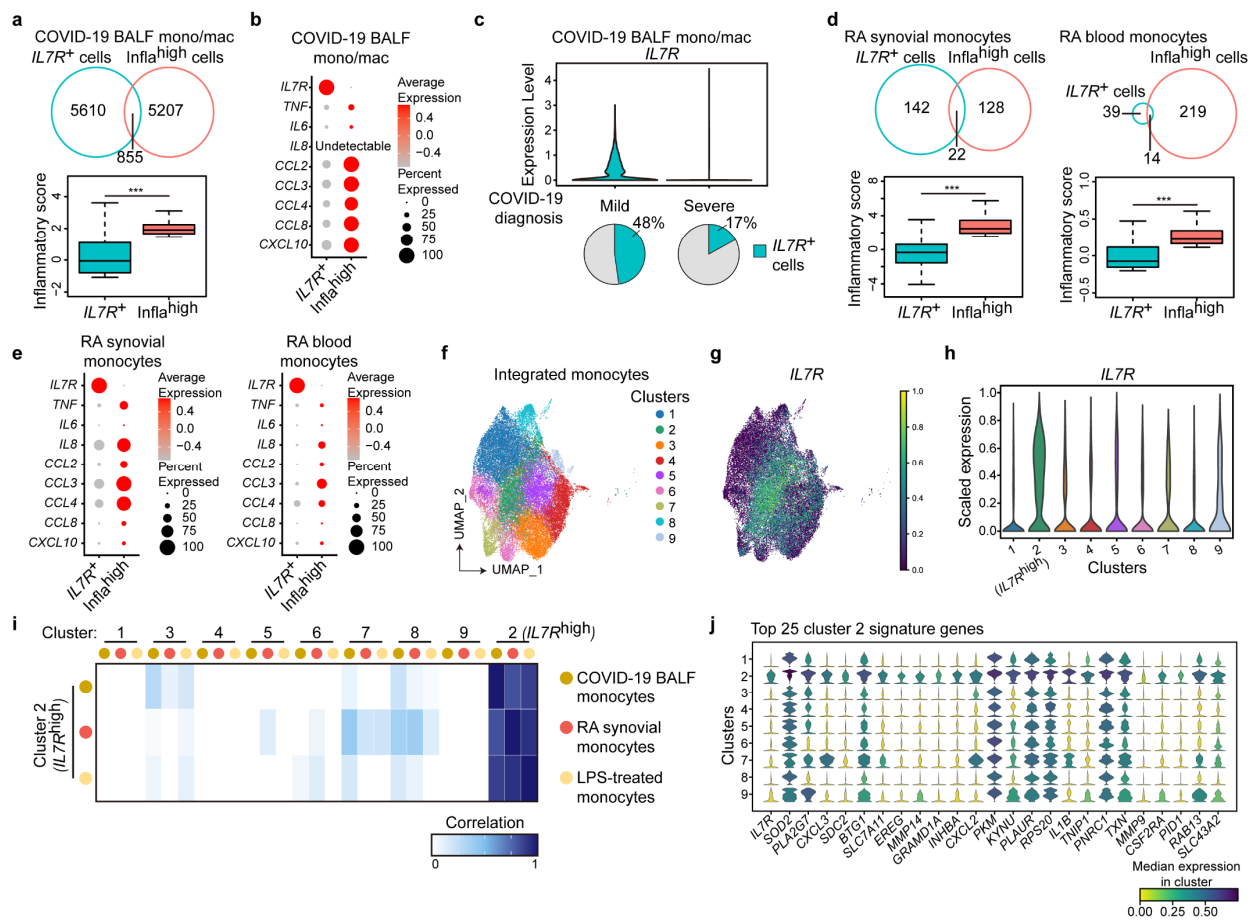
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(a) UMAP projection of LPS 6 h treated human CD14⁺ monocytes that were subgrouped by unsupervised cluster analysis of scRNA-seq data. (b) Pie graph shows the percentages of each monocyte cluster shown in (A) in LPS-treated monocytes. (c) Violin plot shows the expression of *IL7R* among four clusters of LPS-treated monocytes. *** $P < 0.001$ by Wilcoxon rank-sum test. (d) Inflammatory score was defined by average expression of eight inflammatory genes, and box plot shows the inflammatory score distribution among four clusters of LPS-treated monocytes. *** $P < 0.001$ by Wilcoxon rank-sum test. (e) Heat map shows the expression of *IL7R* and eight inflammatory genes for inflammatory score calculation among four clusters of LPS-treated monocytes. The scaled average expression levels for each gene and percentages of cells expressing each gene in each cluster were represented by color and size of the corresponding dots, respectively. (f, g) CD127^{high} and CD127^{low} populations were isolated from 6 h LPS-stimulated CD14⁺ monocytes by FACS (f) and the mRNA levels of *IL6* and *TNF* in two populations were measured by qPCR (g). Relative expression was normalized to internal control (*GAPDH*) and shown relative to CD127^{high} sample. ** $P < 0.01$, *** $P < 0.001$ by paired Student's *t* test. Data are shown as means \pm SD of eight independent experiments for *IL6* and twelve independent experiments for *TNF*. (h) Protein levels of IL-6 and TNF in CD127^{high} and CD127^{low} populations were measured by GolgiStop-utilized intracellular staining and analyzed by flow cytometry. One representative FACS plot (left) and cumulative mean fluorescence

