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Understanding Macrophage Phagocytosis in Pediatric Leukemia

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THE UNIVERSITY OF KANSAS
CANCER CENTER

NCI Comprehensive Cancer Center

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Background

Macrophages are a diverse and widespread type of innate immune cell which play an important role in homeostasis and defense. In a process called phagocytosis, macrophages engulf dying cells and pathogens. If they detect a threat, they will present antigens from phagocytosed cells to initiate an adaptive immune response against remaining cells of the same type. Questions remain about how macrophages recognize, or fail to recognize, cancerous cells for clearance, and how macrophage state in the tumor microenvironment promotes or inhibits an immune response.

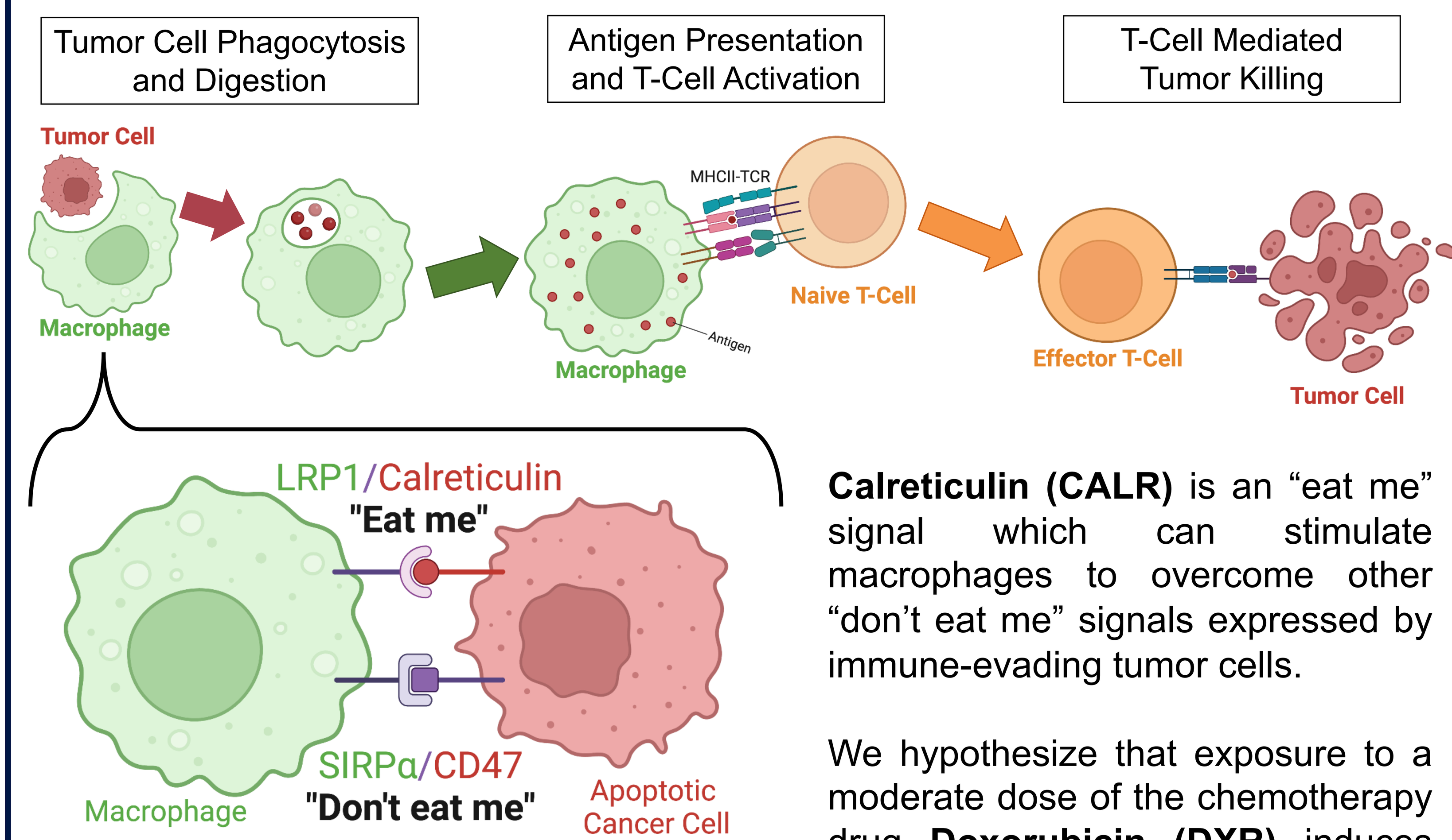


Figure 1. Phagocytosis can initiate an adaptive immune response against cancer cells. *"Eat me" and "don't eat me" signals on cancer cells determine macrophage response.*

Calreticulin (CALR) is an "eat me" signal which can stimulate macrophages to overcome other "don't eat me" signals expressed by immune-evading tumor cells.

We hypothesize that exposure to a moderate dose of the chemotherapy drug **Doxorubicin (DXR)** induces immunogenic cell death in leukemia cells, causing them to express CALR on their surface and initiating a broad immune response beginning with macrophages.

