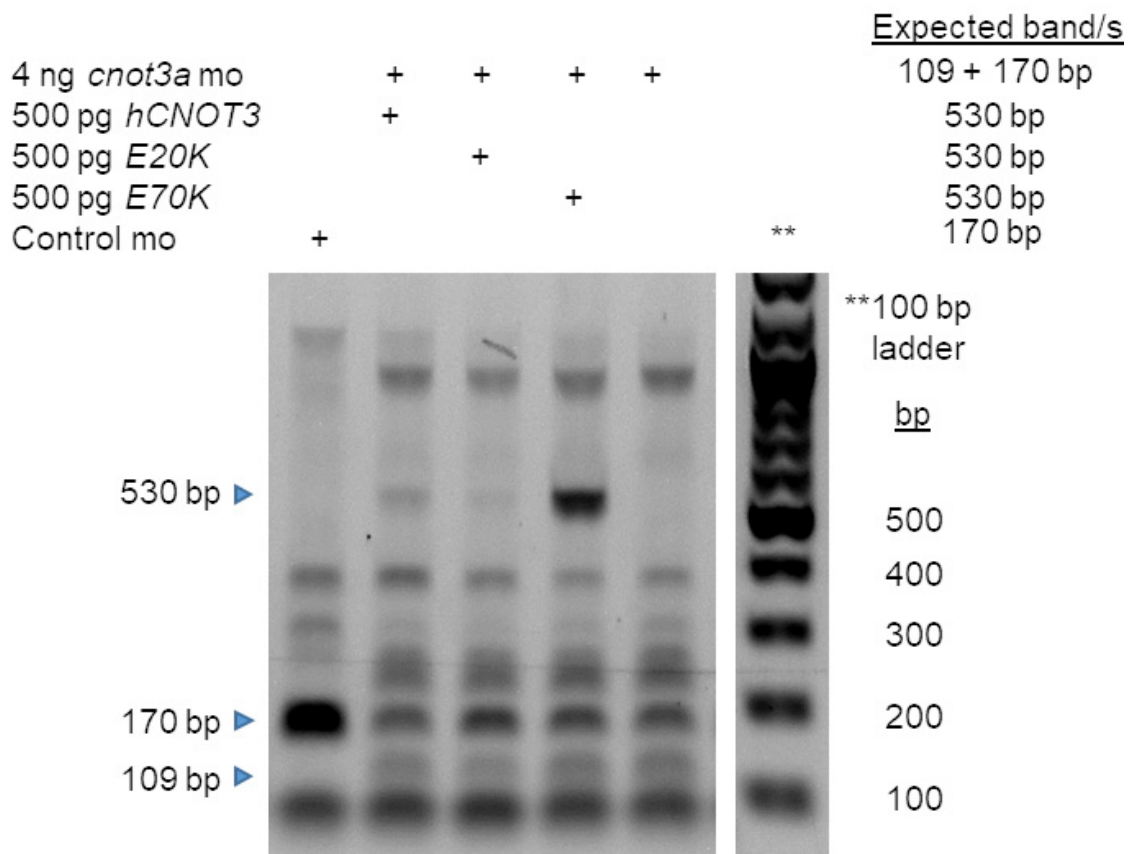


Supplementary Figure 2: Knockdown of *cnot3* gene expression in zebrafish embryos results in head and body developmental defects. (A) Time course development of control, *cnot3a*, and *cnot3b* mo injected zebrafish embryos. Zebrafish *cnot3* deficiency leads to lordosis at 48 hpf that persists at 72 hpf for *cnot3a* knockdown. Knockdown of *cnot3a* also leads to heart (he) and brain edema (be) at 72 hpf. Depletion of *cnot3b* results in only mild lordosis. (B) Graph depicting percentage of *cnot3a* morpholino-injected embryos with observed phenotypic defect/s compared to control mo. Lordosis phenotype is consistently seen in embryos with mild to severe phenotype (>80%). (C) PCR of cDNA prepared from 72 hpf control mo or *cnot3* morpholinos. Arrowhead show confirmatory WT and morpholino (mo) bands.



Supplementary Figure 3: Confirmation of *cnot3a* morpholino and *hCNOT3* mRNA injections. Figure shows gel electrophoresis from PCR run using zebrafish embryos injected with the appropriate reagent (+) as cDNA template, and using the confirmatory primers in Supplementary Table 5. Blue arrowheads show the bands - 530 bp (mRNA), 170 bp (*cnot3a*), 109 bp (mo band) – that confirm morpholino and / or mRNA injections.

