

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

Figure S1: UPD subtype and mechanism

Heterodisomy arises from Meiosis 1 nondisjunction followed by gamete restoration (also termed gamete complementation, where a disomic gamete is rescued by a nullisomic gamete from the other parent) or mitotic rescue, resulting in inheritance of two homologous but non-identical chromosomes from one parent. Isodisomy arises from Meiosis 2 nondisjunction or mitotic error followed by gamete restoration or mitotic rescue, resulting in two identical copies of a single parental chromosome. Mixed UPD involves recombination followed by Meiosis 1 or 2 nondisjunction and gamete restoration or mitotic rescue, producing regions of both heterodisomy and isodisomy along the same chromosome. Segmental UPD results from somatic recombination in the zygote, affecting only a portion of a chromosome. Concepts summarized from: Benn (2021).

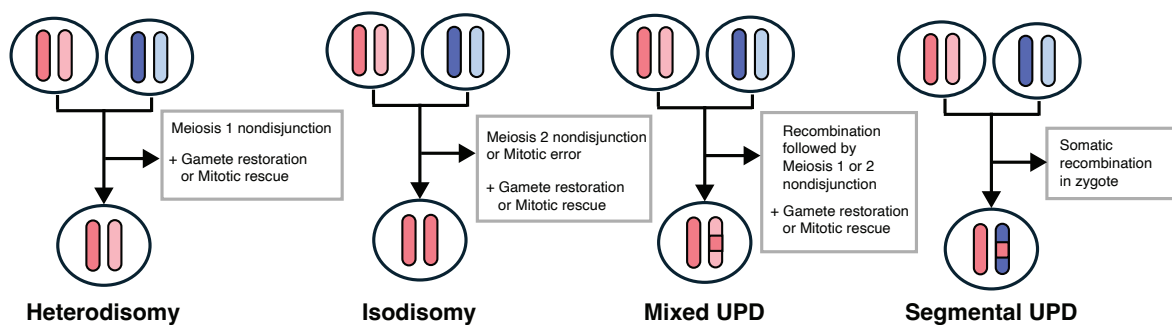


Figure S2: Paternal age at birth

Paternal age at child's birth by cohort and UPD status (PCGC and 23andMe); Result for the 23andMe cohort were obtained from Nakka et al. (2019). Boxplots show median, IQR, and $1.5 \times$ IQR whiskers.

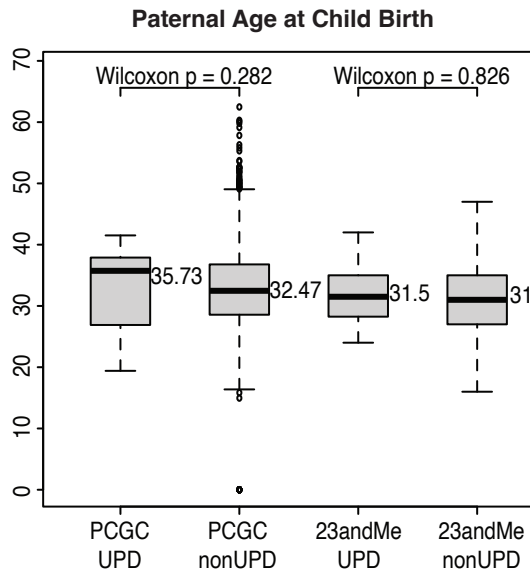


Figure S3. Gene-level burden analysis of rare variants in chromosomal segregation genes.

(A) Case-control gene burden analysis in children. Rare (AF < 0.001) loss-of-function (LoF) or missense variants in predefined candidate genes involved in synaptonemal complex formation, meiotic recombination, cohesin function, spindle assembly checkpoint regulation, centrosome biogenesis, and spindle positioning were compared between UPD cases and controls using Fisher's exact test. Forest plots show odds ratios (ORs) with 95% confidence intervals (CIs), raw P values, and Bonferroni-adjusted P values.

(B) Case-control gene burden analysis in parents, performed as in (A). Forest plots display odds ratios (ORs) with 95% confidence intervals (CIs), raw P values, and Bonferroni-adjusted P values for rare LoF or missense variants identified in the selected candidate genes.

