TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum

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Supplementary Information:

Supplementary Table 1. Collection sites and ethnicities of individuals sequenced at the *TTC21B* locus

		Ethnicity (n=)		
Clinical Diagnosis	**Collection Location**	**N. European**	**N. Africa/Middle East/ S.E.Asia**	**Cohort Totals**
MKS	France[1]	27	22	
MKS	United Kingdom[2]	30	44	
Total MKS		57	66	**123**
JATD	United Kingdom[3,4]	44	8	
JATD	United States[5]	6		
Total JATD		50	8	**58**
BBS	France[6]	203	77	
BBS	Germany[7]	10		
BBS	United States[8]	29	11	
Total BBS		242	88	**330**
JBTS	United States[9,10]	97	3	**100**
NPHP+	United States[10]	29	7	
NPHP	United States[10]	80	26	
Total NPHP		109	33	**142**
Total Ciliopathy		555	198	**753**
Controls	United Kingdom[2]		96	
Controls	United States[8]	192		
CEU	United States[11]	28		
TSI	Italy[11]		21	
YRI	Nigeria[11]		34	
GIH	United States[11]		27	
Total Controls		220	178	**398**

MKS, Meckel-Gruber Syndrome; JATD, Jeune Asphyxiating Thoracic Dystrophy; BBS, Bardet-Biedl Syndrome; JBTS, Joubert Syndrome; SLS, Senior-Loken Syndrome; NPHP, isolated Nephronophthisis; NPHP+, NPHP with extrarenal phenotypes.

[1]Hôpital Necker-Enfants Malades, Paris, France; [2]St. James's University Hospital, Leeds, UK; [3]University College London, London, UK; [4]University of Birmingham, Birmingham, UK; [5]Johns Hopkins University, Baltimore, MD, USA; [6]Université Louis Pasteur, Strasbourg, France; [7]University of Aachen, Aachen, Germany; [8]Baylor College of Medicine, Houston, TX, USA; [9]University of California, San Diego, La Jolla, CA, USA; [10]University of Michigan, Ann Arbor, MI, USA [11]NHGRI Collection/International HapMap Project.

Supplementary Table 2. Summary of *TTC21B* variant pathogenicity.

	Variant counts/Cohort				Functional Assessment						
TTC21B Variant	N. Eur Ciliopathy (n=1110)	Other Ciliopathy (n=396)	N. Eur Controls (n=440)	Other Controls (n=356)	Zebrafish *in vivo*	Zebrafish morphometric	*In vitro* cilia measurement	*In vitro* localization	*In vivo* retinal localization	Western-level relative to WT	Overall pathogenicity**
Nonsense											
K31fsX48	1*	-	-	-	ND	ND	ND	ND	ND	ND	Null- PTC
R411X	1*	-	-	-	ND	ND	ND	ND	ND	ND	Null- PTC
F440fsX443	1*	-	-	-	ND	ND	ND	ND	ND	ND	Null- PTC
C552X	-	1	-	-	ND	ND	ND	ND	ND	ND	Null- PTC
Splice											
c.711G>T	1*	-	-	-	ND	ND	ND	ND	ND	ND	Null- predicted splice mutation-PTC
c.2322+3A>G	3*	-	-	-	ND	ND	ND	ND	ND	ND	Null- predicted Δ2 TPR domains
c.2758-2A>G	-	1	-	-	ND	ND	ND	ND	ND	ND	Null- predicted Δ partial TPR domain
c.3264-3C>G	1*	-	-	-	ND	ND	ND	ND	ND	ND	Null- predicted Δ54 amino acids
Novel Missense											
F60Y	1*	-	-		hypomorph	ND	ND	ND	ND	ND	Hypomorph
K66R	-	-	-	1	benign	ND	ND	ND	ND	ND	Benign
W150R	1*	-	-	-	null	ND	ND	ND	ND	ND	Null
K157E	1*	-	-	-	hypomorph	benign	hypomorph	undetected	ND	ND	Hypomorph
P209L	-	7^	-	-	hypomorph	pathogenic	hypomorph	mislocalized	mislocalized	decreased	Hypomorph
Q222L	1*	-	-	4	hypomorph	pathogenic	hypomorph	undetected	ND	ND	Hypomorph
T231S	3*	5	-	-	hypomorph	pathogenic	hypomorph	normal	ND	decreased	Hypomorph
D242N	6	4	4	1	benign	ND	ND	ND	ND	ND	Benign
Y255C	1*	-	-	-	hypomorph	pathogenic	hypomorph	normal	ND	decreased	Hypomorph
M280V	1*	-	-	4	null	pathogenic	null	normal	ND	decreased	Null
A327S	-	1	-	-	hypomorph	pathogenic	hypomorph	normal	ND	decreased	Hypomorph
Y347C	-	1	-	-	hypomorph	pathogenic	hypomorph	mislocalized	ND	normal	Hypomorph
R411G	-	1	-	1	hypomorph	pathogenic	hypomorph	mislocalized	mislocalized	decreased	Hypomorph
Q412R	-	-	-	1	benign	ND	ND	ND	ND	ND	Benign
D424E	-	-	-	1	benign	ND	ND	ND	ND	ND	Benign
H566R	1*	-	-	-	null	ND	ND	ND	ND	ND	Null
S591N	1*	-	-	-	hypomorph	pathogenic	hypomorph	mislocalized	ND	decreased	Hypomorph
R616C	1	9	2	6	benign	ND	ND	ND	ND	ND	Benign
I624V	-	-	-	1	benign	ND	ND	ND	ND	ND	Benign
H645R	-	-	1	-	benign	ND	ND	ND	ND	ND	Benign
S724T	1	-	-	-	benign	ND	ND	ND	ND	ND	Benign
P753L	-	2	-	5	null	pathogenic	null	normal	ND	increased	Null
D755Y	-	1	-	-	null	ND	ND	ND	ND	ND	Null
L795P	1*	-	-	-	hypomorph	pathogenic	hypomorph	normal	ND	normal	Hypomorph
M844V	-	1	-	-	null	ND	ND	ND	ND	ND	Null
R867C	-	1	-	-	null	ND	ND	ND	ND	ND	Null
R867H	1*	-	-	1	null	ND	ND	ND	ND	ND	Null
Q869R	1*	-	-	-	hypomorph	ND	ND	ND	ND	ND	Hypomorph
R939Q	-	-	-	1	hypomorph	ND	ND	ND	ND	ND	Hypomorph

