

Introduction and Background

- The **glycocalyx** layer is a protective barrier made up of glycans and mucins
- plays an influential role in the **immune cells response** that are against cancer cells (Buffone, 2020)
- Difficulty of treating cancers such as **breast cancer and melanoma**
- This study focuses on enzymes attacking the glycocalyx layer
- experimenting breaking down the glycocalyx using specific enzymes: **chondroitinase, hyaluronidase, heparinase, and neuraminidase**

Hypothesis/Objective

We hypothesize that by attacking the glycocalyx barrier with enzymes, we aspire to enhance immune cell recognition and attacks, potentially improving immunotherapy methods.

Methods

- Cultured breast cancer and melanoma cells.
- Plated 10,000 breast cancer cells and 20,000 melanoma cells per well in a 96-well plate.
- Treated cells with enzymes (chondroitinase, heparinase, neuraminidase, hyaluronidase) across two wells per enzyme.
- After 24 hours, fixed cells with 4% formaldehyde.
- Stained nuclei with DAPI (blue) and glycocalyx layer with WGA (red).
- Acquired images using confocal microscopy and processed using ImageJ/FIJI.

Results

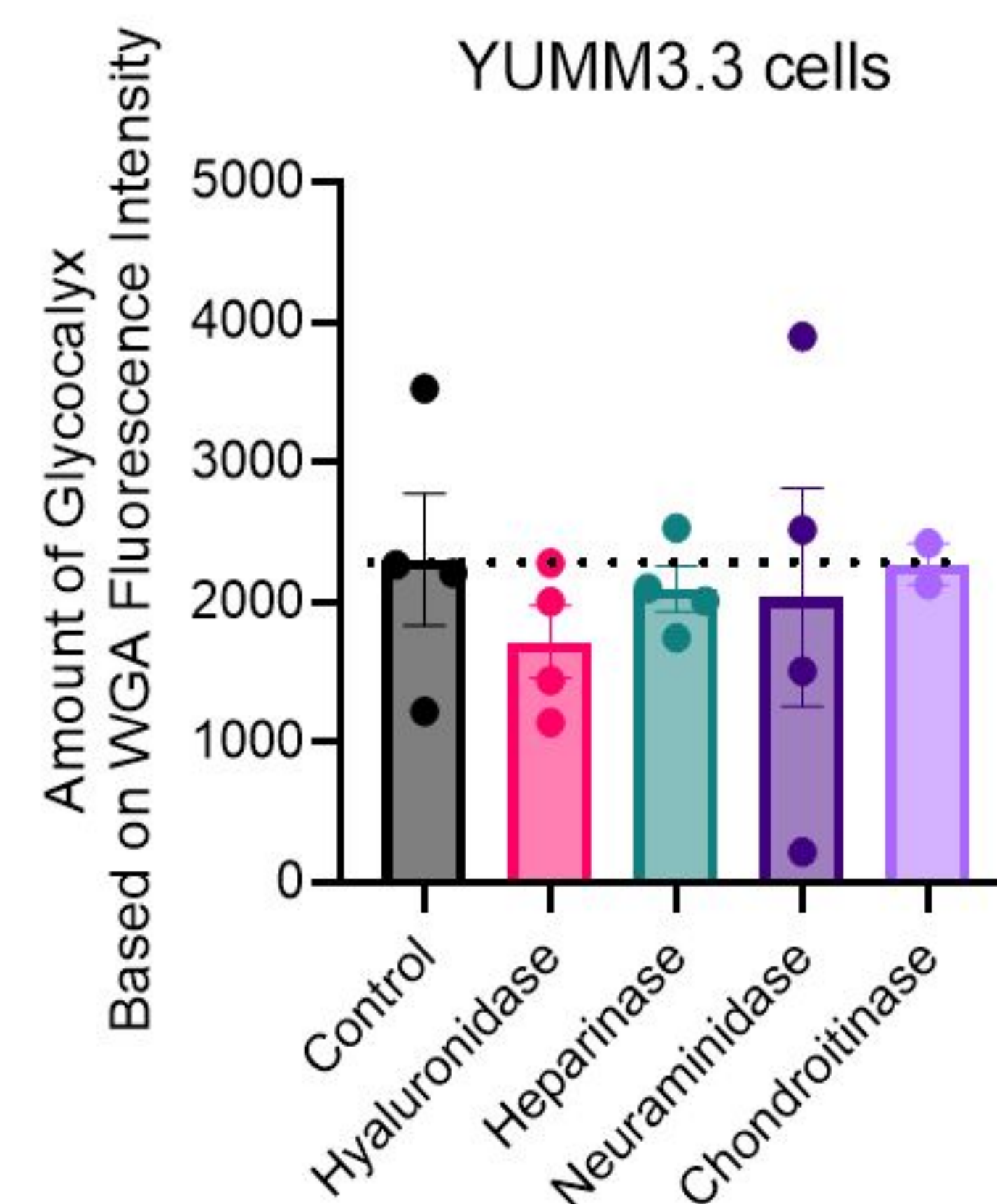


Figure 1. YUMM enzyme treatment results. The Amount of Glycocalyx Based on WGA Fluorescence Intensity on YUMM3.3 mouse melanoma cells (*indicates significance by t-test)

