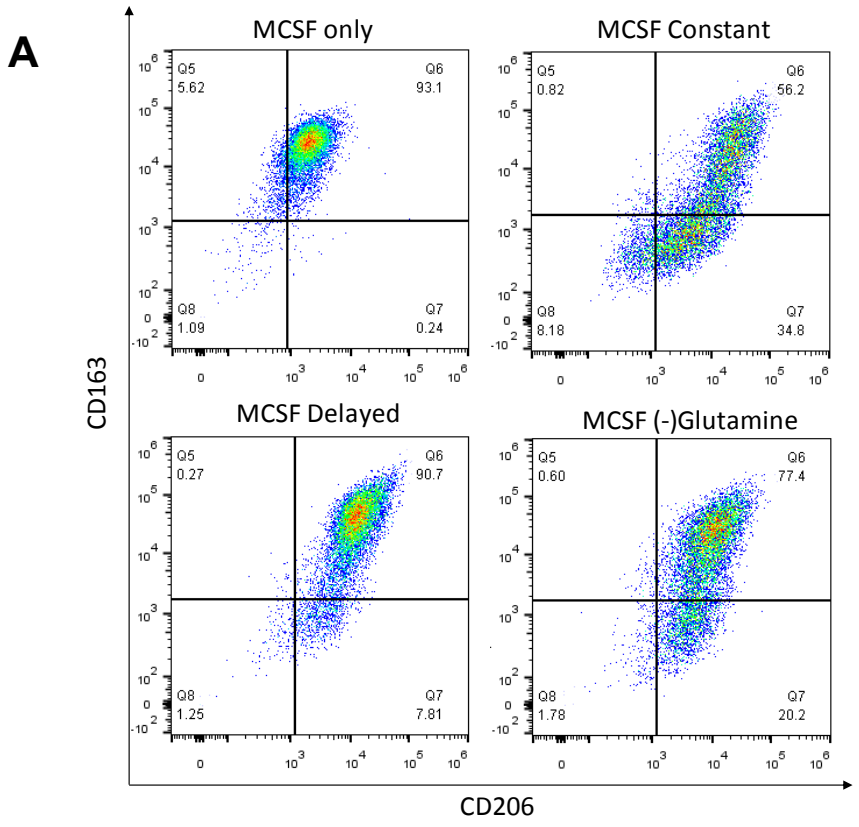
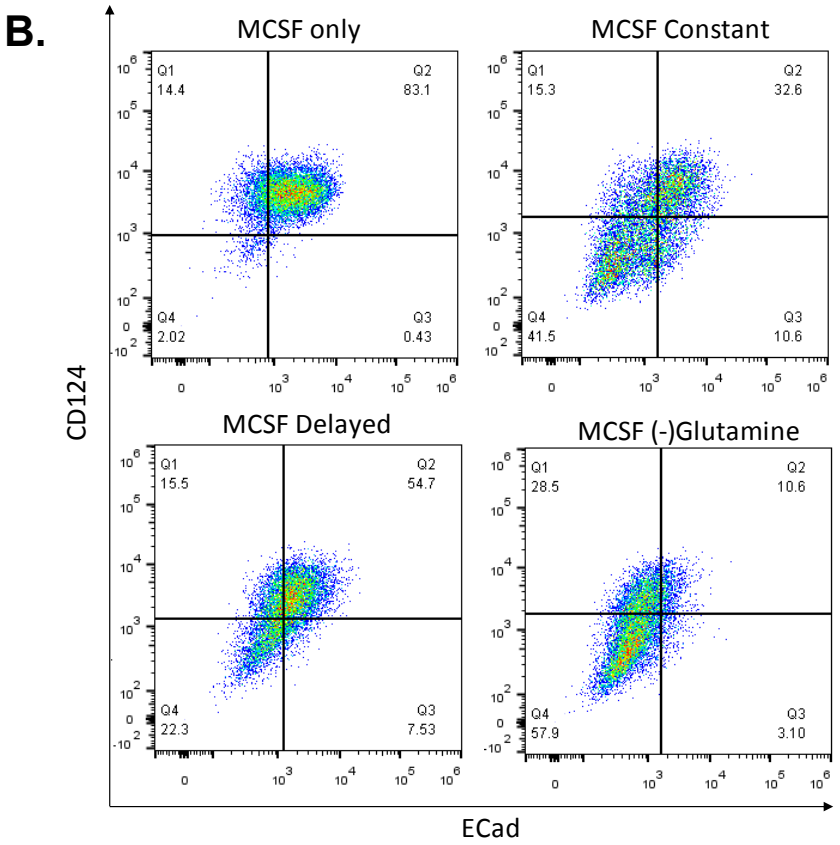
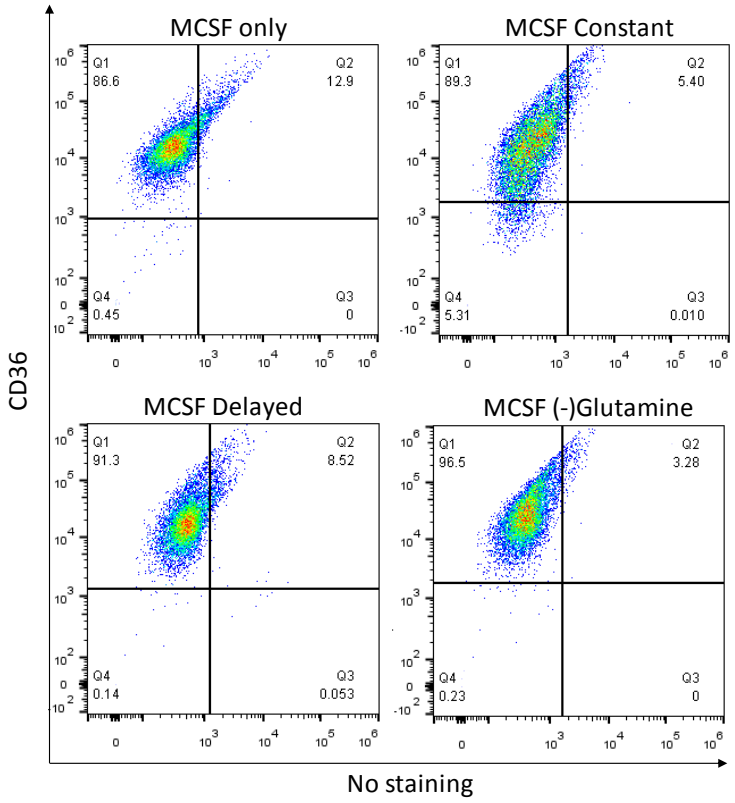
Supplemental Figure 1