R939W	-	-	2*	-	null	pathogenic	null	normal	ND	decreased	Null
L1002V	5*	-	2*	1	hypomorph	ND	hypomorph	ND	mislocalized	decreased	Hypomorph
M1011V	-	-	-	1	benign	ND	ND	ND	ND	ND	Benign
Y1035C	-	-	-	2	benign	ND	ND	ND	ND	ND	Benign
D1041N	-	1	-	-	null	ND	hypomorph	mislocalized	mislocalized	increased	Null
T1103R	1*	-	-	-	hypomorph	pathogenic	hypomorph	mislocalized	mislocalized	normal	Hypomorph
Y1167C	-	1	-	-	hypomorph	ND	ND	ND	ND	ND	Hypomorph
M1186V	-	1	-	-	hypomorph	ND	ND	ND	ND	ND	Hypomorph
I1208S	-	1	-	-	null	pathogenic	null	undetected	undetected	undetected	Null
D1284H	-	-	-	1	hypomorph	ND	ND	ND	ND	ND	Hypomorph
R1311G	2	-	1	-	benign	ND	ND	ND	ND	ND	Benign
HapMap Variants											
M210V (rs1432273)	306	70	141	67	benign	ND	ND	ND	ND	ND	Benign
A276T (rs7592429)	2	43	1	28	benign	ND	ND	ND	ND	ND	Benign
P463S (rs16851307)	-	1	-	1	benign	ND	ND	ND	ND	ND	Benign
L473F (rs2163649)	-	1	-	-	benign	ND	ND	ND	ND	ND	Benign

* Pathogenic variants counted in genetic association studies. ^Likely founder allele. TPR, tetratricopeptide repeat; ND, not done; PTC, premature termination codon.

** Overall pathogenicity is based on *in vivo* zebrafish scoring, which is in agreement with at least one other functional assay for 17/17 variants.

Supplementary Table 3. *In vivo* rescue efficiency of *ttc21b* morphant phenotypes with *TTC21B* mRNA

***TTC21B* Variant**	**Cohort**		**Injection**	**Embryo counts**				**vs. WT rescue**		**vs. MO alone**		**Pathogenicity**
	Case	Cntrl		Normal	Class I	Class II	n=	effect*	P-val**	effect*	P-val**	*in vivo* call***
			Controls	225	21	0	246					
			10ng MO	79	62	35	176					
WT			10ng MO + 50pg WT RNA	139	29	17	185				<0.0001	
F60Y	X		10ng MO + 50pg F60Y RNA	66	17	24	107	-	<0.0001	+	0.0002	hypomorph
K66R		X	10ng MO + 50pg K66R RNA	72	17	13	102	NS	0.3636	+	<0.0001	benign
W150R	X		10ng MO + 50pg W150R RNA	64	39	30	133	-	<0.0001	NS	0.432	null
K157E	X		10ng MO + 50pg K157E RNA	74	27	7	108	-	0.038	+	<0.0001	hypomorph
M201V (rs1432273)	X	X	10ng MO + 50pg M201V RNA	87	22	8	117	NS	0.6004	+	<0.0001	benign
P209L	X		10ng MO + 50pg P209L RNA	48	21	8	77	-	0.0067	+	0.0012	hypomorph
Q222L	X	X	10ng MO + 50pg Q222L RNA	61	29	10	100	-	0.0013	+	0.0029	hypomorph
T231S	X		10ng MO + 50pg T231S RNA	57	32	6	95	-	<0.0001	+	0.0006	hypomorph
D242N	X	X	10ng MO + 50pg D242N RNA	83	26	1	110	+	0.0039	+	<0.0001	benign
Y255C	X		10ng MO + 50pg Y255C RNA	62	41	10	113	-	<0.0001	+	0.0158	hypomorph
A276T (rs7592429)	X	X	10ng MO + 50pg A276T RNA	78	11	6	95	NS	0.2654	+	<0.0001	benign
M280V	X	X	10ng MO + 50pg M280V RNA	68	38	30	136	-	<0.0001	NS	0.3404	null
A327S	X		10ng MO + 50pg A327S RNA	75	29	21	125	-	0.0014	+	0.0084	hypomorph
Y347C	X		10ng MO + 50pg Y347C RNA	72	34	12	118	-	0.0013	+	0.0029	hypomorph
R411G	X	X	10ng MO + 50pg R411G RNA	71	44	12	127	-	<0.0001	+	0.0127	hypomorph
Q412R		X	10ng MO + 50pg Q412R RNA	77	9	8	94	NS	0.2387	+	<0.0001	benign
D424E		X	10ng MO + 50pg D424E RNA	67	9	7	83	NS	0.3407	+	<0.0001	benign
P463S (rs16851307)	X	X	10ng MO + 50pg P463S RNA	68	12	10	90	NS	0.6004	+	<0.0001	benign
L473F (rs2163649)	X	X	10ng MO + 50pg L473F RNA	74	10	2	86	+	0.0178	+	<0.0001	benign
H566R	X		10ng MO + 50pg H566R RNA	32	17	33	82	-	<0.0001	-	<0.0001	null
S591N	X		10ng MO + 50pg S591N RNA	116	45	19	180	-	0.0284	+	0.0006	hypomorph
R616C	X	X	10ng MO + 50pg R616C RNA	91	16	3	110	NS	0.0882	+	<0.0001	benign
I624V		X	10ng MO + 50pg I624V RNA	72	12	3	87	NS	0.078	+	<0.0001	benign
H645R		X	10ng MO + 50pg H645R RNA	92	13	5	110	NS	0.1627	+	<0.0001	benign
S724T	X		10ng MO + 50pg S724T RNA	63	15	10	88	NS	0.7309	+	<0.0001	benign
P753L	X	X	10ng MO + 50pg P753L RNA	46	44	23	113	-	<0.0001	NS	0.6661	null
D755Y	X		10ng MO + 50pg D755Y RNA	42	11	26	79	-	<0.0001	-	<0.0001	null
L795P	X		10ng MO + 50pg L795P RNA	63	23	14	100	-	0.0206	+	0.0014	hypomorph
M844V	X		10ng MO + 50pg M844V RNA	74	40	44	158	-	<0.0001	-	0.0463	null
R867C	X		10ng MO + 50pg R867C RNA	68	37	49	154	-	<0.0001	-	0.0048	null
R867H	X	X	10ng MO + 50pg R867H RNA	64	29	30	123	-	<0.0001	NS	0.069	null
Q869R	X		10ng MO + 50pg Q869R RNA	77	28	16	121	-	0.0397	+	0.0007	hypomorph
R939Q		X	10ng MO + 50pg R939Q RNA	52	18	20	90	-	<0.0001	+	0.0056	hypomorph
R939W		X	10ng MO + 50pg R939W RNA	33	26	17	76	-	<0.0001	NS	0.8562	null

