Supplementary Information

Methods

Materials: 2,5-dioxopyrrolidin-1-yl 4-azido-2-hydroxybenzoate (NHS-ASA) was purchased from ProChem. Inc (Rockford, IL). 6-Methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine and *N*-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-4-(piperazin-1-ylmethyl)benzamide (*N*-desmethyl imatinib) were purchased from ChemPacific Inc (Baltimore, MD). 2,5-dioxopyrrolidin-1-yl 5-((3aS,4S,6aR)-2-oxo-hexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanoate (Biotin-OSu), *N*-(chloro(dimethylamino)methylene)-*N*-methylmethanaminium hexafluorophosphate (TCFH), trifluoroacetic acid (TFA), 1*H*-benzo[d][1,2,3]triazol-1-ol (HOBt) and *N*,*N*-diisopropylethyl amine (DIPEA) were purchased from Sigma-Aldrich (St. Louis, MO). *Tert*-butyl 2-(piperazin-1-yl)ethylcarbamate was purchased from Astatech Inc (Bristol, PA).

Synthesis of an imatinib derived photo-affinity label, G01: DIEPA (63 μl, 0.36 mmol) was added to a solution of NHS-ASA (50 mg, 0.18 mmol), HOBt (25 mg, 0.18 mmol), and 6-Methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (50 mg, 0.18 mmol) in DMF (2 ml). The reaction mixture was stirred at room temperature overnight under argon atmosphere. The generated crude product was purified by a semi-preparative HPLC to give 54 mg of the titled compound with a yield of 68%. The product, **G01**, 4-azido-2-hydroxy-*N*-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide, was confirmed by mass spectral analysis using an ESI-MS in the positive mode $[M+H]^+$, demonstrating a *m/z* of 439.1.

Radioiodination of G01 by ^{125}I was performed without carrier using a modification of a Chloramine-T procedure and the iodinated product was purified by HPLC. Specifically, in a UV protected "V" vial, total volume 0.9 ml, ~10 mCi of ^{125}I stock isotope (volume = 25 μl) was added to 200 μl of 0.2M phosphate buffer, pH 7.2. G01 was dissolved to 1 mg/ml in ethanol and 25 μl of this solution was combined with chloramine-T at 1 mg/mL in water (50 μl) and then added to the V-vial. The reaction proceeded for 1 min and was terminated by the addition of 50 μL of 1 mg/ml meta-bisulfite. The reaction mixture was chromatographed on a 25 cm Waters RP-C18 column, using 0.1% TFA in water as the "A" solvent and 0.1% TFA in acetonitrile as the "B" solvent. A gradient was run at 1 ml/min from 0% B to 50% B for 45 minutes and held at 50% B for 15 minutes. The product demonstrated a retention time of 54.5 min as followed by radiochemical detection, and had a specific activity of 2000 Curies per millimole. The I^{125} labeling experiment was performed by PerkinElmer Life and Analytical Sciences, Inc.

^{3}H-G01 was prepared by ViTrax Radiochemicals via catalytic tritium exchange of G01. The labeled product was purified by HPLC. The composition of the purified product was verified by co-injection of the tritium labeled product with its cold precursor and both compounds co-chromatographed on an analytical HPLC.

Cellular Aβ production assays and incubation with G01.

Neuroblastoma 2a cells stably overexpressing human APP695 were treated with 10 μM G01 for 3 hr. Cells treated with DMSO, or DMSO plus imatinib, were used as controls. After 3 hr, conditioned medium was collected and Aβ immunoprecipitation was conducted using 4G8 antibody. The immunoprecipitated Aβ was separated on 10-20% Tris-tricine gel, transferred to PVDF membrane and detected by 6E10 antibody.

Synthesis and kinase profiling of biotin-imatinib (active and inactive form): Inactive biotin-imatinib, (IC200001) was synthesized by reacting N-desmethyl imatinib with Biotin-OSu. **Active biotin-imatinib, (IC339239)** was synthesized from the key intermediates, tert-butyl 2-(piperazin-1-yl)ethylcarbamate and 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine, via 4 steps, as shown in supplementary figure 1.

The kinase profiling was performed by Millipore Inc. using the standard assays for Abl kinase and PDGF receptor (ATP = 45 μM). Compound IC200001 showed no significant inhibitory activity toward either kinase, while compound IC339239 had an IC50 of 146 nM against Abl kinase (imatinib had an IC50 of 79 nM) and an IC50 of 6.6 μM against PDGF receptor (imatinib had an IC50 of 4.8 μM). Thus, we refer to IC200001 as "inactive biotin-imatinib" and IC339239 as "active biotin-imatinib".

Construction of APP-CTF truncated forms

APP-CTF-T1 is the truncated form of APP-β-CTF spanning from its N-terminus to HHGV[64]; APP-CTF-T2 is the truncated form of APP-β-CTF spanning from its N-terminus to VMLKK[55]. Both truncated forms were generated by introducing a stop codon in related positions of the CT100 (APP-CTF) in pcDNA4. Mutagenesis was performed using QuickChange Site-Directed Mutagenesis kit (Stratagene) according to the manufacturer's instructions. The primers used for APP-CTF-T1 are: Forward: 5' CATTCATCATGGTGTGTAGGAGGTTGACGCCGC 3'. Reverse: 5' GCGGCGTCAACCTCCTACACACCATGATGAATG 3'. The primers used for APP-CTF-T2 are: Forward: 5' CTTGGTGATGCTGAAGAAGTAACAGTACACATCCATTC 3' Reverse: 5'

GAATGGATGTGTACTGTTACTTCTTCAGCATCACCAAG 3'. The presence of the stop codon and integrity of the cDNA were verified by sequencing.