C.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MCSF Delayed
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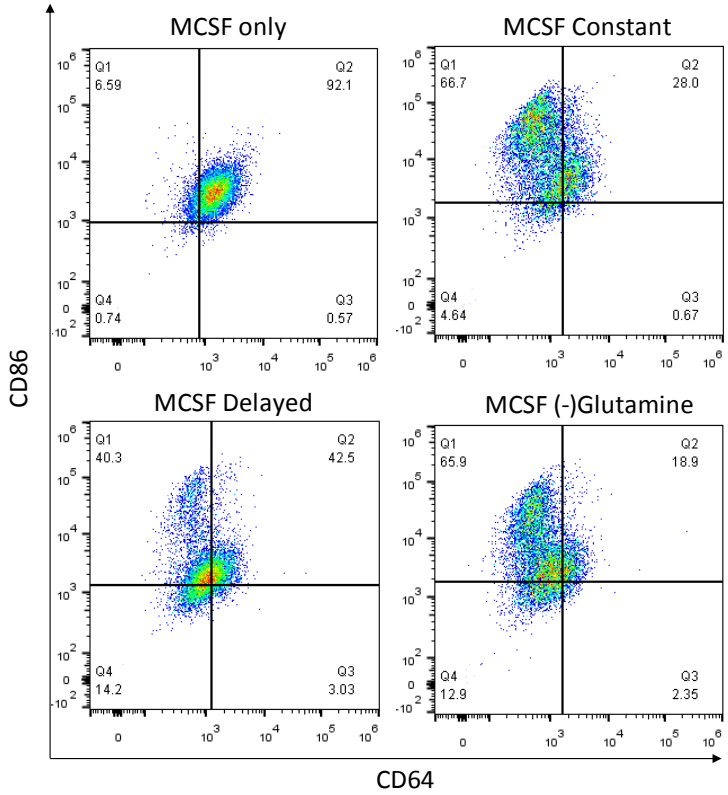
Q2 5.40
Q4 5.31
Q3 0.010
Q1 91.3
Q2 8.52
Q4 0.14
Q3 0.053
Q1 96.5
Q2 3.28
Q4 0.23
Q3 0
CD36
No staining

D.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MCSF Delayed
MCSF (-)Glutamine
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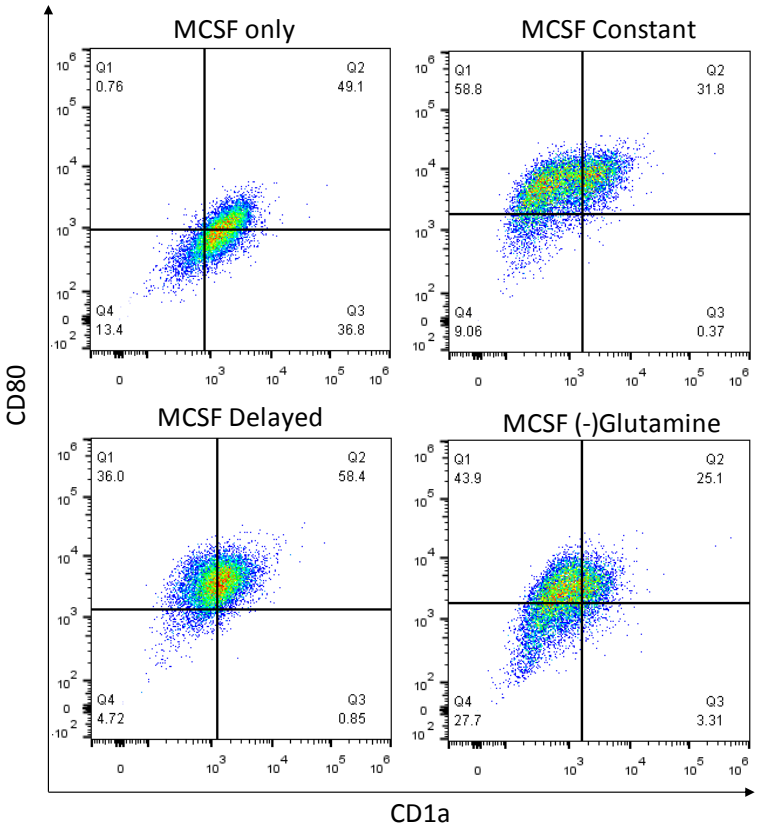
Q2 28.0
Q4 4.64
Q3 0.67
Q1 40.3
Q2 42.5
Q4 14.2
Q3 3.03
Q1 65.9
Q2 18.9
Q4 12.9
Q3 2.35
CD86
CD64

E.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MCSF Delayed
MCSF (-)Glutamine
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Q2 31.8
Q4 9.06
Q3 0.37
Q1 36.0
Q2 58.4
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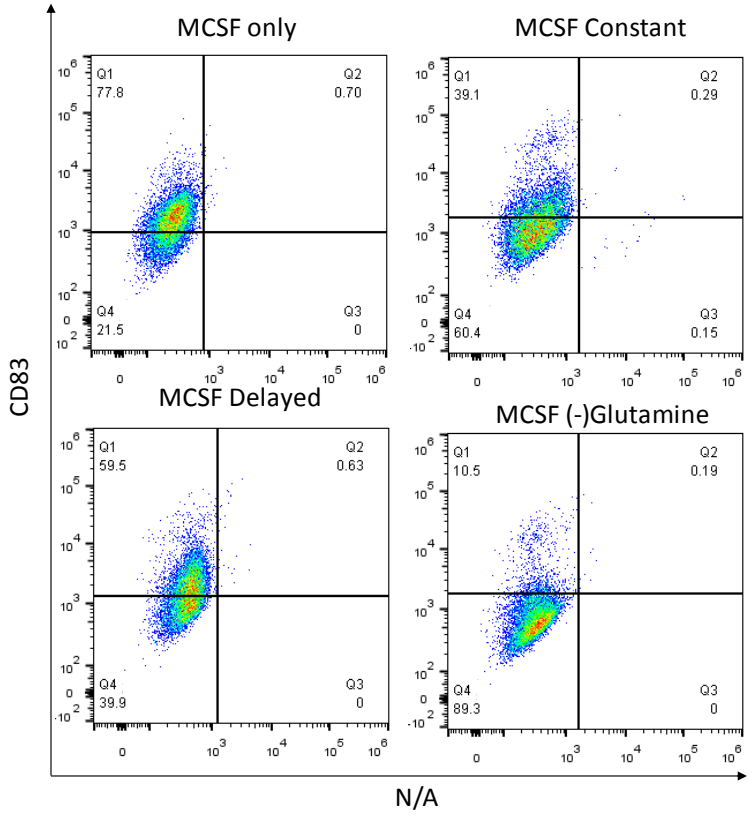
Q1 43.9
Q2 25.1
Q4 27.7
Q3 3.31
CD80
CD1a

