Supporting Information

Lyssand et al. 10.1073/pnas.1010819107

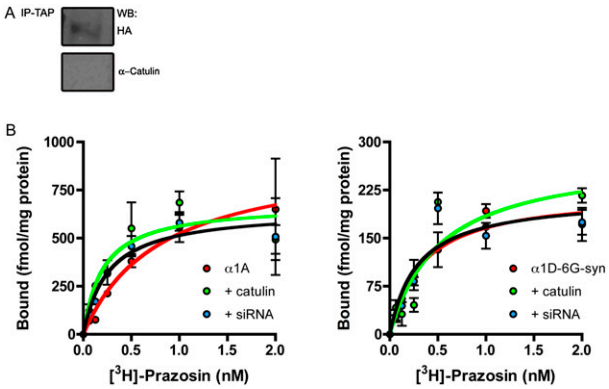

Fig. S1. (*A*) TAP-α1A-AR was immunoprecipitated from HEK293 cells, run on SDS/PAGE, and probed for α1A-AR (HA-tag) and α-catulin. (*B*) Effects of α-catulin overexpression (green trace) and siRNA knockdown (black trace) were compared with receptor alone (red trace) for both the α1A-AR (*Left*) and α1D-AR (*Right*). TAP, tandem-affinity purification; AR, adrenergic receptor.

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Human αDB       SASACSTPTHTPQDSLTGVGGDVQEAFAQSSR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETESNVDSEF
Human βDB       STSAGSTPTHCPQDSLSGVGGDVQEAFAQGTR--RNLRNDLLVAADSITNTMSSLVKELHSAEEGAEEEEEKMQN

α-DB:
Human           SASACSTPTHTPQDSLTGVGGDVQEAFAQSSR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETESNVDSEF
Chimp           SASACSTPTHTPQDSLTGVGGDVQEAFAQSSR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETESNVDSEF
Mouse           SASACPTPTHTPQDSLTGVGGDVQEAFAQSSR--RNLRSDLLVAADSITNTMSSLVKELNSEVASETESTVDSEF
Cow             SASACSTPTPTPQDSLTGVGGDVQEAFAQSSR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETDSNVDSEF
Dog             SASACSSPAHTPQDSLTGVGGDVQEAFAQSSR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETESNVDSEF
Chicken         SASACSTPTHTPQDSLTGVGGDVQEAFAQSAR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETESNVDSEF
Zebrafish       STSGNTTPSQTPQDSLMGLGGEVQQAFAQGSR--RNLRSDLLVAADSITNTMSSLVKELNSEAGNESNSISDFMF
Frog            SASACSTPTPTPQDSLTGVGGDVQEAFAQSSR--RNLRNDLLVAADSITNTMSSLVKELNSEVGSETDSNVDSEF
Worm            SASLSQLPFTASDQQLTGVNSTVANAFRAGSLPATSLQGDLLHAADQITTNMSDLVRQLDLAQSDENGVTING-F
                *:*    *  :.:::.* *:.. * :**  .:    .*:.*** ***.**..**.**::*:   ..*.    :  *

    • - single, fully conserved residue
    • : - conservation of strong groups
    • . - conservation of weak groups
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Fig. S2. The C terminus tail of human α-DB1 (amino acids 578–651) was aligned against the C terminus of human β-DB (*Upper*) and the C terminus tails of α-DB genes across multiple species (*Lower*). DB, dystrobrevin.

