TNF-α and TGF-β Counter-Regulate PD-L1 Expression on Monocytes in Systemic Lupus Erythematosus

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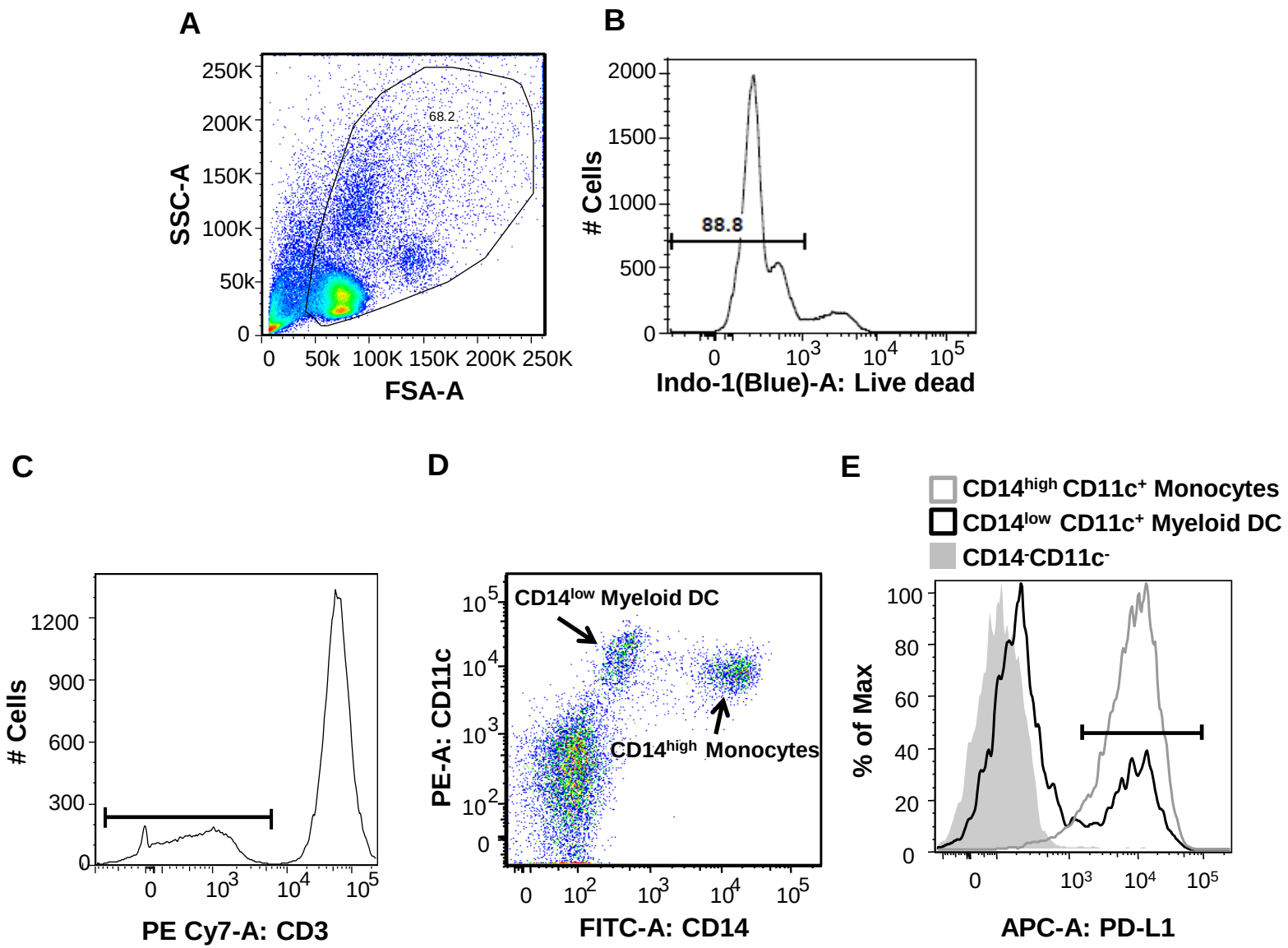
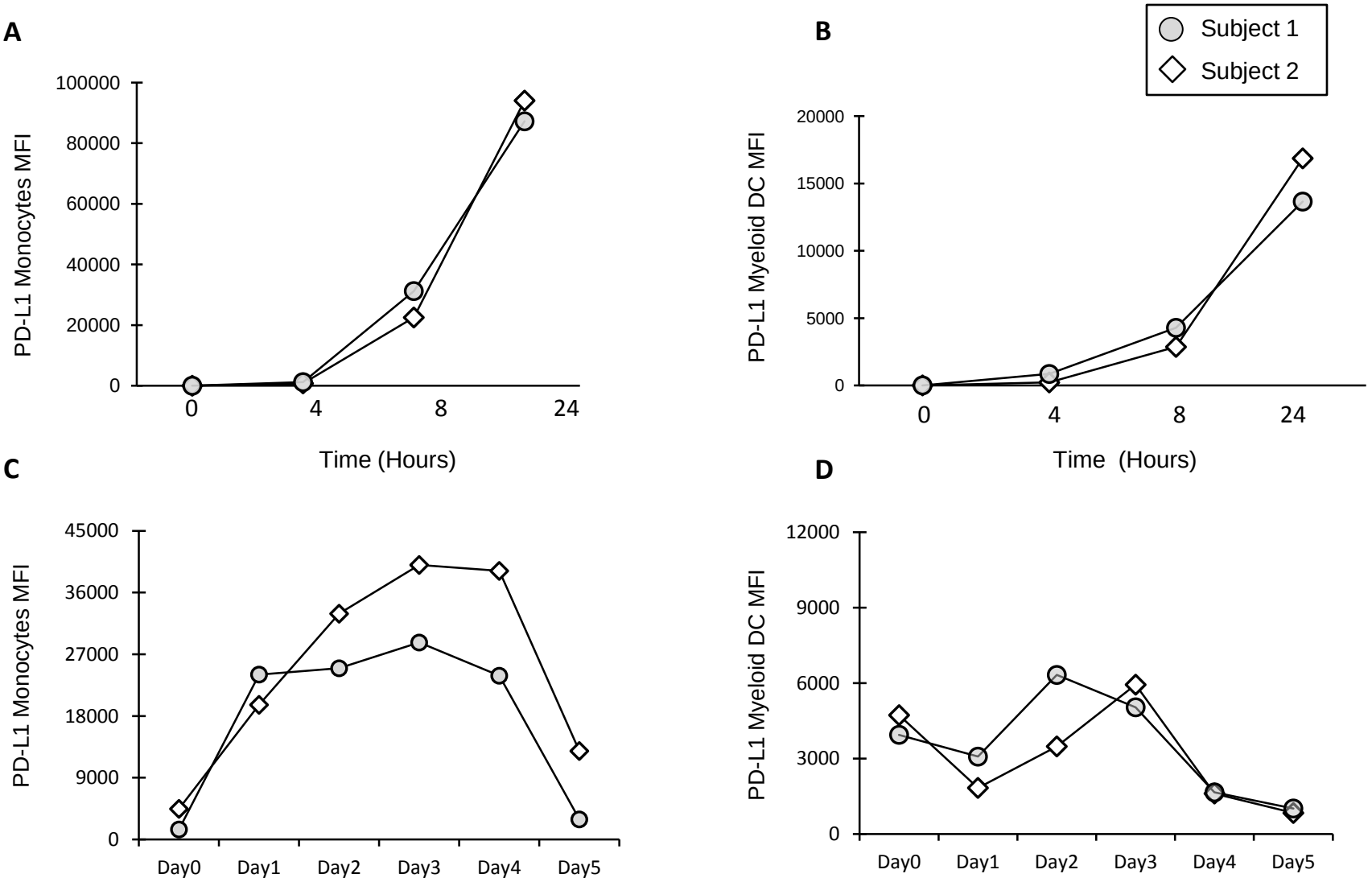
S. Figure-1:


