Supplementary Figure 1. Segregation of c.178Cins in two *SPG12* families. A) Family ROM-05 shows co-segregation of the sequence change with the disease. Pedigree adapted from Orlacchio et al. (16). B) Family 28 also shows co-segregation of the c178Cins variant with the disease. Pedigree adapted from Reid et al. (13).

Supplementary Figure 2. Conservation of S367 in *RTN2* across a wide range of species. Alignment is shown for the RTN2B isoform, in which the residue corresponding to S367 is S294. This residue is conserved within all mammals and available marsupial genomes. It appears not to be conserved in fish species.

Supplementary Figure 3. RNA studies of patient samples. (A) cDNA was produced from random primed RNA from a patient and a control of family 28 (c.178insC). Primer locations spanning an intron are indicated. (B) An agarose gel shows PCR products of expected size and indicates expression of RTN2 in control brain, and peripheral blood of an affected and unaffected individual. (C) Sanger sequencing shows the presence of the mutant *RTN2* allele in the affected patient at appreciable levels. Non-sense mediated decay does not appear to remove the mutated RNA species.

Supplementary Figure 4. Image analysis of ER morphology in HEK293 cells co-expressing the ER marker ER-mKate2 and either WT-, S294-, or R60fs- RTN2B. Live cell imaging was performed with a Zeiss LSM710 confocal microscope and the ImageJ software package (see Methods). Thirty doubly transfected cells (10 for each RTN2B construct) were chosen at random and imaged in 3D according to Nyquist criterion. The images were processed and reduced to a binary skeleton to represent the overall structure of the network. The connectivity of the