594 intensities (MFI) from five independent experiments for IL-6 and eleven independent experiments for TNF
595 (right) are shown. ****** $P < 0.01$, ******* $P < 0.001$ by paired Student's t test. Data are shown as means \pm SD. **(i)** CD14⁺
596 monocytes were transfected with negative control or *IL7R* specific short interfering RNA (siControl or
597 si*IL7R*). Two days post transfection, cells were stimulated with LPS (10 ng/ml) for 3 h, and the mRNA of
598 *IL7R*, *IL6* and *TNF* were measured by using real time qPCR. Relative expression was normalized to internal
599 control (*GAPDH*) and expressed relative to LPS untreated siControl sample. ***** $P < 0.05$ by paired Student's t
600 test. **(j)** Three healthy donors' CD127^{high} and C127^{low} monocytes were sorted as in (F), and generation of
601 ATAC-seq data sets were performed for two monocyte populations from each donor. Pie graph shows the
602 percentages of feature open chromatin regions (OCR) in CD127^{high} and C127^{low} monocytes in total OCRs in
603 LPS-treated monocytes by statistical analyses for three ATAC-seq data sets. **(k)** Three ATAC-seq data sets
604 were combined, and average ATAC-seq signals were calculated in combined ATAC-seq data set for
605 CD127^{high} and C127^{low} monocytes around feature OCRs in CD127^{high} monocytes. **(l)** Motif enrichment
606 analysis in CD127^{high} monocyte featured open chromatin regions. Top 5 most enriched transcription factor
607 binding motifs are shown, and x-axis is $-\log_{10}(\text{p-value})$ for each enriched motif. Binomial distribution was
608 used for p-value calculation. **(m)** CD14⁺ monocytes were pretreated with STAT5 inhibitor (100 μM) for 2 h
609 and then were stimulated with LPS (10 ng/ml). mRNA levels of *IL6* (LPS 6 h) and *TNF* (LPS 3 h) were
610 measured using qPCR. ***** $P < 0.05$ by paired Student's t test. The results from six independent experiments are
611 shown.
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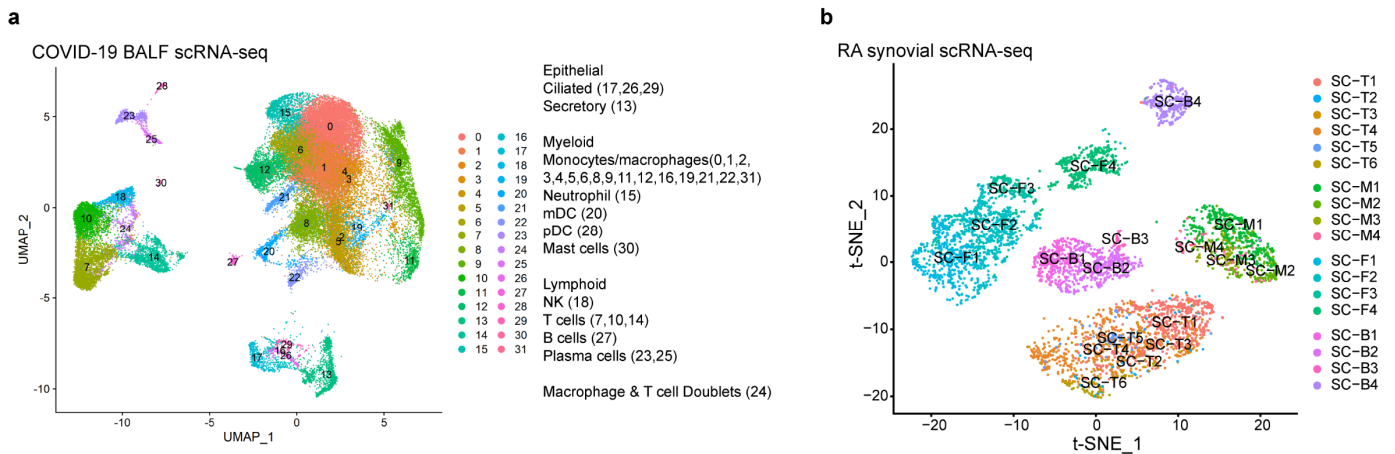
Figure 4. CD127^{high} monocytes are anti-inflammatory in human diseases.

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(a, d) Infla^{high} cells for each disease condition were subgrouped as top 20% inflammatory cells by inflammatory score in mono/mac from COVID-19 BALF, RA synovial monocytes and RA blood monocytes, respectively. Venn diagrams (upper) show the extent of overlap between *IL7R*⁺ cells and infla^{high} cells, and box plots (bottom) show the inflammatory score distribution in *IL7R*⁺ cells and infla^{high} cells in mono/mac from COVID-19 BALF (a), RA synovial monocytes and RA blood monocytes (d) as indicated. ****P*<0.001 by Wilcoxon rank-sum test. (b, e) Heat maps show the expression of *IL7R* and eight inflammatory genes for inflammatory score calculation in *IL7R*⁺ cells and infla^{high} cells in mono/mac from COVID-19 BALF (b), RA synovial monocytes and RA blood monocytes (e) as indicated. The scaled average expression levels for each gene and percentages of cell expressing each gene in each group were represented by color and size of the corresponding dots, respectively. (c) Mono/mac cells from COVID-19 patients' BALF were subgrouped by the diagnosed disease severity. Violin plot (upper) shows the expression of *IL7R* in BALF mono/mac from mild or severe COVID-19 patients. Pie graphs on the bottom show the percentage of *IL7R*⁺ cells in BALF mono/mac from mild or severe COVID-19 patients as indicated. (f) UMAP projection of integrated monocytes from COVID-19 BALF, RA synovial cavity and LPS-treated monocytes. Nine monocyte clusters by unsupervised clustering were indicated by different colors in plot. (g) UMAP projection of integrated monocytes in (f). *IL7R* expression among cells were expressed by color as indicated. (h) Violin plot shows the expression of *IL7R* among nine clusters of integrated-monocytes in (f). Cluster 2 were named as *IL7R*^{high}

