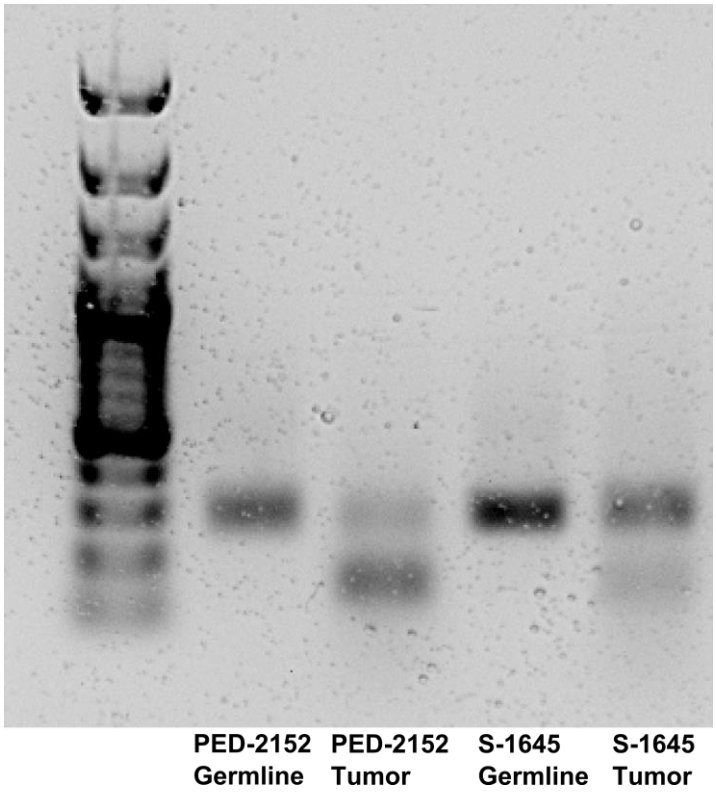
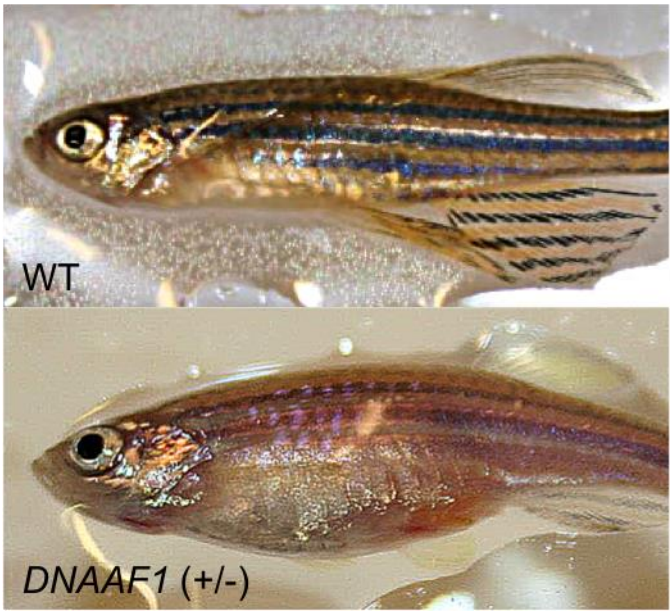
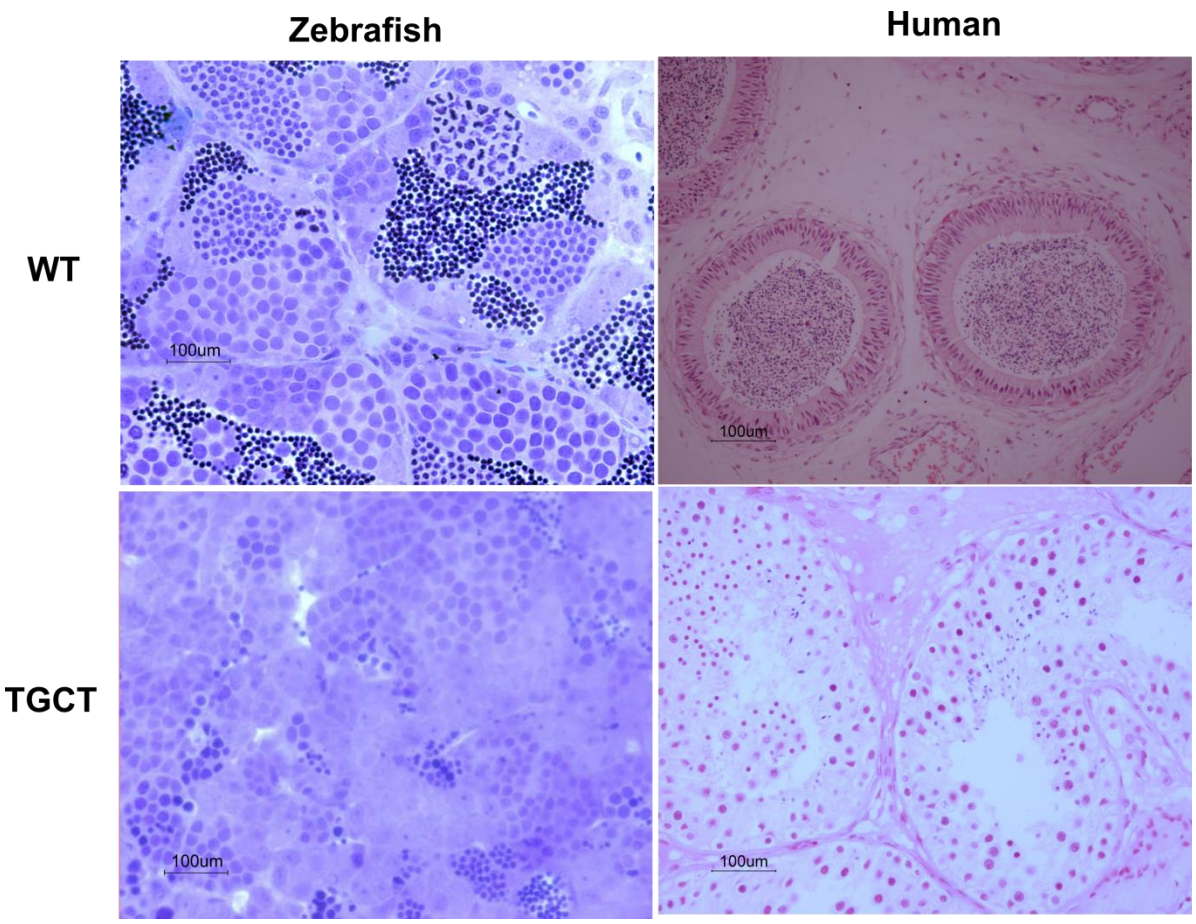
Supplementary Figure 1. Evidence to support biallelic inactivation was observed in tumor material from 2/3 available cases. A 297bp region within exon 4 of DNAAF1 was captured (chr16:84203572-84203869), and germline/tumor product lengths are shown for the 2 cases with biallelic inactivation. In PED-2152 a 23bp deletion was present in this region in the germline, resulting in a germline product length of 274bp. For the PED-2152 tumor sample a second truncated product length is also clearly observed, containing a second somatic 100bp deletion not found in the germline. For S-1645, a sample from our sporadic cases, a nonsense point mutation is present in the germline, meaning a full 297 bp germline product length for this region. For the S-1645 tumor sample again a second truncated product length is detected, caused by a somatic deletion.



