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SelR/MsrB Reverses Mical-mediated Oxidation of Actin to Regulate F-actin Dynamics

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Abstract

Actin's polymerization properties are dramatically altered by oxidation of its conserved methionine (Met)-44 residue. Mediating this effect is a specific oxidation-reduction (Redox) enzyme, Mical, that works with Semaphorin repulsive guidance cues and selectively oxidizes Met-44. We now find that this actin regulatory process is reversible. Employing a genetic approach, we identified a specific methionine sulfoxide reductase enzyme SelR that opposes Mical Redox activity and Semaphorin/Plexin repulsion to direct multiple actin-dependent cellular behaviors in vivo. SelR specifically catalyzes the reduction of the *R*-isomer of methionine sulfoxide (methionine-*R*-sulfoxide) to methionine, and we found that SelR directly reduced Mical-oxidized actin, restoring its normal polymerization properties. These results indicate that Mical oxidizes actin stereo-specifically to generate actin Met-44-*R*-sulfoxide (actin$^{\mathrm{Met}(R)\mathrm{O}\text{-}44}$) – and they also implicate the interconversion of specific Met/Met(*R*)O residues as a precise means to modulate protein function. Our results therefore uncover a specific reversible Redox actin regulatory system that controls cell and developmental biology.

Identifying the factors that shape the actin cytoskeleton, the basic building blocks of cellular form and function, is a critical biomedical goal [1,2]. Interestingly, actin is susceptible to post-translational modification of its amino acid residues but the physiological importance of these covalent modifications is still poorly understood [3]. Recently, we found that actin's polymerization properties are altered by specific oxidation of its conserved methionine (Met)-44 residue on the pointed-end of actin subunits [4]. These observations raise issues of the susceptibility of this residue to pathological modification [3], but we have also identified a specific oxidation-reduction (Redox) enzyme, Mical, that selectively oxidizes Met-44 to disassemble actin filaments (F-actin) and impair actin polymerization [4,5]. Our results reveal that Mical uses F-actin as a direct substrate, employing an oxidation-dependent post-translational mechanism to regulate filament dynamics [4].

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AUTHOR CONTRIBUTIONS

R.-J.H., C.S.S., H.G.Y. and J.R.T. designed/performed experiments and analyzed data, R.-J.H., C.S.S., and J.R.T. prepared the manuscript, R.-J.H. wrote the paper, and C.S.S. and J.R.T. assisted in the writing of the paper.

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MICAL family proteins, which include one Drosophila Mical and three mammalian MICALs, regulate numerous cellular events in different tissues including morphology, motility, navigation, exocytosis, and survival (Reviewed in [6-9]). At least some of these effects occur through MICALs ability to regulate actin cytoskeletal organization [4,5,10-12]. Interestingly, 2 MICALs also directly link one of the largest families of extracellular guidance cues, the Semaphorins and their Plexin cell surface receptors, to changes in the actin cytoskeleton [5,13]. Semaphorins are the largest family of repulsive guidance cues [14,15] and have been characterized for their ability to disassemble F-actin and "collapse" the actin cytoskeleton of multiple different cell types [6,16]. MICALs directly bind to the Semaphorin receptor Plexin through their C-termini [13,17] and employ their actin-binding/regulatory Redox domain to mediate the destabilizing effects of Semaphorins/Plexins on the actin cytoskeleton [5]. These effects include a loss of F-actin, the decreased ability to polymerize new F-actin, a decrease in the number of F-actin bundles, and the regulation of F-actin-rich filopodia/branches [6].

We now find a specific methionine sulfoxide enzyme SelR/MsrB that selectively reverses this Mical-mediated oxidation of actin. SelR counteracts Mical in vivo to direct multiple actin-dependent cellular processes including axon guidance, synaptogenesis, muscle organization, and mechanosensory development. SelR also neutralizes Semaphorin/Plexin repulsion. Thus, Mical and SelR comprise a reversible Redox cellular signaling system that orchestrates proper cytoskeletal-mediated physiology.

RESULTS

SelR Counteracts Mical-mediated F-actin Alterations In Vivo

Mical directs the organization of actin in a number of different cell types [4,5,10-12] including within developing bristle processes, which are akin to mammalian mechanotransducing inner ear hair cells that detect sound [18,19]. Bristles have also long served as a simple, single cell model for characterizing actin dependent events in vivo [5,20,21]. Raising the levels of Mical specifically in bristle cells using the GAL4-UAS [22] system (bristle-specific GAL4/UAS:Mical) results in F-actin disassembly and bristle branching (compare **Figure 1a and b**) that is dependent on Mical's Redox activity and the Met-44 residue of actin [4,5]. Thus, to better characterize Mical-mediated F-actin alterations, we have initiated a large-scale genetic screen to look for enhancers and suppressors of Mical-mediated bristle branching. One of the mutations that we identified in our genetic screen, the transposable element mutation *EY22443*, strongly suppressed Mical-induced actin-dependent bristle branching (**Figure 1b-d**). Molecular analysis revealed that the *EY22443* transposable element mutation was situated within the Drosophila *SelR* gene (**Figure 1e**). *SelR* codes for a methionine sulfoxide reductase (MsrB) family enzyme, that has been characterized for its ability to reduce oxidized methionine residues [23]. In light of our observations that Mical oxidizes methionine residues on actin [4], we wondered if SelR might play a role in modulating Mical's effects on actin.

The *EY22443* transposable element mutation situated in *SelR* contains a UAS promoter (**Figure 1e**), thereby suggesting that this mutation might be abnormally inducing SelR expression to suppress GAL4/UAS:Mical-dependent bristle branching. To test this

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hypothesis, we generated transgenic flies expressing SelR directly under the UAS promoter. Consistent with our results with *EY22443* (**Figure 1c-d**) and another UAS-containing mutation within SelR, *EP3340* (**Figure 1d**), multiple transgenic lines revealed that raising the levels of SelR specifically in bristles strongly suppressed Mical-induced bristle branching and even generated normal appearing bristles (**Figure 1f**). Moreover, elevating the levels of *SelR* in a wild-type background generated abnormally bent bristles that resembled Mical$^{-/-}$ mutant bristles (**Figure S1**; [5]); and these effects of SelR were genetically enhanced by decreasing the levels of *Mical* (**Figure S1**). Further analysis revealed that SelR localized with Mical at the tips of bristles and suppressed Mical-mediated F-actin disassembly and reorganization (**Figure 1f**). Therefore, SelR counteracts the effects of Mical on actin reorganization in vivo.

SelR Restores the Polymerization of Mical-treated Actin

To better understand the role of SelR in counteracting Mical-mediated actin reorganization, we purified recombinant *Drosophila* SelR protein (**Figure S2**). Using in vitro actin biochemical and imaging assays, we previously observed that purified Mical protein in the presence of its coenzyme NADPH disrupts actin polymerization and induces F-actin disassembly (**Figure 2a**; [4,5]). Strikingly, we found that purified SelR protein rescued the ability of Mical-treated actin to polymerize (**Figure 2a**). This Mical/SelR-treated actin re-polymerized to an extent that was indistinguishable from normal untreated actin (**Figure 2b**). Moreover, while Mical-treated actin failed to polymerize even after removal of Mical and NADPH (**Figure 2c**; [4]), SelR induced the polymerization of this purified Mical-treated actin in a dosage-dependent manner (**Figure 2c**). Thus, SelR restores the polymerization properties of Mical-treated actin.

