MTSS1/Src family kinase Dysregulation Underlies Multiple Inherited Ataxias

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Abstract (153/250 words)

The genetically heterogeneous Spinocerebellar ataxias (SCAs) are caused by Purkinje neuron dysfunction and degeneration, but their underlying pathological mechanisms remain elusive. The Src family of non-receptor tyrosine kinases (SFK) are essential for nervous system homeostasis and are increasingly implicated in degenerative disease. Here we reveal that the SFK suppressor Missing-in-Metastasis (MTSS1) is an ataxia locus that links multiple SCAs. MTSS1 loss results in increased SFK activity, reduced Purkinje neuron arborization, and low basal firing rates, followed by cell death. Surprisingly, mouse models for SCA1, SCA2, and SCA5 show elevated SFK activity, with SCA1 and SCA2 displaying dramatically reduced MTSS1 protein levels through reduced gene expression and protein translation, respectively. Treatment of each SCA model with a clinically-approved Src inhibitor corrects Purkinje basal firing, and delays ataxia progression in MTSS1 mutants. Our results identify a common SCA therapeutic target and demonstrate a key role for MTSS1/SFK in Purkinje neuron survival and ataxia progression.

Keywords: Neurodegeneration, Src Kinase, MTSS1, Bar Domain Proteins, Actin Cytoskeleton, Spinocerebellar ataxia, SCA1, SCA2, Src kinase Inhibitor, RNA binding protein, Translation
The Src family of non-receptor tyrosine kinases (SFK) are essential for nervous system function, and may contribute to neurodegeneration. Spinocerebellar ataxias (SCAs) are neurodegenerative diseases where Purkinje neurons fire irregularly and degenerate leading to motor problems. We show that the SFK suppressor Missing-in-Metastasis (MTSS1) is an ataxia gene that links multiple SCAs. MTSS1 loss results in increased SFK activity, degenerating Purkinje neurons with low firing rates, and cell death. Surprisingly, mouse models for three different SCAs show elevated SFK activity, with SCA1 and SCA2 models displaying dramatically reduced MTSS1 protein levels. Treatment of each SCA model with SFK inhibitor corrects Purkinje basal firing, and delays ataxia progression in MTSS1 mutants. Our results identify a common link among disparate neurodegenerative diseases.
Introduction

Neurons are non-dividing cells that depend on homeostatic regulation of protein, RNA, and metabolite turnover to permit dynamic synaptic connections that allow adaptation to changing environments. Loss of such mechanisms result in one of several hundred neurodegenerative disorders. Over 40 loci form the genetic basis for human Spinocerebellar Ataxia (SCA), a progressive motor disorder characterized by cerebellar atrophy and pervasive Purkinje neuron degeneration where patients experience poor coordination and balance, hand-eye coordination, dysarthria, and abnormal saccades.

One common phenotype prominent in multiple SCA animal models is the altered Purkinje neuron firing rates that precede motor impairment and cell death (1-3), with restoration of the normal firing rates reducing Purkinje neuron death and improving motor function (4, 5). Defects in many cell functions lead to SCA including effectors of transcription (6), translation (7), proteostasis (8, 9), calcium flux (10, 11), and cytoskeletal/membrane interactions (12, 13). An open question remains how the many SCA genes interact to control firing rates and cell survival, with a common target emerging as an ideal treatment for the genetically diverse etiologies.

One such therapeutic target is the class of Src family of non-receptor tyrosine kinases (SFKs). Several SFKs are expressed in the nervous system and have partially overlapping functions. While single mutants for Src or Yes kinase have no overt neuronal phenotype (14, 15), Fyn loss of function leads to increased Src activity and hippocampal learning and memory deficits (16, 17) Moreover, Fyn;Src double mutants rarely survive past birth and have severely disorganized cortical and cerebellar layers (15, 18). SFKs are post-translationally regulated through activating and inhibitory phosphorylation marks deposited by inhibitory kinases and removed by receptor tyrosine phosphatases in a context dependent manner (19, 20). SFK activation occurs rapidly in response to extracellular signals and in response to a variety of cellular stresses ranging from osmotic pressure (21) to tetanic stimulation (22). Additionally, SFKs are inappropriately active in disease states including Amyotrophic lateral sclerosis (23), Alzheimer disease (24), and Duchenne muscular dystrophy (25).

Missing-in-Metastasis (MTSS1) is one of the defining members of the I-BAR family of negative membrane curvature sensing proteins first identified as being deleted in metastatic bladder cancer (26). Although MTSS1 biochemically interacts with membranes and regulates the actin cytoskeleton (27), genetic studies reveal that MTSS1 functions in an evolutionarily conserved signaling cassette to antagonize Src
kinase activity (28, 29). Disruption of the MTSS1/Src regulatory cassette results in endocytosis and polarization abnormalities demonstrated by defects in primary cilia
dependent hedgehog signaling, and hair follicle epithelial migration (28). In tissues requiring MTSS1 function, levels of active MTSS1 are critical, as loss (26) or gain (30) of MTSS1 has been associated with metastasis and invasion. Regardless of the particular phenotype, an evolutionarily conserved property of MTSS1 mutants is that loss of MTSS1 function can be reversed through the removal or inhibition of Src kinases. This property was first demonstrated through double mutant analysis in the fly ovary, and subsequently in mammalian tissue culture using Src family kinase inhibitors (28, 29). The availability of FDA-approved Src kinase inhibitors has led to the investigation of clinically relevant MTSS1 phenotypes with the hope of using SFK inhibitors to ameliorate them.

Although SFKs have been shown to regulate multiple classes of neurotransmitter receptors (31) they also function to control basic cytoskeletal components. Src regulates local actin polymerization (32) and endocytic receptor internalization (32-35). The actin cytoskeleton plays a critical role in cell signaling, proliferation, motility, and survival. Local, rather than global, actin dynamics control homeostatic synaptic signaling, and abnormalities in actin regulation underlie a diversity of psychiatric and neuronal diseases including Amyotrophic lateral sclerosis (36), Schizophrenia, Autism Spectrum Disorders (37), and motor dysfunction such as spinocerebellar ataxia (SCA) (38). A major challenge remains to understand how actin cytoskeletal regulation controls synaptic function and to develop improved therapeutics for these common and poorly-treated diseases.

Here we reveal that actin regulator and SFK antagonist Mtss1 is an ataxia locus regulated by multiple SCA alleles that subsequently result in SFK hyper-activation. We show that clinically-available Src inhibitors correct Purkinje neuron firing rates and delay ataxia progression, demonstrating a novel and druggable role for the evolutionarily conserved MTSS1/SFK network in Purkinje neuron survival and ataxia progression.