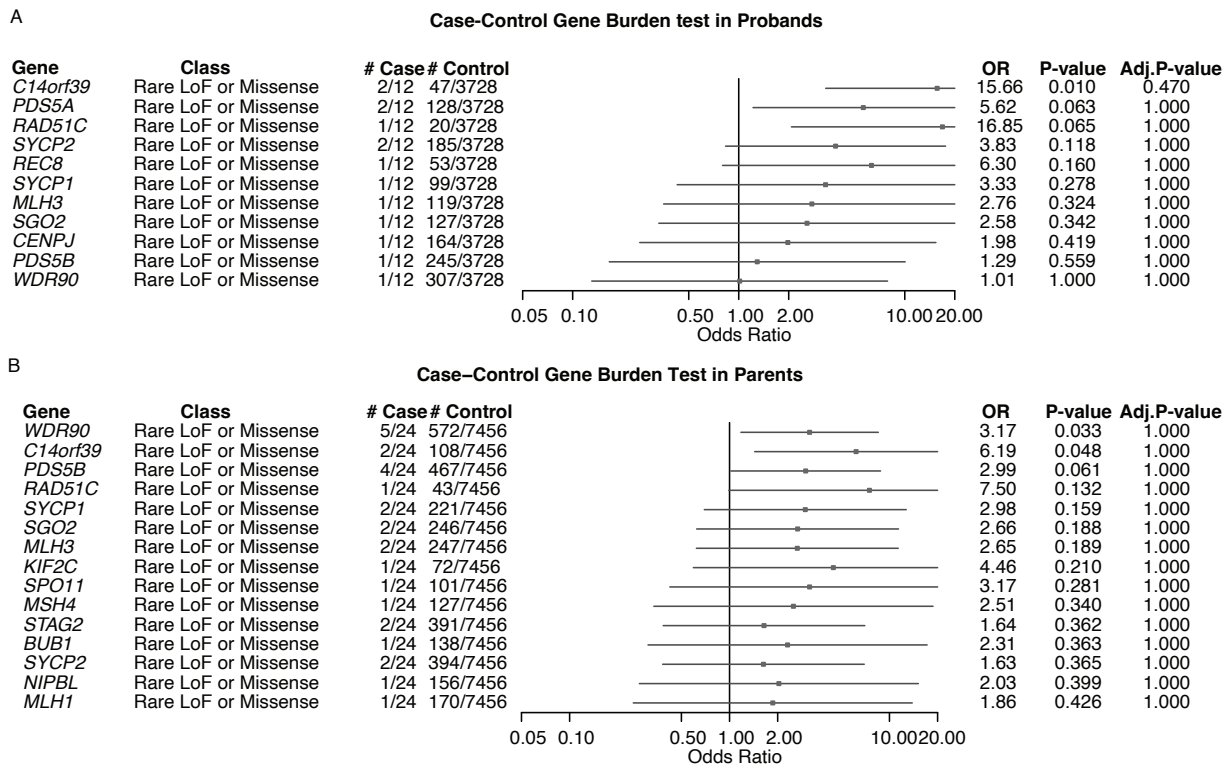


Figure S4: PIEZO1 Patch-Clamp and GCaMP6f analysis

(A) Whole-cell voltage clamp following activation by cellular indentation. Quantification of max current density and channel inactivation kinetics are shown following a 5 μm deep cellular indentation. Each point

represents the response of an individual cell.

(B) Transfection of *Piezo1* WT and p.P42L GCaMP6f plasmids into human ECs (HUVECs). Cells were fixed and immunostained with GFP antibody to enhance the GCaMP6f signal. Both WT and p.P42L channels show active channels; however, the density of channel puncta is decreased in the p.P42L condition. (Scale bar, 5 μm).

