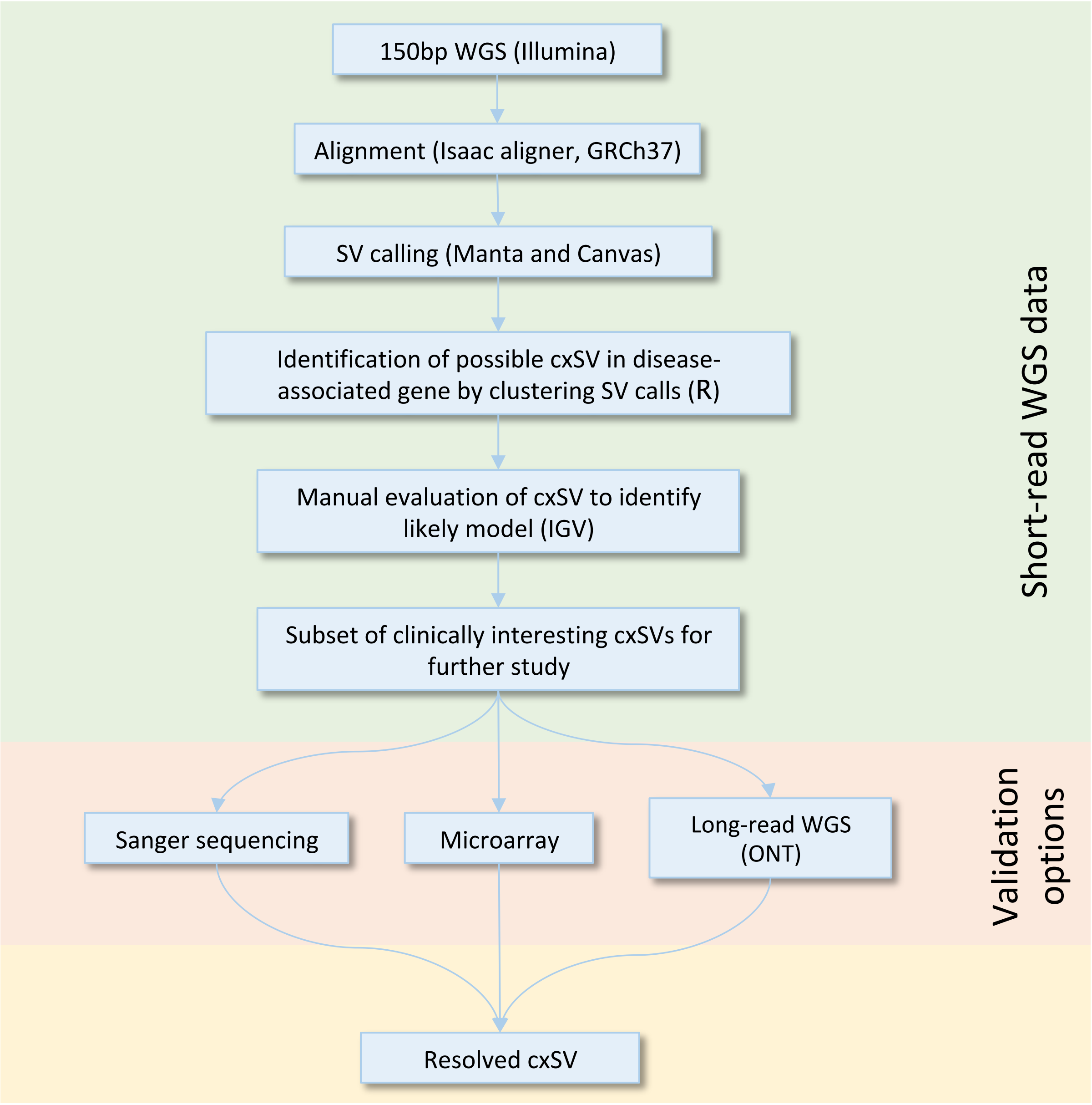
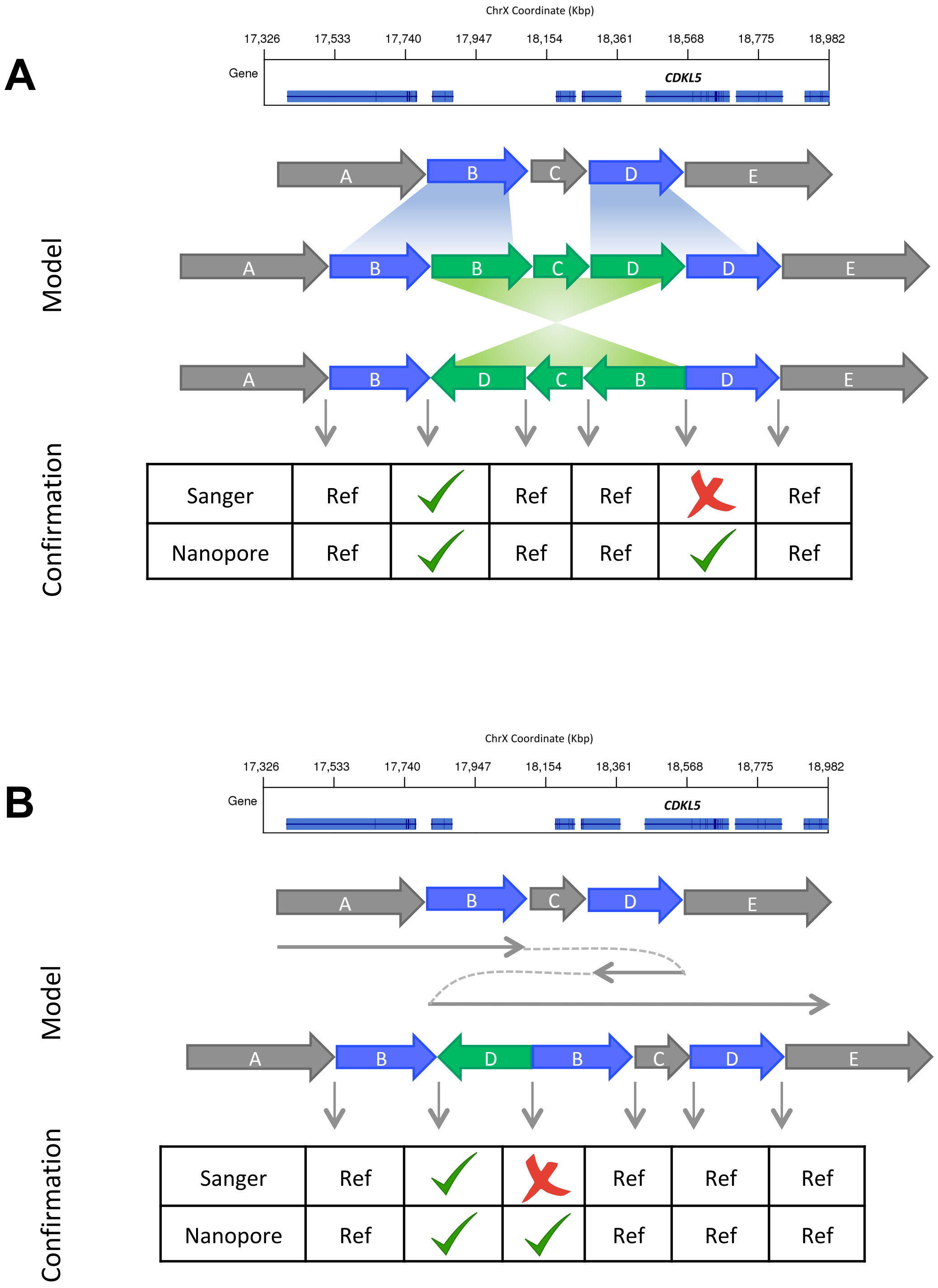