Results
Mtss1 null mice display a progressive ataxia
Mtss1 functions in many tissues, and previous mutant alleles disrupting 5’ exons resulted in mild lymphmagenesis (39), progressive kidney disease (40), mild neurological phenotypes (41) and cerebellar dysfunction(42). However, Mtss1 has
several possible internal promoters (43), and multiple splice variants with differing sub-
cellular localization (44), and existing mutant lines display MTSS1 proteins (40, 45). As 
an alternative approach we generated a conditional mutant allele targeting the 
endophilin/Src interacting domain located in the final exon \(\text{MIM}^{\text{Ex15}}, \text{Fig 1A}\) (28, 29).

Germline deletion with HPRT-cre resulted in the loss of MTSS1 protein as detected by 
an antibody specific to the N-terminal IMD domain (30) (Fig 1B).

To our surprise, homozygous \(\text{MIM}^{\text{Ex15}}\) mutants appear normal for cilia dependent 
processes with no observed instances of holoprosencephaly or polydactyly after multiple 
generations. Additionally, \(\text{MIM}^{\text{Ex15}}\) mutant males are fertile. Instead, \(\text{MIM}^{\text{Ex15}}\) mutants 
display a striking and progressive ataxia. To better understand the nature of \(\text{MIM}^{\text{Ex15}}\) 
ataxia, we characterized \(\text{MIM}^{\text{Ex15}}\) mutants using an open field test to evaluate gross 
motor control. \(\text{MIM}^{\text{Ex15}}\) mutants had reduced velocity (Fig 1C) and rearing behavior (Fig 
1D), consistent with overall movement defects. To uncouple possible motor and 
behavioral abnormalities we evaluated \(\text{MIM}^{\text{Ex15}}\) mutants with rotarod assay and 
observed coordination abnormalities in as early as 4 weeks of age (Fig 1E). Many 
spinocerebellar ataxias display progressive neurologic phenotypes. To determine 
whether \(\text{MIM}^{\text{Ex15}}\) animals showed progressive deterioration we employed a composite 
test measuring gait, grip strength and balance (46). We found \(\text{MIM}^{\text{Ex15}}\) animals 
performed consistently worse than controls, with severity increasing with age (Fig 1F). 
\(\text{MIM}^{\text{Ex15}}\) heterozygous animals displayed 75% of normal protein levels (SI Appendix, Fig 
S1C), giving no overt phenotype.

Reduced Mtss1 levels are associated with a variety of cellular phenotypes 
including reduced presentation of receptors on the cell membrane (47), and altered 
Purkinje neuron morphology (41, 44). To determine the basis of the motor abnormalities 
and to distinguish among these possibilities we performed histological analysis. At 4 
weeks, \(\text{MIM}^{\text{Ex15}}\) mice are ataxic, yet their cerebella appeared grossly normal with intact 
granule, Purkinje neuron, and molecular layers. However, \(\text{MIM}^{\text{Ex15}}\) mutants displayed a 
progressive loss of Purkinje neurons in all cerebellar lobes readily seen by 8 weeks of 
age (Fig S1A). Whereas wild type cerebella contain approximately 8 Purkinje neurons in 
a 250 μm linear distance, 8-week old mice retained only 25% of wild type, and 36 week 
\(\text{MIM}^{\text{Ex15}}\) mutants contained only 5% of the total number of Purkinje neurons (Fig 1G).

While ataxia genes can act in many cell types to regulate Purkinje cell function, 
MTSS1 is highly expressed in Purkinje cells, suggesting it is required in these cells for 
normal Purkinje cell function and survival. To confirm the Purkinje neuron defects seen
in MIM\textsuperscript{EX15} animals are due to a cell autonomous requirement for Mtss1, we conditionally inactivated Mtss1 using the Purkinje neuron specific L7-Cre (MIM\textsuperscript{cko}) then compared Purkinje neuron morphology and loss to the global MIM\textsuperscript{EX15} mutant. MIM\textsuperscript{cko} Purkinje neurons were mosaic for MTSS1 expression likely due to inefficient LoxP recombination as the MTSS1 antibody showed high specificity (SI Appendix Fig S1B). At 20 weeks MIM\textsuperscript{cko} had a significant reduction in Purkinje neurons. In remaining Purkinje neurons, those lacking MTSS1 protein displayed thickened dendritic branches and reduced arbor volume, while neighboring Purkinje neurons with MTSS1 protein appeared normal (Fig 1H). We conclude that Mtss1 acts cell autonomously in Purkinje neurons to maintain dendritic structure, with loss of MTSS1 resulting in abnormalities and eventual cell death.

**Mtss1 mutant neurons display limited autophagic markers**

An emergent mechanism of cell loss during neurodegeneration is aberrant macroautophagy. Autophagy is essential for Purkinje neuron survival, as loss of autophagy (48, 49) results in cell death. Increased levels of early autophagy markers have been described in multiple neurodegenerative diseases including Huntington’s disease (50), Alzheimer disease (51), and SCA3 (52). MIM\textsuperscript{EX15} mutants partially fit this pattern of disease as we observed some signs of autophagy. As early as 4 weeks, we observed increased Complex V/ATP synthase staining indicative of fused mitochondria as well as dramatically reduced staining for the Golgi body marker Giantin (Fig 2A). We also observed increased transcript abundance for the early autophagy effector VMP1 (53). By 8 weeks of age we could detect increased LC3-II species (Fig 2B, SI Appendix S2A), and electron microscopy revealed several autophagy related morphologies including swollen mitochondria, fragmented golgi bodies, lamellar bodies and double membrane autophagic vacuoles (Fig S2C). Interestingly, we were unable to detect increased Sqstm1 (p62) transcript or protein levels in MIM\textsuperscript{EX15}, an autophagic adapter protein associated with protein aggregation neurodegenerative disease (54) (Fig S2B). MIM\textsuperscript{EX15} animals displayed increased neuroinflammation shown by increased Aif1 transcript levels (Fig 2D), a readout of microglial infiltration. MIM\textsuperscript{EX15} animals also show increased GFAP positive glial infiltration (Fig 2E, 2F, SI Appendix S1A) consistent with reactive astroglyosis. Consistent with signs of autophagocytic cell death and neuroinflammation, we failed to see increased DNA breaks in MIM\textsuperscript{EX15} Purkinje neurons with TUNEL stain (Fig 2G).
Mtss1 prevents SFK dependent Purkinje neuron firing defects and ataxia

To characterize cellular changes associated with the ataxia present in 4-week old MIM\textsuperscript{EX15} mice, we examined the dendritic tree of individual biocytin injected Purkinje neurons (Fig 3A). Purkinje neuron dendritic arbor collapse has been observed in several SCA models including SCA1 (2), SCA5 (3), while many other models have shown thinned molecular layer including SCA2(1), SCA3 (55), that likely reflects reduced Purkinje dendritic volume. Similarly, MIM\textsuperscript{EX15} mutants showed a 60% reduction in the expansiveness of the dendritic tree (Fig 3B) and a significant decrease in the number of dendritic spines (Fig 3C), although no significant difference was detected in spine length (Fig 3D) or width (Fig 3E).

