Supporting Information

Ono et al. 10.1073/pnas.1411959111

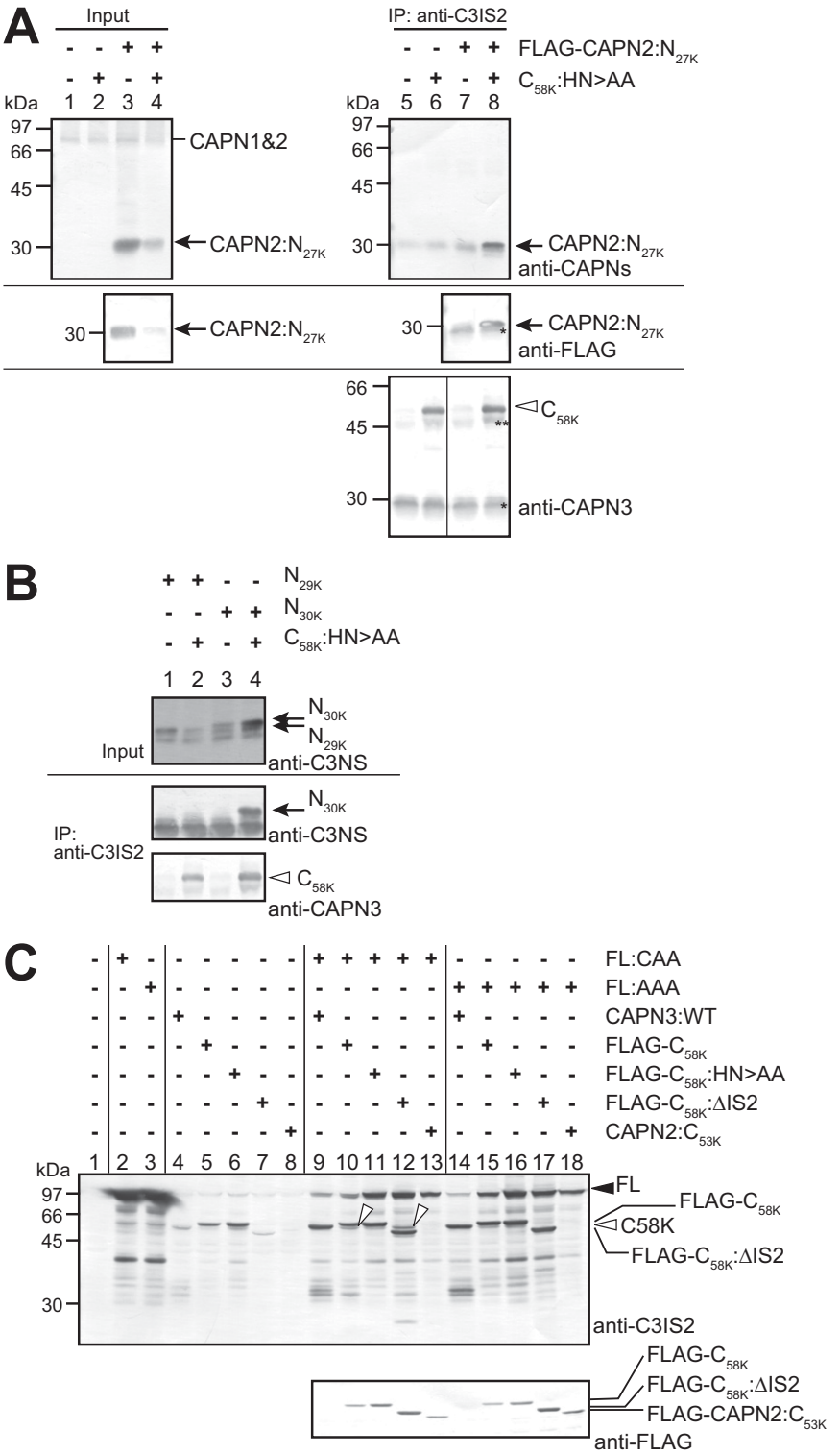

Fig. S1. iMOC between C_{58K} and the N-terminal region of CAPN2 or FL-CAPN3. (*A*) Coexpression of CAPN2:N_{27K} and CAPN3 C_{58K}:HN > AA in COS7 cells. Both fragments were efficiently coimmunoprecipitated using the anti-C3IS2 antibody. (*B*) Coexpression of CAPN3 N_{29K} or N_{30K} and C_{58K}:HN > AA in COS7 cells. The

