The effect of mismatch repair proteins in a Huntington's disease cellular model

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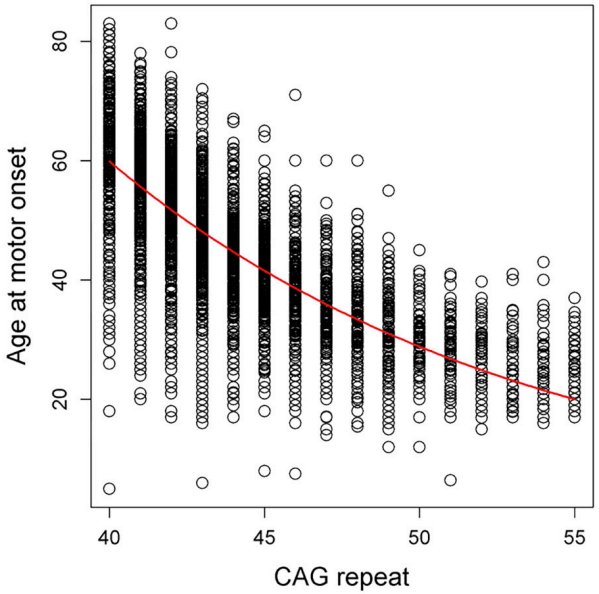
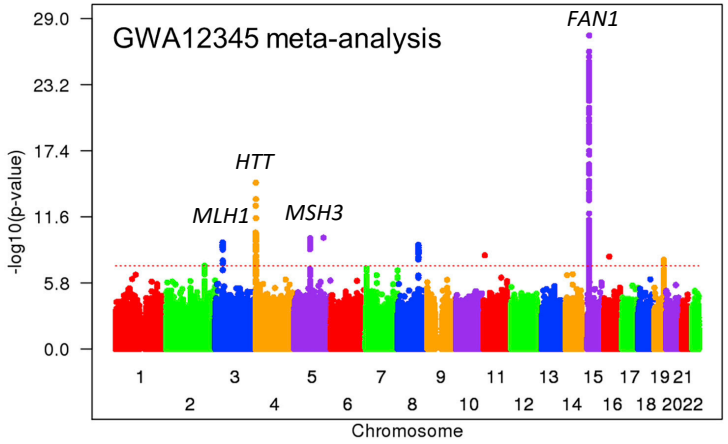
Introduction

- Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disease caused by an expanded CAG repeat in exon 1 of the Huntingtin gene (*HTT*). Repeats greater than 35 CAGs in length cause disease and the age at disease onset is inversely correlated with CAG repeat length[1].
- Although about 60% of the variation in age at onset of disease can be attributed to the CAG repeat length, other genetic factors have been found to modify age at disease onset[2]. Recent genome wide association studies (GWAS) have identified several SNPs in genes encoding DNA damage repair factors that modify age at disease onset. There is a particular enrichment in mismatch repair genes with *MLH1 and MSH3* implicated[3, 4].

