

Using ELISA to Quantify β -Catenin Levels by GSK-3 β Inhibition in HEK293T Cells

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Background

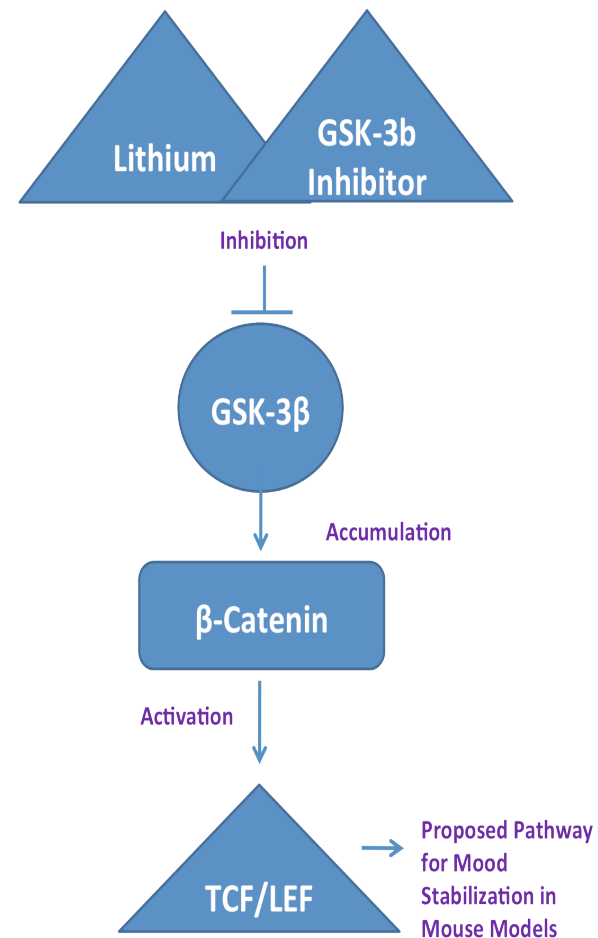
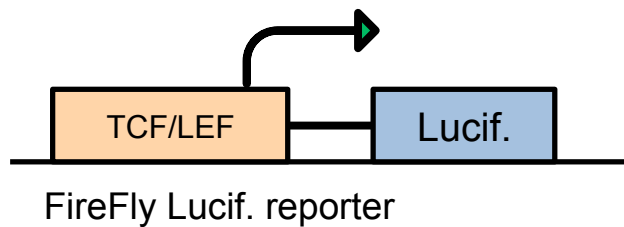
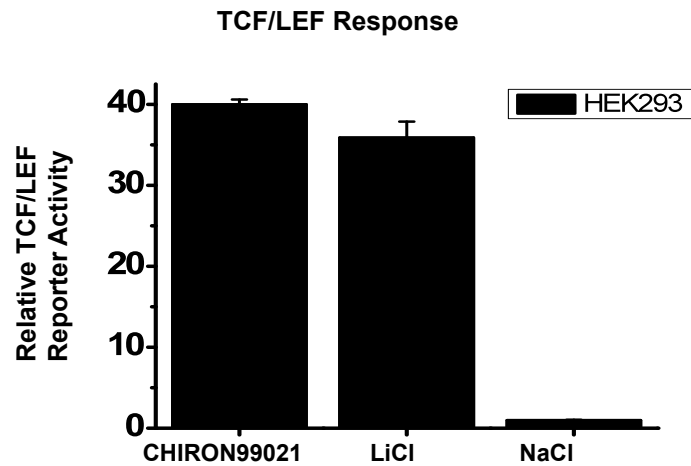
- ❖ Bipolar disorder
 - ❖ Recurrence of mania and depression cycles
 - ❖ Affecting 2% of the world population¹
 - ❖ Pathogenesis not well understood²

Background

- ❖ Lithium (Li^+)

- ❖ First-line treatment for bipolar patients²
- ❖ Less than half of patients respond to treatment²
- ❖ Unclear how lithium exerts its clinical efficacy

Background



CHIRON99021- GSK-3 β Inhibitor

Purpose

- ❖ Li^+ and CHIRON99021
 - ❖ If the accumulation of the β -catenin causing the activation of TCF/LEF reporter
 - ❖ The dose- and time-dependent effects on levels of β -catenin
- ❖ Characterize novel GSK-3 β inhibitors
 - ❖ TCF/LEF transcriptional activation
 - ❖ B-catenin stabilization