F.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MCSF Constant
MCSF Delayed
MCSF (-)Glutamine
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Q2 0.70
Q4 21.5
Q3 0
Q1 39.1
Q2 0.29
Q4 60.4
Q3 0.15
Q1 59.5
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Q1 10.5
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Q3 0
CD83
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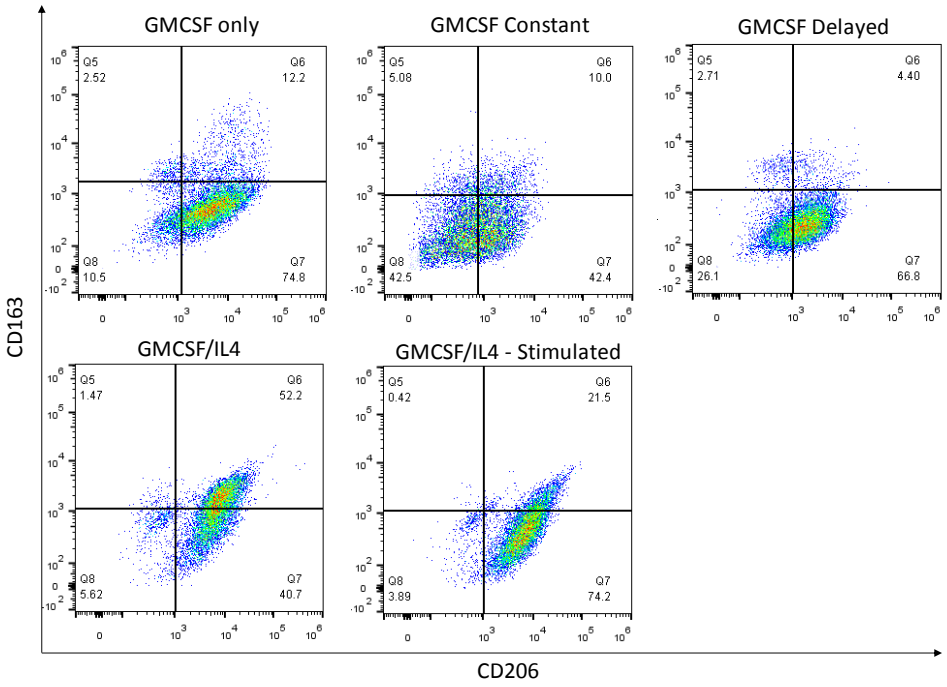
G.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GMCSF Constant
GMCSF Delayed
GMCSF/IL4
GMCSF/IL4 - Stimulated
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Q6 12.2
Q8 10.5
Q7 74.8
Q5 5.08
Q6 10.0
Q8 42.5
Q7 42.4
Q5 2.71
Q6 4.40
Q8 26.1
Q7 66.8
Q5 1.47
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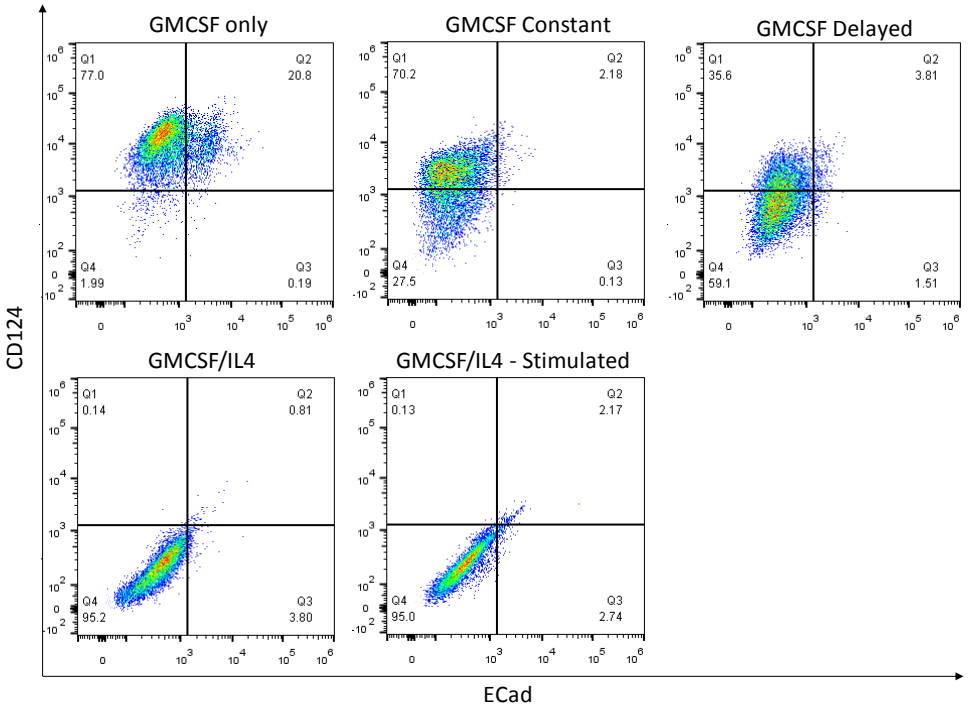
Q6 21.5
Q8 3.89
Q7 74.2
CD163
CD206

H.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GMCSF Constant
GMCSF Delayed
GMCSF/IL4
GMCSF/IL4 - Stimulated
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Q1 70.2
Q2 2.18
Q4 27.5
Q3 0.13
Q1 35.6
Q2 3.81
Q4 59.1
Q3 1.51
Q1 0.14
Q2 0.81
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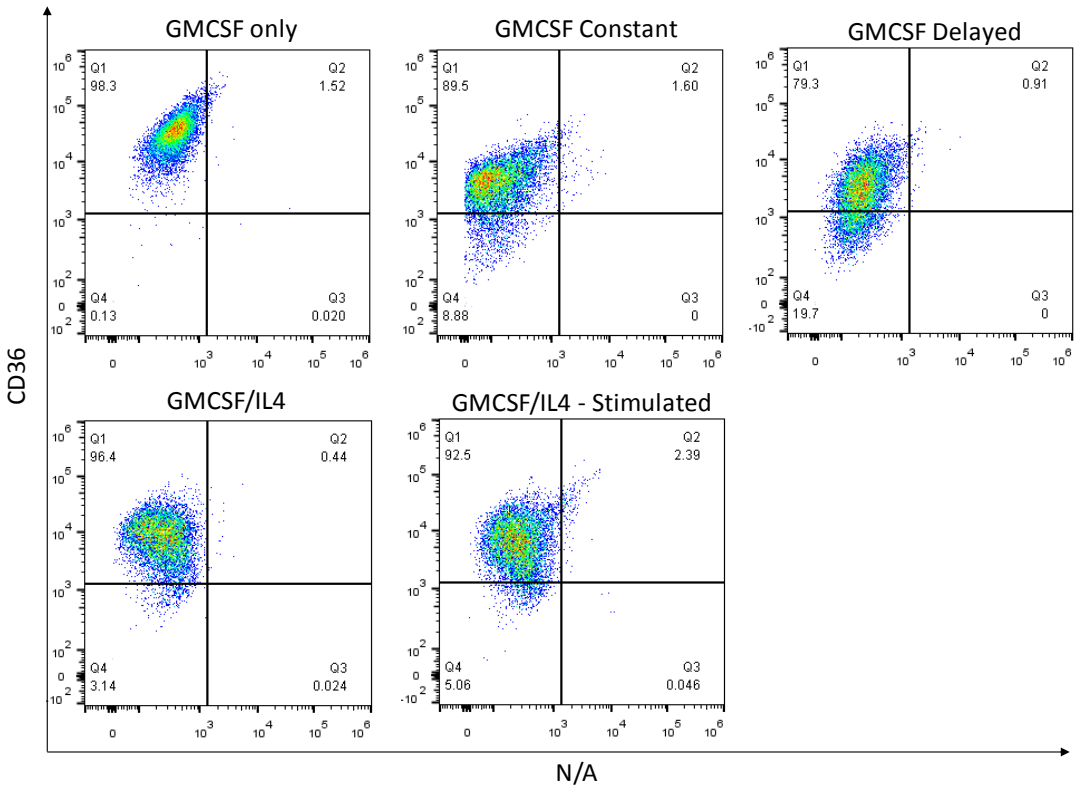
Q3 3.80
Q1 0.13
Q2 2.17
Q4 95.0
Q3 2.74
CD124
ECad