Figure S1. Complex structural variant analysis workflow. Schematic describing the general stages of complex structural variant analysis. Each variant was validated by at least two of the validation options. WGS = whole genome sequencing; SV = structural variant; cxSV = complex structural variant; IGV = Integrative Genomics Viewer; ONT = Oxford Nanopore Technologies.

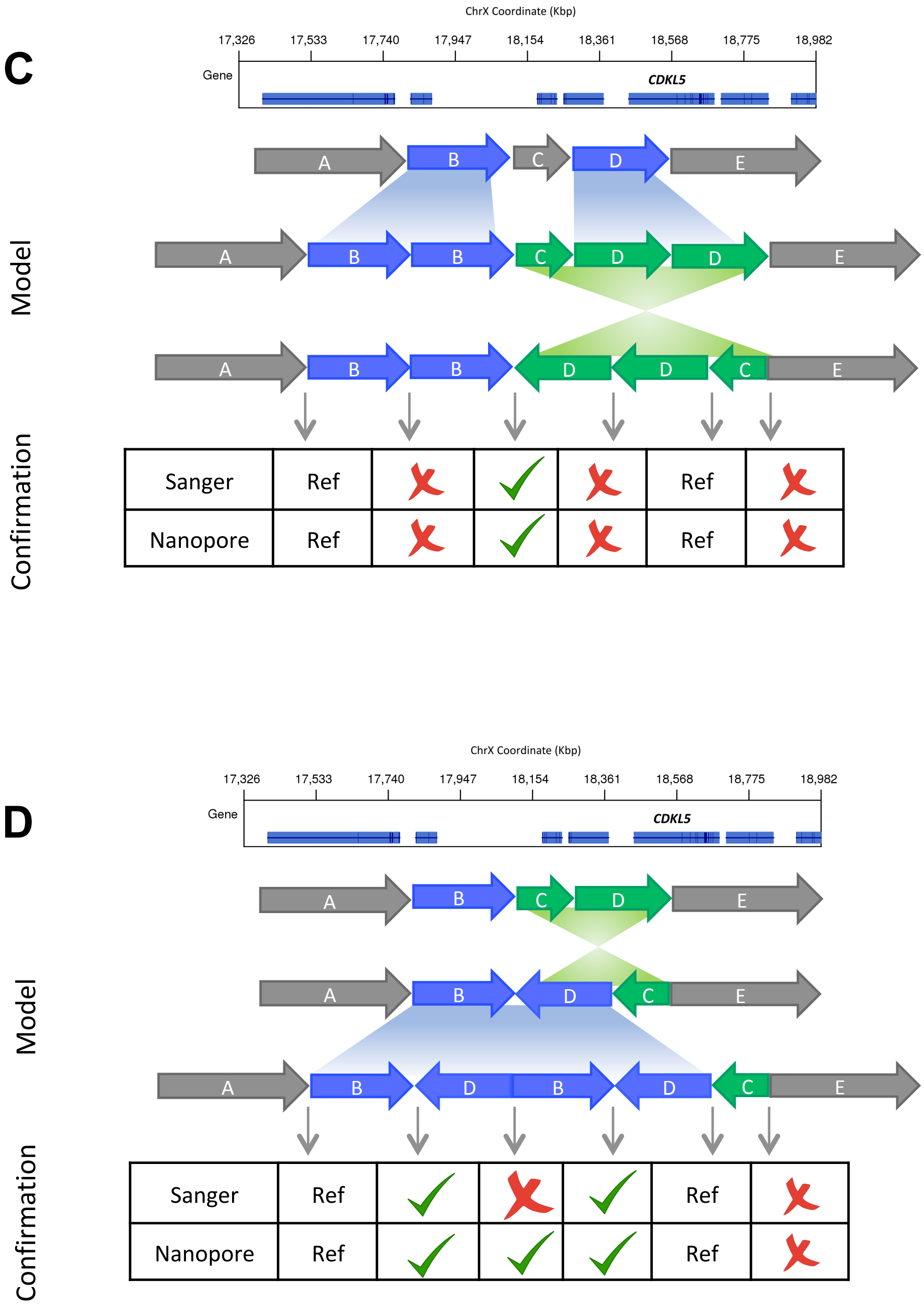


A

Sanger	Ref	✓	Ref	Ref	✗	Ref
Nanopore	Ref	✓	Ref	Ref	✓	Ref

B

Sanger	Ref	✓	✗	Ref	Ref	Ref
Nanopore	Ref	✓	✓	Ref	Ref	Ref



Sanger	Ref	✗	✓	✗	Ref	✗
Nanopore	Ref	✗	✓	✗	Ref	✗

Sanger	Ref	✓	✗	✓	Ref	✗
Nanopore	Ref	✓	✓	✓	Ref	✗

