

SUPPLEMENTARY MATERIAL

Reduced levels of ALS gene *DCTN1* induce motor defects in *Drosophila*

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Supplementary Figure 1.

Conservation of Dctn1 and Dred proteins.

Supplementary Figure 2.

Survival of adult flies with muscle-specific knockdown of *Dred* or *Dctn1*.

Supplementary Figure 3.

Muscle- or neuron-specific knockdown of *Dred* or *Dctn1* does not disrupt larval mobility.

Supplementary Figure 4.

Genes that are alternatively spliced in response to *Dctn1* silencing.

Dataset S1 (separate Excel file).

DEGs identified by RNA-seq on *Dctn1* knockdown.

Dataset S2 (separate Excel file).

DSGs identified by RNA-seq on *Dctn1* knockdown.

Dataset S3 (separate Excel file).

DSGs with a synaptic function downstream of *Dcct1* knockdown.

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dm -----MSEFNKLVGARVELTGKDLLGTVAIVGMTSFVAGKVGWV
hs MAQSKRHVYSRTPSGSRMSAEASAPLRVGSRVVEIGKGRGTVAIVGATLFAATGKVGWV

dm VLDEPKGNSSGSIKQQYFQCDENCGMVFRTQLRLLEAAPGSRSSIEDVSGATPTAAQP
hs ILDEAGKNDGTVQGRKYFTCDGEGHIFVRQSQIQVFEDGADITSPET-----PDSSAS

dm TKARLSSSRSTLSSSRSQSLGSRQLTTLTSLERTASSSSIGPRKSLAPQN-SKDKESSPT
hs KV--LKREGTDTAKTSKLR-----GLKPKKAPTARKTTTRPKPTR

dm SL---AEGAPAAASGGNGAASHASSKRASFVETGFLLELKQPTTSPQPLRSPSTMPNSNG
hs PASTGVAGASSSILGPGSGAS-----AGELSSSEPTPAQTPLAAPIIPTVLTLS

dm AEDKVALLEAQKTAELAQALADLTEKLETLKQRNEDKERLREFDMMKIQFEQLQEFRT
hs PGAVPPLPSPSKEEGLRAQVRDLEKELETLRLKRAEDKAKLKEKHKIQLEQVQEWKS

dm KIMGQAASLQKELLRAKQEAADAEAKEQHAQEMADLADNVEMITLDKEMAEKADTLQL
hs KMQEQQADLQRRLKEARKEAKEALEAKERYMEEMADTADATEMATLDKEMAEERAEALQQ

dm ELESSEMERIEVDLELLRESEMOMKAEASAINISGGGDSPLSTYEFKQLEQVQEWKS
hs EVEALKERVDELTDLEILKAEIEEK-----GSDGAASSYQLKLEQVQEWKS

dm TLVRDLSDAHDHDIQKLSKELEMKRSEVTELETKELSAKIDLELAIVADLQEQVDA
hs ALVRDLSSSEKQEHVKLQKLEKKEKQLEVVRQQRERLQELQSAESTIDELQEQVDA

dm ALGAEMVQLEAKEMLEEDKVKLLEEEIAQLEALEEVHEQVLENEHLELDRELDIA
hs ALGAEMVEMITDRNLNLEKVELERETVGLEAMNEMDLQENARETELELEQVDA

dm NGAKKEVLEERDAIETIYDRDQTIKVFRELQVQKINDQLTELRDNRSSNEKESLQDPSLK
hs GARVSEADQVRVEAEQVQVQTTIKYRQLTAHLQDVNRRLNQEQEASV-----ERQQQ

dm MVTETIDYKMFASKEYATRAIDVQLRQIELSQANEHVQMLTAFMPESFMSRGDDHSL
hs PPPETFDKIFKFAETKAHAKAIEMLEKQVEAQRNMSLITAFMPDSFLRPGDDHCVL

dm VILLISRVFKCDIVVSQTRERFPVDAITREAVTQGHAVQYAFKCRLLHYVHSLQCAL
hs VILLMPRLIKAEILRKQAEKFELESENCSERFGLGAAGQLSFAAGLVYSILSLQATL

