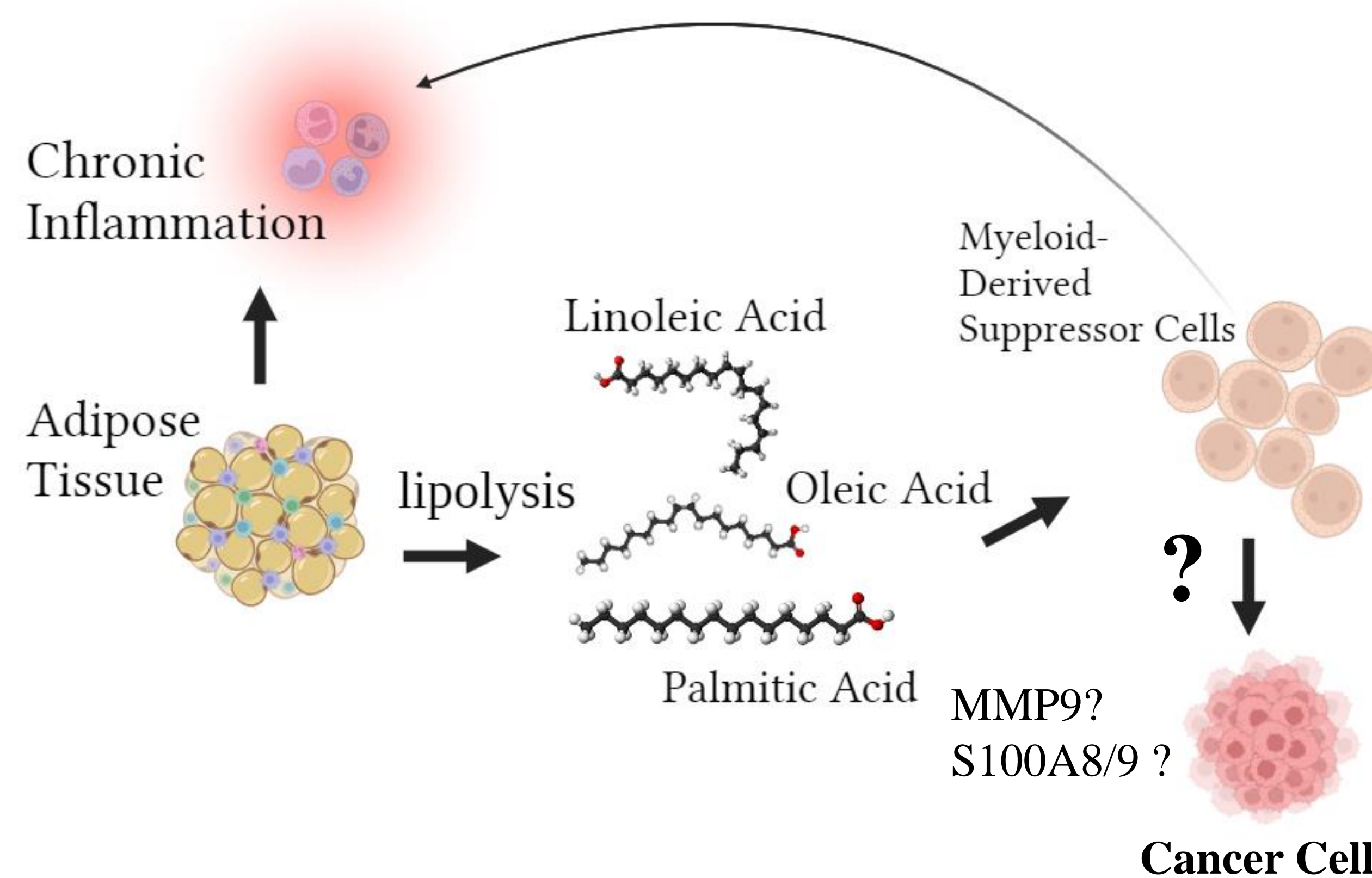


Introduction

Background

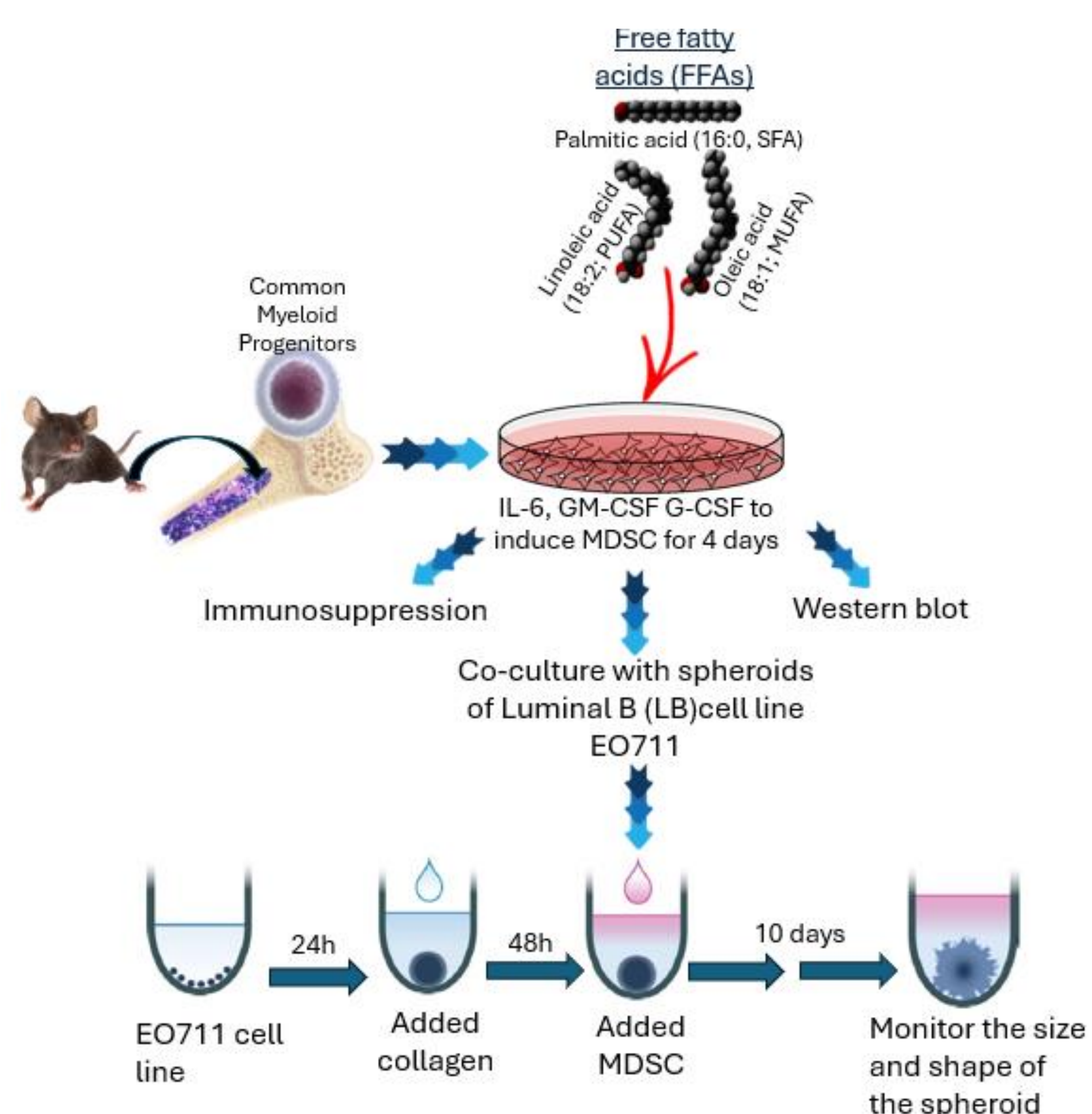
- In 2023, 49.3% of the American population were considered obese. Obesity has been associated with the development of at least 13 types of cancers, including breast cancer in post-menopausal women.
- However, the mechanisms behind this association between Obesity and cancer are unknown.
- In patients with severe obesity (BMI>40) Oleic Acid (OA) and Palmitic Acid (PA) predominate the circulation (~25%) followed by Linoleic Acid (LA) (~21%).



