SUPPLEMENTAL DATA

CutA Divalent Cation Tolerance Homolog (*E. coli*) (CUTA) Regulates β-Cleavage of β-Amyloid Precursor Protein (APP) through Interacting with β-Site APP Cleaving Protein 1 (BACE1)

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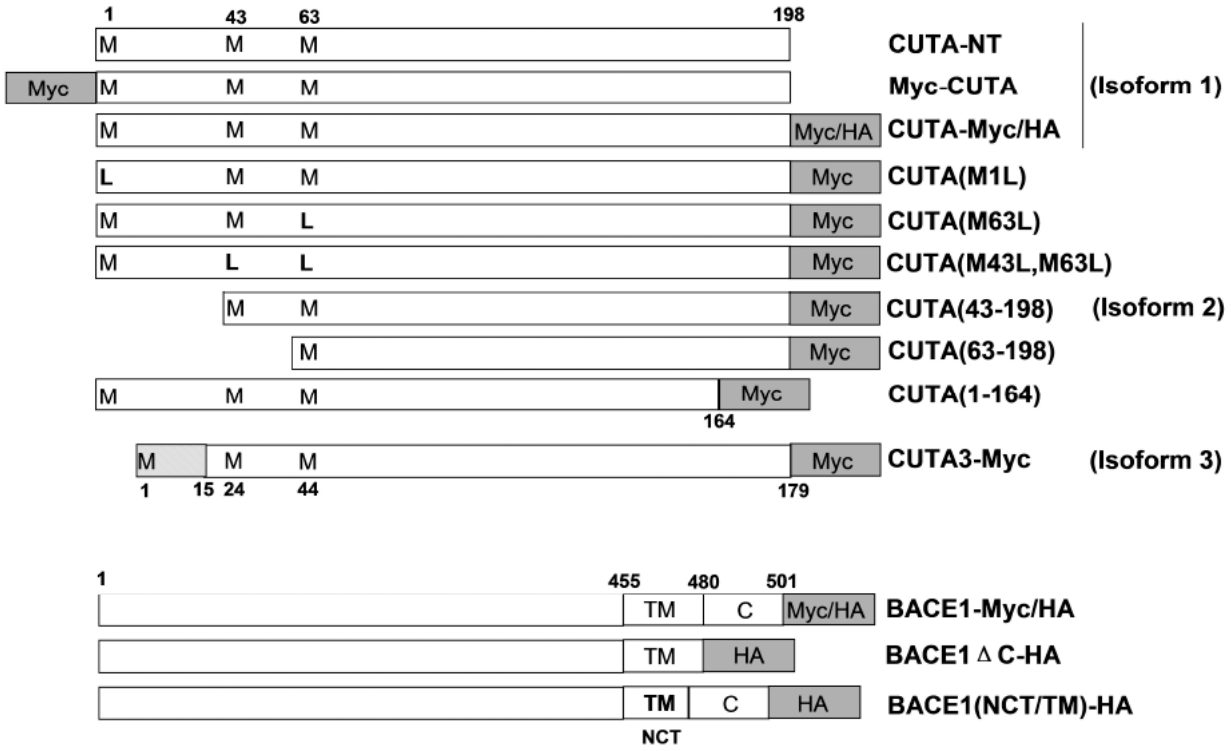
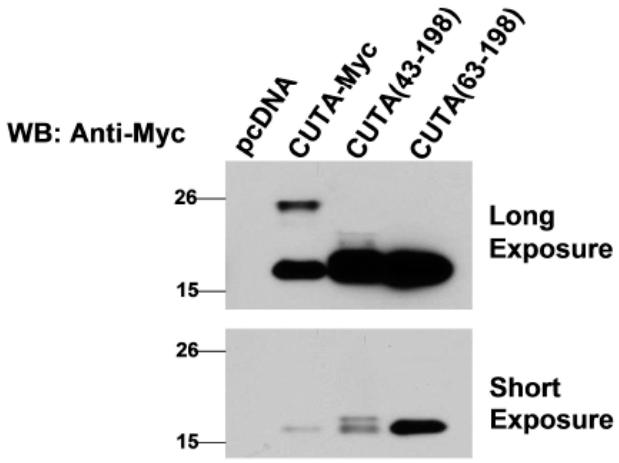
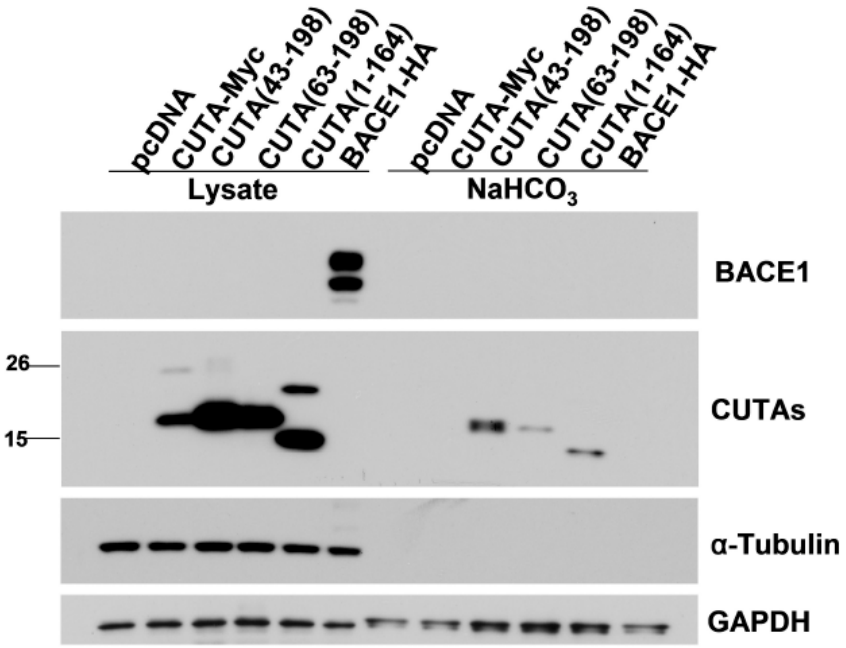


