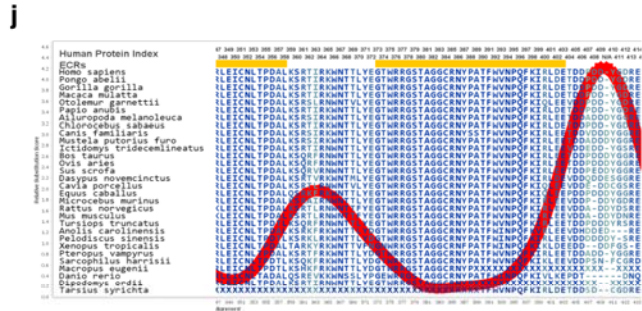
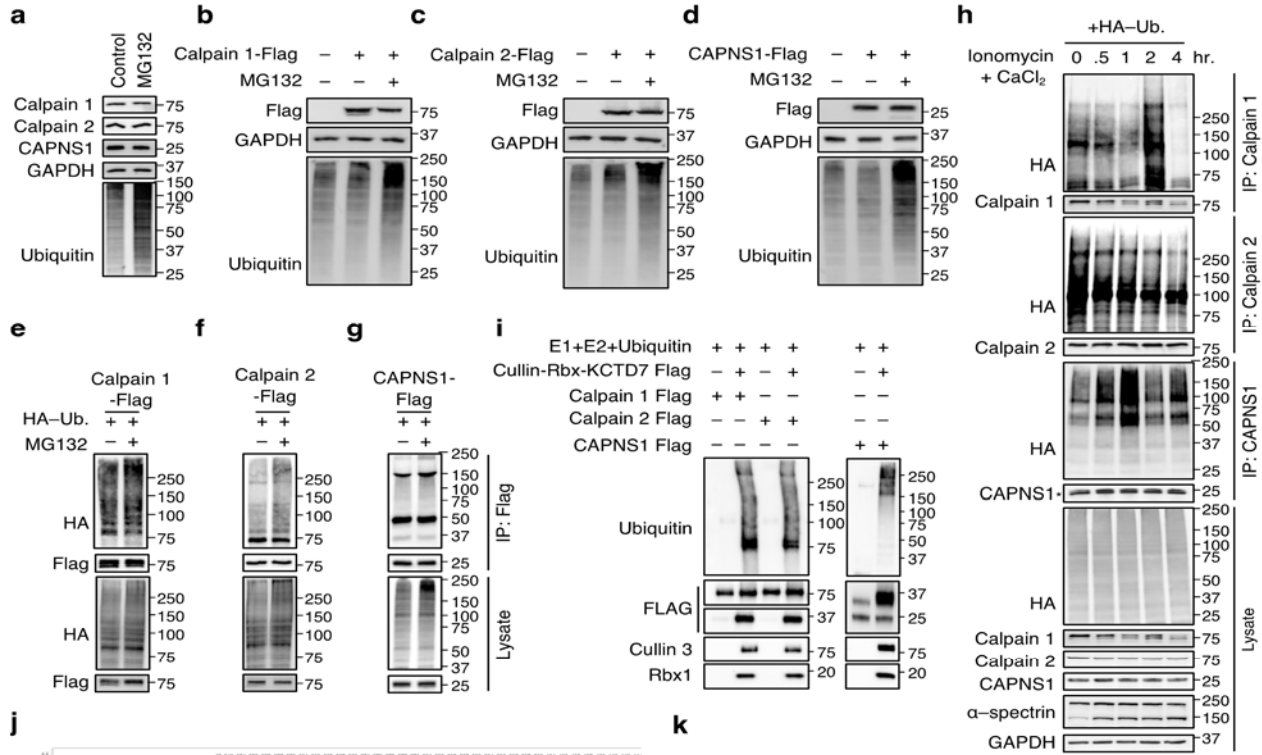
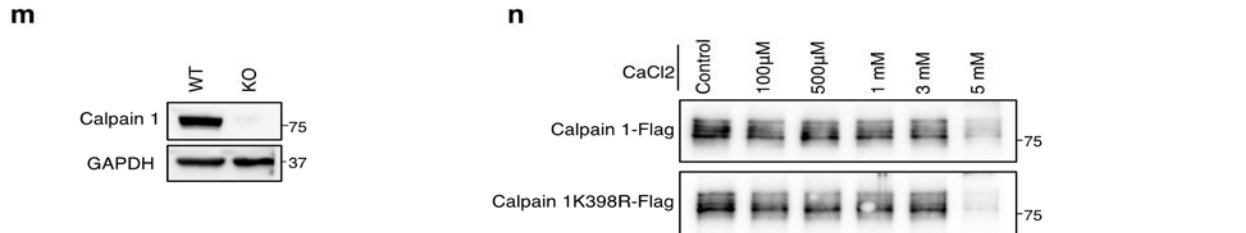


Fig. S1: Interaction of KCTD7 with Cullin-3 and calpains. **a**, Schematic representation of the pGLUE-KCTD7 expression vector. Shown are the streptavidin-(streptag) and calmodulin-(CBP) binding affinity tags placed in tandem, along with an HA epitope for protein detection, placed at the N-terminus of full length human KCTD7. **b**, Immunoblot analysis of HEK293 cell clones stably expressing pGLUE-KCTD7 using α -HA antibody. **c**, Silver staining gel analysis of affinity purified proteins. **d**, Immunoblot analysis of affinity purified proteins using α -HA antibody to detect KCTD7. **e**, Co-IP analysis of KCTD7 deletion constructs and calpain 1-Myc in HEK293 cells. **f**, Co-IP analysis of calpain 1 deletion constructs and KCTD7-Myc in HEK293 cells. **g**, Western blot analysis of subcellular fractions of HeLa cell lysates after differential velocity centrifugation. Equal amounts of protein from each fraction were separated by SDS-PAGE. **h-j**, Calpain 1 (h), calpain 2 (i), and CAPNS1 (j) were knocked down in HEK293 cells and, 24 h later, cells were transfected with plasmids expressing KCTD7 and Cullin-3 for 48 h. Shown are co-IP analyses of KCTD7 and Cullin-3 in the tested conditions. **k, l**, Evaluation of shRNAs and siRNAs for KCTD7. KCTD7-Flag was transfected in HEK293 cells and, 24 h later, cells were transfected with the indicated shRNAs (k) or siRNAs (l) for 48 h. Cell lysates were immunoblotted to detect KCTD7 expression using α -Flag antibody. **m, n**, Immunoblot analysis of KCTD7 knock-out HeLa cells. **o, p**, KCTD7-Flag was expressed in HEK293 cells for 36 h and α -spectrin cleavage was evaluated by immunoblotting using α -spectrin antibody. Data represent mean \pm SEM, $n = 3$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, ns = not significant.



l

Gene name	GlyGly (K) site	GlyGly (K) Probabilities	Sequence window	n=1		n=2	
				pcDNA	KCTD7	pcDNA	KCTD7
CAPNS1	398	NYPATFWVNPQF(K)IR	GCRNYPATFWVNPQFKIRLDEDDP DDYGDR	0	68607	0	40721



Sup. 2

Fig. S2: Calpains are regulated via ubiquitin-mediated modification. **a**, Immunoblot analysis of HEK293 cells treated with MG132 (10 μ M) or vehicle for 4 h using antibodies for calpain 1, calpain 2, and CAPNS1. GAPDH was used as a loading control. **b-d**, Immunoblot analysis of HEK293 cells expressing calpain 1 (b), calpain 2 (c), or CAPNS1 (d) and treated with MG132 (10 μ M) or vehicle for 4 h before protein collection. GAPDH was used as a loading control. **e-g**, In vivo ubiquitination assay for calpain1 (e), calpain 2 (f), and CAPNS1 (g) performed by coexpressing either protein with HA-Ub in HEK293 cells for 36 h and treating with MG132 (10 μ M) or vehicle for 4 h before protein collection. **h**, In vivo ubiquitination assay for calpain1, calpain 2, and CAPNS1 performed after treatment with 1 μ M Ionomycin along with 5 mM CaCl₂ for the indicated times. The lysates were also blotted for α -spectrin to evaluate calpain activity. **i**, In vitro ubiquitination assay of calpain 1, calpain 2, and CAPNS1. **j**, Evolutionarily constrained region analysis of calpain1. Shown is a multi-alignment of calpain1 protein sequences along with a plot of local evolutionary rates. Evolutionarily constrained regions (ECRs) are indicated with yellow lines. **k-l**, Mass spectrometry data of enzymatic digested peptides from CAPN1-Flag was acquired on nanoElute coupled to a timsTOF Pro2 Mass Spectrometer and analyzed using MaxQuant v2.1.0.0. The ubiquitination site of CAPN1-Flag on K398 is highlighted and shown on the peptide sequence. Trypsin digestion of ubiquitin conjugates generates a diGly tag that is formed at the ubiquitinated lysine residue. **m**, Immunoblot analysis of calpain 1 knock out HeLa cells. **n**, Immunoblot analysis of calpain autolysis by incubating Flag tagged WT calpain 1 or calpain 1-K398R immunopurified proteins, from HEK293 cells, with indicated calcium concentrations.