dm HQILYGLNSQCDPTLLFAGSSLEPMVAQEKIVDGIIELLKSNQDENSTTONIEKVAFF
hs HRYEHALSQCSVDVYKVGSLYPEMSAHERSLDFIELLHKDQLEDTVNVPLTKAIKY

dm NAMNSVLLAGEQLNEIQMIRDCV-ASLGAACESILSDTAIAKVIIEAGATSVDLSLLIQ
hs QHLYSHLAEQPEDCTMQLADHIKFTQSALDCMSV--EVGRRLRAFQGGQEQATDIALLLR

dm FLNENMESVRQVKLIRRLPSDQHVIK-SGLSQ--HKVEAMRGLAQNISRIMSAMHAT
hs DLETSCSDIRQFCKKIRRRMPGTDAFGIPAAALAFGPQVSDTLLDCRGLHTWVAVLQVEA

dm KQSLAAIVSTIESDNAAEHTLPQEKYIALLTASCERIEYQDDRGPTQNFKTLAQANSDL
hs -AAAQILAPLAEN-----EGLVLAALAEAFKASEQIYTPSSSPYECLRQSCNLISTM

dm QLIAQHLLDKEYDIISAANNASNQKSGAHSPTITQRAQLIKQLEQKIVLAATLENREA
hs NKLATAMQEGEYDA-----ERPPSKPPVELRAALAEITDAEGLGLKLEDRET

dm DVKQLKVAARKQNELSEMQRKDLAEKLSVLQNEYEHAVDKWRQYETSLSQLQLEKE
hs VIKELRKSILKIGEELESEANVRLSILEKKLDSAAKADERIEKVQTLRETTQALLRKKEK

dm EFEETMDHLQSDIDALESEKSDRLKILNISTGKVGQPSSESHSPHNS----LSGNTS
hs EFEETMDALQADIDQLEAEKAEKRLQSLNSQSKR--TIEGLRGPPPSGIATLVSGIAGEEQ

dm ---TAPGISNVYSAP--AGTAPVVAEVELLNANFNQRNQRLRLQAQDMKAKLSQFEP
hs QRGAIPOQAPGSPGVPGFLVQSLPLLLQQISAMPLHISQLQHENSILKGAQMKAASLASLPP

dm LHVPOPDQORITA-LE-SELTRMKHAWVLSLLQVRSQDSVNSGTRIDAVALQRR--NQP
hs LHVVALSHGPGSELPAAGALYRKTSQLETLNQSLSTHVVDTITRTSPAASPSAQIMEQ

dm VPLKEISSKASQASDILTEYLQKPHRATHGQFASFPPTVDVKVLIQI-----
hs VAQLKLSLSDTVKELKDEVLKETVSQRPGATVPTDFATFPSSAFRLAKEEQDDTVYMGKV

dm ----- 1265
hs TFSCAAGFGQRHRLVLTQEQHLQHSRLIS 1278

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dm -----MMSPRELKLQGVREVTGKNLQGVKAYVGRTNFAAGLWYGV
hs MAQSKRHVYSRTPSGSRMSAEASARPLRVGSRVVEIGKGRGTVAIVGATLFAATGKVGWV

dm VLDEPLKGNSSVHSGSIYFKCPTNCGLFVRAQQLVRIAEPLKGGDNKKADEMQRDGRARAK
hs ILDEAGKNDGTVQGRKYFTCDGEGHIFVRQSQIQVFED--GADTTSPET--PDSSASK

