

# The effects of arylamine N-acetyltransferase 1 on tumor immune response

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## Introduction

Arylamine N-acetyltransferase 1 (NAT1) is an enzyme that is often upregulated in subtypes of breast cancer. Using two guide RNAs (numbered 2 and 5) via CRISPR-Cas9, we had knocked out NAT1 in MDA-MB-231 cells. Our lab's previous proteomics studies have shown that the NAT1 knockouts have increased Major Histocompatibility Complex I (MHC I) and associated proteins production<sup>1</sup>. MHC I presents peptides from within the cell to cytotoxic T cells and inhibits Natural Killer cell response. Thus, we expected a difference in the immune response for NAT1 knockout cells and the wild types. This project aims to show how NAT1's effect on MHC I affects the immune response to breast cancers. This may open new avenues for treatment in the future as we manipulate how both sides of the immune system respond to cancer.

## Methods

We used three breast cancer cell lines: MDA-MB-231 (an ER-, PR-, HER2- or "Triple Negative" line), MCF-7 (an ER+, PR+, HER2- line), and ZR-75-1 (an ER+, PR+, HER2+ line). By having three lines with different receptors, we insured that our results were not due to a specific receptor if they were the same and that we would have other potential variables to investigate further if they reacted differently. To remove the effect of NAT1 in these cell lines, we knocked it out using CRISPR-Cas9. Two different guide RNAs (#2 and #5) were used in each line to isolate any NAT1 unrelated effects and confirm which effects were NAT1 related. Unfortunately, the ZR-75-1 KO5 line failed to grow.

To test the effect of the adaptive immune system, we used an immortal cytotoxic T cell line called Jurkat. To test the effect of natural killer cells, we used an immortal natural killer cell line called NK-92. These cells were grown on top of the cancer cells for 24 hours before they were washed away and the viability of remaining cancer cells was measured with alamar Blue. To avoid any remaining immune cells adding to viability readings, they were dyed with Trypan blue prior to being added to the cancer cells. Then the ratio of glowing to non-glowing cells would show what amount of viability was from the surviving cancer cells. Unfortunately, the glowing immune cells were not detectable on microscope after co-culture.