Figure S2. Complex structural variant models for Participant 4 (P4). Multiple possible models for the cxSV in P4 were considered, of which four are shown here. (**A**) Formed by two duplications and one inversion. Mechanism suggested by Brand et al. 2015[1]. Resulting junctions of this model were confirmed by Sanger and/or ONT sequencing. **(B)** Mechanism suggested by Gu et al. 2015[2], formed by one-step event. Resulting junctions of this model were confirmed by Sanger and/or ONT sequencing. (**C**) Rejected model. (**D**) Rejected model. Mechanism suggested by Gu et al. 2015[2]

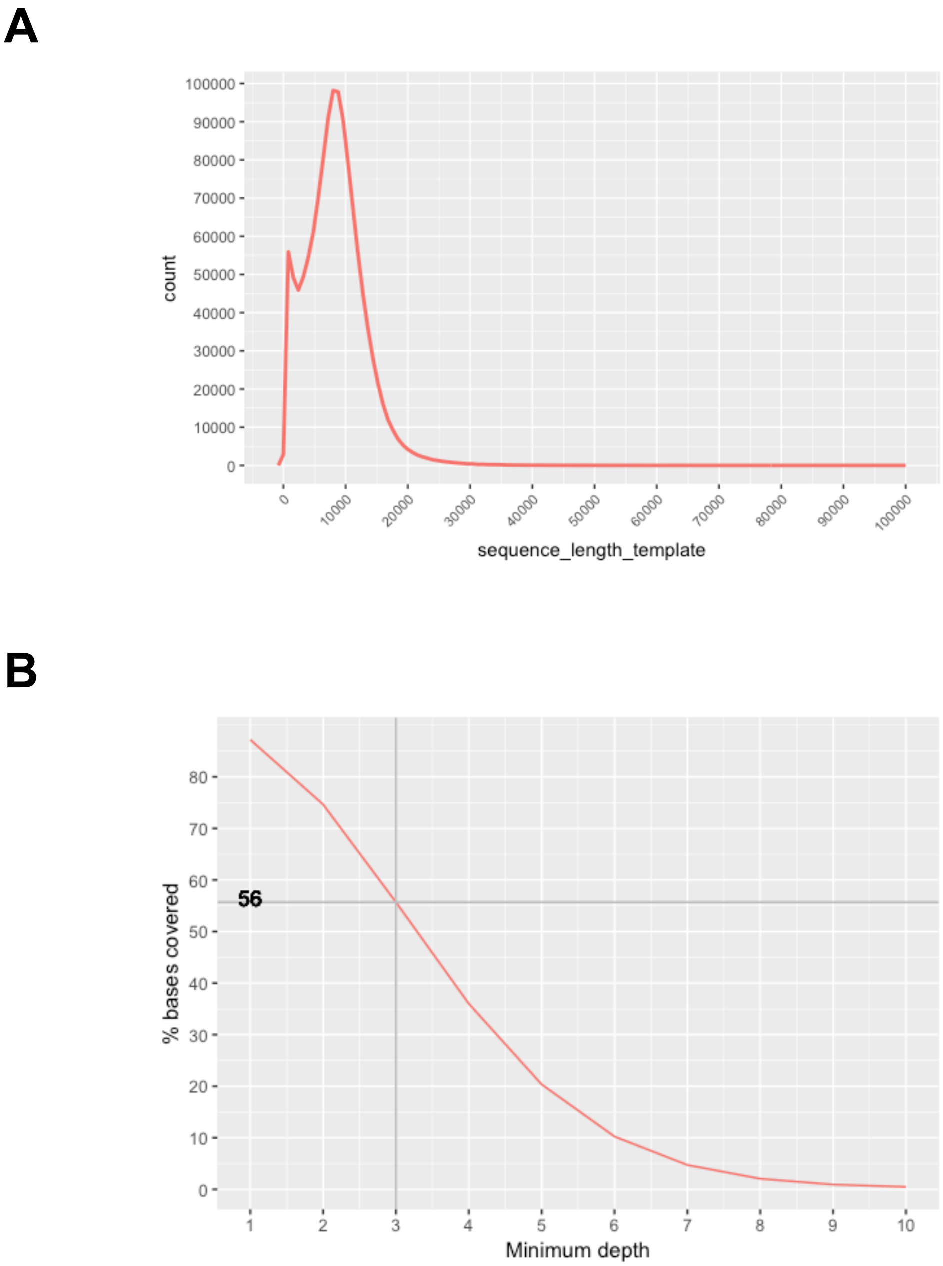
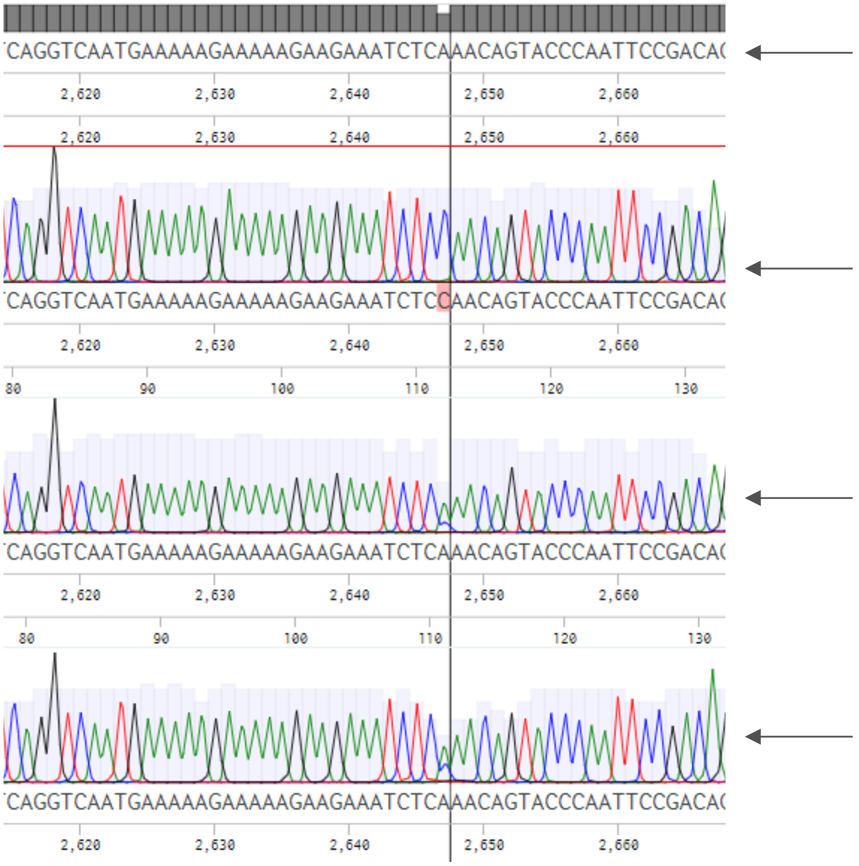