SelR converts methionine sulfoxide (MetO) to methionine [23,24], requiring a redox active cysteine (Cys_{124}) residue (**Figure 2d-e**; [25]) and also utilizing reducing agents to cycle back to its reduced form (**Figures 2d, S3**; [24,25]). In some cases methionine oxidation is also reversed by general reducing agents [26], so we wondered if Mical-treated actin was specifically reversed by SelR. In contrast to SelR, neither chemical reducing agents such as DTT (**Figures 2a** [buffer only contains DTT]; **S3**) nor other reducing enzymes including thioredoxins/thioredoxin reductases altered Mical-mediated effects on actin in vitro (**Figure S3)** or in vivo (**Figure 1d**). Furthermore, SelR did not restore the normal polymerization properties of other oxidized forms of actin (e.g., H_2O_2-treated actin; **Figure S3**), indicating that SelR selectively affects Mical- modified actin. Mutating SelR's critical catalytic cysteine (Cys_{124}) to generate an enzymatically dead SelR (SelR^{C124S}; **Figure 2e**; [25]), abolished SelR's effects on Mical-treated actin in vitro (**Figures 2b, f**) and in vivo (**Figure 2g**). Moreover, consistent with such a role for SelR's reductase activity in counteracting Mical's oxidative effects on actin, elevating the levels of wild-type SelR not only phenocopied the in vivo effects of disrupting Mical's monooxygenase (Redox) domain (**Figures S1, S4**), but it also rescued the severe bristle/F-actin alterations that result from hyperactive Mical Redox signaling (**Figure S4**; Mical^{redoxCH}; [5]). Thus, SelR specifically employs its catalytic activity to restore Mical-treated actin polymerization and counteract the in vivo effects of Mical.

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SelR Reverses Mical-mediated Actin^{Met-44} Oxidation

In many organisms, including Drosophila and mammals, two main types of methionine sulfoxide reductases have been identified: SelR (MsrB family proteins) and Drosophila Eip71CD (MsrA) (**Figure 3a**; [27]). Interestingly, SelR and MsrA/Eip71CD are both methionine sulfoxide reductases, but they do not exhibit similarity in their sequence, domain organization, or substrate specificity (**Figure 3a-b;** [27]). In particular, methionine has a unique oxidation pattern in that two stereoisomers can be produced by oxidation [27]. SelR/MsrB family proteins catalyze the reduction of the *R*-isomer of methionine sulfoxide (methionine-*R*-sulfoxide; **Figure 3b, top**) to methionine, while MsrA/Eip71CD catalyzes the reduction of the *S*-isomer of methionine sulfoxide (methionine-*S*-sulfoxide; **Figure 3b, bottom**) to methionine [23,25,27]. Therefore, to further test the specificity of SelR in restoring the polymerization properties of Mical-treated actin, we purified recombinant MsrA/Eip71CD protein (**Figure S2**; [25]). Unlike SelR, MsrA/Eip71CD did not restore the polymerization properties of Mical-treated actin in vitro (**Figure 3c**), nor did it counteract Mical-mediated actin reorganization/bristle branching in vivo (**Figure 1d**). These results further reveal that Mical-treated actin polymerization is specifically restored by SelR. Moreover, in light of the isomer-specific nature of the methionine sulfoxide enzymes SelR and MsrA/Eip71CD, these results also indicate that Mical oxidizes actin in a stereo-specific manner.

Mical oxidizes actin on its Met-44 and Met-47 residues, although it is the oxidation of the Met-44 residue through which Mical induces F-actin disassembly [4]. Thus, we wondered if SelR directly reverses Mical-mediated oxidation of actin. Previously, we determined the conditions to purify Mical-treated actin, which is polymerization impaired and exhibits a mass increase of two oxygens (32 Daltons) [4]. SelR, but not the enzymatically dead SelR^{C124S} protein, restored the polymerization properties of purified Mical-treated actin (**Figure S3**), an effect that was maintained even after removal of SelR (**Figure 3d**). Subjecting both purified Mical/SelR-treated and Mical/SelR^{C124S}-treated actin to mass spectrometry revealed that SelR, but not the enzymatically dead SelR^{C124S} protein, eliminated the Mical-catalyzed two oxygen (32 Dalton) mass increase on actin (**Figure 3e**).

Mical's ability to effect actin in vitro and in vivo is dependent on the presence of the Methionine (M) 44 residue of actin [4]. To further examine a physiological role for SelR in reducing Mical-mediated oxidation of Met-44, we turned to in vivo assays. We first noted that overexpression of either a non-Mical oxidizable Met44Leu (M44L) version of actin [4] or wild-type SelR generated the same effects: suppression of Mical-mediated actin/bristle morphology and *Mical* loss-of-function-like defects (**Figures 1f, 2g, S1, S4**; [4]). Furthermore, we found that actin^{M44L} worked in combination with SelR to generate *Mical* loss-of-function-like bristle defects (**Figure S4**). Moreover, actin^{M44L} prevented the enhanced Mical-mediated bristle branching/actin reorganization that occured with expression of the reductase dead SelR^{C124S} (**Figure 2g**). Thus, SelR reverses Mical-mediated oxidation of actin, including using its catalytic activity to directly reduce Mical-induced MetO-44 actin to Met-44 actin (**Figure 3f**) – and these observations with purified proteins are supported by our in vivo genetic assays.

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The Mical/SelR System Regulates Actin Organization in Multiple Cell Types

In addition to bristle cells, Mical regulates the organization of actin in multiple other cell types including mammalian cells in vitro and muscles in vivo [4-6,10,11]. Thus, we wondered if SelR could also counteract the effects of Mical on actin in these other cellular systems. Our initial examination revealed that as in bristle cells, SelR rescued Mical-dependent changes in morphology and actin organization in cultured cells (**Figure 4a**). Further examination revealed that overexpression of SelR in muscles in vivo phenocopied the muscle actin defects found in *Mical*$^{-/-}$ mutants (**Figure 4b**; [10]). Moreover, SelR could even rescue the lethality and changes in actin organization associated with overexpression of Mical in muscles (**Figure 4c) –** as well as the lethality that results when Mical is broadly expressed using an actin promoter (**Figure 4c**).

Drosophila SelR, like Mical, is broadly expressed (**Figure S5**; [5,10,13,25,28-31]) and thus to better examine these Mical-SelR interactions and their physiological effects on actin, we characterized *SelR*$^{-/-}$ mutants (**Figure S6**). Strikingly, loss of *SelR* generated bristle and muscle defects that resembled overexpression of Mical (**Figure 5a and 5e**). Moreover, loss of *SelR* specifically enhanced Mical-mediated effects on actin organization/bristle morphology (**Figures 5b-c, S4**) and phenocopied overexpression of the SelR^{C124S} reductase mutant protein (**Figure 5d**). Thus, SelR, like Mical, plays both important and selective roles in regulating actin organization in vivo in different cell types. Likewise, an equilibrium between Mical and SelR activities underlies normal actin-directed cell biology.