## Results

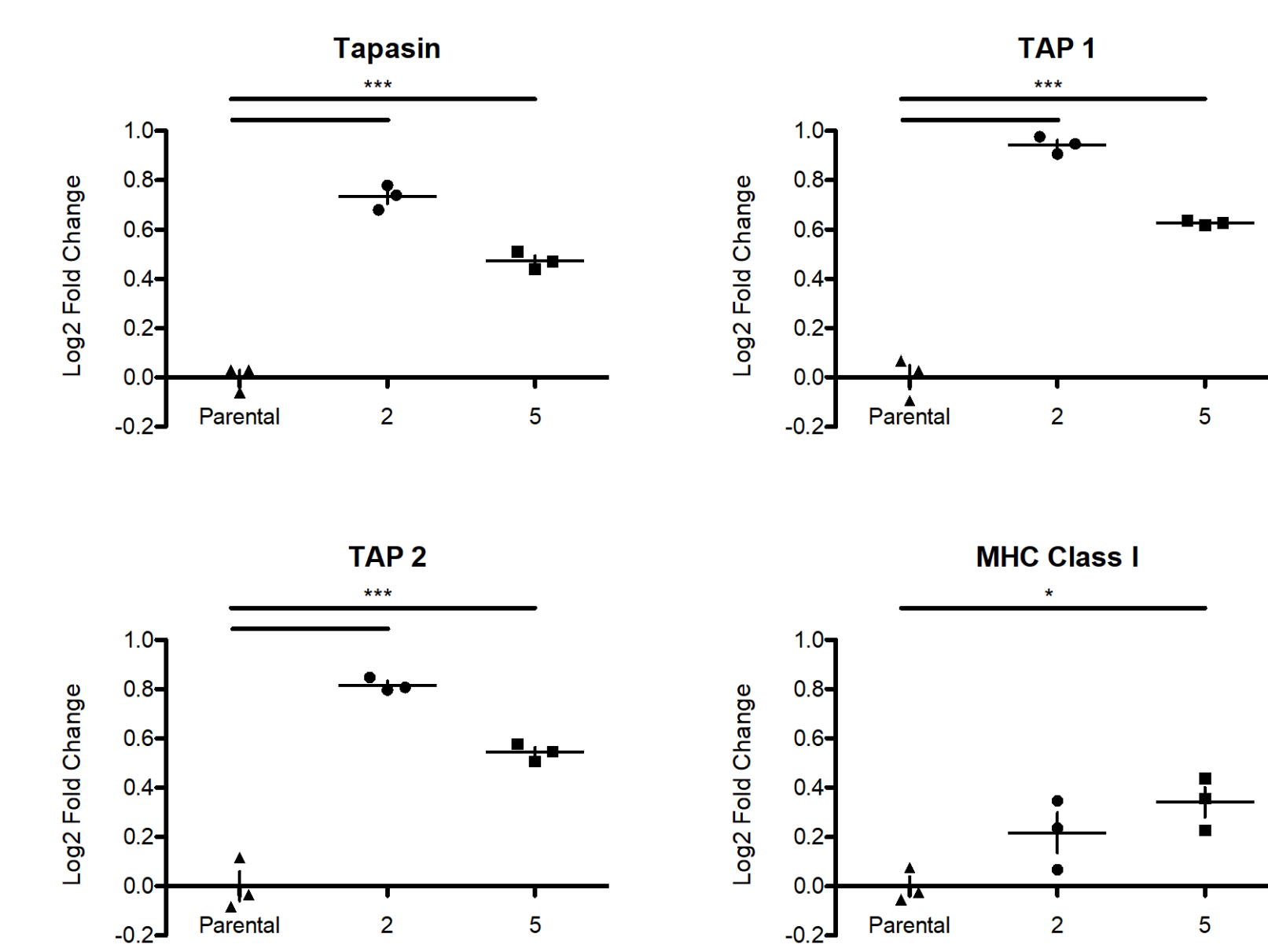


Figure 2: Proteomics data showing the difference in expression of MHC I and related proteins for wild-type and NAT1 KO MDA-MB-231 cell lines. The relationships seen here imply that NAT1 may inhibit expression of these proteins.

