

Additional file 1: Supplementary Figures

Kabuki syndrome type 1 and type 2 converge on a shared chromatin and transcriptional state in hiPSC-derived neuroectoderm

Emile Danvin, Patricia Respuela, Eleonora Di Venere, Paolo Salerno, Giorgia Gemma, Gabriella Maria Squeo, Alvaro Rada-Iglesias, Giuseppe Merla

Figure S1. Reported pathogenic *KMT2D* and *KDM6A* variants around the edited positions.

Figure S2. Sanger verification of the three highest-probability off-target sites per guide.

Figure S3. eSNP-karyotyping from RNA-seq detects no arm-level allelic imbalance.

Figure S4. RNA-seq library quality.

Figure S5. ATAC-seq library quality.

Figure S7. Expression level and differential expression of WNT pathway genes.

Per-read evidence at the edited positions is provided separately as Additional file 2 (Figure S6).

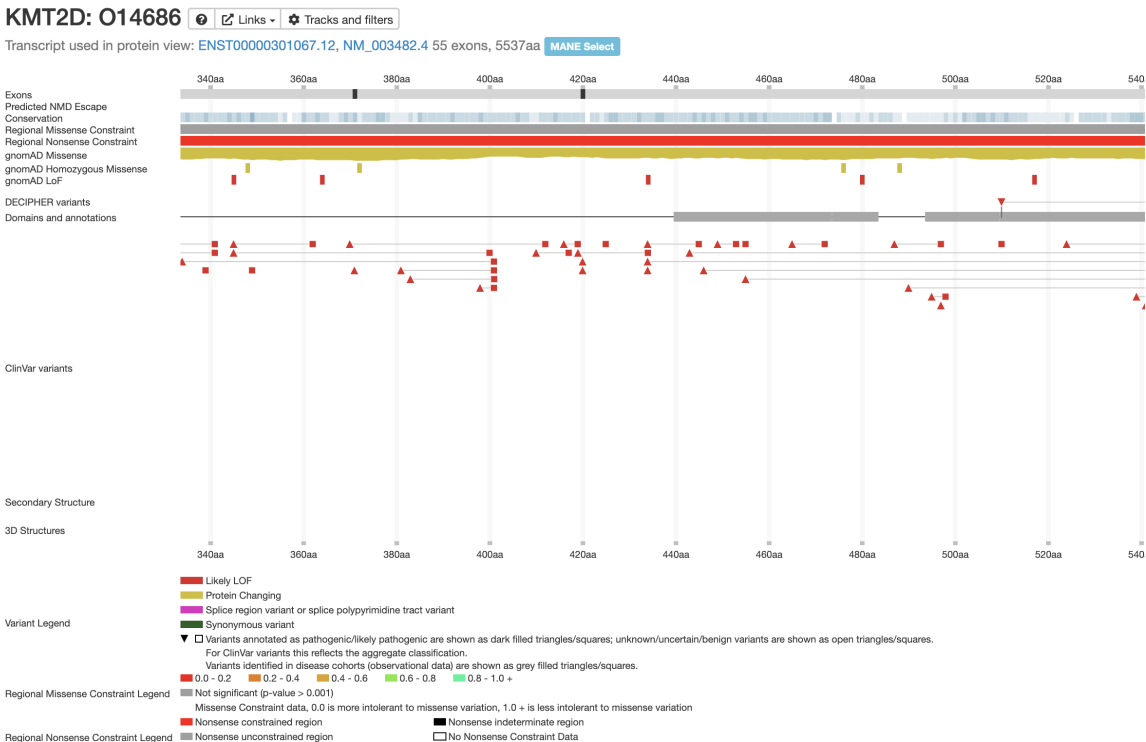
Figure S1. Reported pathogenic KMT2D and KDM6A variants around the edited positions.

Protein-level views from DECIPHER showing reported pathogenic and likely pathogenic variants, ClinVar variants, gnomAD constraint and annotated domains. **(A)** *KMT2D*, full protein (5,537 aa). **(B)** *KMT2D*, residues 335-540, the window containing the engineered p.(Asn435LysfsTer11) allele. **(C)** *KDM6A*, full protein (1,401 aa). **(D)** *KDM6A*, residues 1-270, the window containing the engineered p.(Asn103Ter) allele. Neither engineered variant has itself been reported in a patient; both create a premature termination codon of the same class as, and close to, variants reported in Kabuki syndrome.

