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Supplemental Information

Mutations in Subunits of the Activating Signal

Cointegrator 1 Complex Are Associated with Prenatal

Spinal Muscular Atrophy and Congenital Bone Fractures

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Morphometric analysis of the sural nerve

Figure S2. Morphometric analysis of myelinated axon diameters of sural nerve biopsies. Sural nerve biopsies were fixed in glutaraldehyde, embedded in Epon[®]. Semithin sections were stained with methylene blue or with paraphenylene diamine to visualize the myelin sheaths. From each nerve five full visual fields that were entirely filled with myelinated axons were photographed, together with an object micrometer scale using a 100x objective on a Leica DML3000 inverted microscope. The images were recorded in bright field with a cooled SPOT RT3 CCD-camera (1,600 x 1,200 px). Axon diameters were measured morphometrically using the free ImageJ v1.37a program. The axon diameters were depicted in a multiple dot/box blot. The boxes depict the median, 10th, 25th, 75th, and 90th percentiles; p-values were calculated with the non-parametric Man-Whitney U-test.



Results of the autozygosity mapping in three families





Figure S4. Autozygosity mapping of Family B Three affected and two unaffected individuals were used for the analysis. The red bars depict the regions that were only homozygous in the patients and not in the unaffected family members at Chr15:63,594,231-68 726 159 (rs12910654-rs12915814). A *shared autozygous region* between Families A and B comprised an interval on Chr15:63,594,231-66,260,894 (rs12910654-rs333556) that contained 38 protein-coding genes.



Chr10:124,114,432-135,422,505 (rs7905027-rs4498943) comprising 196 protein coding genes.



In-situ hybridization of whole E17.5 mouse embryos

Figure S6. Comparison of in-situ intensities for *Trip4* antisense and sense probes. In-situ hybridization of a parasagittal section of an E17.5 C57BL6/J embryo with a *Trip4* antisense probe (left) and the corresponding sense probe (right). Both DIG-labeled RNA-probes were generated from the same plasmid via transcription from the T3 promoter (antisense) or the T7 promoter (sense) of the pCR-Script plasmid. Due to the large size of the embryo single sub-images are stitched together to represent the entire embryo on a single image. Both images have been recorded with the identical illumination settings.



Figure S7. Comparison of in-situ intensities for *Ascc1* antisense and sense probes. In-situ hybridization of a parasagittal section of an E17.5 C57BL6/J embryo with an *Ascc1* antisense probe (left) and the corresponding sense probe (right). Both DIG-labeled RNA-probes were generated from the same plasmid *via* transcription from the T3 promoter (antisense) or the T7 promoter (sense) of the pCR-Script plasmid. Due to the large size of the embryo single sub-images are stitched together to represent the entire embryo on a single image. Both images have been recorded with the identical illumination settings.



Quantification of morpholino injection into zebrafish



Figure S9. Morpholino mediated knockdown of *ascc1* and *trip4*. (A) Effect of control, *trip4* and *ascc1* MO (5 ng each) mediated knockdown on the ability of the 30 hpf zebrafish embryos for "full coil" or "partial coil". (B) *Left panel:* Location of the MOs at *trip4* and *ascc1* splice junctions and position of the oligonucleotide primers used for RT-PCR. *Right panels:* Agarose gel electrophoresis of *trip4* and *ascc1* RT-PCR products in differently injected zebrafish embryos verifying the specific knockdown of the intended targets. β -actin mRNA was taken as reference.



Figure S10. Use of alternative MO2 constructs for *trip4* and *ascc1* knockdown. (A) Effect of control, *trip4* and *ascc1* MO2 (5 ng each) mediated knockdown on the ability of the 30 hpf zebrafish embryos for "full coil" or "partial coil". (B) The alternative *trip4* and *ascc1* MO2s have the same specificity to generate the morphologic abnormalities of the α -motoneuron and the neuromuscular junction as the respective MOs (see Figure 3C for comparison), hence verifying the specificity of the effect. (C) *Left panel:* Location of the MO2s at *trip4* and *ascc1* splice junctions and position of the oligonucleotide primers used for RT-PCR. *Right panels:* Agarose gel electrophoresis of *trip4* and *ascc1* RT-PCR products in differently injected zebrafish embryos verifying the specific knockdown of the intended targets. β -actin mRNA was taken as reference.



morpholinos and rescue mRNA into zebrafish larvae rescues (A) the clinical phenotype ("coiling" behavior) and (B) the morphological abnormalities of the α -motoneuron projections only if wildtype mRNA is injected and not by injection of mRNA carrying the zebrafish equivalents of the patient mutations.



Expression of SRF responsive genes after serum challenge

Figure S12. Expression of known SRF responsive target genes after serum challenge. Human skin fibroblasts were grown in DMEM + 15% FBS, then serum starved for 24 hours and re-challenged with 15% FBS for 30 and 60 minutes. The lines depict the fold change of gene expression from baseline (serum starvation). The red lines depict the patients, the black lines the controls. There was no significant and persistent difference in the response of the SRF downstream target genes.



Subcellular location of the ASC-1 complex and of the CSRP1 protein

Figure S13. Subcellular localization of the ASC-1 complex and of the CSRP1 protein after serum starvation and challenge. The members of the ASC-1 complex TRIP4, ASCC1, and ASCC2 as well as the CSRP1 protein remain in the nucleus independently of the status of serum depletion or challenge of fibroblasts. As control for the subcellular fractionation, transcription factor SP1, a nuclear maker protein, is enriched in the nuclear fraction and the Calreticulin protein (CALR), a marker protein for the endoplasmatic reticulum (ER) is depleted in the nuclear fraction.

