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Combinatorial ShcA Docking Interactions Support Diversity in Tissue Morphogenesis

W. Rod Hardy^{1,2}, Lingying Li³, Zhi Wang³, Jiri Sedy⁴, James Fawcett¹, Eric Frank³, Jan Kucera^{5,6,*}, and Tony Pawson^{1,2,*}

¹Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada.

²Department of Molecular and Medical Genetics, 4388 Medical Sciences Building, 1 King's College Circle, University of Toronto, Ontario M5S 1A8, Canada.

³Department of Physiology, Tufts University School of Medicine, Boston, MA 02111, USA.

⁴Institute of Anatomy, Charles University, First Faculty of Medicine, Prague 12000, Czech Republic.

⁵Department of Neurology, Boston University School of Medicine, Boston, MA 02118, USA.

⁶Veterans Administration Medical Center, Boston, MA 02130, USA.

Abstract

Changes in protein-protein interactions may allow polypeptides to perform unexpected regulatory functions. Mammalian ShcA docking proteins have amino-terminal phosphotyrosine (pTyr) binding (PTB) and carboxyl-terminal Src homology 2 (SH2) domains, which recognize specific pTyr sites on activated receptors, and a central region with two phosphorylated tyrosine-X-asparagine (pYXN) motifs (where X represents any amino acid) that each bind the growth factor receptor-bound protein 2 (Grb2) adaptor. Phylogenetic analysis indicates that ShcA may signal through both pYXN-dependent and -independent pathways. We show that, in mice, cardiomyocyte-expressed ShcA directs mid-gestational heart development by a PTB-dependent mechanism that does not require the pYXN motifs. In contrast, the pYXN motifs are required with PTB and SH2 domains in the same ShcA molecule for the formation of muscle spindles, skeletal muscle sensory organs that regulate motor behavior. Thus, combinatorial differences in ShcA docking interactions may yield multiple signaling mechanisms to support diversity in tissue morphogenesis.

Docking proteins with multiple interaction domains and motifs act downstream of receptor tyrosine kinases (RTKs) and other cell-surface receptors (1,2). Although discrete protein-protein interactions are known to exert specific biological effects in RTK signaling (3,4), there is little information as to the combinatorial effects of these docking interactions in vivo. We have used the 46- and 52-kD protein products of the mouse *ShcA* gene (5) (p46^{ShcA} and p52^{ShcA}, collectively ShcA) as a model to investigate this issue.

*To whom correspondence should be addressed. E-mail: pawson@mshri.on.ca (T.P.); jfkucera@bu.edu (J.K.)

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The PTB and SH2 domains of ShcA bind phosphorylated motifs with the consensus sequences Φ -X-Asn-Pro-X-pTyr (Φ -X-N-P-X-pY) and pTyr- Φ -X-Ile/Leu (pY- Φ -X-I/L) (6), respectively (where Φ represents any large hydrophobic amino acid), on various cell-surface receptors (7). The collagen homologous 1 (CH1) region contains two YXN motifs (Y²³⁹Y²⁴⁰N and Y³¹³VN of mouse p52^{ShcA}) (7). Upon phosphorylation, these motifs can each bind the SH2 domain of the adaptor Grb2, which recruits activators of the Ras and phosphatidylinositol 3-kinase signaling pathways through its SH3 domains (7-10). Although Shc proteins in metazoans are defined by their PTB-CH1-SH2 structure, the pYXN motifs are absent in some invertebrates (fig. S1), and additional CH1 pTyr motifs are present in certain vertebrate family members (11) (fig. S2). This suggests that ShcA and its homologs have alternative pYXN-dependent and -independent signaling functions, which are variably used in evolution (12).

Mutant mice selectively missing a third ShcA isoform, p66^{ShcA}, are long-lived (13), whereas mutants lacking all three ShcA proteins die around E11.5 (embryonic day 11.5) with cardiovascular defects (14). To explore the biological roles of the various pTyr-dependent interactions of ShcA, we generated mice with targeted *ShcA* knock-in (KI) alleles expressing either wild-type (WT) ShcA proteins with a single Flag (1×Flag) epitope tag (*ShcA*^{KI-WT} allele) or ShcA-1×Flag variants with substitutions that (i) disrupt pTyr binding by the PTB domain [*ShcA*^{δPTB} allele, Arg¹⁷⁵→Gln¹⁷⁵ (R175Q) substitution] or by the SH2 domain (*ShcA*^{δSH2}, R397K mutation) or (ii) alter the CH1 pTyr sites to phenylalanine, either separately (*ShcA*^{2F} allele, Y239/240F substitutions; *ShcA*^{313F} allele, Y313F substitution) or in combination (*ShcA*^{3F} allele, Y239/240/313F substitutions) (Fig. 1, A to C, and fig. S3). Mutant embryos expressed similar levels of ShcA-1×Flag proteins that were slightly reduced as compared with endogenous ShcA in WT (*ShcA*^{+/+}) littermates (fig. S4). Heterozygotes all appeared normal and were fertile.