(A) KMT2D, full protein



(B) KMT2D, residues 335-540



(C) KDM6A, full protein

KDM6A: O15550


 Links
  Tracks and filters

Transcript used in protein view: [ENST00000377967.9](#) 29 exons, 1401aa



(D) KDM6A, residues 1-270

KDM6A: O15550

 Links
 Tracks and filters

Transcript used in protein view: [ENST00000377967.9](#) 29 exons, 1401aa

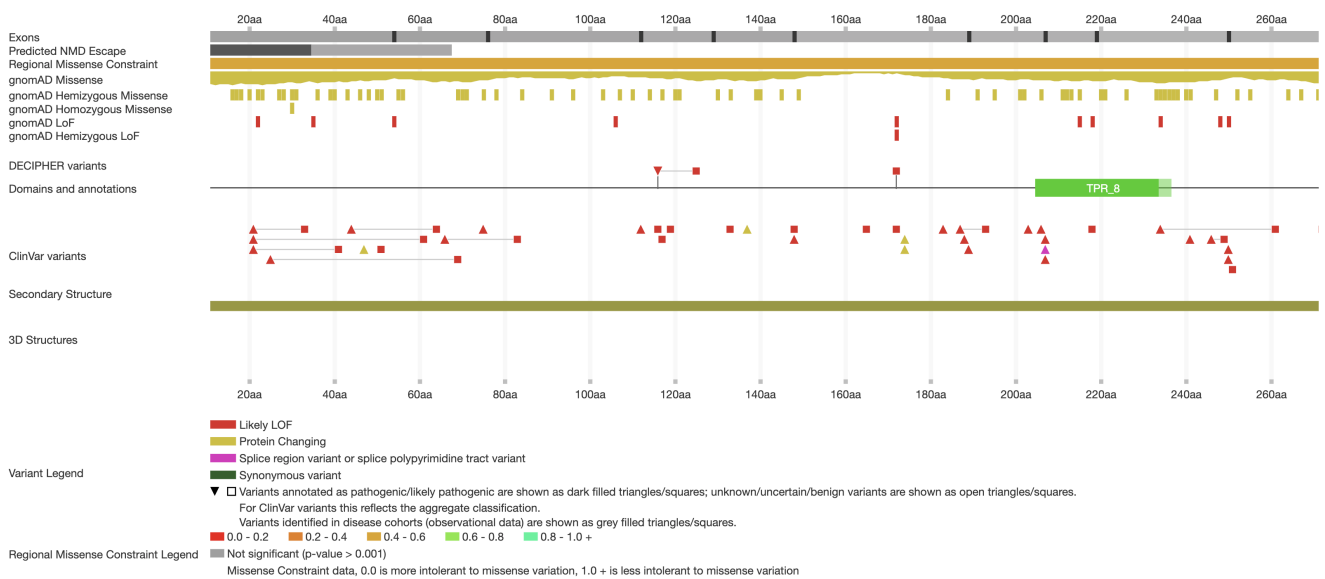
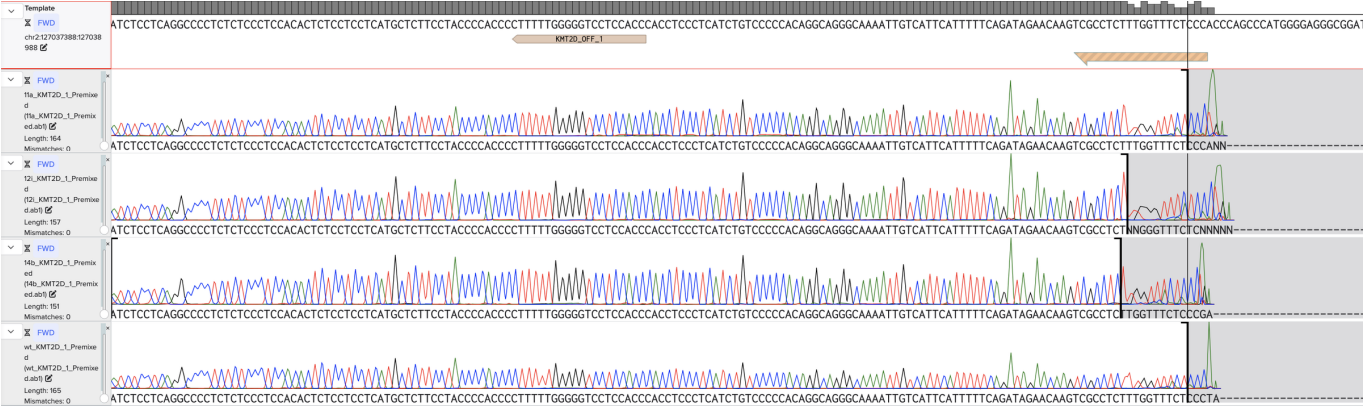