L1002V	X	X	10ng MO + 50pg L1002V RNA	51	26	6	83	-	0.0002	+	0.0006	hypomorph
M1011V		X	10ng MO + 50pg M1011V RNA	100	13	10	123	NS	0.3407	+	<0.0001	benign
Y1035C		X	10ng MO + 50pg Y1035C RNA	71	19	9	99	NS	0.6792	+	0.0001	benign
D1041N	X		10ng MO + 50pg D1041N RNA	39	17	15	71	-	<0.0001	NS	0.057	null
T1103R	X		10ng MO + 50pg T1103R RNA	65	38	14	117	-	<0.0001	+	0.0463	hypomorph
Y1167C	X		10ng MO + 50pg Y1167C RNA	49	15	22	86	-	<0.0001	+	0.0008	hypomorph
M1186V	X		10ng MO + 50pg M1186V RNA	51	19	22	92	-	<0.0001	+	0.0134	hypomorph
I1208S	X		10ng MO + 50pg I1208S RNA	48	38	39	125	-	<0.0001	-	0.019	null
D1284H		X	10ng MO + 50pg D1284H RNA	78	16	18	112	-	0.0491	+	<0.0001	hypomorph
R1311G	X	X	10ng MO + 50pg R1311G RNA	55	11	12	78	NS	0.1073	+	<0.0001	benign

* indicates effect of test injection vs. WT rescue or MO alone. "NS", not significantly different; "+," ameliorated phenotype; "-," exacerbated phenotype

** P-value for χ^2 test; $p<0.05$ is considered significant

*** benign- significantly ameliorated in comparison to MO alone and either improved or not significantly different from WT rescue; hypomorph- significantly exacerbated in comparison to WT rescue, but significantly ameliorated in comparison to MO alone; null- significantly exacerbated in comparison to WT rescue, but not significantly different or exacerbated in comparison to MO alone.

Supplementary Table 4. *In vivo* phenotypic data for *TTC21B* mRNA injected alone

***TTC21B* Variant**	**Cohort**		**Injection**	**Embryo counts**				**versus WT mRNA**		**versus MO alone**	
	Case	Cntrl		Normal	Class I	Class II	n=	effect*	P-val**	effect*	P-val**
			Controls	225	21	0	246				
			10ng MO	79	62	35	176				
WT			50pg WT RNA	68	10	3	81			+	<0.0001
F60Y	X		50pg F60Y RNA	102	14	2	118	NS	0.5923	+	<0.0001
K66R		X	50pg K66R RNA	78	16	3	97	NS	0.4103	+	<0.0001
W150R	X		50pg W150R RNA	77	15	4	96	NS	0.4668	+	<0.0001
K157E	X		50pg K157E RNA	104	12	1	117	NS	0.2368	+	<0.0001
M201V (rs1432273)	X	X	50pg M201V RNA	112	12	2	126	NS	0.4482	+	<0.0001
P209L	X		50pg P209L RNA	68	10	2	80	NS	0.8471	+	<0.0001
Q222L	X	X	50pg Q222L RNA	70	10	6	86	NS	0.3077	+	<0.0001
T231S	X		50pg T231S RNA	98	10	4	112	NS	0.6309	+	<0.0001
D242N	X	X	50pg D242N RNA	110	18	1	129	NS	0.2732	+	<0.0001
Y255C	X		50pg Y255C RNA	92	16	6	114	NS	0.7081	+	<0.0001
A276T (rs7592429)	X	X	50pg A276T RNA	112	10	4	126	NS	0.3904	+	<0.0001
M280V	X	X	50pg M280V RNA	80	9	6	95	NS	0.4153	+	<0.0001
A327S	X		50pg A327S RNA	70	5	4	79	NS	0.1697	+	<0.0001
Y347C	X		50pg Y347C RNA	108	6	4	118	NS	0.0783	+	<0.0001
R411G	X	X	50pg R411G RNA	108	21	4	133	NS	0.4295	+	<0.0001
Q412R		X	50pg Q412R RNA	68	9	6	83	NS	0.3041	+	<0.0001
D424E		X	50pg D424E RNA	74	10	3	87	NS	0.8442	+	<0.0001
P463S (rs16851307)	X	X	50pg P463S RNA	84	6	1	91	NS	0.0783	+	<0.0001
L473F (rs2163649)	X	X	50pg L473F RNA	94	8	1	103	NS	0.1245	+	<0.0001
H566R	X		50pg H566R RNA	55	10	2	67	NS	0.5923	+	<0.0001
S591N	X		50pg S591N RNA	112	10	2	124	NS	0.2513	+	<0.0001
R616C	X	X	50pg R616C RNA	109	8	2	119	NS	0.1498	+	<0.0001
I624V		X	50pg I624V RNA	75	9	5	89	NS	0.5134	+	<0.0001
H645R		X	50pg H645R RNA	88	12	6	106	NS	0.5783	+	<0.0001
S724T	X		50pg S724T RNA	78	6	1	85	NS	0.0783	+	<0.0001
P753L	X	X	50pg P753L RNA	56	4	2	62	NS	0.1568	+	<0.0001
D755Y	X		50pg D755Y RNA	67	7	2	76	NS	0.5514	+	<0.0001
L795P	X		50pg L795P RNA	104	8	4	116	NS	0.2513	+	<0.0001
M844V	X		50pg M844V RNA	64	7	5	76	NS	0.2231	+	<0.0001
R867C	X		50pg R867C RNA	66	5	2	73	NS	0.8471	+	<0.0001
R867H	X	X	50pg R867H RNA	58	9	4	71	NS	0.5741	+	<0.0001
Q869R	X		50pg Q869R RNA	92	6	5	103	NS	0.1697	+	<0.0001
R939Q		X	50pg R939Q RNA	74	12	6	92	NS	0.2831	+	<0.0001
R939W		X	50pg R939W RNA	90	18	6	114	NS	0.3904	+	<0.0001
L1002V	X	X	50pg L1002V RNA	68	5	2	75	NS	0.2372	+	<0.0001

M1011V		X	50pg M1011V RNA	92	10	4	106	NS	0.6514	+	<0.0001
Y1035C		X	50pg Y1035C RNA	72	11	5	88	NS	0.5741	+	<0.0001
D1041N	X		50pg D1041N RNA	80	17	7	104	NS	0.1379	+	<0.0001
T1103R	X		50pg T1103R RNA	92	19	4	115	NS	0.1245	+	<0.0001
Y1167C	X		50pg Y1167C RNA	90	12	8	110	NS	0.3041	+	<0.0001
M1186V	X		50pg M1186V RNA	78	10	4	92	NS	0.9535	+	<0.0001
I1208S	X		50pg I1208S RNA	63	11	6	80	NS	0.1015	+	<0.0001
D1284H		X	50pg D1284H RNA	96	18	6	120	NS	0.5514	+	<0.0001
R1311G	X	X	50pg R1311G RNA	58	10	3	71	NS	0.8266	+	<0.0001