From intercrosses, *ShcA*^{KI-WT/KI-WT} mutants were recovered at the expected Mendelian frequency of ~25% (24.6%; 58 *ShcA*^{KI-WT/KI-WT} mice out of 236 mice in the total progeny), as were *ShcA*^{2F/2F} mice (24.4%; 60 out of 245). In contrast, the numbers of *ShcA*^{δSH2/δSH2}, *ShcA*^{3F/3F}, and *ShcA*^{313F/313F} mutant animals were reduced, representing 7.9% (30 out of 380), 12.2% (57 out of 466), and 17.5% (44 out of 251) of viable progeny, respectively ($P < 0.05$, chi-square test). No viable *ShcA*^{δPTB/δPTB} mice were identified (0 out of 155). Whereas viable *ShcA*^{KI-WT/KI-WT}, *ShcA*^{2F/2F}, and *ShcA*^{313F/313F} mice appeared normal, *ShcA*^{3F/3F} and *ShcA*^{δSH2/δSH2} mutants exhibited defects in limb coordination (movies S1 to S6). Thus, the PTB and SH2 domains and CH1 pTyr sites of ShcA are variably required for embryonic development and postnatal motor control.

Many E11.5 *ShcA*^{δPTB/δPTB} embryos were dead (13 out of 27), while live mutants exhibited enlarged and irregularly beating hearts with poorly developed cardiac trabeculae (Fig. 1D). By E12.5, no live *ShcA*^{δPTB/δPTB} embryos were present (0 out of 19). In contrast, between E12.5 and E13.5, virtually all *ShcA*^{3F/3F} mutants were alive (27 out of 29), with regularly beating hearts in which cardiac trabeculae appeared grossly normal (Fig. 1E). As expected, ShcA-1×Flag proteins in *ShcA*^{3F/3F} mouse embryo fibroblasts (MEFs) were not tyrosine-phosphorylated and selectively failed to coimmunoprecipitate with Grb2, in response to an activated ErbB2 RTK; conversely, ShcA-1×Flag proteins from *ShcA*^{δPTB/δPTB} MEFs selectively failed to associate with an NPXpY phosphopeptide (fig. S5). These results indicate that ShcA regulates mid-gestational heart development by a PTB-dependent mechanism, whereas phosphorylation of the YXN motifs is not essential at this stage.

To test whether ShcA is intrinsically required in the heart, we produced mice with a protein-null *ShcA* allele (*ShcA*^{flxβ}) that can be reactivated to express ShcA by Cre recombinase (Cre)-mediated DNA recombination (Fig. 1F and fig. S6). We then generated *ShcA*^{flxβ/KO}; *MHCα*-

Cre mice (KO, knock out; *MHCα*, myosin heavy chain α), in which the *MHCα-Cre* transgene selectively expresses Cre in cardiomyocytes beginning before E9 (15) and the *ShcA^{KO}* allele is irreversibly protein-null (Fig. 1G and fig. S6). Polymerase chain reaction (PCR) analysis revealed Cre-mediated reactivation of the *ShcA^{flxβ}* allele in the hearts, but not in the heads, of *MHCα-Cre* carriers (fig. S6). Between E12.5 and E13.5, 21 out of 21 *ShcA^{flxβ/KO}* mutants were dead, whereas 8 out of 19 *ShcA^{flxβ/KO}; MHCα-Cre* embryos were alive with regularly beating hearts and grossly normal cardiac trabeculae (Fig. 1, H and I). Thus, PTB-dependent *ShcA* signaling is required in cardiomyocytes for mid-gestational heart development.