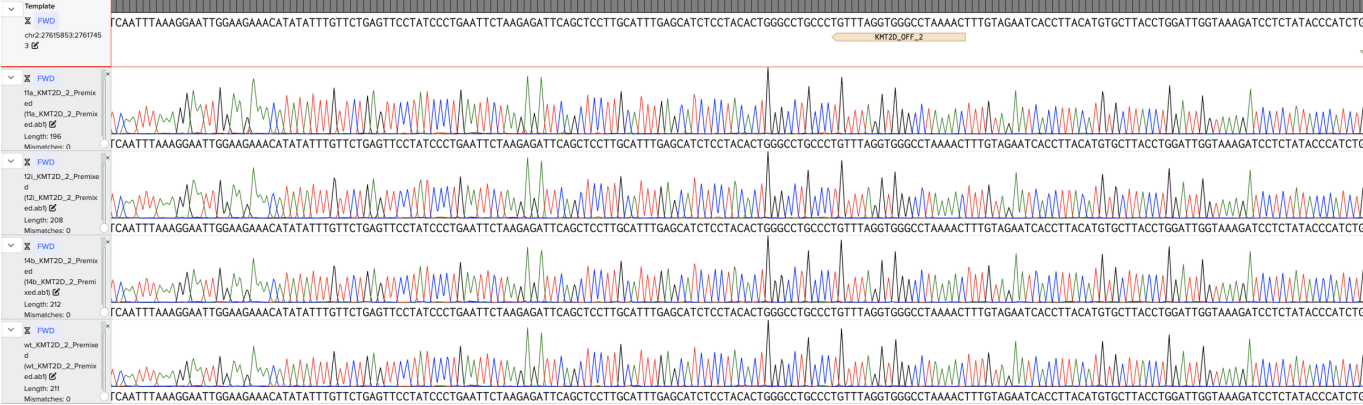
Figure S2. Sanger verification of the three highest-probability off-target sites per guide.

Chromatograms at the three top-ranked predicted off-target sites for each guide RNA, aligned to the reference template. Each panel shows the edited clones and the parental wild type; no editing was detected at any site in any clone (0 mismatches to the template in every trace). The *KDM6A* guide's third-ranked site lies in *UTY* on chromosome Y; the sequence variant seen there is a known benign single-nucleotide polymorphism and is unrelated to Cas9 editing.

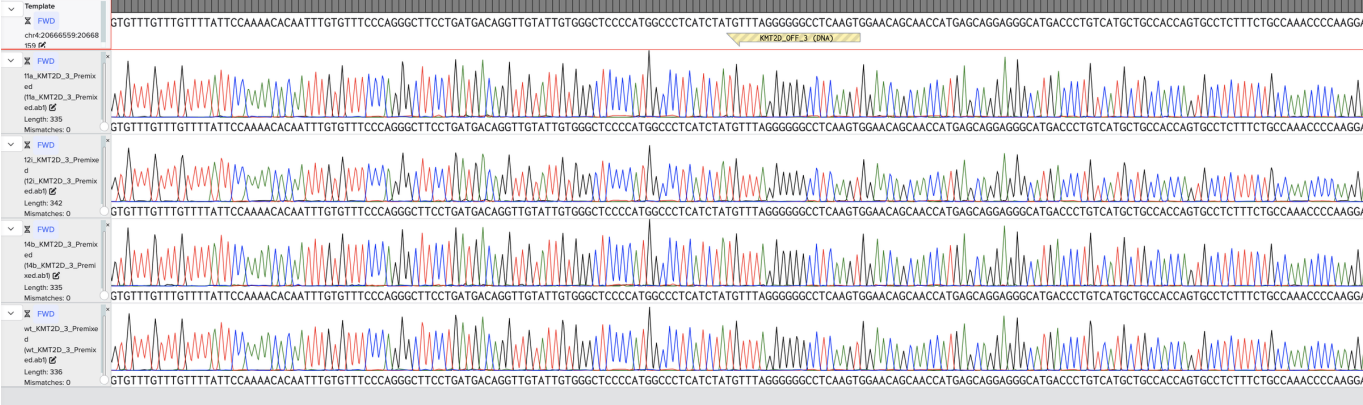
(A) KMT2D off-target 1, chr2:127,037,388-127,038,988



