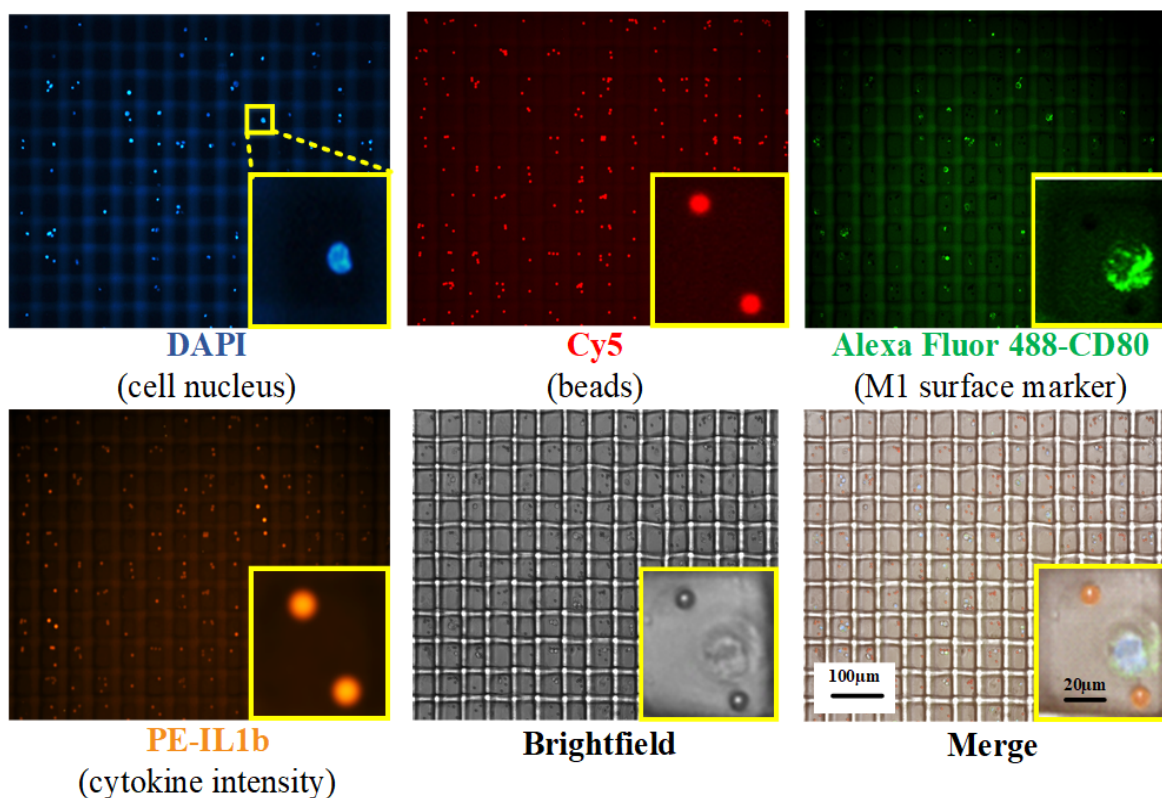**Supplemental Information**

Fig. S1. Image analysis pipeline. M1 macrophages were used as a model analyte. The nanowell images were captured in blue, red, green and orange channels to identify cell nucleus (DAPI), beads (Cy5), cell phenotype (Alexa fluor 488-CD80) and cytokine (PE-IL1b), respectively. Four channel-images were merged. Cells and beads were then analyzed using NIS software to determine cell surface intensity and bead intensity, respectively.