Construction, expression, and analysis of APP/NICD and NotchΔE/AICD

APP/NICD and NotchΔE coding sequences were synthesized by Genscript Inc. and are illustrated in supplementary Fig. 10. Both sequences were then incorporated into a pcDNA3.1 vector coding for a C-terminal Myc tag. APP-CTF/NICD, APP-CTF, and NotchΔE/AICD were overexpressed separately in HEK293 cells. Immunoprecipitation was conducted using gSAP antibody. APP/NICD and NotchΔE/AICD were detected by c-myc antibody. APP-CTF was detected by 369 antibody.

APP-CTF/NICD, APP-CTF, and NotchΔE/AICD were transfected into N2a cells with/without gSAP siRNA knockdown. NICD was detected by both myc antibody and cleavage specific Val1844 antibody (Cell Signaling); AICD was detected by myc antibody. AICD production from APP-CTF was detected by 369 antibody.

Mouse administration of a γ-secretase inhibitor dibenzazepine (DBZ)

gSAP RNAi mice (6 months old) were administered dibenzazepine (DBZ) (10 μmol/kg) once daily for 5 days by intraperitoneal injection. DBZ was suspended finely in 0.5% (w/v) hydroxypropylmethylcellulose and 0.1% (w/v) Tween 80. Mice were treated with DBZ or with vehicle, with 4 mice in each group. After sacrificing, mouse brain was removed for Aβ ELISA assays (Invitrogen); Mouse intestine was processed for H&E and PAS staining.

Supplementary Figure Legends

Supplementary Figure 1. gSAP action on APP processing. Ternary complex of gSAP, APP and γ-secretase (top) is associated with elevated γ-cleavage (Aβ production) and reduced ε-cleavage (AICD production). In the absence of gSAP (bottom), the binary complex of APP and γ-secretase is associated with decreased γ-cleavage and increased ε-cleavage.

Supplementary Figure 2. **a**: structures of imatinib, G01, and ^{125}I-G01. **b**: G01 significantly reduces levels of Aβ in N2a cells.

Supplementary Figure 3. **a**: Procedures for the synthesis of biotinylated imatinib derivative, IC339239: reagents and conditions: **(a)** 4-(bromomethyl) benzoic acid, K_2CO_3, DMF, room temperature, 2 h. **(b)** 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine, TCFH, DIPEA, DMF, room temperature, overnight. **(c)** TFA, CH_2Cl_2, room temperature, 30 min. **(d)**Biotin-OSu, HOBt, DIPEA, room temperature, overnight, and then HPLC purification. Compound IC200001 was synthesized by reacting N-desmethyl imatinib with Biotin-OSu.

b: Kinase profiling results shows that IC339239 has activities comparable to those of imatinib, while IC200001 showed no activity. Therefore, IC339239 is designated as "active biotin-imatinib" and IC200001 as "inactive biotin-imatinib".

Supplementary Figure 4. Alignments of gSAP sequences among species. Red: identical residues; Blue/Green: conservative substitutions. The C-terminal region of gSAP is highly conserved. The gSAP-16K region is underlined.

Supplementary Figure 5. gSAP mRNA expression levels in different tissues. Tissues from 3 month old wild type BL/6 mice were harvested and gSAP levels were quantitated

using real time PCR. Both actin and GAPDH served as internal controls (n=6). Tissue extracts were adjusted to the same protein levels prior to analysis.

Supplementary Figure 6. gSAP knockdown does not influence the generation of α-, or β-APP-CTF from full length APP. N2a cells overexpressing APP695 were pre-treated with the γ-secretase inhibitor, L685,458 [1 μM] and the cleavages of APP were monitored by pulse-chase labeling (^{35}S-methionine) followed by immunoprecipitation. Proteins were separated by SDS-PAGE and transferred to PVDF membrane for autoradiography.

Supplementary Figure 7. Regulation of AICD production by gSAP. **a.** Either gSAP knockdown or imatinib treatment increases AICD levels in N2a cells overexpressing APP695 (**$p < 0.01$; n=3). **b.** Transfection of gSAP into HEK293 cells overexpressing APP-CTF led to reduced AICD production and increased Aβ production. Cleavage of APP-CTF was monitored by pulse-chase labeling at indicated time point. AICD and Aβ levels after the 3 hr chase were quantitated (**$p < 0.01$; n=3).

