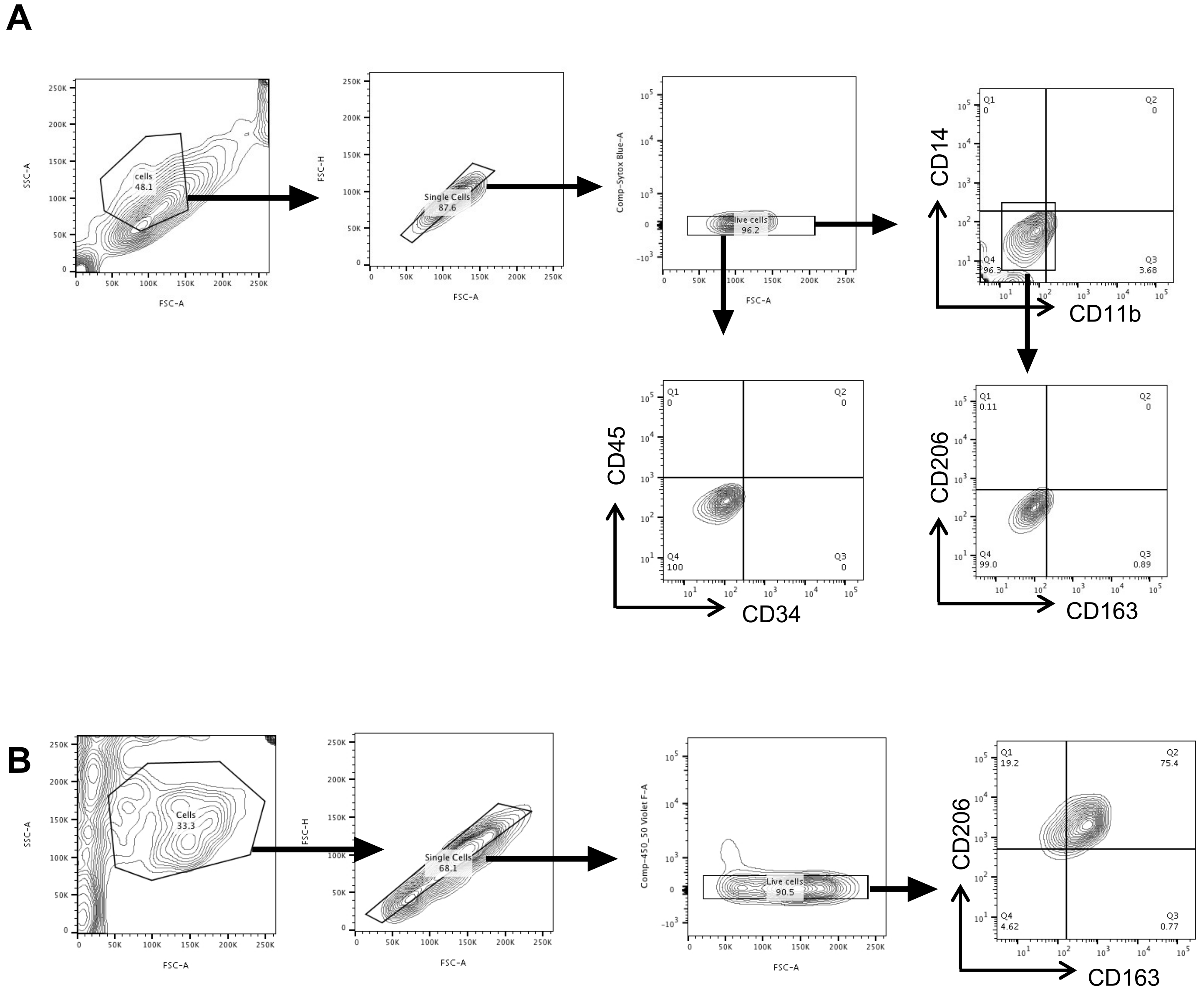
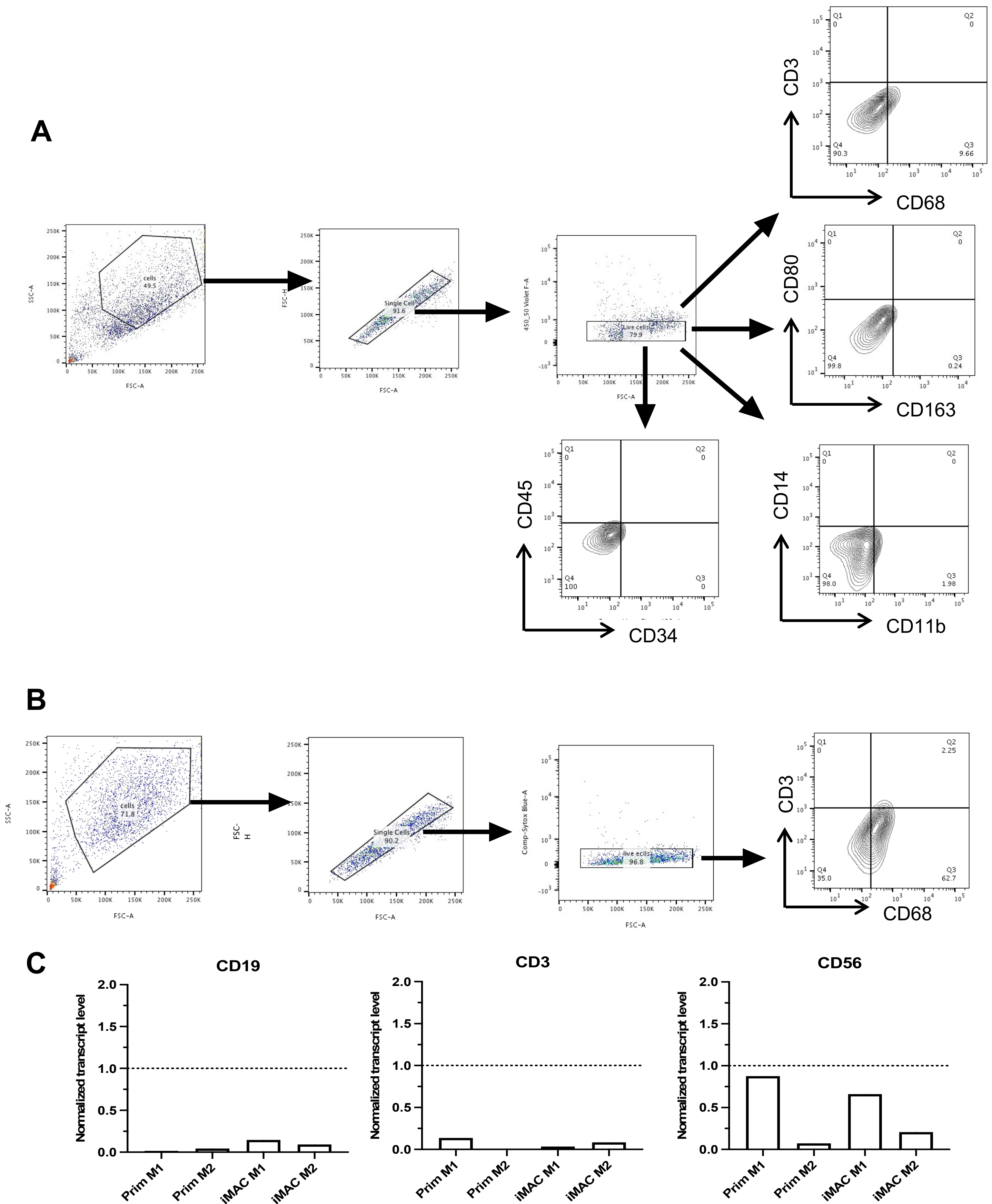


Supplementary Fig. 1. FACS analysis of unstained 3D-iMACs as a control and primary monocyte-derived M2-like macrophages as a reference.