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fragment N_{30K} but not N_{29K} was coimmunoprecipitated with C_{58K}:HN > AA. (*C*) Coexpression of the inactive CAPN3 mutant, FL:CAA, with either C_{58K} or C_{58K}:ΔIS2 N-terminally FLAG-tagged fragments reconstituted the autolytic reaction (lanes 10 and 12, open arrowhead). Mutation of His334 and N358D in C_{58K} (lane 11) or of Cys129 in FL:CAA (lanes 15 and 17) abrogated the reconstitution of the autolytic reaction, and C_{58K} was not detected. Under the same context, FLAG-CAPN2: C_{53K} was also unable to complement mutant forms of FL-CAPN3 (lanes 13 and 18).

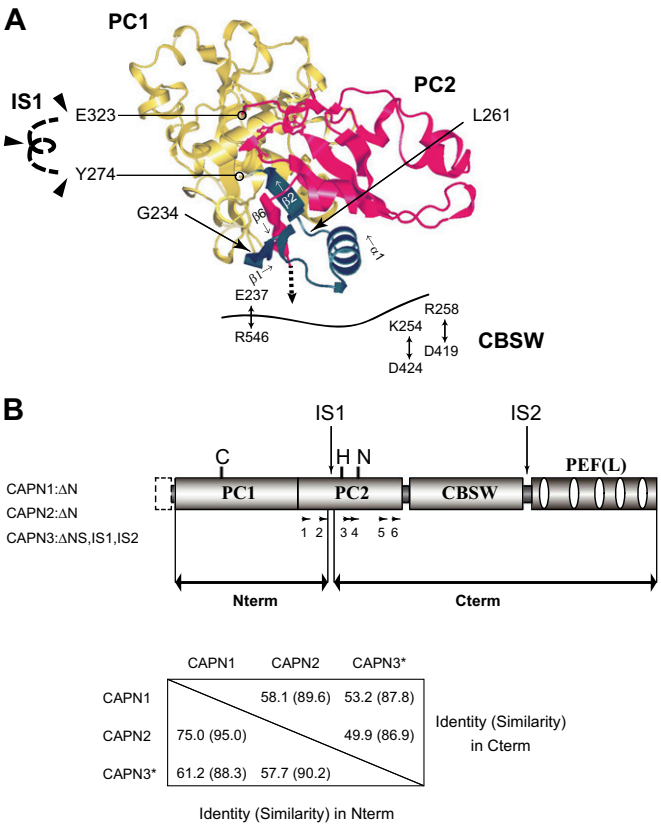


Fig. S2. Contribution of the PC2 structure in CAPN3 iMOC. (*A*) Structure of the Ca^{2+}-bound form of the rat CAPN2 CycPc domain (Protein Data Bank 1MDW), showing the positions of the amino acid residues N_{26K} and N_{29K} in Fig. 1 and Fig. S3. Bidirectional vertical arrows indicate electrostatic interactions between amino acid residues in PC2 and those in the linker and CBSW regions identified in the rat CAPN2 structure; the numbers reflect the corresponding amino acid residues in the CAPN3 sequence. (*B*) Sequence homology comparisons of the N- and C-terminal regions in CAPN1–3. Given that NS, IS1, and IS2 were demonstrated to be dispensable for iMOC, the CAPN3 sequence is represented by that of FL:ΔNS/IS1/IS2, and the N-terminal regions are omitted from the CAPN1 and -2 sequences.