SelR Neutralizes Semaphorin/Plexin/Mical Repulsive Signaling

Besides its Redox region that Mical uses to oxidize actin, Mical has several other domains and protein interaction motifs including a region that interacts with the cytoplasmic portion of Plexin (**Figure 6a**; [13,17]). Plexins are receptors for Semaphorin guidance cues and play critical roles in regulating multiple actin-dependent events in vivo [6,32,33]. Semaphorins/ Plexins signal through Mical to induce changes in bristle morphology and F-actin disassembly [5], so we wondered if SelR also counteracted the effects of Semaphorin/Plexin/ Mical signaling. Employing loss and gain-of-function genetics in the bristle system, we found that similar to our results with Mical, SelR counteracted Semaphorin/Plexin effects on actin-dependent bristle morphology (**Figure S1**). Next, we turned to in vivo axon guidance assays, where Semaphorins/Plexins have been characterized as repulsive axon guidance molecules [15] and were first linked to MICAL family proteins [13]. Interestingly, one of the *SelR* mutants that we found in our screen (*EP3340*, **Figure 1d-e**) recently emerged from a genetic screen as an uncharacterized regulator of axon guidance [34]. Employing our SelR transgenic lines, we found that overexpression of SelR generated axon guidance and synaptogenic defects that phenocopy *Mical*$^{-/-}$ mutants (**Figures 6b, S6**; [5,10,13]). Furthermore, *SelR*$^{-/-}$ mutants generated axon guidance defects that phenocopy increased Semaphorin/Plexin/Mical-mediated repulsive axon guidance (**Figures 6c, S6**; [5,35-37]). Moreover, increasing the levels of SelR rescued these Semaphorin/Plexin/Mical-triggered repulsive axon guidance defects (**Figures 6d, S6**). Thus, SelR also plays critical roles in axon guidance and synaptogenesis and counteracts the effects of Semaphorin/Plexin/Mical repulsive signaling in vivo.

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DISCUSSION

Our results reveal that Mical-mediated actin alterations – a selective means to post-translationally regulate F-actin dynamics and cellular behaviors – are reversible. This Micalcatalyzed reaction is directly reversed by a specific methionine sulfoxide reductase enzyme, SelR/MsrB, which we also find selectively controls actin-dependent cellular events in vivo and regulates specific neuronal, muscular, and mechanosensory developmental processes. We also find that SelR counteracts Semaphorins, which are one of the largest families of extracellular guidance cues and play a critical role in the formation and function of multiple tissues [6,32]. Thus, our results demonstrate an important role for these methionine sulfoxide reductases – enzymes thought to function primarily in the repair of oxidatively "damaged" methionine residues [24,38] – in modulating normal signaling events. Moreover, our genetic data, which reveals that *SelR* and *Mical* loss and gain-of-function phenotypes are opposite in appearance, indicate that SelR has a specific, primary, and regulated role in counteracting Mical during development.

The Mical substrate Met-44 residue of actin is conserved in all actin family members from yeast to humans [4] and a dominant (hetereozygous) mutation in the Met-44 residue (M44T) of skeletal muscle actin underlies a human musculoskeletal disease associated with actin accumulation and aggregation (nemaline myopathy [39]). This Met-44 mutant version of human skeletal muscle actin would be predicted to prevent Mical from having effects on skeletal muscle actin – and generally phenocopies both $Mical^{-/-}$ mutants and SelR muscle overexpression. However, the Met-44 residue is well-conserved and is at a subunit interface in filaments [4,40,41] and thus mutating it may influence F-actin organization for reasons other than that it is non oxidizable. It should be noted however, that our previous results indicate that Met-44 mutant actin (M44L) appears to polymerize normally in vitro and in vivo, but is resistant to Mical-mediated F-actin disassembly [4].

It is also interesting to note the differences in the cellular localization we see between SelR and different forms of Mical. For example, in bristles, SelR shows overlapping localization with Mical, but is more broadly distributed than full-length Mical, which strongly localizes to bristle tips (**Figure 1f**; [5]). The broader cellular localization of SelR is similar to that seen when the hyperactive $Mical^{redoxCH}$ is expressed in bristles and other cells (**Figure 4a**; [5]). One of the differences between full-length Mical and the hyperactive $Mical^{redoxCH}$ is the presence of the Plexin-interacting region (**Figure 6a**; [13]). Our results indicate that full-length Mical is susceptible to regulation by Plexin, whereas the $Mical^{redoxCH}$ protein (which does not have the Plexin-interacting region) is not regulated by Plexin [5] (see also [11,17]). Interestingly, the *MICALs* express multiple different transcripts, including versions that may be similar to $Mical^{redoxCH}$ [6]. Thus, there may be roles for both endogenous Sema/Plexin-regulated and perhaps, non Sema/Plexin-regulated forms of Mical (which appear to be more generally localized in cells). In any case, it should be noted that we find that SelR rescues both the lethality and F-actin defects associated with overexpression of either full-length Mical or $Mical^{redoxCH}$. Likewise, we find that SelR counteracts Semaphorin/Plexin effects in vivo.

