Characterizing infiltrating monocytes in anti-PD-1/CTLA-4 immunotherapy resistant NSCLC tumors

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Introduction

Lung cancer is the leading cause of cancer-related mortality in the world cancers because of its propensity to metastasize and its late-stage diagnosis. Small-cell and non-small-cell lung cancer (NSCLC) represent over 80% of lung cancer cases. NSCLC treatment options include chemotherapy, radiation therapy, hormone therapy, biological therapy, and immunotherapy. Instead of targeting the tumor cells, immunotherapy directly targets a host's immune system by mobilizing immune cells to recognize and eliminate tumor cells. Because of primary and acquired resistance to immunotherapy we investigated the potential immune cells that may be contributing to resistance.

Background

Fig 1. anti-PD1/CTLA-4 combination resistant tumors are eliminated with anti-Ly6C.
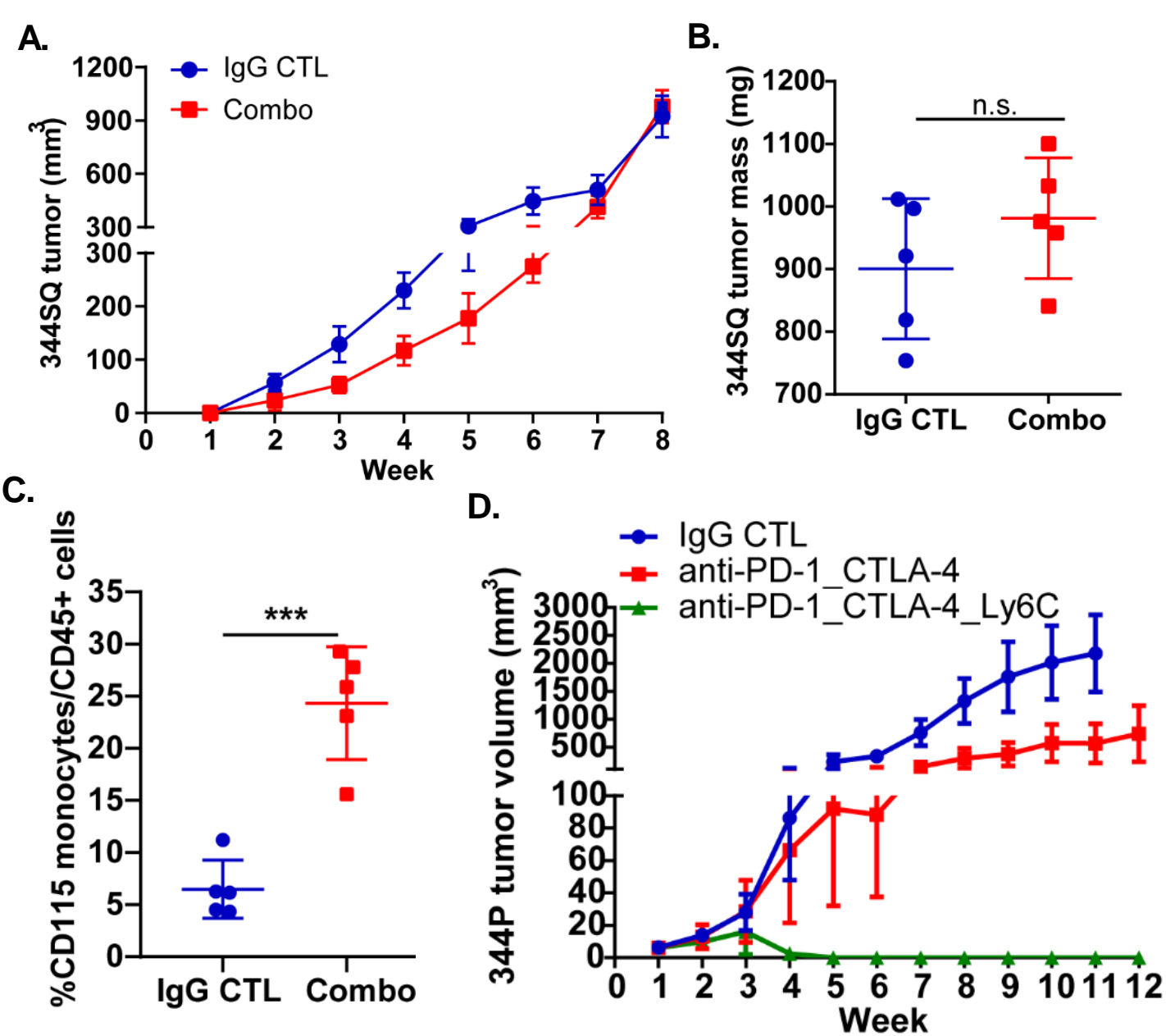

