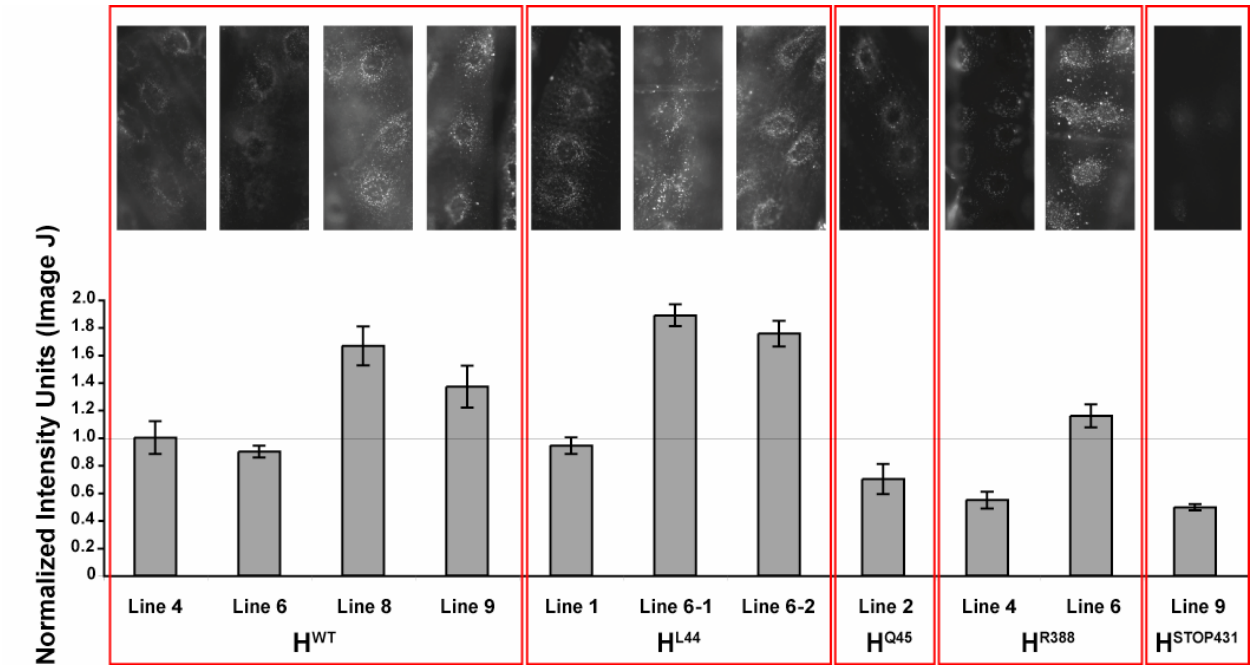

Figure S1. Three-fold variation in expression levels is observed between independent *spastin* transgene insertion lines, but does not correlate with the effects of the alleles. *Spastin* transgenes were overexpressed using the muscle driver twist, 24B-GAL4. Larval fillets were dissected and immunostained under identical conditions, and the muscles imaged using a Zeiss AxioImager with 20X objective under identical exposure settings. Fluorescence was quantified in ImageJ by measuring the average intensity along a line of uniform length drawn across the same muscle fiber in each image. Average intensity and standard error are shown for each insertion line, with representative images of the muscle staining depicted above. H^{L44}(6-1) and H^{L44}(6-2) are larvae from the same insertion line (6) but fixed and stained on different days, demonstrating consistency of signal intensity even across experiments. Within an allele or mutational class (i.e., wild type, amino terminus, or catalytic domain), however, expression levels could be high or low (e.g., lines 8 versus 6 of H^{WT}, or lines 6 versus 8 of H^{L44}, and line H^{Q45}(2)), and did not correlate with the phenotypic changes observed.

