



Examining Protein Expression as a Biomarker in Cancer-associated Fibroblasts activated by HPV Negative Cancer Cell Exosomes

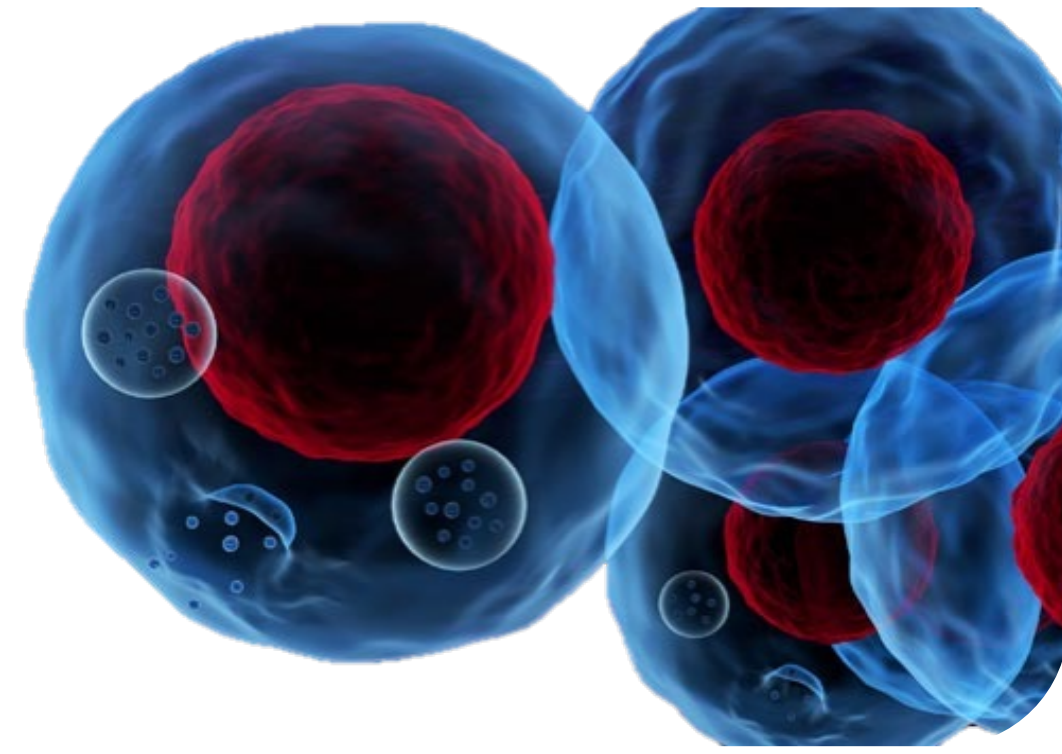
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INTRODUCTION

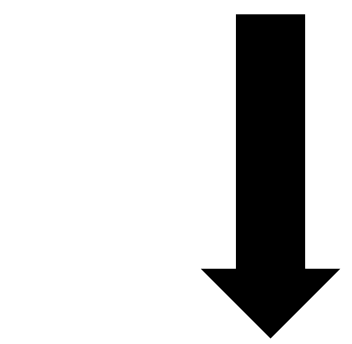
HPV Negative Cervical Cancer

- Until a decade ago, cervical cancer was the leading cause of death in American women.
- 10% of cervical cancer accounts are HPV negative, but they are under-recognized and under-reported.



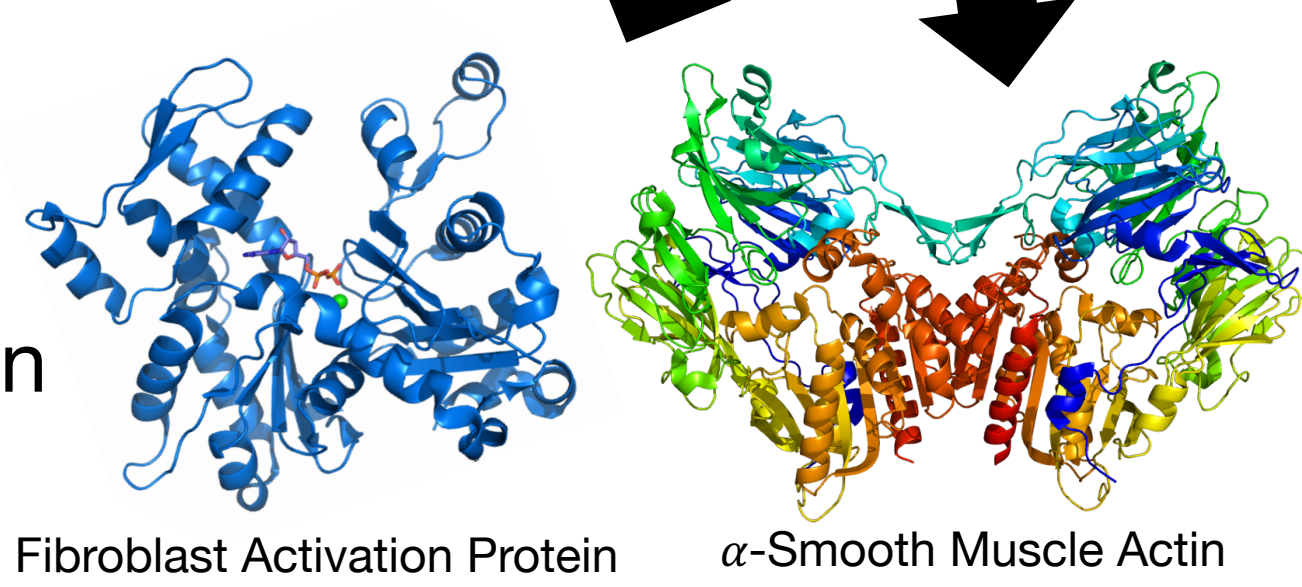
Exosomes

- Small extracellular vesicles that are crucial to intercellular communication.
- Exosomes carry DNA, RNA, and proteins to be delivered to a target cell.