skeleton network was analyzed by reassigning each pixel value of the skeleton a gray level that described the number of surrounding neighbors plus one. Histograms of the processed images were recorded. The scores for this analysis range from 1-9 where a score of 1 represents a single pixel with no neighbors and a score of 9 represents a pixel completely surrounded by neighbors. In this scheme the given scores would be associated with the following morphological features: 1 = fractioned/non-connected to network; 2 = end of tube; 3 = tube/line; 4 = 3-

way junction; 5 = 4-way junction and so on. To compare ER network between cells, each image was normalized by dividing the raw value of each morphological score by the total number of measured pixels. The resulting ratios were averaged and their means and standard deviations were plotted. No difference in ER morphology was identified in cells expressing the different RTN2 constructs.

Supplementary Figure 5. Schematic diagram showing known interactions between mammalian RTNs, spastin, atlastin-1 and REEPs1 and 5, based on references (2, 5, 8-10, 20). Other REEPs and atlastins are not shown for clarity. Bold lines indicate an interaction confirmed by co-immunoprecipitation, dashed line is for an interaction demonstrated only by yeast two-hybrid. The red line indicates the interaction verified in the present study.

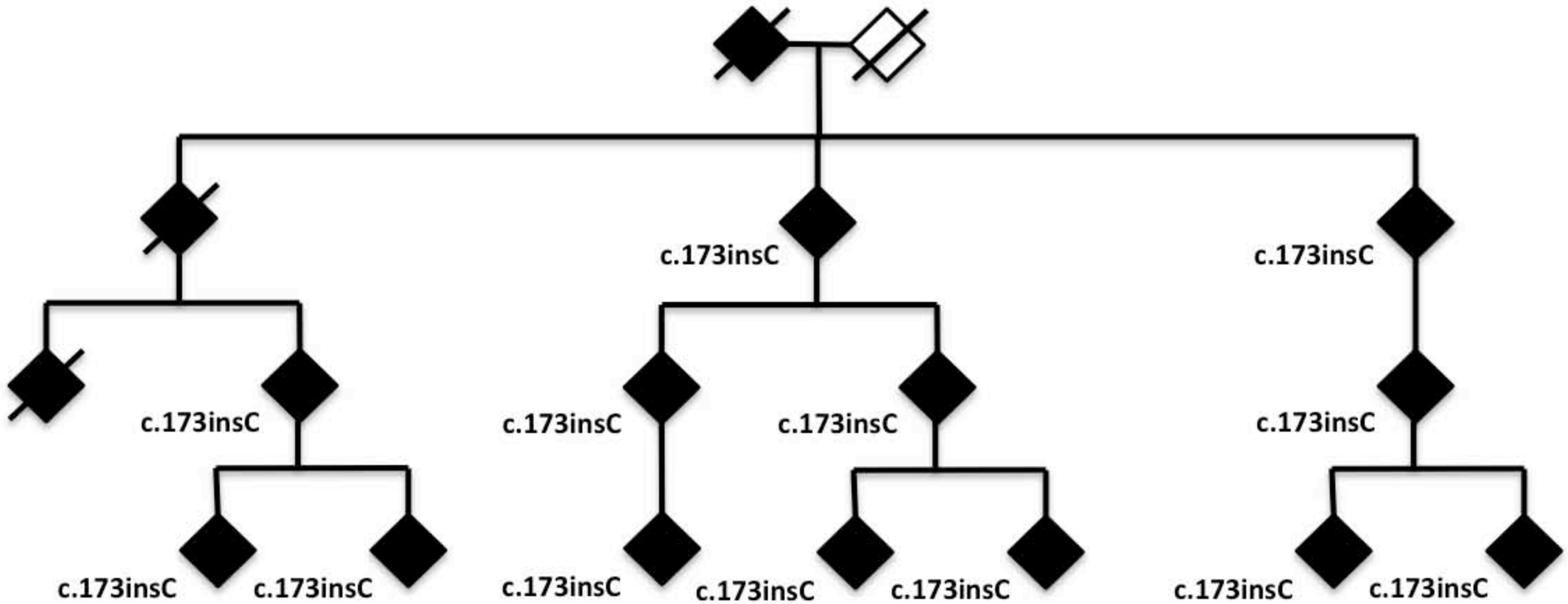
Supplementary Figure 6. Control RTN2B-spastin IP experiments. HeLa cells were transfected with the constructs indicated, or were untransfected, then immunoprecipitated (IP) with spastin86-340 antibody, or pre-immune serum (PI). The immunoprecipitates were then immunoblotted with the antibodies indicated.