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632 cluster by highest *IL7R* expression among clusters. **(i)** For populations of COVID-19 BALF monocytes, RA
633 synovial monocytes and LPS-treated monocytes, heat map shows the correlation between cluster 2 cells in
634 each monocytes population and all nine clusters in each monocytes population, respectively. **(j)** The stacked
635 violin plot shows the expression of top 25 cluster 2 signature genes in all nine monocyte clusters in **(f)**.
636 Median expression levels for each gene in each cluster were expressed by color as indicated.
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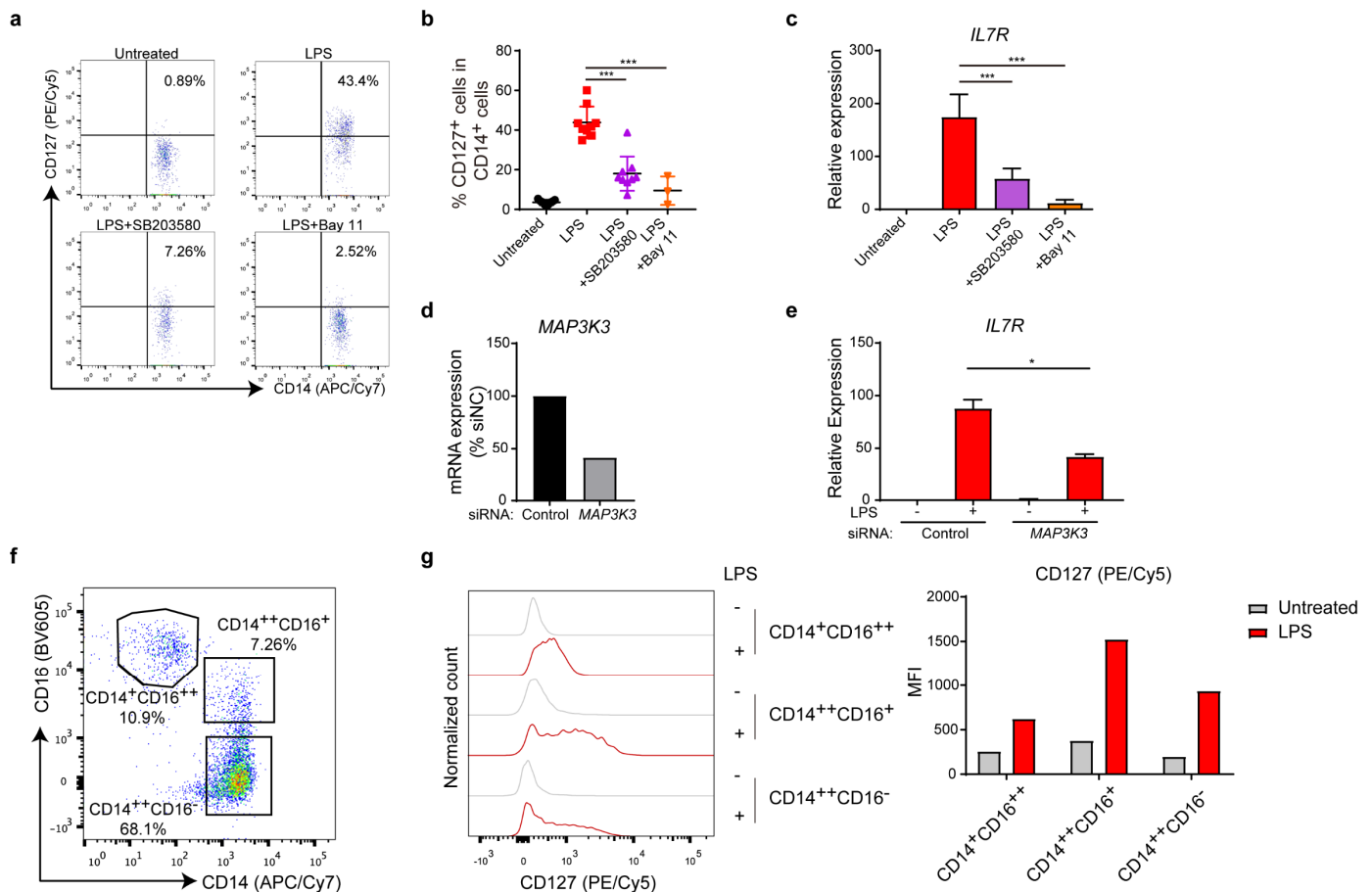


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639 **Extended Data Figure 1. Clustering analyses of scRNA-seq data sets**

640 **(a)** UMAP projection of broncho-alveolar lavage fluid (BALF) cells from COVID-19 patients. Cell type
 641 annotations were labeled for each cluster. Monocyte/macrophage clusters ($CD14^{high} CD68^{high}$) were used for
 642 the subsequent analyses. **(b)** t-SNE projection of synovial cells from RA patients. Cell type annotations were
 643 labeled for each cluster. T, M, F and B represent T cell, monocytes, fibroblasts and B cells, respectively.
 644 Monocyte clusters ($CD14^{high}$) were used for the subsequent analyses.

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Extended Data Figure 2. CD127 upregulation in activated human monocytes is dependent on canonical TLR signaling and observed in all monocyte subsets.