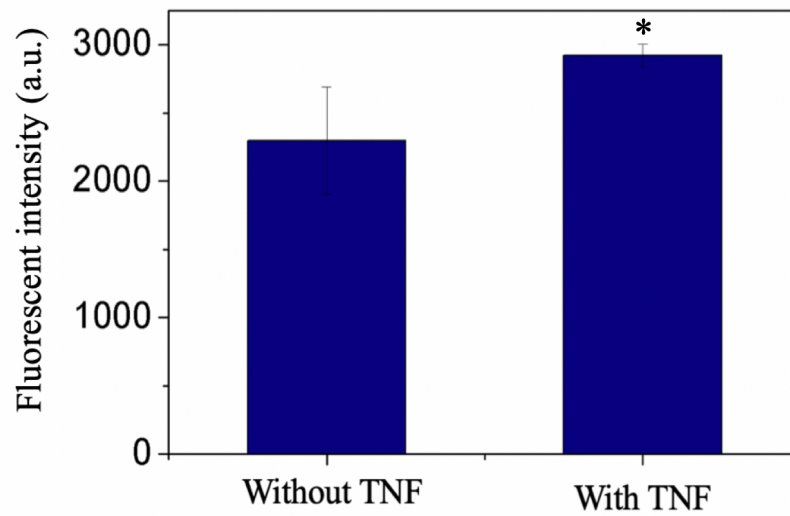
Fig. S2. Bulk IL8 secretion assay for MDA-MB-231 cells with and without TNF stimulation. Secretion of bulk MDA-MB-231 cells were detected. Stimulated cells secrete more IL8 compared to unstimulated cells. (*$p<0.05$ relative to untreated cell)
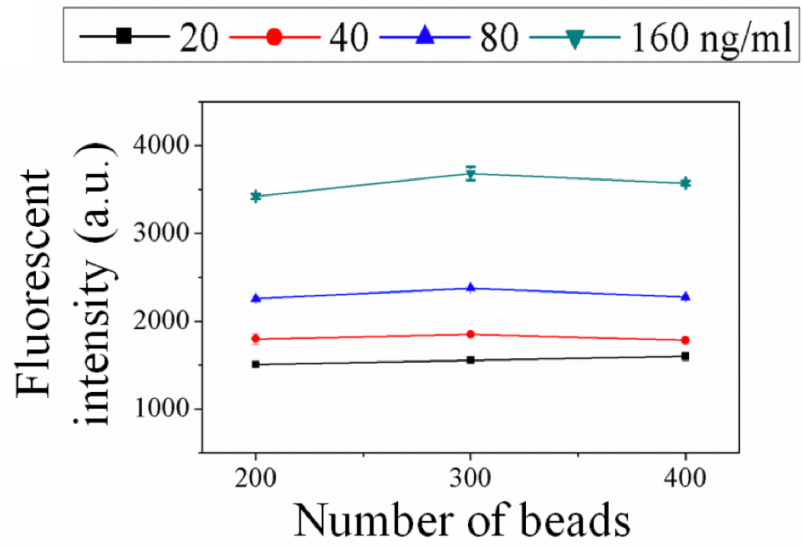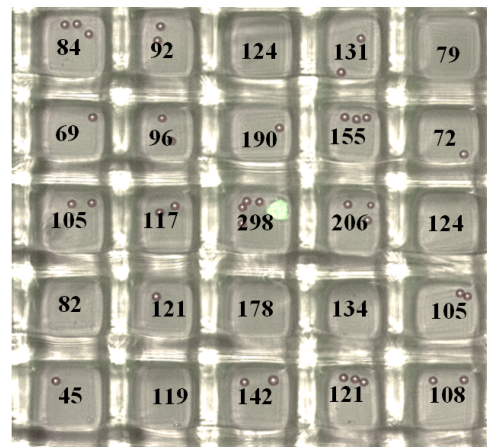
Fig. S3. Dependence of bead fluorescence intensity on the total number of beads. The measured IL-8 concentration was independent of the number of beads.