Multiple species alignment of TRIP4 and ASCC1 amino acid sequences

Human Chimpanzee Rat Mouse Clawfrog Zebrafish	1 1 1 1 1	MAVAGAVSGEPLVHWCTQQLRKTFGLDVSEEIIQYVLSIESAEEIREYVTDLLQGNEGKK MAVAGAVSGEPLVHWCTQQLRKTFGLDVSEEIIQYVLSIESAEEIREYVTDLLQGNEGKK MAVAGAASGEPLVHWCTQQLRKTFALDVSEEIIQYVLSIESAEEIRDYVTDLLQGNEGKK MAVAGAAYREPLVHWCTQQLQKTFALDVSEEIIQYVLSIENAEEIREYVTDLLQGNEGKK VFSCCSDMAADLLGWCVEELEKRFGLGVSEDVVKYILSIDKEEEIDPYINDLVQAPEDTK MSDSLLRWTVDKLKHGFGLDASDDVVQYLSIDNADEIVEYVGDLLQGIEGNK
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	61 61 61 61 54	GQ <mark>FIEELITKWQKNDQELISDPLQQCFKKDEILD</mark> GQKSG <mark>DHLKRGRKKGRNRQEV</mark> PA. GQ <mark>FIEELITKWQKNDQELISD</mark> PLQQCFKKDEILDGQKSGDHLKRGRKKGRNRQEVPA. GQ <mark>FIEDLITKWQKNDQECISD</mark> SFQQCWKKDESLDGQRSVDQLKRSRRKGRNKQEVPA. GQ <mark>FIEDLITKWQKNDQEFISD</mark> SFQQCLRKDEILDGQRSVDQLKRSRRKGRNKQEVPA. SL <mark>FTRELKLRWHRIRQ</mark> PPASRTTSAFQRKDDKLLFGDQG <mark>KQRRKGRNKQEI</mark> VT. QE <mark>FVDELVQRWQR</mark> C.QTQ TSD GLGGVL <mark>RKEV</mark> VMBELDTAPK <mark>U</mark> TQKKSKRKGRNKQEIVT.
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	118 118 118 118 118 116 112	ETEPDT.TAEVKTPFDLAK.AQE.NS.NSVKKKTKFVNLYTREGODELAVLIPGRHP FTEPDT.TAEVKTPFDLAK.AQE.NS.NSVKKKTKFVNLYTREGODELAVLIPGRHP FPEPDV.TVEVKTPLDLAK.AQE.GN.SSVKKKTRFVNLYTREGODKLAVLIPGRHP FPEPDV.AVEVKTPLDLAK.AQE.SN.NSVKKKTRFVNLYTREGODKLAVLIPGRHP VYPTSNTSAEEVKTPMDLAKKAQEVVGL.SSSKKKGKFVNLYTKEGODKLAVLIPGRYP VSQAE.PEPVRTPIDLMK.AQE.SSNSSVKKKSNFVNLYAREGKDKLAVLIPGRQA zinc-finger motif
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	171 171 171 171 174 166	CDCLGQKHKLINNCLICGRIVCEQEGSGPCHFCGTLVCTHEEODILQRDSNKSQKLLKKL CDCLGQKHKLINNCLICGRIVCEQEGSGPCHFCGTLVCTHEEODILQRDSNKSQKLLKKL CDCLGQKHKLINNCLVCGRIVCEQEGSGPCHFCGSLVCTNEEODILQRDSNKSQKLLKKL CDCLGQKHKLINNCLVCGRIVCEQEGSGPCHFCGSLVCTNEEODILQRDSNKSQKLLKKL CECLGQKHKLINNCMTCGRIVCEQEGSGPCHFCGSLVCTNEEDDILQRDSNKSQKLRKKL CECLAQKHRLINNCMTCGRIVCEQEGSGPCHFCGSLVCTNEEDDILQRDSNKSQKLRKKL CECLAQKHRLINNCLSGRIVCEQEGSGPCHFCGSLVCTNEEODILQRDSNKSQKLRKKL
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	231 231 231 231 234 226	Vp.Arg254* Vp.Arg278* MSGVENSGKWDISTKDLLPHQELRIKSGLEKAIKHKDKLLEFDRTSIRRTQVIDDESD MSGAENSGKVDISTKDLLPHQELRIKSGLEKAIKHKDKLLEFDRTSIRRTQVIDDESD MSGAENSGKVDISTKDLLPHQESRMKSGLEKAIKHKDKLLEFDRTSIRRTQVIDDESD MSGAETSGKVDVSTKDLLPHQESRMKSGLEKAIKHKDKLLEFDRTSIRRTQVIDDESD MSGAETSGKVDVSTKDLLPHQESRMKSGLEKAIKHKDKLLEFDRTSIRRTQVIDDESD MGE