Methods

Cell Culture

HEK293T-pBARLS cells



Total Protein Measurements

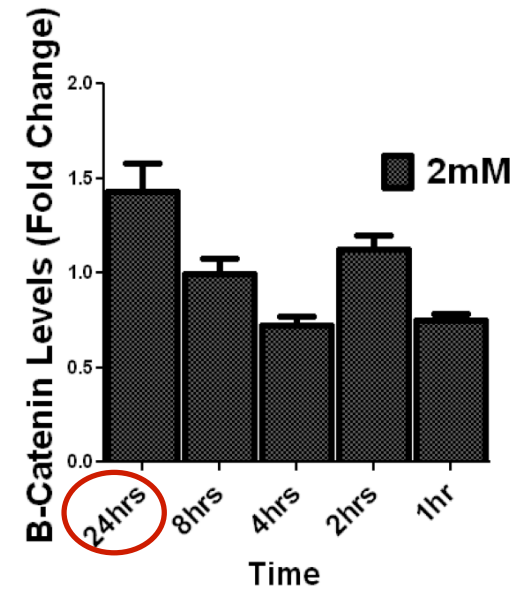
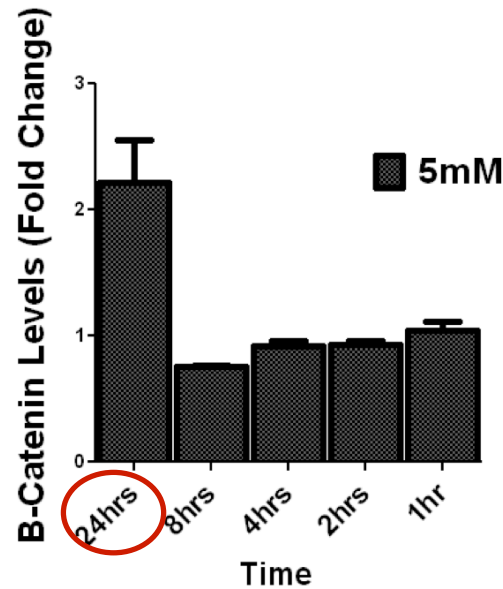
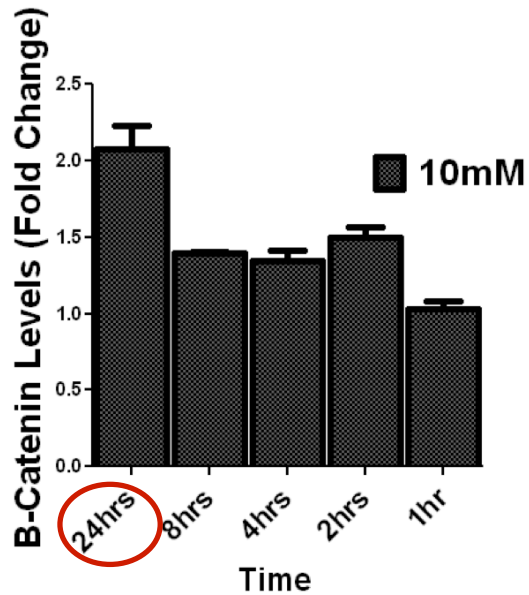
Lowry Assay