Fig 1. (A) 344SQ tumor growth curves 129/SV mice were treated with IgG control or anti-PD1/CTLA-4 once a week starting on day 7 for 8 weeks. **(B)** 344SQ tumor mass from (A) at week 8 from mice treated with IgG control or anti-PD1/CTLA-4 **(C)** Percentage of CD115 Ly6C+ monocytes from 344SQ tumors processed at week 8. **(D)** 344P tumor growth curve from mice treated with IgG control, anti-PD1/CTLA-4, or anti-PD1/CTLA-4/Ly6C starting on day 7 after tumor cell implantation.

Fig 2. Ly6C+ mouse monocyte cluster 1 is equivalent to human monocyte cluster 1.
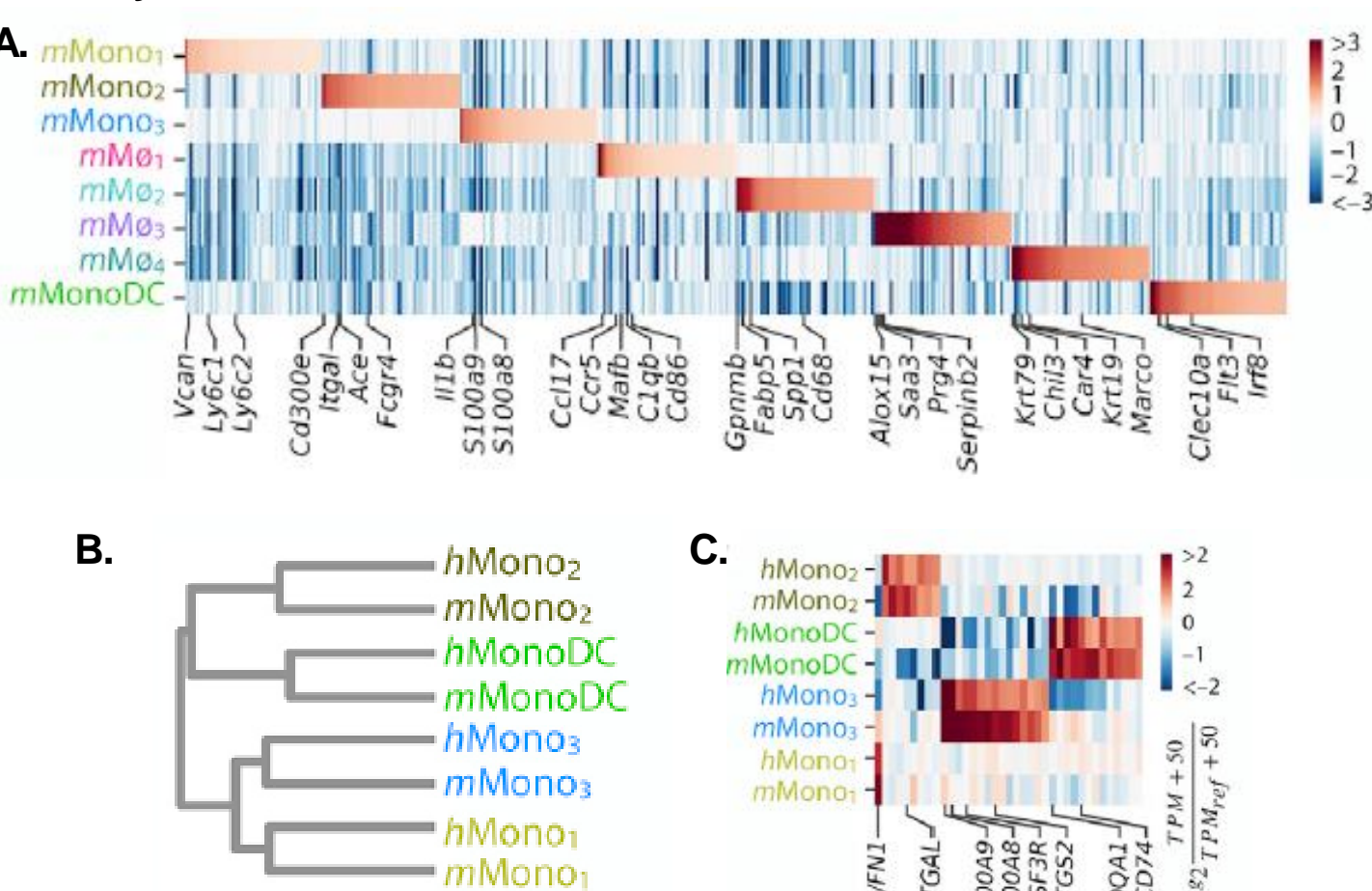

