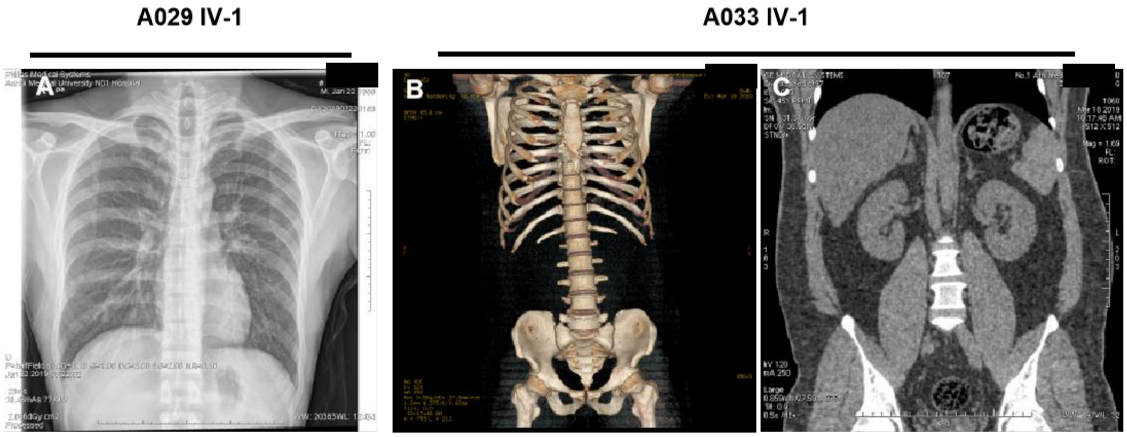
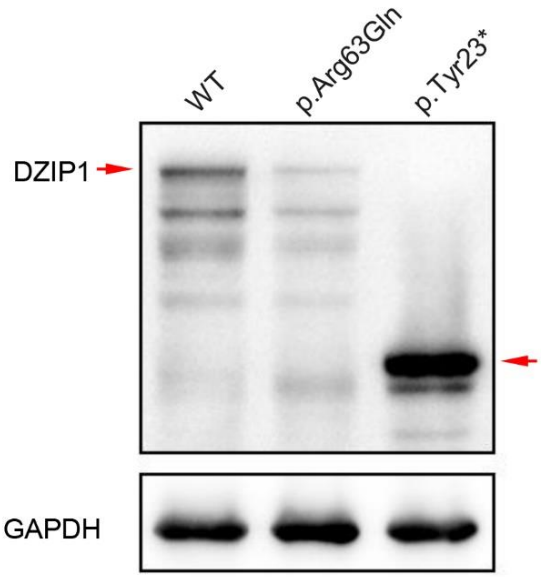
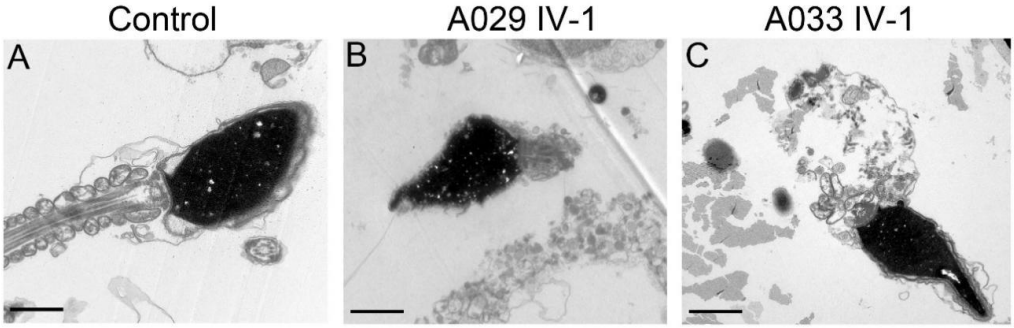