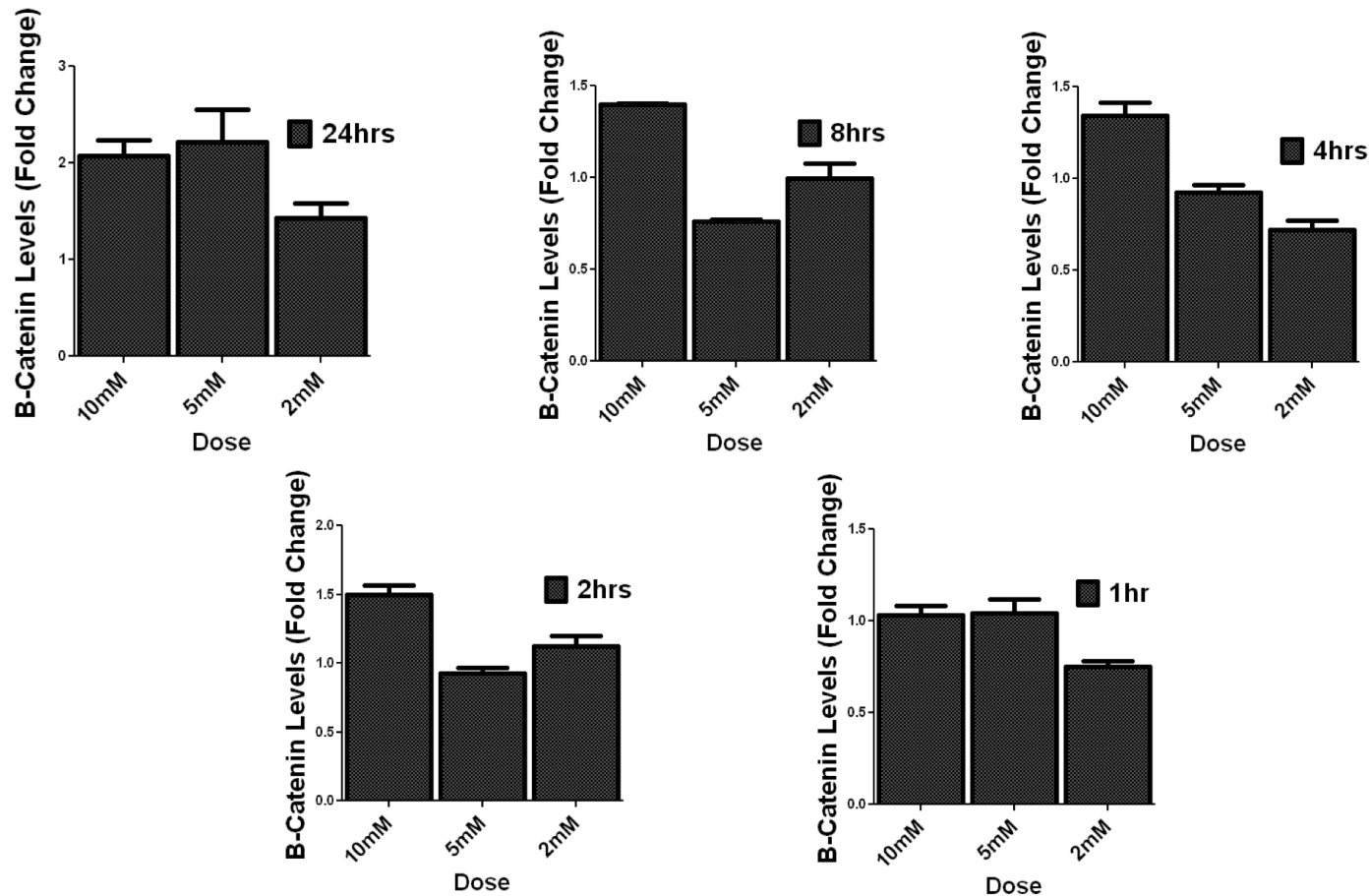
B-Catenin Detection

Sandwich Enzyme-linked Immuno-absorbent Assay (ELISA)