Figure S3. Quality control results of the long-read sequencing performed on P4. (**A**) Sequence length template distribution (**B**) Percentage of bases of the genome covered at a specific minimum coverage.

Informative SNP rs35478150 (GRCh37) Xg.18638082A>C



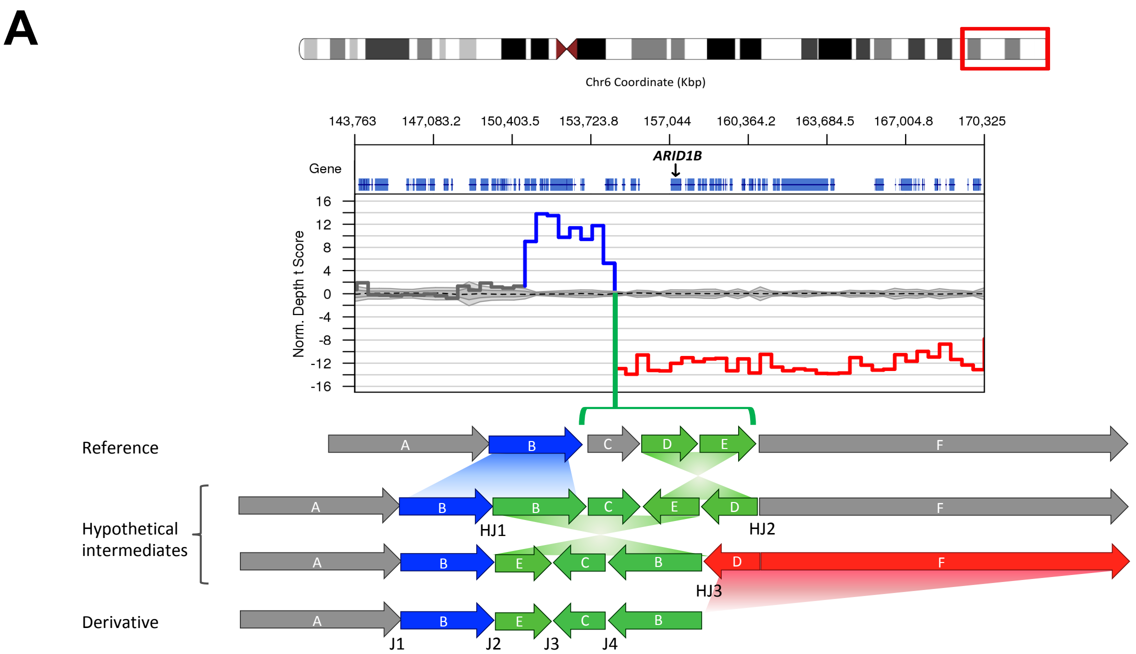
Ref Sequence
(NM_003159)
rs35478150
A>T

Father
Hemi for
rs35478150

Mother
Het for
rs35478150

Child
Het for
rs35478150

Figure S4. RNA gene expression of *CDKL5* from P4 and both parents. PCR amplification and Sanger sequencing of the informative SNP rs35478150 in the cDNA of *CDKL5* demonstrated that P4 expresses both alleles of *CDKL5* in blood cells.



```
HJ1

6:151443283-151443342     GAGAGAGCCAGACAGAAGCTGTATCCTTGTTATGACCTAACCTGGAGGTCACCCAGCACT  LTR/ERVL-MaLR
                           | |        |||   |  | | |       ||   |        |  ||||||||||
HJ1                  1-60     TATAATCGGTCTCAGGTACCCTCTACCCAGAGAGAGGAAGAAAAATTGGGACCCAGCACT
                          ||||||||||||||||||||||||||||||||||||||||||||||||||   |    ||
6:154768521-154768580     TATAATCGGTCTCAGGTACCCTCTACCCAGAGAGAGGAAGAAAAATTGGGTACAGATTCT

6:151443343-151443382     GTTTCCACCAAACTTCACTGACCAAGCTGATCACAAGCCT  LTR/ERVL-MaLR
                          ||||||||||||||||||||||||||||||||||||||||
HJ1                61-100     GTTTCCACCAAACTTCACTGACCAAGCTGATCACAAGCCT
                            ||| | |          | ||  |         |   |
6:154768581-154768620     AATTCAATCTGGGAAAGTGGTCCTGGTCTCATCATATAAT

J2

HJ1                  1-60     TATAATCGGTCTCAGGTACCCTCTACCCAGAGAGAGGAAGAAAA-ATTGGGACCCAGCACT