* indicates effect of test injection vs. WT mRNA or MO alone. "NS", not significantly different; "+," ameliorated phenotype

** P-value for χ^2 test; <0.05 is considered significant

Supplementary Table 5. Morphometric measurements of embryos labeled *in situ* with *krox20*, *pax2*, and *myoD*

***TTC21B* Variant**	**Injection**	**W/L ratio**			**versus WT rescue**	**versus MO alone**	**Pathogenicity**
		n=	Mean	St. Dev	P-val	P-val	
	Controls	10	0.164	0.014			
	10ng MO	10	0.214	0.034			
WT	10ng MO + 50pg WT RNA	10	0.169	0.028			
K157E	10ng MO + 50pg K157E RNA	9	0.184	0.017	0.0905	0.014	benign
P209L	10ng MO + 50pg P209L RNA	7	0.189	0.018	0.0500	0.049	pathogenic
Q222L	10ng MO + 50pg Q222L RNA	9	0.193	0.019	<0.0001	0.054	pathogenic
T231S	10ng MO + 50pg T231S RNA	9	0.229	0.060	0.0058	0.263	pathogenic
Y255C	10ng MO + 50pg Y255C RNA	9	0.266	0.088	0.0020	0.051	pathogenic
M280V	10ng MO + 50pg M280V RNA	10	0.200	0.066	0.0008	0.278	pathogenic
A327S	10ng MO + 50pg A327S RNA	9	0.193	0.022	0.0250	0.068	pathogenic
Y347C	10ng MO + 50pg Y347C RNA	10	0.205	0.026	0.0036	0.249	pathogenic
R411G	10ng MO + 50pg R411G RNA	10	0.212	0.019	0.0004	0.415	pathogenic
S591N	10ng MO + 50pg S591N RNA	10	0.207	0.020	0.0012	0.285	pathogenic
P753L	10ng MO + 50pg P753L RNA	9	0.276	0.108	0.0038	0.052	pathogenic
L795P	10ng MO + 50pg L795P RNA	10	0.198	0.017	0.0059	0.088	pathogenic
R939W	10ng MO + 50pg R939W RNA	9	0.215	0.062	0.0240	0.484	pathogenic
T1103R	10ng MO + 50pg T1103R RNA	10	0.206	0.015	0.0007	0.244	pathogenic
I1208S	10ng MO + 50pg I1208S RNA	10	0.226	0.053	0.0039	0.286	pathogenic

Supplementary Table 6. Cilia length measurements for *in vitro* complementation assays in IMCD3-*Ttc21b* shRNA cells.

***TTC21B* Variant**	**Cell line**	**Cilia length (mm)**			**versus WT rescue**	**versus shRNA alone**	**Pathogenicity**
		n=	Mean	St. Dev	P-val	P-val	
	IMCD3-EV	102	4.08	1.04			
	IMCD3-*Ttc21b*-shRNA	104	2.07	0.69			
WT	IMCD3- *Ttc21b* -shRNA	96	4.10	1.65			
K157E	IMCD3- *Ttc21b* -shRNA	122	2.33	0.59	<0.0001	0.0026	hypomorph
P209L	IMCD3- *Ttc21b* -shRNA	90	2.45	0.91	<0.0001	0.0011	hypomorph
Q222L	IMCD3- *Ttc21b* -shRNA	122	2.58	0.82	<0.0001	<0.0001	hypomorph
T231S	IMCD3- *Ttc21b* -shRNA	133	2.40	0.59	<0.0001	0.0001	hypomorph
Y255C	IMCD3- *Ttc21b* -shRNA	126	2.47	0.69	<0.0001	<0.0001	hypomorph
M280V	IMCD3- *Ttc21b* -shRNA	131	2.03	0.52	<0.0001	0.0454	null
A327S	IMCD3- *Ttc21b* -shRNA	155	2.68	0.75	<0.0001	<0.0001	hypomorph
Y347C	IMCD3- *Ttc21b* -shRNA	116	2.37	0.61	<0.0001	0.0008	hypomorph
R411G	IMCD3- *Ttc21b* -shRNA	102	2.45	0.87	<0.0001	0.0006	hypomorph
S591N	IMCD3- *Ttc21b* -shRNA	114	2.47	0.58	<0.0001	<0.0001	hypomorph
P753L	IMCD3- *Ttc21b* -shRNA	135	2.02	0.49	<0.0001	0.5452	null
L795P	IMCD3- *Ttc21b* -shRNA	115	2.54	0.68	<0.0001	<0.0001	hypomorph
R939W	IMCD3- *Ttc21b* -shRNA	114	2.09	0.62	<0.0001	0.8241	null
L1002V	IMCD3- *Ttc21b* -shRNA	154	2.56	0.60	<0.0001	<0.0001	hypomorph
D1041N	IMCD3- *Ttc21b* -shRNA	117	2.54	0.70	<0.0001	<0.0001	hypomorph
T1103R	IMCD3- *Ttc21b* -shRNA	85	2.84	1.35	<0.0001	<0.0001	hypomorph
I1208S	IMCD3- *Ttc21b* -shRNA	89	2.05	0.74	<0.0001	0.8555	null

Supplementary Table 7. Densitometry analysis of TTC21B variants transiently transfected and tested for protein levels by Western blot.

Variant	TTC21B-V5	N^+/K^+-ATPase	TTC21B/ N^+/K^+ATPase	Normalized to WT	Effect
WT	1.45	25.34	0.057	1	
K157E	0.03	17.61	0.002	0.030	decrease
P209L	0.30	23.48	0.013	0.203	decrease
Q222L	0.04	25.43	0.002	0.028	decrease
T231S	0.60	15.44	0.039	0.682	decrease
Y255C	0.24	20.26	0.012	0.208	decrease
M280V	0.76	19.38	0.039	0.688	decrease
A327S	0.94	27.14	0.035	0.489	decrease
Y347C	1.20	22.38	0.054	0.941	normal
R411G	0.98	26.74	0.037	0.582	decrease
S591N	0.31	13.91	0.022	0.391	decrease
P753L	1.76	16.10	0.109	1.918	increase
L795P	1.04	19.26	0.054	0.947	normal
R939W	0.37	21.10	0.018	0.308	decrease
L1002V	0.90	20.81	0.043	0.759	decrease
D1041N	1.79	19.55	0.092	1.606	increase
T1103R	1.78	25.14	0.071	1.124	normal
I1208S	0.01	24.00	0	0	undetected

Values expressed as arbitrary fluorescent units.

