DCTN1 mutations in Perry syndrome

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Supplementary figure 1. The N-terminal CAP-Gly domain of *DCTN1*.

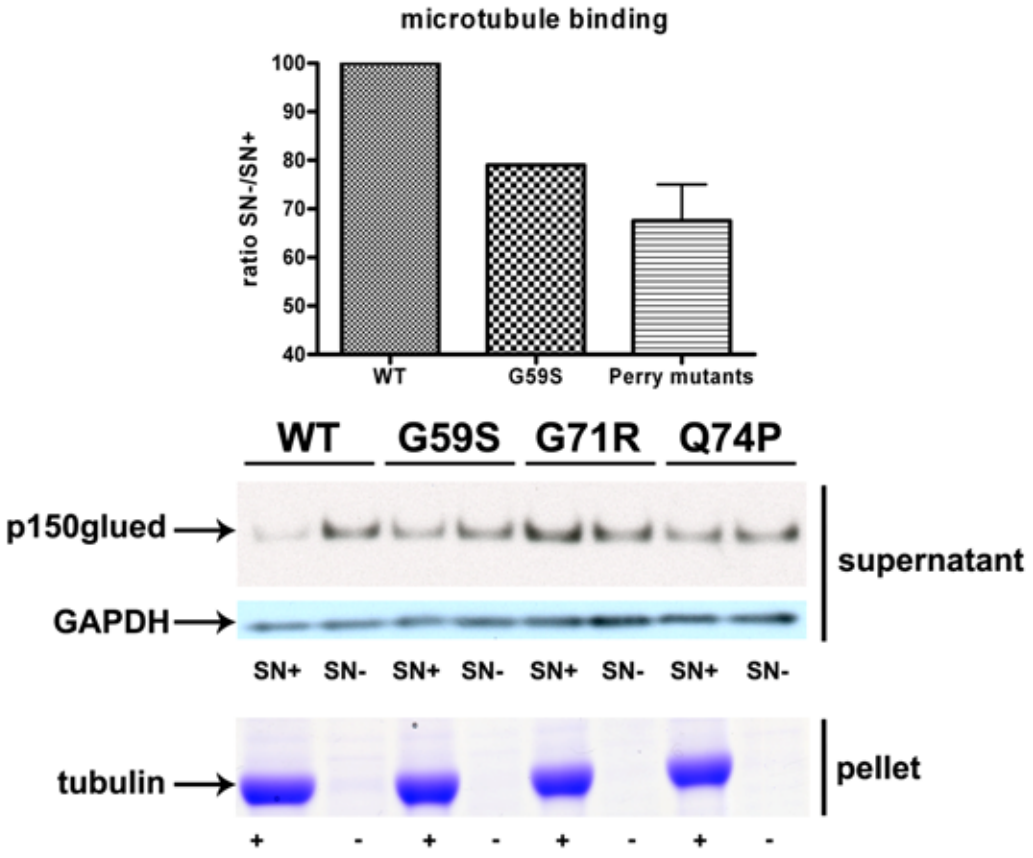
Homo sapiens	VILDEAK<u>GKNDG</u>TVQGRKYFTCDEGHG
Gly71Arg	VILDEAK<u>GKND**R**</u>TVQGRKYFTCDEGHG
Gly71Ala	VILDEAK<u>GKND**A**</u>TVQGRKYFTCDEGHG
Gly71Glu	VILDEAK<u>GKND**E**</u>TVQGRKYFTCDEGHG
Thr72Pro	VILDEAK<u>GKNDG**P**</u>VQGRKYFTCDEGHG
Gln74Pro	VILDEAK<u>GKNDGTV**P**</u>GRKYFTCDEGHG
Mus musculus	VILDEAK<u>GKNDG</u>TVQGRKYFTCDEGHG
Danio rerio	VILDE+K<u>GKNDG</u>TVQG++YF+C+E+HG
Xenopus laevis	VILD++K<u>GKNDG</u>TVQGR+YFTC+E+HG
Drosophila pseudoobscura	VILDE++<u>GKN+G</u>T+QG++YF+C++++G
Caenorhabditis elegans	VILD+A+<u>GKN+G</u>TVQ+++YF+C++++G

Amino acid sequence conservation across species. The CAP-Gly 'GKNDG' binding motif is underlined with the position of mutations in Perry syndrome highlighted in bold.