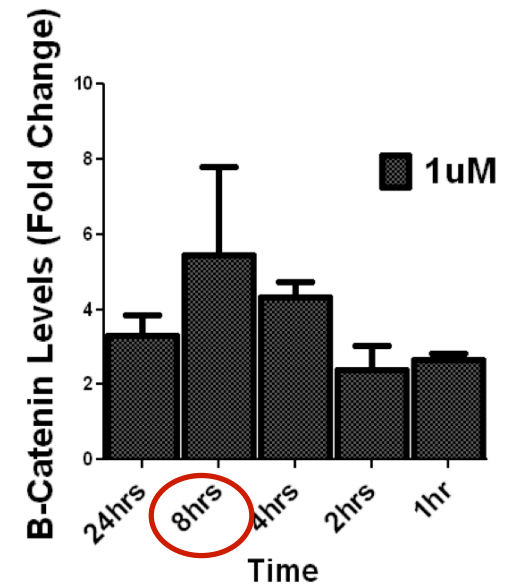
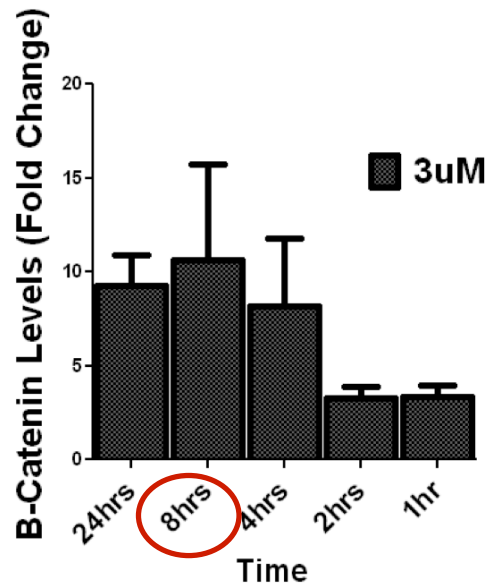
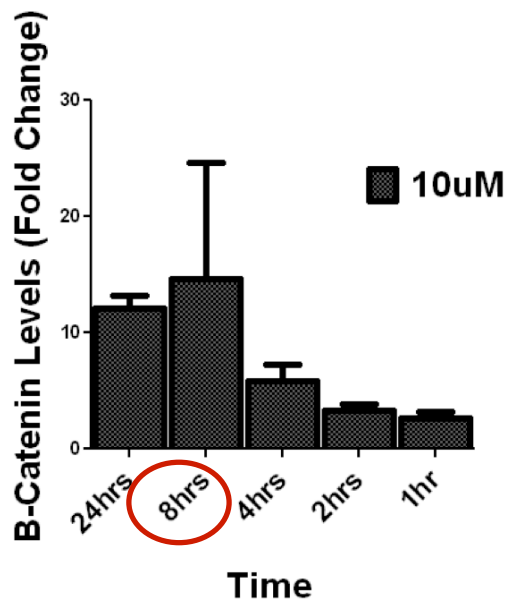
Over All Doses Tested, Lithium Shows the Greatest Effect on Total Levels of β -Catenin After 24hrs



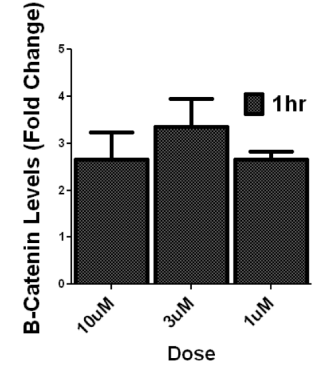
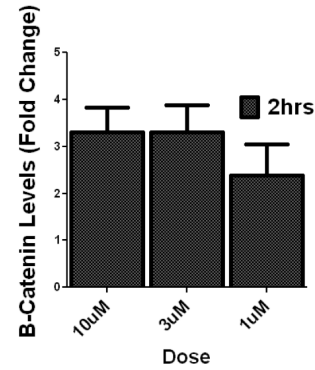
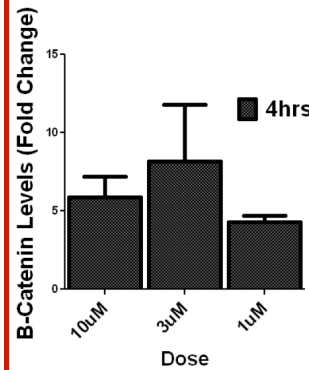
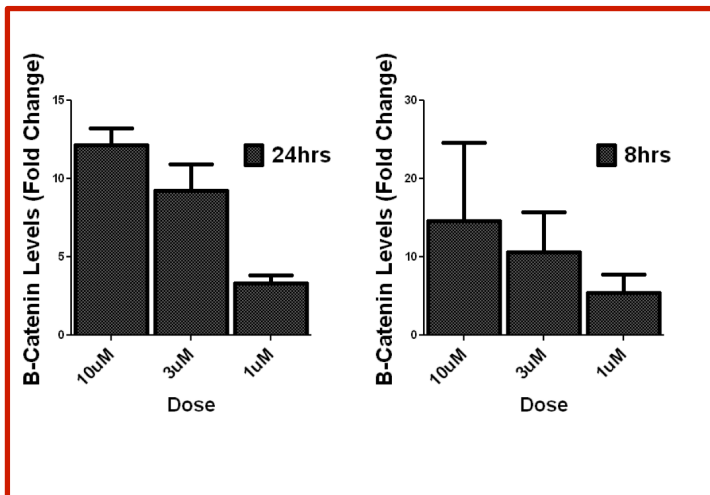
Of All the Concentrations Collected, 10mM has Generated the Highest β -Catenin Levels



