Sedimentation Assay Screening of TRIMs on Polyglutamine-Expanded ATXN1

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INTRODUCTION

When their linear amino acid sequence is translated and folded into the correct three-dimensional conformation, proteins play crucial roles in various biological processes. However, proteins can undergo misfolding for various reasons, including genetic mutations and translational errors. One prevalent consequence of misfolded proteins is insoluble aggregates. The intracellular propagation of such aggregates underly pathogenesis in numerous **neurodegenerative diseases**.

To counteract the detrimental effects of protein misfolding, organisms across diverse kingdoms have evolved sophisticated protein quality control systems that eliminate such aggregates. Recent studies have suggested that the **tripartite motif proteins (TRIM),** may possess the ability to fulfill this function.

Spinocerebellar Ataxia Type 1 (SCA1) is a neurodegenerative disease caused by the aggregation of the **polyglutamine-expanded ATXN1 protein**. In this study, we undertake a systematic analysis of virtually all known human TRIM proteins and their potential to mitigate Atxn1 82Q aggregation in cultured HEK293T cells.

MATERIALS AND METHODS

CELL CULTURE

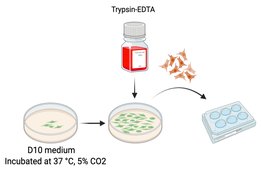


Figure 1. HEK293T cells are cultured in D10 medium and passaged onto 6-well plates after reaching 100% confluency on 15cm culture dishes.

GFP-ATXN82Q + TRIM CO-TRANSFECTION

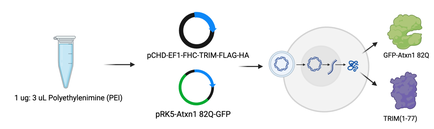


Figure 2. 293T cells that reaches 80% confluency are transfected with 0.5 ug of pRK5-Ataxin-1[82Q]-GFP plasmid and 2 ug of pCDH-EF1-FHC-TRIM-FLAG-HA plasmid.

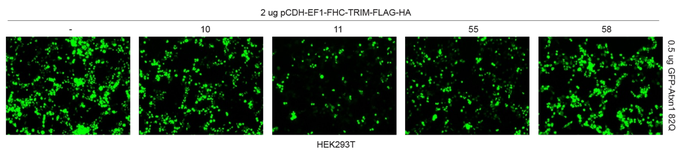


Figure 3. Detection of GFP fluorescence signal after co-transfection under an inverted fluorescence microscope (Revolve, Echo Laboratories).

SAMPLE COLLECTION & PREPARATION FOR SDS-PAGE

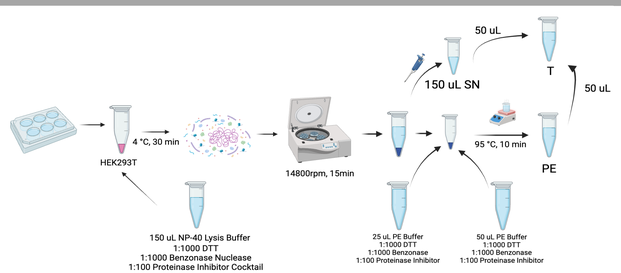


Figure 4. 36 hours after co-transfection, cells are harvested and lysed with NP-40. Insoluble and soluble components of the lysates are separated and designated by the PE (insoluble) and SN (soluble) faction, with a mixture of the two designated as the T (total) faction.

PROTEIN DETECTION AND QUANTIFICATION

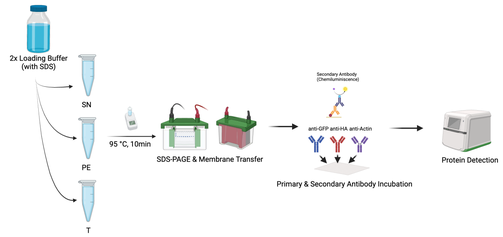


Figure 5. All samples are resolved through SDS-PAGE with controlled sample volume and transferred onto NC membrane, which is then incubated with anti-HA, GFP, and Actin antibodies. Proteins are then detected with chemiluminescence.

TRANSCRIPTION LEVEL QUANTIFICATION

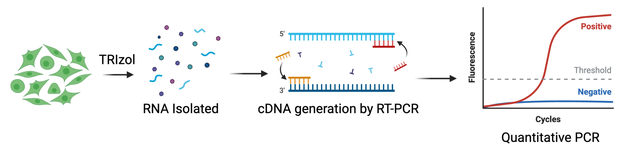


Figure 6. After co-transfected cell samples go through lysis and RNA extraction by TRIzol, cDNA of the encoding mRNA of Atxn1 82Q, GAPDH, and TRIM proteins were generated with corresponding forward and reverse primers. Then gene expression is determined by SYBR Green-based RT-qPCR and is normalized to GAPDH expression.

