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Supplementary Information (SI) for:

On the cause of sleep: protein fragments, the concept of sentinels, and links to epilepsy

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This PDF includes:

Figures S1-S6 and their legends.

References for SI.

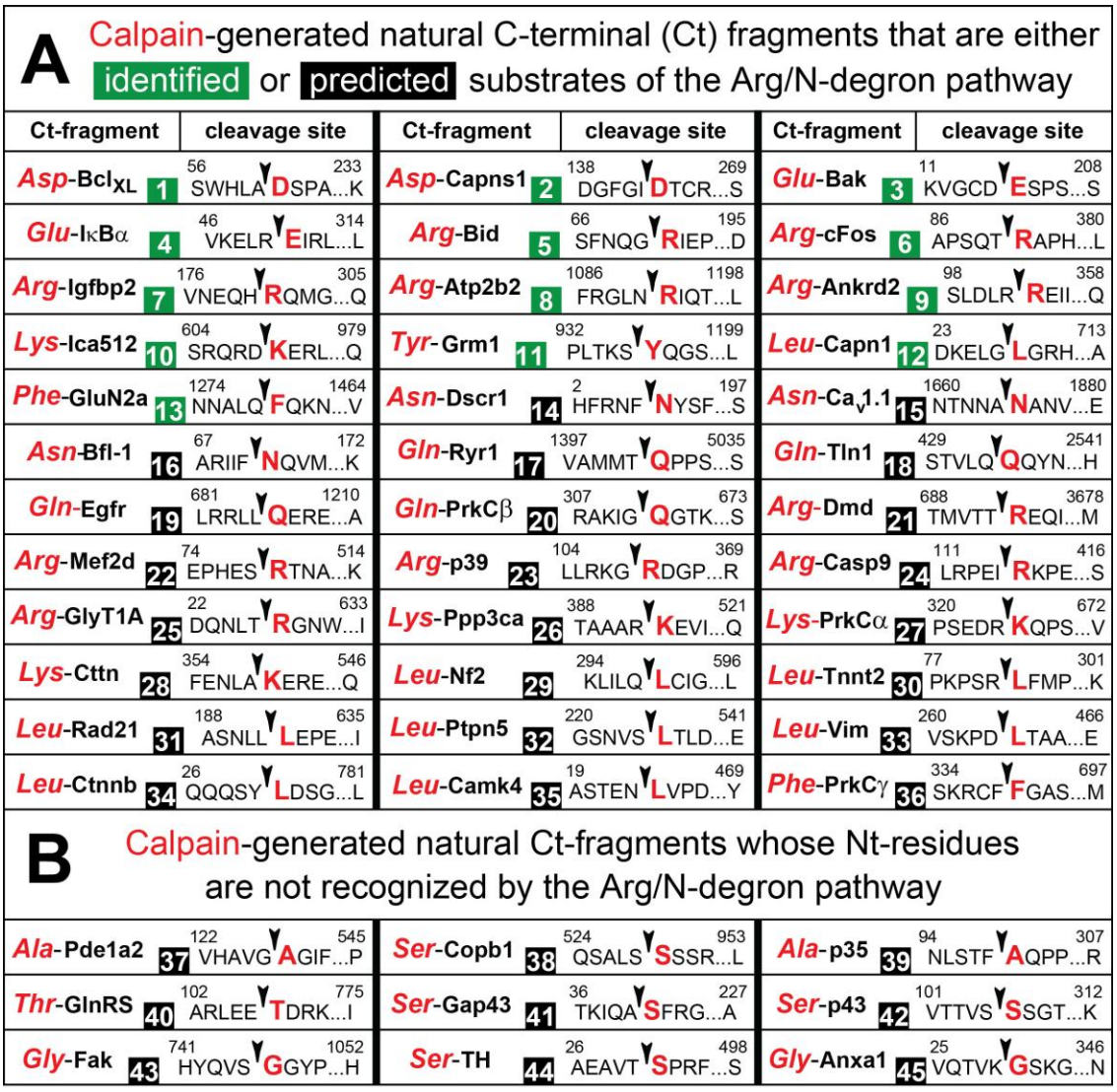

Fig. S1. Calpain-generated natural C-terminal (Ct) fragments of mammalian proteins. This list is but a small subset of natural calpain substrates in a mammal.

(*A*) Ct-fragments with numbers in green are experimentally characterized and validated substrates of the Arg/N-degron pathway (Fig. 2) (1, 2). Ct-fragments with numbers in black are predicted Arg/N-degron substrates. Each entry cites a calpain-generated Ct-fragment and the fragment's N-terminal (Nt) residue (in red), using 3-letter abbreviations for amino acids. A calpain cleavage site, indicated by an arrowhead, is denoted using single-letter abbreviations for amino acids. A P1' residue (in red and enlarged) becomes Nt-residue upon the cleavage. The indicated residue numbers are, respectively, the number of the first shown residue of a full-length protein precursor and the number of its last residue. Residue numbers of proteins are counted from their initially present Nt-Met residue, irrespective of whether or not Nt-Met is removed by Met-aminopeptidases. All entries are mouse proteins, save for #14 and #24, which are human proteins.

(*B*)Same as in *A* but examples of calpain-generated Ct-fragments whose Nt-residues are not recognized by the Arg/N-degron pathway (Fig. 2).

#1. *Asp*-Bcl_{XL}. Bcl_{XL} is a 26 kDa antiapoptotic regulatory protein (3, 4). Under conditions that include glucose and oxygen deprivation, Bcl_{XL} can be cleaved by activated calpain-1, resulting in the 21 kDa Asp^{61}-Bcl_{XL} Ct-fragment. In contrast to full-length Bcl_{XL}, Asp^{61}-Bcl_{XL} has proapoptotic activity (5), and has been shown to be a short-lived substrate of the Arg/N-degron pathway (6).