A. Expression profiles of CD34, CD45, CD11b, CD14, CD206 and CD163 in unstained 3D-iMACs.
B. Primary monocyte-derived M2-like macrophages were analyzed as a reference for 3D-iMACs. Primary M2-like macrophages show expression both of CD163/CD206, which is similar to that of 3D-iMACs shown in Fig.1C.

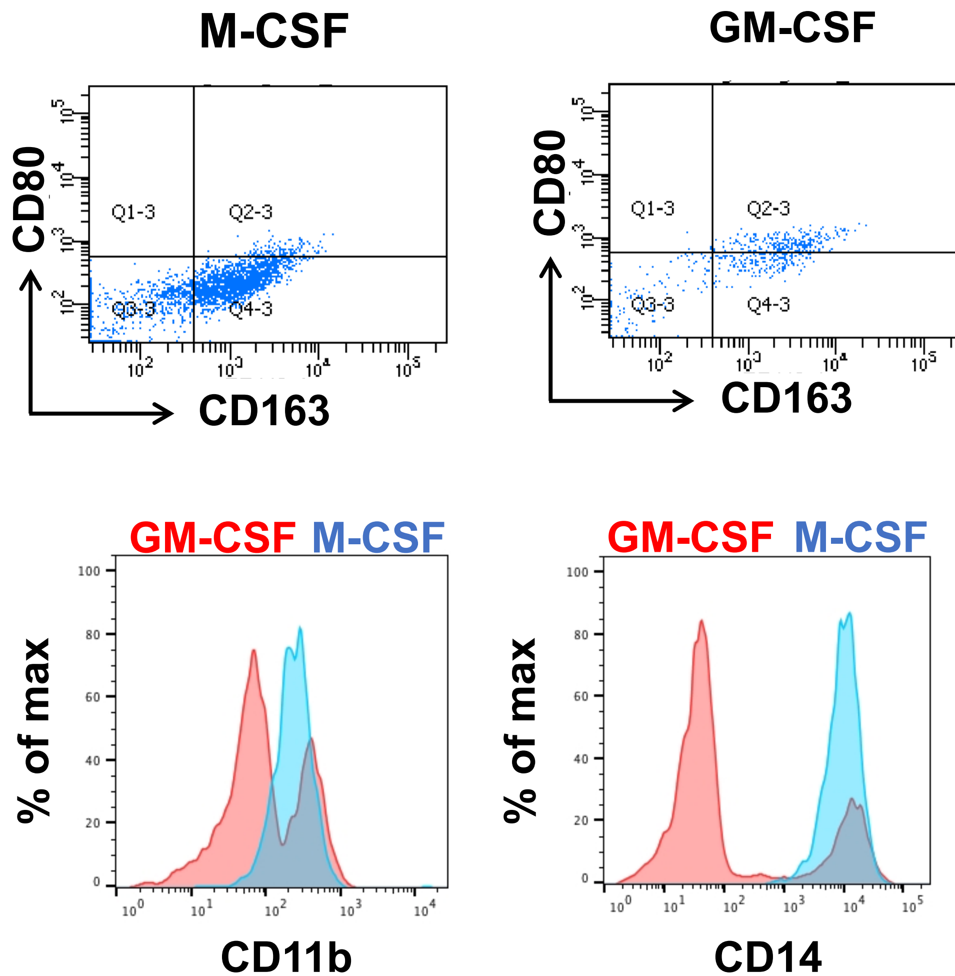


Supplementary Fig. 2. FACS and qPCR analysis of unstained 2D-iMACs.

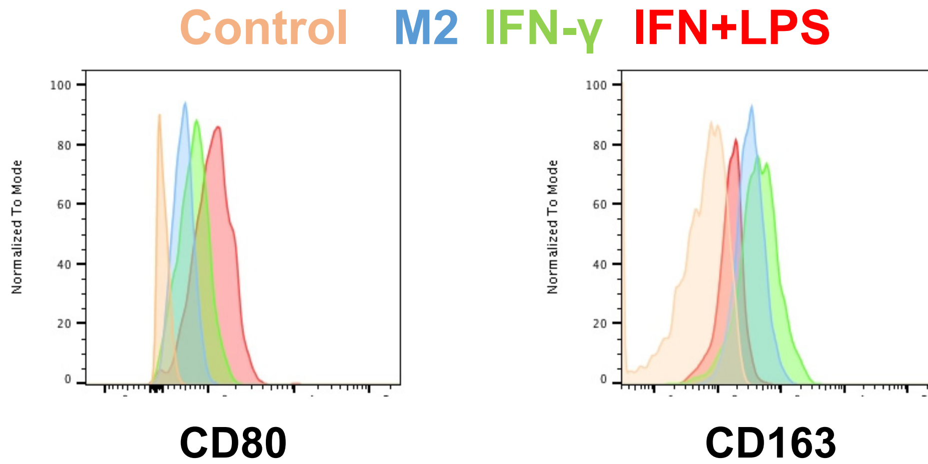
A. Unstained control samples were analyzed to set the threshold for each surface marker.