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(a, b) PBMCs from healthy donors were pretreated with DMSO or 10 μ M SB203580 or 10 μ M Bay 11-7082 (Bay 11) for 30 min and subsequently stimulated with or without 10 ng/ml LPS for 6 hours as indicated. The protein levels of CD127 were measured by flow cytometry and was shown as representative FACS distribution (a) and cumulative percentages (b) in CD14⁺ monocytes. Each data point represents the result from an independent experiment with cells obtained from a healthy donor. *** P <0.001 by unpaired Student's t test. Data are shown as means \pm SD of multiple independent experiments as listed respectively: Untreated n = 9, LPS n = 9, SB203580 n = 9, Bay 11-7082 n = 3. (c) CD14⁺ monocytes were isolated from healthy donors' PBMCs, and were pretreated with DMSO or 10 μ M SB203580 or 10 μ M Bay 11-7082 (Bay 11) for 30 min and subsequently stimulated with or without 10 ng/ml LPS for 6 h as indicated. The mRNA levels of *IL7R* were measured by qPCR. The relative expression was normalized to internal control (*GAPDH*) and expressed relative to the untreated sample. *** P <0.001 by unpaired Student's t test. Data are shown as means \pm SD of multiple independent experiments as listed respectively: Untreated n = 9, LPS n = 9, SB203580 n = 6, Bay 11-7082 n = 3. (d, e) CD14⁺ monocytes were transfected with negative control or *MAP3K3* specific short interfering RNAs. Two days post transfection, cells were stimulated with LPS (10 ng/ml) for 3 h. Knock down efficiency of *MAP3K3* was examined (d), and mRNA induction of *IL7R* by LPS stimulation in siControl and si*MAP3K3* transfected cells was measured by qPCR (e). Relative expression was normalized

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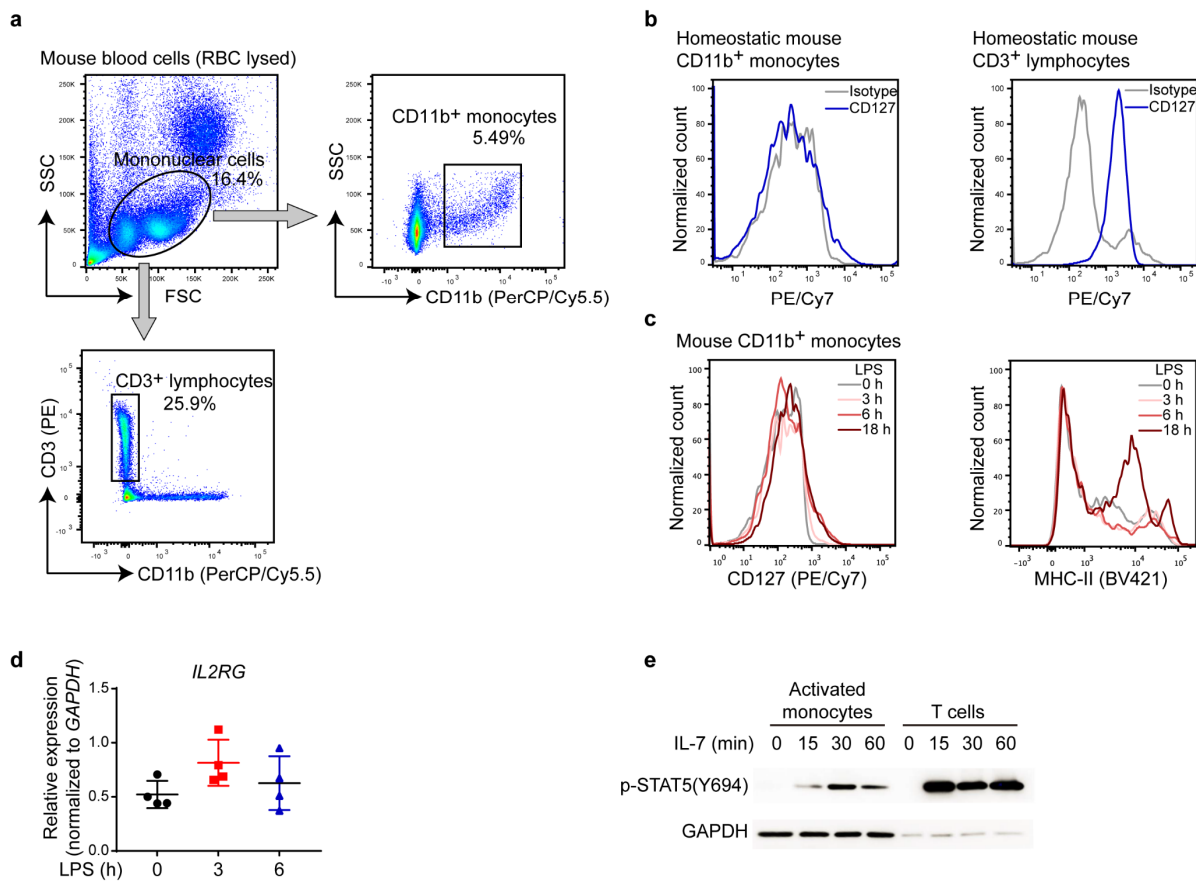
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665 to internal control (*GAPDH*) and expressed relative to LPS untreated siControl sample. * $P < 0.05$ by paired
666 Student's *t* test. *IL7R* expression data are shown as means \pm SD of three independent experiments with cells
667 from one healthy donor for each data set. **(f, g)** Three monocyte populations were gated by CD14 and CD16
668 expression in flow cytometry analysis of human PBMCs. CD127 was also stained in PBMCs with or without
669 LPS stimulation, and CD127 upregulation in three human monocyte populations was analyzed by gating
670 strategy shown in **(f)**. Representative FACS distribution **(g, left)** and mean fluorescence intensity (MFI) **(g,**
671 right) for CD127 expression are shown.

