Supplemental Information

Constitutional chromothripsis of the APC locus as a cause of genetic predisposition to colon cancer

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Figure S1 Heterozygous deletion within chromosome 5q22.1 region

Chromosomal microarray profile of deletion 5q22.1 is shown under view of chromosome bands 5q22.1 and 5q22.2 (BlueFuse Multi 4.5, Illumina).
**Figure S2 Insertion of 5q22.1q22.3 into chromosome 10q21.3**

(A) Bionano optical mapping of the 5q22.1q22.3 region. The genomic fragments involved in the rearrangement and their orientations and relative locations are shown. The middle panel shows the chromosome 5 reference. Top and bottom panels show der5 and der10.

(B) Confirmation of the insertion of chromosome 5q22.2 into chromosome 10q21.3 by fluorescent in situ hybridization (FISH). Metaphase chromosomes were hybridized with fluorescently labeled probe RP11-3B10 mapping to the APC gene in band 5q22.2. The green arrow indicates chromosome 5, the red arrow chromosome 10 (inverted DAPI image).
Figure S3 Circos plot of structural variants predicted by Nanovar showing insertion of 5q22.1q22.3 into chromosome 10q21.3.
Figure S4 Integrative Genomics Viewer representation of APC mRNA expression

(A) Sashimi Plot for APC promoter/exon 1B in a control individual and the FAP patient harbouring the complex genomic rearrangement identified in this study. Plotted bars represent the coverage for specific regions with higher bars corresponding to higher coverage, arcs represent possible splice junctions connecting exons. (B) Fusion transcripts between APC exon 1 and EPB41L4A exon 2.
Table S1 PCR and sequencing primers used

| BP | Primer 1                           | Primer 2                          | Junction   |
|----|------------------------------------|-----------------------------------|------------|
| 1  | GGTTTGCTCTGTATCCCACACTC (CGAAGCTTAAATCCCCCACATGTC)* | GACATGAGGGAGGAAAATGACTGC          | chr5:i     |
| 2  | GATCTATGTCAATGGAGGTGGCA            | TGATGACCCCTGAGGCATCT              | 1:chr5     |
| 3  | TGCTGAAATGTGCTATCCACT              | AGGCAGACAAACCGTTCACAGA            | E:F        |
| 4  | ATCTCTAGTTGCTTGGCGGGA              | GGCATGTGGAAGAAATGTTG              | D:L        |
| 5  | AGATGCTAAGTCTCTTCTCAGTTGTAT        | CATTGACATTGGGAGGACTGAC            | G:C        |
| 6  | CCCCAAGGGAACCAACTCCTTA             | ATCCAGGCGAGCATACACAGT             | G:H        |
| 7  | CACGTTCAGCAGCCAAGCTACTCA           | AGGCGAAAGGTTTCCCCAGGTTCA          | G:J        |
| 8  | TGAGGGCCCCATCGGAAAGTTAT            | GTGAGATAATCCTGTGGGTTGGA           | chr10:K    |
| 9  | CCCAAAGCTGGAGGTAGAAG              | TGCCCTTCTCCGAGATCAGTT             | A:chr10    |

* Primer used to sequence BP1. All other breakpoints were sequenced using the indicated upstream and downstream PCR primers. BP are numbered according to Figure 1A.

Table S2 Allelic ratios of coding SNVs in genomics DNA as determined by NGS and in cDNA based on long-read RNA-seq data

| APC variant | Exon 12 c.1458T>C p.(Tyr486=) | Exon 14 c.1635G>A p.(Ala545=) | Exon 16 c.4479G>A p.(Thr1493=) | Exon 16 c.5034G>A p.(Gly1678=) | Exon 16 c.5268T>G p.(Ser1756=) | Exon 16 c.5880G>A p.(Pro1960=) | Exon 16 c.6921A>G p.(Ser2307=) |
|-------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|             | gDNA Allele Ratio   | mRNA Allelic Ratio   | w/o Puromycin   | with Puromycin   | w/o Puromycin   | with Puromycin   | w/o Puromycin   | with Puromycin   |
|             | Allele 1 | Allele 2 | Allele 1 | Allele 2 | Allele 1 | Allele 2 | Allele 1 | Allele 2 |
| Exon 12 c.1458T>C p.(Tyr486=) | 51 | 49 | 34 | 63 | 17 | 78 | 17 | 78 |
| Exon 14 c.1635G>A p.(Ala545=) | 56 | 44 | 30 | 70 | 20 | 80 | 20 | 80 |
| Exon 16 c.4479G>A p.(Thr1493=) | 51 | 49 | 30 | 70 | 37 | 59 | 37 | 59 |
| Exon 16 c.5034G>A p.(Gly1678=) | 52 | 48 | 31 | 69 | 32 | 64 | 32 | 64 |
| Exon 16 c.5268T>G p.(Ser1756=) | 51 | 49 | 22 | 70 | 22 | 73 | 22 | 73 |
| Exon 16 c.5880G>A p.(Pro1960=) | 51 | 49 | 18 | 77 | 20 | 79 | 20 | 79 |
| Exon 16 c.6921A>G p.(Ser2307=) | 53 | 47 | 7 | 93 | 27 | 73 | 27 | 73 |
### Table S3 Nucleotide sequences at the fragment junctions

| Fragment Junction | Overlap (Microhomology) | Insertion | Other |
|-------------------|--------------------------|-----------|-------|
| chr5/K           | N/A                      |           |       |
| K/chr5           | GCCAA                    |           |       |
| chr10/M          | ATT[C/-]TTCAGQ[T/-]CCCTGQ[TG/CT]AGA |            |       |
| M/G              |                          | GAGTG     |       |
| G/H              | N/A                      |           |       |
| H/E              |                          | GTTGGTTGACTTACTTAAGACTTATCTGTTATT |       |
| E/N              | ACC                      |           |       |
| N/C              | N/A                      |           |       |
| C/J              | G -> A at junction       |           |       |
| J/I              | GG                       |           |       |
| I/L              | GCCTG                    |           |       |
| L/A              | N/A                      |           |       |
| A/chr10          | GCA[T/G]G               |           |       |

*a Nomenclature based on Figure 1A*

*b Not applicable: No homologies or insertions at junction*