                          |||||||||||||||||||||||||||||||||||||||||||| |||||| ||     |
P1                   1-60     TATAATCGGTCTCAGGTACCCTCTACCCAGAGAGAGGAAGAAAA-ATTGGGTCCTCTATCA
                             |    |     |||  ||     |                  |||   ||||||||||
6:154778852-154778911     ATAACCTAGAAAGTGGTGGCCATGCTCACTCCCTTCTTCCTCTTAATTCA-TCCTCTATCA  LINE/L2

HJ1                61-100     GTTTCCACCAAACTTCACTGACCAAGCTGATCACAAGCCT
                           |       |       |   |    ||   || |  ||
P1                 61-100     TTCCATCTGAGCAAAACCATCCTGTCCTTTACAGATCCCA
                          ||||||||||||||||||||||||||||||||||||||||
6:154778912-154778951     TTCCATCTGAGCAAAACCATCCTGTCCTTTACAGATCCCA

J3

6:154774097-154774040     CAAAGTTCCATAGACTGGGAGGCCGGAACAACAGAAATTTATCTCATCACC--ATTTTGG  LTR/ERVL-MaLR
                          || |   |  |  | | |     |  | || |  || |   |      |||  |||||||
P1                   1-58     CAGATCCCAGTCCATTAGCTCAACACAGCATCTCAAGTCCTTTGGG--ACCCCATTTTGG
                          ||||||||||||||||||||||||||||||||||||||||||||||  ||||    |
6:154778943-154779000     CAGATCCCAGTCCATTAGCTCAACACAGCATCTCAAGTCCTTTGGG--ACCCGTCCTCTT

6:154774039-154773998     AGGCTAGAAGTCTGAGATCGAGGTGTCAGCAGGGTAGGTTTC  LTR/ERVL-MaLR
                          ||||||||||||||||||||||||||||||||||||||||||
P1                 59-100     AGGCTAGAAGTCTGAGATCGAGGTGTCAGCAGGGTAGGTTTC
                                        ||  | |     | | |    |   | |
6:154779001-154779042     CCAAACCTCCCTGCAGTCCCATCATCCTGTAACTCACCCTAC
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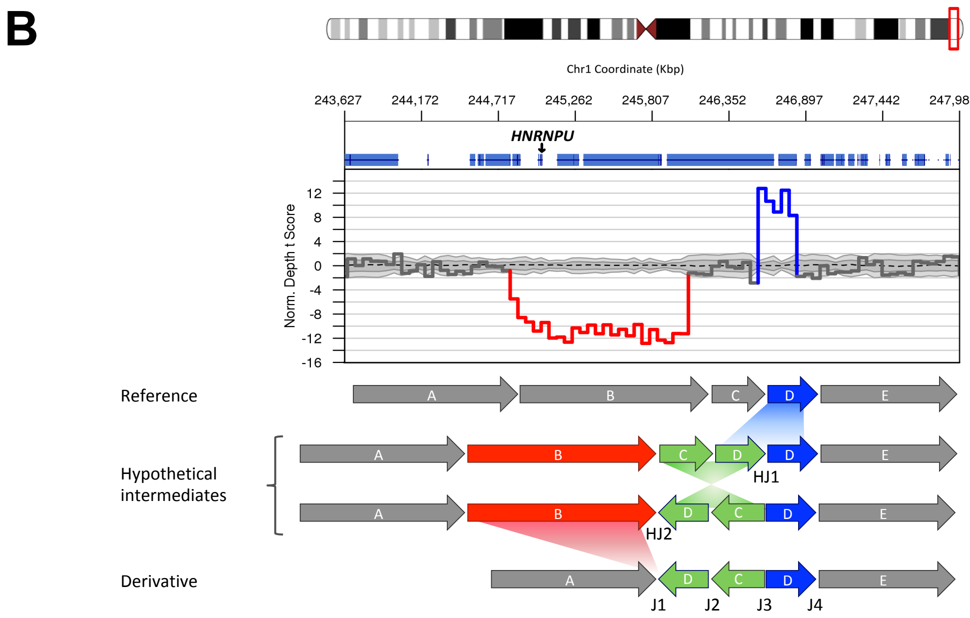