Supplementary Figure 7. M87 spastin does not co-localize with RTN2B. Confocal micrographs of representative HeLa cell co-transfected with RTN2B-V5 and Myc-M87 spastin. Scale bar=10μm.

Supplementary Figure 8. RTN2B-S294F co-localizes with wild-type and ATPase-defective M1 spastin. A and B) Confocal micrographs of representative HeLa cells co-transfected with RTN2B-S294F-V5 and either Myc-M1 spastin (A) or Myc-M1 spastin K388R (B). Some of the smaller co-localized structures are indicated with arrowheads in the zoomed boxes shown below the main panels. Scale bars=10μm.

Supplementary Figure 1

A



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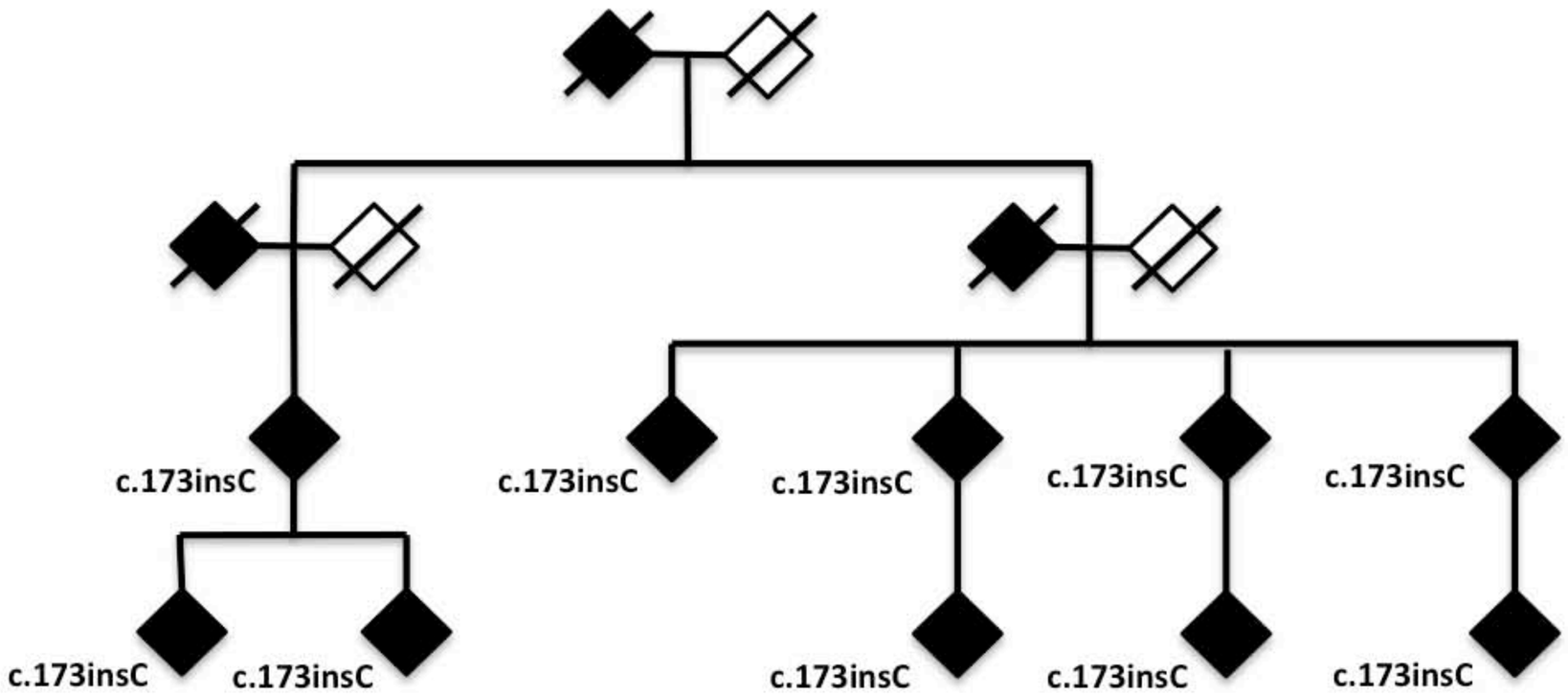
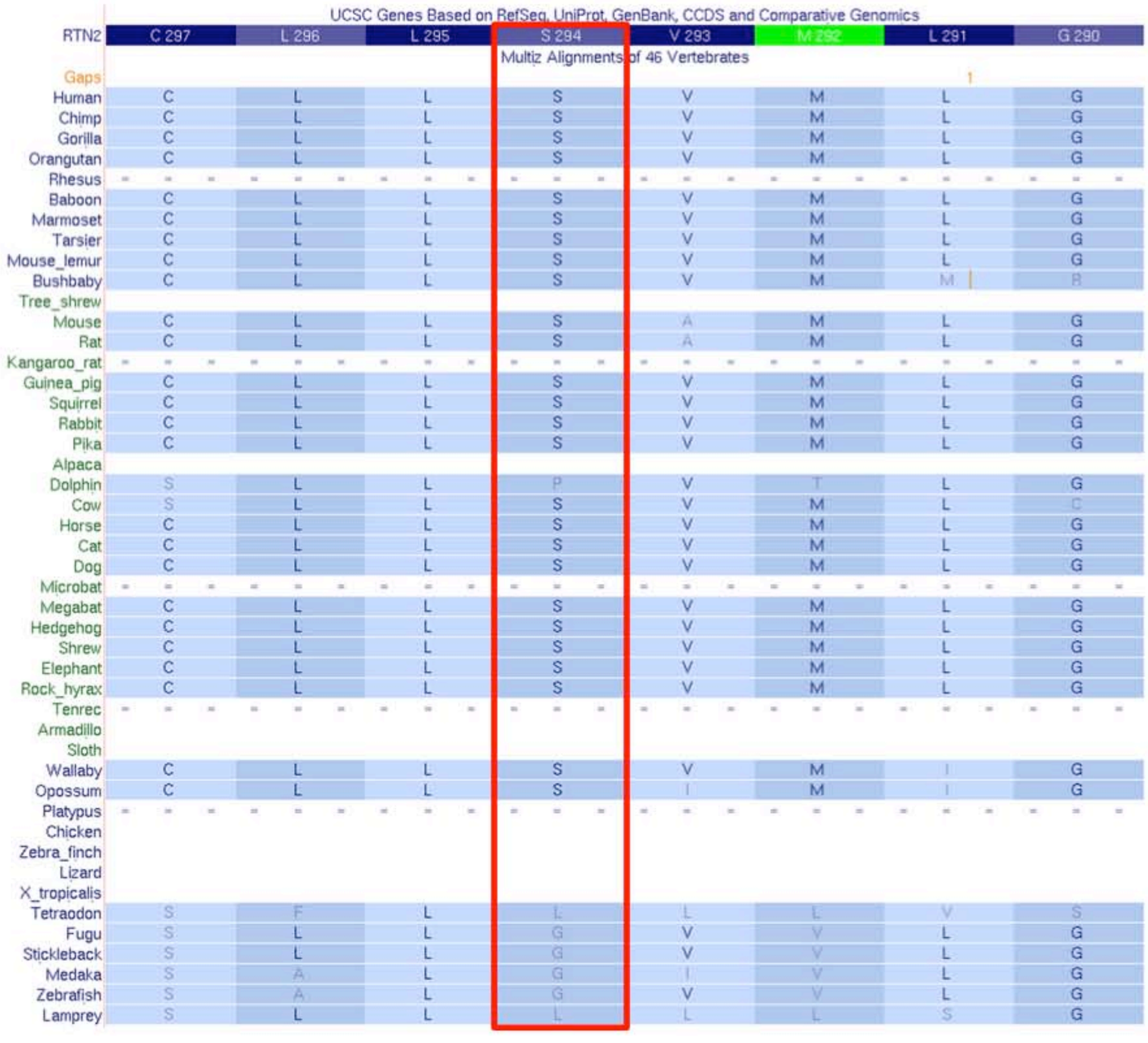
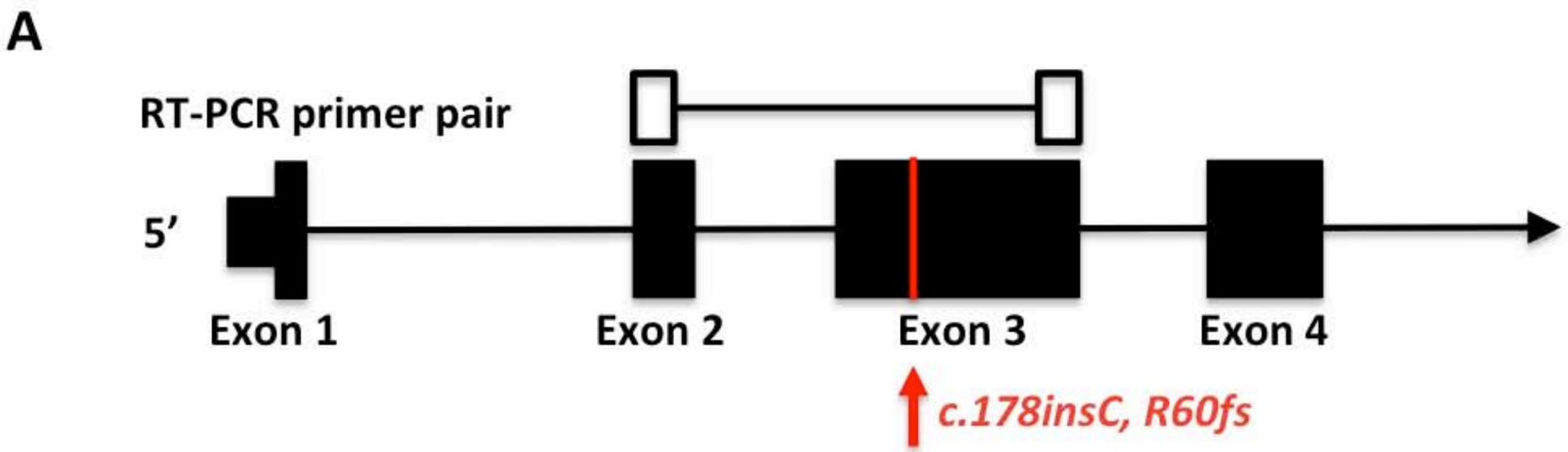