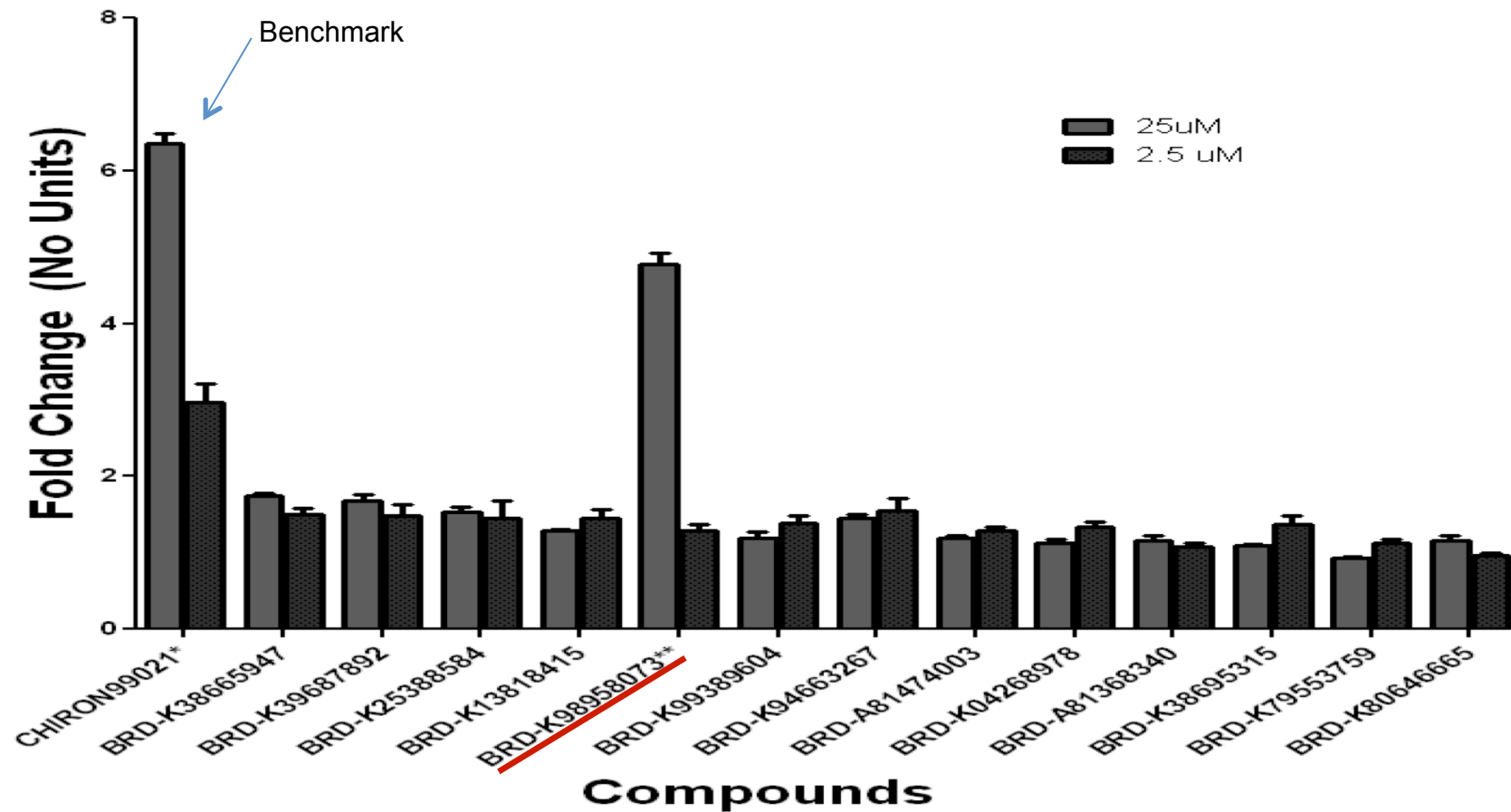
CHIRON99021 dramatically increased β -Catenin Levels as early as 8hrs