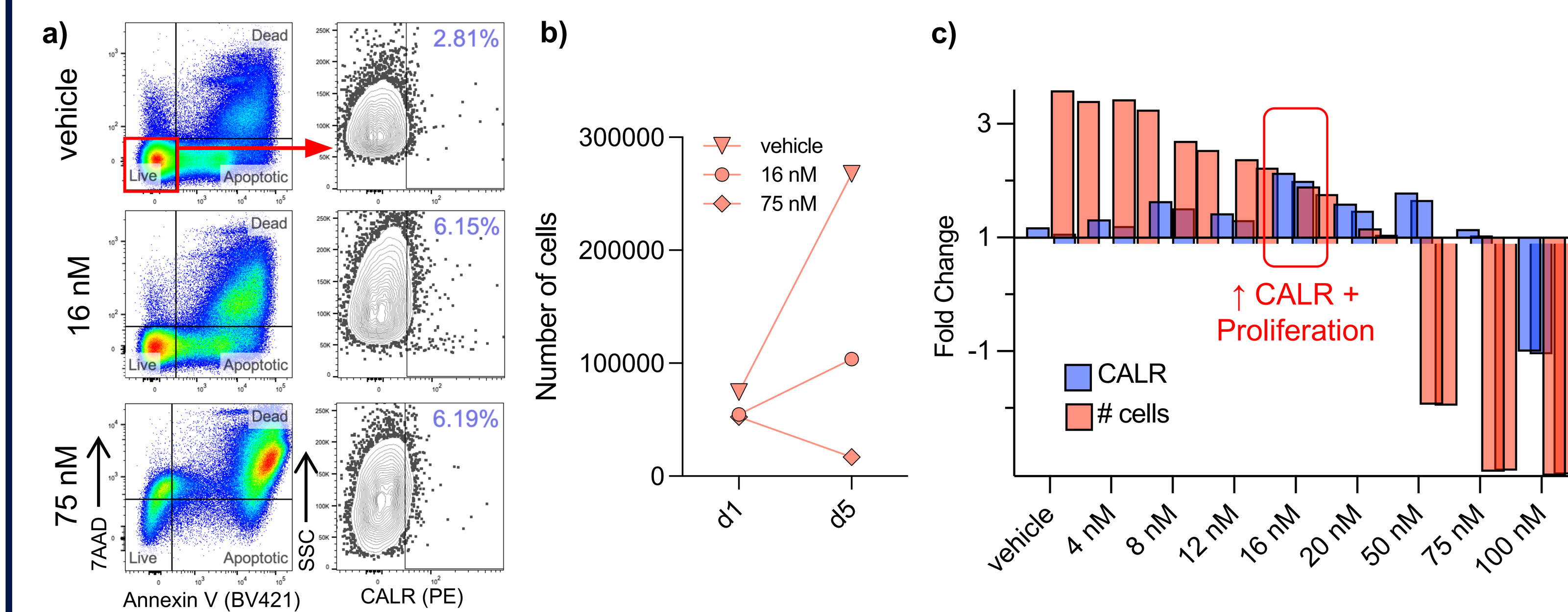


Figure 2. Moderate dose DXR induces pre-mortem calreticulin expression. *Data on Kasumi-1 pediatric leukemia cells from Jackie Nemechek.* ***a)*** *DXR treatment leads to increased CALR surface expression.* ***b)*** *Moderate dose DXR treatment supports cell proliferation while high dose is toxic.* ***c)*** *16 nM (moderate dose) DXR treatment induces peak calreticulin expression while still supporting proliferation.*

Macrophage polarization

Macrophages exhibit phenotypic plasticity based on signals from their environment. Their polarization state affects their interactions with cancer cells. In brief, M1 are shown to be pro-inflammatory (anti-tumor) while M2 are anti-inflammatory (pro-tumor). M2 macrophages are often found in and around tumors where they tolerate or even promote tumor growth. We are interested in exploring ways to activate them against cancer cells. To test macrophage response to various stimuli, we collect mouse bone marrow and differentiate the monocytes into macrophages for experimentation.