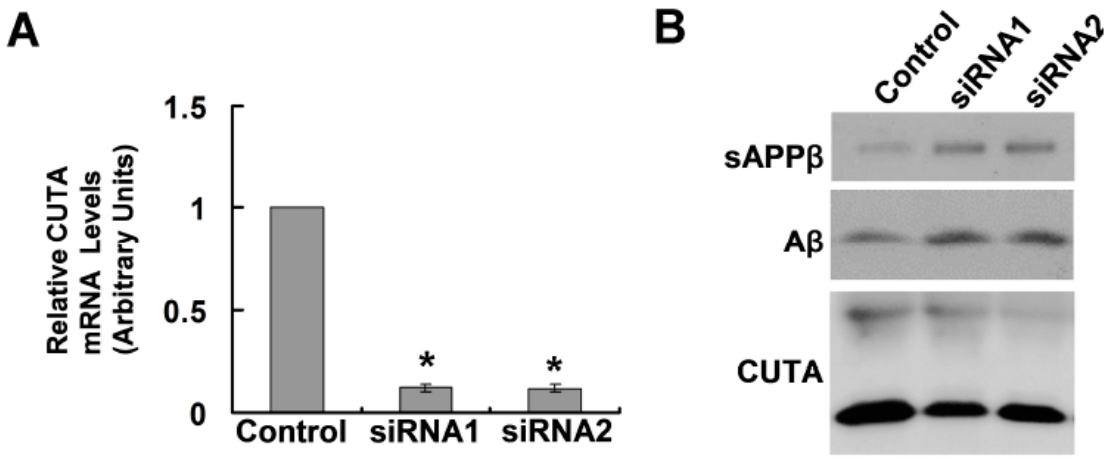
Supplemental FIGURE 1. **Diagram of various vectors used in this study.** CUTA vectors used include non-tagged CUTA isoform 1 (CUTA-NT), Myc-tagged at the N-terminus (Myc-CUTA), Myc- or HA-tagged at the C-terminus (CUTA-Myc or CUTA-HA), mutated at methionine 1 to leucine (M1L), mutated at methionine 63 to leucine (M63L), or mutated at methionines 43 and 63 to leucines (M43L, M63L). CUTA truncated with the N-terminal amino acids 1-42 (43-198, equal to isoform 2) or amino acids 1-62 (63-198), and CUTA isoform 1 truncated with the 34 C-terminal amino acids (1-164) are all Myc-tagged at the C-terminus. CUTA isoform 3, which differs from isoform 1 at the N-terminus, is C-terminal Myc-tagged. BACE1 vectors are C-terminal tagged with Myc or HA. BACE1 truncated at the C-terminus by 21 amino acids and BACE1 that has its transmembrane (TM) domain substituted with the Nicastrin (NCT) TM domain are HA-tagged at the C-terminus.