Fig 2. (A) Heatmap showing gene enrichment in mouse monocytes and macrophages. **(B)** Phylogenetic analysis of mouse and human monocyte and monoDC subsets **(C)** Heatmap showing comparison analysis from (B). From Zilionis *et al*. Immunity 2019 (1).

Methods
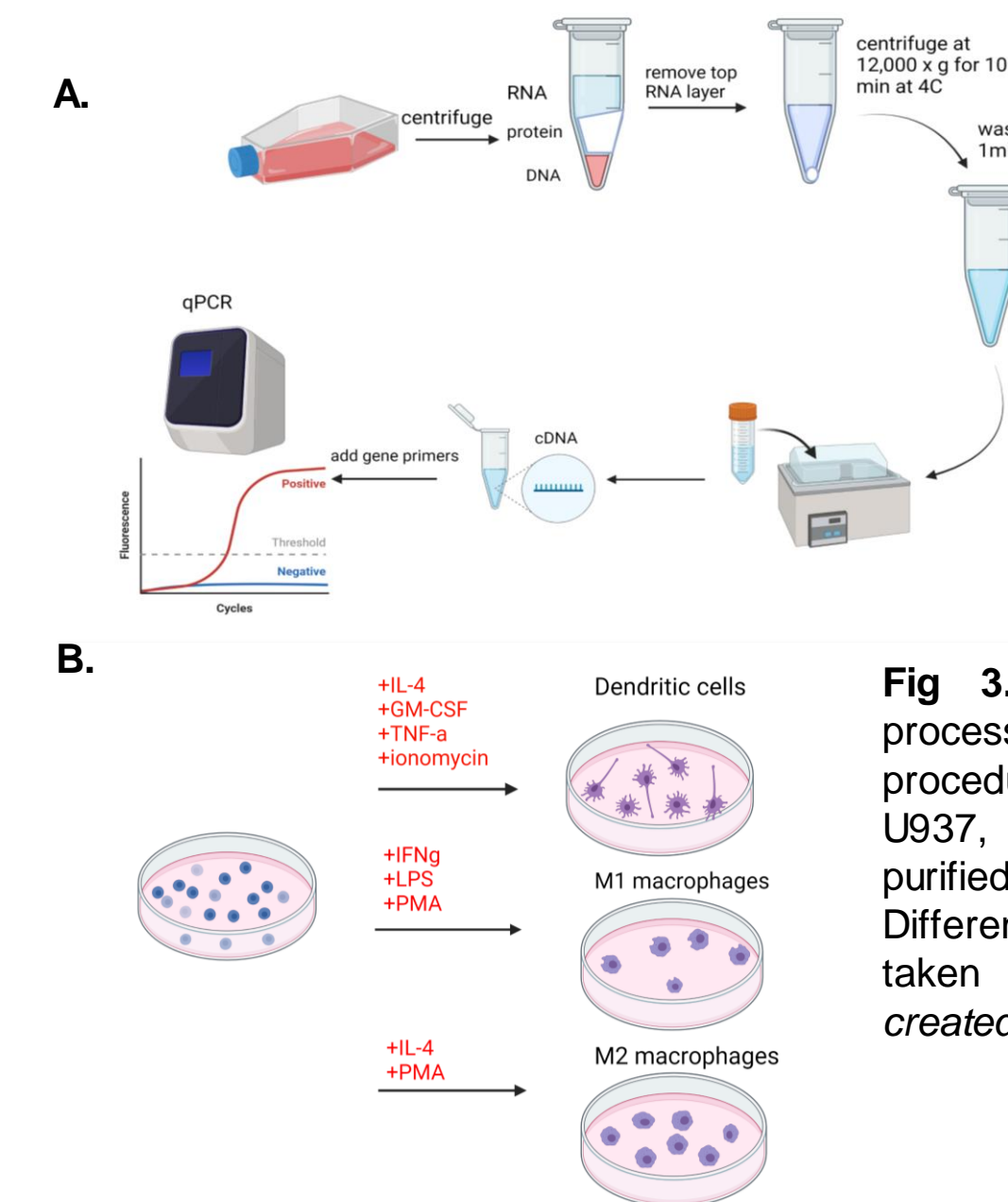
Fig 3. Schematics explaining methodology.