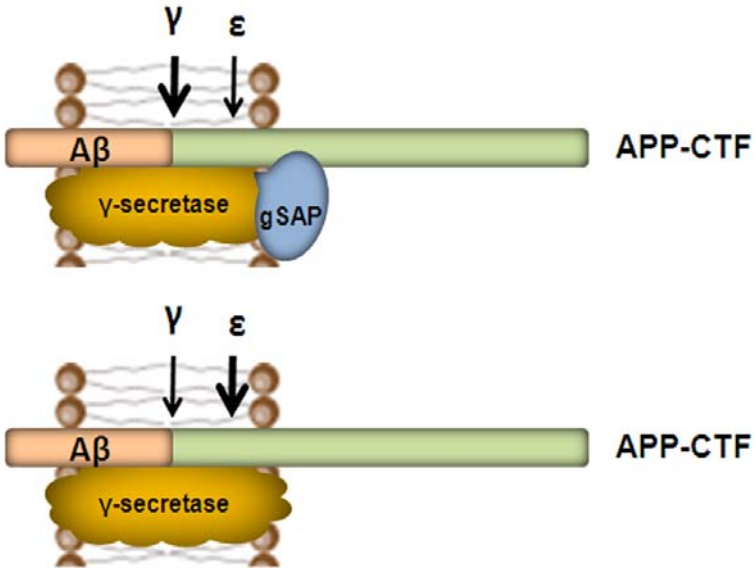
Supplementary Figure 8. After separation of organelles from N2a cells on a continuous sucrose gradient, endogenous gSAP co-localizes with γ-secretase in a Golgi-enriched fraction (No. 6), which also contains endosomes.

Supplementary Figure 9. Truncation of APP-CTF and immunoprecipitation using gSAP antibody through gSAP demonstrates that gSAP interacts with the juxtamenbrane region of APP-CTF. APP-CTF-T1 is the truncated form of APP-β-CTF spanning from its N-terminus to HHGV^{64}; APP-CTF-T2 is the truncated form of APP-β-CTF spanning from its N-terminus to VMLKK^{55}. Truncated forms were overexpressed in HEK293 cells and immunoprecipitated with gSAP antibody. 6E10 antibody was used for immuno-detection.

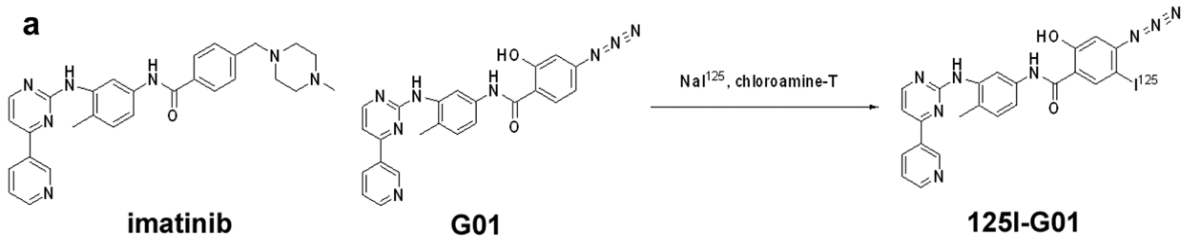
Supplementary Figure 10. Domain exchange studies demonstrate that gSAP regulates ε-cleavage of APP-CTF but not NotchΔE, through selective interaction with AICD. **a.** Design of APP-CTF/NICD and NotchΔE/AICD constructs. **b.** APP-CTF/NICD, APP-CTF, and NotchΔE/AICD were overexpressed separately in HEK293 cells. Both APP-CTF and NotchΔE/AICD interact with gSAP, while APP-CTF/NICD does not. **c.** Effects of gSAP knockdown on the cleavage of APP-CTF/NICD, APP-CTF, and NotchΔE/AICD by γ-secretase. Individual constructs were overexpressed in N2a cells. Upon gSAP knockdown, AICD production from APP-CTF (center panel) as well as from NotchΔE/AICD (right panel) increased, but NICD production from APP-CTF/NICD was not influenced (left panel).

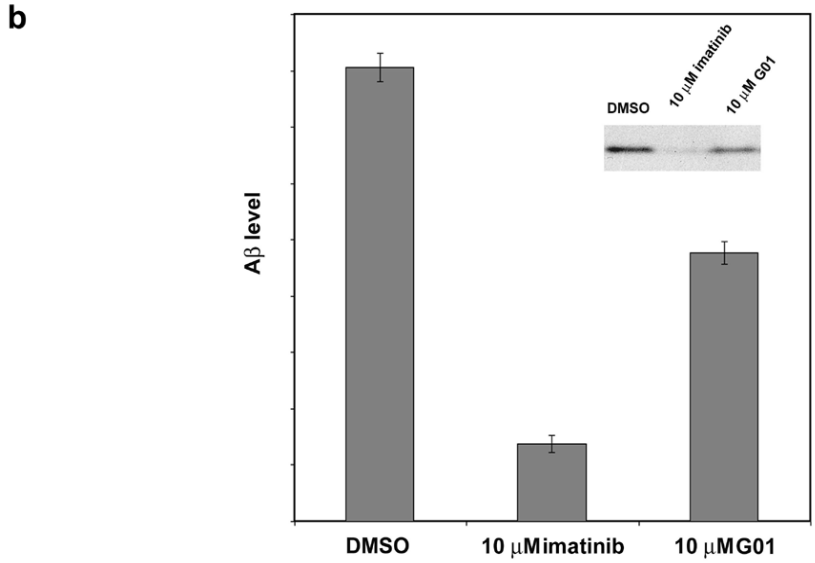
Supplementary Figure 11. Comparison of the effects of either reducing gSAP or of a γ-secretase inhibitor on Aβ levels and histopathology. **a.** Mice were given a γ-secretase inhibitor DBZ (10 μmol/kg) for 5 days. This resulted in reduced Aβ40 and Aβ42 levels of 44±9% and 47±5% respectively. **b.** No histopathological changes were observed in mouse small intestine after gSAP knockdown (H&E and PAS staining). However, increased amounts of violet-stained goblet cells were observed after DBZ administration, a finding typical of Notch inhibition.