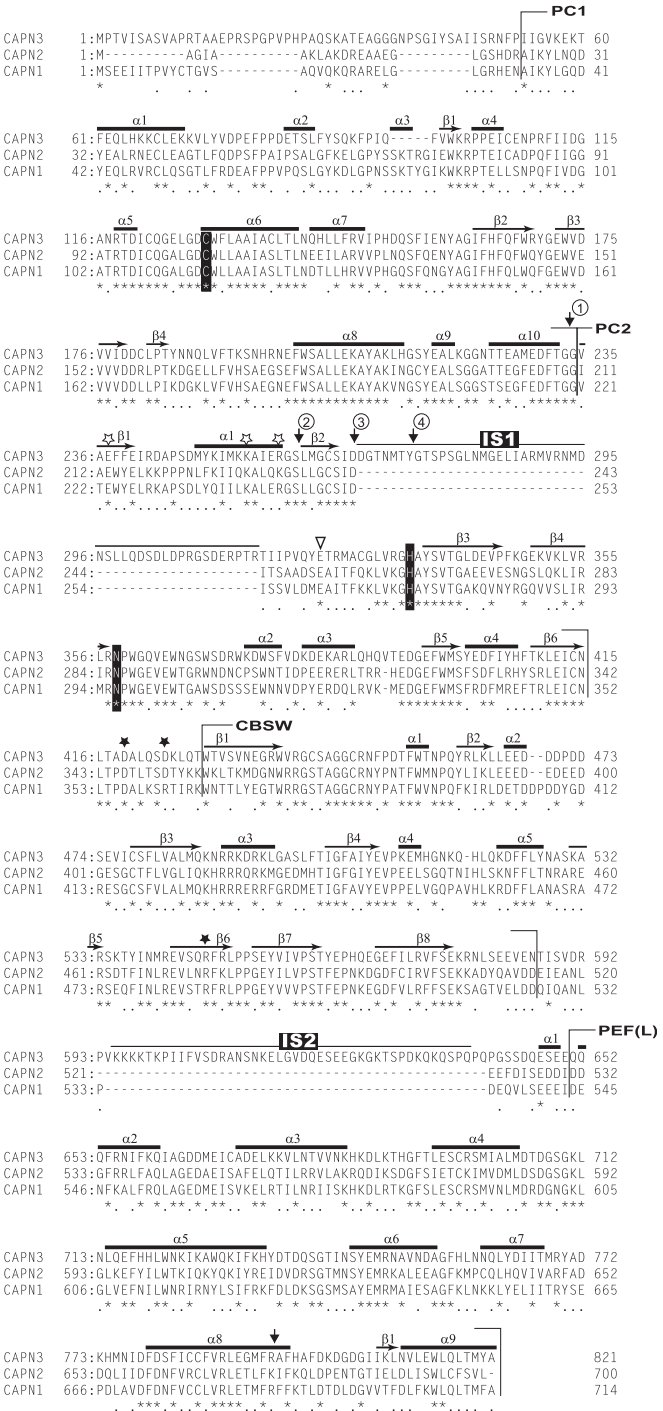
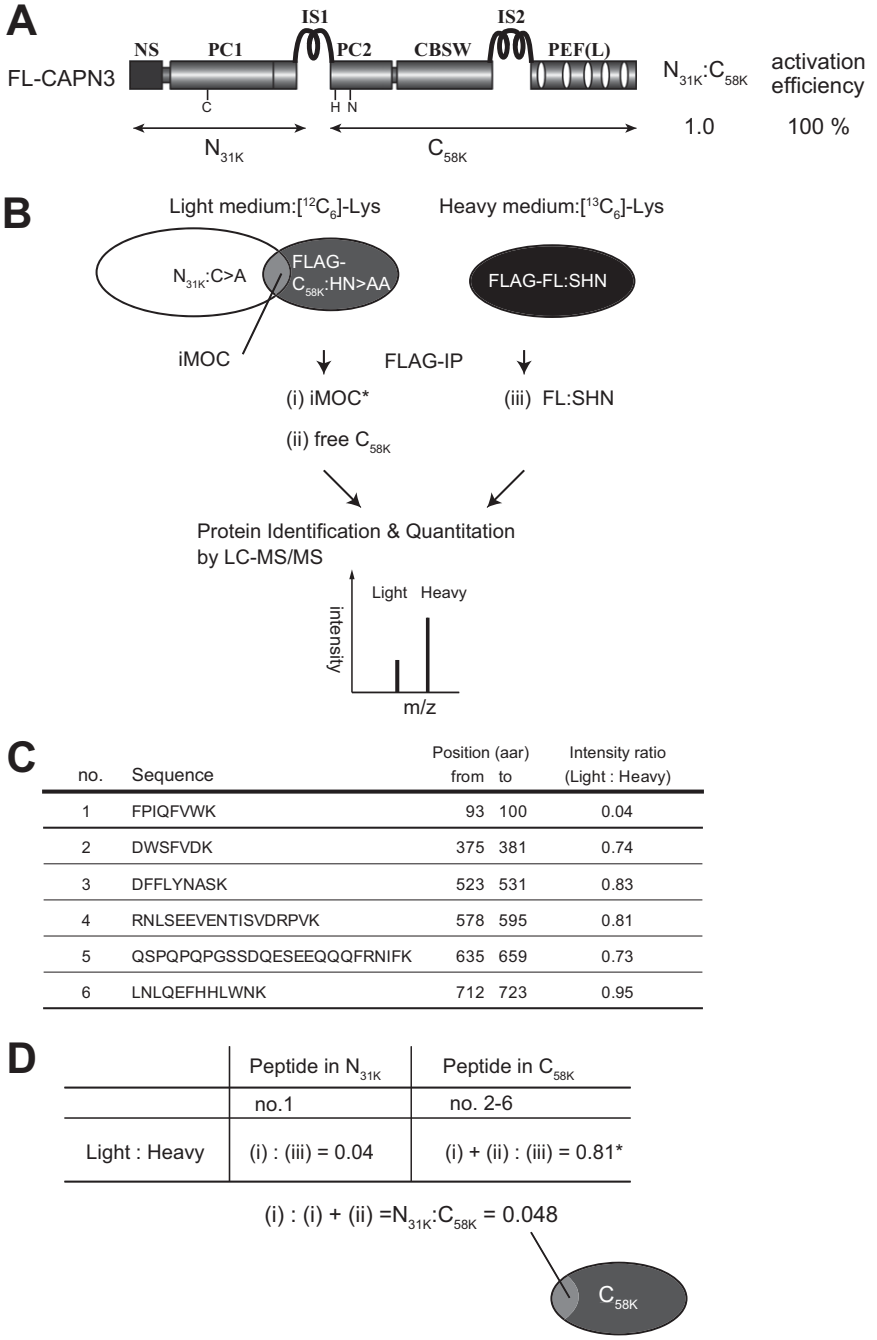


