# Development of a MeCP2 condensate-modifying small molecule for the treatment of Rett syndrome

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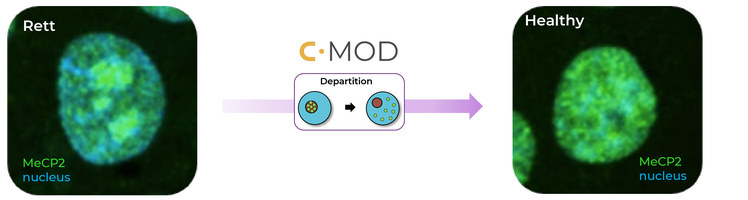
## Introduction

The majority of Rett syndrome (RTT) cases are caused by mutations in MeCP2, a ubiquitous DNA binding protein that is highly expressed in neurons (1). Mutations within the MeCP2 methyl-CPG binding domain that disrupt DNA binding and chromatin regulation are among the most prevalent RTT mutations (2, 3). There are no approved treatments that address the loss of MeCP2 function central to RTT. Moreover, the molecular events linking MeCP2 mutations with diverse neuronal dysfunctions are incompletely understood. Regulation of mutant MeCP2 levels improves RTT phenotypes *in vivo* (4), suggesting that modulation of mutant MeCP2 is a promising avenue for disease-modifying therapeutic intervention.

We identified a novel mechanistic node of dysfunction that is shared across common RTT-causing MeCP2 mutations: these mutations result in pathological mislocalization of MeCP2 to nucleolar condensates in human neuronal disease models. We hypothesize that this mislocalization disrupts MeCP2 chromatin localization and results in cellular dysfunction, including ribosomal dysfunction. Here, we propose to restore MeCP2 nuclear function and neuronal health by using a small molecule condensate-modifying drug (c-mod) that reduces the nucleolar mislocalization of mutant MeCP2 and normalizes MeCP2 chromatin localization.

**Disease mechanism**
RTT mutations in MeCP2 lead to its nucleolar mislocalization, disrupting MeCP2 chromatin localization and resulting in cellular dysfunction

**Therapeutic approach**
C-mod reduces nucleolar mis-localization of MeCP2, normalizes MeCP2 chromatin localization, and restores cellular function




## Background

Condensates are dynamic, membraneless organelles that regulate diverse cellular processes. Condensatopathies are aberrations in condensates that drive cellular dysfunction and disease (5).

MeCP2 normally localizes to chromatin, where it binds methyl DNA and recruits the co-repressor complex. We observe mislocalization of RTT mutant MeCP2 to the nucleolus in human neuronal cells; the same nucleolar mislocalization has been previously reported in RTT murine models alongside RTT phenotypes (6, 7).

The nucleolus is a nuclear condensate that is the site of ribosomal biogenesis and maturation. Defects in nucleolar structure and ribosomal maturation and activity are reported in RTT models and in patient brain (8). We hypothesize that the mutant MeCP2 condensatopathy contributes to nucleolar and ribosomal dysfunction in RTT. Reversing this condensatopathy with c-mods promises to restore MeCP2 function and the complex, systemic defects that drive RTT.

## Methods

Development of a high content imaging-based assay using human neuronal cells to monitor the nucleolar localization of mutant MeCP2

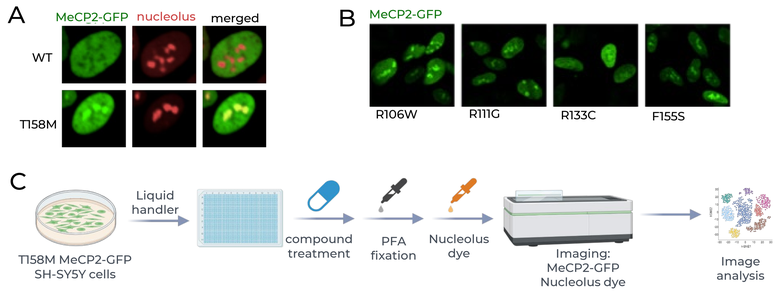



Figure 1. (A) Scalable human neuroblastoma cells expressing T158M MeCP2-GFP recapitulate nucleolar mislocalization of MeCP2 observed in patient iPSCs. (B) MeCP2 nucleolar mislocalization is observed with additional RTT mutations. T158M is the most common RTT-causing mutation and was chosen as the representative model for high throughput screening (HTS). (C) Assay workflow to identify compounds that can departition mutant MeCP2 out of the nucleolus.