All viable *ShcA^{3F/3F}* mice were severely uncoordinated (Fig. 2A and movie S5). Mice with disruptions in signaling by tyrosine kinase C (TrkC) or ErbB2, two RTKs known to bind *ShcA* (16,17), display similar motor defects, whose physiological cause is abnormal stretch reflex development (18–20). The monosynaptic stretch reflex circuit detects alterations in skeletal muscle length and induces compensatory muscle contraction and relaxation (21). It has three major components: group Ia sensory neurons located in the dorsal root ganglia (DRG), α -motor neurons in the ventral spinal cord, and skeletal muscle sensory organs known as muscle spindles (Fig. 2B). Spindles differentiate from type I myotubes that are innervated by group Ia sensory neurons during embryogenesis (21). In newborn mice, skeletal muscles have their full complements of spindles, which reach structural maturity around the second postnatal week (22). Between postnatal days 2 and 9 (P2 and P9), between P22 and P35, and in adults (older than P56), spindle numbers in the soleus and medial gastrocnemius muscles of *ShcA^{3F/3F}* mutants were, respectively, 70, 50, and 11.5% of those in age-matched controls (Fig. 2C and table S1). Properly developed spindles contain stereotypical numbers of intrafusal fibers (22). Between P2 and P9, between P22 and P35, and in adults, the numbers of intrafusal fibers in *ShcA^{3F/3F}* spindles were, respectively, 38.5, 53.8, and 27.5% of those in controls (Fig. 2, C and D, and table S1). In contrast, extrafusal muscle fibers in adult *ShcA^{3F/3F}* mice had WT morphologies and normal distributions of type I and type II myofibers, indicating that neuromuscular function was not overtly disrupted (fig. S7). Supporting this view, the ventral motor roots of adult *ShcA^{3F/3F}* mice contained normal numbers of large-diameter ($>3.5 \mu\text{m}$) α -motor axons, which contact extrafusal fibers, whereas the numbers of small-diameter ($\leq 3.5 \mu\text{m}$) γ -motor axons, which contact muscle spindles, were only 20% of those of controls (*ShcA^{3F/3F}*, 53.3 ± 10.6 SEM, $n = 3$ mice; controls, 346.6 ± 6.7 SEM, $n = 3$ mice; $P < 0.001$) (fig. S7). The numbers and structures of spindles in adult *ShcA^{KI-WT/KI-WT}* animals were similar to those in WT controls (Fig. 2C and table S1). Thus, muscle spindles develop abnormally and are lost with age in uncoordinated *ShcA^{3F/3F}* mice.

The ataxic behavior of *ShcA^{δSH2/δSH2}* mice was less severe than that of *ShcA^{3F/3F}* mutants (movie S6). Between P2 and P9, and in adults, total spindle numbers in *ShcA^{δSH2/δSH2}* animals were equivalent to those of controls (Fig. 2C and table S1), but their spindles appeared structurally abnormal, with intrafusal fiber numbers that were 66.7 and 62.5% of those in controls, respectively (Fig. 2, C and E, and table S1). Thus, spindle morphogenesis, but not spindle production, is disrupted in *ShcA^{δSH2/δSH2}* mutants. The pYXN motifs and SH2 domain are therefore both required for proper spindle formation.

Abnormal spindle formation could result from defects intrinsic to group Ia sensory neurons (23). Labeling of DRG neurons with fluorescent dextran or DiI (1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate) revealed axons of group Ia sensory neurons reaching their target regions in the ventral horn of the spinal cord in *ShcA^{3F/3F}* and *ShcA^{δSH2/δSH2}* mice (fig. S8). To test the functionality of these neurons, we stimulated DRG neurons electrically and recorded EPSPs (excitatory postsynaptic potentials) evoked in ventral root motor axons (24). The amplitudes of short-latency EPSPs (2 to 3 ms), which result from the monosynaptic input of group Ia sensory neurons, were smaller in *ShcA^{3F/3F}* ($n = 9$ mice) and *ShcA^{δSH2/δSH2}* ($n = 2$ mice) mutants as compared with those in WT mice ($n = 9$) (Fig. 2F). In

the case of *ShcA*^{3F/3F} animals, the amplitude was less than 20% of that in controls ($n = 9$ mice for both groups; $P < 0.001$, Student's t test). Thus, the synaptic conductivity of group Ia sensory neurons is impaired in ataxic *ShcA*^{3F/3F} and *ShcA*^{ΔSH2/ΔSH2} mutants.