Supplementary Figure 1



Supplementary Figure 2





Supplementary Figure 3

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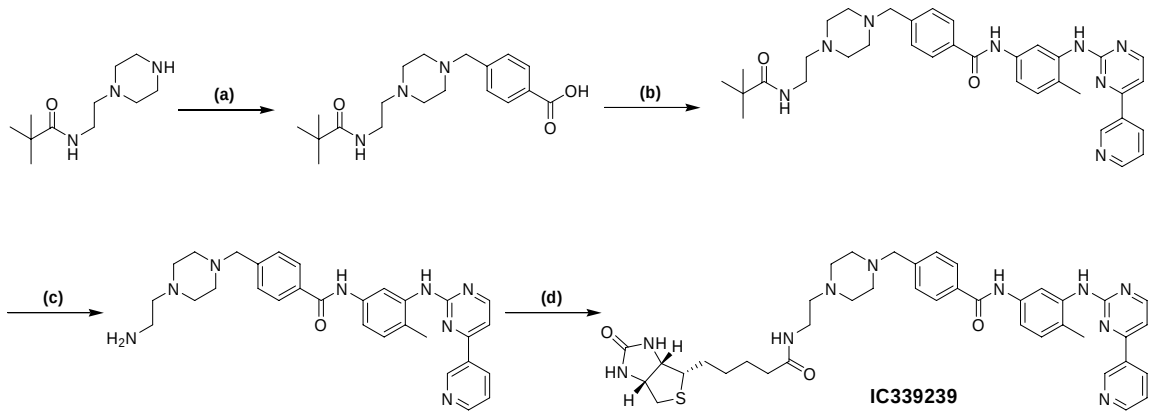

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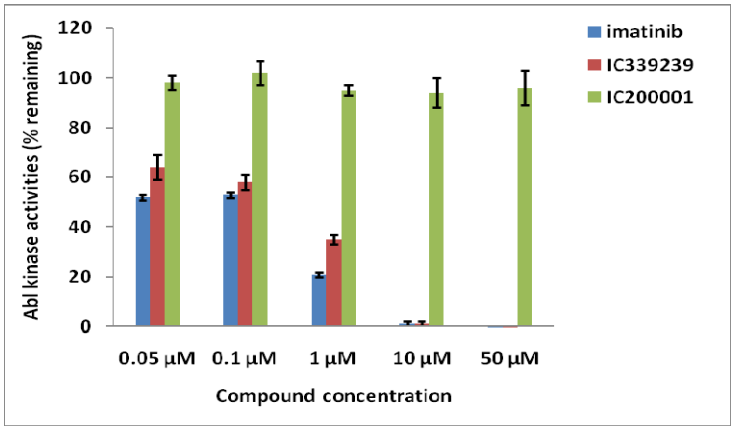