dm LSRRSGSGKSVEEQDNQREQQASTSGKVKATSTSPSPQHKNRNTSHSMETSLAKTSKGFL
hs VLKREGTDTAK-----TSKLR-----

dm ATHQQLQLPKNFVTCESSVRNSQSKETGEVTLSPKSSDIEQSKKHSQQETNETNPAS
hs -----GLKPKKAPTARKTTT--RRPKPTRPAS

dm KEVEPKNENELQSTEAANSMLDTPNPLQTSITQQLPQKASQTLPPPLTCNQRSTSY
hs TGVAG-----A-----SSSLGPGSASAGELSS-----

dm TQLRPTRISQPKPTTAQASSTAQTLAMPVFLALAPKRKSTSMSPSSIKRVPAAPAVE
hs -----SEPST-----PAQTPFLA-----

dm PRFLEILRPQTPGPALRTPSSVAPPLD-----NPELRQLREELQLLRGKSKEDKL
hs -----APIIPTVLTSPGAV-PPLPSPSKKEEGLRAQVRDLEKELETLRLKRAEDKA

dm KLELEHMRHINQIMEFKSQIMAQVLLQELQRSRHLREAEQVSSSLKRLRELDIAES
hs KLELEHMKIQLEQVQEWKSQEQEQADLQRRLKEARKEALEAKERYMEEMADTADA

dm IEILTLDKEMAEERMETLQMLEMAQERNDLSLQVEILKAEQEQQRTEKSKSIGS
hs IEMATLDKEMAEERAEISLQVEALKEVDELTDLEILKAEIEEKSGSGAA-----

dm GVTNQSAGFLRLQEQNRQLRETVRRLDTEAEQIGQRTHEKLETKHSEINELKSKE
hs -----SSYQLKLEQVQVRLKDALVRDLSSSEKQEHVRLQKLEKKEKQVLEVRQEQE

dm LLSRRVDNMEMQMLDLKEQVDASLGAEMVTLQASLKLLEBDRVKLLEEVNELEAEQI
hs RLQELQSAESTIDELQEQVDAALGAEMVEMITDRNLNLEKVELERETVGLEAMNEM

dm QEQLIESNQLETDLREEIDKLGHVILEQKQNAAMESLYDRVITMKFDRVLRLQEQ
hs NDELQENARETELELRQQLDMAGAVRQVLEAEQVLEADYQQTIKYRQLTAHLQVQV

dm LQLRADGTLSEIDFSSANESQEDGSGNQSDTYQHIFSVSKAYGRALEQKIKTVLRLQR
hs NRELTN-----QQEAS---VERQQPPPETDFDKFAETKAHAKAIEMLEKQVEAQRN

dm QHLEHVLAFVPEQFLRGGEGHDVVLMLLERNEKLTIVCQAINKEFTACEFGRDAIF
hs RHMSLITAFMPDSFLRPGDDHCVLWVAVLQVEVAAQAQIAPLANEGILVAAL-----EE

dm EGYSVQPIYFRSQCLYLLKSLQLVQQFRHGLTHCDYELCTHAAYRSOLDQAQEQQLDEF
hs RGAAGEQLSFAAGLVYSLSLQATLHRYEHALSQCSVDVYKVGSLYPEMSAHERSLDFL

dm VRLKTKQLDEHTNCEPIQRVLHYVSLGNLNM---PPQTLVELDEQQLYEALIEVYEA
hs IELLHKDQLEDTVNVPEPLTKAIKYQHLYSHLAEQPEDCTMQLAD--HIKFTQS

dm GLDAVNANAGLMHTIIQLGHEQTASFSCQMLQEQSCAHKQ-KLKKLQRLKSGSKTASWT
hs ALDCMSVEVGRRLRAFQGGQEQATDIA-LILRLDLESCSDIRQFCKKIRRRMPGTDAFGIP

dm GMQCAR---YQIMEANEALGTILIRLIGCTARE----ASKDSNGGIAHEKLWRMLVNY
hs AALAFGPQVSDTLLDCRHLTWVAVLQVEVAAQAQIAPLANEGILVAAL-----EE

dm NKFAPSQEA---EEPREVDAYSQRCMLLEEQFDELFAALLDSTVNTVEYVRHPT-CNTLQ
hs LAFKASEQIYGTSPSSPYECLRQSCNLIST-MNKLATAMQEGEYDA--ERPPSKPPVVE

dm ERAAQVRHYEDVKNLELTVAREKEIKSLKYTAKMKQDYQSELQVRKEMAEKQSKQCH
hs LRAAALFAEITDAEGLGLKLEDRETVIRKELKKSILKIGEELESEANVRLSILEKKLDSAAK

