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Supplemental Data

TTC25 Deficiency Results in Defects of the Outer Dynein Arm Docking Machinery and Primary Ciliary Dyskinesia with Left-Right Body Asymmetry Randomization

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Supplemental Material:

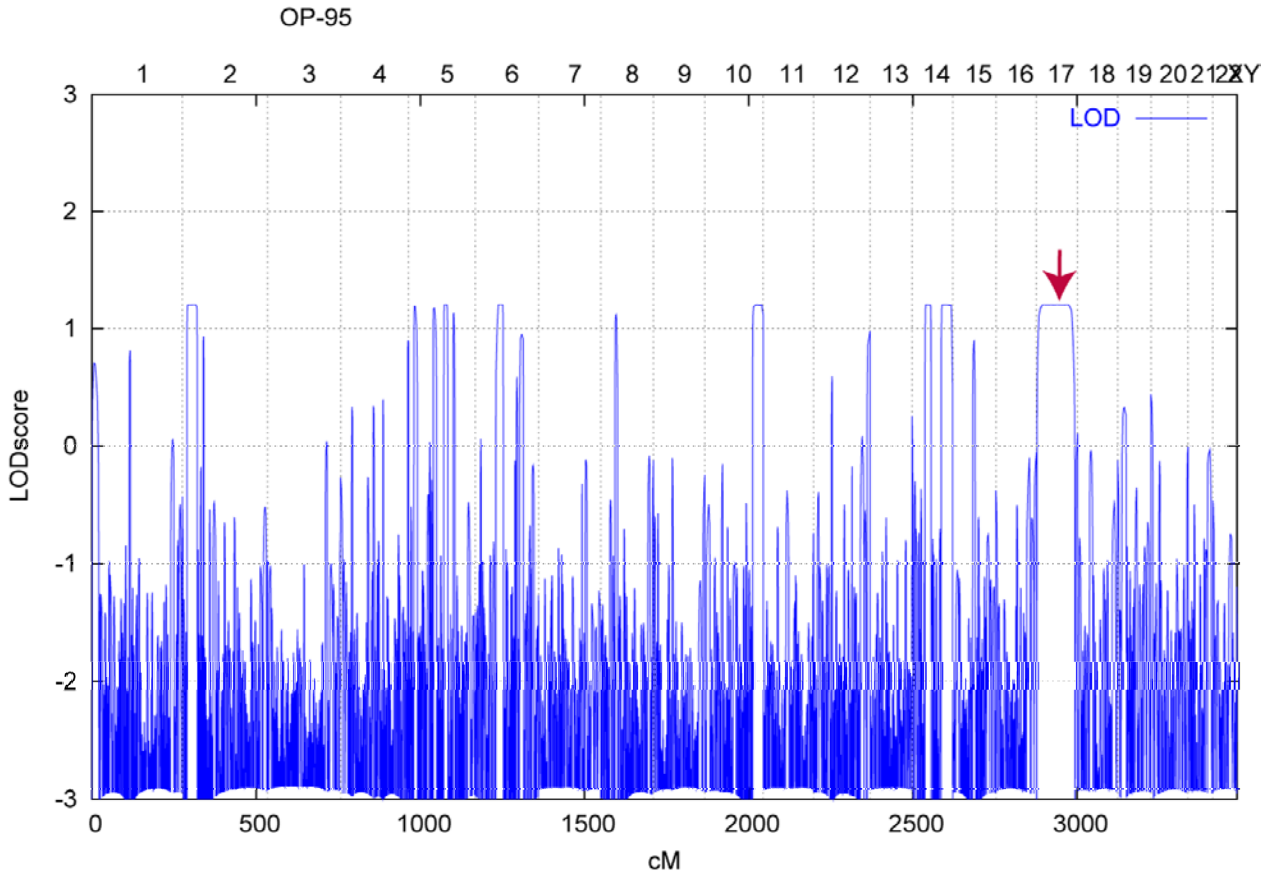

Figure S1: SNP-Haplotype analysis of OP-95 II1; cM: centimorgan, Allegro LOD score: logarithm of the odds