The development of spindles and group Ia sensory neurons is mutually dependent (23,24), and ShcA is expressed in both skeletal muscles and DRG neurons (25,26). To investigate where ShcA acts in the stretch reflex circuit, we generated mice with a targeted *ShcA* allele (*ShcA*^{flx}) encoding WT ShcA isoforms with a triple Flag (3×Flag) tag, which could be inactivated by Cre (Fig. 3A and fig. S9). For this purpose, we used the *Mlc1f-Cre* allele, which is expressed in developing skeletal muscles starting before the onset of spindle formation (27), or the *Isl1-Cre* allele that expresses Cre in embryonic motor and sensory neurons, including group Ia sensory neurons (28). *ShcA*^{flx/flx} mice were viable and appeared normal. All *ShcA*^{flx/flx}; *Mlc1f-Cre* animals exhibited *ShcA*^{3F/3F}-like motor coordination defects, whereas *ShcA*^{flx/flx}; *Isl1-Cre* and *ShcA*^{flx/KO}; *Isl1-Cre* mutants appeared normal (movies S7 and S8). PCR analysis revealed Cre-mediated deletion of ShcA-3×Flag coding sequences specifically in skeletal muscles of *Mlc1f-Cre* carriers (Fig. 3B), and immunoblotting showed that ShcA-3×Flag was specifically ablated from skeletal muscles (Fig. 3C). Cre-mediated excision of ShcA-3×Flag coding sequences was detected in DRG and hindlimb tissues of *Isl1-Cre* carriers (Fig. 3D), where this allele is known to be active in sensory neurons and mesenchymal cells, respectively (29). Between P22 and P35, spindle numbers in *ShcA*^{flx/flx}; *Mlc1f-Cre* animals were 50% of those of controls (table S2). *ShcA*^{flx/flx}; *Mlc1f-Cre* spindles were morphologically abnormal (Fig. 3E), with intrafusal fiber numbers that were 59% of those of controls (table S2). By contrast, spindles in *ShcA*^{flx/flx}; *Isl1-Cre* mice were present in proper numbers and appeared structurally normal (Fig. 3E and table S2). Thus, ShcA is required in developing skeletal muscles and/or spindles for proper motor behavior and spindle formation, and the defective synaptic conductivity of group Ia sensory neurons in *ShcA*^{3F/3F} and *ShcA*^{ΔSH2/ΔSH2} mutants is likely a non-cell-autonomous effect.

The preceding data did not discount the possibility that the spindle phenotypes of germline *ShcA*^{3F/3F} and *ShcA*^{ΔSH2/ΔSH2} mutants resulted from the effects of these alleles in a combination of spindle, muscle, and nonmuscle cell types. Furthermore, the role of the ShcA PTB domain in spindle formation could not be analyzed in *ShcA*^{ΔPTB/ΔPTB} mutants because of their embryonic lethality. To address these issues, we generated *ShcA*^{ΔPTB/flx}; *Mlc1f-Cre* mutants, *ShcA*^{3F/flx}; *Mlc1f-Cre* mutants, and *ShcA*^{ΔSH2/flx}; *Mlc1f-Cre* mutants, anticipating that the Cre-mediated inactivation of the *ShcA*^{flx} allele would selectively restrict the effects of the *ShcA*^{ΔPTB}, *ShcA*^{3F}, and *ShcA*^{ΔSH2} alleles to developing skeletal muscles and spindles (Fig. 4A). *ShcA*^{ΔPTB/flx}; *Mlc1f-Cre* mice, *ShcA*^{3F/flx}; *Mlc1f-Cre* mice, and *ShcA*^{ΔSH2/flx}; *Mlc1f-Cre* mice were fully viable, with the former two mutants exhibiting *ShcA*^{3F/3F}-like motor abnormalities and the latter mutant displaying *ShcA*^{ΔSH2/ΔSH2}-like motor defects (movies S9 to S11). Between P22 and P35, the spindle and intrafusal fiber numbers in *ShcA*^{ΔPTB/flx}; *Mlc1f-Cre* and *ShcA*^{3F/flx}; *Mlc1f-Cre* mutants were reduced, whereas only intrafusal fiber numbers were decreased in *ShcA*^{ΔSH2/flx}; *Mlc1f-Cre* mice (Fig. 4, B and C, and table S2). Immunoblot analysis confirmed that the slower-migrating ShcA-3×Flag proteins of the *ShcA*^{flx} allele were selectively eliminated from the skeletal muscles of *ShcA*^{ΔPTB/flx}; *Mlc1f-Cre* mutants (Fig. 4D). Thus, the pTyr-binding functions of the ShcA PTB and SH2 domains, and phosphorylation of the YXN sites, are all required in skeletal muscles and/or spindles for proper motor coordination and spindle development.