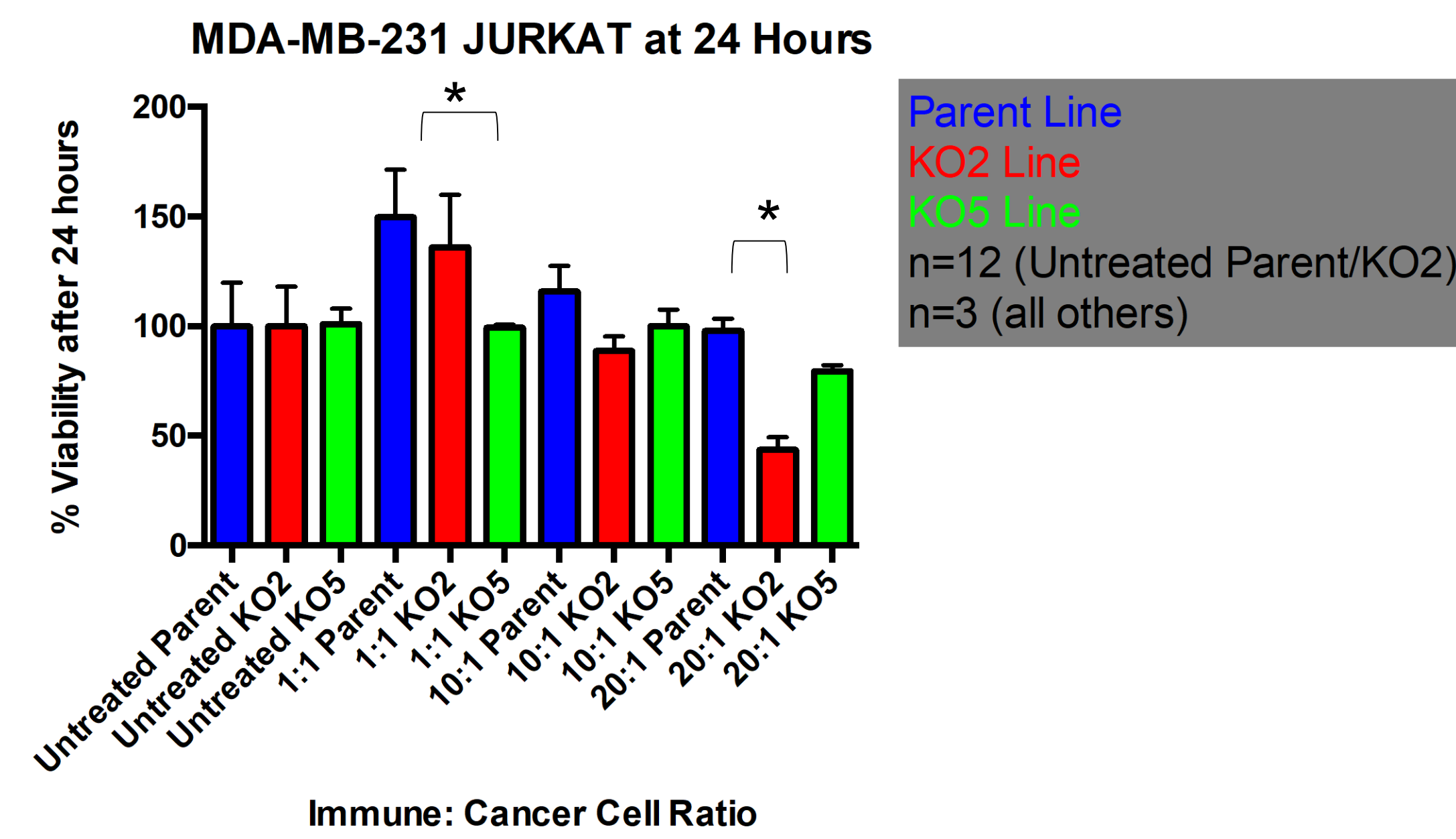


Figure 3: The relative viability of MDA-MB-231 breast cancer cells after 24 hours of Jurkat T cell treatment. The 1:1 KO5 and the 20:1 KO2 were both significantly less viable than their parent equivalents at  $p < 0.05$ .