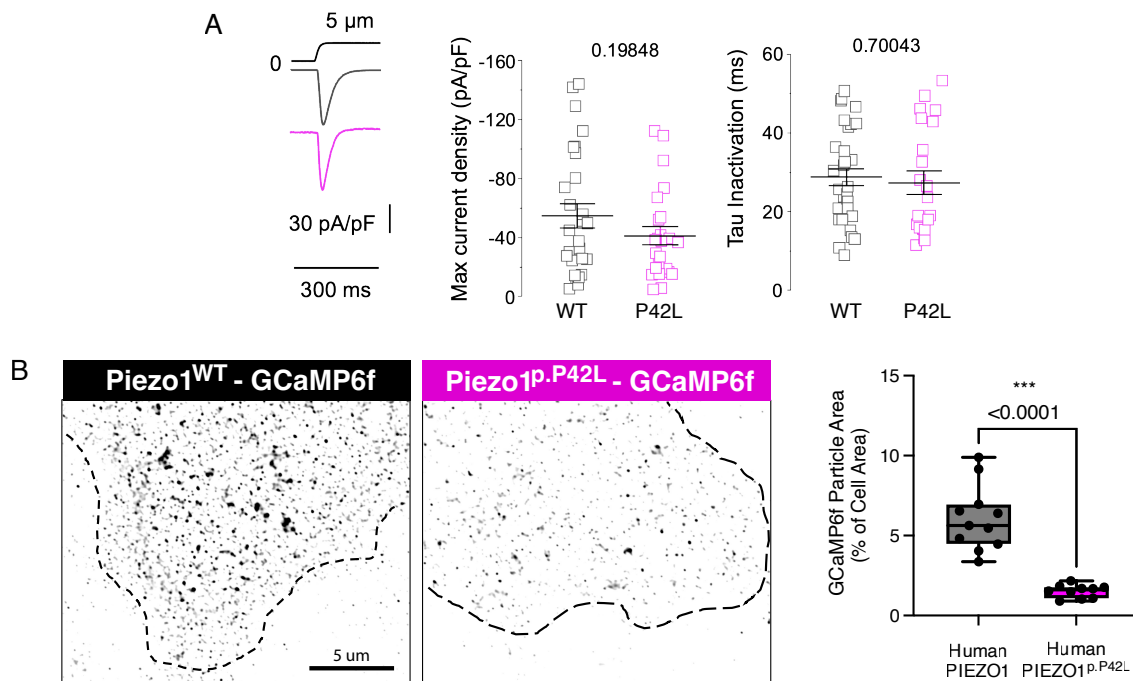


Figure S5: Effect of *MESD* Pro29Ser variant on LRP6 localization in HUVECs

Representative immunostaining images of total LRP6, phospho-LRP6, and phalloidin (actin) in non-transfected (NT) control, *MESD* WT, and *MESD* Pro29Ser variant transfected HUVECs. Cells were fixed 24 hours after transfection of the indicated plasmid. Arrows highlight plasma membrane boundaries. (Scale bar, 50 μ m).