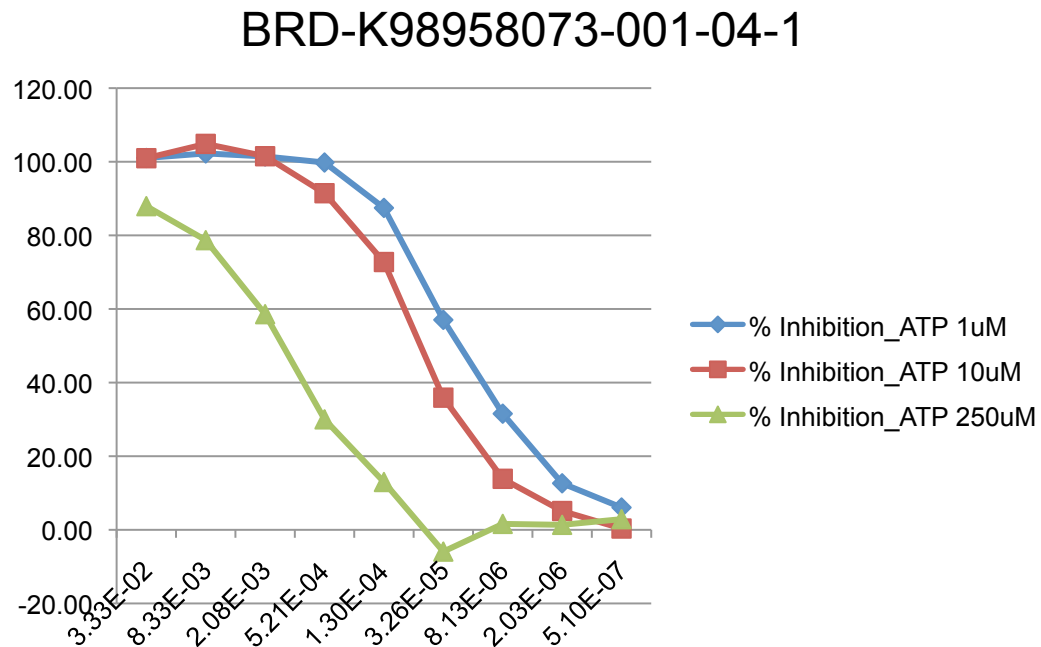
A Clear Correlation Exists between Treatment Concentrations and β -Catenin Response After 8hrs



At 25uM Novel Compound BRD-K98958073 Significantly Increases β -Catenin Levels Even Compared to CHIRON99021



Novel GSK-3 β Inhibitor



Summary

- ❖ Lithium shows a higher accumulation of β -catenin after 24 hours
- ❖ CHIRON99021 shows its higher accumulation of β -catenin as early as 8 hours

Summary

- ❖ CHIRON99021 dose-dependently increases β -catenin levels more consistently than Li^+ .
 - ❖ The mechanism by which Li^+ activates TCF/LEF transcription may differ from that of CHIRON99021.

Summary

- ❖ BRD-K98958073**

- ❖ Most effective novel inhibitor of GSK-3 β assayed

- ❖ ATP Competitive

Future Applications

- ❖ Understanding how Li^+ activates TCF/LEF transcription
 - ❖ Potential new biological targets for bipolar therapeutics.
- ❖ More effective mood stabilizers/GSK-3 β inhibitors.

Acknowledgements

- ❖ Special thanks to Joshua Ketterman for his instrumental mentorship and to Jen Pan for her guidance.
- ❖ Thanks to the Summer Research Program in Genomics, the Broad Institute, the Stanley Center for Psychiatric disease.

References

- (1) Bipolar disorder in adults. National Institute of Mental Health booklet; 2010 Aug 31
- (2) Pan J, et al. AKT kinase activity is required for lithium to modulate mood-related behaviors in mice.
Neuropsychopharmacology. 2011 Jun;36(7): 1397-411.
- (3) Beaulieu JM, et al. Akt/GSK3 signaling in the action of psychotropic drugs. *Trends Pharmacol Sci* 2007a;28: 166-172.