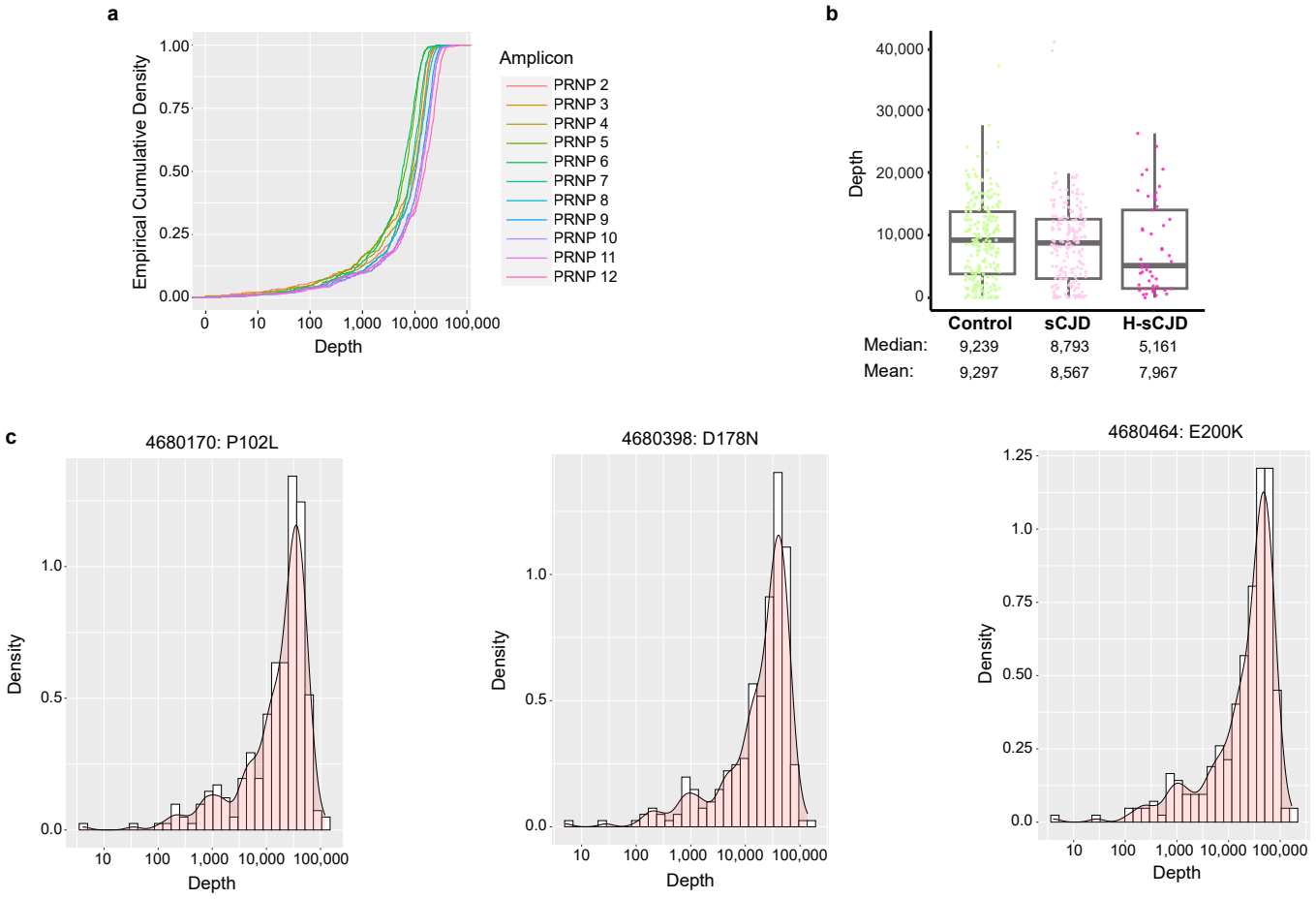
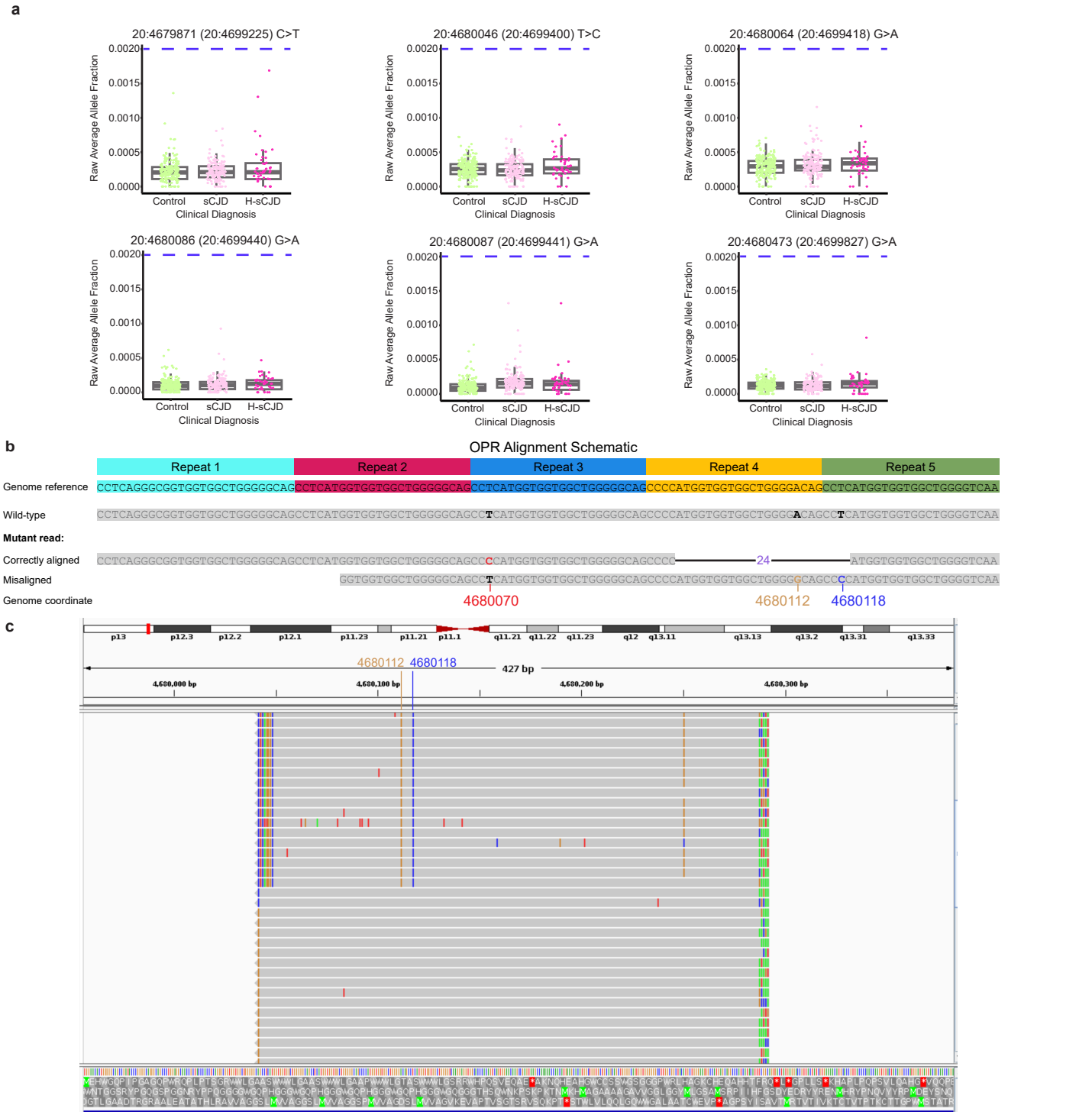
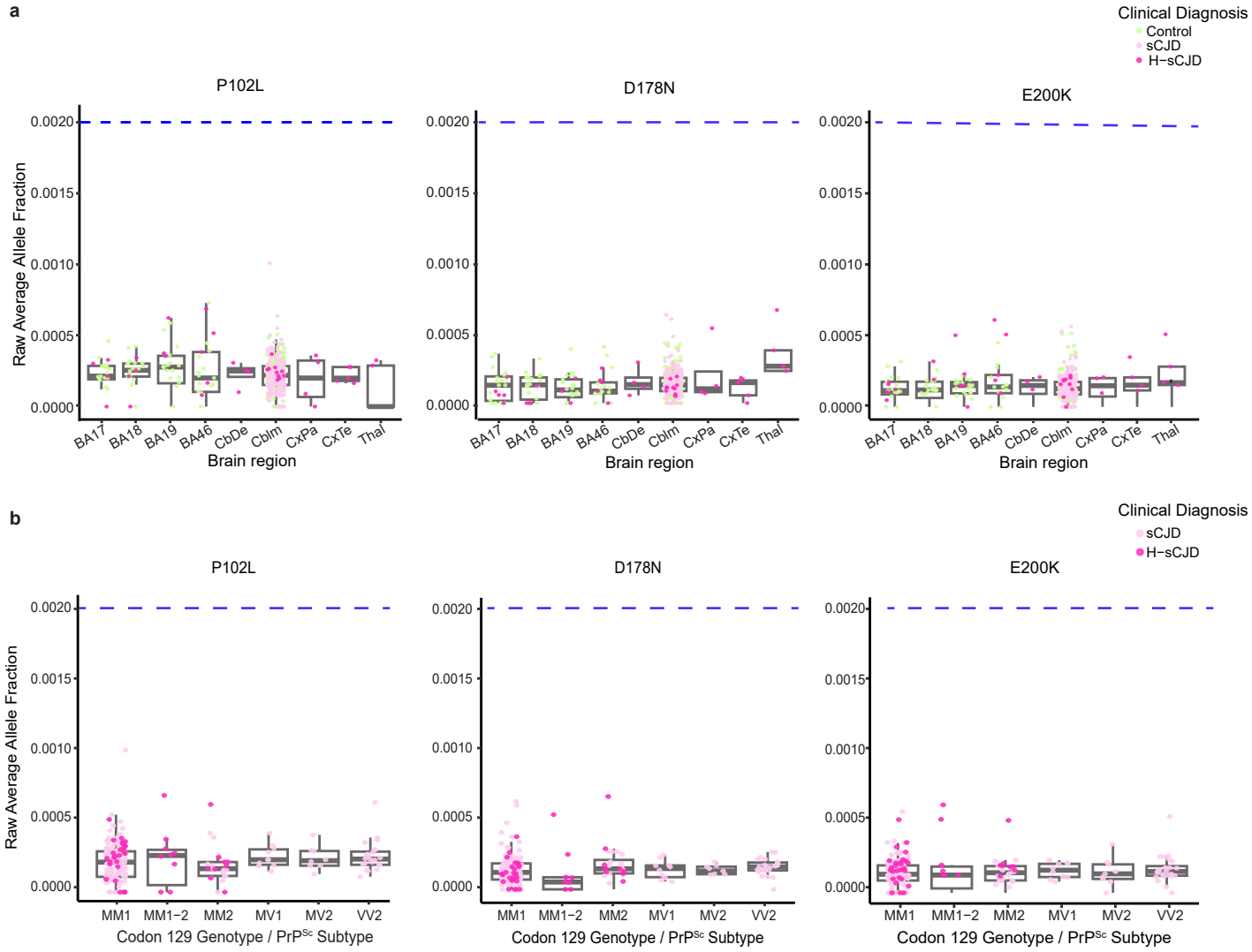
Supplemental Information for McDonough et al.
"Neuropathologically-directed profiling of *PRNP* somatic and germline variants in sporadic human prion disease"



Supplemental Fig. 1. Sequencing coverage. **a**) Sequencing coverage by amplicon. We obtained a median depth of coverage of >5,000X in the PRNP protein coding region, with 88% of all amplicons obtaining at least 1,000X coverage. An originally designed amplicon 1 was not studied, as its 5' PRNP coverage area was not necessary for triple amplicon coverage of the mature PrP sequence, thus the studied amplicons are named 2-12. **b**) Mean and median depths of coverage are displayed for each cohort: Control in green, non-Heidenhain variant sCJD in light pink, and Heidenhain-variant sCJD (H-sCJD) in dark pink. **c**) Overall coverage of P102L, D178N, and E200K germline disease-causing variant loci across all profiled samples in this study, as determined by the density of amplicon reads (y-axes) at a given depth of coverage (x-axes).