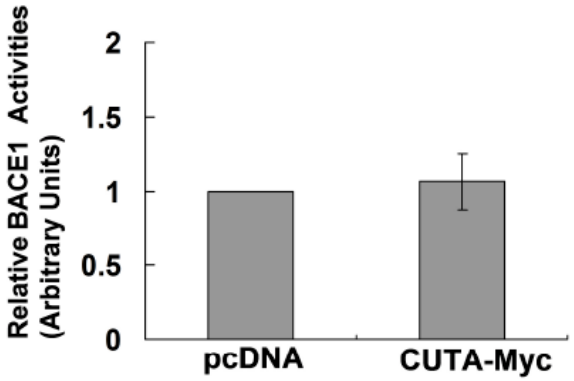
Supplemental FIGURE 2. **Human CUTA variants are separated into heavy and light components.** HEK 293T cells were transfected with control pcDNA, CUTA-Myc, CUTA(43-198) or CUTA(63-198). Equal protein amounts of cell lysates were subjected to SDS-PAGE and Western blot using the Myc antibody 9E10.



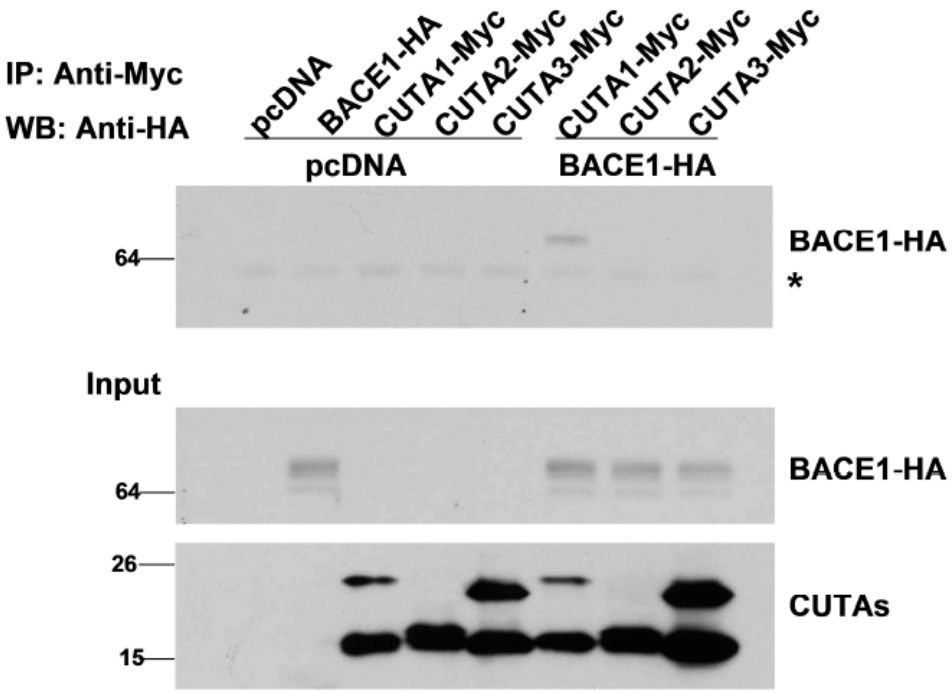
Supplemental FIGURE 3. **The heavy component of CUTA containing the N-terminus is integrated with the membrane.** Cells were transfected with pcDNA, full length CUTA, CUTA(43-198), CUTA(63-198), CUTA(1-164), or BACE1-HA. After homogenization of cells, a small sample was used to analyze total lysates and the rest was subjected to centrifuge to separate the cytosolic and membrane fractions. The membrane pellet was rinsed with $NaHCO_3$ (pH 11.3) to dissociate peripheral membrane proteins. The washing buffer was subjected to Western blot to detect CUTA (using the Myc antibody 9E10), GAPDH (to indicate membrane-bound protein), BACE1 (using an HA antibody to indicate the membrane fraction) and α-Tubulin (to indicate the cytosolic fraction).