In dermal fibroblasts and Drosophila border cells MTSS1 functions to locally prevent ectopic Src kinase activity and Mtss1 mutant phenotypes can be rescued by genetically removing Src kinase (28, 29). To determine if Mtss1 acts similarly in Purkinje neurons we evaluated SFK activity levels in cerebellar lysates from MIM\textsuperscript{EX15} mutants and found elevated levels of SFK\textsuperscript{Y416} (Fig 3F) indicative of increased SFK activity. Previous work has shown strong functional interactions between SFK and metabotropic glutamate receptor type I (mGluR1) neurotransmission at parallel fiber synapse (56). To investigate whether MTSS1/SFK modulation of mGluR1 signaling forms the basis of the ataxia, we performed electrophysiological analysis of Purkinje neurons in cerebellar slices from MIM\textsuperscript{EX15} mice. We evaluated Purkinje neuron response to parallel fiber stimulation using calcium imaging. We found MIM\textsuperscript{EX15} mutant Purkinje neurons responded with a comparable increase of calcium dependent fluorescence to controls, while adding the mGluR1 antagonist CPCCOEt abolished these responses (Fig 3G). These data support MTSS1 acting post-synaptically to control Purkinje cell function.

Purkinje neurons maintain a cell autonomous tonic firing rate that is essential for their function (57, 58). Since MIM\textsuperscript{EX15} Purkinje neurons responded normally to parallel fiber stimulation suggesting normal synaptic transmission, we assayed basal firing rate. Purkinje neuron tonic firing rate is highly sensitive to temperature and may vary slightly between investigators (59). In our assays, wild type cells had a mean firing rate of 43±2Hz (n=2 animals, 62 cells), while 4-week old MIM\textsuperscript{EX15} mutants exhibited a 12±1Hz mean rate (n=2 animals, 55 cells) (Figs 3H, 3I). Previous studies of SCA mouse models demonstrated reduced tonic firing is a basis for ataxia (1, 3, 5). Since basal firing is reduced at an age when MIM\textsuperscript{EX15} mice possess a normal number of Purkinje neurons, our results suggest neuron malfunction rather than loss underlies the initial ataxia phenotype.
MTSS1/Src double mutants rescue MTSS1 phenotypes in Drosophila and vertebrate cell culture. To test the hypothesis that reducing SFK activity would ameliorate the MIM<sup>EX15</sup> ataxia phenotype, we added the FDA-approved SFK inhibitor dasatinib to cerebellar slice preparations and measured basal firing rate, using a concentration approximately 2-fold over in vivo IC50 (200nM, Fig 3H, 3I). Dasatinib significantly increased the MIM<sup>EX15</sup> basal firing rate from baseline to 29±1Hz (n=2 animals, 62 cells). We also observed that dasatinib slightly reduced the wild type basal firing rate to 35±1Hz (n=2 animals, 79 cells). Time course experiments showed the increase in basal firing rate occurred over 5 hours (SI Appendix Fig S3), consistent with a low concentration, high affinity mechanism of action. Direct modulation of ion channel or mGluR1 activity raises basal firing within minutes (4, 60), suggesting that dasatinib works through a distinct mechanism. To determine whether SFK inhibition ameliorates ataxia in vivo we administered dasatinib directly to the cerebellum via minipumps to overcome poor CNS bioavailability (61). Over 4 weeks, dasatinib treated MIM<sup>EX15</sup> mice were protected from disease progression while untreated mice showed progressively worsening rotarod performance (Fig 3J) (n=2 drug, 3 control). These results demonstrate that Src family kinases act downstream of MTSS1 and that SFK inhibitors rescue Mtss1-dependent basal firing rate defects to slow disease progression.

**Mtss1 is a translation target of ATXN2**

The slow basal firing and ataxia preceding cell death seen in the MIM<sup>EX15</sup> mutants resembles that seen in other SCA models such as SCA1, SCA2, and SCA5, prompting us to investigate whether MTSS1/SFK dysregulation occurs in other ataxias. SCA2 is caused by an expansion in the polyglutamine (polyQ) tract of the RNA binding protein ATAXIN-2 (ATXN2) to more than 34 repeats (62). The exact molecular defects that drive SCA2 pathogenesis remain unclear, as loss of function mice do not recapitulate the SCA2 phenotype (63), while intermediate expansion alleles are associated with increased risk for frontotemporal dementia (64). Atxn2 has an ancestral role in translation control (7, 65), which may be altered with the SCA2 mutation, but the exact targets have yet to be described.

MTSS1 protein abundance is heavily regulated by metastasis-associated miRs which bind to the Mtss1 3’ untranslated region and reduce steady-state MTSS1 protein levels (66-70). To determine whether MTSS1 protein accumulation is sensitive to Atxn2 we examined the ATXN2<sup>Q127</sup> mouse model of SCA2 (1). We found MTSS1 abundance
was progressively reduced by 90% at 24 weeks, a level far greater than the 50%
reduction in Purkinje neuron marker Calbindin (Fig 4A upper band, SI Appendix Fig
S4). Cerebellar SFK activity was increased nearly 8-fold in ATXN2Q127 animals compared
to wild type littermates (Fig 4B).

We sought to determine whether the age-dependent reduction in Purkinje neuron
basal firing frequency seen in ATXN2Q127 mice is due to elevated SFK activity.
Remarkably, addition of dasatinib to ATXN2Q127 cerebellar slices restored the basal firing
rate from an average of 14±1Hz (n=2 animals, 100 cells) to nearly normal levels of
32±2Hz (n=2 animals, 72 cells; Fig 4C, 4D). As in the MIMEX15 mutants, the firing rate
reached maximal effect at 5-6 hours of SFK inhibition (SI Appendix Fig S3), leading us
to conclude that inappropriate SFK activity underlies both the ATXN2 and MTSS1-
mediated firing phenotype.