I.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Q3 0.020
GMCSF Constant
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Q2 1.60
Q4 8.88
Q3 0
GMCSF Delayed
Q1 79.3
Q2 0.91
Q4 19.7
Q3 0
GMCSF/IL4
Q1 96.4
Q2 0.44
Q4 3.14
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GMCSF/IL4 - Stimulated
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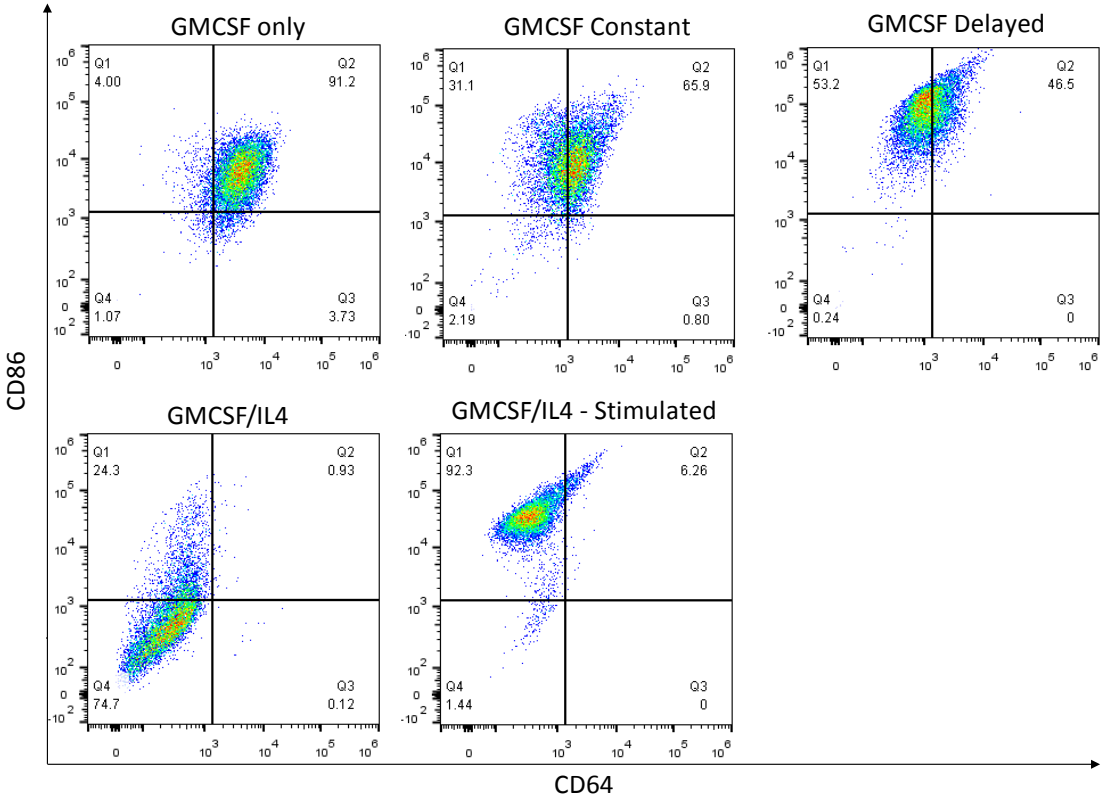
Q2 2.39
Q4 5.06
Q3 0.046
CD36
N/A

J.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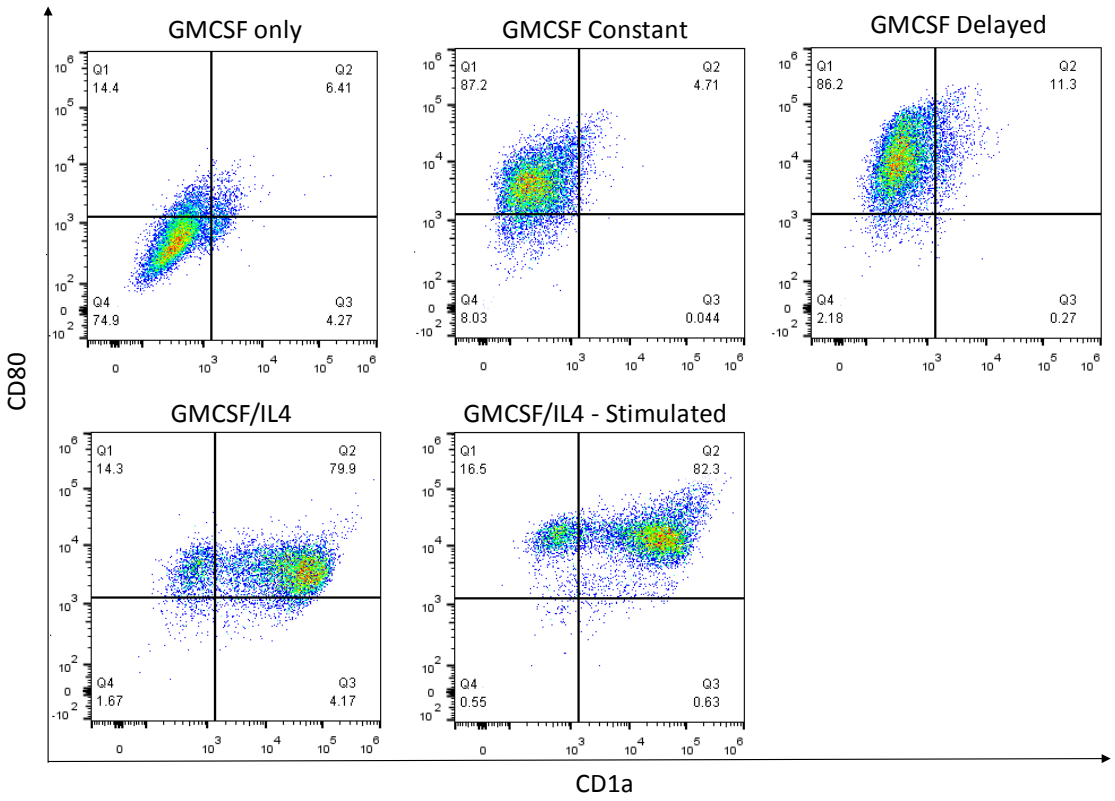
Q2 65.9
Q4 2.19
Q3 0.80
GMCSF Delayed
Q1 53.2
Q2 46.5
Q4 0.24
Q3 0
GMCSF/IL4
Q1 24.3
Q2 0.93
Q4 74.7
Q3 0.12
GMCSF/IL4 - Stimulated
Q1 92.3
Q2 6.26
Q4 1.44
Q3 0
CD86
CD64