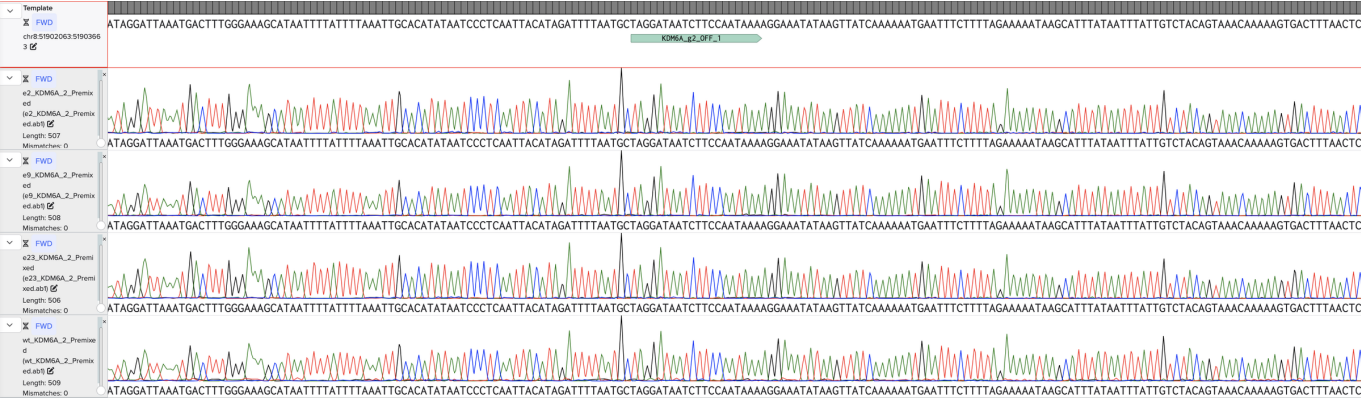
(B) KMT2D off-target 2, chr2:27,615,853-27,617,453



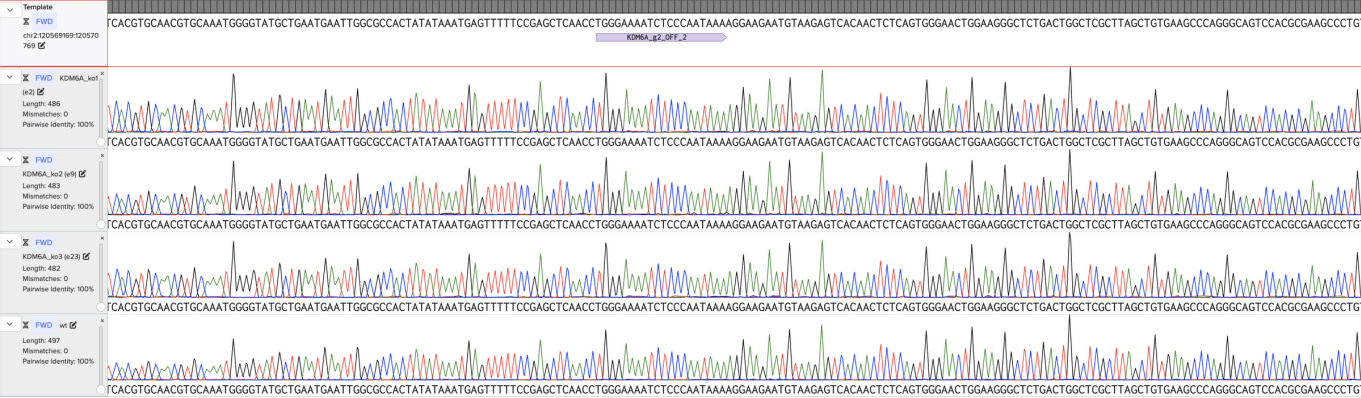
(C) KMT2D off-target 3, chr4:20,666,559-20,668,159