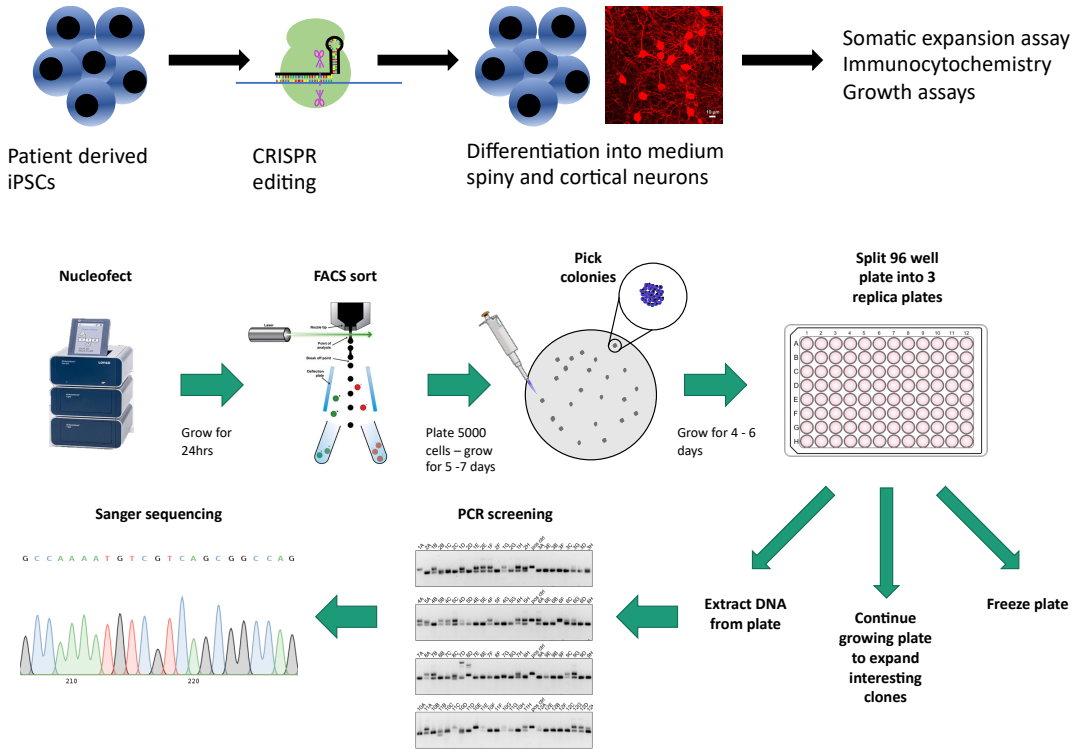




Gusella, J. F. (2019). CAG Repeat Not Polyglutamine Length Determines Timing of Huntington's Disease Onset Article CAG Repeat Not Polyglutamine Length Determines Timing of Huntington's Disease Onset. *Cell*, *178*, 887-900.e14. https://doi.org/10.1016/j.cell.2019.06.036

Methods

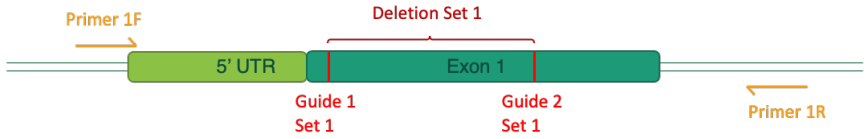
- Induced pluripotent stem cell (iPSC) culture and differentiation to striatal medium spiny neurons.
- CRISPR Cas9 genome editing.
- PCR followed by capillary gel electrophoresis.
- Immunocytochemistry.
- Western blotting

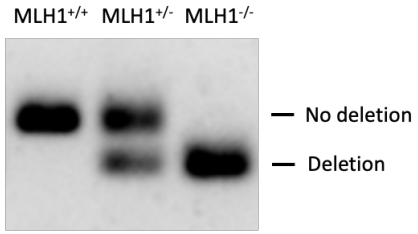


MLH1 KO in HD109 iPSCs

Two guides were used to generate a 68bp deletion in exon 1 of *MLH1* in HD109 iPSCs.