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	289 289 289 289 294 276	YFASDSNQWLSKLERETLOKREEELRELRHASRLSKKUTIDFAGRKILEEENSLAEYHSR YFASDSNQWLSKLERETLOKREEELRELRHASRLSKKUTIDFAGRKILEEENSLAEYHSR YFASDSNQWLSKVERDMLOKREEELRELRHASRLSKWTIDFAGRKILEEENPLAEYHSR YFASDSNQWLSKVERMLOKREEELRELRHASRLSKWTIDFAGRKILEEENPLAEYHSR YFSTDSNQWLSQTERETLRKKEOELOELRHASRLSRKETIDFAGRKVFEEGEDLKEYNKL YFATDSNQWLSQTERETLRKKEOELOELRHASRLSRKETIDFAGRKVFEEGEDLKEYNKL YFATDSNQWLSPGEREALRKREEELRELRASRLSRKETIDFAGRRVLDEGENLSPYQK
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	349 349 349 349 354 354	LDETIQAIANGTLNQPLTKLDRSSEEPLGVLVNPNMYQSPPOWVDHTGAASQKKAFRSSG LDETIQAIANGTLNOPLTKLDRSSEEPLGVLVNPNMYQSPPOWVDHTGAASQKKAFRSSG LDETIQAIANGTLNQSLVTSDRSSEEPLGVLVNPNMYQASPQWVDNTGSAPPKKTSLSVG LDETIQAIASGTLNQSLVTLDRSCEEPLGVLVNPNMYQASPQWVDNTGSTPQKKTSLSAG LDETVQSIHSGTVVFPSGS.PGERSNTSSLVNPSLLQPSPVWVDQVSSGSYKKKASSE FDETVKAINTGSFGQKAKHSAPADRQHFRELVNPNLLQSABEWVDVASSNPSEKSSKAA
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	409 409 409 409 411 396	FGLEFNSFQHQLRIQDQEFQEGFDGGWCLSWHQPWASLLVRGIKEVEGRSWYTPHRGRLW FGLEFNSFQHQLRIQDQEFQEGFDGGWCLSWHQPWASLLVRGIKEVEGRSWYTPHRGRLW PRLELSLHPHQLRIQDQEFQEGFDGGWCLSWHQPWASLLVRGIKEVEGRSWYTPHRGRLW PRLEPSLHQHQLRIQDQEFQEGFDGGWCLSWHQPWASLLVRGIKEVEGRSWYTPHRGRLW .ETKPCTERNRLRIQDRELQEISDLGMCLSWHQPWASLLVAGIKOVEGRTWYSAHRGRLW SGKET.SERSRLRIQDKELQEIADGGWCLSWHQPWASLLVKGIKEVEGRTWYSAHRGRLW
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	469 469 469 469 470 455	IAATAKKPSPQEVSELQATYRILRGKDVEFPNDYPSGCLLGCVDIIDCISGKOFKEQEPD IAATAKKPSPQEVSELQATYRILRGKDVEFPNDYPSGCLLGCVDIIDCISGKOFKEQEPD IAATGKRPSTQEVSELQATYRILRGKDVEFPNDYPSGCLLGCVDIIDCISGKOFGEGEPD IAATGKRPSPQEVSELQATYRILRGKDVEFPNDYPSGCLLGCVDIIDCISGKOFGEGEPD IAAASKRPSPQEISELETSYRVLLGKDIHFPKDYPISCLLGCVDVNDCUSGEGFKEQYPN IAAAAKRPPTQEIAEVEAMYRHLYKHEPOFPASYPIGCLLGCVNVSDCLSGEGFREQYPQ
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	529 529 529 529 529 530 515	ISQESDSPFVFICKNPQEMVVKFPIKGNPKIWKLDSKIHQGAKKGLM.KQNKAV ISQESDSPYVFICKNPQEMVVKFPIKGNPKIWKLDSKIHQGAKKGLM.KQNKAV ISQESDSPFVFICKNPQEMVVKFPIKGNPKIWKLDSKIHQGAKKGLM.KQNKAV ISQESDSSFVFICKNPQEMVVKFPIKGPKIWKLDSKIHQGAKKGLM.KQNKAV LNQESASPFIFICSNPQELLIKFPIKGPKKIWKLDSKIHQGAKKGLMACQEQAR ISEESTSPFVFICSNPQELLVKFPMKGKHKIWKLSSOSHQSAKKGLM.QPA