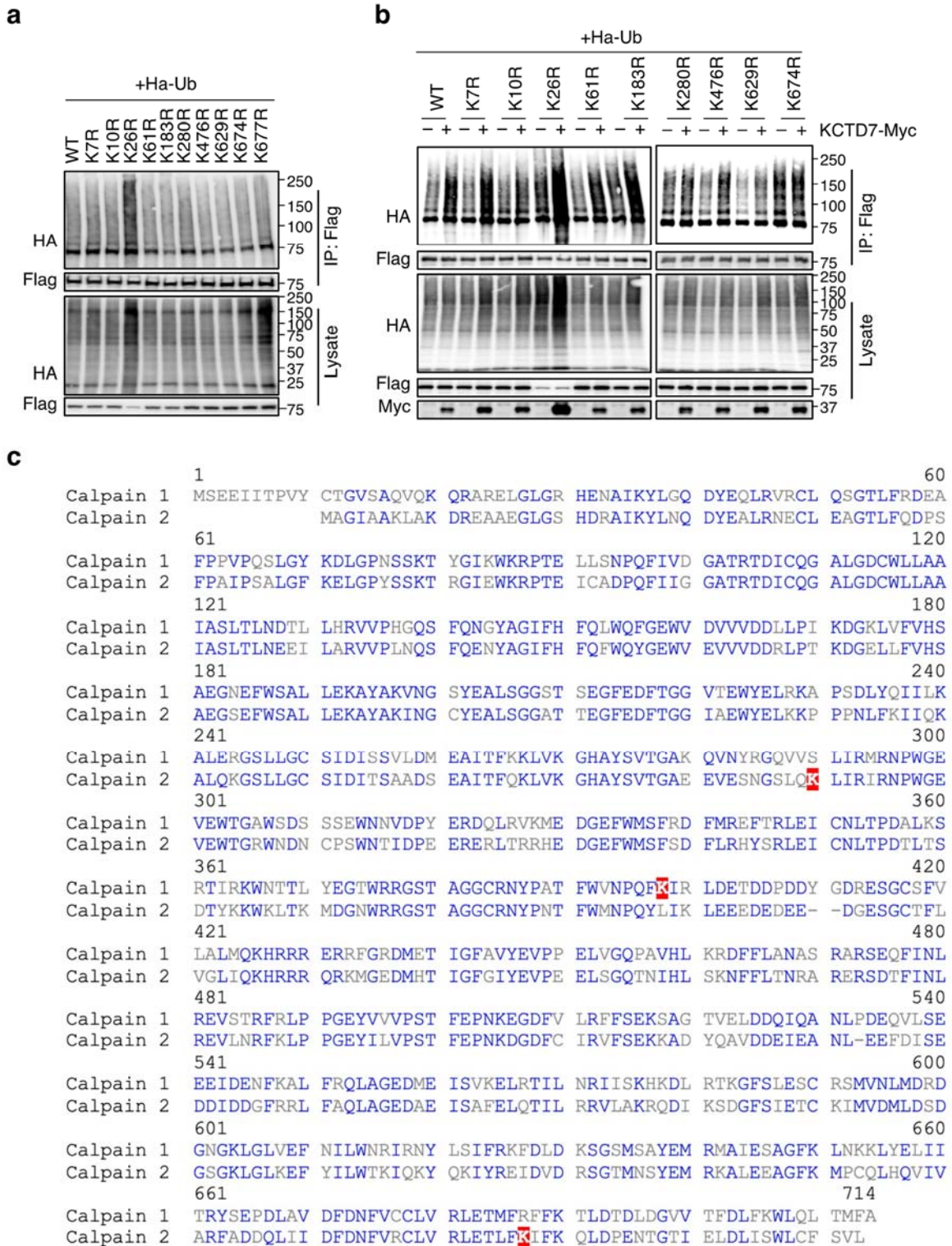


Fig. S3: Determination of KCTD7 mediated ubiquitination site in calpain 2. a, In vivo

ubiquitination assay in HEK293 cells for calpain 2 K—R mutants. **b**, In vivo ubiquitination assay

for calpain2 K—R mutants performed with or without KCTD7 in HEK293 cells. **c**, Multiple alignment of calpain 1 and calpain 2 proteins. Lysine residues ubiquitinated by KCTD7 are highlighted.