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Our results herein, coupled with our previous observations [4], also indicate that unlike diffusible oxidants that induce random protein modifications [24,38,42], Mical-mediated oxidation is substrate, residue, and stereo-specific. Our results indicate that Mical oxidizes the methioinine-44 residue of actin stereospecifically to generate actin methionine-44-*R*-sulfoxide (actin$^{Met(R)O\text{-}44}$) to alter F-actin dynamics. These observations contend that the enzyme-driven interconversion of specific Met/Met(*R*)O residues, similar to the reversible phosphorylation of specific serine, threonine, and tyrosine residues [43], provides a selective means to precisely modulate protein function. Moreover, in contrast to a view that oxidation simply plays a destructive role in cell health and protein function, our results indicate that the site specific and reversible oxidation of proteins is critical for proper cellular physiology. Thus, together, our results uncover a specific reversible Redox cellular signaling system that dynamically regulates multiple actin cytoskeletal-mediated events and controls Semaphorin/Plexin repulsion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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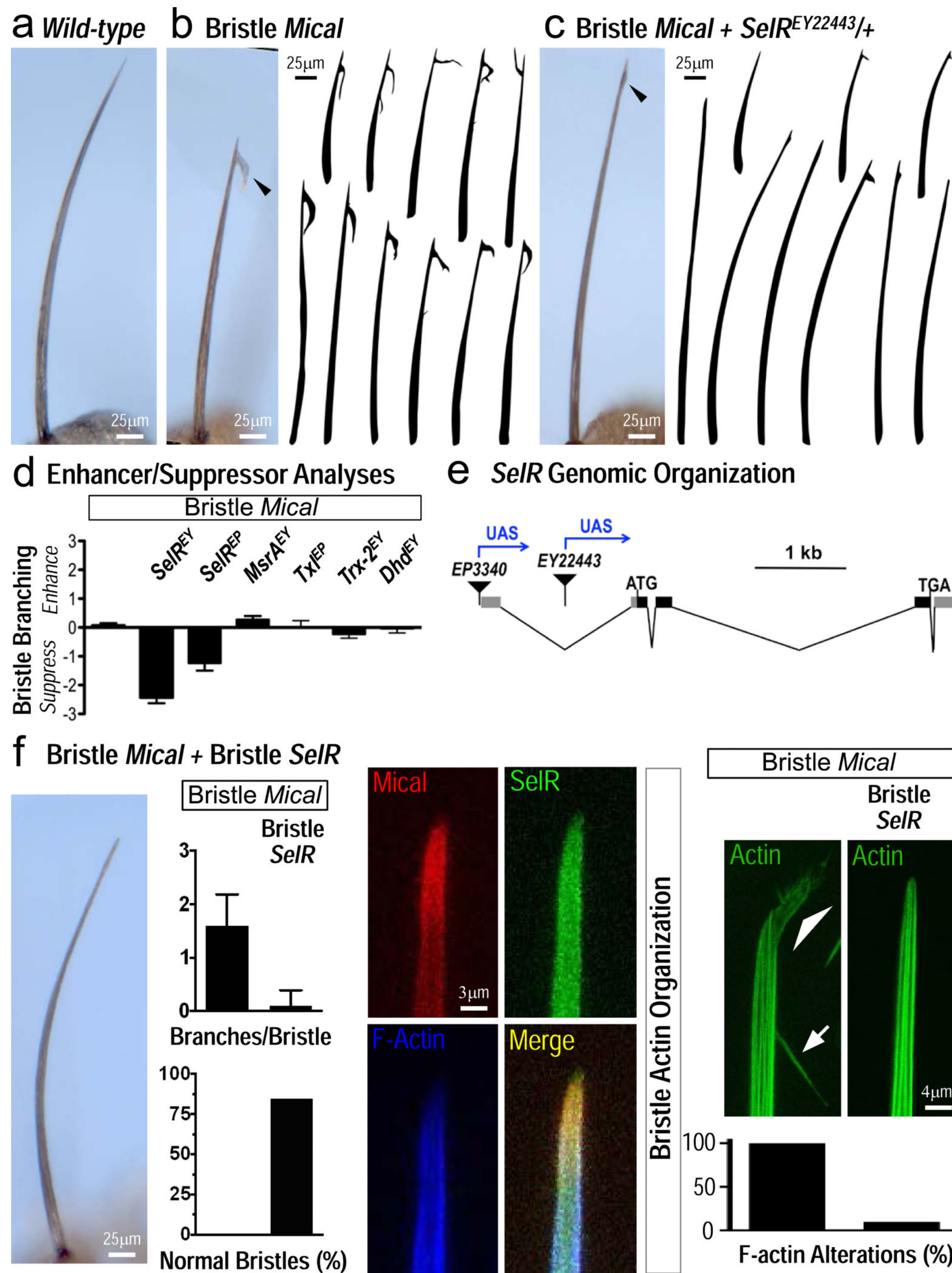

Figure 1. SelR counteracts Mical-mediated actin-dependent changes in vivo

a-b, *Wild-type* Drosophila bristles are unbranched (**a**) but become branched (**b**; arrowhead) when Mical is overexpressed specifically within them using the GAL4-UAS system (*B11-GAL4/+; UAS:Mical / +)*. **c-d**, A dominant genetic screen identifies that SelR (*SelR^{EY} [EY22443])* and *SelR^{EP} [EP3340]),* but not other specific reductase enzymes, strongly decrease/suppress Mical-induced bristle branching. Txl (thioredoxin-like), Trx-2 (thioredoxin-2), Dhd (deadhead/thioredoxin-like). All genotypes are heterozygous (*B11-GAL4, UAS:Mical* /+ and *mutations*/+). *n*=20 animals per genotype. Mean + standard error

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of the mean (SEM). Replicated in at least 2 independent experiments (separate crosses) per genotype. **e**, Both the *EY22443* and *EP3340* mutations contain *UAS* sequences that are directed towards *SelR*. **f**, Bristle specific expression of SelR localizes with Mical (middle, ^{GFP}SelR and ^{mCherry}Mical) and F-actin (phalloidin), suppresses Mical-induced bristle branching (left and upper graph; *n*=40 bristles assessed in 10 animals per genotype; Mean + standard error of the mean [SEM]), and generates wild-type appearing bristles (left and lower graph; *n*=40 bristles assessed in 10 animals per genotype). Likewise, the F-actin alterations (right), including areas of decreased F-actin (arrowhead) and actin-rich branches (arrow) that occur upon bristle specific expression of Mical [5], are suppressed by co-expression of SelR. *n*=30 bristles assessed in 8 animals per genotype. All quantitative data in f was replicated in at least 2 independent experiments (separate crosses) per genotype.

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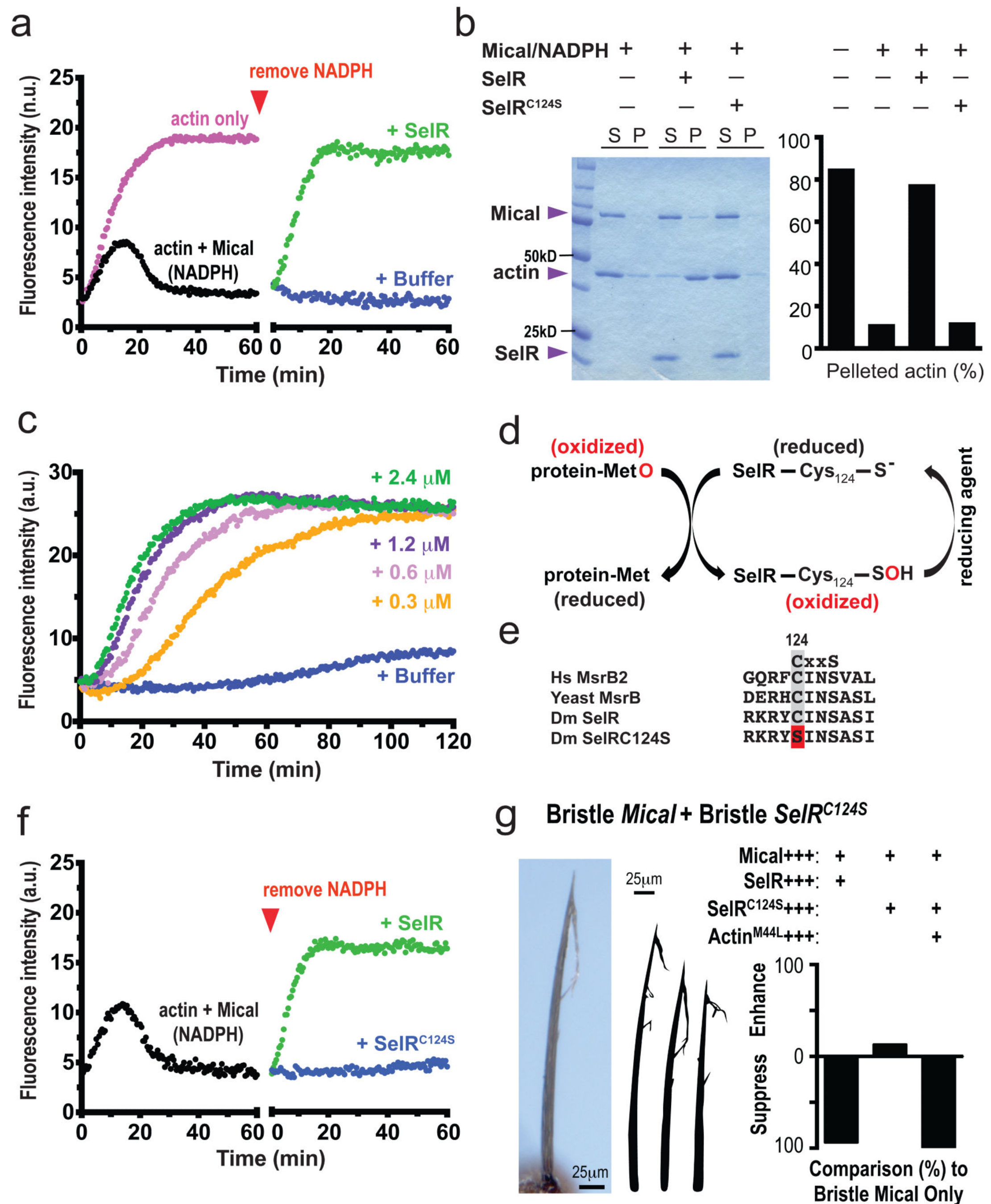