Fig 3. (A) Schematic of process taken for qPCR procedure used on cell lines, U937, THP, and mouse purified monocytes. **(B)** Differentiation assay process taken for images. *Images created using BioRender (2).*

Results

Fig 4. Identification of gene markers associated with monocyte cluster 1.
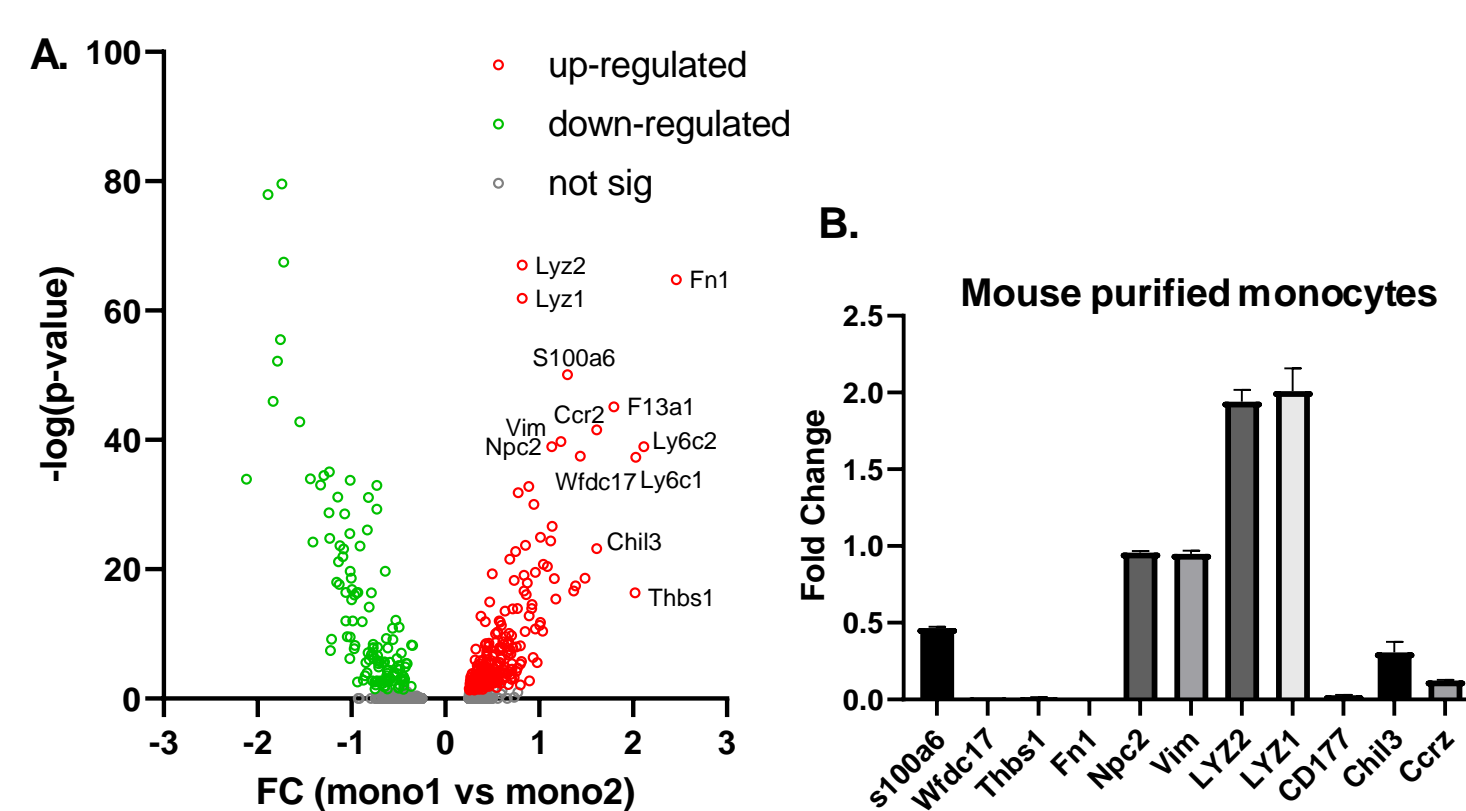