#2. *Asp*-Capns1 is the Ct-fragment of the calpain regulatory subunit that is cleaved by activated calpains (7, 8).

#3. *Glu*-Bak is the proapoptotic Ct-fragment of the apoptotic regulator BAK. Glu-BAK is generated by calpain-1 in vitro and is apparently formed in vivo as well (9).

#4. *Glu*-IκBα is the Ct-fragment of the IκBα subunit of the NFκB-IκBα complex in which the NFκB transcriptional regulator is inhibited by IκBα. The IκBα subunit is targeted for degradation either through a conditional phosphodegron or through a calpain-mediated cleavage (10).

#5. *Arg*-Bid. Bid is a 22 kDa member of the BCL-2 family of apoptosis regulators (3, 11, 12). Although full-length Bid is a proapoptotic protein, its Ct-fragments, which can be naturally produced by activated caspases, calpains or granzyme B, can be even more active than intact Bid as proapoptotic protein fragments (13). The cleavage of Bid by calpains produces the 14 kDa Arg^{71}-Bid fragment (13-16) that has been shown to be a short-lived substrate of the Arg/N-degron pathway (6).

#6. *Arg*-c-Fos is the Ct-fragment of the c-Fos transcriptional regulator. c-Fos is targeted for conditional degradation through several degrons, including the path that involves the cleavage by calpains (17).

#7. *Arg*-Igfbp2 is the calpain-generated Ct-fragment of the insulin-like growth factor binding protein-2 (18).

#8. *Arg*-Atp2b2 is the Ct-fragment of the transmembrane Atp2b2 plasma membrane Ca^{2+} pump (PMCA) that ejects Ca^{2+} from cells. This pump is activated either by the binding of Ca^{2+}/calmodulin or by the calpain-mediated truncation of Atp2b2 that generates the *Arg*-Atp2b2 fragment and thereby activates the pump (19).

#9. *Arg*-Ankrd2. Ankrd2 (Marp2, Arpp), a member of the MARP (muscle ankyrin repeat protein) family, functions as a negative regulator of muscle differentiation (20). Calpains can produce the 30 kDa Arg^{103}-Ankrd2 Ct-fragment (21).

#10. *Lys*-Ica512. Ica512 (Ptprn) is a member of the transmembrane receptor protein phosphatase family (22). The 43 kDa calpain-generated mouse Lys^{609}-Ica512 Ct-fragment enters the nucleus and acts as a transcriptional regulator (22, 23).

#11. *Tyr*-Grm1. Grm1 is the Ct-fragment of the mGluR1α transmembrane metabotropic glutamate receptor (24). Receptors containing the calpain-truncated mGluR1α Ct-fragment can elevate cytosolic Ca^{2+} but cannot activate PI_3K-Akt signaling pathways, in contrast to uncleaved receptors (24, 25).

#12. *Leu*-Capn1 is the auto-generated, catalytically active Ct-fragment of calpain-1 (26, 27).

#13. *Phe*-GluN2a. GluN2a (NMDA-R2a) is a subunit of the N-methyl-D-aspartate receptor (NMDAR), a glutamate receptor that can function as a ligand-gated Ca^{2+} channel (28, 29). The GluN2b subunit of NMDAR can also be cleaved by calpains (30). Ct-fragments of NR2A and NR2B contain domains required for the association of these subunits with other synaptic proteins. NMDAR receptors lacking a Ct-region of GluN2a could function as

glutamate-gated Ca^{2+} channels but the intracellular traffic of cleaved receptors and their electrophysiological properties were altered (31).

#14. *Asn*-DSCR1 (Rcan1) is the calpain-generated Ct-fragment of the Down syndrome critical region 1 protein Dscr1, which binds to Raf1, inhibits the phosphatase activity of calcineurin, and enhances its degradation. The *Asn*-DSCR1 fragment does not bind to the Raf1 kinase (32).

#15. *Asn*-$Ca_v1.1$ is the Ct fragment of the voltage-gated transmembrane Ca^{2+} channel. This (apparently) calpain-generated fragment is noncovalently associated with the rest of the channel and can inhibit its activity. Upon dissociation from the channel, the *Asn*-$Ca_v1.1$ fragment enters the nucleus and acts as a transcriptional regulator (33-36).

#16. *Asn*-Bfl-1. Bfl-1 is antiapoptotic regulatory protein whose cleavage by calpain-1 generates the *Asn*72-Bfl-1 proapoptotic Ct-fragment (37).

#17. *Gln*-Ryr1 is the Ct-fragment of the Ryr1 ryanodine receptor, a Ca^{2+} channel in the ER (38) that mediates the efflux of Ca^{2+} from the ER into the cytosol. Calpain-mediated cleavage of Ryr1 increases Ca^{2+} efflux (39).

#18. *Gln*-talin is the calpain-generated Ct-fragment of talin, an adaptor protein that interacts with the integrin family of cell adhesion transmembrane proteins (19, 40, 41).

#19. *Gln*-Egfr is one of calpain-generated Ct-fragments of the transmembrane epidermal growth factor (EGF) receptor protein kinase (42). Remarkably, all 7 calpain cleavage sites in the cytosol-exposed domain of the 170-kDa EGFR contain P1' residues that are recognized as destabilizing by the Arg/N-degron pathway (42).

#20. *Gln*-PrkCβ is the Ct-fragment of PrkCβ, a Ser/Thr PKC kinase (43).

#21. *Arg*-dystrophin is the calpain-generated Ct-fragment of a major cytoskeletal protein in the skeletal muscle (44).