Fig. S3. Structure-based amino acid sequence alignment of calpains. Secondary structures, α-helixes and β-strands, identified in the structure of a Ca^{2+}-bound form of rat CAPN2/CAPNS1, are indicated above the sequence of human CAPN1–3. Vertical arrows show the ends of the CAPN3 N-terminal constructs: N_{26K}, N_{29K}, N_{30K}, and N_{31K}. Open arrowhead shows the first amino acid residue in C_{58K} and the corresponding CAPN2:C_{53K} construct. Open and closed stars show amino acid residues involved in electrostatic interactions in PC2 and CBSW, respectively. Horizontal arrows show β1–β6 strands in PC2, corresponding to those depicted in Fig. S2*B*. The following sequences were used: CAPN1, P07384; CAPN2, P17655; and CAPN3, P20807.



no.	Sequence	Position (aar) from	to	Intensity ratio (Light : Heavy)
1	FPIQFVWK	93	100	0.04
2	DWSFVDK	375	381	0.74
3	DFFLYNASK	523	531	0.83
4	RNLSEEVENTISVDRPVK	578	595	0.81
5	QSPQPQPGSSDQESEEQQQFRNIFK	635	659	0.73
6	LNLQEFHHLWNK	712	723	0.95

Fig. S4. SIALC-based quantition of N_{31K}–C_{58K} interaction. (*A*) It is hypothesized that N_{31K}:C_{58K} = 1.0 corresponds to 100% activation, as in the case of FL-CAPN3. (*B*) Schematic illustration of the experimental procedure. Under the condition used, N_{31K}:C > A is expressed in excess of C_{58K}:HN > AA. *N_{31K} is coimmunoprecipitated. (*C*) The peak intensity ratio (light:heavy) was calculated for selected peptides. (*D*) Intensity ratios for N_{31K} and C_{58K} were used to calculate the molar ratio of N_{31K}:C_{58K} in the immunoprecipitated sample from light medium. *Average of peptides nos. 2–6.