To test whether the ShcA PTB domain, SH2 domain, and YXN phosphorylation sites act independently or in combination in spindle development, we tested for genetic complementation in animals with two different mutant alleles of ShcA. Transheterozygous *ShcA*^{3F/ΔSH2}, *ShcA*^{ΔPTB/ΔSH2}, and *ShcA*^{3F/ΔPTB} mutants displayed motor defects of increasing severity. Between P22 to P35, spindle numbers in *ShcA*^{ΔPTB/3F}, *ShcA*^{ΔPTB/ΔSH2}, and

ShcA^{3F/ΔSH2} mutants were 13, 83.3, and 86.6%, respectively, of those in controls (Fig. 4C and table S2). Spindles in *ShcA*^{3F/ΔPTB}, *ShcA*^{ΔPTB/ΔSH2}, and *ShcA*^{3F/ΔSH2} animals were structurally abnormal, with intrafusal fiber numbers that were 35.9, 66.6, and 48.7% of those in controls, respectively (Fig. 4C and table S2). This lack of complementation indicates that the PTB domain, SH2 domain, and phosphorylated YXN motifs are likely required in the same ShcA molecule (i.e., in cis) for proper spindle development and motor coordination.

Our findings imply that by combining the modular functions of a limited set of domains and motifs in alternate ways, ShcA is able to provide multiple signaling mechanisms to support diversity in tissue morphogenesis (Fig. 4E). This likely extends to other Shc family members and unrelated docking proteins with modular functions (1,11). The ShcA PTB domain is required in both spindle and midgestational heart formation, whereas the CH1 YXN motifs are only essential in the former case. This result indicates an important role for ShcA signaling in mammalian development that is independent of direct pYXN-mediated Grb2 binding. Genetic data from *Drosophila* suggest that this scheme is conserved in evolution, because the ShcA ortholog, DSHC, requires the PTB domain for signaling by the DER and Torso RTKs but can mediate RTK-dependent Ras activation in cells devoid of the Grb2 ortholog, DRK (30). By contrast, the pYXN motifs are required in cis with the PTB and SH2 domains for ShcA to elicit proper spindle formation, suggesting that pYXN-mediated ShcA-Grb2 interactions may be critical in this process. Based on the presence of both YXN motifs in some invertebrate Shc proteins (fig. S1), we speculate that this functional combination of ShcA domains and motifs, possibly acting in a signaling pathway downstream from ErbB RTKs (19,20,23), was co-opted in evolution toward the development of muscle spindles as a novel tissue in vertebrates (31, 32).

Supplemental Material

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6. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Fig. 1.

Cardiomyocyte-expressed *ShcA* supports mid-gestational viability and heart formation by a PTB-dependent mechanism not requiring the CH1 pTyr residues. **(A)** Common structure of targeted *ShcA^{KI}* alleles generated by gene targeting in embryonic stem cells. Exons are indicated by boxes, introns by horizontal lines, and LoxP sites by arrowheads. ATG and TGA are start and stop codons, respectively. pA, polyA; Neo, neomycin resistance gene. **(B)** The WT *ShcA*-1xFlag protein product of the *ShcA^{KI-WT}* allele. Mutated amino acids are indicated. **(C)** Summary of *ShcA^{KI}* alleles introduced into mice. Amino acid changes and their effects on *ShcA*-1xFlag function are indicated. **(D)** Hematoxylin and eosin (H&E)-stained transverse sections showing that the formation of cardiac trabeculae (arrows) is severely impaired in E11.5 *ShcA^{δPTB/δPTB}* hearts. **(E)** H&E-stained transverse sections revealing the similar extent of trabeculation in E13.5 *ShcA^{3F/3F}* and WT hearts. **(F)** Depiction of the protein-null *ShcA^{flxβ}* allele and its Cre-mediated conversion into the *ShcA*-expressing *ShcA^{Δβ}* allele. sa-IRES-βgeo, splice acceptor–internal ribosomal entry site–β-galactosidase/neomycin fusion gene. **(G)** Structure of the irreversibly protein-null *ShcA^{KO}* allele. **(H)** Phenotypic rescue of lethality in an E12.5 *ShcA^{flxβ}/KO; MHCα-Cre* embryo, as compared with a dead and necrotic *ShcA^{flxβ}/KO* littermate. **(I)** H&E-stained transverse sections showing the comparable extent of trabeculation in E12.5 *ShcA^{flxβ}/KO; MHCα-Cre* and WT hearts.

Fig. 2.