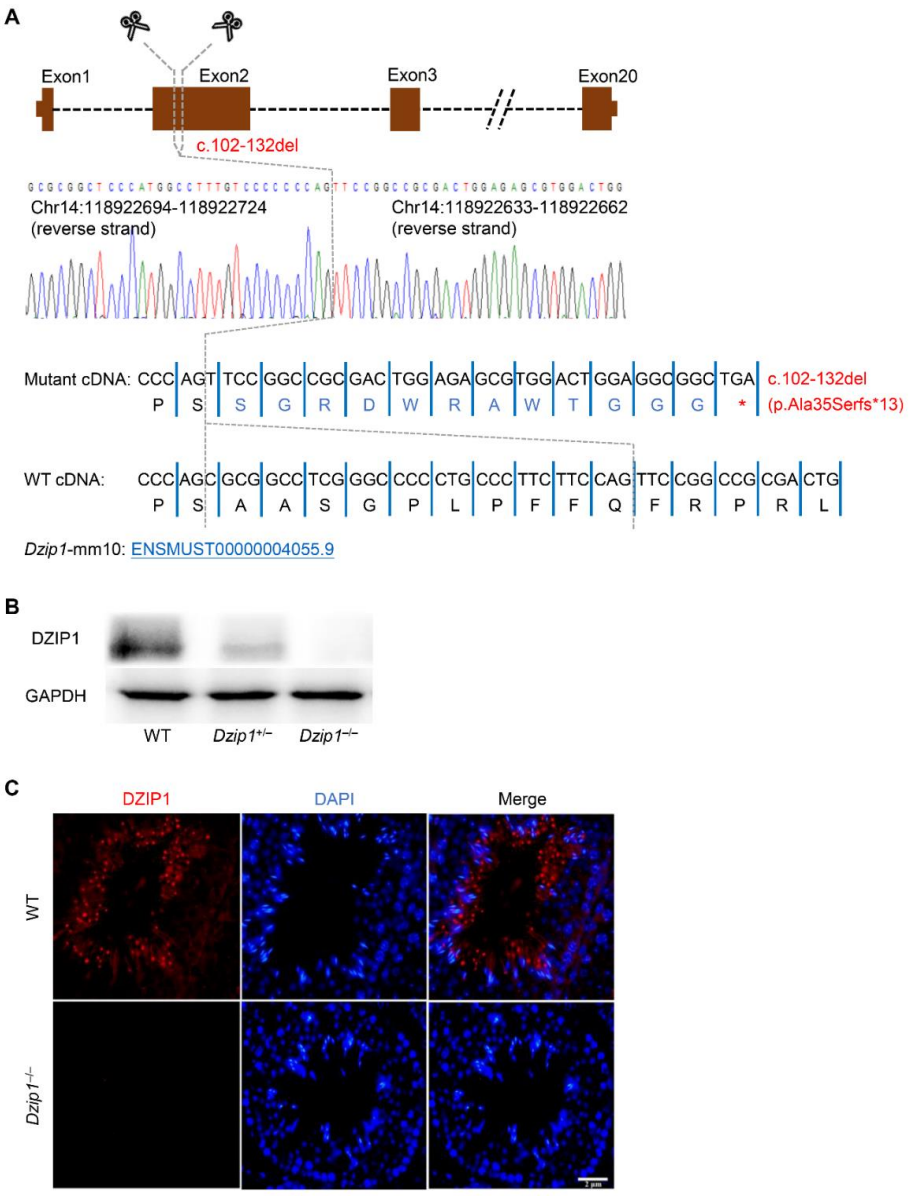
Supplementary figure S1 Clinical examinations of the two *DZIP1*-mutated men. **(A)** Chest X-ray image indicated the normal pulmonary structure, heart and ribs of subject A029 IV-1. **(B, C)** Computed reconstructed images of the ribs, pelvis, liver and kidneys of subject A033 IV-1. No obvious abnormalities were observed. The names of the two subjects were anonymized.