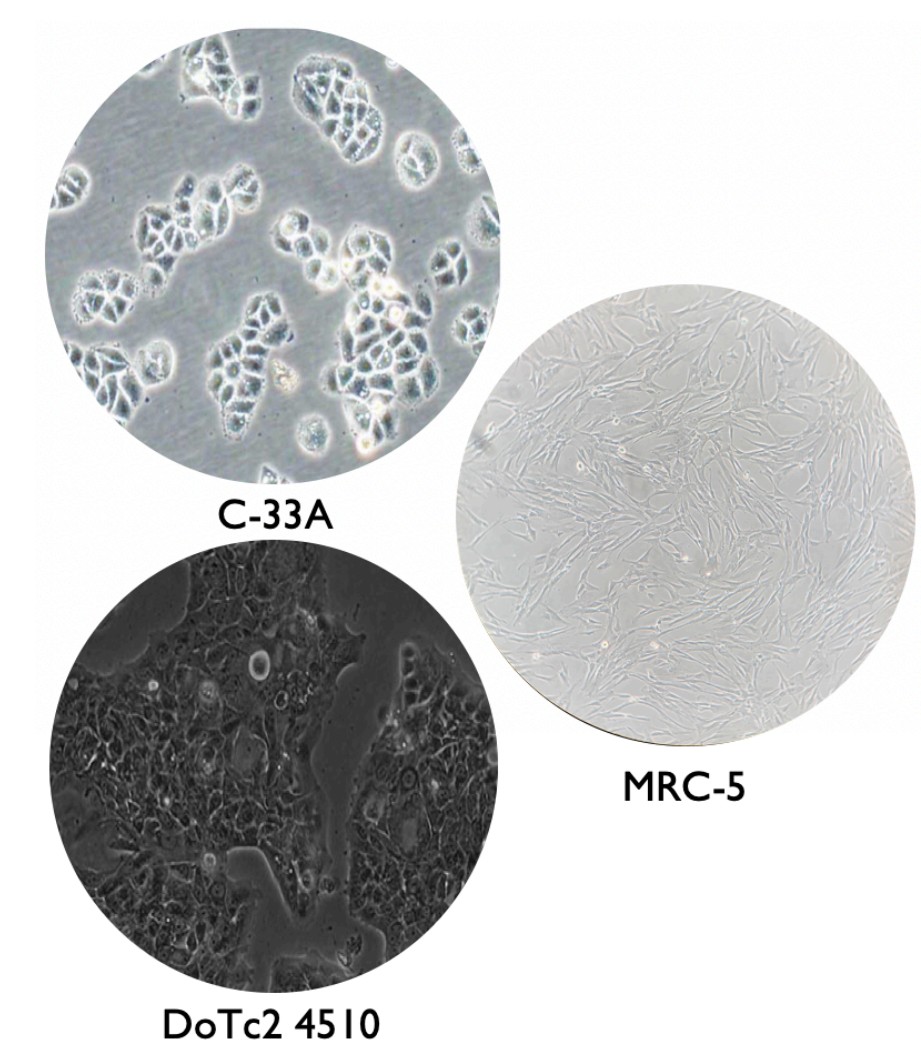


Proteins in Cancer-associated Fibroblasts

- Fibroblast Activation Protein is often studied as a marker for pro-tumorigenic stroma.
- α -Smooth Muscle Actin plays an important role in fibrogenesis.