Fig 4. (A) Volcano plot generated from gene expression data found in Zilionis *et al*. Immunity 2019. Genes defining independent monocyte clusters 1 (mono1) & 2 (mono2) were plotted as fold change (FC) to identify those associated specifically with mono1. **(B)** Genes identified in panel A were analyzed in mouse monocytes purified from spleen cells by qPCR.

Fig 5. Cluster analysis of THP1 and U937 human monocyte cell lines revealed a heterogeneous population.
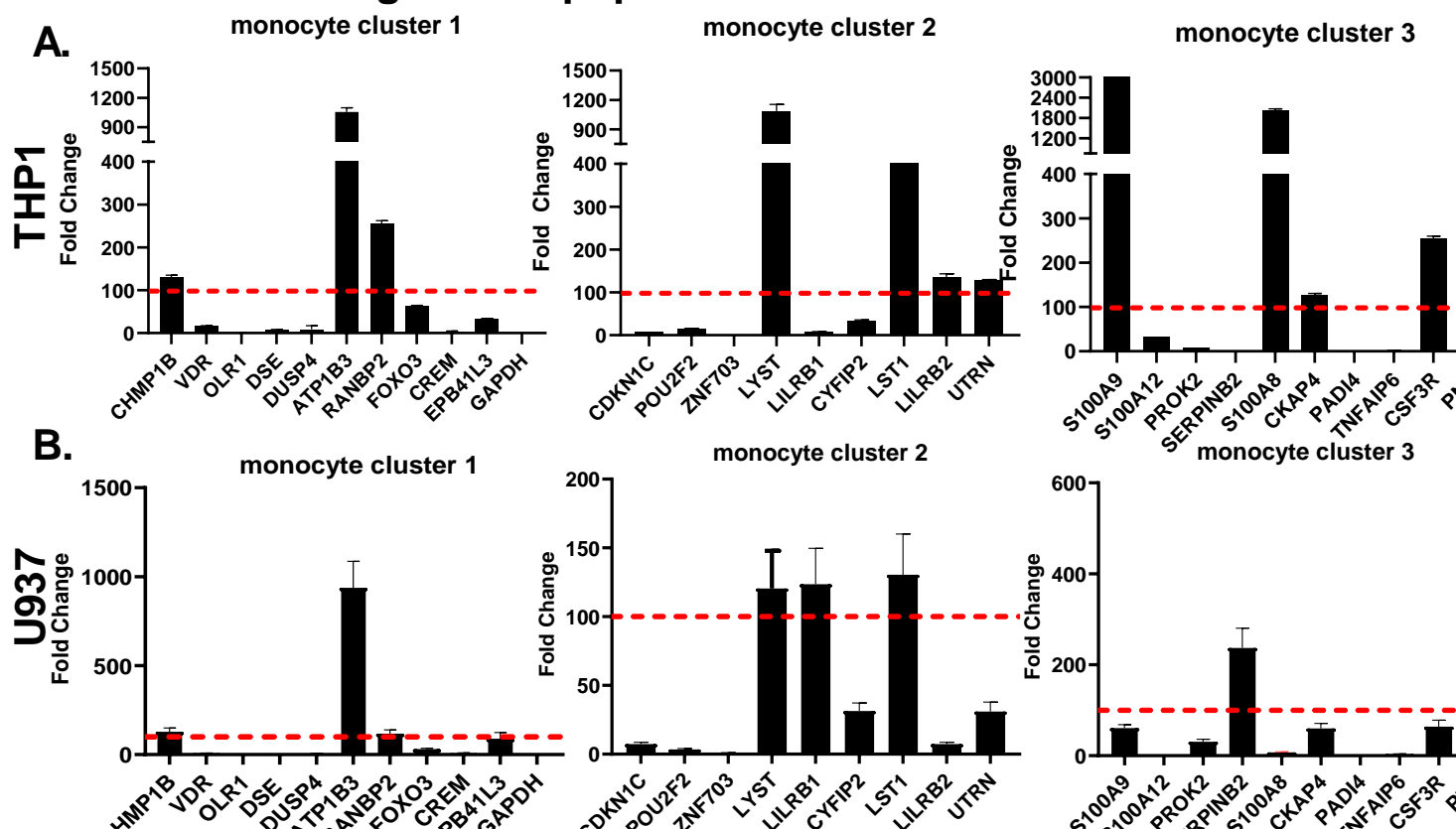

Fig 5. (A) Genes identified as markers for each monocyte cluster were analyzed in THP1 human monocyte cells at baseline using qPCR. Relative gene expression is graphed using L32 and GAPDH normalization. **(B)** U937 cells were analyzed as described in panel A.