dm VLAGFAEA----VEQLQSLILAKAALGQALNMLADKLTSLEQSQEHWKEQQ-----
hs DADERIEKVQTRLEETQALLRKKEKEFEETMDALQADIDQLEAEKAEKRLQSLNSQSKRTI

dm -----ADSAEL-----TSTTSSN-----RENNMLHQALR
hs EGLRGPPPSGIATLVSGIAGEEQQGAIPGQAPGSPVPGPLVQDPSPLLLQQISAMRLHIS

dm QERSLRVELQSGEMKTFAALEPLHVPAQSGELTD-LE-----KDLRLSKNQ
hs QLQHENSILKGAQMKAASLASLPLHVAKLSHGGPGSELPAAGALYRKTSQLETLNQSLSTH

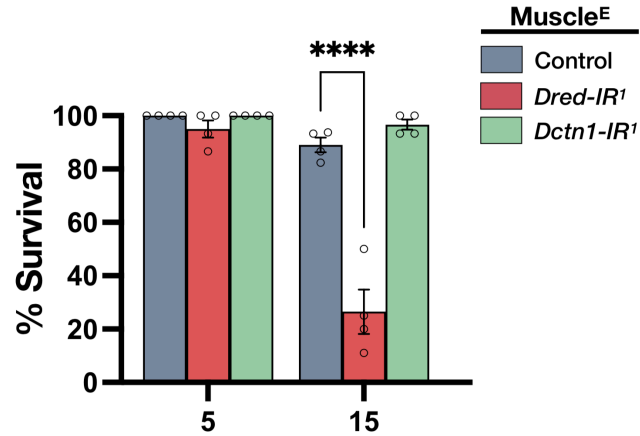
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hs THVVDITRTSPAASPSAQIMEQVQVQLKSLSDTVKELKDEVLKETVSQRPGATVPTDFAT