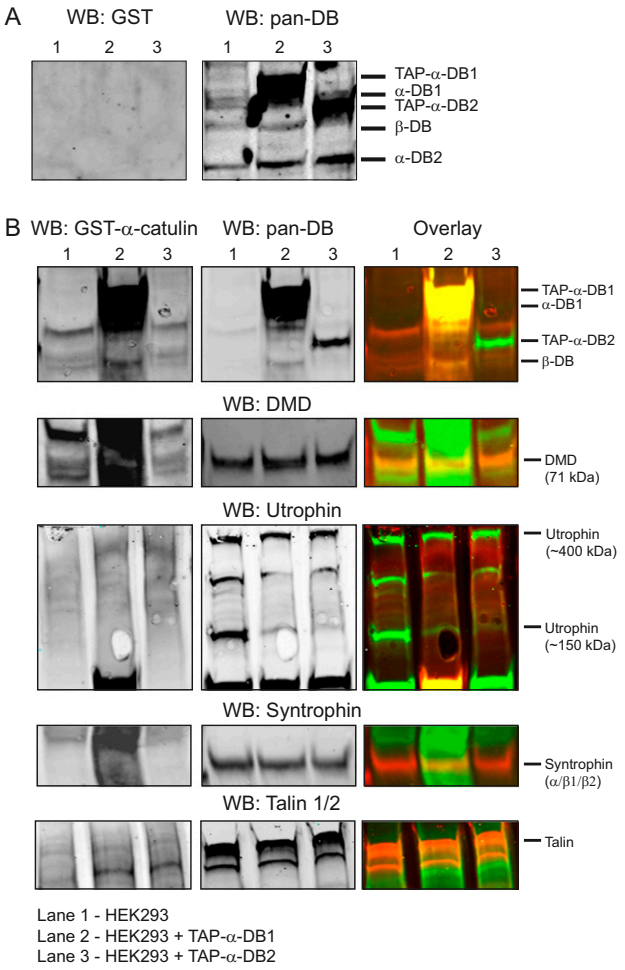

Fig. S3. Untransfected (lane 1), TAP-α-DB1–expressing (lane 2), and TAP-α-DB2–expressing (lane 3) cell lysates were run on SDS/PAGE gels, transferred to nitrocellulose, and overlaid with either (*A*) GST or (*B*) GST-α-catulin. The GST Western blot on the right indicates which protein bands GST or GST-α-catulin bound to, center Western blots are as indicated, and left images are the combined images. For dystrophin, syntrophin, and talin combined images, GST-α-catulin is in green and unknowns are in red. For all remaining images, GST-α-catulin is in red and unknowns are in green. TAP, tandem-affinity purification; DB, dystrobrevin.

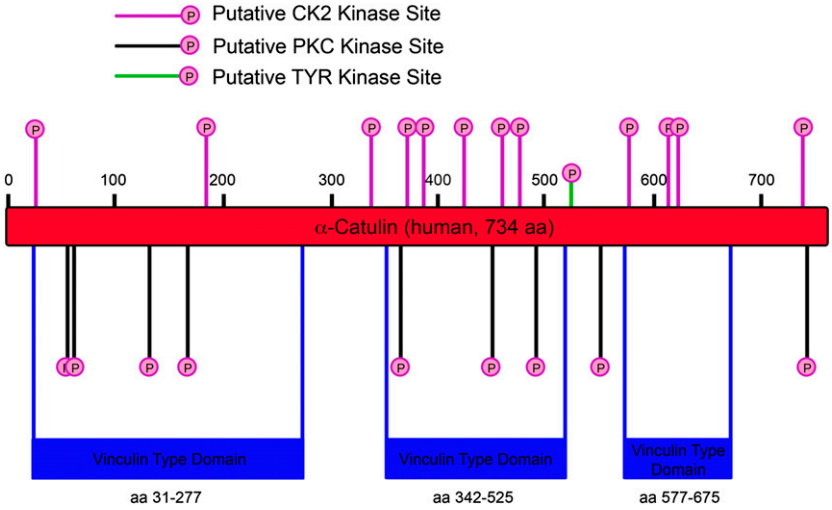

Fig. S4. A pfam protein domain analysis indicated that α-catulin has three domains homologous to vinculin (domains are highlighted in blue). A PROSEARCH motif search found consensus caseine kinase (CK2, pink), protein kinase C (PKC, black), and receptor tyrosine kinase (TYR, green) sites in α-catulin. Approximate locations of phosphorylation sites are indicated.