Supplemental Fig. 2. Evaluation of previously reported somatic SNVs in PRNP, through evaluation of read misalignment at OPR region and raw allele fractions. **a**) Raw average allele fractions of reported somatic SNVs in PRNP. Genomic positions that were covered with >1,000X depth across all covered amplicons were analyzed for previously reported somatic SNVs. Stringent variant calling and filtering criteria that were implemented for standard analysis were not performed, apart from exclusion of gnomAD variants in at least 0.1% population allele frequency. Genomic coordinates of each locus are shown with the GRCh37 position listed first, followed by the GRCh38 position in parenthesis. Each clinical diagnosis cohort is shown with control samples in green, non-Heidenhain sCJD samples (sCJD) in light pink and Heidenhain sCJD (H-sCJD) samples in dark pink. Each data point represents a different sample from each individual case. Boxplots show median and interquartile range. Dashed blue lines show the 0.2% VAF detection threshold for the variant calling pipeline. **b-c**) Two reported variants showed evidence in our data that their potential detection resulted from read misalignment in the OPR region. **b**) Schematic depicting observed misalignment of reads containing one OPR deletion and resulting artifactual appearance of SNVs. The OPR region is represented at the top, color coded by each OPR repeat. The reference genome sequence and the wild-type allele are also represented, with no SNVs or deletions. Below is the alignment of a read derived from the mutant allele, which contains a germline 24 bp deletion (1-OPRD) and a germline SNV at 4680070, in both the correctly aligned and misaligned form. While both mutant read alignments possess the same sequence, only the correctly aligned read reflects the appropriate SNV calls, as the misaligned read misses the deletion and the 4680070 germline SNV while introducing two false SNV calls at 4680112 and 4680118. **c**) IGV screenshot depicting reads of the wild-type (lower) and mutant (upper) alleles, where false SNV calls at 4680112 and 4680118 were introduced by the misaligned mutant reads.



Supplemental Fig. 3. Raw average allele fractions of known pathogenic germline PRNP alleles, analyzed by brain location and case subtypes. **a**) Raw average allele fractions of known pathogenic germline alleles in brain region subsets (BA: Brodmann area; CbDe: dentate nucleus; Cblm: cerebellum; CxPa: parietal cortex; CxTe: temporal cortex; Thal: thalamus). **b**) Raw average allele fractions of known pathogenic germline alleles in prion disease subsets. Each prion disease cohort that was present within our two disease cohorts is represented on the x-axes. No VV1 cases were present, while one case had intermediate 1-2 PrP protease-resistant core size and is designated as such here. In both parts, PRNP amplicon raw reads were analyzed for single nucleotide variants, upon removal of stringent variant calling and filtering that were implemented for standard analysis, as in Figure 4c. Control cases are represented in green, non-Heidenhain sCJD cases are represented in light pink, and Heidenhain sCJD cases are represented in dark pink. As with Supplemental Figure 2, each data point represents a different sample from each individual case. Dashed blue lines show the 0.2% VAF detection threshold for the variant calling pipeline.

Supplemental Table 1. Case information for all samples used in the study and MIPP-Seq primer information (found in Excel file). Sheets **a-c**) Sample ID, individual case ID, age measured in years at death, sex (M: Male; F: Female), brain regions included from each individual (Cblm: cerebellum; BA: Brodmann area; CxPa: parietal cortex; CxTe: temporal cortex; Thal: thalamus; CbDe: dentate nucleus), clinical diagnosis, codon 129 genotype/ PrPSc subset, and postmortem interval (PMI) measured in hours are included for each sample. Samples are separated by clinical diagnosis on each tab of the spreadsheet, **a**) controls, **b**) non-Heidenhain sCJD, **c**) Heidenhain sCJD. Sheet **d**) Forward and reverse primer sequences used for each of the 11 amplicons targeting PRNP are included along with corresponding GRCh37/hg19 genomic start sites and melting temperatures.