Hypothesis

We hypothesized that LA, OA, and PA increase the capacity of MDSC to facilitate spheroid growth of the mouse Luminal B cell line EO711

Methods



3D multicellular (EO711 cell line and MDSC) culture was successfully implemented for assessing spheroid growth for 10 days

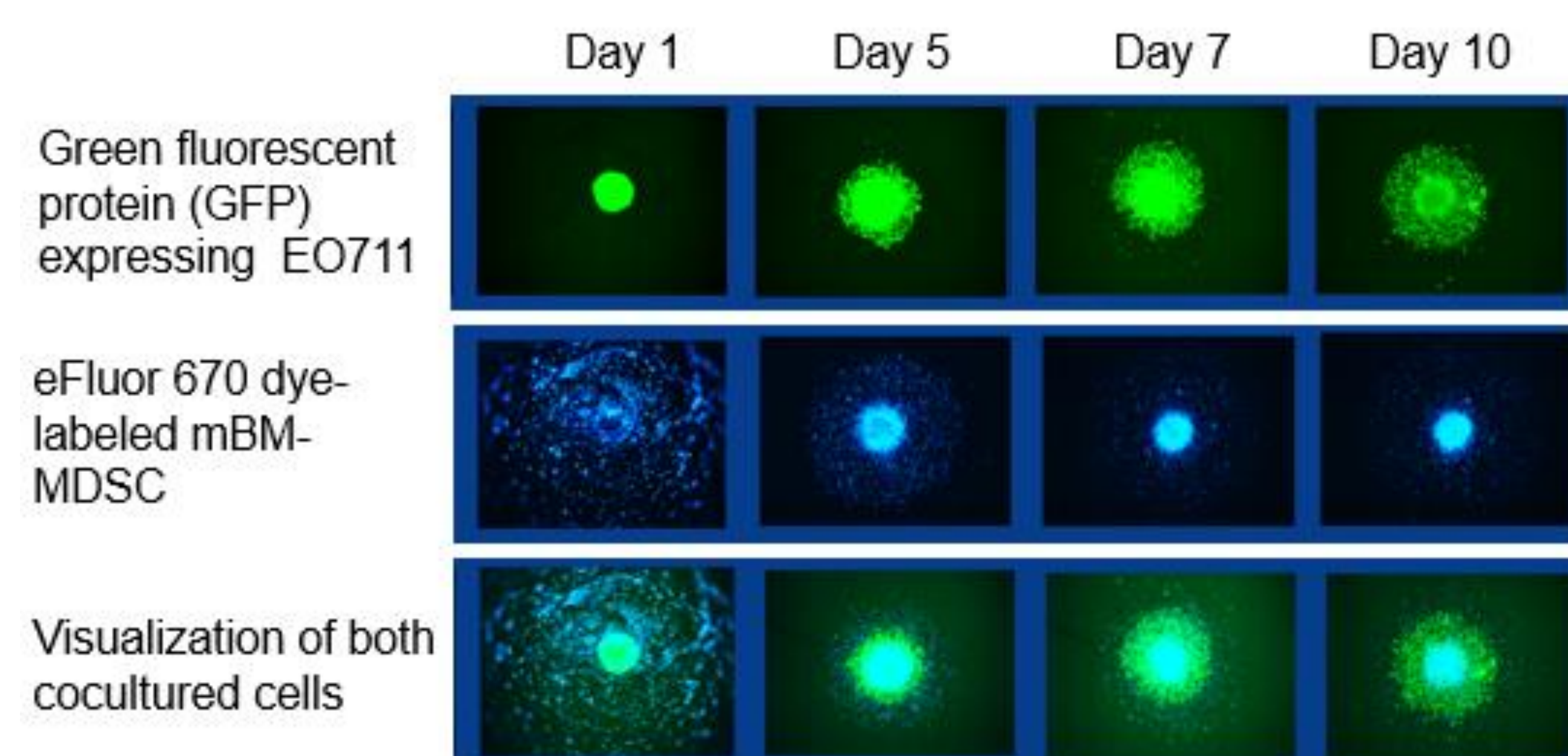


Figure 1: Representative pictures of EO711 spheroids and MDSC during coculture of 10 days. EO711-GFP (10,000 cells) were seeded in a 96-well ultra-low attachment plate. After the formation of a single spheroid, 40,000 eFluor 670 dye-labeled MDSC were added. The spheroid size was monitored in the Incucyte SX5 for 10 days. Half of the culture media was changed with fresh media at day 6th.