Supplementary figure 2. Microtubule binding properties of wild type and mutant p150^{glued}

Equal amounts of lysates from HEK293T cells transfected with cDNA encoding either wild type or mutant (G59S, G71R or Q74P) p150^{glued} were incubated in the presence or absence of previously assembled microtubule at room temperature. To separate microtubule bound and unbound p150^{glued} the suspension was placed on a glycerol cushion and centrifuged at 100,000g at room temperature for 40min. The resulting supernatants and pellets were subjected to SDS-PAGE and subsequently analyzed using Coomassie staining (pellets +/- microtubule) and Western blot (supernatants) probed with p150^{glued} or GAPDH antibodies (goat anti p150^{glued} 1:1000, Abcam; mouse anti GAPDH 1:50 000, Biodesign). While the supernatant incubated with microtubule

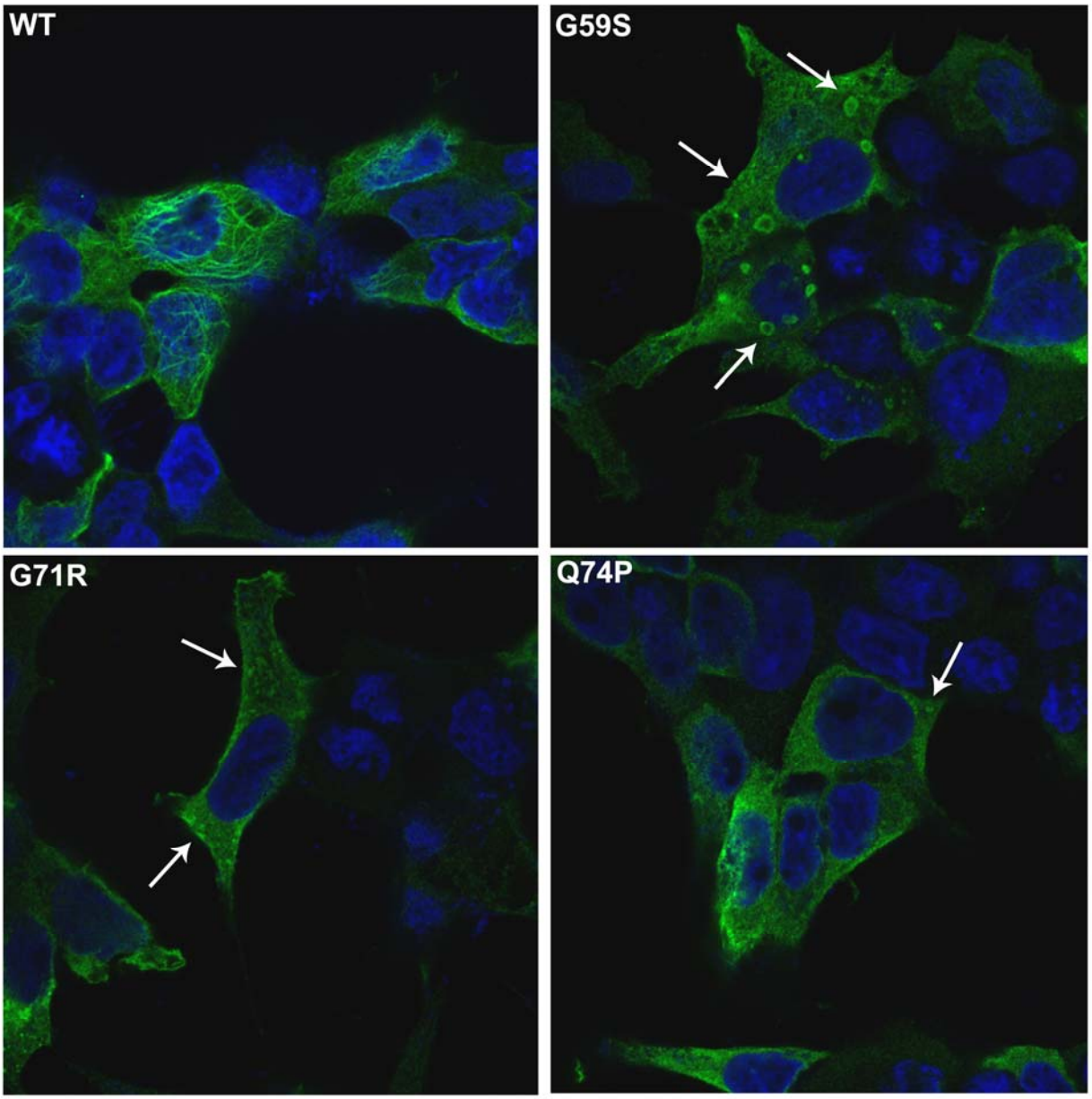
(SN+) is almost depleted for p150^{glued} wild type protein, considerable amounts are found for the G59S and Perry mutations (G71R and Q74P), and indicative of reduced microtubule binding. The ratio of p150^{glued} in the supernatant of the sample incubated with microtubule (SN+) compared to the supernatant incubated without microtubule (SN-) was arbitrarily set as 100 for wild type p150^{glued} protein. An average and SEM is given for the two Perry syndrome mutations, G71R and Q74P. The blots shown from one experiment are representative of three replicates.