(D) KDM6A off-target 1, chr8:51,902,063-51,903,663



(E) KDM6A off-target 2, chr2:120,569,169-120,570,769



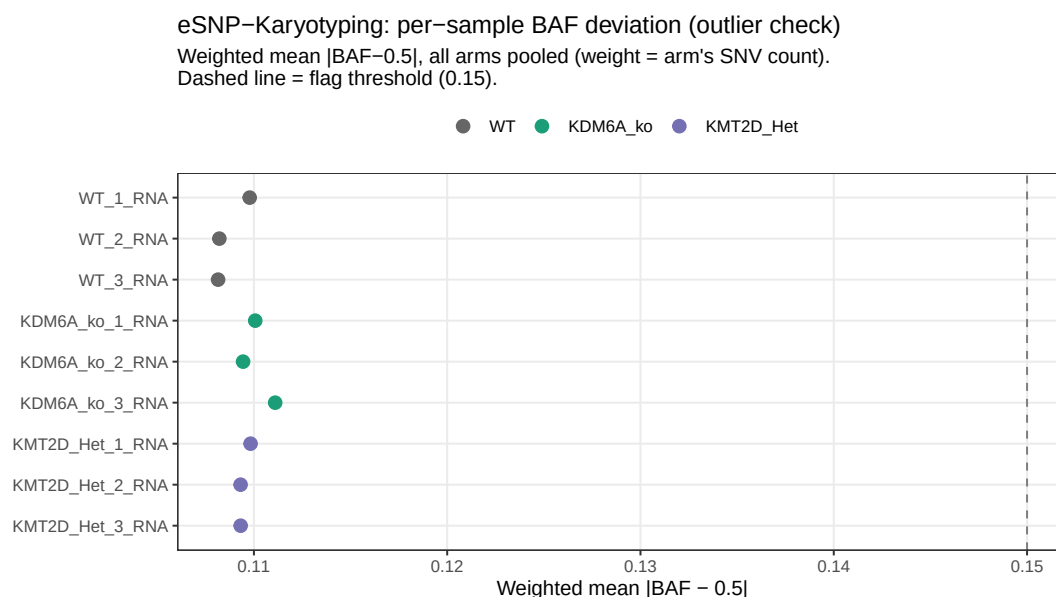
(F) KDM6A off-target 3, chrY:13,469,341-13,470,941 (UTY)



Figure S3. eSNP-karyotyping from RNA-seq detects no arm-level allelic imbalance.

Allelic imbalance was assessed from the RNA-seq data by measuring the B-allele frequency (BAF) of heterozygous single-nucleotide variants. **(A)** SNV-weighted mean $|BAF - 0.5|$ per sample, pooled across chromosome arms and weighted by each arm's SNV count, against the 0.15 flagging threshold (dashed line); no sample is flagged. **(B)** Genome-wide B-allele frequency by chromosome, all nine samples overlaid and coloured by genotype (heterozygous PASS biallelic SNVs, depth ≥ 20 ; dashed line, BAF = 0.5; vertical lines mark centromeres). A clonal gain or loss would appear as a coherent shift of one chromosome arm away from 0.5; none is present. The single X chromosome of this male line yields almost no heterozygous SNVs, hence the sparse chrX panel.

(A) Per-sample BAF deviation



(B) Genome-wide B-allele frequency by chromosome

eSNP-Karyotyping: genome-wide B-allele frequency by chromosome
Heterozygous PASS biallelic SNVs, depth >= 20. Vertical line = centromere.