#22. *Arg*-Mef2d is the Ct-fragment of the Mef2d myocyte enhancer factor 2d, a transcriptional regulator that contributes to neuronal survival, development, and synaptic plasticity (45).

#23. *Arg*-p39 is the calpain-generated Ct-fragment of the p39 activator of the Cdk5 protein kinase (46). The indicated cleavage site is located immediately downstream of two other closely spaced (and strongly conserved) calpain cleavage sites in p39. A cleavage at any one of these sites yields a predicted Arg/N-degron substrate.

#24. *Arg*-caspase-9 is the Ct-fragment of caspase-9, which can be inactivated by calpains (47), followed by the (predicted) degradation of the Arg-caspase-9 Ct-fragment by the Arg/N-degron pathway.

#25. *Arg*-Glyt1a is the Ct-fragment of the transmembrane Glyt1a glycine transporter (48). Another Gly transporter, Glyt1b, is also cleaved by calpains, yielding the *Arg*-Glyt1b fragment (48). These Ct fragments are still active as transporters but are impaired in their ability to remove Gly (an inhibitory neurotransmitter) from synaptic clefts (48).

#26. *Lys*-Ppp3ca is the calpain-generated Ct-fragment if the Ser/Thr protein phosphatase 2B (catalytic subunit, alpha isoform) (49, 50).

#27. *Lys*-PkCα is the calpain-generated Ct-fragment of PkCα, a broadly expressed Ser/Thr kinase of the PKC family (51). Being catalytically active but no longer controlled by the regulatory Nt-domain of the full-length PkCα, the Lys-PkCα fragment can be toxic, for example, upon its formation in an ischemic heart (52).

#28. *Lys*-cortactin is the Ct-fragment of cortactin, an actin-binding protein that regulates actin polymerization (53).

#29. *Leu*-Nf2 is the calpain-generated Ct-fragment of NF2 (merlin), a tumor suppressor and cytoskeletal protein. Loss of function NF2 mutants result in autosomal-dominant neurofibromatosis, a predisposition to specific kinds of brain tumors (54).

#30. *Leu*-troponin T2 is the Ct-fragment of the cardiac troponin T that is produced by calpain-1 in the troponin-containing cardiac myofibril complex (55).

#31. *Leu*-Rad21 is the calpain-generated Ct-fragment of the Scc1/Rad21 subunit of the chromosome-associated cohesin complex (56). The calpain-mediated generation of *Leu*-Rad21 contributes to the control of chromosome cohesion/segregation, together with processes that include the separase-mediated cleavage of the same Rad21 subunit of cohesin (56-59).

#32. *Leu*-$STEP_{33}$ is the Ct-fragment of the striatal-enriched $STEP_{61}$ phosphatase, a brain-specific Tyr-phosphatase whose substrates include the MAPK-family kinases Erk1/2 and p38. The calpain-generated *Leu*-$STEP_{33}$ fragment lacks phosphatase activity (60).

#33. *Leu*-vimentin is the calpain-generated Ct-fragment of vimentin, a component of intermediate filaments (61).

#34. *Leu*-β-catenin (Ctnnb)is the calpain-generated Ct-fragment of β-catenin, a conditionally short-lived cytoskeletal protein and transcriptional regulator. The *Leu*-β-catenin fragment is a nuclear protein that activates specific genes in conjunction with other transcription factors (62).

#35. *Leu*-Camk4 is the calpain-generated Ct-fragment of the Ca^{2+}/calmodulin-dependent kinase-IV. This fragment lacks kinase activity (63).

#36. *Phe*-PrkCγ is the calpain-generated Ct-fragment of PkCγ, a Ser/Thr kinase of the PKC family (51). The *Phe*-PrkCγ fragment is constitutively active as a kinase, because it lacks the regulatory Nt-domain of the full-length PrkCγ kinase (51).

#37. *Ala*-Pde1a2 is the calpain-generated Ct-fragment of Pde1a2, an isoform of calmodulin-dependent cyclic nucleotide phosphodiesterase (64).

#38. *Ser*-Copb1 is the is the calpain-generated Ct-fragment of the Copb1 coatomer, a component of the cytosolic protein complex that binds to dilysine motifs and reversibly associates with Golgi non-clathrin-coated vesicles.

#39. *Ala*-p35 is the calpain-generated Ct-fragment of p35, a neuron-specific activator Cdk5, a cyclin-dependent kinase (65).

#40. *Thr*-GlnRS is the calpain-generated Ct-fragment of GlnRS, a Thr-tRNA synthetase (66).

#41. *Ser*-Gap43 is the calpain-generated Ct-fragment of Gap43, a protein with functions in axon guidance, synaptic plasticity and regulation of neuronal death and survival (67, 68).

#42. *Ser*-p43 is the calpain-generated Ct-fragment of p43, a component of specific complex containing several aminoacyl-tRNA synthetases (66).

#43. *Gly*-Fak is the calpain-generated Ct-fragment of the focal adhesion kinase Fak, which regulates adhesive properties of cells (69, 70).

#44. *Ser*-TH is the calpain-generated Ct-fragment of tyrosine hydroxylase (71, 72).

#45. *Gly*-Anxa1 is the calpain-generated Ct-fragment of annexin-1, a member of the annexin family of Ca^{2+}-binding/phospholipid binding proteins. Annexin-1 acts, in particular, as a mediator of glucocorticoid action in inflammation and in the control of anterior pituitary hormone release (73, 74).