Figure S14. Multiple species alignment of TRIP4 Red boxes depict identical, yellow boxes similar amino acids. The zinc-finger motif (aa125-237) is depicted by a green line. The positions of the two nonsense mutations are depicted by a red triangle. The Ensembl accession number for the amino acid sequences are: Human [ENSP00000261884], Chimpanzee [ENSPTRP00000012241], Rat [ENSRNOP0000021863], Mouse [ENSMUSP00000112385], Clawfrog [ENSXETP00000023326], Zebrafish [ENSDARP00000107782]

		p.Glu53Glyfs*V
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	1 1 1 1 1	MEVLRPOLIRIDGRNYRKNPVOEOTYOHEEDEEDFYOGGSM.ECADEPCDAYEVEOTPOGF MEVLRPOLIRIDGRNYRKNPVOEOTYOHEEDEEDFYRGSM.ECADEPCDAYEVEOTPOGF MDVLRPOLYTFDGRNYRKNPVOEKOPOHEEE.EDFYODSM.EYSDEPCGAYEVEOTPHGF MDVLRPOIVTFDGRNYRKNPIOEKOYOHEED.EDFYPDSM.EYSDEPCGAYEVAOTPHGF MEVLRPTLINIGGRIYRKNAVREASHONEEDEEDFYRESV.OCLDEPCDATEVEETEKGF MEVLRPPLINIGGRIYRKNPVOEETYEEEEEDCSYSEAAVDOCLDEPCDAHNIEOTDRGF
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	60 59 59 60 61	RSTLRAPSLLYKHIVGKRGDTRKKIEMETKTSISIPKPGODGEIVITGOHRNGVISARTR RSTLRAPSLLYKHIVGKRGDTRKKIEMETKTSISIPKPGODGEIVITGOHRNGVISARTR RATVSAPSLLYKHIVGKRGDTKKKIEVETKTSINIPKLGHEGEIVITGHHRNGVVSARTR RATVSAPSLLYKHIVGKRGDTKKKIEVETKTSINIPKHGHEGEIVITGOHRNGVVSARTR QCTIDLPSOLFKYIIGKKGETKRNLESETRTSIIIPRPGVEGDIIITGORNGVISARTR RCALDVPSVLYKYIIGKKGETRRRLESETKTSINIPKQOVEGOIVVTGAHRPSVSSAVTR
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	120 120 119 119 120 121	IDVLLDTFRRKQPFTHFLAFFLNEVEVQEGFLRFQEEVLAKCSMDHGVDSSIFQNPKKLH IDVLLDTFRRKQPFTHFLAFFLNEVEVQEGFLRFQEEVLAKCSMDHGVDSSIFQNPKKLH IDVLLDTFRRRQPFTHFLSFFLNEVEVQERFLEFQAEVLKKCSKDRGVDSTIFQNPKKLH IDVLLDTFRRRQPFTHFLSFFLNEVEVQERFLMFQEEVLRKCSKDRGVDSTIFQNPKKLH IELLAESFRRKQPFTHFLSFALNHPEIQEKVLLFKEEVLAKCSKDRGVESSIFQNPAKLH IEVLIDSFRRKQPFTHFLSFALNHAQVREGFLRFREEVLEHCSQDSGVDVSIFQNPDKLH
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	180 180 179 179 180 181	LTIGMLVLLSEEIQQTCEMLQQCKEEFINDISGGKPLEVEMAGIEYMNDDPGMVDVLYA LTIGMLVLLSEEIQQTCEMLQQCKEEFINDISGGKPLEVEMAGIEYMNDDPGMVDVLYA LTIGMLVLLSEQEIQQTCEILQRCKEEFINDISGGKPLEVEMAGIEYMNDDPAMVDVLYA LTIGMLVLLSEQEIQQTCEILQRCKEEFINDISGGRPLEVEMAGIEYMNDDPAMVDVLYA LTIGTMVLLSEKEVMQANEILQKCKEEFLDKITGGKSLQLQVVGIEYMNDDPAMVDVLYA LTIGTLVLLNQQEVTRANELLHQCQD.TIREITGAEALPVEVRGVEYMNDDPSMVDVLYA
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	240 240 239 239 240 240	KVHMKDGSNRLQELVDRVLERFQASGLIVKEWNSVKLHATVMNTLFRKDPNAEGRYNLYT KVHMKDGSNRLQELVDRVLERFQASGLIVKEWNSVKLHATVMNTLFRKDPNAEGRYNLYT KVHMKDGSNRLQELVDRVLERFQSMGLVMKEWTSVKLHATVMNTLLRKDPNAEGRYNLYT KVHMKDGSNRLQELVDRVLERFQSLGLIVKEWTSVKLHATVMNTLLRKDPNAEGRYNLYT KVEMKDGSERLQLIADRLMQRFVSSGLMLKEWDRVKLHATVMNTLFRRDPLGKERSSI. KVS <mark>VQ</mark> DGSDRLQQ <mark>ISDRLVEC</mark> FVSAGLMERERDRVKIHATVMNTLFRRDPLGKERSSI.
Human Chimpanzee Rat Mouse Clawfrog Zebrafish	300 300 299 299 298 298	AEGKYIF <mark>KERESFDGRNILKLFENFYFG</mark> SLKLNSIHISQRFTVDSFGNYASCGQIDFS ADGKCIF <mark>KERESFDGRNILKLFENFYFGSL</mark> KLNSIHISQRFTVDSFGNYASCGQIDFS ADGKYIF <mark>KERESFDGRNILKTFENFYFGSLKLNSIHISQRFTVDSFGNYASCGHVDFS</mark> ADGKYIF <mark>KERESFDGRNILKTFENFYFGSLRLNSIHISQRFTVDSFGNYASCGHVDFS</mark> SA <mark>GK</mark> PGQ <mark>RERESFDARNVLKIFGNFCFGDLSMDTVHLSQRFSADSSGYYASSGHVSLS . <mark>AR</mark>ANP<mark>RDREAFNGKNILQ</mark>MFGDFYFGAFE<u>LNSVQISQRFSTDS</u>SGYYSSAGHITFS</mark>
Figure S15. Multiple	e spe	cies alignment of ASCC1 Red boxes depict identical, yellow boxes similar aming
acids. The position	of th	e frameshift mutation is depicted by a red triangle. The Ensembl/GenBank ac

Figure S15. Multiple species alignment of ASCC1 Red boxes depict identical, yellow boxes similar amino acids. The position of the frameshift mutation is depicted by a red triangle. The Ensembl/GenBank accession number for the amino acid sequences are: Human [ENSP00000320810], Chimpanzee [ENSPTRP00000004528], Rat [ENSRNOP0000000701], Mouse [ENSMUSP00000052351], Clawfrog [ENSXETP00000013525], Zebrafish [NP_001017610]. The multiple amino acid sequence alignment was performed with T-Coffee and the coloring /printout with ESPript.¹



Co-immunoprecipitation with antibodies against three subunits of the ASC-1 complex



Figure S17. Immunoprecipitation with an anti-ASCC1 antibody. Co-precipitation with ASCC1 can be seen for TRIP4 and ASCC2 only in the controls, not in the patients. SRF is not co-precipitated ASCC1 (or the ASC-1 complex) and can only be detected in the unbound flow-through.



