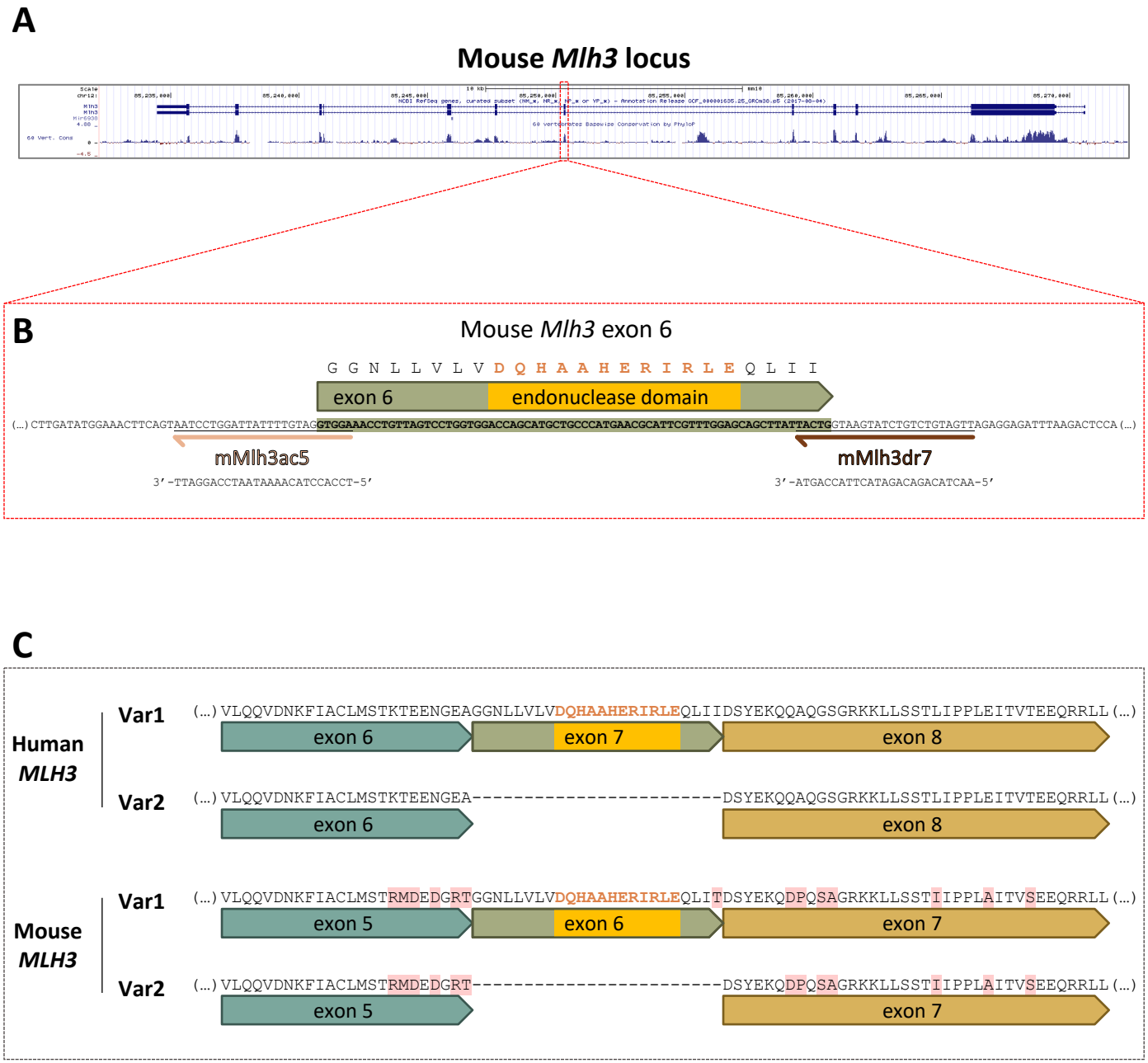
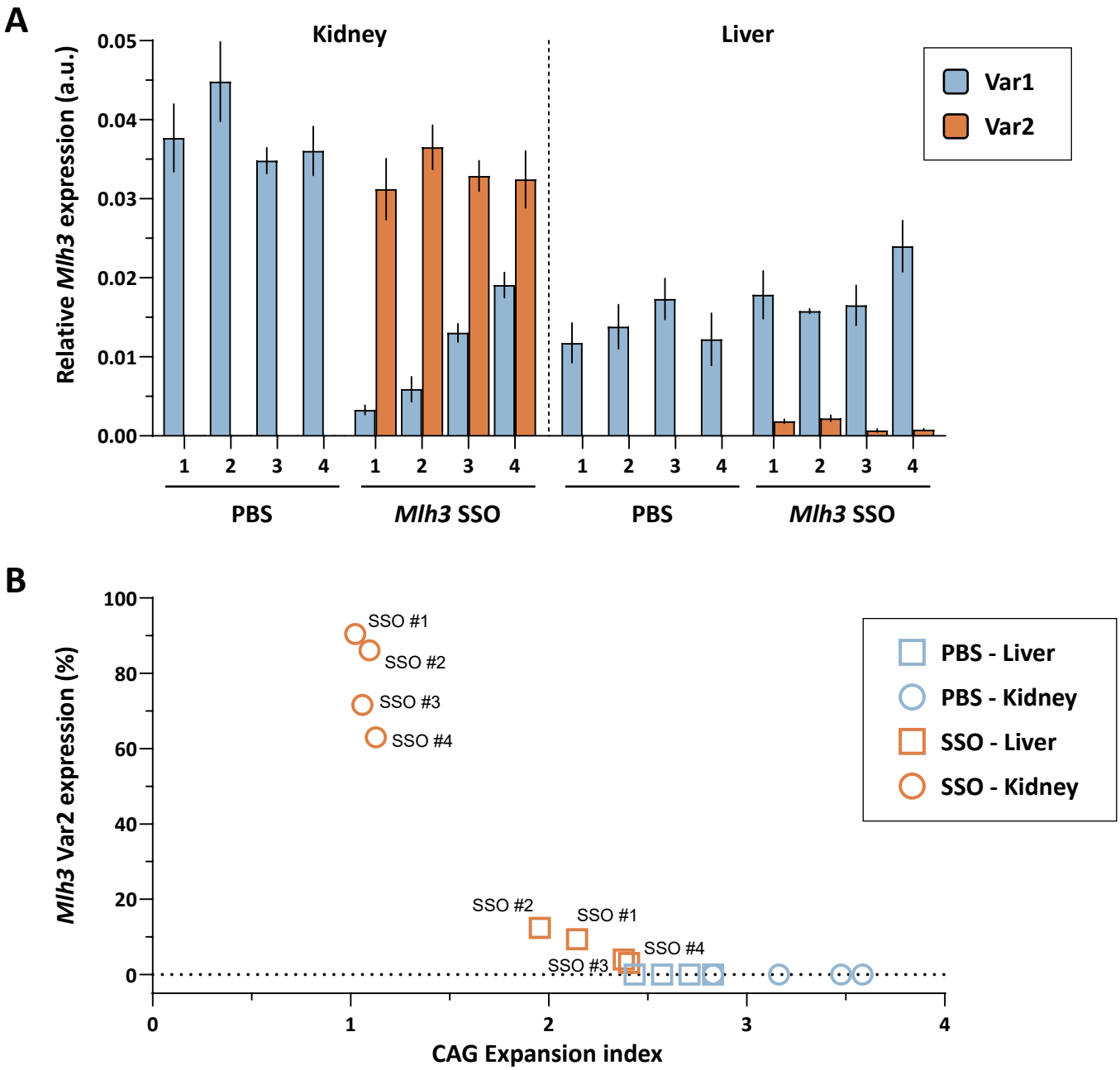
Figure S1. Mouse *Mlh3* exon 6 splice exclusion results in a variant that lacks the endonuclease domain but otherwise maintains the same translational frame.