MDSC treated with palmitic acid increases the size of EO711 spheroid

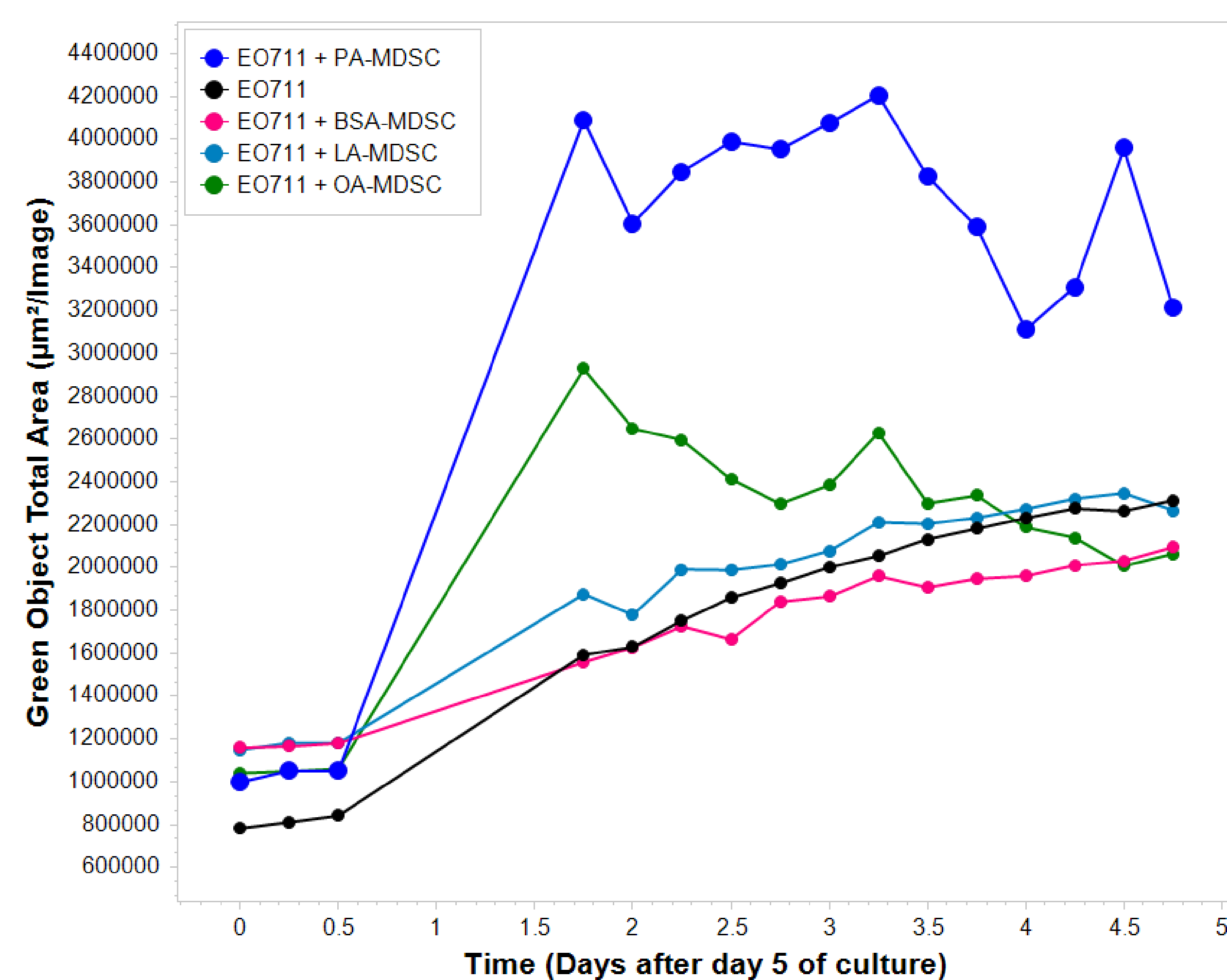


Figure 2: Measurements of the green (EO711-GFP) spheroid area from day 5 to 10. Data points were taken by Incucyte SX5 every 6 hours after coculture of EO711 spheroid with MDSC induced from mouse bone marrow with recombinant cytokines in the absence (Bovine Serum Albumin [BSA]) or presence of the FFAs BSA-Oleic (OA), BSA-Linoleic (LA), and BSA-Palmitic (PA) acids