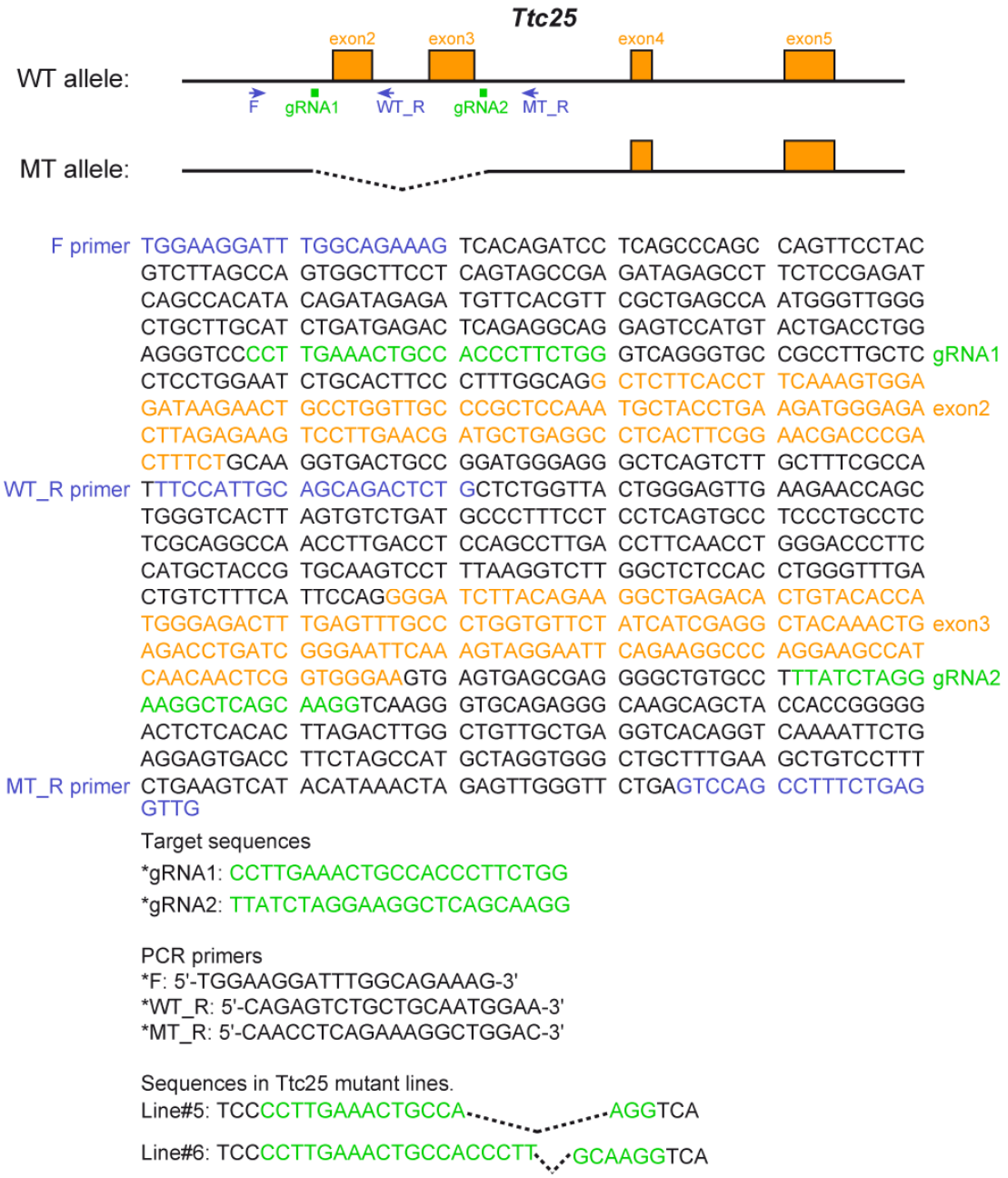

Figure S2: Generation of mutant mice by CRISPR/Cas9 system.

Ttc25 mutant mice were generated with the use of the clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 system. Two small guide RNAs (sgRNAs) designed to delete *Ttc25* exons 2 and 3 were produced by *in vitro* transcription (IVT) with the use of a MEGA short script T7 kit (Ambion, AM1354) essentially as previously described. Capped synthetic mRNA for Cas9 was transcribed from the Cas9/pSP64T vector with the use of an SP6 mMessage mMachine Kit (Ambion, AM1340). Cas9 mRNA and the two sgRNAs were injected into C57BL/6 fertilized eggs as previously described[24]. The pups were genotyped by polymerase chain reaction (PCR) and subsequent sequence analysis. The primers used were F: 5'-TGGAAGGATTTGGCAGAAAG and MT_R: 5'-CAACCTCAGAAAGGCTGGAC.