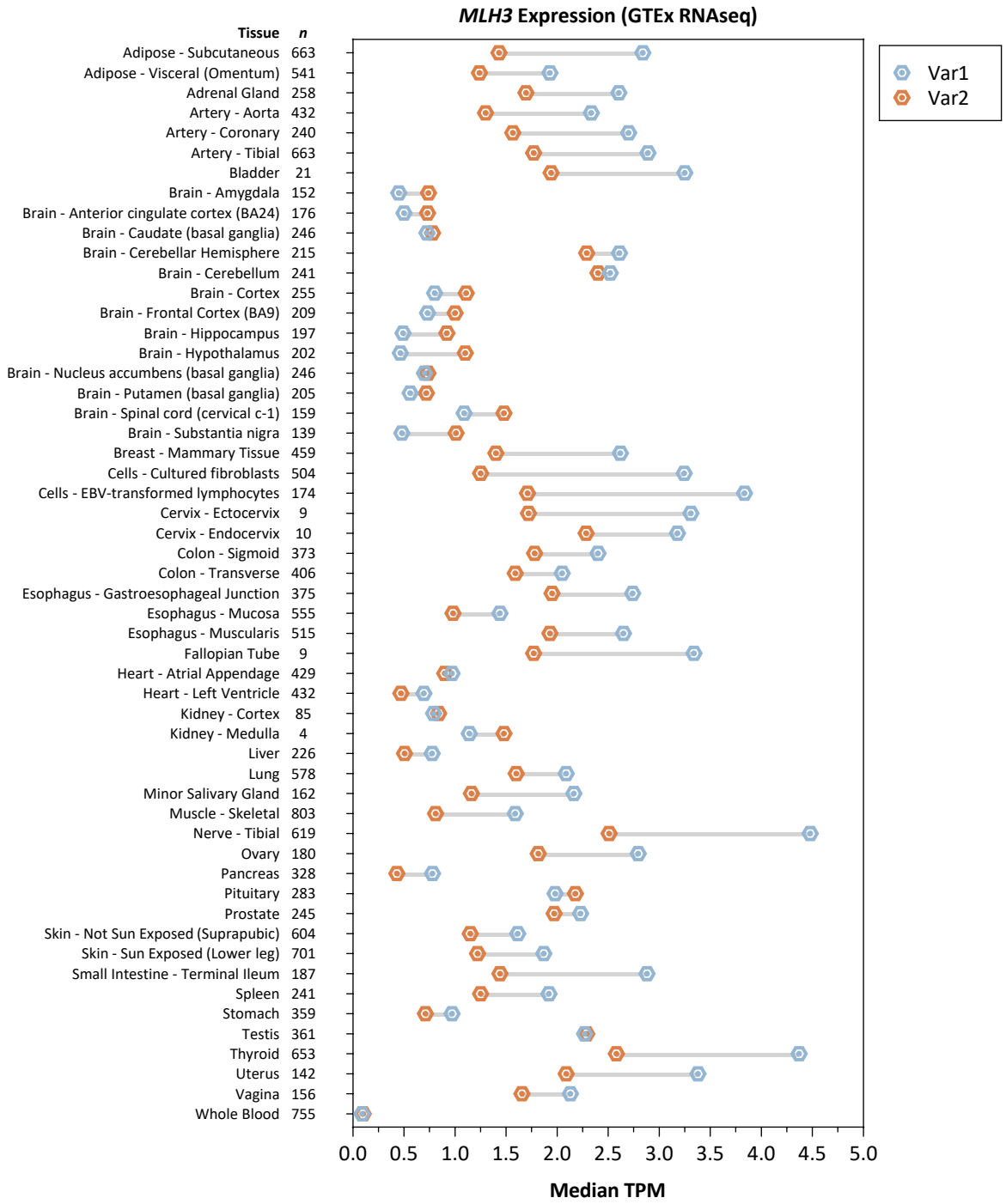
(**A**) Mouse *Mlh3* locus: chr12:85,232,251-85,272,250; GRCm38/mm10 assembly; NCBI RefSeq genes, curated subset; UCSC Genome Browser. (**B**) Splice redirection of mouse *Mlh3* using SSOs (mMlh3ac5 and mMlh3dr7) to exclude exon 6, which encodes the endonuclease domain (DQHAAHERIRLE). (**C**) Alignment of MLH3 protein isoforms encoded by human and mouse splice variants 1 and 2 (Var1 and Var2, respectively).