Figure 2. SelR restores the polymerization properties of Mical-treated actin

a, Pyrene-actin assays, where the fluorescence is higher in the polymerized state, reveal that SelR (green dots) restores the polymerization of Mical-treated (600nM Mical, 100 μM NADPH; [4,5]) actin (1.15 μM actin), while buffer only (blue dots, containing 20 mM of DTT) does not. n.u. (normalized units between the 2 graphs). **b**, Mical-treated actin polymerizes to a normal extent following addition of SelR, but not the enzymatically inactive SelR^{C124S}. Sedimentation assay and Coomassie-stained gel. Actin monomers/G-actin is in the supernatant (S); actin polymers/F-actin is in the pellet (P). Right, quantification of pelleted

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actin from *n*=2 separate experiments per condition. See also Figure S3e for the uncropped gel. **c**, SelR (0.3 – 2.4 μM) restores the polymerization of purified Mical-treated actin in a concentration dependent manner. a.u. (arbitrary units). **d**, SelR/MsrB family proteins use the conserved Cysteine(Cys)$_{124}$ residue to reduce MetO to Met [23,25]. **e**, Catalytically inactive Cys (C) to serine (S) mutation (SelR^{C124S}; [25]). Hs, human; Dm, *Drosophila*. **f-g**, Unlike wild-type SelR, SelR^{C124S} does not restore Mical-treated actin polymerization in vitro (**f**) or suppress Mical-induced actin reorganization/bristle branching (**g**). **g,** Note that in contrast to bristle overexpression of SelR (SelR+++), which suppresses bristle branching due to bristle overexpression of Mical (Mical+++), bristle-specific expression of SelR^{C124S} (SelR^{C124S}++ +) enhances Mical-dependent bristle branching (increasing both the number and length of branches). Mutating Mical's substrate residue on actin, the Met-44 residue, and expressing this mutant actin in bristles (Actin^{M44L}+++), suppresses the effects of SelR^{C124S} on Mical. *n*=40 bristles assessed in 10 animals per genotype. Replicated in at least 2 independent experiments (separate crosses) per genotype.

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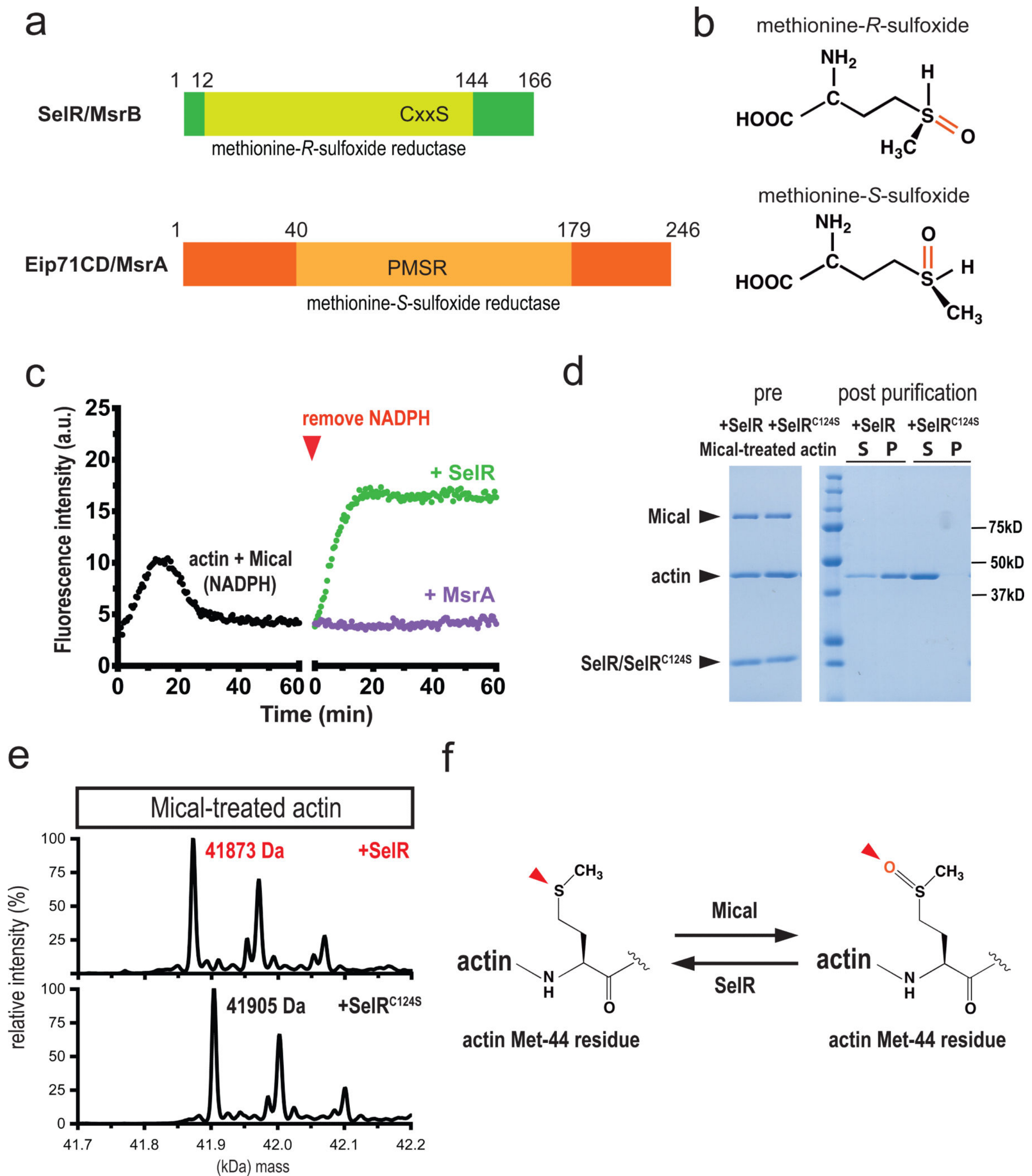