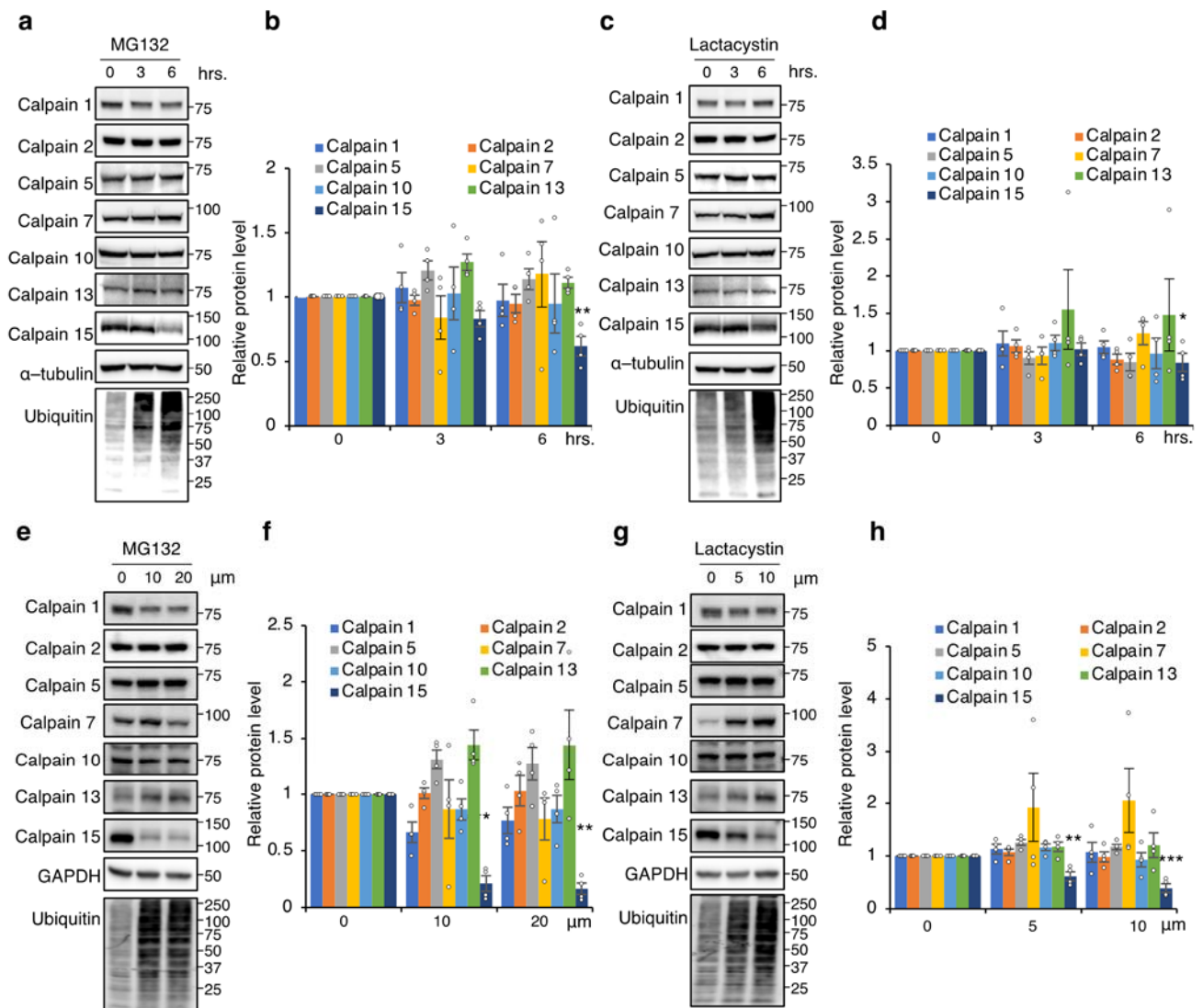


Fig. S4: Calpains are regulated via ubiquitin-mediated modification. **a, b**, Immunoblot analysis of HEK293 cells treated with MG132 (10 μM) or vehicle using antibodies for ubiquitous expressing calpains. **c, d**, Immunoblot analysis of HEK293 cells treated with lactacystin (20 μM) or vehicle using antibodies for ubiquitous expressing calpains. **e, f**, Immunoblot analysis of HEK293 cells treated with MG132 or vehicle for 24 h using antibodies for ubiquitous expressing calpains. **g, h**, Immunoblot analysis of HEK293 cells treated with lactacystin or vehicle for 24 h using antibodies for ubiquitous expressing calpains. Data represent mean ± SEM, $n = 5$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, ns = not significant.

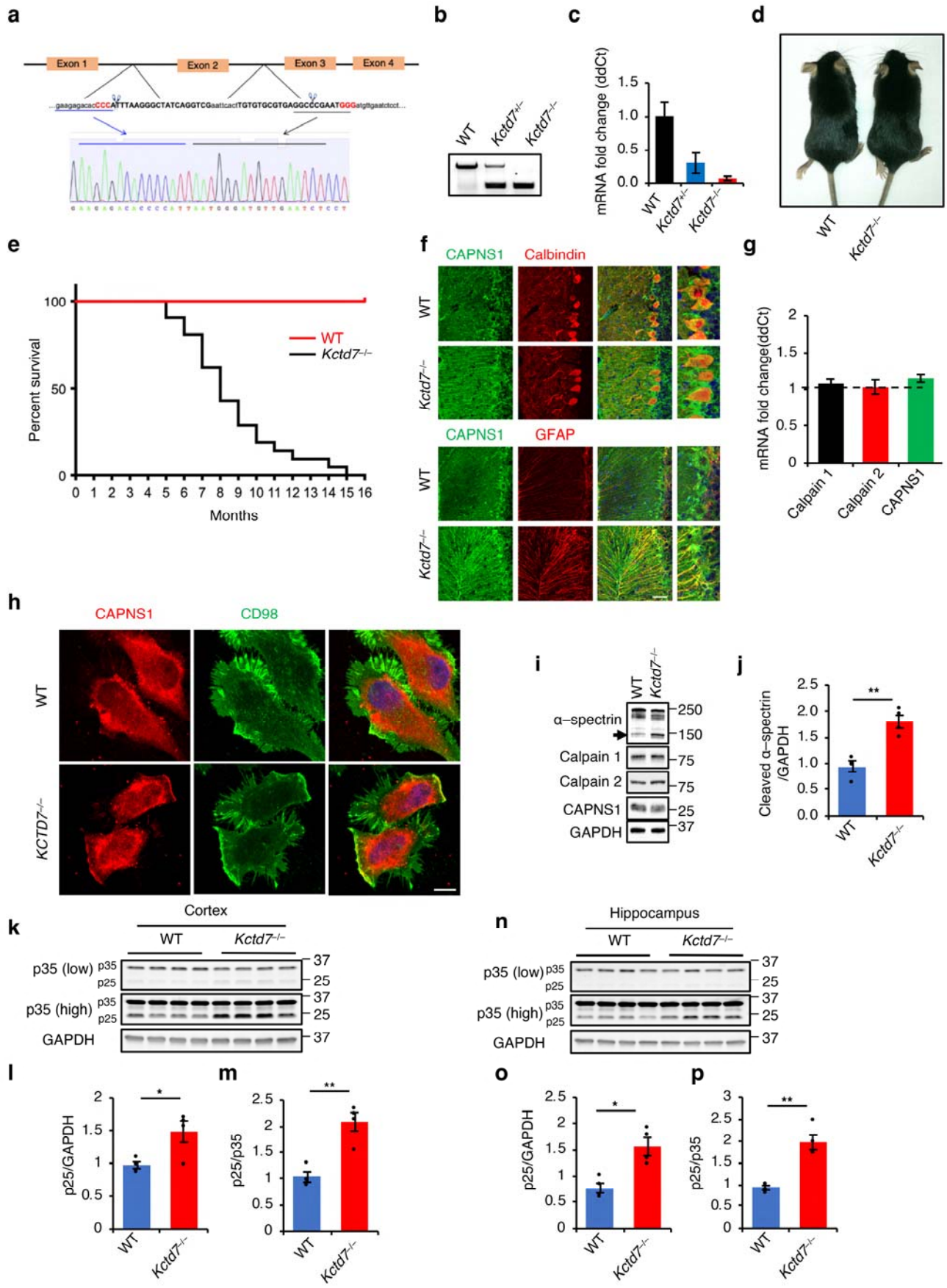


Fig. S5: Generation of a *Kctd7*^{-/-} mouse line using CRISPR/Cas9 technology. **a**, Two gRNAs were designed to cut both ends of exon 2 of the *KCTD7* gene. **b**, Agarose gel electrophoresis analysis of PCR products using genomic DNA from a WT mouse and heterozygous (*Kctd7*^{+/-}) and homozygous (*Kctd7*^{-/-}) mice from the selected *Kctd7*^{-/-} mouse line. **c**, RT-qPCR analysis of mRNAs extracted from WT, *Kctd7*^{+/-} and *Kctd7*^{-/-} mouse brain homogenates showing transcriptional levels of *Kctd7* ($n = 3$ per genotype). **d**, Representative pictures of 5-week-old WT and *Kctd7*^{-/-} mice. **e**, Survival curve of *Kctd7*^{-/-} mice (median lifespan = 9 months; $n = 22$ for WT mice, $n = 20$ for *Kctd7*^{-/-} mice). **f**, Confocal microscopy of 5-week-old WT and *Kctd7*^{-/-} mouse cerebellum showing CAPNS1 localization in Purkinje cells and astrocytes. α -calbindin and α -GFAP antibodies were used to mark Purkinje cells and astrocytes, respectively. **g**, RT-qPCR analysis of mRNAs extracted from 5-week-old WT and *Kctd7*^{-/-} mouse brain homogenates showing no differences in transcriptional levels of calpain 1, calpain 2, and CAPNS1 ($n = 3$ per genotype). **h**, Confocal microscopy analysis of CAPNS1 localization in WT or *KCTD7* knock-out HeLa cells. CD98 was used as a plasma membrane marker. Scale bar, 10 μ m. **i**, **j**, Four-week-old astrocyte cultures were lysed in RIPA buffer and α -spectrin cleavage was evaluated by immunoblotting. **k-p**, Cortical and hippocampal lysates from 5-week-old WT and *Kctd7*^{-/-} mice were lysed in NP-40 lysis buffer and immunoblotted for p35 to evaluate calpain-mediated cleavage. Data represent mean \pm SEM, ($n = 4$ per genotype); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, ns = not significant.