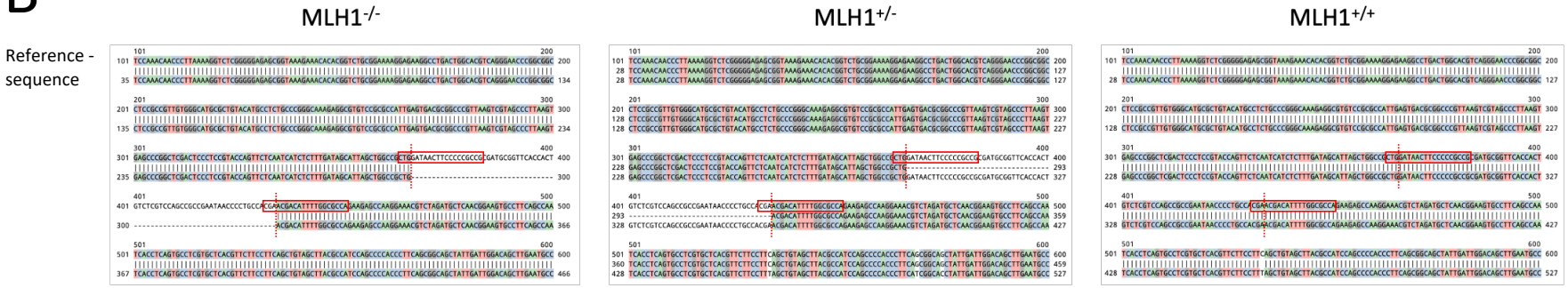
A





MLH1 KO, guide set 1, primers 1F and 1R

B



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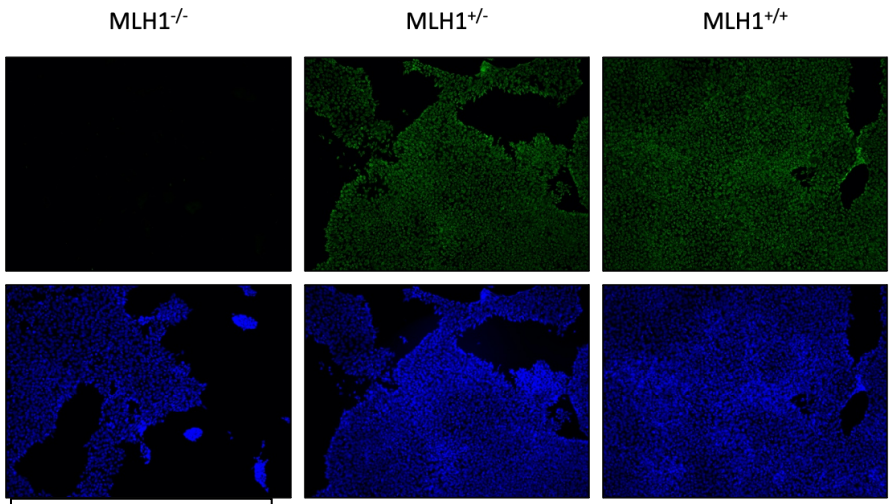
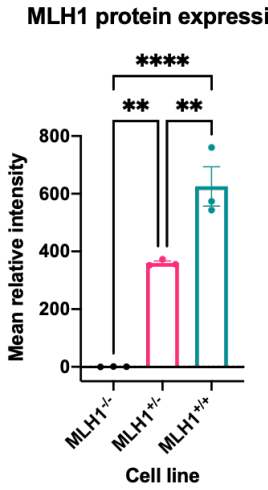


Anti-MLH1, Mouse, 1:100
#BD Biosciences, 550838



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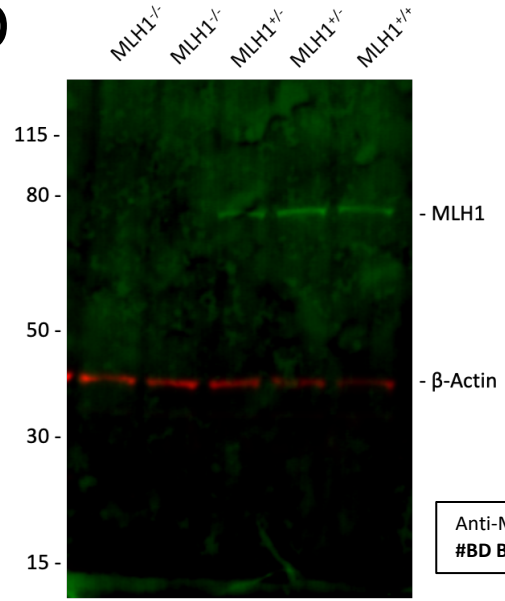