METHODS

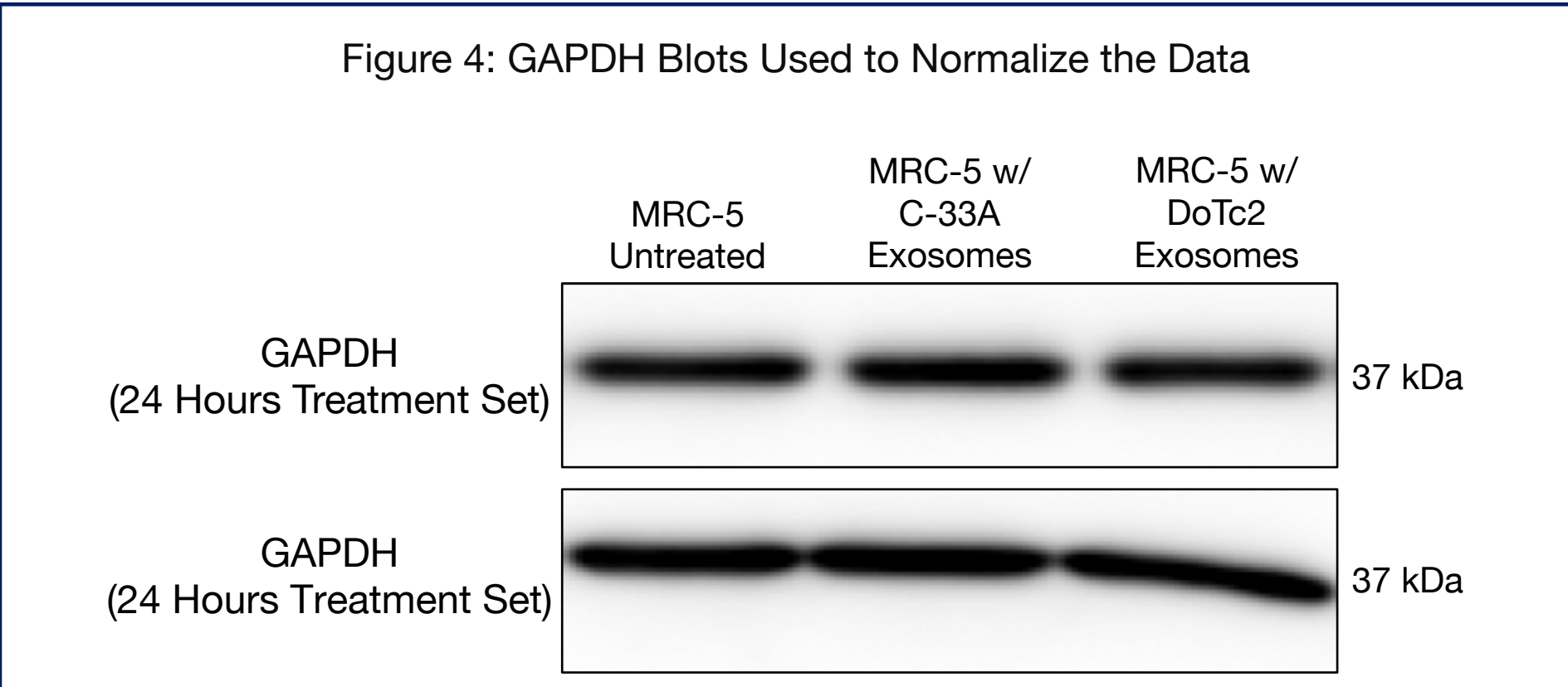
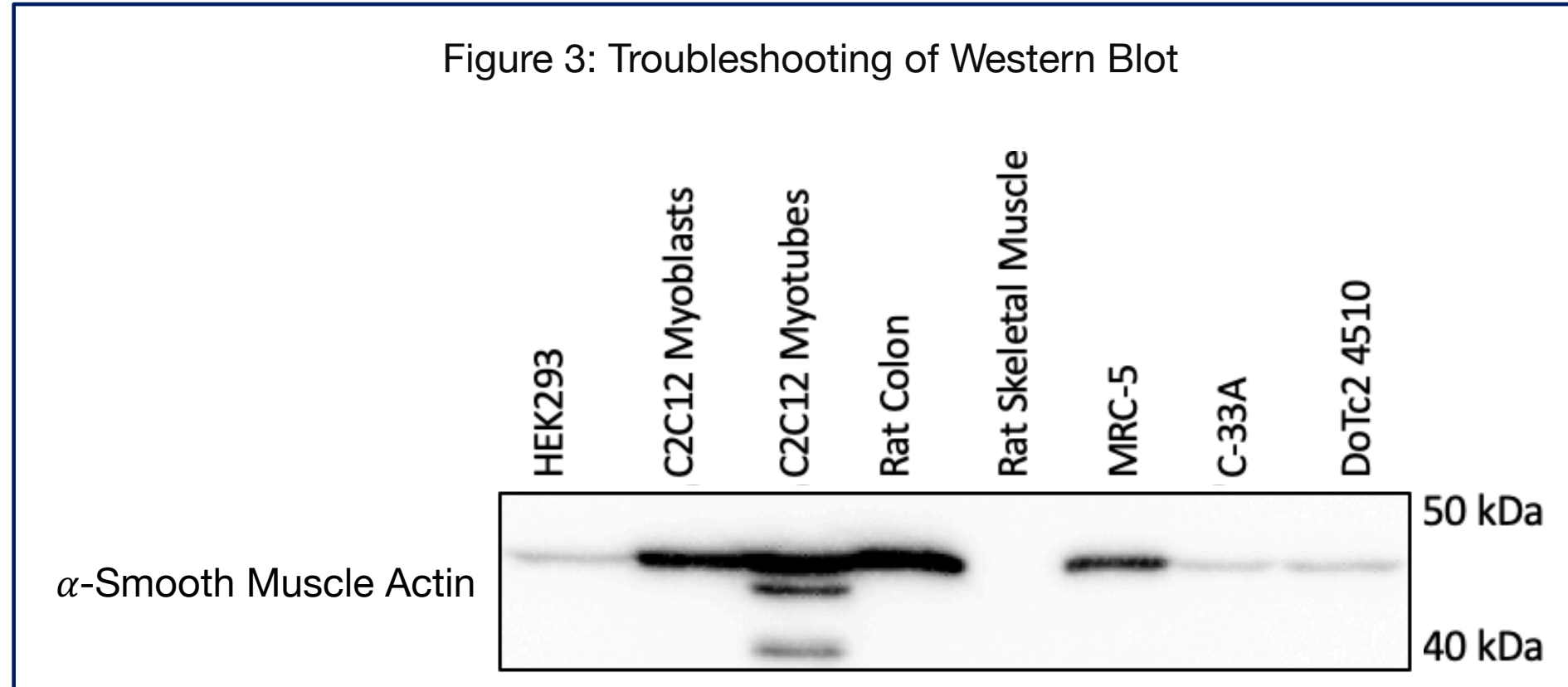