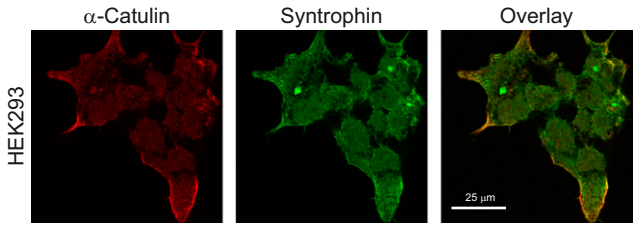

Fig. S5. Syntrophin (green) and α-catulin (red) colocalize in HEK293 cells.

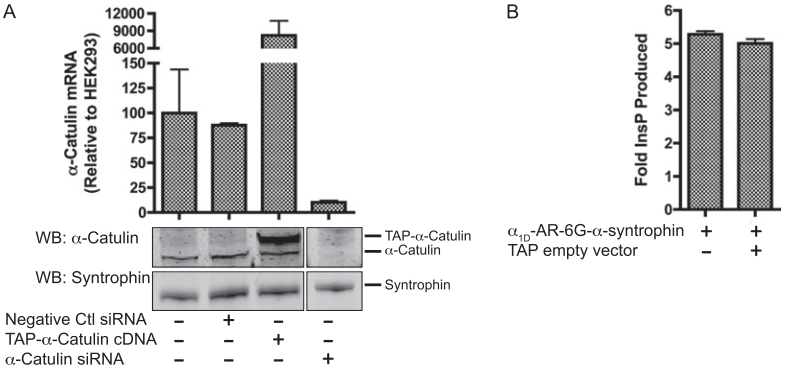

Fig. S6. (*A*) α-Catulin mRNA and protein levels are measured after treatment for 48 h with siRNA directed against α-catulin. (*B*) An empty TAP vector was cotransfected with α1D-AR-6G-α-syntrophin to assess the affect of the TAP vector on α1D-AR-6G-α-syntrophins IP3 production in response to 100 μM phenylephrine. Data are graphed as fold over basal measurements. TAP, tandem-affinity purification.

Table S1. TAP/MS results for bait proteins

	DMD		UTR		SNTA1		SNTB1		SNTB2		DTNA		DTNB		CTNNAL1		PPFIA1	
Bait	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
α_{1D}-AR	—	—	3	2	—	—	10	22	6	12	2	4	—	—	1	3	—	—
α-Syntrophin	34	26	220	41	73	28	2	6	16	29	44	43	9	19	18	24	—	—
β_1-Syntrophin	10	3	49	17	1	5	7	15	—	—	8	12	—	—	2	2	—	—
β_2-Syntrophin	21	4	105	34	—	—	—	—	15	22	28	35	1	1	15	20	—	—
α-DB1	7	2	12	5	28	53	63	64	41	43	158	38	—	—	60	44	14	17
α-DB2	8	3	18	7	28	51	64	61	56	48	110	44	—	—	—	—	6	5

Total spectral counts (*n*) and percentage amino acid coverage of spectral counts are shown for each protein hit. DMD, dystrophin; UTR, utrophin; SNTA1, α-syntrophin; SNTB1, β1-syntrophin; SNTB2, β2-syntrophin; DTNA, α-dystrobrevin; DTNB, β-dystrobrevin; CTNNAL1, α-catulin; PPFIA1, liprin-α-1.