Figure S18. Immunoprecipitation with an anti-ASCC2 antibody. (A) Co-precipitation with ASSC2 can be seen for TRIP4 and ASCC1 only in the controls, not in the patients. SRF is not co-precipitated ASCC2 (or the ASC-1 complex) and can only be detected in the unbound flow-through.





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Morphometric analysis of the sural nerve

Patient	Median	10 th percentile	90 th percentile
Control	22,059 mm ²	18,971 mm²	25,111 mm ²
B:II_05 (<i>TRIP4</i>)	22,752 mm ²	21,781 mm ²	23,169 mm ²
D:II_02 (ASCC1)	21,781 mm ²	16,509 mm ²	28,857 mm ²

Table S1. Fiber density of myelinated fibers in patients and control

Coverage details of the exome sequencing

Individual	Mean coverage	Coverage	100 bp paired-end		
	[fold]	>3x [%]	>10x [%]	>20x [%]	fragments [n]
B.II_01	175.99	99.8	99.4	98.7	77.3 Mio
D.II_02	112.91	99.5	98.7	97.0	53.2 Mio
D.II_03	230.12	99.8	99.5	98.9	106.1 Mio

Table S2. Coverage details of three WES datasets

Quantification of morpholino injection into zebrafish

Table S	53.	Quantification	of the	"coiling"	behavior	of mor	nhants :	and	control	S
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Morpholino [amount injected]		"partial coil"/total number of examined embryos (p-value in comparison to <i>bactin</i> MO injection; χ^2 -test)	Affected embryos [%]
Control [5 ng]		0/43 (<i>p</i> <0.001)	0
trip4 MO [5 ng]		49/62 (<i>p</i> <0.001)	79
ascc1 MO [5 ng]		46/55 (<i>p</i> <0.001)	84
Morpholino [amount injected]	Primer set	Specific/bactin RT-PCR band density [mean±SD; t-test]	N
trip4 MO [5 ng]	trip4	0.15±0.09 (p<0.01)	4
trip4 MO [5 ng]	ascc1	0.92±0.07 (<i>p</i> >0.05)	4
ascc1 MO [5 pg]		1.02+0.21 (+ 0.00)	4
	trip4	1.03 ± 0.31 (p>0.80)	4

Table S4. Quantification of the "coiling" behavior using an alternative morpholino

Morpholino [amount injected]		"partial coil"/total number of examined embryos (p-value in comparison to <i>bactin</i> MO injection; χ^2 -test)	Affected embryos [%]
Control [5 ng]		0 /19 (p<0.001)	0
<i>trip4</i> MO2 [5 ng]		24/28 (p<0.001)	86
ascc1 MO2 [5 ng]		35/43 (<i>p</i> <0.001)	81
Morpholino [amount injected]	Primer set	Specific/bactin RT-PCR band density [mean±SD; t-test]	N
<i>trip4</i> MO2 [5 ng]	trip4	0.19±0.11 (p<0.001)	4
<i>trip4</i> MO2 [5 ng]	ascc1	1.00±0.09 (<i>p</i> >0.9)	4
ascc1 MO2 [5 ng]	trip4	0.92±0.09 (<i>p</i> >0.15)	4
ascc1 MO2 [5 ng]	ascc1	0.14±0.10 (p<0.001)	4

Table S5. Quantification of the density of neuromuscular junctions

Morpholino	Amount injected [ng]	Cumulative tion in com	relative density of the neuromuscular junc- parison to CTRL MO [± SD]
CTRL MO	5	1.00±0.26	(n=4)
ascc1 MO	5	0.61±0.12	(n=4, p<0.05)
trip4 MO	5	0.56±0.18	(n=4, p<0.05)

Quantification of gene expression in patient and control fibroblasts

Table S6. Differentially regulated genes in ASCC1 mutant versus control skin fibroblasts

(The PubMed link can be directly clicked and connects to the PubMed abstract of the cited article)

