# CRISPR/Cas9-Mediated Genome Editing of Y705 Residue of STAT3 for Genetic Validation of Small Molecule Inhibitors In Breast Cancer Cells

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THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
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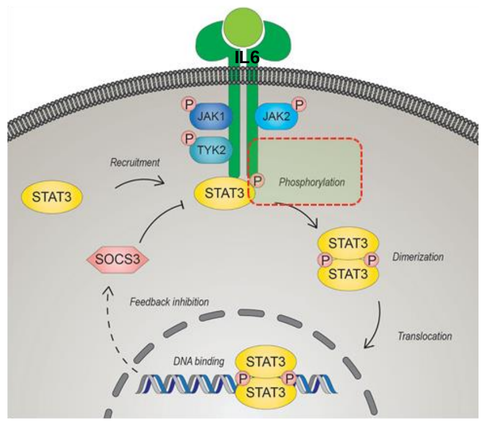
## Introduction

- Signal transducer and activator of transcription 3 (STAT3), is a transcription factor that plays a crucial role in the signal transduction pathway of various cytokines, growth factors.
- STAT3 deregulation results in serious diseases, including inflammatory diseases and cancer
- Stat3 is considered an oncogene that is constitutively active in as many as 70% of the primary human tumors.

**The classical STAT3 signaling pathway**

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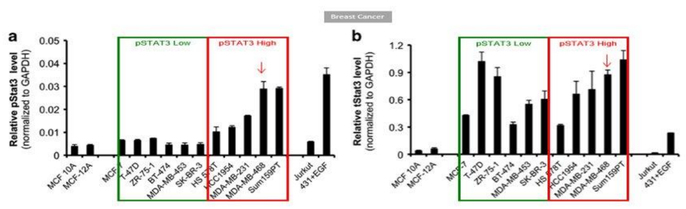
Cytokine/peptide hormone binds to the membrane-bound receptor. The receptor complex activates the phosphorylation of kinases, followed by STAT3 phosphorylation and activation.

## Objective

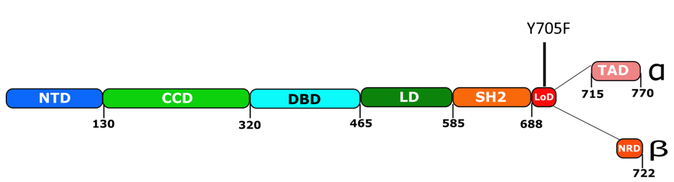
- Generate Y705F mutations in TNBC cell lines (using CRISPR/Cas-9 system)
- Test for specific inhibition of pY705 STAT3 Activity.

## pYSTAT3 In Breast Cancer Cell Lines




Luminex bead assay data showing constitutive levels of pYSTAT3 in breast cancer cell lines. The red boxes show cells with high constitutive levels of pSTAT3 from which MDA-MB-468 was selected

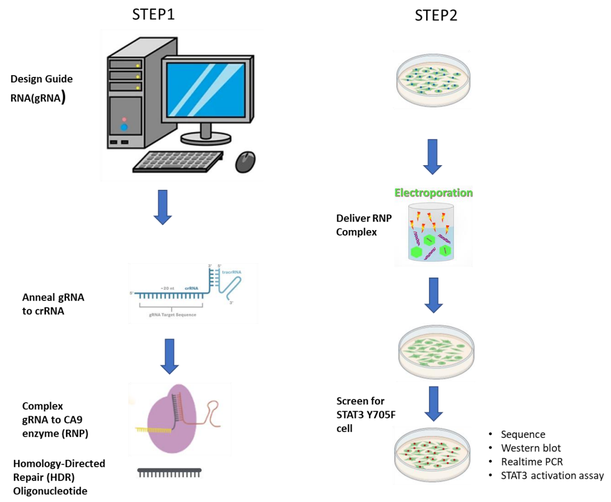
## Domain Organization of STAT3




Organization of STAT3 and Y705F amino acid edit.