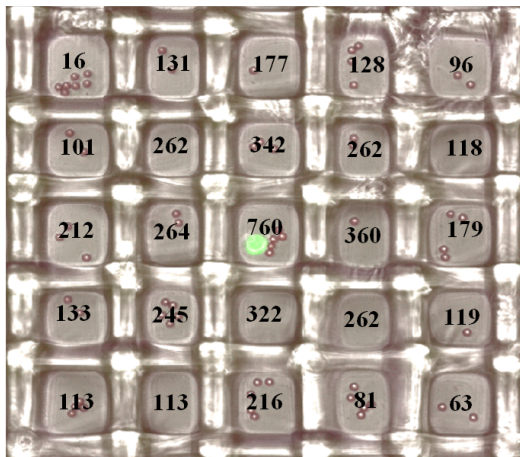
Fig. S4. Measurement of bead fluorescence intensity with different concentration of methylcellulose. Compared to (A) standard medium and (B) 0.005% MC, greater concentrations of methylcellulose e.g., (C) 0.01% & (D) 0.05% have higher capability of confining cytokine within nanowells.
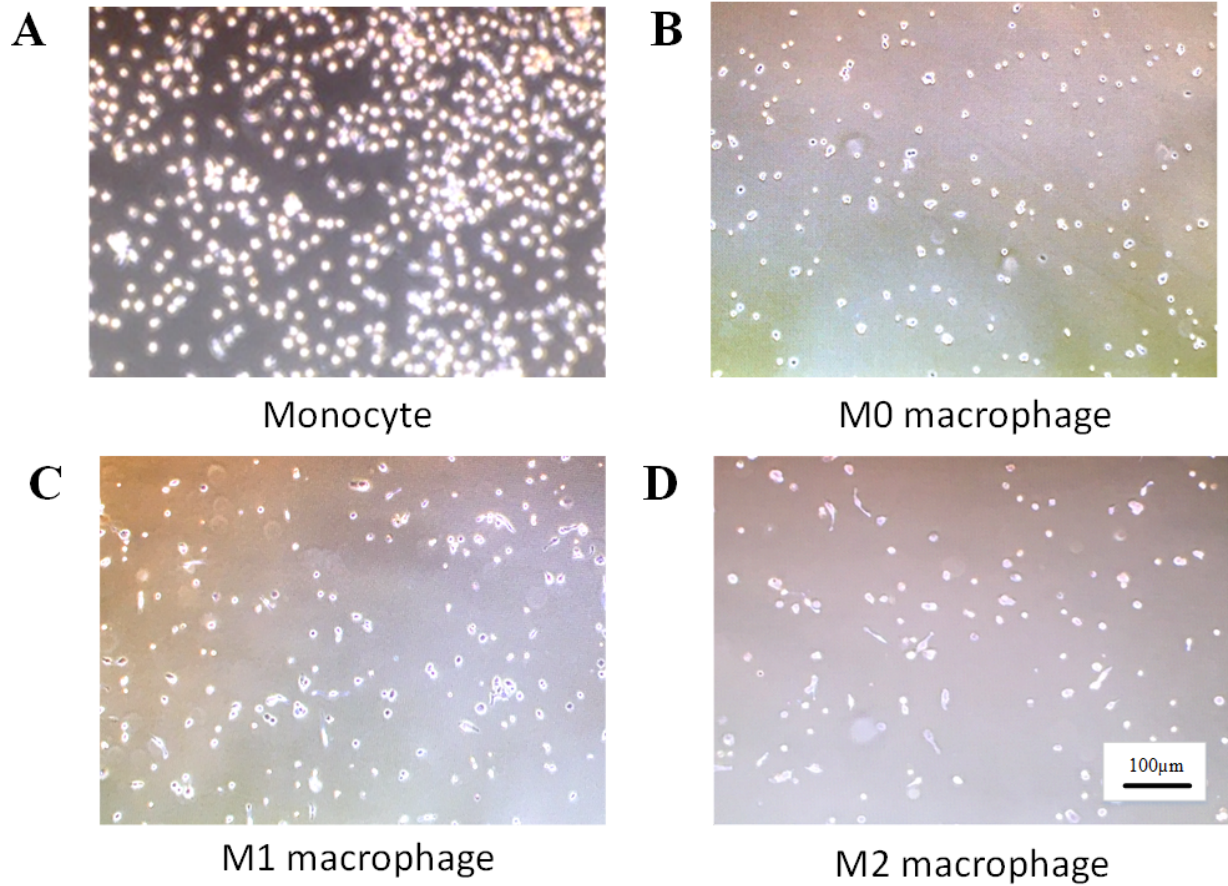
A Monocyte

B M0 macrophage

C M1 macrophage

D M2 macrophage

100μm

Fig. S5. Morphology of THP-1 monocytes, M0, M1 and M2 macrophages. **(A)** THP-1 monocytes are suspension cells which became adherent cells once they were differentiated to **(B)** M0 macrophages. **(C)** M1 and **(D)** M2 macrophages appear as adherent cells.