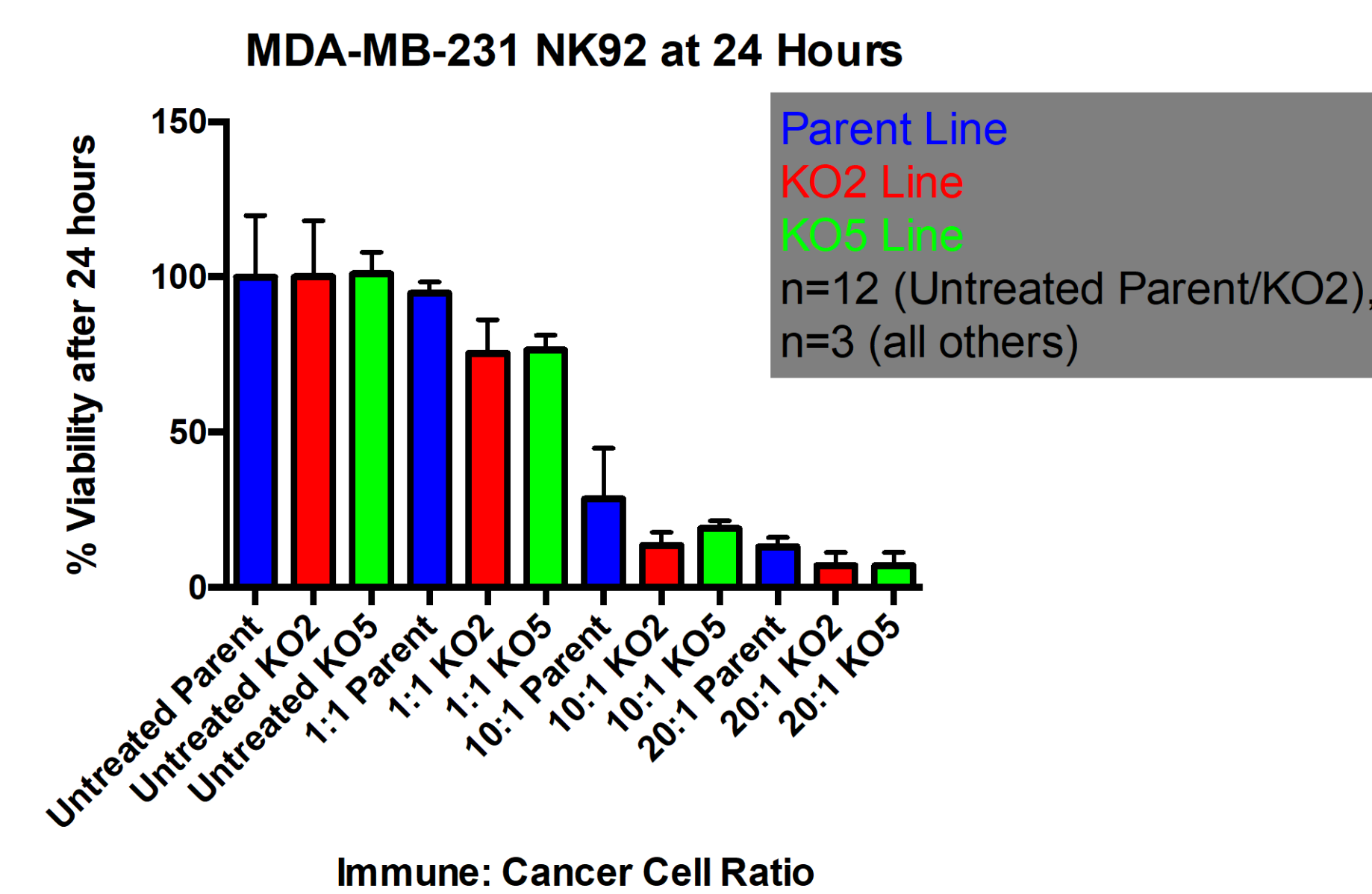


Figure 4: The relative viability of MDA-MB-231 breast cancer cells after 24 hours of NK-92 natural killer cell treatment. No KO lines had significantly different responses than their parent.

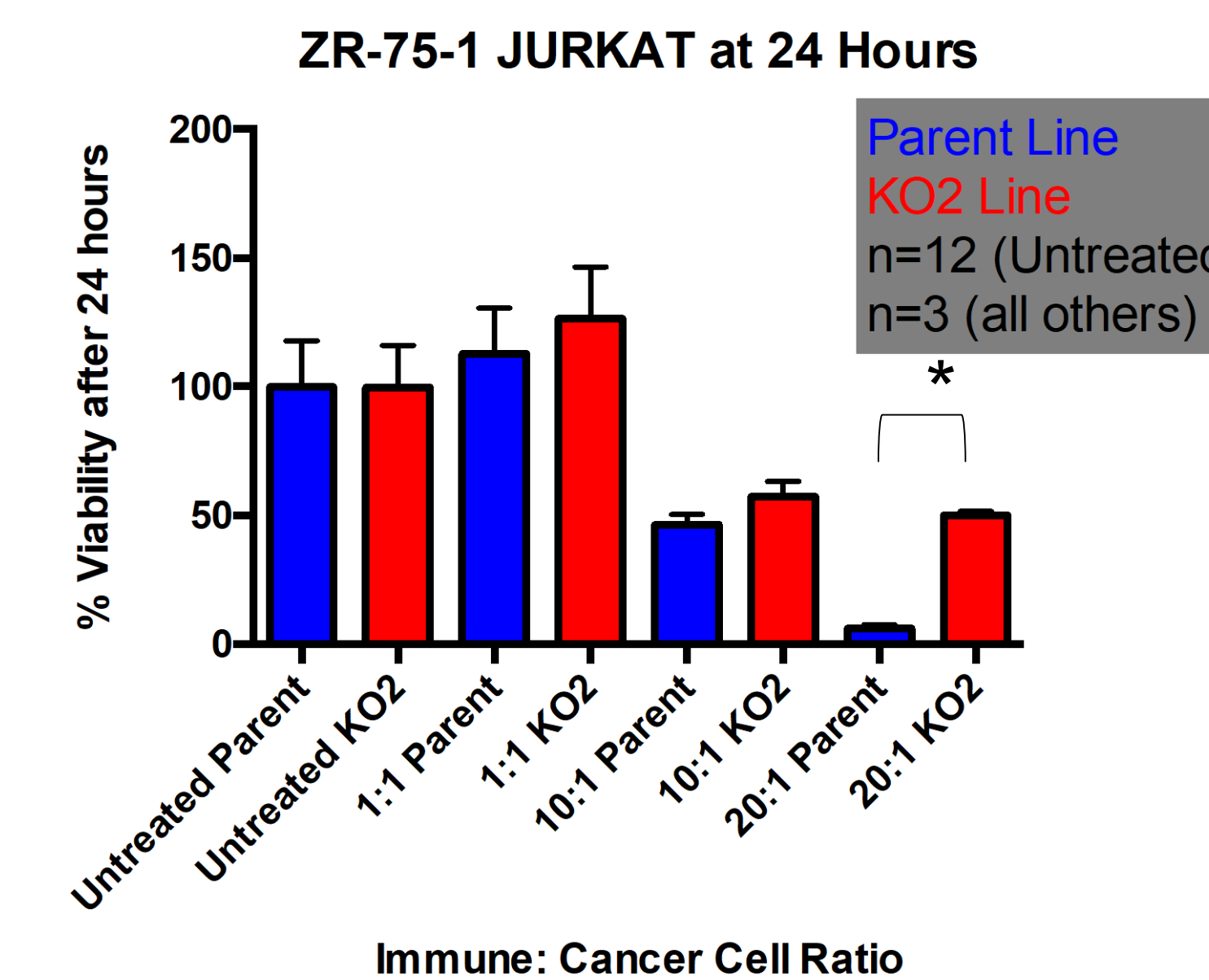


Figure 5: The relative viability of ZR-75-1 breast cancer cells after 24 hours of Jurkat T cell treatment. The 20:1 ratio showed significantly more viability when NAT1 is knocked out with  $p < 0.05$ .