```
HJ1
1:246569822-246569881     TAAAAAGCTTGTCCTTGCCTTTTCGCTCTTCTTGTAAGTCTCTCTTCAGTGCACCGTGTC
                                   || |     | | | |  |    |    |     ||   | ||||||||||
HJ1                 1-60     CGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCGCACCGTGTC
                          ||||||||||||||||||||||||||||||||||||||||||||||||||
1:246816162-246816221     CGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGG   SINE/Alu

1:246569882-246569920     CATAGTGCTACGTGTTAGGCACTATTCAAAGCACATGACA   SINE/MIR
                          ||||||||||||||||||||||||||||||||||||||||
HJ1               61-100     CATAGTGCTACGTGTTAGGCACTATTCAAAGCACATGACA
                          | |    ||  | |   |    |      |||  |
1:246816222-246816261     CGTGAACCTGGGAGGCGGAGCTTGCAGTGAGCCGAGATTG   SINE/Alu

HJ2
1:246064189-246064244     TCCCAGGGCAGTTTCCCATGATTTCTGTCATTCTTGCCTGTGGCAAAAGG----CTAACA
                          ||||||||||||||||||||||||||||||||||||||||||||||||||    |
HJ2                 1-57     TCCCAGGGCAGTTTCCCATGATTTCTGTCATTCTTGCCTGTGGCAAAAGG---GCCTCAG
                          |  || |    ||    |     |    | |         ||| | | ||   |||||||
HJ1:-1              1-57     TGTCATGTGCTTTGAATAGTGCCTAACACGTAGCACTA--TGG-ACACGGTGCGCCTCAG

1:246064245-246064287     ATAAAGTAGATTGAATCTTCAGGAGCTATTAGAATATTCAACT
                                 ||           |   ||       |       |
HJ2               58-100     CCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCACGCCCG
                          |||||||||||||||||||||||||||||||||||||||||||
HJ1:-1            58-100     CCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCACGCCCG

J1
HJ2                 1-54     TCCCAGGGCAGTTTCCCATGATTTCTGTCATTCTTGCCTGTGGCAAAAGG------GCCT
                                      |  |    |     |||    |  ||||     |          ||||
J1                  1-59     GGGGTTTCACCGTGTCAGCCAGGATGGTCTCGATCTCCTGACCTCATGATTCGCC-GCCT
                          |||||||||| |||| ||||||||||||||||||||||||||||||||||  ||| ||||
1:244867151-244867210     GGGGTTTCACTGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCATGATCTGCCCGCCT   SINE/Alu

HJ2                55-94     CAGCCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCA
                          ||||||||| |   ||||||| ||||||||||||||||||
J1                 60-99     CAGCCTCCCAAAGTGCTGGGATTACAGGCGCCCGCCACCA
                          | ||||||||||||||||||| |    |       |  |
1:244867211-244867250     CGGCCTCCCAAAGTGCTGGGAATTACAGGTGTGAGCCACC   SINE/Alu

J3
HJ1                 1-50     CGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTG--AGGC--------
                            |  |      |      ||   |      | |          ||  |||
J3                  1-54     AAGTTGAATATTCTAATAGCTCCTGAAGATTCAATCTACTTTATTGTTAGGGGT------
                          ||||||||||||||||||||||||||||||||||||||||||||||||||   |
1:246064288-246064229     AAGTTGAATATTCTAATAGCTCCTGAAGATTCAATCTACTTTATTGTTAGCCTTTTGCCA

HJ1                51-66     --------------------------------------------GCACCGTGTCCATAGT
                                                                      ||||||||||||||||
J3                55-114     CCCAGAAGAAGGCCCTCCTAGCCGACTACAACAGCAATCTCTAGGCACCGTGTCCATAGT
                          |    ||||| | |         |   |      |      |   | | |         |
1:246064228-246064169     CAGGCAAGAATGACAGAAATCATGGGAAACTGCCCTGGGATTCCTCCCAGACCATGATCT

HJ1               67-106     GCTACGTGTTAGGCACTATTCAAAGCACATGACATGTACC
                          ||||||||||||||||||||||||||||||||||||||||
J3               115-154     GCTACGTGTTAGGCACTATTCAAAGCACATGACATGTACC
                          |  | ||            |     |      | |
1:246064168-246064129     GGAATGTTAGCAAGGTAGCTTTCCACCAGCATCCTTGGTT
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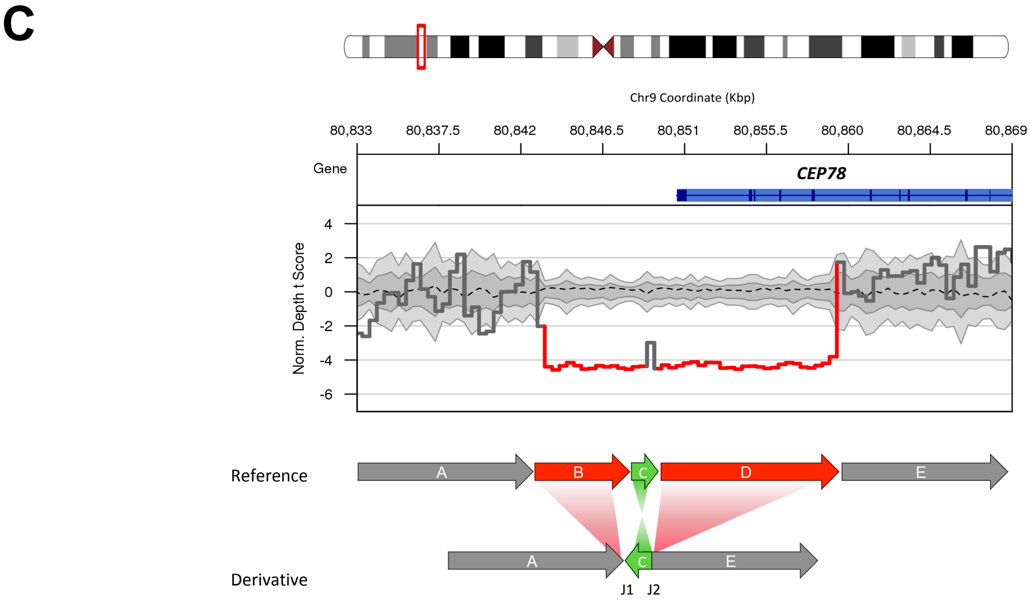