Supplementary Table 8. Evolutionary conservation of TTC21B missense variants

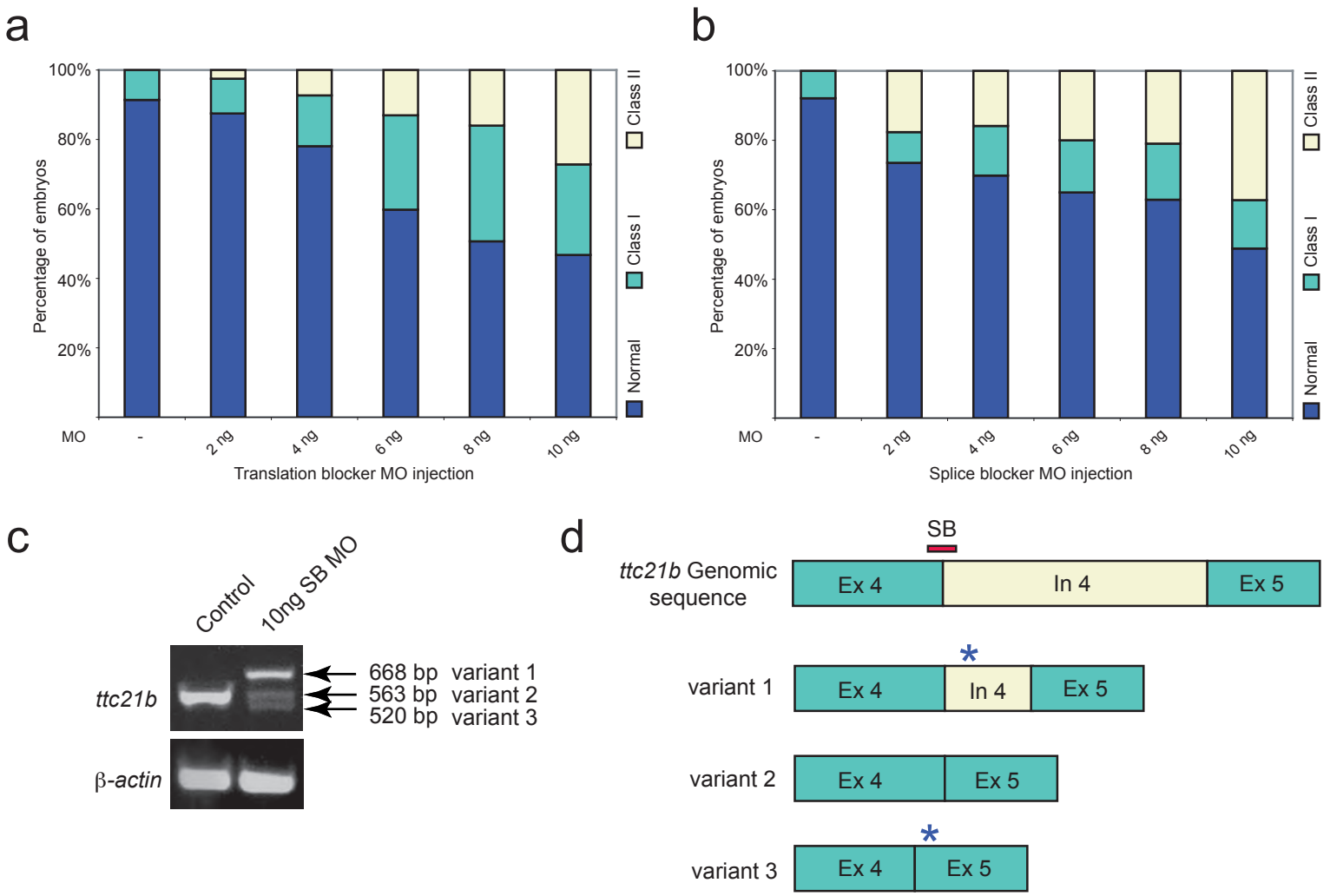
	Cohort		Evolutionary conservation																	
***TTC21B* Variant**	**Case**	**Cntrl**	**Hsa**	**Mmu**	**Bta**	**Eca**	**Cfa**	**Mmus**	**Rno**	**Mdo**	**Oan**	**Gga**	**Tgu**	**Xla**	**Dre**	**Spu**	**Cel**	**Cre**	**Protein domains**	**ClustalW2 Conservation Score**	**BLOSUM62 Substitution Matrix Score**	**Overall pathogenicity**
F60Y	X		F	F	F	F	F	F	F	L	L	L	L	L	L	L	L	L		8	3	Hypomorph
K66R		X	K	K	K	K	K	K	K	K	K	K	K	D	R	K	D	N		6	2	Benign
W150R	X		W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W		11	-3	Null
K157E	X		K	K	K	K	K	K	K	K	K	K	K	K	K	R	K	Q		7	1	Hypomorph
P209L	X		P	P	P	P	P	P	P	P	P	P	P	P	P	P	I	G	TPR	5	-3	Hypomorph
Q222L	X	X	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	F	L	Q	S	L		4	-2	Hypomorph
T231S	X		T	T	T	T	T	T	T	T	T	A	T	T	T	T	V	V		6	1	Hypomorph
D242N	X	X	D	D	D	D	D	D	D	D	N	D	D	D	E	E	E	D		8	1	Benign
Y255C	X		Y	Y	Y	Y	Y	Y	Y	Y	H	H	H	H	H	Q	H	I		4	-2	Hypomorph
M280V	X	X	M	M	V	M	M	M	M	M	V	L	L	A	Q	F	N	Q		3	-2	Null
A327S	X		A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	V	TPR	3	1	Hypomorph
Y347C	X		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	S	F	TPR	5	-2	Hypomorph
R411G	X	X	R	R	R	R	P	R	R	G	R	R	R	E	P	P	S	-		0	-2	Hypomorph
Q412R		X	Q	Q	Q	Q	R	Q	Q	Q	Q	Q	Q	E	P	A	F	-		0	1	Benign
D424E		X	D	D	D	D	D	N	N	D	D	D	D	E	D	T	E	A		4	2	Benign
H566R	X		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	TPR	11	0	Null
S591N	X		S	S	S	S	S	N	N	S	S	N	N	S	S	A	Q	N	TPR	5	1	Hypomorph
R616C	X	X	R	R	R	R	R	R	R	R	R	R	R	K	C	R	K	R		5	-3	Benign
I624V		X	I	I	V	V	V	V	V	V	V	V	V	A	A	A	I	A		7	1	Benign
H645R		X	H	H	H	H	H	H	H	N	N	D	N	N	N	N	A	R		2	0	Benign
S724T	X		S	S	S	S	S	S	S	T	T	T	T	T	S	T	S	S	TPR	9	1	Benign
P753L	X	X	P	P	P	P	P	P	P	P	P	P	P	P	P	P	S	P	TPR	7	-3	Null
D755Y	X		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	TPR	11	-3	Null
L795P	X		L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	TPR	11	-3	Hypomorph
M844V	X		M	M	V	V	M	M	M	M	M	M	M	Y	V	G	M	K	TPR	3	-2	Null
R867C	X		R	R	R	R	R	R	R	R	R	R	R	R	R	R	K	K		9	-3	Null
R867H	X	X	R	R	R	R	R	R	R	R	R	R	R	R	R	R	K	K		9	0	Null
Q869R	X		Q	L	Q	Q	Q	Q	Q	Q	Q	Q	Q	P	Q	Q	L	R		3	1	Hypomorph
R939Q		X	R	R	R	R	R	R	R	R	R	H	H	N	Q	H	Q	A		3	1	Hypomorph
R939W		X	R	R	R	R	R	R	R	R	R	H	H	N	Q	H	Q	A		3	-3	Null
L1002V	X	X	L	L	L	L	L	L	L	L	L	L	L	L	L	L	Q	L		7	3	Hypomorph
M1011V		X	M	M	M	M	M	M	M	M	M	M	M	K	M	Q	R	L		5	-2	Benign
Y1035C		X	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		11	-2	Benign
D1041N	X		D	D	D	D	D	D	D	D	D	D	D	D	D	L	E	E		4	1	Null
T1103R	X		T	T	T	T	T	T	T	T	T	T	T	T	T	T	K	T		6	-1	Hypomorph
Y1167C	X		Y	Y	H	Y	Y	Y	Y	F	F	Y	Y	Y	Y	Y	H	F		7	-2	Hypomorph
M1186V	X		M	M	M	M	M	M	M	M	M	M	M	V	M	M	R	I		4	-2	Hypomorph
I1208S	X		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	TPR	11	-2	Null
D1284H		X	D	D	D	D	D	D	D	D	N	D	D	D	D	D	A	E	TPR	4	-1	Hypomorph