Figure S2. Relative expression of mouse *Mlh3* splice variants 1 and 2 after splice redirection with SSOs.



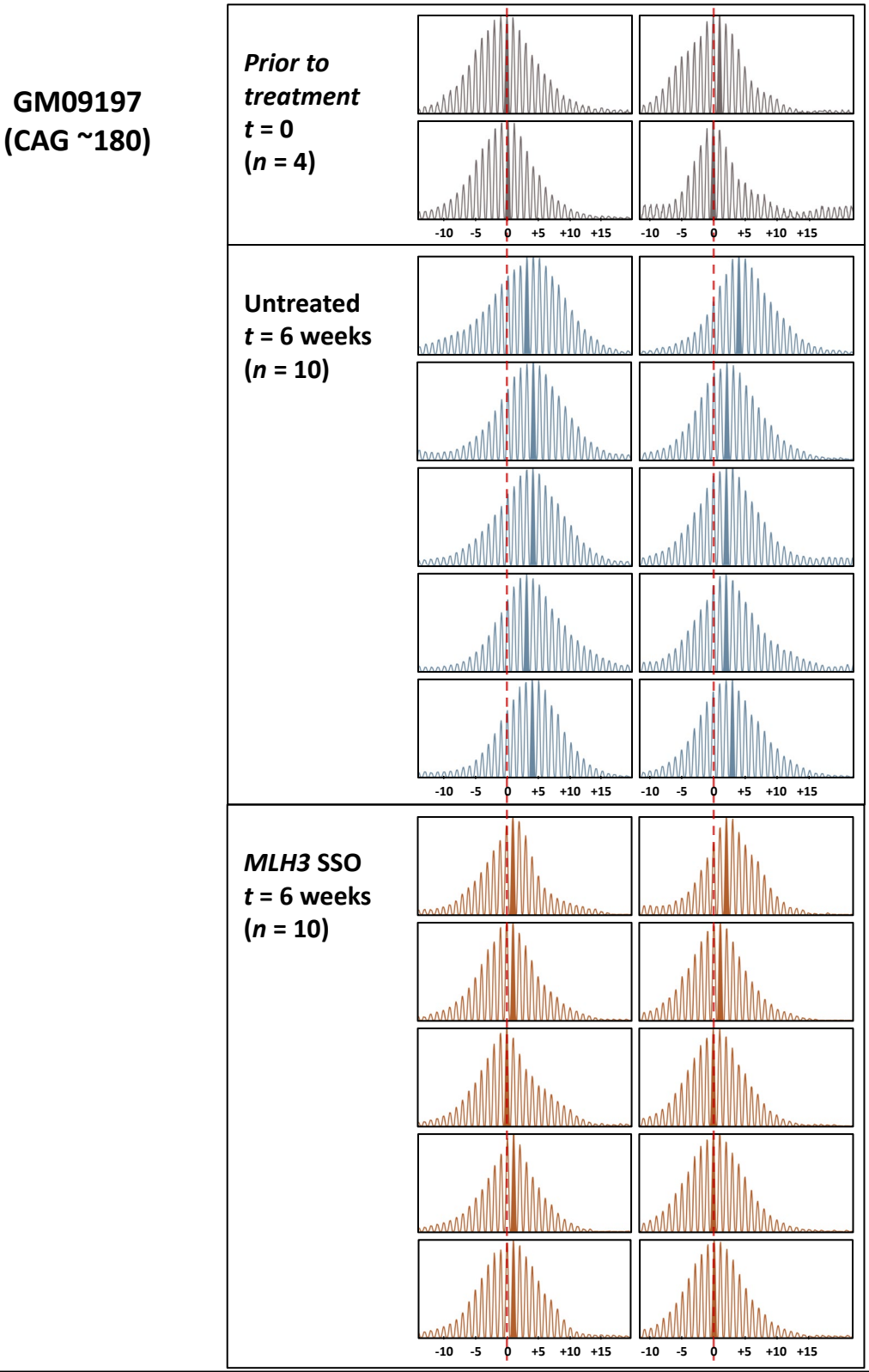
(**A**) Quantification of *Mlh3* splice variants by RT-qPCR in *Htt*^{Q111} mice (CAG 104-109) at 12 weeks of age, following 8-week-long treatment with either 25 mg/kg *Mlh3* SSOs (mMLH3ac5 and mMLH3dr7, 1:1 ratio) or PBS. Tissue collection was performed 24hrs after the final tail-vein injection. *Mlh3* SSO treatment successfully induced splice redirection from variant 1 (Var1) to variant 2 (Var2) in the kidney and, to a lesser degree, in the liver. *Mlh3* expression is relative to endogenous mouse β-actin (*Actb*). Since *Actb* mRNA levels can vary across different mouse tissues, relative *Mlh3* expression cannot be directly compared between kidney and liver. Biological replicates, $n = 4$ (two males and two females per group); technical replicates, $n = 3$; error bars represent standard deviation of the mean. (**B**) Relative abundance of *Mlh3* Var2 (% Var2 relative to total Var1+Var2) correlates negatively with CAG expansion.

Figure S3. Expression of human *MLH3* splice variants 1 and 2 across multiple tissues.



GTEx RNA-Seq data (analysis release V8, dbGaP Accession phs000424.v8.p2) was downloaded from the GTEx Portal on 07/08/2020 (GTEx_Analysis_2017-06-05_v8_RSEMv1.3.0_transcript_tpm.gct.gz). The median transcript TPM (Transcripts Per Kilobase Million) was calculated for all annotated *MLH3* transcripts (Ensembl gene version ENSG00000119684.15) across all available samples (*n* = 17,382 total samples; *n* = 4-803 samples per tissue). *MLH3* variant 1 (Var1) was calculated by combining two transcripts (ENST00000355774.6 and ENST00000556740.5), both of which, encode the full-length MLH3 protein isoform with the endonuclease domain (CCDS32123.1). *MLH3* variant 2 (Var2) represents a single transcript (ENST00000380968.6) that encodes the MLH3 protein isoform lacking the endonuclease domain (CCDS9837.1). The remaining *MLH3* transcripts detected do not represent any human consensus coding sequence (CCDS) and were excluded from this analysis.

Figure S4. *HTT* CAG repeat size distributions in HD patient-derived fibroblasts before and after six weeks of *MLH3* SSO treatment.



GeneMapper traces of *HTT* CAG repeat size distributions in primary HD fibroblasts (GM09197, CAG ~180) before (t = 0) and after six weeks of CAG expansion induction by ectopic MSH3 expression (t = 6weeks). During this period, cells were either treated with *MLH3* SSOs two times per week, or left untreated. Solid peaks indicate the modal CAG allele. Red lines represent the modal CAG at t = 0.