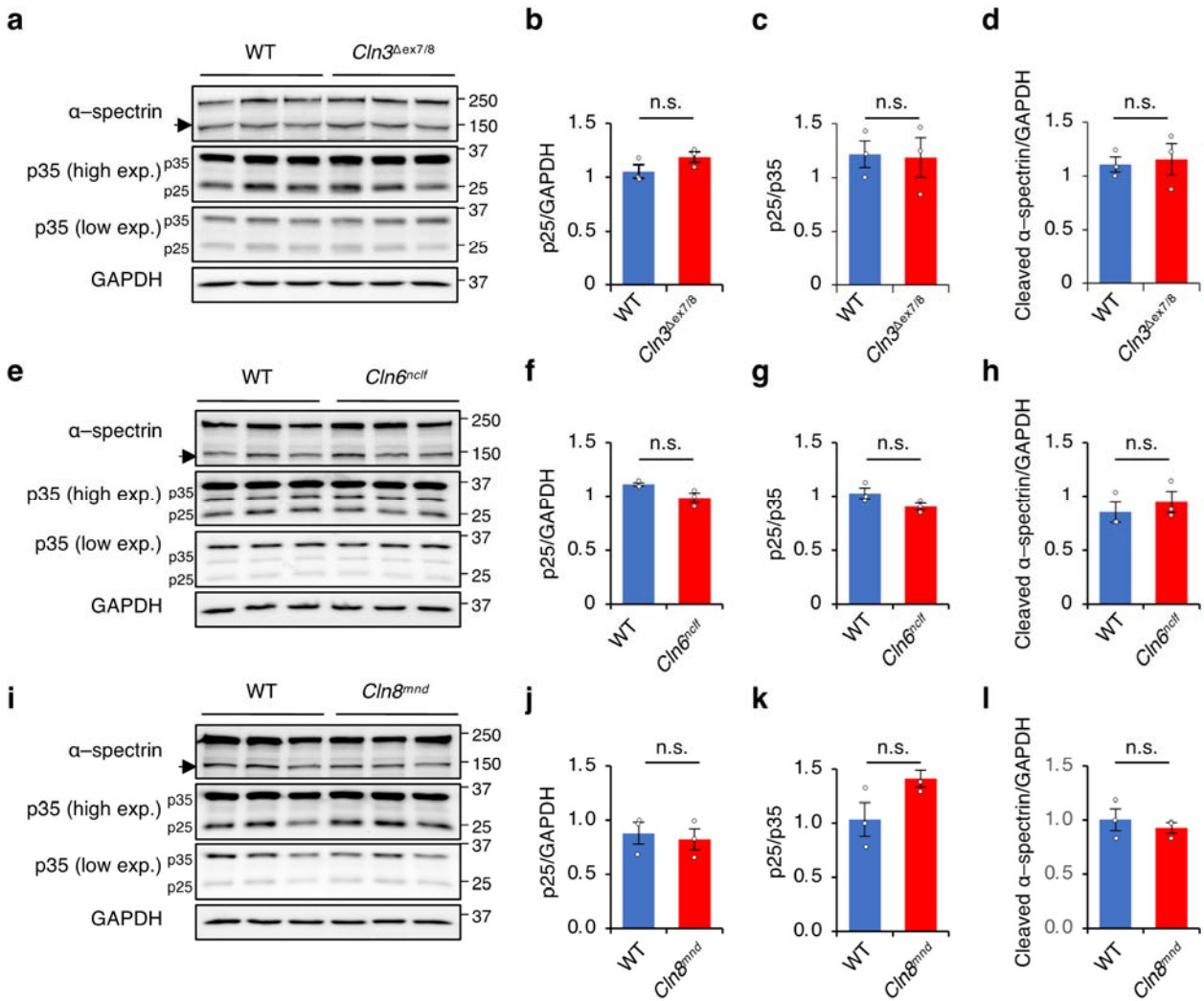


Fig. S6: Analysis of calpain activity in various mouse models of NCL. a-l, Calpain activity was evaluated in *Cln3^{Δex7/8}* mice (a-d), *Cln6^{ncfl}* mice (e-h), and *Cln8^{mnd}* mice (i-l) by performing immunoblot analysis of whole brain lysates collected at 2-3 months of age with antibodies for α-spectrin and p35. GAPDH was used as a loading control. $n = 3$ per genotype. Data represent mean \pm SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, ns = not significant.

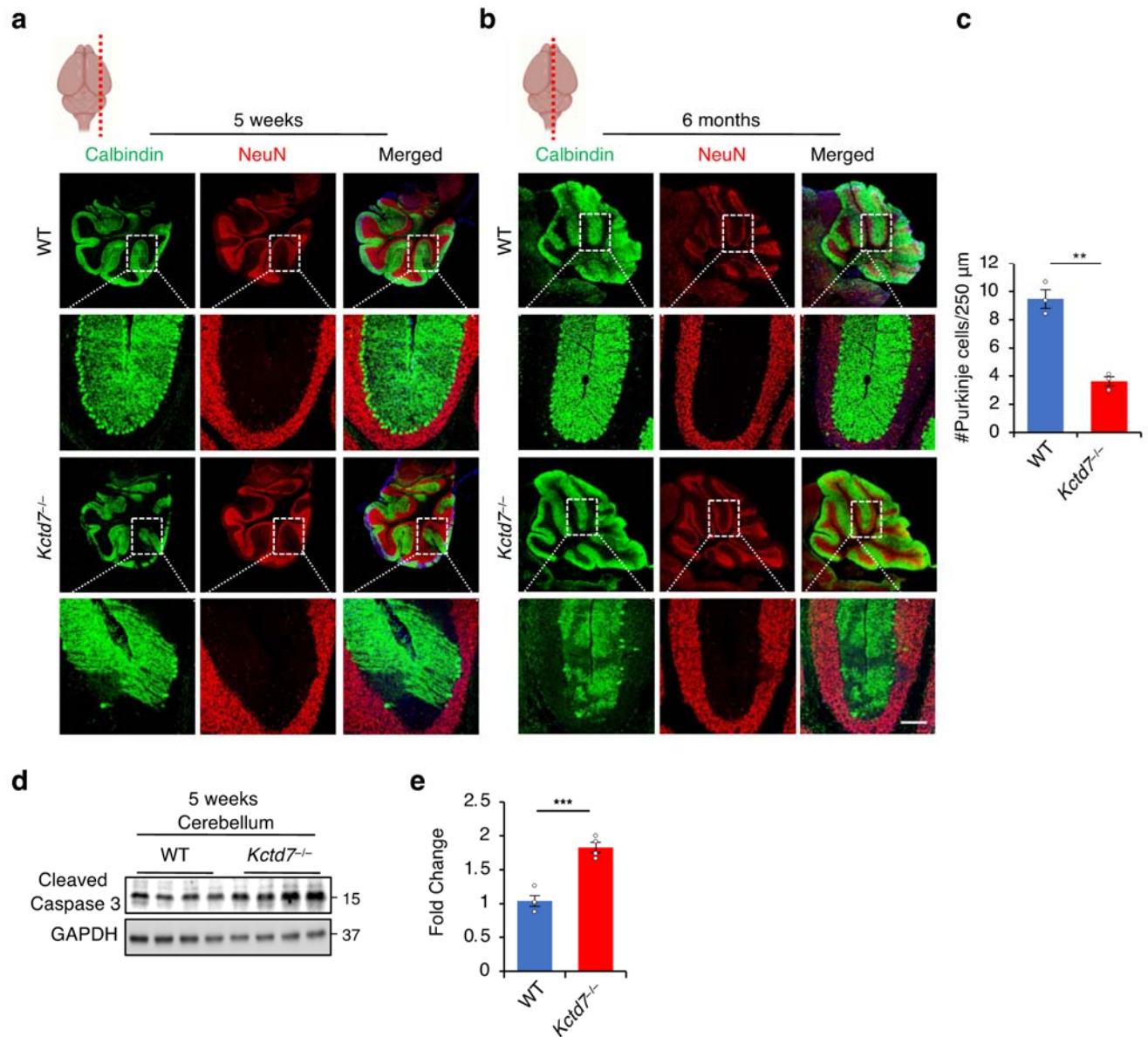


Fig. S7: KCTD7 deficiency leads to neurodegeneration. **a-c**, Confocal microscopy analysis of the cerebellum from 5-week-old and 6-month-old WT and *Kctd7^{-/-}* mice. α -calbindin antibody was used to label Purkinje cells. Scale bars, 200 μ m. Quantification of Purkinje cell numbers in 6-month-old animals shown in (c), $n = 5$ per genotype. Scale bars, 200 μ m. **d**, **e**, Immunoblot analysis of cerebellar tissue from 5-week-old WT and *Kctd7^{-/-}* mice using cleaved caspase 3 antibody. Data represent mean \pm SEM, $n = 4$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, ns = not significant.