B. CD68 and CD3 expression levels were analyzed in 2D-iMACs.

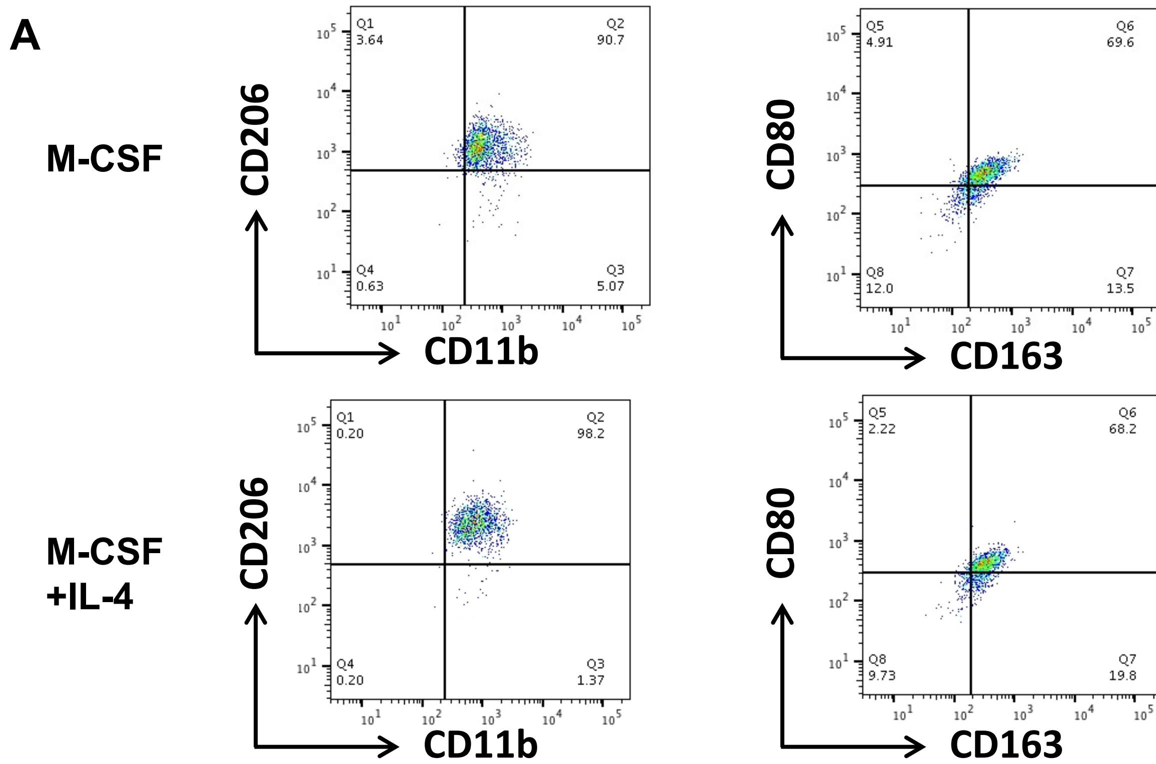
C. CD19, CD3, and CD56 expression of M1-like and M2-like 2D-iMACs are similar to that seen in the M1-like and M2-like macrophages from primary blood monocytes. N=1 biological replicate with technical triplicates, normalized to expression levels in undifferentiated 2D-iMACs.

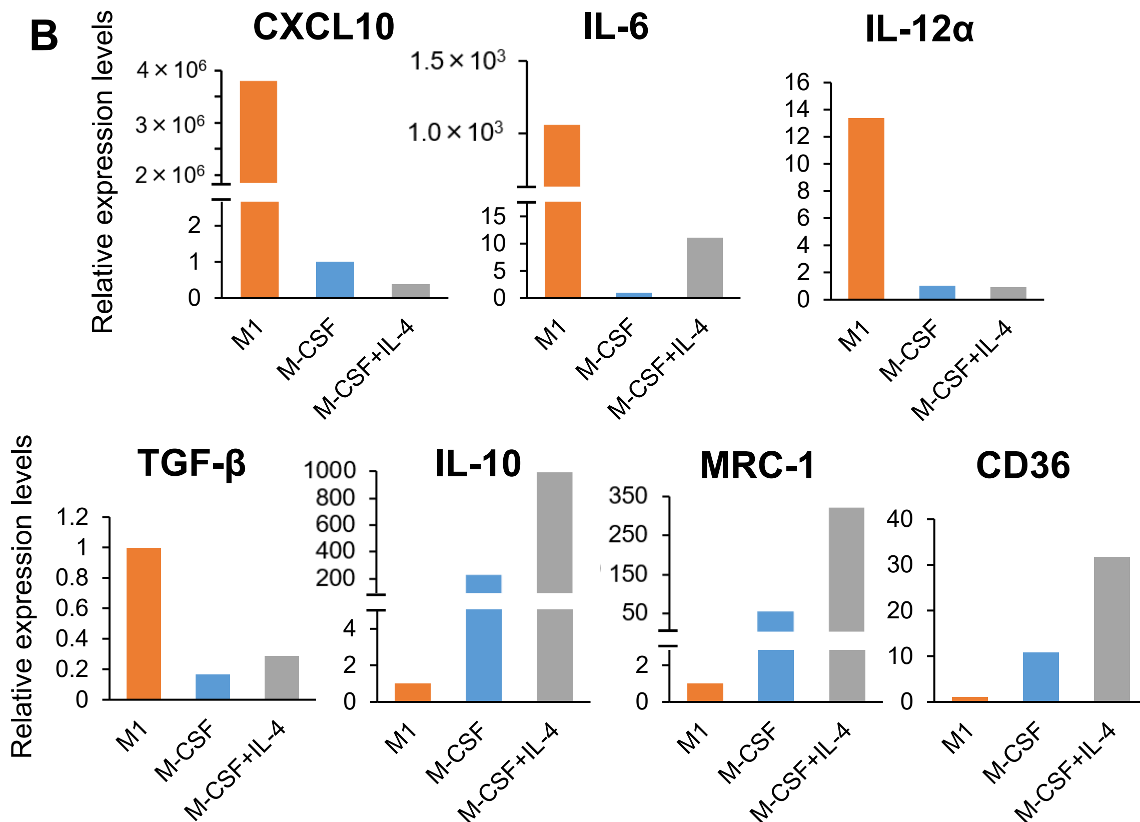


Supplementary Fig. 3. Characters of 2D-iMACs differentiated with GM-CSF
HSCs were cultured with 50 ng/ml M-CSF or 50 ng/ml GM-CSF for 7 days and analyzed with FACS. A total of 10,000 cells were analyzed in both samples. While GM-CSF increased the expression level of CD80 (upper figures), it generated CD11b$^-$/CD14$^-$ populations as well (lower figures), resulting in low final cell numbers of differentiated macrophages.