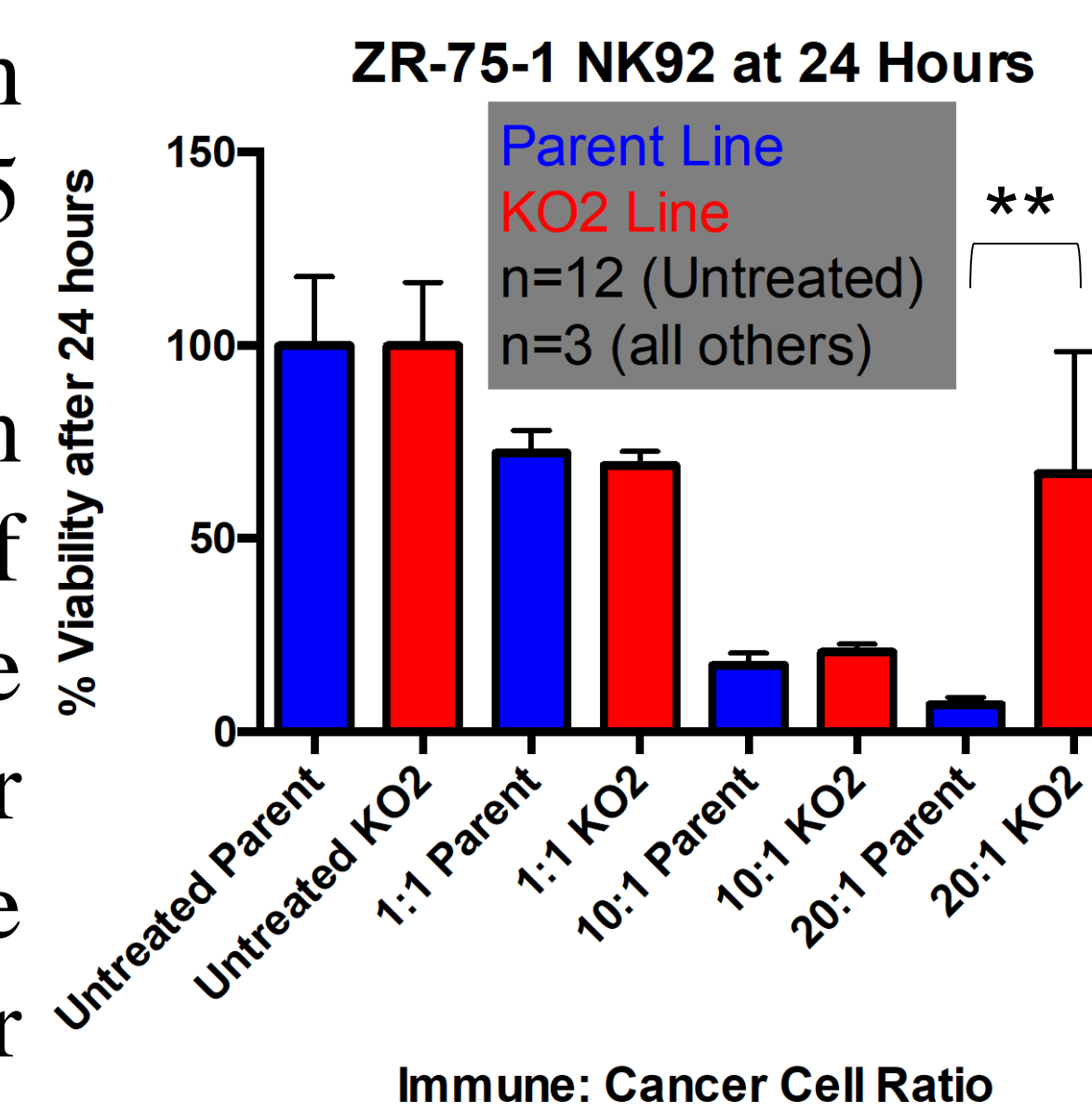


Figure 6: The relative viability of ZR-75-1 breast cancer cells after 24 hours of NK-92 treatment. The 20:1 ratio showed significantly more viability when NAT1 is knocked out with  $p < 0.01$ .