Anti-MLH1, Mouse, 1:100
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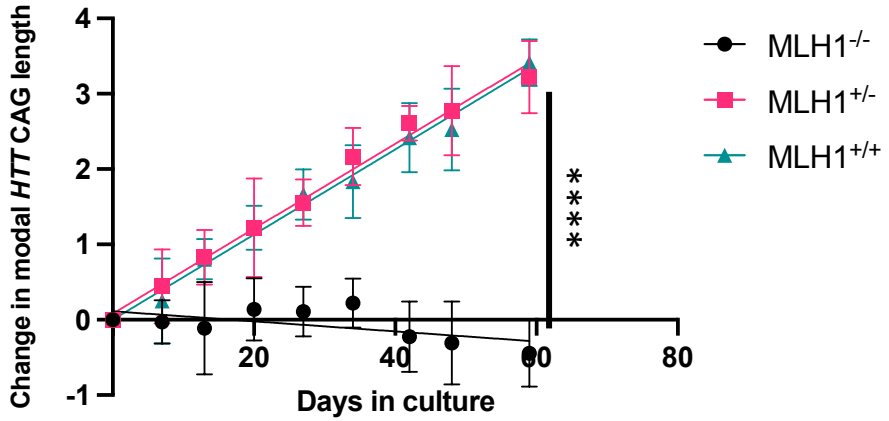
MLH1 KO using CRISPR Cas9 gene editing. (A) Two guides were used to introduce 2 double strand breaks into exon 1 of *MLH1*. The resulting 68bp deletion led to the introduction of a frameshift mutation and a premature STOP codon. This led to ablation of protein expression. Clones were screened for deletions using PCR followed by gel electrophoresis. (B) Sanger sequencing across the deletion was used to validate the genotype of the clones with several homozygous deletion ($MLH1^{-/-}$), heterozygous deletion ($MLH1^{+/-}$) and unedited ($MLH1^{+/+}$) clones identified. The red boxes mark the guide binding sites with the cut sites marked by a dashed red line. Alignments were performed using CRISP-ID[5]. Loss of MLH1 protein expression was validated using immunocytochemistry (C) and Western blotting (D). (C) Top panels show MLH1 staining (green), lower panels show Hoechst 33342 nuclear staining (blue). Nuclear MLH1 intensity was quantified using Image J (n=3, one way ANOVA P<0.0001; error bars = S.E.M; **** P <0.0001, ** P <0.01.

HTT CAG expansion in MLH1 KO iPSCs

A



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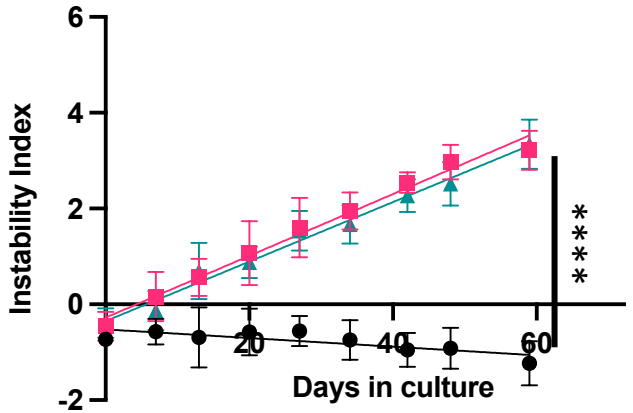