Caspase-generated C-terminal (Ct) protein fragments that are either identified or predicted substrates of the Arg/N-degron pathway

Ct-fragment	cleavage site	Ct-fragment	cleavage site
Proapoptotic caspase-generated Ct-fragments that are identified Arg/N-degron substrates			
***Cys*-RIPK1**; S/T kinase, regulator of apoptosis	321 SLQHD**C**VPL...S 656 **1**	***Cys*-TRAF1**; regulator of apoptosis	152 DLEVD**C**YRA...A 409 **2**
***Asp*-BRCA1**; multifunctional tumor suppressor	1118 DDLLD**D**VEI...D 1812 **3**	***Leu*-LIMK1**; Ser/Thr protein kinase	236 LDEID**L**LIQ...D 647 **4**
***Tyr*-NEDD9**; regulator of cell adhesion	626 MDDYD**Y**VHL...F 834 **5**	***Arg*-BIM$_{EL}$**; regulator of apoptosis	9 SSECD**R**EGG...H 196 **6**
***Asp*-EPHA4**; transmembrane dependence receptor	769 RVLED**D**PEA...V 986 **7**	***Tyr*-MET**; transmembrane dependence receptor	996 NESVD**Y**RAT...S 1379 **8**
Proapoptotic caspase-generated Ct-fragments that are predicted Arg/N-degron substrates			
***Asn*-PKCδ**; Ser/Thr protein kinase	325 EDMQD**N**SGT...D 676 **9**	***Lys*-PKCθ**; Ser/Thr protein kinase	350 LDEVD**K**MCH...S 706 **10**
***Trp*-ETK**; Tyr protein kinase	236 EDFPD**W**WQV...H 675 **11**	***Gln*-SLK**; S/T kinase, regulator of actin	432 PDTQD**Q**QTV...S 1202 **12**
***Ile*-HPK1**; Ser/Thr protein kinase	381 YDDVD**I**PTP...L 833 **13**	***Ile*-MLH1**; DNA repair protein	414 EDKTD**I**ISS...C 756 **14**
Other caspase-generated Ct-fragments that are predicted Arg/N-degron substrates			
***Tyr*-CYLD;** deubiquitylase (DUB)	211 ALESD**Y**AGP...K 952 **15**	***Leu*-p21**; p21$^{Clp1/Waf1}$ inhibitor of cell division	107 EDHVD**L**SLS...P 164 **16**
***Arg*-IP$_3$R**; inositol 1,4,5-triphosphate receptor	1887 DDEVD**R**DAP...A 2749 **17**	***Asn*-LMN1**; lamin-A, a component of nuclear lamina	295 LVEID**N**GKQ...M 476 **18**
***Arg*-ETS-1**; a transcription factor	258 YDSCD**R**LTQ...E 441 **19**	***Tyr*-TOP1**; DNA topoisomerase I	142 EDDAD**Y**KPK...F 765 **20**
***Leu*-MEFD2**; a transcription factor	287 EDHLD**L**NNA...K 521 **21**	***Asn*-DNA-PK**; DNA-dependent protein kinase	2709 GDEVD**N**KVK...M 5028 **22**
***Asn*-CAD1**; E-cadherin, an adhesion receptor	746 DDTRD**N**VYY...D 882 **23**	***Gln*-synphilin-1**; a ligand of α–synuclein	450 LDEVD**Q**DGN...A 919 **24**
***Tyr*-ACINUS**; a mediator of chromatin condensation	1089 QDELD**Y**HRG...R 1341 **25**	***Lys*-PLECTIN**; a cytoskeletal protein	2390 LILRD**K**DNT...A 4684 **26**
***Cys*-CCNE1**; a G1-S cyclin	271 VLDVD**C**LEF...A 395 **27**	***His*-PMCA4b**; a Ca^{2+} extrusion pump	1076 LDEID**H**AEM...P 1170 **28**
***Asp*-CDC42**; a RAS superfamily member	117 IDLRD**D**PST...F 191 **29**	***Tyr*-iPLA$_2$**; Ca-independent phospholipase	179 MDVTD**Y**KGE...P 752 **30**

Fig. S2. Caspase-generated natural C-terminal (Ct) fragments of intracellular proteins that are either experimentally confirmed (marked in green) or predicted (marked in black) substrates of the Arg/N-degron pathway. As indicated in the diagram, some of these Ct-fragments have proapoptotic activities (6). This list of confirmed or putative Arg/N-degron substrates of caspases is but a small subset of known putative Arg/N-degron substrates of this kind, most of which remain to be analyzed. The list contains both human and mouse proteins; all of them are denoted by all-capital notations for human proteins.

Caspase recognition sites are underlined. Each entry cites a caspase-generated Ct-fragment of a protein and the fragment's N-terminal (Nt) residue (in red), using 3-letter abbreviations for amino acids. A caspase cleavage site, indicated by an arrowhead, is denoted using single-letter abbreviations for amino acids. A P1' residue (in red and enlarged) becomes Nt-residue upon a cleavage by a caspase. Residue numbers are, respectively, the number of the first shown residue of a full-length protein precursor and the number of its last residue. Residue numbers are counted from their initially present Nt-Met, irrespective of whether or not Nt-Met is removed by Met-aminopeptidases.

The first section of this diagram describes 8 previously identified natural proapoptotic Ct-fragments, specifically Cys-Ripk1, Cys-TRAF1, Asp-BRCA1, Leu-LIMK1, Tyr-NEDD9, Arg-BIM_{EL}, Asp-EPHA4, and Tyr-MET. They were found to be short-lived substrates of the Arg/N-degron pathway (6).

The diagram's second section describes six other previously identified proapoptotic Ct-fragments (all of them are produced by caspases) bearing destabilizing Nt-residues that can be recognized by the Arg/N-degron pathway. These fragments remain to be verified as Arg/N-degron substrates.