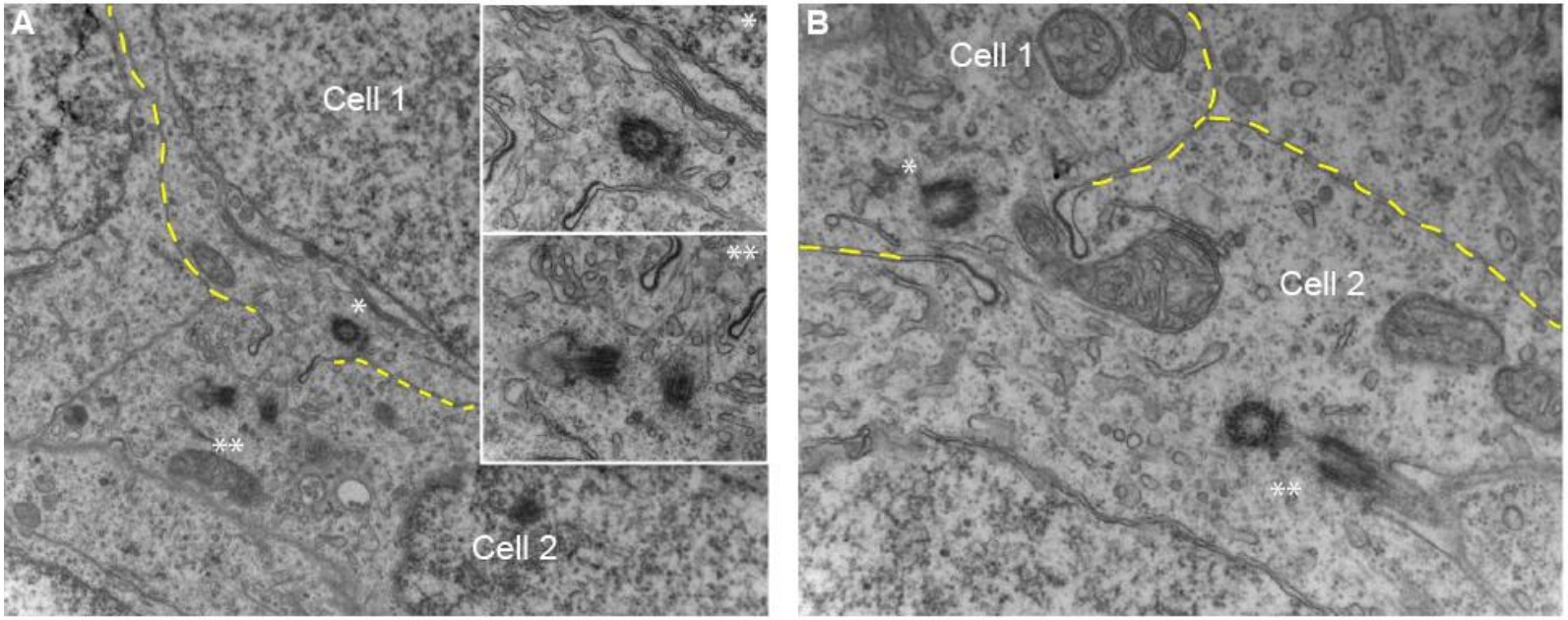
Supplementary Figure 2a. Comparison of wild-type male zebrafish and mutant *DNAAF1*^{Hu255h}(+/-) zebrafish. On top is the healthy wild-type zebrafish compared to mutant *DNAAF1*^{Hu255h}(+/-) zebrafish with visible tumor mass on bottom. TGCT was confirmed through histological characterization.