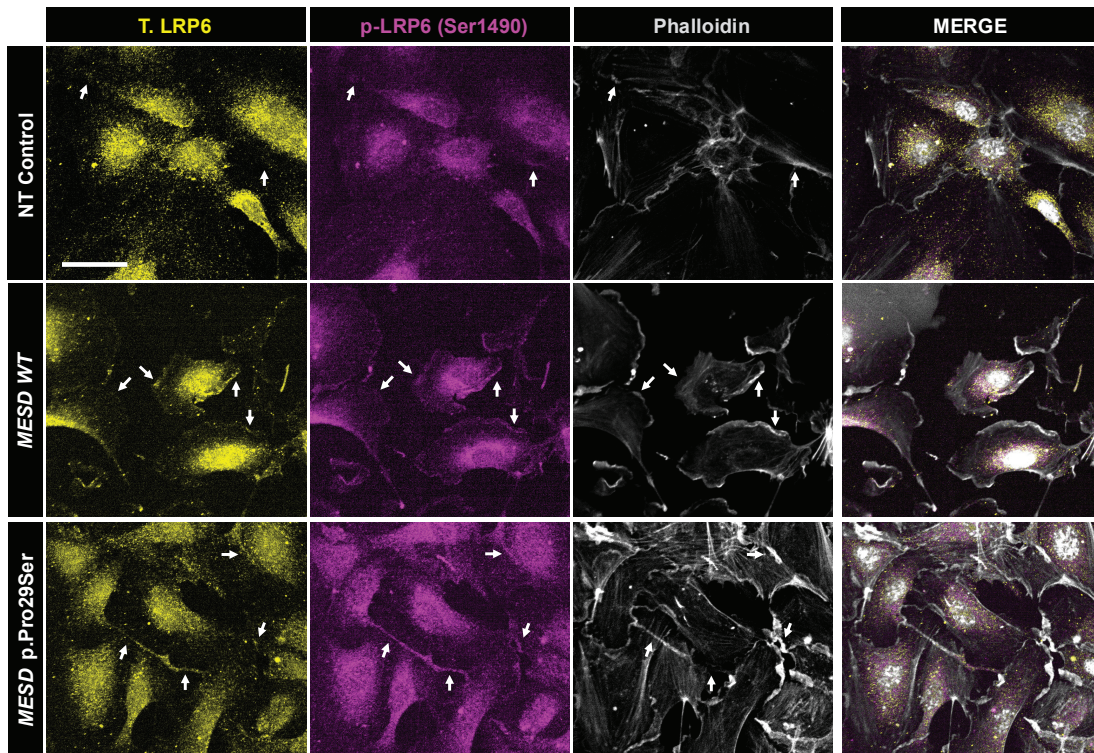


Figure S6. CRISPR targeting strategy for *mesd* and *glyr1*.

Gene structures of (A) *mesd* and (B) *glyr1* showing exon organization and CRISPR sgRNA target sites used for genome editing. sgRNAs were designed to target coding exons to generate loss-of-function alleles.

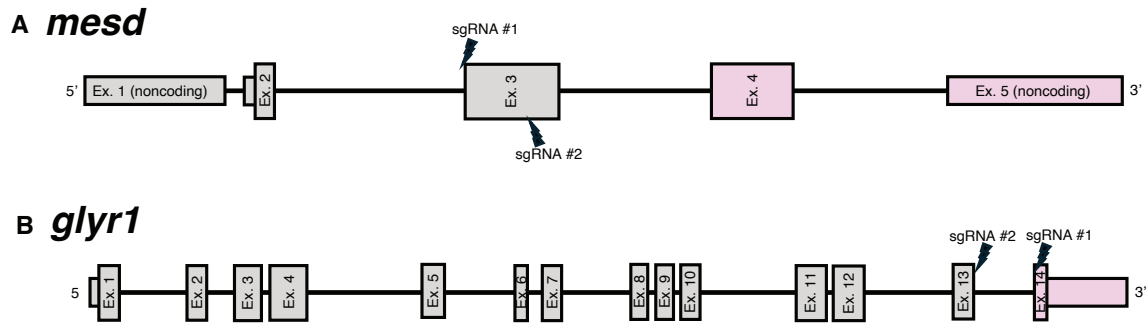


Figure S7: TrioMix-UPD workflow

Overview of the TrioMix-UPD pipeline for detecting uniparental disomy (UPD) from trio sequencing data. Variants are first called using Samtools mpileup and then grouped based on parental genotypes: Group A (one parent 0/0, the other 1/1) and Group B (one parent 0/0, the other 0/1). Expected variant allele frequency (VAF) patterns in the child are then evaluated to distinguish normal inheritance, UPD isodisomy, and UPD heterodisomy. The diagrams illustrate these expected patterns using an example of maternal UPD. The resulting VAF signatures across informative variants are used to classify UPD events.