#9. *Asn*-PkCδ is the Ct-fragment of the protein kinase Cδ (PkCδ) that can be generated by (in particular) caspase-3. This fragment bears Nt-Asn and is proapoptotic, in contrast to the full-length PkCδ kinase (75-79).

#10. *Lys*-PkCθ is the Ct-fragment of the protein kinase Cθ (PkCθ). This fragment can be generated by (in particular) caspase-3, bears Nt-Lys, and is proapoptotic, in contrast to the full-length PkCθ kinase (80).

#11. *Trp*-Etk is the Ct-fragment of the Etk/Bmc tyrosine kinase, a member of the Btk/Tek family of kinases, at least some of which regulate apoptosis. The Trp-Etk fragment can be generated by (in particular) caspase-3, bears Nt-Trp, and is proapoptotic, in contrast to the full-length Etk kinase (81).

#12. *Gln*-Slk is the Ct-fragment of Slk, a Ste20-related protein kinase that plays a role in regulation of actin fibers. The Gln-Slk fragment can be generated by (in particular) caspase-3, bears Nt-Gln, and is proapoptotic. The concomitantly produced Nt-fragment of Slk is also proapoptotic (82).

#13. *Ile*-Hhp1 is the Ct-fragment of the hematopoietic progenitor kinase 1 (Hpk1), a Ste20-related protein kinase whose functions include stimulation of the stress-activated protein kinases SAPKs/JNKs and the NF-κB transcriptional regulon. The Ile-Hpk1 fragment can be generated by (in particular) caspase-3, bears Nt-Ile, and is proapoptotic, in contrast to the full-length Hk1 kinase (83).

#14. *Ile*-Mlh1 is the Ct-fragment of the mismatch repair Mhl1 protein that can be generated by (in particular) caspase-3, bears Nt-Ile, resides in the cytosol (in contrast to the full-length nuclear MLH1) and is proapoptotic, unlike full-length MLH1 (84).

The next 16 caspase-generated Ct-fragments and predicted substrates of the Arg/N-degron pathway that are not necessarily proapoptotic.

#15. *Tyr*-CYLD is the Ct-fragment of a deubiquitylase that regulates apoptosis and necroptosis (85).

#16. *Leu*-$p21^{Cip1/Waf1}$ is the Ct-fragment of p21, an inhibitor of cell division (86).

#17. *Arg*-IP_3R is the Ct-fragment of the inositol 1,4,5-triphosphate receptor (87).

#18. *Asn*-LMN1 is the Ct-fragment of lamin-A, a subunit of nuclear lamina (88).

#19. Arg-ETS-1 is the Ct-fragment of a transcription factor (89).

#20. *Tyr*-TOP1 is the Ct-fragment of a type I DNA topoisomerase (90).
#21. *Leu*-MEFD2 is the Ct-fragment of a transcription factor (91).
#22. *Asn*-DNA-PK is the Ct-fragment of the DNA-dependent protein kinase (92).
#23. *Asn*-CAD1 is the Ct-fragment of E-cadherin, an adhesion receptor (93).
#24. *Gln*-Synphilin-1 is the Ct-fragment of synphilin-1, a ligand of α-synuclein (94).
#25. *Tyr*-ACINUS is the Ct-fragment of a mediator of apoptotic chromatin condensation (95).
#26. *Lys*-PLECTIN is the Ct-fragment of a cytoskeletal protein (96).
#27. *Cys*-CCNE1 is the Ct-fragment of a specific G1/S cyclin (97).
#28. *His*-PMCA4b is the Ct-fragment of a Ca^{2+} extrusion pump (98).
#29. *Asp*-CDC42 is the Ct fragment of CDC42, a RAS superfamily member (99).
#30. *Tyr*-iPLA$_2$ is the Ct-fragment of the phospholipase A$_2$ (100).