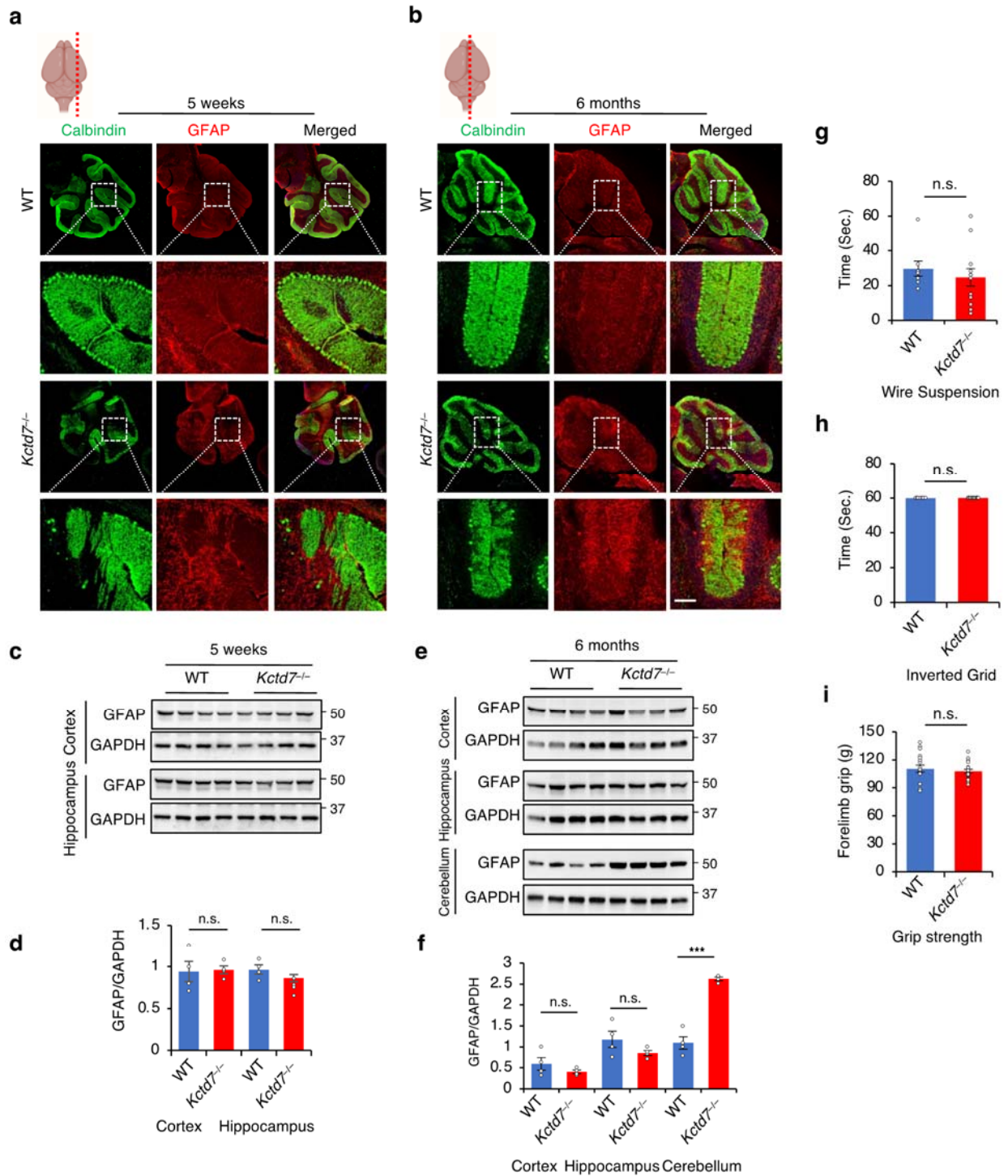


Fig. S8: KCTD7 deficiency leads to neurodegeneration and behavioral impairments. a, b, Confocal microscopy analysis of the cerebellum from 5-week-old and 6-month-old WT and *Kctd7*^{-/-} mice. α -GFAP antibody was used to label astrocytes. **c, d,** Immunoblot analysis of

cortical and hippocampal tissue from 5-week-old WT and *Kctd7*^{-/-} mice using α -GFAP antibody. GAPDH is used as a loading control. **e, f**, Immunoblot analysis of cortical, hippocampal, and cerebellar tissue from 6-month-old WT and *Kctd7*^{-/-} mice using α -GFAP antibody. GAPDH is used as a loading control. **g, h**, WT and *Kctd7*^{-/-} mice were assessed for latency to fall in a wire suspension test (**g**) and inverted grid tests (**h**) at 10 weeks of age. $n = 10$ for WT mice; $n = 16$ for *Kctd7*^{-/-} mice. **i**, WT and *Kctd7*^{-/-} mice were assessed for grip strength in a forepaw grip strength at 10 weeks of age using a digital force gauge. $n = 10$ for WT mice; $n = 16$ for *Kctd7*^{-/-} mice. Data represent mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, ns = not significant.

Supplementary Table 1. List of KCTD7-interacting proteins as detected by LC-MS/MS.

Accession No.	Name	No. Peptides
	<i>Replicate #1</i>	
gi 825635	calmodulin	634
gi 62897129	heat shock 70kDa protein 8 isoform 1 variant	143
gi 18088719	Tubulin, beta	102
gi 269784642	BTB/POZ domain-containing protein KCTD7 isoform 2	82
gi 297691739	Tubulin alpha-1B chain isoform 2	51
gi 111305821	Valosin-containing protein	19
gi 187281	M4 protein	19
gi 4502565	calpain small subunit 1	48
gi 302129652	annexin A6 isoform 2	19
gi 20149594	heat shock protein HSP 90-beta	17
gi 62897075	heat shock 70kDa protein 9B precursor variant	17
gi 3139079	cullin 3	10
gi 47115317	VIM	8
gi 6682361	talin	9
gi 119619622	reticulocalbin 2, EF-hand calcium binding domain	8
gi 375314781	keratin 1	8
gi 28277071	Importin 5	11
gi 5107666	Chain A, Structure Of Importin Beta Bound To The Ibb Domain Of Importin Alpha	8
gi 19111150	angiominin isoform 2	8
gi 48145697	CPNE1	8
gi 62897625	beta actin variant	8
gi 49168528	ANXA5	9
gi 62898293	programmed cell death 6 variant	6
gi 5734603	KARP-1-binding protein 2 (KAB2)	6
gi 31221	protein serine/threonine kinase	8
gi 38571606	Ribosomal protein L23	7
gi 9963810	NRAGE	5
gi 662841	heat shock protein 27	12
gi 48145673	HNRPH1	6
gi 73915357	Protein transport protein Sec16A;	5
gi 12017959	C1orf28	6
gi 49065418	BAG2	9
gi 119596821	myosin light chain kinase 2, skeletal muscle, isoform CRA_b	13
gi 68533039	DSP variant protein	4
gi 31544959	TRIM28 protein	4
gi 40788339	KIAA0723 protein	4