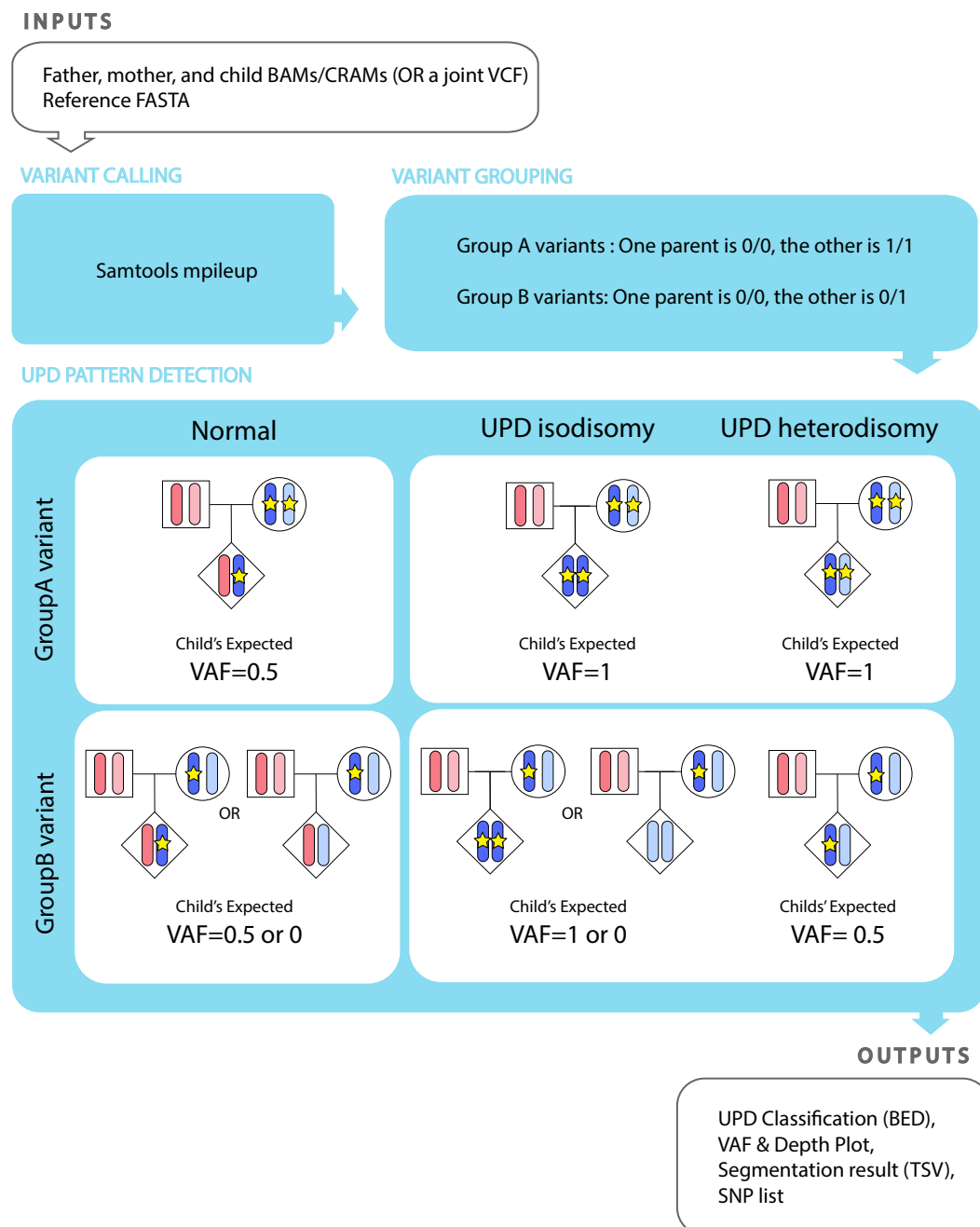


Figure S8: Long read QC.

Quality metrics for PacBio long-read sequencing across four trios with UPD children (1-02917, 1-03820, 1-06216 and 1-14785) and 82 additional control parents from PCGC cohort. (A) Mean sequencing coverage per sample. (B) Mean read length per sample. (C) Read length N50 per sample.