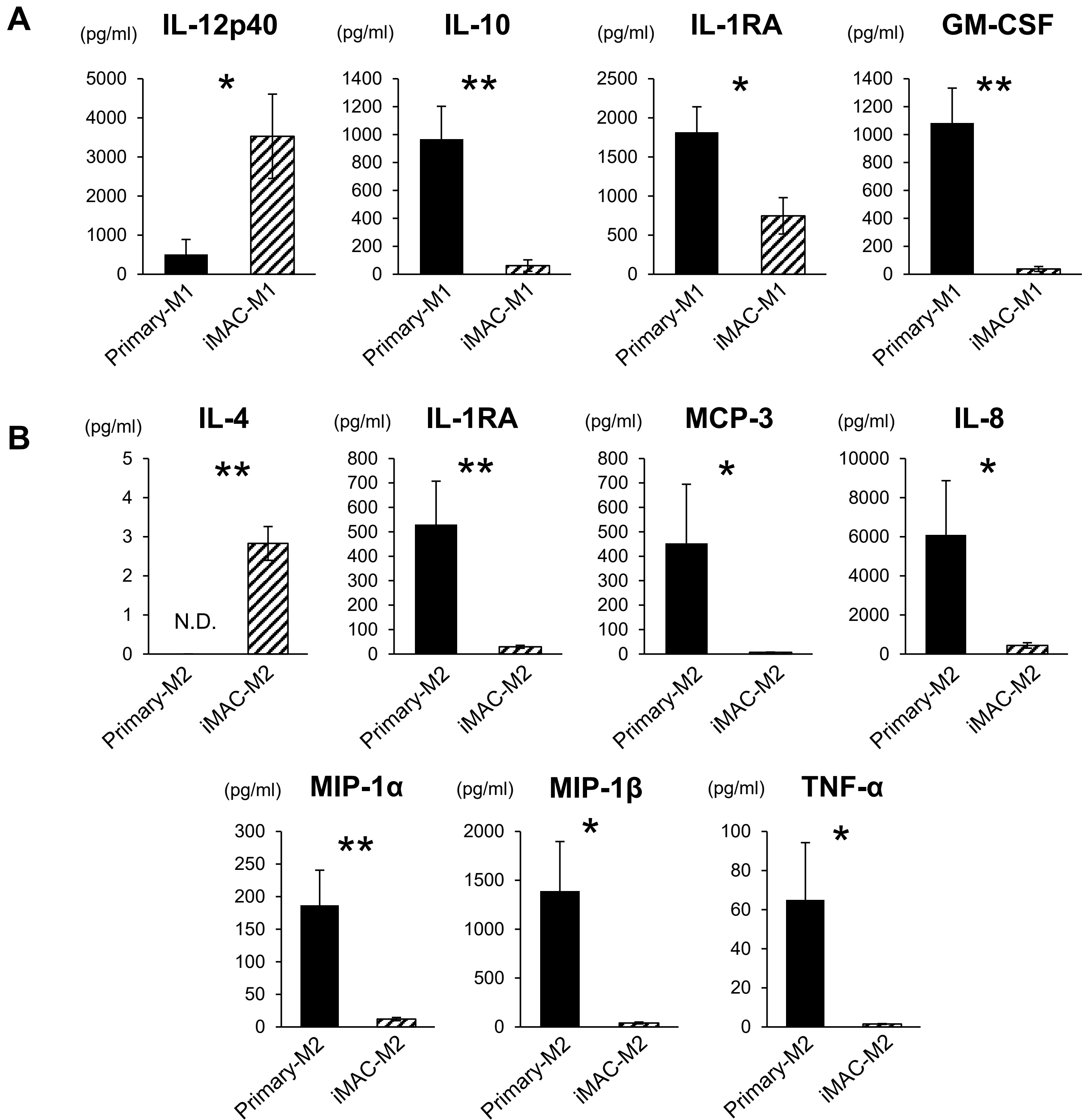


Supplementary Fig. 4. Characteristics of iMACs polarized with IFN-γ alone.
A. Surface marker expression of CD80 and CD163. IFN-γ treated iMACs showed higher expression of CD80 and CD163 than M2-iMACs. IFN-γ-treated iMACs were compared with M2-iMACs and IFN-γ/LPS-treated iMACs. WTc-11 was used for this analysis. B. mRNA expression levels of macrophage-related genes. IFN-γ+LPS-treated iMACs showed higher expression of M1-related genes (upper) and lower M2-related genes (lower). Gene expression levels were normalized to those of β-actin. WTc-11 was used for this analysis (n=1) with technical triplicates.