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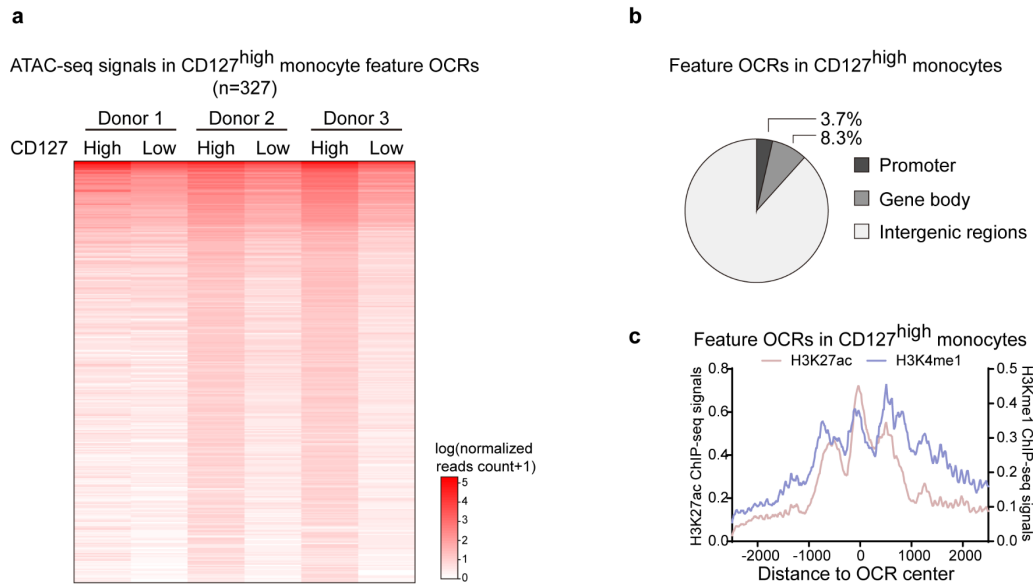


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674 **Extended Data Figure 3. Human but not mouse activated monocytes are CD127 positive and**
 675 **functionally competent for IL-7 receptor signaling.**

676 (a) Gating strategy for CD11b⁺ mouse monocytes and CD3⁺ mouse lymphocytes from red blood cell (RBC)
 677 lysed mouse peripheral blood cells. (b, c) Mouse blood cells (RBC lysed) were treated with or without 100
 678 ng/ml LPS for different time points as indicated. The expression of CD127 (b) and MHC-II (c) on mouse
 679 CD11b⁺ monocytes was analyzed by flow cytometry. (d) Human CD14⁺ monocytes were treated with 10
 680 ng/ml LPS for 3 h and 6 h, the mRNA levels of *IL2RG* were measured by qPCR. Each data point represents
 681 the result from an independent experiment with cells obtained from a healthy donor. Relative expression was
 682 normalized to *GAPDH* as internal control. Data are shown as means ± SD of four independent experiments.
 683 (e) Human CD14⁺ monocytes were pretreated with 10 ng/ml LPS for 6 h, and subsequently stimulated with
 684 recombinant human IL-7 (10 ng/ml) for the indicated time. Meanwhile, CD3⁺ T cells from the same donor
 685 were treated with 10 ng/ml IL-7 for the indicated time. The protein levels of p-STAT5(Y694) were detected
 686 by western blotting. GAPDH was used as a loading control.

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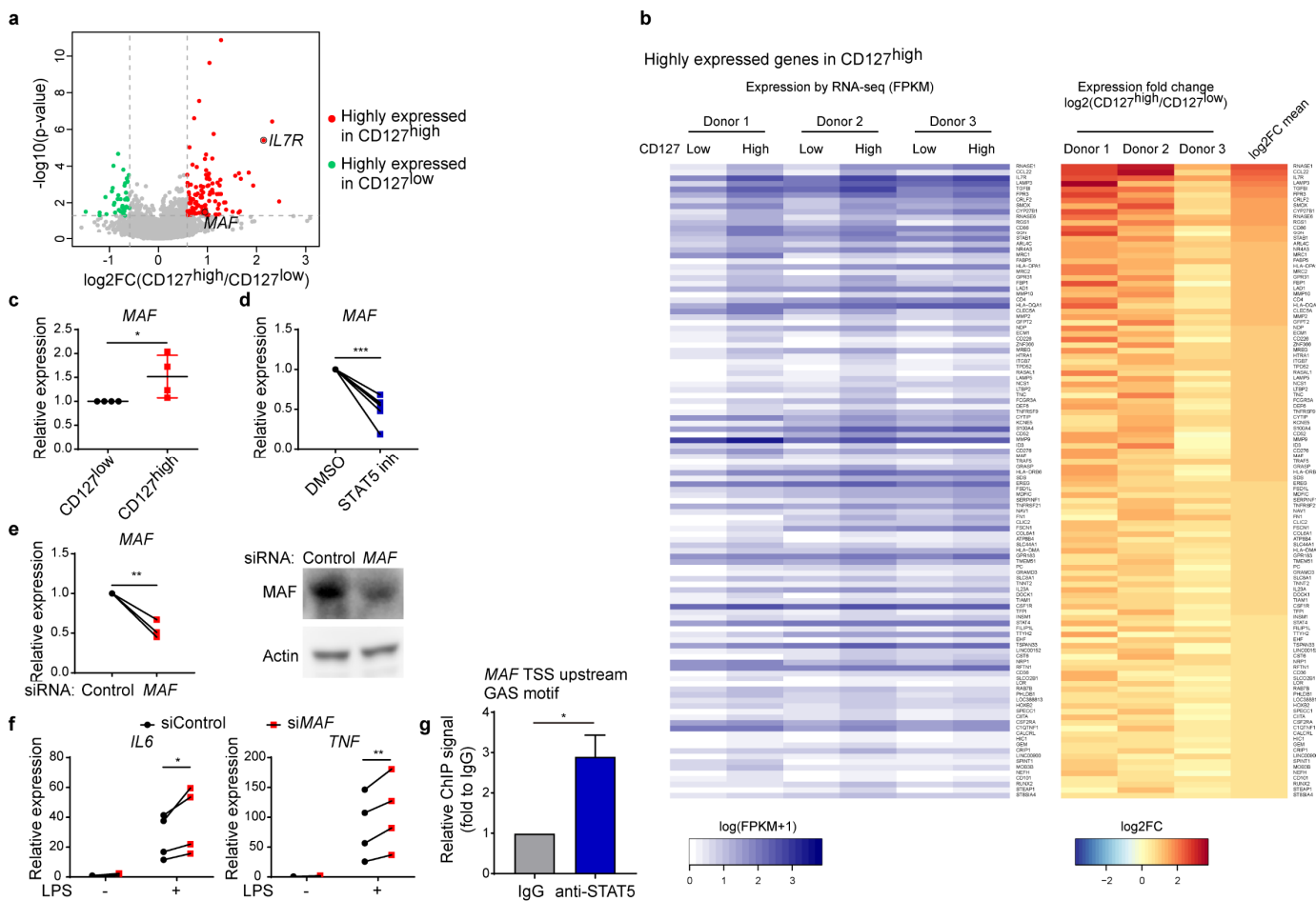