Table S2. Complete TAP/MS results for α-catulin

Total	Unique	AA coverage (%)	Best gene name	Description
232	44	60.1	*CTNNAL1*	α-Catulin
67	30	41.7	*DTNA*	α-Dystrobrevin
47	41	15.2	*UTRN*	Utrophin
37	18	31.2	*DMD*	Dystrophin
27	20	36.5	*SNTB2*	β2-Syntrophin
26	17	40.7	*SNTB1*	β1-Syntrophin
12	6	17.9	*DTNB*	β-Dystrobrevin
7	7	8.8	*PPFIA1*	Liprin-α-1
7	7	10.2	*HRNR*	Hornerin
5	3	2.8	*COL1A1*	Collagen α-1(I) chain precursor
4	4	21.6	*CAPNS1*	Calpain small subunit 1
4	4	12.4	*TUBB*	Tubulin, β polypeptide
3	3	6.6	*HSPA5*	HSPA5 protein
3	3	16.0	*RPS3*	40S ribosomal protein S3
3	3	7.1	*SNTA1*	α1-Syntrophin
3	3	11.8	*RPS4X*	40S Ribosomal protein S4
3	2	1.8	*MYLK2*	Myosin light chain kinase
2	2	12.1	*C1QBP*	Complement component 1 Q
2	2	2.4	*PPFIBP1*	Liprin-β-1
2	1	6.8	*HBE1*	HBE1 protein
2	2	9.6	*PPP1CC*	Serine/Threonine Protein Phos.
2	1	6.1	*SBSN*	Suprabasin
1	1	0.8	*PLCB2*	PLC β2
1	1	3.4	*PLEKHM1*	PH domain containing, family M1
1	1	5.5	*NDUFA12*	13-kDa differentiation-associated protein
1	1	4.6	*ITPA*	Inosine triphos. pyrophosphatase
1	1	1.9	*ALDH5A1*	Succinic semialdehyde dehydrogenase
1	1	2.0	*SGSH*	N-sulphoglucosamine sulphohydrolase
1	1	0.8	*ARHGEF5*	RhoGEF5
1	1	1.2	*EDD1*	E3 ubiquitin-protein ligase EDD1
1	1	3.3	*TUFM*	Tu translation elongation factor
1	1	10.0	*PIF*	Proteolysis inducing factor
1	1	1.4	*MAIL*	Nuclear factor of kappa light polypeptide
1	1	9.9	*S100A4*	Protein S100-A4
1	1	2.6	*PLCXD2*	Phosphatidylinositol phospholipase CX
1	1	15.6	*HEJ1*	HEJ1
1	1	5.5	*DIRAS2*	GTP-binding protein Di-Ras2
1	1	7.4	*CALM3*	Calmodulin
1	1	8.7	*PATE*	PATE
1	1	4.6	*TUBA1C*	Tubulin, α 1C
1	1	3.8	*RBMY1D*	RNA binding motif protein 2
1	1	0.9	*SYCP1*	Synaptonemal complex protein 1
1	1	10.8	*RPS15A*	40S ribosomal protein S15a
1	1	16.3	*RPS17*	40S ribosomal protein S17
1	1	3.7	*GAD1*	Putative uncharacterized protein GAD1
1	1	23.9	*S100B*	Protein S100-B
1	1	1.9	*ZHX3*	Zinc fingers and homeoboxes protein 3
1	1	0.3	*NEB*	Nebulin
1	1	0.7	*ITIH5*	Interalpha inhibitor H5
1	1	1.1	*NLRC5*	Nucleotide-binding oligomerization domains 27
1	1	0.6	*PTPRD*	Protein tyrosine phosphatase, receptor type, D
1	1	2.1	*IFPS*	Ifapsoriasin
1	1	9.7	*FLJ46266*	Hypothetical protein FLJ46266
1	1	1.3	*GTF3C1*	General transcription factor IIIC, polypeptide 1
1	1	5.4	*C3orf33*	Uncharacterized protein C3orf33
1	1	20.0	*ZNF14*	Zinc finger protein 14
1	1	2.1	*KLC3*	Kinesin light chain 3
1	1	1.1	*XPO5*	Exportin-5
1	1	6.2	*TUBA8*	Tubulin α-8 chain

Total spectral (peptide) counts, unique spectral (peptide) counts, percentage amino acid coverage (AA coverage), gene name (Best gene name), and a brief description of each hit are shown.

Other Supporting Information Files

Dataset S1 (XLS)