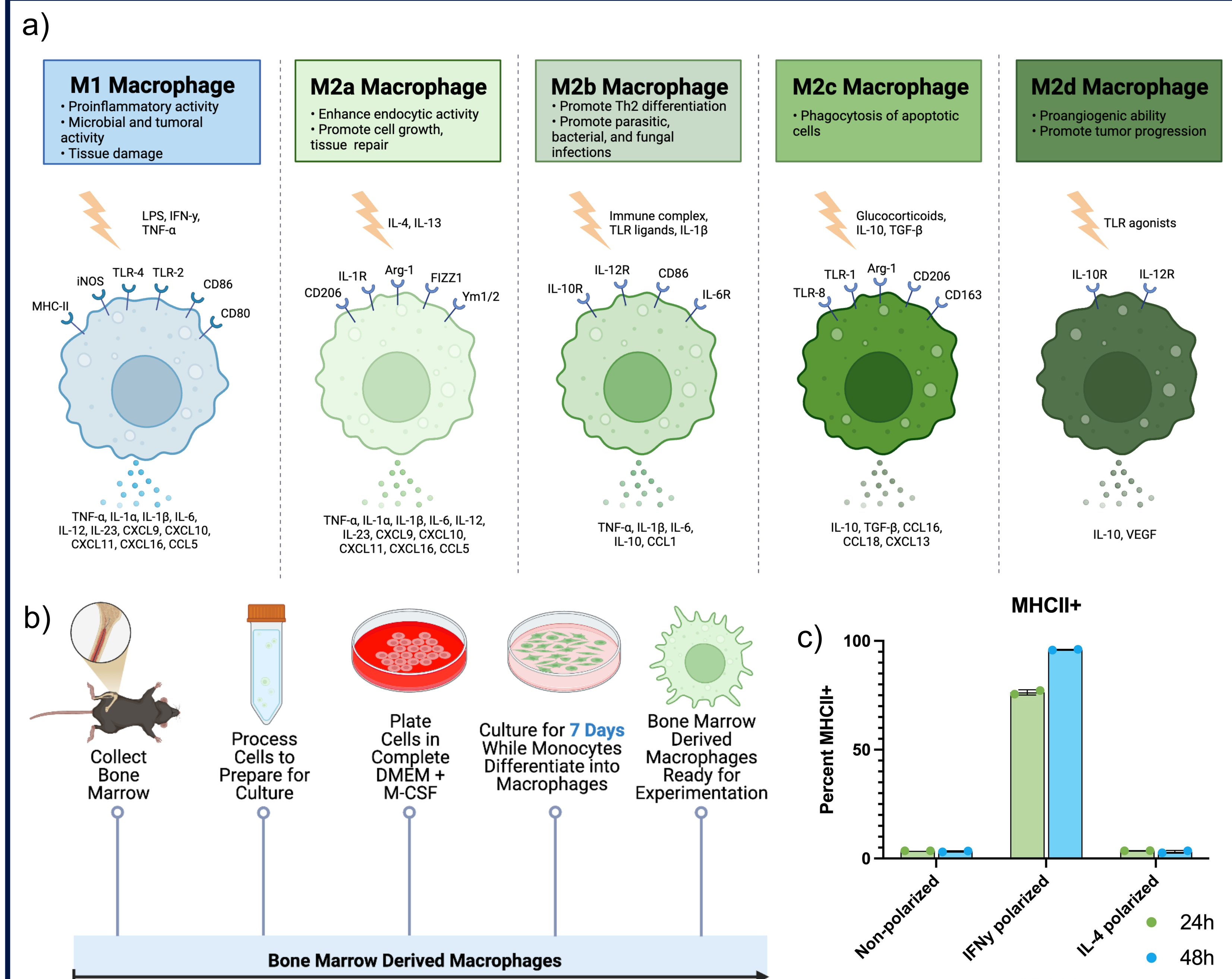


Figure 3. Primary macrophages for *in vitro* polarization studies. ***a)*** *Macrophage structure and function is variable in response to signals from their microenvironment.* ***b)*** *Method for collecting and differentiating primary murine Bone Marrow Derived Macrophages (BMDMs).* ***c)*** *BMDMs cultured with IFNγ exhibit an M1 phenotype as indicated by increased MHCII surface expression.* ***d)*** *BMDMs cultured with IL-4 exhibit an M2 phenotype as indicated by increased CD206 surface expression.*