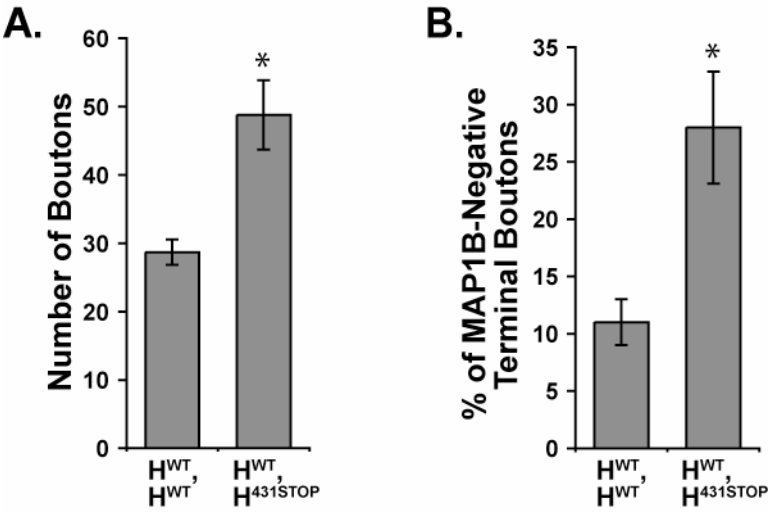

Figure S2. Expression of the catalytic domain mutant R431STOP phenocopies K388R. Transgenic flies were generated to enable expression of an YFP-tagged spastin nonsense allele, R431STOP. Although identified in a human AD-HSP pedigree, we do not consider this a pathogenic mutation representative of the disease in the context of our *Drosophila* model. This is because the protein is likely never produced in humans due to nonsense-mediated decay, but GFP fluorescence confirms expression of the cDNA construct. Predicted to be catalytically inactive, this mutant protein serves as an independent test of the R388 results. **(A, B)** Bouton morphology and microtubule distribution are similarly affected by the $H^{431STOP}$ mutation as compared to H^{R388} spastin. $H^{WT},H^{431STOP}$ heterozygotes exhibit a significant increase in synaptic bouton number ($p < 2x10^{-4}$) and a concomitant decrease in microtubule distribution within terminal boutons compared to H^{WT},H^{WT} controls ($p < 0.02$), and similar to H^{WT},H^{R388} heterozygotes.

Movie S1. Motor behavior in "AD-HSP" flies recapitulates the effects of *spastin* mutations in humans. As shown in the Supplementary Movie in (25), WCS control flies in an enclosed dish crawl very rapidly, move their wings, and are fully stable when standing still or hanging upside-down. *Spastin* null flies, in contrast, move slowly and have weak and unsteady legs whether walking or standing. Here, control flies heterozygous for the *spastin* deletion (*spastin*$^{5.75}$/+) are the first genotype shown, moving in a Petri dish. Like WCS controls, these flies are highly agile, walking rapidly (often upside-down), exhibiting wing movement, and standing or hanging in place stably. Human wild type *spastin* (genotype H^{WT},H^{WT}) rescues the severe behavioral defects of *spastin* null flies such that they are indistinguishable from controls in walking ability. Similarly, L44 heterozygotes (genotype H^{WT},H^{L44}) exhibit normal leg and wing movement, crawling rapidly about the dish. R388 heterozygotes (genotype H^{WT},H^{R388}), however, are slower-moving and seldom move their wings; they are unable to fly and rarely hang or walk upside-down. Further addition of the L44 allele, in compound heterozygous mutants (genotype H^{L44},H^{R388}), yields severe movement defects that are comparable those seen in *spastin* nulls, with extremely weak legs that slip frequently, difficulty walking and standing, and rare wing movement. Shown for this genotype are a male, and then a female, fly.

Table S1. Eclosion rates are equivalent for different insertions of the H^{WT} transgene.

Genotype	% Eclosion	N (# trials)	Total animals scored	p-value
$H^{WT}(4)$	59.0 ± 10.7	4	523	
$H^{WT}(6)$	57.9 ± 3.2	6	3392	$p > 0.1$ for all
$H^{WT}(8)$	49.6 ± 3.7	5	3826	combinations
$H^{WT}(9)$	55.3 ± 6.0	7	2273	

Four to ten independent insertions lines (denoted by "(1)," "(2)," etc.) were recovered for each transgene. *Geneswitch-elav-GAL4*-driven expression of different H^{WT} lines bearing the same transgene showed similar eclosion rates, despite differences in transgene expression levels (see Figure S1). N = the number of experimental trials (parental crosses) performed. Total number of flies scored across experiments is shown.

Table S2. Lines 4 versus 6 of H^{R388} may differentially affect eclosion.

Genotype	% Eclosion	N (# trials)	Total animals scored	p-value
$H^{WT}(4),H^{R388}(4)$	25.7 ± 5.2	3	798	$p > 0.05$
$H^{WT}(4),H^{R388}(6)$	43.0 ± 3.5	3	230	

Comparison of two recombinant heterozygous genotypes expressing either line 4 or line 6 of H^{R388} show different eclosion rates, although statistically, $p > 0.05$. Since the H^{WT} line in both cases is the same insertion (line 4), these data suggest that positional effects of the H^{R388} transgene may influence eclosion although it does not affect bouton number (see Table S3). Specific insertion lines were therefore kept consistent in eclosion analyses of different genotypic combinations.

Table S3. Synaptic bouton numbers are equivalent for different insertion lines of the same transgene.

Genotype	# of Boutons	N (# muscles)	# trials	p-value
$H^{WT}(4),H^{R388}(4)$	38.2 ±3.0	20	2	p > 0.3
$H^{WT}(4),H^{R388}(6)$	41.5 ± 2.0	16	3	
$H^{L44}(6),H^{R388}(1)$	50.7 ± 2.1	30	2	p > 0.1
$H^{L44}(6),H^{R388}(6)$	55.2 ± 2.2	31	4	

Comparison of two recombinant lines each for the single and compound heterozygous mutant genotypes shows that the observed changes in bouton number are specific to the genotype rather than a specific insertion. N = the total number of muscle 4's scored; each trial represents a separate dissection and staining day.