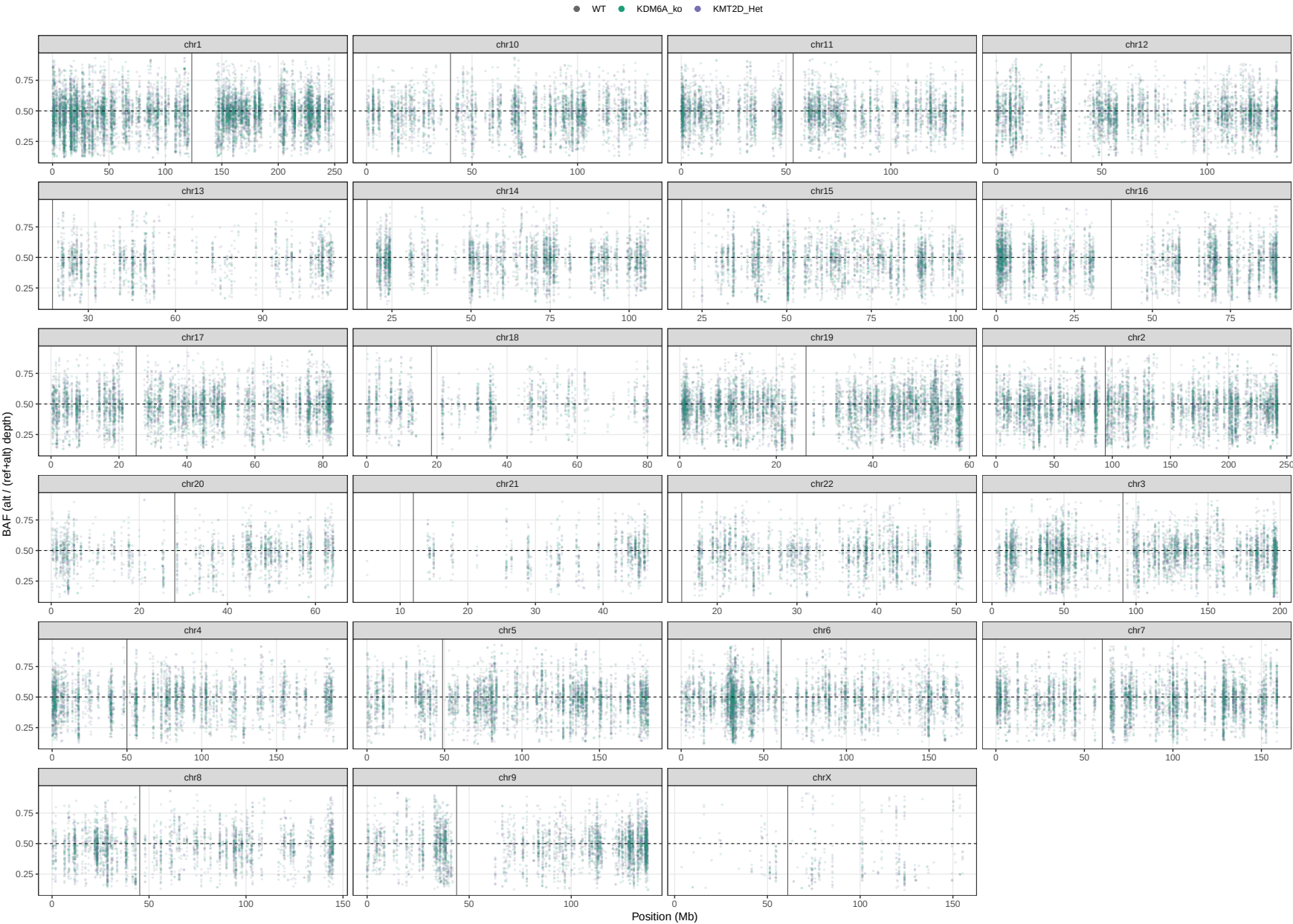


Figure S4. RNA-seq library quality.

Quality metrics for the nine RNA-seq libraries (three independent clones for each of wild type, KMT2D_Het and KDM6A_ko). Top row: sequencing depth in million read pairs, percentage of uniquely mapped reads (STAR), GC content, 5'-3' coverage bias (Qualimap), and principal component analysis of variance-stabilised counts. Bottom row: distribution of aligned reads by genomic feature, normalised gene body coverage, and mean per-base sequence quality of the raw reads. Grey, wild type; orange, KMT2D_Het; blue, KDM6A_ko.

RNA-seq library quality (n = 9)
sequencing depth, alignment, GC content, 5'-3' bias, sample clustering, read origin, gene body coverage, and per-base quality