The convergence of Mtss1 and ATXN2 on SFK activity suggested they work in a
common or parallel molecular pathway. To distinguish between these possibilities, we
further interrogated MTSS1 protein levels in ATXN2Q127 cerebella. While we found
reduction of MTSS1 protein (Fig S4A) and RNA in ATXN2Q127 Purkinje neurons (Fig
S4B), we failed to see comparable changes in ATXN2 levels in 4-week old MIMEX15
mice (Fig 4E). Because ATXN2 possesses RNA binding activity, and Mtss1 contains a
long 3’UTR, we hypothesized that ATXN2 controls Mtss1 translation in Purkinje neurons.
RNA-IP followed by QPCR in cells expressing tagged versions of either WT (ATXN2Q22)
or SCA2 (ATXN2Q108) demonstrated both proteins specifically bound MTSS1 mRNA
compared to GAPDH control. (Fig 4F). Using a luciferase reporter fused to the MTSS1
3’ UTR we were able to map the ATXN2 interacting domain to a central 500bp region
that was sufficient for both RNA-protein interaction and translation control (SI Appendix
Fig S4C,D). Furthermore, polyribosome fractionation experiments revealed that
pathogenic ATXN2Q108 was sufficient to block the translation of reporter mRNA fused to
the MTSS1 3’UTR shifting the transcript from the polyribosome fractions to a detergent
resistant fraction consistent with stress granules (Fig 4G). These results suggest the
pathogenic ATXN2 acts directly as a dominant negative RNA binding protein preventing
MTSS1 translation. Notably, we observed MTSS1 abundance is reduced in human SCA
patient cerebellum, bolstering the evolutionary conservation of the ATXN2/MTSS1
interaction (Fig 4H).

SFK inhibition rescues Purkinje neuron firing across SCA
Two other SCA mouse models have been shown to have slow basal firing rates, SCA1 (2) and SCA5 (3). Much like SCA2, SCA1 is due to a polyQ expansion in the RNA binding protein ATAXIN-1 (ATXN1) (71). One observed result of the SCA1 allele is changed ATXN1 association with transcriptional regulatory complexes (72), leading to vastly different Purkinje neuron mRNA profiles (73). However, the exact targets that drive SCA1 pathogenesis are still being determined. Unlike SCA1 and SCA2, SCA5 is a more pure cerebellar ataxia due to lesions in the structural protein β-III spectrin (13). β-III spectrin directly binds to and controls the cell membrane localization of EAAT4 (excitatory amino acid transporter 4), a protein involved in the synaptic clearance of glutamate (12, 74).

If SCA1 or SCA5 arises similarly to SCA2 by dysregulation of the MTSS1/SFK cassette, we would expect decreased MTSS1 abundance. Indeed, in the ATXN1Q82 mouse model of SCA1 (75) we observed a 95% decrease in MTSS1 protein abundance (Fig 5A) with only a 50% reduction in calbindin, suggesting the loss of MTSS1 is not solely due to loss of Purkinje neurons.

Atxn1 pathogenicity is partially driven by phosphorylation at serine 776 (72), which was unchanged in 4-week old MIMEx15 mice, suggesting MTSS1 is a target of the SCA1 allele (Fig 5B). Additionally, Mtss1 transcript abundance is reduced at multiple ages in ATXN1Q8 mice (73) (Fig 5C). We found treating ATXN1Q82 slices with dasatinib increased the basal firing rate from a baseline of 15±1Hz (n=3 animals, 21 cells) to 23±2Hz (n=3 animals, 21 cells), a level statistically indistinguishable from dasatinib-treated controls (Fig 5D).

By contrast, the Sptbn2 knockout model of SCA5 (βIII−−/−) (3), showed no change in MTSS1 protein abundance at 3 weeks yet demonstrated a clear increase in SFKY416 phosphorylation (Fig 5E). We also observe increased basal firing from 25±1Hz (n=2 animals, 31 cells) to 30±2Hz (n=3 animals, 43 cells) over a 7-hour period of dasatinib treatment (Fig 5F). We fail to see changes in β-III spectrin abundance in MIMEx15 mice, and detect a 40% decrease in β-III spectrin levels in 24-week ATXN2Q127 mice that is likely due to reduced Purkinje neuron dendritic arbor size, correlating with calbindin levels (Fig 5G, 5H). Together these data suggest that β-III spectrin and MTSS1 may work in parallel, through different mechanisms, to modulate SFK activity (Fig 5I).

Discussion

While SCA gene functions appear heterogeneous, our study establishes a genetic framework to understand how several SCA loci regulate SFK activity to ensure
neuronal homeostasis and survival. We identify β-III spectrin and MTSS1, proteins that link the cell membrane and actin cytoskeleton, as negative regulators of Src family kinases. We show that MTSS1 is a target of the SCA genes *ATXN1* and *ATXN2* (Fig 5I), and that increased SFK activity from lesions in *MTSS1*, *SPTNB2* (SCA5), *ATXN1* (SCA1), and *ATXN2* (SCA2) reduces Purkinje neuron basal firing, an endophenotype that underlies multiple ataxias, providing support for the clinical use of SFK inhibitors in many SCA patients.

Our results reveal a central role for the MTSS1/SFK regulatory cassette in controlling neuronal homeostasis and survival. MTSS1 regulation of SFKs has been demonstrated in several migratory cell types including metastatic breast cancer and *Drosophila* border cells. This is the first demonstration of the regulatory cassette functioning in non-migratory post-mitotic cells. MTSS1 integrates the cell membrane and cytoskeletal response to local signals by serving as a docking site for the kinases and phosphatases that control actin polymerization (76), a process essential for dendritic spine assembly, maintenance and function. In fly border cells, MTSS1-regulated SFK activity polarizes the membrane to spatially detect guidance cues. Similarly, MTSS1 functions in neurons to promote dendritic arborization and spine formation, structures that were shown to be essential for maintaining basal firing frequencies by electrically isolating increasing areas of Purkinje neuron dendrites (59). Other members of the I-BAR family of membrane/cytoskeletal signaling proteins have been implicated in human neurological disorders such as microcephaly (77), but it remains to be determined how they interact with MTSS1.