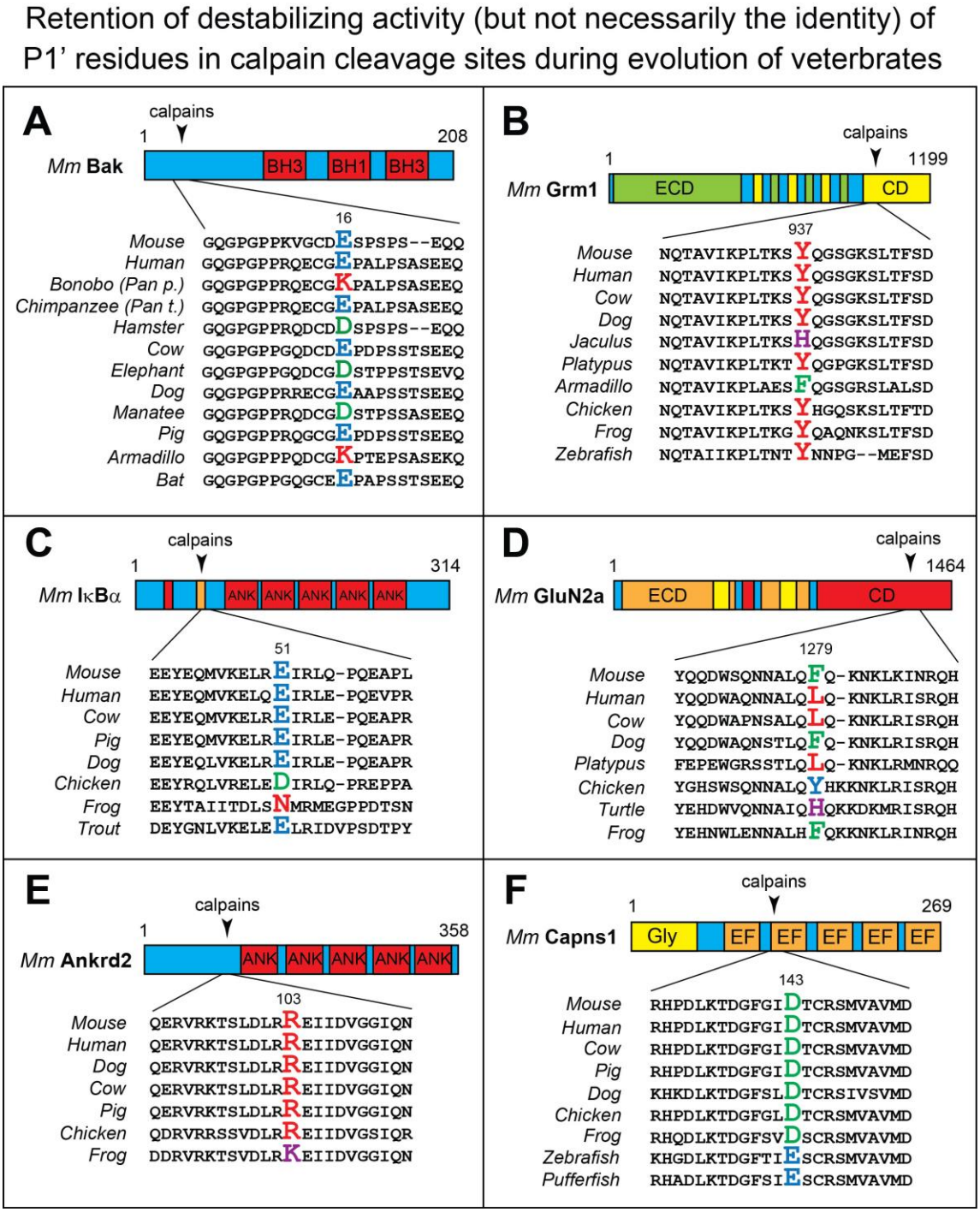

Fig. S3. Retention of *destabilizing* activity (but not necessarily the *identity*) of P1' residues in calpain cleavage sites during evolution of vertebrates.

Arrowheads indicate calpain cleavage sites. P1' residues, which become N-terminal upon the cleavage, are larger and colored. The diagrams and indicated residue numbers are of mouse [*Mus musculus* (*Mm*)] caspase substrates, some of which are cited in Fig. S1.

(*A*) Bak. The P1' residue (future Nt-residue) is Glu in mouse, human, chimpanzee, and bat, is Lys in bonobo (a close relative of chimpanzee) and armadillo; and is Asp in hamster,

elephant, and manatee. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*B*) Grm1. The P1’ residue (future Nt-residue) is Tyr in all cited species save for jaculus (a rodent) and armadillo, in which P1’ residues are, respectively, His and Phe. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*C*) IκBα. The P1’ residue (future Nt-residue) is Glu in all cited species except chicken and frog, in which P1’ residues are, respectively, Asp and Asn. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*D*) GluN2a. The P1’ residue (future Nt-residue), is Phe in mouse, dog, and frog, is Leu in human, cow, and platypus, is Tyr in chicken, and is His in turtle. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*E*) Ankrd2. The P1’ residue (future Nt-residue) is Arg in all cited species except frog, in which P1’ is Lys. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*F*) Capsn1. The P1’ residue (future Nt-residue) is Asp in all cited species except zebrafish and pufferfish, in which P1’ is Glu. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