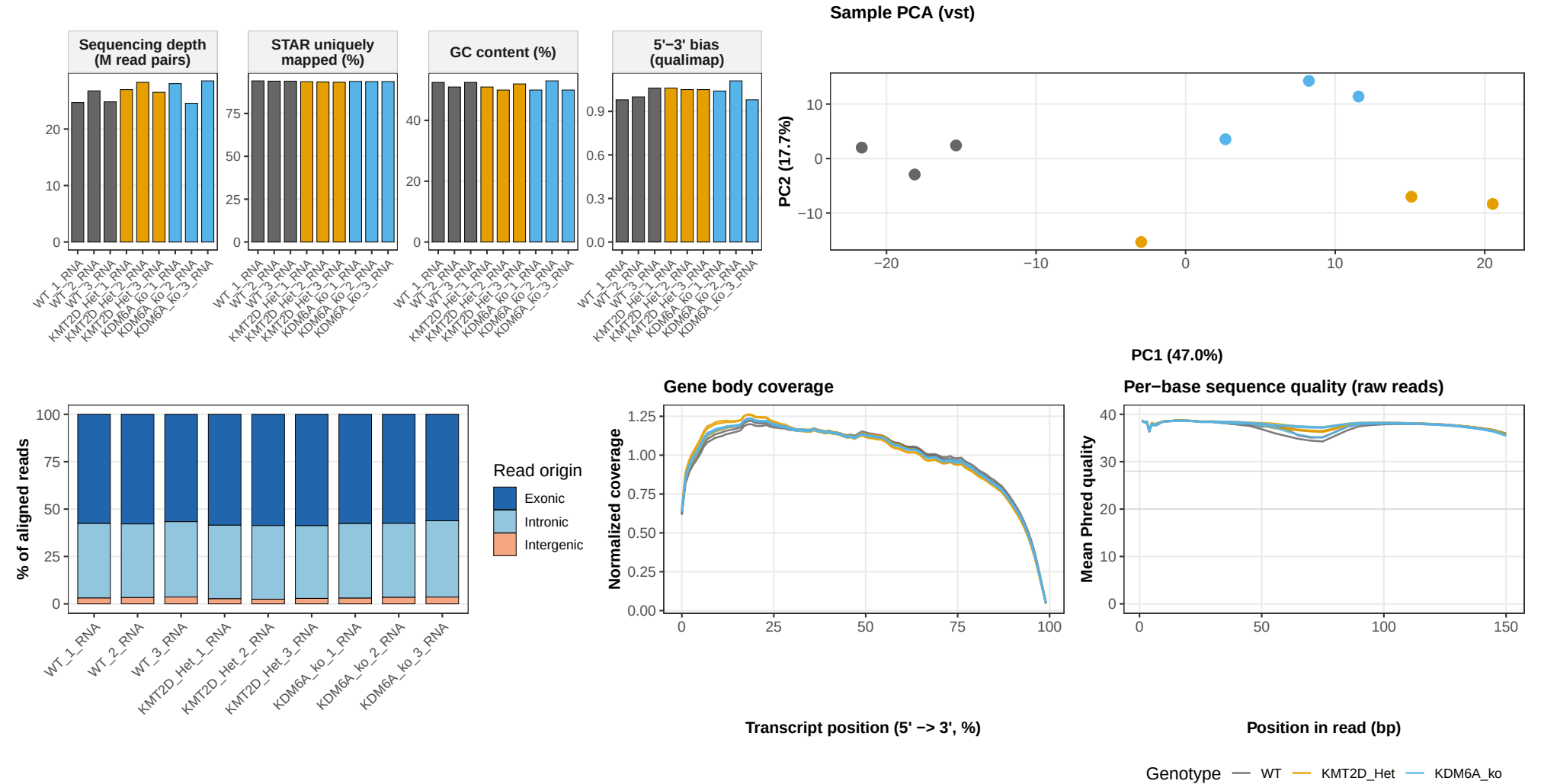


Figure S5. ATAC-seq library quality.

Quality metrics for the nine ATAC-seq libraries (three independent clones per genotype). Top row: TSS enrichment score computed with the ENCODE definition, mean ATAC signal around transcription start sites, and principal component analysis of variance-stabilised, loess-normalised counts. Bottom row: usable reads after filtering, number of peaks called per sample, mean per-base sequence quality of the raw reads, and the fragment-size distribution showing nucleosome periodicity. Grey, wild type; orange, KMT2D_Het; blue, KDM6A_ko.