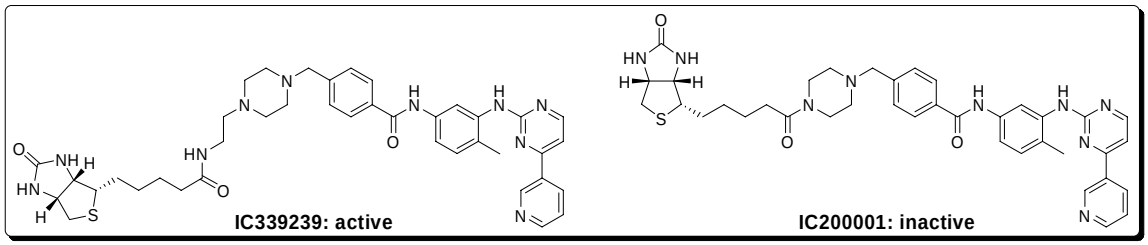

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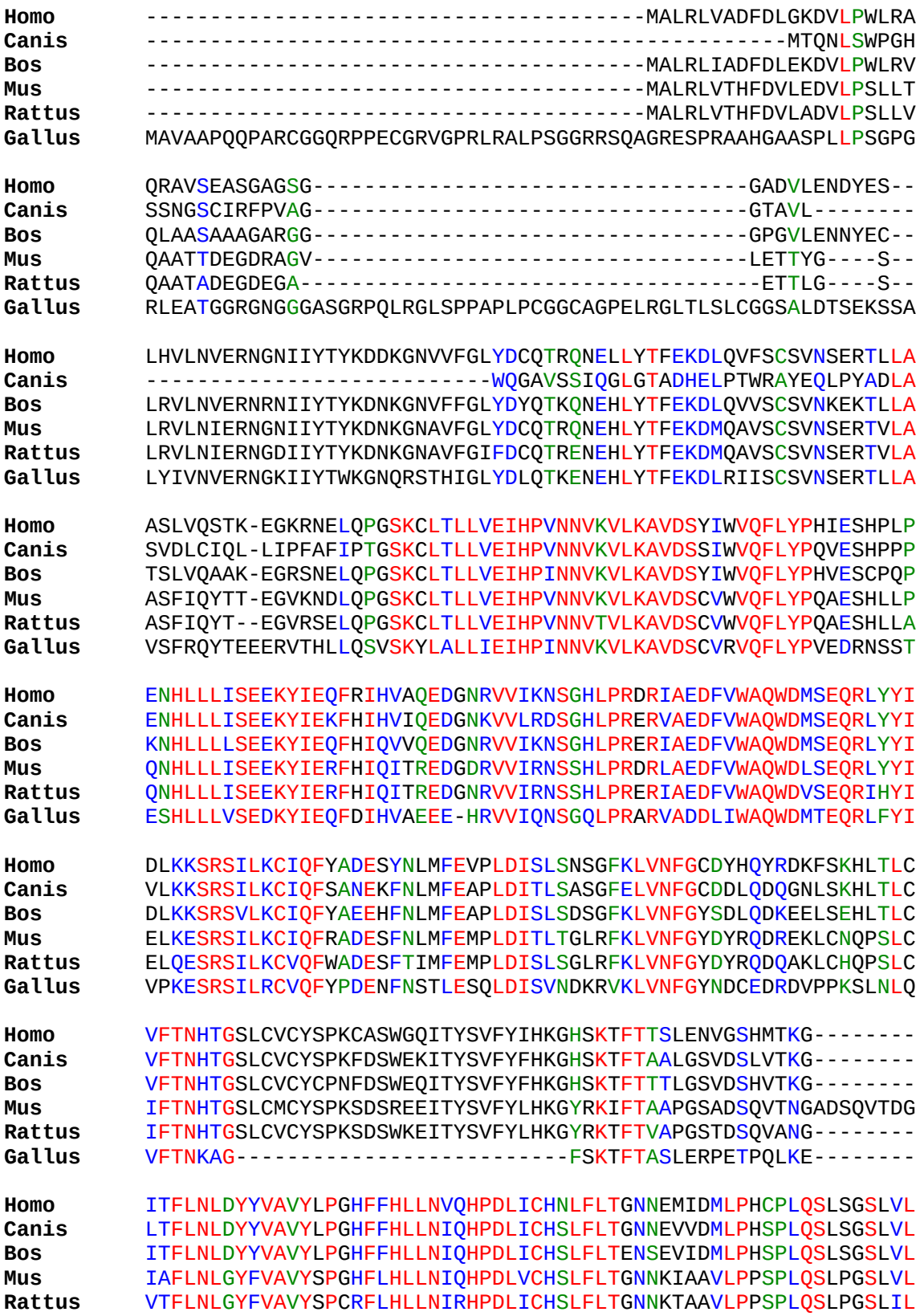

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Canis      DWCSGKLYRALLNQSYLLQFLWNTQLDCEKMAVLHCVLSCGRDPRFLEAKIIQWISENIS
Bos        DSRSGKLYRVLLNQSYLVEFLRSARLDCERMALLHCALSHGRDPRRLEAKIIQWISENIS
Mus        DCYSGKVYRVTLDQSYLLRFLWNAHLDCERMAALHCILSCSQDPGFPEEQIIQWISEHVS
Rattus     DCSSGKVYRATLDQSYLMGFLWNAQLDCEKMAALHCALSCDSDPGFPE-QIVQWVSERVS
Gallus     DCCIGRLYAMSISDSALLKYLQNSKRDSERLAALHCALLCVRRTTDLEMKIIWWISENLS

