Supporting Information

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Fig. S1. The lack of BACE1 S-palmitoylation does not markedly affect prominent BACE1 localization in mossy fibers. (*A*) Coronal brain sections of WT and 4CA mice were immunolabeled using BACE1 (green) and MAP2 (magenta) antibodies to visualize mossy fiber localization of BACE1. (*B, Left*) Schematic representation of infrapyramidal bundle (IPB) and suprapyramidal bundle (SPB) to stratum lucidum (slu) length measurement. Quantification of IPB length, SPB-slu length, and IPB/SPB-slu ratio in WT and 4CA mice (*n* = 6 WT and 7 4CA).



Fig. S2. The lack of BACE1 S-palmitoylation does not markedly affect BACE1 localization in the olfactory bulb. Olfactory bulb sections of WT and 4CA mice were immunolabeled using BACE1 (green), phospho-Neurofilament H (pNF, magenta), and CHL-1 (blue) antibodies to visualize BACE1 accumulation in the glomeruli.

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Fig. S3. Analysis of neuronal substrate processing. (*A*) Two-month-old WT and 4CA mouse brains were separated into a soluble protein fraction (0.2% DEA sol.) and a detergent-soluble membrane protein fraction (RIPA sol.). Aliquots of each fraction were analyzed by immunoblotting with N- and C-terminal antibodies to APP or APLP to confirm separation of soluble and membrane-bound proteins. (*B*) The levels of BACE1 at P7 and P60 in RIPA extracts were quantified by immunoblotting and normalized to the levels of actin. (*C*) DEA and RIPA lysates were analyzed by immunoblotting with the indicated antibodies. The results shown are representative blots from P7 mice. (*D*) The levels of indicated proteins in the DEA homogenates (normalized to actin) were quantified from P7 (n = 5 WT and 7 4CA) and 2-mo-old mice (n = 6 WT and 6 4CA). *P < 0.05.



Fig. S4. Cerebral CTF and amyloid levels in 5XFAD mice in the absence of BACE1 S-palmitoylation. (*A*) APP and APP-CTFs were immunoprecipitated from brain homogenates of 5XFAD and 5X4CA mice, subject to dephosphorylation, and analyzed by immunoblotting. (*B*) The relative abundance of $+1 \beta$ -CTF, $+11\beta$ -CTF, and α -CTF were quantified as the percentage of total APP CTFs (n = 6 5XFAD and 6 5X4CA). (*C*) Forebrain tissue harvested from female 5XFAD and 5X4CA mice (n = 17 and 18 animals, respectively) were sequentially homogenized in PBS and guanidine to extract soluble and insoluble A β . The levels of A β 40 and A β 42 in each fraction were quantified by ELISA. (*D*) A z-score analysis shows lower insoluble A β levels in 5X4CA animals.