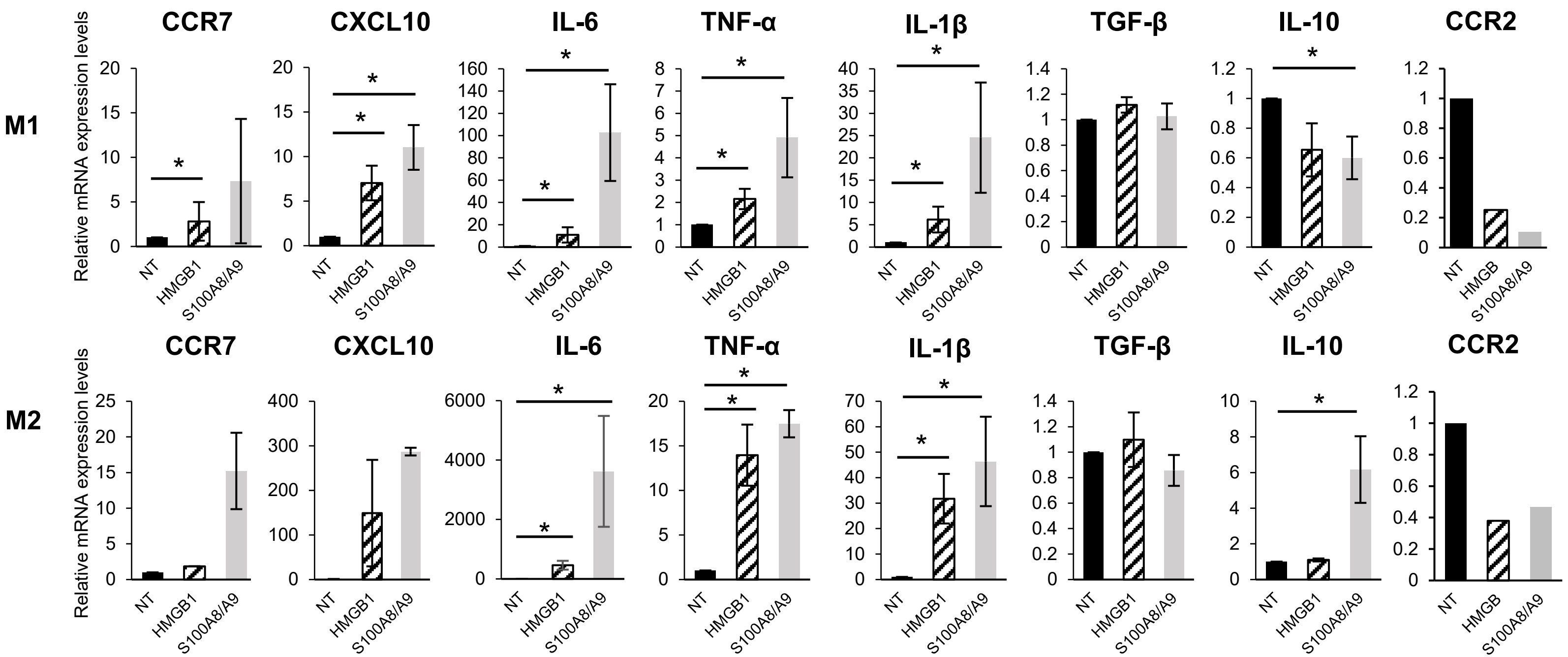


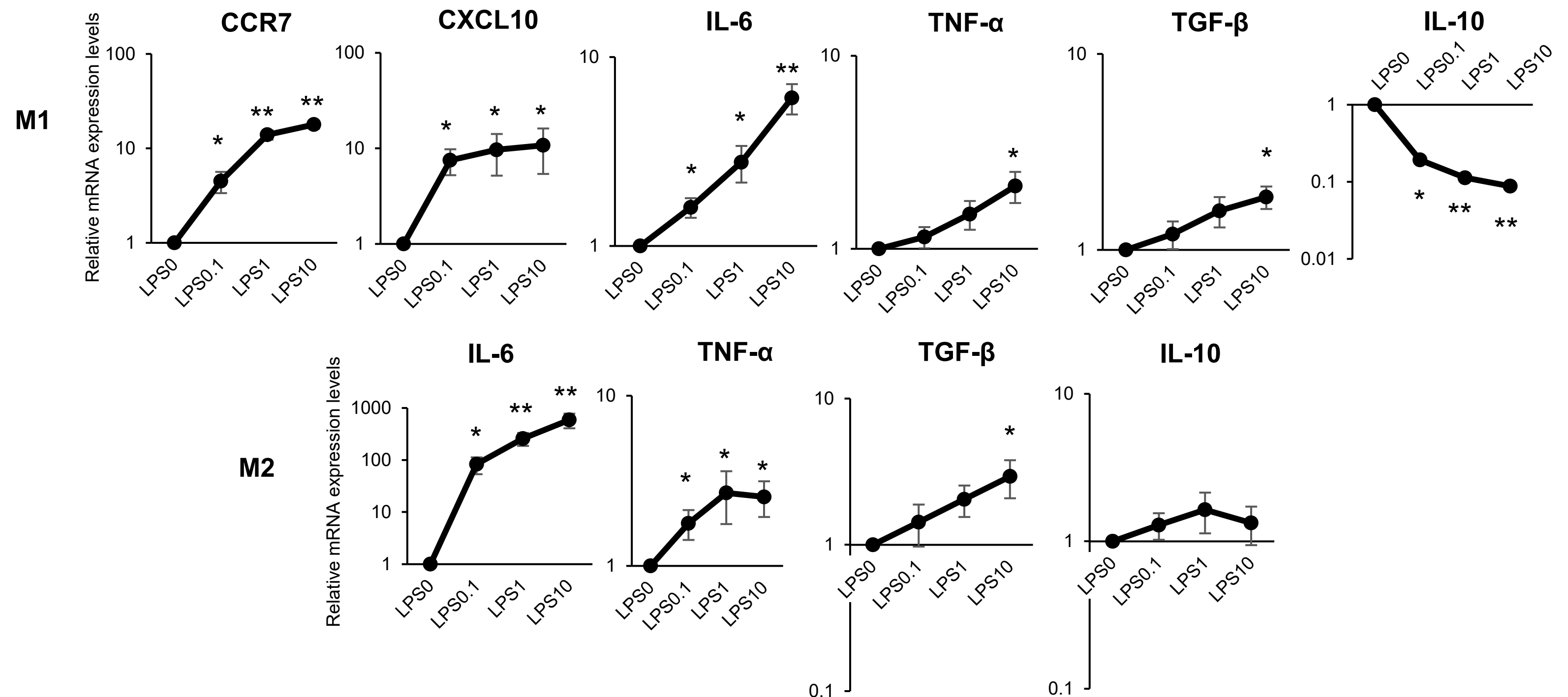
Supplementary Fig. 5. Characteristics of 2D-iMACs polarized with IL-4.
A. Surface marker expression patterns of iMACs treated with IL-4. CD206 expression was slightly elevated but neither CD80 nor CD163 were changed. WTc-11 was used for this analysis. B. mRNA expression levels of macrophage-related genes. IL-4 upregulated the M2-related gene expressions including IL-10, MRC-1 and CD36, but the effect was relatively small compared with that of M1-polarization. Gene expression levels were normalized to those of β-actin. Expression levels of upper three gene and lower four genes were shown as relative expression levels to M-CSF group and M1 group, respectively. WTc-11 was used for this analysis (n=1) with technical triplicates.



Supplementary Fig. 6. Cytokine concentrations showing significant differences between primary macrophages and 2D-iMACs.

A. Comparison between primary M1 macrophages and M1-like 2D-iMACs are show. Student's *t*-test was used for comparison of two groups. *$p < 0.05$. **$p < 0.01$. Data represent mean ± SEM of four to five independent experiments (n = 4-5) with technical triplicates. B. Comparison between primary M2-like macrophages and M2-like 2D-iMACs are shown. Student's *t*-test was used for comparison of two groups. *$p < 0.05$. **$p < 0.01$. Data represent mean ± SEM of independent experiments (n=4-6) with technical triplicates. N.D.; not detectable.