Gene sym-	Fold	FDR	Gene name	Gene Ontology (GO)	Direct link to
bol (HGNC)	change	(BH)		annotation	PubMed
	Ge	nes in <mark>ne</mark> r	vous system develo	pment (DOWN-REGULATED)	
SERPINF1	-5.7	0.0045	Serpin peptidase inhibitor, clade F	positive regulation of neuron projection development	PMID:23825416
			(alpha-2	neuronal cell body	PMID:10411342
			antiplasmin, pig- ment epithelium	negative regulation of neu- ron death	PMID:22410737
			derived factor), member 1	positive regulator of neuro- genesis	PMID:8226833
GSTM3	-2.1	0.0045	Glutathione S- transferase mu 3 (brain)	establishment of blood- nerve barrier	PMID:2345169
DAB1	-6.4	0.0045	Dab, reelin signal transducer, hom-	lateral motor column neuron migration	PMID:20711475
			olog 1 (Drosophi- la)	positive regulation of neuron differentiation	PMID:18848628
				neuron migration	<u>PMID:15703280</u> ,
					PMID:15091337
					, <u>PMID:1122631</u>
					<u>4, PMID:128829</u>
					86
				neuron projection	<u>BMID:14961563</u>
				neuronal cell body	PMID:14961563
LAMA4	-2.8	0.0045	Laminin, alpha 4	muscle attachment	PMID:22859503
FHOD3	-2.1	0.0045	Formin homology	sarcomere organization	PMID:19706596,
			2 domain contain-	_	PMID:22509354
			ing 3		
CRLF1	-2.2	0.0045	Cytokine recep-	negative regulation of neu-	PMID:10966616
			tor-like factor 1	ron apoptotic process	
				negative regulation of motor	PMID:14523086
654430	2.2	0.0045	Comontonia 2D	neuron apoptotic process	
SEIVIA3D	-2.3	0.0045	Semaphorin 3D	axon guidance	PIVID:15456815,
				nerinheral nervous system	PMID:17231203
				development	<u>1 WID:10200355</u>
				retinal ganglion cell axon	PMID:14724229.
				guidance	PMID:16467361
				Neural crest development	PMID:16971468
				regulation of axon extension	PMID:16280593
				neural crest cell differentia-	PMID:16860789,
				tion	PMID:16971468

SEMA3A	-5.1	0.0045	Semaphorin 3A	retinal ganglion cell axon	PMID:16467361,
				guidance	PMID:14724229
				neural crest cell develop-	PMID:16971468
				ment	
				regulation of axon extension	PMID:16280593
				pathway involved in axon	PMID:18804103
				guidance	
				axogenesis involved in in-	PMID:22790009
				nervation	
PPAP2B	-1.9	0.0045	Phosphatidic acid	Bergmann glial cell differen-	PMID:21319224
			phosphatase type	tiation	
			28	ossification	PMID:12933688
		0.0070		response to vitamin D	PMID:11/02003
CRABP2	-1.6	0.0078	Cellular retinoic	embryonic forelimb mor-	PMID://205/5
			acid binding pro-	pnogenesis	DMUD-22640200
			tein z	nindbrain morphogenesis	PMID:22619388
				positive regulation of collat-	PIVIID:18052984
EZD4	2.1	0.0045	Frizzlad class ro		
FZD4	-2.1	0.0045	centor A	morphogenesis	PIVILD.13035969,
ΛςΦΛ	_2 7	0.0078	Aspartoaculase	nositive regulation of	PMID:12230312
AJFA	-2.7	0.0078	Aspartoacylase	oligodendrocyte differentia-	<u>FIMID.10034033</u>
				tion	
NOV	-2.6	0.0078	Nephroblastoma	axon development	PMID:19286457
_			overexpressed	neuronal cell development	PMID:19286457
				dendrite formation	PMID:19286457
	. (Genes in <mark>r</mark>	ervous system deve	lopment (UP-REGULATED)	
	+6.7	0.0045	Hyaluropan and	Suppression of neuronal	
HAPLNI	+0.7	0.0045	nroteoglycan link	plasticity	<u>PIVIID.20300464</u>
			protein	Memory function	PMID:23595763
			protein	Extracellular matrix	PMID:23595763
EDIL3	+3.1	0.0045	FGF-like repeats	Neural plate development	PMID:20823067
		0.0015	and discoidin I-		111112120020007
			like domains 3		
NTN4	+3.1	0.0045	Netrin 4	Neuron remodeling	PMID:11038171
ITGA	+1.7	0.0045	Integrin alpha-3	Neuron migration	PMID:15091337
				Excitatory synapse	PMID:23595732
				Regulation of neuron projec-	PMID:2223092
				tion development	
CDH13	+2.0	0.0045	Cadherin-13	Negative regulation of neu-	PMID:10737605
				ron projection	
				Negative regulation of cell	PMID:10737605
				proliferation	

Gene sym-	Fold	FDR	Gene name	Gene Ontology (GO)	Direct link to
bol (HGNC)	change	(BH)		annotation	PubMed
		Genes	in <mark>bone</mark> development (DC	OWN-REGULATED)	
TNFRSF11B	-4.8	0.0045	Tumor necrosis factor	negative regulation of	PMID:16283633
			receptor superfamily,	odontogenesis of den-	PMID:14981127
			member 11b	tin-containing tooth	
				negative regulation of	PMID:11839361
				bone resorption	PMID:16912914
				skeletal system devel-	PMID:9108485
				opment	
FAM20A	-1.9	0.0045	Family with sequence	tooth eruption	PMID:23434854
			similarity 20, member		PMID:23468644
			A		PMID:23697977
				calcium ion homeostasis	PMID:23434854
RASSF2	-2.5	0.0078	Ras association	bone remodeling	PMID:22227519
			(RalGDS/AF-6) domain	ossification	PMID:22227519
			family member 2	regulation of osteoblast	PMID:22227519
				differentiation	
				regulation of osteoclast	PMID:22227519
				differentiation	
				skeletal system devel-	PMID:22227519
				opment	
TSHZ2	-2.2	0.0045	Teashirt zinc finger	embryonic cranial skele-	PMID:23559552
			homeobox 2	ton morphogenesis	
STC1	-3.0	0.0078	Stanniocalcin 1	positive regulation of	PMID:17032941
				calcium ion import	
DPT	-2.5	0.0045	Dermatopontin	collagen fibril organiza-	PMID:16877395
				tion	