Disruption of post-transcriptional gene regulation leading to altered proteostasis has recently emerged as a key contributor to neurodegeneration. In the cerebellum, reducing the abundance of the RNA-binding protein Pumilio leads to SCA1-like neurodegeneration through a specific increase in ATXN1 protein levels (78, 79). Yet Pumilio binds hundreds of transcripts to control protein levels (80, 81), suggesting that changing protein abundance of a few key effector genes post-transcriptionally leads to disease. Our data demonstrate that MTSS1 is a key effector gene whose activity is tightly regulated to prevent Purkinje neuron malfunction. Post-transcriptional control of MTSS1 is disrupted in many disease states such as cancer, where MTSS1 levels are reduced by locus deletion or miRNA overexpression and are associated with increased metastasis and poorer prognosis (67, 82). In Purkinje neurons, the SCA1 ATXN1Q82 allele reduces MTSS1 transcript levels. ATXN1 is thought to act as a transcriptional
regulator by associating with the transcriptional repressor *Capicua* (CIC) (72), though it remains to be shown whether the ATXN1/CIC complex occupies the *MTSS1* promoter. By contrast, the SCA2 allele ATXN2\textsuperscript{Q58} binds the *MTSS1* 3’ UTR to prevent ribosome binding and *MTSS1* translation, ultimately leading to increased SFK activity. ATXN2 (and the redundant gene ATXN2L) have recently been identified in a large complex of 3’ UTR binding proteins that regulate networks of genes controlling epithelial differentiation and homeostasis (83). Our results suggest other ataxia disease genes that control proteostasis may also regulate *MTSS1* abundance, and the strong role for miRNAs controlling *MTSS1* abundance in cancer suggest they may also function as effectors of as yet undescribed ataxia loci.

The identification of the *MTSS1*/SFK regulatory cassette in multiple ataxias further reinforces the pathological consequences associated with inappropriate SFK activation in response to a variety of cellular stresses. While the cytoskeletal regulator *MTSS1* is an evolutionarily-conserved SFK inhibitor, SFK effects on Purkinje neuron basal firing may derive from the fundamental roles SFKs play in cell homeostasis outside cytoskeletal control. For example, SFK control of translation is implicated in Alzheimer disease, as reducing SFK activity proves beneficial for Alzheimer disease progression (24) due to SFK control of pathogenic \( \alpha \beta \) translation (84). SFK impairment of autophagy is seen in models of Amyotrophic lateral sclerosis and Duchenne muscular dystrophy (23, 85). Additionally, reduction of Src kinase expression was identified as a suppressor of SCA1 toxicity in *Drosophila* ommatidia (86), supporting the need for moderating SFK activity. The pleiotropic effects of inappropriate SFK activity suggest that SFK inhibition may be a critical therapeutic node to slow the progression of multiple neurodegenerative disorders including SCAs. Our work points out the need for future development of neuro-active SFK inhibitor variants, as currently approved Src inhibitors were designed for oncology targets and lack potent central nervous system activity. Further, while we provide data for kinase inhibition to suppress *MTSS1* loss, we have previously shown that SFK regulation by regulatory receptor tyrosine phosphatases, or deletion of endocytic adapter proteins can also revert the effects of *MTSS1* loss. Given the challenge of developing specific kinase inhibitors, our work opens additional therapeutic classes to alleviate the progression of neurodegenerative diseases.

In summary, the identification of *Mtss1* as a novel recessive ataxia locus extends the physiologic functions requiring the *MTSS1*/SFK signaling cassette, which include cell polarity, migration, and cancer metastasis. Each of these disparate processes highlight
the common role MTSS1 plays integrating the cell membrane and cytoskeletal response
to local signals, as the dendritic spine defects seen in MIM<sup>EX15</sup>-mutant Purkinje neurons
(Fig 3A-E) recalls the loss of directional cell extensions in migrating Drosophila border
cells (29). They also reinforce the critical need to suppress inappropriate SFK activity,
and provide a therapeutic opportunity for otherwise devastating and debilitating
diseases.

Author Contributions

AEO and SXA conceived the project. ASB, SXA, BA, JM performed and
interpreted most experiments. PM performed and interpreted all electrophysiology in
Mtss1<sup>EX15</sup> and ATXN2<sup>Q127</sup> mice. EP and MJ performed and interpreted all
electrophysiology and western blots in βIII-spectrin<sup>−/−</sup> mice. RC, HH and VS performed
and interpreted all electrophysiology and western blots in ATXN1<sup>Q82</sup> mice. SP and DS
performed and interpreted MTSS1 western blot and QPCR in ATNX2<sup>Q127</sup> mice and HEK-293 cell RNAIP. SP performed and interpreted MTSS1 staining in human samples. ET
quantified biocytin-filled Purkinje data. TSO and SMP contributed ideas and interpreted
results. ASB and AEO wrote the manuscript with input from all authors.

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**Figure Legends**

**Figure 1.** MIM\textsuperscript{EX15} mutants develop progressive spinocerebellar ataxia

A: The structure of the Mtss1 locus with alternative promoters and Src interacting domain deleted in MIM\textsuperscript{EX15} mutants. B: Loss of MTSS1 protein in MIM\textsuperscript{EX15} cerebellum lysate shown with MTSS1 antibody against N-terminal IMD domain. C,D MIM\textsuperscript{EX15} show slower movement velocity and reduced rearing frequency in open field tests. E: Impaired rotarod performance in MIM\textsuperscript{EX15} mutants shown as reduced duration (time to fall). F: A composite test of gait, balance, and grip strength to measure spinocerebellar ataxia symptoms. Increased score reflects reduced function with an age dependent increase in severity in MIM\textsuperscript{EX15} mutants. G: Age dependent loss of Purkinje neurons in MIM\textsuperscript{EX15} mutants occurs after the onset of ataxia. H: At 20 weeks MIM\textsuperscript{Loxp/-};Pcp2-Cre and MIM\textsuperscript{Loxp/Loxp};Pcp2-Cre mutants show dramatic reduction in Purkinje neurons that stain with MTSS1. Many Purkinje neurons persist, as there is a less dramatic reduction in calbindin positive Purkinje cell number. *p<0.05, **p<0.005, ***p<5E-5, one-way ANOVA with Tukey post-hoc test. ns not significant. Error bars, s.e.m.

**Figure 2.** MIM\textsuperscript{EX15} mutant Purkinje neurons undergo autophagy

A: MIM\textsuperscript{EX15} mutants display fused mitochondria shown by increased Complex 5 ATP-synthase immuno-staining and collapsed Golgi shown by reduced Giantin immune-staining at 4 weeks. B: 8 week old MIM\textsuperscript{EX15} mutants show increased LC3-II abundance (*P<0.005 student’s t-test), C: MIM\textsuperscript{EX15} mutants show increased levels of mRNA for the autophagocytic marker VMP1 (*p<0.05 student’s t-test). D: MIM\textsuperscript{EX15} mutants show increased microglial infiltration shown by Aif1 transcript. E: MIM\textsuperscript{EX15} mutants show GFAP+ glial infiltration during disease progression. F: Western blots quantifying increased cerebellar GFAP. G: MIM\textsuperscript{EX15} mutant cerebella do not have increased TUNEL stain at 4, 8 or 16 weeks of age.