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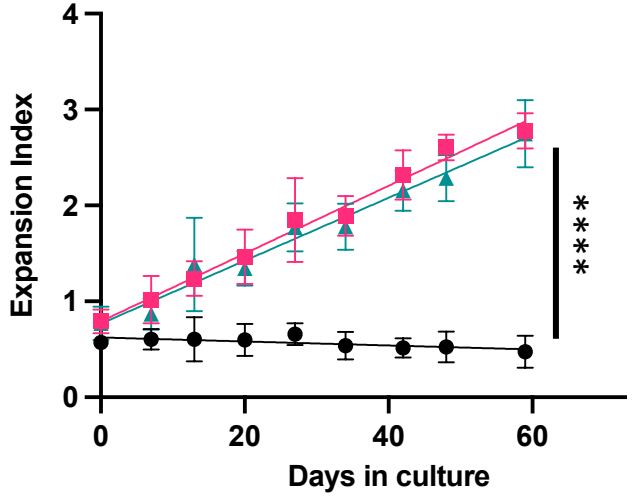

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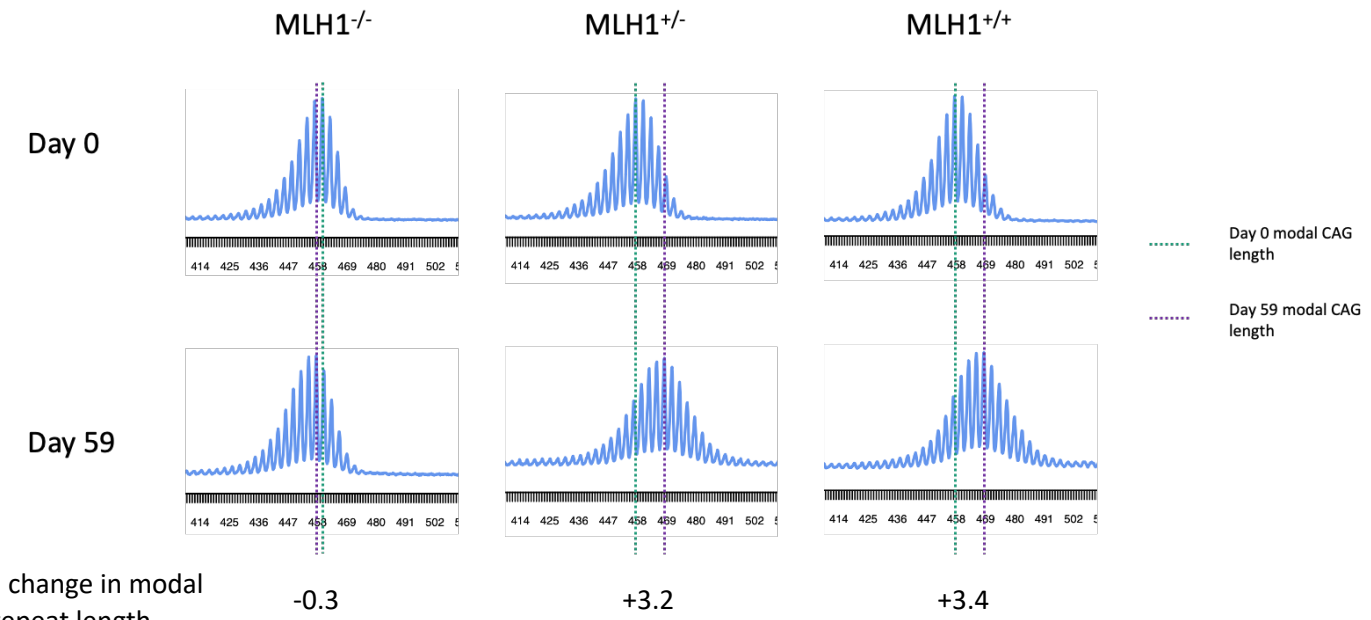
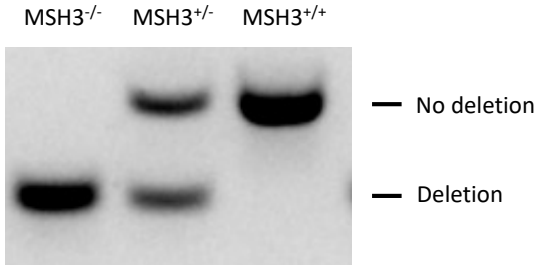