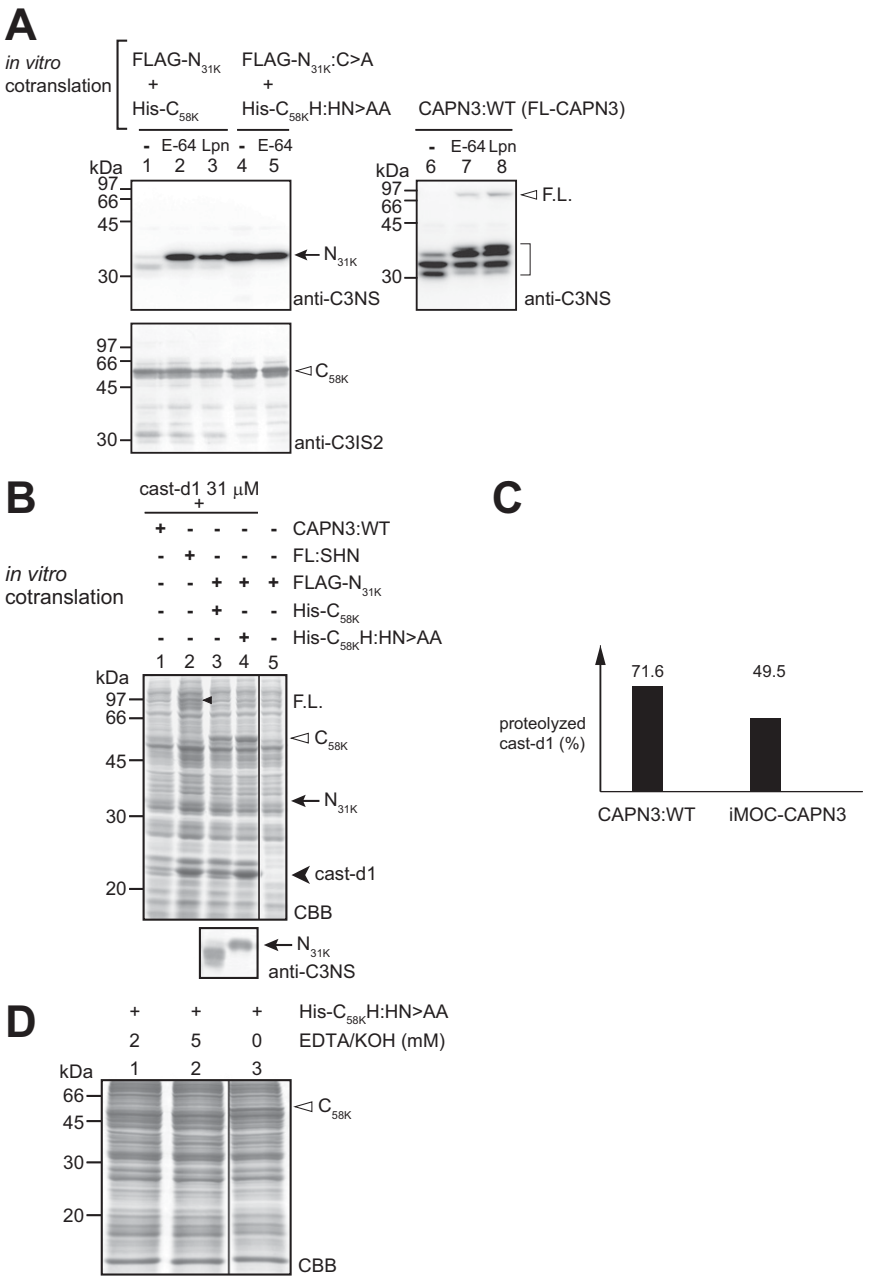


Fig. S5. Expression of CAPN3 fragments in in vitro translation system. (*A*) Effect of protease inhibitors. In the presence of 100 μM of E-64-c or leupeptin, iMOC-mediated autolysis of CAPN3 was significantly inhibited (anti-C3NS, lanes 2 and 3 vs. lane 1). Autolytic activity of FL-CAPN3 was hardly inhibited (lanes 7 and 8 vs. lane 6). (*B*) Proteolysis of CAST. Translation reaction was carried out in the presence of cast-d1. Protease activity-dependent decrease of cast-d1 was observed (CBB, lane 1 vs. lanes 2 and 3 vs. lane 4). Generation of autolytic fragment N_{31K} was not inhibited (anti-C3NS, lane 3). (*C*) CAST-proteolytic activity of FL-CAPN3 and