RESULTS

SEDIMENTATION ASSAY SCREENING OF TRIMS ON ATXN1 82Q

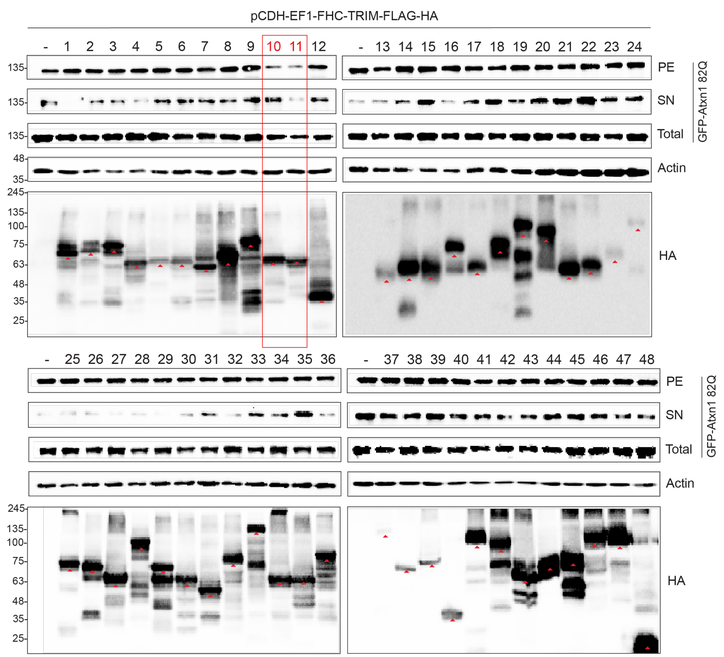
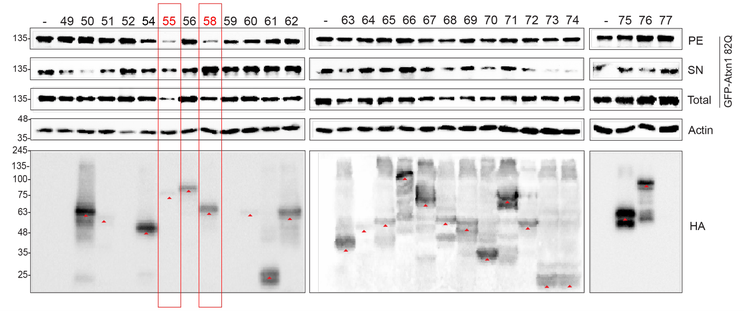


Figure 4. Sedimentation Assay screening of 77 TRIM proteins on Atxn1 82Q. TRIM 10, 11, 55, 58 reduces signal of GFP-Atxn1 82Q in the NP-40 insoluble faction of the lysates sample and are identified as potential disaggregase of Atxn1 82Q.



DENSITOMETRIC ANALYSIS & TRANSCRIPTION QUANTIFICATION

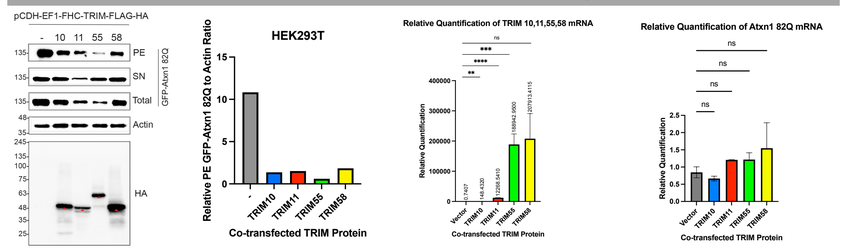


Figure 5 & 6. Sedimentation Assay of GFP-Atxn1 82Q consisted of the 4 hit TRIM proteins as treatments (left) and densitometric analysis of the western blot. TRIM10, 11, 55, 58 decreases detection of GFP-Atxn1 82Q detected by 87%, 86%, 94% and 83%.

Figure 7 & 8. Quantification of TRIM 10, 11, 55, 58 and Atxn1 82Q protein mRNA level. None of the TRIM proteins result in a statistically significant (unpaired T-test) difference in Atxn1 82Q transcript level vs. controll, indicating the 4 TRIM proteins does not affect transcription regulation of the substrate. Quantification of the 4 TRIM proteins mRNA level in the sample indicates adequate transfection efficiency.

CONCLUSIONS

When co-transfected in HEK293T cells, **TRIM10, 11, 55, 58 are potent disaggregases of GFP-Atxn1 82Q**. Moreover, these four TRIM proteins do not reduce Atxn1 82Q mRNA level and interact with GFP-Atxn1 82Q on a **protein-protein level**. These results indicate these four TRIM proteins are potentially guardians against polyglutamine-expanded Atxn1 protein aggregation and SCA1 pathogenesis.

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