Fig 6. Cluster analysis of murine bone marrow derived monocytes demonstrates variable expression patterning across clusters.
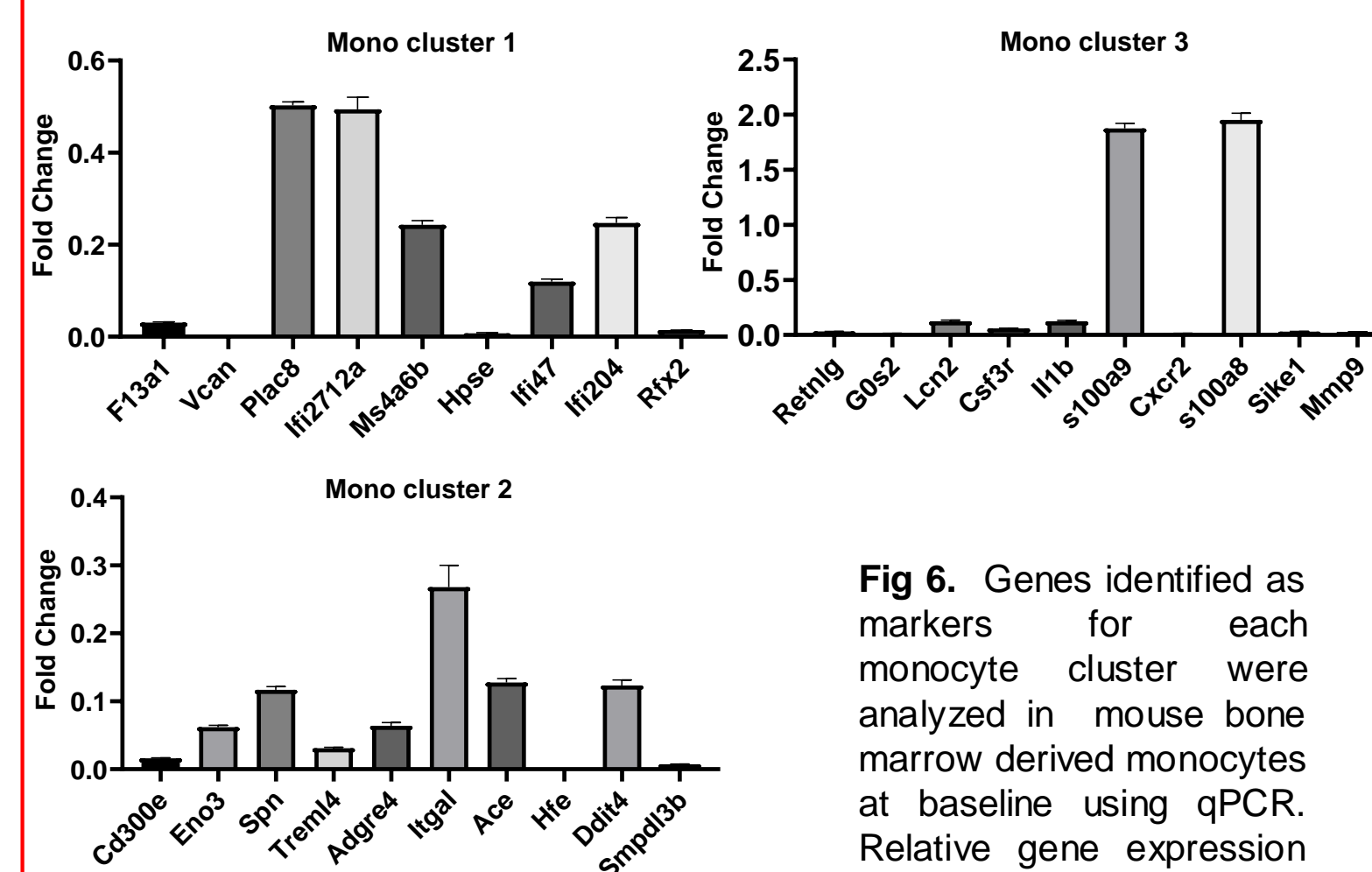

Fig 6. Genes identified as markers for each monocyte cluster were analyzed in mouse bone marrow derived monocytes at baseline using qPCR. Relative gene expression is graphed using L32 and GAPDH normalization.