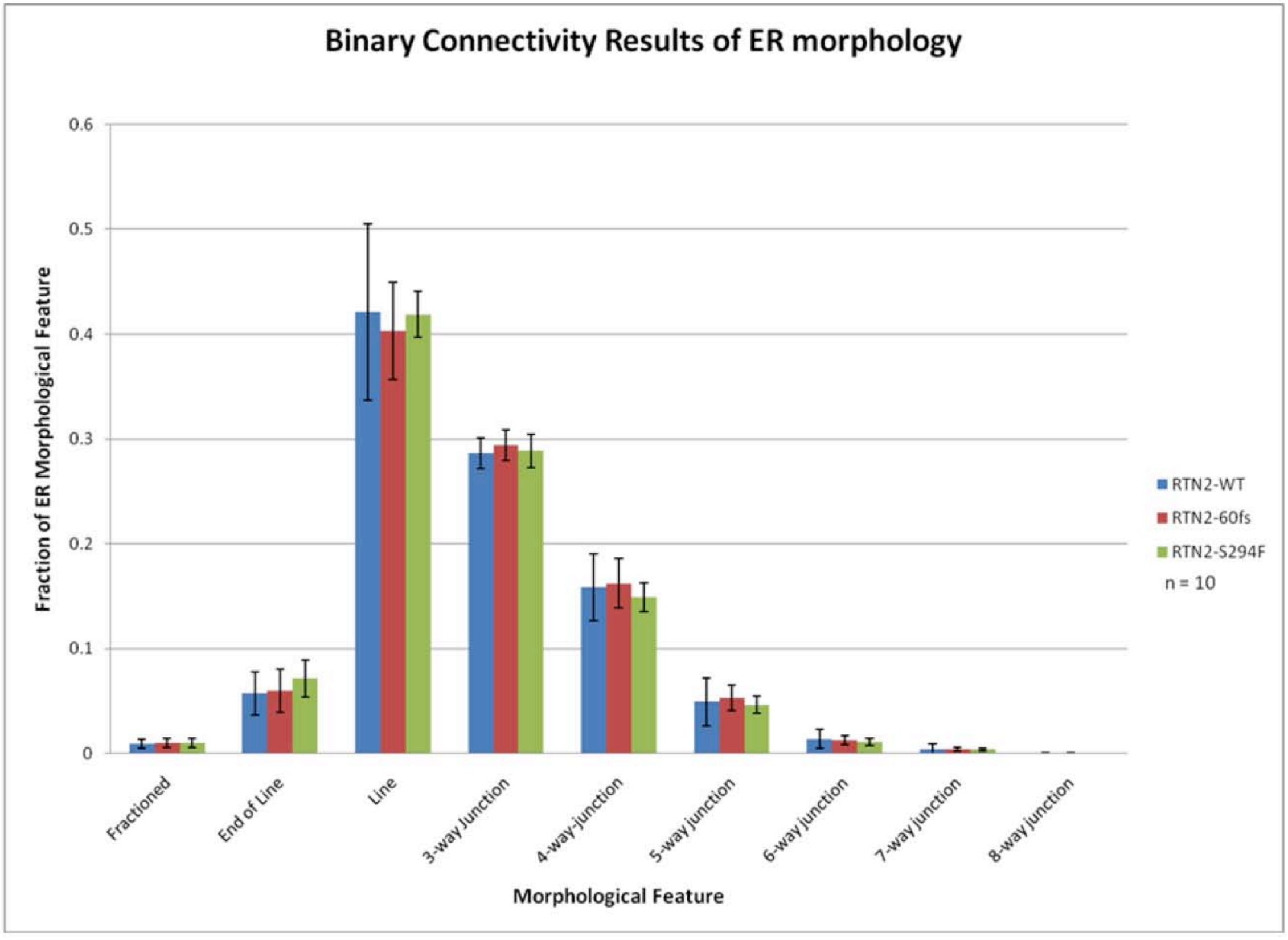
Supplementary Figure 2



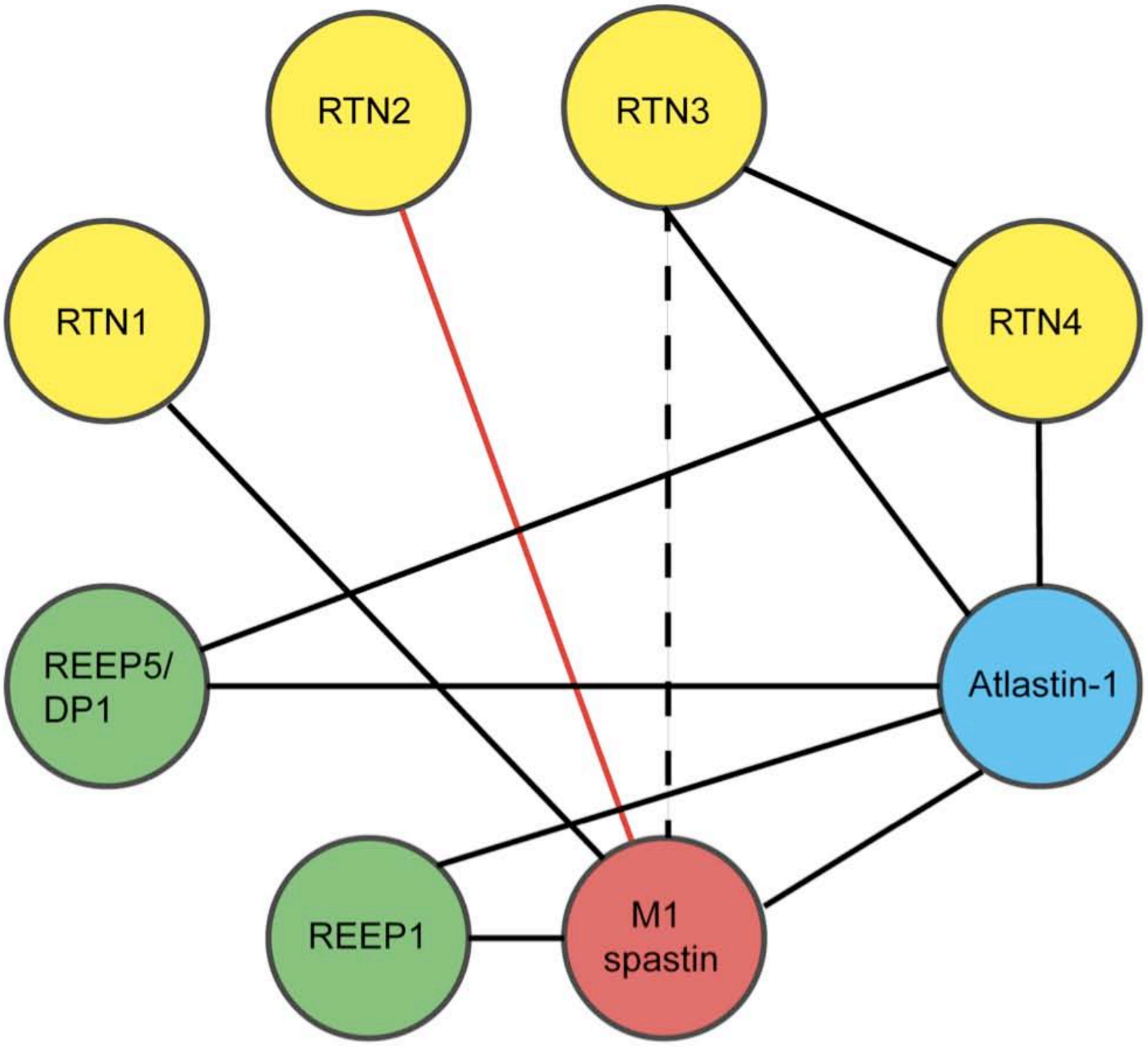
Supplementary Figure 3



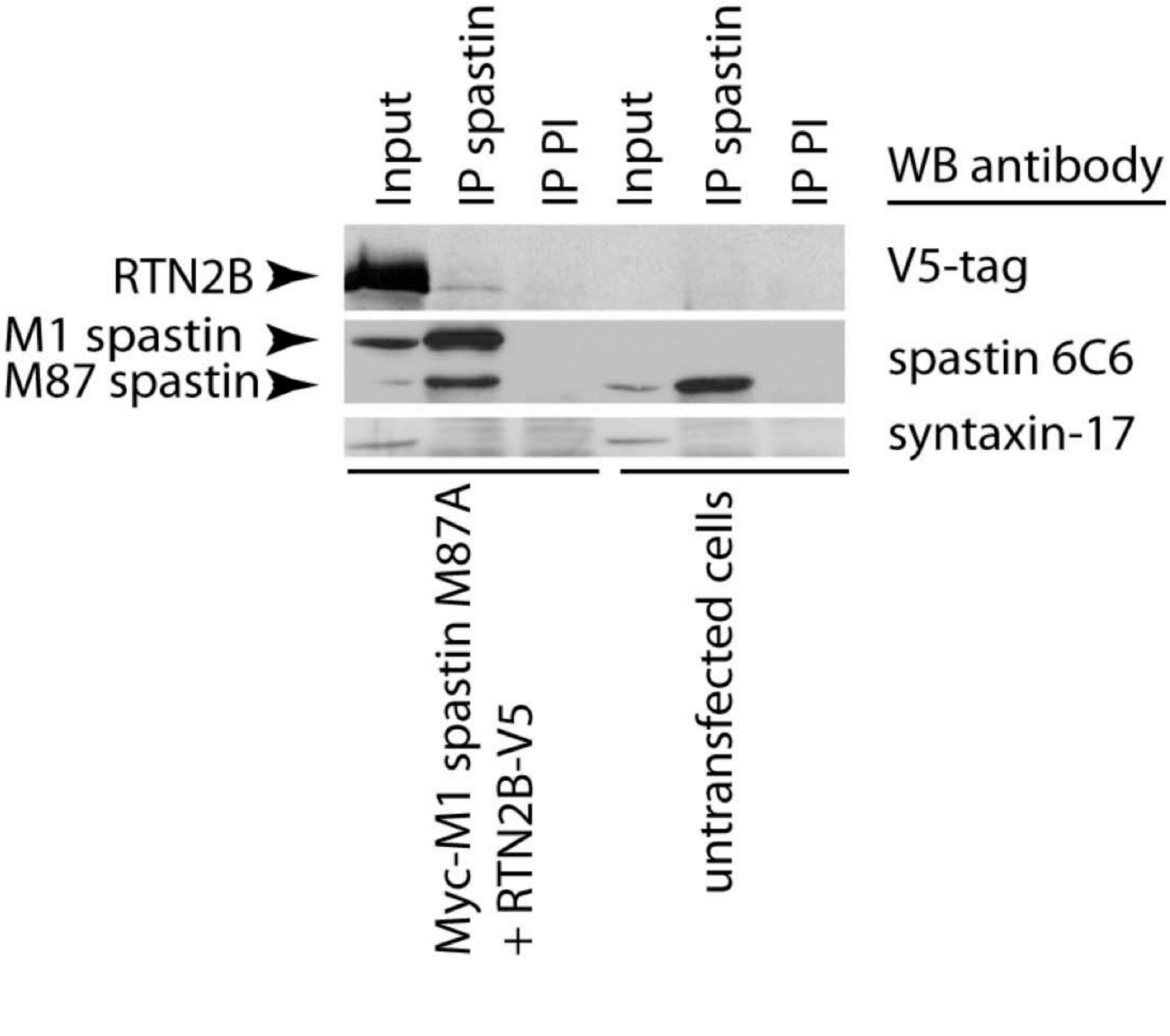
Supplementary Figure 4



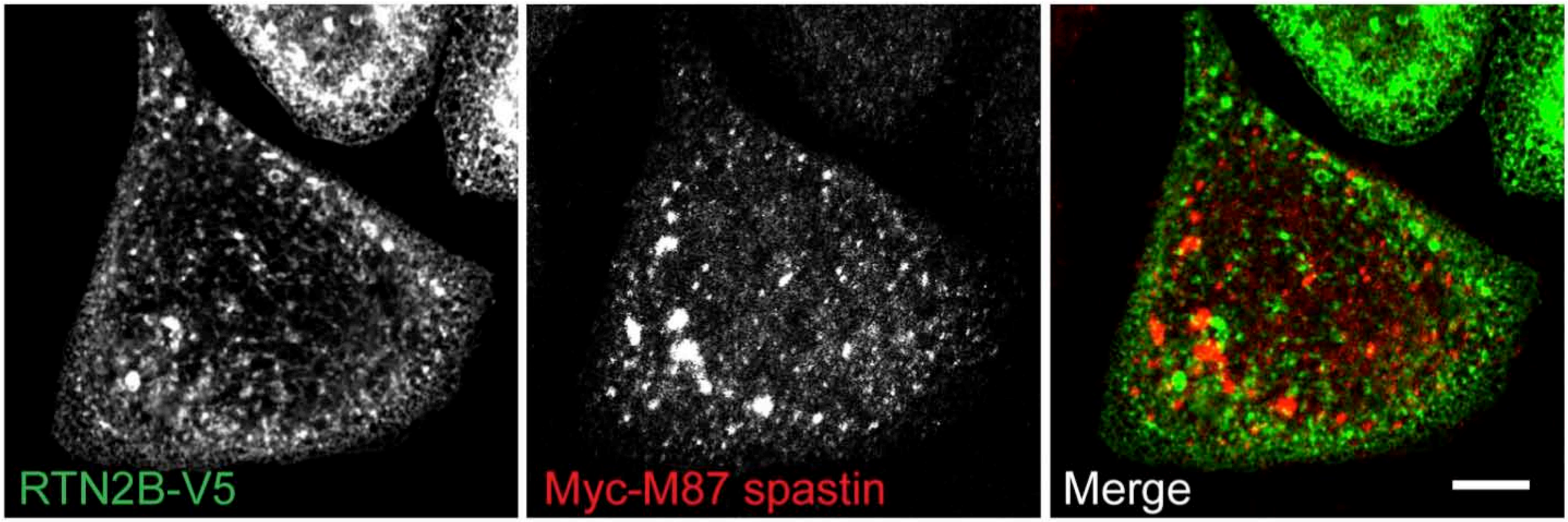
Supplementary Figure 5



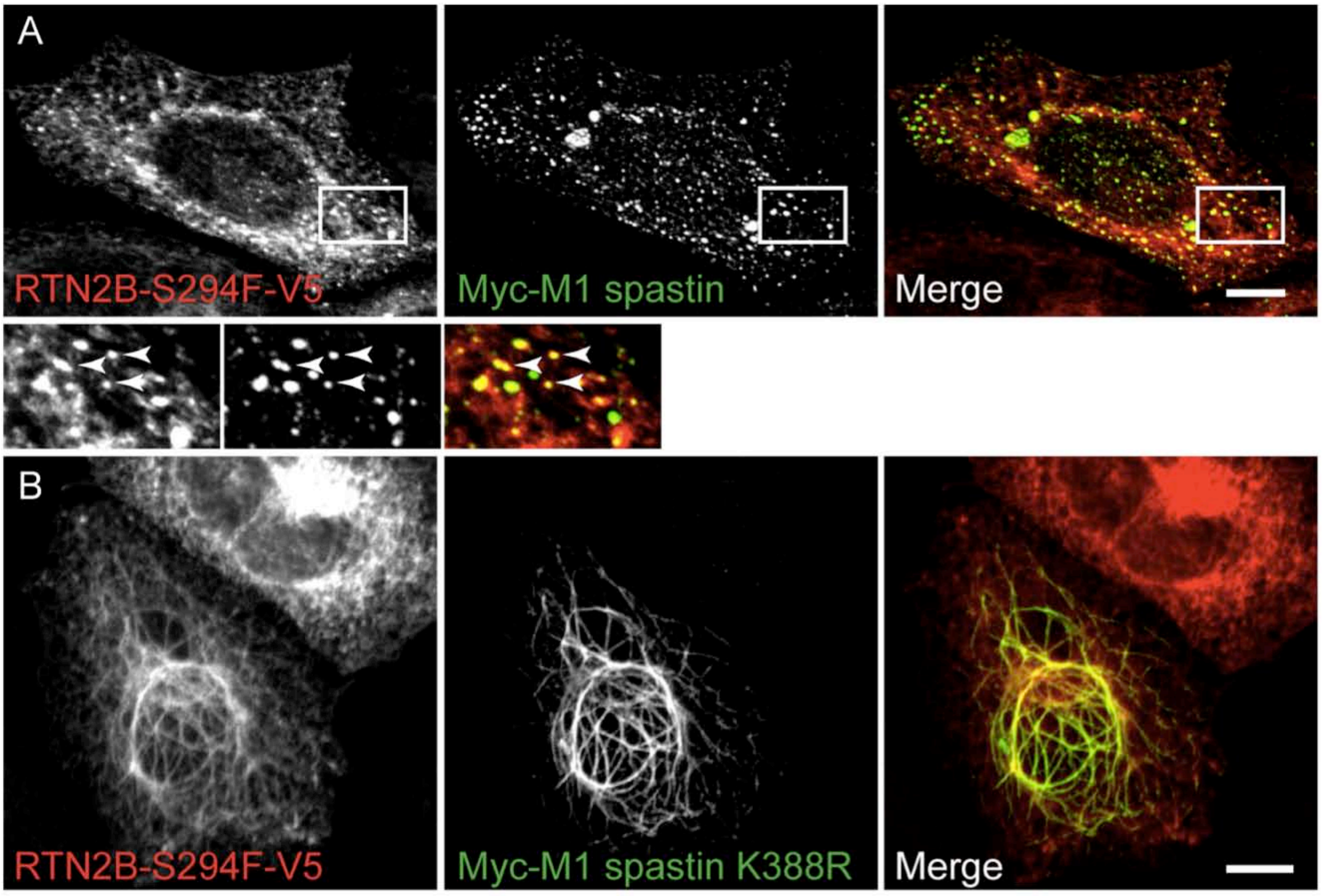
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Table 1. Genotyping supports a shared haplotype between the Italian and British Family suggesting a founder effect for the identified mutation. Red indicates affected individuals and shared haplotype. Green indicates unaffected individual. III:5 and III:4 are parents of IV:5.

position	variant ID	Family 28; IV:5		Family 28; III:5		Family 28; III:4		ROM05; index	
chr19:41313202	rs3736329	C	A	C	C	C	A	C	C
chr19:43979595	rs1055099	C	A	C	C	C	A	C	C
chr19:45814947	rs17875617	A	G	A	G	G	G	A	G
chr19:45815033	rs17875616	A	C	A	C	C	C	A	C
chr19:45815248	rs1042747	G	A	G	G	A	A	G	G
chr19:45836204	rs12973246	A	G	A	A	G	G	A	A
chr19:45854919	rs13181	G	T	G	G	T	T	G	G
chr19:45983183	rs34197957	ref	indel	ref	ref	ref	indel	ref	ref
chr19:45998163	RTN2, c.178insC	ins	ref	ins	ref	ref	ref	ins	ref
chr19:46289503	rs8110017	G	A	G	G	A	A	G	G
chr19:46338492	rs7256192	C	T	C	C	T	T	C	C