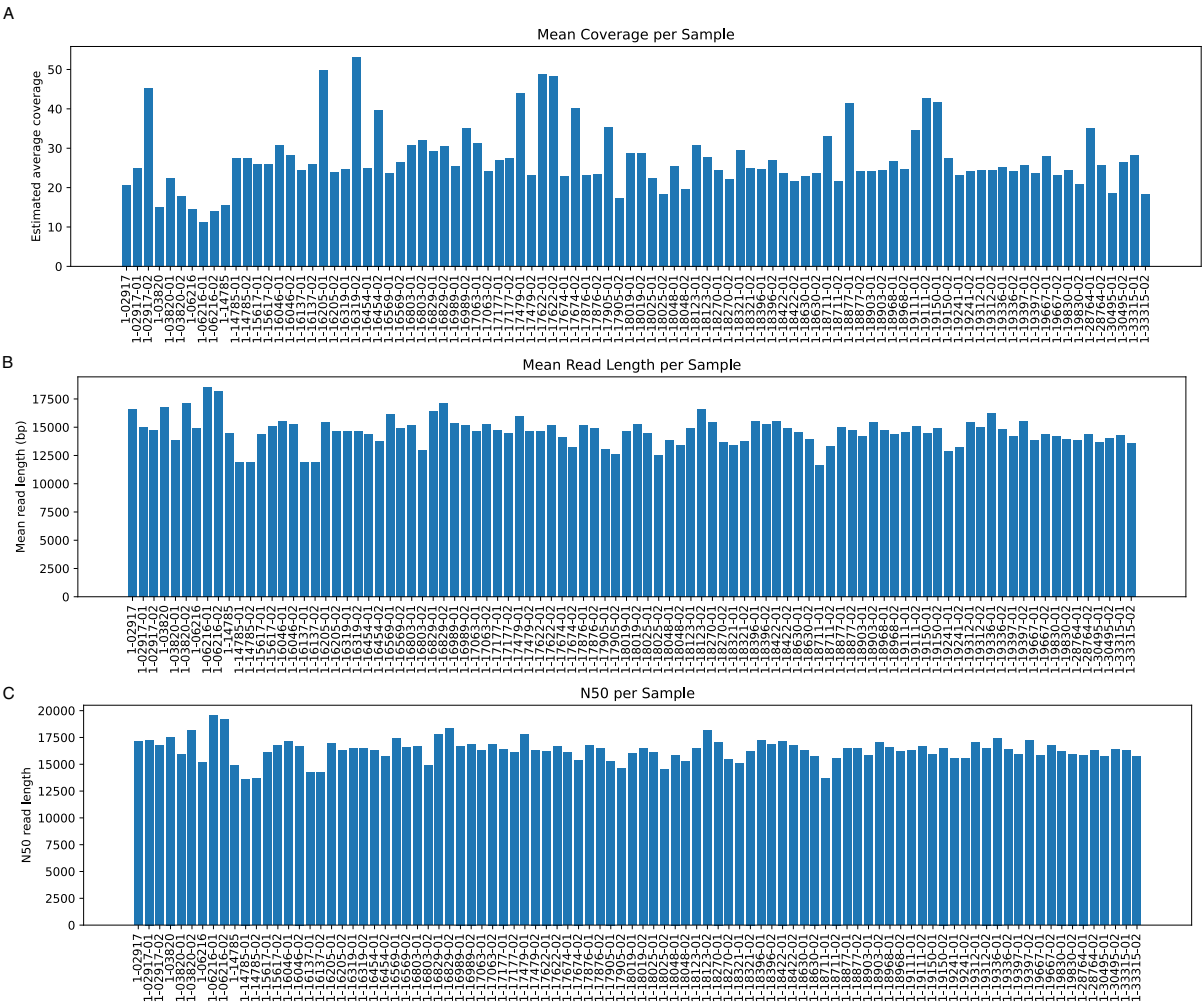


Figure S9: Long read SV pipeline

PacBio HiFi reads from the UPD child and both parents were aligned to hg38 using pbmm2, and structural variants (SVs) were called with Sniffles2. Using bcftools and Truvari, SVs that were homozygous in the child, heterozygous in the UPD-inheriting parent, and absent in the other parent were identified. Common variants (minor allele frequency (MAF) > 0.01) were removed using the 1000 Genomes long-read SV database, and the remaining SVs were annotated with AnnotSV. Candidate SVs were prioritized if they were located within the isodisomy region, overlapped genes or regulatory elements, and passed ACMG-based filtering, followed by IGV inspection.