K.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Q3 4.27
GMCSF Constant
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Q2 4.71
Q4 8.03
Q3 0.044
GMCSF Delayed
Q1 86.2
Q2 11.3
Q4 2.18
Q3 0.27
GMCSF/IL4
Q1 14.3
Q2 79.9
Q4 1.67
Q3 4.17
GMCSF/IL4 - Stimulated
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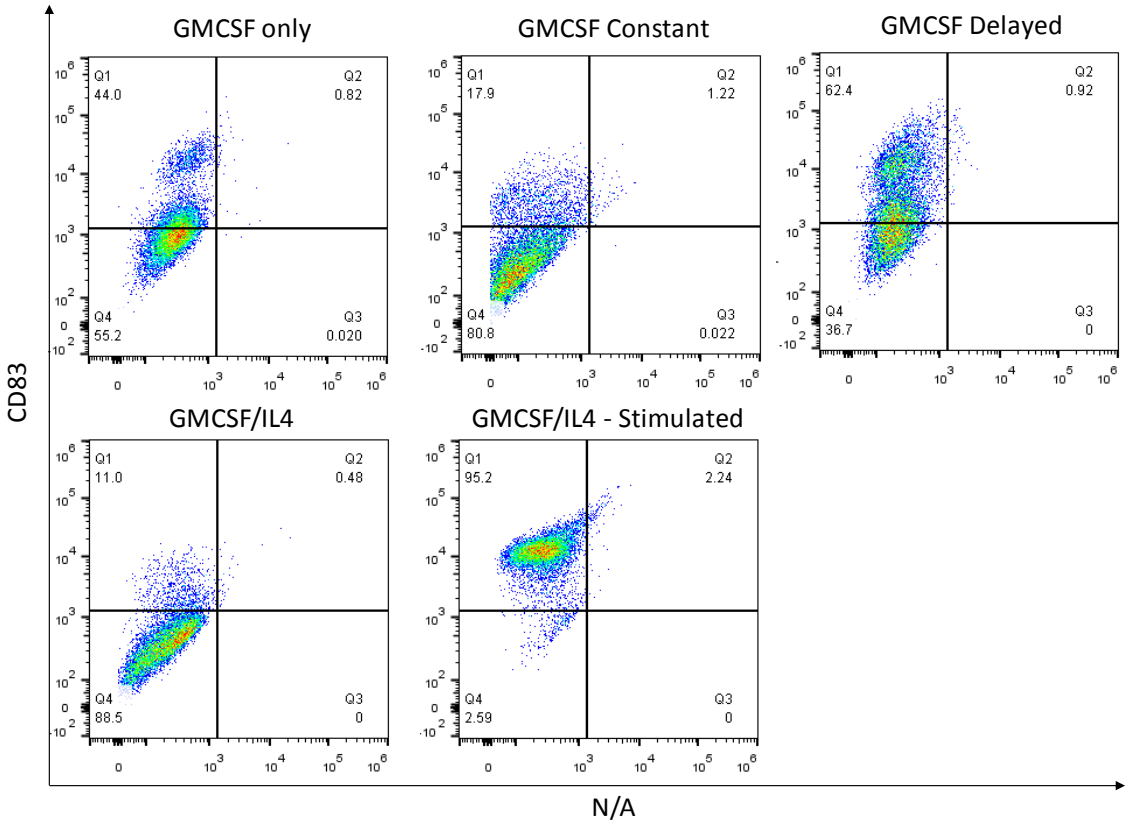
Q2 82.3
Q4 0.55
Q3 0.63
CD80
CD1a

L.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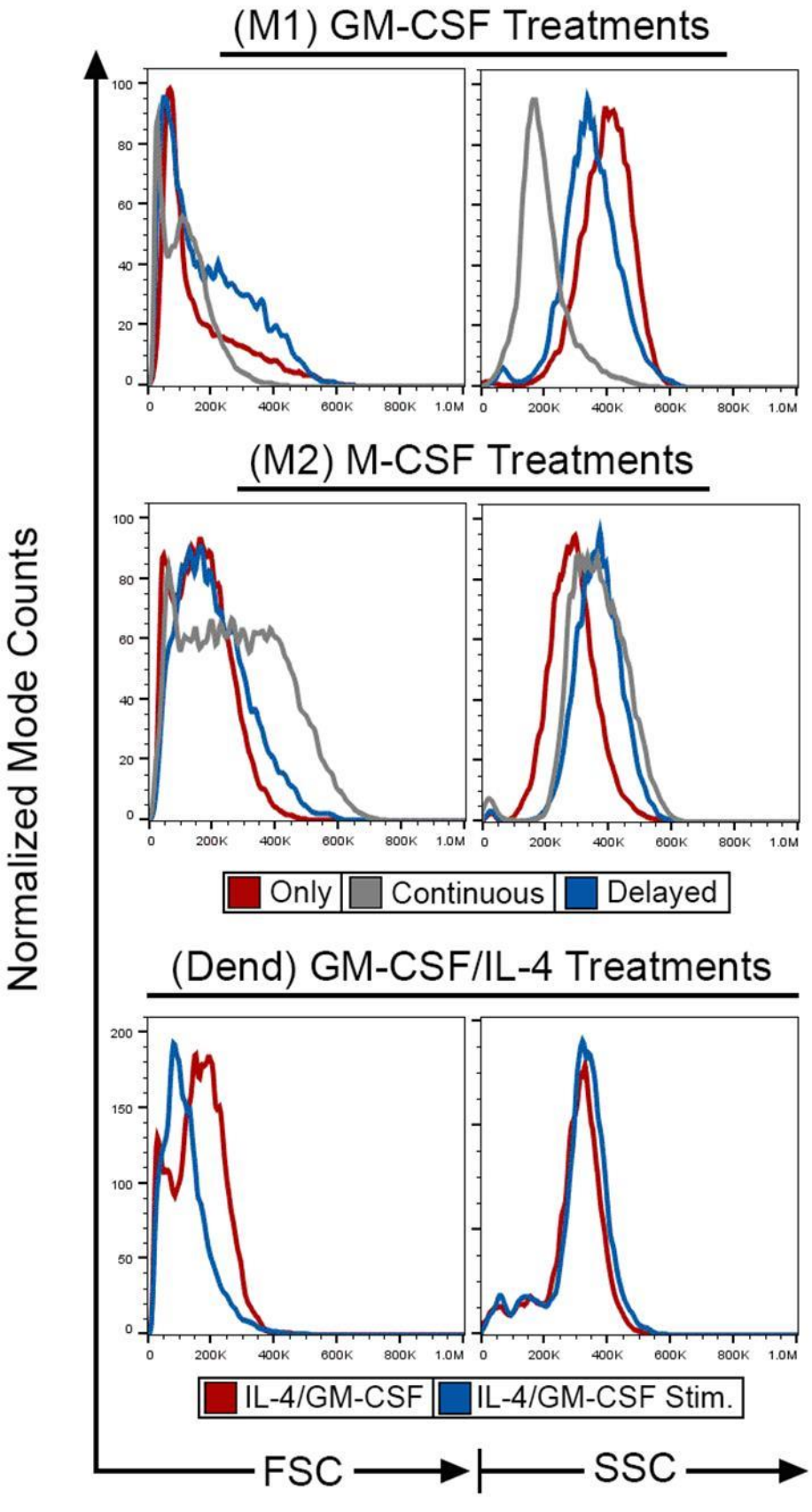
Q4 55.2
Q3 0.020
GMCSF Constant
Q1 17.9
Q2 1.22
Q4 80.8
Q3 0.022
GMCSF Delayed
Q1 62.4
Q2 0.92
Q4 36.7
Q3 0
GMCSF/IL4
Q1 11.0
Q2 0.48
Q4 88.5
Q3 0
GMCSF/IL4 - Stimulated
Q1 95.2
Q2 2.24
Q4 2.59
Q3 0
CD83
N/A