A
250K
200K
150K
100K
50k
0
SSC-A
68.2
0 50k 100K 150K 200K 250K
FSA-A
B
2000
1500
1000
500
0
Cells
88.8
0 10^3 10^4 10^5
Indo-1(Blue)-A: Live dead
C
1200
900
600
300
0
Cells
0 10^3 10^4 10^5
PE Cy7-A: CD3
D
CD14low Myeloid DC
CD14high Monocytes
10^5
10^4
10^3
10^2
0
PE-A: CD11c
0 10^2 10^3 10^4 10^5
FITC-A: CD14
E
CD14high CD11c+ Monocytes
CD14low CD11c+ Myeloid DC
CD14-CD11c-
100
80
60
40
20
0
% of Max
0 10^3 10^4 10^5
APC-A: PD-L1

Supplemental Figure 1. Gating strategy to assay PD-L1 expression on $CD14^+$ monocytes and myeloid dendritic cells. A, A forward and side scatter plot was used to identify leukocyte subpopulations. **B**, Cell viability was determined by LIVE⁄DEAD staining. **C**, Myeloid cells were differentiated from T lymphocytes by CD3 staining. **D**, Phenotyping analyses segregate $CD3^-$ cells into monocytes ($CD3^-CD14^{high}CD11c^+$), and myeloid DC ($CD3^-CD14^{low}CD11c^+$). **E**, Representative cytometry histogram shows PD-L1 expression was induced on myeloid DC and monocytes. The background PD-L1 expression is represented by $CD14^-CD11c^-$ cells.

S. Figure-2


A
PD-L1 Monocytes MFI
100000
80000
60000
40000
20000
0
0
4
8
24
Time (Hours)
B
Subject 1
Subject 2
PD-L1 Myeloid DC MFI
20000
15000
10000
5000
0
0
4
8
24
Time (Hours)
C
PD-L1 Monocytes MFI
45000
36000
27000
18000
9000
0
Day0
Day1
Day2
Day3
Day4
Day5
D
PD-L1 Myeloid DC MFI
12000
9000
6000
3000
0
Day0
Day1
Day2
Day3
Day4
Day5

Supplemental Figure 2. Time course of PD-L1 surface protein expression *ex vivo*. PBMC were cultured over 24 hours without stimulation. PD-L1 protein expression was assayed by flow cytometry at indicated time point. **A**, PD-L1 mean fluorescence intensity (MFI) on monocytes ($CD3^{-}CD14^{high}CD11c^{+}$); **B**, PD-L1 expression on myeloid DC ($CD3^{-}CD14^{low}CD11c^{+}$), subtracting background ($CD3^{-}CD14^{-}CD11c^{-}$) MFI. Expression of PD-L1 in cultured PBMC from two healthy subjects over a five day time course. **C**, PD-L1 MFI on monocytes; **D**, PD-L1 expression on myeloid DC, subtracting background MFI.