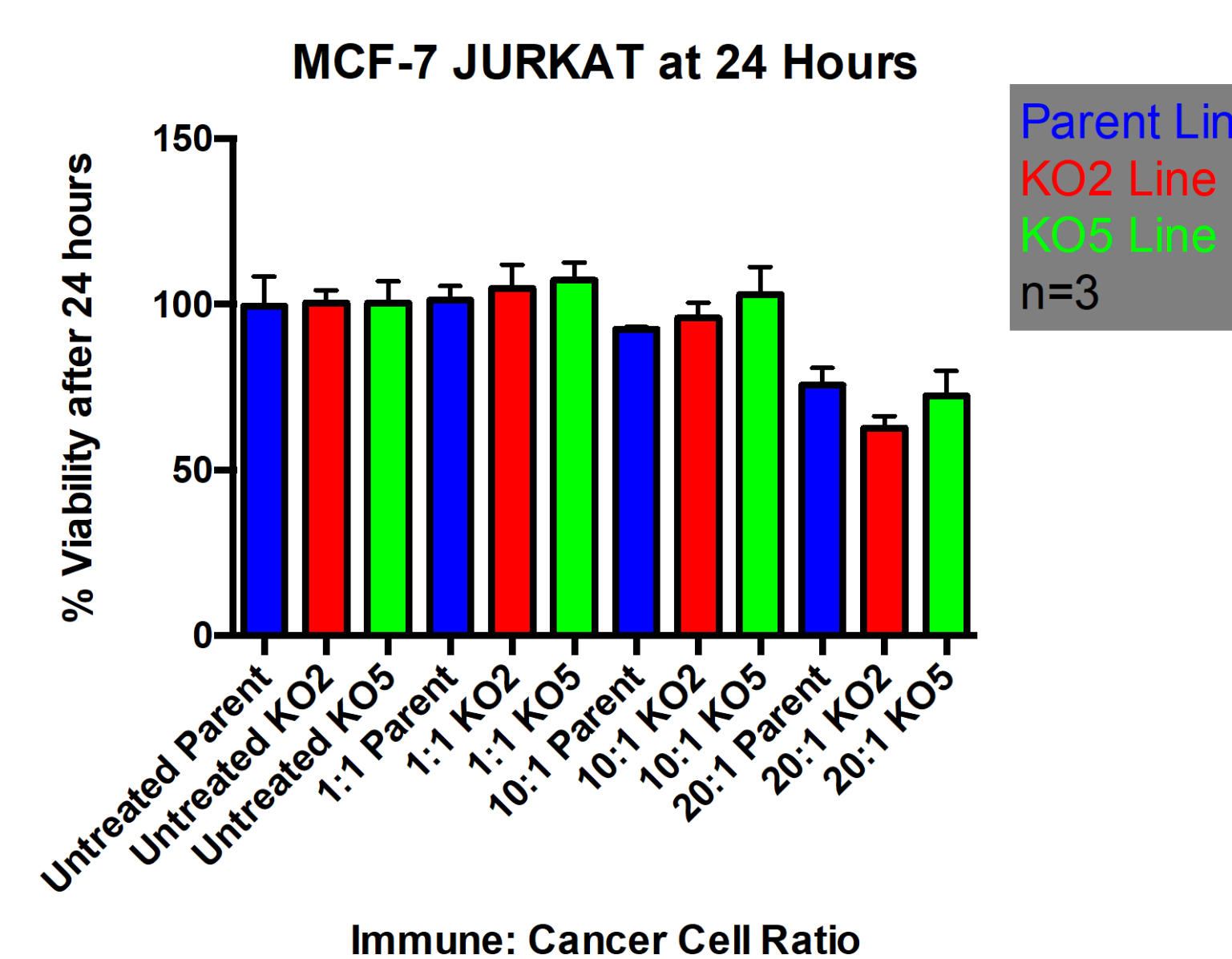


Figure 7: The relative viability of MCF-7 breast cancer cells after 24 hours of Jurkat T cell treatment. No KO lines had significantly different responses than their parent.