R1311G	X	X	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	C	**R**	H	Q	**R**	**R**	K	**R**	4	-2	Benign
HapMap Variants																					
V201M (rs1432273)	X	X	**V**	**V**	**V**	**V**	**V**	**V**	**V**	**V**	**V**	**V**	**V**	I	I	I	A	L	7	1	Benign
T276A (rs7592429)	X	X	**T**	A	A	A	A	A	A	**T**	A	A	A	A	N	L	S	S	4	-1	Benign
P463S (rs16851307)	X	X	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	S	**P**	**P**	**P**	7	-1	Benign
L473F (rs2163649)	X	X	**L**	**L**	**L**	**L**	**L**	V	V	**L**	**L**	**L**	**L**	**L**	Q	A	C	**L**	5	0	Benign

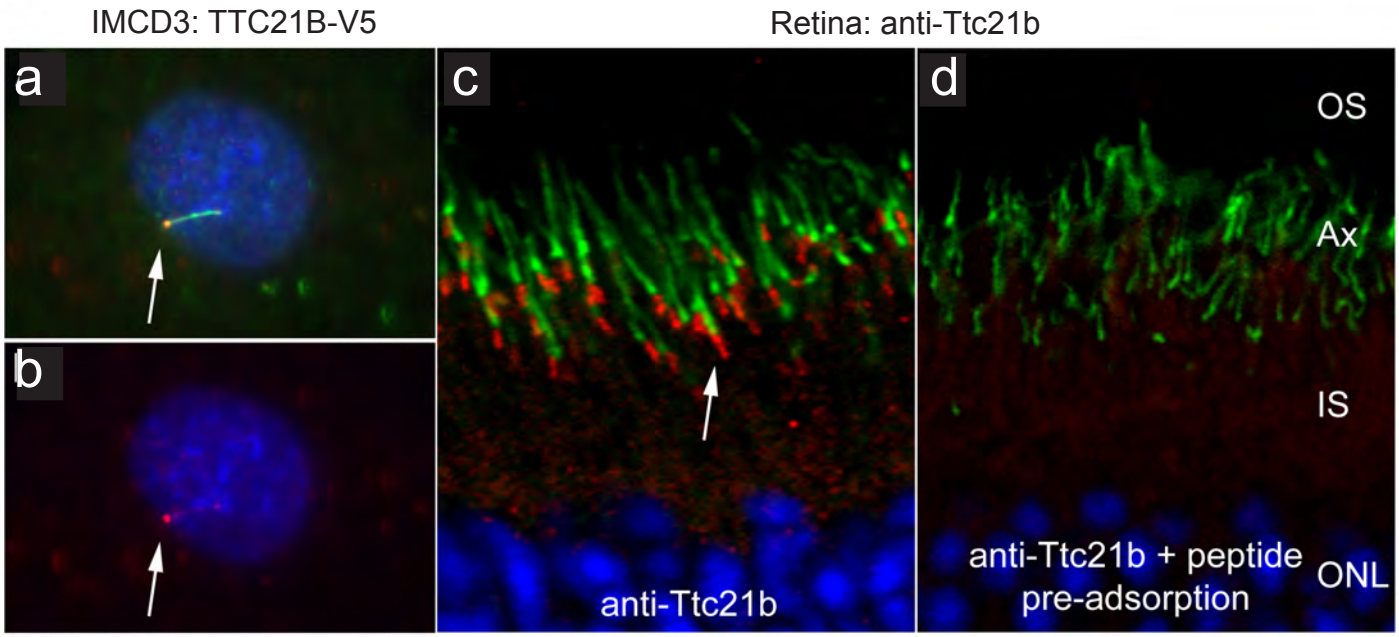
Evolutionary conservation species abbreviated as follows: Hsa, H. Sapiens; Mmul, M. mulatta; Bta, B. taurus; Eca, E. caballus; Cfa, C. familiaris; Mmus, M. musculus; Rno, R. norvegicus; Mdo, M. domesticus; Oan, O. anatinus; Gga, G. gallus; Tgu, T. guttata; Xla, X. laevis; Dre, D. rerio; Spu, S. purpuratus; Cel, C. elegans; Cre, C. reinhardtii. Nucleotides indicated in yellow or gray; Amino acids coded for AVFPMILW (red), small (small+ hydrophobic (including aromatic -Y)); DE (blue), acidic; RK (magenta), basic; STYHCNGQ (green), hydroxyl + amine + basic – Q. Domains: TPR, tetratricopeptide repeat.