MLH1 KO in HD109 iPSCs ablates expansion of the CAG repeat. Heterozygous KO of MLH1 does not reduce somatic expansion of the CAG repeat in HD109 iPSCs. Somatic expansion of the *HTT* CAG repeat was assayed in iPSCs over 59 days. Pellets were taken at each passage and DNA was extracted. The CAG repeat was sized using PCR followed by capillary gel electrophoresis and analysis was performed as in Lee et al., 2010[6] with an R script written by Branduff McAllister (Cardiff University). (A) Change in modal CAG length is plotted against the time in culture for homozygous ($MLH1^{-/-}$) and heterozygous ($MLH1^{+/-}$) *MLH1* KO lines as well as unedited controls ($MLH1^{+/+}$). (B) The instability index is a measure of the instability of the CAG repeat with positive values showing a bias towards expansions and a negative value showing a bias towards contractions. (C) The expansion index is a measure of the number of peaks to the right of the modal peak. Each data point represents the mean ± SD (n=6); one-way ANOVA performed on regression slope values (**** = $P < 0.0001$). (D) Representative electropherograms showing CAG lengths following 59 days in culture.

MSH3 KO in HD109 iPSCs

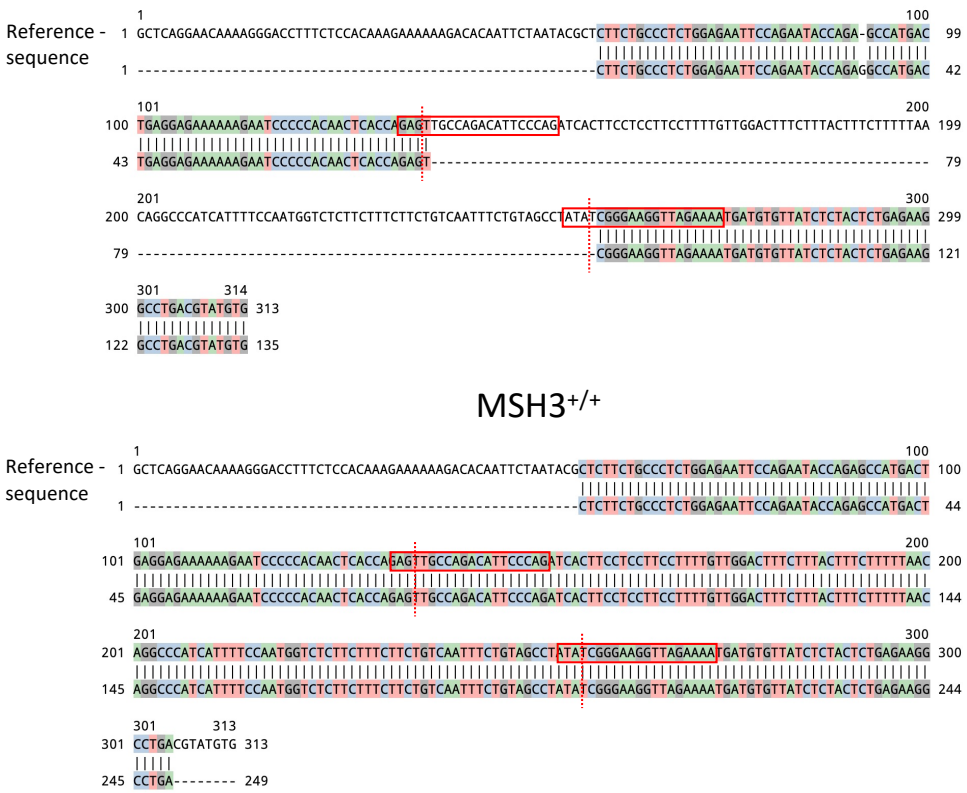
A

Two guides were used to generate a 121bp deletion in exon 2 of *MSH3* in HD109 iPSCs.