Supplementary Table 1: European Bioinformatics Institute Global and Local Protein Alignment to compare zebrafish *cnot3a* and *cnot3b* amino acid sequence to human CNOT3 protein sequence

Alignment Score versus Human CNOT3 amino acid sequence		
EBI TOOL:	<i>cnot3a</i>	<i>cnot3b</i>
GLOBAL ALIGNMENT:		
NEEDLE	2574	2173.5
STRETCHER	2286	1876
LOCAL ALIGNMENT:		
WATER	2574	2190
MATCHER	1970	1673
LALIGN	3062	2626

Larger score means better alignment.

Supplementary Table 2: Clinical characteristics of patients analyzed

Patient			Germline information			Ion Torrent (Target sequencing)
Patient ID	Gender	Age	Gene	cDNA	protein	
MDAC02	M	33	<i>APC</i>	c.3810T>A	p.Cys1270*	2
MDAC03	F	63	<i>APC</i>	del exons 11-12	p.?	3
MDAC10	F	22	<i>APC</i>	c.3440dupA	p.Ser1148Thrfs*18	1
MDAC14	F	40	<i>APC</i>	del 8-9	p.?	2
MDAC17	M	37	<i>APC</i>	c.1658G>A	p.Trp553*	2
MDAC18	M	27	<i>APC</i>	c.4393_4394del	p.Ser1465Trpfs*3	1
MDAC20	M	65	<i>APC</i>	c.477C>G	p.Tyr159*	1
MDAC21	F	70	<i>APC</i>	c.487C>T	p.Gln163*	1
MDAC24	F	25	<i>APC</i>	c.4733_4734del	p.Cys1578Tyrfs*12	5
MDAC26	F	28	<i>APC</i>	c.847C>T	p.Arg283*	5
MDAC29	F	25	<i>APC</i>	c.3810T>A	p.Cys1270*	2
MDAC32	F	58	<i>APC</i>	c.1620insA	p.Gln541Thrfs*19	3
MDAC33	M	43	<i>APC</i>	c.2894del	p.Asn965Ilefs*15	5
MDAC34	M	29	<i>APC</i>	c.5936del	p.Asn1979Thrfs*65	4

Abbreviations: M, male; F, female.

Supplementary Table 3: Background information and histopathology of FAP adenoma samples

Sample ID	Location	Type of Polyp	Size	Sample ID	Location	Type of Polyp	Size
MDAC02_P02	Rectum	N/A	N/A	MDAC26_P02	Rectum	N/A	< 5 mm
MDAC02_P03	Rectum	N/A	N/A	MDAC26_P03	Rectum	N/A	< 5 mm
MDAC03_P03	Rectum	N/A	> 5 mm	MDAC26_P04	Rectum	N/A	< 5 mm
MDAC03_P01	Rectum	N/A	> 5 mm	MDAC26_P05	Rectum	N/A	< 5 mm
MDAC03_P02	Rectum	N/A	> 5 mm	MDAC29_P01	N/A	Tubular Adenoma	> 5 mm
MDAC10_P02	Rectum	Tubular Adenoma	< 5 mm	MDAC29_P02	N/A	Tubular Adenoma with HGD	> 5 mm
MDAC14_P04	Rectum	N/A	< 5 mm	MDAC32_P03	Ascending colon	Tubular Adenoma	< 5 mm
MDAC14_P05	Rectum	N/A	< 5 mm	MDAC32_P01	Ascending colon	Tubular Adenoma	< 5 mm
MDAC17_P03	Rectum	Tubular Adenoma	< 5 mm	MDAC32_P02	Sigmoid	Tubular Adenoma	< 5 mm
MDAC17_P04	Rectum	Tubular Adenoma	< 5 mm	MDAC33_P03	Rectosigmoid	N/A	N/A
MDAC18_P02	Rectum	N/A	> 5 mm	MDAC33_P01	Ascending colon	N/A	N/A
MDAC20_P02	Rectum	Tubular Adenoma	< 5 mm	MDAC33_P04	Ascending colon	N/A	N/A
MDAC21_P01	Rectum	Tubular Adenoma	< 5 mm	MDAC33_P05	Ascending colon	N/A	N/A
MDAC24_P01	Rectum	Tubular Adenoma	< 5 mm	MDAC33_P02	Ascending colon	N/A	N/A
MDAC24_P02	Sigmoid	Tubular Adenoma	< 5 mm	MDAC34_P02	Ascending colon	Tubular Adenoma	< 5 mm
MDAC24_P03	Transverse colon	Tubular Adenoma	< 5 mm	MDAC34_P03	Splenic flexure	Hyperplastic polyp	< 5 mm
MDAC24_P04	Hepatic flexure	Tubular Adenoma	< 5 mm	MDAC34_P01	Descending colon	Tubular Adenoma	< 5 mm
MDAC24_P05	Transverse colon	Tubular Adenoma	< 5 mm	MDAC34_P04	Sigmoid	Tubular Adenoma	< 5 mm
MDAC26_P01	Rectum	N/A	< 5 mm				