Supplementary figure 3. Mutations in p150^{glued} protein change its cellular distribution and result in inclusions.

HEK293T cells were transiently transfected with plasmids encoding wild-type or mutant (G59S, G71R or Q74P) p150^{glued} protein. One day after transfection cells were fixed in 4% formaldehyde, blocked in 3% BSA and subsequently stained for p150^{glued} (green) and DAPI (blue). A polyclonal goat antibody directed against C' terminal p150^{glued} was

used (1266-1278 aa, Abcam, 1:200 in 1%BSA in PBS) as *DCTN1* mutations may affect N' terminal epitopes and antibody affinity.Confocal pictures were taken using the 100x oil immersion objective of a Zeiss Axivert 200M microscope equipped with LSM510META technology. White arrows indicate representative examples of inclusion bodies. In *DCTN1* G59S transfected cells the intra-cytoplasmic inclusions appear larger and more numerous than with Perry mutations.



Supplementary table 1. Two-point LOD scores (θ=0) for families with Perry syndrome.

	France 395	Turkey 513	Japan 537	Canada 605	Japan 711	US 730	Sum
D2S3061	-2.94	1.81	0.21	4.07	0.49	0.98	
D2S3035	-0.56	1.81	-0.1	0.6	1.44	1.49	
D2S3062	-0.47	1.81	0.27	0.9	1.42	1.34	
D2S3063	-0.28	0.22	0.24	-0.01	1.46	-0.16	
D2S3064	0.44	1.71	0.14	3.17	0.51	1.04	
D2S1389	-1.42	0.48	0.15	1.19	0.81	1.04	
D2S3065	2.95	0.57	0.12	2.34	-0.14	0.41	6.25
D2S291	3.5	1.81	0	4.2	0.5	0.35	10.36
D2S3039	3.52	-0.01	0.04	0.52	0.45	0.39	4.91
D2S3066	2.36	0.9	0.29	-0.06	0.3	1.19	4.98
D2S3040	0.21	-0.21	0.25	3.16	1.38	2.39	7.18
D2S3041	1.96	0.65	0.21	4.26	0.9	2.31	10.29
D2S3067	3.51	1.71	0.21	3.7	0.51	0.07	9.71
D2S3068	3.41	0.71	-0.09	3.93	0.54	0.86	9.36
D2S2110	3.74	1.81	0.07	3.37	0.84	1.06	10.89
D2S1394	1.23	0.53	0.14	4.17	1.35	1.3	8.72
D2S2111	3.33	1.81	0.13	3.25	1.15	0.59	10.26
D2S145	0.55	1.81	0.22	3.54	-0.05	0.17	6.24
D2S3069	0.55	1.81	0.28	4.21	1.37	1.32	9.54
D2S2109	0.43	0.29	0.21	2.85	1.33	1.53	6.64
D2S3070	3.23	1.49	0.12	3.14	1.35	-0.15	9.18
D2S3042	0.33	-0.21	0.07	2.52	0.02	0.02	2.75
D2S3071	0.15	0.92	0.25	1.8	0.74	0.6	4.46
D2S3043	0.22	1.81	0.02	2.92	1.46	-0.05	6.38
D2S3044	-0.23	1.81	-0.01	3.61	0.25	2.1	7.53
D2S3072	3.6	0.9	0.17	4.22	1.45	1.18	**11.52**
D2S3045	2.52	1.81	0.28	1.64	1.19	1.47	8.91
D2S3046	3.45	0.9	0.08	4.14	1.24	0.96	10.77
D2S3047	3.56	1.81	0.19	2.67	0.55	0.81	9.59
D2S3048	0.28	0.92	0.21	-0.13	1.31	1.31	3.9
D2S3049	3.28	1.81	0.2	2.34	0.54	- infinity	

Only six of eight families have two or more affected subjects and were informative for linkage. LOD scores were generated using founder allele frequencies, due to the diverse ethnic origin of families, however similar scores can be generated using CEPH marker allele frequencies. The disease allele frequency was set at 0.001, and with complete penetrance for males and females as all asymptomatic mutation carriers were younger than the average age of onset. Inter-marker recombination fractions were obtained from the Marshfield genetic map (http://research.marshfieldclinic.org/genetics). D2S3072 generated the highest two-point LOD score (θ=0) summed across families

(bold). The most parsimonious haplotypes within each pedigree were estimated using SIMWALK2 [4]. Of note, obligate recombinants were observed centromeric of D2S1389 in family 395, and telomeric of D2S3049 in family 730 (indicated in gray).