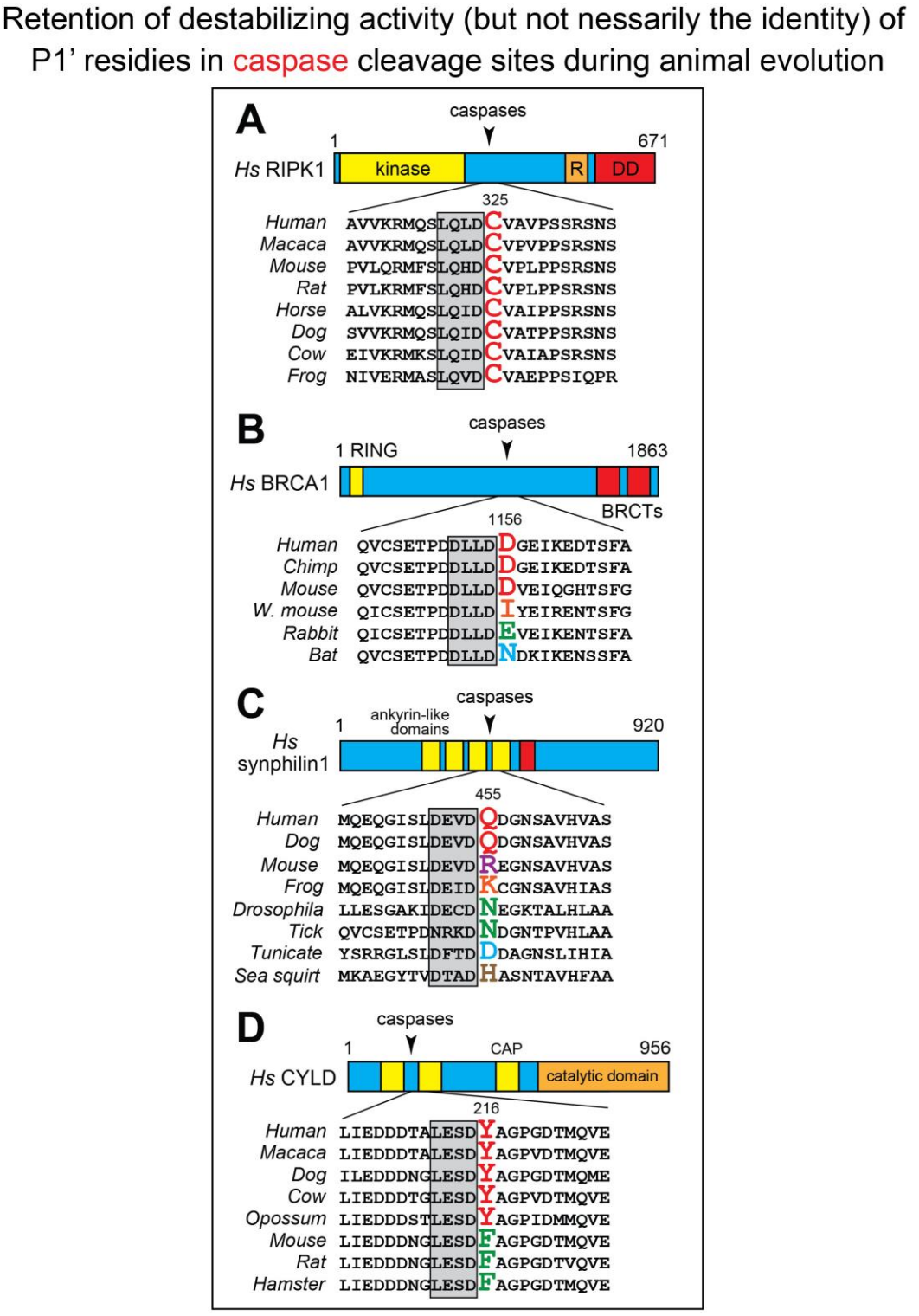

Fig. S4. Retention of *destabilizing* activity (but not necessarily the *identity*) of P1' residues in caspase cleavage sites during animal evolution.

Arrowheads indicate caspase cleavage sites, which are highlighted by grey rectangles. P1' residues, which become N-terminal upon cleavage by a caspase, are larger and colored. The diagrams and indicated residue numbers are of human caspase substrates, which are cited in Fig. S2.

(*A*) RIPK1. The P1' residue (future Nt-residue) is Cys in all cited species. Nt-Cys is destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*B*) BRCA1. The P1' residue (future Nt-residue) is Asp in human, chimpanzee and mouse, is Ile in western mouse (an Australian rodent), is Glu in rabbit, and is Asn in bat. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*C*) Synphilin1. The P1' residue (future Nt-residue) is Gln in human and dog, is Arg in mouse, is Lys in frog, is Asn in a tick and fruit fly (*Drosophila*), is Asp in a tunicate (a marine invertebrate), and is His in sea squirt. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

(*D*) GluN2a. The P1' residue (future Nt-residue) is Tyr in human, macaca, dog and cow, and is Phe in mouse, rat and hamster. All of these Nt-residues are destabilizing in the Arg/N-degron pathway (see the main text and Fig. 2).