Differential regulation between different FFA on Arg1 expression without affecting MMP9 and S100A8 protein levels in MDSC

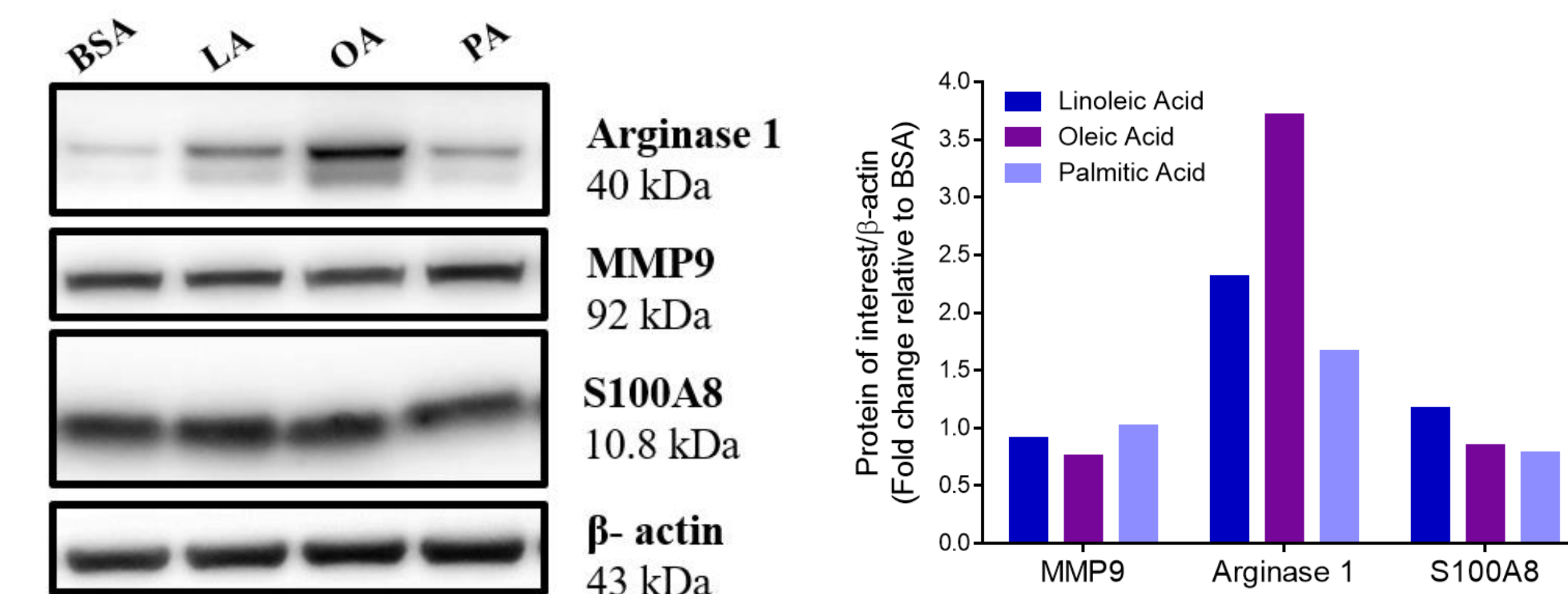


Figure 3: Expression levels of MDSC markers by western blot and densitometry. Cell extract of MDSC treated or not with different FFAs was used to determine the intracellular expression levels of Arg1, MMP9, and S100A8