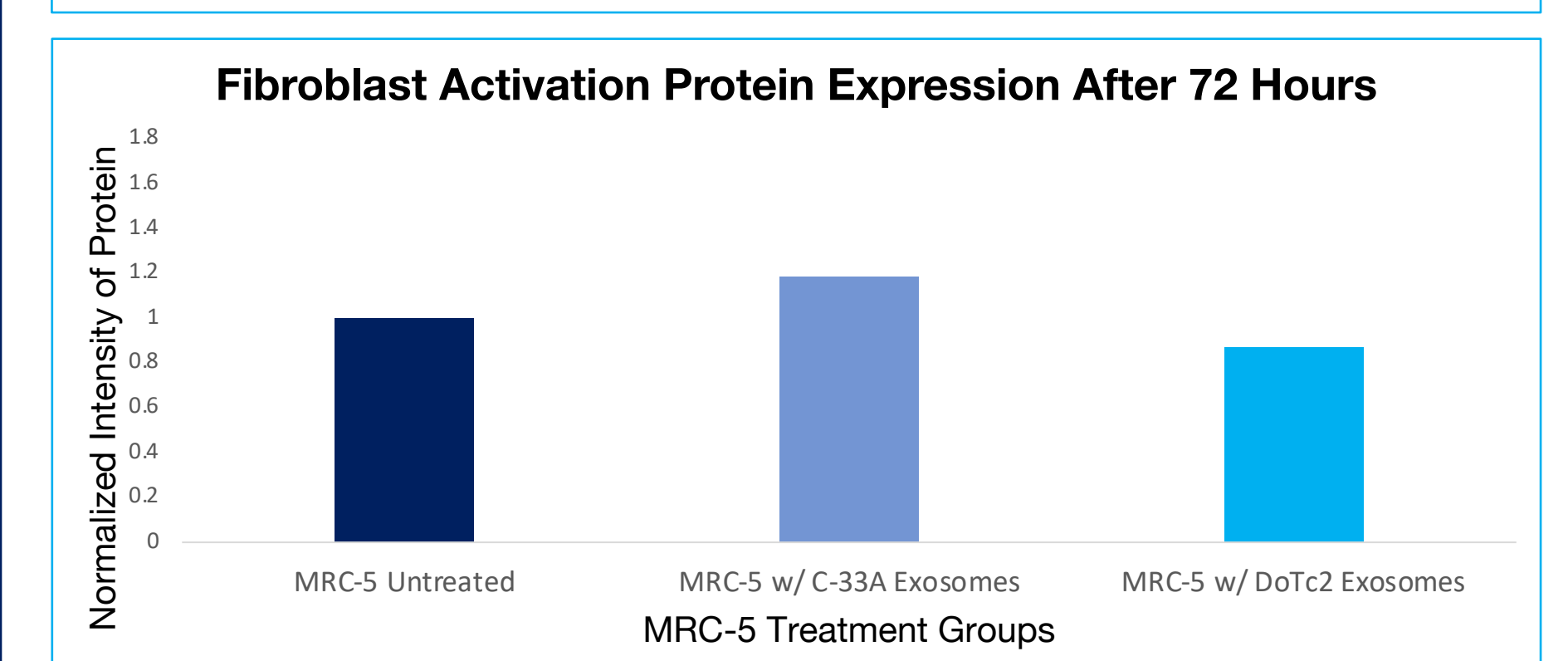
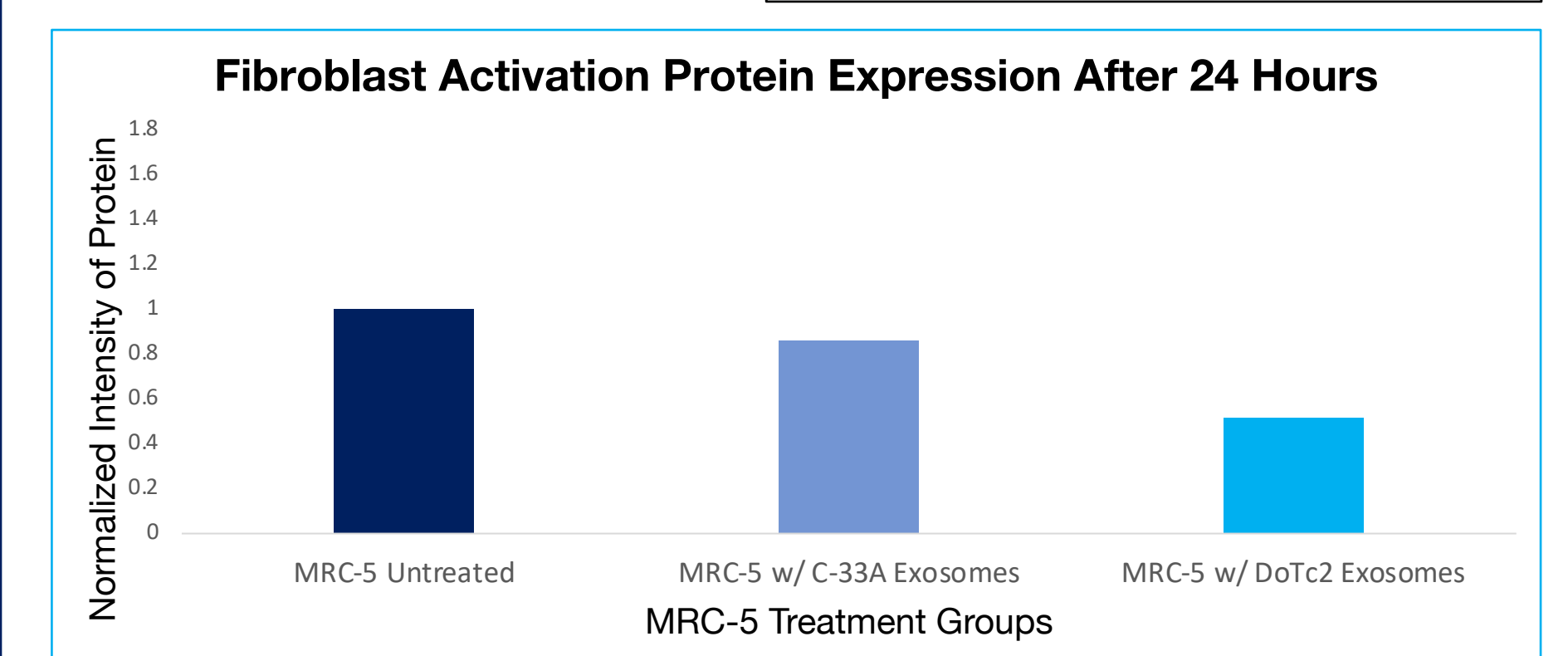