Fig 7. Human THP-1 cells can differentiate into dendritic cells, M1 macrophages, or M2 macrophages with various cytokine additions.
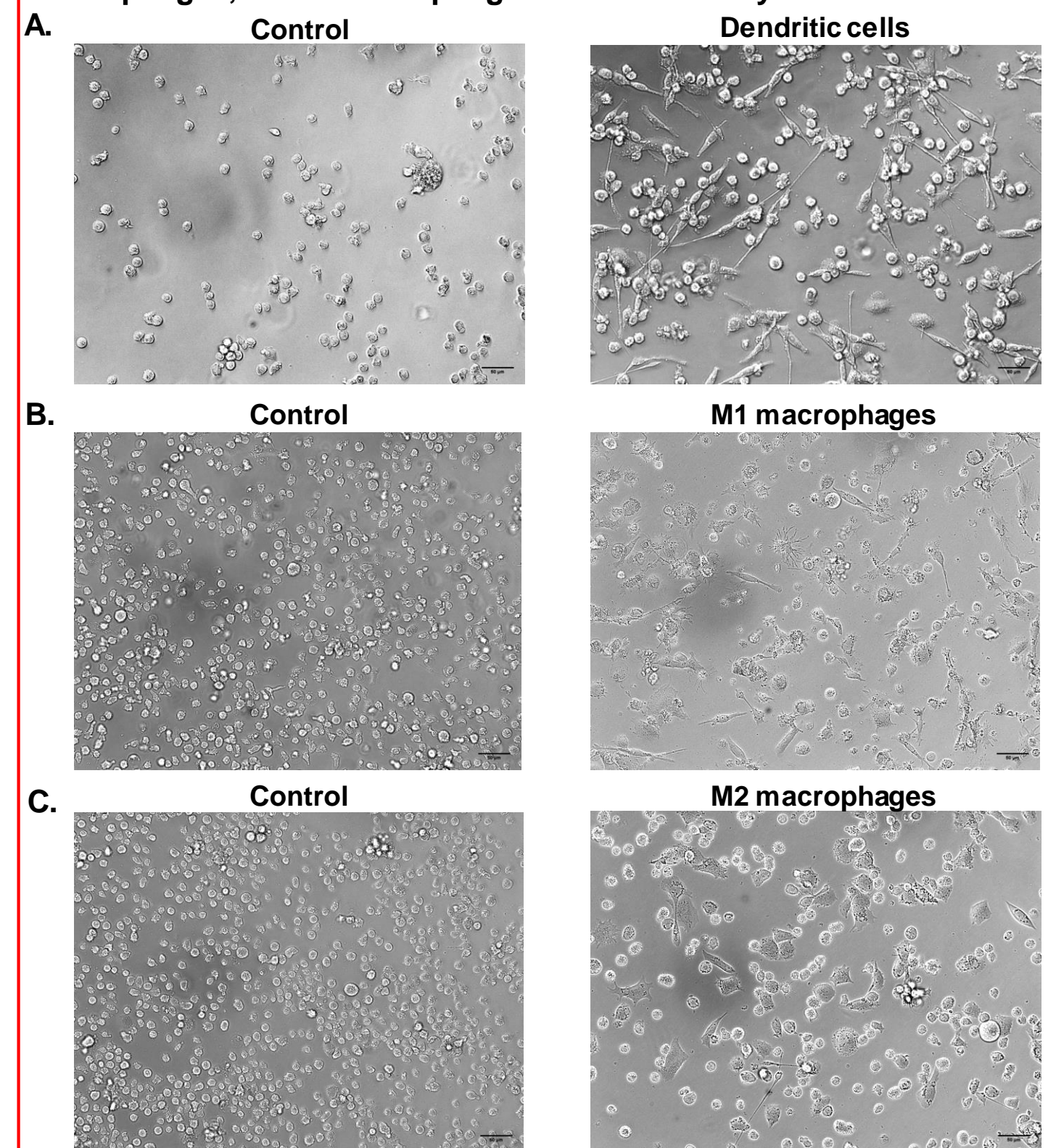

Fig 7. (A) Brightfield microscopy images depicting the contrast from the control THP-1 cells and those cultured with IL-4, GM-CSF, TNF-a and ionomycin for 3 days to induce dendritic cell differentiation. **(B)** M1 macrophage differentiation was induced in THP-1 cells by addition of IFNγ, LPS and PMA for 3 days. **(C)** M2 macrophage differentiation was induced in THP-1 cells by addition of IL-4 and PMA for 3 days. Scale bar = 50µm.

Conclusions

Cluster analysis of THP-1 and U937 had varied expression. Due to the large variability in expression between genes, additional genes will need to be queried for further confirmation of cluster. Identification of the monocyte clusters will contribute to understanding how they are able to differentiate and how they are contributing to immunotherapy resistance.

References

Zilionis R, et al. *Immunity*. 2019 May 21;50(5):1317-1334.e10. PMCID: PMC6620049.
1) BioRender.com