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689 **Extended Data Figure 4. Open chromatin regions (OCRs) in CD127^{high} monocytes display enhancer-**
690 **like features.**

691 (a) Heat map shows the ATAC-seq signals in OCRs featured in CD127^{high} monocytes and in CD127^{low}
692 monocytes from three independent experiments with three blood donors. The ATAC-seq reads count was
693 normalized (per 10 million mapped reads) and then $\log_{10}(n + 1)$ transformed for expression in heat map. (b)
694 Pie graph shows the genomic distribution of CD127^{high} monocyte-featured OCRs. (c) Average ChIP-seq
695 signals of H3K27ac and H3K4me1 in LPS activated monocytes were assessed around CD127^{high} monocyte-
696 featured OCRs and expressed by different colors as indicated in the plot.

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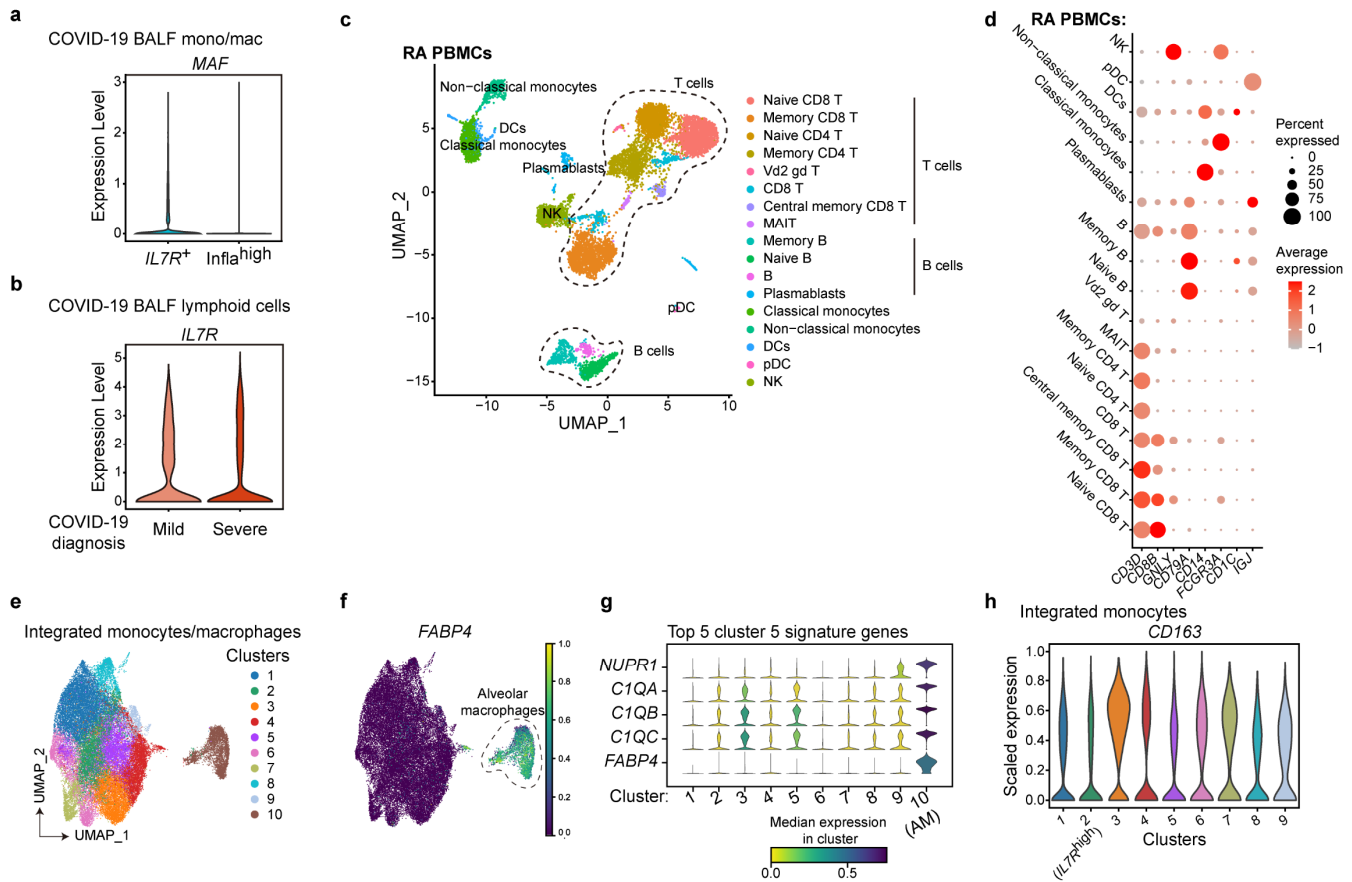
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699 **Extended Data Figure 5. CD127-STAT5-c-Maf axis imposes an anti-inflammatory loop in activated**
 700 **monocytes**