Supplementary table 2. *DCTN1* haplotypes of families with Perry syndrome.

			G71R		**G71E**	**G71A**			**T72P**	**Q74P**
			c.211G>A		**c.212G>A**	**c.212G>C**			**c.214A>C**	**c.221A>C**
			Turkey	**Canada**	**France**	**England**	**Hawaii**	**Japan**	**US**	**Japan**
	Marker	**Position**	**513**	**605**	**395**	**396**	**5**	**711**	**730**	**537**
	D2S1389	71,613,395	341	341	X	341	345	345	341	337/341
	D2S291	71,788,451	182	204	186	184	184	184/192	184	184
	D2S3039	71,791,851	159	159	147	159	159	159	159	159
	D2S3072	74,112,948	330	386	386	390	336	336	324	328
	D2S3045	74,299,783	204	208	202	202	204	204	200	212
Int 2	rs3815241	74,458,403	A	A	A	A	A	A	G	G
Ex 2	**Q74P**	74,458,693	A	A	A	A	A	A	A	**C**
Ex 2	**T72P**	74,458,700	A	A	A	A	A	A	**C**	A
Ex 2	**G71A**	74,458,702	G	G	G	**C**	**C**	**C**	G	G
Ex 2	**G71E**	74,458,702	G	G	**A**	G	G	G	G	G
Ex 2	**G71R**	74,458,703	**A**	**A**	G	G	G	G	G	G
	rs6713611	74,465,477	C	C	C	C	C	C	G	G
	D2S3047	74,556,561	300	300	302	296	296	296	294	298
	D2S3049	74,891,515	121	125	117	117	117	117	X	121

Pedigrees are grouped by nucleotide mutation and the consequent amino acid substitution. Mutant haplotypes are shown, if phase is unknown both alleles are indicated; allele sizes are consistent with CEPH standards. STR and SNP markers are shown with their physical locations (NCBI Build 36.1), the *DCTN1* gene (RefSeq identifier NM_004082; Genbank accession BC071583) spans 74,441,790-74,461,472 bp of chromosome 2p12-14 and relevant introns/exons are shown adjacent. Specific mutations are highlighted in bold text and obligate recombinants are indicated in gray with an X.

Supplementary table 3. *DCTN1* exon 2 (p150^{Glued} CAP-Gly domain) sequence variants

NM_004082	Nucleotide	Amino acid	Accession no.	Positive	Origin/Ethnicity
exon 2	c.43G>A*	G15S	ss102711322	1 case	Germany
exon 2	c.45C>T*	G15G	ss102711325	1 control	UK
exon 2	c.177C>T	G59G	ss102711328	1 control	Ireland
exon 2	c.211G>A*	**G71R**	ss102711331	2 cases	Turkey, Canada
exon 2	c.212G>A*	**G71E**	ss102711334	1 case	France
exon 2	c.212G>C*	**G71A**	ss102711337	3 cases	Hawaii, Japan, UK
exon 2	c.214A>C*	**T72P**	ss102711340	1 case	US (Ireland)
exon 2	c.221A>C*	**Q74P**	ss102711344	1 case	Japan
exon 2	c.260T>C*	I87T	ss102711348	1 control	US

*DCTN1 e*xon 2 encoding the p150^{Glued} CAP-Gly domain was sequenced in 949 control subjects free of neurological disease, all US Caucasians of European ancestry, and 475 probands with familial parkinsonism including patients from continental America, Europe, Asia and N.Africa. The table lists genetic variants identified, the number of samples that screened positive and the origin/ethnicity of these subjects. Nucleotides evolutionarily conserved within *Eutherian* mammals are asterisked; consequent amino acid substitutions are noted with reference to Genbank accession BC071583. In addition to five mutations identified in 8 probands with Perry syndrome (highlighted in gray), four novel variants were discovered: c.43G>A (G15S) was found in a proband with familial parkinsonism 49 years at symptom onset, for which DNA samples from other affected family members were not available. c.260C>T (I87T) was identified in a control subject of 83 years. Two synonymous variants, c.45C>T (G15G) and c.177C>T (G59G) were found in two other control subjects.