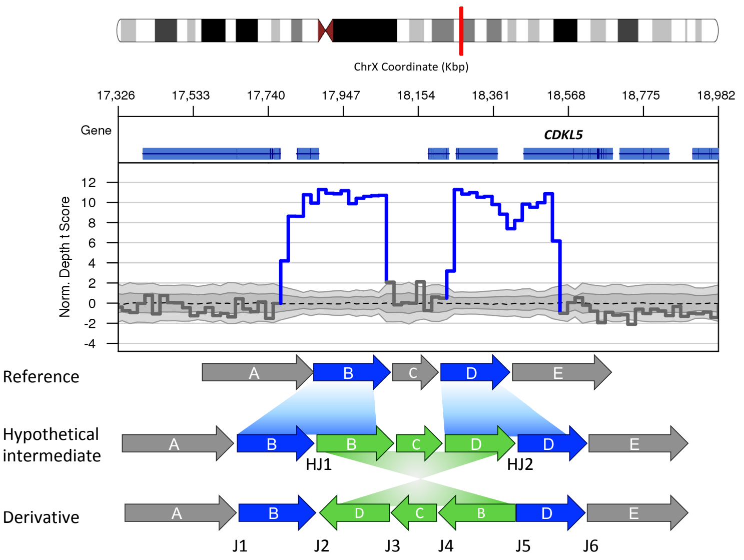
J1

```
9:80843648-80843707    TGTAATTGTGTTAATAATCTCTAATTGTATTTCTTTAGTTAATAGTTGCCCTAAAGCTTA    LINE/L1
                       ||||||||||||||||||||||||||||||||||||||||||||||||||  |   ||
J1             1-58    TGTAATTGTGTTAATAATCTCTAATTGTATTTCTTTAGTTAATAGTTGCC--ATTCCTGT
                       |   |     ||    ||   |||      ||  | | | ||   |||||  ||||||||
9:80849808-80849751    TAGTAAACGCTTGTATATTCATAAACCAGCTTAATAAATGAAACATTACC--ATTCCTGT    LINE/L1

9:80843708-80843747    TGGTATTCATCTTATCAGAATCTACTTTAGATTTATACTA    LINE/L1
                       ||     | |  | | |  | || |   |     |
J1            59-98    TGAAGAGCTTTGTGTAACCACCTTCCCAATCACGACTACT
                       ||||||||||||||||||||||||||||||||||||||||
9:80849750-80849711    TGAAGAGCTTTGTGTAACCACCTTCCCAATCACGACTACT    LINE/L1
```

J2

```
9:80849512-80849453    GGAGTTCCATGTAGAAACATACAGTAATGTGTCTGTTTTTCTGTTGATGGTAATTTAAAT    LINE/L1
                       ||||||||||||||||||||||||||||||||||||||||||||||||||    |||
J2             1-57    GGAGTTCCATGTAGAAACATACAGTAATGTGTCTGTTTTTCTGTTGATGG---CTTATCC
                         |||  |   |               |   | ||   |  |     |||   |||||||
9:80859632-80859688    TTAGTGACTCTTCAGGGTTCCATTATGTTAATATGCAATAGTACACTTGG---CTTATCC    LINE/L1

9:80849452-80849413    TGTTTCCAACTTTACGATTTCTTTGTTGCTCTATCCATTT    LINE/L1
                         |         ||      || | |     | | ||||
J2            58-97    GTTCCTATTAGATATATAAGCTATTTCCAGTTTTTCATTG
                       ||||||||||||||||||||||||||||||||||||||||
9:80859689-80859728    GTTCCTATTAGATATATAAGCTATTTCCAGTTTTTCATTG    LINE/L1
```

D



HJ1

```
X:17792960-17793019      ACATGATCTGCCTCTGCCCATGTCTTACTGTCTTTCTGACATTCGTACCCACACATGATG
                         | | ||    |         | | ||       |        |   |   ||||||||||
HJ1                 1-60 AGAAGACTAACTGAAAAAAGTTTGTTTTGTGTCTATATTTGGTGCCAGTTACACATGATG
                         ||||||||||||||||||||||||||||||||||||||||||||||||||      ||
X:18073956-18074015      AGAAGACTAACTGAAAAAAGTTTGTTTTGTGTCTATATTTGGTGCCAGTTTGTTGAGAAA

X:17793020-17793059      TGGCTCTGACCATCTCTTCCTGTCTTCCTTTCATTGGTAC
                         ||||||||||||||||||||||||||||||||||||||||
HJ1               61-100 TGGCTCTGACCATCTCTTCCTGTCTTCCTTTCATTGGTAC
                            || |     |   |     ||   | | |       |
X:18074016-18074055      ACCCTATCTAACTTGATGTTATTCAAGCATGCCCAACAGC
```