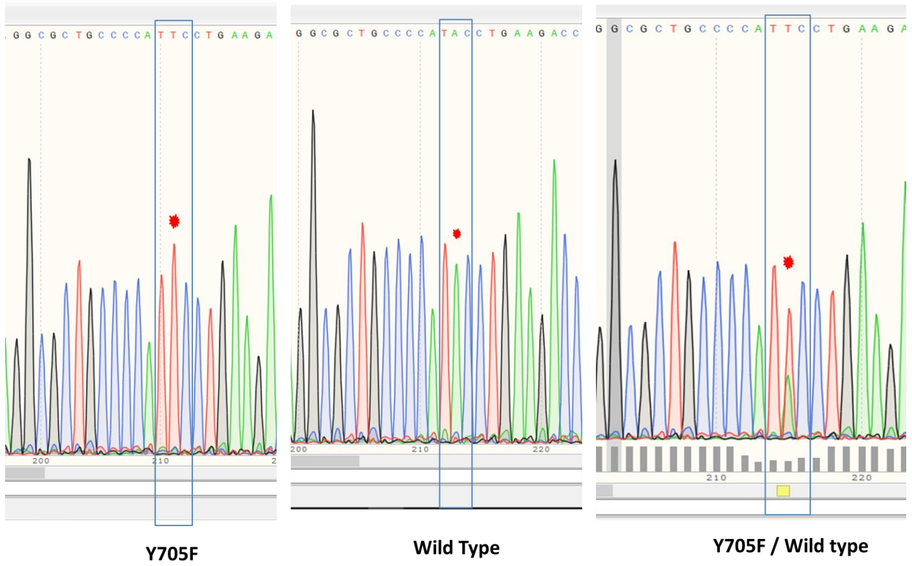
## Methods

### *CRISPR Cas-9 Genome Editing*

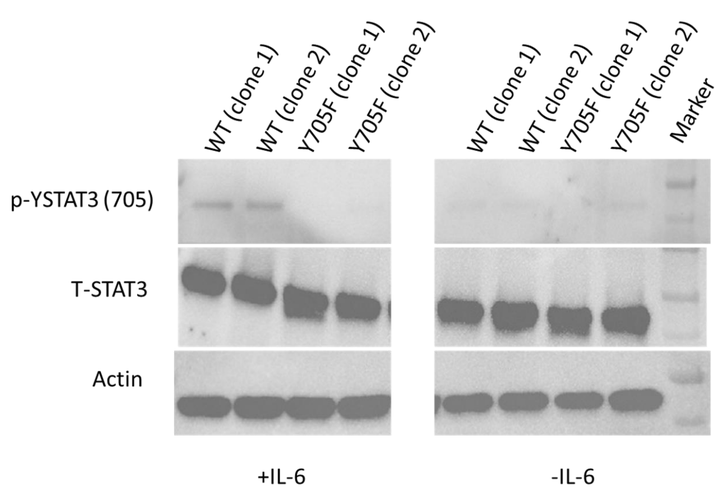



Schematic showing the generation of Y705F mutations in breast cancer cells (MDA MB-468) using cas9 mediated homology-directed repair with DNA donor Oligonucleotide

## Results



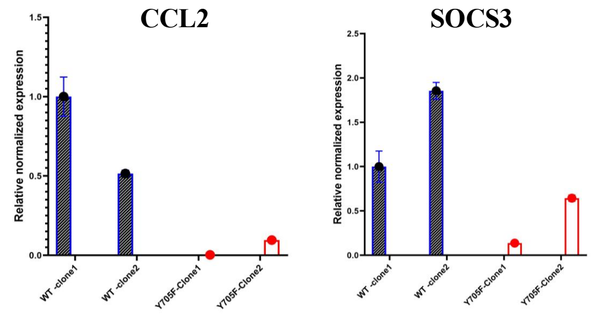

Sequencing results showing selected clones with edits at position 705 of STAT3. Homozygous (Y705F), wild type, and heterozygous mutations (Y705F/Wild type)




MDA MB-468 cells were treated with 100ng/ml of IL6 for 30 minutes. Clones with homozygous Y705F mutations show no phosphorylation of STAT3, as indicated by the absence of pSTAT3 bands in lanes 3 and 4

## Results

### *Realtime PCR Data*




Effects of IL-6–induced STAT3 activation on gene expression of CCL2 and SOCS3 in WT vs Y705F MDA-MB-468 Cells.

## Summary

- CRISPR/Cas9 strategy generated stable and complete edits of tyrosine (Y) to phenylalanine (F) at position 705 of STAT3 in MDA-MB-468 cell lines

## Future Direction

- Design RNA-Seq experiments to compare Y705F mutants and TTI-101 treated cells
- Confirm effect on proliferation
- Compare effect on proliferation with C188-9/TTI-101 treated cells

## References

1. Kasembeli, Moses, et al. "Contribution of STAT3 to Inflammatory and Fibrotic Diseases and Prospects for Its Targeting for Treatment." *International Journal of Molecular Sciences*, vol. 19, no. 8, 2018, p. 2299., doi:10.3390/ijms19082299.
2. Bharadwaj, Uddalak, et al. "STAT3 Inhibitors in Cancer: A Comprehensive Update." *Cancer Drug Discovery and Development STAT Inhibitors in Cancer*, 2016, pp. 95–161., doi:10.1007/978-3-319-42949-6_5.

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