dm FITEDLRRRA-----FGQNF-----
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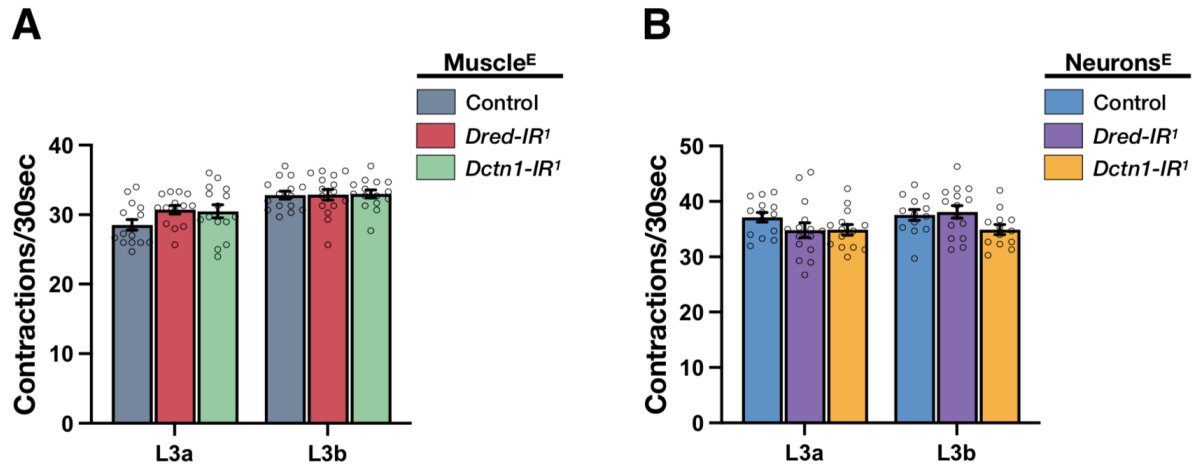
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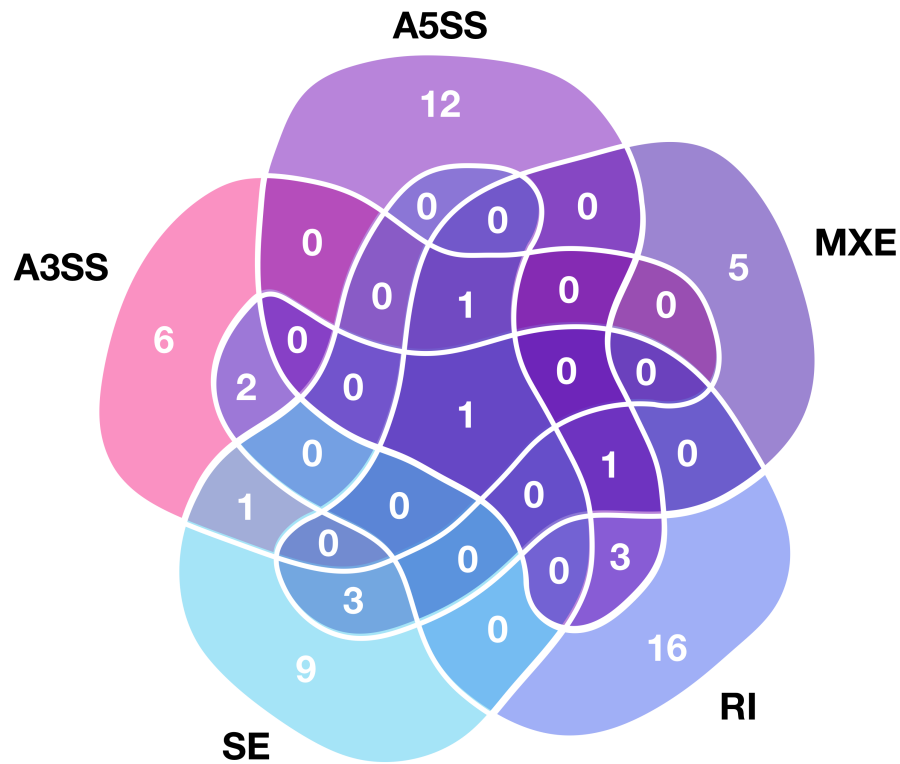
Supplementary Figure 1. Conservation of Dctn1 and Dred proteins. Protein alignment of human (hs) DCTN1 and *Drosophila* (ds) orthologues Dctn1 (A) or Dred (B).



Supplementary Figure 2. Survival of adult flies with muscle-specific knockdown of *Dred* or *Dctn1*. Flies with loss of *Dred* function in muscles have reduced survival as early as day 15 post-eclosion. Each bar represents the mean $\pm$ SEM of 4 independent experiments superimposed on the bars (for each genotype, $n \geq 15$ flies/replicate). Significance was tested by two-way ANOVA with Dunnett's *post hoc* test (**** $p < 0.0001$). Enhanced knockdown was achieved by co-expression of *Dcr-2* (indicated by E in superscript). Control expressed both driver (*Mef2-GAL4*) and *Dcr-2*.



Supplementary Figure 3. Muscle- or neuron-specific knockdown of *Dred* or *Dctn1* does not disrupt larval mobility. **(A)** Body wall contraction rate of L3 larvae with muscle-selective downregulation of *Dred* or *Dctn1* assessed at 72 hours (L3a) and, subsequently, 96 hours (L3b) after egg laying ($n \geq 13/\text{genotype}$). **(B)** Body wall contraction rate of L3 larvae with neuron-selective downregulation of *Dred* or *Dctn1* assessed at 72 hours (L3a) and, subsequently, 96 hours (L3b) after egg laying ($n \geq 13/\text{genotype}$). For both **(A)** and **(B)**, each bar represents the mean $\pm$ SEM of several independent experiments superimposed on the bars.



Supplementary Figure 4. Genes that are alternatively spliced in response to *Dctn1* silencing. Venn diagram showing overlap of differentially spliced genes (DSGs) across the five modes of alternative splicing including alternative 3' splice site (A3SS), alternative 5' splice site (A5SS), mutually exclusive exon (MXE), retained intron (RI) and skipped exon (SE).