Supplementary Table 9. Haplotype analysis of *TTC21B* P209L homozygotes

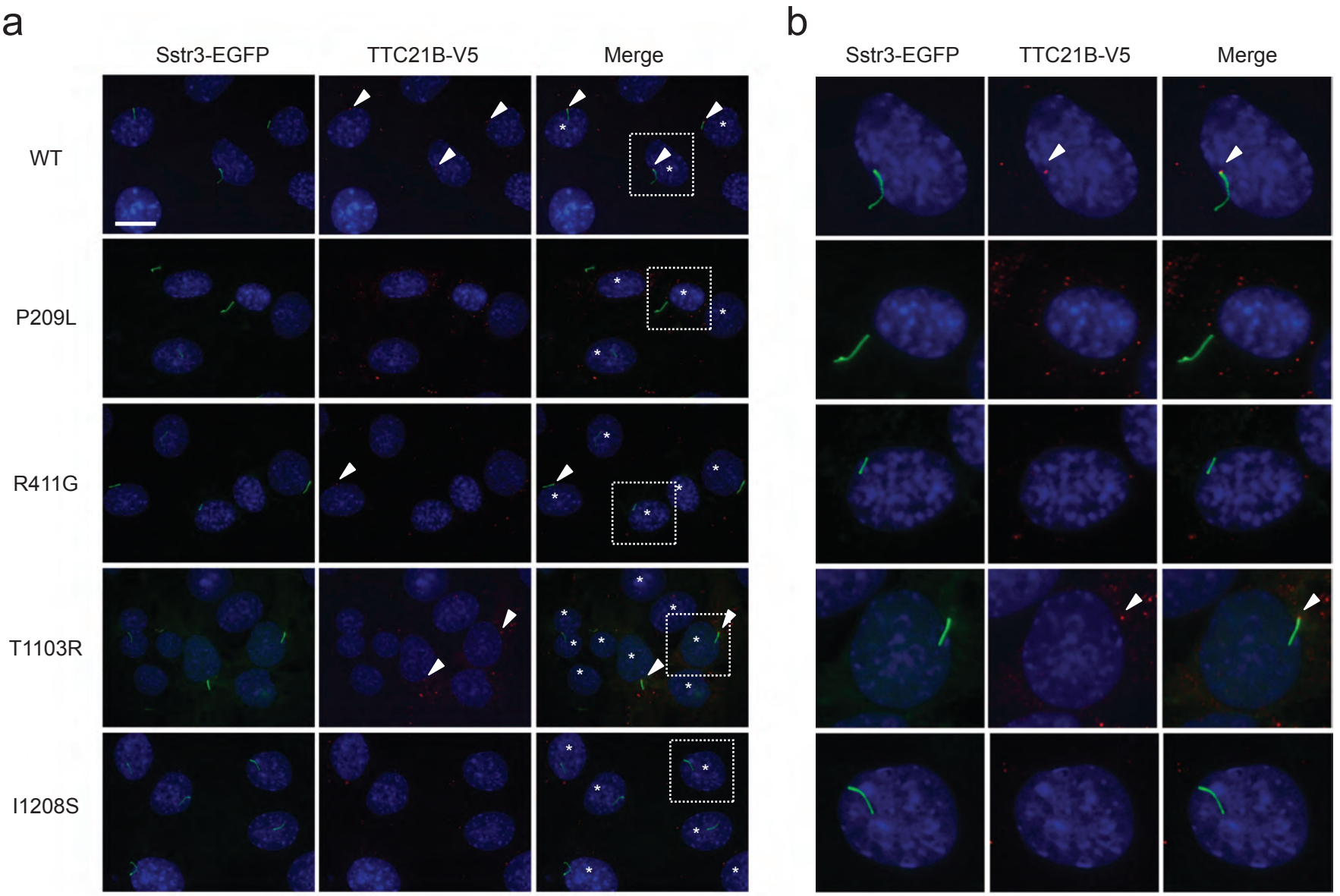
			Genotype	
***TTC21B* Exon**	**SNP ID**	**Genomic coordinate**	**A3214-II1**	**F623-II1**
2	rs34486024	chr2:166,806,142	C/C	C/C
6	rs1432273	chr2:166,797,646	A/A	A/A
8	rs7592429	chr2:166,788,336	G/G	G/G
10	rs62177816	chr2:166,786,137	C/C	C/C
12	rs16851307	chr2:166,781,188	C/C	C/C
12	rs2163649	chr2:166,781,158	C/C	C/C
14	rs6750044	chr2:166,773,971	C/C	C/C
15	rs16822802	chr2:166,771,884	G/G	G/G
15	rs7590559	chr2:166,771,663	G/G	G/G
16	rs10176588	chr2:166,770,120	T/T	T/T
16	rs73969727	chr2:166,770,071	A/A	A/A
20	rs7595100	chr2:166,764,281	C/C	C/C
20	rs7595010	chr2:166,764,219	C/C	C/C
24	rs34925776	chr2:166,747,029	G/G	G/G
26	rs3749031	chr2:166,740,323	G/G	G/G
27	rs71426786	chr2:166,737,306	T/T	T/T