Figure 3. SelR/MsrB reverses Mical-mediated actin^{Met-44} oxidation

a, SelR/MsrB and Eip71CD/MsrA family proteins including catalytically active cysteine (CxxS) and PMSR (peptide methionine sulfoxide reductase) motifs. **b**, Methionine-*R*-sulfoxide (top) and methionine-*S*-sulfoxide (bottom) are reduced by SelR/MsrB family proteins and Eip71CD/MsrA, respectively. **c**, MsrA (purple dots) does not restore polymerization of Mical-treated actin (pyrene-actin assay). **d**, Mical-oxidized actin was treated with SelR or SelR^{C124S} (left) and then purified to reveal that SelR-treated, but not SelR^{C124S}-treated, Mical-oxidized actin polymerizes (right; Pellet [P]). Coomassie-stained

gel. See also Figure S3f for the uncropped gel. **e**, Mass spectrometry of Mical/SelR-treated purified actin reveals that SelR, but not SelR^{C124S}, reverses the Mical-catalyzed 32 Dalton (two oxygen) increase [4] in the mass of actin. Note that the different peaks are different modified versions of actins that have been purified from rabbit. **f**, Mical oxidizes the Met-44 residue of actin and SelR reverses this Mical-catalyzed Met-44 oxidation (compare arrowheads).

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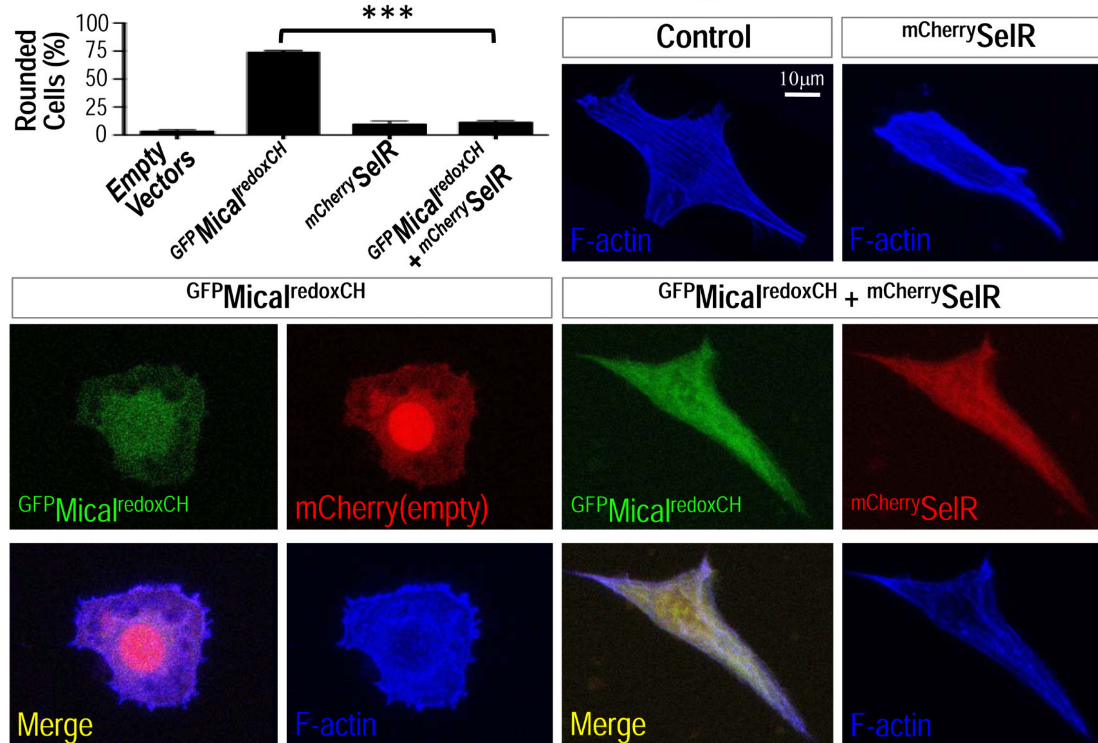

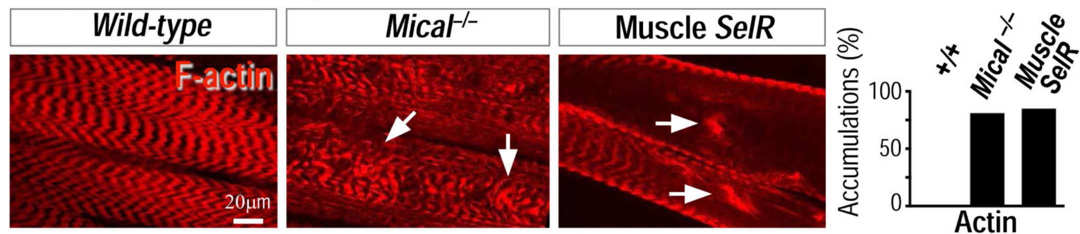

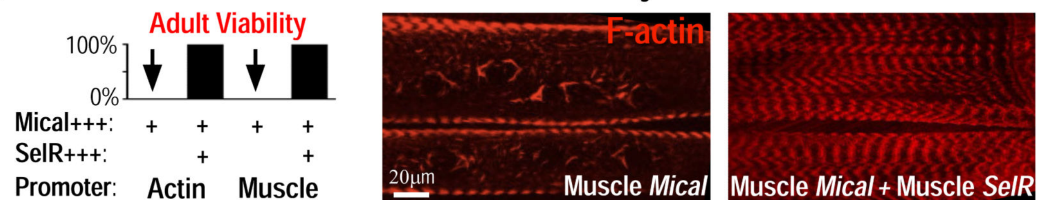

Figure 4. SelR opposes Mical-mediated effects in different cell types

a, Expression of Mical (^{GFP}Mical^{redoxCH}) in 3T3 cells results in a loss of F-actin stress fibers [4] and generates an abnormal rounded cell morphology. SelR localizes together with Mical when it is co-expressed with Mical (^{GFP}Mical^{RedoxCH} + ^{mCherry}SelR) and significantly rescues this Mical-mediated rounded cell morphology. Note also the localization of ^{GFP}Mical^{RedoxCH} and ^{mCherry}SelR with F-actin. ***$P<0.0001$; one way ANOVA with multiple comparison correction; $n=79$ cells assessed from 2 independent experiments including a total of 4 different transfected plates per condition. Scale bar