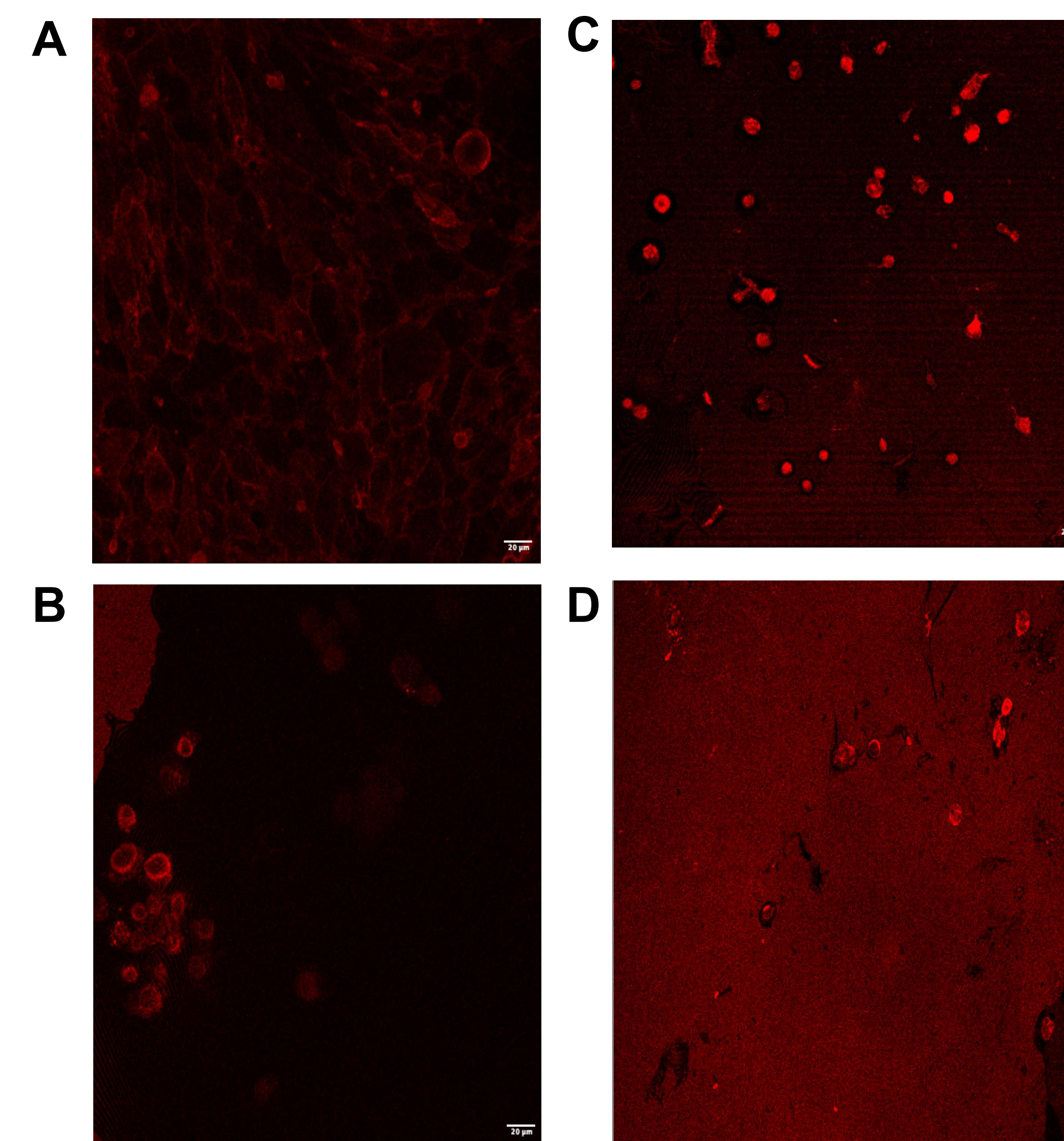


Figure 2. YUMM confocal imaging (A: control; B: hyaluronidase; C: neuraminidase; D: heparinase). Staining is for WGA.