Supplemental Figure 1. Flow Cytometric Analysis of Surface Marker Expression. Differentiated $CD14^+$ monocytes were treated with the (delayed) phased, only or continuous conditions and subject to flow cytometric analysis. Dual staining of cells treated with M-CSF, GM-CSF, or GM-CSF and IL-6 was performed (**C-H**). Each experiment was repeated in triplicate. M2 Macrophages display higher expression of cell surface markers associated with M2 Macrophage and glutamine-free media attenuate M2 markers and efficient polarization (**A, D**) groups relative to M1 treatment groups. Additionally, within the M2 groups (blue) there is substantial increases in CD163 and E-Cadherin (Ecad) expression between phased conditions and IL-6 exposure (**A**). Mature dendritic cell marker, CD83 expression suggests that the phased or stimulated conditions increase both M2 and mature dendritic cell surface marker expression (**B, K, L**). CD1a and CD86 surface expression is increased in dendritic cells (**K, L**).

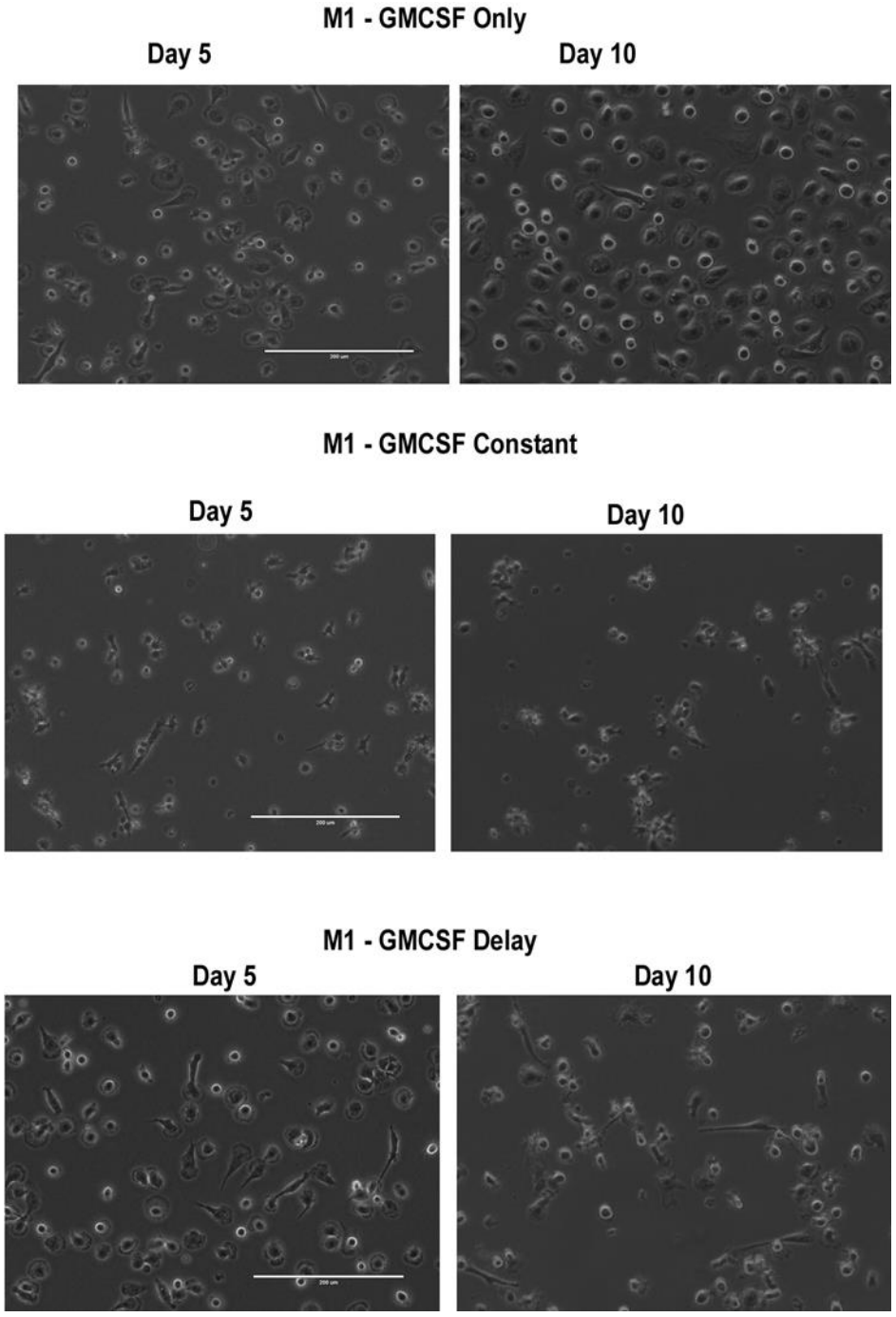
Supplemental Figure 2



Supplemental Figure 2. Analysis of Cell size and granularity under different polarization conditions. Histograph plots compare differences in cell size (FSC) and complexity (SSC) within moDC (dend), M1 and M2 macrophages observed after different treatment strategies. M1 macrophages treated with GM-CSF only (only) appear to be larger in size (FSC), the internal complexity (SSC) data reveals minimal differences between the treatment groups. M2 macrophages display minimal internal complexity and size differences among the treatment groups. The moDC (dend) groups (IL-4/GM-CSF and IL-4/GM-CSF stim.) had minimal changes in cell granularity and size across treatment groups.