Palmitic Acid moderately reduces immunosuppressive function

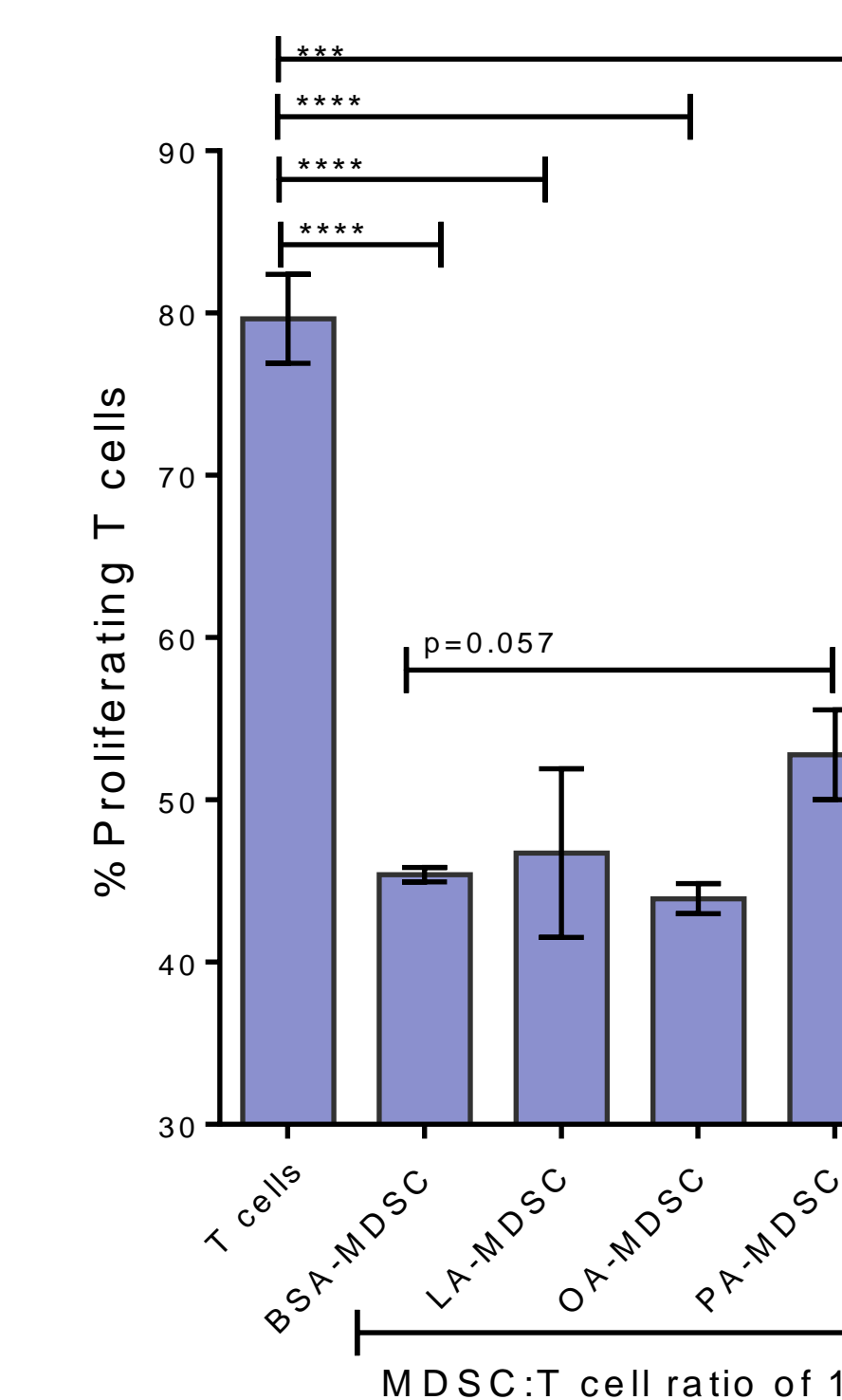


Figure 4: Suppression Assay to evaluate the effect of FFAs on the immunosuppressive function of MDSC on T cell proliferation. Induced MDSC with and without FFAs were cocultured with Cell-Trace violet dye-labeled T-cells for 3 days. Activation of T cells was performed using Dynabeads Mouse T-Activator CD3/CD28 kit in the presence of mouse recombinant IL-2. CellTrace™ dilution on T cell proliferation was measured by flow cytometry

Conclusion & Future Research

- The 3D multicellular culture was successfully established; however, further optimization is needed including 1) the number of seeded cells (EO711 and MDSC), 2) avoiding perturbing spheroids by changing media and 3) not using wells in the borders of the plate due to media evaporation.
- MDSC treated with PA showed a higher capacity for increasing spheroid growth. This PA reduced the expression of Arg1, a marker of immunosuppression, aligns with a decreased capacity to suppress T cell proliferation
- OA, LA, and PA differentially impact the expression of the MDSC marker Arg1 but not on MMP9 and S100A8.
- Additional assessments need to be performed to determine the effect of FFA on these proteins in supernatant since MMP9 and S100A8 are secreted after activation, as well as other secreted proteins such as cytokines.

Acknowledgments

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