Homo       ACHSFDLIQEFIIASSYWSVYSETSNMDKLLPHSSVLTWNTEIPGITLVTEDIALPLMKV
Canis      TCHSFDLIQEFIIASSYWSIYPETSNIDKLLPYSSVLTWNTEIPGITLVTEEITLPFMKV
Bos        ACHSFDLIQEFIIASSYWSIYPETSNMDKLLPYSSLLTWDTEIPGITLVTEEIPLPLMKV
Mus        ACHSFDLIQEFLIASSYWSVYAELDDMGMLLQYSSVLTWNTEIPGIKFTTEELPLPLMKV
Rattus     ACHSFDLIQEFLIASSYWSVYPGLDDVDLLLPYSSVLTWDTEIPGMKLVTEELPLPLMKV
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Homo       LSFKGYWEKLNSNLEYVKYAKPHFHYNNSVVRREWHNLISEEKTGKRRSAAYVRNILDNA
Canis      HSFKGYWEKLNSNLEYVKCSKPCLLYNNSMVKREWHSLISEEKTGRRRSMVYVRNIFDNA
Bos        HSFKGYWEKLNSNLEYVKYSKPHLHYNNSVVRREWHNLISEEKTGKRRSTVYVRNILDNA
Mus        YGLKGYWAKLNSNLEYIKYTKPHLHYHNSVVRREWHNLISEERTGKRRSTMYVRNILENA
Rattus     YSLKGYWAKLNSNLEYIKYTKPHLHYHNSVVRREWHNLISEERTGKRRSTMYVRNILDNA
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Homo       VKVISNLEARNLGPRLTPLLQEEDSHQRLLMGLMVSELKDHFLRHLQGVEKKKIEQMVLD
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Bos        YVSKLLDLICQILEASWRKHNLHPWALHFNRQASAAEFAVFHIMTRILEATNTLFLPLPP
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Canis      GFHTLHTILGVHCLPLHNLLHYIDSGVLLLTETAVIRLMKDLDNSENNEKLKFSIIVRLP
Bos        GFHTLHMILGVRCLPLHNLLHYIDHGVLLLTEAAVTRLMKDLDNTEKNEKLKFSIIMRLP
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Rattus     PL----IGQKVCRLWDHPMSSNIISRNHVAQLLKNY-KKEPQSSMIDKSSFPVEFLPLNY
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Bos        FIHILTDIESSNPALYAFEGHDNVDAKFVEEAALKHTAMLLGL---
Mus        FIEILMGLESSNQALYGFEGHDNVDAEFVEEAALKHTTMLLGL---
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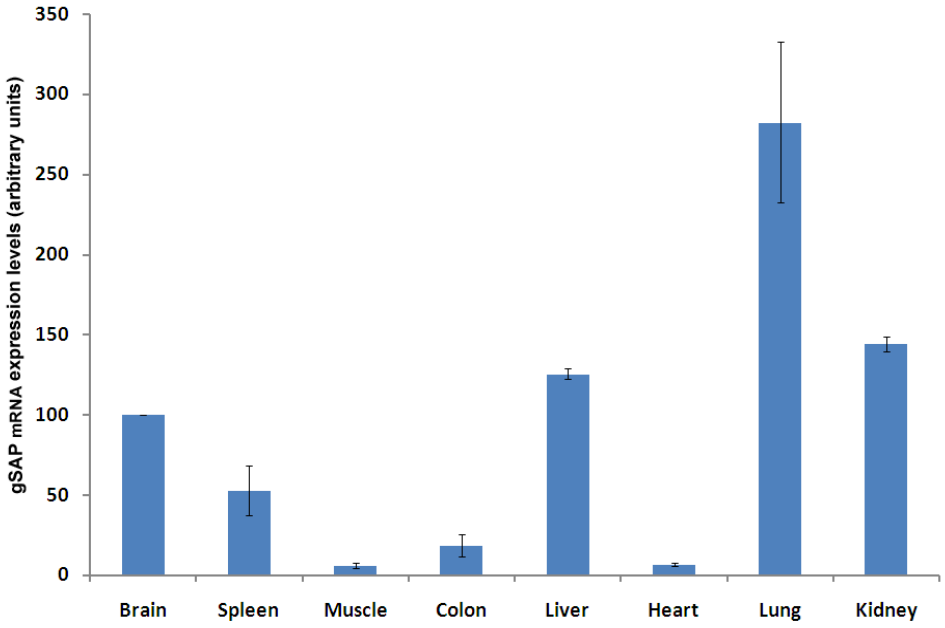
Supplementary Figure 5.



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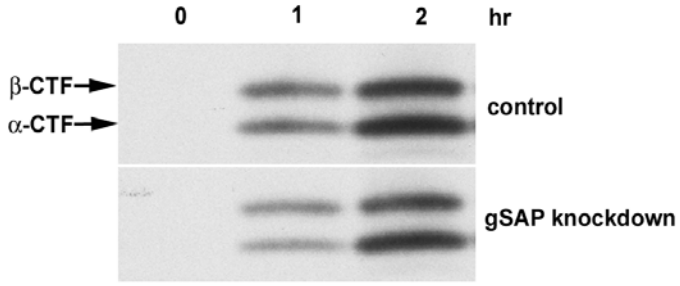