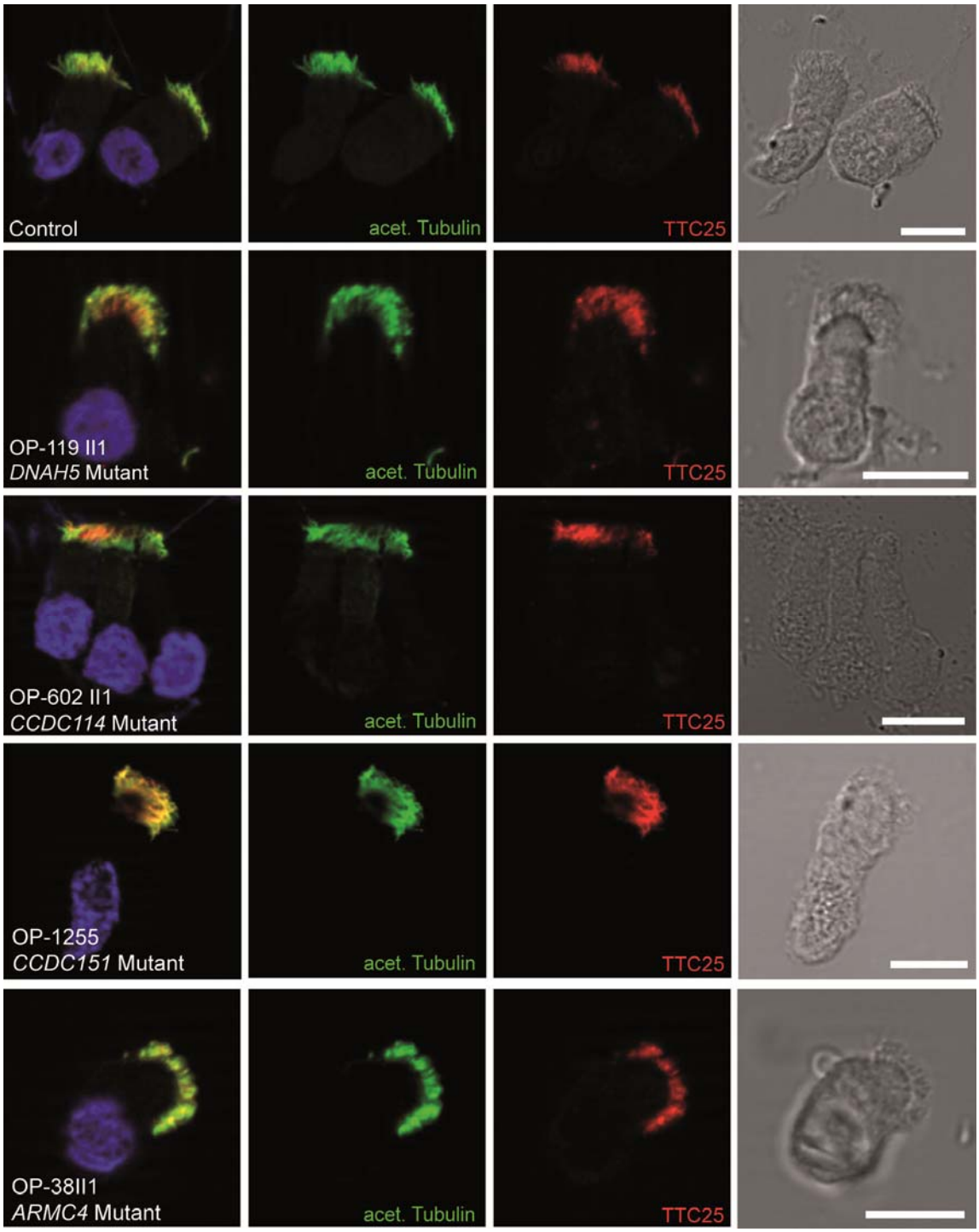

Figure S3: TTC25 is detectable in ODA-HC and ODA-DC mutant cilia.

Respiratory cilia from control and PCD individual (OP-119 II1) carrying *DNAH5* LOF-mutations were double labeled with antibodies directed against acetylated Tubulin and TTC25. Respiratory cilia from control and PCD individuals with ODA-DC defects carrying LOF-mutations in *CCDC114* (OP-602 II1), *CCDC151* (OP-1255) and *ARMC4* (OP-38 II1) respectively were double labeled with antibodies directed against acetylated tubulin and TTC25. Yellow color represents co-localization of TTC25 and acetylated tubulin. Nuclei (blue) were stained with Hoechst33342. Scale bars represent 10µm.