MSH3 KO primers 1F and 1R

B



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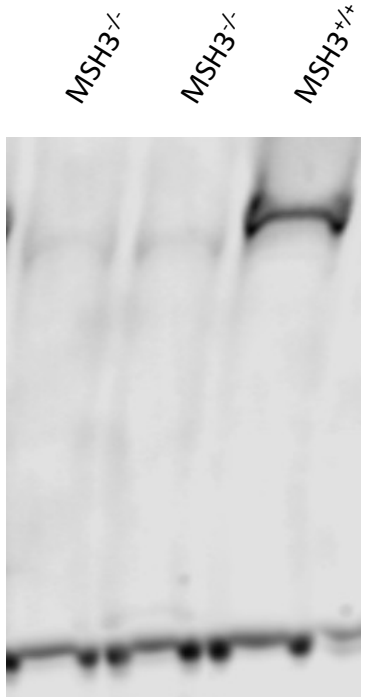
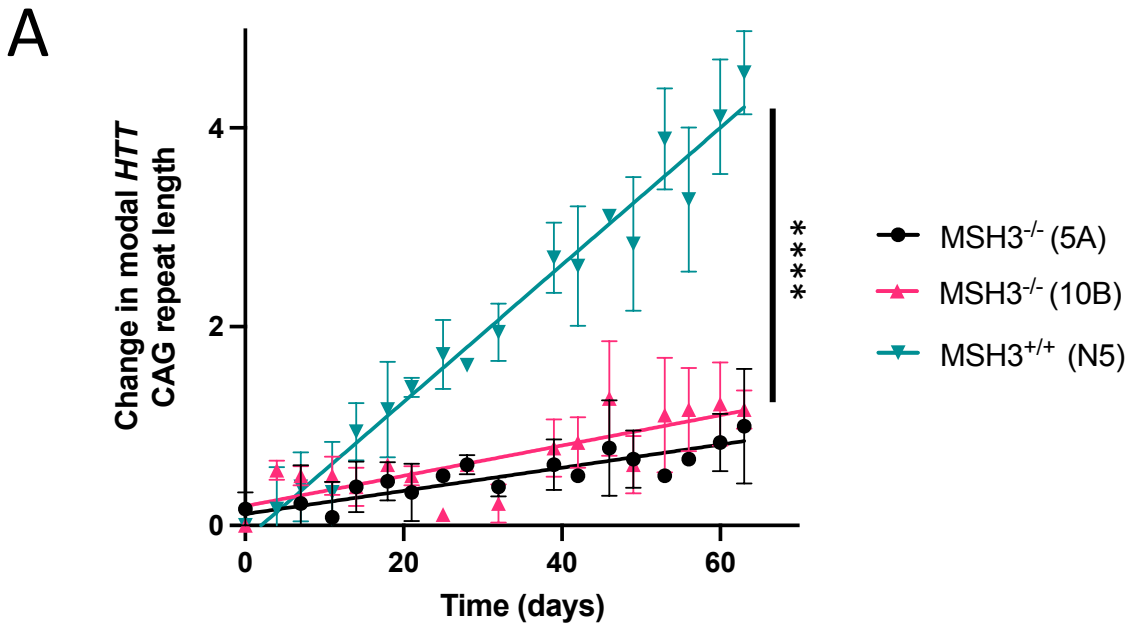