Supplemental Figure 3

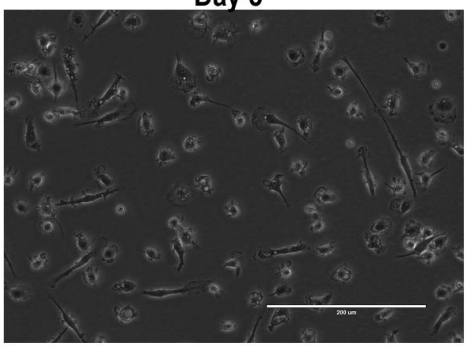
A



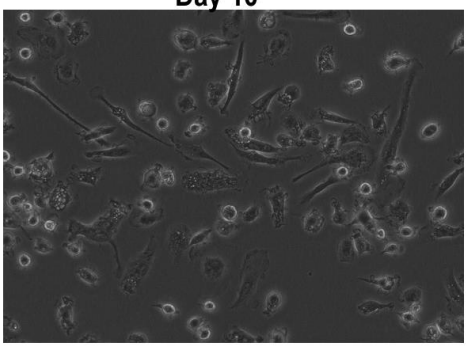
B

M2 - MCSF Only

Day 5

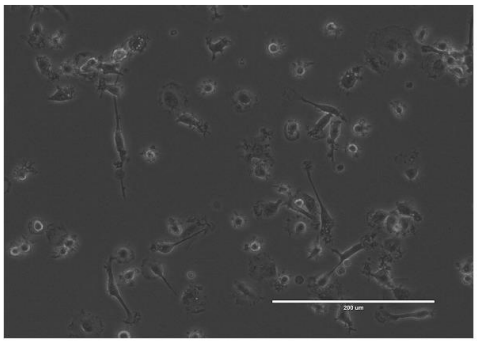


Day 10

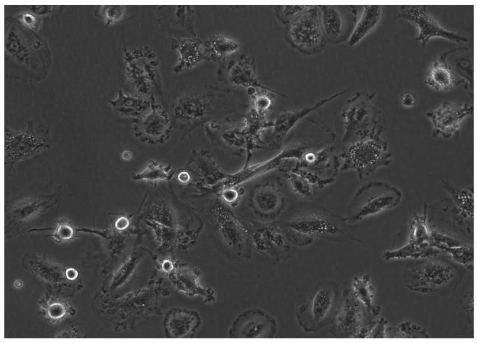


M2 - MCSF Constant

Day 5

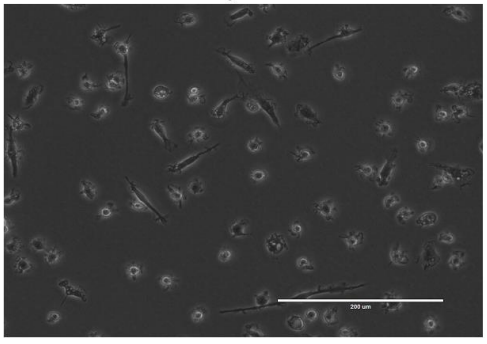


Day 10

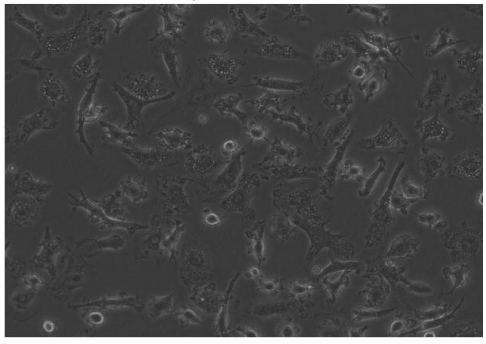


M2 - MCSF Delay

Day 5



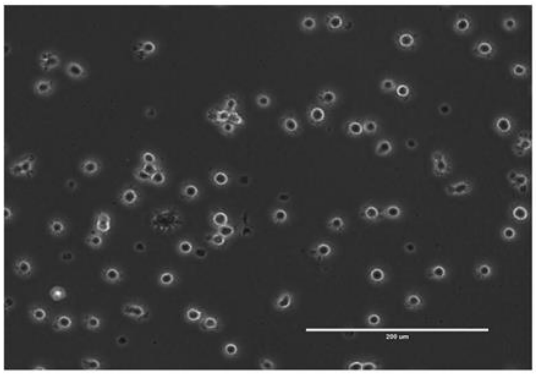
Day 10



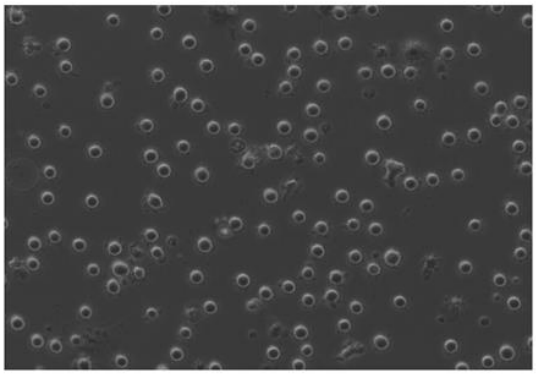
C

DC - GMCSF/IL-4 Only

Day 5

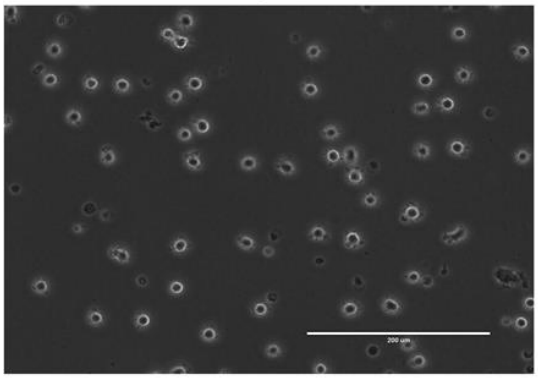


Day 10

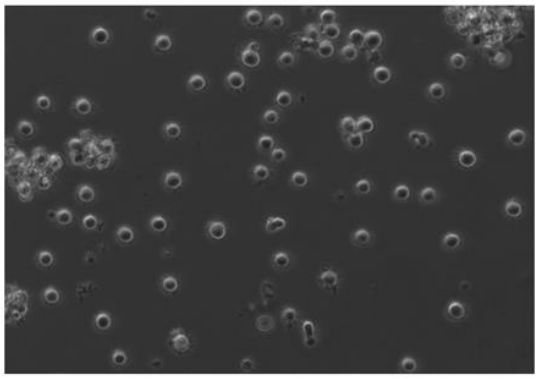


DC - IL-6/LPS Stimulated

Day 5

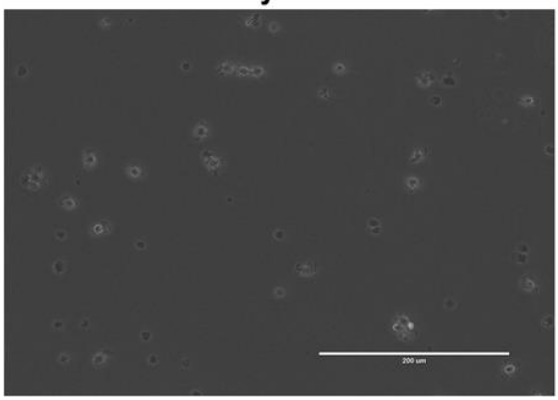


Day 10

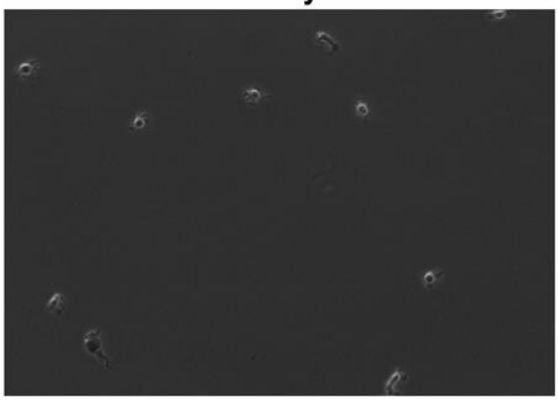


Untreated

Day 5

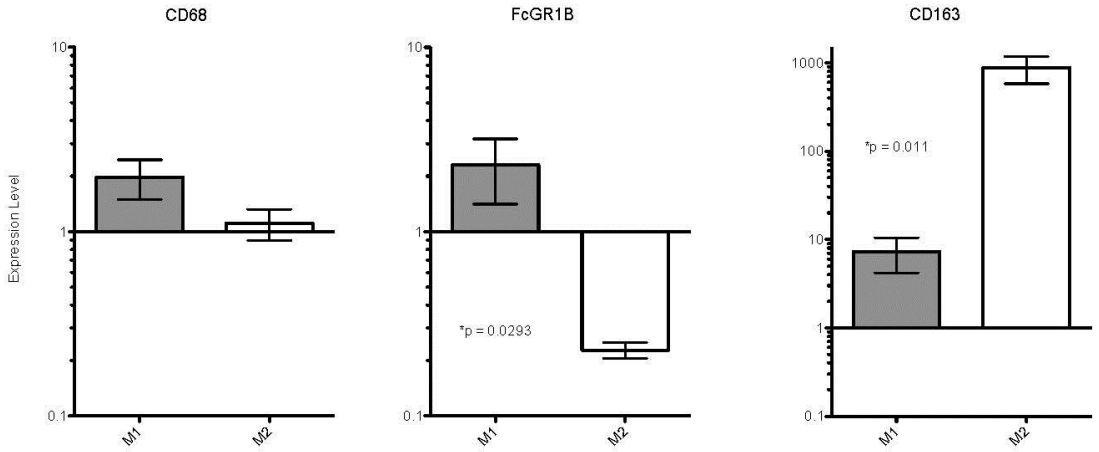


Day 10



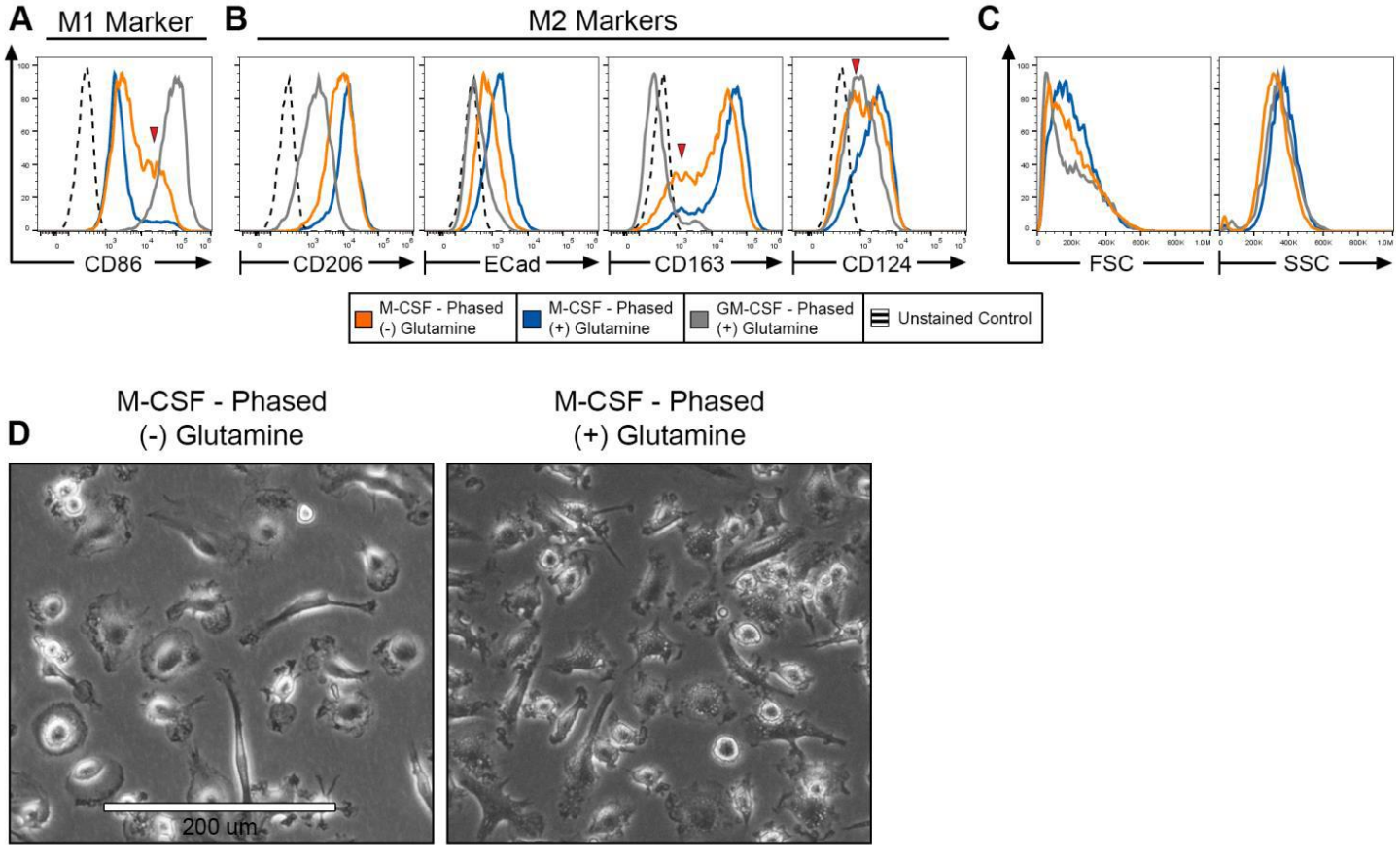
Supplemental Figure 3. Morphological Differences in Adherent Cells. $CD14^{+}$ monocytes treated with GM-CSF only or GM-CSF/IFN-γ/LPS/IL-6 (**A**), M-CSF alone or M-CSF/IL-4/IL-13/IL-6 (**B**) or GM-CSF/IL-4 (**C**) continuously (constant) or phased (delay). Cells were examined using phase contrast microscopy at day 5 (just before phased treatment began) and day 10 (when flow cytometry is performed) (**A-D**). Notice the dramatic phenotypic differences between M1, M2 and Dendritic cell populations under continuous and phased treatment (**A-C**). It should also be noted the dendritic (GM-CSF/IL-4) cells do not adhere to the surface of the plate and remain in suspension (**A**).