Supplementary Figure 2b. Histological characterisation of zebrafish and human tissue. On top left is age-matched (24–44 months old) wild-type zebrafish and on bottom left is the zebrafish *DNAAF1*^{Hu255h}(+/-) tumor tissue, morphological tissue analysis of toluidine blue stained sections shows a dramatic loss of differentiated germ cells. On the top right for comparison is a healthy human testis and bottom right is a human seminoma.



Supplementary Figure 3. Ciliary structures adjacent to germ cell syncytium intercellular bridges from high resolution images of wild-type zebrafish male gonads. Spermatogonial stem cells commit to the development of spermatozoa via sequential steps of proliferation and differentiation after initial asymmetric cell division. A hallmark feature of spermatogonial derived early germ cell is the presence of intercellular bridges, concomitant from prematurely blocked cytokinesis followed by intercellular bridge stabilization. Presented are two gonadal sections displaying two adjacent male germ cell associated through intercellular bridges. Intriguingly, we observed presence of ciliary structures in the immediate vicinity of intercellular bridges. As the sections are ultrathin, only one of either cilium is visible as the corresponding cilium or centrosome is not in the same plane. A: The lower cell 2 shows mother and daughter centrioles where microtubules emanating from the mother centriole extend into a structure reminiscent of the primary vesicle (insert**). In cell 1, one centriole can be observed in the same plane B: Cell 2 shows presence of a cilium clearly featured by an axonemal structure composed of a membrane wrapped microtubule structure. Cell membranes are indicated in yellow.



Supplementary Table 1. **Gene Set Enrichment Analysis (GSEA) of rare disruptive mutations, which shows a significant association for familial TGCT with cilia-microtubule function**. Shown are the genes contributing to the leading edge of the top 5 pathways ranked based on enrichment score. Only the cilia-microtubule gene set was significant (*i.e*. *Q* value < 0.1). The genes displayed are those that contribute to the leading edge of each gene set, the value in each cell is the -$\log_{10}$(T1 test *P*-value) used in the ranking for GSEA. Genes with values greater than 1.0 are shown. Please note while gene sets "Reactome loss of nlp from mitotic centrosomes" and "Reactome recruitment of mitotic centrosome" do not differ in terms of leading edge genes, they are different gene sets overall, with the latter being a larger set and hence the association is less significant.

