Title: Genome-wide CRISPR screens to identify modulators of TDP-43 aggregation in ALS

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The formation of inclusions comprised of TAR DNA-binding protein-43 (TDP-43) throughout the brain and spinal cord in ALS is strongly correlated with motor neuron degeneration. However, the molecular processes that initiate the aggregation of TDP-43 and mediate its toxicity remain unknown. The objective of this study is to identify key genes and biological processes that modulate TDP-43 inclusion formation and toxicity using a pooled genome-wide CRISPR knockout screen. Analysis of the genome-wide CRISPR knockout screen data revealed seven distinct groups of “TDP-43 modifier genes” that have functionally distinct roles in the cell, including inhibiting or enhancing TDP-43 aggregation and protecting or sensitising to TDP-43 toxicity. Gene knockouts that sensitise or protect cells against mutant TDP-43 toxicity were enriched with genes associated with endoplasmic reticulum to cytosol transport and autonomic nervous system development, respectively. Gene knockouts that enhance or inhibit mutant TDP-43 aggregation were enriched in genes associated with– neuron projection organisation and response to interferon-beta, respectively. Using this combination of state-of-the-art analysis algorithms for genome-wide CRISPR knockout data (e.g. MAGeCK) and top gene ontology pathways (WebGestalt), we short-listed 323 “TDP-43 modifiers”. These gene targets are the subject of further investigation for prioritisation of genes that decrease TDP-43 aggregation and enhance neuronal survival. Using arrayed CRISPR knockout tools combined with multi-parametric high-content imaging, we have developed sophisticated analytical pipelines to cluster targets that cause statistically similar phenotypic effects. This has allowed us to rapidly identify and triage our highest confidence targets. This research will lead to a greater understanding of the pathogenesis of ALS and identify avenues for better therapeutic interventions aimed at protecting neurons against TDP-43 pathology.