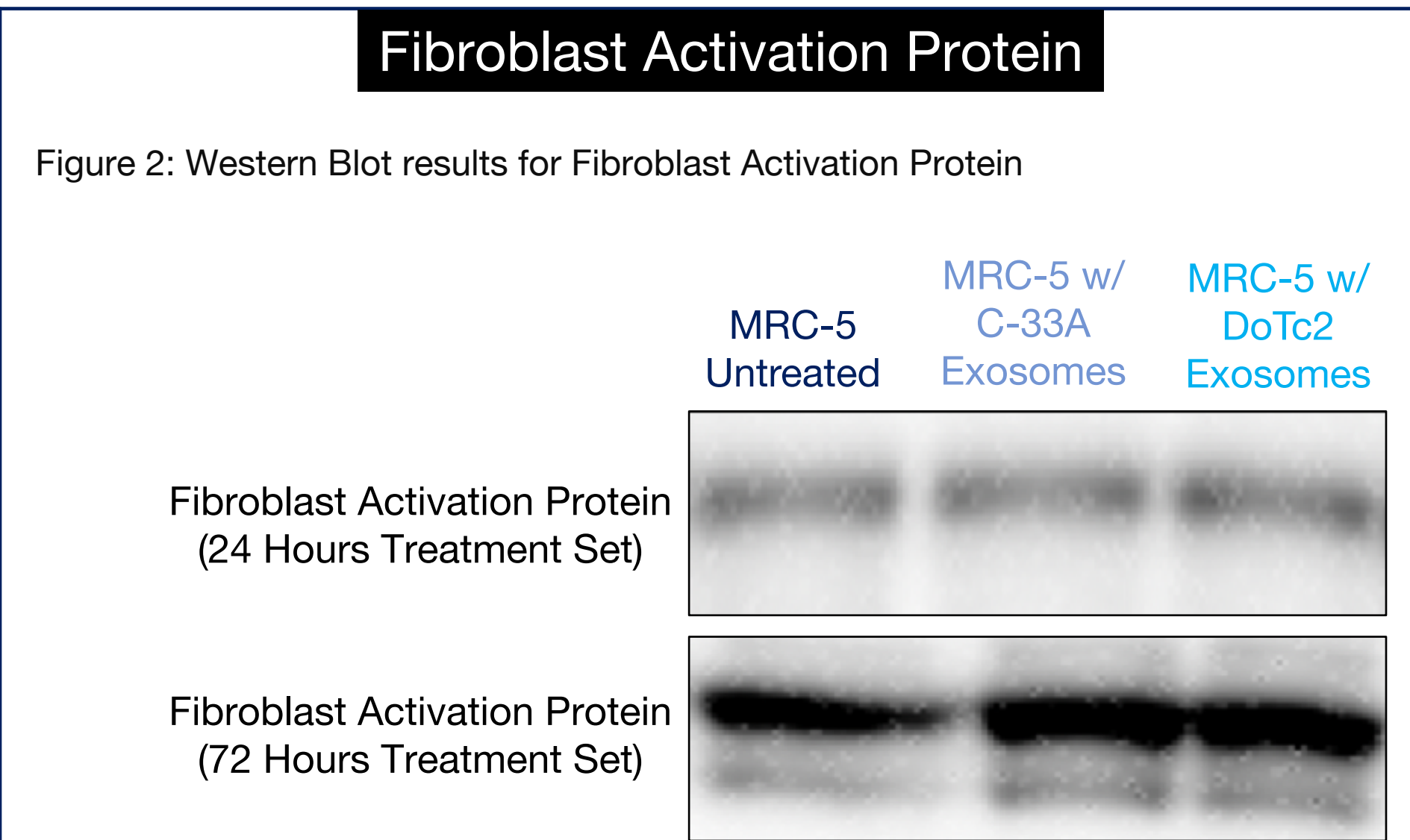
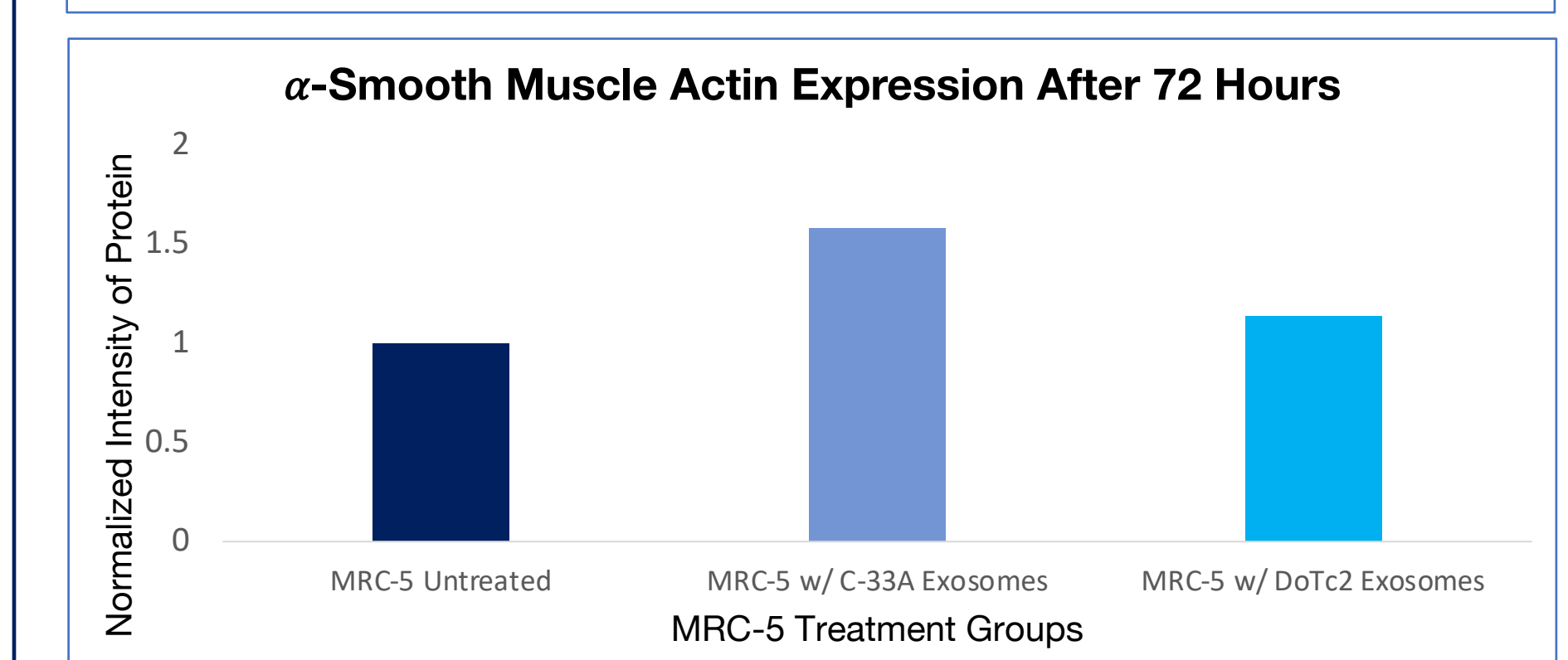