gi 62089432	DnaJ (Hsp40) homolog, subfamily A, member 3 variant	4
gi 78042577	MAP7 domain-containing protein 1	3
gi 158256510	unnamed protein product	5
gi 62089254	DnaJ (Hsp40) homolog, subfamily C, member 7 variant	3
gi 18105007	CAD protein	4
gi 62898934	DnaJ (Hsp40) homolog, subfamily B, member 6 isoform a variant	3
gi 56122599	leukemia multidrug resistance associated protein	4
gi 187609788	truncated plakophilin-2	3
gi 119617041	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types), isoform CRA_d	3
gi 8925970	eIF4E-transporter	3
gi 49168458	DNAJB1	3
gi 904032	p48	3
gi 119600190	ribosomal protein L24, isoform CRA_e	4
gi 9246427	RNA helicase A binding protein 95	3
gi 88759348	RING finger protein 219	4
gi 63252886	prolyl 4-hydroxylase subunit alpha-1 isoform 1 precursor	2
gi 310750364	ensconsin isoform 6	2
gi 62896517	ribosomal protein S4, X-linked X isoform variant	2
gi 62088004	EGF-containing fibulin-like extracellular matrix protein 1 isoform b variant	2
gi 790817	microfibril-associated glycoprotein 4, partial	4
gi 62897071	oxygen regulated protein precursor variant	2
gi 453155	keratin 9	2
gi 62896605	eukaryotic translation elongation factor 1 alpha 1 variant	2
gi 169159111	tubulin tyrosine ligase-like family, member 12	2
gi 8394343	sperm surface protein Sp17	2
gi 62896539	chaperonin containing TCP1, subunit 8 (theta) variant	2
gi 71891685	KIAA0829 protein	2
gi 340026	tyrosine kinase, partial	2
gi 253735647	HsMcm7	2
gi 3088356	ribosomal protein L38	2
gi 71533987	General transcription factor II, i	2
gi 197692147	T-complex protein 1 subunit beta	2
gi 42476299	PERQ amino acid-rich with GYF domain-containing protein 2 isoform b	3
gi 23242517	Copine III	4
gi 317373596	Calpain-2 catalytic subunit	2
gi 12804313	Similar to expressed sequence 2 embryonic lethal, partial	2
gi 397524998	Tescalcin isoform 1	2
gi 61656607	tumor rejection antigen (gp96) 1	3
gi 45709422	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	2
gi 5031579	A-kinase anchor protein 8	2

gi 495126	ribosomal protein L11	2
gi 194390416	eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa, isoform CRA_b	2
gi 119623811	hCG2005638, isoform CRA_c	2
gi 452269	glucose-6-phosphate dehydrogenase	2
gi 194374093	unnamed protein product	2
gi 8099630	protein kinase CDK9	3
gi 49456297	PRDX4	2
gi 13661193	fibulin-1 isoform D precursor	2
gi 15147337	E3 ubiquitin-protein ligase UBR5	2
gi 15667255	calpain small subunit 2	2
gi 643016	T cell receptor alpha chain Mb11	2
<i>Replicate #2</i>		
gi 825635	calmodulin	267
gi 62897129	heat shock 70kDa protein 8 isoform 1 variant	54
gi 27368062	class IVb beta tubulin	44
gi 269784642	BTB/POZ domain-containing protein KCTD7 isoform 2	68
gi 375314781	keratin 1	24
gi 297691739	tubulin alpha-1B chain isoform 2	21
gi 119581085	keratin 10 (epidermolytic hyperkeratosis; keratosis palmaris et plantaris), isoform CRA_b	14
gi 28277071	Importin 5	14
gi 20149594	heat shock protein HSP 90-beta	15
gi 5107666	Chain A, Structure Of Importin Beta Bound To The Ibb Domain Of Importin Alpha	13
gi 302129652	annexin A6 isoform 2	18
gi 4502565	calpain small subunit 1	22
gi 89059026	extracellular signal-regulated kinase-2 splice variant	11
gi 62898934	DnaJ (Hsp40) homolog, subfamily B, member 6 isoform a variant	7
gi 15277503	ACTB protein, partial	8
gi 197101391	40S ribosomal protein S3	5
gi 48734966	Eukaryotic translation elongation factor 1 alpha 1	7
gi 49065418	BAG2	7
gi 14141152	heterogeneous nuclear ribonucleoprotein M isoform a	5
gi 119611832	hCG2027326	6
gi 150439331	copine I	5
gi 47115317	VIM	6
gi 435476	cytokeratin 9	7
gi 62088004	EGF-containing fibulin-like extracellular matrix protein 1 isoform b variant	5
gi 18105007	CAD protein	5
gi 119613655	calpain 2, (m/II) large subunit, isoform CRA_a	5
gi 62897075	heat shock 70kDa protein 9B precursor variant	6

gi 66365795	Histone cluster 1, H1e	4
gi 15126735	Heat shock 27kDa protein 1	5
gi 20521736	KIAA1027 protein	4
gi 119571370	hCG1985580, isoform CRA_a	3
gi 3139079	cullin 3	3
gi 15080163	DnaJ (Hsp40) homolog, subfamily A, member 3	3
gi 38571606	Ribosomal protein L23	3
gi 49456627	CALU	3
gi 3088356	ribosomal protein L38	3
gi 62089254	DnaJ (Hsp40) homolog, subfamily C, member 7 variant	3
gi 1916622	HAX-1	4
gi 17391477	Annexin A5	3
gi 119600195	hCG1777176, isoform CRA_b	2
gi 119618532	hCG2016250, isoform CRA_a	2
gi 60299991	aging-associated protein 14b	3
gi 14043538	MYLK2 protein	6
gi 23242517	Copine III	4
gi 194389120	unnamed protein product	2
gi 158260761	unnamed protein product	3
gi 119619622	reticulocalbin 2, EF-hand calcium binding domain	2
gi 5174661	protein S100-A2	2
gi 31418053	CCT8 protein	2
gi 116283604	SPA17 protein	2
gi 114306812	BAT1 protein	2
gi 111305821	Valosin-containing protein	2
gi 109389365	glucose-6-phosphate 1-dehydrogenase isoform a	3
gi 386803	40-kDa keratin protein, partial	3
<i>Replicate #3</i>		
gi 4529893	HSP70-1	112
gi 66360457	Chain O, Crystal Structure Of Anthrax Edema Factor (Ef) Truncation Mutant, Ef-Delta 64 In Complex With Calmodulin	186
gi 20809886	Tubulin, beta 2C	75
gi 269784642	BTB/POZ domain-containing protein KCTD7 isoform 2	81
gi 111305821	Valosin-containing protein	35
gi 119612249	E3 ubiquitin protein ligase, HECT domain containing, 1, isoform CRA_a	23
gi 297691739	tubulin alpha-1B chain isoform 2	42
gi 3139079	cullin 3	19
gi 1147813	desmoplakin I	23
gi 18105007	CAD protein	20
gi 4502565	calpain small subunit 1	28
gi 49065418	BAG2	14