Mass spectrometric analysis of peptides from immunoprecipitations

Table S7. Mass spectrometric analysis of peptides from immunoprecipitations (TableS7.xlsx)

Immunoprecipitated samples were boiled at 95°C for 5 min, reduced in 50 mM DTT and alkylated with a final concentration of 5.5 mM chloroacetamide for 30 min. Proteins were digested by 250 ng of trypsin overnight at 37°C and desalted with C18 columns. Each sample fraction was dissolved in 2 µL of 5% ACN and 2% FA for subsequent MS analysis. LC-MS/MS was carried out by nanoflow reverse-phase liquid chromatography (Dionex Ultimate 3000, Thermo Scientific; Waltham, MA) coupled online to a Q-Exactive Plus Orbitrap mass spectrometer (Thermo Scientific). Briefly, LC separation was performed using a PicoFrit analytical column (75 µm ID × 25 cm long, 15 µm Tip ID (New Objectives, Woburn, MA)) in -house packed with 3 µm C18 resin (Reprosil-AQ Pur, Dr. Maisch, Germany). Peptides were eluted using a gradient from 3.8 to 98% solvent B over 46 min at a flow rate of 300 nL/min (solvent A: 0.1% formic acid in water; solvent B: 80% acetonitrile and 0.08% formic acid). Three kilovolts were applied for nanoelectrospray generation. A cycle of one full FT scan mass spectrum (300–1700 m/z, resolution of 35000 at m/z 200) was followed by 12 data-dependent MS/MS scans with a normalized collision energy of 25 eV. Raw MS data were processed with MaxQuant software (version 1.5.0.0) and searched against the human proteome database UniProtKB with 88,717 entries, released 2014-11. A false discovery rate (FDR) of 0.01 for proteins and peptides and a minimum peptide length of 7 amino acids were required. A maximum of two missed cleavages was allowed for the tryptic digest. Cysteine carbamidomethylation was set as fixed modification, whereas N-terminal protein acetylation and methionine oxidation were set as variable modifications.

Oligonucleotides used for sequencing, cloning, in vitro mutagenesis and morpholino mediated knockdown

Table S8. Oligonucleotides used for molecular genetics experiments

Gene	Exon	Forward	Reverse	Product
				size [bp]
		Sequencing of genon	nic DNA	
ASCC1	Ex 3	5-CCCAATTCAGACCTGCATAA-3	5-TTGATCCTACAATACACAGGACA-3	296
ASCC1	Ex 4	5-AGCGGTTGACAGCTGAGG-3	5-TCAAGCACTGCAATAAGTATGGA-3	275
ASCC1	Ex 5	5-TCTCTTGTCACAAAGGATTTAAACA-3	5-CCCATAGCTAGAACCCCACA-3	299
ASCC1	Ex 6	5-TCAGAACTGCTTTAGCTCCTCA-3	5-TTGGTGGGGGAAGAAGTTTA-3	390
ASCC1	Ex 7	5-TGGGCTGAGTTTCTTGTTTT-3	5-CTGAGAATTACTCACATACCAAAAAG-3	249
ASCC1	Ex 8	5-TTTTGAAATTAGAAGGGCAATTAGA-3	5-TCAAAGAAAAGGAATCAAAATGAA-3	381
ASCC1	Ex 9	5-GCAGGGCTTTGTATGGTGAT-3	5-TTGCCCACCTCTACTATGCTC-3	395
ASCC1	Ex 10	5-TCATTATGCCACTTTCCAGGT-3	5-TGAAGAAATATACGGAGAACTACTTG-3	298
ASCC1	Ex 11	5-GGGATTGTTGTGGTTTGGTG-3	5-CCCTGCTTGGCAATTAAAAC-3	300
		Sequencing of genon	nic DNA	
TRIP4	Ex 1	5-GGGATCTGCACTGGAGGC-3	5-GCACATCCCAGAGCTCCTT-3	289
TRIP4	Ex 2	5-CTCTGTTAAGATTACTGAGAAACAGTG-3	5-AGCCTGCCCACTTACCAATA-3	291
TRIP4	Ex 3	5-TTCACCAGGAATCCTCTTATCAA-3	5-TCACTTCCTCTTCTCCAATAGTCA-3	248
TRIP4	Ex 4	5-GGAGCATTTTCTCCCTTGAC-3	5-AGATCTGACTGGCTCTGCGT-3	352
TRIP4	Ex 5	5-GCAATATAATTTAACTGTTGGATCAC-3	5-ACAAGGTACCCATCATCCCA-3	178
TRIP4	Ex 6	5-TTGTTCTTTGTTGCACACTGG-3	5-ATGGCCAGAATCTGTGGACT-3	267
TRIP4	Ex 7	5-AATCGGTCCTTATTTGTCAACAG-3	5-GACCCTTTCTAGTGCTGCTCA-3	298
TRIP4	Ex 8	5-TGTTGGTGGTAAGCATTCTGAG-3	5-AAATTGGGGCCAAACTTCTT-3	244
TRIP4	Ex 9	5-ACCTACCCAGATTCTTGCC-3	5-TCTAAGATCTTCCACTGCCCA-3	285
TRIP4	Ex 10	5-TGGGATATTCCAGGAAACTAGA-3	5-AACGTCAATGACTTCTCTCCA-3	241
TRIP4	Ex 11	5-TCCTGAGTTCCAAGATCACAAA-3	5-ATGGCGAAACCCTGTCTCTA-3	199
TRIP4	Ex 12	5-GCTTGTCCCAACTATCCTGAA-3	5-TCCCCATTCCTTACTACTGCAC-3	204
TRIP4	Ex 13	5-TAACCAGAAGACCTGGGAGG-3	5-TTTCCAACTTGGTCCAATCTC-3	445