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applies to each image. **b,** Expression of SelR specifically in muscles generates actin accumulation defects that resemble *Mical*$^{-/-}$ mutant muscles [10]. The percentage (%) of muscles exhibiting abnormal accumulations of actin is shown (*n*=24 muscles assessed in 9 animals per genotype). Replicated in at least 2 independent experiments (separate crosses) per genotype. *Mical*$^{-/-}$=*Mical*^{G56}/*Mical*^{I666}. The scale bar applies to each image. **c,** Mical overexpression (Mical+++) using either an actin promoter (*Actin5C-GAL4*) or a muscle-specific promoter (*24B-GAL4*) is lethal (graph). SelR (SelR+++) co-expression completely rescues this Mical-induced lethality (*n*=100 animals examined per cross) and also rescues the changes in actin organization that result from Mical overexpression in muscles (*n*=24 muscles assessed in 9 animals per genotype). Both experiments were replicated in at least 2 independent experiments (separate crosses) per genotype. The scale bar applies to both images.

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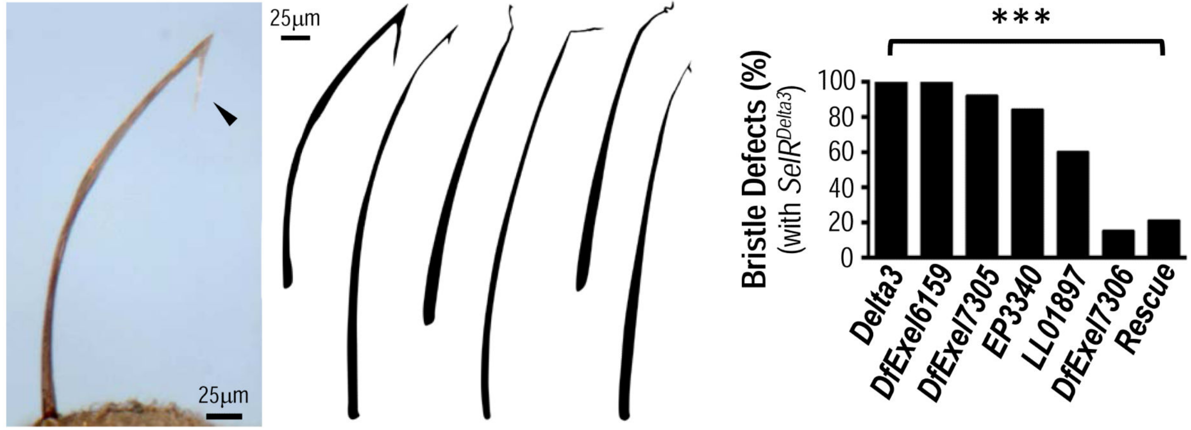

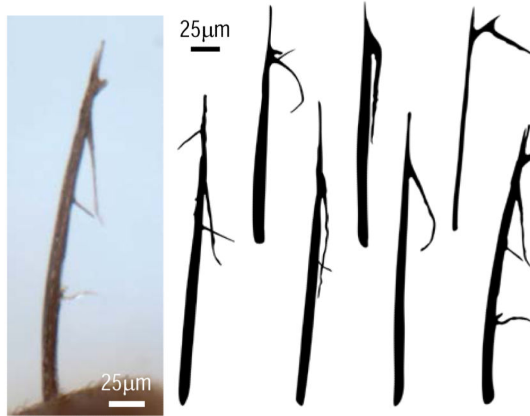

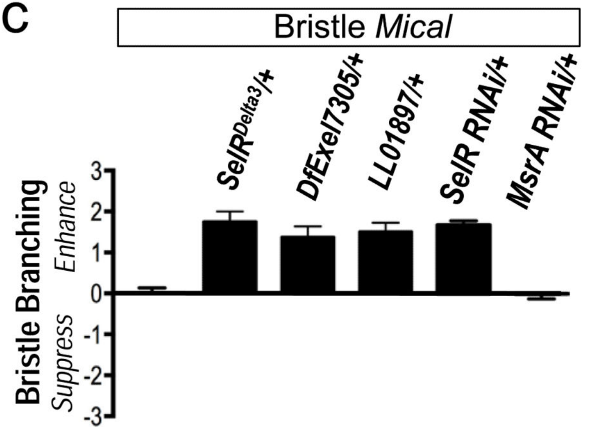

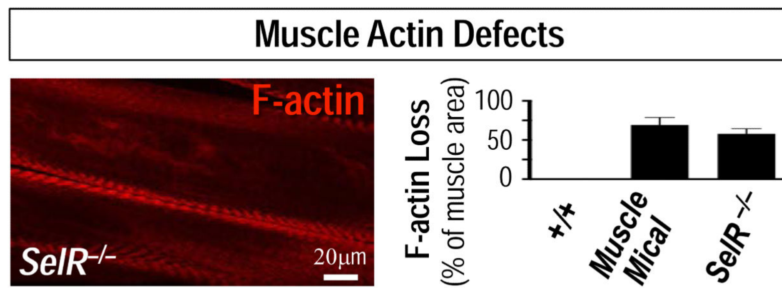

Figure 5. SelR is required in vivo for normal actin organization and cellular morphology

a, *SelR* loss-of-function (*SelR*$^{-/-}$) mutants exhibit bristle defects that phenocopy Mical overexpression bristles, with branches emerging from the bristle tip (arrowhead). All combinations of these *SelR*$^{-/-}$ mutant alleles and deficiencies removing *SelR* generate bristle defects. *DfExel7306* does not remove *SelR*. The bristle defects present in SelR$^{-/-}$ mutants (*SelR*^{Delta3}/*SelR*^{Delta3}) are significantly rescued by bristle expression of *SelR* (*Rescue* genotype = *UAS:SelR/+; SelR*^{Delta3}/*SelR*^{Delta3}, *B11-GAL4*). Chi-Square Test; ***P<0.0001; n=20 animals per genotype. Replicated in at least 2 independent experiments

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(separate crosses) per genotype. **b-c**, Multiple different heterozygous loss-of-function or RNAi mutations of *SelR* (*SelR* Mutant/+), but not *MsrA*, enhance Mical-dependent actin reorganization/bristle branching. *n*=40 bristles assessed in 10 animals per genotype. Mean + standard error of the mean (SEM). Replicated in at least 2 independent experiments (separate crosses) per genotype. **d**, Expressing the catalytically dead $SelR^{C124S}$ specifically in bristles in a wild-type background generates bristle branches (arrowhead) that phenocopy both $SelR^{-/-}$ mutant and Mical overexpression bristles. **e**, $SelR^{-/-}$mutants exhibit muscle actin defects that phenocopy Mical overexpression muscles with a paucity of F-actin. *n*=20 muscles assessed in 7 animals per genotype. Mean + standard error of the mean (SEM). Replicated in at least 2 independent experiments (separate crosses) per genotype. Image of $SelR^{-/-}$ from $SelR^{Delta3}/SelR^{Delta3}$.