Phagocytosis assay

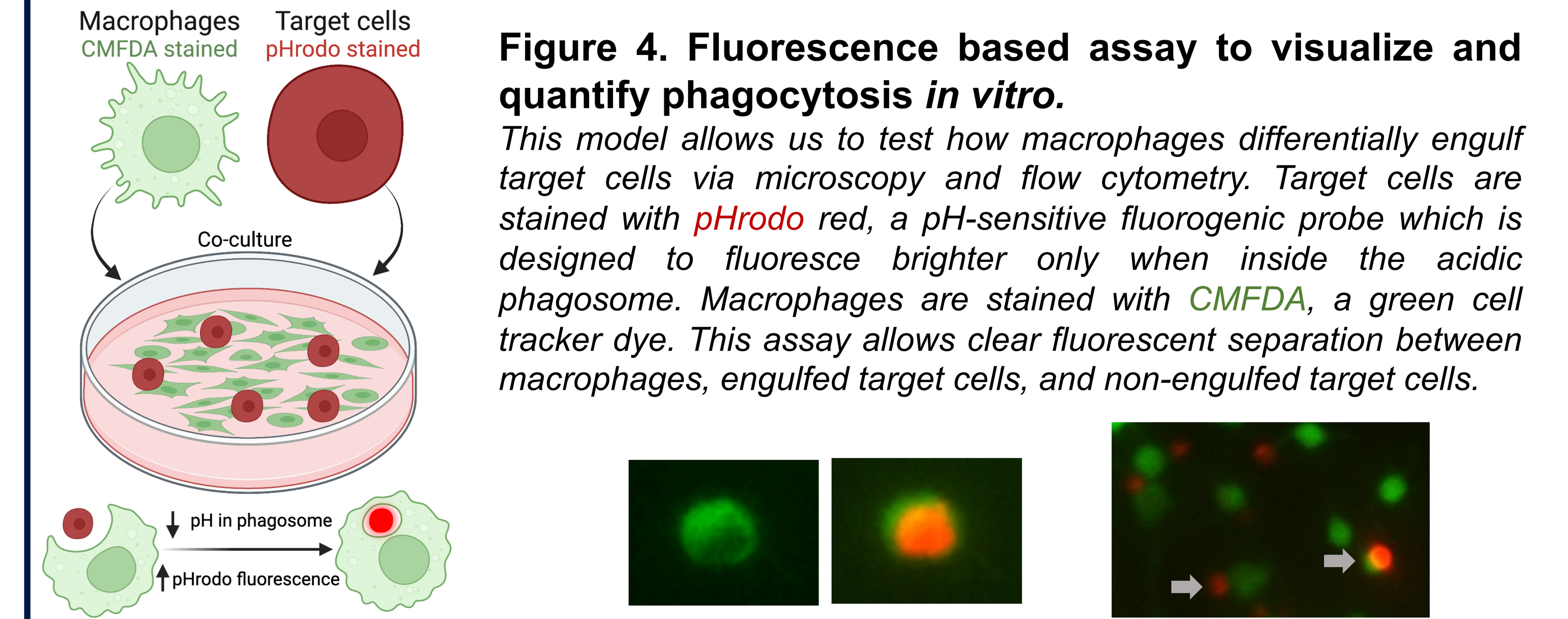
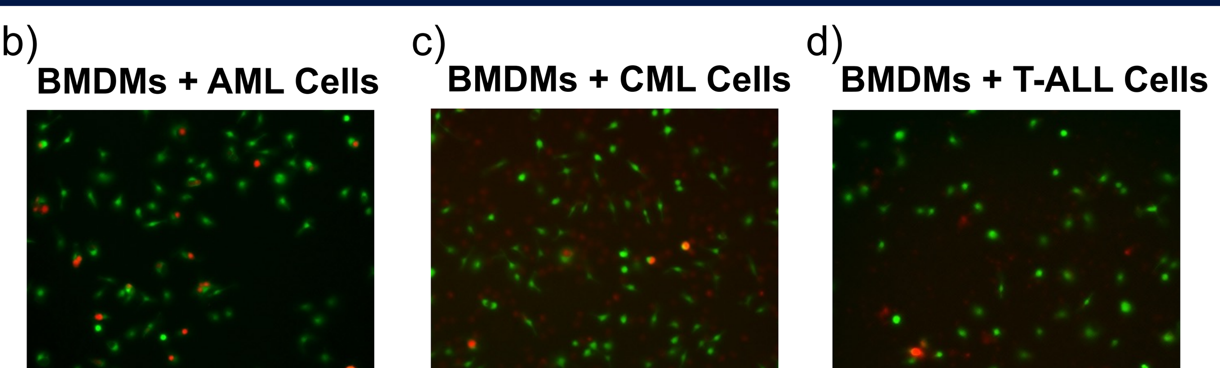


Figure 4. Fluorescence based assay to visualize and quantify phagocytosis *in vitro*. *This model allows us to test how macrophages differentially engulf target cells via microscopy and flow cytometry. Target cells are stained with pHrodo red, a pH-sensitive fluorogenic probe which is designed to fluoresce brighter only when inside the acidic phagosome. Macrophages are stained with CMFDA, a green cell tracker dye. This assay allows clear fluorescent separation between macrophages, engulfed target cells, and non-engulfed target cells.*