Supplementary Table 4: Summary of detected CNOT3 mutations using the ion torrent platform and their functional *in silico* analysis

chr	hg19 pos	ref	alt	region type	gene	mutation type	Uniprot aa pos	aa ref	aa alt	PhyloP	SIFT	Polyphen2 HDIV
19	54647842	A	C	exonic	CNOT3	nonsynonymous SNV	120	E	A	1.998	0.56	D;D;D
19	54650354	T	G	exonic	CNOT3	nonsynonymous SNV	.	I	R	0.144	.	.
19	54650355	A	G	exonic	CNOT3	nonsynonymous SNV	286	K	E	2.043	0.74	D;D;D
19	54650355	A	G	exonic	CNOT3	nonsynonymous SNV	286	K	E	2.043	0.74	D;D;D
19	54650355	A	G	exonic	CNOT3	nonsynonymous SNV	286	K	E	2.043	0.74	D;D;D
19	54650355	A	G	exonic	CNOT3	nonsynonymous SNV	286	K	E	2.043	0.74	D;D;D
19	54653350	-	C	exonic	CNOT3	frameshift insertion

chr	hg19 pos	Polyphen2 HVAR	LRT	Mutation Taster	Mutation Assessor	Sample ID	Frequency	Annovar change	Depth
19	54647842	D;D;D	D	D	L	MDAC14_P05	0.037145	CNOT3:NM_014516:exon6:c. A359C:p.E120A	5840
19	54650354	.	.	D	N	MDAC34_P04	0.027923	CNOT3:NM_014516:exon10:c. T855G:p.D285E	563
19	54650355	D;D;D	D	D	N	MDAC24_P02	0.043937	CNOT3:NM_014516:exon10:c. A856G:p.K286E	531
19	54650355	D;D;D	D	D	N	MDAC29_P01	0.040477	CNOT3:NM_014516:exon10:c. A856G:p.K286E	1789
19	54650355	D;D;D	D	D	N	MDAC32_P01	0.059016	CNOT3:NM_014516:exon10:c. A856G:p.K286E	570
19	54650355	D;D;D	D	D	N	MDAC34_P02	0.074935	CNOT3:NM_014516:exon10:c. A856G:p.K286E	724
19	54653350	MDAC24_P01	0.12126	CNOT3:NM_014516:exon13:c. .1462_1463insC:p.G488fs	629

Abbreviations: chr, chromosome; hg19 pos, human genome position; ref, reference, alt, alteration; AA, amino acid; SNV, single nucleotide polymorphism; N, neutral; D, probably damaging, B, benign, P, possibly damaging; H, higher impact; M, medium impact; L, lower impact.

Supplementary Table 5: Morpholino and primer sequences

MORPHOLINOS	5' - 3' sequence
cnot3a	GTTACACCGTGTGTCTTACCTTGA
ctbp1	TCCTTATGACGATACCTTCTCATGG
cnot3b	TTCTCCTGTTACCATACCTTGAAGT
PCR PRIMERS	5' - 3' sequence
cnot3a F	GGTCCTGGCTGACACAC
cnot3a R	GTCTTCAAACGTTCGACGC
CNOT3 F	CACCAAGGAGCAGCTCTATC
CNOT3 R	CCTTATCGTCGTCATCCTTG
cnot3b F	TGAAAAGTTGGAGGAATCGTG
cnot3b R	TTTCTTCATGCCTTCTTCTGC
RT-PCR PRIMERS	5' - 3' sequence
cnot3a F	CGTCCGATTGAGAGAGAATCA
cnot3a R	GAATGATGTGGGGGCATCT
cnot3b F	TGAAAAGTTGGAGGAATCGTG
cnot3b R	TTTCTTCATGCCTTCTTCTGC
β -ACTIN_F	CCCAGAGCAAGAGAGGTATC
β -ACTIN_R	AGAGCATAGCCCTCGTAGAT
CNOT3_RT_F	CGCAAATGGAACGGTTCAAAG
CNOT3_RT_R	GTATTCGTGAGCCACTGGCCA