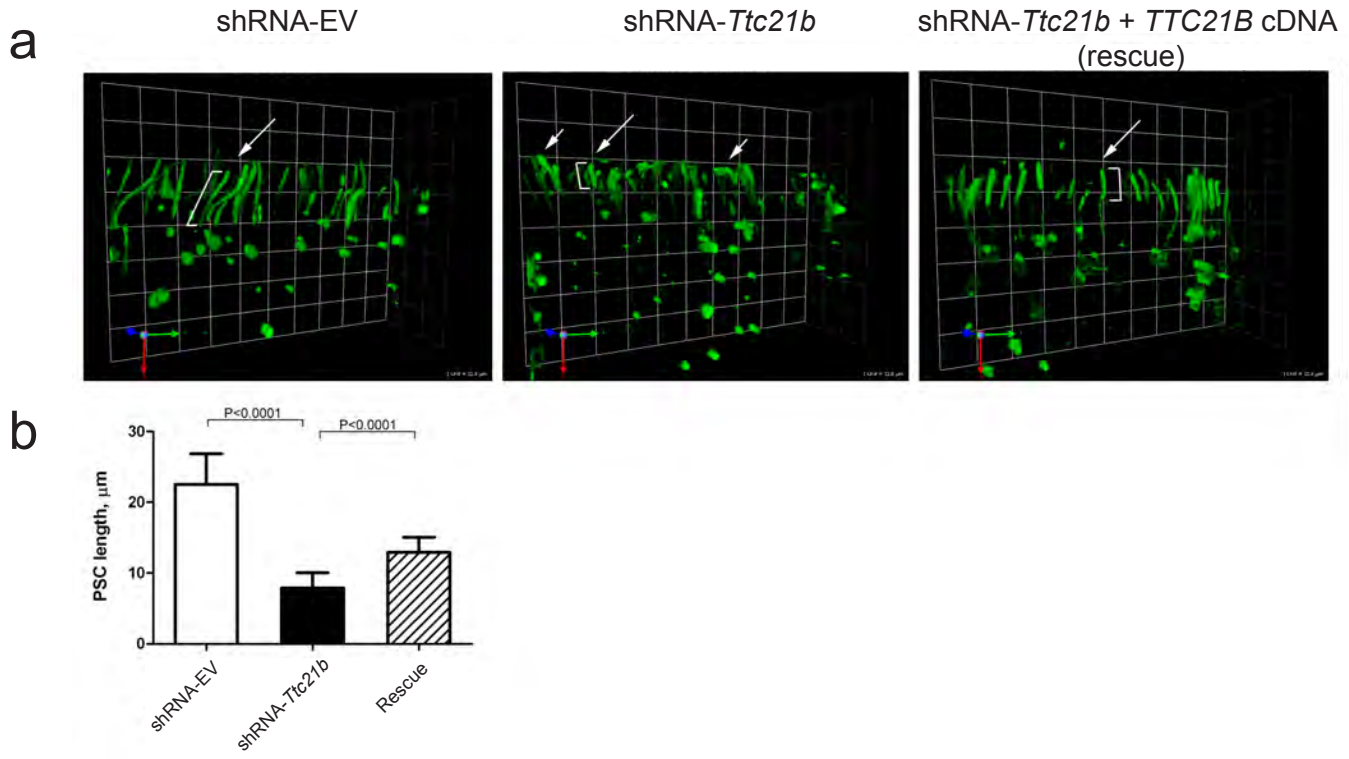
Supplementary Figure 1. Characterization of *ttc21b* morpholinos. (**a**) Dose response curve for *ttc21b* translation-blocking (tb) morpholino (MO). Wild type zebrafish embryos were injected at the two-cell stage with increasing concentrations of tb-MO (2ng, 4ng, 6ng, 8ng, and 10ng) and scored at the 9-10 somite stage for gastrulation defects. Class I, shortened embryonic body axis with small head/eye and mild somite defects; Class II, severely shortened anterior-posterior axis, severely affected anterior structures, broadening and kinking of the notochord, thinning and widening of the somites, and tail extension defects. (**b**) Dose response curve for *ttc21b* splice-blocking (sb) morpholino (MO); injection concentrations and phenotypic criteria are described in (a). Both *ttc21b* tb and sb MOs give rise to similar phenotypic dose responses. (**c**) Determination of *ttc21b* sb-MO efficiency. Total RNA was extracted from whole embryos at the 10-somite stage and oligo dT primed cDNA was generated by reverse transcription. Subsequent PCR of zebrafish *ttc21b* and agarose gel electrophoresis resulted in three different amplification products in morphant embryos; variant 1, 668bp, results in a premature termination codon (PTC); variant 2, 563bp, represents the correctly spliced transcript; variant 3, 520bp, results in a PTC. (**d**) Schematic of the amplification products produced by the *ttc21b* sb-MO. SB, splice-blocking MO targeting the splice acceptor of exon 4; Ex, exon; In, intron; blue asterisks indicate PTCs.



Supplementary Figure 2. Localization of endogenous and V5-tagged TTC21B in mIMCD3 cells and retina. (**a-b**) Transiently transfected V5-tagged TTC21B (red, arrow) is located at the base of cilia in transfected mIMCD3 cells that stably express the ciliary marker Sstr3-EGFP (green) (a, merged image; b, V5 only). A faint TTC21B signal is also detected along the axoneme of the cilium. (**c**) Antibodies to Ttc21b (red) show that the protein is localized to the transition zone of rodent photoreceptor sensory cilia (PSC; arrow). The axonemes of PSCs are shown by staining with antibodies to Rp1 (green). (**d**) Pre-adsorption of the anti-Ttc21b antibodies with peptide demonstrates the specificity of Ttc21b signal. Blue, nuclei; Ax, axoneme; IS, inner segment; ONL, outer nuclear layer; OS outer segment.



Supplementary Figure 3. *In vitro* analysis of V5-tagged TTC21B protein in ciliated mIMCD3 cells. Immunofluorescent staining of mIMCD3-Sstr3-EGFP cells transfected transiently with plasmids encoding wild-type (WT) or mutant versions of TTC21B cDNA demonstrate mislocalization of mutant proteins. (**a**) Tagged proteins were detected using anti-V5 antibody (red), Sstr3-EGFP signal demarcates primary cilia (green), and nuclei are stained with Hoechst dye (blue). WT TTC21B -V5 localizes primarily to the basal body and also to the cilium, however some mutants show mislocalization in >70% of cells scored (P209L, R411G, T1103R), or are undetected in this assay (I1208S; 50-70 transfected cells scored per construct). White arrows indicate localization of tagged protein to the base of the cilium; *, transfected cells; scale bar 10μm. (**b**) Magnified images of single cells in panel (a) are shown to demonstrate mislocalization of mutant proteins in comparison to the basal body localization of WT TTC21B -V5. Panel origin is boxed with dashed lines in Merged views of panel (a).



Supplementary Figure 4. Ttc21b is required for normal photoreceptor sensory cilia (PSC) formation (**a**) Photoreceptor cells in neonatal rat retinas were transfected via *in vivo* electroporation with shRNA-EV (empty vector; left panel), shRNA-*Ttc21b* (2923) (center panel), or shRNA-*Ttc21b* plus shRNA-resistant TTC21B cDNA (right panel). A plasmid encoding arrestin-EGFP was included in all transfections to demonstrate PSC structure. Suppression of *Ttc21b* expression results in shortened, abnormally shaped PSCs (small arrows indicate examples). shRNA-resistant TTC21B partially rescues PSC length and structure. Images are 3D reconstructions of confocal image stacks; the grids are included to show perspective; in each image, grid size is 12.8 µm. (**b**) PSC length was measured using Volocity Quantitation software; mean ± SD are shown. Long arrows in a. show example PSCs measured (white brackets). ONL, outer nuclear layer; OS, outer segment.