# Investigating the Reversibility of Rett Syndrome Pathology by Altering MeCP2 Condensate with C-mods.

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## Introduction

Rett Syndrome (RTT) is a severe and progressive neurodevelopmental disorder that affects approximately 1 in 10,000 live female births, resulting in profound cognitive and physical disabilities (5). Most RTT cases are linked to mutations in the methyl-CpG-binding protein 2 (MeCP2) gene, with a notable pattern where increases in the frequency of these mutations correspond to a higher incidence of the disorder (3). MeCP2 plays a pivotal role in gene regulation, turning genes on and off in a manner essential for normal brain development (4). These mutations arise de novo, meaning they occur spontaneously and are not inherited. As MeCP2 is an X-linked gene, RTT predominantly affects females with two X chromosomes, compared to males, who typically experience more severe outcomes and early mortality due to their single X chromosome lacking a backup copy of the gene.

One of the most common mutations associated with RTT is the T158M missense mutation, found in approximately 12% of cases (4). T158M mice demonstrate a loss of localization to heterochromatic foci and a redistribution of mutant MeCP2 to the nucleolus (2). This mutation disrupts the methyl-CpG binding domain (MBD) of MeCP2, impairing its ability to bind to methylated DNA. Normally, MeCP2 binds to DNA in heterochromatin regions. These heterochromatin regions exhibit liquid-like properties, forming condensates, which compartmentalize and concentrate molecules to facilitate complex gene regulatory processes. This mislocalization impairs chromatin regulation and leads to significant cellular dysfunction, contributing to the neurological symptoms seen in RTT.

## Background

Condensate-modifying drugs (C-mods) offer a promising therapeutic approach aimed at redistributing mutant MeCP2 from the nucleolus back to its proper location in the chromatin-rich regions of the nucleus. A key dysfunction in RTT appears to be the pathological mislocalization of MeCP2 to nucleolar condensates, which presents a promising therapeutic target.

Although the exact mechanisms by which C-mods restore MeCP2's regulatory functions are not yet fully understood, it is hypothesized that redistributing MeCP2 to chromatin improves its DNA-binding capacity, potentially compensating for some of the functional deficiencies caused by the mutation. In doing so, C-mods may potentially reverse or reduce the neurological symptoms associated with RTT. This highlights the importance of precise MeCP2 regulation in maintaining proper neuronal function and brain development (1).

## Acknowledgments

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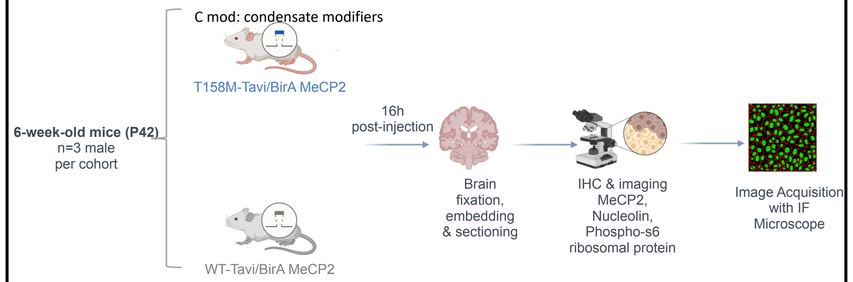
## Methods & Procedures

<u>in vivo condensate Mechanism of Action Studies:</u>




<u>Immunohistochemistry: Primary Antibodies Used (1:1000 dilutions):</u>

- Rabbit anti-MeCP2 (1:1000 dilutions): Reduced MeCP2 levels in mutant samples (less intense staining indicates lower MeCP2).
- Rabbit anti-nucleolin (1:1000 dilutions): Detect nucleolus location as nucleolin serves as a nucleolar marker.
- Rabbit anti-phospho-riboprotein S6 (1:1000 dilutions): Serves as a direct readout for MeCP2 function.