HJ2

```
X:18249005-18248946      CATGAGCCACCATGCCCAGCCTATGCCTTATTGTATTACACTCAATGCCAACACATGATG     SINE/Alu
                         ||||||||||||||||||||||||||||||||||||||||||||||||||    || |
HJ2                 1-60 CATGAGCCACCATGCCCAGCCTATGCCTTATTGTATTACACTCAATGCCACTGGATCAGC
                             |   | ||     |   |  |     | |          |    ||||||||||
X:18532362-18532303      TGAAATTGAGCAGCATTAATTTCAGTAAATTAGGAAGTATGATCTTTGGTCTGGATCAGC

X:18248945-18248906      TGGCTCTGACCATCTCTTCCTGTCTTCCTTTCATTGGTAC
                               | |   ||| | |       |     |    ||
HJ2               61-100 CCAACATCATATTCTATGCACAAAAGCAAAATACACCTAT
                         ||||||||||||||||||||||||||||||||||||||||
X:18532302-18532263      CCAACATCATATTCTATGCACAAAAGCAAAATACACCTAT
```

J2

```
HJ1                 1-60 AGAAGACTAACTGAAAAAAGTTTGTTTT-GTGTCTATATTTGGTGCCAGTTACACATGATG
                         |||||||||||||||||||||||||||| |||||||||||||||||||||| ||||
J2                  1-60 AGAAGACTAACTGAAAAAAGTTTGTTTT-GTGTCTATATTTGGTGCCAGTTGCACAATCAC    SINE/Alu
                              || |     |     |   || | || ||| | |    |||||
HJ2                 3-50 GAGCCACCATGCCCAGCCTATGCCTTATTGTATTACACTCAA-TGCCA-------------

HJ1               61-120 TGGCTCTGACCATCTCTTCCTGTCTTCCTTTCATTGGTACTCACACAGGATCTGCCCCCC
                          | |||    || | |||     |||||     |         | |         || ||
J2                61-115 AG-CTCACTGCAGC-CTTGA---CTTCCCAGGCTCAAGTGATGCTCCCACCTCAGCCTCC     SINE/Alu
HJ2                50-50 ------------------------------------------------------------

HJ1              121-180 CACCCCCCTTCTCTTCTGTCTTTCTGTGCTCCATCCTCACACCTCTTCTGCTCCTGTCCG
                                ||     | |     |       |   |||    |  |      || | | |
J2               116-175 TGAGTAGCTGGGACTATACACATGAACCACCACACCTGGATCAGCCCAACATCATATTCT     SINE/Alu
                                                            |||||||||||||||||||||||||
HJ2                51-75 -----------------------------------CTGGATCAGCCCAACATCATATTCT

HJ1              181-200 TGTCTTTCTGTTTTAGTAGG
                            |          | ||
J2               176-175 ATGCACAAAAGCAAAATACA
                         ||||||||||||||||||||
HJ2                76-95 ATGCACAAAAGCAAAATACA
```

J5

```
HJ1               100-40 GTACCAATGAAAGGAAGACAGGAAGAGATGGTCAGAGCCACATCATGTGTAACTGGCACC
                         ||||||||||||||||||||||||||||||||||||||||||||||||||
J5                  1-60 GTACCAATGAAAGGAAGACAGGAAGAGATGGTCAGAGCCACATCATGTGTTGGCATTGAG
                         |    | |        |          ||| |    | |  |||  |||   ||||||||
HJ2               100-43 GGTGTATTTTGCTTTTGTGCATAGAATATGATGTTGGGCTGATCCAGTG---GCATTGAG

HJ1                 39-1 AAATATAGACACAAAACAAACTTTTTTCAGTTAGTCTTCT
                             ||| |   |     |   |      | | |
J5                61-100 TGTAATACAATAAGGCATAGGCTGGGCATGGTGGCTCATG
                         ||||||||||||||||||||||||||||||||||||||||
HJ2                 42-3 TGTAATACAATAAGGCATAGGCTGGGCATGGTGGCTCATG
```

Figure S5. Proposed mechanisms of cxSV formation and breakpoint junction alignments for P1 (A), P2 (B), P3 (C) and P4 (D). Breakpoint junction sequence is aligned to the proximal and distal genomic reference sequence or sequence of breakpoint junction in hypothetical intermediate, as shown. Alignment is only shown for novel breakpoint junctions in the derivative chromosome, and the precursor hypothetical intermediate, if any. Microhomology at the breakpoint is indicated in red. Sequence in blue indicates inserted sequences at the breakpoint junction. Underline indicates repetitive elements in the reference, specified in *Italic*. J=Junction; HJ=Hypothetical junction.

References

1. Brand H, Collins RL, Hanscom C, Rosenfeld JA, Pillalamarri V, Stone MR et al **Paired-Duplication Signatures Mark Cryptic Inversions and Other Complex Structural Variation.** *Am J Hum Genet 2015, 97(1)170-176.*

2. Gu S, Yuan B, Campbell IM, Beck CR, Carvalho CMB, Nagamani SCS et al **Alu-mediated diverse and complex pathogenic copy-number variants within human chromosome 17 at p13.3**. *Hum Mol Genet. 2015 Jul 15; 24(14) 4061–4077.*