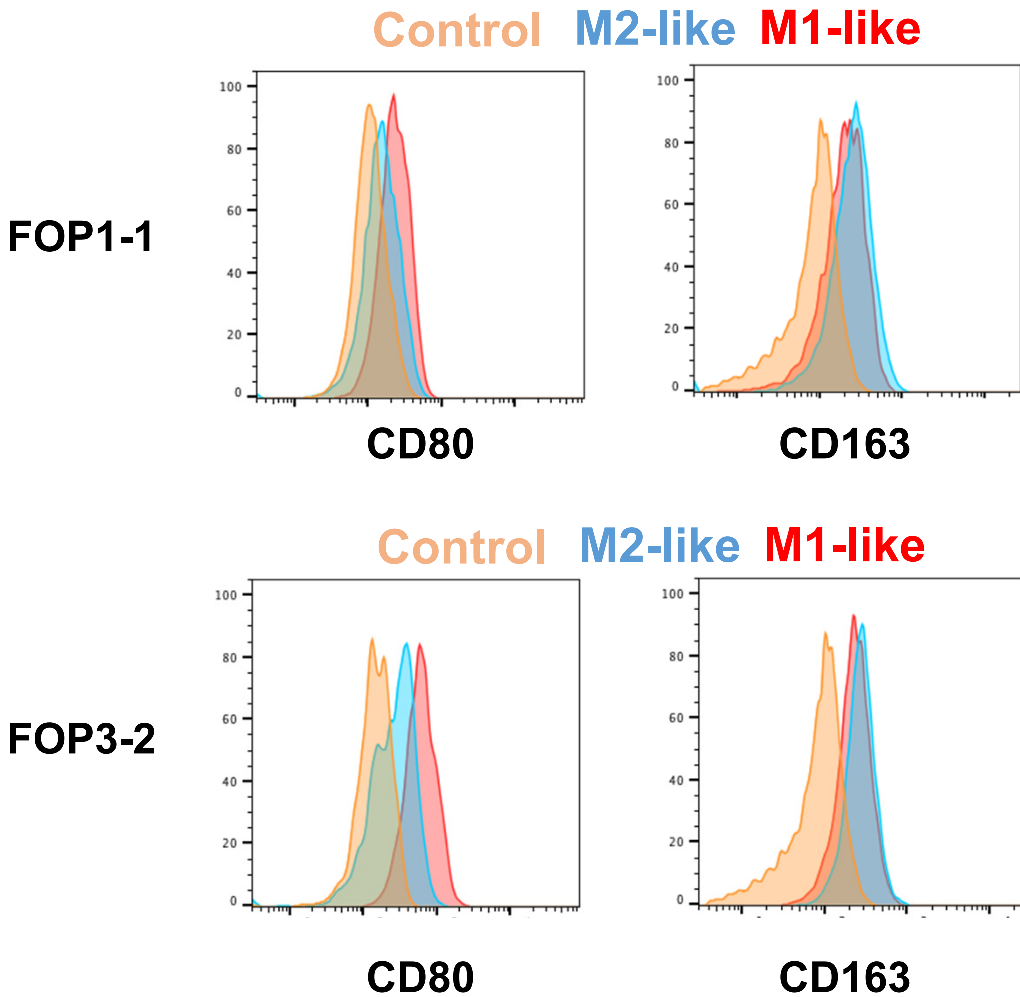


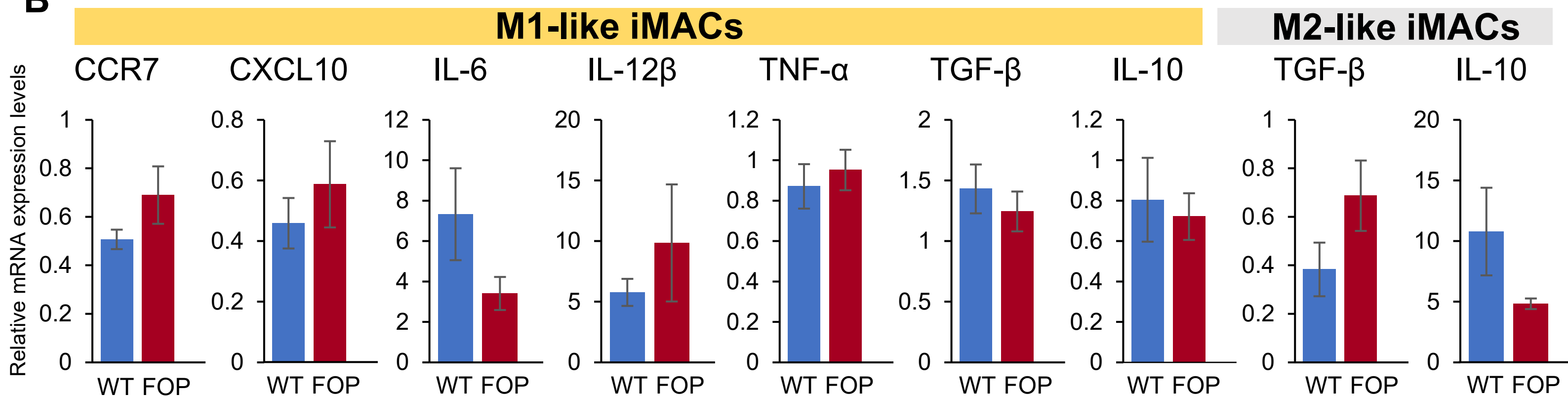


Supplementary Fig. 7. Response to PAMP (LPS) and DAMPs (HMGB1, S100A8/A9) stimulations in 2D-iMACs.

A. qPCR analysis of representative cytokine genes in M1-like and M2-like 2D-iMACs stimulated with HMGB1 or S100A8/A9. Both M1-like and M2-like iMACs showed significant changes in various pro-inflammatory cytokines including IL-6, TNF-α, IL-1β. Gene expression changes of IL-10 were opposite between M1-like and M2-like iMACs. Expression levels are normalized to levels of a housekeeping gene, β-actin. Steel-Dwass test was used to compare each group. *$p<0.05$, **$p<0.01$. Data represent mean ± SEM of independent experiments (n=4) with technical triplicates.

B. qPCR analysis of representative cytokine genes in M1-like and M2-like 2D-iMACs stimulated with different concentrations of LPS (0.1, 1, and 10 ng/ml). Pro-inflammatory cytokine genes in M1-iMACs were significantly upregulated after stimulation. WTc11 was used for this experiment. Expression levels are normalized to levels of a housekeeping gene, β-actin. Shirley-Williams test was used to compare each group to control. *$p<0.05$, **$p<0.01$. Data represent mean ± SEM of independent experiments (n=3) with technical triplicates.