Tailed primers for the cloning of the in-situ hybridization probes								
Trip4	Notl-Sall	5-GGAGGA <u>GCGGCCGC</u> CGTACCGGGAGCCGCTAGTA-3	5-GGAGGA <u>GTCGAC</u> CTTCTGGCCCAGGCAATCAC-3	512				
		(<i>Not</i> l site underlined)	(Sall site underlined)					
Ascc1	Notl-Sall	5-GGAGGA <u>GCGGCCGC</u> TCCAGGAGGAGGTGCTGAGG-3	5-GGAGGA <u>GTCGAC</u> TCCAAAGCTGTCCACCGTGA-3	581				
		(<i>Not</i> l site underlined)	(<i>Sal</i> l site underlined)					
Two different sets of morpholinos used for <i>trip4</i> and <i>ascc1</i> knockdown								
trip4	МО	5-ATTGGTGCACACCTGAATGATGTGC-3						
ascc1	МО	5-TGCACCTGTGACCACTGAGAGAAC-3						
trip4	MO2	5-AGTGCGAGCTCACCAGACTGCCGCA-3						
ascc1	MO2	5-ATACACTGACTTGTAAAGCACACTG-3						
bactin	МО	5-CCTCTTACCTCAGTTACAATTTATA-3						
Primers used for RT-PCR quantification of trip4, ascc1, and control (bactin) mRNA abundance after MO and MO2-knockdown								
trip4	Ex4-5	5-CTCCCATTGACTTAATGAAGGCAC-3	5-GGTTCCCTCTCCCATCAG-3					
ascc1	Ex2-4	5-GCAGCAGTGGATCAGTGTC-3	5-CCCTGAGTCCTGTGAGC-3					
bactin	Ex1-2	5-CCTTCCTTCCTGGGTATGG-3	5-GGGGGAGCAATGATCTTGATC-3					
Primers used for cloning of full length trip4 and ascc1 constructs and for site directed mutagenesis								
trip4	cloning	5-GGAATTCGCCGCCACCATGAGCGATTCTTTGCTTCGCT	5-GCTCGAG <u>TCA</u> CGCTGGCTGCATTAAACACTT	1784				
		GGAC-3 (start codon underlined)	CTTG-3 (stop codon underlined)					
trip4	p.K265*	5-GAATTTGACAAGAACAGTGTT <u>T</u> AGAGAACACAAGTCT	5-CAAGACTTGTGTTCTCT <u>A</u> AACACTGTTCTTGT					
		TG-3 (mutant residue underlined)	CAAATTC -3 (mutant residue underlined)					
ascc1	cloning	5-GAATCGATGCCGCCACCATGGAGGTCTTACGGCCGCC-3	5-GTCTAGA <u>TCA</u> TGAGAATGTGATGTGTCCGGCTG-3	1165				
		(start codon underlined)	(stop codon underlined)					
ascc1	fs	5-CGATGCTCATAACATAG <u>G</u> AGCAGACGGAC-3	5-GTCCGTCTGCT <u>C</u> CTATGTTATGAGCATCG-3					
		(inserted nucleotides underlined)	(inserted nucleotides underlined)					

Antibodies for immunostaining, immunoprecipitation, and Western blot

Table S9. List of used antibodies

AB against	Species	Raised in species	Company	Order number
TRIP4	Humans	Rabbit-pAB (IgG)	ATLAS	HPA016605
ASCC1	Humans	Goat-pAB (IgG)	Santa Cruz	sc-160156
ASCC2	Humans	Rabbit-pAB (IgG)	Sigma/Prestige	HPA001439
CSRP1	Humans	Rabbit-pAB (IgG)	Abcam	ab70010
CALR	Humans	Rabbit-mAB (IgG), clone EPR3924	Millipore	MABT145
SP1	Humans	Rabbit-pAB	Millipore	07-645
MHC _{fast}	Humans	Rabbit-mAB (IgG1)	Novocastra/Leica	Clone: WB-MHCf
MHC _{slow}	Humans	Rabbit-mAB	Novocastra/Leica	Clone: WB-MHCs
MHC _{neo}	Humans	Rabbit-mAB (IgG1)	Novocastra/Leica	Clone: WB-MHCn
MHC _{dev}	Humans	Rabbit-mAB (IgG1)	Novocastra/Leica	Clone: WB-MHCd
GAPDH	Humans	Mouse-mAB (IgG1)	Ambion/Applied Biosystems	AM4300, Clone: 6C5
β-Tubulin	Humans	Rabbit-pAB (IgG)	Abcam	ab6046
pan-Actin	Humans	Mouse-mAB (IgG1), clone C4	Chemicon international	MAB1501R
znp-1	Zebrafish	Mouse-mAB (IgG2a,κ)	DSHB	znp-1
		AlexaFluor594-α-Bungarotoxin	Life Technologies/Molecular Probes	B-13423
		Protein G Sepharose 4B	Life Technologies	101241