Motor behavior and stretch reflex development are disrupted in viable *ShcA*^{3F/3F} and *ShcA* ^{$\delta SH2/\delta SH2$} mice. **(A)** Adult WT and *ShcA*^{3F/3F} mice. Note the inability of the *ShcA*^{3F/3F} mutant to maintain normal limb position. **(B)** Diagram depicting the monosynaptic stretch reflex circuit. **(C)** Histograms representing the numbers of muscle spindles per muscle and numbers of intrafusal fibers per muscle spindle in *ShcA*^{+/+}, *ShcA*^{KI-WT/KI-WT}, *ShcA*^{3F/3F}, and *ShcA* ^{$\delta SH2/\delta SH2$} mice. Error bars indicate SEM; numbers and statistics are presented in table S1. **(D)** Toluidine-stained transverse sections through the equatorial regions of muscle spindles in adult WT and *ShcA*^{3F/3F} mice. Note the structural differences between the stereotypical WT spindle with its multiple intrafusal fibers (arrow-heads) and the atrophied *ShcA*^{3F/3F} spindle with its single intrafusal fiber. **(E)** Toluidine-stained transverse sections demonstrating the abnormal structure of spindles in adult *ShcA* ^{$\delta SH2/\delta SH2$} mice. **(F)** Ventral root recordings of synaptic input to motor neurons evoked by electrical stimulation of DRG neurons in *ShcA*^{+/+}, *ShcA*^{3F/3F}, and *ShcA* ^{$\delta SH2/\delta SH2$} mice. Red arrows indicate short-latency EPSPs induced by group Ia sensory neurons.

Fig. 3.

Cre-mediated ablation of *ShcA* specifically from skeletal muscles disrupts motor behavior and spindle formation. (A) The *ShcA^{flx}* allele. P1/P2 and P1/P3 primer combinations were used to identify unrecombined (active) *ShcA^{flx}* alleles and Cre-recombined (inactive) *ShcA^{KO}* alleles, respectively. (B) PCR analysis showing that *ShcA*-3xFlag coding sequences are deleted from skeletal muscles but not other tissues in adult *Mlc1f-Cre* carriers. He, heart; Li, liver; Lu, lung; Sp, spleen. Skeletal muscles are also indicated: Ga, medial gastrocnemius; Ti, tibialis; Qu, quadriceps; So, soleus. (C) Immunoblot (IB) analysis showing that *ShcA*-3xFlag is specifically ablated from the skeletal muscles in adult *ShcA^{flx/flx}; Mlc1f-Cre* mice. Med Gastroc, medial gastrocnemius muscle; RasGAP, guanosine triphosphatase activating protein of Ras. (D) PCR evidence of Cre activity in the DRGs of *ShcA^{flx/+}; Isl1-Cre* mice. (E) Toluidine-stained transverse sections demonstrating the disrupted structure of spindles in *ShcA^{flx/flx}; Mlc1f-Cre* mutants, as compared with the WT appearance of those in *ShcA^{flx/flx}; Isl1-Cre* mice, between P22 and P35.

Fig. 4.

The PTB and SH2 domain and CH1 pTyr residues are required in the same ShcA molecule for proper motor behavior and spindle development. **(A)** Genetic strategy to reveal intrinsic requirements for the ShcA PTB domain in spindle formation. **(B)** Toluidine-stained transverse sections demonstrating the disrupted structure of spindles in *ShcA*^{flx/δPTB}; *Mlc1f-Cre* mutants between P22 and P35. The single intrafusar fiber in the *ShcA*^{δPTB/flox}; *Mlc1f-Cre* spindle has taken on the appearance of an extrafusar fiber (arrow). **(C)** Histograms showing the average numbers of spindles per muscle, the average numbers of intrafusar fibers per spindle, and percentages of the average numbers of spindles with greater than three intrafusar fibers in muscle-restricted and transheterozygous *ShcA* mutants between P22 and P35. Mutants are grouped by whether they exhibit normal or impaired motor behavior. Error bars indicate SEM; numbers and statistics are presented in table S2. **(D)** Immunoblot analysis demonstrating that ShcA-1xFlag protein from the *ShcA*^{δPTB} allele is selectively expressed in the skeletal muscles of *ShcA*^{δPTB/flox}; *Mlc1f-Cre* mice. **(E)** Diagram depicting combinatorial differences in the roles of the PTB and SH2 domains and CH1 pTyr residues of ShcA downstream of protein tyrosine kinases in spindle morphogenesis (green arrows and box) and embryonic heart formation (blue arrows and box). ???, presumptive CH1 pTyr-independent ShcA function.