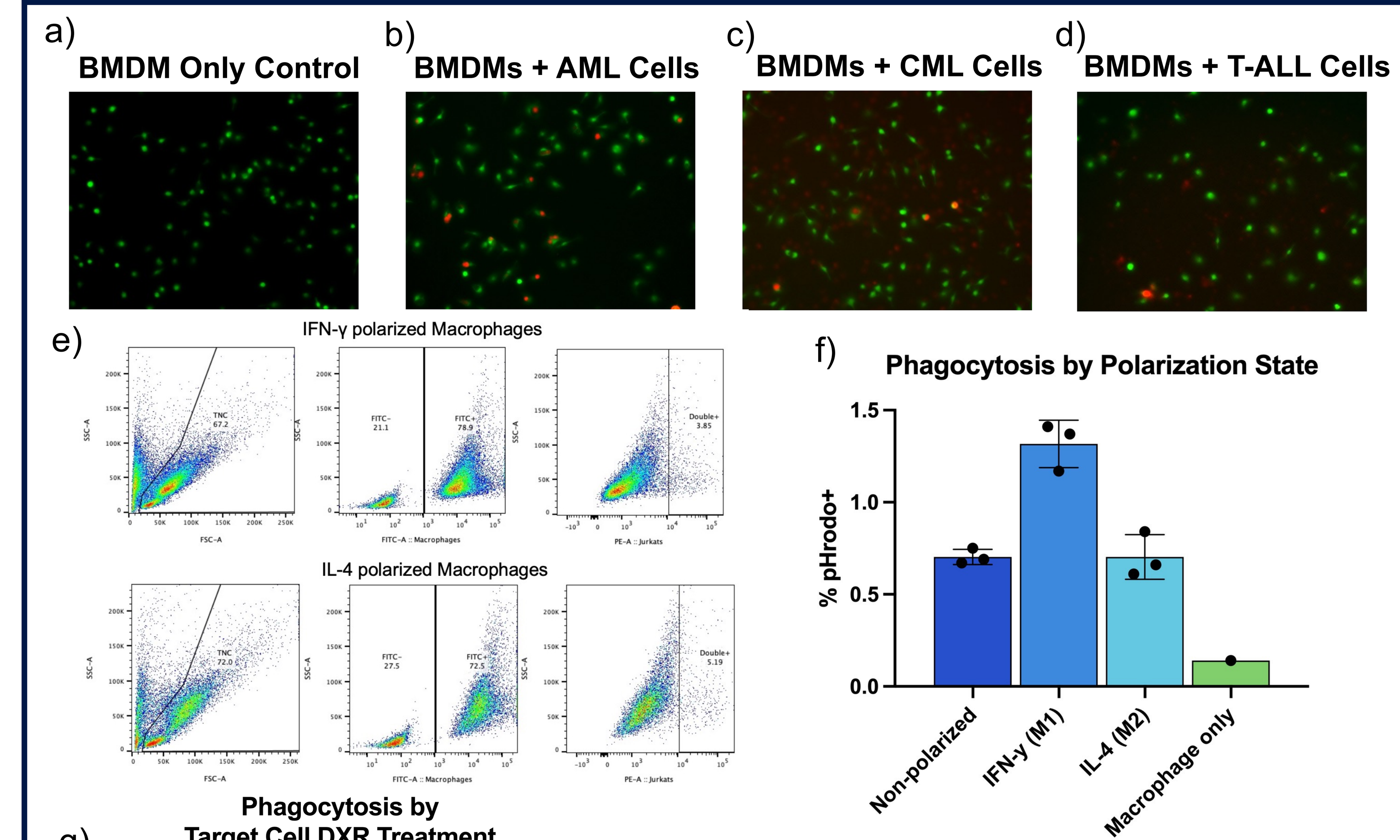


Figure 5. Macrophages differentially engulf cancer cells. ***a)*** *Fluorescence microscopy of macrophages only.* ***b)*** *Phagocytosis of THP-1, an acute monocytic leukemia (AML) cell line.* ***c)*** *Phagocytosis of K562, a chronic myelogenous leukemia (CML) cell line.* ***d)*** *Phagocytosis of Jurkat, a pediatric T-cell leukemia (T-ALL) cell line.* ***e)*** *Flow cytometry strategy for quantifying phagocytosis.* ***f)*** *M1 polarized macrophages show increased phagocytosis of Jurkat cells.* ***g)*** *Phagocytosis of K562 cells increases with moderate DXR treatment compared to irradiation.*

Acknowledgements

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Future directions

- Additional CALR expression and phagocytosis assays to investigate how CALR expression alters macrophage behavior.
- Further test polarization state using cytokine assays and additional flow markers.
- Utilize 3D bioprinting to test macrophages in a more complex microenvironment.

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