**Figure 3.** Mtss1 prevents SFK dependent firing defects and ataxia

A: Confocal projection of an individual Purkinje cell filled with biocytin and with fluorescent dye to visualize morphology (50μm, 5μm, 1μm scale bars). B: Measurement of dye filled Purkinje neurons show MIM\textsuperscript{EX15} mutants have reduced arbor volume (n=3 each genotype), C: reduced dendritic spine density, but D: no change in dendritic spine length and E: no change in dendritic spine width (MIM\textsuperscript{EX15/+} n=3, 1720 spines; MIM\textsuperscript{EX15} n=3, 1454 spines, *p<0.05 student’s t-test). Error bars, s.e.m F: Western blot for active SFK-Y416 phosphorylation with actin loading control. Cerebellar lysate from MIM\textsuperscript{EX15} and age matched controls collected at indicated times between post-natal day 15 (P15) and MTSS1/SFK Ataxia
post-natal day 30 (P30). G: Slow excitatory post synaptic potential (EPSP) spikes in wild type (WT) and MIM<sup>EX15</sup> (top) elicited by stimulation of parallel fibers with 10 pulse trains at 100 Hz in the presence AMPA, NMDA and GABA receptor antagonists (control conditions). Corresponding intra-cellular Ca<sup>2+</sup> signals (ΔF/F) for responses for WT and MIM<sup>EX15</sup> mGluR EPSPs are illustrated. EPSPs and corresponding Ca<sup>2+</sup> signals are blocked by mGluR1 antagonist CPCCOEt (bottom). Summary data of intracellular Ca<sup>2+</sup> signals (ΔF/F) for responses for WT and MTSS1<sup>EX15</sup> in control conditions and in presence of CPCCOEt are shown (right). H: Percent histograms of Purkinje neuron mean firing frequencies (left), examples of extracellular recording of 1 second duration of a spontaneously spiking Purkinje neuron in respective condition (center), and histograms of inter-spike intervals calculated for the 2 minute recording periods of the same neuron (right) are shown for WT, MIM<sup>EX15</sup>, WT+dasatinib, or MIM<sup>EX15</sup>+dasatinib conditions. I: Summary of data presented in H *p=6.1E-14 **p=1E-13, one-way ANOVA, Tukey post-hoc J: Direct cerebellar administration of dasatinib maintains rotarod performance, slowing the progressive ataxia in MIM<sup>EX15</sup> mice. q=0.006, two-stage step-up Benjamini, Krieger, Yekutieli method, Error bars, s.e.m.

**Figure 4. MTSS1 is an Atxn2 translation target**

A: Western blot of 24-week whole cerebellum lysate shows 90% reduction of upper band (arrow) that corresponds MTSS1 in ATXN2<sup>Q127</sup> mice, while calbindin was reduced 50%. Actin is included as a loading control *p<0.01, **p<0.001, Student’s t-test. B: Western blot for active SFK-Y416 phosphorylation and total Src, with tubulin loading control using cerebellar lysate from 24 week Atxn2<sup>Q127</sup> mice show 8-fold increase in SFK-Y416 abundance. C: Percent histograms of Purkinje neuron mean firing frequencies (left), examples of extracellular recording of 1 second duration of a spontaneously spiking Purkinje neuron in respective condition (center), and histograms of inter-spike intervals calculated for the 2 minute recording periods of the same neuron for ATXN2<sup>Q127</sup> and ATXN2<sup>Q127</sup>+dasatinib D: Mean firing rates **p=3.77E-8, one-way ANOVA, Tukey post-hoc E: Western blot for Atxn2 with tubulin loading control. Cerebellar lysate from 4-week old MIM<sup>EX15</sup> cerebellum and age matched controls. F: RNA-IP in HEK-293 cells for flag-ATXN2<sup>Q22</sup> and flag-ATXN2<sup>Q108</sup> show enrichment for MTSS1 but not GAPDH mRNA, error bars are SD. G: Polyribosome fractionation in 293T cells transfected with MTSS1-UTR reporter and pCDNA, ATXN2<sup>Q22</sup>, ATXN2<sup>Q108</sup>, or ATXN2<sup>Q22</sup>+ATXN2<sup>Q108</sup>. Green line indicates UV254nm absorbance (nucleic acids) with 40S, 60S, 80S, polyribosome peaks.
labeled. **H**: Remaining Purkinje neurons in human SCA2 cerebellum (Atxn2Q22/Q41) show reduced MTSS1 staining compared to age matched control (Atxn2Q22/Q22).

**Figure 5.** SFK dysregulation occurs in multiple SCA

**A**: Western blot of 15-week whole cerebellum lysate shows 95% reduction of upper band that corresponds MTSS1 in ATXN1Q82 mice with only a 50% reduction in calbindin. Tubulin is included as a loading control. **B**: Western blot of 4-week old MIMEx15 cerebellum lysate shows no change in phospho-Serine776 ATXN1 levels. **C**: RNA-seq from ATXN1Q82 cerebella show reduced FPKM for Mtss1 mRNA in 12 and 28 week samples, * q<0.005. **D**: Mean firing frequency values in Hz for WT and ATXN1Q82 mice, with and without dasatinib treatment. Error bars, s.e.m. (*p=0.0094, one-way ANOVA with Tukey post-hoc) **E**: Western blot of 3-week whole cerebellum lysate shows no change MTSS1 in βIII-spectrin-/- mice, yet active SFK-Y416 phosphorylation is increased. Calbindin and total Src are included as a loading controls. **F**: SPTNB2 abundance is not changed in 4-week old MIMEx15 mice. **G**: βIII-spectrin levels are reduced 40% in 24-week ATXN2Q127 mice. **H**: Mean firing frequency values in Hz for WT and βIII-spectrin-/- mice, with and without dasatinib treatment. Error bars, s.e.m. (*p<0.05, 1-way ANOVA, Tukey posthoc ) **I**: A model where pathogenic alleles of ATNX1 (ATXN1Q82) and ATXN2 (ATXN2Q42) prevent the accumulation of MTSS1 and SPTBN2 which restrain SFK activity to prevent abnormal firing patterns and neurodegeneration.

**Supplementary Figure Legends**

**Figure S1** associated with Figure 1. Sagittal sections of MIMEx15 mutants and antibody validation **A**: mosaic images of 16 week old WT and MIMEx15 demonstrate widespread Purkinje neuron loss and increased GFAP staining intensity. **B**: MTSS1 antibody specificity is show by lack of signal on MIMEx15 tissue. **C**: Western blot of 4 week cerebella show MIMEx15+ have 25% reduction in MTSS1 compared to WT *p<0.05, **p<0.0001, 1-way ANOVA, Tukey post-hoc test.