	Q	*DNAAF1*	*DRC1*	*MAP4*	*DYNC2H1*	*CEP290*	*CNTRL*	*DYNC1H1*	*HSP90AA1*	*CEP135*	*ALMS1*	*CDK5RAP2*	*MIS12*	*CENPE*	*SRPK1*	*PDS5B*	*PAPD7*	*GSTM4*	*GSTO1*	*GSS*
Cilia-microtubule function	0.01	2.8	2.3	1.8	1.7	1.4														
Reactome loss of nlp from mitotic centrosomes	0.11					1.4	2.6	1.8	1.7	1.4	1.1	1.1								
Reactome recruitment of mitotic centrosome proteins and complexes	0.34					1.4	2.6	1.8	1.7	1.4	1.1	1.1								
Chromosome segregation	0.37												1.9	1.8	1.7	1.7	1.4			
Reactome glutathione conjugation	0.34																	3.9	1.4	1.1

Supplementary Table 2. *DNAAF1* missense allele frequencies from Basten et al. (2013).

Mutation	ExAC Minor Allele Frequency	UK Control Minor Allele Frequency	*P*-Value for association with TGCT in UK Study
Gln307Glu	2.0%	2.4%	0.8
Asp435Asn	2.5%	2.8%	0.1
Thr590Met	1.0%	2.1%	0.3

Supplementary Table 3. Primer sequences used to confirm rare disruptive variants in cilia microtubule genes in germline samples and to examine for loss of heterozygosity in tumour samples.

Oligo sequence (5'-3')	Oligo name	Gene
AAGGAAAGCTTTGAGGCCAA	DNAAF1_1F	*DNAAF1*
AGGCGGTGACAGTAGCAG	DNAAF1_1R	*DNAAF1*
TGCCTTTATCGTGCCTATCAG	DNAAF1_1F	*DNAAF1*
GGAACATTTTACTTTCAAGTTTAGGA	DNAAF1_1R	*DNAAF1*
GTCACTCCCTGTGCTGGAAA	DNAAF1_1F	*DNAAF1*
AGTGGTGCAGCATCTCTTTG	DNAAF1_1R	*DNAAF1*
GGGGTTCCTCATAGAGAGCA	DRC1_1F	*DRC1*
AAGGATGTGACAGGGAGCAG	DRC1_1R	*DRC1*
GCACCGAGTTGGTGACAAAT	DRC1_2F	*DRC1*
GAGGGAGGGAGGGAGAAGTT	DRC1_2R	*DRC1*
TTTTTCTAGTTGATCCTGATTTTCTAA	DYNC2H1_1F	*DYNC2H1*
ACCCGCAACATAGCATTGTG	DYNC2H1_1R	*DYNC2H1*
AGGTGGGGGTTCAAGTTAGG	MAP4_1F	*MAP4*
ACACAGTCCCTGGCTCAAAG	MAP4_1R	*MAP4*
TGGTCACAAAAATCAATAAAGAATG	CEP290_1F	*CEP290*
GGTCTTCAAAATGTGAATTTGTT	CEP290_1R	*CEP290*
CCTTTGTTGAACCACCACAACT	CEP290_2F	*CEP290*
GGTGGGAGAATTGCTTGA	CEP290_2R	*CEP290*
AACTTACCAATTTTCCCAATAAGA	LRRC6_1F	*LRRC6*
TTGAACAATGGGGACAGAAA	LRRC6_1R	*LRRC6*
CAAGAGCAGCATGAGGTGAA	CNTRL_1F	*CNTRL*
GCACAGGGTCCTTAGTTTGC	CNTRL_1R	*CNTRL*
AGCCTGGTAGGCAATGTGAT	CNTRL_2F	*CNTRL*
TGGGTACCAACACTGACCTG	CNTRL_2R	*CNTRL*

Supplementary Note 1. The UK Testicular Cancer Collaboration (UKTCC).

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Supplementary Note 2. **International Testicular Cancer Linkage Consortium (ITCLC) centers from which samples were used in this study.**

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