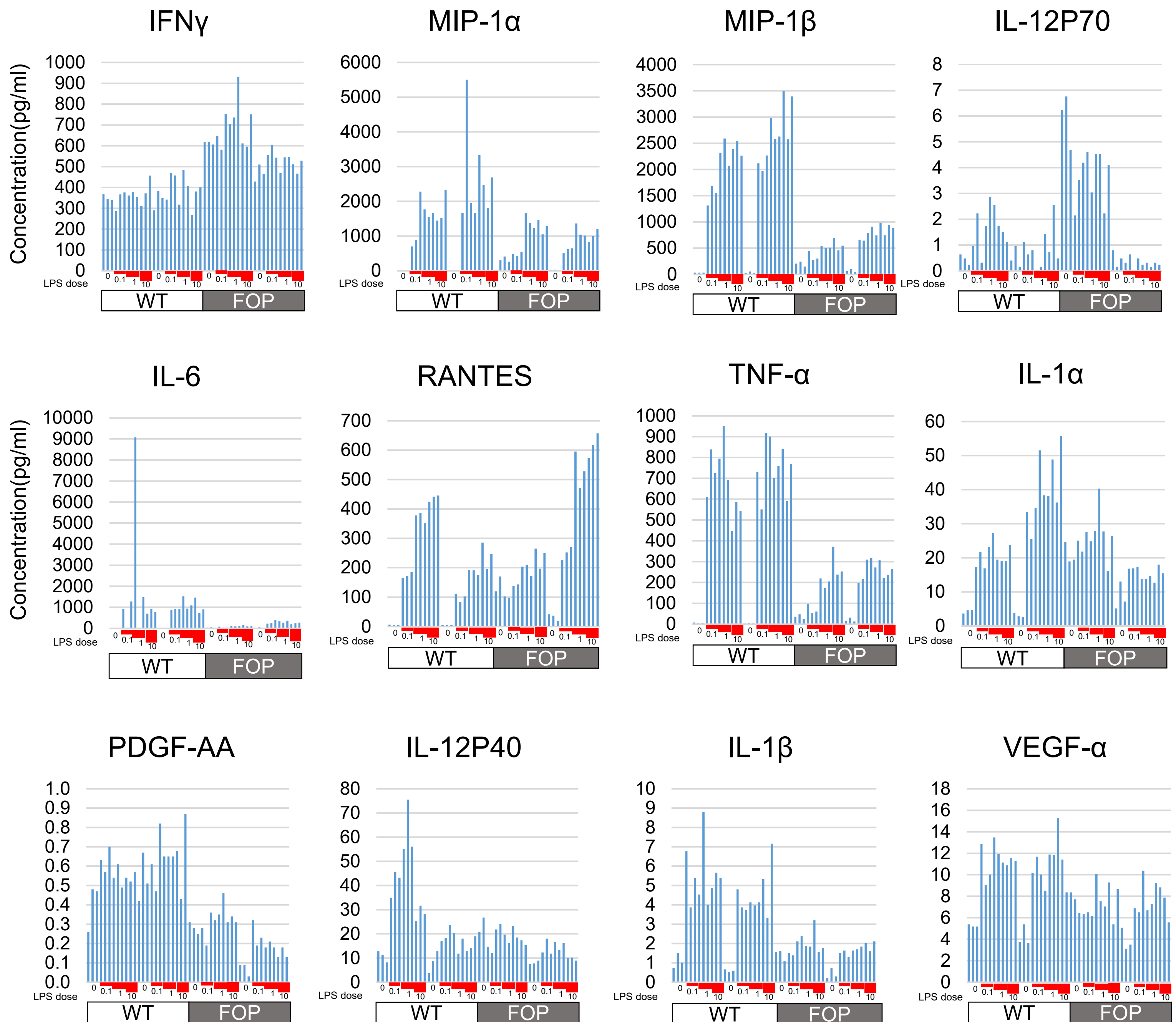




Supplementary Fig. 8. Surface marker and gene expression of polarized FOP-2D-iMACs

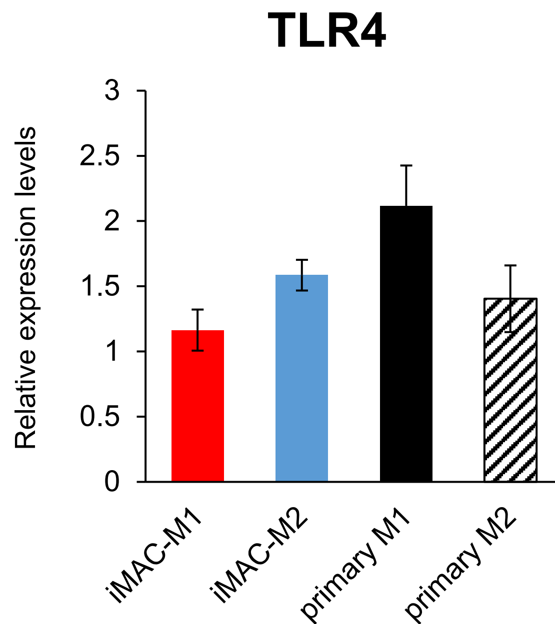
A. iMACs were generated using 2D-culture protocol and polarized with or without IFN-gamma + LPS for 24 hours. After M1 polarization, expression levels of CD80 were increased and that of CD163 were decreased in both FOP-derived cell lines in the same manner as WT-iMACs.

B. qPCR analysis of representative cytokine genes in WT- and FOP-2D-iMACs. Cells were harvested and analyzed just after their polarization into M1-like or M2-like phenotype. There were no significant differences between two groups. Expression levels are normalized to levels of a housekeeping gene, β-actin. Student's t-test was used for comparison of two groups. *$p<0.05$. Data represent mean ± SEM of independent experiments (n=8) with technical triplicates.

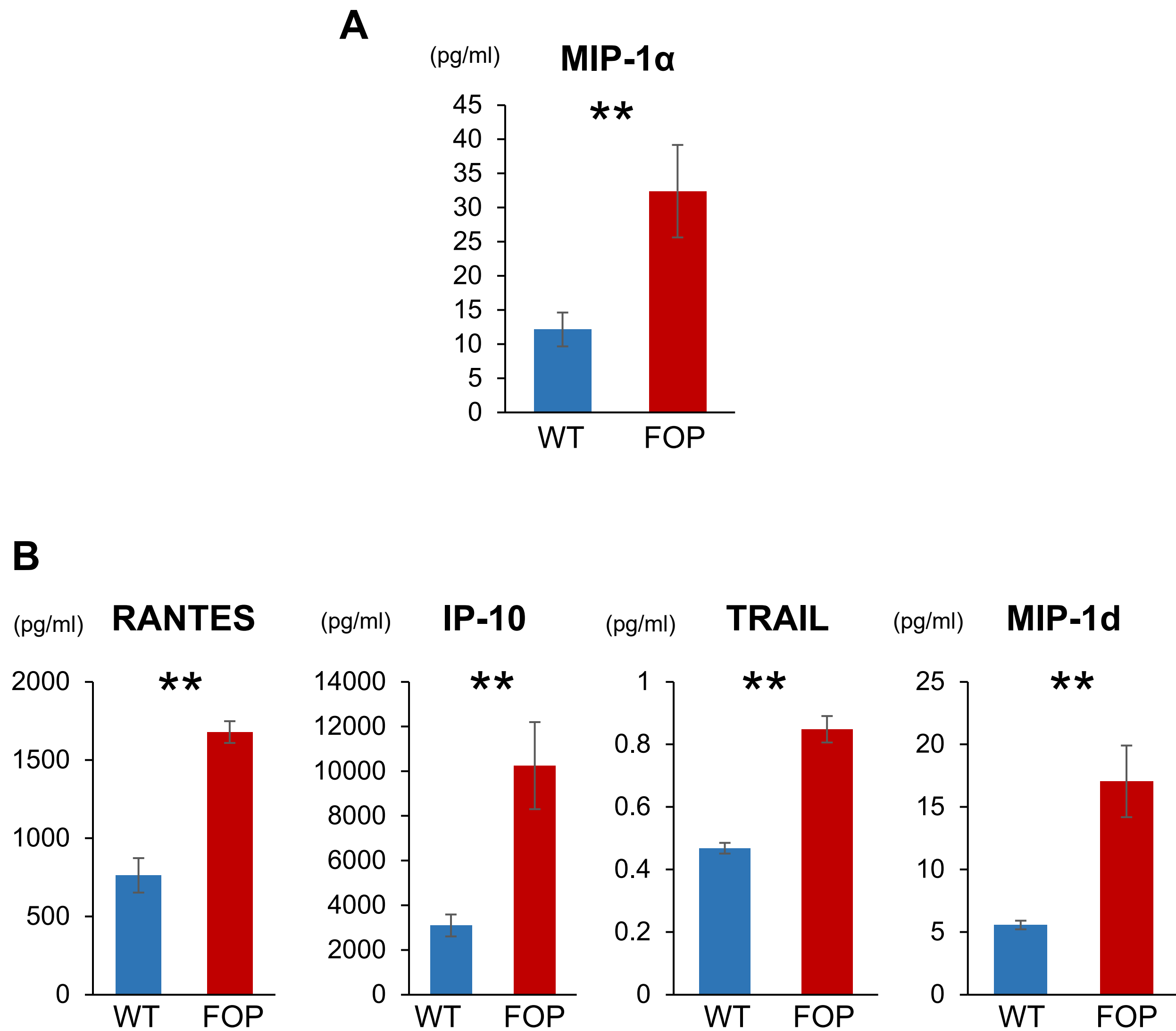


Supplementary Fig. 9. Cytokine concentrations secreted by M1-like iMACs stimulated with 0.1-10 ng/ml LPS.