ATAC-seq library quality (n = 9)

TSS enrichment (score + profile), sample clustering, depth/peak yield, and read quality

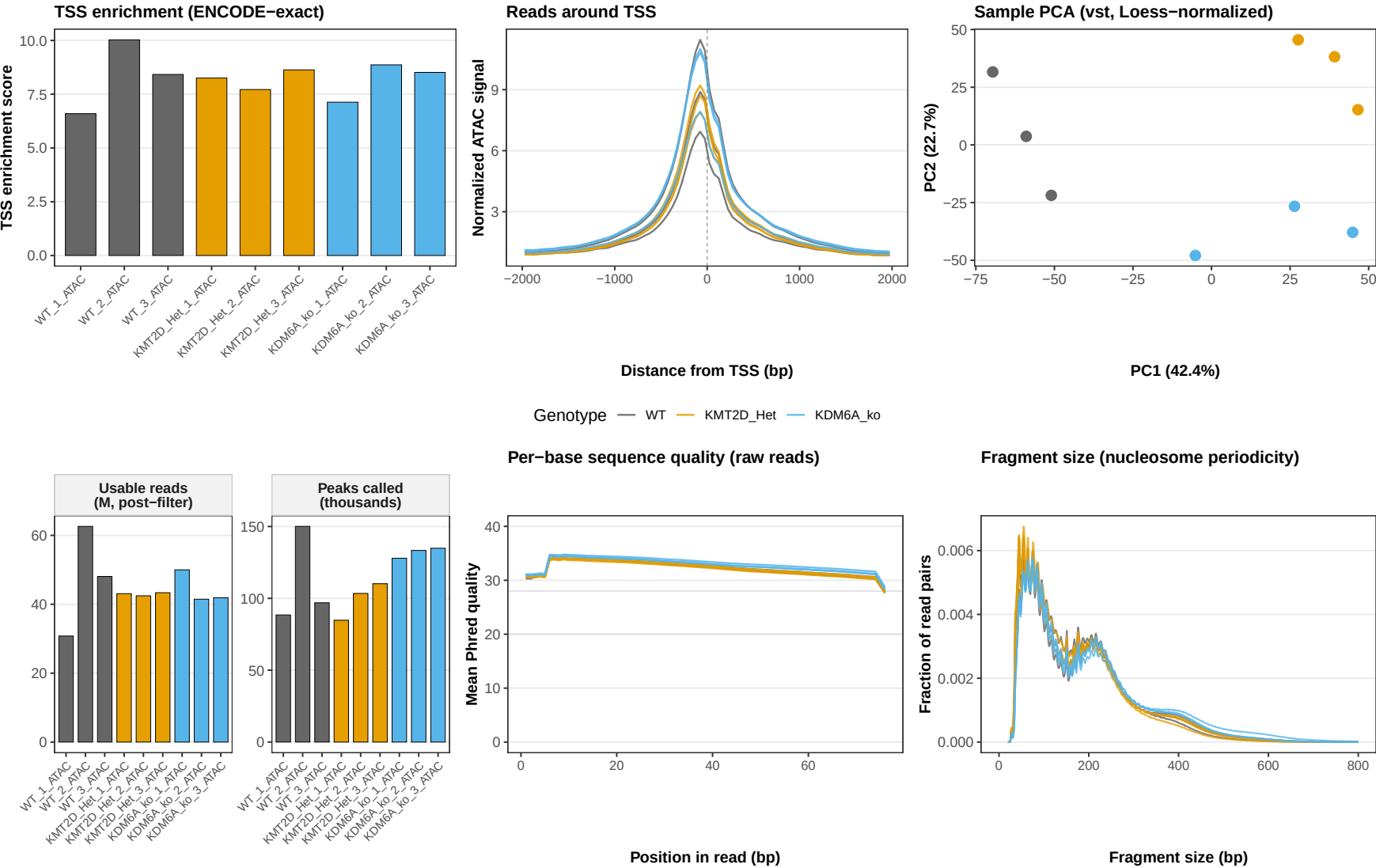


Figure S7. Expression level and differential expression of WNT pathway genes.

Left, mean normalised RNA-seq counts per genotype (log scale). Right, log₂ fold change versus wild type for the same genes in the same order; filled circles, adjusted p < 0.05; open circles, not significant. No fold-change threshold is applied in this figure, unlike the differential expression calls in the main text. Genes are grouped as canonical ligands, other ligands, receptors, secreted antagonists, cytoplasmic and nuclear components, and target genes. *WNT1* and *WNT10B* are expressed above *WNT3A* in wild-type cells and are not differentially expressed in either mutant. Grey, wild type; orange, KMT2D_Het; blue, KDM6A_ko.