Supplementary figure S2 Western blotting assay of the *DZIP1* mutations identified in men with asthenoteratospermia. The *in vitro* effects of the mutations on DZIP1 protein level were investigated using western blotting in HEK293T cells transfected with wild-type (WT) or *DZIP1*-mutated constructs. Red arrows: WT or mutated DZIP1 proteins.



Supplementary figure S3 Ultra-structure of the sperm flagella from a control subject and the two *DZIP1*-mutated men. **(A)** The control sample showed a regular flagellum with a normal connecting piece and axoneme, which was surrounded with mitochondrial sheath. **(B, C)** The longitudinal section of sperm head and flagellar mid-piece of the D*ZIP1*-mutated men presented with a very short axoneme structure (B) or complete lack of axoneme structure (C). Scale bar: 2 μm.



Supplementary figure S4 Generation of *Dzip1*-knockout mice using CRISPR-Cas9. (**A**) A frameshift deletion (c.102_132del) was generated in mouse ortholog *Dzip1* using the CRISPR-Cas9 technology. This mutation was predicted to cause premature translational termination (p.Ala35Serfs*13). (**B**) Western blotting analysis of the whole testis lysates from wild-type (WT), heterozygous mutated (*Dzip1*$^{+/–}$) and homozygous mutated (*Dzip1*$^{–/–}$) male mice. The DZIP1 protein expression levels were obviously reduced in the *Dzip1*$^{+/–}$ mice, and was absent in the *Dzip1*$^{–/–}$ mice. (**C**) Immunofluorescence staining of DZIP1 was conducted in mouse testicular tissues. The DZIP1 staining was detected in germ cells (especially in elongated spermatids) of the WT male mice, but was absent in the *Dzip1*$^{–/–}$ male mice. Scale bar: 20 μm.

Supplementary table S1 Primers used for amplification and verification of *DZIP1* mutations.

Primer name	Primer sequence (5'-3')	Tm
M1-F	AAGAAAGAGTGGTGATACGGGACAG	62°C
M1-R	GCCCTTCCAGAAGCATGTCTACTAC	
M2-F	AAAATGCAATCATCTTGTCAAATTC	58°C
M2-R	AAGCACCCTGTATTATTCCTTGTTA	

Supplementary table S2 Primers used for full-length PCR and segmental PCR.

Primer name	Primer sequence (5'-3')	Tm
WT-F	ACAAGTCCGGACTCAGATCTCCTATGCAAGCTGAGGCAGC	72°C
WT-R	TATCTAGATCCGGTGGATCCTTAGACATCTGAAGTGTCGCTC	
M1-cDNA-F	AGCTGGAGAGTGTGGACTGG	70°C
M1-cDNA-R	CAGTCCACACTCTCCAGCTGCGGCCTGAACTGGAAGAAG	
M2-cDNA-F	CAGAAAAATGCACAGATTGAG	65°C
M2-cDNA-R	CAATCTGTGCATTTTTCTGCTACTCAAAATGAGAATT	

Supplementary table S3 Primers used for verification of mouse *Dzip1* mutation.

Primer name	Primer sequence (5'-3')	Tm
Mouse-F	TGCCATAGCAACGTCCTGGAC	55°C
Mouse-R	AGCTGCGAGGTGAGGAACTC	

Supplementary table S4 Homozygous *DZIP1* mutations identified in men with asthenoteratospermia.

Individual	**A029 IV-1**	**A033 IV-1**
DZIP1 cDNA mutation	c.188G>A	c.690T>G
Protein alteration	p.Arg63Gln	p.Tyr230*
Mutation type	missense	stop-gain
Allele frequency in human populations		
ExAC	0	0
gnomAD	0	0
1000 Genomes Project	0	0
Han Chinese controls [a]	0	0

[a] The Han Chinese controls consist of 300 fertile individuals and 668 individuals affected by non-reproductive disorders.