iMACs were stimulated with different concentrations of LPS for 24 hours. Representative pro-inflammatory cytokine genes are shown here. Cytokine levels with the lowest dose of LPS were mostly equivalent to those with the highest dose of LPS. While RANTES and MIP-1α seem to have mild dose-dependencies, they don't have significant differences regarding the trend between WT- and FOP-iMACs.



Supplementary Fig. 10. Comparison of TLR4 expressions between 2D-iMACs and primary macrophages
mRNA expression levels of TLR4 are shown. No significant differences were found between 2D-iMACs and primary macrophages regarding their expression levels of TLR4. Gene expression levels were normalized to those of β-actin. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. Data represent mean ± SEM of four to five independent experiments with technical triplicates.

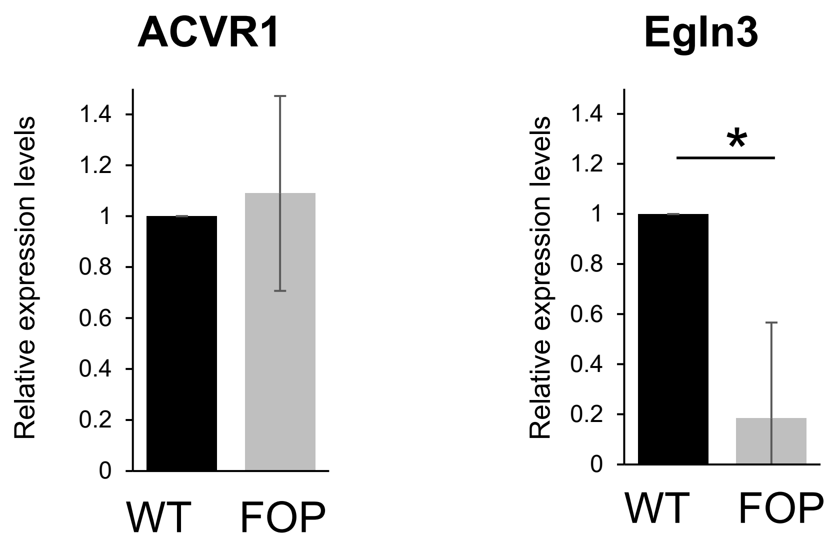


Supplementary Fig. 11. Cytokine concentrations showing significant differences between WT- and FOP-M2-like iMACs.

A. The concentrations of MIP-1α was significantly higher in FOP-M2-like iMACs without LPS stimulation (NT). Student's *t*-test was used for comparison of two groups. **p< 0.01 . Data represent mean ± SEM of six independent experiments with technical triplicates.

B. The concentrations of RANTES, IP-10, TRAIL, and MIP-1d were significantly higher in FOP-M2-like iMACs when stimulated with 10ng/ml LPS. Student's *t*-test was used for comparison of two groups. **p< 0.01. Data represent mean ± SEM of independent experiments (n = 6) with technical triplicates.

Supplementary Fig. 12. Response to DAMPs (HMGB1, S100A8/A9) stimulation in FOP-M1-like and M2-like iMACs. A. mRNA expression levels of key cytokine genes in FOP-M1-like iMACs were shown. B. mRNA expression levels of key cytokine genes in FOP-M2-like iMACs were shown. Gene expression levels were normalized to those of β-actin. Statistical analysis was performed by Steel-Dwass test. Data represent mean ± SEM of independent experiments (n = 4) with technical triplicates. *$p < 0.05$, **$p < 0.01$.



Supplementary Fig. 13. Comparison of ACVR1 and Egln3 expression between WT- and FOP-iMACs. ACVR1 expression was not significantly different between WT- and FOP-iMACs. In addition, Egln3 expression level was downregulated in FOP-iMACs. Student's t-test was used for comparison. *$p<0.05$. Data represent mean ± SEM of independent experiments (n=3) with technical triplicates

Name	Company	Catalog number	Conjugate	Dilution
CD34	eBioscience	11-0349-42	FITC	1:100
CD45	eBioscience	12-9459-42	PE	1:100
CD14	eBioscience	25-0149-42	PE-Cyanine7	1:100
CD11b	eBioscience	11-0118-42	FITC	1:100
CD11b	eBioscience	17-0118-42	APC	1:100
CD163	eBioscience	12-1639-42	PE	1:100
CD206	eBioscience	17-2069-42	APC	1:100
CD80	eBioscience	46-0809-42	PerCP-eFlour 710	1:100
CD68	eBioscience	11-0689-42	FITC	1:100
CD3	eBioscience	17-0038-42	APC	1:100

Supplementary Table 1. Antibodies used for Flow Cytometry

Gene	Assays ID
ACTB	Hs01060665_g1
INHBA	Hs1081598_m1
CCR7	Hs01013469_m1
CD36	Hs00169627_m1
CXCL10	Hs01124251_g1
IL10	Hs00961622_m1
IL1B	Hs01555410_m1
IL6	Hs00174131_m1
IL12A	Hs01073447_m1
IL12B	Hs01011518_m1
MRC1	Hs00267207_m1
TGFB1	Hs00998133_m1
TNF-α	Hs00174128_m1
TLR4	Hs00152939_m1
CCR2	Hs00704702_s1
Egln3	Hs00222966_m1

Gene	Sybr Green
CD3e F	TGC TGC TGG TTT ACT ACT GGA
CD3e R	GGA TGG GCT CAT AGT CTG GG
CD19 F	GGC CCG AGG AAC CTC TAG T
CD19 R	TAA GAA GGG TTT AAG CGG GGA
CD56 F	GGC ATT TAC AAG TGT GTG GTT AC
CD56 R	TTG GCG CAT TCT TGA ACA TGA
GAPDH F	ATG TTT GTG ATG GGT GTGAA
GAPDH R	ATG CCA AAG TTG TCA TGG AT

Supplementary Table 2. Taqman and Sybr Green primers for human gene expression