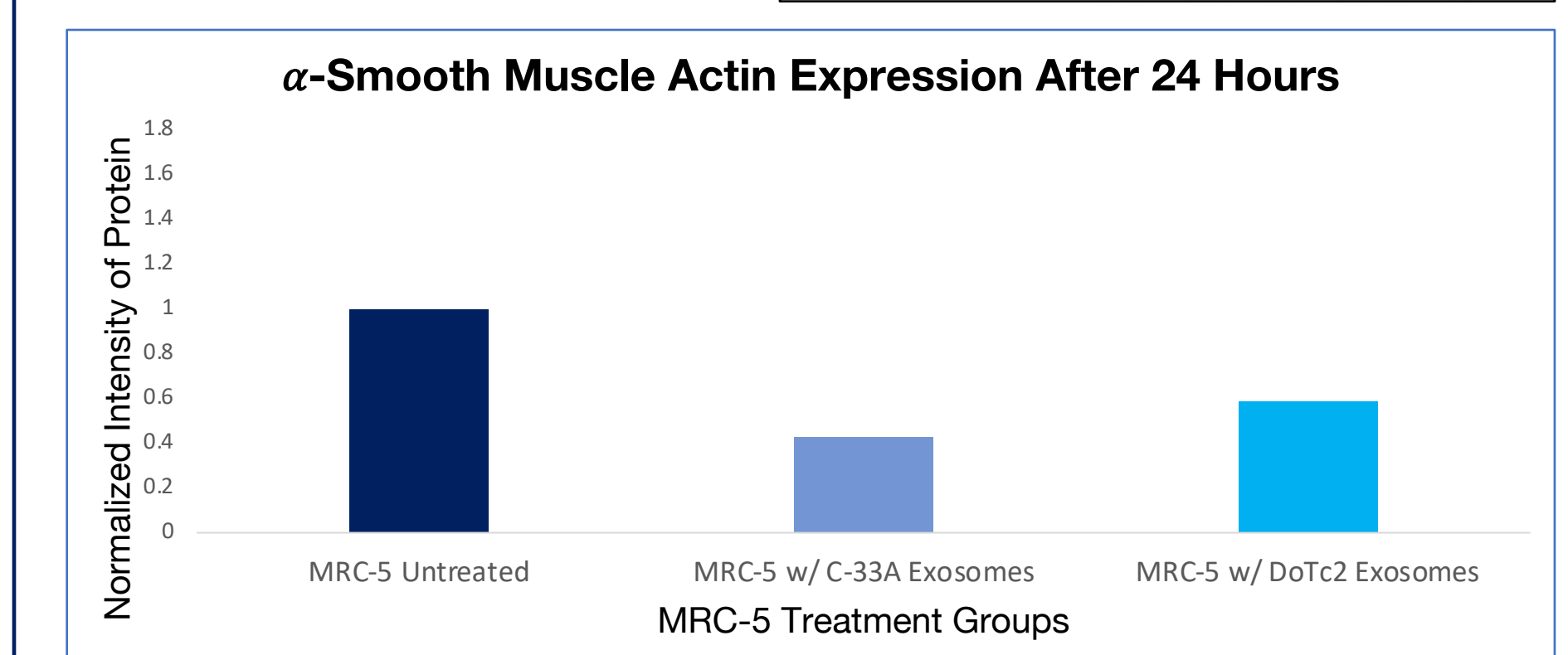
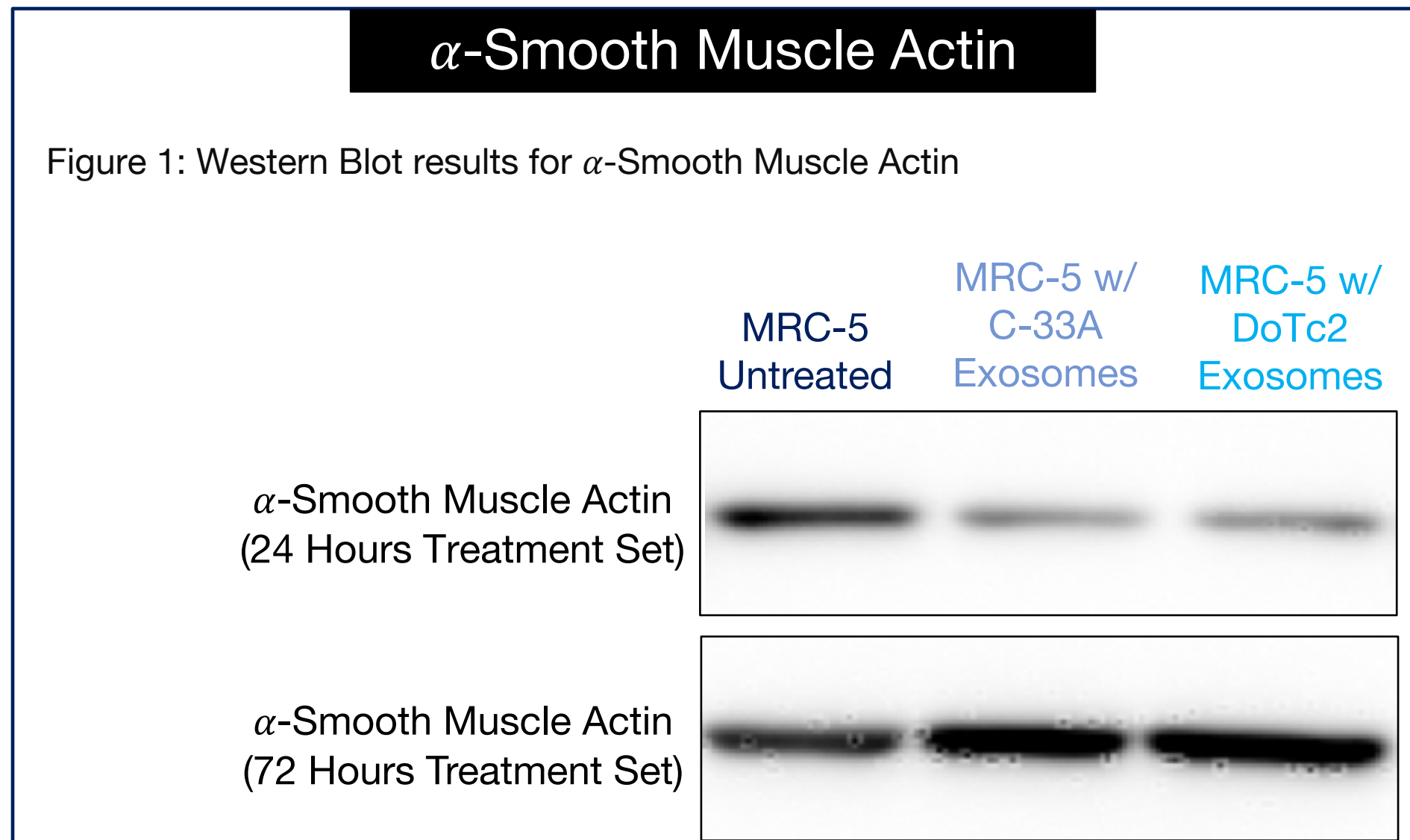