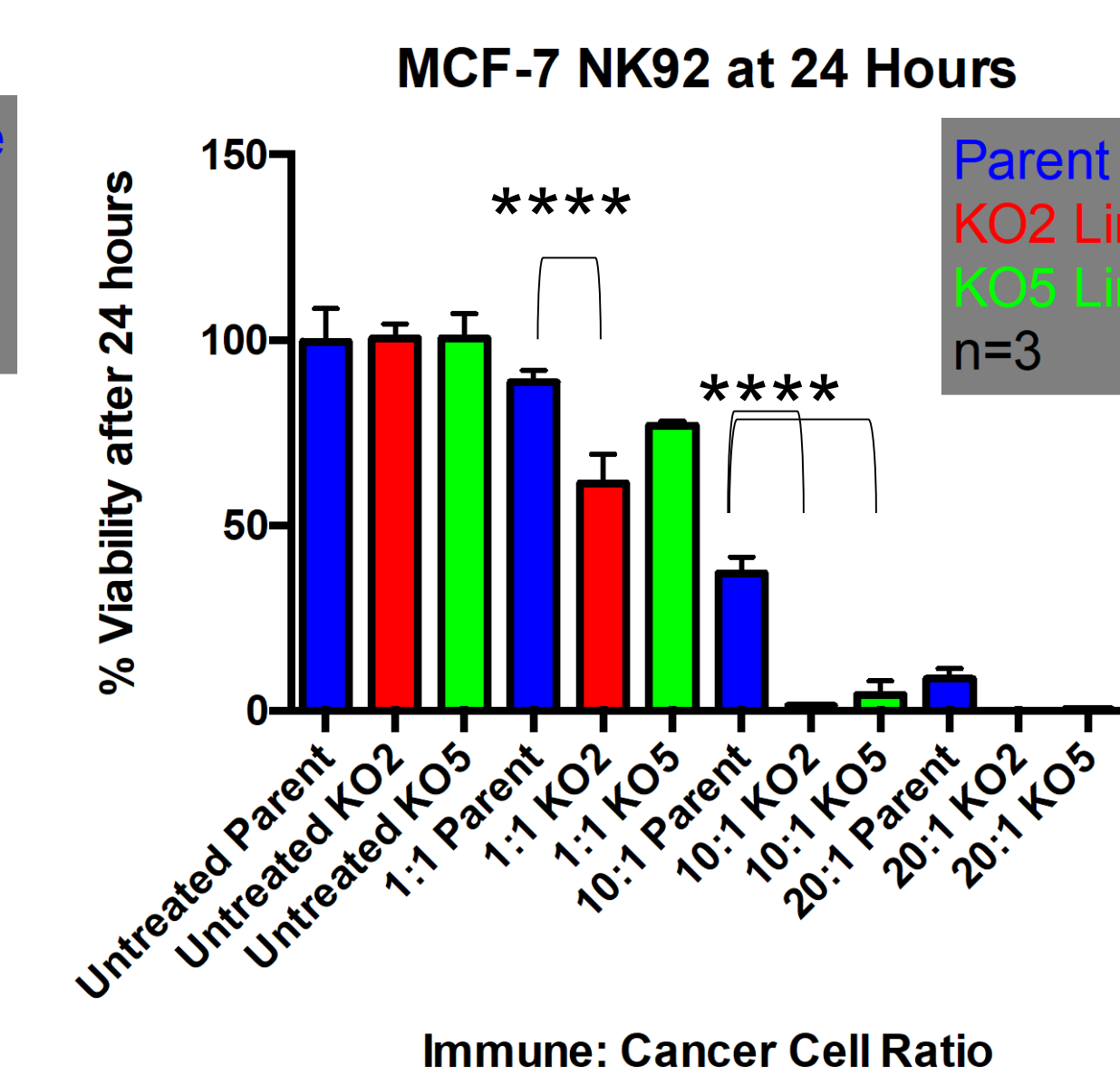


Figure 8: The relative viability of MCF-7 breast cancer cells after 24 hours of NK-92 natural killer cell treatment. The 1:1 Parent and KO2 lines were significantly different at  $p < 0.0001$ . Both 10:1 KO lines were significantly different from the parent at  $p < 0.0001$ .

