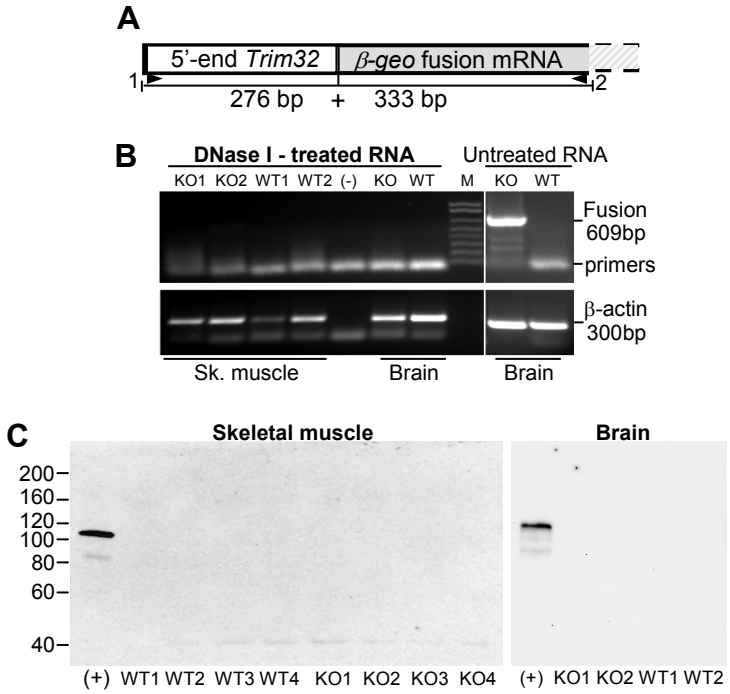

Figure S1. TRIM32-β-geo fusion protein is not produced in T32KO

(**A**) Schematic of hypothetical *Trim32-β-geo* fusion mRNA.

(**B**) *Trim32-β-geo* fusion cDNA was not detected in T32KO muscles or brain by RT-PCR. DNase I untreated RNA from KO tissue was used as a positive control to assure capability of the primers (shown as arrows 1 and 2 in **A**). M – markers (DNA ladder), Sk. muscle – skeletal muscle, (-) – negative control in which no DNA was added into the PCR reaction. β-actin PCR is shown for loading control.

(**C**) β-galactosidase immunoblotting of skeletal muscle (left panel) and brain (right panel) extracts confirmed the absence of TRIM32-β-geo fusion protein in KO tissues. Lane (+) – positive control (bacterial lysate expressing β-galactosidase – 116 kDa).