1. Isolated the C-33A and DoTc2 4510 exosomes by a series of centrifugations.
2. Treated the MRC-5 cells with the HPV negative cervical cancer cell exosomes for a 72 hour period with spiking every 24 hours. When the cell culture was spiked, the existing media was aspirated and fresh media and exosomes were added.
3. Purified the proteins in the samples by cell lysate preparation. The samples were C-33A, DoTc2 4510, MRC-5, HEK293, the 24 hour MRC-5 test set, and the 72 hour MRC-5 test set. The MRC-5 test sets included MRC-5 Untreated, MRC-5 treated with C-33A exosomes, and MRC-5 treated with DoTc2 4510 exosomes.

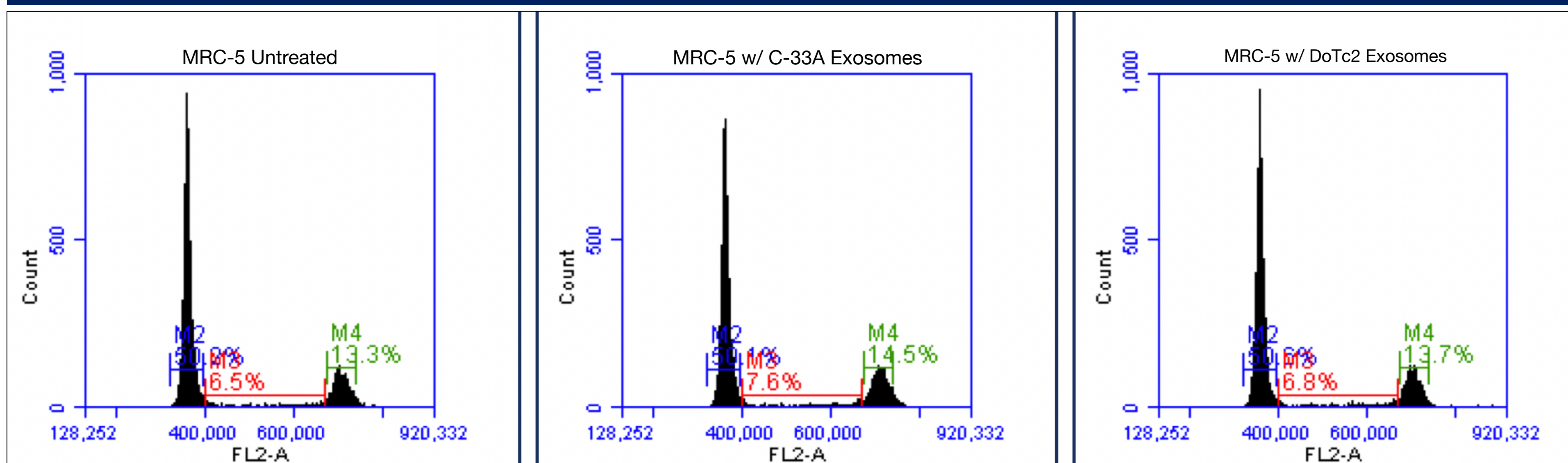
4. Western Blotting was conducted for the cell lysate sample for α -SMA, FAP, and GAPDH.