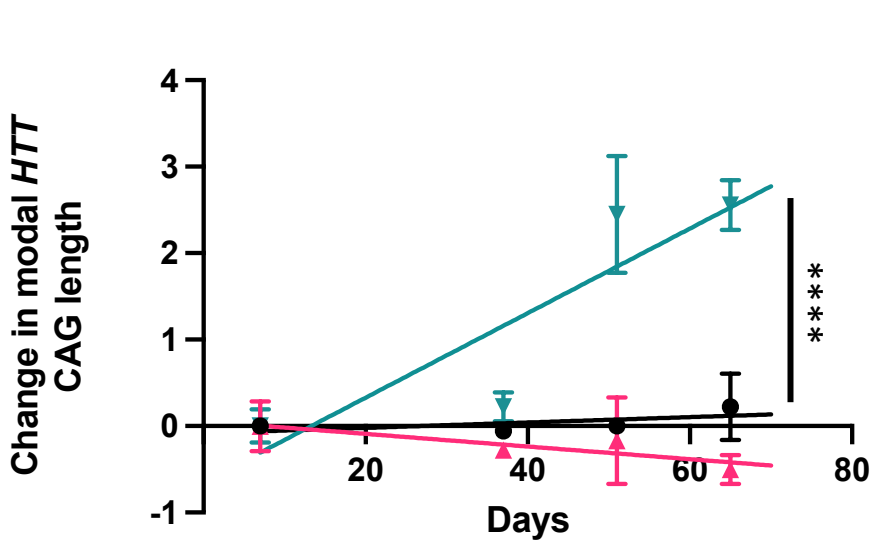
Anti-MSH3
Mouse, 1:100
#SC-271079

Jasmine Donaldson, Sophie Powell

MSH3 KO using CRISPR Cas9 gene editing. (A) – As with the MLH1 KO, 2 guides were used to introduce an out of frame deletion resulting in loss of protein expression. PCR followed by visualization on an agarose gel was used to screen for deletions. (B) – Sanger sequencing across the deletion identified several homozygous KO ($MSH3^{-/-}$) and unedited ($MSH3^{+/+}$) clones. Sanger sequencing to identify heterozygous KO clones is ongoing. The red boxes mark the guide binding sites with the cut sites marked by a dashed red line. Alignments were performed using CRISP-ID[5]. (C) Loss of protein expression was confirmed using Western blotting.

Preliminary MSH3 somatic expansion data





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Preliminary somatic expansion data suggest that MSH3 KO reduces CAG repeat expansion in iPSCs and ablates expansion in neurons. Somatic expansion of the *HTT* CAG repeat was assayed in iPSCs over 63 days and in differentiated striatal medium spiny neurons over 65 days. Analysis of the CAG repeat was performed as in the MLH1 KO somatic expansion assay. (A) Change in modal CAG length is plotted against the time in culture for 2 homozygous ($MSH3^{-/-}$) MSH3 KO iPSC clones and an unedited control iPSC clone ($MSH3^{+/+}$). (B) *HTT* CAG repeat somatic expansion was assayed in differentiated medium spiny neurons for 2 homozygous ($MSH3^{-/-}$) MSH3 KO iPSC clones and an unedited control iPSC clone ($MSH3^{+/+}$). Each data point represents the mean ± SD (n=3); one-way ANOVA performed on regression slope values (**** = P < 0.0001).

Conclusions

- MLH1 KO ablates somatic expansion of the CAG repeat in an iPSC model of HD.
- Heterozygous KO of MLH1 does not reduce the rate of expansion of the CAG repeat in an iPSC model of HD.
- Preliminary data suggest that MSH3 KO reduces expansion of the CAG repeat in iPSCs and ablates expansion of the CAG repeat in differentiated striatal neurons.

Acknowledgements and References

I would like to thank the CHDI Foundation and Professor Lesley Jones for funding this work.

1. Langbehn et al. (2004), *Clinical Genetics*, *65*(4), 267–277.
2. Gusella et al. (2014), *Movement Disorders*, *29*(11), 1359–1365.
3. GeM-HD Consortium (2015), *Cell*, *162*(3), 516–526.
4. GeM-HD Consortium (2019), *Cell*, *178*, 887-900.e14.
5. Dehairs et al. (2016), *Scientific Reports*, *6*(1), 28973.
6. Lee et al. (2010), *BMC Systems Biology*, *4*(1), 29.