**Figure S2**, associated with Figure 2. Purkinje cell autophagy in MIMEx15 mutants **A**: Western blot shows no increase in LC3-ii levels in 4 week old MIMEx15 mutants. **B**: Sqstm1 (P62) transcript is not increased in MIMEx15 mutants and protein levels are not elevated. **C**: Electron micrographs of 8 week old animals showing defects present in 3 MIMEx15 mutants but absent in WT animals: swollen mitochondria where the inner
matrix is poorly resolved and dissociated from the outer matrix, electron dense autophagic bodies (AV), lamellar bodies in Purkinje neuron dendrites.

**Figure S3, associated with Figures 3 and 4. Acute Src inhibition restores tonic firing rates**

Average firing frequency measured at the end of each incubation hour of Dasatinib in MIM\textsuperscript{EX15} (A) and ATXN2\textsuperscript{Q127} (B) is plotted. A respective mean value with number of Purkinje neurons (PN) at indicated incubations is given in the figure. Note that baseline firing frequency of ATXN2\textsuperscript{Q127} (14±1 Hz, n=100 PNs) mice of this age is similar to the MIM\textsuperscript{EX15} (12±1 Hz, n=55 PNs). Basal firing frequency of PNs from wild type mouse are given.

**Figure S4, associated with Figure 4. ATXN2 regulates translation through the MTSS1 3’UTR**

A: MTSS1 immuno-fluorescence in ATXN2\textsuperscript{Q127} and age matched control mice show reduced Purkinje neuron signal at 4, 12 and 24 week time points. B: Quantitative RT-PCR (QPCR) shows Mtss1 transcript abundance is reduced in ATXN2\textsuperscript{Q127} compared to age matched controls \( *p<0.05, **p<0.01 \), student’s t-test, SD error bars. C: RNA-IP followed by QPCR shows ATXN2 binding to the MTSS1 3’UTR is mediated by a central 500bp region \( *p<0.05, **p=0.015 \), one-way ANOVA with Tukey post-hoc test. D: Luciferase reporter assay shows ATXN2 translation is strongly mediated by the 3’UTR, and ATXN2\textsuperscript{Q108} allele is sufficient to block activation ns not significant, all other interactions \( p<0.005 \). 2way ANOVA finds reporter constructs explain 56% of total variance, differences in response to transfection (pcDNA, ATXN2\textsuperscript{Q22}, ATXN2\textsuperscript{Q22+Q108}) between reporter constructs explains 30% of variance, and different response to transfection (pcDNA, ATXN2\textsuperscript{Q22}, ATXN2\textsuperscript{Q22+Q108}) within a reporter explain 14% of total variance.
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Anthony Oro (oro@stanford.edu)

Generation of MIM^EX15 allele:
To generate the MIM^EX15 conditional allele exon15 was cloned into the PGK-gb2 targeting vector between the 5' LoxP site and the 3' LoxP/FRT flanking neomycin cassette. The targeting vector contained a 5.97kb 5' homology arm that included exons 12, 13, 14 and a 2.34kb 3' homology arm that included the 3'-UTR. The targeting vector was electroporated into C57bl6xSV129 embryonic stem cells, and Neo-resistant colonies were screened by PCR. Chimeric mice were generated by injecting ES cells into blastocysts, and chimeras were mated to a FLP deleter strain. To generate MIM^EX15 null animals, mice with the MIM^EX15 conditional allele were crossed to HPRT-Cre mice. Mice were maintained on a mixed C57bl6 SV129 background and examined at listed ages.

Western blot:
Isolated tissues were lysed in RIPA buffer supplemented with complete mini protease inhibitor (Roche) and PhosStop (Roche). Protein concentrations were normalized by using the BCA assay (Pierce). Proteins were electrophoresed on Novex 4-12%, 3-8%, 10-20% gradient gels or 16% gels. Rabbit anti-Src-Y416 (CST 2101S or CST 6943S), mouse anti-beta actin (Sigma), rabbit anti-Sptbn2 (Thermo PA1-46007), rabbit anti-Atxn2 (Sigma HPA021146), mouse anti-Atxn1 (abcam ab63376), rabbit anti-LC3A/B (CST 4108), rabbit anti-P62 (CST 23214) rabbit anti-Src (CST 2123 or CST 2108), primary antibodies were detected with LICOR secondary antibodies.

Antibodies and Immunofluorescence:
Isolated cerebella were immersion fixed in 4% paraformaldehyde and embedded in paraffin. 7μm sections were cut and deparaffinized using standard conditions before staining. Sections were blocked with 20% horse serum 0.3% Triton X-100 in PBS. The following antibodies were used at 1:1000 dilutions: Rabbit anti-Mtss1(30), Rabbit anti-Calbindin (CST 13176), mouse anti-Calbindin-D-28K monoclonal (Sigma), mouse anti-Complex V (Novex 459240), rabbit anti-Ubiquitin (CST 3933), rabbit anti-Giantin (Abcam ab 24586), Chicken anti-GFAP (Abcam ab4674).
Alexafluor conjugated secondary antibodies were purchased from Invitrogen. Images
were acquired either on a Leica SP2 AOBS laser scanning microscope or a Zeiss
axioplan widefield scope.

Human samples:
Paraffin-embedded brain slices from SCA2 patient were provided by Prof. Arnulf H.
Koeppen, M.D., Albany Medical College, New York, USA. Non-SCA2 control paraffin-
embedded brain slices were provided by Dr. Sonnen, Pathologist, University of Utah.
Human tissues were maintained and processed under standard conditions consistent
with National Institutes of Health guidelines and conformed to an approved University of
Utah IRB protocol. Sections were deparaffinized using standard conditions and
blocked/permeabilized with 5% donkey serum 0.3% Triton X-100 in PBS and processed
for immunostaining. The nuclei were stained with DAPI followed by mounting with
Fluoromount-G (Southern Biotech, Cat# 0100-01). Antibody dilutions for tissue
immunostainings were custom-designed MTSS1 antibody (1: 500) and fluorescent
secondary antibody: goat anti-rabbit IgG (H+L) antibody, DyLight-488 [(1:1,000)
(ThermoFisher Scientific, Cat# 35552)]. Images were acquired using confocal
microscope (Nikon Eclipse Ti microscopy) in University of Utah cell imaging core lab,
and analyzed by NIS-Elements AR 4.5 software. As massive degeneration of cerebellum
is seen in SCA2 brain tissue, the lobe can’t be verifiable.