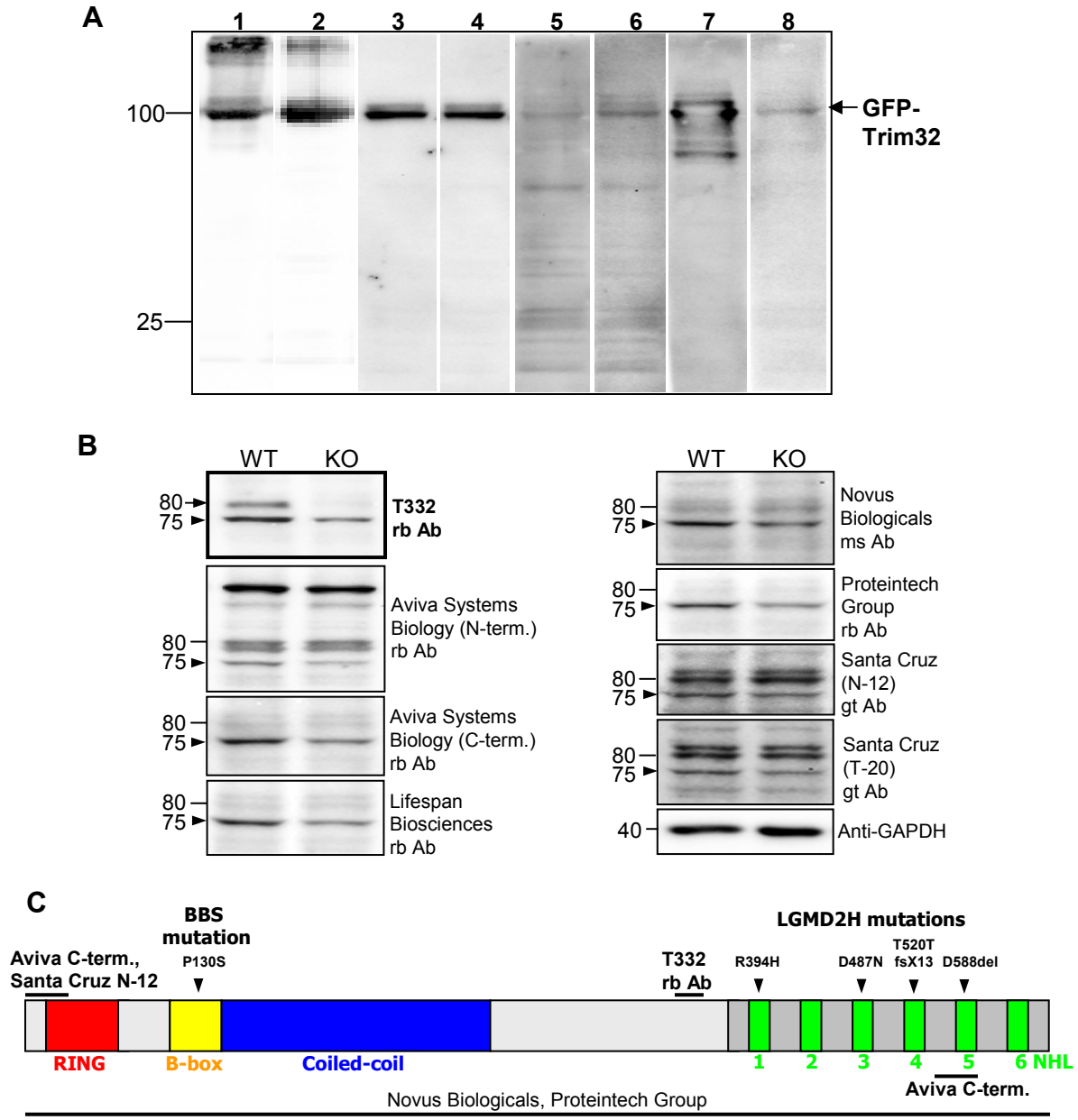

Figure S2. Western blotting analysis using a panel of anti-TRIM32 antibodies

(**A**) Immunoblotting of recombinant TRIM32 fused to GFP using a panel of anti-TRIM32 antibodies: 1 – T332, 2 – Aviva Systems Biology (N-term.), 3 – Aviva Systems Biology (C-term.), 4 – LifeSpan Biosciences, 5 – Novus Biologicals, 6 – Proteintech Group, 7 – Santa Cruz (N-12), 8 – Santa Cruz (T-20).

(**B**) Immunoblotting of brain lysates using TRIM32 antibody from different sources. Note absence of the 80 kDa band in T32KO only with T332 antibody. Anti-GAPDH blot is shown as a loading control.

(**C**) Schematic of TRIM32 domain structure with known pathogenic mutations and position of antibody epitopes (where available).

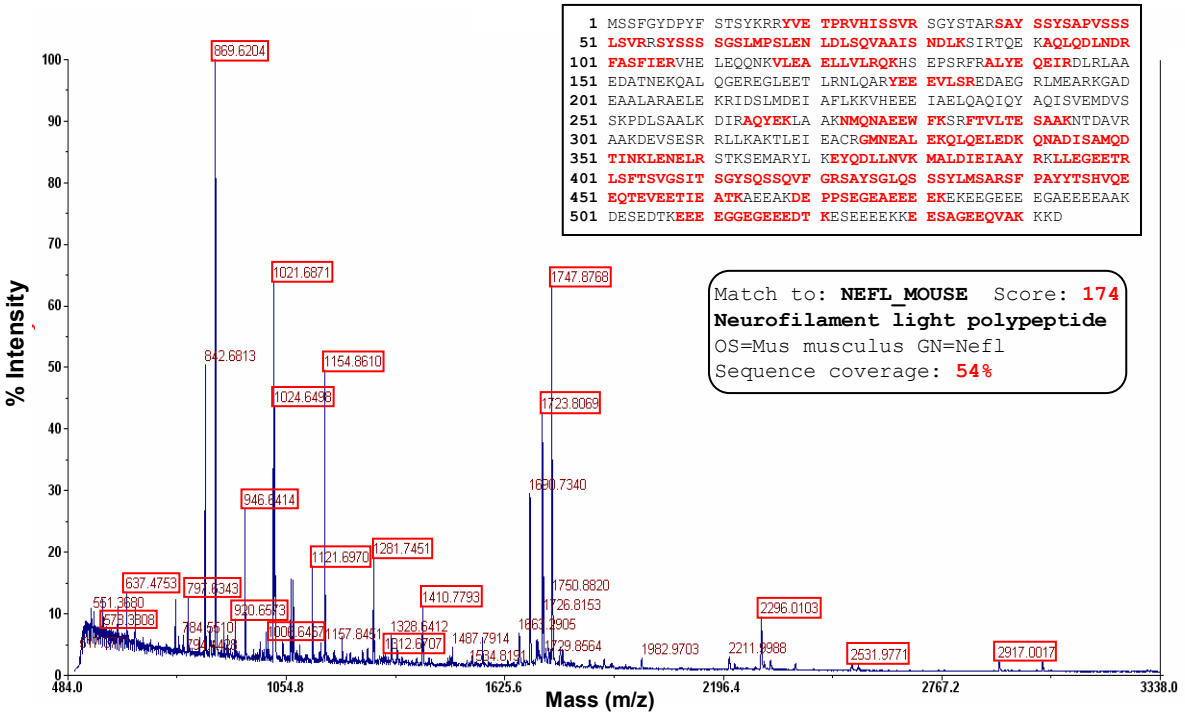

Figure S3. MALDI-TOF MS

Mass spectrum of the in-gel trypsin-digested immunoprecipitated 75 kDa protein detected in brain lysates by anti-TRIM32 antibodies. Mass signals in red boxes correspond to predicted trypsin-digested peptides of NEFL. Sequence coverage is shown in the inset (matched peptides are shown in red).