<u>Immunostaining</u>:

- Brain sections were collected at the thickness of 50 um using the Cryostat
- Staining was followed using 1x PBS for washes, and 10% NGS for blocking
- Slices were stained with an anti-GFP antibody, conjugated with Goat anti-rabbit Alexa Fluor 488 and Steptavidin Dylight 650, at 1:1000 dilution in 1% NGS
- DAPI staining was conducted at 1:1000 dilution in 1x PBS
- Slices were transferred onto microscope slides, followed by mounting and seal

<u>Image Acquisition</u>:

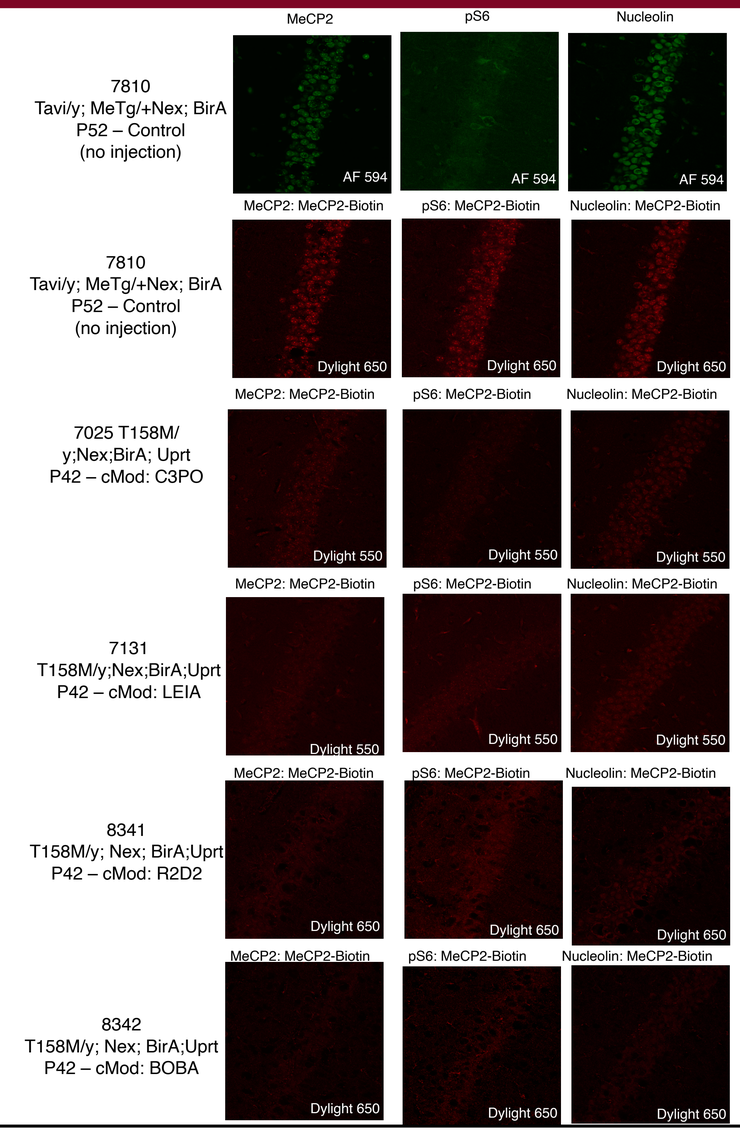
- Immunofluorescence (IF) microscope was used to capture images of stained tissue slides.
- For each corresponding antibody, the captured images were split into separate channels, adjusted brightness/contrast, and merged into adjusted layers.

## Conclusion

Currently, no approved therapies directly address the loss of MeCP2 function central to Rett Syndrome (RTT). We hypothesize that, despite the T158M mutation impairing MeCP2's DNA-binding ability, higher protein levels may retain sufficient functionality to alleviate RTT pathology (3).

The reduced levels of MeCP2 and phospho-riboprotein S6 observed in T158M mutants, compared to wild-type, further suggest that phospho-riboprotein S6 could serve as a direct readout of MeCP2 function due to its positive correlation with MeCP2 activity. C-mods may also help relocate mutant MeCP2 from nucleoli to chromatin, potentially mitigating the loss of function and restoring normal cellular processes. Biotin tagging of MeCP2 has proven to be a reliable method for assessing MeCP2 localization, and this research highlights the therapeutic potential of c-mods in treating RTT by improving MeCP2 chromatin localization and function.

## Results

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