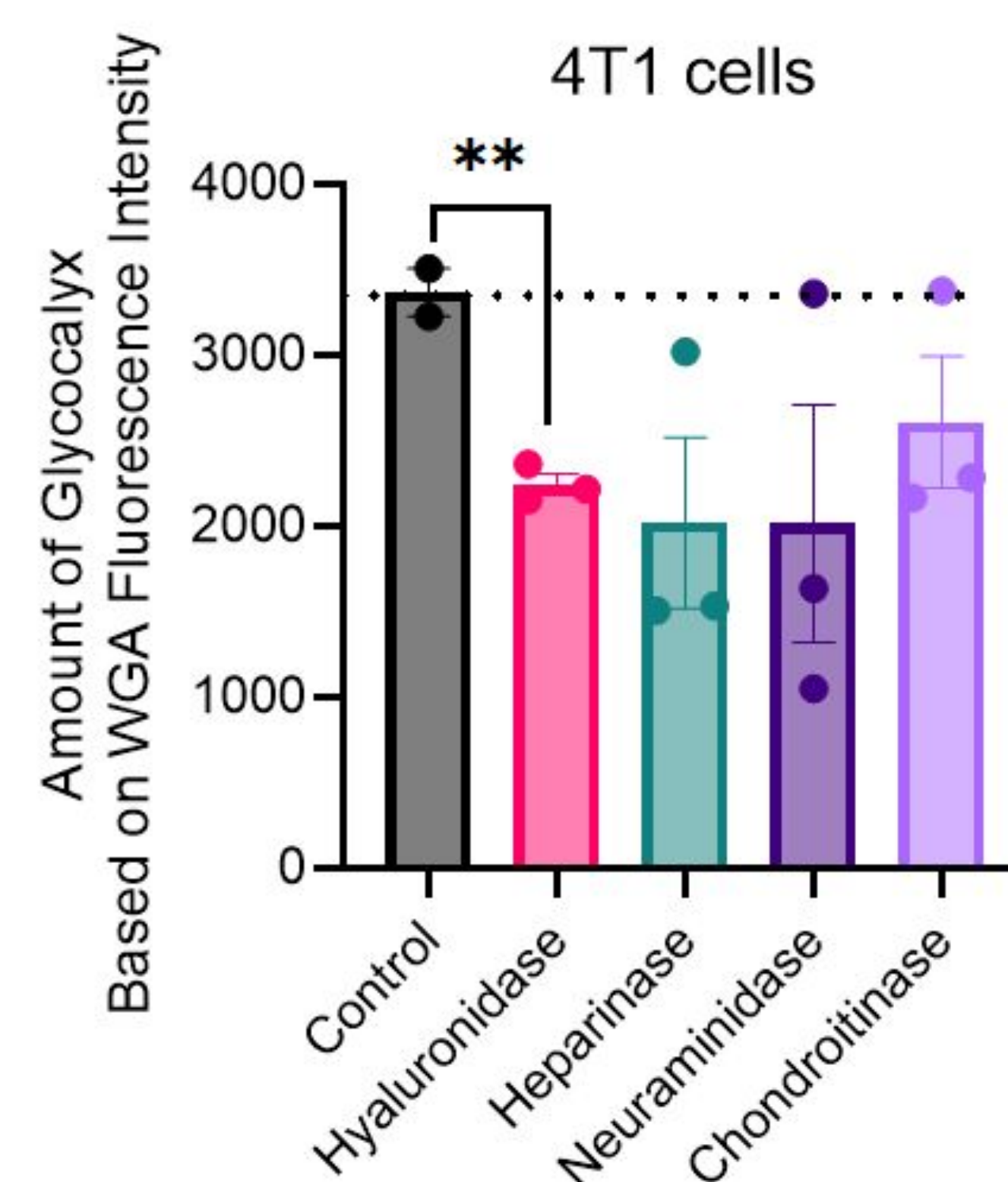


Figure 3. 4T1 enzyme treatment results. The Amount of Glycocalyx Based on WGA Fluorescence Intensity on 4T1 mouse model of stage IV breast cancer cells (*indicates significance by t-test)