Table S1. Primers used for PCR and genotyping

Name	Application	Primer Sequence (5'->3')
(Seqtag up) forward (1)	Genotyping (Figure 1A,B)	GGCCATACCATCTGCCGCCAGTGTC
(beta-gal dn) reverse (3)	Genotyping (Figure 1A,B)	GGGGACGACGACAGTATCGGCCTCAGG
(noCC dn) reverse(2)	Genotyping (Figure 1A,B)	CCGCTCCTCAGCTGCCTCCTTGACC
(1) forward	RT-PCR (Figure 1C,D)	ATGGCTGCGGCTGCAGCAGCTTCT
(2) forward	RT-PCR (Figure 1C,D)	CTGTGGCCATACCATCTGCCG
(3) reverse	RT-PCR (Figure 1C,D)	CTGTGTCAATGATCTTCAGCA
(4) reverse	RT-PCR (Figure 1C,D)	CCGCTCCTCAGCTGCCTCCTTGACC
(5) reverse	RT-PCR (Figure 1C,D)	GCTTCTTGAGAAACAGACACTG
(6) forward	RT-PCR (Figure 1C,D)	GGGGCGAAAGGCAGCACTCCCGGC
(7) reverse	RT-PCR (Figure 1C,D)	CCCGCCTTCCACGTTAGTCACCTCAAAC
(8) reverse	RT-PCR (Figure 1C,D)	TTAAGGGGTGGAATATCTTCTCAG
(ASTN2F) forward	Q-PCR (Figure 1F,G)	CGAGGCTTTATACGGTTCTGAGC
(ASTN2R) reverse	Q-PCR (Figure 1F,G)	GGTGTGGGGCTCAGCTCTTTGC
(ASTN2Fex3) forward	RT-PCR (Figure 1E)	CCGATCCTCCAGACTTCAGA
(ASTN2Rex16) reverse	RT-PCR (Figure 1E)	GCCTGCTGAGAGGGTGATAG
(Fusion) forward (1)	RT-PCR (Figure S1A,B)	ATGGCTGCGGCTGCAGCAGCTTCT
(Fusion) reverse (2)	RT-PCR (Figure S1A,B)	GGGGACGACGACAGTATCGGCCTCAGG
beta-actin forward	RT-PCR (Figure 1D,E and S1B)	CGTTGACATCCGTAAAGACCTCTA
beta-actin reverse	RT-PCR (Figure 1D,E and S1B)	TAAAACGCAGCTCAGTAACAGTCCG
Trim32 forward	Q-PCR (Figure 5A,B)	GTGGACTCGCGTCGGAGCTG
Trim32 reverse	Q-PCR (Figure 5A,B)	GGTTCAGGTGAGAAGCTGCTGC
GAPDH forward	Q-PCR (Figure 5A,B)	ACTCCACTCACGGCAAATTC
GAPDH reverse	Q-PCR (Figure 5A,B)	TCTCCATGGTGGTGAAGACA
(NEFL up) forward	Q-PCR (Figure 6C)	AGCATAACCAGCGGCTACTC
(NEFL dn) reverse	Q-PCR (Figure 6C)	TCCTTGGCAGCTTCTTCCTCCTCA
(NEFM up) forward	Q-PCR (Figure 6C)	AGAACATGCACCAGGCCGAAGAGT
(NEFM dn) reverse	Q-PCR (Figure 6C)	GCAAATGACGAGCCATTTCCCACT
(NEFH up) forward	Q-PCR (Figure 6C)	GGAGGCCCTGAAAAGCACCAA
(NEFH dn) reverse	Q-PCR (Figure 6C)	CAGGAGCTTTCTGTAAGCGGC
(PRPH up) forward	Q-PCR (Figure 6C)	GCTCAAGCAGAGGTTAGAAG
(PRPH dn) reverse	Q-PCR (Figure 6C)	TCTACGCTCACCTGCAGGTCTC
(INA up) forward	Q-PCR (Figure 6C)	GAGGAGATCCACGAGTACCGGC
(INA dn) reverse	Q-PCR (Figure 6C)	TGCTAAACCGCGTCTCTTCAC
(GAPDH up) forward	Q-PCR (Figure 6C)	GACTTCAACAGCAACTCCCAC
(GAPDH dn) reverse	Q-PCR (Figure 6C)	TCCACCACCCTGTTGCTGTA