701 (a) Volcano plot shows the differentially expressed genes by RNA-seq between CD127^{high} and CD127^{low}
 702 populations from CD14⁺ monocytes upon 6 h LPS stimulation. Red colors indicate highly expressed genes
 703 in CD127^{high} population, and green colors indicate highly expressed genes in CD127^{low} population. Two
 704 CD127^{high} highly expressed genes, *IL7R* and *MAF*, were highlighted with black circles. The dash lines
 705 indicate the threshold of p-value ($p\text{-value} < 0.05$) and fold change (≥ 1.5 fold for upregulation and ≤ 0.67 fold
 706 for downregulation) for identifying differentially expressed genes. Fold changes are shown as mean value of
 707 CD127^{high}/ CD127^{low} ratio from three healthy donors. (b) Heat map shows the highly expressed genes in
 708 CD127^{high} monocytes in (A). The original expression (\log_{10} transformed FPKM+1, left) and expression fold
 709 change (\log_2 transformed CD127^{high}/CD127^{low}, right) were shown. Fold changes are shown as the individual
 710 value in each donor and the mean value from three donors. (c) The mRNA expression of *MAF* in CD127^{high}
 711 and CD127^{low} monocytes was measured by qPCR. Relative expression was normalized to internal control
 712 (*GAPDH*) and expressed relative to CD127^{low} sample. Each data point represents the result from an
 713 independent experiment with cells obtained from a healthy donor. * $P < 0.05$ by paired Student's *t* test. Data
 714 are shown as means \pm SD of four independent experiments. (d) CD14⁺ monocytes were pretreated with
 715 STAT5 inhibitor (100 μM) for 2 h and then were stimulated with LPS (10 ng/ml) for 6 h. mRNA of *MAF*

716 was measured by qPCR. Relative expression is normalized to internal control (*GAPDH*) and expressed
717 relative to DMSO treated sample. Each data point represents the result from an independent experiment with
718 cells obtained from a healthy donor. *** $P < 0.001$ by paired Student's *t* test. The results from six independent
719 experiments are shown. (e, f) CD14⁺ monocytes were transfected with negative control or *MAF* specific short
720 interfering RNA (siControl or si*MAF*). Two days post transfection, knockdown efficiency was assessed by
721 measuring *MAF* mRNA with qPCR (e left, relative expression is normalized to internal control (*GAPDH*)
722 and expressed relative to siControl group) and *MAF* protein levels with immunoblotting (e right). Two days
723 post transfection, cells were stimulated with LPS (10 ng/ml) for 3 h and mRNA levels of *IL6* and *TNF* were
724 measured by qPCR (f). Relative expression was normalized to internal control (*GAPDH*) and expressed
725 relative to LPS untreated siControl sample. Each data point represents the result from an independent
726 experiment with cells obtained from a healthy donor. * $P < 0.05$, ** $P < 0.01$ by paired Student's *t* test. The
727 results from four independent experiments are shown. (g) Occupancy of STAT5 on the *MAF* transcription
728 start site (TSS) upstream GAS motif was assessed by ChIP-qPCR in THP-1 cells with 6 h LPS stimulation
729 prior to 30 min IL-7 stimulation. IgG ChIP was used as negative control, and relative STAT5 ChIP signals
730 were expressed relative to IgG ChIP control sample. * $P < 0.05$ by paired Student's *t* test. Data are shown as
731 means \pm SD of three independent experiments.

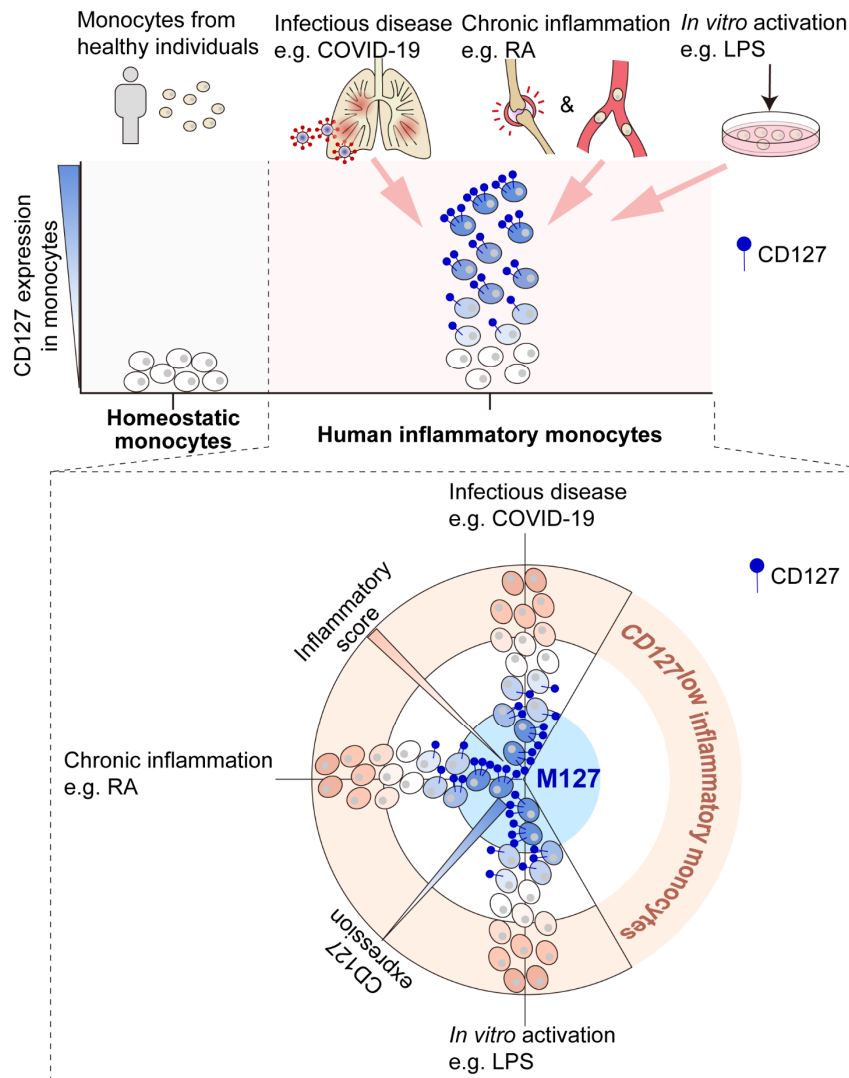
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734 **Extended Data Figure 6. CD127 expression designates a functionally distinct monocyte subset**