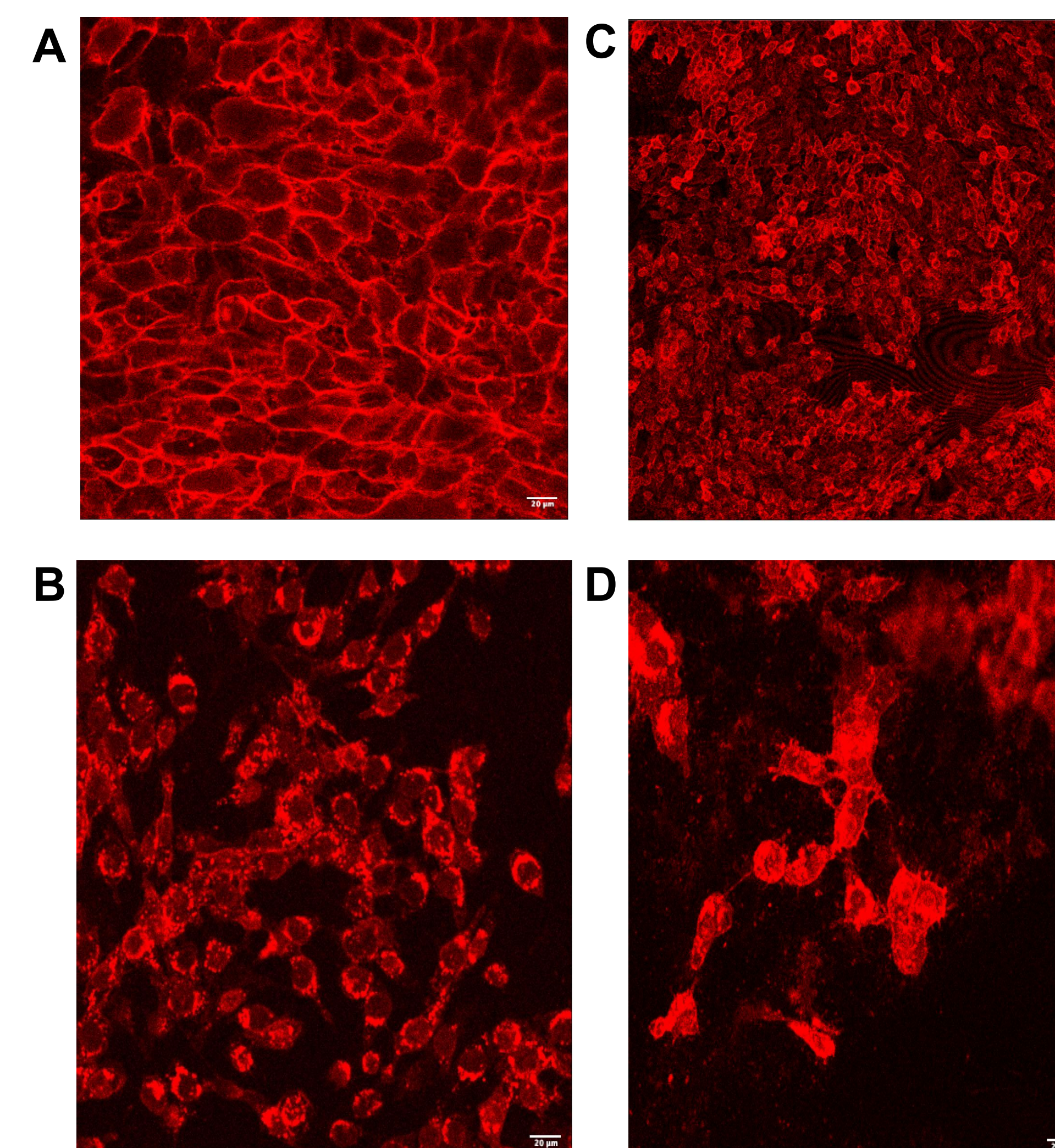


Figure 4. 4T1 confocal imaging (A: control; B: neuraminidase; C: hyaluronidase; D: heparinase). Staining is for WGA.

Conclusions

- **Hyaluronidase** and **heparinase** showing the highest overall effectiveness.
- These findings highlight the potential of targeting the glycocalyx as a strategy to enhance immunotherapy outcomes. Limitations of this study include variability across different cell lines. However, advancing our understanding of the glycocalyx and refining methods to disrupt it could pave the way for more effective cancer treatments.

Future Work

- Conduct longer-term experiments to evaluate the longevity and long-term effects of enzymatic treatments on cancer cells.
- T cell co culture experiments
- In vivo analysis of effect of enzymes on tumor growth

Bibliography

Buffone A, Weaver VM. Don't sugarcoat it: How glycocalyx composition influences cancer progression. J Cell Biol. 2020 Jan 6;219(1):e201910070. doi: 10.1083/jcb.201910070. PMID: 31874115; PMCID: PMC7039198.

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