gi 262206280	dnaJ homolog subfamily C member 7 isoform 1	14
gi 119619622	reticulocalbin 2, EF-hand calcium binding domain	13
gi 19111150	angiominin isoform 2	11
gi 48734966	Eukaryotic translation elongation factor 1 alpha 1	15
gi 15080163	DnaJ (Hsp40) homolog, subfamily A, member 3	11
gi 14141152	heterogeneous nuclear ribonucleoprotein M isoform a	16
gi 6470150	BiP protein, partial	24
gi 119578931	DnaJ (Hsp40) homolog, subfamily A, member 1, isoform CRA_d	13
gi 62897075	heat shock 70kDa protein 9B precursor variant	20
gi 1699027	nuclear corepressor KAP-1	11
gi 197692465	ATP-dependent RNA helicase DDX5	9
gi 28277071	Importin 5	14
gi 49168458	DNAJB1	9
gi 42406316	FAM29A protein, partial	9
gi 20149594	heat shock protein HSP 90-beta	12
gi 71891790	KIAA0310 protein	9
gi 332808466	40S ribosomal protein S27-like	7
gi 662841	heat shock protein 27	13
gi 197101391	40S ribosomal protein S3	8
gi 47115317	VIM	6
gi 48146259	CCT2	6
gi 55962085	centrosomal protein 170kDa	7
gi 38571606	Ribosomal protein L23	13
gi 20521736	KIAA1027 protein	6
gi 119582464	hCG1982388, isoform CRA_d	8
gi 1916622	HAX-1	5
gi 302129652	annexin A6 isoform 2	12
gi 219519019	5-methyltetrahydrofolate-homocysteine methyltransferase	7
gi 62898934	DnaJ (Hsp40) homolog, subfamily B, member 6 isoform a variant	7
gi 3170178	antigen NY-CO-7	7
gi 119618532	hCG2016250, isoform CRA_a	5
gi 119599729	RuvB-like 1 (E. coli)	5
gi 217272894	116 kDa U5 small nuclear ribonucleoprotein component isoform b	6
gi 119574194	heterogeneous nuclear ribonucleoprotein H1 (H), isoform CRA_b	8
gi 6822170	zinc finger protein	6
gi 31873894	hypothetical protein	9
gi 33112885	acetyl-CoA carboxylase 1	5
gi 150439331	copine I	4
gi 12275876	tripartite motif protein TRIM27 beta	5
gi 49472841	A-kinase anchor protein 8-like	6

gi 15030240	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	5
gi 119623458	DiGeorge syndrome critical region gene 14, isoform CRA_a	5
gi 62898013	F-actin capping protein alpha-1 subunit variant	4
gi 385719194	caseinolytic peptidase B protein homolog isoform 2	4
gi 51476906	hypothetical protein	3
gi 5107666	Chain A, Structure Of Importin Beta Bound To The Ibb Domain Of Importin Alpha	4
gi 4337109	BAT3	7
gi 20384797	extracellular signal-related kinase 1c	6
gi 119594339	damage-specific DNA binding protein 1, 127kDa, isoform CRA_a	6
gi 1870688	Bruton's tyrosine kinase-associated protein-135	3
gi 119568002	t-complex 1, isoform CRA_b	5
gi 62896517	ribosomal protein S4, X-linked X isoform variant	7
gi 3088356	ribosomal protein L38	5
gi 68533031	FASN variant protein	3
gi 15277503	ACTB protein, partial	3
gi 119580514	eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa, isoform CRA_b	5
gi 14043538	MYLK2 protein	8
gi 119600993	chromosome 13 open reading frame 7, isoform CRA_b	3
gi 28175596	FARSA protein	3
gi 33150546	D-myo-inositol-3-phosphate synthase	3
gi 119614756	hCG2000485	4
gi 4507677	endoplasmic precursor	5
gi 62131678	14-3-3 protein epsilon isoform transcript variant 1	4
gi 20521013	KIAA0360	4
gi 119583300	melanoma antigen family D, 1, isoform CRA_a	5
gi 56205679	ribosomal protein L5	2
gi 4757732	apoptosis-inducing factor 1, mitochondrial isoform 1 precursor	4
gi 3283882	BDP-1 protein	3
gi 34782987	CAND1 protein, partial	2
gi 38079	75 kDa subunit NADH dehydrogenase precursor	3
gi 197692279	cyclin-dependent kinase 9	3
gi 63102091	AMOT protein, partial	6
gi 55960506	chaperonin containing TCP1, subunit 3 (gamma)	4
gi 119619424	hCG18053	3
gi 40788224	KIAA0355	3
gi 38607488	breast cancer-associated antigen SGA-56M	2
gi 12017959	C1orf28	2
gi 21619217	C1orf142 protein	2
gi 157311635	protein FAM83H	3

gi 17391477	Annexin A5	5
gi 119608918	plakophilin 2, isoform CRA_b	2
gi 2150046	26S proteasome subunit 9	2
gi 1620018	heat shock protein 90	2
gi 14424674	CDC42 effector protein (Rho GTPase binding) 1	3
gi 119627021	hook homolog 1 (Drosophila), isoform CRA_b	2
gi 119588620	glutamine and serine rich 1	3
gi 49456627	CALU	2
gi 31418053	CCT8 protein	2
gi 189306	nucleolin	2
gi 119584552	IQ motif and Sec7 domain 1, isoform CRA_b	2
gi 114432124	activating molecule in beclin-1-regulated autophagy	2
gi 1890050	cysteine protease	2
gi 19851923	CLL-associated antigen KW-14	2
gi 13111995	Ubiquitin associated protein 2-like	2
gi 11967975	myeloid leukemia factor 1 isoform 1	2
gi 119600195	hCG1777176, isoform CRA_b	2
gi 291868	ATPase	2
gi 119610743	complement component 1, q subcomponent binding protein, isoform CRA_c	2
gi 5802968	protein AF1q	3
gi 187957150	KIAA0841	2
gi 40807091	GANAB protein	2
gi 306890	chaperonin (HSP60)	3
gi 119617721	nucleosome assembly protein 1-like 1, isoform CRA_b	3
gi 3005758	RNA polymerase II 140 kDa subunit	2
gi 330864767	dnaJ homolog subfamily B member 5 isoform 2	5
gi 62089430	DnaJ (Hsp40) homolog, subfamily B, member 4 variant	2
gi 23270929	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	2
gi 758423	zinc-finger protein	3
gi 124001558	inactive ubiquitin carboxyl-terminal hydrolase 54	2
gi 58257735	KIAA0651 protein	3
gi 2209374	HsCdc7	2
gi 119620390	chaperonin containing TCP1, subunit 4 (delta), isoform CRA_a	2
gi 332836269	reticulocalbin-1	2
gi 5771523	3-phosphoglycerate dehydrogenase	2
gi 3170190	antigen NY-CO-25	2
gi 8885790	filamin 2	3
gi 197692141	ATP-dependent RNA helicase DDX3X	3
gi 26051235	nuclear pore complex protein Nup133	3
gi 207029415	histone-binding protein RBBP4 isoform b	2

gi 214829673	ADP-ribosylation factor GTPase-activating protein 3 isoform 1	2
gi 226342869	TBC1 domain family member 15 isoform 2	2
gi 15126760	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	3
gi 33869643	EEF2 protein, partial	2
gi 119610102	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide, isoform CRA_d	2
gi 62088004	EGF-containing fibulin-like extracellular matrix protein 1 isoform b variant	3
gi 109389365	glucose-6-phosphate 1-dehydrogenase isoform a	2
gi 39963074	PRP8 pre-mRNA processing factor 8 homolog (<i>S. cerevisiae</i>)	2
gi 58761504	HAUS augmin-like complex subunit 8 isoform a	3
gi 52545638	hypothetical protein	3
gi 119570975	ubiquinol-cytochrome c reductase core protein II, isoform CRA_a	2
gi 397524998	tescalcin isoform 1	4
gi 226246671	40S ribosomal protein S20 isoform 1	2
gi 103471999	serine/threonine-protein kinase 3 isoform 1	2
gi 553640	ribosomal protein S13	2
gi 119570641	hCG1994130, isoform CRA_b	2
gi 119611476	centrosomal protein 350kDa, isoform CRA_a	2
gi 5689435	KIAA1049 protein	2
gi 20067381	ALMS1 protein	3
gi 62897195	glutamate dehydrogenase 1 variant	2
gi 16876910	HNRPF protein	7
gi 4885079	ATP synthase subunit gamma, mitochondrial isoform H (heart) precursor	2
gi 48146309	DNAJB11	2
gi 51094698	MAD1 mitotic arrest deficient-like 1 (yeast)	2
gi 50949411	hypothetical protein	2
gi 119617974	PCTAIRE protein kinase 2, isoform CRA_c	2
gi 47938913	HYOU1 protein	2
gi 119608880	hCG1979429, isoform CRA_a	2
gi 62548856	BAG family molecular chaperone regulator 5 isoform a	2
gi 23242517	Copine III	3
gi 14017863	KIAA1823 protein	2
gi 45861372	200 kDa U5 snRNP-specific spliceosomal protein	2
gi 2370178	P73	2
gi 395132436	60S ribosomal protein L18 isoform 2	2
gi 21280444	AF15q14	2
gi 1419564	cytokeratin	2
gi 156181166	DnaJ	4
gi 2078529	Hlark	2
gi 180928	core protein II precursor	2
gi 89059026	extracellular signal-regulated kinase-2 splice variant	4

gi 19353009	Similar to Elongation factor 2b, partial	2
gi 3170190	antigen NY-CO-25	2

Supplementary Table S2. Putative target sites of ubiquitination in CAPN1 and CAPN2 as calculated by UbiNet.