Table S1: Rare variants in the homozygous region (17:5961695-78901893) of OP-95II1 (Frameshift, Splice site-Substitution, Non-Synonymous Substitution, Variation frequency <0.005)

Gene	Ensembl gene number	cDNA level	Protein level	Variation	Variation frequency
MYH4	ENSG00000264424	c.3833C>T	p.S1278L	rs145453135	0.0003
NT5M	ENSG00000205309	c.604_615del(CTGCAG)2ins(CTGCAG)3	p.Q205_P206insLQ	n.a.	n.a.
PHF12	ENSG00000109118	c.1534C>G	p.H512D	n.a.	n.a.
GIT1	ENSG00000108262	c.1489G>A	p.A497T	n.a.	n.a.
SLFN11	ENSG00000172716	c.766G>A	p.E256K	n.a.	n.a.
SLFN12	ENSG00000172123	c.42G>C	p.L14F	rs202085233	0.0002
KRTAP29-1	ENSG00000212658	c.833A>G	p.K278R	n.a.	n.a.
KRT32	ENSG00000108759	c.665C>G	p.S222C	n.a.	n.a.
TTC25	**ENSG00000204815**	**c.114+1G>T**	**Splice site**	**n.a.**	**n.a.**
INTS2	ENSG00000108506	c.380C>T	p.T127M	rs370752436	0.0002
CASKIN2	ENSG00000177303	c.2635G>A	p.V879I	n.a.	n.a.
GALR2	ENSG00000182687	c.1163G>C	p.*388S	n.a.	n.a.

n.a.: not available

Table S2: Rare variants in the shared homozyous region (17:16479171-66815637) of OP-1331 II1 and II2 (Frameshift, Splicesite-Substitution, Non-Synonymous Substitution, Variation frequency <0.005).

Gene	Ensembl gene number	cDNA level	Protein level	Variation	Variation frequency
EVI2A	ENSG00000126860	c.494C>A	p.S165Y	rs147909684	0.0003
C17orf50	ENSG00000154768	c.374G>A	p.R125Q	n.a	n.a
KRTAP1-1	ENSG00000188581	c.125C>T	p.T42I	n.a	n.a
TTC25	**ENSG00000204815**	**c.425_426insT**	**p.K142Nfs*12**	**n.a**	**n.a**
KCNH4	ENSG00000089558	c.2696G>A	p.R899Q	n.a	n.a
FAM187A	ENSG00000214447	c.-1466+6T>A	Splice site	n.a	n.a
COPZ2	ENSG00000005243	c.16-1delCins(C)2	Splice site	n.a	n.a
XYLT2	ENSG00000015532	c.1942-8_23del(TTTA)4ins(TTTA)3	Splice site	n.a	n.a
EME1	ENSG00000154920	c.567T>A	p.N189K	rs150118812	0.0012
LRRC59	ENSG00000108829	c.126A>T	p.N43I	rs150118812	0.0012
MTMR4	ENSG00000108389	c.3029A>G	p.D1010G	rs61742345	0.000099

n.a.: not available

Table S3: Phenotype in TTC25 mutant mice (2 weeks old)

	1	**2**	**3**	**4**	**5**	**6**
Heart apex on the right side	x	-	x	x	x	-
Reversed lung lobation	x	-	x	x	x	-
Aortic arch on the right side	x	-	x	x	x	-
Azygos vein on the right side	x	-	x	x	x	-
Stomach on the right side	x	-	x	-	x	-
Abnormal liver lobation	ND	x	ND	-	x	-
Vena cava located to the left of the aorta	-	x	ND	-	-	-
Slow moving of tracheal cilium	ND	x	ND	x	x	x
Hydrocephalus	ND	ND	ND	x	x	-
Small body	ND	x	x	x	x	x

X: defect is present; -: defect is absent; ND: not determined

Table S4: Genotypes from intercross of *Ttc25*+/- mice (2 weeks-old)

Line No.	*Ttc25* +/+ (%)	*Ttc25* +/- (%)	*Ttc25* -/- (%)	Total
#5	13 (33.3%)	22 (56.4%)	4 (10.3%)	39 (100%)
#6	5 (35.7%)	7 (50.0%)	2 (14.3%)	14 (100%)

+/+: wild type; *Ttc25* +/-: heterozygous;*Ttc25* -/-: homozygous

Supplemental References:

24. Saijoh, Y., Adachi, H., Mochida, K., Ohishi, S., Hirao, A., and Hamada, H. (1999). Distinct transcriptional regulatory mechanisms underlie left-right asymmetric expression of lefty-1 and lefty-2. Genes Dev. *13*, 259–269.