## Phenotypic discovery of MeCP2-correcting c-mods

A 370K compound condensate phenotypic screen identifies c-mods that selectively departition mutant MeCP2 from nucleoli

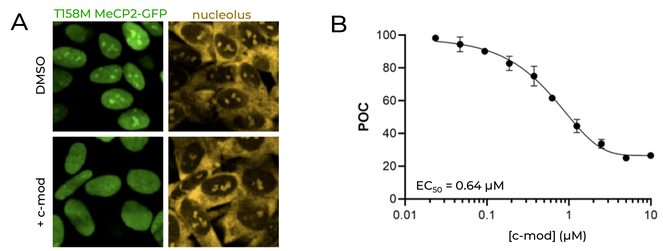



Figure 2. (A) Early c-mod hit departitions MeCP2 from nucleoli without interfering with nucleolar integrity. (B) Early c-mod hit departitions MeCP2 from the nucleoli in a concentration-dependent manner.

C-mods correct MeCP2-T158M localization to heterochromatin

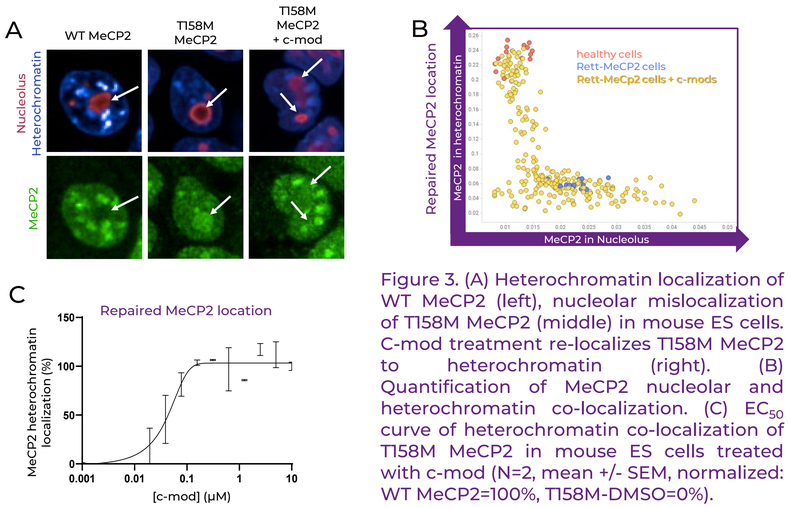



Figure 3. (A) Heterochromatin localization of WT MeCP2 (left), nucleolar mislocalization of T158M MeCP2 (middle) in mouse ES cells. C-mod treatment re-localizes T158M MeCP2 to heterochromatin (right). (B) Quantification of MeCP2 nucleolar and heterochromatin co-localization. (C) $EC_{50}$ curve of heterochromatin co-localization of T158M MeCP2 in mouse ES cells treated with c-mod (N=2, mean +/- SEM, normalized: WT MeCP2=100%, T158M-DMSO=0%).

## Functional validation of the c-mod

C-mods correct ribosomal dysfunction in patient neuron progenitors based on quantitative phospho-ribosomal protein S6 readout

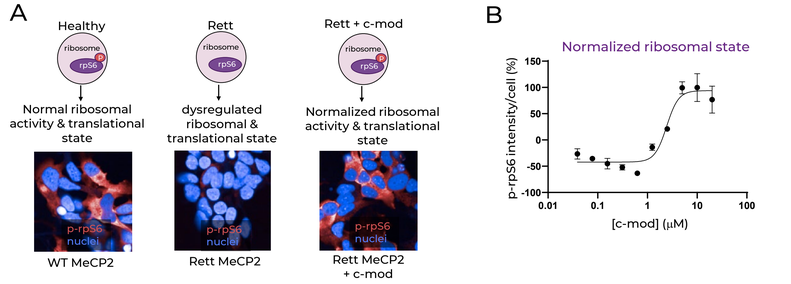



Figure 4. (A) Patient-derived T158M MeCP2 neuronal progenitors display reduced phospho-ribosomal protein S6 (p-rpS6) levels compared to isogenic control. C-mod treatment rescues p-rpS6 levels in the T158M cells. (B) Concentration-dependent normalization of p-rpS6 in Rett patient neuronal progenitor cells.

## Conclusions

We developed a high content imaging-based assay monitoring the nucleolar localization of mutant MeCP2 and performed a small molecule HTS to identify compounds that departition mutant MeCP2 out of the nucleolus. We identified condensate-modifying small molecules, or c-mods, that reduce the nucleolar mislocalization of MeCP2, normalize MeCP2 chromatin localization, and reverse a readout of ribosomal dysfunction in patient induced pluripotent stem cell (iPSC)-derived neuronal progenitors. These findings suggest that a c-mod can departition mutant MeCP2 from nucleolar condensates and repair MeCP2 chromatin localization, correcting the loss of MeCP2 function and gain of cellular dysfunction that drive disease. Our results demonstrate the therapeutic potential of c-mods to restore MeCP2 function to treat RTT.

## References

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