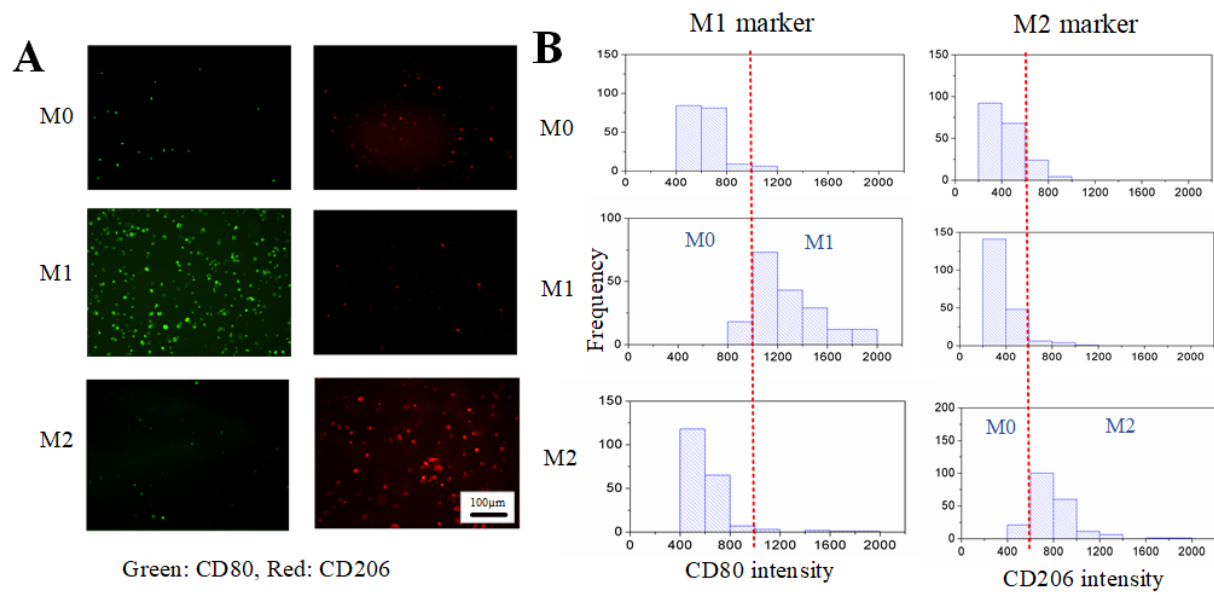
Fig. S6. Surface marker expression of M0, M1 and M2 macrophages. The M1 and M2 cells exhibit characteristic increase in CD80 and CD206 expression, respectively based on **(A)** image observation and **(B)** histogram.