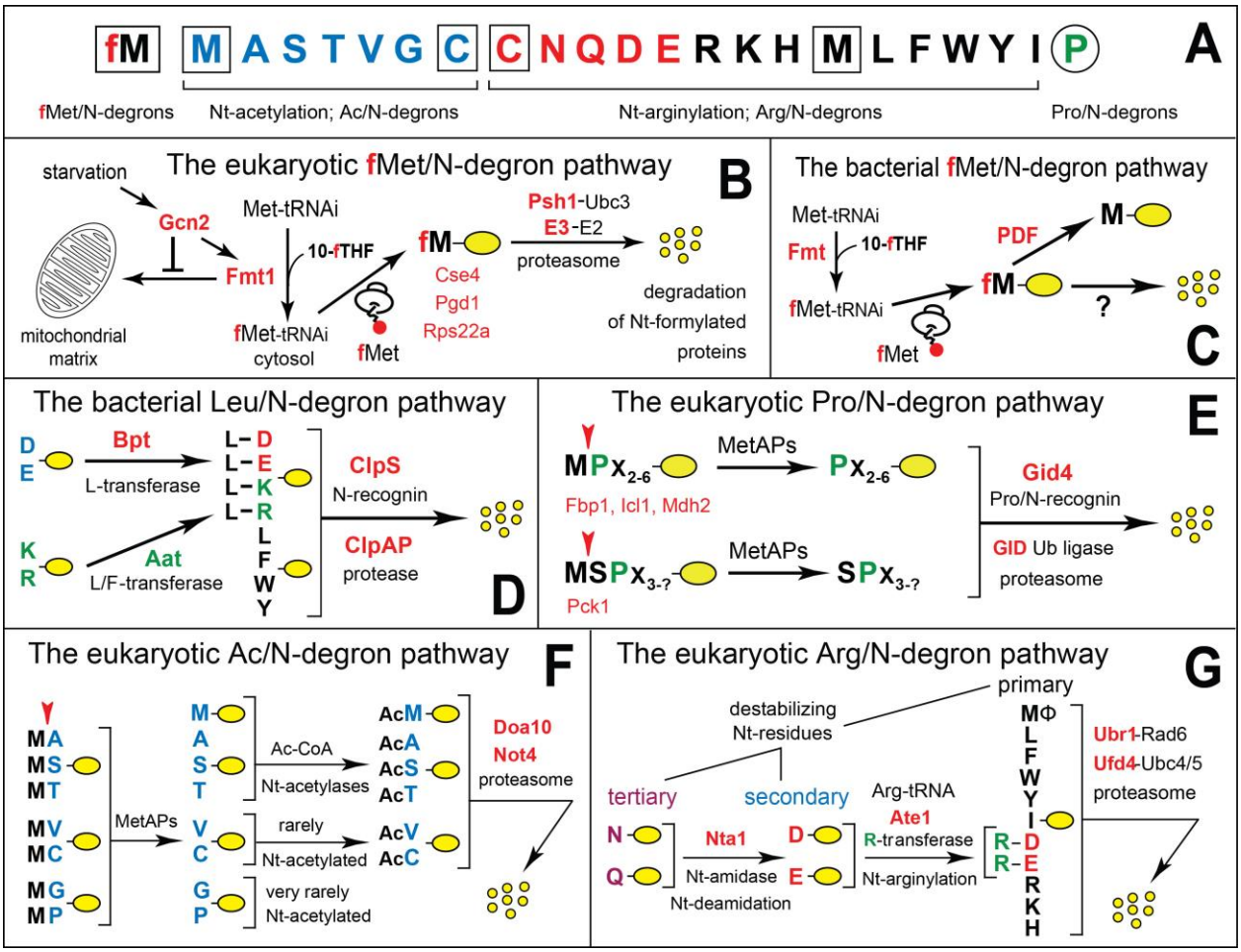

Fig. S5. N-degron pathways. N-terminal (Nt) residues are indicated by single-letter abbreviations. A yellow oval denotes the rest of a protein substrate.

(*A*) Twenty amino acids of the genetic code are arranged to delineate specific N-degrons. Nt-Met is cited thrice, since it can be recognized by the Ac/N-degron pathway (as Nt-acetylated Ac-Met), by the Arg/N-degron pathway (as unacetylated Nt-Met), and by the fMet/N-degron pathway (as Nt-formylated fMet). Nt-Cys is cited twice, since it can be recognized by the Ac/N-degron pathway (as Nt-acetylated Cys) and by the Arg/N-degron pathway (as an oxidized, arginylatable Nt-Cys sulfinate or sulfonate, formed in multicellular eukaryotes but apparently not in unstressed *S. cerevisiae*).

(*B*) The eukaryotic (*S. cerevisiae*) fMet/N-degron pathway (101). 10-fTHF, 10-formyltetrahydrofolate.

(*C*) The bacterial (*E. coli*) fMet/N-degron pathway (102).

(*D*) The bacterial (*V. vulnificus*) Leu/N-end rule pathway (103).

(E) The eukaryotic (*S. cerevisiae*) Pro/N-degron pathway (104-106).

(*F*) The eukaryotic (*S. cerevisiae*) Ac/N-degron pathway (107-110).

(G) The eukaryotic (*S. cerevisiae*) Arg/N-degron pathway (2, 111, 112). Modified with permission from ref. (2).

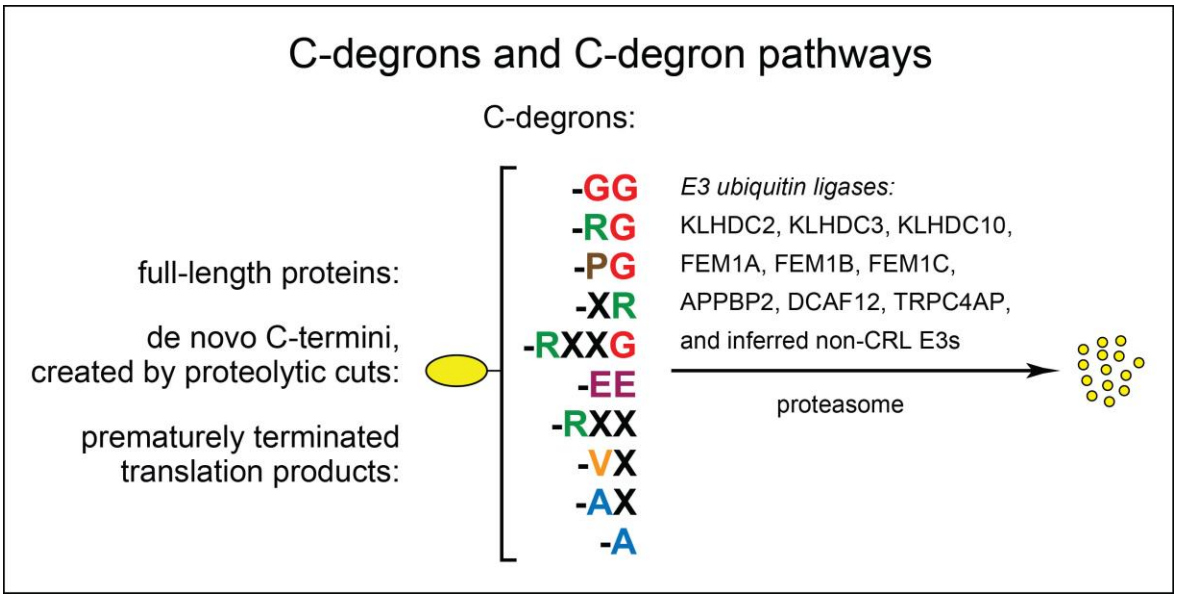

Fig. S6. C-degrons and C-degron pathways in human cells. This diagram is a simplified summary of the 2018 discovery, by the laboratories of Elledge and Yen, of a large set of C-degrons in human proteins (113-115). Amino acid residues are denoted by single-letter abbreviations. A yellow oval denotes a protein substrate upstream of its C-terminus. The indicated C-terminal (Ct) sequences and individual Ct-residues, referred to as C-degrons, are targeted, in conjunction with internal Lys residues (ubiquitylation sites) of individual C-degron substrates, by a broad range of Ub ligases, largely but not solely of the CRL class (113-115). See the main text for a brief discussion of C-degron pathways.

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