735 (a) Violin plot shows the *MAF* expression distribution in *IL7R*⁺ cells and infla^{high} cells in
 736 monocytes/macrophages from COVID-19 patients' BALF in Fig. 4a. (b) Lymphoid cells from COVID-19
 737 patients' BALF were subgrouped by the diagnosed disease severity. Violin plot shows the expression of *IL7R*
 738 in BALF lymphoid cells from mild or severe COVID-19 patients. (c) UMAP projection of PBMCs from RA
 739 patient. Cell type annotations were labeled for each cluster. (d) Heat map shows the expression of hallmark
 740 genes in different cell clusters from RA PBMCs. The scaled average expression levels of marker genes and
 741 percentage of cell expressing marker genes were expressed by color and size of each dot corresponding to
 742 cell clusters, respectively. (e) UMAP projection of integrated monocytes/macrophages from COVID-19
 743 BALF, RA synovial cavity and LPS-activated monocytes. Ten clusters by unsupervised clustering were
 744 indicated by different colors in plot. (f) UMAP projection of integrated monocytes/macrophages in (e).
 745 *FABP4* expression in cells was quantitatively visualized by the indicated colors. Cells corresponding to
 746 Cluster 5 were highlighted by dotted line as alveolar macrophages given the specific *FABP4* expression
 747 pattern. (g) The stacked violin plot shows the expression of top 5 cluster 5 signature genes in all ten clusters
 748 shown in (e). Median expression levels for each gene in each cluster were expressed by colors as indicated.
 749 (h) Violin plot shows the expression of *CD163* among nine clusters of integrated monocytes in Fig. 4f, in
 750 which cluster 2 was designated as *IL7R*^{high} cluster.



751

752 **Extended Data Figure 7. M127 represents a unique hypo-inflammatory monocyte population in**
753 **human inflammatory diseases**

754 CD127 is minimally expressed on homeostatic monocytes from healthy individuals. Upregulation of CD127
755 is a unified hallmark event for human monocyte activation under various inflammatory conditions including
756 COVID-19, RA and stimulated blood monocytes. CD127⁺ inflammatory monocytes, M127, display unique
757 gene signatures and subdued inflammatory phenotypes imposed by CD127-STAT5 signaling. As a result,
758 CD127 levels inversely correlate with the inflammatory capacity of human monocytes. Identification and
759 characterization of M127 highlight the previously underappreciated functional diversity among human
760 monocytes in inflammatory diseases.

761 **Extended Data Table 1. qPCR primers**

Gene	Forward (5'-- 3')	Reverse (5'-- 3')
<i>IL7R</i>	TCCAACCGGCAGCAATGTAT	TGACCAACAGAGCGACAGAG
<i>IL2RG</i>	GTGCAGCCACTATCTATTCTCTG	GTGAAGTGTTAGGTTCTCTGGAG
<i>IL6</i>	ACCCCCAATAAATATAGGACTGGA	TTCTCTTTCGTTCCCGGTGG
<i>TNF</i>	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
<i>MAP3K3</i>	ACCAGCATCAACAGTGAGGG	TGATTTCCGGAAGGATGGGC
<i>MAF</i>	ACTGGCAATGAGCAACTCCG	CACTGGCTGATGATGCGGTC

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764 **Extended Data Table 2. Antibodies used for the experiments**

Antibodies	Manufacturer	Catalog number
Anti-CD68 antibody	Abcam	ab201340
Anti-human CD127 antibody	Invitrogen	PA5-97870
Goat anti-rabbit IgG (H&L)-HRP conjugated	Bioeasy (Beijing) Technology CO. Ltd	BE0101
Goat anti-mouse IgG (H&L)-HRP conjugated	Bioeasy (Beijing) Technology CO. Ltd	BE0102
Alexa Fluor™ 488 goat anti-mouse IgG	Life Technologies	B40941
Alexa Fluor™ 555 goat anti-rabbit IgG	Invitrogen	A27039
Anti-mouse CD3ε antibody PE	Biolegend	100307
Anti-mouse CD11b PerCP-Cyanine5.5	eBioscience	45-0112-82
Anti-mouse CD127 PE-Cyanine7	Biolegend	135013
PE-Cy7 Rat IgG2a, κ isotype control antibody	Biolegend	400521
Anti-mouse MHC class II BV421	eBioscience	48-5321-82
Anti-human CD127 antibody PE-Cyanine5	Biolegend	351323
Anti-human CD14 antibody APC-Cyanine7	Biolegend	301820
Anti-human IL-6 antibody PE	Biolegend	501106
Anti-human TNF-α antibody PE	Biolegend	502908
PE Rat IgG2b, κ isotype control antibody	Biolegend	400607
Anti-human CD16 antibody BV605	Biolegend	302039
Anti-GAPDH Mouse Monoclonal Antibody	Bioeasy (Beijing) Technology CO. Ltd	BE0023
Anti-β-Actin Mouse Monoclonal Antibody	Abclonal	AC026
Anti-Phospho-Stat5 (Tyr694) Rabbit Monoclonal Antibody	Cell Signaling Technology	9314S
Anti-Stat5 Antibody	Cell Signaling Technology	9363S
Anti-c-Maf Antibody	Santa Cruz Biotechnology	sc-518062

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