## Immune Schema

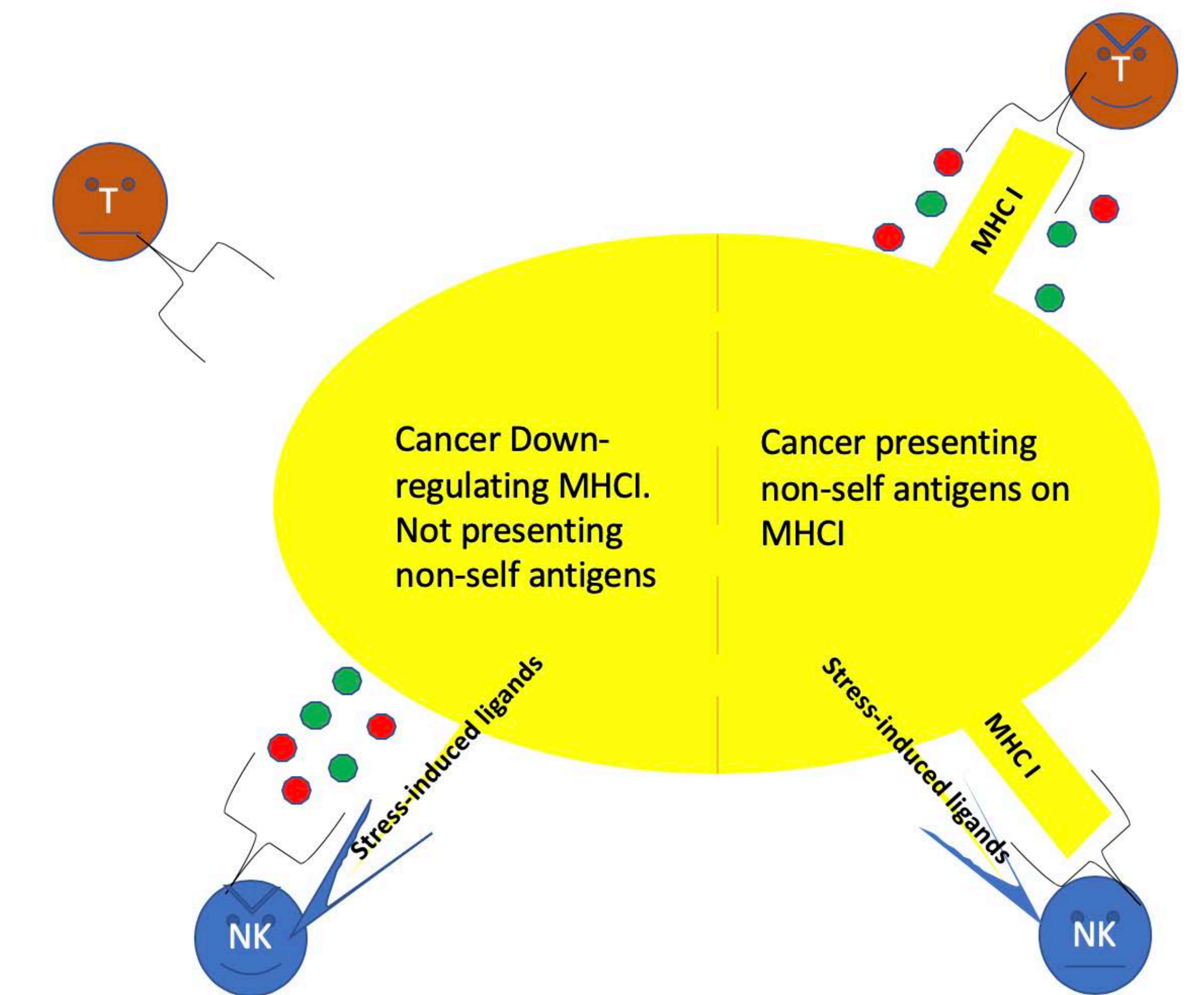


Figure 1: The effect MHC I has on immune cells. The active cells release perforin and other chemicals to kill the cancer cell.

## Conclusions

NAT1 knockout exhibited a clear effect on the immune response in the MDA-MB-231, MCF-7, and ZR-75-1 breast cancer cell lines. NAT1 knockout improved the Jurkat response to MDA-MB-231 at  $\alpha = 0.05$ . It also decreased both the Jurkat and NK-92 response to ZR-75-1 at  $\alpha = 0.05$  and  $\alpha = 0.01$  respectively. NAT1 knockout improved the NK-92 response at  $\alpha = 0.0001$ . While the data for MCF-7 Jurkat response and MDA-MB-231 NK-92 response hints at correlations, the correlation was not statistically significant due to our small sample size ( $n = 3$ ). Going forward, we plan to further investigate why the T cell and natural killer cell response vary between these cell lines. Once the source(s) of the differences are understood, they may provide evidence that the regulation of NAT1 is an important aspect of immunotherapy in cancer patients.

## References

1. Gardner, J., Stepp, M.W., Wilkey, D., Merchant, M. and Hein, D.W. Identification of the endogenous role of arylamine N-acetyltransferase 1 in cancer related cellular processes through proteomic analysis. Proceedings of Research!Louisville, Abstract MED-DT-9, Louisville, Kentucky, September, 2017.

## Acknowledgements

Research supported by a grant from NCI R25- CA134283 and the School of Medicine Summer Research Scholar Program