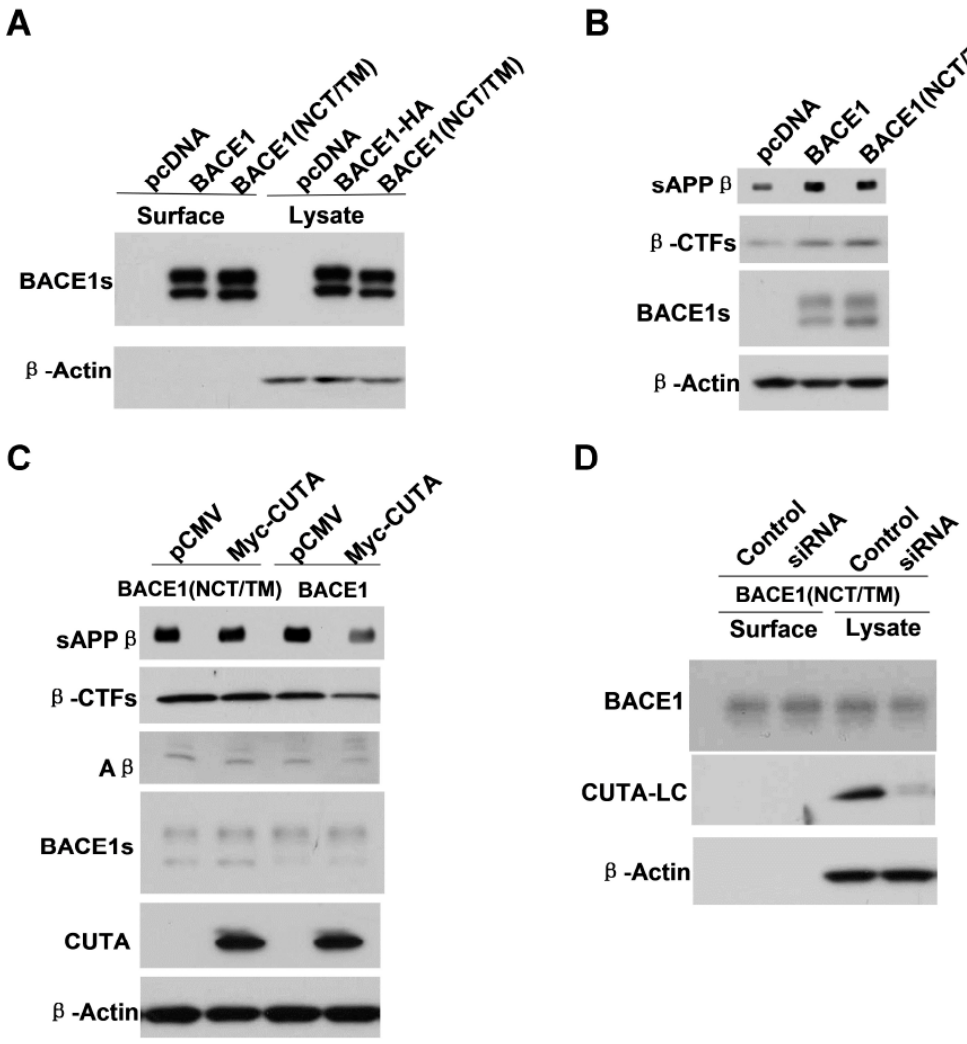
Supplemental FIGURE 4. **Downregulation of CUTA increases the levels of sAPPβ and Aβ.** Cells were transfected with CUTA siRNA 1, siRNA 2, or control siRNA. (*A*) Cells were subjected to RNA extraction, reverse transcription and RT-PCR to detect mRNA levels of *CUTA*, which were normalized to those of *GAPDH* for comparison. *: $p<0.05$, n=3. (*B*) The levels of sAPPβ and Aβ in conditioned media were analyzed by Western blot or immunoprecipitation-Western blot. The level of CUTA in cell lysates was analyzed by immunoprecipitation-Western blot.