5. Flow Cytometry was conducted for the 72 hour MRC-5 test set.

WESTERN BLOT OUTCOMES



FLOW CYTOMETRY OUTCOMES



	G1	S	G2/M
MRC-5 Untreated	50.2%	6.5%	13.3%
MRC-5 w/ C-33A Exosomes	50.1%	7.6%	14.5%
MRC-5 w/ DoTc2 Exosomes	50.6%	6.8%	13.7%

RESULTS

- The Western Blots produced distinct protein bands for qualitative analysis.
- Both the expression of α -Smooth Muscle Actin and Fibroblast Activation Protein in MRC-5 cells increased over a span of 72 hours.
- Cell cycle of MRC-5 cells remained normal even after the exosome treatments.

CONCLUSION

- The results suggest that the normal MRC-5 cells were activated by the exosomes of the C-33A and DoTc2 4510 cell lines, as both the levels of expression for α -SMA and FAP had increased after 72 hours of treatment. Further, that the cells under investigation were growing healthily, as the cell cycle progression was normal.
- Therefore, these results reveal that these two proteins can be studied to provide insight on the microenvironment of HPV negative cervical cancer and how HPV negative cells become malignant.

FUTURE WORK

- Conduct a proliferation assay.
- Investigate the Fibroblast Specific Protein.
- Obtain more data to calculate statistical significance.

REFERENCES

Boelens Mirjam C, Wu Tony J, Nabet Barzin Y, Xu B, Qiu Y, Yoon T et al. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. *Cell* 159: 499–513.

"CDC - What Do My Cervical Cancer Screening Test Results Mean?" *Centers for Disease Control and Prevention, Centers for Disease Control and Prevention*, www.cdc.gov/cancer/cervical/basic_info/test-results.htm.

"Cervical Cancer." *American Cancer Society*, www.cancer.org/cancer/cervical-cancer.html.

Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. *J Extracell Vesicles* 2014; 3: 10.3402/jev.v3.23743

"Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic Revision of Rochefortia Sw. (Ehretiaceae, Boraginales). *Biodiversity Data Journal* 4: e7720. <https://doi.org/10.3897/BDJ.4.e7720>." doi:10.3897/BDJ.4.e7720.figure2f.

Learn: *Method Western Blot - The Human Protein Atlas*, www.proteinatlas.org/learn/method/western-blot.

"Negative HPV Screening Test Predicts Low Cervical Cancer Risk Better than Negative Pap Test." *National Cancer Institute*, www.cancer.gov/news-events/press-releases/2014/HPVscreeningPredictor.

Räsänen K, Vaheri a. Activation of fibroblasts in cancer stroma. *Exp Cell Res*. 2101; 316 (17): 2713-2722. Doi: 10.1016/j.yexcr.2010.04.032

Robertson, Sally. "What Is Flow Cytometry?" *News-Medical.net*, News Medical, 23 Aug. 2018, www.news-medical.net/life-sciences/What-is-Flow-Cytometry.aspx.

"What Do We Know about HPV-Negative Cervical Cancer?" *The Medical Republic*. *The Medical Republic*, 21 Feb. 2017, medicalrepublic.com.au/know-hpv-negative-cervical-cancer/5616.

Zhou, Y. et al. Hepatocellular carcinoma-derived exosomal miRNA-21 contributes to tumor progression by converting hepatocyte stellate cells to cancer-associated fibroblasts. *Journal of Experimental & Clinical Cancer Research* 37, (2018).

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