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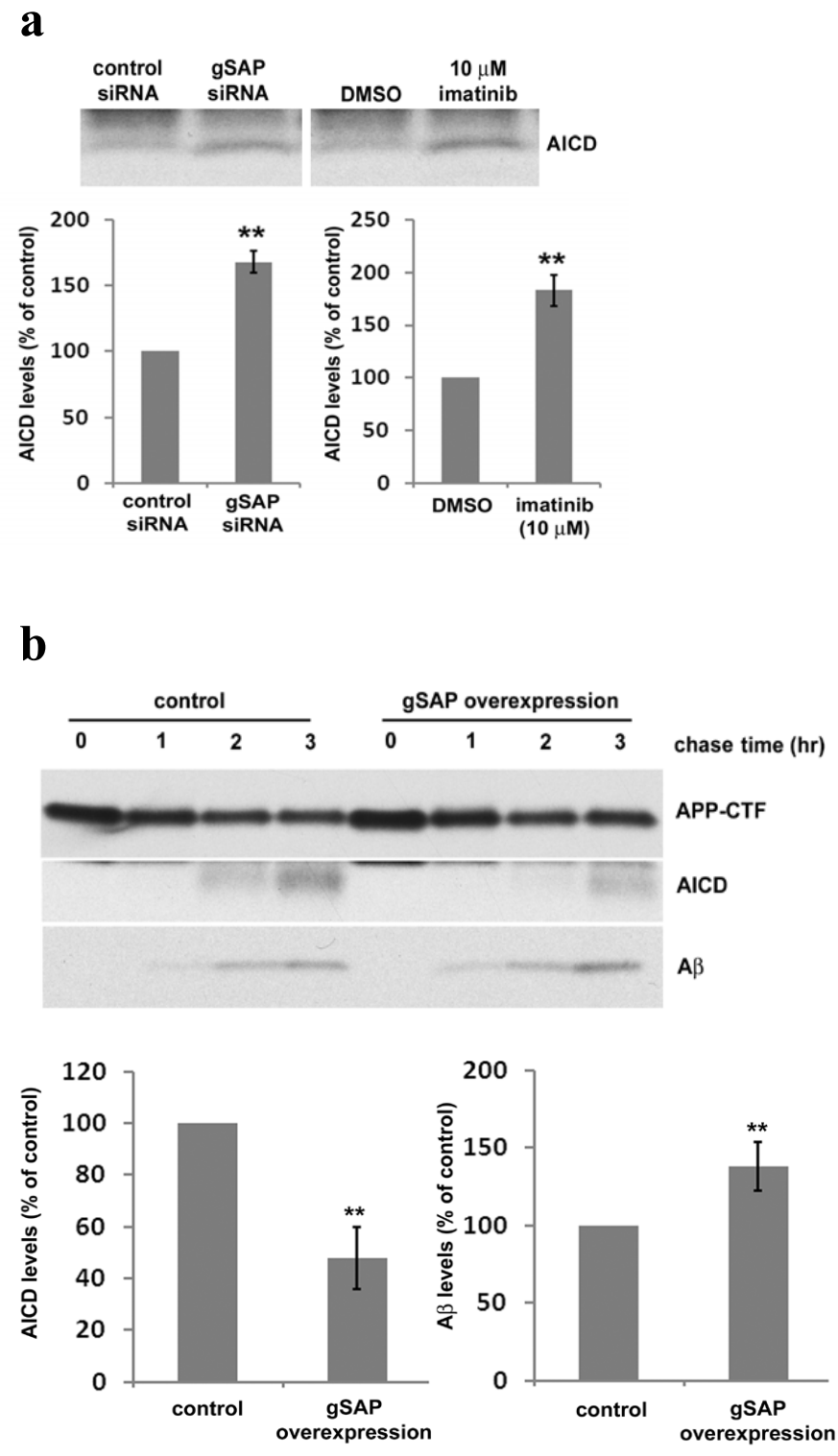
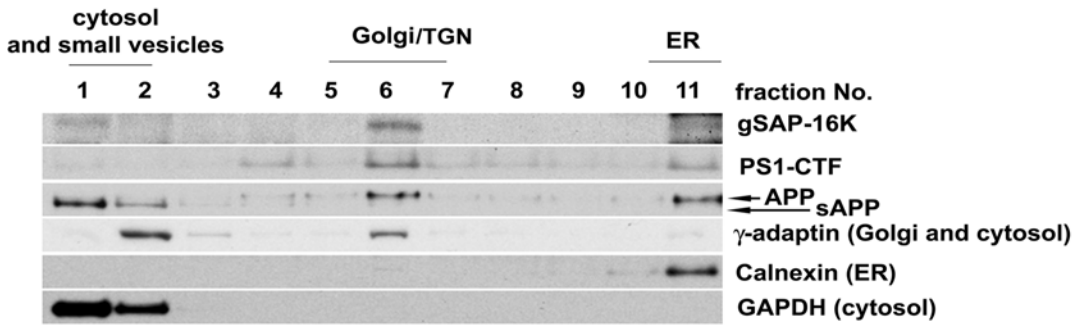
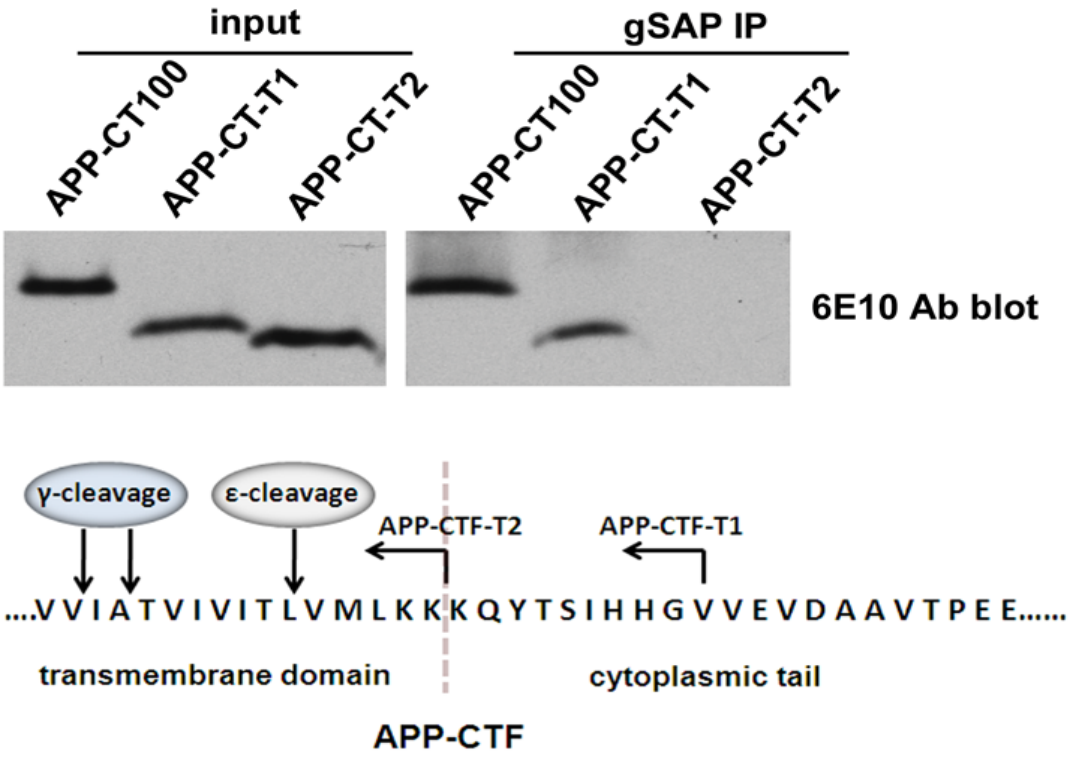