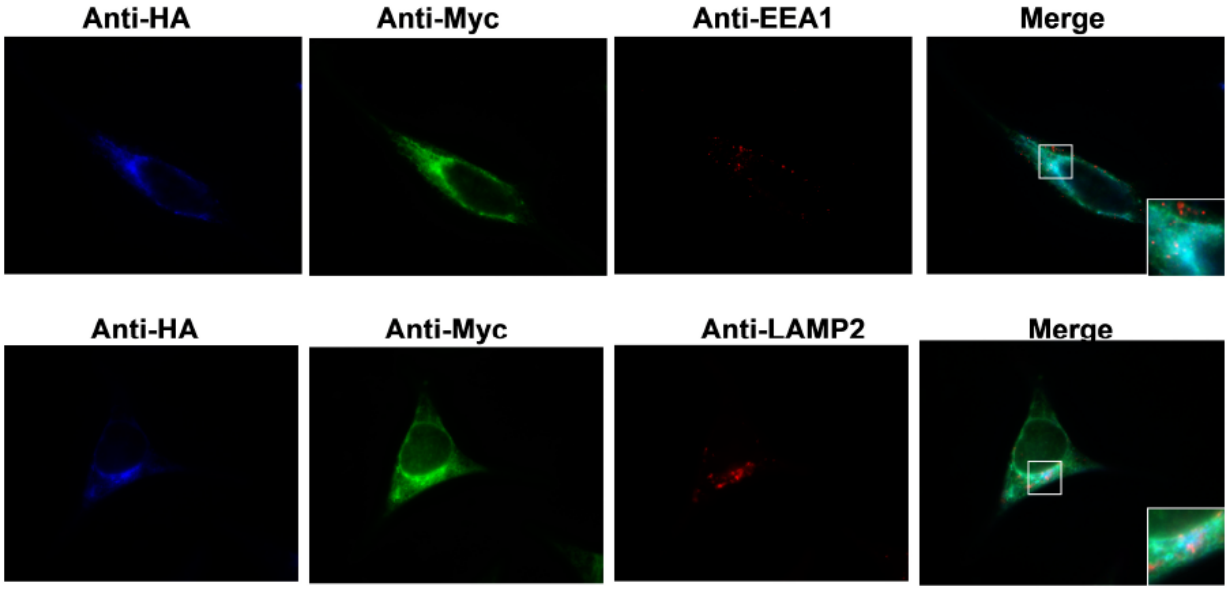
Supplemental FIGURE 5. **Overexpression of CUTA does not affect *in vitro* enzymatic activity of BACE1.** SH-SY5Y cells were transiently transfected with control pcDNA or CUTA-Myc. Cell lysates were assayed for BACE1 activity using a commercial kit, following the manufacturer's protocol.