Electrophysiology:
Preparation of Cerebellar Slices (SCA2 and Mtss1)
Acute parasagittal slices of 285μm thickness were prepared from the cerebella of 4- to 8-
week-old mutant and control littermates following published methods(1). In brief, brains
were removed quickly and immersed in an ice-cold artificial cerebrospinal fluid (ACSF or
extracellular) solution consisting of: 119 mM NaCl, 26 mM NaHCO₃, 11 mM glucose, 2.5
mM KCl, 2.5 mM CaCl₂, 1.3 mM MgCl₂ and 1 mM NaH₂PO₄, pH 7.4 when gassed with
5% CO₂ / 95% O₂. Cerebella were dissected and sectioned using a vibratome (Leica VT-
1000). Slices were initially incubated at 35 °C for 35 min, and then at room temperature
before recording in the same ACSF. Dasatinib (200nM) was added during cerebellar
sectioning and remained on the slices for recording.

Recordings (SCA2 and Mtss1)
Non-invasive extracellular recordings were obtained from Purkinje neurons in voltage-
clamp mode at 34.5 ± 1°C. The temperature was maintained using a dual channel
heater controller (Model TC-344B, Warner Instruments) and slices were constantly
perfused with carbogen-bubbled extracellular solution alone or with 200 nM dasatinib.
Cells were visualized with an upright Leica microscope using a water-immersion 40×
objective. Glass pipettes were pulled with Model P-1000 (Sutter instruments). Pipettes
had 1 to 3 MΩ resistance when filled with extracellular solution and were used to record
action potential-associated capacitative current transients near Purkinje neuron axon
hillock with the pipette potential held at 0 mV. Data was acquired at 20 kHz using a
Multiclamp 700B amplifier, Digidata 1440 with pClamp10 (Molecular Devices), filtered at
4 kHz. A total of 50 to 100 Purkinje neurons were measured from each genotype and
each recording was of 2 minutes in duration. The experimenter was blinded to the
mouse genotype and 2 to 4 mice were used per genotype. Simultaneous mGluR EPSPs
and calcium were measured in the presence of GABA_A receptor antagonist, picrotoxin
(PTX at 100 µM), AMPA receptor blockers (5 µM NBQX and 10 µM DNQX) using a two-
photon microscope and a standard electrophysiology set-up. The patch pipettes had 4 to
5 MΩ resistance when filled with internal solution (135 mM KMS0, NaCl, 10 mM
HEPES, 3 mM MgATP, 0.3 mM Na2GTP) containing 200 µM Oregon Green Bapta1 and
20 µM Alexa 594. The stimulating electrode was filled with ACSF containing 20 µM
Alexa 594, placed in the dendritic region to minimally stimulate PF synaptic inputs. Slow
mGluR EPSPs in control littermate and mutant were elicited by stimulation of PFs with
100 Hz trains, and 10 pulses in the presence of receptor antagonists that block AMPA,
NMDA, GABA_A receptors. Corresponding intracellular Ca^{2+} signals (ΔF/F) for responses
for wild type and mutant mGluR EPSPs were blocked by the mGluR1 antagonist
CPCCOET.

Experiments were analyzed using both the Clampfit and Igor algorithms, and
were further analyzed using Microsoft Excel. Figures were made in Igor program.
Calcium signals were analyzed using Slidebook (Intelligent Imaging Innovations, Inc.).
Results are presented as mean ±SEM. All chemicals were purchased either from Sigma
Aldrich, Tocris and Invitrogen, USA.)

**Biocytin fills of Purkinje neurons or Intracellular labeling of Purkinje neurons with
**Biocytin:

Biocytin filling of Purkinje neurons was performed using recording pipettes filled with 1%
Biocytin (Tocris). Purkinje neurons were filled for 15 to 30 minutes and then the pipette
was removed slowly for enabling the cell membrane to reseal. Slices were then fixed in
4% Paraformaldehyde overnight and washed 3 times with phosphate-buffered saline (PBS). Slices were then incubated with Alexa Fluor 488 streptavidin (1:500, Life S11223) in PBS, 0.5% Triton X-100, and 10% normal goat serum for 90min. After another 3 PBS washes, the slices were then mounted onto a slide with prolong gold. Individual biocytin-filled Purkinje cells were visualized on a Leica SP2 AOBS laser scanning microscope at a 0.5um step size. Dendritic arbor volume was measured by calculating the biocytin-filled area in each confocal optical section using ImageJ, adding the areas in each z-stack, and multiplying by the step size.
Figure 1

A. Mts1 Protein

B. Wild-type

C. Open field movement

D. Open field rearing

E. 16rpm Rotarod

F. Gait, Ledge, Limb tests

G. 2 weeks 4 weeks 8 weeks 16 weeks 36 weeks

H. Calbindin MTSS1 DNA

MTSS1 29 weeks

MTSS1 50μm
Figure 3
Figure 4

A

B

C

D

E

F

G

H

MTSS1/SFK Ataxia
SCA1

A

MTSS1

Intensity

0.49
0.38
0.12
0.03
0.04
0.02

SFK-Y

Intensity

0.91
1.21
0.71
0.98
0.89
0.98
1.49

Tubulin

1.31
0.71
0.79
0.8
0.8
1.15
1.81

WT

ATXN1Q82

B

ATXN1-S76

Intensity

0.06
0.77
1.35
0.81
1
1.06

Tubulin

0.08
0.74
1.37
0.81
1
1.06

WT

MIMPQ15

C

Mts1 mRNA levels

ATXN1

ATXN1 + Das.

Mean Firing Frequency (Hz)

0
10
20
30
40

D

Firing Frequency, Hz

WT

ATXN1

ATXN1 + Das.

SCA5

E

MTSS1

Intensity

1.33
1.04
0.72
1.38
1.62
1.39

CALB1

Intensity

1.01
1.33
1.33
2.71
3.86
1.88

SFK-Y

Intensity

1.04
1.02
1.03
0.78
0.89
0.57

Total SRC

0.95
1
0.60
0.63
0.58

WT

βIII-

F

SPTNB2

Intensity

1.04
1.02
1.03
0.78
0.89
0.57

Tubulin

0.95
1
0.60
0.63
0.58

WT

MIMPQ15

G

SPTNB2

Intensity

0.95
1
0.60
0.63
0.58

Actin

0.95
1
0.60
0.63
0.58

WT

ATXN2Q72

H

Firing Frequency, Hz

0
10
20
30
40
50

WT

βIII-

βIII-

I

WT

SCA / Mts1

Normal Firing

Slow Firing

Rescued Firing

MTSS1/SFK Ataxia