iMOC-CAPN3. Decrease of signal intensity of cast-d1 was expressed using protease-inactive mutant as a negative control (lane 1 vs. lanes 2 and 3 vs. lane 4). (*D*) Inhibition of in vitro translation by EDTA/KOH. In the presence of 2 mM or 5 mM EDTA/KOH, translation was significantly inhibited (lanes 1 and 2 vs. lane 3).

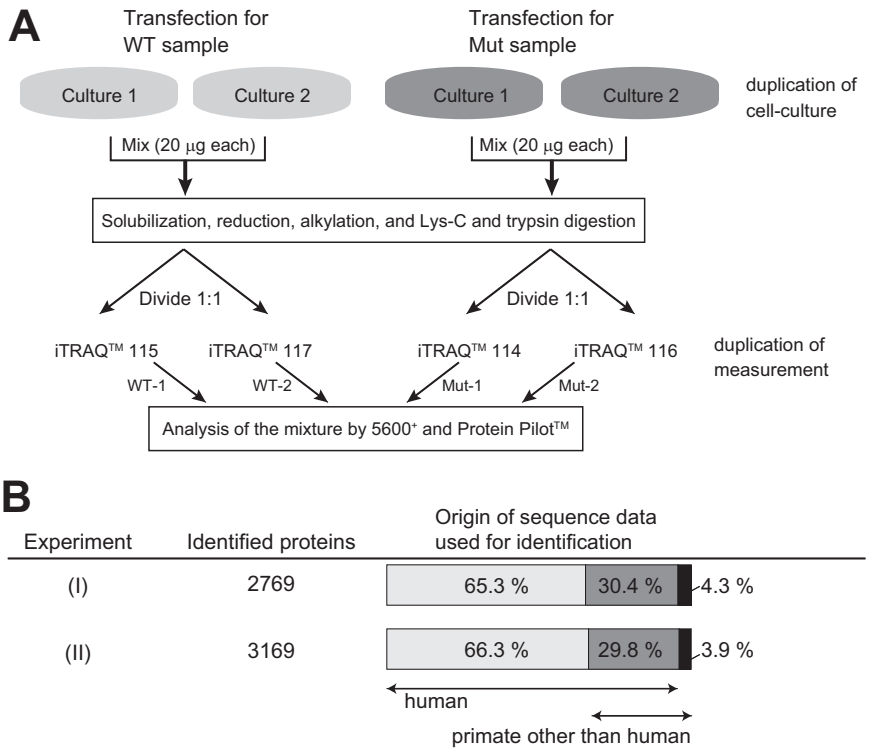


Fig. S6. Proteomic analysis of COS7 cells expressing CAPN3. (*A*) Schematic illustration of the experimental procedure. (*B*) Because COS7 cells were established from monkey, protein identification was performed using the human database supplemented with the sequences for other primates. More than 65% of proteins were found only in the human database.