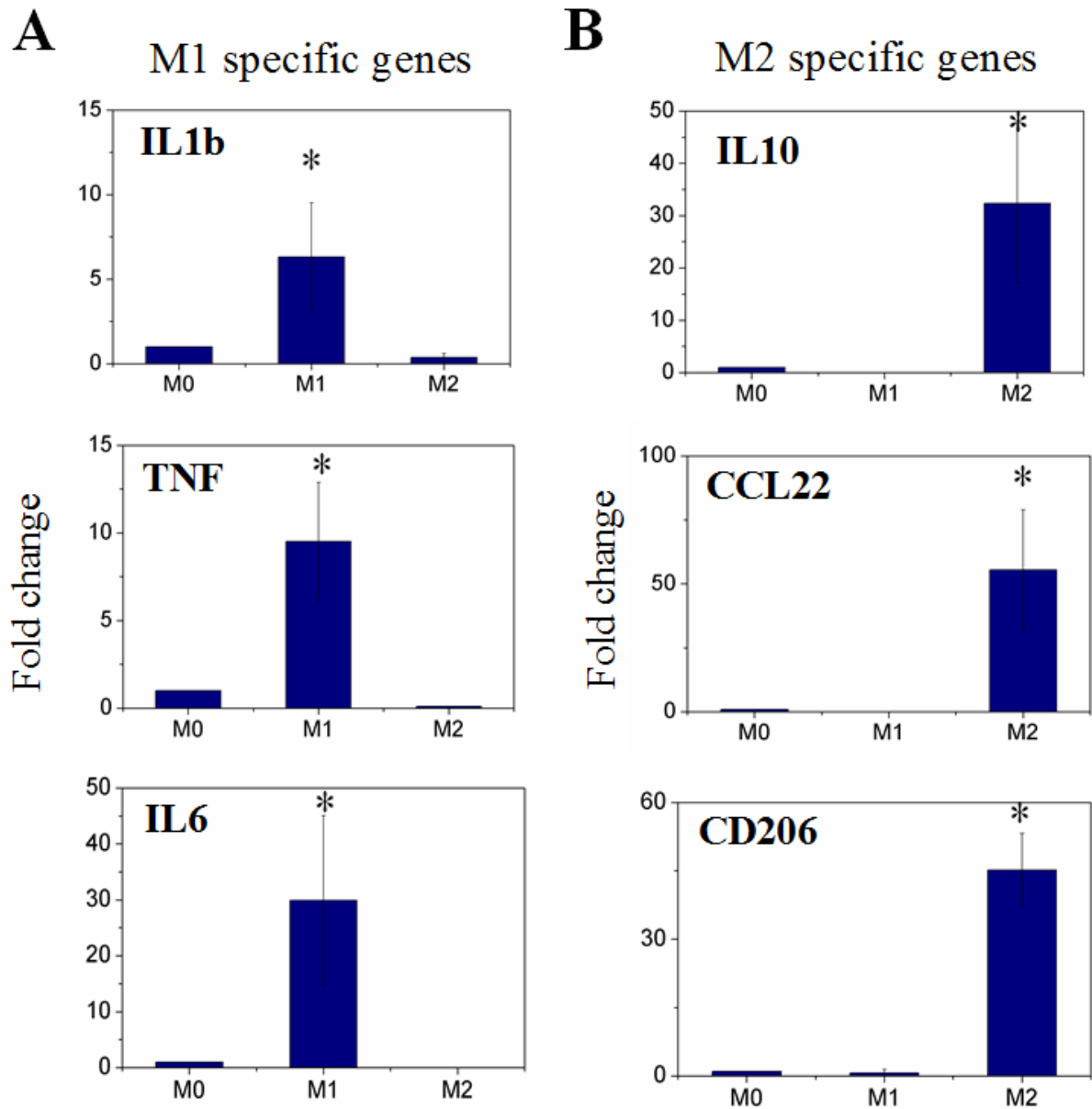
Fig. S7. Gene expression analysis of M0, M1 and M2 macrophages. The result shows that most M1 cells express **(A)** M1 specific genes (*i.e.,* IL1b, TNF and IL6) while **(B)** M2 cells express M2 specific genes (*i.e.,* IL10, CCL22 and CD206), which further prove the efficiency of polarization. (*$p < 0.05$ relative to M0)