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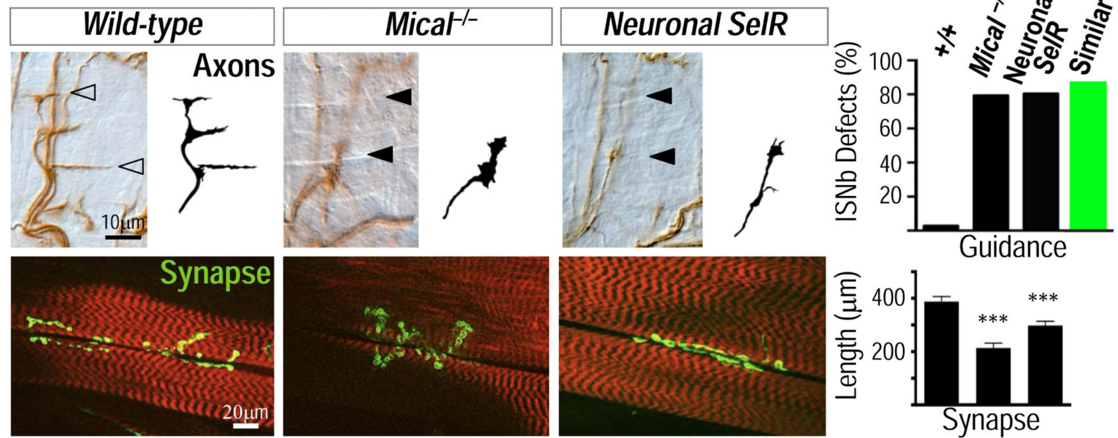

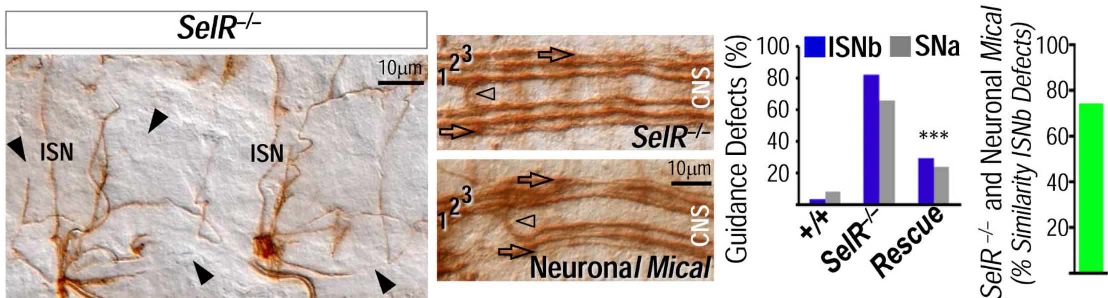

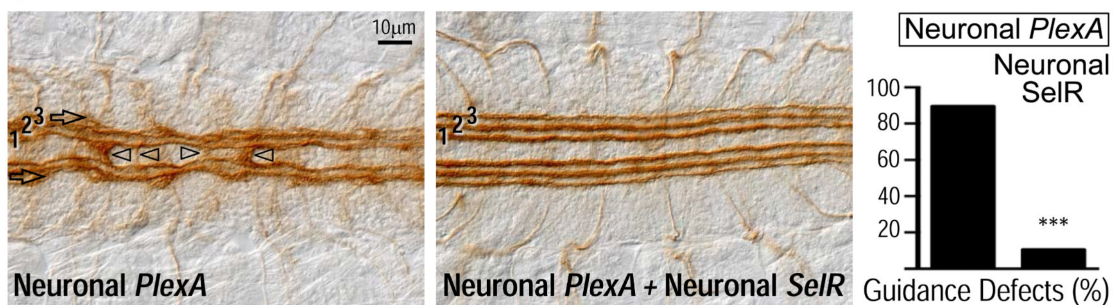

Figure 6. SelR counteracts Semaphorin/Plexin/Mical repulsive signaling

a, Drosophila and mammalian MICAL proteins are characterized by multiple domains including their actin regulatory Redox domain and Plexin receptor interacting C terminus (Plexin IR). CH, Calponin homology domain; LIM, LIM domain; PxxPs, proline (P)-rich motifs. **b**, **Top Row**: Neuronal overexpression of *SelR* generates *Semaphorin-1a*$^{-/-}$ [44], *PlexinA*$^{-/-}$ [35], and *Mical*$^{-/-}$–like intersegmental nerve b (ISNb) axon guidance defects that are characterized by decreased axonal defasciculation/repulsion and a failure of axons to reach their correct targets (closed arrowheads). Wild-type innervation (open arrowheads).

Similar = percent of the neuronal *SelR* ISNb guidance defects that resemble $Mical^{-/-}$ mutants ($Mical^{K1496}/Mical^{Df(3R)swp2}$). *n*=100 hemisegments assessed in 10 animals per genotype. **b**, **Bottom Row:** Neuronal overexpression of SelR generates $Mical^{-/-}$– like synaptogenesis defects, with a decreased length of synaptic innervation. One Way ANOVA with Correction for Multiple Comparisons; ****P*<0.0001; Mean + standard error of the mean [SEM]; *n*=20 synapses assessed in 7 animals per genotype. $Mical^{-/-}=Mical^{G56}/Mical^{I666}$. **c**, $SelR^{-/-}$($SelR^{Delta3}/DfExel7305$) mutants exhibit ISNb and segmental nerve a (SNa) axon guidance defects that are significantly rescued by neuronal (*ELAV-GAL4*) expression of SelR. Both $SelR^{-/-}$ ISNb (green bar) and CNS (see image) axon guidance defects resemble the increased axonal defasciculation/repulsion seen with neuronal Mical overexpression. Note motor axons projecting into abnormal areas (closed arrowheads), discontinuous/thin/missing CNS longitudinal connectives (arrows), and CNS axons abnormally crossing the midline (open arrowheads). See Figure S6d for the ISNb guidance defects from this Neuronal *Mical* image. Chi-Square Test; ****P*<0.0001; *n*=94 hemisegments assessed in 10 animals per genotype. The intersegmental nerve (ISN) and CNS longitudinal connectives (1, 2, and 3) are labeled for reference. **d,** The repulsive guidance defects seen when Plexin A (PlexA) is overexpressed in neurons (Neuronal *PlexA*; [35-37,45]) resembles the CNS axon guidance defects that are present in $SelR^{-/-}$ mutants. Note the discontinuous/thin/missing CNS longitudinal connectives (arrows) and CNS axons abnormally crossing the midline (open arrowheads). These PlexA axon guidance defects are dependent on both Semaphorin-1a and Mical [37] and raising the levels of SelR in neurons (Neuronal *PlexA* + Neuronal *SelR*) significantly rescues these Semaphorin-1a/PlexA/Mical-dependent axon guidance defects. Chi-Square Test; ****P*<0.0001; *n*=47 animals per genotype.

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