Serial Number	Amino Acid	Probability Score	Ubiquitination site		
Calpain 1					
1	461	0.99849	QPAVHL	K	RDFFLA
2	36	0.664366	RHENAI	K	YLGQDY
3	398	0.634094	WVNPQF	K	IRLDET
4	564	0.998944	DMEISV	K	ELRTIL
5	631	0.601169	RKFDDL	K	SGSMSA
6	71	0.710379	PQSLGY	K	DLGPNS
7	365	0.998614	KSRTIR	K	WNTTLY
8	193	0.515752	WSALLE	K	AYAKVN
9	197	0.510909	LEKAYA	K	VNGSYE
10	20	0.577844	VSAQVQ	K	QRAREL
11	626	0.659654	YLSIFR	K	FDLDKS
12	171	0.608432	DDLLPI	K	DGKLVF
13	240	0.558482	LYQIIL	K	ALERGS
14	359	0.595199	LTPDAL	K	SRTIRK
15	328	0.582712	RDQLRV	K	MEDGEF
16	280	0.520307	YSVTGA	K	QVNYRG
Calpain 2					
1	7	0.56294	MAGIAA	K	LAKDRE
2	10	0.572412	IAAKLA	K	DREAAE
3	26	0.998295	SHDRAI	K	YLNQDY
4	61	0.582818	PSALGF	K	ELGPYS
5	183	0.52848	WSALLE	K	AYAKIN
6	280	0.532409	SNGSLQ	K	LIRIRN
7	476	0.545334	EVLNRF	K	LPPGEY
8	629	0.512513	NSYEMR	K	ALEEAG
9	674	0.998452	RLETLF	K	IFKQLD
10	677	0.661898	TLFKIF	K	QLDPEN

Supplementary Table S3. List of primary and secondary antibodies.

Antibody	Supplier	Catalog No.	Dilution
<i>Primary Antibodies</i>			
Anti-Rabbit CAPN1	Cell Signaling	2556	WB 1:1000
Anti-Rabbit CAPN2	Cell Signaling	2539	WB 1:1000
Anti-Rabbit CAPNS1	Sigma	HPA006872	WB 1:1000
Anti-Rabbit CAPNS1	Sigma	HPA006872	IF 1:250
Anti-Mouse CAPN1	Santa Cruz	sc-390677	WB 1:250
Anti-Mouse CAPN1	Sigma	C0355	WB 1:1000
Anti-Mouse CAPN2	Santa Cruz	sc-373967	WB 1:1000
Anti-Mouse CAPNS1	Santa Cruz	sc-32325	WB 1:500
Anti-Mouse alpha spectrin	Millipore	MAB1622	WB 1:2000
Anti-Rabbit p35	Cell Signaling	2680	WB 1:1000
Anti-Rabbit c-Myc	Sigma	C3956	WB 1:5000
Anti-Rabbit FLAG	Sigma	F7425	WB 1:5000
Anti-Mouse FLAG	Sigma	F1804	WB 1:5000
Anti-Rabbit Cullin 3	Abcam	ab108407	WB 1:500
Anti-Mouse HA	BioLegend	901514	WB 1:5000
Anti-Rabbit KCTD7	ProSci Inc.	61-770	WB 1:500
Anti-Mouse GAPDH	Proteintech	60004-1-Ig	WB 1:20000
Anti-Mouse GFAP	Abcam	ab7260	WB 1:10000
Anti-Mouse GFAP	Sigma	G6171	IF 1:500
Anti-Rabbit HA-Tag (C29F4)	Cell Signaling	3724S	WB 1:1000
Anti-Rabbit Sodium Potassium ATPase	Abcam	ab76020	WB 1:1000
Anti-Rabbit Calreticulin	Cell Signaling	12238	WB 1:1000
Anti-Rabbit Tom20	Santa Cruz	sc-11415	WB 1:1000
Anti-Mouse Calbindin	Swant	300	IF 1:5000
Anti-Mouse Calbindin	Swant	CB38	IF 1:5000
Anti-Rabbit GFP (D5.1)	Cell Signaling	2956S	WB 1:5000
Anti-Rabbit GFP (D5.1)	Cell Signaling	2956S	IF 1:1000
Anti-Rabbit Ubiquitin	Cell Signaling	3933	WB 1:1000
Anti-Rabbit LAMP1 (D2D11)	Cell Signaling	9091	IF 1:250
Anti-Mouse NeuN	Millipore	MAB377	IF 1:500
<i>Secondary Antibodies</i>			
Alexa Fluor® 488 Anti-Rat IgG	Invitrogen	A-11006	1:1000
Alexa Fluor® 488 Anti-Chicken IgG	Invitrogen	A-11039	1:1000
Alexa Fluor® 488 Anti-Mouse IgG	Invitrogen	A-21200	1:1000
Alexa Fluor® 594 Anti-Mouse IgG	Invitrogen	A-21201	1:1000
Alexa Fluor® 594 Anti-Rabbit IgG	Invitrogen	A-21207	1:1000

Alexa Fluor® 633 Anti-Mouse IgG	Invitrogen	A-21052	1:1000
Alexa Fluor® 633 Anti-Goat IgG	Invitrogen	A-21082	1:1000
ECL anti-rabbit IgG-HRP	GE Healthcare	NA9340-1ML	1:5000
ECL anti-chicken IgY-HRP	Santa Cruz	sc-2428	1:5000
ECL anti-goat IgG-HRP	Santa Cruz	sc-2020	1:5000
ECL anti-rat IgG-HRP	GE Healthcare	NA9350-1ML	1:5000
ECL anti-mouse IgG-HRP	GE Healthcare	NA9310-1ML	1:5000

Supplementary Table S4. Oligos used in this study.

Primer name	Forward Primer	Reverse Primer
<i>Cloning and site-directed mutagenesis</i>		
KCTD7 WT	gccggaattcaccatggtgtagtcacgggg	ccgggtaccgccaccatgtgatcttg
KCTD7 R84W	ccatgttcagtgggtggcactacatcccc	ggggatgtagtgccaccactgaacatgg
KCTD7 R94W	eggactccgagggctggtacttcacgac	gtc gatgaagtaccagccctcggagtccg
KCTD7 L108M	cacactttggagatgtgatgaattcctgcgctca	tgagcgcaggaaattcatcacatctccaaagtgtg
KCTD7 D115Y	cctgcgctcagggatctcccaccagg	ccctgggtgggagataccctgagcgcagg
KCTD7 R184C	ccagcgggaaggcctgctttgccaagct	agcttggcaaagcaggccttccgctgg
CAPNS1	cgactctagaggatccatgttctggtaactcgtt	agtcagcccgggatccggaatacatagtcagctgc
<i>Mouse genotyping</i>		
mKCTD7_F1	aacatctgtggccaaaaagc	
mKCTD7_R1	gctggacttcagaggacac	
mKCTD7_F2	cttgacgtgagatggaggg	
mKCTD7_R2	ccaaagtgtgtgccatctcgatcg	
<i>qRT-PCR</i>		
Cyclophilin	ggcaaatgctggaccaaacacaa	gtaaaatgcccgaagtcaaaag
CAPN1	gctggcctcatgcagaaac	cgagggacctggtactcgc
CAPN2	gagaggaggggaaggaggga	ggaggagccctctctgaca
CAPNS1	gaatgtgctcggaggcctga	cctcgtgatggcgtaatg
KCTD7	gagaacatgcagccactgaa	tccgctccaagtggctttt