Supplemental Figure 4.



Supplemental Figure 4. Phased polarized M1 and M2 macrophage mRNA expression. mRNA expression levels as measured by qRT-PCR and calculated using $^{\Delta\Delta}$Ct method as previously described [24]. P-values were calculated using unpaired, two-tailed student t-tests comprising the $^{\Delta\Delta}$Ct value for each qRT-PCR trial performed in the M1 and M2 phased populations for each gene. For CD68 and FcGR1B, equal variance was assumed, whereas for CD163 unequal variance was assumed.

Supplemental Figure 5



Supplemental Figure 5. Glutamine deprivation alters M2 Macrophage polarization. Phased polarized macrophages were cultivated in glutamine rich or deprived conditions (**A-D**). A comparison of the expression of M1 macrophage marker CD86 (**A**), and M2 macrophage markers CD206, E-Cadherin (ECad), CD163, CD124 (**B**) were analyzed by flow cytometry. Red arrow denote shift in cell surface expression of CD86 (**A**), CD163 (**B**), and CD124 (**B**) on the cell surface of M2 macrophages in glutamine-deprived conditions. Cells size (FSC) and internal complexity (SSC) was also determined by flow cytometric analysis (**C**). Phase contrast images illustrating morphological changes were taken the prior to performing the flow cytometry (**D**).