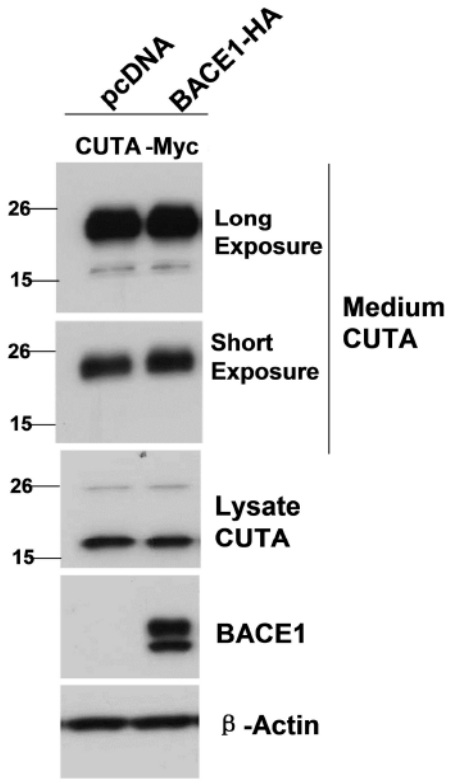
Supplemental FIGURE 6. **The N-terminus of CUTA isoform 3 does not interact with BACE1.** BACE1-HA was co-transfected with the three isoforms of CUTA, 1, 2 and 3, all of which have a Myc-tag at the C-terminus. Equal protein amounts of cell lysates were used for immunoprecipitation with a Myc antibody and Western blotted with an HA antibody. Ten percent of cell lysates were used as input. *: non-specific band.



Supplemental FIGURE 7. **The transmembrane domain of BACE1 is important for its interaction with CUTA.** (*A*) HEK 293T cells were transfected with control pcDNA, wild type BACE1 or BACE1(NCT/TM), and then subjected to biotinylation. Cell lysates were affinity-precipitated with streptavidin-agarose beads. The levels of biotinylated BACE1 and BACE1(NCT/TM), as well as their total protein levels, were analyzed by Western blot. The intracellular protein β-Actin that can not be biotinylated was used as a negative control. (*B*) HEK-Swe cells were transfected with control pcDNA, wild type BACE1 or BACE1(NCT/TM). APP β-CTFs in cell lysates and sAPPβ in conditioned media were detected by Western blot. (*C*) HEK-Swe cells were first transfected with wild type BACE1 or BACE1(NCT/TM). After equal splitting, cells were transfected with control pCMV or Myc-CUTA. The levels of Aβ and sAPPβ in conditioned media, as well as the levels of APP β-CTFs in cell lysates were analyzed. (*D*) HEK 293T cells were first transfected with BACE1(NCT/TM). After equal splitting, cells were transfected with control siRNA or CUTA siRNA 3. Cells were then subjected to biotinylation, affinity precipitation, and Western blot.



Supplemental FIGURE 8. **The colocalization site of CUTA-BACE1 is not in the endosome compartment.** HeLa cells were co-transfected with BACE1-HA and Myc-CUTA. Cells were then fixed, permeabilized and immunostained with primary antibodies against HA (indicating BACE1, in blue), Myc (indicating CUTA, in green), and EEA1 (indicating early endosome, in red) or LAMP2 (indicating late endosome, in red), and respective secondary antibodies conjugated with Alexa Fluor 350, 488, or 594. Samples were examined by immunofluorescence microscopy.



Supplemental FIGURE 9. **BACE1 does not affect CUTA secretion.** HEK 293T cells were transfected with CUTA-Myc and split equally. Cells were then transfected with control pcDNA or BACE1. CUTA in conditioned media and in cell lysates was analyzed by Western blot.