Supplementary Figure 8



Supplementary Figure 9



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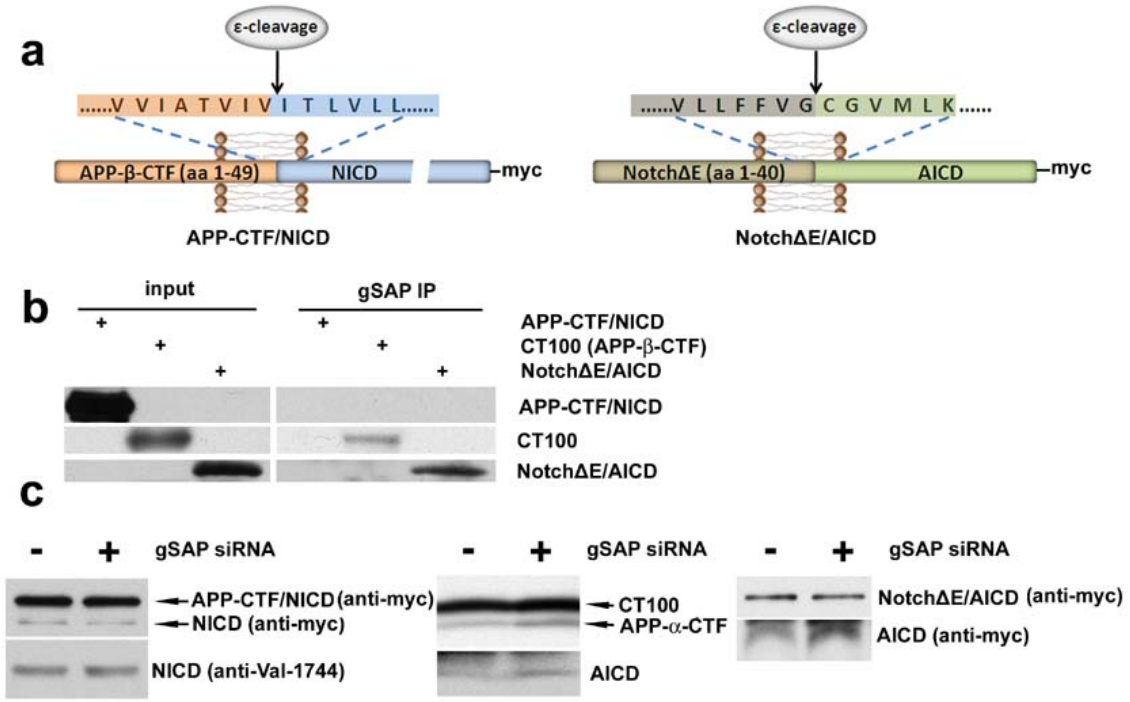

Supplementary Figure 11

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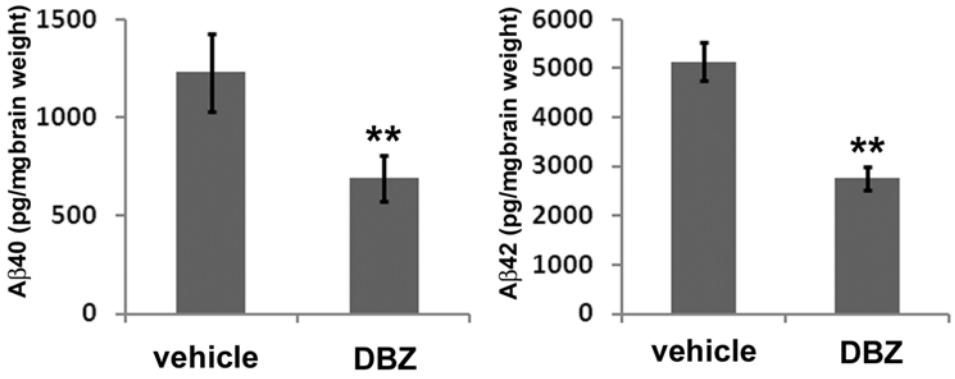

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