Direct recognition of LPS drive TLR4 expressing CD8$^+$ T cell activation in patients with rheumatoid arthritis.

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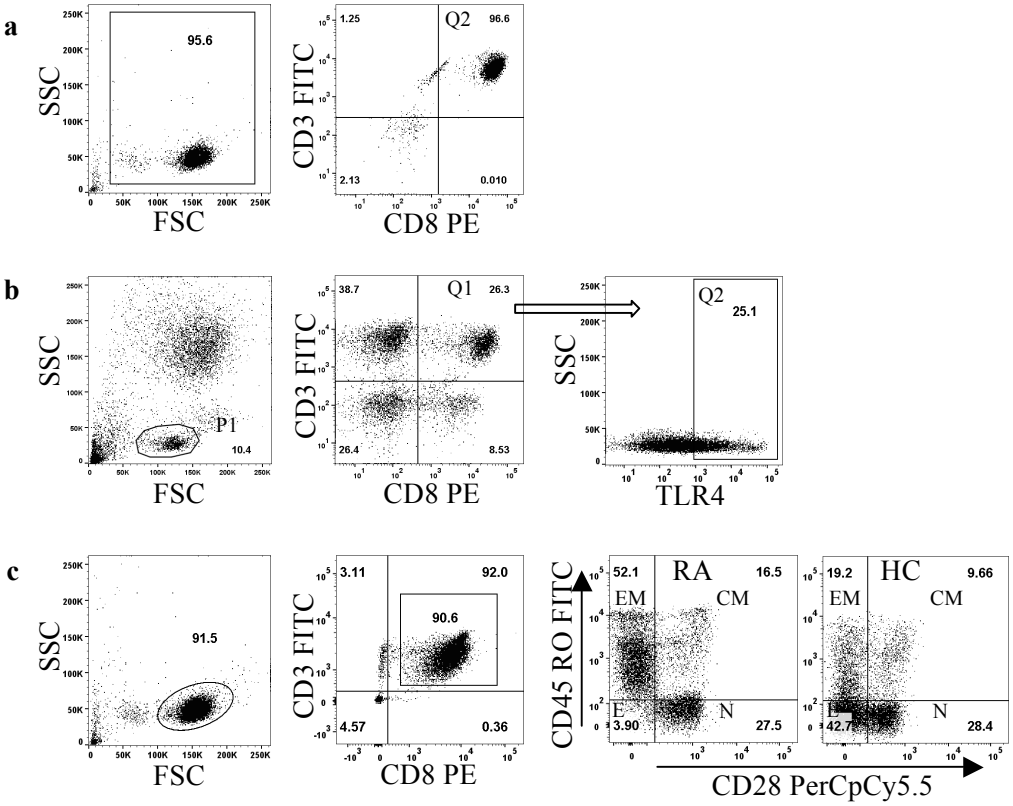
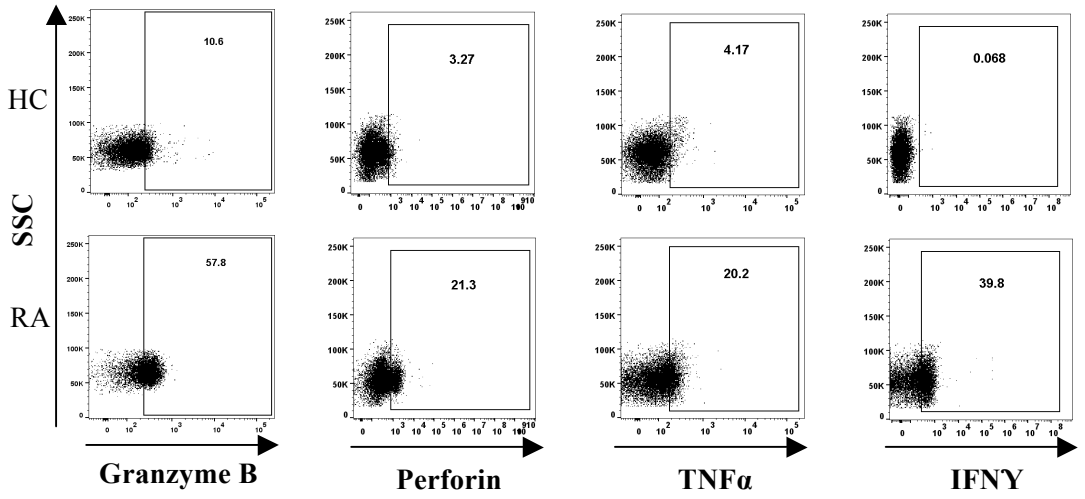
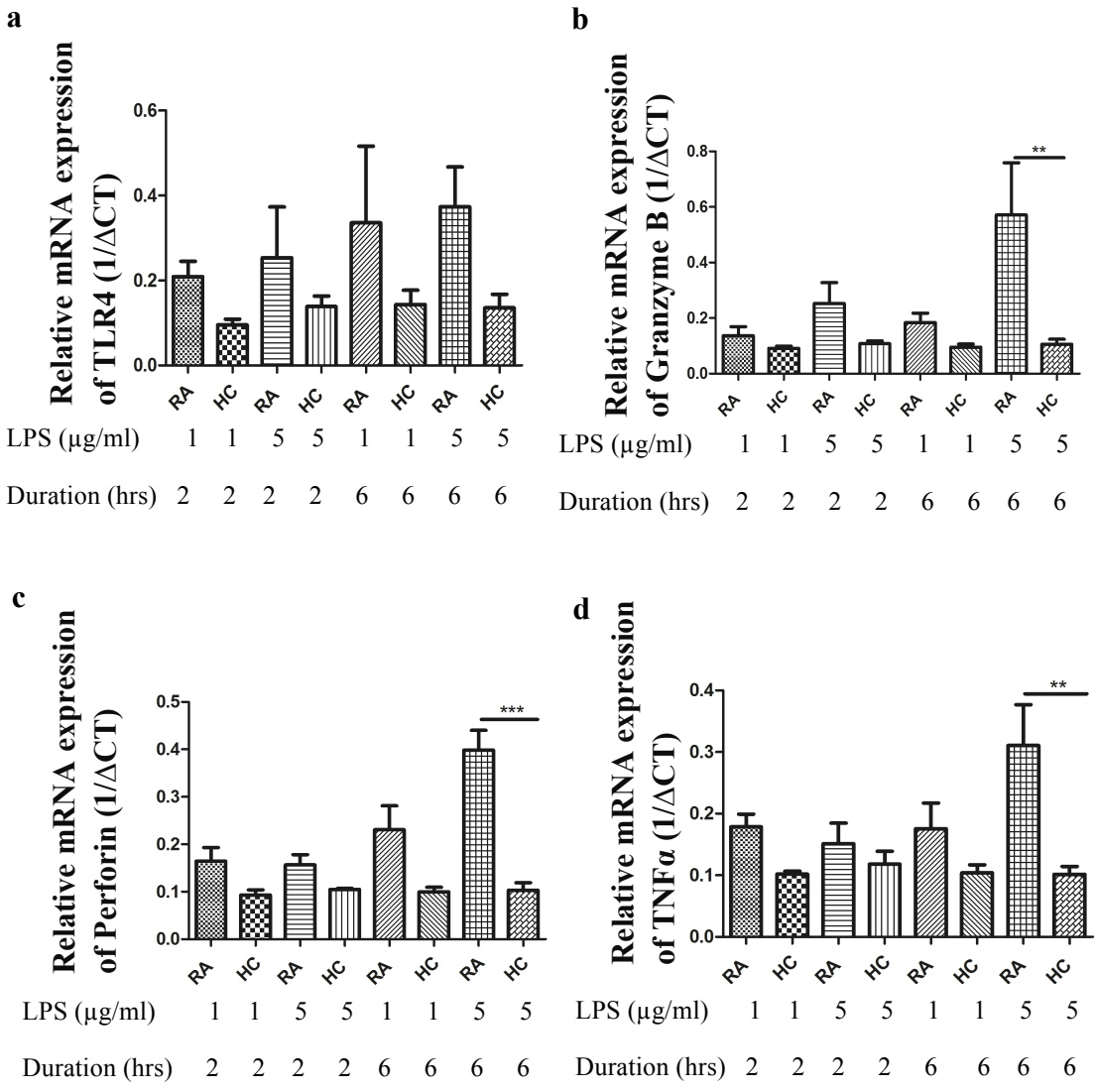
Supplementary Figures:

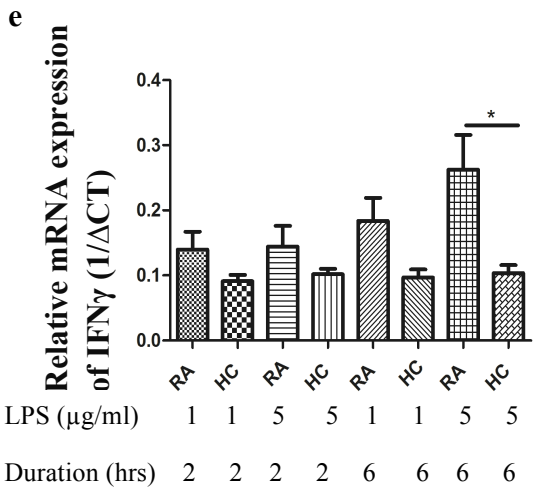


Supplementary Figure 1: (a) Flow cytometry gating strategy for isolated CD8$^+$ T cells using RosetteSep CD8$^+$ T cells isolation kit. Quadrant 2 (Q2) represents percentage of CD3 and CD8 double positive cells from enriched samples. (b) Flow cytometry gating strategy for identification of TLR4 expressing CD3$^+$CD8$^+$ T cells from PBMCs. Lymphocyte population was gated and named as P1. Q1 population represents CD3$^+$ CD8$^+$ T cells and Q2 population shows TLR4$^+$ CD3$^+$CD8$^+$ T cells. (c) Gating strategy for identification of CD8$^+$ T cells subpopulations (N: Naïve cells, E: Effector cells, EM: Effector memory cells, CM: Central memory cells).

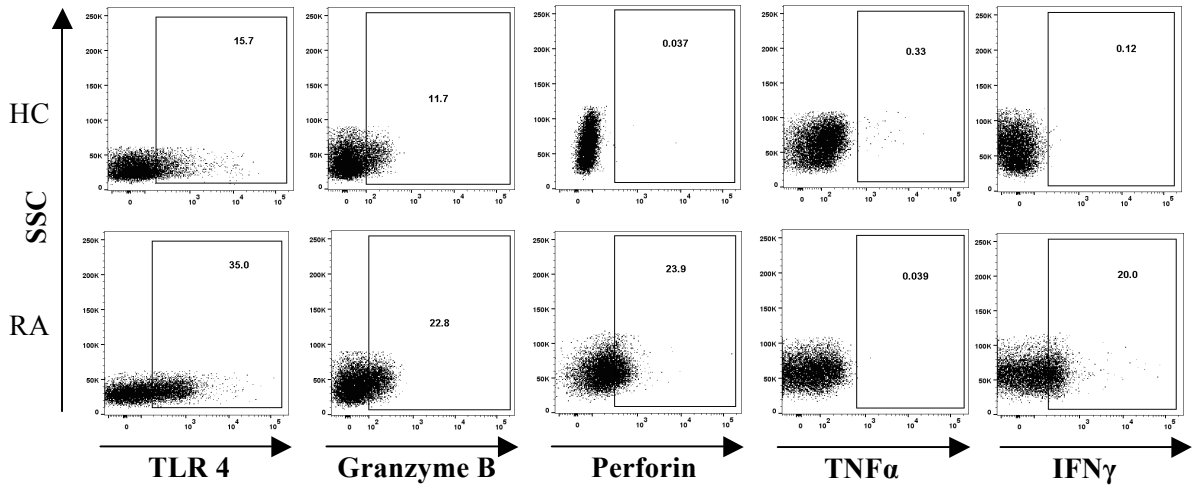


Supplementary Figure 2: Dot plots show a representative figure of Granzyme B, Perforin, TNFα and IFNγ expressing $CD8^+$ T cells in a HC and RA patient. Upper panels represent for healthy control and lower panels represent for RA patients.





Supplementary Figure 3: Different stimulation conditions of $CD8^+$ T cells isolated from RA patients to observe expression pattern of TLR4, Granzyme B, Perforin, TNFα and IFNγ mRNA transcripts. $CD8^+$ T cells were isolated from both RA patients (n=6) and healthy controls (n=6) and cultured for 2hrs and 6hrs with 1 µg/ml and 5 µg/ml LPS in RPMI-1640 media. Results are expressed as normalized RNA values ($1/\Delta C_t$) at 2hrs and 6hrs after culture. (b, c, d, e). A significant increase in mRNA expression of Granzyme B, Perforin, TNFα and IFNγ in $CD8^+$ T cells of RA patients incubated with 5µg/ml concentration of LPS for 6hrs in comparison to HC was observed. Bars represent mean ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Figure 4: Representative dot plots for TLR 4, Granzyme B, Perforin, TNFα and IFNγ expressing $CD8^+$ T cells after stimulating with 5 μg/ml of LPS-EB ultrapure for 6 hrs at 37°C and 5% CO_2 in a HC and RA patient. Upper panels represent healthy control and lower panels represent RA patient.